# Supporting Information

# The synthesis and biological evaluation of sanguinarine derivatives as anti-non-small cell lung cancer agents

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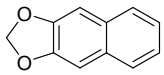
**Contents of SI**



## 1. General information

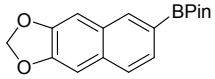
All chemicals were purchased from commercial vendors and used without further purification, unless indicated otherwise. All reactions requiring anhydrous conditions were carried out under argon atmosphere using oven-dried glassware. AR-grade solvents were used for all reactions. Reaction progress was monitored by TLC on pre-coated silica plates (Merck 60 F254 nm, 0.25 μm) and spots were visualized by UV, iodine or other suitable stains. Flash column chromatography was carried out using silica gel (Qingdao Ocean company). $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz and 101 MHz, respectively. Coupling constants (*J*) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to internal control (TMS). Mass spectra were obtained on Agilent LC-ESI-MS system. High resolution ESI-MS were recorded on an AB SCIEX X500r QTOF mass spectrometer. Purity of compounds was determined by reverse-phase high performance liquid chromatography (HPLC) analysis to be >95%. HPLC instrument: Dionex Summit HPLC (Column: Diamonsil C18, 5.0μm, 4.6×250 mm (Dikma Technologies); detector: PDA-100 photodiode array; inJector: ASI-100 autoinJector; pump: p-680A). A flow rate of 1.0 mL/min was used with mobile phase of MeOH in $H_2O$.

## 2 Synthetic procedures and compound characterization



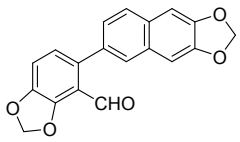
**4**

**naphtho[2,3-d][1,3]dioxole (4).** Naphthalene-2,3-diol **2** (20 g, 125 mmol, 1.00 eq), $CH_2Br_2$ (25.4 mL, 361.8 mmol, 2.89 eq) and $K_2CO_3$ (50 g, 361.8 mmol, 2.89 eq) were suspended in DMF (600mL). The reaction mixture was stirred at 100 °C for 10 h. After cooling to rt, the reaction was poured into a mixture of EtOAc (800 mL) and $H_2O$ (200 mL). The aqueous layer was removed and the organic layer was washed with $H_2O$ (250 mL) and saturated NaCl (250 mL). The organic layer was dried over $NaSO_4$ and the solvents were removed under reduced pressure. Further column chromatography on silica gel (PE) afforded **4** (12.5 g, yield 58%) as a white solid. $R_f$ = 0.25 (PE). $^1$H NMR (400 MHz, Chloroform-d) δ 7.69 – 7.63 (m, 2H), 7.34 – 7.29 (m, 2H), 7.12 (s, 2H), 6.03 (s, 2H). $^{13}$C NMR (101 MHz, Chloroform-d) δ 147.68, 130.59, 127.13, 124.49, 104.01, 101.11. Dept-135 (101 MHz, Chloroform-d) δ 127.03, 124.38, 103.90, 101.00.



**5**

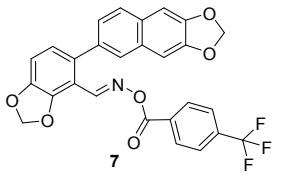
**4,4,5,5-tetramethyl-2-(naphtho[2,3-d][1,3]dioxol-6-yl)-1,3,2-dioxaborolane (5).** Two-neck bottle was charged with naphtho[2,3-d][1,3]dioxole **4** (10 g, 58.2 mmol, 1 equiv), bis(pinacolato)diboron (14.78 g, 58.2 mmol, 1 equiv), $[Ir(OMe)cod]_2$ (580 mg, 0.87 mmol, 1.5 mol %), and 4,4'-di-tert-buty2,2'-bipyridine (470 mg, 1.75 mmol, 3 mol %) and flushed with argon. Then cyclohexane (160 mL) was added and the reaction was heated to reflux for 24 h and quenched by adding water (100 mL) dropwise. The mixture was then extracted with EtOAc (200 mL) twice and the combined organic phase was dried with $Na_2SO_4$ and concentrated under vacuum. Further column chromatography on silica gel (EtOAc: PE = 1: 50) afforded **5** (5.4 g, yield 31.1%) as a white solid. 1H NMR (400 MHz, Chloroform-d) δ 8.17 (s, 1H), 7.70 (dd, J = 8.1, 1.2 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.15 (s, 1H), 7.11 (s, 1H), 6.04 (s, 2H), 1.38 (s, 12H). MS (ESI): m/z Calcd for For $C_{17}H_{20}BO_4$ $[M + H]^+$: 299.15, found 299.1.



**6**

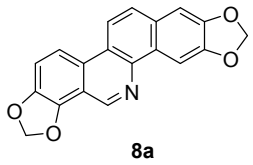
**5-(naphtho[2,3-d][1,3]dioxol-6-yl)benzo[d][1,3]dioxole-4-carbaldehyde (6).** Compound **5** (3.2g, 18.7 mmol, 1.1 equiv), 5-bromobenzo[d][1,3]dioxole-4-carbaldehyde (3.9g, 17 mmol , 1.0 equiv) and $Pd(PPh_3)_2Cl_2$ (600 mg, 0.857 mmol, 0.05 equiv) were added together, then the tube was evacuated and backfilled with Argon for 3 times. DME (160 mL), 2.0 M aq. $K_2CO_3$ (33 mL) was then added by syringe under Argon. The mixture was stirred at 80℃ for 4h and cooled to rt. EtOAc (200 mL) was added to the mixture and extracted with saturated NaCl twice. The organic phase was dried with $Na_2SO_4$ and concentrated under vacuum. Further column chromatography on silica gel (EtOAc: PE = 1:10) afforded **6** (4.2 g, yield 77.1%) as a faint yellow solid. $^1$H NMR (400 MHz, Chloroform-*d*) δ 9.87 (s, 1H), 7.70 (d, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 1.8 Hz, 1H), 7.31 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.26 (s, 1H),

7.16 (s, 1H), 7.12 (s, 1H), 7.06 (d, $J$ = 8.0 Hz, 1H), 6.96 (d, $J$ = 8.0 Hz, 1H), 6.21 (s, 2H), 6.07 (s, 2H). MS (ESI): m/z Calcd for For $C_{19}H_{13}O_5$ $[M + H]^+$: 321.08, found 321.1.



7

**(E)-5-(naphtho[2,3-d][1,3]dioxol-6-yl)benzo[d][1,3]dioxole-4-carbaldehyde O-(4-(trifluoromethyl) benzoyl) oxime (7).** A mixture of compound **6** (1.81 g, 5.66 mmol, 1.0 equiv), $H_2NOH$-HCl (472 mg, 6.8 mmol, 1.2 equiv) and pyridine (683 mg, 8.5 mmol, 1.5 equiv) in MeOH (60 mL) was stirred under reflux for 6 h. MeOH was then removed under vacuo and the residue was dissolved in EtOAc (150 mL) and extracted with 1.0 M aq. HCl (60 mL). Organic phase was then extracted with saturated NaCl (100 mL) twice, dried with $Na_2SO_4$ and concentrated under vacuum. The residue was then dissolved in dry $CH_2Cl_2$ (100 mL) and added $Et_3N$ (860 mg, 8.5 mmol, 1.5 equiv). 4-(trifluoromethyl)-benzoylchlorid (1.42g, 6.8 mmol, 1.2 equiv) was then added dropwise at 0℃ and stirred at 0℃ for another 30 min. Then, the mixture was poured into saturated $NaHCO_3$ (100 mL) and extracted with $CH_2Cl_2$ (100 mL) twice. The combined organic phases were then dried with $Na_2SO_4$ and concentrated under vacuum. Further column chromatography on silica gel (EtOAc : PE = 1:20) afforded compound **7** (1.4 g, yield 49.3%) as a white solid. $^1$H NMR (400 MHz, Chloroform-d) δ 8.40 (s, 1H), 8.14 (d, J = 8.1 Hz, 2H), 7.72 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 1.8 Hz, 1H), 7.28 (d, J = 1.8 Hz, 1H), 7.15 (d, J = 8.3 Hz, 2H), 7.02 – 6.93 (m, 2H), 6.25 (s, 2H), 6.06 (s, 2H). MS (ESI): m/z Calcd for For $C_{27}H_{16}F_3NNaO_6$ $[M + H]^+$: 530.08, found 529.7



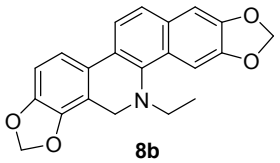
8a

**[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridine (8).** A 25 mL round bottom flask was equipped with a rubber septum and magnetic stir bar and was charged with **7** (1.3g, 2.56 mmol, 1.0 equiv), fac-Ir(ppy)$_3$ (50 mg, 0.0256 mmol, 0.01 equiv). The flask was evacuated and backfilled with Ar for 3 times. DMF (20 mL) was then added by syringe under Argon. The mixture was then irradiated by a 5W white LEDs strip. After the reaction was complete (as judged by TLC analysis), the mixture was concentrated under vacuum to remove DMF and dissolved in $CHCl_3$ (200 mL). The solution was extracted with 1.0 M $K_2CO_3$ (100 mL) twice. The organic phase was dried with $Na_2SO_4$ and concentrated under vacuum. Further column chromatography on silica gel (EtOAc : PE = 1:10) afforded compound **8a** (700 mg, 86.3%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.41 (s, 1H), 8.55 (d, $J$ = 8.9 Hz, 1H), 8.51 (s, 1H), 8.41 (d, $J$ = 8.7 Hz, 1H), 8.00 (d, $J$ = 8.9 Hz, 1H), 7.69 (d, $J$ = 8.7 Hz, 1H), 7.53 (s, 1H), 6.39 (s, 2H), 6.22 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 148.73, 148.53, 145.65, 145.01, 143.56, 139.22, 129.94, 128.82, 128.03, 127.66, 120.55, 119.32, 116.74, 114.71, 112.35, 104.93, 103.39, 102.04, 101.41. HRMS (ESI): m/z Calcd For $C_{19}H_{12}NO_4$ $[M + H]^+$: 318.0766, found 318.0748. HPLC analysis: MeOH : $H_2O$ (90 : 10), 11.78 min, 97.08% purity.

**General Procedure for synthesis of compounds 8b and 8c.**

Sodium borohydride (378mg, 10 mol, 20 equiv) was added portionwise to a stirred solution of the

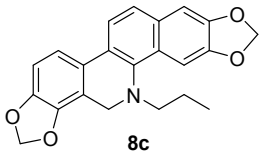
compound **8a** (108mg, 0.5 mol, 1.0 equiv) in AcOH or $CH_3CH_2COOH$ at room temperature. After being stirred at rt for 30 min, the reaction mixture was made weakly alkaline with 10% aq. NaOH and extracted with EtOAc. The organic phase was dried with $Na_2SO_4$ and concentrated under vacuum. Further column chromatography on silica gel (EtOAc : PE = 1:20) afforded compound **8b** (49 mg, yield 28.7%) and compound **8c** (54 mg, yield 30%) as white solid. (for **8b**: $NaBH_4$, AcOH, rt, 30min; for **8c**: $NaBH_4$, $CH_3CH_2COOH$, rt, 30min.)



**8b**

**13-ethyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5- i]phenanthridine (8b)**

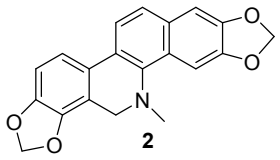
$^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.76 (d, $J$ = 8.6 Hz, 1H), 7.54 (d, $J$ = 8.6 Hz, 1H), 7.47 (s, 1H), 7.38 (d, $J$ = 8.2 Hz, 1H), 7.31 (s, 1H), 6.93 (d, $J$ = 8.2 Hz, 1H), 6.13 (s, 2H), 6.10 (s, 2H), 4.15 (s, 2H), 2.69 (q, $J$ = 6.9 Hz, 2H), 1.11 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 147.86, 147.26, 146.84, 144.03, 141.87, 130.52, 126.91, 125.89, 124.22, 123.76, 120.19, 116.26, 113.42, 107.21, 104.28, 101.38, 101.23, 99.75, 46.67, 42.85, 13.52. HRMS (ESI): m/z Calcd For $C_{21}H_{18}NO_4$ $[M + H]^+$: 348.1230, found 348.1223. HPLC analysis: MeOH : $H_2O$ (90 : 10), 13.75 min, 96.35% purity.



**8c**

**13-propyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridine (8c)**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.76 (d, $J$ = 8.7 Hz, 1H), 7.55 (d, $J$ = 8.7 Hz, 1H), 7.52 (s, 1H), 7.39 (d, $J$ = 8.2 Hz, 1H), 7.31 (s, 1H), 6.93 (d, $J$ = 8.2 Hz, 1H), 6.14 (s, 2H), 6.10 (s, 2H), 4.14 (s, 2H), 2.63 – 2.57 (m, 2H), 1.62 – 1.48 (m, 2H), 0.86 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, Chloroform-$d$) δ 148.14, 147.59, 147.14, 144.51, 142.91, 131.03, 127.93, 126.79, 124.95, 123.87, 120.45, 116.25, 114.44, 107.24, 104.50, 101.45, 101.14, 100.95, 54.74, 43.75, 21.68, 11.69. HRMS (ESI): m/z Calcd For $C_{22}H_{20}NO_4$ $[M + H]^+$: 362.1387, found 362.1370. HPLC analysis: MeOH : $H_2O$ (90 : 10), 17.10 min, 96.42% purity.



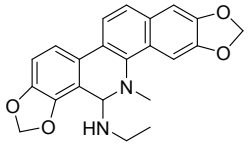
**2**

**13-methyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5] benzo [1,2-c][1,3]dioxolo[4,5 -i] phenanthridine (2).** To the solution of sanguinarine chloride **(1)** (0.10 g, 0.27 mmol,1.0 equiv) in 15mL of MeOH, of $NaBH_4$ (82 mg, 2.17 mmol, 8 equiv) was added. The reaction solution was stirred for 30 min at rt. The solvent was evaporated to dryness under reduced pressure. The residue was subjected to column chromatography over silica gel using petroleum ether-ethyl acetate (20 : 1) as eluent, and recrystallized from petroleum ether-ethyl acetate (20 : 1) as a red-white granule crystal ( 33 mg, yield 36%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.77 (d, $J$ = 8.6 Hz, 1H), 7.56 (d, $J$ = 8.6 Hz, 1H), 7.52 (s, 1H), 7.40 (d, $J$ = 8.2

Hz, 1H), 7.31 (s, 1H), 6.95 (d, $J$ = 8.2 Hz, 1H), 6.14 (s, 2H), 6.11 (s, 2H), 4.13 (s, 2H), 2.51 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 147.92, 147.27, 146.92, 144.29, 141.87, 130.44, 126.38, 125.73, 123.94, 123.85, 120.29, 116.29, 112.82, 107.27, 104.23, 101.36, 101.24, 99.74, 47.90, 41.30. HRMS (ESI): m/z Calcd For $C_{20}H_{16}NO_4$ $[M + H]^+$: 334.1074, found 334.1064. HPLC analysis: MeOH: $H_2O$ : TEA (80 : 20 : 0.02), 17.78 min, 96.82% purity.

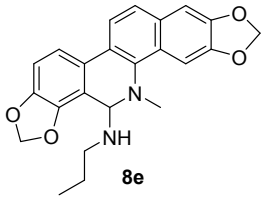
**General procedure for synthesis of compounds 8d-8i.**

A reaction mixture of chelerythrine chloride **(1)** (40 mg, 0.10 mmol, 1.0 equiv) and amines (1.0 mmol, 10 equiv) in acetonitrile (3 mL) is stirred at rt overnight. The off-white solid is filtered, washed with acetonitrile and dried to afford compounds **8d-8i**.



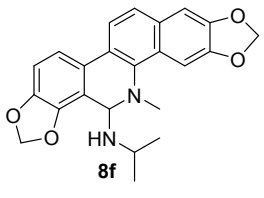
**8d**

***N*-ethyl-13-methyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5i] phenanthridine-14-amine (8d).** A reaction mixture of chelerythrine chloride **(1)** (40 mg, 0.10 mmol) and ethylamine (45 mg, 1.0 mmol) in acetonitrile (3 mL) is stirred at rt overnight. The off-white solid is filtered, washed with acetonitrile and dried to afford off-white solid (15 mg, 39 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.81 (d, $J$ = 8.6 Hz, 1H), 7.56 (s, 1H), 7.54 (d, $J$ = 12.6 Hz, 1H), 7.44 (d, $J$ = 8.2 Hz, 1H), 7.30 (s, 1H), 6.98 (d, $J$ = 8.2 Hz, 1H), 6.11 (d, $J$ = 20.0 Hz, 4H), 4.96 (s, 1H), 2.76 (dt, $J$ = 14.1, 7.2 Hz, 1H), 2.65 (dd, $J$ = 12.1, 6.6 Hz, 1H), 2.56 (s, 3H), 1.61 (s, 1H), 0.90 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 147.68, 147.08, 146.74, 144.49, 138.87, 130.48, 126.79, 124.99, 123.29, 122.86, 120.09, 116.19, 115.20, 107.74, 104.19, 101.34, 101.10, 99.68, 69.30, 40.68, 38.73, 14.70. HPLC analysis: MeOH : $H_2O$ (90: 10), 6.88 min, 99.95% purity.



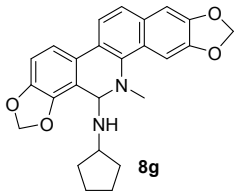
**8e**

**13-methyl-N-propyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridin-14-amine (8e).** A reaction mixture of chelerythrine chloride **(1)** (40 mg, 0.10 mmol) and propylamine (59 mg, 1.0 mmol) in acetonitrile (3 mL) is stirred at room temperature overnight. The off-white solid is filtered, washed with acetonifrile and dried to afford off-white solid (25 mg, yield 64 %) .$^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.81 (d, $J$ = 8.7 Hz, 1H), 7.56 (s, 1H), 7.53 (d, $J$ = 8.7 Hz, 1H), 7.44 (d, $J$ = 8.2 Hz, 1H), 7.30 (s, 1H), 6.98 (d, $J$ = 8.2 Hz, 1H), 6.16 – 6.07 (m, 4H), 4.94 (d, $J$ = 6.9 Hz, 1H), 2.65 (t, $J$ = 6.7 Hz, 2H), 2.56 (s, 3H), 1.58 (d, $J$ = 6.8 Hz, 1H), 1.41 – 1.19 (m, 2H), 0.74 (t, $J$ = 7.4 Hz, 3H).$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 148.23, 147.63, 147.30, 145.05, 139.46, 131.03, 127.37, 125.55, 123.84, 123.42, 120.63, 116.75, 115.80, 108.30, 104.74, 101.91, 101.66, 100.22, 69.93, 46.92, 41.25, 22.72, 12.25. HPLC analysis: MeOH : $H_2O$ (90 : 10), 6.99 min, 99.77% purity.



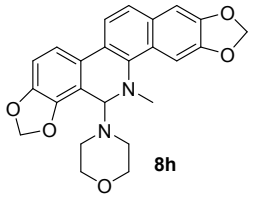
**8f**

**N-isopropyl-13-methyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridin-14-amine (8f).** A reaction mixture of chelerythrine chloride **(1)** (40 mg, 0.10 mmol) and Isopropylamine (59 mg, 1.0 mmol) in acetonitrile (3 mL) is stirred at room temperature overnight. The off-white solid is filtered, washed with acetonitrile and dried to afford off-white solid (13 mg, yield 33 %) . $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 7.82 (d, $J$ = 8.7 Hz, 1H), 7.54 (t, $J$ = 4.3 Hz, 2H), 7.44 (d, $J$ = 8.2 Hz, 1H), 7.31 (s, 1H), 6.98 (d, $J$ = 8.1 Hz, 1H), 6.15 (d, $J$ = 6.2 Hz, 2H), 6.14 – 6.07 (m, 2H), 5.05 (d, $J$ = 8.4 Hz, 1H), 3.27 – 3.10 (m, 1H), 2.56 (s, 3H), 1.41 (s, 1H), 1.09 (d, $J$ = 6.1 Hz, 3H), 0.76 (d, $J$ = 6.4 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 147.67, 147.07, 146.77, 138.65, 130.47, 126.77, 124.89, 123.35, 122.86, 120.08, 116.22, 115.27, 107.71, 104.21, 101.35, 101.10, 99.57, 66.93, 43.00, 40.62, 40.03, 23.80, 21.43. HPLC analysis: MeOH : $H_2O$ (90 : 10), 6.92 min, 99.81% purity.



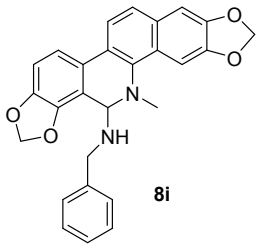
**8g**

**N-cyclopentyl-13-methyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridin-14-amine (8g).** A reaction mixture of chelerythrine chloride **(1)** (40 mg, 0.10 mmol) and cyclopentylamine (85 mg, 1.0 mmol) in acetonitrile (3 mL) is stirred at room temperature overnight. The off-white solid is filtered, washed with acetonifrile and dried to afford off-white solid (21 mg, yield 50 %). $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 7.81 (d, J = 8.7 Hz, 1H), 7.55 (s, 1H), 7.53 (d, J = 8.7 Hz, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.30 (s, 1H), 6.97 (d, J = 8.3 Hz, 1H), 6.16 – 6.14 (m, 2H), 6.14 – 6.06 (m, 2H), 4.98 (d, J = 7.6 Hz, 1H), 3.45 (tt, J = 8.5, 4.3 Hz, 1H), 2.57 (s, 3H), 1.85 (dt, J = 12.4, 6.3 Hz, 1H), 1.65 – 1.32 (m, 7H), 1.06 (dq, J = 13.5, 7.1 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 148.20, 147.63, 147.32, 144.95, 139.49, 131.05, 127.33, 125.45, 123.84, 123.37, 120.64, 116.73, 115.84, 108.28, 104.76, 101.94, 101.67, 100.30, 68.95, 55.05, 41.42, 33.73, 32.28, 24.01, 23.94. HPLC analysis: MeOH : $H_2O$ (90 : 10), 6.84 min, 100% purity.



**8h**

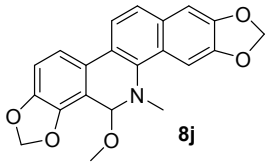
**13-methyl-14-morpholino-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridine (8h).** A reaction mixture of chelerythrine chloride **(1)** (40 mg, 0.10 mmol) and morpholine (87 mg, 1.0 mmol) in acetonitrile (3 mL) is stirred at room temperature overnight. The off-white solid is filtered, washed with acetonifrile and dried to afford off-white solid (21 mg, yield 50 %).$^{1}$H NMR (400 MHz, Chloroform-$d$) δ 7.72 (d, $J$ = 8.5 Hz, 1H), 7.64 (s, 1H), 7.46 (d, $J$ = 8.5 Hz, 1H), 7.37 (d, $J$ = 8.1 Hz, 1H), 7.11 (s, 1H), 6.93 (d, $J$ = 8.2 Hz, 1H), 6.15 – 5.97 (m, 4H), 4.65 (s, 1H), 3.46 (dt, $J$ = 6.3, 3.1 Hz, 4H), 2.83 (dt, $J$ = 10.4, 4.2 Hz, 2H), 2.69 (s, 3H), 2.38 – 2.24 (m, 2H). $^{13}$C NMR (101 MHz, Chloroform-$d$) δ 148.16, 147.56, 146.94, 146.17, 140.59, 131.22, 126.93, 126.58, 123.64, 123.46, 120.12, 116.44, 112.79, 108.46, 104.71, 101.59, 101.18, 100.92, 76.69, 67.11, 49.16, 42.49. HPLC analysis: MeOH : $H_2O$ (90 : 10), 7.14 min, 99.36% purity.



8i

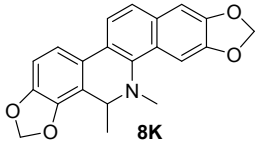
**N-benzyl-13-methyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridin-14-amine (8i).**

A reaction mixture of chelerythrine chloride **(1)** (40 mg, 0.10 mmol) and benzylamine (107 mg, 1.0 mmol) in acetonitrile (3 mL) is stirred at room temperature overnight. The off-white solid is filtered, washed with acetonifrile and dried to afford off-white solid (25 mg, yield 57 %). $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 7.84 (d, $J$ = 8.6 Hz, 1H), 7.65 (s, 1H), 7.56 (d, $J$ = 8.6 Hz, 1H), 7.46 (d, $J$ = 8.2 Hz, 1H), 7.35 – 7.26 (m, 5H), 7.24 – 7.17 (m, 1H), 6.99 (d, $J$ = 8.2 Hz, 1H), 6.16 (d, $J$ = 3.5 Hz, 2H), 6.10 (dd, $J$ = 31.7, 1.1 Hz, 2H), 4.87 (s, 1H), 3.97 (d, $J$ = 13.9 Hz, 1H), 3.76 (d, $J$ = 13.9 Hz, 1H), 2.51 (s, 3H), 2.28 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 147.72, 147.06, 146.74, 140.38, 138.66, 130.50, 127.95, 127.92, 126.31, 125.13, 123.34, 122.93, 120.09, 116.19, 115.08, 107.80, 104.21, 101.35, 101.10, 99.72, 67.96, 47.32, 40.48. HPLC analysis: MeOH : $H_2O$ (90 : 10), 6.94 min, 100% purity.



8j

**14-methoxy-13-methyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridine (8j).** To the solution of chelerythrine chloride **(1)** (0.12 g, 0.33 mmol,1.0 equiv.) in 40mL of MeOH, 4mL of the solution of $CH_3ONa$ in MeOH was added for **(1)** to pH 10, and refluxed for 6h. 15 ml of $H_2O$ were added to the reaction solution, and extracted with $CHCl_3$. The solvent was evaporated to dryness under reduced pressure, and the residue was recrystallised in MeOH. 6-Methoxysanguinarine **8j** as a white crystal (45 mg, yield 37 %). $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 7.84 (d, $J$ = 8.7 Hz, 1H), 7.59 (s, 1H), 7.55 (d, $J$ = 8.7 Hz, 1H), 7.51 (d, $J$ = 8.3 Hz, 1H), 7.33 (s, 1H), 7.06 (d, $J$ = 8.3 Hz, 1H), 6.19 – 6.11 (m, 4H), 5.34 (s, 1H), 3.32 (s, 3H), 2.71 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 148.39, 147.71, 147.37, 145.35, 138.34, 131.05, 126.59, 125.52, 123.92, 122.77, 120.42, 116.60, 113.40, 109.24, 104.86, 102.19, 101.76, 100.25, 85.55, 53.72, 41.06. HPLC analysis: MeOH : $H_2O$ (90 : 10), 6.71 min, 98.76% purity.

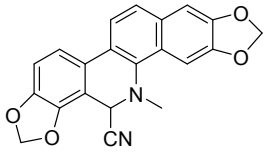


8K

**13,14-dimethyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridine (8k).**

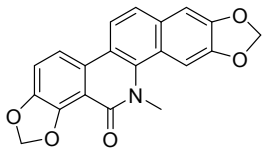
In a dry and inert 25 mL Schlenk tube, chelerythrine chloride **(1)** (0.12 g, 0.33 mmol,1.0 equiv) was suspended in anhydrous THF (10 mL, purged with argon) and cooled to 0 °C in an ice bath. $CH_3MgBr$ (1M in THF, 1 mL, 0.99 mmol, 3.00 eq) was added dropwise. The reaction mixture was allowed to reach rt and stirring was continued until a clear solution was obtained. Afterwards, the reaction mixture was quenched with water and extracted with EA (40 mL, three times). The combined organic layers were washed with brine, dried over magnesium sulfate. The solvent was removed under reduced

pressure. Further column chromatography on silica gel (EtOAc : PE = 1:25) afforded a white solid **8k** (40mg, yield 29 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.79 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.54 (s, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.30 (s, 1H), 6.94 (d, J = 8.1 Hz, 1H), 6.15 – 6.07 (m, 4H), 4.33 (q, J = 6.9 Hz, 1H), 2.52 (s, 3H), 1.05 (d, J = 6.9 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 147.92, 147.29, 146.80, 143.97, 139.40, 130.53, 126.98, 124.63, 123.80, 122.93, 120.08, 118.05, 116.53, 107.20, 104.20, 101.40, 101.22, 99.76, 52.30, 42.53, 20.05. HRMS (ESI): m/z Calcd For $C_{21}H_{18}NO_4$ $[M + H]^+$: 348.1230, found 348.1219. HPLC analysis: MeOH : $H_2O$ (90 : 10), 12.41 min, 98.92% purity.




**8l**

**13-methyl-13,14-dihydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridine-14-carbonitrile (8l).** Add $CH_3CN$ to a mixture of chelerythrine chloride **(1)** (0.12 g, 0.33 mmol,1.0 equiv), CsF (55 mg, 0.36 mmol,1.1 equiv), trimethylsilyl cyanide (36mg, 0.36 mmol,1.1 equiv) in a ball flask at rt for 3h. Afterwards, the reaction mixture was quenched with water and extracted with EA (three times). The combined organic layers were washed with brine, dried over magnesium sulfate. The solvent was removed under reduced pressure. Further column chromatography on silica gel (EtOAc : PE = 1:20) afforded **8l** as a white solid (70mg, yield 59 %). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.87 (d, J = 8.7 Hz, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.52 (s, 1H), 7.38 (s, 1H), 7.13 (d, J = 8.1 Hz, 1H), 6.23 (d, J = 26.1 Hz, 2H), 6.18 (d, J = 2.7 Hz, 2H), 5.95 (s, 1H), 2.60 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 148.32, 147.65, 147.61, 144.66, 138.09, 130.69, 125.75, 125.01, 124.83, 122.56, 120.07, 118.08, 117.17, 109.34, 107.20, 104.29, 102.26, 101.39, 99.33, 47.30, 40.79. HRMS (ESI): m/z Calcd For $C_{21}H_{15}N_2O_4$ $[M + H]^+$: 359.1026, found 359.1026. HPLC analysis: MeOH : $H_2O$ (90 : 10), 5.38 min, 96.12% purity.




**8m**

**13-methyl-[1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridin-14(13H)-one (8m).** To a hot solution (90°C) of chelerythrine chloride **(1)** (0.12 g, 0.33 mmol,1.0 equiv) in 50mL of 0.2% HCl in water was added a hot solution (80°C) of $K_3Fe(CN)_6$ (0.8 g) in $H_2O$ (10 mL) with stirring. Stirring was continued for 3 h at 90°C. During the reaction, 5mL of 3% KOH in water was added every 30 min. After cooling, the precipitate was collected. The solid was dissolved in $CHCl_3$, washed with water and dried over $Na_2SO_4$. The residue was recrystallised from $CHCl_3$-acetone. compound **8m** as a grey amorphous powder (50 mg, yield 43%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.19 (d, $J$ = 8.7 Hz, 1H), 7.99 (d, $J$ = 8.6 Hz, 1H), 7.72 (s, 1H), 7.64 (d, $J$ = 8.7 Hz, 1H), 7.43 (d, $J$ = 8.5 Hz, 1H), 7.41 (s, 1H), 6.27 (s, 2H), 6.18 (s, 2H), 3.76 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-$d$) δ 162.83, 147.90, 147.79, 147.66, 147.25, 135.70, 131.97, 128.93, 123.68, 121.28, 118.83, 117.42, 115.58, 113.34, 111.02, 104.85, 103.03, 102.65, 101.68, 40.99. HRMS (ESI): m/z Calcd For $C_{20}H_{13}NO[M + H]^+$: 348.0866, found 348.0867. HPLC analysis: MeOH : $H_2O$ (90 : 10), 7.28 min, 100% purity.

**3. Materials and methods of biological studies**

Antibodies

The antibodies used in this study were as follows: anti-GAPDH (Beyotime); anti-AKT, anti-p-AKT, anti-caspase3, anti-cleaved-caspase3, anti-caspase9, anti-cleaved-caspase9, anti-PARP, goat anti-rabbit IgG-HRP and goat anti-mouse IgG-HRP antibody (Cell Signaling Technology).

Cell Culture and Treatment

A549 and H1975 cell lines were obtained from the Cell Research Institute of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in 1640 medium (Gibco, CA, USA) containing 10% fetal bovine serum (Gibco), penicillin (100U/ml, Gibco), and streptomycin (100μg/ml, Gibco) under a humid 5% $CO_2$ atmosphere at 37 °C. For drug treatment, A549 and H1975 cells were treated with D3896 for 72h. **8h** with a purity of ≥98% according to HPLC was dissolved in DMSO to prepare a stock solution.

Cell Counting Kit-8 Assay

The viability of A549 and H1975 was measured by using a cell counting kit-8 (Dojindo, Japan). In brief, $3 \times 10^3$ cells were seeded into each well of the 96-well plates and incubated overnight for attachment. After 72 h treatments of **8h**, the cells were incubated with CCK-8 solution at 37°C for 2 hr. The absorbance under 450 nm was detected by using a microplate reader (Bio-TEK instruments, VT, USA). The results were expressed as the mean percentage of absorbance in treated versus control cells. .

Cell Cycle and Cell Apoptosis Assay

Flow cytometry was conducted to determine the cell cycle distribution and apoptosis of A549 and H1975 cells after **8h** treatment. Briefly, A549 and H1975 cells were seeded in six-well plates and cultured overnight, then the cells were incubated with **8h** for 24h or 48h to test cell cycle and apoptosis respectively. The cells were prepared according to instruction of the BD Cycletest Plus DNA Reagent kit (BD Biosciences) and FITC-Annexin V Apoptosis Detection Kit (BD Pharmingen, USA) and then detected by a flow cytometer (Guawa easyCyte, USA ).

Western Blotting.

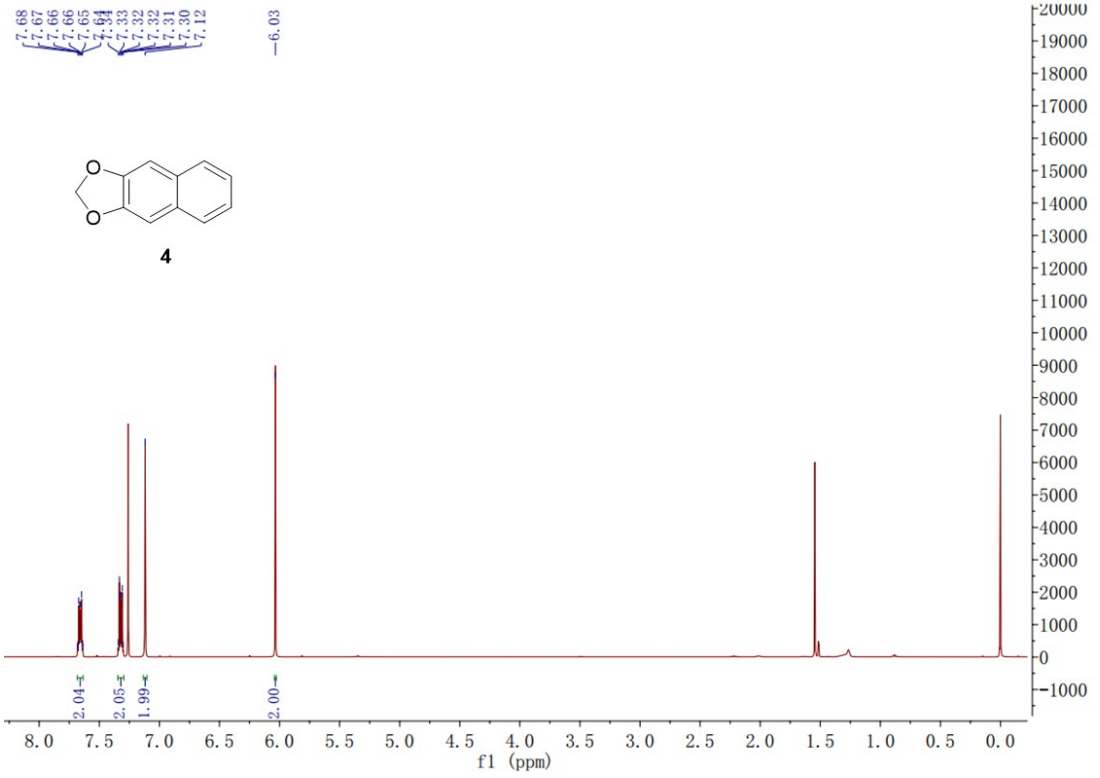
At the end of treatment, the cells were harvested and lysed using RIPA lysis buffer. Equal amounts of protein per sample were loaded in each lane and separated by 12% SDS-PAGE and then transferred to PVDF membranes. The membranes were blocked with 5% milk for 2 h at room temperature and incubated with the indicated antibodies overnight at 4 °C. The PVDF membranes were further incubated with HRP-conjugated secondary antibodies for 2 h at room temperature. The protein bands were visualized using a Super Signal West Pico Chemiluminescent Substrate Trial Kit (Pierce, Rockford, IL). Images were obtained using an Amersham Imager 600 (GE, UK).

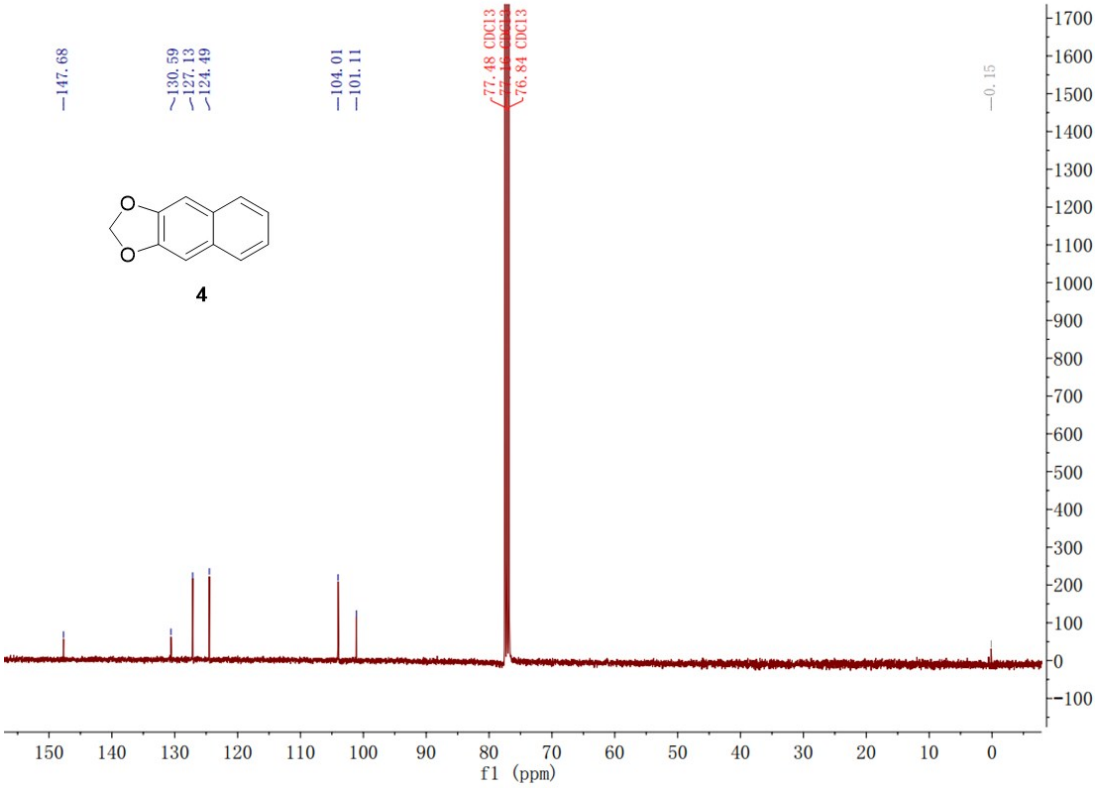
Determination of ROS Production

Flow cytometry was conducted to determine ROS production in A549 and H1975 cells after **8h** treatment. The intracellular ROS level was measured using a 2',7'-dichlorofluorescin diacetate (DCFH-DA) fluorescent probe. Briefly, 20 minutes later after **8h** treatment, A549 and H1975 cells were washed with PBS and incubated with 10μM DCFH-DA in phenol red-free DMEM medium for 30 min at 37 °C in the dark. DCFH-DA will be cleaved by intracellular esterases and be oxidized into the highly fluorescent dichlorofluorescein (DCF) by ROS. The cells were then washed with PBS for 3 times to remove the residual DCFH-DA. ROS-positive cells were monitored by a flow cytometer (Guawa easyCyte, USA ) with excitation at 488 nm and emission

at 530 nm.

**$^{1}$H and $^{13}$C NMR Spectra**

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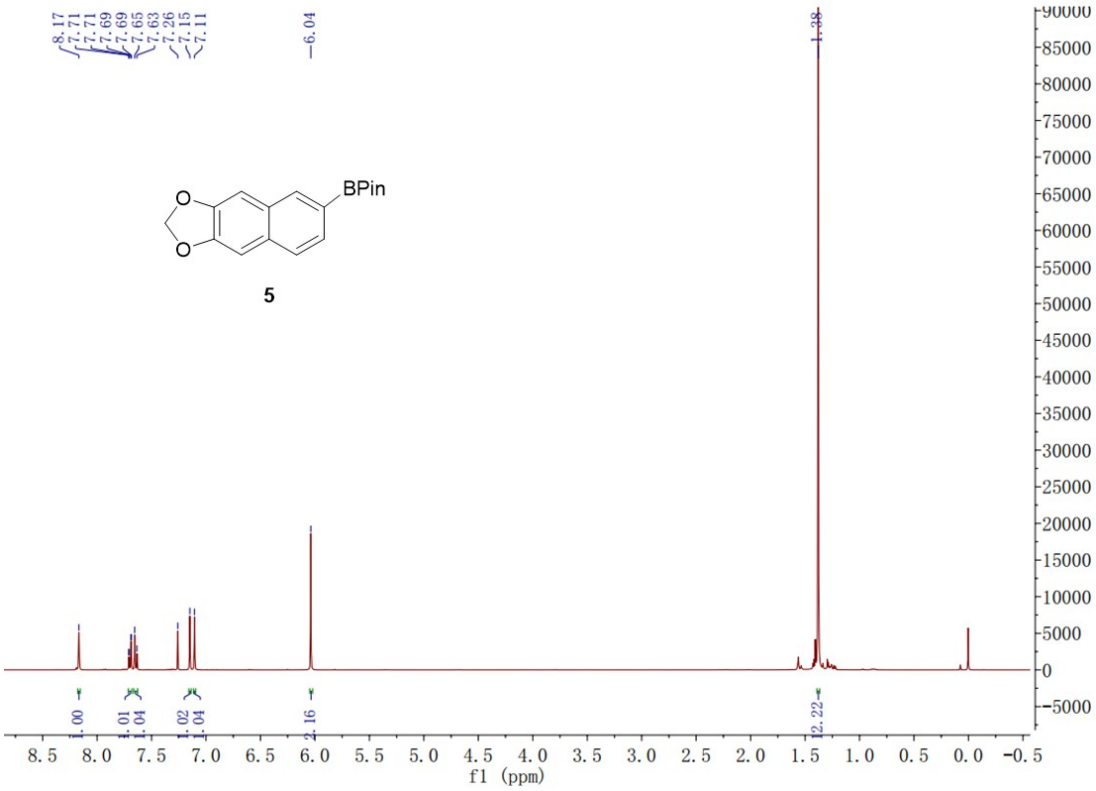
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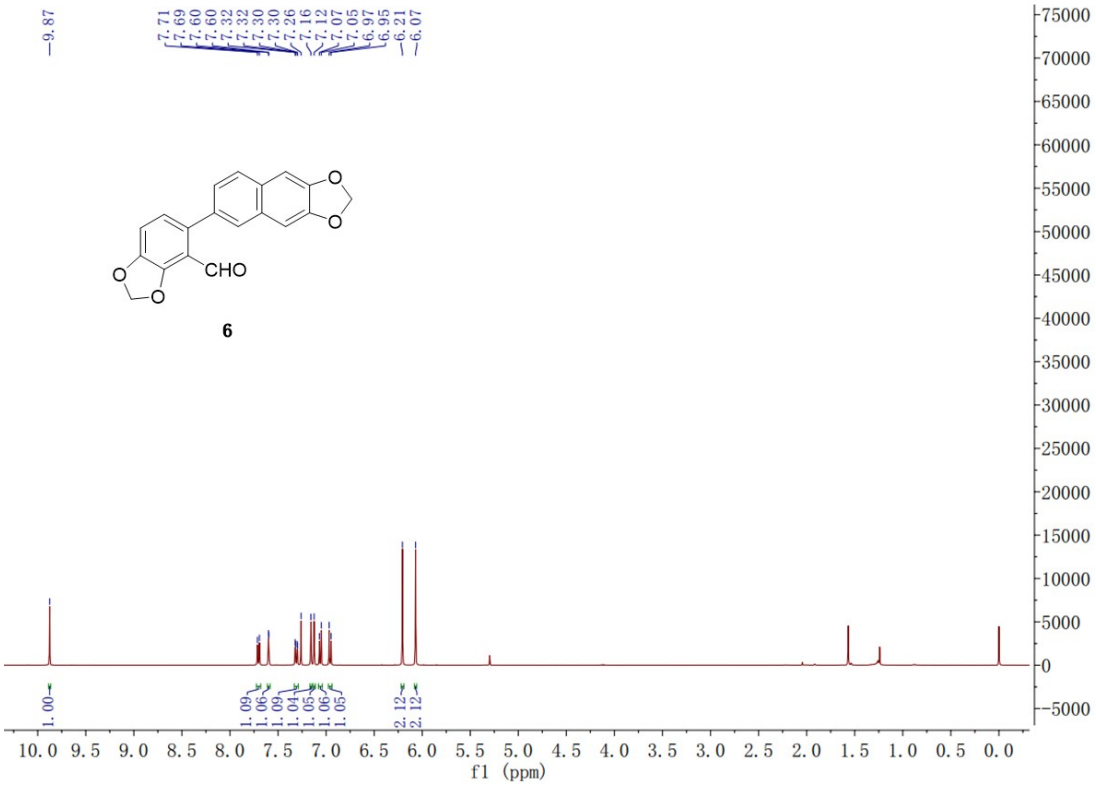
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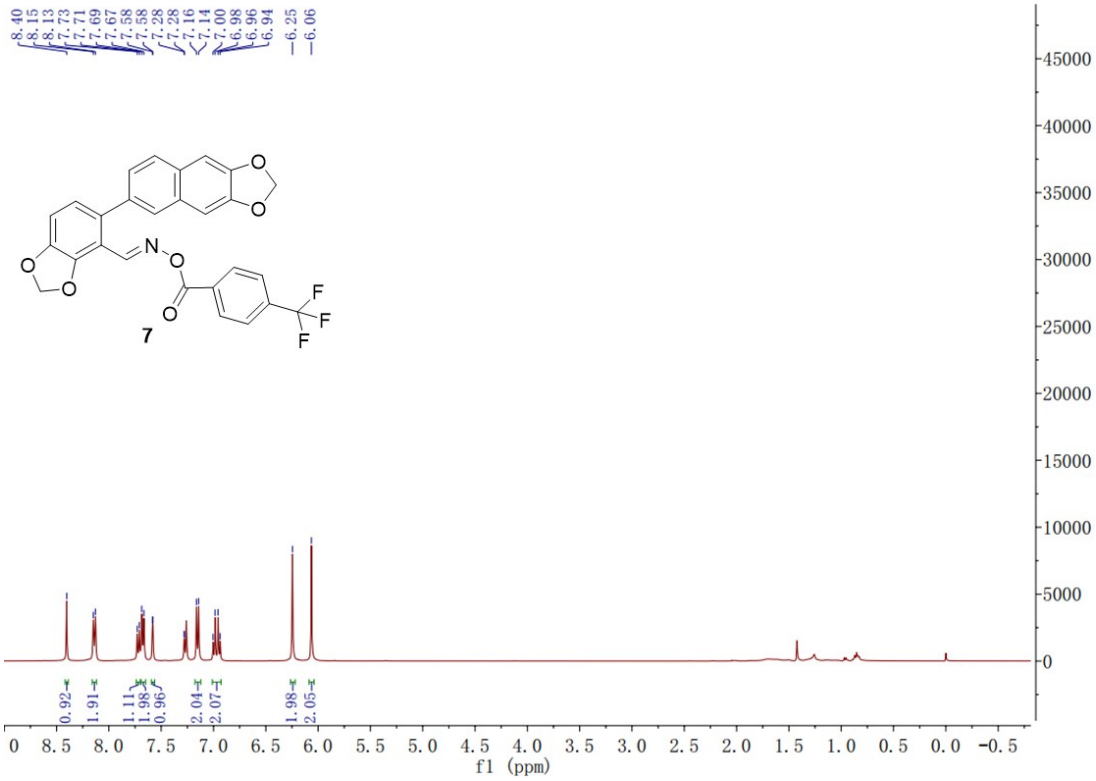
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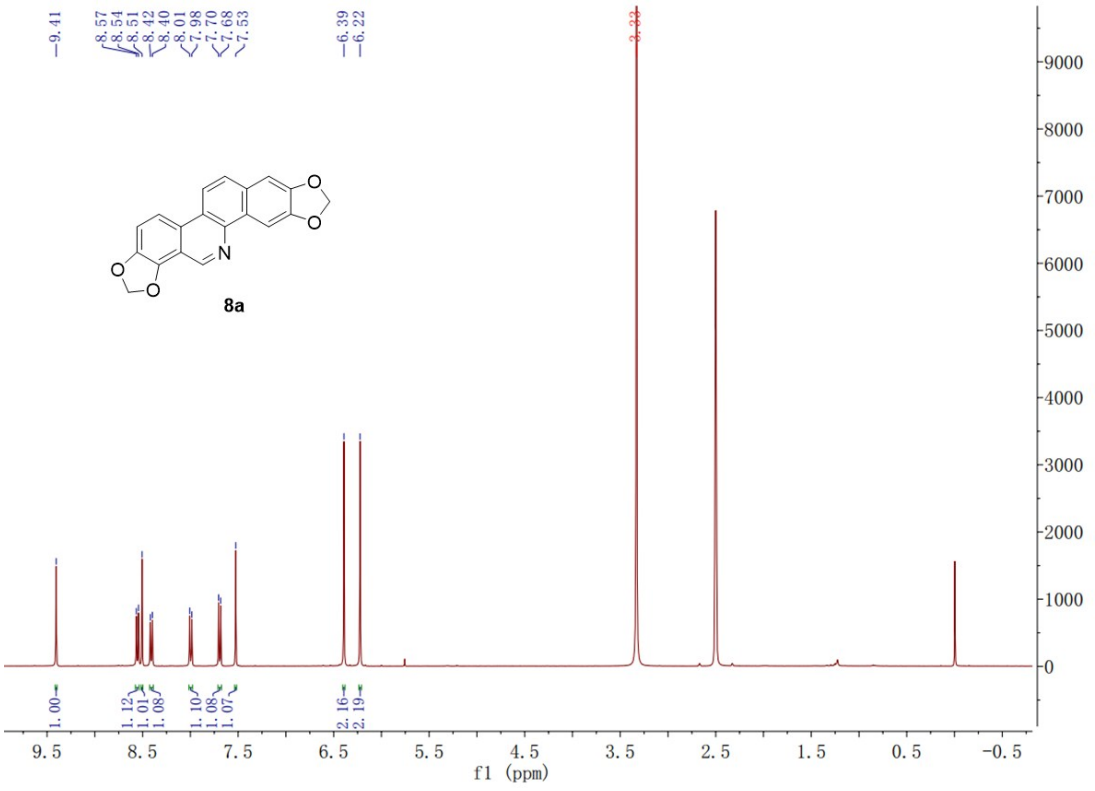
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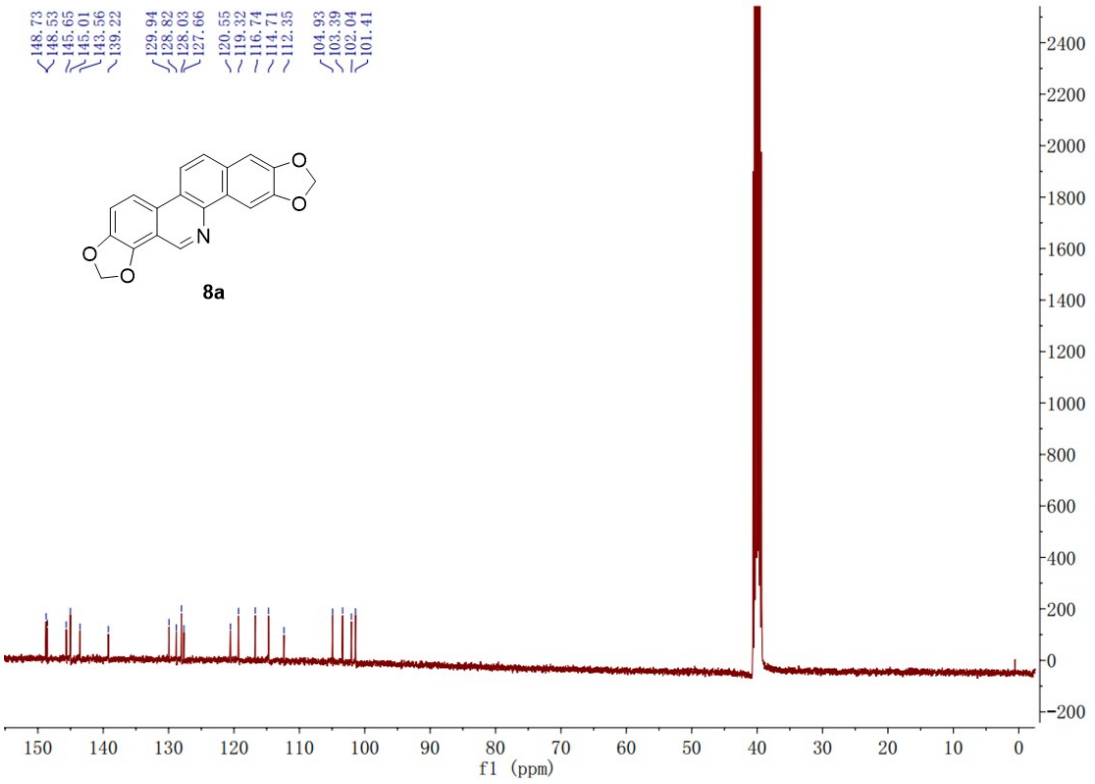
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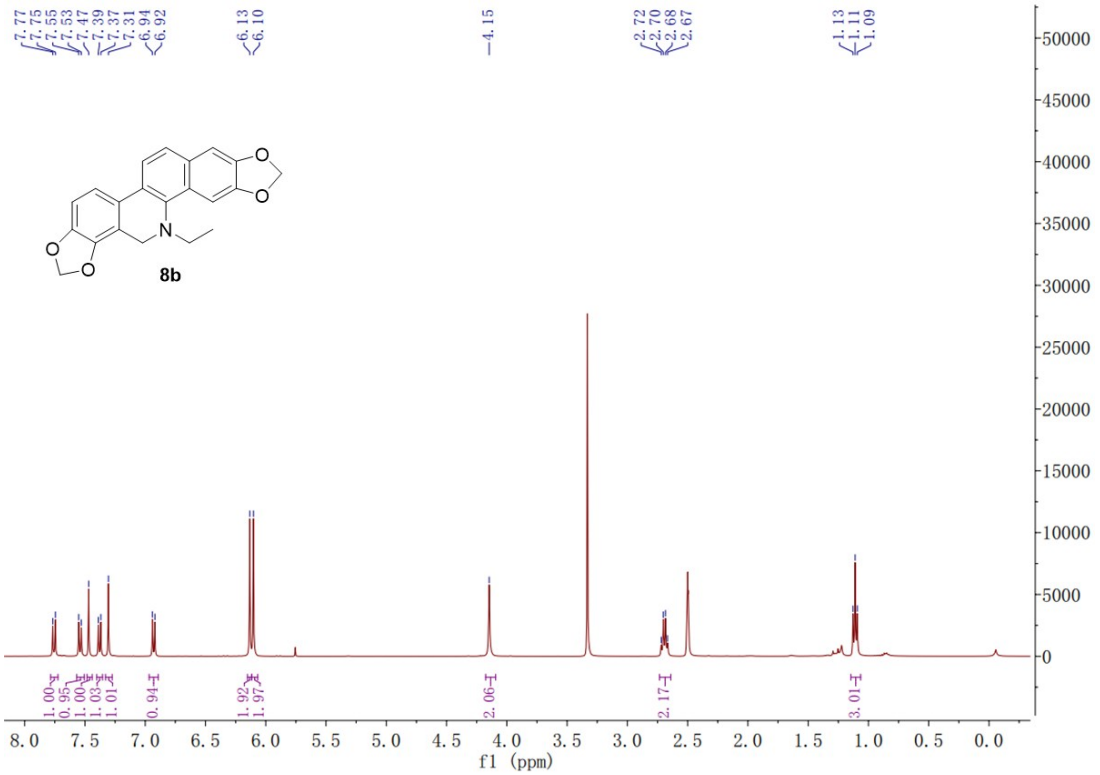
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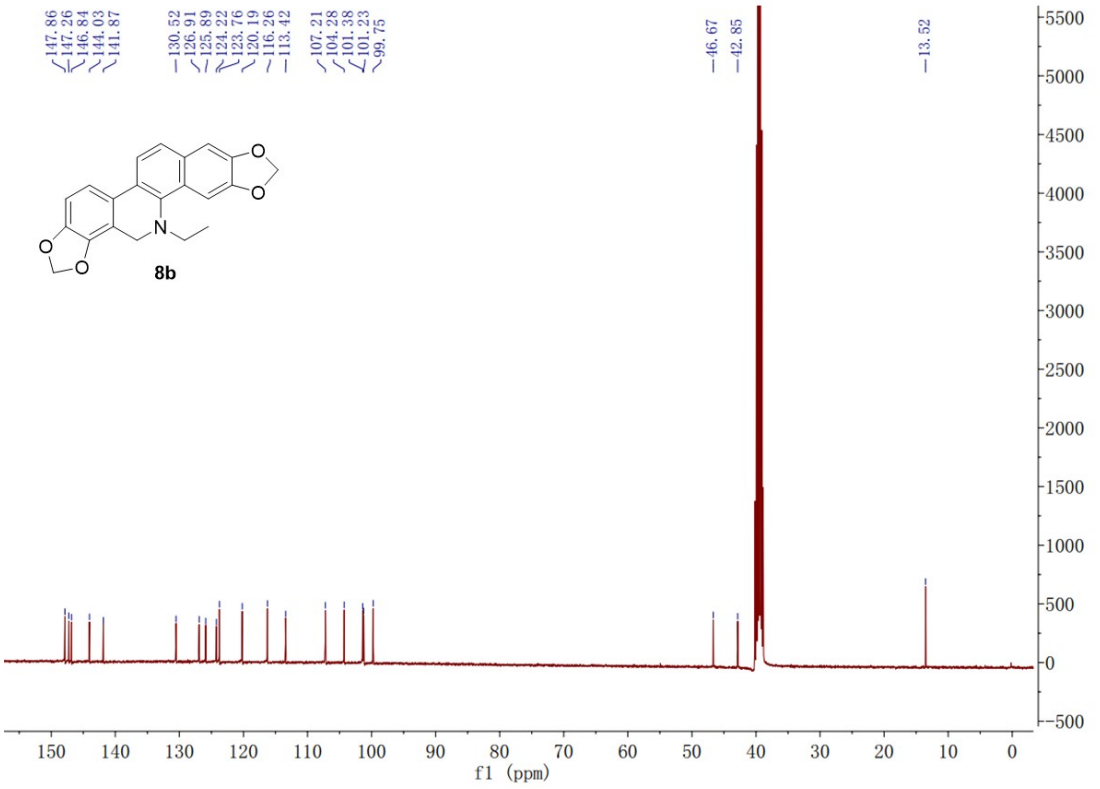
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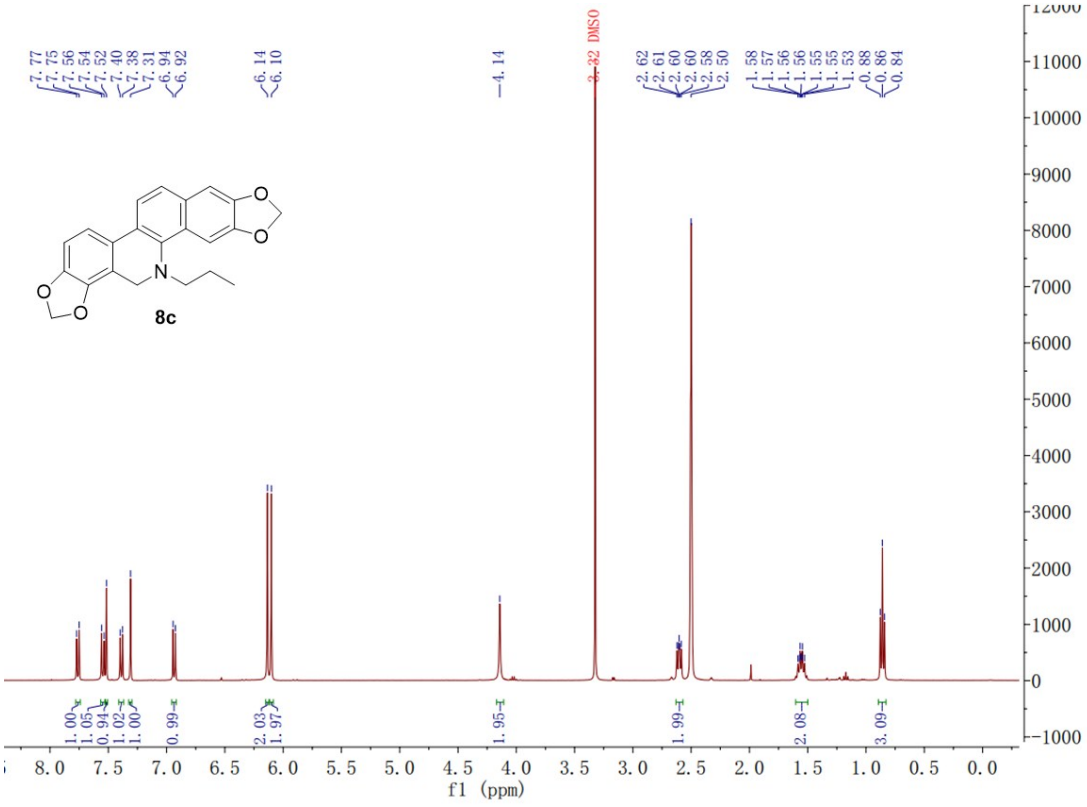
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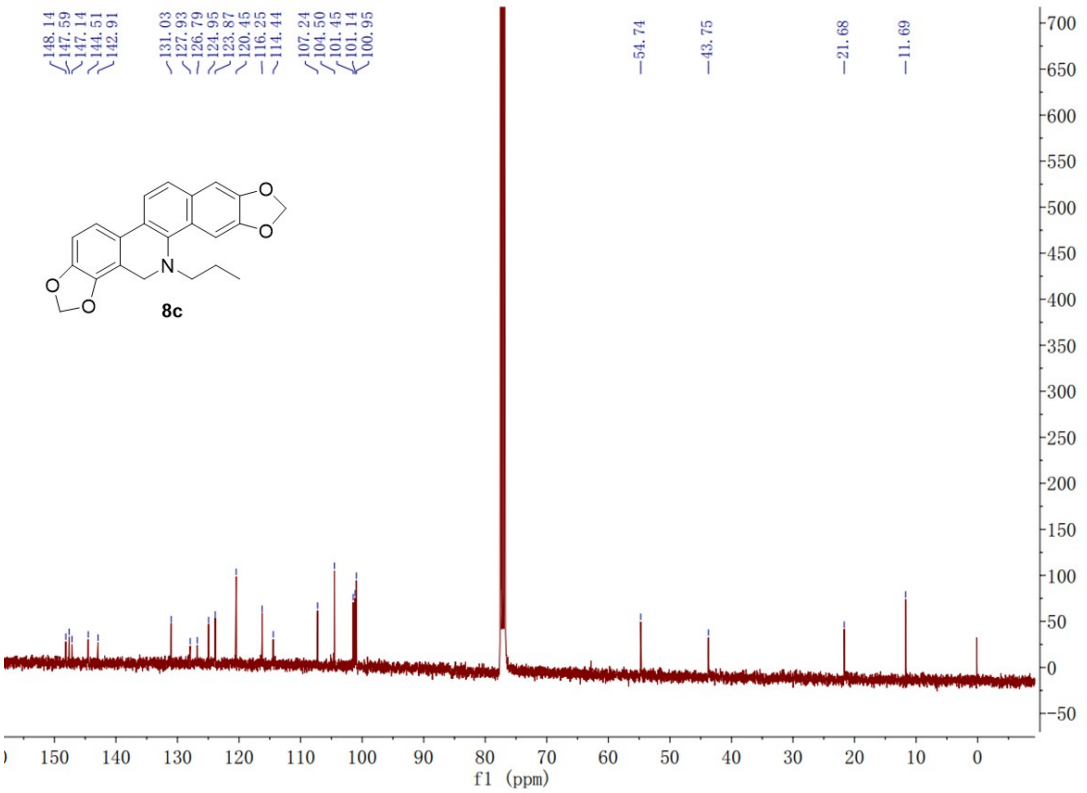
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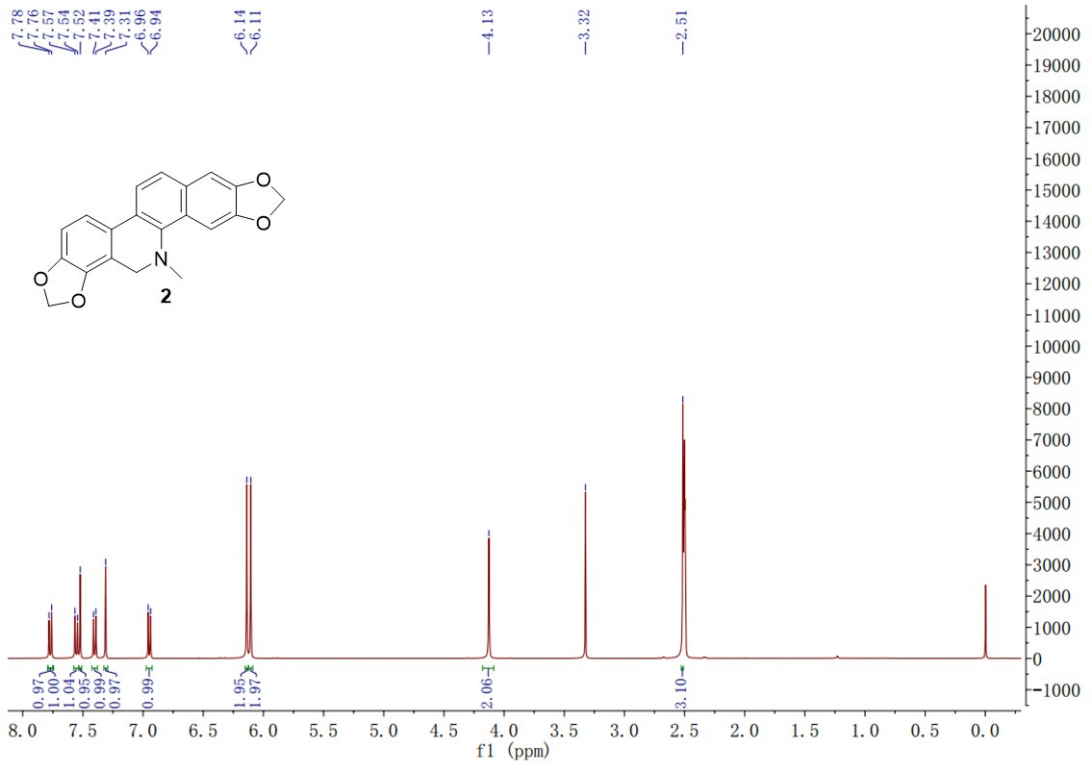
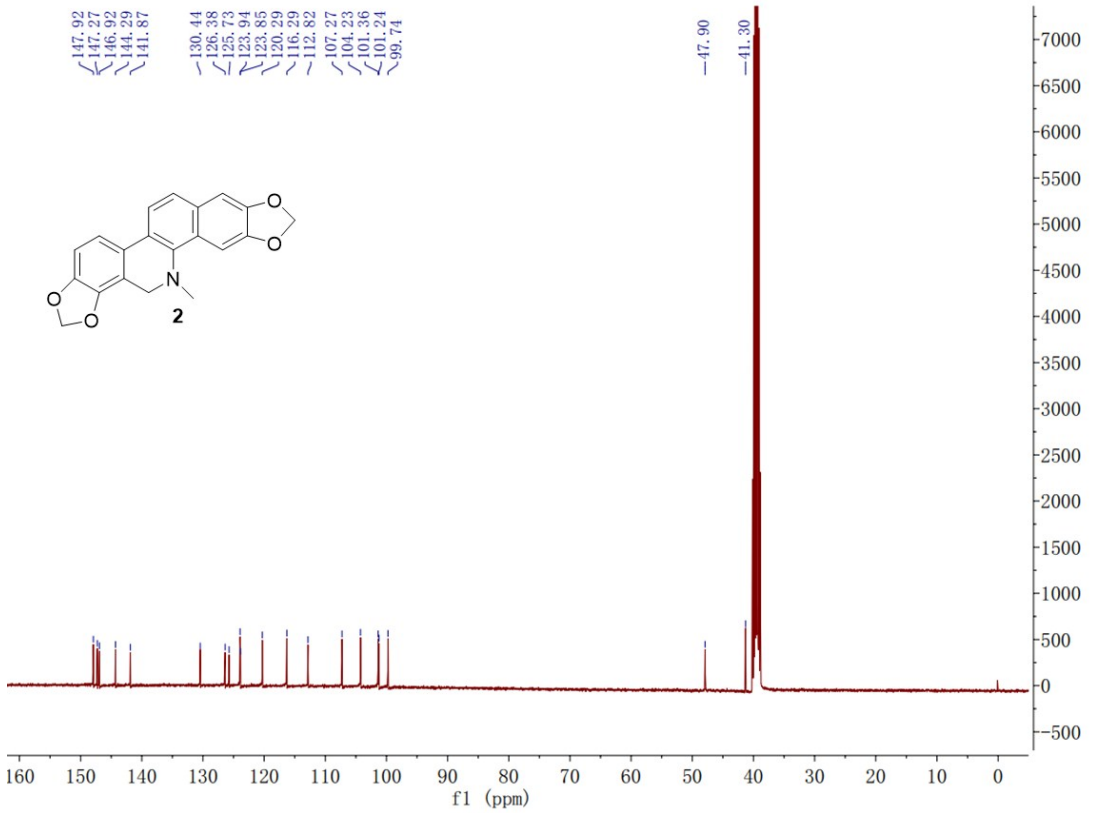
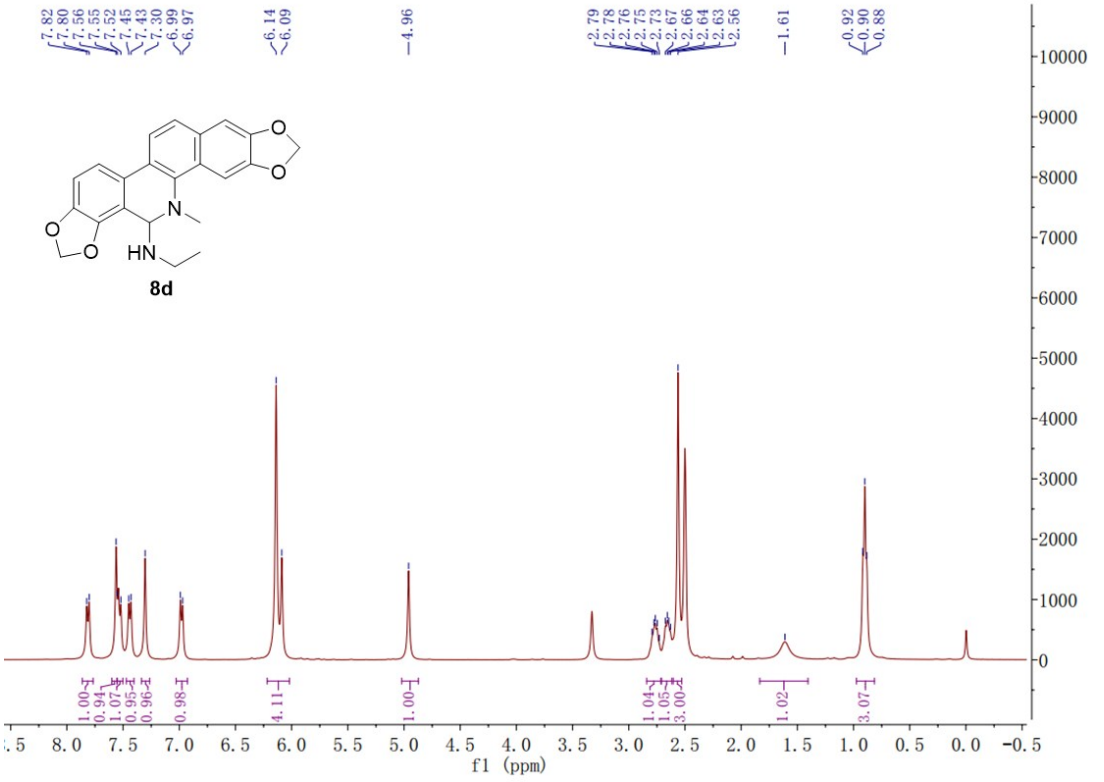
8c
f1 (ppm)


7.78
7.76
7.57
7.54
7.52
7.41
7.39
7.31
6.96
6.94
6.14
6.11
4.13
3.32
2.51
2
0.97
1.00
1.04
0.95
0.99
0.97
0.99
1.95
1.97
2.06
3.10
f1 (ppm)


147.92
147.27
146.92
144.29
141.87
130.44
126.38
125.73
123.94
123.85
120.29
116.29
112.82
107.27
104.23
101.36
101.24
99.74
47.90
41.30
2
f1 (ppm)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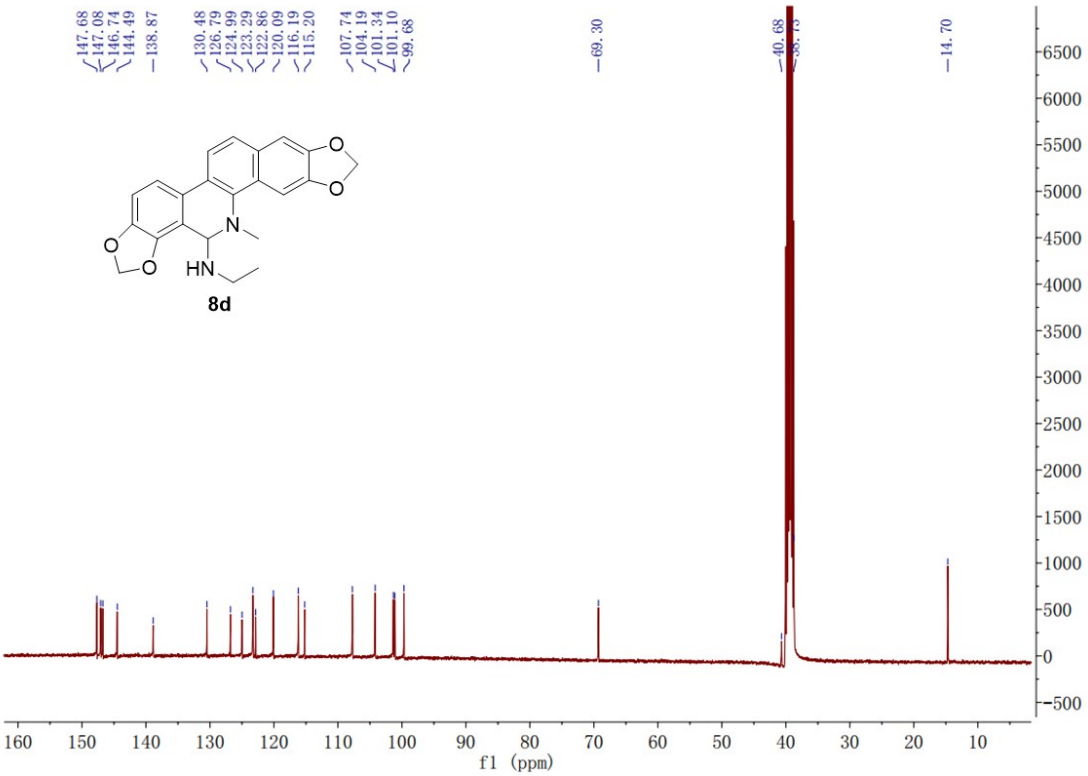
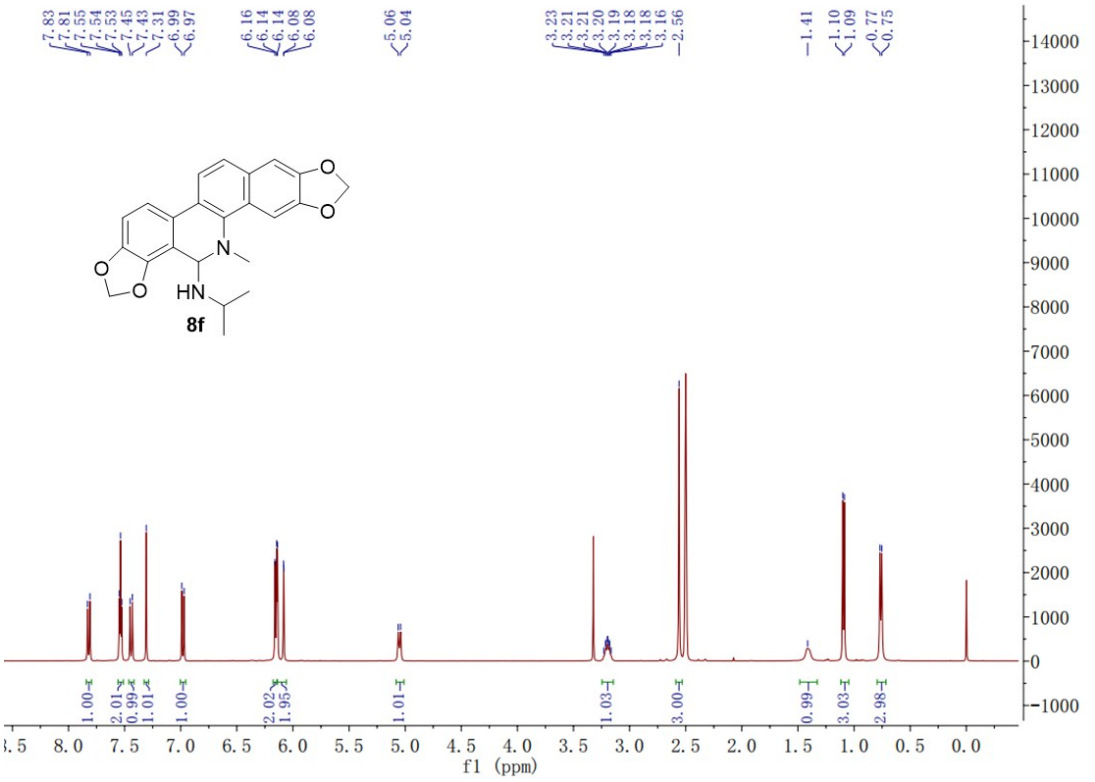
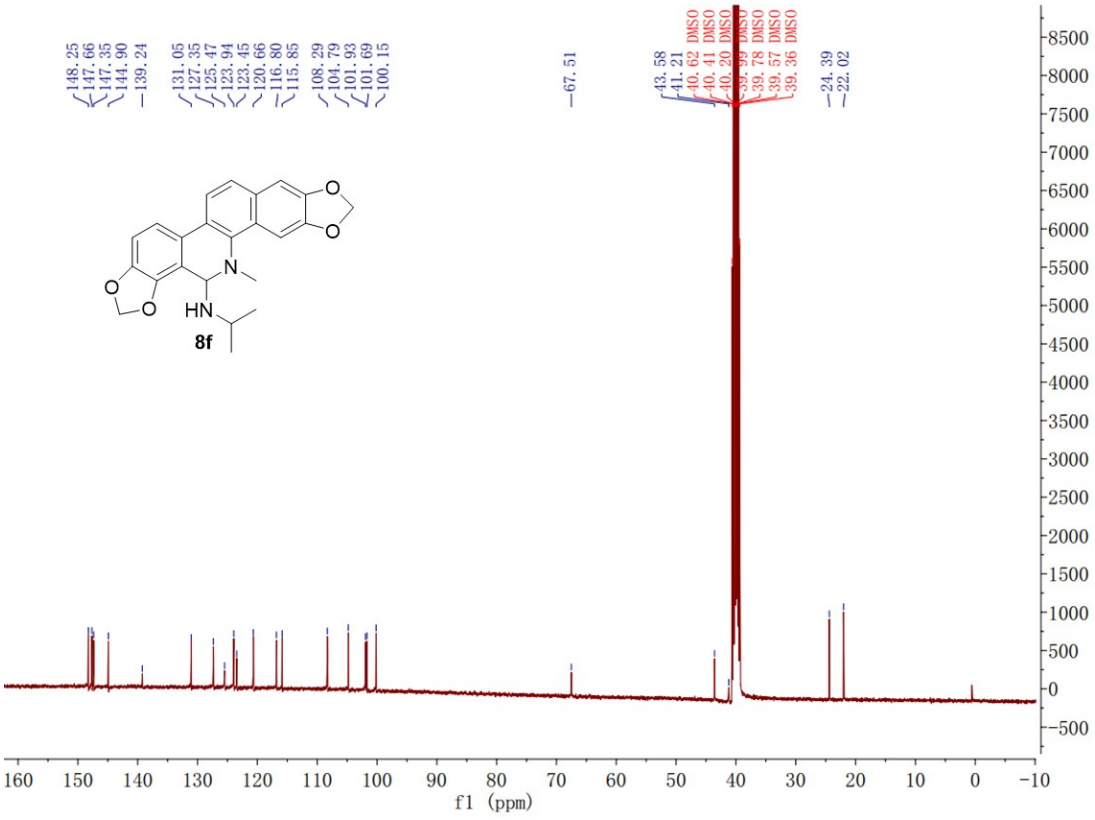
7.80
7.56
7.55
7.52
7.45
7.43
7.30
6.99
6.97
6.14
6.09
4.96
2.79
2.78
2.76
2.75
2.73
2.67
2.66
2.64
2.63
2.56
1.61
0.92
0.90
0.88
8d
1.00
0.94
1.07
0.95
0.96
0.98
4.11
1.00
1.04
1.05
3.00
1.02
3.07
f1 (ppm)


147.68
147.08
146.74
144.49
138.87
130.48
126.79
124.99
123.29
122.86
120.09
116.19
115.20
107.74
104.19
101.34
101.10
99.68
69.30
40.68
38.73
14.70
8d
f1 (ppm)


7.83
7.81
7.55
7.54
7.53
7.45
7.43
7.31
6.99
6.97
6.16
6.14
6.14
6.08
6.08
5.06
5.04
3.23
3.21
3.21
3.20
3.19
3.18
3.16
2.56
1.41
1.10
1.09
0.77
0.75
O
O
N
O
O
HN
8f
1.00
2.01
0.99
1.01
1.00
2.02
1.95
1.01
1.03
3.00
0.99
3.03
2.98
f1 (ppm)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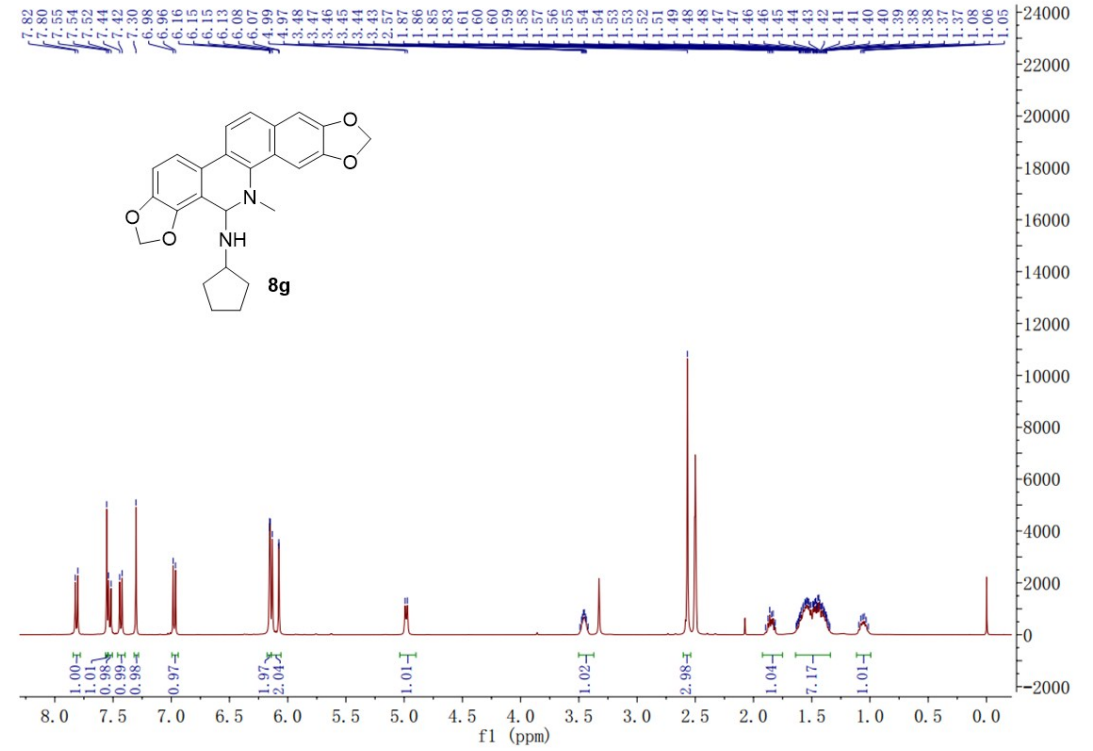
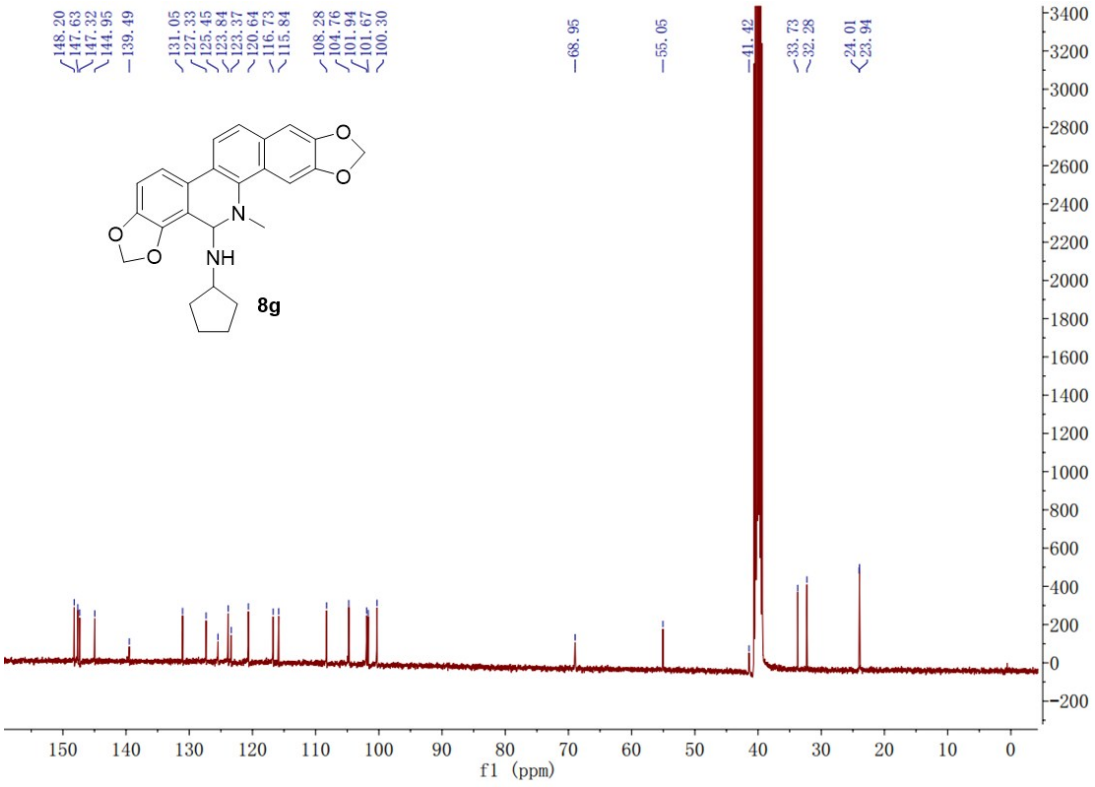
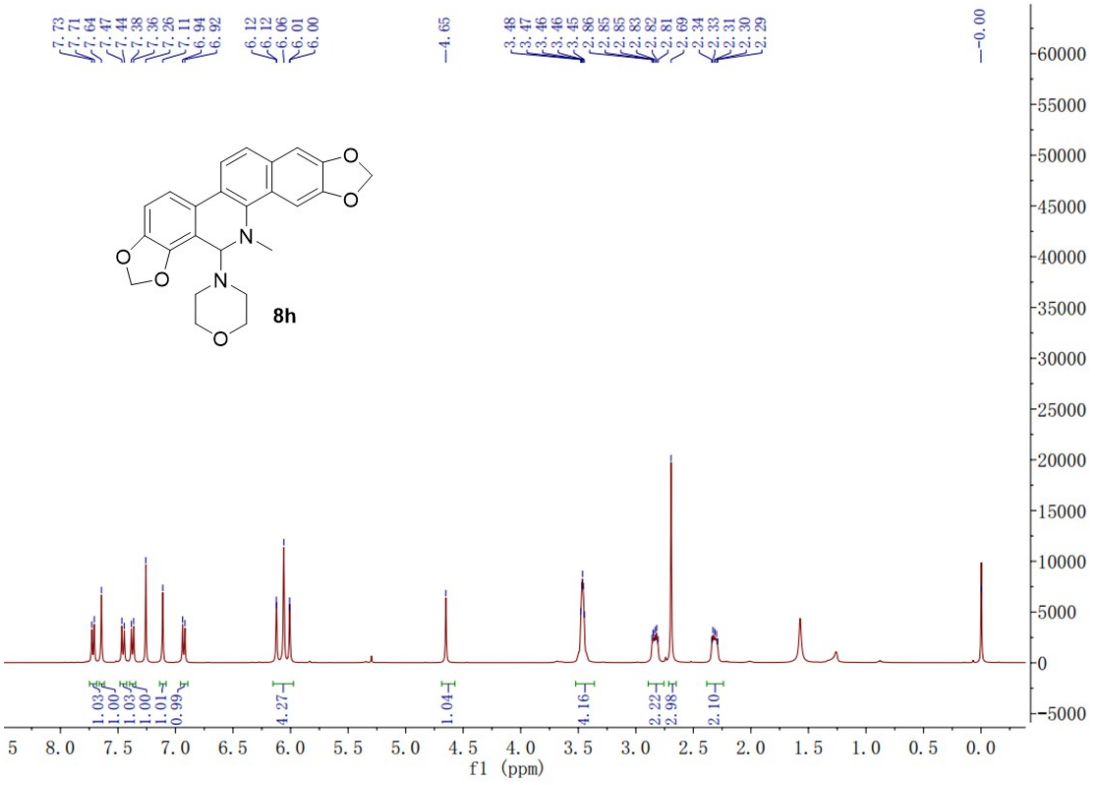
147.66
147.35
144.90
139.24
131.05
127.35
125.47
123.94
123.45
120.66
116.80
115.85
108.29
104.79
101.93
101.69
100.15
67.51
43.58
41.21
40.62 DMSO
40.41 DMSO
40.20 DMSO
39.99 DMSO
39.78 DMSO
39.57 DMSO
39.36 DMSO
24.39
22.02
O
O
N
O
O
HN
8f
f1 (ppm)


8g
f1 (ppm)


8g
f1 (ppm)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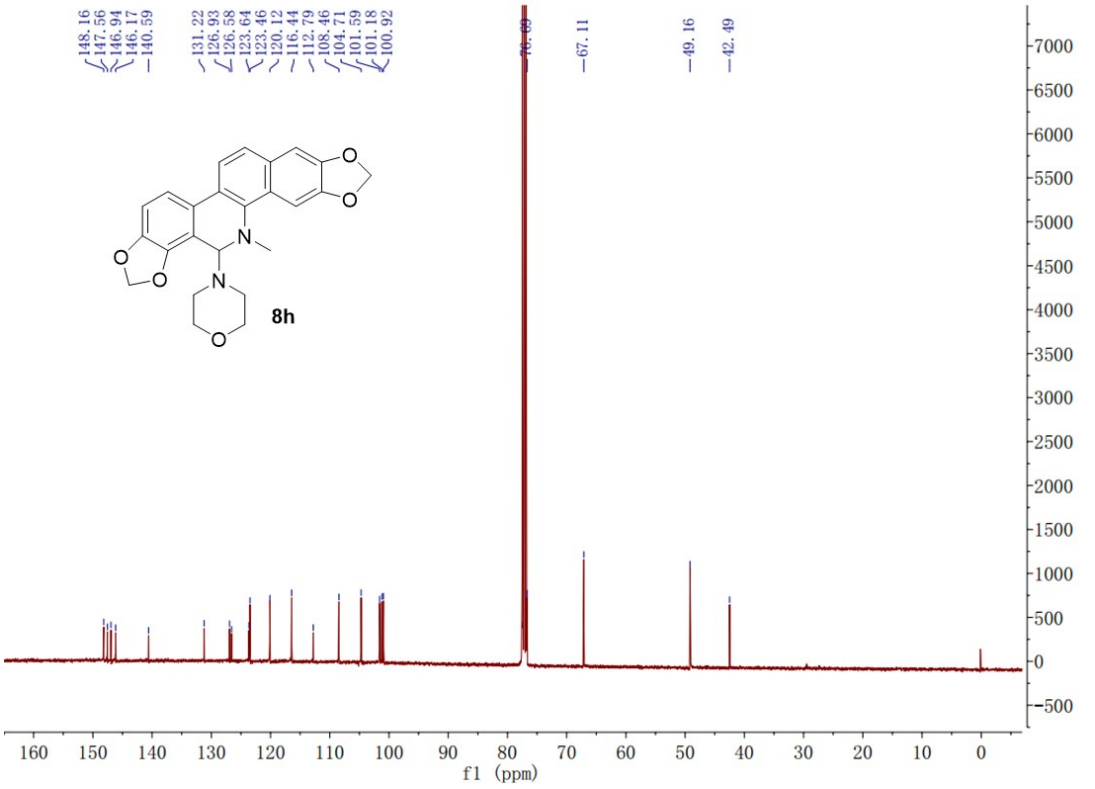
7.36
7.26
7.11
6.94
6.92
6.12
6.12
6.06
6.01
6.00
4.65
3.48
3.47
3.46
3.46
3.45
2.86
2.85
2.85
2.83
2.82
2.81
2.69
2.34
2.33
2.31
2.30
2.29
0.00
8h
1.03
1.00
1.03
1.00
1.01
0.99
4.27
1.04
4.16
2.22
2.98
2.10
f1 (ppm)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131.22
126.93
126.58
123.64
123.46
120.12
116.44
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101.59
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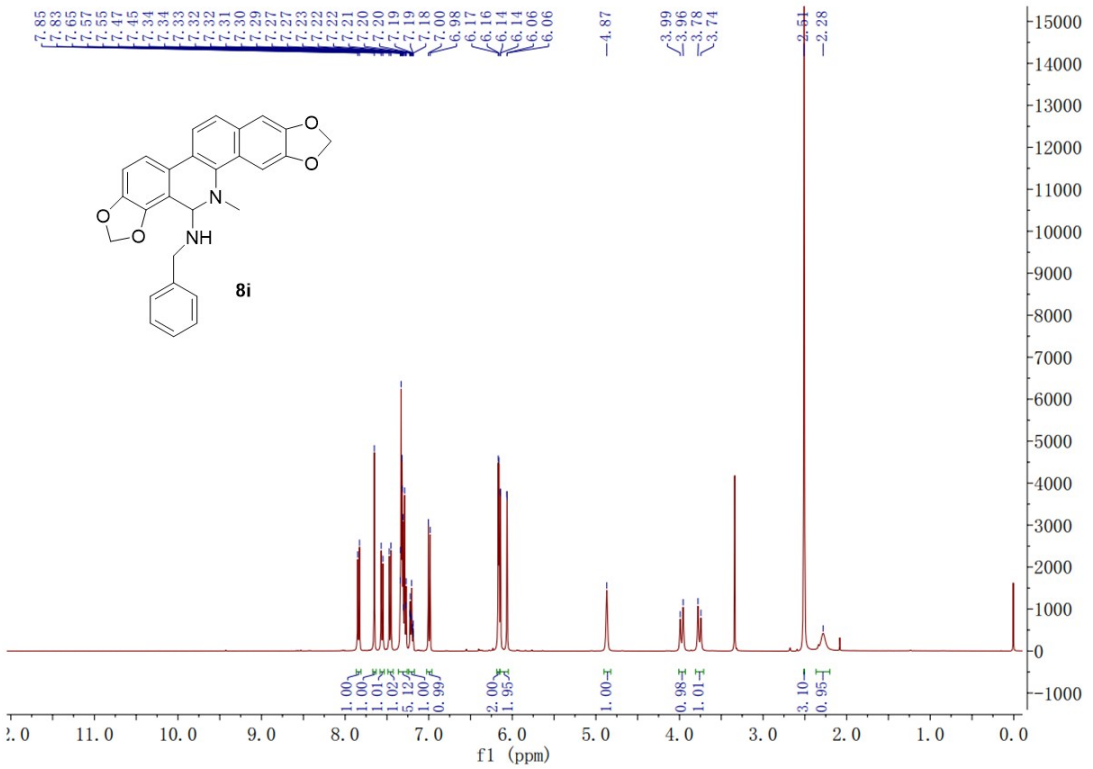
67.11
49.16
42.49
8h
f1 (ppm)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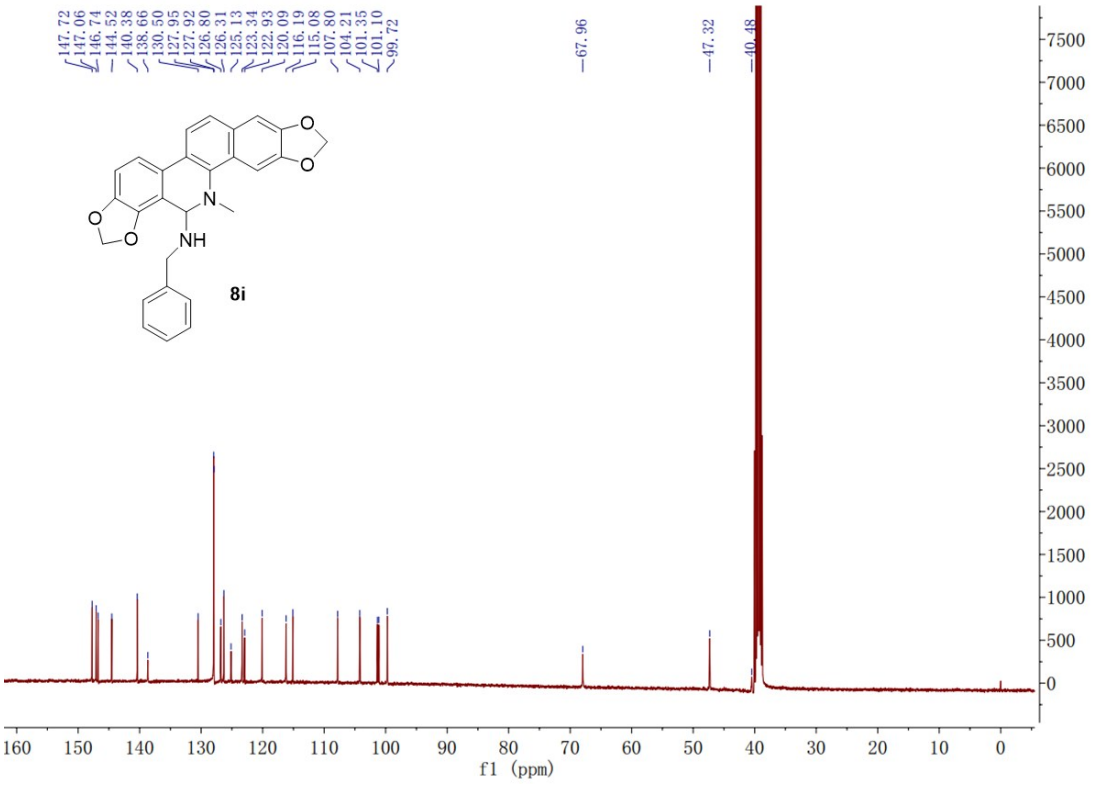
7.57
7.55
7.47
7.45
7.34
7.34
7.33
7.32
7.32
7.31
7.30
7.29
7.27
7.27
7.23
7.22
7.22
7.21
7.20
7.20
7.19
7.19
7.18
7.00
6.98
6.17
6.16
6.14
6.14
6.06
6.06
4.87
3.99
3.96
3.78
3.74
2.61
2.28
O
O
O
O
N
NH
8i
1.00
1.00
1.01
1.02
5.12
1.00
0.99
2.00
1.95
1.00
0.98
1.01
3.10
0.95
f1 (ppm)


147.72
147.06
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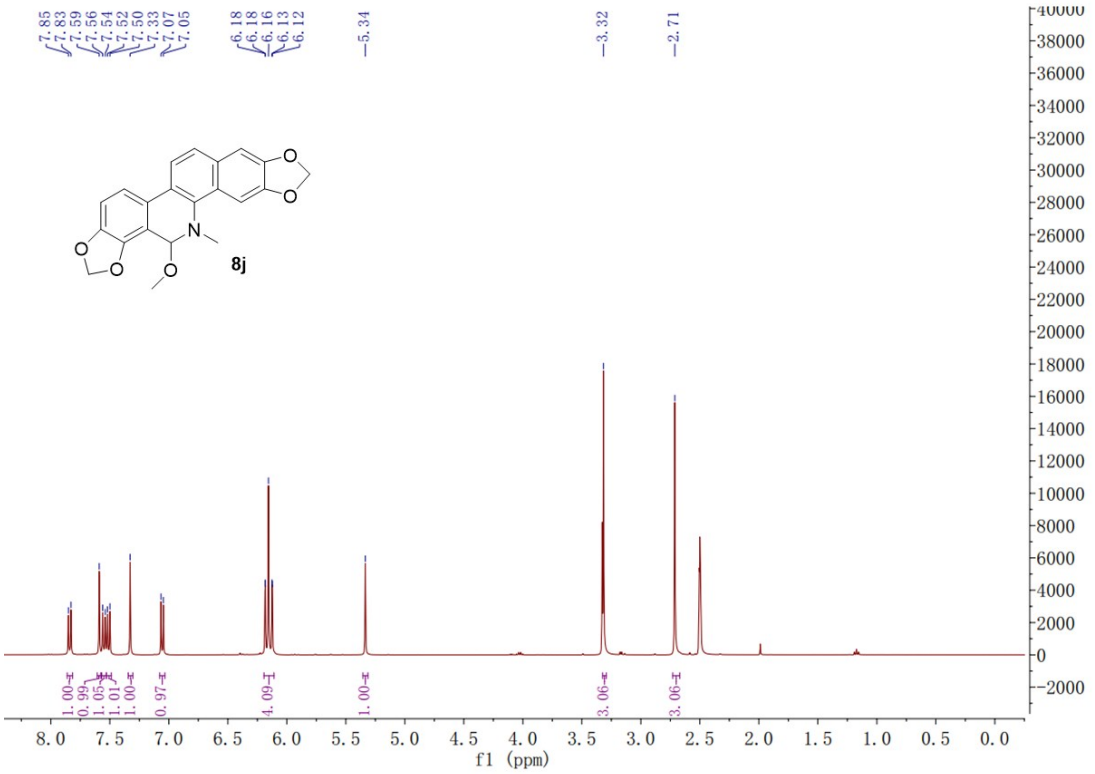
144.52
140.38
138.66
130.50
127.95
127.92
126.80
126.31
125.13
123.34
122.93
120.09
116.19
115.08
107.80
104.21
101.35
101.10
99.72
67.96
47.32
40.48
O
O
O
O
N
NH
8i
f1 (ppm)


7.85
7.83
7.59
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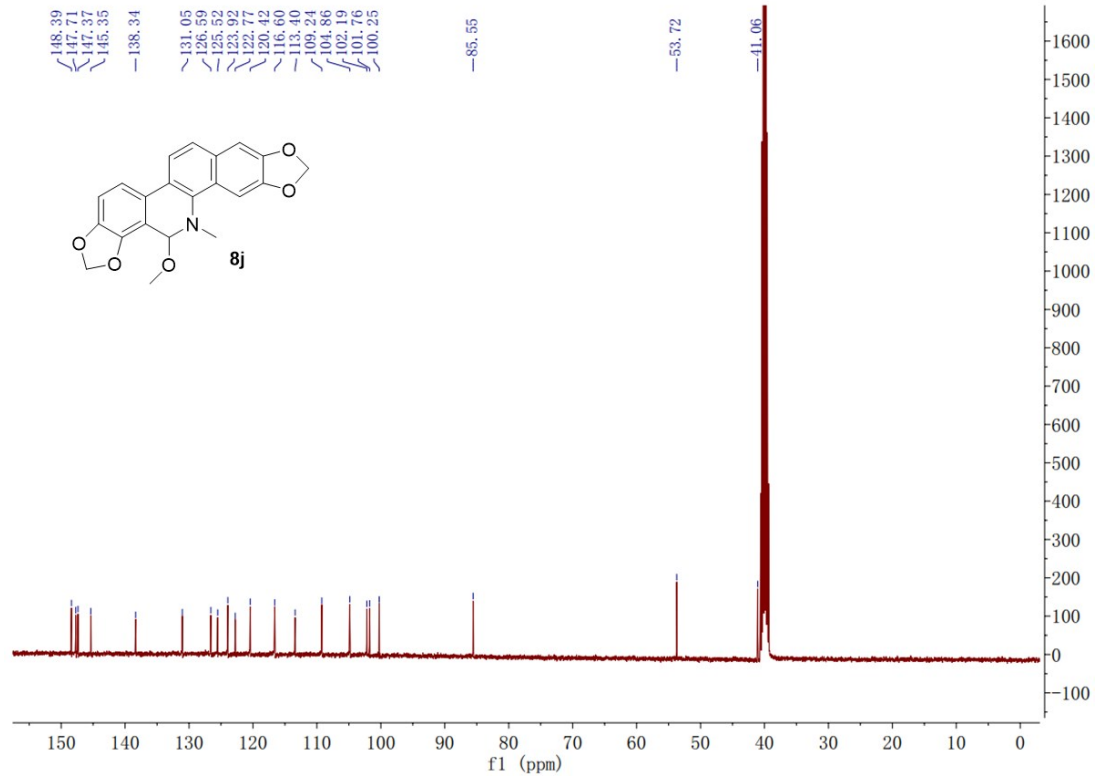
7.54
7.52
7.50
7.33
7.07
7.05
6.18
6.18
6.16
6.13
6.12
5.34
3.32
2.71
8j
1.00
0.99
1.05
1.01
1.00
0.97
4.09
1.00
3.06
3.06
f1 (ppm)


148.39
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126.59
125.52
123.92
122.77
120.42
116.60
113.40
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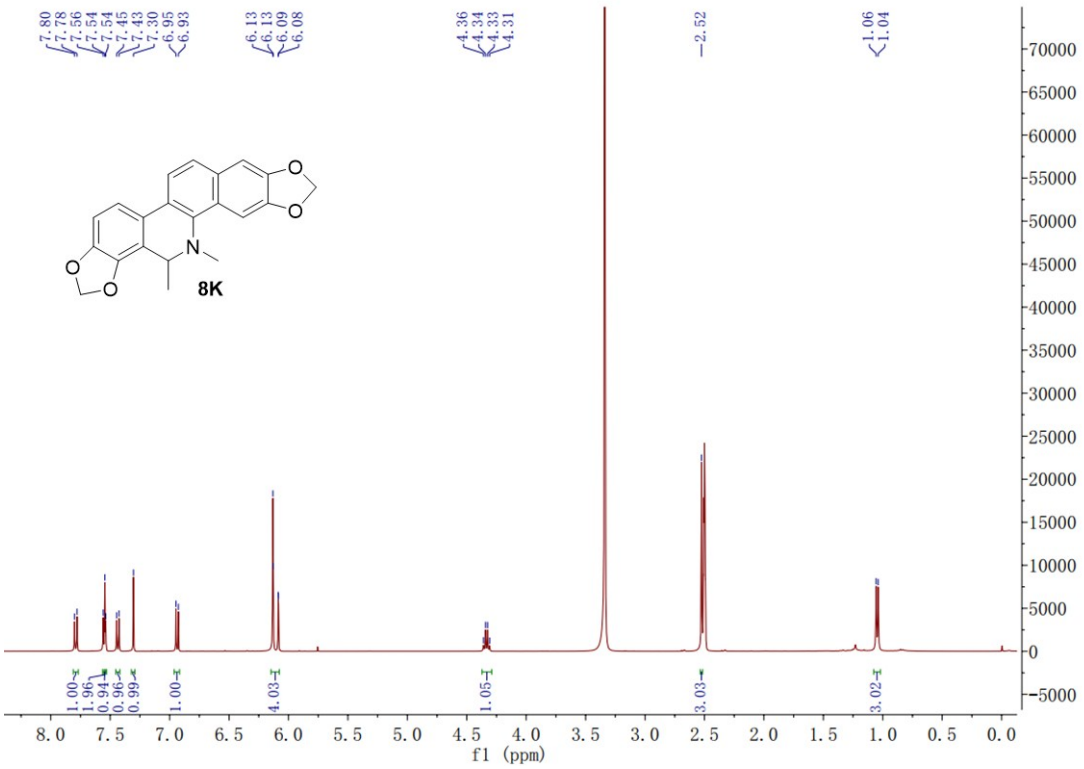
104.86
102.19
101.76
100.25
85.55
53.72
41.06
8j
f1 (ppm)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7.78
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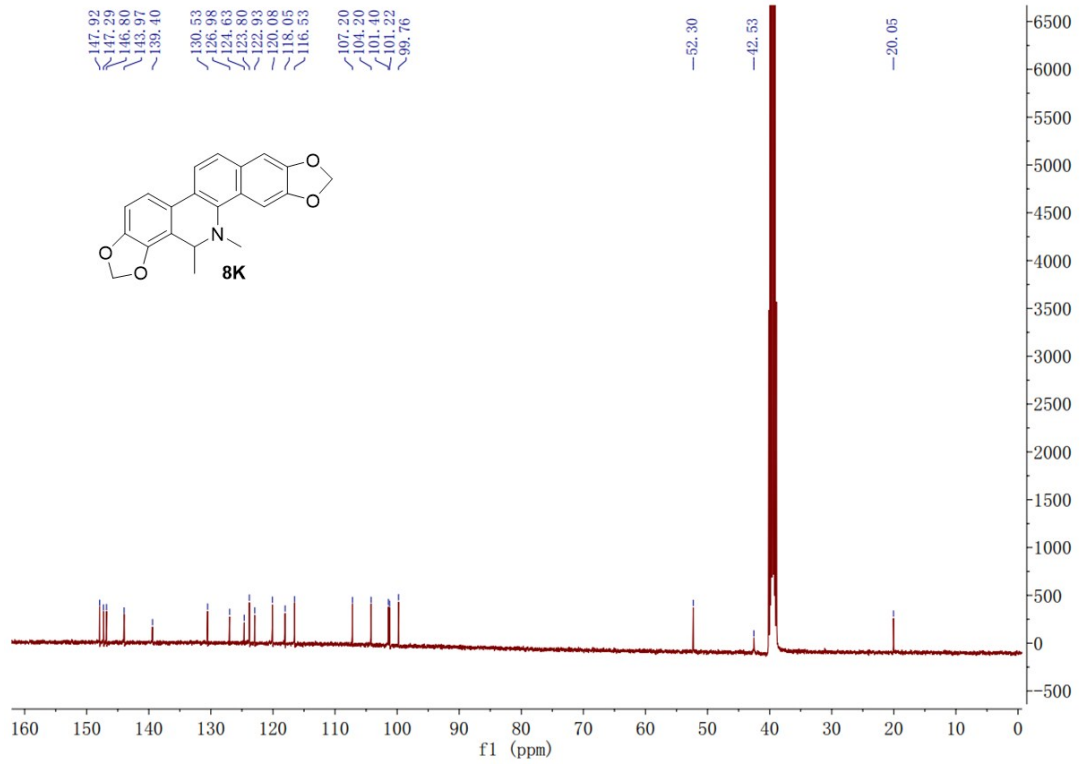
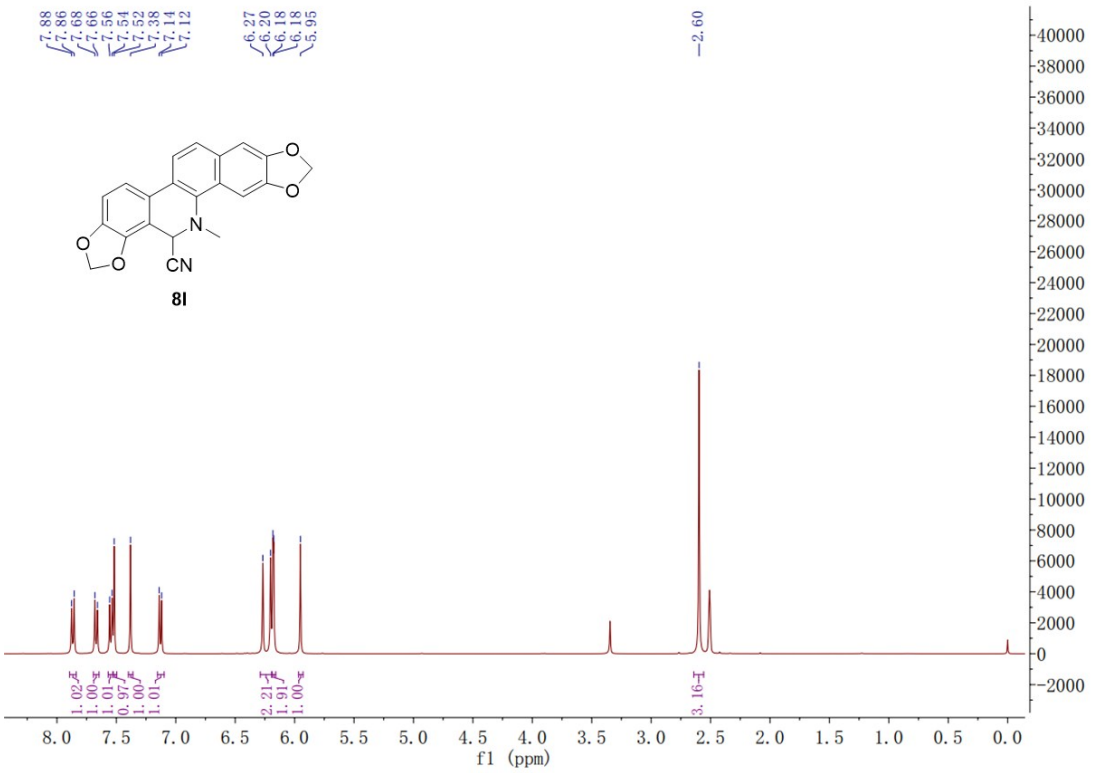
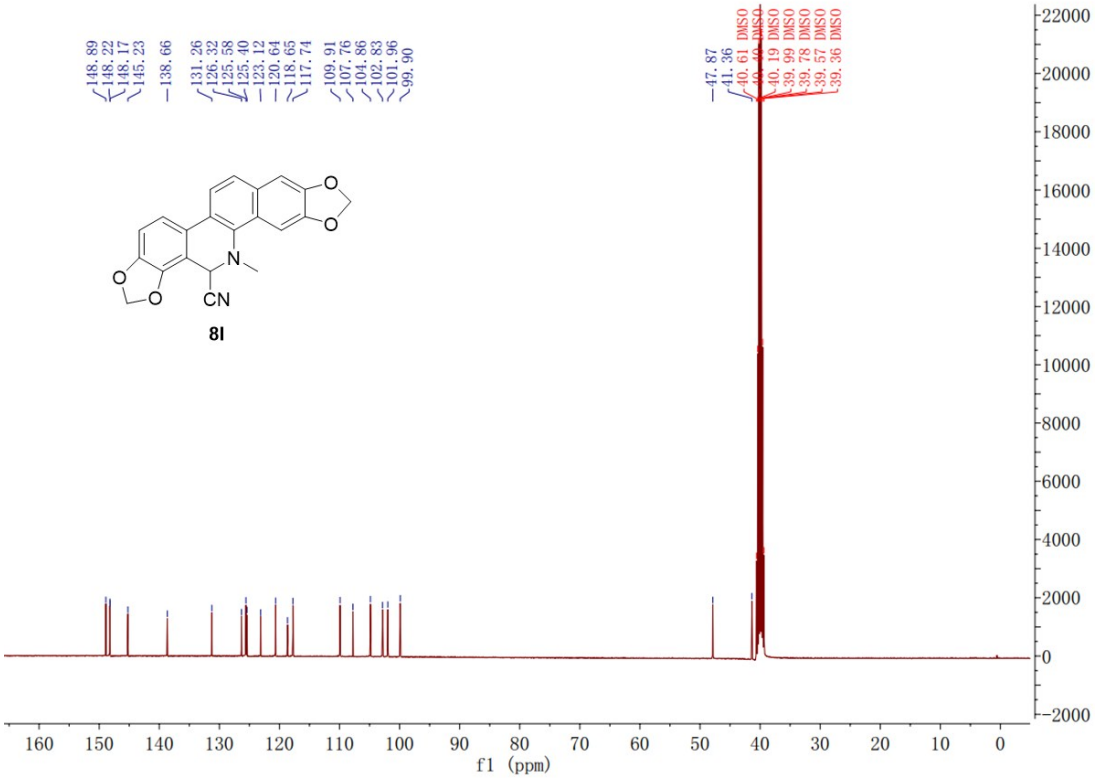
7.54
7.45
7.43
7.30
6.95
6.93
6.13
6.13
6.09
6.08
4.36
4.34
4.33
4.31
2.52
1.06
1.04
8K
1.00
1.96
0.94
0.96
0.99
1.00
4.03
1.05
3.03
3.02
f1 (ppm)


147.92
147.29
146.80
143.97
139.40
130.53
126.98
124.63
123.80
122.93
120.08
118.05
116.53
107.20
104.20
101.40
101.22
99.76
52.30
42.53
20.05
8K
f1 (ppm)


7.88
7.86
7.68
7.66
7.56
7.54
7.52
7.38
7.14
7.12
6.27
6.20
6.18
6.18
5.95
2.60
O
O
O
O
N
CN
8l
1.02
1.00
1.01
0.97
1.00
1.01
2.21
1.91
1.00
3.16
f1 (ppm)


148.89
148.22
148.17
145.23
138.66
131.26
126.32
125.58
125.40
123.12
120.64
118.65
117.74
109.91
107.76
104.86
102.83
101.96
99.90
47.87
41.36
40.61 DMSO
40.40 DMSO
40.19 DMSO
39.99 DMSO
39.78 DMSO
39.57 DMSO
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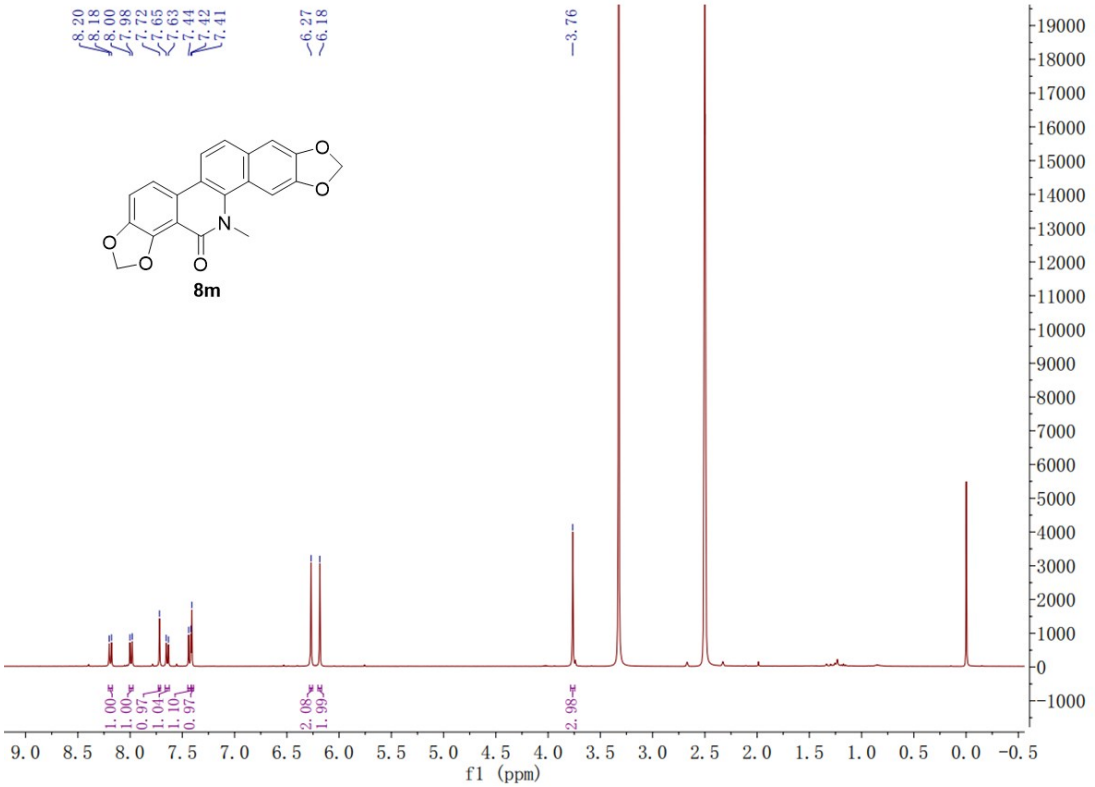
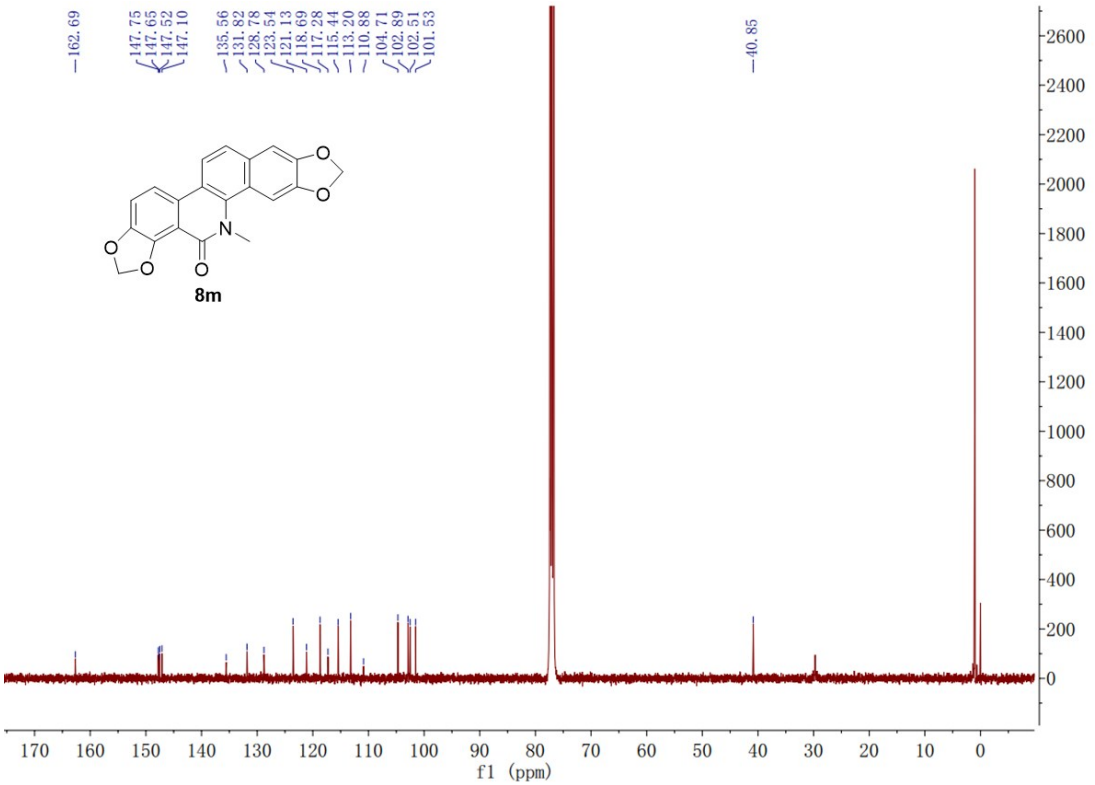
O
O
O
O
N
CN
8l
f1 (ppm)


8.20
8.18
8.00
7.98
7.72
7.65
7.63
7.44
7.42
7.41
6.27
6.18
3.76
1.00
1.00
0.97
1.04
1.10
0.97
2.08
1.99
2.98
f1 (ppm)

8m


162.69
147.75
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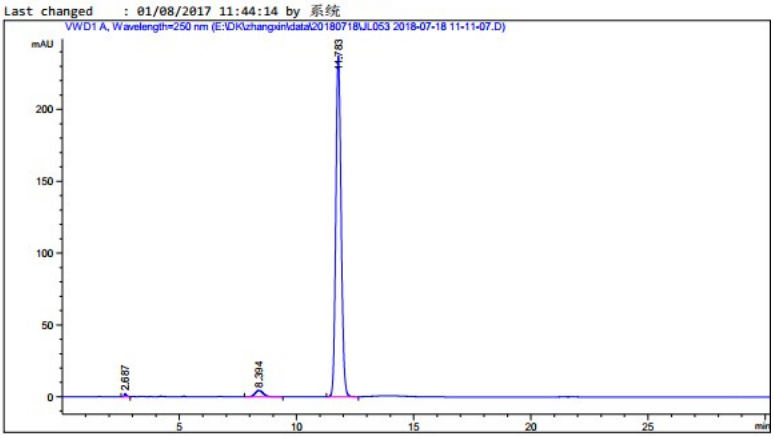
147.52
147.10
135.56
131.82
128.78
123.54
121.13
118.69
117.28
115.44
113.20
110.88
104.71
102.89
102.51
101.53
40.85
f1 (ppm)

8m

## HPLC Purity Analysis

## HPLC Purity Analysis of 8a

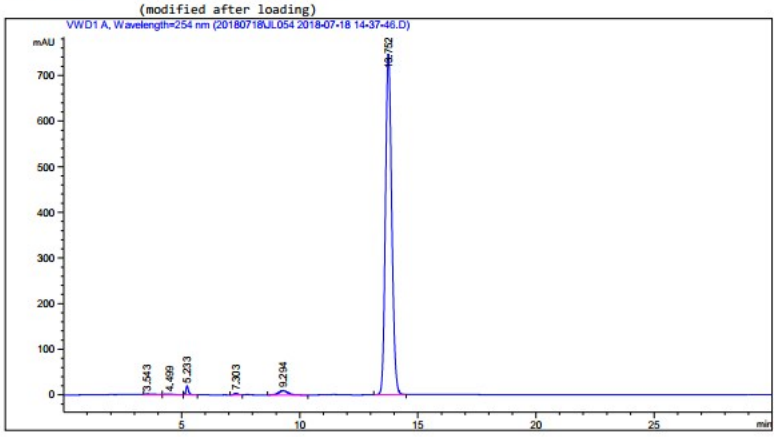
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   11
Injection Date  : 18/07/2018 11:11:46
                                                Inj Volume : 5.000 µl
Acq. Method     : C:\Chem32\1\Methods\DEF_LC.M
Last changed    : 18/07/2018 11:17:28 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      2.0000
Dilution              :      1.0000
Sample Amount:                :     10.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.687 BB    0.0966   14.79608    2.19736   0.3829
   2   8.394 BB    0.3710  108.04200    4.50100   2.7960
   3  11.783 BB    0.2439 3741.26123  237.34528  96.8210

Totals :                  3864.09931  244.04363
```

## HPLC Purity Analysis of 8b

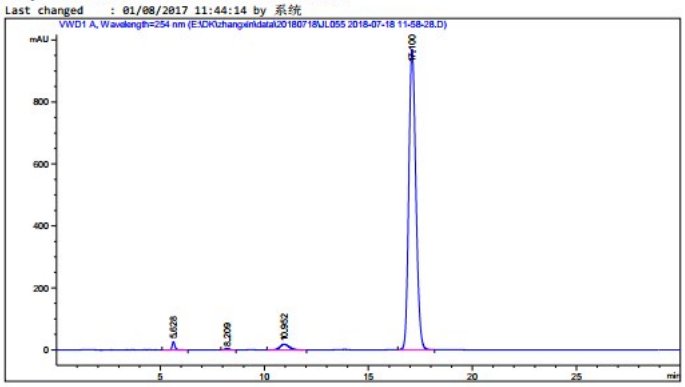
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   22
Injection Date  : 18/07/2018 14:38:35
                                                Inj Volume : 15.000 µl
Method          : E:\DK\TL\方法\90C-10A-30min-1u.M
Last changed    : 18/07/2018 14:37:40 by 系统
                  (modified after loading)
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      2.0000
Dilution              :      1.0000
Sample Amount:        :     10.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.543 VV    0.3394   48.96468    1.81678   0.3412
   2   4.499 VB    0.3099   41.13912    1.72063   0.2866
   3   5.233 BV R  0.1121  146.29930   19.81963   1.0193
   4   7.303 BB    0.1660   34.27641    3.19803   0.2388
   5   9.294 BB    0.4175  253.01122    9.35623   1.7629
   6  13.752 BB    0.2870 1.38286e4   746.84760  96.3512

Totals :                  1.43523e4   782.75889
```

## HPLC Purity Analysis of 8c

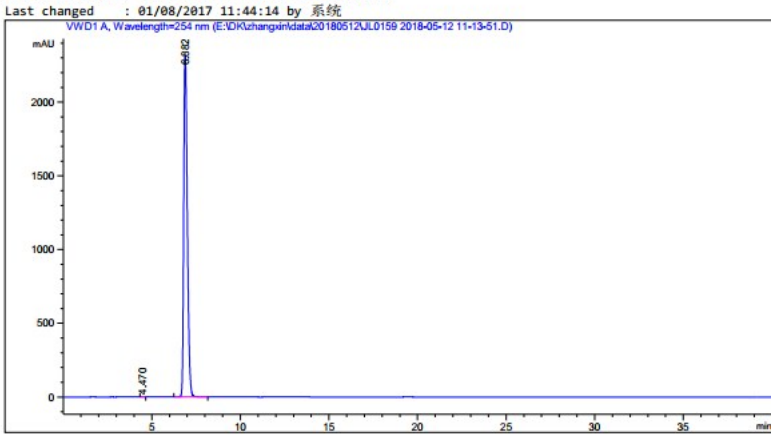
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   21
Injection Date  : 18/07/2018 11:59:15
                                                Inj Volume : 25.000 µl
Acq. Method     : E:\DK\TL\方法\90C-10A-30min-1u.M
Last changed    : 18/07/2018 11:57:57 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      2.0000
Dilution              :      1.0000
Sample Amount:        :     10.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.628 BV R  0.1364  241.19193   26.94648   1.0134
   2   8.209 BB    0.1987   58.34347    4.51754   0.2451
   3  10.952 BB    0.4710  552.49304   18.10527   2.3214
   4  17.100 BB    0.3683 2.29477e4   969.06537  96.4200

Totals :                  2.37997e4  1018.63465
```

## HPLC Purity Analysis of 8d

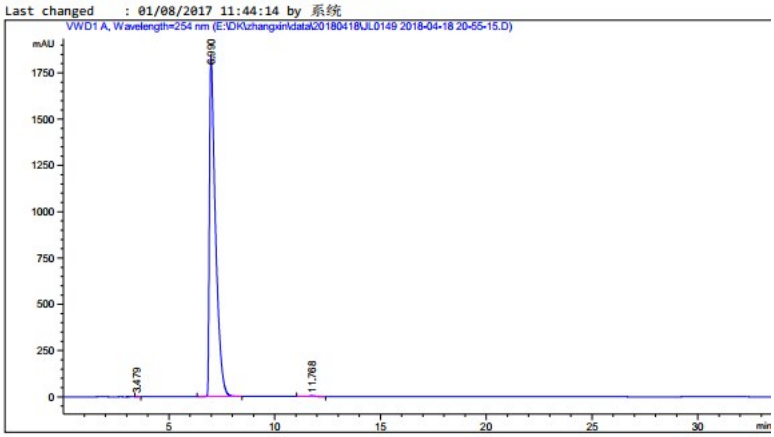
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   12
Injection Date  : 12/05/2018 11:14:35
                                                Inj Volume : 15.000 µl
Acq. Method     : E:\DK\ZHANGXIN\METHOD\90C-10A-40Min-5uL.M
Last changed    : 12/05/2018 10:21:57 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      5.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.470 VB    0.1173   14.28067    1.80530   0.0435
   2   6.882 BB    0.2183 3.28071e4  2315.02637  99.9565

Totals :                  3.28214e4  2316.83167
```

## HPLC Purity Analysis of 8e

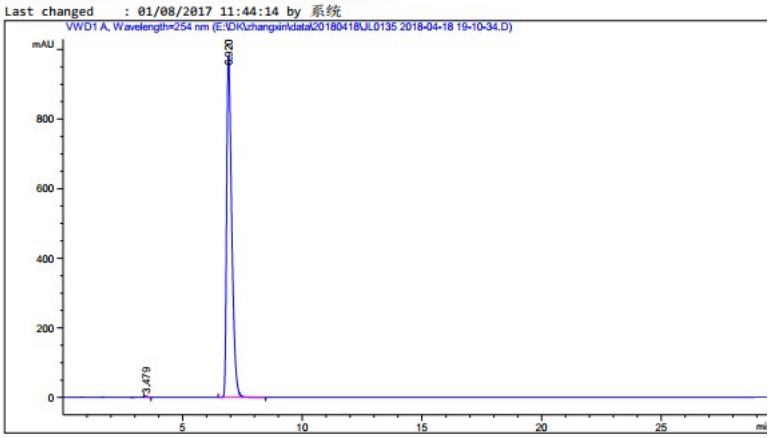
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   13
Injection Date  : 18/04/2018 20:56:03
                                                Inj Volume : 15.000 µl
Acq. Method     : E:\DK\ZHANGXIN\METHOD\90C-10A-40Min-5uL.M
Last changed    : 18/04/2018 18:34:59 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      5.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.479 VB    0.0988   16.01152    2.40145   0.0434
   2   6.990 BB    0.2870 3.68041e4  1842.73682  99.7766
   3  11.768 VB R  0.2694   66.40449    3.70020   0.1800

Totals :                  3.68865e4  1848.83847
```

## HPLC Purity Analysis of 8f

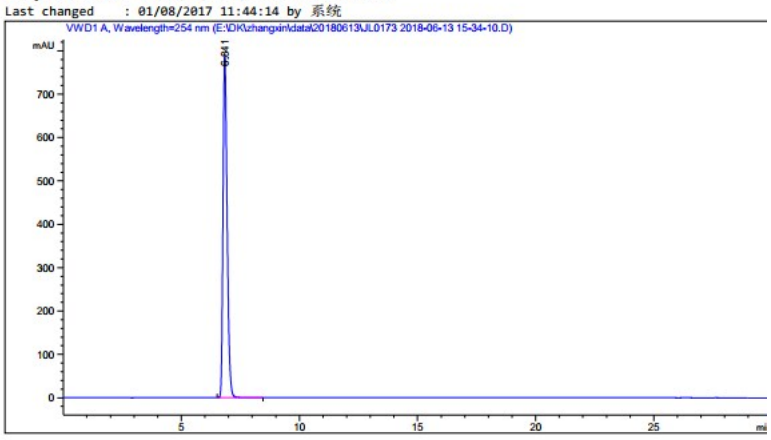
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   3
Injection Date  : 18/04/2018 19:11:21
                                                Inj Volume : 15.000 µl
Acq. Method     : E:\DK\ZHANGXIN\METHOD\90C-10A-40Min-5uL.M
Last changed    : 18/04/2018 18:34:59 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      5.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.479 VB    0.0964   26.38414    4.13907   0.1804
   2   6.920 BB    0.2256 1.45986e4   980.88928  99.8196

Totals :                  1.46250e4   985.02835
```

## HPLC Purity Analysis of 8g

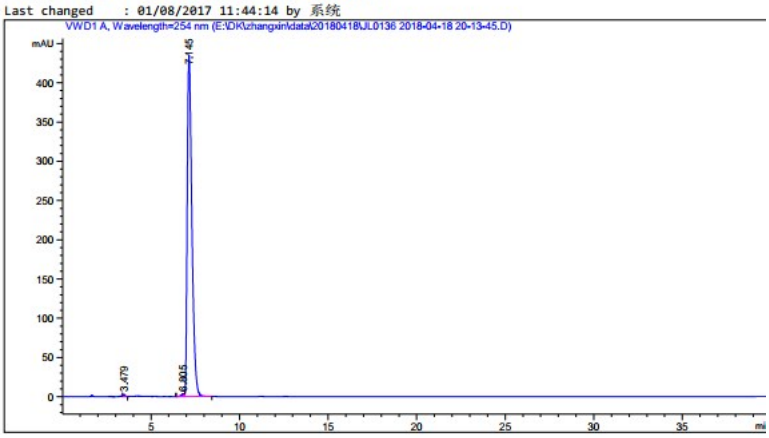
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   3
Injection Date  : 13/06/2018 15:34:55
                                                Inj Volume : 5.000 µl
Acq. Method     : E:\DK\TL\方法\90C-10A-30min-1u.M
Last changed    : 04/07/2017 19:18:29 by 系统
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      2.0000
Dilution              :      1.0000
Sample Amount:        :      5.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.841 BB    0.1886 9619.76270  786.90442 100.0000

Totals :                  9619.76270  786.90442
```

## HPLC Purity Analysis of 8h

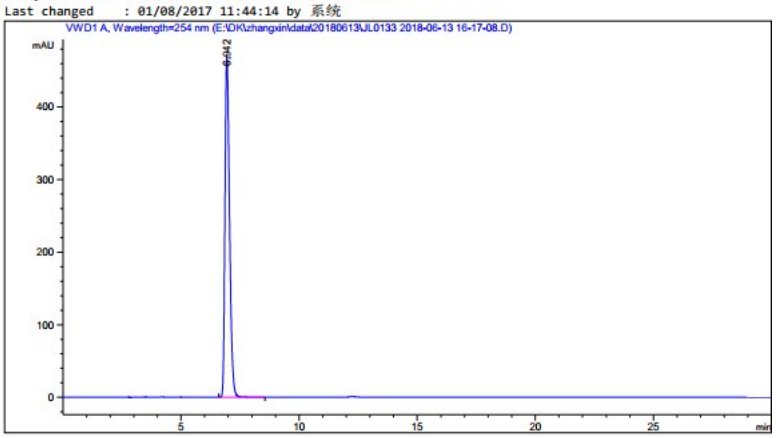
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   12
Injection Date  : 18/04/2018 20:14:30
                                                Inj Volume : 15.000 µl
Acq. Method     : E:\DK\ZHANGXIN\METHOD\90C-10A-40Min-5uL.M
Last changed    : 18/04/2018 18:34:59 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      5.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.479 VB    0.0948   18.06529    2.85508   0.2257
   2   6.805 BV E  0.1571   32.98967    3.10186   0.4122
   3   7.145 VB R  0.2814 7952.07910  434.64624  99.3621

Totals :                  8003.13406  440.60318
```

## HPLC Purity Analysis of 8i

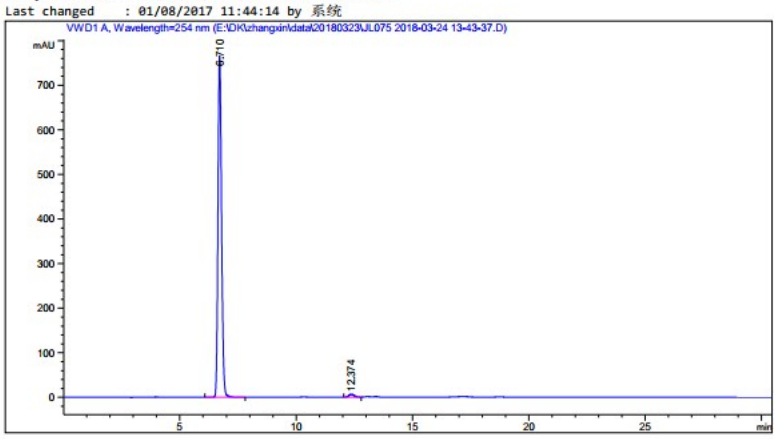
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   2
Injection Date  : 13/06/2018 16:17:49
                                                Inj Volume : 5.000 µl
Acq. Method     : E:\DK\TL\方法\90C-10A-30min-1u.M
Last changed    : 04/07/2017 19:18:29 by 系统
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      2.0000
Dilution              :      1.0000
Sample Amount:                :      5.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.942 BB    0.2081 6328.11084  469.86807 100.0000

Totals :                  6328.11084  469.86807
```

## HPLC Purity Analysis of 8j

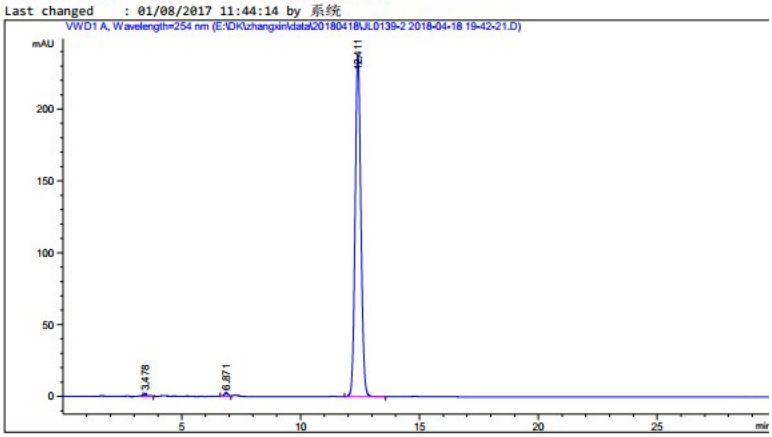
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   1
Injection Date  : 24/03/2018 13:44:21
                                                Inj Volume : 5.000 µl
Acq. Method     : E:\DK\DYX\90C-10A-40MIN-5uL.M
Last changed    : 27/01/2018 20:34:38 by 系统
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:        :     20.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.710 VB R  0.1657 8176.02734  764.93518  98.7660
   2  12.374 BB    0.2478  102.15154    6.41491   1.2340

Totals :                  8278.17889  771.35009
```

## HPLC Purity Analysis of 8k

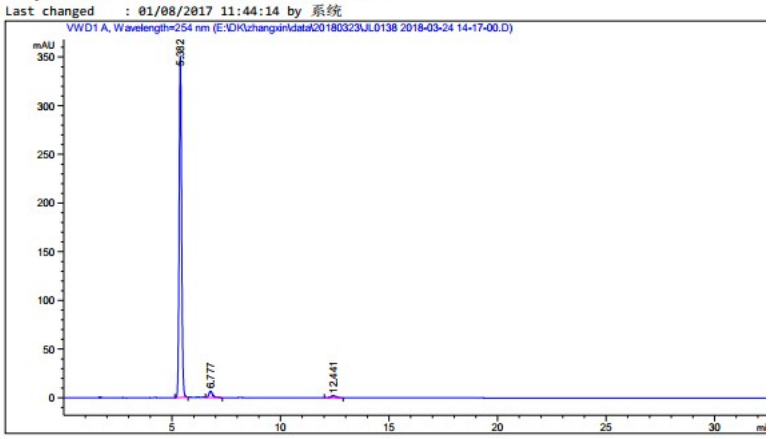
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   11
Injection Date  : 18/04/2018 19:43:07
                                                Inj Volume : 15.000 µl
Acq. Method     : E:\DK\ZHANGXIN\METHOD\90C-10A-40Min-SuL.M
Last changed    : 18/04/2018 18:34:59 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      5.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.478 VV R  0.1091   16.61591    2.20112   0.4084
   2   6.871 BV    0.1691   27.18153    2.49476   0.6680
   3  12.411 BB    0.2627 4025.15674  237.49684  98.9236

Totals :                  4068.95418  242.19272
```

## HPLC Purity Analysis of 8l

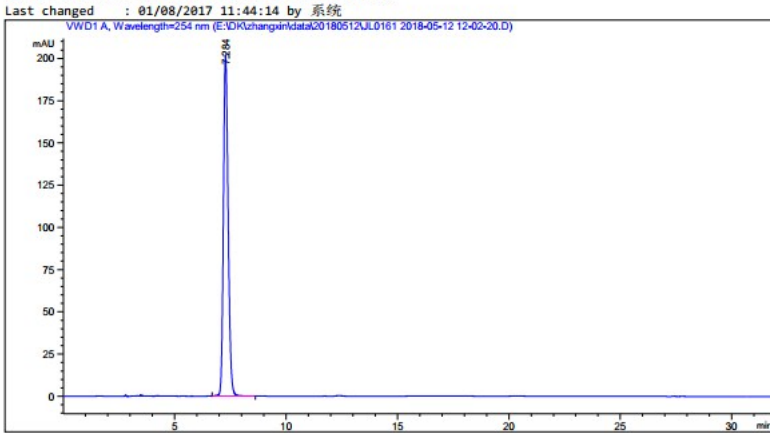
```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   2
Injection Date  : 24/03/2018 14:17:39
                                                Inj Volume : 5.000 µl
Acq. Method     : E:\DK\DYX\90C-10A-40MIN-5uL.M
Last changed    : 27/01/2018 20:34:38 by 系统
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:        :     20.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.382 BB    0.1232 2768.77344  350.45569  96.1217
   2   6.777 BV R  0.1846   80.14971    6.55704   2.7825
   3  12.441 BB    0.2520   31.56367    1.92786   1.0958

Totals :                  2880.48682  358.94059
```

## HPLC Purity Analysis of 8m

```
Acq. Operator   : 系统
Sample Operator : 系统
Acq. Instrument : 1260LC                        Location :   21
Injection Date  : 12/05/2018 12:03:09
                                                Inj Volume : 5.000 µl
Acq. Method     : E:\DK\ZHANGXIN\METHOD\90C-10A-40Min-5uL.M
Last changed    : 12/05/2018 12:02:15 by 系统
                  (modified after loading)
Analysis Method : E:\DK\TL\方法\80C-20D-20MIN-20UL.M
Last changed    : 01/08/2017 11:44:14 by 系统
```




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      5.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.284 BB    0.2277 2985.99316  201.73741 100.0000

Totals :                  2985.99316  201.73741
```