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Design and Discovery of Novel Chiral Antifungal Amides with 2-(2-Oxazolinyl)aniline as a Promising Pharmacophore

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ABSTRACT: Inspired by established succinate dehydrogenase inhibitors (SDHIs), our continuing efforts toward the discovery of chiral antifungal amides turned to the optimization of their polar regions with 2-(2-oxazolinyl)aniline as a known pharmacophore. Scaffold hopping and bioactivity-guided convergent synthesis enabled the identification of promising antifungal categories. Fine tuning of the substituents and chirality furnished 7 amides (**1s**, **1t**, **2d**, **2h**, **2j**, **3k**, and **2l**) as antifungal candidates, with EC_{50} values lower than 5 mg/L. The first investigation of chiral amides of acyclic acids as SDHIs was conducted, and **2d** was selected as a promising candidate against *B. cinerea*, with a preventative efficacy of up to 93.9% at 50 mg/L, which is better than that of boscalid. The different binding models between compounds with different configuration were simulated for **2d** and its diastereoisomers. The benefits of synthetic accessibility and cost-effectiveness highlight the practical potential for **2d** as a good alternative to known SDHIs fungicides.

KEYWORDS: chiral pesticide, oxazoline alkaloids, respiratory chain, fungicide, structure-activity relationship

INTRODUCTION

The respiratory electron transport chain (ETC) can create an electrochemical proton gradient across the membrane via a series of redox reactions,[1] which transfer electrons from NADH to O_2 with concomitant production of adenosine triphosphate (ATP). Many fungicides have been developed and launched based on this crucial biological process, mainly targeting mitochondrial redox carrier protein complexes I to IV. The respiratory inhibitors are ranked first for disease control in modern plant protection, in terms of either commercial species or sales volume, mainly addressing the protein targets SDH (complex II) and cytochrome bc1 (Complex III).[2, 3] Among different respiratory chain inhibitors, SDHIs have been attracting increasing attention especially after the commercialization of boscalid in 2003. Significant advances have been achieved for this kind of fungicides as exemplified by the more recent researches[4-9]. Succinate dehydrogenase was also chosen as a potential target for exploring natural products as probes for the discovery of novel antibiotics.[10]

The importance of the ubiquinone-binding pocket was clearly established during the discovery of new SDHIs. [11] The continuing interest in the agrochemicals targeting the respiratory chain prompted us to consider the possibility of chiral ligands for the ubiquinone binding pocket. The rationality was confirmed by a survey of the chiral SDHIs launched since 2010, including sedaxane,[12] benzovindiflupyr,[13] and pydiflumetofen.[14] We envisioned that the binding pocket was sensitive to the innate chirality of the small ligands, and our efforts were therefore devoted to chiral drimane terpenoids[15-17] and chiral carboxamides[9] which simulated the natural ubiquinone and commercial respiratory inhibitors, respectively. With the hypothesis that

dearomatization of the "substituent" in the SDHIs model can be used to optimize the system (Figure 1),[9] novel chiral antifungal nicotinamides were synthesized, and 2-(2-oxazolinyl)aniline was validated as a promising pharmacophore.

As shown in Figure 1, 23 SDHIs have been released as commercial fungicides .[2] The core moiety (the polar part) is essential for both binding and in *vivo* potency. The core is speculated to be inserted deeply into the cavity of SDH, and mainly interact with the Arg87 and His267 in SDHc and SDHb, respectively (Figure 1).[11] We envision that the cavity and the interactions of amino acid residues with small molecules are enantioselective.

Structure analysis demonstrated that with the exception of oxathiin carboxamides (carboxin and oxycarboxin), aromatic acids are common in the cyclic fragments attached to the amide functionality.[2] 2-(2-Oxazolinyl)aniline is embodied in numerous bioactive compounds of relevance to crop protection and medicinal chemistry.[18, 19] Our previous investigation with this motif for the dearomatization of boscalid culminated in the discovery of **(*R*)-LE001** as an antifungal candidate (Figure 1). With 2-(2-oxazolinyl)aniline as a pharmacophore (inserted into the groove formed by SDHb and SDHc), novel molecules were designed to diversify and optimize the polar cores. All of the prepared compounds can potentially form hydrogen bonds and π-π interaction with the amino acid residues in the cavity. Considering the privileged subunits, synthetic accessibility, aforementioned potential interactions and cost-effectiveness, our study featured the introduction of versatile polar parts for the establishment of novel amides of 2-(2-oxazolinyl)aniline (Figures 1 and 2).

MATERIALS AND METHODS

Instruments, Chemicals and Related Materials. Unless otherwise mentioned, all solvents and reagents were analytically pure and were purchased from commercial sources (Energy, Shanghai, China). Anhydrous solvents were dried and distilled by standard techniques before use. Silica gel GF_{254} and silica gelfor column chromatography (200-300 mesh) were both purchased from Qingdao Broadchem Industrial Co., Ltd. (Qingdao, Shandong, China). The ^{1}H NMR and ^{13}C NMR spectra were recorded on an AV 400 spectrometer (Bruker, Switzerland) with $CDCl_3$ or d_6-DMSO as the solvent and tetramethylsilane (TMS) as the internal standard. Elemental analyses were performed on a Vario EL instrument (Elementar, Hesse, Germany). Melting points (m.p.) were recorded on a WRS-1B melting point apparatus (Shenguang, Shanghai, China) and are uncorrected. Electrospray ionization mass spectrometry (ESI-MS) data were obtained with aXevo TQ-S Micro-Spectrometer (Waters, Milford, MA). The single-crystal diffraction was carried out on an AXS Smart APEX CCD diffractometer (Bruker, Switzerland).

General Procedures for the Condensation of Acids and Amines. Method 1A: To a dried Schlenk flask charged with 4-(difluoromethyl)-1-methyl-1*H*-pyrazole-3-carboxylic acid (1 mmol, 176 mg) and the synthesized (*R*)-2-(4-methyl-4,5-dihydrooxazol-2-yl)aniline (1.2 mmol, 211 mg) was added anhydrous CH_2Cl_2 (5 mL) for dissolution. 4-Dimethylaminopyridine(DMAP) (0.06 g, 0.5 mmol) and *N*-(3-(dimethyl amino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI−HCl) (0.268 g, 1.4 mmol) were then added while the reaction flask was in an ice bath. The mixture was allowed to gradually warm to room temperature, and it was stirred overnight until full

consumption of the carboxylic acid was detected by thin layer chromatography (TLC). The mixture was quenched by the addition of a saturated aqueous solution of $NaHCO_3$ (20 mL) and separated. The water phase was extracted with dichloromethane (10 mL × 3), and the combined organic phase was sequentially washed with water (10 mL × 2) and saturated aqueous NaCl (10 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum. Purification by silica gel column chromatography (254 mm x 17 mm i.d.) on silica gel with hexane/EtOAc (5:1, v/v) as the eluent gave (*R*)-4-(difluoromethyl)-1-methyl-*N*-(2-(4-methyl-4,5-dihydrooxazol-2-yl)phenyl)-1*H*-pyrazole-3-carboxamide (**1a**).

Data for **1a**: yield 66%; white solid; m.p. 146.8-147.7 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (d, J = 6.7 Hz, 3H, CH_3), 3.69 (dd, J_1 = 11.2 Hz, J_2 = 3.6 Hz, 1H, OCH_2), 3.82 (dd, J_1 = 11.2 Hz, J_2 = 4.4 Hz, 1H, OCH_2), 4.02 (s, 3H, N-CH_3), 4.55 (m, 1H, N-CH), 6.47 (m, br, CHF_2), 7.11 (m, 1H, H in phenyl ring), 7.48-7.54 (m, 2H, H in phenyl ring), 7.91 (s, 1H, H in pyrazole), 8.67 (d, J = 8.80 Hz, 1H, H in phenyl ring), 11.77 (s, br, 1H, NH). Elemental anal. calcd for $C_{16}H_{16}F_2N_4O_2$: C, 57.48; H, 4.82; N, 16.76. Found: C, 57.51; H, 4.86; N, 16.63. ESI-MS: calcd for $C_{16}H_{17}F_2N_4O_2$ $[M+H]^+$: 335.13, found: 335.23.

Compounds **1b**, **1c**, **1d**, **1e**, **1f**, **1q**, **1r** were prepared similarly by the variation of either acid parts or chiral amine moieties.

Method 1B: To a dried Schlenk flask charged with (*R*)-mandelic acid (0.152 g, 1 mmol) and the synthesized (*R*)-2-(4-methyl-4,5-dihydrooxazol-2-yl)aniline (0.176 g, 1 mmol) was added anhydrous CH_2Cl_2 (5 mL) for dissolution. This mixture was chilled

with an ice-bath, and DMAP (0.024 g, 0.2 mmol) and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (0.493 g, 1.3 mmol) was added sequentially under a N_2 atmosphere. The reaction progress was monitored by TLC until the full conversion of (*R*)-2-(4-methyl-4,5-dihydrooxazol-2-yl)aniline was observed. The mixture was quenched by the addition of a saturated aqueous solution of NH_4Cl (10 mL) and separated. The aqueous phase was extracted with dichloromethane (10 mL × 3), and the combined organic phase was sequentially washed with water (10 mL × 2) and saturated aqueous NaCl (10 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum. Purification by column chromatography (254 mm x 17 mm i.d.) on silica gel with hexane/EtOAc (2:1, v/v) gave the desired product (*R*)-2-hydroxy-*N*-(2-((*R*)-4-methyl-4,5-dihydrooxazol-2-yl)phenyl)-2-phenylacetamide (**2b**).

Data for **2b**: yield 46%; pale yellow solid; m.p. 115.4-116.9 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (d, J = 6.24 Hz, 3H, CH_3), 3.87 (m, 1H, OCH_2), 4.25 (d, J = 4.6 Hz, 1H, OH), 4.39-4.49 (m, 2H, NCH and OCH_2), 5.20 (d, J = 4.6 Hz, 1H, Ph-CH-OH), 7.09 (m, 1H, H in phenyl ring), 7.30-7.41 (m, 4H, H in phenyl ring), 7.46 (m, 1H, H in phenyl ring), 7.50-7.57 (m, 2H, H in phenyl ring), 7.81 (dd, J_1 = 7.9 Hz, J_2 = 1.7 Hz, 1H, H in phenyl ring), 8.72 (dd, J_1 = 8.5 Hz, J_2 = 1.1 Hz, 1H, H in phenyl ring), 12.56 (s, br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.5 (CH_3), 61.8 (CH), 72.7 (CH_2), 74.8 (CH), 113.7 (C), 119.8 (CH), 122.9 (CH), 127.4 (2 × CH), 128.6 (CH), 128.6 (2 × CH), 129.1 (CH), 132.5 (CH), 139.1 (C), 139.4 (C), 163.0 (C), 171.8 (C). Elemental anal. calcd for $C_{18}H_{18}F_2N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.71; H, 5.90; N, 9.11. ESI-MS calcd for $C_{18}H_{19}N_2O_3$ $[M+H]^+$: 311.14, found: 311.17. The structure of

2b was confirmed by single-crystal diffraction, and the data was given the CCDC number 1838207.

The compounds listed in Figure 2B were prepared similarly by variation of the mandelic acid or chiral amines.

Compounds **4a**, **4b**, **4c**, and **4d** were prepared according to a similar manipulation. As an example, the spectroscopic data of compound **4d** are as follows: (*R*)-N-(2-(4-benzyl-4,5-dihydrooxazol-2-yl)phenyl)-2-oxo-2-phenylacetamide: yield 61%, white solid, m.p. 129.8-30.9 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83 (dd, J_1 = 13.9 Hz, J_2 = 8.2 Hz, 1H, Ph-CH_2), 3.27 (dd, J_1 = 13.9 Hz, J_2 = 5.4 Hz, 1H, Ph-CH_2), 4.12 (dd, J_1 = 8.5 Hz, J_2 = 7.6 Hz, 1H, OCH_2), 4.35 (dd, J_1 = 8.5 Hz, J_2 = 8.4 Hz, 1H, OCH_2), 4.75 (m, 1H, =NCH), 7.19 (m, 1H, H in phenyl ring), 7.23-7.29 (m, 4H, H in phenyl ring), 7.50-7.57 (m, 3H, H in phenyl ring), 7.66 (m, 1H, H in phenyl ring), 7.88 (dd, J_1 = 7.9 Hz, J_2 = 1.6 Hz, 1H, H in phenyl ring), 8.31-8.36 (m, 2H, H in phenyl ring), 8.89 (dd, J_1 = 8.4 Hz, J_2 = 1.1 Hz, 1H, H in phenyl ring), 13.46 (s, br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.6 (CH_2), 67.8 (CH_2), 70.6 (CH), 114.6 (C), 120.1 (CH), 123.6 (CH), 126.6 (CH), 128.5 (2 × CH), 128.6 (2 × CH), 129.3 (2 × CH), 129.5 (CH), 131.1 (2 × CH), 132.6 (CH), 133.4 (C), 134.3 (CH), 137.5 (C), 138.7 (C), 161.3 (C), 163.5 (C), 188.0 (C). Elemental anal. calcd for $C_{24}H_{20}N_2O_3$: C, 74.98; H, 5.24; N, 7.29. Found: C, 74.95; H, 5.33; N, 7.31. ESI-MS calcd for $C_{24}H_{21}N_2O_3$ $[M+H]^+$: 385.16, found: 385.20.

General Procedures for the Synthesis of Amides from Acids with an NH group (Method 2). The synthesis of **1h** is presented as an example (Figure 4).

Step 1: To the stirred solution of L-proline(2.0 g, 17.39 mmol) in tetrahydrofuran (THF) (30 mL) and water (15 mL) was added $NaHCO_3$ (2.8 g, 33.3 mmol) slowly in portions while in an ice-bath, and then di-*tert*-butyl dicarbonate($(Boc)_2O$) (4.06 g, 18.62 mmol) was added. The mixture was allowed to gradually warm to room temperature and was stirred overnight until full consumption of the carboxylic acid was observed. The volatile solvent was evaporated under reduced pressure, and the inorganic phase was extracted with dichloromethane (20 mL × 3). The combined organic phase was washed sequentially with water (15 mL × 2) and saturated aqueous NaCl (15 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum to furnish the crude **Boc-L-Proline** as a colorless oil in 96% yield. This intermediate was used for the next step without further purification.

Step 2: **Boc-1h** was successfully synthesized in 72% yield following Method 1.

Step 3: To a solution of **Boc-1h** (373 mg, 1 mmol) in CH_2Cl_2 (5 mL) in an ice bath was added trifluoroacetic acid (TFA) (1.5 mL, 20 mmol), and the mixture was stirred until full conversion was reached as judged by TLC (~ 30 min). Saturated aqueous $NaHCO_3$ (15 mL) was added while the mixture was in an ice bath, and the organic phase was separated. The aqueous layer was extracted with dichloromethane (10 mL × 3), and the organic extracts were combined and washed with saturated aqueous NaCl (10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was subjected to flash chromatography (254 mm x 17 mm i.d.) on silica gel with petroleum ether/EtOAc (1:1, v/v) as the eluent to give (*R*)-*N*-(2-((*S*)-4-methyl-4,5-dihydrooxazol-2-yl)phenyl)pyrrolidine-3-carboxamide (**1h**).

213 Data for **1h**: yield 64%; white solid; m.p.: 104.5-105.1 °C; ^{1}H NMR (400 MHz,
214 $CDCl_3$) δ 1.39 (d, J = 6.3 Hz, 3H, CH_3), 1.72-1.86 (m, 2H, H in pyrrolidine ring), 2.02
215 (m, 1H, H in pyrrolidine ring), 2.15-2.31 (m, 2H, H in pyrrolidine ring), 3.07-3.11 (m,
216 2H, H in pyrrolidine ring), 3.88-3.96 (m, 2H, 1H in OCH_2 and 1H in CH-CO), 4.44-
217 4.50 (m, 2H, 1H in OCH_2 and 1H in CH-N), 7.08 (dd, J_1 = 7.9 Hz, J_2 = 7.3 Hz, H in
218 phenyl ring), 7.45 (m, 1H, H in phenyl ring), 7.84 (dd, J_1 = 7.9 Hz, J_2 = 1.7 Hz, H in
219 phenyl ring), 8.81 (d, J = 8.4 Hz, H in phenyl ring), 12.65 (s, br, 1H, H in phenyl ring).
220 Elemental anal. calcd for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37. Found: C, 65.89; H,
221 7.05; N, 15.43. ESI-MS calcd for $C_{15}H_{20}N_3O_2$ $[M+H]^+$: 274.16, found: 274.26.

222 Compounds **1g**, **1i**, **1j**, **1k**, **1l**, **1m**, **1n**, **1s**, **1t**, **1u**, and **1v** were prepared similarly
223 by variation of either the acid or chiral amine moieties.

224 **Synthesis of Amides from Isoquinoline-3-Carboxylic Acid (Method 3)**. The
225 synthesis of **1o** is selected as an example (Figure 5).

226 *Step 1*: To a stirred suspension of (*S*)-phenylalanine (10 g, 60.6 mmol) and 37%
227 formalin (23 mL) was added concentrated HCl (77 mL). The reaction mixture was
228 immersed in a preheated oil bath (95 °C) for 1 h. Second portions of formalin (10 mL)
229 and concentrated HCl (20 mL) were added, and the reaction mixture was stirred until
230 the full conversion of the phenylalanine. The resulting mixture was chilled with an ice
231 bath and filtered. The precipitate was dissolved in hot water, concentrated ammonium
232 hydroxide was added to neutralize the system and then the mixture was chilled with an
233 ice-bath. The precipitate was filtered, washed with cold water, collected and dried under
234 vacuum. This crude (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, **I** (75% yield)

was used for the next step without further purification.

Step 2: Distilled thionyl chloride ($SOCl_2$) (1.1mL, 15 mmol) was added dropwise to a stirred solution of compound **I** (1.77 g, 10 mmol) in anhydrous methanol (15 mL) in an ice-bath under a nitrogen atmosphere. The reaction mixture was heated in a preheated oil bath (80 ℃) for 6 h. The solvent was evaporated under reduced pressure to give the crude residue, which was partitioned into dichloromethane (50 mL) and a saturated aqueous solution of $NaHCO_3$ (30 mL). The organic phase was separated and dried over anhydrous Na_2SO_4, filtered and concentrated. The crude residue was subjected to flash column chromatography (305 mm x 32 mm i.d.) on silica gel (200-300 m) with 1% NEt_3 in petroleum ether/dichloromethane (1:5, v/v) as the eluent to give (*S*)-methyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**II**) in 81% yield.

Step 3: To a stirred solution of compound **II** (1.11 g, 5.8 mmol) in *N*,*N*-dimethylformamide (DMF) (5 mL) and xylene (20 mL) was added Pd/C (0.9 g), and the reaction mixture was immersed in a preheated oil bath (150 ℃) and refluxed until full conversion was achieved as judged by TLC. The heterogeneous system was filtered and concentrated under vacuum. The crude residue was purified by flash chromatography (305 mm x 32 mm i.d.) on silica gel with 1% NEt_3 in petroleum ether/dichloromethane = 1:5 (v/v) as the eluent to provide methyl isoquinoline-3-carboxylate (**III**) in 68% yield.

Step 4: To a stirred solution of compound **III** (0.374 g, 2 mmol) in methanol (2 mL) was added 2 M aqueous NaOH solution (2 mL), and the mixture was immersed in a preheated oil-bath (100 ℃) and refluxed until full hydrolysis was reached as detected

by TLC. The volatile solvent was removed through rotary evaporation and the system was adjusted to pH 6 by the addition of 0.5 N aqueous HCl. The resulting inorganic phase was extracted with dichloromethane (15 mL × 3), and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated to give crude isoquinoline-3-carboxylic acid (**IV**) in 82% yield, which was directly used in the next step.

Step 5: The synthesis of (*R*)-N-(2-(4-methyl-4,5-dihydrooxazol-2-yl)phenyl)-isoquinoline-3-carboxamide (**1o**, 73%) was conducted according to Method 1 as described for **1b**.

Data for compound **1o**: yield 73 %; pale yellow solid; m.p. 105.9-107.1 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (d, J = 6.5 Hz, 3H, CH_3), 3.96 (dd, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 1H, OCH_2), 4.55 (dd, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 1H, OCH_2), 4.64 (m, 1H, N-CH), 7.15 (m, 1H, H in phenyl ring), 7.55 (m, 1H, H in phenyl ring), 7.72 (m, 1H, H in phenyl ring), 7.77 (m, 1H, H in phenyl ring), 7.90 (dd, J_1 = 7.84 Hz, J_2 = 1.72 Hz, 1H, H in phenyl ring), 8.02 (d, J = 7.6 Hz, 1H, H in phenyl ring), 8.07 (d, J = 8.0 Hz, 1H, H in phenyl ring), 8.72 (s, 1H, H in pyridine ring), 9.09 (dd, J_1 = 8.5 Hz, J_2 = 1.2 Hz, 1H, H in phenyl ring), 9.30 (s, 1H, H in pyridine ring), 13.93 (s, br, 1H, NH). Elemental anal. calcd for $C_{20}H_{17}N_3O_2$: C, 72.49; H, 5.17; N, 12.68; Found: C, 72.53; H, 5.21; N, 12.71. ESI-MS calcd for $C_{20}H_{18}N_3O_2$ $[M+H]^+$: 332.14, found: 332.23.

(*S*)-N-(2-(4-methyl-4,5-dihydrooxazol-2-yl)phenyl)isoquinoline-3-carboxamide (**1p**) was successfully prepared in 68% yield.

Synthesis of Amides from *β*-Carboline-3-Carboxylic Acid (Method 4). The synthesis of **1x** is presented as an example (Figure 6).

279 *Step 1*: To a mixture of L-tryptophan (10.2 g, 50 mmol) and acetaldehyde (8.5 mL,
280 150 mmol) in water (80 mL) was added 0.5 M H_2SO_4 (1.5 mL), and the mixture was
281 stirred at room temperature. The precipitate was isolated by filtration, washed with cold
282 water and dried under vacuum. The crude *β*-carboline acid, **V** (82% yield) was directly
283 used in the next step without further purification.

284 *Step 2*: (3*S*)-Methyl 1-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-
285 carboxylate (**VI**) was prepared in 89% yield from precursor **V** following Method 3.

286 *Step 3*: To the stirred solution of compound **VI** (1.8 g, 7.4 mmol) in DMF (20 mL)
287 was added potassium permanganate ($KMnO_4$) (1.58 g, 10 mmol) in portions while the
288 mixture was in an ice bath. The reaction mixture was stirred vigorously and allowed to
289 gradually warm to room temperature until the full consumption of the starting material
290 was detected by TLC. The heterogeneous mixture was filtered and rinsed with methanol.
291 The combined organic phase was concentrated under vacuum. The crude residue was
292 subjected to column chromatography (305 mm x 32 mm i.d.) with 1% NEt_3 in
293 petroleum ether/dichloromethane (1:8, v/v) as the eluent to provide methyl 1-methyl-
294 9*H*-pyrido[3,4-b]indole-3-carboxylate (**VII**) in 62% yield.

295 *Step 4*: Compound **VII** was hydrolyzed under basic conditions to produce acid
296 **VIII**, which was then condensed with (*R*)-2-(4-methyl-4,5-dihydrooxazol-2-yl)aniline
297 or (*S*)-2-(4-methyl-4,5-dihydrooxazol-2-yl)aniline according to Method 3, to give
298 desired amides **1w** and **1x**, respectively.

299 Data for **1x**: (*S*)-1-methyl-*N*-(2-(4-methyl-4,5-dihydrooxazol-2-yl)phenyl)-9H-
300 pyrido[3,4-b]indole-3-carboxamide; yield 63%; yellow solid; m.p. 288.3-288.9°C; ^{1}H

NMR (400 MHz, d_6-DMSO) δ 1.45 (d, J = 6.4 Hz, 3H, CH_3), 2.94 (s, 3H, pyridine-CH_3), 3.99 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, OCH_2), 4.56 (dd, J_1 = 9.3 Hz, J_2 = 7.5 Hz, 1H, OCH_2), 4.63 (m, 1H, N-CH), 7.18 (dd, J_1 = 7.8 Hz, J_2 = 7.3 Hz, 1H, H in phenyl ring), 7.31 (dd, J_1 = 7.4 Hz, J_2 = 7.3 Hz, 1H, H in phenyl ring), 7.56-7.63 (m, 2H, H in phenyl ring), 7.66 (d, J = 8.1 Hz, 1H, H in phenyl ring), 7.88 (dd, J_1 = 7.8 Hz, J_2 = 1.6 Hz, 1H, H in phenyl ring), 8.39 (d, J = 7.8 Hz, 1H, H in phenyl ring), 8.84 (s, 1H, H in pyridine), 9.04 (d, J = 8.3 Hz, 1H, H in phenyl ring), 12.06 (s, 1H, NH), 13.77 (s, 1H, NH). Elemental anal. calcd for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found: C, 71.89; H, 5.21; N, 14.61. ESI-MS calcd for $C_{23}H_{21}N_4O_2$ $[M+H]^+$: 385.17, found:385.24.

In vitro and in vivo antifungal activity. All the plant pathogens were provided by the Department of Pesticide, College of Plant Protection, Nanjing Agricultural University (Nanjing, China). The in *vitro* antifungal activities of the target compounds were tested using the mycelium growth rate test as in our previous report.[9] The in *vivo* antifungal activities of the prepared compounds against *Botrytis cinerea* and *Sclerotinia sclerotiorum* were determined on strawberry fruits[9] of and oilseed rape leaves,[15] respectively. The statistical analyses were performed by SPSS software (SPSS Statistic 22.0).

Molecular Docking. The molecular docking studies of all the synthesized compounds were performed with the assistance of Tripos SYBYL X 2.0 software. The crystal structure of succinate dehydrogenase (SDH, respiratory complex II) was acquired from the RCSB Protein Data Bank (PDB code 2FBW). [20] The ligand carboxin (P/CBE 202) was extracted and all water molecules were eliminated from this crystal

complex. The 3D structures of the synthesized chiral amides were built in ChemBio 3D Ultra software, Version 12.0, before being imported to Tripos SYBYL-X 2.0 and optimized by the Powell method to determine lowest energy geometry. Surflex-Dock was applied for simulating and evaluating the interactions between the amides and the target protein by an empirical scoring function.[4]

RESULTS AND DISCUSSIONS

Design and Synthesis. A scaffold-hopping tactic was applied for the discovery of novel antifungal amides. As depicted in Figure 2, the optimization of the polar regions described herein can be categorized into cyclic and acyclic acids. The 2-(2-oxazolinyl)aniline core and chiral analogues were successfully synthesized following the procedure described in our previous report. [9] As shown in Figure 3, either EDCI or HBTU can be used in Steglich-type amidation. Heterocyclic acids, mandelic acid, and related mimics were selected for preparation of chiral amides in moderate to good yields. The common subunits in fungicides, including pyrazolyl carboxylic acid, pyrazine-2-carboxylic acid, indazolyl and quinolyl carboxylic acids, and mandelic acid, are commercially available.

The free NH groups of L-proline and homoproline were protected with $(Boc)_2O$ in excellent yield (> 95%) before condensation with 2-(2-oxazolinyl)aniline. Deprotection of Boc-group was efficiently conducted with superstiochiometric trifluoroacetic acid (TFA). The related chiral amides can be prepared in fair to good yields (Figure 4).

Isoquinoline-3-carboxylic acids and β-carboline-3-carboxylic acids were synthesized via Pictet-Spengler cyclization. Tiny modifications of pH were necessary to cyclize different amino acids. L-phenylalanine was suspended in excess formalin and

346 treated by refluxing concentrated HCl to furnish tetrahydro-3-isoquinoline carboxylic
347 acid (**I**, Figure 5).[21] The Pictet-Spengler cyclization could be much milder for the
348 construction of the *β*-carboline ring with *L*-tryptophan and acetaldehyde (**V**, Figure 6).
349 The ester of isoquinoline-3-carboxylic acids (**III**) was successfully prepared through
350 oxidation of the precursor **II** with Pd/C in refluxing xylene. Hydrolysis of **III** furnished
351 isoquinoline-3-carboxylic acid (**IV**) for the preparation of **1o** and **1p**. Notably, the
352 chirality of tetrahydro-*β*-carboline-3-carboxylic acid **V** can be controlled through
353 simple treatment with aqueous sulfuric acid.[22] Intermediate **VI** underwent oxidation,
354 hydrolysis, and condensation to smoothly provide desired chiral amides **1w** and **1x**.

355 **Structure and Activity Relationship (SAR).** A bioactivity-guided mixed
356 synthesis [16] was applied for the discovery of antifungal candidates against *Magnaporthe*
357 *oryzae* and *Botrytis cinerea*, the top two fungal plant pathogens based on scientific and
358 economic importance. [23] The new chiral amides of 2-(2-oxazolinyl)aniline with a range
359 of acids can be divided into five groups: (1) heterocyclic acids that are commercially
360 available and ubiquitous in SDHIs, (2) biologically important isoquinoline and *β*-
361 carboline analogues, (3) inexpensive aliphatic acids, (4) benzoic acids, and (5) mandelic
362 acid and its analogues. The initial screening indicated the amides of heterocyclic or
363 mandelic acids were promising candidates. These compounds have the potential to
364 interact with amino acid residues via H-bonds and π-π interaction, which is consistent
365 with initial design. Typical antifungal profiles are provided in Table 1 and Table 2.

366 Amides of cyclic carboxylic acids (Figure 2) were initially screened at 10 mg/L
367 against up to eight kinds of plant pathogens (Table 1). The introduction of a pyrazole

subunit (embedded in bixafen, penthiopyrad, benzovindiflupyr, sedaxane, etc.), only provided modest activities (**1a** and **1b**). This unexpected result may demonstrate the unique properties of chiral oxazolines as the replacement for "substituent" in SDHIs. The benzopyrazole counterparts of **1a** and **1b** (**1e** and **1f**) gave improved bioactivities. Neither dearomatization of the pyridine (**1i** and **1j**) nor ring-contraction (**1g** and **1h**) improved the antifungal activities. The tetrahydroisoquinoline counterparts (**1k** and **1l**, **1m** and **1n**) possessed enhanced activity against *M. oryzae*, showing that the presence of fused rings may be beneficial for the bioactivity. Notably, ring-fusing position is crucial for the antifungal effect (**1o** vs **1q**, and **1p** vs **1r**). Interestingly, further enhancement of the activities against *R. solani* and *G. graminis* was driven by the introduction of a carboline ring, which was inspired by the harmine natural products. The bioactivity against *G. graminis* is dependent on the chirality of either the oxazoline or the carboline ring. Compound **1s** was a prominent candidate against *G. graminis* (EC_{50} = 3.53 mg/L) and showed superior efficacy in *vitro* to boscalid (Figure 7).

The amides from mandelic acid demonstrated good effects against both *M. oryzae* and *B. cinerea* at 50 mg/L. Detailed and specific information is listed in Table 2. Interestingly, the chirality of both mandelic and 2-(2-oxazolinyl)aniline subunits showed pronounced effects on the biological results. An ethyl substituent in the *R*-configuration on the oxazoline ring gave amides **2d** and **3d** broad-spectrum antifungal activities. Synergistic effects against all the fungi except for *P. capsica* were detected when using (*R*)-mandelic acid. This may be attributed to the special characteristics of oomycetes. The antifungal effects of the four diastereoisomers of **2d** against *R. solani*,

390 *S. scleotiorum*, and *B. cinerea* were in the following order: (*R*,*R*)-isomer (**2d**) > (*R*,*S*)-
391 isomer (**2e**) > (*S*, *S*)-isomer (**3e**) > (*S*, *R*)-isomer (**3d**). We speculated that the chirality
392 of mandelic acid on bioactivity may be more remarkable since the (*R*,*R*)-isomer (**2d**)
393 was >10-fold more potent than the (*S*,*R*)-isomer (**3d**). Fine-tuning of the oxazoline gave
394 notable enhancements in the antifungal activities. Chiral amides **2h**, **2l**, and **2j** were
395 identified as promising candidates against *R. solani*, *B. cinerea* and *M. oryzae,* with the
396 EC_{50} values of 2.98, 2.95 and 3.01 mg/L, respectively.

397 As shown in Figure 8, the hydroxyl group in mandelic acid is crucial for the
398 antifungal activities. The inhibitory effect against *B. cinerea* and *S. scleotiorum* dropped
399 sharply or were even lost when the hydroxyl group was formally reduced to a methylene
400 group (**4a** and **4b**) or oxidized to a ketone group (**4c** and **4d**).

401 **In vivo Antifungal Activity and Discussion.** The in *vivo* antifungal efficacies of
402 promising candidates **2d** and **2l** were evaluated against *B. cinerea*, with boscalid as a
403 positive control. As shown in Figure 9, both **2d** and **2l** showed good preventative
404 efficacies against *B. cinerea*. Gratifyingly, **2d** displayed a stronger effect in *vivo* than
405 boscalid; the preventative efficacy of **2d** was up to 93.9% at 50 mg/L. The disparity
406 with the in *vitro* results may be caused by their innate physicochemical characteristics.
407 Hydrophilicity was speculated to be a key factor, and the calculated ClogP values of **2d**
408 and **2l** were 3.3506 and 4.3896, respectively. Compound **3k** possessed good
409 preventative efficacy (72.7%) against *S. scleotiorum* at 200 mg/L. Considering its
410 synthetic accessibility, cost-effectiveness, and tunability of the chirality, compound **2d**
411 demonstrates practical potential as a novel antifungal ingredient for crop protection.

Molecular Docking Study. To determine the plausible binding model and elucidate the chiral deviation thereof, the docking of **2d**, its stereoisomers (**2e**, **3d**, and **2e**) and analogue (**2l**) with the potential target protein (PDB code 2FBW) was conducted. Major hydrogen bonds between the chiral amides and amino acid residues are shown in Figure 10. Notably, the chirality of both the mandelic acid and the oxazoline ring had distinct effects on the mode and length of the hydrogen bonds. The substituents on the oxazoline showed a significant effect on the "composition" of the hydrogen bonds (**2d** vs **2l**). The sensitivity of the protein cavity to the size of the ligand is also noticeable. The presence of a sterically bulky pendant on the oxazoline ring may force the attached phenyl ring partially out of the binding pocket (**2l**). This effect could be mitigated by docking the 4-benzyl-4,5-dihydrooxazole substructure properly into the active "groove" of SDH. The order of total scores of **2d** and isomers was well correlated with their antifungal bioactivities against *B. cinerea*.

In summary, novel chiral amides were conceived and synthesized with 2-(2-oxazolinyl)aniline as a promising pharmacophore. Heterocyclic acids and mandelic acids were demonstrated as building blocks for the optimization of the polar region in the SDHIs. The chiral amides of acyclic acids have been discussed in the discovery of novel SDHIs for the first time. Intentional fine-tuning of polar and hydrophobic regions was accomplished. The resulting novel amides **1s**, **1t**, **2d**, **2h**, **2j**, **3k** and **2l** demonstrated significant *in vitro* antifungal activities. *In vivo* test confirmed our hypothesis and showed the practical potential of **2d** as a promising treatment for destructive *B. cinerea*. Molecular docking showed the binding difference between **2d** and its stereoisomers in

the interaction with the potential SDH (PDB code: 2FBW). Synthesis of new chiral amides and explorations of related biological mechanisms are underway, to discover novel antifungal ingredients with practical potentials.

AUTHOR CONTRIBUTIONS

S. Li conceived this work; L. Zhang, W. Li, and T. Xiao performed the chemical synthesis, biological tests, and molecular docking; Z. Song conducted structure detection; S. Li wrote the paper; R. Csuk checked the manuscript.

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SUPPORTING INFORMATION

X-ray single-crystal diffraction for compound **2b (**deposited in the Cambridge Structural Database with the CCDC number of 1838207**)**, physical data of the target compounds, molecular docking of compound **2d** and its diastereoisomers, and in *vivo* antifungal profiles. The Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

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FIGURE CAPTIONS

Figure 1. Overview of SDHIs and design of novel chiral antifungal ingredients.

Figure 2. New chiral amides of 2-(2-oxazolinyl)aniline with different polar moieties.

Figure 3. General synthesis of target amides.

Figure 4. Synthesis of **1h** and related amides.

Figure 5. Synthesis of amides **1o** and **1p**.

Figure 6. Synthesis of amides **1w** and **1x**.

Figure 7. Antifungal activity of cyclic amides against *G. graminsis*.

Figure 8. Importance of hydroxyl group on antifungal activities

Figure 9. In *vivo* antifungal activities of **2d** and **2l** against *B. cinerea* (1% DMSO as CK)

Figure 10. Molecular docking of **2d** and its isomers and analogue

553 # TABLE CAPTIONS

554 Table 1, Antifungal Activities of Chiral Amides from Heterocyclic Acids

555 Table 2, Antifungal Activities of Chiral Mandelic Amides in *vitro* (EC_{50}, mg/L)

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575 Table 1, Antifungal Activities of Chiral Amides from Heterocyclic Acids (inhibitory rate at 10 mg/L)

Compd.	*R. S.*	*B. C.*	*S. S.*	*A. S.*	*F. F.*	*G. G.*	*P. C.*	*M. O.*
1a	8.8	9.0	13.6	8.6	10.1	24.3	5.6	< 5.0
1b	< 5.0	7.9	8.2	6.4	10.1	30.7	< 5.0	< 5.0
1c	37.4	21.7	24.6	33.6	20.7	12.4	11.3	5.1
1d	29.2	31.8	20.3	28.2	9.0	13.9	11.3	12.8
1e	**45.9**	**44.4**	17.5	**48.2**	34.0	**61.1**	< 5.0	19.5
1f	**45.8**	**42.9**	24.1	30.9	27.1	**57.9**	< 5.0	17.7
1g	< 5.0	< 5.0	9.6	8.2	5.9	17.9	< 5.0	8.5
1h	11.9	< 5.0	9.6	9.5	5.0	23.4	< 5.0	6.0
1i	< 5.0	< 5.0	< 5.0	8.2	6.4	20.7	3.2	10.4
1j	13.9	< 5.0	< 5.0	9.1	7.4	14.3	< 5.0	5.1
1k	10.0	7.1	7.7	35.0	35.1	21.9	14.5	35.4
1l	< 5.0	< 5.0	12.7	27.3	19.1	12.4	10.5	39.6
1m	8.6	8.7	14.1	27.3	10.5	36.3	7.7	**59.1**
1n	6.3	6.7	8.5	15.5	8.5	35.5	< 5.0	**50.0**
1o	38.2	32.3	**42.9**	**41.8**	38.3	39.7	8.5	31.7
1p	37.0	35.9	37.5	32.7	34.0	27.6	14.5	36.6
1q	< 5.0	< 5.0	< 5.0	9.1	8.0	10.5	< 5.0	< 5.0
1r	< 5.0	< 5.0	5.8	6.4	9.0	10.4	< 5.0	< 5.0
1s	**53.3**	16.7	10.6	< 5.0	6.4	**72.2**	12.3	12.8
1t	**46.8**	30.4	9.9	28.2	12.8	**65.8**	12.3	19.5
1u	**55.2**	5.6	14.1	27.3	6.9	**52.1**	5.3	10.4
1v	39.2	23.6	27.8	27.3	6.9	**49.1**	< 5.0	23.2
1w	3.7	5.2	< 5.0	< 5.0	8.0	14.5	< 5.0	10.0
1x	21.9	< 5.0	< 5.0	5.4	6.4	12.4	< 5.0	8.5

576 Data are given as the mean of triplicate experiments, promising candidates with inhibitory rate > 40% are showed with a green
577 background. *R.s.* : *Rhizoctonia solani*, *B.c.* : *Botrytis cinerea*, *S.s.* : *Sclerotinia scleotiorum*, *A.s.* : *Alternaria solani*, *F.f.*: *Fusarium*
578 *fujikuroi*, *G.g.* : *Gaeumanomyces graminis*, *P.c.* : *Phytophthora capsici*, *M.o.* : *Magnaporthe oryzae*

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589 Table 2, Antifungal Activities of Chiral Mandelic Amides in *vitro* (EC_{50}, mg/L)

Compd.	*R. solani*	*S.sclerotiorum*	*B.cinerea*	*P. capsici*	*M. oryzae*
2a	14.92	>50	>50	>50	>50
3a	>50	>50	9.21	>50	>50
2b	13.76	>50	>50	>50	>50
3b	>50	32.96	>50	>50	>50
2c	5.32	>50	>50	>50	18.85
3c	8.74	40.71	>50	17.25	>50
2d	5.76	12.41	**3.83**	15.52	10.54
3d	15.76	42.56	>50	13.31	16.62
2e	8.94	16.97	9.73	9.76	18.93
3e	9.98	18.74	12.73	9.93	>50
2f	8.12	>50	7.32	>50	>50
3f	7.76	15.59	8.09	12.27	>50
2g	7.23	>50	13.35	>50	>50
3g	8.14	>50	5.38	19.97	>50
2h	**2.98**	>50	>50	>50	>50
3h	9.76	>50	>50	>50	>50
2i	>50	>50	>50	>50	>50
3i	>50	>50	>50	18.27	>50
2j	6.47	>50	6.57	>50	**3.01**
3j	9.37	13.75	5.98	16.52	9.73
2k	7.71	>50	>50	>50	5.75
3k	7.98	**3.95**	>50	10.33	12.98
2l	>50	9.07	**2.95**	>50	5.56
3l	7.23	31.76	>50	>50	15.52
2m	>50	35.58	>50	>50	6.91
3m	6.77	>50	>50	>50	14.38
Boscalid	1.59	0.29	1.53	2.53	1.12

590 Data are given as the means of triplicate experiments, promising candidates with EC_{50} values < 5 mg/L are highlighted in bold red
591 with a green background.

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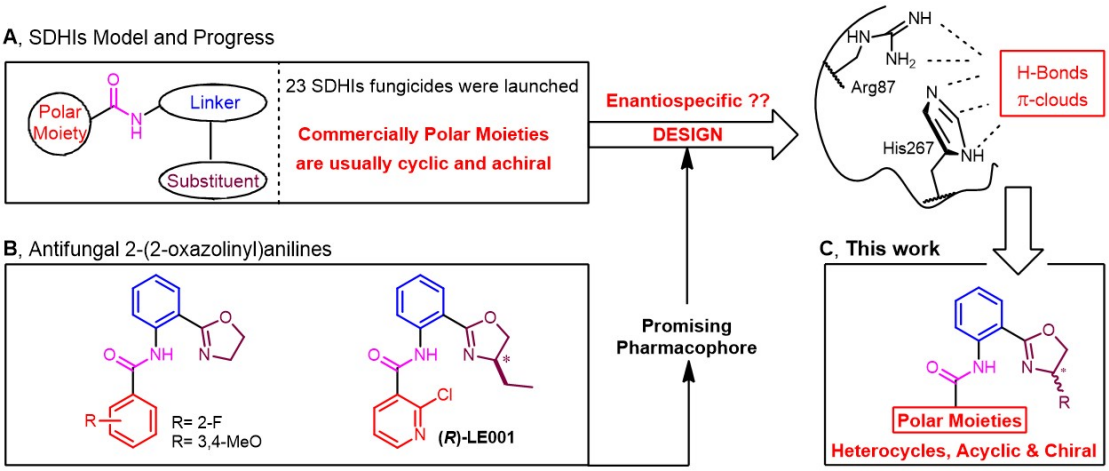

A, SDHIs Model and Progress
Polar Moiety
Linker
Substituent
23 SDHIs fungicides were launched
Commercially Polar Moieties are usually cyclic and achiral
Enantiospecific ??
DESIGN
B, Antifungal 2-(2-oxazolinyl)anilines
R= 2-F
R= 3,4-MeO
(R)-LE001
Promising Pharmacophore
C, This work
Arg87
His267
H-Bonds
π-clouds
Polar Moieties
Heterocycles, Acyclic & Chiral

Figure 1.

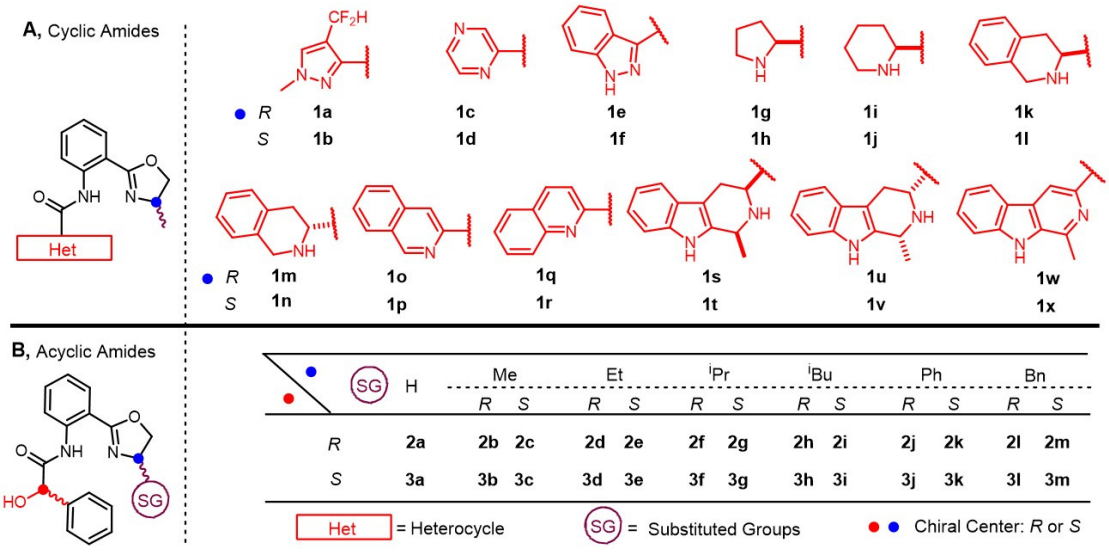

A, Cyclic Amides
Het
R 1a 1c 1e 1g 1i 1k
S 1b 1d 1f 1h 1j 1l
R 1m 1o 1q 1s 1u 1w
S 1n 1p 1r 1t 1v 1x
B, Acyclic Amides
SG
H Me Et iPr iBu Ph Bn
R S R S R S R S R S R S
R 2a 2b 2c 2d 2e 2f 2g 2h 2i 2j 2k 2l 2m
S 3a 3b 3c 3d 3e 3f 3g 3h 3i 3j 3k 3l 3m
Het = Heterocycle
SG = Substituted Groups
Chiral Center: R or S

Figure 2.

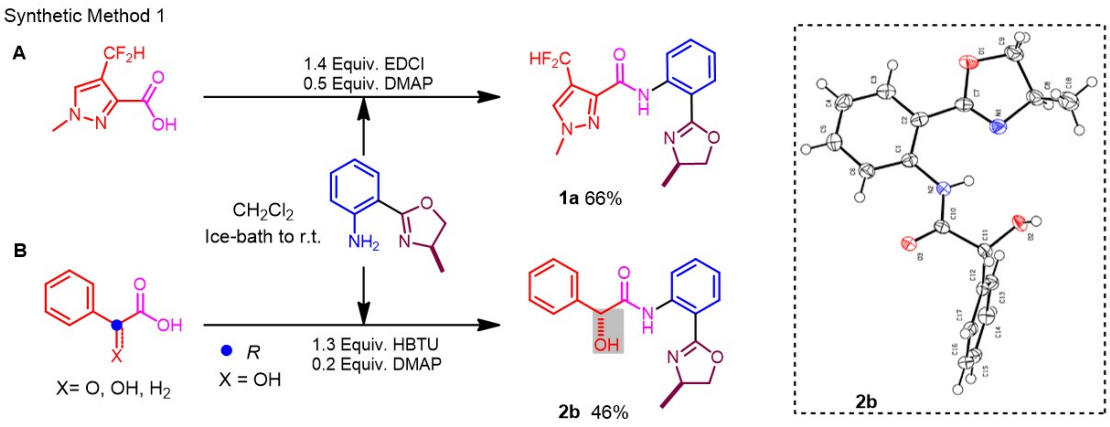
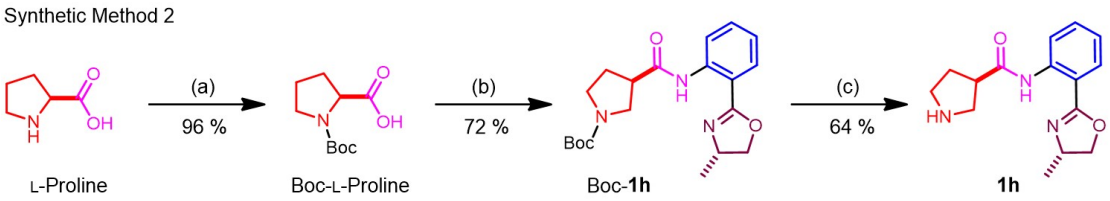

Synthetic Method 1
A
B
1.4 Equiv. EDCI
0.5 Equiv. DMAP
CH2Cl2
Ice-bath to r.t.
1.3 Equiv. HBTU
0.2 Equiv. DMAP
R
X = OH
X= O, OH, H2
1a 66%
2b 46%
2b

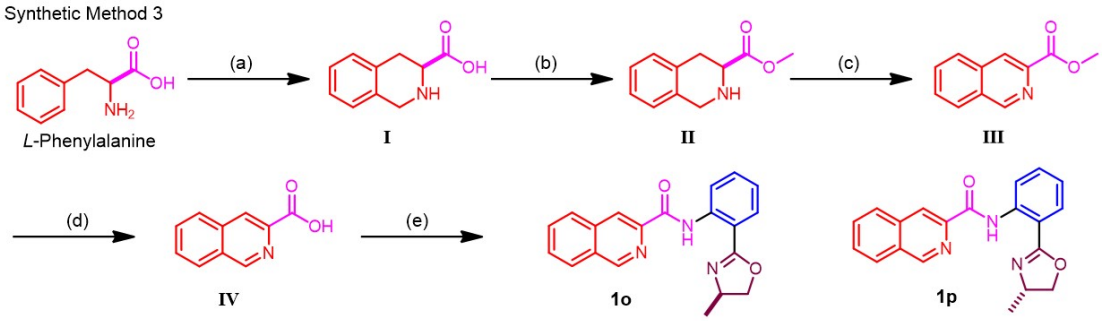
Figure 3.



Similar Procedure: **1g**, 70%; **1i**, 65%; **1j**, 60%; **1k**, 63%;**1l**, 64%;**1m**, 70%; **1n**, 65%; **1s**, 54%; **1t**, 53%; **1u**, 65%; **1v**, 63%

Reagents and conditions: (a) 1.1 Equiv.$(Boc)_2O$, 2.0 Equiv. Na_2CO_3 ,THF/H_2O, r.t.; (b) 1.4 Equiv. EDCI, 0.5 Equiv. DMAP, CH_2Cl_2, Ice-bath to r.t.; (c) TFA,CH_2Cl_2, Ice-bath to r.t.

Figure 4.



Reagents and conditions: (a) 37% HCHO, Conc. HCl, 95 ^{o}C; (b) $SOCl_2$, MeOH; (c) Pd/C, DMF, Xylene, reflux; (d) 2M NaOH, MeOH/H_2O, reflux; (e) 1.4 Equiv. EDCI, 0.5 Equiv. DMAP,Chiral amine, CH_2Cl_2, Ice-bath to r.t.;

Figure 5.

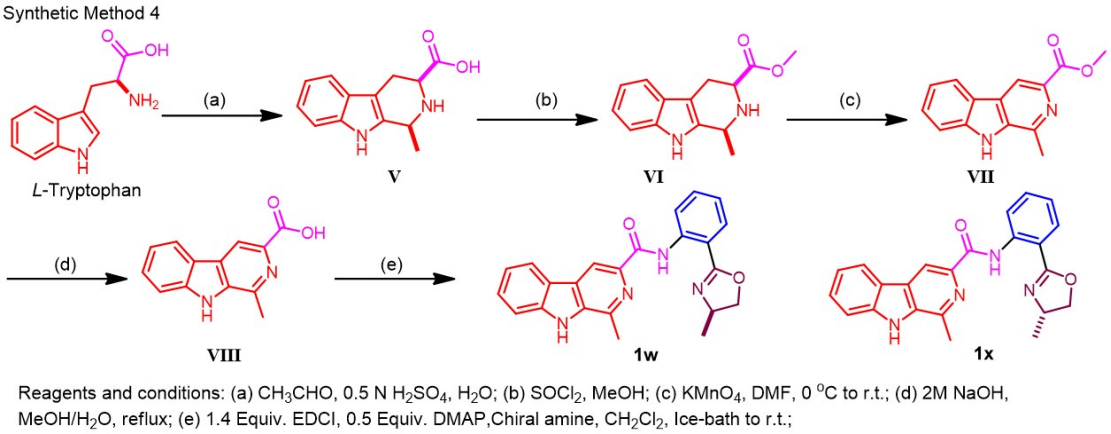

Synthetic Method 4
L-Tryptophan
(a)
V
(b)
VI
(c)
VII
(d)
VIII
(e)
1w
1x
Reagents and conditions: (a) CH3CHO, 0.5 N H2SO4, H2O; (b) SOCl2, MeOH; (c) KMnO4, DMF, 0 °C to r.t.; (d) 2M NaOH, MeOH/H2O, reflux; (e) 1.4 Equiv. EDCI, 0.5 Equiv. DMAP,Chiral amine, CH2Cl2, Ice-bath to r.t.;

Figure 6.

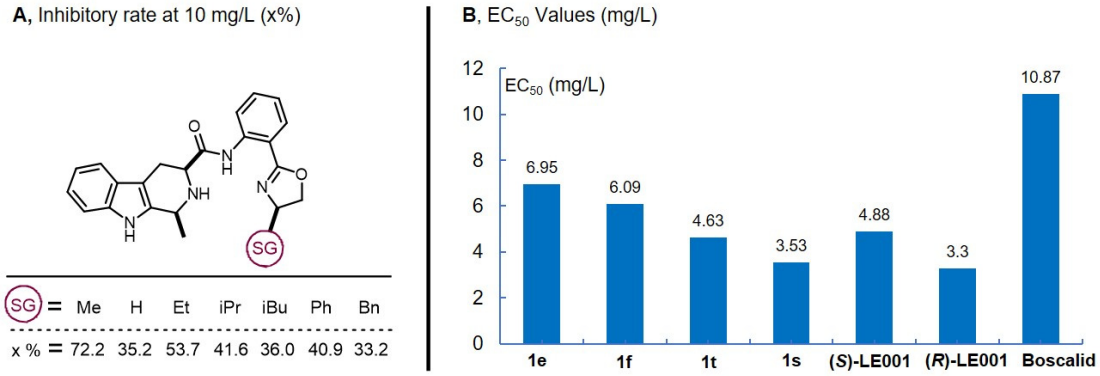
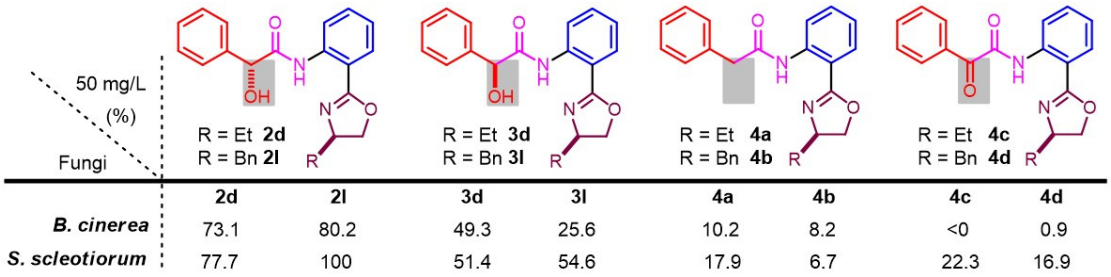
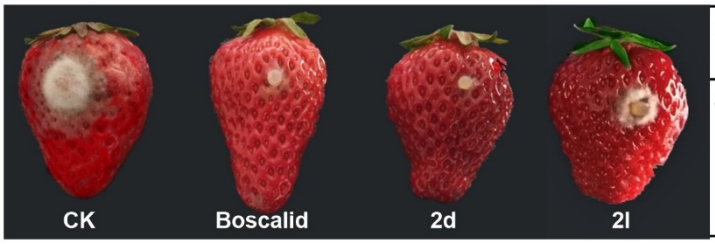

A, Inhibitory rate at 10 mg/L (x%)
SG = Me H Et iPr iBu Ph Bn
x % = 72.2 35.2 53.7 41.6 36.0 40.9 33.2
B, EC50 Values (mg/L)
EC50 (mg/L)
12
10
8
6
4
2
0
6.95
6.09
4.63
3.53
4.88
3.3
10.87
1e
1f
1t
1s
(S)-LE001
(R)-LE001
Boscalid

Figure 7.



Fungi	2d	2l	3d	3l	4a	4b	4c	4d
B. cinerea	73.1	80.2	49.3	25.6	10.2	8.2	<0	0.9
S. scleotiorum	77.7	100	51.4	54.6	17.9	6.7	22.3	16.9

Figure 8.



Compound (50 mg/L)	Preventative Efficacy (%)
CK (1% DMSO)	-
Boscalid	90.6
2d	93.9
2l	73.5

Figure 9.

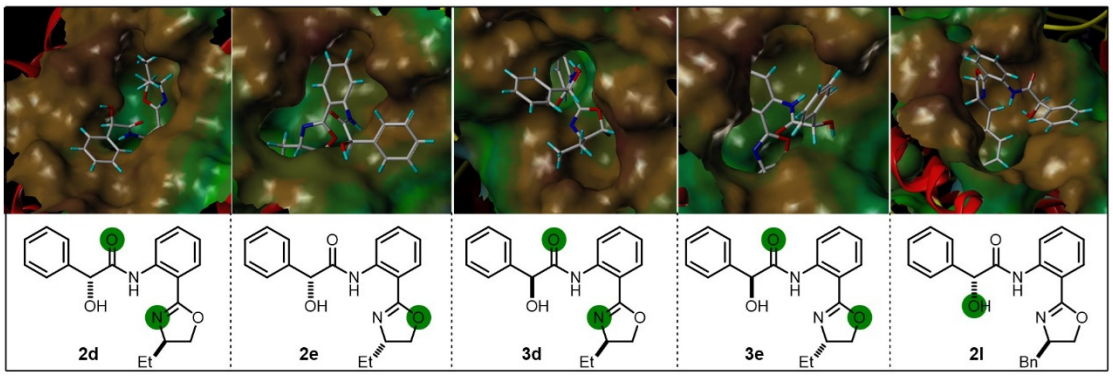


Figure 10.

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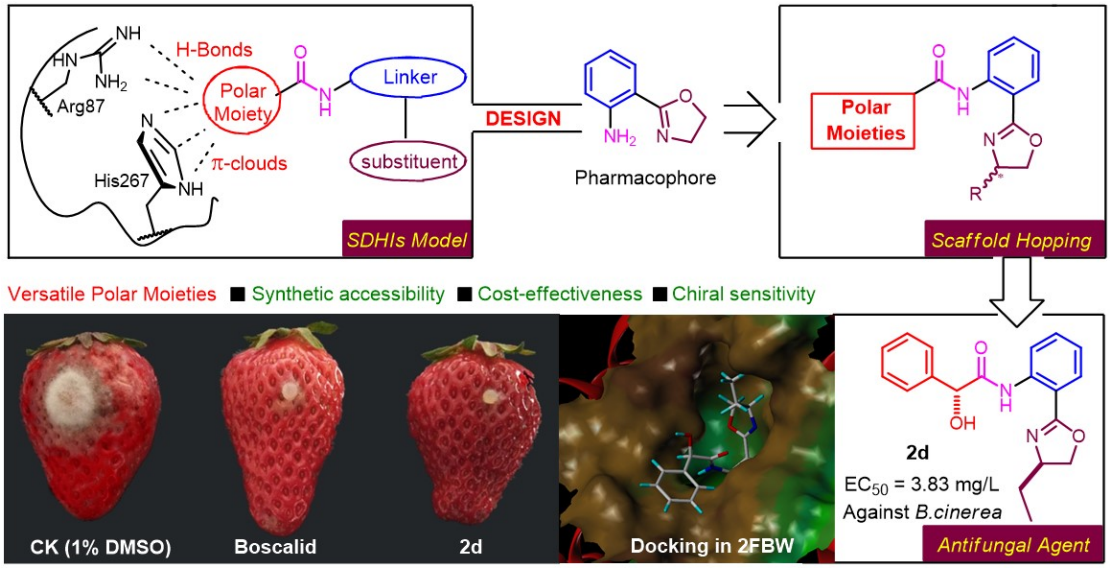

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