JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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# Expedient Discovery for Novel Antifungal Leads Targeting Succinate Dehydrogenase: Pyrazole-4-formylhydrazide Derivatives Bearing a Diphenyl Ether Fragment

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Cite This: https://dx.doi.org/10.1021/acs.jafc.0c03736 Read Online

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**ABSTRACT:** The pyrazole-4-carboxamide scaffold containing a flexible amide chain has emerged as the molecular skeleton of highly efficient agricultural fungicides targeting succinate dehydrogenase (SDH). Based on the above vital structural features of succinate dehydrogenase inhibitors (SDHI), three types of novel pyrazole-4-formylhydrazine derivatives bearing a diphenyl ether moiety were rationally conceived under the guidance of a virtual docking comparison between bioactive molecules and SDH. Consistent with the virtual verification results of a molecular docking comparison, the *in vitro* antifungal bioassays indicated that the skeleton structure of title compounds should be optimized as an *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide scaffold. Strikingly, *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives **11o** against *Rhizoctonia solani*, **11m** against *Fusarium graminearum*, and **11g** against *Botrytis cinerea* exhibited excellent antifungal effects, with corresponding $EC_{50}$ values of 0.14, 0.27, and 0.52 μg/mL, which were obviously better than carbendazim against *R. solani* (0.34 μg/mL) and *F. graminearum* (0.57 μg/mL) as well as penthiopyrad against *B. cinerea* (0.83 μg/mL). The relative studies on an *in vivo* bioassay against *R. solani*, bioactive evaluation against SDH, and molecular docking were further explored to ascertain the practical value of compound **11o** as a potential fungicide targeting SDH. The present work provided a non-negligible complement for the structural optimization of antifungal leads targeting SDH.

**KEYWORDS:** *pyrazole-4-formylhydrazine, diphenyl ether, crop protection, fungicide, lead optimization, succinate dehydrogenase inhibitor*



## 1. INTRODUCTION

The electron transfers along mitochondrial respiratory enzyme complexes generate a vital proton gradient across the cellular membrane, which activates adenosine 5′-triphosphate (ATP) synthase that translates adenosine 5′-diphosphate into ATP.[1,2] The above oxidative phosphorylation on cellular mitochondria produces essential energies for maintaining normal physiological and biochemical reactions within aerobic eukaryotes.[3,4] In this pivotal biochemical process, succinate dehydrogenase (SDH), recognized as the crucial component of respiratory enzyme complexes, catalyzes succinate oxidation coupling with electron transfers from succinate to ubiquinone.[5−7] Over the past half a century, practical explorations have confirmed that inhibiting the normal physiological function of SDH within phytopathogenic fungi could be an important strategy to effectively control the fungal infections in grains, vegetables, and fruits.[8−10] During the development process of succinate dehydrogenase inhibitors (SDHIs) that structurally possess a common carboxamide framework, a stable pyrazole-4-carboxamide fragment has emerged as the indispensable dominant scaffold (Figure 1) that leads to a significant improvement in the antifungal effects of constructed carboxamides against phytopathogens.[9,11−15] Currently, introducing a flexible amide chain into pyrazole-4-carboxamide templates (e.g., isoflucypram, pyrapropoyne, and pydiflumetofen) is considered as an innovative exploitation strategy for SDHI structural optimization, which implicitly indicates the optimization importance of amide chain fragments for developing novel carboxamide fungicides with higher efficacies and broader biological spectra.[16−18]

Hydrazides exist as nitrogenous fragments in numerous bioactive compounds that present antibacterial,[19] antifungal,[20] antiviral,[21] insecticidal,[22] herbicidal,[23] anticancer,[24] anticoagulant,[25] anti-inflammatory,[26] and antimalarial[27] properties. Among them, formylhydrazide skeletons are widely utilized as the ideal bioisosteres of carboxamide scaffolds for developing novel agrochemical candidates due to their more effective combinations with various biological enzymes than a carboxamide moiety within living organisms.[28−30] As representative fungicides bearing a formylhydrazide fragment, miexiuyihao and famoxadone (Figure 2) were applied as efficacious agricultural measures to control fungal diseases and reduce crop losses.[31,32] During the last decade, extensive studies on the structural optimizations of carboxamide derivatives documented that replacing the formylamide

Received: June 14, 2020
Revised: October 25, 2020
Accepted: November 10, 2020

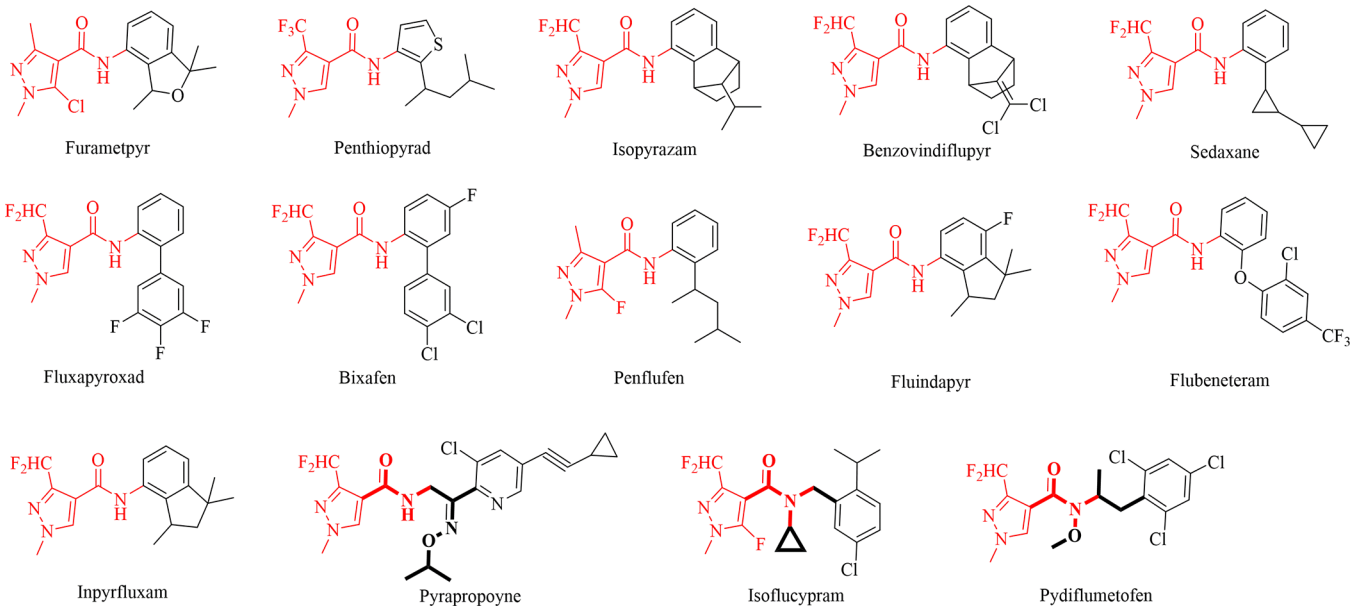



**Figure 1.** Commercialized SDHIs bearing a pyrazole-4-carboxamide fragment as agricultural fungicides.

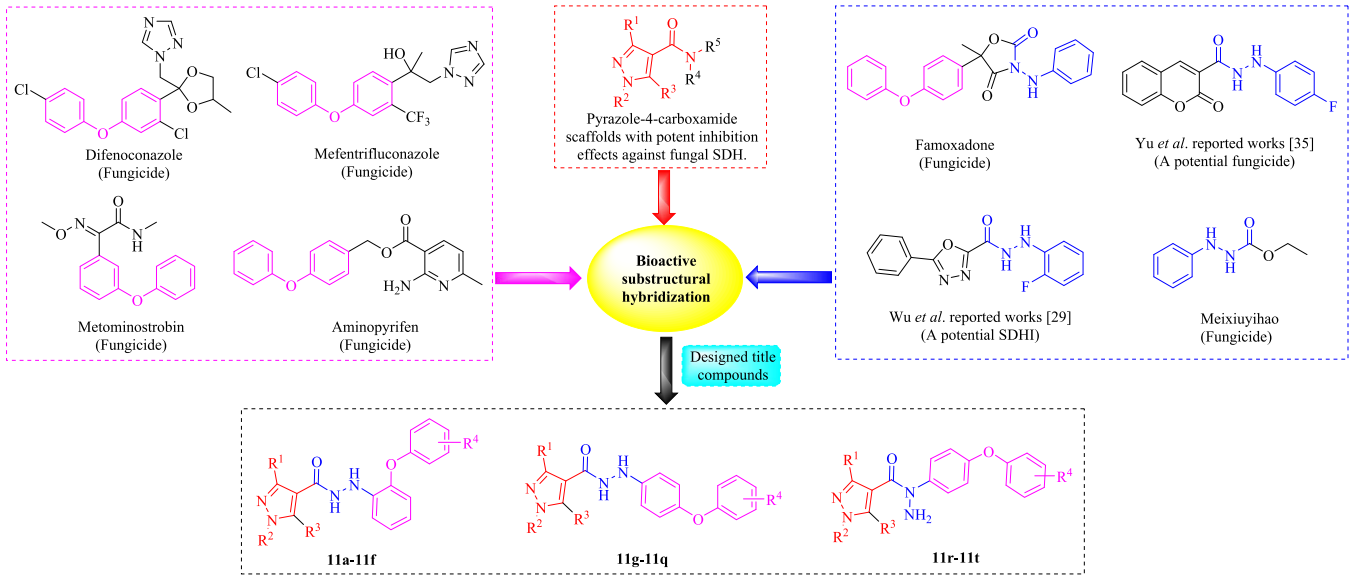



**Figure 2.** Design strategy of title compounds.

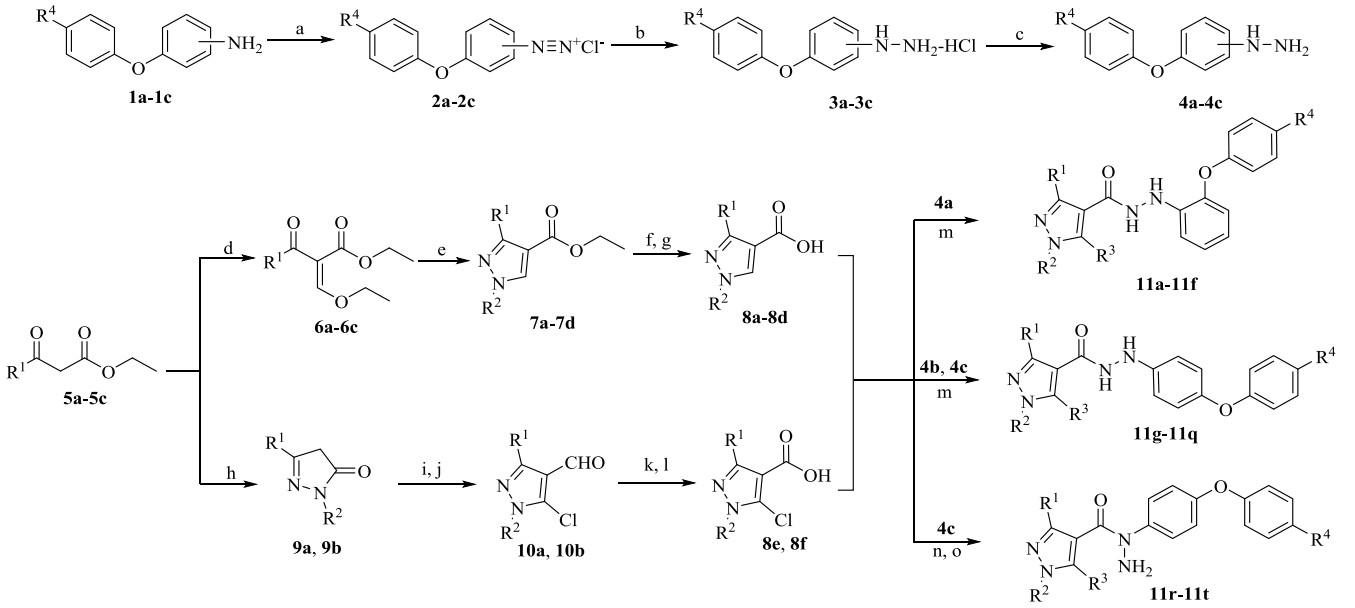
fragment in bioactive leads with a formylhydrazide skeleton could significantly improve their inhibition effects and greatly broaden their biological spectra against phytopathogenic fungi.[29,30,33−35] Recently, inhibition of the normal physiological function of cellular SDH was revealed as the underlying action mechanism of formylhydrazide derivatives against agricultural fungi.[29,36]

Diphenyl ether derivatives have not only emerged as versatile secondary metabolites exhibiting antibacterial,[37] anticancer,[38] anti-inflammatory,[39] antidiabetic,[40] and osteogenic[41] bioactivities, but also are proverbially utilized as valuable industrial products, including flame retardants, fungicides, herbicides, insecticides, perfumes, coatings, preservatives, and so on.[42−45] Remarkably, subsequent explorations on the application developments of diphenyl ethers unraveled that introducing this special lipophilic group into bioactive leads could effectively improve the permeability of obtained molecules within the cellular membranes of phytopathogenic fungi.[46] As a bioactive diphenyl ether derivative blocking electron transfers along the cellular respiratory chain, metominostrobin (Figure 2) was arduously developed as an agricultural fungicide after long-term studies on structural optimizations of natural strobilurins.[47] Meanwhile, integrations of lipophilic diphenyl ether and bioactive 1,2,4-triazole fragments in a single molecular structure ingeniously constructed the commercialized fungicides difenoconazole and mefentrifluconazole (Figure 2), which inhibit ergosterol biosynthesis within agricultural fungi.[48,49] Recently, diphenyl ether fragments have aroused our interest again due to their existence in the novel fungicide aminopyrifen (Figure 2), which perturbs the remodeling and formation of cell wall polymers in phytopathogenic fungi.[50]

Considering the structural features of the commercialized SDHIs bearing a pyrazole-4-carboxamide scaffold as well as the


https://dx.doi.org/10.1021/acs.jafc.0c03736
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

**Scheme 1. Synthesis of Title Compounds 11a−t**[a]




**11a**: $R^1$=$CH_3$, $R^2$=$CH_3$, $R^3$=H, $R^4$=H; **11b**: $R^1$=$CHF_2$, $R^2$=$CH_3$, $R^3$=H, $R^4$=H; **11c**: $R^1$=$CF_3$, $R^2$=$CH_3$, $R^3$=H, $R^4$=H; **11d**: $R^1$=$CF_3$, $R^2$=H, $R^3$=H, $R^4$=H;
**11e**: $R^1$=$CF_3$, $R^2$=$CH_3$, $R^3$=Cl, $R^4$=H; **11f**: $R^1$=$CH_3$, $R^2$=$CH_3$, $R^3$=Cl, $R^4$=H; **11g**: $R^1$=$CH_3$, $R^2$=$CH_3$, $R^3$=H, $R^4$=H; **11h**: $R^1$=$CHF_2$, $R^2$=$CH_3$, $R^3$=H, $R^4$=H;
**11i**: $R^1$=$CF_3$, $R^2$=$CH_3$, $R^3$=H, $R^4$=H; **11j**: $R^1$=$CF_3$, $R^2$=H, $R^3$=H, $R^4$=H; **11k**: $R^1$=$CF_3$, $R^2$=$CH_3$, $R^3$=Cl, $R^4$=H; **11l**: $R^1$=$CH_3$, $R^2$=$CH_3$, $R^3$=Cl, $R^4$=H;
**11m**: $R^1$=$CHF_2$, $R^2$=$CH_3$, $R^3$=H, $R^4$=Cl; **11n**: $R^1$=$CF_3$, $R^2$=$CH_3$, $R^3$=H, $R^4$=Cl; **11o**: $R^1$=$CF_3$, $R^2$=H, $R^3$=H, $R^4$=Cl; **11p**: $R^1$=$CF_3$, $R^2$=$CH_3$, $R^3$=Cl, $R^4$=Cl;
**11q**: $R^1$=$CH_3$, $R^2$=$CH_3$, $R^3$=Cl, $R^4$=Cl; **11r**: $R^1$=$CH_3$, $R^2$=$CH_3$, $R^3$=H, $R^4$=Cl; **11s**: $R^1$=$CHF_2$, $R^2$=$CH_3$, $R^3$=H, $R^4$=Cl; **11t**: $R^1$=$CF_3$, $R^2$=$CH_3$, $R^3$=Cl, $R^4$=Cl.

[a]Reagents and conditions: (a) $NaNO_2$ (1.2 equiv), HCl, and 0 °C; (b) $SnCl_2$ (3.0 equiv), HCl, and 0 °C; (c) NaOH (1.2 equiv), $H_2O$, and reflux; (d) triethyl orthoformate (2.0 equiv), acetic anhydride, and reflux; (e) $R_2NHNH_2$ (3.0 equiv), reflux; (f) LiOH (1.5 equiv), THF, and reflux; (g) 5% HCl, pH = 2.0; (h) $R_2NHNH_2$ (1.0 equiv), EtOH, and reflux; (i) $POCl_3$ (25.0 equiv), dimethylformamide (DMF), and reflux; (j) NaOH, pH = 7.0; (k) $KMnO_4$ (1.5 equiv), $H_2O$, and 80 °C; (l) 5% HCl, pH = 2.0; (m) TBTU (1.5 equiv), $NEt_3$ (1.5 equiv), and $CH_2Cl_2$; (n) $SOCl_2$ (5.0 equiv), reflux; and (o) $NEt_3$ (1.5 equiv), $CH_2Cl_2$.

vital applications of formylhydrazide and diphenyl ether fragments on searching for novel agricultural fungicides, the crucial aims of this work are mainly to: (i) construct novel potential SDHIs by ingeniously integrating formylhydrazide and diphenyl ether fragments in the molecular structures of commercialized pyrazole-4-carboxamides, as shown in Figure 2, (ii) generate a series of pyrazole-4-formylhydrazine derivatives bearing a diphenyl ether fragment (Scheme 1) and evaluate their *in vitro* and *in vivo* antifungal effects against *Rhizoctonia solani*, *Fusarium graminearum*, *Botrytis cinerea*, and *Colletotrichum capsici*, and (iii) analyze the structure−activity relationship of title compounds against the above-tested strains and further investigate the interaction modes of title compounds against SDH. To the best of our knowledge, this is the first report on the design, synthesis, and antifungal activity of pyrazole-4-formylhydrazine derivatives bearing a diphenyl ether fragment as potential SDHIs.

## 2. MATERIALS AND METHODS

**2.1. Chemicals and Instruments.** All used solvents and reagents without further purification were analytical reagent grade. Thin-layer chromatography (TLC) on silica gel $GF_{254}$ was used to monitor chemical reactions. The melting points (mp) of all synthesized compounds were tested using an uncorrected SMP50 Automatic Melting Point Apparatus from Bibby Scientific LTD (Staffordshire, United Kingdom). The synthesized compounds dissolved in a DMSO-$d_6$ or $CDCl_3$ solvent were detected on a BRUKER 400 NMR spectrometer (Bruker Corporation, Germany) to generate corresponding $^1H$ and $^{13}C$ NMR spectra at room temperature. Mass spectral studies on pyrazole-4-formylhydrazine derivatives were performed on a Triple TOF 5600 plus LC/MS/MS spectrometer (AB Sciex, America). A Thermo Nicolet 380 FT-IR spectrometer (Thermo Nicolet Corporation, America) scanned the KBr flake carrier containing a title compound to obtain a relevant infrared (IR) spectrum.

**2.2. General Synthesis Procedures for Intermediates 4.** In an ice bath, sodium nitrite (12.00 mmol) dissolved in distilled water (3.00 mL) was dropwise added into concentrated hydrochloric acid (5.00 mL) containing a substituted phenoxyaniline (10.00 mmol) and then, the resulting mixture was stirred for 1 h. Subsequently, the above solution containing an intermediate **2** was slowly added into concentrated hydrochloric acid (7.50 mL) containing stannous chloride (30.00 mmol) in an ice bath. After the above mixture was stirred at 0 °C for 1 h, the solid that emerged was filtered and washed with distilled water (30.00 mL), cooled alcohol (30.00 mL), and cooled ether (30.00 mL) to obtain a substituted phenoxy phenylhydrazine hydrochloride **3**. The intermediate **3** (10.00 mmol) mixed with sodium hydroxide (12.00 mmol) in distilled water (50.00 mL) was extracted with dichloromethane (30.00 mL × 3), and the obtained organic phase was removed under reduced pressure to generate a substituted phenoxy phenylhydrazine **4**.

**2.3. General Synthesis Procedures for Intermediates 8.** Intermediates **8a−d** were successfully synthesized using the reported procedures, with minor modifications.[4] Acetic anhydride (30 mL) containing a substituted ethyl acetoacetate (100.00 mmol) and triethyl orthoformate (200.00 mmol) was stirred under reflux for 7 h and then, superfluous acetic anhydride was removed under reduced pressure. After the above yellow liquid including an intermediate **6** was slowly added into the ethyl acetate (50.00 mL) containing substituted hydrazine (300.00 mmol) in an ice bath, the obtained solution was stirred under reflux for 3 h and was then extracted with ethyl acetate (50.00 mL × 3). After that, the obtained organic phase was removed under reduced pressure to obtain a substituted ethyl 1*H*-pyrazole-4-carboxylate **7**. Subsequently, the tetrahydrofuran (20.00


https://dx.doi.org/10.1021/acs.jafc.0c03736
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

mL) containing an intermediate 7 (10.00 mmol) and 10% lithium hydroxide (15.00 mmol) was stirred under reflux for 3 h and then, superfluous tetrahydrofuran was removed under reduced pressure. After the remaining solution was acidified with 5% hydrochloric acid until the pH values reached 2.0, the yellow solid formed in water was filtered and washed with distilled water to generate a substituted 1*H*-pyrazole-4-carboxylic acid **8**. Meanwhile, the relevant synthetic procedures for intermediates **8e** and **8f** were reported in detail in our previous work.[51]

**2.4. General Synthesis Procedures for Title Compounds 11a−q.** Dichloromethane (30 mL) containing a substituted 1*H*-pyrazole-4-carboxylic acid **8** (2.00 mmol), a substituted phenoxy phenylhydrazine **4** (2.00 mmol), triethylamine (3.00 mmol), and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumtetrafluoroborate (TBTU, 3.00 mmol) was stirred for 2 h at room temperature. After the above reaction was completed, the superfluous solvent was removed under reduced pressure and the resulting mixture was separated by column chromatography ($V_{\text{petroleum ether}}$:$V_{\text{ethyl acetate}}$ = 1:2) to produce a title compound. The obtained pyrazole-4-formylhydrazine derivatives bearing a diphenyl ether fragment were structurally characterized by Fourier-transform infrared spectroscopy (FT-IR), $^{1}$H NMR, $^{13}$C NMR, and high-resolution mass spectrometry (HRMS) spectra that are collected and presented in the Supporting Information.

**2.5. General Synthesis Procedures for Title Compounds 11r−t.** Thionyl chloride (10.00 mmol) containing a substituted 1*H*-pyrazole-4-carboxylic acid **8** (2.00 mmol) was stirred under reflux for 3 h. After the superfluous thionyl chloride was removed under reduced pressure, the obtained residue dissolved in dichloromethane (15 mL) was stirred with dichloromethane (15 mL) containing (4-(4-chlorophenoxy)phenyl)hydrazine (10.00 mmol) and triethylamine (15.00 mmol) for 1 h in an ice bath. Then, the superfluous solvent was removed under reduced pressure, and the resulting mixture was subsequently separated by column chromatography ($V_{\text{petroleum ether}}$:$V_{\text{ethyl acetate}}$ = 2:1) to produce a title compound. The obtained pyrazole-4-formylhydrazine derivatives bearing a diphenyl ether fragment were structurally characterized by FT-IR, $^{1}$H NMR, $^{13}$C NMR, and HRMS spectra, which are collected and presented in the Supporting Information.

**2.6. Antifungal Effects of Title Compounds *In Vitro* and *In Vivo*.** The tested strains *B. cinerea*, *R. solani*, *F. graminearum*, and *C. capsici* were provided by the State & Local Joint Engineering Research Center of Green Pesticide Invention and Application at Nanjing Agricultural University. In the *in vitro* bioassays conducted in our present work, the commercial agricultural fungicides carbendazim, boscalid, and penthiopyrad were selected as the positive controls to evaluate the practical value of constructed molecules as potential fungicide candidates. Carbendazim is the broad-spectrum systemic fungicide that is currently used for effectively controlling the fungal diseases caused by *R. solani* and *F. graminearum* (two important fungi that were bioassayed in our present work).[52−54] As the widely used SDHI fungicides that were registered for effectively controlling the fungal diseases caused by *B. cinerea* (an important fungus that was bioassayed in our present work),[55,56] boscalid and penthiopyrad are widely utilized as positive controls to evaluate the application potential of the constructed molecules as potential fungicides targeting fungal SDH.[2,7,9−11,13]

The *in vitro* antifungal effects of title compounds against the above phytopathogenic fungi were evaluated using a mycelium growth rate method that is briefly described as follows:[2,5,7−16,29−36] 100 μL dimethylsulfoxide (DMSO) dissolved in a tested compound was added into 45 mL of potato dextrose agar (PDA). After shaking well, the obtained mixture was equally divided and poured into three nine-centimeter Petri plates. Equal DMSO and the commercial agricultural fungicides carbendazim, boscalid, and penthiopyrad were used as the blank and positive controls, respectively. Then, a mycelia dish with a diameter of 5 mm was aseptically inoculated in the center of the above PDA plate with three replicates. The inoculated plates were incubated at 25 ± 1 °C for 3−7 days in a dark environment. After the mycelium diameter of the blank control reached 7.0−7.5 cm, the radial growth of the fungal colonies was measured and the data were statistically analyzed. The title compounds, which had inhibitions that exceeded 50% at 10 μg/mL, were further tested for their antifungal effects at five double-declining concentrations to calculate the corresponding $EC_{50}$ values using SPSS 11.5 software. The *in vivo* anti-*R. solani* effects of title compounds were determined on rice leaves (yiyou 186) using a detached leaf assay, which is a widely used testing method for investigating the practical application of bioactive molecules as potential agricultural fungicides.[31,34,36,57] Although the SDHI fungicides boscalid and penthiopyrad exhibited outstanding anti-*R. solani* effects in the *in vitro* bioassays, they are not registered and recommended for effectively controlling the fungal diseases caused by *R. solani*. Therefore, the agricultural fungicides validamycin and carbendazim, which are registered and recommended for effectively controlling the fungal diseases caused by *R. solani*,[53,58] were selected as positive controls to investigate the practical application of constructed compounds as potential anti-*R. solani* fungicides.

**2.7. *In Vitro* SDH Inhibition Assay.** The succinate dehydrogenase assay kit (BC0955) purchased from Beijing Solarbio Science & Technology Co., Ltd was used to determine the *in vitro* inhibitory effects of title compound **11o** against SDH, with the commercialized SDHIs boscalid and penthiopyrad as positive controls.[5,13] The mitochondrial suspension containing fungal SDH was collected from the mycelia of *R. solani* that had been incubated in potato dextrose medium for 5 days.[14,17] An inhibitor dissolved in 2 μL DMSO was added into 190 μl specific substrates that are provided by the succinate dehydrogenase assay kit (BC0955). After adding 10 μL mitochondrial suspensions into the above specific substrates, the absorbance values of the resulting mixtures were monitored at 595 nm to determine the inhibition effects of corresponding treatments against fungal SDH.[9−11,14,29]

**2.8. Molecular Docking Study.** The three-dimensional conformations of title compounds were drawn using SYBYL 2.0 software (Shanghai Tri-I. Biotech. Inc., China).[2,4,30,51] Molecular energy optimizations of three-dimensional conformations with a convergence criterion of 0.01 kcal/mol were obtained using Powell and Conj Grad conjugate gradient algorithms, Tripos force field, and the Gasteiger−Huckel charge.[2,4,30,51,59] The SDH crystal structure (PDB code: 2FBW) was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org) and was treated using Discovery Studio 4.5 to remove the superfluous nonprotein compositions from the crystal structure of 2FBW.[9−11,17] The above three-dimensional structures of the title compounds and the crystal structure of 2FBW were further treated using AutoDockTools 1.5.6 to generate docking input files.[4,5,7,8] The binding energies between the title compounds and 2FBW were calculated using Autodock 4.2.6.[4,12,14] The obtained three-dimensional binding modes between title compounds and 2FBW were determined using PyMol 1.7.6 and were further treated using Discovery Studio 4.5 to obtain the corresponding two-dimensional binding modes.[4,7−15,60]

## 3. RESULTS AND DISCUSSION

**3.1. Molecular Design and Virtual Verification.** Numerous studies focusing on the structural optimizations of fungal SDHIs have been carried out during the past half a century. These studies have shown that highly efficient and broad-spectrum SDHIs bearing a carboxamide framework should feature two vital molecular characteristics in chemical structures. One structural feature is that the aromatic acid part of carboxamide structures should be optimized as a pyrazole-4-carboxylic acid fragment; the other is the existence of a flexible amide chain on pyrazole-4-carboxamide templates, which was perfectly incorporated into some commercialized agricultural SDHIs, including isoflucypram, pyrapropoyne, and pydiflumetofen. Considering the above two important features in SDHI structures, in the present work, we tactfully conceived a pyrazole-4-formylhydrazine scaffold that has better structural flexibility than the aryl amide fragment in commercialized


https://dx.doi.org/10.1021/acs.jafc.0c03736
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

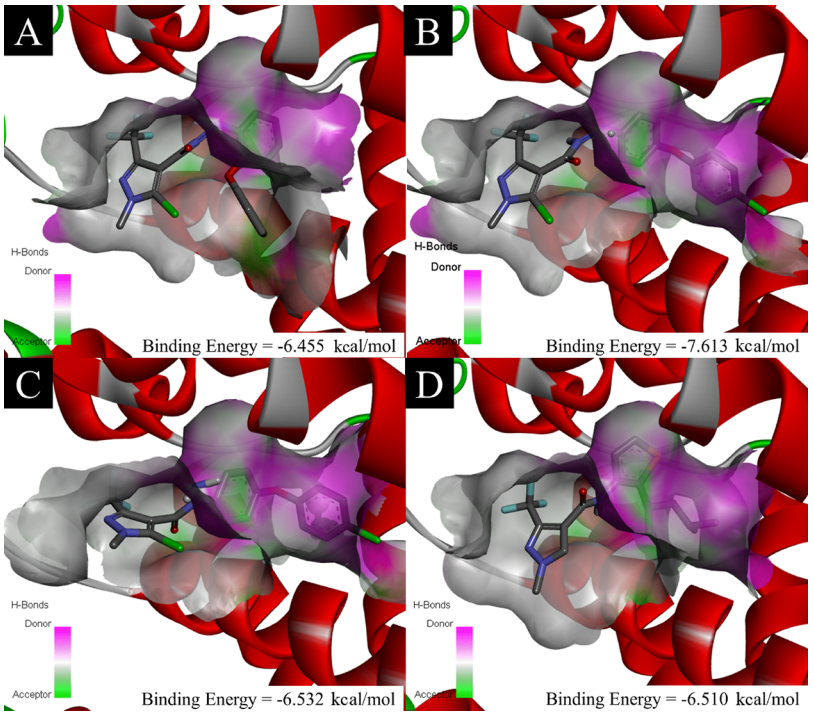



**Figure 3.** Virtual molecular docking comparisons of title compound **11e** (A), title compound **11p** (B), title compound **11t** (C), and penthiopyrad (D) with SDH (PDB code: 2FBW).

carboxamide derivatives. Concurrently, a lipophilic diphenyl ether fragment, which facilitates the permeability of existing fungicides within the cellular membranes of phytopathogens, was ingeniously introduced into the obtained pyrazole-4-formylhydrazine scaffold to construct three types of potential SDHIs that are different in molecular structures (Figure 2). Compared with all commercialized SDHI fungicides bearing a pyrazole-4-carboxamide fragment, the antifungal molecules constructed in our present work display two crucial characteristics in molecular structures. One feature is creatively turning the amide link in the SDHI leads into a novel hydrazide fragment; the other is the location difference of the diphenyl ether fragment that was introduced into the hydrazide group of the constructed pyrazole-4-carbohydrazine scaffold.

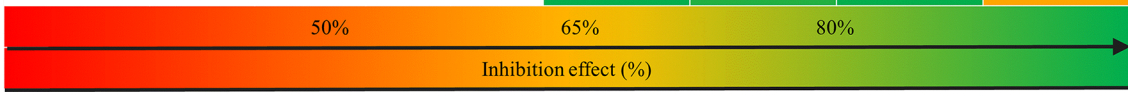
Increasing studies have documented that the discovery of bioactive leads targeting functional proteins was greatly promoted by the verification of virtual molecular docking against constructed molecular skeletons.[4,11,61−64] Therefore, before being synthesized, the feasibility of designed compounds as potential SDHIs was rationally verified by a virtual molecular docking comparison. As shown in Figure 3A−C, the pyrazole-4-formylhydrazine fragment in three types of designed molecules tightly bound with SDH in an identical pocket by approximately the same conformations, which closely resembled the binding conformation of the pyrazole-4-carboxamide fragment in the commercialized SDHI penthiopyrad (Figure 3D). As shown in Figure 3A, the phenoxy fragment of the diphenyl ether moiety in title compound **11e** did not interact obviously with the ambient amino acid residues at the binding pocket, which gave a weaker binding energy of −6.455 kcal/mol. Meanwhile, the binding conformation of the diphenyl ether moiety in Figure 3B was very similar to that of the 2-(4-methylpentan-2-yl)thiophene fragment that is shown in Figure 3D. Additionally, as shown in Figure 3B,C, although the diphenyl ether moiety of title compound **11t** possessed similar binding conformation to that of title compound **11p**, the distorted conformation of the pyrazole ring in Figure 3C led to the weaker interaction of title compound **11t** with SDH than that of title compound **11p** with SDH. Notably, the binding energy of title compound **11p** with SDH was calculated as −7.613 kcal/mol, which was superior to that of penthiopyrad (−6.510 kcal/mol) with SDH, which implicitly indicated that the title compound **11p** had a better inhibition effect against SDH than penthiopyrad. Although the above results generated by virtual molecular docking are not exactly suitable for real situations, it still greatly encouraged us to explore the practical value of constructed molecules as the potential fungicides targeting SDH.

**3.2. Effective Construction.** The synthetic route to pyrazole-4-formylhydrazine derivatives bearing a diphenyl ether fragment is shown in Scheme 1. A substituted phenoxyaniline was oxidized by sodium nitrite to generate an intermediate **2** that was then reacted with stannous chloride in concentrated hydrochloric acid to synthesize a substituted phenoxy phenylhydrazine hydrochloride **3**. The above intermediate **3** was reacted with sodium hydroxide in distilled water to produce a substituted phenoxy phenylhydrazine **4**. The nucleophilic reaction of substituted ethyl acetoacetate with triethyl orthoformate created an intermediate **6** that was reacted with substituted hydrazine to obtain a substituted ethyl 1*H*-pyrazole-4-carboxylate **7**. After the reaction of an intermediate **7** with 10% lithium hydroxide was completed in tetrahydrofuran, the resulting residue was acidized with hydrochloric acid to obtain a substituted 1*H*-pyrazole-4-carboxylic acid **8**. The cyclization reaction of substituted ethyl acetoacetate with substituted hydrazine was carried out under reflux to generate a substituted 2,4-dihydro-3*H*-pyrazol-3-one **9** that was reacted with phosphorus oxychloride under reflux to synthesize a substituted 5-chloro-1*H*-pyrazole-4-


https://dx.doi.org/10.1021/acs.jafc.0c03736
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1. Inhibition Effects (%) of Title Compounds 11a−t against Phytopathogenic Fungi at 10 μg/mL[a]

| compound | $R^1$ | $R^2$ | $R^3$ | $R^4$ | *R. solani* | *B. cinerea* | *F. graminearum* | *C. capsici* |
|---|---|---|---|---|---|---|---|---|
| **11a** | $CH_3$ | $CH_3$ | H | H | 73.6 ± 0.4 | 20.5 ± 0.1 | 59.4 ± 1.0 | 1.0 ± 0.3 |
| **11b** | $CHF_2$ | $CH_3$ | H | H | 41.5 ± 0.1 | 18.1 ± 0.2 | 44.1 ± 1.0 | 10.4 ± 0.8 |
| **11c** | $CF_3$ | $CH_3$ | H | H | 50.5 ± 1.8 | 30.1 ± 0.1 | 40.4 ± 0.5 | 14.0 ± 0.3 |
| **11d** | $CF_3$ | H | H | H | 48.9 ± 0.3 | 24.3 ± 0.3 | 38.9 ± 0.7 | 32.8 ± 0.6 |
| **11e** | $CF_3$ | $CH_3$ | Cl | H | 21.2 ± 1.0 | 42.7 ± 0.2 | 7.3 ± 0.3 | 9.4 ± 0.3 |
| **11f** | $CH_3$ | $CH_3$ | Cl | H | 68.1 ± 0.6 | 38.6 ± 0.2 | 35.6 ± 0.4 | 20.1 ± 0.5 |
| **11g** | $CH_3$ | $CH_3$ | H | H | 81.3 ± 0.3 | 74.4 ± 0.6 | 92.9 ± 0.3 | 52.4 ± 0.3 |
| **11h** | $CHF_2$ | $CH_3$ | H | H | 85.2 ± 0.4 | 75.5 ± 0.7 | 97.4 ± 0.2 | 43.8 ± 0.3 |
| **11i** | $CF_3$ | $CH_3$ | H | H | 85.1 ± 0.3 | 68.0 ± 0.4 | 96.5 ± 0.1 | 43.8 ± 0.1 |
| **11j** | $CF_3$ | H | H | H | 91.2 ± 0.1 | 64.8 ± 0.3 | 96.5 ± 0.1 | 47.6 ± 0.2 |
| **11k** | $CF_3$ | $CH_3$ | Cl | H | 84.3 ± 0.3 | 58.7 ± 0.5 | 92.9 ± 0.3 | 39.7 ± 0.2 |
| **11l** | $CH_3$ | $CH_3$ | Cl | H | 81.1 ± 0.5 | 69.2 ± 0.1 | 94.7 ± 0.2 | 44.5 ± 0.2 |
| **11m** | $CHF_2$ | $CH_3$ | H | Cl | 90.5 ± 0.2 | 82.2 ± 0.1 | 93.1 ± 0.3 | 45.6 ± 0.5 |
| **11n** | $CF_3$ | $CH_3$ | H | Cl | 83.5 ± 0.2 | 77.9 ± 0.4 | 91.5 ± 0.3 | 45.5 ± 0.6 |
| **11o** | $CF_3$ | H | H | Cl | 94.5 ± 0.4 | 79.7 ± 0.4 | 87.1 ± 0.1 | 52.2 ± 0.2 |
| **11p** | $CF_3$ | $CH_3$ | Cl | Cl | 86.7 ± 0.1 | 40.0 ± 0.2 | 88.1 ± 0.1 | 36.6 ± 0.6 |
| **11q** | $CH_3$ | $CH_3$ | Cl | Cl | 76.5 ± 0.1 | 82.5 ± 0.1 | 88.6 ± 0.2 | 57.0 ± 0.4 |
| **11r** | $CH_3$ | $CH_3$ | H | Cl | 67.3 ± 0.2 | 32.5 ± 0.2 | 43.8 ± 0.3 | 37.2 ± 0.1 |
| **11s** | $CHF_2$ | $CH_3$ | H | Cl | 76.9 ± 0.8 | 31.7 ± 0.2 | 24.9 ± 0.2 | 33.6 ± 0.6 |
| **11t** | $CF_3$ | $CH_3$ | Cl | Cl | 73.1 ± 0.3 | 21.3 ± 0.4 | 3.5 ± 0.1 | 37.2 ± 1.0 |
| boscalid[b] | – | – | – | – | 80.3 ± 0.4 | 94.2 ± 0.2 | 30.6 ± 0.1 | 38.6 ± 0.2 |
| penthiopyrad[b] | – | – | – | – | 88.5 ± 0.3 | 79.0 ± 0.2 | 30.9 ± 0.2 | 76.7 ± 0.4 |
| carbendazim[b] | – | – | – | – | 100.0 ± 0.1 | 95.3 ± 0.2 | 100.0 ± 0.1 | 63.6 ± 0.3 |




[a]Average of three replicates. [b]Agricultural fungicides boscalid, penthiopyrad, and carbendazim were used to compare antifungal effects with title compounds.

carbaldehyde **10**. After the above intermediate **10** was oxidized by potassium permanganate, the resulting mixture was acidized with hydrochloric acid to obtain a substituted 5-chloro-1*H*-pyrazole-4-carboxylic acid **8e** or **8f**. The condensation of an intermediate **4** with an intermediate **8** was catalyzed by TBTU in the dichloromethane containing triethylamine to generate a substituted *N*′-(2-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide or *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide. The chlorination of an intermediate **8** with thionyl chloride synthesized a substituted 1-methyl-1*H*-pyrazole-4-carbonyl chloride that expediently reacted with (4-(4-chlorophenoxy)-phenyl)hydrazine to yield a substituted *N*-(4-(4-chlorophenoxy)phenyl)-1-methyl-1*H*-pyrazole-4-carbohydrazide.

**3.3. Spectral Characteristic of Title Compounds.** Three categories of designed compounds were structurally characterized by FT-IR, $^1$H NMR, $^{13}$C NMR, and HRMS spectra. Their structural characteristics were carefully analyzed and are described below. In the FT-IR spectra of title compounds, the obvious absorption peaks located at 3411−2956 cm$^{-1}$ were attributed to the stretching vibrations of an −NHNH− or $-NH_2$ fragment. Simultaneously, the FT-IR signals ranging from 1682 to 1612 cm$^{-1}$ confirmed the presence of a C═O fragment in title compounds. In the $^1$H NMR spectra of title compounds, the protons located at benzene rings and methyl groups were, respectively, recognized by the signals at 7.56−6.73 ppm and 3.95−2.25 ppm. Meanwhile, the characteristic signals of the −CONHNH− group in title compounds **11a−q** were clearly presented by the two peaks at 10.27−9.73 and 8.16−7.36 ppm in their $^1$H NMR spectra and the singlet at 163.61−159.85 ppm in their $^{13}$C NMR spectra. Moreover, the $-NH_2$ fragment in title compounds **11r−t** yielded a singlet with 2H integral areas at 5.57−5.43 ppm in their corresponding $^1$H NMR spectra. In the HRMS spectra of title compounds, the detection values of $[M + H]^+$ ion absorption signals were consistent with their calculated values.

**3.4. *In Vitro* Antifungal Activity Screening of Title Compounds.** A reported mycelium growth rate technique was used as a typical tested method to explore the *in vitro*


https://dx.doi.org/10.1021/acs.jafc.0c03736
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

antifungal effects of title compounds against the phytopathogenic fungi including *R. solani*, *F. graminearum*, *B. cinerea*, and *C. capsici*.[2,5,7−16,29−36] Aiming to contrast the antifungal effects of title compounds against the tested fungi, the agricultural fungicides carbendazim, boscalid, and penthiopyrad were used as positive controls under the same conditions. The preliminary bioassay results in Table 1 show that most of the title compounds, especially those derivatives bearing an *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide skeleton, effectively inhibited the *in vitro* mycelium growth of *R. solani*, *B. cinerea*, and *F. graminearum*, with the inhibition rates surpassing 50% at 10 μg/mL. For example, the anti-*R. solani* effects of title compounds **11g−p** ranged from 81.1 to 94.5% at 10 μg/mL, which were obviously better than that of boscalid (80.3%). Meanwhile, title compounds **11g**, **11h**, **11m**, **11n**, **11o**, and **11q** exhibited impressive anti-*B. cinerea* effects at 10 μg/mL, with the corresponding inhibition rates of 74.4, 75.5, 82.2, 77.9, 79.7, and 82.5%, which were similar to penthiopyrad (79.0%). Remarkably, the anti-*F. graminearum* effects of all *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives (**11g−q**) exceeded 87.1% at 10 μg/mL, which was superior to those of other designed compounds (3.5−59.4%), boscalid (30.6%), and penthiopyrad (30.9%).

Subsequently, the $EC_{50}$ values of some title compounds with significant antifungal activities against *R. solani*, *F. graminearum*, and *B. cinerea* were further evaluated and are presented in Tables 2 and 3. As shown in Table 2, the anti-*R. solani* $EC_{50}$ values of title compounds **11h**, **11i**, **11j**, **11k**, **11n**, **11o**, **11p**, and **11q** were 0.31, 0.25, 0.21, 0.29, 0.31, 0.14, 0.17, and 0.17 μg/mL, respectively, which were observably better than those of the commercialized fungicides boscalid (2.21 μg/mL) and carbendazim (0.34 μg/mL). Meanwhile, as can be seen from Table 3, title compounds **11k**, **11i**, **11m**, and **11q** dramatically inhibited *F. graminearum in vitro*, with corresponding $EC_{50}$ values of 0.32, 0.54, 0.27, and 0.46 μg/mL, which were superior to carbendazim (0.57 μg/mL). In addition, the anti-*B. cinerea* $EC_{50}$ values of title compounds **11g** and **11q** were found to be 0.52 and 0.71 μg/mL, as shown in Table 3, which exceeded that of penthiopyrad (0.83 μg/mL). Interestingly, the antifungal activity screening presented in Tables 1−3 indicates that the skeleton structure of title compounds should be optimized as an *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide scaffold, which is consistent with the virtual verification results that are presented in Figure 3.

**Table 2. Anti-*R. solani* $EC_{50}$ Values of Title Compounds**[a]

| compound | regression equation | *r* | $EC_{50}$ (μg/mL)[b] |
|---|---|---|---|
| **11a** | $y = 1.90x + 3.29$ | 0.99 | 7.91 ± 1.66 |
| **11b** | $y = 1.67x + 3.20$ | 0.94 | 11.87 ± 4.46 |
| **11c** | $y = 1.77x + 3.08$ | 0.94 | 12.17 ± 5.41 |
| **11d** | $y = 1.75x + 2.97$ | 0.88 | 14.37 ± 6.57 |
| **11e** | $y = 1.57x + 3.09$ | 0.93 | 16.31 ± 8.69 |
| **11f** | $y = 1.82x + 3.12$ | 0.98 | 10.67 ± 2.63 |
| **11g** | $y = 1.17x + 5.79$ | 0.99 | 0.38 ± 0.13 |
| **11h** | $y = 1.26x + 5.64$ | 0.99 | 0.31 ± 0.13 |
| **11i** | $y = 1.20x + 5.51$ | 0.96 | 0.25 ± 0.21 |
| **11j** | $y = 1.06x + 5.63$ | 0.99 | 0.21 ± 0.18 |
| **11k** | $y = 1.63x + 5.87$ | 0.98 | 0.29 ± 0.24 |
| **11l** | $y = 1.26x + 5.48$ | 0.98 | 0.41 ± 0.11 |
| **11m** | $y = 1.27x + 5.51$ | 0.99 | 0.39 ± 0.32 |
| **11n** | $y = 1.36x + 5.69$ | 0.99 | 0.31 ± 0.27 |
| **11o** | $y = 0.91x + 5.77$ | 0.98 | 0.14 ± 0.09 |
| **11p** | $y = 0.77x + 5.59$ | 0.98 | 0.17 ± 0.12 |
| **11q** | $y = 0.88x + 5.68$ | 0.99 | 0.17 ± 0.13 |
| **11r** | $y = 1.39x + 3.94$ | 0.99 | 5.83 ± 1.00 |
| **11s** | $y = 1.89x + 3.55$ | 0.99 | 5.85 ± 0.62 |
| **11t** | $y = 2.12x + 3.47$ | 0.97 | 5.30 ± 1.31 |
| boscalid[c] | $y = 1.06x + 4.64$ | 0.99 | 2.21 ± 1.84 |
| penthiopyrad[c] | $y = 0.48x + 5.59$ | 0.98 | 0.06 ± 0.03 |
| carbendazim[c] | $y = 5.09x + 7.38$ | 0.93 | 0.34 ± 0.22 |

[a]Average of three replicates. [b]All results are expressed as the mean ± standard error (SE). [c]The agricultural fungicides boscalid, penthiopyrad, and carbendazim were used to compare antifungal activities with title compounds.

**3.5. Structure−Activity Relationships (SARs).** As can be seen from Tables 1−3, the inhibition effects of title compounds against phytopathogenic fungi were undeniably affected by the structural transformations on molecular skeletons and substituent groups. Based on the *in vitro* bioassay results, some SAR rules were analyzed and are described below. First, Table 1 shows that pyrazole-4-formylhydrazine derivatives containing a 4-phenoxyphenyl fragment, such as **11g**, **11h**, **11i**, **11j**, **11k**, and **11l**, exhibited better antifungal effects against all tested fungi than their isomerides bearing a 2-phenoxyphenyl substructure (**11a**, **11b**, **11c**, **11d**, **11e**, and **11f**). Second, as shown in Table 1, the inhibition effects of *N*′-phenyl-1*H*-pyrazole-4-carbohydrazides against tested fungi declined sharply by converting their molecular skeleton into an *N*-phenyl-1*H*-pyrazole-4-carbohydrazide scaffold. For instance, the antifungal effects of *N*′-phenyl-1*H*-pyrazole-4-carbohydrazides **11m** and **11p** against *R. solani*, *B. cinerea*, and *F. graminearum* at 10 μg/mL were obviously better than those of the corresponding *N*-phenyl-1*H*-pyrazole-4-carbohydrazides **11s** and **11t**. Third, the antifungal $EC_{50}$ values in Tables 2 and 3 indicate that all *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives, except **11m**, presented better antifungal effects against *R. solani* than against *F. graminearum* and *B. cinerea*. Fourth, replacing a $CH_3$ or $CHF_2$ group at the $R^1$ position of *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives with a $CF_3$ fragment will generally enhance the anti-*R. solani* effects of the obtained compounds. For example, the anti-*R. solani* $EC_{50}$ values of title compounds **11i**, **11k**, **11n**, and **11p** ($R^1 = CF_3$), respectively, reached 0.25, 0.29, 0.31, and 0.17 μg/mL, which were obviously better than those of title compounds **11g**, **11l**, and **11q** ($R^1 = CH_3$; 0.38, 0.41, and 0.17 μg/mL) as well as **11h** and **11m** ($R^1 = CHF_2$; 0.31 and 0.39 μg/mL). Fifth, the anti-*R. solani* activities of *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives could be further improved after the methyl group at the $R^2$ position was changed into a hydrogen atom. For instance, the anti-*R. solani* effects of title compounds **11i** and **11n** ($R^2 = CH_3$) with the $EC_{50}$ values of 0.25 and 0.31 μg/mL were lower than those of title compounds **11j** and **11o** ($R^2 = H$), which had $EC_{50}$ values of 0.21 and 0.14 μg/mL. Sixth, when the $R^4$ group of *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives was substituted as a chlorine atom, most of the obtained compounds exhibited better anti-*R. solani* effects than the title compounds bearing a hydrogen atom at the above-mentioned $R^4$ position. As shown in Table 2, the anti-*R. solani* $EC_{50}$ values of title compounds **11o**, **11p**, and **11q** ($R^4 = Cl$) were 0.14, 0.17, and 0.17 μg/mL, which were obviously better than those of title compounds **11j**, **11k**, and **11l** ($R^4 = H$; 0.21, 0.29, and 0.41 μg/mL).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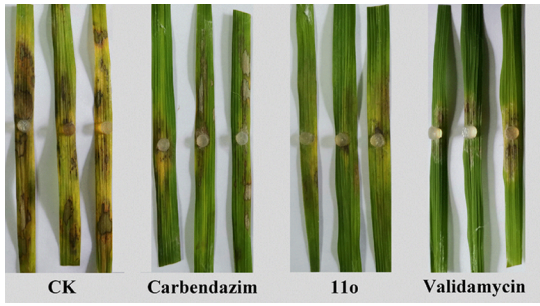
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 3. Antifungal $EC_{50}$ Values of Title Compounds 11g−q against *F. graminearum* and *B. cinerea*[a]

| | *F. graminearum* | | | *B. cinerea* | | |
|---|---|---|---|---|---|---|
| compound | regression equation | $r$ | $EC_{50}$ ($\mu$g/mL)[b] | regression equation | $r$ | $EC_{50}$ ($\mu$g/mL)[b] |
| **11g** | $y = 1.57x + 5.27$ | 0.96 | 0.67 ± 0.48 | $y = 0.96x + 5.27$ | 0.99 | 0.52 ± 0.11 |
| **11k** | $y = 1.58x + 5.79$ | 0.98 | 0.32 ± 0.24 | $y = 0.71x + 4.80$ | 0.98 | 1.89 ± 0.41 |
| **11i** | $y = 2.05x + 5.54$ | 0.98 | 0.54 ± 0.44 | $y = 0.67x + 4.83$ | 0.92 | 1.76 ± 0.73 |
| **11j** | $y = 1.31x + 5.11$ | 0.98 | 0.82 ± 0.65 | $y = 0.90x + 4.71$ | 0.99 | 2.10 ± 0.44 |
| **11k** | $y = 1.64x + 5.24$ | 0.99 | 0.71 ± 0.58 | $y = 0.61x + 4.59$ | 0.98 | 4.59 ± 3.64 |
| **11l** | $y = 1.36x + 5.10$ | 0.97 | 0.84 ± 0.62 | $y = 0.82x + 4.76$ | 0.99 | 1.97 ± 1.60 |
| **11m** | $y = 1.56x + 5.86$ | 0.99 | 0.27 ± 0.25 | $y = 1.20x + 4.69$ | 0.99 | 1.81 ± 1.51 |
| **11n** | $y = 1.46x + 5.28$ | 0.95 | 0.64 ± 0.44 | $y = 0.97x + 4.68$ | 0.99 | 2.11 ± 1.81 |
| **11o** | $y = 1.40x + 5.19$ | 0.87 | 0.73 ± 0.38 | $y = 0.85x + 4.87$ | 0.98 | 1.41 ± 0.48 |
| **11p** | $y = 1.23x + 4.22$ | 0.93 | 4.26 ± 0.52 | / | / | / |
| **11q** | $y = 1.30x + 5.43$ | 0.97 | 0.46 ± 0.34 | $y = 0.91x + 5.14$ | 0.99 | 0.71 ± 0.58 |
| boscalid[c] | / | / | / | $y = 1.22x + 5.32$ | 0.99 | 0.54 ± 0.50 |
| penthiopyrad[c] | / | / | / | $y = 0.70x + 5.06$ | 0.99 | 0.83 ± 0.67 |
| carbendazim[c] | $y = 8.64x + 7.10$ | 0.99 | 0.57 ± 0.51 | $y = 3.54x + 6.35$ | 0.98 | 0.42 ± 0.31 |

[a]Average of three replicates. [b]All results are expressed as the mean ± SE. [c]The agricultural fungicides boscalid, penthiopyrad, and carbendazim were used to compare antifungal activities with title compounds.




| Compound | Preventative efficacy (%) |
|---|---|
| CK (1% DMSO) | – |
| Carbendazim | 59.81 |
| 11o | 73.25 |
| Validamycin | 88.48 |

**Figure 4.** *In vivo* anti-*R. solani* preventative efficacies of bioactive compounds at 200 $\mu$g/mL.

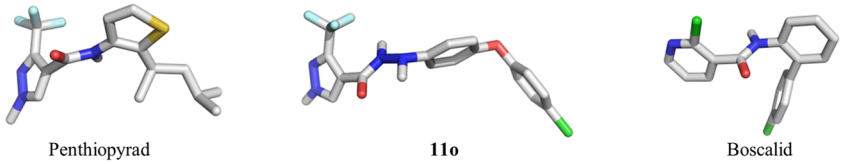



**Figure 5.** Inhibition effects of bioactive molecules against fungal SDH.

**3.6. *In Vivo* Anti-*R. solani* Control Efficacies of Bioactive Compounds on Rice Leaves.** The bioassay results in Tables 1−3 clearly show that the *in vitro* mycelium growth of *R. solani*, *B. cinerea*, and *F. graminearum* could be effectively inhibited by the title compounds (**11g−q**) that utilized an *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide scaffold as their skeleton structure. In the *in vitro* bioassays conducted in our present work, the antifungal effect of title compound **11o** against *R. solani* was evaluated as the best among that of all title compounds. Although lower than that of penthiopyrad (0.06 $\mu$g/mL), the *in vitro* anti-*R. solani* $EC_{50}$ value of title compound **11o** remarkably reached 0.14 $\mu$g/mL, which was approximately 2- and 15-fold higher than those of the commercialized fungicides carbendazim (0.34 $\mu$g/mL) and boscalid (2.21 $\mu$g/mL), respectively. Encouraged by the above results, the *in vivo* anti-*R. solani* effect of title compound **11o** was evaluated on rice leaves (yiyou 186) using a detached leaf assay to investigate its practical application as a potential agricultural fungicide.[31,34,36,57] As shown in Figure 4, the *in vivo* anti-*R. solani* control efficacy of title compound **11o** was 73.25% at 200 $\mu$g/mL, which was obviously better than that of carbendazim under the same conditions (59.81%). The above *in vivo* bioassay result laid a significant foundation for the structural optimization of *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives as potential agricultural fungicides.

**3.7. Inhibitory Effects against Fungal SDH *In Vitro*.** The title compound **11o** exhibiting excellent anti-*R. solani* effects *in vitro* and *in vivo* was selected as a representative molecule to determine its inhibitory effects against the SDH that was collected from the mycelia of *R. solani*. Under the same conditions, the commercialized SDHIs boscalid and penthiopyrad were used as positive controls for comparing the inhibitory effects of title compound **11o** against the above-mentioned SDH. As shown in Figure 5, the $IC_{50}$ values of penthiopyrad, title compound **11o**, and boscalid were, respectively, calculated as 0.79 $\mu$M (0.29 $\mu$g/mL), 3.99 $\mu$M (1.58 $\mu$g/mL), and 13.37 $\mu$M (4.57 $\mu$g/mL). The above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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

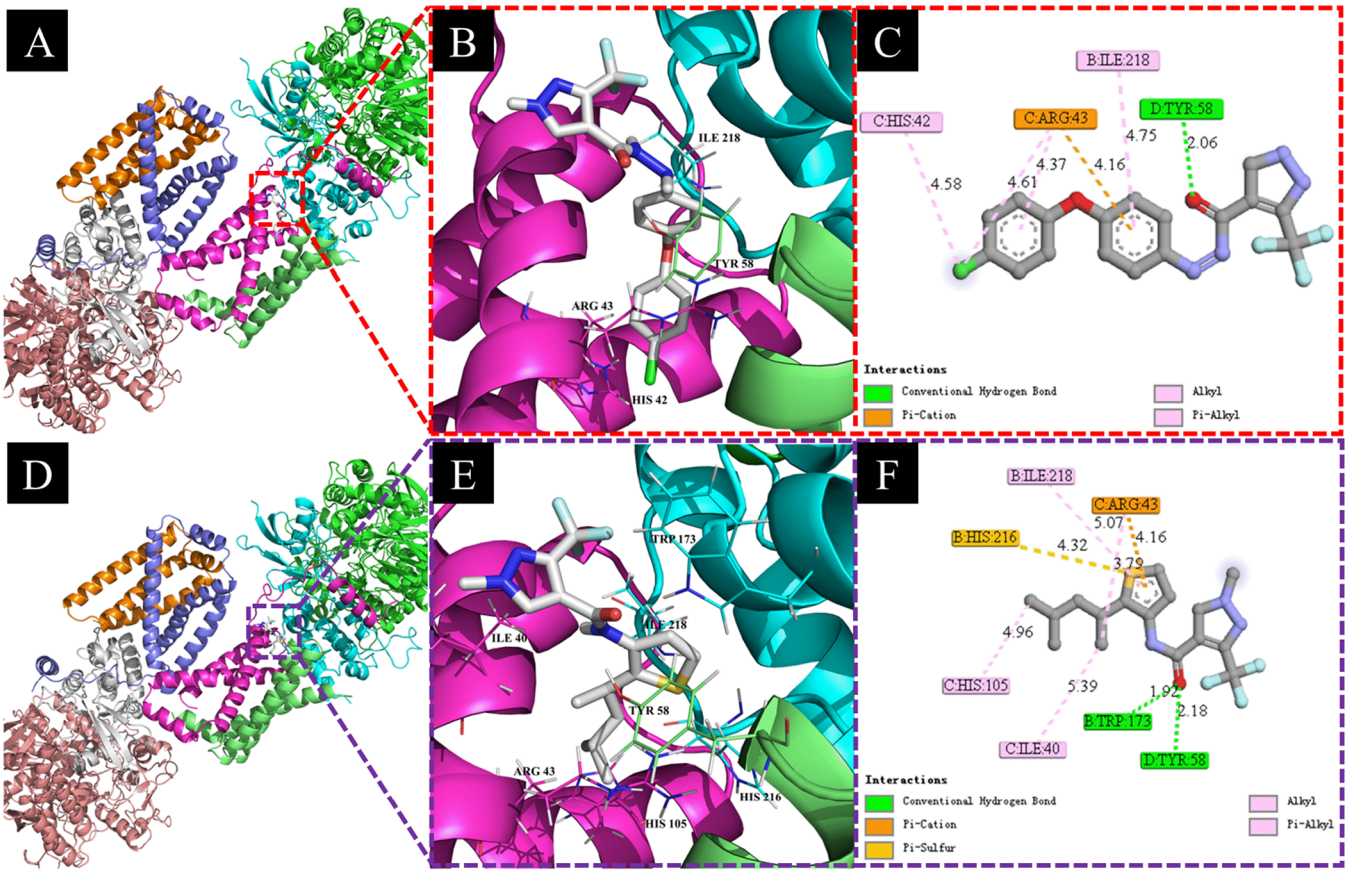


**Figure 6.** Molecular docking modes of title compound **11o** (A−C) and penthiopyrad (D−F) with SDH (PDB code: 2FBW).

bioactive evaluations against SDH provided a non-negligible basis that powerfully ascertains the feasibility of designed compounds as potential SDHIs.

**3.8. Molecular Docking Study.** A semi-open ellipsoid region on succinate dehydrogenase was formed by several crucial residues including tyrosine at the 58 position (TYR 58), histidine at the 42 position (HIS 42), isoleucine at the 218 position (ILE 218), arginine at the 43 position (ARG 43), histidine at the 216 position (HIS 216), tyrosine at the 173 position (TYR 173), isoleucine at the 40 position (ILE 40), histidine at the 105 position (HIS 105), and so on. Numerous crystallographic studies have confirmed that the above-mentioned protein cavity has emerged as the active pocket of the commercialized pyrazole-4-carboxamides targeting fungal SDH.[2−4,6−15,17,18] Based on the important structural features of bioactive SDHI, the title compound **11o** was conveniently synthesized as the potential SDHI that exhibited excellent antifungal effects against phytopathogenic fungi *in vitro* and *in vivo*. To further explore their plausible binding modes with fungal SDH, molecular docking of title compound **11o** with the potential targeting protein (PDB code: 2FBW) was conducted with the commercialized SDHI penthiopyrad as a reference compound for comparison in the same investigation.

As can be seen from Figure 6, title compound **11o** and penthiopyrad were embedded well in the active protein pocket on SDH via approximately the same conformations that connected with circumjacent residues by a conventional hydrogen bond, $\pi$−cation, $\pi$−alkyl, alkyl, and $\pi$−sulfur interactions. For example, TYR 58 showed a strong hydrogen bond with the title compound **11o** (2.06 Å) and penthiopyrad (2.18 Å), and ILE 218 connected the aromatic nucleus in title compound **11o** (4.75 Å) and penthiopyrad (5.07 Å) by a $\pi$−alkyl interaction. Meanwhile, title compound **11o** and penthiopyrad both interacted with ARG 43 by a $\pi$−cation interaction (4.16 and 4.16 Å) and a $\pi$−alkyl interaction (4.37 and 3.79 Å). To some extent, the above-mentioned similar interactions of title compound **11o**/penthiopyrad with the crucial residues on SDH might be pivotal factors to guarantee their inhibition efficiencies against phytopathogenic fungi *in vitro* and *in vivo*. Furthermore, as clearly shown in Figure 6C, the para-substituted chlorine atom at the benzene ring of title compound **11o** formed two alkyl interactions with the residues ARG 43 (4.61 Å) and HIS 42 (4.58 Å), which might be the potential reason why their antifungal bioactive expression is different from other pyrazole-4-formylhydrazine derivatives and penthiopyrad.

In summary, based on the vital structural features of bioactive molecules targeting fungal SDH, 20 pyrazole-4-formylhydrazine derivatives with three categories of structural skeletons were conceived, synthesized, and characterized by FT-IR, $^{1}$H NMR, $^{13}$C NMR, and HRMS analyses. The feasibility of designed compounds as potential SDHIs was subsequently verified by a virtually molecular docking comparison and bioactive evaluations against phytopathogenic fungi *in vitro* and *in vivo*. Consistent with the virtual verification results of a molecular docking comparison, the *in vitro* antifungal bioassays indicated that the skeleton structure of title compounds should be optimized as an *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide scaffold. Among *N*′-


https://dx.doi.org/10.1021/acs.jafc.0c03736
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives, compounds **11o** against *R. solani*, **11m** against *F. graminearum*, and **11g** against *B. cinerea* exhibited striking antifungal effects, with corresponding $EC_{50}$ values of 0.14, 0.27, and 0.52 μg/mL, which were obviously better than those of carbendazim against *R. solani* (0.34 μg/mL) and *F. graminearum* (0.57 μg/mL) as well as penthiopyrad against *B. cinerea* (0.83 μg/mL). Furthermore, the relative studies on an *in vivo* anti-*R. solani* bioassay, bioactive evaluation against SDH, and molecular docking were further explored to ascertain the practical value of compound **11o** as a potential fungicide targeting SDH. The present work laid a non-negligible foundation for further structural optimization of *N*′-(4-phenoxyphenyl)-1*H*-pyrazole-4-carbohydrazide derivatives targeting fungal SDH.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c03736.

Physical and spectroscopic data of all conceived pyrazole-4-formylhydrazine derivatives bearing a diphenyl ether moiety; all of the copies of FT-IR, $^1$H NMR, $^{13}$C NMR, and HRMS spectrogram for title compounds **11a−t**; and binding energies and binding modes of constructed compounds **11a−t** (PDF)

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors gratefully acknowledge the grants from the National Natural Science Foundation of China (No. 31772209), the Fundamental Research Funds for the Central Universities of China (No. KYTZ201604), and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (No. KYCX19_0602).

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