

Supporting Information

Discovery of 5-cyano-6-phenylpyrimidin derivatives containing an acylurea moiety as orally bioavailable reversal agents against P-glycoprotein-mediated multidrug resistance

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Solubility of compounds 7,8,9,10,16,17,18,19

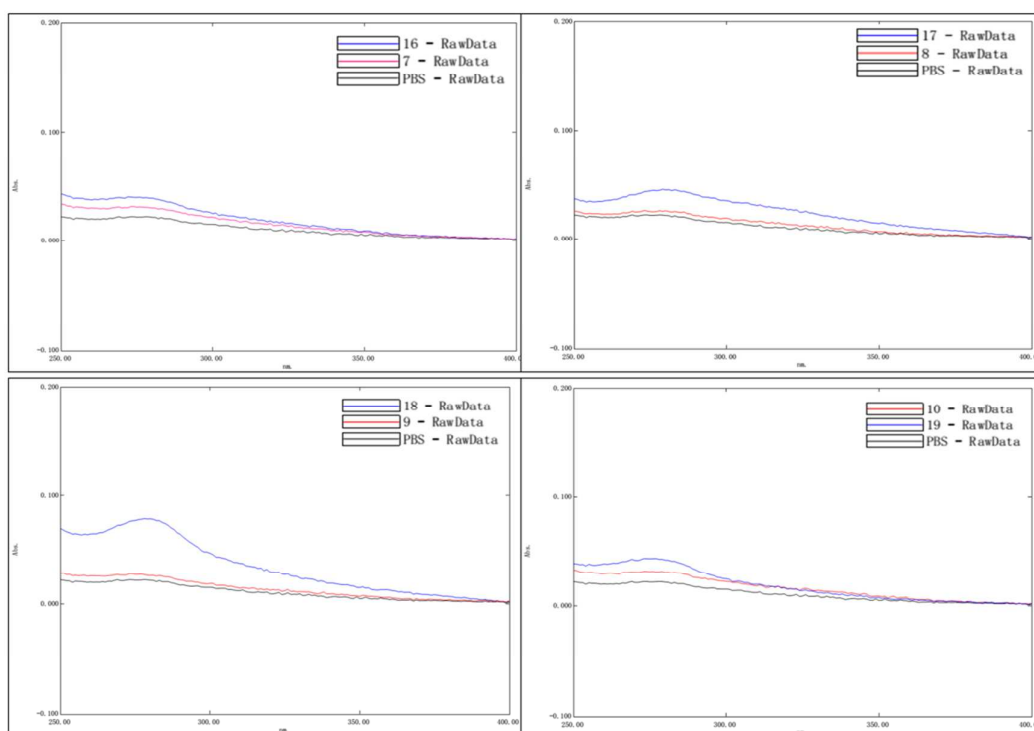


Figure S1. Solubility of compounds **16-19** and **7-10**. UV-Vis absorption spectra of eight compounds at pH value 7.4.

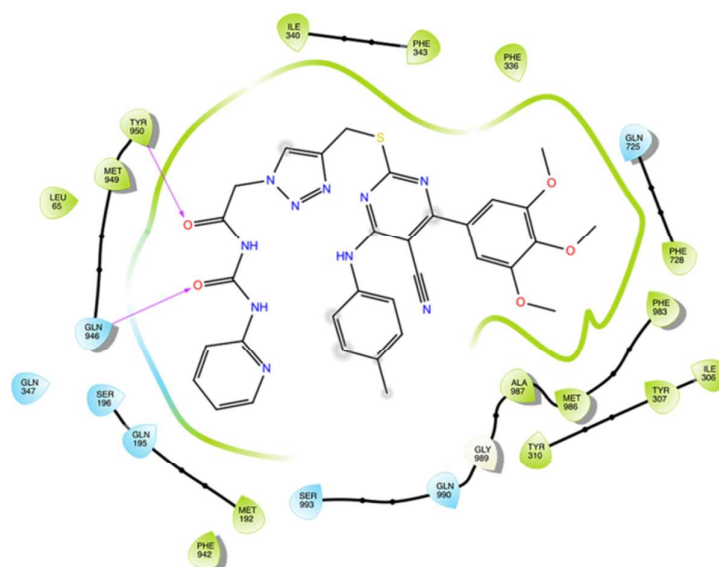


Figure S2. XP Glide docking predicted binding mode of compound **18** to human ABCB1 homology model. The two-dimensional ligand–receptor interaction diagram of 18 with human ABCB1. The amino acids within 4 Å are shown as colored bubbles, blue indicates polar residues, and green indicates hydrophobic residues. Grey circles indicate solvent exposure. Hydrogen bonds are shown by the purple arrow.

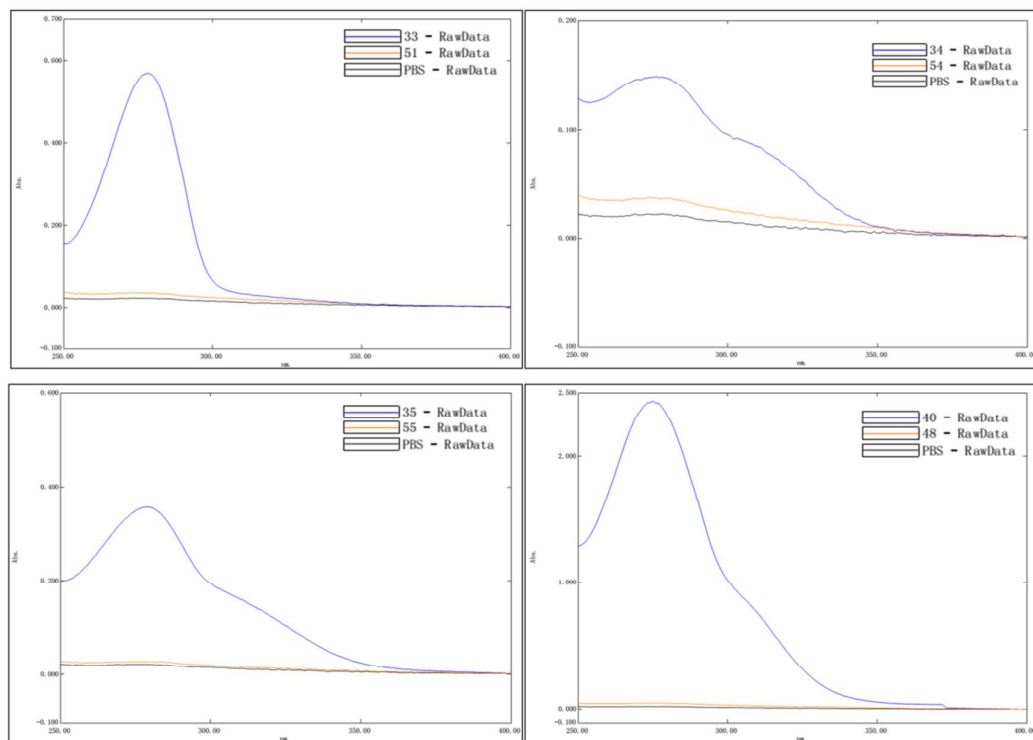


Figure S3. Solubility of compounds **33-35**, **40** and **51**, **54**, **55**, **48**. UV-Vis absorption spectra of eight compounds at pH value 7.4.

Associated assay about ABCB1 of compound 52 and 60

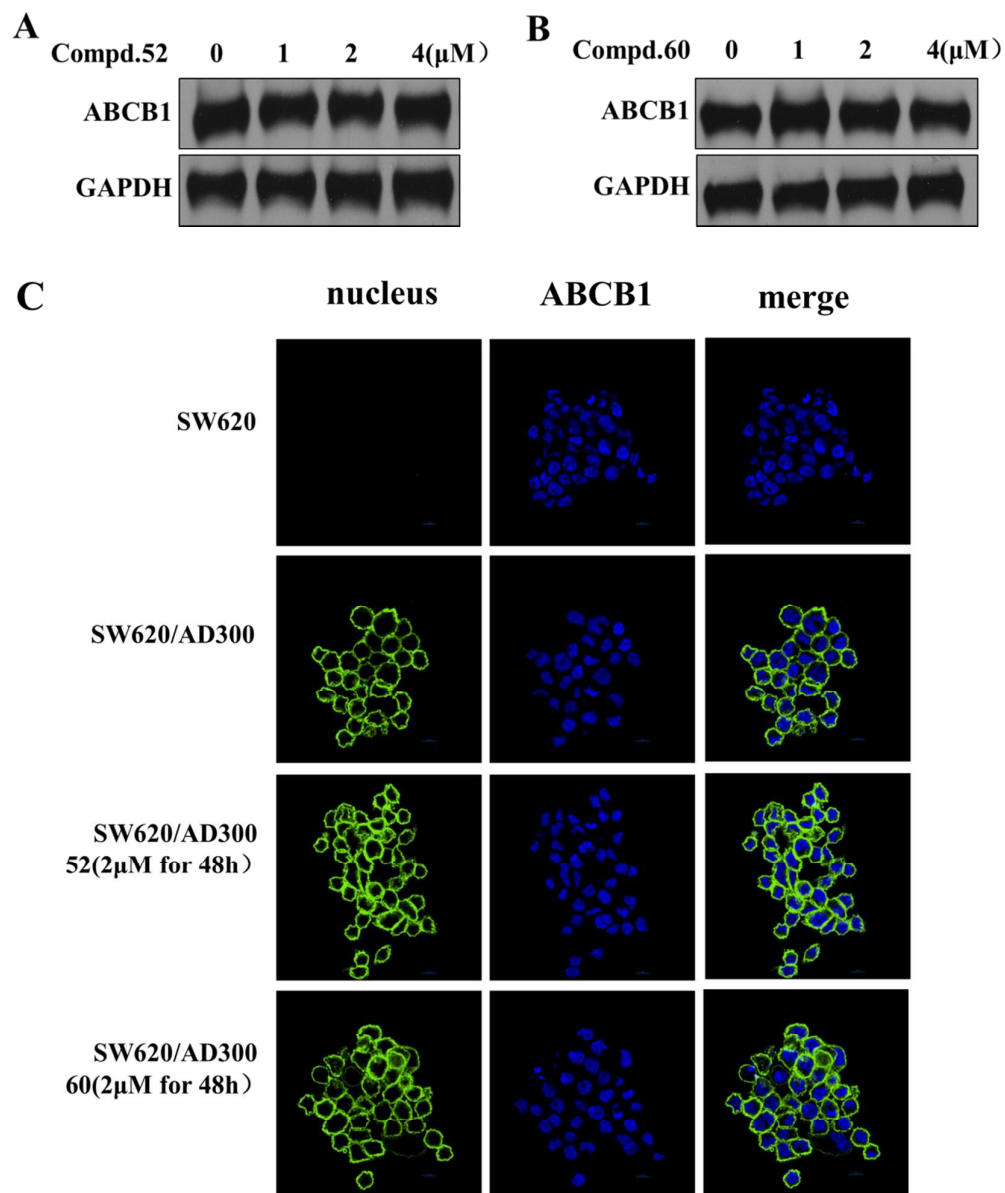
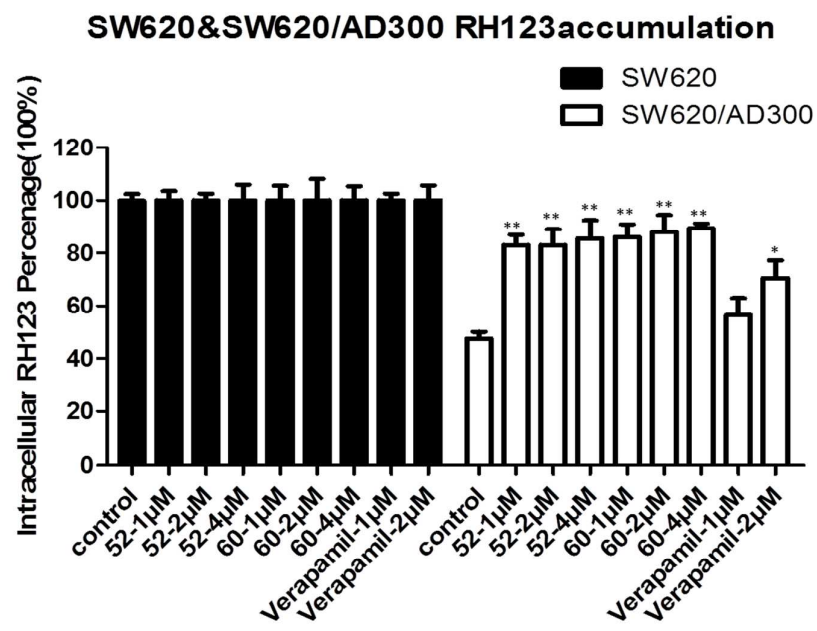


Figure S4. Effects of compound **52** and **60** on the expression level and subcellular localization of ABCB1. Effect of compound **52** (A) and **60** (B) on the expression of ABCB1 after treatment for 48 h, and the GAPDH was used as control. (C) Effect of compound **52** and **60** on the expression and subcellular localization of ABCB1 (green) after treatment for 48 h. Hoechst 33258 (blue) was stained for the cell nuclei. The

experiments were performed three times, and a representative experiment was shown.

A



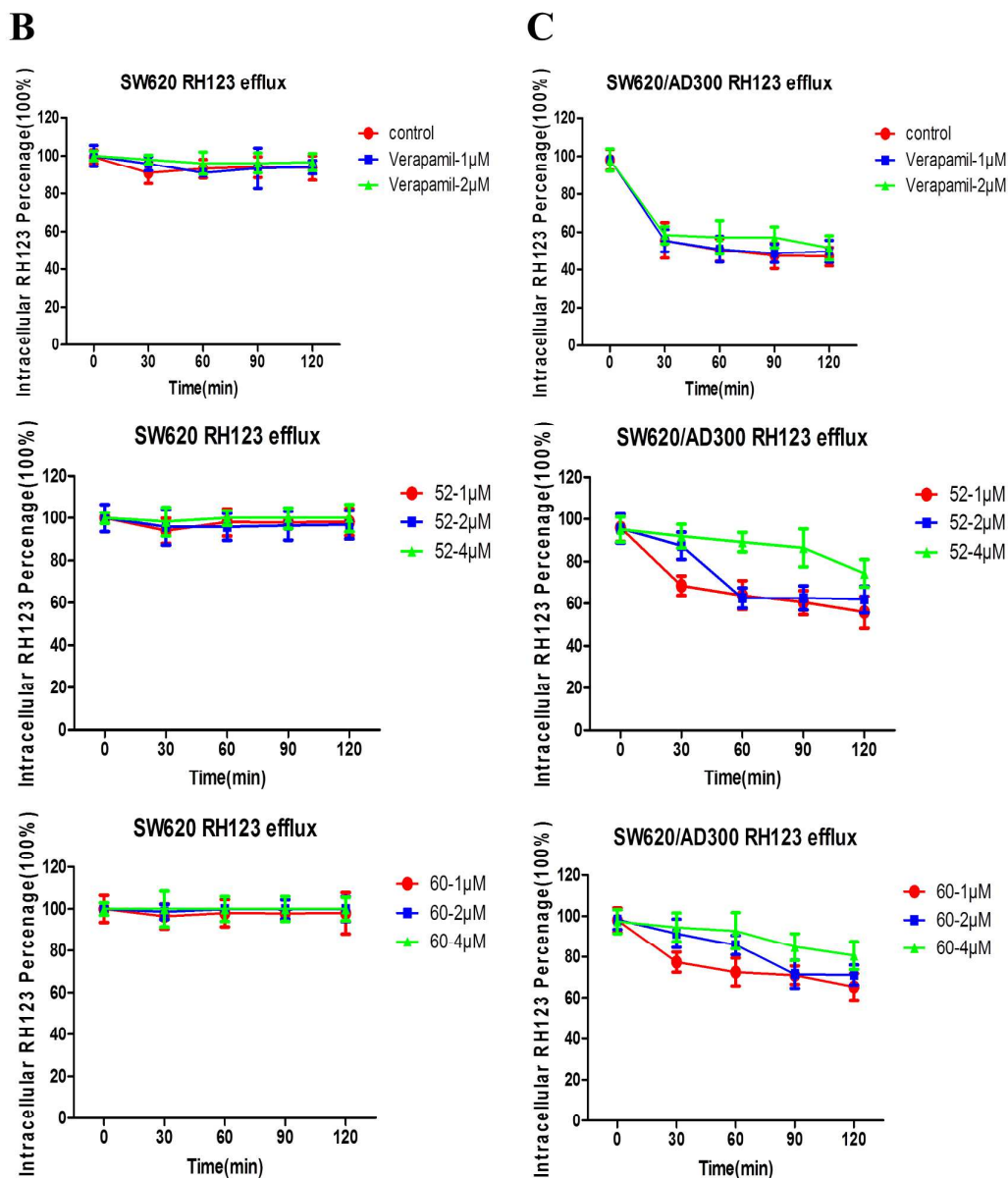


Figure S5. Effect of compound **52** and **60** on ABCB1-mediated Rh123 accumulation and efflux. (A) Effect of compound **52**, **60** and VRP on accumulation of Rh123 in SW620 and SW620/AD300 cell lines. A time course versus percentage of intracellular Rh123 retaining was plotted (0, 30, 60, 90, 120 min) to show effects of compound **52**, **60** or VRP in SW620 (A) and SW620/AD300 (B) cell lines. * $p < 0.05$ and ** $p < 0.01$ were considered statistically significant compared with the control. Data are mean $\pm$

SD. All experiments were carried out at least three times and a representative experiment is shown.

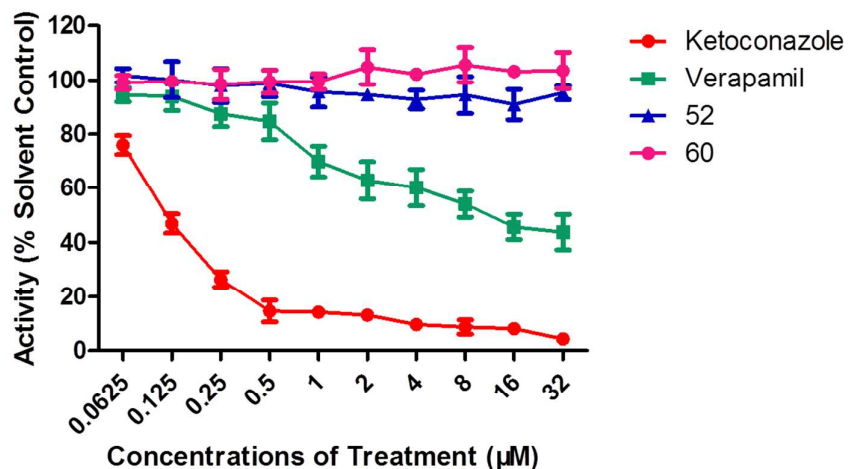


Figure S6. Dose-response curves after different treatment: the canonical competitive CYP3A4 inhibitor Ketoconazole, positive reversal modulator of ABCB1-mediated resistance Verapamil, compound **52** and **60**. The data represent the mean $\pm$ SD of three independent experiment.

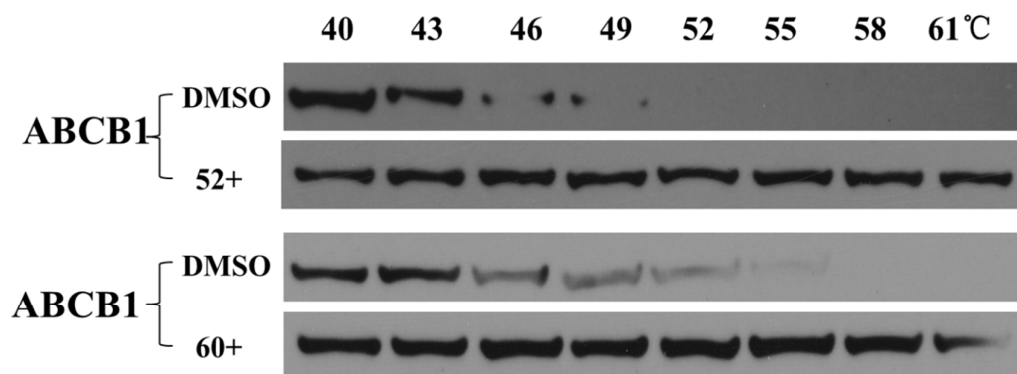


Figure S7. Cellular thermal shift assay. Target engagement of compound **52** (upper) and **60** (lower) to ABCB1 in SW620/AD300.

IC₅₀ and survival rate (at 2μM) of all the target compounds toward SW620 and SW620/AD300 cells.

Table S1. IC₅₀ and survival rate (at 2μM) of target compounds toward SW620 and SW620/AD300 cells.

Compd.	IC ₅₀ (μM)		survival rate (%)	
	SW620	SW620/AD300	SW620	SW620/AD300
7	18.146±1.259	17.973±1.255	92.03±3.26	>99.00
8	>32	>32	94.62±5.67	>99.00
9	>32	>32	94.68±2.13	90.55±6.54
10	16.895±0.839	19.030±1.279	96.56±5.32	>99.00

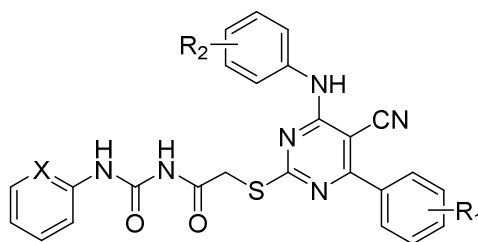
Table S2. IC₅₀ and survival rate (at 2μM) of target compounds toward SW620 and SW620/AD300 cells.

Compd.	IC ₅₀ (μM)		Survival Rate (%)	
	SW620	SW620/AD300	SW620	SW620/AD300
16	>32	>32	98.30±3.55	95.47±1.92

17	>32	>32	>99.00	>99.00
18	>32	>32	>99.00	95.36±3.61
19	15.659±0.573	>32	92.13±1.69	97.43±4.79
20	>32	12.532±1.098	98.81±5.96	>99.00
21	11.118±1.046	>32	94.29±5.56	>99.00
22	13.562±1.132	>32	90.57±3.65	>99.00
23	23.277±1.367	>32	95.48±5.69	94.94±6.38
24	>32	>32	94.65±6.54	>99.00
25	>32	>32	92.83±1.69	91.71±1.69
26	15.317±1.014	18.287±1.132	93.60±5.65	>99.00
27	>32	>32	>99.00	90.73±2.97
28	>32	>32	94.01±9.86	>99.00
29	14.858±1.172	17.886±1.253	97.96±5.36	98.89±1.67
30	>32	>32	95.86±5.69	>99.00
31	16.354±0.803	16.531±0.815	91.49±1.65	92.38±5.19
32	>32	>32	93.03±6.54	>99.00
33	>32	>32	>99.00	>99.00
34	>32	>32	91.17±3.98	>99.00
35	>32	>32	95.12±4.65	>99.00
36	26.917±1.430	17.693±0.886	>99.00	>99.00
37	>32	26.238±1.419	96.34±5.98	>99.00
38	18.941±0.951	12.814±1.108	91.13±4.79	>99.00

39	>32	>32	95.92±5.98	>99.00
40	>32	17.162±1.235	95.33±4.65	93.00±5.89
41	13.589±0.555	>32	91.56±5.89	97.29±6.54
42	>32	>32	96.36±7.95	>99.00

Table S3. IC₅₀ and survival rate (at 2μM) of target compounds toward SW620 and SW620/AD300 cells.



Compd.	IC ₅₀ (μM)		survival rate (%)	
	SW620	SW620/AD300	SW620	SW620/AD300
44	17.824±0.893	11.034±0.015	92.56±2.31	>99.00
45	30.372±1.482	11.205±0.081	>99.00	92.63±6.87
46	16.461±0.773	12.335±1.091	92.15±1.67	95.26±5.69
47	16.952±0.842	12.650±0.178	93.02±3.19	>99.00
48	16.366±0.804	17.582±0.880	93.89±3.49	95.38±2.34
49	16.111±1.207	16.435±0.809	>99.00	90.22±4.96
50	28.265±1.175	>32	94.31±0.65	>99.00
51	>32	>32	>99.00	>99.00
52	>32	>32	>99.00	92.54±4.39
53	15.648±1.194	12.296±1.090	>99.00	93.14±6.87
54	12.103±0.279	13.099±0.491	>99.00	94.22±2.37

55	13.768±0.247	>32	94.10±4.79	97.56±4.19
56	12.551±0.407	11.905±0.280	94.93±2.68	95.61±6.97
57	>32	>32	>99.00	>99.00
58	>32	>32	>99.00	>99.00
59	>32	>32	>99.00	>99.00
60	18.256±0.917	16.797±0.832	>99.99	>99.00
61	14.839±0.685	12.229±0.348	95.07±1.67	94.69±6.79
62	12.563±0.409	10.524±0.028	93.28±2.64	93.94±6.87
63	15.339±0.727	13.215±0.507	96.83±3.91	>99.00
64	>32	18.045±1.256	91.50±4.69	91.99±7.69
65	>32	12.864±0.457	98.59±5.76	>99.00
66	15.895±0.770	14.999±0.316	95.69±3.49	98.10±5.89
67	17.406±0.870	13.046±0.484	93.19±5.19	>99.00
68	15.839±1.200	12.050±0.312	>99.00	>99.00
69	>32	12.370±0.375	>99.00	>99.00
70	12.755±0.890	15.359±1.238	>99.00	>99.00
71	16.895±1.687	>32	>99.00	>99.00
72	13.895±0.496	>32	>99.00	>99.00

Characterization of compounds 44-72

2-((5-cyano-4-(4-isopropylphenyl)-6-(p-tolylamino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (44) Yield 78.5%. Yellow solid. Mp: 216–217°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.04 (s, 1H, NH, D₂O exchangeable), 10.63 (s, 1H, NH, D₂O exchangeable), 9.77 (s, 1H, NH, D₂O exchangeable), 8.30 (s, 1H, ArH), 8.00 (s, 1H, ArH), 7.93 – 7.68 (m, 3H, ArH), 7.36 (s, 4H, ArH), 7.13 (d, *J* = 6.1 Hz, 3H, ArH), 4.10 (s, 2H, CH₂), 2.94 (s, 1H, CH), 2.26 (s, 3H, CH₃), 1.21 (d, *J* = 6.0 Hz, 6H, CH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.77, 170.77, 168.18, 160.72, 152.43, 151.42, 150.72, 148.70, 139.03, 135.29, 134.74, 133.78, 129.33, 129.26, 126.91, 124.24, 120.09, 116.52, 114.64, 113.41, 85.03, 36.02, 33.86, 24.04, 21.06. HRMS (ESI) calcd for C₂₉H₂₈N₇O₂S [M+H]⁺: 538.2025, found: 538.2023.

2-((5-cyano-4-(4-isopropylphenyl)-6-((4-methoxyphenyl)amino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (45) Yield 86.7%. Yellow solid. Mp: 175–176°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.05 (s, 1H, NH, D₂O exchangeable), 10.66 (s, 1H, NH, D₂O exchangeable), 9.72 (s, 1H, NH, D₂O exchangeable), 8.30 (d, *J* = 4.2 Hz, 1H, ArH), 7.99 (d, *J* = 7.2 Hz, 1H, ArH), 7.91 – 7.68 (m, 2H, ArH), 7.36 (dd, *J* = 10.3, 8.8 Hz, 4H, ArH), 6.89 (d, *J* = 8.9 Hz, 1H, ArH), 6.65 (dd, *J* = 34.8, 8.7 Hz, 2H, ArH), 4.07 (s, 2H, CH₂), 3.73 (s, 3H, OCH₃), 2.94 (dt, *J* = 13.7, 6.8 Hz, 1H, CH), 1.20 (d, *J* = 6.9 Hz, 6H, CH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.73, 170.84, 168.11, 160.78, 157.17, 152.41, 151.36, 150.72, 148.70, 139.05, 133.79, 130.56, 129.23, 126.90, 125.98, 120.14, 116.48, 114.98, 114.02, 113.46, 84.74, 55.54, 35.95, 33.85, 24.03. HRMS (ESI) calcd for

C₂₉H₂₈N₇O₃S [M+H]⁺: 554.1974, found: 554.1972.

2-((5-cyano-4-(4-isopropylphenyl)-6-(o-tolylamino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (46) Yield 81.0%. Yellow solid. Mp: 156–157°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.06 (s, 1H, NH, D₂O exchangeable), 10.63 (s, 1H, NH, D₂O exchangeable), 9.78 (s, 1H, NH, D₂O exchangeable), 8.30 (d, *J* = 4.7 Hz, 1H, ArH), 7.99 (d, *J* = 7.1 Hz, 1H, ArH), 7.90 – 7.70 (m, 3H, ArH), 7.49 – 7.09 (m, 6H, ArH), 6.97 (d, *J* = 7.5 Hz, 1H, ArH), 4.10 (s, 2H, CH₂), 2.94 (dt, *J* = 13.7, 6.9 Hz, 1H, CH), 2.27 (s, 3H, CH₃), 1.21 (d, *J* = 6.9 Hz, 6H, CH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.69, 170.90, 168.24, 160.72, 152.47, 151.37, 150.69, 148.69, 139.04, 138.23, 137.83, 133.75, 129.27, 128.68, 126.91, 126.26, 124.75, 121.43, 120.11, 116.46, 113.44, 85.23, 35.89, 33.85, 24.03, 21.44. HRMS (ESI) calcd for C₂₉H₂₈N₇O₂S [M+H]⁺: 538.2025, found: 538.2022.

2-((5-cyano-4-(4-isopropylphenyl)-6-((2-(trifluoromethyl)phenyl)amino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (47) Yield 88.6%. Yellow solid. Mp: 194–195°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.08 (s, 1H, NH, D₂O exchangeable), 10.63 (s, 1H, NH, D₂O exchangeable), 10.10 (s, 1H, NH, D₂O exchangeable), 8.29 (d, *J* = 4.0 Hz, 1H, ArH), 8.06 – 7.89 (m, 3H, ArH), 7.89 – 7.77 (m, 2H, ArH), 7.60 (t, *J* = 7.8 Hz, 1H, ArH), 7.54 – 7.40 (m, 1H, ArH), 7.35 (d, *J* = 8.3 Hz, 2H, ArH), 7.14 (dd, *J* = 6.9, 5.3 Hz, 1H, ArH), 4.14 (s, 2H, CH₂), 2.94 (dt, *J* = 13.7, 6.9 Hz, 1H, CH), 1.21 (d, *J* = 6.9 Hz, 6H, CH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.74, 170.91, 168.36, 160.69, 152.61, 151.35, 150.67, 148.67, 138.99, 138.96, 133.63, 129.98, 129.31, 127.66, 126.95, 125.84, 123.13, 121.54, 120.33,

120.10, 116.26, 113.45, 85.89, 35.91, 33.85, 24.01. HRMS (ESI) calcd for $C_{29}H_{25}F_3N_7O_2S$ $[M+H]^+$: 592.1743, found: 592.1741.

2-((5-cyano-4-(p-tolyl)-6-((2-(trifluoromethyl)phenyl)amino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (48) Yield 72.3%. Yellow solid. Mp: 181–182°C.

1H NMR (400 MHz, DMSO- d_6 , δ , ppm) δ 11.09 (s, 1H, NH, D_2O exchangeable), 10.62 (s, 1H, NH, D_2O exchangeable), 10.12 (s, 1H, NH, D_2O exchangeable), 8.28 (d, $J = 4.4$ Hz, 1H, ArH), 7.95 (dd, $J = 18.1, 8.7$ Hz, 3H, ArH), 7.82 (dd, $J = 20.8, 8.0$ Hz, 3H, ArH), 7.60 (t, $J = 7.8$ Hz, 1H, ArH), 7.49 (d, $J = 7.7$ Hz, 1H, ArH), 7.30 (d, $J = 8.0$ Hz, 2H, ArH), 7.19 – 7.04 (m, 1H, ArH), 4.14 (s, 2H, CH_2), 2.37 (s, 3H, Ar- CH_3). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm) δ 172.72, 170.90, 168.39, 151.30, 150.65, 148.65, 142.03, 139.00, 138.94, 133.21, 129.96, 129.56, 129.17, 127.67, 126.15, 125.84, 123.13, 121.53, 120.33, 120.10, 116.25, 113.47, 85.87. HRMS (ESI) calcd for $C_{27}H_{21}F_3N_7O_2S$ $[M+H]^+$: 564.1430, found: 564.1428.

2-((4-(4-chlorophenyl)-5-cyano-6-((2-(trifluoromethyl)phenyl)amino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (49) Yield 87.5%. Yellow solid. Mp: 202–203°C. 1H NMR (400 MHz, DMSO- d_6 , δ , ppm) δ 11.07 (s, 1H, NH, D_2O exchangeable), 10.58 (s, 1H, NH, D_2O exchangeable), 10.18 (s, 1H, NH, D_2O exchangeable), 8.28 (s, 1H, ArH), 8.12 – 7.69 (m, 6H, ArH), 7.69 – 7.34 (m, 4H, ArH), 7.13 (s, 1H, ArH), 4.14 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): δ 172.92, 170.83, 167.37, 160.54, 151.27, 150.63, 148.63, 138.99, 138.82, 136.72, 134.81, 131.02, 129.97, 129.52, 129.09, 127.73, 125.81, 123.11, 121.69, 120.41, 120.10, 115.93, 113.46, 86.33, 35.91. HRMS (ESI) calcd for $C_{26}H_{18}ClF_3N_7O_2S$

$[M+H]^+$: 584.0883, found: 584.0882.

2-((5-cyano-4-(3,4-difluorophenyl)-6-((2-(trifluoromethyl)phenyl)amino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (50) Yield 86.7%. Yellow solid. Mp: 134–135°C. ^1H NMR (400 MHz, DMSO- d_6 , δ , ppm) δ 11.08 (s, 1H, NH, D₂O exchangeable), 10.58 (s, 1H, NH, D₂O exchangeable), 10.22 (s, 1H, NH, D₂O exchangeable), 8.27 (d, J = 4.4 Hz, 1H, ArH), 8.04 – 7.72 (m, 6H, ArH), 7.56 (dt, J = 27.0, 8.0 Hz, 3H, ArH), 7.25 – 7.03 (m, 1H, ArH), 4.15 (s, 2H, CH₂). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): δ 172.94, 170.83, 166.29, 160.45, 151.26, 150.56, 148.59, 138.97, 138.75, 133.40, 130.00, 129.84, 127.78, 126.68, 125.80, 123.10, 121.76, 120.42, 120.08, 118.71, 118.52, 118.37, 118.19, 115.82, 113.46, 86.53, 35.92. HRMS (ESI) calcd for C₂₆H₁₇F₅N₇O₂S $[M+H]^+$: 586.1085, found: 586.1086

2-((5-cyano-4-((2-(trifluoromethyl)phenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (51) Yield 72.0%. Yellow solid. Mp: 145–146°C. ^1H NMR (400 MHz, DMSO- d_6 , δ , ppm) δ 11.18 (s, 1H, NH, D₂O exchangeable), 10.59 (s, 1H, NH, D₂O exchangeable), 10.14 (s, 1H, NH, D₂O exchangeable), 8.27 (s, 1H, ArH), 8.07 – 7.73 (m, 4H, ArH), 7.70 – 7.39 (m, 2H, ArH), 7.16 (d, J = 38.9 Hz, 3H, ArH), 4.21 (s, 2H, CH₂), 3.83 (s, 6H, OCH₃), 3.74 (s, 3H, OCH₃). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): δ 172.64, 170.62, 168.16, 160.64, 153.15, 151.31, 150.63, 148.58, 140.64, 138.97, 138.93, 131.01, 130.02, 129.82, 129.50, 127.67, 125.83, 123.12, 121.54, 120.30, 120.03, 116.39, 113.42, 106.94, 86.05, 60.65, 56.49, 36.03. HRMS (ESI) calcd for C₂₉H₂₅F₃N₇O₅S $[M+H]^+$: 640.1590, found: 640.1588.

2-((4-((4-bromophenyl)amino)-5-cyano-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (52) Yield 75.0%. Yellow solid. Mp: 216–217°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.08 (s, 1H, NH, D₂O exchangeable), 10.58 (s, 1H, NH, D₂O exchangeable), 9.92 (s, 1H, NH, D₂O exchangeable), 8.29 (d, *J* = 4.3 Hz, 1H, ArH), 7.95 (d, *J* = 7.2 Hz, 1H, ArH), 7.83 (t, *J* = 7.2 Hz, 1H, ArH), 7.62 – 7.40 (m, 4H, ArH), 7.20 (s, 2H, ArH), 7.17 – 7.07 (m, 1H, ArH), 4.18 (s, 2H, CH₂), 3.83 (s, 6H, OCH₃), 3.75 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.67, 170.64, 168.13, 160.52, 153.15, 151.30, 150.64, 148.62, 140.60, 139.04, 137.41, 131.73, 131.07, 125.92, 120.11, 117.54, 116.49, 113.50, 106.94, 85.86, 60.66, 56.49, 36.10. HRMS (ESI) calcd for C₂₈H₂₅BrN₇O₅S [M+H]⁺: 650.0821, found: 650.0820.

2-((4-((2-chlorophenyl)amino)-5-cyano-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (53) Yield 87.5%. Yellow solid. Mp: 206–207°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.17 (s, 1H, NH, D₂O exchangeable), 10.60 (s, 1H, NH, D₂O exchangeable), 9.97 (s, 1H, NH, D₂O exchangeable), 8.28 (d, *J* = 4.1 Hz, 1H, ArH), 8.03 – 7.71 (m, 2H, ArH), 7.75 – 7.45 (m, 2H, ArH), 7.39 (t, *J* = 8.1 Hz, 1H, ArH), 7.30 – 7.04 (m, 4H, ArH), 4.21 (s, 2H, CH₂), 3.83 (s, 6H, OCH₃), 3.75 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.64, 170.63, 168.14, 160.61, 153.15, 151.34, 150.67, 148.60, 140.65, 139.59, 138.99, 133.15, 131.02, 130.46, 125.08, 123.54, 122.50, 120.04, 116.43, 113.46, 106.96, 85.97, 60.66, 56.50, 36.04. HRMS (ESI) calcd for C₂₈H₂₅ClN₇O₅S [M+H]⁺: 606.1326, found: 606.1324.

2-((4-((4-chlorophenyl)amino)-5-cyano-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (54) Yield 80.0%. Yellow solid. Mp: 128–129°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.07 (s, 1H, NH, D₂O exchangeable), 10.57 (s, 1H, NH, D₂O exchangeable), 9.93 (s, 1H, NH, D₂O exchangeable), 8.29 (s, 1H, ArH), 8.09 – 7.72 (m, 2H, ArH), 7.55 (d, *J* = 7.9 Hz, 2H, ArH), 7.39 (d, *J* = 8.0 Hz, 2H, ArH), 7.17 (d, *J* = 26.0 Hz, 3H, ArH), 4.17 (s, 2H, CH₂), 3.83 (s, 6H, OCH₃), 3.75 (s, 2H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.67, 170.63, 168.13, 160.59, 153.15, 151.29, 150.64, 148.62, 140.59, 139.04, 136.94, 131.08, 129.37, 128.81, 125.66, 120.11, 116.50, 113.48, 106.92, 85.78, 60.66, 56.48, 36.12. HRMS (ESI) calcd for C₂₈H₂₅ClN₇O₅S [M+H]⁺: 606.1326, found: 606.1324.

2-((4-((3-chlorophenyl)amino)-5-cyano-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (55) Yield 76.5%. Yellow solid. Mp: 194–195°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.17 (s, 1H, NH, D₂O exchangeable), 10.60 (s, 1H, NH, D₂O exchangeable), 9.97 (s, 1H, NH, D₂O exchangeable), 8.28 (d, *J* = 4.1 Hz, 1H, ArH), 8.03 – 7.71 (m, 2H, ArH), 7.75 – 7.45 (m, 2H, ArH), 7.39 (t, *J* = 8.1 Hz, 1H, ArH), 7.30 – 7.04 (m, 4H, ArH), 4.21 (s, 2H, CH₂), 3.83 (s, 6H, OCH₃), 3.75 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.64, 170.63, 168.14, 160.61, 153.15, 151.34, 150.67, 148.60, 140.65, 139.59, 138.99, 133.15, 131.02, 130.46, 125.08, 123.54, 122.50, 120.04, 116.43, 113.46, 106.96, 85.97, 60.66, 56.50, 36.04. HRMS (ESI) calcd for C₂₈H₂₄ClN₇NaO₅S [M+H]⁺: 628.1146, found: 628.1147.

2-((5-cyano-4-((3-nitrophenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (56) Yield 79.0%. Yellow solid. Mp: 213–214°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.12 (s, 1H, NH, D₂O exchangeable), 10.52 (s, 1H, NH, D₂O exchangeable), 10.28 (s, 1H, NH, D₂O exchangeable), 8.48 (s, 1H, ArH), 8.27 (s, 1H, ArH), 7.91 (ddd, *J* = 36.8, 22.0, 7.6 Hz, 4H, ArH), 7.64 (t, *J* = 7.9 Hz, 1H, ArH), 7.17 (d, *J* = 43.4 Hz, 3H, ArH), 4.21 (s, 2H, CH₂), 3.79 (d, *J* = 35.6 Hz, 9H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.71, 170.46, 168.19, 160.67, 153.15, 151.30, 150.59, 148.56, 148.10, 140.63, 139.36, 138.96, 130.96, 130.18, 130.06, 120.01, 119.70, 118.24, 116.37, 113.37, 106.92, 86.18, 60.66, 56.49, 36.22. HRMS (ESI) calcd for C₂₈H₂₅N₈O₇S [M+H]⁺: 617.1567, found: 617.1564.

2-((5-cyano-4-((4-(trifluoromethyl)phenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (57) Yield 87.5%. Yellow solid. Mp: 201–202°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.10 (s, 1H, NH, D₂O exchangeable), 10.57 (s, 1H, NH, D₂O exchangeable), 10.14 (s, 1H, NH, D₂O exchangeable), 8.29 (d, *J* = 4.2 Hz, 1H, ArH), 7.82 (ddd, *J* = 46.5, 37.2, 8.6 Hz, 6H, ArH), 7.37 – 7.05 (m, 3H, ArH), 4.21 (s, 2H, CH₂), 3.84 (s, 6H, OCH₃), 3.75 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.78, 170.56, 168.31, 160.56, 153.17, 151.27, 150.61, 148.63, 143.55, 141.92, 140.66, 139.02, 130.98, 126.10, 123.41, 120.12, 116.43, 113.42, 106.97, 86.47, 60.67, 56.49, 36.34. HRMS (ESI) calcd for C₂₉H₂₅F₃N₇O₅S [M+H]⁺: 640.1590, found: 640.1603.

2-((5-cyano-4-((2-methoxyphenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)

***thio*-N-(pyridin-2-ylcarbamoyl)acetamide (58)** Yield 74.2%, Yellow solid. Mp: 204-205 °C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.12 (s, 1H, NH, D₂O exchangeable), 10.52 (s, 1H, NH, D₂O exchangeable), 10.28 (s, 1H, NH, D₂O exchangeable), 8.48 (s, 1H, ArH), 8.27 (s, 1H, ArH), 7.91 (ddd, *J* = 36.8, 22.0, 7.6 Hz, 4H, ArH), 7.64 (t, *J* = 7.9 Hz, 1H, ArH), 7.17 (d, *J* = 43.4 Hz, 3H, ArH), 4.21 (s, 2H, CH₂), 3.79 (d, *J* = 35.6 Hz, 9H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm) δ 172.71, 170.46, 168.19, 160.67, 153.15, 151.30, 150.59, 148.56, 148.10, 140.63, 139.36, 138.96, 130.96, 130.18, 130.06, 120.01, 119.70, 118.24, 116.37, 113.37, 106.92, 86.18, 60.66, 56.49, 36.22. HRMS (ESI) calcd for C₂₉H₂₈N₇O₆S [M+H]⁺: 602.1822, found: 602.1820.

2-((5-cyano-4-((2-methoxyphenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(phenylcarbamoyl)acetamide (59) Yield 81.2%. Yellow solid. Mp: 231–232°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.01 (s, 1H, NH, D₂O exchangeable), 10.58 (s, 1H, NH, D₂O exchangeable), 9.78 (s, 1H, NH, D₂O exchangeable), 8.30 (s, 1H, ArH), 7.89 (d, *J* = 42.5 Hz, 2H, ArH), 7.58 – 6.84 (m, 7H, ArH), 4.13 (s, 2H, CH₂), 3.79 (d, *J* = 33.4 Hz, 9H, OCH₃), 2.26 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm) δ 172.61, 170.58, 168.04, 160.69, 153.13, 151.48, 150.66, 148.66, 140.37, 139.06, 135.25, 134.80, 131.22, 129.33, 124.29, 120.09, 116.62, 113.41, 106.86, 85.28, 60.65, 56.47, 36.06, 21.07. HRMS (ESI) calcd for C₃₀H₂₉N₆O₆S [M+H]⁺: 601.1869, found: 601.1866.

2-((4-((3-chlorophenyl)amino)-5-cyano-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(phenylcarbamoyl)acetamide (60) Yield 86.0%. Yellow solid. Mp: 184-185°C.

¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 10.86 (s, 1H, D₂O exchangeable), 10.25 (s, 1H, D₂O exchangeable), 9.98 (s, 1H, D₂O exchangeable), 7.66 (s, 1H, ArH), 7.56 (d, *J* = 8.1 Hz, 1H, ArH), 7.50 (d, *J* = 7.9 Hz, 2H, ArH), 7.39 (t, *J* = 8.1 Hz, 1H, ArH), 7.32 (t, *J* = 7.8 Hz, 2H, ArH), 7.23 (d, *J* = 8.1 Hz, 3H, ArH), 7.09 (t, *J* = 7.3 Hz, 1H, ArH), 4.16 (s, 2H, S-CH₂), 3.84 (s, 6H, O-CH₃), 3.75 (s, 3H, O-CH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm) δ 172.66, 170.58, 168.19, 160.58, 153.16, 150.88, 140.62, 139.59, 137.91, 133.18, 131.03, 130.46, 129.40, 129.22, 125.12, 124.20, 123.55, 122.46, 120.90, 120.14, 116.45, 106.91, 85.99, 60.64, 56.45, 35.73. HRMS (ESI) calcd for C₂₉H₂₅ClN₆NaO₅S [M+Na]⁺: 627.1193, found: 627.1193.

2-((5-cyano-4-((3-nitrophenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(phenylcarbamoyl)acetamide (61)

Yield 79.2%. Yellow solid. Mp: 132–134°C.

¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 10.80 (s, 1H, D₂O exchangeable), 10.26 (s, 1H, D₂O exchangeable), 10.18 (s, 1H, D₂O exchangeable), 8.51 (s, 1H, ArH), 8.02 (dd, *J* = 15.7, 5.7 Hz, 2H, ArH), 7.64 (t, *J* = 8.2 Hz, 1H, ArH), 7.47 (d, *J* = 7.9 Hz, 2H, ArH), 7.32 (t, *J* = 7.8 Hz, 2H, ArH), 7.22 (d, *J* = 6.7 Hz, 2H, ArH), 7.08 (t, *J* = 7.3 Hz, 1H, ArH), 4.16 (s, 2H, S-CH₂), 3.84 (s, 6H, O-CH₃), 3.75 (s, 3H, O-CH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm) δ 172.71, 170.44, 168.24, 160.66, 153.17, 150.79, 148.18, 140.66, 139.37, 137.88, 130.96, 130.17, 129.99, 129.38, 124.18, 120.09, 119.75, 118.28, 116.36, 106.92, 86.23, 60.65, 56.47, 35.88. HRMS (ESI) calcd for C₂₉H₂₆N₇O₇S [M+H]⁺: 616.1614, found: 616.1611.

2-((5-cyano-4-((4-fluorophenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(phenylcarbamoyl)acetamide (62)

Yield 75.0%. Yellow solid. Mp: 208–209°C.

¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 10.84 (s, 1H, D₂O exchangeable), 10.23 (s, 1H, D₂O exchangeable), 9.88 (s, 1H, D₂O exchangeable), 7.67 – 7.43 (m, 4H, ArH), 7.34 (dd, *J* = 19.7, 12.0 Hz, 2H, ArH), 7.25 – 7.13 (m, 4H, ArH), 7.09 (t, *J* = 7.3 Hz, 1H), 4.11 (s, 2H, S-CH₂), 3.84 (s, 6H, O-CH₃), 3.75 (s, 3H, O-CH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm) δ 172.68, 170.43, 168.04, 160.80, 153.15, 150.88, 140.53, 137.93, 134.14, 131.14, 129.41, 126.50, 126.42, 124.20, 120.15, 116.57, 115.72, 115.50, 106.86, 85.32, 60.64, 56.46, 35.82. HRMS (ESI) calcd for C₂₉H₂₅FN₆NaO₅S [M+Na]⁺: 611.1489, found: 611.1488.

2-((5-cyano-4-((4-isopropylphenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-ylthio)-N-(phenylcarbamoyl)acetamide (63) Yield 84.5%. Yellow solid. Mp: 151–152°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 10.87 (s, 1H, D₂O exchangeable), 10.28 (s, 1H, D₂O exchangeable), 9.76 (s, 1H, D₂O exchangeable), 7.52 (d, *J* = 7.9 Hz, 2H, ArH), 7.44 (d, *J* = 8.4 Hz, 2H, ArH), 7.34 (dd, *J* = 15.8, 8.1 Hz, 2H, ArH), 7.27 – 7.17 (m, 4H, ArH), 7.09 (t, *J* = 7.4 Hz, 1H, ArH), 4.14 (s, 2H, S-CH₂), 3.84 (s, 6H, O-CH₃), 3.75 (s, 3H, O-CH₃), 3.01 – 2.70 (m, 1H, -CH(CH₃)₂), 1.19 (d, *J* = 6.9 Hz, 6H, -CH(CH₃)₂). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm) δ 172.70, 170.54, 168.06, 160.51, 153.14, 150.91, 145.62, 140.50, 137.97, 135.62, 131.21, 129.41, 126.67, 124.18, 123.92, 120.07, 116.64, 106.87, 85.39, 60.63, 56.46, 35.82, 33.42, 24.29. HRMS (ESI) calcd for C₃₂H₃₃N₆O₅S [M+H]⁺: 613.2233, found: 613.2231.

2-((4-((2-chlorophenyl)amino)-5-cyano-6-(3,4-difluorophenyl)pyrimidin-2-ylthio)-N-(pyridin-2-ylcarbamoyl)acetamide (64) Yield 75.5%. Yellow solid. Mp: 106–107°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 10.88 (s, 1H, NH, D₂O

exchangeable), 10.56 (s, 1H, NH, D₂O exchangeable), 10.05 (s, 1H, NH, D₂O exchangeable), 8.29 (d, $J = 4.3$ Hz, 1H, ArH), 8.08 – 7.68 (m, 4H, ArH), 7.63 – 7.20 (m, 5H, ArH), 7.20 – 7.02 (m, 1H, ArH), 3.99 (s, 2H, CH₂). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 173.06, 170.44, 166.02, 161.18, 151.35, 150.56, 148.67, 139.01, 134.91, 133.39, 131.39, 130.08, 129.66, 129.03, 128.03, 126.73, 120.07, 118.73, 118.54, 118.37, 118.19, 115.85, 113.44, 85.21, 35.96. HRMS (ESI) calcd for C₂₅H₁₇ClF₂N₇O₂S [M+H]⁺: 552.0821, found: 552.0818.

2-((4-((3-chlorophenyl)amino)-5-cyano-6-(3,4-difluorophenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (65) Yield 85.0%. Yellow solid. Mp: 192–193°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.08 (s, 1H, NH, D₂O exchangeable), 10.60 (s, 1H, NH, D₂O exchangeable), 10.08 (s, 1H, NH, D₂O exchangeable), 8.28 (d, $J = 4.4$ Hz, 1H, ArH), 8.05 – 7.69 (m, 4H, ArH), 7.67 – 7.48 (m, 3H, ArH), 7.38 (t, $J = 8.1$ Hz, 1H, ArH), 7.28 – 7.03 (m, 2H, ArH), 4.15 (s, 2H, CH₂). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.95, 170.83, 166.28, 160.42, 151.29, 150.60, 148.62, 139.41, 138.98, 133.16, 130.45, 126.72, 126.68, 125.27, 123.70, 122.64, 120.09, 118.71, 118.53, 118.36, 118.18, 115.86, 113.48, 86.42, 35.93. HRMS (ESI) calcd for C₂₅H₁₇ClF₂N₇O₂S [M+H]⁺: 552.0821, found: 552.0820.

2-((5-cyano-4-(3,4-difluorophenyl)-6-((2-methoxyphenyl)amino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (66) Yield 78.5%. Yellow solid. Mp: 198–199°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.00 (s, 1H, NH, D₂O exchangeable), 10.57 (s, 1H, NH, D₂O exchangeable), 9.32 (s, 1H, NH, D₂O exchangeable), 8.28 (d, $J = 4.3$ Hz, 1H, ArH), 8.09 – 7.69 (m, 4H, ArH), 7.68 – 7.44

(m, 2H, ArH), 7.28 – 6.82 (m, 4H, ArH), 4.08 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.96, 170.66, 165.54, 160.57, 153.08, 151.33, 150.59, 148.63, 139.00, 133.46, 127.61, 126.56, 126.11, 126.05, 120.69, 120.06, 118.64, 118.45, 118.36, 118.18, 115.99, 113.45, 112.14, 100.00, 85.53, 56.19, 36.02. HRMS (ESI) calcd for C₂₆H₂₀F₂N₇O₃S [M+H]⁺: 548.1316, found: 548.1314.

2-((4-((4-chlorophenyl)amino)-5-cyano-6-(3,4-difluorophenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (67) Yield 80.9%. Yellow solid. Mp: 213–214°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.09 (s, 1H, NH, D₂O exchangeable), 10.58 (s, 1H, NH, D₂O exchangeable), 10.03 (s, 1H, NH, D₂O exchangeable), 8.28 (d, *J* = 4.1 Hz, 1H, ArH), 8.09 – 7.71 (m, 4H, ArH), 7.71 – 7.45 (m, 3H, ArH), 7.39 (d, *J* = 8.8 Hz, 2H, ArH), 7.20 – 7.00 (m, 1H, ArH), 4.15 (s, 2H, CH₂). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 173.01, 170.71, 166.20, 160.37, 151.29, 150.60, 148.55, 139.01, 136.77, 133.41, 129.53, 128.80, 126.66, 125.73, 120.08, 118.69, 118.51, 118.35, 118.17, 115.93, 113.51, 86.15, 36.09. HRMS (ESI) calcd for C₂₅H₁₇ClF₂N₇O₂S [M+H]⁺: 552.0821, found: 552.0819.

2-((4-(4-chlorophenyl)-6-((4-chlorophenyl)amino)-5-cyanopyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (68) Yield 74.5%. Yellow solid. Mp: 212–213°C. ¹H NMR (400 MHz, DMSO-d₆, δ, ppm) δ 11.07 (s, 1H, NH, D₂O exchangeable), 10.57 (s, 1H, NH, D₂O exchangeable), 9.99 (s, 1H, NH, D₂O exchangeable), 8.29 (d, *J* = 4.1 Hz, 1H, ArH), 8.10 – 7.71 (m, 4H, ArH), 7.56 (dd, *J* = 8.7, 7.2 Hz, 4H, ArH), 7.39 (d, *J* = 8.8 Hz, 2H, ArH), 7.13 (dd, *J* = 6.9, 5.3 Hz, 1H, ArH), 4.14 (s, 2H, CH₂). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): δ 172.99, 170.71, 167.31, 160.46, 151.31,

150.66, 148.63, 139.02, 136.84, 136.66, 134.86, 131.01, 129.47, 129.08, 128.80, 125.68, 120.11, 116.05, 113.51, 85.98, 36.08. HRMS (ESI) calcd for $C_{25}H_{18}Cl_2N_7O_2S$ $[M+H]^+$: 550.0620, found: 550.0617.

2-((5-cyano-4-phenyl-6-(p-tolylamino)pyrimidin-2-yl)thio)-N-(pyridin-2-ylcarbamoyl)acetamide (69) Yield 70.5%. Yellow solid. Mp: 206–207°C. 1H NMR (400 MHz, DMSO- d_6 , δ , ppm) δ 11.03 (s, 1H, NH, D_2O exchangeable), 10.63 (s, 1H, NH, D_2O exchangeable), 9.81 (s, 1H, NH, D_2O exchangeable), 8.30 (d, $J = 4.1$ Hz, 1H, ArH), 8.09 – 7.77 (m, 4H, ArH), 7.54 (dt, $J = 32.2, 7.3$ Hz, 3H, ArH), 7.36 (d, $J = 8.3$ Hz, 2H, ArH), 7.14 (dd, $J = 10.5, 4.9$ Hz, 3H, ArH), 4.10 (s, 2H, CH_2), 2.25 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): δ 172.85, 170.73, 168.42, 160.65, 151.38, 150.70, 148.70, 139.07, 136.21, 135.24, 134.80, 131.68, 129.33, 129.13, 128.94, 124.28, 120.11, 116.35, 113.44, 85.43, 36.01, 21.06. HRMS (ESI) calcd for $C_{26}H_{22}N_7O_2S$ $[M+H]^+$: 496.1556, found: 496.1553.

2-(benzylthio)-4-((4-chlorophenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidine-5-carbonitrile (70) Yield 60.5%. Yellow solid. Mp: 206–207°C. 1H NMR (400 MHz, DMSO- d_6 , δ , ppm) δ 7.31 – 7.15 (m, 5H, ArH), 7.15 – 7.04 (m, 6H, ArH), 4.14 (s, 2H, CH_2), 3.80 (s, 6H, OCH_3), 3.72 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): δ 169.44, 165.59, 158.53, 152.23, 139.38, 138.57, 133.19, 128.72, 128.06, 127.34, 126.43, 124.94, 122.37, 120.48, 105.75, 86.84, 60.05, 55.86, 33.52. HRMS (ESI) calcd for $C_{27}H_{23}ClN_4O_3S$ $[M+H]^+$: 519.1258, found: 519.1259.

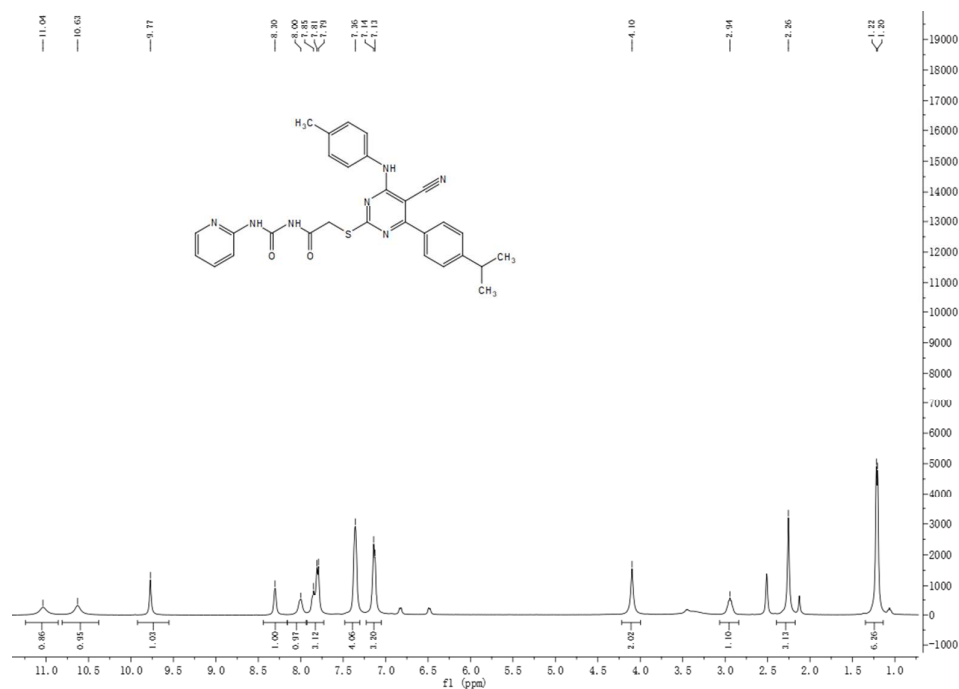
2-((4-((3-chlorophenyl)amino)-5-cyano-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-yl)thio)-N-(pyridin-2-yl)acetamide (71) Yield 60.5%. White solid. Mp: 206–207°C. 1H

NMR (400 MHz, DMSO- d_6 , δ , ppm) δ 10.67 (s, 1H, NH, D₂O exchangeable), 9.94 (s, 1H, NH, D₂O exchangeable), 8.32 (dd, J = 4.9, 1.0 Hz, 1H, ArH), 8.00 (d, J = 7.9 Hz, 1H, ArH), 7.80 – 7.71 (m, 1H, ArH), 7.68 (t, J = 2.0 Hz, 1H, ArH), 7.63 – 7.53 (m, 1H, ArH), 7.32 (t, J = 8.1 Hz, 1H, ArH), 7.21 (s, 2H, ArH), 7.17 (dd, J = 8.0, 1.2 Hz, 1H, ArH), 7.13 – 7.07 (m, 1H, ArH), 4.16 (s, 2H, CH₂), 3.76 (s, 6H, OCH₃), 3.74 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO- d_6 , δ , ppm): δ 172.58, 167.62, 166.59, 160.00, 152.62, 151.76, 147.95, 140.10, 139.14, 138.18, 132.63, 130.57, 129.93, 124.44, 122.90, 121.82, 119.46, 115.99, 113.25, 106.48, 85.31, 60.15, 55.91, 35.35. HRMS (ESI) calcd for C₂₇H₂₃ClN₆O₄S [M-H]⁻: 561.11, found: 561.06.

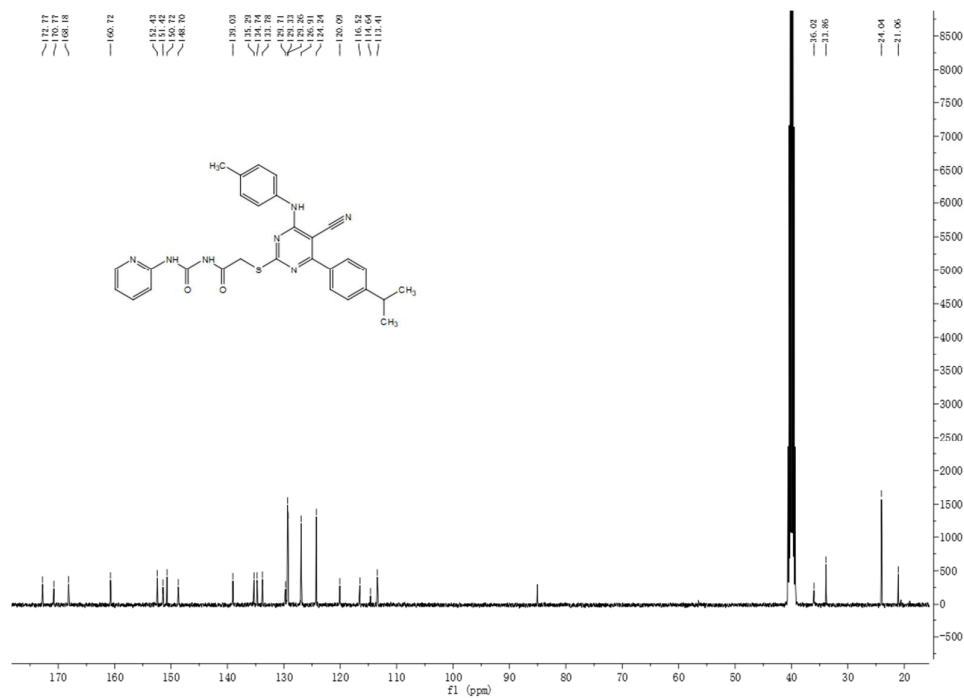
2-(benzylthio)-4-((3-chlorophenyl)amino)-6-(3,4,5-trimethoxyphenyl)pyrimidine-5-carbonitrile (72) Yield 60.5%. White solid. Mp: 206–207°C. ¹H NMR (400 MHz, DMSO- d_6 , δ , ppm) δ 10.00 (s, 1H, NH, D₂O exchangeable), 7.74 (t, J = 2.0 Hz, 1H, ArH), 7.54 (dd, J = 8.2, 1.0 Hz, 1H, ArH), 7.40 (t, J = 8.1 Hz, 1H, ArH), 7.31 – 7.15 (m, 8H, ArH), 4.34 (s, 2H, CH₂), 3.84 (s, 6H, OCH₃), 3.77 (s, 3H, OCH₃). ¹³C NMR (100 MHz, DMSO- d_6 , δ , ppm): δ 172.83, 167.70, 160.29, 152.64, 140.04, 139.23, 137.42, 132.65, 130.67, 130.01, 128.68, 128.32, 127.04, 124.75, 123.74, 122.55, 116.05, 106.42, 84.99, 60.18, 56.03, 34.24. LC-MS (ESI) calcd for C₂₇H₂₃ClN₄O₃S [M-H]⁻: 517.11, found: 517.07.

¹H and ¹³C NMR spectra, and HPLC chromatograms of compounds 44-72

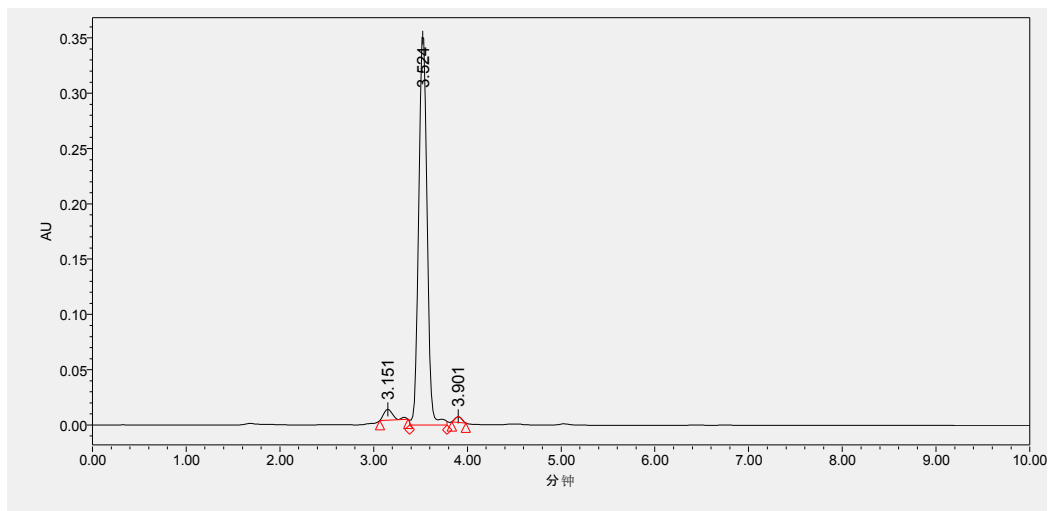
¹H, ¹³C NMR spectra, and HPLC chromatograms of compounds 44-72.



¹H NMR spectrum of compound 44

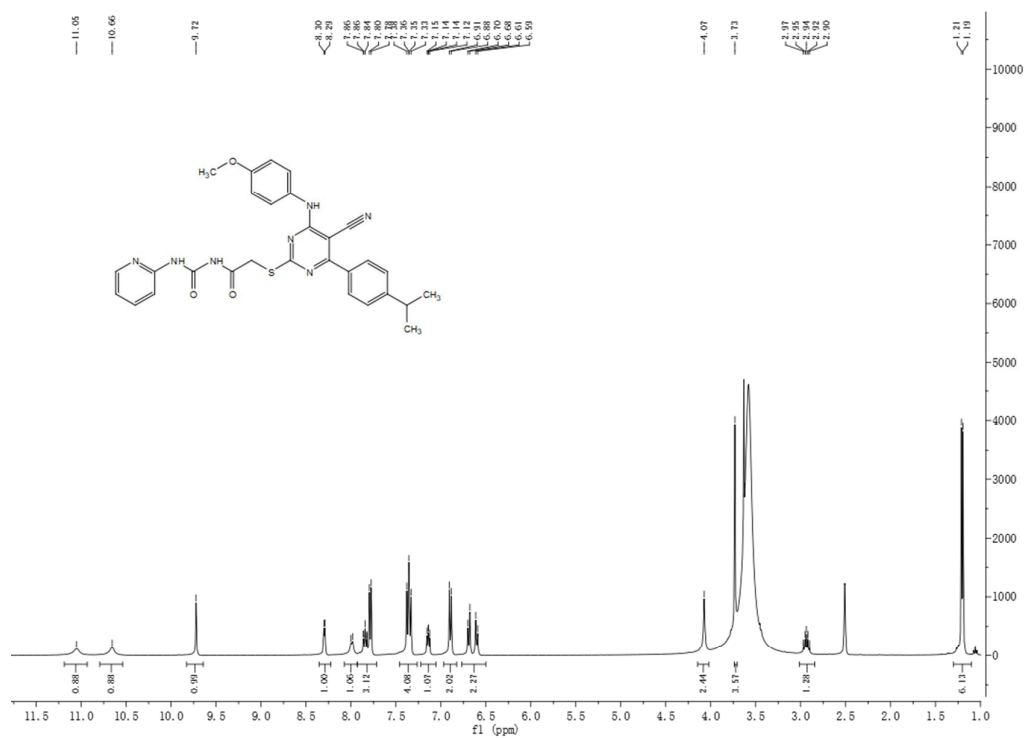


¹³C NMR spectrum of compound 44

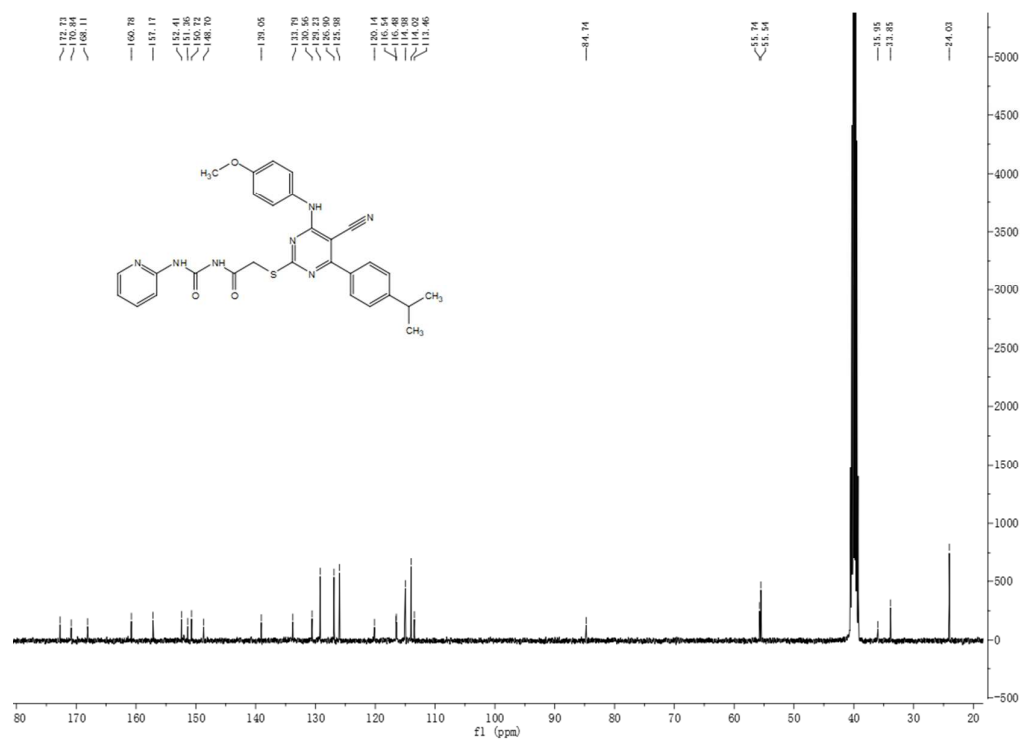


Peak	Retention Time	Area	% Area
1	3.151	66974	2.96
2	3.524	2172601	95.87
3	3.901	26519	1.17

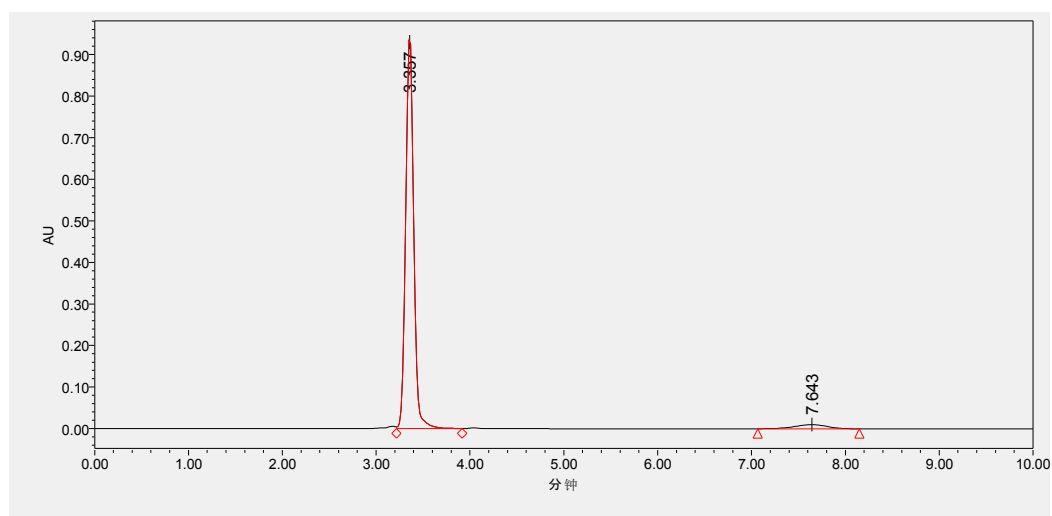
HPLC chromatogram of compound **44**



^1H NMR spectrum of compound **45**

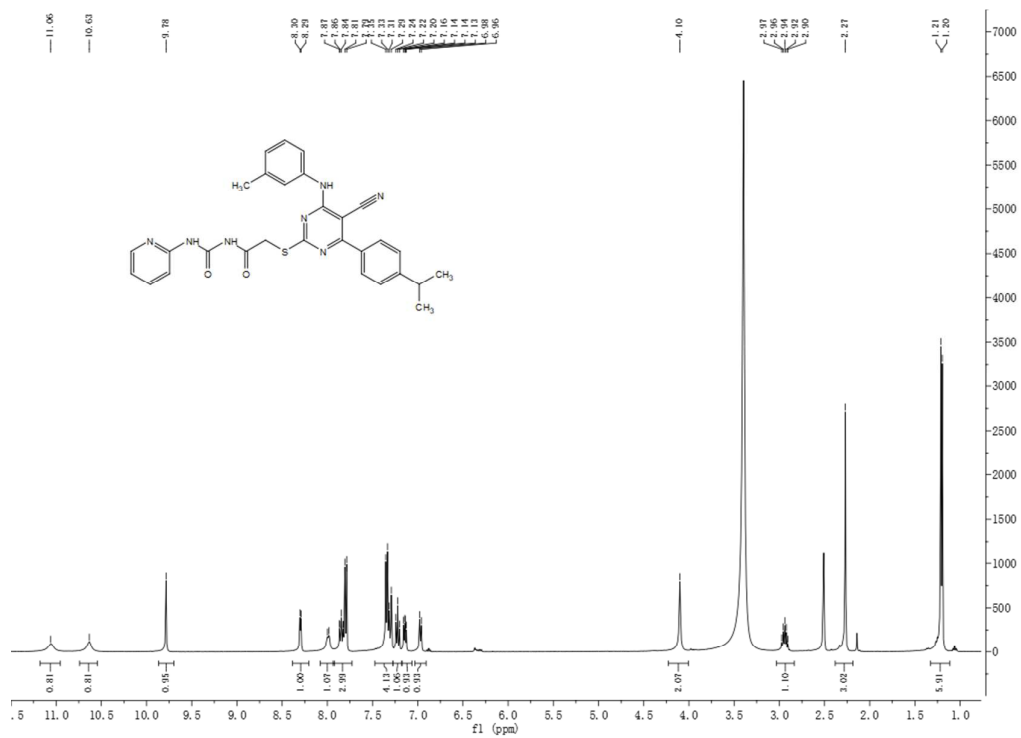


^{13}C NMR spectrum of compound **45**

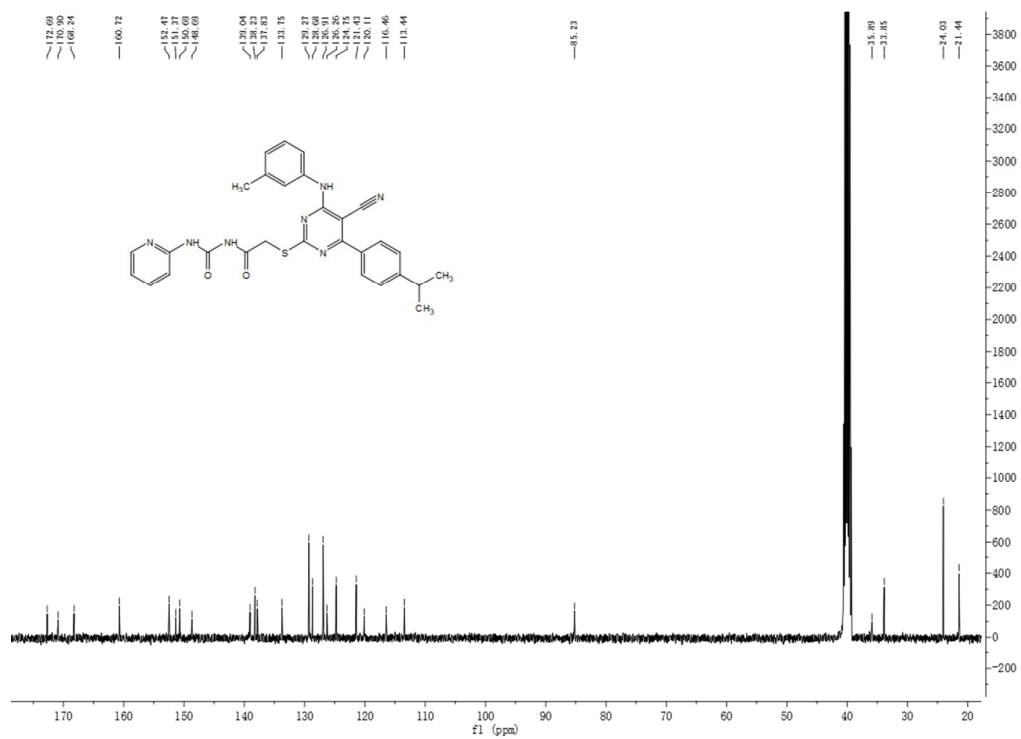


Peak	Retention Time	Area	% Area
1	3.357	5649178	95.84
2	7.643	245061	4.16

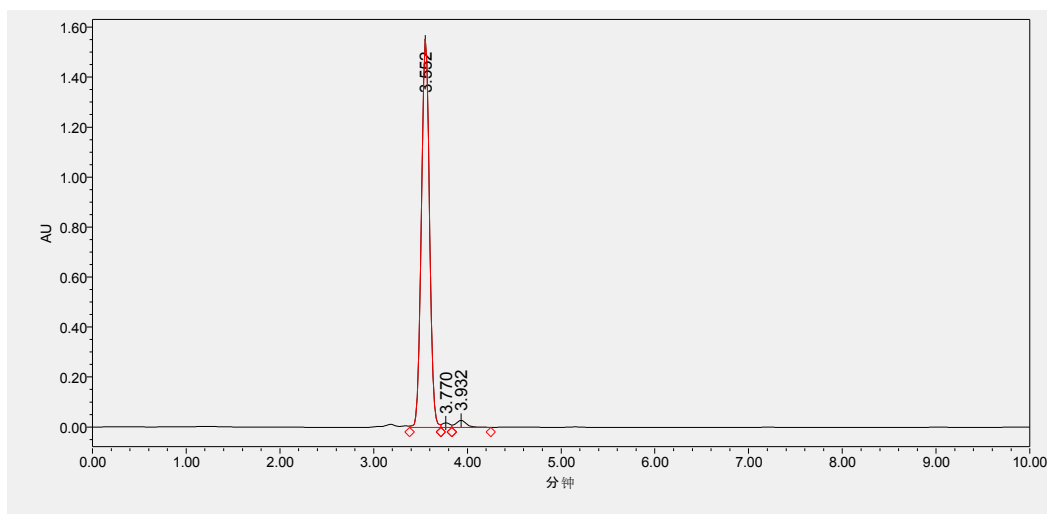
HPLC chromatogram of compound **45**



¹H NMR spectrum of compound **46**

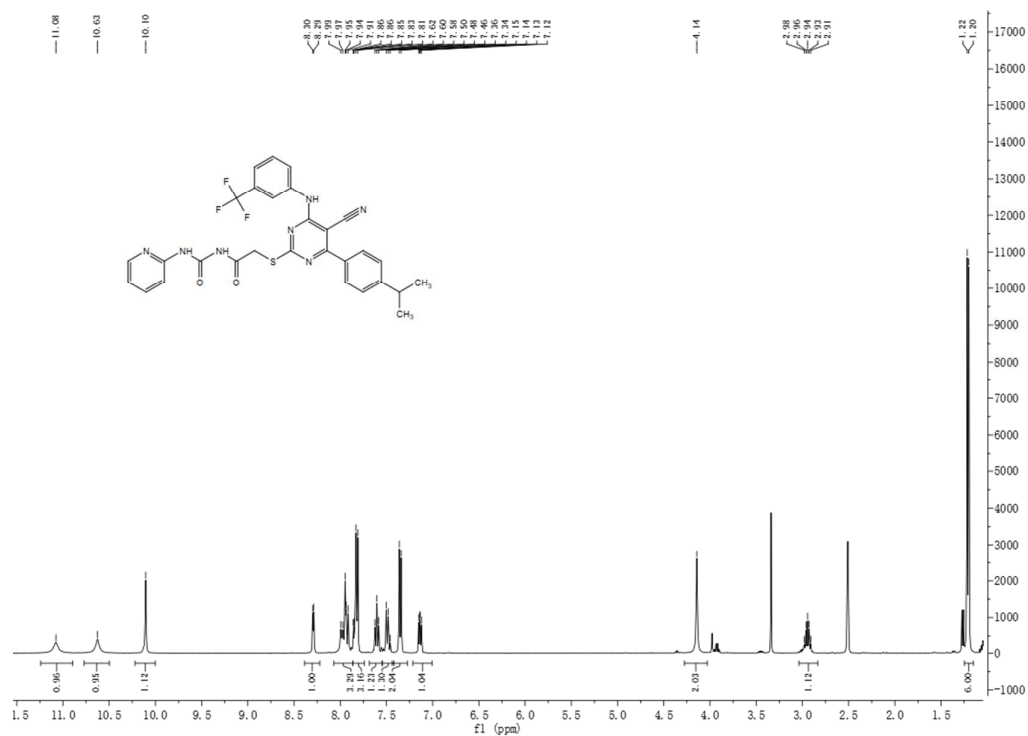


¹³C NMR spectrum of compound **46**

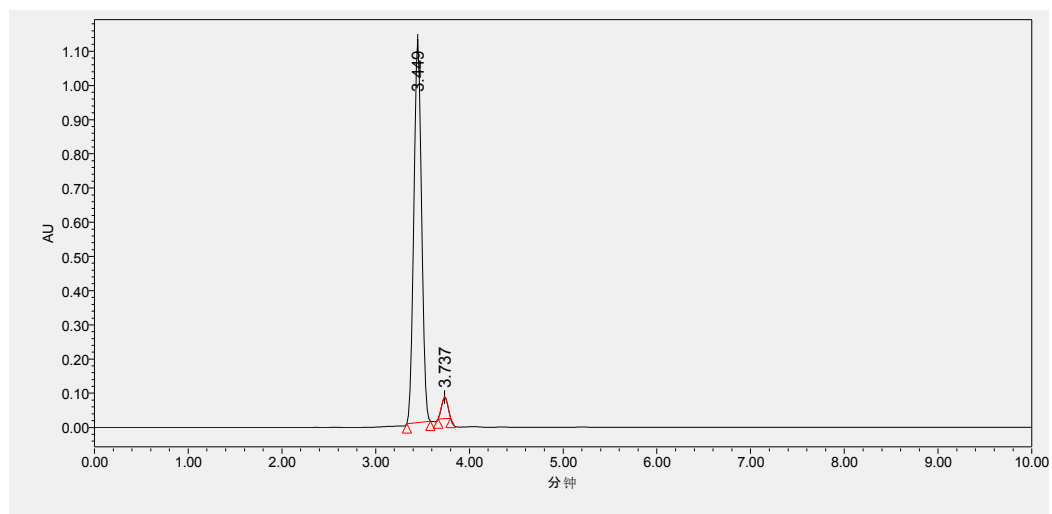
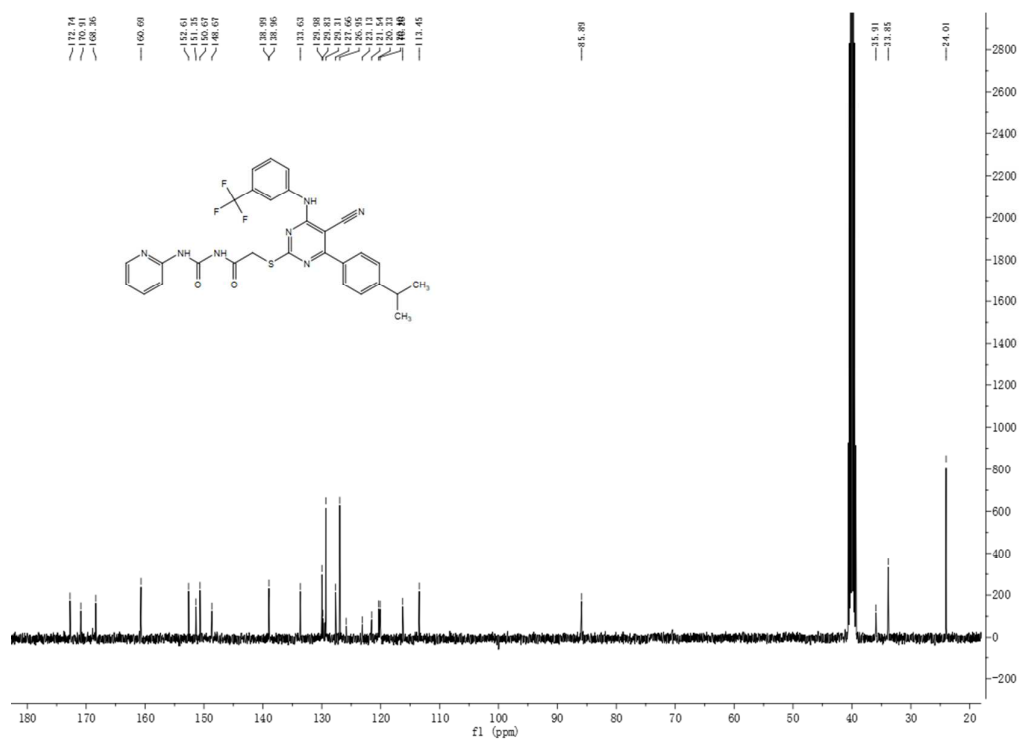


Peak	Retention Time	Area	% Area
1	3.552	9283223	96.75
2	3.770	98366	1.03
3	3.932	213001	2.22

HPLC chromatogram of compound 46

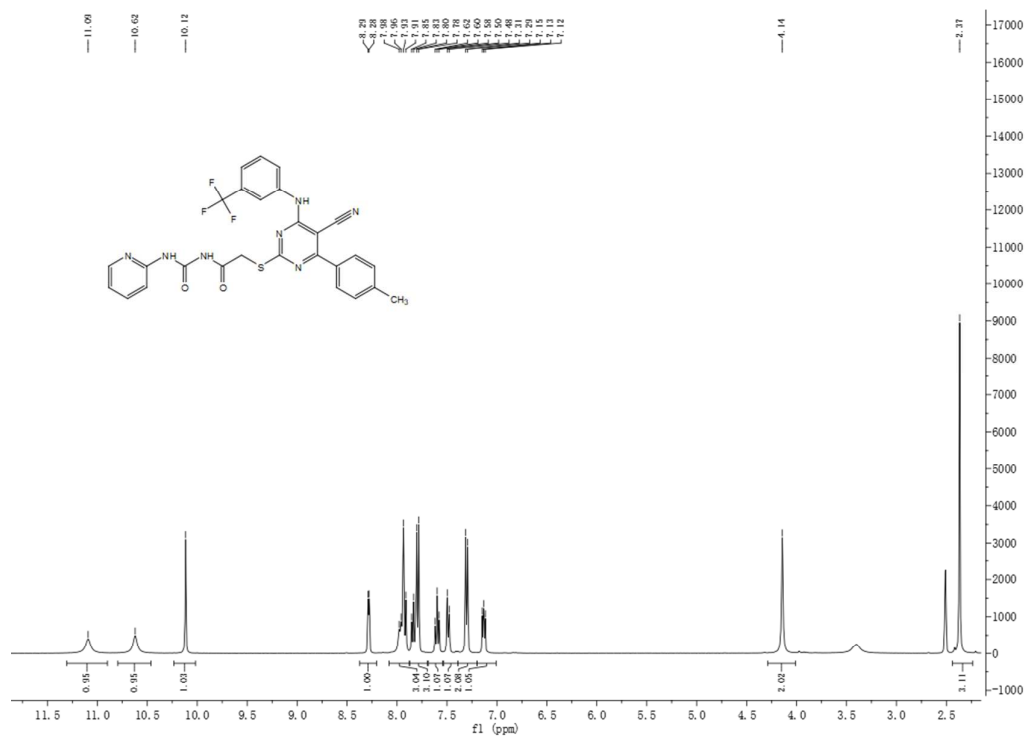


¹H NMR spectrum of compound 47

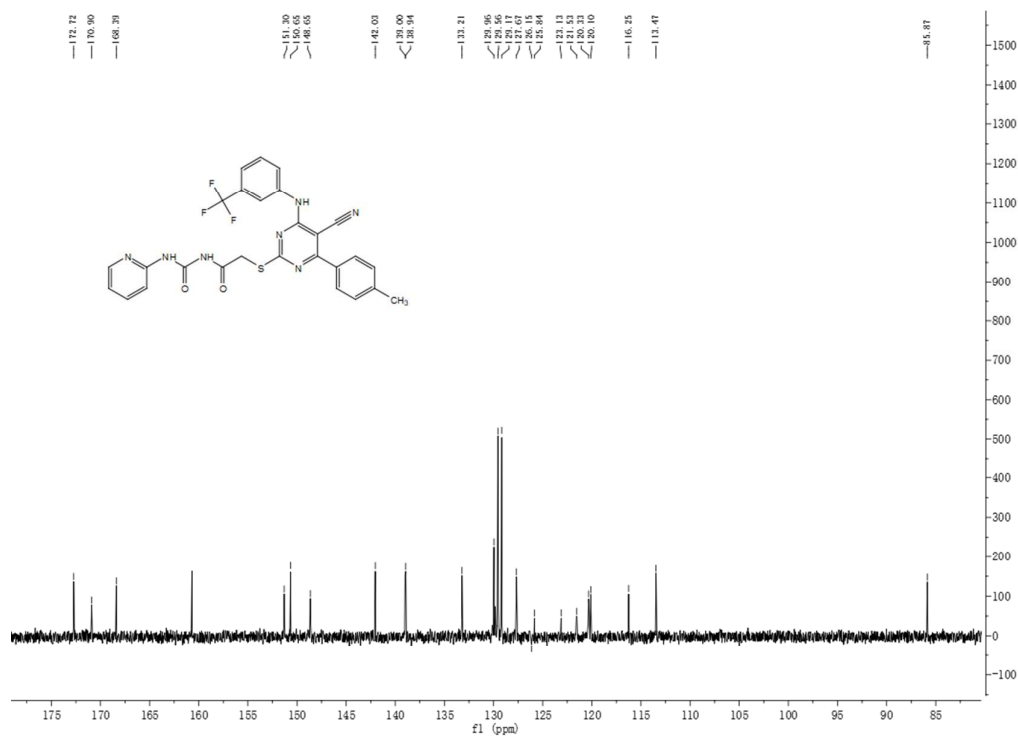


Peak	Retention Time	Area	% Area
1	3.449	6442727	95.71
2	3.737	288695	4.29

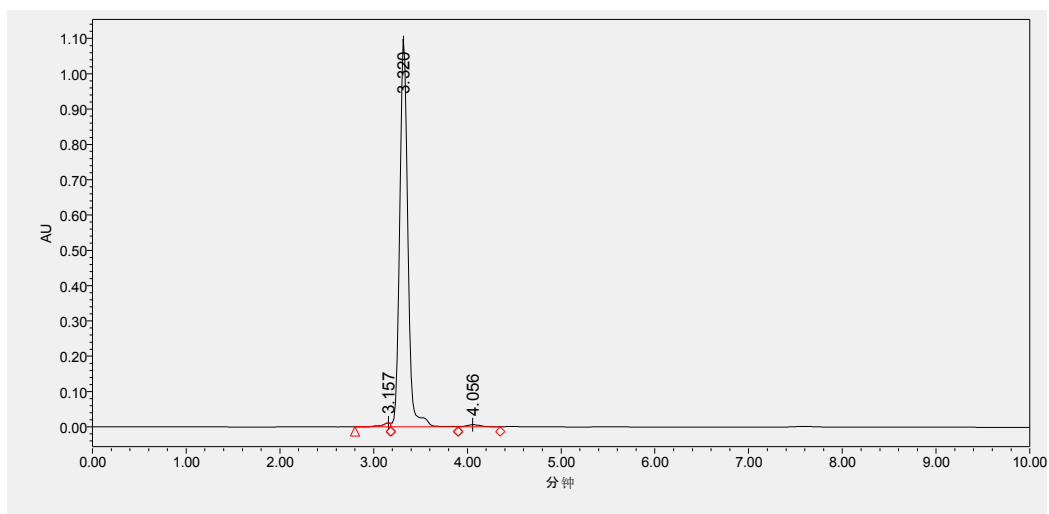
HPLC chromatogram of compound 47



¹H NMR spectrum of compound **48**

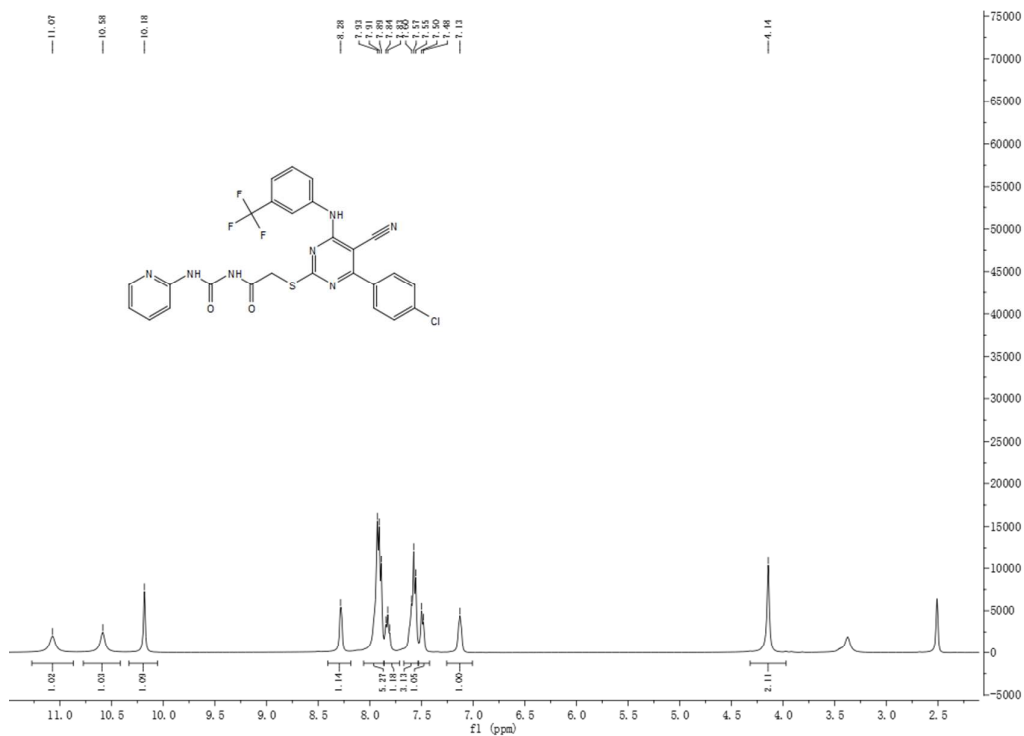


¹³C NMR spectrum of compound **48**

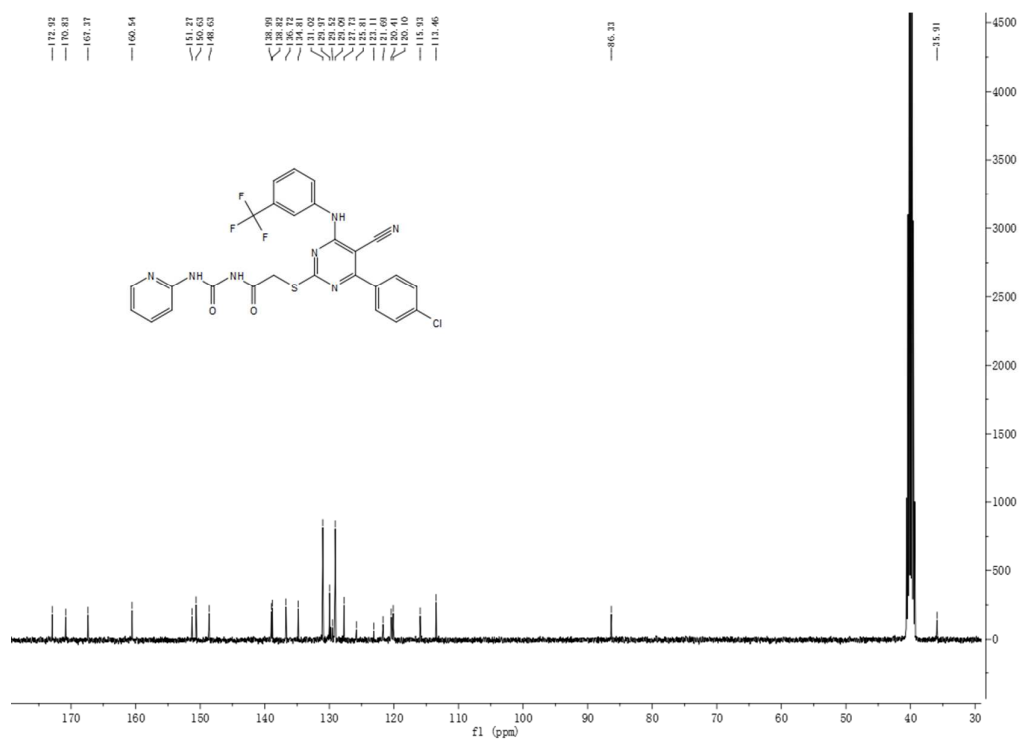


Peak	Retention Time	Area	% Area
1	3.157	75088	1.10
2	3.320	6661372	97.92
3	4.056	66400	0.98

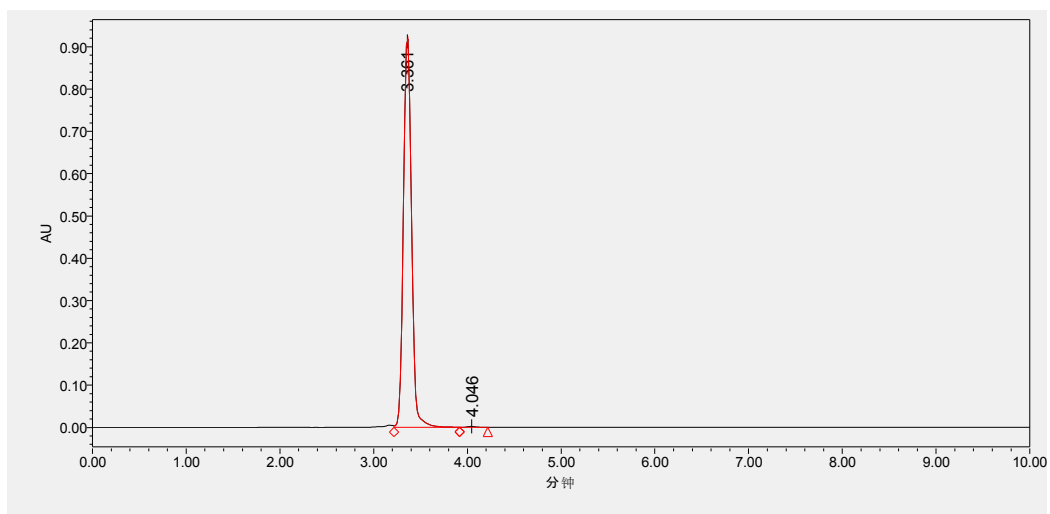
HPLC chromatogram of compound **48**



^1H NMR spectrum of compound **49**

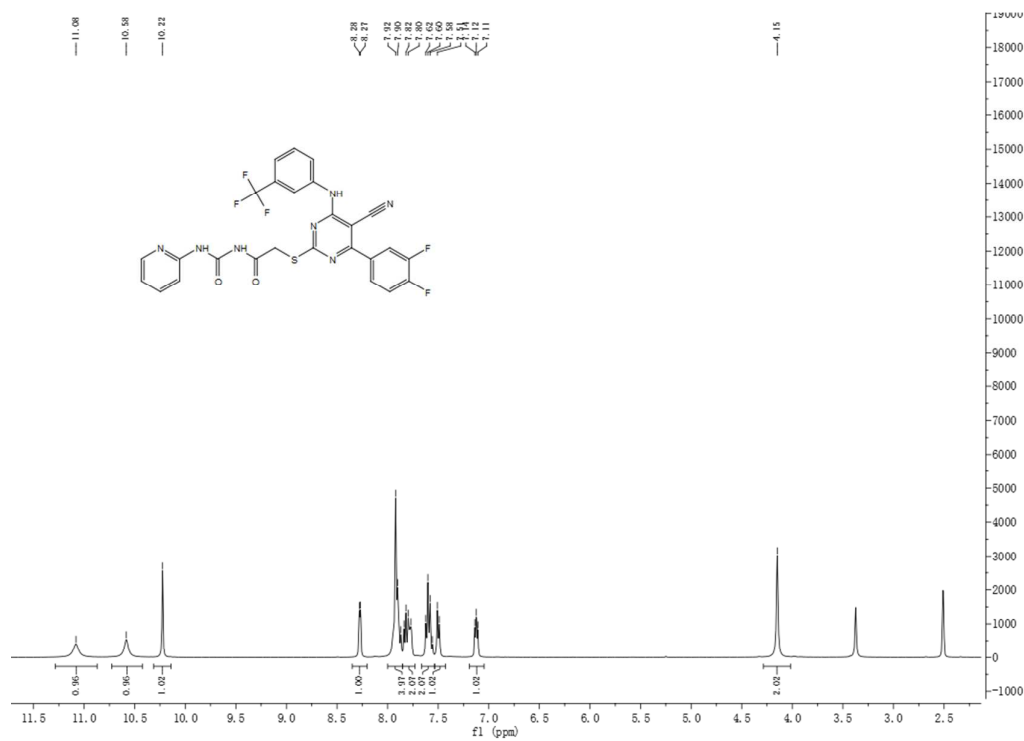


¹³C NMR spectrum of compound 49

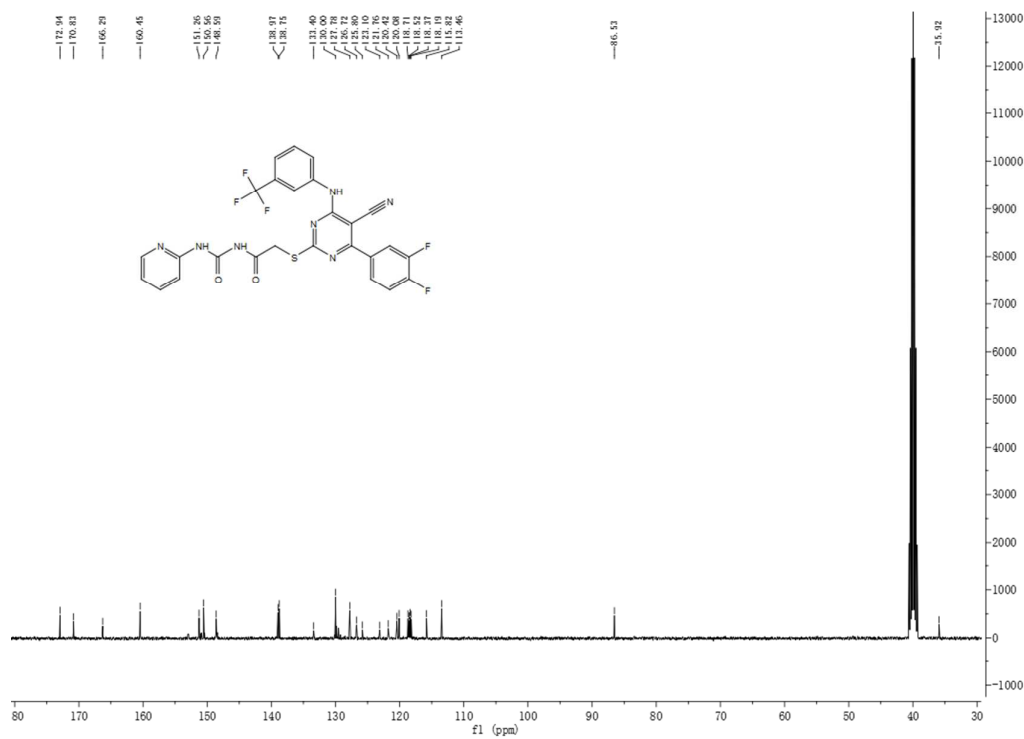


Peak	Retention Time	Area	% Area
1	3.361	5541166	99.76
2	4.046	13342	0.24

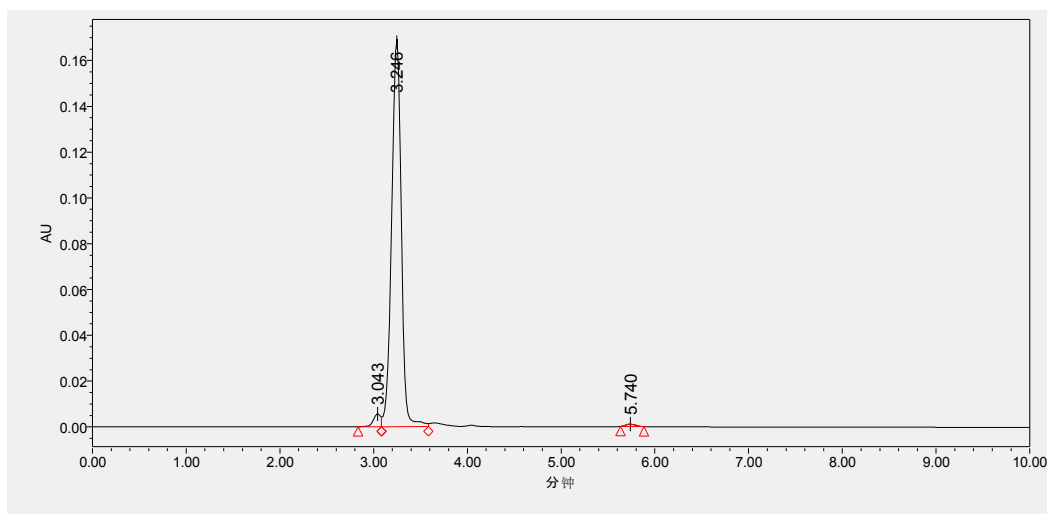
HPLC chromatogram of compound 49



^1H NMR spectrum of compound 50

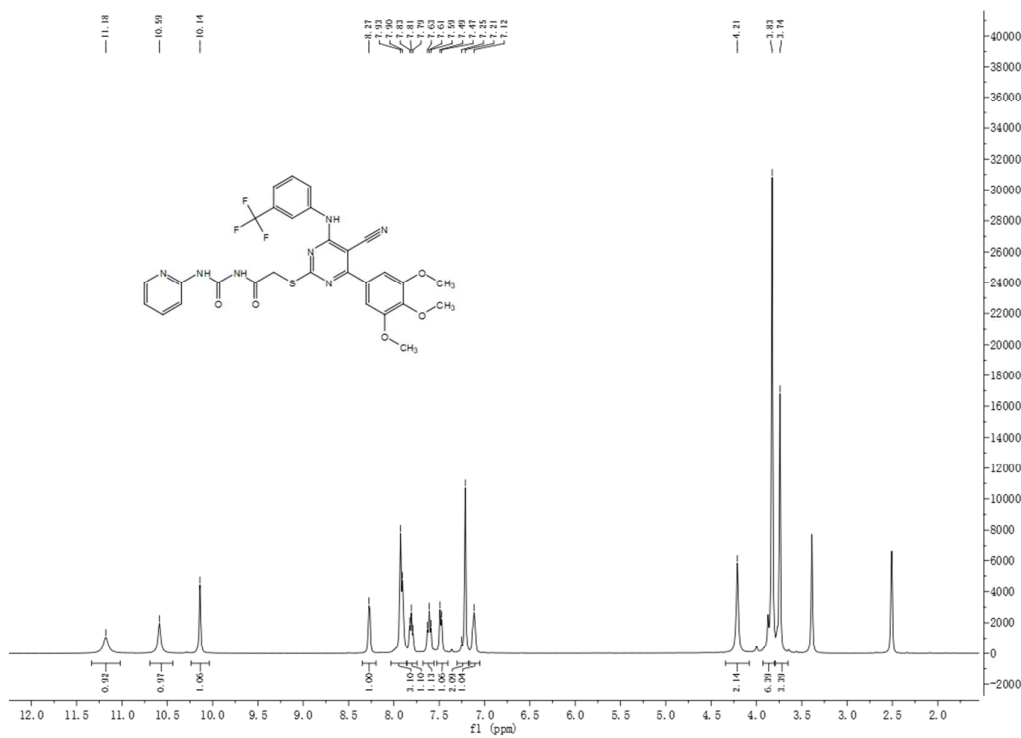


^{13}C NMR spectrum of compound 50

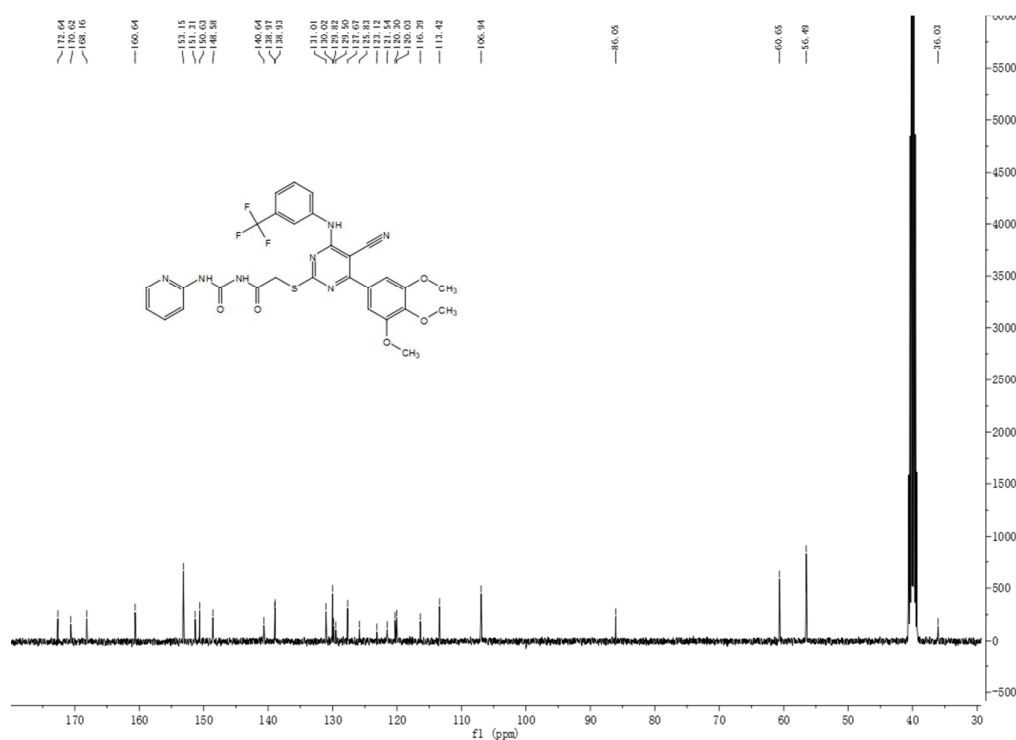


Peak	Retention Time	Area	% Area
1	3.043	29898	2.40
2	3.246	1205711	96.97
3	5.740	7795	0.63

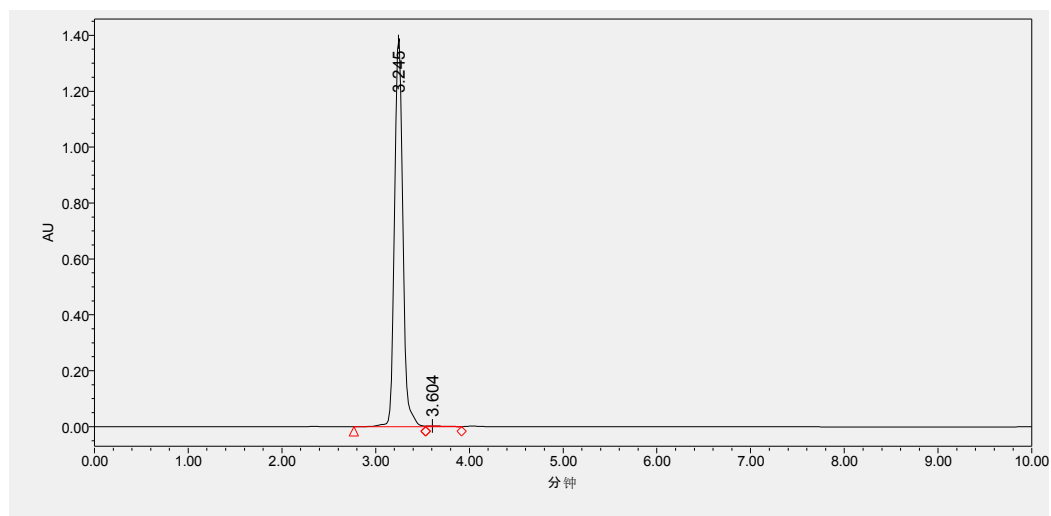
HPLC chromatogram of compound **50**



^1H NMR spectrum of compound **51**

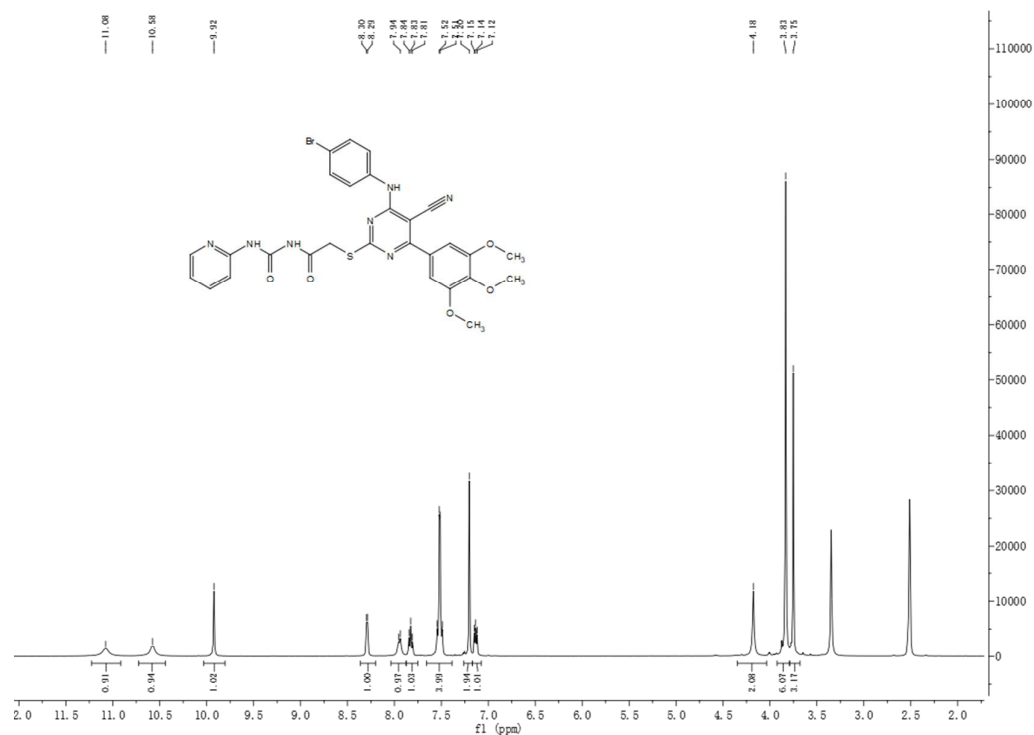


^{13}C NMR spectrum of compound **51**

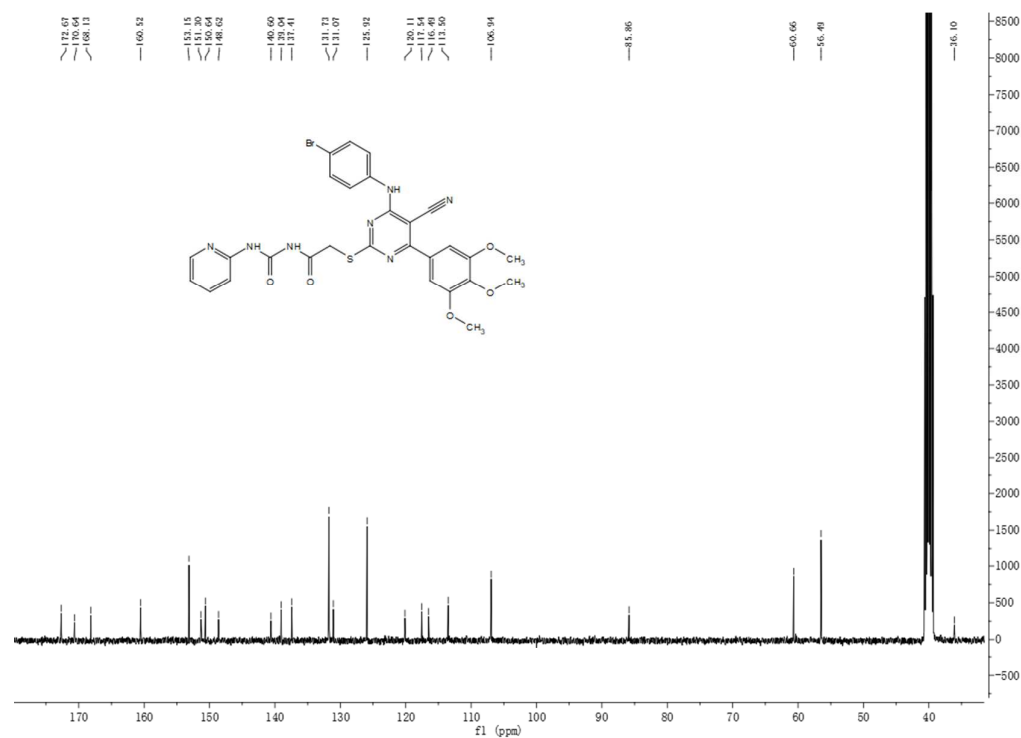


Peak	Retention Time	Area	% Area
1	3.245	8546286	99.68
2	3.604	27669	0.32

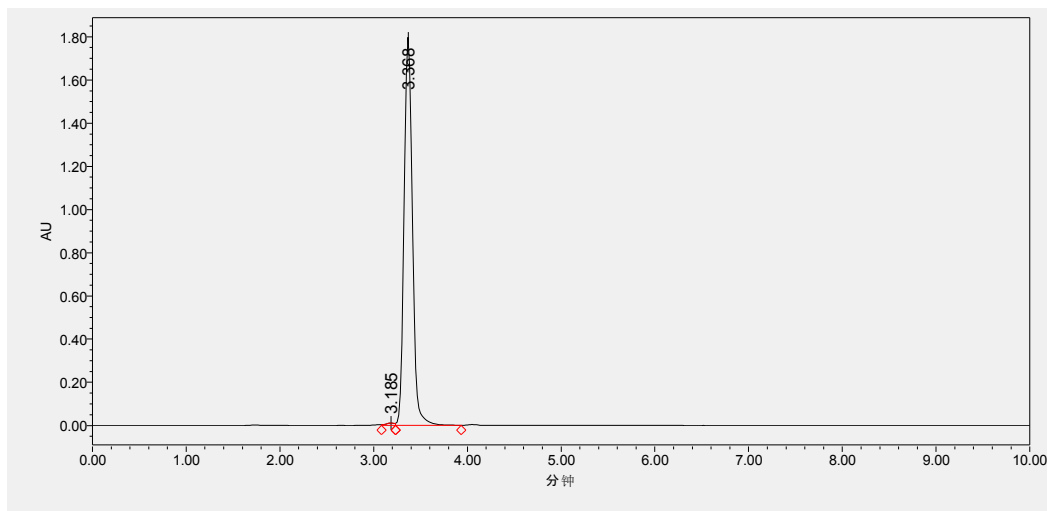
HPLC chromatogram of compound **51**



¹H NMR spectrum of compound **52**

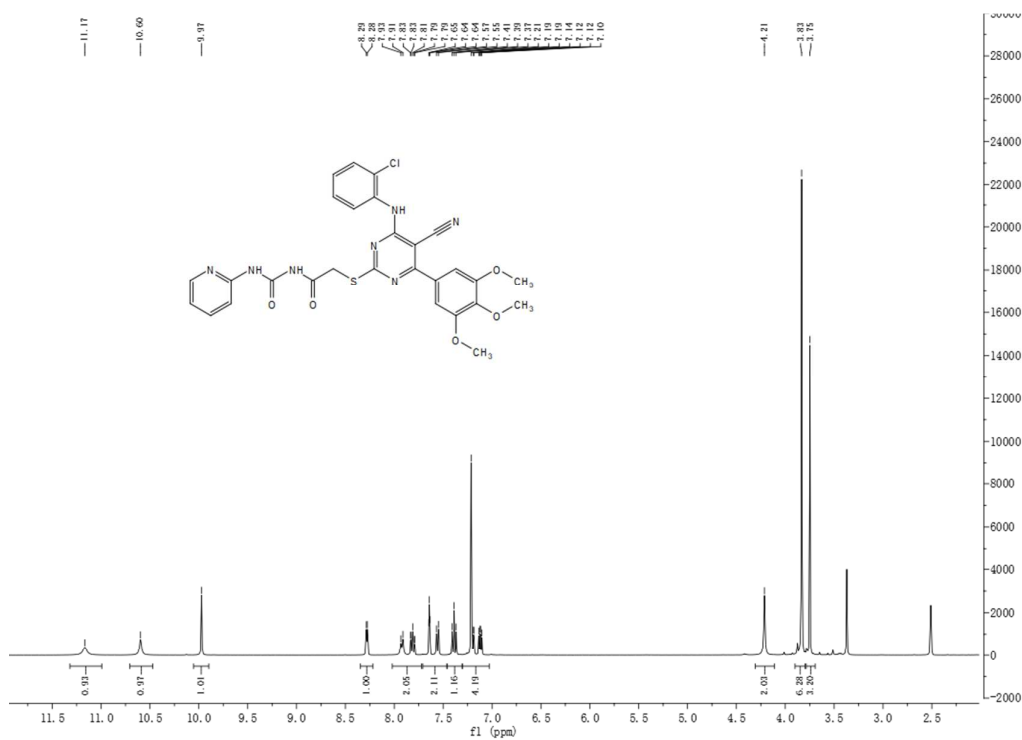


¹³C NMR spectrum of compound **52**

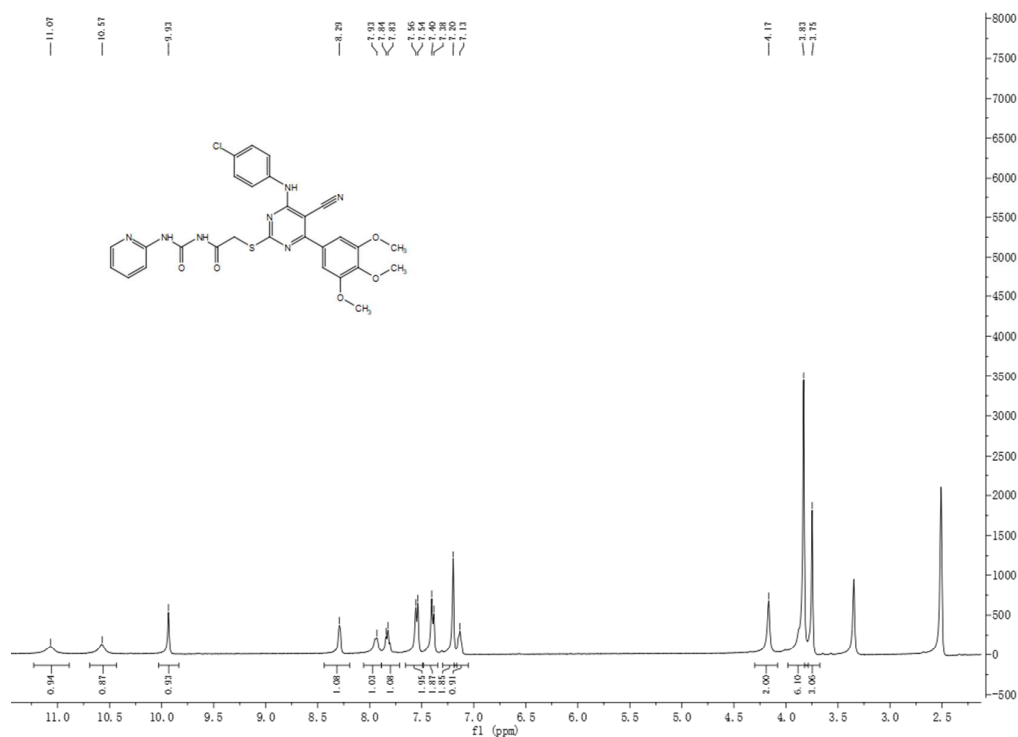


Peak	Retention Time	Area	% Area
1	3.185	70695	0.63
2	3.368	11122311	99.37

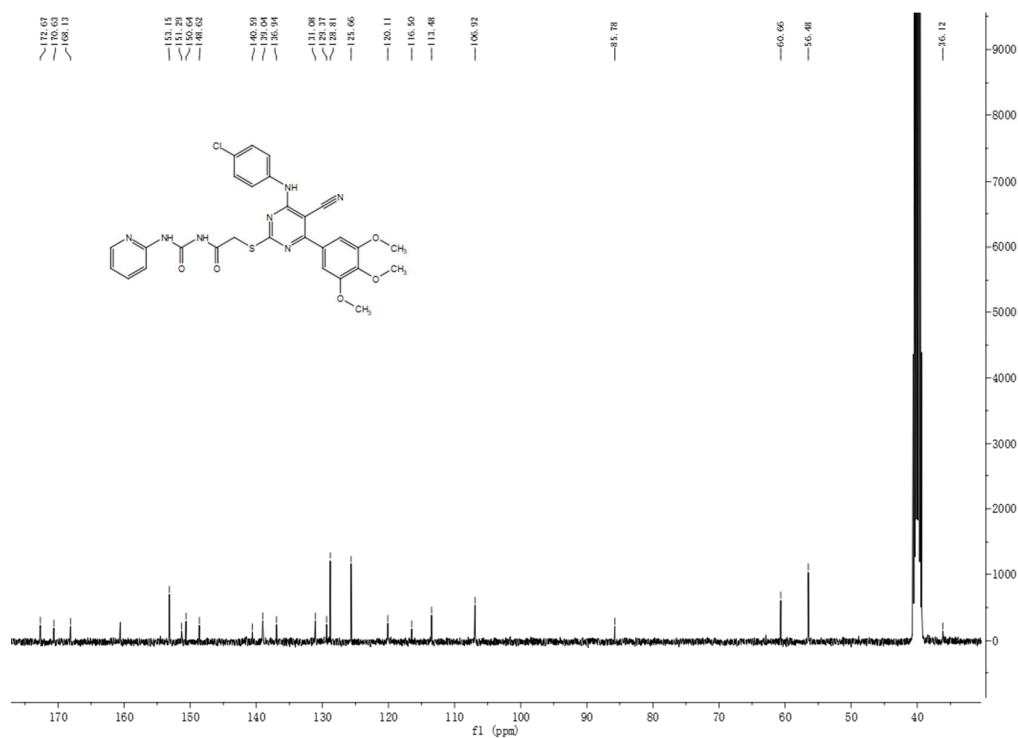
HPLC chromatogram of compound **52**



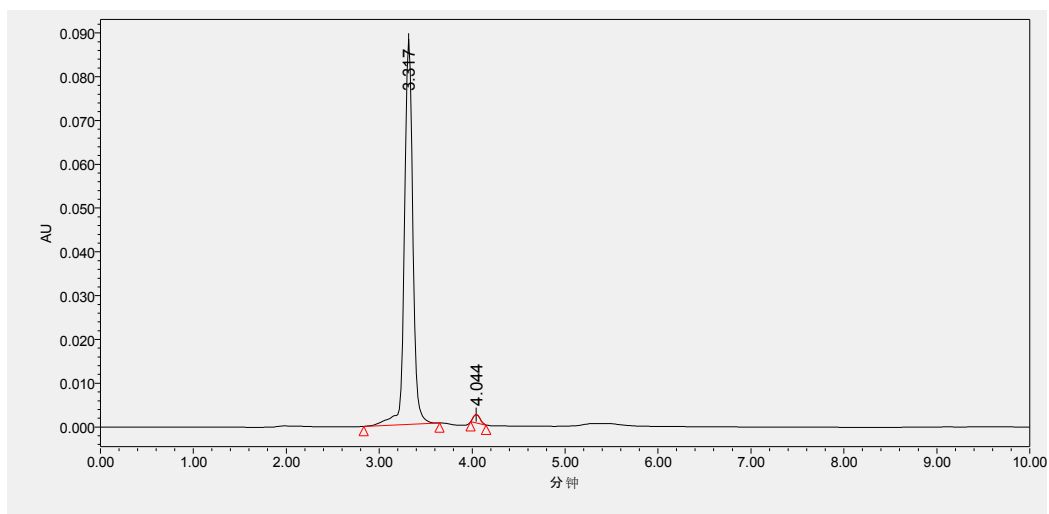
¹H NMR spectrum of compound **53**



¹H NMR spectrum of compound **54**

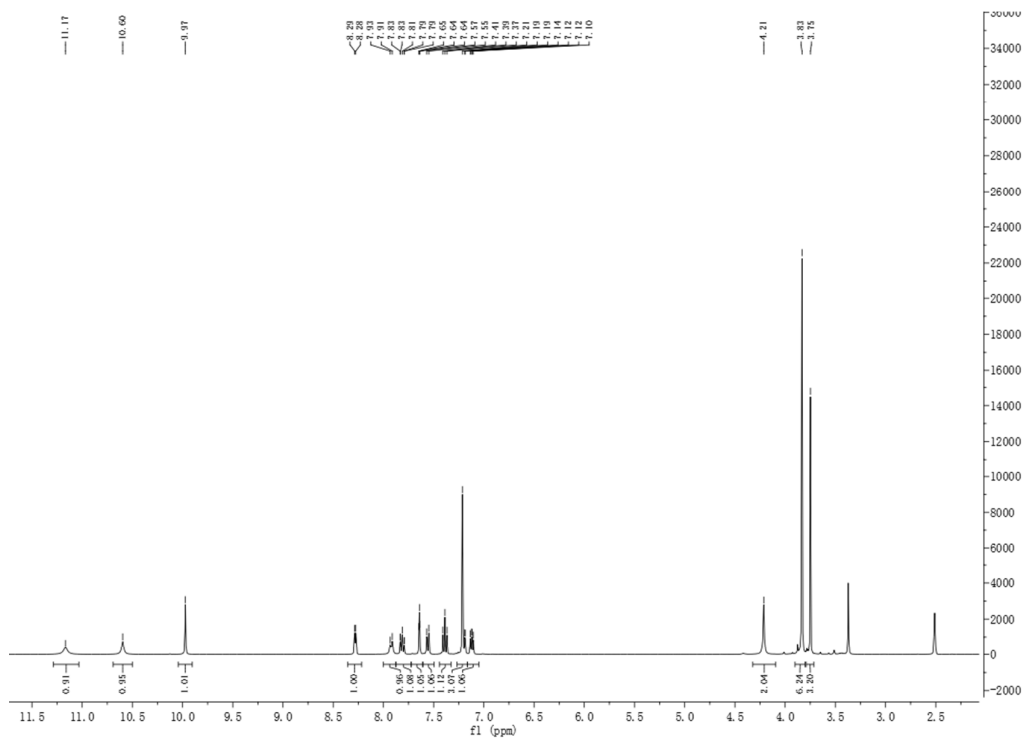


¹³C NMR spectrum of compound **54**

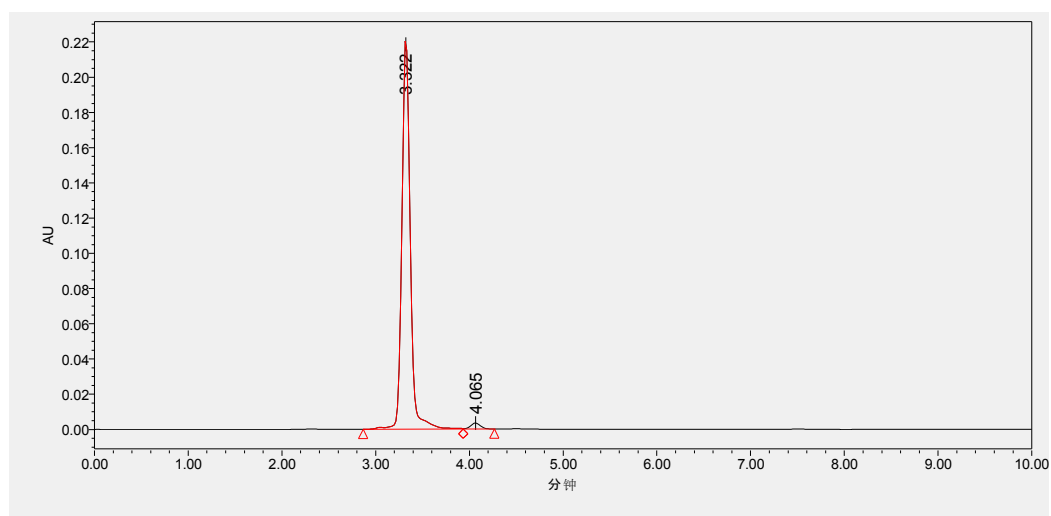
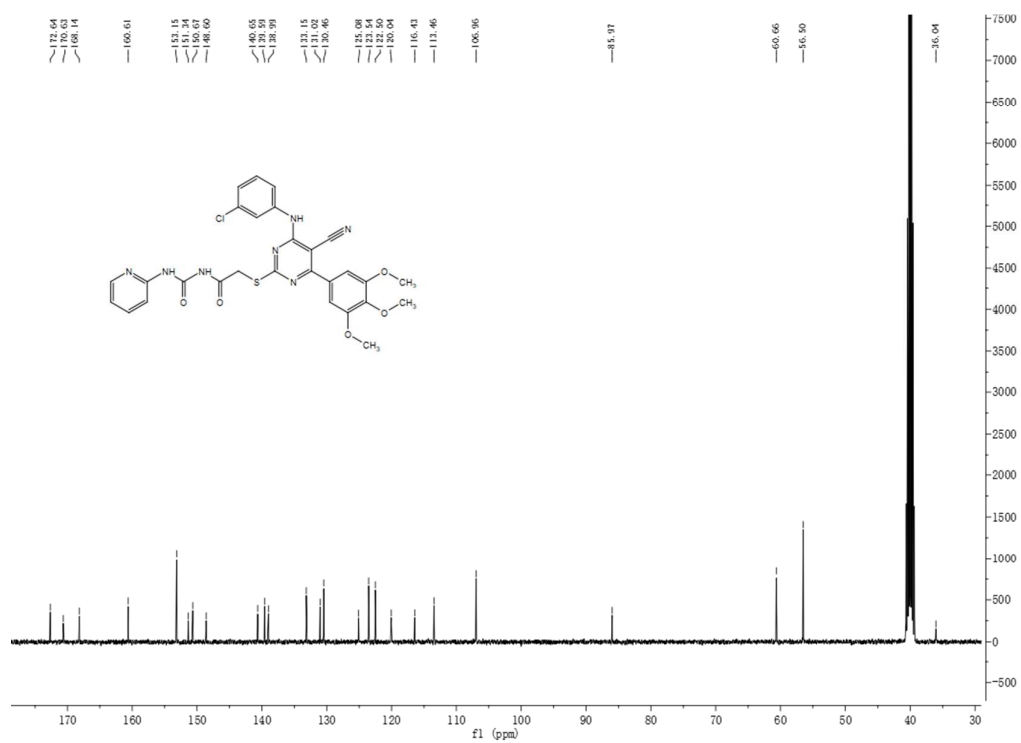


Peak	Retention Time	Area	% Area
1	3.317	552786	98.29
2	4.044	9605	1.71

HPLC chromatogram of compound **54**

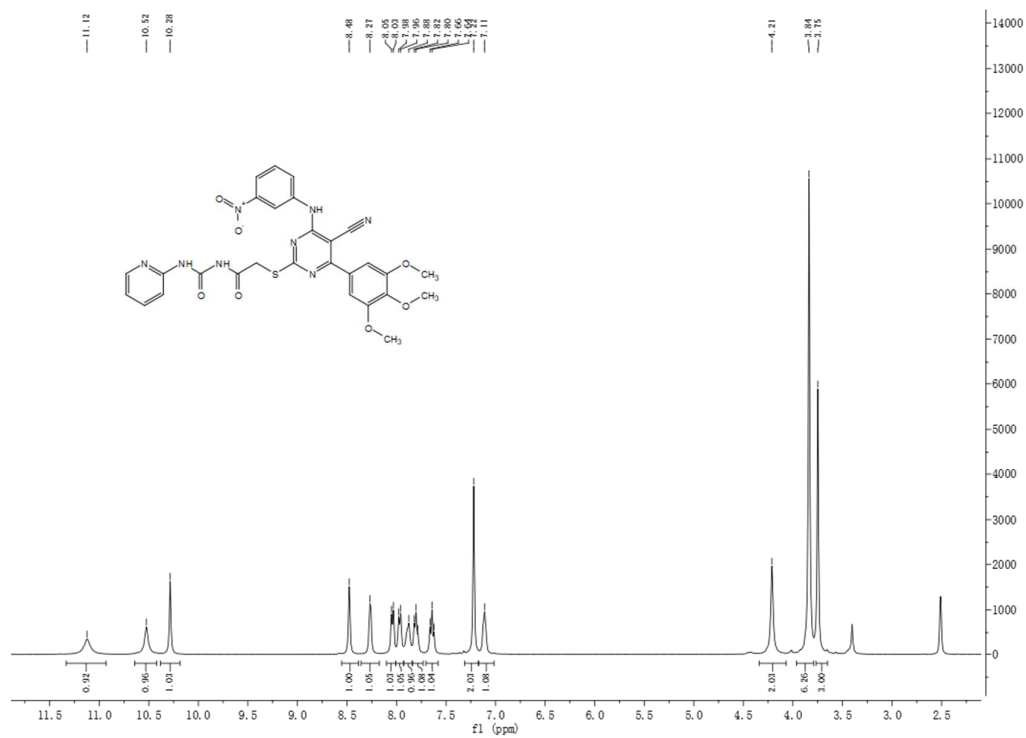


¹H NMR spectrum of compound **55**

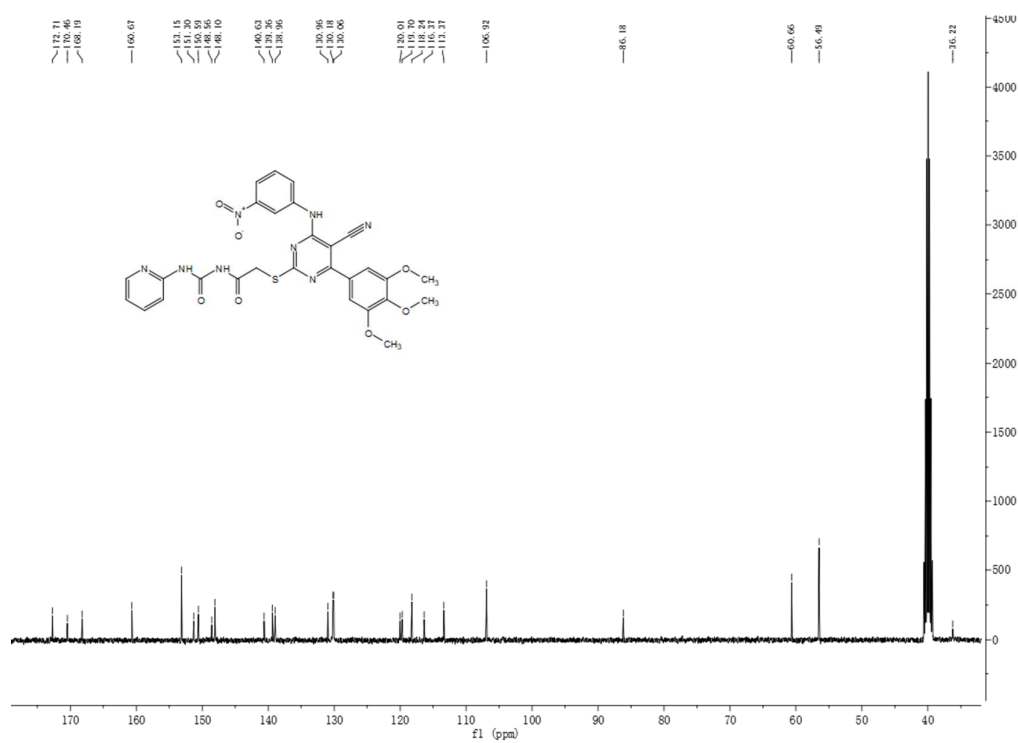


Peak	Retention Time	Area	% Area
1	3.322	1391499	98.20
2	4.065	25496	1.80

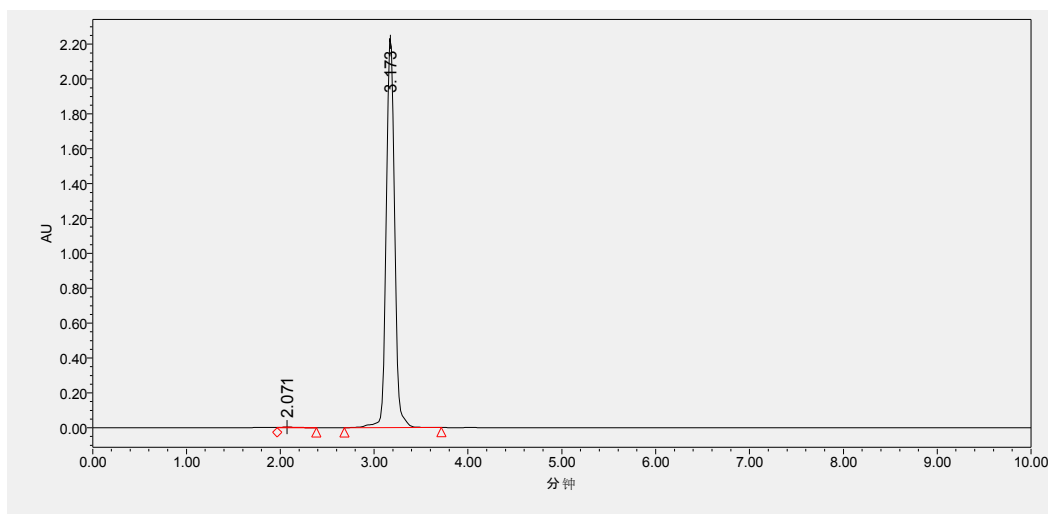
HPLC chromatogram of compound 55



^1H NMR spectrum of compound **56**



^{13}C NMR spectrum of compound **56**

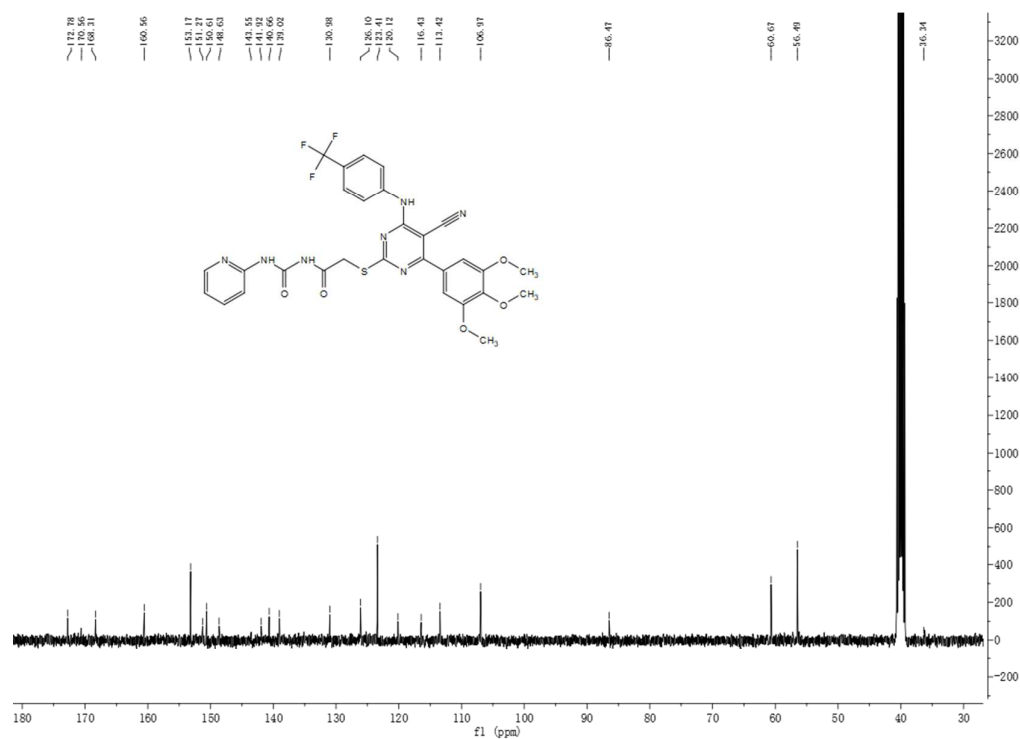


Peak	Retention Time	Area	% Area
1	2.071	37716	0.27
2	3.173	13845636	99.73

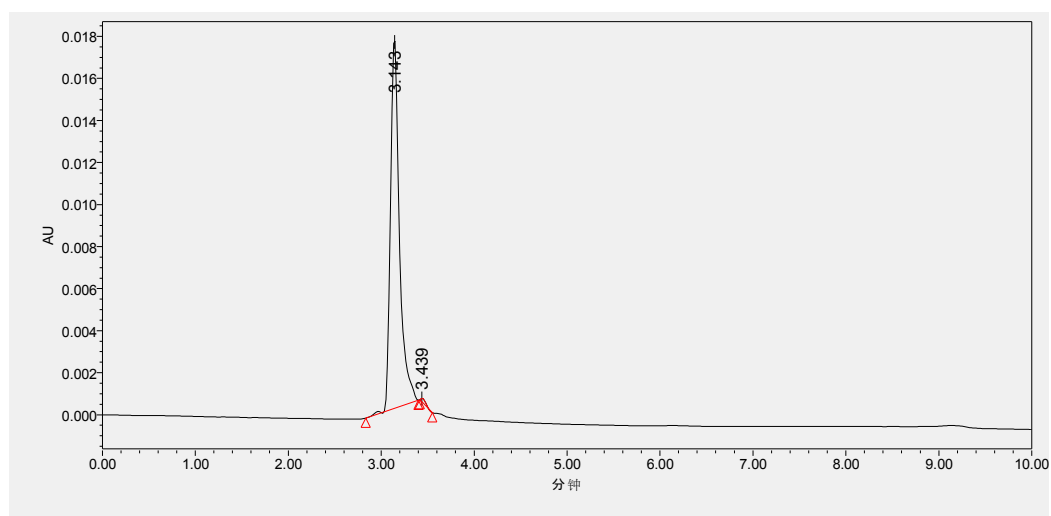
HPLC chromatogram of compound **56**



¹H NMR spectrum of compound **57**

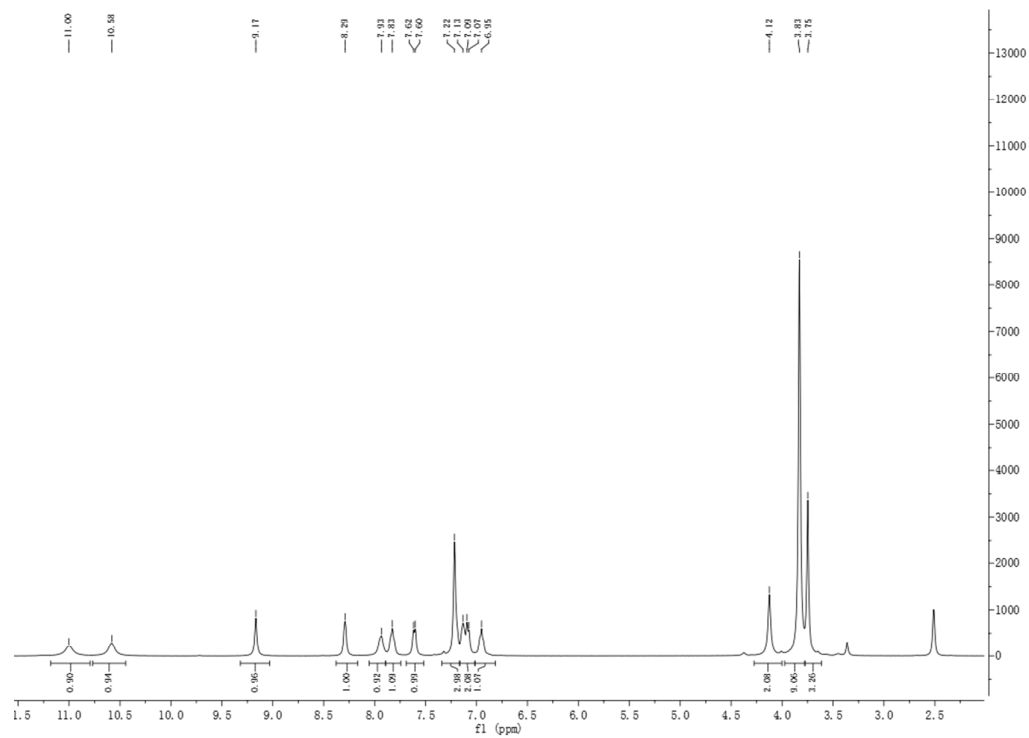


^{13}C NMR spectrum of compound **57**

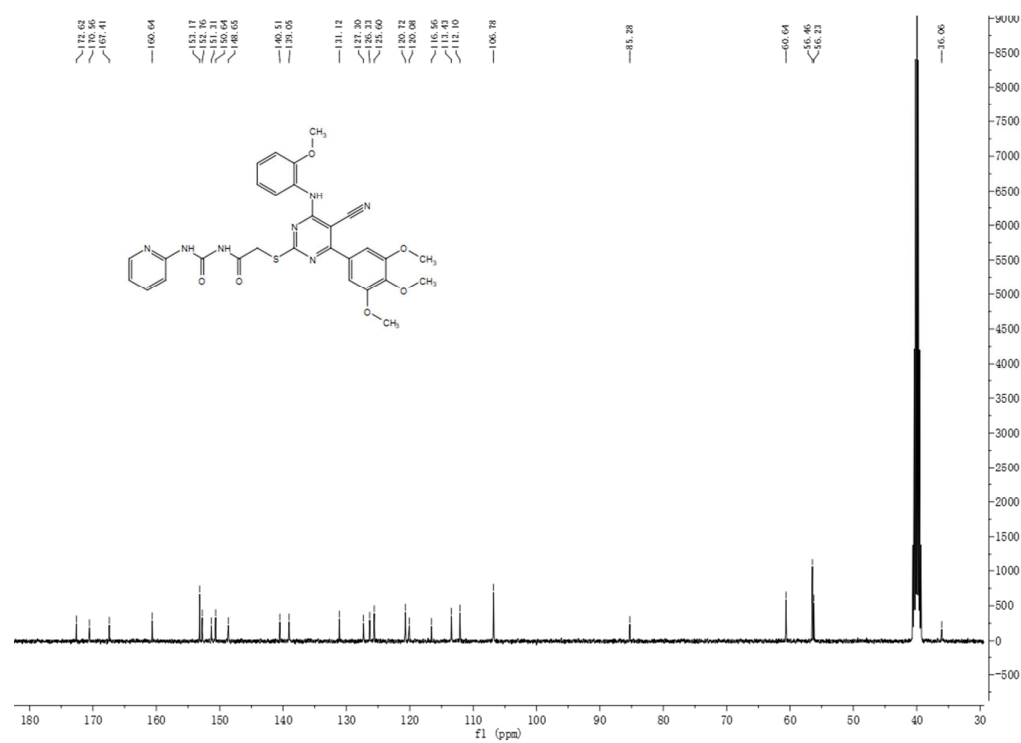


Peak	Retention Time	Area	% Area
1	3.143	118693	99.38
2	3.439	744	0.62

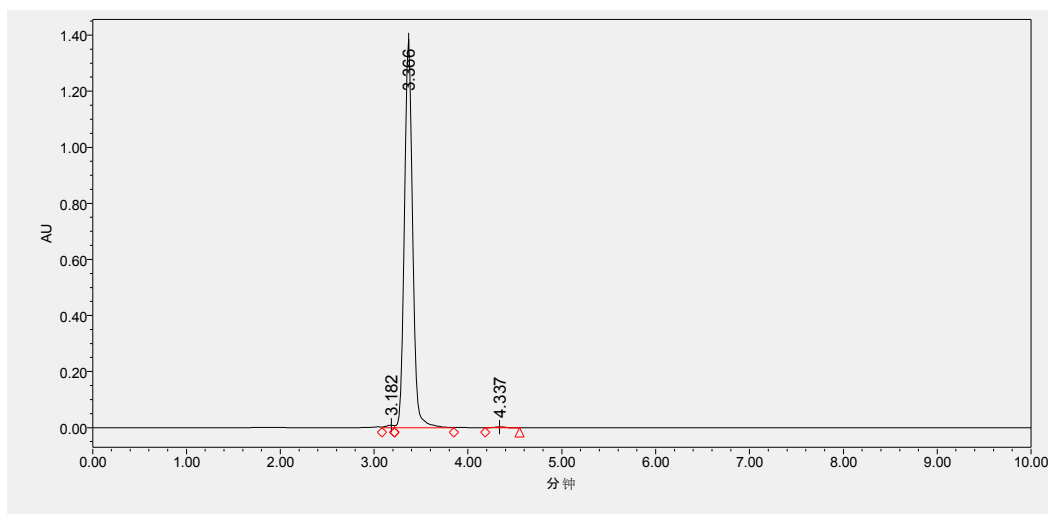
HPLC chromatogram of compound **57**



¹H NMR spectrum of compound **58**

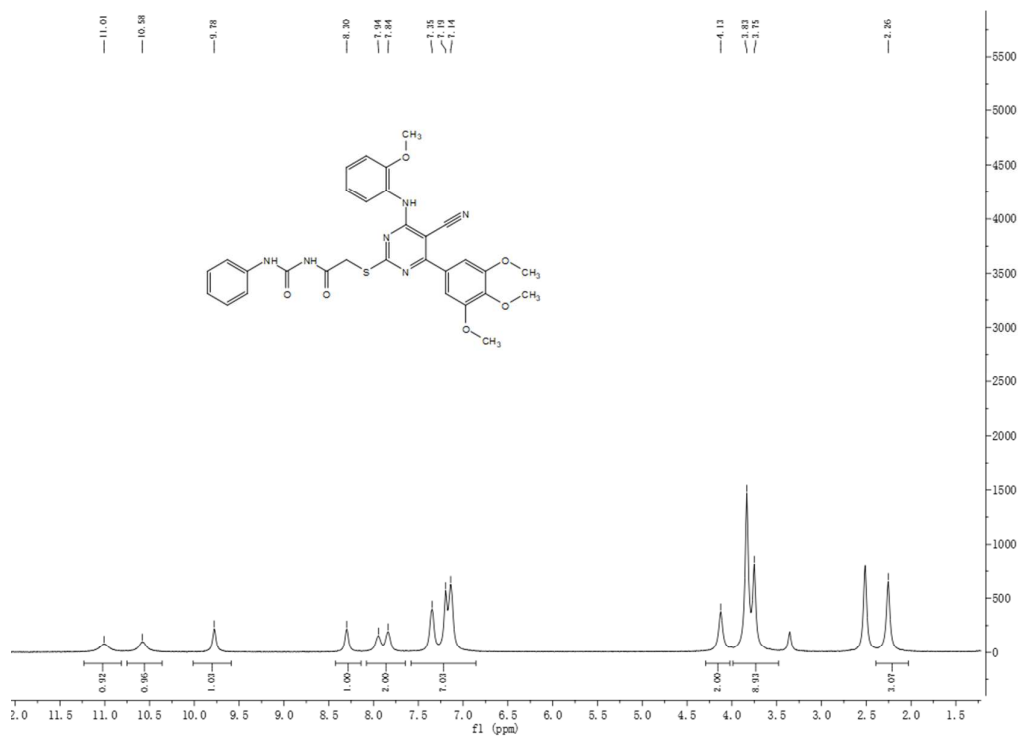


¹³C NMR spectrum of compound **58**

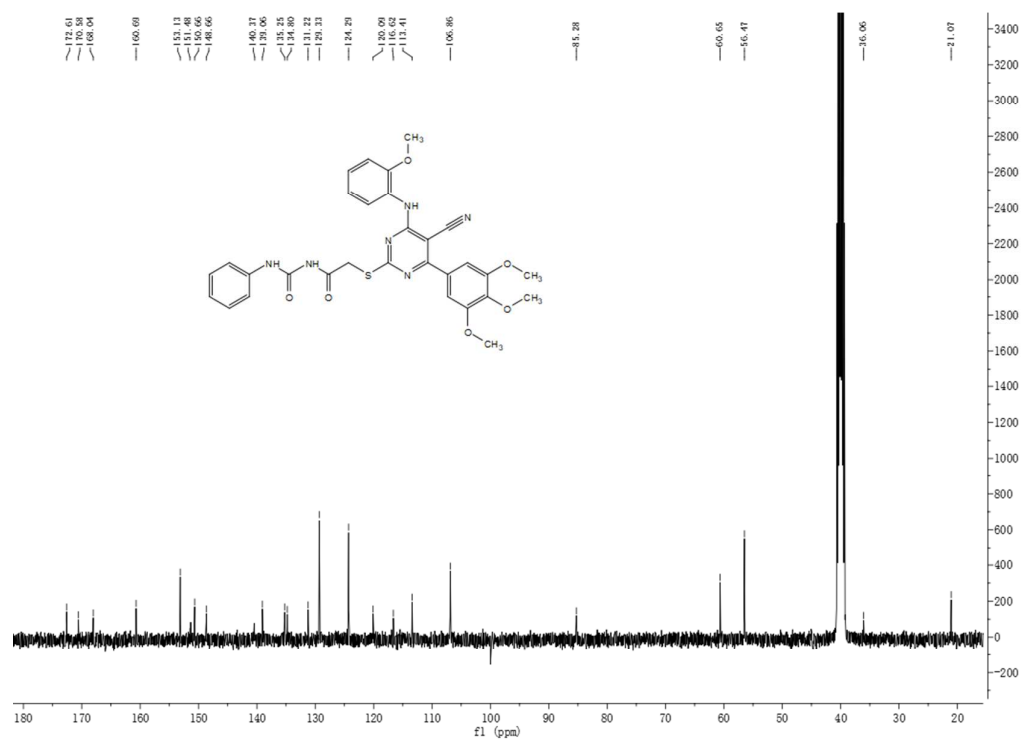


Peak	Retention Time	Area	% Area
1	3.182	47852	0.56
2	3.366	8470422	99.23
3	4.337	17465	0.20

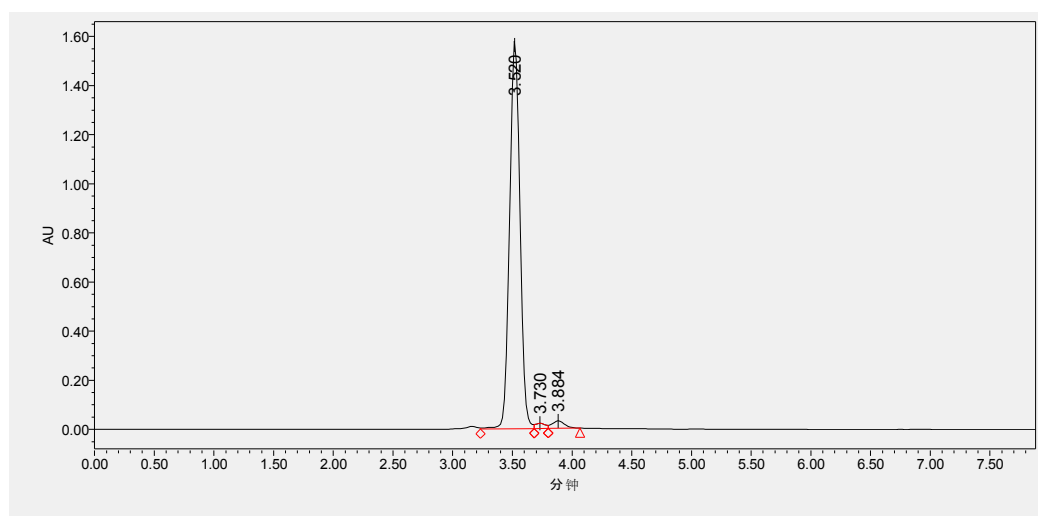
HPLC chromatogram of compound **58**



^1H NMR spectrum of compound **59**

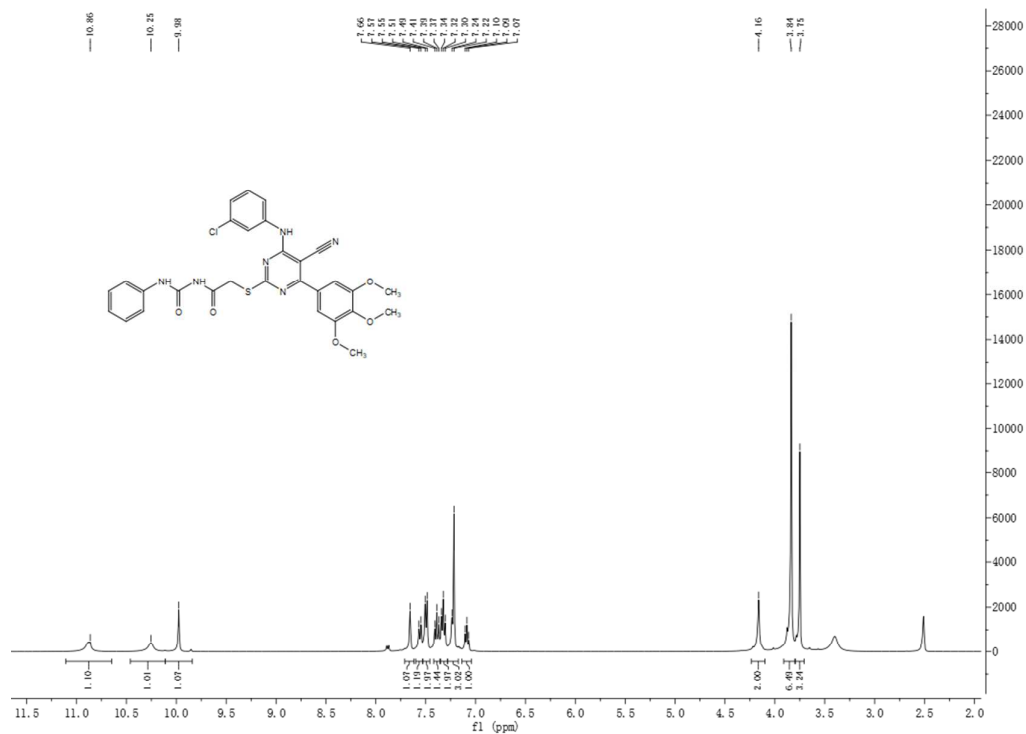


^{13}C NMR spectrum of compound **59**

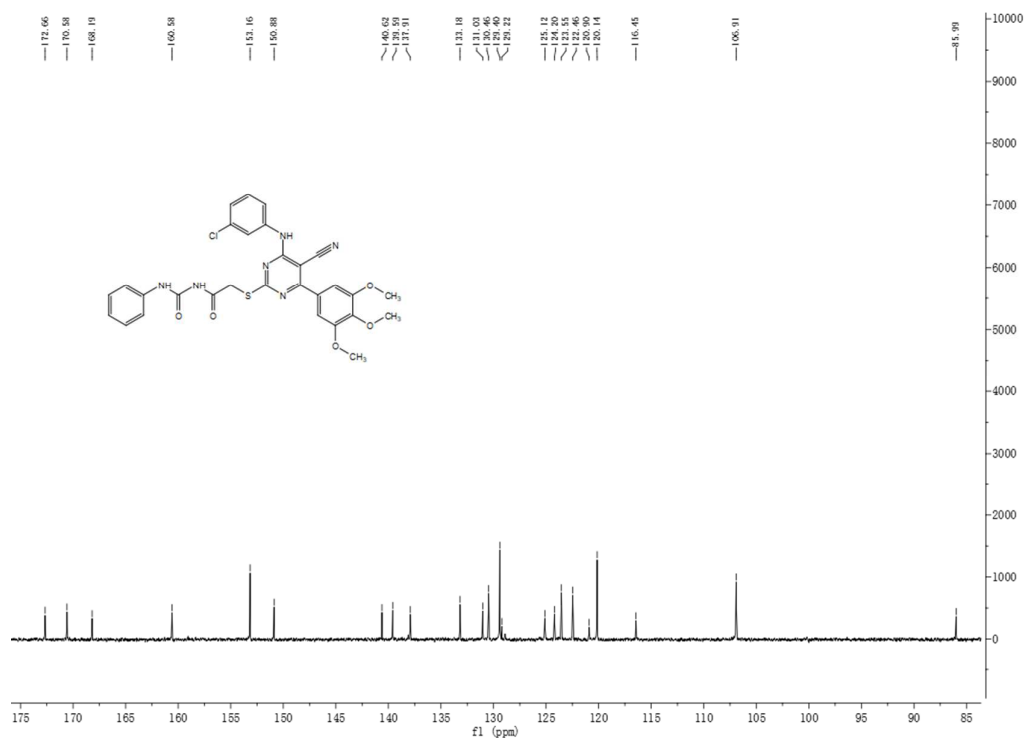


Peak	Retention Time	Area	% Area
1	3.520	9499785	96.50
2	3.730	123059	1.25
3	3.884	221223	2.25

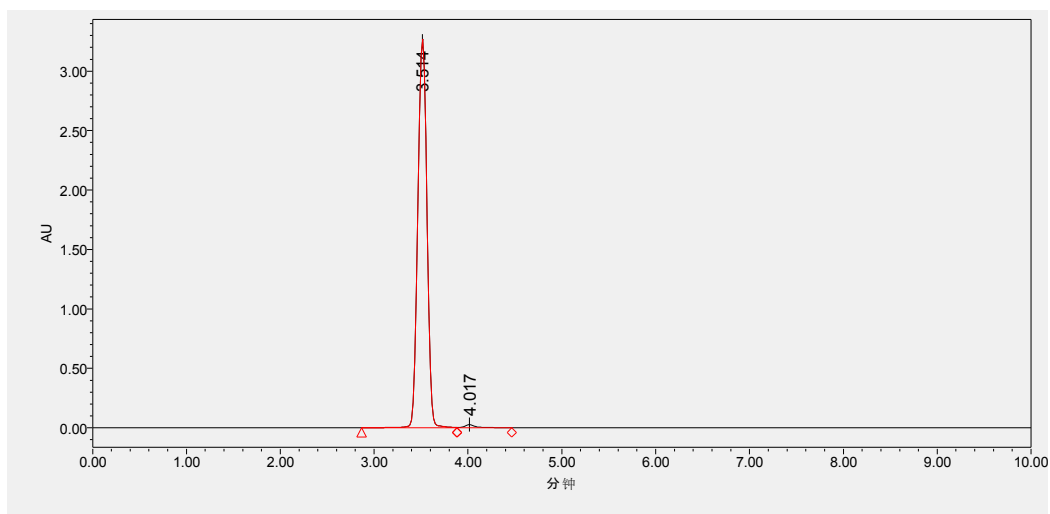
HPLC chromatogram of compound **59**



¹H NMR spectrum of compound **60**

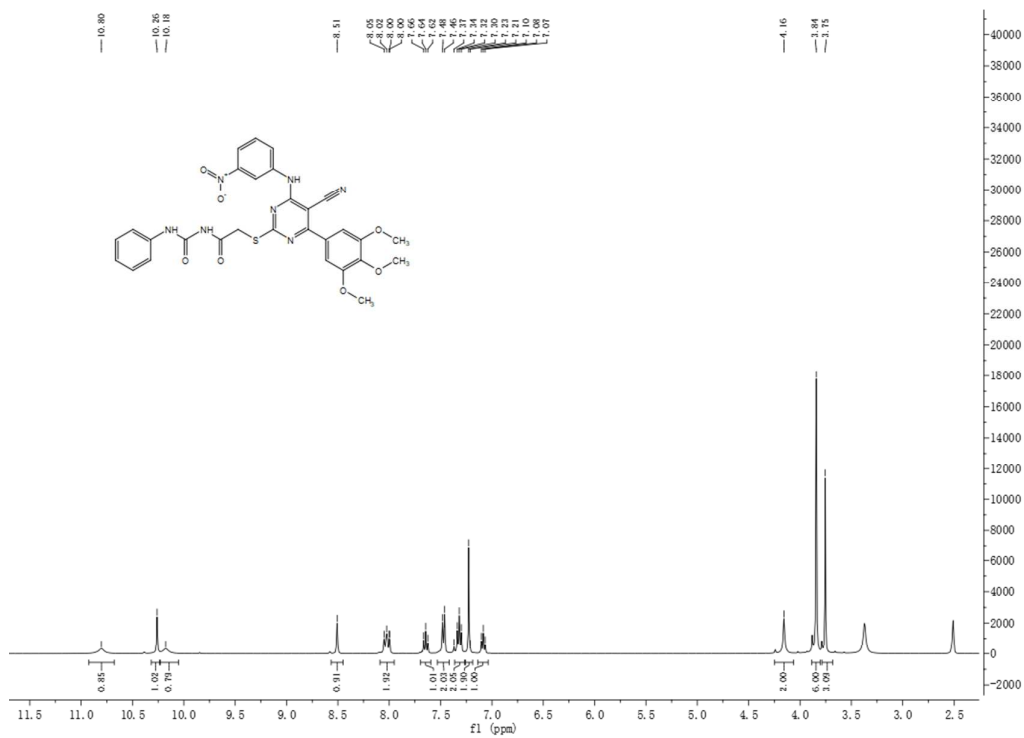


¹³C NMR spectrum of compound **60**

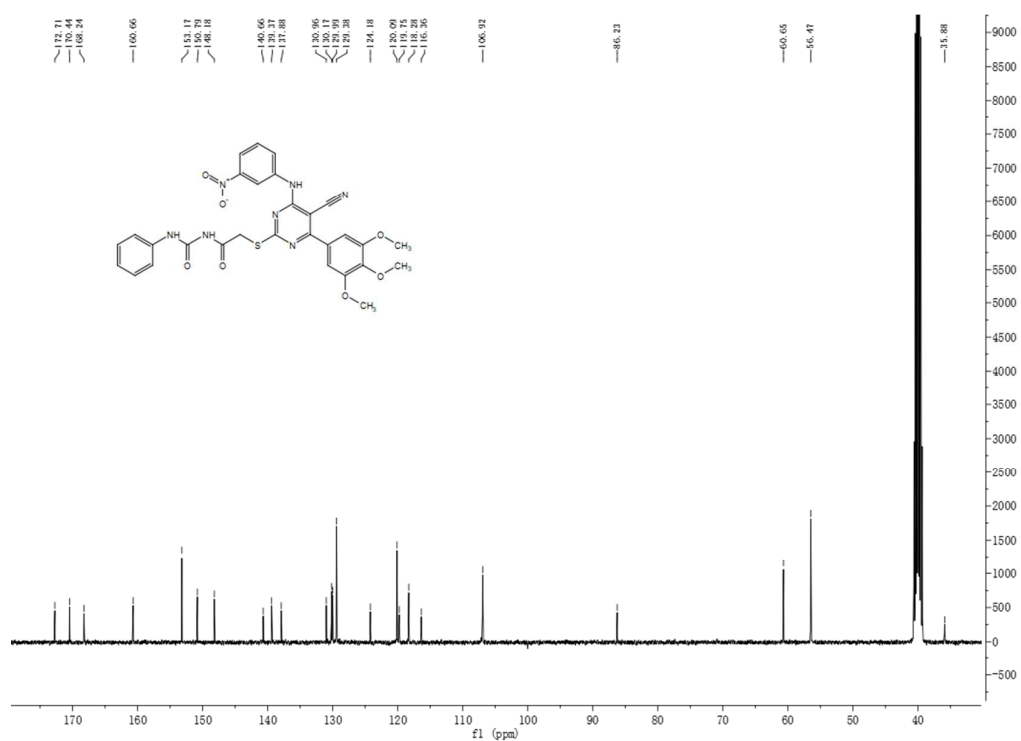


Peak	Retention Time	Area	% Area
1	3.514	22109575	99.01
2	4.017	221289	0.99

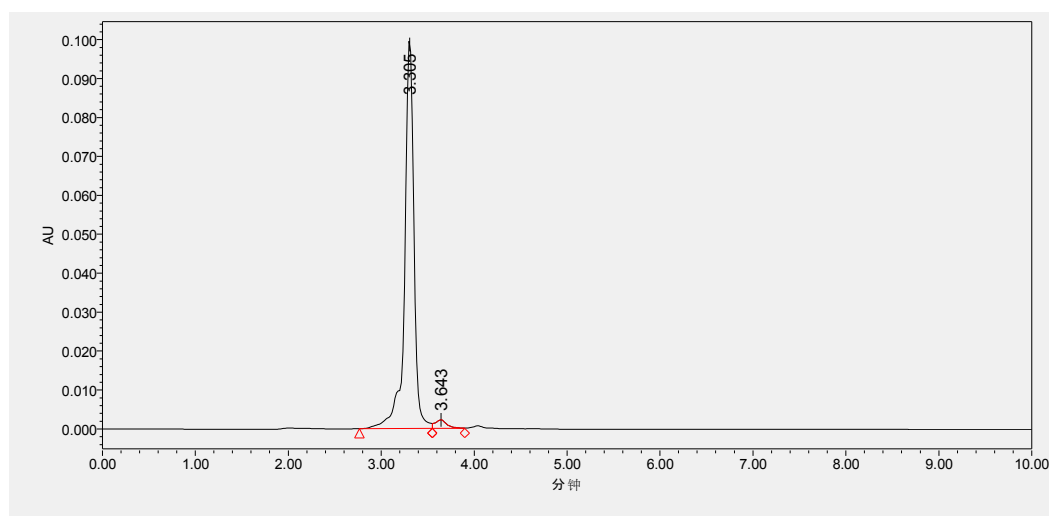
HPLC chromatogram of compound **60**



¹H NMR spectrum of compound **61**

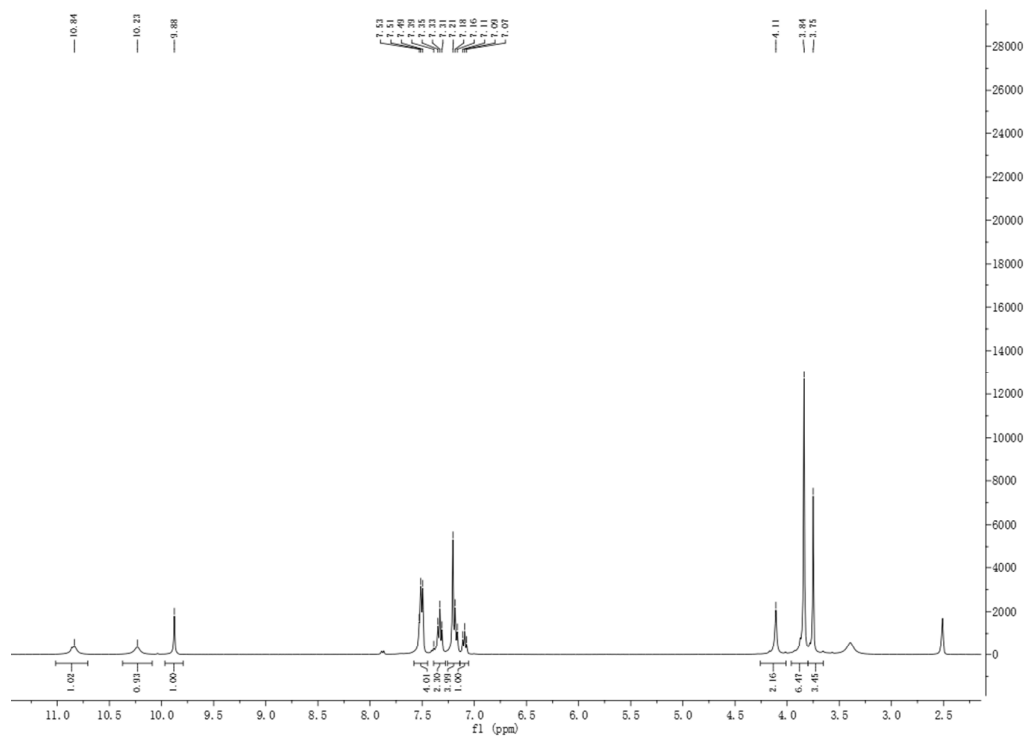


^{13}C NMR spectrum of compound **61**

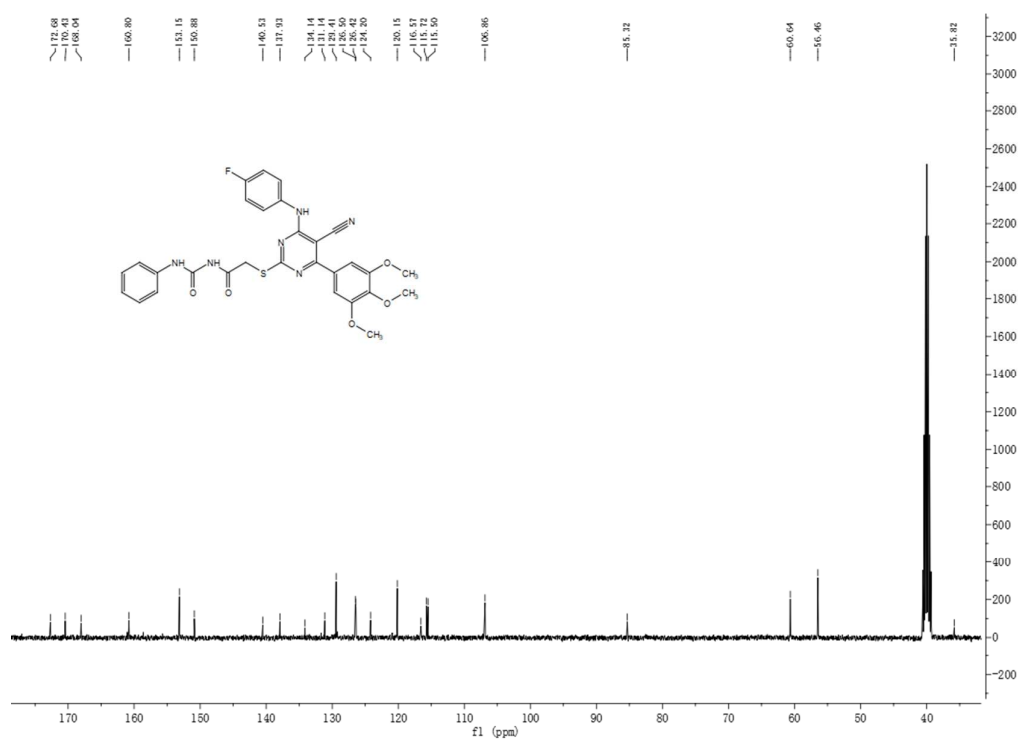


Peak	Retention Time	Area	% Area
1	3.305	693749	97.19
2	3.643	20055	2.81

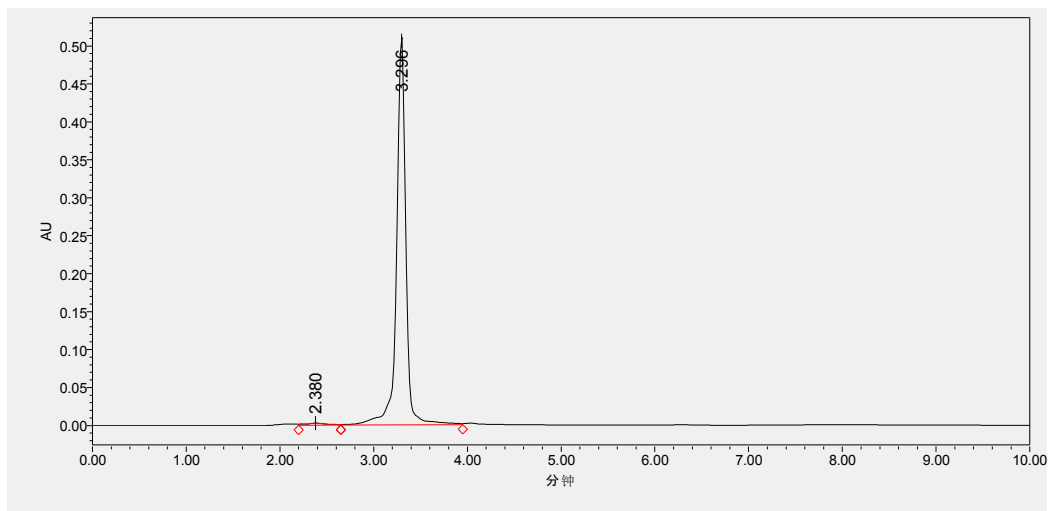
HPLC chromatogram of compound **61**



¹H NMR spectrum of compound **62**

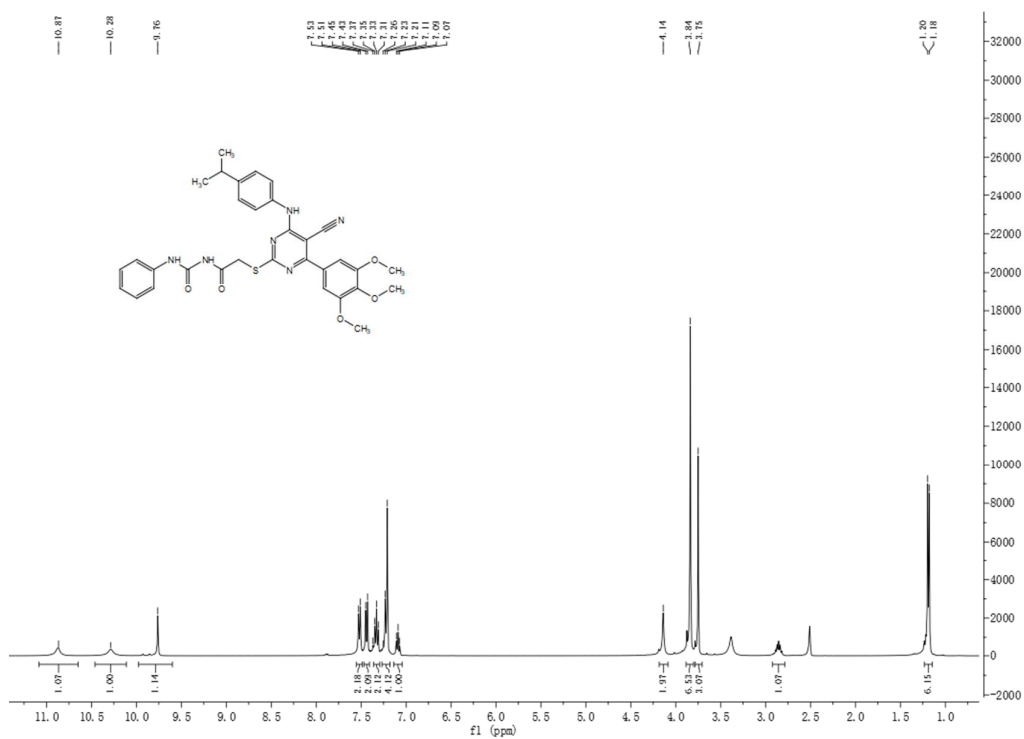


¹³C NMR spectrum of compound **62**

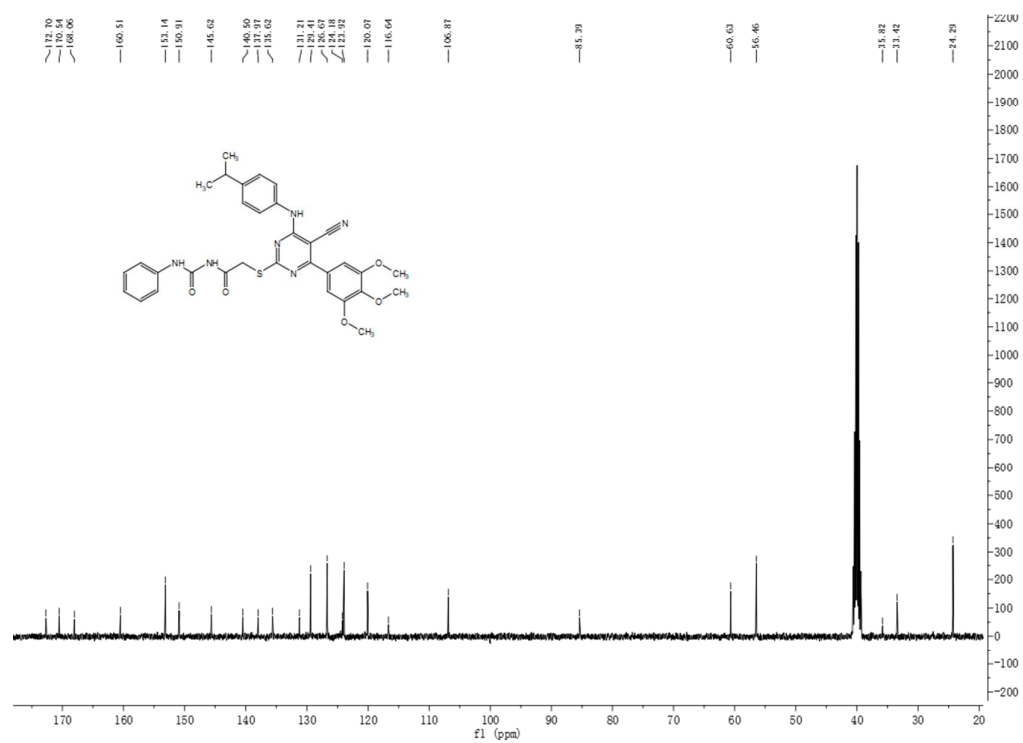


Peak	Retention Time	Area	% Area
1	2.380	44591	1.24
2	3.296	3547873	98.76

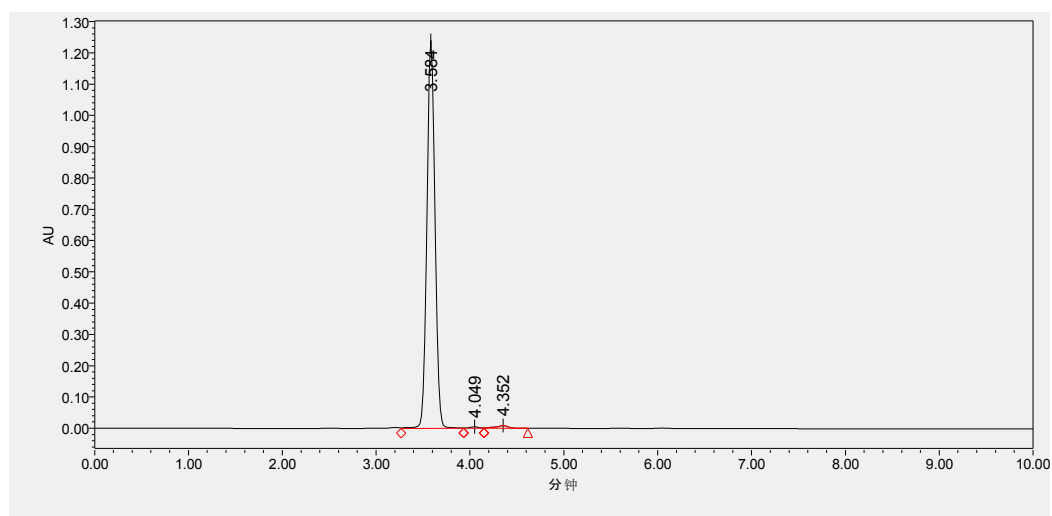
HPLC chromatogram of compound **62**



^1H NMR spectrum of compound **63**

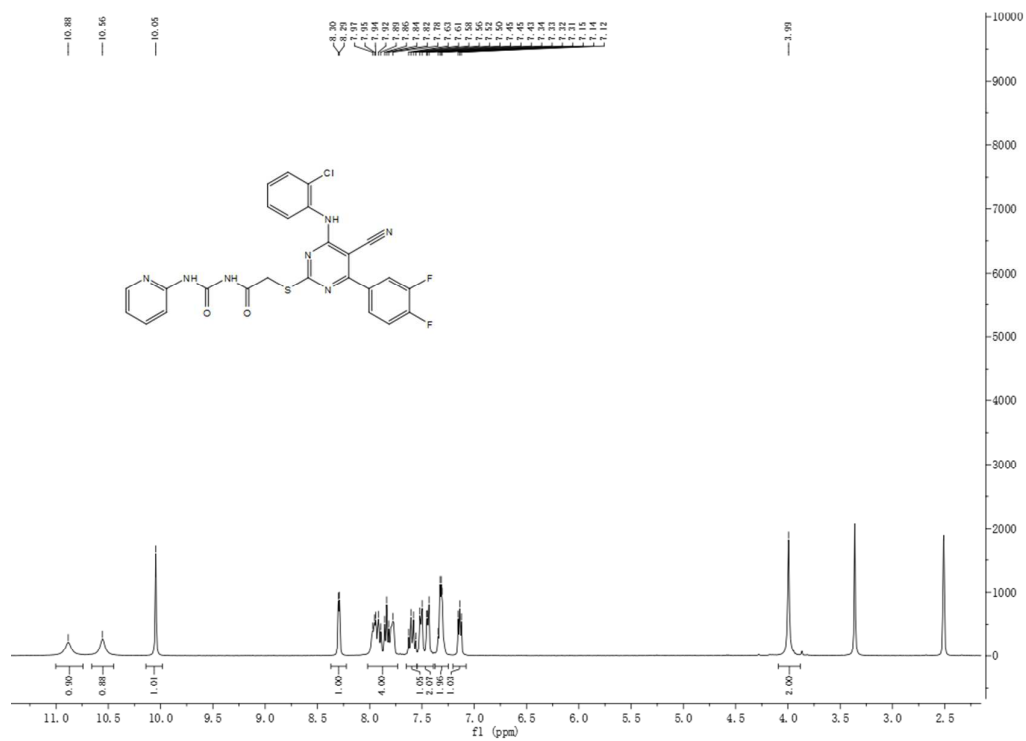


¹³C NMR spectrum of compound **63**

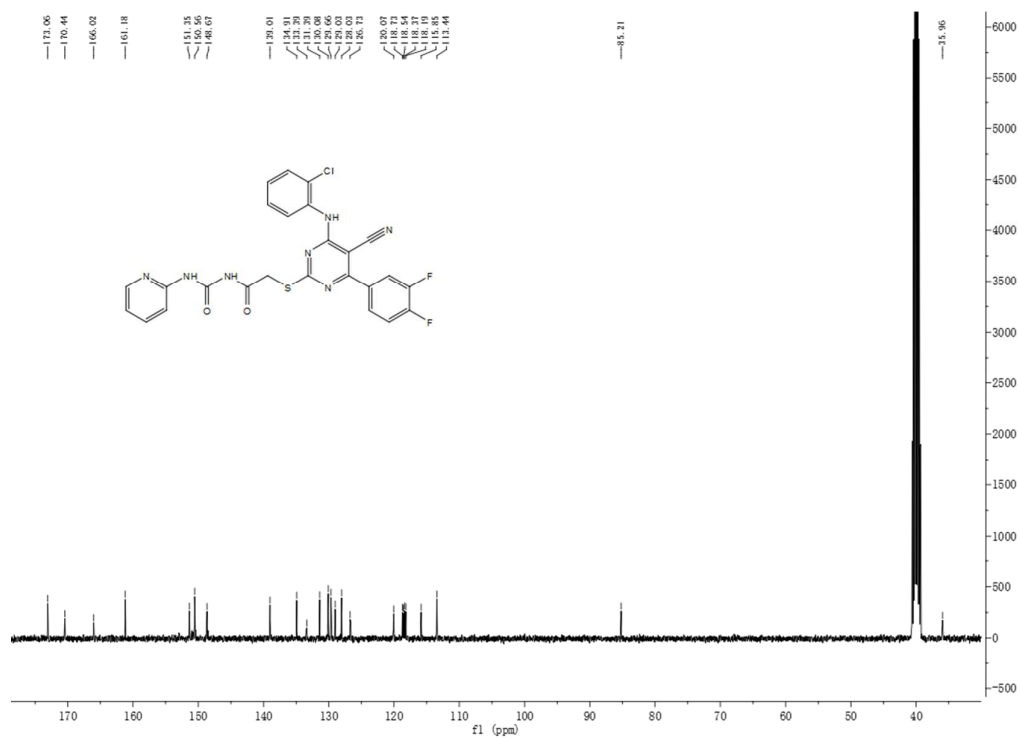


Peak	Retention Time	Area	% Area
1	3.584	7452518	98.38
2	4.049	36650	0.48
3	4.352	85923	1.13

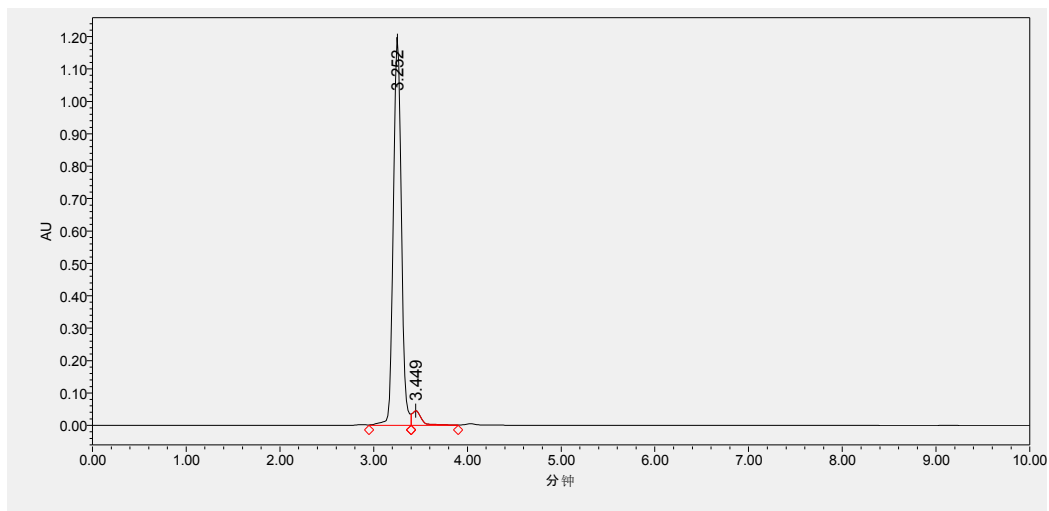
HPLC chromatogram of compound **63**



¹H NMR spectrum of compound 64

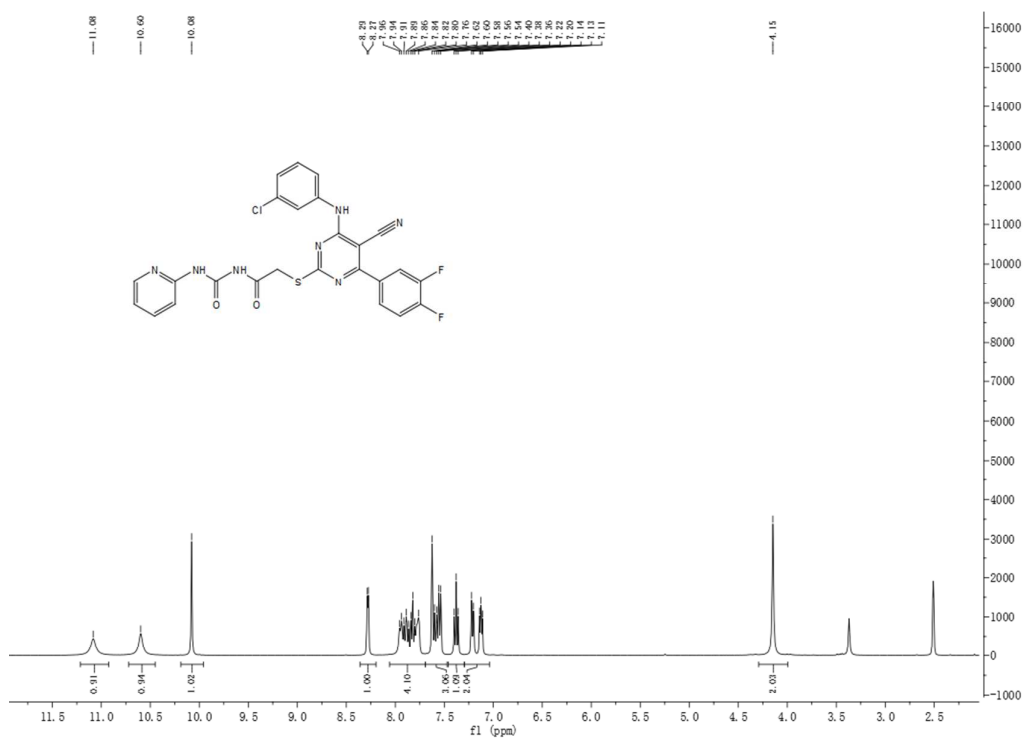


¹³C NMR spectrum of compound 64

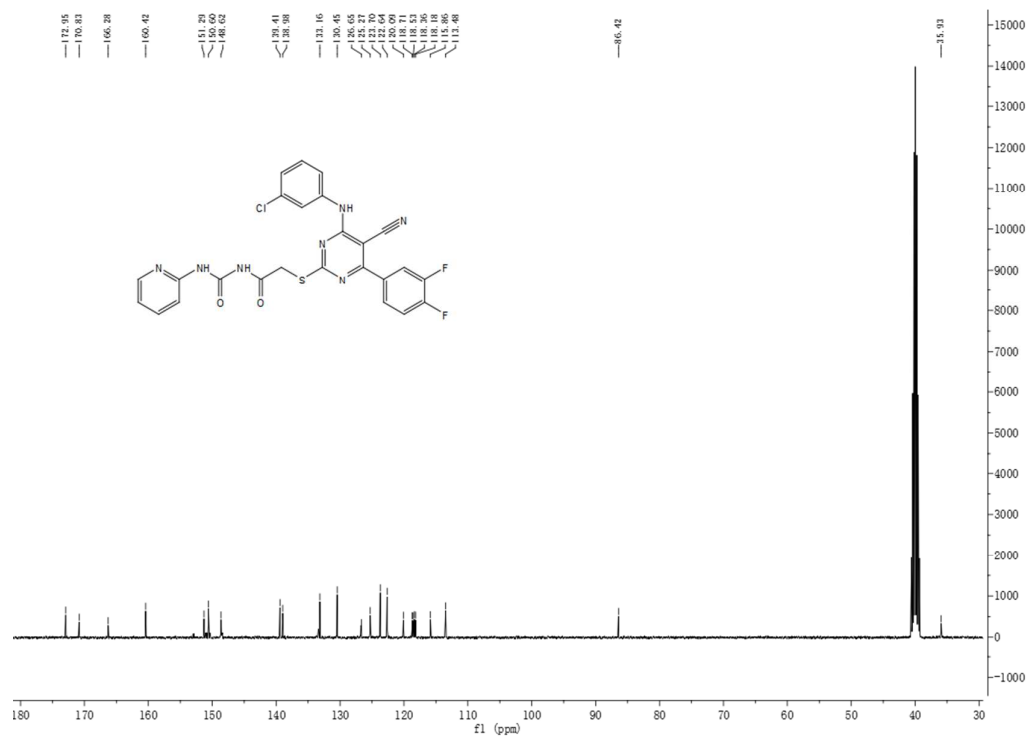


Peak	Retention Time	Area	% Area
1	3.252	7075320	95.73
2	3.449	315474	4.27

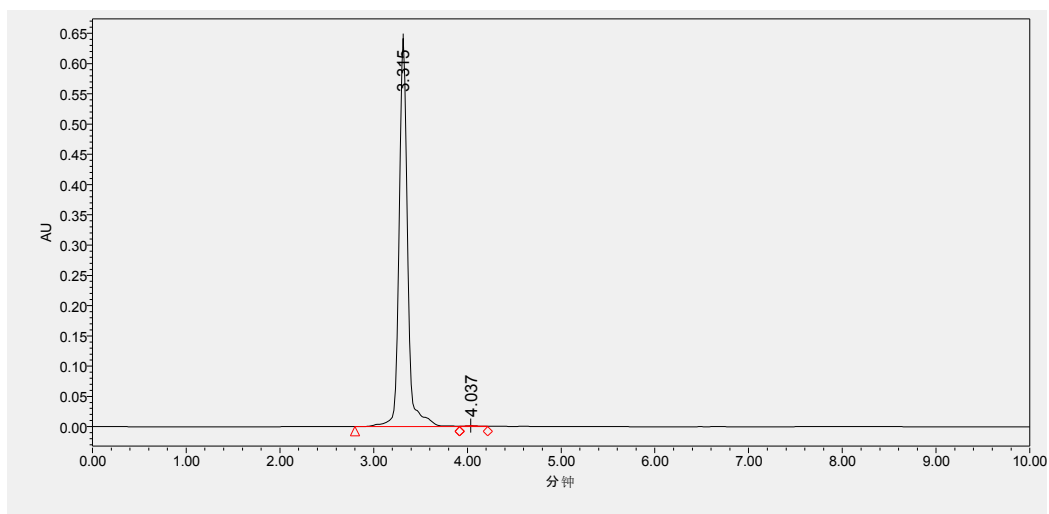
HPLC chromatogram of compound **64**



¹H NMR spectrum of compound **65**

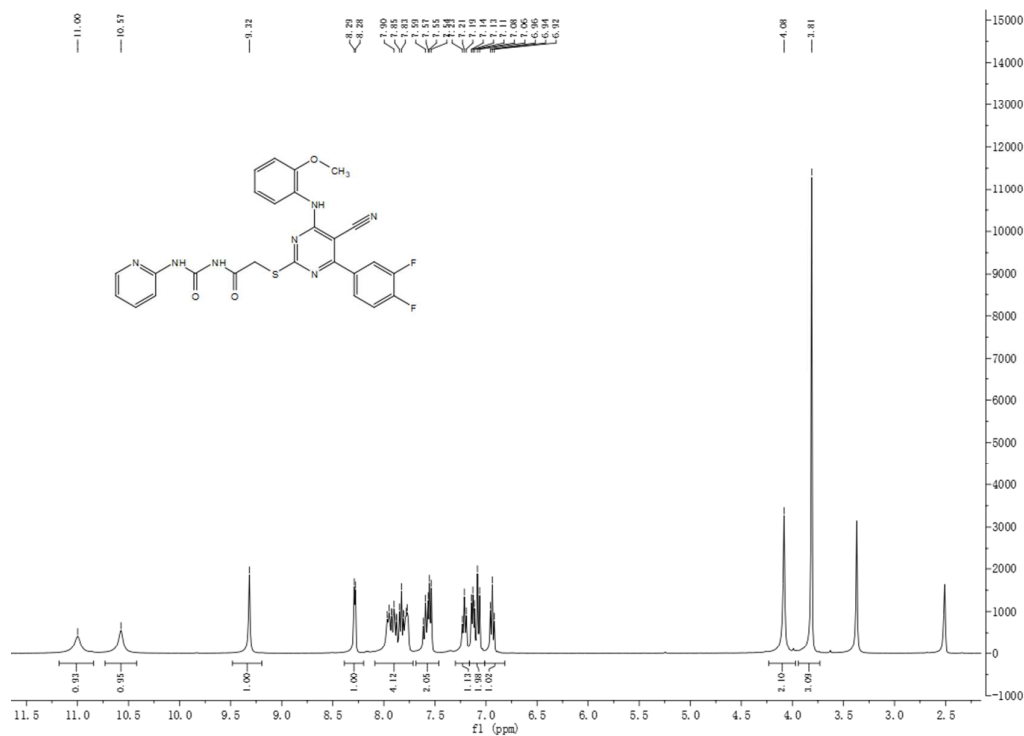


¹³C NMR spectrum of compound **65**

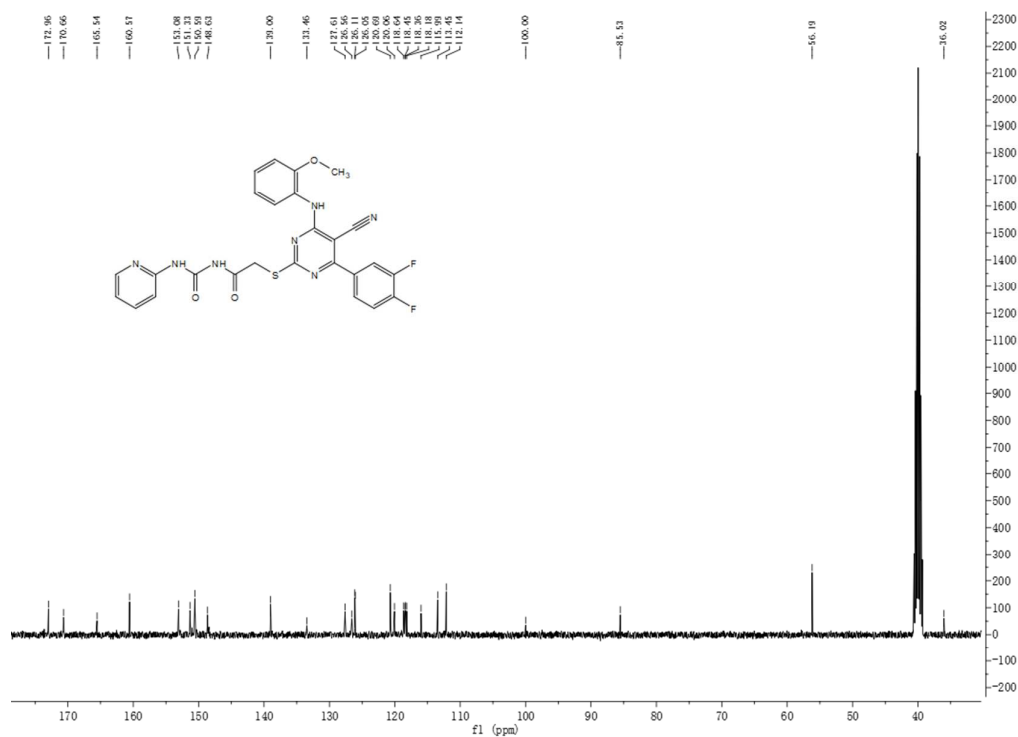


Peak	Retention Time	Area	% Area
1	3.315	4081945	99.61
2	4.037	15920	0.39

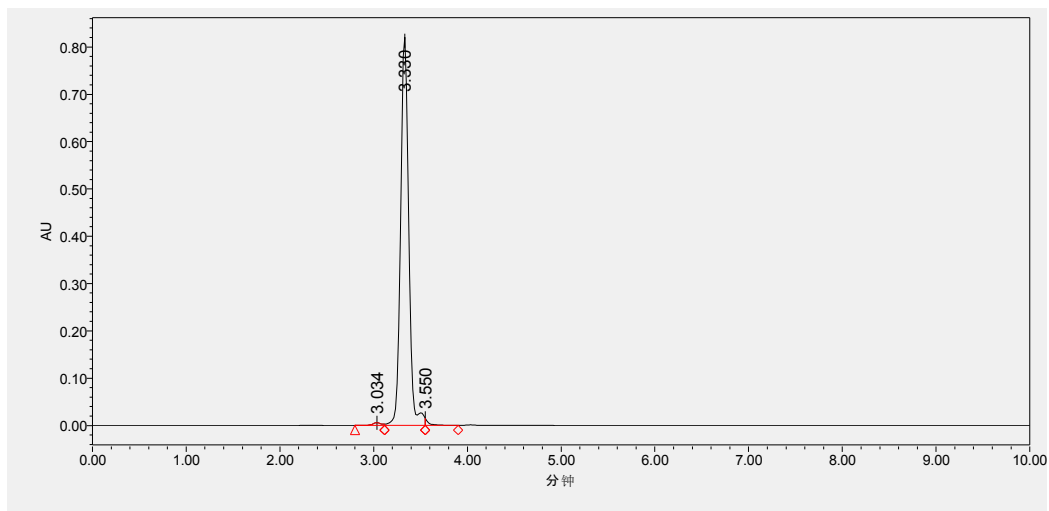
HPLC chromatogram of compound **65**



¹H NMR spectrum of compound 66

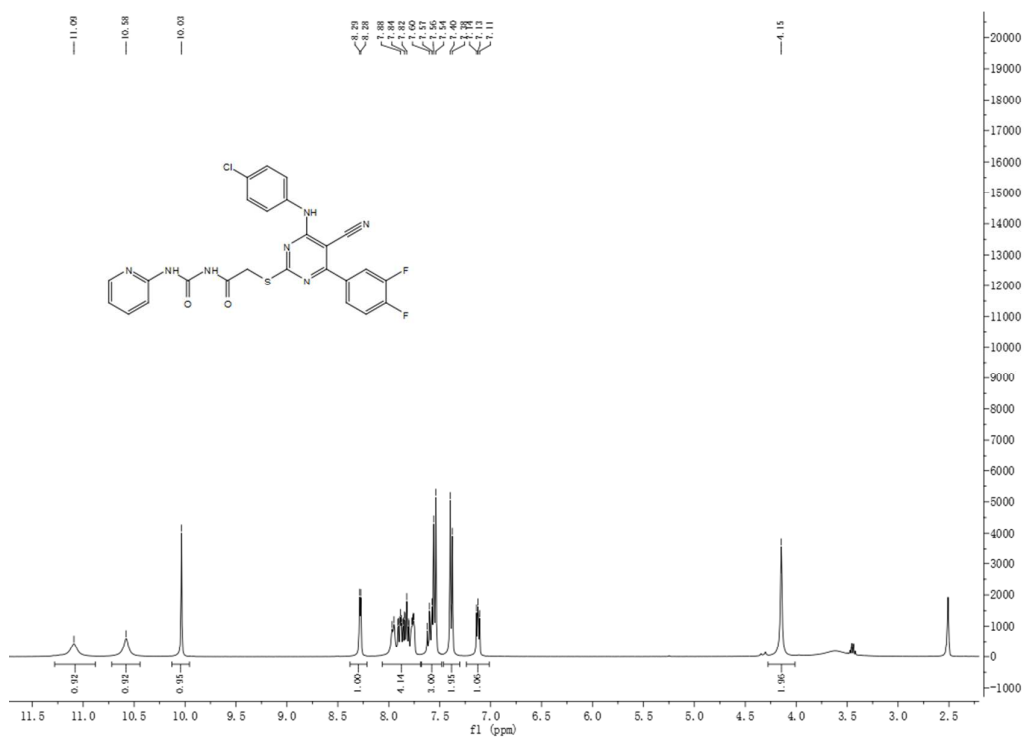


¹³C NMR spectrum of compound 66

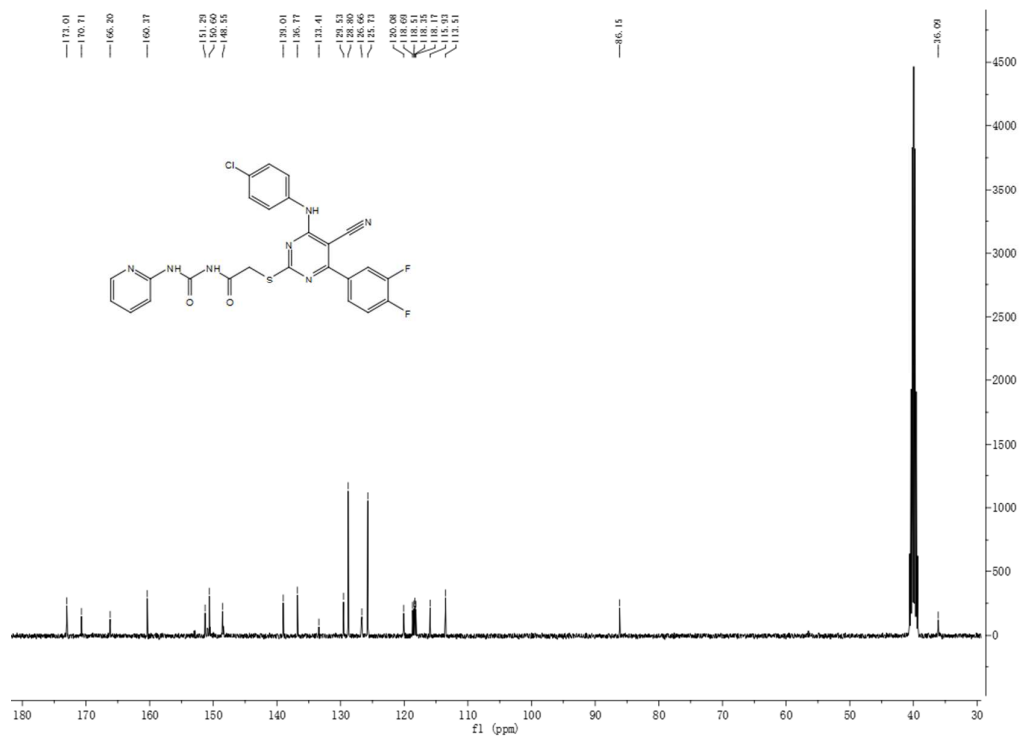


Peak	Retention Time	Area	% Area
1	3.034	38104	0.75
2	3.330	4973060	98.47
3	3.550	38976	0.77

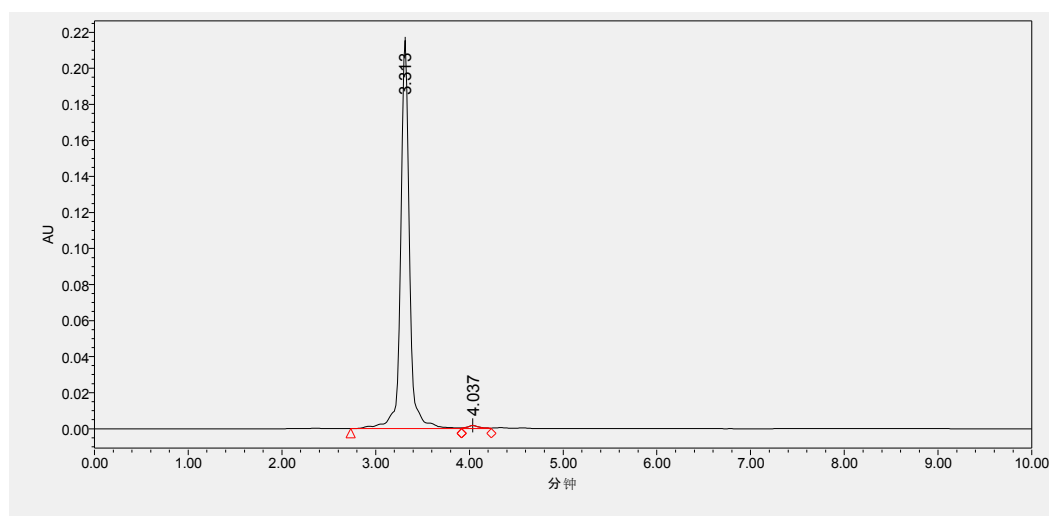
HPLC chromatogram of compound **66**



¹H NMR spectrum of compound **67**

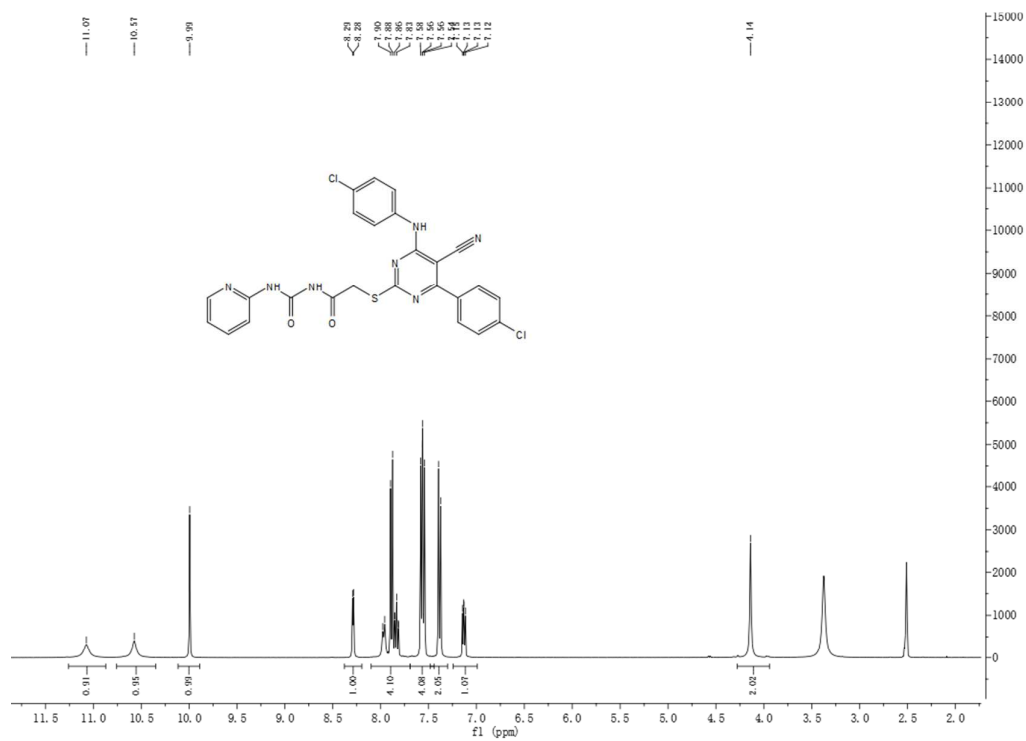


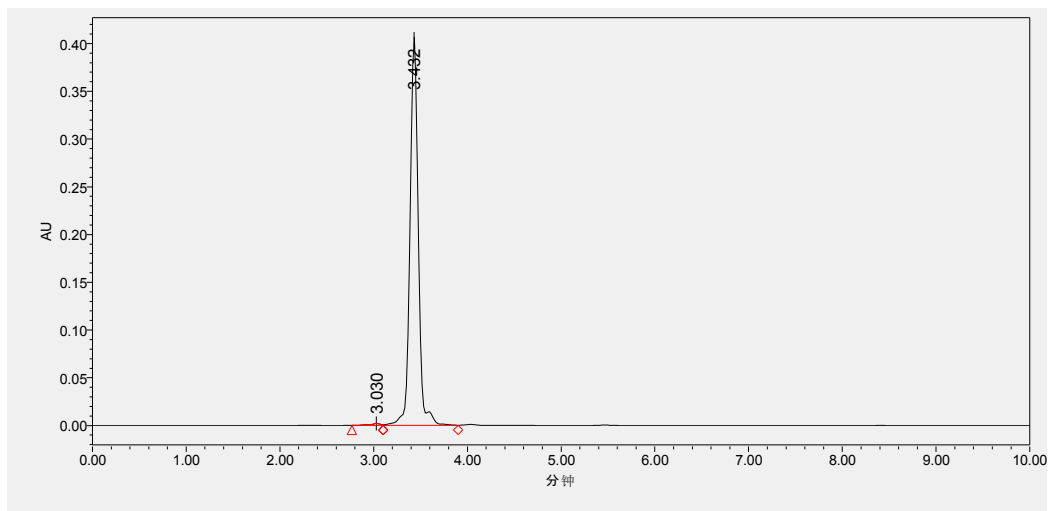
¹³C NMR spectrum of compound **67**



Peak	Retention Time	Area	% Area
1	3.313	1417824	98.91
2	4.037	15693	1.09

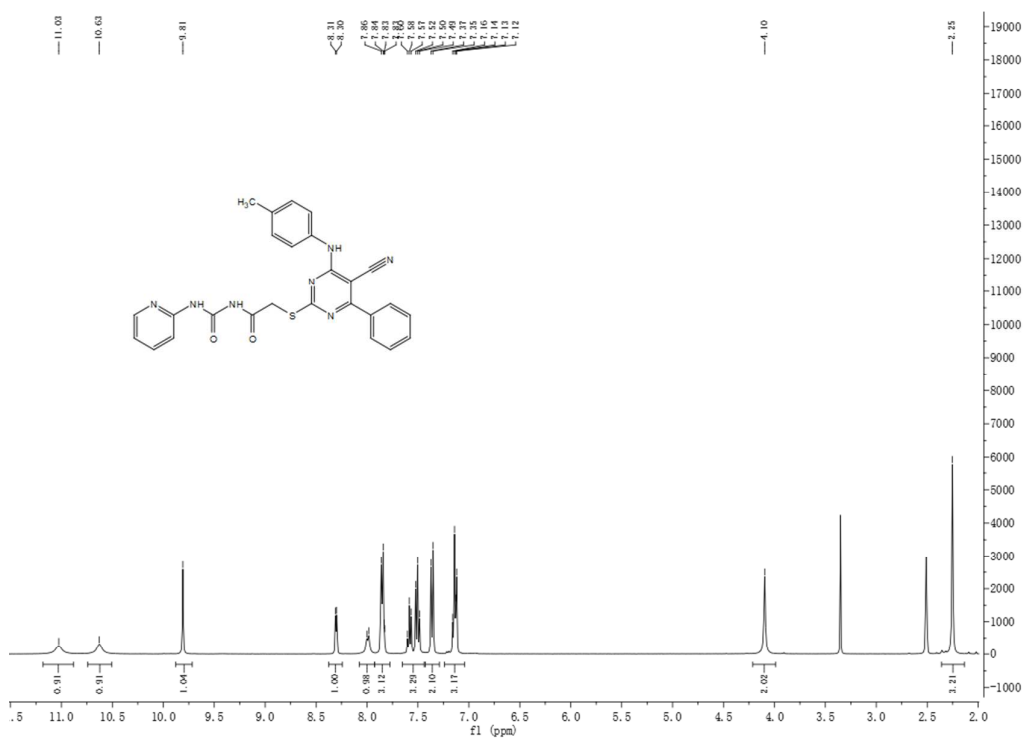
HPLC chromatogram of compound **67**



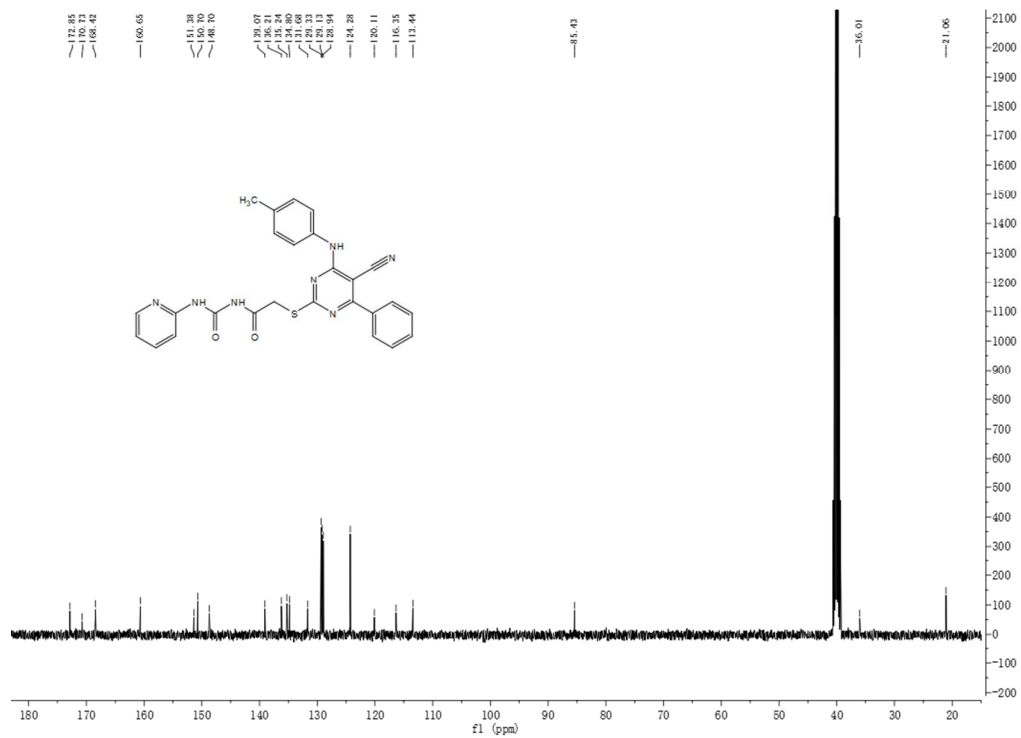


Peak	Retention Time	Area	% Area
1	3.030	16775	0.66
2	3.432	2532368	99.34

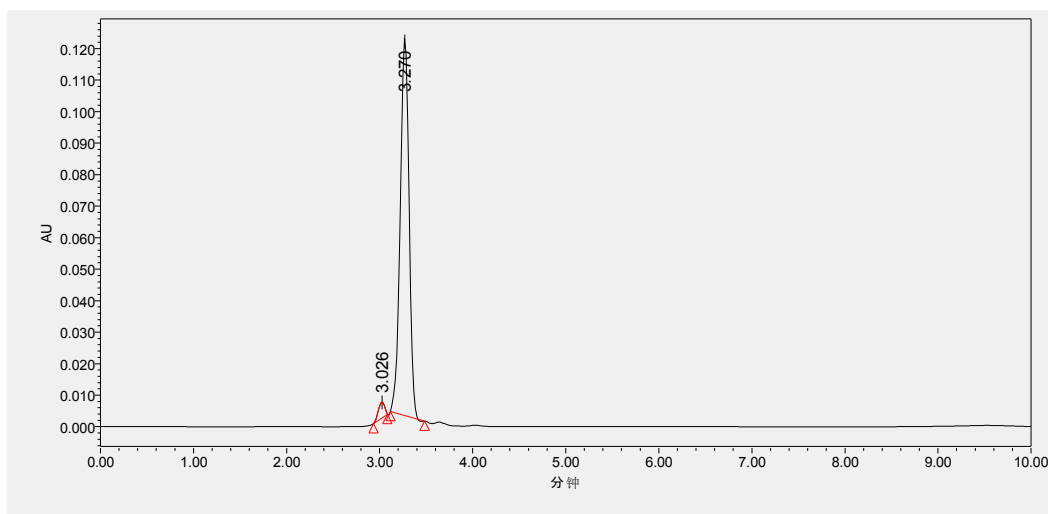
HPLC chromatogram of compound **68**



^1H NMR spectrum of compound **69**

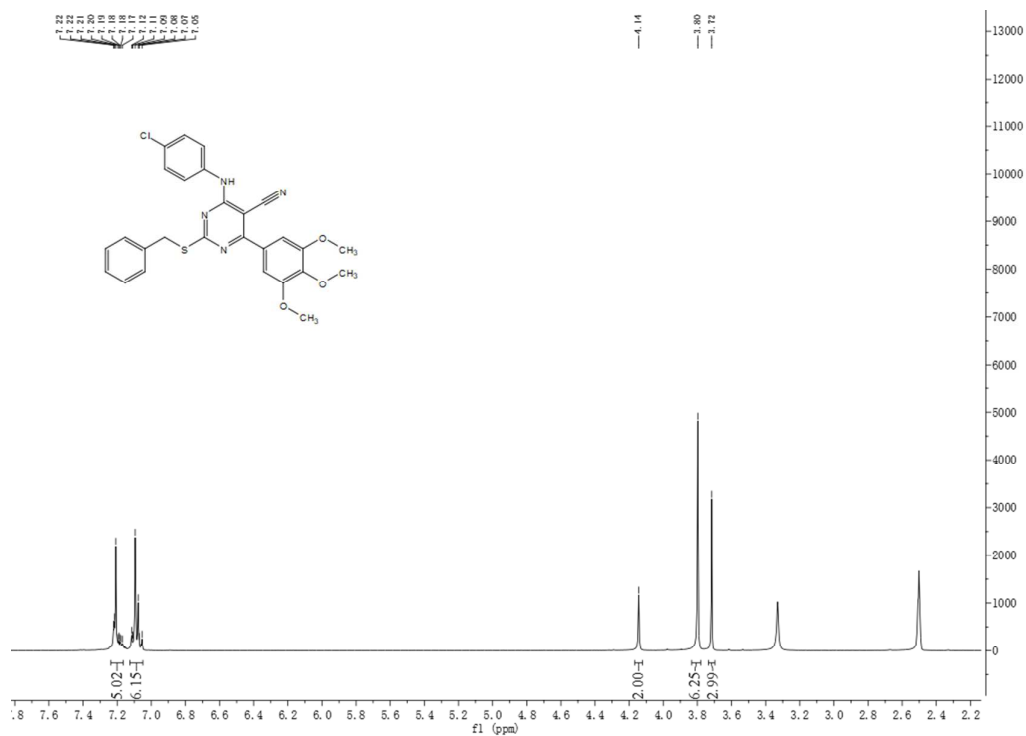


¹³C NMR spectrum of compound **69**

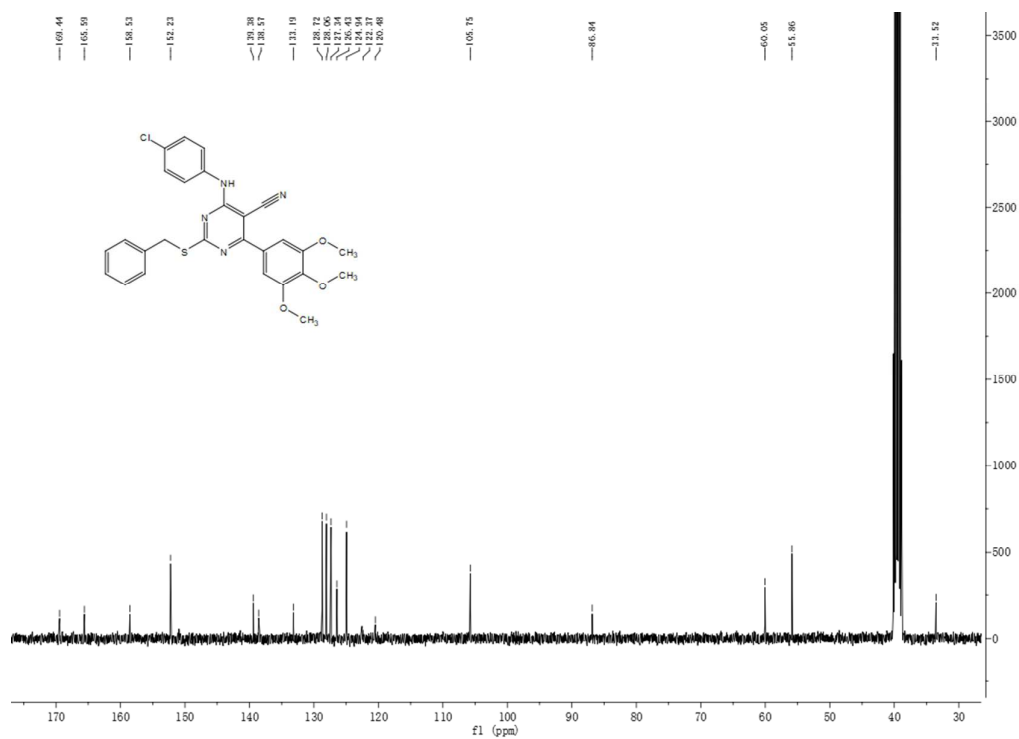


Peak	Retention Time	Area	% Area
1	3.026	23537	2.93
2	3.270	780928	97.07

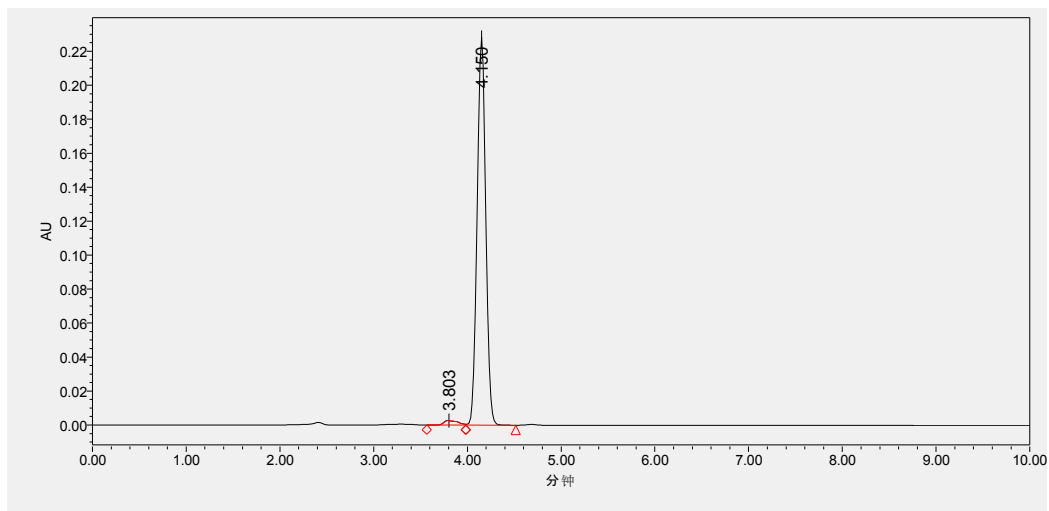
HPLC chromatogram of compound **69**



¹H NMR spectrum of compound **70**



¹³C NMR spectrum of compound **70**

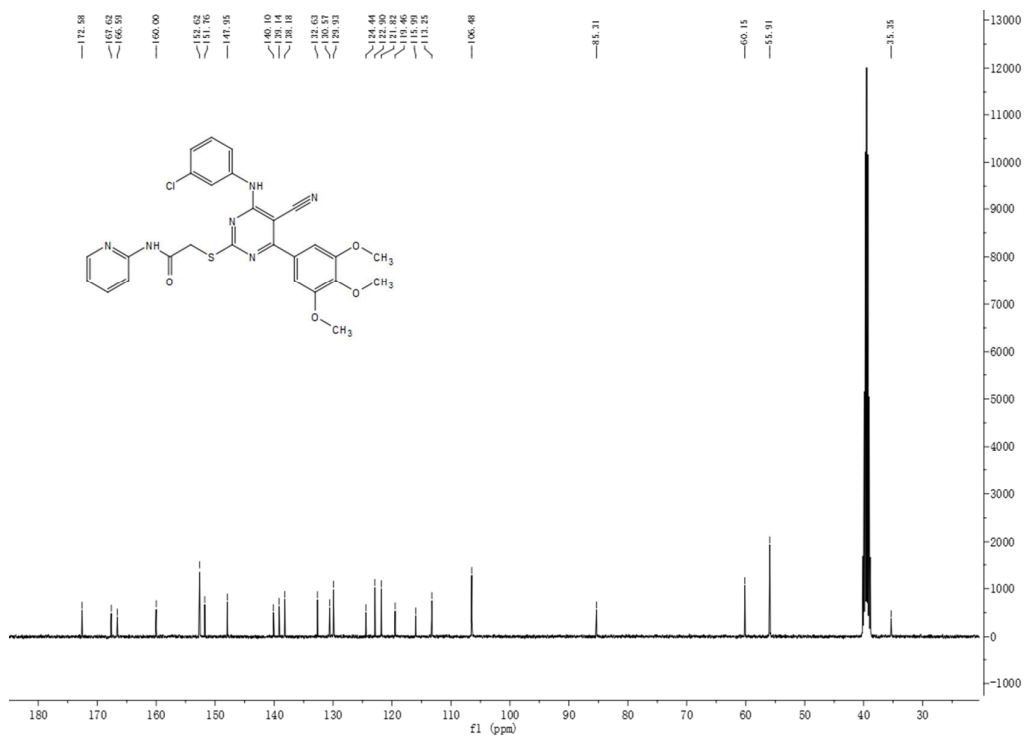


Peak	Retention Time	Area	% Area
1	3.803	29268	1.96
2	4.150	1464052	98.04

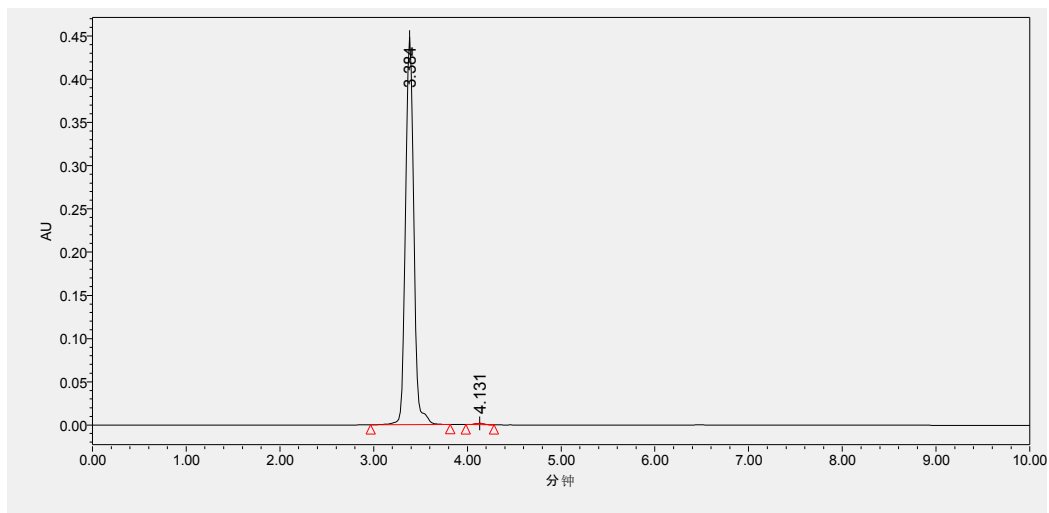
HPLC chromatogram of compound 70



¹H NMR spectrum of compound 71

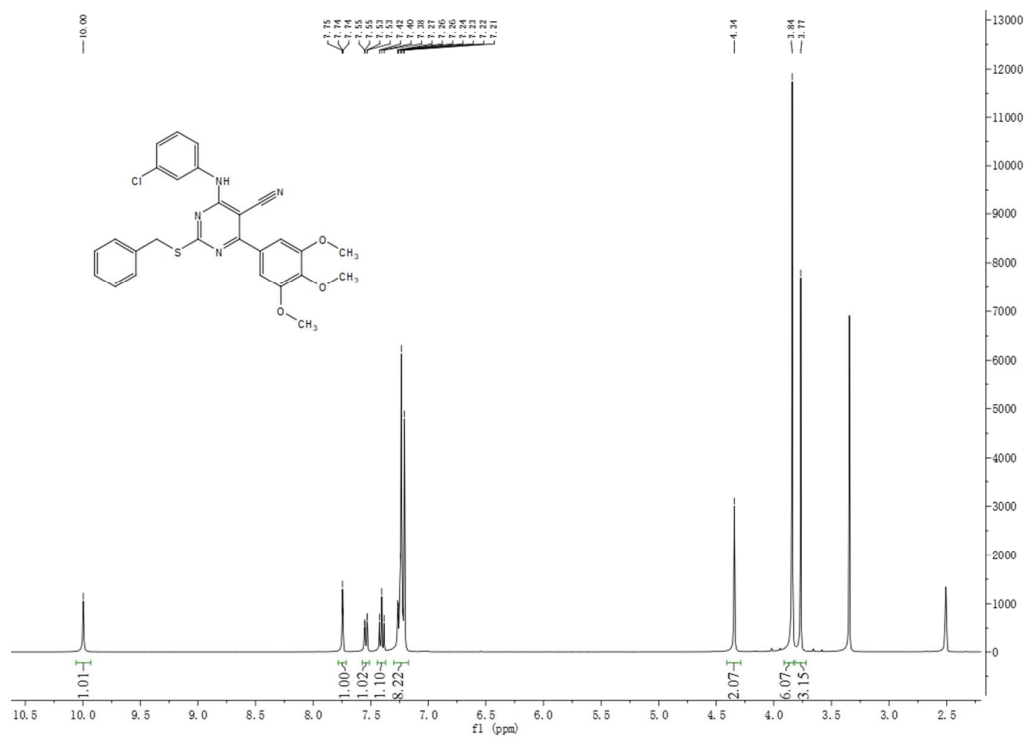


¹³C NMR spectrum of compound **71**

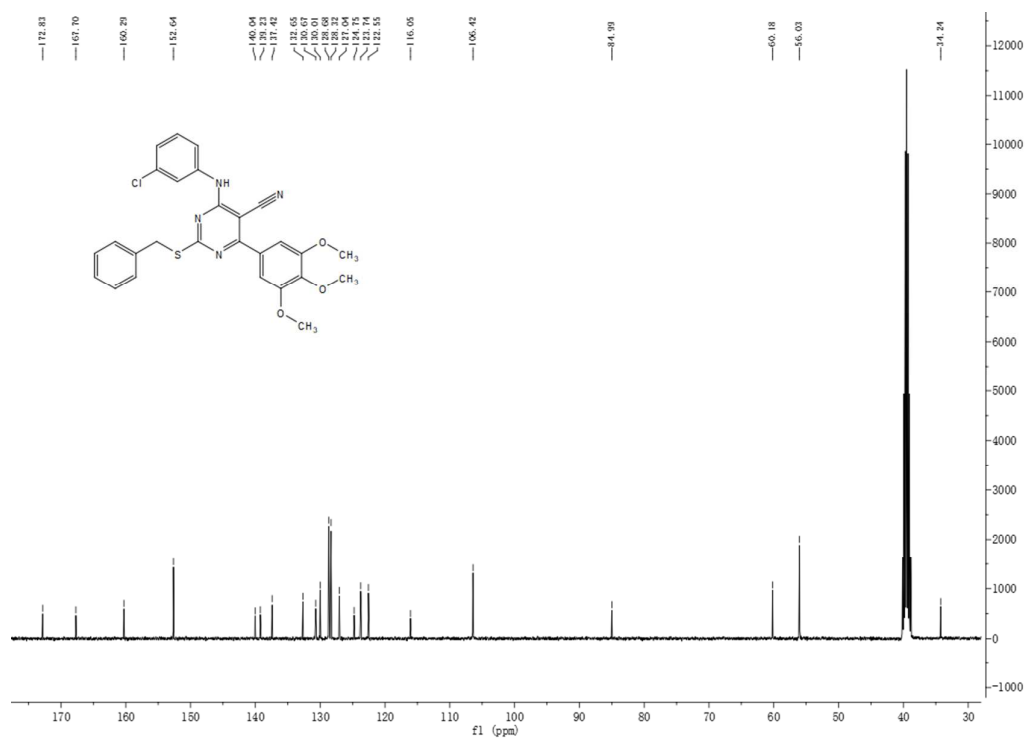


Peak	Retention Time	Area	% Area
1	3.384	2741565	99.59
2	4.131	11260	0.41

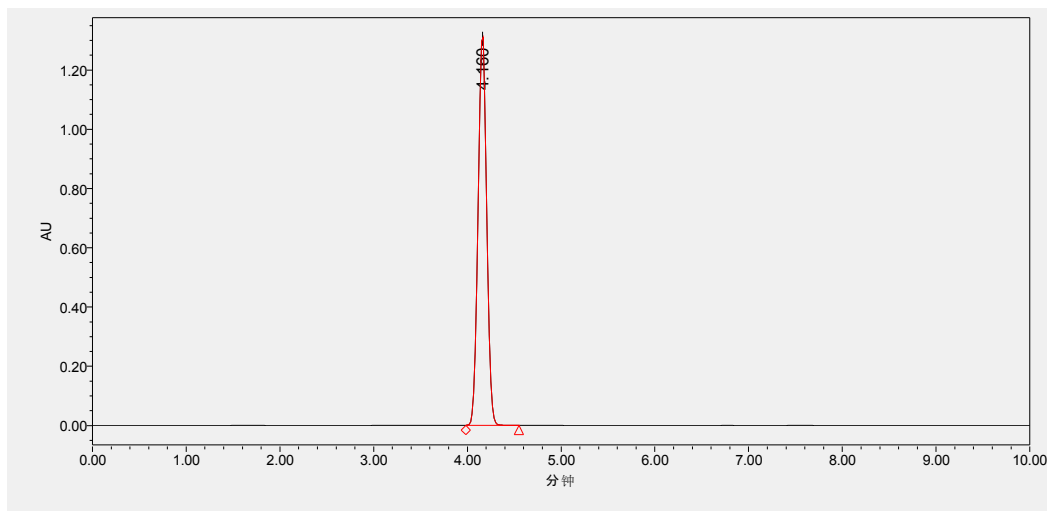
HPLC chromatogram of compound **71**



¹H NMR spectrum of compound 72

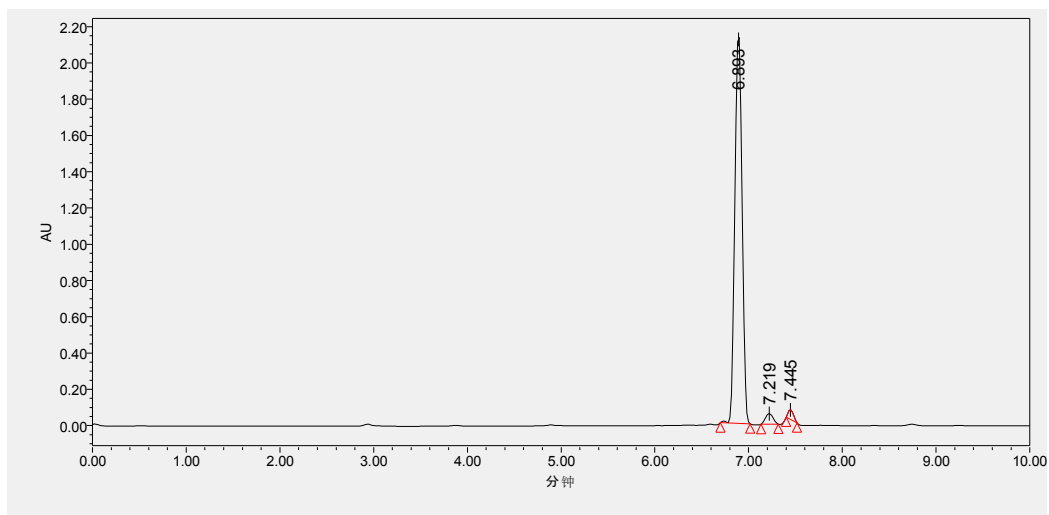


¹³C NMR spectrum of compound 72



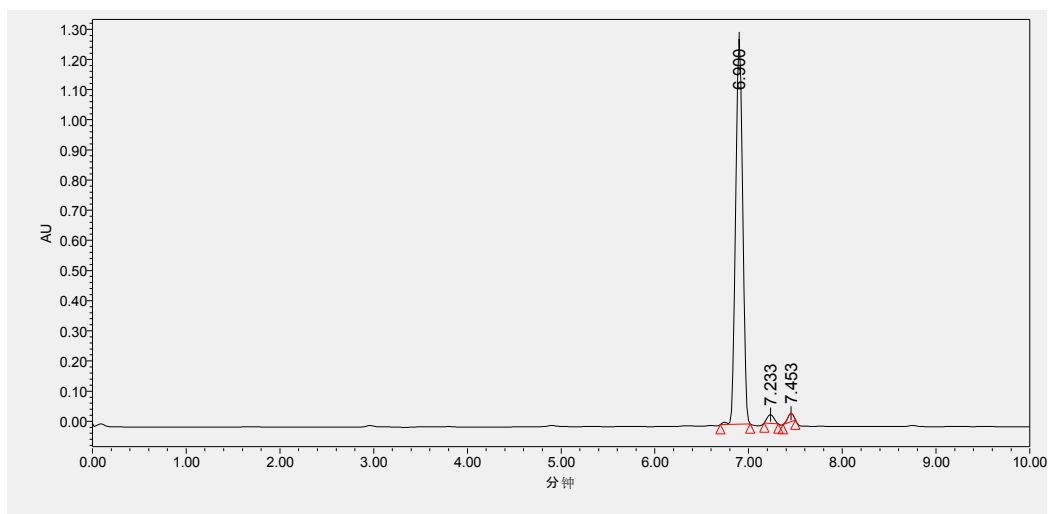
Peak	Retention Time	Area	% Area
1	4.160	8483392	100.00

HPLC chromatogram of compound **72**



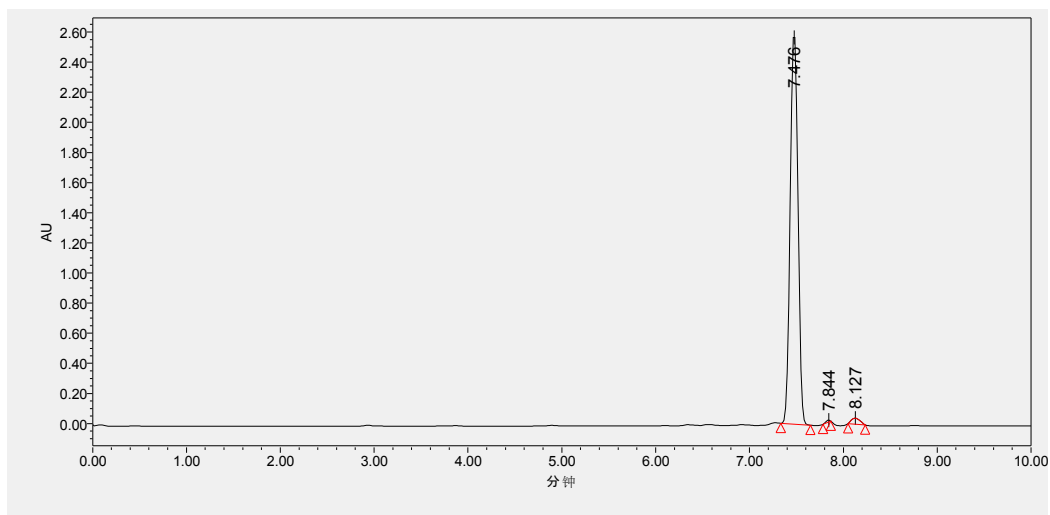
Peak	Retention Time	Area	% Area
1	6.893	11344774	95.42
2	7.219	326644	2.75
3	7.445	217297	1.83

HPLC chromatogram of compound **52** (binary gradient)



Peak	Retention Time	Area	% Area
1	6.900	6407464	98.69
2	7.232	60048	0.92
3	7.458	25117	0.39

HPLC chromatogram of compound **55** (binary gradient)



Peak	Retention Time	Area	% Area
1	7.476	14937706	98.05
2	7.844	36976	0.24
3	8.127	259615	1.70

HPLC chromatogram of compound **60** (binary gradient)