Supporting Information for

Discovery of cytochrome bc_1 complex inhibitors inspired by the natural product Karrikinolide

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Contents:

1. The synthesis and characterization of the reported compounds:

1.1 The synthesis and characterization of 5 and 6.

To a stirred solution of compound **1** (18.0 g, 120 mmol) in acetone (600 mL) was added slowly anhydrous $CuSO_4$ (40.0 g, 250 mmol), followed by dropwise addition of concentrated H_2SO_4 (2 ml). The reaction mixture was filtered through celite and concentrated to afford a yellow oil after being stirred at room temperature for 12 h. Then 0.2% HCl aqueous solution (400 mL) was added, and the solution was stirred at room temperature for 15 h. Afterwards, the resulted mixture was netrualized by saturated $NaHCO_3$ (400 mL), extracted with EtOAc (2 x 400 mL), dried over $MgSO_4$, concentrated under reduced pressure to give crude **5** as a yellow oil.

Et_3N (25.0 mL, 180 mmol) was added to a solution of crude **5** in dry CH_2Cl_2 (180 mL), and the mixture was stirred at room temperature for 1 h. Then a solution of triphenylmethyl chloride (50.2 g, 180 mmol) in CH_2Cl_2 (180 mL) was added dropwisely, and the resulted solution was stirred at room temperature for 3 h. Afterwards, the solvents was evaporated and water (400 mL) was added. The mixture was extracted with Et_2O (3 x 400 mL), and the organic layer was washed with water (3 x 400 mL), brine (400 mL), dried over $MgSO_4$, and concentrated. The crude mixture was purified by column chromotography to afford **6** as a white solid (44.0 g, 85% in two steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47-7.43 (m, 6H), 7.34-7.29 (m, 6H), 7.27-7.24 (m, 3H), 6.01 (d, *J* = 3.6 Hz, 1H), 4.53 (d, *J* = 3.6 Hz, 1H), 4.27 (s, 2H), 3.57 (dd, *J* = 10.2, 5.1 Hz, 1H), 3.47 (dd, *J* = 10.2, 2.7 Hz, 1H), 3.20 (s, 1H), 1.49 (s, 3H), 1.33 (s, 3H). The ^{1}H NMR data is in accordance with a previous publication.[1]

1.2 The synthesis and characterization of 7.

Acetic anhydride (37.8 mL, 400 mmol) was added to a solution of **6** (43.2 g, 100 mmol) in anhydrous dimethyl sulfoxide (200 ml). After 22 h at room temperature, the reaction mixture was poured into an aqueous solution of 10% $NaHCO_3$ (1 L). This mixture was stirred for 1 h and then extracted with CH_2Cl_2 (4 x 500 ml). The

combined organic layers were washed with water (5 x 500 ml), dried over $MgSO_4$, and evaporated. The residue was purified by column chromotography to afford **7** as a white solid (32.2 g, 75%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42-7.20 (m, 15H), 6.33 (d, *J* = 4.5 Hz, 1H), 4.55 (d, *J* = 4.4 Hz, 1H), 4.41 (s, 1H), 3.50 (dd, *J* = 10.1, 2.3 Hz, 1H), 3.31 (dd, *J* = 10.1, 2.4 Hz, 1H), 1.47 (s, 6H). The ^{1}H NMR data is in accordance with a previous publication.[1]

1.3 The synthesis and characterization of 8.

Triethyl phosphonoacetate (33.2 g, 148 mmol) was added dropwise to NaH (5.92 g, 148 mmol, 60% dispersion in mineral oil) in THF (150 mL) at -10 °C, and the mixture stirred for 0.5 h. Then **7** (31.8 g, 74 mmol)] in THF (150 mL) was added dropwisely and the red solution stirred for 1 h. The mixture was concentrated and purified by column chromotography to afford **8** as a pale yellow oil (27.4 g, 74%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47-7.22 (m, 15H), 6.05 (d, *J* = 4.1 Hz, 1H), 5.74 (d, *J* = 4.0 Hz, 1H), 5.72 (d, *J* = 1.5 Hz, 1H), 4.94 (s, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.38 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.22 (dd, *J* = 10.0, 4.0 Hz, 1H), 1.50 (s, 3H), 1.45 (s, 3H), 1.29 (t, *J* = 7.1 Hz, 3H). The ^{1}H NMR data is in accordance with the literature.[2]

1.4 The synthesis and characterization of 9.

Trifluoroacetic acid/H_2O (270 mL, 4:1) was added to **8** (27.0 g, 54.0 mmol) in CH_2Cl_2 (150 mL) and the yellow solution was stirred at room temperature for 30 min. The solvent was removed, and H_2O (300 mL) was added. Then the aqueous solution was washed with EtOAc (3 x 300 mL), concentrated and purified by column chromotography to afford **9** as a white solid (7.43 g, 80%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.00 (s, 1H), 5.88 (s, 1H), 5.44 (d, *J* = 4.1 Hz, 1H), 4.96 (d, *J* = 4.0 Hz, 1H), 4.55-4.47 (m, 1H), 3.76 (dd, *J* = 10.0, 7.5 Hz, 1H), 3.45-3.42 (m, 1H). The ^{1}H NMR data is in accordance with a previous publication.[2]

1.5 The synthesis and characterization of 10 and 11.

Acetic anhydride (15.1 mL, 160.0 mmol) was added to **9** (6.88 g, 40.0 mmol) in pyridine (160 mL) at 0 °C and the mixture was stirred at room temperature for 2 h. Then 1 M HCl solution was added slowly to the reaction mixture at 0 °C, and EtOAc (300 mL) was added. The organic layer was washed with water (5 x 300 mL), brine (300 mL), dried over $MgSO_4$, and concentrated to afford crude **10** as a yellow oil.

Triethylamine (60.0 mL, 44 mmol) was added to crude **10** in CH_2Cl_2 (200 mL) and the solution was stirred at room temperature for 30 min. Concentration of the mixture and flash column chromatography gave compound **11** as a pale yellow oil (7.13 g, 91 % in two steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.08 (d, *J* = 1.2 Hz, 1H), 5.94 (s, 1H), 5.85 (t, *J* = 3.7 Hz, 1H), 4.34 (dd, *J* = 12.6, 4.1 Hz, 1H), 4.20 (dd, *J* = 12.5, 3.3 Hz, 1H), 2.14 (s, 3H). The ^{1}H NMR data is in accordance with a previous publication.[2]

1.6 The synthesis of 2.

$Pd(PPh_3)_4$ (8.32 g, 7.20 mmol) was added to **11** (7.06 g, 36.0 mmol) in THF (300 mL) and the solution was heated at reflux for 48 h. Concentration of the mixture and flash column chromatography gave compound **2** as a white solid (3.92 g, 80 %).

1.7 The synthesis of 3.

Phosphoryl chloride (1.40 mL, 15.0 mmol) was added dropwise to **2** (0.136 g, 1.00 mmol) in DMF (3 mL) and the solution stirred at 50 °C for 15 min. The cooled solution was diluted with CH_2Cl_2 (10 mL), poured into saturated aqueous $NaHCO_3$ (10 mL) and the mixture was stirred for 15 min. The mixture was extracted with CH_2Cl_2 (3 x 10 mL), the combined organic layers were dried over $MgSO_4$, filtered and concentrated. Flash column chromatography gave compound **3** as a white solid (0.148 g, 90%).

1.8 The synthesis of Karrikinolide.

Aluminium(III) chloride (0.30 g, 2.25 mmol) was added to *t*$BuNH_2 \cdot BH_3$ (0.40 g, 4.5 mmol) and **3** (0.75 mmol) in CH_2Cl_2 (15 mL) and the mixture was heated to reflux and stirred for 20 min. Additional $AlCl_3$ (100 mg, 0.75 mmol) was added periodically

(every 10 min) until the reaction was complete (as monitored by TLC). The mixture was cooled to 0 °C, and 1 M HCl (25 mL) was added dropwise with stirring. The mixture was extracted with CH_2Cl_2 (3 x 25 mL), the combined organic layers were dried over $MgSO_4$, filtered and concentrated. Flash chromatography gave the natural product **Karrikinolide** as a white solid (91.1 mg, 81%).

1.9 The synthesis of 4.

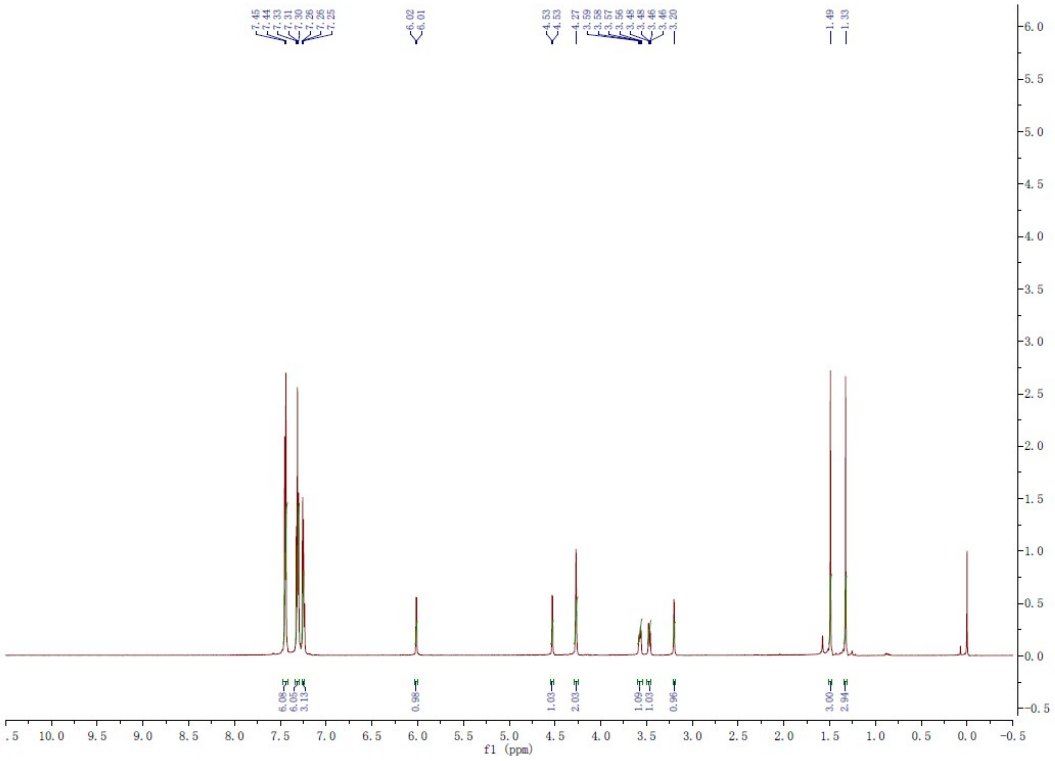
To a stirred solution of **2** (3.67 g, 27.0 mmol) in MeOH (250 mL) was added dropwisely *N*-bromosuccinimide (NBS, 2.54 mL, 30.0 mmol), and the reaction mixture was stirred at room temperature for 20 min. The solvent was removed under reduced pressure, and water (200 mL) was added. Then the mixture was extracted with EtOAc (3 x 200 mL), the combined organic layers were dried over $MgSO_4$, concentrated, and purified by flash column chromatography to afford compound **4** as a yellow solid (4.35 g, 75%).

1.10 References

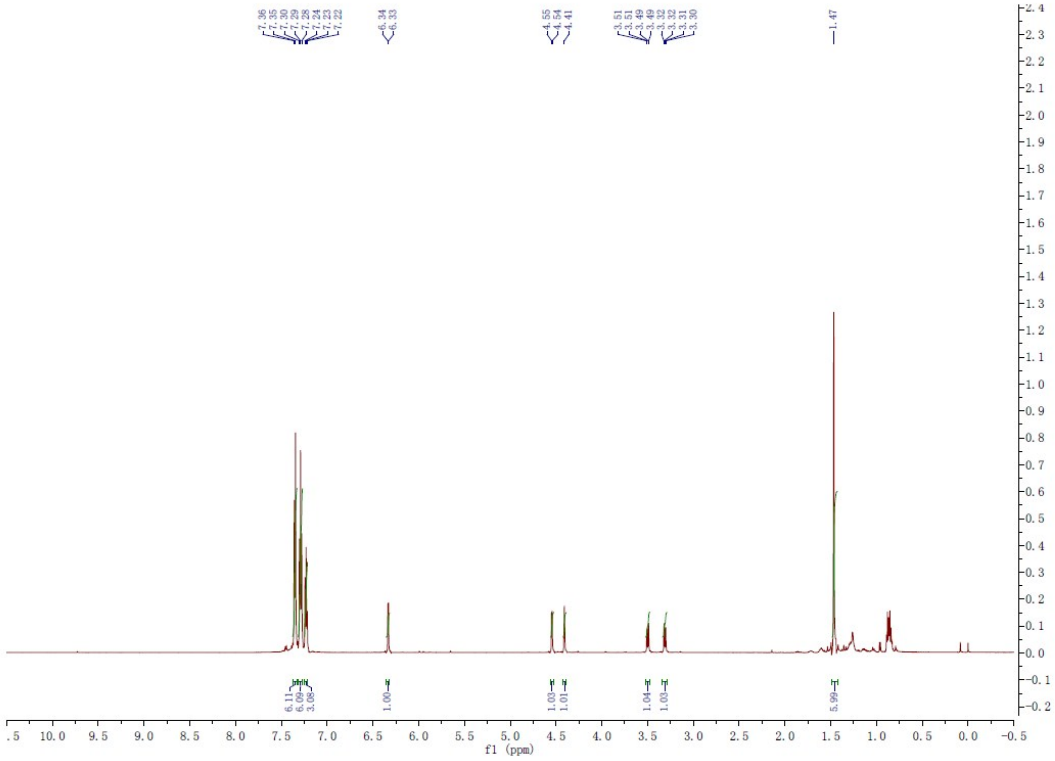
1. W. Sowa, *Can. J. Chem.*, 1968, **46**, 1586-1589.
2. E. D. Goddard‐Borger, E. L. Ghisalberti and R. V. Stick, *Eur. J. Org. Chem.*, 2007, 3925-3934.

2. The original spectra for the reported compounds:

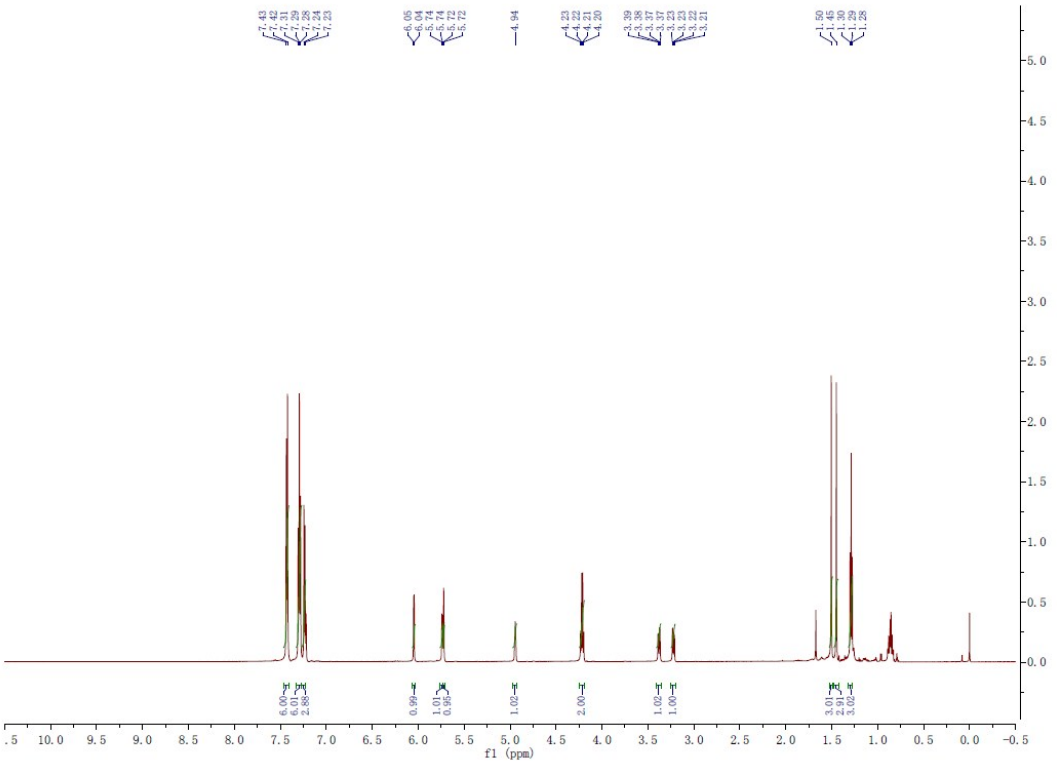
- ^{1}H NMR spectrum for compound **6**



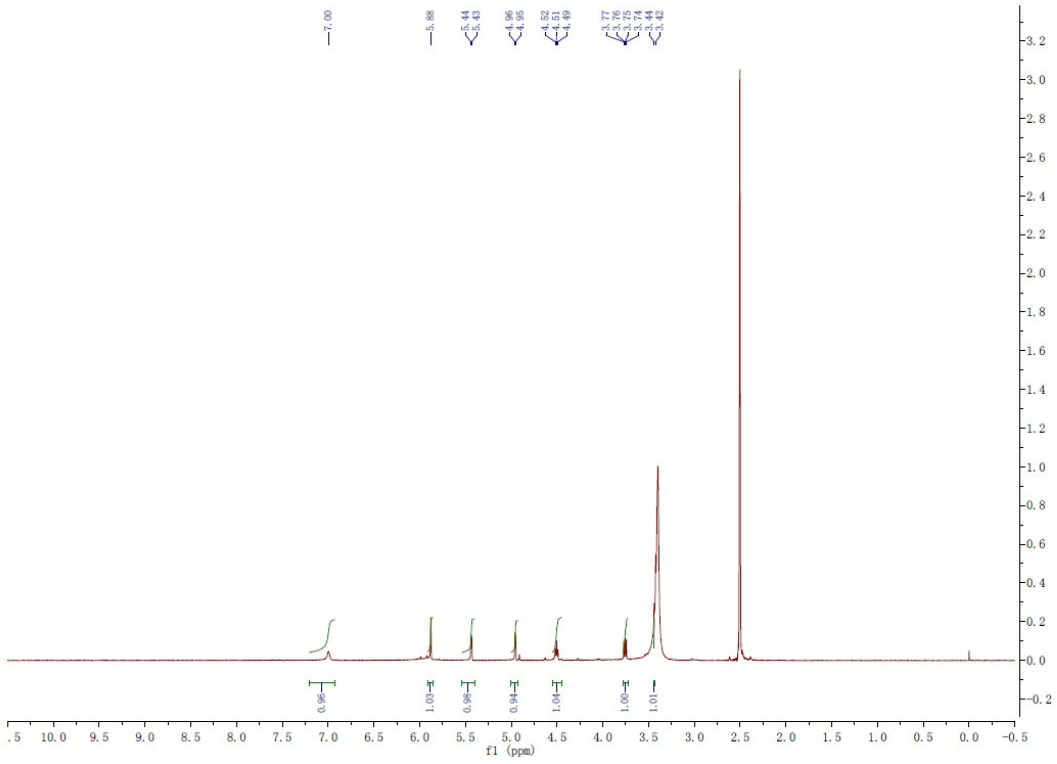
- ^{1}H NMR spectrum for compound **7**



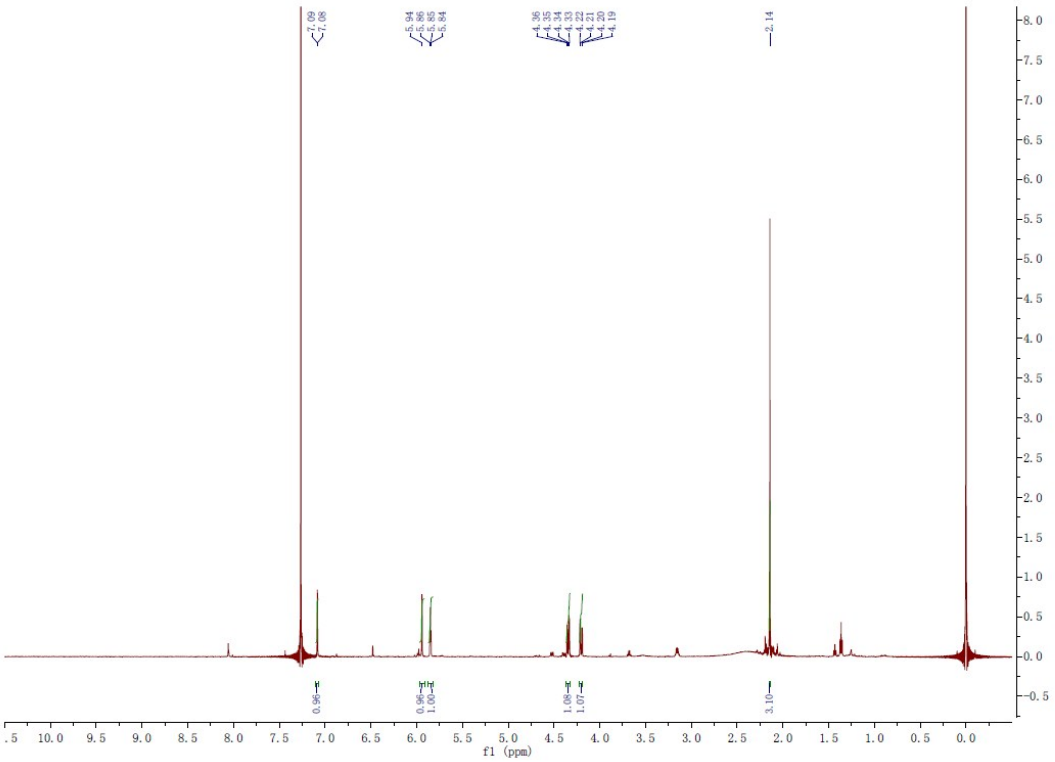
- ^{1}H NMR spectrum for compound **8**



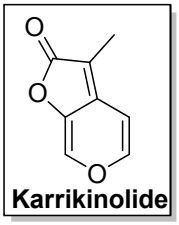
- ^{1}H NMR spectrum for compound **9**



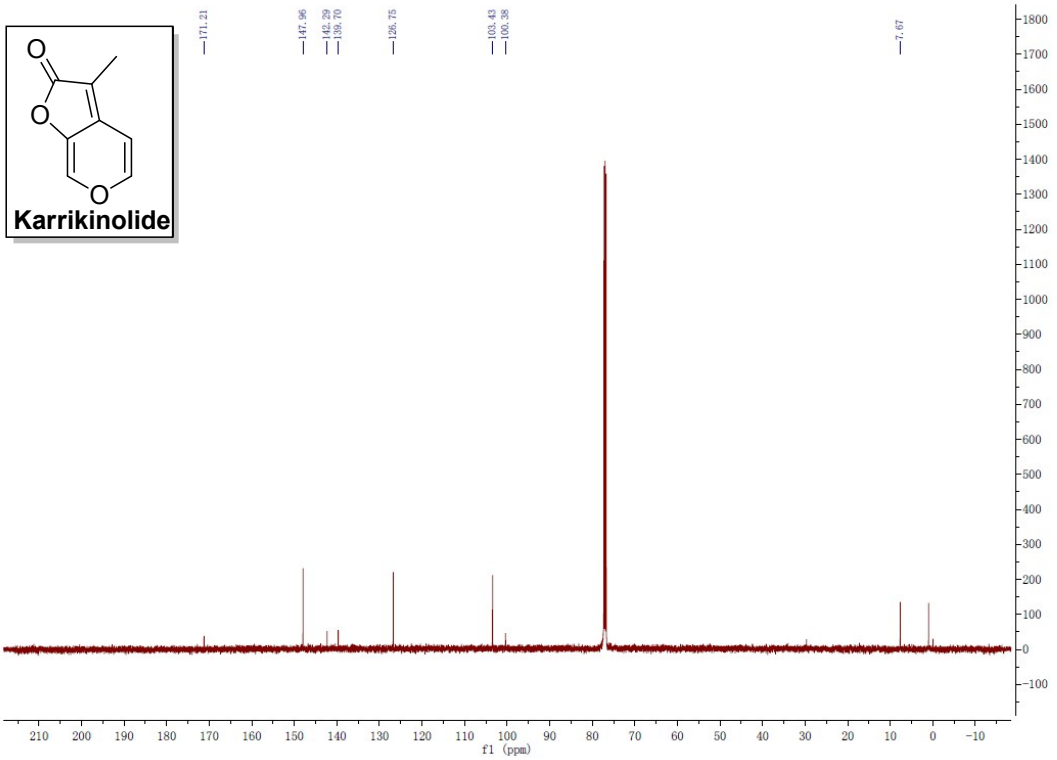
- ^{1}H NMR spectrum for compound **11**



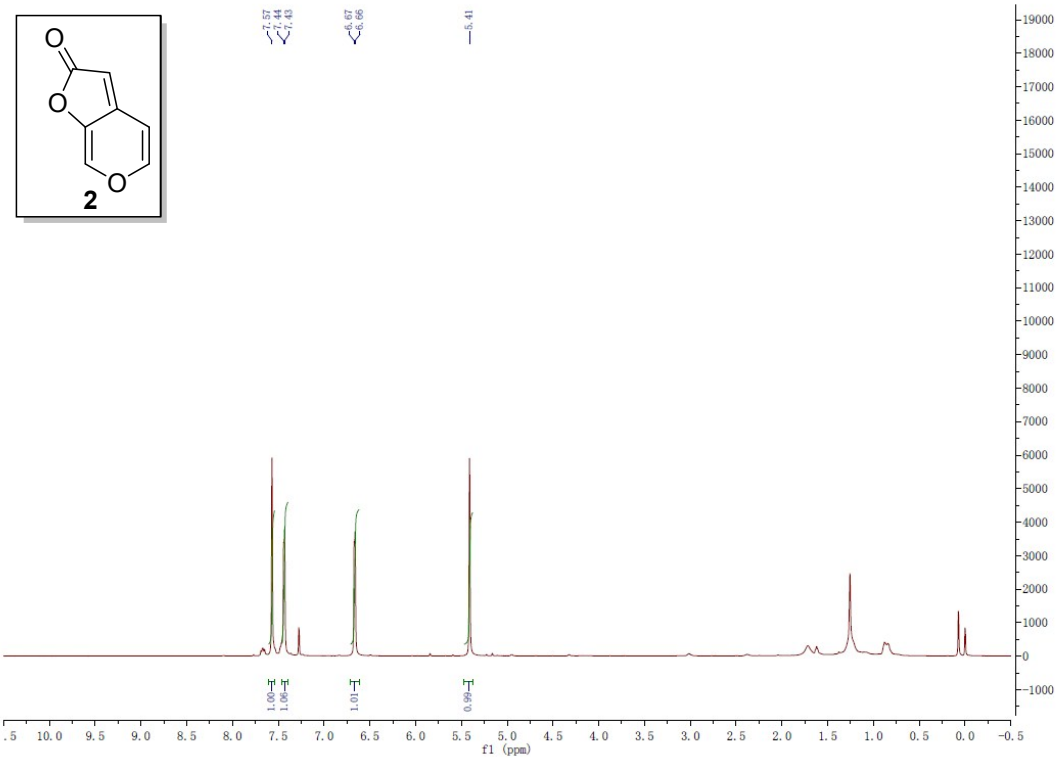
- ^{1}H NMR spectrum for **Karrikinolide**



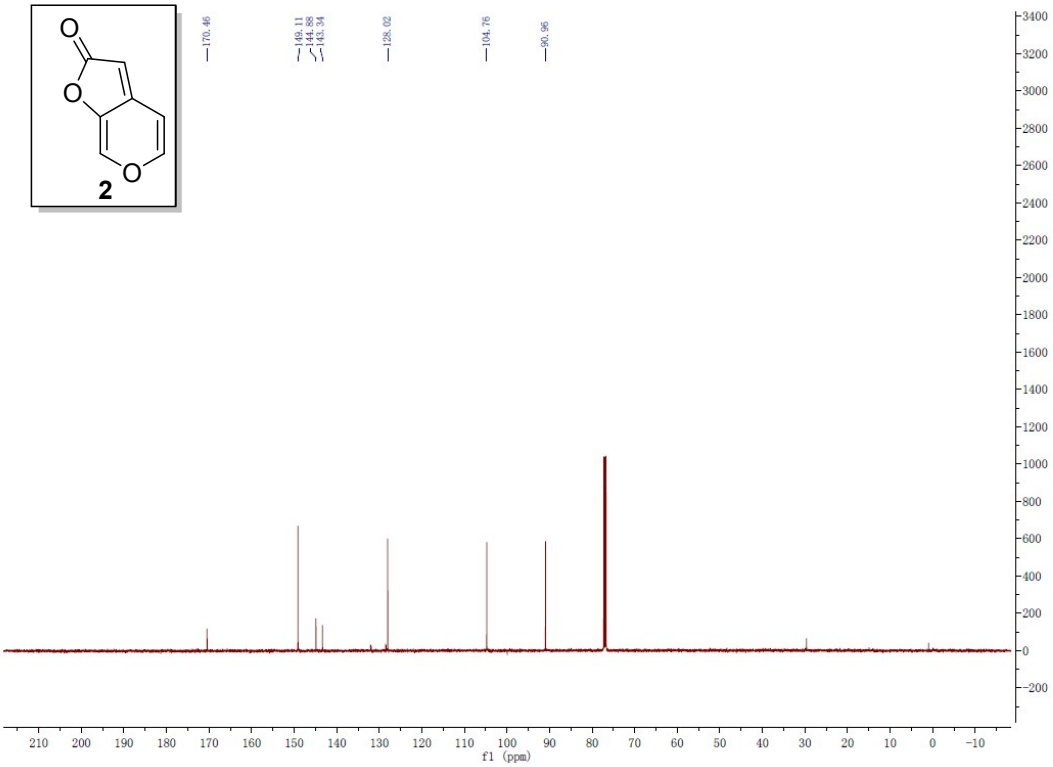
- ^{13}C NMR spectrum for **Karrikinolide**



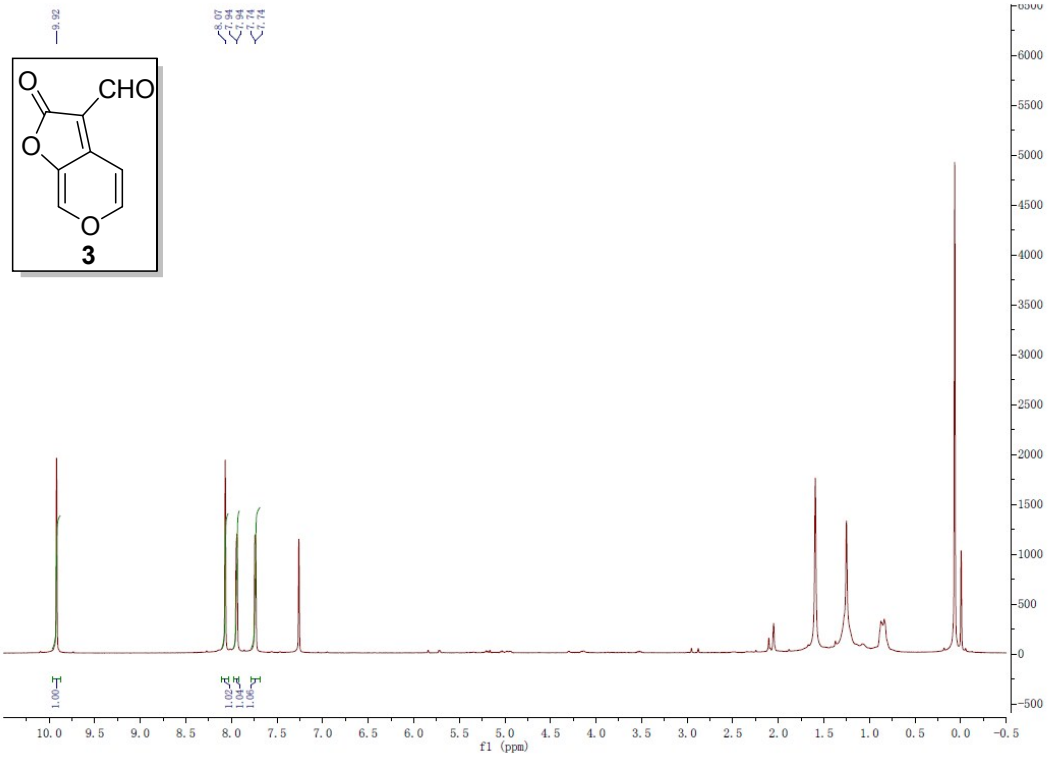
- ^{1}H NMR spectrum for compound **2**



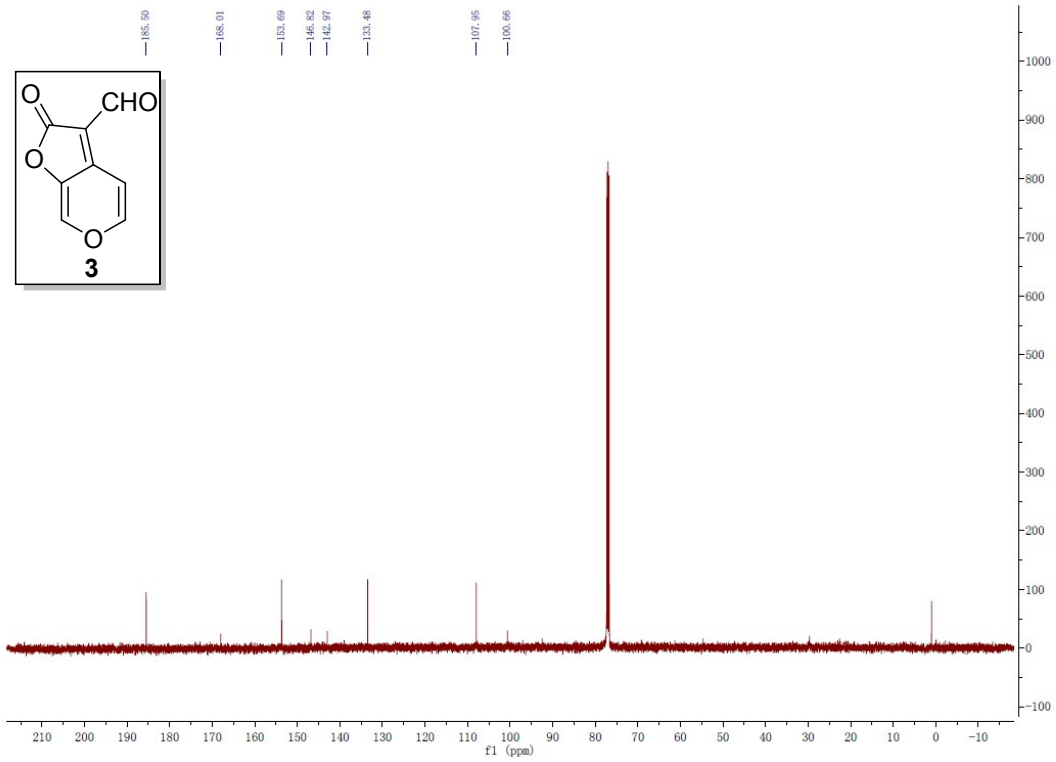
➢ ^{13}C NMR spectrum for compound **2**



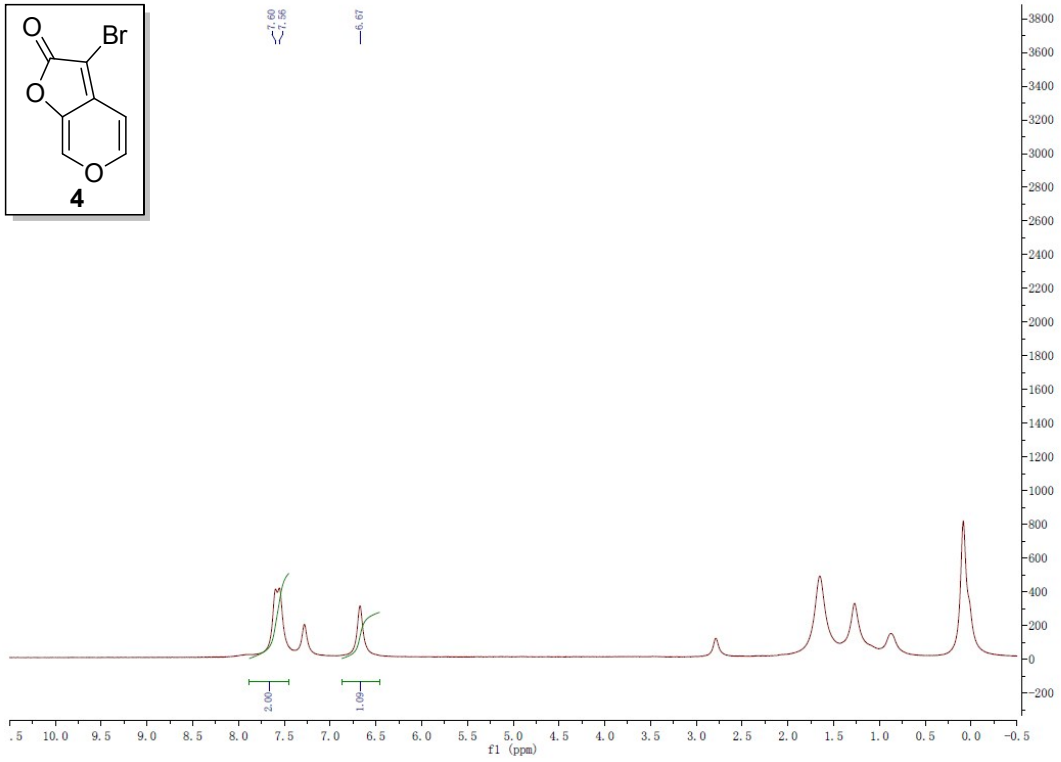
➢ ^{1}H NMR spectrum for compound **3**



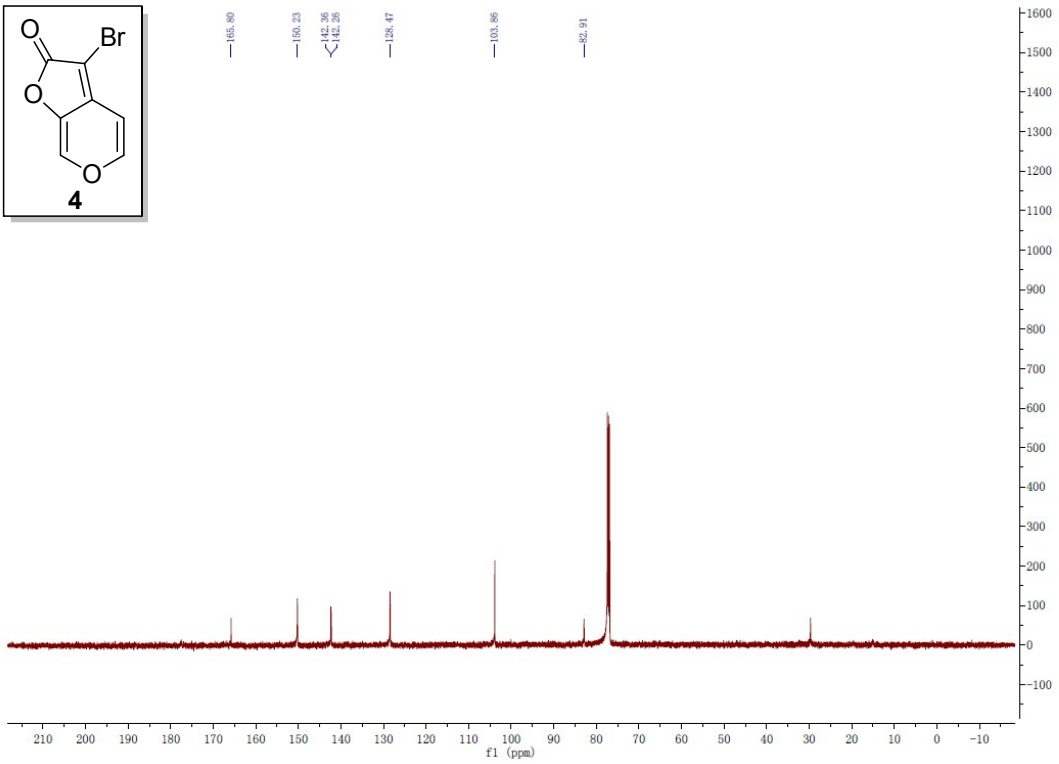
➢ ^{13}C NMR spectrum for compound **3**



➢ ^{1}H NMR spectrum for compound **4**

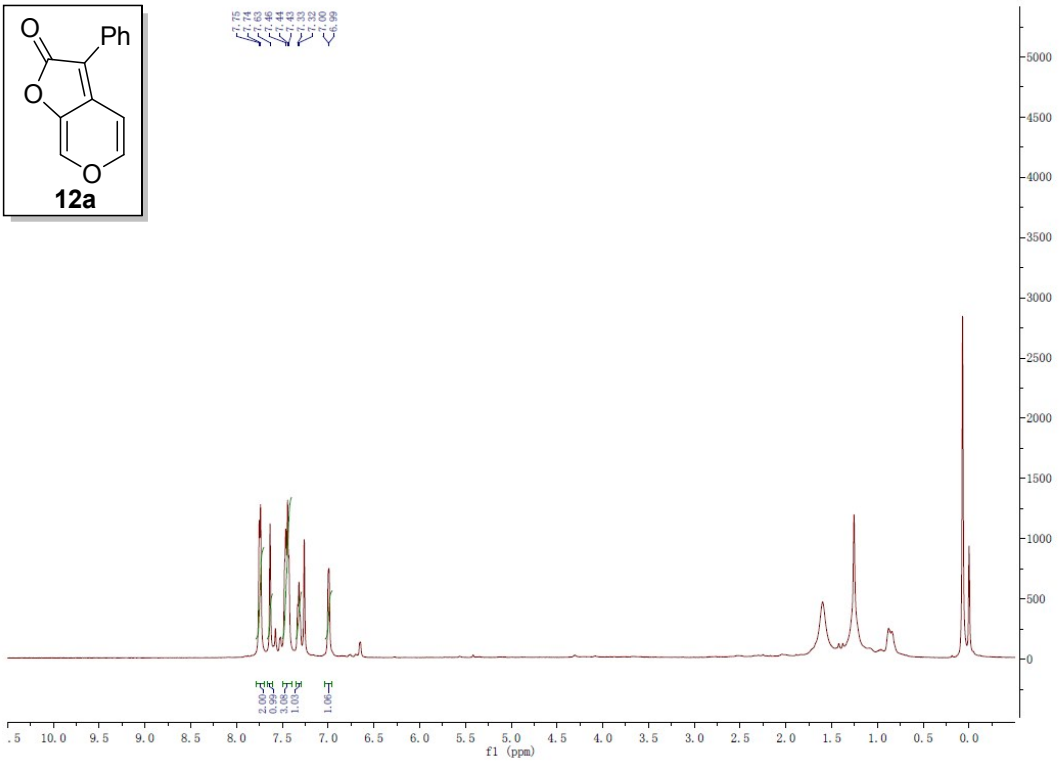


➢ ^{13}C NMR spectrum for compound **4**

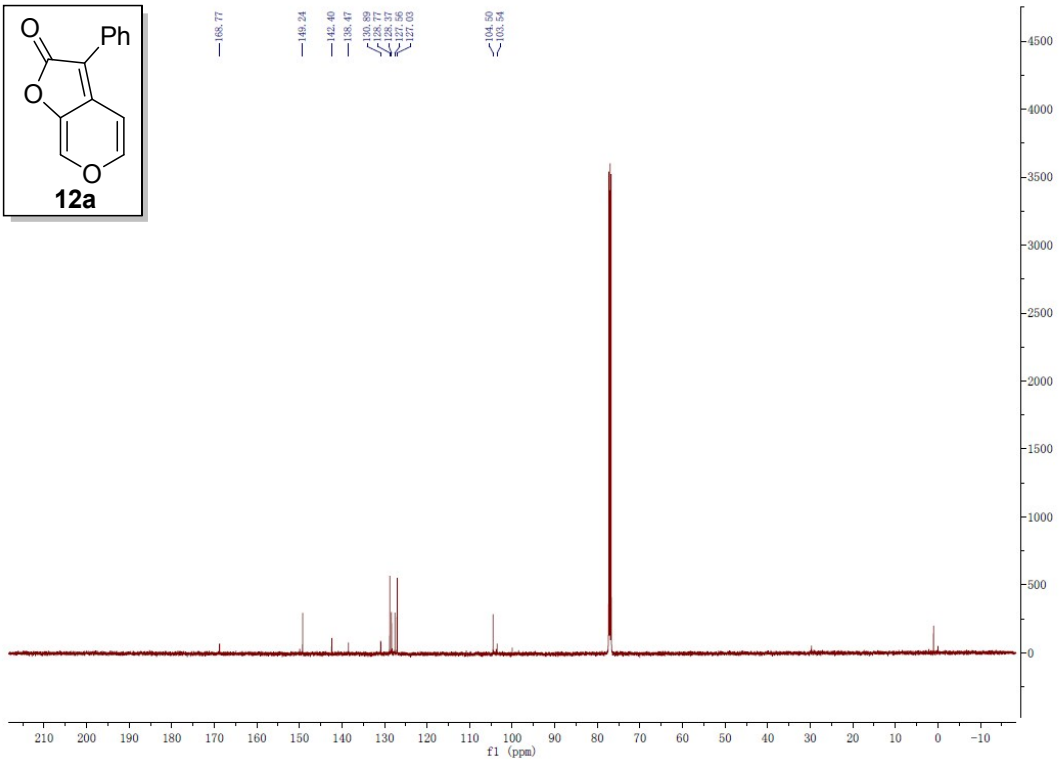


3. The original ^{1}H, ^{13}C NMR, MS and HR-MS spectra for compounds 12a-12q:

- ^{1}H NMR spectrum for **12a**

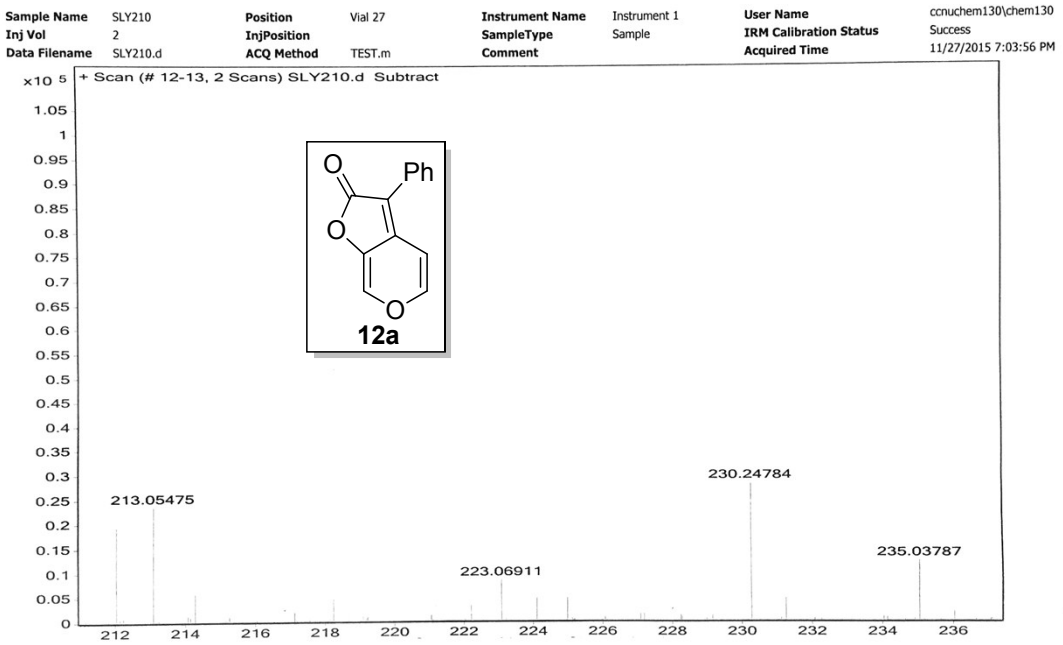


- ^{13}C NMR spectrum for **12a**

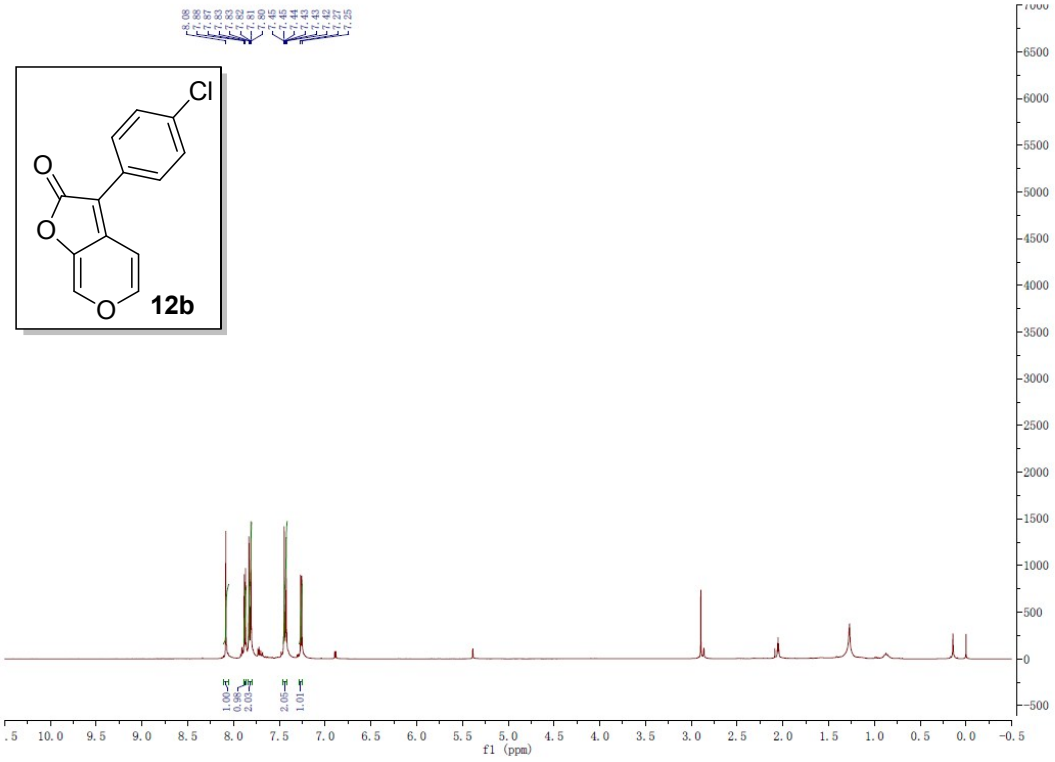


- HRMS (ESI): m/z calcd. for $C_{13}H_9O_3$ $[M+H]^+$: 213.05462; Found 213.05475.

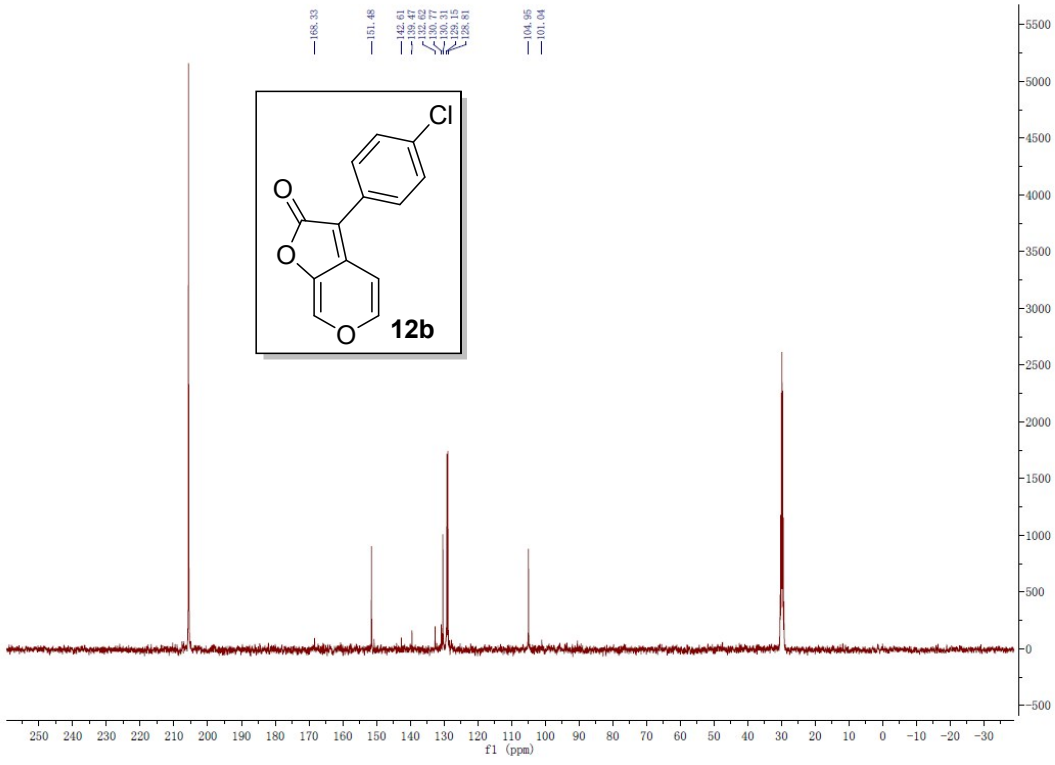
- HRMS (ESI) spectrum for **12a**



➢ ^{1}H NMR spectrum for **12b**

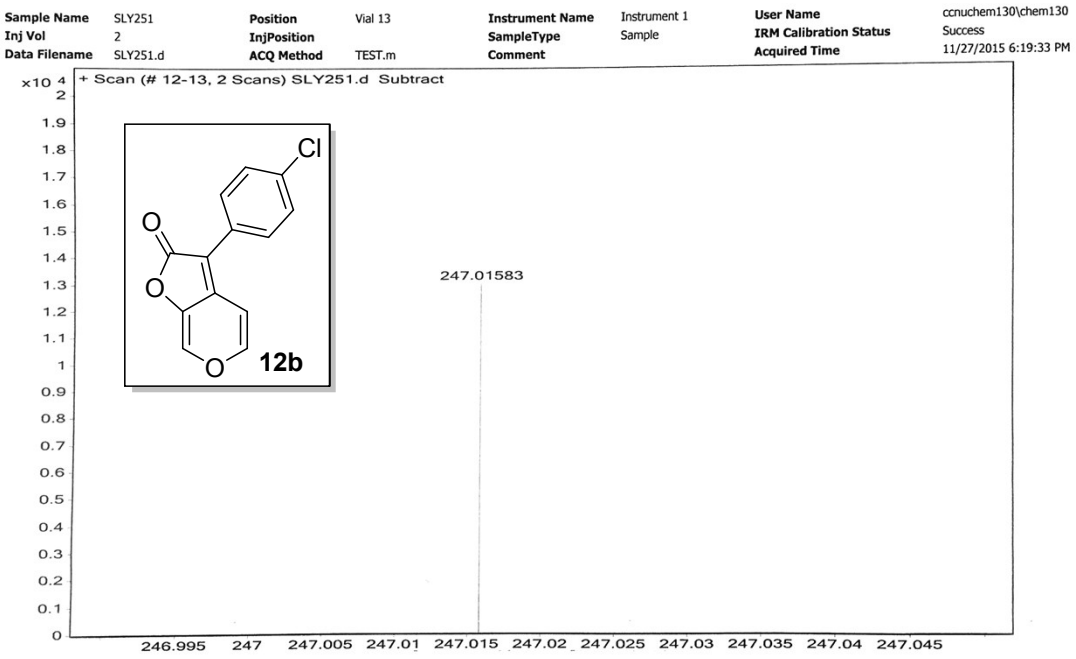


➢ ^{13}C NMR spectrum for **12b**

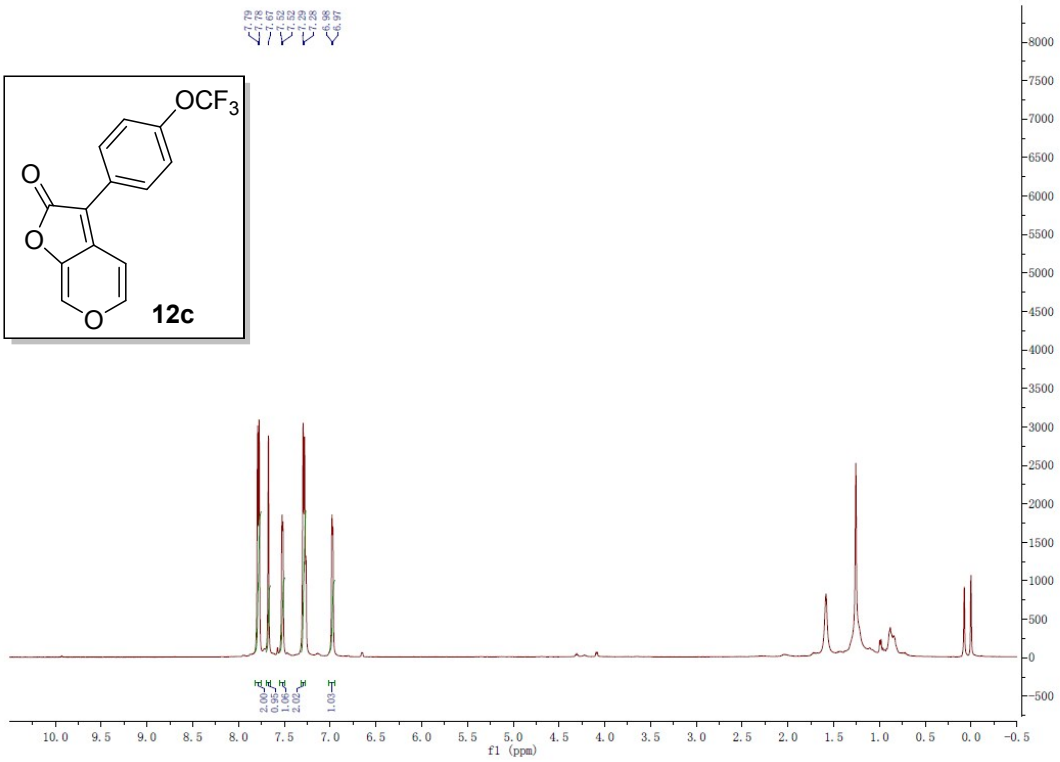


- HRMS (ESI): m/z calcd. for $C_{13}H_8ClO_3$ $[M+H]^+$: 247.01565; Found 247.01583.

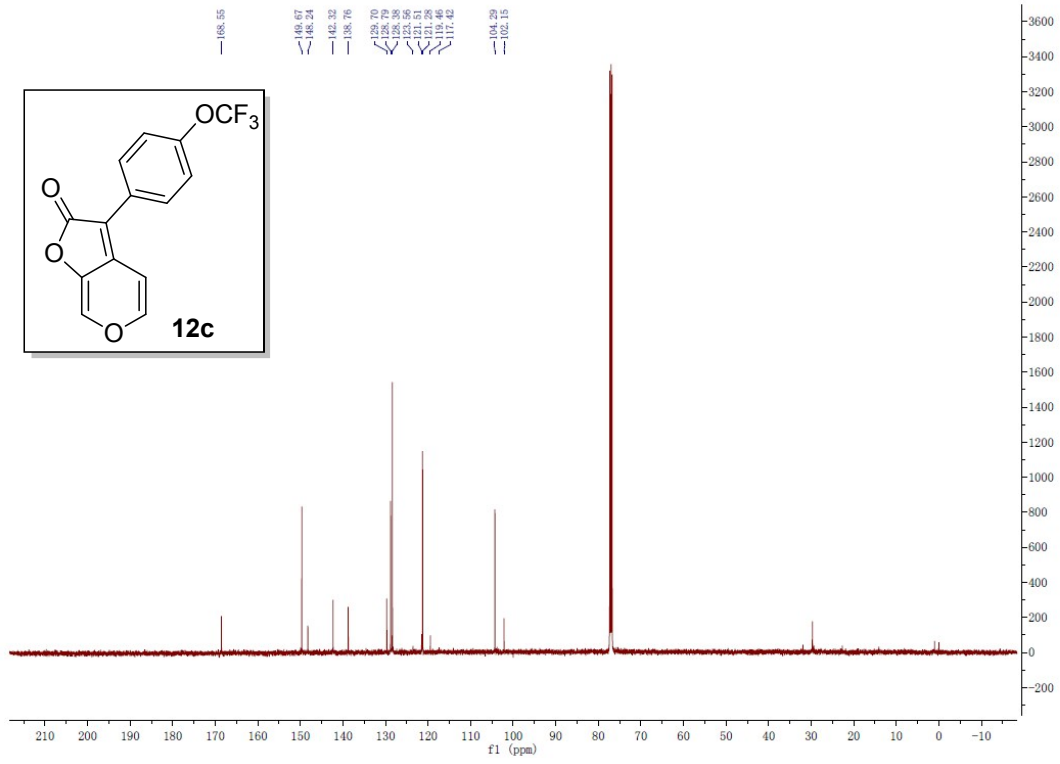
- HRMS (ESI) spectrum for **12b**



➢ ^{1}H NMR spectrum for **12c**

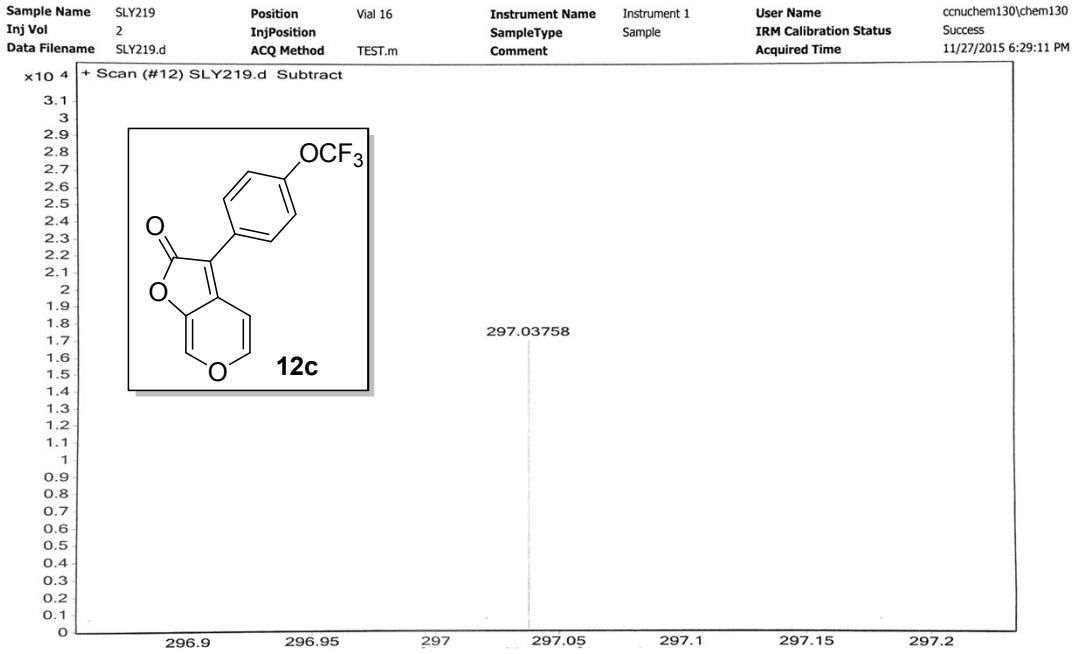


➢ ^{13}C NMR spectrum for **12c**

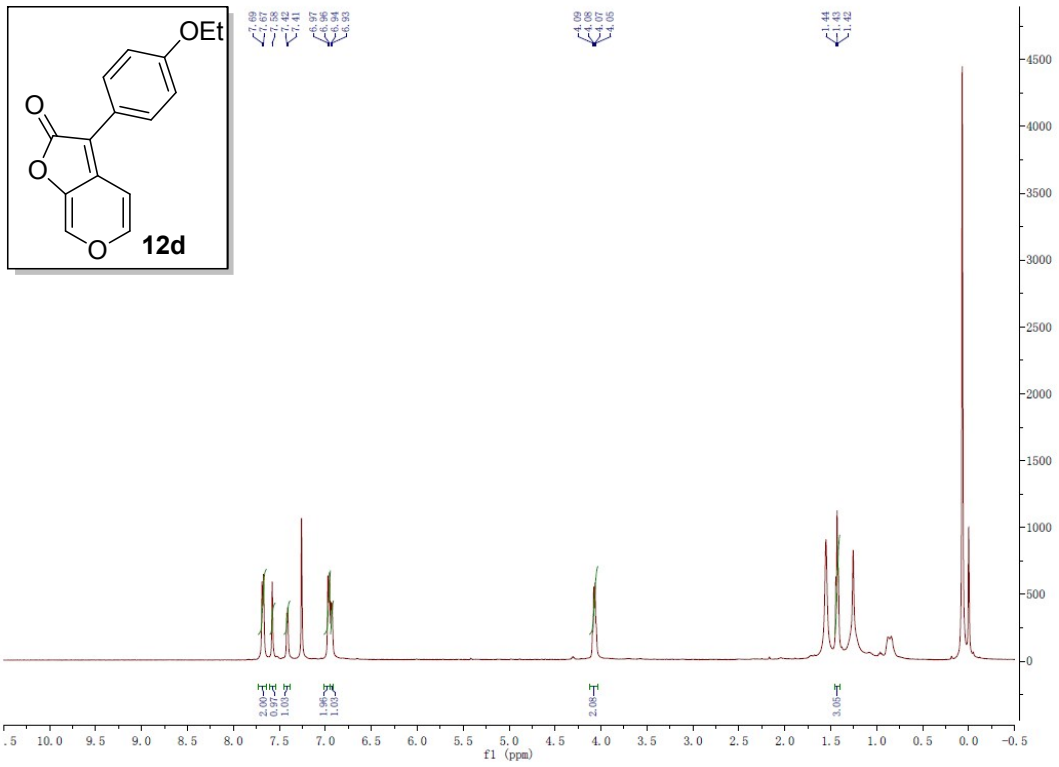


- HRMS (ESI): m/z calcd. for $C_{14}H_8F_3O_4$ $[M+H]^+$: 297.03692; Found 297.03758.

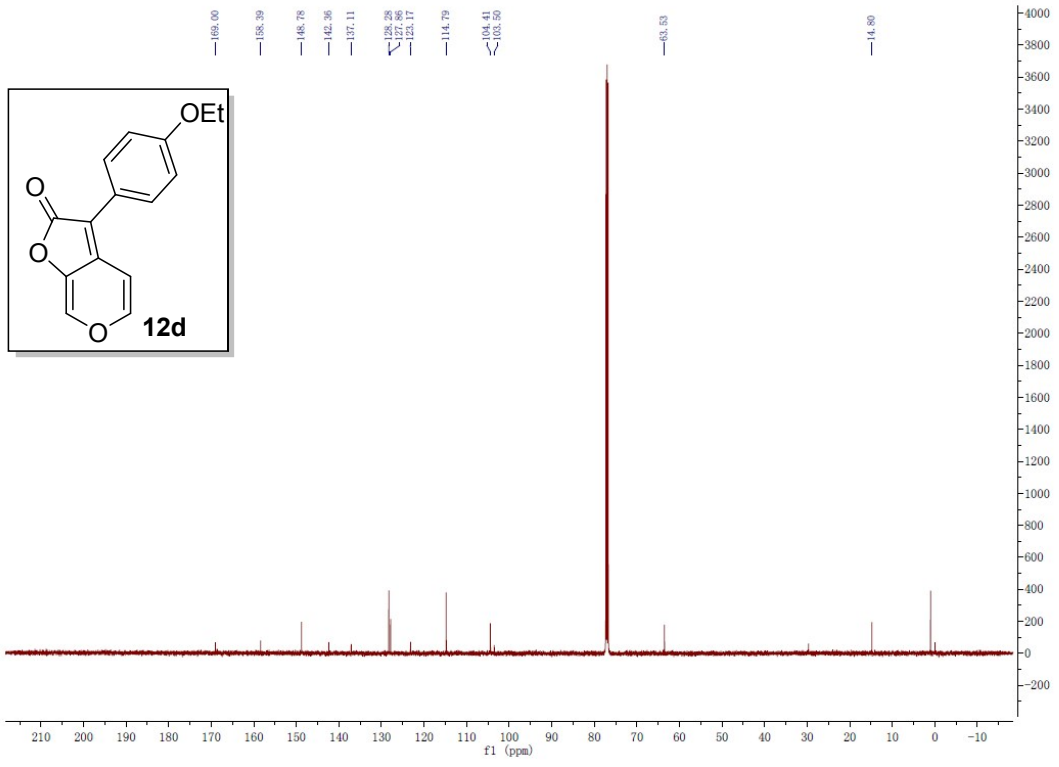
- HRMS (ESI) spectrum for **12c**



➢ ^{1}H NMR spectrum for **12d**

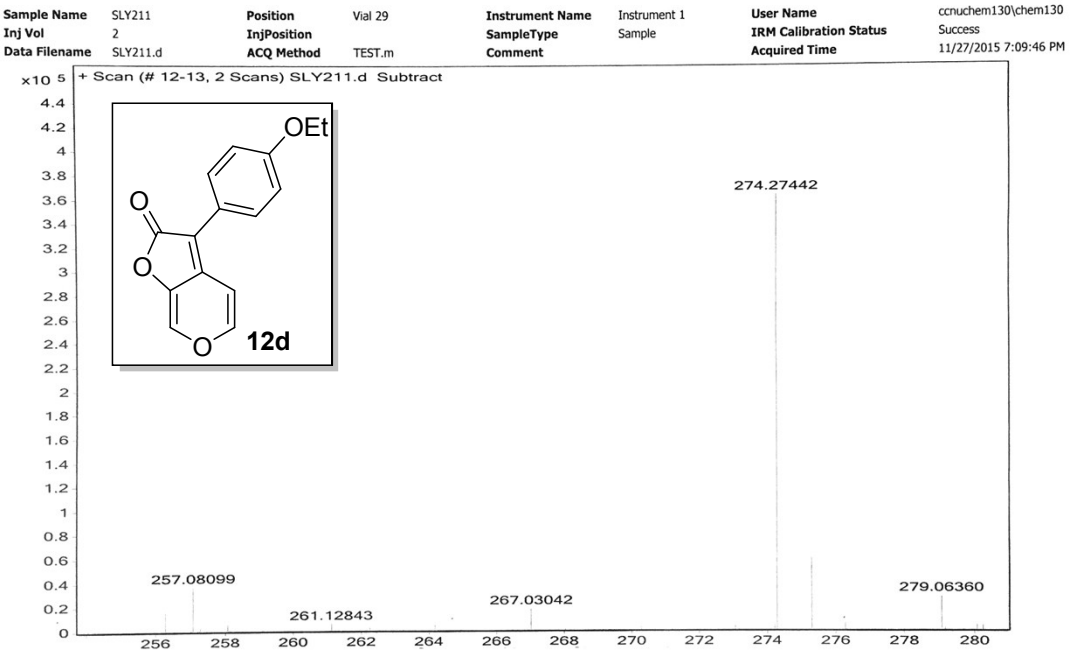


➢ ^{13}C NMR spectrum for **12d**

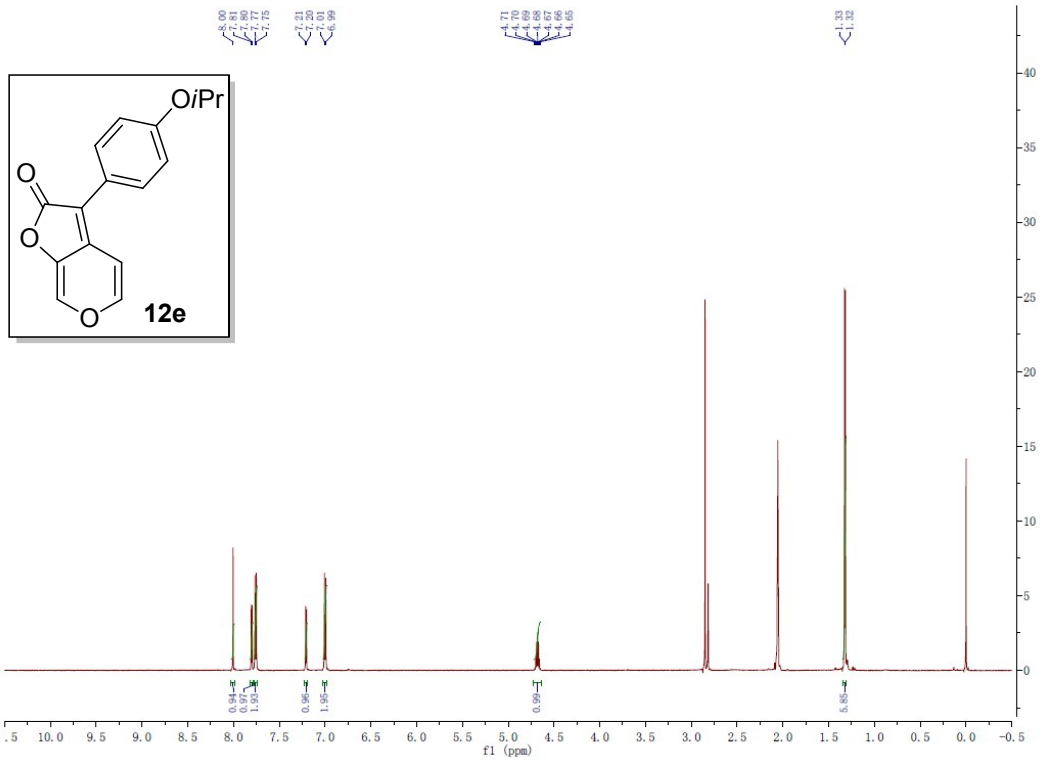


- HRMS (ESI): m/z calcd. for $C_{15}H_{13}O_4$ $[M+H]^+$: 257.08084; Found 257.08099.

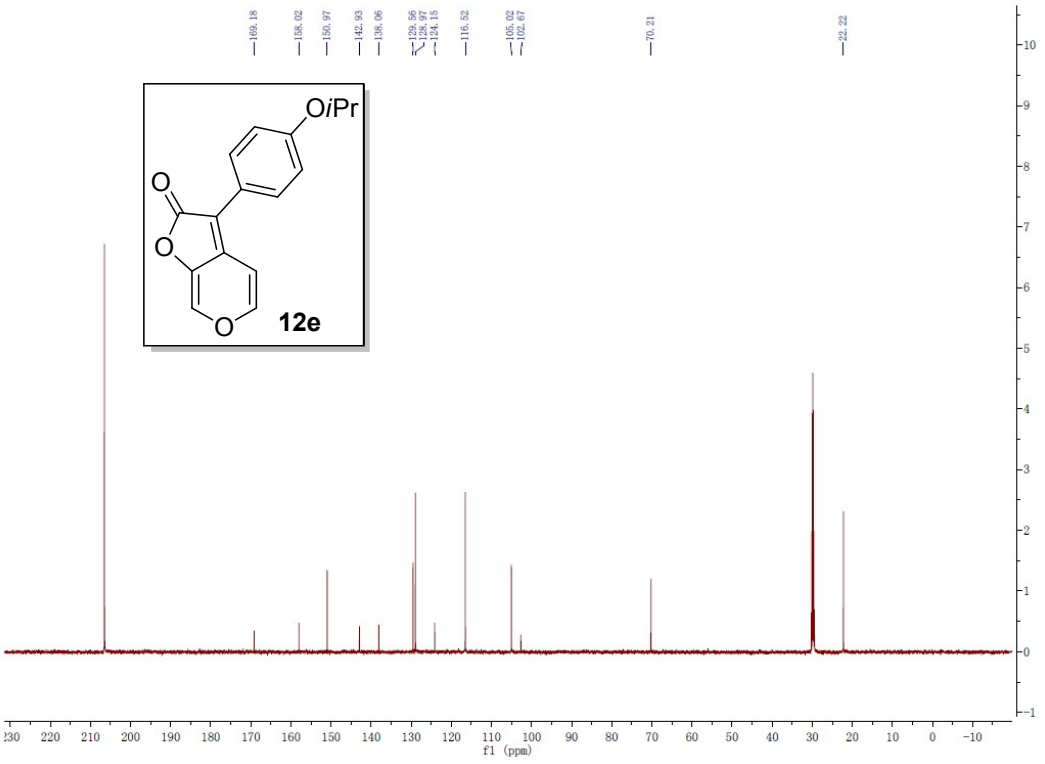
- HRMS (ESI) spectrum for **12d**



➢ ^{1}H NMR spectrum for **12e**

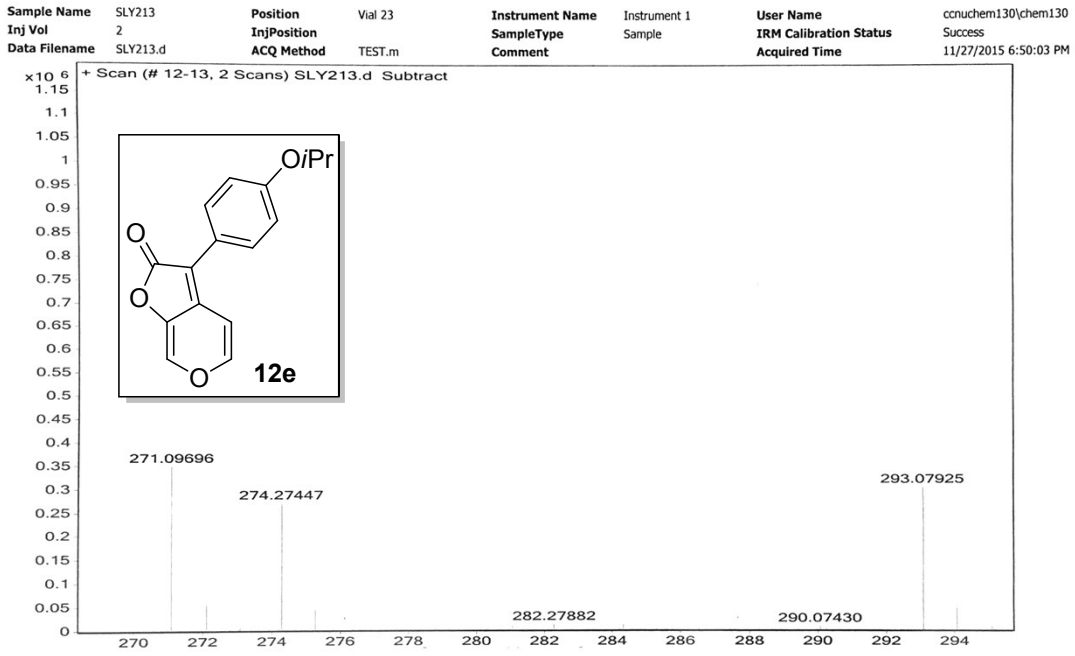


➢ ^{13}C NMR spectrum for **12e**

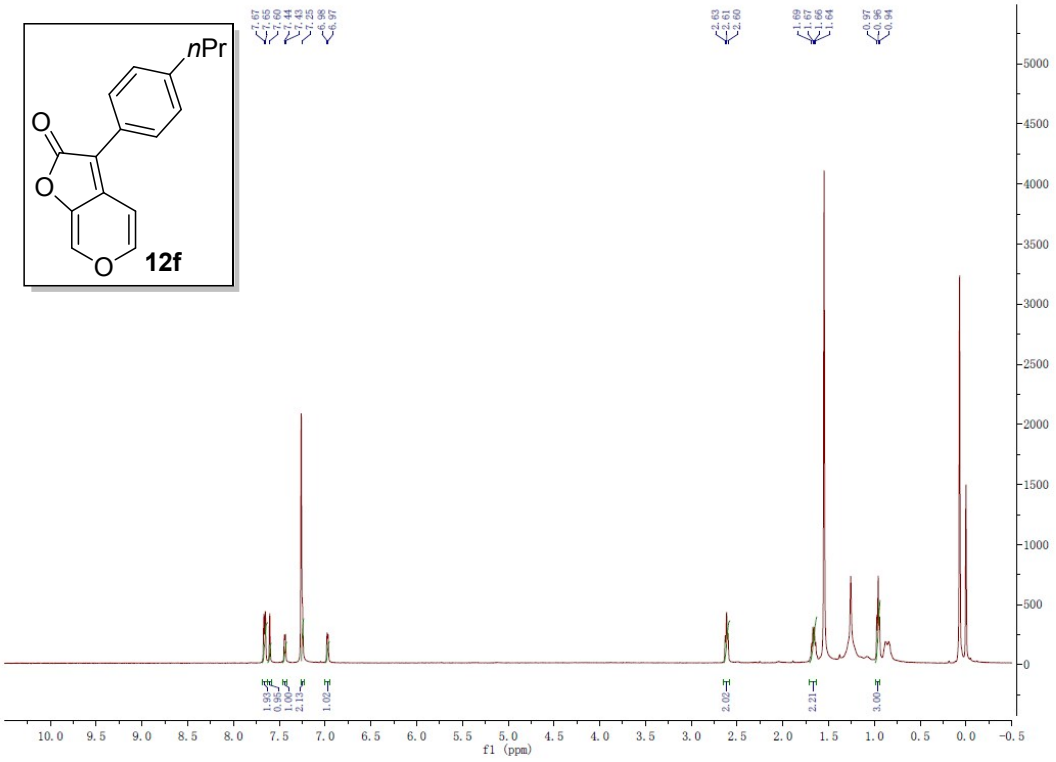


- HRMS (ESI): m/z calcd. for $C_{16}H_{15}O_4$ $[M+H]^+$: 271.09649; Found 271.09696.

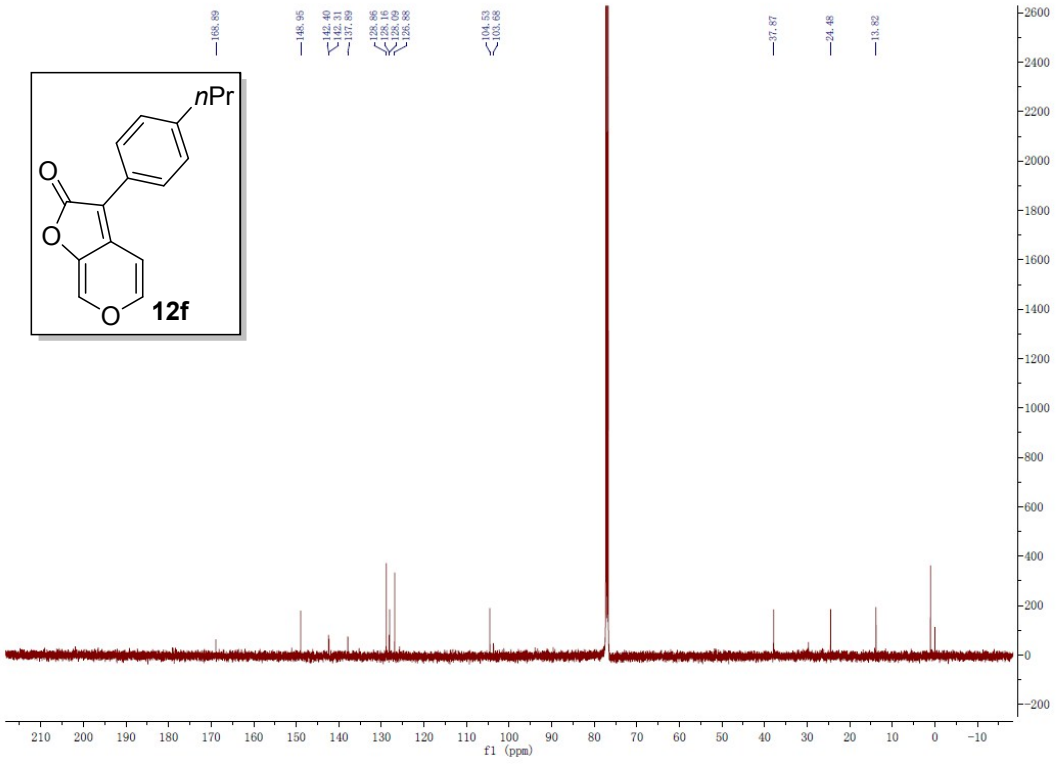
- HRMS (ESI) spectrum for **12e**



➢ ^{1}H NMR spectrum for **12f**

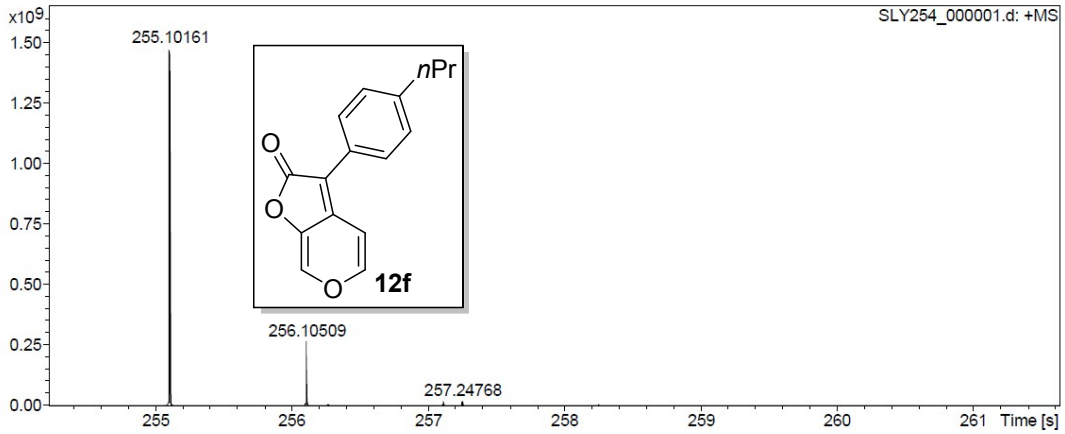


➢ ^{13}C NMR spectrum for **12f**

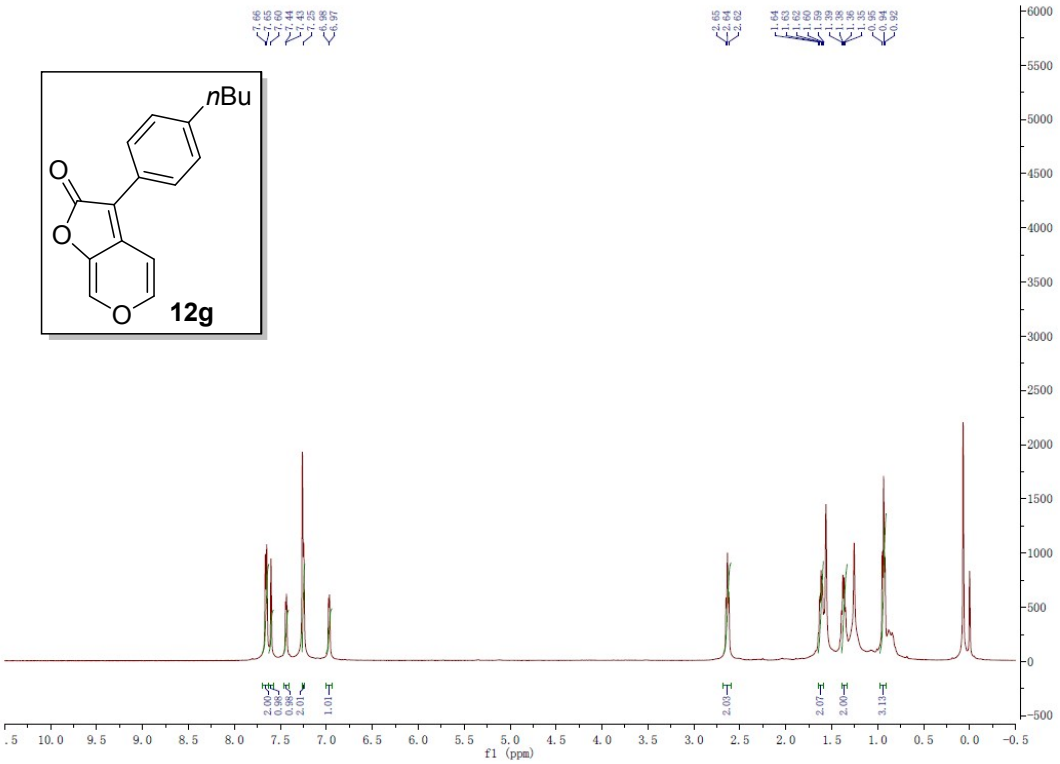


- HRMS (APCI): m/z calcd. for $C_{16}H_{15}O_3$ $[M+H]^+$: 255.10157; Found 255.10161.

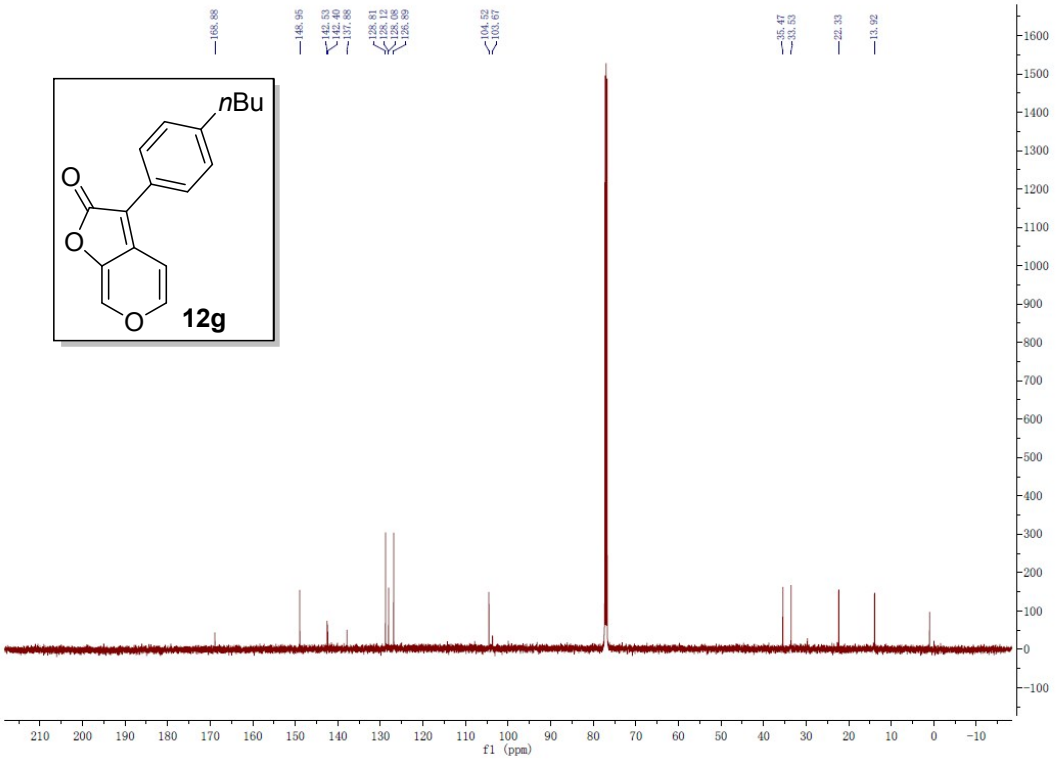
- HRMS (APCI) spectrum for **12f**



➢ ^{1}H NMR spectrum for **12g**

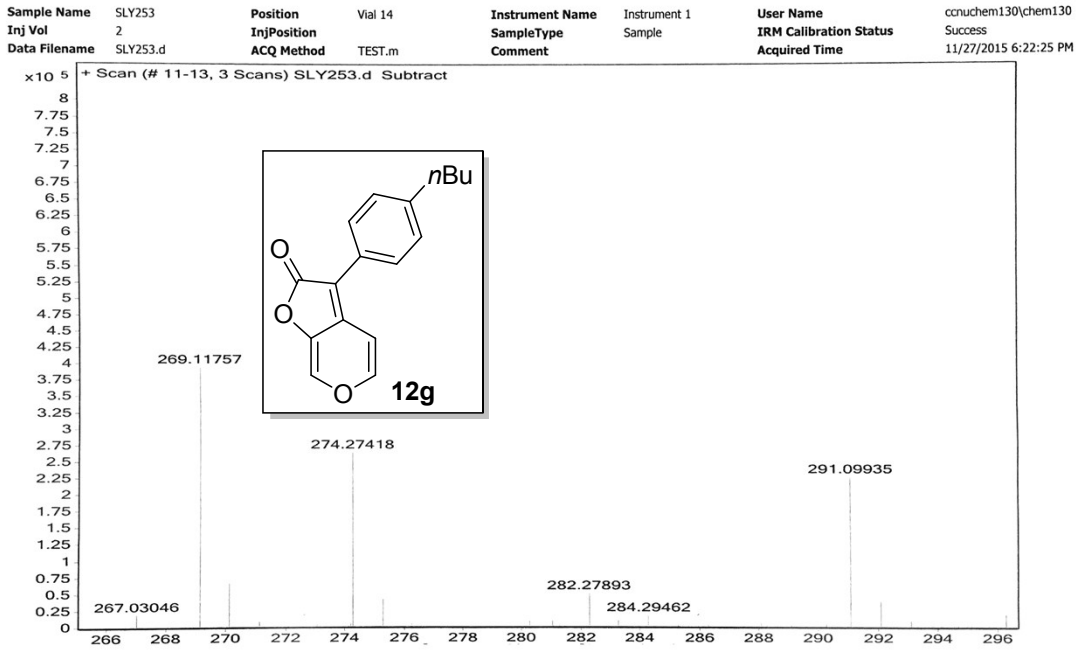


➢ ^{13}C NMR spectrum for **12g**

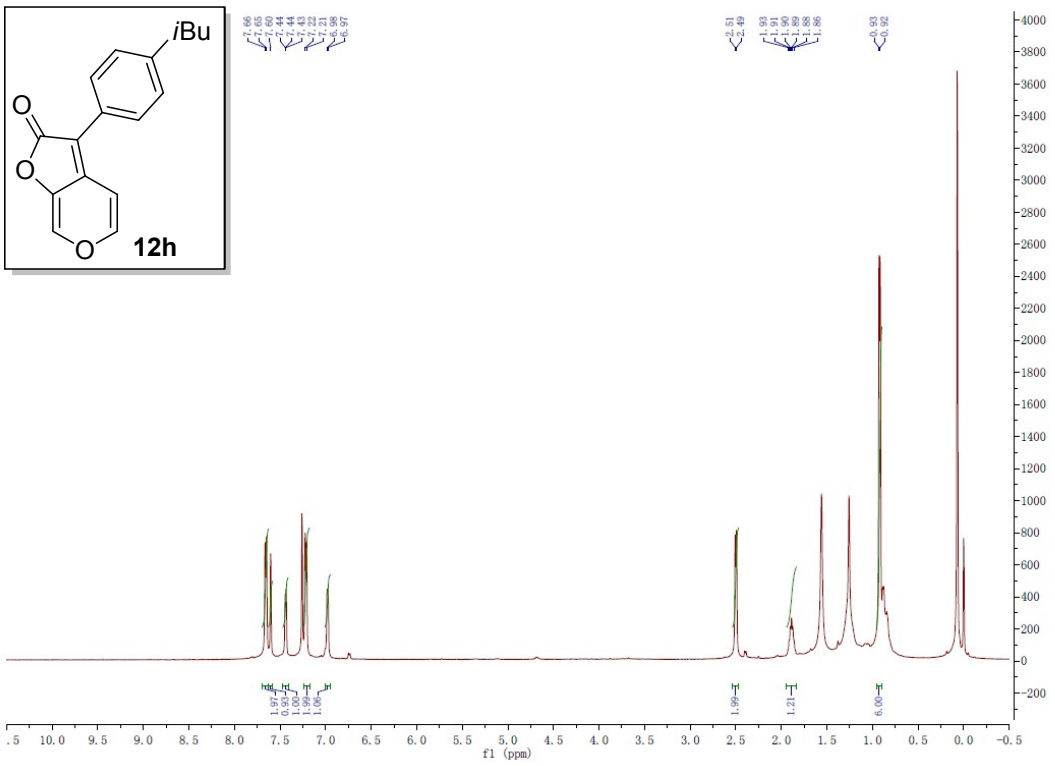


- HRMS (ESI): m/z calcd. for $C_{17}H_{17}O_3$ $[M+H]^+$: 269.11722; Found 269.11757.

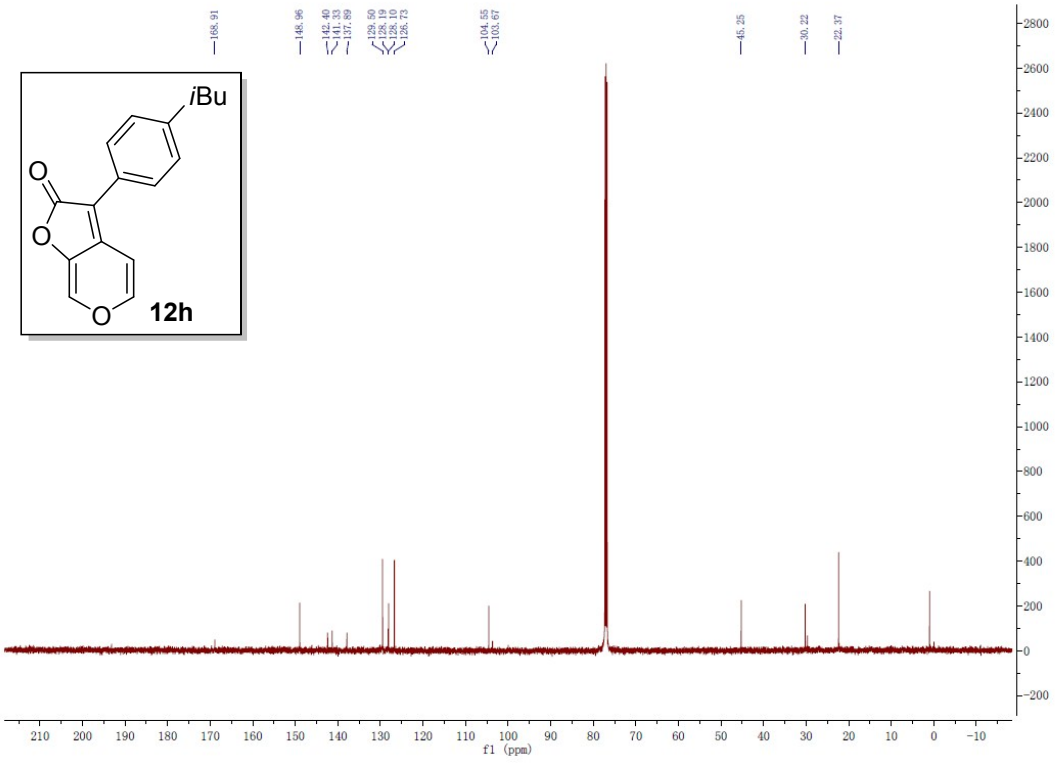
- HRMS (ESI) spectrum for **12g**



➢ ^{1}H NMR spectrum for **12h**

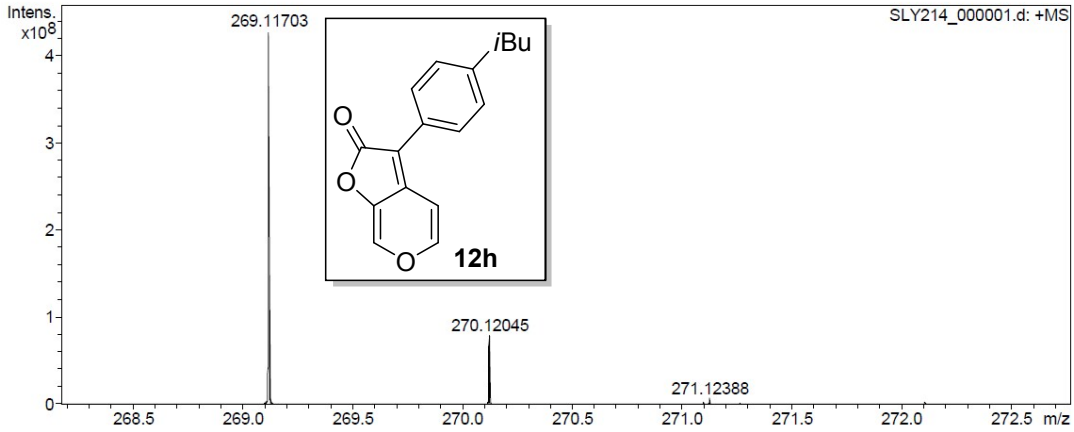


➢ ^{13}C NMR spectrum for **12h**

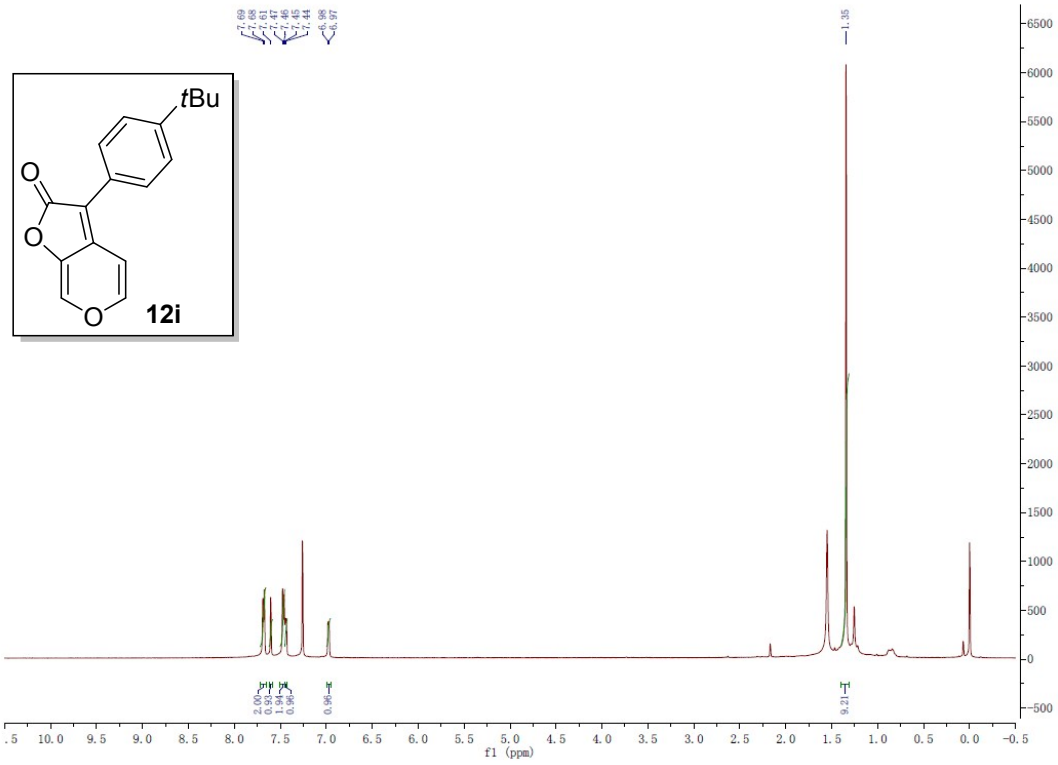


- HRMS (APCI): m/z calcd. for $C_{17}H_{17}O_3$ $[M+H]^+$: 269.11722; Found 269.11703.

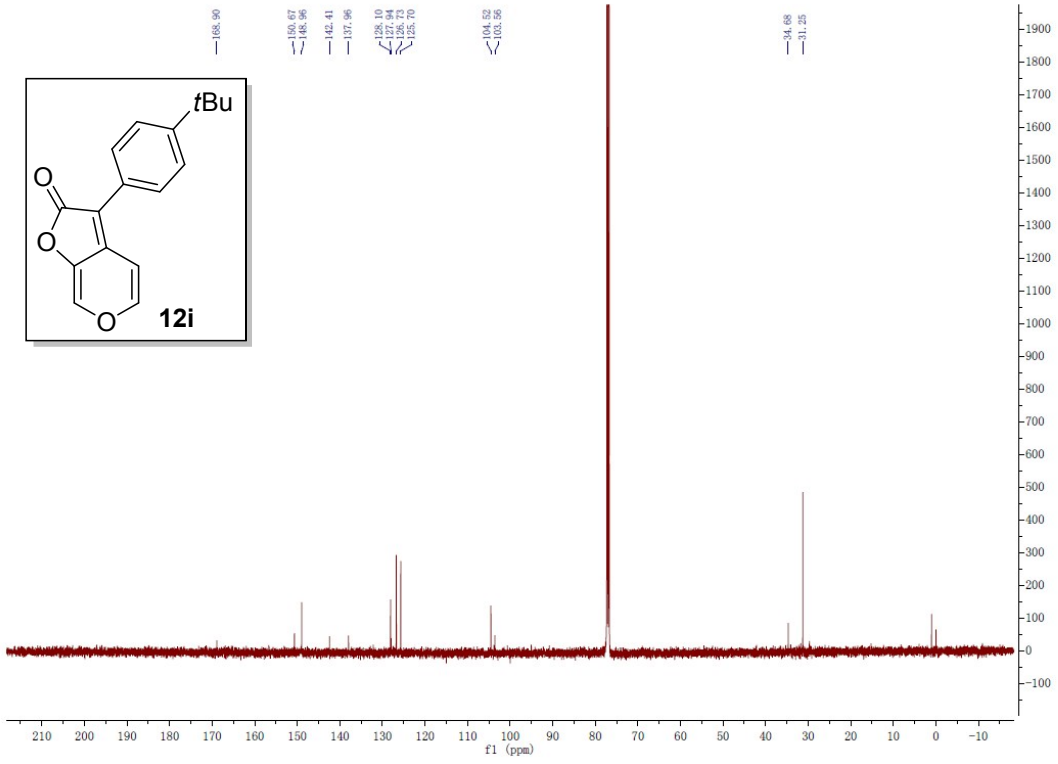
- HRMS (APCI) spectrum for **12h**



➢ ^{1}H NMR spectrum for **12i**

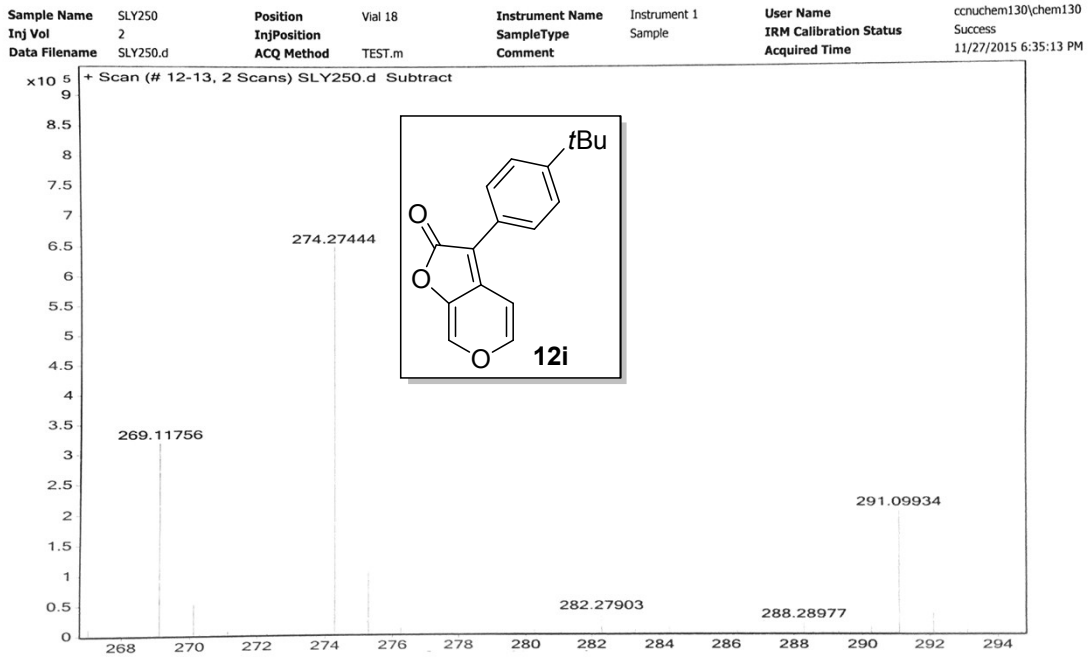


➢ ^{13}C NMR spectrum for **12i**

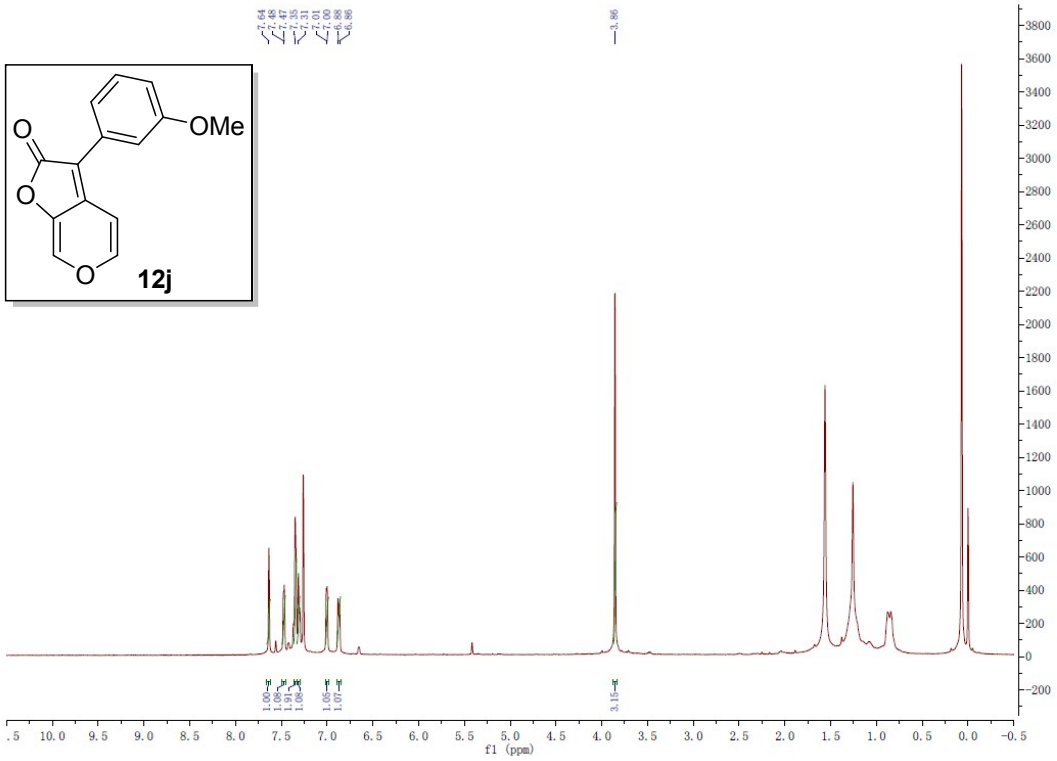


- HRMS (ESI): m/z calcd. for $C_{17}H_{17}O_3$ $[M+H]^+$: 269.11722; Found 269.11756.

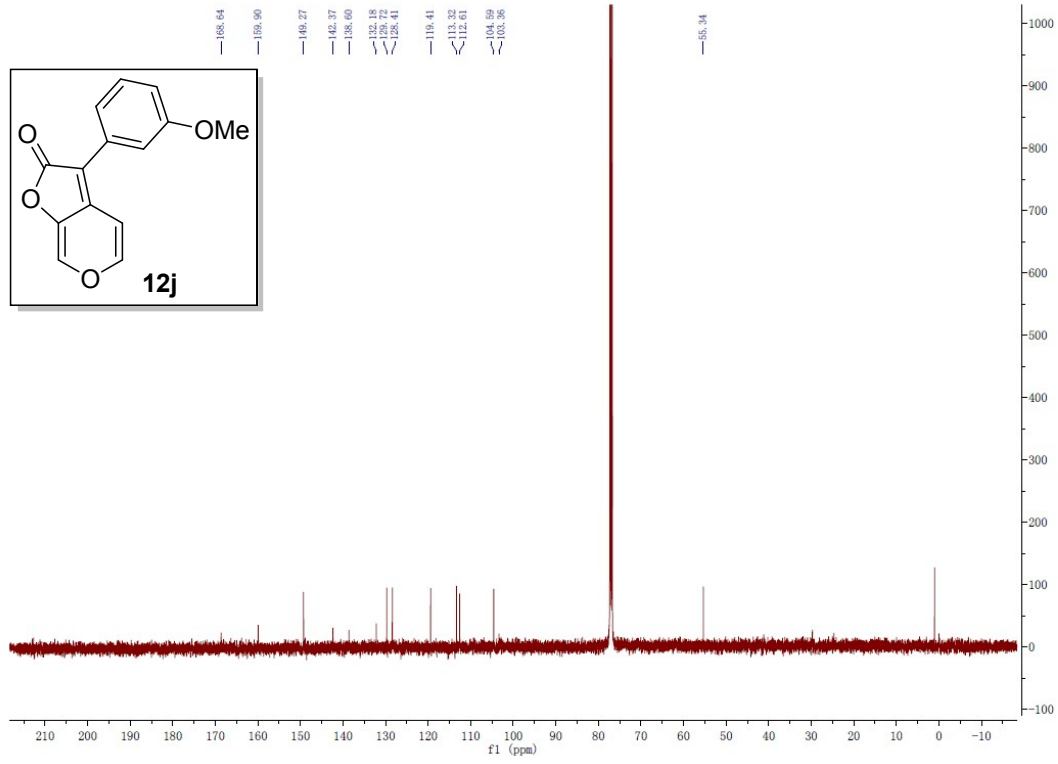
- HRMS (ESI) spectrum for **12i**



➢ ^{1}H NMR spectrum for **12j**

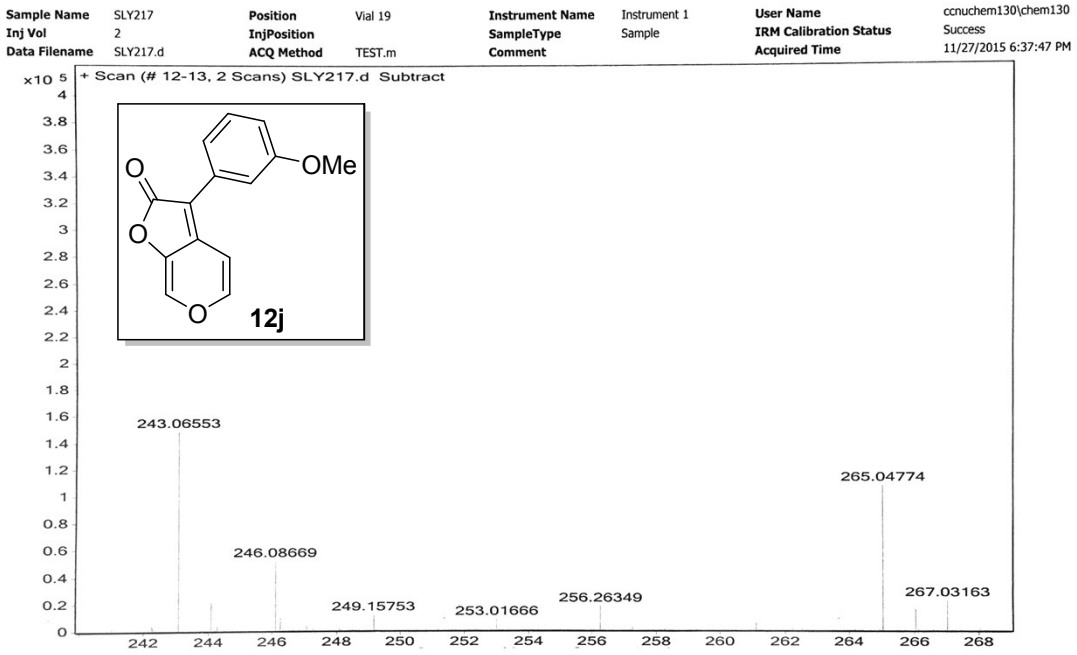


➢ ^{13}C NMR spectrum for **12j**

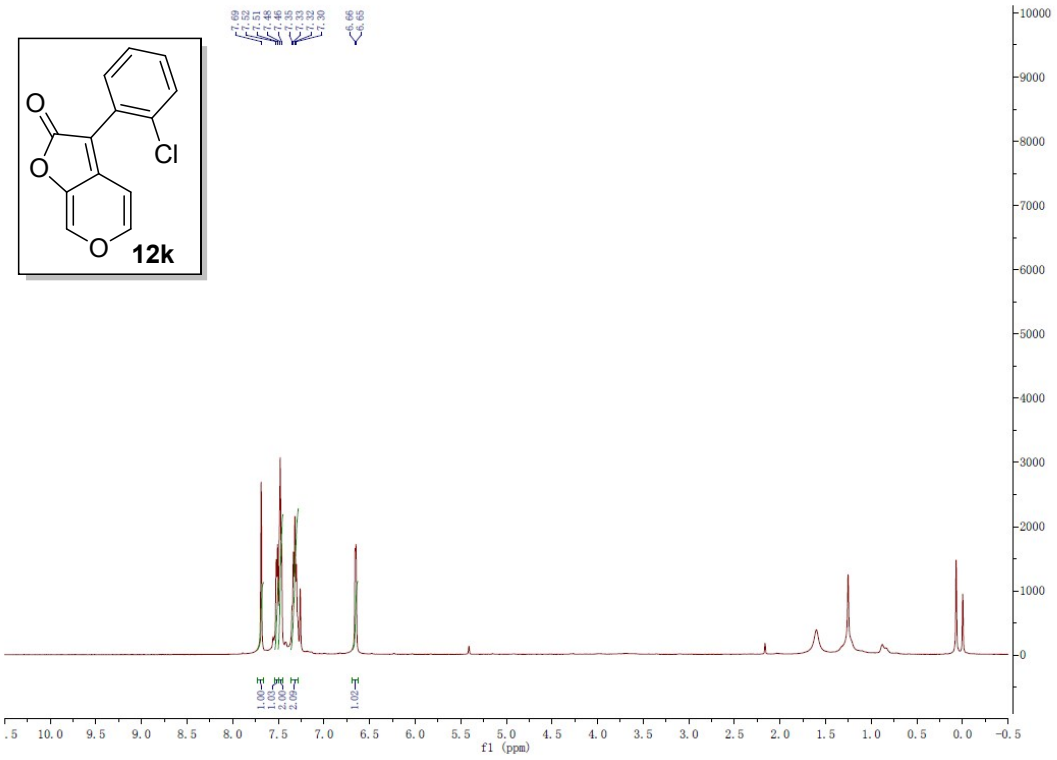


- HRMS(ESI): m/z calcd. for $C_{14}H_{11}O_4$ $[M+H]^+$: 243.06519; Found 243.06553.

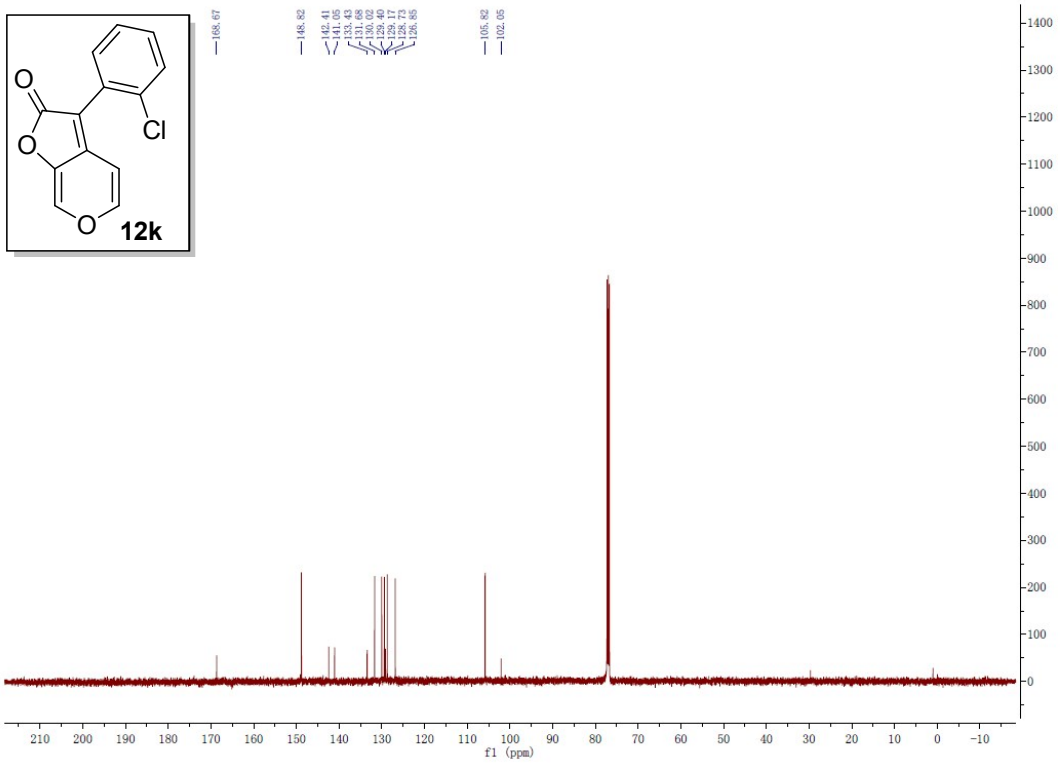
- HRMS (ESI) spectrum for **12j**



➢ ^{1}H NMR spectrum for **12k**

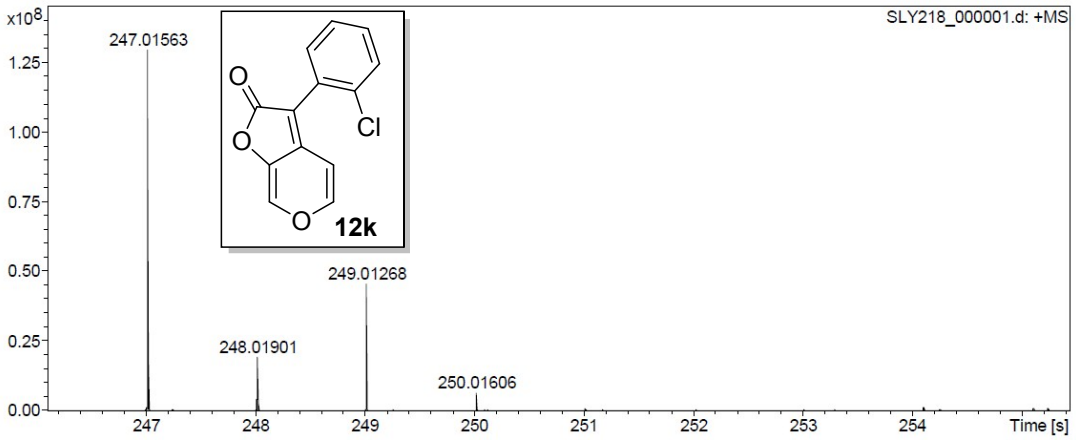


➢ ^{13}C NMR spectrum for **12k**

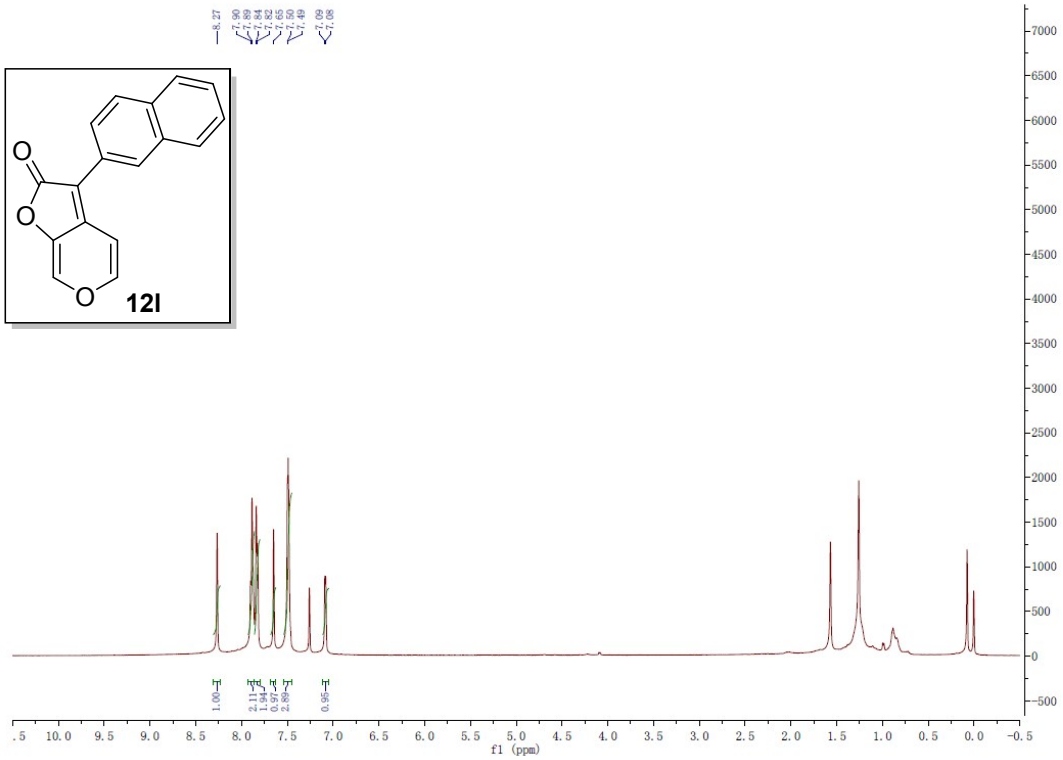


- HRMS(APCI): m/z calcd. for $C_{13}H_8ClO_3$ $[M+H]^+$: 247.01565; Found: 247.01563.

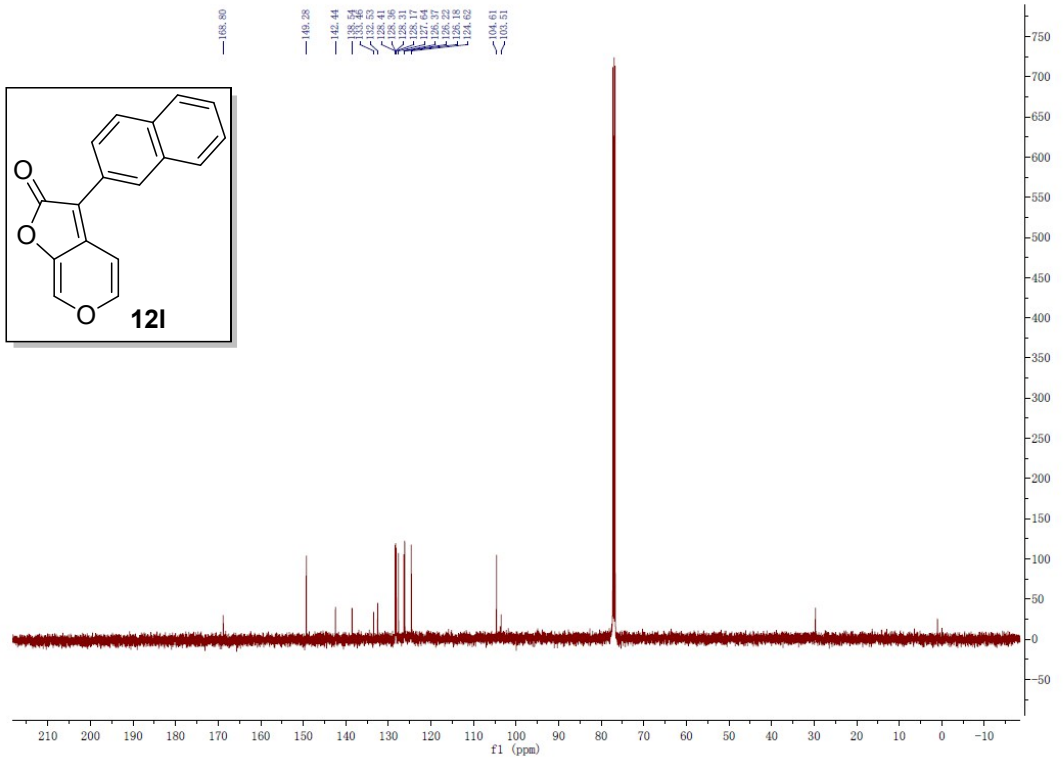
- HRMS (APCI) spectrum for **12k**



➢ ^{1}H NMR spectrum for **12l**

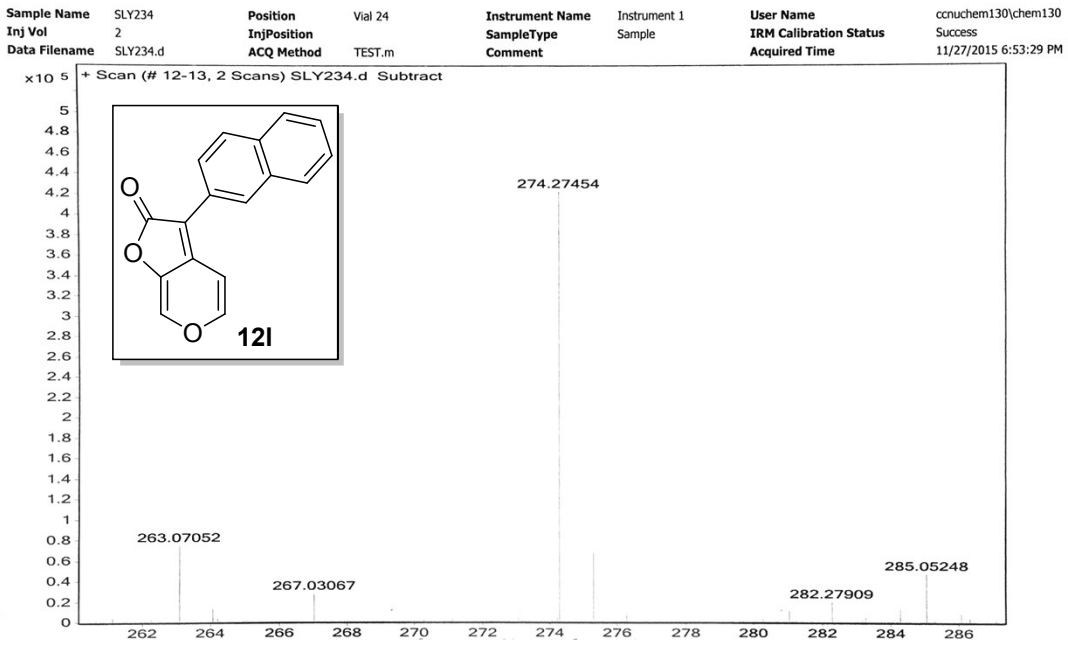


➢ ^{13}C NMR spectrum for **12l**

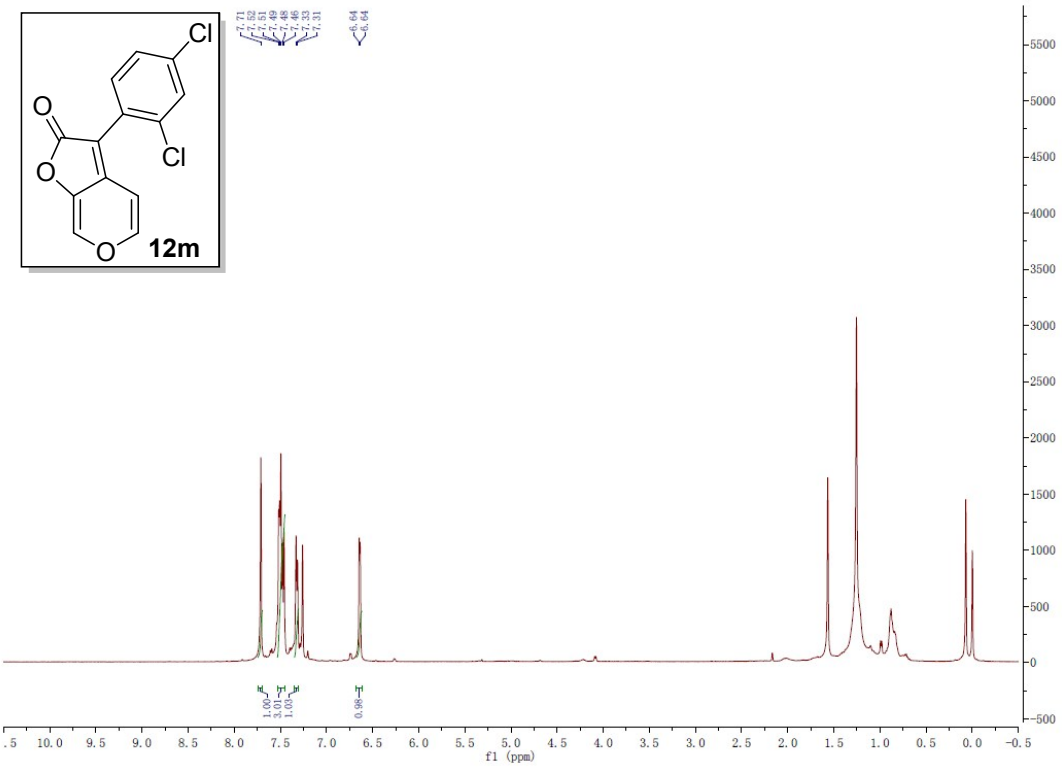


- HRMS(ESI): m/z calcd. for $C_{17}H_{11}O_3$ $[M+H]^+$: 263.07027; Found 263.07052.

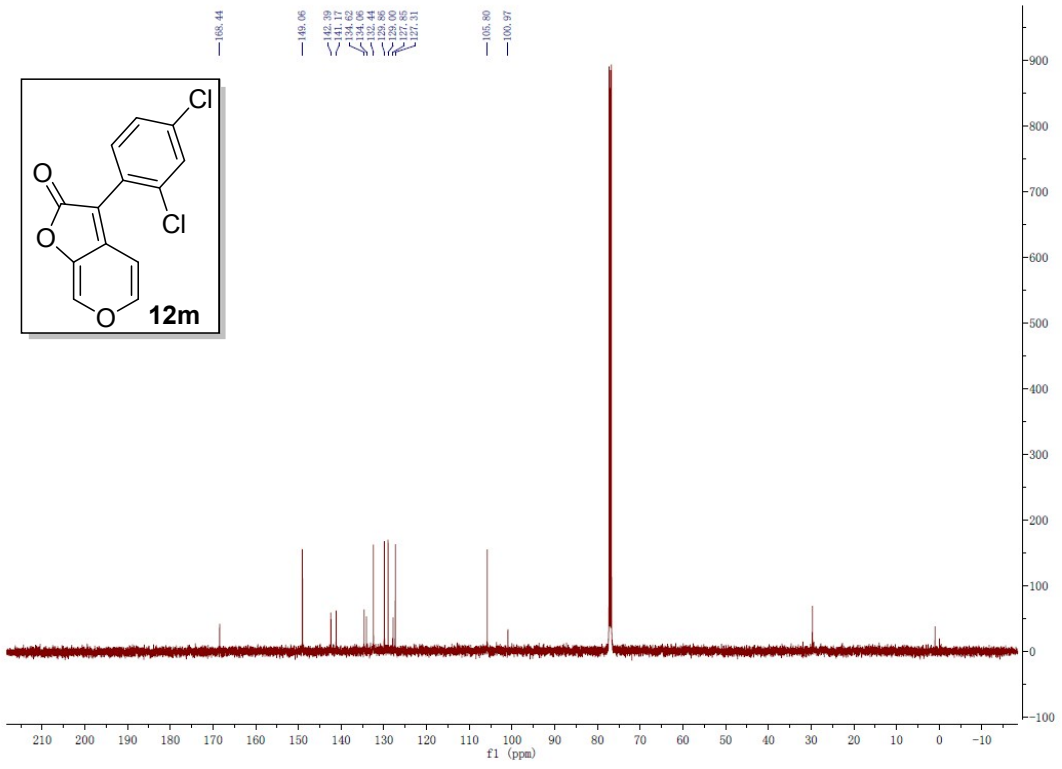
- HRMS (ESI) spectrum for **12l**



➢ ^{1}H NMR spectrum for **12m**

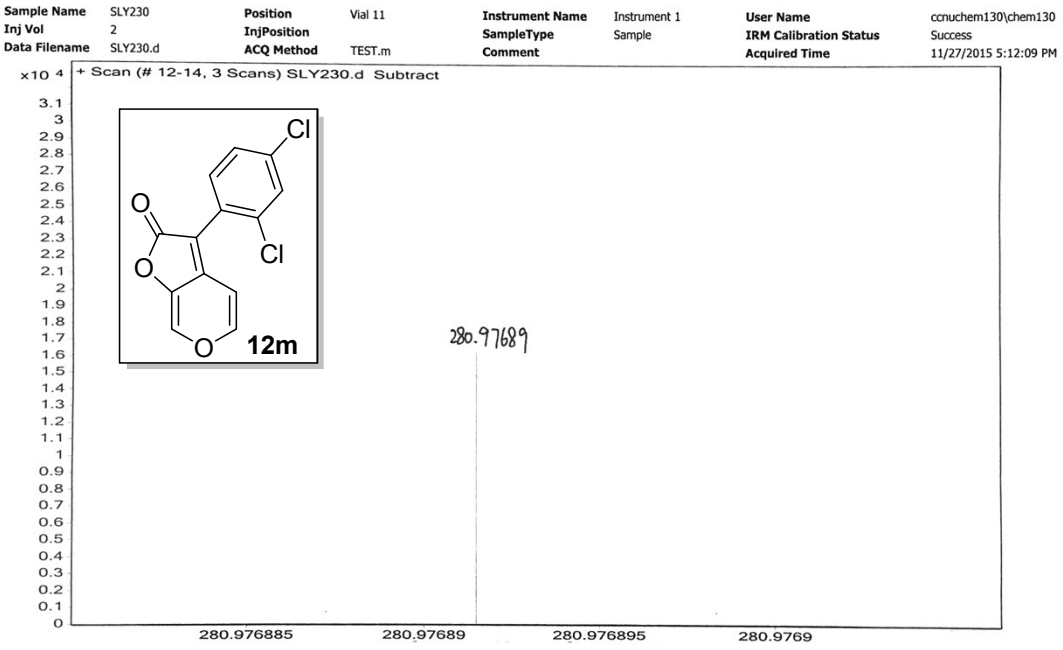


➢ ^{13}C NMR spectrum for **12m**

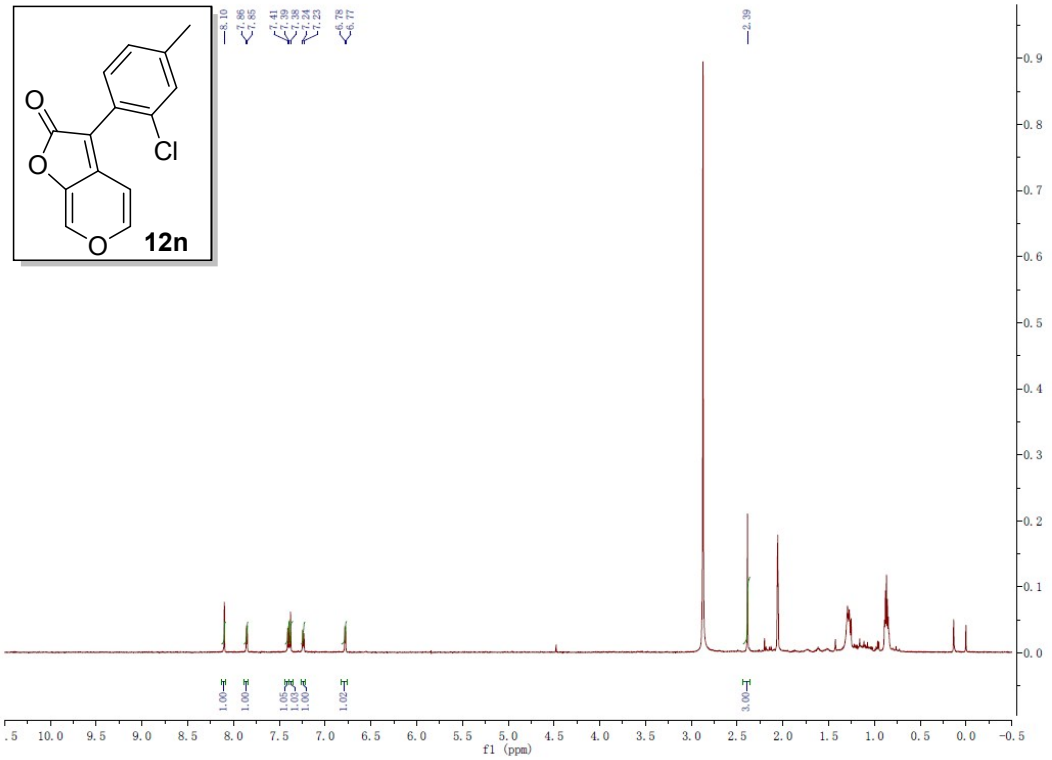


- HRMS(ESI): m/z calcd. for $C_{13}H_7Cl_2O_3$ $[M+H]^+$: 280.97668; Found 280.97689.

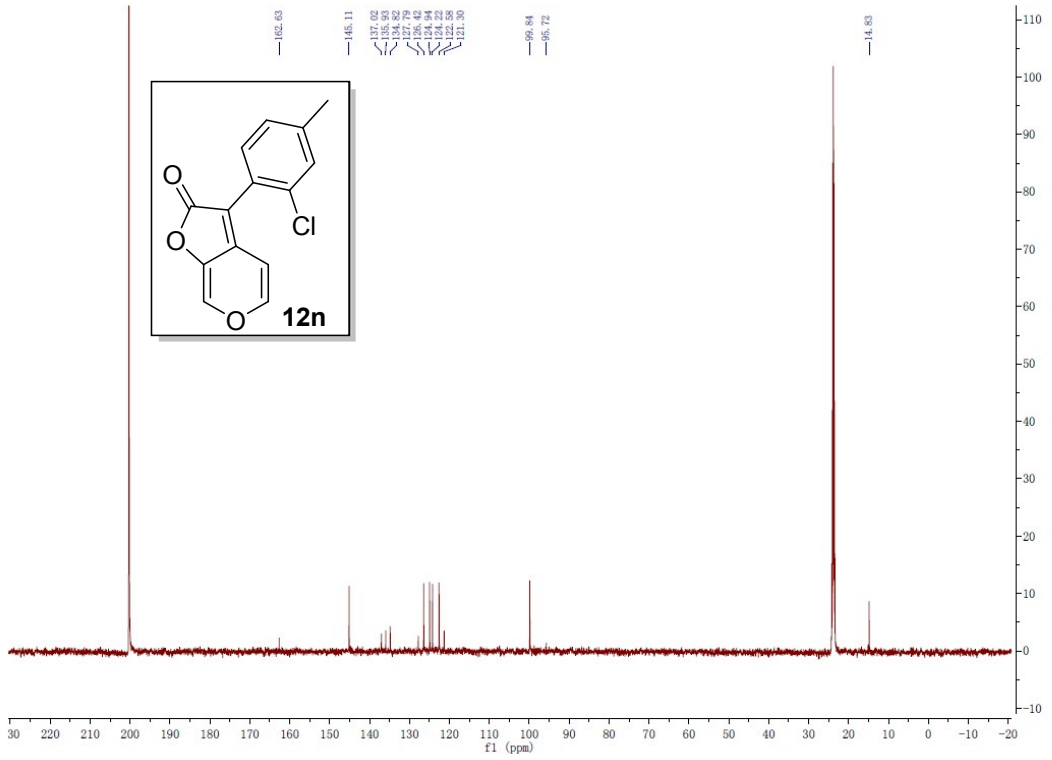
- HRMS (ESI) spectrum for **12m**



➢ ^{1}H NMR spectrum for **12n**

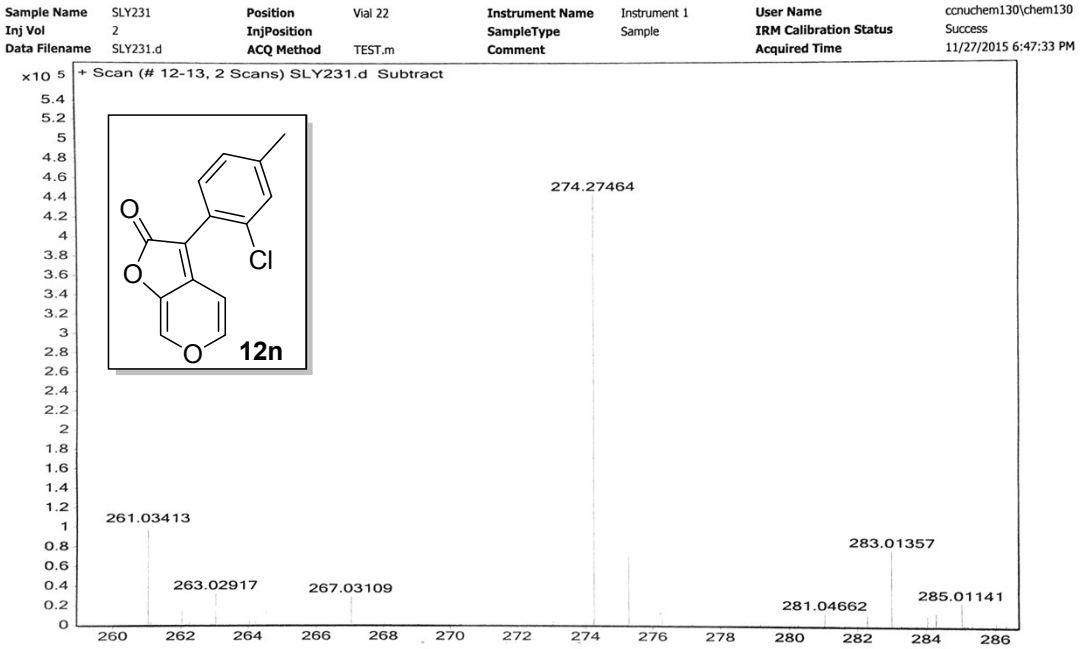


➢ ^{13}C NMR spectrum for **12n**

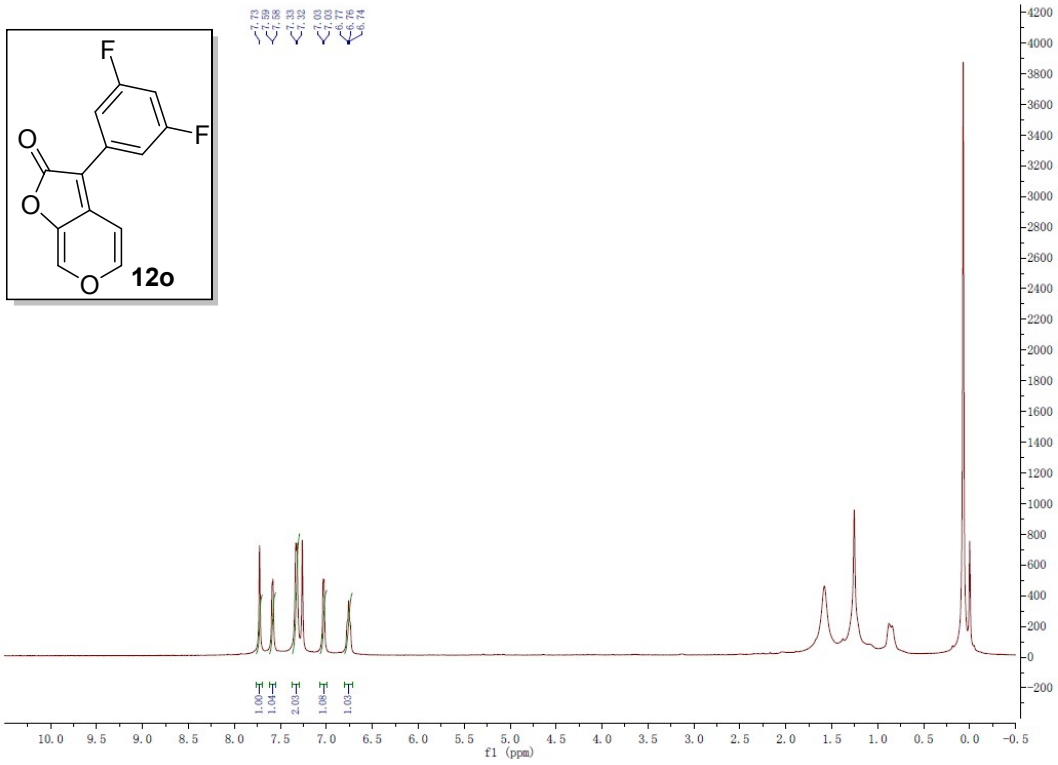


- HRMS(ESI): m/z calcd. for $C_{14}H_{10}ClO_3$ $[M+H]^+$: 261.03130; Found 261.03413.

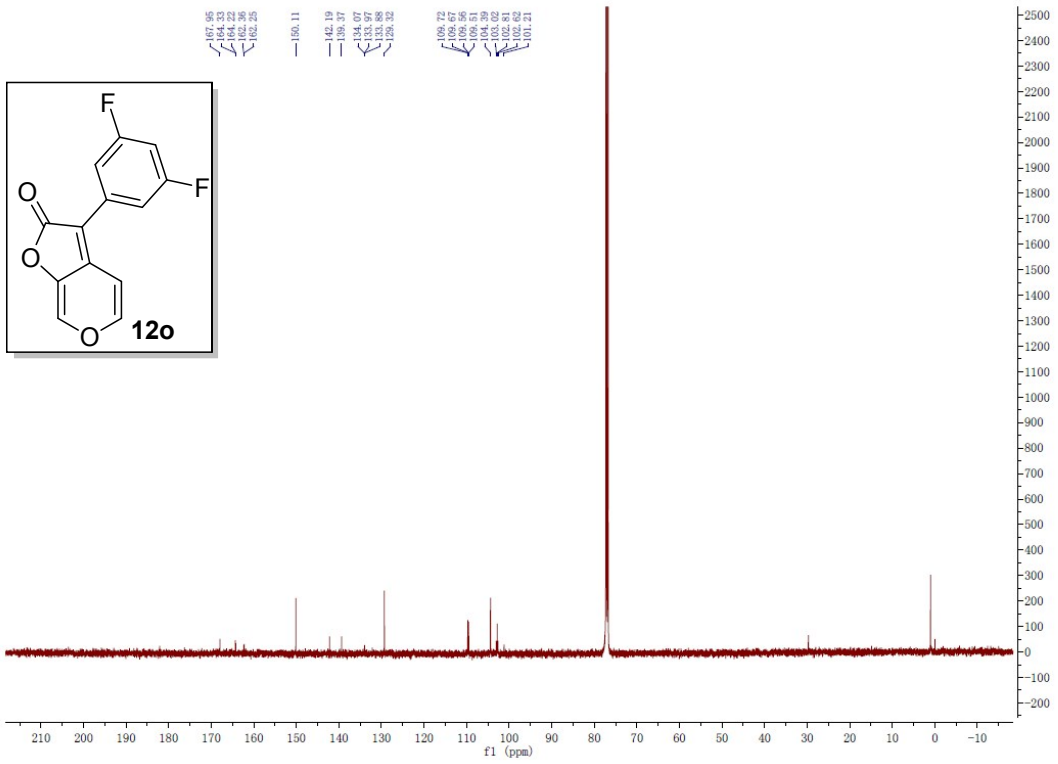
- HRMS (ESI) spectrum for **12n**



➢ ^{1}H NMR spectrum for **12o**

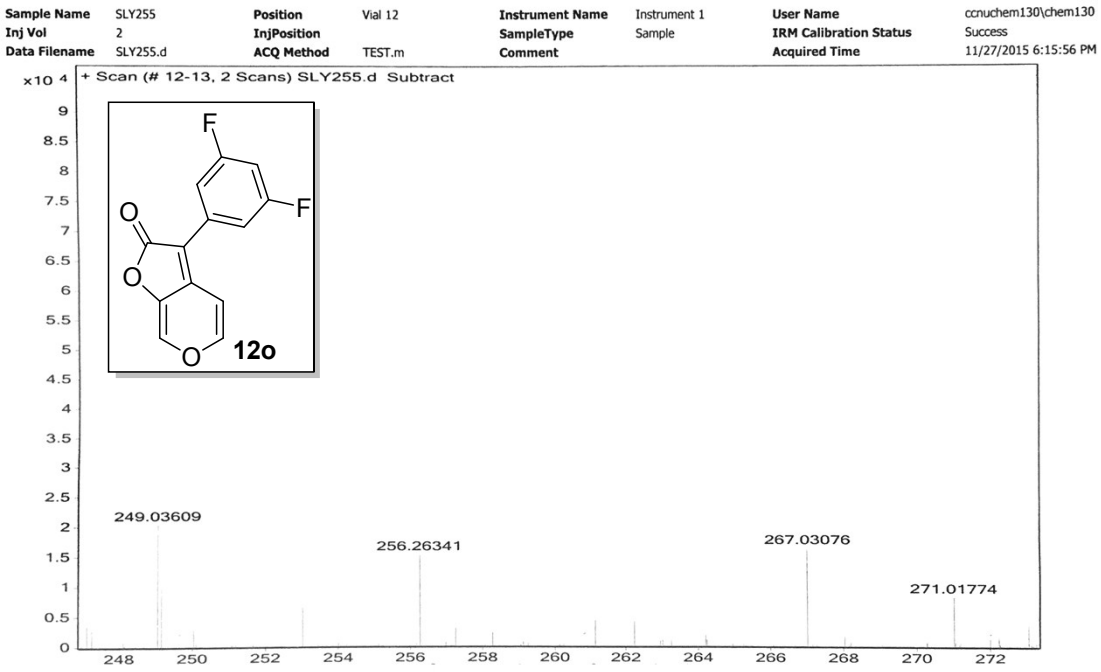


➢ ^{13}C NMR spectrum for **12o**

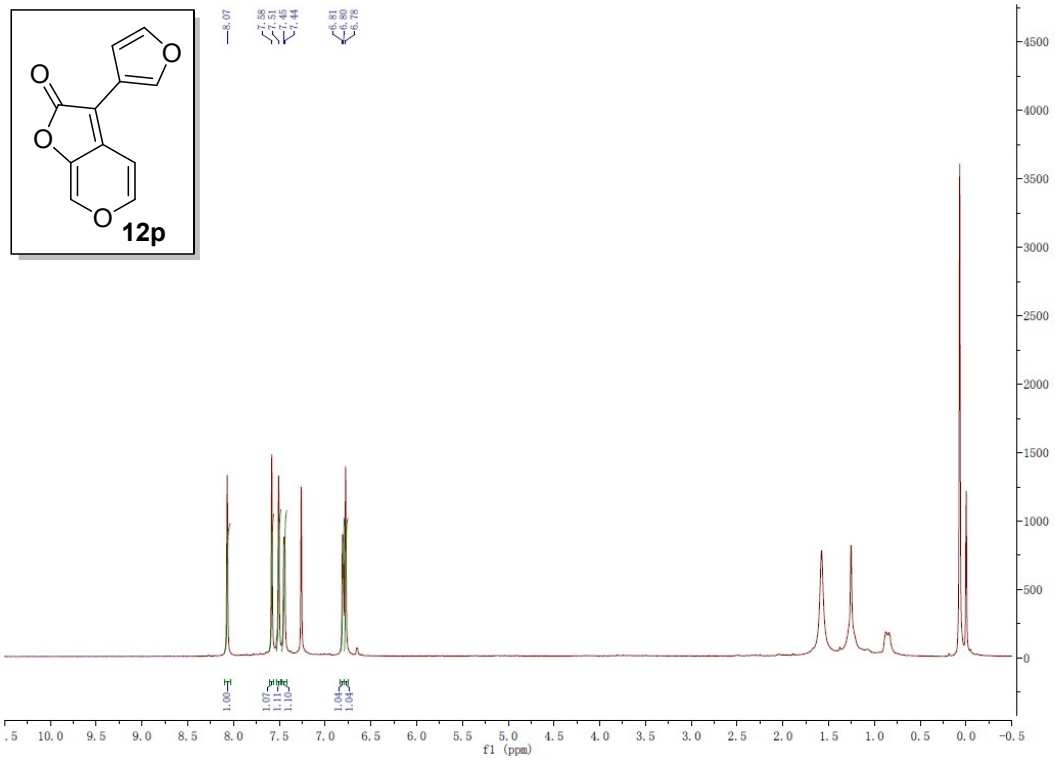


- HRMS(ESI): m/z calcd. for $C_{13}H_7F_2O_3$ $[M+H]^+$: 249.03578; Found 249.03609.

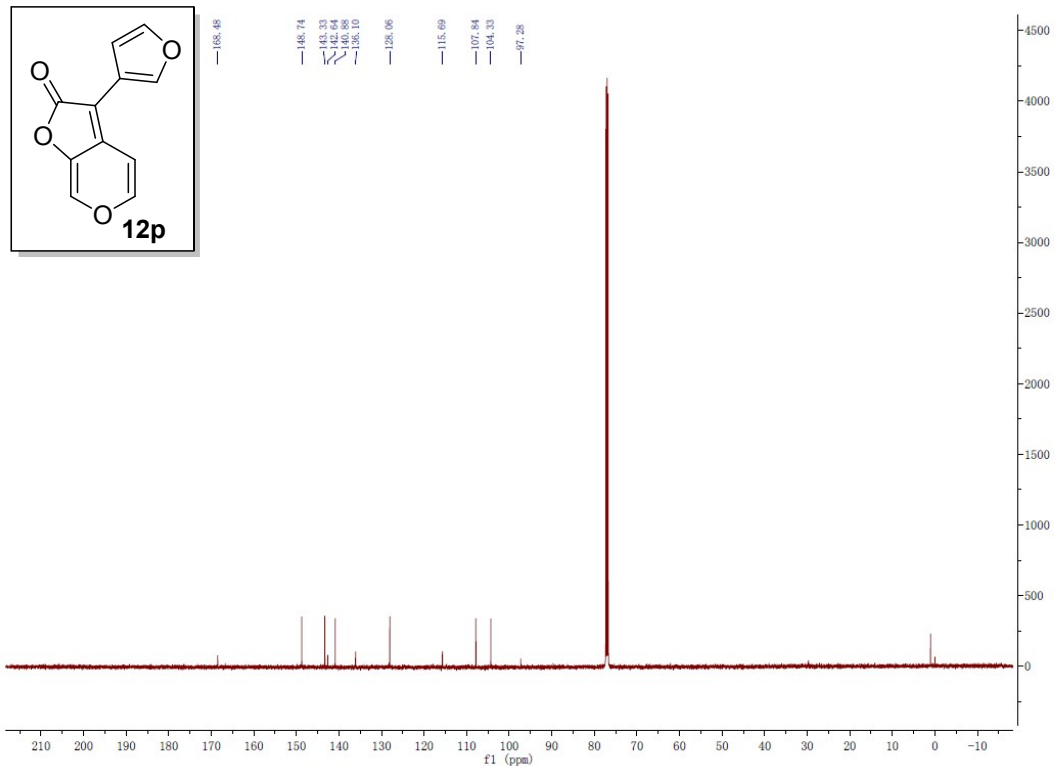
- HRMS (ESI) spectrum for **12o**



➢ ^{1}H NMR spectrum for **12p**

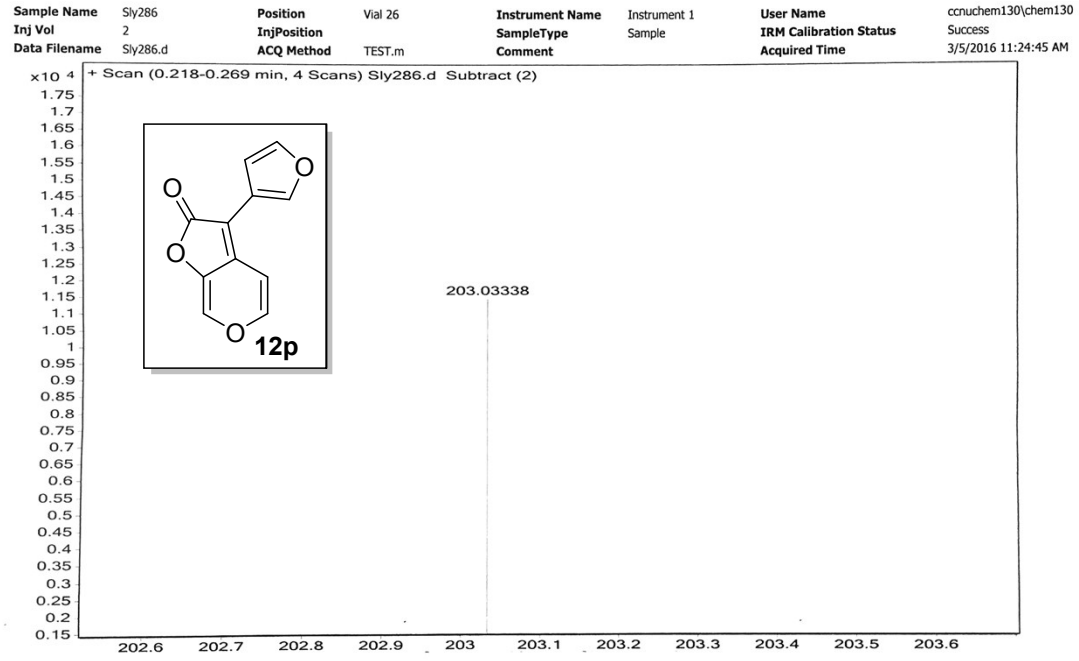


➢ ^{13}C NMR spectrum for **12p**

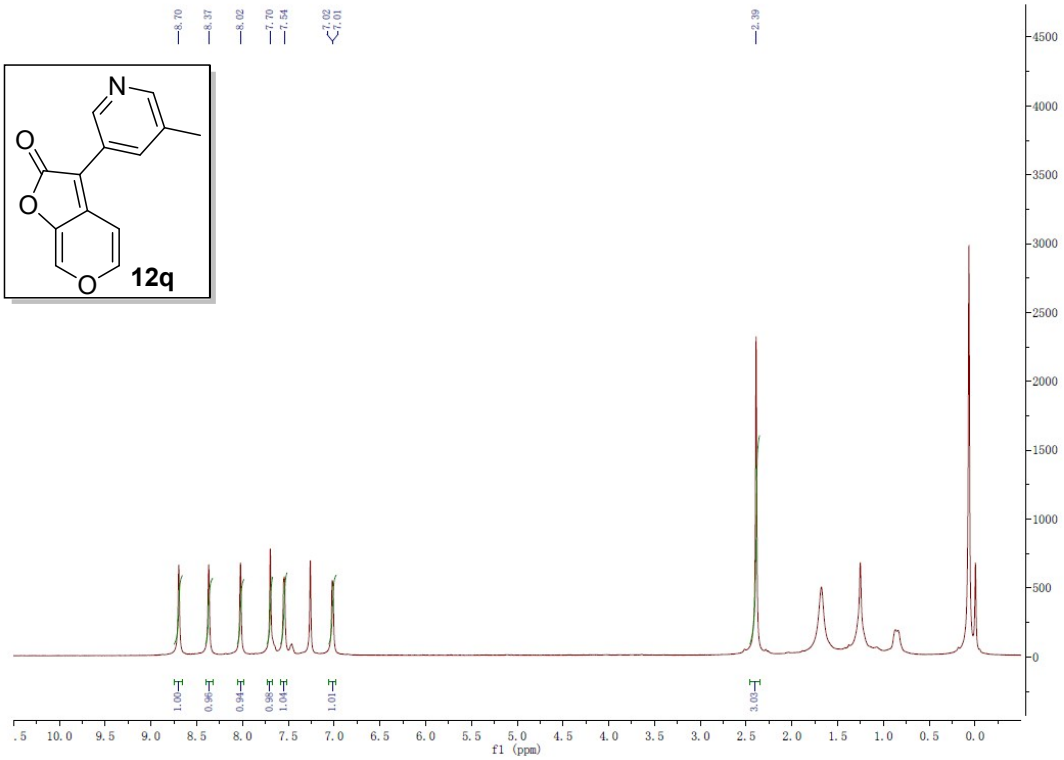


- HRMS (ESI): m/z calcd. for $C_{11}H_7O_4$ $[M+H]^+$: 203.03389; Found 203.03338.

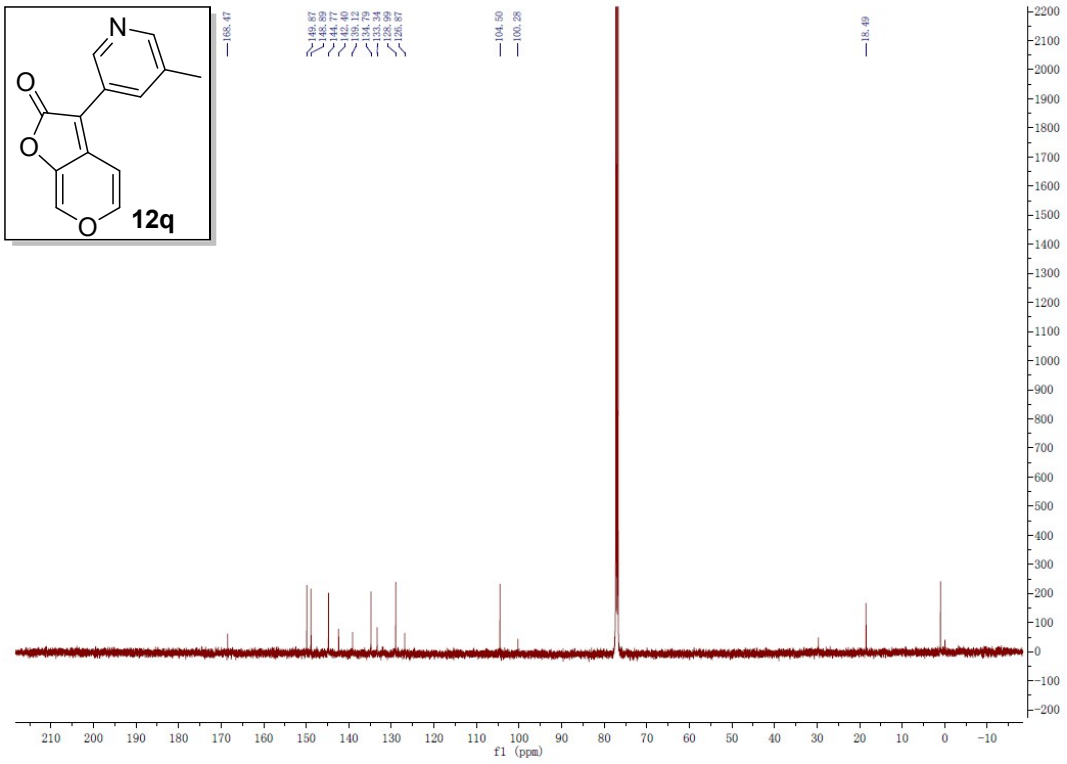
- HRMS (ESI) spectrum for **12p**



➢ ^{1}H NMR spectrum for **12q**



➢ ^{13}C NMR spectrum for **12q**



- HRMS (ESI): m/z calcd. for $C_{13}H_{10}NO_3$ $[M+H]^+$: 228.06552, Found 228.06548.

- HRMS (ESI) spectrum for **12q**