Supporting Information

Furanyl Acryl Conjugated Coumarinas Efficient Inhibitor and Highly Selective Off-On Fluorescent Probe for Covalent Labelling of ThioredoxinReductase

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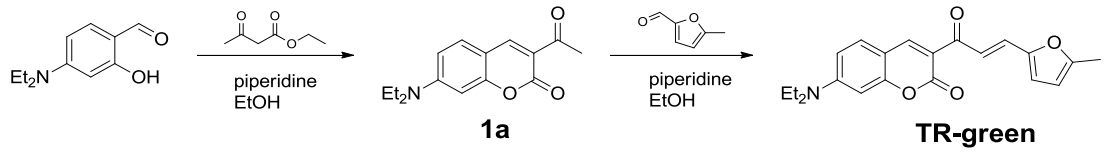
1. General

All reagents used were commercially available. Solvents were treated using standard techniques. Reactions were monitored by TLC on a glass plate coated with silica gel with fluorescent indicator (GF254). Column chromatography was performed on silica gel(200-300 mesh).^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as an internal standard with a BurkerBioSpinUltrashield 400 NMR system at 400 MHz and 100 MHz, respectively. The Purity of target compounds (>95%) was determined on a DIONEX Ultimate 3000 HPLC System(Chromeleon SR9 Build 2673); column, Acclaim 120®C18, 5μm, 4.6×250 mm; mobile phase, solvent A:water, solvent B: Methanol, flow rate 1 ml/min; UV wavelength, 254 nm; temperature, ambient; compound purities were calculated as the percentage peak area of the analyzed compound, retention times (t_R) were calculated in minutes. High resolution mass spectra (HRMS) were recorded on The Pro-Orbitrap High Resolution LC-MC (ThermoFisher).

2. Synthesis



Scheme S1 Synthesis of **TR-green**

Compound 1a

Ethyl acetoacetate (1 g, 5.17 mmol) and 4-(diethylamino) salicylaldehyde (1.35 g, 10.38 mmol) was dissolved in absolute EtOH (20 mL), and then 100 μl of piperidine was added as catalyst. The mixture was heated to reflux for 5 h, and then cool down, a bright yellow precipitate formed. It was collected by filter and washed with cold absolute ethanol to give compound **1a** as a yellow solid (1.2 g, 4.6 mmol) in 90 % yield:^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.40 (d, J = 9.0 Hz, 1H), 6.63 (dd, J = 9.0, 2.3 Hz, 1H), 6.48 (d, J = 2.2 Hz, 1H), 3.47 (q, J = 7.1 Hz, 1H), 2.69 (s, 1H), 1.25 (t, J = 7.1 Hz, 1H).

Compound TR-green

3-Acetyl-7-diethylaminocoumarin (**1a**; 250.0 mg, 0.96 mmol) and 5-methylfurfural (106.0 mg, 0.96 mmol) were dissolved in ethanol (20 mL), and then 100 μl of piperidine were added as a catalyst. The mixture was heated to reflux overnight, and the solvent was evaporated under the reduced pressure. The resulting residue was then purified by chromatography on silica gel to give **TR-green** as a red solid (135 mg, 0.38 mmol) in 40% yield:HPLC purity >99.9% t_R=15.51 min.^{1}H NMR (400 MHz, CDCl3) δ 8.51 (s, 1H), 7.87 (d, J = 15.4 Hz, 1H), 7.55 (d, J = 15.3 Hz, 1H), 7.43 (d, J = 8.9 Hz, 1H), 6.68 (d, J = 8.9 Hz, 1H), 6.62 (s, 1H), 6.55 (d, 1H), 6.10 (s, 1H), 3.47 (q, J = 6.6 Hz, 4H), 2.38 (s, 3H), 1.25 (t, J = 7.1 Hz, 6H).^{13}C NMR (101 MHz, CDCl3) δ 186.15, 160.69, 158.53, 155.89, 152.71, 150.88, 148.26, 131.63, 129.76, 120.72, 117.76, 117.22, 109.86, 109.19, 108.75, 96.81, 45.21, 14.04, 12.44. HRMS calcd for $C_{21}H_{22}O_4N$ $[M+H]^+$: 352.15433, found 352.15494.

Compound 1

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 150.0 mg, 0.58mmol), 2-Pyrrolecarbaldehyde (82.0 mg, 0.86 mmol) and piperidine (100μl) were dissolved

in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **1** as a redsolid (50 mg, 0.15 mmol) of in 26% yield: HPLC purity 99.50%, t_R=9.72 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (s, 1H), 8.58 (s, 1H), 7.76 (s, 2H), 7.42 (d, *J* = 8.9 Hz, 1H), 6.97 (s, 1H), 6.67 – 6.60 (m, 2H), 6.49 (d, *J* = 1.7 Hz, 1H), 6.30 (d, *J* = 2.7 Hz, 1H), 3.46 (q, *J* = 7.1 Hz, 4H), 1.25 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.64, 161.19, 158.44, 152.82, 148.47, 133.33, 131.66, 129.88, 123.20, 117.99, 116.95, 116.63, 111.11, 109.83, 108.74, 96.57, 45.12, 12.46. HRMS calcd for $C_{20}H_{21}O_3N_2$ $[M+H]^+$: 337.15467, found 337.15508.

Compound 2

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 200.0 mg, 0.77mmol), 5-methyl-2-thiophenecarbaldehyde (126.0 mg, 1.0 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **2** as a redsolid (80 mg, 0.22 mmol) of in 28% yield: HPLC purity 98.23%, t_R=19.21 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.88 (d, *J* = 15.3 Hz, 1H), 7.81 (d, *J* = 15.3 Hz, 1H), 7.41 (d, *J* = 8.9 Hz, 1H), 7.17 (d, *J* = 3.5 Hz, 1H), 6.73 (d, *J* = 3.5 Hz, 1H), 6.62 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.49 (d, *J* = 2.2 Hz, 1H), 3.46 (q, *J* = 7.1 Hz, 4H), 2.51 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.95, 160.73, 158.59, 152.87, 148.37, 144.49, 139.16, 136.26, 132.31, 131.68, 126.68, 122.59, 116.84, 109.78, 108.63, 96.64, 45.14, 15.89, 12.47. HRMS calcd for $C_{21}H_{22}O_3NS$ $[M+H]^+$: 368.13149, found 368.13221.

Compound 3

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 150.0 mg, 0.58mmol),3-Pyridinecarboxaldehyde (93.0 mg, 0.86 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **3**as a redsolid (60 mg, 0.17 mmol) of in 30% yield: HPLC purity 99.11%, t_R=10.82 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.68 – 8.49 (m, 2H), 8.23 (d, *J* = 15.8 Hz, 1H), 8.02 (s, 1H), 7.79 (d, *J* = 15.8 Hz, 1H), 7.44 (d, *J* = 9.0 Hz, 1H), 7.34 (dd, *J* = 8.0, 4.5 Hz, 1H), 6.64 (d, *J* = 8.9 Hz, 1H), 6.50 (s, 1H), 3.48 (q, *J* = 5.7 Hz, 4H), 1.26 (t, *J* = 9.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.98, 160.91, 158.81, 153.20, 150.74, 150.53, 149.00, 139.02, 134.42, 131.95, 131.22, 127.00, 123.65, 116.15, 110.01, 108.67, 96.65, 45.20, 12.46. HRMS calcd for $C_{21}H_{21}O_3N_2$ $[M+H]^+$: 349.15467, found 349.15531.

Compound 4

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 150.0 mg, 0.58mmol), 1-Benzothiophene-3-carbaldehyde (140.0 mg, 0.86 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **4** as a redsolid (60 mg, 0.15 mmol) of in 26% yield: HPLC purity 96.07%, t_R=23.47 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.29 (d, *J* = 15.8 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 15.8 Hz, 1H), 7.94 (s, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.54 – 7.35 (m, 3H), 6.64 (d, *J* = 9.0 Hz, 1H), 6.50 (s, 1H), 3.47 (q, *J* = 6.7 Hz, 4H), 1.26 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.45, 160.98, 158.73, 153.02, 148.71, 140.56, 137.48, 134.72, 132.89, 131.83, 129.07, 125.33, 125.01, 124.94, 122.90, 122.59, 116.72, 109.89, 108.71, 96.66, 45.16, 12.47. HRMS calcd for $C_{24}H_{22}O_3NS$ $[M+H]^+$: 404.13149, found 404.13219.

Compound 5

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 200.0 mg, 0.77mmol), 2-Benzofurancarboxaldehyde (146.0 mg, 1.0 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **5** as a redsolid (150 mg, 0.39 mmol) of in 50% yield: HPLC purity >99.9%, t_R=21.01 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.22 (d, *J* = 15.4 Hz, 1H), 7.70 (d, *J* = 15.4 Hz, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.43 (d, *J* = 9.0 Hz, 1H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.23 (t, *J* = 7.5 Hz, 1H), 7.02 (s, 1H), 6.63 (dd, *J* = 9.0, 2.1 Hz, 1H), 6.51 (d, *J* = 2.2 Hz, 1H), 3.47 (q, *J* = 7.1 Hz, 4H), 1.26 (t, *J* = 7.1 Hz, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 185.98, 160.72, 158.74, 155.67, 153.71, 153.08, 148.69, 131.84, 129.56, 128.64, 126.39, 125.39, 123.18, 121.60, 116.53, 111.77, 111.59, 109.91, 108.67, 96.67, 45.17, 12.47. HRMS calcd for $C_{24}H_{22}O_4N$ $[M+H]^+$: 388.15433, found 388.15503.

Compound 6

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 150.0 mg, 0.58mmol), 3-methoxy-4-hydroxybenzaldehyde (105.0 mg, 0.86 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **6** as a redsolid (60 mg, 0.15 mmol) of in 26% yield: HPLC purity 95.42%, t_R=11.87 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.09 – 7.96 (m, 1H), 7.89 – 7.73 (m, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 6.94 (d, *J* = 7.9 Hz, 1H), 6.64 (d, *J* = 8.9 Hz, 1H), 6.50 (s, 1H), 5.93 (s, 1H), 3.97 (s, 1H), 3.47 (dd, *J* = 6.8, 2.5 Hz, 1H), 1.36 – 1.13 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.37, 160.99, 158.61, 152.91, 148.56, 148.17, 146.79, 143.75, 131.72, 128.09, 124.10, 122.53, 116.97, 114.69, 109.92, 109.84, 108.68, 96.63, 56.04, 45.13, 12.47. HRMS calcd for $C_{23}H_{24}O_5N$ $[M+H]^+$: 394.16490, found 394.16564.

Compound 7

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 200.0 mg, 0.77mmol), 3-methoxybenzaldehyde (136.0 mg, 1.0 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **7** as a redsolid (60 mg, 0.16 mmol) of in 21% yield: HPLC purity 95.18%, t_R=18.67 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.12 (d, *J* = 15.7 Hz, 1H), 7.79 (d, *J* = 15.6 Hz, 1H), 7.43 (d, *J* = 9.0 Hz, 1H), 7.36 – 7.27 (m, 2H), 7.19 (s, 1H), 6.94 (d, *J* = 7.0 Hz, 1H), 6.63 (d, *J* = 9.0 Hz, 1H), 6.49 (s, 1H), 3.85 (s, 3H), 3.47 (dd, *J* = 6.8 Hz, 4H), 1.25 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.50, 160.85, 159.85, 158.71, 153.01, 148.69, 143.14, 136.82, 131.80, 129.72, 125.25, 121.46, 116.71, 116.26, 113.40, 109.88, 108.64, 96.65, 55.31, 45.15, 12.46. HRMS calcd for $C_{23}H_{24}O_4N$ $[M+H]^+$: 378.16998, found 378.17073.

Compound 8

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 150.0 mg, 0.58mmol), 2,3-Dimethoxybenzaldehyde (123.0 mg, 0.86 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **8** as a redsolid (150 mg, 0.37 mmol) of in 64% yield: HPLC purity >99.9%, t_R=16.35 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.15 (s, 2H), 7.43 (d, *J* = 9.0 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.07 (t, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.63 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.49 (d, *J* =

2.2 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.47 (q, *J* = 7.1 Hz, 4H), 1.25 (t, *J* = 7.1 Hz, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 186.70, 160.84, 158.66, 153.09, 152.96, 149.01, 148.62, 137.75, 131.76, 129.63, 126.20, 124.06, 119.83, 116.88, 113.97, 109.86, 108.61, 96.62, 61.48, 55.88, 45.14, 12.46. HRMS calcd for $C_{24}H_{26}O_5N$ $[M+H]^+$: 408.18055, found 408.18132.

Compound 9

The reaction was carried out as described above. 3-Acetyl-7-diethylaminocoumarin (**1a**; 150.0 mg, 0.58mmol), 2-(Trifluoromethyl)benzaldehyde (131.0 mg, 0.86 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **9** as a redsolid (100 mg,0.24 mmol) of in 43% yield: HPLC purity 99.10%, t_R=19.88 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.15 (d, *J* = 5.6 Hz, 2H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 7.7 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.50 – 7.41 (m, 2H), 6.64 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.50 (d, *J* = 1.9 Hz, 1H), 3.47 (q, *J* = 7.1 Hz, 4H), 1.26 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.91, 160.94, 158.81, 153.18, 149.03, 138.00, 134.41, 131.97, 129.29, 128.93, 128.41, 126.02, 125.97, 125.45, 122.72, 116.23, 110.00, 108.67, 96.64, 45.19, 12.45. HRMS calcd for $C_{23}H_{21}O_3NF_3$ $[M+H]^+$: 416.1468, found 416.14754.

Compound 10

The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 150.0 mg, 0.58mmol), 3,5-Bis(trifluoromethyl) benzaldehyde (210.0 mg, 0.86 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gel to give **10** as a redsolid (30 mg, 0.06 mmol) of in 11% yield: HPLC purity >99.9%, t_R=15.39 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.25 (d, *J* = 15.8 Hz, 1H), 8.06 (s, 2H), 7.86 (s, 1H), 7.79 (d, *J* = 15.8 Hz, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 6.65 (d, *J* = 9.0 Hz, 1H), 6.51 (s, 1H), 3.49 (q, *J* = 7.0 Hz, 4H), 1.27 (t, *J* = 7.0 Hz, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 185.84, 160.98, 158.92, 153.38, 149.16, 138.98, 137.66, 132.43, 132.07, 128.56, 128.10, 128.08, 124.47, 123.00, 121.75, 115.86, 110.12, 108.70, 96.69, 45.23, 12.45. HRMS calcd for $C_{24}H_{20}O_3NF_6$ $[M+H]^+$: 484.13419, found 484.13488.

Compound 11

The reaction was carried out as described above. 3-Acetyl-7-diethylaminocoumarin (**1a**; 150.0 mg, 0.58mmol), 4-(dimethylamino)-Benzaldehyde (129.0 mg, 0.86 mmol) and piperidine (100 µl) were dissolved in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **11** as a redsolid (60 mg, 0.15 mmol) of in 27% yield: HPLC purity >99.9%, t_R=19.27 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 7.96 (d, *J* = 15.5 Hz, 1H), 7.84 (d, *J* = 15.5 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 2H), 7.41 (d, *J* = 8.9 Hz, 1H), 6.68 (d, *J* = 7.7 Hz, 2H), 6.61 (d, *J* = 8.9 Hz, 1H), 6.49 (s, 1H), 3.46 (q, *J* = 6.9 Hz, 4H), 3.03 (s, 6H), 1.24 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.17, 160.90, 158.45, 152.64, 151.89, 148.10, 144.57, 131.51, 130.72, 123.39, 119.79, 117.57, 111.79, 109.67, 108.69, 96.62, 45.09, 40.13, 12.48. HRMS calcd for $C_{24}H_{27}O_3N_2$ $[M+H]^+$: 391.20162, found 391.20225.

Compound 12

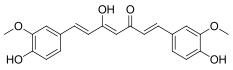
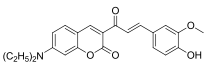
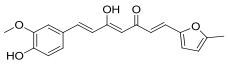
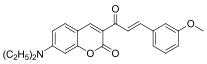
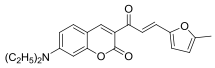
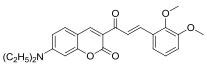
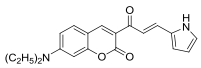
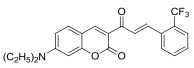
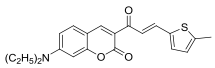
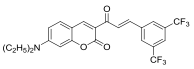
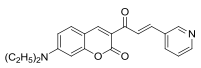
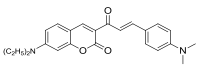
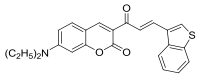
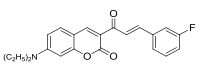
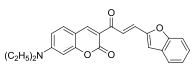
The reaction was carried out as described above.3-Acetyl-7-diethylaminocoumarin (**1a**; 200.0 mg, 0.77mmol), 3-fluorobenzaldehyde (114.0 mg, 0.91 mmol) and piperidine (100 µl) were dissolved

in ethanol (15 mL) refluxed overnight.Purification by chromatography on silica gelto give **12** as a redsolid (30 mg, 0.08 mmol) of in 11% yield: HPLC purity 98.30%, t_R=19.01 min.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.14 (d, *J* = 15.7 Hz, 1H), 7.76 (d, *J* = 15.6 Hz, 1H), 7.48 – 7.31 (m, 4H), 7.07 (t, *J* = 8.0 Hz, 1H), 6.64 (d, *J* = 8.5 Hz, 1H), 6.50 (s, 1H), 3.47 (q, *J* = 6.8 Hz, 4H), 1.26 (t, *J* = 6.9 Hz, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 186.28, 164.23, 161.78, 160.86, 158.78, 153.14, 148.85, 141.57, 137.81, 137.73, 131.89, 130.27, 130.19, 126.28, 124.60, 117.00, 116.79, 116.41, 114.91, 114.69, 109.95, 108.66, 96.66. HRMS calcd for $C_{22}H_{21}O_3NF$ $[M+H]^+$: 366.15000, found 366.15056.

3. Evaluation of the TrxR inhibitory activities

For determining the TrxR inhibitory activity of the compounds, the DTNB reduction assay wasemployed. All assays were conducted at 25℃ in a total volume of 40μl. In each measurement, 0.3 μl of TrxR was added to an assay buffer containing 1 M potassium phosphate (pH 7.0), 500 mM EDTA (pH 7.4), NADPH (0.48mM) and 1μl of inhibitor at various concentrations. After 5 min pre-incubation, the reaction was initiated with the addition of 3.2μl of DTNB. The control was incubated with the same amount of DMSO. The increase in absorbance at 412 nm (Δε412 =13.6 mM^{-1} cm^{-1}) was monitored in the initial 120s. The IC_{50} values were calculated to represent the TrxR inhibitory effect of compounds.

Table. S1 The TrxR inhibitory activity of different compounds (IC_{50}, μM) [a]

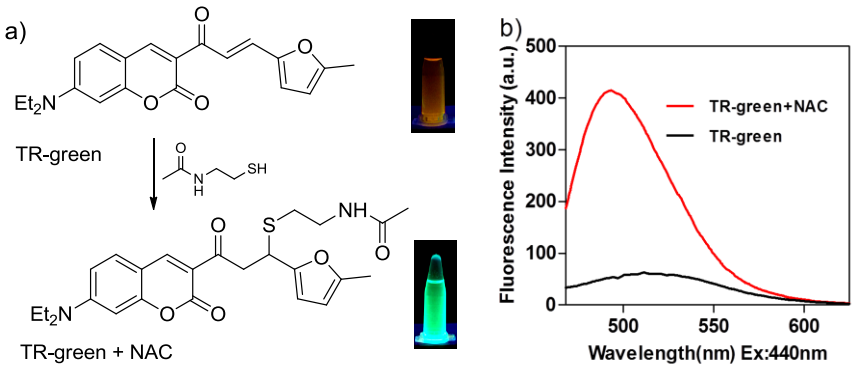
Entry	Structure	Mean±SD	Entry	Structure	Mean±SD
curcumin		38.3	**6**		23.86±1.88
2a		0.82±0.17	**7**		32.51±2.65
TR-green		0.52±0.15	**8**		38.95±3.54
1		42.39±3.69	**9**		>50
2		>50	**10**		>50
3		>50	**11**		>50
4		>50	**12**		>50
5		9.93±1.58			

[a], For determining the TrxR inhibitory activity of compounds,the DTNB reduction assay was employed.

4. TR-green treated with N-acetylcysteamin

To a solution of **TR-green**(5 mg, 0.014 mmol) in 10 mL of MeOH was added excess amount of N-acetylcysteamin (33 mg, 0.55 mmol) and NaOH (2.8 mg, 0.07 mmol), the reaction mixture was further stirred for 24 h at room temperature. The Fluorescence change assay was tested in PBS buffer (pH=7.4), Fluorescence spectra

and the mass result was shown in Scheme S2 and Fig.S1.



Scheme S2 a) Covalent modification of **TR-green** by NAC. The fluorescence change was shown in tubules. b) Fluorescence spectra of TR-green (1 μM) in the absence (black) and presence (red) of NAC (20 μM). The spectra was acquired 24h after NAC addition at room temperature.

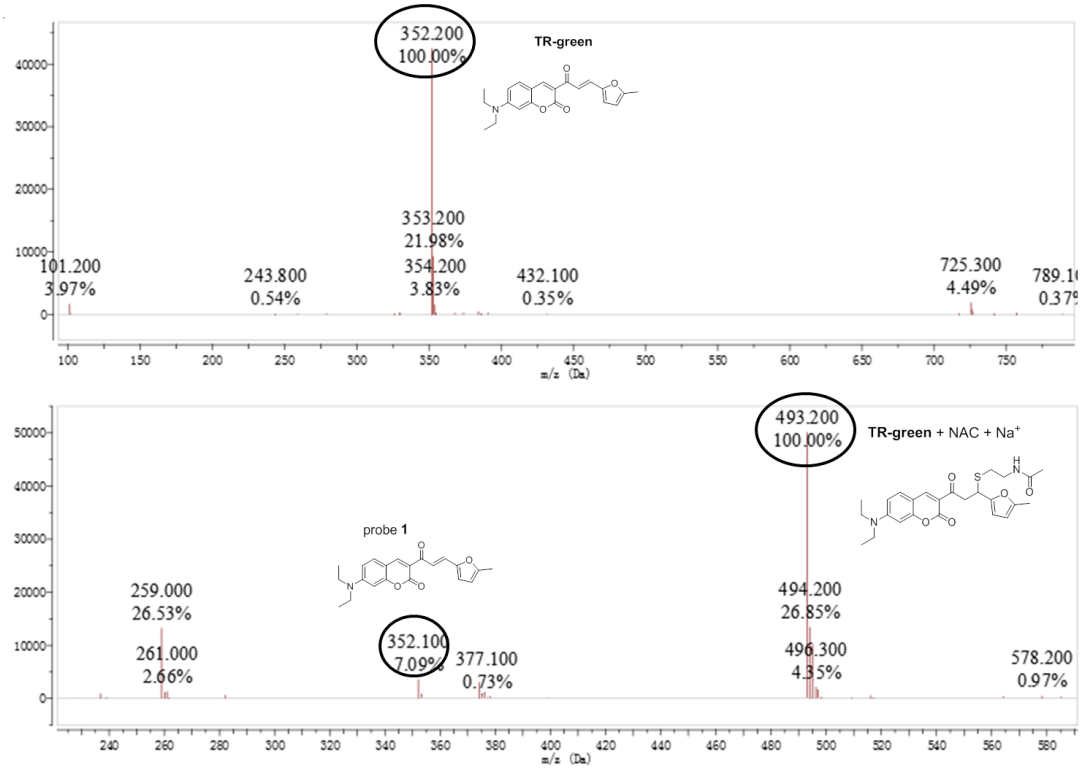


Fig.S1 Mass spectra of **TR-green** and **TR-green** +NAC

5. Protein fluorescence labelling by SDS-PAGE

Three same samples (1,2,3) with concentrations of TrxR (3.8 μM) were pretreated with NADPH(38μM) in PBS buffer (pH 7.4) for 20 minutes at 37 ℃. Then, sample 2 incubated with IAM(50 μM) for 20 minutes at 37 ℃. The sample 2 and 3 were incubated with **TR-green** (95 μM) at 37 ℃ for 2h in 200μl reaction volume. The samples were directly employed for gel analysis after concentrate through the overspeed centrifugal. Addition 8 μL 5 × SDS-PAGE buffer (0.2 MTris / 8% SDS / 40% glycerol / 0.4% bromophenol blue) into 32 μl reaction solution for gel electrophoresis. All samples were heated at 95 ℃ for 5 min before using for gel electrophoresis. Bands were visualized by staining with Coomassie blue staining. For fluorescent labeling, the protein bands were visualized with an excitation λ = 365 nm.

6. UV/Vis, Fluorescence Spectroscopy and Kinetic Data

Preparation for fluorescent study: stock solution of **TR-green** (1 mM) in DMSO was prepared and used by dilution in aqueous PBS solution for fluorescence experiments. In the case of TrxR and Trx, reduced TrxR and Trx was prepared by incubating 5 μM TrxR with 25 μM DTT and 5 μM Trx with 25 μM DTT for 1 h at 37 ℃[S1].The time dependences of the response of **TR-green** (1.0 μM) to thiols (TrxR, BSA, GSH, Cys, and DTT) were determined at 500 nm, with a time interval of 10 s.For the IAM treated samples, the TrxR (1.0 μM) pre-treated with IAM (1.0 mM) in PBS at 37 ℃ for 20min. All the measurements were performed using a Teacen M1000. The fluorescence changes were measured at room temperature in PBS buffer (pH 7.4), with an excitation λ = 440 nm (excitation and emission slit widths at 2.5 nm).

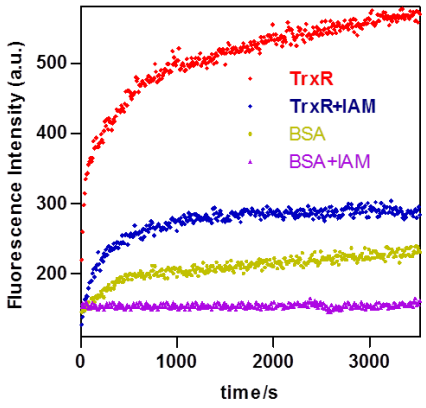


Fig. S2 Time course fluorescence response of **TR-green** (1.0 μM) towards TrxR (1.0 μM), BSA (1.0 μM) treated with IAM (1 mM) or not. All fluorescence changes were measured at room temperature in PBS buffer (pH 7.4), with an excitation λ = 440 nm.

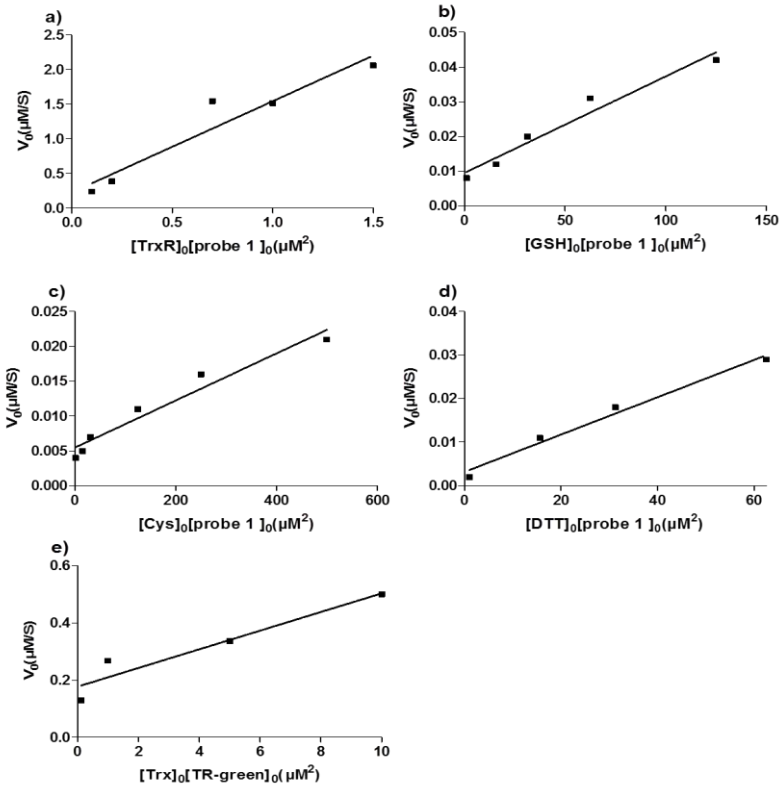


Fig. S3 Plot of V_0 vs $[thiols]_0[\textbf{TR-green}]_0$ of TrxR, Trx, GSH,Cys,DTT in various concentrations and probe 1 (1.0μM). The reaction rate constant (k) for reaction between**TR-green**with TrxR,GSH,Cys,DTT were obtained from $V_0 = k[thiols]_0[\textbf{TR-green}]_0$. All kinetic experiments were performed under physiological conditions (PBS buffer, pH 7.4).

References

S1. a) Q. Cheng, L. Johansson, J.O. Thorell, A. Fredriksson, E. Samén, S. Stone-Elander, E. S. J. Arnér, *ChemBioChem*,2006, **7**, 1976-1981. b) E. H. Chew, J. Lu, T. D. Bradshaw, A. Holmgren,*The FASEB Journal*,2008, **22**, 2072-2083. c) M. H. Lee, J. H. Han, J.-H. Lee, H. G. Choi, C. Kang, J. S. Kim, *Journal of the American Chemical Society*,2012, **134**, 17314-17319.

7. The selectivity of TR-green

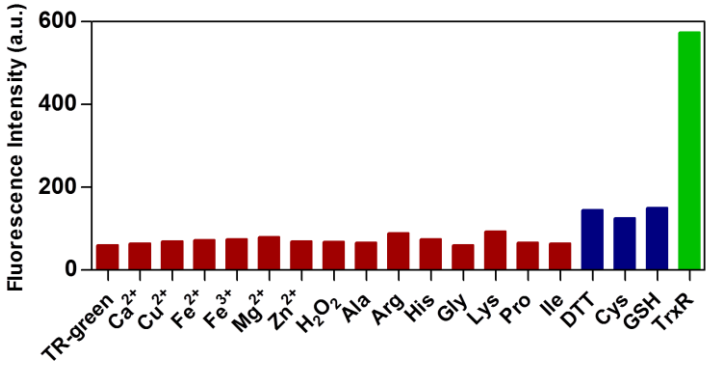


Fig.S4 Reactivity of **TR-green** with TrxR and other putative interferants. Fluorescence responses of **TR-green** (1.0 μM) toward 1.0 mM of amino acids, metals, H_2O_2 and 1.0 μM of TrxR. The bars represent the fluorescence intensity at 500 nm. All fluorescence changes were acquired 30 min after the addition of the analytes at room temperature in PBS buffer (pH 7.4), with an excitation λ = 440 nm.

8. Inhibition of Trx by TR-green[S2]

Trx was incubated with different concentrationsof **TR-green** for 0.5 h at 37 ℃. The final concentration of DMSO in the experiment was 1%, and the control contained the same amount of DMSO. The enzyme activity was measured at room temperature by monitoring the increase of A650, which is caused by the precipitation of reduced insulin. [DTT] 2 mM, [Trx] 4μM, and [insulin] 1 mg/mL.(a) Positive control; (b-e) incubation with 100, 50, 25 and 12.5μM **TR-green**; (e) negative control (only DTT and insulin).

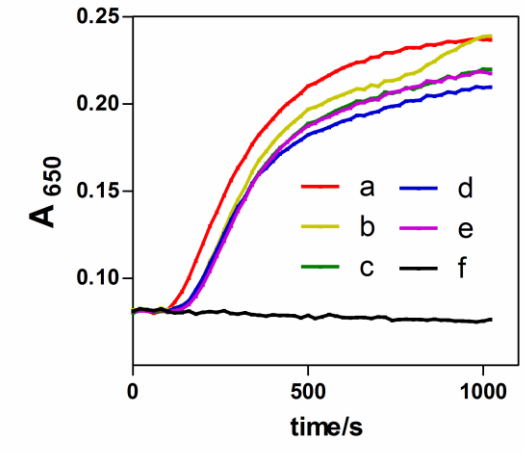


Fig.S5 Inhibition of Trx by TR-green. (a) Positive control; (b-e) incubation with 100, 50, 25 and 12.5μM TR-green; (e) negative

control (only DTT and insulin).

References

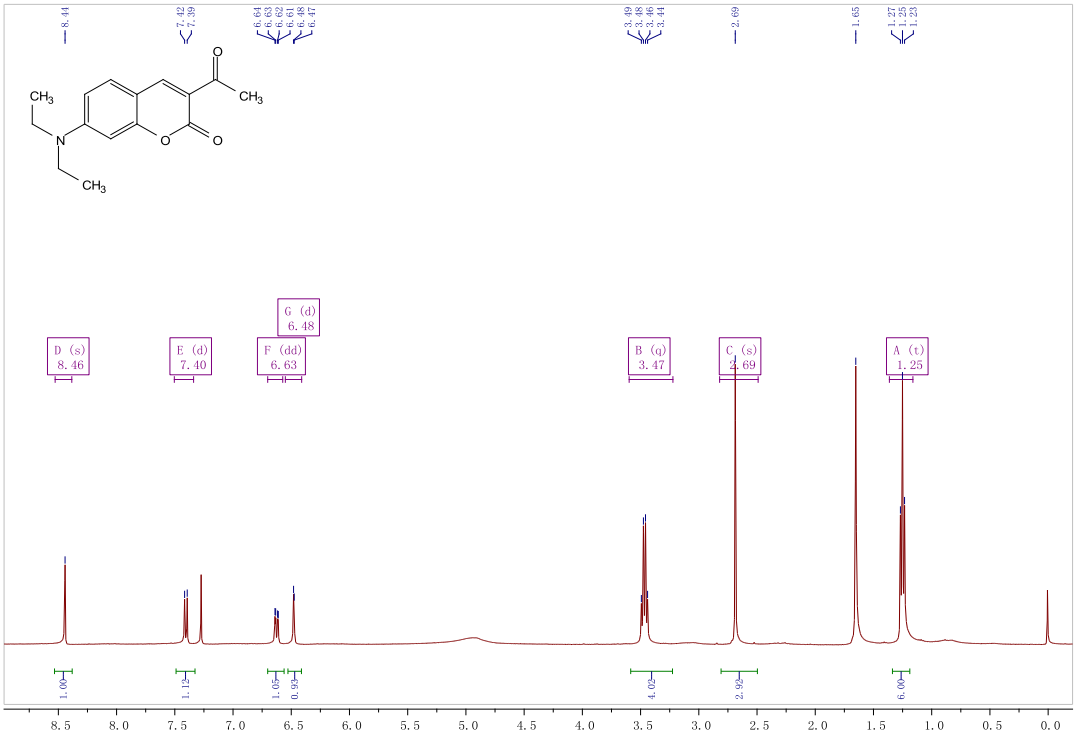
S2. J Fang , A Holmgren. *Journal of the American Chemical Society*, 2006, **128**, 1879-1885.

9. Cell culture and fluorescence imaging.

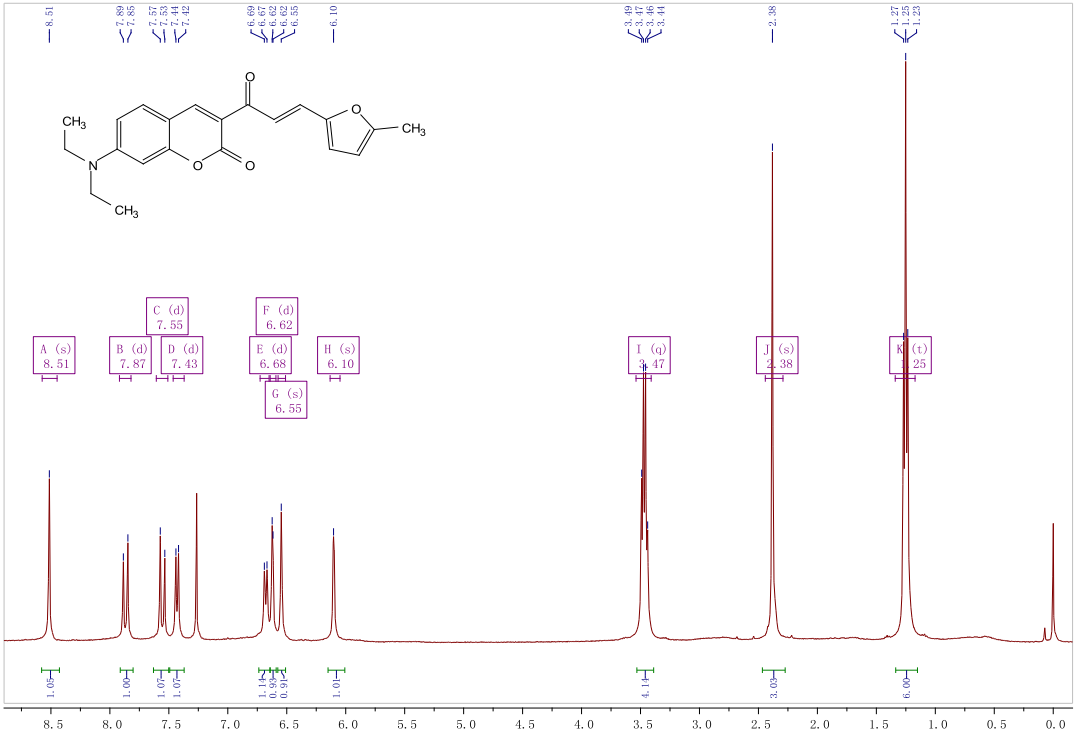
A human cancer cell line, cisplatin-resistant A549 (A549/CDDP) cell, was grown inDMEM supplemented with 10% fetal calf serum, 1% penicillin, and 10,000 Unit/mL of streptomycin at 37 ℃ under humidified air containing 5% CO_2. Cells (1.0 x 10^5) were locatedand stabilized in confocal plate. When 80% confluence was reached, the cells werewashed with 1.0 mL of phosphate buffered saline (PBS) twice and finally incubated with 1.0 mL of DMEMcontaining **TR-green** (final concentration of 2.5μM) for the following confocal experiment (LSM710 Zeiss). For the NEM (N-ethylmaleimide) treated samples, before the media were finally replaced with PBS containing**TR-green**, the cells were incubated withthe media containing NEM (50μM) for 0.5 h at 37 ℃. The cellular TrxR were stained with TrxR1 (B-2) mouse antibody and Dylight 549-Goat Anti-Mouse IgG (red). The fluorescence images inconfocal experiments were obtained at 20 min after the cellswere treated with **TR-green**.

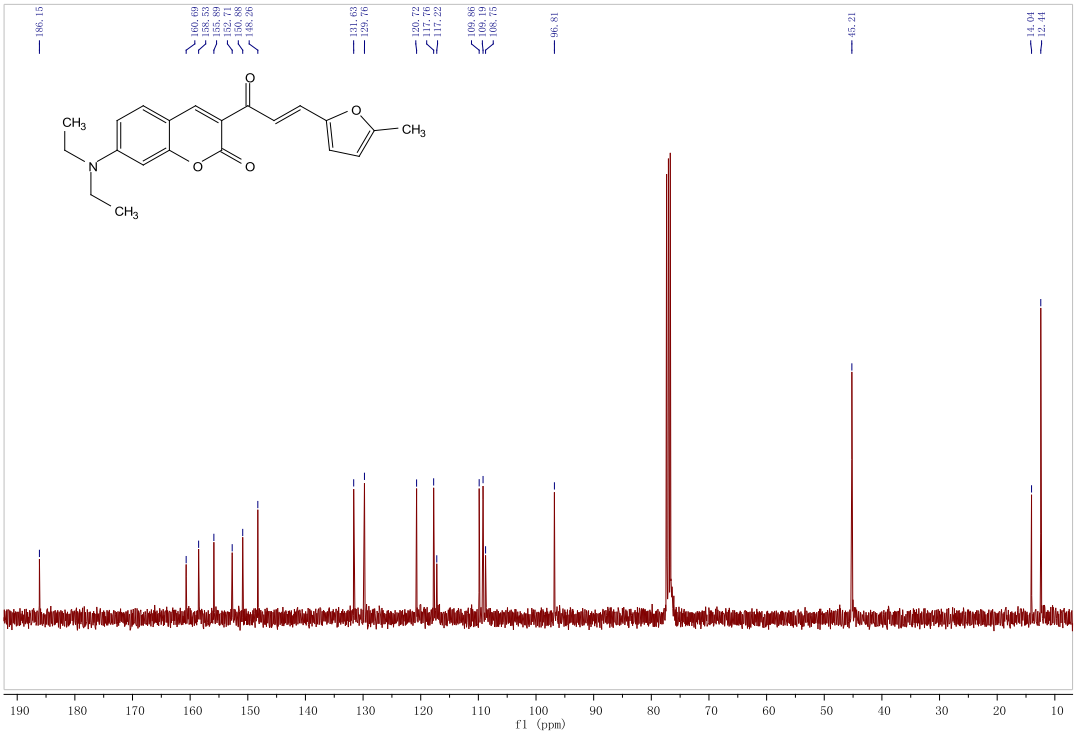
10. ^{1}HNMR ^{13}CNMR spectra

Compound 1a

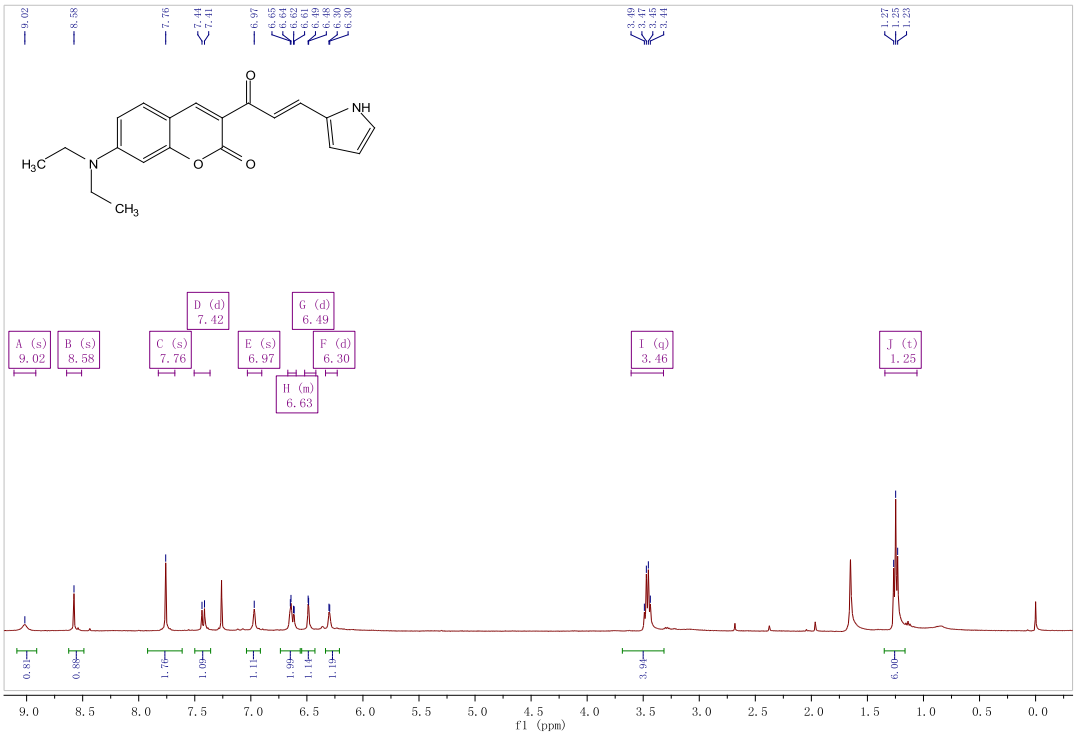


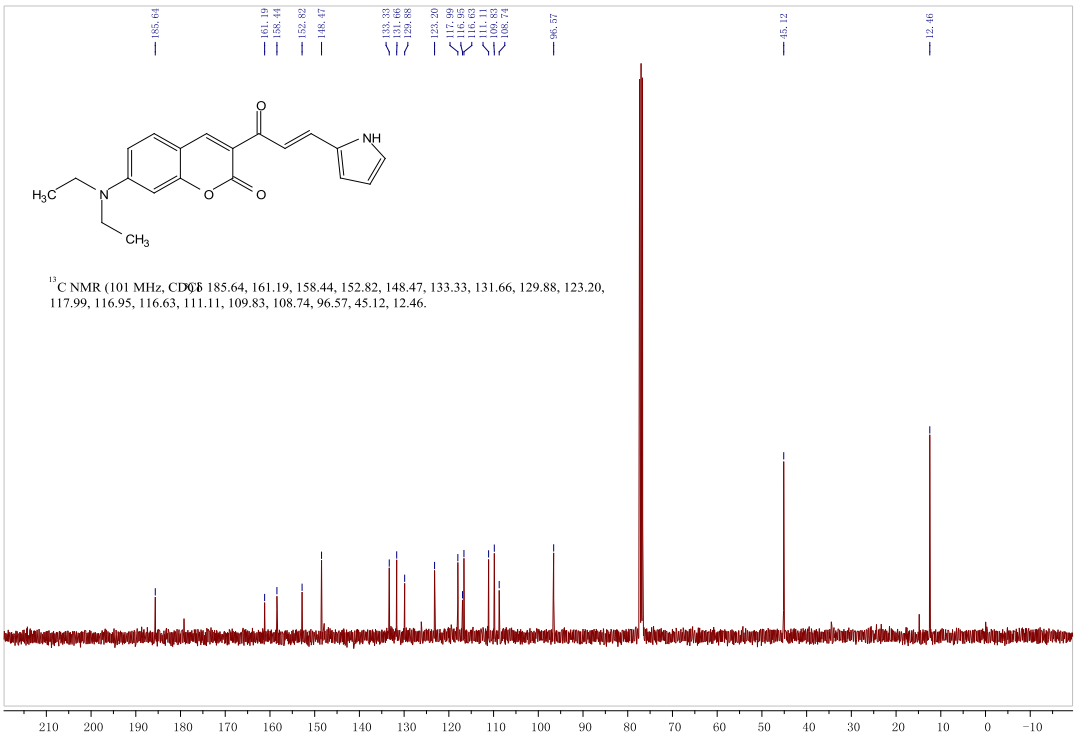
TR-green


8.51
7.89
7.85
7.57
7.53
7.44
7.42
6.69
6.67
6.62
6.62
6.55
6.10
3.49
3.47
3.46
3.44
2.38
1.27
1.25
1.23
A (s) 8.51
B (d) 7.87
C (d) 7.55
D (d) 7.43
E (d) 6.68
F (d) 6.62
G (s) 6.55
H (s) 6.10
I (q) 3.47
J (s) 2.38
K (t) 1.25
1.00
1.00
1.07
1.07
1.14
0.99
2.92
1.01
4.14
3.09
6.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


186.15
160.69
158.53
155.89
152.71
150.88
148.26
131.63
129.76
120.72
117.76
111.22
109.86
108.19
106.75
96.81
45.21
14.04
12.44
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

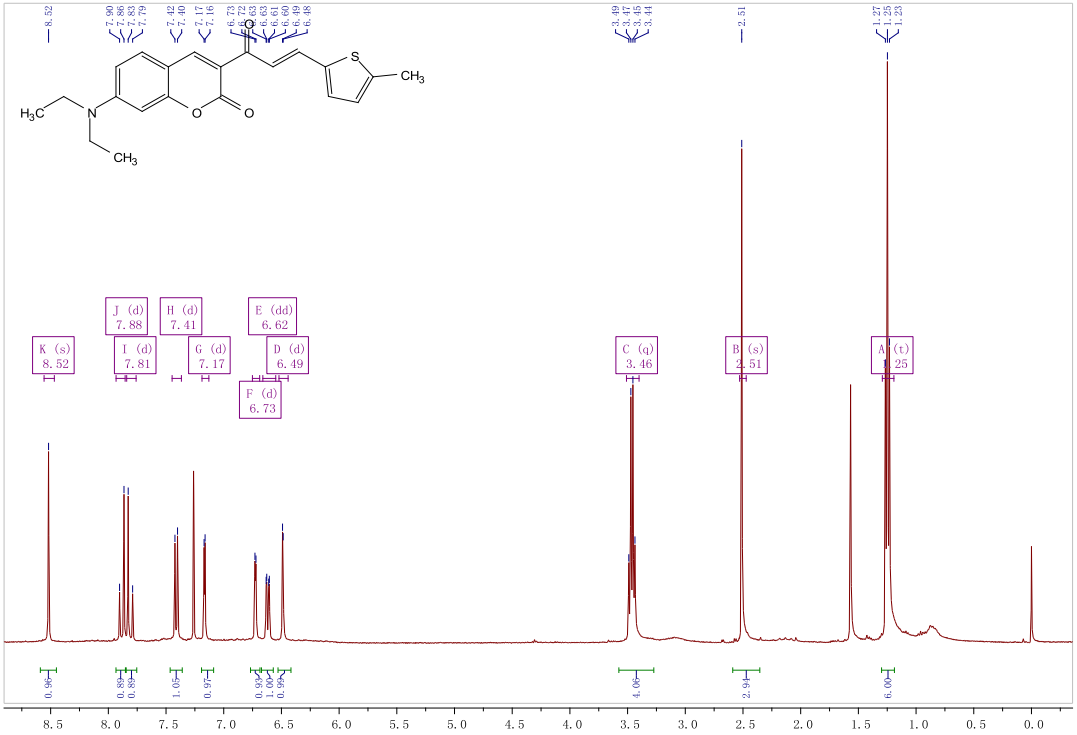
Compound 1





^{13}C NMR (101 MHz, CDCl$_3$) δ 185.64, 161.19, 158.44, 152.82, 148.47, 133.33, 131.66, 129.88, 123.20, 117.99, 116.95, 116.63, 111.11, 109.83, 108.74, 96.57, 45.12, 12.46.

Compound 2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7.90
7.86
7.83
7.79
7.42
7.40
7.17
7.16
6.73
6.72
6.63
6.61
6.60
6.49
6.48
3.49
3.47
3.45
3.44
2.51
1.27
1.25
1.23
J (d) 7.88
H (d) 7.41
E (dd) 6.62
K (s) 8.52
I (d) 7.81
G (d) 7.17
D (d) 6.49
F (d) 6.73
C (q) 3.46
B (s) 2.51
A (t) 1.25
0.96
0.88
0.88
1.06
0.97
0.97
1.00
0.98
4.06
2.94
6.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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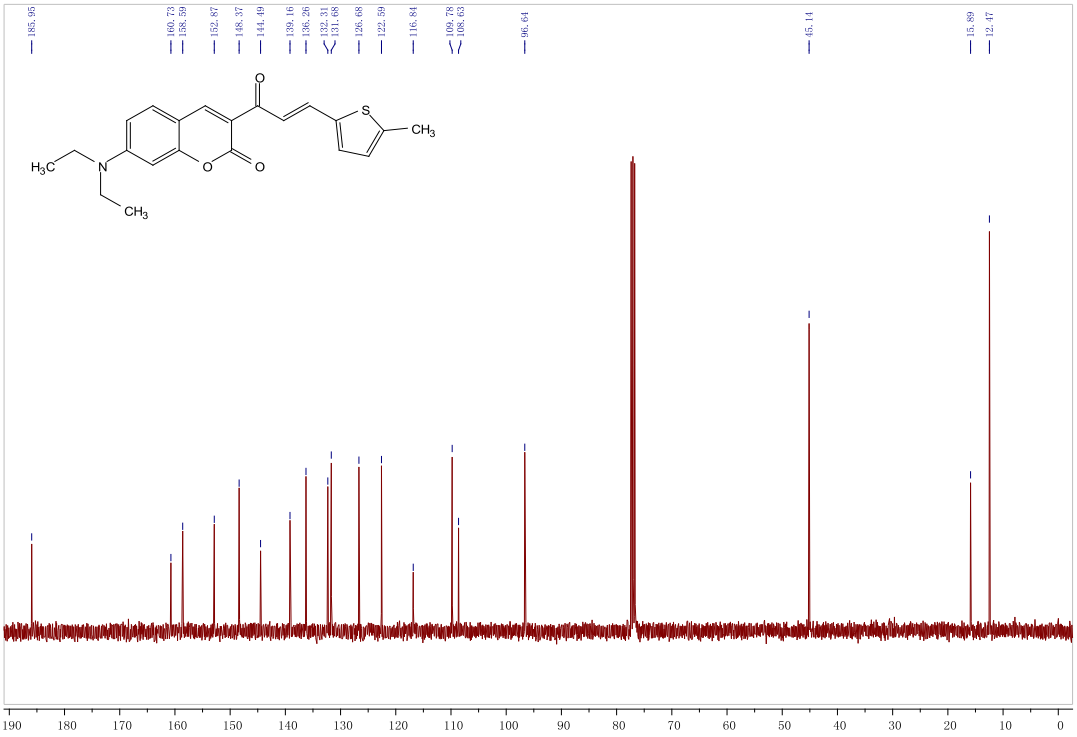
1.0
0.5
0.0


185.95
160.73
158.59
152.87
148.37
144.49
139.16
136.26
132.31
131.68
126.68
122.59
116.84
109.78
108.63
96.64
45.14
15.89
12.47
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

Compound 3


8.83
8.59
8.58
8.57
8.25
8.21
8.02
7.81
7.77
7.45
7.43
7.35
7.34
7.33
7.32
6.65
6.63
6.50
3.49
3.48
3.47
3.46
CH3
N
O
O
O
CH3
K (s) 8.83
J (m) 8.58
I (d) 8.23
H (s) 8.02
G (d) 7.79
F (dd) 7.34
E (d) 7.44
D (d) 6.64
C (s) 6.50
B (q) 3.48
A (t) 1.26
1.14
1.95
1.01
0.98
0.96
1.00
0.98
0.99
0.96
4.17
6.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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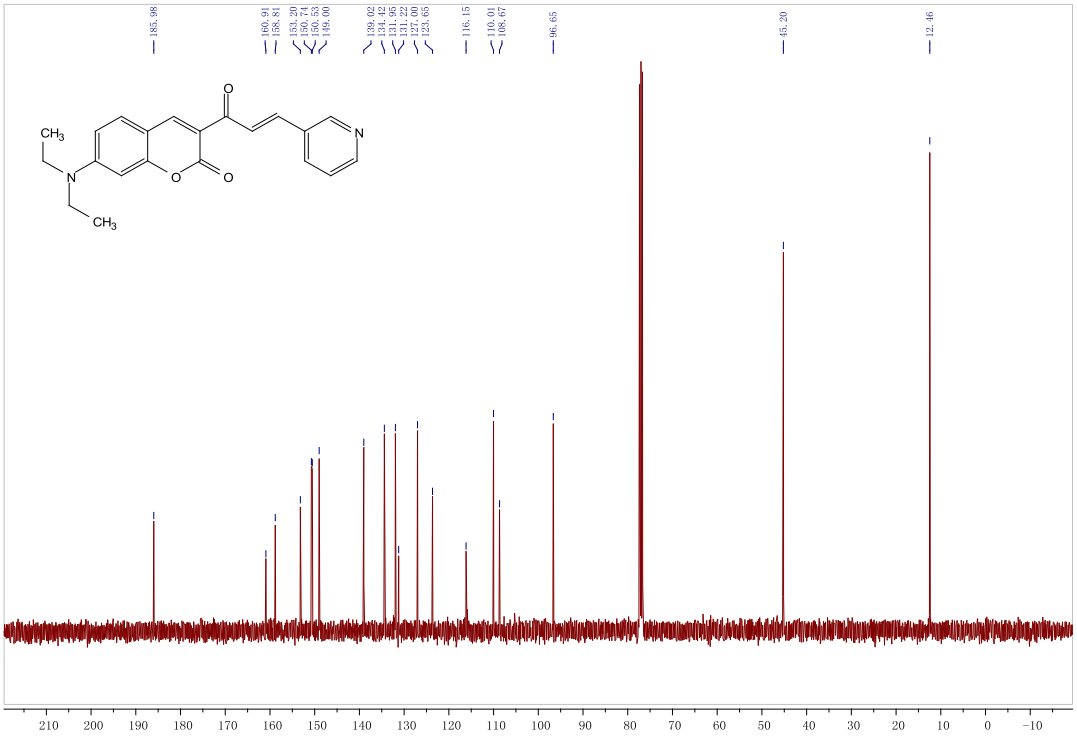
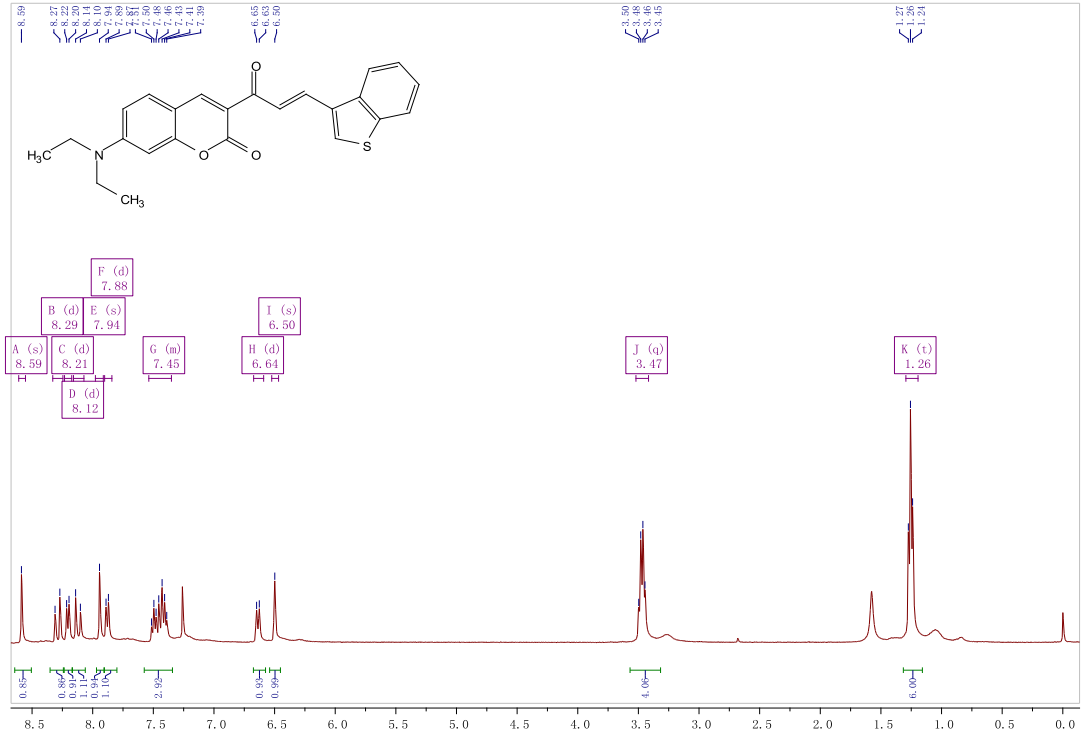
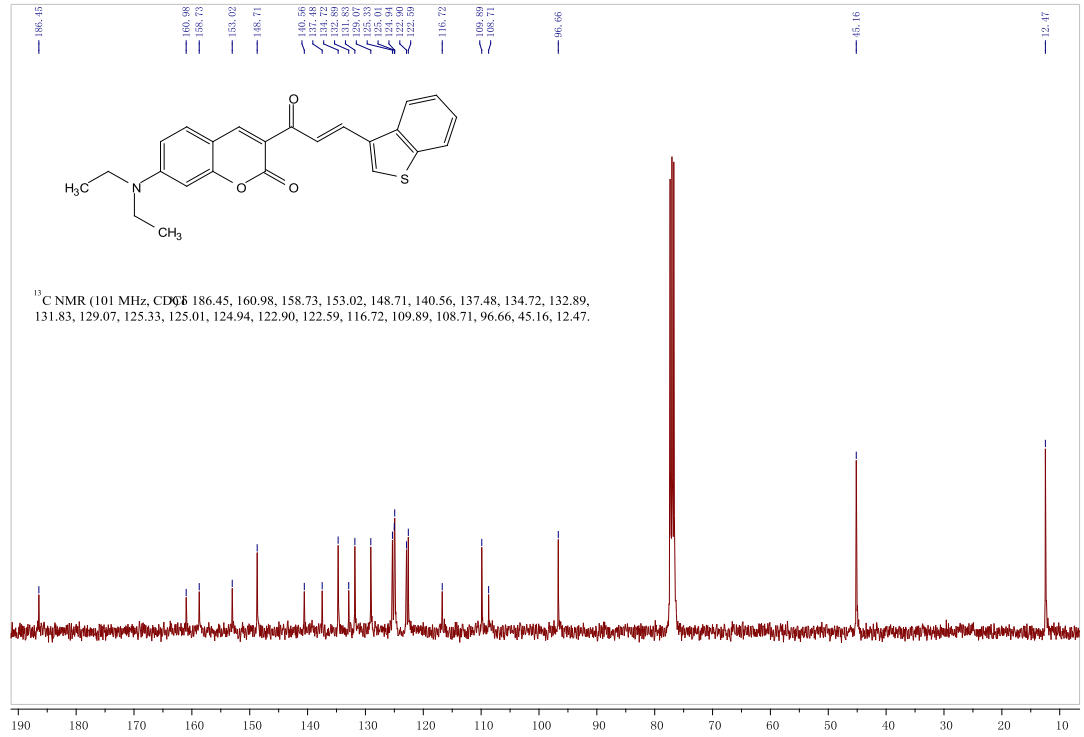

185.98
160.91
158.81
153.20
150.74
150.53
149.00
139.02
134.42
131.95
131.22
127.00
123.65
116.15
110.01
108.67
96.65
45.20
12.46
CH3
N
O
O
O
CH3
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10

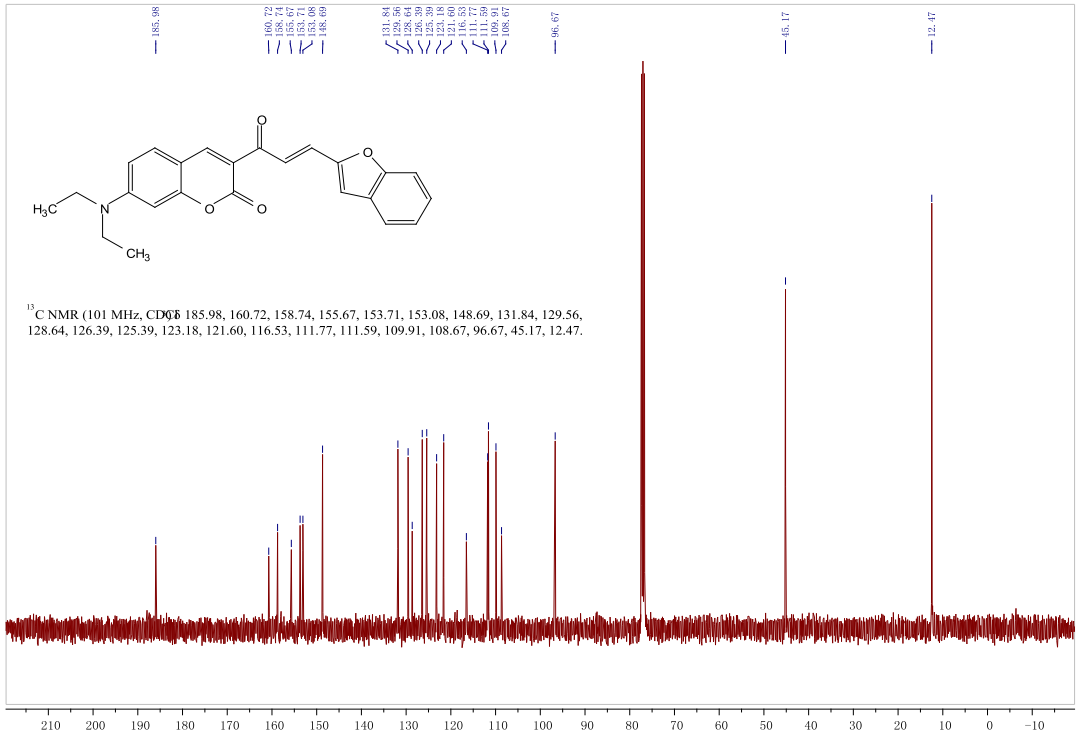
Compound 4





^{13}C NMR (101 MHz, CDCl$_3$) δ 186.45, 160.98, 158.73, 153.02, 148.71, 140.56, 137.48, 134.72, 132.89, 131.83, 129.07, 125.33, 125.01, 124.94, 122.90, 122.59, 116.72, 109.89, 108.71, 96.66, 45.16, 12.47.

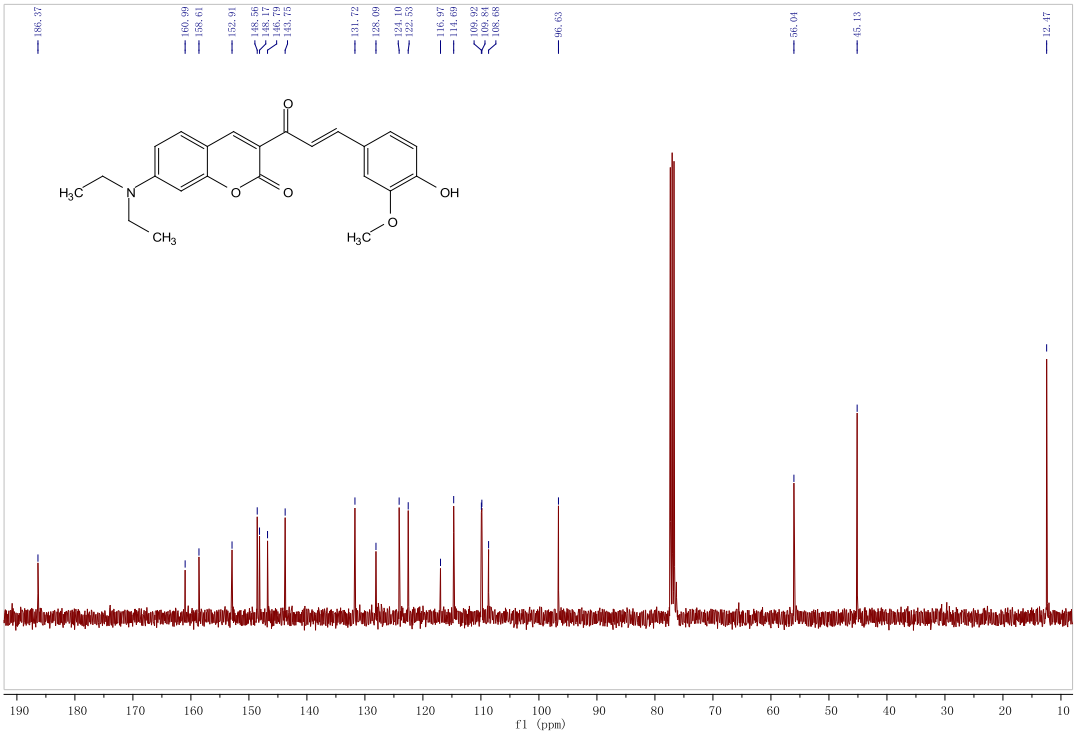
Compound 5



^{13}C NMR (101 MHz, CDCl$_3$) δ 185.98, 160.72, 158.74, 155.67, 153.71, 153.08, 148.69, 131.84, 129.56, 128.64, 126.39, 125.39, 123.18, 121.60, 116.53, 111.77, 111.59, 109.91, 108.67, 96.67, 45.17, 12.47.

Compound 6


8.56
7.45
7.42
6.95
6.93
6.65
6.62
6.50
5.93
3.97
3.48
3.47
3.46
1.26
1.25
1.24
K (s) 8.56
J (m) 8.01
I (m) 7.77
H (d) 7.22
G (d) 6.94
F (d) 6.64
E (s) 6.50
D (s) 5.93
C (s) 3.97
B (dd) 3.47
A (m) 1.25
0.91
0.80
1.01
1.11
1.82
1.04
0.94
0.94
0.88
2.98
3.95
6.00
f1 (ppm)


186.37
160.99
158.61
152.91
148.56
148.17
146.79
143.75
131.72
128.09
124.10
122.53
116.97
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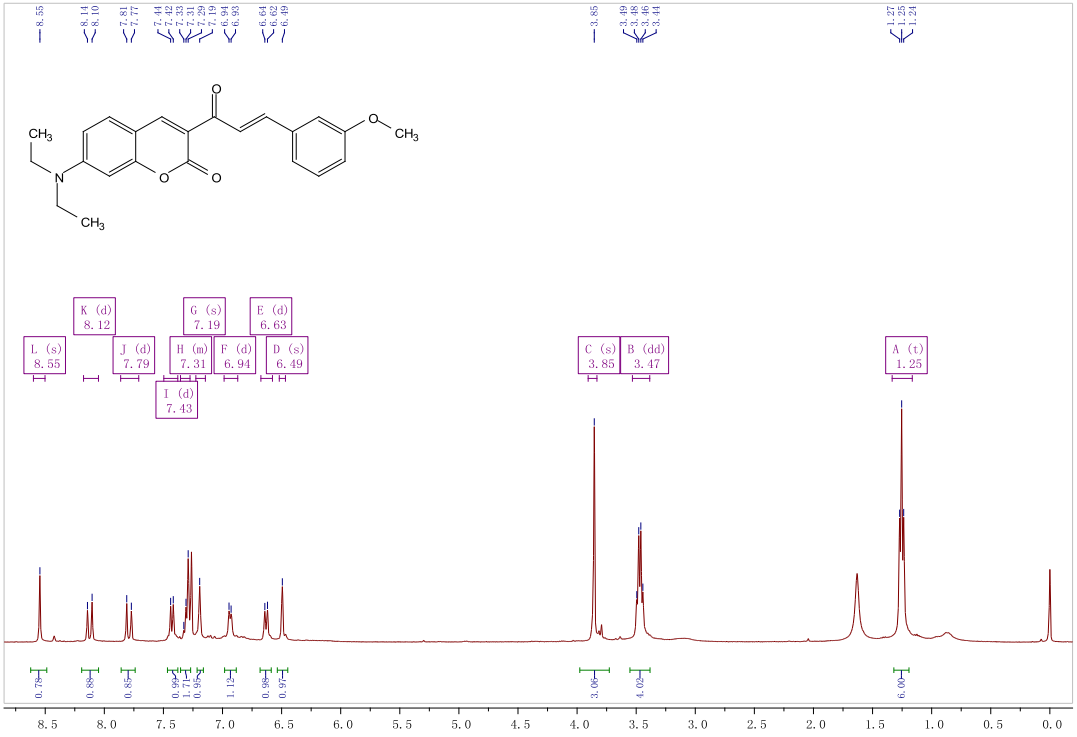
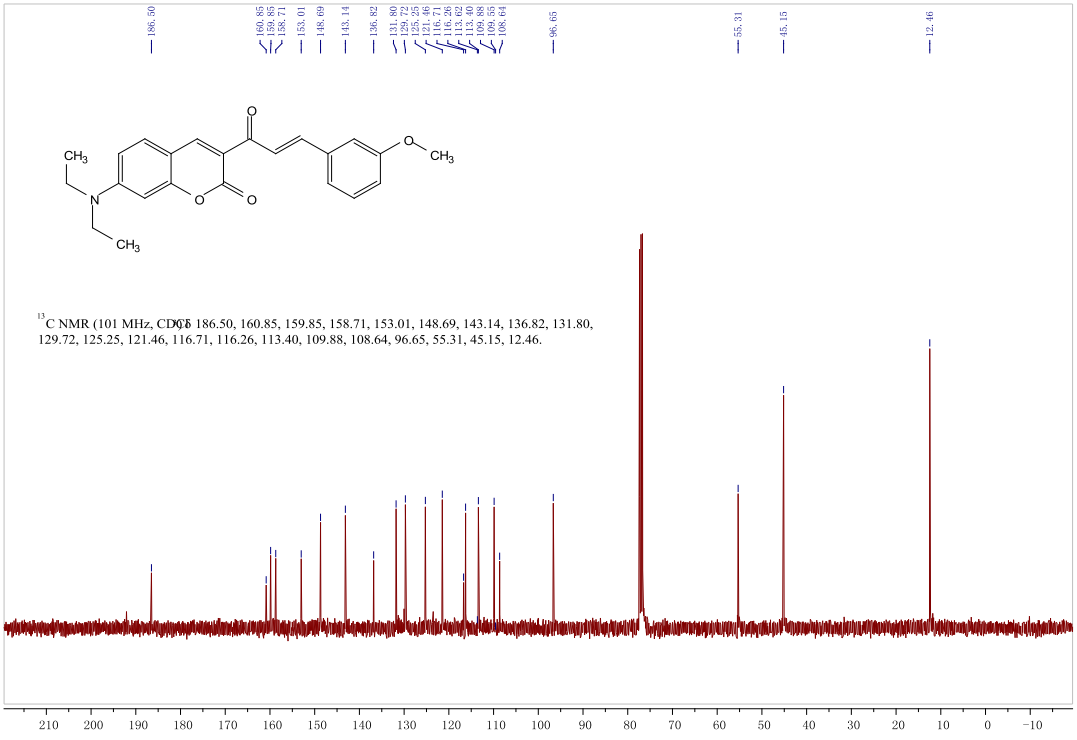
109.92
109.84
108.66
96.63
56.04
45.13
12.47
f1 (ppm)

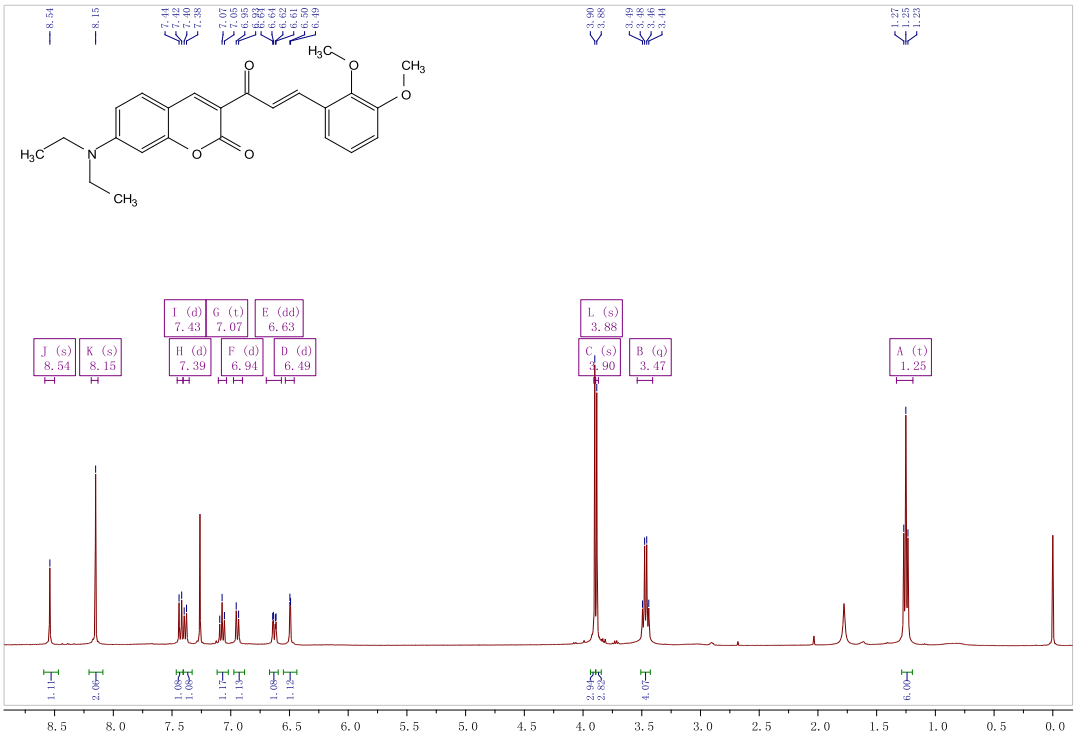
Compound 7

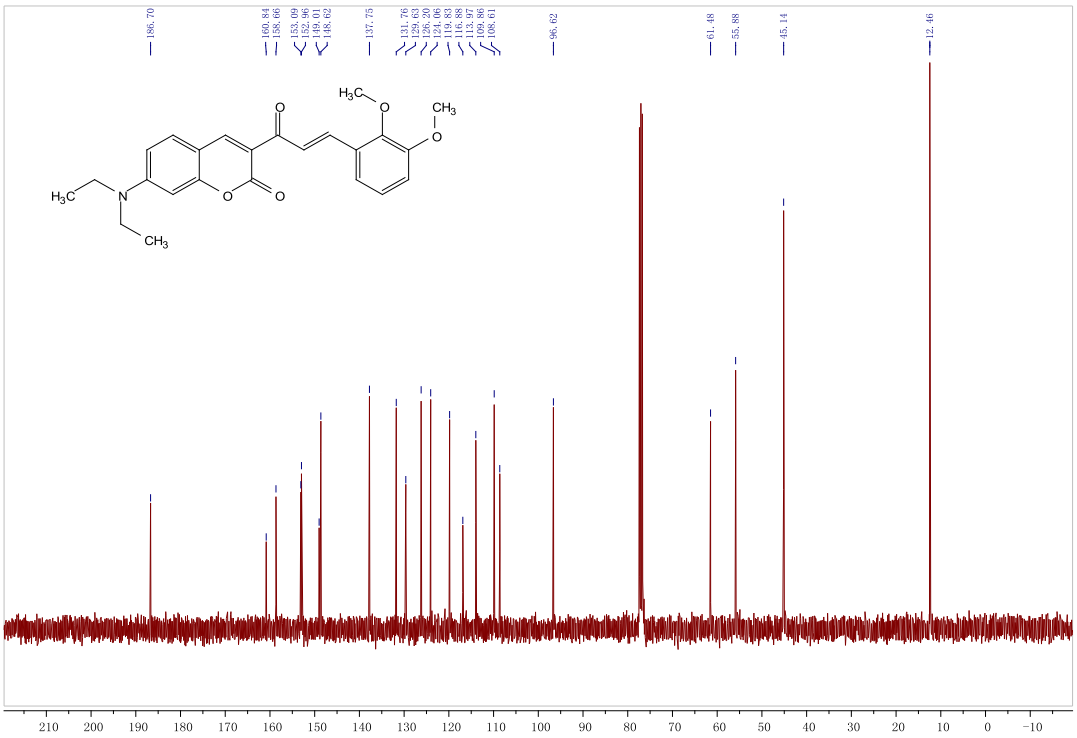




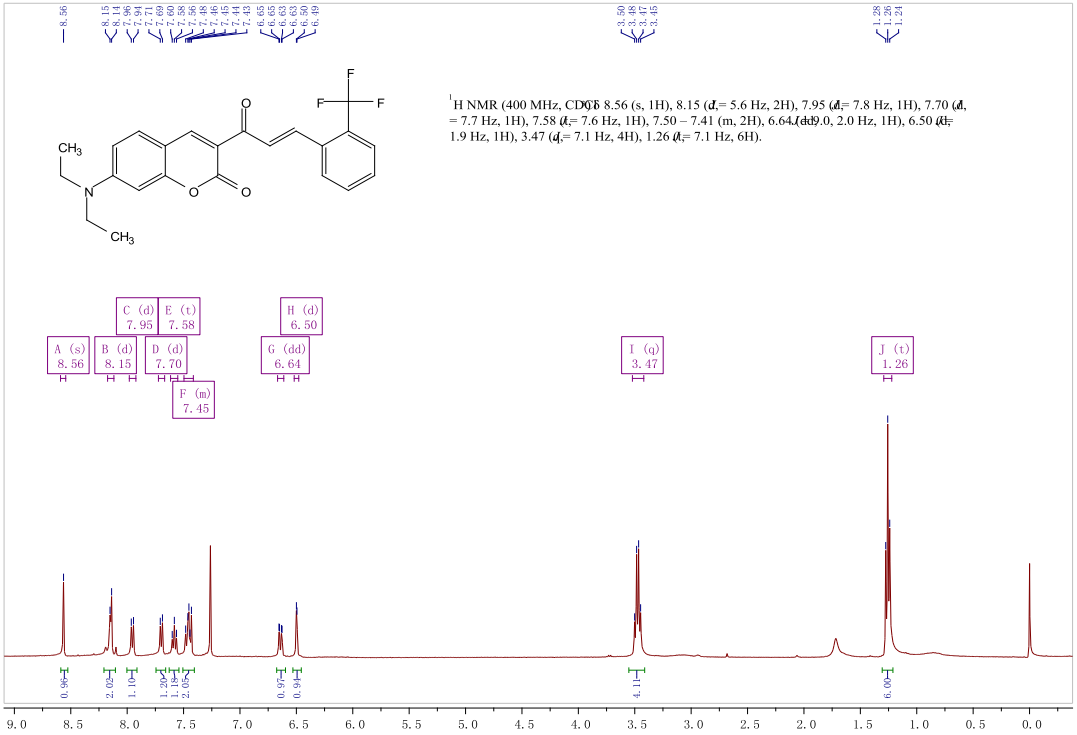
^{13}C NMR (101 MHz, CDCl$_3$) δ 186.50, 160.85, 159.85, 158.71, 153.01, 148.69, 143.14, 136.82, 131.80, 129.72, 125.25, 121.46, 116.71, 116.26, 113.40, 109.88, 108.64, 96.65, 55.31, 45.15, 12.46.

Compound 8

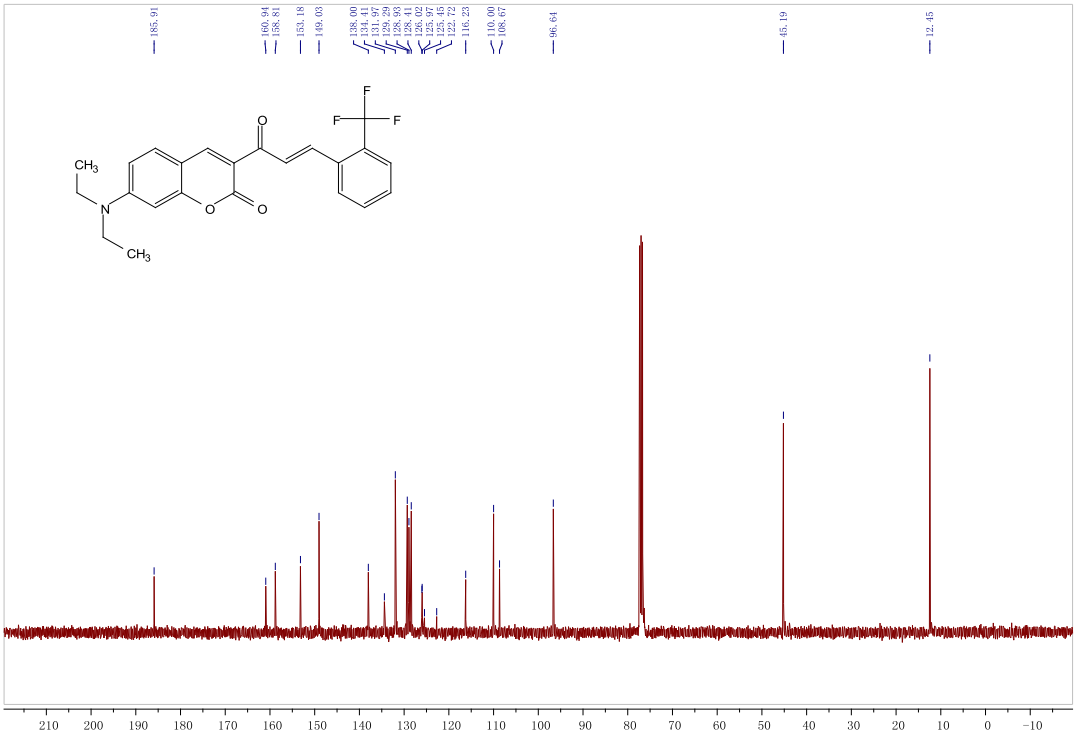




Compound 9

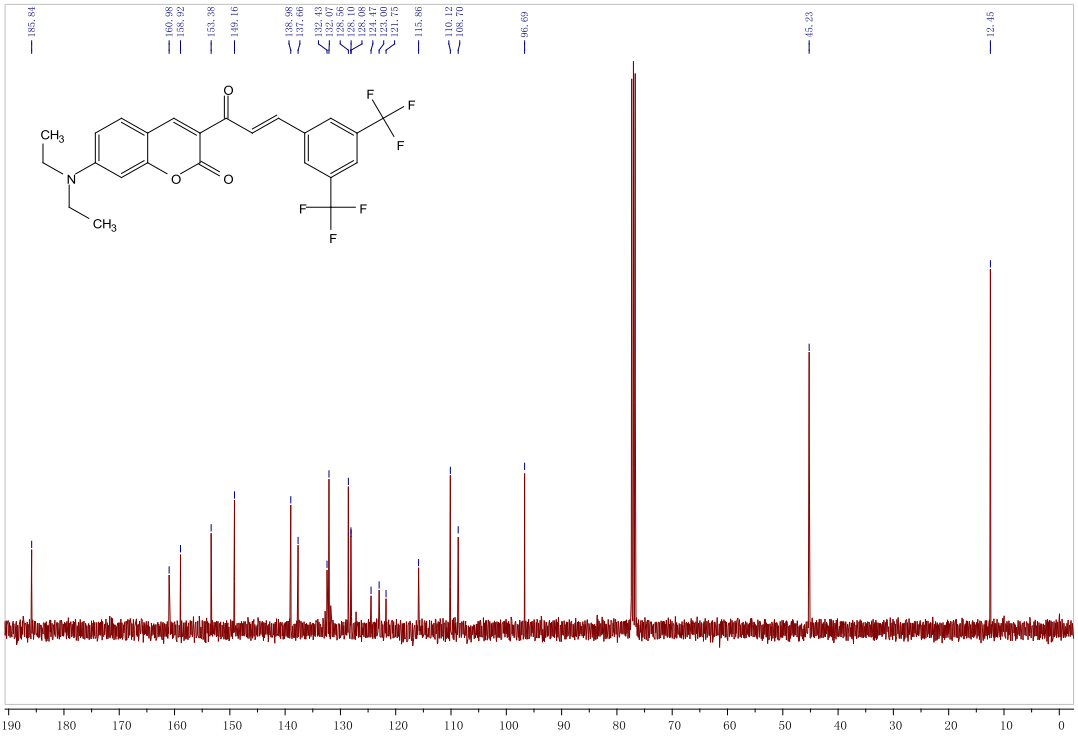


^{1}H NMR (400 MHz, CDCl$_3$) δ 8.56 (s, 1H), 8.15 (*d*, *J* = 5.6 Hz, 2H), 7.95 (*d*, *J* = 7.8 Hz, 1H), 7.70 (*d*, *J* = 7.7 Hz, 1H), 7.58 (*t*, *J* = 7.6 Hz, 1H), 7.50 – 7.41 (m, 2H), 6.64 (*dd*, *J* = 9.0, 2.0 Hz, 1H), 6.50 (*d*, *J* = 1.9 Hz, 1H), 3.47 (*q*, *J* = 7.1 Hz, 4H), 1.26 (*t*, *J* = 7.1 Hz, 6H).

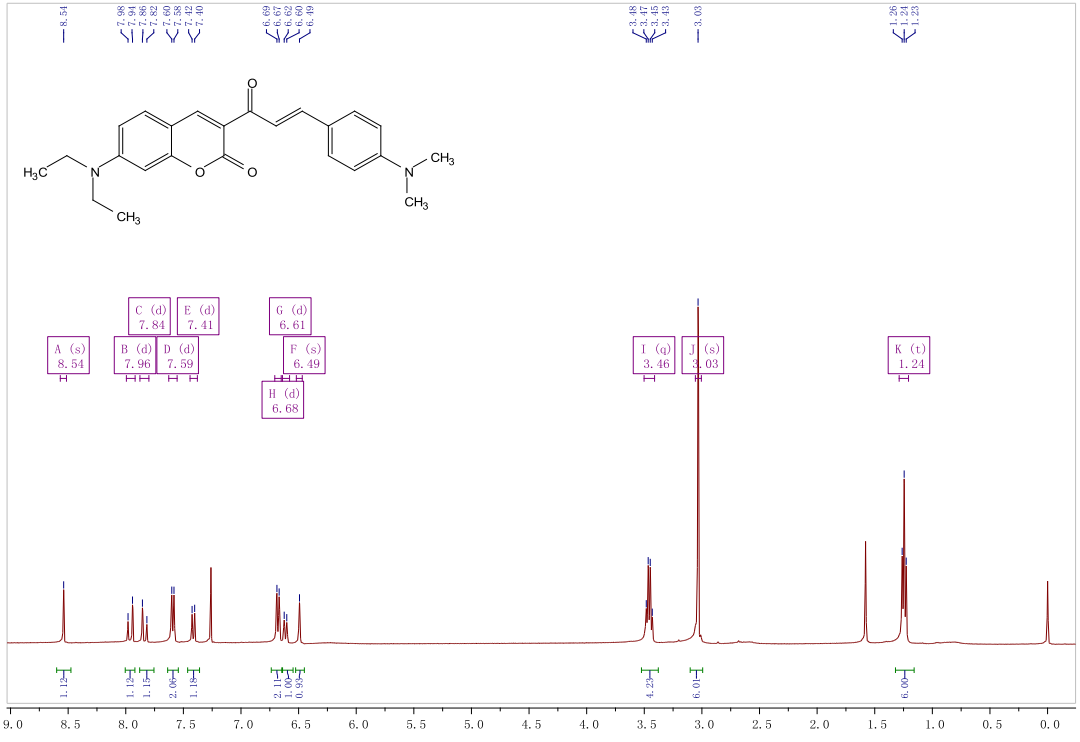


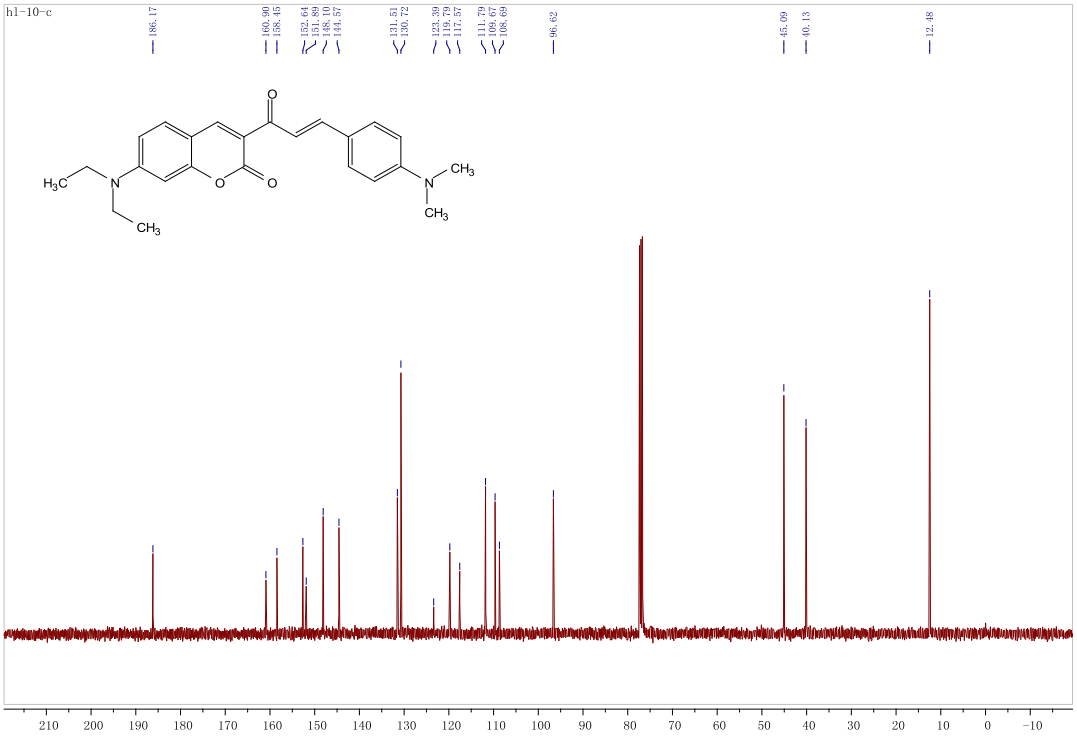
Compound 10


8.57
8.26
8.23
8.06
7.86
7.81
7.77
7.46
7.44
6.66
6.64
6.51
3.51
3.50
3.48
3.46
1.29
1.27
1.25
CH3
N
CH3
O
O
O
F
F
F
F
F
F
B (d) 8.25
D (s) 7.86
H (s) 6.51
A (s) 8.57
C (s) 8.06
F (d) 7.45
G (d) 6.65
I (q) 3.49
J (t) 1.27
E (d) 7.79
1.11
1.12
2.00
1.04
1.01
1.02
1.01
0.96
4.01
6.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


185.84
160.98
158.92
153.38
149.16
138.98
137.66
132.43
132.07
125.56
128.10
128.08
124.47
123.60
121.75
115.86
110.12
108.70
96.69
45.23
12.45
CH3
N
CH3
O
O
O
F
F
F
F
F
F
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

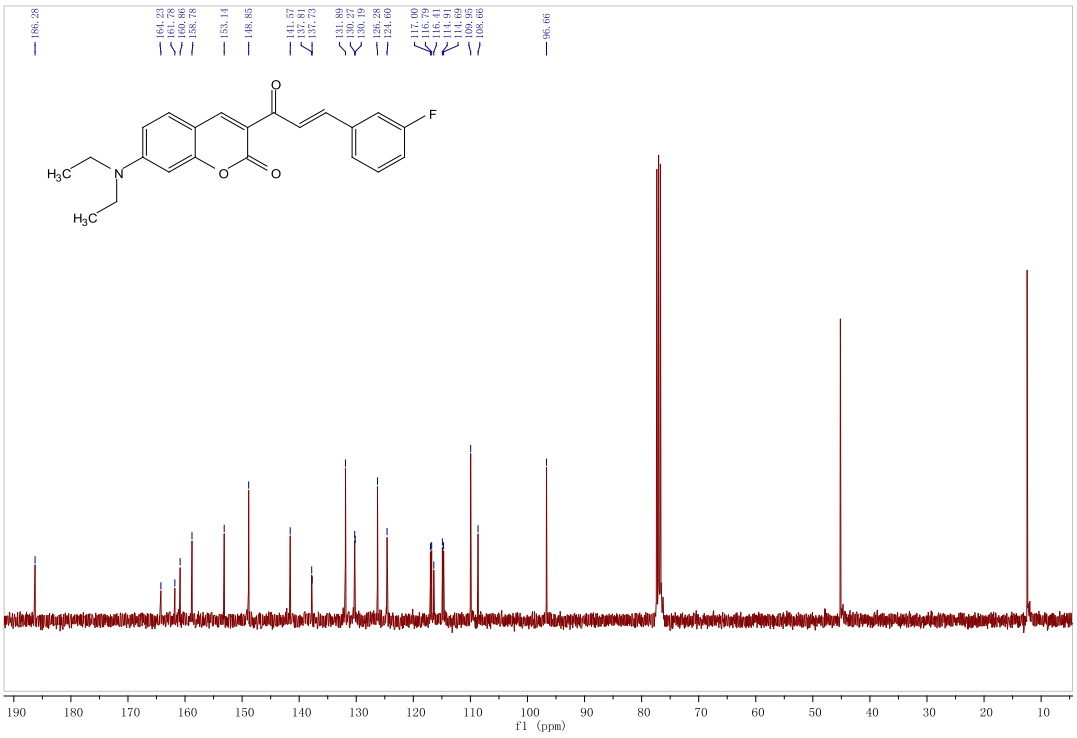
Compound 11


A (s) 8.54
B (d) 7.96
C (d) 7.84
D (d) 7.59
E (d) 7.41
F (s) 6.49
G (d) 6.61
H (d) 6.68
I (q) 3.46
J (s) 3.03
K (t) 1.24


h1-10-c

Compound 12


8.55
8.16
8.12
7.78
7.74
7.44
7.43
7.42
7.41
7.07
7.05
6.65
6.63
6.50
3.50
3.48
3.46
3.45
1.27
1.26
1.24
A (s) 8.55
B (d) 8.14
C (d) 7.76
D (m) 7.40
E (t) 7.07
F (d) 6.64
G (s) 6.50
H (q) 3.47
I (t) 1.26
0.89
0.99
0.97
3.85
1.06
0.98
0.96
3.81
6.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


186.28
164.23
161.78
160.86
158.78
153.14
148.85
141.57
137.81
137.73
131.89
130.27
130.19
126.28
124.60
117.00
116.79
116.41
114.91
114.69
109.95
108.66
96.66
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)