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Supporting Information

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,*]

1. ^{1}H NMR spectra of **12a~12r**

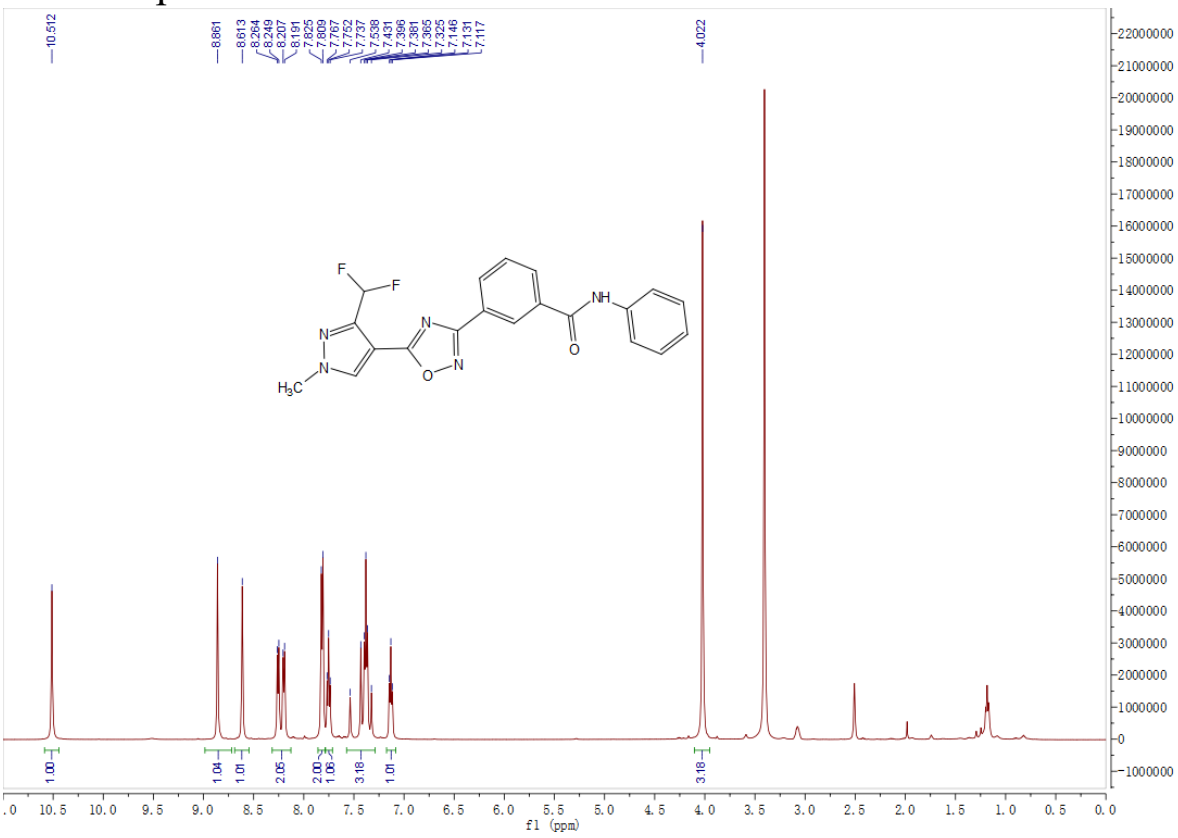

Figure S1 ^{1}H NMR spectra of **12a**

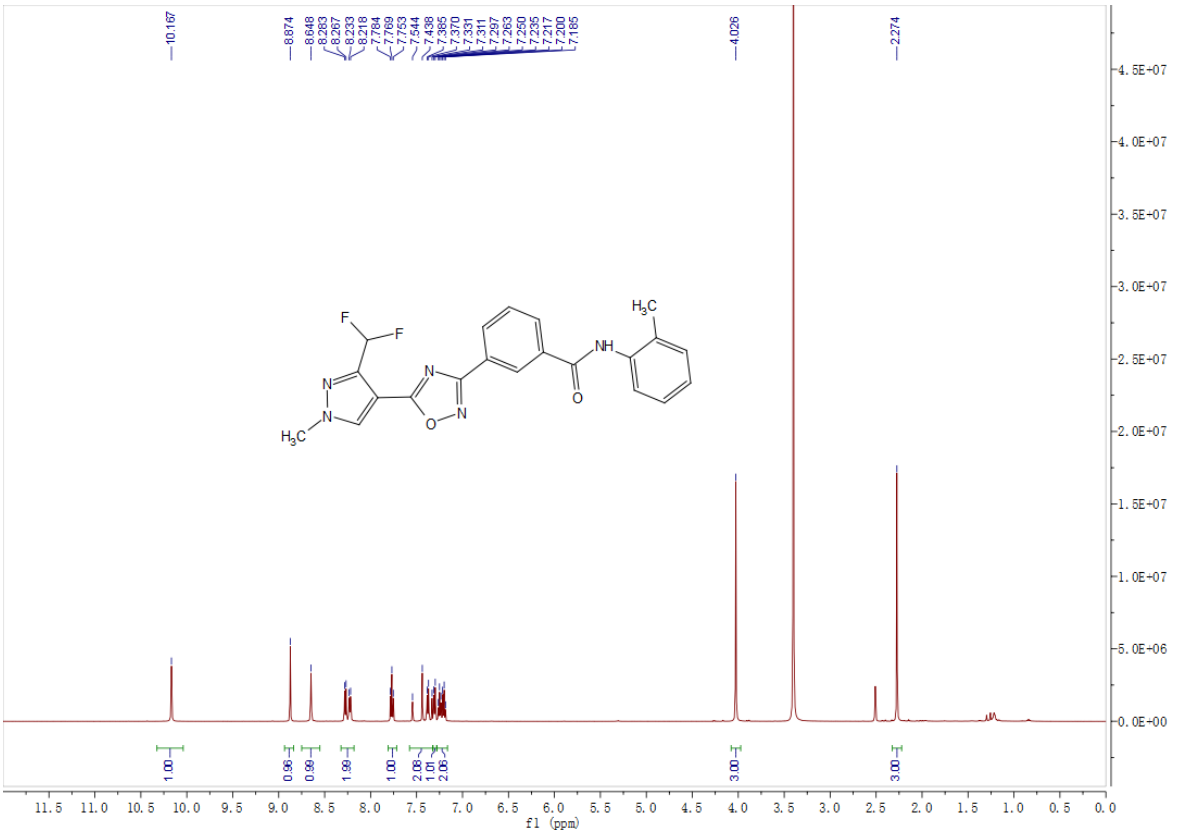

Figure S2 ^{1}H NMR spectra of **12b**

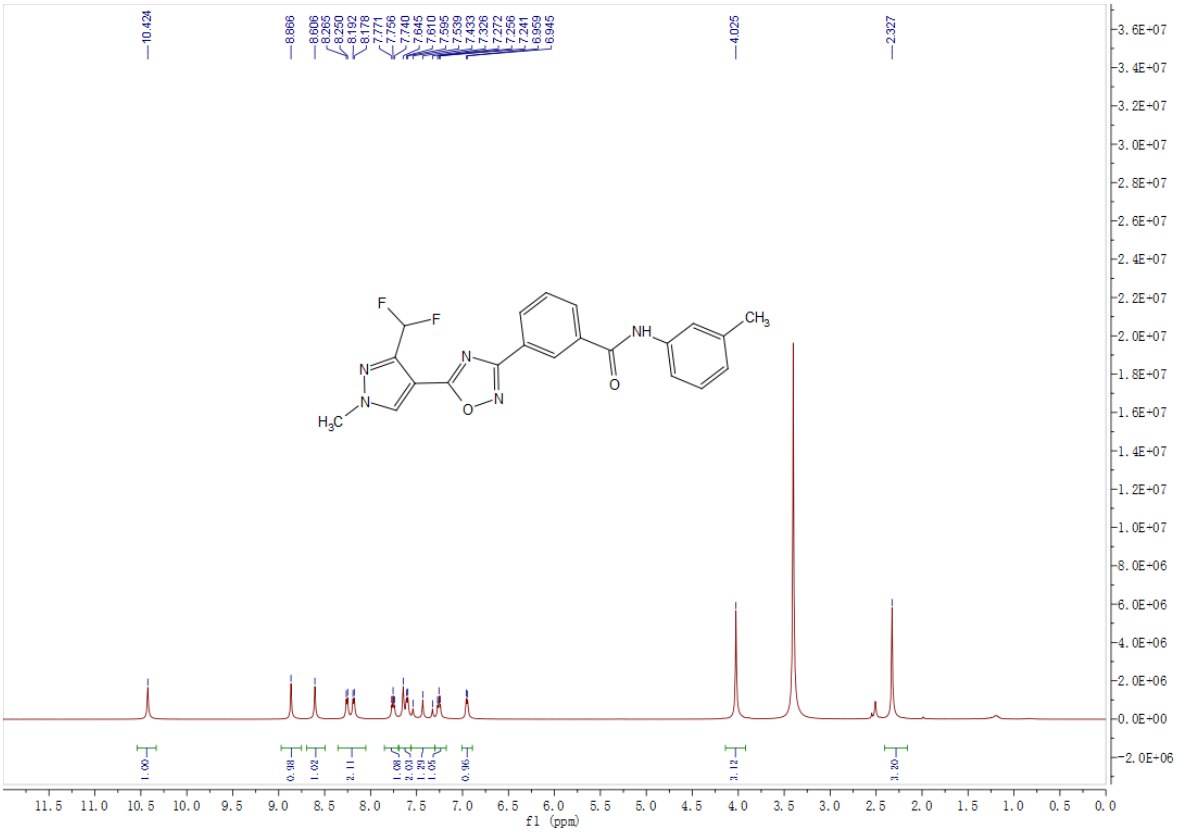

Figure S3 ^{1}H NMR spectra of **12c**

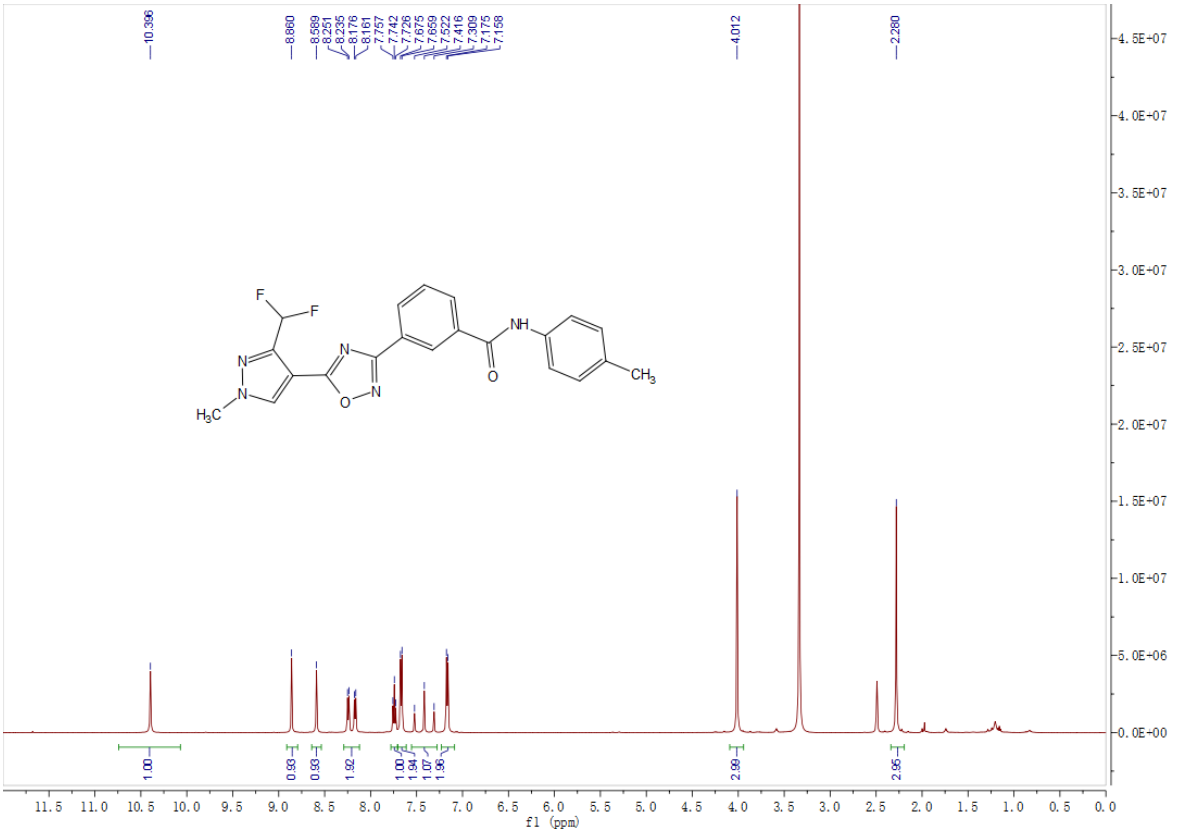

Figure S4 ^{1}H NMR spectra of **12d**

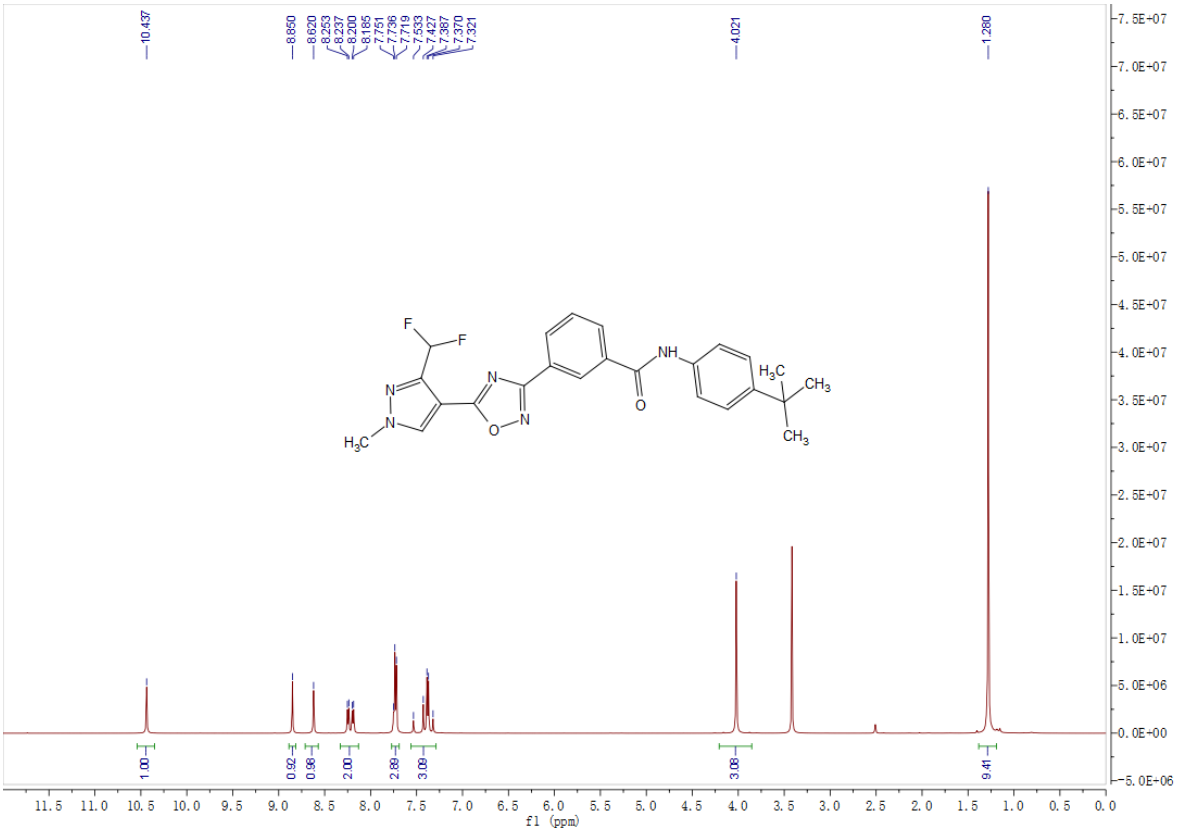

Figure S5 ^{1}H NMR spectra of **12e**

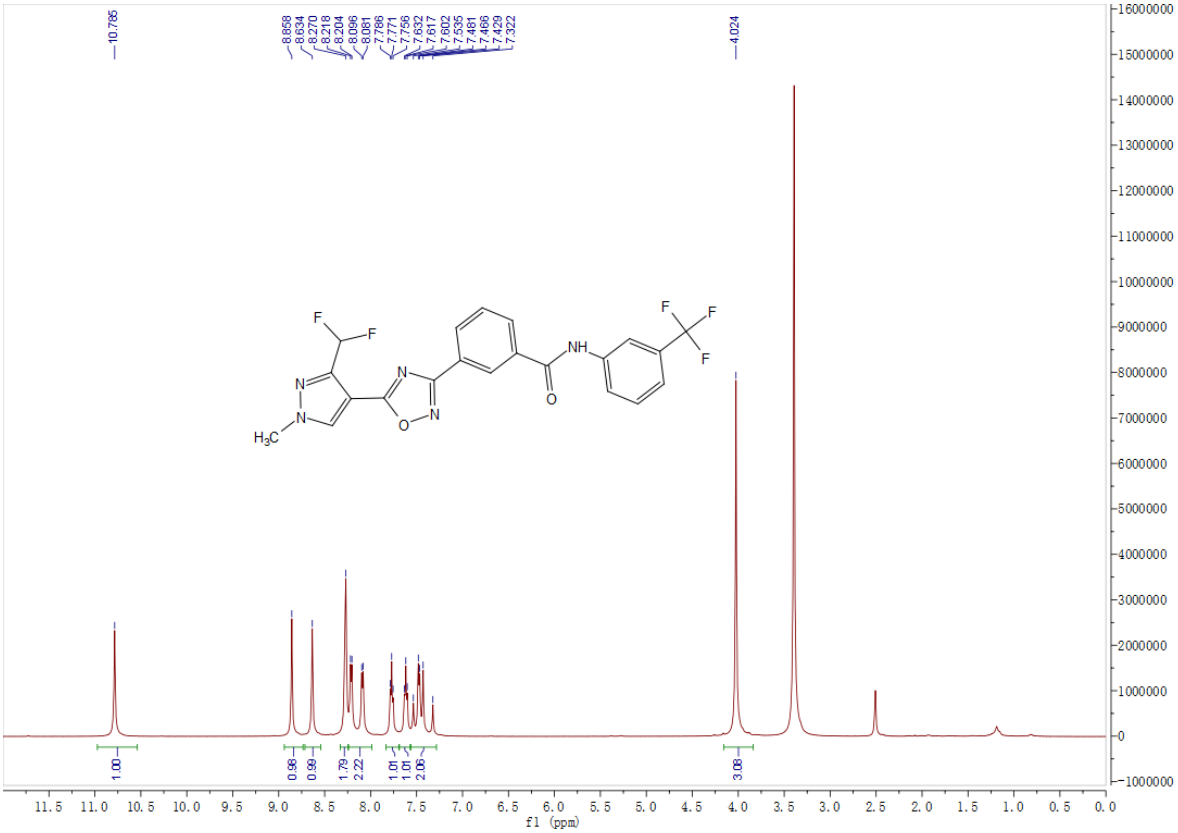

Figure S6 ^{1}H NMR spectra of **12f**

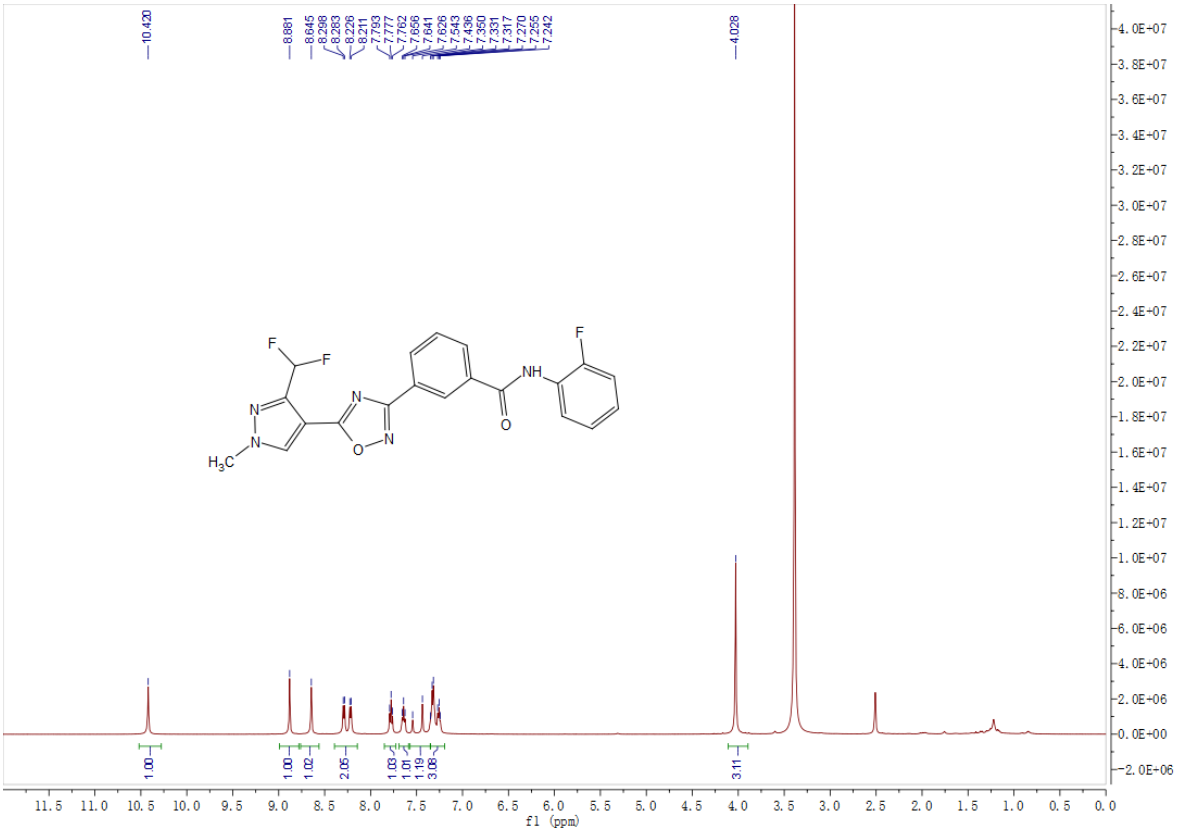

Figure S7 ^{1}H NMR spectra of **12g**

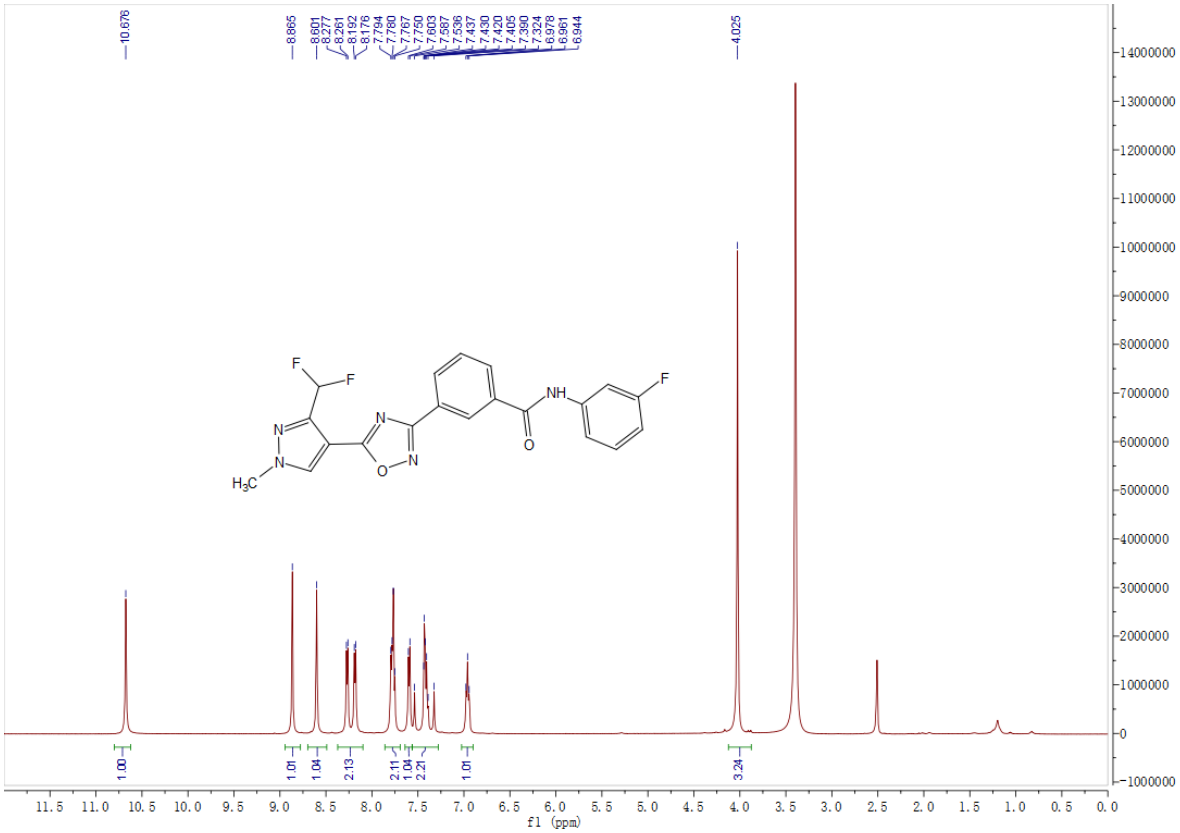

Figure S8 ^{1}H NMR spectra of **12h**

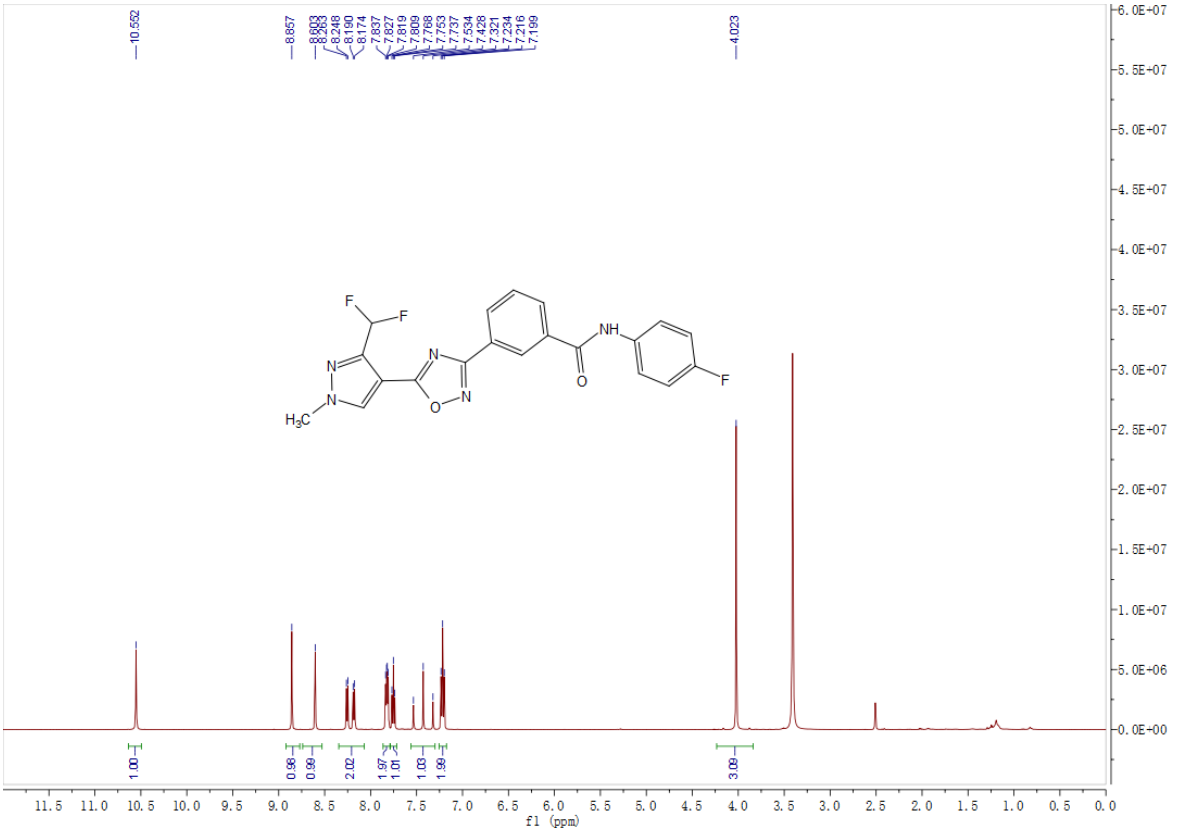

Figure S9 ^{1}H NMR spectra of **12i**

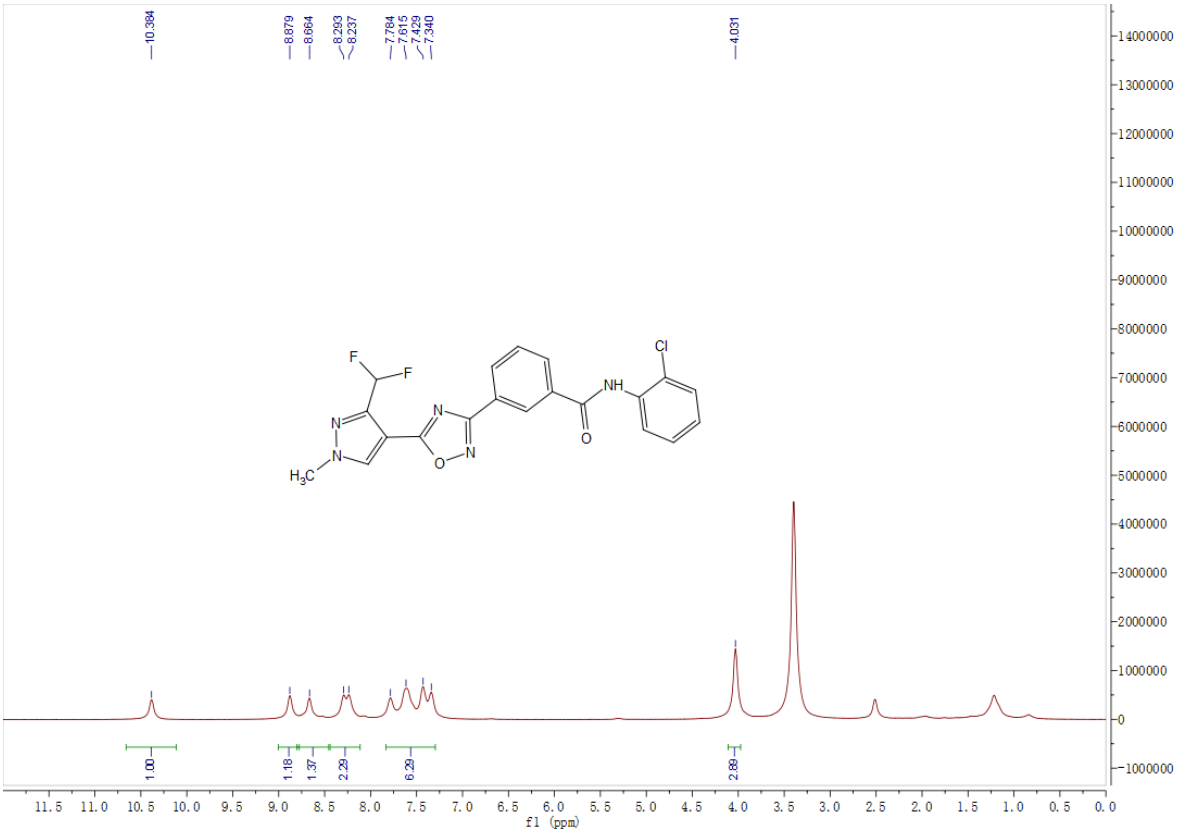

Figure S10 ^{1}H NMR spectra of **12j**

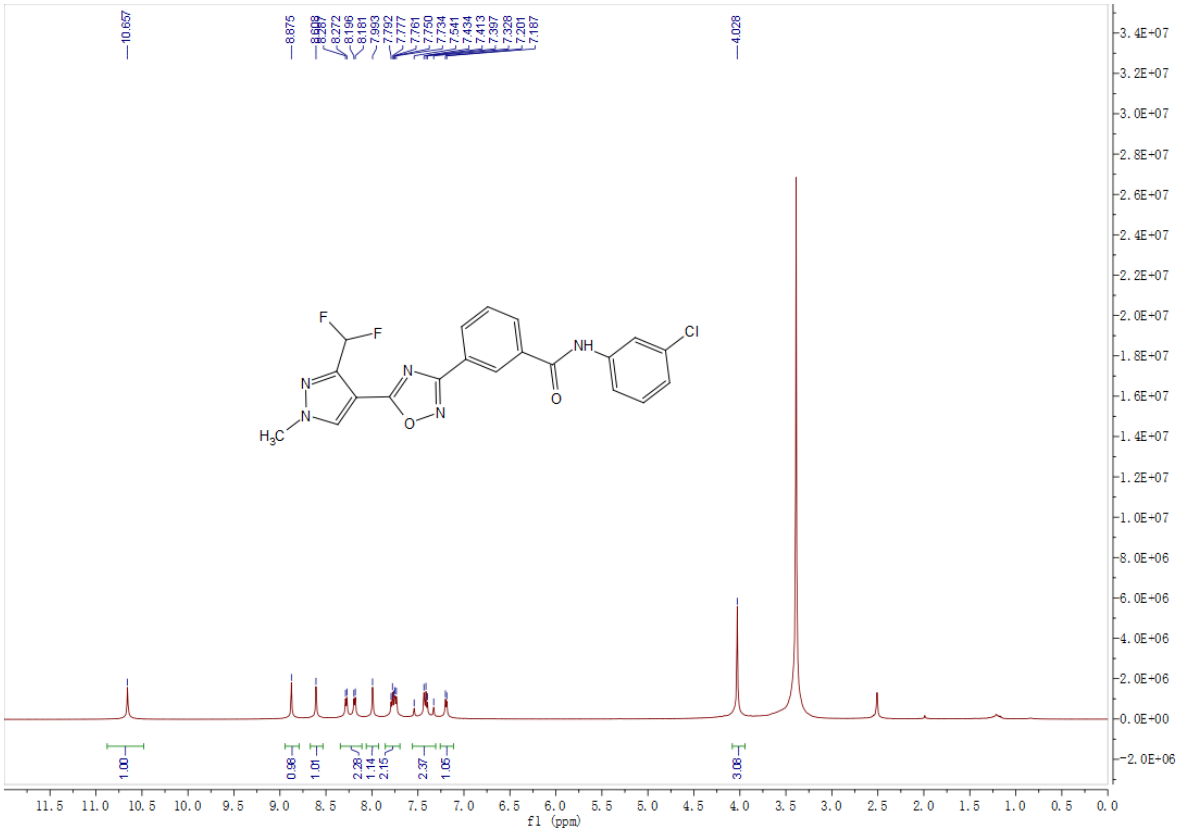

Figure S11 ^{1}H NMR spectra of 12**k**

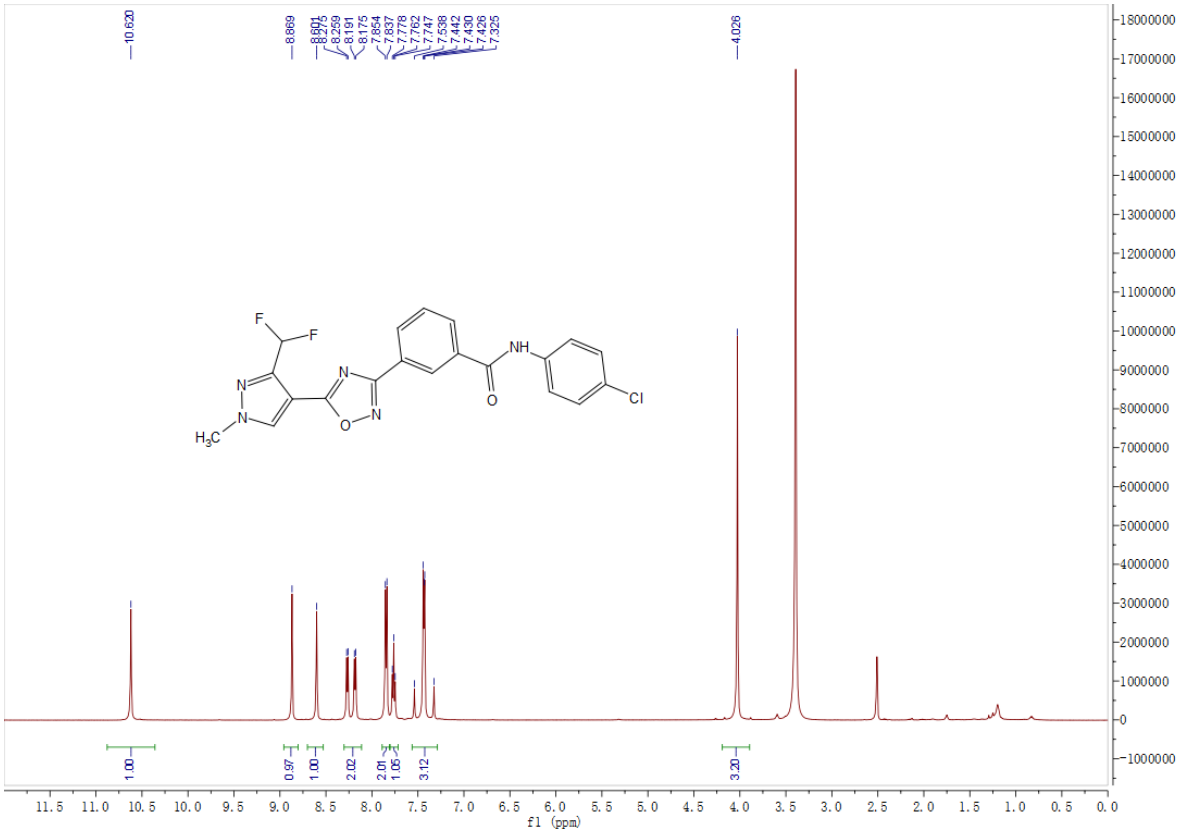

Figure S12 ^{1}H NMR spectra of **12l**

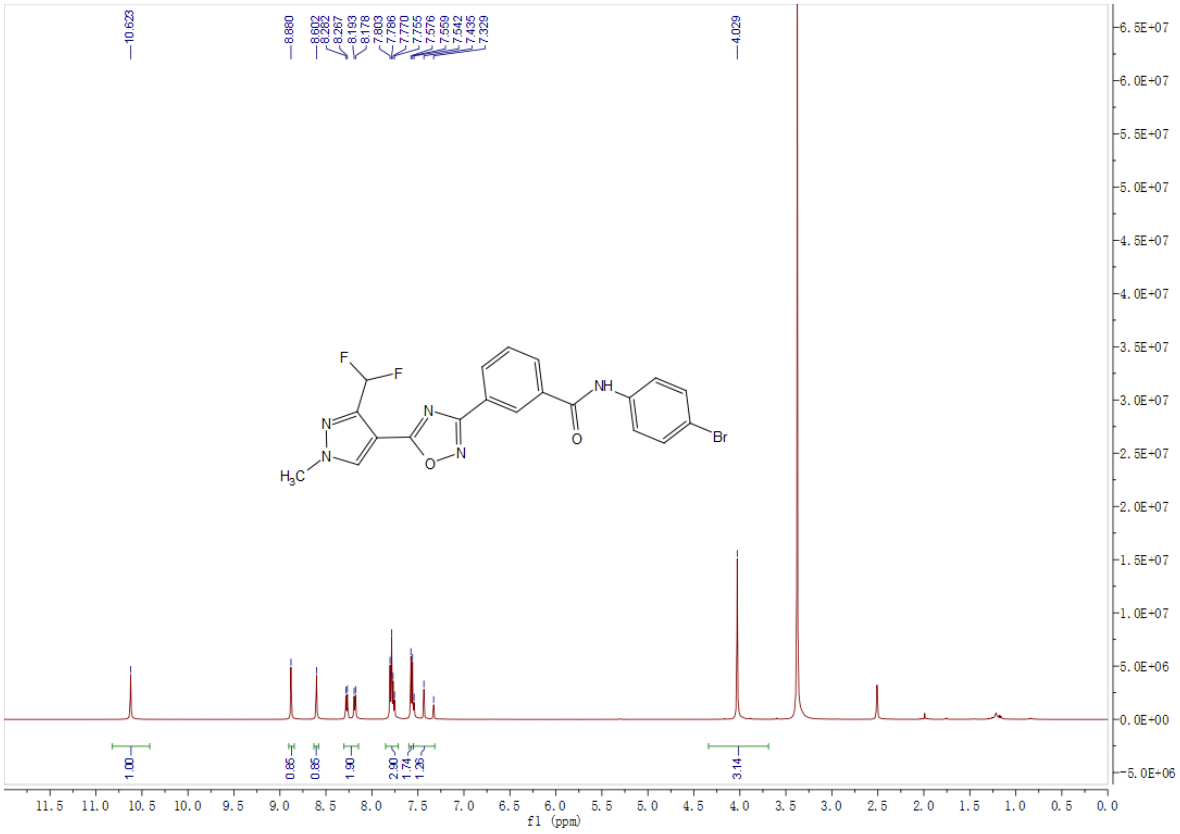

Figure S13 ^{1}H NMR spectra of **12m**

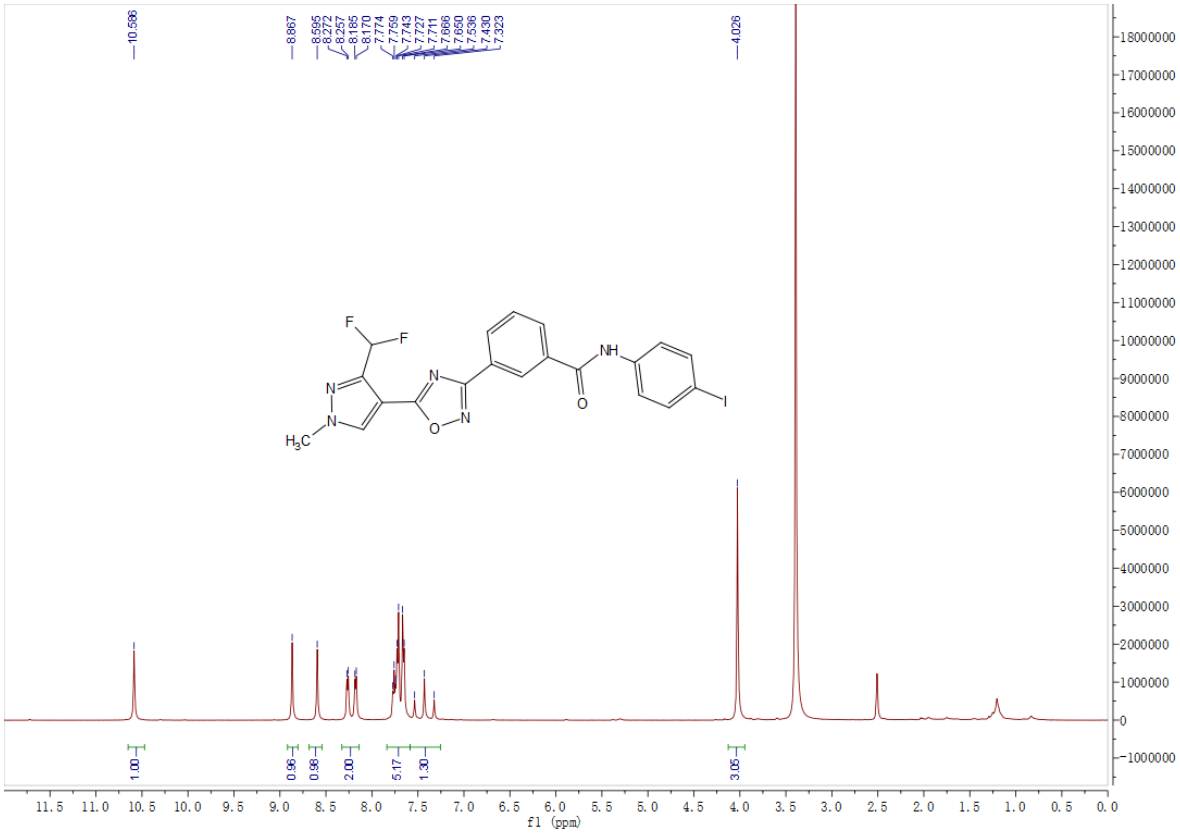

Figure S14 ^{1}H NMR spectra of **12n**

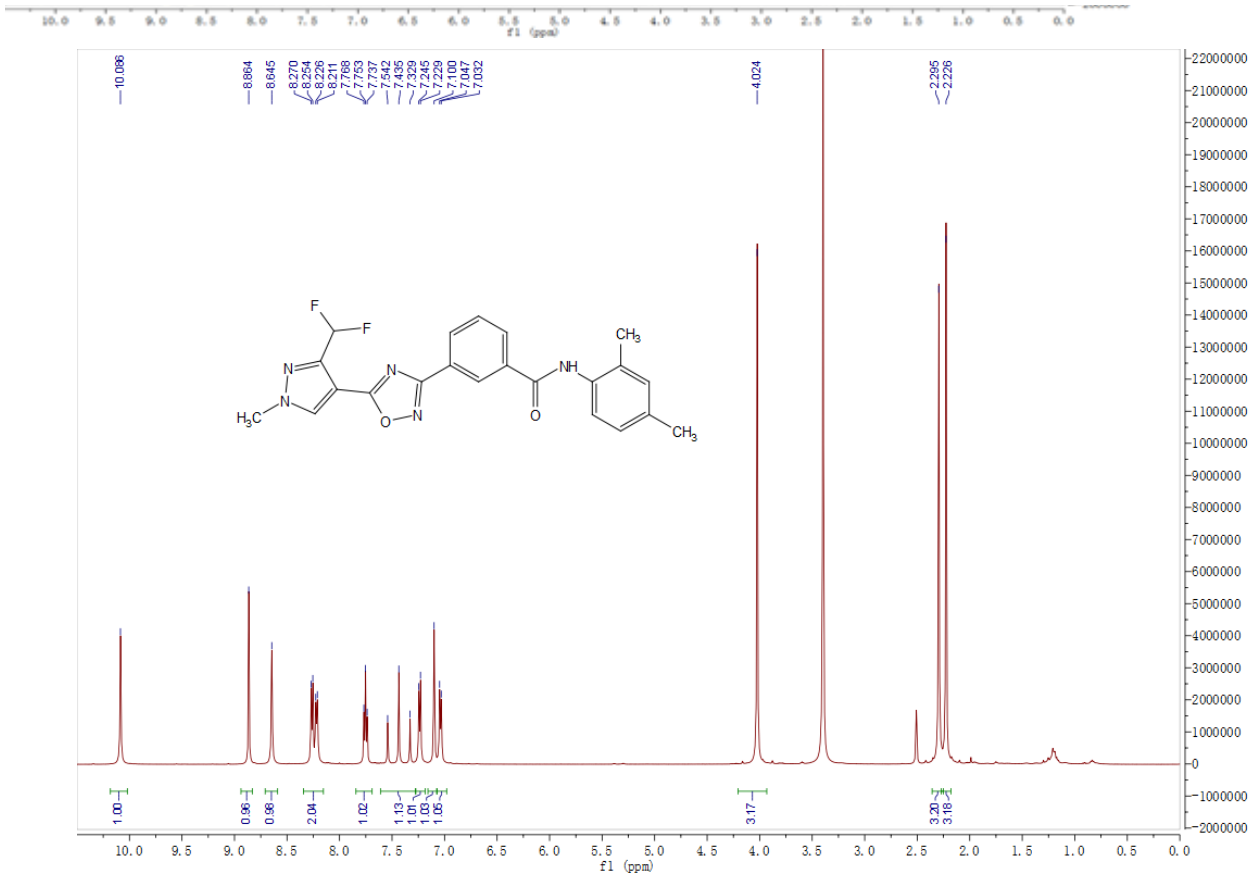

Figure S15 ^{1}H NMR spectra of **12o**

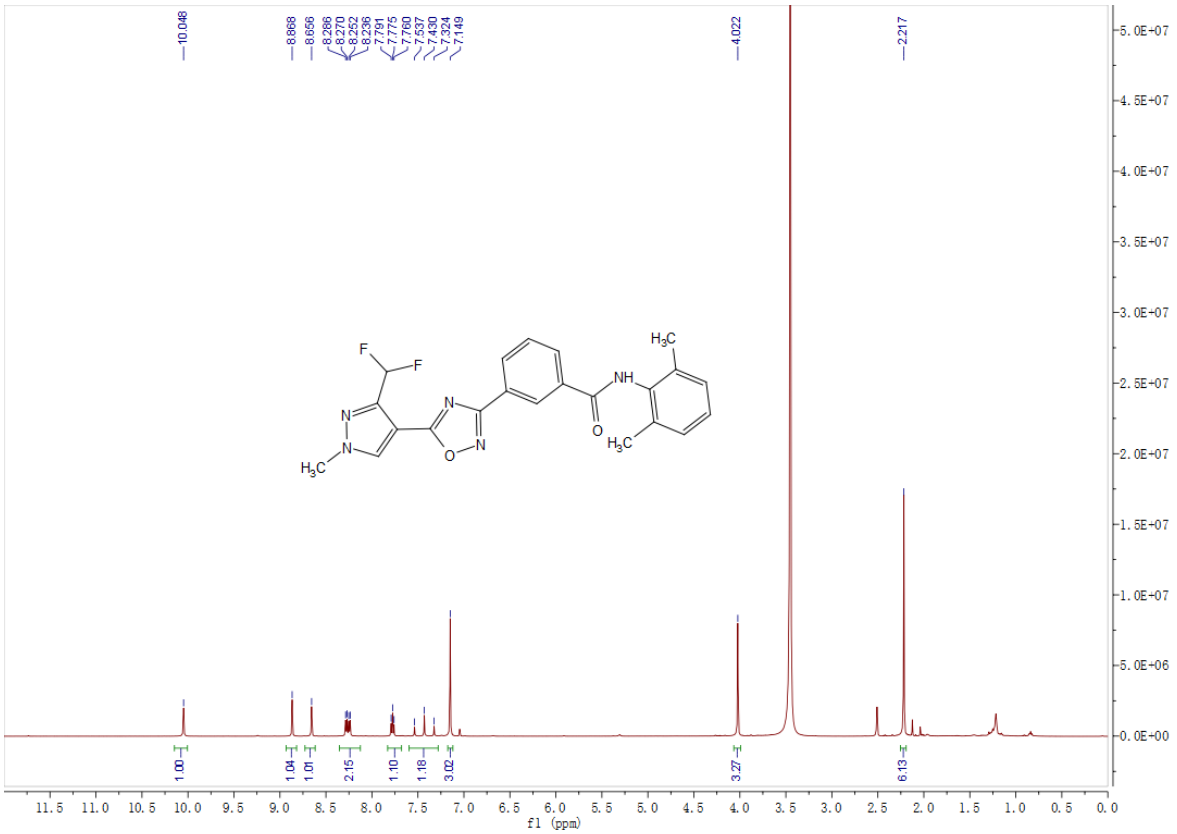

Figure S16 ^{1}H NMR spectra of **12p**

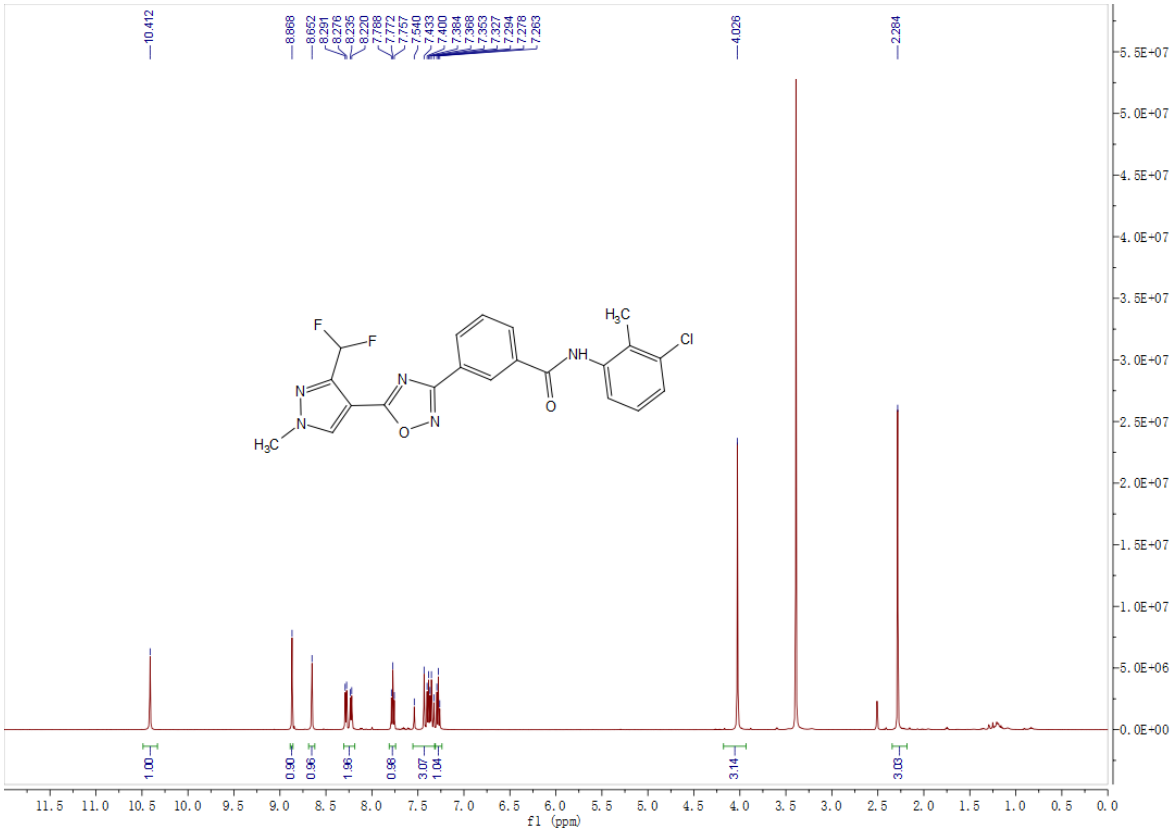

Figure S17 ^{1}H NMR spectra of **12q**

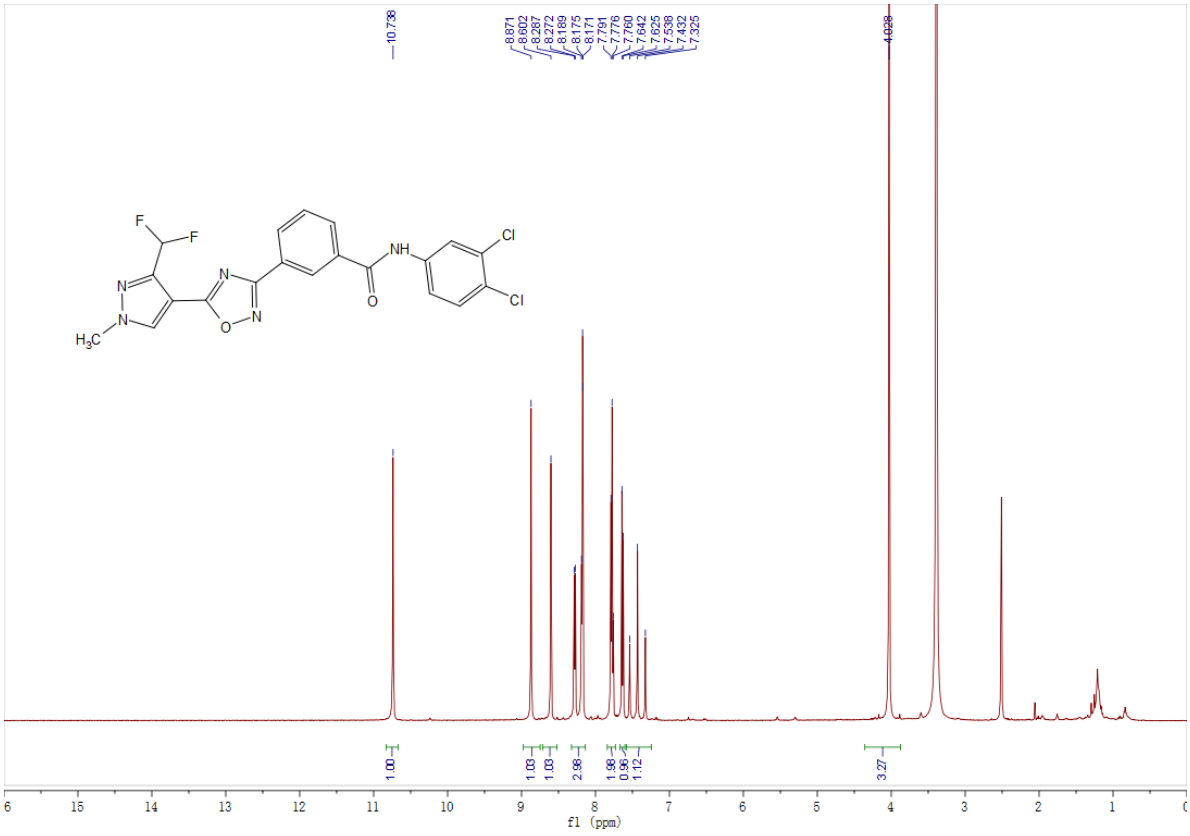

Figure S18 ^{1}H NMR spectra of **12r**

2. ^{13}C NMR spectra of **12a~12r**

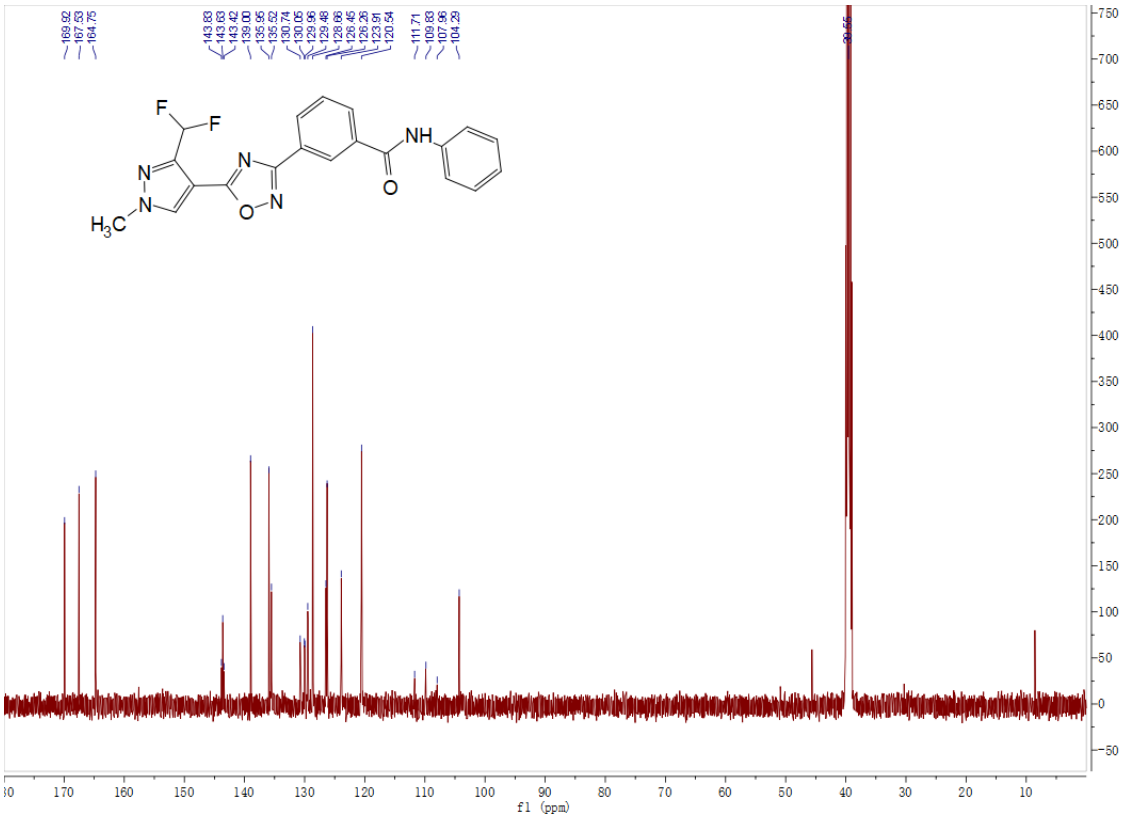

Figure S19 ^{13}C NMR spectra of **12a**

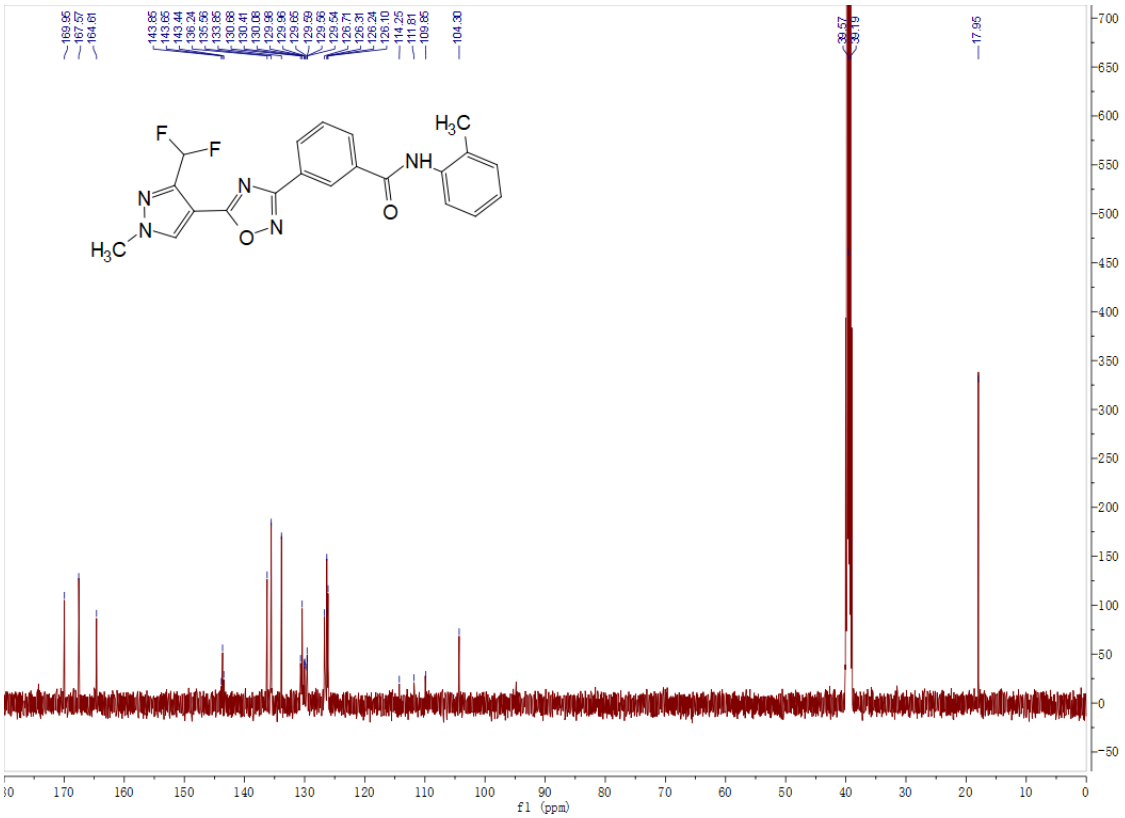

Figure S20 ^{13}C NMR spectra of **12b**

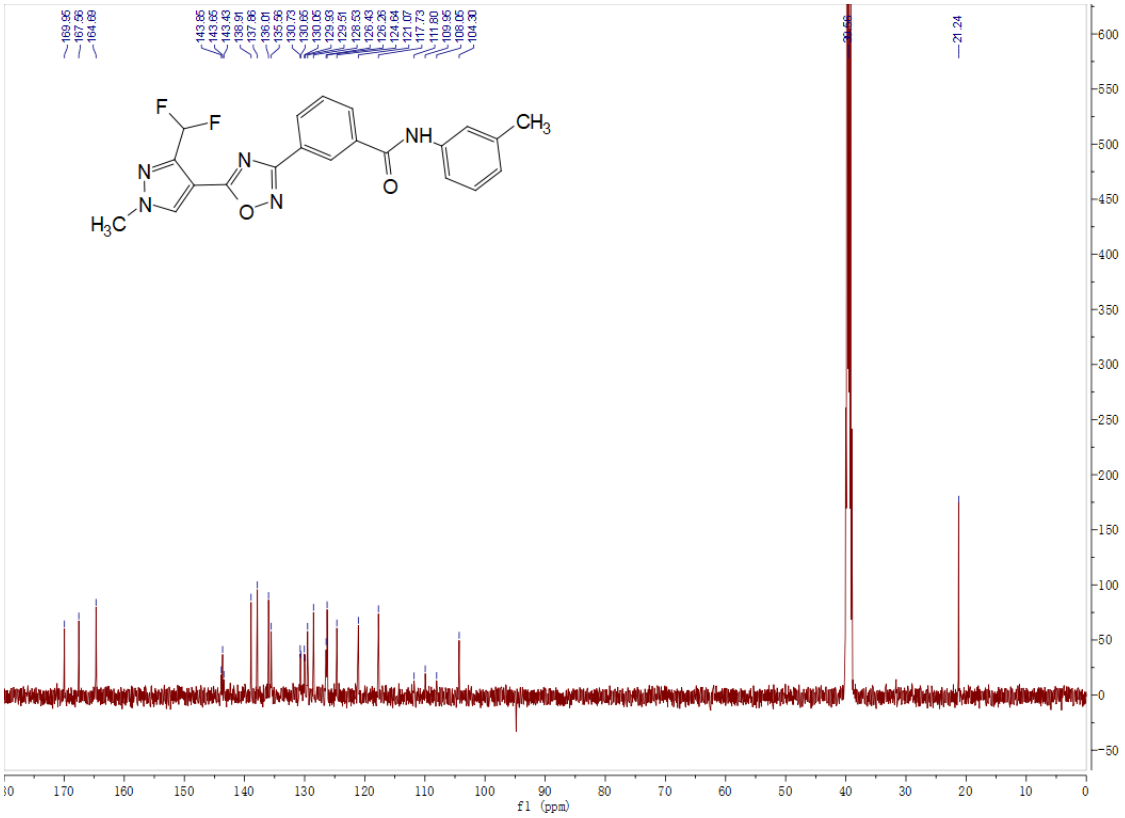

Figure S21 ^{13}C NMR spectra of **12c**

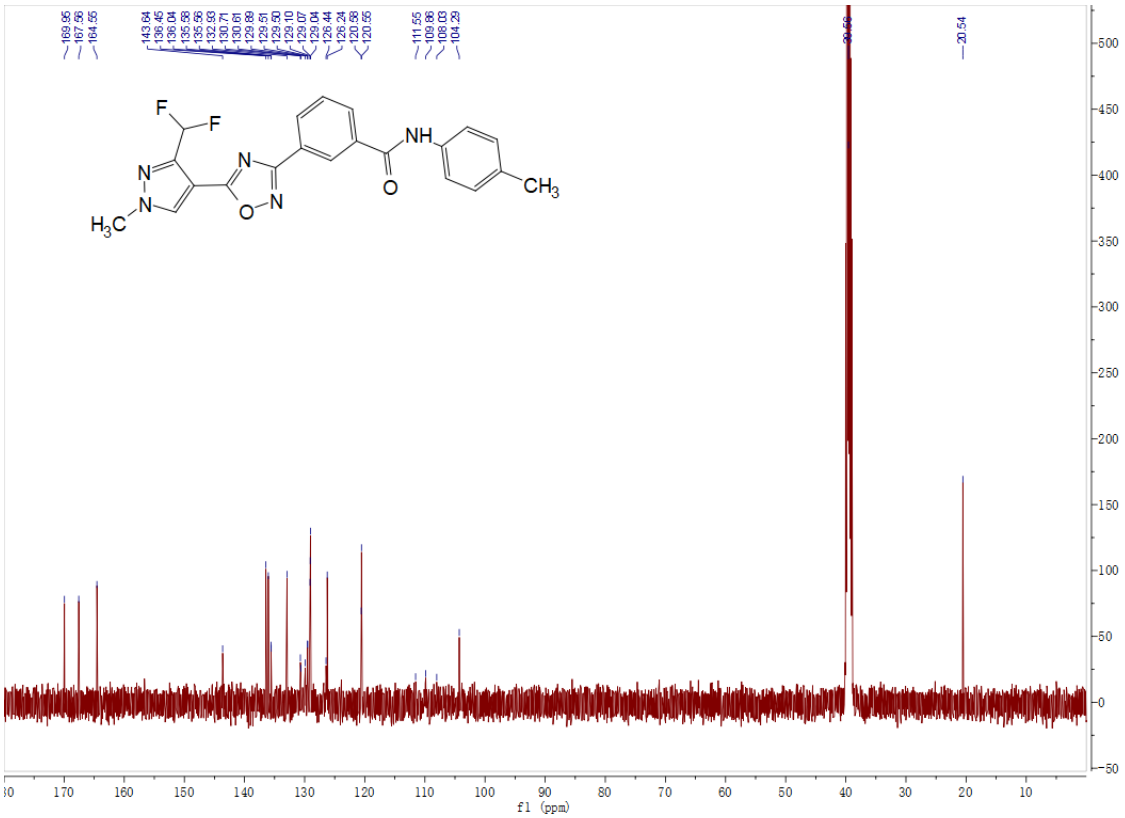

Figure S22 ^{13}C NMR spectra of **12d**

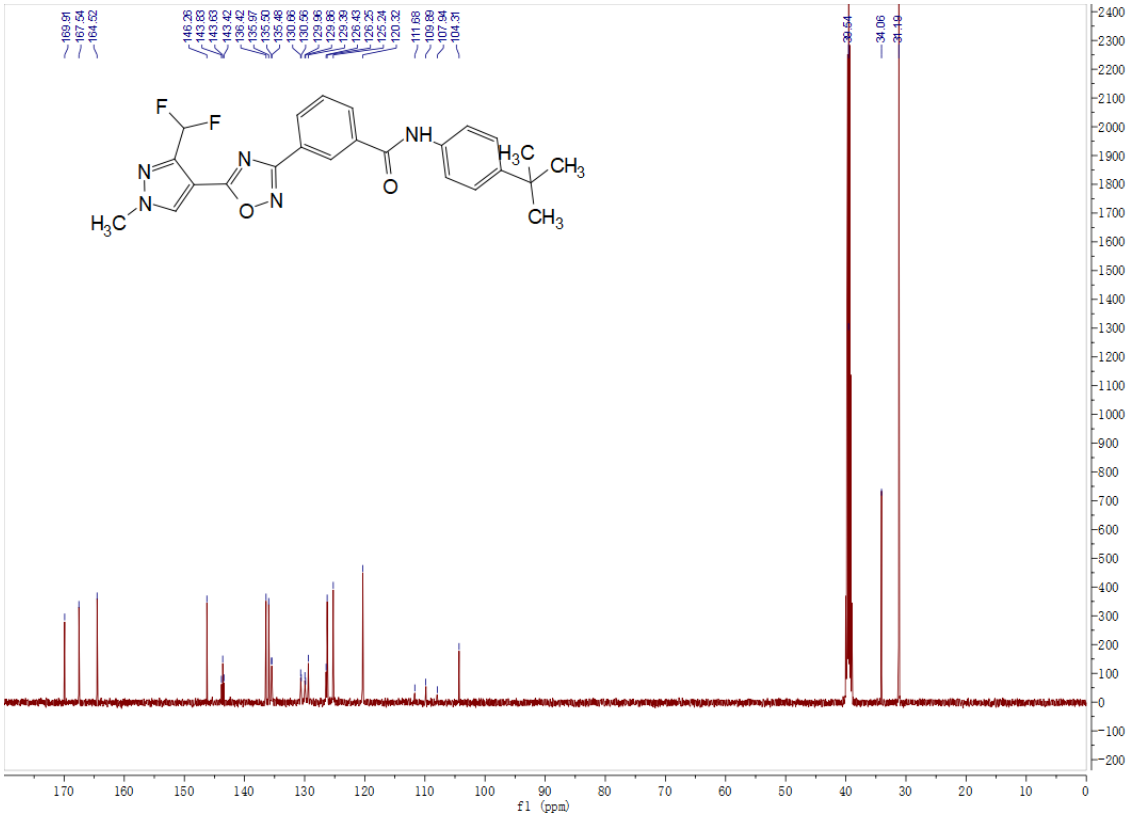

Figure S23 ^{13}C NMR spectra of **12e**

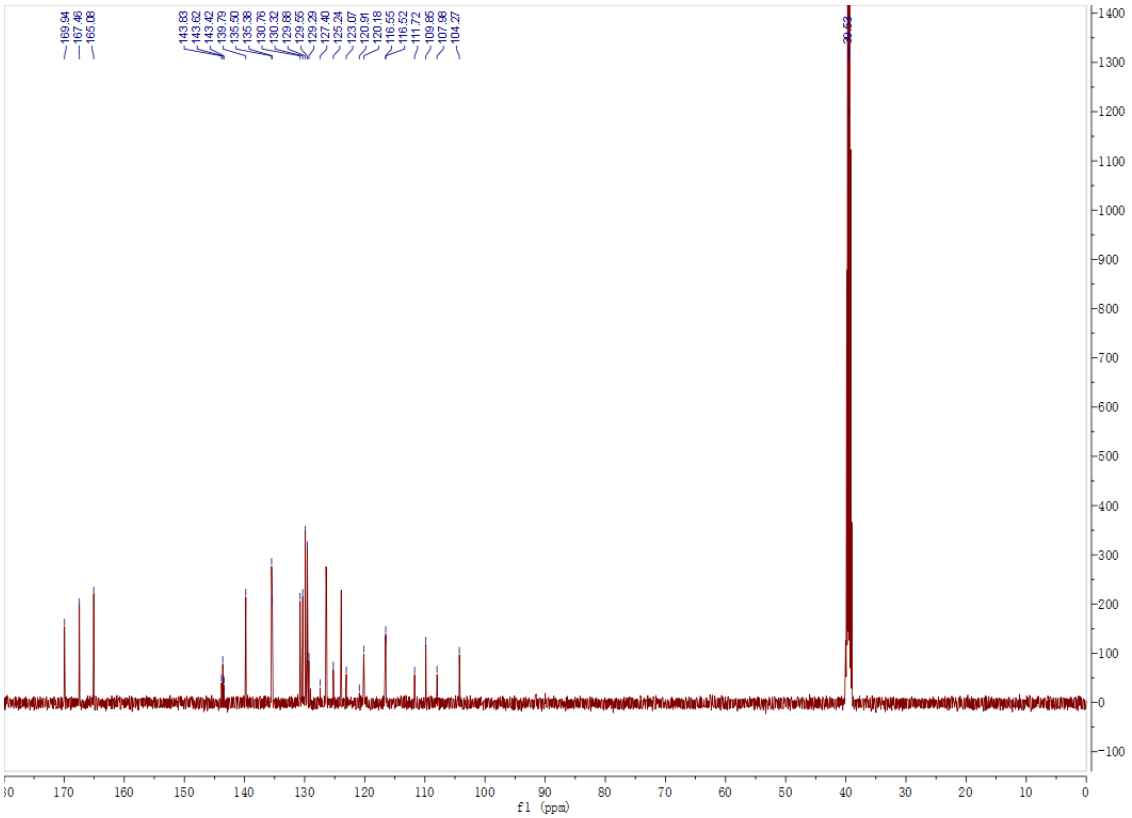

Figure S24 ^{13}C NMR spectra of **12f**

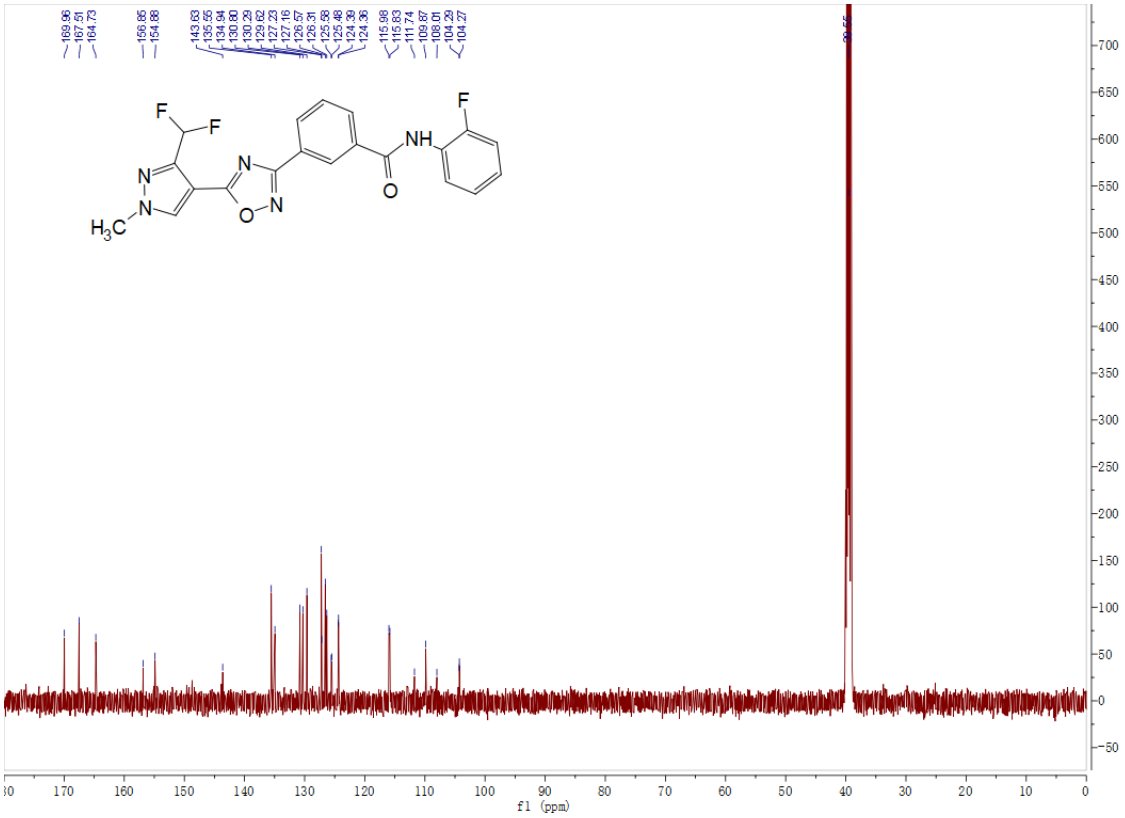

Figure S25 ^{13}C NMR spectra of **12g**

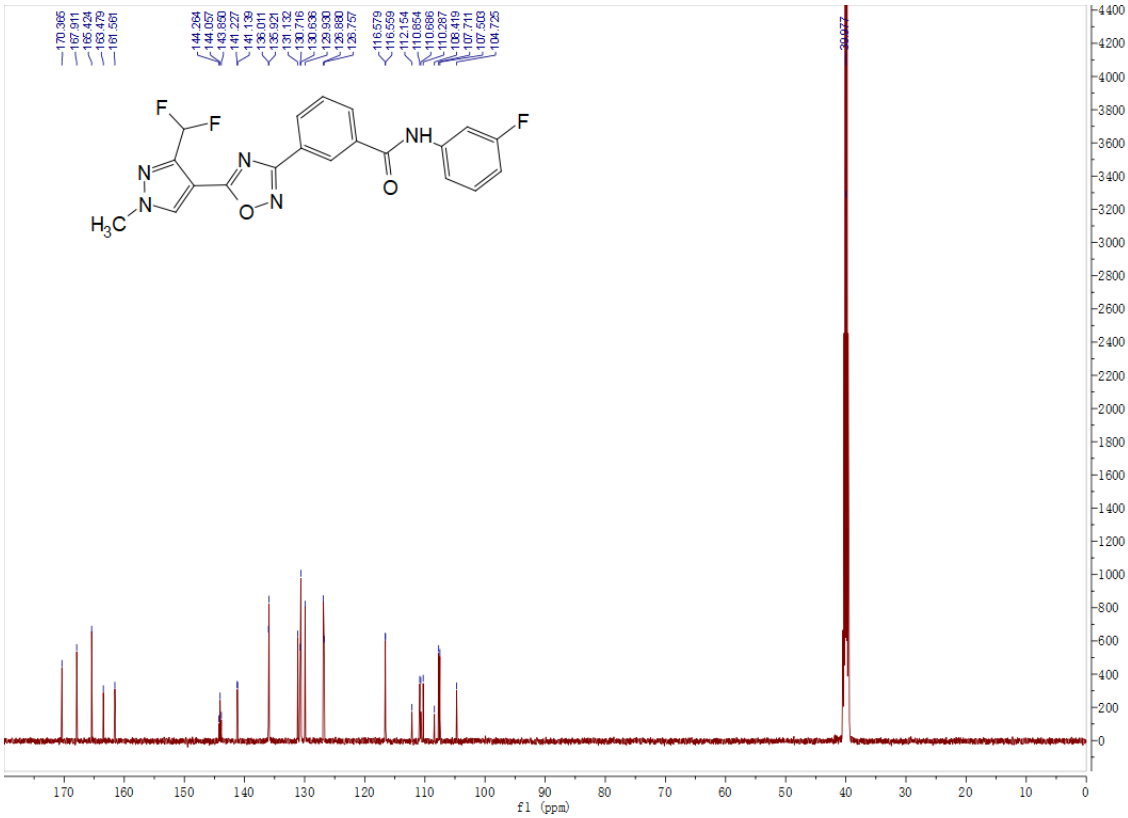

Figure S26 ^{13}C NMR spectra of **12h**

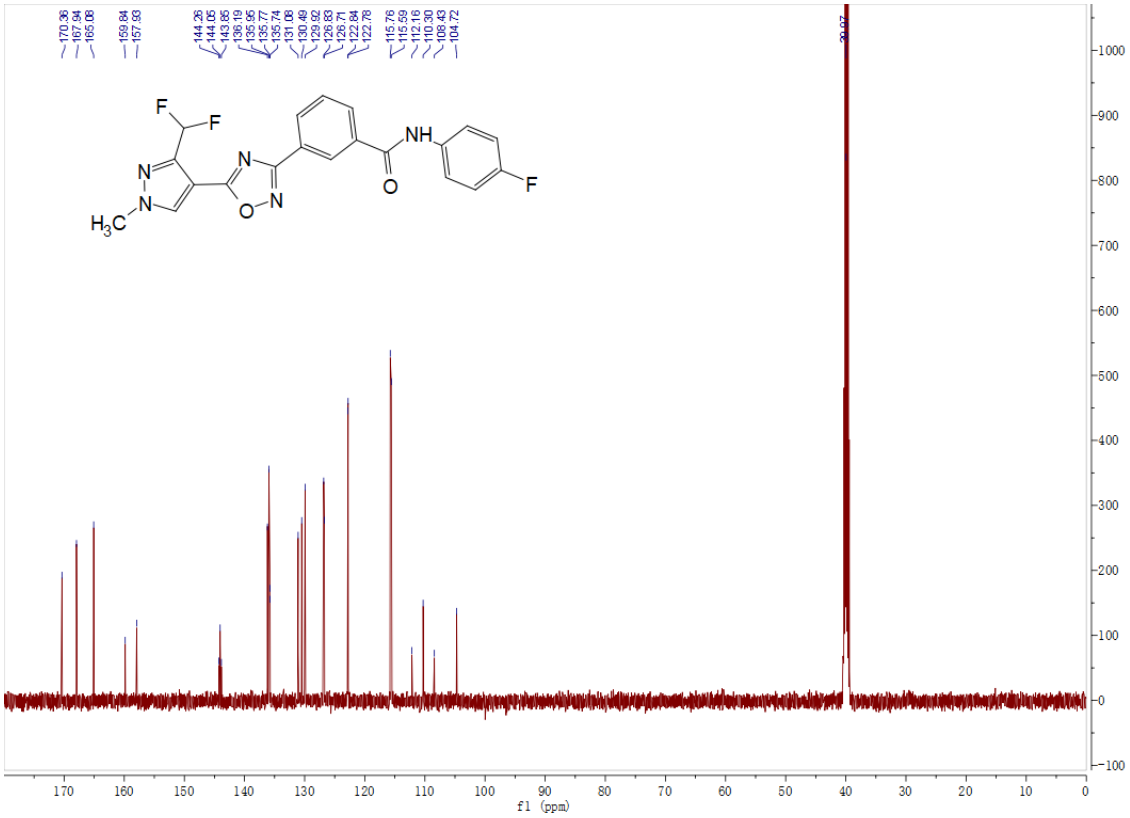

Figure S27 ^{13}C NMR spectra of **12i**

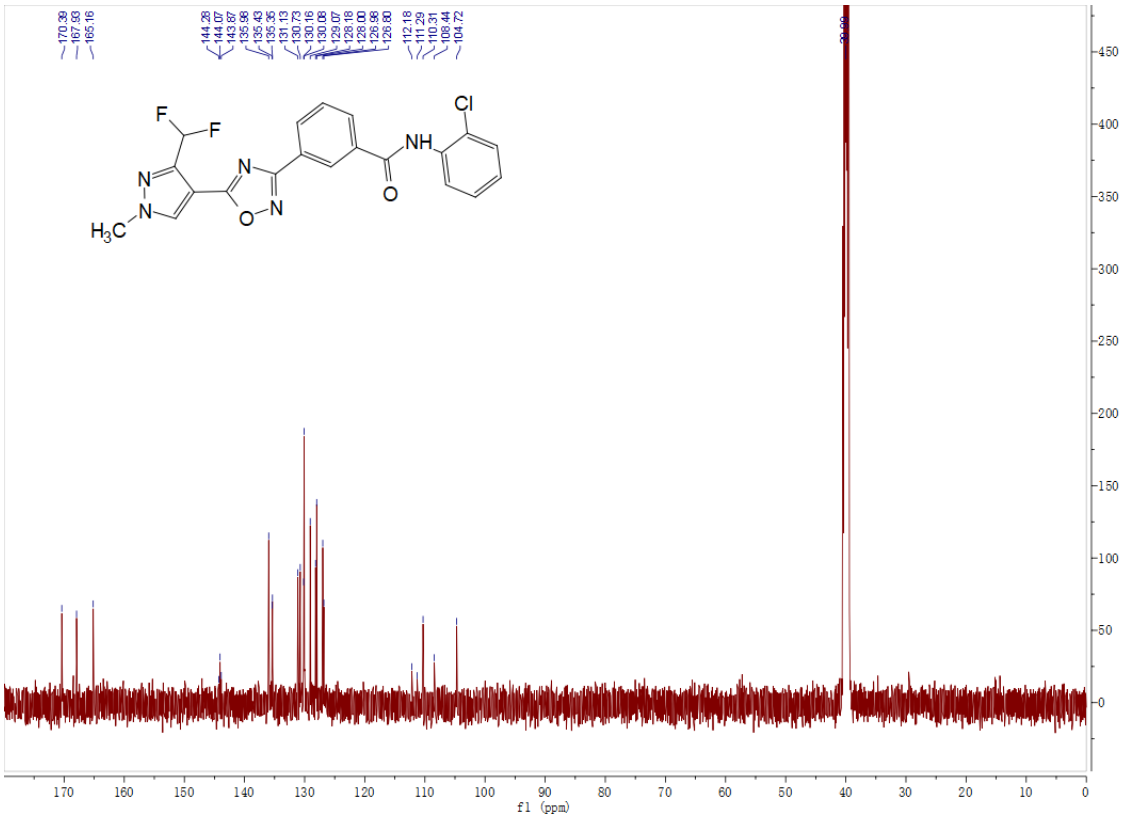

Figure S28 ^{13}C NMR spectra of **12j**

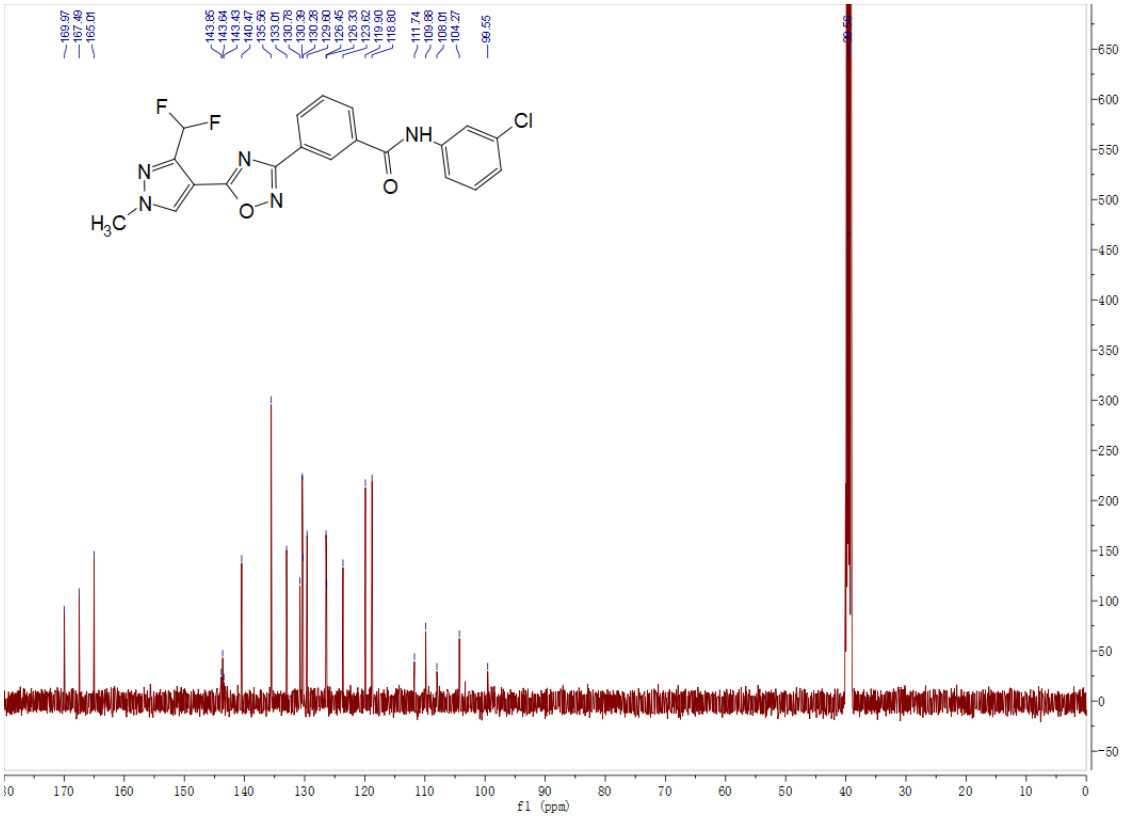

Figure S29 ^{13}C NMR spectra of **12k**

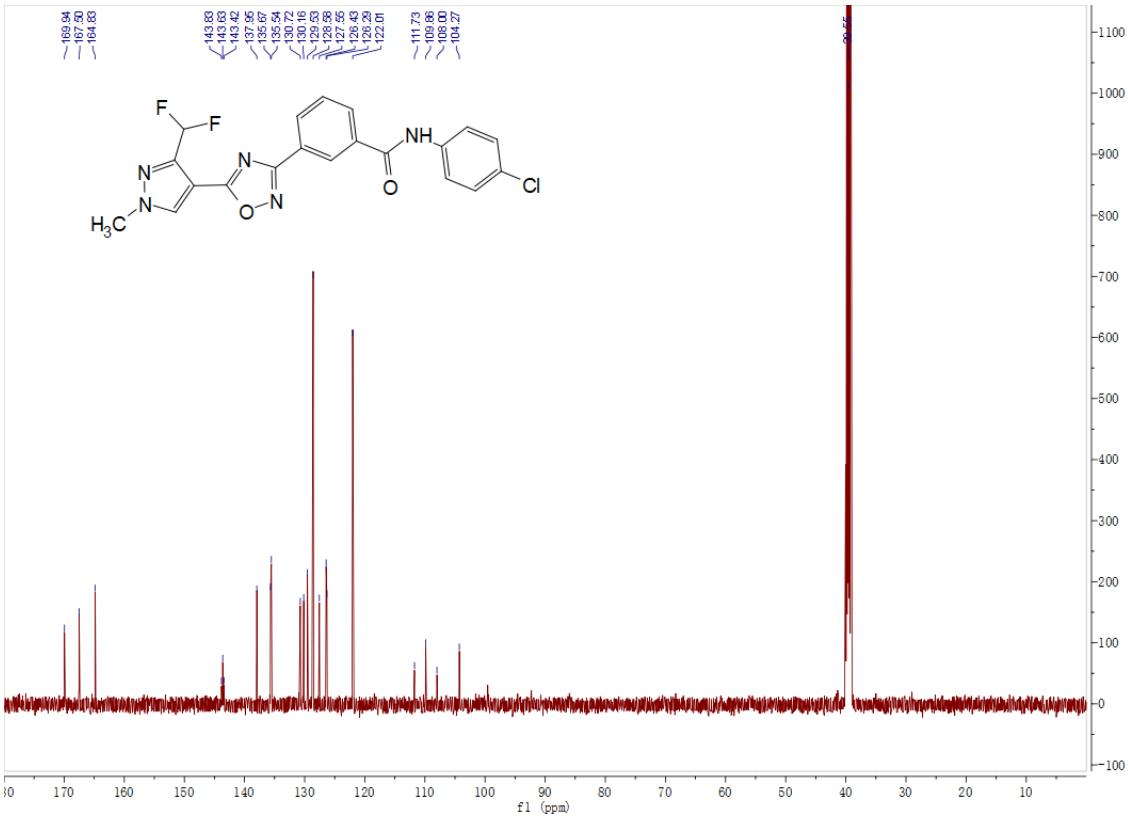

Figure S30 ^{13}C NMR spectra of **12l**

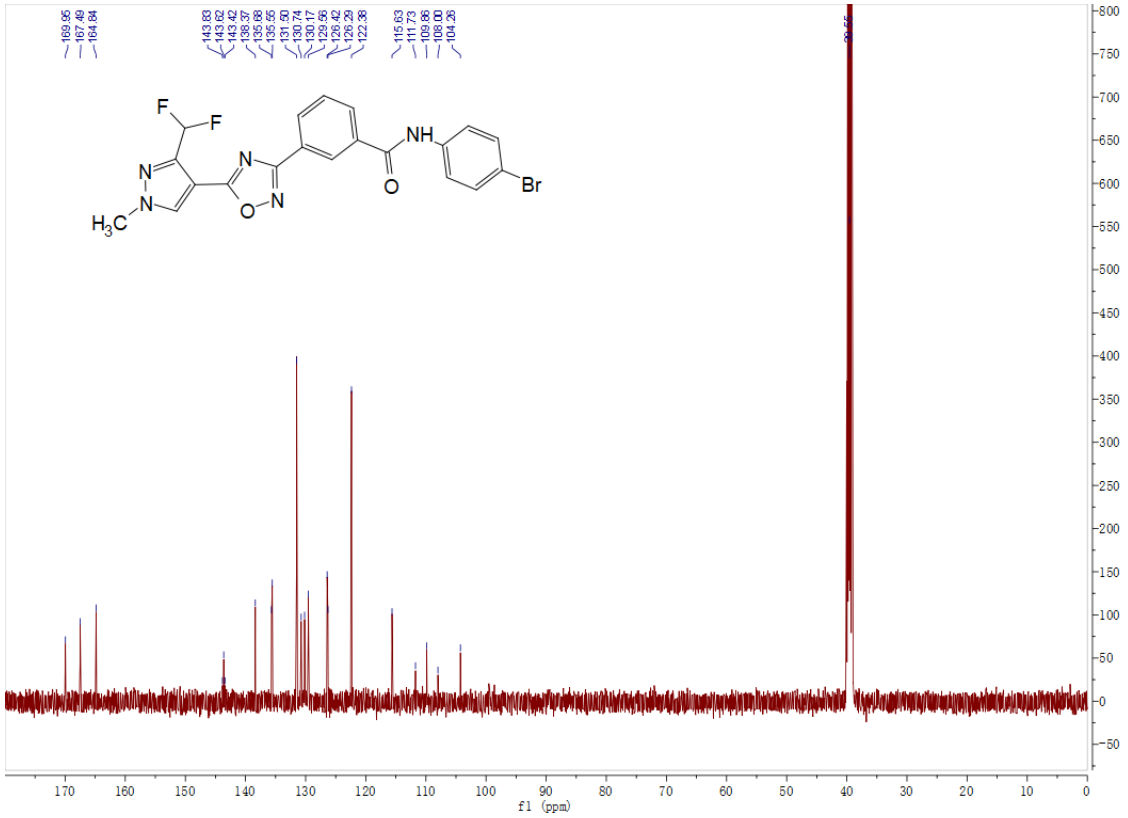

Figure S31 ^{13}C NMR spectra of **12m**

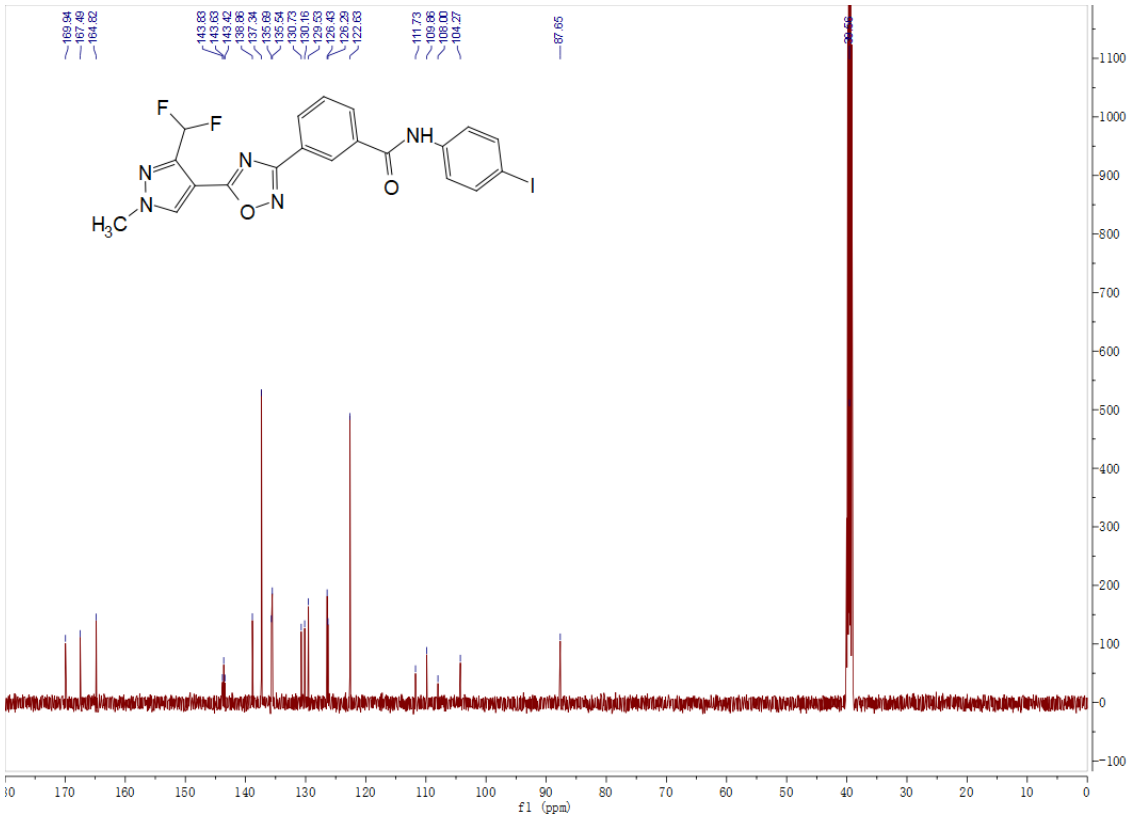

Figure S32 ^{13}C NMR spectra of **12n**

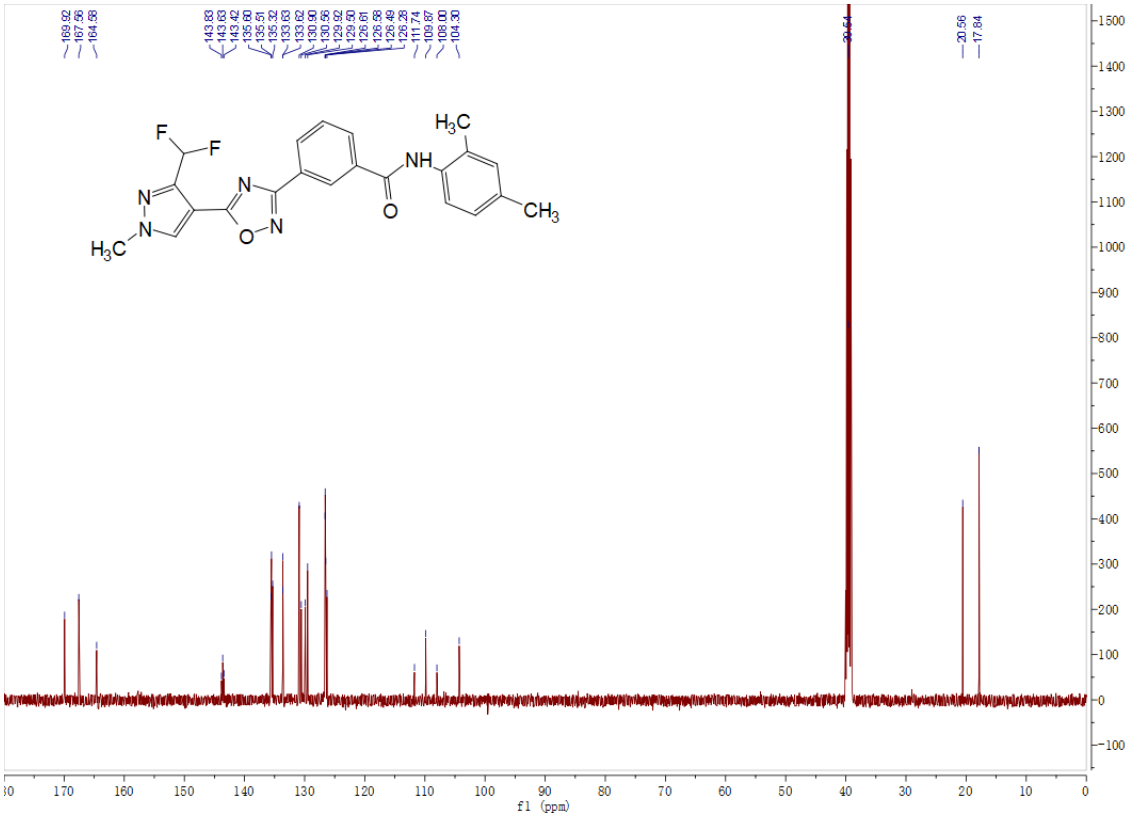

Figure S33 ^{13}C NMR spectra of **12o**

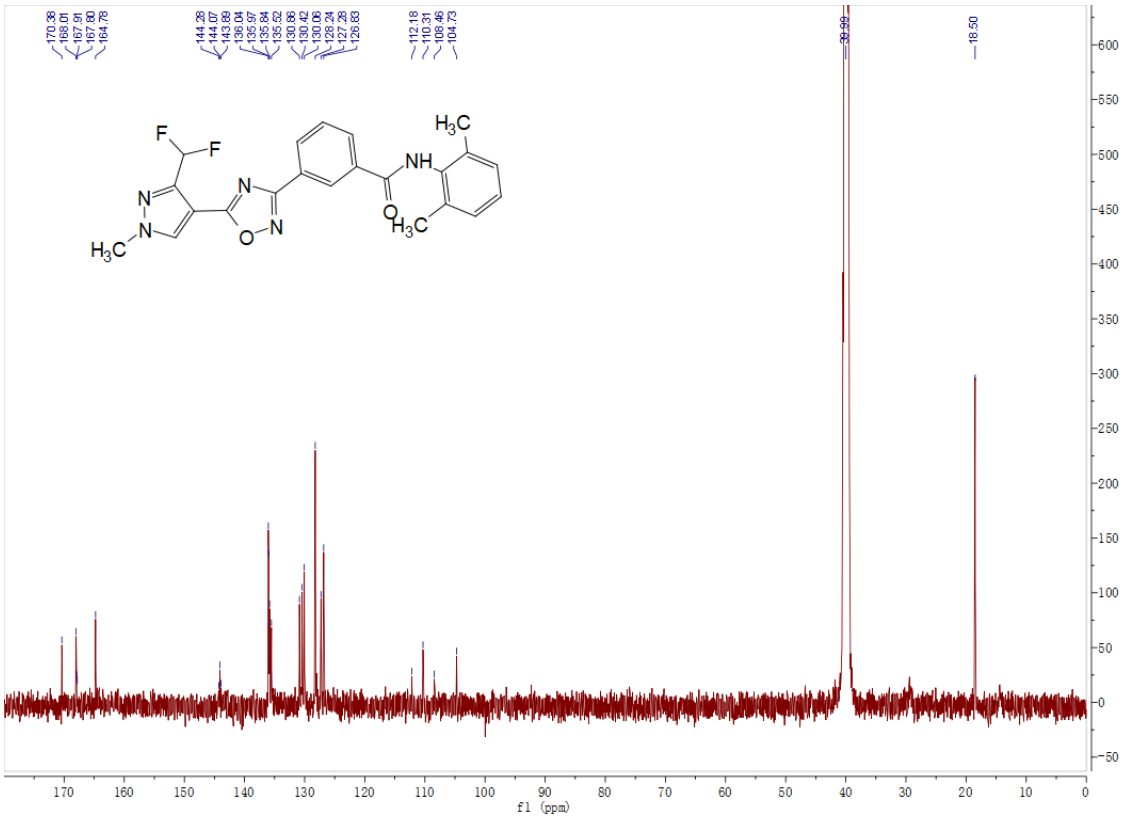

Figure S34 ^{13}C NMR spectra of **12p**

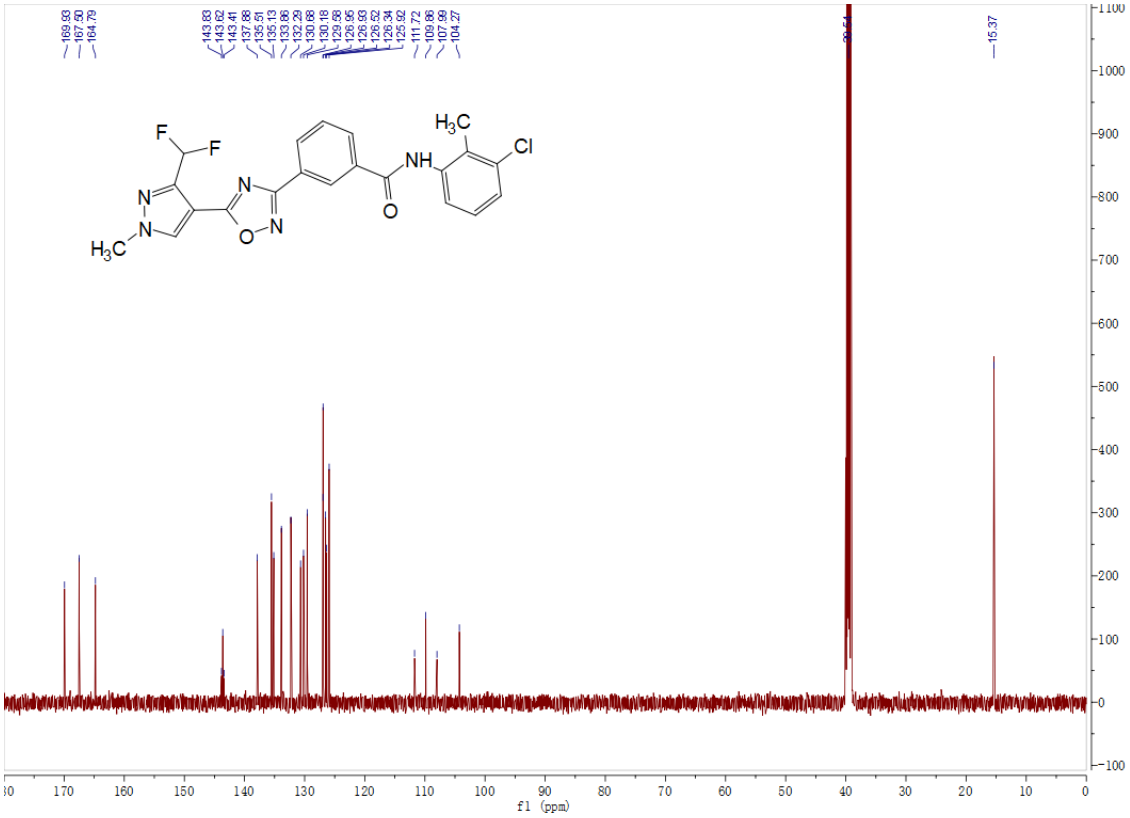

Figure S35 ^{13}C NMR spectra of **12q**

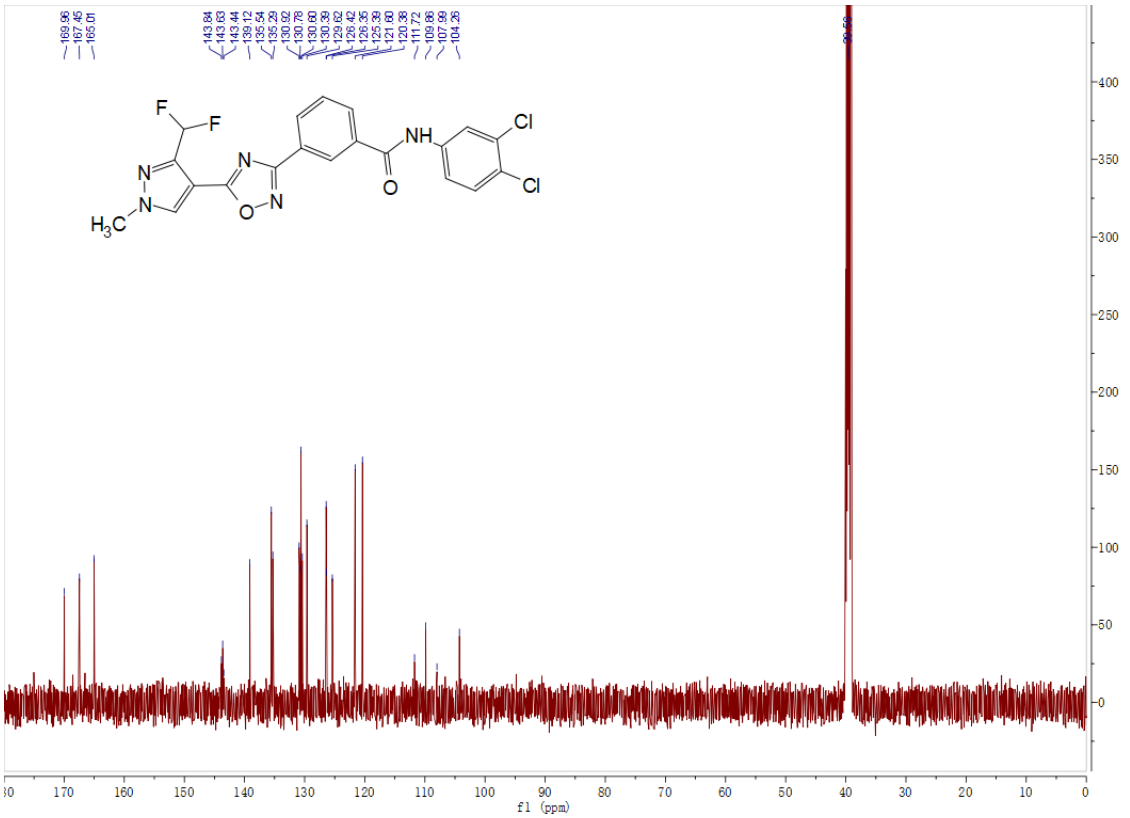

Figure S36 ^{13}C NMR spectra of **12r**

3. ESI-HRMS spectra of **12a~12r**

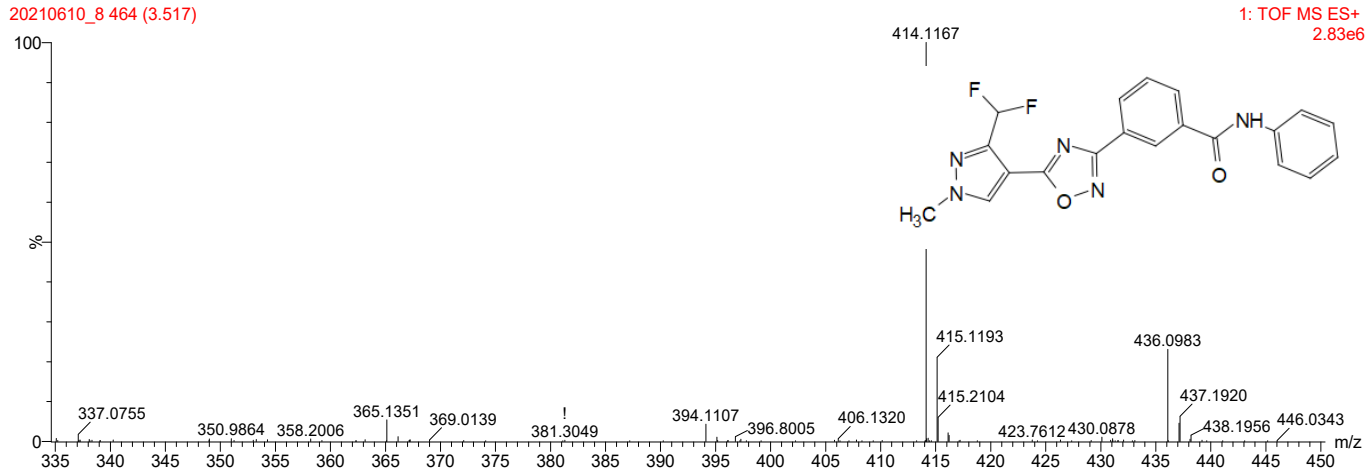

Figure S37 ESI-HRMS spectra of **12a**

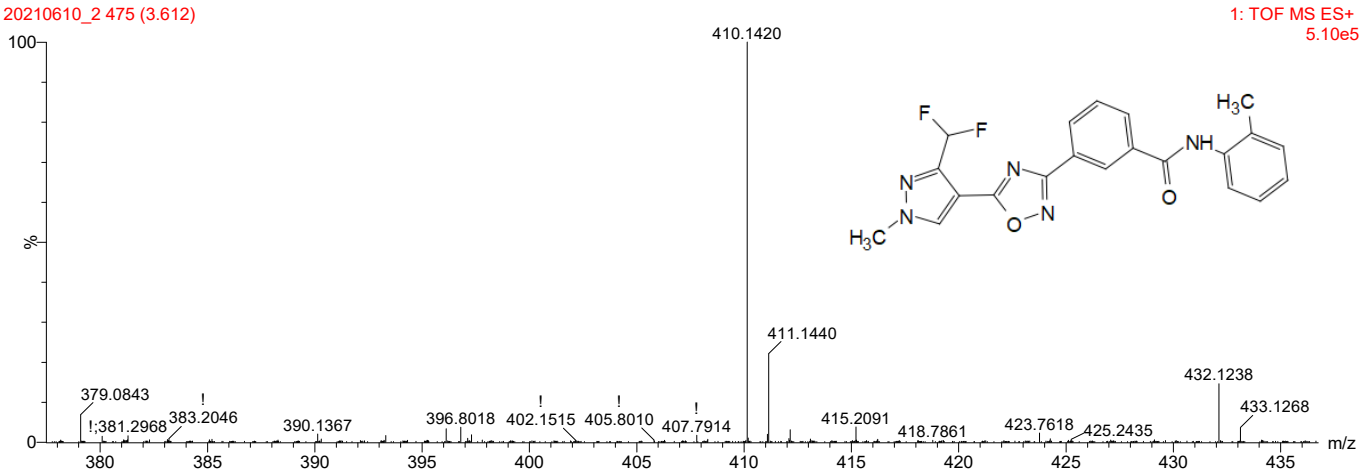

Figure S38 ESI-HRMS spectra of **12b**

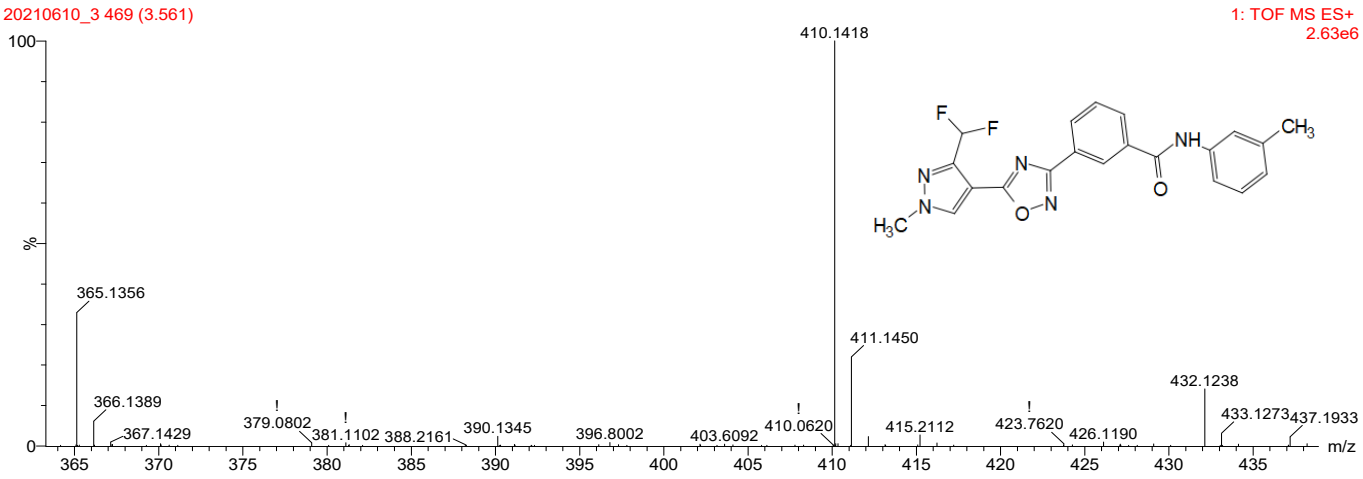

Figure S39 ESI-HRMS spectra of **12c**

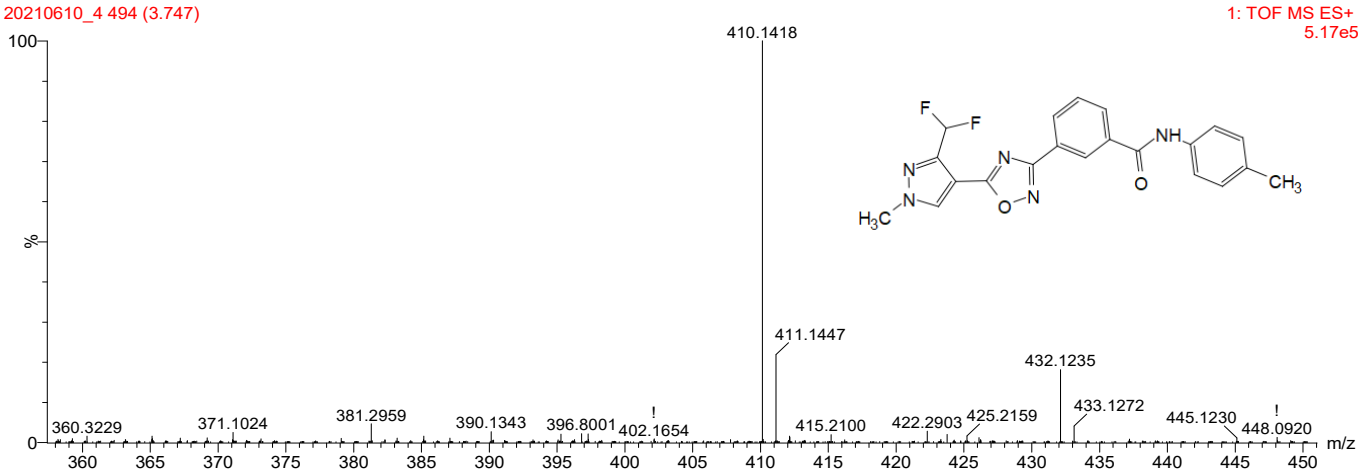

Figure S40 ESI-HRMS spectra of **12d**

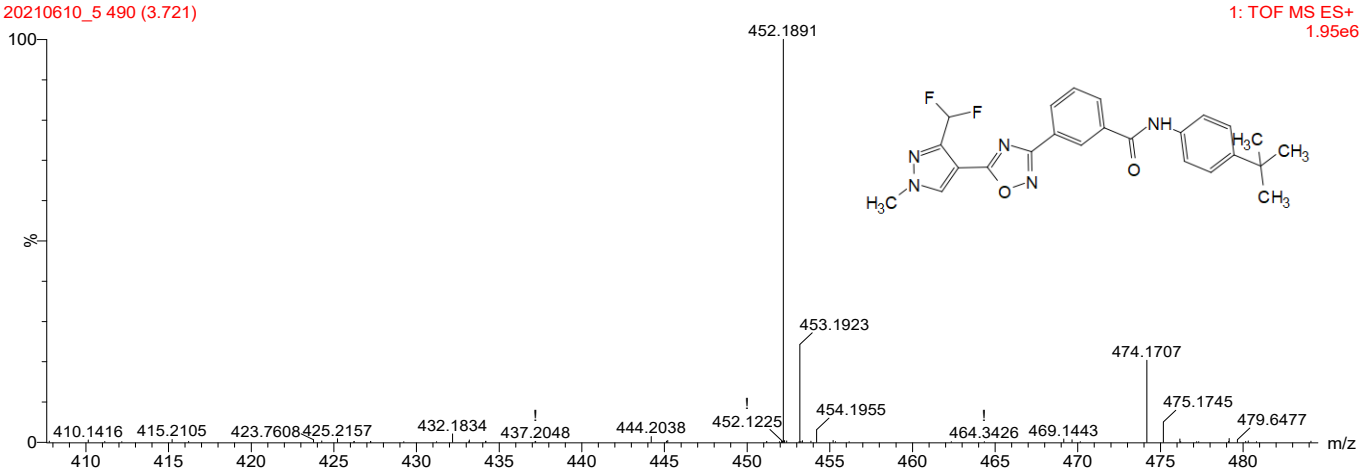

Figure S41 ESI-HRMS spectra of **12e**

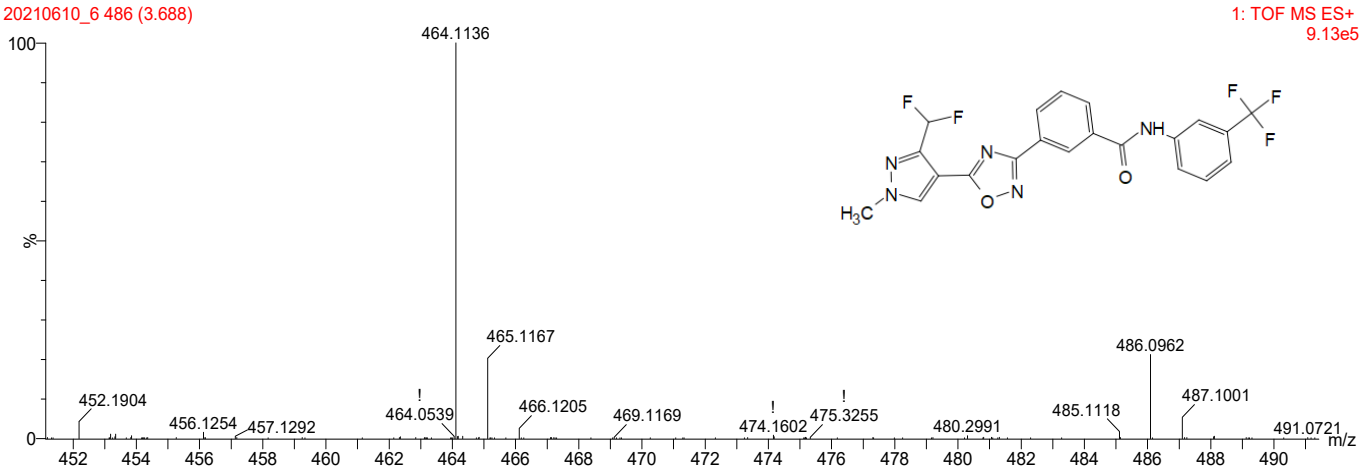

Figure S42 ESI-HRMS spectra of **12f**

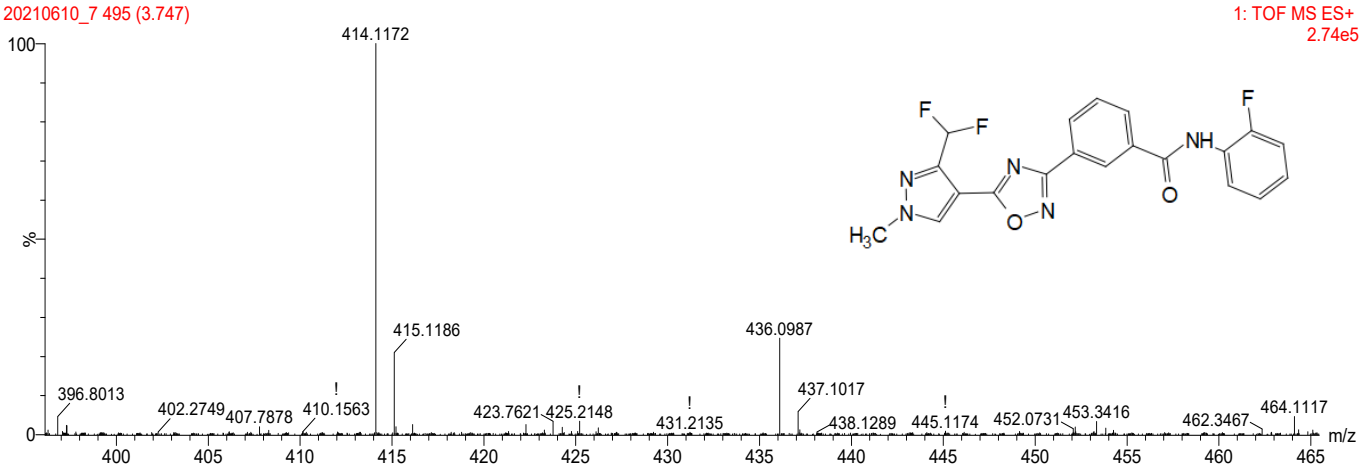

Figure S43 ESI-HRMS spectra of **12g**

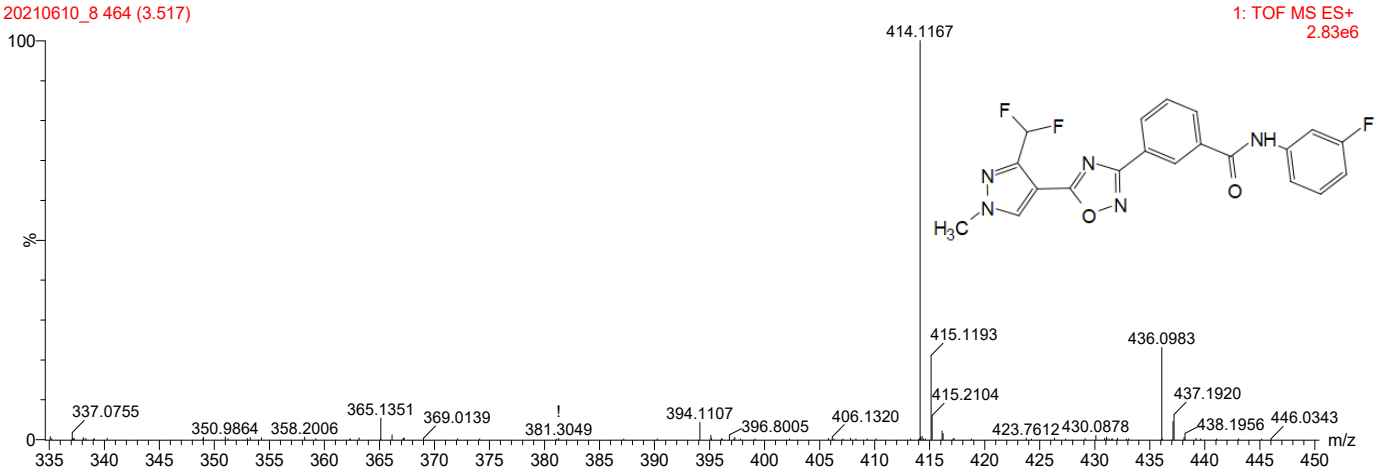

Figure S44 ESI-HRMS spectra of **12h**

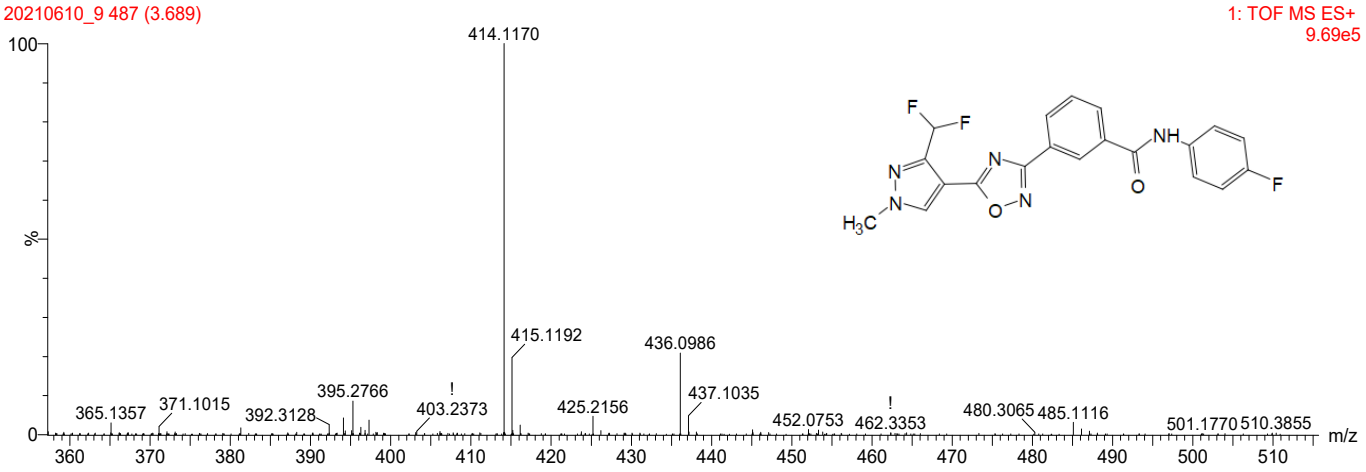

Figure S45 ESI-HRMS spectra of **12i**

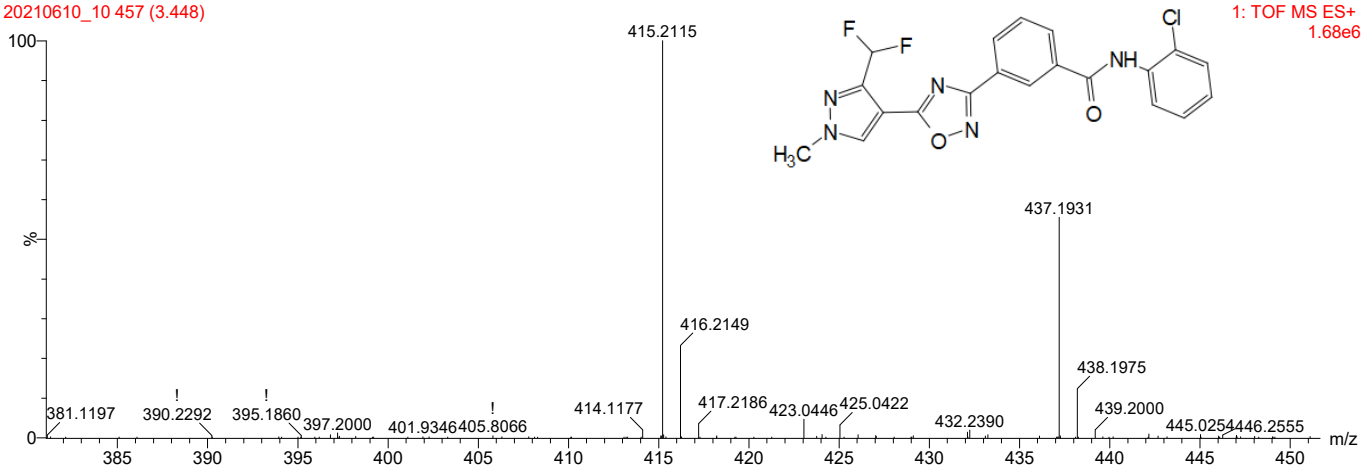

Figure S46 ESI-HRMS spectra of **12j**

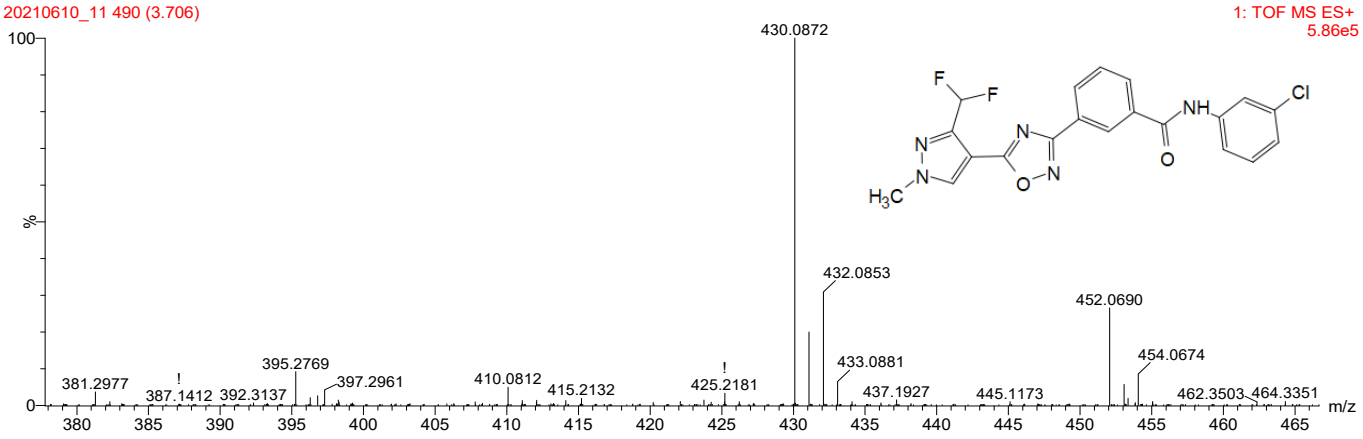

Figure S47 ESI-HRMS spectra of **12k**

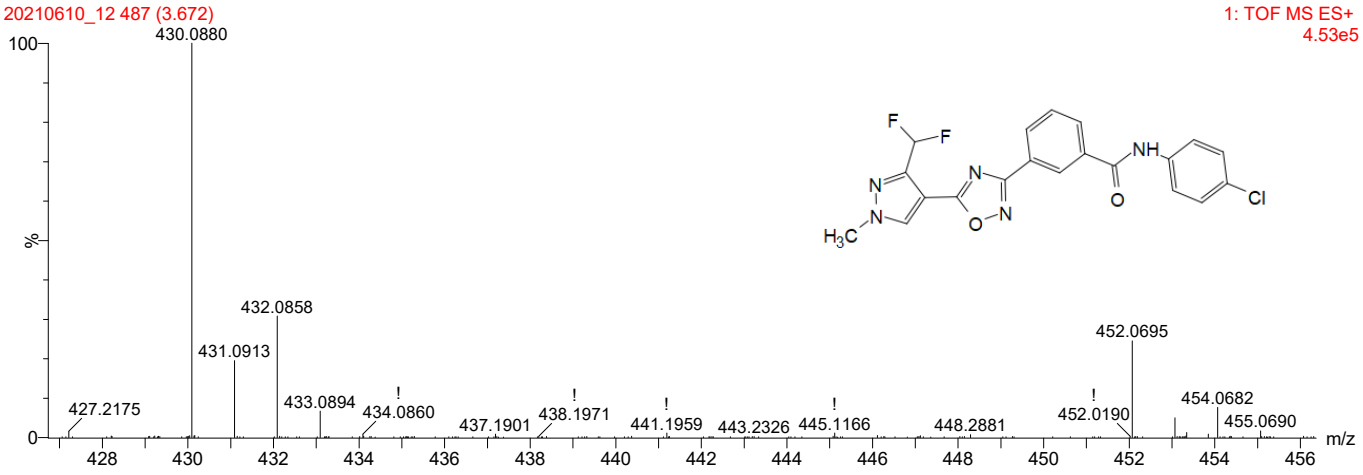

Figure S48 ESI-HRMS spectra of **12l**

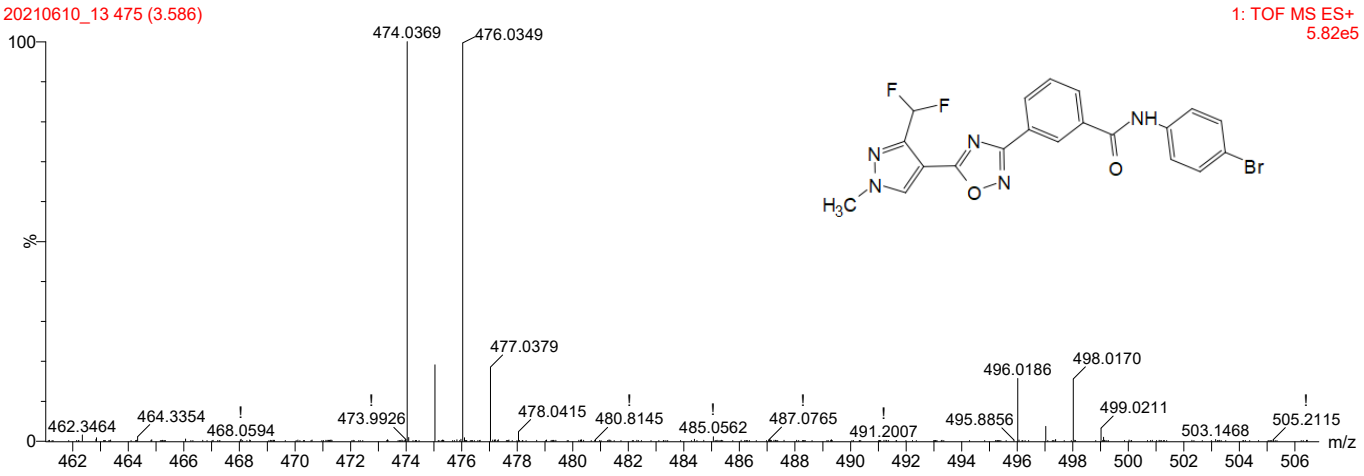

Figure S49 ESI-HRMS spectra of **12m**

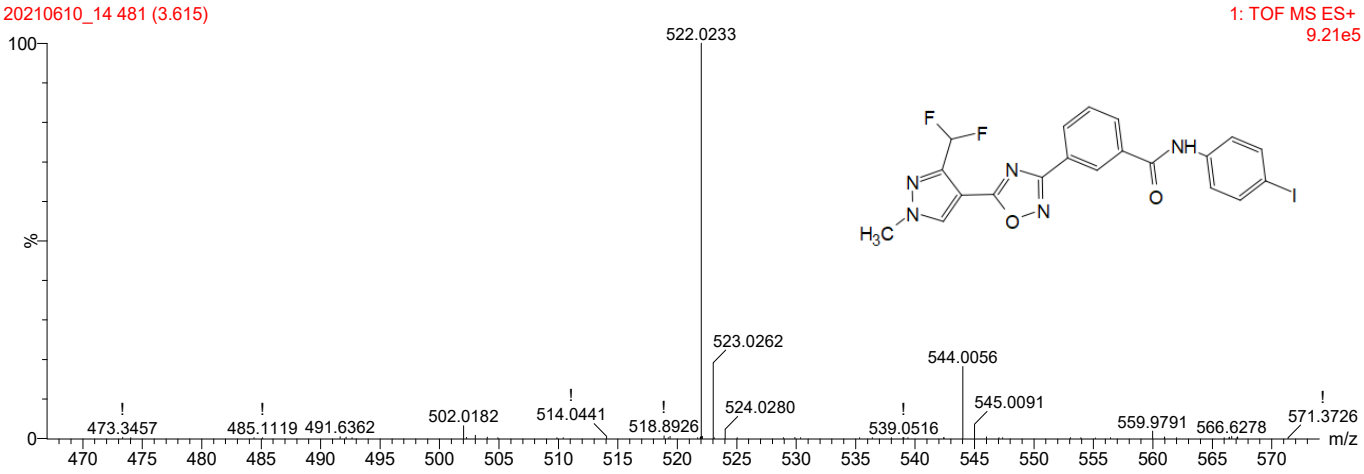

Figure S50 ESI-HRMS spectra of **12n**

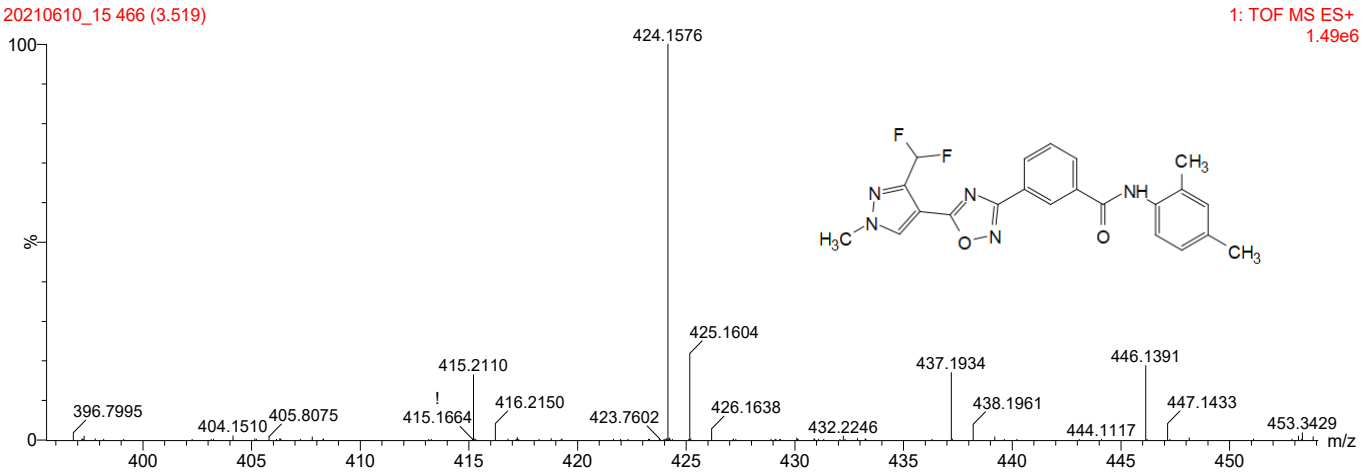

Figure S51 ESI-HRMS spectra of **12o**

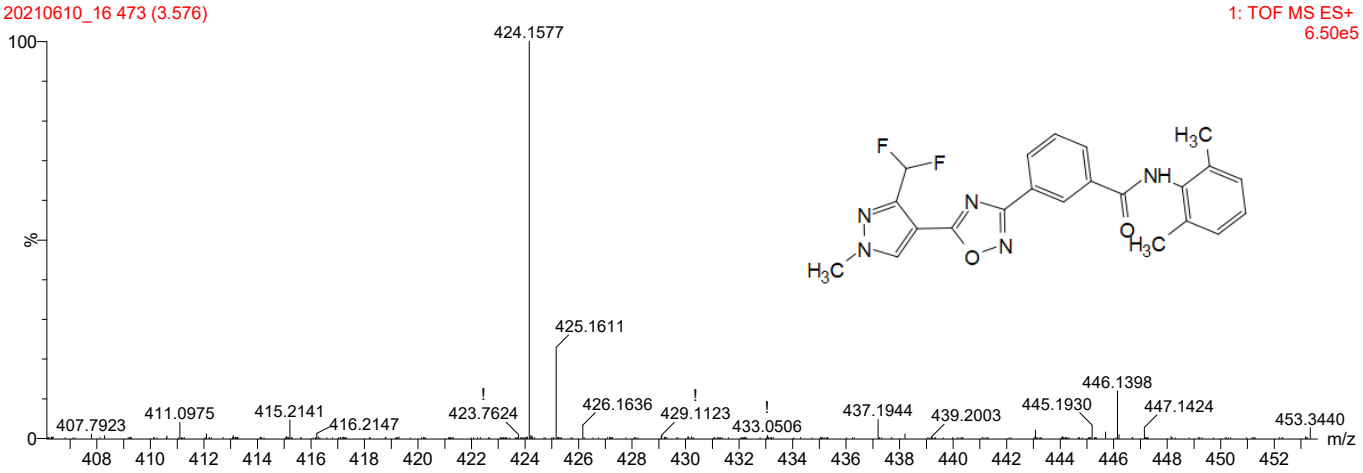

Figure S52 ESI-HRMS spectra of **12p**

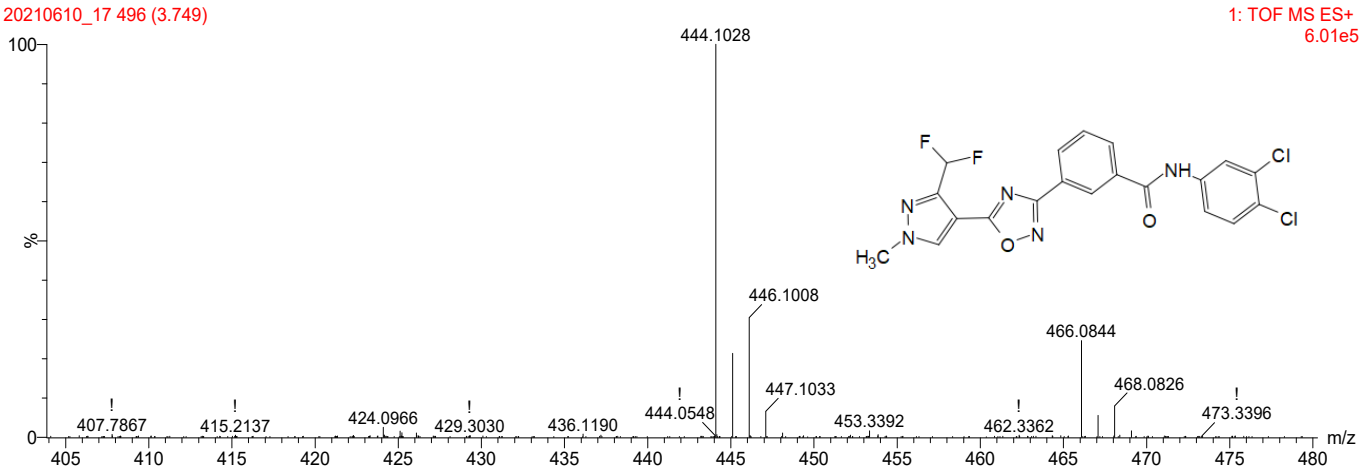

Figure S53 ESI-HRMS spectra of **12q**

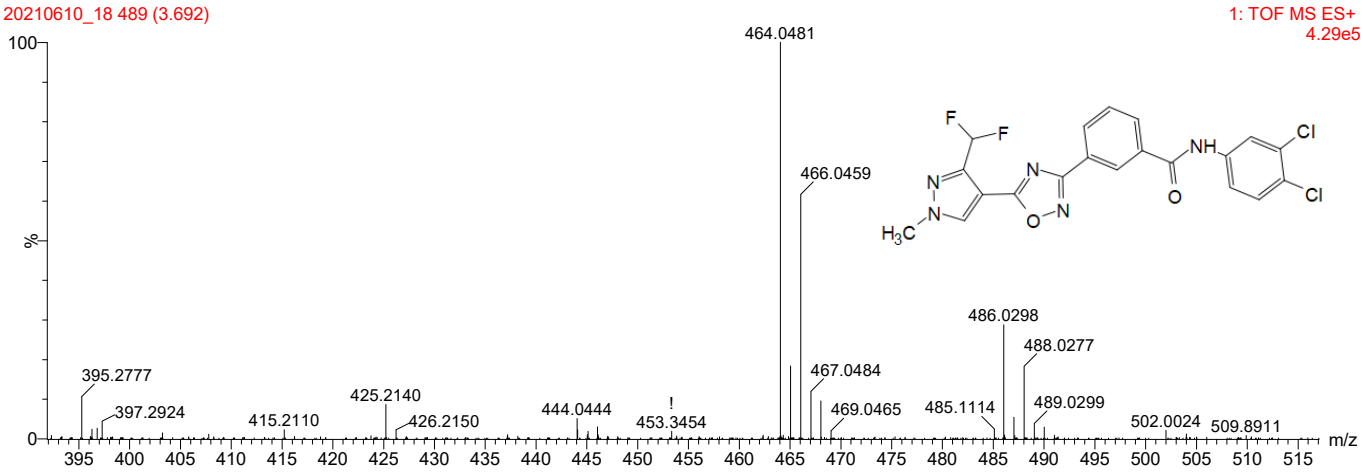

Figure S54 ESI-HRMS spectra of **12r**

4. Biological activity and toxicity assays

Fungicide Bioassay

Insecticidal activities against *Mythimna sepatara, Pyrausta nubilalis* and *Helicoverpa armigera*. Tested compounds were dissolved in N,N-dimethylformamide (DMF), and diluted to the required concentration (500 mg/L) with distilled water containing TW-80 (1%). A leaf discs of approximately 2 cm diameter cut from corn leaf was dipped in the test solution for 10 seconds. The dipped leaf discs were took out, dry naturally, and then place them in a petri dish with absorbent paper. Each petri dish infected with 10 the third stage larvae of *Mythimna sepatara (Pyrausta nubilalis* and *Helicoverpa armigera)*, and the inoculated petri dishes were incubated at 27 °C for 48h. Mortality was assessed after 48 h. Etoxazole, broflanilide were used as positive controls, and a solution of equal DMF and TW-80 concentration was used as a negative control agent (CK). For each treatment, three replicates were conducted.

Insecticidal activities against *Spodoptera frugiperda.* Tested compounds were dissolved in N,N-dimethylformamide (DMF), and diluted to the required concentration (500 mg/L) with distilled water containing TW-80 (1%). A leaf discs of approximately 4 cm diameter cut

from corn leaf was dipped in the test solution for 10 seconds. The dipped leaf discs were took out, dry naturally, and then place them in a petri dish. Each petri dish infected with 10 the second stage larvae of *Spodoptera frugiperda* (starvation for 4 h), and the inoculated petri dishes were incubated at 25 ± 5 °C for 72 h. Mortality was assessed after 72 h. Etoxazole, broflanilide were used as positive controls, and a solution of equal DMF and TW-80 concentration was used as a negative control agent (CK). For each treatment, four replicates were conducted.

Larvicidal Activity against Mosquito (Culex pipiens pallens). Tested compounds were dissolved in N,N-dimethylformamide (DMF), and diluted to the required concentration (10, 5 and 2 mg/L) with distilled water containing TW-80 (1%). Then 10 second-instar mosquito larvae were put into 10 mL of the test solution and raised for 72h. Mortality was measured after 72 h. Etoxazole was used as a positive control, and a solution of equal DMF and TW-80 concentration was used as a negative control agent (CK). Each treatment was performed three times.

Antifungal Bioassay

Antifungal activities assay in vitro: The tested compounds were screened in vitro for their antifungal activities against nine phytopathogenic fungi by the mycelial growth inhibitory rate method. Nine phytopathogenic fungi such as *Alternaria solani (AS), Fusarium graminearum (FG), Cercospora arachidicola (CA), Pyricularia oryae (PO), Sclerotinia Sclerotiorum (SS), Botrytis cinerea (BC), Thanatephorus cucumeris (TC), Fusarium oxysporum (FO), Physalospora piricola (PP)* were used for the assays. Potato dextrose agar (PDA) medium was prepared in the flasks and sterilized. All tested compounds were dissolved in dimethyl sulfoxide (DMSO) before mixing with PDA, and the concentration of test compounds in the medium was fixed at 50 μg/mL. Subsequently, 50% effective concentration (EC_{50}) values of some selected compounds were further calculated. The medium was then poured into sterilized petri dishes. All types of fungi were incubated in PDA at 27 °C for 5 days to get new mycelium for the antifungal assays, and a mycelia disk of approximately 4 mm diameter cut from culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA petri dishes. The inoculated Petri dishes were incubated at 27 °C for 48-72h. DMSO without any compounds mixed with PDA was served as a control, while bixafen, a commercial agricultural fungicide, was used as a positive control. For each treatment, three replicates were conducted. The radial growths of the fungal colonies were measured, and the data were statistically analyzed. The inhibitory effects of the test compounds on these fungi in vitro were calculated by the formula:

$$\text{Inhibition rate (\%)} = (C - T) \times 100/(C - 4\ \text{mm})$$

Where C represents the diameter of fungi growth on untreated PDA, and T represents the diameter of fungi on treated PDA.

Finally, the linear regressions of inhibition rates (%) versus seven concentrations of some selected compounds and bixafen were obtained, and the EC_{50} values were calculated. Statistical analysis was processed by the DPS v16.05 (Data Processing System) software.

Zebrafish (Danio Rerio) Toxicity Bioassay

AB strain of zebrafish (Danio rerio) acquired from China Zebrafish Resource Center (CZRC), was used in this study. Before the spawning, adult zebrafish (two female and three males) were kept separately in a spawning box overnight, and the isolation boards were removed when the light was switched on. Embryos were collected within 30 min. The fertilized and normal embryos were inspected and staged for subsequent experiments under a stereomicroscope. All tested compounds were dissolved in DMSO, and the concentration of test compounds was fixed at 2 mg/L. At 6 hpf, these embryos were distributed into 6-well plates (one embryos per well) for exposure to the tested compounds solution as described above. All exposure solutions were renewed every 24 h, and dead embryos were immediately removed. After continuous exposure for 5 d, statistical related experimental data were recorded every 24 h, and images were taken with a digital camera. Each treatment included three biological replicates.