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J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.9b05751 • Publication Date (Web): 07 Nov 2019

Downloaded from pubs.acs.org on November 8, 2019

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Synthesis and Biological Activity of Novel Succinate Dehydrogenase Inhibitor Derivatives as Potent Fungicide Candidates

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Abstract

In searching for novel fungicidal leads, the novel bioactive succinate dehydrogenase inhibitor (SDHI) derivatives were designed and synthesized by the inversion of carbonyl and amide groups. Bioassay indicated that compound **5i** was outstood with broad-spectrum of in vitro activity against five fungi, its EC_{50} value (0.73 μg/mL) was comparable to boscalid (EC_{50} of 0.51 μg/mL) and fluxapyroxad (EC_{50} of 0.19 μg/mL) against *Sclerotinia sclerotiorum.* For *Rhizoctonia cerealis*, **5i** and **5p** with the EC_{50} of 4.61 μg/mL and 6.48 μg/mL respectively, showed significant higher activity than fluxapyroxad with the EC_{50} of 16.99 μg/mL. In vivo fungicidal activity of **5i** exhibited excellent inhibitory rate (100%) against *Puccinia sorghi* at 50 μg/mL, while the positive control boscalid showed only 70% of inhibitory rate. Moreover, **5i** showed promising fungicidal activity with 60% inhibitory rate against *Rhizoctonia solani* at 1 μg/mL, which was better than boscalid (30%). Compound **5i** possessed better in vivo efficacy against *P. sorghi* and *R. solani* than boscalid. Molecular docking showed, even the carbonyl oxygen atom of **5i** was far from pyrazole ring, it could also form hydrogen bonds towards the hydroxyl hydrogen, amino hydrogen of TYR58 and TRP173 on SDH, respectively, which consisted with the positive control fluxapyroxad. Fluorescence quenching analysis and SDH enzymatic inhibition studies also validated its mode of action. Our studies showed that **5i** was worthy for further investigation as a promising fungicide candidate.

Key words：SDHIs, amide inversion, molecular docking, fungicidal activity, rice disease

Introduction

Plant diseases cause a dramatic decline in crop quality and yield, farmers suffer huge economic losses.[1, 2] Treating crops by fungicides with different mode of action to against plant disease damage is always necessary.[3] Succinate dehydrogenase (SDH) inhibitors are widely used to protect crops from all kinds of plant pathogens attack.[4-7] As an important membrane complex in the tricarboxylic acid cycle in mitochondrial respiratory chain, SDH is an ideal fungicide target.[8, 9] SDHIs disrupt the respiration of the pathogen and inhibit its growth by inhibiting the oxidation of succinate to fumarate, and thus cause the pathogen death.[10, 11]

Among the SDHIs, pyrazole amide derivatives have a central place in fungicidal chemistry because of their broad spectrum of high efficiency, low toxicity and structure diversity. As shown in **Figure 1**, furametpyr was invented by Sumitomo Chemical in 1986,[12] and then, penflufen was developed by Bayer Cropscience in 2006.[13] In recent decades, several pyrazole contained fungicides, such as sedaxane,[14] bixafen,[15] fluxapyroxad,[16] benzovindiflupyr,[17] pydiflumetofen[18] and so on, have made a significant contribution to the crop protection against plant diseases. All these representative compounds containing pyrazole moiety show outstanding fungicidal activity. The wide practical interest in this important pyrazole moiety motivates continuous efforts of the exploitation of efficient compounds.[19-21]

In research for some commercially available aromatic amide fungicides, we found mebenil and benodanil, which could be seen as a couple of mirror compounds.[22] Many documents reported the lead modification of SDHIs, but the design strategy by inversion of carbonyl and amide groups was rarely used in the studies of novel pyrazole fungicide development. On the basis of the development of fungicides targeted at SDH and prompted by the excellent biological activity of pyrazole carboxamide fungicides, we attempted to investigate

the structure of pyrazole-contained derivatives, and target compounds were designed and synthesized by inversion of carbonyl and amide groups of fluxapyroxad **(Figure 2**). All of the target compounds were identified by ^{1}H NMR, elemental analysis and HRMS. The in vitro fungicidal activities of these compounds were evaluated against eight common plant pathogenic fungi and in vivo fungicidal activities of these compounds were tested against three different crop diseases. Furthermore, the enzymatic activity and fluorescence quenching analysis of **5i** to SDH were investigated to validate its mode of action.

MATERIALS AND METHODS

Equipment and Materials

Melting points were determined using an X-4 melting point apparatus and were uncorrected (Beijing Tech Instruments Co., Beijing, China). ^{1}H NMR spectra was obtained using a Bruker Avance 400 MHZ spectrometer at 400 MHz in deuterochloroform ($CDCl_3$) and tetramethylsilane (TMS) as internal standards (Bruker, Switzerland). Elemental analyses were performed on a Vario EL III elemental analysis instrument (Elementar, Germany). High resolution mass spectrometry data were obtained with an Agilent 6520 Q-TOF LC/MS instrument equipped with electrospray ionization (ESI) source (Agilent, America). Crystal structure was recorded by a Rigaku 007 Saturn 70 diffraction meter (Rigaku MSC, Japan). All solvents were of analytical grade. All of the yields were not optimized. The synthesis procedure of intermediate **4** and all the corresponding data could be found in the Supplementary Information.

Molecular Docking. The molecular structures were drawn by ChemBioDraw Ultra 14.0 and energetically minimized by using Chemdraw 3D Pro MM2 minimized procedure, then saved

as mol2 file. The structure of SDH from *Gallus gallus* (pdb code: 2FBW)[23] was downloaded from RCSB PDB database and the docking program was performed with SYBYL-X 6.91. The detail docking procedure was conducted·according to the reported literature.[24]

Synthetic Procedures

General Synthesis Procedure for Intermediate (**1**). The Methyl 2-iodobenzoate (3.6mmol), substituted phenylboronic acid (4.5mmol) and dichloro-bis(triphenyl-phosphine)palladium(II) (5%mmol) were added to a three-neck round-bottom flask equipped with a magnetic stir bar and a reflux condenser. Under an atmosphere of argon, 15 mL of THF was injected to the mixture by syringe, and then 1mol/L Na_2CO_3 was added in one portion. The flask was put into an oil bath and heated to 65 °C for 12h. After completion, the flask was cooled to room temperature; 30ml water was added and then extracted with CH_2Cl_2 (3×15 mL). The organic layer was combined and dried over anhydrous Na_2SO_4. Solvent was removed under the reduced pressure. The residue was subjected to silica gel chromatography using PE and EtOAc (50:1, v/v) as an eluent to afford intermediate (**1**) and used for the next step without further purification.

General Synthesis Procedure for Intermediate (**2**).

A solution of **1** (2.8mmol) dissolving in 10 mL THF was added in NaOH (28mmol, in 10mL H_2O). The mixture was heated to 80℃ and monitored by TLC until the reaction was complete. Solvent was removed under the reduced pressure. The residue was acidified by 2mol/L HCl and precipitation was appeared. The precipitated solid was filtered out and recrystallized in CH_2Cl_2 and hexane to give pure products in an 88-99% yield.

Data for 3',4',5'-trifluoro-[1,1'-biphenyl]-2-carboxylic acid (**2a**). Yield 95%; white solid; mp 155-156 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.80 (s, 1H), 8.03 (dd, *J*=7.8, 1.4 Hz, 1H), 7.60 (td, *J* =7.5, 1.4 Hz, 1H), 7.49 (td, *J* =7.7, 1.3 Hz, 1H), 7.30 (dd, *J* =7.6, 1.3 Hz, 1H), 6.99 – 6.87 (m, 2H).

Data for 3',4'-dichloro-[1,1'-biphenyl]-2-carboxylic acid (**2b**). Yield 96%; white solid; mp 173-174 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.59 (s, 1H), 7.97 (d, *J* =7.8 Hz, 1H), 7.60 (t, *J* =7.5 Hz, 1H), 7.47 (t, *J* =7.6 Hz, 1H), 7.41 (d, *J* =8.4 Hz, 2H), 7.32 (d, *J* =7.6 Hz, 1H), 7.13 (dd, *J* =8.2, 2.0 Hz, 1H).

Data for 4'-chloro-[1,1'-biphenyl]-2-carboxylic acid (**2c**). Yield 99%; white solid; mp 170-171°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.92 (s, 1H), 7.98 (dd, *J* =7.8, 1.4 Hz, 1H), 7.57 (td, *J* =7.6, 1.4 Hz, 1H), 7.44 (td, *J* =7.6, 1.3 Hz, 1H), 7.39 – 7.30 (m, 3H), 7.29 – 7.22 (m, 2H).

Data for 4'-(tert-butyl)-5-fluoro-[1,1'-biphenyl]-2-carboxylic acid (**2d**). Yield 88%; white solid; mp 149-150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (dd, *J* =8.4, 5.9 Hz, 1H), 7.40 (d, *J* =8.1 Hz, 2H), 7.27 (s, 1H), 7.25 (d, *J* =1.8 Hz, 1H), 7.12 – 7.03 (m, 2H), 1.35 (s, 9H).

Data for 3',4',5,5'-tetrafluoro-[1,1'-biphenyl]-2-carboxylic acid (**2e**). Yield 95%; white solid; mp 186-187 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (dd, *J* =8.8, 5.7 Hz, 1H), 7.20 (ddd, *J* =8.8, 7.7, 2.6 Hz, 1H), 7.03 (dd, *J* =8.9, 2.6 Hz, 1H), 6.99 – 6.90 (m, 2H).

Data for 3',4'-dichloro-5-fluoro-[1,1'-biphenyl]-2-carboxylic acid (**2f**). Yield 94%; white solid; mp 182-183°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (td, *J* =8.3, 6.9, 3.5 Hz, 1H), 7.46 (dd, *J* =8.4, 3.5 Hz, 1H), 7.41 (q, *J* =2.2 Hz, 1H), 7.15 (dddd, *J* =10.5, 6.8, 4.8, 2.0 Hz, 2H), 7.02 (dq, *J* =9.2, 2.6 Hz, 1H).

Data for 5-fluoro-[1,1'-biphenyl]-2-carboxylic acid (**2g**). Yield 98%; white solid; mp 111-112°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ11.34 (s, 1H), 7.99 (dd, *J* =8.6, 5.8 Hz, 1H), 7.38 (dd, *J* =5.5, 1.8 Hz, 3H), 7.30 (dt, *J* =6.7, 1.6 Hz, 2H), 7.14 – 7.02 (m, 2H).

Data for 4'-chloro-5-fluoro-[1,1'-biphenyl]-2-carboxylic acid (**2h**). Yield 93%; white solid; mp 150-151 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.87 (s, 1H), 8.06 (dd, *J* =8.7, 5.8 Hz, 1H), 7.42 – 7.36 (m, 2H), 7.28 – 7.22 (m, 2H), 7.15 (ddd, *J* =8.8, 7.8, 2.6 Hz, 1H), 7.04 (dd, *J* =9.2, 2.6 Hz, 1H).

General Synthesis Procedure for Intermediate (**3**).

Compound **2** (3 mmol) and CH_2Cl_2 (30 mL) was added to a 100 mL single-neck round bottom flask and stirred in an ice bath. To the mixture, 2 drops of DMF was added and 6 mmol of oxalyl chloride solution was added dropwise. The mixture was slowly rise to room temperature, after stirring at room temperature for 2 h, the mixture was concentrated under the reduced pressure to remove excess oxalyl chloride solution and solvent to afford intermediate (**3**) and used for the next step without further purification.

General Synthesis Procedure for target compound (**5**).

In a 100 mL one-neck round bottom flask, 3 mL of compound **3** was added and dissolved in 30 mL of dry CH_2Cl_2. After the addition of 6 mmol of triethylamine, the mixture was stirred for 10 min. in an ice bath. Compound **4** (3 mmol) was dissolved in 5 mL of dry CH_2Cl_2 and added dropwise to the reaction system, then the mixture was stirred for 30 min in an ice bath, and stirred at room temperature for 4 h. After the reaction was completed, 15 mL water was added to the reaction solution and extracted with CH_2Cl_2 (3×15 mL). The organic layer was combined and washed with brine, dried over anhydrous sodium sulfate, filtered. The solvent was removed

under reduced pressure. The residue was subjected to silica gel chromatography using PE and EtOAc (10:1-2:1, v/v) as an eluent to afford the target compounds **5a**-**5u** (46-90%).

Data for N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-4'-(trifluoromethyl)-[1,1'-biphenyl]-2-carboxamide (**5a**). Yield 49%; white solid; mp 153-154 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.81 (dd, *J* =7.6, 1.5 Hz, 1H), 7.67 (d, *J* =8.1 Hz, 2H), 7.60 (td, *J* =7.5, 1.5 Hz, 1H), 7.56 – 7.49 (m, 3H), 7.43 (dd, *J* =7.6, 1.4 Hz, 1H), 7.11 (s, 1H), 3.90 (s, 3H). Elemental anal. calcd for $C_{19}H_{13}F_6N_3O$: C, 55.21; H, 3.17; N, 10.17. Found: C, 54.98; H, 3.52; N, 9.92. HRMS (ESI) m/z calcd for $C_{19}H_{14}F_6N_3O$ $(M+H)^+$ 414.1036, found 414.1035.

Data for N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-4'-(trifluoromethyl)-[1,1'-biphenyl]-2-carboxamide (**5b**). Yield 61%; white solid; mp 132-133°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.80 (dd, *J*=7.6, 1.5 Hz, 1H), 7.66 (d, *J*=8.1 Hz, 2H), 7.59 (td, *J*=7.5, 1.5 Hz, 1H), 7.55 – 7.47 (m, 3H), 7.42 (dd, *J*=7.5, 1.4 Hz, 1H), 7.30 (s, 1H), 6.48 (t, *J*=54.6 Hz, 1H), 3.86 (s, 3H). Elemental anal. calcd for $C_{19}H_{14}F_5N_3O$: C, 57.73; H, 3.57; N, 10.63. Found: C, 57.09; H, 3.26; N, 10.63. HRMS (ESI) m/z calcd for $C_{19}H_{15}F_5N_3O$+ $(M+H)^+$ 396.1130, found 396.1131.

Data for 4'-methyl-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5c**). Yield 68%; white solid; mp 115-116°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.82 (dd, *J*=7.7, 1.5 Hz, 1H), 7.51 (td, *J*=7.5, 1.5 Hz, 1H), 7.43 (td, *J*=7.5, 1.4 Hz, 1H), 7.38 (dd, *J*=7.6, 1.4 Hz, 1H), 7.28 – 7.22 (m, 2H), 7.18 (d, *J*=7.9 Hz, 2H), 7.08 (s, 1H), 3.86 (s, 3H), 2.33 (s, 3H). Elemental anal. calcd for $C_{19}H_{16}F_3N_3O$: C, 63.51; H, 4.49; N, 11.69. Found: C, 63.06; H, 4.64; N, 11.56. HRMS (ESI) m/z calcd for $C_{19}H_{17}F_3N_3O$ $(M+H)^+$ 360.1318, found 360.1322.

Data for N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-4'-methyl-[1,1'-biphenyl]-2-carboxamide (**5d**). Yield 79%; white solid; mp 140-141 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.82 (dd, *J*=7.7, 1.5 Hz, 1H), 7.53 (td, *J*=7.5, 1.5 Hz, 1H), 7.49 – 7.36 (m, 2H), 7.34 – 7.25 (m, 3H), 7.21 (d, *J*=7.9 Hz, 2H), 6.39 (t, *J*=54.7 Hz, 1H), 3.85 (s, 3H), 2.36 (s, 3H). Elemental anal. calcd for $C_{19}H_{17}F_2N_3O$: C, 66.85; H, 5.02; N, 12.31. Found: C, 66.93; H, 5.43; N, 12.23. HRMS (ESI) m/z calcd for $C_{19}H_{18}F_2N_3O$ $(M+H)^+$ 342.1412, found 342.1414.

Data for 3',4',5'-trifluoro-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5e**). Yield 73%; white solid; mp 150-151 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.73 (dd, *J*=7.6, 1.5 Hz, 1H), 7.55 (dtd, *J*=22.9, 7.5, 1.5 Hz, 2H), 7.37 (dd, *J*=7.5, 1.4 Hz, 1H), 7.22 (s, 1H), 7.09 – 6.96 (m, 2H), 3.92 (s, 3H). Elemental anal. calcd for $C_{18}H_{11}F_6N_3O$: C, 54.14; H, 2.78; N, 10.52. Found: C, 54.20; H, 2.96; N, 10.35. HRMS (ESI) m/z calcd for $C_{18}H_{12}F_6N_3O$ $(M+H)^+$ 400.0879, found 400.0882.

Data for N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-3',4',5'-trifluoro-[1,1'-biphenyl]-2-carboxamide (**5f**). Yield 88%; white solid; mp 142-143°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.76 (dd, *J* =7.5, 1.6 Hz, 1H), 7.62 – 7.48 (m, 2H), 7.42 (s, 1H), 7.38 (dd, *J* =7.5, 1.4 Hz, 1H), 7.11 – 6.99 (m, 2H), 6.61 (t, *J* =54.5 Hz, 1H), 3.89 (s, 3H). Elemental anal. calcd for $C_{18}H_{12}F_5N_3O$: C, 56.70; H, 3.17; N, 11.02. Found: C, 56.52; H, 3.27; N, 11.02. HRMS (ESI) m/z calcd for $C_{18}H_{13}F_5N_3O$ $(M+H)^+$ 382.0973, found 382.0975.

Data for N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5g**). Yield 90%; white solid; mp 87-88 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.84 (dd, *J*=7.7, 1.4 Hz, 1H), 7.57 (td, *J*=7.5, 1.5 Hz, 1H), 7.49 (td, *J*=7.5, 1.4 Hz, 1H), 7.46 – 7.34 (m, 6H), 7.27 (s, 1H), 6.41 (t, *J*=54.8 Hz, 1H), 3.86 (s, 3H). Elemental anal. calcd for $C_{18}H_{15}F_2N_3O$:

C, 66.05; H, 4.62; N, 12.84. Found: C, 65.68; H, 4.63; N, 12.86. HRMS (ESI) m/z calcd for $C_{18}H_{16}F_2N_3O$ $(M+H)^+$ 328.1256, found 328.1258.

Data for 3',4'-dichloro-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5h**). Yield 66%; white solid; mp 117-118 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.77 (dd, *J*=7.7, 1.4 Hz, 1H), 7.65 – 7.37 (m, 5H), 7.28 – 7.14 (m, 2H), 3.93 (s, 3H). Elemental anal. calcd for $C_{18}H_{12}Cl_2F_3N_3O$: C, 52.20; H, 2.92; N, 10.14.Found: C,51.90; H,2.78; N,10.21. HRMS (ESI) m/z calcd for $C_{18}H_{13}Cl_2F_3N_3O$ $(M+H)^+$ 414.0382, found 414.0381.

Data for 3',4'-dichloro-N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5i**). Yield 72%; white solid; mp 100-101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.76 (dd, *J*=7.6, 1.4 Hz, 1H), 7.60 – 7.47 (m, 3H), 7.44 (d, *J*=8.3 Hz, 1H), 7.41 – 7.34 (m, 2H), 7.21 (dd, *J*=8.3, 2.1 Hz, 1H), 6.56 (t, *J*=54.6 Hz, 1H), 3.86 (s, 3H). Elemental anal. calcd for $C_{18}H_{13}Cl_2F_2N_3O$: C, 54.57; H, 3.31; N, 10.61. Found: C, 54.16; H, 3.50; N, 10.45. HRMS (ESI) m/z calcd for $C_{18}H_{14}C_{l2}F_2N_3O$ $(M+H)^+$ 396.0477, found 396.0470.

Data for 4'-chloro-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5j**). Yield 46%; white solid; mp 139-140 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.79 (dd, *J* =7.6, 1.4 Hz, 1H), 7.52 (dtd, *J* =30.2, 7.6, 1.4 Hz, 2H), 7.42 – 7.35 (m, 3H), 7.35 – 7.29 (m, 2H), 7.14 (s, 1H), 3.90 (s, 3H). Elemental anal. calcd for $C_{18}H_{13}ClF_3N_3O$: C, 56.93; H, 3.45; N, 11.06. Found: C, 56.83; H, 3.95; N, 10.82. HRMS (ESI) m/z calcd for $C_{18}H_{14}ClF_3N_3O$ $(M+H)^+$ 380.0772, found 380.0772.

Data for 4'-chloro-N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5k**). Yield 82%; white solid; mp 128-129°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.77 (dd, *J*=7.6, 1.5 Hz, 1H), 7.51 (dtd, *J*=28.9, 7.5, 1.4 Hz, 2H), 7.41 – 7.29 (m, 6H), 6.50 (t, *J*=54.6 Hz, 1H), 3.85 (s, 3H). Elemental anal. calcd for $C_{18}H_{14}ClF_2N_3O$: C, 59.76; H,

3.90; N, 11.62. Found: C, 59.28; H, 4.02; N, 11.48. HRMS (ESI) m/z calcd for $C_{18}H_{15}ClF_2N_3O$ $(M+H)^+$ 362.0866, found 362.0872.

Data for 5-fluoro-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5l**). Yield 61%; white solid; mp 82-83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.86 (dd, *J* =8.6, 5.8 Hz, 1H), 7.40 (qq, *J* =7.1, 2.6, 1.8 Hz, 5H), 7.20 – 7.00 (m, 3H), 3.88 (s, 3H). Elemental anal. calcd for $C_{18}H_{13}F_4N_3O$: C, 59.51; H, 3.61; N, 11.57. Found: C, 59.47; H, 4.03; N, 11.60. HRMS (ESI) m/z calcd for $C_{18}H_{14}F_4N_3O$ $(M+H)^+$ 364.1068, found 364.1070.

Data for N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-5-fluoro-[1,1'-biphenyl]-2-carboxamide (**5m**). Yield 66%; white solid; mp 95-96°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.86 (dd, *J* =8.6, 5.7 Hz, 1H), 7.52 – 7.33 (m, 5H), 7.25 (s, 1H), 7.22 – 7.10 (m, 2H), 6.41 (t, *J* =54.8 Hz, 1H), 3.86 (s, 3H). Elemental anal. calcd for $C_{18}H_{14}F_3N_3O$: C, 62.61; H, 4.09; N, 12.17. Found: C, 62.03; H, 3.93; N, 12.27. HRMS (ESI) m/z calcd for $C_{18}H_{15}F_3N_3O$ $(M+H)^+$ 346.1162, found 346.1164.

Data for 3',4',5,5'-tetrafluoro-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5n**). Yield 61%; white solid; mp 155-156 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.76 (dd, *J*=8.6, 5.6 Hz, 1H), 7.22 (td, *J*=8.2, 2.6 Hz, 1H), 7.16 (s, 1H), 7.08 (dd, *J*=9.0, 2.6 Hz, 1H), 7.03 (dd, *J*=7.6, 6.2 Hz, 2H), 3.92 (s, 3H). Elemental anal. calcd for $C_{18}H_{10}F_7N_3O$: C, 51.81; H, 2.42; N, 10.07. Found: C, 51.86; H, 2.60; N, 9.98. HRMS (ESI) m/z calcd for $C_{18}H_{11}F_7N_3O$ $(M+H)^+$ 418.0785, found 418.0778.

Data for N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-3',4',5,5'-tetrafluoro-[1,1'-biphenyl]-2-carboxamide (**5o**). Yield 60%; white solid; mp 146-147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.79 (dd, *J*=8.6, 5.6 Hz, 1H), 7.37 (s, 1H), 7.22 (td, *J*=8.3, 2.6 Hz, 1H), 7.07 (ddd, *J*=17.8, 8.3, 4.4 Hz, 3H), 6.61 (t, *J*=54.5 Hz, 1H), 3.89 (s, 3H). Elemental anal. calcd

for $C_{18}H_{11}F_6N_3O$: C, 54.14; H, 2.78; N, 10.52. Found: C, 54.01; H, 2.85; N, 10.69. HRMS (ESI) m/z calcd for $C_{18}H_{12}F_6N_3O$ $(M+H)^+$ 400.0879, found 400.0871.

Data for 3',4'-dichloro-5-fluoro-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5p**). Yield 48%; white solid; mp 117–118 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.80 (dd, *J*=8.6, 5.6 Hz, 1H), 7.54 – 7.44 (m, 2H), 7.21 (ddd, *J*=8.3, 6.5, 2.3 Hz, 2H), 7.10 (dd, *J*=9.1, 2.6 Hz, 2H), 3.91 (s, 3H). Elemental anal. calcd for $C_{18}H_{11}Cl_2F_4N_3O$: C, 50.02; H, 2.57; N, 9.72. Found: C, 49.25; H, 2.59; N, 9.73. HRMS (ESI) m/z calcd for $C_{18}H_{12}Cl_2F_4N_3O$ $(M+H)^+$ 432.0288, found 432.0285.

Data for 3',4'-dichloro-N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-5-fluoro-[1,1'-biphenyl]-2-carboxamide (**5q**). Yield 53%; white solid; mp 111-112 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.77 (dd, *J*=8.6, 5.6 Hz, 1H), 7.51 (d, *J*=2.1 Hz, 1H), 7.46 (d, *J*=8.2 Hz, 1H), 7.35 (s, 1H), 7.23 – 7.14 (m, 2H), 7.08 (dd, *J*=9.1, 2.6 Hz, 1H), 6.55 (t, *J*=54.5 Hz, 1H), 3.86 (s, 3H).Elemental anal. calcd for $C_{18}H_{12}Cl_2F_3N_3O$: C, 52.20; H, 2.92; N, 10.14. Found: C, 51.80; H, 4.06; N, 10.17. HRMS (ESI) m/z calcd for $C_{18}H_{13}Cl_2F_3N_3O$ $(M+H)^+$ 414.0382, found 414.0386.

Data for 4'-chloro-5-fluoro-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5r**). Yield 50%; white solid; mp 132-133°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.81 (dd, *J*=8.6, 5.7 Hz, 1H), 7.46 – 7.28 (m, 4H), 7.17 (td, *J*=8.3, 2.6 Hz, 1H), 7.12 – 7.04 (m, 2H), 3.90 (s, 3H). Elemental anal. calcd for $C_{18}H_{12}ClF_4N_3O$: C, 54.35; H, 3.04; N, 10.56. Found: C, 54.18; H, 2.95; N, 10.59. HRMS (ESI) m/z calcd for $C_{18}H_{13}ClF_4N_3O$ $(M+H)^+$ 398.0678, found 398.0672.

Data for 4'-chloro-N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-5-fluoro-[1,1'-biphenyl]-2-carboxamide (**5s**). Yield 61%; white solid; mp 129-130 °C. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.17 (s, 1H), 7.79 (dd, *J*=8.6, 5.7 Hz, 1H), 7.42 – 7.27 (m, 5H), 7.16 (td, *J*=8.2, 2.6 Hz, 1H), 7.08 (dd, *J*=9.3, 2.6 Hz, 1H), 6.49 (t, *J*=54.6 Hz, 1H), 3.85 (s, 3H). Elemental anal. calcd for $C_{18}H_{13}ClF_3N_3O$: C, 56.93; H, 3.45; N, 11.06. Found: C, 57.03; H, 3.55; N, 11.12. HRMS (ESI) m/z calcd for $C_{18}H_{14}ClF_3N_3O$ $(M+H)^+$ 380.0772, found 380.0769.

Data for 4'-(tert-butyl)-5-fluoro-N-(1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)-[1,1'-biphenyl]-2-carboxamide (**5t**). Yield 73%; white solid; mp 125-126 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.90 (dt, *J*=8.5, 4.2 Hz, 1H), 7.45 (dd, *J*=8.5, 2.2 Hz, 2H), 7.31 (dd, *J*=8.6, 2.3 Hz, 2H), 7.19 – 6.99 (m, 3H), 3.89 (s, 3H), 1.32 (s, 9H). Elemental anal. calcd for $C_{22}H_{21}F_4N_3O$: C, 63.00; H, 5.05; N, 10.02 Found: C, 62.72; H, 5.29; N, 9.95. HRMS (ESI) m/z calcd for $C_{22}H_{22}F_4N_3O$ $(M+H)^+$ 420.1694, found 420.1699.

Data for 4'-(tert-butyl)-N-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-5-fluoro-[1,1'-biphenyl]-2-carboxamide (**5u**). Yield 75%; white solid; mp 115-116 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.90 (dd, *J*=8.6, 5.8 Hz, 1H), 7.47 (d, *J*=8.1 Hz, 2H), 7.35 (d, *J*=8.1 Hz, 2H), 7.27 (s, 1H), 7.20 – 7.08 (m, 2H), 6.36 (t, *J*=54.8 Hz, 1H), 3.87 (s, 3H), 1.35 (s, 9H).Elemental anal. calcd for $C_{22}H_{22}F_3N_3O$: C, 65.82; H, 5.52; N, 10.47. Found: C, 65.65; H, 5.47; N, 10.55. HRMS (ESI) m/z calcd for $C_{22}H_{23}F_3N_3O$ $(M+H)^+$ 402.1788, found 402.1792.

X-ray Diffraction. Compound **5i** was recrystallized by a slow evaporation from a solution of dichloromethane/n-hexane (υ/υ=2:5) to afford a single crystal suitable for X-ray crystallography for structure validation. Cell dimensions and intensities were measured using a Rigaku 007 Saturn 70 diffractometer with graphite monochromated Mo Kα radiation. Compound **5i**: Monoclinic, *a*=10.994 (2) Å, *b*=19.344 (4) Å, *c*=8.4094 (17) Å, *U*=1759.4(6) Å^3, *T*=113(2), space group P2(1)/c. A total of 18736 reflections were measured, of which 4184 were unique (R_{int}=0.0768) in the range of 1.883 <2Θ< 27.893°(-14 ≤ h ≤ 13, -25 ≤ k ≤ 25, -11 ≤ l ≤ 11), and

2882 observed reflections with $I > 2\sigma(I)$ were used in the refinement on F^2. The structure of **5i** was solved by direct method with the SHELXTL-97 program and all of the non-H atoms were refined anisotropically by full matrix least-squares to give the Final R indices R_1=0.0599, wR_2=0.1903. The atomic coordinates of **5i** were deposited at the Cambridge Crystallographic Data Centre (CCDC) and CCDC-1942098 contained the supplementary crystallographic data for this paper. The crystallographic data of **5i** can be download from the CCDC and the Supporting Information here.

Bioassays

In vitro fungicidal activity. The fungicidal activity of the target compounds was tested in vitro against *Alternaria solani, Botrytis cinerea, Gibberella zeae, Phytophthora infestans* (Mont) de Bary, *Physalospora piricola, Pellicularia sasakii, Rhizoctonia cerealis* and *Sclerotinia sclerotiorum* by using mycelium growth rate test.[25] The commercial SDHIs boscalid and fluxapyroxad were selected as positive control. The synthesized compounds and controls were dissolved in DMSO to prepare the 20 mg/mL stock solution before diluting by PDA. Target compounds at a concentration 50 μg/mL were used for the initial preliminary screening with three replicates. Their relative inhibitory rate (I, %) was calculated according to the following equation:

$$I\ (\%) = [(C-T)/(C-4)]*100$$

I, inhibitory rate; C, colony diameter of control (mm); T, colony diameter of treatment (mm).

The compounds with excellent inhibitory rate were selected for the determination of EC_{50} values. The 20 mg/mL stock solutions were diluted by PDA to obtain a series of concentrations for the same procedures as described in the above experiments. The EC_{50} data were calculated

according to the concentration and the probit of the corresponding inhibitory rate respectively. The results were summarized in **Table 1** and **Table 2**.

In vivo fungicidal activity. The in vivo fungicidal activities of the target compounds against *Erysiphe graminis* (wheat white powder), *Puccinia sorghi* Schw (corn rust) and *Rhizoctonia solani* (rice sheath blight) were evaluated with three replicates by using pot bioassay as reported previously.[26,27] The biological assay was carry out by Shenyang Sinochem Agrochemicals R&D Co. Ltd. and the results were summarized in **Table 3** and **Table 4**.

Fluorescence quenching analysis of succinate dehydrogenase. Succinate dehydrogenase of *R. cerealis* was extracted according to operation instructions (BC0955, solarbio, Beijing) for the assay. Fluorescence quenching analysis was performed with F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Fluorescence emission spectra was recorded at excitation wavelength of 280 nm with a wavelength ringing from 300-390 nm at 4°C, and the emission band-width was set at 10 nm with medium sensitive. Compound **5i** with 0 to 4 mg/L was added into the succinate dehydrogenase for fluorescence determination, with boscalid, fluxapyroxad and tricyclazole as control. The fluorescence intensity under the treatment was equal which in SDH and compound interaction minus fluorescence intensity of compound itself.

SDH enzymatic activity. The SDH enzyme activity of **5i** was determined by using a succinate dehydrogenase assay kit (Solabio, BC0950) and assessed as reported previously.[28] *R. cerealis* was grown in sterilized Fries medium for 5 d and then treated with different concentrations of **5i** and fluxapyroxad, respectively. The SDH enzyme activity was measured after 24 h of treatment with the selected compounds, and the absorbance value was recorded at 600 nm by using a microplate reader. Compounds were tested at 6 different concentrations with three replicates and the IC_{50} value was calculated by GraphPad Prim version 6.02.

RESULTS AND DISCUSSION

Molecular Docking Analysis. Novel SDHI derivatives are rationally designed by reversing of –CO-NH– group and we speculate whether these compounds also target at SDH and form hydrogen bonds at the pocket. Molecular docking of selected compounds and fluxapyroxad were carried out for evaluation its potent mode of action of target compounds. All reported SDHs are highly reserved in spatial structure, subunit and electron transport pathway, and even the ubiquinone binding site in the prokaryotes and eukaryotes is also highly reserved.[29] For these reasons, avian (*Gallus gallus*) respiratory complex II with carboxin bound (pdb code: 2FBW) was chose for molecular docking study. In order to illustrate the binding mode between ligand and receptor, **5i** was selected as an example. As shown in **Figure 3**, **5i** located in the same site and adopted a similar binding conformation as compared to fluxapyroxad in the three-dimensional schematic diagrams. Compound **5i** bounded to the receptor protein, the carboxyl oxygen formed two hydrogen bonds with the amino acid residue TRP173 and TYR58, respectively. The binding mode was similar to that of fluxapyroxad, this indicated that, the target compounds also possessed strong interaction with SDH.

Chemical Synthesis. The synthetic procedures for the target compounds are described in **Scheme 1**. The Suzuki-Miyaura cross-coupling of aryl halide and aryl boronic acid was conducted under argon at 80 ℃ to produce the key intermediate **1**.[30] Intermediate **1** was then reacted with NaOH via a direct ester hydrolysis under 80 ℃ to yield the compound **2**. Compound **2** was reacted with oxalyl chloride in CH_2Cl_2 with DMF to afford intermediate **3**. Intermediate **3** was then reacted with pyrazole amine **4** in CH_2Cl_2 in the presence of Et_3N to afford target compound **5** with moderate to good yields. To further validate the structure of the title compounds, the structure of **5i** was further identified by X-ray diffraction studies (see **Figure. 4**).

Fungicidal Activity. The results of in vitro and in vivo fungicidal activities of the target compounds (**5a** to **5u**) were summarized in **Table 1-4**. In general, most of the compounds displayed considerable to excellent fungicidal activities against eight phytopathogens in vitro at 50 μg/mL. (**Table 1**). No obvious difference of fungicidal activity between 3-CF_3 substituent prazole compounds and 3-CF_2H substituent prazole compounds was observed. Most of these compounds were highly active against *B. cinerea*, *R. cerealis* and *S. sclerotiorum*. As shown in **Table 1**, **5c, 5d** and **5j** showed over 90% of inhibitory rate against *B. cinereal*, which were comparable to boscalid and fluxapyroxad. For *G. zeae,* **5i** and **5j** showed better activity than positive controls. Compound **5i, 5p, 5q, 5s** displayed higher activity against *P. infestans* than boscalid and fluxapyroxad, **5h, 5i, 5j, 5p, 5q, 5r, 5s** exhibited excellent activities against *P. sasakii*, which were better than boscalid and fluxapyroxad. Eight compounds showed over 86% of inhibitory rate against *R. cerealis*, boscalid and fluxapyroxad only showed 35% and 86% of inhibitory rate, respectively. All compounds exhibited excellent activities against *S. sclerotiorum.*

Several compounds with superior fungicidal activities were selected for the calculation of EC_{50} value. As shown in **Table 2**, several compounds showed good activity against *Botrytis cinerea,* but not as effective as that of the positive controls. Compounds **5i** and **5q** displayed excellent activities against *P. infestans* and *P. sasakii* with the EC_{50} values lower than the positive control fluxapyroxad. Nine compounds exhibited better fungicidal activity against *R. cerealis* than fluxapyroxad, especially, **5i** showed excellent fungicidal activity with the EC_{50} value of 4.61 μg/mL, which was more active than that of fluxapyroxad with its EC_{50} value of 16.99 μg/mL. The EC_{50} values of all target compounds were tested against *S. sclerotiorum,* **5i** also showed the highest fungicidal activity with its EC_{50} value of 0.73 μg/mL, which was comparable to that of boscalid with its EC_{50} value of 0.51 μg/mL. Therefore, **5i** exhibited

promising fungicidal activity against five fungi with broader spectrum of fungicidal activity and could be an alternative to SDHIs candidate.

The in vivo fungicidal activity of the target compounds was tested against *E. graminis*, *P. sorghi* and *R. solani* for comparison with the commercial fungicide boscalid and fluxapyroxad (**Table 3**). Most of the compounds exhibited excellent fungicidal activity against *P. sorghi* and *R. solani* at the concentration 200 μg/mL. Compound **5c, 5l, 5o, 5t** displayed 100% of inhibitory rate against *E. graminis* and similar activity as that of boscalid and fluxapyroxad. Compound **5d, 5f, 5g, 5h, 5i, 5g** exhibited more than 90% of excellent fungicidal activity against *P. sorghi*, which was comparable to those of boscalid and fluxapyroxad. Compound **5g, 5h, 5i, 5t** displayed over 80% of inhibitory rate against *R. solani*.

Some compounds with higher inhibitory rate were selected for further greenhouse in vivo fungicidal activity evaluation. As shown in **Table 4**, **5g** and **5h** were not as effective as **5i** against *P. sorghi* and *R. solani*. Compound **5i** and fluxapyroxad exhibited 100% inhibitory rate against *P. sorghi* at the concentration of 50 μg/mL, they were better than boscalid (70%). Furthermore, compound **5i** showed excellent inhibitory rate (60%) against *R. solani* at 1μg/mL, which was better than boscalid (30%) at the same concentration. In general, **5i** presented excellent activity against *R. cerealis* in vitro and was highly effective against *R. solani* in vivo. Both in vitro and in vivo activity determination indicated that, **5i** could be a promising candidate against *R. solani* for rice disease control.

SDH Enzymatic Inhibition Activity. Compound **5i** with promising fungicidal activity was selected and evaluated for SDH enzymatic inhibition determination to its target site validation. As shown in **Table 5**, **5i** exhibited very good SDH inhibition with an IC_{50} of 0.60 μg/mL, while the IC_{50} of fluxapyroxad was 0.29 μg/mL, **5i** showed the same level of inhibitory

activity as fluxapyroxad. Compound **5i** with carbonyl group adjacent to benzene ring contrast to fluxapyroxad which carbonyl group was adjacent to pyrazole could also exhibit excellent SDH enzymatic inhibition activity and formed strong interaction with SDH.

Fluorescence Quenching Analysis of SDH. The interaction of protein and small molecules could be revealed by fluorescence quenching analysis. Fluorescence quenching of the SDH by **5i**, boscalid and fluxapyroxad were conducted to validate interactions between protein and compounds. As shown in **Figure 5C**, with the increase of the concentration of **5i**, the fluorescence intensity for SDH gradually quenched, it was similar to that of the positive control boscalid and fluxapyroxad (**Figure 5A, 5B**), there showed no significant fluorescence changes when treated by a negative control tricyclazole, a melanin synthesis inhibitor[31] (**Figure 5D**). Boscalid and fluxapyroxad, carboxamide fungicides, were SDHIs.[32] Pesticide target discovery and validation is a most important basis for novel pesticide development.[33] Compound **5i** exhibited the similar fluorescence quenching rules as that of boscalid and fluxapyroxad, which indicated that they might act at the same target site.

In summary, a series of novel SDHIs derivatives were designed and synthesized by inversion of carbonyl and amide groups of fluxapyroxad. Bioassay discovered highly active **5i** with broad-spectrum of outstanding in vitro fungicidal activity and better in vivo efficacy against *P. sorghi* and *R. solani*. Fluorescence quenching analysis for SDH target validation indicated that, **5i** exhibited the similar properties as that of the two SDHI positive controls boscalid and fluxapyroxad, these results revealed that, the designed compounds might also act at SDH. Our results provided a new clue for the molecular design and development of the novel highly active SDHIs.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

^{1}H NMR spectra for all compounds and HRMS spectra for target compounds

Crystal data of **5i** (CIF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported in part by the National Natural Science Foundation of China (No. 31872007 and No. 31571991), the Tianjin Natural Science Foundation (No. 18JCZDJC33500), the International Science & Technology Cooperation Program of China (No. 2014DFR41030),

The Fundamental Research Funds for the Central Universities, Nankai University (No. 63191743), The Tianjin Development Program for Innovation and Entrepreneurship and The Russian Foundation for Basic Research (No. 18-316-20018).

Dedicated to the 100th anniversary of Nankai University and the 100th birthday anniversary of Professor Ruyu Chen.

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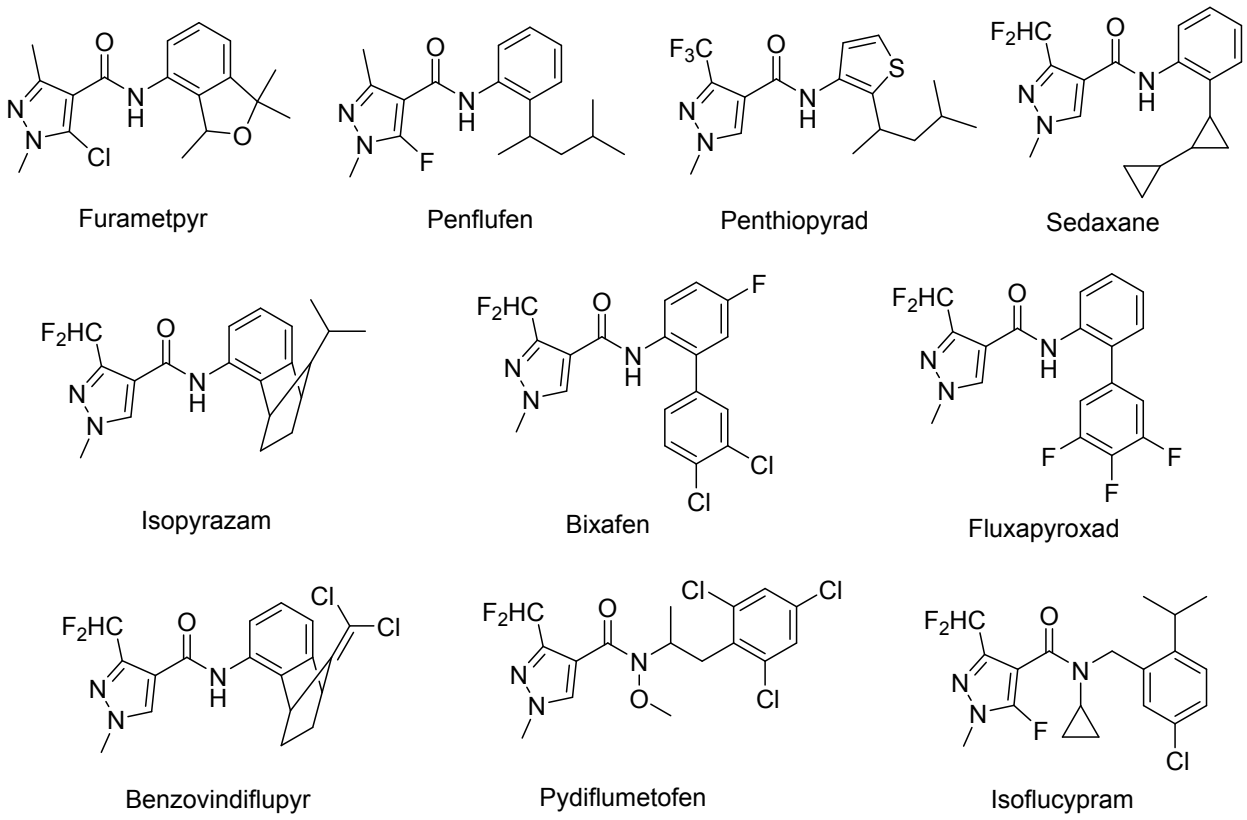

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537 **Figure 1**. Representative chemical structures of pyrazole contained SDHIs

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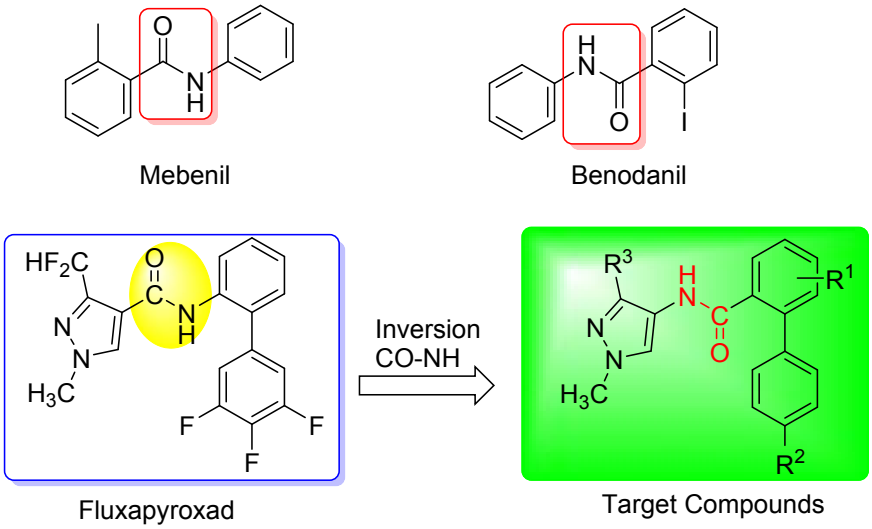

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540 **Figure 2**. The design strategy of the target compounds.

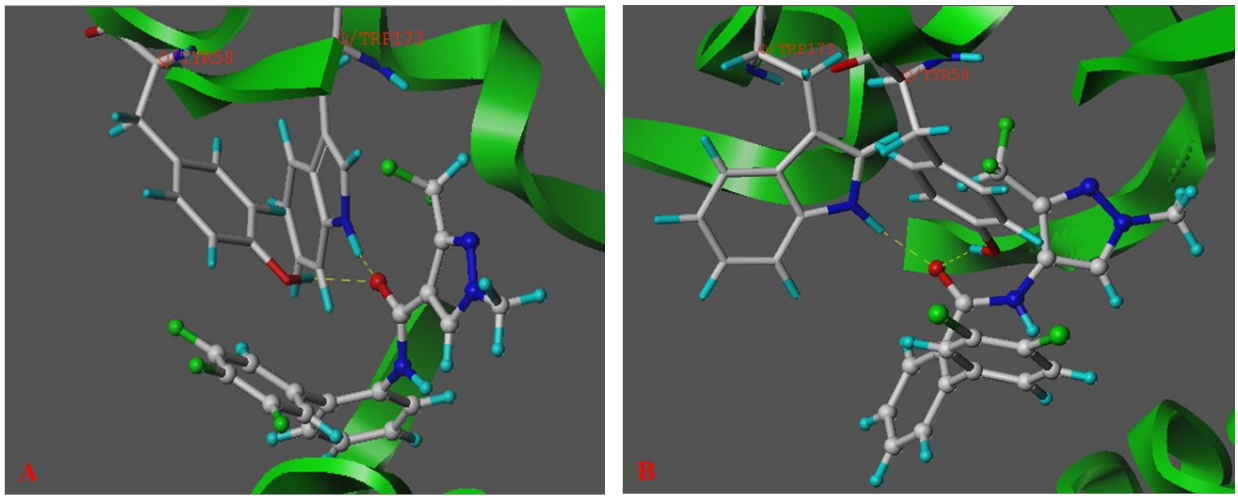


Figure 3. Binding modes of **5i** (A) and fluxapyroxad (B) with SDH.

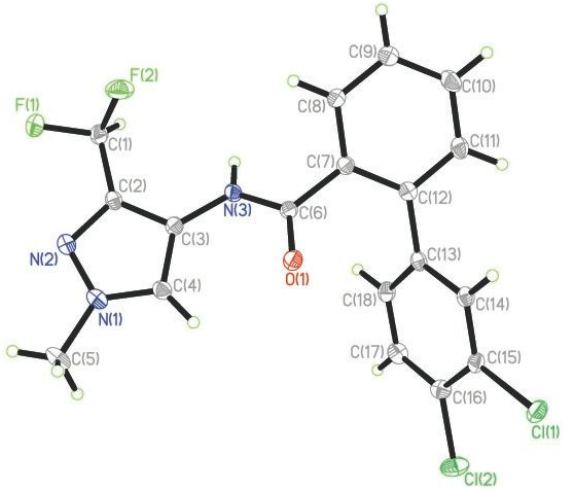
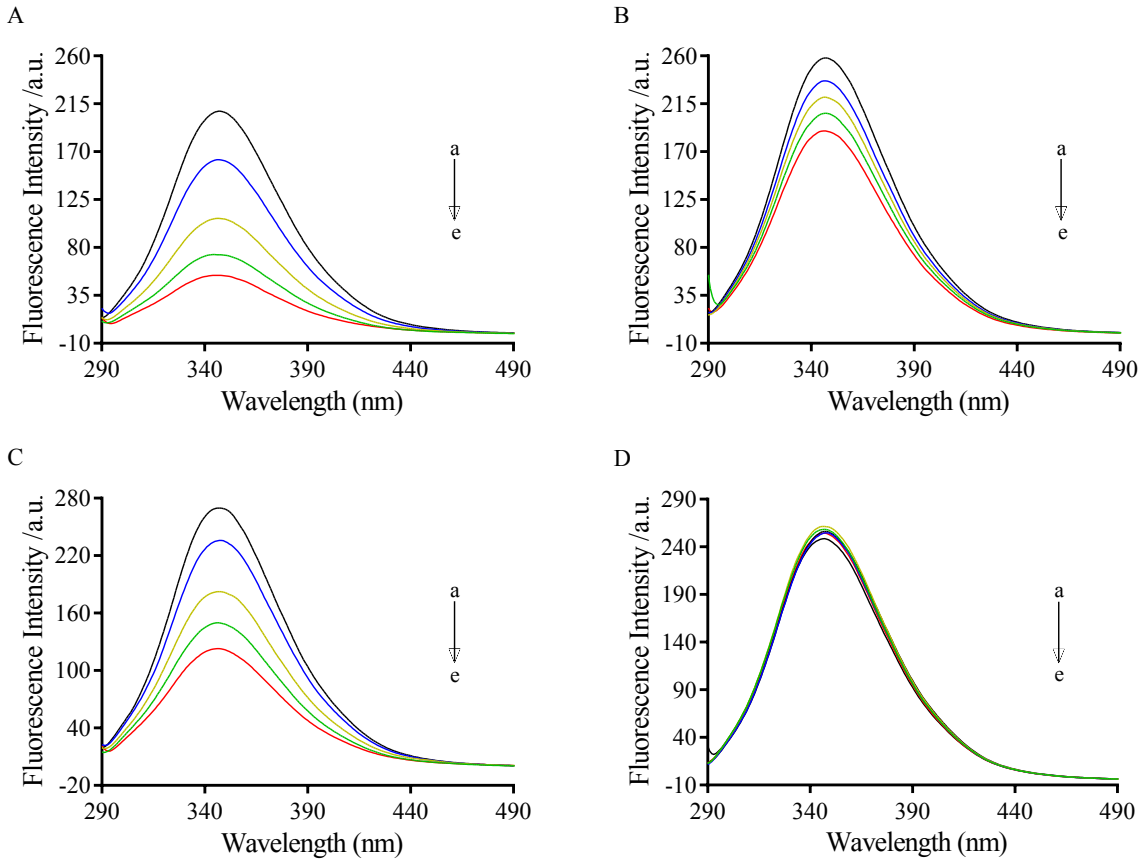


Figure 4. Crystal structure for **5i** by X-ray diffraction determination. (CCDC number: 1942098)



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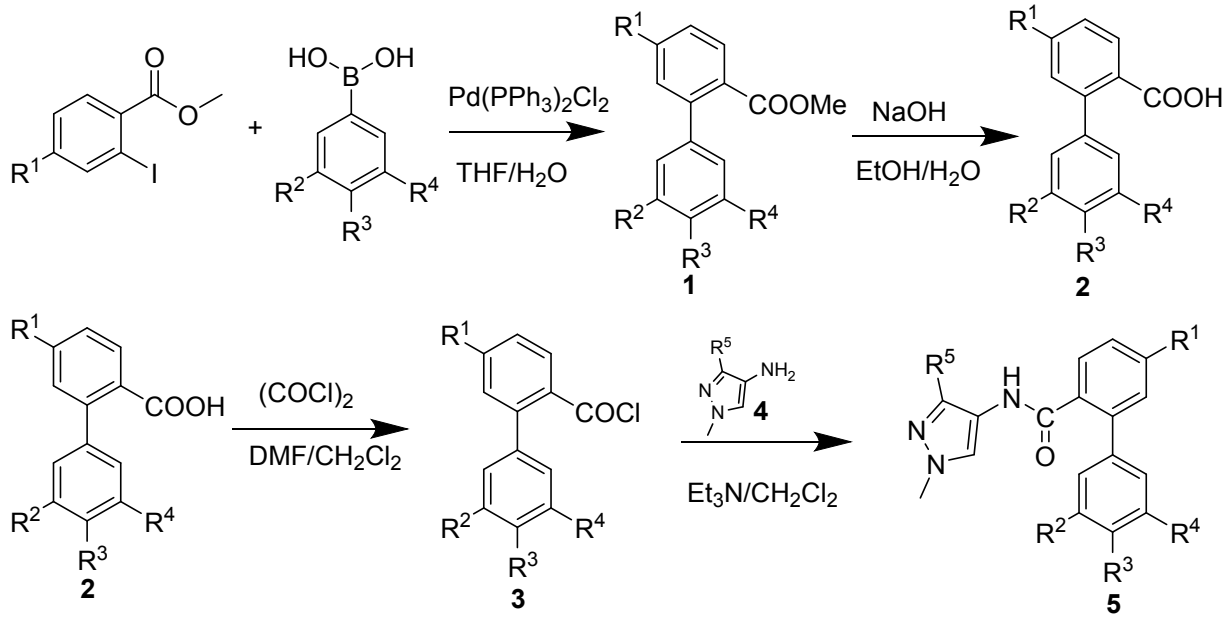
549 **Figure 5**. Fluorescence spectra of succinate dehydrogenase: A, boscalid; B, fluxapyroxad; C, compound **5i**; D,
550 tricyclazole "a to e" were "0 to 4 mg/L".



Compd	R^1	R^2	R^3	R^4	R^5	Compd	R^1	R^2	R^3	R^4	R^5	Compd	R^1	R^2	R^3	R^4	R^5
5a	H	H	CF_3	H	CF_3	**5h**	H	H	Cl	Cl	CF_3	**5o**	F	F	F	F	CHF_2
5b	H	H	CF_3	H	CHF_2	**5i**	H	H	Cl	Cl	CHF_2	**5p**	F	H	Cl	Cl	CF_3
5c	H	H	CH_3	H	CF_3	**5j**	H	H	Cl	H	CF_3	**5q**	F	H	Cl	Cl	CHF_2
5d	H	H	CH_3	H	CHF_2	**5k**	H	H	Cl	H	CHF_2	**5r**	F	H	Cl	H	CF_3
5e	H	F	F	F	CF_3	**5l**	F	H	H	H	CF_3	**5s**	F	H	Cl	H	CHF_2
5f	H	F	F	F	CHF_2	**5m**	F	H	H	H	CHF_2	**5t**	F	H	tBu	H	CF_3
5g	H	H	H	H	CHF_2	**5n**	F	F	F	F	CF_3	**5u**	F	H	tBu	H	CHF_2

Scheme 1 The synthetic route of target compounds

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557 **Table 1** In vitro fungicidal activity of target compounds against Phytopathogens at 50 μg/mL

Compd	Mycelium growth inhibitory rate (%)							
	AS[a]	BC	GZ	PI	PP	PS	RC	SS
5a	36±2	49 ±1	35 ±0	21 ±0	13 ±1	22 ±2	52 ±1	100
5b	54 ±1	76 ±1	59 ±1	49 ±1	30 ±0	56 ±1	80 ±0	91 ±1
5c	56±3	93 ±0	61 ±2	46 ±2	25 ±0	54 ±2	100	96 ±0
5d	46 ±1	90 ±0	44 ±2	36 ±2	35 ±0	46 ±1	71 ±0	95 ±0
5e	44 ±0	66 ±1	57 ±1	26 ±2	11 ±2	32 ±1	65 ±1	89 ±2
5f	49 ±1	68 ±0	56 ±1	28 ±1	32 ±1	27 ±2	68 ±0	88 ±1
5g	41±0	63 ±1	30 ±2	33 ±2	21 ±1	24 ±2	56 ±2	91 ±0
5h	59±1	71 ±2	65 ±1	51 ±1	35 ±1	61 ±0	95 ±0	96 ±0
5i	62±0	71 ±0	67 ±1	62 ±0	49 ±0	71 ±0	92 ±0	100
5j	56±1	90 ±1	69 ±1	51 ±0	38 ±0	61 ±1	100	95 ±0
5k	44±1	83 ±1	52 ±1	46 ±1	25 ±1	54 ±0	80 ±0	91 ±0
5l	46±0	76 ±0	44 ±1	28 ±1	28 ±0	27 ±1	83 ±1	89 ±3
5m	46 ±1	56 ±1	35 ±2	21 ±1	21 ±0	24 ±1	68 ±1	86 ±2
5n	51 ±1	68 ±0	59 ±1	36 ±1	10 ±2	46 ±1	74 ±1	96 ±1
5o	49 ±0	59 ±1	61 ±0	51 ±0	17 ±0	54 ±1	76 ±1	100
5p	59±1	68 ±1	61 ±0	59 ±1	13 ±2	66 ±0	91 ±0	100
5q	59±0	78 ±1	59 ±0	72 ±0	39 ±1	76 ±0	89 ±0	100
5r	56±3	88 ±1	59 ±1	51 ±1	32 ±1	66 ±0	100	96 ±1
5s	56 ±1	80 ±1	59 ±1	56 ±1	21 ±1	68 ±0	86 ±1	100
5t	46 ±3	49 ±3	44 ±0	33 ±3	21 ±1	44 ±1	68 ±1	100
5u	54 ±2	59 ±2	57 ±1	41 ±1	32 ±2	54 ±1	79 ±2	100
Boscalid	90 ±0	95 ±1	57 ±2	23 ±2	58 ±1	22 ±2	35 ±1	100
Fluxapyroxad	95 ±1	100	67 ±2	54 ±0	82 ±0	54 ±1	86 ±1	100

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559 [a]: AS： *Alternaria solani*、BC： *Botrytis cinerea*、GZ： *Gibberella zeae*、PI： *Phytophthora infestans*

560 (Mont.) de Bary、PP： *Physalospora piricola*、PS： *Pellicularia sasakii*、RC： *Rhizoctonia cerealis*、SS

561 ： *Sclerotinia sclerotiorum*.

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567 **Table 2** The in vitro EC_{50} value (μg/mL) of selected compounds

Fungi	Compd.	EC_{50} (95% confidence interval)	Regression equation	R^2
B. cinerea	**5b**	16.11 (11.58-23.34)	y=3.9205+0.9050 x	0.9649
	5c	7.90 (6.65-9.32)	y=3.5335+1.6762 x	0.9887
	5d	14.91 (11.67-19.41)	y=3.0082 +1.7329 x	0.9765
	5h	19.88 (14.43-24.93)	y=3.6833+1.0469 x	0.9802
	5i	17.22 (14.27-19.81)	y=3.0418+1.6371 x	0.9919
	5j	16.21 (11.52-21.06)	y=4.2262+0.6529 x	0.9694
	5k	24.38 (19.50-27.69)	y=2.7646+1.6426 x	0.9911
	5l	43.29 (24.00-73.56)	y=2.2335+1.7245 x	0.9636
	5q	35.34 (26.97-45.26)	y=2.6208+1.5635 x	0.9884
	5r	25.56 (18.78-32.85)	y=4.2162+0.5721 x	0.9792
	5s	8.19 (6.07-11.51)	y=3.6524+1.4798 x	0.9628
	Boscalid	0.45 (0.36-0.53)	y=5.2928+0.7623 x	0.9907
	Fluxapyroxad	0.10 (0.08-0.11)	y=6.0470+1.0169 x	0.9908
P. infestans	**5i**	24.22 (20.09-30.66)	y=3.0215+1.4420 x	0.9839
	5q	24.45 (23.22-29.23)	y=2.9475+1.4804 x	0.9976
	Fluxapyroxad	38.84 (27.58-60.05)	y=3.3449+1.0493 x	0.9643
P. sasakii	**5i**	19.74 (15.44-27.77)	y=3.2913+1.3284 x	0.9652
	5q	18.79 (15.37-24.32)	y=3.4240+1.2413 x	0.9864
	Fluxapyroxad	38.18 (30.54-51.26)	y=2.5966+1.5260 x	0.9881
R. cerealis	**5b**	17.75 (15.43-19.82)	y=3.5447+1.1907 x	0.9926
	5c	14.60 (10.38-22.73)	y=2.8779+1.8342 x	0.9408
	5d	25.85 (17.71-39.23)	y=2.3516+1.8853 x	0.9566
	5h	15.45 (12.14-19.43)	y=3.4256+1.3460 x	0.9820
	5i	4.61 (3.95-5.36)	y=3.8287+1.7728 x	0.9921
	5j	9.80 (6.31-14.84)	y=3.2247+1.8335 x	0.9410
	5k	20.75 (17.45-23.34)	y=3.0136+1.5349 x	0.9934
	5l	31.52 (18.66-56.03)	y=3.3679+1.0929 x	0.9475
	5n	12.52 (10.89-14.03)	y=3.7241+1.1842 x	0.9950
	5o	11.03 (9.11-11.61)	y=3.7202+1.2741 x	0.9937
	5p	6.48 (5.41-7.76)	y=4.2499+0.9598 x	0.9917
	5q	12.13 (9.96-15.41)	y=3.7561+1.1587 x	0.9812
	5r	16.96 (14.89-18.83)	y=1.7841+2.6537 x	0.9964
	5s	19.91 (16.72-23.42)	y=1.8063+2.4936 x	0.9897
	5u	17.78 (16.70-19.55)	y=3.0593+1.5796 x	0.9970
	Fluxapyroxad	16.99 (14.06-20.62)	y=2.7777+1.8349 x	0.9881
S. sclerotiorum	**5a**	12.15 (10.78-13.01)	y=4.4216+0.5519 x	0.9966
	5b	20.55 (14.67-32.31)	y=3.4946+1.1542 x	0.9643
	5c	8.55 (4.99-14.19)	y=4.2416+0.8353 x	0.9184
	5d	34.28 (21.89-59.91)	y=3.0072+1.3091 x	0.9535
	5e	15.64 (10.40-24.95)	y=1.9826+2.5505 x	0.9327
	5f	6.93 (4.86-9.20)	y=4.3711+0.7671 x	0.9661
	5g	14.26 (13.14-16.86)	y=3.6601+1.1822 x	0.9936
	5h	1.08 (0.73-1.50)	y=4.9935+0.6235 x	0.9606
	5i	0.73 (0.49-1.04)	y=5.1180+0.6857 x	0.9659
	5j	8.20 (4.69-14.08)	y=4.0936+1.0227 x	0.9248

5k	7.57 (5.24-11.15)	y=3.8165+1.3999 x	0.9552
5l	24.20 (15.74-34.15)	y=2.5859+1.7845 x	0.9657
5m	3.98 (2.74-5.87)	y=4.5779+0.7132 x	0.9860
5n	16.50 (13.56-21.11)	y=3.2996+1.4007 x	0.9830
5o	13.21 (12.40-15.93)	y=4.0860+0.8218 x	0.9941
5p	8.46 (7.00-10.29)	y=3.6722+1.4763 x	0.9866
5q	5.98 (4.60-7.87)	y=4.0054+1.3166 x	0.9776
5r	8.87 (7.43-10.66)	y=4.0090+1.0814 x	0.9881
5s	3.72 (2.35-6.05)	y=4.5508+0.8075 x	0.9575
5t	3.94 (2.48-6.06)	y=4.6745+0.5696 x	0.9621
5u	5.53 (4.18-7.14)	y=4.5031+0.7048 x	0.9769
Boscalid	0.51 (0.36-0.67)	y=5.3117+0.9572 x	0.9760
Fluxapyroxad	0.19 (0.15-0.23)	y=5.6713+0.8878 x	0.9805

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570 **Table 3** In vivo fungicidal activities of the target compound

Compd	Fungicidal activity (%) at 200 μg/mL		
	E. graminis [a]	*P. sorghi*	*R. solani*
5a	0	0	0
5b	0	0	0
5c	100	20	50
5d	20	90	10
5e	0	0	0
5f	0	90	60
5g	0	98	90
5h	0	90	90
5i	0	100	100
5j	0	20	40
5k	0	0	0
5l	100	20	40
5m	0	40	50
5n	0	0	20
5o	100	0	30
5p	0	30	0
5q	0	98+[b]	0
5r	0	20	50
5s	0	20	80
5t	100	0	100
5u	90	30	50
Boscalid	100	100	80
Fluxapyroxad	100	100	70

571 [a]: *E. graminis* ,wheat white powder; *P. sorghi*, corn rust; *R. solani*, Rice Sheath Blight.

572 [b]: Existing phytotoxicity.

573 **Table 4**. Greenhouse in vivo fungicidal activity validation

Compd	*P. sorghi*			*R. solani*		
	50μg/mL	10μg/mL	1μg/mL	50μg/mL	10μg/mL	1μg/mL
5g	30	0	0	30	20	0
5h	85	0	0	20	10	0
5i	100	40	0	80	70	60
Boscalid	70	30	0	90	60	30
Fluxapyroxad	100	100	85	100	100	100

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576 **Table 5** Inhibitory effect of **5i** against *R. cerealis* SDH

Compd	IC_{50}(μg/mL)	Regression equation	R^2
5i	0.60	y=5.2854+1.2844 x	0.9806
Fluxapyroxad	0.29	y=5.7100+1.3059 x	0.9529

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578 # Graphic for table of contents

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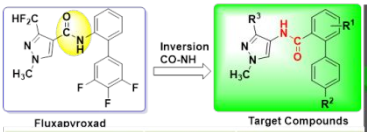
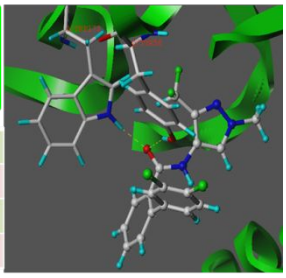


EC_{50} (μg/mL)	5i	fluxapyroxad
SDH	0.60	0.29
R.cerealis	4.61	16.99
S.sclerotiorum	0.73	0.19



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