SUPPORTING INFORMATION

Visualization of mercury (II) accumulation *in vivo* using bioluminescence imaging with a highly selective probe

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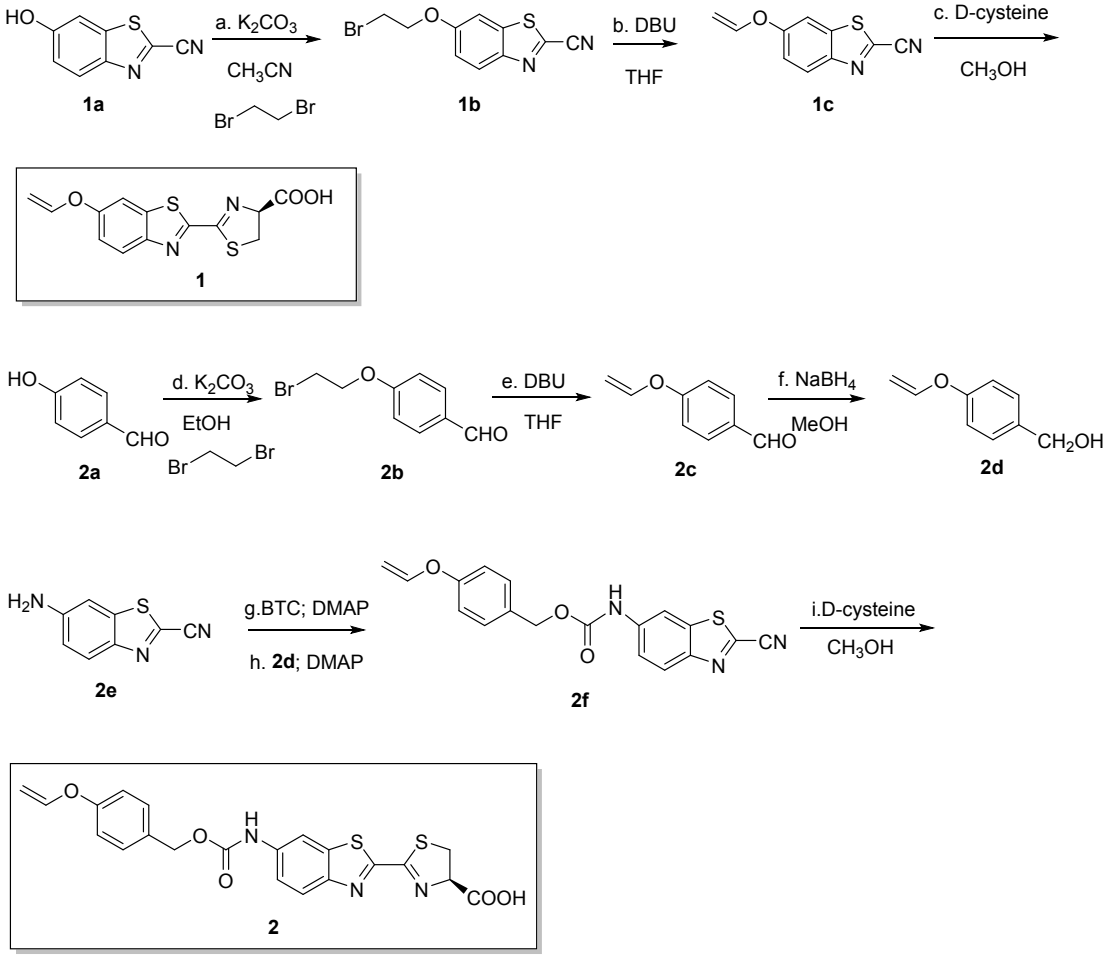
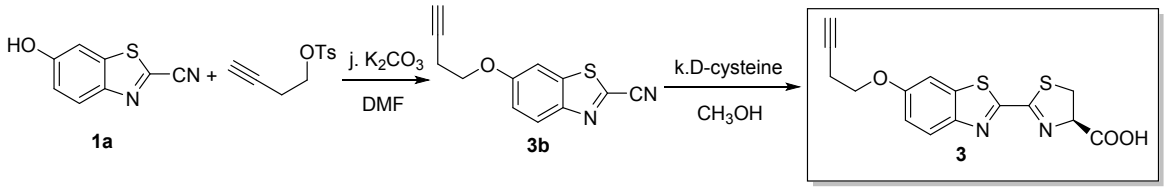
CONTENT

1. General methods

All chemicals and solvents were used without purification unless otherwise noted. Column chromatography was carried out on silica gel (200-300 mesh) using an eluent of ethyl acetate and cyclohexane ether. TLC analyses were conducted on silica gel plates; products were visualized using UV light (ZF-2 UV254) and I_2. Mass spectral analyses were performed on an API 4000 (ESI-HRMS). NMR spectra were recorded at ^1H (400 MHz) and ^{13}C (100 MHz) on a Bruker instrument. Chemical shifts (δ values) and coupling constants (*J* values) are given in ppm and hertz respectively, using solvents (^1H NMR, ^{13}C NMR) as the internal standard in DMSO and $CDCl_3$ solution. Melting points were determined on a Mel-Temp apparatus.

2. Synthesis





Scheme S1: The synthesis procedure of mercury probe **1-3**. a) K_2CO_3, CH_3CN, 75.7%; b) DBU, THF, 20.9%; c) D-cysteine·HCl, H_2O, MeOH, 66%; d) K_2CO_3, EtOH, 87.5%; e) DBU, THF, 43%; f) $NaBH_4$, MeOH, 76.8%; g) BTC, DMAP, toluene; h) **2d**, DMAP, 23.2% for two steps; i) D-cysteine·HCl, H_2O, MeOH, 74.2%; j) K_2CO_3, DMF, 71.5% ; k) D-cysteine·HCl, H_2O, MeOH, 74.2%.

6-(2-bromoethoxy) benzo[*d*]thiazole-2-carbonitrile (1b)

To a solution of compound **1a** (500 mg, 2.86mmol) and K_2CO_3 (790 g, 5.72 mmol) in 25 ml CH_3CN was added the solution of 2.5 mL 1,2-dibromoethane (5.37 g, 28.6 mmol) dropwise, and the reaction mixture was reflux overnight. Then the mixture was filtered, and the filter cake was rinsed three times by CH_3CN and filtrate was concentrated and purified by silica gel column chromatography (cyclohexane: EtOAc = 5:1) to afford **1b** as a white solid (600 mg, 75.7 % yield), mp 95.1–96.4 ℃; The purity confirmed using HPLC is 98.8 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 9.1 Hz, 1 H), 7.38 (d, *J* = 2.5 Hz, 1 H), 7.27 (dd, *J* = 9.1, 2.5 Hz, 1H), 4.40 (t, *J* = 6.2 Hz, 2 H), 3.70 (t, *J* = 6.2 Hz, 2 H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 147.3, 137.3, 133.9, 126.1, 118.7, 113.1, 104.2, 88.5, 28.4.

6-(vinyloxy) benzo[*d*]thiazole-2-carbonitrile (1c)

To a solution of compound **1b** (200 mg, 0.71 mmol) in 20ml THF was added DBU (216 mg, 1.42 mmol) dropwise. The mixture was stirred at 100 ℃ under Ar atmosphere for 4h, after which it was diluted with DCM (20 mL), washed with brine (3*10 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated and purified with silica gel chromatography (10% EtOAc/hexane) to afforded **1c** as a white solid (30 mg, 20.9% yield): mp 79.1–80.1 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 9.1 Hz, 1H), 7.52 (d, *J* = 2.4 Hz, 1H), 7.34 (dd, *J* = 9.1, 2.4 Hz, 1H), 6.69 (dd, *J* = 13.6, 6.0 Hz, 1H), 4.95 (dd, *J* = 13.6, 2.0 Hz, 1H), 4.65 (dd, *J* = 6.0, 2.0 Hz,

1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.4, 148.2, 146.9, 137.0, 134.9, 126.2, 119.4, 113.0, 107.7, 98.2.

(*S*)-2-(6-(vinyloxy) benzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid (1)

To a solution of compound **1c** (50 mg, 0.25 mmol) in the mixture of MeOH and CH_2Cl_2 (5 mL, v/v = 2:3) was added D-Cysteine hydrochloride (43 mg, 0.25 mmol) and K_2CO_3 (34 mg, 0.25 mmol) in the mixture of MeOH and water (2 mL, *v/v* = 1:1). The reaction mixture was stirred at rt(room temperature) under nitrogen atmosphere for 1 h. Then the mixture was acidified with 1 M HCl solution to afford compound 1 as yellow solid (49 mg, 66% yield): mp 143.4–145.0 ℃; ^1H NMR (400 MHz, DMSO) δ 8.14 (d, *J* = 9.0 Hz, 1H), 7.93 (d, *J* = 2.5 Hz, 1H), 7.34 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.00 (dd, *J* = 13.5, 6.0 Hz, 1H), 5.44 (dd, *J* = 9.7, 8.4 Hz, 1H), 4.88 (dd, *J* = 13.5, 1.6 Hz, 1H), 4.62 (dd, *J* = 6.0, 1.6 Hz, 1H), 3.85 – 3.65 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ 171.1, 164.3, 159.4, 155. 5, 148.6, 147.8, 136.9, 125.1, 117.8, 108.8, 96.7, 78.2, 34.8. HRMS (ESI) calcd. for $C_{13}H_{10}N_2O_3S_2$ $[M+Na]^+$ *m/z*: 306.0133； found:329.0032.

4-(2-bromoethoxy) benzaldehyde（2b)

To a solution of p-hydroxybenzaldehyde **2a** (3 g, 0.025 mol) and anhydrous K_2CO_3 (6.6 g, 0.05 mol) in 30 ml ethanol, 1, 2-dibromoethane (46 g, 0.25 mol) was added and then stirred at 70℃ overnight. After the p-hydroxybenzaldehyde was consumed (monitored by TLC), the reaction mixture was cooled to room temperature and Filter to remove sediment. The mixture was then diluted with DCM and the organic layer was washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum to afford a yellowish compound **2b** (4.9 g, 87.5% yield). mp 50.3–51.6℃; 1H NMR (82 MHz,) δ 9.88 (s, 1H), 7.83 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 4.36 (t, J = 6.0 Hz, 3H), 3.64 (t, J = 6.1 Hz, 2H).

4-(vinyloxy) benzaldehyde (2c)

To a solution of **2b** (2.9 g, 0.013 mol) in DMF (30 mL), t-BuOK (2.8 g, 0.026 mol) was added. The reaction mixture was stirred at room temperature for 2 h, after which it was diluted with DCM (50 mL), washed with H_2O (3*20 mL) and then brine (20 mL). The organic layer was dried, filtered, and concentrated. The crude product was purified by column chromatography (EtOAc/petroleum ether = 1:5 as eluent) to

afford compound **2c** as a colorless oil (0.8 g, 43% yield). ^{1}H NMR (82 MHz,) δ 9.91 (s, 1H), 7.85 (d, *J* = 8.6 Hz, 2H), 7.37 – 6.93 (m, 4H), 6.68 (dd, *J* = 13.6, 6.0 Hz, 1H), 4.92 (dd, *J* = 13.6, 1.6 Hz, 1H), 4.61 (dd, *J* = 6.0, 1.6 Hz, 1H).

(4-(vinyloxy) phenyl) methanol (2d)

To a solution of **2c** (0.2 g, 0.014 mol) in MeOH (10 mL), $NaBH_4$ (0.055 g, 0.014 mol) was added. The reaction was stirred at room temperature for 2h until **2c** was consumed completely. The reaction was quenched with saturated NH_4Cl and then diluted with DCM and the mixture was washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum to afford a yellowish oil **2d** (0.156 g, 76.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.29 (m, 1H), 7.03 – 6.94 (m, 1H), 6.63 (dd, *J* = 13.7, 6.1 Hz, 1H), 4.76 (dd, *J* = 13.7, 1.7 Hz, 1H), 4.63 (s, 1H), 4.44 (dd, *J* = 6.1, 1.7 Hz, 1H).

4-(vinyloxy) benzyl (2-cyanobenzo [d] thiazol-6-yl) carbamate (2f)

To 6-amino-2-cyanobenzothiazole **2e** (100 mg, 1.14 mmol) in 5 mL toluene was added BTC (bis (trichloromethyl) carbonate) (350 mg, 1.14 mmol) and DMAP (280 mg, 2.28 mmol). The reaction mixture was stirred at 120 ℃ for 3 hours under the nitrogen atmosphere. After cooled to 50 ℃, **2b** (514 mg, 3.42 mmol) and DMAP (280 mg, 2.28 mmol) were added. The reaction mixture was stirred overnight under a nitrogen atmosphere. After cooled to room temperature, the reaction mixture was washed by saline and water. The residue was purified by flash chromatography on silica gel (petroleum ether: EtOAc= 4:1) to afford **2f** as a yellow solid (94 mg, 23.2 % yield) : mp 129.8-130.8 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.09 (d, *J* = 8.9 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.33 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.08 – 6.97 (m, 3H), 6.64 (dd, *J* = 13.7, 6.1 Hz, 1H), 5.20 (s, 2H), 4.80 (dd, *J* = 13.7, 1.7 Hz, 1H), 4.48 (dd, *J* = 6.1, 1.7 Hz, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 157.1, 153.0, 148.3, 147.7, 138.6, 137.2, 134.9, 130.3, 130.2, 125.5, 119.7, 117.2, 113.1, 109.7, 95.9, 67.2.

(R)-2-(6-((((4-(vinyloxy) benzyl) oxy) carbonyl) amino) benzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid (2)

To a solution of compound **2f** (50 mg, 0.14 mmol) in the mixture of MeOH and CH_2Cl_2 (5 mL, v/v = 2:3) was added D-Cysteine hydrochloride (25 mg, 0.14 mmol)

and K_2CO_3 (20 mg, 0.14 mmol) in the mixture of MeOH and water (2 mL, *v/v* = 1:1). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 30 min. Then the mixture was acidified with 1 M HCl solution to afford compound **2** as yellow solid (52 mg, 72% yield): mp 164.5—167.3; The purity confirmed using HPLC is 98.8 %; ^{1}H NMR (400 MHz, DMSO) δ 10.22 (s, 1H), 8.38 (d, *J* = 1.9 Hz, 1H), 8.07 (d, *J* = 9.0 Hz, 1H), 7.58 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.13 – 7.04 (m, 2H), 6.88 (dd, *J* = 13.6, 6.0 Hz, 1H), 5.43 (dd, *J* = 9.7, 8.3 Hz, 1H), 5.16 (s, 2H), 4.75 (dd, *J* = 13.6, 1.5 Hz, 1H), 4.50 (dd, *J* = 6.0, 1.5 Hz, 1H), 3.84 – 3.61 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 171.2, 163.9, 158.7, 156.1, 153.4, 148.1, 138.6, 136.5, 131.0, 130.3, 124.2， 118.8, 116.5, 110.0, 95.4, 78.6, 65.6, 34.8. HRMS (ESI) calcd. For $C_{21}H_{17}N_3O_5S_2$ $[M+Na]^+$ *m/z*: 455.0610; found: 478.0508.

6-(but-3-yn-1-yloxy) benzo[d]thiazole-2-carbonitrile (3b)

To a solution of compound **1a** (100 mg, 0.57 mmol) and K_2CO_3 (228 mg, 1.71 mmol) in 10 ml DMF was added the solution of 3-Butynyl 4-methylbenzenesulfonate (324 mg, 1.71 mmol) dropwise, and the reaction mixture was react at 84 ℃ overnight. Then the mixture was filtered, and the filter cake was rinsed three times by CH_3CN and filtrate was concentrated and purified by silica gel column chromatography (cyclohexane: EtOAc = 5:1) to afford **1b** as a white solid (93 mg, 71.5 % yield): mp 112.4-114.2 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 9.1 Hz, 1H), 7.38 (d, *J* = 2.5 Hz, 1 H), 7.27 (dd, *J* = 9.1, 2.5 Hz, 1H), 4.4 (t, *J* = 6.2 Hz, 2 H), 3.7 (t, *J* = 6.2 Hz, 2 H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 147.3, 137.3, 133.9, 126.1, 118.7, 113.1, 104.2, 88.5, 28.4.

2-(6-(but-3-yn-1-yloxy) benzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid (3)

To a solution of compound **3b** (50 mg, 0.22 mmol) in the mixture of MeOH and CH_2Cl_2 (5 mL, v/v = 2:3) was added D-Cysteine hydrochloride (39 mg, 0.22 mmol) and K_2CO_3 (30 mg, 0.22 mmol) in the mixture of MeOH and water (2 mL, *v/v* = 1:1). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 30 min. Then the mixture was acidified with 1 M HCl solution to afford the product as yellow solid (54 mg, 74.2% yield), mp 158.7–159.3 ℃; The purity confirmed using

HPLC is 98.2 %; ^{1}H NMR (400 MHz, DMSO) δ 13.23 (s, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.80 (d, *J* = 2.5 Hz, 1H), 7.22 (dd, *J* = 9.0, 2.6 Hz, 1H), 5.43 (dd, *J* = 9.7, 8.4 Hz, 1H), 4.18 (t, *J* = 6.5 Hz, 2H), 3.74 (m, 2H), 2.93 (t, *J* = 2.6 Hz, 1H), 2.71 (td, *J* = 6.5, 2.6 Hz, 2H), 2.49 (dd, *J* = 18.3, 16.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 171.2, 164.4, 158.0, 157.70, 147.2, 137.1, 124.8, 117.3, 105.5, 81.20, 78.1, 72.6, 66.4, 34.7, 18.8. HRMS (ESI) calcd. For $C_{15}H_{12}N_2O_3S_2$ $[M+Na]^+$ *m/z*: 332.0289; found: 355.0186.

3. Experiment procedure of bioluminescence determination

Various Hg^{2+} prepared with the Tris-HCl buffer was added to a black 96-well plate, and incubated with probes 1-3 (probes 1-2: 25 μM, room temperature, 70 min; probe 3: 25 μM, 60 °C, 60 min). The enzyme was then added and subsequently detected by the living optical imaging system.

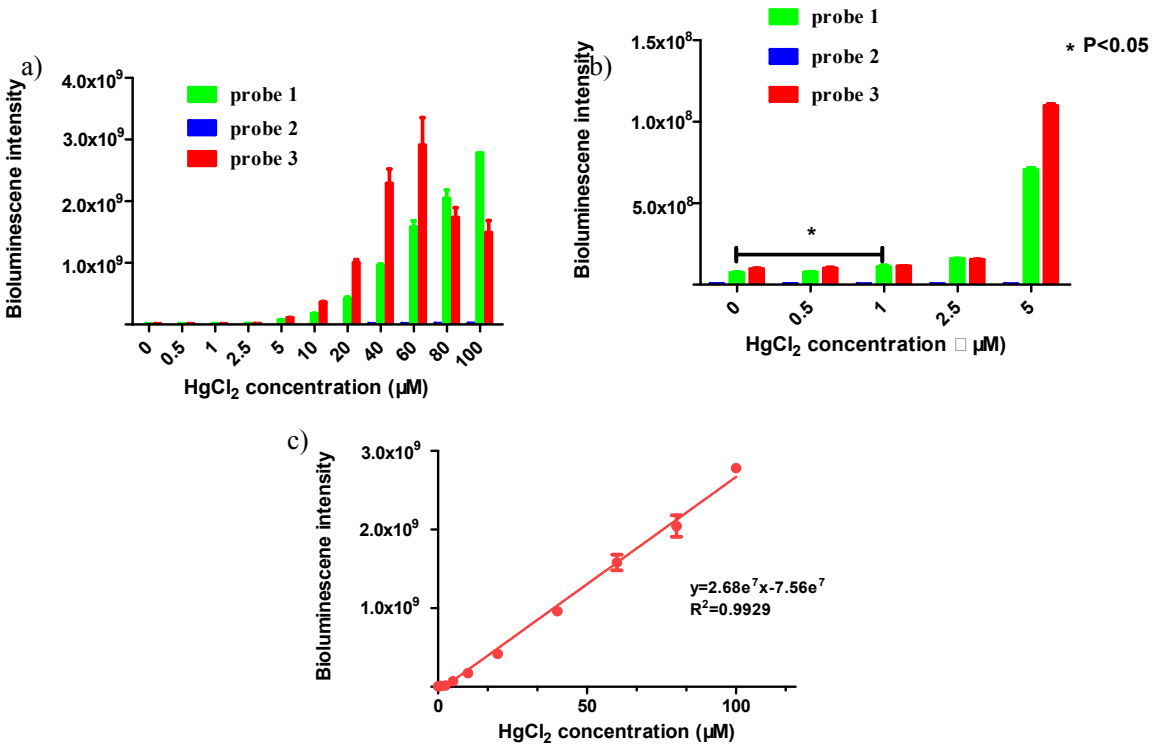
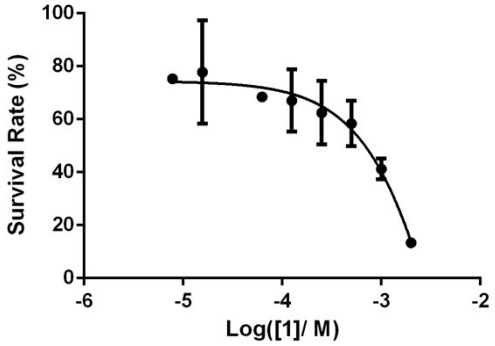


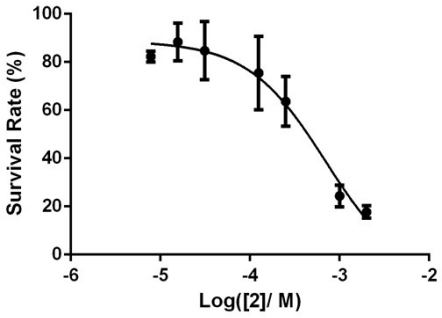
Figure S1. The bioluminescence intensity of probes **1-3** after incubated with various concentrations of Hg^{2+}. quantification of the bioluminescent intensity of (**a**) and calculating the relative bioluminescence intensity; (**b**) the relative bioluminescence intensity of a small range of Hg^{2+} concentrations (0, 0.5, 1, 2.5, 5 μM). *P<0.05; (**c**)

linear relationship between the relative bioluminescence intensity of the probe **1** and the concentration of Hg^{2+} (R^2 = 0.9929).

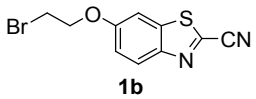
The cytotoxicity assay of probe 1 and 2

Cytotoxicity studies were performed using the MTT assay. ES-2-Fluc cells were incubated with different concentrations of our probes (2000 μM, 1000 μM, 500 μM, 250 μM, 125 μM, 62.5 μM, 31.25 μM, 15.625 μM, 7.8125 μM, 0 μM) for 24 h before the cell viability measured by MTT method. Fig. S3 demonstrated that our probes showed no cytotoxicity below 500 μM.



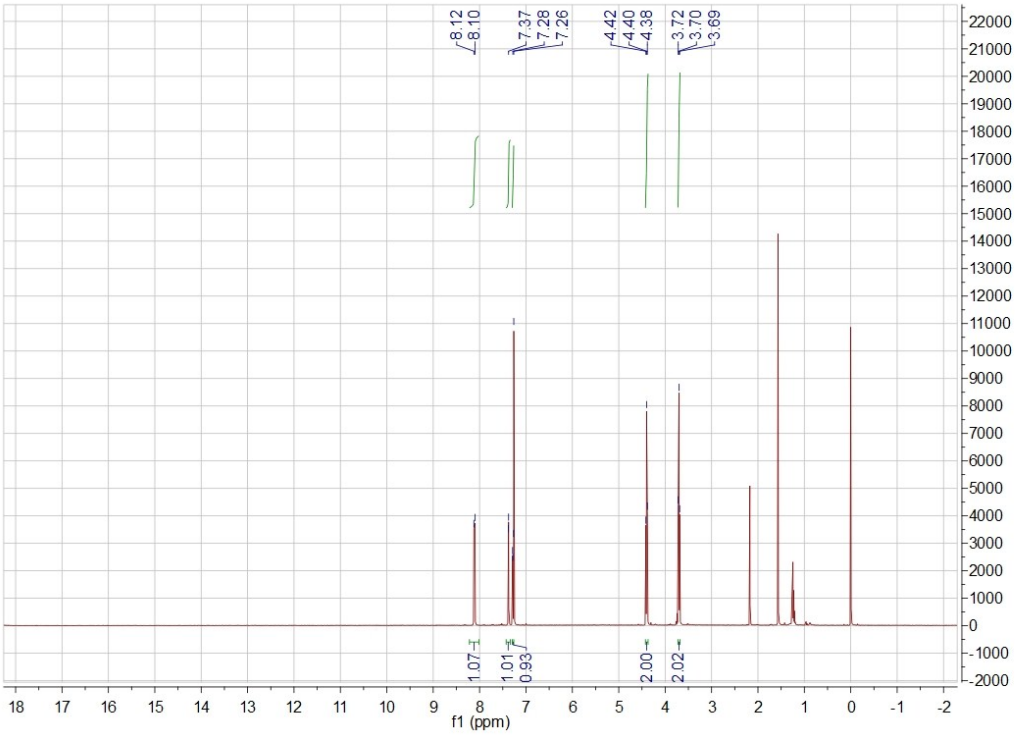


4. NMR，MS and HPLC spectra

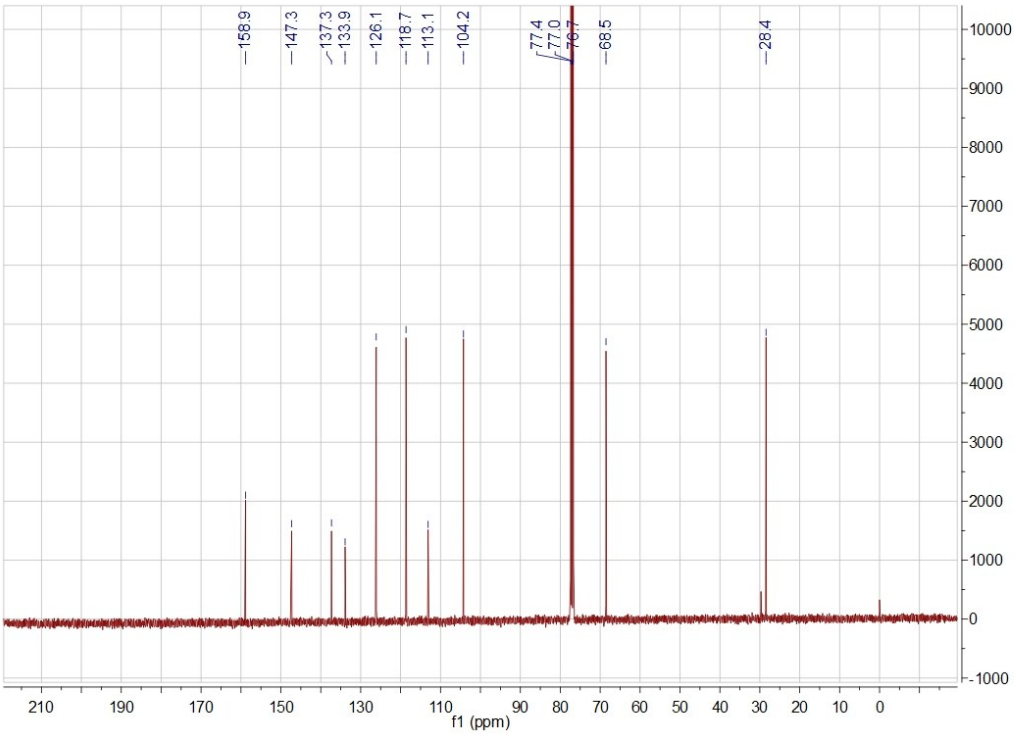


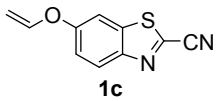
1b

^{1}H NMR



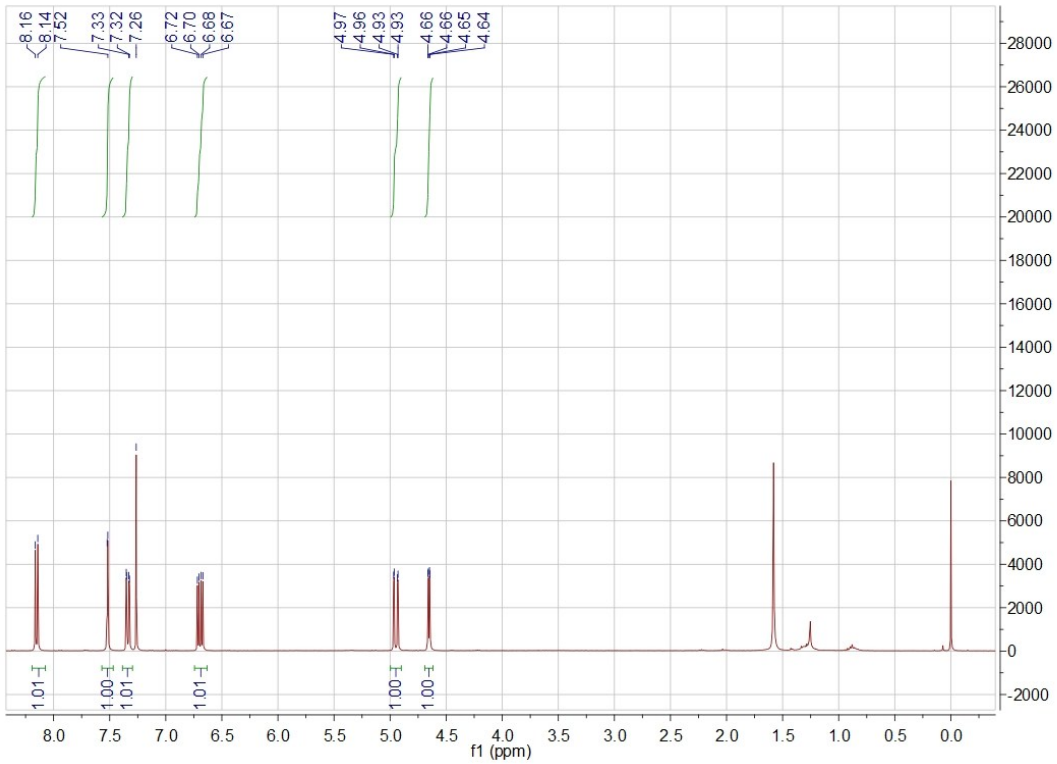
^{13}C NMR





1c

^{1}H NMR



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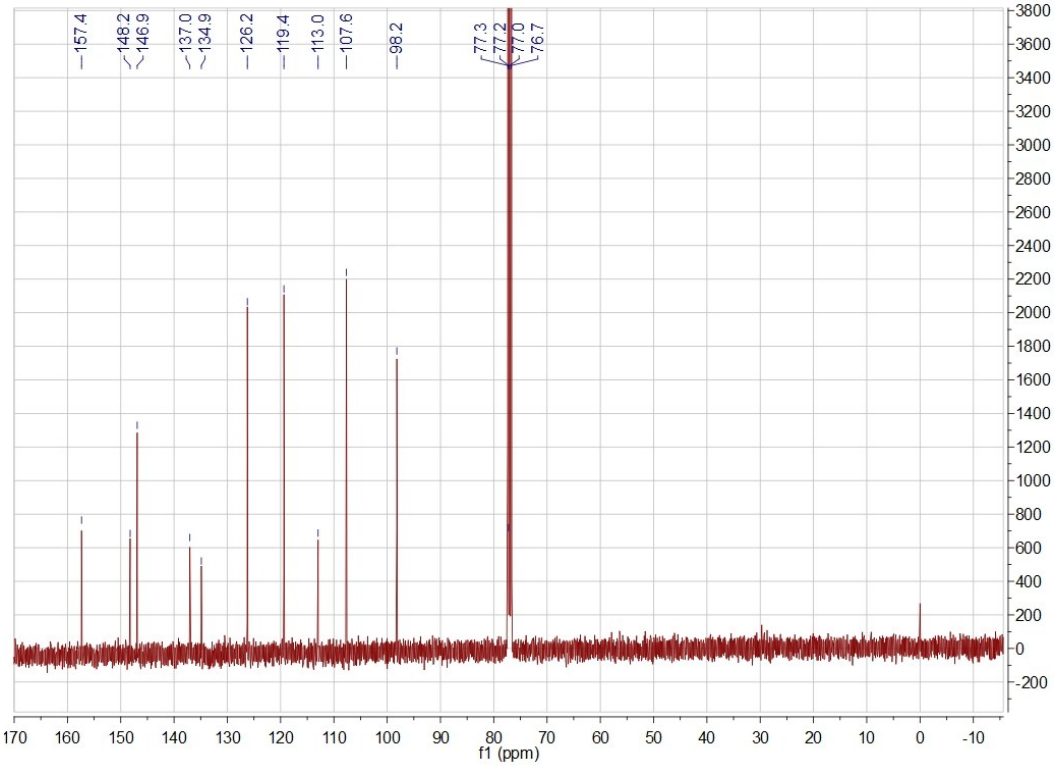
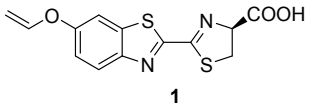
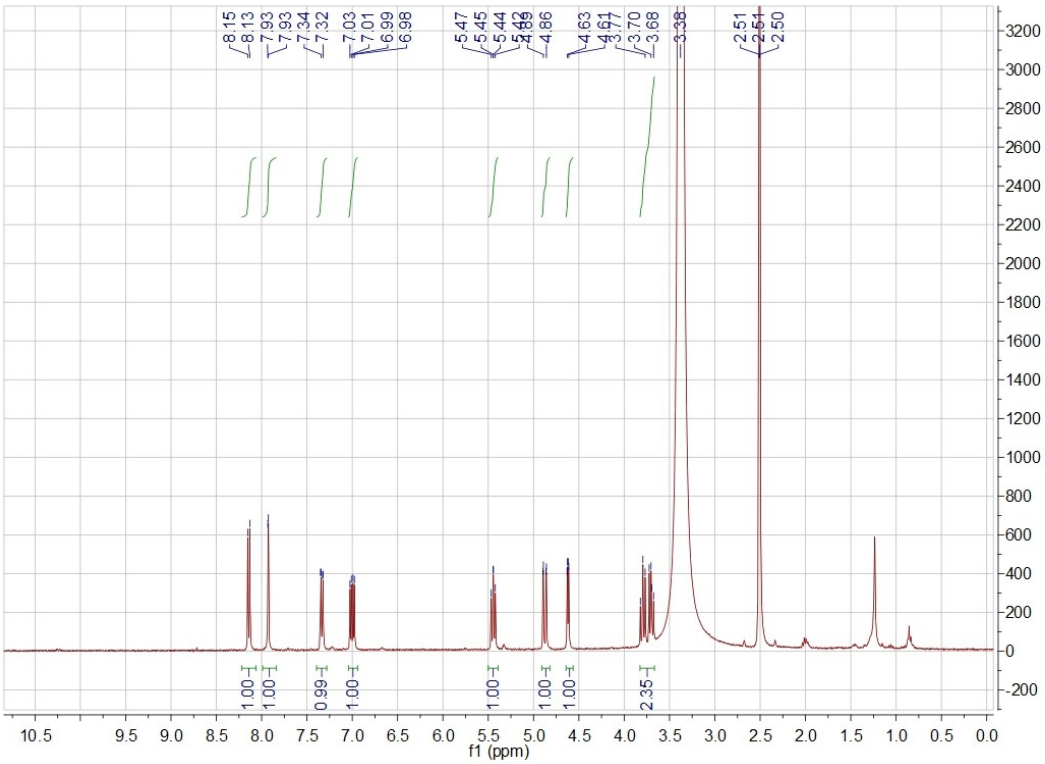




1

^{1}H NMR



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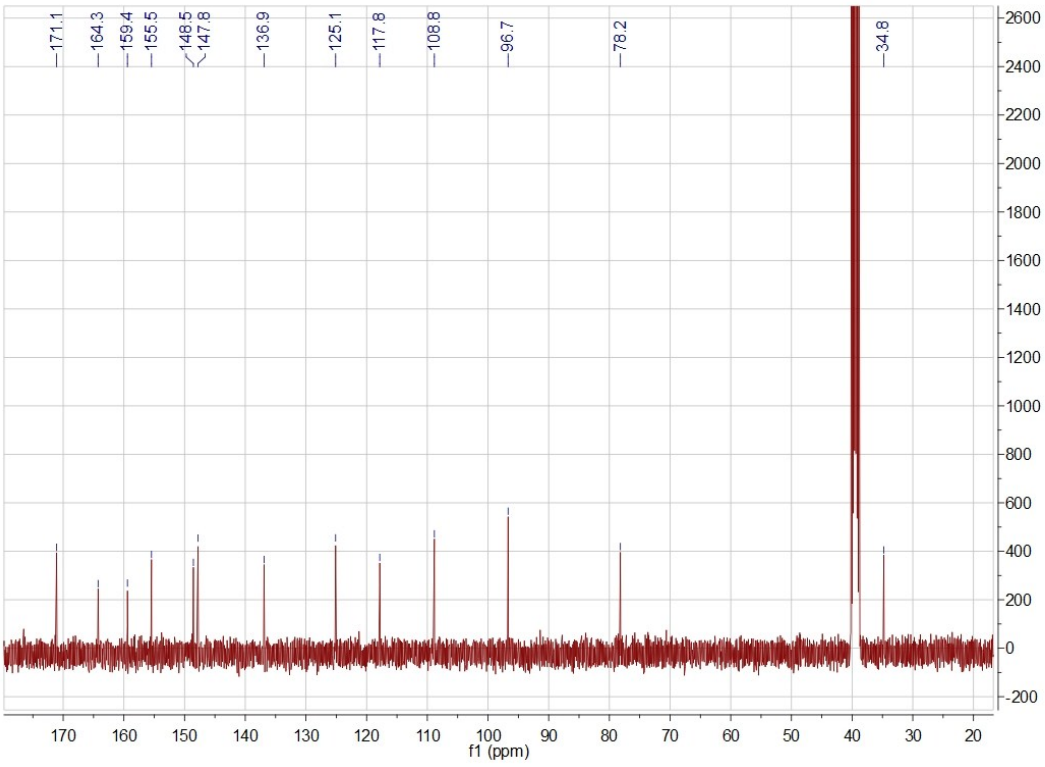
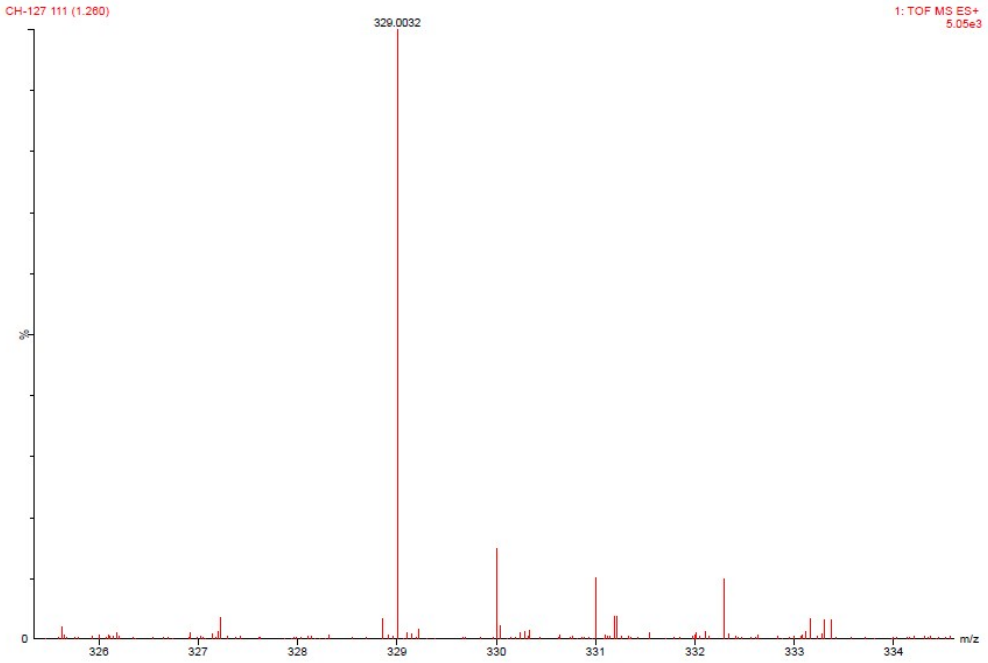


MS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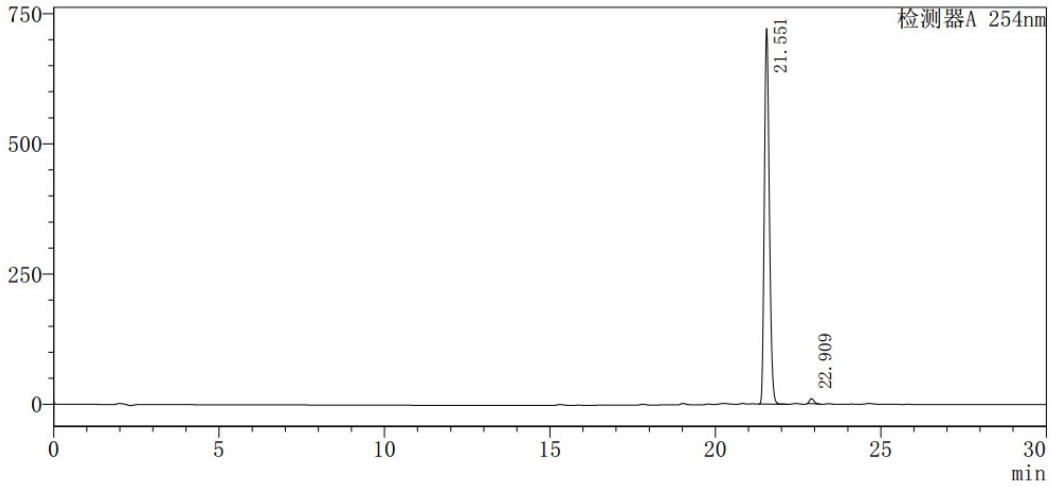
HPLC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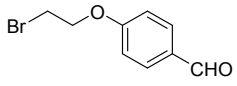
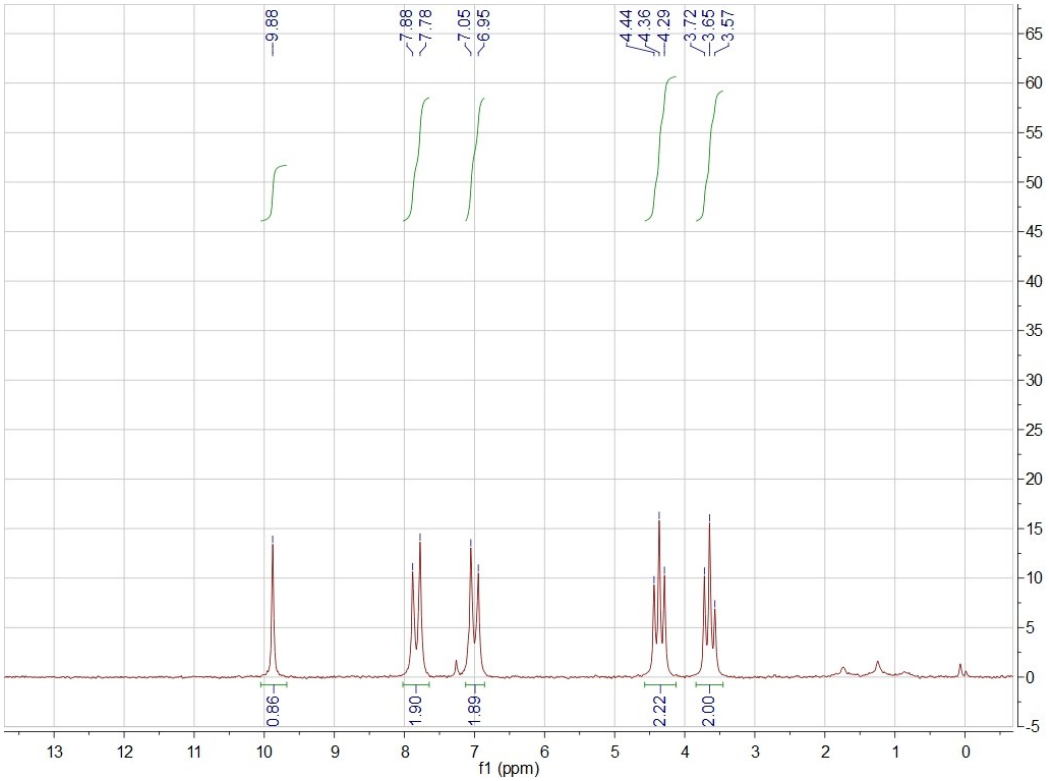
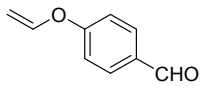


2b

^{1}HNMR





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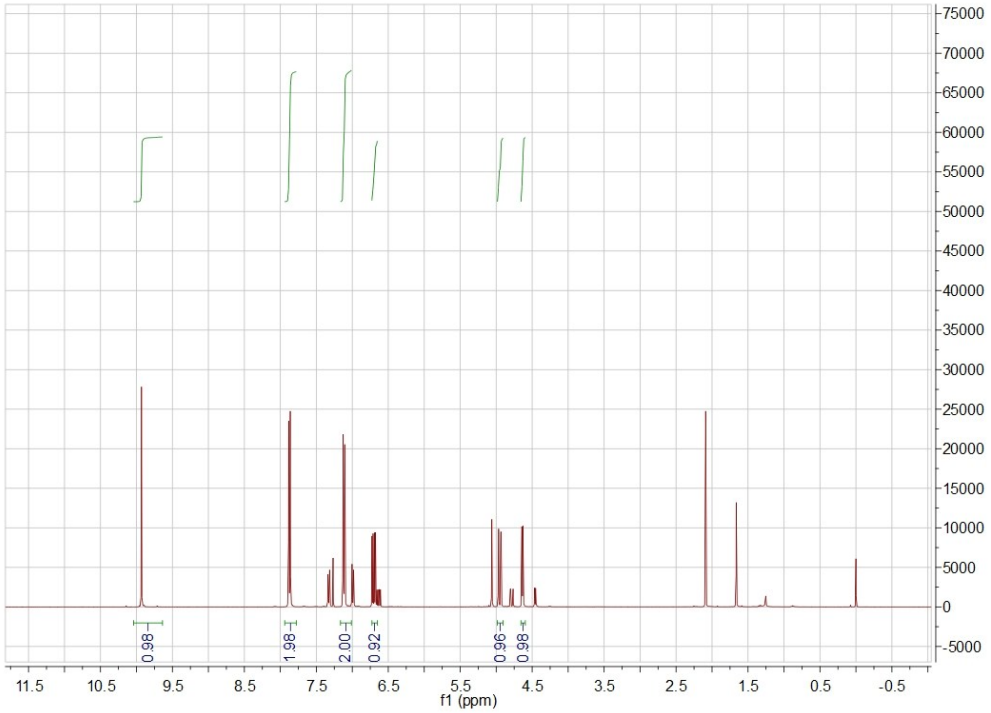
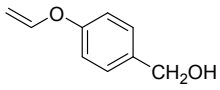
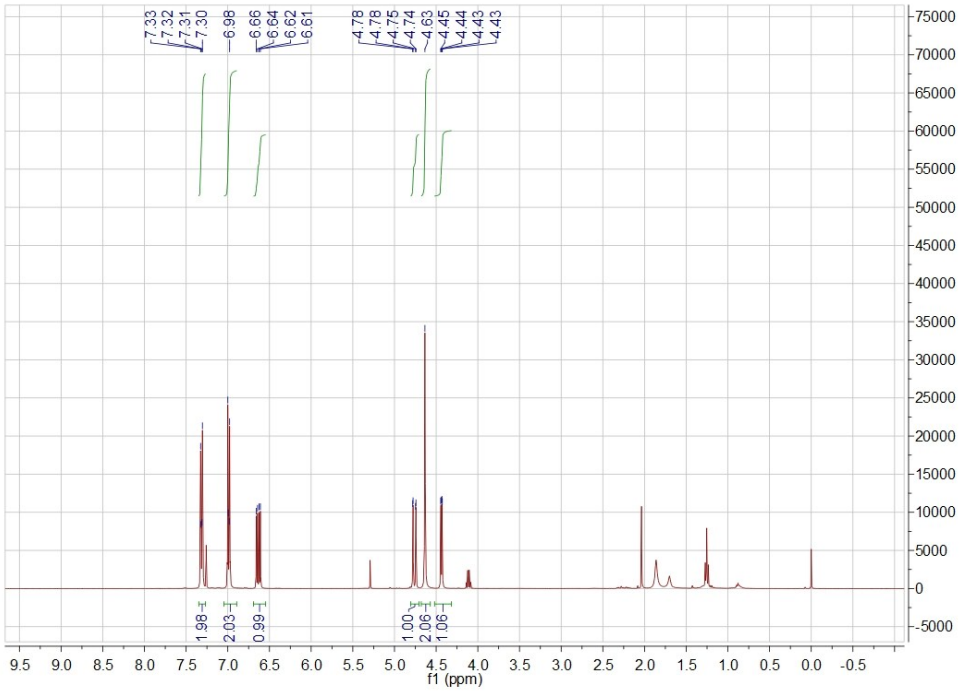
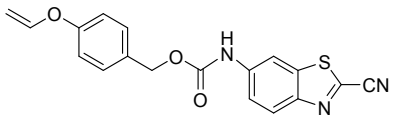




2d

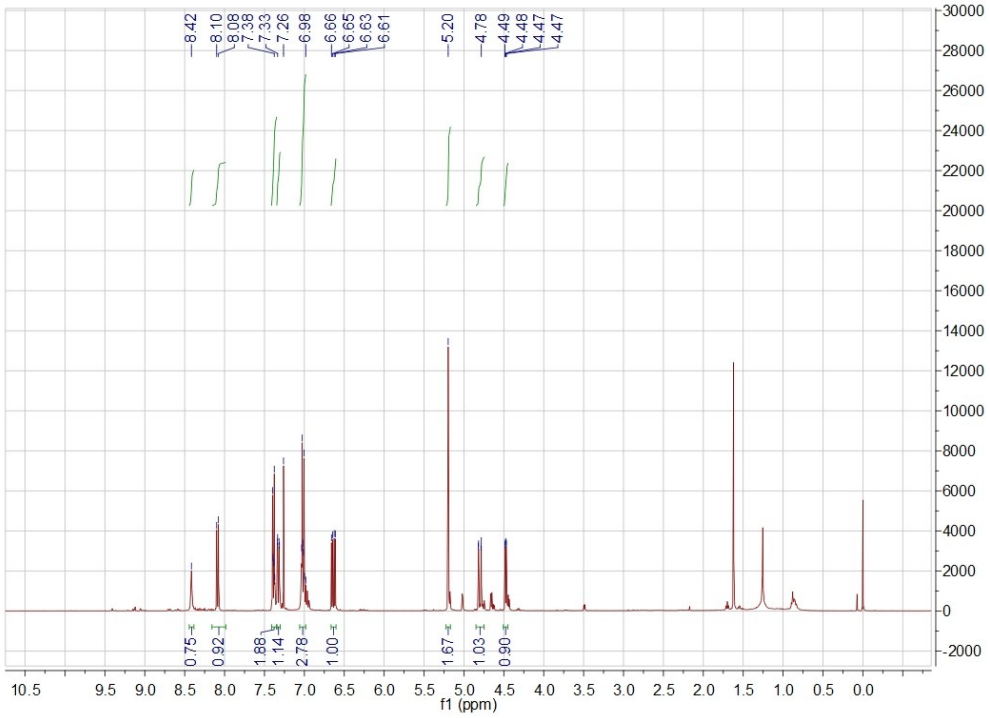
^{1}H NMR



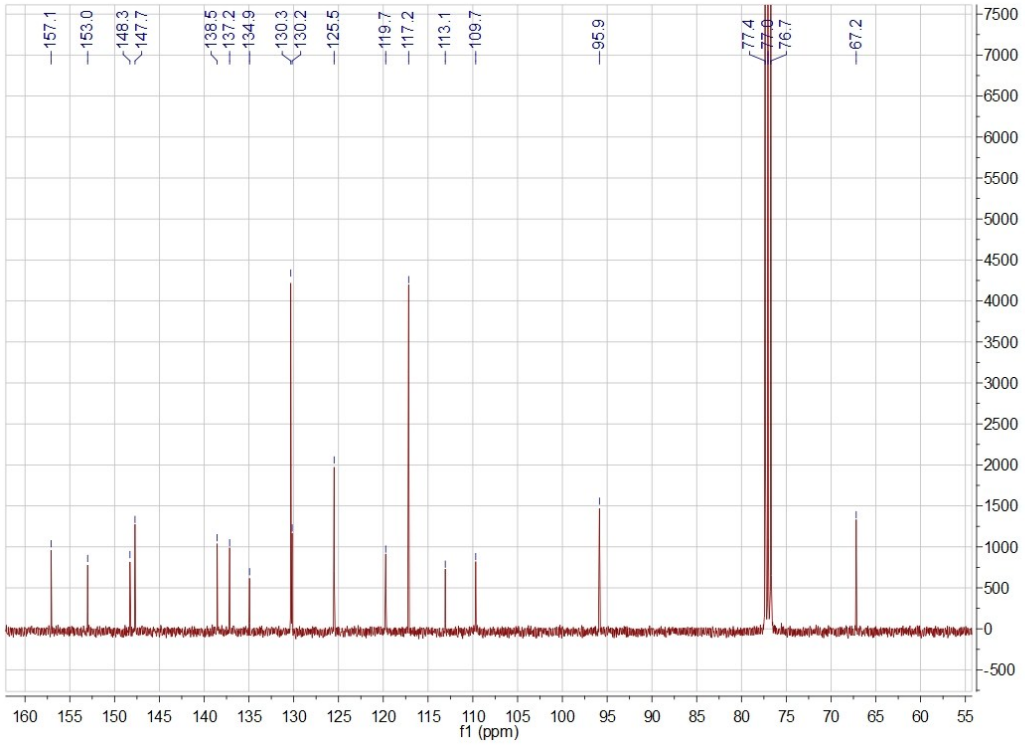


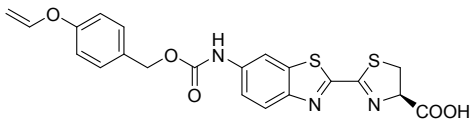
2f

^{1}H NMR



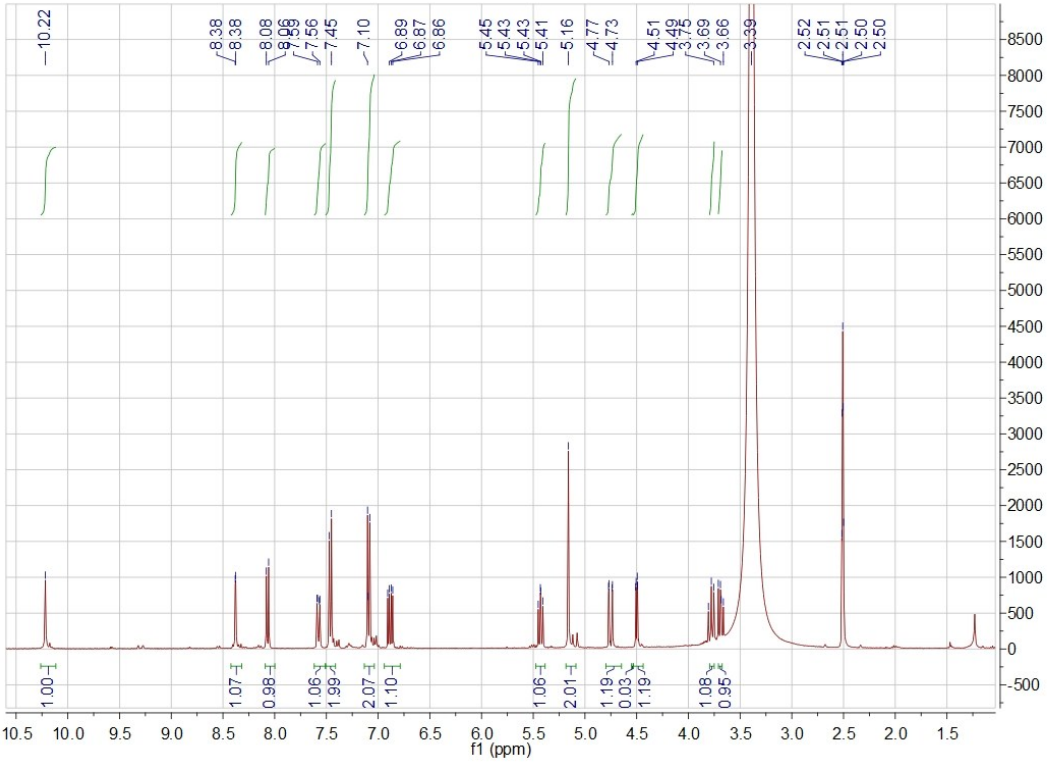
^{13}C NMR



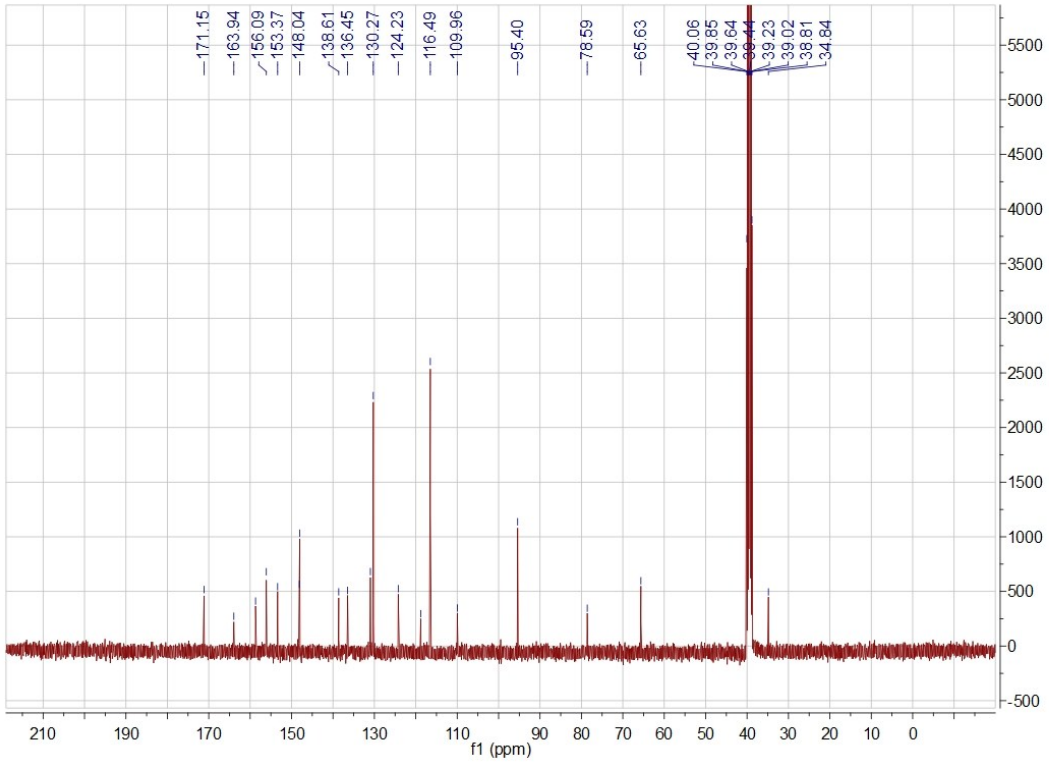


2

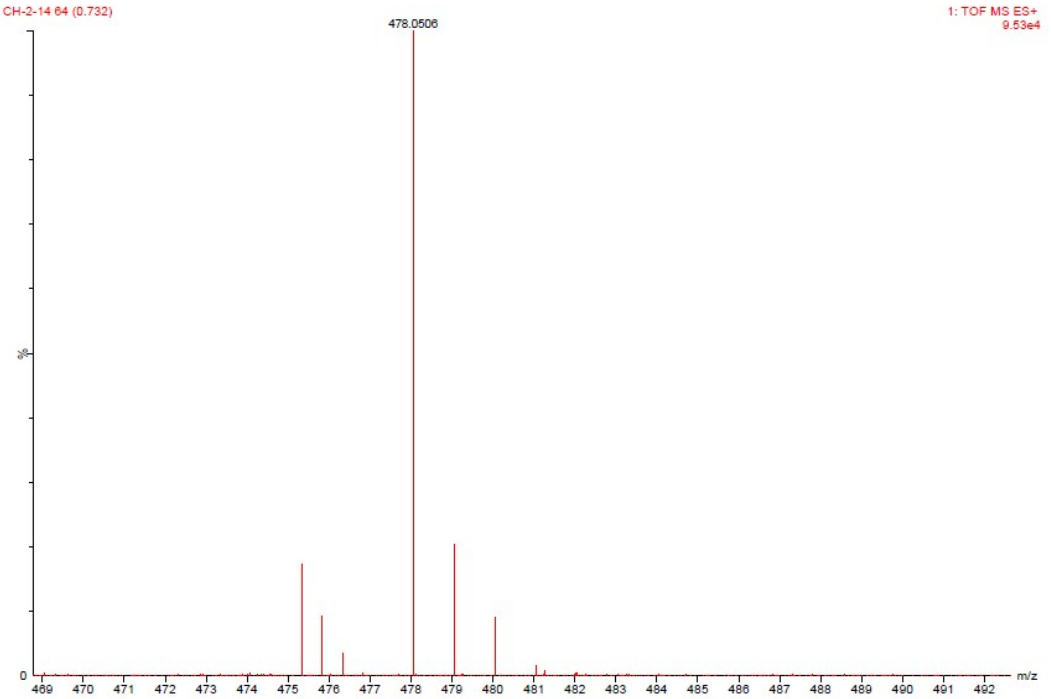
^{1}H NMR



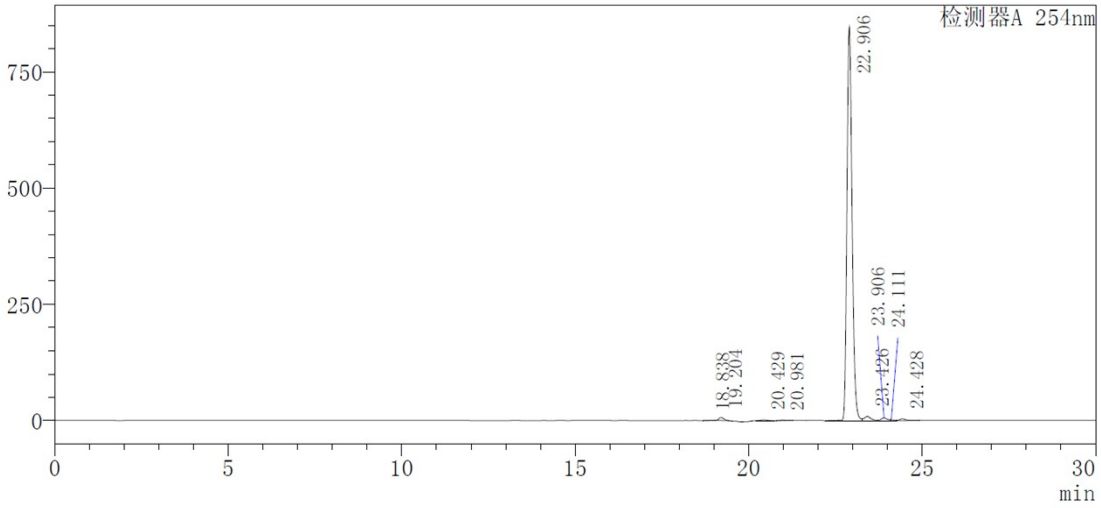
^{13}C NMR

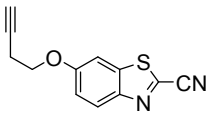

171.15
163.94
156.09
153.37
148.04
138.61
136.45
130.27
124.23
116.49
109.96
95.40
78.59
65.63
40.06
39.85
39.64
39.44
39.23
39.02
38.81
34.84
f1 (ppm)

MS


CH-2-14 64 (0.732)
1: TOF MS ES+
9.53e4
478.0506
%
m/z

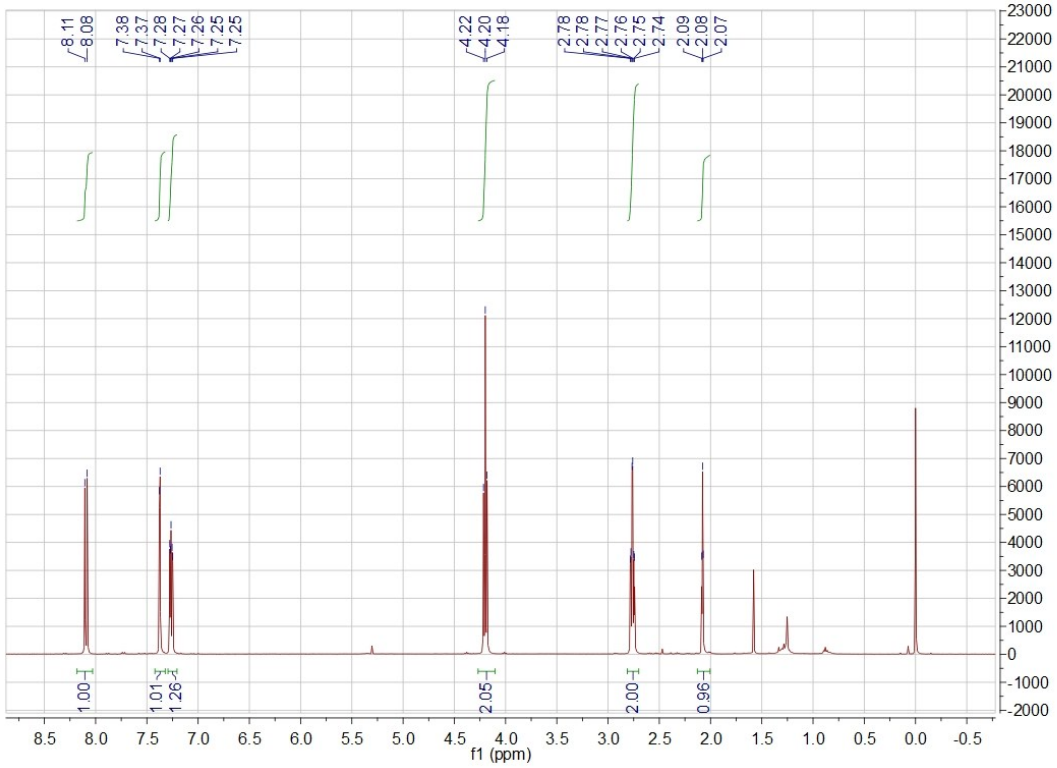
HPLC



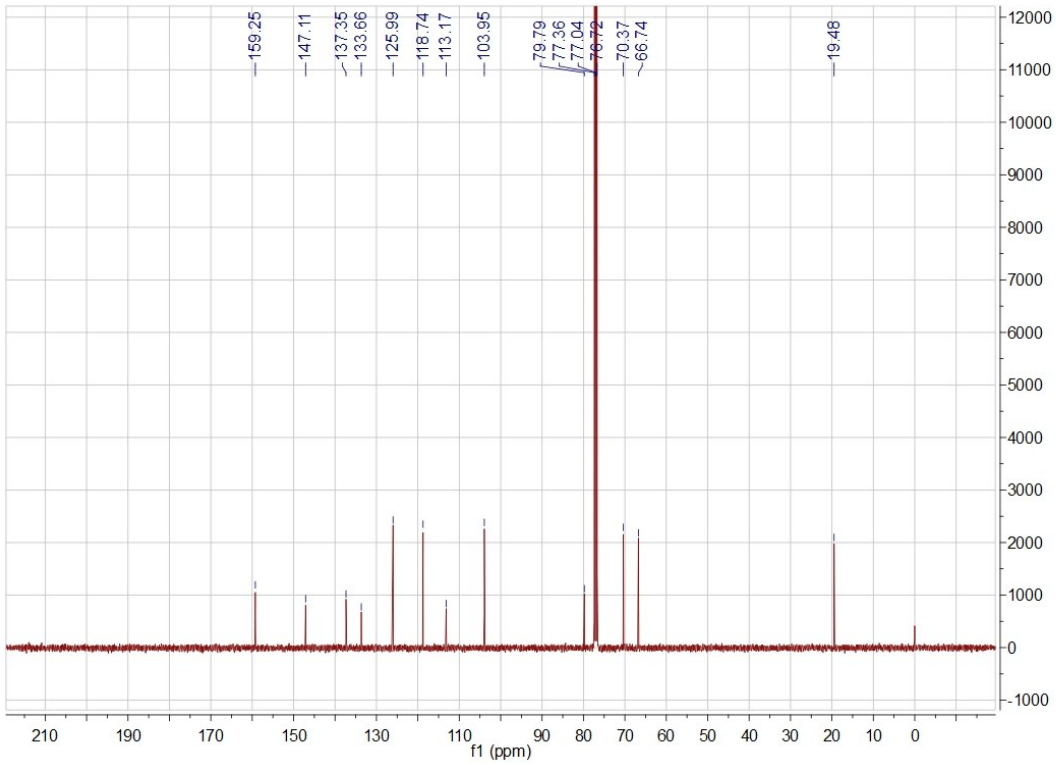


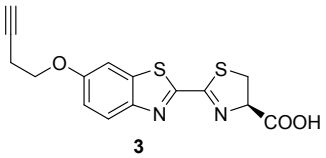
3b

^{1}H NMR



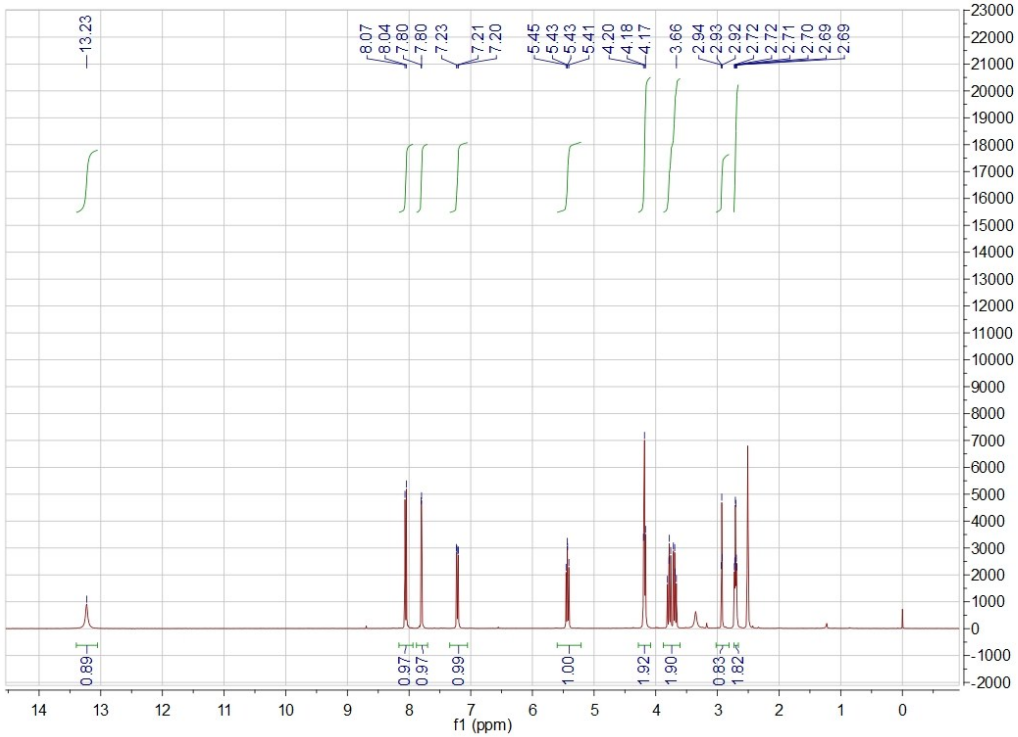
^{13}C NMR



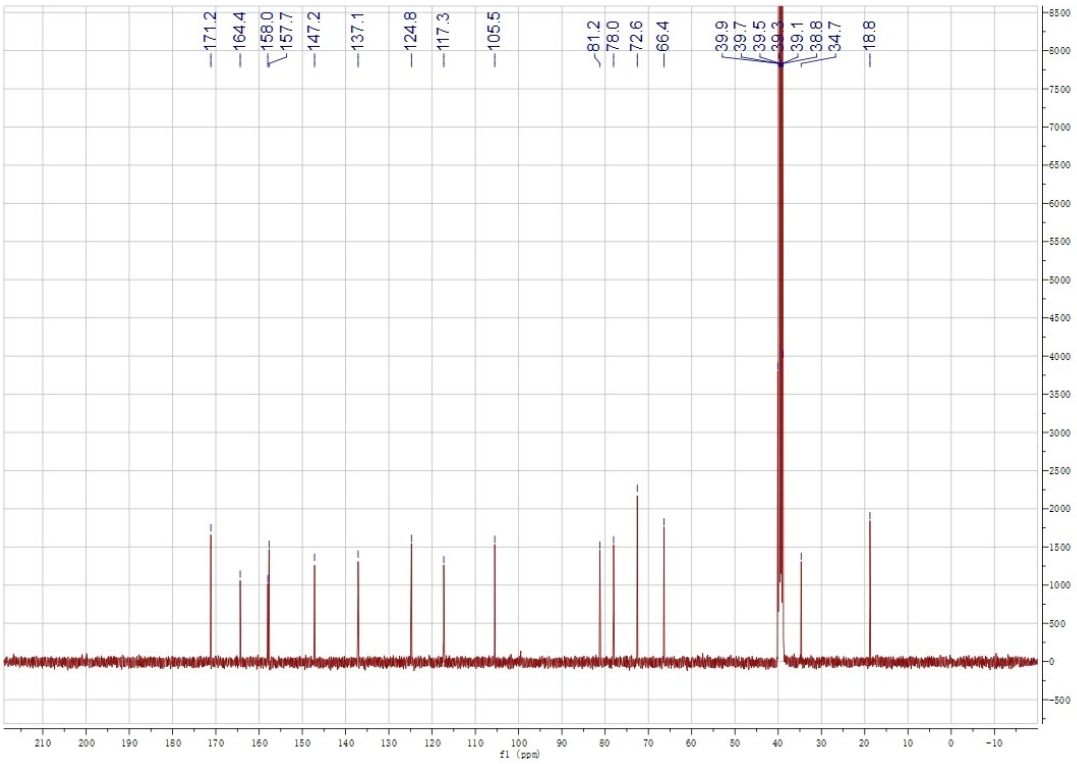


3

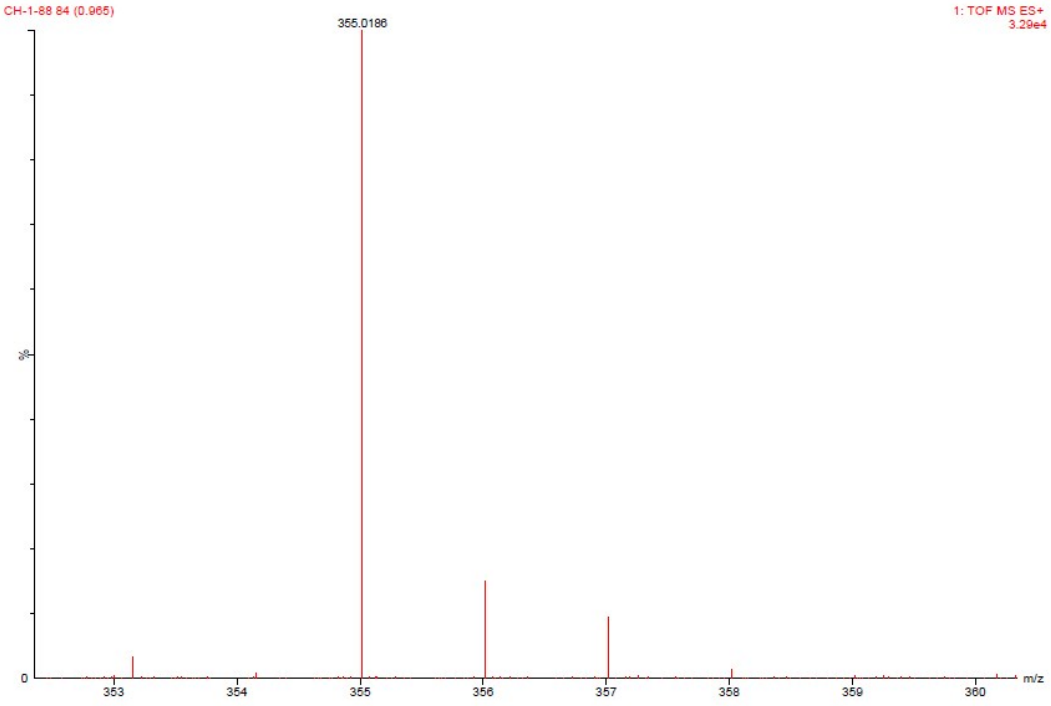
^{1}H NMR



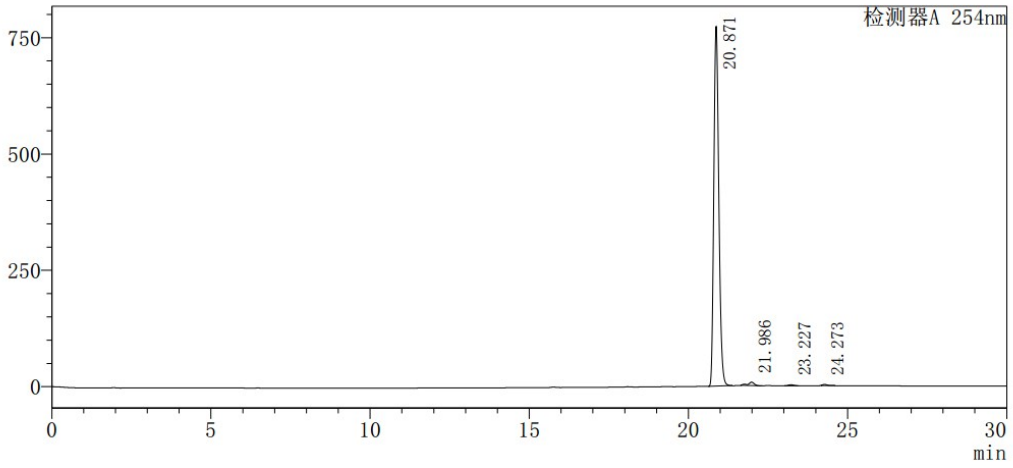
^{13}C NMR


171.2
164.4
158.0
157.7
147.2
137.1
124.8
117.3
105.5
81.2
78.0
72.6
66.4
39.9
39.7
39.5
39.3
39.1
38.8
34.7
18.8
f1 (ppm)

MS


CH-1-88 84 (0.965)
1: TOF MS ES+
3.29e4
355.0186
%
m/z

HPLC


检测器A 254nm
750
500
250
0
20.871
21.986
23.227
24.273
0
5
10
15
20
25
30
min