SUPPLEMENTARY INFORMATION

Late-stage functionalization of 5-Nitrofurans derivatives and their antibacterial activities.

*Geshuyi Chen,[a] Zhe Chang,[b] Pei Yuan,[a] Si Wang,[b] Yongxiu Yang,**[a,c,d] Xiaolei Liang,*[a,c] and Depeng Zhao*[b]*

a. The First Clinical Medical College, Lanzhou University, Lanzhou, China.
b. Guangdong Provincial Key Laboratory of Chiral Molecule and Drug Discovery, School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China.
c. Department of Obstetrics and Gynecology, the First Hospital of Lanzhou University, Key Laboratory for Gynecologic Oncology Gansu Province, China.
d. Lead contact.

**Corresponding author at: The First Clinical Medical College, Lanzhou University. Department of Obstetrics and Gynecology, The First Hospital of Lanzhou University, Key Laboratory for Gynecologic Oncology, Lanzhou,730000, Gansu Province, China. E-mail address: yxyanglzu@163.com.

*Corresponding author. E-mail address: liangxl07@lzu.edu.cn (Xiao-lei Liang)
zhaodp5@mail.sysu.edu.cn(Depeng Zhao)

Supplementary Methods

1. Materials

All reactions were performed under anhydrous conditions and under N_2 atmosphere. All chemicals used were of analytical grade and were used as received without any further purification. All anhydrous solvents used in reactions were purchased in SureSeal bottles or dried over molecular sieves. Flash column chromatography was performed on Biotage Isolelera One with prepacked columns. *Staphylococcus aureus* (*S. aureus*, ATCC 29213), *Escherichia coli* (*E. coli*, ATCC 25922) and *Candida albicans* (*C. albicans*, ATCC 14053) were kindly provided by School of Basic Medical Sciences, Lanzhou University, Gansu Province. *Helicobacter pylori* (*H. pylori*, SS1) was kindly provided by School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou Province. HepaRG cells were obtained from Key Laboratory of Biotherapy and Regenerative Medicine, Gansu Province.

2. General remarks

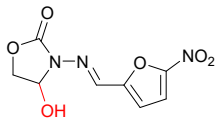
Column chromatography was performed on silica gel (Silica-P flash silica gel from Silicycle, size 40-63 μm). TLC was performed on silica gel 60/ Kieselguhr F254. Mass spectra were recorded on an AEI-MS-902 mass spectrometer (EI+) or a LTQ Orbitrap XL (ESI+). ^{1}H, ^{13}C NMR were recorded on a Varian AMX400 (400 and100.6 MHz, respectively) or a Varian Unity Plus Varian-500 (500 and 125 MHz, respectively). Chemical shift values for ^{1}H and ^{13}C NMR are reported in ppm with the solvent resonance as the internal standard (CHCl3: δ 7.26 ppm for ^{1}H, δ77.0 ppm for ^{13}C; DMSO: δ2.50 ppm for ^{1}H, δ39.52 ppm for ^{13}C). Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), and integration.

3. General procedure for synthesis of compounds 1-16 and characterization.

Synthesis of analogue 1-3

In a dried Schlenk tube, **FZD** (0.2 mmol), NFSI (0.3 mmol), ligand (0.024 mmol, 12 mol%) and CuOAc (0.02 mmol, 10 mol%) were dissolved in CH_3CN (analytical grade without drying, 1.0 mL) under a N_2 atmosphere, then ROH or TMS-N_3 (0.6 mmol) was added. The reaction mixture was stirred at 35 ˚C for 24 h. Upon completion, saturated sodium bicarbonate (30 mL) was added, and the reaction was extracted with DCM (2 x 30 mL), washed with and brine (30 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel with a gradient eluent of petroleum ether and ethyl acetate (petroleum ether/ethyl acetate 10% -50%).

(E)-4-hydroxy-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***1***



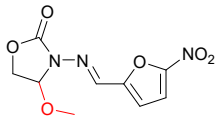
1: yellow solid; yield 91%.

^{1}H NMR (400 MHz, DMSO) δ 8.26 (s, 1H), 7.79 (d, *J* = 3.9 Hz, 1H), 7.24 (d, *J* = 3.9 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 1H), 5.92 (ddd, *J* = 8.3, 6.4, 2.0 Hz, 1H), 4.61 (dd, *J* = 9.9, 6.3 Hz, 1H), 4.16 (dd, *J* = 9.8, 2.0 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 152.4, 151.9, 151.3, 134.1, 115.5, 114.5, 76.2, 70.1.

HRMS (ESI+, *m/z*) calculated for $C_8H_7N_3O_6$ $[M + Na]^+$: 264.0227; found: 264.0239.

(E)-4-methoxy-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***2***



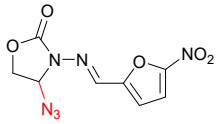
2: yellow solid; yield 93%.

^{1}H NMR (400 MHz, DMSO) δ 8.29 (s, 1H), 7.78 (d, *J* = 3.9 Hz, 1H), 7.28 (d, *J* = 3.9 Hz, 1H), 5.89 (dd, *J* = 6.0, 1.6 Hz, 1H), 4.56 (dd, *J* = 10.5, 5.9 Hz, 1H), 4.47 (dd, *J* = 10.5, 1.5 Hz, 1H), 3.28 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 152.5, 152.1, 151.0, 135.1, 116.3, 114.4, 82.4, 66.5, 51.8.

HRMS (ESI+, *m/z*) calculated for $C_9H_9N_3O_6$ $[M + Na]^+$: 278.0384; found: 278.0394.

(E)-4-azido-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***3***



3: yellow solid; yield 83%.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 7.38 (d, *J* = 3.8 Hz, 1H), 6.98 (d, *J* = 3.8 Hz, 1H), 5.76 (dd, *J* = 7.0, 2.7 Hz, 1H), 4.60 (dd, *J* = 10.1, 7.0 Hz, 1H), 4.21 (dd, *J* = 10.1, 2.7 Hz, 1H).

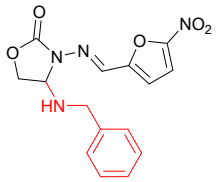
^{13}C NMR (126 MHz, $CDCl_3$) δ 152.6, 151.5, 151.3, 139.3, 114.0, 113.0, 73.1, 67.0.

HRMS (ESI+, *m/z*) calculated for $C_8H_6N_6O_5$ [M + Na]$^+$: 289.0292; found: 289.0296.

Synthesis of analogues 4-5

In a dried Schlenk tube, FZD (0.1 mmol), NFSI (0.15 mmol), ligand (0.012 mmol, 12 mol%) and CuOAc (0.01 mmol, 10 mol%), H2O (0.3 mmol) in CH3CN (1.0 mL) under N_2, 35 ˚C for 24 h; amine (0.3 mmol) added under N_2, 35 ˚C for 24h. Upon completion, the reaction was added 10 mL H_2O for quenching. The aqueous layer was extracted with DCM (2 x 10 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (eluting with petroleum ether/ethyl acetate 10% -20%).

(E)-4-(benzylamino)-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***4***



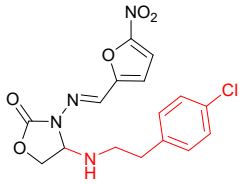
4: yellow solid; yield 89%.

^{1}H NMR (400 MHz, DMSO) δ 8.33 (s, 1H), 7.78 (d, *J* = 3.9 Hz, 1H), 7.35 – 7.26 (m, 4H), 7.26 – 7.19 (m, 1H), 7.12 (d, *J* = 3.9 Hz, 1H), 5.51 (ddd, *J* = 9.1, 5.9, 3.2 Hz, 1H), 4.58 (dd, *J* = 9.5, 8.1 Hz, 1H), 4.24 (dd, *J* = 9.5, 3.3 Hz, 1H), 3.83 – 3.68 (m, 2H), 3.51 (dd, *J* = 12.6, 4.1 Hz, 1H).

^{13}C NMR (126 MHz, DMSO) δ 152.9, 151.9, 151.8, 139.6, 133.7, 128.2, 128.0, 126.9, 114.8, 114.6, 67.7, 67.1, 45.1.

HRMS (ESI+, *m/z*) calculated for $C_{15}H_{14}N_4O_5$ [M + Na]$^+$: 353.0856; found: 353.0861.

(E)-4-((4-chlorophenethyl) amino)-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***5***



5: yellow solid; yield 87%.

^{1}H NMR (400 MHz, DMSO) δ 8.15 (s, 1H), 7.78 (d, *J* = 3.9 Hz, 1H), 7.25 – 7.16 (m, 4H), 7.06 (d, *J* = 4.0 Hz, 1H), 5.45 (ddd, *J* = 8.6, 5.5, 2.9 Hz, 1H), 4.54 (dd, *J* = 9.5, 8.0 Hz, 1H), 4.16 (dd, *J* = 9.5, 3.0 Hz, 1H), 3.24 (p, *J* = 4.9 Hz, 1H), 2.75 (dd, *J* = 17.2, 8.9 Hz, 1H), 2.64 (dp, *J* = 20.5, 6.9 Hz, 2H), 2.49 – 2.41 (m, 1H).

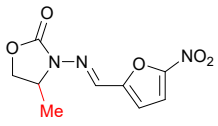
^{13}C NMR (126 MHz, DMSO) δ 153.0, 151.8, 151.8, 138.9, 133.9, 130.5, 130.5, 128.0, 114.7, 114.5, 67.6, 66.9, 41.8, 34.7.

HRMS (ESI+, *m/z*) calculated for $C_{16}H_{15}N_4O_5Cl$ $[M + Na]^+$: 401.0623; found: 401.0641.

Synthesis of analogue 6

In a dried Schlenk tube, **1** (0.1 mmol) was dissolved in 1.0 mL of DCM under nitrogen and cooled to -78 ˚C. DAST (0.1 mmol) was added, and the solution was stirred for 1 h at room temperature. The reaction was then placed back into -78 ˚C cold bath, where $AlMe_3$ (0.3 mmol) was then added dropwise. The reaction mixture was stirred at -78 ˚C for 2 h, then allowed to warm to room temperature while stirring for 1 h. Upon completion, the reaction was added 10 mL H_2O for quenching. The aqueous layer was extracted with DCM (2 x 10 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (eluting with petroleum ether/ethyl acetate 30% - 40%).

(E)-4-methyl-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***6***



6: yellow solid; yield 71%.

^{1}H NMR (400 MHz, DMSO) δ 8.22 (s, 1H), 7.78 (d, *J* = 3.9 Hz, 1H), 7.17 (d, *J* = 3.9 Hz, 1H), 4.81 – 4.33 (m, 2H), 4.14 (d, *J* = 5.0 Hz, 1H), 1.46 – 0.99 (m, 3H).

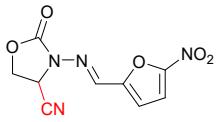
^{13}C NMR (101 MHz, DMSO) δ 152.9, 151.8, 151.7, 133.3, 115.2, 114.6, 68.7, 50.0, 16.3.

HRMS (ESI+, *m/z*) calculated for $C_9H_9N_3O_5$ $[M + Na]^+$: 262.0434; found: 262.0440.

Synthesis of analogues 7, 9-16

In a dried Schlenk tube, **1** (0.1 mmol) was dissolved in 1.0 mL of DCM under a N_2 atmosphere and cooled to -40 °C, acid (0.2 mmol, $BF_3 \cdot OEt_2$ for **7**, **9-13**; TFA for **14-16**) and nucleophilic reagent (0.3 mmol) was added, and the solution was stirred for 1 hour. The reaction was allowed to warm to room temperature while stirring for 2 h. Upon completion, the reaction was added 10 mL H_2O for quenching. The aqueous layer was extracted with DCM (2 x 10 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (eluting with petroleum ether/ethyl acetate 30% -50%).

*(E)-3-(((5-nitrofuran-2-yl) methylene) amino)-2-oxooxazolidine-4-carbonitrile, **7***



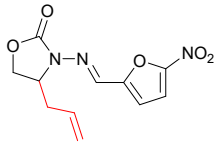
7: yellow solid; yield 45%.

^{1}H NMR (400 MHz, DMSO) δ 8.33 (s, 1H), 7.79 (d, *J* = 3.9 Hz, 1H), 7.32 (d, *J* = 3.9 Hz, 1H), 5.62 (dd, *J* = 8.8, 3.3 Hz, 1H), 4.83 (dd, *J* = 9.4, 3.2 Hz, 1H), 4.74 (t, *J* = 9.0 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 152.2, 151.7, 150.3, 135.1, 116.6, 115.5, 114.3, 64.6, 44.9.

HRMS (ESI+, *m/z*) calculated for $C_9H_6N_4O_5$ $[M + Na]^+$: 273.0230; found: 273.0231.

*(E)-4-allyl-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, **9***



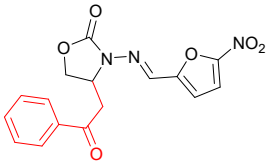
9: yellow solid; yield 90%.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.37 (d, *J* = 3.8 Hz, 1H), 6.93 (d, *J* = 3.9 Hz, 1H), 5.73 (dddd, *J* = 16.9, 10.4, 7.7, 6.5 Hz, 1H), 5.32 – 5.14 (m, 2H), 4.50 (t, *J* = 8.6 Hz, 1H), 4.34 (tdd, *J* = 8.4, 5.2, 3.4 Hz, 1H), 4.21 (dd, *J* = 8.9, 5.2 Hz, 1H), 2.71 (dddt, *J* = 14.2, 6.2, 3.1, 1.3 Hz, 1H), 2.49 (dt, *J* = 14.2, 7.9 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 153.2, 152.4, 152.1, 136.9, 130.6, 120.7, 113.3, 113.2, 66.2, 56.6, 36.2.

HRMS (ESI+, *m/z*) calculated for $C_{11}H_{11}N_3O_5$ $[M + Na]^+$: 288.0591; found: 288.0605.

(E)-3-(((5-nitrofuran-2-yl) methylene) amino)-4-(2-oxo-2-phenylethyl) oxazolidin-2-one, ***10***



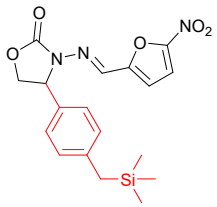
10: yellow solid; yield 53%.

^{1}H NMR (500 MHz, DMSO) δ 8.23 (s, 1H), 7.99 (d, *J* = 7.7 Hz, 2H), 7.77 (d, *J* = 3.9 Hz, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 2H), 7.10 (d, *J* = 4.0 Hz, 1H), 4.90 (d, *J* = 9.2 Hz, 1H), 4.74 (t, *J* = 8.6 Hz, 1H), 4.32 (dd, *J* = 8.9, 3.7 Hz, 1H), 3.90 (d, *J* = 18.3 Hz, 1H), 3.49 (dd, *J* = 18.5, 9.9 Hz, 1H).

^{13}C NMR (126 MHz, DMSO) δ 197.5, 153.0, 152.0, 151.8, 136.2, 133.5, 133.4, 128.7, 128.1, 114.9, 114.7, 67.8, 50.4, 38.4.

HRMS (ESI+, *m/z*) calculated for $C_{16}H_{13}N_3O_6$ $[M + Na]^+$: 366.0697; found: 366.0702.

(E)-3-(((5-nitrofuran-2-yl) methylene) amino)-4-(4-((trimethylsilyl)methyl) phenyl) oxazolidin-2-one, ***11***



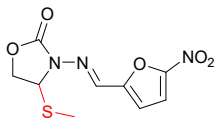
11: yellow solid; yield 85%.

^{1}H NMR (400 MHz, DMSO) δ 7.84 – 7.56 (m, 2H), 7.19 (d, *J* = 8.1 Hz, 2H), 7.11 – 6.95 (m, 3H), 5.58 (dd, *J* = 8.7, 4.1 Hz, 1H), 4.87 (t, *J* = 8.7 Hz, 1H), 4.20 (dd, *J* = 8.8, 4.1 Hz, 1H), 2.08 (s, 2H), -0.07 (s, 9H).

^{13}C NMR (101 MHz, DMSO) δ 153.3, 151.8, 151.0, 141.0, 133.0, 131.8, 128.7, 126.1, 115.0, 114.4, 70.1, 57.0, 26.0, -2.0.

HRMS (ESI+, *m/z*) calculated for $C_{18}H_{21}N_3O_5Si$ $[M + Na]^+$: 410.1143; found: 410.1152.

*(E)-4-(methylthio)-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one,****12***



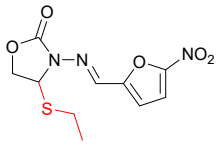
12: yellow solid; yield 95%.

^{1}H NMR (400 MHz, DMSO) δ 8.22 (s, 1H), 7.79 (d, *J* = 3.9 Hz, 1H), 7.24 (d, *J* = 3.9 Hz, 1H), 5.72 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.91 (dd, *J* = 9.8, 8.1 Hz, 1H), 4.63 (dd, *J* = 9.8, 2.4 Hz, 1H), 1.98 (s, 3H).

^{13}C NMR (101 MHz, DMSO) δ 152.3, 152.0, 151.0, 135.5, 115.6, 114.5, 68.6, 57.4, 8.9.

HRMS (ESI+, *m/z*) calculated for $C_9H_9N_3O_5S$ $[M + Na]^+$:294.0155; found: 294.0163.

(E)-4-(ethylthio)-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***13***



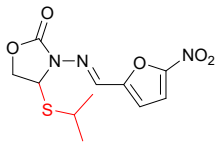
13: yellow solid; yield 93%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.38 (d, *J* = 3.8 Hz, 1H), 7.01 (d, *J* = 3.9 Hz, 1H), 5.32 (dd, *J* = 8.5, 4.3 Hz, 1H), 4.84 (dd, *J* = 9.8, 8.5 Hz, 1H), 4.43 (dd, *J* = 9.8, 4.3 Hz, 1H), 2.75 (qd, *J* = 7.5, 5.8 Hz, 2H), 1.32 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 152.5, 152.1, 151.7, 136.8, 113.2, 113.2, 68.6, 60.7, 23.8, 14.5.

HRMS (ESI+, *m/z*) calculated for $C_{10}H_{11}N_3O_5S$ $[M + Na]^+$: 308.0312; found: 308.0331.

(E)-4-(isopropylthio)-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***14***



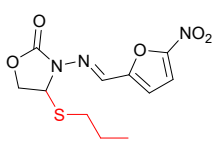
14: yellow solid; yield 86%.

^{1}H NMR (400 MHz, DMSO) δ 8.21 (s, 1H), 7.79 (d, *J* = 3.9 Hz, 1H), 7.27 (d, *J* = 3.9 Hz, 1H), 5.75 (dd, *J* = 7.9, 2.4 Hz, 1H), 4.93 (dd, *J* = 9.7, 7.8 Hz, 1H), 4.59 (dd, *J* = 9.7, 2.4 Hz, 1H), 3.20 (p, *J* = 6.7 Hz, 1H), 1.26 (d, *J* = 6.8 Hz, 3H), 1.19 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 152.1, 152.0, 151.0, 115.7, 114.5, 69.9, 57.9, 33.7, 24.4, 23.9.

HRMS (ESI+, *m/z*) calculated for $C_{11}H_{13}N_3O_5S$ $[M + Na]^+$: 322.0468; found:322.0481.

(E)-3-(((5-nitrofuran-2-yl) methylene) amino)-4-(propylthio) oxazolidin-2-one, ***15***



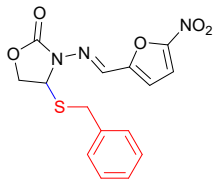
15: yellow solid; yield 87%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.38 (d, *J* = 3.8 Hz, 1H), 7.01 (d, *J* = 3.9 Hz, 1H), 5.31 (dd, *J* = 8.5, 4.3 Hz, 1H), 4.84 (dd, *J* = 9.8, 8.5 Hz, 1H), 4.42 (dd, *J* = 9.8, 4.2 Hz, 1H), 2.79 – 2.55 (m, 2H), 1.66 (qd, *J* = 7.4, 1.8 Hz, 2H), 1.00 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 152.5, 152.2, 151.7, 136.7, 113.2, 113.2, 68.7, 60.7, 31.6, 22.8, 13.6.

HRMS (ESI+, *m/z*) calculated for $C_{11}H_{13}N_3O_5S$ [M + Na]$^+$: 322.0468; found: 322.0464.

(E)-4-(benzylthio)-3-(((5-nitrofuran-2-yl) methylene) amino) oxazolidin-2-one, ***16***



16: yellow solid; yield 81%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.44 – 7.27 (m, 6H), 6.95 (d, *J* = 3.8 Hz, 1H), 5.19 (dd, *J* = 8.5, 4.5 Hz, 1H), 4.67 (dd, *J* = 9.9, 8.5 Hz, 1H), 4.27 (dd, *J* = 9.8, 4.5 Hz, 1H), 4.13 (d, *J* = 13.6 Hz, 1H), 3.91 (d, *J* = 13.6 Hz, 1H).

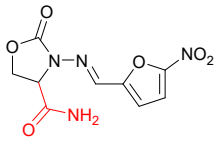
^{13}C NMR (126 MHz, $CDCl_3$) δ 152.6, 152.0, 151.6, 137.2, 136.5, 129.3, 129.0, 127.9, 113.7, 113.1, 67.9, 60.3, 34.8.

HRMS (ESI+, *m/z*) calculated for $C_{15}H_{13}N_3O_5S$ [M + Na]$^+$: 370.0468; found: 370.0477.

Synthesis of analogues 8

In a dried Schlenk tube, **7** (0.1 mmol), $LiOH{\cdot}H_2O$ (0.2 mmol) were dissolved in THF (3.0 mL) and H_2O (3.0 mL) under a N_2 atmosphere. Then H_2O_2 (36% wt 100 μl) was added. The reaction mixture was stirred at rt for 1 h. Upon completion, the reaction was added Na_2SO_3 (1.0 mmol) for quenching. The aqueous layer was extracted with DCM (2 x 10 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (eluting with petroleum ether/ethyl acetate 10% -20%).

(E)-3-(((5-nitrofuran-2-yl) methylene) amino)-2-oxooxazolidine-4-carboxamide, ***8***



8: yellow solid; yield 43%.

^{1}H NMR (500 MHz, DMSO) δ 7.94 (s, 1H), 7.78 (d, *J* = 4.0 Hz, 1H), 7.74 (s, 1H), 7.64 (s, 1H), 7.22 (d, *J* = 4.0 Hz, 1H), 4.94 (dd, *J* = 9.1, 3.2 Hz, 1H), 4.67 (t, *J* = 9.0 Hz, 1H), 4.36 (dd, *J* = 8.9, 3.2 Hz, 1H).

^{13}C NMR (126 MHz, DMSO) δ 168.6, 153.2, 151.9, 151.3, 131.8, 115.2, 114.6, 65.9, 55.8.

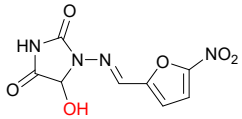
HRMS (ESI+, *m/z*) calculated for $C_9H_8N_4O_6$ [M + Na]$^+$: 291.0336; found: 291.0345.

4. General procedure for synthesis of compounds 17-18 and characterization.

Synthesis of analogue 17

In a dried Schlenk tube, **NFT** (0.2 mmol), NFSI (0.3 mmol), ligand (0.024 mmol, 12 mol%) and CuOAc (0.02 mmol, 10 mol%) were dissolved in CH_3CN (analytical grade without drying, 1.0 mL) under a N_2 atmosphere, then H_2O (0.6 mmol) was added. The reaction mixture was stirred at 35 ˚C for 24 h. Upon completion, saturated sodium bicarbonate (30 mL) was added, and the reaction was extracted with DCM (2 x 30 mL), washed with and brine (30 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel with a gradient eluent of petroleum ether and ethyl acetate (petroleum ether/ethyl acetate 60%) to provide the product **17** in a yield of 72%.

(E)-5-hydroxy-1-(((5-nitrofuran-2-yl) methylene) amino) imidazolidine-2,4-dione, ***17***



17: yellow solid; yield 72%.

^{1}H NMR (400 MHz, DMSO) δ 11.54 (s, 1H), 8.18 (s, 1H), 7.77 (d, *J* = 3.9 Hz, 1H), 7.49 (d, *J* = 9.8 Hz, 1H), 7.22 (d, *J* = 3.9 Hz, 1H), 5.70 (d, *J* = 9.6 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 169.9, 151.9, 151.9, 151.5, 132.7, 115.5, 114.6, 78.1.

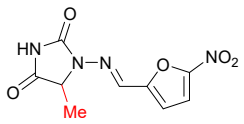
HRMS (ESI+, *m/z*) calculated for $C_8H_6N_4O_6$ [M + Na]$^+$: 277.0180; found: 277.0188.

Synthesis of analogue 18

In a dried Schlenk tube, **17** (0.1 mmol) was dissolved in 1.0 mL of DCM under nitrogen and cooled to -78 ˚C.

DAST (0.1 mmol) was added, and the solution was stirred for 1 h at room temperature. The reaction was then placed back into -78 ˚C cold bath, where $AlMe_3$ (0.3 mmol) was then added dropwise. The reaction mixture was stirred at -78 ˚C for 2 h, then allowed to warm to room temperature while stirring for 1 h. Upon completion, the reaction was added 10 mL H_2O for quenching. The aqueous layer was extracted with DCM (2 x 10 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (eluting with petroleum ether/ethyl acetate 20% - 30%).

(E)-5-methyl-1-(((5-nitrofuran-2-yl) methylene) amino) imidazolidine-2,4-dione, ***18***



18: yellow solid; yield 40%.

^{1}H NMR (400 MHz, DMSO) δ 8.23 (s, 1H), 7.77 (d, *J* = 3.9 Hz, 1H), 7.13 (d, *J* = 3.9 Hz, 1H), 4.55 (q, *J* = 6.8 Hz, 1H), 1.42 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 174.5, 155.0, 152.3, 151.7, 131.4, 114.8, 114.4, 56.1, 14.5.

HRMS (ESI+, *m/z*) calculated for $C_9H_8N_4O_5$ $[M + Na]^+$: 275.0387; found: 275.0398.

5. In vitro biological activity evaluation

Bacteria strains and culture conditions

The following standard bacterial and fungal strains were used in this study: *Staphylococcus aureus* (*S. aureus* ATCC 29213), *Escherichia coli* (*E. coli* ATCC 25922), *Candida albicans* (*C. albicans* ATCC 14053) and *Helicobacter pylori* (*H. pylori* SS1). *S. aureus* strain and *E. coli* strain were cultured in Mueller–Hinton (MH) medium (Hopebio, Qingdao, China) at 37 °C [1, 2]. *C. albicans* strain was cultured in Sabouraud medium (Hopebio, Qingdao, China) at 35 °C. *H. pylori* strain SS1 was cultured on Columbia blood plates supplemented with fetal bovine serum (FBS) under microaerophilic conditions at 37 °C [3].

Minimal inhibitory concentration (MIC) assays

MIC of *S. aureus*, *Escherichia coli* and *C. albicans*: Clinical and Laboratory Standards Institute (CLSI) microdilution method were referred to measure the MICs of analogs with minor modification [4]. The bacteria strains were cultured in MH medium or Sabouraud medium at 37 °C or 35 °C overnight. Afterwards, we diluted mid-log phase bacteria to 1×10^5 colony forming units (CFU)/ml in the same medium before using. Diluted analogue solution were added into 96-well plates from 50 μg/mL to 0.39065 μg/mL by typical serial two-fold dilution method. After incubation at 37 °C or 35 °C for 18 h, the lowest concentration at which no visible bacterial growth observed in the well was determined as the MIC. Medium without antibiotic served as the negative control. All tests were performed three times independently.

MIC of *H. pylori*: the strain was cultured on Columbia blood plates supplemented with FBS (200 μl) under microaerophilic conditions at 37 °C for 3–4 days. The bacteria were diluted with 10% FBS-containing Brain Heart Infusion (BHI) and then measured by reading optical density (OD) at 600 nm (OD 600). OD 600 was adjusted to 0.1. Then 200 μl of bacteria suspension with the dilution of 1:10 was inoculated in the 96-well plates. Then analogue solution with the same final concentrations (50 to 0.0976 μg/ml, two-fold dilution) were added subsequently. The inoculated 96-well plates were incubated at 37 °C for 48 h with shaking (100 rpm) under microaerobic conditions and the OD 600 of the cultures were measured by Flex Station 3 microplate reader (Molecular devices, USA).

Cell Culture

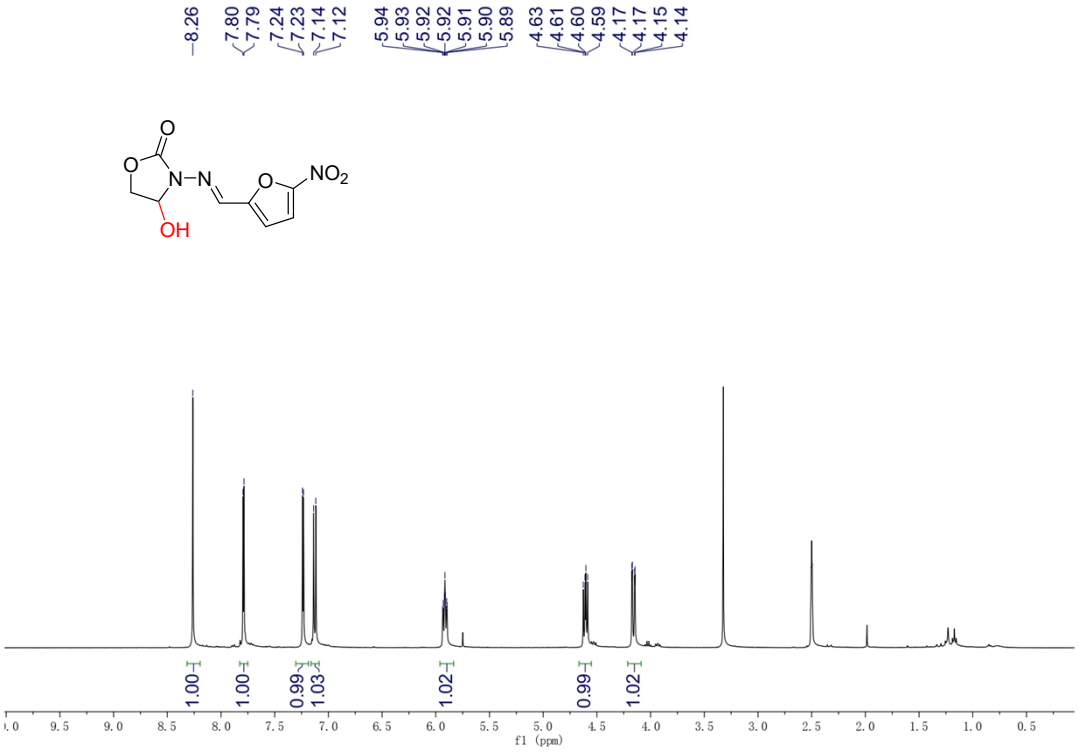
The HepaRG cells were cultivated in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, Serana, Germany) and 1% streptomycin and penicillin (Hyclone, USA) and maintained under standard conditions

at 37 °C in a humidified air containing 5% CO_2. For cells treatment, **FZD** and potent compounds **1**, **3**, **4**, **5** and **18** were freshly diluted with DMSO, the stock solution (100 mM) was stored at -20 °C until used. To evaluate the effect of compounds on HepaRG cells, the stock solution was further diluted into the final concentration of 0.01~100 μM.

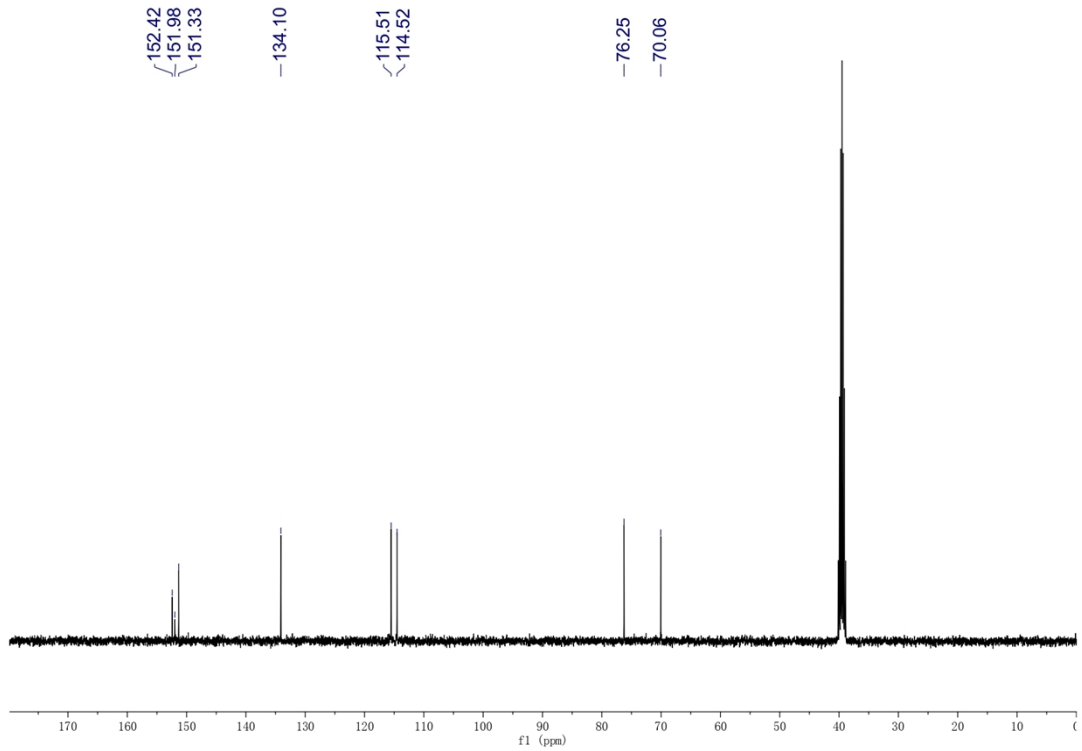
Cytotoxicity Assay

Cytotoxicity of **FZD** and the potent compounds were performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Beyotime, China) assays. Cells were seeded onto 96-well plates, incubated overnight, cultured with increasing concentrations of **FZD** and potent compounds **1**, **3**, **4**, **5** and **18** (0.01~100 μM) for 24 h. Next, fresh MTT solution (0.5 mg/ml) was added and re-incubated for 4 h at 37 °C. Then, DMSO (150 μl) were added to dissolve the formazan crystals product and measured the optical density at a wavelength of 490 nm with a microplate reader (ThermoFisher Scientific, USA). GraphPad prism v 8.0 software (GraphPad Software, USA) was used to deal with all data, which were presented as the mean±standard deviation. Statistical analysis between different groups were determined using student's *t*-test, and $p<0.05$ was considered as significant.

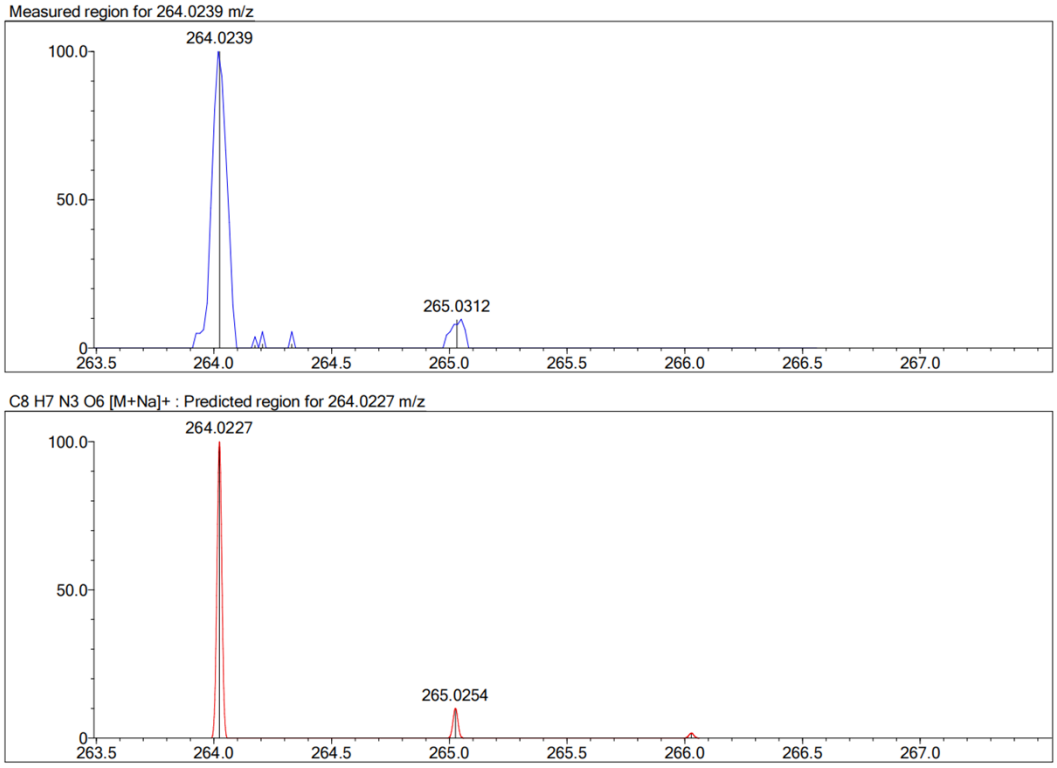
6. NMR and HRMS Spectra



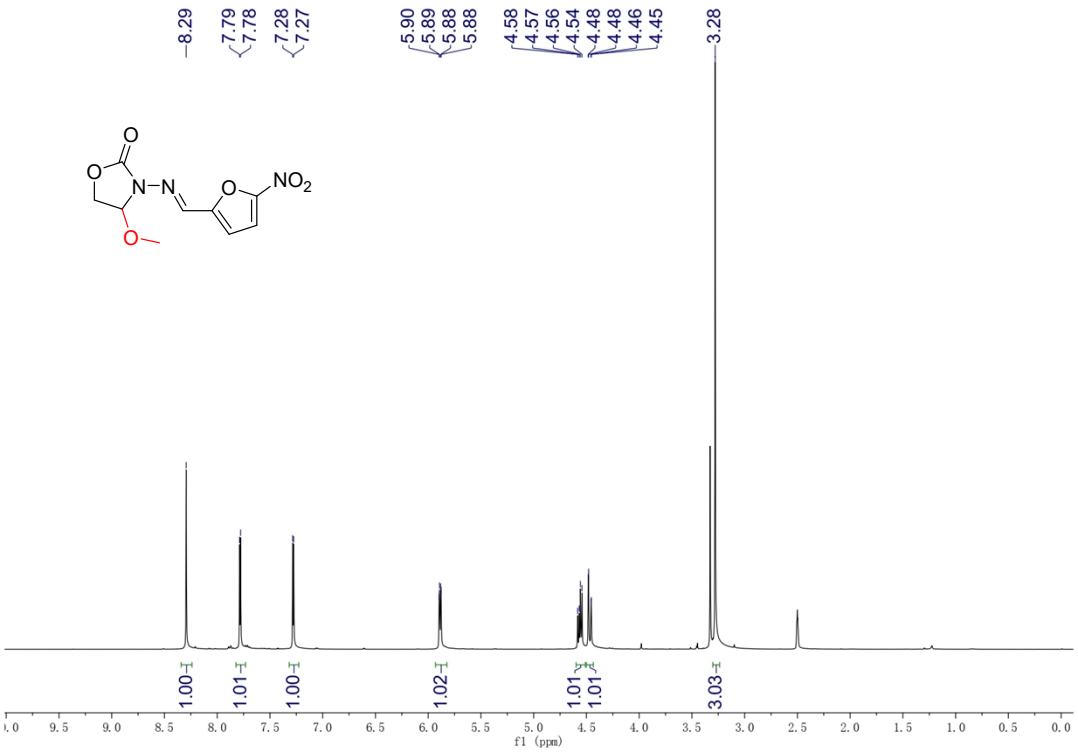
Supplementary Fig. 1. ^{1}H NMR spectrum of compound **1**.



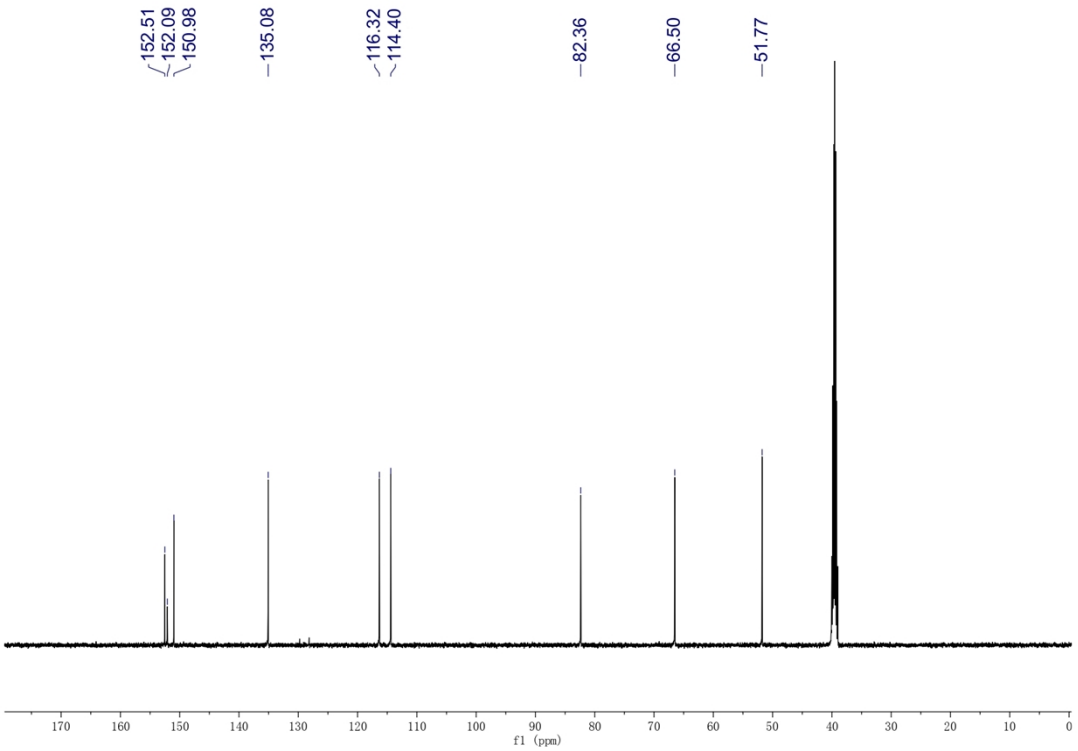
Supplementary Fig. 2. ^{13}C NMR spectrum of compound **1**.



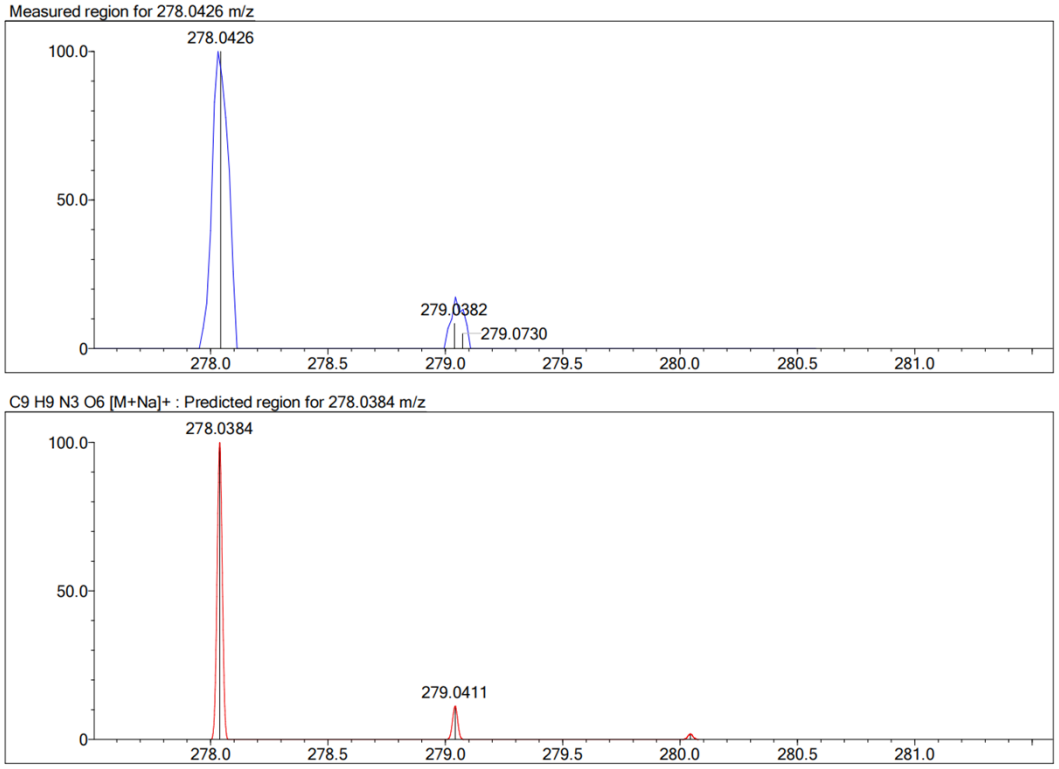
Supplementary Fig. 3. HRMS spectrum of compound **1**.



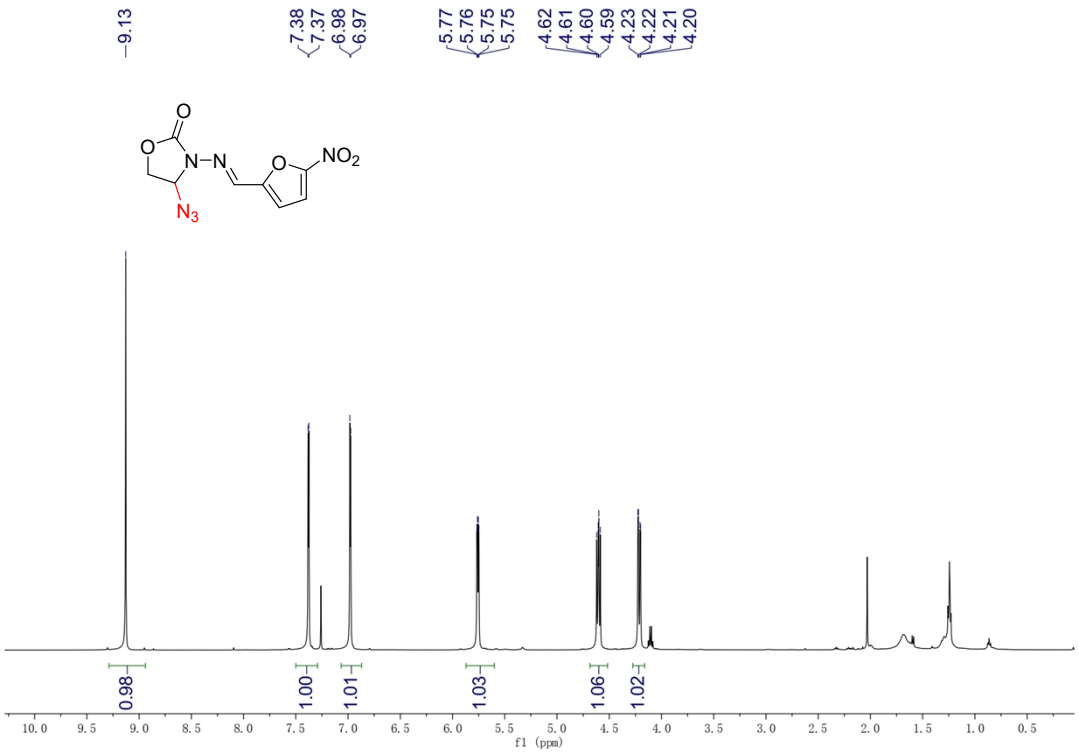
Supplementary Fig. 4. ^{1}H NMR spectrum of compound **2**.



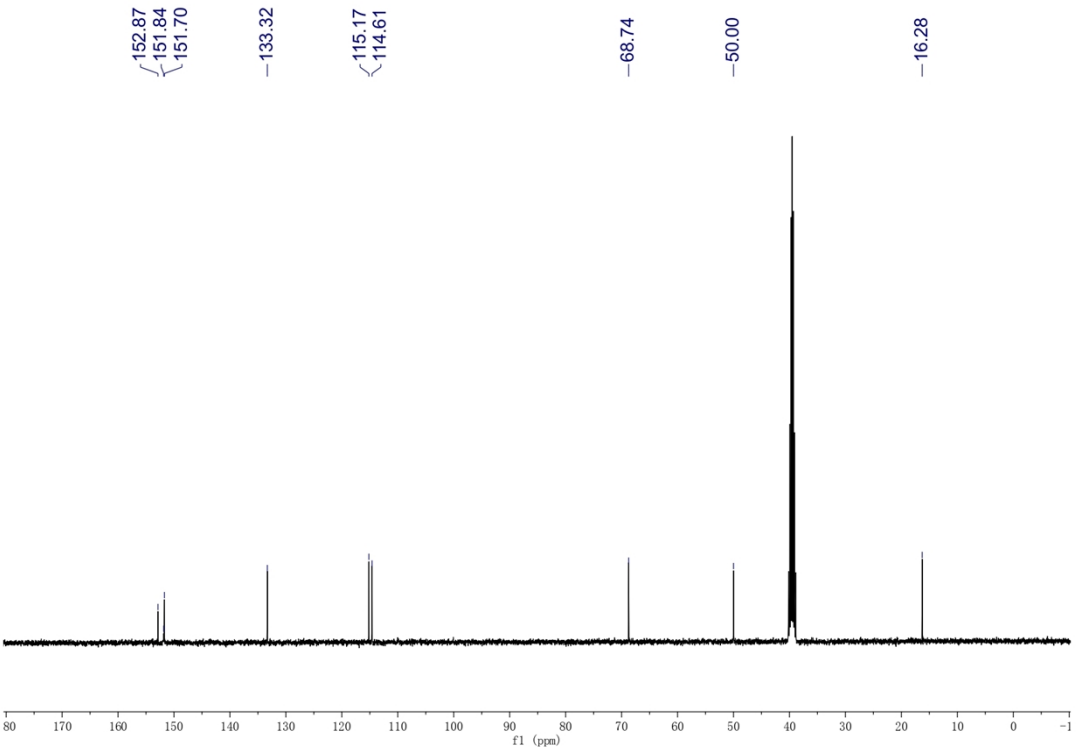
Supplementary Fig. 5. ^{13}C NMR spectrum of compound **2**.



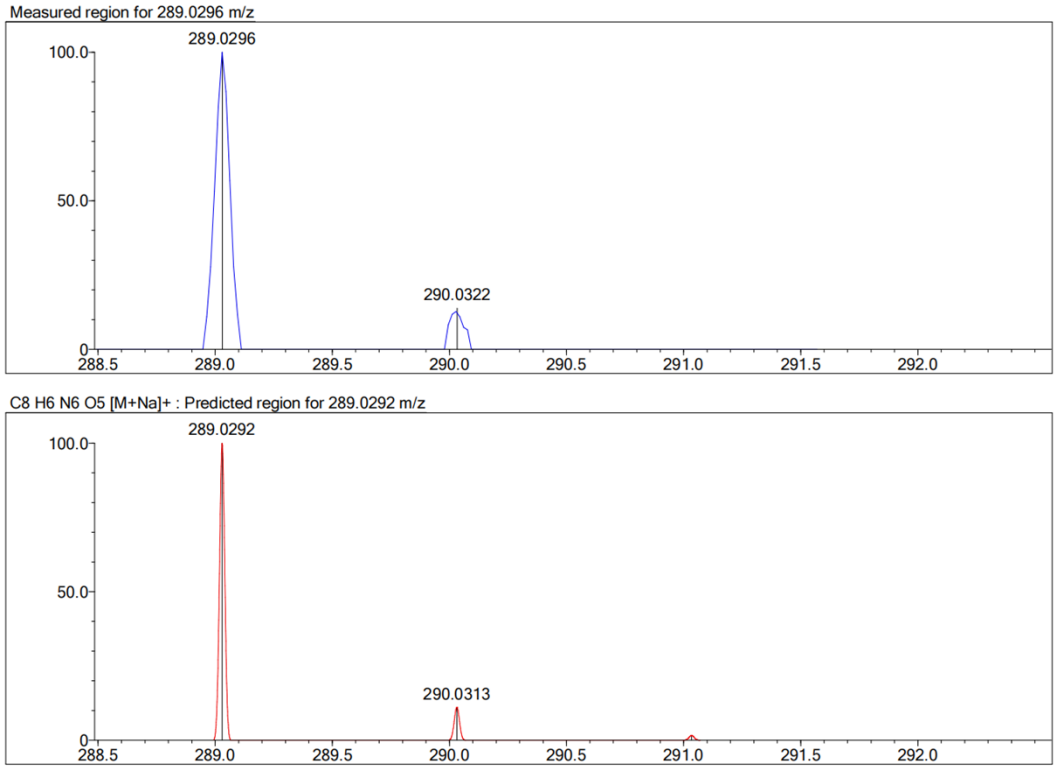
Supplementary Fig. 6. HRMS spectrum of compound **2**.



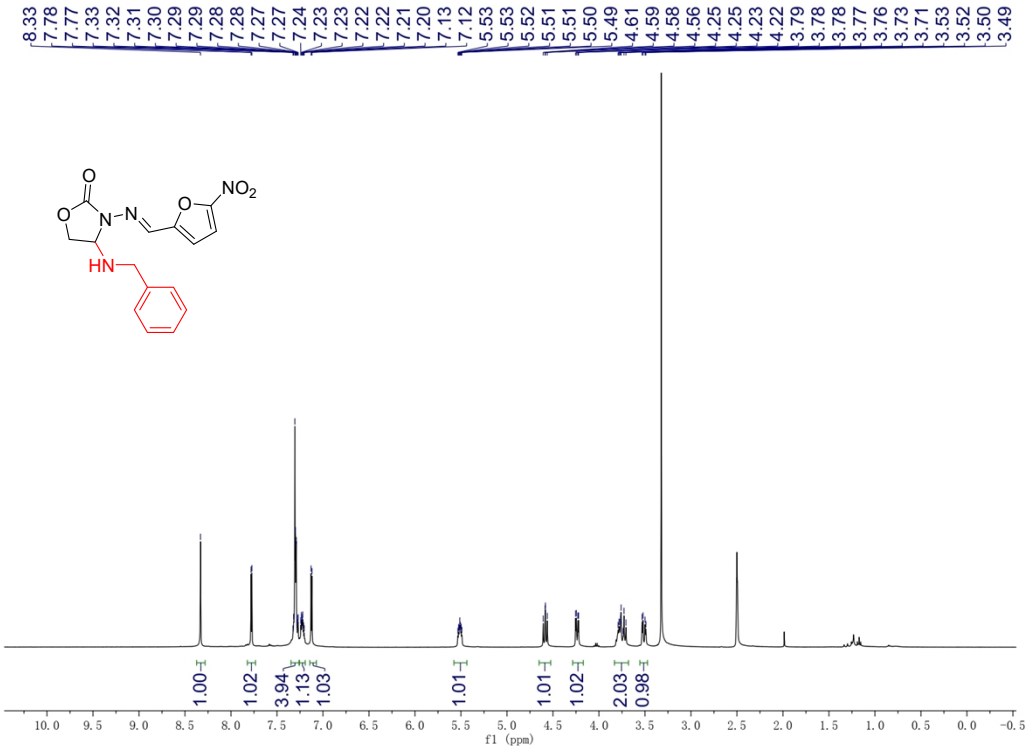
Supplementary Fig. 7. ^{1}H NMR spectrum of compound **3**.



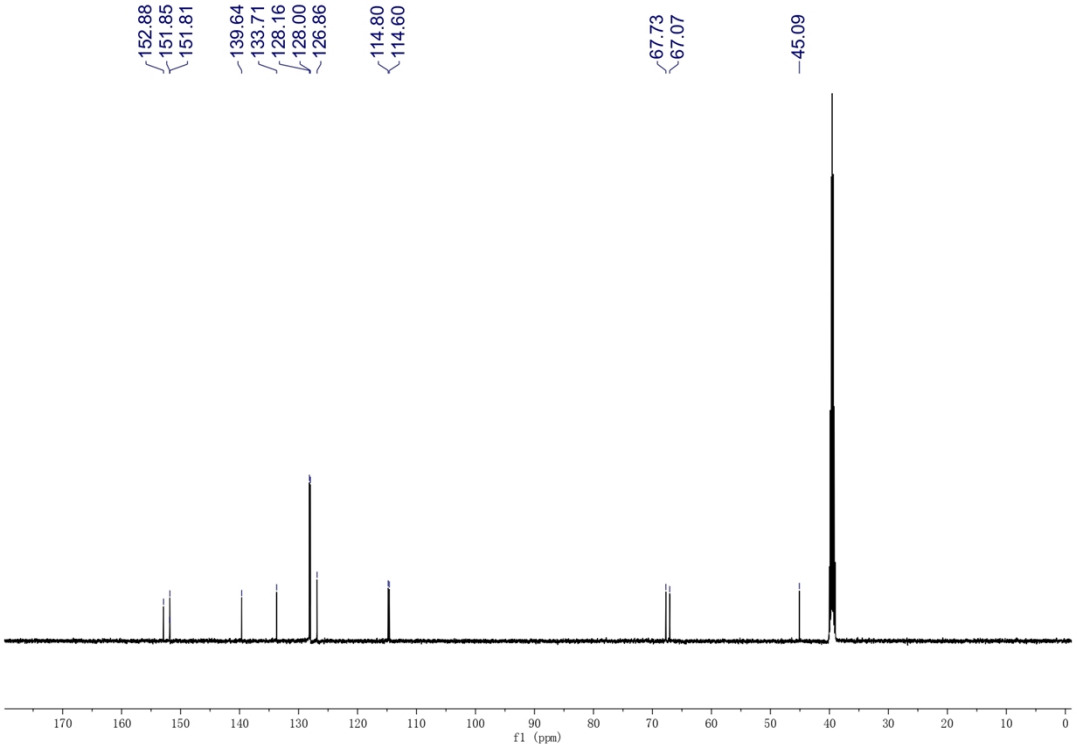
Supplementary Fig. 8. ^{13}C NMR spectrum of compound **3**.



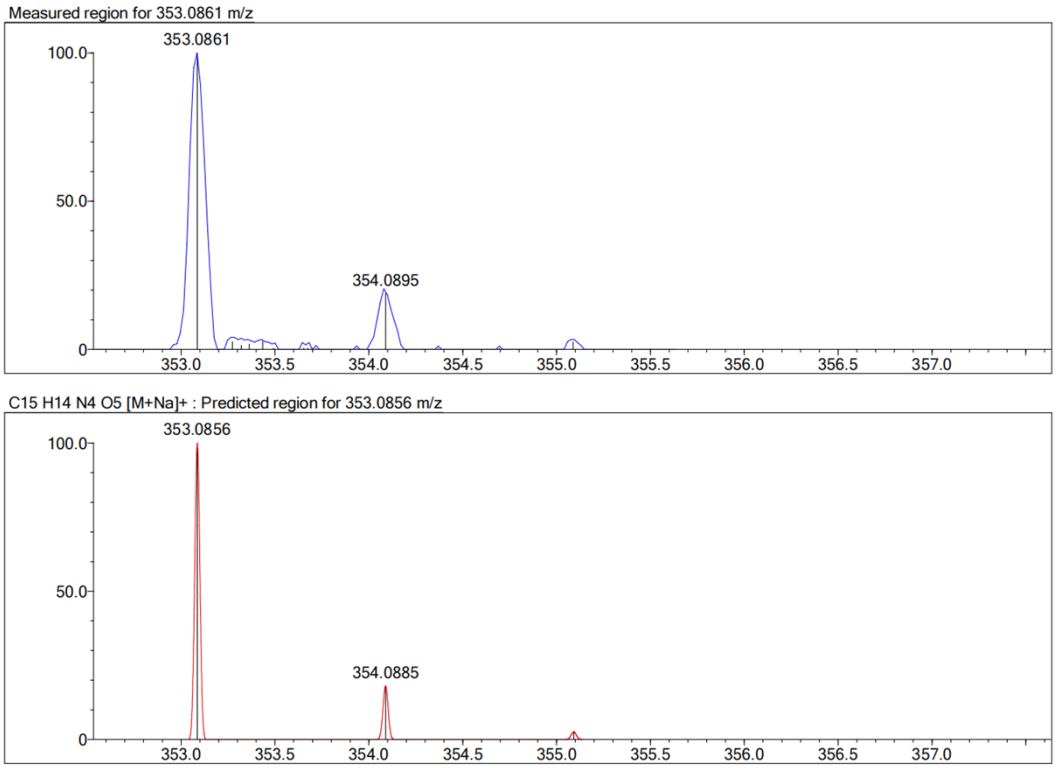
Supplementary Fig. 9. HRMS spectrum of compound **3**.



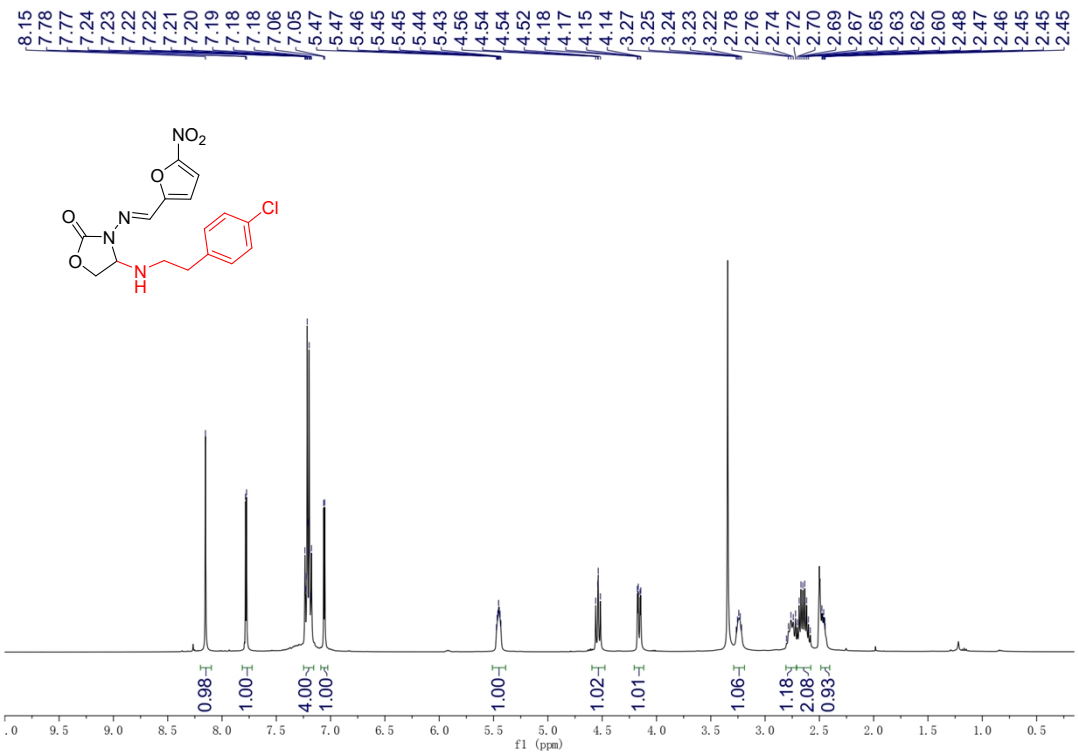
Supplementary Fig. 10. ^{1}H NMR spectrum of compound **4**.



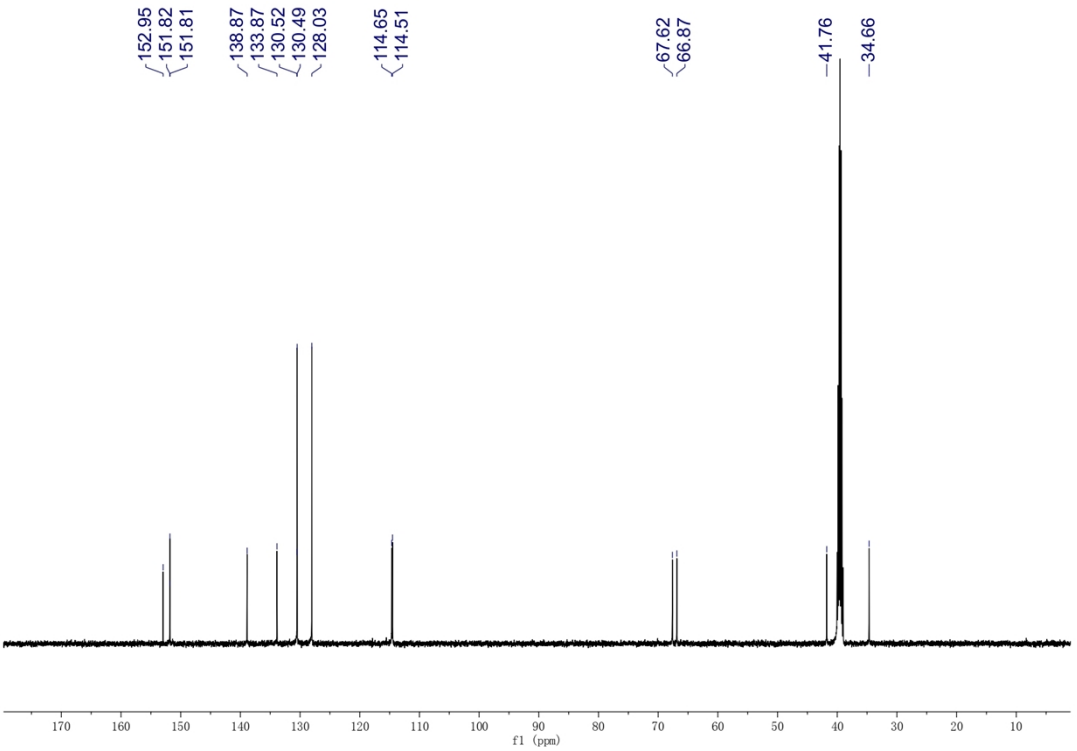
Supplementary Fig. 11. ^{13}C NMR spectrum of compound **4**.



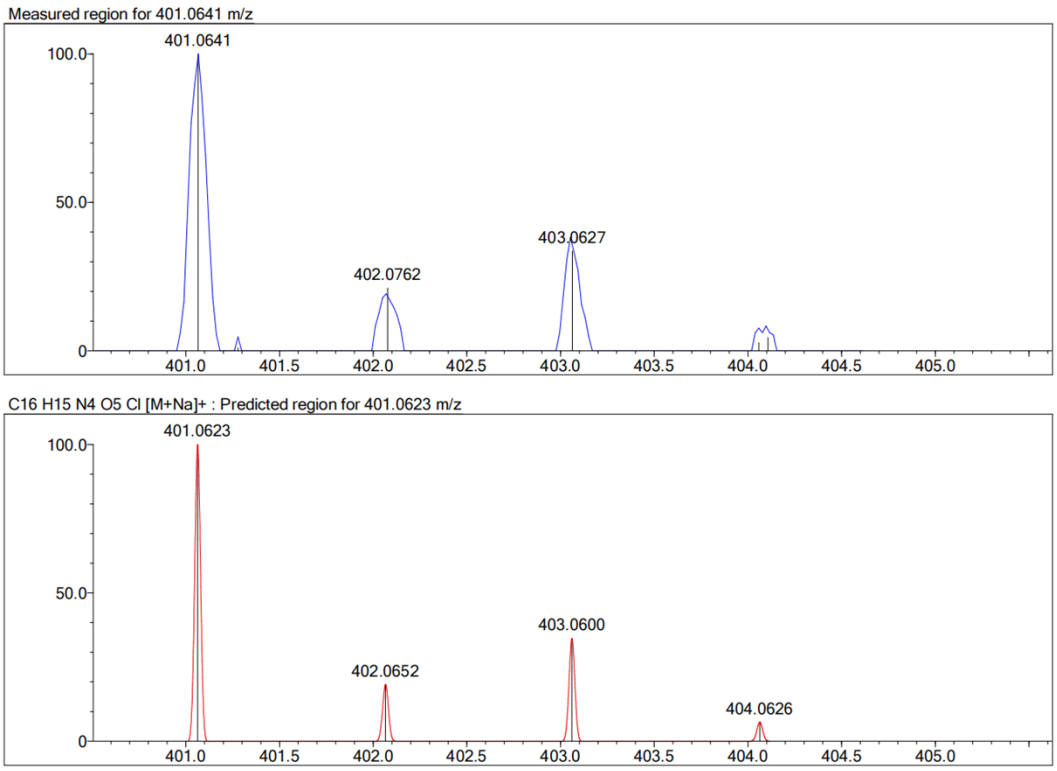
Supplementary Fig. 12. HRMS spectrum of compound **4**.



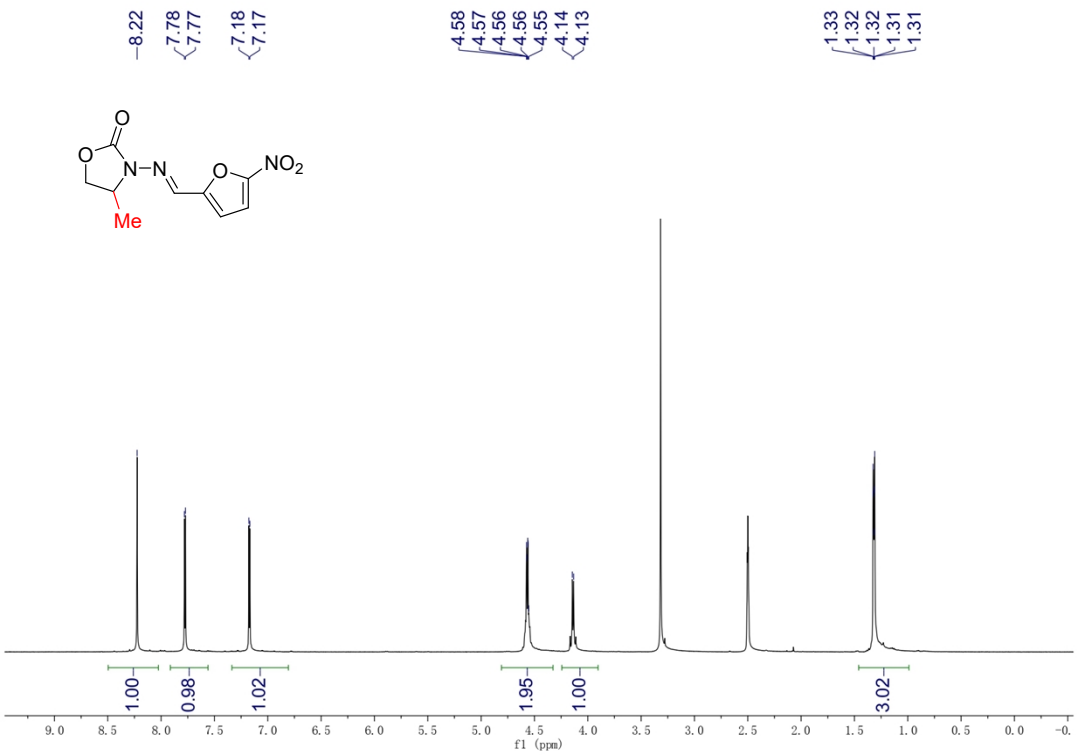
Supplementary Fig. 13. ^{1}H NMR spectrum of compound **5**.



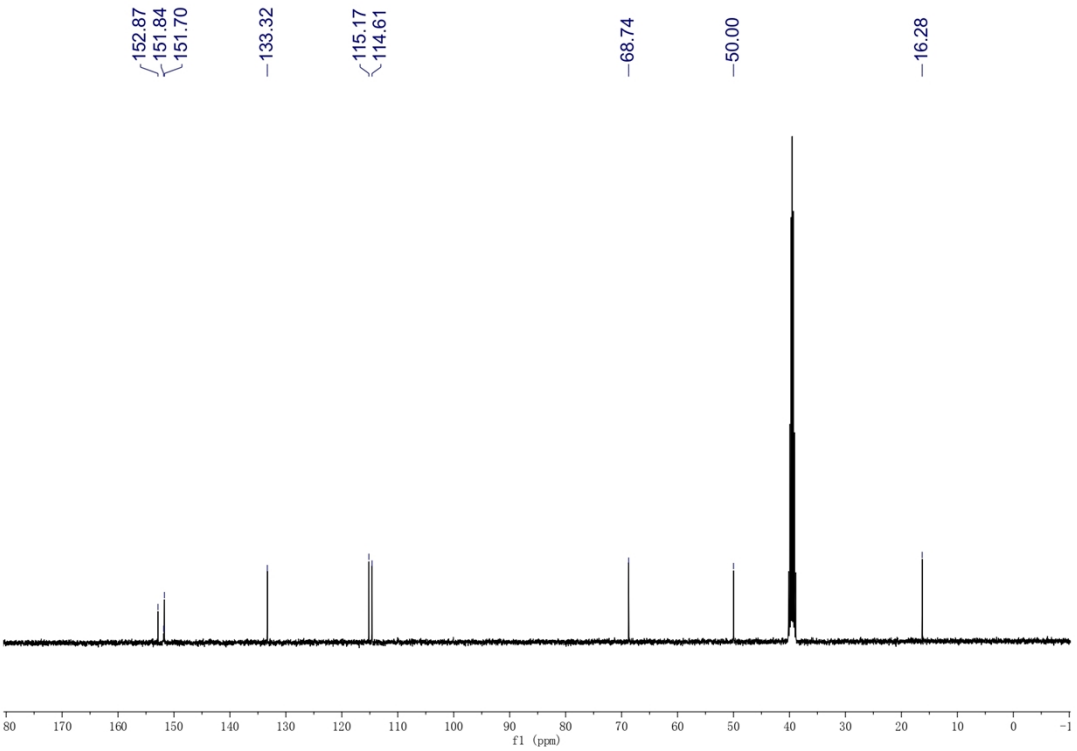
Supplementary Fig. 14. ^{13}C NMR spectrum of compound **5**.



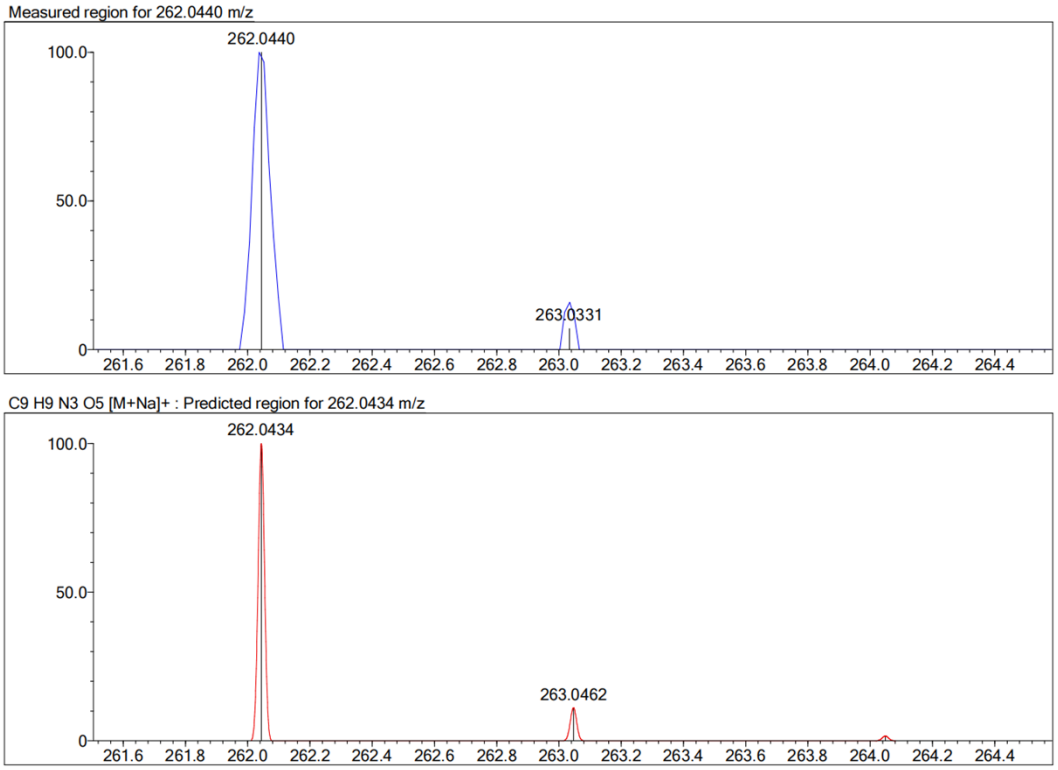
Supplementary Fig. 15. HRMS spectrum of compound **5**.



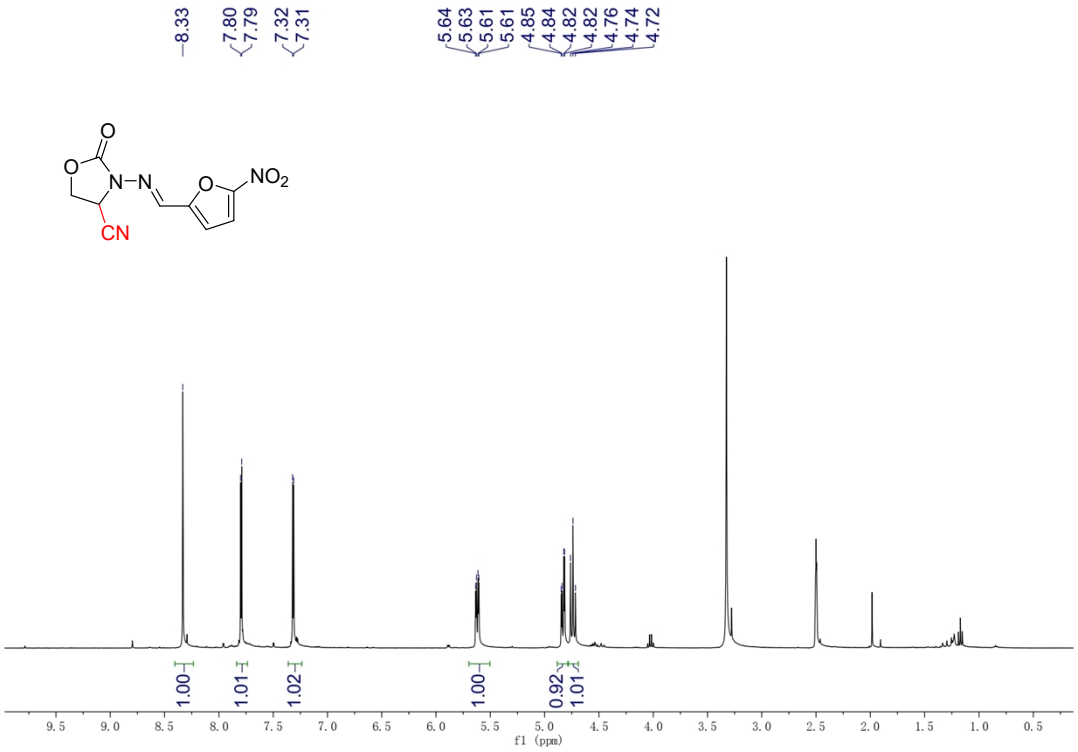
Supplementary Fig. 16. ^{1}H NMR spectrum of compound **6**.



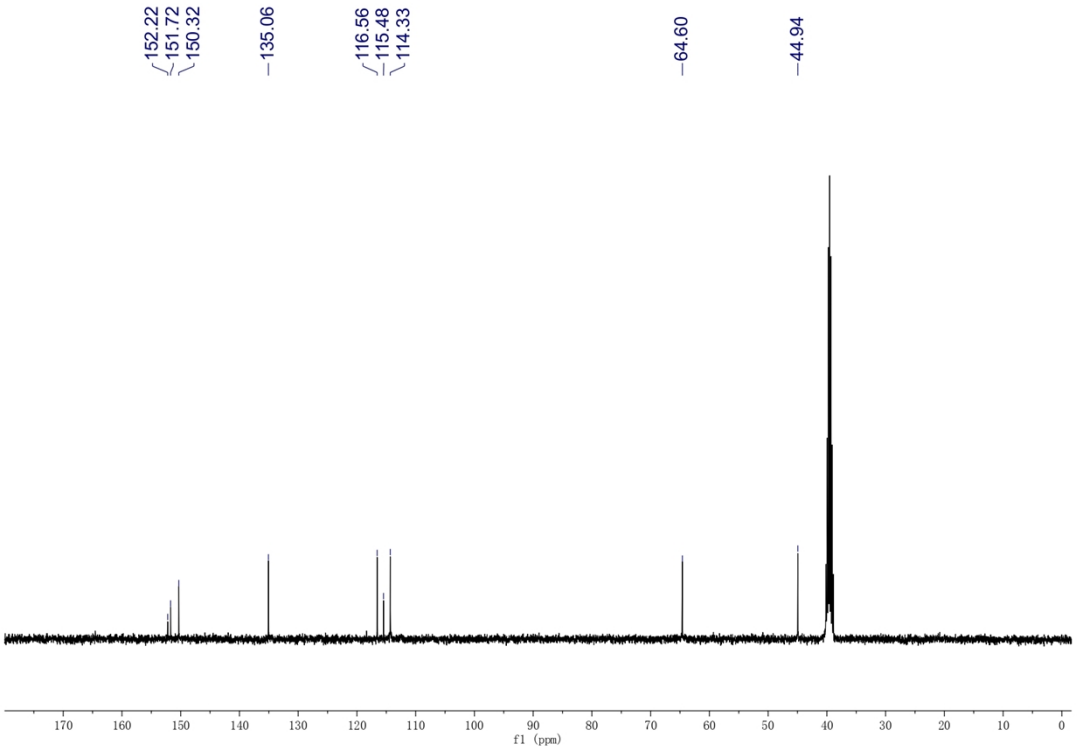
Supplementary Fig. 17. ^{13}C NMR spectrum of compound **6**.



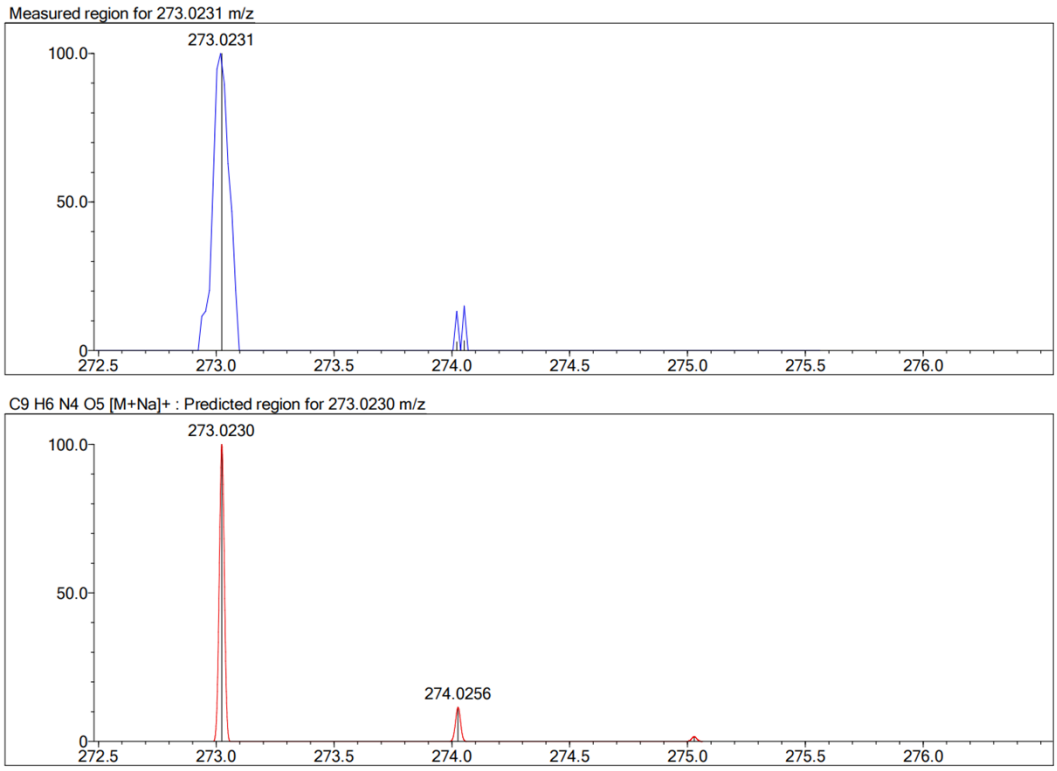
Supplementary Fig. 18. HRMS spectrum of compound **6**.



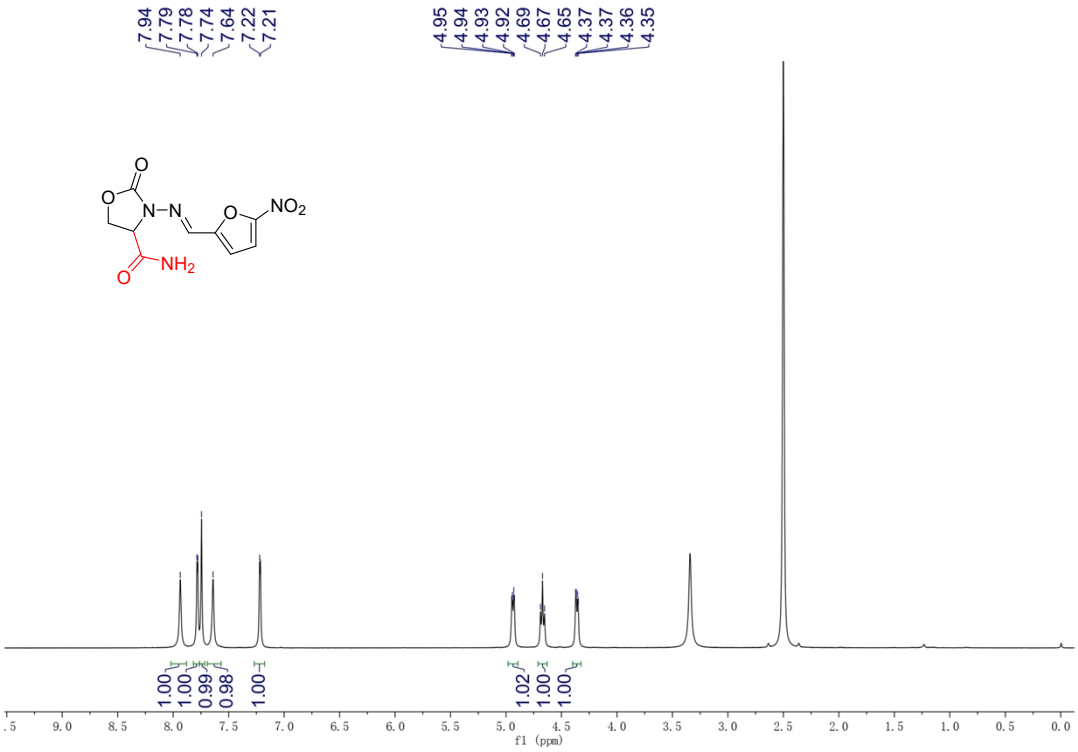
Supplementary Fig. 19. ^{1}H NMR spectrum of compound **7**.



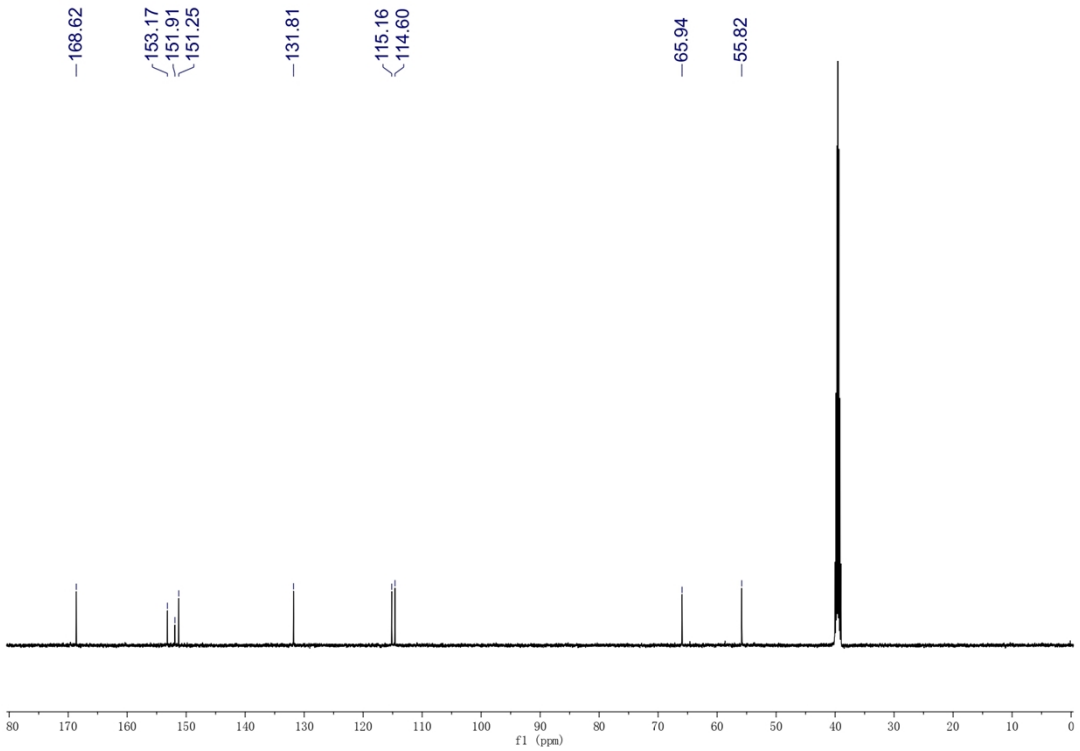
Supplementary Fig. 20. ^{13}C NMR spectrum of compound **7**.



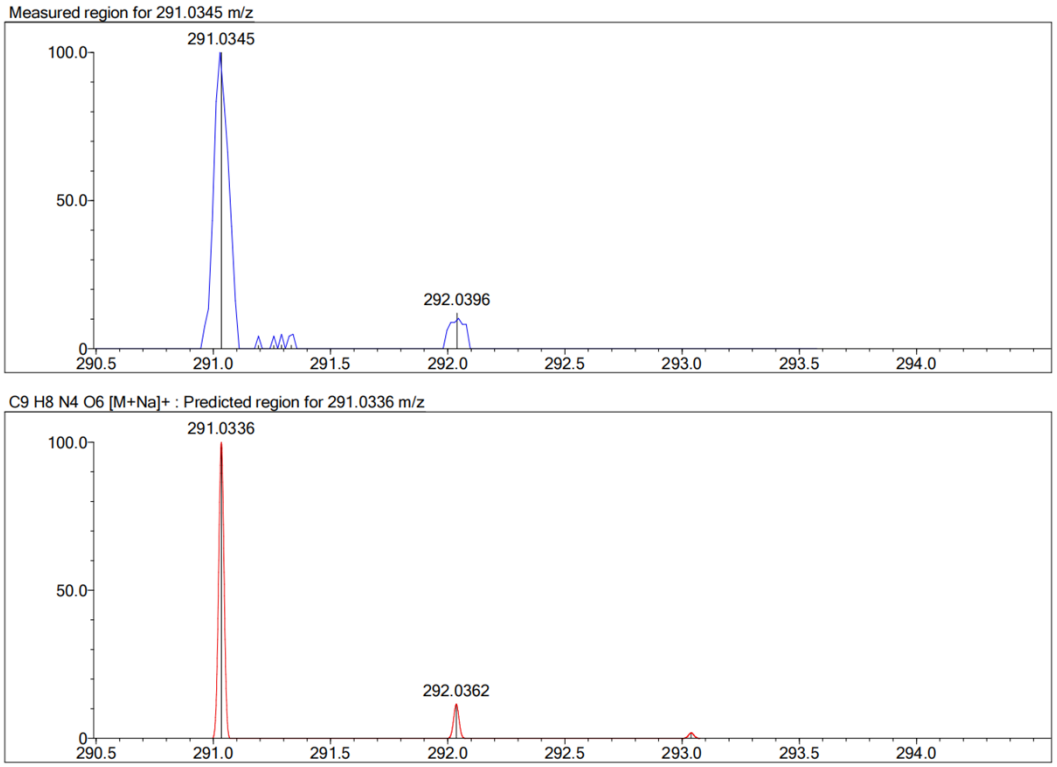
Supplementary Fig. 21. HRMS spectrum of compound **7**.



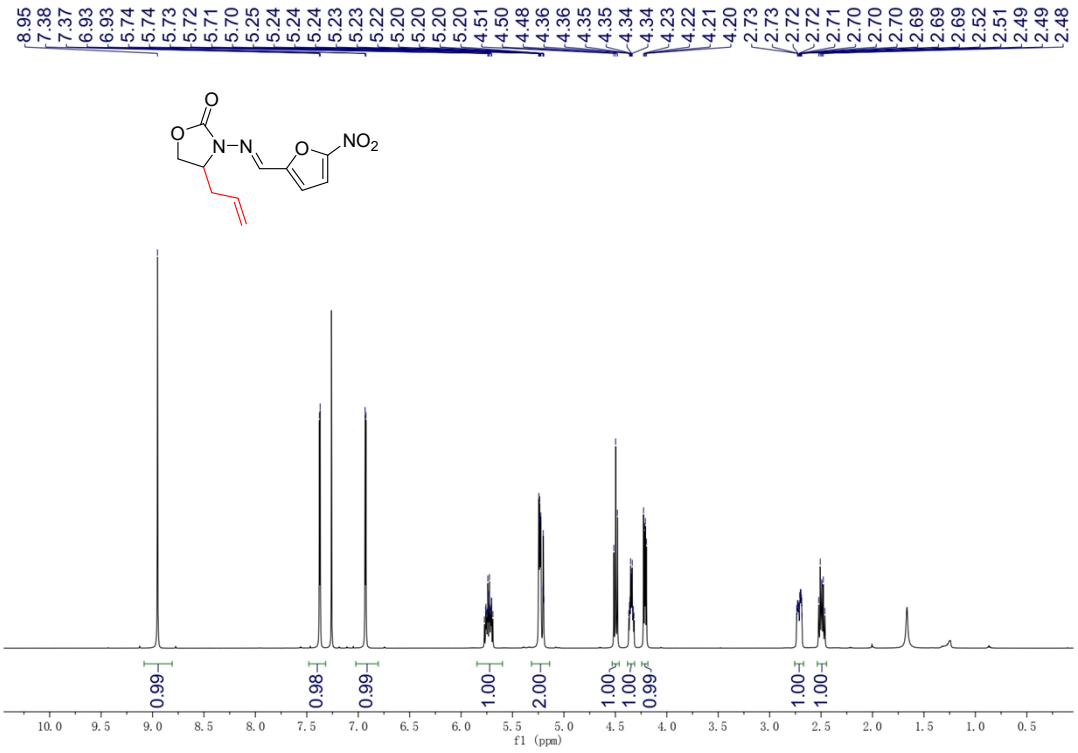
Supplementary Fig. 22. ^{1}H NMR spectrum of compound **8**.



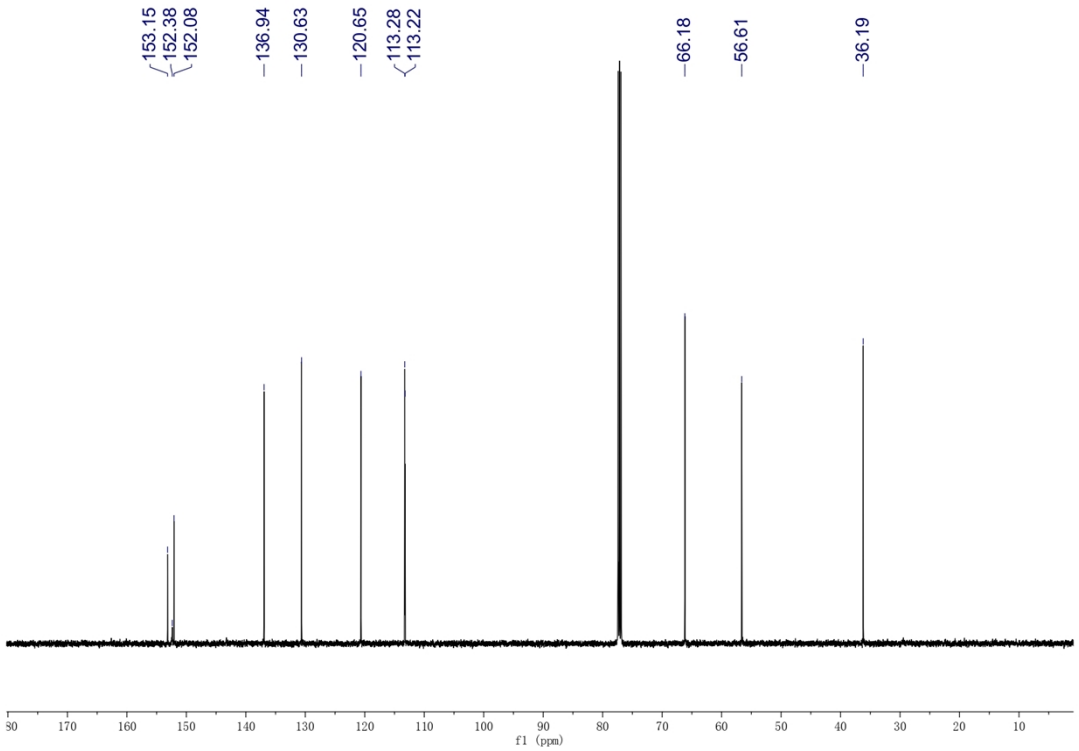
Supplementary Fig. 23. ^{13}C NMR spectrum of compound **8**.



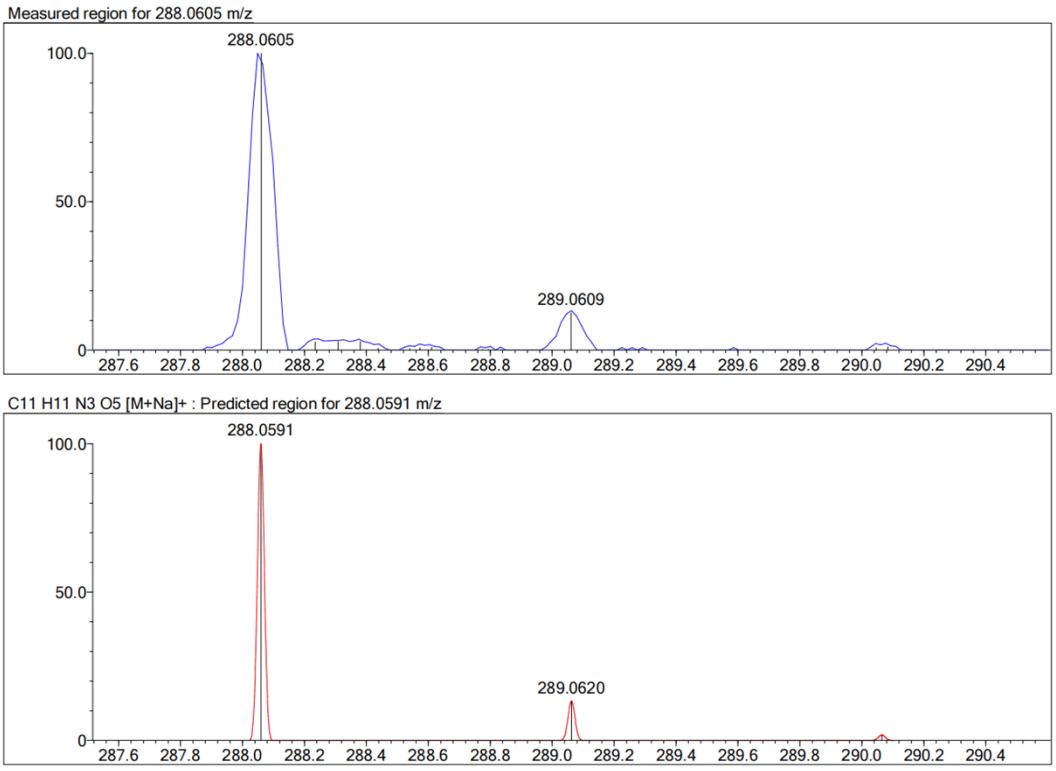
Supplementary Fig. 24. HRMS spectrum of compound **8**.



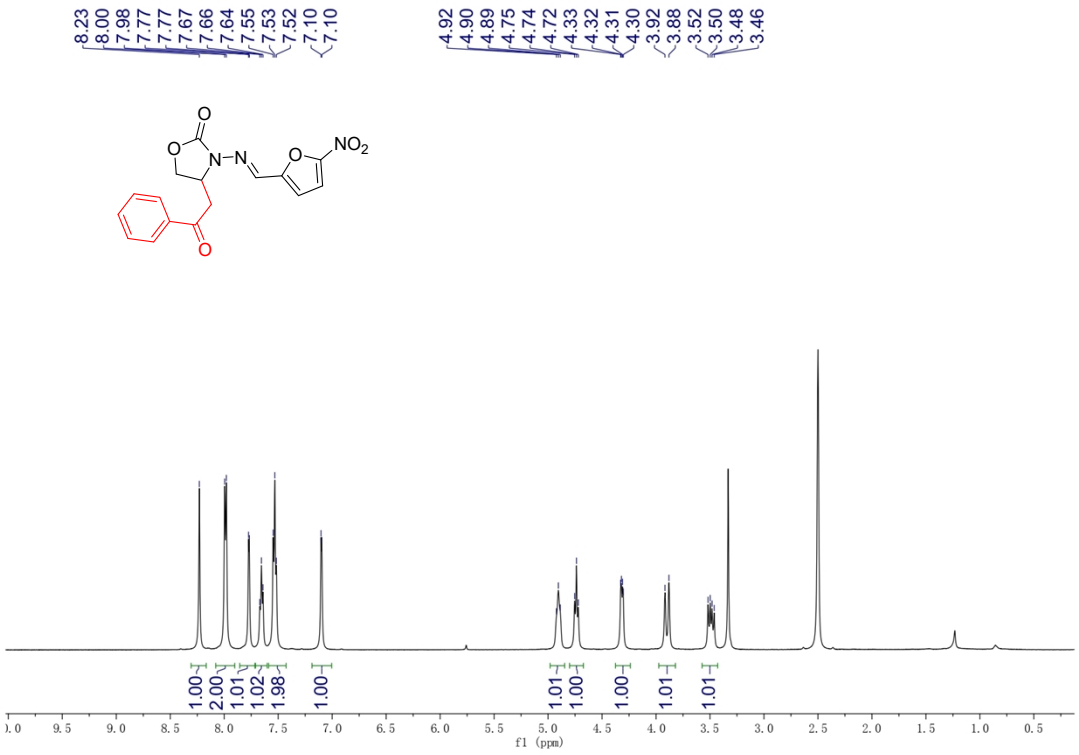
Supplementary Fig. 25. ^{1}H NMR spectrum of compound **9**.



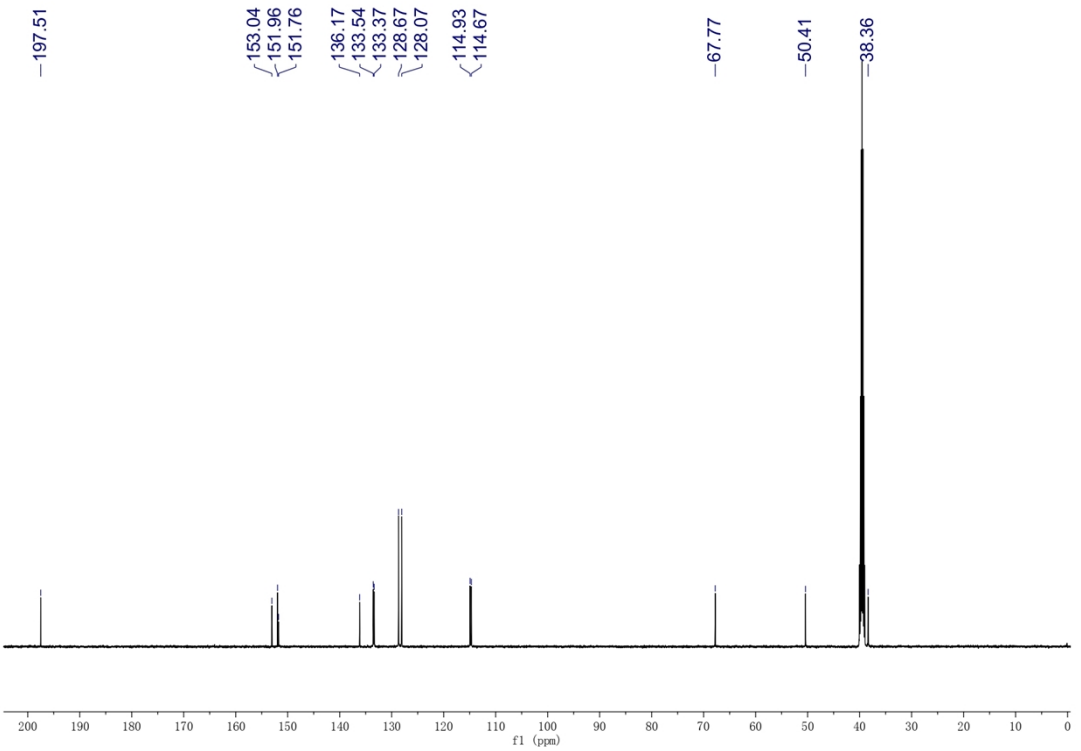
Supplementary Fig. 26. ^{13}C NMR spectrum of compound **9**.



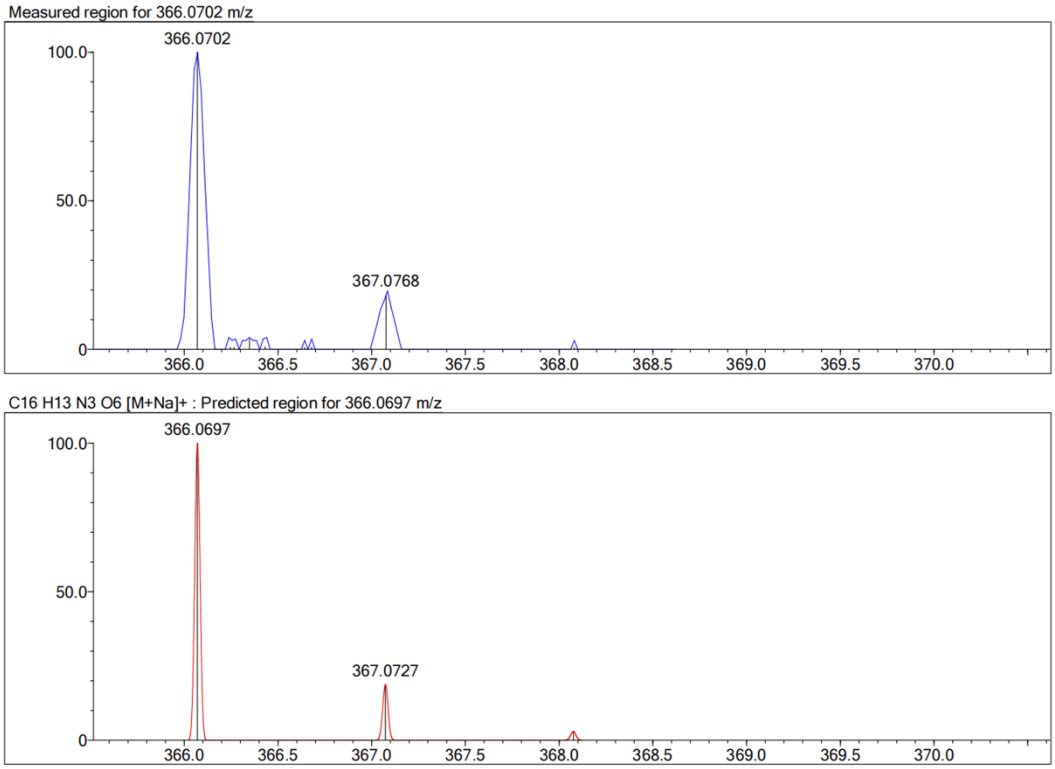
Supplementary Fig. 27. HRMS spectrum of compound **9**.



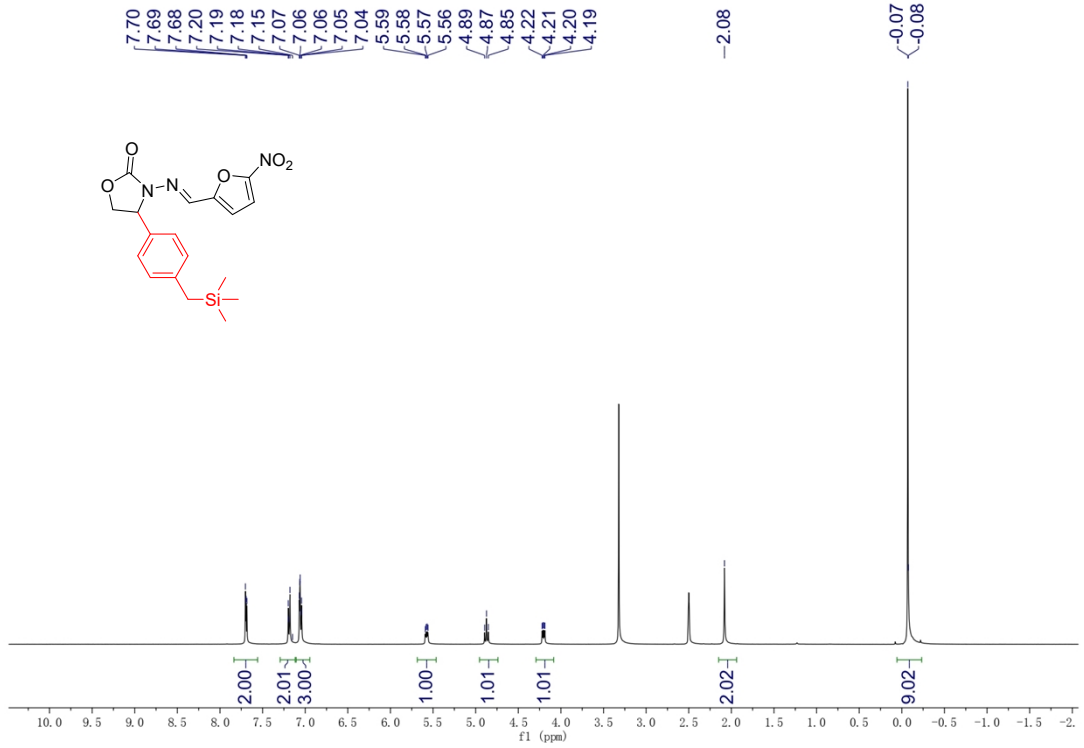
Supplementary Fig. 28. ^{1}H NMR spectrum of compound **10**.



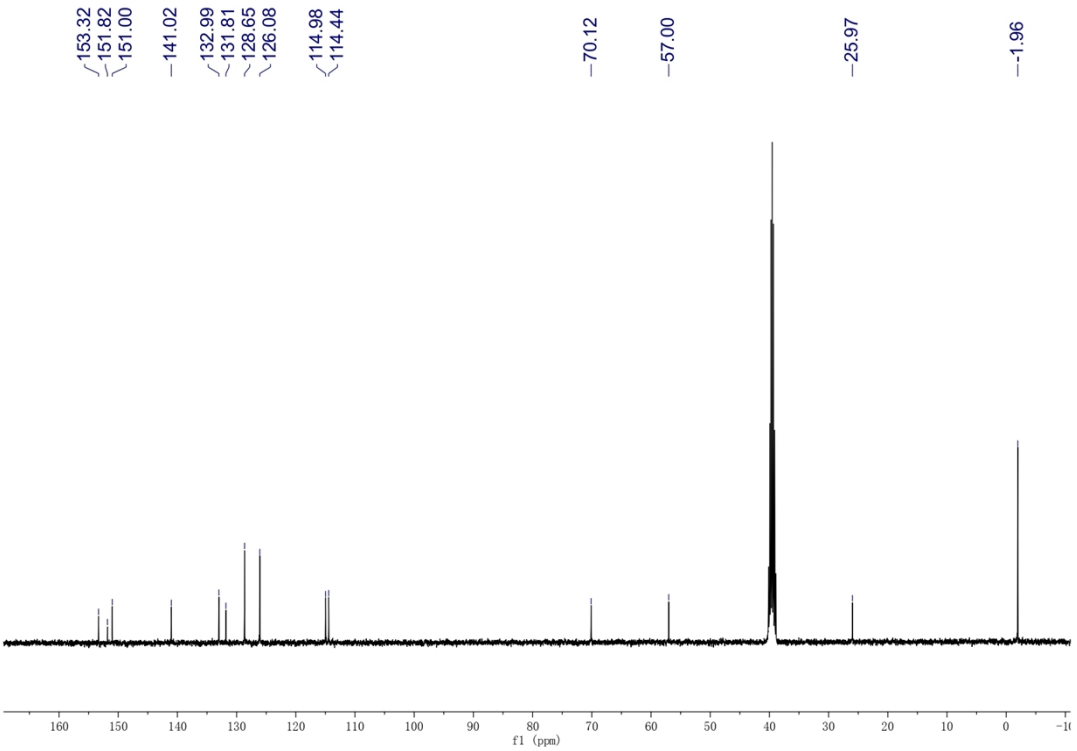
Supplementary Fig. 29. ^{13}C NMR spectrum of compound **10**.



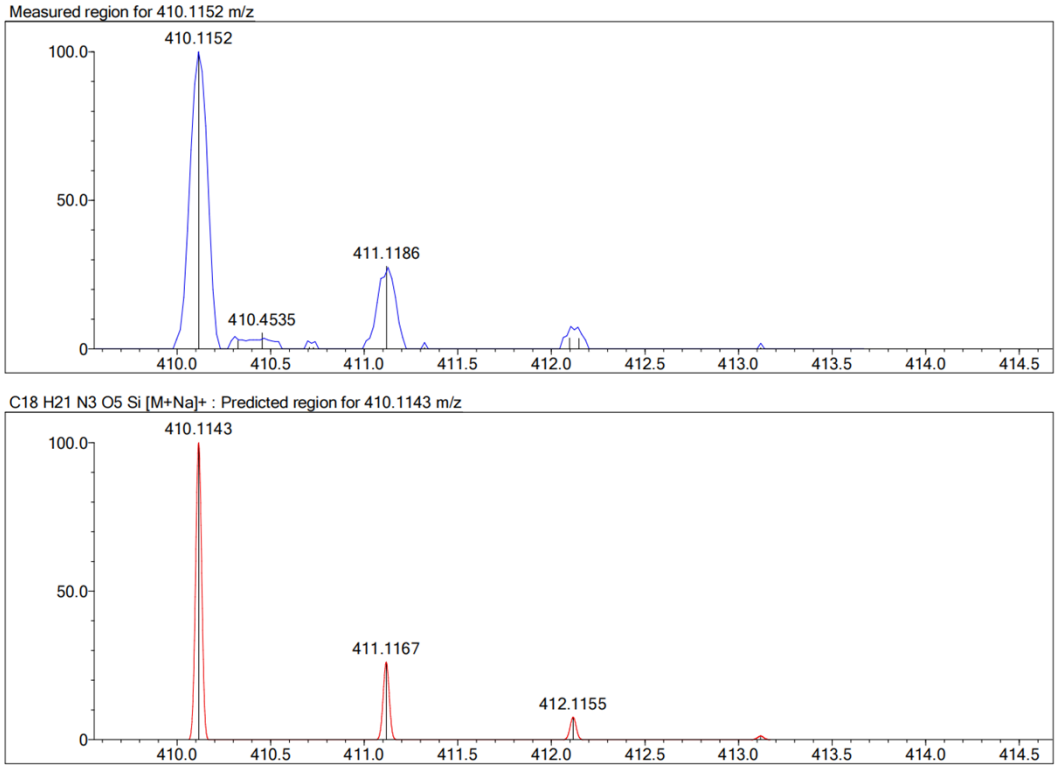
Supplementary Fig. 30. HRMS spectrum of compound **10**.



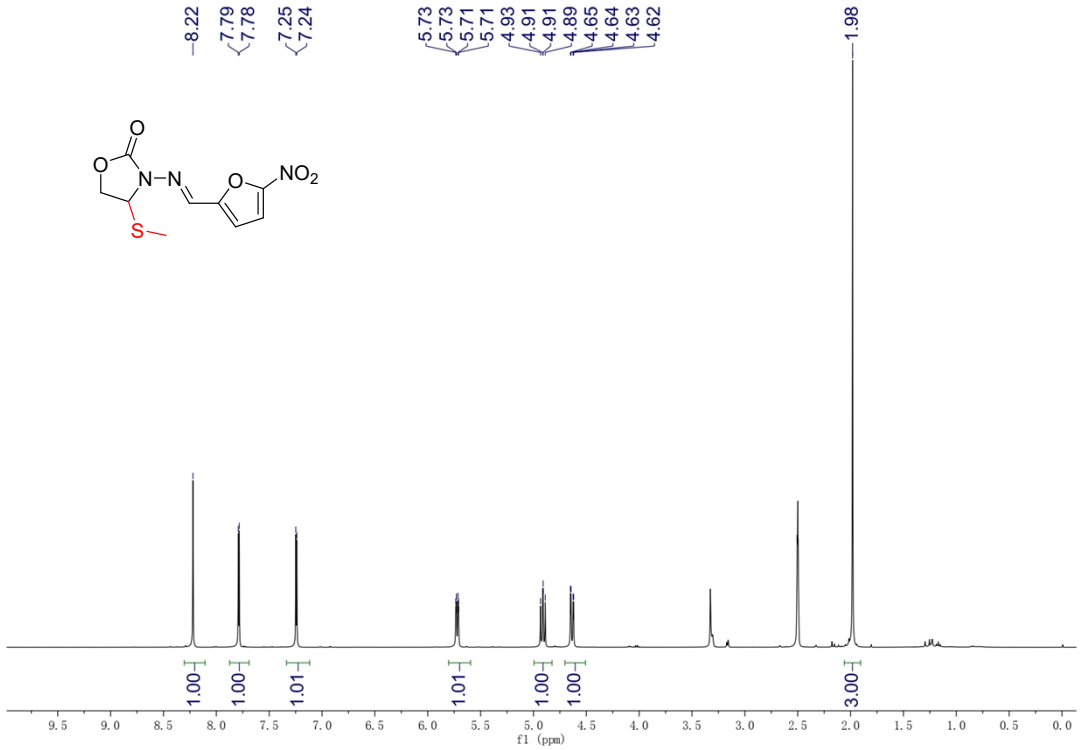
Supplementary Fig. 31. ^{1}H NMR spectrum of compound **11**.



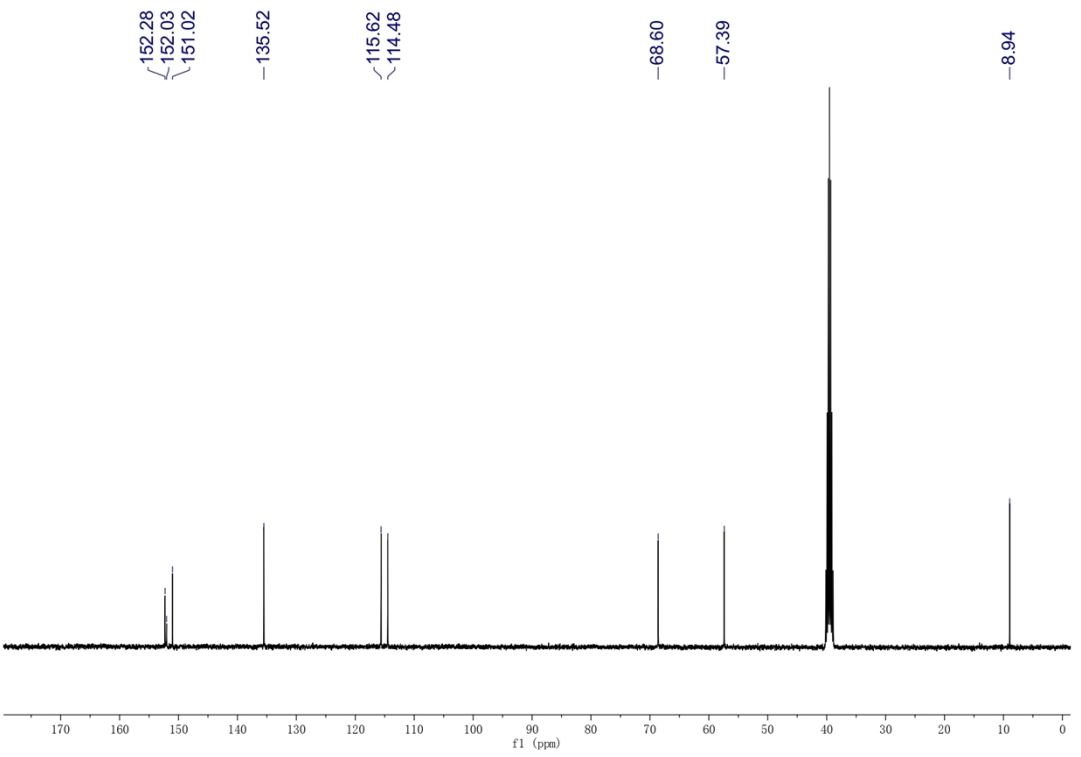
Supplementary Fig. 32. ^{13}C NMR spectrum of compound **11**.



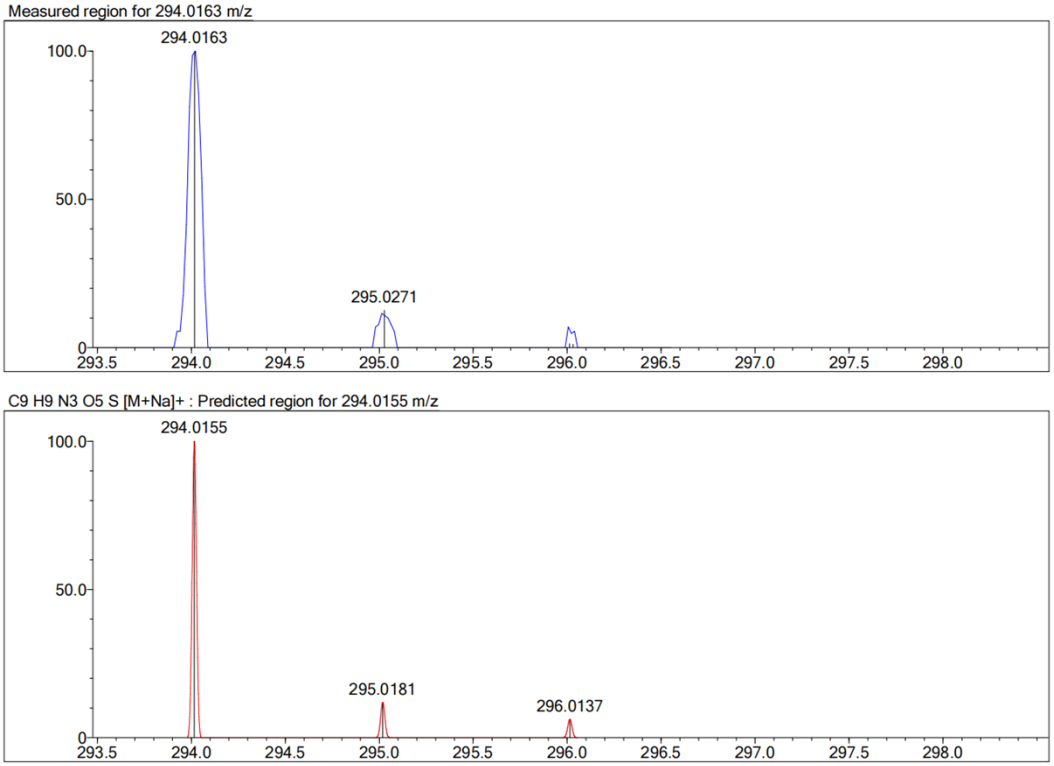
Supplementary Fig. 33. HRMS spectrum of compound **11**.



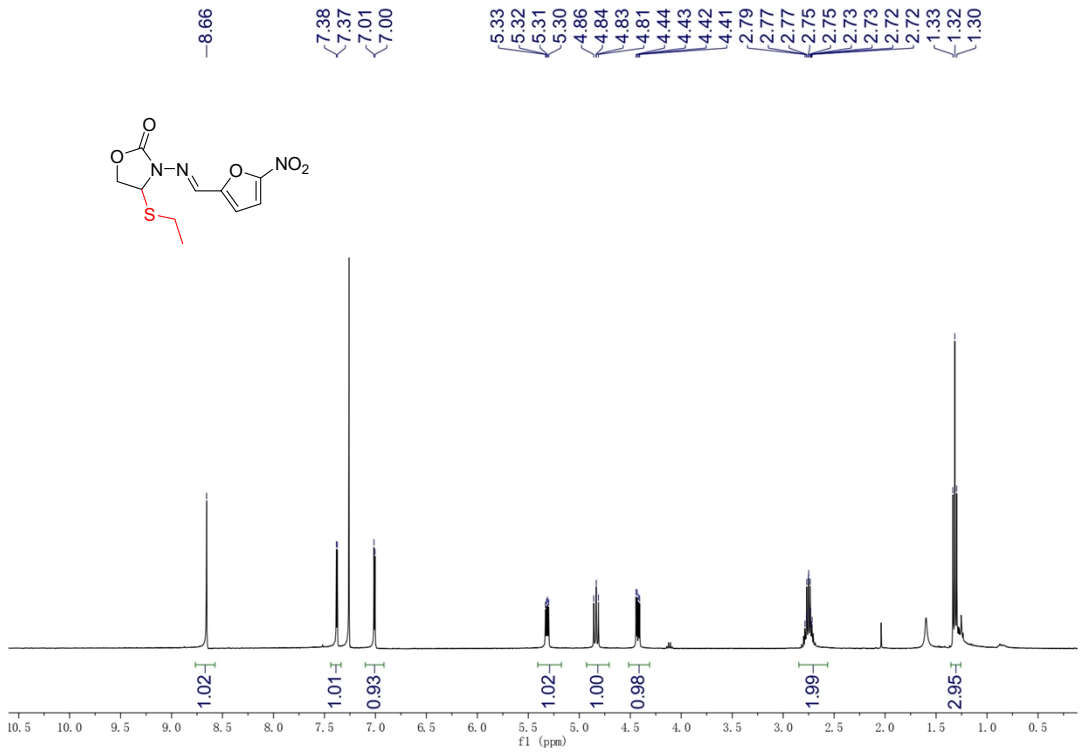
Supplementary Fig. 34. ^{1}H NMR spectrum of compound **12**.



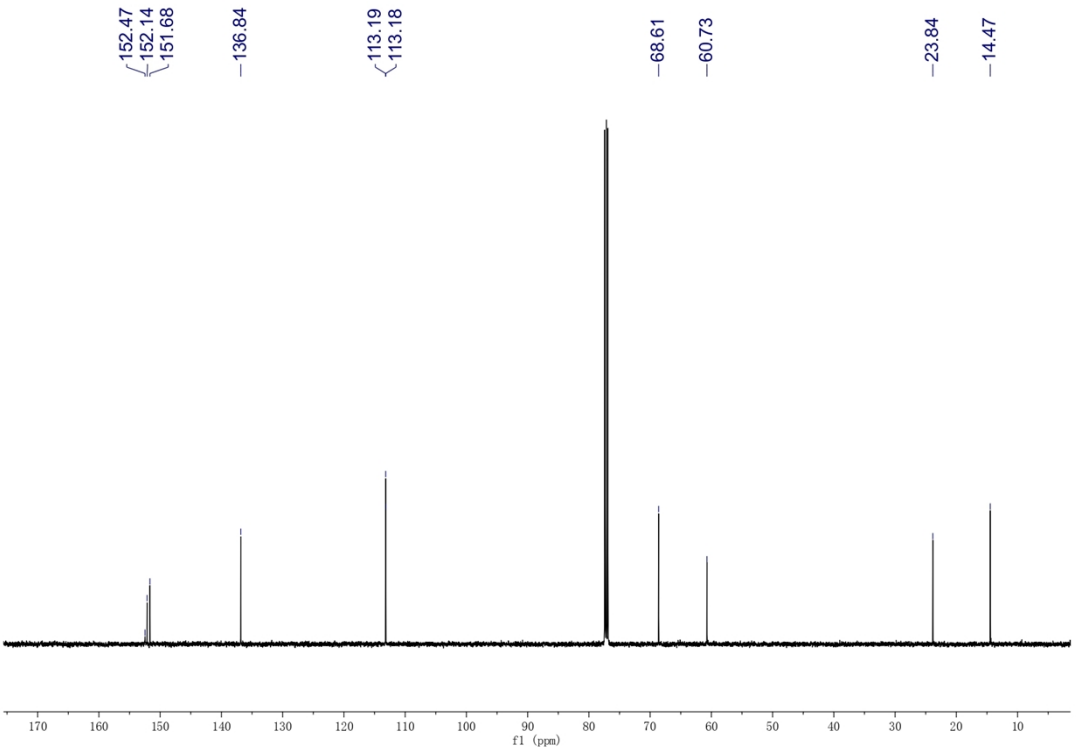
Supplementary Fig. 35. ^{13}C NMR spectrum of compound **12**.



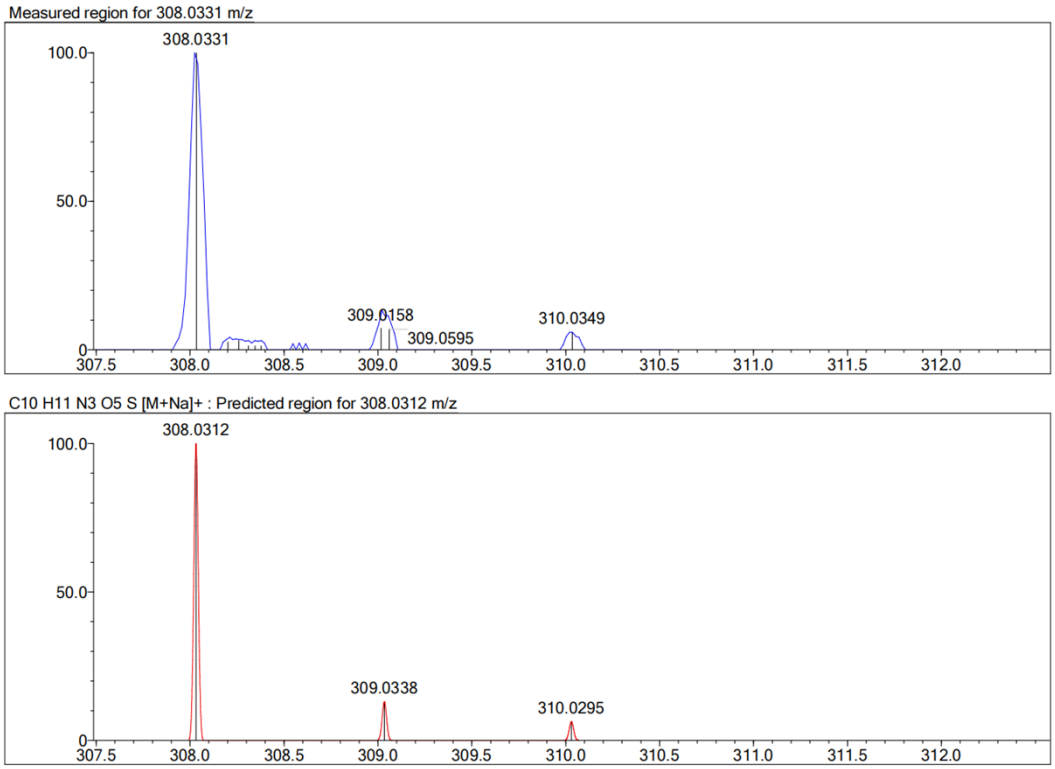
Supplementary Fig. 36. HRMS spectrum of compound **12**.



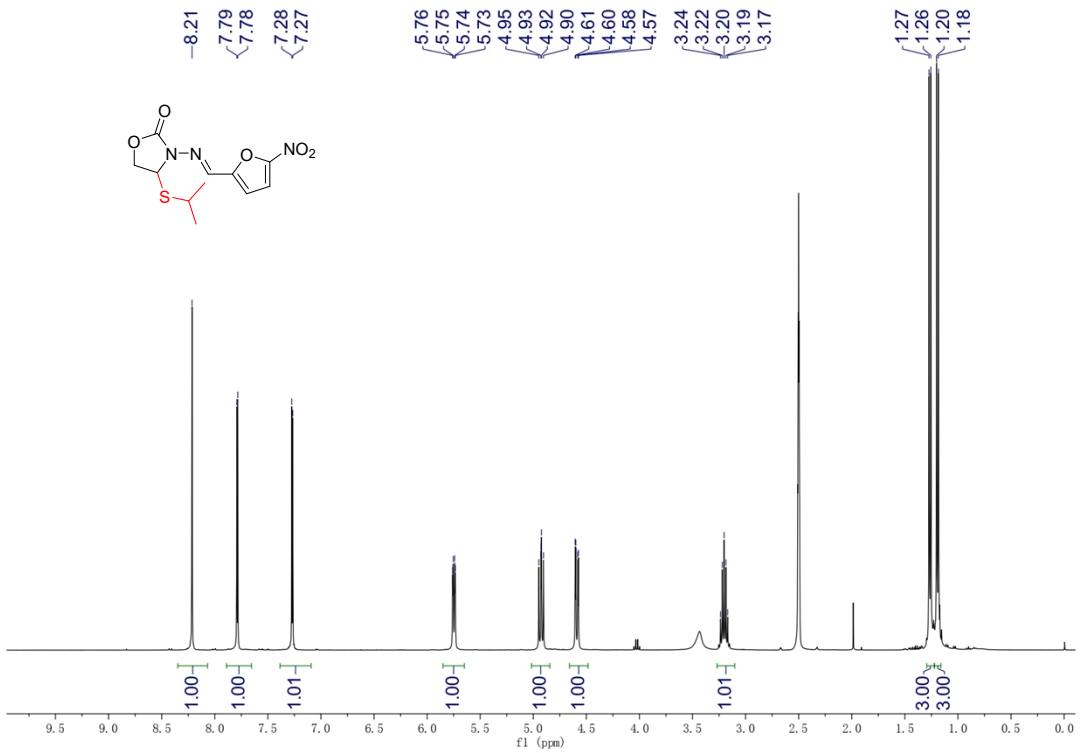
Supplementary Fig. 37. ^{1}H NMR spectrum of compound **13**.



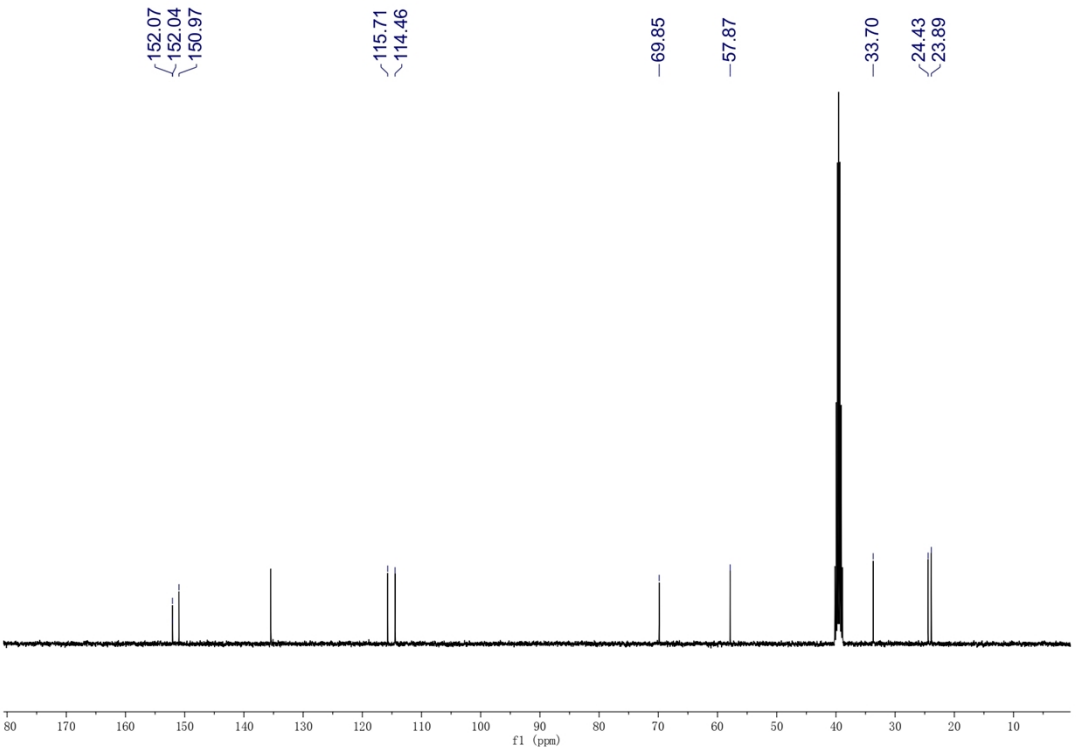
Supplementary Fig. 38.^{13}C NMR spectrum of compound **13**.



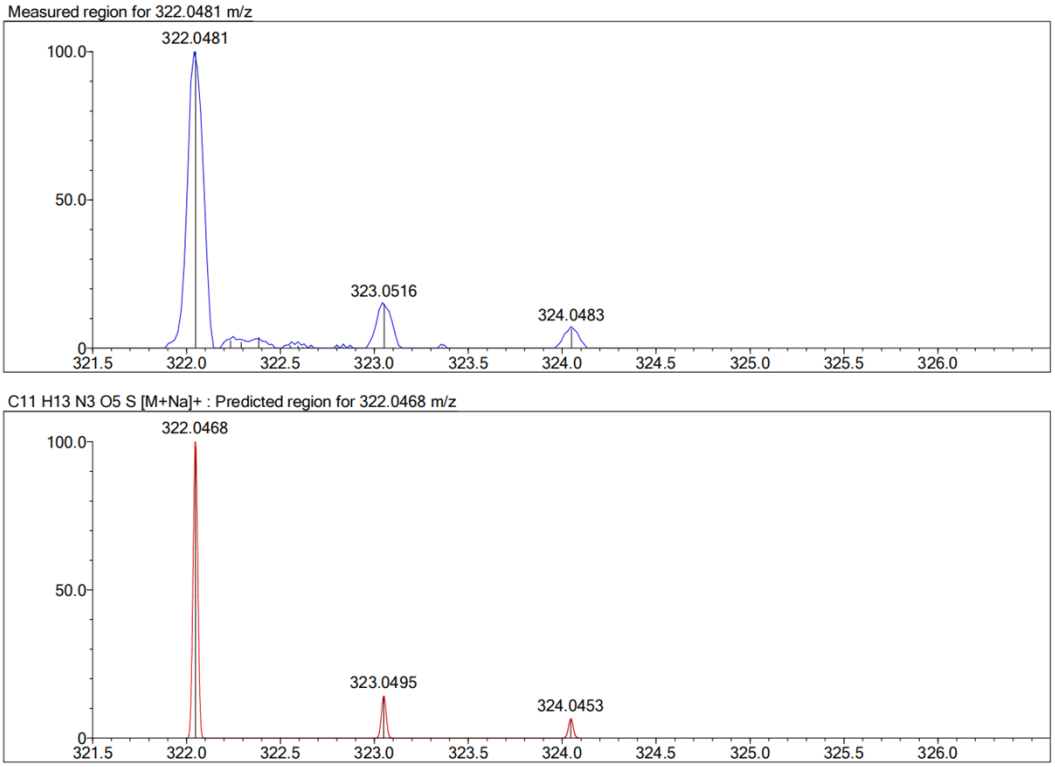
Supplementary Fig. 39. HRMS spectrum of compound **13**.



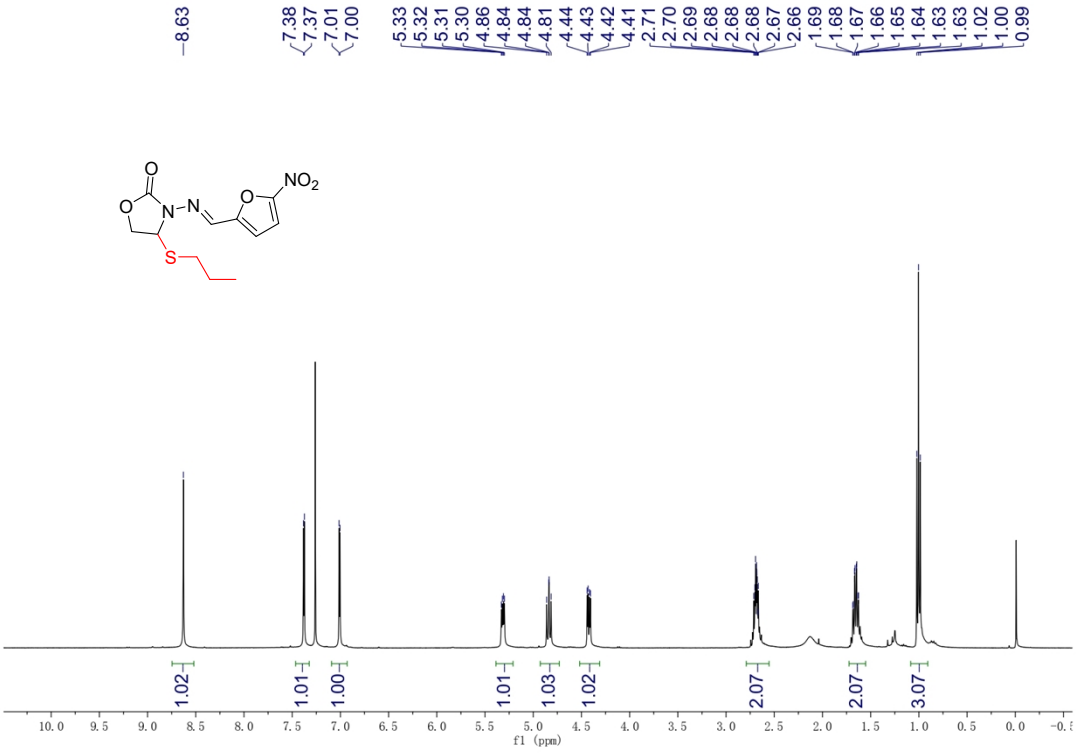
Supplementary Fig. 40. ^{1}H NMR spectrum of compound **14**.



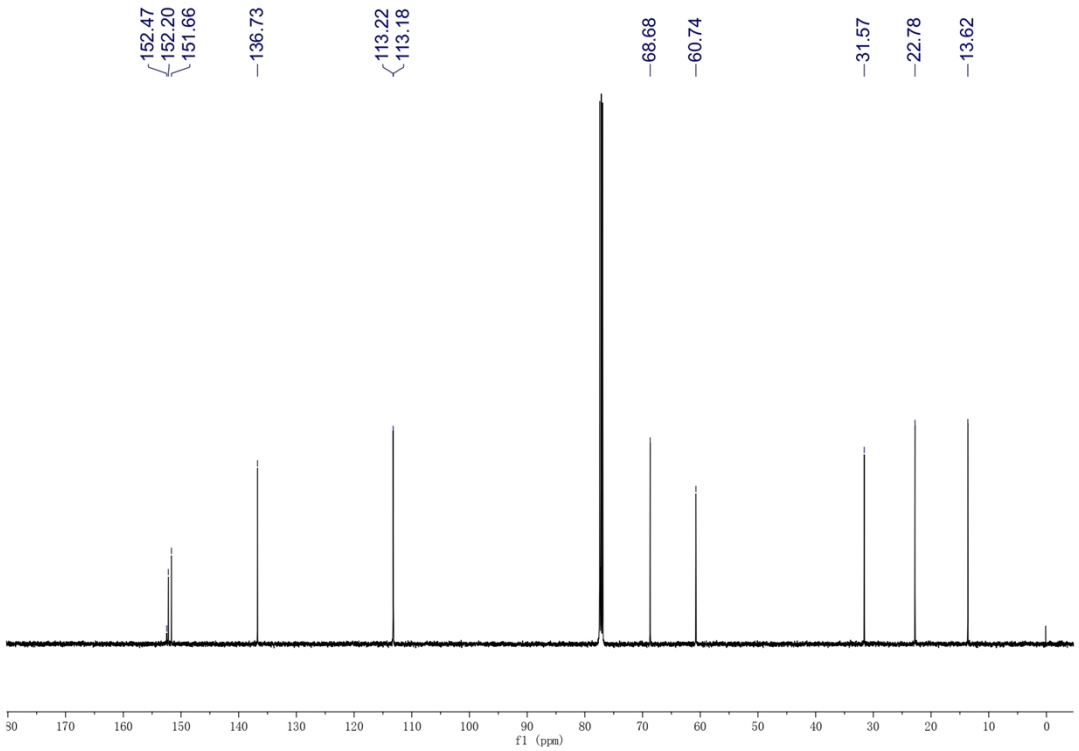
Supplementary Fig. 41.^{13}C NMR spectrum of compound **14**.



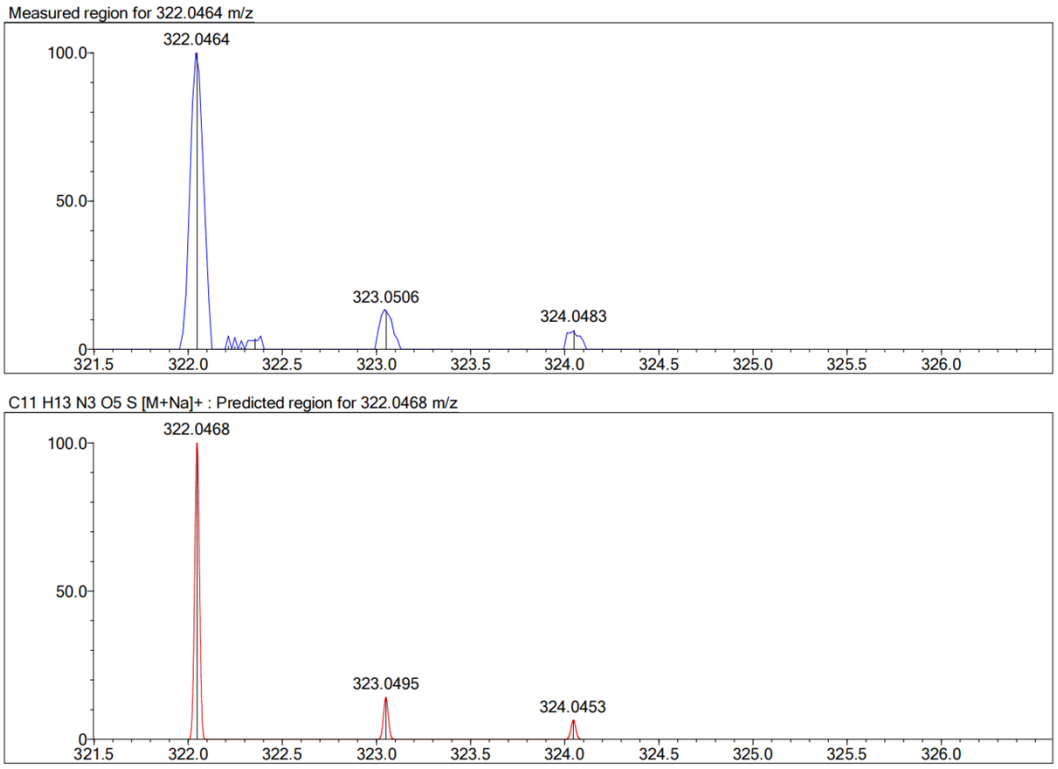
Supplementary Fig. 42. HRMS spectrum of compound **14**.



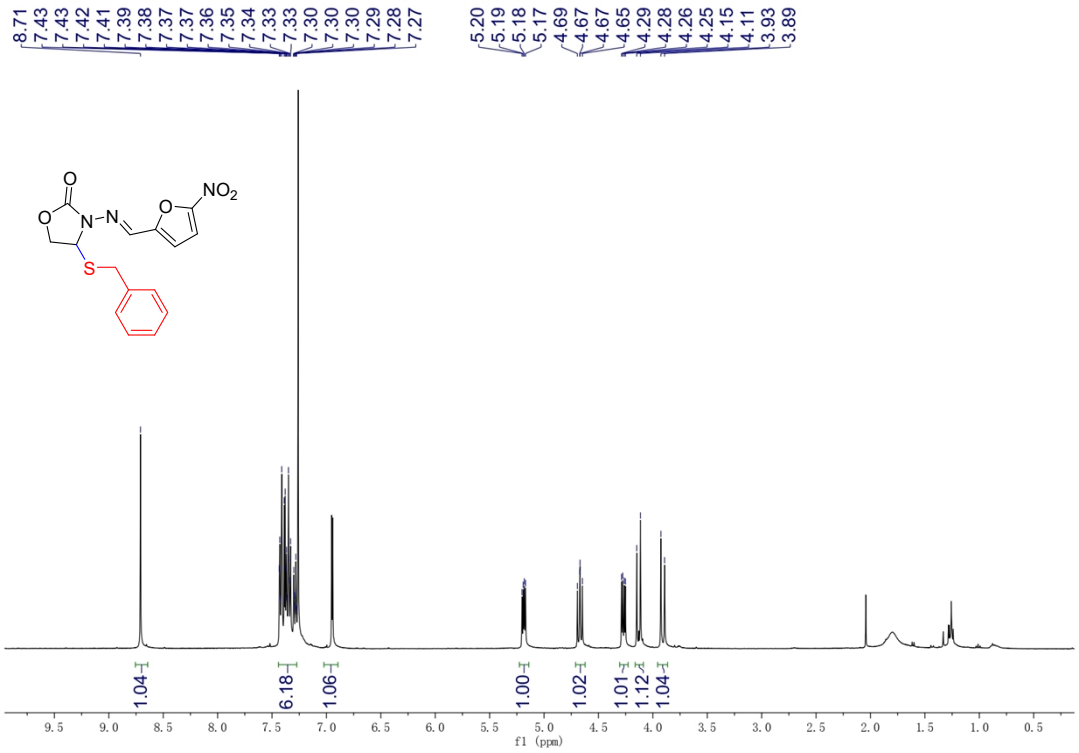
Supplementary Fig. 43. ^{1}H NMR spectrum of compound **15**.



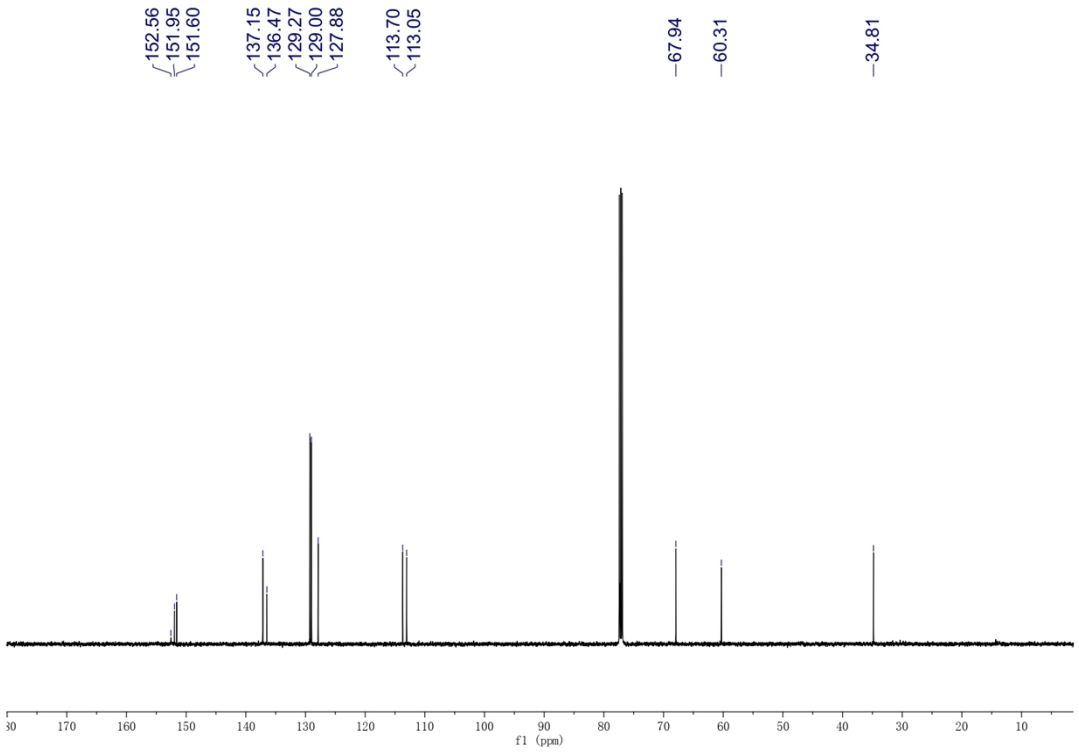
Supplementary Fig. 44.^{13}C NMR spectrum of compound **15**.



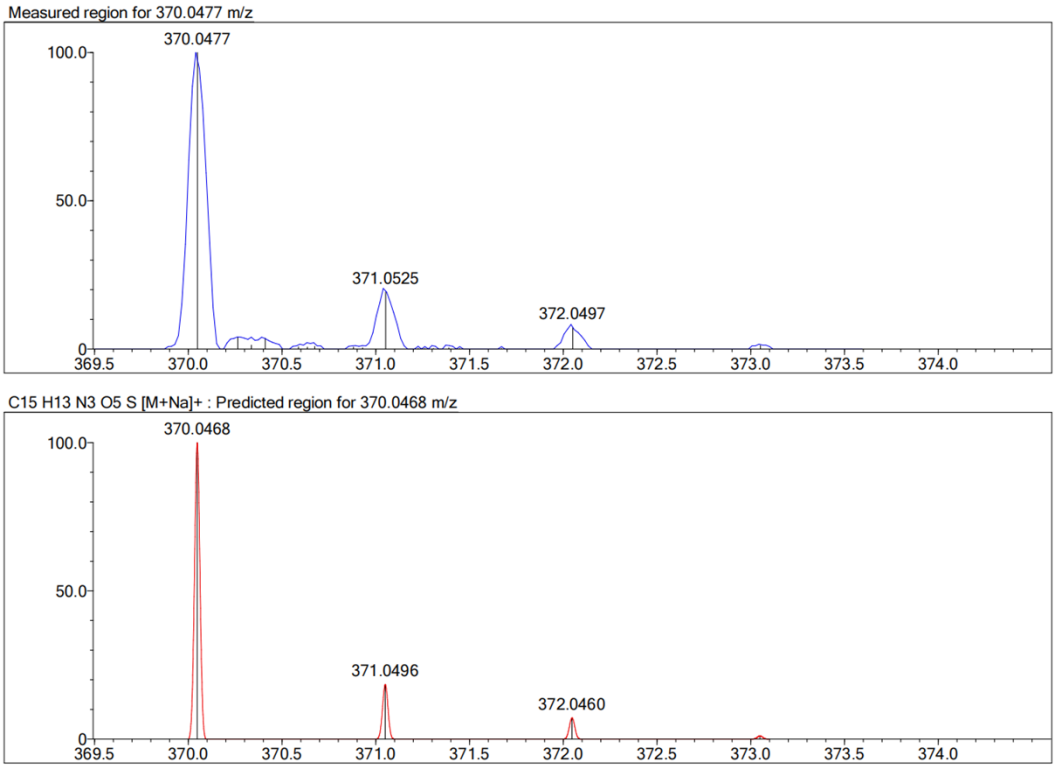
Supplementary Fig. 45. HRMS spectrum of compound **15**.



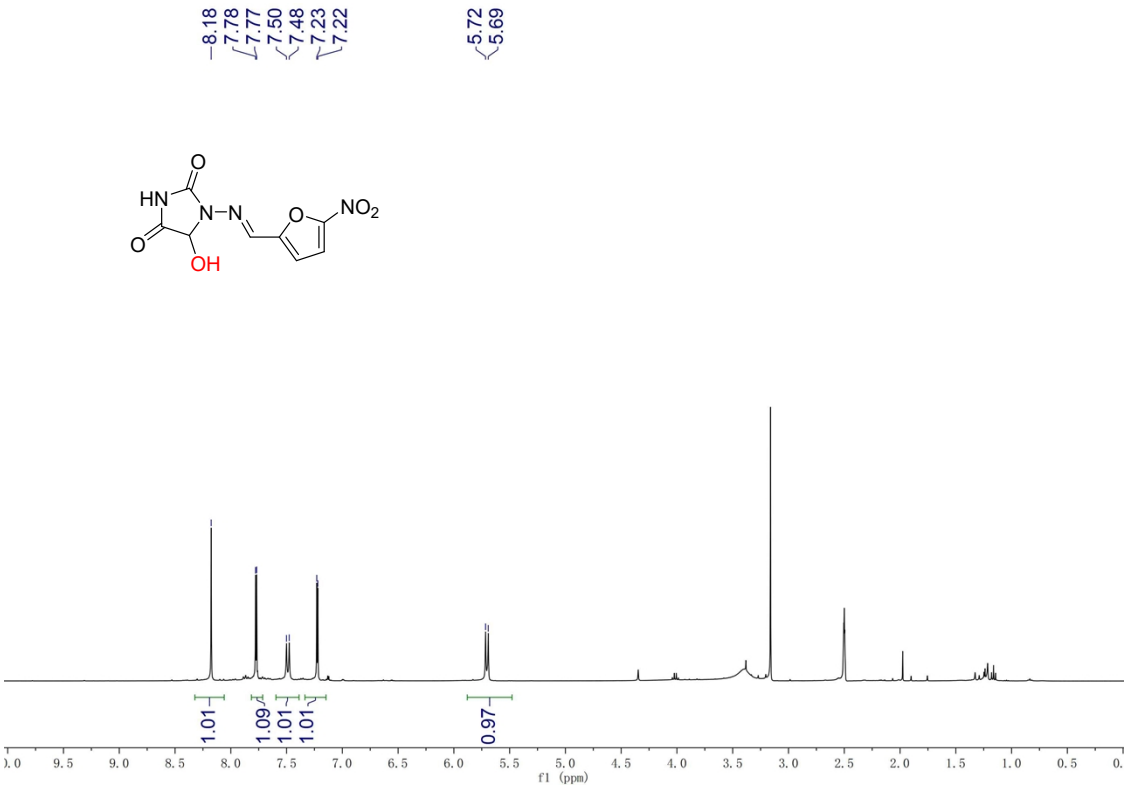
Supplementary Fig. 46. ^{1}H NMR spectrum of compound **16**.



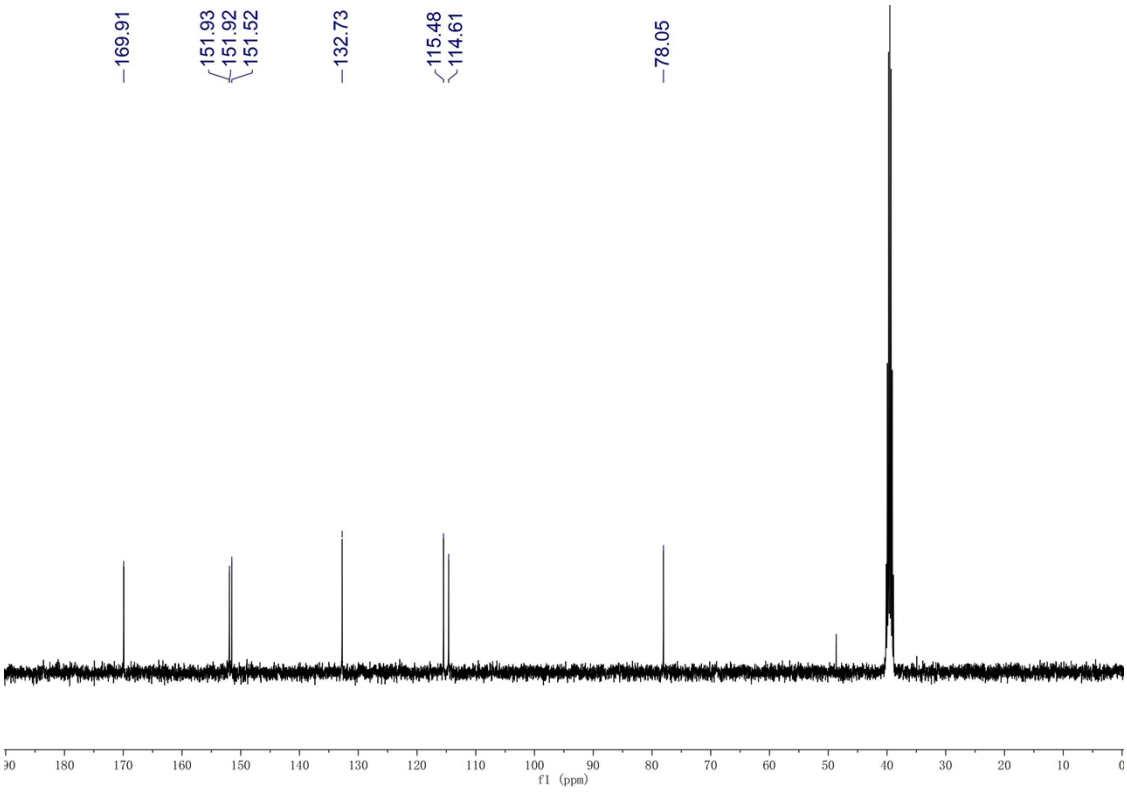
Supplementary Fig. 47.^{13}C NMR spectrum of compound **16**.



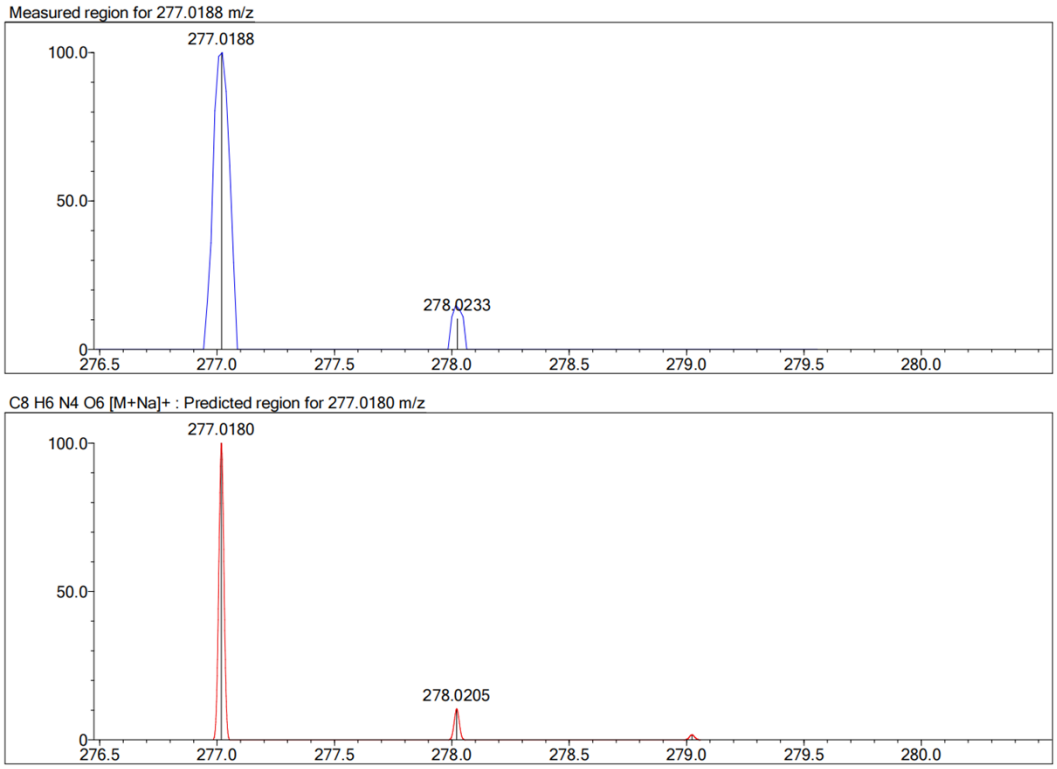
Supplementary Fig. 48. HRMS spectrum of compound **16**.



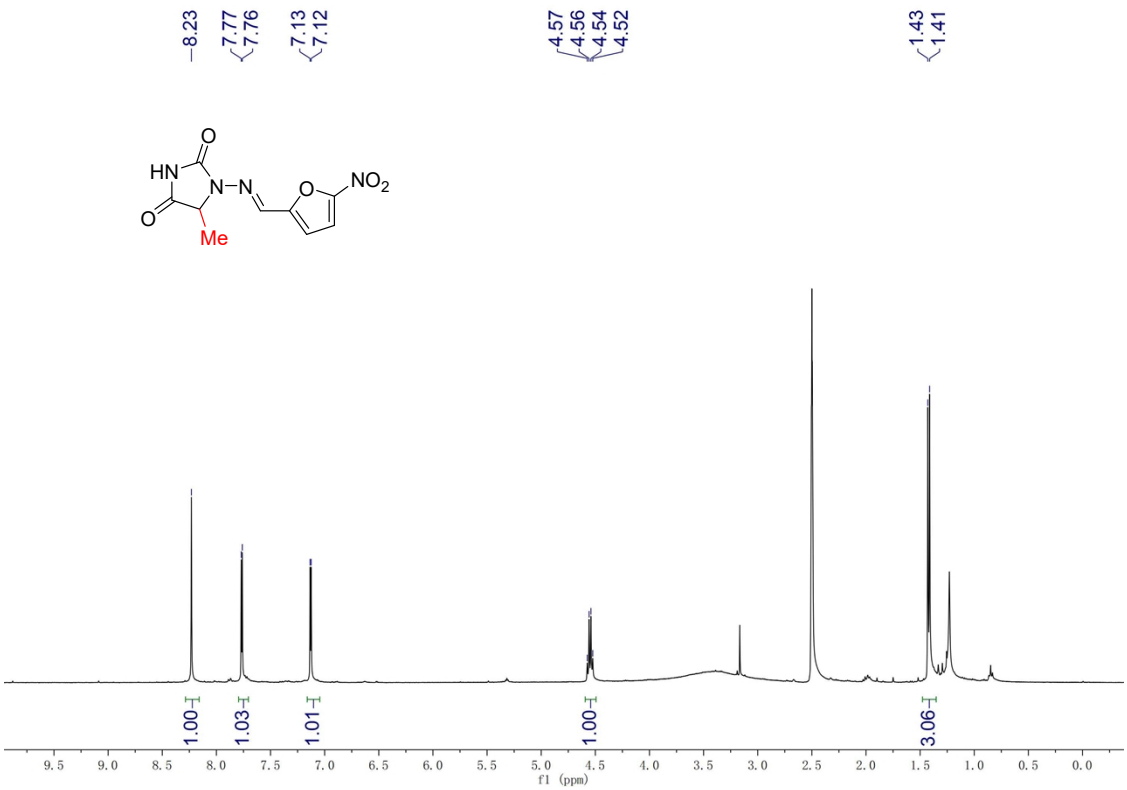
Supplementary Fig. 49. ^{1}H NMR spectrum of compound **17**.



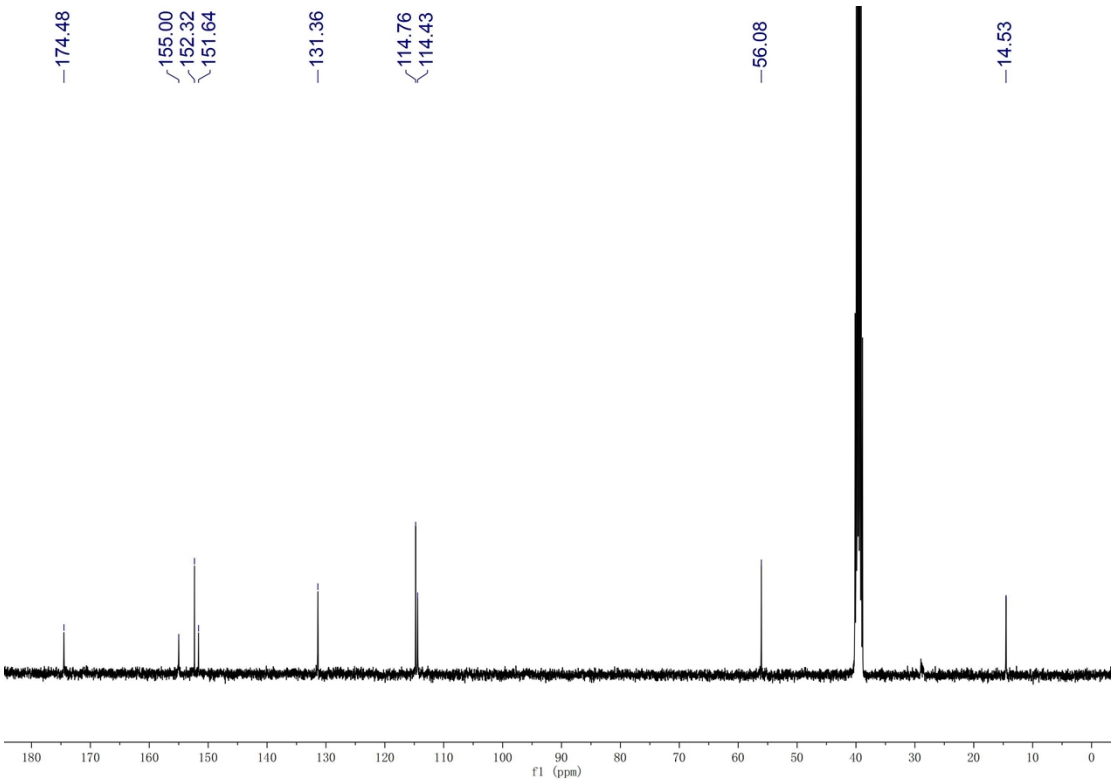
Supplementary Fig. 50. ^{13}C NMR spectrum of compound **17**.



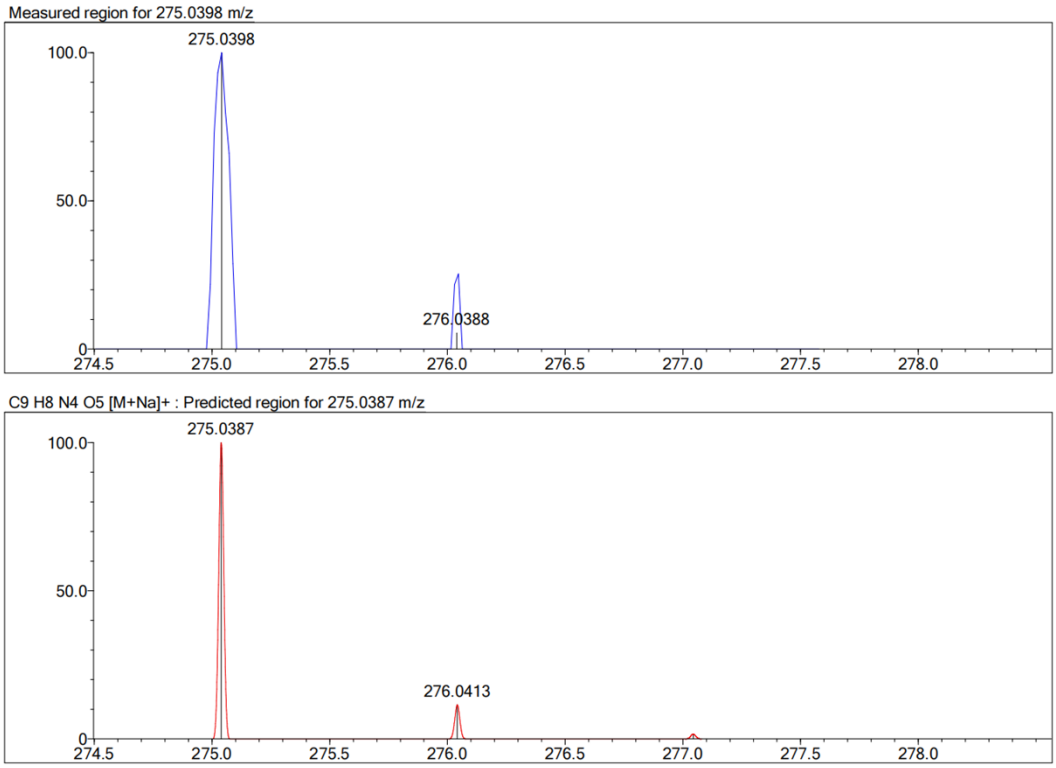
Supplementary Fig. 51. HRMS spectrum of compound **17**.



Supplementary Fig. 52. ^{1}H NMR spectrum of compound **18**.



Supplementary Fig. 53. ^{13}C NMR spectrum of compound **18**.



Supplementary Fig. 54. HRMS spectrum of compound **18**.

References

1. J. Zhou, L. Zhang, Y. He, K. Liu, F. Zhang, H. Zhang, Y. Lu, C. Yang, Z. Wang, M. S. Fareed, X. Liang, W. Yan and K. Wang, *Eur. J. Med. Chem.*, 2021, **219**, 113433.
2. X. Liang, K. Liu, P. Zhao, J. Zhou, F. Zhang, Y. He, H. Zhang, M. S. Fareed, Y. Lu, Y. Xu, Z. Zhang, W. Yan and K. Wang, *Amino acids*, 2022, **54**, 123-135.
3. P. Li, X. Chen, Y. Shen, H. Li, Y. Zou, G. Yuan, P. Hu and H. Hu, *J. Control. Release,* 2019, **300**, 52-63.
4. M. Cuenca-Estrella, A. Gomez-Lopez, A. Alastruey-Izquierdo, L. Bernal-Martinez, I. Cuesta, M. J. Buitrago and J. L. Rodriguez-Tudela, *J. Clin. Microbiol.*, 2010, **48**, 1782-1786.