Supporting Information

Construction of Benzofuranone Library via Metal-Free, One-Pot Intermolecular Condensation and Their Application as Efficient Estrogen Receptor β Modulators

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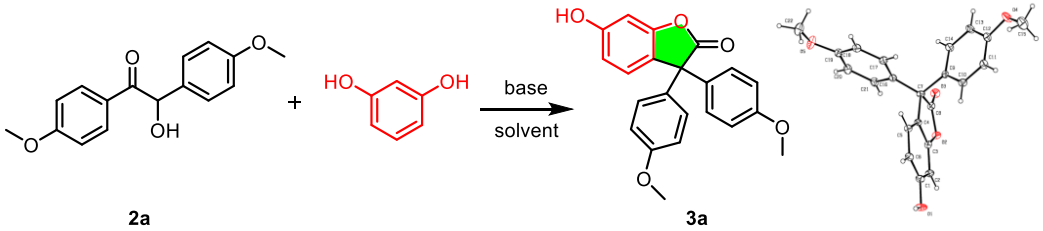
1. Optimization of reaction conditions

Table S1. Optimization of reaction conditions[a]

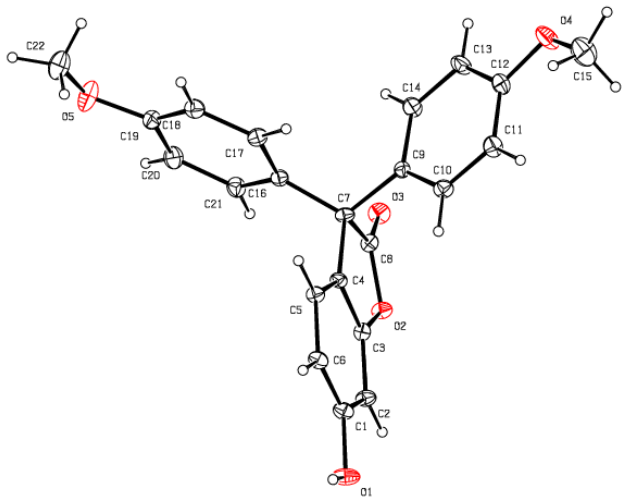


2a **3a**

Entry	Base (equiv)	Solvent	Temperature	Time	Yield [b]
1	NaOH (2.0)	ethylene glycol	110 °C	12 h	Trace[c,d]
2	NaOH (2.0)	DMSO	110 °C	12 h	0%[c,d]
3	NaOH (2.0)	DME	110 °C	12 h	0%[c,d]
4	NaOH (2.0)	1-butanol	110 °C	12 h	0%[c,d]
5	NaOH (2.0)	glycerol	110 °C	12 h	Trace[d]
6	NaOH (2.0)	glycerol	110 °C	12 h	23%
7	NaOH (2.0)	glycerol	100 °C	12 h	10%
8	NaOH (2.0)	glycerol	120 °C	12 h	51%
9	NaOH (2.0)	glycerol	130 °C	12 h	39%
10	NaOH (2.0)	glycerol	140 °C	12 h	16%
11	NaOH (2.0)	glycerol	150 °C	12 h	12%
12	NaOH (2.0)	glycerol	120 °C	6 h	33%
13	NaOH (2.0)	glycerol	120 °C	18 h	47%
14	NaOH (2.0)	glycerol	120 °C	24 h	50%
15	KOH (2.0)	glycerol	120 °C	12 h	38%
16	Na_2CO_3 (2.0)	glycerol	120 °C	12 h	Trace
17	Cs_2CO_3 (2.0)	glycerol	120 °C	12 h	Trace
18	*t*BuOK (2.0)	glycerol	120 °C	12 h	Trace
19	NaOH (1.0)	glycerol	120 °C	12 h	Trace
20	NaOH (3.0)	glycerol	120 °C	12 h	28%

[a] Reaction conditions: **2a** (1.0 equiv), resorcinol (1.0 equiv), and base. [b] Isolated yield. [c] 100% conv. messy reaction. [d] Instead of **2a**, the corresponding diketone **2a'** was used.

2. X-ray crystallography structure of 3a (CCDC 1918835)



Bond precision: C-C = 0.0125 A Wavelength=0.71073

Cell: a=9.176(5) b=10.351(5) c=11.107(6)
alpha=96.843(9) beta=108.663(9) gamma=113.730(8)
Temperature: 296 K

	Calculated	Reported
Volume	876.8(8)	876.9(8)
Space group	P -1	P-1
Hall group	-P 1	?
Moiety formula	C22 H18 O5	?
Sum formula	C22 H18 O5	C22 H18 O5
Mr	362.36	362.36
Dx,g cm-3	1.372	1.372
Z	2	2
Mu (mm-1)	0.097	0.097
F000	380.0	380.0
F000'	380.21	
h,k,lmax	11,12,13	11,12,13
Nref	3625	3598
Tmin,Tmax	0.972,0.978	0.972,0.978
Tmin'	0.972	

Correction method= # Reported T Limits: Tmin=0.972 Tmax=0.978
AbsCorr = MULTI-SCAN

Data completeness= 0.993 Theta(max)= 26.500

R(reflections)= 0.1305(2802) wR2(reflections)= 0.4022(3598)

S = 1.005 Npar= 248

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3. Synthesis of benzofuranone derivatives

General. Unless otherwise noted, starting materials were purchased from commercial suppliers and were used without further purification. Ethanol was dried over Na and distilled prior to use, and dichloromethane was distilled with anhydrous CaH_2. Glassware was oven-dried, assembled while hot, and cooled under an inert atmosphere. All reactions were performed under an argon atmosphere unless otherwise specified. Reaction progress was monitored using analytical thin-layer chromatography (TLC). Visualization was achieved by UV light (254 nm). ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AVANCE III 400 (400 MHz) instrument. Chemical shifts are reported in ppm (parts per million) and are referenced to either tetramethylsilane or the solvent. Melting points were determined on an X-4 Beijing Tech melting point apparatus, and the data were uncorrected.

3.1 General procedure for condensation reaction.

A mixture of α-hydroxyl ketone derivative **2** (0.37 mmol, 1.0 equiv), resorcinol (81.5 mg, 0.37 mmol, 1.0 equiv) and sodium hydroxide (30 mg, 0.74 mmol, 2.0 equiv) in glycerol was stirred at 120 °C for 12 h. The reaction was monitored by TLC for the completion. The reaction mixture was poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated in vacuum. The crude product was purified by column chromatography.

3.2 General procedure for sequential process of condensation and demethylation reaction.

A mixture of α-hydroxyl ketone derivative **2** (0.37 mmol, 1.0 equiv), resorcinol (81.5 mg, 0.37 mmol, 1.0 equiv) and sodium hydroxide (30 mg, 0.74 mmol, 2.0 equiv) in glycerol was stirred at 120 °C for 12 h. The reaction was monitored by TLC for the completion. The reaction mixture was poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous

sodium sulfate. The organic layer was filtered through a short silica pad. The filtrate was concentrated in vacuum to afford crude product, which can be directly used in the next step. Under argon atmosphere, to a solution of crude benzofuranone derivatives (1.0 equiv) in dry dichloromethane at 0 °C, boron tribromide (6.0 equiv) was added dropwise. The reaction was performed for 8 h and monitored by TLC. After the reaction was completed, the mixture was quenched by water. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate. The filtrate was concentrated in vacuum. The crude product was purified by column chromatography.

4. Compounds characterization

6-Hydroxy-3,3-bis(4-methoxyphenyl)benzofuran-2(3*H*)-one (3a).

Compound **3a** was prepared by 1,2-bis(4-methoxyphenyl)-2-hydroxyethan-1-one, resorcinol and sodium hydroxide according to the general procedure **3.1**. The product was purified by column chromatography (petroleum ether : ethyl acetate = 10 : 1). Yield, 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, J = 8.9 Hz, 4H), 7.07 (d, J = 8.2 Hz, 1H), 6.92 – 6.77 (m, 4H), 6.70 (d, J = 2.3 Hz, 1H), 6.65 (dd, J = 8.3, 2.4 Hz, 1H), 5.84 (s, 1H), 3.78 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) (101 MHz, Acetone-d_6) δ 177.39, 159.21, 158.52, 153.25, 133.39, 129.07, 126.79, 122.24, 113.90, 111.70, 98.48, 59.50, 54.71. HRMS (ESI) calcd for $C_{22}H_{18}O_5$ $[M + H]^+$ 363.1232; found 363.1241.

6-Hydroxy-3,3-di-p-tolylbenzofuran-2(3*H*)-one (3b).

Compound **3b** was prepared by 2-hydroxy-1,2-di-p-tolylethan-1-one, resorcinol and sodium hydroxide according to the general procedure for cyclization reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 10 : 1). Yield: 36%, brown solid (mp 142-145 °C). ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.06 (s, 1H), 7.22 – 7.09 (m, 16H), 6.74 (d, J = 8.0 Hz, 3H), 2.30 (s, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.93, 138.72, 137.37, 136.86, 135.62, 134.91, 133.36, 129.26, 128.89, 128.84, 128.55, 128.47, 126.83, 94.11, 79.32, 46.94, 21.04, 21.01. HRMS (ESI) calcd for $C_{22}H_{18}O_3$ $[M + H]^+$ 331.1329; found 331.1383.

6-Hydroxy-3,3-di-*m*-tolylbenzofuran-2(3*H*)-one (3c).

Compound **3c** was prepared by 2-hydroxy-1,2-di-*m*-tolylethan-1-one, resorcinol and sodium hydroxide according to the general procedure for cyclization reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 10 : 1). Yield: 43%, yellow wax. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, J = 12.5 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H), 7.11 – 7.04 (m, 5H), 7.03 (d, J = 8.0 Hz, 2H), 6.71 (d, J = 2.2 Hz, 1H), 6.65 (dd, J = 8.3, 2.3 Hz, 1H), 6.33 (s, 1H), 2.27 (s, 6H). ^{13}C NMR

(100 MHz, $CDCl_3$) δ 178.14, 156.85, 153.27, 140.85, 138.50, 128.65, 128.59, 126.83, 125.23, 122.98, 111.89, 99.25, 61.15, 21.56. HRMS (ESI) calcd for $C_{22}H_{18}O_3$ $[M + H]^+$ 331.1329; found 331.1363.

6-Hydroxy-3,3-di-*o*-tolylbenzofuran-2(3*H*)-one (3d).

Compound **3d** was prepared by 2-hydroxy-1,2-di-*o*-tolylethan-1-one, resorcinol and sodium hydroxide according to the general procedure for cyclization reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 10 : 1). Yield: 47%, yellow wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.07 (s, 1H), 7.27 (d, J = 3.9 Hz, 2H), 7.24 – 7.20 (m, 6H), 7.16 (dd, J = 5.8, 2.7 Hz, 4H), 7.13 (s, 1H), 6.99 (d, J = 8.0 Hz, 2H), 6.88 (dd, J = 19.9, 8.0 Hz, 4H), 6.77 (d, J = 2.1 Hz, 2H), 6.75 – 6.71 (m, 1H), 2.13 (s, 4H), 2.09 (s, 4H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 177.23, 159.67, 153.71, 139.69, 139.53, 134.04, 133.33, 130.30, 129.13, 129.01, 128.57, 128.41, 128.03, 127.80, 127.19, 127.04, 126.70, 112.89, 99.04, 62.24, 22.95, 20.80. HRMS (ESI) calcd for $C_{22}H_{18}O_3$ $[M + H]^+$ 331.1329; found 331.1341.

3,3-Bis(4-chlorophenyl)-6-hydroxybenzofuran-2(3*H*)-one (3e).

Compound **3e** was prepared by 1,2-bis(4-chlorophenyl)-2-hydroxyethan-1-one, resorcinol and sodium hydroxide according to the general procedure for cyclization reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 10 : 1). Yield: 44%, yellow wax. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.43 (m, 4H), 7.15 – 7.09 (m, 4H), 7.07 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 2.2 Hz, 1H), 6.70 (dd, J = 8.3, 2.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.80, 157.14, 153.33, 138.98, 134.23, 130.33, 129.39, 129.05, 128.71, 126.61, 121.94, 112.07, 99.52, 60.06. HRMS (ESI) calcd for $C_{20}H_{12}Cl_2O_3$ $[M + H]^+$ 371.0236; found 371.0211.

3,3-Bis(4-bromophenyl)-6-hydroxybenzofuran-2(3*H*)-one (3f).

Compound **3f** was prepared by 1,2-bis(4-bromophenyl)-2-hydroxyethan-1-one, resorcinol and sodium hydroxide according to the general procedure for cyclization reaction. The product was purified by column chromatography (petroleum ether :

ethyl acetate = 10 : 1). Yield: 42%, yellow solid (mp 240-242 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 8.7 Hz, 4H), 7.12 (d, J = 8.7 Hz, 4H), 7.07 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 2.2 Hz, 1H), 6.70 (dd, J = 8.3, 2.3 Hz, 1H), 5.89 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.52, 157.24, 153.35, 139.50, 132.00, 129.72, 126.58, 122.41, 121.70, 120.74, 112.07, 99.52, 60.18. HRMS (ESI) calcd for $C_{20}H_{12}Br_2O_3$ $[M + H]^+$ 460.9205; found 460.9201.

3,3-Bis(4-chlorophenyl)-6-hydroxy-4-methylbenzofuran-2(3*H*)-one (3g).

Compound **3g** was prepared by 1,2-bis(4-chlorophenyl)-2-hydroxyethan-1-one, 5-methylbenzene-1,3-diol and sodium hydroxide according to the general procedure for cyclization reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 10 : 1). Yield: 40%, yellow wax. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 4H), 7.26 – 7.11 (m, 4H), 6.65 (d, J = 2.1 Hz, 1H), 6.48 (d, J = 1.7 Hz, 1H), 5.95 (d, J = 8 Hz, 1H), 1.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.75, 157.17, 153.47, 137.57, 136.31, 134.28, 130.26, 128.83, 120.53, 113.83, 97.22, 60.69, 18.81. HRMS (ESI) calcd for $C_{21}H_{14}Cl_2O_3$ $[M + H]^+$ 385.0393; found 385.0346.

6-Hydroxy-3,3-bis(4-hydroxyphenyl)benzofuran-2(3*H*)-one (4a).

Compound **4a** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 46%, brown solid (mp 286-288 °C). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.96 (s, 1H), 8.53 (s, 2H), 7.16 (d, J = 8.9 Hz, 1H), 7.10 – 7.03 (m, 4H), 6.85 – 6.78 (m, 4H), 6.75 – 6.70 (m, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 177.59, 158.42, 156.93, 153.22, 132.38, 129.14, 126.78, 115.29, 115.20, 111.58, 98.38, 59.46. HRMS (ESI) calcd for $C_{20}H_{14}O_5$ $[M + H]^+$ 335.0914; found 335.0973.

6-Hydroxy-3,3-bis(3-hydroxyphenyl)benzofuran-2(3*H*)-one (4b).

Compound **4b** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether: ethyl acetate = 6 : 1). Overall yield: 41%, yellow wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.10 (s, 1H), 8.54 (s, 2H), 7.23 (d, J = 2.9 Hz, 1H), 7.20 (d, J = 1.6 Hz, 1H), 7.18 (s, 1H), 6.82 (dd, J = 2.3, 0.9 Hz, 1H), 6.80 (dd, J = 2.4, 0.8 Hz, 1H), 6.79 – 6.75 (m, 5H), 6.74 (dd, J = 1.7, 0.9 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 176.66, 158.66, 157.56, 153.33, 142.66, 129.63, 126.97, 121.65, 119.03, 115.14, 114.69, 111.67, 98.43, 60.61. HRMS (ESI) calcd for $C_{20}H_{14}O_5$ $[M + H]^+$ 335.0914; found 335.0971.

6-Hydroxy-3,3-bis(2-hydroxyphenyl)benzofuran-2(3*H*)-one (4c).

Compound **4c** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether: ethyl acetate = 6 : 1). Overall yield: 50%, yellow wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.17 (s, 1H), 8.88 (s, 1H), 8.11 – 8.01 (m, 1H), 7.55 – 7.46 (m, 1H), 7.33 (dd, J = 8.5, 2.5 Hz, 1H), 7.10 (s, 1H), 7.04 (dd, J = 6.6, 2.0 Hz, 1H), 7.01 – 6.86 (m, 4H), 6.74 (d, J = 7.9 Hz, 1H), 6.68 (d, J = 18.2 Hz, 1H), 5.64 (s, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 188.81, 176.69, 162.95, 158.83, 154.89, 153.94, 136.99, 136.38, 133.24, 132.05, 131.09, 130.23, 129.66, 128.12, 59.65. HRMS (ESI) calcd for $C_{20}H_{14}O_5$ $[M + H]^+$ 335.0914; found 335.0971.

3-(4-Chlorophenyl)-6-hydroxy-3-(4-hydroxyphenyl)benzofuran-2(3*H*)-one (4d).

Compound **4d** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 36%, yellow wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.05 (s, 1H), 8.50 (s, 1H), 7.42 (d, J = 8.7 Hz, 2H), 7.34 – 7.28 (m, 2H), 7.26 – 7.15 (m, 2H), 6.81 (ddd, J = 8.2, 2.3, 1.1 Hz, 1H), 6.77 (d, J = 7.0 Hz, 2H), 6.73 – 6.69 (m, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 176.95, 170.06, 158.75, 157.21, 153.29, 140.76, 133.12, 131.48, 129.69, 129.18,

128.66, 126.84, 121.54, 115.51, 115.42, 111.88, 98.59, 59.67. HRMS (ESI) calcd for $C_{20}H_{13}ClO_4$ $[M + H]^+$ 353.0575; found 353.0522.

3-(4-Chlorophenyl)-6-hydroxy-3-(3-hydroxyphenyl)benzofuran-2(3*H*)-one (4e). Compound **4e** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 61%, yellow wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.19 (s, 1H), 8.61 (s, 1H), 7.44 – 7.39 (m, 2H), 7.32 – 7.27 (m, 2H), 7.25 – 7.21 (m, 1H), 7.20 – 7.15 (m, 1H), 6.82 – 6.78 (m, 1H), 6.77 – 6.75 (m, 2H), 6.71 – 6.69 (m, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 176.53, 158.91, 157.72, 153.34, 142.49, 140.03, 133.29, 129.88, 129.82, 128.68, 126.92, 121.04, 118.86, 114.94, 114.92, 111.96, 98.63, 60.27. HRMS (ESI) calcd for $C_{20}H_{13}ClO_4$ $[M + H]^+$ 353.0575; found 353.0584.

3-(4-Bromophenyl)-6-hydroxy-3-(4-hydroxyphenyl)benzofuran-2(3*H*)-one (4f). Compound **4f** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 53%, brown wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.03 (s, 1H), 8.60 (s, 1H), 7.56 (d, J = 8.3 Hz, 2H), 7.26 – 7.16 (m, 3H), 7.08 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 6.75 (d, J = 2.5 Hz, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 176.91, 158.79, 157.24, 153.28, 141.29, 131.66, 131.37, 130.00, 129.18, 126.83, 121.28, 115.52, 111.90, 98.59, 59.77. HRMS (ESI) calcd for $C_{20}H_{13}BrO_4$ $[M + H]^+$ 397.0070; found 397.0006.

3-(4-Bromophenyl)-6-hydroxy-3-(3-hydroxyphenyl)benzofuran-2(3*H*)-one (4g). Compound **4g** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 64%, yellow wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.20 (s, 1H), 8.62 (s, 1H), 7.62 – 7.51 (m,

2H), 7.28 – 7.21 (m, 3H), 7.21 – 7.14 (m, 1H), 6.84 – 6.80 (m, 1H), 6.79 – 6.75 (m, 2H), 6.72 (dd, J = 7.1, 1.1 Hz, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 176.46, 158.90, 157.71, 153.34, 142.41, 140.54, 131.69, 130.13, 129.89, 126.93, 121.48, 120.98, 118.88, 114.94, 111.96, 98.64, 60.35. HRMS (ESI) calcd for $C_{20}H_{13}BrO_4$ [M + H]$^+$ 397.0070; found 397.0002.

6-Hydroxy-3-(4-hydroxyphenyl)-3-phenylbenzofuran-2(3*H*)-one (4h).

Compound **4h** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 61%, yellow solid (mp 288-290 °C). ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.03 (s, 1H), 8.60 (s, 1H), 7.38 – 7.32 (m, 2H), 7.30 (dt, J = 5.6, 2.3 Hz, 1H), 7.27 – 7.22 (m, 2H), 7.19 (d, J = 8.4 Hz, 1H), 7.11 – 7.04 (m, 2H), 6.86 – 6.79 (m, 2H), 6.74 (dd, J = 10.2, 2.2 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 177.26, 158.59, 157.09, 153.29, 141.98, 131.79, 129.31, 128.63, 127.83, 127.54, 126.90, 122.03, 115.40, 111.73, 98.50, 60.18. HRMS (ESI) calcd for $C_{20}H_{14}O_4$ [M + H]$^+$ 319.0965; found 319.0906.

6-Hydroxy-3-(4-hydroxyphenyl)-3-isopropylbenzofuran-2(3*H*)-one (4i).

Compound **4i** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 47%, yellow wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.92 (s, 1H), 8.46 (s, 1H), 7.31 (dd, J = 12.4, 8.5 Hz, 3H), 6.90 – 6.74 (m, 4H), 6.72 (d, J = 2.2 Hz, 1H), 2.80 (p, J = 6.8 Hz, 1H), 0.91 (d, J = 6.9 Hz, 3H), 0.74 (d, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 178.38, 158.45, 156.90, 154.33, 129.19, 128.32, 127.11, 118.09, 115.33, 110.99, 98.36, 58.88, 36.60, 17.47, 16.45. HRMS (ESI) calcd for $C_{17}H_{16}O_4$ [M + H]$^+$ 285.1121; found 285.1130.

6-Hydroxy-3-(3-hydroxyphenyl)-3-isopropylbenzofuran-2(3*H*)-one (4j).

Compound **4j** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 51%, brown wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.95 (s, 1H), 8.42 (s, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.19 (t, J = 7.9 Hz, 1H), 7.00 – 6.92 (m, 2H), 6.78 (td, J = 7.7, 7.1, 2.1 Hz, 2H), 6.72 (d, J = 2.3 Hz, 1H), 2.86 – 2.78 (m, 1H), 0.92 (d, J = 6.9 Hz, 4H), 0.76 (d, J = 6.6 Hz, 4H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 177.83, 158.56, 157.65, 154.29, 140.15, 129.57, 127.12, 118.19, 117.93, 114.51, 114.28, 111.05, 98.34, 59.52, 36.69, 17.44, 16.53. HRMS (ESI) calcd for $C_{17}H_{16}O_4$ $[M + H]^+$ 285.1121; found 285.1122.

6-Hydroxy-3-(2-hydroxyphenyl)-3-isopropylbenzofuran-2(3*H*)-one (4k).

Compound **4k** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 6 : 1). Overall yield: 56%, brown wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.68 (s, 1H), 8.36 (s, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.29 – 7.21 (m, 1H), 7.09 – 7.00 (m, 4H), 6.44 (dd, J = 8.6, 2.5 Hz, 1H), 6.33 (d, J = 2.5 Hz, 1H), 2.99 (dq, J = 13.4, 6.7 Hz, 2H), 1.12 (d, J = 6.6 Hz, 4H), 0.77 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.20, 163.34, 157.21, 129.00, 110.74, 59.32, 36.98, 32.79, 31.87, 29.72, 17.56. HRMS (ESI) calcd for $C_{17}H_{16}O_4$ $[M + H]^+$ 285.1121; found 285.1109.

6-Hydroxy-3-(4-hydroxyphenyl)-3-isopropyl-4-methylbenzofuran-2(3*H*)-one (4l).

Compound **4l** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 8 : 1). Overall yield: 39%, yellow wax. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.01 (s, 1H), 8.38 (s, 1H), 7.37 (d, J = 8.7 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 6.61 (s, 1H), 6.56 (s, 1H), 3.01 (dt, J = 14.6, 7.5 Hz, 1H), 2.31 (s, 3H), 1.00 (d, J = 6.9 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.31, 155.33, 154.55, 152.17, 140.94, 128.87, 128.56, 115.68,

112.99, 104.48, 60.47, 36.00, 21.08, 18.84, 18.04. HRMS (ESI) calcd for $C_{18}H_{18}O_4$ $[M + H]^+$ 299.1278; found 299.1279.

6-Hydroxy-3-(3-hydroxyphenyl)-3-isopropyl-4-methylbenzofuran-2(3*H*)-one (4m).

Compound **4m** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 8 : 1). Overall yield: 42%, yellow solid (mp 145-147 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.43 (s, 1H), 7.32 – 7.08 (m, 1H), 6.85 – 6.80 (m, 1H), 6.78 – 6.73 (m, 2H), 6.54 (d, *J* = 2.2 Hz, 1H), 6.48 – 6.44 (m, 1H), 3.11 (dt, *J* = 13.6, 6.8 Hz, 1H), 3.05 (s, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 177.65, 157.94, 157.51, 154.10, 139.48, 136.49, 129.44, 119.72, 119.00, 115.06, 114.36, 113.44, 95.92, 60.74, 32.20, 18.84, 18.41, 17.15. HRMS (ESI) calcd for $C_{18}H_{18}O_4$ $[M + H]^+$ 299.1278; found 299.1293.

6-Hydroxy-3-(2-hydroxyphenyl)-3-isopropyl-4-methylbenzofuran-2(3H)-one (4n).

Compound **4n** was prepared according to the general procedure for sequential process of condensation and demethylation reaction. The product was purified by column chromatography (petroleum ether : ethyl acetate = 8 : 1). Overall yield: 33%, brown wax. ^{1}H NMR (400 MHz, Acetone-d6) δ 8.80 (s, 1H), 8.61 (s, 1H), 7.66 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.15 – 6.94 (m, 2H), 6.81 (dd, *J* = 8.0, 1.3 Hz, 1H), 6.42 (d, *J* = 2.2 Hz, 1H), 6.30 (d, *J* = 2.2 Hz, 1H), 3.21 – 3.16 (m, 1H), 1.88 (s, 3H), 1.26 (d, *J* = 6.6 Hz, 3H), 0.74 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, Acetone-d6) δ 176.47, 157.37, 155.08, 155.02, 134.72, 129.50, 128.18, 124.89, 120.32, 118.65, 115.89, 112.22, 95.17, 55.90, 29.76, 17.11, 16.72, 16.69. HRMS (ESI) calcd for C18H18O4 $[M + H]^+$ 299.1278; found 299.1278.

5. Experimental procedure for biological assays

Estrogen Receptor Binding Affinity Relative binding affinities were determined by a competitive fluorometric binding assay as previously described. Briefly, 40 nM of a fluorescence tracer (coumestrol, Sigma-Aldrich, MO) and 0.8 μM purified human ERα or ERβ ligand binding domain (LBD) were diluted in 100 mM potassium phosphate buffer (pH 7.4), containing 100 μg mL^{-1} bovine gamma globulin (Sigma-Aldrich, MO). Incubations were for 2 h at room temperature (25 °C). Fluorescence polarization values were then measured. The binding affinities are expressed as relative binding affinity (RBA) values with the RBA of 17β-estradiol set to 100%. The values given are the average ± range of two independent determinations. IC_{50} values were calculated according to equations described previously.[2]

Gene Transcriptional Activity The human embryonic kidney cell line HEK 293T was maintained in Dulbecco's Minimum Essential Medium (DMEM) (Gibco by Invitrogen Corp., CA) with 10% fetal bovine serum (FBS) (Hylcone by Thermo Scientific, UT). Cells were plated in phenol red-free DMEM with 10% FBS. HEK 293T cells were transfected with 25 μL of the mixture per well, containing 300 ng of the 3 × ERE-luciferase reporter, 100 ng of the ERα or ERβ expression vector, 125 mM calcium chloride (GuoYao, China) and 12.5 μL 2 × HBS. On the next day, the cells were treated with increasing doses of ER ligands diluted in phenol red-free DMEM with 10% FBS. After 24 h, luciferase activity was measured using a Dual-Luciferase Reporter Assay System (Promega, MI) according to the manufacturer's protocol.[3]

Cell Culture and Cell Viability Assay The human breast cancer cell line MCF-7 was obtained from ATCC. DU-145 and U-87, and Vero cells were obtained from the cell bank of Chinese Academy of Science (Shanghai, China). Cells were maintained in DMEM with 10% FBS. For all experiments, cells were grown in 96-well microtiter plates (Nest Biotech Co., China) with appropriate ligand triplicates for 72 h. MTT

colormetric tests (Biosharp, China) were employed to determine cell viability per the manufacturer's instructions. IC_{50} values were calculated according to the following equation using Origin software: Y = 100% inhibition + (0% inhibition − 100% inhibition)/(1 + $10^{[(LogIC50-X)\times Hillslope]}$), where Y = fluorescence value, and X = log[inhibitor].[2,4]

Flow cytometry analysis of apoptosis U-87 cells were plated in 6-well plates 24h and then treated with diffident concentrations of **4n** for 24h. All cells were harvested and then washed by PBS. The following steps were based on the manufacturer's instructions by the Annexin V-FITC/ Propidium Iodide (PI) Apoptosis Detection Kit (BD Biosciences, USA). After being stained with 5μL Annexin V-FITC and 5 μL PI for 15 min, fluorescence was measured by a flow cytometer (Beckman Coulter, USA) with 10,000 events per sample and analysis by Summit 4.3 software (Beckman Coulter, USA).

6. Effects of 4e, 4i, 4m, 4n on the Transcriptional Activities of *ERα and ERβ*

	ERα (Agonist mode)		ERβ (Antagonist mode)	
Compound	EC_{50} (μM)	Eff (% E_2)	EC_{50} (μM)	Eff (% E_2)
4e	2.99	39 ± 4	0.073	61 ± 3
4i	0.284	70 ± 3	0.831	36 ± 2
4m	0.51	45 ± 7	0.428	83 ± 10
4n	-	16 ± 5	3.01	68 ± 6

[a] Luciferase activity was measured in HEK293T cells transfected with 3 × ERE-driven luciferase reporter and expression vectors encoding ERα or ERβ and treated in triplicate with increasing doses (up to 10^{-5} M) of the compounds.

7 The annexin V-FITC/PI staining assays on compound 4n

To elucidate the association between compound 4n and apoptosis, Annexin V-FITC/PI staining assays were then conducted. The results showed that compound 4n could strongly induce apoptosis of U87 cancer cells compared to the control group and apoptosis was induced in a concentration dependent manner (12.57% in 5 μM, 14.72% in 10 μM, 36.66% in 50 μM). These phenomena suggest that the effect of compound 4n on U87 cells is associated with apoptosis

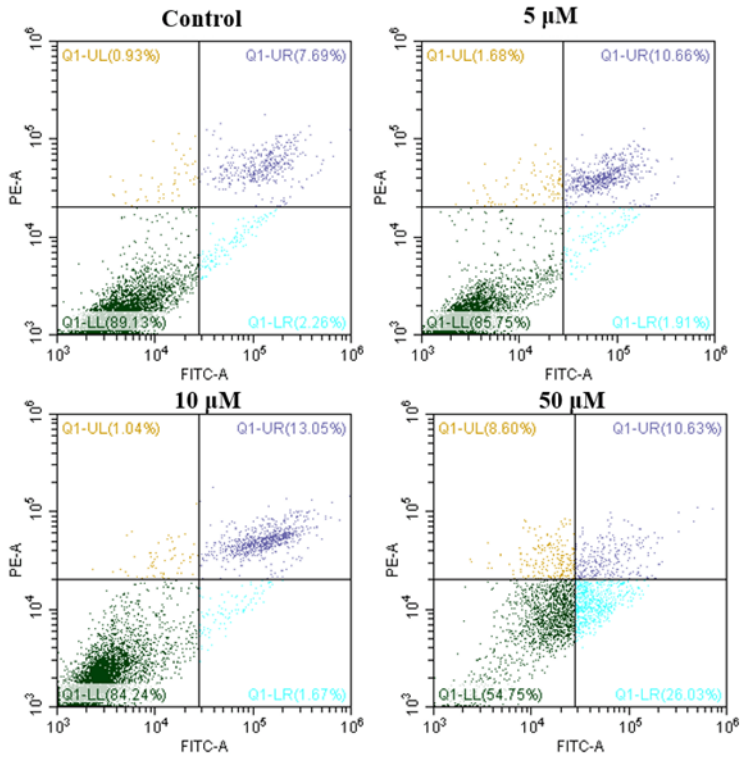
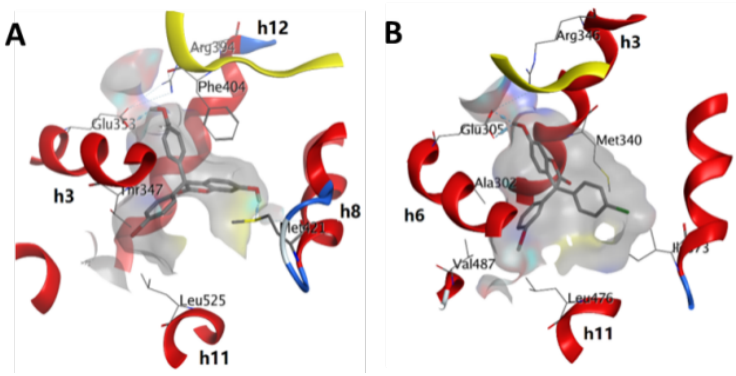


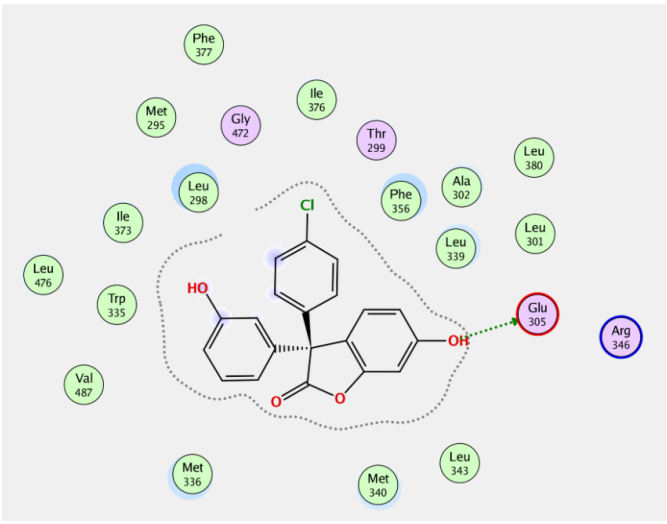
Figure S1. Compound **4n** induced apoptosis of U87 cancer cells. U87 cells were treated with different concentrations (0 μM, 5 μM, 10 μM, 50 μM) of compound **4n** for 24 h.

8 Molecular modeling

The crystal structures of ERα and ERβ were downloaded from the protein data bank (ERα PDB code: 3ERD[5] and ERβ PDB code: 2I0G,[6] respectively), and all water molecules were removed. Compounds **4f** and **4e** were respectively docked into the three-dimensional structure of ERα and ERβ LBD with AutoDock Vina software.[7] Crystallographic coordinates of **4f** and **4e** were created by Biochemoffice. Preparations of all ligands and the protein were performed with AutoDockTools (ADT). A docking cube with points of 40 Å, 40 Å, 40 Å in the X, Y, and Z dimensions was performed center on these compounds, and the grid spacing is 0.375 Å. The figures were prepared using PyMOL.



Models of benzofuranone compounds bound to ERα and ERβ. (A) Computer-developed model of **4f** bound to ERα (PDB: 3ERD) with the conserved H-bond to Arg394; (B) Computer-developed model of **4e** bound to ERβ (PDB: 2I0G) with the conserved H-bond to Glu305.

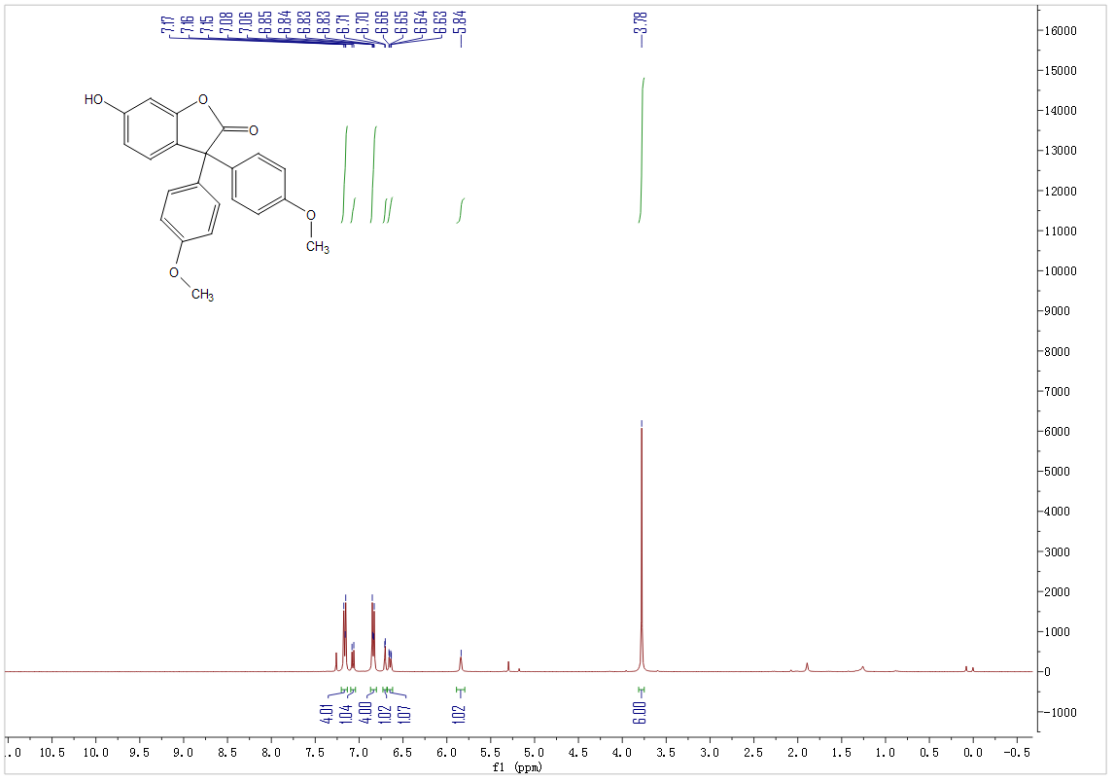


Computer-developed model of **4e** bound to ERβ

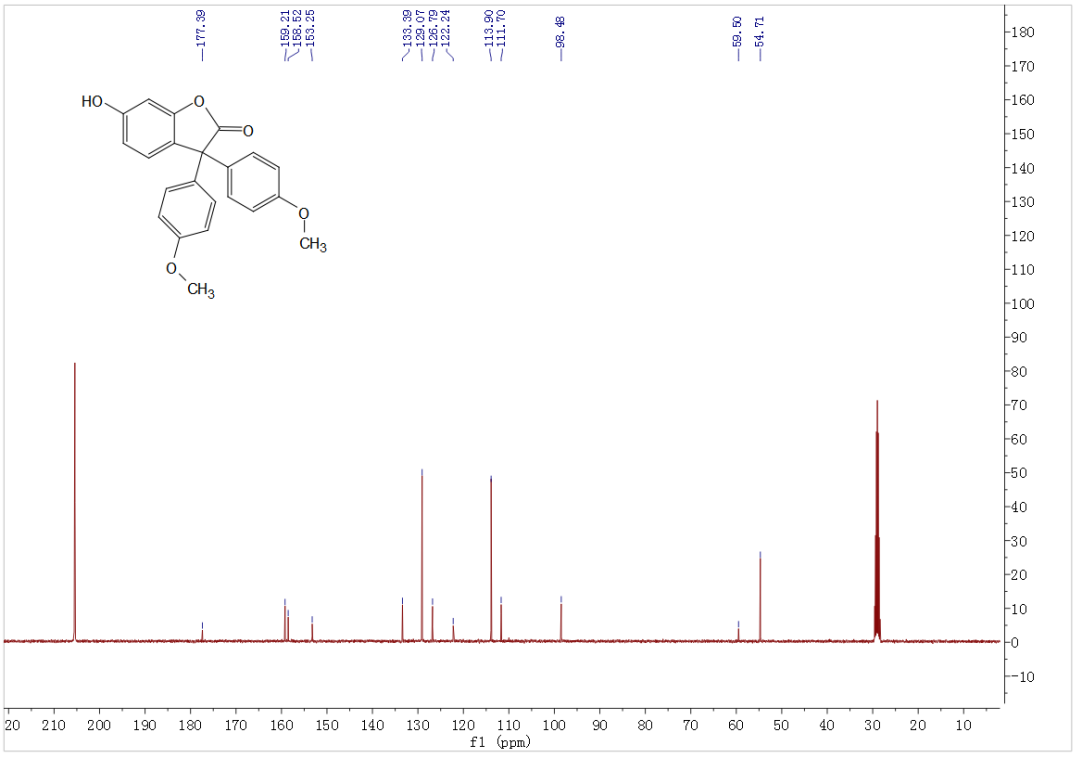
9 ^{1}H NMR and ^{13}C NMR spectra of final products

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer (400 MHz, ^{1}H NMR; 101 MHz, ^{13}C NMR) at room temperature. NMR spectra were calibrated to the solvent signals of $CDCl_3$ (δ 7.26 and 77.00), Acetone-d_6 (δ 2.05 and 29.84, 206.26). The chemical shifts are provided in ppm and the coupling constants in Hz. The following abbreviations for multiplicities are used: s, singlet; d, doublet; t, triplet; m, multiplet. Glassware was oven-dried, assembled while hot, and cooled under an inert atmosphere. Unless otherwise noted, all reactions were conducted in an inert atmosphere. Reaction progress was monitored using analytical thin-layer chromatography (TLC). Visualization was achieved by UV light (254 nm). Chromatography was performed with silica gel (0.040-0.063 mm) packing.

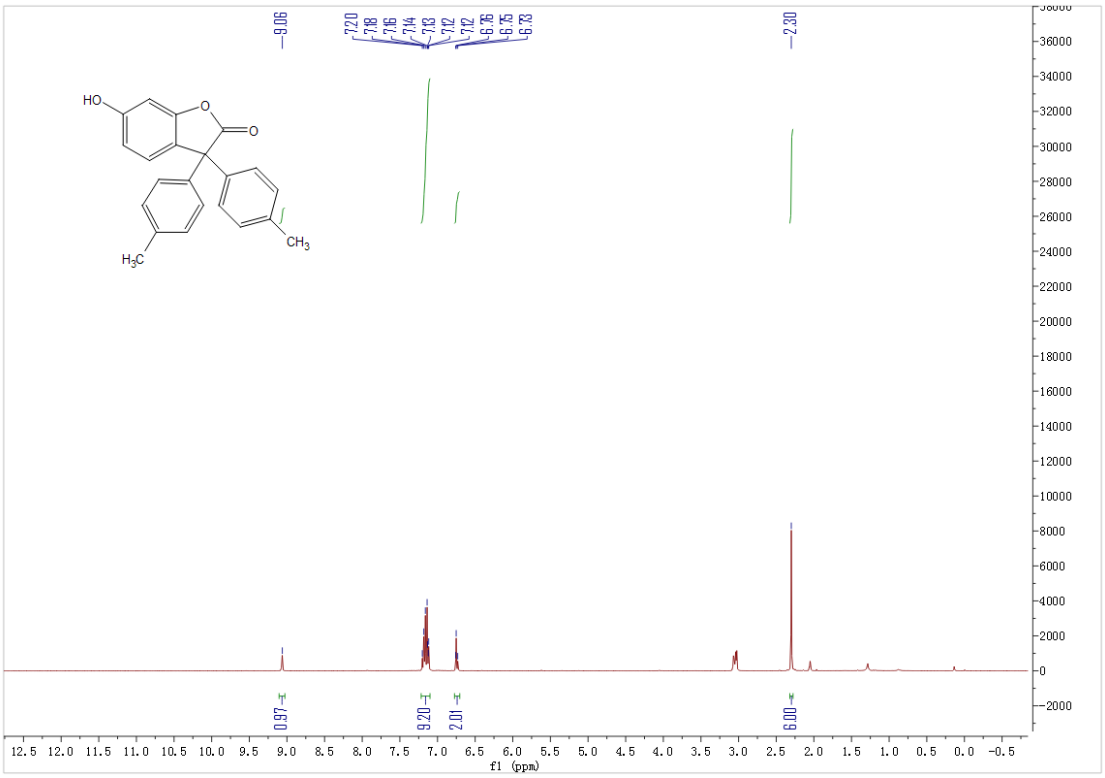
^{1}H NMR of compound **3a**



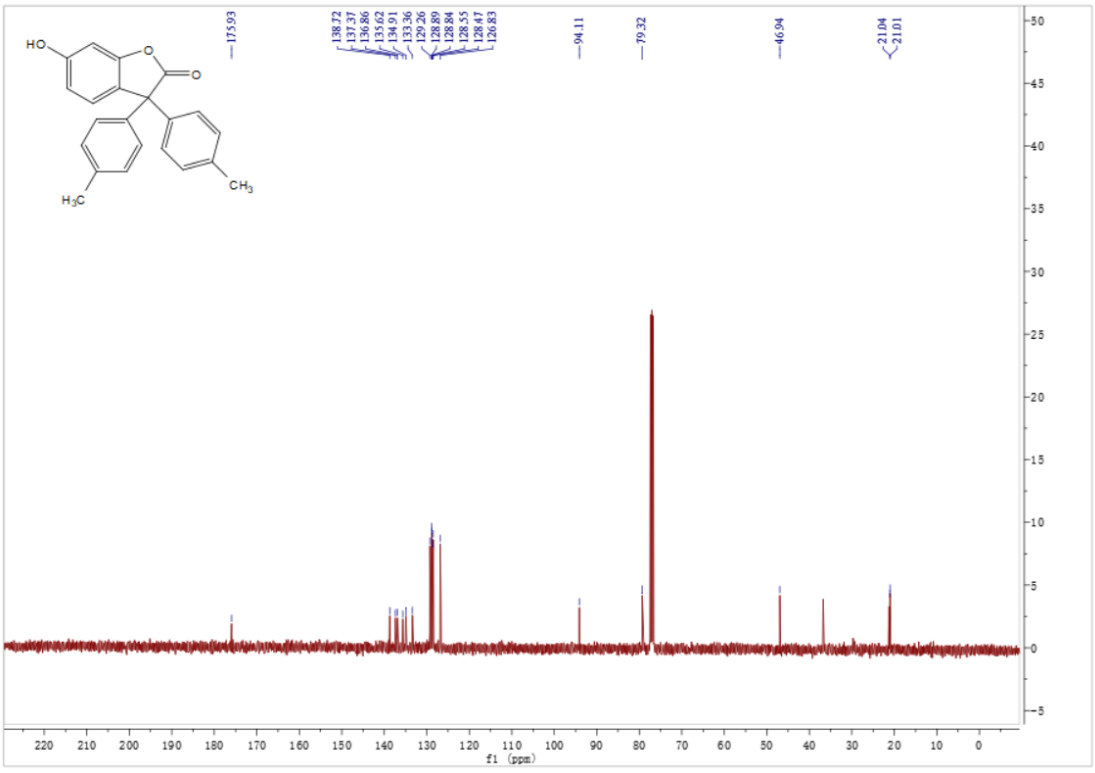
^{13}C NMR of compound **3a**



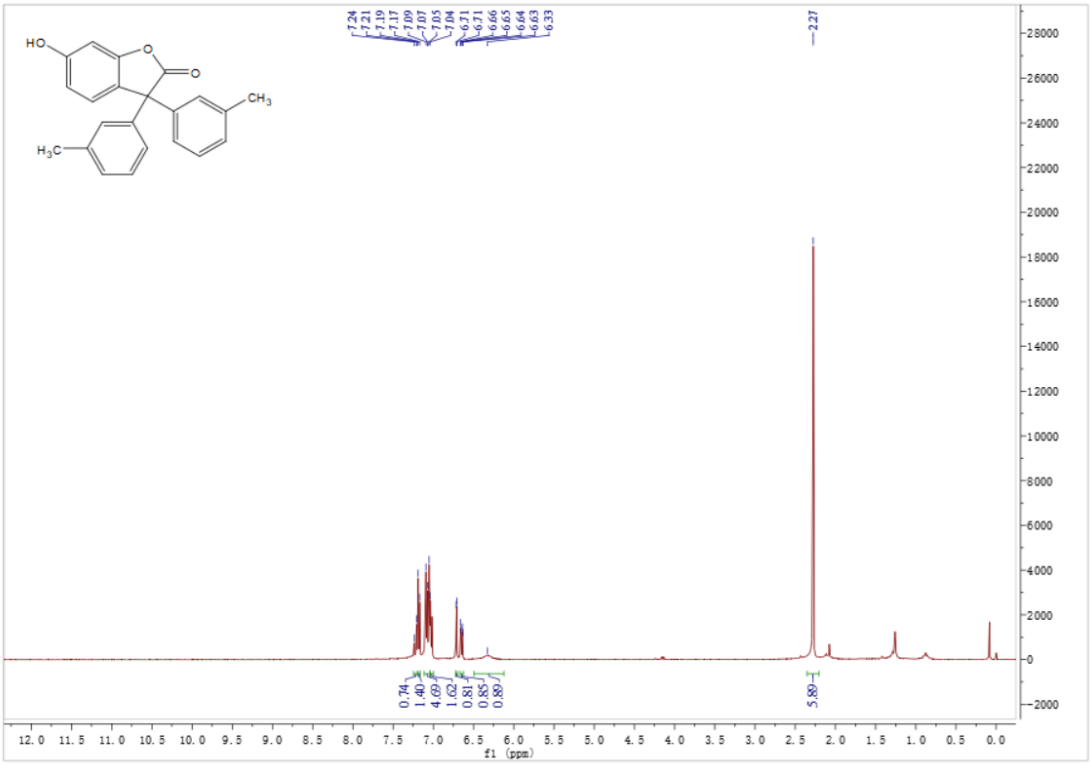
^{1}H NMR of compound **3b**



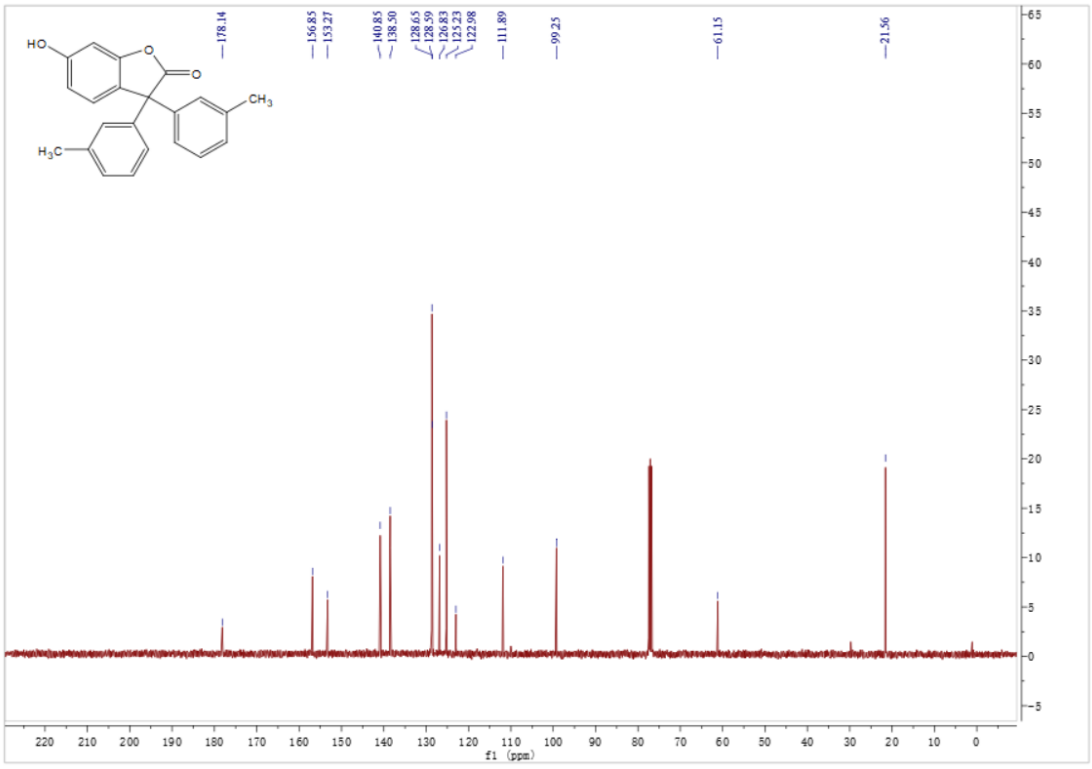
^{13}C NMR of compound **3b**



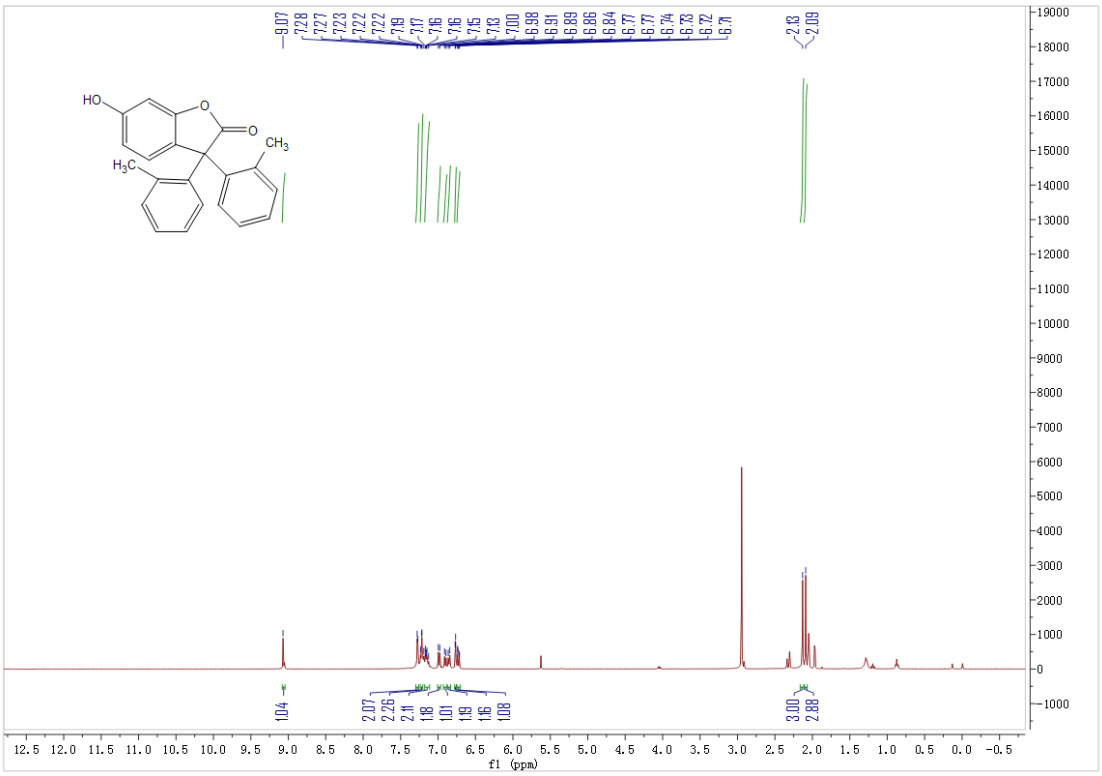
^{1}H NMR of compound **3c**



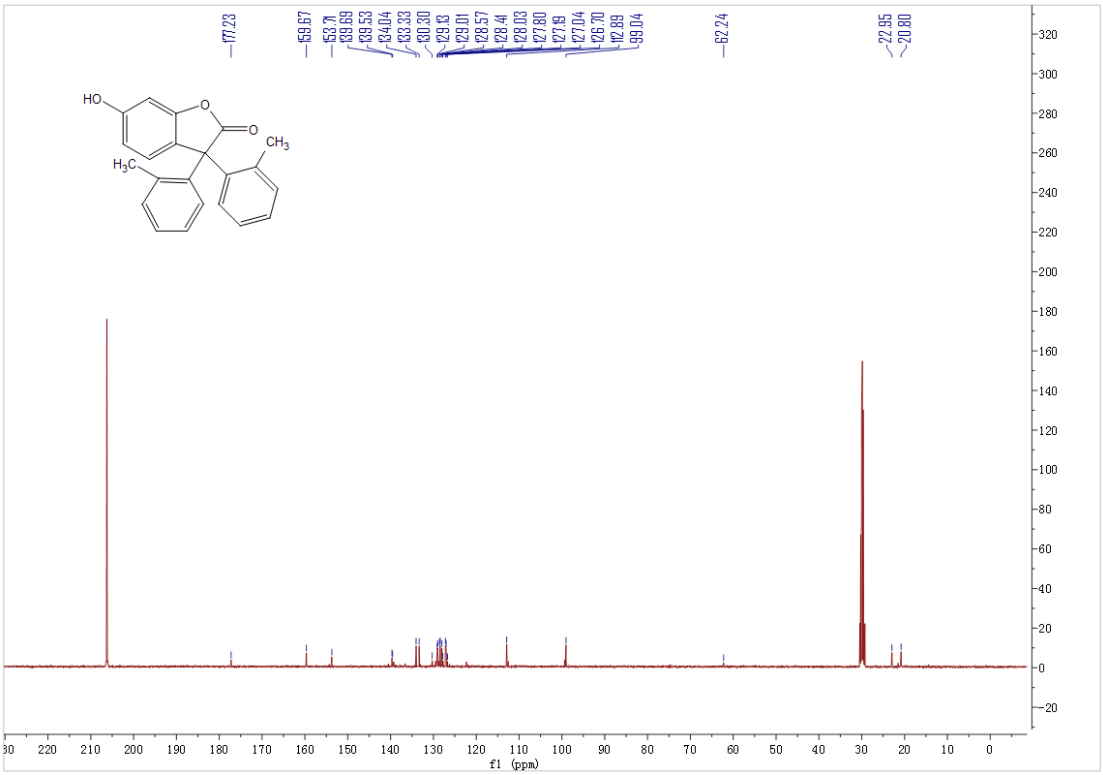
^{13}C NMR of compound **3c**



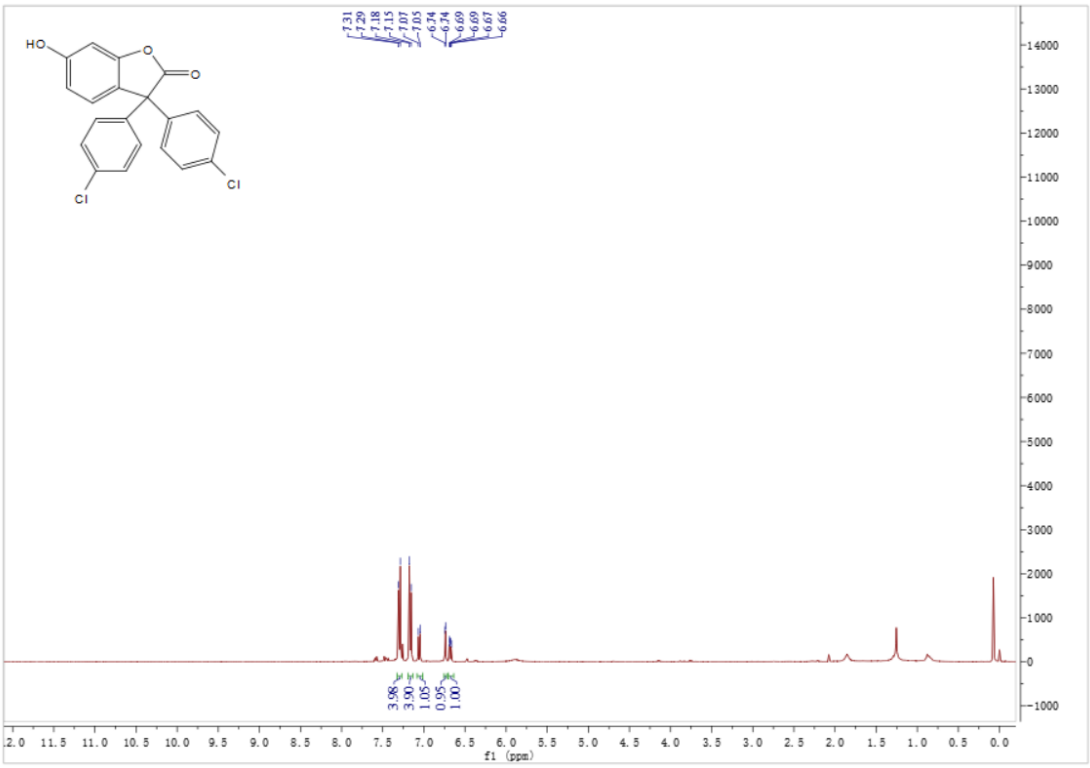
^{1}H NMR of compound **3d**



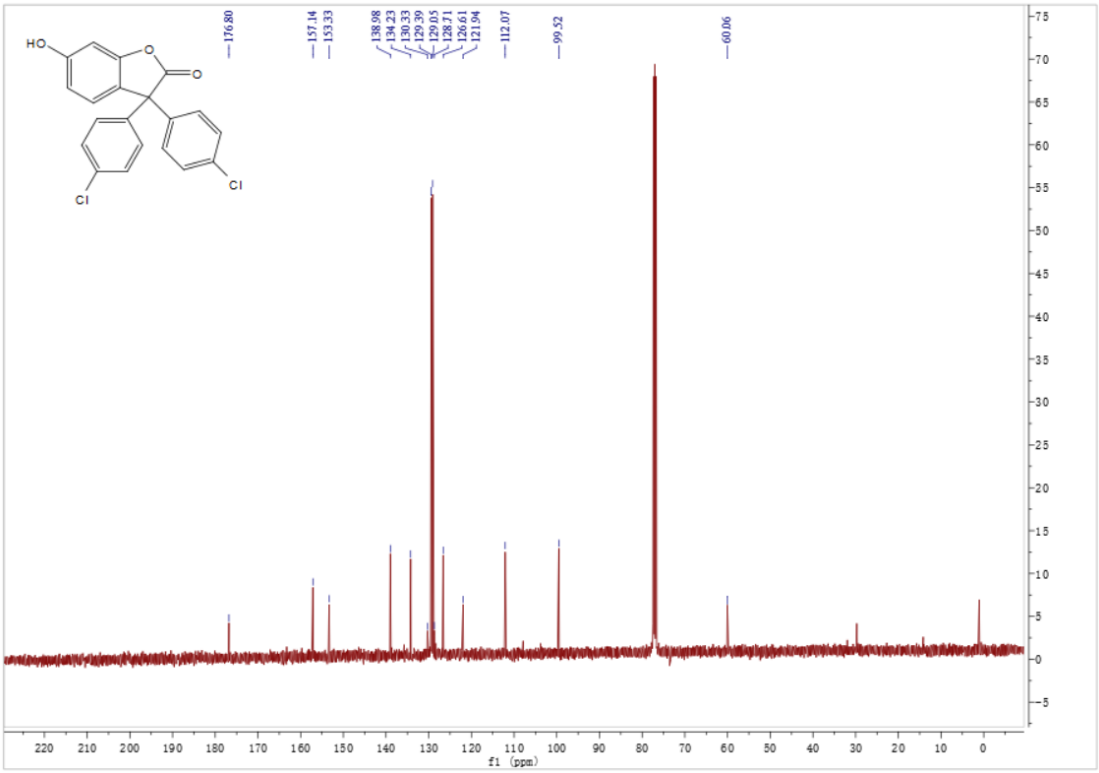
^{13}C NMR of compound **3d**



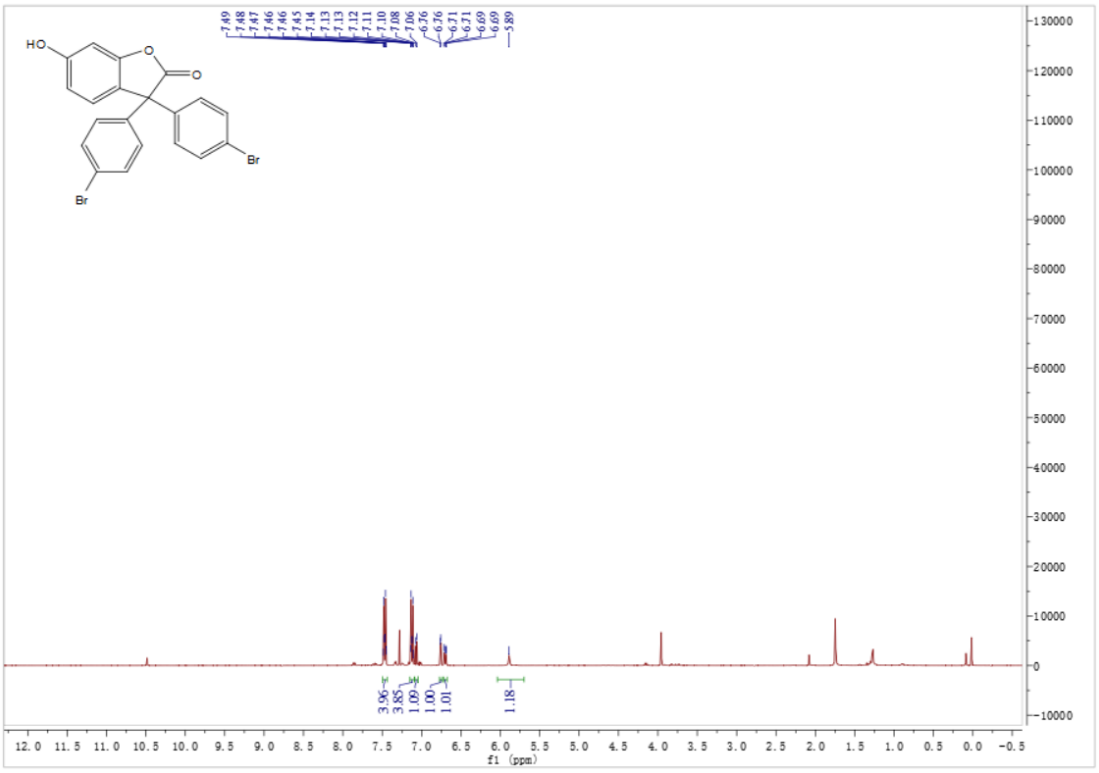
^{1}H NMR of compound **3e**



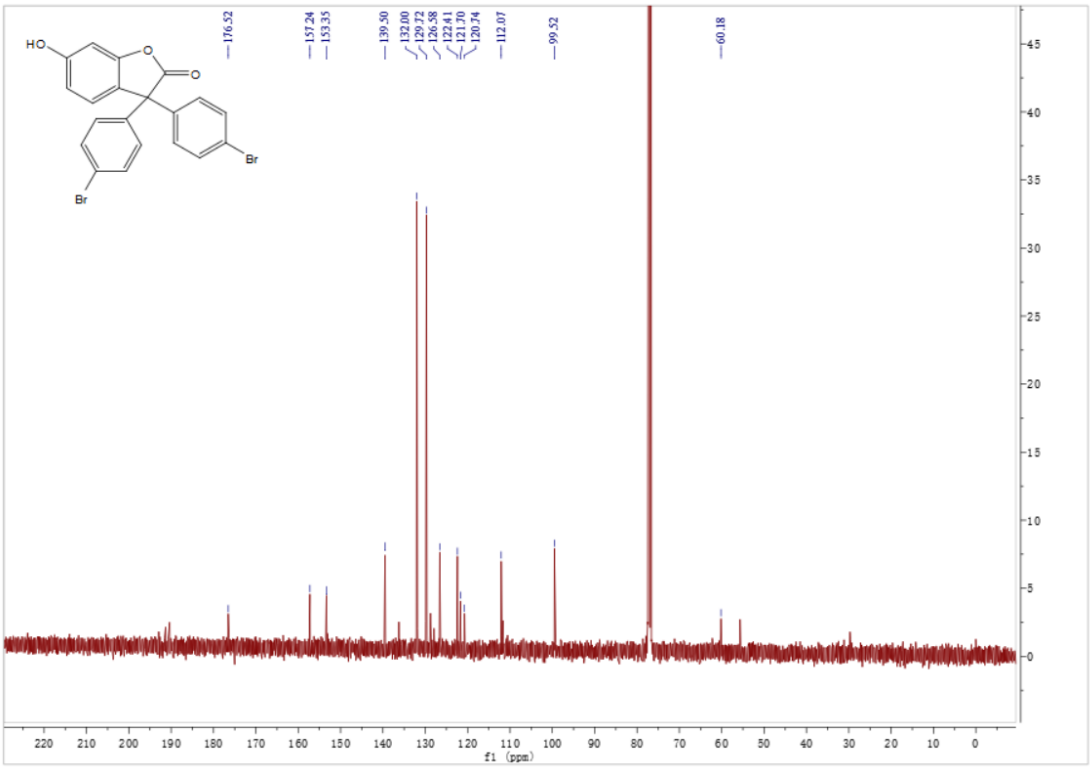
^{13}C NMR of compound **3e**



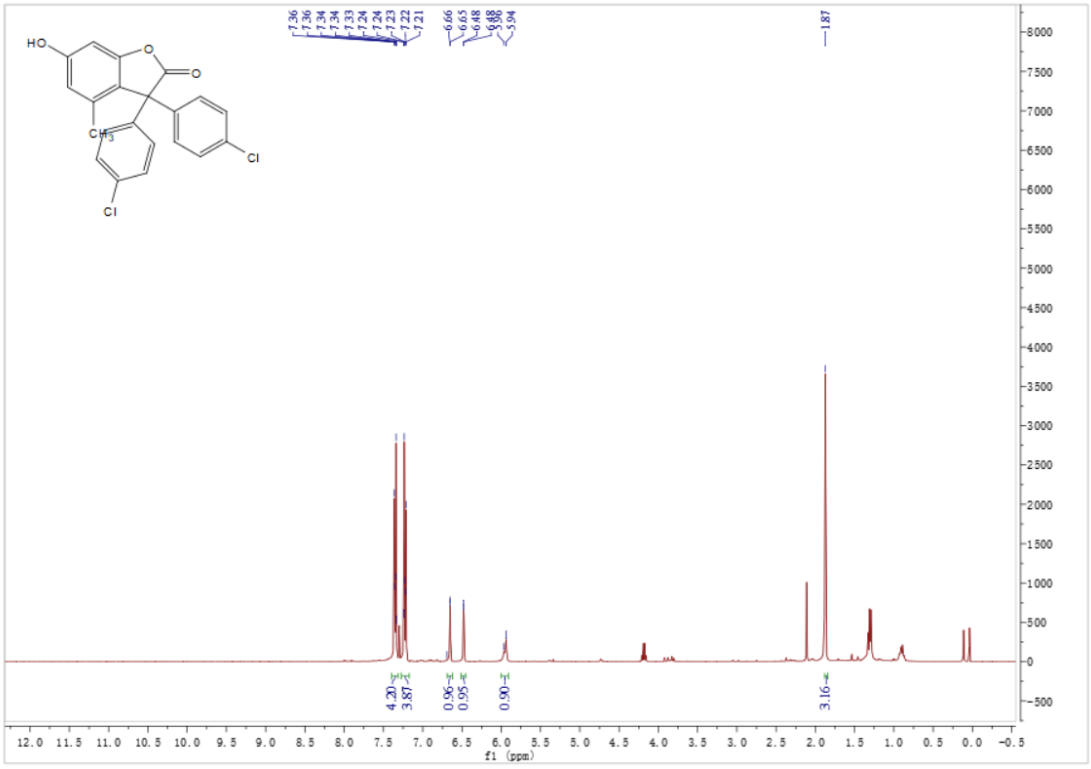
^{1}H NMR of compound **3f**



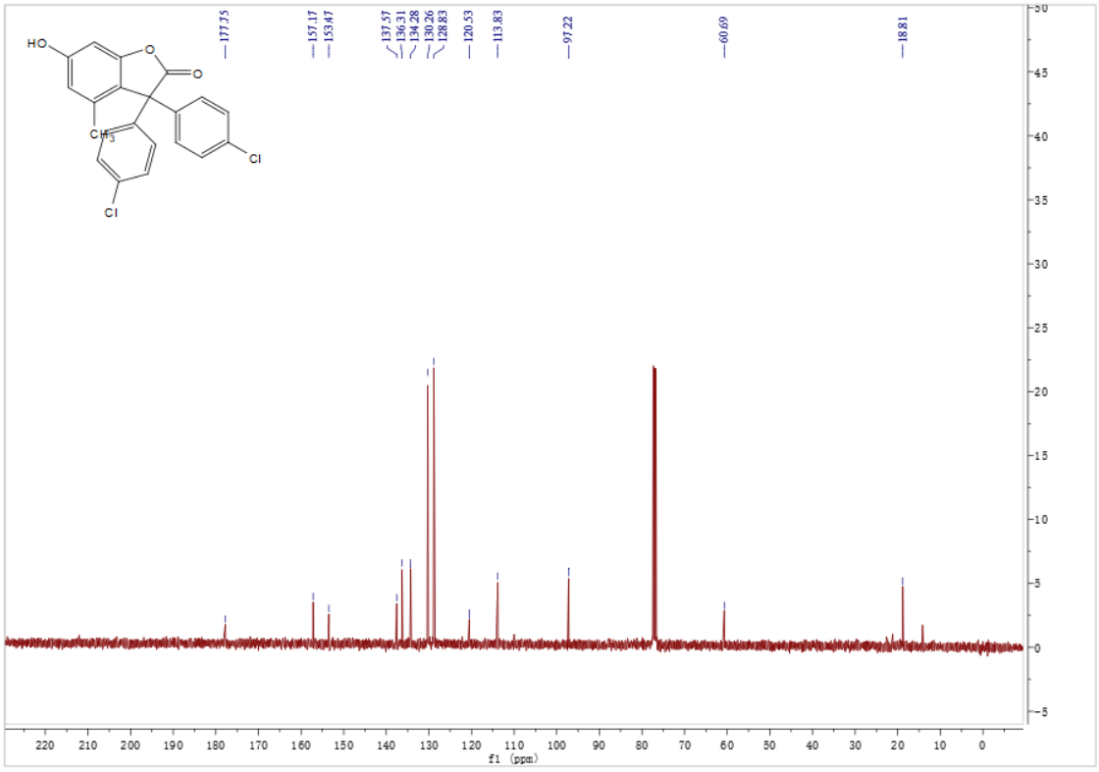
^{13}C NMR of compound **3f**



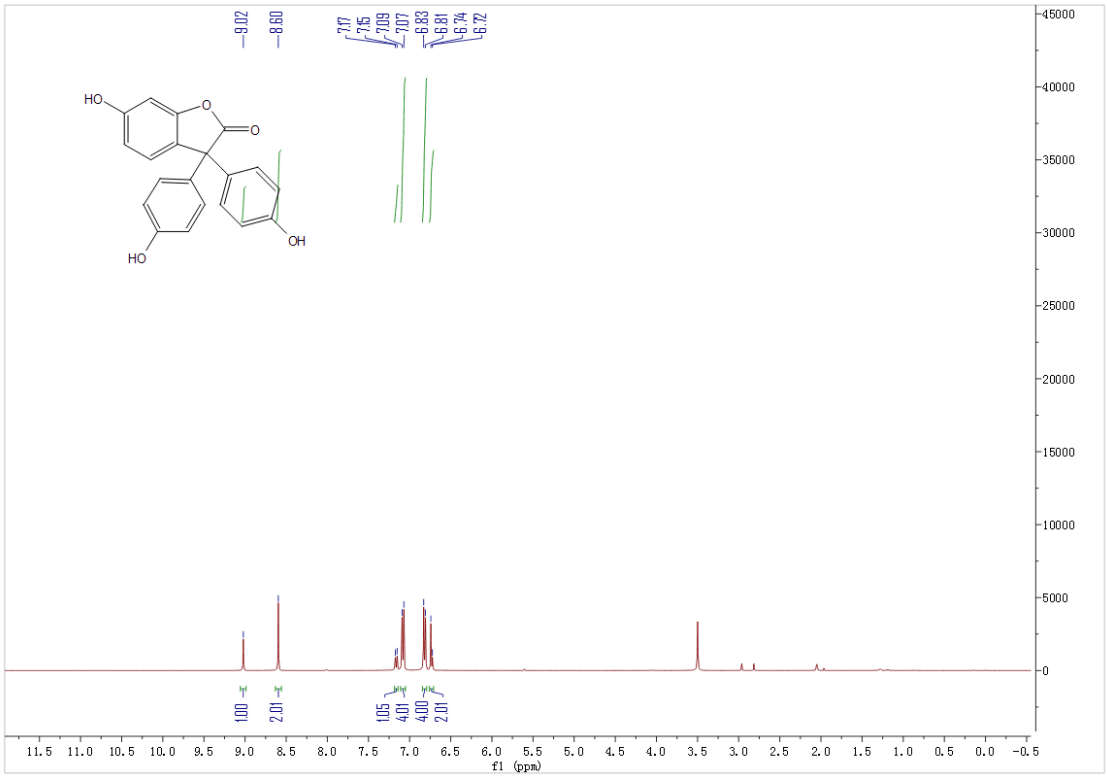
^{1}H NMR of compound **3g**



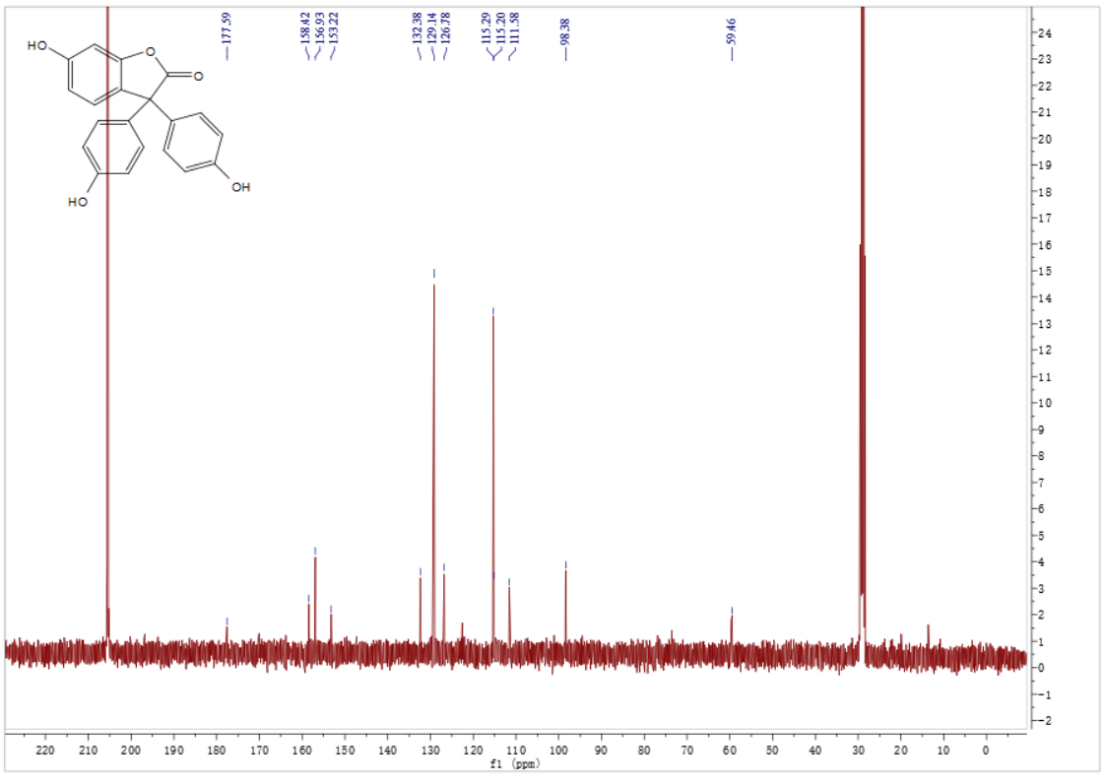
^{13}C NMR of compound **3g**



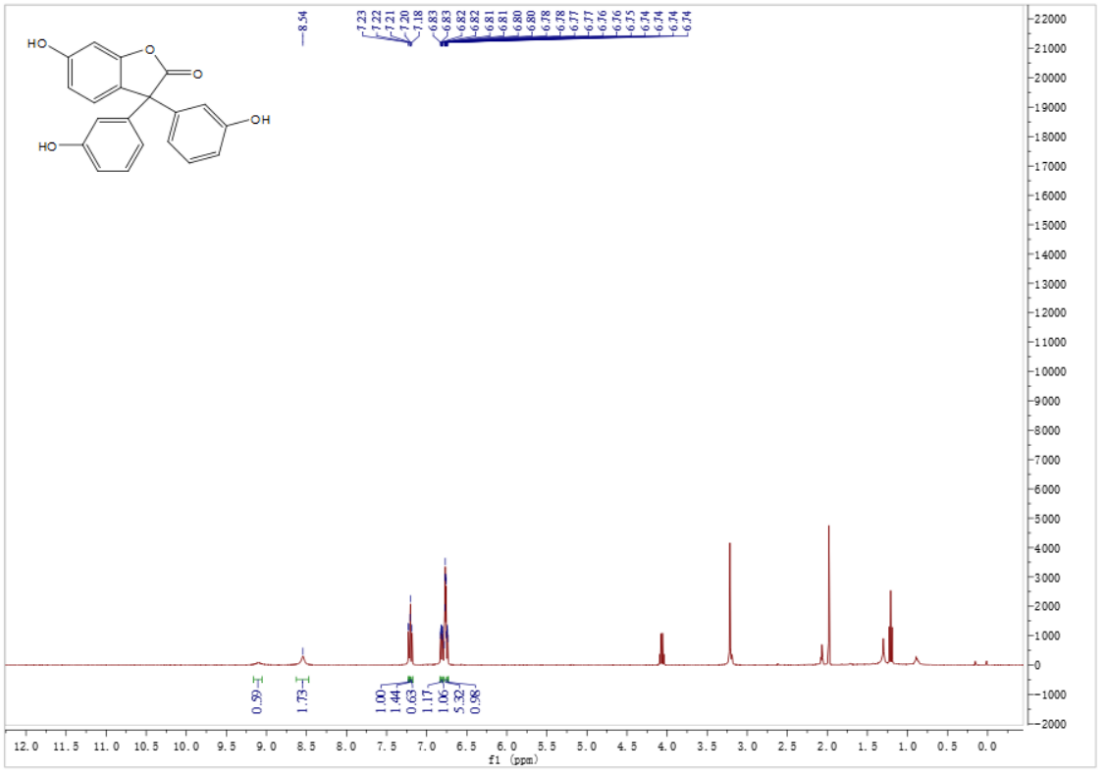
^{1}H NMR of compound **4a**



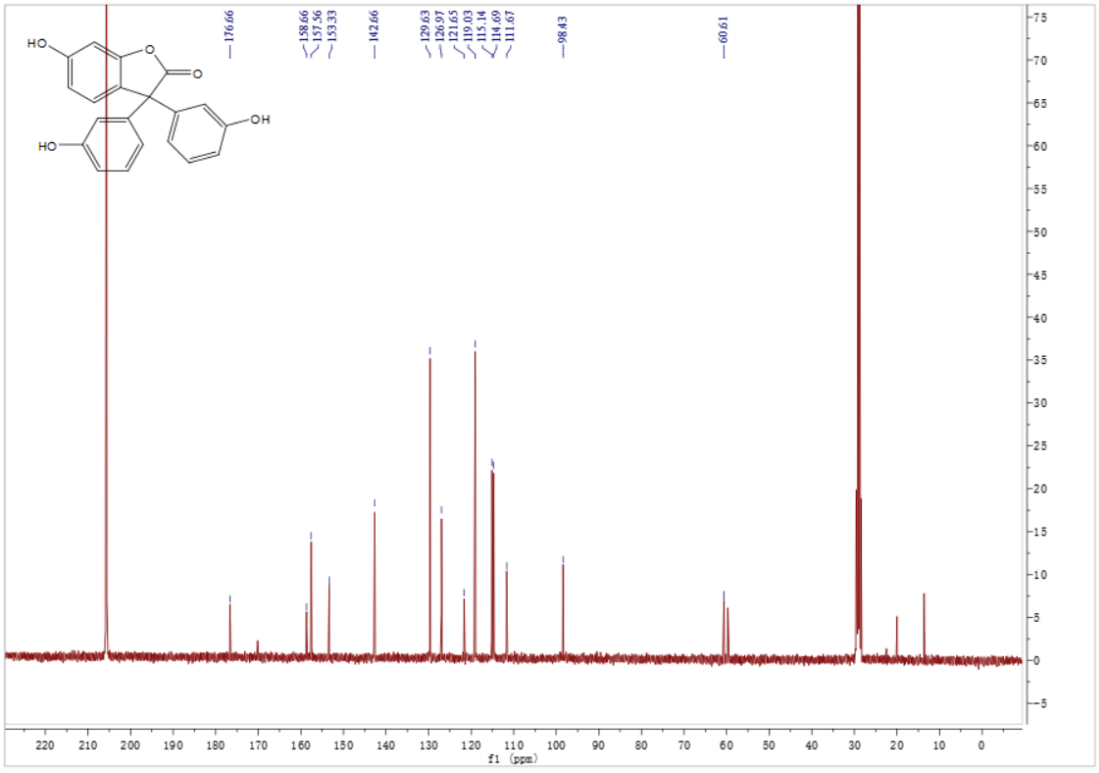
^{13}C NMR of compound **4a**



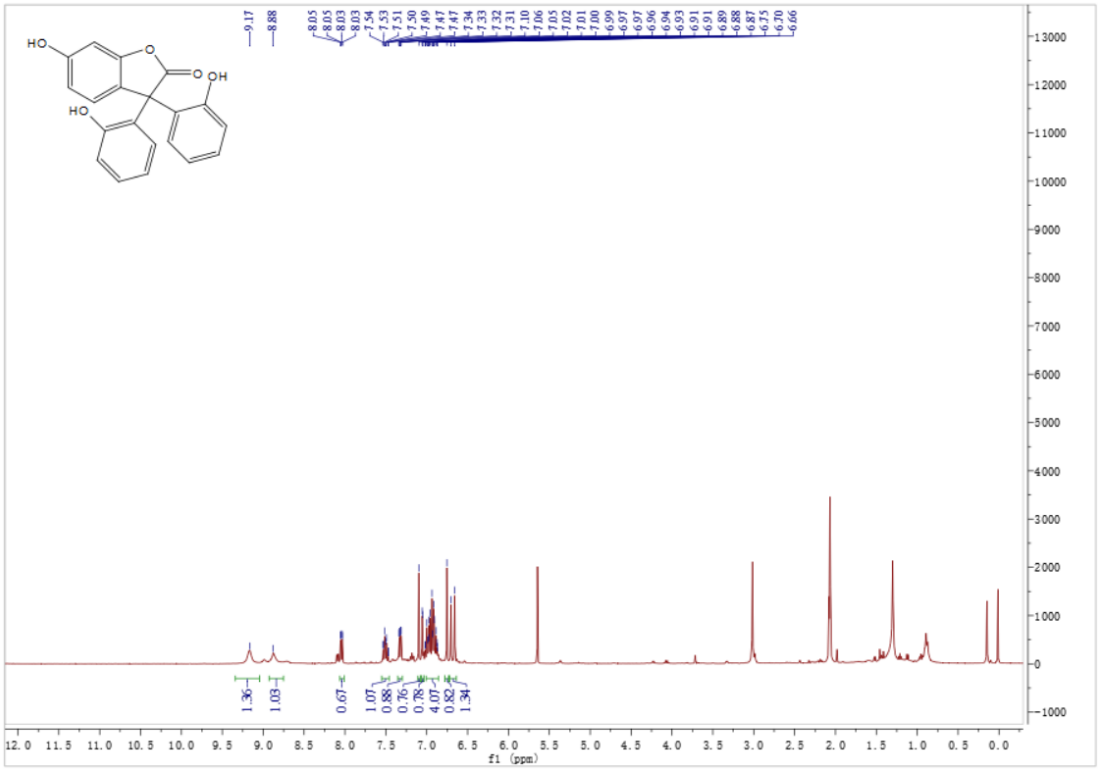
^{1}H NMR of compound **4b**



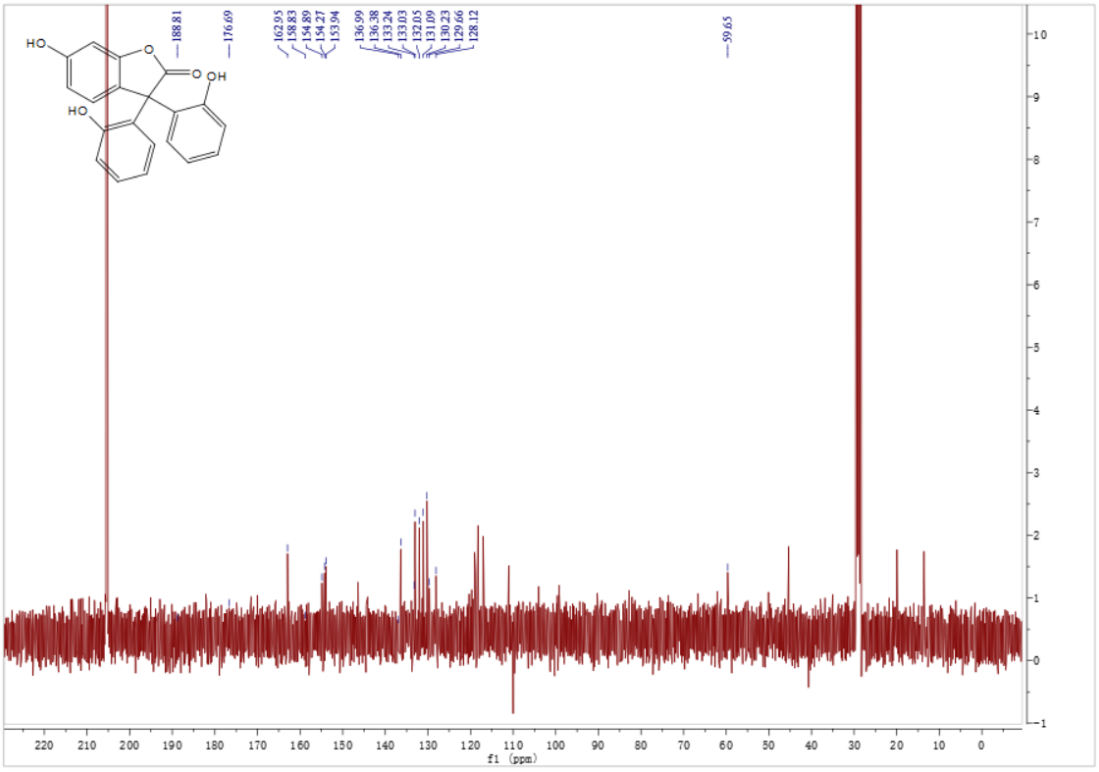
^{13}C NMR of compound **4b**



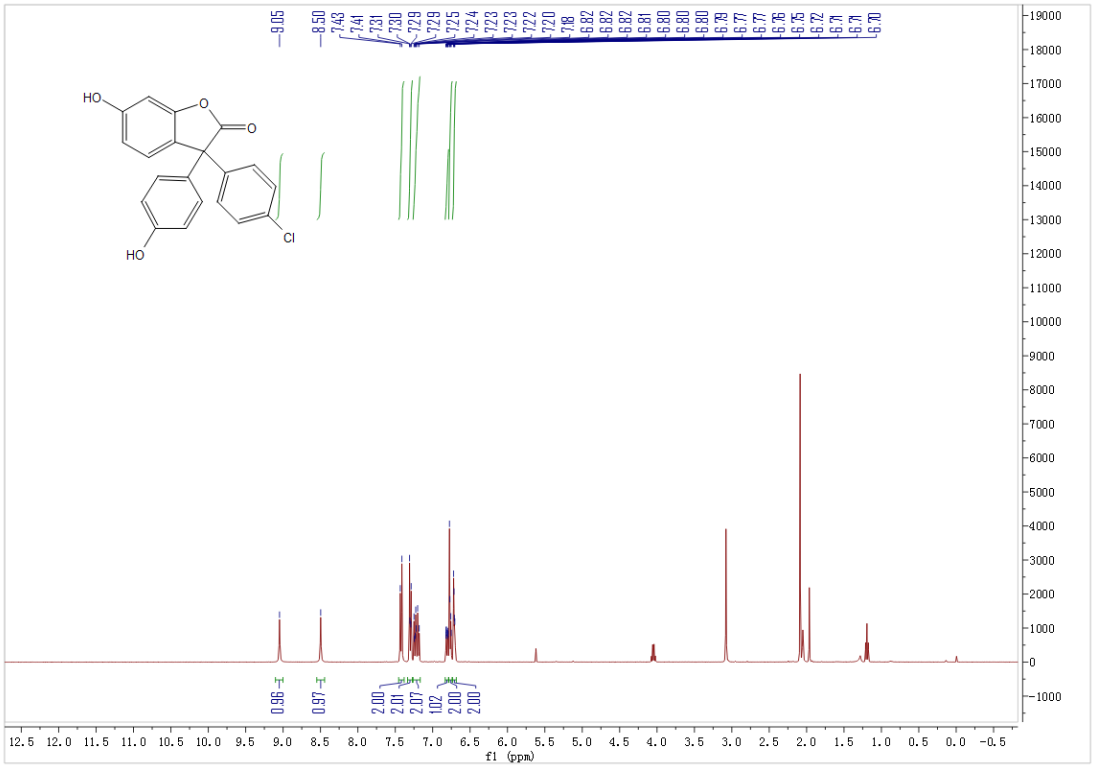
^{1}H NMR of compound **4c**



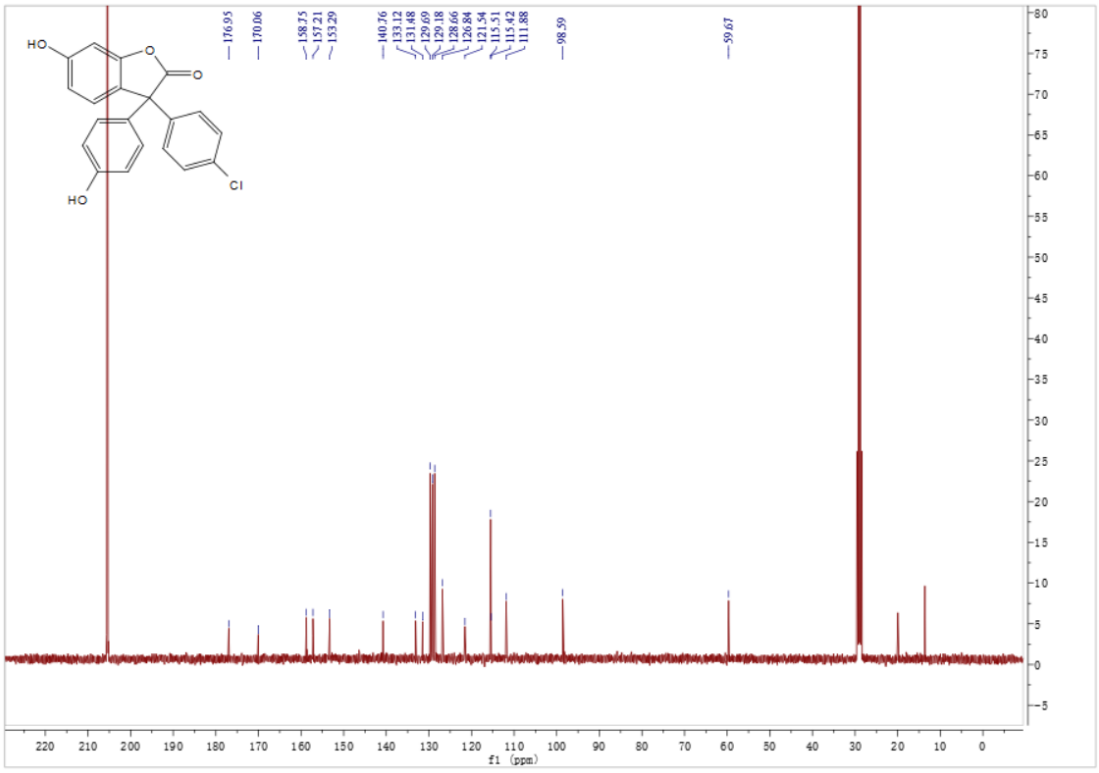
^{13}C NMR of compound **4c**



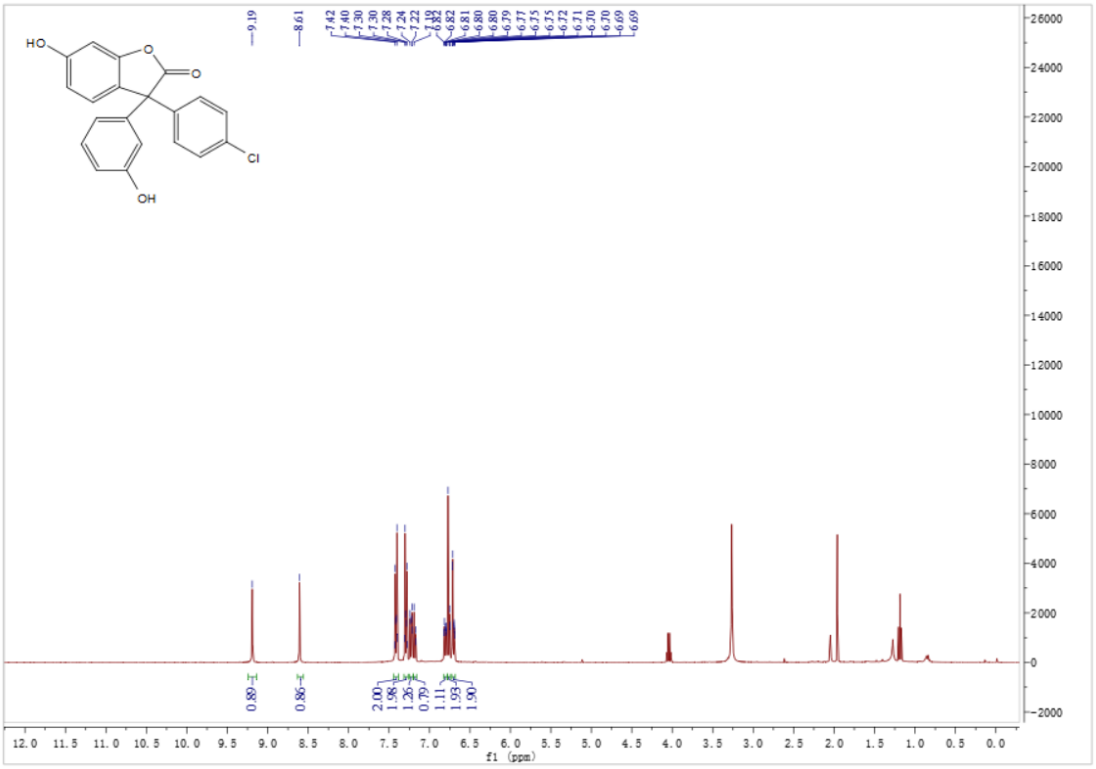
^{1}H NMR of compound **4d**



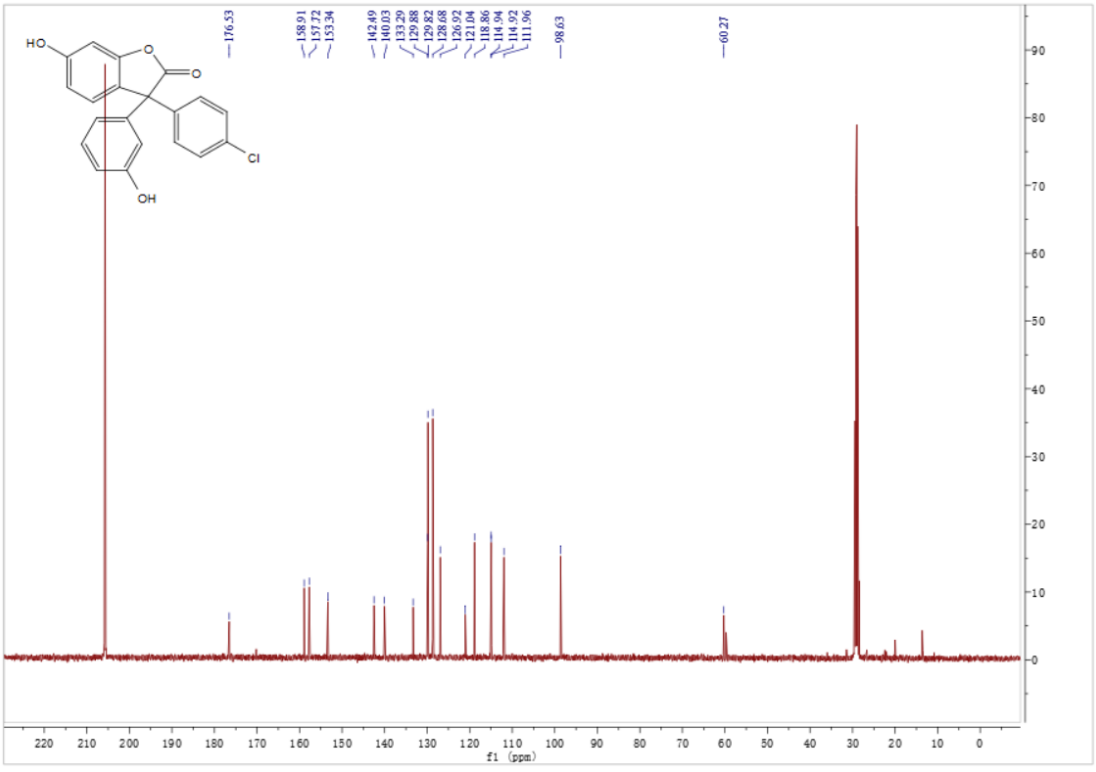
^{13}C NMR of compound **4d**



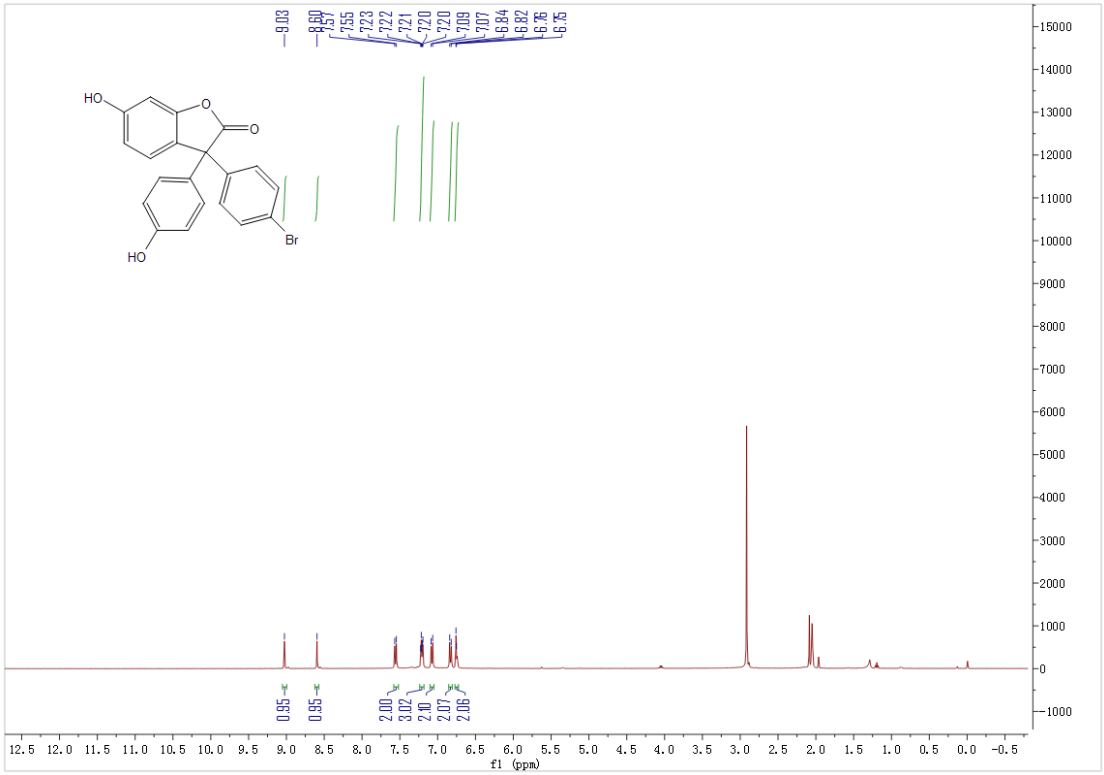
^{1}H NMR of compound **4e**



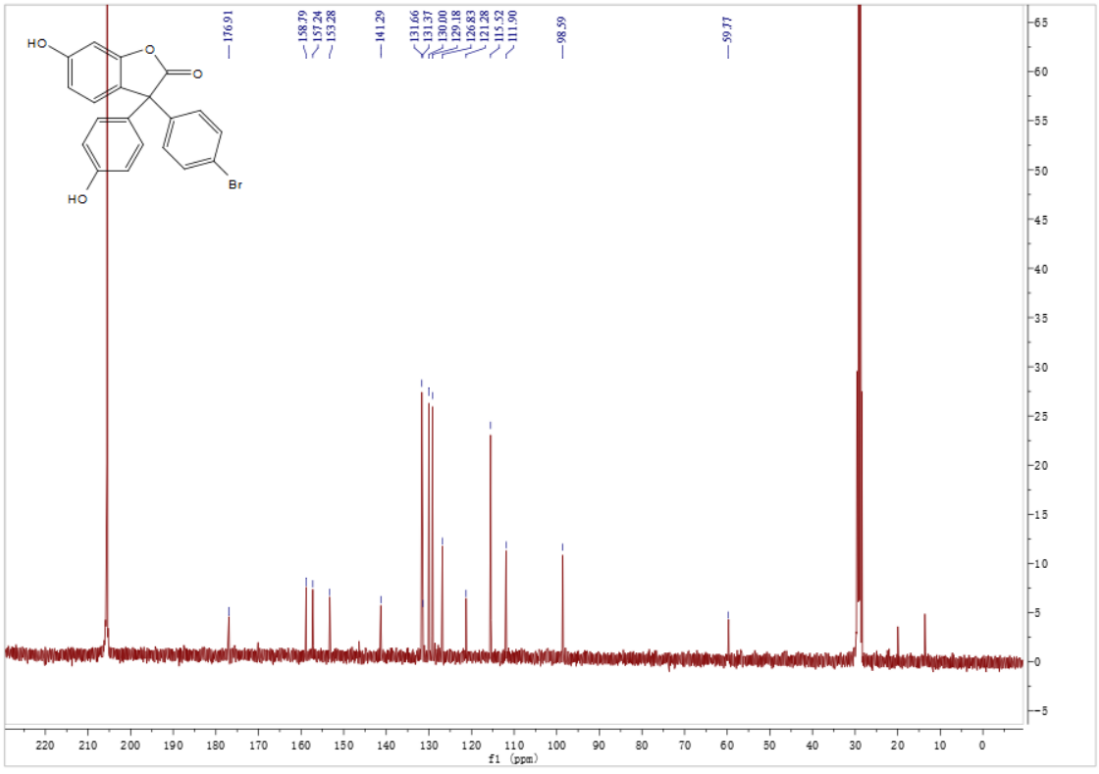
^{13}C NMR of compound **4e**



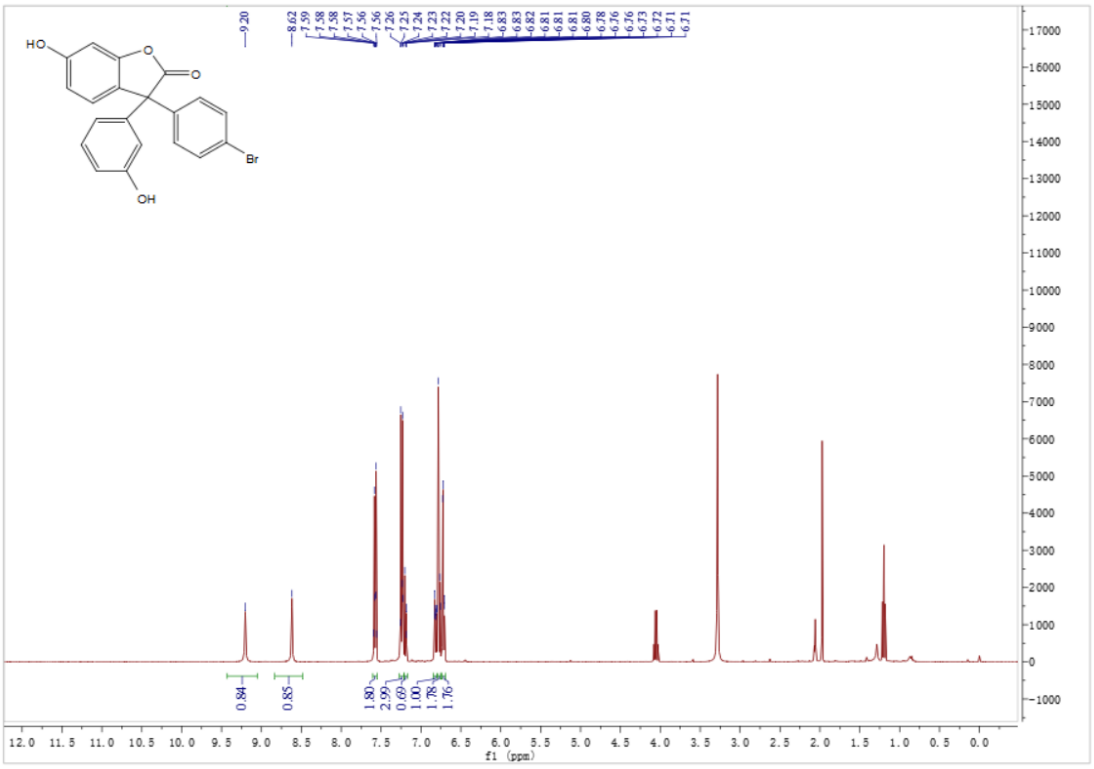
^{1}H NMR of compound **4f**



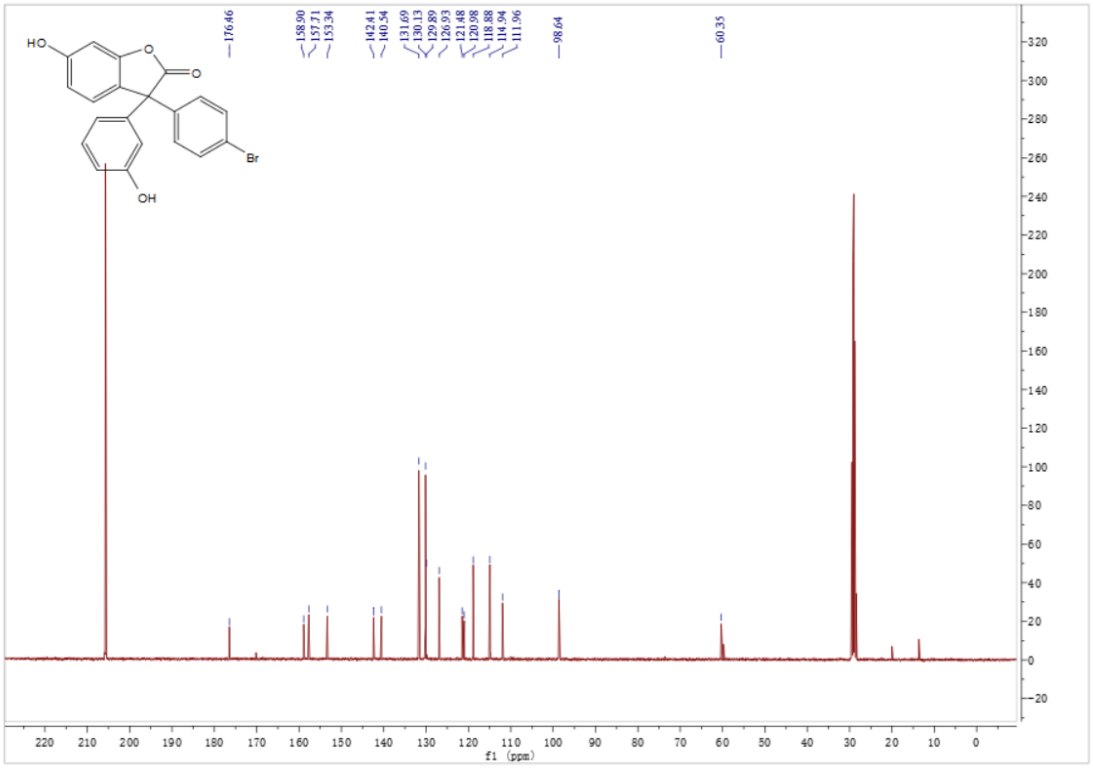
^{13}C NMR of compound **4f**



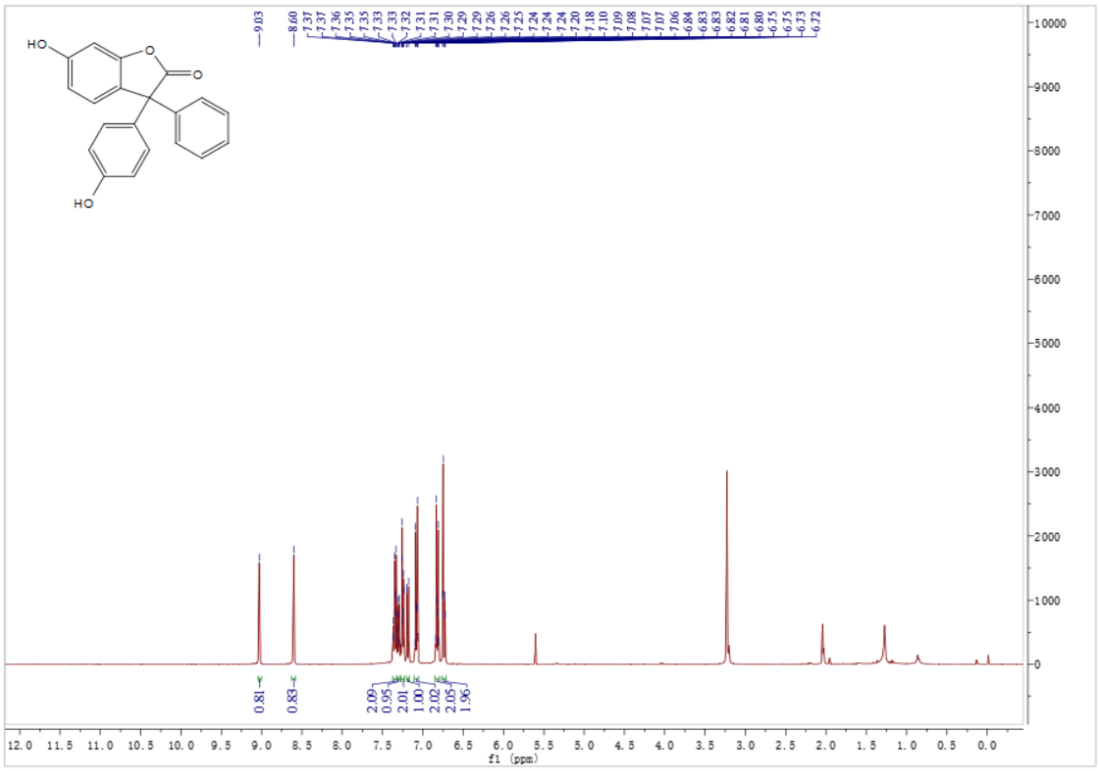
^{1}H NMR of compound **4g**



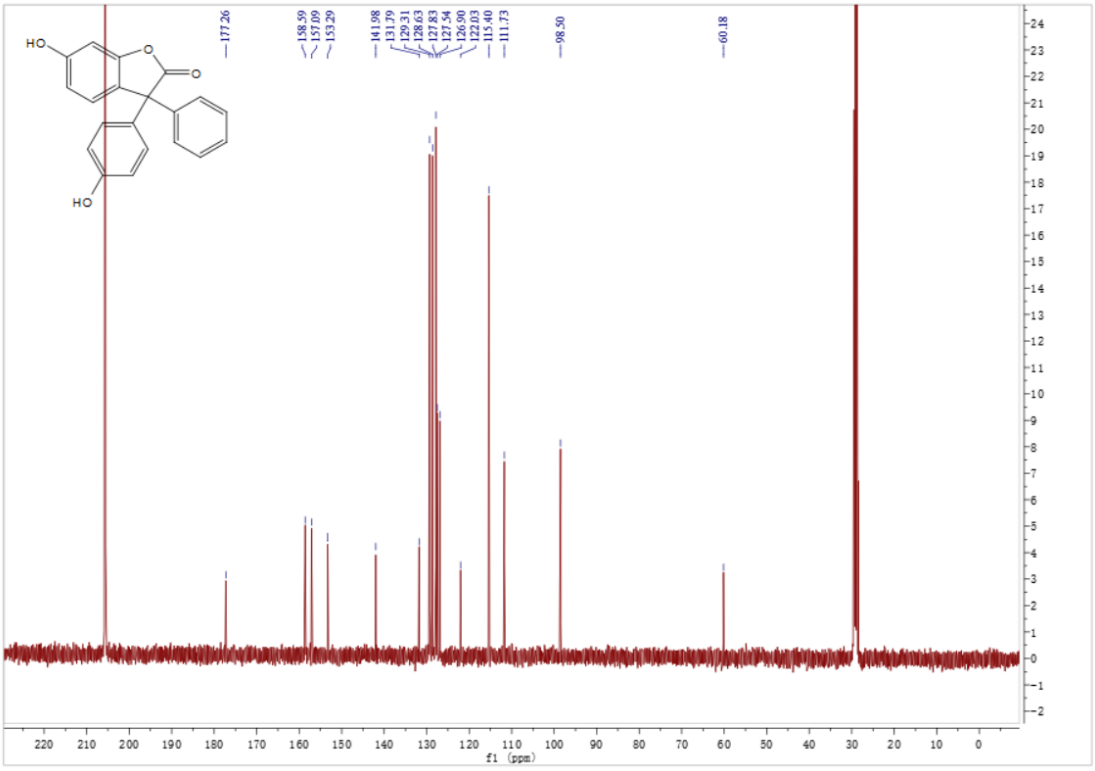
^{13}C NMR of compound **4g**



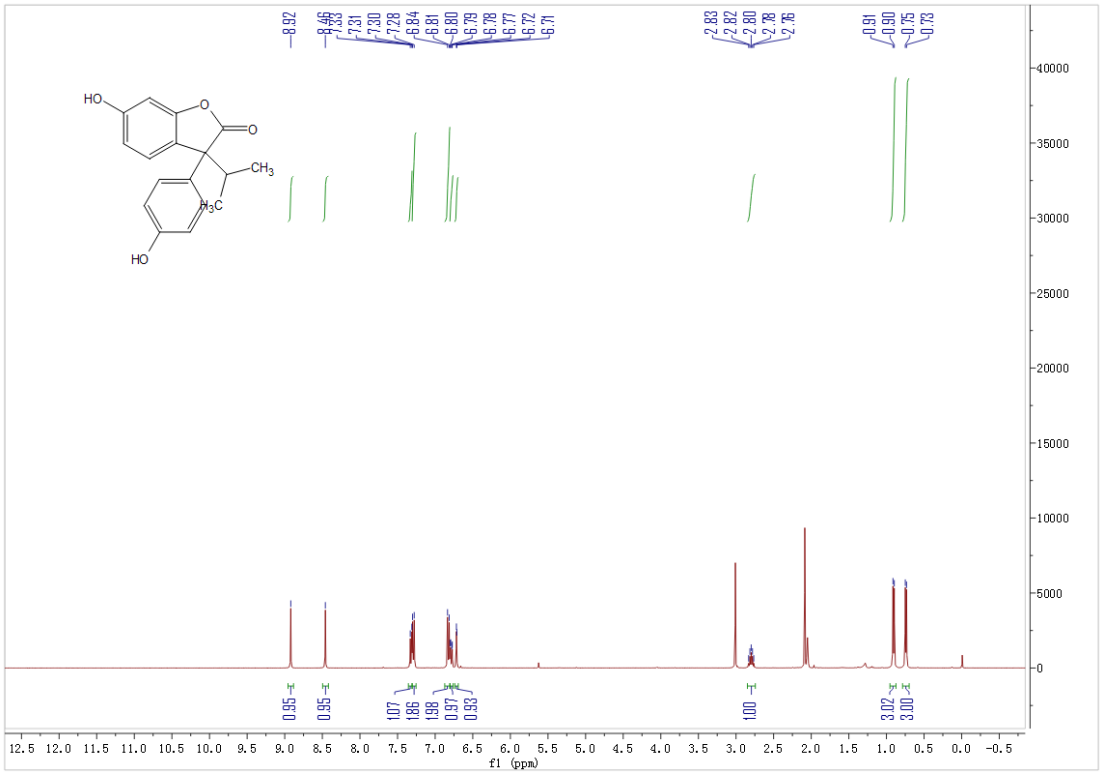
^{1}H NMR of compound **4h**



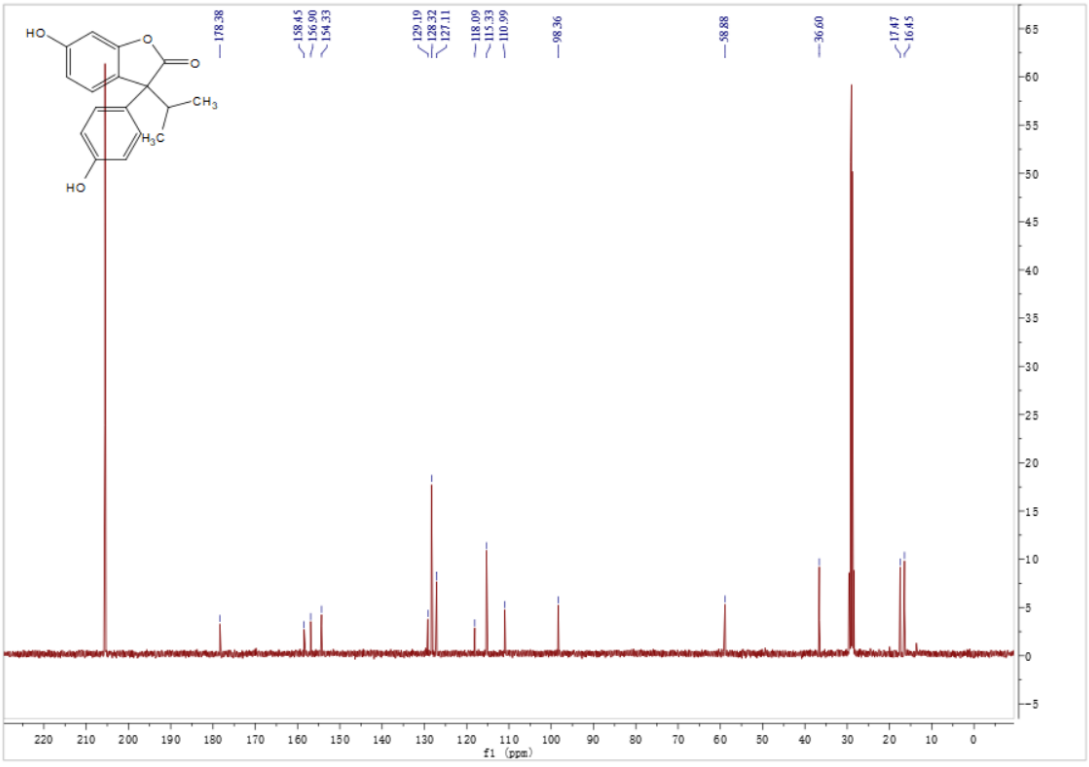
^{13}C NMR of compound **4h**



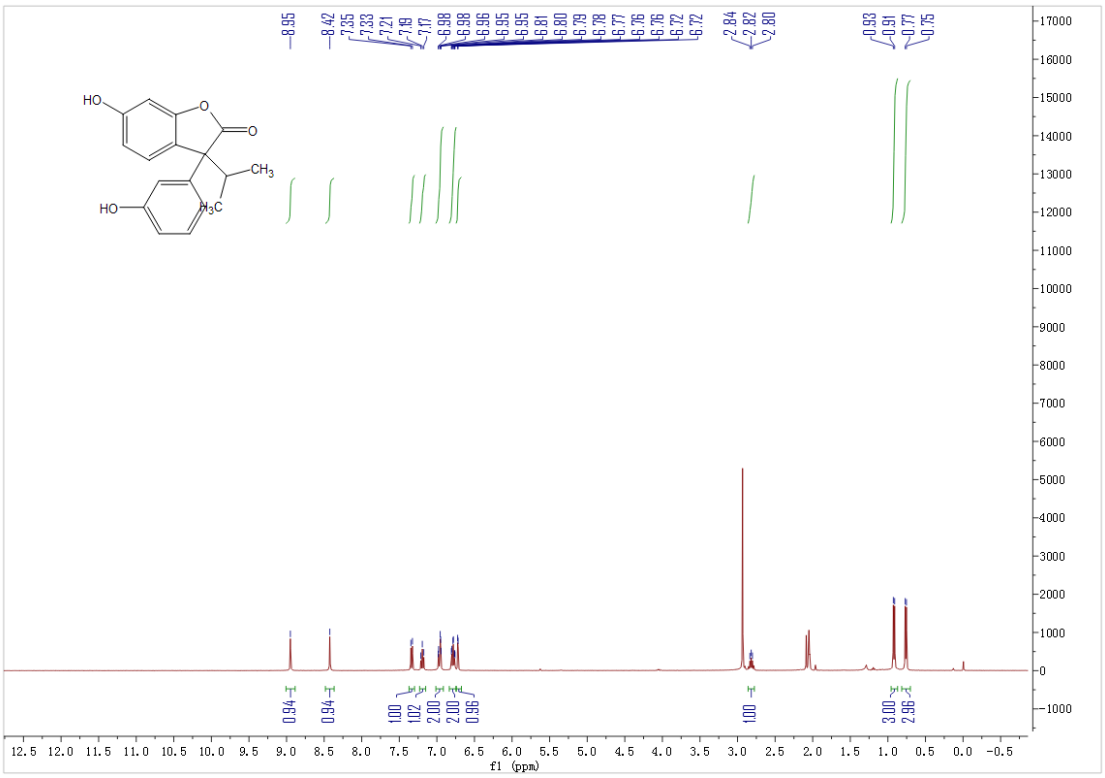
^{1}H NMR of compound **4i**



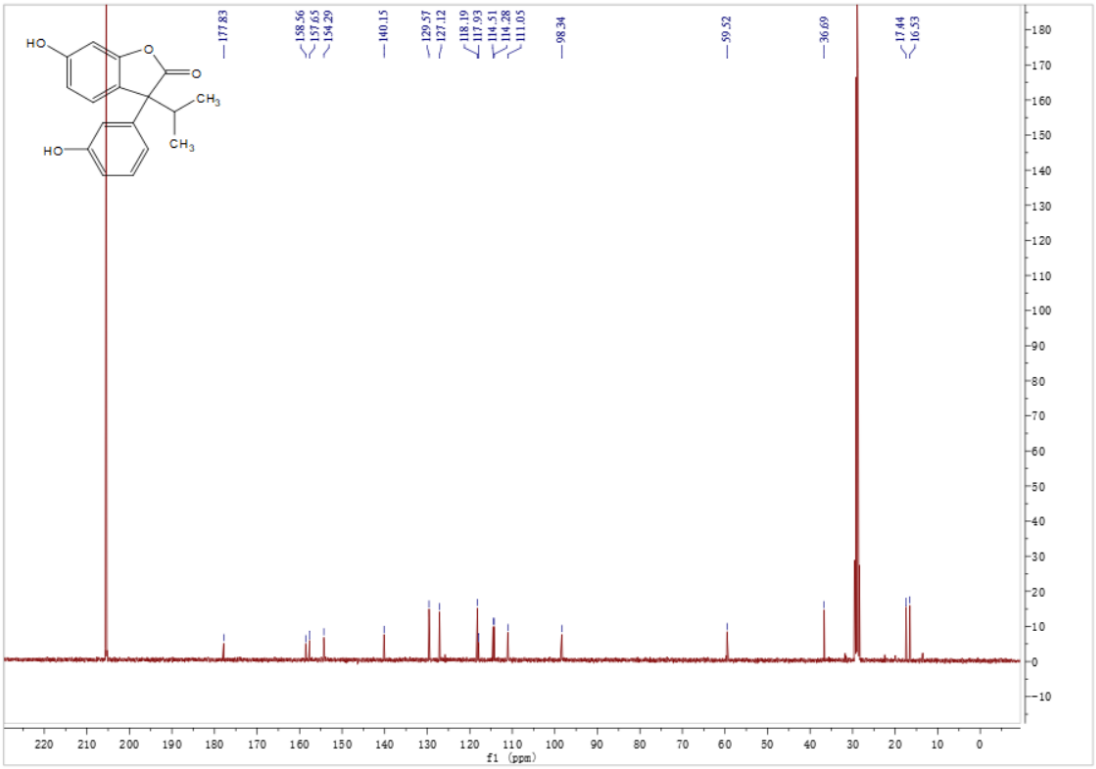
^{13}C NMR of compound **4i**



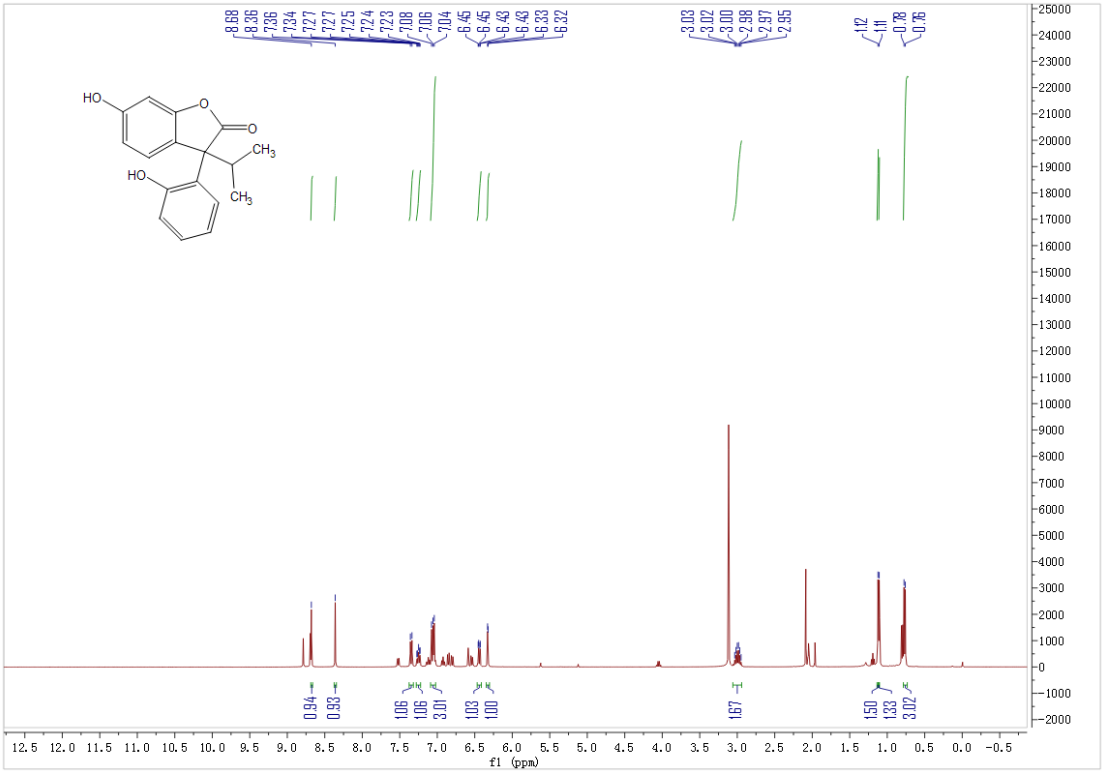
^{1}H NMR of compound **4j**



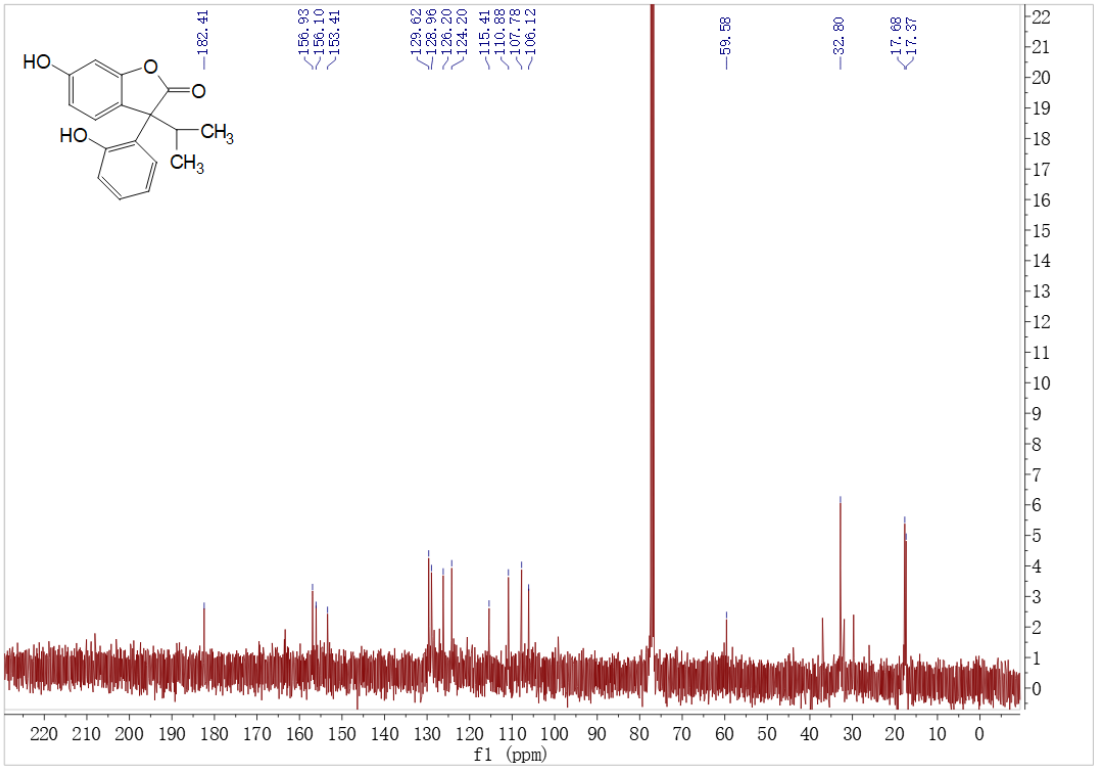
^{13}C NMR of compound **4j**



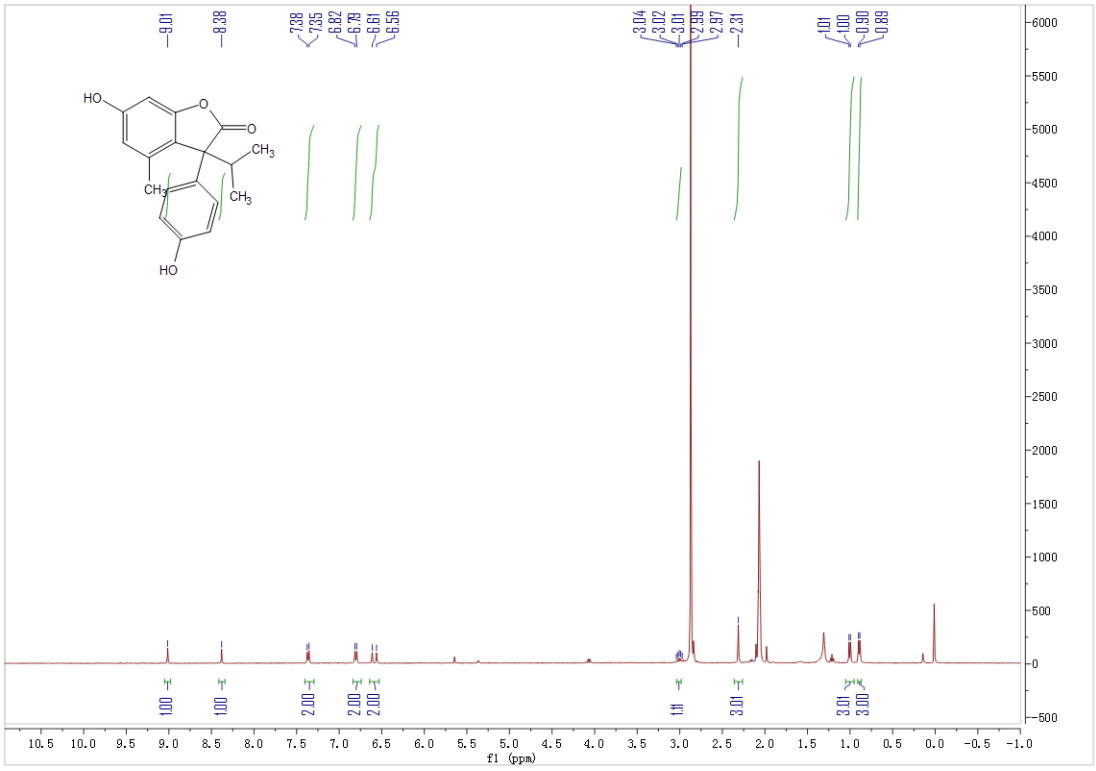
^{1}H NMR of compound **4k**



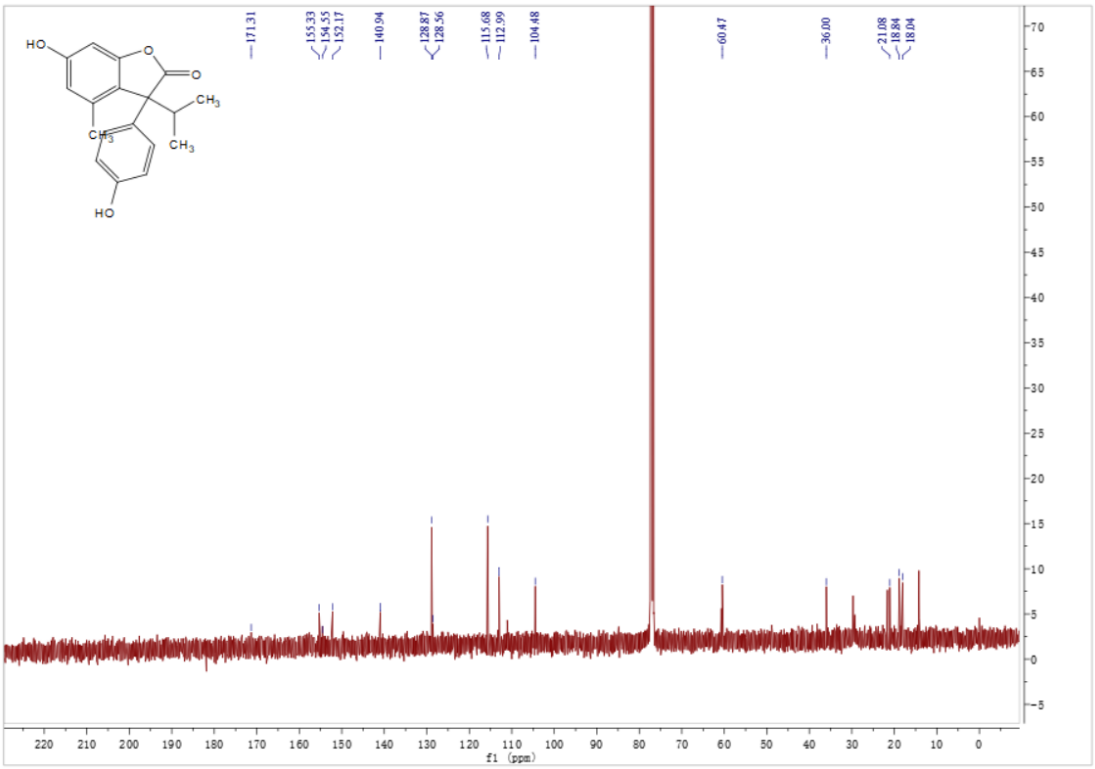
^{13}C NMR of compound **4k**



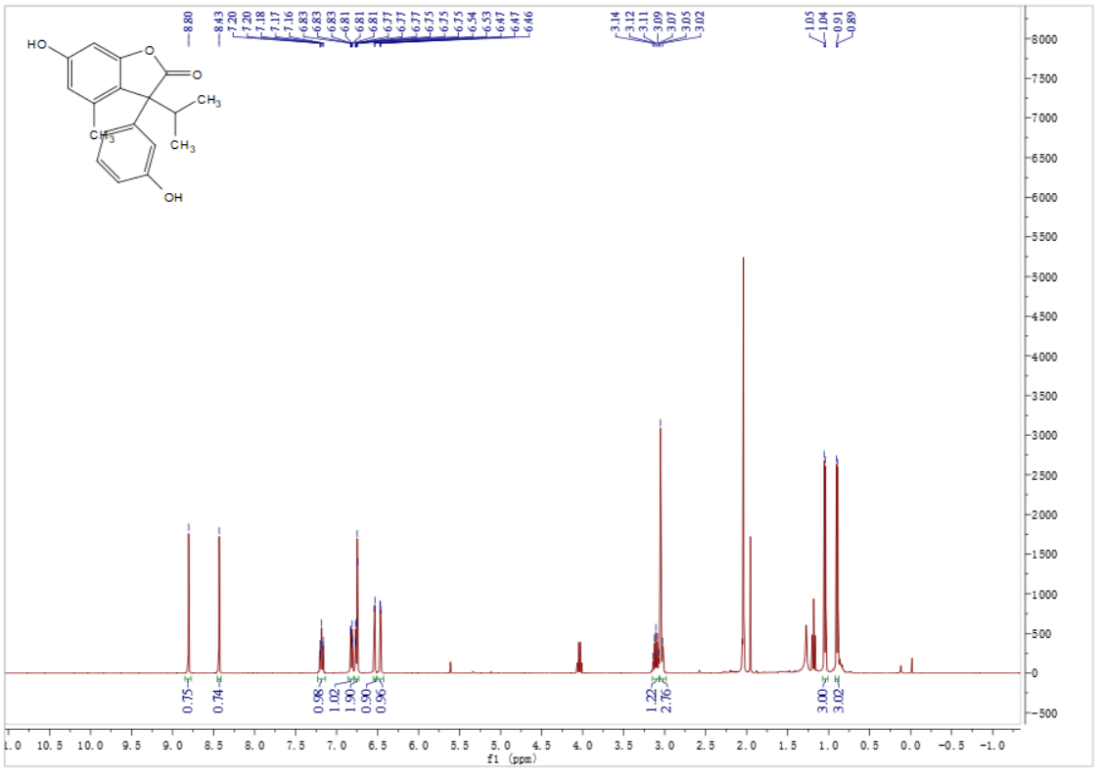
^{1}H NMR of compound **4l**



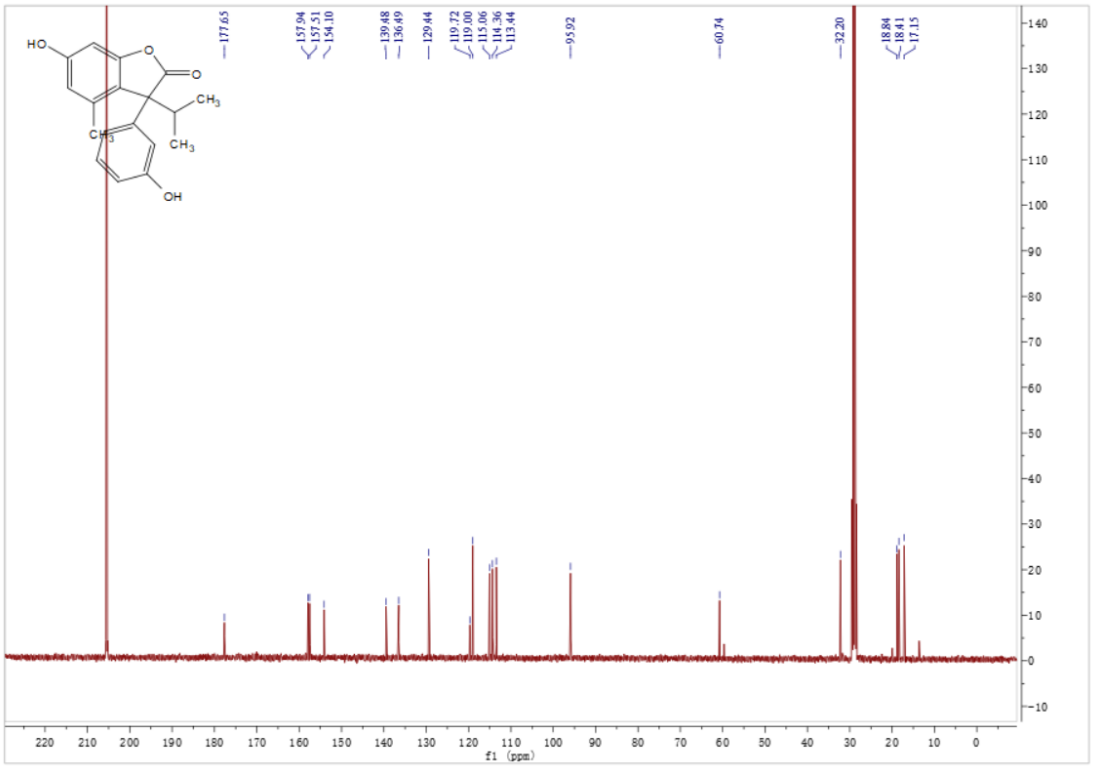
^{13}C NMR of compound **4l**



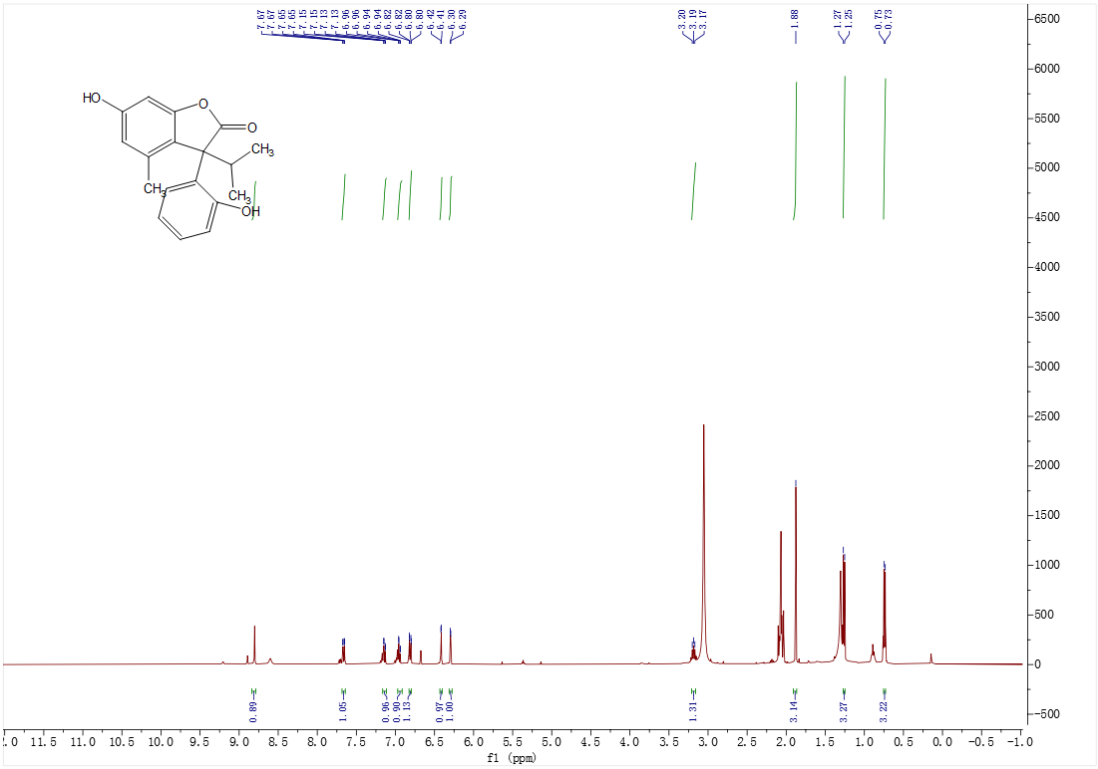
^{1}H NMR of compound **4m**



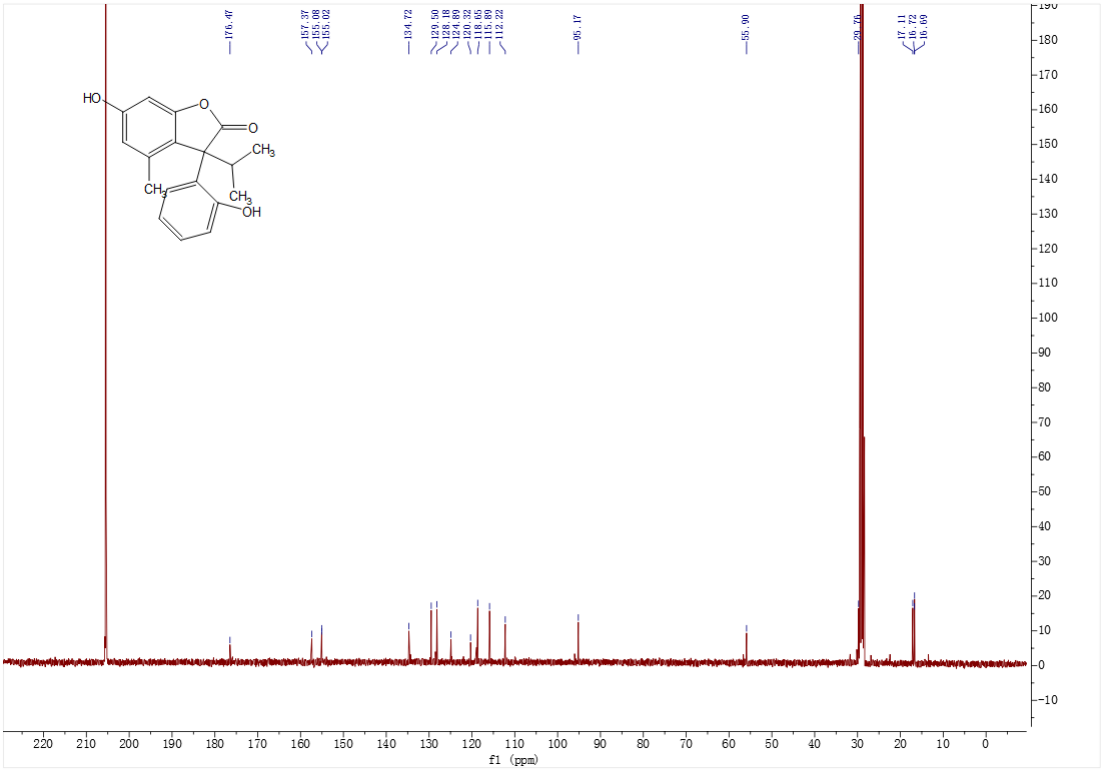
^{13}C NMR of compound **4m**



^{1}H NMR of compound **4n**



^{13}C NMR of compound **4n**



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