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Synthesis, biological activity and toxicity to zebrafish of benzamides substituted with pyrazole-linked 1,2,4-oxadiazole†

 Yingying Shao,^a Minting Tu,^a Sen Yang,^a Yingying Wang,^a Binlong Sun,^a Jianjun Shi,^c Chengxia Tan ^{*a} and Xuedong Wang ^{*b}

To find pesticidal lead compounds with high activity, a series of novel benzamides substituted with pyrazole-linked 1,2,4-oxadiazole was designed and synthesized by using the splicing principle of active substructures. The chemical structures of the target compounds were confirmed by ¹H NMR, ¹³C NMR and HRMS. The preliminary bioassay showed that most compounds displayed good larvicidal activities against mosquito larvae at 10 mg L⁻¹. In particular, compound **12g** exhibited obvious activity; its lethal rate reached up to 100% (at 5 mg L⁻¹) and 55% (at only 2 mg L⁻¹). Furthermore, compound **12f** (70.6%) and **12h** (100%) showed good fungicidal activities against *Pyricularia oryzae*, with EC₅₀ values of 8.28 and 5.49 μg mL⁻¹, respectively, which were superior to that of the control drug bixafen (9.15 μg mL⁻¹). Finally, the LC₅₀ of compound **12h** to zebrafish embryo was 0.39 mg L⁻¹, so it was classified as a high-toxic compound. Thus, this compound may be used as a potential lead compound for further structural optimisation to develop new compounds with high activity and low toxicity.

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1. Introduction

Nitrogenous heterocyclic compounds play an important role in the process of new pesticide development. Among them, possessing the advantages of variable structure, high-selectivity, high-efficiency, and low-toxicity and in line with the development concept of modern green pesticides, pyrazole derivatives have become the focus in the field of pesticides. In addition, pyrazole amide derivatives have good insecticidal,¹⁻⁷ fungicidal⁸⁻¹⁶ and other biological activities.¹⁷ Developed in recent years, pyrazole amide derivatives, such as furametpyr, pen thiopyrad, bixafen, sedaxane, penflufen, chlorantraniliprole, cyantraniliprole and tebufenpyrad (Fig. 1), have important shares in the pesticide market. However, the skeleton structure changes of them are mainly the replacement and modification of the pyrazole amide acid parent nucleus and aromatic amine, which is prone to cross-resistance.¹⁸⁻²³ Therefore, through introducing a new skeleton, there is room for pyrazole amide derivatives to further improve their biological activities.

The 1,2,4-oxadiazole heterocycle, as a bio-isostere of the amide bond, not only has biological activities such as insecticidal,^{24,25} fungicidal²⁶⁻³⁰ and herbicidal,^{31,32} but also has anti-inflammatory,³³⁻³⁷ hypotensive³⁸ and other physiological activities. Our previous work showed that benzamides substituted with 1,2,4-oxadiazole compounds have good insecticidal and fungicidal activities.³⁹ Therefore, in this study, taking bixafen as lead compound, introducing benzoic acid substituted with 1,2,4-oxadiazole between the pyrazole ring and the amide bond, 18 novel benzamides substituted with pyrazole-linked 1,2,4-oxadiazole compounds were designed and synthesized (Fig. 2). The chemical structures of all the target compounds were confirmed by ¹H NMR, ¹³C NMR and HRMS. Their insecticidal activities and fungicidal activities were studied and a toxicity

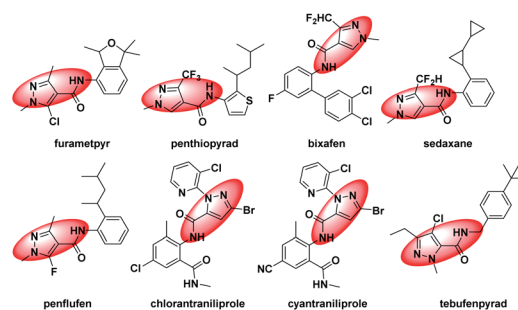


Fig. 1 Chemical structures of furametpyr, penthiopyrad, bixafen, sedaxane, penflufen, chlorantraniliprole, cyantraniliprole, tebufenpyrad.

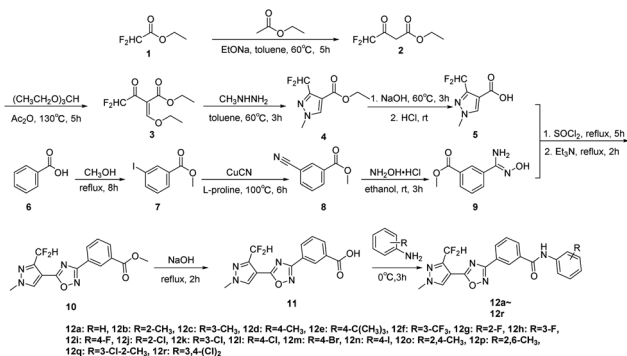
^aCollege of Chemical Engineering, Zhejiang University of Technology, Hangzhou 310014, China. E-mail: tanchengxia@zjut.edu.cn

^bSchool of Environmental Science and Engineering, Suzhou University of Science and Technology, Suzhou 215009, China. E-mail: zjuwxd@163.com

^cSchool of Chemistry and Chemical Engineering, Huangshan University, Huangshan 245041, China

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Scheme 1 Synthetic route of target compounds 12a–12r.

test of zebrafish embryo was performed. The synthetic route of the target compounds is shown in Scheme 1.

2. Results and discussion

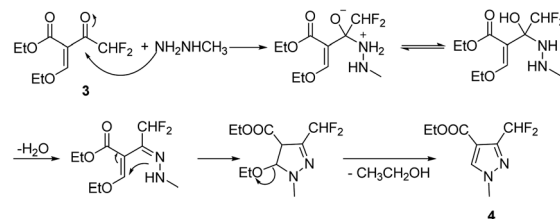
2.1. Synthesis of target compounds

The target compounds 12a–12r were synthesized taking ethyl difluoroacetate 1 and 3-iodobenzoic acid 6 as starting materials. The compound 1 underwent Claisen condensation, addition–elimination, cyclization and hydrolysis reaction to give 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid 5. The compound 6 experienced esterification, cyanidation reaction, then reacted with hydroxylamine to give methyl (Z)-3-(N'-hydroxycarbamimidoyl) benzoate 9. Subsequently, the cyclization reaction of compound 5 and compound 9 yielded intermediate 10. Finally, the compound 10 was hydrolyzed under alkaline condition and acidified, then condensation reaction of intermediate 11 and substituted aniline obtained target compounds 12a–12r.

The mechanism of step 3, cyclization reaction, is shown in Scheme 2. From this mechanism, it could be seen that the late stage of the reaction was a nucleophilic reaction of intermediate hydrazone, however, its nucleophilic activity was not strong enough to react at a high rate. Thus, to improve the reaction rate and yield, through increasing the temperature appropriately (from 0 to 80 °C), the effect of temperatures on reaction yield was studied and the results showed that its yield reached up to highest (85.3%) at 60 °C.

2.2. Biological activities of target compounds and SAR

The insecticidal activities of the target compounds against *Mythimna separata*, *Pyrausta nubilalis*, *Helicoverpa armigera* and *Spodoptera frugiperda* are listed in Table 1. And, the larvicidal activities of target compounds against Mosquito larvae are shown in Table 2. Etoxazole and broflanilide was used as the control drug. As shown in Table 1, most of the target compounds were found to exhibit certain insecticidal activities. Among them, compounds 12h (55%) and 12l (65%) exhibited moderate insecticidal activities against *Mythimna separata* at 500 mg L⁻¹, which was inferior than that of etoxazole (100%) and broflanilide (100%). In addition, some of them showed



Scheme 2 Synthetic mechanism of the intermediate 4.

good lethal activities against Mosquito larvae, and it was worth mentioning that compound 12g exhibited obvious activity, its lethal rate reached up to 100% (at 5 mg L⁻¹) and even at 2 mg L⁻¹, the lethal activity of compound 12g was 55%, which was definitely superior than etoxazole.

The fungicidal activity of target compounds 12a–12r against 9 tested fungi at 50 mg L⁻¹ are listed in Table 3. Bixafen was used as the control drug. Overall, most of the target compounds were found to exhibit certain antifungal activities. Among them, the target compounds exhibited better fungicidal activities against *Pyricularia oryzae* and *Sclerotinia sclerotiorum*. For example, compounds 12f, 12h, 12k and 12r exhibited an inhibition rate of 52.9–100% against *Pyricularia oryzae*, especially compound 12h showed an inhibition rate of 100% against *Pyricularia oryzae*, which was comparable to that of bixafen (100%). For *Sclerotinia sclerotiorum*, compounds 12f (50.0%), 12h (50.0%), 12k (69.2%) and 12o (60.6%) also showed moderate fungicidal activities.

On the basis of the preliminary fungicidal activity results, compounds 12f, 12h, 12k (>70% inhibitory) and bixafen (control drug) were selected for further EC₅₀ bioassays, and the

Table 1 Insecticidal activities of target compounds 12a–12r

| Compound | Insecticidal activities (death rates%, 500 mg L ⁻¹) | | | |
|--------------|-----------------------------------------------------------------|---------------------------|-----------------------------|------------------------------|
| | <i>Mythimna separata</i> | <i>Pyrausta nubilalis</i> | <i>Helicoverpa armigera</i> | <i>Spodoptera frugiperda</i> |
| 12a | 40 | 0 | 15 | 0 |
| 12b | 10 | 0 | 0 | 20 |
| 12c | 0 | 20 | 0 | 0 |
| 12d | 25 | 10 | 15 | 10 |
| 12e | 10 | 0 | 0 | 0 |
| 12f | 15 | 0 | 5 | 0 |
| 12g | 45 | 25 | 35 | 0 |
| 12h | 55 | 0 | 10 | 0 |
| 12i | 20 | 0 | 15 | 0 |
| 12j | 30 | 0 | 0 | 0 |
| 12k | 30 | 10 | 25 | 10 |
| 12l | 65 | 30 | 35 | 0 |
| 12m | 0 | 10 | 5 | 15 |
| 12n | 0 | 0 | 0 | 0 |
| 12o | 0 | 5 | 10 | 0 |
| 12p | 10 | 10 | 0 | 0 |
| 12q | 20 | 0 | 10 | 0 |
| 12r | 5 | 30 | 5 | 10 |
| Etoxazole | 100 | 100 | 100 | 100 |
| Broflanilide | 100 | 100 | 100 | 100 |
| QCK | 0 | 0 | 0 | 0 |



Table 2 Larvicidal activities of target compounds 12a–12r

| Compound | Larvicidal activities (death rates%) | |
|----------|--------------------------------------|-----------------|
| | Concentration (mg L ⁻¹) | Mosquito larvae |
| 12a | 10 | 0 |
| 12b | 10 | 0 |
| 12c | 10 | 100 |
| | 5 | 50 |
| 12d | 10 | 0 |
| 12e | 10 | 0 |
| 12f | 10 | 5 |
| 12g | 10 | 100 |
| | 5 | 100 |
| | 2 | 55 |
| 12h | 10 | 0 |
| 12i | 10 | 25 |
| 12j | 10 | 0 |
| 12k | 10 | 0 |
| 12l | 10 | 15 |
| 12m | 10 | 0 |
| 12n | 10 | 10 |
| 12o | 10 | 0 |
| 12p | 10 | 0 |
| 12q | 10 | 5 |
| 12r | 10 | 35 |
| Etoazole | 10 | 100 |
| | 5 | 35 |
| QCK | 0 | 0 |

Table 3 Fungicidal activities (inhibition rate/%) of target compounds 12a–12r at 50 mg L^{-1a}

| Compound | Fungicidal activities (inhibition rate%, 50 mg L ⁻¹) | | | | | | | | |
|----------|------------------------------------------------------------------|------|------|-------|-------|------|------|------|------|
| | AS | FG | CA | PO | SS | BC | TC | FO | PP |
| 12a | 21.4 | 30.8 | 16.1 | 17.6 | 47.1 | 21.1 | 6.5 | 9.1 | 25.0 |
| 12b | 14.3 | 3.8 | 9.7 | 17.6 | 23.5 | 15.8 | 6.5 | 4.5 | 7.1 |
| 12c | 28.6 | 30.8 | 16.1 | 29.4 | 49.0 | 31.6 | 43.5 | 13.6 | 7.1 |
| 12d | 28.6 | 19.2 | 9.7 | 5.9 | 23.5 | 15.8 | 10.9 | 13.6 | 10.7 |
| 12e | 35.7 | 23.1 | 16.1 | 41.2 | 37.3 | 26.3 | 10.9 | 4.5 | 17.9 |
| 12f | 28.6 | 42.3 | 29.0 | 70.6 | 70.6 | 42.1 | 10.9 | 18.2 | 35.7 |
| 12g | 21.4 | 15.4 | 22.6 | 5.9 | 43.1 | 5.3 | 10.9 | 4.5 | 10.7 |
| 12h | 28.6 | 38.5 | 35.5 | 100.0 | 68.6 | 36.8 | 43.5 | 27.3 | 17.9 |
| 12i | 28.6 | 3.8 | 16.1 | 41.2 | 39.2 | 21.1 | 26.1 | 22.7 | 7.1 |
| 12j | 21.4 | 19.2 | 22.6 | 17.6 | 39.2 | 26.3 | 10.9 | 4.5 | 7.1 |
| 12k | 21.4 | 38.5 | 22.6 | 76.5 | 62.7 | 42.1 | 30.4 | 13.6 | 35.7 |
| 12l | 21.4 | 11.5 | 22.6 | 5.9 | 19.6 | 15.8 | 6.5 | 13.6 | 10.7 |
| 12m | 21.4 | 30.8 | 16.1 | 29.4 | 37.3 | 10.5 | 23.9 | 18.2 | 17.9 |
| 12n | 21.4 | 30.8 | 16.1 | 17.6 | 23.5 | 10.5 | 8.7 | 13.6 | 7.1 |
| 12o | 14.3 | 19.2 | 22.6 | 5.9 | 64.7 | 21.1 | 10.9 | 9.1 | 21.4 |
| 12p | 21.4 | 23.1 | 22.6 | 5.9 | 47.1 | 5.3 | 26.1 | 4.5 | 25.0 |
| 12q | 21.4 | 3.8 | 16.1 | 17.6 | 29.4 | 5.3 | 17.4 | 9.1 | 17.9 |
| 12r | 21.4 | 38.5 | 22.6 | 52.9 | 47.1 | 15.8 | 34.8 | 22.7 | 25.0 |
| Bixafen | 92.9 | 70.6 | 86.7 | 100.0 | 100.0 | 72.7 | 92.7 | 73.9 | 77.8 |
| QCK | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

^a *Alternaria solani* (AS), *Fusarium graminearum* (FG), *Cercospora arachidicola* (CA), *Pyricularia oryzae* (PO), *Sclerotinia sclerotiorum* (SS), *Botrytis cinerea* (BC), *Thanatephorus cucumeris* (TC), *Fusarium oxysporum* (FO), *Phytophthora blight* (PP).

Table 4 EC₅₀ of compounds 12f, 12h and 12k to *Pyricularia oryzae* and *Sclerotinia sclerotiorum*

| Compound | Fungus | $y = a + bx$ | r^2 | EC ₅₀ (μg mL ⁻¹) |
|----------|--------|------------------------|--------|-----------------------------------------|
| 12f | PO | $y = 1.3031x + 3.8036$ | 0.9846 | 8.2819 |
| 12f | SS | $y = 1.1444x + 3.6775$ | 0.9843 | 14.3106 |
| 12h | PO | $y = 1.0839x + 4.1985$ | 0.9809 | 5.4879 |
| 12k | PO | $y = 1.2952x + 3.5555$ | 0.9986 | 13.0409 |
| Bixafen | PO | $y = 1.7973x + 3.2715$ | 0.9766 | 9.1549 |
| | SS | $y = 1.3923x + 3.5714$ | 0.9707 | 6.8965 |

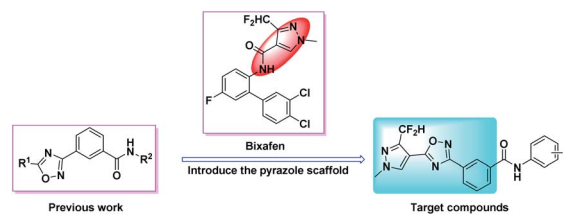


Fig. 2 Design strategy of target compounds.

results are shown in Table 4. The results showed that compounds 12f and 12h possessed excellent fungicidal activities against *Pyricularia oryzae* with EC₅₀ of 8.28 and 5.49 μg mL⁻¹, respectively, and their EC₅₀ values were significantly superior than that of bixafen (9.15 μg mL⁻¹).

From Table 3, it was concluded that substituent on the benzene ring can greatly influence the activity. Structure–activity relationship (SAR) results for these target compounds showed that the order of fungicidal activity of target compounds was F > CF₃ > CH₃ on the benzene ring, such as the high, middle and low active compounds 12h, 12f and 12c. Moreover, in the target compounds substituted with F (12g–12i), the meta substitution of benzene ring can increase the fungicidal activity. And, when the meta-directing of the benzene ring was electron-withdrawing group, its inhibitory activity against *Pyricularia oryzae* was better than others. Overall, compared compound 12q (3-Cl-2CH₃) with 12b (2-CH₃) and 12k (3-Cl), the insecticidal activities (against *Mythimna separata*, *Pyrausta nubilalis*, and *Spodoptera frugiperda*) and fungicidal activities showed that their biological activities were inhibited (3-Cl > 3-Cl-2CH₃ > 2-

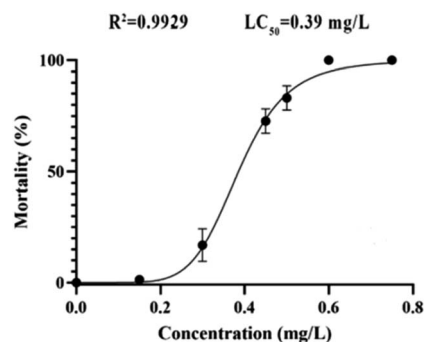


Fig. 3 Zebrafish embryo mortality rates after exposure to compound 12h.



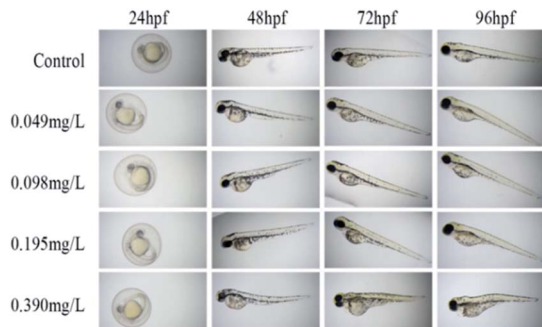


Fig. 4 Zebrafish embryo malformation after exposure to compound 12h.

CH₃) when methyl group was introduced into the ortho position. Meanwhile, these experimental data provide ideas for further exploration and optimization of such compounds.

2.3. Toxicity to zebrafish (*Danio rerio*)

According to the test results of fungicidal activity, we selected compound 12h with higher fungicidal activity to further detect its zebrafish toxicity. After exposure to its gradient concentrations (0–0.80 mg L⁻¹), the LC₅₀ calibration curve was achieved by plotting the mortality percentages vs. the concentrations of analyte (Fig. 3). Obviously, the mortality rate increased dramatically with increases in the exposure concentrations (<0.60 mg L⁻¹). However, over 0.60 mg L⁻¹, the mortality rate reached as high as 100%. As a consequence, the LC₅₀ value of compound 12h was calculated to be 0.39 mg L⁻¹, which should be attributable to a highly toxic compound according to ISO 15088-2007.

From 24 to 96 hpf (hours post fertilization), the embryonic-larval zebrafish were acutely exposed to compound 12h across the concentration range of 0.049–0.390 mg L⁻¹ (Fig. 4). The concentration selection of compound 12h was based on its LC₅₀ value. In the 0.049 and 0.098 mg L⁻¹ treatment groups, zebrafish body length significantly increased when compared to the control group, demonstrating the low-level exposure promoted larval growth and development. In sharp contrast, in the 0.195 and 0.390 mg L⁻¹ treatments, a series of typical abnormalities were observed, including spinal curvature, head deformity, pericardial edema, yolk cyst, and melanin loss. Especially in the 0.390 mg L⁻¹ treatment, typical yolk cyst and swim sac closure were observed for the 96 hpf larvae, reflecting in that their swim speed and activity were substantially decreased.

3. Materials and methods

3.1. General information

¹H NMR and ¹³C NMR spectra were measured on BRUKER Avance 500 MHz spectrometer (Bruker 500 MHz, Fällanden, Switzerland) using DMSO-*d*₆ or CDCl₃ as the solvent. High-resolution electrospray mass spectra (HR-ESI-MS) were determined using an UPLC H CLASS/QTOF G2 XS mass spectrometer (Waters, Milford, CT, USA). The HPLC analysis was conducted

on Shimadzu LC-20AT. Melting points were determined using an M-565 melting point (Buchi, Switzerland). All the reagents were analytical grade or synthesized in our laboratory. The characterization data for all synthetic compounds are provided in the ESI.†

Ethics statement: The Institutional Animal Care and Use Committee (IACUC) at Wenzhou Medical University (SYXK 2019-0009, April 4, 2019 to April 4, 2024) approved our study plan for proper use of zebrafish. All studies were carried out in strict accordance with the guidelines of the IACUC. All dissections were performed on ice, and all efforts were made to minimize suffering.

3.2. Synthesis

3.2.1 Synthesis of intermediate 2. Under nitrogen, toluene (20 mL), sodium (1.10 g, 48.00 mmol) and absolute ethanol (4.42 g, 0.11 mol) were added to a three-necked flask, and stirred to dissolve the Na completely. Afterwards, the solution of ethyl difluoroacetate (4.96 g, 0.04 mol) in ethyl acetate (17.60 g, 0.20 mol) was added dropwise to the newly prepared EtONa toluene suspension under nitrogen and ice bath. The mixture was reacted at 65 °C for 3 h and then cooled to room temperature. The solution was distilled under reduced pressure to obtain a yellow viscous substance. Subsequently, adjusted the pH to acidic, extracted by ethyl acetate (15 mL × 3), dried with MgSO₄ and filtered, and desolvated to obtain 5.89 g yellow oily liquid intermediate 2. Yield 88.5%. ¹H NMR (500 Hz, CDCl₃) δ 5.78–6.13 (t, *J* = 5.20 Hz 1H), 4.25 (q, *J* = 8.0 Hz 2H), 3.64 (s, 2H), 1.33–1.28 (t, *J* = 6.0 Hz, 3H).

3.2.2 Synthesis of intermediate 3. Intermediate 2 (9.96 g, 0.06 mol), acetic anhydride (18.37 g, 0.18 mol) and triethyl orthoformate (11.60 g, 78.00 mol) were added to a round-bottomed flask successively. The mixture was reacted at reflux. Cooled to room temperature after the reaction was completed, then the solvent was removed under reduced pressure to obtain 13.35 g light yellow liquid intermediate 3. The crude residue was subjected to the next step without further isolation or purification. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.44–8.06 (m, 1H), 6.64 (m, *J* = 105.5, 53.2 Hz, 1H), 4.59–3.45 (m, 4H), 1.33–1.28 (m, *J* = 7.1 Hz, 2H), 1.26–1.20 (m, 3H), 1.09–1.04 (m, *J* = 7.0 Hz, 1H).

3.2.3 Synthesis of intermediate 4. The 40% aqueous solution of methylhydrazine (3.00 g, 26.00 mmol) was added dropwise to the solution of intermediate 3 (4.46 g, 0.02 mol) in toluene (30 mL) under ice bath. Then, the mixture was reacted at 60 °C for 3 h. After the reaction was completed, extracted with water (30 mL), the organic phase was directly used in the next step. ¹H NMR (500 Hz, CDCl₃) δ 7.89 (s, 1H), 7.09 (t, *J* = 54.0 Hz, 1H), 4.32 (q, *J* = 7.0 Hz, 2H), 3.96 (s, 3H), 1.35 (t, *J* = 7.0 Hz, 3H).

3.2.4 Synthesis of intermediate 5. NaOH (5%, 16.70 g) was added to the above solution, then heated to 60 °C and reacted for 3 h. After the mixture was cooled to room temperature, the solvent was removed, water (100 mL) was added and then the pH was adjusted to 2–3 with hydrochloric acid to precipitate white solid. Finally, filtered and recrystallized filter cake to obtain 2.75 g white solid intermediate 5. Yield 78.1%, mp 203–



205 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 12.83 (s, 1H), 8.33 (s, 1H), 7.20 (t, $J = 53.8$ Hz, 1H), 3.91 (s, 3H).

3.2.5 Synthesis of intermediate 7. 3-Iodobenzoic acid (2.53 g, 0.01 mol) and methanol (50 mL) were added to a three-necked flask, followed by the dropwise addition of conc. H_2SO_4 (0.5 mL). Afterwards, the mixture was reacted at reflux for 8 h. Then, cooled it to room temperature, removed the solvent under reduced pressure, EtOAc (50 mL) was added and the pH was adjusted to 7–8 with saturated aqueous Na_2CO_3 . At last, the organic layer was dried with Na_2SO_4 , filtered, and the solvent was removed to give 2.39 g white solid. Yield 90.4%, mp 112–114 °C.

3.2.6 Synthesis of intermediate 8. Methyl 3-iodobenzoate 7 (0.34 g, 1.30 mmol), CuCN (0.18 g, 2.0 mmol), L-proline (0.15 g, 1.30 mmol) and DMF (15 mL) were added to a three-necked flask. After being dissolved, the mixture was reacted at 70 °C for 2 h. Then, the temperature was heated to 100 °C. The reaction was completed after 9 h. After cooled to room temperature, the mixture was filtered with diatomite. The filtrate was extracted by water (100 mL) and EtOAc (100 mL). The organic layer was washed with water (50 mL \times 3), dried with NaSO_4 and filtered. Finally, EtOAc was removed under reduced pressure and 0.17 g yellow solid was obtained. Yield 81.9%, mp 52–56 °C.

3.2.7 Synthesis of intermediate 9. Methyl 3-cyanobenzoate 8 (1.16 g, 7.20 mmol) was added to a three-necked flask and dissolved by ethanol (45 mL). Mechanical stirring was started at room temperature, hydroxylamine hydrochloride (0.75 g) and triethylamine (1.10 g) were gradually added. The mixture was stirred for 3 h. Then the solvent was removed under reduced pressure and the remnant was dissolved in EtOAc (50 mL) and saturated NaCl (50 mL). The organic layer was dried with Na_2SO_4 , filtered, and evaporated to give 1.26 g light yellow solid. Yield 90.3%. ^1H NMR (400 MHz, DMSO- d_6) δ 9.91 (s, 1H), 7.97 (s, 1H), 7.95–7.94 (m, 1H), 7.85–7.80 (m, 2H), 5.94 (s, 2H), 3.86 (s, 3H).

3.2.8 Synthesis of intermediate 10. 3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid 5 (0.88 g, 5.00 mmol) and SOCl_2 (10 mL) were added to a three-necked flask, then reacted at reflux for 3 h. The SOCl_2 was removed under reduced pressure to obtain 3-difluoromethyl-1-methyl-1H-pyrazole-4-carbonyl chloride. Afterwards, intermediate 9 (0.97 g, 5.00 mmol), triethylamine (1.20 g, 12.00 mmol) and anhydrous toluene (100 mL) were added to a three-necked flask, and stirred at 0 °C for 2 h. Subsequently, the newly prepared 3-difluoromethyl-1-methyl-1H-pyrazole-4-carbonyl chloride was added dropwise to the above solution, stirred at 0 °C for 3 h, and then heated to reflux for 2 h. The mixture was washed with water (150 mL) and saturated sodium chloride solution successively. At last, the organic layer was dried by Na_2SO_4 , filtered, and the solvent was removed to give 1.06 g intermediate 10. Yield 63.8%, mp 168–169 °C. ^1H NMR (500 MHz, DMSO- d_6) δ 8.86 (s, 1H), 8.56 (s, 1H), 8.30 (d, $J = 7.5$ Hz, 1H), 8.16 (d, $J = 7.5$ Hz, 1H), 7.75 (t, $J = 8.0$ Hz, 1H), 7.39 (t, $J = 53.0$ Hz, 1H), 4.02 (s, 3H), 3.92 (s, 3H); HRMS calcd for $\text{C}_{15}\text{H}_{13}\text{F}_2\text{N}_4\text{O}_3$ [$\text{M} + \text{H}$] $^+$ 335.0950, found 335.0946.

3.2.9 Synthesis of intermediate 11. Intermediate 10 (0.74 g, 2.00 mmol) and THF (40 mL) were added to a three-necked

flask. After it was dissolved completely, NaOH (40%, 5 mL) was added and refluxed for 2 h. Afterwards, THF was removed, and water (50 mL) was added to dissolve the solids, the pH was adjusted to 2–3 with hydrochloric acid to precipitate 0.66 g white solid. Yield 93.1%, mp 286–289 °C. ^1H NMR (400 MHz, DMSO- d_6) δ 13.33 (s, 1H), 8.89 (s, 1H), 8.61 (t, $J = 1.7$ Hz, 1H), 8.30 (dd, $J = 7.8, 1.5$ Hz, 1H), 8.17 (dd, $J = 7.8, 1.5$ Hz, 1H), 7.74 (t, $J = 7.7$ Hz, 1H), 7.41 (t, $J = 53.2$ Hz, 1H), 4.03 (s, 3H).

3.2.10 Synthesis of target compounds 12a–12r. Intermediate 11 (2.00 mmol) and SOCl_2 (10 mL) were added to a three-necked flask and then heated to reflux for 3 h. After SOCl_2 was removed, THF (30 mL) was added, and the mixture (substituted aniline (2.20 mmol), triethylamine (5 mmol), THF (2 mL)) was added dropwise under ice bath. Finally, stirred overnight, the target compound 12a–12r were obtained by column chromatography.

3.2.10.1 3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-phenylbenzamide (12a). White solid, yield 63.8%, mp 217–219 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.51 (s, 1H), 8.86 (s, 1H), 8.61 (s, 1H), 8.23 (dd, $J = 28.5, 7.5$ Hz, 2H), 7.82 (d, $J = 8.0$ Hz, 2H), 7.75 (t, $J = 7.5$ Hz, 1H), 7.58–7.28 (m, 3H), 7.13 (t, $J = 7.5$ Hz, 1H), 4.02 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.92, 167.53, 164.75, 143.62 (t, $J = 26.0$ Hz), 139.00, 135.95, 135.52, 130.70, 130.00 (d, $J = 11.2$ Hz), 129.48, 128.66, 126.45, 126.26, 123.91, 120.54, 110.77 (t, $J = 237.1$ Hz), 104.29, 39.55; HRMS calcd for $\text{C}_{20}\text{H}_{16}\text{F}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 396.1267, found 396.1261.

3.2.10.2 3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(2-tolyl)benzamide (12b). White solid, yield 61.4%, mp 211–214 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.17 (s, 1H), 8.87 (s, 1H), 8.65 (s, 1H), 8.25 (dd, $J = 25.0, 8.0$ Hz, 2H), 7.77 (t, $J = 7.5$ Hz, 1H), 7.56–7.32 (m, 2H), 7.30 (d, $J = 7.0$ Hz, 1H), 7.28–7.16 (m, 2H), 4.03 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.95, 167.57, 164.61, 143.55 (t, $J = 26.0$ Hz), 136.24, 135.56, 133.85, 130.68, 130.41, 130.08, 129.97 (d, $J = 2.4$ Hz), 129.65, 129.59, 129.55 (d, $J = 2.7$ Hz), 126.71, 126.27 (d, $J = 9.4$ Hz), 126.10, 110.83 (t, $J = 246.5$ Hz), 104.30, 39.57, 17.95; HRMS calcd for $\text{C}_{21}\text{H}_{18}\text{F}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 410.1423, found 410.1420.

3.2.10.3 3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(3-tolyl)benzamide (12c). White solid, yield 69.3%, mp 170–173 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.42 (s, 1H), 8.87 (s, 1H), 8.61 (s, 1H), 8.22 (dd, $J = 36.5, 7.5$ Hz, 2H), 7.76 (t, $J = 7.5$ Hz, 1H), 7.68–7.57 (m, 2H), 7.43 (t, $J = 53.0$ Hz, 1H), 7.26 (t, $J = 8.0$ Hz, 1H), 6.95 (d, $J = 7.0$ Hz, 1H), 4.03 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.95, 167.56, 164.69, 143.75 (t, $J = 25.8$ Hz), 138.91, 137.86, 136.01, 135.56, 130.69 (d, $J = 11.0$ Hz), 129.99 (d, $J = 14.8$ Hz), 129.51, 128.53, 126.43, 126.26, 124.64, 121.07, 117.73, 109.93 (t, $J = 233.1$ Hz), 104.30, 39.56, 21.24; HRMS calcd for $\text{C}_{21}\text{H}_{18}\text{F}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 410.1423, found 410.1418.

3.2.10.4 3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(4-tolyl)benzamide (12d). White solid, yield 61.4%, mp 225–229 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.40 (s, 1H), 8.86 (s, 1H), 8.59 (s, 1H), 8.21 (dd, $J = 37.5, 8.0$ Hz, 2H), 7.74 (t, $J = 7.5$ Hz, 1H), 7.67 (d, $J = 8.0$ Hz, 2H), 7.42 (t, $J = 53.0$ Hz, 1H), 7.17 (d, $J = 8.5$ Hz, 2H), 4.01 (s, 3H), 2.28 (s, 3H);



^{13}C NMR (126 MHz, DMSO- d_6) δ 169.95, 167.56, 164.55, 143.64, 136.45, 136.04, 135.57 (d, $J = 2.5$ Hz), 132.93, 130.66 (d, $J = 11.9$ Hz), 129.89, 129.51 (d, $J = 2.3$ Hz), 129.08 (d, $J = 3.7$ Hz), 129.04, 126.24, 120.56 (d, $J = 4.2$ Hz), 109.86 (t, $J = 212.94$ Hz), 104.29, 39.56, 20.54; HRMS calcd for $\text{C}_{21}\text{H}_{18}\text{F}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 410.1423, found 410.1418.

3.2.10.5 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(4-(tert-butyl)phenyl)benzamide (12e)*. White solid, yield 69.2%, mp 146–149 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.44 (s, 1H), 8.85 (s, 1H), 8.62 (s, 1H), 8.22 (dd, $J = 26.5, 8.0$ Hz, 2H), 7.79–7.66 (m, 3H), 7.58–7.23 (m, 3H), 4.02 (s, 3H), 1.28 (s, 9H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.91, 167.54, 164.52, 146.26, 143.63 (t, $J = 25.9$ Hz), 136.42, 135.97, 135.49 (d, $J = 2.8$ Hz), 130.61 (d, $J = 12.8$ Hz), 129.91 (d, $J = 13.2$ Hz), 129.39, 126.43, 126.25, 125.24, 120.32, 109.84 (t, $J = 225.54$ Hz), 104.31, 39.54, 34.06, 31.19; HRMS calcd for $\text{C}_{24}\text{H}_{24}\text{F}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 452.2893, found 452.1891.

3.2.10.6 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(3-(trifluoromethyl)phenyl)benzamide (12f)*. White solid, yield 76.8%, mp 191–194 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.78 (s, 1H), 8.86 (s, 1H), 8.63 (s, 1H), 8.27 (s, 2H), 8.15 (dd, $J = 61.0, 7.5$ Hz, 2H), 7.77 (t, $J = 7.5$ Hz, 1H), 7.62 (t, $J = 7.5$ Hz, 1H), 7.56–7.29 (m, 2H), 4.02 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.94, 167.46, 165.08, 143.62 (t, $J = 26.1$ Hz), 139.79, 135.50, 135.38, 130.76, 130.32, 129.88, 129.55, 129.29, 126.43, 126.34, 124.16 (q, $J = 272.2$ Hz), 123.92, 120.18, 116.53 (d, $J = 3.8$ Hz), 109.85 (t, $J = 234.9$ Hz), 104.27, 39.53; HRMS calcd for $\text{C}_{21}\text{H}_{15}\text{F}_5\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 464.1140, found 464.1136.

3.2.10.7 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(2-fluorophenyl)benzamide (12g)*. White solid, yield 59.7%, mp 221–224 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.42 (s, 1H), 8.88 (s, 1H), 8.64 (s, 1H), 8.25 (dd, $J = 36.0, 7.5$ Hz, 2H), 7.78 (t, $J = 8.0$ Hz, 1H), 7.64 (t, $J = 7.5$ Hz, 1H), 7.44 (t, $J = 53.5$ Hz, 1H), 7.34–7.21 (m, 3H), 4.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.96, 167.51, 164.73, 156.85, 154.88, 143.63, 135.55, 134.94, 130.80, 130.29, 129.62, 127.19 (d, $J = 8.0$ Hz), 126.57, 126.31, 125.53 (d, $J = 12.6$ Hz), 124.37 (d, $J = 3.2$ Hz), 115.91 (d, $J = 19.8$ Hz), 109.87 (t, $J = 234.6$ Hz), 104.28 (d, $J = 2.1$ Hz), 39.55; HRMS calcd for $\text{C}_{20}\text{H}_{15}\text{F}_3\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 414.1172, found 414.1172.

3.2.10.8 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(3-fluorophenyl)benzamide (12h)*. White solid, yield 62.5%, mp 192–195 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.68 (s, 1H), 8.87 (s, 1H), 8.60 (s, 1H), 8.23 (dd, $J = 42.5, 8.0$ Hz, 2H), 7.86–7.70 (m, 2H), 7.60 (d, $J = 8.0$ Hz, 1H), 7.55–7.30 (m, 2H), 6.96 (t, $J = 8.5$ Hz, 1H), 4.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 170.37, 167.91, 165.42, 163.48, 161.56, 144.06 (t, $J = 26.0$ Hz), 141.18 (d, $J = 11.0$ Hz), 135.97 (d, $J = 11.3$ Hz), 131.13, 130.72, 130.64, 129.93, 126.88, 126.76, 116.57 (d, $J = 2.4$ Hz), 110.77 (d, $J = 20.16$ Hz), 110.29 (t, $J = 234.36$ Hz), 107.61 (d, $J = 26.2$ Hz), 104.73, 39.98; HRMS calcd for $\text{C}_{20}\text{H}_{15}\text{F}_3\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 414.1172, found 414.1167.

3.2.10.9 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(4-fluorophenyl)benzamide (12i)*. White solid, yield 61.6%, mp 241–245 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.55 (s, 1H), 8.86 (s, 1H), 8.60 (s, 1H), 8.22 (dd, $J = 36.5, 7.5$ Hz, 2H), 7.82 (dd, $J = 9.0, 5.0$ Hz, 2H), 7.75 (t, $J = 7.5$ Hz, 1H),

7.43 (t, $J = 53.0$ Hz, 1H), 7.22 (t, $J = 9.0$ Hz, 2H), 4.02 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 170.36, 167.94, 165.08, 159.84, 157.93, 144.05 (t, $J = 26.0$ Hz), 136.19, 135.95, 135.75 (d, $J = 2.6$ Hz), 131.08, 130.49, 129.92, 126.77 (d, $J = 14.6$ Hz), 122.81 (d, $J = 7.9$ Hz), 115.67 (d, $J = 22.2$ Hz), 110.30 (t, $J = 234.7$ Hz), 104.72, 39.97; HRMS calcd for $\text{C}_{20}\text{H}_{15}\text{F}_3\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 414.1172, found 414.1170.

3.2.10.10 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(2-chlorophenyl)benzamide (12j)*. White solid, yield 62.8%, mp 208–211 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.38 (s, 1H), 8.88 (s, 1H), 8.66 (s, 1H), 8.27 (d, $J = 28.0$ Hz, 2H), 7.88–7.21 (m, 6H), 4.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 170.39, 167.93, 165.16, 144.07 (t, $J = 25.9$ Hz), 135.98, 135.43, 135.35, 131.13, 130.73, 130.16, 130.08, 129.07, 128.18, 128.00, 126.98, 126.80, 110.31 (t, $J = 235.0$ Hz), 111.29, 104.72, 39.99; HRMS calcd for $\text{C}_{20}\text{H}_{15}\text{ClF}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 430.0877, found 430.0880.

3.2.10.11 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(3-chlorophenyl)benzamide (12k)*. White solid, yield 65.0%, mp 194–197 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.66 (s, 1H), 8.88 (s, 1H), 8.61 (s, 1H), 8.23 (dd, $J = 45.5, 7.5$ Hz, 2H), 7.99 (s, 1H), 7.82–7.71 (m, 2H), 7.58–7.29 (m, 2H), 7.19 (d, $J = 7.0$ Hz, 1H), 4.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.97, 167.49, 165.01, 143.64 (t, $J = 26.2$ Hz), 140.47, 135.56, 133.01, 130.78, 130.39, 130.28, 129.60, 126.45, 126.33, 123.62, 119.90, 118.80, 110.81 (t, $J = 234.6$ Hz), 104.27, 99.55, 39.56; HRMS calcd for $\text{C}_{20}\text{H}_{15}\text{ClF}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 430.0877, found 430.0872.

3.2.10.12 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(4-chlorophenyl)benzamide (12l)*. White solid, yield 65.4%, mp 228–230 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.62 (s, 1H), 8.87 (s, 1H), 8.60 (s, 1H), 8.23 (dd, $J = 42.0, 8.0$ Hz, 2H), 7.85 (d, $J = 8.5$ Hz, 2H), 7.76 (t, $J = 8.0$ Hz, 1H), 7.64–7.26 (m, 3H), 4.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.94, 167.50, 164.83, 143.63 (t, $J = 26.2$ Hz), 137.95, 135.67, 135.54, 130.72, 130.16, 129.53, 128.58, 127.55, 126.43, 126.29, 122.01, 109.86 (t, $J = 234.7$ Hz), 104.27, 39.55; HRMS calcd for $\text{C}_{20}\text{H}_{15}\text{ClF}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 430.0877, found 430.0880.

3.2.10.13 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(4-bromophenyl)benzamide (12m)*. White solid, yield 67.3%, mp 218–223 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.62 (s, 1H), 8.88 (s, 1H), 8.60 (s, 1H), 8.23 (dd, $J = 44.5, 7.5$ Hz, 2H), 7.79 (m, 3H), 7.57 (d, $J = 8.5$ Hz, 2H), 7.44 (t, $J = 53.5, 11.0$ Hz, 1H), 4.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.95, 167.49, 164.84, 143.73 (t), 138.37, 135.68, 135.55, 131.50, 130.74, 130.17, 129.56, 126.42, 126.29, 122.38, 115.63, 110.80 (t, $J = 234.8$ Hz), 104.26, 39.55; HRMS calcd for $\text{C}_{20}\text{H}_{15}\text{BrF}_2\text{N}_5\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 474.0372, found 474.0369.

3.2.10.14 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(4-iodophenyl)benzamide (12n)*. White solid, yield 68.8%, mp 241–245 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 10.59 (s, 1H), 8.87 (s, 1H), 8.59 (s, 1H), 8.22 (dd, $J = 43.5, 7.5$ Hz, 2H), 7.81–7.58 (m, 5H), 7.43 (t, $J = 53.0$ Hz, 1H), 4.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.94, 167.49, 164.82, 143.63 (t, $J = 26.0$ Hz), 138.86, 137.34, 135.69, 135.54, 130.73, 130.16, 129.53, 126.43, 126.29, 122.63, 110.80 (t, $J = 234.6$ Hz), 104.27,



87.65, 39.56; HRMS calcd for $C_{20}H_{15}F_2N_5O_2 [M + H]^+$ 522.0233, found 522.0233.

3.2.10.15 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(2,4-dimethylphenyl)benzamide (12o)*. White solid, yield 65.0%, mp 218–222 °C; 1H NMR (500 MHz, DMSO- d_6) δ 10.09 (s, 1H), 8.86 (s, 1H), 8.65 (s, 1H), 8.24 (dd, $J = 22.0, 8.0$ Hz, 2H), 7.75 (t, $J = 7.5$ Hz, 1H), 7.44 (t, $J = 53.5$ Hz, 1H), 7.24 (d, $J = 8.0$ Hz, 1H), 7.10 (s, 1H), 7.04 (d, $J = 7.8$ Hz, 1H), 4.02 (s, 3H), 2.29 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.92, 167.56, 164.58, 143.63 (t, $J = 26.0$ Hz), 135.60, 135.51, 135.32, 133.63, 133.62, 130.90, 130.56, 129.92, 129.50, 126.61, 126.58, 126.49, 126.28, 109.87 (t, $J = 234.8$ Hz), 104.30, 39.54, 20.56, 17.84; HRMS calcd for $C_{22}H_{20}F_2N_5O_2 [M + H]^+$ 424.1580, found 424.1576.

3.2.10.16 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(2,6-dimethylphenyl)benzamide (12p)*. White solid, yield 65.4%, mp 259–264 °C; 1H NMR (500 MHz, DMSO- d_6) δ 10.05 (s, 1H), 8.87 (s, 1H), 8.66 (s, 1H), 8.26 (dd, $J = 17.0, 8.0$ Hz, 2H), 7.78 (t, $J = 8.0$ Hz, 1H), 7.43 (t, $J = 53.5$ Hz, 1H), 7.15 (s, 3H), 4.02 (s, 3H), 2.22 (s, 6H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 170.38, 168.01, 167.91, 167.80, 164.78, 143.98 (t, $J = 23.2$ Hz), 136.04, 135.97, 135.84, 135.52, 130.86, 130.42, 130.06, 128.24, 127.28, 126.83, 111.25 (t, $J = 235.0$ Hz), 104.73, 39.99, 18.50; HRMS calcd for $C_{22}H_{20}F_2N_5O_2 [M + H]^+$ 424.1580, found 424.1577.

3.2.10.17 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(3-chloro-2-methylphenyl)benzamide (12q)*. White solid, yield 67.3%, mp 214–217 °C; 1H NMR (500 MHz, DMSO- d_6) δ 10.41 (s, 1H), 8.87 (s, 1H), 8.65 (s, 1H), 8.26 (dd, $J = 28.0, 7.5$ Hz, 2H), 7.77 (t, $J = 8.0$ Hz, 1H), 7.56–7.31 (m, 3H), 7.31–7.25 (m, 1H), 4.03 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.93, 167.50, 164.79, 143.62 (t, $J = 26.1$ Hz), 137.88, 135.51, 135.13, 133.86, 132.29, 130.68, 130.18, 129.58, 126.95, 126.93, 126.52, 126.34, 125.92, 109.86 (t, $J = 234.8$ Hz), 104.27, 39.54, 15.37; HRMS calcd for $C_{21}H_{17}ClF_2N_5O_2 [M + H]^+$ 444.1033, found 444.1028.

3.2.10.18 *3-(5-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)-N-(3,4-dichlorophenyl)benzamide (12r)*. White solid, yield 68.8%, mp 204–208 °C; 1H NMR (500 MHz, DMSO- d_6) δ 10.74 (s, 1H), 8.87 (s, 1H), 8.60 (s, 1H), 8.28 (m, 3H), 7.79–7.76 (m, 2H), 7.63 (d, $J = 8.5$ Hz, 1H), 7.43 (t, $J = 53.0$ Hz, 1H), 4.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 169.96, 167.45, 165.01, 143.74 (t, $J = 26.4$ Hz), 139.12, 135.54, 135.29, 130.92, 130.78, 130.60, 130.39, 129.62, 126.42, 126.35, 125.39, 121.60, 120.38, 109.86 (t, $J = 234.6$ Hz), 104.26, 39.56; HRMS calcd for $C_{20}H_{14}Cl_2F_2N_5O_2 [M + H]^+$ 464.0487, found 464.0481.

3.3. Biological activity and toxicity determination

The insecticidal and fungicidal activities were investigated in the National Pesticide Engineering Research Centre, Nankai University, according to ref. 40 and 41, and the results of the activity test are shown in Tables 1–3. Through acute exposure, we assessed the toxicity of compounds **12h** on zebrafish embryo. According to the preliminary exposure experiments, a series of gradient concentrations of compounds **12h** was set on the basis of mortality rates in the range of 10–95%. LC_{50}

values for zebrafish embryos exposed to compound **12h** from 24 to 96 hpf: control (0 mg L^{-1} of **12h**), 0.049, 0.098, 0.195, 0.390 mg L^{-1} of **12h**. The LC_{50} (median lethal concentration) value was computed by the Boltzmann equation.^{42,43} The observational indexes included mortality rate and teratogenic effects. Full experimental details are available in the ESI.†

4. Conclusions

In conclusion, taking ethyl difluoroacetate and 3-iodobenzoic acid as starting materials, a total of 18 unreported pyrazole-linked 1,2,4-oxadiazole substituted benzamides were synthesized by a multi-step reaction based on the splicing principle of active substructures. The structures of the target compounds were confirmed by 1H NMR, ^{13}C NMR and HRMS. The preliminary insecticidal activity results showed that compound **12g** had excellent lethal activity against mosquito larvae (100% inhibition at 5 mg L^{-1}). Furthermore, the fungicidal activity results showed that compounds **12f** (70.6%) and **12h** (100%) exhibited obvious activities against *Pyricularia oryzae*, with EC_{50} of 8.28 and 5.49 $\mu g mL^{-1}$, respectively, which were lower than bixafen (9.15 $\mu g mL^{-1}$). Although, the resulting LC_{50} value for compound **12h** was 0.39 mg L^{-1} , which was classified as a high-toxic compound, it may be used as a potential leading compound for further structural optimisation to develop new compounds with high activity and low toxicity.

Author contributions

Y. S., M. T., S. Y., Y. W., B. S. and J. S. carried out experimental work; Y. S. prepared the manuscript; C. T. designed the material and supervised the project; And C. T. revised the paper. X. W. acquired toxic data and revised the section of zebrafish toxicity. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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