Supporting Information

Efficient synthesis of 1-Azakenpaullone, a selective inhibitor of glycogen synthase kinase-3β for cellular regeneration

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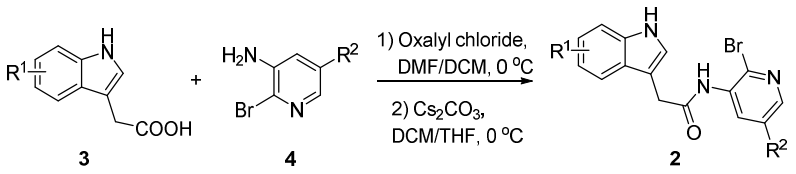
General Information

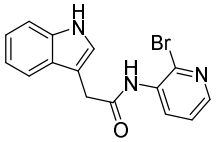
All commercially available reagents and solvents (ACS grade) were purchased from commercial sources and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck silica gel 60 F-254 thin layer plates using UV light for visualization and an ethanolic solution of phosphomolybdic acid under heat or powdered iodine for developing. Flash column chromatography was generally performed on silica gel (200-300 mesh). LC-MS analyses were performed on the Agilent 1200 HPLC/MCD electrospray mass spectrometer in positive/negative ion mode. The scan range was 100−1000d. The yields refer to chromatographically homogeneous materials. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-500 or AV-400 spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet. HRMS (High-resolution mass spectra) were conducted by a an Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer under the condition of electrospray ionization (ESI). Compound purity was determined by reverse-phase high-performance liquid chromatography (HPLC) with 5% solvent A (H_2O) and 95% solvent B (MeOH) as eluents. The purity of all the final compounds was determined by HPLC to be >95%.

Experimental Procedures and Characterization Data

General Procedure for the synthesis of 2a-2k:



***N*-(2-bromopyridin-3-yl)-2-(1*H*-indol-3-yl)acetamide (2b)**

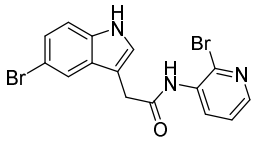


2-(1H-indol-3-yl)acetic acid (176 mg, 1.005 mmol) was dissolved in DCM (10 ml) containing a catalytic amount of DMF. Then andoxalyl chloride (255 mg, 2.010 mmol) was added slowly into the mixture at 0 ℃. The resulting mixture was stirred at room temperature for 30 minutes, and evaporated to obtain the intermediate 2-(1H-indol-3-yl)acetyl chloride.

Bromopyridin-3-amine (348 mg, 2.010 mmol) was dissolved in THF (15 ml) and added CS_2CO_3 (655 mg, 2.010 mmol). A DCM solution of 2-(1H-indol-3-yl)acetyl chloride (15 ml) was added slowly onto the mixture at 0 ℃. The resulting mixture was stirred at 0 ℃ for 4 hours. The resulting mixture was then quenched by water and extracted by DCM for three times. The organic layer was collected and washed with saturated $NaHCO_3$ solution and saturated NaCl solution for once. Then the organic layer was concentrated under vacuum and added a small amount of DCM

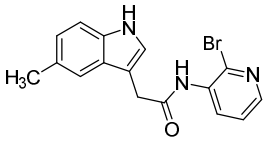
for recrystallization. The precipitated solid was collected by filtration and dried to afford the product **2b** (277 mg, 84% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (d, J = 8.1 Hz, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 7.96 (d, J = 4.1 Hz, 1H, Ar-H), 7.58 (d, J = 7.9 Hz, 1H, Ar-H), 7.39 (d, J = 8.1 Hz, 1H, Ar-H), 7.24 (d, J = 4.9 Hz, 1H, Ar-H), 7.22 (d, J = 6.7 Hz, 1H, Ar-H), 7.20–7.16 (m, 1H, Ar-H), 7.14 (t, J = 7.5 Hz, 1H, Ar-H), 3.92 (s, 2H, CH_2); ESI-MS *m/z* 330.1 $[M + H]^+$.

2-(5-Bromo-1*H*-indol-3-yl)-*N*-(2-bromopyridin-3-yl)acetamide (2a)



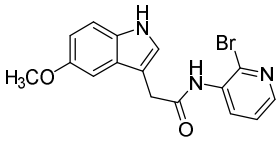
Compound **2a** (79% yield) was prepared according to the procedure of **2b** from 2-(5-bromo-1H-indol-3-yl)acetic acid . ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.20 (s, 1H, NH), 9.63 (s, 1H, NH), 8.18 (dd, J = 4.5, 1.6 Hz, 1H, Ar-H), 8.08 (dd, J = 8.0, 1.5 Hz, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.44 (dt, J = 17.9, 9.0 Hz, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.35 (d, J = 8.6 Hz, 1H, Ar-H), 7.20 (dd, J = 8.6, 1.8 Hz, 1H, Ar-H), 3.85 (s, 2H, CH_2); ESI-MS *m/z* 407.9 $[M + H]^+$.

***N*-(2-bromopyridin-3-yl)-2-(5-methyl-1*H*-indol-3-yl)acetamide (2c)**



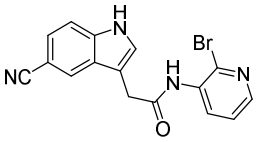
Compound **2c** (81% yield) was prepared according to the procedure of **2b** from 2-(5-methyl-1H-indol-3-yl)acetic acid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (d, J = 8.1 Hz, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 8.01 (d, J = 4.2 Hz, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.33 (d, J = 8.6 Hz, 1H, Ar-H), 7.09 (d, J = 8.4 Hz, 1H, Ar-H), 3.93 (s, 2H, CH_2), 2.45 (s, 3H, CH_3); ESI-MS *m/z* 334.1 $[M + H]^+$.

***N*-(2-bromopyridin-3-yl)-2-(5-methoxy-1*H*-indol-3-yl)acetamide (2d)**



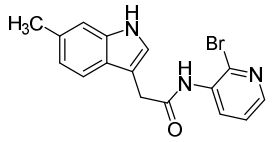
Compound **2d** (80% yield) was prepared according to the procedure of **2b** from 2-(5-methoxy-1H-indol-3-yl)acetic acid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (d, J = 8.1 Hz, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 8.01 (d, J = 4.4 Hz, 1H, Ar-H), 7.33 (d, J = 8.9 Hz, 1H, Ar-H), 7.27 (d, J = 6.7 Hz, 1H, Ar-H), 7.23 (dd, J = 7.8, 4.7 Hz, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 6.93 (d, J = 8.8 Hz, 1H, Ar-H), 3.92 (d, J = 7.0 Hz, 2H, CH_2), 3.84 (s, 3H, OCH_3); ESI-MS *m/z* 360.0 $[M + H]^+$.

***N*-(2-bromopyridin-3-yl)-2-(5-cyano-1*H*-indol-3-yl)acetamide (2e)**



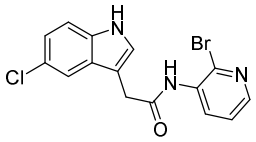
Compound **2e** (72% yield) was prepared according to the procedure of **2b** from 2-(5-cyano-1H-indol-3-yl)acetic acid**.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.56 (s, 1H, NH), 9.71 (s, 1H, NH), 8.22 (s, 1H, Ar-H), 8.18 (d, J = 4.4 Hz, 1H, Ar-H), 8.06 (d, J = 8.0 Hz, 1H, Ar-H), 7.54 (d, J = 9.0 Hz, 2H, 2×Ar-H), 7.44 (d, J = 8.2 Hz, 2H, 2×Ar-H), 3.90 (s, 2H, CH_2); ESI-MS *m/z* 355.2 $[M + H]^+$.

***N*-(2-bromopyridin-3-yl)-2-(6-methyl-1*H*-indol-3-yl)acetamide (2f)**



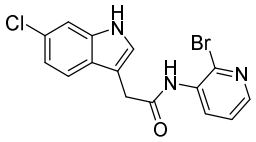
Compound **2f** (83% yield) was prepared according to the procedure of **2b** from 2-(6-methyl-1H-indol-3-yl)acetic acid**.** ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (d, J = 8.2 Hz, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 8.04 (d, J = 4.4 Hz, 1H, Ar-H), 7.52 (d, J = 8.1 Hz, 1H, Ar-H), 7.29 (d, J = 8.5 Hz, 1H, Ar-H), 7.05 (d, J = 8.0 Hz, 1H, Ar-H), 3.97 (s, 2H, CH_2), 2.51 (s, 3H, CH_3); ESI-MS *m/z* 344.1 $[M + H]^+$.

***N*-(2-bromopyridin-3-yl)-2-(5-chloro-1*H*-indol-3-yl)acetamide (2g)**



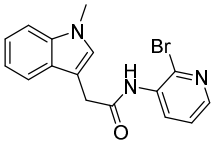
Compound **2g** (81% yield) was prepared according to the procedure of **2b** from 2-(5-chloro-1H-indol-3-yl)acetic acid**.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (s, 1H, NH), 9.59 (s, 1H, NH), 8.17 (dd, J = 4.6, 1.8 Hz, 1H, Ar-H), 8.08 (dd, J = 7.9, 1.8 Hz, 1H, Ar-H), 7.71 (d, J = 2.1 Hz, 1H, Ar-H), 7.43 (dd, J = 8.0, 4.6 Hz, 1H, Ar-H), 7.40 (d, J = 2.4 Hz, 1H, Ar-H), 7.38 (d, J = 8.6 Hz, 1H, Ar-H), 7.08 (dd, J = 8.6, 2.1 Hz, 1H, Ar-H), 3.84 (s, 2H, CH_2); ESI-MS *m/z* 364.1 $[M + H]^+$.

***N*-(2-bromopyridin-3-yl)-2-(6-chloro-1*H*-indol-3-yl)acetamide (2h)**



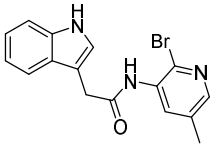
Compound **2h** (78% yield) was prepared according to the procedure of **2b** from 2-(6-chloro-1H-indol-3-yl)acetic acid**.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1H, NH), 9.54 (s, 1H, NH), 8.16 (dd, J = 4.6, 1.8 Hz, 1H, Ar-H), 8.08 (dd, J = 8.1, 1.8 Hz, 1H, Ar-H), 7.63 (d, J = 8.4 Hz, 1H, Ar-H), 7.45 – 7.42 (m, 1H, Ar-H), 7.41 (d, J = 1.8 Hz, 1H, Ar-H), 7.38 (d, J = 2.3 Hz, 1H, Ar-H), 7.02 (dd, J = 8.5, 1.9 Hz, 1H, Ar-H), 3.85 (s, 2H, CH_2); ESI-MS *m/z* 364.1 $[M + H]^+$.

***N*-(2-bromopyridin-3-yl)-2-(1-methyl-1*H*-indol-3-yl)acetamide (2i)**



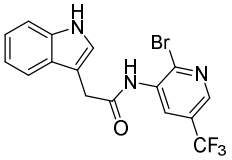
Compound **2i** (76% yield) was prepared according to the procedure of **2b** from 2-(1-methyl-1H-indol-3-yl)acetic acid **.** ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (d, J = 8.1 Hz, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 8.02 (d, J = 4.5 Hz, 1H, Ar-H), 7.61 (d, J = 7.9 Hz, 1H, Ar-H), 7.38 (d, J = 8.2 Hz, 1H, Ar-H), 7.30 (t, J = 7.6 Hz, 1H, Ar-H), 7.23–7.19 (m, 1H, Ar-H), 7.17 (d, J = 7.4 Hz, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 3.94 (s, 2H, CH_2), 3.84 (s, 3H, CH_3); ESI-MS *m/z* 344.0 $[M + H]^+$.

***N*-(2-bromo-5-methylpyridin-3-yl)-2-(1*H*-indol-3-yl)acetamide (2j)**



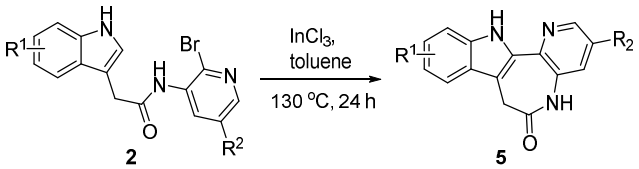
Compound **2j** (75% yield) was prepared according to the procedure of **2b** from 2-bromo-5-methylpyridin-3-amine . ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.98 (s, 1H, NH), 9.53 (s, 1H, NH), 8.06 (d, J = 15.2 Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 7.63 (d, J = 7.9 Hz, 1H, Ar-H), 7.37 (t, J = 10.1 Hz, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 7.10 (t, J = 7.5 Hz, 1H, Ar-H), 7.01 (t, J = 7.4 Hz, 1H, Ar-H), 3.87 (s, 2H, CH_2), 2.29 (d, J = 14.6 Hz, 3H, CH_3); ESI-MS *m/z* 344.0 $[M + H]^+$.

***N*-(2-bromo-5-(trifluoromethyl)pyridin-3-yl)-2-(1*H*-indol-3-yl)acetamide (2k)**



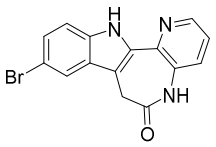
Compound **2k** (73% yield) was prepared according to the procedure of **2b** from 2-bromo-5-(trifluoromethyl)pyridin-3-amine .^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (s, 1H, NH), 8.34 (s, 1H, NH), 8.28 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 7.59 (t, J = 14.2 Hz, 1H, Ar-H), 7.46 (d, J = 8.2 Hz, 1H, Ar-H), 7.32–7.27 (m, 2H, 2×Ar-H), 7.23–7.14 (m, 1H, Ar-H), 4.00 (s, 2H, CH_2); ESI-MS *m/z* 398.2 $[M + H]^+$.

General Procedure for the synthesis of 5a-5k:



2 **5**

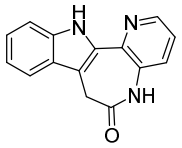
9-Bromo-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5a, 1-Akp)



2a (205 mg, 0.501 mmol) and $InCl_3$ (111 mg, 0.501 mmol) were placed in the sealed tube and dissolved in dry toluene (10 ml). The reaction mixture was heated at 130 ℃ for 24 hours. After cooling to room temperature, the suspension was quenched with saturated $NaHCO_3$ aqueous solution and extracted with EA. The organic layer was collected and concentrated under vacuum. Purification by column chromatography on silica gel (eluting with DCM-MeOH, 100:1), afforded

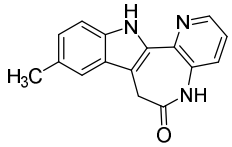
the white solid product **5a(1-Akp)** (115 mg, 70% yield) . The characterization data are consistent with the reported in literature[1]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.95 (s, 1H, NH), 10.30 (d, J = 26.6 Hz, 1H, NH), 8.56–8.41 (m, 1H, Ar-H), 7.98 (d, J = 1.5 Hz, 1H, Ar-H), 7.70–7.54 (m, 1H, Ar-H), 7.47–7.39 (m, 2H, Ar-H), 7.31 (dd, J = 8.6, 1.8 Hz, 1H, Ar-H), 3.68 (s, 2H, CH_2); ESI-MS *m/z* 328.1 $[M + H]^+$; 96.5% purity.

7,12-Dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5b)



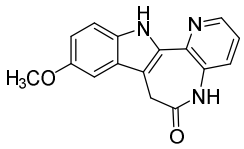
Compound **5b** (65% yield) was prepared according to the procedure of **5a** from **2b**. The characterization data are consistent with the reported in literature[1]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.72 (s, 1H, NH), 10.24 (s, 1H, NH), 8.48 (d, J = 4.2 Hz, 1H, Ar-H), 7.70 (d, J = 7.9 Hz, 1H, Ar-H), 7.61 (d, J = 8.1 Hz, 1H, Ar-H), 7.47 (d, J = 8.2 Hz, 1H, Ar-H), 7.44–7.36 (m, 1H, Ar-H), 7.19 (t, J = 7.5 Hz, 1H, Ar-H), 7.07 (t, J = 7.5 Hz, 1H, Ar-H), 3.66 (s, 2H, CH_2); ESI-MS *m/z* 250.1 $[M + H]^+$; 95.7% purity.

9-Methyl-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5c)



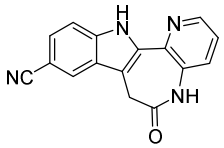
Compound **5c** (67% yield) was prepared according to the procedure of **5a** from **2c**. The characterization data are consistent with the reported in literature[1]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.58 (s, 1H, NH), 10.21 (s, 1H, NH), 8.46 (s, 1H, Ar-H), 7.60 (d, J = 8.2 Hz, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.35 (d, J = 8.8 Hz, 1H, Ar-H), 7.02 (d, J = 8.0 Hz, 1H, Ar-H), 3.61 (s, 2H, CH_2), 2.41 (s, 3H, CH_3); ESI-MS *m/z* 264.1 $[M + H]^+$; 99.1% purity.

9-Methoxy-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5d)



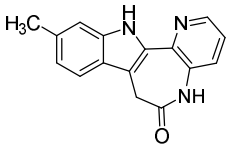
Compound **5d** (66% yield) was prepared according to the procedure of **5a** from **2d**. The characterization data are consistent with the reported in literature[1]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.54 (s, 1H, NH), 10.20 (s, 1H, NH), 8.46 (d, J = 4.3 Hz, 1H, Ar-H), 7.60 (d, J = 8.1 Hz, 1H, Ar-H), 7.42–7.37 (m, 1H, Ar-H), 7.35 (d, J = 9.1 Hz, 1H, Ar-H), 7.21 (s, 1H, Ar-H), 6.83 (d, J = 8.6 Hz, 1H, Ar-H), 3.80 (s, 3H, OCH_3), 3.64 (s, 2H, CH_2); ESI-MS *m/z* 279.2 $[M + H]^+$; 99.8% purity.

6-Oxo-5,6,7,12-tetrahydropyrido[3',2':2,3]azepino[4,5-b]indole-9-carbonitrile (5e)



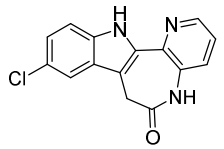
Compound **5e** (53% yield) was prepared according to the procedure of **5a** from **2e**. The characterization data are consistent with the reported in literature[1]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.33 (s, 1H, NH), 10.33 (s, 1H, NH), 8.51 (s, 1H, Ar-H), 8.39 (s, 1H, Ar-H), 7.65 (d, J = 8.3 Hz, 1H, Ar-H), 7.60 (d, J = 8.6 Hz, 1H, Ar-H), 7.53 (d, J = 8.5 Hz, 1H, Ar-H), 7.46 (d, J = 4.6 Hz, 1H, Ar-H), 3.76 (s, 2H, CH_2); ESI-MS *m/z* 274.8 $[M + H]^+$; 97.4% purity.

10-Methyl-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5f)



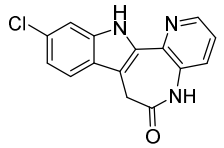
Compound **5f** (64% yield) was prepared according to the procedure of **5a** from **2f**.^{1}H NMR (500 MHz, DMSO-d_6) δ 11.55 (s, 1H, NH), 10.20 (s, 1H, NH), 8.46 (s, 1H, Ar-H), 7.67–7.50 (m, 2H, 2×Ar-H), 7.38 (s, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 6.91 (d, J = 8.1 Hz, 1H, Ar-H), 3.62 (s, 2H, CH_2), 2.42 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.53, 144.72, 141.14, 138.47, 132.68, 132.39, 132.29, 129.74, 124.88, 122.70, 121.48, 118.61, 112.13, 109.40, 32.46, 22.04; HRMS calcd for $C_{16}H_{13}N_3O^+$ $[M + H]^+$ 264.1131, found 264.1130; 99.8% purity.

9-Chloro-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5g)



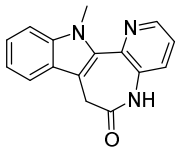
Compound **5g** (62% yield) was prepared according to the procedure of **5a** from **2g**. The characterization data are consistent with the reported in literature[1]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.91 (s, 1H, NH), 10.25 (s, 1H, NH), 8.49 (d, J = 4.1 Hz, 1H, Ar-H), 7.81 (d, J = 12.5 Hz, 1H, Ar-H), 7.61 (t, J = 10.0 Hz, 1H, Ar-H), 7.49–7.39 (m, 2H, 2×Ar-H), 7.18 (t, J = 9.9 Hz, 1H, Ar-H), 3.65 (d, J = 18.4 Hz, 2H, CH_2); ESI-MS *m/z* 284.1 $[M + H]^+$; 99.7% purity.

10-Chloro-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5h)



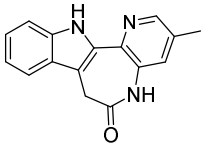
Compound **5h** (64% yield) was prepared according to the procedure of **5a** from **2h**.^{1}H NMR (500 MHz, DMSO-d_6) δ 11.86 (s, 1H, NH), 10.27 (s, 1H, NH), 8.48 (d, J = 4.4 Hz, 1H, Ar-H), 7.75 (d, J = 8.7 Hz, 1H, Ar-H), 7.62 (d, J = 8.2 Hz, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 7.42 (dd, J = 7.8, 4.6 Hz, 1H, Ar-H), 7.09 (d, J = 8.5 Hz, 1H, Ar-H), 3.67 (s, 2H, CH_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.37, 144.85, 140.45, 138.25, 134.01, 132.72, 129.89, 127.92, 125.67, 123.31, 120.51, 120.03, 111.87, 109.44, 32.28; HRMS calcd for $C_{15}H_{10}ClN_3O^+$ $[M + H]^+$ 283.0585, found 283.0584; 95.9% purity.

12-Methyl-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5i)



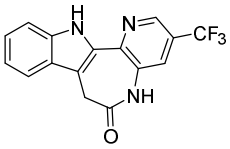
Compound **5i** (60% yield) was prepared according to the procedure of **5a** from **2i**. The characterization data are consistent with the reported in literature[1]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (d, J = 4.5 Hz, 1H, Ar-H), 8.09 (s, 1H, NH), 7.73 (d, J = 7.9 Hz, 1H, Ar-H), 7.50 (d, J = 8.2 Hz, 1H, Ar-H), 7.44 (d, J = 8.3 Hz, 1H, Ar-H), 7.35 (t, J = 7.6 Hz, 1H, Ar-H), 7.31 (dd, J = 8.1, 4.6 Hz, 1H, Ar-H), 7.20 (t, J = 7.4 Hz, 1H, Ar-H), 4.08 (s, 3H, CH_3), 3.64 (s, 2H, CH_2); ESI-MS *m/z* 264.0 $[M + H]^+$; 99.8% purity.

3-Methyl-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5j)



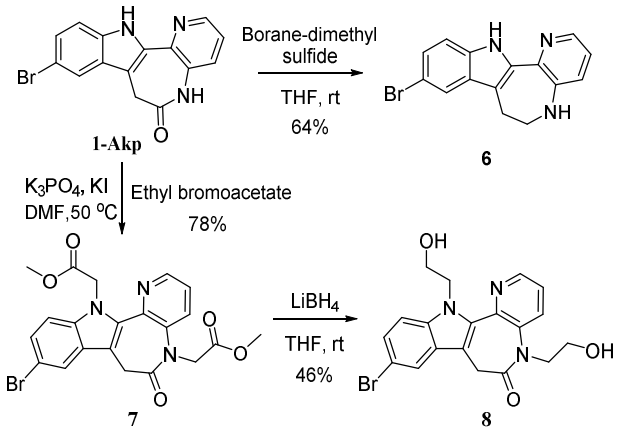
Compound **5j** (57% yield) was prepared according to the procedure of **5a** from **2j**.^{1}H NMR (500 MHz, DMSO-d_6) δ 11.67 (s, 1H, NH), 10.18 (s, 1H, NH), 8.35 (s, 1H, Ar-H), 7.69 (d, J = 7.9 Hz, 1H, Ar-H), 7.47 (d, J = 8.1 Hz, 1H, Ar-H), 7.43 (s, 1H, Ar-H), 7.19 (t, J = 7.5 Hz, 1H, Ar-H), 7.08 (t, J = 7.4 Hz, 1H, Ar-H), 3.64 (s, 2H, CH_2), 2.38 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.47, 145.38, 138.57, 137.86, 133.15, 132.56, 132.19, 129.80, 126.84, 123.12, 119.53, 118.71, 112.35, 108.61, 32.35, 18.04; HRMS calcd for $C_{16}H_{13}N_3O^+$ $[M + H]^+$ 264.1131, found 264.1129; 95.7% purity.

3-(Trifluoromethyl)-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (5k)



Compound **5k** (54% yield) was prepared according to the procedure of **5a** from **2k**.^{1}H NMR (500 MHz, DMSO-d_6) δ 11.91 (s, 1H, NH), 10.47 (s, 1H, NH), 8.83 (s, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 7.77 (d, J = 8.0 Hz, 1H, Ar-H), 7.51 (d, J = 8.2 Hz, 1H, Ar-H), 7.26 (t, J = 7.5 Hz, 1H, Ar-H), 7.12 (t, J = 7.5 Hz, 1H, Ar-H), 3.78 (s, 2H, CH_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.61, 144.25, 140.66, 140.63, 138.49, 132.13, 131.74, 127.32, 126.72, 126.50, 125.15, 124.33, 123.68, 123.43, 122.99, 119.95, 119.36, 112.67, 111.70, 32.47; HRMS calcd for $C_{16}H_{10}F_3N_3O^+$ $[M + H]^+$ 318.0848, found 318.0851; 97.4% purity.

General Procedure for the synthesis of 6-8:



9-Bromo-5,6,7,12-tetrahydropyrido[3',2':2,3]azepino[4,5-b]indole (6)

1-Akp (30 mg, 0.091 mmol) was dissolved in THF (dry, 1 ml), and borane dimethyl sulfide (21 mg, 0.274 mmol) was added slowly onto the mixture at 0 ℃. The reaction mixture was stirred at room temperature overnight. The reaction mixture was quenched with methanol and added 0.5 ml of 6N HCl solution, then stirred at room temperature for 3 hours. Adjusted the pH of the reaction mixture to alkaline with the saturated $NaHCO_3$ solution and extracted with EA. The organic layer was collected and concentrated under vacuum. Purification by column chromatography on silica gel (eluting with PE/EA = 3/1), afforded the white solid product **6** (17.9 mg, 64% yield).^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (s, 1H, NH), 8.04 (d, J = 4.0 Hz, 1H, NH), 7.62 (s, 1H, Ar-H), 7.39 (d, J = 8.5 Hz, 1H, Ar-H), 7.19 (t, J = 9.0 Hz, 2H, 2×Ar-H), 7.07 (dd, J = 7.8, 4.4 Hz, 1H, Ar-H), 6.48 (s, 1H, Ar-H), 3.35 (s, 2H, CH_2), 3.05 (s, 2H, CH_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 145.08, 138.95, 135.63, 135.34, 134.82, 130.97, 125.04, 124.81, 122.69, 121.14, 113.96, 113.61, 111.36, 43.46, 28.71; HRMS calcd for $C_{15}H_{12}BrN_3^+$ $[M + H]^+$ 314.0287, found 314.0284.

Dimethyl
2,2'-(9-bromo-6-oxo-6,7-dihydropyrido[3',2':2,3]azepino[4,5-b]indole-5,12-diyl)diacetate (7)

1-Akp (60 mg, 0.183 mmol), methyl 2-bromoacetate (84 mg, 0.549 mmol), K_3PO_4 (117 mg, 0.549 mmol), KI (91 mg, 0.549 mmol) were dissolved in DMF (1 ml). The reaction mixture was heated at 50 ℃ overnight. After cooling to room temperature, extracted with EA and washed by water. The organic layer was collected and concentrated under vacuum. Purification by column chromatography on silica gel (eluting with DCM/MeOH = 100/1), afforded the product **7** (67.4 mg, 78% yield).^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, J = 3.5 Hz, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.79 (d, J = 8.4 Hz, 1H, Ar-H), 7.42 (d, J = 8.5 Hz, 1H, Ar-H), 7.34–7.30 (m, 1H, Ar-H), 7.20 (d, J = 8.7 Hz, 1H, Ar-H), 5.45 (s, 1H, CH_2-1), 5.23 (d, J = 14.1 Hz, 1H, CH_2-1), 4.61 (s, 1H, CH_2-1), 4.03 (s, 2H,CH_2), 3.79 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.68 (d, J = 9.2 Hz, 1H, CH_2-1); HRMS calcd for $C_{21}H_{18}BrN_3O_5^+$ $[M + H]^+$ 472.0502, found 472.0498.

9-Bromo-5,12-bis(2-hydroxyethyl)-7,12-dihydropyrido[3',2':2,3]azepino[4,5-b]indol-6(5*H*)-one (8)

7 (63.7 mg, 0.135 mmol) was dissolved in THF (1 ml), and $LiBH_4$ (9 mg, 0.405 mmol) was added slowly onto the mixture at 0 ℃. The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was quenched by water and extracted by EA. The organic layer was collected and concentrated under vacuum. Purification by column chromatography on silica gel (eluting with DCM/MeOH = 100/1 to 80/1), afforded the white solid product **8** (25.7 mg, 46%

yield).^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (d, J = 4.4 Hz, 1H, Ar-H), 8.09 (d, J = 8.3 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.41 (d, J = 2.5 Hz, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.29 (d, J = 9.2 Hz, 1H, Ar-H), 4.96 (dd, J = 32.1, 13.6 Hz, 1H, CH_2-1), 4.76–4.67 (m, 1H, CH_2-1), 4.63 (d, J = 15.5 Hz, 1H, CH_2-1), 4.13 (t, J = 10.4 Hz, 1H, CH_2-1), 4.06 (dd, J = 12.6, 8.4 Hz, 2H, CH_2), 3.97 (d, J = 14.0 Hz, 1H, CH_2-1), 3.86–3.79 (m, 1H, CH_2-1), 3.75 (d, J = 3.4 Hz, 2H, CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.45, 145.31, 143.62, 138.10, 137.28, 133.99, 133.05, 127.02, 126.92, 122.56, 121.93, 114.18, 113.60, 111.68, 61.66, 60.91, 53.26, 46.84, 29.70; HRMS calcd for $C_{19}H_{18}BrN_3O_3^+$ $[M + H]^+$ 416.0604, found 416.0600.

Chemically Induced Totipotent Stem Cells (ciTotiSCs) Induced From Mouse Embryonic Stem Cells (mESCs)[2]

ciTotiSCs were induced from mESCs on inactivated MEF feeder layers using KSR basal medium composed of KnockOut DMEM, 5% KSR, CDL (CD lipid concentrate, 500×), 1% N2, 1× l-glutaMAX, 1× penicillin-streptomycin, 1× non-essential amino acids, 1× sodium pyruvate, 55 μM 2-mercaptoethanol and 1,000 U ml−1 mLIF, 50 ng ml−1 sodium l-ascorbyl-2-phosphate, 2.5 μM 1-azakenpaullone, 0.5 μM WS6 and 0.2 μM TTNPB. The medium was changed every day until day 2. All cells were cultured at 37℃, 5% CO_2 and 21% O_2.

mESCs were maintained on inactivated MEF feeder layers using Serum/LIF/2i medium composed of KnockOut DMEM supplemented with 15% FBS, 1× l-glutaMAX, 1× penicillin-streptomycin, 1× non-essential amino acids (NEAA), 1× sodium pyruvate, 55 μM 2-mercaptoethanol, 1,000 U ml−1 mouse leukaemia inhibitory factor (mLIF), 3 μM CHIR-99021 and 1 μM PD0325901.

Biological activity of 1-Akp and analogues in inducing ciTotiSCs from mESCs

For evaluating the biological activity of 1-Akp and analogues in inducing ciTotiSCs from mESCs, the ciTotiSCs induction procedure using either 1-Akp or equivalent analogue was conducted. The resultant ciTotiSCs were collected and resuspended in 1× DPBS, of which the MERVL-Tdtomato signal were analyzed using FACSAria-II flow cytometer (BD Biosciences). 30,000 cells were analyzed per sample. Three replicate experiments were performed for each compound. Data analysis was performed using FlowJo vX.0.7 and GraphPad Prism 9. The data are shown below.

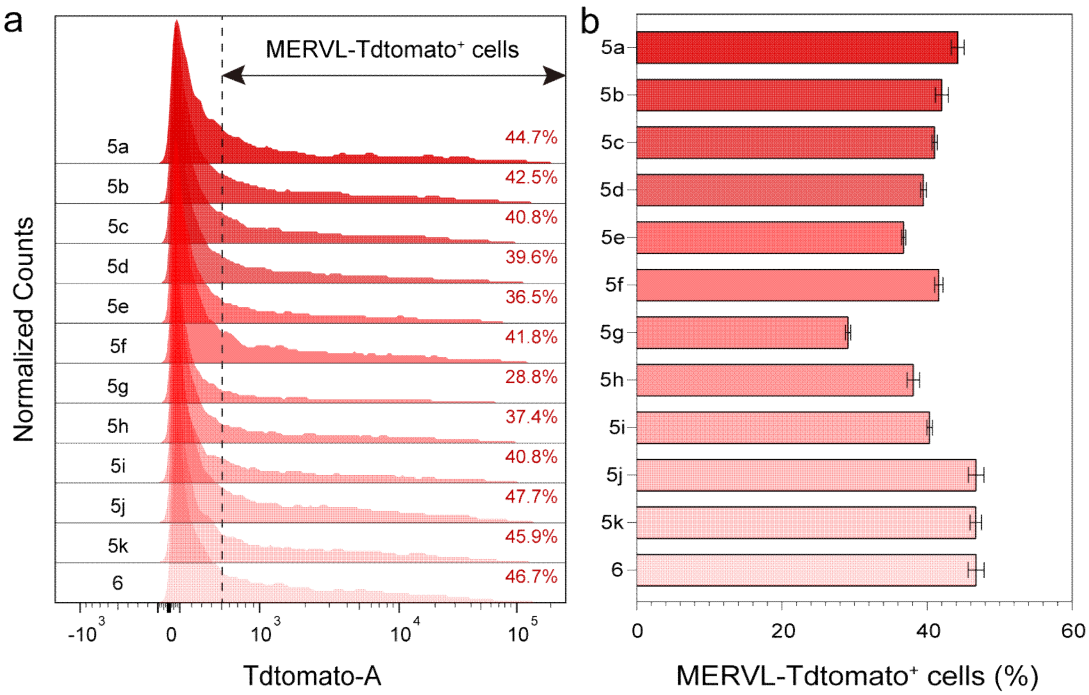
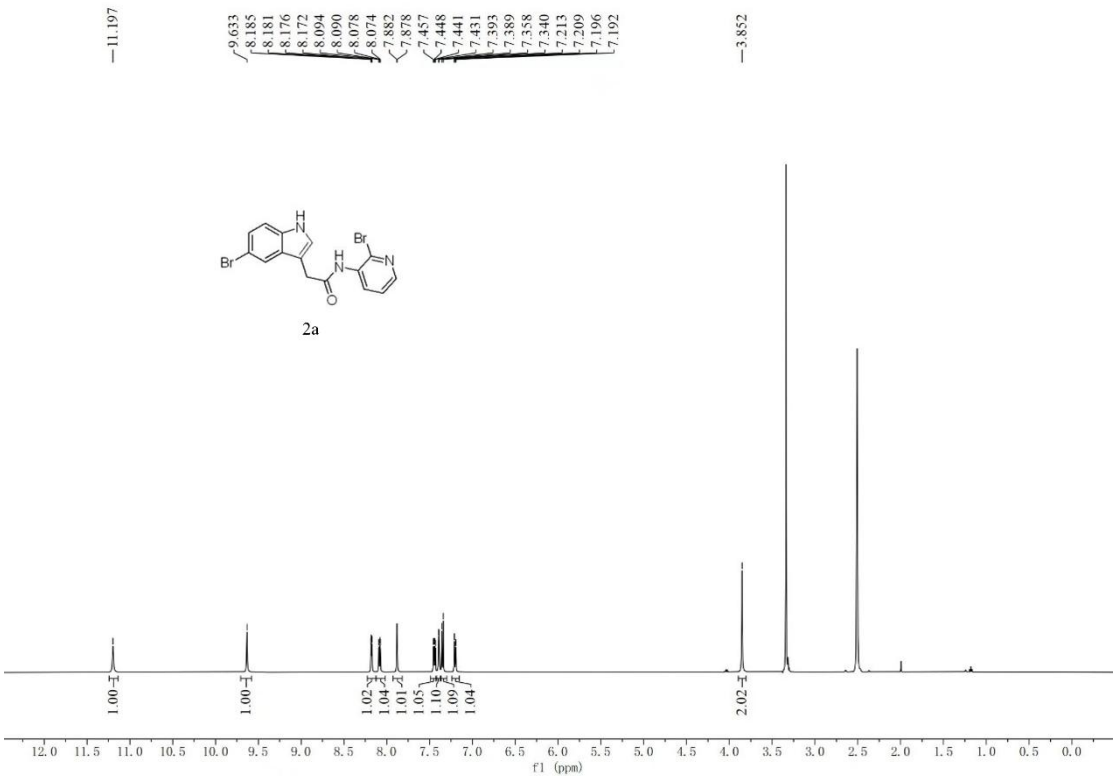


Figure caption: The activation efficiency of totipotency-marked MERVL-Tdtomato by 1-AKP and its analogs. (**a**) FACS-derived histograms and (**b**) MERVL activation efficiencies for mESCs after 2-day induction by 1-AKP and its analogs. Error bars indicate the SD of three replicating measurements.

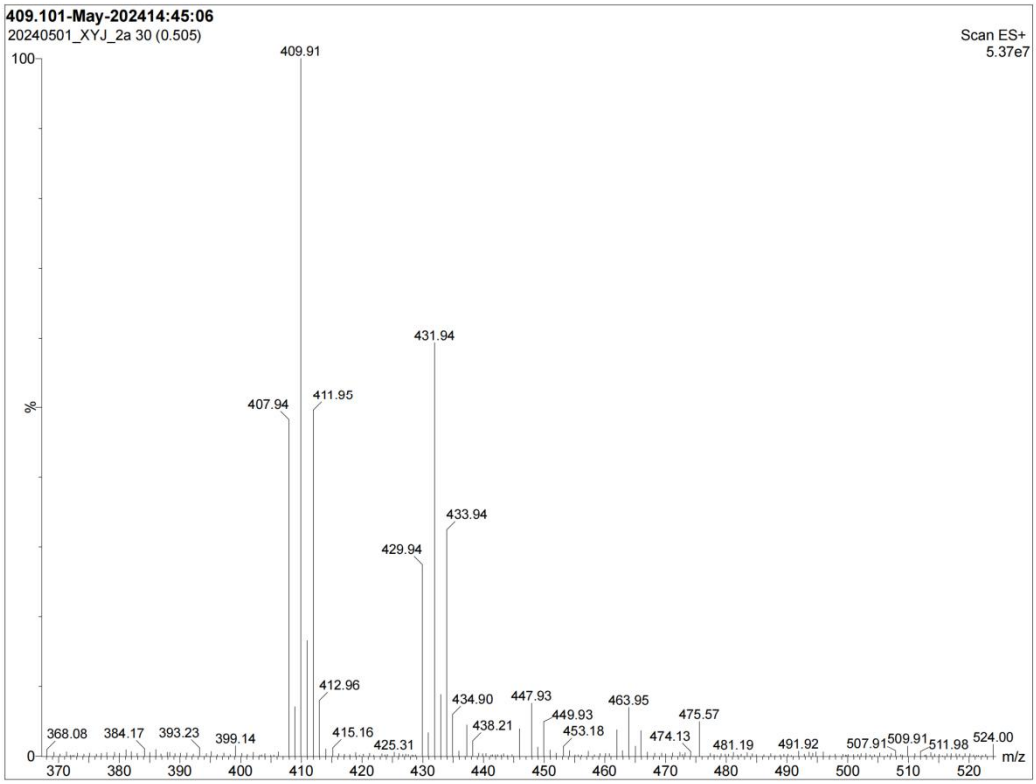
References

1. H. Stukenbrock, R. Mussmann, M. Geese, Y. Ferandin, O. Lozach, T. Lemcke, S. Kegel, A. Lomow, U. Burk, C. Dohrmann, L. Meijer, M. Austen and C. Kunick, *Journal of Medicinal Chemistry*, 2008, **51**, 2196-2207.
2. Y. Hu, Y. Yang, P. Tan, Y. Zhang, M. Han, J. Yu, X. Zhang, Z. Jia, D. Wang, K. Yao, H. Pang, Z. Hu, Y. Li, T. Ma, K. Liu and S. Ding, *Nature*, 2023, **617**, 792-797.

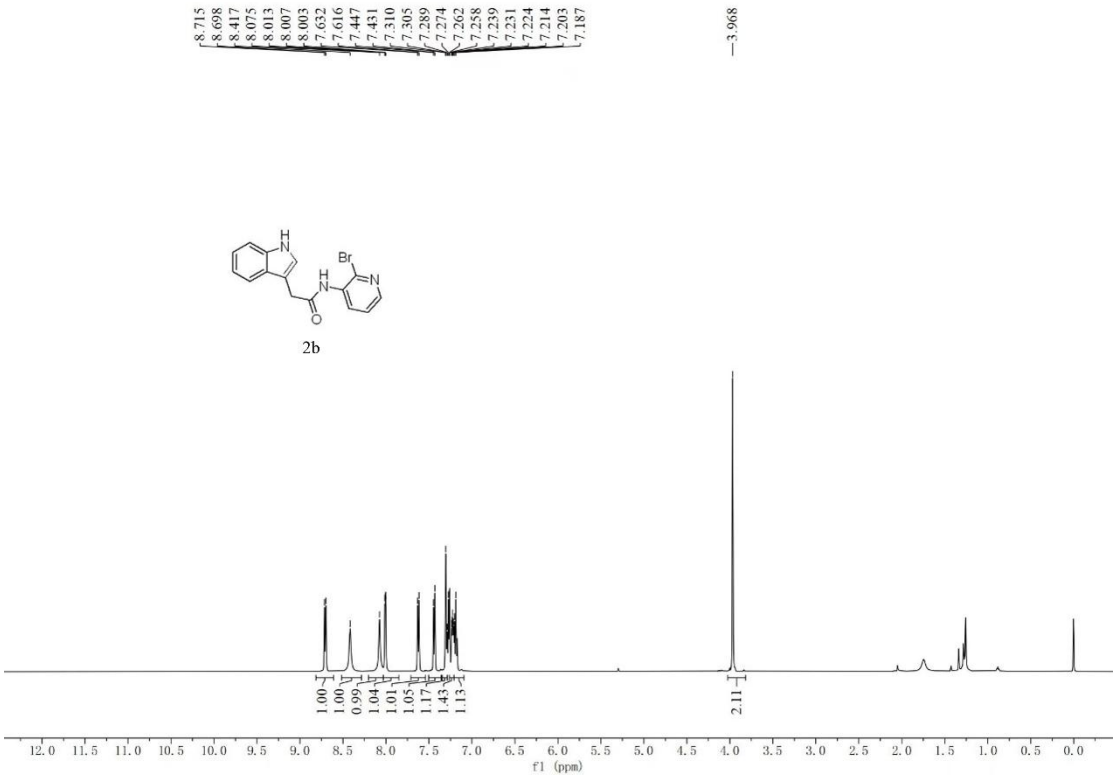
^{1}H NMR, ^{13}C NMR, MS and HRMS Spectra



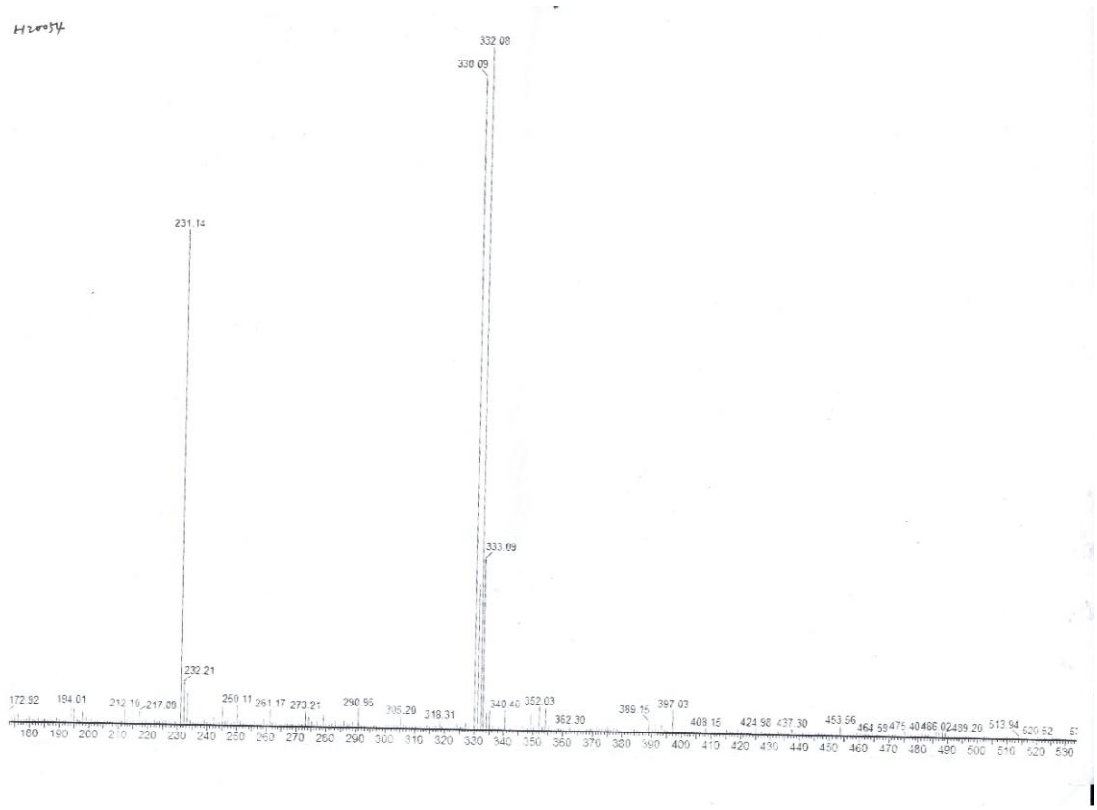
^{1}H-NMR spectrum of compound 2a



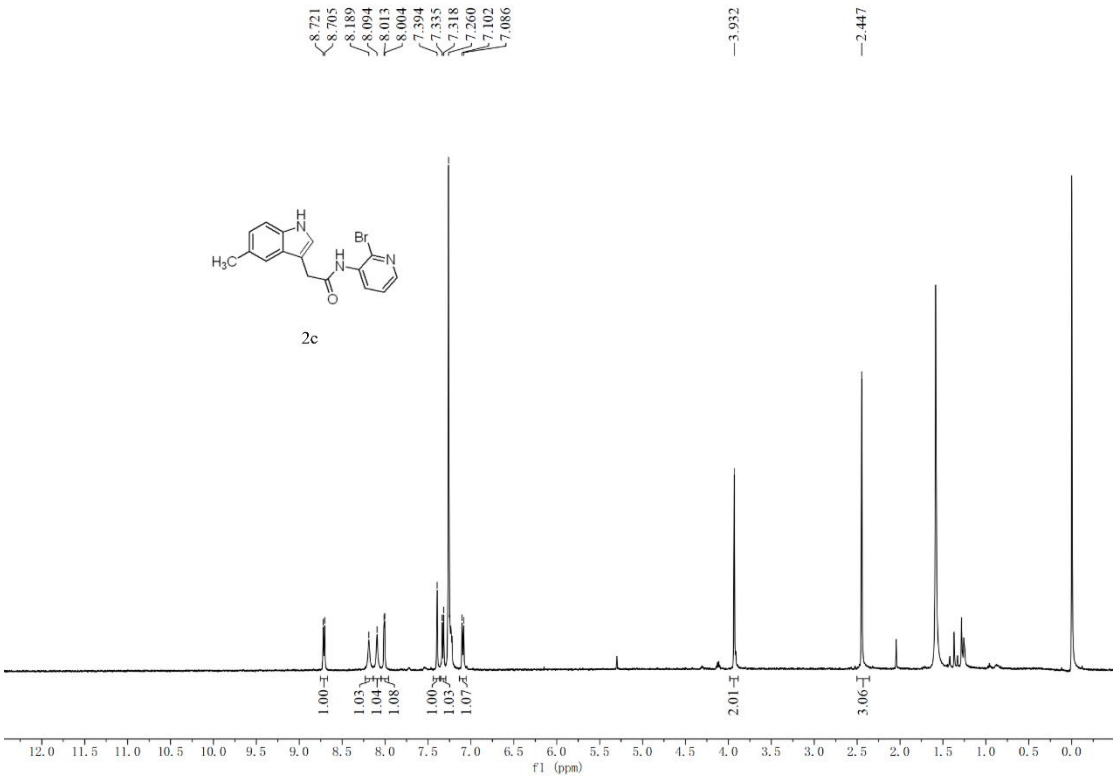
MS spectrum of compound 2a



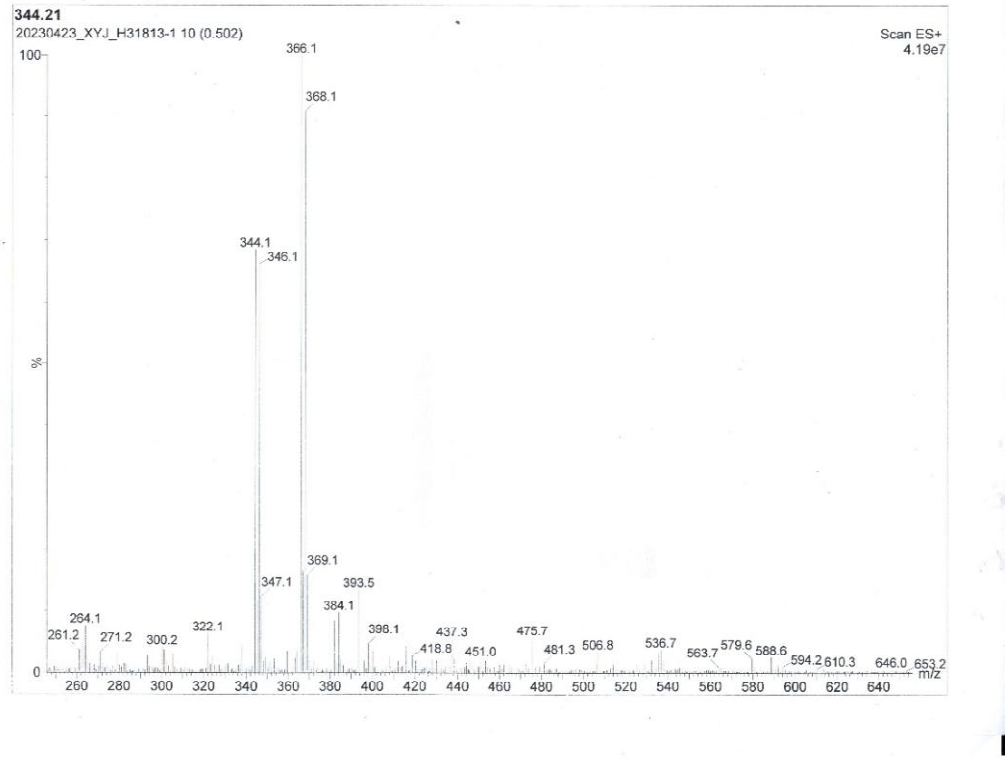
^{1}H-NMR spectrum of compound 2b



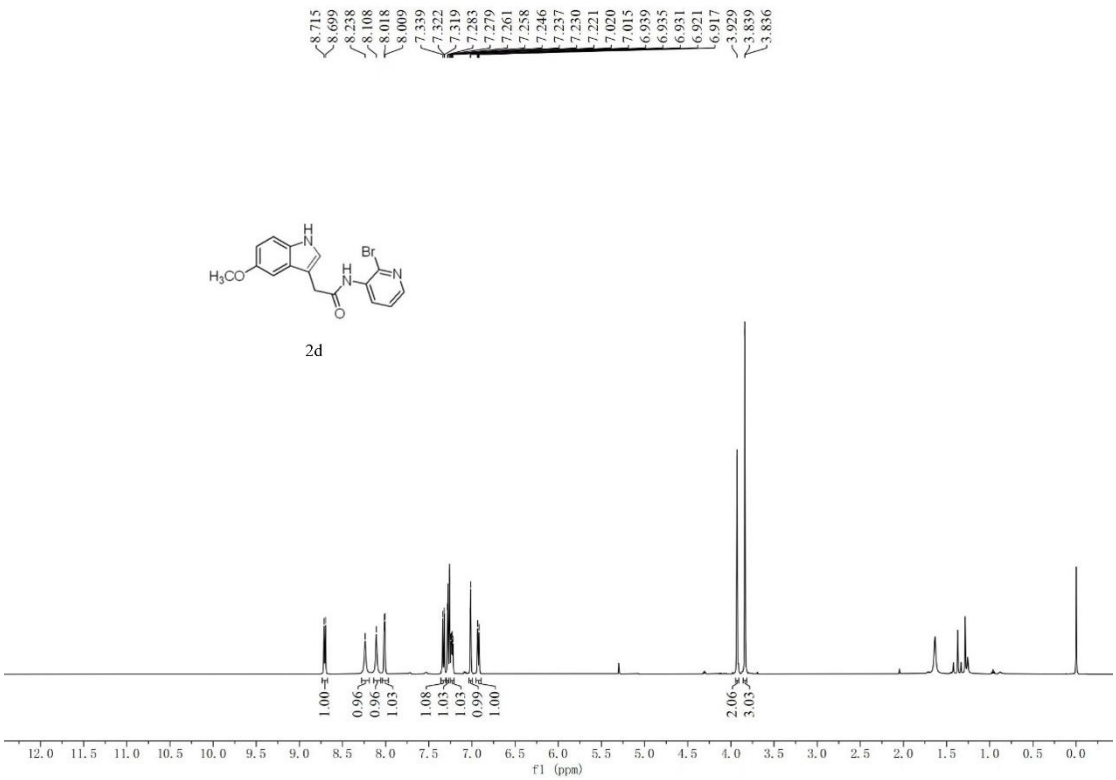
MS spectrum of compound 2b



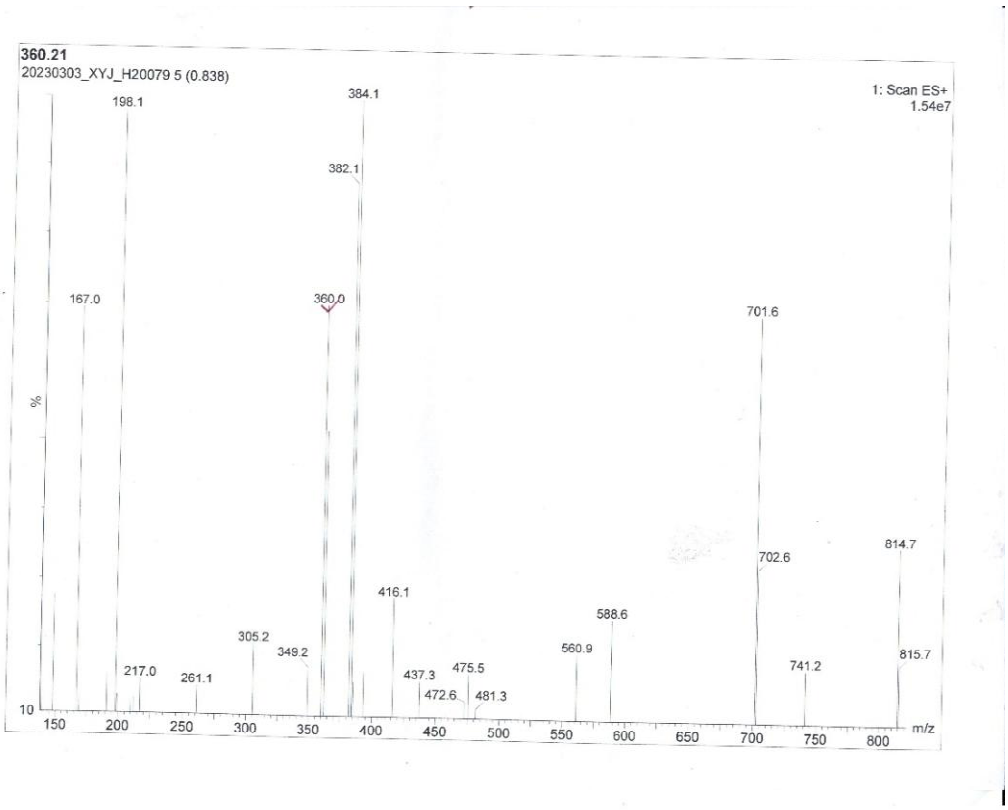
^{1}H-NMR spectrum of compound 2c



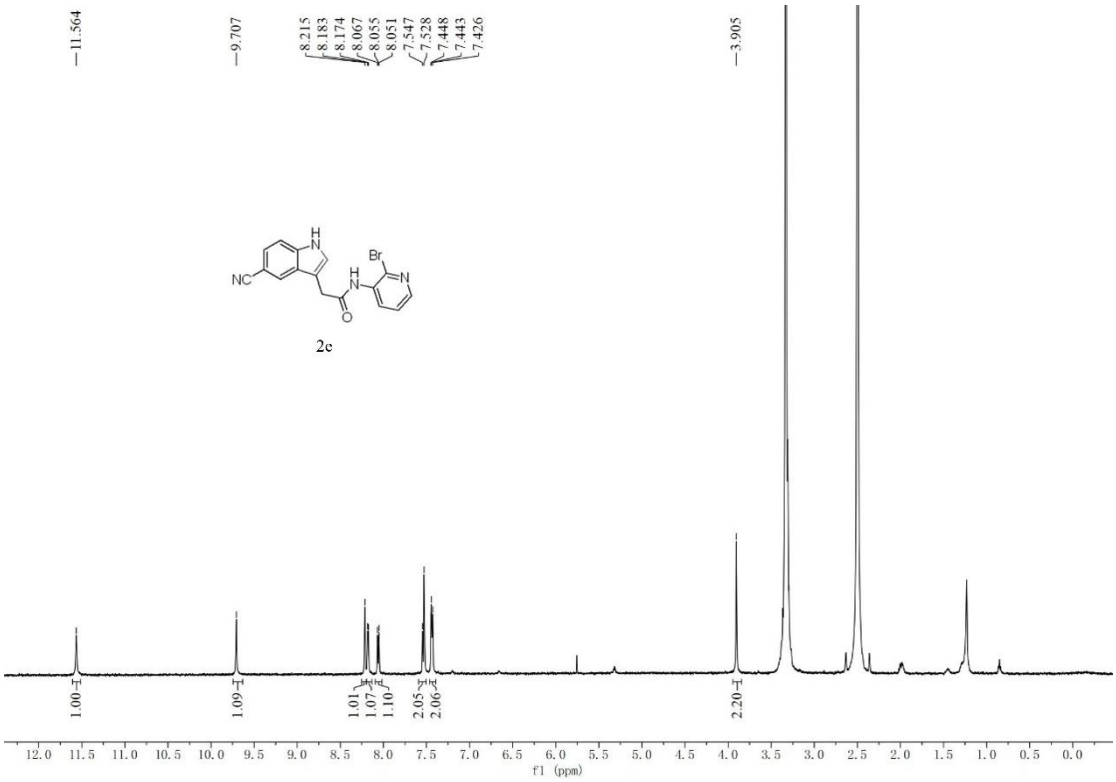
MS spectrum of compound 2c



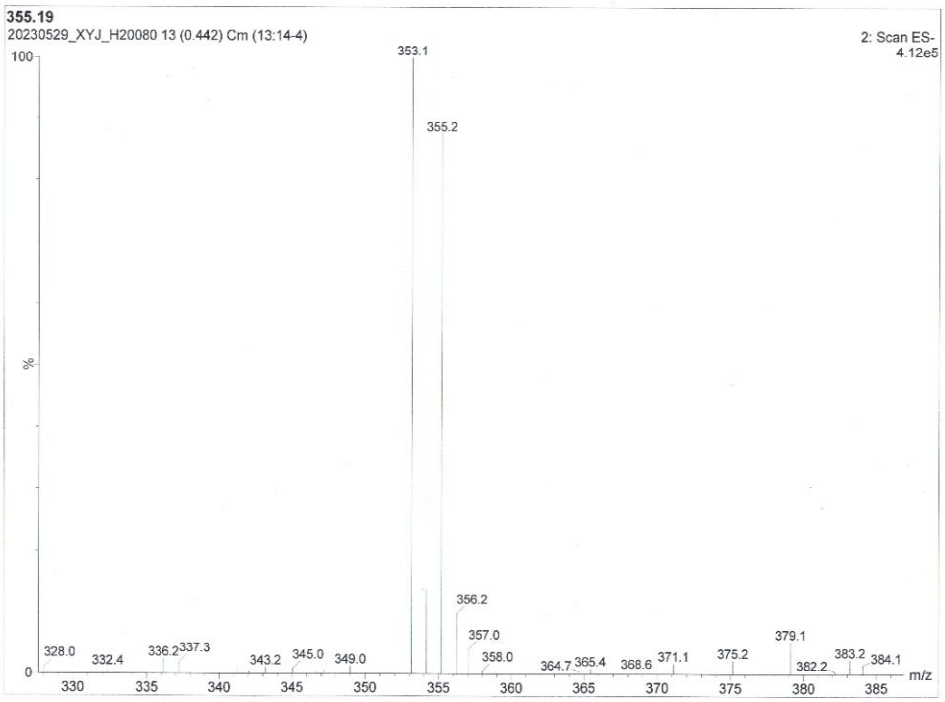
^{1}H-NMR spectrum of compound 2d



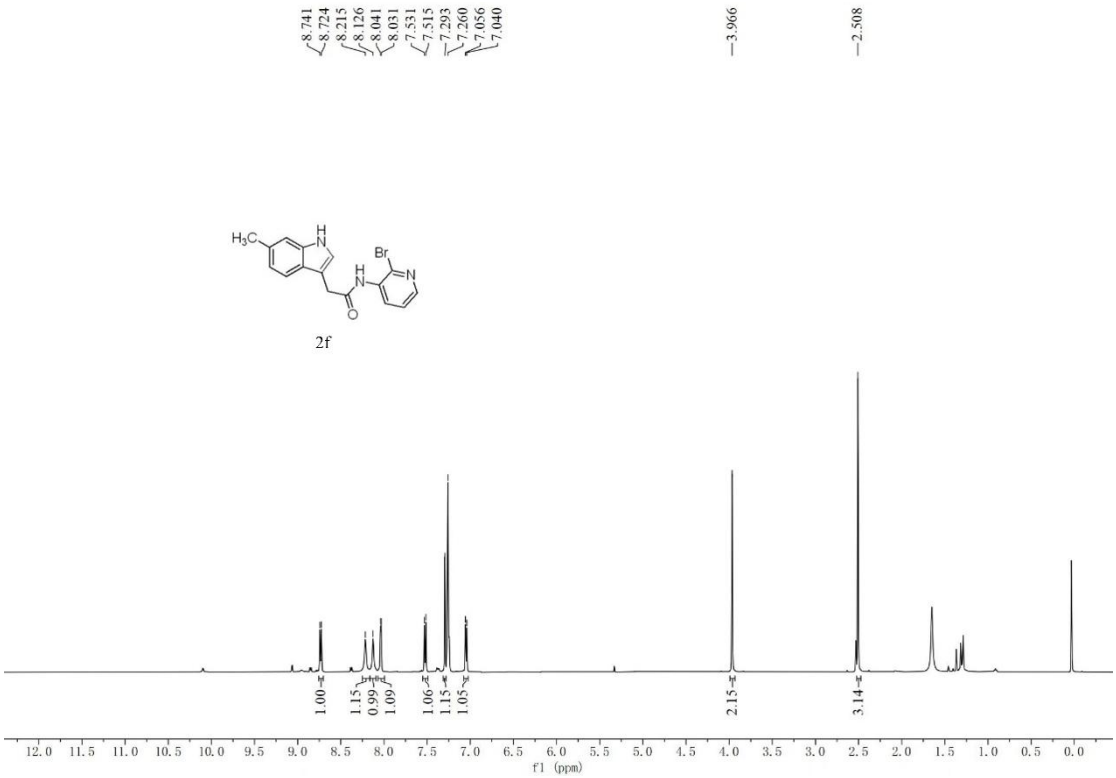
MS spectrum of compound 2d



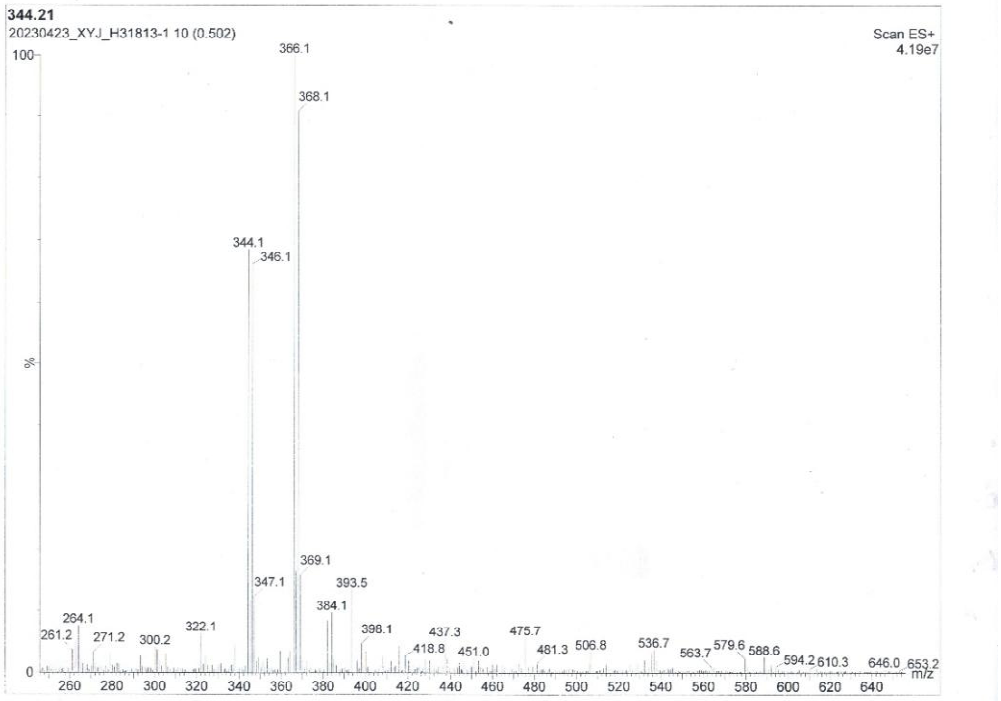
^{1}H-NMR spectrum of compound 2e



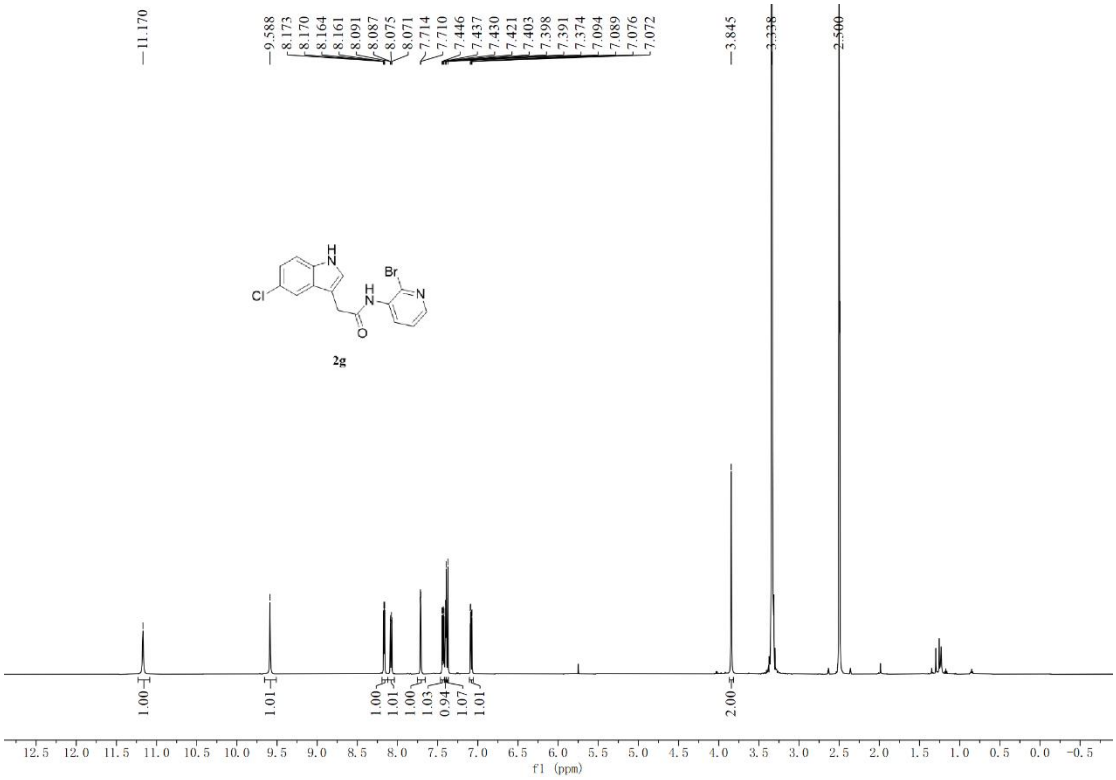
MS spectrum of compound 2e



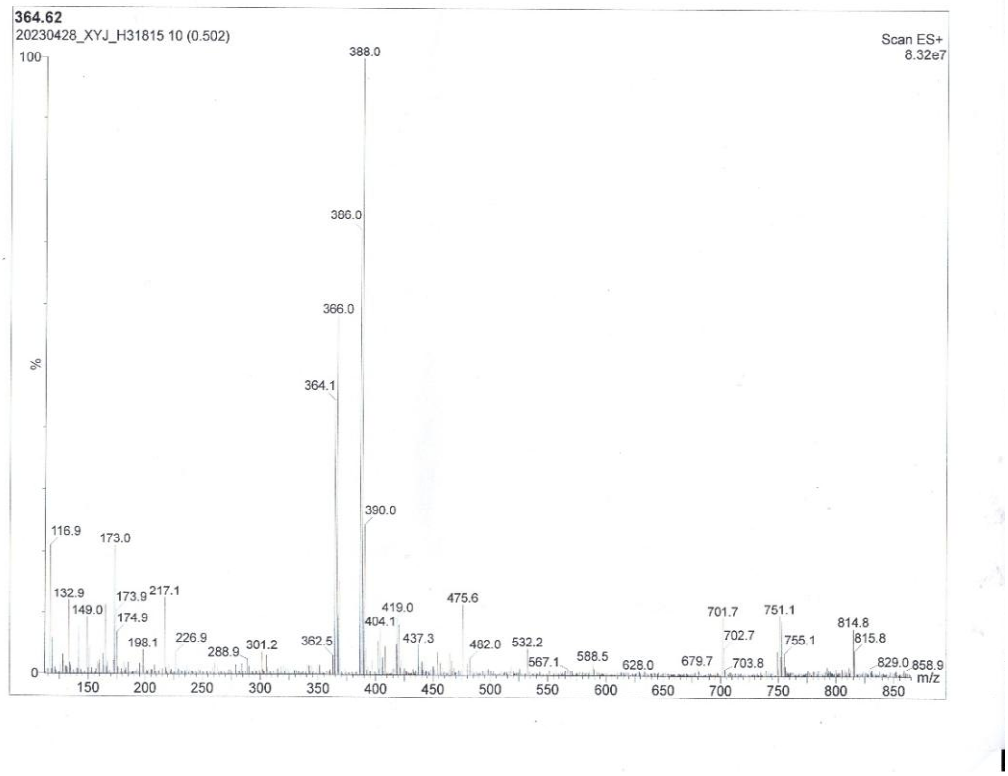
^{1}H-NMR spectrum of compound 2f



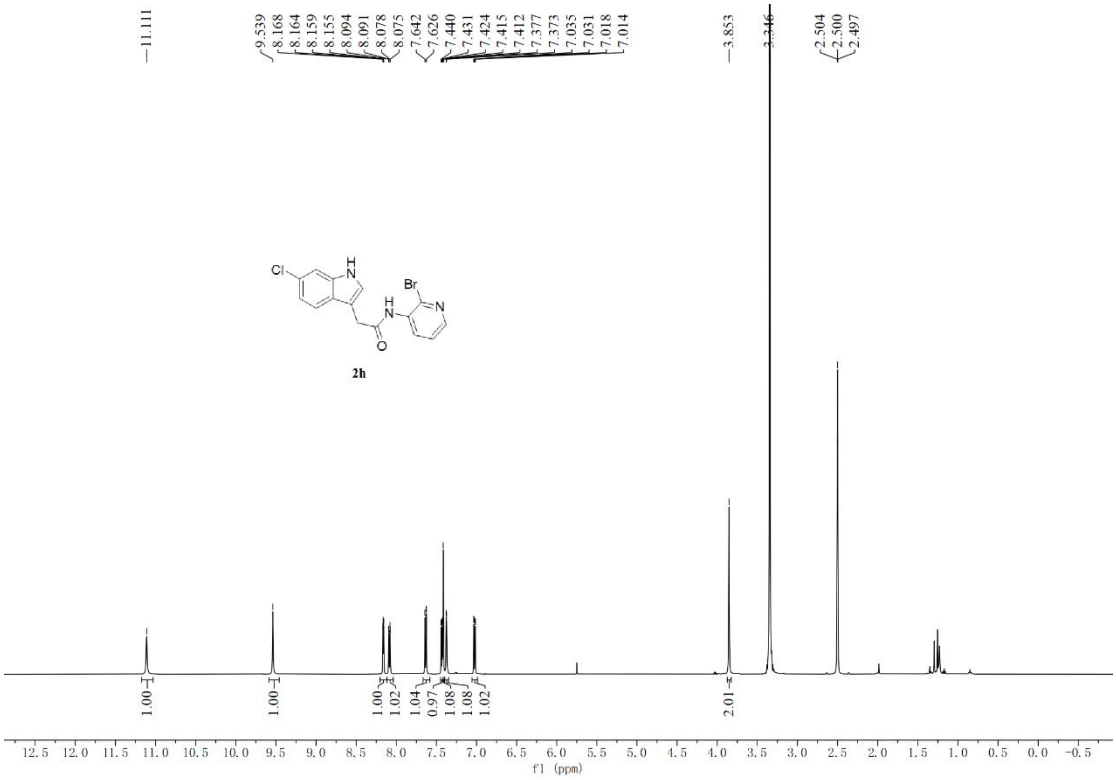
MS spectrum of compound 2f



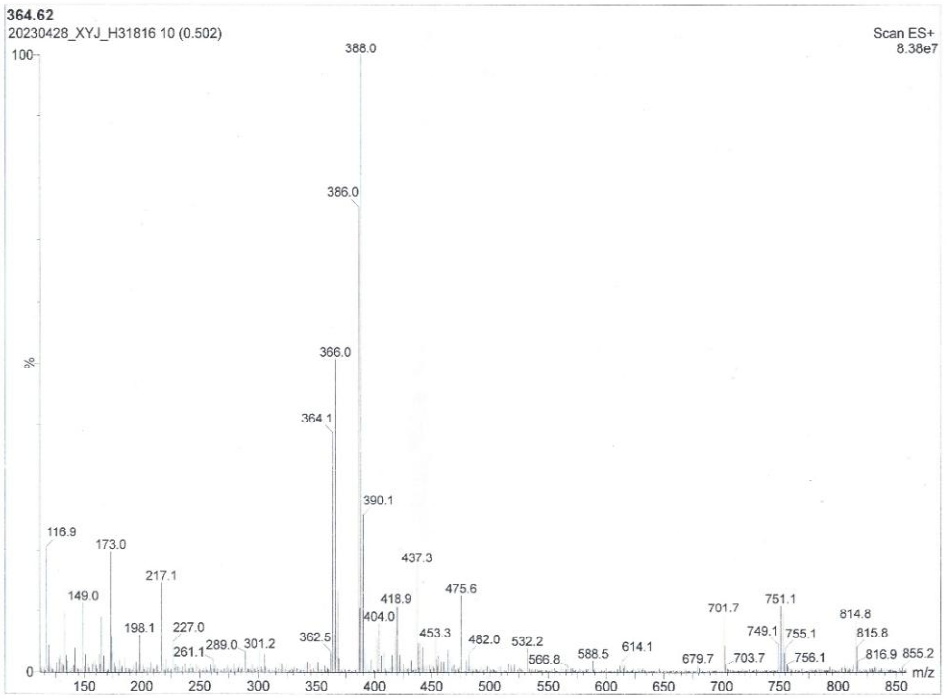
^{1}H-NMR spectrum of compound 2g



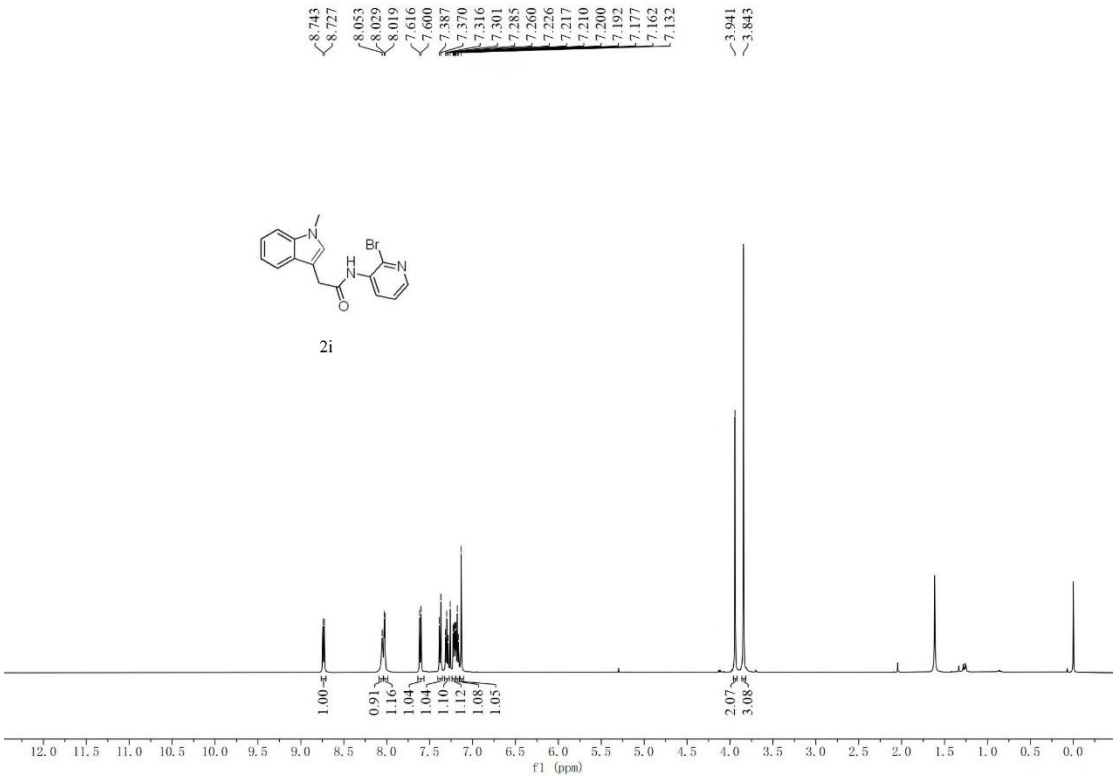
MS spectrum of compound 2g



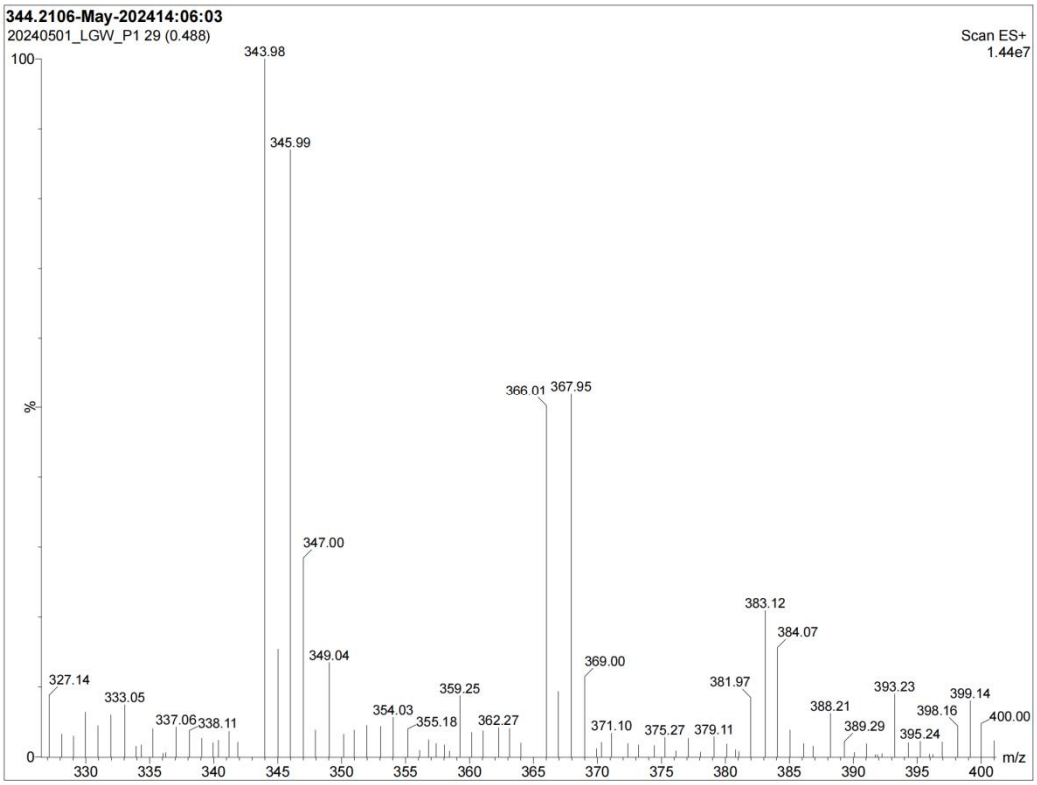
^{1}H-NMR spectrum of compound 2h



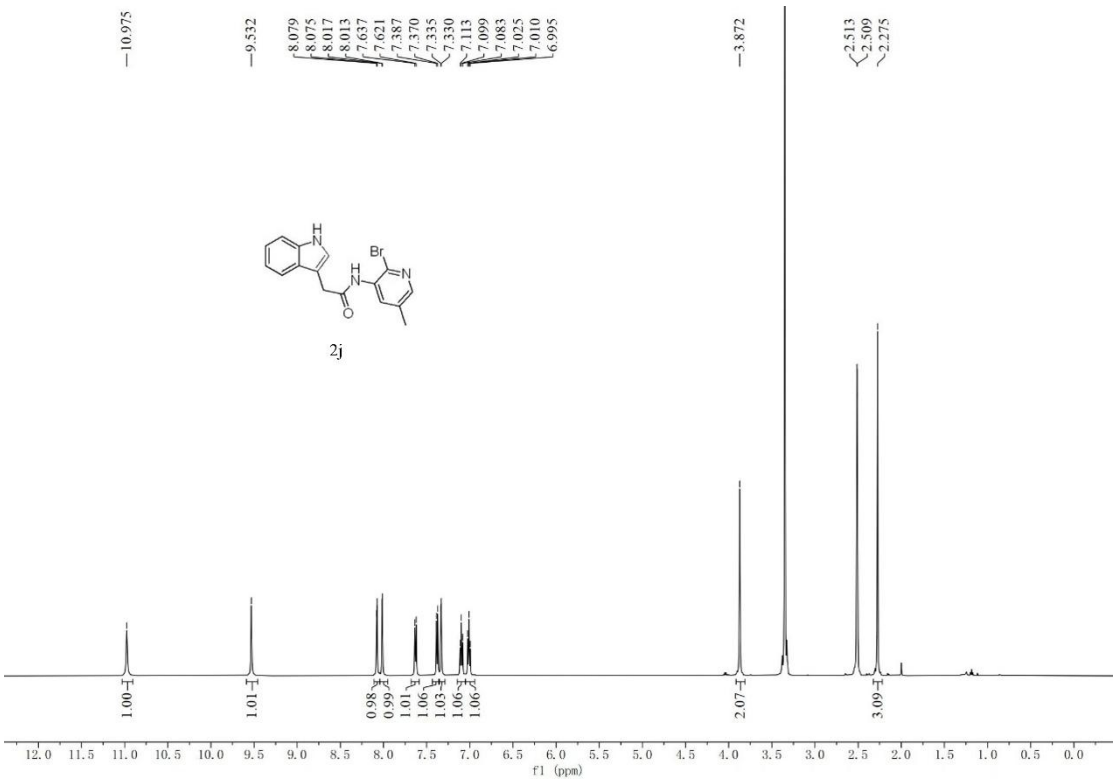
MS spectrum of compound 2h



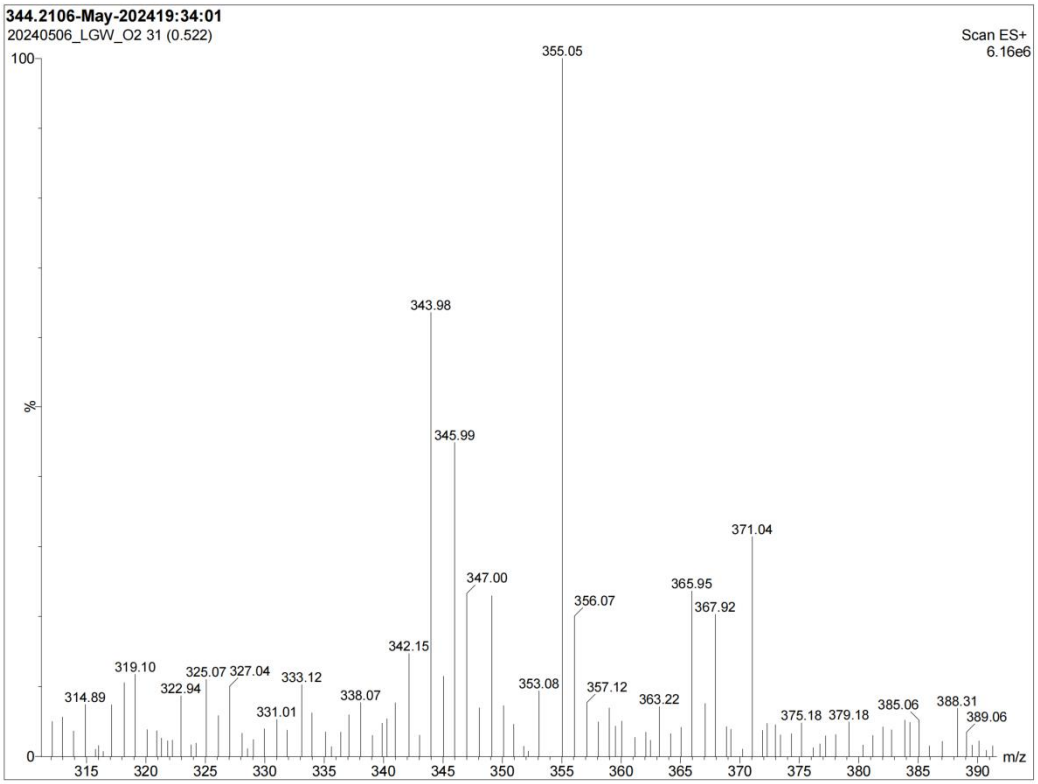
^{1}H-NMR spectrum of compound 2i



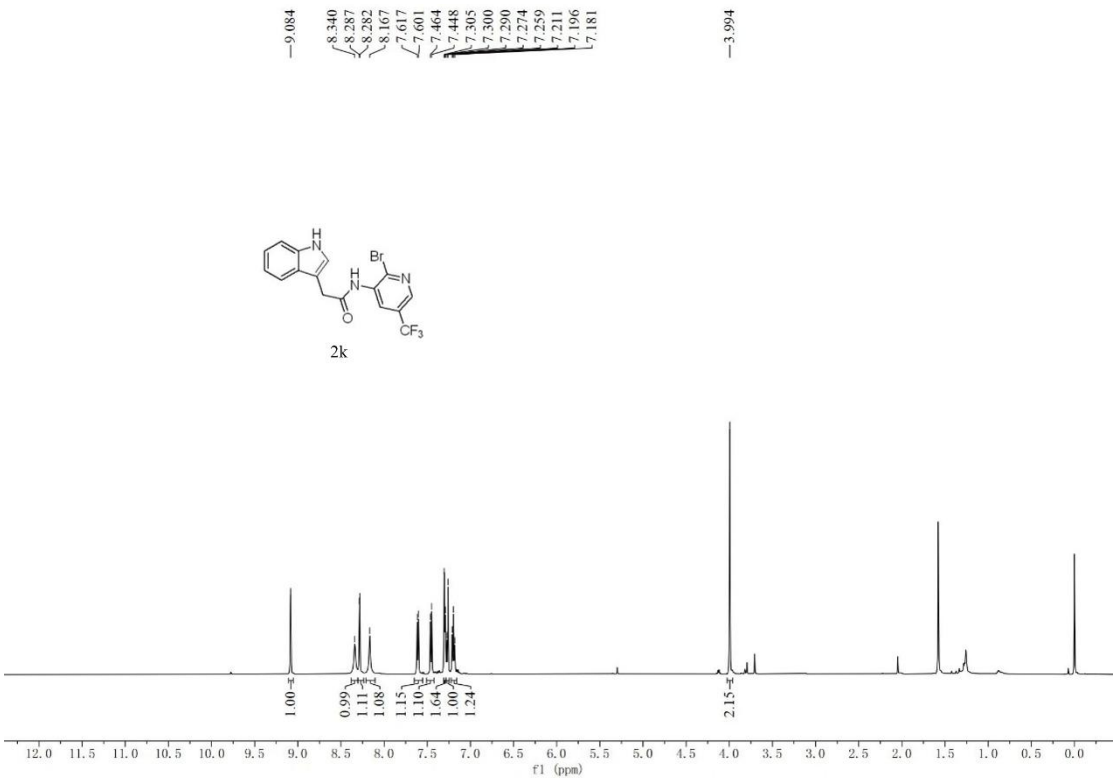
MS spectrum of compound 2i



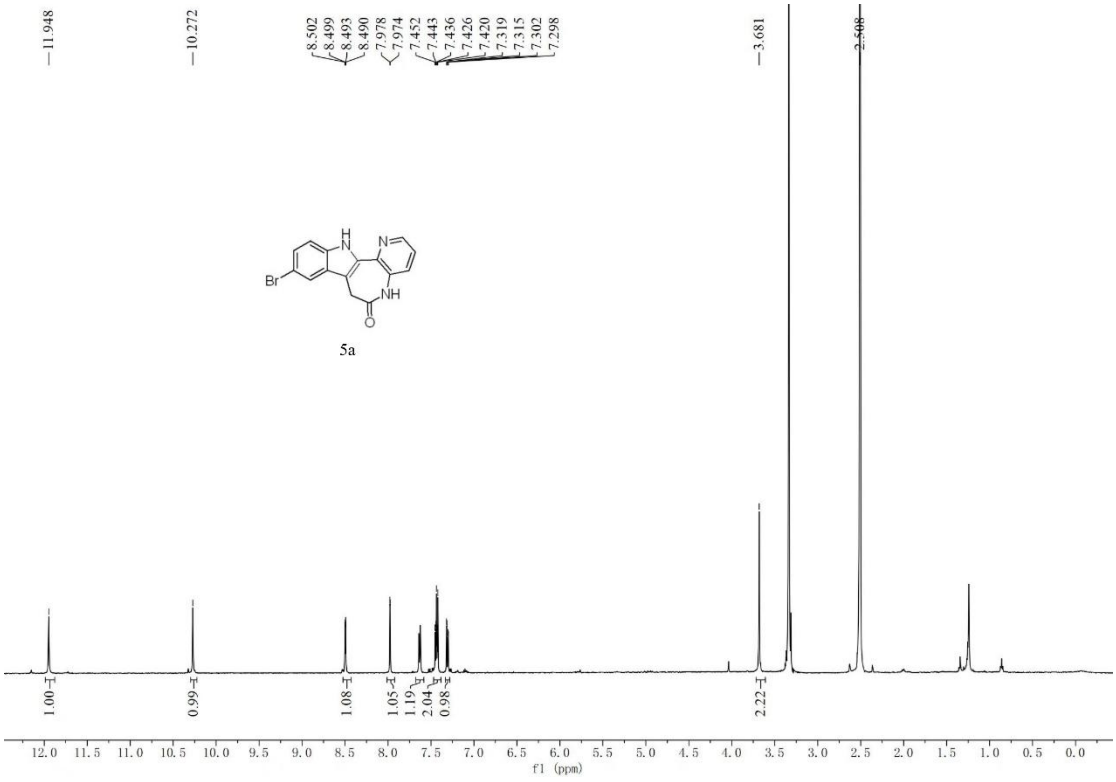
^{1}H-NMR spectrum of compound 2j



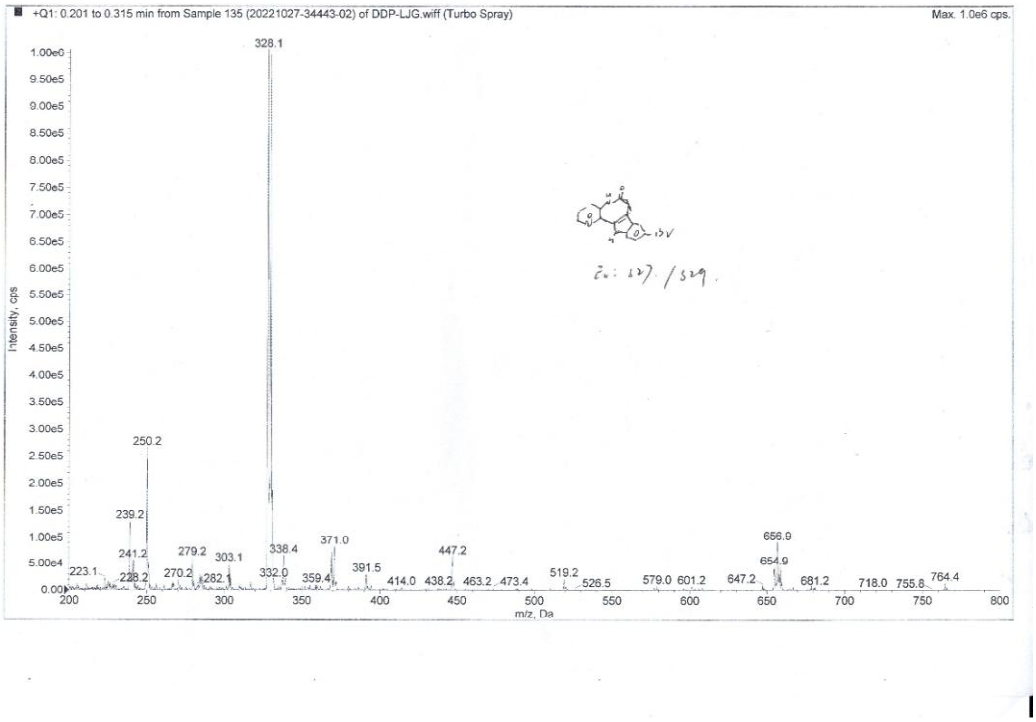
MS spectrum of compound 2j



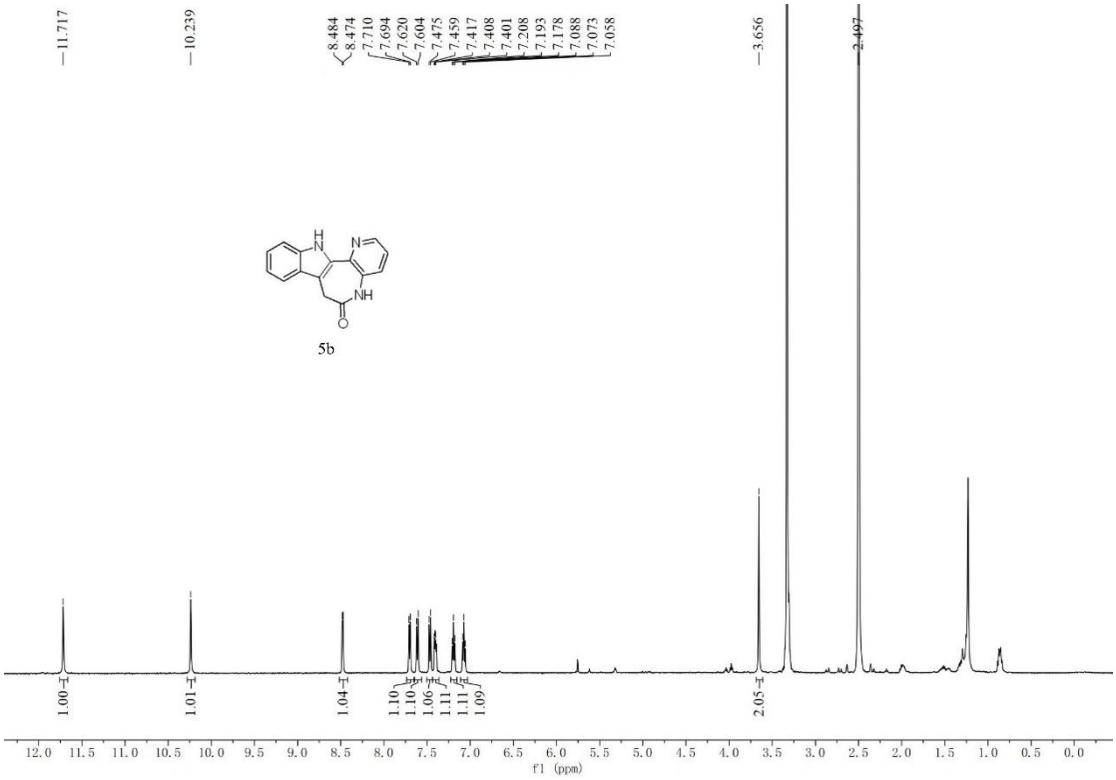
[1]H-NMR spectrum of compound 2k



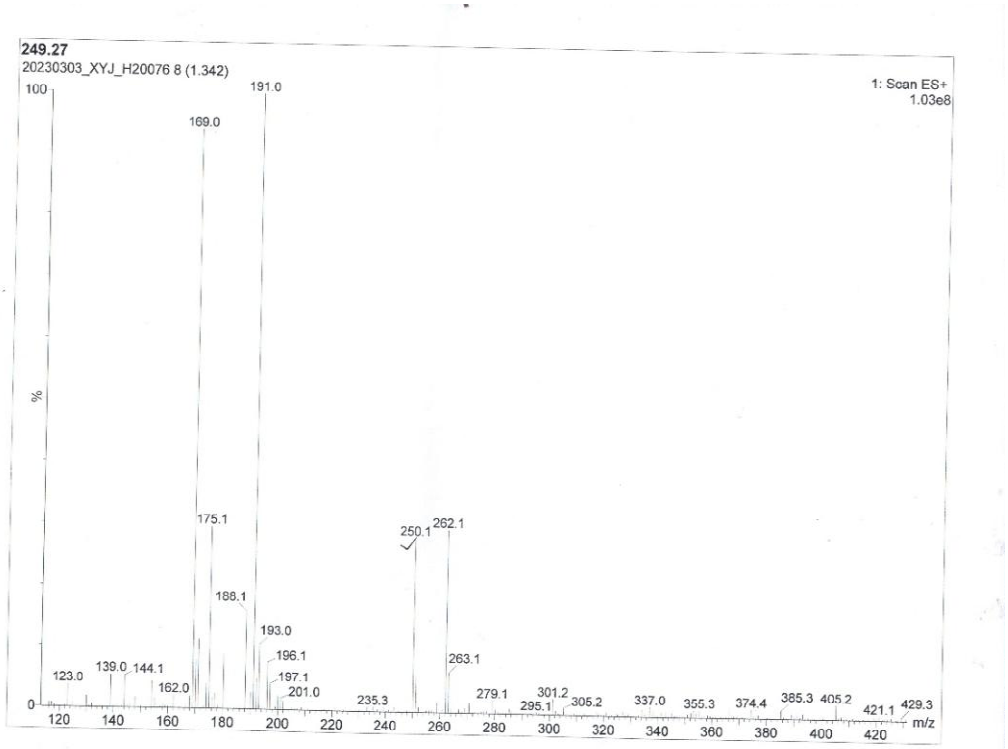
[1]H-NMR spectrum of compound 5a(1-Azakenpaullone)



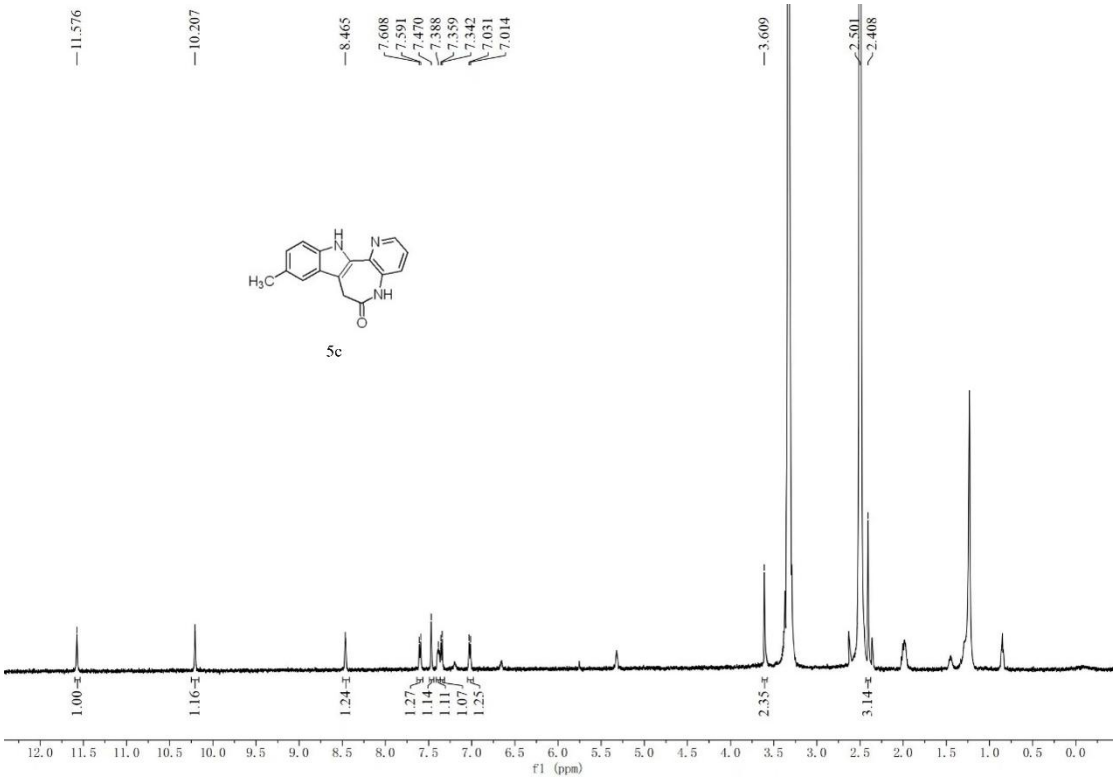
MS spectrum of compound 5a(1-Azakenpaullone)



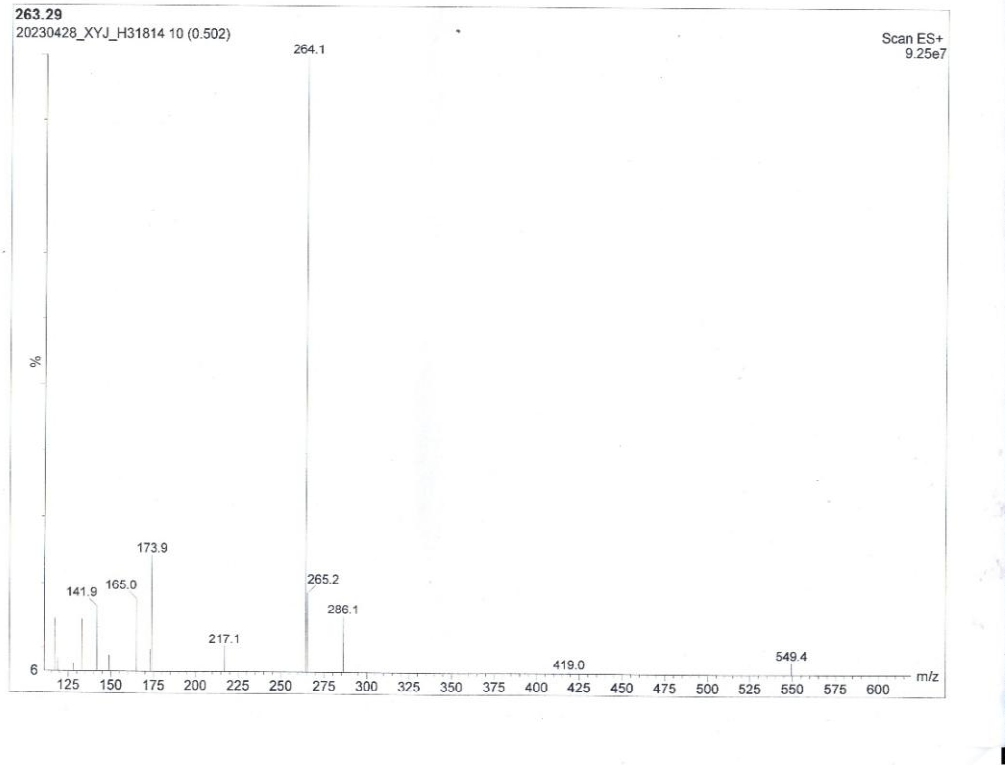
^{1}H-NMR spectrum of compound 5b



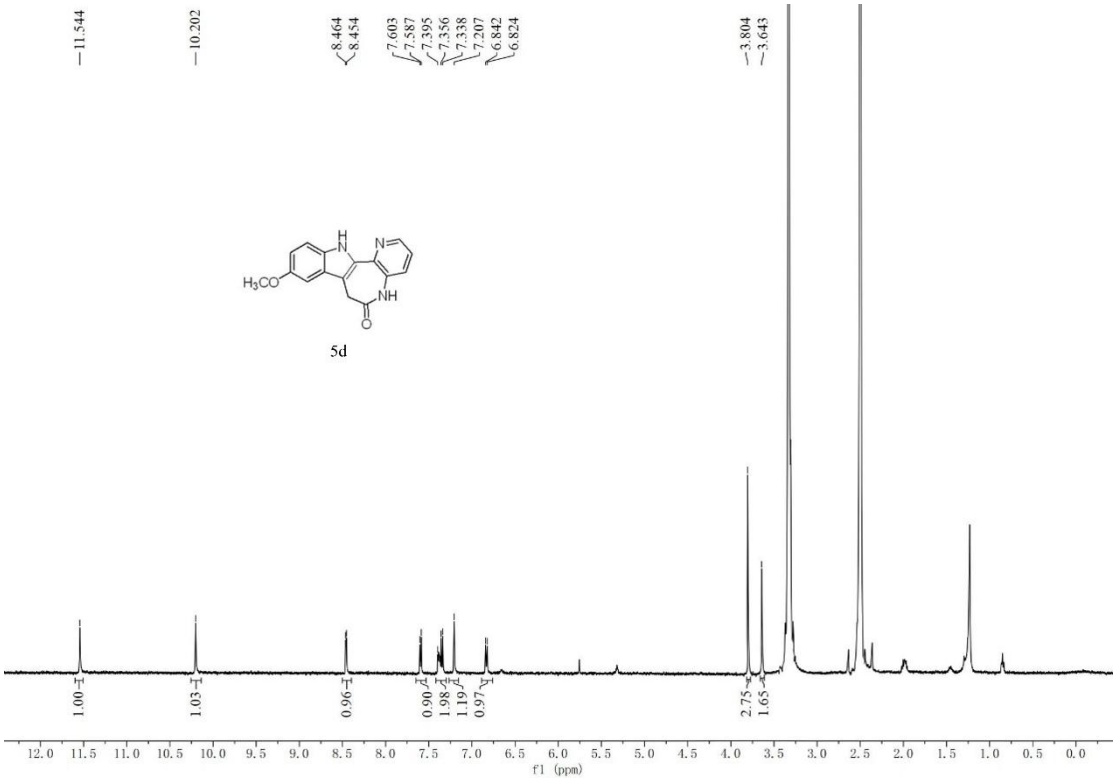
MS spectrum of compound 5b



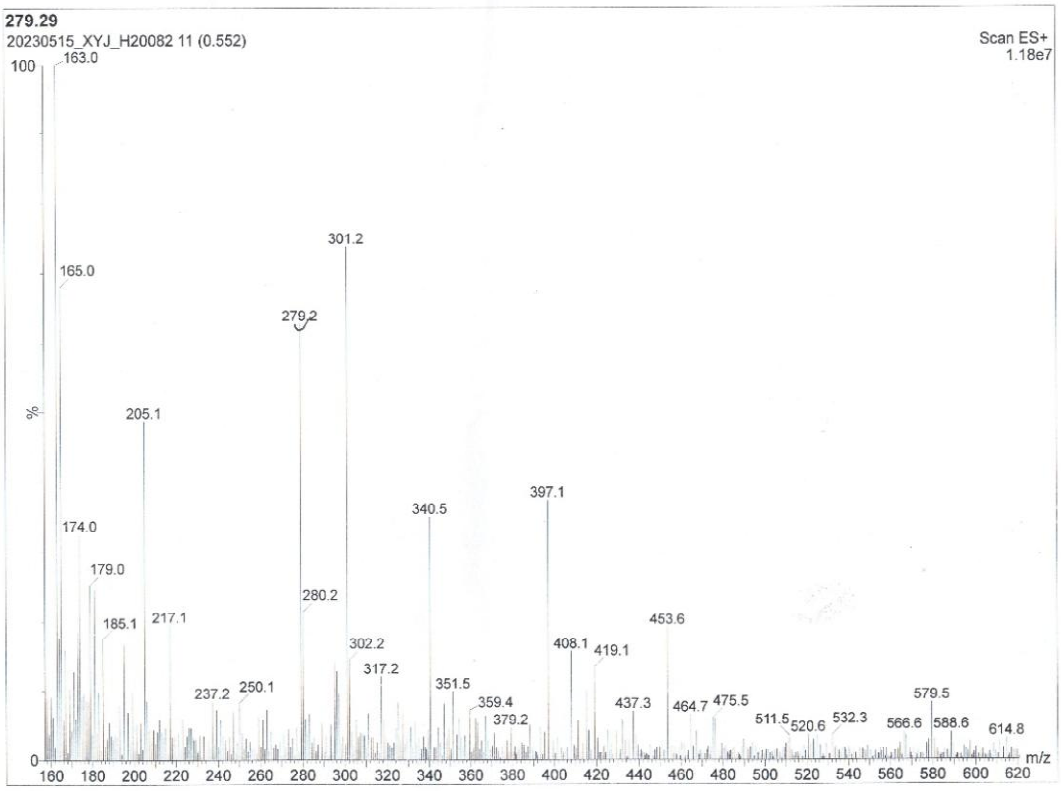
^{1}H-NMR spectrum of compound 5c



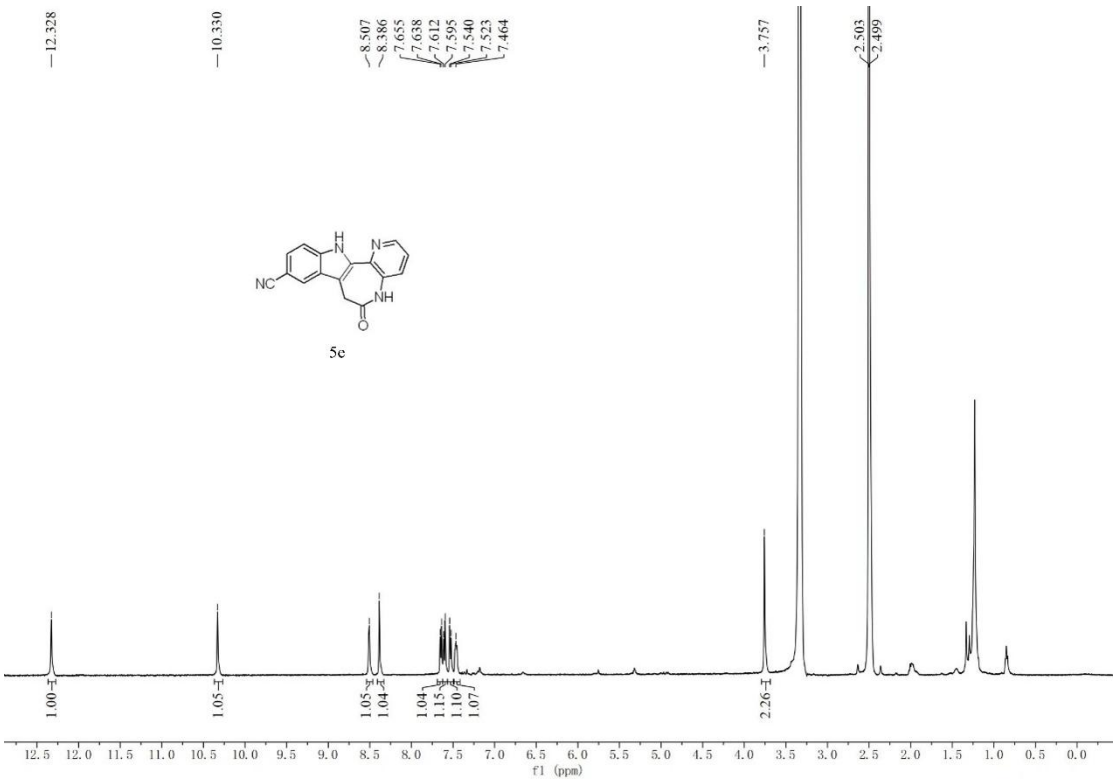
MS spectrum of compound 5c



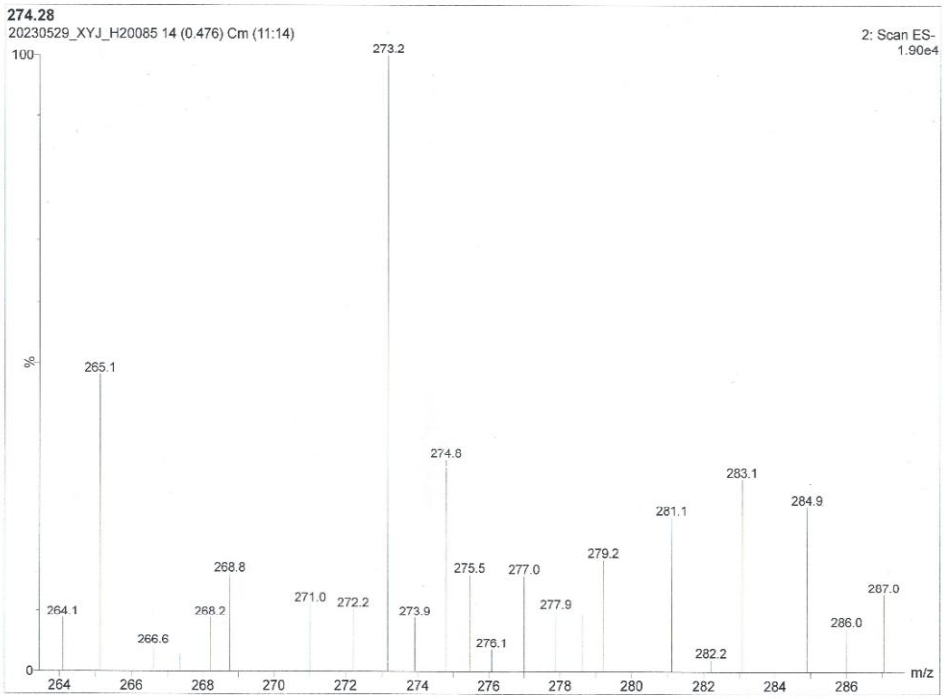
^{1}H-NMR spectrum of compound 5d



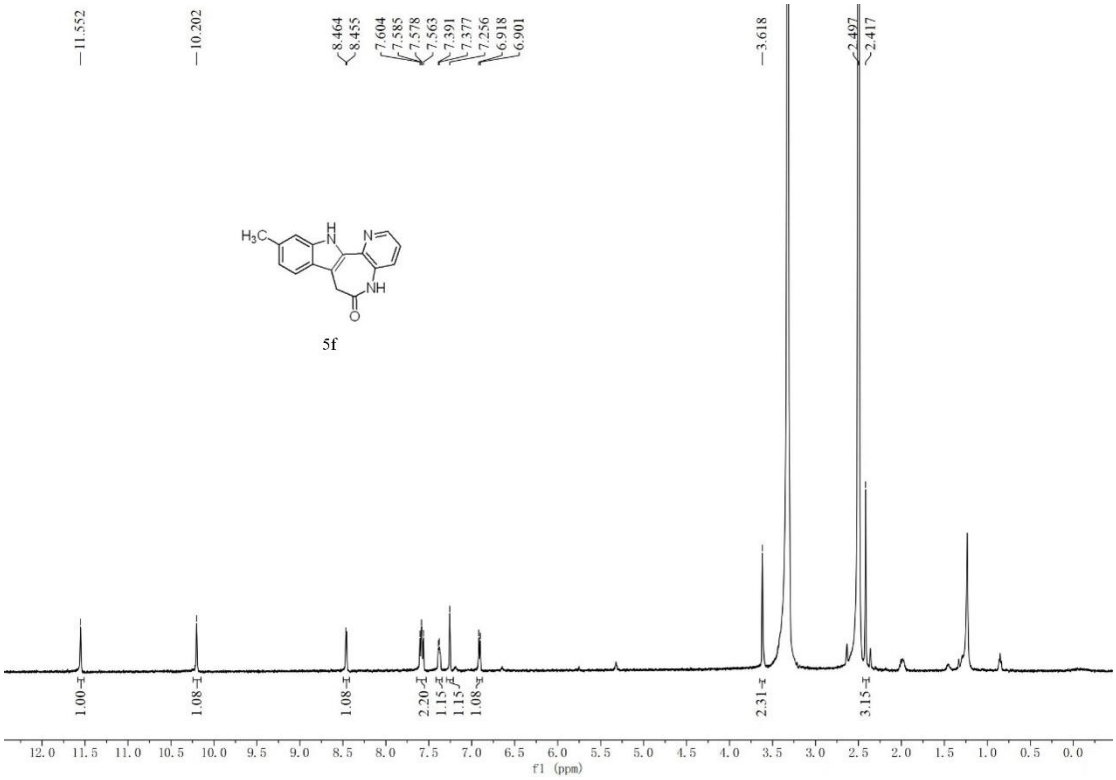
MS spectrum of compound 5d



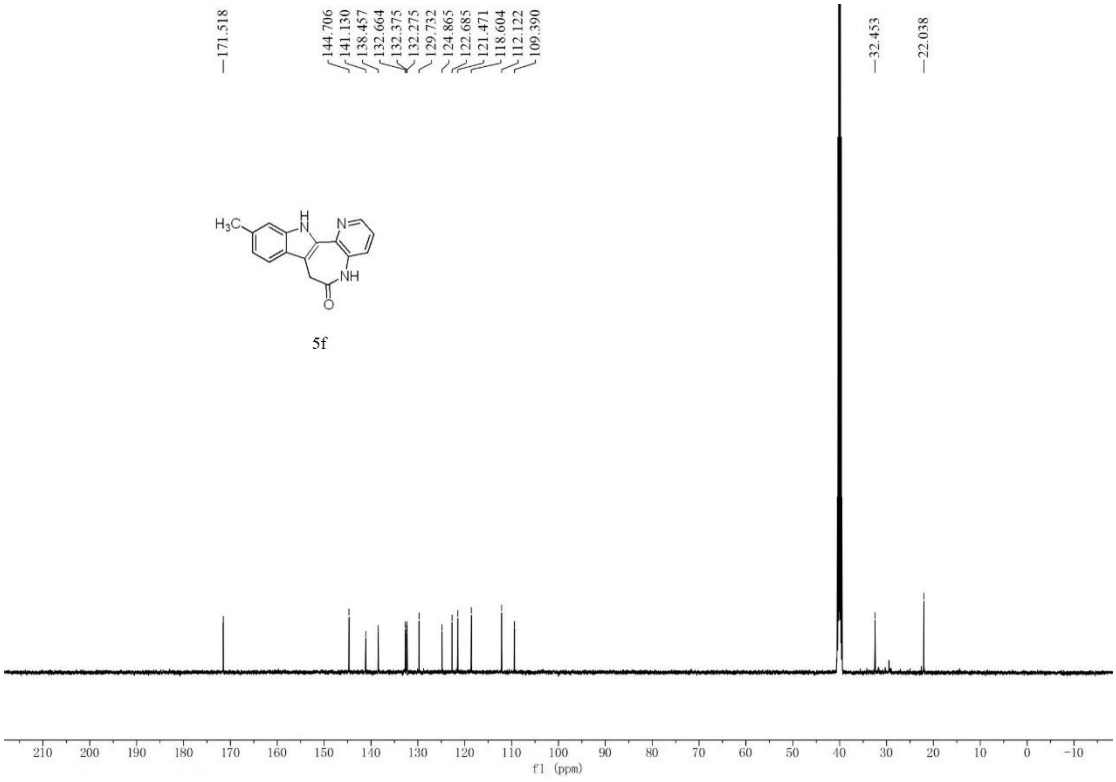
^{1}H-NMR spectrum of compound 5e



MS spectrum of compound 5e

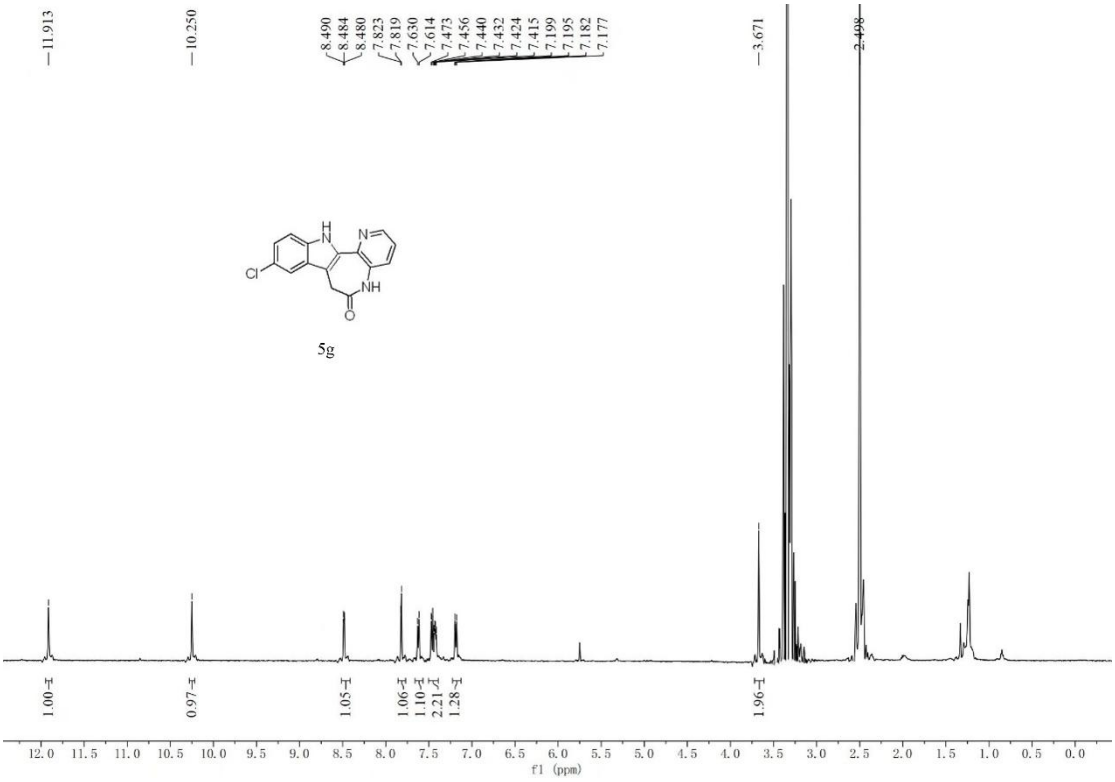


^{1}H-NMR spectrum of compound 5f

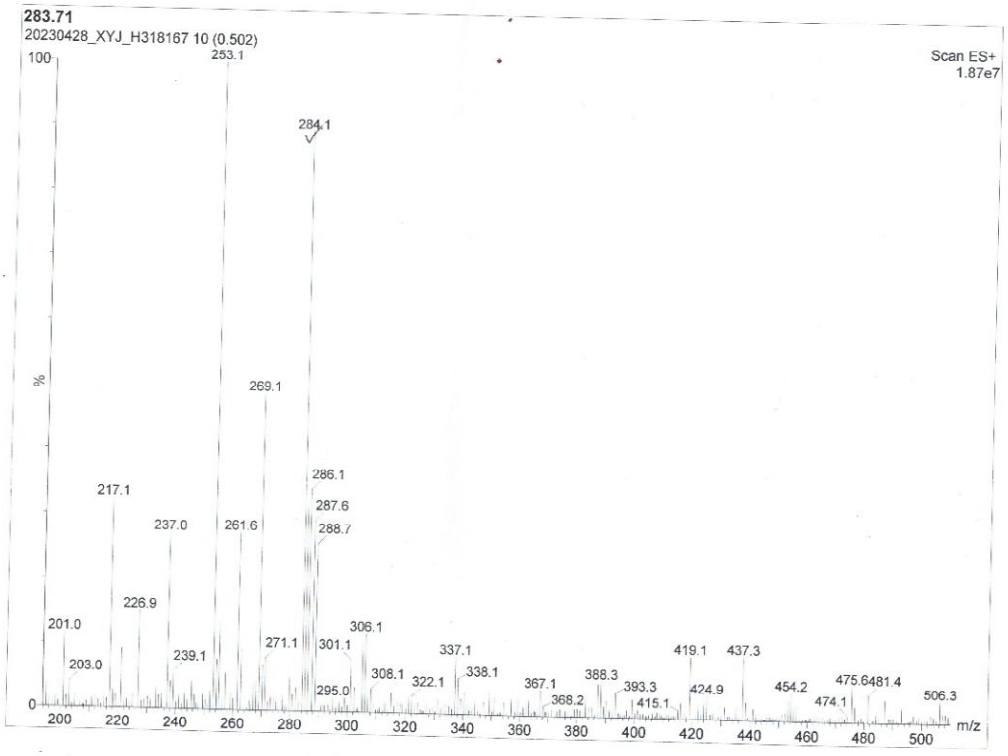


^{13}C-NMR spectrum of compound 5f

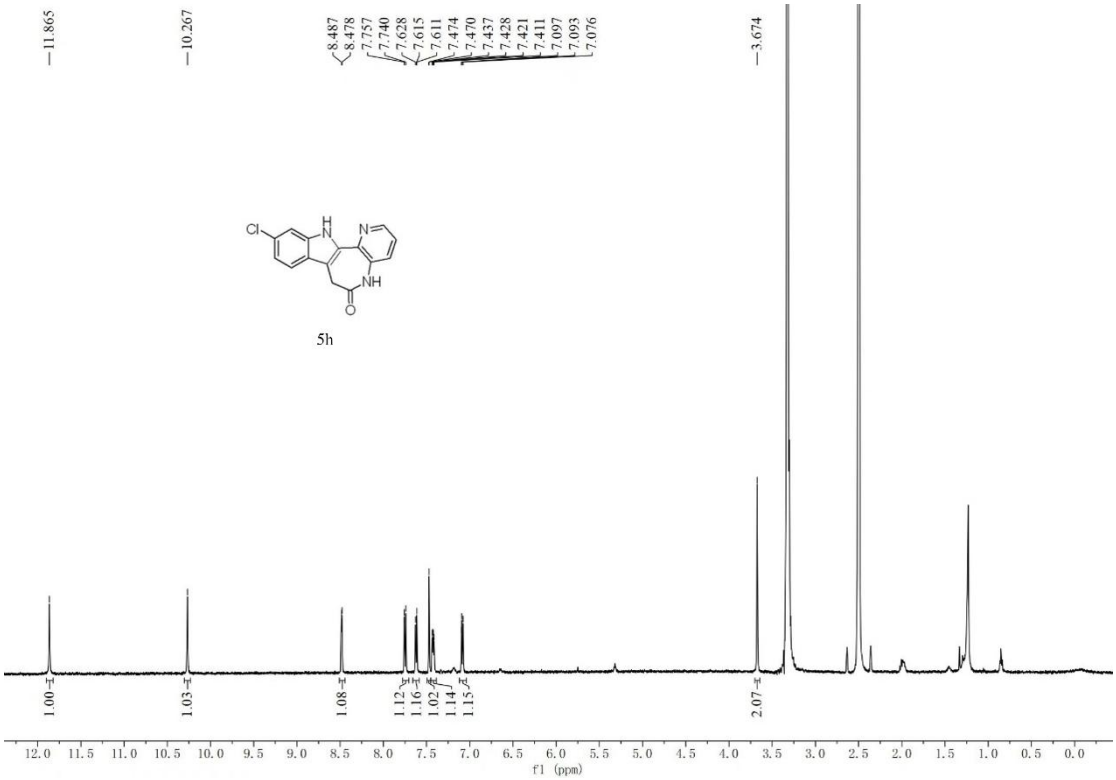
HRMS spectrum of compound 5f



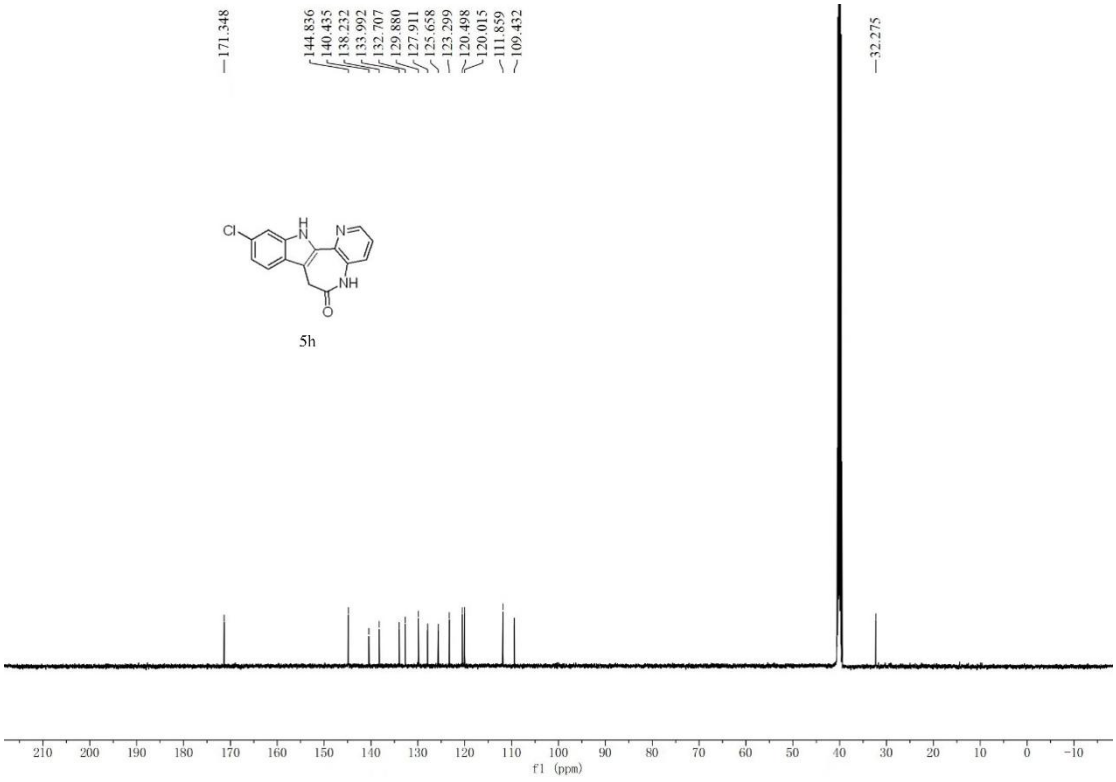
^{1}H-NMR spectrum of compound 5g



MS spectrum of compound 5g

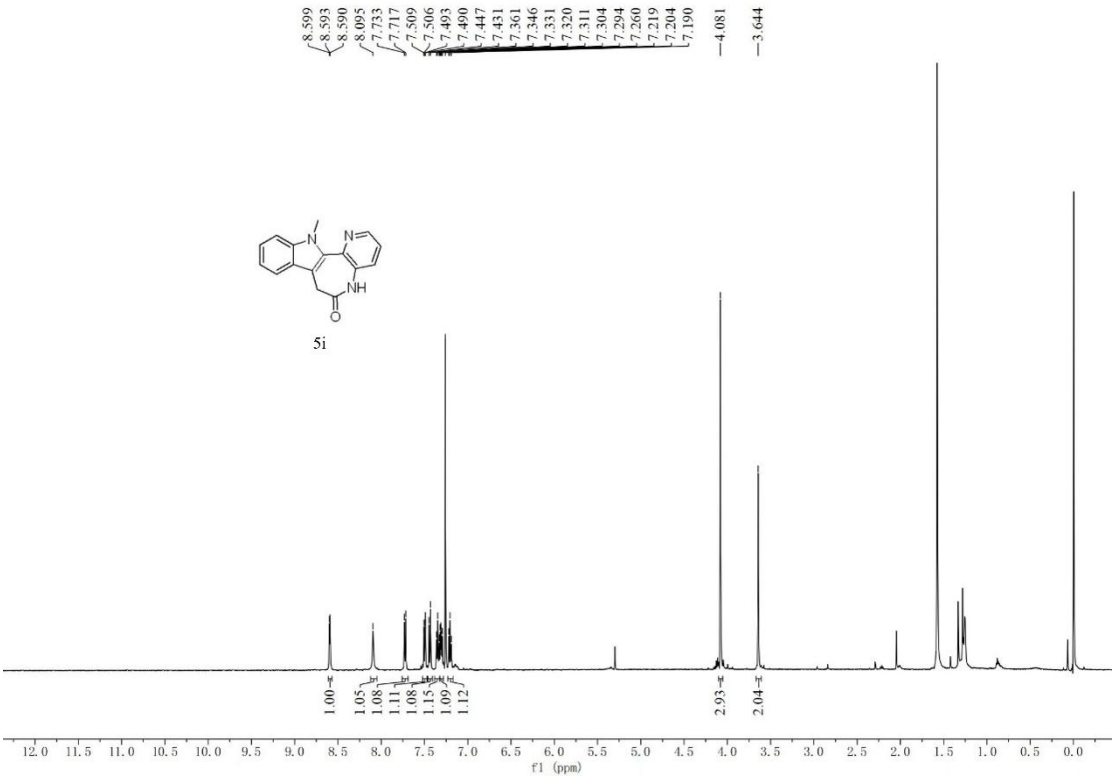


^{1}H-NMR spectrum of compound 5h

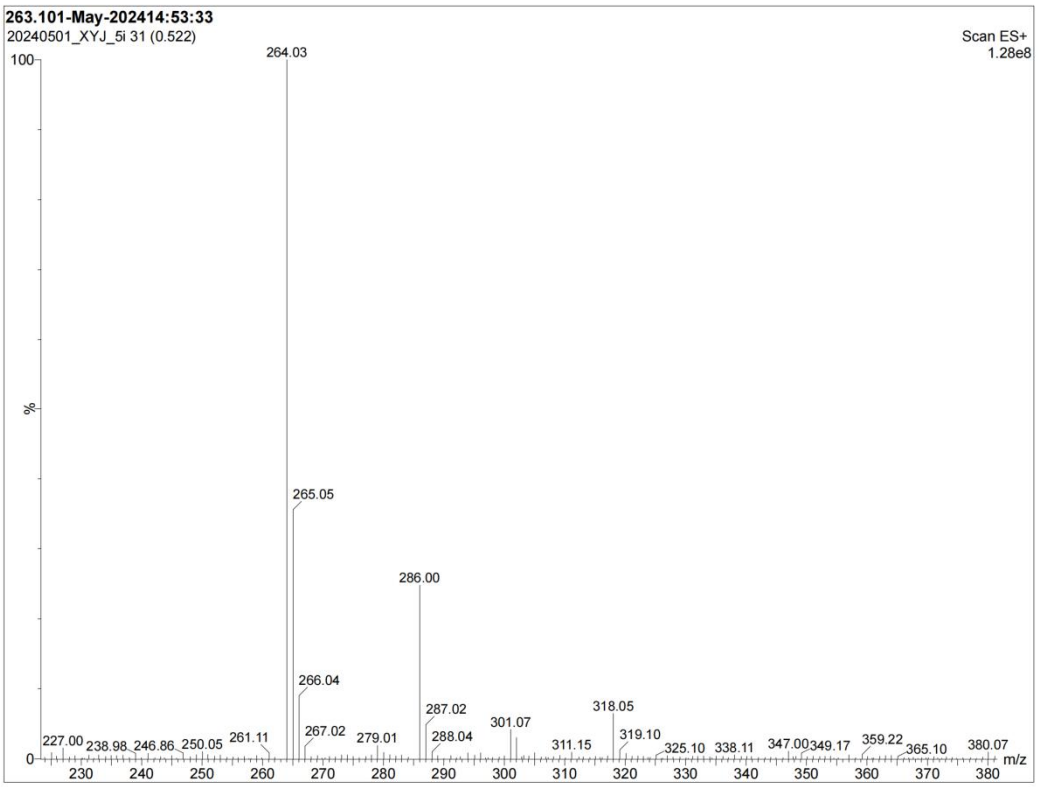


^{13}C-NMR spectrum of compound 5h

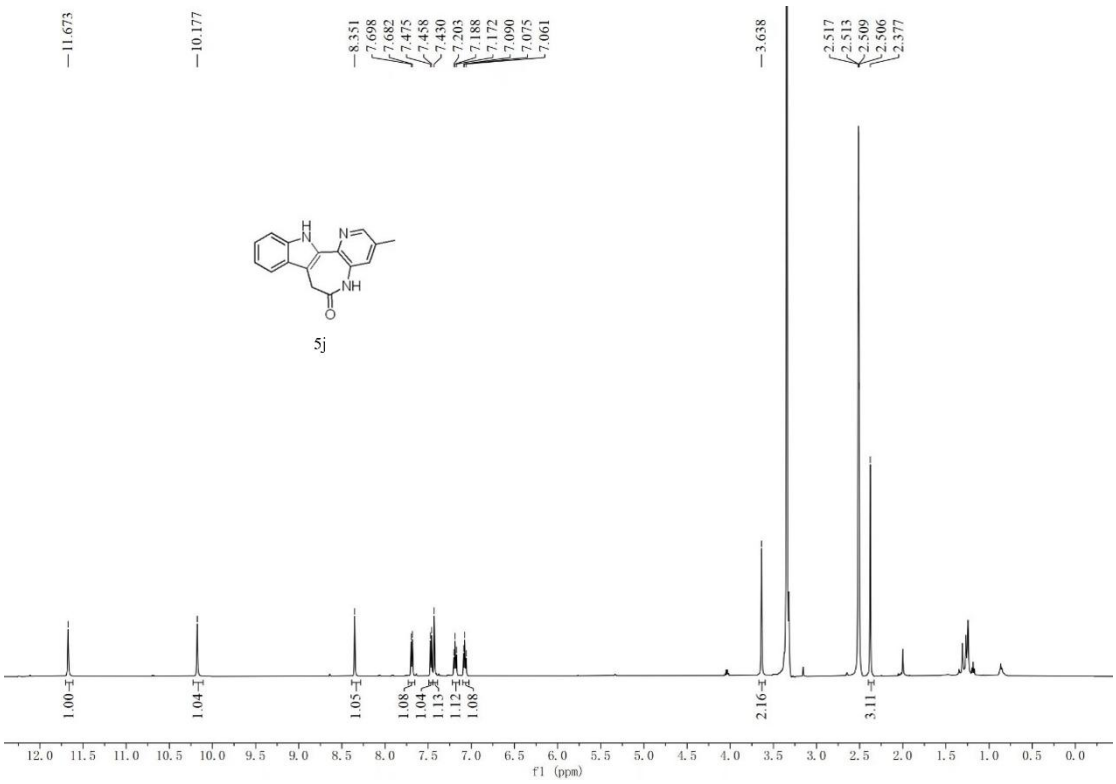
HRMS spectrum of compound 5h



[1]H-NMR spectrum of compound 5i

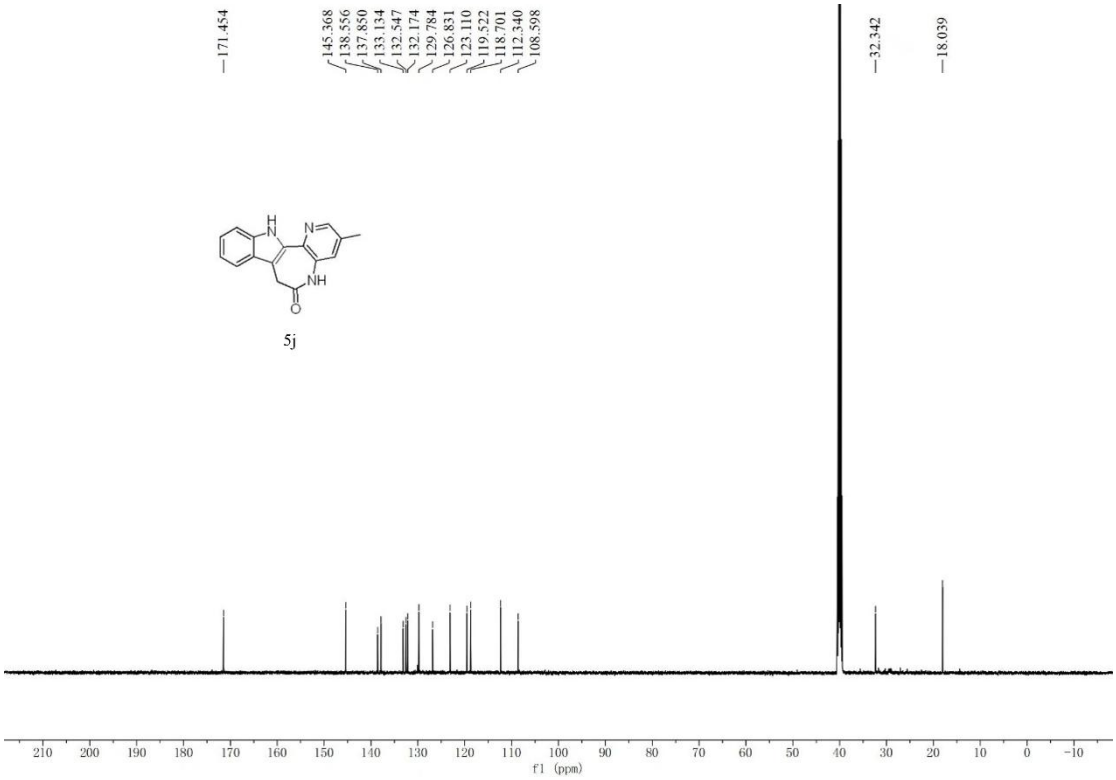


MS spectrum of compound 5i



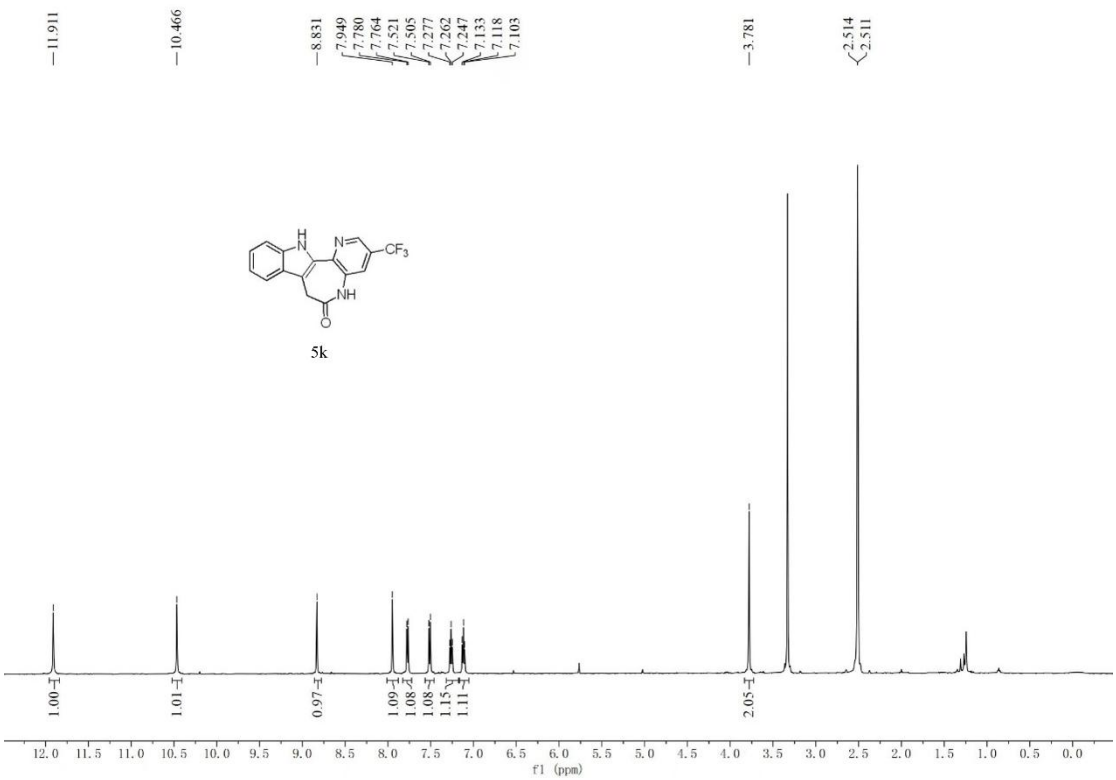
5j

^{1}H-NMR spectrum of compound 5j

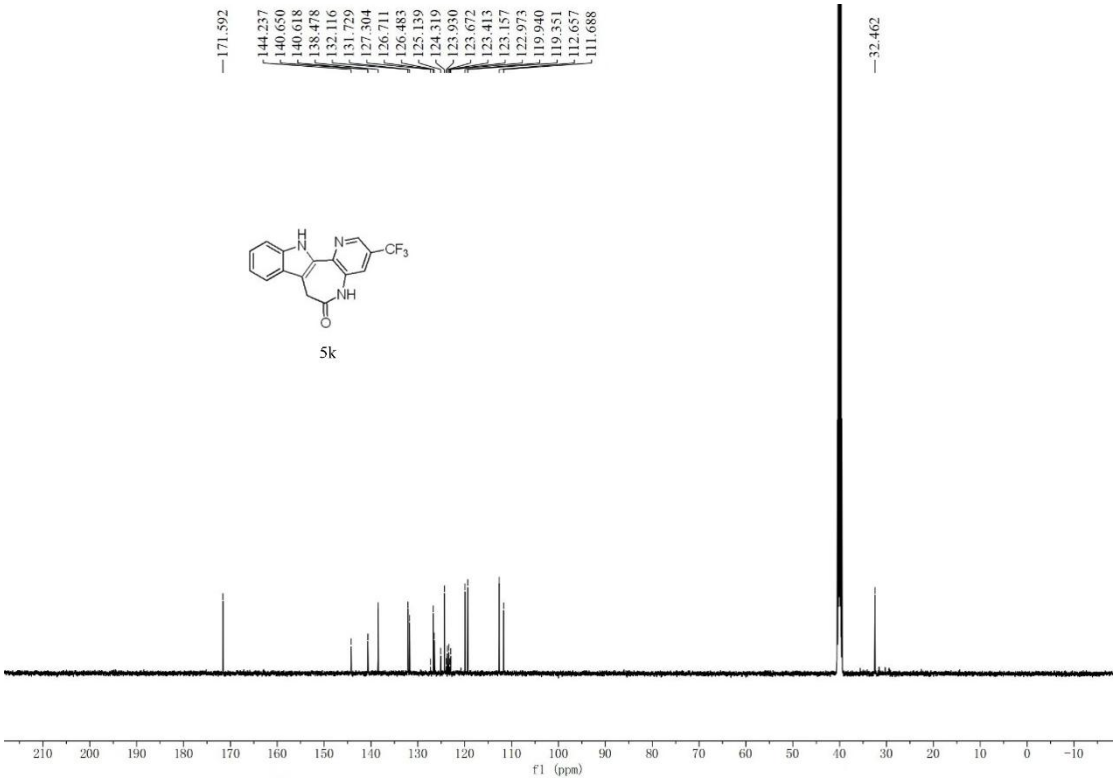


^{13}C-NMR spectrum of compound 5j

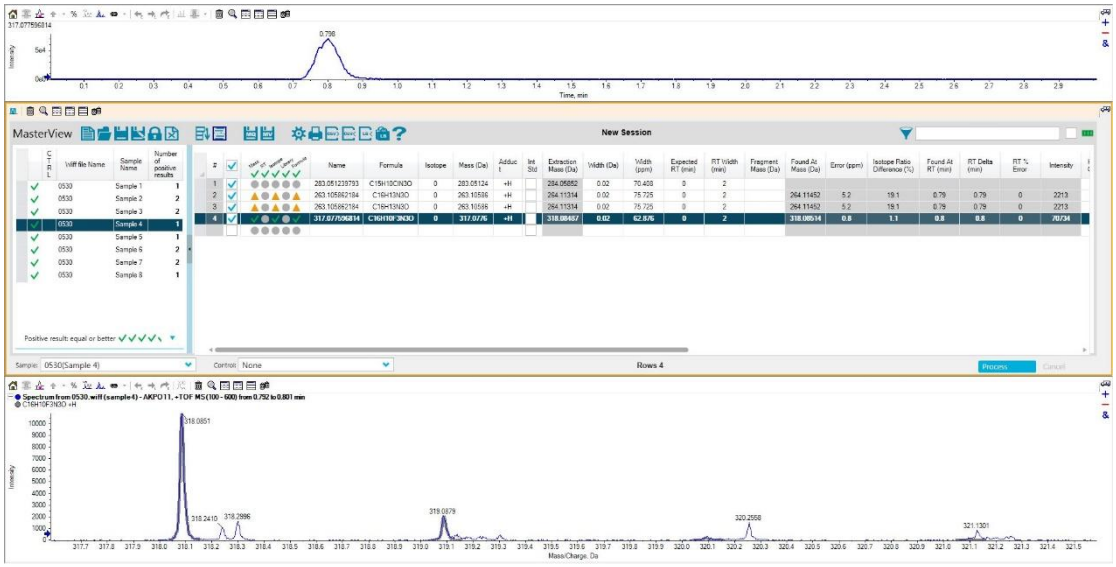
HRMS spectrum of compound 5j



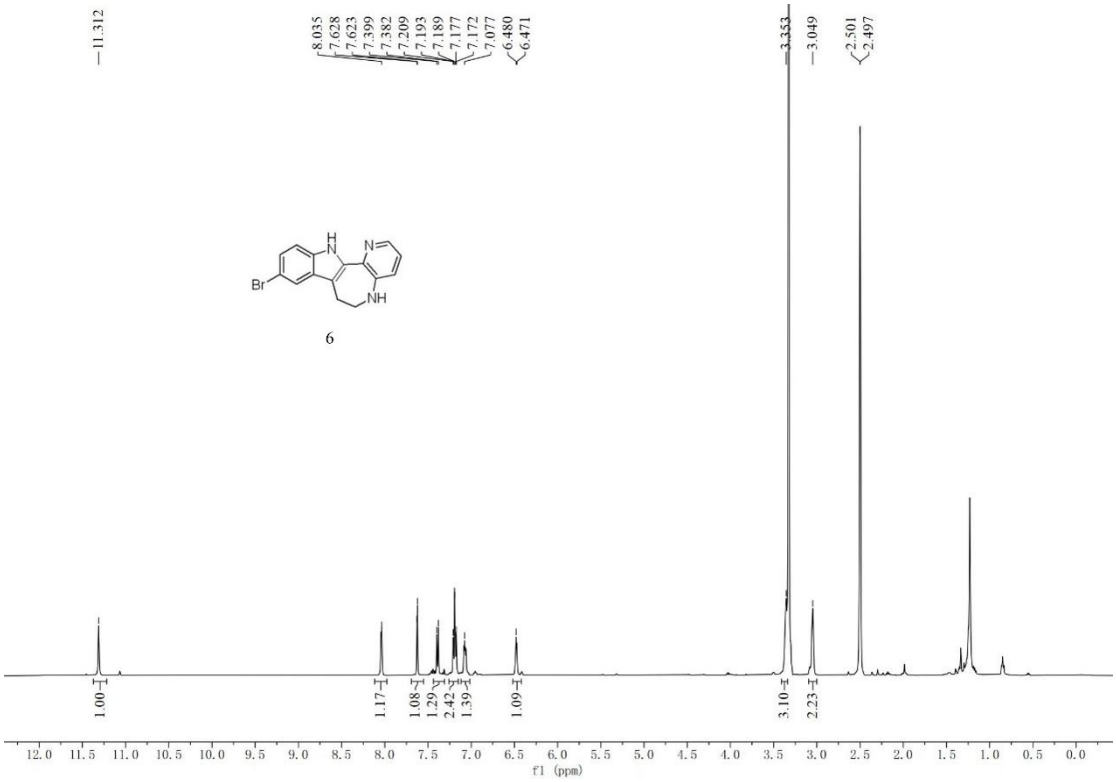
[1]H-NMR spectrum of compound 5k



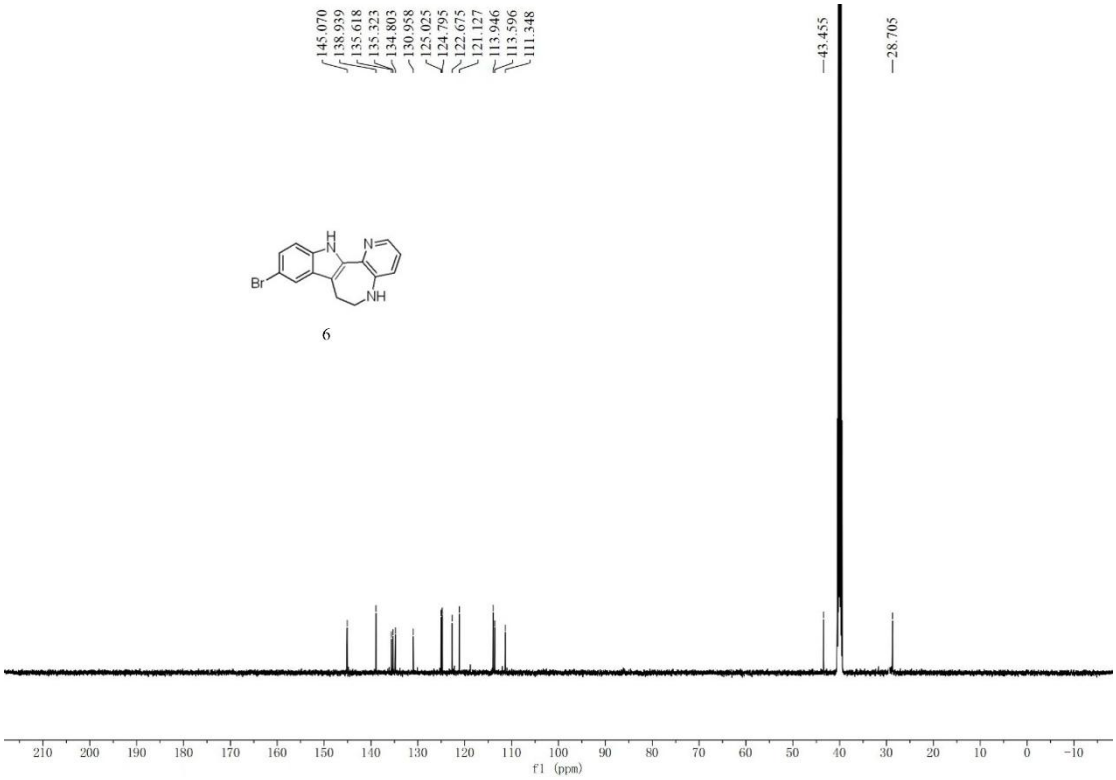
[13]C-NMR spectrum of compound 5k



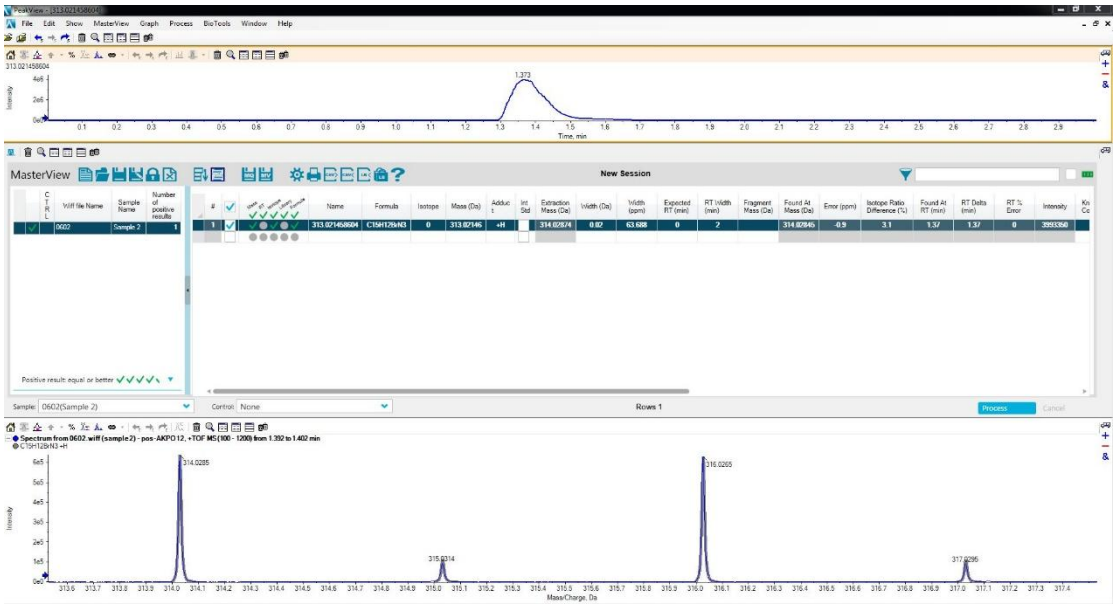
HRMS spectrum of compound 5k



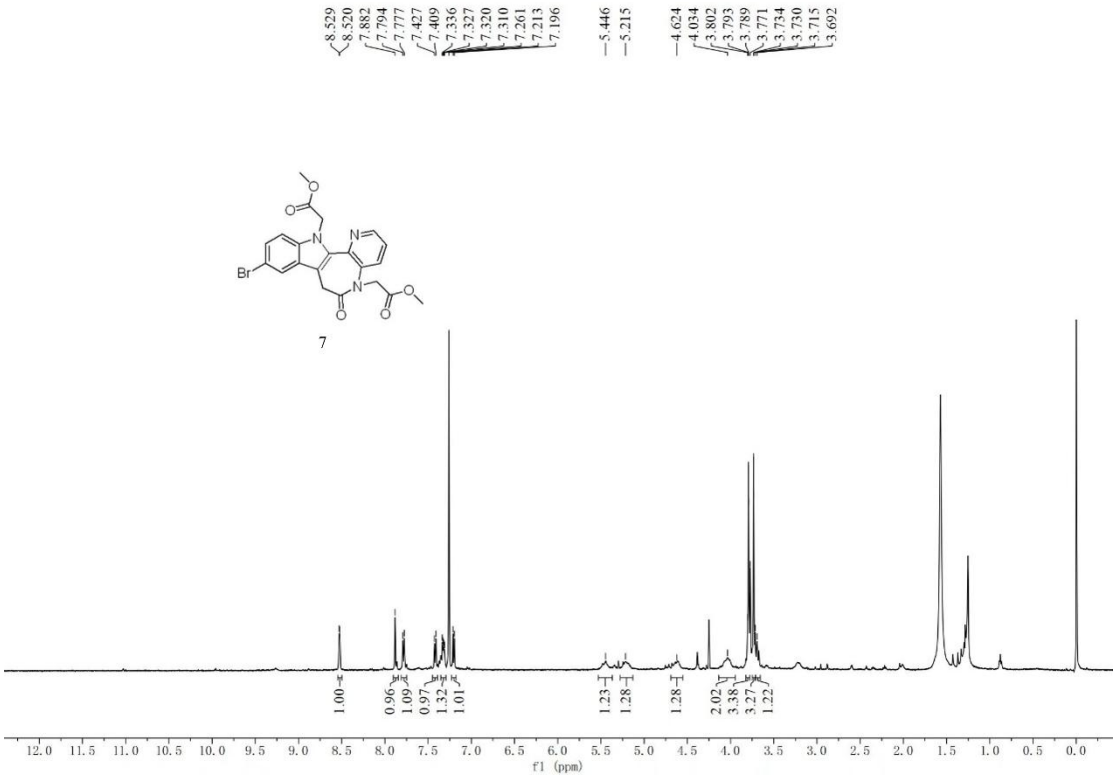
^{1}H-NMR spectrum of compound 6



^{13}C-NMR spectrum of compound 6

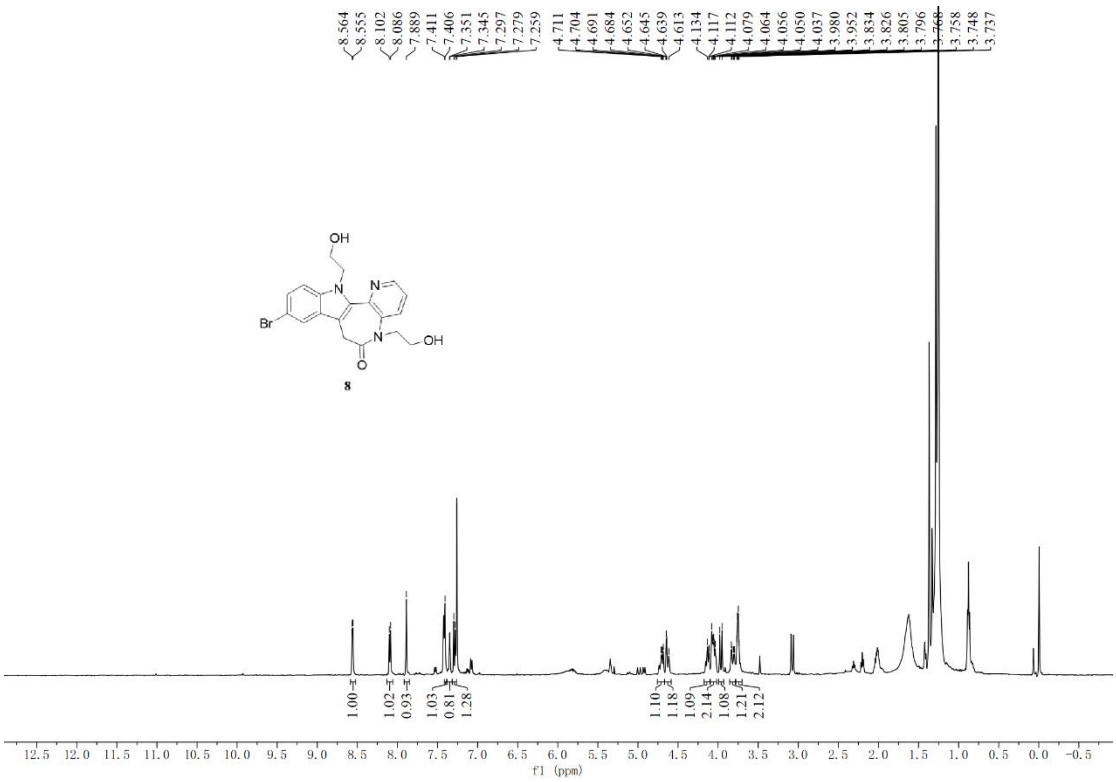


HRMS spectrum of compound 6

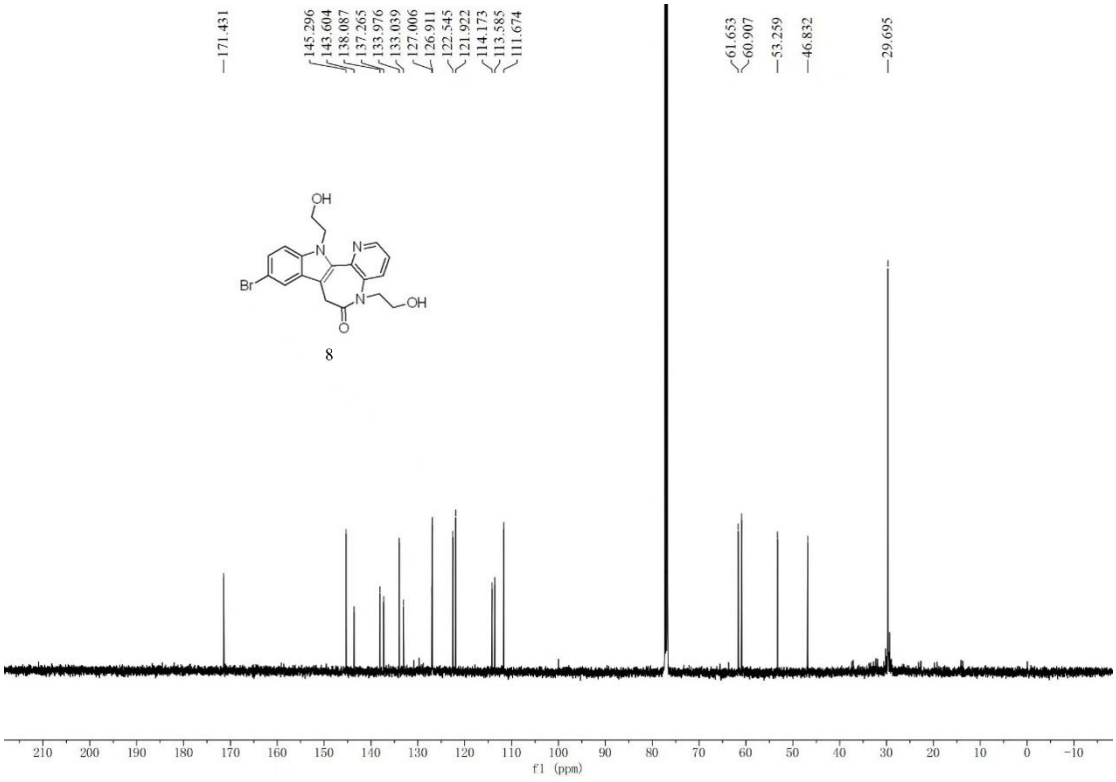


^{1}H-NMR spectrum of compound 7

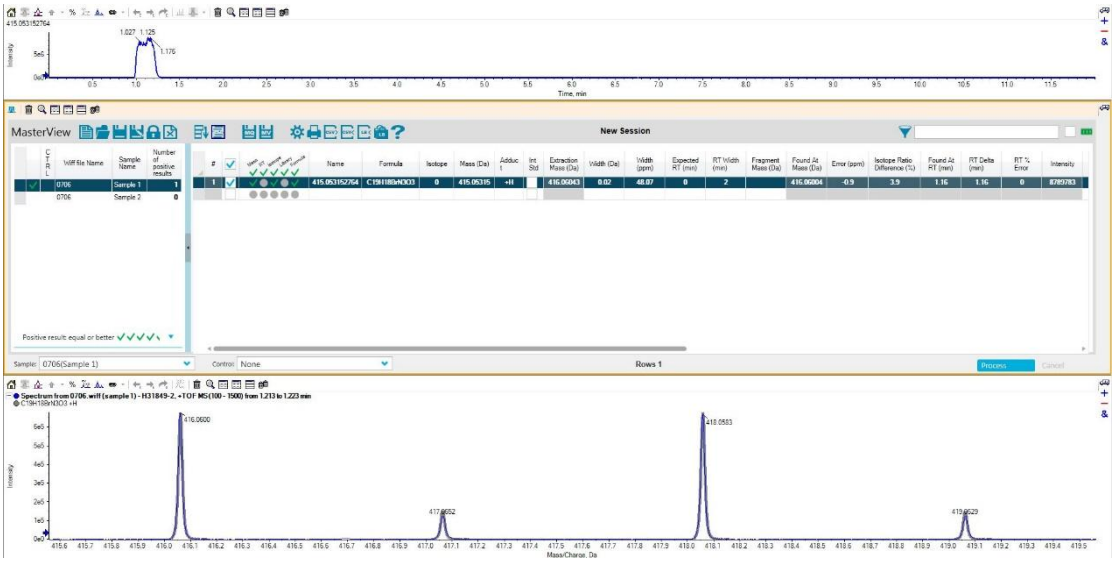
HRMS spectrum of compound 7



^{1}H-NMR spectrum of compound 8



^{13}C-NMR spectrum of compound 8



HRMS spectrum of compound 8