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Design, Synthesis, and Structure–Activity Relationship of Quinazolinone Derivatives as Potential Fungicides

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ABSTRACT: Plant diseases caused by phytopathogenic fungi reduce the yield and quality of crops. To develop novel antifungal agents, we designed and synthesized eight series of quinazolinone derivatives and evaluated their anti-phytopathogenic fungal activity. The bioassay results revealed that compounds **KZL-15**, **KZL-22**, **5b**, **6b**, **6c**, **8e**, and **8f** exhibited remarkable antifungal activity *in vitro*. Especially, compound **6c** displayed the highest bioactivity against *Sclerotinia sclerotiorum*, *Pellicularia sasakii*, *Fusarium graminearum*, and *Fusarium oxysporum*, displaying appreciable IC_{50} values (50% inhibitory concentration) of 2.46, 2.94, 6.03, and 11.9 μg/mL, respectively. A further mechanism interrogation revealed abnormal mycelia, damaged organelles, and changed permeability of cell membranes in *S. sclerotiorum* treated with compound **6c**. In addition, the *in vivo* bioassay indicated that compound **6c** possessed comparable curative and protective effects (87.3 and 90.7%, respectively) to the positive control azoxystrobin (89.5 and 91.2%, respectively) at 100 μg/mL concentration against *S. sclerotiorum*. This work validated the potential of compound **6c** as a new and promising fungicide candidate, contributing to the exploration of potent antifungal agents.

KEYWORDS: *phytopathogenic fungi, quinazolinone, Sclerotinia sclerotiorum*

INTRODUCTION

Plant diseases caused by phytopathogenic fungi reduce crop yields and dampen the quality and safety of agricultural products, causing severe production losses of agricultural and horticultural crops worldwide and threatening global food security and public health.[1,2] For instance, *Sclerotinia sclerotiorum*, a phytopathogenic fungus distributed globally, invades hundreds of crop species and is responsible for *Sclerotinia* stem rot of oilseed rape (*Brassica napus* L.), which is most devastating for its production.[3−5] The immense loss caused by phytopathogenic fungi led to the wide application of various antifungal agents. However, the misuse and overuse of many traditional fungicides for a long time have caused toxicity, altered pharmacokinetics, and ever-rising resistance.[6] Thus, it is urgently needed to discover novel fungicides featuring a unique acting mechanism, optimized targeting ability, and safety to the environment.[7]

Natural products are highly efficient, low-toxic, environmentally friendly, and meet the requirements of the Environmental Action Plan (EAP). For example, validamycin isolated from *Streptomyces* shows significant activity against some phytopathogenic fungi.[8] Nevertheless, many of these products are difficult to obtain, poorly soluble, and instable during metabolism, which severely hinders their further application,[9] availability, solubility, and instability; these problems can be addressed by structural optimization of natural products, which is an effective strategy for discovering new antifungal agents.

The 4(3*H*)-quinazolinone skeleton belongs to the N-containing heterocyclic building block and is composed of a benzene ring and a pyrimidine ring,[10] This skeleton is widely present in natural products, including luotonin A,[11] tryptanthrin,[12] evodiamine,[13] rutaecarpine,[14] quinazolinone, and 2-methylquinazolinone (Figure 1). Its derivatives display various pharmacological properties, such as antifungal,[15] antibacterial,[16] antimalarial,[17] anticancer,[18] and antiviral[19] activities. In addition, the backbone of 4(3*H*)-quinazolinone is also critical for the prevention and treatment of agricultural diseases; commercial fungicides fluquinconazole and proquinazid are two representative drugs (Figure 1). Because of the significant value in pharmaceuticals, the synthesis and bioactivity of 4(3*H*)-quinazolinone and its derivatives have drawn considerable interest. On the other hand, hydrazide has been widely used as a functional fragment in drug design owing to a lot of its derivatives exhibiting various bioactivities, such as anticancer,[20] antibacterial,[21] antifungal,[22] insecticidal,[23] and herbicidal[24] activities. Tebufenozide and chromafenozide (Figure 1) are two compounds bearing a hydrazide group, which have been launched as commercial insecticides.[25]

Based on the above considerations and our consistent efforts in developing antimicrobial natural alkaloid derivatives for agricultural applications,[26−28] herein, we used the structure–activity relationship (SAR) obtained from screening the antifungal activities of the six natural products containing the 4(3*H*)-quinazolinone skeleton in Figure 1 to guide the design and synthesis of eight series of 4(3*H*)-quinazolinone

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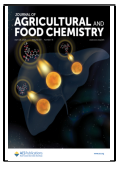

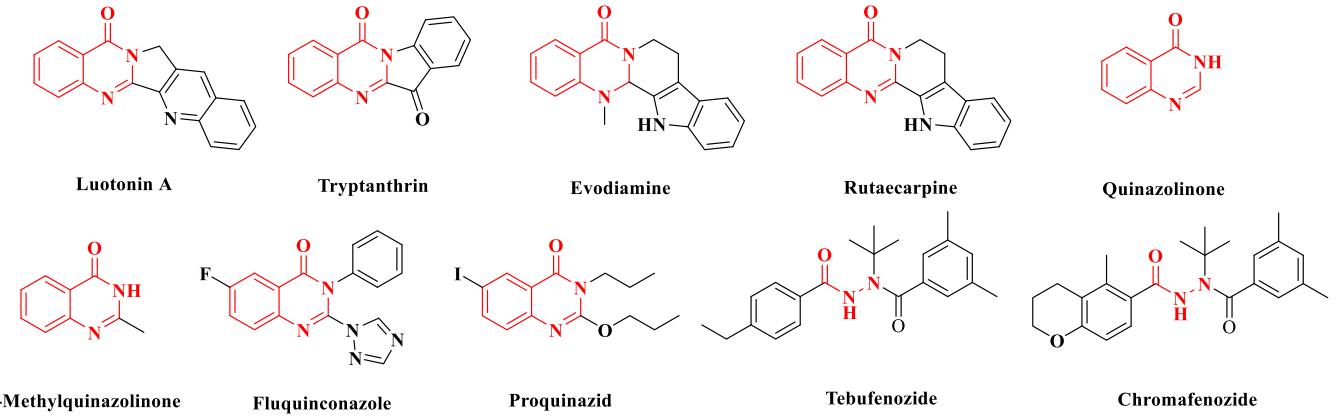

Figure 1. Chemical structures of several natural products and commercial drugs containing the quinazolinone backbone, hydrazide.

derivatives. Subsequently, these synthesized compounds were tested against *S. sclerotiorum*, *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium graminearum*, *Pellicularia sasakii*, and *Fusarium oxysporum*. Afterward, a pot experiment was performed with *S. sclerotiorum* to further evaluate the prospective application of developing 4(3*H*)-quinazolinone derivatives as agricultural fungicides. Furthermore, preliminary action mechanism investigations were conducted for evaluating morphological changes, relative electric conductivity, and cytoplasmic content leakage.

MATERIALS AND METHODS

Instruments and Chemicals. A WRS-2U melting point apparatus (Shanghai Precision Instrument Co., Ltd., China) was used to determine the uncorrected melting points (mp). A Bruker AM-400 spectrometer (Bruker Company, Billerica, MA, US.) was used to obtain the ^{1}H and ^{13}C NMR spectra of compounds (with tetramethylsilane as the internal standard). Mass spectra were recorded using a Bruker Daltonics APEXII49e spectrometer (Bruker Daltonics Inc., Billerica, MA, USA) with an ESI or EI ionization source. A scanning electron microscope (Hitachi, S-3400N, Japan) and a transmission electron microscope (Hitachi, HT7700, Japan) were used for morphological observation. Hypha relative conductivity and cytoplasmic content leakage were obtained with a conductivity meter (Leici DDS-307, China) and a UV−vis spectrophotometer (UV1902 PC, China). Commercial solvents and reagents used were of reagent grade, and the commercial fungicide azoxystrobin was used as positive control in the assay.

Fungi. *S. sclerotiorum*, *R. solani*, *B. cinerea*, *F. graminearum*, and *F. oxysporum* were provided by Gansu Academy of Agricultural Sciences and *P. sasakii* was obtained from Huazhong Agricultural University. These fungi were maintained in a potato dextrose agar medium at 4 °C.

Synthetic Procedures. The synthetic route of target compounds **KZL-1**−**KZL-34** and **1a**−**8i** is outlined in Schemes 1−3 (Figure 1).

*Synthesis of Key Intermediate **1(A)**.* A stirred solution of substituted aminobenzoic acid (0.500 g, 3.65 mmol) in trifluoroacetic anhydride or acetic anhydride (6 mL) was refluxed for 4 h. The reaction was concentrated under reduced pressure to a brown oil.[29]

*Synthesis of Quinazolinone **KZL-1**.* In a 25 mL flask, the mixture of 2-aminobenzoic acid (0.500 g, 3.65 mmol) and formamide (2.90 mL, 73.0 mmol) was refluxed at 150 °C for 7 h. Then, a white precipitate was formed, filtered, washed with water, and dried to give **KZL-1**.

*General Methods for the Preparation of Compounds **KZL-2**−**KZL-16**.* In a 50 mL flask, ammonium acetate (1.90 g, 24.7 mmol) was added to the corresponding intermediate **A** (0.700 g, 2.47 mmol) and refluxed for 60 min. Then, the reaction mixture was transferred into stirred ice-water, filtered, and the precipitate was obtained. The crude product was washed with water and dried to obtain **KZL-2**−**KZL-16**.[30]

*General Synthetic Procedure for Compounds **KZL-17**−**KZL-34**.* Hydrazine hydrate (0.0600 mL, 1.27 mmol) was added dropwise to a stirred suspension of intermediate **A** (0.300 g, 1.06 mmol) in ethanol (5 mL). The solution was refluxed and monitored by thin-layer chromatography (TLC). After cooling and in vacuo concentration, H_2O (20 mL) was added and ethyl acetate (EtOAc, 3 × 20 mL) was extracted, which was dried under $MgSO_4$ and concentrated in vacuo to obtain a crude product. Purification by column chromatography (with 9:1 petroleum ether/EtOAc) afforded **KZL-17**−**KZL-34**.[31]

*Synthesis of Key Intermediate **2**.* In a 50 mL flask, an appropriate amount of triethylamine was added dropwise to a mixture solvent of glycine methyl ester hydrochloride (0.310 g, 2.47 mmol) and intermediate **1** in benzene (20 mL). After refluxing for 10 h, water was added and extracted with EtOAc (3 × 20 mL), dried with $MgSO_4$, and concentrated. Target compounds were harvested after purification by flash chromatography (using 8:1 petroleum ether/EtOAc).[32]

*Synthesis of Key Intermediate **3**.* In a 50 mL flask, a solution of lithium hydroxide (0.190 g, 5.00 mmol) in H_2O was dropped into intermediate **2** (0.700 g, 2.47 mmol) in tetrahydrofuran (THF) (20 mL). The solution was maintained at room temperature for 3 h and monitored by TLC, followed by reduced pressure evaporation. The residue was diluted with 2 N hydrochloric acid and extracted with dichloromethane (DCM). Target compounds were finally harvested after drying with $MgSO_4$ and evaporated and purified by flash chromatography (using 10:1 DCM /methanol).

*General Methods for the Preparation of Compounds **1a**−**1g**, **2a**.* At 0 °C, to a suspension of intermediate **2** (5.00 g, 28.4 mmol) in dry DCM, the corresponding amine (0.210 mL, 2.00 mmol), hydroxybenzotriazole (0.270 g, 2.00 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.380 g, 2.00 mmol) were added successively. The reaction mixture was stirred for 24 h at room temperature. After confirmation of the completion of the reaction by TLC, the residue was washed with brine, dried over anhydrous $MgSO_4$, and after removing the solvent was purified by silica gel column chromatography (6:1 petroleum ether/EtOAc) to give **1a**−**1g** and **2a**.[33]

*Synthesis of Key Intermediate **4**.* In a 50 mL flask, hydrazine hydrate (0.360 mL, 7.41 mmol) was dropped into intermediate **2** (0.700 g, 2.47 mmol) solution in methanol (20 mL) at room temperature while stirring and then refluxed for a short time; a white solid was formed and that was collected by filteration.[34]

*General Synthetic Procedure for Compounds **3a**−**3g**.* In a 25 mL flask, intermediate **4** (0.350 g, 1.00 mmol) was mixed with a corresponding benzaldehyde (0.110 mL, 1.05 mmol) solution in ethanol (10 mL) and was refluxed for 2 h. After cooling, the crude product was collected by filtration, washed, and recrystallized from hot ethanol to yield **3a**−**3g**.[35]

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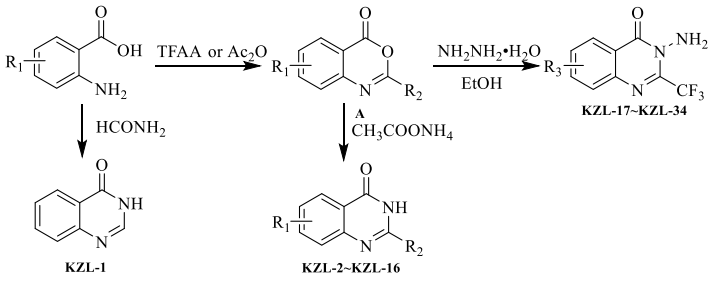
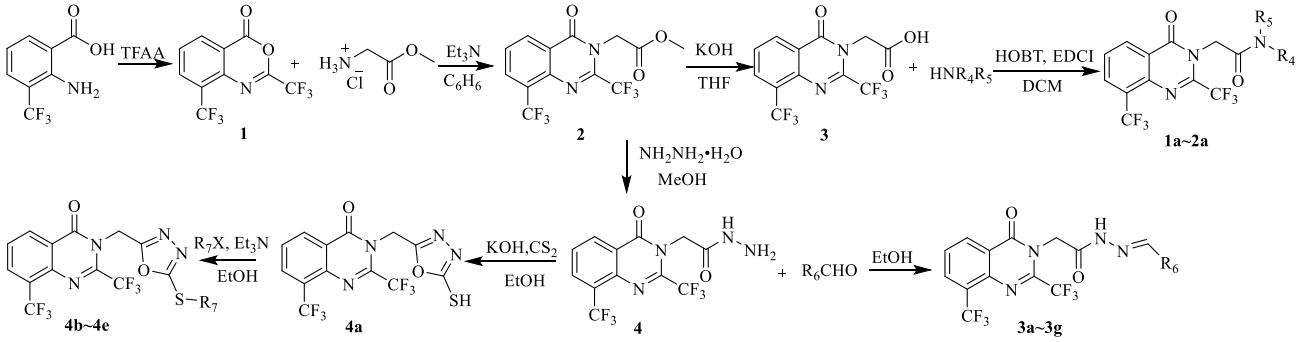
Scheme 1. Synthesis for compounds **KZL-1**, **KZL-2**~**KZL-16**, **KZL-17**~**KZL-34.**

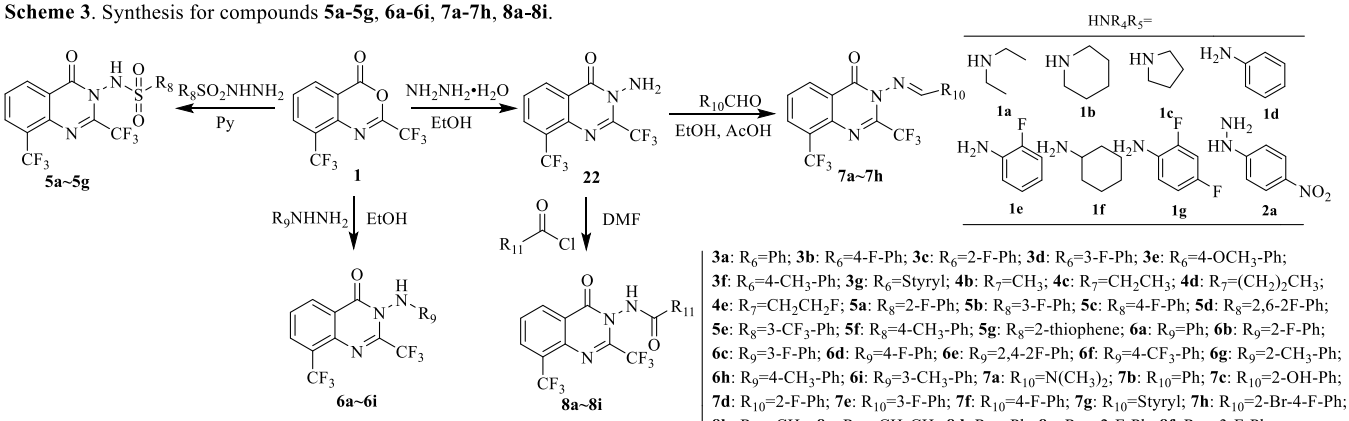


KZL-2: R_1=H, R_2=CH_3; **KZL-3**: R_1=H, R_2=CF_3;
KZL-4: R_1=6-F, R_2=CH_3;**KZL-5**: R_1=8-F, R_2=CH_3;
KZL-6: R_1=8-CH_3, R_2=CH_3; **KZL-7**: R_1=6-F, R_2=CF_3;
KZL-8: R_1=8-F, R_2=CF_3; **KZL-9**: R_1=5-F, R_2=CF_3;
KZL-10: R_1=7-F, R_2=CF_3; **KZL-11**: R_1=5-CH_3, R_2=CF_3;
KZL-12: R_1=6-CH_3, R_2=CF_3; **KZL-13**: R_1=7-CH_3, R_2=CF_3;
KZL-14: R_1=8-CH_3, R_2=CF_3; **KZL-15**: R_1=8-CF_3, R_2=CF_3;
KZL-16: R_1=8-CF_3, R_2=CH_3; **KZL-17**: R_3=H; **KZL-18**: R_3=7-F;
KZL-19: R_3=6-F; **KZL-20**: R_3=8-F;**KZL-21**: R_3=5-F;
KZL-22: R_3=8-CF_3; **KZL-23**: R_3=8-CH_3; **KZL-24**: R_3=7-CH_3;
KZL-25: R_3=6-CH_3; **KZL-26**: R_3=5-CH_3; **KZL-27**: R_3=8-Cl;
KZL-28: R_3=7-Cl; **KZL-29**: R_3=6-Cl; **KZL-30**: R_3=5-Cl;**KZL-31**: R_3=6-OCH_3;
KZL-32: R_3=6,7-2-OCH_3; **KZL-33**: R_3=6-Cl, 8-Br; **KZL-34**: R_3=6-Cl, 8-CH_3

Scheme 2. Synthesis for compounds **1**, **2**, **3**, **4**, **1a-2a**, **3a-3g**, **4a-4e**.



Scheme 3. Synthesis for compounds **5a-5g**, **6a-6i**, **7a-7h**, **8a-8i**.



3a: R_6=Ph; **3b**: R_6=4-F-Ph; **3c**: R_6=2-F-Ph; **3d**: R_6=3-F-Ph; **3e**: R_6=4-OCH_3-Ph;
3f: R_6=4-CH_3-Ph; **3g**: R_6=Styryl; **4b**: R_7=CH_3; **4c**: R_7=CH_2CH_3; **4d**: R_7=$(CH_2)_2CH_3$;
4e: R_7=CH_2CH_2F; **5a**: R_8=2-F-Ph; **5b**: R_8=3-F-Ph; **5c**: R_8=4-F-Ph; **5d**: R_8=2,6-2F-Ph;
5e: R_8=3-CF_3-Ph; **5f**: R_8=4-CH_3-Ph; **5g**: R_8=2-thiophene; **6a**: R_9=Ph; **6b**: R_9=2-F-Ph;
6c: R_9=3-F-Ph; **6d**: R_9=4-F-Ph; **6e**: R_9=2,4-2F-Ph; **6f**: R_9=4-CF_3-Ph; **6g**: R_9=2-CH_3-Ph;
6h: R_9=4-CH_3-Ph; **6i**: R_9=3-CH_3-Ph; **7a**: R_{10}=$N(CH_3)_2$; **7b**: R_{10}=Ph; **7c**: R_{10}=2-OH-Ph;
7d: R_{10}=2-F-Ph; **7e**: R_{10}=3-F-Ph; **7f**: R_{10}=4-F-Ph; **7g**: R_{10}=Styryl; **7h**: R_{10}=2-Br-4-F-Ph;
8b: R_{11}=CH_3; **8c**: R_{11}=CH_2CH_3;**8d**: R_{11}=Ph; **8e**: R_{11}=2-F-Ph; **8f**: R_{11}=3-F-Ph;
8g: R_{11}=4-F-Ph; **8h**: R_{11}=2,4-2F-Ph; **8i**: R_{11}=4-CH_3-Ph

Figure 2. Synthesis route of target compounds.

*Synthesis of Target Compound **4a**.* In a 25 mL flask, to a suspension of KOH (0.170 g, 3.00 mmol) in ethanol (10 mL), **4** (0.350 g, 1.00 mmol) and CS_2 (0.150 mL, 2.50 mmol) were mixed. After 4 h of refluxing, the solvent was concentrated under reduced pressure, extracted with DCM (3 × 20 mL), dried with $MgSO_4$, and concentrated. Purification by column chromatography (50:1 DCM /methanol) gave **4a**.[36]

*Synthesis of Compounds **4b-4e**.* In a 25 mL flask, the corresponding alkyl halide (0.0600 mL; 1.00 mmol) was dissolved in a suspension of compound **4a** (0.400 g, 1.00 mmol) and triethylamine (0.140 mL, 1.00 mmol) in EtOH (15 mL). After refluxing for 8 h, evaporation was performed, followed by purification of the residue by column chromatography (80:1 DCM /methanol) to harvest **4b**−**4e**.[37]

*General Synthetic Procedure for Compounds **5a**−**5g**.* In a 25 mL flask, a solution of **1** (0.300 g, 1.06 mmol) in pyridine (3 mL) was added to substituted benzenesulfonyl hydrazide (0.200 g, 1.17 mmol), stirred at 78 °C for 7 h; after that, the solution was concentrated, poured into ice-cold water, and acidified with 2 N hydrochloric acid; then, the solution was extracted with EtOAc (3 × 20 mL), dried with $MgSO_4$, and evaporated under reduced pressure. The target compound was harvested after purification by column chromatography (120:1 DCM /methanol).[38]

*General Synthetic Procedure for Compounds **6a**−**6i**.* In a 25 mL flask, the corresponding substituted phenylhydrazine (0.200 mL, 2.12 mmol) was added to a stirred solution of intermediate **A** (0.300 g, 1.06 mmol) in ethanol (5 mL) and monitored by TLC for completion. The reaction mixture was refluxed for 7 h and concentrated under vacuum. Then, it was poured into H_2O (20 mL), extracted with EtOAc, and dried over anhydrous $MgSO_4$; the solvent was removed and purification of the residue by Column chromatography (15:1 petroleum ether/EtOAc) gave **6a**−**6i**.[39]

*General Synthetic Procedure for Compounds **7a**−**7h**.* Compound **22** (0.300 g, 1.01 mmol) was mixed with aromatic aldehydes (0.100 mL, 1.01 mmol) in ethanol (8 mL) that contained glacial acetic acid (0.580 mL, 10.1 mmol) and refluxed for 7 h with TLC monitoring. The solution was concentrated, dissolved in EtOAc (10 mL), washed with H_2O (20 mL), dried under $MgSO_4$, and concentrated. Compounds **7a**−**7h** were obtained after purification of the residue by column chromatography (10:1 petroleum ether/EtOAc).[40]

*General Synthetic Procedure for Compounds **8a**−**8i**.* In a 25 mL flask, a solution of **22** (0.300 g, 1.06 mmol), dry dimethyl formamide

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Table 1. Antifungal Activity of Six Natural Products Containing a Quinazolinone Skeleton

compd.[a]	conc.[b] (μg/mL)	average inhibition rate ± SD (%) (n = 3)					
		S. s.[c]	*R. s.*[c]	*B. c.*[c]	*F. g.*[c]	*P. s.*[c]	*F. o.*[c]
luotonin A	100	56.3 ± 0.3	0.00 ± 0.00	66.7 ± 0.2	0.00 ± 0.00	0.00 ± 0.00	12.5 ± 0.3
	50	40.0 ± 0.1	0.00 ± 0.00	34.2 ± 0.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
tryptanthrin	100	36.7 ± 0.0	31.5 ± 0.5	0.00 ± 0.00	2.92 ± 0.05	0.00 ± 0.00	35.3 ± 0.3
	50	0.00 ± 0.00	21.7 ± 0.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	19.0 ± 0.2
evodiamine	100	0.00 ± 0.00	9.03 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
rutaecarpine	100	0.00 ± 0.00	5.90 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.48 ± 0.21
	50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
quinazolinone	100	0.00 ± 0.00	41.7 ± 0.2	51.5 ± 0.3	8.97 ± 0.180	44.2 ± 0.4	31.7 ± 0.4
	50	0.00 ± 0.00	9.67 ± 0.27	23.0 ± 0.4	8.04 ± 0.26	22.2 ± 0.3	12.9 ± 0.1
2-methyl quinazolinone	100	0.00 ± 0.00	32.8 ± 0.3	15.5 ± 0.5	30.0 ± 0.2	27.9 ± 0.1	28.5 ± 0.3
	50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.6 ± 0.2	19.4 ± 0.2	24.2 ± 0.3

[a]Compd.: compound. [b]Conc.: concentration. [c]*S. s.*: *Sclerotinia sclerotiorum*. *R. s.*: *Rhizoctonia solani*. *B. c.*: *Botrytis cinerea*. *F. g.*: *Fusarium graminearum*. *P. s.*: *Pellicularia sasakii*. *F. o.*: *Fusarium oxysporum*.

(3 mL), and substituted acid chloride (0.230 mL, 3.18 mmol) was stirred at 0 °C for 30 min. Then, after overnight reaction at room temperature with TLC monitoring, the reaction was transferred into ice-cold water with stirring. The mixture was then subjected to EtOAc (3 × 20 mL) extraction, washed with water, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. Compounds **8a**−**8i** were obtained after purification of the residue by column chromatography (50:1 petroleum ether/EtOAc).[41]

The spectral data of all compounds are provided in the Supporting Information.

Antifungal Bioassay *In Vitro*. The antifungal activity of six natural products and synthetic compounds was assayed against six pathogenic fungi (*S. sclerotiorum*, *R. solani*, *B.cinerea*, *F. graminearum*, *P. sasakii*, and *F. oxysporum*) using the mycelial growth inhibitory rate method.[42] The potato dextrose agar (PDA) medium was sterilized at 121 °C for 20 min. Test compounds were dissolved in dimethyl sulfoxide (DMSO) before mixing with PDA at 40−50 °C, with compound concentrations in the medium of 100 and 50 μg/mL, respectively. The mixture was then poured into sterilized Petri dishes. After cooling, a sterilized inoculation needle was used to pick up test fungi with a mycelial disk of approximately 5 mm diameter, which were then inoculated in the center of PDA Petri dishes. 0.5% DMSO was used as blank control, while different concentrations of azoxystrobin served as positive control. When the Petri dish of blank control was completely occupied by fungi, mycelium diameters were measured and used to determine the inhibition rate using the formula

$$\text{inhibition rate (\%)} = (C - T)/(C - 5\ \text{mm}) \times 100$$

where C represents the fungal growth diameter on untreated PDA and T represents the fungal growth diameter on treated PDA. The colony diameter of all samples was measured in triplicate and determined by the cross-bracketing method. Otherwise, anti-bioassay compounds were prepared in fresh PDA Petri dishes with concentrations of 100, 50, 25, 10, 5, 2.5, 1, and 0.5 μg/mL, and the IC_{50} values (median inhibitory concentration) were further determined.

Antifungal Bioassay *In Vivo*. Based on the *in vitro* results of antifungal activity, compound **6c** was further tested for protective and curative activity against *S. sclerotiorum* according to our previous work.[26] Cole leaves with similar shapes were picked for testing. Afterward, the leaves were soaked in 1% sodium hypochlorite for 2 min for disinfection, which was followed by rinsing with running water and blotting with sterile filter paper. The difference between the protective activity assay and the curative activity assay was the time of inoculation. The 5 mm agar disk containing mycelia was placed carefully at the widest central part of the rapeseed leaf while avoiding placing it on the main vein. To evaluate the protective activity, the leaves were sprayed with **6c** of different concentrations. Inoculation was conducted when the liquid flowed on the surface at 24 h. To test the curative activity, inoculation was conducted after the leaves were sprayed with **6c** of different concentrations with liquid flowing at 24 h. DMSO and azoxystrobin were used as negative control and positive control, respectively. After inoculation, the leaves were incubated at 25 °C with a photoperiod of 16 h and a relative humidity of 85%. After 72 h, the lesion in two perpendicular directions was measured for calculating the average lesion diameter. The control efficacy was calculated according to the following formula

$$\text{Control efficacy (\%)} = [(D_{CK} - D_T)/(D_{CK} - 5)] \times 100$$

where control efficacy represents the disease control efficacy, DCK represents the lesion diameter in water control, and DT represents the lesion diameter in treatment. The experiment was performed twice with 10 replicates per treatment.

Scanning Electron Microscopy Observations.[43] Samples treated with DMSO control and 5 μg/mL compound **6c** were prepared according to reported methods. Then, a scanning electron microscope (Hitachi, S-3400N, Japan) was used for observation.

Transmission Electron Microscopy Observations.[27] Mycelium plug samples treated with 0.5% DMSO or compound **6c** (5 μg/mL) were prepared according to reported methods. A transmission electron microscope (Hitachi, HT7700, Japan) was used for observation.

Determination of Cell Membrane Permeability.[44] The permeability of *S. sclerotiorum* membrane was analyzed by determining the relative electric conductivity of mycelium suspension. The PD medium (80 mL) containing *S. sclerotiorum* mycelial disks (5 mm) was shaken at 150 rpm for 72 h at 25 °C for complete growth of mycelia. Then, after filtration, mycelia (200 mg) were treated with compound **6c** of different concentrations, with deionized water as blank control. The data were obtained using a conductivity detector (Leici DDS-307, China) within 24 h.

Determination of Cytoplasmic Content Leakage.[7] The general procedures were followed for the determination of cytoplasmic content leakage according to cell membrane permeability; afterward, the absorbance value was determined with a UV spectrophotometer (UV 1902PC, INESA Scientific, China) within 10 h.

Statistical Analysis. The statistical analysis of all antifungal activity assays was carried out using SPSS 24.0. The IC_{50} values, 95% CI, regression equation, and R^2 are provided in the Supporting Information.

RESULTS AND DISCUSSION

Chemistry. The syntheses of all compounds are outlined in schemes 1−3 (Figure 2), and their structures were confirmed using ^1H NMR, ^{13}C NMR, and MS analyses.

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Table 2. Antifungal Activity of a Series of Quinazolinone Derivatives at 100 μg/mL

compound	average inhibition rate ± SD (%) (n = 3)					
	S. s.[a]	*R. s.*[a]	*B. c.*[a]	*F. g.*[a]	*P. s.*[a]	*F. o.*[a]
KZL-01	0.00 ± 0.00	41.7 ± 1.2	51.5 ± 0.9	8.97 ± 0.88	44.2 ± 0.7	31.7 ± 0.4
KZL-02	0.00 ± 0.00	32.8 ± 0.3	15.5 ± 1.5	30.0 ± 0.7	27.9 ± 0.7	28.5 ± 0.3
KZL-03	88.2 ± 0.2	84.0 ± 0.4	82.0 ± 0.7	70.1 ± 0.2	84.6 ± 0.6	70.4 ± 0.1
KZL-04	0.00 ± 0.00	29.5 ± 0.8	28.9 ± 0.5	21.4 ± 2.0	41.5 ± 0.6	29.1 ± 0.6
KZL-05	0.00 ± 0.00	17.6 ± 4.3	0.00 ± 0.00	0.00 ± 0.00	32.1 ± 0.9	30.9 ± 0.5
KZL-06	13.7 ± 1.1	41.1 ± 3.7	32.8 ± 2.0	26.0 ± 1.0	67.1 ± 1.3	45.5 ± 0.3
KZL-07	93.9 ± 0.1	88.5 ± 1.0	85.5 ± 1.3	50.1 ± 0.1	87.8 ± 0.4	69.0 ± 0.3
KZL-08	75.3 ± 0.5	56.5 ± 1.2	78.7 ± 0.2	33.2 ± 0.6	86.9 ± 0.3	56.6 ± 0.6
KZL-09	58.4 ± 1.6	52.5 ± 0.3	59.0 ± 2.8	39.0 ± 1.2	63.5 ± 0.5	39.4 ± 0.1
KZL-10	95.9 ± 0.1	95.2 ± 0.5	93.4 ± 0.3	71.7 ± 0.8	98.2 ± 0.5	70.4 ± 0.2
KZL-11	19.0 ± 2.5	82.0 ± 1.3	30.7 ± 0.3	70.6 ± 0.7	80.4 ± 0.2	44.4 ± 0.6
KZL-12	87.1 ± 2.2	75.7 ± 0.7	67.9 ± 0.5	72.8 ± 0.5	77.5 ± 0.5	69.1 ± 0.4
KZL-13	95.5 ± 0.6	76.6 ± 1.1	71.8 ± 0.7	67.9 ± 0.5	75.1 ± 0.7	71.2 ± 0.5
KZL-14	87.0 ± 0.6	98.7 ± 0.0	84.7 ± 1.1	85.6 ± 1.5	93.4 ± 0.3	63.0 ± 0.6
KZL-15	100 ± 0	98.8 ± 0.1	99.7 ± 0.1	96.2 ± 0.6	99.9 ± 0.1	90.5 ± 0.6
KZL-16	11.2 ± 2.4	45.5 ± 1.8	36.7 ± 1.7	29.2 ± 0.0	51.2 ± 0.0	51.0 ± 1.4
KZL-17	53.4 ± 1.5	85.9 ± 0.3	9.07 ± 1.05	0.00 ± 0.00	94.1 ± 0.1	18.2 ± 0.5
KZL-18	45.0 ± 0.2	79.4 ± 0.8	32.1 ± 1.0	20.4 ± 0.4	94.3 ± 0.6	27.6 ± 0.6
KZL-19	31.7 ± 0.1	80.0 ± 0.7	21.1 ± 1.5	8.87 ± 1.33	81.1 ± 0.0	30.4 ± 0.0
KZL-20	36.3 ± 0.6	47.8 ± 1.5	40.0 ± 0.5	20.9 ± 0.6	77.5 ± 0.5	29.7 ± 0.2
KZL-21	17.6 ± 0.6	58.1 ± 0.0	42.4 ± 0.7	10.6 ± 0.7	93.1 ± 0.4	28.9 ± 1.7
KZL-22	72.1 ± 1.0	97.8 ± 0.3	97.2 ± 0.6	94.3 ± 0.6	100 ± 0	84.3 ± 0.1
KZL-23	74.8 ± 0.9	100 ± 0	95.2 ± 0.7	88.3 ± 1.1	100 ± 0	79.4 ± 0.1
KZL-24	57.1 ± 0.5	63.9 ± 0.9	45.2 ± 1.3	35.8 ± 1.2	100 ± 0	39.2 ± 0.9
KZL-25	54.4 ± 0.0	61.4 ± 0.8	57.9 ± 0.8	32.6 ± 1.3	85.0 ± 0.4	16.5 ± 0.4
KZL-26	60.1 ± 1.5	100 ± 0	85.3 ± 0.8	51.2 ± 1.3	100 ± 0	33.3 ± 1.3
KZL-27	88.7 ± 0.5	97.5 ± 0.8	94.2 ± 1.06	71.9 ± 0.6	99.7 ± 0.0	48.2 ± 0.2
KZL-28	66.2 ± 0.0	96.4 ± 0.1	91.6 ± 0.4	60.9 ± 1.0	100 ± 0	19.6 ± 0.4
KZL-29	68.2 ± 0.2	99.3 ± 0.5	77.5 ± 1.2	65.0 ± 2.3	100 ± 0	95.8 ± 0.5
KZL-30	58.8 ± 0.3	96.0 ± 0.8	74.1 ± 2.0	53.4 ± 0.9	97.6 ± 0.1	33.0 ± 0.8
KZL-31	49.7 ± 0.4	53.1 ± 0.7	51.4 ± 0.4	19.6 ± 1.1	88.8 ± 0.9	0.00 ± 0.00
KZL-32	72.7 ± 2.2	59.9 ± 0.1	63.4 ± 0.4	13.1 ± 0.7	78.8 ± 0.2	15.8 ± 0.1
KZL-33	31.8 ± 0.6	77.6 ± 0.2	72.2 ± 1.5	30.8 ± 0.2	93.6 ± 0.9	43.5 ± 0.6
KZL-34	31.1 ± 0.2	75.3 ± 0.8	69.6 ± 4.5	29.6 ± 1.8	77.0 ± 0.2	6.2 ± 0.2
1a	13.4 ± 1.0	30.7 ± 0.4	0.00 ± 0.00	13.0 ± 1.9	0.00 ± 0.00	0.00 ± 0.00
1b	42.6 ± 4.6	57.9 ± 1.4	48.8 ± 1.9	47.9 ± 0.4	46.6 ± 0.2	36.9 ± 0.2
1c	43.6 ± 4.8	49.8 ± 0.8	50.2 ± 1.6	50.7 ± 1.6	40.8 ± 1.8	49.2 ± 2.9
1d	0.00 ± 0.00	13.7 ± 1.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1e	0.00 ± 0.00	24.4 ± 0.8	34.7 ± 3.4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1f	0.00 ± 0.00	11.2 ± 0.3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1g	28.2 ± 1.6	16.5 ± 0.7	51.0 ± 1.6	15.6 ± 0.5	20.6 ± 0.1	0.00 ± 0.00
2a	73.7 ± 0.9	99.6 ± 0.0	100 ± 0	51.0 ± 0.5	40.9 ± 1.7	50.0 ± 2.0
3a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3b	8.84 ± 1.76	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3c	27.5 ± 1.3	0.00 ± 0.00	70.2 ± 1.3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3d	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.3 ± 0.0	0.00 ± 0.00
3e	0.00 ± 0.00	9.47 ± 0.37	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.15 ± 0.21
3f	0.00 ± 0.00	5.22 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3g	0.00 ± 0.00	8.85 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4a	89.7 ± 0.94	14.0 ± 0.6	59.0 ± 0.2	28.9 ± 0.8	43.0 ± 0.2	18.0 ± 0.7
4b	43.9 ± 0.0	75.6 ± 0.1	60.4 ± 0.2	58.9 ± 0.3	64.0 ± 0.3	32.0 ± 0.7
4c	43.2 ± 0.2	68.7 ± 1.0	49.2 ± 0.6	51.7 ± 0.1	67.1 ± 0.1	29.6 ± 0.6
4d	19.0 ± 0.0	39.6 ± 0.9	26.3 ± 0.6	41.6 ± 1.8	40.1 ± 1.2	20.3 ± 0.1
4e	30.3 ± 0.0	49.3 ± 0.2	49.4 ± 1.6	53.8 ± 0.1	44.7 ± 0.0	34.5 ± 0.1
5a	89.7 ± 0.8	88.1 ± 0.0	75.2 ± 0.3	56.9 ± 0.4	57.0 ± 0.0	78.5 ± 0.0
5b	93.0 ± 0.2	81.8 ± 0.9	77.9 ± 0.2	61.9 ± 0.0	71.6 ± 1.6	76.5 ± 0.2
5c	96.4 ± 0.1	94.5 ± 0.4	73.9 ± 1.1	69.6 ± 1.0	80.2 ± 1.0	71.1 ± 1.0
5d	86.7 ± 0.1	69.7 ± 0.5	68.7 ± 1.6	43.5 ± 0.5	55.4 ± 0.0	31.4 ± 0.1
5e	84.3 ± 0.1	55.9 ± 1.0	39.2 ± 0.1	63.3 ± 0.1	65.7 ± 0.6	36.9 ± 0.0

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Table 2. continued

compound	average inhibition rate ± SD (%) (n = 3)					
	S. s.[a]	*R. s.*[a]	*B. c.*[a]	*F. g.*[a]	*P. s.*[a]	*F. o.*[a]
5f	71.5 ± 0.0	16.9 ± 0.1	10.2 ± 0.0	18.8 ± 0.1	27.4 ± 0.2	0.00 ± 0.00
5g	96.4 ± 0.0	91.3 ± 0.2	77.5 ± 0.3	64.0 ± 1.0	78.0 ± 0.1	72.2 ± 0.5
6a	89.1 ± 0.3	53.8 ± 1.0	23.0 ± 0.1	48.0 ± 0.5	67.7 ± 0.6	27.3 ± 0.0
6b	94.4 ± 0.2	86.2 ± 0.1	81.6 ± 0.4	79.0 ± 0.1	92.4 ± 0.7	65.2 ± 0.2
6c	95.0 ± 0.6	86.8 ± 0.6	89.2 ± 0.2	78.9 ± 0.1	94.2 ± 0.8	74.4 ± 0.2
6d	18.3 ± 0.4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24.8 ± 0.4	11.0 ± 0.5
6e	8.55 ± 0.15	12.7 ± 0.7	10.9 ± 0.9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6f	37.1 ± 0.0	22.2 ± 0.1	15.5 ± 0.2	0.00 ± 0.00	25.9 ± 0.7	0.00 ± 0.00
6g	91.7 ± 0.0	77.0 ± 0.4	75.4 ± 0.2	84.8 ± 0.4	89.7 ± 0.2	63.0 ± 0.0
6h	17.0 ± 0.2	19.0 ± 0.5	20.7 ± 1.0	0.00 ± 0.00	19.8 ± 0.2	0.00 ± 0.00
6i	7.97 ± 0.30	12.9 ± 0.4	13.7 ± 1.2	0.00 ± 0.00	6.18 ± 0.45	0.00 ± 0.00
7a	29.8 ± 0.2	53.8 ± 0.8	14.8 ± 0.2	36.4 ± 0.9	47.6 ± 0.4	0.00 ± 0.00
7b	14.3 ± 0.2	31.8 ± 0.4	57.5 ± 0.8	11.1 ± 0.1	32.1 ± 0.2	0.00 ± 0.00
7c	14.0 ± 0.1	30.0 ± 0.7	40.1 ± 0.7	21.3 ± 0.6	28.0 ± 0.2	0.00 ± 0.00
7d	0.00 ± 0.00	16.4 ± 0.1	0.00 ± 0.00	0.00 ± 0.00	28.7 ± 0.3	0.00 ± 0.00
7e	25.4 ± 0.7	38.7 ± 0.6	46.1 ± 0.4	10.8 ± 0.9	38.3 ± 1.5	15.4 ± 0.0
7f	8.07 ± 0.34	33.8 ± 0.4	0.00 ± 0.00	0.00 ± 0.00	34.6 ± 0.6	4.16 ± 0.47
7g	12.0 ± 0.1	18.8 ± 0.4	11.8 ± 0.4	6.90 ± 0.27	19.9 ± 1.0	9.83 ± 0.01
7h	25.3 ± 1.0	32.0 ± 0.2	31.8 ± 0.2	17.9 ± 0.0	48.7 ± 2.1	0.00 ± 0.00
8a	79.6 ± 0.7	83.1 ± 1.0	12.1 ± 0.7	44.7 ± 1.8	29.2 ± 0.4	32.8 ± 0.6
8b	72.7 ± 3.7	49.5 ± 1.1	73.6 ± 1.0	49.5 ± 1.1	35.9 ± 0.2	10.1 ± 0.1
8c	23.8 ± 0.5	48.7 ± 0.4	51.6 ± 0.8	29.1 ± 0.5	64.8 ± 0.2	22.2 ± 0.4
8d	16.8 ± 0.2	46.0 ± 0.4	51.7 ± 0.0	46.4 ± 1.2	55.6 ± 0.2	8.89 ± 0.01
8e	93.0 ± 0.4	83.7 ± 0.5	78.3 ± 0.2	68.8 ± 0.5	75.9 ± 0.0	63.8 ± 1.1
8f	96.2 ± 0.6	89.1 ± 0.4	85.3 ± 0.3	71.9 ± 0.2	85.9 ± 0.5	73.4 ± 0.5
8g	33.4 ± 0.4	36.6 ± 0.7	38.1 ± 0.1	35.5 ± 0.2	11.9 ± 0.3	0.00 ± 0.00
8h	29.3 ± 0.4	39.8 ± 0.2	64.6 ± 0.2	20.5 ± 0.3	28.6 ± 1.2	7.15 ± 0.41
8i	25.9 ± 0.0	53.0 ± 0.8	67.5 ± 0.2	40.7 ± 0.1	85.9 ± 0.0	10.0 ± 0.9
azoxystrobin	55.7 ± 1.0	59.1 ± 0.7	46.4 ± 0.3	71.8 ± 1.4	54.8 ± 0.3	50.5 ± 0.9

[a]*S. s.*: *Sclerotinia sclerotiorum*. *R. s.*: *Rhizoctonia solani*. *B. c.*: *Botrytis cinerea*. *F. g.*: *Fusarium graminearum*. *P. s.*: *Pellicularia sasakii*. *F. o.*: *Fusarium oxysporum*.

Table 3. IC_{50} of a Series of Quinazolinone Derivatives Against Six Phytopathogenic Fungi (μg/mL)

compound	IC_{50}					
	S. s.[a]	*R. s.*[a]	*B. c.*[a]	*F. g.*[a]	*P. s.*[a]	*F. o.*[a]
KZL-15	6.42	4.06	9.99	12.5	4.50	12.7
KZL-22	10.7	11.6	23.7	24.3	3.36	>25.0
5b	5.01	14.3	7.76	>25.0	>25.0	24.0
6b	3.60	6.21	8.01	8.41	3.06	>25.0
6c	2.46	5.14	7.10	6.03	2.94	11.9
8e	7.85	5.38	8.66	>25.0	11.1	17.6
8f	4.07	3.45	6.51	20.3	17.3	15.8

[a]*S. s.*: *Sclerotinia sclerotiorum*. *R. s.*: *Rhizoctonia solani*. *B. c.*: *Botrytis cinerea*. *F. g.*: *Fusarium graminearum*. *P. s.*: *Pellicularia sasakii*. *F. o.*: *Fusarium oxysporum*.

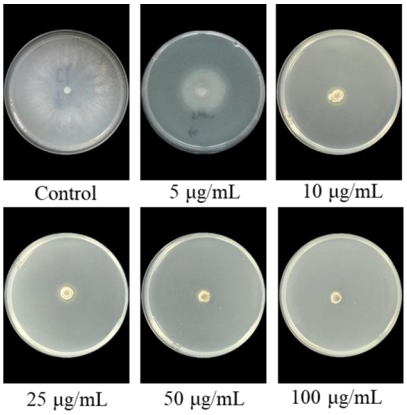

Figure 3. Antifungal activities of compound **6c** against S. sclerotiorum *in vitro*.

Intermediate **1** was prepared by the reaction of trifluoroacetic anhydride (or acetic anhydride) and the corresponding aminobenzoic acid. Intermediate **2** was obtained by the reaction of intermediate **1** and glycine methyl ester hydrochloride. Intermediate **2** was acidified by alkalization in the mixture of THF solution of potassium hydroxide to afford intermediate **3**. Then, intermediate **4** was obtained by the mixture of intermediate **2** and hydrazine hydrate without further purification.

Compounds **KZL-01**−**KZL-34** were obtained from intermediate **1** through a one-step reaction in good yields, and compounds **1a**−**2a** were obtained through the reaction of intermediate **3** with the corresponding amine. Compounds **3a**−**3g** were obtained through the reaction of intermediate **4** with the corresponding benzaldehyde, and intermediate **4** was transformed into oxadiazoles **4a**−**4e** by reacting with iodobenzene diacetate. The intermediate derivatization method was often used to find active candidate compounds; compounds **5a**−**5g**, **6a**−**6i**, **7a**−**7h**, and **8a**−**8i** were synthesized by this method, including intermediates **1** and **22**.

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Table 4. Protective and Curative Activities of Compound 6c *In Vivo*

		curative effect		protective effect	
compd.[a]	concentration (μg/mL)	lesion length (mm ± SD)	control efficacy (%)	lesion length (mm ± SD)	control efficacy (%)
6c	25	15.4 ± 0.6	49.9	8.34 ± 0.70	72.9
	50	10.7 ± 0.5	72.3	7.52 ± 0.43	79.5
	100	7.62 ± 0.65	87.3	6.15 ± 0.10	90.7
ASB[b]	25	9.30 ± 0.35	79.2	7.28 ± 0.41	81.5
	50	7.75 ± 0.28	86.7	6.78 ± 0.80	85.5
	100	7.18 ± 0.80	89.5	6.09 ± 0.14	91.2
Control		25.7 ± 1.5		17.3 ± 0.6	

[a]Compd.: compound. [b]ASB: azoxystrobin.

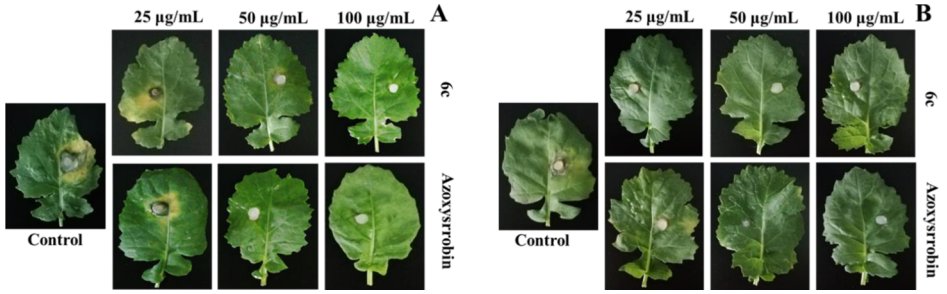

Figure 4. Curative and protective activities of compound **6c** against *S. sclerotiorum*. (A) Curative effects of compound **6c** against *S. sclerotiorum*. (B) Protective effects of compound **6c** against *S. sclerotiorum*.

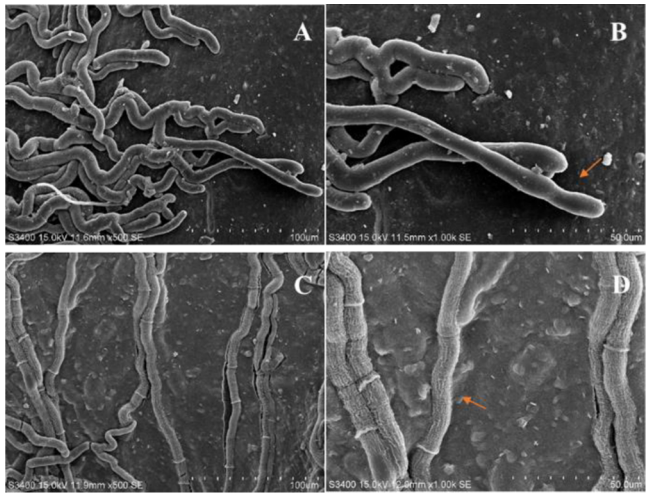

Figure 5. SEM patterns of *S. sclerotiorum* hyphae treated with DMSO or compound **6c**. (A,B) Control treatment with 0.5% DMSO, ×500, and ×1000. (C,D) Treatment with compound **6c** at 2.46 μg/mL (IC_{50}), ×500, and ×1000.

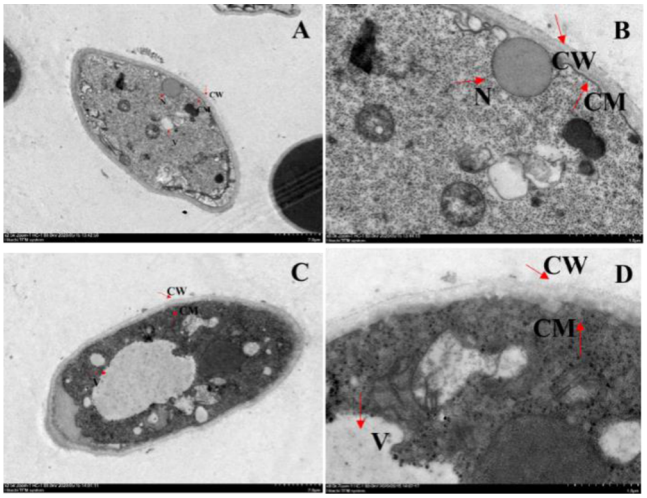

Figure 6. TEM observation of the cellular structure of *S. sclerotiorum* treated with (A,B) 0.5% DMSO control and (C,D) 0.5% DMSO and compound **6c** at 2.46 μg/mL (IC_{50}). CW: cell wall; CM: cell membrane; N: nucleus; V: vacuole.

Antifungal Bioassay *In Vitro* and SAR. First, we evaluated the antifungal efficacy of six natural products containing a quinazolinone skeleton [luotonin A, tryptanthrin, evodiamine, rutaecarpine, quinazolinone (**KZL-01**), and 2-methylquinazolinone (**KZL-02**)] at 100 and 50 μg/mL against *S. sclerotiorum*, *R. solani*, *B. cinerea*, *F. graminearum*, *P. sasakii*, and *F. oxysporum*, which are critical for agriculture. The data in Table 1 show that quinazolinone and 2-methylquinazolinone had a broader antifungal spectrum than luotonin A, tryptanthrin, evodiamine, and rutaecarpine, which were more complex in structure. Therefore, we synthesized a series of quinazolinone derivatives **KZL-01**−**KZL-34** with a single quinazolinone skeleton and tested their antifungal activity at 100 μg/mL, whose results are summarized in Table 2. The results show that when the N-2 point of quinazolinone was substituted by trifluoromethyl (−CF_3), most of the target compounds exhibited potent activity against the six phytopathogenic fungi at 100 μg/mL, and compounds **KZL-15** and **KZL-22** with strong antifungal activity were selected as the lead compounds for the next structure derivatization.

The results in Table 2 suggest that when the quinazolinone nucleus was substituted by an electron-withdrawing group, the compound possessed stronger antifungal activity. In order to further investigate the antifungal effect of side-chain-substituent quinazolinone derivatives against phytopathogenic fungi, eightseries of quinazolinone 3-substituted derivatives were designed and synthesized based on compounds **KZL-15** and **KZL-22** (compounds **1a**−**4e** and **5a**−**8i**). From the data

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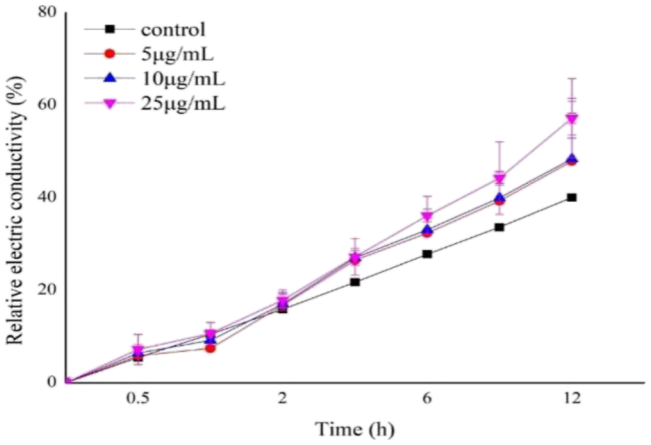

Figure 7. Changes in cell membrane permeability of *S. sclerotiorum* treated with compound **6c**.

in Table 2, we can observe that compound **2a** possessed higher antifungal activity against the six test fungi than compounds **1a**−**1g**; so, we can speculate that the hydrazide fragment had a better antifungal potential than the amide fragment in our modification strategy. Moreover, the antifungal activity of the derivatives disappeared when the hydrazide fragment was converted to acylhydrazone (**3a**−**3g**), but the activity still existed when the hydrazide fragment was converted to 1,3,4-oxadiazole (**4a**−**4e**). Interestingly, we observed that compounds **5a**, **5b**, **5c**, **6b**, **6c**, **8e**, and **8f** containing the same substituent group, fluorophenyl, showed significant antifungal activities. Then, we extrapolated that fluorophenyl could maintain or increase the antifungal activity of the lead compound **KZL-22**.

For further exploration of the quinazolinone derivatives, Table 3 outlines the IC_{50} values of target compounds with remarkable antifungal activity at 100 μg/mL concentration. From the values shown in Table 3, we can find that compound **6c** displayed the highest bioactivity against *S. sclerotiorum*, *P. sasakii*, *F. graminearum*, and *F. oxysporum*, displaying appreciable IC_{50} values of 2.46, 2.94, 6.03, and 11.9 μg/mL, respectively. Also, compound **8f** showed the highest antifungal activity against *R. solani* and *B. cinerea* with IC_{50} values of 3.45 and 6.51 μg/mL, respectively. In addition, compounds **5b**, **6b**, and **8e** also exhibited moderate to significant activity against the six tested fungal strains.

Notably, the highly active compounds **5b**, **6b**(**6c**), and **8e**(**8f**) were fluorobenzenesulfonyl-, fluorophenyl-, and fluorobenzoyl-substituted derivatives of **KZL-22** at the 3-position, respectively. Therefore, it was implied that compared with compound **KZL-15**, **KZL-22** was more suitable for structural derivatization, and the hydrazine fragment at the 3-position of quinazolinone was crucial to maintain or improve the antifungal activity of the derivative. On the other hand, these compounds contained a common substituent group: fluorophenyl. Therefore, it can be concluded that fluorophenyl is an important pharmacophore for the derivatization of **KZL-22**. Furthermore, compounds **5f**, **6h**, and **8i** were 4-methylbenzenesulfonyl-, 4-methylphenyl-, and 4-methylbenzoyl-substituted derivatives of **KZL-22** at 3-position, respectively, and exhibited poor antifungal activity at 100 μg/mL concentration. Compared with compounds **5a**, **5b**, **5c**, **6b**, **6c**, **8e**, and **8f**, it was therefore extrapolated that the derivatives possessed better antifungal activity when the benzene ring was replaced by an electron-withdrawing group (−F).

***In Vivo* Activity.** The antifungal activity of the most active compound **6c** was assessed *in vitro* against *S. sclerotiorum* (Figure 3), and compound **6c** exhibited excellent curative and protective effects, as determined using pot experiments *in vivo* (Table 4). The following conclusions could be drawn from Table 4: first, compound **6c** possessed stronger protective effects (72.9, 79.5, and 90.7% at 25, 50, and 100 μg/mL, respectively) than curative effects (49.9, 72.3, and 87.3% at 25, 50, and 100 μg/mL, respectively); second, the curative effect and the protective effect of compound **6c** at 100 μg/mL concentration (87.3 and 90.7%, respectively) were comparable to those of azoxystrobin (89.5 and 91.2%, respectively); third, the curative and protective efficacies of compound **6c** were concentration-dependent. The images in Figure 4 validate the negligible phytotoxicity of compound **6c** on oilseed rape leaves even at a high concentration of 100 μg/mL, which was benign to the cole leaves.

Preliminary Exploration of Antifungal Mechanism. *Effects of **6c** on S. sclerotiorum Morphology.* The effects of compound **6c** on the morphology of *S. sclerotiorum* were investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). As shown in Figure 5, the mycelia showed a smooth, uniform, and normal morphology in the control group. In sharp contrast, treatment with compound **6c** led to extensive hyphal branching of

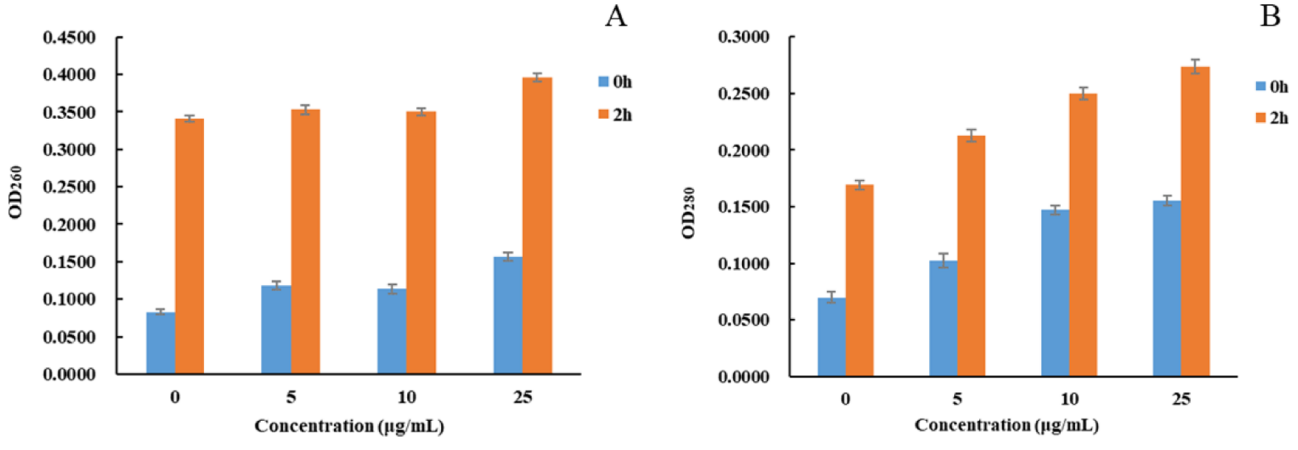

Figure 8. Cellular content release from *S. sclerotiorum* treated with compound **6c**.

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mycelia and other abnormalities including bent, shrunk, collapsed, and deformed structures. The ultrastructural alterations of *S. sclerotiorum* were also analyzed by TEM, as shown in Figure 6 organelles in the control group were arranged regularly with complete cell walls and obvious diaphragms. However, after treatment with compound **6c**, the volume of vacuoles increased, the number of organelles decreased, the nucleus disappeared, the color of the cytoplasmic matrix became darker, and the cell wall became thinner. SEM observation proved the mycelium damage of *S. sclerotiorum* caused by compound **6c**, and TEM results validated that the compound could destroy the organelles and nucleus of *S. sclerotiorum*.

*Effect of **6c** on Cell Membrane Permeability.* In order to determine whether compound **6c** acted on the membrane of *S. sclerotiorum*, we measured the cell membrane permeability of mycelia treated with different concentrations of compound **6c**. Treatment with compound **6c** significantly increased the relative electric conductivities of the mycelia 12 h post-treatment (Figure 7). The values of the high-concentration treatment group were higher than those of the low-concentration treatment group.

*Effects of **6c** on Cellular Content Release.* The concentrations of nucleic acids and proteins in *S. sclerotiorum* mycelial suspensions were evaluated by determining the characteristic absorbance at 260 and 280 nm. However, there were no significant changes in absorbance after treatment with compound **6c**, according to Figure 8.

CONCLUSIONS

In conclusion, compounds **KZL-01**−**KZL-34** were designed and synthesized based on diversity-oriented synthesis; *in vitro* antifungal activity screening found that most of the target compounds have potent antifungal activity against six phytopathogenic fungi, and two lead compounds **KZL-15** and **KZL-22** were obtained. Furthermore, eight novel series of quinazolinone hybrids were synthesized, and the antifungal results revealed that compound **6c** displayed the highest antifungal activity among all synthetic compounds against *S. sclerotiorum*, *P. sasakii*, *F. graminearum*, and *F. oxysporum* with IC_{50} values of 2.46, 2.94, 6.03, and 11.9 μg/mL respectively. In addition, an *in vivo* bioassay was carried out to explore the potential of compound **6c** in practical applications; the results indicated that compound **6c** exhibited comparable curative effect and protective effect (87.3 and 90.7%, respectively) to those of azoxystrobin (89.5 and 91.2%, respectively) at 100 μg/mL, respectively. To further explore the application value of compound **6c** in controlling *S. sclerotiorum*, we have studied its preliminary mechanism of action. The results showed that compound **6c** could cause obvious hyphal malformation and organelle damage of *S. sclerotiorum* and an increase in cell membrane permeability. Based on the above findings, we can say that these quinazoline derivatives with superior antifungal activity *in vivo* and *in vitro* are promising candidate compounds, and investigation of the specific action mechanism of compound **6c** is in progress.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c05475.

IC_{50} values of quinazolinone derivatives against six phytopathogenic fungi in detail; physical properties of the target compounds; and spectra of representative compounds (PDF)

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Notes

The authors declare no competing financial interest.

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