HETEROCYCLES, Vol. 96, No. 1, 2018, pp. 74 - 85. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 23rd October, 2017, Accepted, 27th November, 2017, Published online, 5th December, 2017
DOI: 10.3987/COM-17-13826

SYNTHESIS, ANTIFUNGAL ACTIVITY AND QSAR OF NOVEL PYRAZOLE AMIDES AS SUCCINATE DEHYDROGENASE INHIBITORS

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Abstract – We design and synthesize a series of novel pyrazole amides based on the commercialized fungicides and our previous work. The antifungal activity was tested *in vitro* by mycelial growth inhibition assay. The results show that all the compounds are of antifungal activities against the tested fungi at different levels. Among them, *N-(2-(7-bromo-5-chloro-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide* (**Vk**) exhibited higher antifungal activity than boscalid against two fungi. Molecular docking study revealed that the carbonyl oxygen atom of **Vk** forms two hydrogen bonds toward the hydroxyl hydrogens of TYR58 and TRP173.

INTRODUCTION

Succinate dehydrogenase inhibitors (SDHIs) are a class of fungicides with good inhibition effect of spore germination, germ tube elongation and other negative effects on fungal growth. As the bactericidal mechanism of SDHIs was illustrated in detail, pyrazole amide fungicides are becoming much more popular. In recent years, there are many new products being developed, including boscalid (BASF, 2004), penthiopyrad (Mitsui Corporation, 2010), isopyrazam (Syngenta, 2010), sedaxane (Syngenta, 2011), bixafen (Bayer, 2011) penflufen (Bayer, 2012) and fluopyram (BASF, 2012). Boscalid (*2-chloro-N-(4'-chloro-[1,1'-biphenyl]-2-yl)nicotinamide*) is an efficient fungicide with good inhibition of spore germination, germ tube elongation and other effects on fungal growth. Some studies have shown that the resistance observed in certain species towards SDHIs is due to mutations in the succinate dehydrogenase genes. These results imply that the resistance mutations affecting specific SDHIs could be controlled by structurally different SDHIs.[1] Therefore, the introduction of further structurally diversity to SDHIs

represents a potential strategy for delaying the onset of resistance.

In our previous work, we designed and synthesized a series of amide compounds based on introducing N atom to replace the C atom of the amine moiety of amide.[2] Bioassays have shown that some target molecules exhibited good antifungal activities against *Rhizoctonia solani*, *Botrytis cinerea* and *Pythium aphanidermatum*. By comparing the structure and activity, we found that amides with indazole groups have high antibacterial activity. In this paper, we introduce a series of substituted indazole groups into the target molecule (Figure 1). Some have shown much better activities compared to the previous compounds.

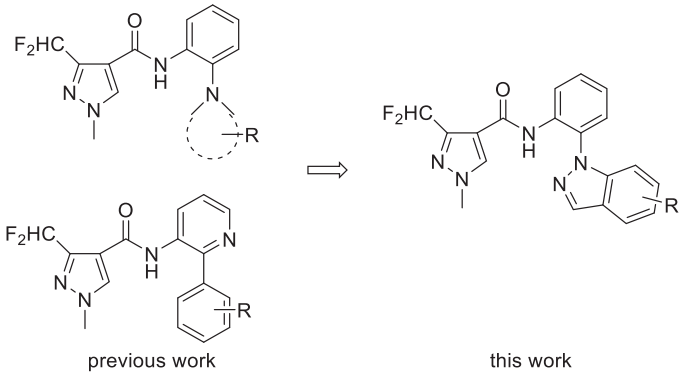
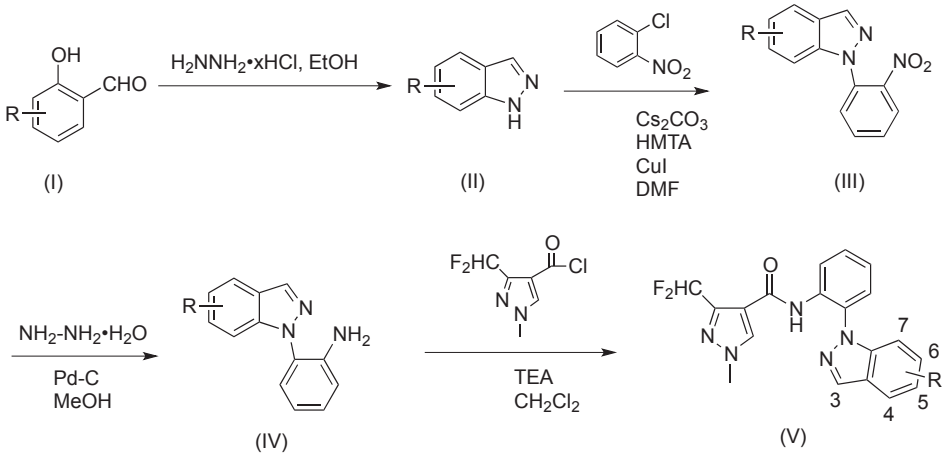


Figure 1. Design strategy of target compound

RESULTS AND DISCUSSION

The synthetic route to the target compounds is shown in **Scheme 1**. The substituted indazole ring was constructed by *o*-hydroxybenzaldehyde and hydrazine hydrate. The chlorine atom in 1-chloro-2-nitrobenzene was replaced by an amino group via an aromatic nucleophilic substitution reaction, giving the compound **III**. Then the nitro group in compound **III** was reduced with hydrazine hydrate to provide the key intermediates **IV**, which were subsequently acylated to produce the target amides **V**.



Scheme 1. The synthetic routes of the title compounds

The EC_{50} of the *in vitro* fungistatic activity of compounds **Va**–**Vs** and boscalid against fungi *Rhizoctonia solan* and *Colletotrichum orbiculare* are listed in **Table 1**. All of the synthetic compounds exhibited antifungal effects on the two fungi. Most compounds of the series showed strong antifungal activity with EC_{50} values <100 μg·mL^{-1}. Among them, the EC_{50} of compounds **Vk** and **Vd** against *Colletotrichum orbiculare* were all below 10 μg·mL^{-1}, the inhibition rates of compounds **Vf**, **Ve** and **Vk** against *Colletotrichum orbiculare* were all below 20 μg·mL^{-1}. The common structural feature of the outstanding compounds is the R in the indazole with a bromine and a chlorine substitution, suggesting that the moiety at this site plays a positive role in the mechanism of their antifungal action. Removing the 5-chlorine of the indazole did not increase any activity against *Rhizoctonia solan* significantly, but was found to be favorable to increase the activity against *Colletotrichum orbiculare*. With the R replaced with 5,7-di-*tert*-butyl, 6-diethylamino, 5-nitro and 6-benzyloxy, it was more unfavorable for the activity. According to the different substituents, the activity decreased according to the following order: bromine > chlorine > fluorine > aliphatic hydrocarbon group > nitro > benzyl. Careful comparison of the activity of compounds **Vk**, **Vl** and **Vf** showed the following order: 5-substituent > 6-substituent > 7-substituent. Small substitutent compounds were better than bulky substitutent in same position, e.g., **Va** > **Vo** > **Vj**. The activities of compounds **Ve** and **Vk** exhibited stronger *in vitro* antifungal activity than boscalid against *Colletotrichum orbiculare*. The EC_{50} value indicated that **Vk** had stronger *in vitro* antifungal activity than boscalid to these two fungi. The EC_{50} values of the compound against *Rhizoctonia solan* and *Colletotrichum orbiculare* were 2.38 μg·mL^{-1} and 6.51 μg·mL^{-1}, respectively. Therefore, it is more reasonable that we mainly synthesized these compounds on **Table 1**, which were more likely to be developed as potential fungicides.

Table 1. Structures and EC_{50} values against *R. solani* and *C. orbiculare*

Compound	Structure	EC_{50} (μg·mL^{-1})	
	R	*R. solani*	*C. orbiculare*
Va	7-methyl	26.51	68.25
Vb	7-ethyl	37.22	78.52
Vc	7-vinyl	39.84	52.31
Vd	7-bromo	5.32	21.63
Ve	7-fluoro	36.40	5.27
Vf	7-chloro	24.55	65.32
Vg	5-fluoro	65.26	89.51
Vh	5,7-dimethyl	78.33	41.52
Vi	5,7-di-*tert*-butyl	186.31	236.51
Vj	7-allyl	104.64	125.60
Vk	**5-chloro-7-bromo**	**2.38**	**6.51**
Vl	6- chloro	15.63	26.31
Vm	6-diethylamino	196.37	241.25

Vn	6-benzyloxy	389.55	>400
Vo	6-ethyl	85.15	102.56
Vp	5-nitro	325.61	245.1
Vq	7-ethoxy	75.42	85.98
Vr	6-methyl	41.80	65.82
Vs	5-methoxy	102.72	126.35
Boscalid		4.85	8.66

In an effort to elucidate the possible antifungal mechanism of the fungicidal activities induced by these compounds, molecular docking of compounds boscalid and **Vk** to the binding site of succinate dehydrogenase (SDH, pdb code: 2FBW[3]) pdb was performed. The crystal structure of SDH was obtained from Protein Data Bank for docking studies. The molecular docking operations in our studies were performed by the Discovery Studio 3.5 Client to investigate the interactions between 2FBW and two molecules. During the whole docking process, drug molecules were flexible, while the protein molecule was kept rigid. 3D and 2D schematic diagrams clearly explained the possible optimal combination between the ligands and receptor protein (Figure 2).

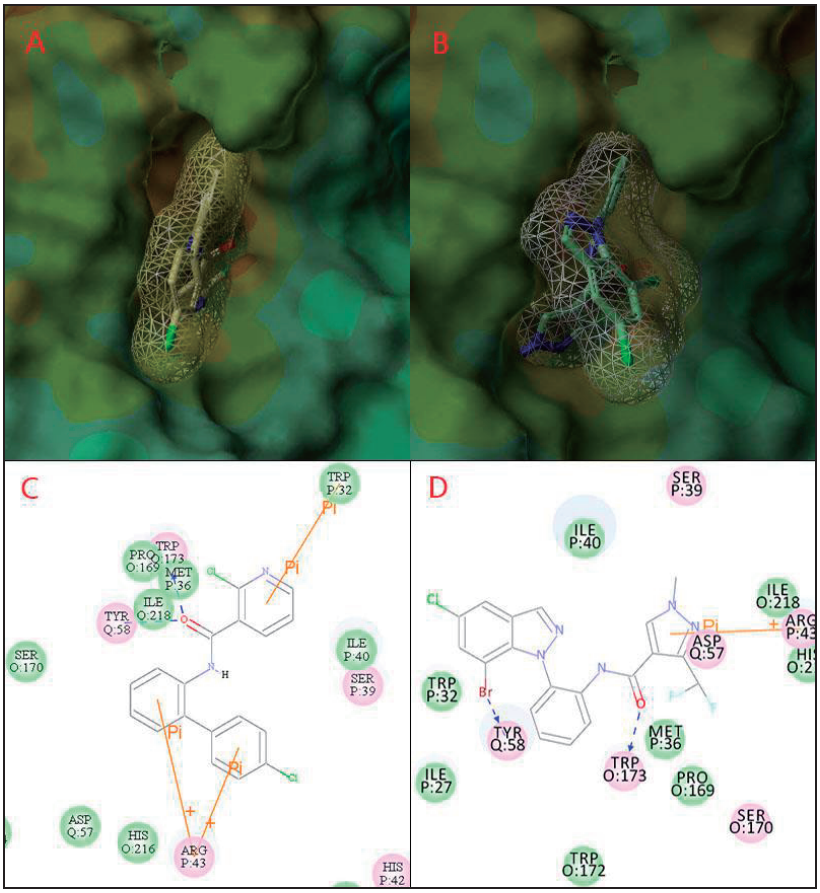


Figure 2. Docking model of boscalid and compounds **Vk**

Boscalid and **Vk** were embedded well in the same protein groove established by extraction of carboxin from the original protein 2FBW. Both were bound to the active site Q_p of SDH and two hydrogen bonds were formed between them to the amino acid residues. Interestingly, although skeleton units are similar, the embedded subunits are different (structures shown in brown in Figure 2; (A) connolly surface of SDH with boscalid shown as a stick model; (B) connolly surface of SDH with **Vk** shown as a stick model; (C) interaction of boscalid and amino acid residues near the ligands (2D diagram); (D) interaction of **Vk** and amino acid residues near the ligands (2D diagram). Two hydrogen bonds were formed between carboxyl oxygen of boscalid and amino acid residues (the amino hydrogen of TRP173 and the hydroxyl hydrogen of TYR58). Three d-d conjugations are simultaneously formed between boscalid to ARG 43 and TRP32. Unlike the previous case, the carboxyl oxygen of **Vk** formed a hydrogen bond with the amino hydrogen of TRP173. Another hydrogen bond was formed between bromine atom of the compound **Vk** and the hydroxyl hydrogen of TYR58. The CDOCKER score (the scoring function value of docking program) of boscalid and compound **Vk** is 78.05 and 86.33, respectively. The docking result appears to be quite harmonious, which may be due to the sensitivity of the protein cavity to the size of the ligand. Resistant fungal genotype analysis verified that most of those key residues involved in forming the binding cavity were related to resistance formation.[4] Thus, a stable complex between compound **Vk** and SDH was formed based on these interactions. The results of this molecular docking could support the postulation that our active compounds may act on the same enzyme target where SDH inhibitor acts, confirming the molecular design of the reported class of antifungal agents.

Nineteen novel pyrazole amides had been synthesized. Two plant pathogenic fungi were used to measure antifungal activities of the compounds. Compounds **Vk** exhibited broad-spectrum and potent antifungal activity. By comparing the activities and structures of the compounds **Vk** with former synthesized lead compounds, it could be found that the indazole ring with bromine atoms introduced to indazole ring play a significant role in improving the activities of the molecules. Molecular docking studies further explained the strong inhibitory activity of **Vk** compared to boscalid of the same series and enabled understanding of the various interactions between the ligands and SDH active sites in detail. Further studies on structural derivation and biometrics are in progress in our laboratory.

EXPERIMENTAL

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-500 spectrometer using TMS as the internal standard. All melting points were obtained on an X-4 binocular microscope melting point apparatus. The HR-MS spectra were acquired on an Agilent 6520 Accurate-Mass apparatus with an ESI source. Solvents and reagents were obtained from commercial suppliers and were used without further purification.

Synthesis of Compounds IIa: To a solution of 2-hydroxy-3-methylbenzaldehyde (5 g, 36.7 mmol) in EtOH (30 mL) was added hydrazine hydrochloride (in excess) in a 250 mL flask. The solution was refluxed for 3 h and the solvent was distilled under reduced pressure.[5] Purification of the residue by column chromatography (EtOAc:PE=10:1) on silica gel was performed to give 3.5 g of 7-methyl-*1H*-indazole, yellow crystals (73% yield). mp 122-124 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.50 (s, 1H, NH), 8.43 (d, *J* = 1.4 Hz, 1H), 7.82 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.13 (dd, *J* = 7.5, 1.4 Hz, 1H), 2.50 (s, 3H-CH_3).

Synthesis of Compounds IIIa: In a 250 mL flask, 1-chloro-2-nitrobenzene (3.5 g, 22.3 mmol), 4-methyl-1*H*-indazole (3.0 g, 23 mmol), cesium carbonate (7.4 g, 23 mmol), HMTA (0.1 g) and CuI (0.1 g) were dissolved in DMF (100 mL). The mixture was heated to reflux for 26 h. After the complete disappearance of the substrates, the reaction was stopped and the mixture was cooled to room temperature. The reaction mixture was passed through a plug of celite and the filtrate was slowly added to the same volume of water, extracted three times with EtOAc and the organic phase was combined.[6] The combined organic layer was dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give a crude product. After the organic layer was concentrated, the residue was purified by column chromatography (EtOAc:PE=8:1) on silica gel to give 4-methyl-1-(2-nitrophenyl)-1*H*-indazole (3.8 g, yellow crystals. yield 68%); mp 172-174 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (d, *J* = 1.4 Hz, 1H), 8.51 (dd, *J* = 7.5, 1.4 Hz, 1H), 8.29 (td, *J* = 7.4, 1.4 Hz, 1H), 8.21 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.98 – 7.86 (m, 1H), 7.82 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.27 (dd, *J* = 7.5, 1.5 Hz, 1H), 2.55 (s, 3H-CH_3).

Synthesis of Compounds IVa: 50 mL EtOH and palladized charcoal (0.1 g, 5%) was added to a 100 mL three-necked flask equipped with a dropping funnel containing 4-methyl-1-(2-nitrophenyl)-1*H*-indazole (3 g, 11.8 mmol), and then 80% hydrazine hydrate (10 mL) from a dropping funnel was added and kept for 30 min.[7] The reaction was then stirred for 8 h and then cooled. The solid was filtered off and the filtrate was evaporated under vacuum to afford the corresponding crude amide product. The residue was purified by column chromatography (EtOAc:PE=4:1) on silica gel to give 2-(4-methyl-1*H*-indazol-1-yl)aniline (2.1 g, white crystals. yield 82%); mp 121-123 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (d, *J* = 1.4 Hz, 1H), 7.80 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.73 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.47 – 7.29 (m, 3H), 7.24 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.02 (dd, *J* = 7.5, 1.4 Hz, 1H), 5.46 (s, 2H, -NH_2), 2.49 (s, 3H, -CH_3).

Synthesis of Compounds Va: A solution of 2-(4-methyl-1*H*-indazol-1-yl)aniline (0.5 g, 2.2 mmol), 25 mL CH_2Cl_2 and 0.5 mL triethylamine was added to a 50 mL flask and cooled to 0 °C in an ice bath. After 10 min, 0.84 g 3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carbonyl chloride was added. The mixture

was stirred for 30 min, and then was concentrated under reduced pressure to give a crude product. The pure 2-chloro-*N*-(2-(piperidin-1-yl)phenyl)nicotinamide (**Va**) was obtained by column chromatography (EtOAc:PE=10:1) purification. Compounds **Vb–Vs** were synthesized according to this way.

***3-(Difluoromethyl)-1-methyl-N-(2-(7-methyl-1H-indazol-1-yl)phenyl)-1H-pyrazole-4-carboxamide* (Va).** white powder. yield 82%; mp 128-130 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (s, 1H, NH), 8.91 (d, *J* = 1.5 Hz, 1H), 8.71 (s, 1H, Pyrazole-H), 8.09 – 8.02 (m, 1H), 8.02 – 7.95 (m, 1H), 7.82 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.79 – 7.67 (m, 2H), 7.48 – 7.42 (m, 1H), 7.30 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.10 (t, *J* = 54 Hz, 1H, -CF_2H), 3.81 (s, 3H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.11, 158.95, 138.05, 137.50, 134.84, 134.40, 134.02, 130.20, 130.19, 126.66, 125.93, 124.29, 123.80, 122.59, 122.36, 117.86, 108.60, 107.12, 40.56, 18.04. HRMS (ESI), *m/z* calcd for $C_{20}H_{17}F_2N_5O$ $(M+H)^+$ 382.1435, found 382.1343.

***3-(Difluoromethyl)-N-(2-(7-ethyl-1H-indazol-1-yl)phenyl)-1-methyl-1H-pyrazole-4-carboxamide* (Vb).** white crystals. yield 75%; mp 143-146 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H, NH), 8.87 (d, *J* = 1.4 Hz, 1H), 8.69 (s, 1H, Pyrazole-H), 8.08 – 8.03 (m, 1H), 8.01 – 7.96 (m, 1H), 7.82 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.77 – 7.69 (m, 2H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.32 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.19 (d, *J* = 54 Hz, 1H, -CF_2H), 3.81 (s, 3H), 2.71 (q, *J* = 6.6 Hz, 2H), 1.18 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.09, 158.94, 138.03, 137.51, 135.68, 134.83, 134.01 130.34, 129.07, 128.82, 126.66, 125.93, 123.45, 122.59, 122.36, 119.38, 108.60, 107.12, 40.56, 28.09, 13.41. HRMS (ESI), *m/z* calcd for $C_{21}H_{19}F_2N_5O$ $(M+H)^+$ 396.1591, found 396.1594.

***3-(Difluoromethyl)-1-methyl-N-(2-(7-vinyl-1H-indazol-1-yl)phenyl)-1H-pyrazole-4-carboxamide* (Vc).** white powder. yield 71%; mp 175-177 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (s, 1H, NH), 8.93 (d, *J* = 1.5 Hz, 1H), 8.73 (s, 1H, Pyrazole-H), 8.10 – 8.03 (m, 1H), 8.02 – 7.96 (m, 1H), 7.84 – 7.77 (m, 1H), 7.76 – 7.70 (m, 2H), 7.56 (dd, *J* =7.5, 1.5 Hz, 1H), 7.50 – 7.41 (m, 1H), 7.33 (d, *J* = 54.6 Hz, 1H, -CF_2H), 7.18 – 7.00 (m, 1H), 5.89 (dd, *J* = 15.0, 7.2 Hz, 1H), 5.44 (dd, *J* = 15.0, 7.2 Hz, 1H), 3.78 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.08, 158.92, 138.05, 137.51, 135.66, 134.84, 134.02, 133.43, 126.66, 126.61, 126.34, 125.98, 125.93, 123.04, 122.59, 122.36, 116.55, 115.05, 108.60, 107.12, 40.56. HRMS (ESI), *m/z* calcd for $C_{21}H_{17}F_2N_5O$ $(M+H)^+$ 394.1435, found 394.1436.

***N-(2-(7-Bromo-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide* (Vd).** white powder. yield 69%; mp 162-164 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (s, 1H, NH), 8.92 (d, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.10 – 8.02 (m, 1H), 8.02 – 7.95 (m, 1H), 7.86 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.81 – 7.69 (m, 2H), 7.68 – 7.61 (m, 1H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 56 Hz, 1H, -CF_2H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.13, 158.97, 138.06, 137.53, 135.44, 134.84, 134.02, 130.55, 130.07, 126.66, 126.11, 125.93, 122.59, 122.36, 121.97, 113.62, 108.60, 107.12, 40.56. HRMS (ESI), *m/z* calcd for $C_{19}H_{14}BrF_2N_5O$ $(M+H)^+$ 447.0329, found 447.0333.

3-(Difluoromethyl)-N-(2-(7-fluoro-1H-indazol-1-yl)phenyl)-1-methyl-1H-pyrazole-4-carboxamide **(Ve).** white powder. yield 86%; mp 153-155 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (s, 1H, NH), 9.11 (d, *J* = 1.6 Hz, 1H), 8.75 (s, 1H), 8.13 – 8.06 (m, 1H), 8.04 – 7.98 (m, 1H), 7.89 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.85 – 7.73 (m, 2H), 7.73 – 7.68 (m, 1H), 7.38 (d, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 56 Hz, 1H, -CF_2H), 3.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.11, 158.99, 143.95, 138.05, 137.50, 134.84, 134.02, 129.78, 127.42, 126.66, 125.93, 125.86, 122.59, 122.36, 118.48, 115.58, 108.64, 107.16, 40.58. HRMS (ESI), *m/z* calcd for $C_{19}H_{14}F_3N_5O$ $(M+H)^+$ 386.1184, found 386.1185.

N-(2-(7-Chloro-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vf).** white crystals. yield 77%; mp 127-129 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (s, 1H, NH), 8.93 (d, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.12 – 7.94 (m, 2H), 7.86 – 7.77 (m, 1H), 7.72 (dd, *J* = 7.5, 1.6 Hz, 2H), 7.49 (dd, *J* = 7.5, 1.6 Hz, 2H), 7.45 – 7.40 (m, 1H), 7.16 (d, *J* = 54 Hz, 1H, -CF_2H), 3.85 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.16, 158.55, 138.05, 137.37, 134.79, 134.08, 132.66, 129.24, 128.79, 126.66, 125.93, 124.85, 122.59, 122.36, 121.94, 120.14, 108.60, 107.12, 40.87. HRMS (ESI), *m/z* calcd for $C_{19}H_{14}F_3N_5O$ $(M+H)^+$ 403.0825, found 403.0824.

3-(Difluoromethyl)-N-(2-(5-fluoro-1H-indazol-1-yl)phenyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vg).** light yellow crystals. yield 81%; mp 180-188 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (s, 1H, NH), 8.93 (d, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.30 (dd, *J* = 7.5, 1.5 Hz, 1H), 8.11 – 7.93 (m, 2H), 7.84 – 7.67 (m, 2H), 7.60 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.27 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.10 (d, *J* = 56 Hz, 1H, -CF_2H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.04, 160.82, 158.95, 137.88, 136.69, 136.53, 134.81, 133.28, 131.01, 126.09, 125.90, 122.76, 122.60, 117.08, 114.16, 109.14, 107.57, 106.45, 40.32. HRMS (ESI), *m/z* calcd for $C_{19}H_{14}F_3N_5O$ $(M+H)^+$ 386.1184, found 386.1185.

3-(Difluoromethyl)-N-(2-(5,7-dimethyl-1H-indazol-1-yl)phenyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vh).** white crystals. yield 73%; mp 156-158 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H, NH), 8.89 (d, *J* = 1.5 Hz, 1H), 8.69 (s, 1H), 8.09 – 7.92 (m, 2H), 7.78 – 7.64 (m, 2H), 7.57 (t, *J* = 1.5 Hz, 1H), 7.31 (d, *J* = 54 Hz, 1H, -CF_2H), 7.15 (d, *J* = 1.5 Hz, 1H), 3.80 (s, 3H), 2.49 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.70, 157.05, 139.11, 137.95, 134.84, 134.02, 132.28, 132.03, 131.05, 130.54, 126.66, 125.93, 125.23, 122.59, 122.36, 120.61, 108.60, 107.12, 40.56, 21.83, 18.73. HRMS (ESI), *m/z* calcd for $C_{21}H_{19}F_2N_5O$ $(M+H)^+$ 396.1591, found 396.1589.

N-(2-(5,7-Di-tert-butyl-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vi).** yellow crystals. yield 78%; mp 161-163 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (s, 1H, NH), 8.95 (d, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.14 – 8.02 (m, 1H), 8.02 – 7.96 (m, 1H), 7.81 – 7.65 (m, 3H), 7.51 (d, *J* = 1.6 Hz, 1H), 7.26 (t, *J* = 56 Hz, 1H, -CF_2H), 3.81 (s, 3H), 1.35 (s, 9H), 1.32 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.11, 158.95, 150.47, 141.67, 140.04, 138.05, 137.95, 134.84, 134.73, 134.02, 126.66, 125.87, 125.54, 122.59, 122.36, 119.30, 108.60, 107.12, 40.56, 38.64, 35.51, 31.36, 31.15.

HRMS (ESI), *m/z* calcd for $C_{27}H_{31}F_2N_5O$ $(M+H)^+$ 480.2530, found 480.2529.

N-(2-(7-Allyl-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vj).** white crystals. yield 71%; mp 147-148 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.26 (s, 1H, NH), 8.93 (d, *J* = 1.4 Hz, 1H), 8.71 (s, 1H), 8.10 – 8.02 (m, 1H), 8.02 – 7.96 (m, 1H), 7.92 (dt, *J* = 7.3, 1.5 Hz, 1H), 7.82 – 7.66 (m, 2H), 7.54 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.48 (t, *J* = 7.4 Hz, 1H), 7.25 (t, *J* = 57.2 Hz, 1H, -CF_2H), 5.92 (ddt, *J* = 16.2, 7.2, 6.1 Hz, 1H), 5.06 (d, 1H, *J* = 6.1 Hz), 4.82 (d, 1H, *J* = 16.2 Hz), 3.81 (s, 3H), 3.33 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.15, 157.55, 137.12, 137.58, 136.82, 135.36, 134.84, 134.02, 128.54, 127.69, 126.66, 126.38, 125.93, 122.59, 122.36, 121.34, 119.07, 115.06, 108.60, 107.12, 40.56, 38.75. HRMS (ESI), *m/z* calcd for $C_{22}H_{19}F_2N_5O$ $(M+H)^+$ 408.1591, found 408.1591.

N-(2-(7-Bromo-5-chloro-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vk).** white crystals. yield 69%; mp 164-166 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H, NH), 8.96 (d, *J* = 1.5 Hz, 1H), 8.71 (s, 1H), 8.11 – 8.02 (m, 1H), 8.02 – 7.96 (m, 1H), 7.84 (t, *J* = 1.6 Hz, 1H), 7.76 – 7.70 (m, 2H), 7.69 (d, *J* = 1.6 Hz, 1H), 7.25 (t, *J* = 56 Hz, 1H, -CF_2H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.45, 157.58, 138.05, 136.82, 134.79, 134.34, 134.02, 132.01, 131.48, 131.26, 126.66, 125.93, 122.59, 122.36, 122.28, 114.68, 108.62, 107.19, 45.86. HRMS (ESI), *m/z* calcd for $C_{19}H_{13}BrClF_2N_5O$ $(M+H)^+$ 480.9940, found 480.9942.

N-(2-(6-Chloro-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vl).** yellow crystals. yield 74%; mp 151-153 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (s, 1H, NH), 8.90 (d, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.09 – 8.02 (m, 1H), 8.02 – 7.97 (m, 1H), 7.96 (d, *J* = 1.5 Hz, 1H), 7.86 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.79 – 7.67 (m, 2H), 7.34 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.10 (d, *J* = 55.4 Hz, 1H, -CF_2H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.79, 159.25, 138.77, 137.65, 136.17, 134.81, 133.28, 130.09, 128.25, 126.09, 125.90, 123.79, 122.76, 122.60, 122.03, 112.28, 108.60, 107.12, 40.56. HRMS (ESI), *m/z* calcd for $C_{19}H_{14}ClF_2N_5O$ $(M+H)^+$ 403.0825, found 403.0823.

N-(2-(6-(Diethylamino)-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vm).** white crystals. yield 77%; mp 174-176 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.35 (s, 1H, NH), 8.83 (d, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.09 – 8.02 (m, 1H), 8.02 – 7.96 (m, 1H), 7.79 – 7.67 (m, 3H), 7.37 (d, *J* = 56 Hz, 1H, -CF_2H), 7.24 (d, *J* = 1.5 Hz, 1H), 6.69 (dd, *J* = 7.5, 1.5 Hz, 1H), 3.81 (s, 3H), 3.40 (q, *J* = 7.2 Hz, 4H), 1.12 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.13, 158.82, 148.37, 145.10, 138.05, 136.17, 134.81, 133.28, 126.09, 125.90, 124.44, 122.86, 122.76, 122.60, 113.87, 108.60, 107.12, 96.99, 46.85, 41.78, 12.99. HRMS (ESI), *m/z* calcd for $C_{23}H_{24}F_2N_6O$ $(M+H)^+$ 439.2013, found 439.2015.

N-(2-(6-(Benzyloxy)-1H-indazol-1-yl)phenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vn).** white powder. yield 73%; mp 138-140 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.40 (s, 1H, NH), 8.88 (d, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.09 – 8.03 (m, 1H), 8.02 – 7.96 (m, 1H), 7.95 – 7.88 (m, 1H), 7.80 – 7.67 (m, 2H), 7.54 (d, *J* = 1.6 Hz, 1H), 7.51 – 7.45 (m, 2H), 7.44 – 7.29 (m, 3H), 7.14 (d, *J* = 56 Hz, 1H,

-CF_2H), 6.96 (dd, *J* = 7.5, 1.6 Hz, 1H), 5.14 (s, 2H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.21, 159.05, 157.93, 139.24, 138.05, 137.56, 136.17, 134.81, 133.28, 129.01, 128.19, 128.16, 126.09, 125.90, 124.13, 122.76, 122.60, 119.91, 112.03, 108.60, 107.12, 97.21, 70.96, 40.56. HRMS (ESI), *m/z* calcd for $C_{26}H_{21}F_2N_5O_2$ $(M+H)^+$ 474.1697, found 474.1699.

3-(Difluoromethyl)-N-(2-(6-ethyl-1H-indazol-1-yl)phenyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vo).** white powder. yield 75%; mp 133-135 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 9.19 (s, 1H, NH), 8.88 (d, *J* = 1.5 Hz, 1H), 8.71 (s, 1H), 8.10 – 8.02 (m, 1H), 8.02 – 7.97 (m, 1H), 7.96 – 7.89 (m, 1H), 7.84 (d, *J* = 1.6 Hz, 1H), 7.79 – 7.65 (m, 2H), 7.23 (d, *J* = 1.5 Hz, 1H), 7.15 (d, *J* = 55.8 Hz, 1H, -CF_2H), 3.81 (s, 3H), 2.72 (q, *J* = 6.2 Hz, 2H), 1.18 (t, *J* = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.51, 162.37, 142.08, 138.05, 136.17, 135.42, 134.81, 133.28, 126.09, 125.90, 123.12, 122.76, 122.60, 122.60, 120.81, 108.60, 108.46, 107.12, 40.56, 28.44, 13.19. HRMS (ESI), *m/z* calcd for $C_{21}H_{19}F_2N_6O$ $(M+H)^+$ 396.1591, found 396.1591.

3-(Difluoromethyl)-1-methyl-N-(2-(5-nitro-1H-indazol-1-yl)phenyl)-1H-pyrazole-4-carboxamide **(Vp).** white powder. yield 84%; mp 159-161 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 9.36 (s, 1H, NH), 9.01 (d, *J* = 1.6 Hz, 1H), 8.79 (t, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.58 (d, *J* = 7.5 Hz, 1H), 8.30 (dd, *J* = 7.5, 1.6 Hz, 1H), 8.12 – 8.03 (m, 1H), 8.03 – 7.95 (m, 1H), 7.80 – 7.65 (m, 2H), 7.25 (t, *J* = 56 Hz, 1H, -CF_2H), 3.66 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.51, 160.15, 144.01, 142.09, 138.05, 136.69, 134.81, 133.28, 126.09, 125.90, 122.76, 122.60, 121.82, 121.14, 117.58, 113.96, 108.60, 107.12, 42.51. HRMS (ESI), *m/z* calcd for $C_{19}H_{14}F_2N_6O_3$ $(M+H)^+$ 413.1129, found 413.1128.

3-(Difluoromethyl)-N-(2-(7-ethoxy-1H-indazol-1-yl)phenyl)-1-methyl-1H-pyrazole-4-carboxamide **(Vq).** yellow crystals. yield 82%; mp 182-184 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 9.23 (s, 1H, NH), 8.94 (d, *J* = 1.4 Hz, 1H), 8.71 (s, 1H), 8.13 – 8.02 (m, 1H), 7.99 (dt, *J* = 7.5, 1.6 Hz, 1H), 7.79 – 7.68 (m, 2H), 7.60 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.29 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.20 (d, *J* = 56.3 Hz, 1H, -CF_2H), 4.07 (q, *J* = 5.9 Hz, 2H), 3.81 (s, 3H), 1.34 (t, *J* = 5.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.13, 160.05, 147.82, 138.05, 137.50, 134.84, 134.02, 130.20, 128.50, 126.66, 125.93, 124.58, 122.59, 122.36, 118.16, 112.85, 108.60, 107.12, 64.46, 40.56, 13.80. HRMS (ESI), *m/z* calcd for $C_{21}H_{19}F_2N_5O_2$ $(M+H)^+$ 412.1540, found 412.1539.

3-(Difluoromethyl)-1-methyl-N-(2-(6-methyl-1H-indazol-1-yl)phenyl)-1H-pyrazole-4-carboxamide **(Vr).** white powder. yield 76%; mp 147-148 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 9.11 (s, 1H, NH), 8.87 (d, *J* = 1.6 Hz, 1H), 8.71 (s, 1H), 8.10 – 8.02 (m, 1H), 8.02 – 7.96 (m, 1H), 7.87 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.79 (t, *J* = 1.6 Hz, 1H), 7.72 (dd, *J* = 7.5, 3.6 Hz, 2H), 7.37 (d, *J* = 56 Hz, 1H, -CF_2H), 7.17 (dd, *J* = 7.5, 1.5 Hz, 1H), 3.81 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.84, 161.78, 138.29, 138.65, 136.17, 135.81, 134.81, 133.28, 126.09, 125.90, 124.08, 122.79, 122.76, 122.60, 122.07, 111.68, 108.62, 107.12, 45.33, 21.23. HRMS (ESI), *m/z* calcd for $C_{20}H_{17}F_2N_5O$ $(M+H)^+$ 382.1435, found 382.1437.

***3-(Difluoromethyl)-N-(2-(5-methoxy-1H-indazol-1-yl)phenyl)-1-methyl-1H-pyrazole-4-carboxamide* (Vs).** white powder. yield 69%; mp 140-142 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.17 (s, 1H, NH), 8.90 (d, *J* = 1.5 Hz, 1H), 8.71 (s, 1H), 8.32 (d, *J* = 7.5 Hz, 1H), 8.13 – 8.03 (m, 1H), 8.02 – 7.93 (m, 1H), 7.82 – 7.61 (m, 2H), 7.43 (t, *J* = 1.5 Hz, 1H), 7.33 (d, *J* = 56 Hz, 1H, -CF_2H) , 7.06 – 6.96 (m, 1H), 4.42 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.05, 162.14, 159.38, 139.42, 136.69, 135.08, 134.81, 133.28, 130.49, 126.09, 125.90, 122.76, 122.60, 114.15, 110.39, 108.60, 106.05, 105.95, 56.48, 48.26. HRMS (ESI), *m/z* calcd for $C_{20}H_{17}F_2N_5O_2$ $(M+H)^+$ 398.1384, found 398.1386.

The fungicidal activity of the target compounds was tested *in vitro* against the *Rhizoctonia solani* and *Colletotrichum orbiculare* using the method of mycelia growth inhibition.[8] The synthesized compounds were dissolved in DMSO to prepare a 10 mg·mL^{-1} solution and further diluted to 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 μg·mL^{-1}. Each test was repeated three times. The antifungal activities were tested according to the methods in reference.[9] We used SPSS 19.0 to perform the statistical analysis.

The automated docking was performed with CDOCKER implemented in Discovery Studio 3.5. The three-dimensional structures of picked compounds were constructed using Chem. 3D ultra 14.0 software. Geometrical structures were energetically optimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger–Hückel charges of ligands were assigned. All bound water and ligands were eliminated from the protein, and the polar hydrogens and the Kollman-united charges were added to the proteins.

ACKNOWLEDGEMENTS

This work was supported by the Guizhou Engineering Research Center (QJHKYZ[2017]024) and Tongren University Dr. fund (trxyDH1509).

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