Design, Synthesis and Molecular Mechanisms of Novel Dual Inhibitors of Heat Shock Protein 90/Phosphoinositide 3-Kinase alpha (Hsp90/PI3Kα) Against Cutaneous Melanoma

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Supporting Information

Experimental section

Melting points (not corrected) were determined using a Reichert thermobaric apparatus. Proton nuclear magnetic resonance (^{1}H NMR, 400 MHz) spectra and carbon nuclear magnetic resonance (^{13}C NMR, 100 MHz) spectra were recorded on a Bruker Avance III NMR spectrometer. Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent ($CHCl_3$: δ 7.26; DMSO-d_6: δ 2.50). Chemical shifts for carbon are reported in parts per million downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent ($CHCl_3$: δ 77.0; DMSO-d_6: δ 39.5). Mass spectra were run using a Waters ESI-QTOF mass spectrometer (Waters, USA) equipped with an interchangeable ESI Z-spray source. Samples were dissolved in methanol which were infused by a syringe pump at a flow rate of 5 mL/min. Nitrogen was used as nebulizing gas, desolvation gas and cone curtain gas. Chemicals received from commercial sources were used without further purification.

Kinase assays

Kinase activity assay was performed by using ADP-Glo^{TM} kinase assay for Hsp90, EGFR, VEGFR2 and PI3Kα kinase system (Promega, Madison, WI, USA) as previously described. In brief, the kinase protein, substrate, ATP, and compound were diluted in kinase buffer (40 mM Tris, pH 7.5; 20 mM MgCl2; 0.1 mg/mL BSA; 50 μM DTT). Then 1μL of compound or 5% DMSO, 2 μL of kinase protein (10 ng), 2 μL of MBP (0.1 μg/μL)/ATP (10 μM) mix were added to the 384-well plate. After incubation at room temperature for 60 min, 5 μL of ADP-Glo reagent was added to each well. The plates were incubated at room temperature for 40 min, then an amount of 10μL of kinase detection reagent was added. After incubation at room temperature for 30 min, the luminescence was recorded.

Flow cytometry analysis

Following treatment for 24 hours with 8m, B16 cells were stained with propidium iodide (PI) to monitor cell-cycle progression. The percentage of cells undergoing apoptosis was measured by Annexin V-FITC Apoptosis Detection Kit (Keygen) per manufacturer's instructions.

Colony formation assay

Cell colony formation ability was measured by plate colony formation assay. 300 cells were added to each well of a 6-well plate. After about 2 days of incubation, the cells were treated with DMSO or 8m at various concentrations and incubated until colony was obviously formed. Then the plate was gently washed and stained with crystal violet. The number of colonies was counted according to cell numbers in each colony using a microscope.

Fluorescence microscopy

Morphological assessment of apoptotic cells was processed by Hoechst 33258 staining method as described earlier. Briefly, cells were cultured in 6-well plate overnight then treated with various concentrations of 8m or DMSO (vehicle control) for 24 hours. After washing with PBS for twice and incubating with fixative solution for 10 min, the cells were treated with 500 μL staining solution of Hoechst 33258 (10 μg/ml) for 5-10 min, followed by washing with PBS for twice again for reducing the background. Finally, the nuclear morphological changes of apoptotic cells were observed under a fluorescent microscope at ×200 magnifications.

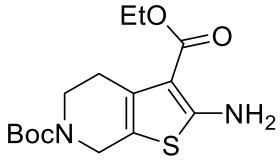
Statistical analysis

Tumor growth kinetics experiments were tested using the nonparametric Mann–Whitney *U* test. Data from all other experiments were tested by *t*-test, using the Prism 6.0 statistical program (GraphPad Prism 6.0, San Diego, CA, USA) and presented as mean ± standard deviation (SD) or

as indicated. A difference at $p < 0.05$ was considered as statistical significance (marked as *) and higher significance level was set at $p < 0.01$ (marked as **).

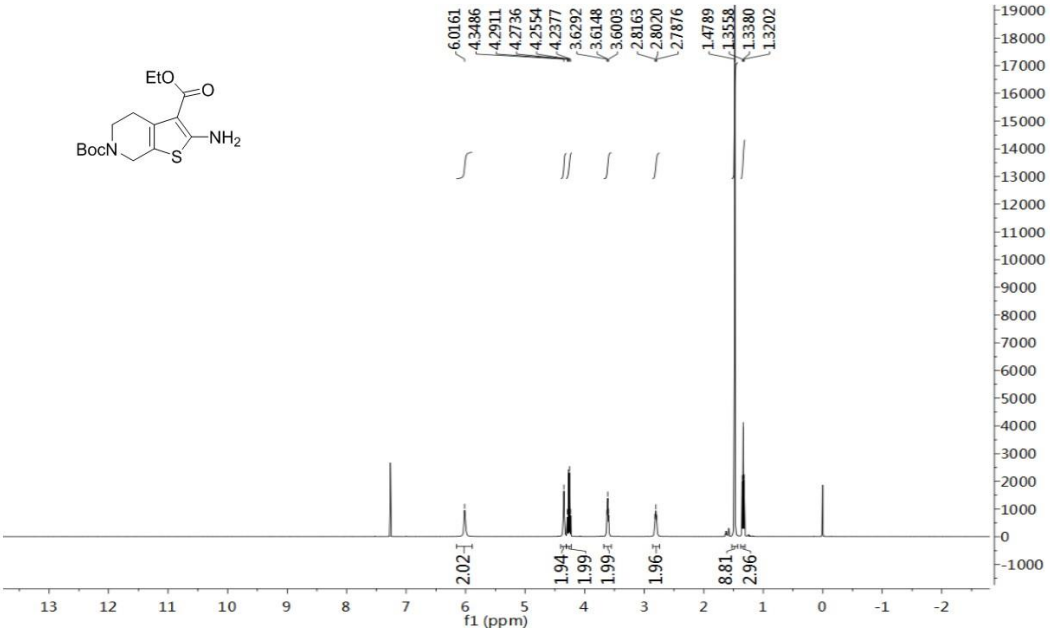
2. Characterization of key intermediates and compounds 8a-n and NMR Spectra

Compound 1

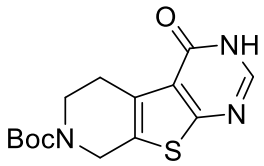


Yield: 86%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.02 (s, 2H), 4.35 (s, 2H), 4.26 (q, *J* = 7.0 Hz, 2H), 3.61 (t, *J* = 5.7 Hz, 2H), 2.80 (t, *J* = 5.7 Hz, 2H), 1.48 (s, 9H), 1.34 (t, *J* = 7.0 Hz, 3H).

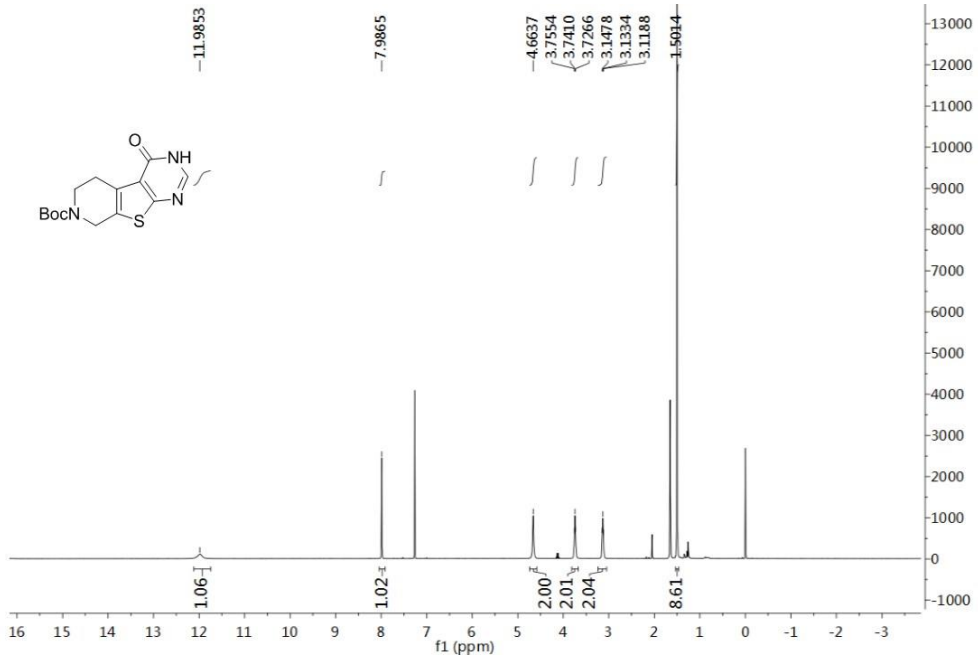


Compound 2

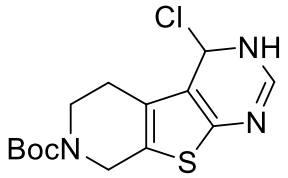


Yield: 90%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.99 (s, 1H), 7.99 (s, 1H), 4.66 (s, 2H), 3.74 (t, *J* = 5.8 Hz, 2H), 3.13 (t, *J* = 5.8 Hz, 2H), 1.50 (s, 9H).

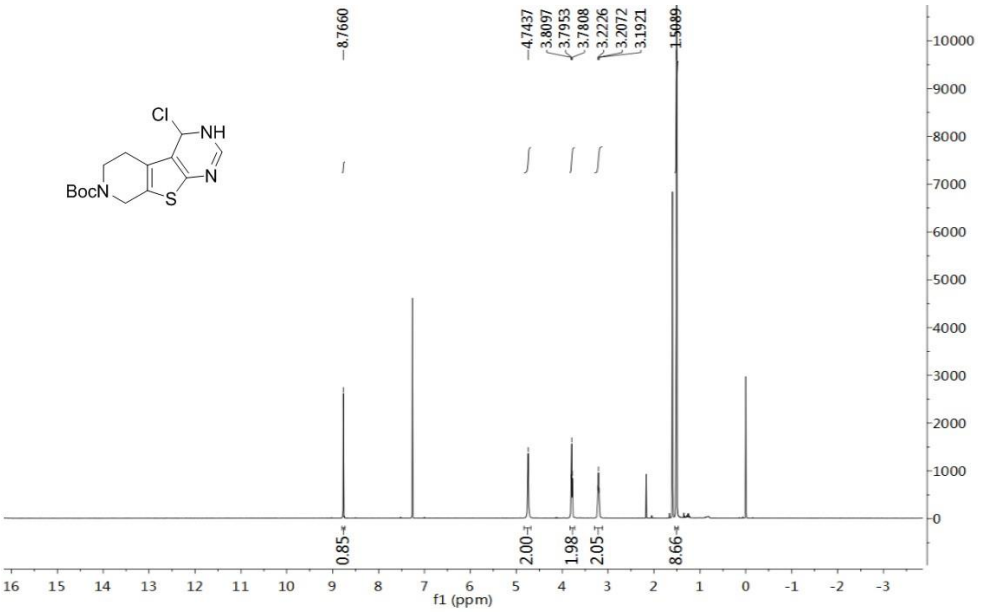


Compound 3

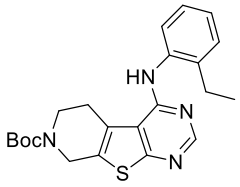


Yield: 85%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.77 (s, 1H), 4.74 (s, 2H), 3.80 (t, *J* = 5.8 Hz, 2H), 3.21 (t, *J* = 5.8 Hz, 2H), 1.51 (s, 9H).

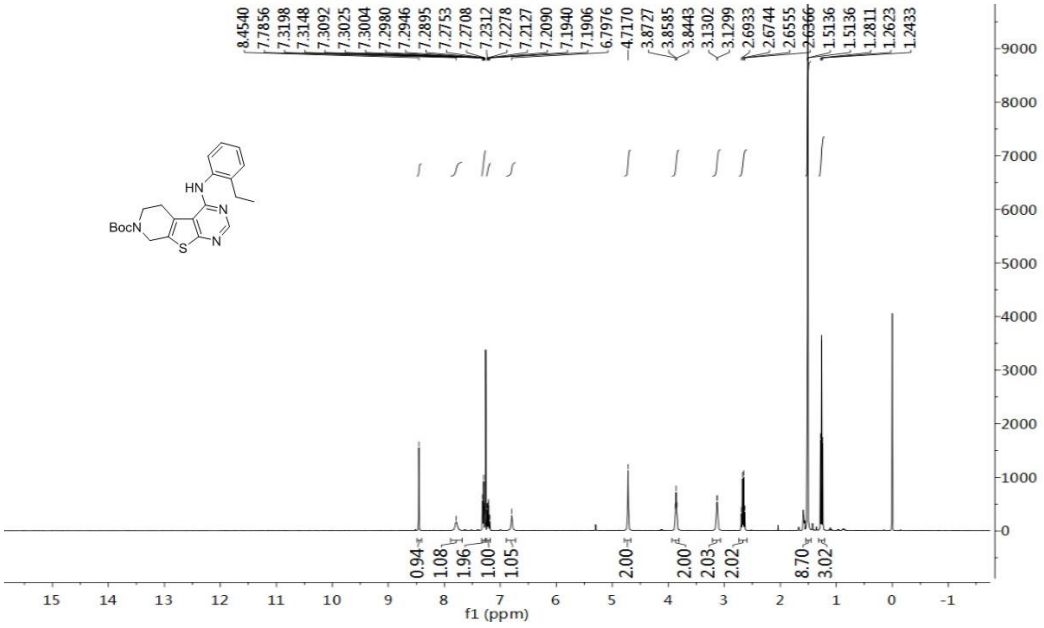


Compound 4a

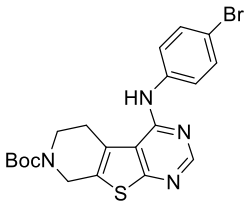


Light yellow powder, m. p. 159.0 – 160.4℃, yield: 55%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.45 (s, 1H), 7.34 – 7.27 (m, 2H), 7.21 (td, *J* = 7.4, 1.4 Hz, 1H), 4.72 (t, *J* = 2.0 Hz, 2H), 3.86 (t, *J* = 5.7 Hz, 2H), 3.13 (t, *J* = 5.7 Hz, 2H), 2.66 (q, *J* = 7.6 Hz, 2H), 1.51 (s, 9H), 1.26 (t, *J* = 7.6 Hz, 3H).

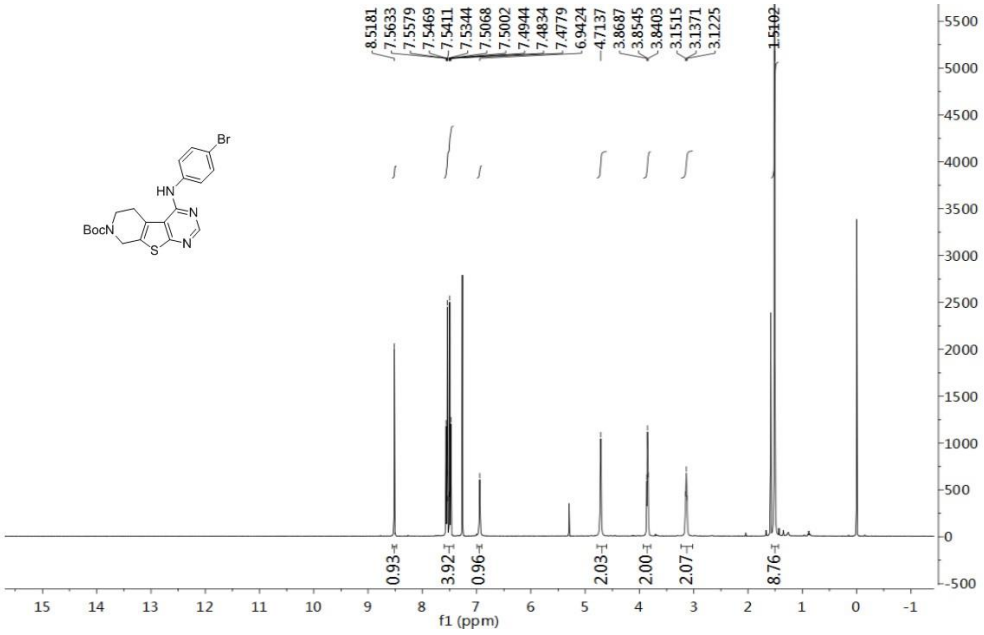


Compound 4b

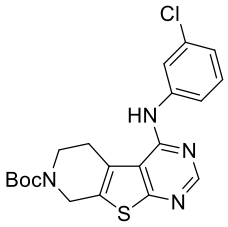


Light yellow powder, m. p. 178.9 – 181.7℃, yield: 66%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.52 (s, 1H), 7.58 – 7.46 (m, 4H), 6.94 (s, 1H), 4.71 (s, 2H), 3.85 (t, *J* = 5.7 Hz, 2H), 3.14 (d, *J* = 5.7 Hz, 2H), 1.51 (s, 8H).

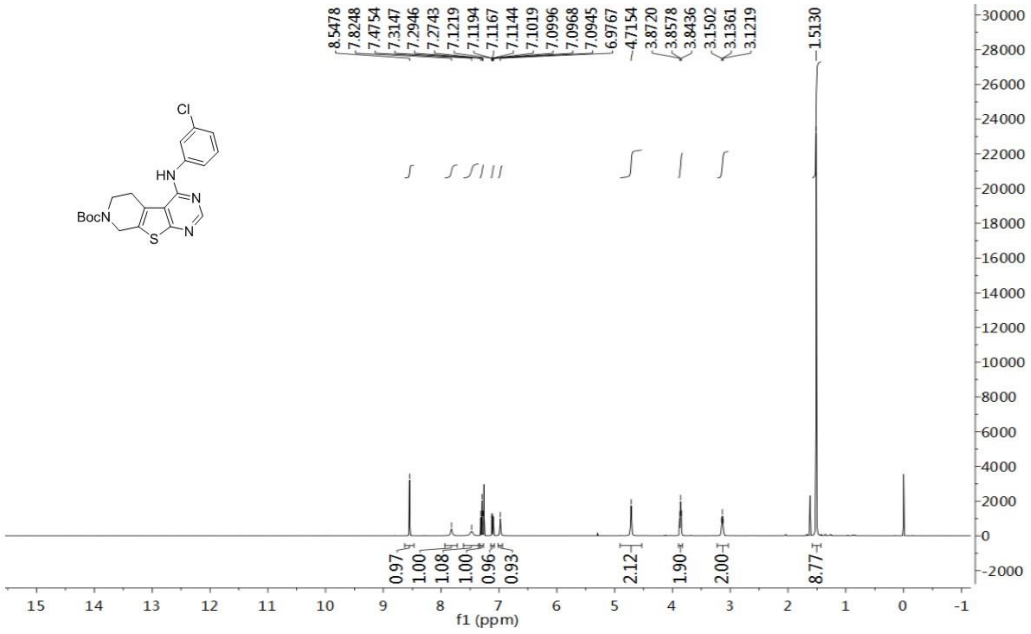


Compound 4c

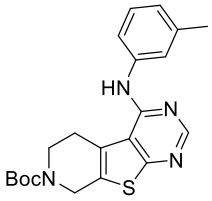


Light yellow powder, m. p. 180.4 – 181.5℃, yield: 65%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.55 (s, 1H), 7.82 (s, 1H), 7.48 (s, 1H), 7.29 (t, *J* = 8.1 Hz, 1H), 7.11 (ddd, *J* = 8.0, 2.1, 1.0 Hz, 1H), 6.98 (s, 1H), 4.72 (s, 2H), 3.86 (t, *J* = 5.7 Hz, 2H),3.14 (t, *J* = 5.7 Hz, 2H), 1.51 (s, 9H).

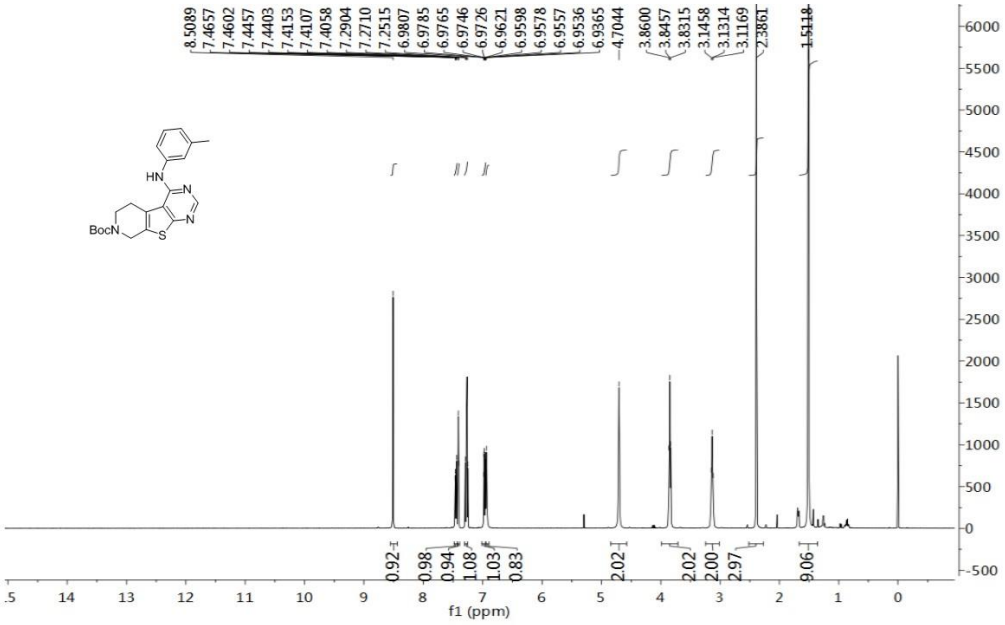


Compound 4d

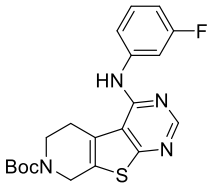


Light yellow powder, m. p. 150.7 – 152.4℃, yield: 59%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.51 (s, 1H), 7.45 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.41 (d, *J* = 1.8 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 6.97 (ddt, *J* = 7.6, 1.7, 0.9 Hz, 1H), 6.94 (s, 1H), 4.70 (s, 2H), 3.85 (t, *J* = 5.7 Hz, 2H), 3.13 (t, *J* = 5.7 Hz, 2H), 2.39 (s, 3H), 1.51 (s, 9H).

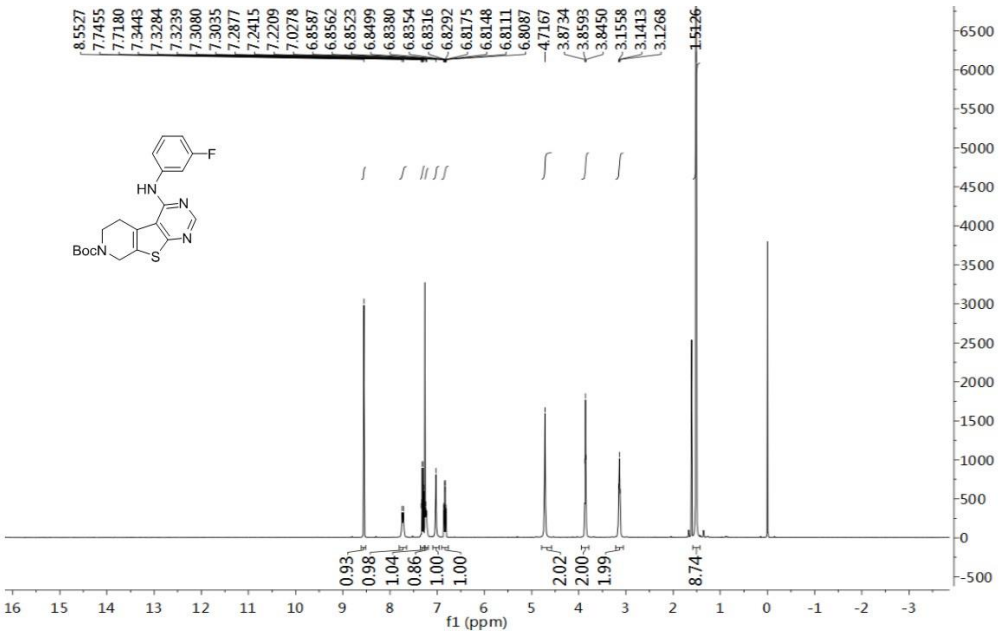


Compound 4e

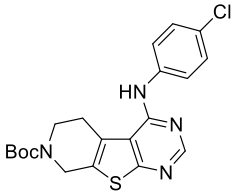


Light yellow powder, m. p. 164.4 – 165.0℃, yield: 69%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.55 (s, 1H), 7.73 (d, *J* = 11.0 Hz, 1H), 7.32 (td, *J* = 8.1, 6.3 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.03 (s, 1H), 6.83 (tdd, *J* = 8.2, 2.5, 1.0 Hz, 1H), 4.72 (s, 2H), 3.86 (t, *J* = 5.7 Hz, 2H), 3.14 (t, *J* = 5.7Hz, 2H), 1.51 (s, 9H).

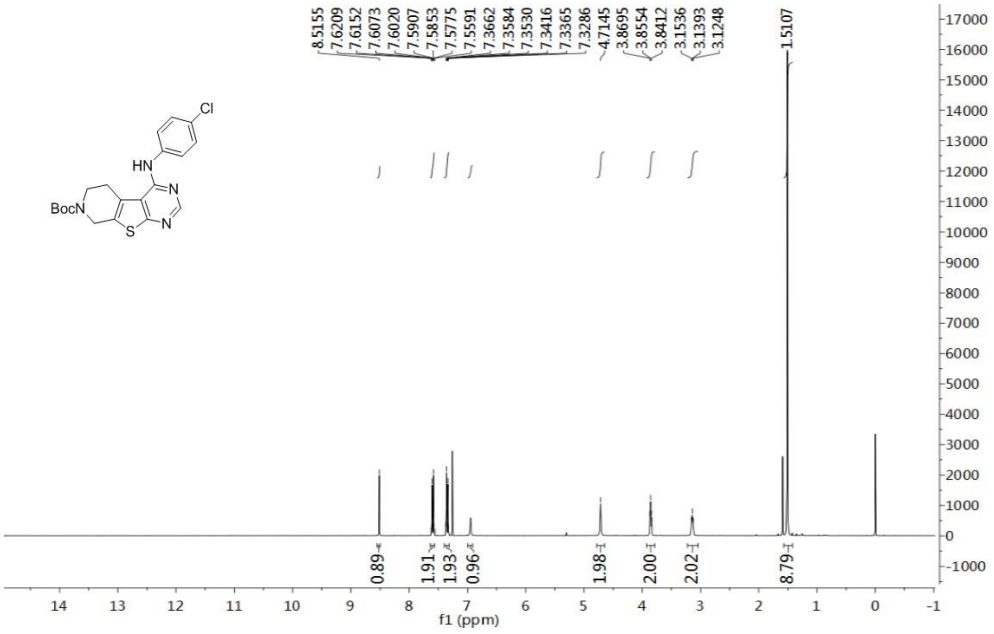


Compound 4f

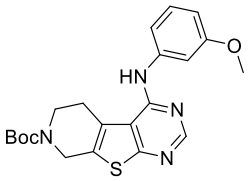


Light yellow powder, m. p. 183.7 – 185.6℃, yield: 68%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.52 (s, 1H), 7.64 – 7.57 (m, 2H), 7.40 – 7.32 (m, 2H), 6.95 (s, 1H), 4.71 (s, 2H), 3.86 (t, *J* = 5.7 Hz, 2H), 3.15 (t, *J* = 5.7 Hz, 2H), 1.51 (s, 9H).

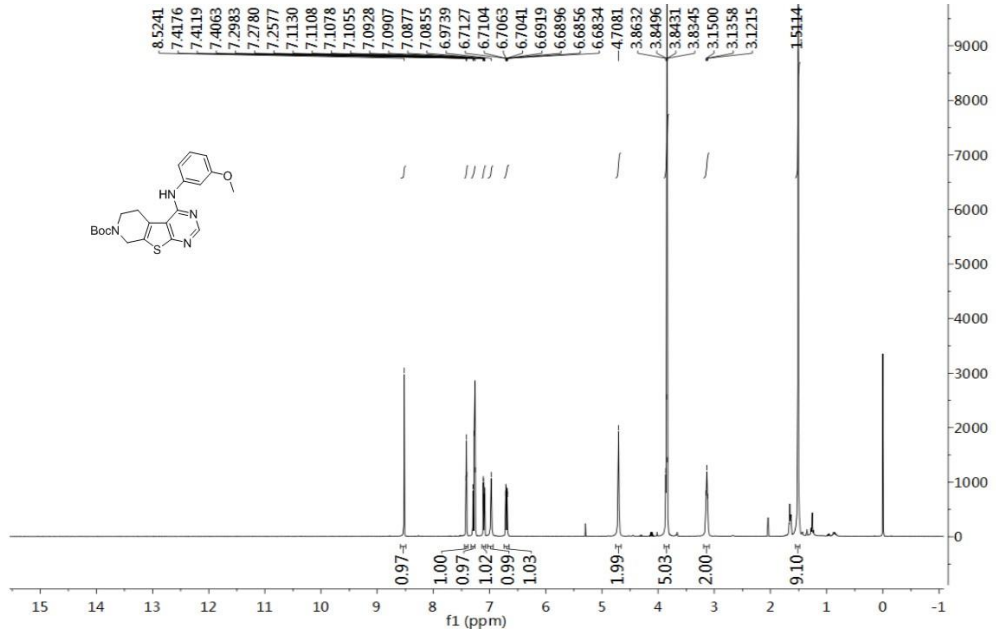


Compound 4g

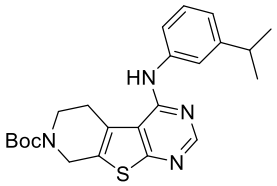


Light yellow powder, m. p. 136.3 – 139.6℃, yield: 58%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.52 (s, 1H), 7.41 (t, *J* = 2.3 Hz, 1H), 7.29 (t, *J* = 8.1 Hz, 1H), 7.10 (ddd, *J* = 8.0, 2.1, 0.9 Hz, 1H), 6.97 (s, 1H), 6.70 (ddd, *J* = 8.3, 2.5, 0.9 Hz, 1H), 4.71 (s, 2H), 3.89 – 3.81 (m, 5H), 3.14 (d, *J* = 5.7 Hz, 2H), 1.51 (s, 9H).

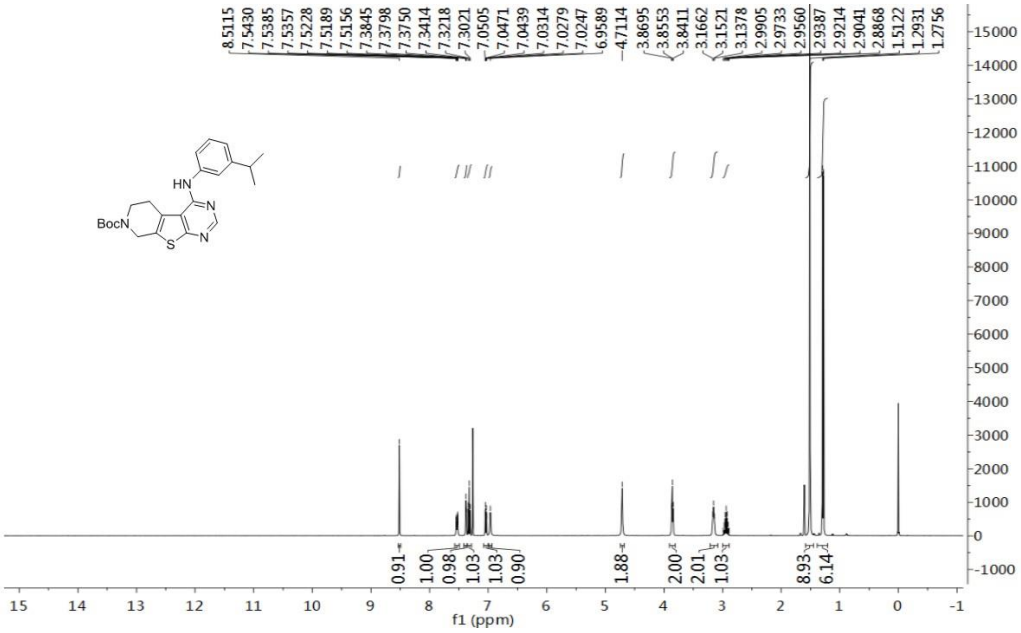


Compound 4h

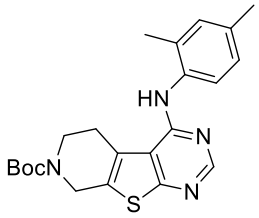


White powder, m. p. 115.7 – 117.2℃, yield: 56%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.51 (s, 1H), 7.53 (dt, *J* = 8.1, 1.6 Hz, 1H), 7.38 (t, *J* = 1.9 Hz, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.04 (dt, *J* = 7.7, 1.3 Hz, 1H), 6.96 (s, 1H), 4.71 (s, 2H), 3.86 (t, *J* = 5.7 Hz, 2H), 3.16 (d, *J* = 5.6 Hz, 2H), 2.94 (hept, *J* = 6.9 Hz, 1H), 1.51 (s, 9H), 1.28 (d, *J* = 7.0 Hz, 6H).

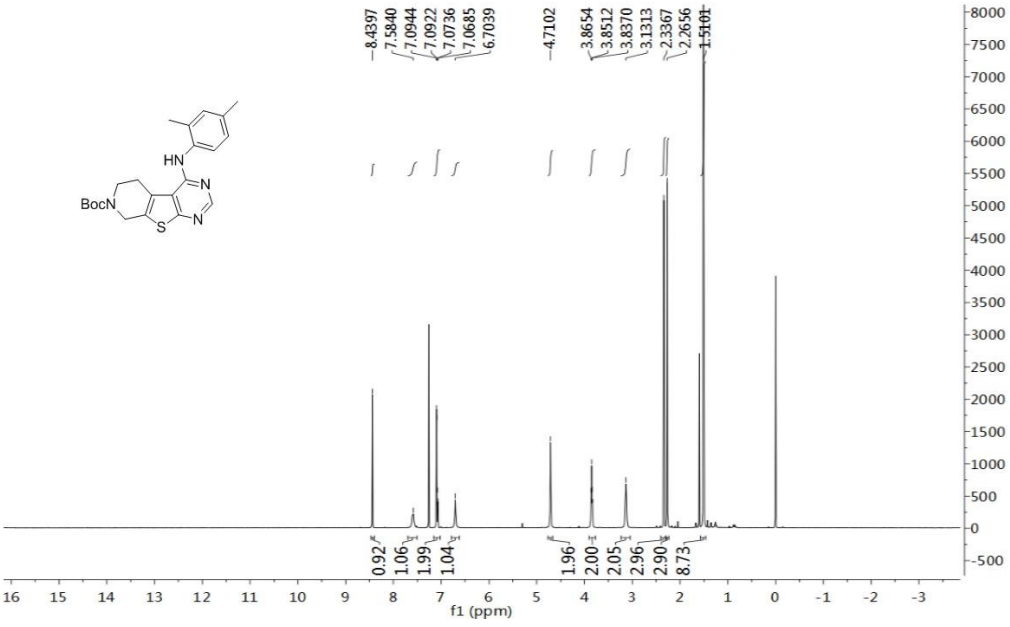


Compound 4i

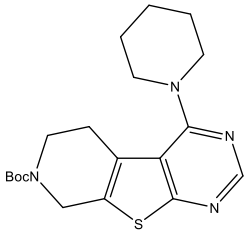


Yellow powder, m. p. 159.6 – 161.3℃, yield: 61%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.44 (s, 1H), 7.58 (s, 1H), 7.16 – 7.02 (m, 2H), 6.70 (s, 1H), 4.71 (s, 2H), 3.85 (t, *J* = 5.7 Hz, 2H), 3.13 (t, *J* = 5.7 Hz, 2H), 2.34 (s, 3H), 2.27 (s, 3H), 1.51 (s, 9H).

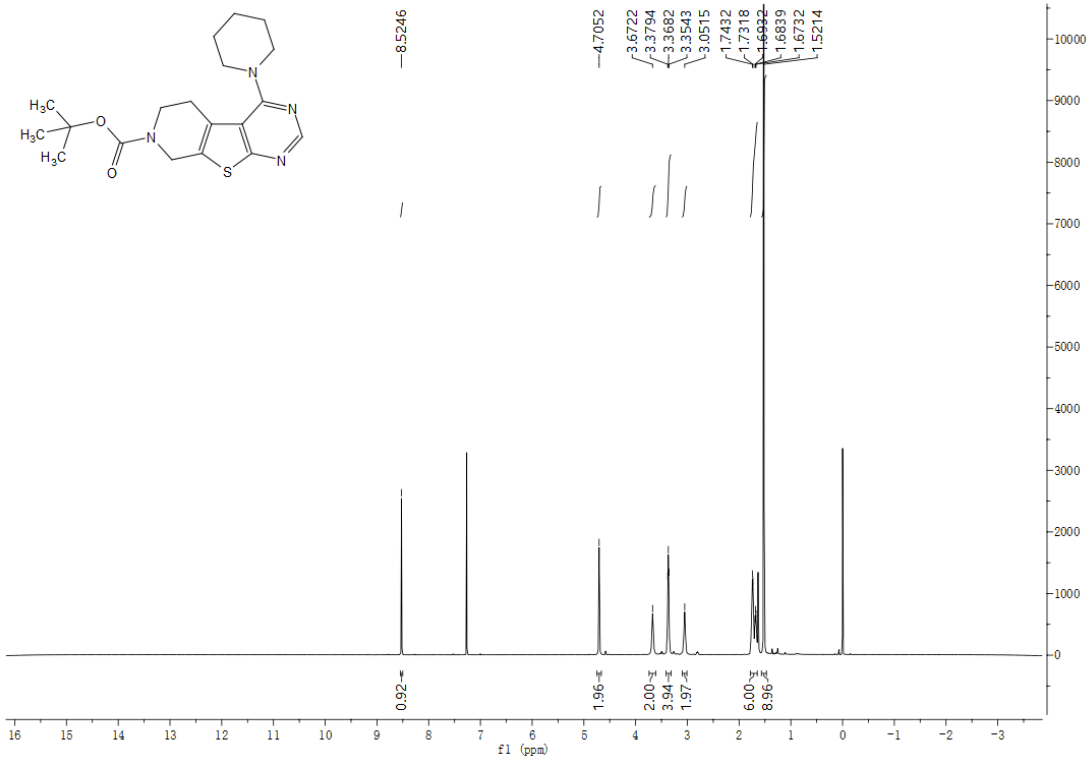


Compound 4k

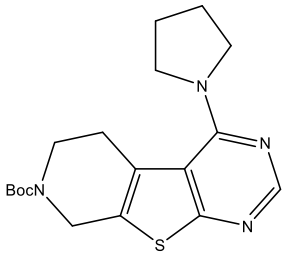


White powder, yield:91%

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 4.71 (s, 2H), 3.67 (s, 2H), 3.41 – 3.31 (m, 4H), 3.05 (s, 2H), 1.78 – 1.65 (m, 6H), 1.52 (s, 9H).

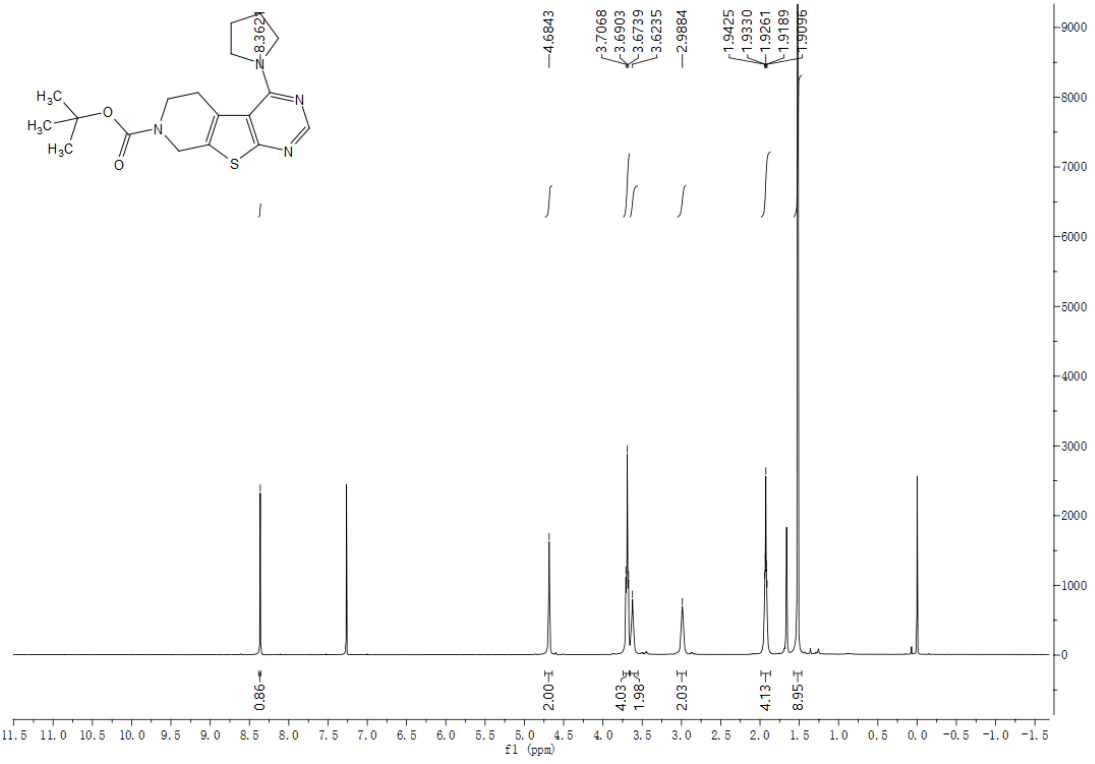


Compound 4l

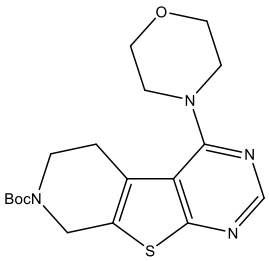


White powder, yield: 93%

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 4.68 (s, 2H), 3.69 (t, *J* = 6.6 Hz, 4H), 3.62 (s, 2H), 2.99 (s, 2H), 1.99 – 1.87 (m, 4H), 1.52 (s, 9H).

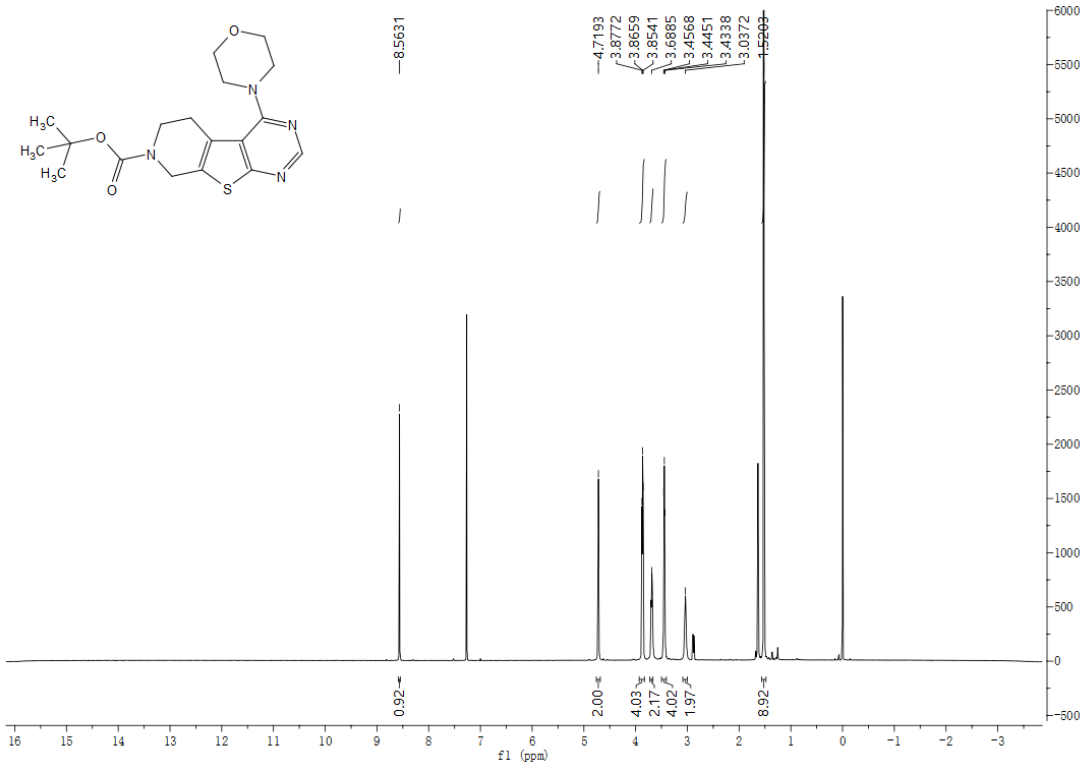


Compound 4m

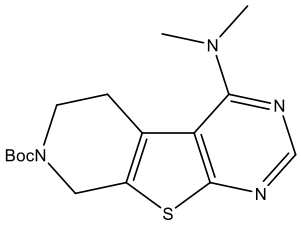


White powder, yield: 95%

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 4.72 (s, 2H), 3.93 – 3.83 (m, 4H), 3.69 (s, 2H), 3.50 – 3.41 (m, 4H), 3.04 (s, 2H), 1.52 (s, 9H).

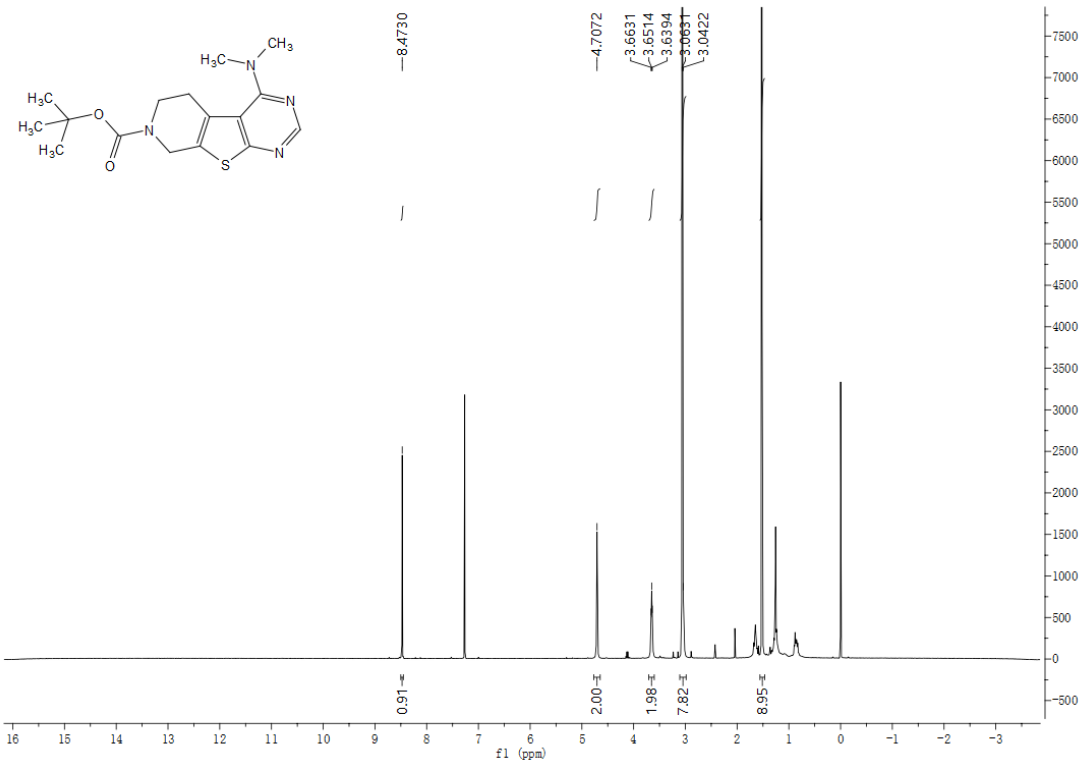


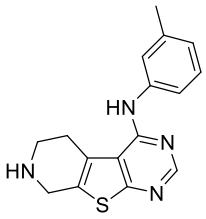
Compound 4n



White powder, yield: 89%

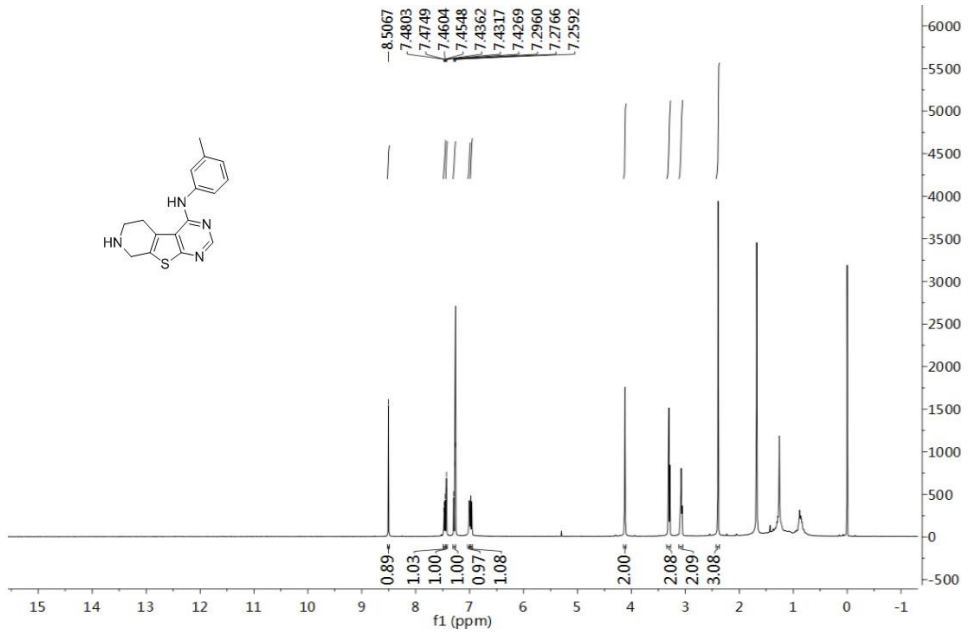
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 4.71 (s, 2H), 3.65 (t, *J* = 4.7 Hz, 2H), 3.06 (s, 6H), 3.04 (s, 2H), 1.52 (s, 9H).

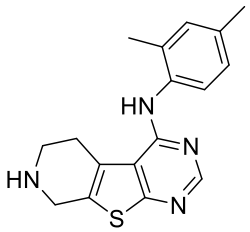




White powder, m. p. 174.9 – 178.0℃

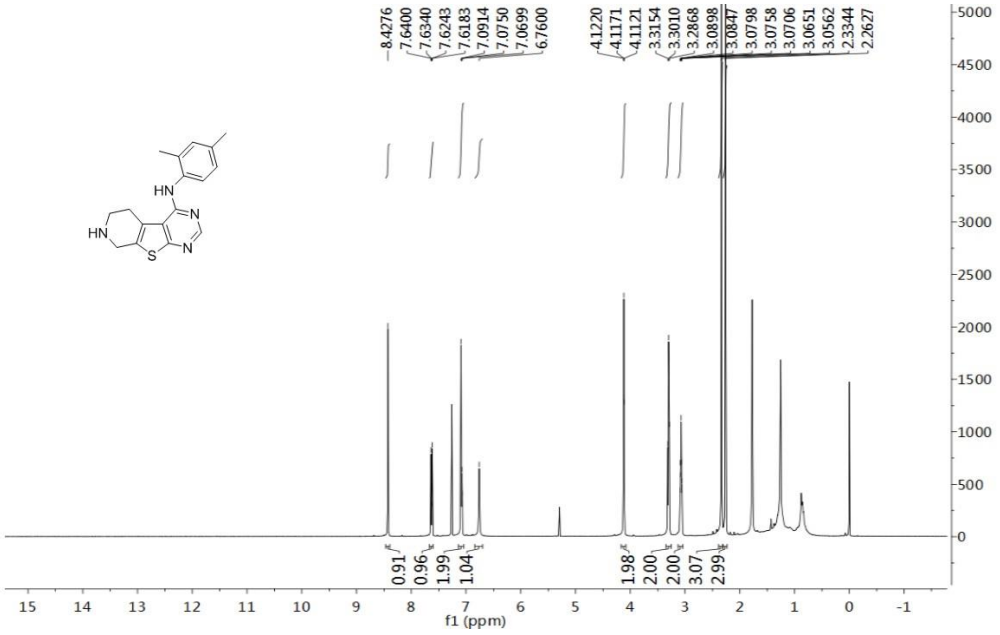
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.51 (s, 1H), 7.47 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.43 (d, *J* = 1.8 Hz, 1H), 7.29 (d, *J* = 7.8 Hz, 1H), 7.00 (s, 1H), 6.97 (ddt, *J* = 7.6, 1.7, 0.8 Hz, 1H), 4.12 (t, *J* = 2.0 Hz, 2H), 3.31 (t, *J* = 5.7 Hz, 2H), 3.08 (tt, *J* = 5.7, 2.0 Hz, 2H), 2.39 (s, 3H).

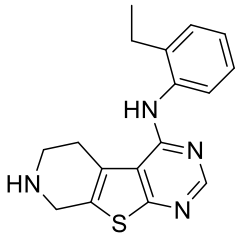




Light yellow powder, m. p. 164.6 – 169.0℃

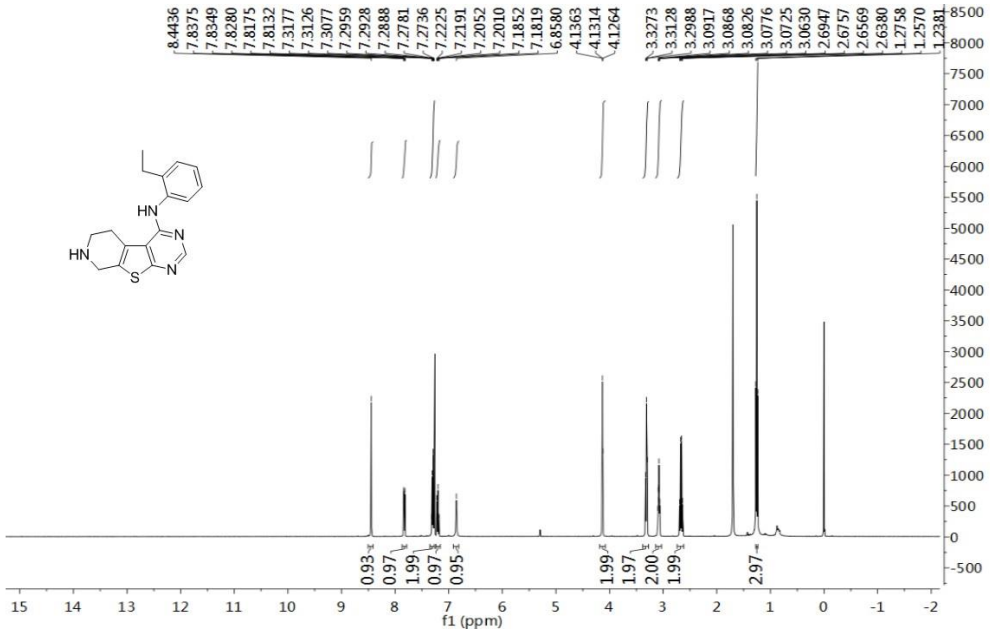
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.43 (s, 1H), 7.67 – 7.60 (m, 1H), 7.09 – 7.07 (m, 2H), 6.76 (s, 1H), 4.12 (t, *J* = 2.0 Hz, 2H), 3.30 (t, *J* = 5.7 Hz, 2H), 3.07 (tt, *J* = 5.7, 3.6, 2.0 Hz, 2H), 2.33 (s, 3H), 2.26 (s, 3H).

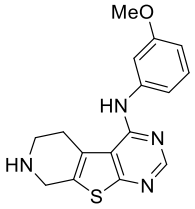




White powder, m. p. 178.8 – 180.9℃

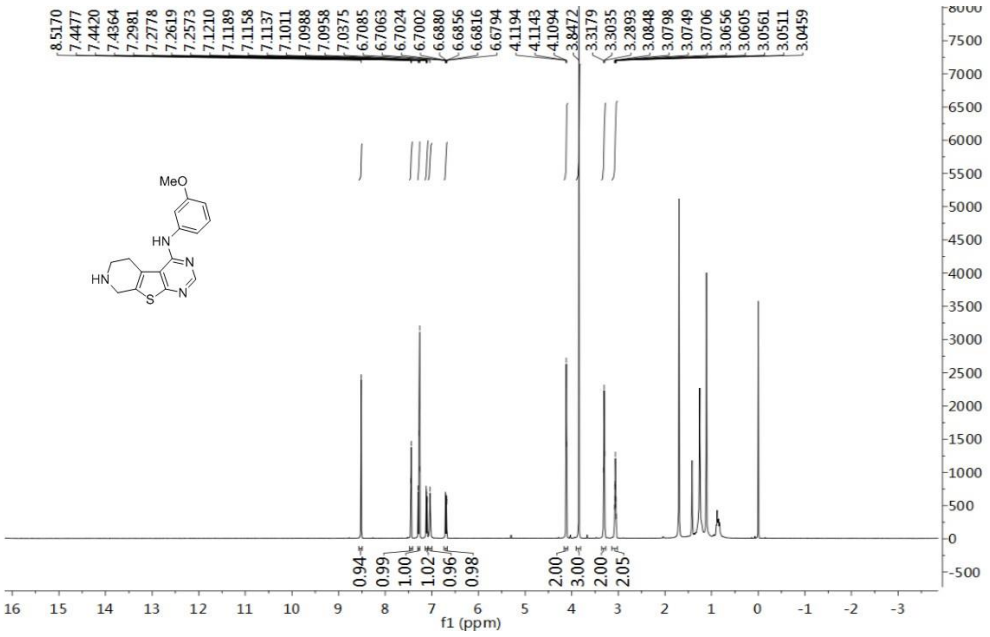
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.44 (s, 1H), 7.83 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.22 – 7.18 (m, 2H), 7.20 (td, *J* = 7.3, 1.4 Hz, 1H), 6.86 (s, 1H), 4.13 (t, *J* = 2.0 Hz, 2H), 3.31 (t, *J* = 5.7 Hz, 2H), 3.08 (tt, *J* = 5.8, 2.0 Hz, 2H), 2.67 (q, *J* = 7.6 Hz, 2H), 1.25 (d, *J* = 7.6 Hz, 3H).

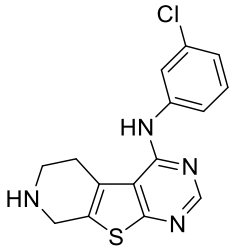




White powder, m. p. 179.2 – 180.5℃

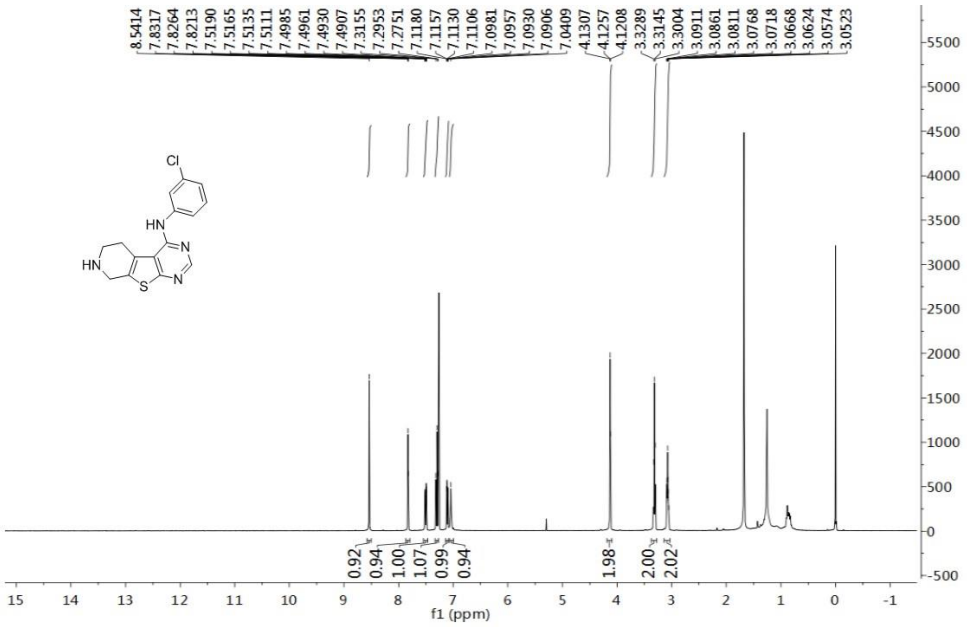
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.52 (s, 1H), 7.44 (t, *J* = 2.3 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.11 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 7.04 (s, 1H), 6.69 (ddd, *J* = 8.3, 2.5, 0.9 Hz, 1H), 4.11 (t, *J* = 2.0 Hz, 2H), 3.85 (s, 3H), 3.30 (t, *J* = 5.7 Hz, 2H), 3.07 (tt, *J* = 5.7, 2.0 Hz, 2H).

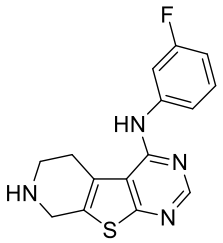




White powder, m. p. 157.6 – 159.6℃

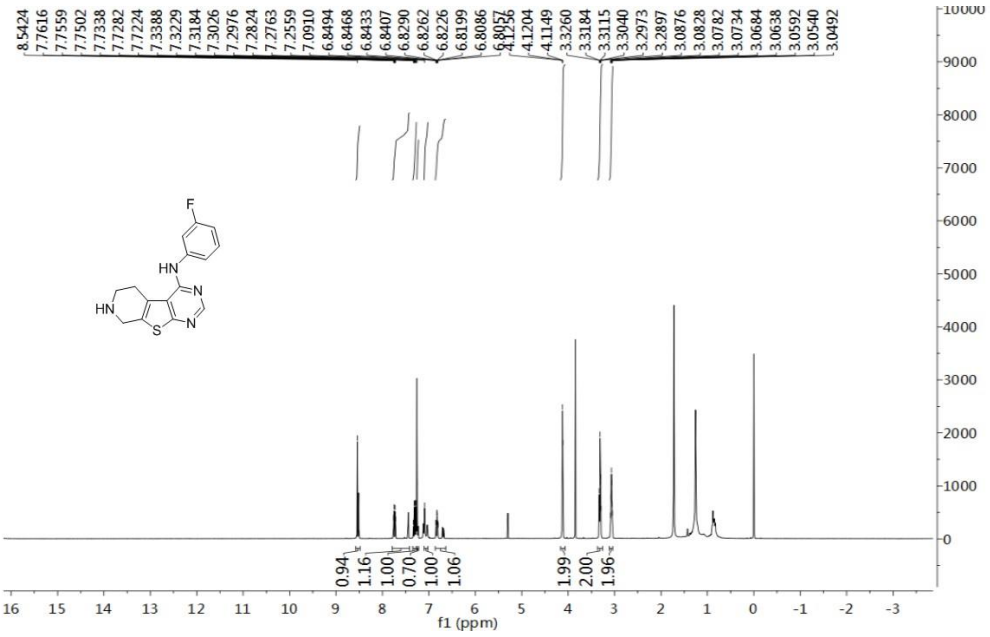
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.54 (s, 1H), 7.83 (t, *J* = 2.1 Hz, 1H), 7.50 (ddd, *J* = 8.2, 2.2, 1.0 Hz, 1H), 7.30 (t, *J* = 8.1 Hz, 1H), 7.10 (ddd, *J* = 8.0, 2.0, 0.9 Hz, 1H), 7.04 (s, 1H), 4.13 (t, *J* = 2.0 Hz, 2H), 3.31 (t, *J* = 5.7 Hz, 2H), 3.07 (tt, *J* = 5.7, 2.0 Hz, 2H).

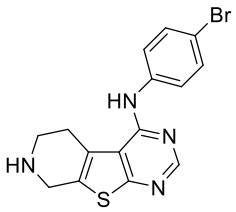




White powder, m. p. 146.6 – 150.9℃

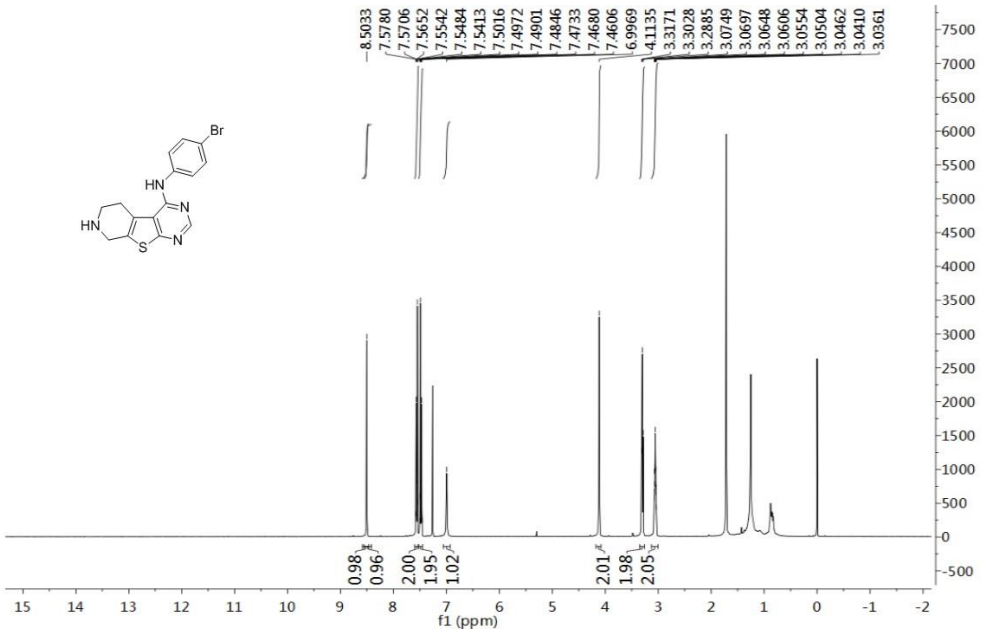
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.54 (s, 1H), 7.74 (dt, *J* = 11.1, 2.3 Hz, 1H), 7.35 – 7.27 (m, 1H), 7.25 (s, 1H), 7.09 (s, 1H), 6.82 (tdd, *J* = 8.2, 2.5, 1.1 Hz, 1H), 4.12 (t, *J* = 2.2 Hz, 2H), 3.31 (td, *J* = 5.8, 3.0 Hz, 2H), 3.07 (tt, *J* = 5.8, 1.9 Hz, 2H).

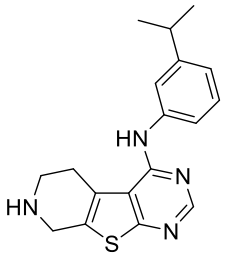




White powder, m. p. 176.4 – 179.4℃

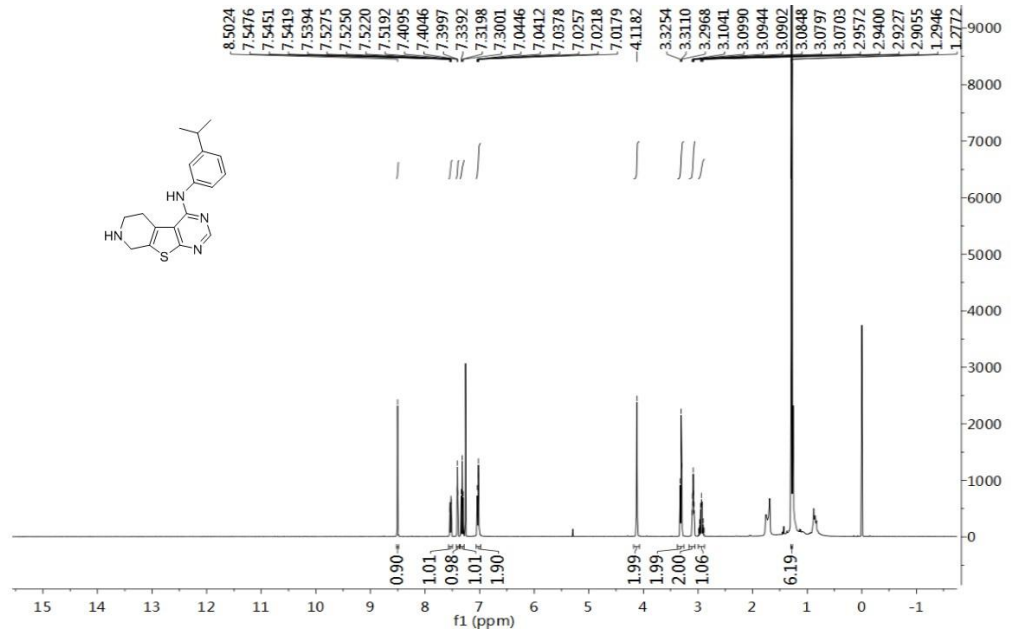
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.50 (s, 1H), 7.60 – 7.53 (m, 2H), 7.52 – 7.45 (m, 2H), 7.00 (s, 1H), 4.11 (s, 2H), 3.30 (t, *J* = 5.7 Hz, 2H), 3.06 (tt, *J* = 5.7, 2.0 Hz, 2H).

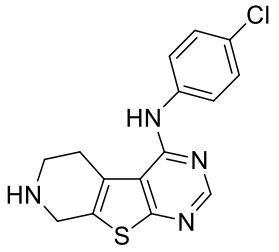




Light yellow powder, m. p. 144.7 – 151.6℃

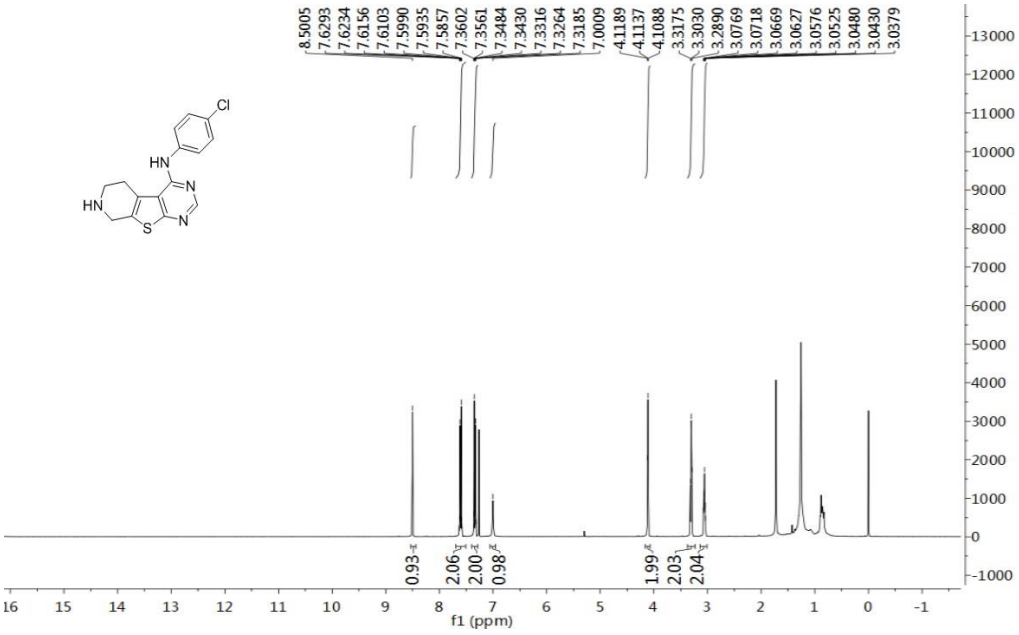
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.50 (s, 1H), 7.53 (ddd, *J* = 8.1, 2.3, 1.1 Hz, 1H), 7.40 (t, *J* = 2.0 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.03 (dt, *J* = 8.0, 1.5 Hz, 2H), 4.12 (s, 2H), 3.31 (t, *J* = 5.7 Hz, 2H), 3.09 (tt, *J* = 5.7, 2.0, 2H), 2.94 (hept, *J* = 7.0 Hz, 1H), 1.29 (d, *J* = 7.0 Hz, 6H).



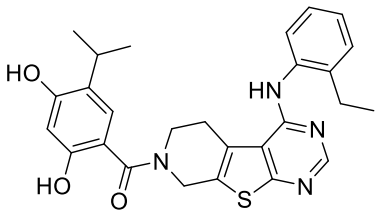


Light yellow powder, m. p. 171.3 – 176.0℃

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.50 (s, 1H), 7.69 – 7.51 (m, 2H), 7.40 – 7.28 (m, 2H), 7.00 (s, 1H), 4.11 (t, *J* = 2.0 Hz, 2H), 3.30 (t, *J* = 5.7 Hz, 2H), 3.06 (tt, *J* = 5.9, 2.0 Hz, 2H).



Compound 8a

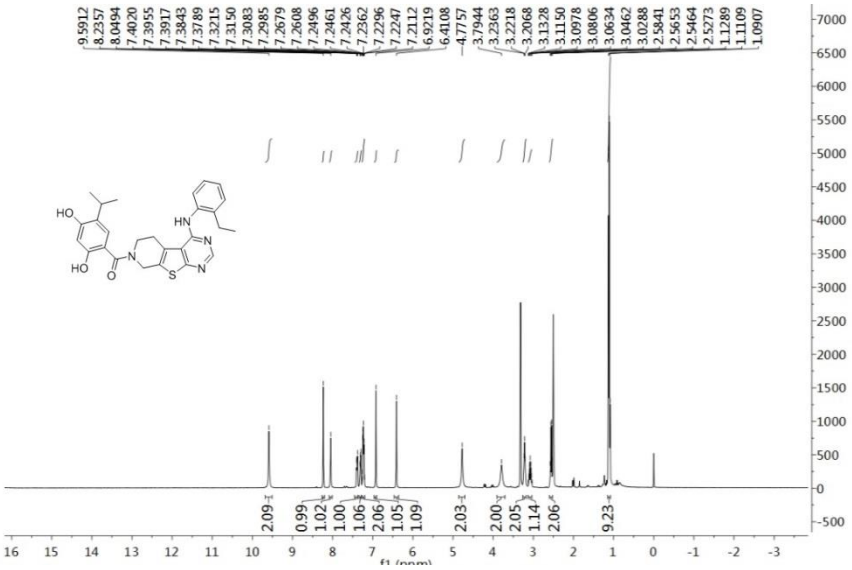


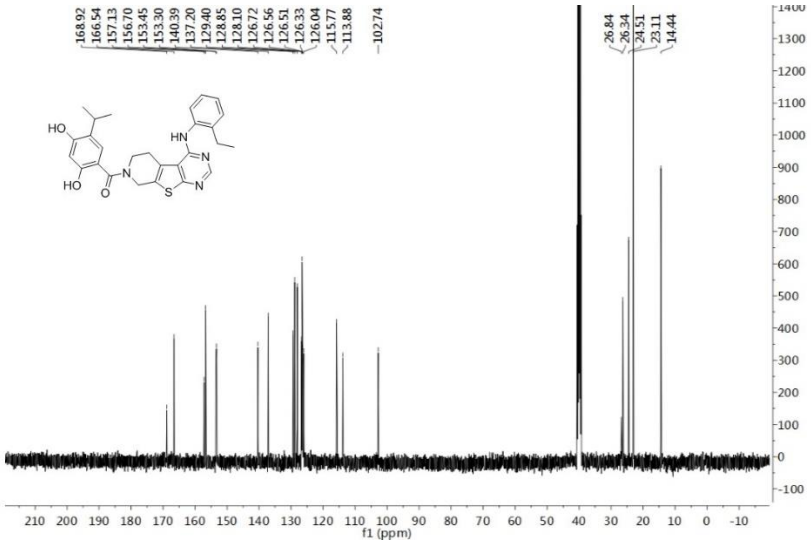
White powder, m. p.272.2 – 230.1℃, yield: 66%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.59 (s, 2H), 8.24 (s, 1H), 8.05 (s, 1H), 7.45 – 7.37 (m, 1H), 7.34 – 7.28 (m, 1H), 7.27 – 7.21 (m, 2H), 6.92 (s, 1H), 6.41 (s, 1H), 4.78 (s, 2H), 3.79 (s, 2H), 3.23 (t, J = 5.8 Hz, 2H), 3.08 (hept, J = 6.9 Hz, 1H), 2.56 (q, J = 7.6 Hz, 2H), 1.12 – 1.09 (m, 9H).

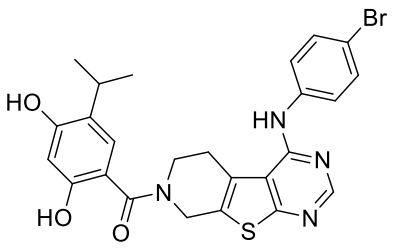
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.92 , 166.54 , 157.13 , 156.70 , 153.45 , 153.30 , 140.39 , 137.20 , 129.40 , 128.85 , 128.10 , 126.72 , 126.56 , 126.51 , 126.33 , 126.04 , 115.77 , 113.88 , 102.74 , 26.84 , 26.34 , 24.51 , 23.11(2C) , 14.44 .

HRMS(ESI) calcd for $C_{27}H_{28}N_4O_3S$ $[M+H]^+$ 489.1955, found 489.1953.





Compound 8b

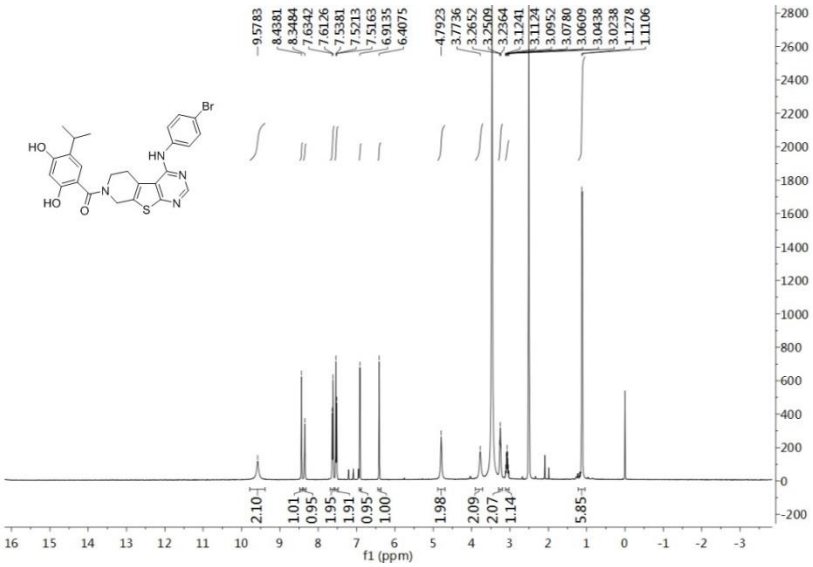


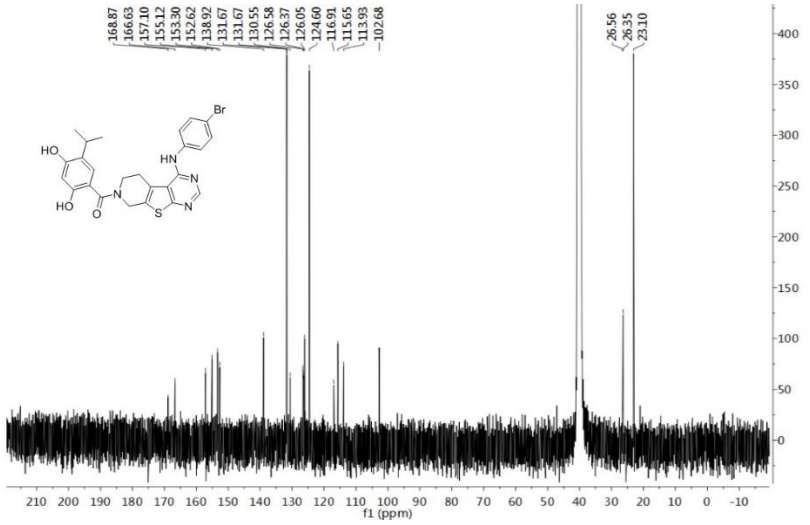
Brown powder, m. p.248.8 – 250.2℃, yield: 33%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 2H), 8.44 (s, 1H), 8.35 (s, 1H), 7.62 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.7 Hz, 2H), 6.91 (s, 1H), 6.41 (s, 1H), 4.79 (s, 2H), 3.77 (s, 2H), 3.26 (t, J = 5.7 Hz, 2H), 3.08 (hept, J = 6.9 Hz, 1H), 1.12 (d, J = 6.9 Hz, 6H).

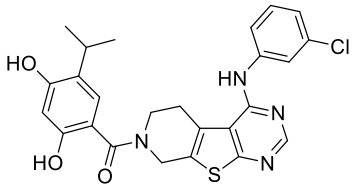
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.87 , 166.63 , 157.10 , 155.12 , 153.30 , 152.62 , 138.92 , 131.67(2C) , 130.55 , 126.58 , 126.37 , 126.05 , 124.60(2C) , 116.91 , 115.65 , 113.93 , 102.68 , 26.56 , 26.35 , 23.10(2C) .

HRMS(ESI) calcd for $C_{25}H_{23}BrN_4O_3S$ $[M+H]^+$ 539.0747, found 539.0742.





Compound 8c

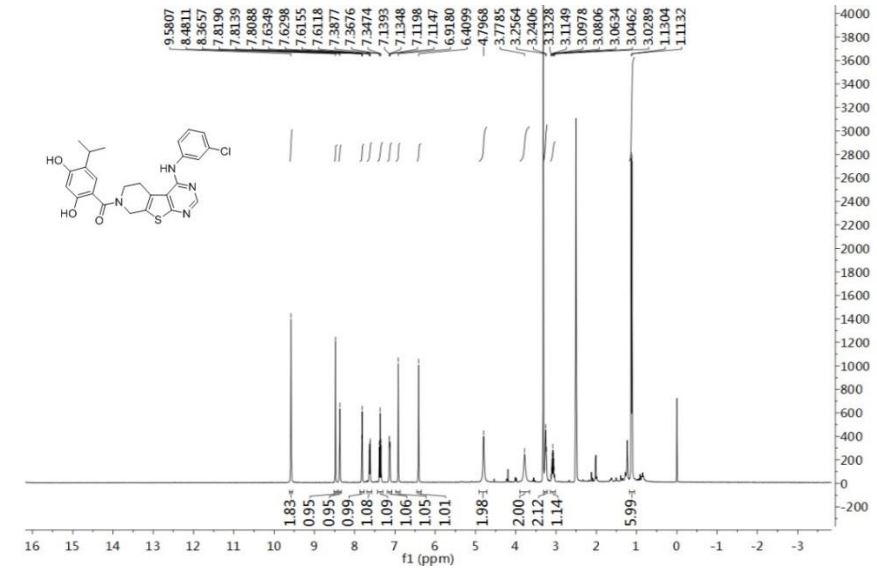


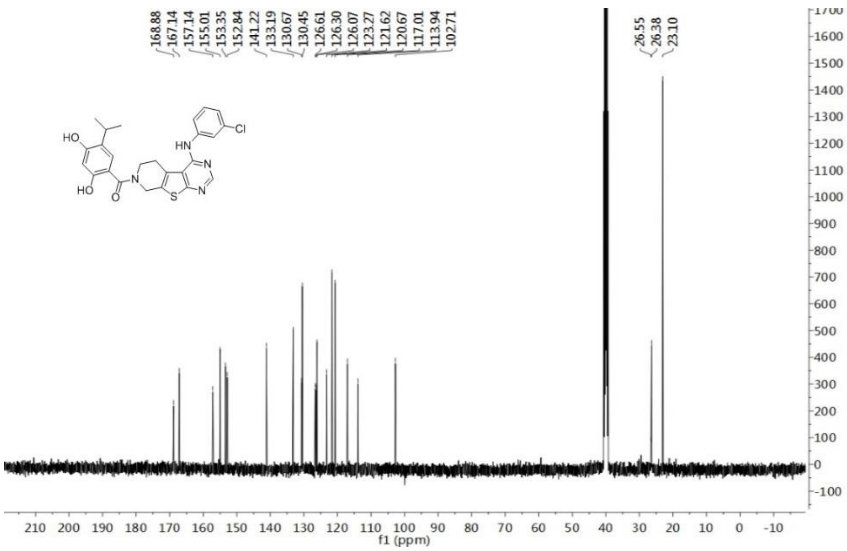
White powder, m. p.189.9 – 190.6℃, yield: 35%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 2H), 8.48 (s, 1H), 8.37 (s, 1H), 7.81 (t, J = 2.0 Hz, 1H), 7.62 (dd, J = 7.5, 1.8 Hz, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.13 (dd, J = 7.9, 1.9 Hz, 1H), 6.92 (s, 1H), 6.41 (s, 1H), 4.80 (s, 2H), 3.78 (s, 2H), 3.25 (t, J = 5.7 Hz, 2H), 3.08 (hept, J = 7.0 Hz, 1H), 1.12 (d, J = 6.9 Hz, 6H).

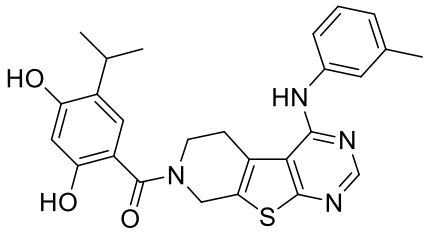
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.88 , 167.14 , 157.14 , 155.01 , 153.35 , 152.84 , 141.22 , 133.19 , 130.67 , 130.45 , 126.61 , 126.30 , 126.07 , 123.27 , 121.62 , 120.67 , 117.01 , 113.94 , 102.71 , 26.55 , 26.38(2C) , 23.10 .

HRMS(ESI) calcd for $C_{25}H_{23}ClN_4O_3S$ $[M+H]^+$ 495.1252, found 495.1253.





Compound 8d

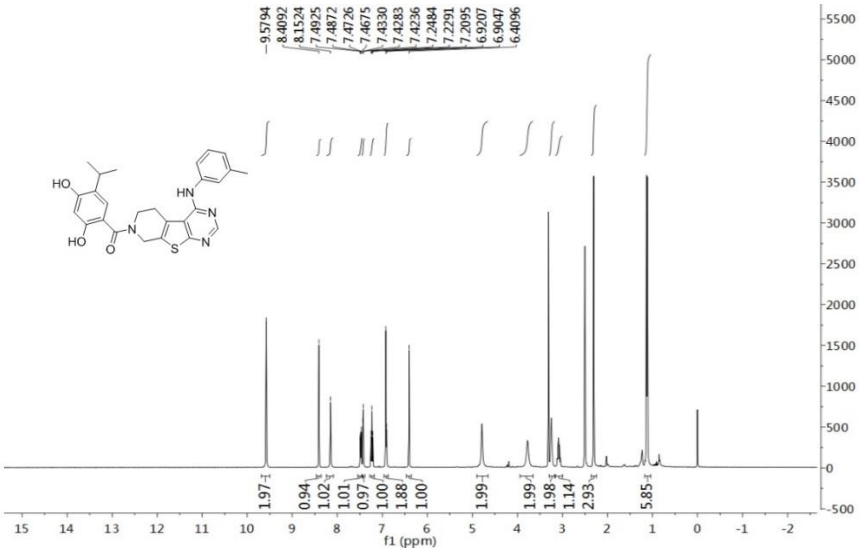


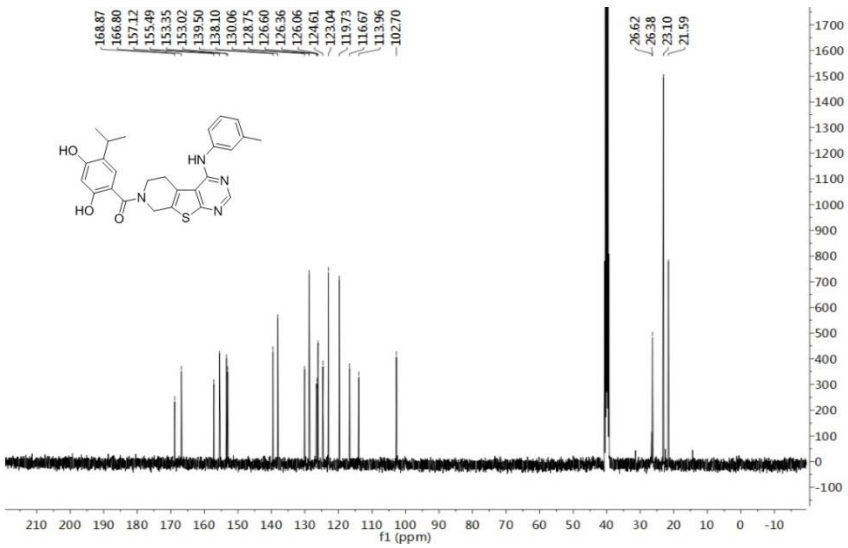
White powder, m. p.157.6 – 160.2℃, yield: 65%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 2H), 8.41 (s, 1H), 8.15 (s, 1H), 7.48 (dd, J = 7.9, 2.1 Hz, 1H), 7.43 (t, J = 1.9 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 6.92 – 6.90 (m, 2H), 6.41 (s, 1H), 4.79 (s, 2H), 3.77 (s, 2H), 3.26 (t, J = 5.7 Hz, 2H), 3.08 (hept, J = 6.9 Hz, 1H), 2.31 (s, 3H), 1.12 (d, J = 6.9 Hz, 6H).

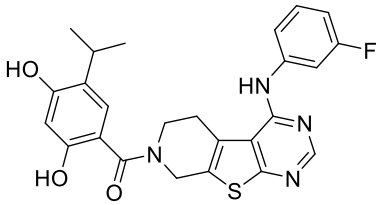
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.87 , 166.80 , 157.12 , 155.49 , 153.35 , 153.02 , 139.50 , 138.10 , 130.06 , 128.75 , 126.60 , 126.36 , 126.06 , 124.61 , 123.04 , 119.73 , 116.67 , 113.96 , 102.70 , 26.62 , 26.38 , 23.10(2C) , 21.59 .

HRMS(ESI) calcd for $C_{26}H_{26}N_4O_3S$ $[M+H]^+$ 475.1798, found 475.1796.





Compound 8e

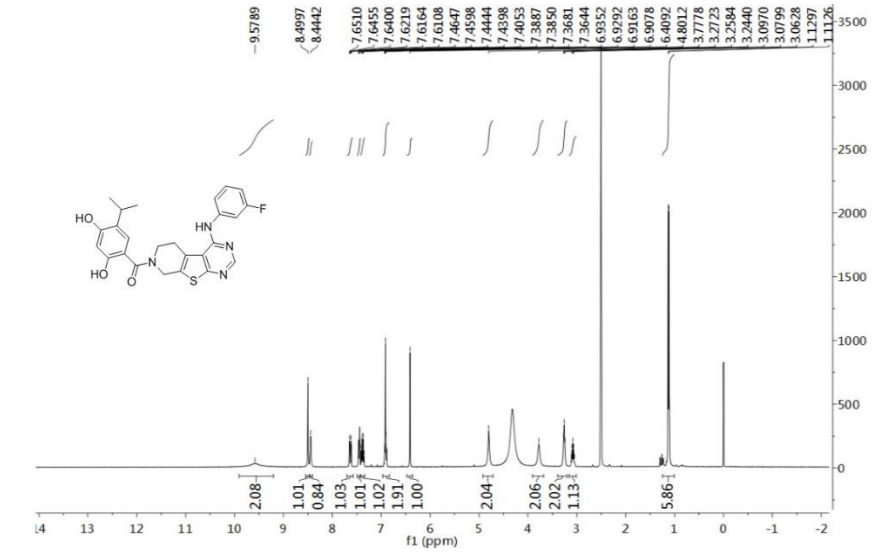


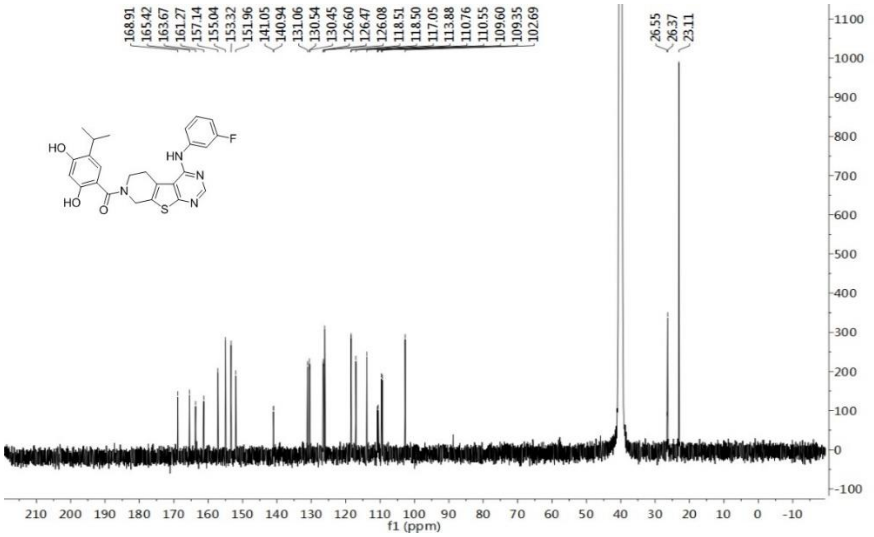
White powder, m. p.218.1 – 219.2℃, yield: 34%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 2H), 8.50 (s, 1H), 8.44 (s, 1H), 7.63 (dt, *J* = 11.7, 2.2 Hz, 1H), 7.45 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.38 (td, *J* = 8.1, 6.6 Hz, 1H), 6.94 – 6.89 (m, 2H), 6.41 (s, 1H), 4.80 (s, 2H), 3.78 (s, 2H), 3.27 (t, *J* = 5.7 Hz, 2H), 3.08 (hept, *J* = 6.9 Hz, 1H), 1.12 (d, *J* = 6.9 Hz, 6H).

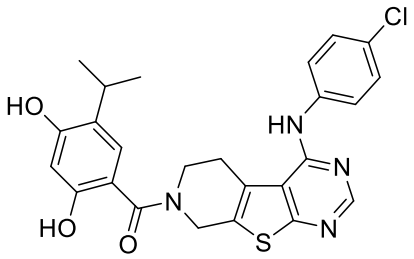
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.91 , 165.42 , 162.47(d, *J* = 240 Hz, 1C) , 157.14 , 155.04 , 153.32 , 151.96 , 141.00(d, *J* = 11 Hz, 1C) , 131.06 , 130.50(d, *J* = 9 Hz, 1C) , 126.54(d, *J* = 13 Hz, 1C) , 126.08 , 118.51 , 118.50 , 117.05 , 113.88 , 110.65(d, *J* = 21 Hz, 1C) , 109.48(d, *J* = 25 Hz, 1C) , 102.69 , 26.55 , 26.37 , 23.11(2C) .

HRMS(ESI) calcd for $C_{25}H_{23}FN_4O_3S$ $[M+H]^+$ 479.1548, found 479.1550.





Compound 8f

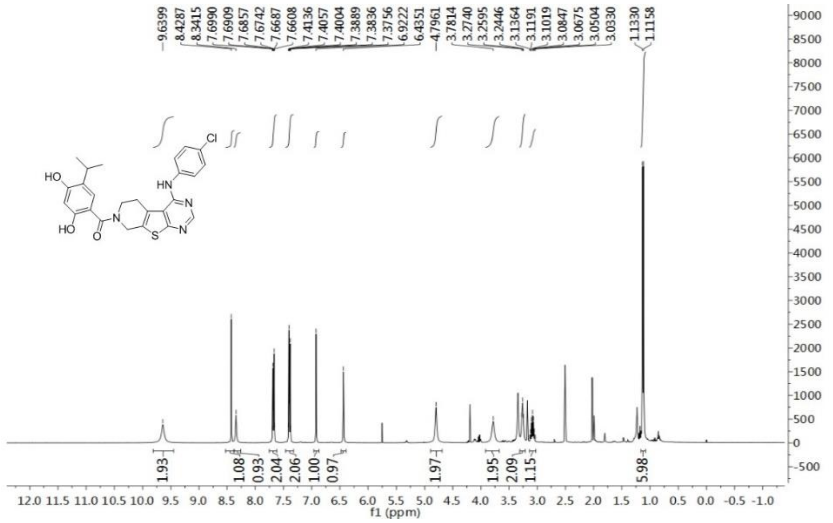


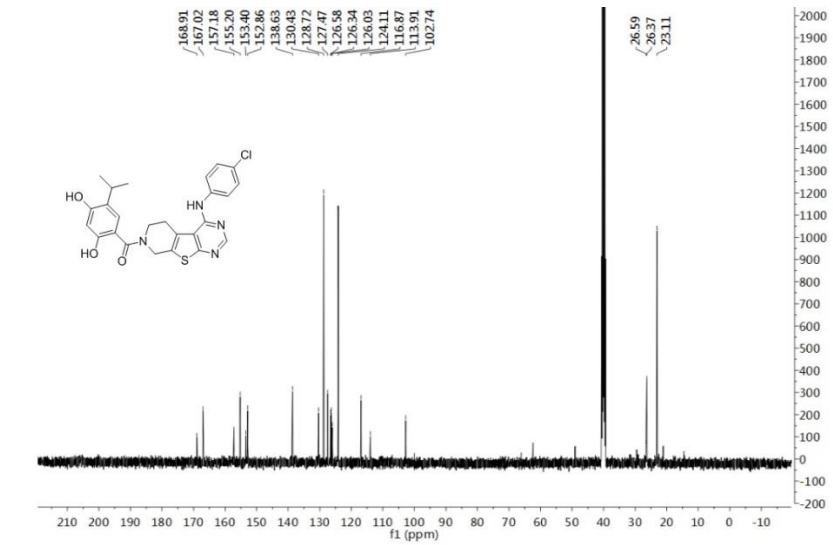
White powder, m. p.178.0 – 182.0℃, yield: 36%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (s, 2H), 8.43 (s, 1H), 8.34 (s, 1H), 7.75 – 7.62 (m, 2H), 7.46 – 7.32 (m, 2H), 6.92 (s, 1H), 6.44 (s, 1H), 4.80 (s, 2H), 3.78 (s, 2H), 3.27 (t, J = 5.8 Hz, 2H), 3.08 (hept, J = 6.9 Hz, 1H), 1.12 (d, J = 6.9 Hz, 6H).

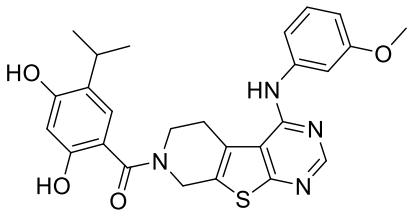
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.91 , 167.02 , 157.18 , 155.20 , 153.40 , 152.86 , 138.63 , 130.43 , 128.72(2C) , 127.47 , 126.58 , 126.34 , 126.03 , 124.11(2C) , 116.87 , 113.91 , 102.74 , 26.59 , 26.37 , 23.11(2C) .

HRMS(ESI) calcd for $C_{25}H_{23}ClN_4O_3S$ $[M+H]^+$ 495.1252, found 495.1249.





Compound 8g

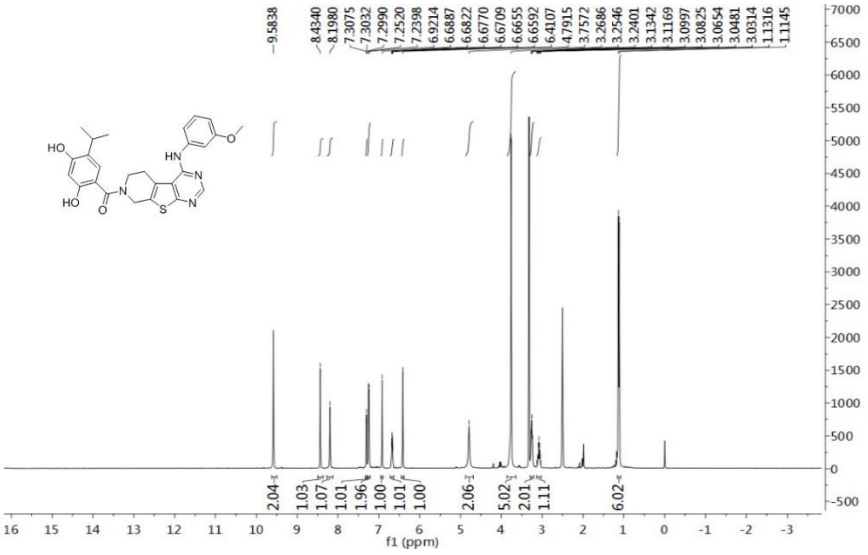


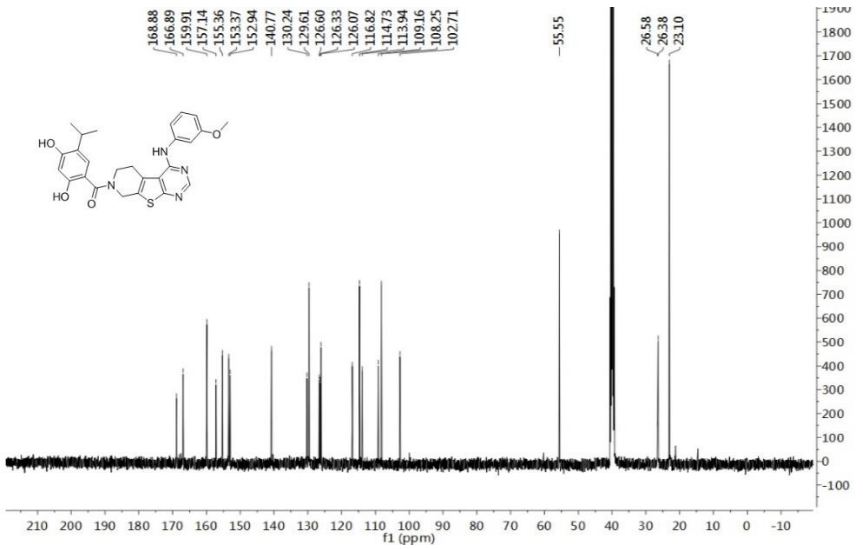
White powder, m. p.220.4 – 221.2℃, yield: 67%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 2H), 8.43 (s, 1H), 8.20 (s, 1H), 7.34 – 7.28 (m, 1H), 7.25 (d, J = 4.9 Hz, 2H), 6.92 (s, 1H), 6.67 (td, J = 4.7, 2.6 Hz, 1H), 6.41 (s, 1H), 4.79 (s, 2H), 3.86 – 3.66 (m, 5H), 3.26 (t, J = 5.7 Hz, 2H), 3.08 (hept, J = 6.8 Hz, 1H), 1.12 (d, J = 6.8 Hz, 6H).

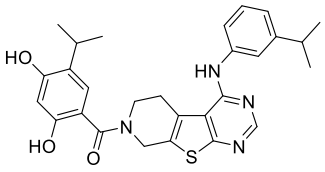
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.88 , 166.89 , 159.91 , 157.14 , 155.36 , 153.37 , 152.94 , 140.77 , 130.24 , 129.61 , 126.60 , 126.33 , 126.07 , 116.82 , 114.73 , 113.94 , 109.16 , 108.25 , 102.71 , 55.55 , 26.58 , 26.38 , 23.10(2C) .

HRMS(ESI) calcd for $C_{26}H_{26}N_4O_4S$ $[M+H]^+$ 491.1748, found 491.1744.





Compound 8h

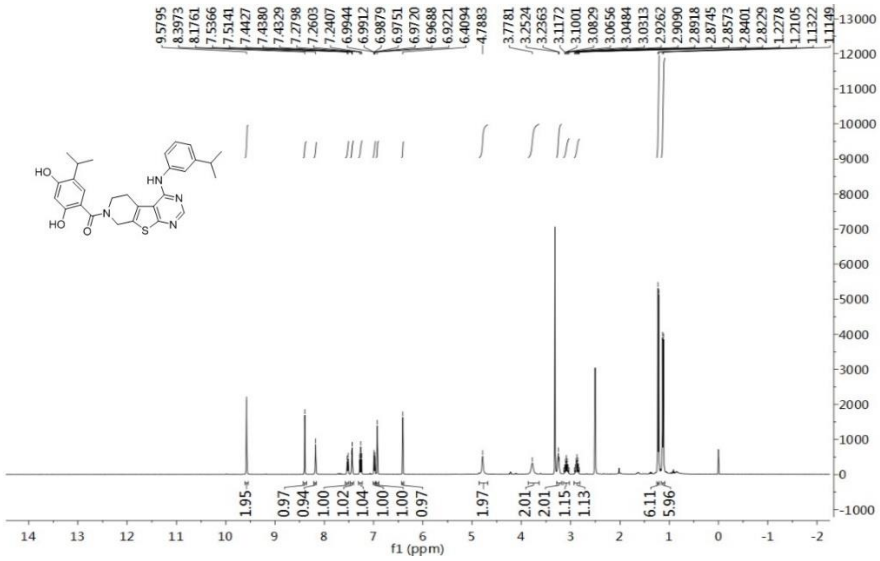


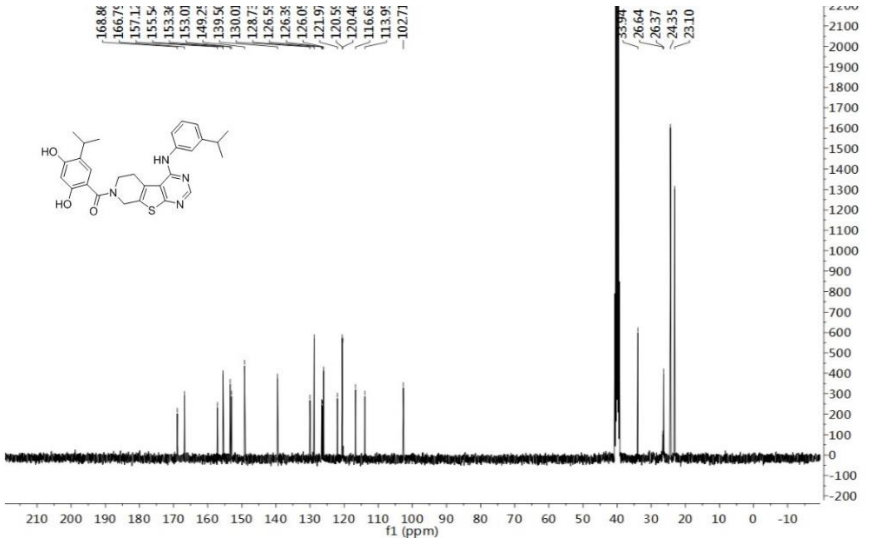
White powder, m. p. 209.5 – 212.4℃, yield: 60%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 2H), 8.40 (s, 1H), 8.18 (s, 1H), 7.53 (d, J = 9.0 Hz, 1H), 7.44 (t, J = 2.0 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 6.98 (dt, J = 7.7, 1.3 Hz, 1H), 6.92 (s, 1H), 6.41 (s, 1H), 4.79 (s, 2H), 3.78 (s, 2H), 3.24 (t, J = 6.5 Hz, 2H), 3.08 (hept, J = 6.9 Hz, 1H), 2.87 (hept, J = 6.9 Hz, 1H), 1.22 (d, J = 6.9 Hz, 6H), 1.12 (d, J = 6.9 Hz, 6H).

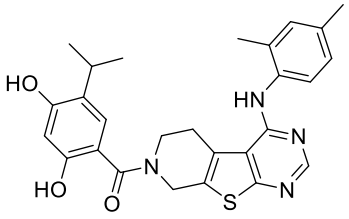
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.86 , 166.79 , 157.12 , 155.54 , 153.36 , 153.01 , 149.25 , 139.50 , 130.01 , 128.73 , 126.59 , 126.39 , 126.05 , 121.97 , 120.59 , 120.40 , 116.63 , 113.95 , 102.71 , 33.94 , 26.64 , 26.37 , 24.35(2C) , 23.10(2C) .

HRMS(ESI) calcd for $C_{28}H_{30}N_4O_3S$ $[M+H]^+$ 503.2111, found 503.2112.





Compound 8i

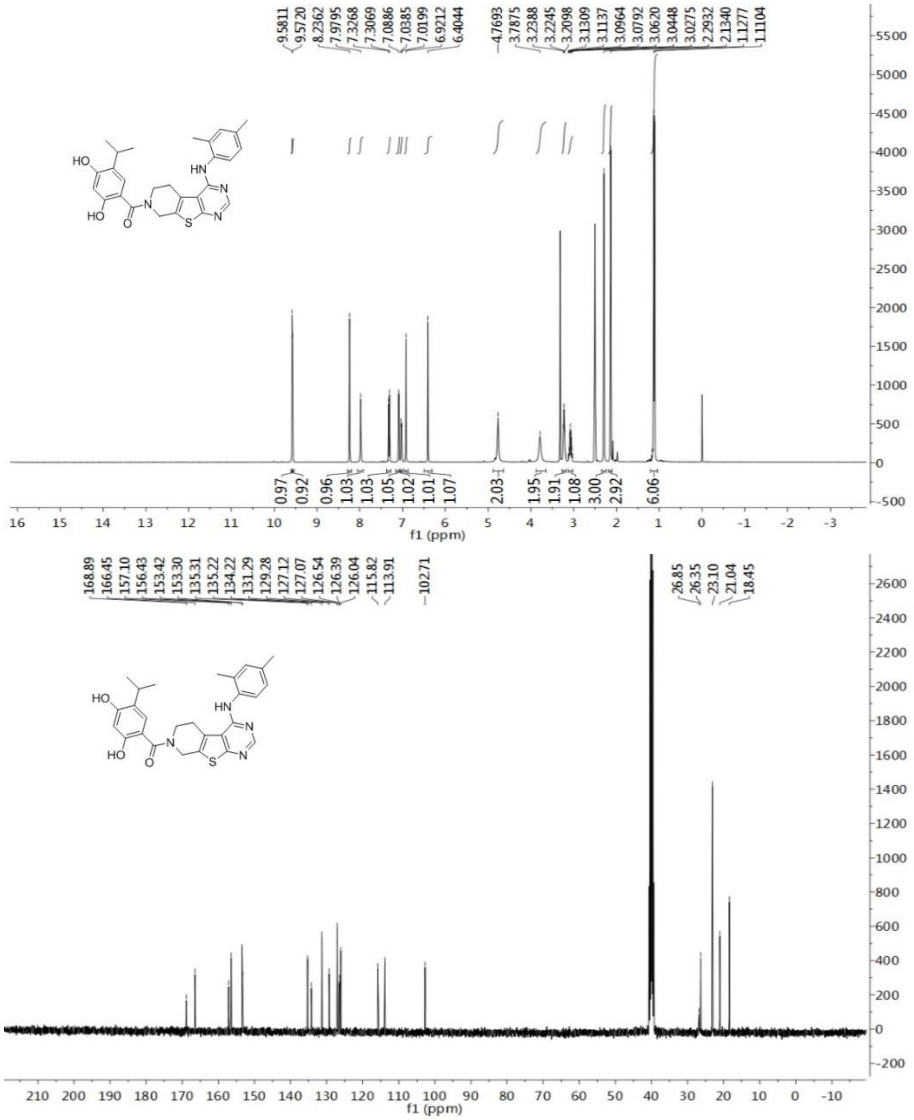


White powder, m. p. 254.3 – 255.4℃, yield: 63%

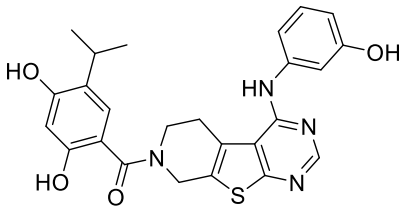
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 9.57 (s, 1H), 8.24 (s, 1H), 7.98 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.09 (s, 1H), 7.03 (d, J = 7.4 Hz, 1H), 6.92 (s, 1H), 6.40 (s, 1H), 4.77 (s, 2H), 3.79 (s, 2H), 3.23 (t, J = 5.7 Hz, 2H), 3.08 (hept, J = 6.9 Hz, 1H), 2.29 (s, 3H), 2.13 (s, 3H), 1.12 (d, J = 6.9 Hz, 6H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 168.89 , 166.45 , 157.10 , 156.43 , 153.42 , 153.30 , 135.31 , 135.22 , 134.22 , 131.29 , 129.28 , 127.12 , 127.07 , 126.54 , 126.39 , 126.04 , 115.82 , 113.91 , 102.71 , 26.85 , 26.35 , 23.10(2C) , 21.04 , 18.45 .

HRMS(ESI) calcd for $C_{27}H_{28}N_4O_3S$ $[M+H]^+$ 489.1955, found 489.1953.



Compound 8j

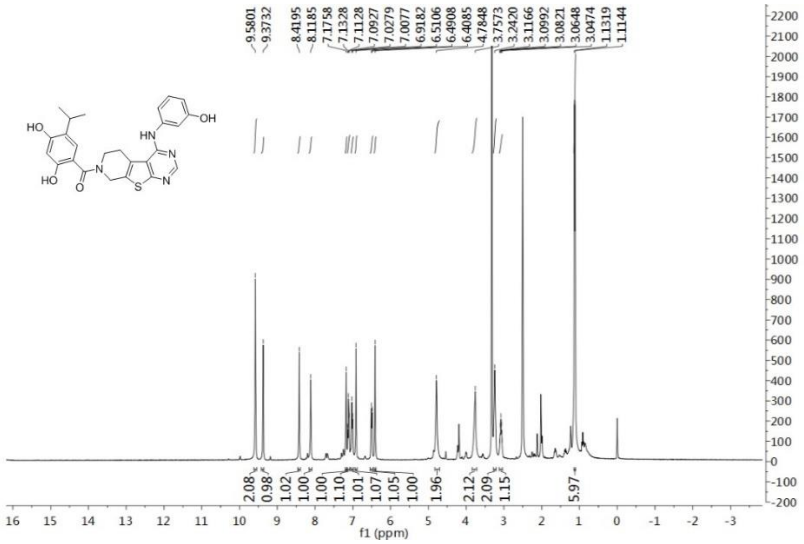


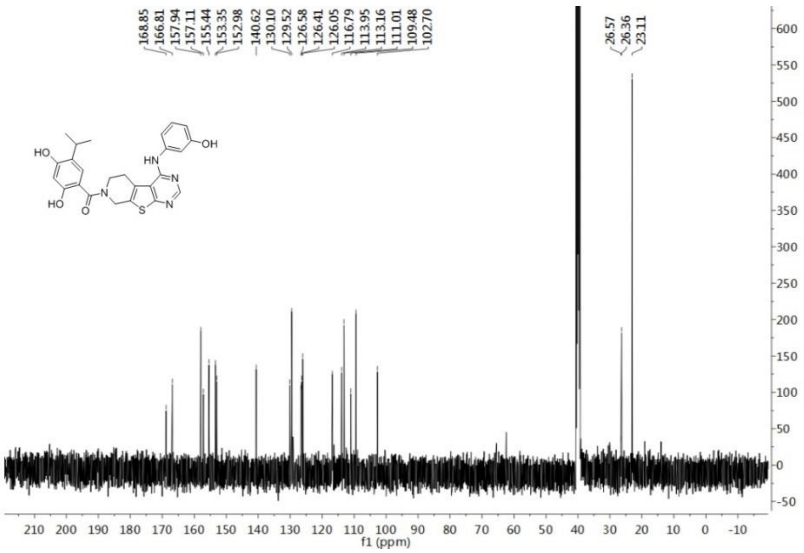
Yellow powder, m. p.146.4 – 148.2℃, yield: 25%

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 2H), 9.37 (s, 1H), 8.42 (s, 1H), 8.12 (s, 1H), 7.18 (s, 1H), 7.11 (t, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 1H), 6.92 (s, 1H), 6.50 (d, *J* = 7.9 Hz, 1H), 6.41 (s, 1H), 4.78 (s, 2H), 3.76 (s, 2H), 3.24 (s, 2H), 3.08 (p, *J* = 7.0 Hz, 1H), 1.12 (d, *J* = 7.0 Hz, 6H).

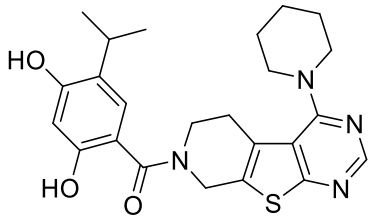
^{13}C NMR (100 MHz, DMSO-d_6) δ 168.85 , 166.81 , 157.94 , 157.11 , 155.44 , 153.35 , 152.98 , 140.62 , 130.10 , 129.52 , 126.58 , 126.41 , 126.05 , 116.79 , 113.95 , 113.16 , 111.01 , 109.48 , 102.70 , 26.57 , 26.36 , 23.11(2C) .

HRMS(ESI) calcd for $C_{25}H_{24}N_4O_4S$ $[M+H]^+$ 477.1591, found 477.1591.





Compound 8k

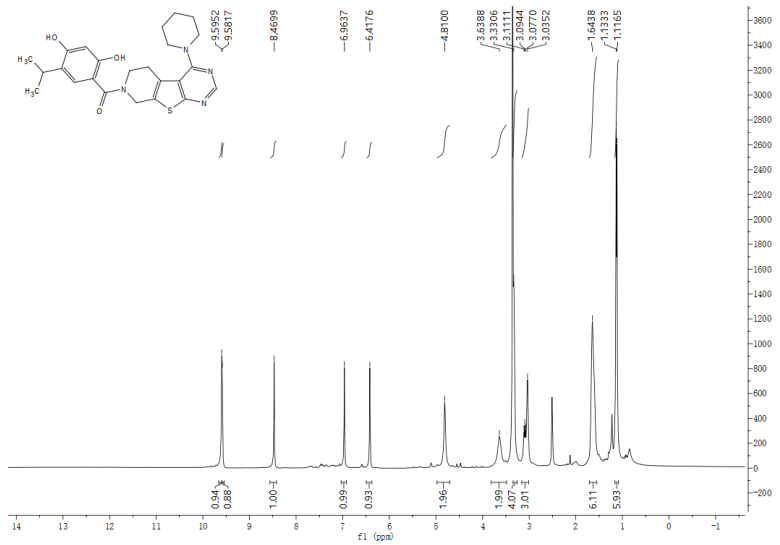


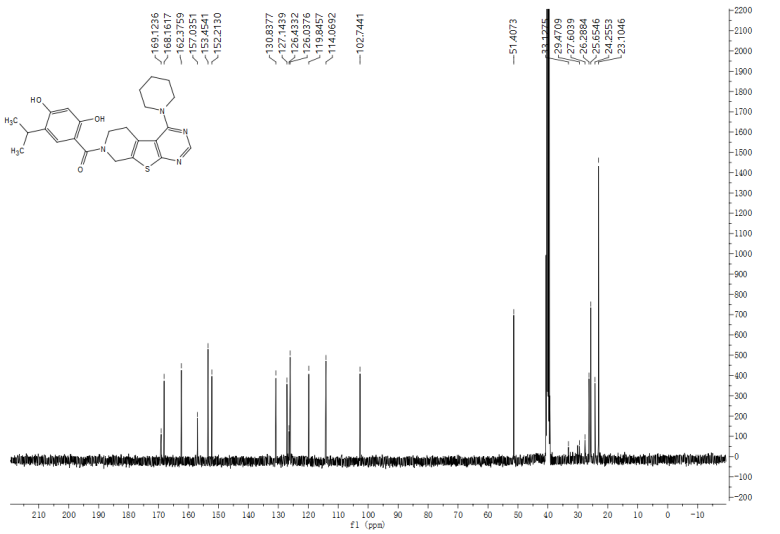
White powder, m. p.196.1 – 198.5℃, yield: 67%

^{1}H NMR (400 MHz, DMSO) δ 9.60 (s, 1H), 9.58 (s, 1H), 8.47 (s, 1H), 6.96 (s, 1H), 6.42 (s, 1H), 4.81 (s, 2H), 3.64 (s, 2H), 3.33 (s, 4H), 3.11 – 3.03 (m, 3H), 1.64 (s, 6H), 1.12 (d, *J* = 6.7 Hz, 6H).

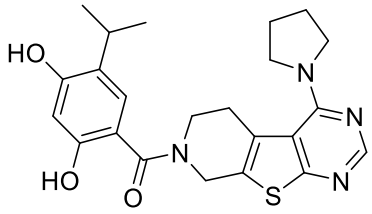
^{13}C NMR (101 MHz, DMSO) δ 169.12, 168.16 , 162.38, 157.04, 153.45, 152.21, 130.84, 127.14, 126.43, 126.03, 119.85, 114.07, 102.74, 51.41 (s, 2C), 33.12, 29.47, 27.60, 26.29, 25.65 (s, 2C), 24.26 (s), 23.10 (s, 2C).

HRMS(ESI) calcd for $C_{25}H_{24}N_4O_4S$ $[M+H]^+$ 452.1882, found 452.1879.





Compound 8l

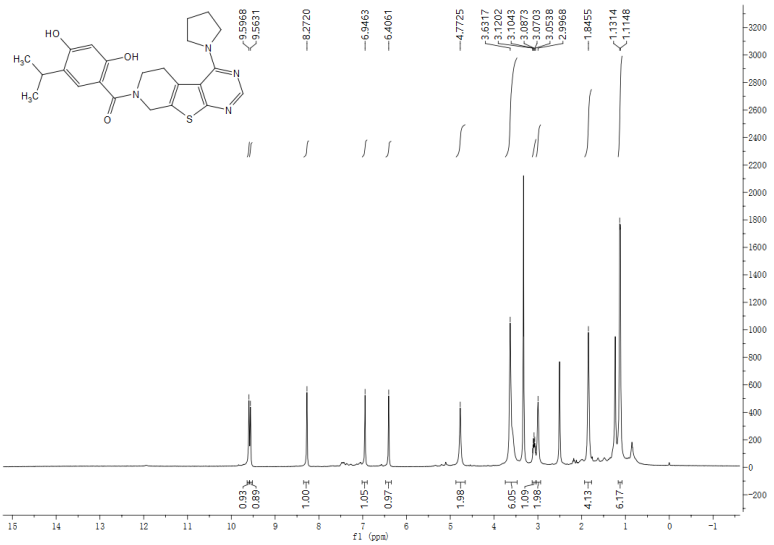


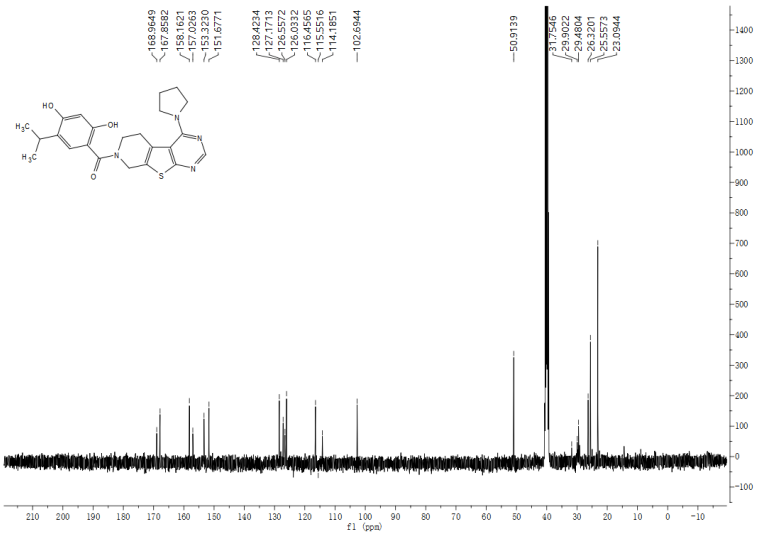
White powder, m. p.185.6 – 188.9℃, yield: 69%

^{1}H NMR (400 MHz, DMSO) δ 9.60 (s, 1H), 9.56 (s, 1H), 8.27 (s, 1H), 6.95 (s, 1H), 6.41 (s, 1H), 4.77 (s, 2H), 3.63 (s, 6H), 3.13 – 3.04 (m, 1H), 3.00 (s, 2H), 1.85 (s, 4H), 1.12 (d, *J* = 6.7 Hz, 6H).

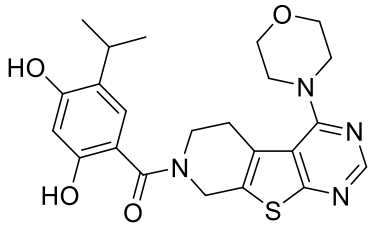
^{13}C NMR (101 MHz, DMSO) δ 168.96, 167.85, 158.16, 157.02, 153.32, 151.68, 128.42, 127.17, 126.56, 126.03, 116.45, 114.18, 102.69, 50.91 (s, 2C), 31.75, 29.90 , 29.48, 26.32 , 25.56 (s, 2C), 23.09 (s, 2C).

HRMS(ESI) calcd for $C_{25}H_{24}N_4O_4S$ $[M+H]^+$ 438.1726, found 438.1730.





Compound 8m

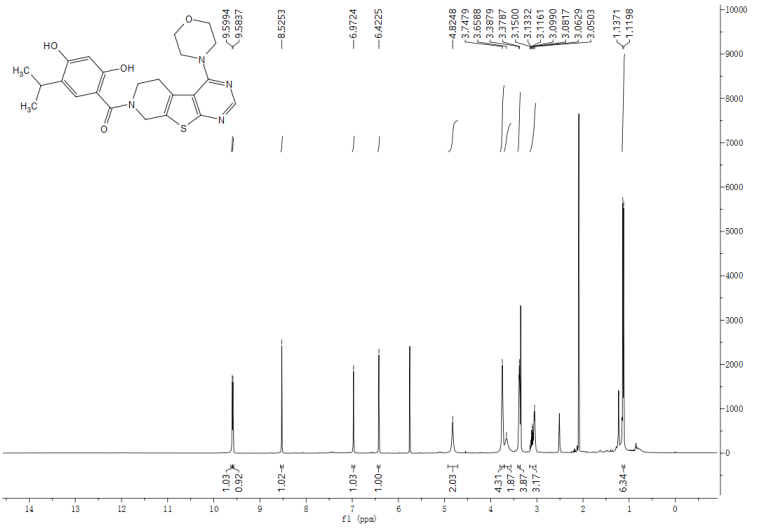


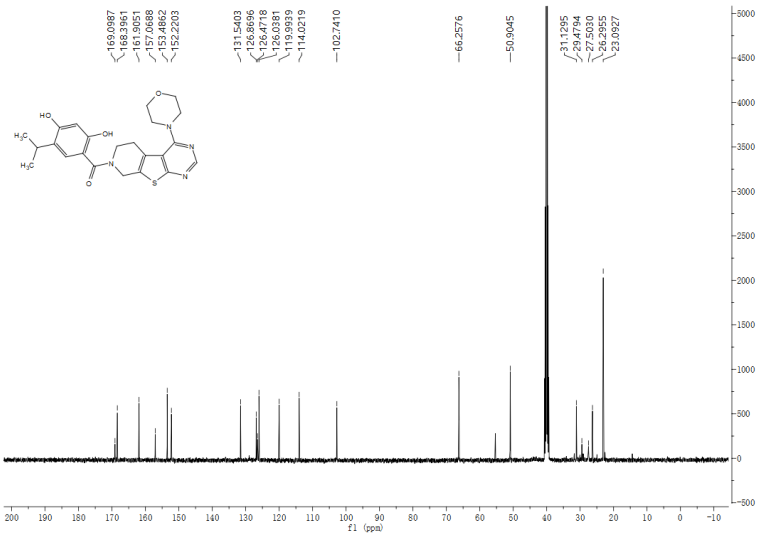
White powder, m. p.183.4 – 185.1℃, yield: 65%

^{1}H NMR (400 MHz, DMSO) δ 9.60 (s, 1H), 9.58 (s, 1H), 8.53 (s, 1H), 6.97 (s, 1H), 6.42 (s, 1H), 4.82 (s, 2H), 3.75 (t, *J* = 3.7 Hz, 4H), 3.66 (s, 2H), 3.38 (t, *J* = 3.7 Hz, 4H), 3.16 – 3.02 (m, 3H), 1.13 (d, *J* = 6.9 Hz, 6H).

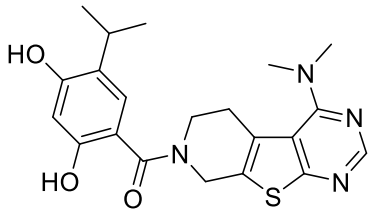
^{13}C NMR (101 MHz, DMSO) δ 169.10, 168.40, 161.91, 157.07, 153.49, 152.22, 131.54, 126.87, 126.47, 126.04, 119.99, 114.02, 102.74, 66.26 (s, 2C), 50.90 (s, 2C), 31.13, 29.48, 27.50, 26.30, 23.09 (s, 2C).

HRMS(ESI) calcd for $C_{25}H_{24}N_4O_4S$ $[M+H]^+$ 454.1675, found 454.1677.





Compound 8n



White powder, m. p.189.1 – 192.2℃, yield: 66%

^{1}H NMR (400 MHz, DMSO) δ 9.59 (s, 1H), 9.56 (s, 1H), 8.40 (s, 1H), 6.95 (s, 1H), 6.40 (s, 1H), 4.80 (s, 2H), 3.60 (s, 2H), 3.14 – 2.99 (m, 9H), 1.12 (d, *J* = 6.9 Hz, 6H).

^{13}C NMR (101 MHz, DMSO) δ 169.05, 168.07, 161.89, 157.01, 153.34, 151.85, 130.02, 127.21, 126.52, 126.03, 118.42, 114.20, 102.70, 41.95 (s, 2C), 31.15, 29.48, 28.43, 26.31, 23.10(s, 2C).

HRMS(ESI) calcd for $C_{25}H_{24}N_4O_4S$ $[M+H]^+$ 412.1569, found 412.1566.