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Synthesis and Antifungal Activity of Nicotinamide Derivatives as Succinate Dehydrogenase Inhibitors

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ABSTRACT: Thirty-eight nicotinamide derivatives were designed and synthesized as potential succinate dehydrogenase inhibitors (SDHI) and precisely characterized by ^{1}H NMR, ESI-MS, and elemental analysis. The compounds were evaluated against two phytopathogenic fungi, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, by mycelia growth inhibition assay in vitro. Most of the compounds displayed moderate activity, in which, **3a-17** exhibited the most potent antifungal activity against *R. solani* and *S. sclerotiorum* with IC_{50} values of 15.8 and 20.3 μM, respectively, comparable to those of the commonly used fungicides boscalid and carbendazim. The structure−activity relationship (SAR) of nicotinamide derivatives demonstrated that the meta-position of aniline was a key position contributing to the antifungal activity. Inhibition activities against two fungal SDHs were tested and achieved the same tendency with the data acquired from in vitro antifungal assay. Significantly, **3a-17** was demonstrated to successfully suppress disease development in *S. sclerotiorum* infected cole in vivo. In the molecular docking simulation, sulfur and chlorine of **3a-17** were bound with PHE291 and PRO150 of the SDH homology model, respectively, which could explain the probable mechanism of action between the inhibitory and target protein.

KEYWORDS: *nicotinamide, antifungal activity, succinate dehydrogenase inhibitors*

INTRODUCTION

As one of the most important categories of agrochemicals,[1,2] carboxamide fungicides have been intensively employed throughout the world to fight against highly destructive plant pathogens, such as *Botrytis cinerea*, *Sclerotinia* spp., *Leveillula taurica*, and *Spherotheca macularis*.[3−5] From the first carboxamide fungicide carboxin for crop protection to those newly discovered, these molecules cover boscalid (BASF), bixafen (Bayer), fluopyram (Bayer), and isopyrazam (Syngenta) (Figure 1). The initial narrow biological spectrum was broadened with progress of the chemical structure modification. These pyridine carboxamide group of pesticides have a common target receptor, succinate dehydrogenase (SDH, EC 1.3.5.1).[6] They act as SDH inhibitors (SDHIs) and disrupt the mitochondrial tricarboxylic acid cycle and respiration chain.

On the basis of the molecular structure of boscalid, a new series of nicotinamide compounds containing an active skeleton, ortho-replaced pyridine amide, have been synthsized. As shown in Figure 2, the *p*-chloride phenyl ring of boscalid was removed, and the chloride atom on the ortho position of pyridine was replaced by sulfur methyl or sulfur benzyl. This modification kept the nicotinamide moiety, which is important to the fungicide activity, and ensured molecular weight and pharmacological properties such as solubility similar to those of boscalid.

In this study, structure-based drug design strategies and homology modeling in the development of novel SDH inhibitors are presented using Discovery Studio 3.1 (Accelrys Inc.).[7,8] To explore the advantage of different substituents at the ortho position of pyridine, we calculated the interaction energy (Table 1) by docking these ligands to the binding site of porcine SDH. On the basis of scores of binding energy, we revealed that sulfur methyl was more suitable than sulfur benzyl for introduction into the ortho position of pyridine. Thus, we focused on sulfur methyl substituted target compounds while synthesizing some sulfur benzyl substituted target compounds to verify our virtual screening.

MATERIALS AND METHODS

Chemicals and Instruments. *Instruments.* All chemicals and reagents used in the current study were of analytical grade (provided by Nanjing Chemical Regent Co., Ltd.). The reactions were monitored by thin layer chromatography (TLC) using precoated silica gel GF_{254} plates from Qingdao Haiyang Chemical, China. Melting points (uncorrected) were determined on an SPSIC WRS-1B digital melting-point apparatus (Shanghai, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer. ^{1}H NMR spectra were collected on a Bruker Avance III 400 NMR spectrometer with $CDCl_3$ as solvent at room temperature. The chemical shifts (δ) are reported in parts per million with reference to internal TMS, and coupling constants (J) are given in hertz. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

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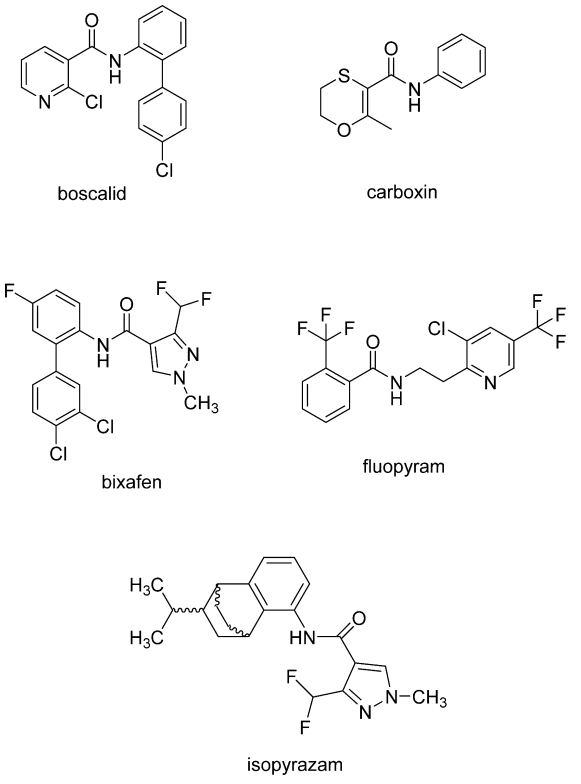

Figure 1. Structural formulas of the studied succinate dehydrogenase inhibitor (SDHI) fungicides.

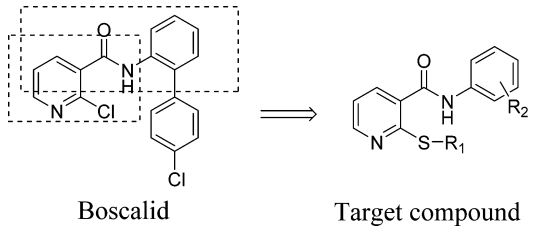

Figure 2. Design of target compounds.

Table 1. Scores of -cdock_interaction_energy for Virtual Screening in Synthesis Design[a]

Compounds		Scores of -cdock_ interaction_engry	Average engry
R_1	R_2		
CH_3-		38.61	38.99
		38.96	
		39.40	
		35.18	35.01
		35.10	
		34.77	
		34.08	34.24
		34.52	
		34.11	

[a]The higher the scores of -cdock_interaction_energy, the easier the bonding between chemical ligands and receptor.

*2-(Methylthio)nicotinic acid (**2a**).* 2-Mercaptonicotinic acid (1.55 g, 10 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF, 40 mL), and K_2CO_3 (4.14 g, 30 mmol) was added. Iodomethane (685 μL, 11 mmol) was added dropwise, and the mixture was stirred for 1 h at room temperature. With stirring, the pH of the solution was adjusted to 2−3 with 5% HCl solution. The precipitated solid was filtered, washed with cold water, and allowed to air-dry overnight to afford 1.61 g of the acid **2a** (9.5 mmol, 95%) as a white solid: mp 171−172 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.59 (s, 3H, CH_3), 7.12 (dd, 1H, J = 7.7, 4.8 Hz, Py−H), 8.34 (dd, 1H, J = 7.8, 1.6 Hz, Py−H), 8.67 (dd, 1H, J = 4.6, 1.6 Hz, Py−H); MS (ESI), 170.02 ($C_7H_7NO_2S$, $[M + H]^+$). Anal. Calcd for $C_7H_7NO_2S$: C, 49.69; H, 4.17; N, 8.28. Found: C, 49.88; H, 4.11; N, 8.32.

*2-((2-Fluorobenzyl)thio)nicotinic Acid (**2b**) and 2-((4-Nitrobenzyl)-thio)nicotinic Acid (**2c**).* 2-Mercaptonicotinic acid (32 mmol, 5.0 g) was dissolved together with potassium hydroxide (20 mol, 1.2 g) in 30 mL of ethanol. Benzyl bromide (1-(bromomethyl)-2-fluorobenzene or 1-(bromomethyl)-4-nitrobenzene) was added dropwise, and the mixture was refluxed for 30 min. After cooling, the reaction mixture was concentrated to approximately 15 mL. The residue was treated with 20 mL of water and slightly acidified with 5% HCl solution. The precipitate was filtered, washed with water, and recrystallized from ethanol to give white crystals of **2b** or **2c** as a white or yellow solid, respectively.

Data for **2b**: yield, 72.5%; mp, 184−185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.40 (s, 2H, S−CH_2−Ar), 7.12−7.16 (m, 2H, Py−H, Ar), 7.26−7.28 (m, 1H, Ph−H), 7.29−7.23 (m, 1H, Ph−H), 7.52 (dt, 1H, J = 7.7, 1.4 Hz, Ph−H), 8.23 (dd, 1H, J = 3.8, 1.8 Hz, Py−H), 8.52 (dd, 1H, J = 7.6, 3.8 Hz, Py−H); MS (ESI), 264.04 ($C_{13}H_{10}FNO_2S$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{10}FNO_2S$: C, 59.31; H, 3.83; N, 5.32. Found: C, 59.48; H, 3.61; N, 5.42.

Data for **2c**: yield, 71.9%; mp, 163−164 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.42 (s, 2H, S−CH_2−Ar), 7.14−7.15 (m, H, Py−H), 7.56 (dd, 2H, J = 8.8 Hz, Ph−H), 8.14 (d, 2H, J = 8.0 Hz, Ph−H), 8.24 (dd, 1H, J = 4.2, 1.7 Hz, Py−H), 8.50 (dd, 1H, J = 7.8, 2.9 Hz, Py−H); MS (ESI), 291.04 ($C_{13}H_{10}N_2O_4S$, $[M + H]^+$). Anal. Calcd for $C_7H_7NO_2S$: C, 53.79; H, 3.47; N, 9.65. Found: C, 53.52; H, 3.50; N, 9.49.

*General Procedure for the Synthesis of Compounds **3**.* A mixture of compound **2** (1 mmol) and $SOCl_2$ (15 mL, acted as both reactant and solvent) was stirred and refluxed at 75 °C for 2 h. Remaining $SOCl_2$ was removed by distillation under reduced pressure. After $SOCl_2$ was completely removed, the solid residue was then directly dissolved in anhydrous CH_2Cl_2 (20−30 mL). Appropriate substituted aniline or alkylamine (1 mmol) was added slowly (finished in 1 min), and the mixture was stirred at room temperature for 2−3 h.

The reaction mixture was washed with HCl solution (5%, 50 mL) and NaOH solution (5%, 50 mL) several times. The CH_2Cl_2 solution was collected and dried by anhydrous Na_2SO_4. Further purification was carried out by silica gel column chromatography with petroleum ether/EtOAc (5:1) as eluent. After recrystallization from EtOAc, final compounds **3a-01**−**3a-27**, **3b-01**−**3b-06**, and **3c-01**−**3c-05** were obtained.

*2-(Methylthio)-N-phenylnicotinamide **3a-01**:* white powder; yield, 80.3%; mp, 151−152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.47 (s, 3H, S−CH_3), 7.12 (t, 1H, J = 7.4 Hz, Ph−H), 7.26 (dd, 1H, J = 7.5, 4.9 Hz, Py−H), 7.36 (t, 2H, J = 7.7 Hz, Ph−H), 7.70−7.72 (m, 2H, Ph−H), 7.91 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.59 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.46 (s, 1H, CONH); MS (ESI), 245.07 ($C_{13}H_{12}N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{12}N_2OS$: C, 63.91; H, 4.95; N, 11.47. Found: C, 63.63; H, 4.54; N, 11.46.

*N-(2-Fluorophenyl)-2-(methylthio)nicotinamide **3a-02**.* white powder; yield, 57.0%; mp, 163−164 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H, S−CH_3), 7.14 (dd, 1H, J = 8.4, 1.6 Hz, Ph−H), 7.29 (dd, 1H, J = 7.6, 4.3 Hz, Py−H), 7.41−7.44 (m, 1H, Ph−H), 7.47 (d, 1H, J = 8.0 Hz, Ph−H), 7.70 (dd, 1H, J = 9.4, 1.8 Hz, Ph−H), 8.02 (dd, 1H, J = 7.5, 1.9 Hz, Py−H), 8.71 (dd, 1H, J = 6.2, 1.7 Hz, Py−H), 10.56 (s, 1H, CONH); MS (ESI), 263.06 ($C_{13}H_{11}FN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}FN_2OS$: C, 59.53; H, 4.23; N, 10.68. Found: C, 59.37; H, 4.45; N, 10.96.

*N-(2-Chlorophenyl)-2-(methylthio)nicotinamide **3a-03**:* white crystal; yield, 85.2%; mp, 196−197 °C; ^{1}H NMR (400 MHz, DMSO-

d_6) δ 2.48 (s, 3H, S−CH_3), 7.26−7.33 (m, 2H, Ph−H, Py−H), 7.41 (dt, 1H, J = 7.6, 1.4 Hz, Ph−H), 7.57 (dd, 1H, J = 8.0, 1.4 Hz, Ph−H), 7.63 (dd, 1H, J = 7.9, 1.3 Hz, Ph−H), 8.00 (dd, 1H, J = 7.1, 1.6 Hz, Py−H), 8.62 (dd, 1H, J = 4.8, 1.7 Hz, Py−H), 10.22 (s, 1H, CONH); MS (ESI), 278.03 ($C_{13}H_{11}ClN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}ClN_2OS$: C, 56.01; H, 3.98; N, 10.05. Found: C, 55.73; H, 3.75; N, 10.26.

N-(2-Bromophenyl)-2-(methylthio)nicotinamide ***3a-04:*** yellow powder; yield, 88.7%; mp, 142−143 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.51 (s, 3H, S−CH_3), 7.23−7.30 (m, 2H, Ph−H, Py−H), 7.41 (dt, 1H, J = 7.6, 1.3 Hz, Ph−H), 7.57 (dd, 1H, J = 7.9, 1.3 Hz, Ph−H), 7.73 (dd, 1H, J = 8.0, 1.3 Hz, Ph−H), 8.01 (dd, 1H, J = 7.6, 1.4 Hz, Py−H), 8.62 (dd, 1H, J = 4.8, 1.7 Hz, Py−H), 10.19 (s, 1H, CONH); MS (ESI), 322.98 ($C_{13}H_{11}BrN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}BrN_2OS$: C, 48.31; H, 3.43; N, 8.67. Found: C, 48.36; H, 3.75; N, 8.30.

2-(Methylthio)-N-(o-tolyl)nicotinamide ***3a-05:*** white powder; yield, 42.1%; mp, 197−198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H, Ar−CH_3), 2.49 (s, 3H, S−CH_3), 7.16−7.28 (m, 4H, 3Ph−H, Py−H), 7.39 (d, 1H, J = 7.3 Hz, Ph−H), 7.95 (d, 1H, J = 7.1 Hz, Py−H), 8.60 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 9.99 (s, 1H, CONH); MS (ESI), 259.08 ($C_{14}H_{14}N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{14}H_{14}N_2OS$: C, 65.09; H, 5.46; N, 10.84. Found: C, 65.18; H, 5.93; N, 10.47.

2-(Methylthio)-N-(2-(trifluoromethyl)phenyl)nicotinamide ***3a-06:*** white crystal; yield, 60.3%; mp, 164−165 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H, S−CH_3), 7.30 (dd, 1H, J = 7.6, 4.8 Hz, Py−H), 7.55−7.59 (m, 2H, Ph−H), 7.76−7.79 (m, 1H, Ph−H), 7.82 (d, 1H, J = 7.9 Hz, Ph−H), 7.92 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.62 (dd, 1H, J = 4.8, 1.7 Hz, Py−H), 10.31 (s, 1H, CONH); MS (ESI), 313.05 ($C_{14}H_{11}F_3N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{14}H_{11}F_3N_2OS$: C, 53.84; H, 3.55; N, 8.97. Found: C, 53.51; H, 3.24; N, 8.64.

N-(2,4-Dichlorophenyl)-2-(methylthio)nicotinamide ***3a-07:*** light yellow powder; yield, 51.7%; mp, 183−184 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H, S−CH_3), 7.20−7.24 (m, 1H, Ph−H), 7.28 (dd, 1H, J = 7.6, 4.8 Hz, Py−H), 7.43 (dd, 1H, J = 8.6, 1.9 Hz, Ph−H), 7.63−7.73 (m, 1H, Ph−H), 8.00 (d, 1H, J = 7.6 Hz, Py−H), 8.63 (dd, 1H, J = 4.8, 1.7 Hz, Py−H), 10.31 (s, 1H, CONH); MS (ESI), 312.99 ($C_{13}H_{10}Cl_2N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{10}Cl_2N_2OS$: C, 49.85; H, 3.22; N, 8.94. Found: C, 49.33; H, 3.10; N, 8.54.

N-(2,6-Difluorophenyl)-2-(methylthio)nicotinamide ***3a-08:*** white powder; yield, 56.1%; mp, 191−192 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.47 (s, 3H, S−CH_3), 7.28 (dd, 1H, J = 7.6, 4.8 Hz, Py−H), 7.50 (dd, 1H, J = 8.6, 2.3 Hz, Ph−H), 7.66 (d, 1H, J = 8.6 Hz, Ph−H), 7.75 (d, 1H, J = 2.4 Hz, Ph−H), 8.00 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.62 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.29 (s, 1H, CONH); MS (ESI), 281.05 ($C_{13}H_{10}F_2N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{10}F_2N_2OS$: C, 55.71; H, 3.60; N, 9.99. Found: C, 55.03; H, 3.38; N, 9.62.

2-(Methylthio)-N-(2,4,5-trichlorophenyl)nicotinamide ***3a-09:*** white powder; yield, 72.8%; mp, 183−184 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.43 (s, 3H, S−CH_3), 7.24 (dd, 1H, J = 7.8, 4.8 Hz, Py−H), 7.67 (dd, 1H, J = 8.2, 3.6 Hz, Ph−H), 8.21−8.23 (m, 2H, Ph−H, Py−H), 8.66 (dd, 1H, J = 4.8, 1.8 Hz, Py−H); MS (ESI), 346.95 ($C_{13}H_9Cl_3N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{10}F_2N_2OS$: C, 44.92; H, 2.61; N, 8.06. Found: C, 45.02; H, 2.41; N, 8.07.

N-(2,5-Dimethoxyphenyl)-2-(methylthio)nicotinamide ***3a-10:*** white−gray powder; yield, 58.2%; mp, 189−190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H, S−CH_3), 3.73 (s, 3H, Ar−OCH_3), 3.78 (s, 3H, Ar−OCH_3), 6.74 (dd, 1H, J = 9.0, 3.0 Hz, Ph−H), 7.01 (d, 1H, J = 9.0 Hz, Ph−H), 7.24 (dd, 1H, J = 7.5, 4.9 Hz, Py−H), 7.60 (s, 1H, Ph−H), 7.91 (d, 1H, J = 7.1 Hz, Py−H), 8.58 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 9.59 (s, 1H, CONH); MS (ESI), 305.09 ($C_{15}H_{16}N_2O_3S$, $[M + H]^+$). Anal. Calcd for $C_{15}H_{16}N_2O_3S$: C, 59.19; H, 5.30; N, 9.20. Found: C, 59.69; H, 5.70; N, 9.01.

N-(3-Fluorophenyl)-2-(methylthio)nicotinamide ***3a-11:*** white powder; yield, 83.2%; mp, 171−172 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, S−CH_3), 7.12 (dt, 1H, J = 9.0, 2.5 Hz, Ph−H), 7.28 (dd, 1H, J = 7.6, 4.9 Hz, Py−H), 7.38−7.42 (m, 1H, Ph−H), 7.46−7.48 (m, 1H, Py−H), 7.69 (td, 1H, J = 11.6, 2.0 Hz, Ph−H), 7.94 (dd, 1H, J = 7.6, 1.7 Hz, Ph−H), 8.60 (dd, 1H, J = 4.9, 1.7 Hz, Py−H), 10.68 (s, 1H, CONH); MS (ESI), 263.06 ($C_{13}H_{11}FN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}FN_2OS$: C, 59.53; H, 4.23; N, 10.68. Found: C, 59.41; H, 4.29; N, 10.10.

N-(3-Bromophenyl)-2-(methylthio)nicotinamide ***3a-12:*** light yellow powder; yield, 82.0%; mp, 168−169 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, S−CH_3), 7.28 (dd, 1H, J = 7.5, 4.8 Hz, Py−H), 7.33−7.35 (m, 2H, Ph−H), 7.63−7.66 (m, 1H, Ph−H), 7.94 (dd, 1H, J = 7.6, 1.7 Hz, Ph−H), 8.05−8.06 (m, 1H, Py−H), 8.61 (dd, 1H, J = 4.9, 1.7 Hz, Py−H), 10.63 (s, 1H, CONH); MS (ESI), 322.98 ($C_{13}H_{11}BrN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}BrN_2OS$: C, 48.31; H, 3.43; N, 8.67. Found: C, 48.26; H, 3.71; N, 8.60.

2-(Methylthio)-N-(3-(trifluoromethyl)phenyl)nicotinamide ***3a-13:*** white powder; yield, 82.0%; mp, 168−169 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, S−CH_3), 7.31 (dd, 1H, J = 7.0, 4.0 Hz, Py−H), 7.36−7.38 (m, H, Ph−H), 7.74 (dd, 1H, J = 8.4, 2.0 Hz, Ph−H), 7.79 (dd, 1H, J = 8.2, 2.0 Hz, Ph−H), 7.91 (dd, 1H, J = 7.6, 1.8 Hz, Ph−H), 8.12 (dd, 1H, J = 8.6, 3.4 Hz, Py−H), 8.61 (dd, 1H, J = 6.0, 1.6 Hz, Py−H), 10.38 (s, 1H, CONH); MS (ESI), 313.05 ($C_{14}H_{11}F_3N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{14}H_{11}F_3N_2OS$: C, 53.84; H, 3.55; N, 8.97. Found: C, 53.91; H, 3.67; N, 9.01.

2-(Methylthio)-N-(3-nitrophenyl)nicotinamide ***3a-14:*** yellow powder; yield, 75.2%; mp, 198−199 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, S−CH_3), 7.31 (dd, 1H, J = 7.6, 4.8 Hz, Py−H), 7.68 (t, 1H, J = 8.2 Hz, Ph−H), 8.00−8.02 (m, 2H, Ph−H), 8.06 (dd, 1H, J = 8.2, 1.0 Hz, Ph−H), 8.64 (dd, 1H, J = 4.9, 1.7 Hz, Py−H), 8.75 (t, 1H, J = 2.0 Hz, Py−H), 10.95 (s, 1H, CONH); MS (ESI), 290.05 ($C_{14}H_{11}F_3N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}N_3O_3S$: C, 53.97; H, 3.83; N, 14.52. Found: C, 53.99; H, 3.50; N, 14.65.

N-(3-Methoxyphenyl)-2-(methylthio)nicotinamide ***3a-15:*** white powder; yield, 90.1%; mp, 165−166 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H, S−CH_3), 3.76 (s, 3H, Ar−OCH_3), 6.70−6.73 (m, H, Ph−H), 7.25−7.28 (m, 3H, 2Ph−H, Py−H), 7.40 (s, H, Ph−H), 7.90 (dd, 1H, J = 7.6, 1.7 Hz, Py−H), 8.06 (dd, 1H, J = 8.2, 1.0 Hz, Py−H), 8.60 (dd, 1H, J = 4.9, 1.7 Hz, Py−H), 10.45 (s, 1H, CONH); MS (ESI), 290.05 ($C_{14}H_{14}N_2O_2S$, $[M + H]^+$). Anal. Calcd for $C_{14}H_{14}N_2O_2S$: C, 53.97; H, 3.83; N, 14.52. Found: C, 53.99; H, 3.50; N, 14.65.

N-(3,4-Dichlorophenyl)-2-(methylthio)nicotinamide ***3a-16:*** white crystal; yield, 88.4%; mp, 151−152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, S−CH_3), 7.29 (dd, 1H, J = 7.6, 4.9 Hz, Py−H), 7.64 (s, 2H, Ph−H), 7.96 (dd, 1H, J = 7.6, 1.7 Hz, Ph−H), 8.09 (s, 2H, Py−H), 8.62 (dd, 1H, J = 4.9, 1.5 Hz, Py−H), 10.75 (s, 1H, CONH); MS (ESI), 290.05 ($C_{13}H_{10}Cl_2N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{10}Cl_2N_2OS$: C, 53.97; H, 3.83; N, 14.52. Found: C, 53.99; H, 3.50; N, 14.65.

N-(3-Chloro-4-fluorophenyl)-2-(methylthio)nicotinamide ***3a-17:*** white powder; yield, 82.0%; mp, 160−161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, S−CH_3), 7.28 (dd, 1H, J = 7.6, 4.8 Hz, Py−H), 7.45 (t, 1H, J = 9.1 Hz, Ph−H), 7.60−7.65 (m, 1H, Ph−H), 7.94 (dd, 1H, J = 7.6, 1.7 Hz, Ph−H), 8.02 (dd, 1H, J = 6.9, 2.5 Hz, Py−H), 8.62 (dd, 1H, J = 4.8, 1.7 Hz, Py−H), 10.68 (s, 1H, CONH); MS (ESI), 297.02 ($C_{13}H_{10}ClFN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{10}ClFN_2OS$: C, 52.62; H, 3.40; N, 9.44. Found: C, 52.87; H, 3.53; N, 9.65.

N-(4-Chloro-3-fluorophenyl)-2-(methylthio)nicotinamide ***3a-18:*** white powder; yield, 82.7%; mp, 150−151 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 3H, S−CH_3), 7.28 (dd, 1H, J = 7.6, 4.9 Hz, Py−H), 7.49 (dd, 1H, J = 8.9, 1.8 Hz, Ph−H), 7.58 (t, 1H, J = 8.6 Hz, Ph−H), 7.86 (dd, 1H, J = 11.9, 2.3 Hz, Ph−H), 7.94 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.62 (dd, 1H, J = 4.8, 0.9 Hz, Py−H), 10.78 (s, 1H, CONH); MS (ESI), 397.02 ($C_{23}H_{40}N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{23}H_{40}N_2OS$: C, 54.62; H, 3.40; N, 9.44. Found: C, 57.47; H, 3.62; N, 9.48.

N-(3,5-Bis(trifluoromethyl)phenyl)-2-(methylthio)nicotinamide ***3a-19:*** white powder; yield, 74.8%; mp, 153−154 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.50 (s, 3H, S−CH_3), 7.32 (dd, 1H, J = 6.5, 4.8 Hz, Py−H), 7.87 (s, 1H, Ph−H), 8.05 (dd, 1H, J = 7.6, 1.7 Hz, Py−H), 8.41 (s, 2H, Ph−H), 8.65 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 11.09 (s, 1H, CONH); MS (ESI), 281.04 ($C_{15}H_{10}F_6N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{15}H_{10}F_6N_2OS$: C, 47.37; H, 2.65; N, 7.37. Found: C, 47.35; H, 2.99; N, 7.15.

2-(Methylthio)-N-(3,4,5-trifluorophenyl)nicotinamide ***3a-20:*** white powder; yield, 60.3%; mp, 146−147 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, S−CH_3), 7.30 (dd, 1H, J = 7.6, 4.9 Hz, Py−H),

7.63 (dd, 2H, J = 10.2, 6.5 Hz, Ph−H), 7.95 (dd, 1H, J = 7.6, 1.7 Hz, Py−H), 8.63 (dd, 1H, J = 4.9, 1.7 Hz, Py−H), 10.82 (s, 1H, CONH); MS (ESI), 299.04 ($C_{13}H_9F_3N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_9F_3N_2OS$: C, 52.35; H, 3.04; N, 9.39. Found: C, 52.12; H, 3.01; N, 9.67.

N-(4-Fluorophenyl)-2-(methylthio)nicotinamide ***3a-21:*** white powder; yield, 51.9%; mp, 153−154 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H, S−CH_3), 7.19−7.28 (m, 3H, 2Ph−H, Py−H), 7.73 (dd, 2H, J = 9.2, 5.0 Hz, Ph−H), 7.92 (dd, 1H, J = 7.6, 1.7 Hz, Py−H), 8.60 (dd, 1H, J = 4.9, 1.7 Hz, Py−H), 10.52 (s, 1H, CONH); MS (ESI), 262.06 ($C_{13}H_{11}FN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}FN_2OS$: C, 59.53; H, 4.23; N, 7.24. Found: C, 59.71; H, 4.65; N, 7.61.

N-(4-Chlorophenyl)-2-(methylthio)nicotinamide ***3a-22:*** white powder; yield, 68.9%; mp, 169−170 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, S−CH_3), 7.27 (dd, 1H, J = 7.6, 4.9 Hz, Py−H), 7.43 (d, 2H, J = 8.9 Hz, Ph−H), 7.75 (d, 2H, J = 8.8 Hz, Ph−H), 7.93 (dd, 1H, J = 7.6, 1.7 Hz, Py−H), 8.60 (dd, 1H, J = 4.8, 1.7 Hz, Py−H), 10.59 (s, 1H, CONH); MS (ESI), 279.03 ($C_{13}H_{11}ClN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}ClN_2OS$: C, 56.01; H, 3.98; N, 10.05. Found: C, 55.63; H, 4.09; N, 10.15.

2-(Methylthio)-N-(p-tolyl)nicotinamide ***3a-23:*** white crystal; yield, 86.9%; mp, 161−162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H, Ar−CH_3), 2.48 (s, 3H, S−CH_3), 7.17 (d, 2H, J = 8.3 Hz, Ph−H), 7.26 (dd, 1H, J = 7.6, 4.8 Hz, Py−H), 7.59 (d, 2H, J = 8.3 Hz, Ph−H), 7.89 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.59 (dd, 1H, J = 4.9, 1.7 Hz, Py−H), 10.37 (s, 1H, CONH); MS (ESI), 259.08 ($C_{14}H_{14}N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{14}H_{14}N_2OS$: C, 65.09; H, 5.46; N, 10.84. Found: C, 65.03; H, 5.26; N, 11.01.

N-(4-Methoxyphenyl)-2-(methylthio)nicotinamide ***3a-24:*** white powder; yield, 67.2%; mp, 175−176 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H, Ar−CH_3), 3.75 (s, 3H, S−CH_3), 6.93 (d, 2H, J = 9.0 Hz, Ph−H), 7.25 (dd, 1H, J = 7.5, 4.9 Hz, Py−H), 7.63 (d, 2H, J = 8.9 Hz, Ph−H), 7.91 (dd, 1H, J = 7.5, 1.4 Hz, Py−H), 8.58 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.37 (s, 1H, CONH); MS (ESI), 275.08 ($C_{14}H_{14}N_2O_2S$, $[M + H]^+$). Anal. Calcd for $C_{14}H_{14}N_2O_2S$: C, 61.29; H, 5.14; N, 10.21. Found: C, 61.12; H, 5.32; N, 10.60.

2-(Methylthio)-N-(4-nitrophenyl)nicotinamide ***3a-25:*** yellow powder; yield, 43.1%; mp, 182−183 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.50 (s, 3H, Ar−CH_3), 7.30 (dd, 1H, J = 7.6, 4.9 Hz, Py−H), 7.96−8.01 (m, 3H, 2 Ph−H, 1 Py−H), 8.29 (d, 2H, J = 9.2 Hz, Ph−H), 8.63 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 11.05 (s, 1H, CONH); MS (ESI), 290.05 ($C_{13}H_{11}N_3O_3S$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{11}N_3O_3S$: C, 53.97; H, 3.83; N, 14.52. Found: C, 54.09; H, 3.99; N, 14.41.

N-Cyclohexyl-2-(methylthio)nicotinamide ***3a-26:*** white powder; yield, 88.4%; mp, 141−142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19−1.79 (m, 10H, cyclohexane), 2.50 (s, 3H, S−CH_3), 3.59−3.62 (m, 1H, N−CH_2-cyclohexane), 7.33 (dd, 1H, J = 7.7, 4.6 Hz, Py−H), 8.02 (dd, 1H, J = 8.0, 1.7 Hz, Py−H), 8.68 (dd, 1H, J = 4.8, 1.6 Hz, Py−H); MS (ESI), 251.11 ($C_{13}H_{18}N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{13}H_{18}N_2OS$: C, 62.37; H, 7.25; N, 11.19. Found: C, 62.47; H, 7.14; N, 11.03.

N-Hexadecyl-2-(methylthio)nicotinamide ***3a-27:*** white powder; yield, 94.1%; mp, 137−138 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (s, 3H, $(CH_2)_{14}C\underline{H}_3$), 1.34 (s, 28H, $(C\underline{H}_2)_{14}CH_3$), 1.67 (s, 2H, N−$C\underline{H}_2$−$(CH_2)_{14}CH_3$), 2.49 (s, 3H, S−$CH_3$), 7.23 (dd, 1H, J = 7.7, 4.8 Hz, Py−H), 8.21 (dd, 1H, J = 7.8, 1.8 Hz, Py−H), 8.65 (dd, 1H, J = 4.8, 1.9 Hz, Py−H); MS (ESI), 393.29 ($C_{23}H_{40}N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{23}H_{40}N_2OS$: C, 70.36; H, 10.27; N, 7.13. Found: C, 70.57; H, 10.40; N, 7.16.

2-((2-Fluorobenzyl)thio)-N-phenylnicotinamide ***3b-01:*** white powder; yield, 54.8%; mp, 181−182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.46 (s, 2H, S−CH_2−Ar), 7.03 (t, 1H, J = 7.5 Hz, Ph−H), 7.12−7.21 (m, 2H, Ph−H), 7.30−7.35 (m, 3H, 2Ph−H, 1 Py−H), 7.52−7.58 (m, 2H, Ph−H), 7.58 (d, 1H, J = 7.8 Hz, Ph−H), 7.99 (d, 1H, J = 7.4 Hz, Py−H), 8.69 (dd, 1H, J = 8.8, 3.2 Hz, Py−H), 10.25 (s, 1H, CONH); MS (ESI), 339.09 ($C_{19}H_{15}FN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{15}FN_2OS$: C, 67.44; H, 4.47; N, 8.28. Found: C, 67.01; H, 4.72; N, 8.39.

2-((2-Fluorobenzyl)thio)-N-(2-fluorophenyl)nicotinamide ***3b-02:*** white powder; yield, 75.1%; mp, 188−189 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.45 (s, 2H, S−CH_2−Ar), 7.11−7.24 (m, 2H, Ph−H), 7.26−7.30 (m, 3H, 2Ph−H, 1 Py−H), 7.40 (t, 1H, J = 8.0 Hz, Ph−H), 7.56−7.61 (m, 2H, Ph−H), 7.63 (d, 1H, J = 8.0 Hz, Ph−H), 8.00 (d, 1H, J = 7.7 Hz, Py−H), 8.67 (dd, 1H, J = 7.9, 1.7 Hz, Py−H), 10.24 (s, 1H, CONH); MS (ESI), 366.08 ($C_{19}H_{14}ClFN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{14}F_2N_2OS$: C, 64.03; H, 3.96; N, 7.86. Found: C, 64.19; H, 4.30; N, 7.44.

N-(2-Chlorophenyl)-2-((2-fluorobenzyl)thio)nicotinamide ***3b-03:*** white powder; yield, 67.1%; mp, 174−175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.45 (s, 2H, S−CH_2−Ar), 7.12−7.21 (m, 2H, Ph−H), 7.28−7.33 (m, 3H, 2Ph−H, 1 Py−H), 7.40 (t, 1H, J = 8.1 Hz, Ph−H), 7.51−7.56 (m, 2H, Ph−H), 7.61 (d, 1H, J = 7.8 Hz, Py−H), 8.04 (d, 1H, J = 7.5 Hz, Py−H), 8.63 (dd, 1H, J = 7.8, 1.7 Hz, Py−H), 10.23 (s, 1H, CONH); MS (ESI), 373.05 ($C_{19}H_{14}ClFN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{14}ClFN_2OS$: C, 61.21; H, 3.78; N, 7.51. Found: C, 61.28; H, 3.70; N, 7.39.

N-(3-Bromophenyl)-2-((2-fluorobenzyl)thio)nicotinamide ***3b-04:*** light yellow powder; yield, 70.4%; mp, 179−180 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.45 (s, 2H, S−CH_2−Ar), 7.12−7.21 (m, 2H, Ph−H), 7.28−7.33 (m, 3H, 2Ph−H, 1 Py−H), 7.40 (t, 1H, J = 8.1 Hz, Ph−H), 7.51−7.56 (m, 2H, Ph−H), 7.61 (d, 1H, J = 7.8 Hz, Py−H), 8.04 (d, 1H, J = 7.5 Hz, Py−H), 8.63 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.67 (s, 1H, CONH); MS (ESI), 373.05 ($C_{19}H_{14}ClFN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{14}ClFN_2OS$: C, 61.21; H, 3.78; N, 7.51. Found: C, 61.28; H, 3.70; N, 7.39.

2-((2-Fluorobenzyl)thio)-N-(3-methoxyphenyl)nicotinamide ***3b-05:*** white powder; yield, 89.3%; mp, 184−185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.74 (s, 3H, O−CH_3), 4.44 (s, 2H, S−CH_2−Ar), 6.68−6.71 (m, 1H, Ph−H), 7.12 (td, 1H, J = 7.5, 1.0 Hz, Ph−H), 7.24 (d, 2H, J = 5.0 Hz, Ph−H), 7.28−7.35 (m, 2H, 1 Ph−H, 1 Py−H), 7.38 (s, 1H, Ph−H), 7.50 (td, 1H, J = 7.7, 1.5 Hz, Ph−H), 7.95 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.62 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.43 (s, 1H, CONH); MS (ESI), 369.10 ($C_{20}H_{17}FN_2O_2S$, $[M + H]^+$). Anal. Calcd for $C_{20}H_{17}FN_2O_2S$: C, 65.20; H, 4.65; N, 7.60. Found: C, 65.31; H, 4.96; N, 7.14.

2-((2-Fluorobenzyl)thio)-N-(4-fluorophenyl)nicotinamide ***3b-06:*** white powder; yield, 81.0%; mp, 163−164 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.45 (s, 2H, S−CH_2−Ar), 7.13 (t, 1H, J = 9.3 Hz, Ph−H), 7.18 (t, H, J = 7.5 Hz, Ph−H), 7.29−7.33 (m, 2H, 1 Ph−H, 1 Py−H), 7.42 (d, 1H, J = 8.9 Hz, Ph−H), 7.51 (td, 1H, J = 7.7, 1.4 Hz, Ph−H), 7.72 (d, 1H, J = 8.8 Hz, Ph−H), 7.98 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.64 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.60 (s, 1H, CONH); MS (ESI), 357.08 ($C_{19}H_{14}F_2N_2OS$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{14}F_2N_2OS$: C, 64.03; H, 3.96; N, 7.86. Found: C, 64.25; H, 3.69; N, 7.54.

2-((4-Nitrobenzyl)thio)-N-phenylnicotinamide ***3c-01:*** light yellow powder; yield, 75.9%; mp, 176−177 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.55 (s, 2H, S−CH_2−Ar), 7.11 (t, 1H, J = 7.8 Hz, Ph−H), 7.26 (d, 2H, J = 8.6 Hz, Ph−H), 7.31 (dd, 1H, J = 7.6, 4.8 Hz, Py−H), 7.69 (d, 2H, J = 8.7 Hz, Ph−H), 7.75 (d, 2H, J = 8.8 Hz, Ph−H), 7.98 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.15 (d, 2H, J = 8.8 Hz, Ph−H), 8.61 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.63 (s, 1H, CONH); MS (ESI), 366.08 ($C_{19}H_{15}N_3O_3S$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{14}BrN_3O_3S$: C, 62.46; H, 4.14; N, 11.50. Found: C, 62.31; H, 4.09; N, 11.74.

N-(3-Bromophenyl)-2-((4-nitrobenzyl)thio)nicotinamide ***3c-02:*** yellow powder; yield, 88.0%; mp, 187−188 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.56 (s, 2H, S−CH_2−Ar), 7.20 (dd, 1H, J = 7.6, 1.8 Hz, Ph−H), 7.32 (dd, 1H, J = 7.6, 4.8 Hz, Py−H), 7.40 (t, 1H, J = 8.1 Hz, Ph−H), 7.59 (d, 1H, J = 9.0 Hz, Ph−H), 7.69 (d, 2H, J = 8.7 Hz, Ph−H), 7.89 (s, 1H, Ph−H), 8.00 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.15 (d, 2H, J = 8.8 Hz, Ph−H), 8.61 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.65 (s, 1H, CONH); MS (ESI), 443.99 ($C_{19}H_{14}BrN_3O_3S$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{14}BrN_3O_3S$: C, 51.36; H, 3.18; N, 9.46. Found: C, 51.74; H, 3.25; N, 9.81.

N-(4-Bromophenyl)-2-((4-nitrobenzyl)thio)nicotinamide ***3c-03:*** yellow powder; yield, 72.9%; mp, 197−198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.55 (s, 2H, S−CH_2−Ar), 7.32 (dd, 1H, J = 7.8, 4.4 Hz, Py−H), 7.38−7.43 (m, 2H, Ph−H), 7.58 (d, 2H, J = 8.2 Hz, Ph−H), 7.72 (d, 2H, J = 9.2 Hz, Ph−H), 7.99 (dd, 1H, J = 7.6, 1.8 Hz, Py−H), 8.14 (d, 2H, J = 8.4 Hz, Ph−H), 8.63 (dd, 1H, J = 4.8, 1.7 Hz, Py−H), 10.66 (s, 1H, CONH); MS (ESI), 443.99 ($C_{19}H_{14}BrN_3O_3S$, $[M + H]^+$).

Table 2. Structures and IC_{50} Values against *R. solani* and *S. sclerotiorum* of 3a, 3b, and 3c

			IC_{50}[a] (μM)	
compd no.	R_1	R_2	*R. solani*	*S. sclerotiorum*
3a-01	CH_3-	C_6H_5-	42.7 ± 1.1	76.2 ± 1.3
3a-02[b]	CH_3-	$2F-C_6H_4-$	201.7 ± 2.9	238.5 ± 2.9
3a-03	CH_3-	$2Cl-C_6H_4-$	105.2 ± 2.1	179.8 ± 1.9
3a-04[b]	CH_3-	$2Br-C_6H_4-$	102.2 ± 1.9	77.7 ± 1.3
3a-05	CH_3-	$2CH_3-C_6H_4-$	124.7 ± 2.0	70.8 ± 1.3
3a-06[b]	CH_3-	$2CF_3-C_6H_4-$	210.8 ± 2.8	205.4 ± 2.0
3a-07[b]	CH_3-	$2,4\text{-}2Cl-C_6H_3-$	147.4 ± 1.5	112.5 ± 1.5
3a-08[b]	CH_3-	$2,6\text{-}2F-C_6H_3-$	164.8 ± 1.5	200.9 ± 2.0
3a-09[b]	CH_3-	$2,4,5\text{-}3Cl-C_6H_2-$	190.2 ± 1.9	122.9 ± 1.7
3a-10[b]	CH_3-	$2,5\text{-}2CH_3O-C_6H_3-$	234.2 ± 2.8	226.4 ± 3.0
3a-11[b]	CH_3-	$3F-C_6H_4-$	85.5 ± 1.2	79.5 ± 1.0
3a-12[b]	CH_3-	$3Br-C_6H_4-$	35.5 ± 1.1	21.4 ± 0.9
3a-13	CH_3-	$3CF_3-C_6H_4-$	195.5 ± 2.0	195.5 ± 1.9
3a-14[b]	CH_3-	$3NO_2-C_6H_4-$	149.9 ± 1.6	114.2 ± 1.4
3a-15	CH_3-	$3CH_3O-C_6H_4-$	38.4 ± 1.0	67.5 ± 1.0
3a-16[b]	CH_3-	$3,4\text{-}2Cl-C_6H_3-$	88.7 ± 1.2	36.0 ± 0.9
3a-17[b]	CH_3-	$3Cl-4F-C_6H_3-$	15.8 ± 0.9	20.3 ± 0.8
3a-18[b]	CH_3-	$3Cl-4F-C_6H_3-$	130.4 ± 2.6	72.5 ± 0.5
3a-19[b]	CH_3-	$3,5\text{-}2CF_3-C_6H_3-$	95.7 ± 1.7	68.6 ± 1.0
3a-20[b]	CH_3-	$3,4,5\text{-}3F-C_6H_2-$	242.7 ± 2.6	249.3 ± 2.8
3a-21	CH_3-	$4F-C_6H_4-$	165.7 ± 1.7	121.5 ± 1.2
3a-22	CH_3-	$4Cl-C_6H_4-$	162.1 ± 1.7	115.5 ± 1.3
3a-23	CH_3-	$4CH_3-C_6H_4-$	101.7 ± 1.1	90.5 ± 1.0
3a-24	CH_3-	$4CH_3O-C_6H_4-$	136.5 ± 1.2	>250
3a-25	CH_3--	$4NO_2-C_6H_4-$	135.0 ± 1.2	>250
3a-26	CH_3-	$C_6H_{11}-$	>500	>500
3a-27	CH_3-	$CH_3\ (CH_2)_{15}-$	>500	>500
3b-01[b]	$2F-C_6H_5-CH_2-$	C_6H_5-	>250	>250
3b-02[b]	$2F-C_6H_5-CH_2-$	$2F-C_6H_4-$	>250	>250
3b-03[b]	$2F-C_6H_5-CH_2-$	$2Cl-C_6H_4-$	199.4 ± 1.8	>250
3b-04[b]	$2F-C_6H_5-CH_2-$	$3Br-C_6H_4-$	146.9 ± 1.5	>250
3b-05[b]	$2F-C_6H_5-CH_2-$	$3CH_3O-C_6H_4-$	135.6 ± 1.4	>250
3b-06[b]	$2F-C_6H_5-CH_2-$	$4F-C_6H_4-$	>250	>250
3c-01[b]	$4NO_2-C_6H_5-CH_2-$	C_6H_5-	187.7 ± 1.5	>250
3c-02[b]	$4NO_2-C_6H_5-CH_2-$	$3Br-C_6H_4-$	171.3 ± 1.6	157.7 ± 1.4
3c-03[b]	$4NO_2-C_6H_5-CH_2-$	$4Br-C_6H_4-$	>250	>250
3c-04[b]	$4NO_2-C_6H_5-CH_2-$	$4F-C_6H_4-$	>250	>250
3c-05[b]	$4NO_2-C_6H_5-CH_2-$	$3CH_3O-C_6H_4-$	158.8 ± 1.4	166.9 ± 1.7
boscalid			3.1 ± 0.5	0.3 ± 0.1
carbendazim			8.4 ± 0.6	1.1 ± 0.1

[a]Values are the mean ± standard deviation (SD) of three replicates. [b]Compounds first reported.

Anal. Calcd for $C_{19}H_{14}BrN_3O_3S$: C, 51.36; H, 3.18; N, 9.46. Found: C, 51.76; H, 3.24; N, 9.30.

N-(4-Fluorophenyl)-2-((4-nitrobenzyl)thio)nicotinamide ***3c-04****:* yellow powder; yield, 61.3%; mp, 174−175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.56 (s, 2H, S−CH_2−Ar), 7.13 (td, 1H, J = 7.5, 0.2 Hz, Ph−H), 7.18 (t, 1H, J = 10.3 Hz, Ph−H), 7.28−7.35 (m, 4H, 3 Ph−H, 1 Py−H), 7.51 (td, 1H, J = 7.7, 1.5 Hz, Ph−H), 7.62−7.65 (m, 1H, Ph−H), 8.01 (dd, 1H, J = 7.6, 1.6 Hz, Py−H), 8.04 (s, 1H, Ph−H), 8.64 (dd, 1H, J = 4.8, 1.6 Hz, Py−H), 10.67 (s, 1H, CONH); MS (ESI), 417.00 ($C_{19}H_{14}BrFN_2OS$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{14}BrFN_2OS$: C, 54.69; H, 3.38; N, 6.71. Found: C, 54.47; H, 3.39; N, 6.34.

N-(3-Methoxyphenyl)-2-((4-nitrobenzyl)thio)nicotinamide ***3c-05****:* white crystal; yield, 58.3%; mp, 180−181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.55 (s, 2H, S−CH_2−Ar), 6.69−6.72 (m, 1H, Ar), 7.26 (d, 2H, J = 5.0 Hz, Ph−H), 7.30 (dd, 1H, J = 7.6, 4.9 Hz, Py−H), 7.39 (s, 1H, Ph−H), 7.69 (d, 2H, J = 8.6 Hz, Ph−H), 7.96 (dd, 1H, J = 7.6, 1.4 Hz, Py−H), 8.15 (d, 2H, J = 8.7 Hz, Ph−H), 8.61 (dd, 1H, J = 4.8, 1.5 Hz, Py−H), 10.44 (s, 1H, CONH); MS (ESI), 396.09 ($C_{20}H_{17}N_3O_4S$, $[M + H]^+$). Anal. Calcd for $C_{20}H_{17}N_3O_4S$: C, 60.75; H, 4.33; N, 10.63. Found: C, 60.22; H, 4.31; N, 10.91.

Biological Testing. *Plant Pathogenic Fungi.* The test fungi, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, were provided by the Laboratory of Plant Disease Control, Nanjing Agricultural University. After retrieval from the storage tube, the strains were incubated in PDA at 25 °C for a week to get new mycelia for the antifungal assay.

Antifungal Bioassay. The fungicidal activity of the synthetic compounds was tested in vitro against two plant pathogenic fungi using a mycelia growth inhibition method.[9] The synthesized compounds were dissolved in DMSO to prepare the 10 mg/mL stock solution before mixing with molten agar below 60 °C. The media containing compounds at a concentration of 25 mg/mL for the initial screening were then poured into sterilized Petri dishes. After appropriate days at 25 °C, the colony diameter of each strain was measured with the original mycelial disk diameter (5 mm) subtracted from this measurement. Percentage inhibition was calculated as $(1 - a/b) \times 100\%$, where a is the colony diameter in Petri dishes with compounds and b is the mean colony diameter in Petri dishes without tested

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compounds. DMSO served as negative control, whereas commercially available agricultural fungicides carbendazim and boscalid were used as positive controls. Each measurement consisted of at least three replicates. The concentration-dependent curve was the values of inhibition rates for the *Y* axis against the test sample concentrations (mg/mL) for the *X* axis. The IC_{50} value (Table 2) was defined as the concentration required for 50% inhibition of mycelial growth.

In Vivo Testing on Cole Leaves Infected by S. sclerotiorum. Strain *S. sclerotiorum* and susceptible cole leaves collected from Pailou Experimental Centre of Nanjing Agricultural University were used to measure the efficacy of compounds in vivo.[10] Healthy cole leaves were sprayed with compounds (0.5 mg/mL) and subsequently cultivated at 20 °C for 24 h before artificial inoculation with strain *S. sclerotiorum*. Results were observed as diameters of symptoms after cultivation at 20 °C for 36 h. Carbendazim (50% WP, Jiangsu Rotam Lanfeng Biochemical Co., Ltd., China) was coassayed as the positive control. The efficacy of disease control was calculated as $(1 - c/d) \times 100$, where c is the diameter of the treatment and d is the diameter of the negative control. The disease control picture is shown in Figure 3.

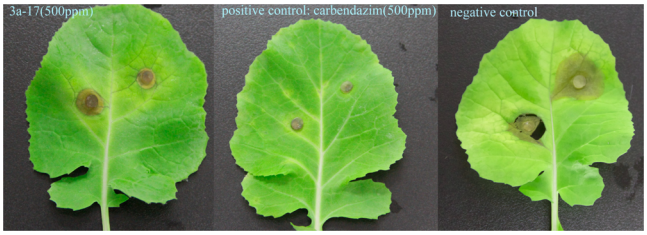


Figure 3. Effects of compound **3a-17** against *S. sclerotiorum* infected cole leaves.

Enzyme Assay in Vitro. *Isolation of R. solani and S. sclerotiorum.* Fungus mitochondrial was isolated according to a previously reported method.[11] Cultures were inoculated at 0.05 $OD_{600\ nm}$ and grown on a reciprocal shaker (180 rpm, 25 °C) for 5 days in Sabouraud maltose broth (SMB) medium. Cells were harvested by vacuum filtration and disrupted in liquid nitrogen using a mortar and pestle. The resultant powder was resuspended to 10% w/v in mitochondrial extraction buffer (10 mM KH_2PO_4, pH 7.2, 10 mM KCl, 10 mM $MgCl_2$, 0.5 M sucrose, 0.2 mM EDTA, 2 mM PMSF). The extract was clarified by centrifugation (5000*g*, 4 °C for 10 min, 2 times), and intact mitochondria were then pelleted at 10000*g* for 20 min at 4 °C and resuspended in the same buffer. Mitochondrial suspensions were brought to a concentration of 10 mg/mL and stored at −80 °C until use. SDH activity was found to remain stable for months.

Succinate:Ubiquinone/DCPIP Activity Inhibition. Mitochondrial suspensions were diluted 1/20 in extraction buffer and preactivated at 30 °C for 30 min in the presence of 10 mM succinate. Succinate:ubiquinone/DCPIP activity inhibition measurements were performed by adding 10 μL of preactivated mitochondria to 200 μL of assay buffer (50 mM phosphate-sodium, pH 7.2, 250 mM sucrose, 3 mM NaN_3, 10 mM succinate) supplemented with 140 μM dichlorophenolindophenol (DCIP) and 1 mM 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q_0). Inhibitor concentrations ranged between 4.4 and 150 μM, with uniform 2× dilution factor steps (six inhibitor concentrations + DMSO control). A total of 96 well plates were pre-equilibrated at reaction temperature (30 °C) for 10 min before the reactions were started by the addition of 10 μL of preactivated *R. solani* mitochondrial suspension. DCPIP reduction was conducted at 30 °C and monitored at 595 nm. Calculated absorbance slopes (OD/h) were used for half-inhibitory concentration (IC_{50}) calculations using GraphPad Prism 5.0 software.

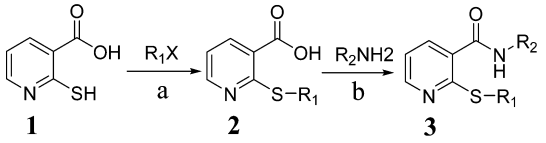
Molecular Docking. *Homology Modeling.* The NCBI protein database (http://www.ncbi.nlm.nih.gov/sites/entrez) was used to search the SDH amino acid sequence of *S. sclerotiorum*. The employed hypothetical protein sequences were XP_001591238, XP_001594577, XP_001597467, and XP_001593251, reported by Birren. The BLAST server (http://blast.ncbi.nlm.nih.gov) was used to search the templates for these four chains. We applied succinate dehydrogenase from porcine heart (PDB code 1ZOY) and avian (PDB code 1YQ3) as two templates, and the homologies of amino acid sequences were aligned. Homology modeling of SDH from *S. sclerotiorum* was carried out using DS MODELER, which is a module integrated in Discovery Studio (version 3.1). The obtained models were evaluated by Ramachandran plots using Discovery Studio software. All of the algorithms and parameters were set as default.

Molecular Docking. The automated docking was performed with DS-CDocker implemented in Discovery Studio (version 3.1).[12] The three-dimensional structures of picked compounds were constructed using Chem. 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft Corp., USA (2009)]. Then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger−Hückel charges of ligands were assigned. All bound water and ligands were eliminated from the protein, and the polar hydrogens and the Kollman-united charges were added to the proteins.

RESULTS AND DISCUSSION

Chemical Synthesis. Scheme 1 and Table 2 describe the synthesis and chemical structures of these nicotinamide

Scheme 1. General Synthesis of Compound 3a, 3b, and 3c[a]



[a]Reagents and conditions: if $R_1X = CH_3I$, (a) DMF, pH 8−9, rt, 16−24 h; 5% $HCl_{(aq)}$, pH 2−3; if R_1X = BnBr, (a) ethanol, K_2CO_3, rt, 1−2 h; 5% $HCl_{(aq)}$, pH 2−3; (b) $SOCl_2$, 75 °C, reflux, 3−4 h; CH_2Cl_2, rt, 3 h.

derivatives. Compound **1** is reacted with RX (iodomethane or benzyl bromide) to afford corresponding compound **2**. Compounds **2** were first transformed into nicotinyl chloride in the presence of $SOCl_2$ and then reacted with appropriate substituted aniline or alkylamine to afford the target compounds **3a**, **3b**, and **3c**. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

Among them, **2b**, **2c** and 26 compounds of **3a**, **3b**, and **3c** series are first reported and others were reported with antifungal activities for the first time (Table 2).

Fungicidal Activities. The antifungal activities of the synthetic molecules, which are expressed as IC_{50} (median inhibitory concentration) values, were determined using a mycelia growth inhibitory rate method. All of the synthetic compounds exhibited antifungal effects to *R. solani* and *S. sclerotiorum*. The results are shown in Table 2.

Compared to **3a**, most compounds of the **3b** and **3c** series showed weak antifungal activity with IC_{50} values >150 μM. However, when other groups replacing sulfur methyl are introduced at the R_1 position, the IC_{50} values of series **3a** compounds decreased at least 4−5-fold, which were observed by comparing the data **3a-01** (IC_{50} = 42.7 μM) and **3b-01** (IC_{50} > 250 μM), **3c-01** (IC_{50} = 187.7 μM); **3a-12** (IC_{50} = 35.5 μM) and **3c-02** (IC_{50} = 171.3 μM); **3a-15** (IC_{50} = 28.4 μM) and **3c-05** (IC_{50} = 185.8 μM) for *R. solani* inhibition activity. The result verified the previous design idea that sulfur methyl had more advantages in antifungal activity than sulfur benzyl at the R_1 position. It seems a small steric hindrance at R_1 position is conducive to combining the nicotinamide ligands and the SDH

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active domain. Therefore, it is more reasonable that we mainly synthesized **3a** series compounds, which were more likely to be developed as potential fungicides.

Most **3a** derivatives exhibited antifungal effects with IC_{50} of <250 μM except **3a-26** and **3a-27**. With the alkane and cyclohexane substitution at the R_2 position, these two compounds show IC_{50} values of >500 μM to both *R. solani* and *S. sclerotiorum*, far larger than the expected activity, indicating that the presence of a nonaromatic group at the R_2 position may cause significant loss of fungicidal activity of **3a** derivatives.

In total, 7 compounds (**3a-01**, **3a-11**, **3a-12**, **3a-15**, **3a-16**, **3a-17**, and **3a-19**) and 11 compounds (**3a-01**, **3a-04**, **3a-05**, **3a-11**, **3a-12**, **3a-15**, **3a-16**, **3a-17**, **3a-18**, **3a-19**, and **3a-23**) displayed IC_{50} values of <100 μM against *R. solani* and *S. sclerotiorum*, respectively. Among them, **3a-17** exhibited the highest activity against *R. solani* with an IC_{50} value of 15.8 μM, which was half-fold that of the positive control carbendazim. Besides, **3a-12** exhibited the second highest activity against the two pathogens. Other compounds exhibited moderate to weak antifungal activities with IC_{50} values ranging from 100 to 250 μM. The best four compounds against two fungi were all substituted in the meta-position, demonstrating that the presence of substitution in the meta-position of the side phenyl ring was favorable for antifungal activity. This finding was similar to the previous Masatsugu ODA's study.[15]

The structure−activity relationships of these compounds can be summarized: (1) Sulfur methyl has more advantages for antifungal activity than sulfur benzyl at the R_1 position. (2) The presence of an aromatic ring at the R_2 position plays an important role in the antifungal activity. (3) Substitution in the meta-position of the side phenyl ring (R_2) may be essential for enhancing antifungal activity.

In Vivo Testing on Cole Leaves Infected by *S. sclerotiorum*. Among the compounds tested for antifungal activity in vitro, **3a-17** was found to be potent against *S. sclerotiorum*. Therefore, it was further evaluated in the greenhouse for the control of *S. sclerotiorum* infected cole. The efficacy of the treatment is shown in Figure 3. The untreated negative control (pathogen only) resulted in 100% disease incidence (0% healthy plant standard) 24 h after transplantation. Treatment with 0.5 mg/mL **3a-17** resulted in 43.9% healthy plant standard after 36 h of treatment (Table 3). In contrast, leaves treated with carbendazim at the same concentration resulted in 64.2% healthy plant standard. Thus, significant differences existed among the treated and untreated groups for disease control experiments in the greenhouse cole leaves.

Table 3. In Vivo Efficacy of 500 μg/mL Compounds on Cole Leaves Infected by *Sclerotinia sclerotiorum*[a]

	diameter of lesions (mm)	protection efficacy (%)
3a-17	8.3 ± 0.8	43.92
carbendazim	5.3 ± 0.4	64.19
negative control	14.8 ± 0.9	

[a]Statistical analysis of the data was performed by analysis of variance (one-way ANOVA). A probability value of $p \leq 0.05$ was considered to denote a statistically significance difference.

Fungal SDH Inhibition Activities. Seven compounds with IC_{50} values of <100 μM were selected and tested against SDH enzymes in vitro. As demonstrated in Table 4, **3a-17** showed the best activity and inhibitory abilities against SDH enzymes of the tested compounds with IC_{50} values from 20 to 50 μM and exhibited the same tendency as the data acquired by using the mycelia growth inhibition assay in vitro. It fully proved that the nicotinamides designed in this paper displayed as good inhibitory effects on SDH enzymes as other successfully developed carboxamide fungicides.

Table 4. IC_{50} Values of Fungal SDH Inhibition Activity (in Vitro)

	IC_{50} (μM)	
compd no.	*R. solani*	*S. sclerotiorum*
3a-01	24.0 ± 0.1	30.7 ± 0.2
3a-11	30.8 ± 0.2	36.3 ± 0.1
3a-12	21.1 ± 0.2	30.0 ± 0.2
3a-15	28.5 ± 0.3	36.8 ± 0.2
3a-16	40.8 ± 0.2	35.7 ± 0.3
3a-17	19.6 ± 0.2	23.9 ± 0.2
3a-19	40.1 ± 0.2	32.6 ± 0.2

Interactions with SDH. *Homology Modeling.* The trace, the templates, and the homology model and the secondary and tertiary structures of the protein are quite similar, all of which is shown in Figure 4A,B). A Ramachandran plot of this minimized model showed that 95.41% of the residues were located in the allowed regions (91.16% most favored) and only 4.59% (54 residues) were outside the allowed regions for the model. As these disallowed residues were far from the succinate dehydrogenase active site, they did not significantly contribute to the function of protein acceptor. Evaluated by the plot, the quality of the homology model is suitable as a molecular docking receptor.

Binding Mode Analysis. In an effort to elucidate the possible mechanism of inducing fungicidal activity by these nicotinamide compounds and explain the previous assumption, molecular docking of **3c-17** and another 10 selected compounds into the homology model according to the binding site of ubiquinone on reported SDH complex structure (1ZOY and 1YQ3, pdb) was performed, respectively. Boscalid was docked as contrast at the same time. The CDOCKER scores are provided in Table 5, in which, compounds got the same tendency with their antifungal and SDH inhibitory activities. Boscalid and **3c-17** showed the best two −cdock_interaction_energy scores of 34.27 and 27.93, respectively. Three-dimensional schematic diagrams clearly explained the possible optimal combination between the ligands and receptor protein. Boscalid is well bound to the receptor protein with its amino hydrogen toward the carboxyl oxygen of GLU171 of chain B, and its amide oxygen interacted with the indole hydrogen of TRP167. Besides, two rings of indole both had pi−pi interactions with 3.8 Å distance to reinforce molecular boscalid near TPR167 (Figure 5A), and the amide group of boscalid interacted well with residues around the binding site. As shown in Figure 5B, sulfur and chlorine of **3a-17** are well bound with the amino hydrogen of PHE291 and the pyrrolidine hydrogen of PRO150, respectively. Two-dimensional diagrams are also displayed as Figure 6, in which all of the amino acid residues interacted with the ligand, including weak interaction such as van der Waals interactions and polar interactions. Thus, a stable complex based on these interactions was formed. Associated with the virtual screening results (Table 1), this binding model indicated that the introduction of a sulfur methyl moiety allowed the ligand to more easily combine with SDH.

The docked compounds mainly interacted by H-bond with B, C, and D chains of receptor protein, which is consistent with previous studies on boscalid and other SDHIs (such as carboxin,

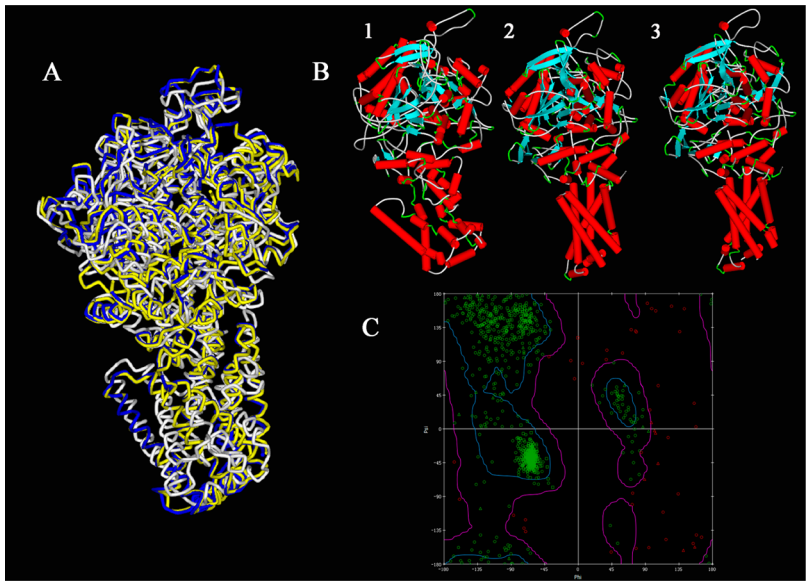

Figure 4. Three-dimensional schematic and Ramachandran plot of *S. sclerotiorum* SDH homology model and templates (1ZOY and 1YQ3): (A) tube trace of the two templates and the model (white color shows model, yellow color shows the template 1YQ3, and blue color shows the template 1ZOY); (B) secondary structures model (1) and templates 1YQ3 (2) and 1ZOY (3); (C) Ramachandran plot of the model (residues distribution plot).

Table 5. -cdock_interaction_energy of Compounds and *S. sclerotiorum* SDH (Homology Model)

compd no.	-cdock_interaction_energy ΔGb (kcal/mol)	compd no.	-cdock_interaction_energy ΔGb (kcal/mol)
3a-01	23.07	**3a-17***	27.93
3a-11*	23.17	**3a-18***	21.63
3a-12*	25.44	**3a-19***	24.59
3a-14*	20.71	**3b-02***	19.54
3a-15	24.09	**3c-02***	20.61
3a-16*	24.93	boscalid	34.27

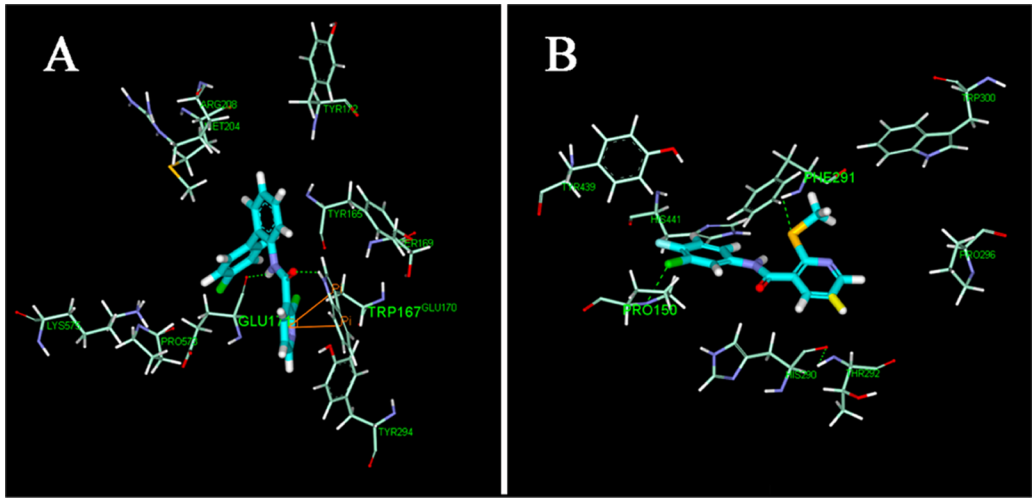

Figure 5. Interaction of boscalid, **3a-17**, and amino acid residues near the ligands (3D diagram).

fluopyram, bixafen, and isopyrazam).[13] Docking studies predicted the activity level and even possibly resistance site through identifying the key amino acid residues that were important for binding of different SDHIs. Resistant fungal genotype analysis verified that most of those key residues involved in forming the binding cavity were related to resistance formation.[14,15]

In conclusion, a series of nicotinamide derivatives as SDHIs were designed, prepared, and evaluated for their antifungal activity against *R. solani* and *S. sclerotiorum*. Some synthetic compounds displayed potent antifungal inhibitory activities with compound **3a-17** being the most effective. In vivo testing demonstrated that **3a-17** could effectively control the disease on *S. sclerotiorum* infected cole leaves. Meanwhile, the structure–activity relationships and molecular modeling study offered further insight into interactions between the receptor and ligand. The binding model of **3a-17** implied that the introduction of a

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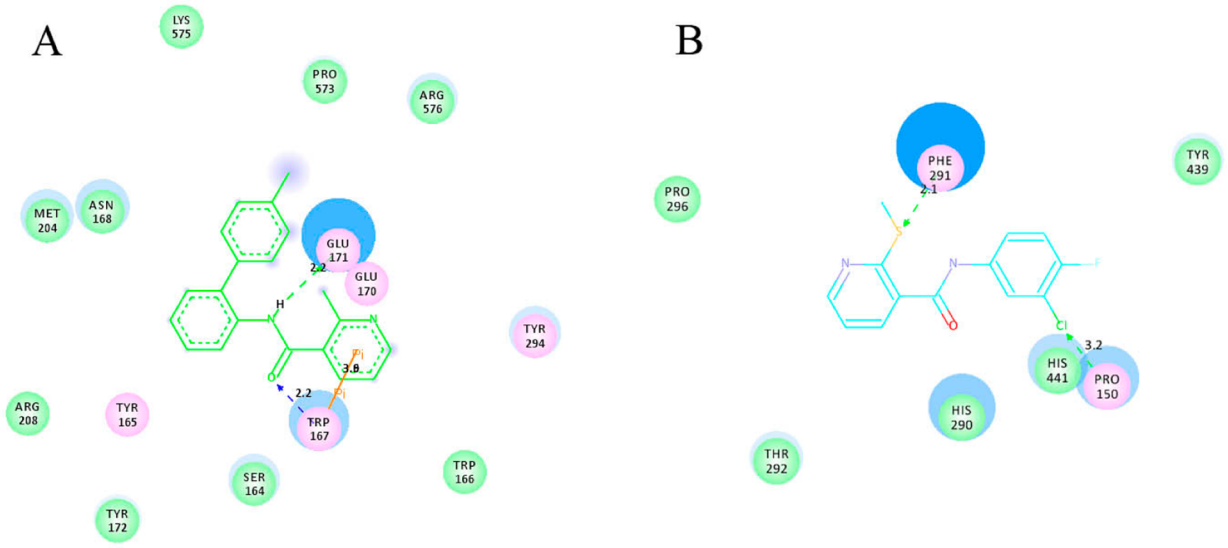

Figure 6. Interaction of boscalid, **3a-17**, and amino acid residues near the ligands (2D diagram). The purple circles show the amino acids that participated in hydrogen bonding or polar interactions. The green circles show the amino acids that participated in the van der Waals interactions. The p-cation interactions are shown as orange lines, and hydrogen bond interactions are represented by a blue or green dashed arrow directed toward the electron donor.

sulfur methyl moiety made the ligand easier to combine with SDH.

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Author Contributions

‖Y.-H.Y. and L.M. contributed equally to this paper.

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Notes

The authors declare no competing financial interest.

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