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Design and Discovery of Novel Antifungal Quinoline Derivatives with Acylhydrazide as a Promising Pharmacophore

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ABSTRACT: Inspired by natural 2-quinolinecarboxylic acid derivatives, a series of quinoline compounds containing acylhydrazine, acylhydrazone, sulfonylhydrazine, oxadiazole, thiadiazole, or triazole moieties were synthesized and evaluated for their fungicidal activity. Most of these compounds exhibited excellent fungicidal activity *in vitro*. Significantly, compound **2e** displayed the superior *in vitro* antifungal activity against *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Botrytis cinerea*, and *Fusarium graminearum* with the EC_{50} values of 0.39, 0.46, 0.19, and 0.18 μg/mL, respectively, and were more potent than those of carbendazim (EC_{50}, 0.68, 0.14, >100, and 0.65 μg/mL, respectively). Moreover, compound **2e** could inhibit spore germination of *F. graminearum*. Preliminary mechanistic studies showed that compound **2e** could cause abnormal morphology of cell walls and vacuoles, loss of mitochondrion, increases in membrane permeability, and release of cellular contents. These results indicate that compound **2e** displayed superior fungicidal activities and could be a potential fungicidal candidate against plant fungal diseases.

KEYWORDS: *quinoline, acylhydrazine, oxadiazole, fungicidal activity, pathogenic fungi*

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INTRODUCTION

Phytopathogenic fungi cause enormous damage to food crops, resulting in diminishing economic quality and quantity of agricultural products.[1,2] Each year, at least 10% decrease of global food production has been attributed to phytopathogenic fungi.[3] For example, *Fusarium graminearum* not only causes loss of germinability, reduced emergence, and post emergence blight of seedlings, but also is the main causal agent of fusarium head blight in adult plants, which is the most important and destructive fungal diseases in wheat worldwide.[4] At present, chemical fungicides are still the most effective method to control fungal infections because of their low cost, high convenience, and fast effect.[5] Unfortunately, the extensive and continuous use of synthetic agrochemicals has resulted in the augmented frequency of severe drug resistance, which drastically reduced the effectiveness of these compounds.[6,7] The above-cited problems have highlighted an urgent need for the development of novel agents to combat these phytopathogenic fungi.

2-Quinolinecarboxylic acid derivatives, distributed in plants,[8,9] insects,[10] marine organisms,[11,12] and bacteria,[13] have attracted much attention as their multiple biological activities. Kynurenic acid, 6-hydroxykynurenic acid, and transtorine were isolated from *Ephedra* species.[8,9] The isomer of kynurenic acid, transtorine, showed moderate antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*.[9] Perspicamide A was first isolated from the Australian ascidian *Botrylloides persipicuum* in 2005 by Quinn *et al.*,[11] and its synthesized analogues exhibited potent activity against *Leishmania donovani*.[14] However, multifarious activities of 2-quinolinecarboxylic acid derivatives have been reported except fungicidal evaluation against plant pathogenic fungi.

Our group has been committed to the discovery of fungicidal agents based on quinoline alkaloids. In our previous work, a series of quinoline derivatives substituted at the 4-position with significant antifungal activity were obtained through structural simplification of quinine alkaloids.[15] Therefore, continuing our efforts on the discovery of fungicidal quinoline compounds for the control of plant diseases and considering that acylhydrazine and oxadiazole moieties, as privileged structures with a broad-range pharmacological potential,[16−20] are widely used in pesticidal and medicinal chemistry, here, we introduced acylhydrazine and oxadiazole groups into the 2-position of quinoline ring to synthesize a series of quinoline derivatives and systematically evaluated their *in vitro* fungicidal activities against four pathogenic fungi. Moreover, the preliminary mechanism of action of the most effective fungicidal candidate against *F. graminearum* was also evaluated.

MATERIALS AND METHODS

General. All reactions were performed with commercially available reagents and solvents without further purification. All reactions were monitored by thin-layer chromatography (TLC), and preparative TLC was performed with silica gel plates using silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd., China). Melting points were determined in an open capillary using a WRS-2U melting point apparatus (Shanghai Precision Instrument Co., Ltd., China) and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE-III HD

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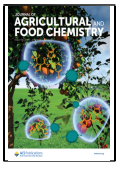

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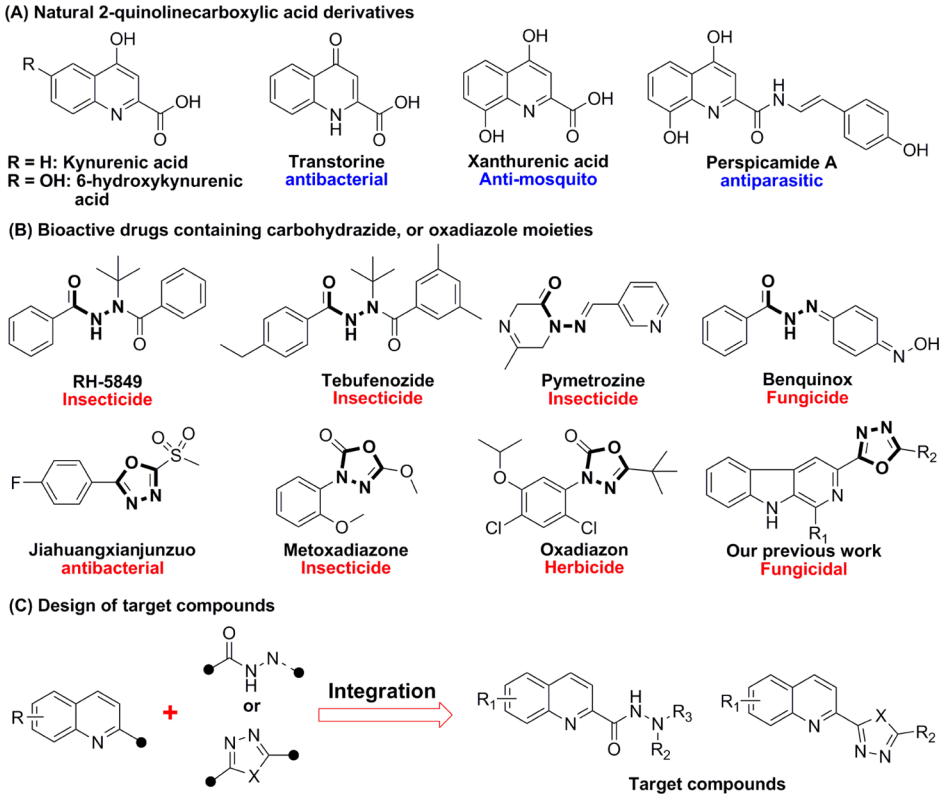

Figure 1. (A) Natural 2-quinolinecarboxylic acid derivatives. (B) Bioactive drugs containing carbohydrazide or oxadiazole moieties. (C) Design of target compounds.

400 MHz or a NEO 500 MHz (Bruker Daltonics Inc., Germany) spectrometer using tetramethylsilane as the reference. Mass spectra were recorded on a Bruker APEXII49e spectrometer (Bruker Daltonics Inc., Germany) with the electrospray ionization (ESI) source as ionization. The commercial fungicide carbendazim (analytical grade, 98% purity) (Shanghai Titan Scientific Co., Ltd., China) was used as a positive control in the *in vitro* experiment.

Fungi. Four pathogenic fungi strains, *Rhizoctonia solani*, *Botrytis cinerea*, *F. graminearum*, and *Sclerotinia sclerotiorum*, were obtained from the Gansu Academy of Agricultural Sciences, Gansu Province of China, and maintained during the experiments on potato dextrose agar medium at 25 °C.

Synthesis of Compound ***1***. To a solution of quinaldic acid (23.10 mmol) in *N*,*N*-dimethylformamide (DMF, 30 mL), K_2CO_3 (23.10 mmol) and CH_3I (46.20 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. The solution was poured into 300 mL of water. The solid was filtered, washed with water, and dried. The resulting solid was dissolved in 50 mL of methanol, and hydrazine hydrate (92.40 mmol) was added dropwise. The reaction mixture was refluxed for 8 h. Then, the solid was filtered, washed with methanol, and recrystallized from methanol to afford the desired compound **1** as a white solid (yield 68%). mp 141−142 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, 1H), 8.55 (d, J = 8.5 Hz, 1H), 8.09−8.05 (m, 3H), 7.86 (t, J = 8.4 Hz, 1H), 7.75−7.66 (t, J = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.37, 150.53, 146.54, 138.16, 130.89, 129.69, 129.12, 128.52, 128.38, 119.10; ESI-MS m/z: 188.13 $[M + H]^+$.

General Synthetic Procedure for Compounds ***2a−2h***. Quinoline-2-carboxylic acid (2.31 mmol) was dissolved in $SOCl_2$ (5 mL), and the reaction mixture was refluxed for 4 h and then concentrated to obtain quinoline-2-carbonyl chloride. To a solution of substituted hydrazine hydrochloride (4.62 mmol) in dichloromethane (DCM, 25 mL) was dropwise added a solution of NaOH (6.93 mmol) in water (5 mL) below 5 °C. After 20 min of stirring, quinoline-2-carbonyl chloride in dry DCM (10 mL) was added dropwise below 5 °C. After stirring in the ice−water bath for 30 min, the mixture was permitted to stand for 3 h at room temperature. The organic layer was washed with water and brine (3 × 50 mL), dried, and concentrated. The residue was purified by column chromatography on silica gel using DCM/acetone mixture as the eluent to afford the target compounds **2a−2h**.

Data for Compound ***2a***. Yellow solid; mp 90−92 °C; yield: 28%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27−8.16 (m, 2H), 8.03 (d, J = 8.5 Hz, 1H), 7.79 (d, J = 8.2 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 7.54 (t, J = 7.4 Hz, 1H), 1.15 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.10, 148.07, 145.54, 136.41, 129.09, 128.79, 128.32, 126.88, 126.71, 117.82, 54.55, 26.32; ESI-MS m/z: 244.21 $[M + H]^+$.

General Synthetic Procedure for Compounds ***3a−3v***. To a solution of compound **1** (2.14 mmol) in DCM (30 mL), substituted acid (2.14 mmol), EDCI (4.27 mmol), HOBt (3.21 mmol), and NMM (4.91 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. After completing the reaction, 30 mL of DCM was added in the mixture. The organic layer was washed with water, 1 M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution, and brine (3 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and removed under vacuum. The residue was purified by column chromatography on silica gel using DCM/acetone mixture as the eluent to afford the target compounds **3a−3v**.

Data for Compound ***3a***. White solid; mp 209−211 °C; yield: 42%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.08 (s, 1H), 10.71 (s, 1H), 10.22 (s, 1H), 8.60 (d, J = 8.4 Hz, 1H), 8.18−8.07 (m, 3H), 7.91 (t, J = 7.6 Hz, 1H), 7.76 (t, J = 7.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.30, 160.16, 149.54, 146.49, 138.44, 131.16, 129.72, 129.39, 128.84, 128.63, 119.29; ESI-MS m/z: 216.14 $[M + H]^+$.

General Synthetic Procedure for Compounds ***4a−4h***. To a solution of compound **1** (1.60 mmol) in pyridine (10 mL), the substituted sulfonylchloride (1.60 mmol) was added below 5 °C. After stirring at room temperature for 3 h, the mixture was removed under vacuum, and the residue was dissolved in 40 mL of DCM. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and brine (3 × 50 mL), dried, and removed under vacuum. The residue was purified by column chromatography on silica gel using DCM/acetone mixture as the eluent to afford the target compounds **4a−4h**.

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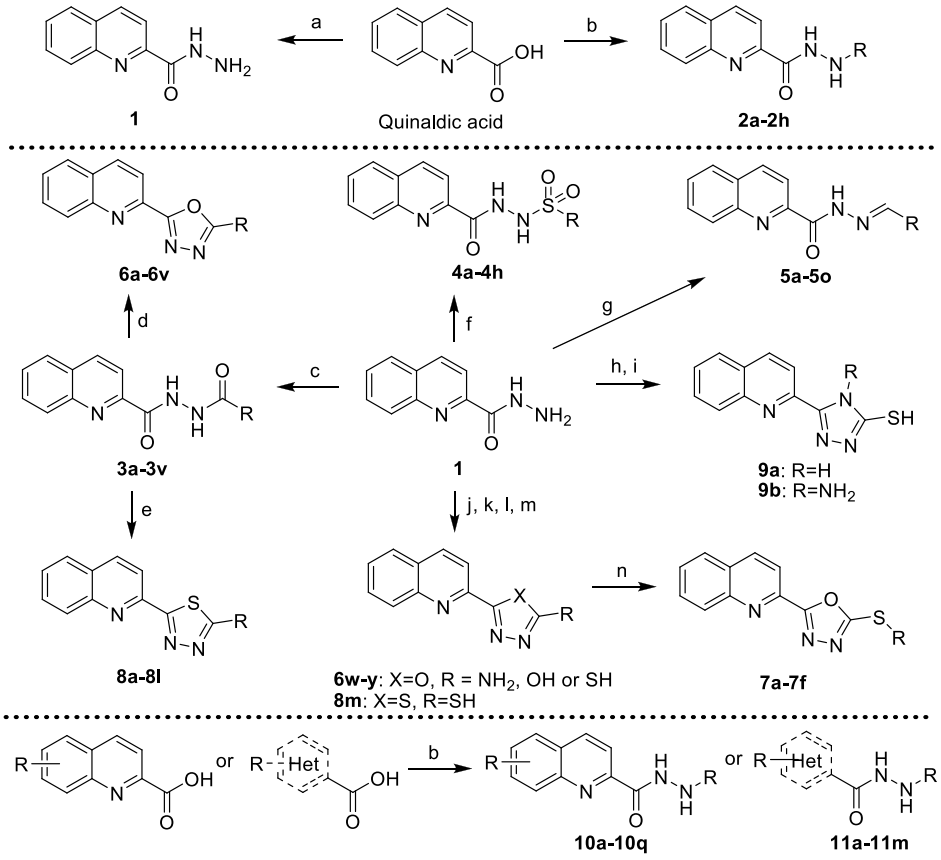

Figure 2. Synthesis of target compounds **2a−2h**, **3a−3v**, **4a−4h**, **5a−5o**, **6a−6y**, **7a−7f**, **8a−8b**, **9a−9m**, **10a−10q**, and **11a−11m**. Reagents and conditions: (a) (i) CH_3I, K_2CO_3, DMF, rt, and 24 h; (ii) $N_2H_4{\cdot}H_2O$, MeOH, reflux, and 8 h; (b) (i) $SOCl_2$, reflux, and 4 h and (ii) $RNHNH_2{\cdot}HCl$, DCM, NaOH, H_2O, 0 °C, 0.5 h then rt, and 3 h; (c) RCOOH, EDCI, HOBt, NMM, DCM, rt, and 24 h; (d) $POCl_3$, reflux, and 12 h; (e) Lawesson's reagent, THF, reflux, and 12 h; (f) RSO_2Cl, Et_3N, DCM, rt, and 3 h; (g) RCHO, EtOH, AcOH, reflux, and 8 h; (h) for compound **9a**: (i) KSCN, HCl, EtOH, reflux, and 48 h and (ii) 2N NaOH, reflux, and 48 h; (i) for compound **9b**: (i) CS_2, KOH, EtOH, reflux, and 48 h and (ii) $N_2H_4{\cdot}H_2O$, reflux, and 4 days; (j) for compound **6w**: CNBr, $NaHCO_3$, dioxane, H_2O, rt, and 8 h; (k) for compound **6x**: triphosgene, DCM, reflux, and 2 h; (l) for compound **6y**: (i) CS_2, KOH, EtOH, reflux, and 8 h and (ii) 2 N HCl; (m) for compound **8m**: (i) CS_2, KOH, EtOH, and 8 h and (ii) conc. H_2SO_4 and 2 h; and (n) RX, K_2CO_3, DMF, rt, and 24 h.

*Data for Compound **4a**.* White solid; mp 203−205 °C; yield: 64%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.98 (s, 1H), 9.69 (s, 1H), 8.61 (d, J = 8.5 Hz, 1H), 8.22−8.07 (m, 3H), 7.91 (t, J = 8.4 Hz, 1H), 7.76 (t, J = 8.1 Hz, 1H), 3.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.56, 149.53, 146.49, 138.41, 131.16, 129.78, 129.42, 128.92, 128.61, 119.45, 41.22; ESI-MS m/z: 266.11 $[M + H]^+$.

*General Synthetic Procedure for Compounds **5a−5o**.* To a solution of compound **1** (1.34 mmol) in ethanol (10 mL) were added the substituted benzaldehyde (2.00 mmol) and acetic acid (0.1 mL), and the reaction mixture was refluxed for 8 h. The target compounds were filtered, washed, and recrystallized from ethanol.

*Data for Compound **5a**.* White solid; mp 136−137 °C; yield: 69%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.06 (s, 1H), 8.33−8.26 (m, 2H), 8.22 (d, J = 8.5 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.71−7.65 (m, 3H), 7.54 (t, J = 7.5 Hz, 1H), 7.19−7.12 (m, 2H), 2.57 (q, J = 7.4 Hz, 2H), 1.16 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.12, 148.07, 148.04, 146.16, 145.28, 136.67, 130.14, 129.28, 128.53, 128.45, 127.19, 127.16, 126.91, 126.82, 118.10, 27.83, 14.25; ESI-MS m/z: 304.20 $[M + H]^+$.

*General Synthetic Procedure for Compounds **6a−6v**.* Compounds **3a−3v** (0.93 mmol) were dissolved in $POCl_3$, and the mixture was refluxed for 12 h. After cooling to room temperature, the reaction mixture was poured into 100 g of crushed ice. The aqueous layer was extracted with DCM (3 × 40 mL). The combined organic layer was washed with saturated $NaHCO_3$ aqueous solution (3 × 50 mL), dried over anhydrous Na_2SO_4, and removed under vacuum. The target compounds were purified by column chromatography on silica.

*Data for Compound **6a**.* White solid; mp 163−165 °C; yield: 40%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (s, 1H), 8.37 (s, 2H), 8.27 (d, J = 8.5 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.83 (t, J = 7.7 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.78, 147.89, 142.97, 137.69, 130.91, 130.76, 130.13, 128.83, 128.53, 127.83, 119.85; ESI-MS m/z: 198.13 $[M + H]^+$.

*General Synthetic Procedure for Compounds **7a−7f**.* To a solution of **6y** (1.31 mmol) in DMF (5 mL) were added K_2CO_3 (1.31 mmol) and haloalkane (2.62 mmol). The reaction mixture was stirred at room temperature for 24 h. The solution was poured into water (100 mL). The solid was filtered, washed with water, dried, and purified by column chromatography on silica gel using petroleum ether/ethyl acetate mixture as the eluent to afford the target compounds.

*Data for Compound **7a**.* White solid; mp 129−130 °C; yield: 96%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25−8.22 (m, 3H), 7.88 (d, J = 8.1 Hz, 1H), 7.80 (t, J = 8.5 Hz, 1H), 7.64 (t, J = 8.1 Hz, 1H), 2.83 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.21, 165.30, 147.83, 143.11, 137.47, 130.58, 130.04, 128.64, 128.25, 127.77, 119.54, 14.73; ESI-MS m/z: 244.12 $[M + H]^+$.

*General Synthetic Procedure for Compounds **8a−8l**.* To a solution of compounds **3a−3v** (1.60 mmol) in tetrahydrofuran (THF) (10 mL) was added Lawesson's reagent (1.60 mmol). The reaction mixture was refluxed for 12 h and then distilled under vacuum. DCM (50 mL) was added into the residue. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and brine (3 × 50 mL), dried, and removed under vacuum. The residue was purified by column chromatography on

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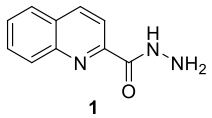
Table 1. Fungicidal Activities of Kynurenic Acid, Quinaldic Acid, and Compounds 1, 2a−2h, and 3a−3v against Four Pathogenic Fungi



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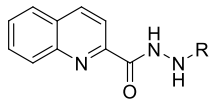
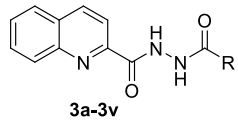


2a-2h



3a-3v

compound	R	inhibition rate ± SD (%) at 100 μg/mL			
		S. sclerotiorum	*R. solani*	*B. cinerea*	*F. graminearum*
kynurenic acid		23.93 ± 2.65	15.71 ± 1.20	37.72 ± 0.09	26.05 ± 1.48
quinaldic acid		30.47 ± 0.31	22.45 ± 1.33	33.62 ± 1.75	23.02 ± 2.10
1		49.23 ± 0.33	34.63 ± 1.73	83.03 ± 1.70	39.07 ± 0.95
2a	*t*-Bu	95.50 ± 0.57	64.58 ± 1.37	18.13 ± 0.52	17.72 ± 1.97
2b	Ph	99.08 ± 0.48	96.25 ± 0.92	90.71 ± 0.17	82.08 ± 1.77
2c	4-CH_3-Ph	99.13 ± 0.27	94.43 ± 0.46	85.38 ± 0.49	85.12 ± 0.95
2d	2-F-Ph	99.80 ± 0.10	98.60 ± 0.58	89.80 ± 0.96	68.54 ± 0.21
2e	4-F-Ph	99.86 ± 0.10	98.85 ± 0.20	91.30 ± 0.61	100 ± 0.00
2f	2,4-F-Ph	99.35 ± 0.20	100 ± 0.00	99.40 ± 0.06	74.38 ± 0.34
2g	4-Cl-Ph	90.83 ± 0.12	85.82 ± 0.51	89.45 ± 0.18	80.64 ± 1.70
2h	4-CF_3-Ph	73.31 ± 1.23	61.49 ± 0.65	81.05 ± 0.45	69.15 ± 0.25
3a	H	23.93 ± 0.65	12.50 ± 1.87	20.13 ± 1.49	15.10 ± 1.36
3b	CH_3	23.12 ± 0.93	12.42 ± 2.58	19.60 ± 1.93	22.12 ± 1.32
3c	CH_2CH_3	37.49 ± 1.33	49.03 ± 1.10	13.41 ± 0.80	36.10 ± 1.21
3d	cyclopropyl	30.30 ± 0.81	52.65 ± 2.63	15.31 ± 1.00	57.95 ± 0.27
3e	Ph	43.31 ± 0.25	38.38 ± 1.68	20.02 ± 0.80	11.59 ± 0.28
3f	4-CH_3-Ph	55.32 ± 1.23	34.53 ± 1.02	68.82 ± 1.10	46.60 ± 0.27
3g	2-OCH_3-Ph	96.12 ± 0.08	87.87 ± 0.56	89.20 ± 0.30	75.00 ± 0.36
3h	3-OCH_3-Ph	33.85 ± 1.03	28.49 ± 1.17	17.14 ± 2.99	11.10 ± 0.36
3i	4-OCH_3-Ph	79.48 ± 0.64	52.40 ± 2.28	37.63 ± 0.71	35.08 ± 0.85
3j	2-F-Ph	91.24 ± 0.46	91.18 ± 0.42	98.88 ± 0.40	85.11 ± 0.81
3k	3-F-Ph	13.00 ± 0.41	25.30 ± 0.79	11.58 ± 0.42	17.93 ± 0.44
3l	4-F-Ph	37.20 ± 0.79	69.32 ± 0.75	26.55 ± 0.50	49.47 ± 0.38
3m	2,6-F-Ph	66.32 ± 0.75	76.29 ± 1.96	52.53 ± 0.60	66.03 ± 2.59
3n	2,3,4,5,6-F-Ph	48.09 ± 2.66	57.19 ± 0.86	27.93 ± 1.19	42.03 ± 1.71
3o	2-Cl-Ph	96.37 ± 0.11	94.20 ± 0.48	98.06 ± 0.49	81.66 ± 1.50
3p	4-Cl-Ph	75.79 ± 1.78	40.46 ± 0.35	17.40 ± 1.60	18.33 ± 0.96
3q	2,6-Cl-Ph	81.60 ± 2.05	91.88 ± 0.32	96.93 ± 0.06	70.54 ± 0.49
3r	2-Br-Ph	87.82 ± 0.90	83.40 ± 0.57	74.73 ± 0.91	65.86 ± 0.18
3s	furan-2-yl	35.30 ± 0.46	88.40 ± 3.95	50.35 ± 0.66	63.54 ± 1.08
3t	thiophene-2-yl	73.79 ± 1.58	80.12 ± 0.80	37.72 ± 0.09	57.49 ± 3.11
3u	pyridine-3-yl	75.12 ± 1.72	14.39 ± 0.54	22.23 ± 0.32	39.08 ± 1.96
3v	CH_2-4-OCH_3-Ph	56.63 ± 0.52	65.38 ± 1.07	18.33 ± 1.11	22.85 ± 0.65
carbendazim		100 ± 0.00	99.91 ± 0.05	0.00 ± 0.00	100 ± 0.00

silica gel using petroleum ether/ethyl acetate mixture as the eluent to afford the target compounds.

*Data for Compound **8a**.* White solid; mp 124−125 °C; yield: 75%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.15 (s, 1H), 8.40 (d, J = 8.4 Hz, 1H), 8.23 (d, J = 8.5 Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.84, 153.90, 148.75, 147.96, 137.41, 130.36, 129.59, 128.89, 127.91, 127.84, 118.59; ESI-MS m/z: 214.10 $[M + H]^+$.

*General Synthetic Procedure for Compounds **10a−10q** and **11a−11m**.* Compounds **10a−10q** and **11a−11m** were synthesized using a similar procedure to compounds **2a−2h**.

*Data for Compound **10a**.* Yellow solid; mp 210−212 °C; yield: 54%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.49 (s, 1H), 8.90 (s, 1H), 8.12 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.89 (t, J = 7.3 Hz, 1H), 7.75 (t, J = 7.5 Hz, 1H), 7.09−6.91 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.30, 157.62, 155.30, 153.35, 145.90, 145.38, 140.56, 131.39, 129.36, 129.30, 129.08, 127.80, 115.80, 115.58, 114.05, 113.63; ESI-MS m/z: 361.97 $[M + H]^+$.

Synthetic details and data of the other quinoline derivatives shown in Figure 1 are provided in the Supporting Information.

Fungicidal Activity Assay. The *in vitro* fungicidal activity of the target compounds against four pathogenic fungi was tested by the mycelium growth rate method.[21,22]

Spore Germination Inhibition Assay. The inhibitory activity of compound **2e** on spore germination of *F. graminearum* was assessed by microscopic observation.[23] Spore suspensions (1×10^5 to 5×10^5 spores/mL) were prepared to inoculate conidia in sterile water. Compound **2e** in DMSO was added to the conidial suspension to obtain different concentrations of 25, 10, and 5 μg/mL. Then, 150 μL of these mixtures were removed, put on concave slides, and incubated in a biochemical incubator at 25 °C. Conidial suspension with 0.5% DMSO in water (v/v) was treated as a blank control. After incubation for 12 h, the number of germinated spores was measured by approximately counting 80 conidia in the blood counting chamber under a biological microscope photographic system at 200× magnification. The experiment was repeated three times, and the inhibitory rates were calculated according to the following formula

$$\text{Inhibition of spore germination (\%)} = [(N_0 - N_1)/N_0] \times 100$$

where N_0 and N_1 are the average values of the spore germination rates of black control and treatment, respectively.

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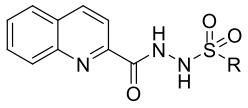
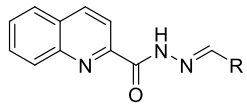
Table 2. Fungicidal Activities of Compounds 4a−4h and 5a−5o against Four Pathogenic Fungi



4a-4h



5a-5o

		inhibition rate ± SD (%) at 100 μg/mL			
compound	R	*S. sclerotiorum*	*R. solani*	*B. cinerea*	*F. graminearum*
4a	CH_3	11.47 ± 0.28	13.80 ± 0.48	6.74 ± 0.61	16.30 ± 1.55
4b	CH_2CH_3	0.00 ± 0.00	20.27 ± 0.48	20.55 ± 0.21	7.13 ± 1.09
4c	2-F-Ph	85.29 ± 0.35	56.43 ± 0.75	27.90 ± 0.85	55.59 ± 1.88
4d	4-F-Ph	91.54 ± 0.12	70.99 ± 0.86	55.46 ± 2.25	75.99 ± 1.23
4e	2-Cl-Ph	29.58 ± 0.65	64.35 ± 1.33	13.47 ± 0.82	23.60 ± 0.88
4f	4-Cl-Ph	84.76 ± 0.22	71.53 ± 1.59	39.90 ± 0.31	48.38 ± 5.72
4g	4-CF_3-Ph	30.47 ± 0.31	53.50 ± 1.41	34.20 ± 0.92	42.13 ± 0.69
4h	4-OCF_3-Ph	66.70 ± 1.20	32.08 ± 0.94	17.36 ± 2.03	32.62 ± 0.32
5a	4-CH_2CH3-Ph	72.49 ± 1.12	65.68 ± 3.08	39.57 ± 1.40	32.87 ± 1.02
5b	4-CN-Ph	0.00 ± 0.00	3.61 ± 1.50	10.55 ± 1.33	0.00 ± 0.00
5c	2-NO_2-Ph	0.00 ± 0.00	10.69 ± 2.05	16.90 ± 0.31	0.00 ± 0.00
5d	4-NO_2-Ph	0.00 ± 0.00	15.71 ± 1.20	19.15 ± 0.41	0.00 ± 0.00
5e	4-OCH_3-Ph	12.26 ± 1.29	8.83 ± 0.66	19.45 ± 0.43	11.17 ± 1.40
5f	2-F-Ph	29.83 ± 0.91	14.54 ± 0.35	14.44 ± 1.08	18.54 ± 1.96
5g	4-F-Ph	9.65 ± 0.12	0.00 ± 0.00	6.54 ± 1.37	13.31 ± 3.22
5h	2-Cl-Ph	45.56 ± 1.72	0.00 ± 0.00	23.49 ± 1.08	13.03 ± 0.82
5i	4-Cl-Ph	51.98 ± 1.64	0.00 ± 0.00	21.59 ± 0.93	12.15 ± 1.81
5j	2,6-Cl-Ph	0.00 ± 0.00	0.00 ± 0.00	16.80 ± 0.63	0.00 ± 0.00
5k	4-Br-Ph	5.68 ± 0.41	11.57 ± 3.30	18.15 ± 1.37	0.00 ± 0.00
5l	2-CF_3-Ph	5.40 ± 1.25	6.74 ± 0.48	11.86 ± 1.20	17.90 ± 0.19
5m	4-CF_3-Ph	0.00 ± 0.00	0.00 ± 0.00	16.16 ± 0.77	11.21 ± 1.11
5n	2-OCF_3-Ph	12.69 ± 0.93	2.92 ± 1.31	6.69 ± 1.43	0.00 ± 0.00
5o	4-OCF_3-Ph	0.00 ± 0.00	0.00 ± 0.00	7.31 ± 0.19	5.25 ± 0.39

Scanning Electron Microscopy Observations. Scanning electron microscopy (SEM) observations were performed according to the described methods to examine the effects of compound **2e** on the microstructure of *F. graminearum*.[24] After treatment with compound **2e** at a concentration of 1 μg/mL, mycelial blocks (5.0 mm × 4.0 mm) were cut from the culture medium. The samples were treated by 4% glutaraldehyde for 4 h at 4 °C and then fixed with 1% osmium tetraoxide solution (w/v) for 2 h. The samples were washed with 0.01 M phosphate-buffered saline and then dehydrated with a series of ethanol solutions. After drying the samples at a critical point and gold-spraying, they were observed with a scanning electron microscope (Hitachi, S-3400N, Japan).

Transmission Electron Microscopy Observations. The dehydrated mycelial blocks were embedded in resin at 70 °C for 24 h and then cut into thin sections. After double-staining the samples with uranyl acetate and lead citrate, they were observed with a transmission electron microscope (Hitachi, HT7700, Japan).

Determination of Cell Membrane Permeability. The cell membrane relative permeability rate of *F. graminearum* was evaluated according to the method with some modifications.[25] The mycelial disk of *F. graminearum* (5 mm) was placed in 60 mL of PD broth medium with 140 rpm shaking at 25 °C for 48 h. Then, the mycelia (500 mg) were filtered and added into compound **2e** solution with different concentrations (25, 10, 5, and 2.5 μg/mL). Finally, the conductivity values were determined with a conductivity detector (0 h was marked as L_0 and 2, 4, 6, 8, 10, 12, 24, and 48 h were marked as L_1, HORIBA, EC1100, Japan). The conductivities of samples treated by boiling water for 0.5 h were marked as L_2. The relative permeability rate of the cell membrane was calculated by the following formula

$$\text{Relative electric conductivity (\%)} = [(L_1 - L_0)/(L_2 - L_0)] \times 100$$

Determination of the Leakage of Cellular Contents. The release of cellular content was determined according to the method with some modifications.[26] The mycelia (400 mg) of *F. graminearum* were added into compound **2e** solutions (25, 10, 5, and 2.5 μg/mL) to obtain mycelia suspension. Then, the samples were incubated at 25 °C for 24 h and then centrifuged. Finally, the absorbance values of the supernatants were measured at 260 and 280 nm with a UV−vis spectrophotometer (Shimadzu, UV-1700, Japan).

Statistical Analysis. All assays were at least performed in triplicate by conventional methods, and the results were presented as means ± standard deviations. Statistical analysis was carried out by SPSS 24.0.

RESULTS AND DISCUSSION

Chemistry. The synthesis of all intermediates and target compounds was performed as shown in Figure 2. Briefly, intermediate **1** and compounds **2a−2h**, **3a−3v**, **4a−4h**, **5a−5o**, **6a−6y**, **7a−7f**, **8a−8b**, **9a−9m**, **10a−10q**, and **11a−11m** were synthesized according to our previously reported procedure.[20] Quinoline-2-carbonyl chloride was reacted with various substituted hydrazine hydrochlorides and NaOH to obtain target compounds **2a−2h**. Intermediate **1** was reacted with various substituted acids to afford compounds **3a−3v** and then further cyclized in $POCl_3$ to obtain target compounds **6a−6v** or reacted with Lawesson's reagent in THF to obtain target compounds **8a−8l**. Intermediate **1** was reacted with various substituted sulfuryl chlorides in pyridine to obtain target compounds **4a−4h** or reacted with various substituted aldehydes in ethanol to afford compounds **5a−5o**. Compound **6y** was reacted with various substituted haloalkanes in DMF to obtain target compounds **7a−7f**. Compounds **10a−10q** and **11a−11m** were synthesized with substituted quinoline-2-carboxylic acid[27] or heteroaromatic carboxylic acid

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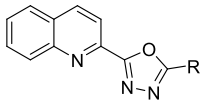
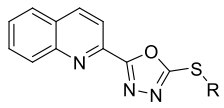
Table 3. Fungicidal Activities of Compounds 6a−6y and 7a−7f against Four Pathogenic Fungi



6a-6y



7a-7f

		inhibition rate ± SD (%) at 100 μg/mL			
compound	R	*S. sclerotiorum*	*R. solani*	*B. cinerea*	*F. graminearum*
6a	H	58.34 ± 0.16	42.57 ± 1.35	66.77 ± 0.97	47.61 ± 0.52
6b	CH_3	16.82 ± 3.24	28.26 ± 0.79	27.27 ± 0.24	23.02 ± 2.10
6c	CH_2CH_3	28.79 ± 1.33	47.73 ± 0.89	29.58 ± 3.48	39.98 ± 1.39
6d	cyclopropyl	50.32 ± 4.71	53.58 ± 1.19	47.61 ± 2.10	53.54 ± 0.33
6e	Ph	23.70 ± 0.57	18.59 ± 1.07	4.25 ± 0.57	0.00 ± 0.00
6f	4-CH_3-Ph	15.19 ± 0.28	13.45 ± 1.31	9.08 ± 0.70	7.33 ± 1.13
6g	2-OCH_3-Ph	14.10 ± 1.10	27.68 ± 0.68	28.11 ± 0.99	9.73 ± 0.22
6h	3-OCH_3-Ph	7.90 ± 0.72	38.02 ± 0.38	18.43 ± 0.37	7.00 ± 0.27
6i	4-OCH_3-Ph	36.56 ± 0.71	41.47 ± 1.62	56.85 ± 0.77	14.95 ± 0.63
6j	2-F-Ph	11.95 ± 1.52	38.13 ± 1.19	21.25 ± 0.15	0.00 ± 0.00
6k	3-F-Ph	7.39 ± 1.54	22.45 ± 3.76	21.03 ± 2.19	0.00 ± 0.00
6l	4-F-Ph	10.99 ± 0.64	22.61 ± 3.05	22.68 ± 4.49	0.00 ± 0.00
6m	2,6-F-Ph	25.49 ± 0.72	12.73 ± 1.13	7.67 ± 0.56	15.21 ± 1.04
6n	2,3,4,5,6-F-Ph	86.85 ± 0.89	48.36 ± 2.68	32.34 ± 2.37	38.59 ± 0.05
6o	2-Cl-Ph	0.00 ± 0.00	15.03 ± 0.92	18.05 ± 0.13	0.00 ± 0.00
6p	4-Cl-Ph	6.18 ± 0.80	23.86 ± 0.35	20.08 ± 0.75	0.00 ± 0.00
6q	2,6-Cl-Ph	0.00 ± 0.00	11.25 ± 1.40	0.00 ± 0.00	0.00 ± 0.00
6r	2-Br-Ph	0.00 ± 0.00	16.18 ± 0.57	0.00 ± 0.00	0.00 ± 0.00
6s	furan-2-yl	27.08 ± 0.33	52.98 ± 1.46	36.19 ± 0.13	20.64 ± 1.02
6t	thiophene-2-yl	23.17 ± 0.81	16.56 ± 0.78	5.04 ± 0.62	0.00 ± 0.00
6u	pyridine-3-yl-	29.49 ± 6.18	18.83 ± 0.93	15.69 ± 1.52	15.52 ± 0.93
6v	4-OCH_3-Ph-CH_2	30.50 ± 2.16	22.63 ± 0.91	46.19 ± 0.90	39.99 ± 1.37
6w	NH_2	14.26 ± 2.64	0.00 ± 0.00	19.08 ± 1.68	13.33 ± 2.56
6x	OH	86.40 ± 0.58	58.35 ± 0.22	74.03 ± 0.64	76.48 ± 0.95
6y	SH	90.72 ± 1.52	45.63 ± 0.77	65.35 ± 0.17	27.28 ± 0.30
7a	CH_3	64.39 ± 0.72	100 ± 0.00	51.23 ± 1.83	43.43 ± 3.63
7b	CH_2CH_3	89.65 ± 0.66	100 ± 0.00	99.40 ± 0.44	82.61 ± 1.44
7c	CH_2CH_2F	93.41 ± 0.32	99.21 ± 0.34	91.26 ± 0.73	70.68 ± 1.01
7d	CH_2CH_2Cl	91.56 ± 0.81	95.70 ± 0.07	79.54 ± 0.19	32.96 ± 1.42
7e	CH_2CH_2Br	74.24 ± 0.26	91.70 ± 0.82	27.39 ± 0.92	35.91 ± 7.88
7f	$CH_2CH_2CH_3$	77.18 ± 0.54	94.54 ± 0.85	90.08 ± 0.63	51.11 ± 1.15

with hydrazine hydrochlorides using a similar procedure to compounds **2a−2h**. All target compounds were purified by recrystallization or column chromatography, and their structures were confirmed on the basis of ^{1}H NMR, ^{13}C NMR, and MS data.

Structure−Activity Relationship Studies. With 132 compounds in hand, we evaluated their fungicidal activity against four pathogenic fungi, *S. sclerotiorum*, *R. solani*, *B. cinerea,* and *F. graminearum*, to probe their structure−activity relationship (SAR). The biological data for all quinoline derivatives are shown in Tables 1−7.

As shown in Table 1, the natural 2-quinolinecarboxylic acid, kynurenic acid, displayed marginal to weak fungicidal activity with the inhibitory rate of <50% against four fungi at a concentration of 100 μg/mL. The dehydroxylated compound, quinaldic acid, showed a similar activity. However, interestingly, quinoline-2-acylhydrazine **1** exhibited significant activity and was more potent than kynurenic acid. Subsequently, the effects of quinoline compounds **2a−2h** and **3a−3v** with a focus on varying substituents at the acylhydrazine and diacylhydrazine groups were investigated. Compared with compound **1**, substitution for a *tert*-butyl group (**2a**) resulted in a 2-fold increase in activity against *S. sclerotiorum* and *R. solani* but diminished potency against *B. cinerea* and *F. graminearum*. Substitution for phenyl ring (**2b−2h**) was favorable for activity that depended significantly on the nature of substituents and their position at the phenyl ring. Of particular note, compound **2e** (*p*-F-C_6H_4) displayed the most effective inhibition among this series with inhibitory rates of 99.86, 98.85, 91.30, and 100% against *S. sclerotiorum*, *R. solani*, *B. cinerea,* and *F. graminearum*, respectively, at a concentration of 100 μg/mL. A possible explanation is that the introduction of a benzene ring and an electron-withdrawing group enhanced the induction and conjugation effect of quinoline acylhydrazine molecules and enhanced the antifungal effect.[28]

In the diacylhydrazine series (**3a−3v**), substitution of alkyl groups (**3a−3d**) led to a substantially reduced fungicidal potency, but in contrast, good activity accompanied substitution with a range of aromatic groups (**3e−3v**); particularly, the substituents on the ortho-position of aromatic ring (**3g**, **3j**, and **3o**) were recommended for the enhancement of activity than the para- (**3i**, **3l**, and **3p**) and meta-counterparts (**3h** and **3k**). The fungicidal activity of ortho-substituted compounds increased following 2-F (**3j**) ≈ 2-Cl (**3o**) > 2-OCH_3 (**3g**) > 2-Br (**3r**). Furthermore, replacement of phenyl ring with furan-2-yl (**3s**) or

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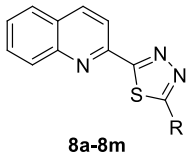
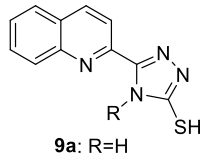
Table 4. Fungicidal Activities of Compounds 8a−8b and 9a−9m against Four Pathogenic Fungi



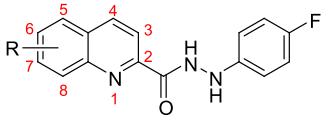
8a-8m



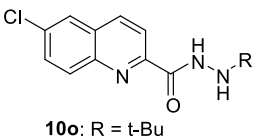
9a: R=H
9b: R=NH_2

compound	R	inhibition rate ± SD (%) at 100 μg/mL			
		S. sclerotiorum	*R. solani*	*B. cinerea*	*F. graminearum*
8a	H	85.83 ± 0.69	80.28 ± 0.67	98.80 ± 0.52	83.01 ± 1.66
8b	CH_3	0.00 ± 0.00	25.81 ± 0.33	11.78 ± 1.19	10.38 ± 1.07
8c	cyclopropyl	17.63 ± 0.33	61.13 ± 2.13	33.62 ± 1.75	44.43 ± 1.84
8d	Ph	0.00 ± 0.00	5.40 ± 0.48	0.00 ± 0.00	8.43 ± 0.82
8e	2-OCH_3-Ph	0.00 ± 0.00	7.98 ± 1.25	0.00 ± 0.00	0.00 ± 0.00
8f	2-F-Ph	21.45 ± 0.69	10.80 ± 2.21	6.29 ± 2.12	0.00 ± 0.00
8g	4-F-Ph	0.00 ± 0.00	4.69 ± 1.94	0.00 ± 0.00	0.00 ± 0.00
8h	2,6-F-Ph	0.00 ± 0.00	8.24 ± 0.23	0.00 ± 0.00	0.00 ± 0.00
8i	2-Cl-Ph	0.00 ± 0.00	20.06 ± 0.11	0.00 ± 0.00	0.00 ± 0.00
8j	4-Cl-Ph	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
8k	2-Br-Ph	0.00 ± 0.00	13.44 ± 5.82	0.00 ± 0.00	0.00 ± 0.00
8l	4-OCH_3-Ph-CH_2	0.00 ± 0.00	24.90 ± 0.73	25.80 ± 4.54	0.00 ± 0.00
8m	SH	96.51 ± 0.20	56.93 ± 3.27	63.08 ± 1.76	58.96 ± 1.32
9a	H	33.90 ± 1.82	32.54 ± 0.88	20.94 ± 1.00	23.60 ± 0.30
9b	NH_2	13.85 ± 0.56	8.48 ± 1.30	13.11 ± 1.43	26.05 ± 1.48

Table 5. Fungicidal Activities of Compounds 10a−10q against Four Pathogenic Fungi



10a-10n



10o: R = t-Bu
10p: R = Ph
10q: R = 2,4-di-F-Ph

compound	R	inhibition rate ± SD (%) at 25 μg/mL			
		S. sclerotiorum	*R. solani*	*B. cinerea*	*F. graminearum*
2e	H	99.34 ± 0.06	95.41 ± 0.64	86.11 ± 0.18	100 ± 0.00
10a	3-Br	73.15 ± 0.80	82.90 ± 0.32	74.97 ± 1.03	66.90 ± 0.73
10b	4-Cl	86.00 ± 0.24	87.22 ± 1.65	79.34 ± 0.96	79.35 ± 0.61
10c	4,7-Cl	0.00 ± 0.00	18.88 ± 1.67	13.90 ± 1.35	18.69 ± 0.22
10d	4-Br	87.45 ± 0.24	83.61 ± 2.66	83.48 ± 0.33	81.44 ± 0.77
10e	5-Cl	80.26 ± 0.76	83.92 ± 1.06	73.99 ± 0.51	66.54 ± 0.50
10f	5-Br	62.67 ± 0.54	67.18 ± 1.62	63.48 ± 0.78	53.21 ± 0.63
10g	6-OCH_3	97.70 ± 0.33	83.12 ± 0.68	85.06 ± 0.38	99.85 ± 0.06
10h	6-F	98.53 ± 0.23	88.26 ± 0.41	88.29 ± 0.39	99.75 ± 0.03
10i	6-Cl	91.06 ± 0.36	86.20 ± 0.80	92.55 ± 0.43	88.70 ± 0.63
10j	6-Br	87.42 ± 0.35	82.42 ± 1.56	82.16 ± 0.71	96.20 ± 0.86
10k	6,8-Br	86.73 ± 0.80	82.19 ± 0.90	85.61 ± 1.10	90.53 ± 0.35
10l	7-Br	90.75 ± 0.38	81.61 ± 1.53	78.35 ± 1.44	98.10 ± 0.18
10m	8-OCH_3	86.11 ± 0.36	87.05 ± 0.39	80.90 ± 0.78	91.07 ± 0.33
10n	8-F	98.05 ± 0.23	89.10 ± 0.38	85.61 ± 0.41	99.60 ± 0.07
10o		86.81 ± 0.47	48.03 ± 1.04	14.80 ± 1.38	42.91 ± 0.65
10p		91.33 ± 0.50	86.82 ± 0.12	81.97 ± 0.47	85.70 ± 0.96
10q		35.95 ± 0.59	40.51 ± 0.14	80.68 ± 1.07	56.28 ± 0.58

thiophene-2-yl (**3t**) resulted in a slight increase in activity. The SAR for the sulfonylhydrazine (**4a−4h**) and acylhydrazone (**5a−5o**) derivatives was probed next, and the results are shown in Table 2. It turned out that extension of diacylhydrazine to sulfonylhydrazine or acylhydrazone led to a substantially reduced fungicidal potency. Taken together, these results implied that the electronegativity and size of substituents at the hydrazine scaffold were crucial.

In synthetic methodology, acylhydrazine is used as an important precursor for building the skeletons in diverse heterocyclic compounds, such as 1,3,4-oxadiazole and 1,3,4-thiadiazole. In addition to acylhydrazines, diacylhydrazines, acylhydrazones, and sulfonylhydrazines, the effects of different five-membered heterocycles at the 2-position on the quinoline scaffold were investigated. As shown in Table 3, cyclization of diacylhydrazines **3a−3v** to obtain 1,3,4-oxadiazole derivatives **6a−6v** bearing alkyl and

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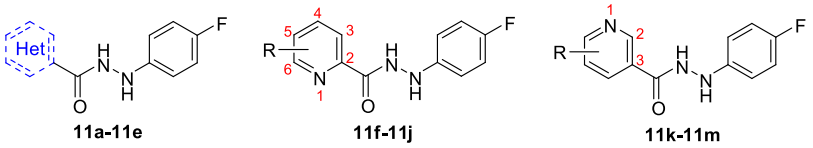
Table 6. Fungicidal Activities of Compounds 11a−11m against Four Pathogenic Fungi



Compound	Het	Inhibition rate ± SD (%) at 25 μg/mL			
		S. sclerotiorum	*R. solani*	*B. cinerea*	*F. graminearum*
2e	-	99.34±0.06	95.41 ± 0.64	86.11±0.18	100±0.00
11a		97.00±0.31	90.78±0.34	88.04±0.46	99.35±0.08
11b		97.75±0.26	82.95±0.62	92.09±0.63	99.89±0.03
11c		98.57±0.16	76.78±0.47	90.58±0.61	99.76±0.05
11d		99.52±0.16	92.87±0.52	92.25±0.33	99.85±0.04
11e		74.40±1.11	57.93±0.45	67.09±1.26	73.84±0.91
11f	H	97.98±0.09	86.65±0.18	91.01±0.37	99.85±0.05
11g	3-F	98.22±0.21	93.67±0.74	93.00±0.48	99.93±0.04
11h	3-Cl	95.05±0.29	91.06±0.97	95.33±0.36	99.83±0.04
11i	5-F	98.45±0.30	97.77±0.51	98.00±0.36	99.53±0.12
11j	5-Cl	98.75±0.27	91.14±0.10	95.76±0.88	99.84±0.05
11k	H	89.46±0.43	76.95±0.19	79.13±0.33	99.29±0.34
11l	2-F	89.65±0.77	82.06±0.21	79.33±0.83	99.46±0.05
11m	2-Cl	96.05±0.45	97.44±0.30	94.70±0.35	99.79±0.05

Table 7. Fungicidal Activities of Selected Compounds against Four Pathogenic Fungi

compound	EC_{50} (μg/mL)			
	S. sclerotiorum	*R. solani*	*B. cinerea*	*F. graminearum*
2b	1.38 ± 0.01	1.22 ± 0.01	0.50 ± 0.02	2.00 ± 0.13
2c	0.71 ± 0.02	4.22 ± 0.23	0.39 ± 0.02	2.69 ± 0.07
2d	1.39 ± 0.04	1.00 ± 0.02	0.51 ± 0.01	23.58 ± 1.02
2e	0.39 ± 0.01	0.46 ± 0.01	0.19 ± 0.01	0.18 ± 0.01
2f	1.04 ± 0.02	0.87 ± 0.03	0.14 ± 0.03	0.46 ± 0.03
2g	0.82 ± 0.01	0.79 ± 0.01	0.29 ± 0.06	0.39 ± 0.03
2h	2.16 ± 0.04	1.88 ± 0.04	0.51 ± 0.01	4.70 ± 0.14
3g	7.73 ± 0.10	10.20 ± 0.11	31.20 ± 0.60	12.40 ± 0.37
3j	21.96 ± 0.51	29.42 ± 0.28	38.74 ± 0.51	31.79 ± 0.11
3o	18.69 ± 0.56	14.82 ± 0.58	48.90 ± 0.08	53.63 ± 1.38
7b	39.91 ± 0.47	5.73 ± 0.14	21.85 ± 0.10	65.96 ± 2.23
7c	36.78 ± 0.06	5.11 ± 0.03	22.73 ± 0.38	70.22 ± 2.67
7d	32.41 ± 0.21	4.92 ± 0.06	19.33 ± 0.13	>100
7f	33.85 ± 1.37	4.96 ± 0.03	28.86 ± 0.63	91.40 ± 3.47
9a	47.15 ± 0.19	36.24 ± 0.63	43.52 ± 0.51	56.79 ± 0.64
10g	0.38 ± 0.01	1.21 ± 0.02	1.39 ± 0.05	0.76 ± 0.01
10h	0.36 ± 0.01	0.97 ± 0.02	1.01 ± 0.05	0.68 ± 0.02
10n	0.45 ± 0.02	0.96 ± 0.05	1.01 ± 0.01	0.89 ± 0.01
11d	0.24 ± 0.01	0.69 ± 0.01	0.87 ± 0.05	0.57 ± 0.01
11g	0.34 ± 0.01	1.07 ± 0.01	0.74 ± 0.01	0.90 ± 0.02
11h	1.07 ± 0.03	1.48 ± 0.03	1.05 ± 0.02	1.39 ± 0.03
11i	0.38 ± 0.01	0.94 ± 0.02	0.83 ± 0.02	0.86 ± 0.02
11j	0.36 ± 0.01	0.71 ± 0.01	0.97 ± 0.02	0.71 ± 0.01
11m	0.78 ± 0.02	1.56 ± 0.02	1.19 ± 0.03	1.04 ± 0.05
carbendazim	0.68 ± 0.01	0.14 ± 0.01	>100	0.65 ± 0.02

aromatic groups resulted in substantially reduced fungicidal potency. Extension of 2-H (**6a**) to 2-oxo (**6x**) or 2-thioxo (**6y**) led to a similar activity, while 2-amino (**6w**) showed inferior activity. However, interestingly, alkylation of compound **6y** was beneficial for the improvement of the fungicidal activity. For example, compounds **7a−7f** exhibited excellent fungicidal activity against *R. solani* with the inhibitory rates of >90% at 100 μg/mL, which were more potent than that of **6y** (45.63%). Likewise, it can be seen that the structural changes in general were detrimental to activity, and no favorable substitution could

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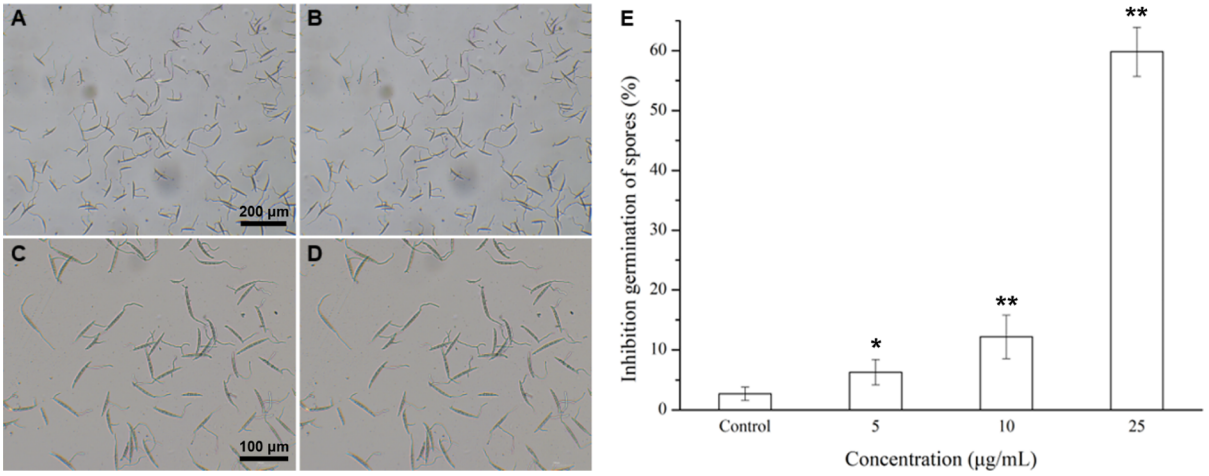

Figure 3. Spore germination inhibition of *F. graminearum* in the untreated control (A) and 5, 10, and 25 μg/mL of compound **2e**-treated control (B–D). The inhibitory rate of **2e** on the spore germination (E). *$P < 0.05$ compared to the control group. **$P < 0.01$ compared to the control group.

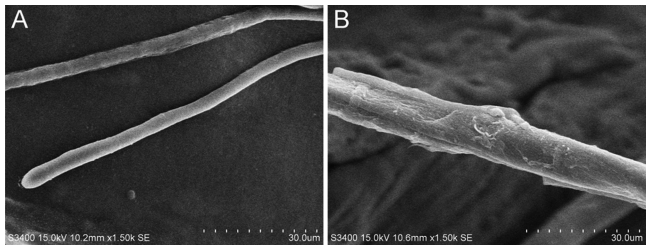

Figure 4. Scanning electron micrographs of *F. graminearum* hyphae in untreated control (A) and 1.0 μg/mL of compounds **2e**-treated control (B).

be found for substitution of 1,3,4-thiadiazole (**8b–8l**) and 1,2,4-triazole (**9a** and **9b**), except for **8a** and **8m** (Table 4). Overall, from the available fungicidal activities, we could obviously find that the compounds containing the acylhydrazine moiety (**2a–h**) showed higher fungicidal activities than the other seven series derivatives (**3a–3v**, **4a–4h**, **5a–5o**, **6a–6y**, **7a–7f**, **8a–8m**, and **9a–9b**).

Finally, the effects of compounds **10a–10q** and **11a–11m** with a focus on varying substituents on the quinoline ring or heterocycle replacement were evaluated, and the biological data are shown in Tables 5 and 6. Compound **2e**, the potent compound against four pathogenic fungi, was utilized as the reference point for relative comparison of substituted quinoline or other heterocycle. Compared with the unsubstituted compound **2e**, substitution of electron-withdrawing groups or electron-donating groups at various positions on the quinoline ring led to a slight decrease in activity against four pathogenic fungi at a concentration of 25 μg/mL (Table 5). Furthermore, replacement of quinoline with various heterocycles, such as isoquinoline, quinoxaline, pyrazine, and pyridine, obtained compounds **11a–11m**, which displayed similar activity relative to **2e** (Table 6). Overall, these findings further underlined that the fungicidal differences of quinoline compounds could be ascribed to a combination of factors, such as a different interaction at the site or the nature of the substituents, which may depend on the size, electronic characteristics of substituents, or other factors.[20]

The EC_{50} values of potent compounds **2b–2h**, **3g**, **3j**, **3o**, **7b–7d**, **7f**, **8a**, **10g**, **10h**, **10n**, **11d**, **11g–11j**, and **11m** were further evaluated, and the results are presented in Table 7. Most of these compounds exhibited significant fungicidal activities against

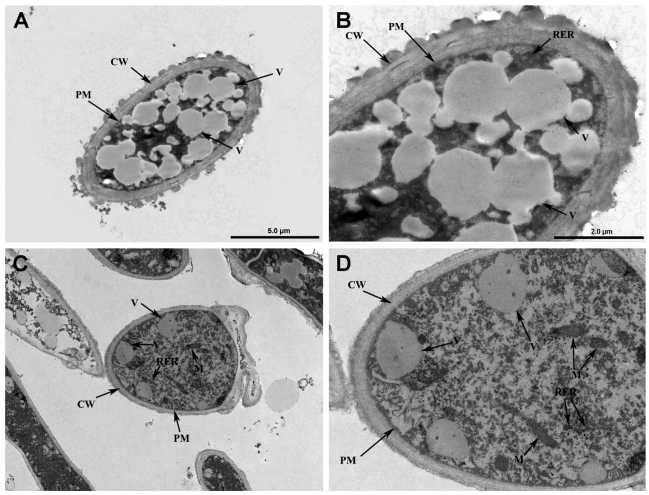

Figure 5. TEM observations of cell structure of *F. graminearum*. Ultrastructure of the hyphae in the untreated control (A,B) and 1 μg/mL of **2e**-treated treated control (C,D). Cell wall (CW), mitochondrion (M), plasma membrane (PM), rough endoplasmic reticulum (RER), and vacuole (V).

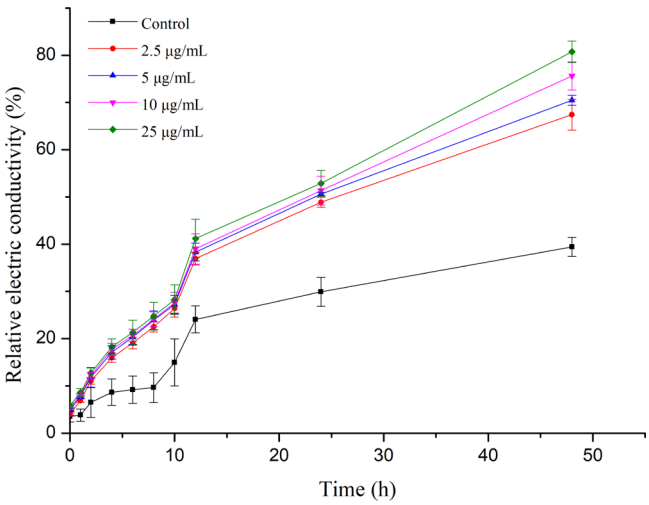

Figure 6. Effect of compound **2e** on the cell membrane permeability of *F. graminearum* mycelia.

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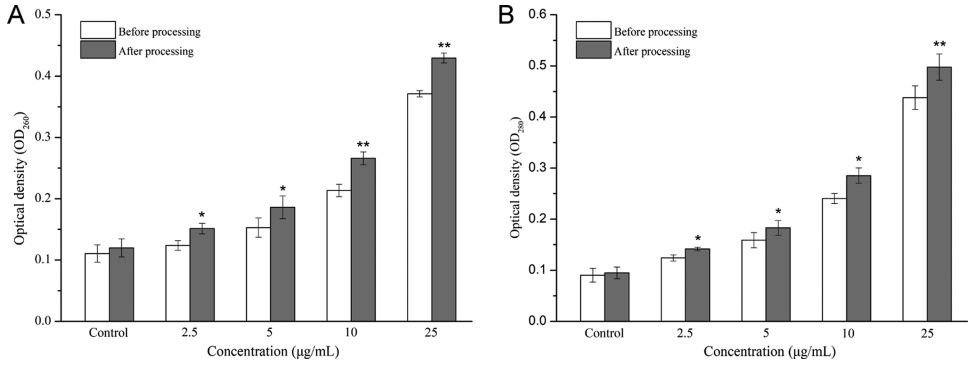

Figure 7. Effect of compound **2e** on release of cellular content *F. graminearum* mycelia. (A) Absorbance value of nucleic acids (OD_{260}) and (B) absorbance value of protein (OD_{280}). *$P < 0.05$ compared to control group. **$P < 0.01$ compared to control group.

S. sclerotiorum, *R. solani*, *B. cinerea*, and *F. graminearum*, with EC_{50} values ranging from 0.24−47.15, 0.46−36.24, 0.14−48.90, and 0.18−91.40 μg/mL, respectively. Especially, compound **2e** exhibited the greatest inhibitory activity against *S. sclerotiorum*, *R. solani*, *B. cinerea*, and *F. graminearum* with the EC_{50} values of 0.39, 0.46, 0.19, and 0.18 μg/mL, respectively, and were more potent than those of carbendazim (EC_{50}, 0.68, 0.14, >100, and 0.65 μg/mL, respectively). Therefore, compound **2e** was selected as the candidate compound to study its preliminary mode of action against *F. graminearum*.

Preliminary Mode of Action of Compound 2e against *F. graminearum*. *Effect of Compound **2e** on the Spore Germination Inhibition.* In Figure 3, the results of spore germination inhibition assay showed that compound **2e** could suppress spore germination of *F. graminearum* in a concentration-dependent manner, with an inhibition rate of 59.82% at a concentration of 25 μg/mL.

*Effect of Compound **2e** on the Hyphae Morphology.* As shown in Figure 4A, untreated mycelia grew well with a smooth surface and an intact structure. However, after treatment with compound **2e** at a concentration of 1 μg/mL, treated mycelia grew abnormally with the phenomenon of rough and incomplete surface (Figure 4B).

The ultrastructural features of the hyphae treated with compound **2e** at a concentration of 1 μg/mL were observed using transmission electron microscopy. The untreated mycelia showed cellular morphology with normal and uniform cell walls (CWs), plasma membranes (PMs), rough endoplasmic reticulum (RER), and cytoplasmic organelles including mitochondria and vacuoles (Figure 5A,B). In contrast, after treatment with compound **2e**, the CWs of mycelia were irregularly thickened, and protrusions existed at the outer layer of CWs. Besides, organelles were partially missing, and no distinct mitochondrion was observed. Moreover, a large number of vacuoles existed in the cytoplasm, which were irregularly shaped and filled most of the cytoplasm (Figure 5C,D).

*Effects of Compound **2e** on the Cell Membrane.* To confirm whether compound **2e** acted on the cell membrane of *F. graminearum*, we next investigated membrane permeability by measuring the change in relative electric conductivity of mycelia exposed to **2e**, and the results are shown in Figure 6. The conductivity of four groups treated with compound **2e** at concentrations of 2.5, 5, 10, and 25 μg/mL and the conductivity of all treated groups were higher than that of the untreated control group and increased in a time- and concentration-dependent manner. Therefore, it indicated that compound **2e** might cause an increase in membrane permeability, result in the release of charged ions such as K^+ and Na^+, and the increase of relative electric conductivity. Moreover, the cellular content leakage assay of *F. graminearum* mycelia such as nucleic acids and proteins also supported the above observation. As shown in Figure 7, after treatment with compound **2e**, macromolecular nucleic acids and proteins were released from the mycelial cells of *F. graminearum* in a concentration-dependent manner. Taken together, compound **2e** could cause damage to the cell membranes of the mycelia and lead to the electrolyte and cellular content leakage.

In summary, a series of quinoline compounds containing acylhydrazine, acylhydrazone, sulfonylhydrazine, oxadiazole, thiadiazole, and triazole moieties were synthesized and evaluated for their fungicidal activity. The results showed that most of these compounds exhibited excellent fungicidal activity *in vitro*. Significantly, compound **2e** displayed the superior *in vitro* antifungal activity against *S. sclerotiorum*, *R. solani*, *B. cinerea*, and *F. graminearum* and were more potent than carbendazim. Moreover, compound **2e** could inhibit spore germination of *F. graminearum*. Although the preliminary mechanistic studies revealed that compound **2e** might exert its antifungal effect through acting on the cell membrane, and influencing the normal functions of PMs, the specific biological target or targets of **2e** remain unknown. Further studies on structural optimization and target identification are in progress.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c00670.

Melting points, ^1H and ^{13}C NMR, and MS spectra for the target compounds and spectra of the representative compounds (PDF)

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Notes

The authors declare no competing financial interest.

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