Engineering 6 (2020) 553–559

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Green Plant Protection Innovation—Article

# Design, Synthesis, and Biological Activity of Novel Aromatic Amide Derivatives Containing Sulfide and Sulfone Substructures

Xuewen Hua [a,*], Nannan Liu [a], Sha Zhou [b], Leilei Zhang [a], Hao Yin [a], Guiqing Wang [a], Zhijin Fan [c], Yi Ma [c]

[a] College of Agronomy, Liaocheng University, Liaocheng 252000, China
[b] Collaborative Innovation Center of Zhejiang Province for Green Pesticide, School of Forestry and Bio-technology, Zhejiang A&F University, Hangzhou 311300, China
[c] State Key Laboratory of Elemento-Organic Chemistry, Tianjin Collaborative Innovation Center of Chemical Science and Engineering, Nankai University, Tianjin 300071, China

ARTICLE INFO

*Article history:*
Received 29 December 2018
Revised 18 July 2019
Accepted 20 September 2019
Available online 24 March 2020

*Keywords:*
Synthesis
Nematicidal activity
Fungicidal activity
Molecular docking

ABSTRACT

In recent years, the damage caused by soil nematodes has become increasingly serious; however, the varieties and structures of the nematicides available on the market are deficient. Fluopyram, a succinate dehydrogenase inhibitor (SDHI) fungicide developed by Bayer AG in Germany, has been widely used in the prevention and control of soil nematodes due to its high efficiency and novel mechanism of action. In this paper, two series of novel target compounds were designed and synthesized with nematicidal and fungicidal fluopyram as the molecular skeleton in order to introduce sulfide and sulfone substructures. The structures were identified and characterized by $^{1}H$ nuclear magnetic resonance (NMR), $^{13}C$ NMR, and high-resolution mass spectrometer (HRMS). The bioassays revealed that most of the compounds showed excellent nematicidal activities at 200 $\mu g \cdot mL^{-1}$ in comparison with fluopyram, while the nematode mortality rate dropped sharply at 100 $\mu g \cdot mL^{-1}$, except for compounds **I-11** and **II-6**. In terms of fungicidal activity, compound **I-9** was discovered to have an excellent inhibitory rate, and a molecular docking simulation was performed that can provide important guidance for the design and exploration of efficient fungicidal lead compounds.



## 1. Introduction

In recent years, the harm caused by soil nematodes has become increasingly serious. In particular, there have been outbreaks of damage from root-knot nematodes in some areas [1,2]. The chemical control agents that are widely available on the market are mainly fosthiazate and avermectin B2a (Fig. 1). Traditional highly toxic or virulent nematicides, such as the carbamates aldicarb, carbofuran, and oxamyl, the organophosphates fenamiphos, cadusafos, fensulfothion, and so forth, have been banned or restricted in China. Early fumigants such as methyl bromide have also been phased out due to the destruction of the ozone layer.

Research on new nematicides is extremely significant in the prevention and control of soil nematodes. At present, nematicidal active ingredients are generally developed by screening existing insecticides, herbicides, or fungicides; however, this process results in the slow development of new nematicides and insufficient control agents for nematodes. Recently, some agrochemical companies have reported several new nematicidal active ingredients (Fig. 2); one of these, fluopyram, is a new amide nematicide that was successfully developed by Bayer AG in Germany and that has also been used as a broad-spectrum fungicide [3–6]. Its mechanism of action is to inhibit succinate dehydrogenase (SDH) in the respiratory electron transport chain of mitochondria [7]. Other nematicidal amide structures have been subsequently reported (Fig. 3) [8–14].

Plant diseases have been recognized as a worldwide threat to crop production, and the use of fungicides has been, is, and will remain critical for the effective control of most plant diseases in agriculture [15]. Among the more than 224 fungicides listed by the Fungicide Resistance Action Committee, the succinate dehydrogenase inhibitor (SDHI) class is the fastest growing in terms of new compounds produced and launched onto the market [16]. Thus far, 23 commercial SDHI fungicides—of which fluopyram possesses a unique amide bridge—have been approved for plant protection since the first launch of carboxin in 1966, and have been extensively applied to combat destructive plant fungi, such as *Sclerotinia sclerotiorum*, *Rhizoctonia solani* (RS), and *Botrytis cinerea* (BC) [17,18].

* Corresponding author.
*E-mail address:* huaxuewen@lcu.edu.cn (X. Hua).

https://doi.org/10.1016/j.eng.2019.09.011

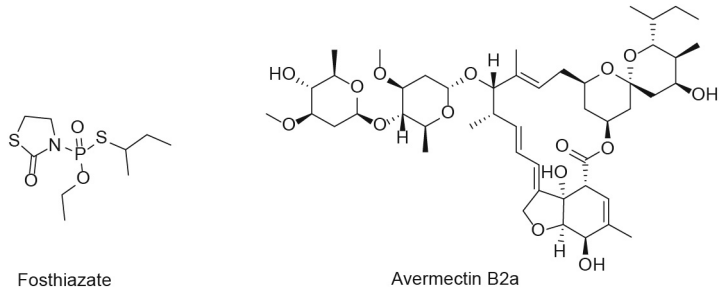



**Fig. 1.** Chemical structures of fosthiazate and avermectin B2a.

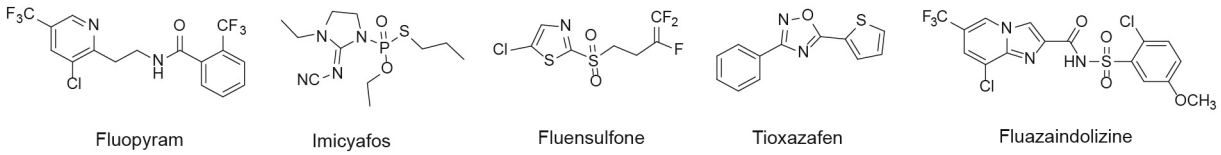



**Fig. 2.** Recently developed nematicidal active ingredients.

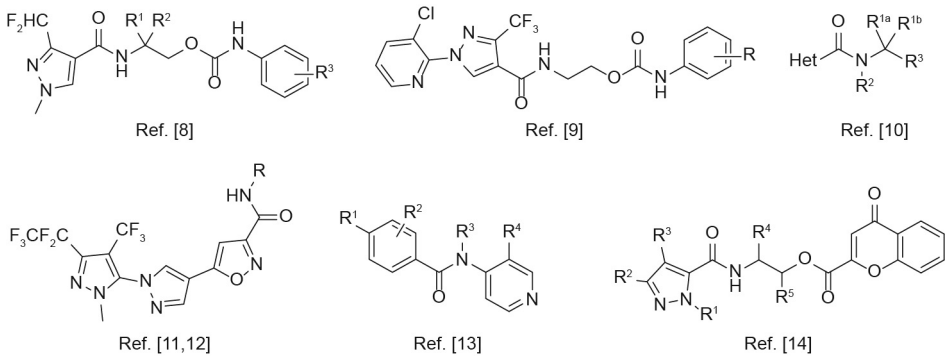



**Fig. 3.** Structures of reported nematicidal amide compounds. Het: substituted aromatic heterocycles.

In the present paper, considering that most of the new nematicidal structures reported above have heterocyclic, sulfide, sulfone, and amide substructures [19,20], while the synthetic procedures of fluopyram involve high-temperature deacidification or high-pressure reduction [21], two series of target compounds were designed and synthesized by introducing sulfide, sulfone, and various aromatic rings into the molecular skeleton of fluopyram (Fig. 4) [19,20]. The synthetic routes for the target compounds **I-1** to **I-12** and **II-1** to **II-12**, and for the intermediate **4a**, are displayed in Fig. 5, and have the advantages of convenient synthesis, simple post-processing, and high yield.

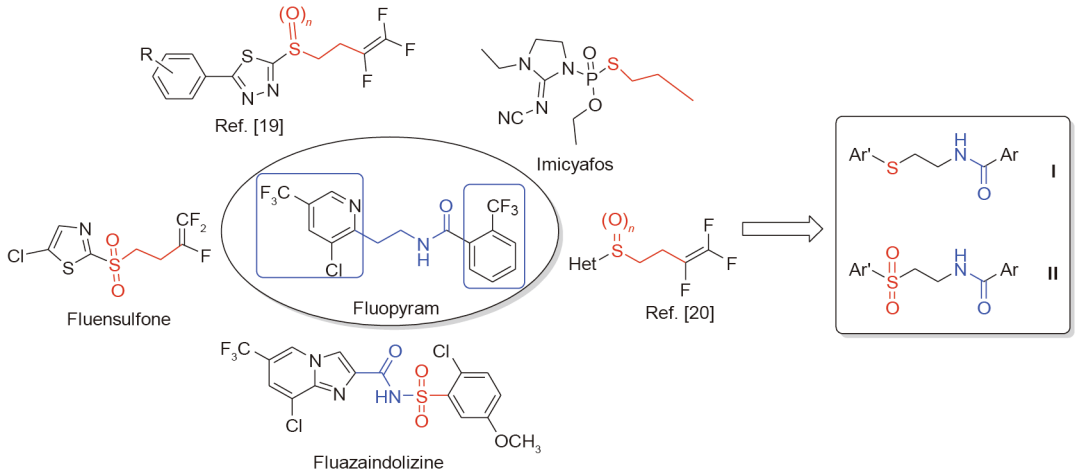
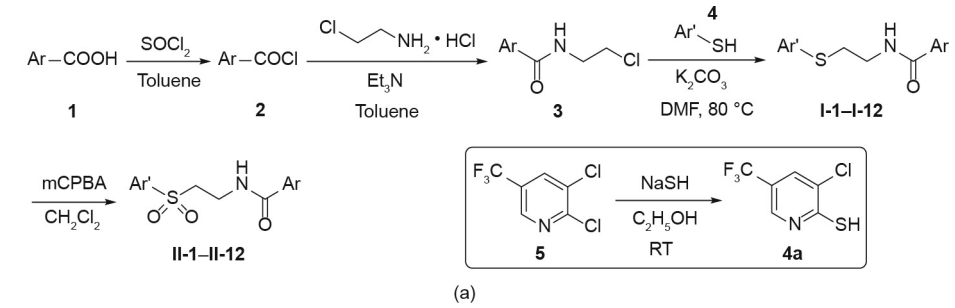
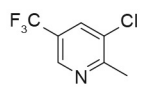
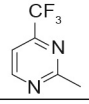
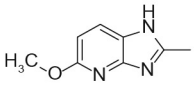
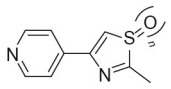
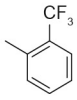
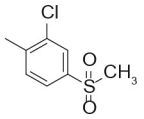
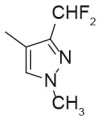



**Fig. 4.** Design strategy of the target compounds. Ar, Ar′: substituted aromatic rings.




(a)

| Ar \ Ar' |  |  |  |  |
|---|---|---|---|---|
|  | **I-1** | **I-2** | **I-3** | **I-4** (*n* = 0) |
| | **II-1** | **II-2** | **II-3** | **II-4** (*n* = 1) |
|  | **I-5** | **I-6** | **I-7** | **I-8** (*n* = 0) |
| | **II-5** | **II-6** | **II-7** | **II-8** (*n* = 1) |
|  | **I-9** | **I-10** | **I-11** | **I-12** (*n* = 0) |
| | **II-9** | **II-10** | **II-11** | **II-12** (*n* = 1) |

(b)

**Fig. 5.** Synthetic route of the target compounds. (a) Organic synthetic route of the target compounds; (b) the structures of different substituted aromatic rings. DMF: *N*,*N*-dimethylformamide; mCPBA: meta-chloroperoxybenzoic acid; RT: room temperature; Et: ethyl.

## 2. Materials and methods

### 2.1. Reagents and instruments

All reaction reagents were of analytical grade. Melting points for target compounds were determined on an X-4 binocular microscope (Gongyi Tech. Instrument Co., China). $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR) was performed using a Bruker AV-400 spectrometer (400 MHz), and chemical-shift values ($\delta$) were reported as parts per million (ppm) with tetramethylsilane as the internal standard. Mass spectra were recorded using a high-resolution mass spectrometer (HRMS) (Varian 7.0 T FTMS, Agilent Technologies, USA). Column chromatography purification was carried out using silica gel (200–300 mesh).

### 2.2. Synthesis of target compounds

The intermediate **3** and **4a** and the target compounds **I-1** to **I-12** and **II-1** to **II-12** were prepared according to previously reported methods [8,22,23]. The corresponding synthetic procedures and characterization data are available in the Supplementary data.

### 2.3. Biological activity screening

The nematicidal activities of the target compounds against *Meloidogyne incognita* were screened and evaluated with reference to the literature [24,25]. Eggs of *Meloidogyne incognita* were extracted from the infected roots of tomato (*Solanum lycopersicum* L.) into a solution of sodium hypochlorite (NaOCl). To obtain second-stage juveniles (J2), the eggs were spread on a mesh nylon filter (openings 30 μm in diameter) in a Petri dish containing water and incubated at 25 °C. Emerging J2 individuals that passed through the filter were collected daily and used for bioassays immediately. The stock solution was prepared by dissolving the target compounds in dimethyl sulfoxide and diluting with 0.1% Tween-80 aqueous solution. The test solutions were introduced into the wells of 24-well tissue culture plates. In each well, the concentration of nematodes was approximately 100 juveniles of *Meloidogyne incognita* per 1 mL of water. The plates were covered and maintained at (25 ± 1) °C, and each treatment was replicated three times. Nematode mortality was observed under a stereomicroscope after 24 h. Nematodes were classified as dead if their bodies were motionless (i.e., straight) even after being transferred to clean water for 12 h.

In addition, considering the fungicidal activity of the reference molecule fluopyram, the *in vitro* fungicidal inhibition rates of the target compounds were investigated using a mycelia growth inhibition method, as previously reported [26]. Common agricultural pathogens, including RS, *Gibberella zeae* (GZ), *Physalospora piricola* (PP), *Cercospora circumscissa Sacc.* (CS), *Alternaria kikuchiana Tanaka* (AK), BC, *Colletotrichum capsici* (CC), and *Phomopsis vexans* (PV), were taken as the test objects.

### 2.4. Molecular docking

The Surflex-Dock method [27] was applied to study the binding mode of the target compound **I-9**, which displayed an excellent fungicidal inhibition rate, with SDH while using the SYBYL 6.9 software package. The literature [7] reports that fluopyram is an SDHI that specifically binds to the ubiquinone-binding site (Q-site) of the mitochondrial SDH. Compound **I-9** and fluopyram were

manually docked into the active Q-site in *Escherichia coli* SDH based on the binding positions at the Q-site for ubiquinone in *Escherichia coli* SDH [28], which were retrieved from the RCSB Protein Data Bank (PDB ID: 1NEK). The receptor and the ligand molecule were prepared using standard procedures.

## 3. Results and discussion

### 3.1. Synthetic chemistry

The key intermediate **3** and the target compounds **I-1** to **I-12** and **II-1** to **II-12** were designed and synthesized according to the procedures reported in the Supporting data. The acyl chloride **2** was prepared through the chlorination reaction of aromatic formic acid, and then converted to amide **3** by a reaction with 2-chloroethylamine hydrochloride. The aromatic thiophenol **4** was obtained from either the market or laboratory preparation; of these compounds, 3-chloro-5-(trifluoromethyl)pyridine-2-thiol (**4a**) was synthesized by the nucleophilic substitution of 2,3-dichloro-5-(trifluoromethyl)pyridine and sodium hydrosulfide. Finally, the *N*-(2-chloroethyl)aromatic amide **3** and thiophenol **4** were reacted to generate the target compounds **I-1** to **I-12**, which were oxidized with meta-chloroperoxybenzoic acid (mCPBA) to yield the products **II-1** to **II-12**. Surprisingly, the sulfur atom on the thiazole ring of compounds **I-4**, **I-8**, and **I-12** was oxidized to sulfoxide to yield **II-4**, **II-8**, and **II-12**, respectively, under excess mCPBA conditions. The advantages of this result were that the introduction of the sulfide substructure made the synthesis of the target compounds more convenient and faster than that of the control fluopyram, and avoided the reaction conditions of high temperature and high pressure. Subsequently, all target compounds were identified and characterized by $^{1}$H NMR, $^{13}$C NMR, and HRMS. Several unique structural characteristics were also revealed via the crystal structure of compound **I-3** (CCDC Number 1830647, Fig. 6).

### 3.2. Biological activity

The nematicidal activities of the target compounds against *Meloidogyne incognita*, with fluopyram as a positive control, are shown in Table 1. According to the data, most compounds displayed excellent nematicidal activity at a concentration of 200 μg·mL$^{-1}$, in comparison with fluopyram, except compound **I-2**. When the test concentration was reduced to 100 μg·mL$^{-1}$, the nematicidal activities of the target compounds changed greatly, and most showed lower mortality. However, compounds **I-11** and **II-6** still exhibited good nematicidal activity at 100 μg·mL$^{-1}$, with mortalities of 75% and 70%, respectively, and therefore provide a valuable guide for the further exploration of potential efficient nematicidal lead compounds. In addition, there was little difference in the mortality rates between the sulfide and sulfone substructures.

Considering the fungicidal activity of the reference molecule, fluopyram, the fungicidal inhibition rates of the target compounds were further measured. The results are shown in Table 2. According to the data, most of the target compounds showed extremely weak fungicidal activity in comparison with fluopyram, except for compound **I-9**, whose inhibition rates were almost comparable to those of the control. Furthermore, similar to the nematicidal activity, there was no significant difference in the inhibitory activity between the sulfide and sulfone substructures. Based on the above results, the introduction of sulfide and sulfone substructures and the replacement of the heterocyclic rings had a great influence on the fungicidal activities of the target compounds, perhaps due to the effect of the change in length of the amide bridge in the compounds' favorable conformations. These results will provide important guidance for subsequent molecular designs of exploring and developing potential fungicidal lead compounds.

To further explore the fungicidal activity of compound **I-9**, the corresponding half maximal effective concentration ($EC_{50}$) values of compound **I-9** and fluopyram were estimated, and are displayed in Table 3. It can be concluded that compound **I-9** and fluopyram have a poor inhibitory effect on *Gibberella zeae*. Compared with fluopyram, compound **I-9** exhibits relatively weak inhibitory activities. However, as a whole, compound **I-9** shows excellent fungicidal activity against BC, CC, and PV, compared with other pathogens.

### 3.3. Molecular docking simulation

The literature [7] reports that the mechanism of action for the fungicidal and nematicidal agent fluopyram involves acting on complex II of the mitochondrial respiratory electron transport chain—namely, SDH or succinate coenzyme Q reductase (SQR). Although the composite crystal structures of fluopyram and the target enzyme SDH have not been reported in the protein database (RCSB PDB), it has been pointed out [28] that amide fungicides acting on SDH specifically bind to the coenzyme Q-site on complex II. Therefore, a careful investigation of the binding pattern of ligands provided a few specific points, which were helpful for correlating *in vitro* fungicidal data.

The Surflex-Dock method (SYBYL software) was used to simulate the interaction between compound **I-9**, fluopyram, and

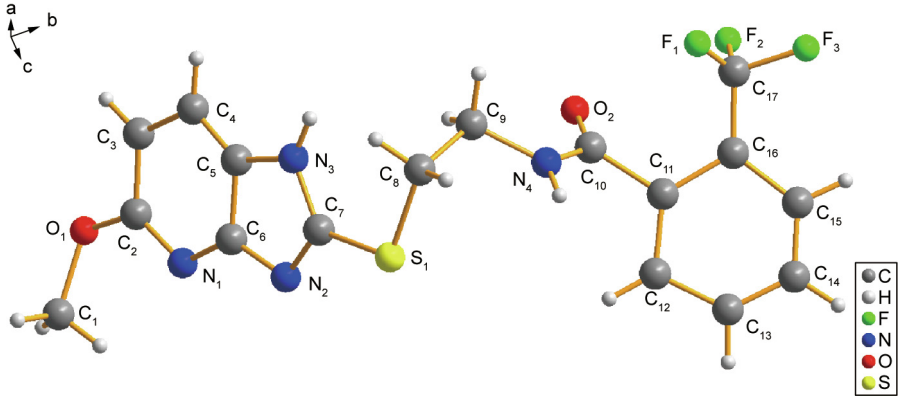



**Fig. 6.** The crystal structure of compound **I-3**.

**Table 1**
Nematicidal activity of target compounds against *Meloidogyne incognita*.

| Compounds | Mortality (%) | | | Compounds | Mortality (%) | | |
|---|---|---|---|---|---|---|---|
| | 200 μg·mL$^{-1}$ | 100 μg·mL$^{-1}$ | 50 μg·mL$^{-1}$ | | 200 μg·mL$^{-1}$ | 100 μg·mL$^{-1}$ | 50 μg·mL$^{-1}$ |
| **I-1** | 100 | 17.9 ± 0.71 | — | **II-1** | 86.2 ± 1.11 | 5.7 ± 0.42 | — |
| **I-2** | 3.9 ± 0.55 | 3.42 ± 0.80 | — | **II-2** | 81.9 ± 1.44 | 14.7 ± 0.72 | — |
| **I-3** | 88.5 ± 0.98 | 17.9 ± 0.83 | — | **II-3** | 91.1 ± 1.05 | 10.5 ± 0.56 | — |
| **I-4** | 94.3 ± 1.43 | 15.4 ± 0.76 | — | **II-4** | 84.4 ± 1.47 | 25.4 ± 2.11 | — |
| **I-5** | 96.7 ± 0.98 | 10.3 ± 0.25 | — | **II-5** | 96.9 ± 0.60 | 15.8 ± 0.01 | — |
| **I-6** | 100 | 5.9 ± 0.95 | — | **II-6** | 94.3 ± 0.06 | 70.2 ± 0.41 | 5.4 ± 0.06 |
| **I-7** | 100 | 7.7 ± 0.61 | — | **II-7** | 96.2 ± 1.56 | 7.6 ± 0.64 | — |
| **I-8** | 94.7 ± 0.67 | 17.1 ± 1.21 | — | **II-8** | 94.6 ± 0.71 | 9.8 ± 0.71 | — |
| **I-9** | 96.4 ± 1.44 | 6.2 ± 0.12 | — | **II-9** | 80.3 ± 1.83 | 10.6 ± 0.24 | — |
| **I-10** | 95.4 ± 0.69 | 13.9 ± 0.74 | — | **II-10** | 92.3 ± 0.62 | 6.7 ± 0.28 | — |
| **I-11** | 97.1 ± 0.95 | 73.0 ± 1.02 | 4.73 ± 0.27 | **II-11** | 95.1 ± 0.96 | 25.6 ± 0.51 | — |
| **I-12** | 91.6 ± 2.18 | 20.8 ± 0.59 | — | **II-12** | 91.9 ± 0.90 | 28.6 ± 2.11 | — |
| Fluopyram | 99.0 ± 0.93 | 99.4 ± 0.55 | 99.4 ± 1.08 | — | — | — | — |

**Table 2**
Fungicidal activity of target compounds at 100 μg·mL$^{-1}$.

| Compounds | Inhibition rate (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | RS | GZ | PP | CS | AK | BC | CC | PV |
| **I-1** | 12.2 ± 0.10 | 5.8 ± 0.11 | 2.9 ± 0.05 | 25.8 ± 0.12 | 36.1 ± 0.04 | 19.6 ± 0.10 | 39.1 ± 0.05 | 25.4 ± 0.02 |
| **I-2** | 24.1 ± 0.05 | 15.4 ± 0.01 | 22.8 ± 0.13 | 30.2 ± 0.11 | 34.9 ± 0.03 | 28.6 ± 0.08 | 19.2 ± 0.05 | 26.9 ± 0.04 |
| **I-3** | 25.8 ± 0.10 | 10.3 ± 0.09 | 14.5 ± 0.15 | 24.2 ± 0.05 | 26.1 ± 0.02 | 24.3 ± 0.05 | 31.6 ± 0.03 | 18.5 ± 0.03 |
| **I-4** | 29.0 ± 0.02 | 20.5 ± 0.10 | 18.7 ± 0.06 | 32.8 ± 0.04 | 20.4 ± 0.02 | 20.5 ± 0.10 | 33.3 ± 0.05 | 16.1 ± 0.03 |
| **I-5** | 21.6 ± 0.08 | 26.3 ± 0.10 | 53.5 ± 0.12 | 17.6 ± 0.06 | 14.8 ± 0.07 | 19.3 ± 0.05 | 24.0 ± 0.08 | 9.0 ± 0.12 |
| **I-6** | 32.6 ± 0.10 | 13.6 ± 0.14 | 2.9 ± 0.13 | 17.9 ± 0.10 | 21.8 ± 0.02 | 16.1 ± 0.06 | 22.7 ± 0.05 | 16.0 ± 0.09 |
| **I-7** | 25.0 ± 0.08 | 11.1 ± 0.18 | 6.2 ± 0.05 | 16.5 ± 0.08 | 27.9 ± 0.07 | 27.5 ± 0.05 | 35.8 ± 0.04 | 22.9 ± 0.05 |
| **I-8** | 35.2 ± 0.08 | 27.5 ± 0.08 | 27.8 ± 0.10 | 25.8 ± 0.01 | 22.4 ± 0.02 | 22.3 ± 0.05 | 29.1 ± 0.04 | 17.0 ± 0.04 |
| **I-9** | 53.1 ± 0.05 | 46.7 ± 0.05 | 85.1 ± 0.17 | 85.0 ± 0.13 | 79.6 ± 0.11 | 80.7 ± 0.06 | 71.6 ± 0.08 | 79.7 ± 0.04 |
| **I-10** | 24.1 ± 0.17 | 22.3 ± 0.03 | 67.6 ± 0.17 | 49.1 ± 0.08 | 26.1 ± 0.06 | 33.0 ± 0.05 | 36.4 ± 0.10 | 28.0 ± 0.06 |
| **I-11** | 21.6 ± 0.14 | 9.9 ± 0.09 | 0.4 ± 0.08 | 4.5 ± 0.12 | 18.1 ± 0.05 | 24.1 ± 0.05 | 20.0 ± 0.12 | 4.7 ± 0.02 |
| **I-12** | 28.4 ± 0.14 | 21.0 ± 0.05 | 21.2 ± 0.12 | 22.7 ± 0.03 | 24.1 ± 0.08 | 31.3 ± 0.05 | 37.3 ± 0.05 | 27.9 ± 0.05 |
| **II-1** | 36.9 ± 0.27 | 26.3 ± 0.10 | 11.3 ± 0.10 | 22.8 ± 0.05 | 20.7 ± 0.19 | 24.1 ± 0.05 | 22.9 ± 0.10 | 14.8 ± 0.06 |
| **II-2** | 37.0 ± 0.05 | 26.1 ± 0.09 | 26.1 ± 0.05 | 37.1 ± 0.05 | 17.1 ± 0.21 | 14.3 ± 0.09 | 27.3 ± 0.08 | 15.1 ± 0.08 |
| **II-3** | 26.7 ± 0.13 | 5.7 ± 0.08 | 3.7 ± 0.00 | 9.6 ± 0.06 | 19.6 ± 0.11 | 24.1 ± 0.05 | 14.5 ± 0.10 | 6.4 ± 0.05 |
| **II-4** | 4.5 ± 0.08 | 10.2 ± 0.07 | 12.0 ± 0.06 | 12.8 ± 0.02 | 9.5 ± 0.03 | 17.3 ± 0.13 | 22.5 ± 0.05 | 10.0 ± 0.04 |
| **II-5** | 27.5 ± 0.15 | 13.4 ± 0.10 | 2.9 ± 0.10 | 16.5 ± 0.14 | 30.9 ± 0.09 | 3.0 ± 0.03 | 7.3 ± 0.10 | 12.0 ± 0.08 |
| **II-6** | 26.7 ± 0.13 | 18.2 ± 0.05 | 50.2 ± 0.00 | 14.5 ± 0.03 | 20.8 ± 0.07 | 17.0 ± 0.05 | 22.5 ± 0.05 | 11.1 ± 0.02 |
| **II-7** | 35.2 ± 0.08 | 21.8 ± 0.08 | 32.8 ± 0.10 | 18.7 ± 0.06 | 17.3 ± 0.03 | 24.6 ± 0.07 | 28.0 ± 0.05 | 15.9 ± 0.05 |
| **II-8** | 20.7 ± 0.13 | 3.8 ± 0.10 | 32.8 ± 0.06 | 30.9 ± 0.01 | 34.1 ± 0.08 | 22.3 ± 0.05 | 38.3 ± 0.01 | 27.1 ± 0.04 |
| **II-9** | 19.0 ± 0.10 | 1.0 ± 0.13 | 12.9 ± 0.10 | 6.6 ± 0.05 | 24.4 ± 0.03 | 23.8 ± 0.05 | 30.8 | 27.9 ± 0.06 |
| **II-10** | 23.3 ± 0.21 | 0.3 ± 0.05 | 15.4 ± 0.24 | 3.8 ± 0.09 | 10.1 ± 0.04 | 18.8 ± 0.05 | 23.1 ± 0.06 | 14.6 ± 0.09 |
| **II-11** | 12.2 ± 0.22 | 12.1 ± 0.10 | 10.4 ± 0.12 | 6.8 ± 0.05 | 11.8 ± 0.03 | 5.9 ± 0.05 | 31.8 ± 0.10 | 14.2 ± 0.26 |
| **II-12** | 23.4 ± 0.12 | 40.8 ± 0.04 | 22.0 ± 0.06 | 29.9 ± 0.13 | 20.8 ± 0.01 | 15.2 ± 0.05 | 8.2 ± 0.05 | 17.3 ± 0.03 |
| Fluopyram | 46.6 ± 0.14 | 57.6 ± 0.06 | 84.5 ± 0.05 | 100 | 75.9 ± 0.06 | 82.0 ± 0.01 | 86.4 ± 0.10 | 61.0 ± 0.06 |

**Table 3**
$EC_{50}$ values of compound **I-9** and fluopyram.

| Fungi | **I-9** | | | Fluopyram | | |
|---|---|---|---|---|---|---|
| | Regression equation | Correlation coefficient, $r$ | $EC_{50}$ (95% confidence interval) (μg·mL$^{-1}$) | Regression equation | Correlation coefficient, $r$ | $EC_{50}$ (95% confidence interval) (μg·mL$^{-1}$) |
| RS | $y = 1.50x + 2.60$ | 0.9842 | 39.13 (31.41–48.75) | $y = 1.32x + 3.02$ | 0.9295 | 31.47 (20.03–49.46) |
| GZ | $y = 0.61x + 3.47$ | 0.9891 | 317.58 (200.69–502.55) | $y = 0.63x + 3.71$ | 0.9831 | 113.31 (77.55–165.57) |
| PP | $y = 1.24x + 3.03$ | 0.9906 | 38.10 (32.26–44.99) | $y = 1.93x + 2.87$ | 0.9367 | 12.68 (7.66–21.00) |
| CS | $y = 0.65x + 3.77$ | 0.9867 | 80.11 (60.34–106.35) | $y = 4.23x + 1.86$ | 0.9529 | 5.54 (2.90–10.58) |
| AK | $y = 1.52x + 2.67$ | 0.9698 | 33.70 (25.18–45.12) | $y = 1.61x + 3.86$ | 0.9877 | 5.08 (3.27–7.89) |
| BC | $y = 1.31x + 3.30$ | 0.9825 | 19.64 (15.82–24.39) | $y = 1.97x + 3.04$ | 0.9461 | 9.83 (5.82–16.60) |
| CC | $y = 1.25x + 4.11$ | 0.9295 | 5.17 (2.25–11.89) | $y = 0.89x + 4.65$ | 0.9877 | 2.47 (1.57–3.87) |
| PV | $y = 1.20x + 3.63$ | 0.9813 | 13.97 (10.85–17.99) | $y = 0.79x + 4.64$ | 0.9523 | 2.81 (1.18–6.70) |

*Escherichia coli* SDH (PDB code: 1NEK), respectively (Fig. 7). From the data, it was concluded that the carbonyl oxygen on the amide and the fluorine atoms on the ortho-trifluoromethyl group of fluopyram facilitated the formation of hydrogen bonds with the amino acid residues B/TRP-164, D/TYR-83, and C/ARG-31 at the Q-site on the target enzyme, which helped to improve the fungicidal activity. Furthermore, the trifluoromethyl group was on the same side of the amide bridge as the carbonyl oxygen, and the two conformed to form hydrogen bonds with the amino acid residue TRP-164 together (Fig. 7(a)).

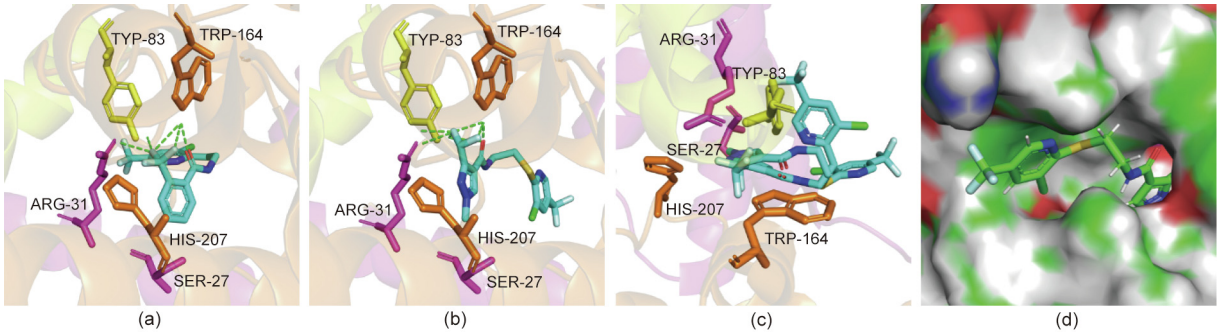



**Fig. 7.** (a) The binding mode of fluopyram to *Escherichia coli* SDH (PDB code: 1NEK); (b) the binding mode of compound **I-9** to *Escherichia coli* SDH; (c) the superposed conformation of fluopyram and compound **I-9**; (d) the docking pocket of compound **I-9** to *Escherichia coli* SDH, TYP, TRP, ARG, HIS, SER: amino acid residue.

When 3-(difluoromethyl)-1-methyl-1*H*-pyrazole was introduced into the amide bridge, the presence of the fluorine atoms on the ortho-difluoromethyl group of the amide contributed to the formation of hydrogen bonds with the amino acid residues, which was consistent with the hydrogen bond interaction when using fluopyram. Considering the excellent fungicidal activity and structural characteristics of fluopyram and the target compound **I-9**, combined with the docking results, it was concluded that the presence of the amide and its ortho-fluorinated groups had an important role in the fungicidal activity. On the other hand, the introduction of different aromatic rings in the aromatic sulfide moiety and the change in the length of the amide bridge had a great influence on the biological activity.

## 4. Conclusion

In summary, 24 novel target compounds were designed and synthesized by introducing sulfide and sulfone substructures into fluopyram. The bioassays indicated that the structural modification of the target compounds had different effects on the compounds' nematicidal and fungicidal activities. Although the synthetic routes for the target compounds were optimized through the introduction of sulfide and sulfone, the biological activities were greatly affected. Through the replacement of various heterocycles, compounds **I-11** and **II-6** with good nematicidal activity and compound **I-9** with excellent fungicidal activity were discovered; combined with the molecular docking results, these results provide important guidance for further structural optimization.

## Acknowledgements

This work was financially supported by the Natural Science Foundation of Shandong Province, China (ZR2017BC053), and the Doctoral Research Startup Foundation of Liaocheng University (318051625).

## Compliance with ethics guidelines

Xuewen Hua, Nannan Liu, Sha Zhou, Leilei Zhang, Hao Yin, Guiqing Wang, Zhijin Fan, and Yi Ma declare that they have no conflict of interest or financial conflicts to disclose.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2019.09.011.

## References

[1] Perfus-Barbeoch L, Castagnone-Sereno P, Reichelt M, Fneich S, Roquis D, Pratx L, et al. Elucidating the molecular bases of epigenetic inheritance in non-model invertebrates: the case of the root-knot nematode *Meloidogyne incognita*. Front Physiol 2014;5:211.
[2] Chitwood DJ. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture-Agricultural Research Service. Pest Manag Sci 2003;59(6–7):748–53.
[3] Hungenberg H, Fürsch H, Rieck H, Hellwege E, inventors; Bayer Intellectual Property Gmbh, assignee. Use of fluopyram for controlling nematodes in crops and for increasing yield. United States patent US 20130253018. 2013 Sep 26.
[4] Raymond RG, Gray RM, inventors; Bayer CropScience Aktiengesellschaft, assignee. Use of the succinate dehydrogenase inhibitor fluopyram for controlling blackleg in Brassicaceae species. WIPO patent, WO 2017013083. 2017 Jan 26.
[5] Slomczynska U, South MS, Bunkers GJ, Edgecomb D, Wyse-Pester D, Selness S, et al. Tioxazafen: a new broad-spectrum seed treatment nematicide. ACS Symp Ser 2015;1204:129–47.
[6] Lahm GP, Desaeger J, Smith BK, Pahutski TF, Rivera MA, Meloro T, et al. The discovery of fluazaindolizine: a new product for the control of plant parasitic nematodes. Bioorg Med Chem Lett 2017;27(7):1572–5.
[7] Avenot HF, Michailides TJ. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Prot 2010;29(7):643–51.
[8] Liu XH, Zhao W, Shen ZH, Xing JH, Xu TM, Peng WL. Synthesis, nematocidal activity and SAR study of novel difluoromethylpyrazole carboxamide derivatives containing flexible alkyl chain moieties. Eur J Med Chem 2017;125:881–9.
[9] Zhao W, Shen ZH, Xing JH, Yang G, Xu TM, Peng WL, et al. Synthesis and nematicidal activity of novel 1-(3-chloropyridin-2-yl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide derivatives. Chem Pap 2017;71(5):921–8.
[10] Lahm GP, Deangelis AJ, Campbell MJ, inventors; E. I. Du Pont DE Nemours and Company, assignee. Nematicidal heterocyclic amides. WIPO patent WO 2017116646. 2017 Jul 6.
[11] El Qacemi M, Bigot A, Edmunds A, Gagnepain JDH, Jeanguenat A, Pitterna T, Stoller A, Sabbadin D, inventors; Syngenta Participations AG, assignee. Pesticidally active pyrazole derivatives. WIPO patent, WO 2017055414. 2017 Apr 6.
[12] Jeanguenat A, Pitterna T, El Qacemi M, Stoller A, Mondiere RJG, Bigot A, et al. Syngenta Participations AG, assignee. Pesticidally active pyrazole derivatives. WIPO patent, WO 2017140771. 2017 Aug 24.
[13] Yoneda T, Yoshida K, Tazawa Y, Kani T, Cho Y, Noujima A, et al. Ishihara Sangyo Kaisha, LTD. assignee. *N*-(4-Pyridyl) benzamide compound or salt thereof, and pest control agent containing compound or salt thereof as active ingredient. WIPO patent WO 2017222037. 2017 Dec 28.
[14] Li W, Li JH, Shen HF, Cheng JG, Li Z, Xu XY. Synthesis, nematicidal activity and docking study of novel chromone derivatives containing substituted pyrazole. Chin Chem Lett 2018;29(6):911–4.
[15] Wang H, Gao X, Zhang X, Jin H, Tao K, Hou T. Design, synthesis and antifungal activity of novel fenfuram-diarylamine hybrids. Bioorg Med Chem Lett 2017;27(1):90–3.
[16] Yan Z, Liu A, Huang M, Liu M, Pei H, Huang L, et al. Design, synthesis, DFT study and antifungal activity of the derivatives of pyrazolecarboxamide containing thiazole or oxazole ring. Eur J Med Chem 2018;149:170–81.
[17] Ren ZL, Liu H, Jiao D, Hu HT, Wang W, Gong JX, et al. Design, synthesis, and antifungal activity of novel cinnamon-pyrazole carboxamide derivatives. Drug Dev Res 2018;79(6):307–12.
[18] Zhang A, Zhou J, Tao K, Hou T, Jin H. Design, synthesis and antifungal evaluation of novel pyrazole carboxamides with diarylamines scaffold as potent succinate dehydrogenase inhibitors. Bioorg Med Chem Lett 2018;28(18):3042–5.
[19] Song BA, Chen XW, Chen YZ, Hu DY, Xue W, Chen JX, et al., inventors; Guizhou University, assignee. The preparation method and application of 1,3,4-oxadiazole (thiadiazole) sulfide (sulfone) derivatives containing trifluorobutene. Chinese patent CN 105646393. 2016 Jun 8.

[20] Bellandi P, Gusmeroli M, Sargiotto C, Bianchi D, inventors; Isagro SPA, assignee. Heterocyclic trifluoroalkenyl compounds having a nematocidal activity, their agronomic compositions and use thereof. WIPO patent WO 2017002100. 2017 Jan 5.
[21] Liu AC, Feng JL, He XL, Zhang SK, Yu CH. Synthesis of novel fungicide fluopyram. Agrochemicals 2015;54:485.
[22] Liu L, Xu P, Zhou L, Lei PS. Synthesis of derivatives of imidazo[4,5-*b*] pyridine: novel sulfur contained side chains for macrolide antibiotics. Chin Chem Lett 2008;19(1):1–4.
[23] Lazer ES, Matteo MR, Possanza GJ. Benzimidazole derivatives with atypical antiinflammatory activity. J Med Chem 1987;30(4):726–9.
[24] Lu H, Xu S, Zhang W, Xu C, Li B, Zhang D, et al. Nematicidal activity of *trans*-2-hexenal against southern root-knot nematode (*Meloidogyne incognita*) on tomato plants. J Agric Food Chem 2017;65(3):544–50.
[25] Liu G, Lai D, Liu QZ, Zhou L, Liu ZL. Identification of nematicidal constituents of *Notopterygium incisum* Rhizomes against *Bursaphelenchus xylophilus* and *Meloidogyne incognita*. Molecules 2016;21(10):1276.
[26] Fan Z, Yang Z, Zhang H, Mi N, Wang H, Cai F, et al. Synthesis, crystal structure, and biological activity of 4-methyl-1,2,3-thiadiazole-containing 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles. J Agric Food Chem 2010;58(5):2630–6.
[27] Jain AN. Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. J Med Chem 2003;46(4):499–511.
[28] Horsefield R, Yankovskaya V, Sexton G, Whittingham W, Shiomi K, Omura S, et al. Structural and computational analysis of the quinone-binding site of complex II (succinate-ubiquinone oxidoreductase): a mechanism of electron transfer and proton conduction during ubiquinone reduction. J Biol Chem 2006;281(11):7309–16.