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Functional Structure/Activity Relationships

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Design, Synthesis and Antifungal Activities of Novel Aromatic Carboxamides Containing Diphenylamine Scaffold

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ABSTRACT: a series of novel *N*-(2-(phenylamino)-4-fluorophenyl)-pyrazole-4-carboxamides **1-15** and aromatic carboxamides with diphenylamine scaffold **16-29** were designed, synthesized and evaluated for their antifungal activities. *In vitro* experiments showed that compound **6** (EC_{50} = 0.03 mg/L) was superior to bixafen (EC_{50} = 0.04 mg/L) against *Rhizoctoinia solani*, and compound **6** (IC_{50} = 1.41 mg/L) was close to bixafen (IC_{50} = 1.22 mg/L) against succinate dehydrogenase from *R. solani*. Additionally, *in vivo* pot experiments showed that compounds **6** (EC_{50} = 1.93 mg/L) was better than bixafen (EC_{50} = 3.72 mg/L) and close to thifluzamide (EC_{50} = 1.83 mg/L) against *R. solani*. And that *in vivo* field trials showed that compound **6** at 200 g ai ha^{-1} had 64.10 % control efficacy against rice sheath blight after 21 days with two sprayings, close to thifluzamide (71.40 %). Furthermore, molecular docking showed that compound **6** anchor in the binding site of SDH.

KEYWORDS: *Aromatic carboxamides, bioisosteric modification, structure-activity relationships, antifungal activity, molecular docking*

INTRODUCTION

Carboxamides inhibiting succinate dehydrogenase (SDH), namely succinate dehydrogenase inhibitors (SDHIs), play an important role in plant protection against many phytopathogenic fungi.[1,2] Among this family, Carboxin was the first commercial product launched by Uniroyal Chemical Co. in 1966.[3] Subsequently a number of Carboxin-related carboxamides had been found with good efficacy in the control of plant diseases, such as Benodanil (BASF), Fenfuram (Bayer), Bixafen (Bayer), Thifluzamide (Dow AgroSciences) and Boscalid (BASF) (Figure 1).[4,5] All of these SDHIs have a common prototypical pharmacophoric scheme, which includes a conserved amide function, a structurally diverse carboxyl "core" and amine moiety (Figure 1). According to the cocrystal structure of SDH from porcine heart,[6] avian,[7] and *Escherichia coli*,[8,9] the carboxyl "core" buries deep into the ubiquinone binding site (Q-site) and contributes predominantly to the binding affinity of SDHIs. Current efforts mainly focused on the modification of the carboxyl "core" and amine moiety of SDHIs.[10-18]

Over the past few years, our group mainly focused on optimization of the amine moiety on SDHIs and discovered some good compounds with antifungal activities.[19-22] For example, by fluxapyroxad being as a lead compound, the diarylamines were introduced to replace the biphenyl group in fluxapyroxad, some pyrazole carboxamides with diarylamine-modified scaffold were synthesized, and compound **1c** presented the highest *in vitro* fungicidal activities against *Rhizoctoinia solani* (*R. solani*) with EC_{50} value of 0.005 mg/L.[19]

Here, to develop the novel carboxamide fungicides, bixafen was applied as a lead compound and the substituted phenylamine group were introduced to replace the biphenyl group by splicing-up. A series of novel N-(2-(phenylamino)-4-fluorophenyl)-pyrazole-4-carboxamides **1-15** containing diphenylamine scaffold were designed and synthesized (Figure 2). Subsequently, *in vitro* antifungal activities were performed to evaluate their antifungal activities against

R. solani, *Phytophthora infestans* (*P. infestans*), *Botryosphaeria dothidea* (*B. dothidea*), *Gibberella zeae* (*G. zeae*), *Fusarium oxysporum f.* sp. *vasinfectum* (*F. oxysporum f.* sp. *vasinfectum*), *Alternaria alternate (A. alternata)* and *Fusarium oxysporum f.* sp. *niveum* (*F. oxysporum f.* sp. *niveum*). In addition, in order to further study antifungal activities of aromatic carboxamides with the diphenylamine scaffold, based on the above selected compound **6** with the highest antifungal activities, some carboxyl "cores" were introduced and a series of novel aromatic carboxamides **16-29** with the diphenylamine scaffold were synthesized and evaluation for their *in vitro* antifungal activities against *R. solani*. And, inhibitory activity against SDH, *in vivo* pot tests against *R. solani*, field trials against rice sheath blight and molecular docking of compound **6** were evaluated.

MATERIALS AND METHODS

Chemicals. All reagents and solvents were commercially available and used directly without further purification.

Fungi. The plant pathogenic fungi, *R. solani, P. infestans, B. dothidea, G. zeae, F. oxysporum f.* sp. *vasinfectum, A. alternata and F. oxysporum f.* sp. *niveum* were kindly provided by College of Life Sciences, Sichuan University, China. These fungi were grown on potato dextrose agar (PDA) plates at 28 °C and maintained at 4 °C with periodic subcultivations.

Instruments. The melting points were determined on an X-4 micro-melting point apparatus (Beijing second optical instrument factory, P.R. China) and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-*d6* as the solvent by using a Bruker 400 MHz NMR spectrometer (Bruker Co., Switzerland), using tetramethylsilane as the internal standard. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 (Qingdao Marine Chemical Ltd., P. R. China). Column chromatography purification was performed over silica gel (300-400 mesh, Qingdao Marine Chemical Ltd., P. R. China). MS data were obtained by using a MALDI-TOF/TOF mass spectrometer (Bruker Co., Switzerland).

Synthesis. The synthetic routes of the target compounds **1-15** and **16-29** were outlined in Figure 3.[19,23]

*General synthetic procedure for intermediate **c***. Anhydrous KF (20 mmol) was added to 2,4-difluoro-1-nitrobenzene **a** (20 mmol) and substituted aniline **b** (30 mmol), and the resulting mixtures were heated at 160 ºC for 14 h. The reaction mixtures were then quenched with water at room temperature, extracted with ethyl acetate (3 x 30 mL). The organic phase was wash with brine, dried over $MgSO_4$, then concentrated under reduced pressure. The residue was subjected to flash column chromatography to give the product **c**.

*General synthetic procedure for intermediate **d***. Compound **c** (15 mmol), reductive iron powder (15 mmol), NH_4Cl (45 mmol) and ethanol aqueous solution (75 %, 60 mL) were added in a flask. The reaction proceeded with refluxing for 3 h at 90 °C. When the reaction finished, the mixtures were cooled to room temperature and extracted with CH_2Cl_2 (3 x 20 mL) and the organic phase was evaporated in vacuum to obtain compound **d**.

*General synthetic procedure for the target compounds **1-15** and **16-29***. Intermediate **d** (10 mmol), EDCI (10 mmol), DMAP (0.1 mmol) and compound **e** (10 mmol) were dissolved in DCM (CH_2Cl_2, 30 mL). The mixture was stirred at room temperature for 6 h. The compounds **1-15** were purified via column chromatography. Similarly, compounds **16-29** were synthesized by using the same methods. The physical data of compounds **1-29** in detail were included in the Supporting Information.

Biological Assay. *In vitro antifungal activities of the target compounds.* All synthesized compounds were screened for their *in vitro* antifungal activities against seven fungi, including *R. solani*, *P. infestans*, *F. oxysporum f.* sp. *vasinfectum*, *B. dothidea*, *G. zeae*, *A. alternate* and *F. oxysporum f.* sp. *niveum* by the mycelium growth rate method.[19,24] The antifungal activities of the target compounds **1-29** in detail were included in the Supporting Information.

Assay of SDH's inhibitory activity. Isolation of R. solani. Fungus mitochondrial was isolated according to a previously reported method.[11,24]The isolation of *R. solani* in detail was included in the Supporting Information.

Activity inhibition of Succinate: Ubiquinone/DCPIP. Activity inhibition of Succinate was carried according to a previously reported method.[11,24]The activity inhibition of the target compound **6** in detail was included in the Supporting Information.

*In vivo pot tests for the target compounds **6**, **9** and **15** against R. solani.* The *in vivo* antifungal activities of **6**, **9** and **15** against *R. solani* were tested according to the procedure described previously.[25]The pot tests of the target compounds **6**, **9** and **15** in detail were included in the Supporting Information.

*Field trials for compound **6** in controlling rice sheath blight.* A rice field naturally infected by *R. solani* was selected to perform the field trial by using the standard method.[25] Each treatment took up in three plots and was distributed randomly in each field. The disease severity was scored by using the following scale.[26,27]The field trials of compound **6** in detail were included in the Supporting Information.

Homology modeling and molecular docking. *Homology modeling.* We applied SDH from avian (PDB ID:1YQ3) as the template, and the homology of the SDH amino acid sequence of *R. solani* was aligned. Homology modeling of SDH from *R. solani* was carried out using MODELER 9.15 (http://salilab. org/modeller/). The homology modeling in detail was included in the Supporting Information.

Molecular docking. Molecular docking studies were performed to investigate the binding mode between compound and the SDH by using Autodockvina 1.1.2.[28] The 3D structure of the compounds were drawn by ChemBioDraw Ultra 14.0 and ChemBio3D Ultra 14.0 softwares and minimized by MM2 using ChemBio3D Ultra 14.0 software. The AutoDockTools 1.5.6 package was employed to generate the docking input files.[29,30]The molecular docking in detail was included in the Supporting Information.

RESULTS AND DISCUSSION

Chemistry. The syntheses of the *N*-(2-(phenylamino)-4-fluorophenyl)-pyrazole-4-carboxamides **1-15** and aromatic carboxamides **16-29** were outline in Figure 3. They were obtained by the reactions i - iii, and MS, ^{1}H NMR and ^{13}C NMR of the target compounds were applied to confirm their structures.

In Figure 3, the syntheses and chemical structures of the target compounds were detailed. Initially, compounds **c** were obtained by condensation reaction of compounds **a** and **b**, and then were transformed into the corresponding compound **d** through reduction reaction. The key aromatic acid **e** reacted with the primary amine **d** under EDCI and DMAP immediately to afford the target compounds **1-15**.[23] Compounds **16-29** were synthesized by using the same method as described.

*In vitro antifungal activities of compounds **1-15** and structure-activity relationships analysis.* The antifungal activities of 15 target compounds were shown in Tables 1 and 2, and bixafen was selected as the positive control at a dosage of 20 mg/L by the mycelium growth rate method.[19,24]The results in Table 1 was the preliminary screening of antifungal activities of the target compounds **1-15** and in Table 2 was the further screening of the target compounds against *R. solani*.

As shown in Table 1, most of the synthesized compounds exhibited good antifungal activities against *R. solani*. Among them, 12 target compounds displayed good antifungal activities (inhibition rate ＞85 %) at 20 mg/L. For example, compounds **2**, **6**, **7**, **9**, **11** and **12** displayed much higher antifungal activities against *R. solani* than bixafen (90.46 %). In addition, the introduced substituents of compounds **1-15** included halogen, methoxyl, trifluoromethoxyl, cyano and so on. As for *F. oxysporum f.* sp. *vasinfectum* (*Fov.*), two compounds **3** (67.46 %) and **12** (64.49 %) have slightly higher antifungal activities than lead compound bixafen (63.49 %). And for the *B. dothidea,* compound **15** (83.73 %) showed relatively higher activities than bixafen (82.54 %). But for the *F. oxysporum f.* sp. *niveum* (*Fon.*), only compound **9** (53.23 %)

displayed higher activities than bixafen (43.55 %).

To analyze the structure-activity relationships (SARs) of the target compounds **1-15** against *R. solani*, all compounds **1-15** were selected for further studies. Their EC_{50} values were listed in Table 2. All compounds showed moderate to good activities with EC_{50} of 0.03 mg/L to 0.88 mg/L. Among the chloro-substituted compounds **2**, **3**, **4**, **5** and **6**, compound **6** (R = 3-Cl-4-Me) showed the best antifungal activity against *R. solani* with EC_{50} value of 0.03 mg/L, higher than that of bixafen (0.04 mg/L). Compound **3** (R = 3,4-Cl_2) also showed good antifungal activity against *R. solani* (EC_{50} = 0.08 mg/L). Compounds **4** (R = 2,4-Cl_2) and **5** (R = 2-Cl-4-Br) exhibited relatively weak activities, even lower than compound **1** (R = H). Bromo-substituted compounds also showed good activities. For example, compounds **9** (R = 2-CH_3-3-Br) displayed higher activity with EC_{50} value of 0.04 mg/L. Meanwhile, compounds **12** (R = 3,5-F_2) and **15** (R = 3,4,5-F_3) also exhibited good activities (0.07 mg/L and 0.05 mg/L, respectively). For the other compounds **10** (R = 3-OCH_3), **11** (R = 3-I), **13** (R = 3-CN) and **14** (R = 3-OCF_3), relatively weak activities were observed.

Inhibitory of SDH. In order to investigate whether the SDH is a potential target enzyme of the target compounds or not, assay of inhibitory activity of the fungal SDH was performed.[24] Compound **6** was selected and tested against SDH from mitochondria of *R. solani.* The lead compound bixafen was used as a control. As demonstrated in Table 3, the selected compound **6** (IC_{50} = 1.41 mg/L) showed good inhibitory activity against SDH, close to bixafen's inhibitory activity (IC_{50} = 1.22 mg/L). It proved that the SDH may be one of the important targets of the compound **6**.

*In vitro antifungal activities of compounds **16-29** and structure-activity relationships analysis.* The carboxyl "core" is one of the prototypical pharmacophoric scheme of aromatic carboxamides and contributes primarily to the binding affinity of the SDHIs. Fortunately, compound **6** showed excellent *in vitro* antifungal activities against *R. solani* and *in vitro* inhibitory activity against SDH. These results indicated that compound **6** could be the novel potential lead compound against *R. solani*. So, in order to further study antifungal activities of aromatic carboxamides with the

diphenylamine scaffold, based on compound **6** as the lead compound**,** some carboxyl "cores" were introduced such as pyridyl, furyl, phenyl and thienyl. Antifungal activities of various aromatic carboxamides **16-29** were evaluated. Preliminary *in vitro* antifungal activities of aromatic carboxamides against *R. solani* were summarized in Table 4. In compounds **16-29**, only compounds **18, 25, 26** and **29** displayed promising antifungal activities (Inhibition rate $\geqslant$ 85 %) against *R. solani* at a dosage of 20 mg/L, and compound **18** (Inhibition rate = 91.67 %) was higher than that of compound **6** (96.17 %) and bixafen (90.44 %)**.**

To analyze the structure-activity relationships (SARs) of the target compounds **16-29** against *R. solani*, all compounds **16-29** were tested for further studies. The results were showed in Table 5. For compounds with pyridyl moieties, compound **18** (R = 2-chloro-3-pyridyl) owned the higher activity than compounds **16** and **17**. For example, compound **18** showed the higher activity (EC_{50} = 0.34 mg/L) than compound **17** (EC_{50} = 0.37 mg/L). In compounds **19-23** with phenyl moieties, compounds **22** (R = 2-methylphenyl) and **23** (R = 2-trifluoromethylphenyl) exhibited the higher activity against *R. solani* with EC_{50} valve of 0.82 mg/L and 0.84 mg/L. In addition, compound **20** (R = 2-chlorophenyl) and **21** (R = 2-iodophenyl) exhibited relatively low activity with EC_{50} values of 1.72 mg/L and 1.66 mg/L, respectively. In compounds **24-29**, compound **29** (R = thiazole) exhibited the highest activity (EC_{50} = 0.31 mg/L) against *R. solani*. But for **27** (R = 2-thiophene) and **28** (R = 3-thiophene), the two compounds revealed lower activities. Similar results also appeared on compounds **24** (R = 2-furyl) and **25** (R = 3-furyl). However, compound **26** (R = 2-methyl-3-furyl) showed relatively good antifungal activity (EC_{50} = 0.35 mg/L). After further analysis of the structure-activity relationships of the compounds **16-29** and **6**, one interesting finding was that compound with ortho-substituent group on the aromatic generally displayed higher antifungal activity than its corresponding compounds. But different substituents influenced their corresponding antifungal activities. This possible reason for this phenomenon might be that ortho-substituent of the aromatic favored the interaction between the compound and the target enzyme

SDH. Unfortunately, no compounds displayed more active than lead compound **6** and bixafen. The results demonstrated that, among all synthesized aromatic carboxamides with the diphenylamine scaffold, compound **6**, 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide, had the best antifungal activity against *R.solani.* One possible answer for this phenomenon may be that the pyrazole unit favor the interaction between ligand and protein.

*In vivo antifungal activities for compounds **6, 9** and **15** against R. solani.* To further know the antifungal activity of the synthesized compounds against *R. solani*, *in vivo* fungicidal activities of compounds **6**, **9** and **15** with high *in vitro* activities were evaluated by pot experiments in a greenhouse environment according to a previously reported procedure.[25-27] The lead compound bixafen and thifluzamide, a commercial fungicide targeting rice sheath blight, were selected as positive control (*in vivo* fungicidal activities were listed in supplementary data). All three compounds exhibited $\geqslant$ 90 % control against tested fungi at 20 mg/L, which was superior to bixafen (80 %), but lower than that of thifluzamide (100 %). Even at a lower dosage of 2.5 mg/L, compounds **6** and **9** still displayed $\geqslant$ 55 % control against *R. solani.* In addition, compound **6** and **9** showed more promising antifungal activity than bixafen at all tested dosages. EC_{50} values of tested compounds against *R.solani* were showed in Table 6. All three compounds exhibited promising activities with EC_{50} values of 1.93 mg/L, 2.29 mg/L and 6.13 mg/L, respectively. Among them, compound **6** and **9** exhibited higher control against *R. solani* than the lead compound bixafen (EC_{50} = 3.72 mg/L). However, compound **6** displayed promising activity (1.93 mg/L), similar to thifluzamide (1.88 mg/L). The results suggested that these molecules could potentially control the disease caused by *R. solani* and field trials were carried out at the next step.

*Field trials for compound **6** controlling rice sheath blight.* To further study the potential of compound **6** controlling rice sheath blight, field trials were carried out in Table 7.[25-27] Rice plants at fruiting stage naturally infected by *R. solani* were used. Applications of compounds **6** and thifluzamide at 200 g ai. ha^{-1} had 48.80 % and

57.19 % control efficacy after the first spraying at the seventh day, respectively. The control efficacy would respectively go up to 64.10 % and 71.40 % after 14 days after the second spraying for compounds **6** and thifluzamide. The result showed that compound **6** was a potential fungicide.

Molecular docking studies. In an effort to explain the possible antifungal mechanism of these aromatic carboxamides containing the diphenylamine scaffold, the compound **6** was docked into the binding site of the SDH and the theoretical binding mode between compound **6** and SDH was shown in Figure 4. The compound **6** fit in the gap composed of subunit B, C and D (Figure 4). The phenyl group in the middle of compound **6** was stretched into the hydrophobic pocket that consisted of the residues B/Pro-202, B/Ile-251, C/Ile-77 and C/Trp-73, while the methylpyrazole group of compound **6** located at another hydrophobic pocket, surrounded by the residues B/Trp-205, B/Trp-206, C/Phe-64 and C/Trp-73, forming a stable hydrophobic binding (Figure 4). Detailed analysis showed that the 3-chloro-4-methylphenyl group of compound **6** formed π-π stacking interaction and cation-π interaction with the residues D/Tyr-128 and C/Arg-80, respectively. In addition, the CH-π interactions were observed between the methylpyrazole group in the terminal of **6** and sidechains of the residues B/Trp-206, C/Phe-64 and C/Trp-73 (Figure 4). Besides, a Cl-π interaction was formed between compound **6** and the residue B/His-249 of SDH (Figure 4). All these interactions helped compound **6** to anchor in the binding site of SDH.

To explain the activity order of bixafen and compound **6** against the SDH, bixafen was then docked into the binding pocket of the SDH, and the theoretical binding mode between bixafen and the SDH were shown in Figure 5. The interaction between bixafen and the SDH was nearly the same as the analog **6** (Figure 5). Compared with bixafen, the methylpyrazole group in the terminal of the compound **6** formed extra CH-π interactions with the residues B/Trp-206, C/Phe-64 and C/Trp-73. Furthermore, the 3-chloro-4-methylphenyl group of compound **6** formed π-π stacking interaction with the residue D/Tyr-128, while bixafen not. Another difference was that

an extra Cl-π interaction was formed between compound **6** and the residue B/His-249 of SDH. Compared with **6,** the oxygen atom in the amido bond of bixafen formed an extra hydrogen bond (bond length: 3.4 Å) interaction with B/Trp-206, however, the hydrogen bond was very weak (Figure 6). All these differences made compound **6** was more active than bixafen (Figure 6).

The estimated binding energies were -8.1 kcal·mol^{-1} for bixafen, and -8.5 kcal·mol^{-1} for compound **6**, respectively. The docking results revealed that compound **6** might be potential SDHI. The above molecular simulations gave us rational explanation of the interactions between bixafen, compound **6** and SDH, which provided valuable information for further discovery of SDHIs.

In summary, a series of *N*-(2-(phenylamino)-4-fluorophenyl)-pyrazole-4-carboxamides **1-15** were designed, synthesized and evaluated for their fungicidal activities against seven phytopathogenic fungi (*R. solani*, *P. infestans*, *F. oxysporum f.* sp. *vasinfectum*, *B. dothidea*, *G. zeae*, *A. alternate* and *F. oxysporum f.* sp. *niveum*). *In vitro* experiments showed that compounds **3**, **6, 9, 12** and **15** showed significant antifungal activities with EC_{50} values of 0.03-0.08 mg/L. Among them, compounds **6** (EC_{50} = 0.03 mg/L) and **9** (EC_{50} = 0.04 mg/L) were superior to the lead compound bixafen (EC_{50} = 0.04 mg/L) and compound **6** had good inhibitory activity against SDH (IC_{50} = 1.41 mg/L), close to bixafen's inhibitory activity (IC_{50} = 1.22 mg/L). Then, based on compound **6**, aromatic carboxamides with diphenylamine scaffold **16-29** were synthesized and evaluated for their fungicidal activities against *R. solani*. Structure-activity relationship analysis showed that pyrazole moiety was the most important carboxyl "core" and displayed high fungicidal activities. In addition, *in vivo* pot experiments showed that compounds **6** and **9** had promising activities against *R. solani* with EC_{50} values of 1.93 mg/L and 2.29 mg/L, respectively, higher than that of the lead compound bixafen (EC_{50} = 3.72 mg/L). And, compound **6** displayed similar activity (EC_{50}= 1.93 mg/L) to thifluzamide (1.88 mg/L). Further field trials showed that compound **6** at 200 g ai. ha^{-1} had 64.10 % control efficacy against rice sheath blight

after the second spraying at the 14th day. Additional computational simulations revealed the possible binding mode of the target compound **6** to the SDH. It was believed that the results of the current studies could support the development of new potent and antifungal drug-like candidates. Further synthesis optimization and toxicological studies are currently in progress.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Supporting Information

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

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Figure captions

Figure 1. Representative SDHIs and their prototypical pharmacophore.

Figure 2. Design of novel *N*-(2-(phenylamino)-4-fluorophenyl)-pyrazole-4-carboxamides **1-15** and aromatic carboxamides **16-29**.

Figure 3. General synthesis of the target compounds **1-15** and **16-29**. Reaction conditions: (i) KF, 160 °C, 14 h; (ii) Fe/NH_4Cl, C_2H_5OH (75 %), 2 h; (iii) EDCI/DMAP, DCM, rt, 6 h.

Figure 4. The theoretical binding mode between compound **6** and SDH and the result was shown by PyMoL 1.7.6.

Figure 5. The theoretical binding mode between bixafen and SDH and the result was shown by PyMoL 1.7.6.

Figure 6. The theoretical binding mode between compound **6**, bixafen and SDH, and the result was shown by PyMoL 1.7.6 (overlap).

Table 1. Preliminary *in vitro* antifungal activities of the target compounds **1-15** at 20 mg/L.

Compd	Inhibition rate (%)						
	Rs	*Pi*	*Fov*	*Bd*	*Gz*	*Aa*	*Fon*
1	86.03±0.25	31.25±0.72	29.36±0.79	64.28±0.79	39.35±0.95	54.97±0.58	29.84±0.81
2	91.91±0.74	50.78±0.78	57.14±0.92	68.25±0.94	38.40±0.00	57.89±0.88	31.45±0.80
3	83.09±0.73	56.72±0.16	67.46±0.79	61.11±1.21	61.32±0.83	60.53±0.87	41.13±0.81
4	89.12±0.31	60.94±0.41	50.00±0.79	77.78±0.46	62.75±0.83	64.91±1.34	37.10±0.93
5	86.77±0.29	27.19±0.48	17.46±00.92	42.86±0.92	48.90±0.48	26.32±0.88	33.87±0.93
6	96.17±0.29	56.87±0.48	61.90±0.92	68.25±0.92	64.66±1.26	49.12±2.03	34.68±0.58
7	94.41±0.22	40.62±0.78	51.59±1.21	68.25±0.92	55.56±2.21	53.51±0.51	30.65±0.93
8	88.53±0.31	64.38±0.65	22.22±1.83	48.78±2.21	63.23±1.26	60.52±0.88	36.29±1.23
9	91.47±0.31	66.10±0.16	61.11±1.21	80.95±0.92	65.62±0.00	68.42±0.35	53.23±0.93
10	86.18±0.30	23.75±0.16	31.75±0.92	43.65±0.79	22.64±3.60	27.00±0.26	28.23±0.81
11	93.23±0.15	50.78±0.78	57.93±0.79	64.28±0.79	71.35±0.29	59.65±1.34	32.16±1.02
12	95.29±0.29	39.84±0.68	64.49±0.40	59.52±0.46	60.84±1.72	58.77±0.16	31.45±0.81
13	83.82±0.73	18.59±0.15	23.80±0.09	30.43±1.32	19.77±2.19	21.93±0.71	19.35±0.93
14	81.17±0.17	22.81±0.31	67.46±1.21	50.79±0.92	47.71±1.24	37.72±0.11	33.18±1.35
15	89.27±0.23	45.00±0.31	42.86±0.46	83.73±0.92	63.23±0.48	72.81±0.13	29.03±0.04
bixafen	90.44±0.25	67.81±0.31	63.49±0.46	82.54±0.46	86.63±1.91	91.89±0.55	43.55±1.86

Rs, *R. solani*; *Pi*, *P. infestans*; *Fon*, *F. oxysporum f.* sp. *vasinfectum*; *Bd*, *B. dothidea*; *Gz*, *G. zeae*; *Aa*, *A. alternate*; *Fov*, F. oxysporum f. sp. niveum.

Table 2. *In vitro* EC_{50} values of the target compounds **1-15** against *R. solani.*

Compd	Regression equation	EC_{50} (mg/L)	95% confidence interval	R
1	Y=0.6389X+5.3657	0.27	0.2098-0.3415	0.9972
2	Y=0.6701X+5.4918	0.18	0.1461-0.2331	0.9977
3	Y=0.4779X+5.5377	0.08	0.0586-0.0959	0.9981
4	Y=0.6785X+5.3570	0.30	0.2411-0.3676	0.9980
5	Y=0.6522X+5.2150	0.47	0.3647-0.6007	0.9974
6	Y=0.6991X+6.0547	0.03	0.0214-0.0449	0.9940
7	Y=0.7481X+5.7382	0.10	0.0659-0.1612	0.9928
8	Y=0.7165X+5.2503	0.45	0.3460-0.5785	0.9968
9	Y=0.5080X+5.7142	0.04	0.0305-0.0505	0.9982
10	Y=0.6159X+5.4744	0.17	0.1629-0.1769	0.9999
11	Y=0.7905X+5.4637	0.26	0.1439-0.4664	0.9846
12	Y=0.7113X+5.8094	0.07	0.0528-0.1005	0.9967
13	Y=0.8024X+5.0461	0.88	0.3911-1.9628	0.9714
14	Y=0.4982X+5.2208	0.36	0.2782-0.4672	0.9968
15	Y=0.4381X+5.5859	0.05	0.0288-0.0733	0.9940
bixafen	Y=0.8124X+6.1105	0.04	0.0357-0.0517	0.9952

Table 3. *In vitro* inhibitory activity of compound **6** against SDH.

Compd	Regression equation	IC_{50} (mg/L)	95% confidence interval	R
6	Y=0.5869X+4.9121	1.41	0.6401-3.1144	0.9427
bixafen	Y=0.6841X+4.9404	1.22	0.6855-2.1783	0.9648

Table 4. Preliminary *in vitro* antifungal activities of aromatic carboxamides **16-29** against *R. solani* at 20 mg/L.

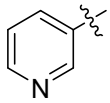
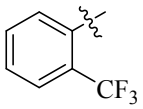
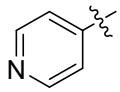
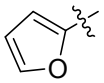
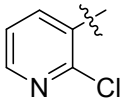
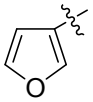
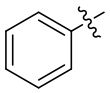
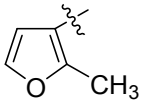
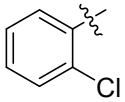
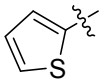
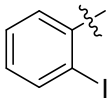
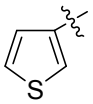
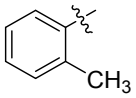
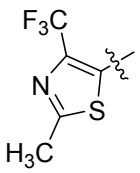
Ar	No.	Inhibition rate (%)	Ar	No.	Inhibition rate (%)
	**16**	77.92±0.50		**23**	78.50±0.48
	**17**	84.07±0.30		**24**	73.97±0.79
	**18**	91.67±0.98		**25**	85.00±0.41
	**19**	73.50±0.44		**26**	85.54±0.18
	**20**	70.54±0.70		**27**	65.00±0.41
	**21**	72.40±0.22		**28**	78.49±0.92
	**22**	79.00±0.41		**29**	87.29±0.58

Table 5. *In vitro* EC_{50} values of aromatic carboxamides **16-29** against *R.solani.*

Compd	Regression equation	EC_{50} (mg/L)	95% confidence interval	R
16	Y=0.6545X+4.9358	1.25	1.0798-1.4548	0.9967
17	Y=0.7770X+5.3367	0.37	0.3012-0.4513	0.9968
18	Y=0.5376X+5.2536	0.34	0.2788-0.4087	0.9972
19	Y=0.7175X+4.7113	2.53	2.1945-2.9077	0.9972
20	Y=0.4888X+4.8850	1.72	1.3389-2.2066	0.9964
21	Y=0.5890X+4.8705	1.66	1.2955-2.1249	0.9964
22	Y=0.5521X+5.0469	0.82	0.6419-1.0534	0.9967
23	Y=0.5300X+5.0392	0.84	0.6636-1.0718	0.9969
24	Y=0.6753X+4.7793	2.12	1.7772-2.5337	0.9954
25	Y=0.8555X+4.9614	1.11	0.9580-1.2847	0.9969
26	Y=0.6204X+5.2857	0.35	0.2835-0.4230	0.9969
27	Y=0.6638X+4.5703	4.44	3.7196-5.2987	0.9964
28	Y=0.6136X+4.9251	1.32	1.1279-1.5555	0.9962
29	Y=0.6095X+5.3140	0.31	0.2620-0.3559	0.9980
6	Y=0.6991X+6.0547	0.03	0.0214-0.0449	0.9940
bixafen	Y=0.8124X+6.1105	0.04	0.0357-0.0517	0.9952

Table 6. *In vivo* EC_{50} values of the target compounds against *R. solani*.

Comp	Regression equation	EC_{50} (mg/L)	95% confidence interval	R
6	Y=1.4566X+4.5841	1.93	1.4034-2.6541	0.9794
9	Y=1.7438X+4.3726	2.29	2.0223-2.5929	0.9962
15	Y=2.5126X+3.0216	6.13	5.4688-6.8682	0.9950
bixafen	Y=1.3155X+4.2488	3.72	3.1194-4.4460	0.9885
thifluzamide	Y=3.8126X+3.9556	1.88	0.8897-3.9685	0.9026

Table 7. Relative control efficacy of compound **6** against rice sheath blight in field trials.

compd	concentration (g ai ha^{-1})	7th day after the first spraying		14th day after the second spraying	
		disease index	control efficacy (%)	disease index	control efficacy (%)
6	200	1.10	48.80±1.21	1.08	64.10±1.55
	100	1.19	44.05±2.46	1.20	59.78±3.56
thifluzamide	200	0.92	57.19±1.73	0.86	71.40±1.60
	100	0.94	56.10±1.68	0.92	69.40±2.48
control	0	2.15	----	3.01	----

Data are means ± SE.

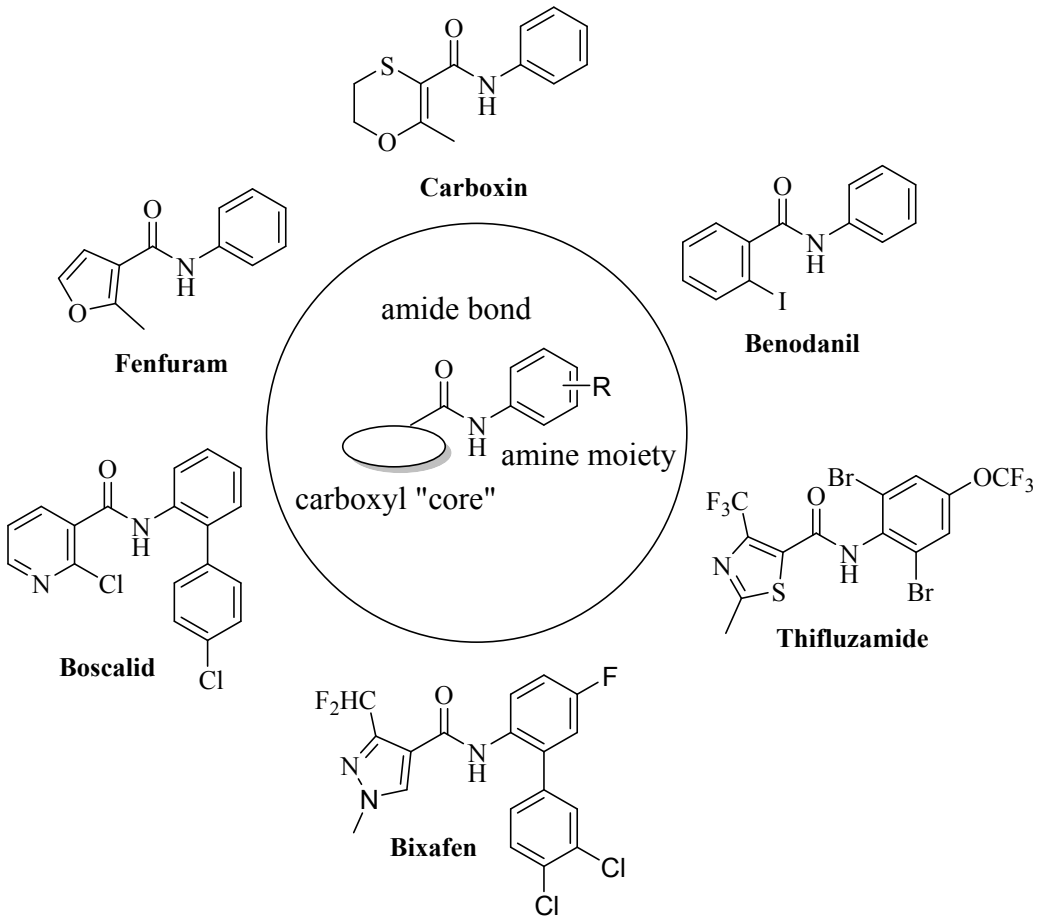


Figure 1. Representative SDHIs and their prototypical pharmacophore.

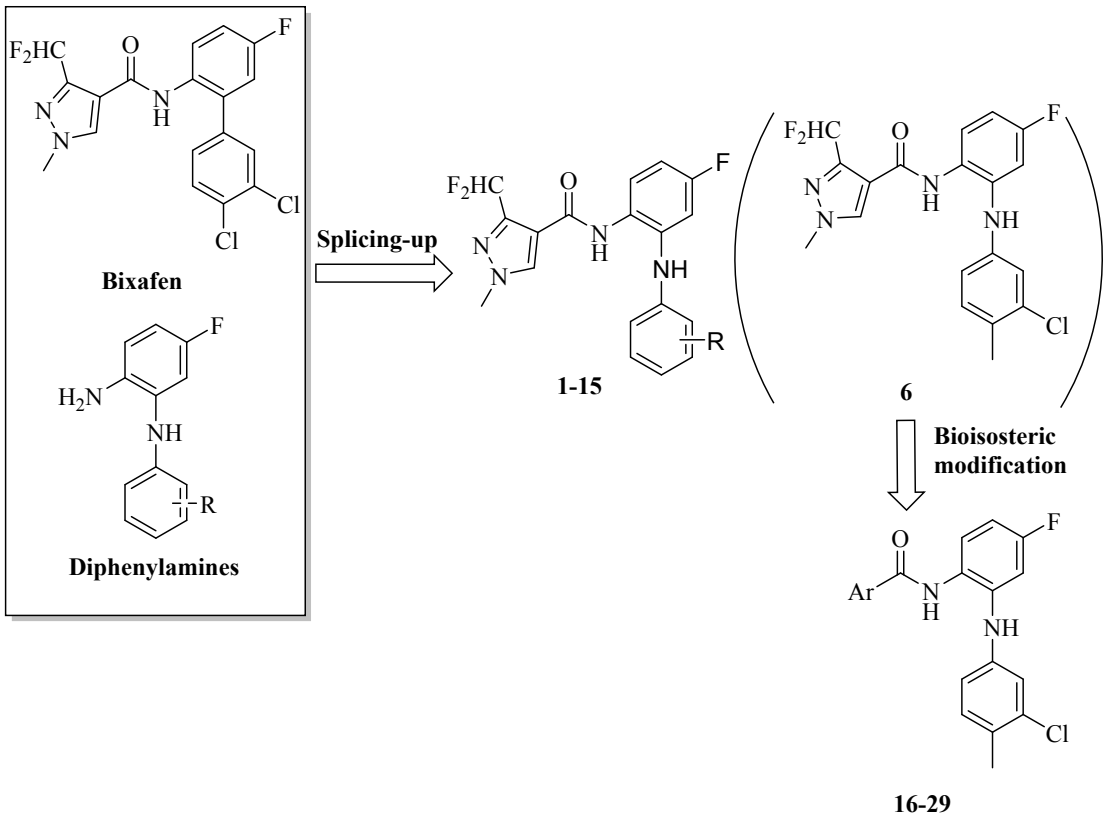


Figure 2. Design of novel *N*-(2-(phenylamino)-4-fluorophenyl)-pyrazole-4-carboxamides **1-15** and aromatic carboxamides **16-29**.

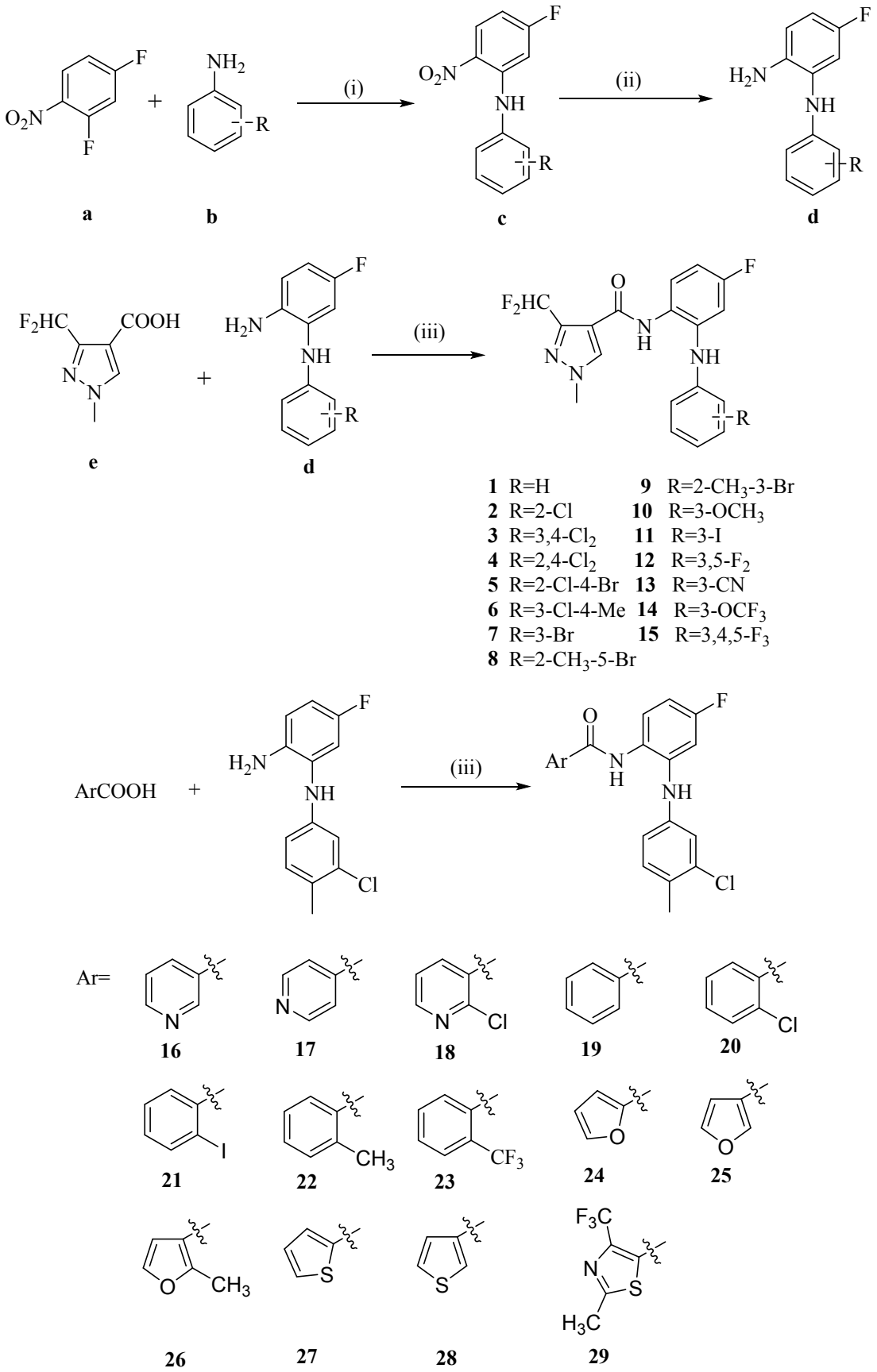


Figure 3. General synthesis of the target compounds **1-15** and **16-29**. Reaction

conditions: (i) KF, 160 °C, 14 h; (ii) Fe/NH_4Cl, C_2H_5OH (75 %), 2 h; (iii) EDCI/DMAP, DCM, rt, 6 h.

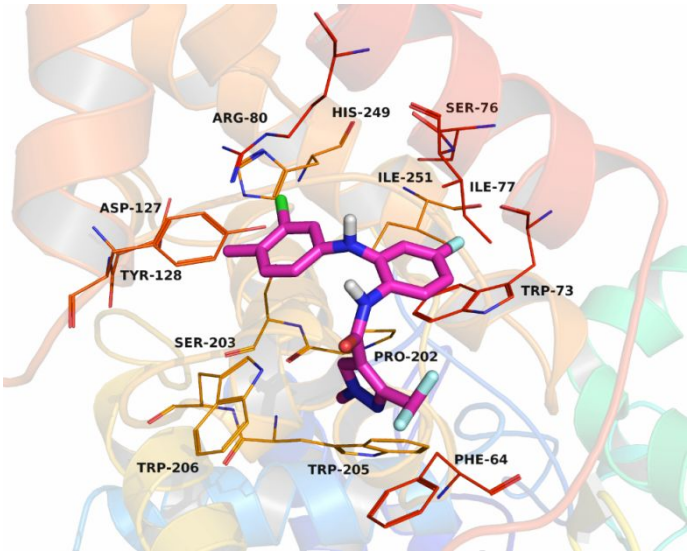


Figure 4. The theoretical binding mode between compound **6** and SDH and the result was shown by PyMoL 1.7.6.

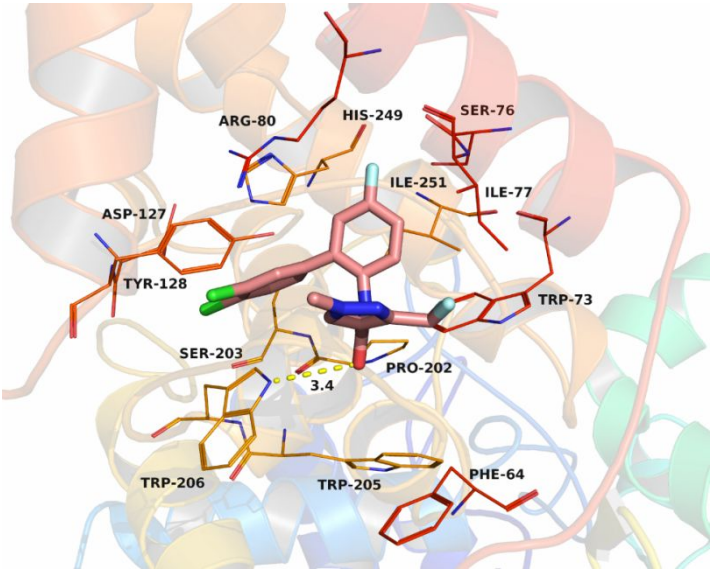


Figure 5. The theoretical binding mode between bixafen and SDH and the result was shown by PyMoL 1.7.6.

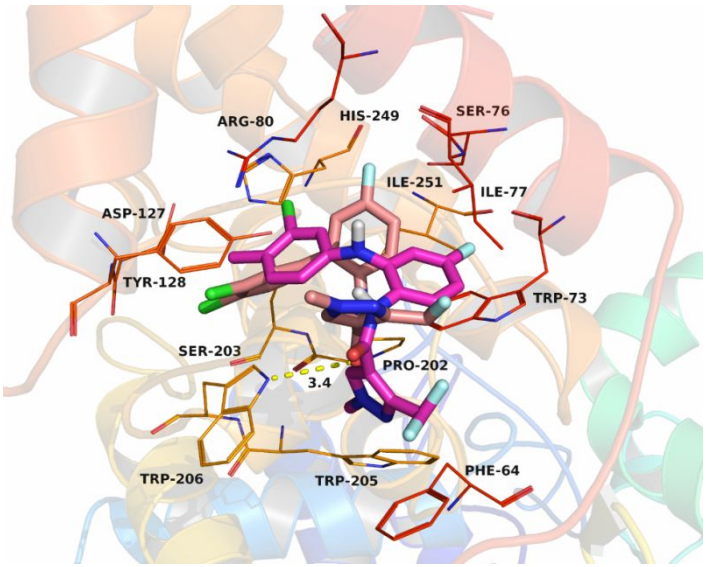
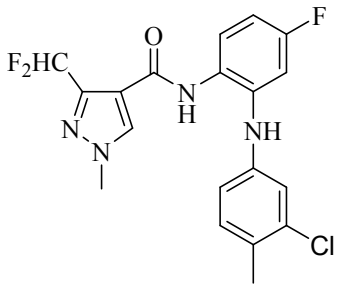


Figure 6. The theoretical binding mode between compound **6**, bixafen and SDH, and the result was shown by PyMoL 1.7.6 (overlap).

TOC graphic



6

Pot tests: EC_{50} = 1.93 mg/L

Field trials: control efficacy = 64.10 %

(200 g ai ha^{-1})