SUPPORTING INFORMATION

Biomass-involved synthesis and biological evaluation of benzofuro[2,3-d]pyrimidine-4-amines as novel EGFR tyrosine kinase inhibitors

Jianfei Sheng,[a] Zhihong Liu,[a] Ming Yan,[a] Xuejing Zhang,[a] Dejian Wang,[a] Jun Xu,[a] Ensheng Zhang[a] and Yong Zou*[a, b, c]

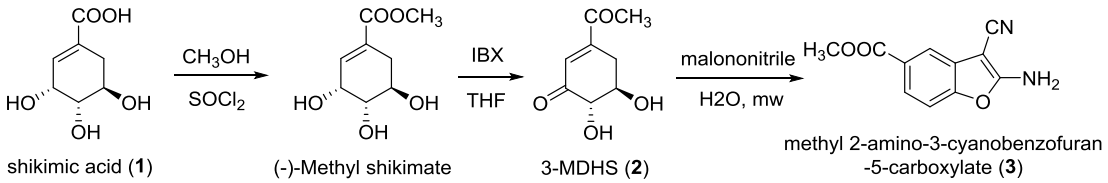
[a] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, 510006, People's Republic of China.

[b] Zhongshan WanYuan New Drug R&D Co., Ltd. Zhongshan City, 528451, People's Republic of China.

[c] Guangdong Provincial Key Laboratory of Brain Function and Disease, Sun Yat-sen University, Guangzhou, 510080, People's Republic of China.

Table of Contents

1. Preparation of methyl 3-dehydroshikimate (3-MDHS, 2) and methyl 2-amino-3-cyanobenzofuran-5-carboxylate (3).



Procedure for the preparation of (-)-Methyl shikimate [1].

A solution of (-)-shikimic acid (17.4 g, 0.10 mol) in MeOH (150 ml) was added $SOCl_2$ (15 ml, 0.20 mol) drop wise at 10-20 ℃ over 1 h. The resulting mixture was heated to 40 ℃ for 3h until completion of the reaction. The mixture was filtered and evaporated under reduced pressure to provide pale yellow oil. This was purified by recrystallizaion from EtOAc to give compound **2** as white powder solid (17.2g ,91%).

Procedure for the preparation of 3-MDHS (2).[1]

To a mixture of (-)-Methyl shikimate (9.4g, 0.05mol) and IBX (16.8g, 0.06mol), THF (220 ml) was added. The resulting mixture was stirred at 10-20 ℃ for the completion of the reaction. The IBA byproduct was filtered off and the filtrate was concentrated under reduced pressure to afford crude 3-MDHS (**2)** as white solid. The crude product was recrystallized from EtOAc to give compound **2** as white crystals (7.31g, 86%).

Procedure for the preparation of methyl 2-amino-3-cyanobenzofuran-5-carboxylate (3).[2]

3-MDHS (**2**, 10 mmol) was treated with malononitrile (15 mmol) in water (50 mL) at 85 ^{0}C for 10 min under microwave conditions (250 W), methyl 2-amino-3-cyanobenzofuran-5-carboxylate (**3**) was precipitated and could be obtained

quantitatively by filtration from the reaction mixture in 93% yield. The product was dried under vacuum and used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (s, 2H), 7.73 (d, J = 1.5 Hz, 1H), 7.69 (dd, J = 8.4, 1.7 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 3.86 (s, 3H).

2. Preparation for Intermediate A

2-Amino-3-cyanobenzofuran-5-carboxylate (**3**, 1.0 mmol) and $HC(OEt)_3$ (1.5 mmol) in toluene (3 ml) and AcOH (1 ml) (v/v = 3:1) at 110 ^{0}C for 3 h. Then, the reaction mixture was concentrated under reduced pressure to afford crude intermediate A as yellow solid. The crude product was recrystallized from EtOAc to give intermediate A as yellow crystals (0.236g, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.10 (d, J = 1.5 Hz, 1H), 7.98 (dd, J = 8.6, 1.6 Hz, 1H), 7.73 (d, J = 8.6 Hz, 1H), 4.47 (q, J = 7.1 Hz, 2H), 1.37 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.02, 164.24, 163.93, 152.76, 127.14, 126.67, 126.63, 120.26, 113.07, 112.14, 80.00, 65.53, 52.77, 14.29. HRMS (ESI-TOF) *m/z* calcd for $C_{14}H_{13}N_2O_4$ $[M+H]^+$ 272.2635, found 272.2633.

3. General procedure for the synthesis of benzofuro[2,3-d]pyrimidine-4-amines 5 and 7.

2-Amino-3-cyanobenzofuran-5-carboxylate (**3**, 1.0 mmol) and $HC(OEt)_3$ (1.5 mmol) in toluene (3 ml) and AcOH (1 ml) (v/v = 3:1) at 110 ^{0}C for 3 h (step 1), after concentration under vacuum, amines (1.5 eq) and AcOH (5ml) were added and stirred at 120 ^{0}C for another 3 h. After completion of the reaction (monitored by TLC), the mixture was poured into water (50 mL). A white solid was obtained and collected by

filtration and recrystallized from EtOAc/petroleum ether to afford the product benzofuro [2,3-d]pyrimidine-4-amines.

4. Method for EGFR tyrosine kinase inhibition assay.[3-6]

Wild type (WT) and the Z′-Lyte Kinase Kit were purchased from Invitrogen. The experiments were performed according to the instructions of the manufacturer. Ten concentration gradients were set for all the tested compounds from 5.1 $\times 10^{-9}$ M to 1 $\times 10^{-4}$ M in DMSO; a 4 × compound solution was prepared. An ATP solution in 1.33 × Kinase Buffer, and a Kinase/Peptide Mixture containing 2 × kinase and Tyr 4 peptide were prepared right before use. The 10 μl Kinase Reactions were consisted of 2.5 μl compound solution, 5 μl Kinase/Peptide Mixture, and 2.5 μl ATP solution. 5 μl phospho-peptide solution instead of Kinase/Peptide Mixture was used as 100 % phosphorylation control. 2.5 μl 1.33 × Kinase Buffer instead of ATP solution was used as 100% inhibition control, while 2.5 μl 4% DMSO instead of compound solution was used as 0% inhibition control. Mixed the plate thoroughly and incubated for 1 h at 25 ℃. 5 μl Development Solutionwas added to each well and the plate was incubated for 1 h at 25 ℃; the non-phospho-peptides were cleaved in this time. In the end, 5 μl Stop Reagent was added to stop reaction. Plate was measured on EnVision Multilabel Reader (PerkinElmer). Curve fitting and data presentations were performed using Graph Pad Prism version 5.0. Every experiment was repeated at least 3 times.

5. Method for MTT assay.

The antiproliferative activity of the target compounds were examined in two human cancer cell lines (A431 and A549) by MTT assay. When the cells grew in the

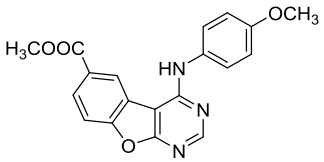
logarithmic phase, 5×10^3 cells per well cells were harvested and plated into the 96-well plates for 24 h, and then the cells were exposed to different concentrations of the test compounds for 48 h in three replicates. Afterward, 20 mL of MTT (5 mg/mL, Sigma) was added and incubated for another 4 h. Then, the suspension was discarded and 150 mL of DMSO was added to each well. After shooked the plateds to dissolve the dark blue crystals (formazan) for 10 min, the absorbance at 570 nm was measured using a multifunction mircoplate reader (Moleculardevices, Flex Station 3). All experiments were repeated at least three times. The data were calculated using Grap Pad Prism version 5.0. The GI_{50} values were fitted using a nonlinear regression model with a sigmodial dose response.

6. Method for molecular docking.[7]

The 3D structure of the protein EGFR was downloaded from RCSB Protein Data Bank (PDB code: 2ITY) and prepared with the Structure Preparation workflow in MOE to correct structural errors. Protonation state of the protein and the orientation of the hydrogens were optimized by LigX, at the PH of 7 and temperature of 300 K. Prior to docking, the force field of AMBER12: EHT and the implicit solvation model of Reaction Field (R-field) were selected. MOE-Dock was used for molecular docking simulations. The docking workflow followed the "induced fit" protocol, in which the side chains of the receptor pocket were allowed to move according to ligand conformations, with a constraint on their positions.

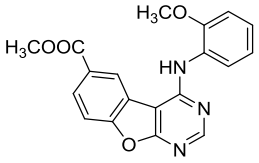
7. Characterizations of Compounds.

Methyl 4-((4-methoxyphenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5a).



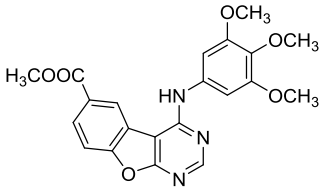
White solid; 87% yield; mp: 195.0–196.7℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.15 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.78 (s, 1H), 7.66 – 7.59 (m, 1H), 7.45 (d, *J* = 8.9 Hz, 2H), 7.40 (s, 1H), 6.97 (d, *J* = 9.0 Hz, 2H), 3.93 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.73, 166.58, 157.99, 157.21, 156.55, 155.11, 130.31, 128.65, 126.12, 125.86, 123.59, 120.88, 114.59, 111.68, 97.49, 55.53, 52.28. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{15}N_3NaO_4$ $[M+Na]^+$ 372.0955, found 372.0956. Purity: 98.893% (by HPLC).

Methyl 4-((2-methoxyphenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5b) .



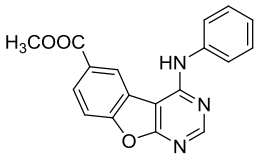
White solid; 82% yield; mp: 229.3–231.0℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.64 (s, 1H), 8.46 (s, 1H), 8.11 (d, *J* = 8.6 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.61 (d, *J* = 7.5 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.16 (d, *J* = 8.1 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 3.91 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.46, 166.48, 157.38, 156.75, 154.72, 154.16, 128.70, 127.56, 127.46, 127.43, 126.26, 124.28, 121.38, 120.90, 112.38, 112.17, 97.30, 56.15, 52.70. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{16}N_3O_4$ $[M+H]^+$ 350.1135, found 350.1123. Purity: 99.628% (by HPLC).

Methyl 4-((3,4,5-trimethoxyphenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5c).



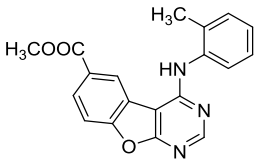
White solid; 86% yield; mp: 217.4–217.8℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.68 (s, 1H), 8.55 (s, 1H), 8.13 (dd, J = 8.6, 1.7 Hz, 1H), 7.86 (dd, J = 8.6, 3.8 Hz, 1H), 6.92 (s, 2H), 3.90 (s, 3H), 3.75 (s, 6H), 3.70 (s, 3H). 13C NMR (100 MHz, DMSO-d_6) δ 169.66, 166.53, 156.82, 156.63, 154.72, 153.20, 135.47, 134.84, 128.73, 126.08, 125.00, 121.22, 112.01, 102.86, 97.71, 60.62, 56.45, 52.72. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{20}N_3O_6$ $[M+H]^+$ 410.1347, found 410.1340. Purity: 98.287% (by HPLC).

Methyl 4-(phenylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5d).



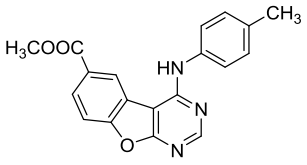
White solid; 75% yield; mp: 180.1–181.7℃;^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.21 (dd, *J* = 8.6, 1.6 Hz, 1H), 8.04 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.46 (t, *J* = 7.9 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 2H), 3.96 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.65, 166.50, 156.58, 156.42, 155.20, 137.62, 129.34, 128.86, 126.22, 125.54, 123.23, 122.89, 120.73, 111.88, 98.02, 52.40. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{14}N_3O_3$ $[M+H]^+$ 320.1030, found 320.1022. Purity: 97.881% (by HPLC).

methyl 4-(o-tolylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5e).



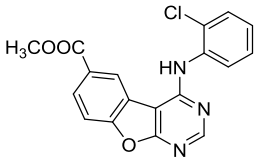
White solid; 76% yield; mp: 203.6–204.1℃;^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.68 (s, 1H), 8.65 (s, 1H), 8.43 (s, 1H), 8.11 (dd, *J* = 8.6, 1.1 Hz, 1H), 7.85 (d, *J* = 8.6 Hz, 1H), 7.40 – 7.35 (m, 1H), 7.35 – 7.25 (m, 3H), 3.90 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.64, 166.52, 157.50, 156.84, 154.66, 137.48, 135.87, 130.97, 128.64, 128.32, 127.33, 126.83, 126.26, 124.66, 121.46, 112.02, 96.88, 52.62, 18.47. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{16}N_3O_3$ $[M+H]^+$ 334.1186, found 334.1190. Purity: 99.486% (by HPLC).

Methyl 4-(p-tolylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5f).



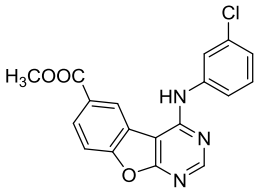
White solid; 85% yield; mp: 224.8–227.5℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H), 8.80 (s, 1H), 8.50 (s, 1H), 8.11 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 3.91 (s, 3H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.59, 166.57, 156.73, 156.65, 154.73, 136.35, 134.47, 129.46, 128.75, 126.13, 124.93, 124.78, 121.27, 112.03, 97.48, 52.74, 21.0; HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{15}N_3NaO_3$ $[M+Na]^+$ 356.1006, found 356.1007. Purity: 98.747% (by HPLC).

methyl 4-((2-chlorophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5g).



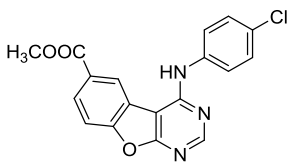
White solid; 76% yield; mp: 238.3–239.8℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.88 (s, 1H), 8.47 (s, 1H), 8.15 (dd, J = 8.7, 1.7 Hz, 1H), 7.89 (d, J = 8.7 Hz, 1H), 7.62 (ddd, J = 16.5, 7.8, 1.6 Hz, 2H), 7.43 (dtd, J = 22.9, 7.5, 1.6 Hz, 2H), 3.92 (s, 3H). 13C NMR (100 MHz, DMSO-d_6) δ 169.60, 166.51, 157.13, 156.76, 154.80, 136.22, 131.94, 130.27, 128.96, 128.74, 128.28, 126.39, 124.56, 121.23, 112.25, 97.39, 52.74. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{13}ClN_3O_3$ $[M+H]^+$ 354.0640, found 354.0626. Purity: 99.560% (by HPLC).

Methyl 4-((3-chlorophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5h).



White solid; 88% yield; mp: 191.0–191.6℃;^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.26 (s, 1H), 8.20 (d, *J* = 8.7 Hz, 1H), 7.80 (s, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.36 (s, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 3.96 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.56, 166.51, 156.49, 155.86, 155.24, 139.01, 134.86, 130.14, 129.07, 126.35, 125.07, 122.95, 122.34, 120.45, 120.24, 112.01, 98.30, 52.48. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{13}ClN_3O_3$ $[M+H]^+$ 354.0640, found 354.0627. Purity: 97.955% (by HPLC).

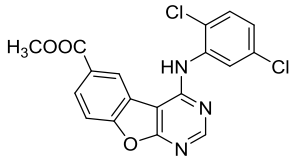
Methyl 4-((4-chlorophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5i).



White solid; 90% yield; mp: 212.0–213.7℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s,

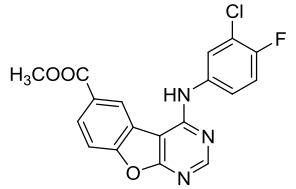
1H), 8.92 (s, 1H), 8.56 (s, 1H), 8.13 (d, J = 8.7 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.68 (d, J = 8.8 Hz, 2H), 7.47 (d, J = 8.7 Hz, 2H), 3.93 (s, 3H);^{13}C NMR (100 MHz, DMSO-d_6) δ 169.56, 166.54, 156.55, 156.19, 154.82, 138.05, 128.99, 128.86, 128.77, 126.18, 125.83, 124.93, 121.02, 112.15, 98.06, 52.79; HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{12}ClN_3NaO_3$ $[M+Na]^+$ 376.0459, found 376.0457. Purity: 99.519% (by HPLC).

methyl 4-((2,5-dichlorophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5j).



White solid; 79% yield; mp: 228.9–230.1℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.93 (s, 1H), 8.52 (s, 1H), 8.17 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.76 (d, *J* = 2.5 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.48 (dd, *J* = 8.6, 2.5 Hz, 1H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.55, 166.44, 156.69, 156.66, 154.85, 137.62, 132.16, 131.54, 130.36, 129.41, 129.10, 128.22, 126.39, 124.51, 121.01, 112.30, 97.84, 52.76. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{12}Cl_2N_3O_3$ $[M+H]^+$ 388.0250, found 388.0239. Purity: 99.844% (by HPLC).

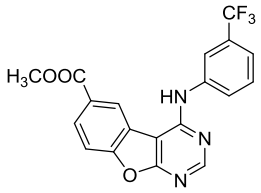
Methyl 4-((3-chloro-4-fluorophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (4k).



White solid; 72% yield; mp: 193.7–194.5℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s,

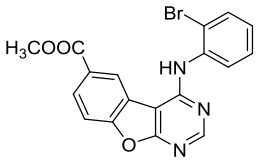
1H), 8.99 (s, 1H), 8.63 (s, 1H), 8.20 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.95 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.71 (ddd, *J* = 8.9, 4.3, 2.7 Hz, 1H), 7.52 (t, *J* = 9.1 Hz, 1H), 3.98 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.45, 166.47, 156.48, 156.02, 154.76, 154.54 (d, *J* = 243.9 Hz), 136.27 (d, *J* = 3.0 Hz), 128.96, 126.15, 125.87, 124.73 (d, *J* = 7.6 Hz), 120.85, 119.34 (d, *J* = 18.5 Hz), 116.95 (d, *J* = 21.8 Hz), 112.06, 97.96, 52.74. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{12}ClFN_3O_3$ $[M+H]^+$ 372.0546, found 372.0538. Purity: 99.461% (by HPLC).

Methyl 4-((3-(trifluoromethyl)phenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5l).



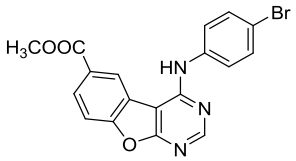
White solid; 85% yield; mp: 228.4–228.9℃;^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.29 (d, *J* = 1.2 Hz, 1H), 8.24 (dd, *J* = 8.6, 1.6 Hz, 1H), 8.01 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.56 (t, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 1H), 7.33 (s, 1H), 3.97 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.42, 166.41, 156.37, 155.82, 154.74, 139.95, 129.92, 129.83, 129.52, 128.96, 127.56, 126.07, 125.99, 124.83, 123.29, 120.91, 120.87, 120.73, 119.94, 119.91, 111.98, 98.26, 52.67, 40.57, 40.36, 40.15, 39.94, 39.74, 39.53, 39.32. Purity: 98.322% (by HPLC).

Methyl 4-((2-bromophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5m).



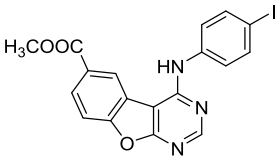
White solid; 80% yield; mp: 240.5–241.4℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.87 (s, 1H), 8.47 (d, J = 2.0 Hz, 1H), 8.27 – 8.07 (m, 1H), 7.88 (d, J = 8.6 Hz, 1H), 7.80 (d, J = 6.8 Hz, 1H), 7.67 – 7.56 (m, 1H), 7.51 (t, J = 6.7 Hz, 1H), 7.34 (t, J = 6.7 Hz, 1H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.61, 166.51, 157.15, 156.76, 154.78, 137.72, 133.41, 130.55, 129.10, 128.92, 126.41, 124.54, 123.03, 121.26, 112.24, 97.33, 52.73. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{13}BrN_3O_3$ $[M+H]^+$ 398.0135, found 398.0128. Purity: 97.895% (by HPLC).

methyl 4-((4-bromophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5n).



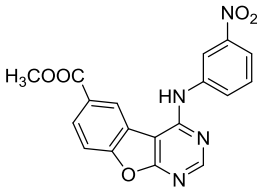
White solid; 83% yield; mp: 224.3–225.2℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 8.94 (s, 1H), 8.56 (s, 1H), 8.14 (dd, J = 8.6, 1.6 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.67 – 7.57 (m, 4H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.57, 166.54, 156.54, 156.11, 154.83, 138.52, 131.76, 129.01, 126.19, 126.11, 124.96, 121.01, 116.84, 112.16, 98.15, 52.81. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{12}BrN_3NaO_3$ $[M+Na]^+$ 419.9954, found 419.9951. Purity: 99.317% (by HPLC).

methyl 4-((4-iodophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5o).



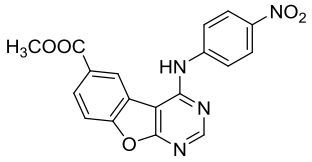
White solid; 84% yield; mp: 230.7–231.9℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H), 8.94 (s, 1H), 8.56 (s, 1H), 8.14 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.87 (d, *J* = 8.7 Hz, 1H), 7.75 (dd, *J* = 9.1, 2.4 Hz, 2H), 7.53 – 7.44 (m, 2H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.54, 166.53, 156.50, 156.04, 154.81, 139.00, 137.61, 129.00, 126.26, 126.16, 124.95, 120.98, 112.13, 98.18, 88.89, 52.83. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{12}IN_3NaO_3$ $[M+Na]^+$ 467.9816, found 467.9816. Purity: 98.814% (by HPLC).

methyl 4-((3-nitrophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5p).



White solid; 83% yield; mp: 258.9–260.1℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 9.09 (s, 1H), 8.64 (s, 2H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 8.6 Hz, 1H), 8.02 (d, *J* = 8.1 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 1H), 7.69 (t, *J* = 8.2 Hz, 1H), 3.94 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.60, 166.56, 156.51, 155.81, 154.99, 148.28, 140.52, 130.22, 129.78, 129.32, 126.32, 125.05, 120.84, 118.98, 117.65, 112.33, 98.77, 52.84. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{13}N_4O_5$ $[M+H]^+$ 365.0880, found 365.0871. Purity: 99.611% (by HPLC).

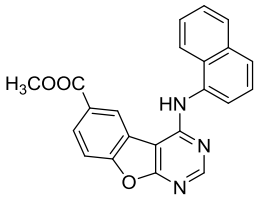
methyl 4-((4-nitrophenyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5q).



White solid; 86% yield; mp: 255.6–256.4℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.99 (s, 1H), 8.70 (s, 1H), 8.28 (d, *J* = 9.1 Hz, 2H), 8.16 (dd, *J* = 8.7, 1.4 Hz,

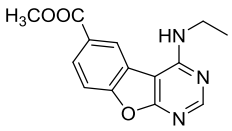
1H), 8.01 (d, *J* = 9.1 Hz, 2H), 7.89 (d, *J* = 8.7 Hz, 1H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.69, 166.46, 156.37, 155.32, 155.07, 146.03, 142.75, 129.51, 126.22, 125.36, 124.95, 122.09, 120.57, 112.34, 99.81, 52.84. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{12}N_4NaO_5$ $[M+Na]^+$ 387.0700, found 387.0699. Purity: 98.831% (by HPLC).

methyl 4-(naphthalen-1-ylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (5r).



White solid; 71% yield; mp: 198.2–198.9℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.60 (s, 1H), 8.38 (s, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 2H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.65 – 7.47 (m, 4H), 3.86 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.71, 166.47, 158.31, 156.77, 154.73, 135.14, 134.52, 130.82, 128.66, 127.56, 126.78, 126.73, 126.16, 125.29, 124.84, 124.06, 121.41, 111.99, 97.16, 52.58. HRMS (ESI-TOF) *m/z* calcd for $C_{22}H_{16}N_3O_3$ $[M+H]^+$ 370.1186, found 370.1184. Purity: 99.512% (by HPLC).

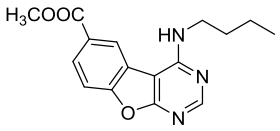
methyl 4-(ethylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (7a).



White solid; 78% yield; mp: 249.4–250.8℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.47 (s, 1H), 8.10 (d, *J* = 7.4 Hz, 2H), 7.81 (d, *J* = 8.6 Hz, 1H), 3.94 (s, 3H), 3.73 – 3.60 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.05, 166.63, 157.66, 157.04, 154.37, 128.19, 126.16, 123.62, 121.68, 111.85, 96.13, 52.69,

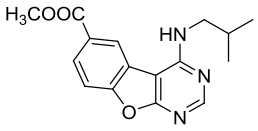
35.98, 15.26. HRMS (ESI-TOF) *m/z* calcd for $C_{14}H_{14}N_3O_3$ $[M+H]^+$ 272.1030, found 272.1030. Purity: 97.575% (by HPLC).

methyl 4-(butylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (7b).



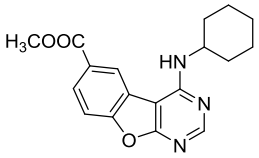
White solid; 63% yield; mp: 189.8–190.3℃;^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (d, *J* = 1.5 Hz, 1H), 8.46 (s, 1H), 8.15 – 8.01 (m, 2H), 7.81 (d, *J* = 8.6 Hz, 1H), 3.94 (s, 3H), 3.63 (dd, *J* = 13.7, 6.7 Hz, 2H), 1.73 – 1.58 (m, 2H), 1.46 – 1.32 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.11, 166.69, 157.86, 157.11, 154.42, 128.29, 126.26, 123.68, 121.75, 111.97, 96.09, 52.75, 31.70, 20.16, 14.30. HRMS (ESI-TOF) *m/z* calcd for $C_{16}H_{18}N_3O_3$ $[M+H]^+$ 300.1343, found 300.1346. Purity: 98.313% (by HPLC).

methyl 4-(isobutylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (7c).



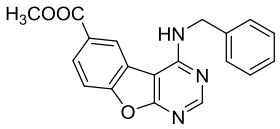
White solid; 64% yield; mp: 140.1–141.4℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (d, *J* = 1.3 Hz, 1H), 8.46 (s, 1H), 8.10 (dd, *J* = 8.6, 1.4 Hz, 2H), 7.81 (d, *J* = 8.6 Hz, 1H), 3.94 (s, 3H), 3.46 (t, *J* = 6.5 Hz, 2H), 2.10 (dp, *J* = 13.6, 6.8 Hz, 1H), 0.95 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.08, 166.67, 157.99, 156.95, 154.39, 128.23, 126.20, 123.71, 121.72, 111.87, 96.01, 52.73, 48.47, 28.26, 20.63. HRMS (ESI-TOF) *m/z* calcd for $C_{16}H_{18}N_3O_3$ $[M+H]^+$ 300.1343, found 300.1353. Purity: 99.989% (by HPLC).

methyl 4-(cyclohexylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (7d).



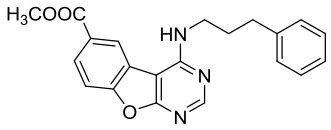
White solid; 69% yield; mp: 126.7–127.3℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.46 (s, 1H), 8.09 (dd, *J* = 8.6, 0.5 Hz, 1H), 7.80 (d, *J* = 8.6 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 4.31 (ddd, *J* = 11.4, 9.6, 6.0 Hz, 1H), 3.94 (s, 3H), 1.96 (d, *J* = 10.5 Hz, 2H), 1.81 (d, *J* = 13.0 Hz, 2H), 1.68 (d, *J* = 12.5 Hz, 1H), 1.59 (qd, *J* = 12.5, 3.1 Hz, 2H), 1.37 (q, *J* = 12.9 Hz, 2H), 1.25 – 1.13 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.22, 166.74, 157.23, 157.00, 154.38, 128.28, 126.10, 124.14, 121.69, 111.86, 96.07, 52.75, 50.44, 32.49, 25.80, 25.69. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{20}N_3O_3$ $[M+H]^+$ 326.1499, found 326.1496. Purity: 99.095% (by HPLC).

methyl 4-(benzylamino)benzofuro[2,3-d]pyrimidine-6-carboxylate (7e).



White solid; 74% yield; mp: 198.8–199.5℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.64 (t, *J* = 5.8 Hz, 1H), 8.46 (s, 1H), 8.12 (dt, *J* = 8.6, 1.9 Hz, 1H), 7.84 (dd, *J* = 8.6, 2.6 Hz, 1H), 7.40 (d, *J* = 7.2 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.24 (t, *J* = 7.2 Hz, 1H), 4.89 (d, *J* = 6.0 Hz, 2H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.15, 166.64, 157.79, 157.05, 154.51, 140.09, 128.76, 128.41, 127.53, 127.20, 126.29, 123.79, 121.62, 111.99, 96.33, 52.73, 44.17. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{16}N_3O_3$ $[M+H]^+$ 334.1186, found 334.1187. Purity: 98.536% (by HPLC).

methyl 4-((3-phenylpropyl)amino)benzofuro[2,3-d]pyrimidine-6-carboxylate (7f).



White solid; 77% yield; mp: 178.5–179.4℃;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.47 (s, 1H), 8.08 (dd, J = 12.5, 3.8 Hz, 2H), 7.80 (d, J = 8.6 Hz, 1H), 7.33 – 7.23 (m, 4H), 7.17 (dd, J = 9.3, 4.3 Hz, 1H), 3.94 (s, 3H), 3.65 (dd, J = 13.9, 6.4 Hz, 2H), 2.71 (t, J = 7.6 Hz, 2H), 2.06 – 1.95 (m, 2H). 13C NMR (100 MHz, DMSO-d_6) δ 169.08, 166.67, 157.85, 157.07, 154.41, 142.19, 128.77, 128.76, 128.28, 126.22, 126.20, 123.72, 121.72, 111.94, 96.19, 52.74, 40.97, 33.18, 31.15. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{20}N_3O_3$ $[M+H]^+$ 362.1499, found 362.1495. Purity: 99.863% (by HPLC).

8. X-ray crystal structures

To ascertain the structural correctness of these products, and exclude the possible yielding of the corresponding regio-isomers, a crystallizing form of **5a** was obtained and the structure was undisputedly confirmed by single crystal X-ray analysis.

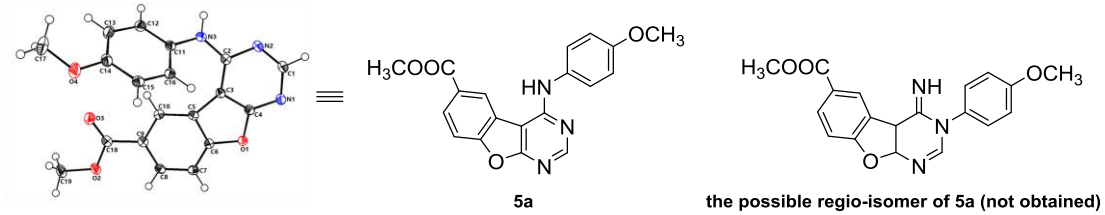
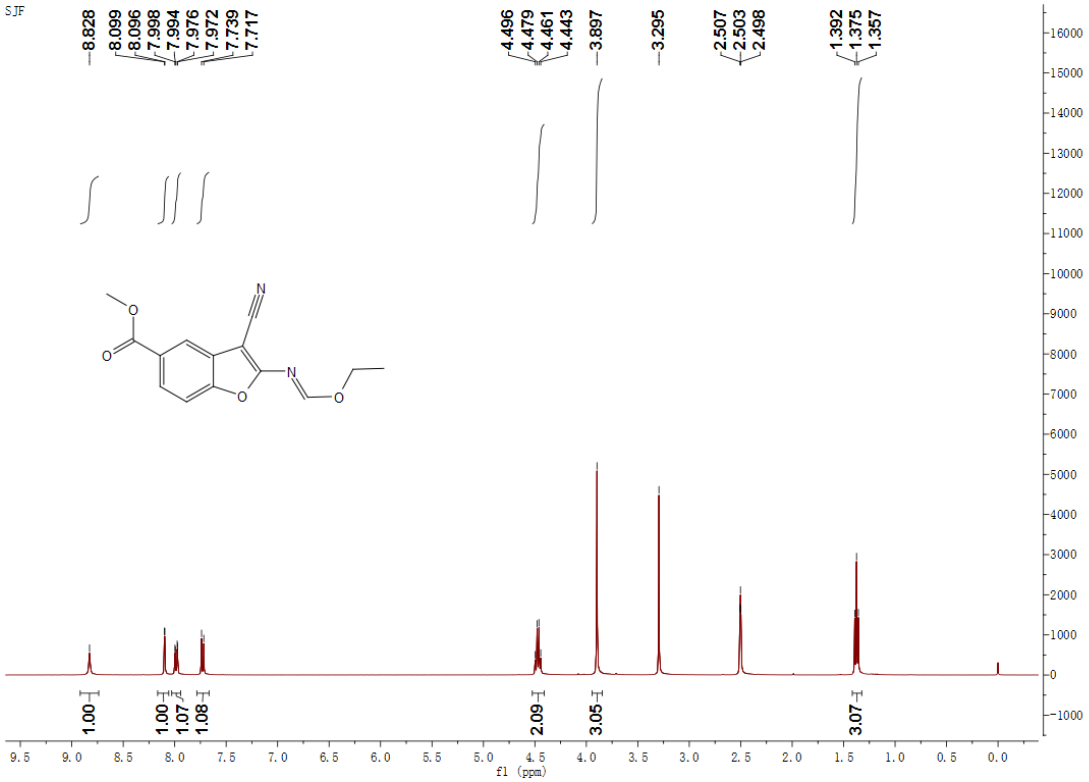


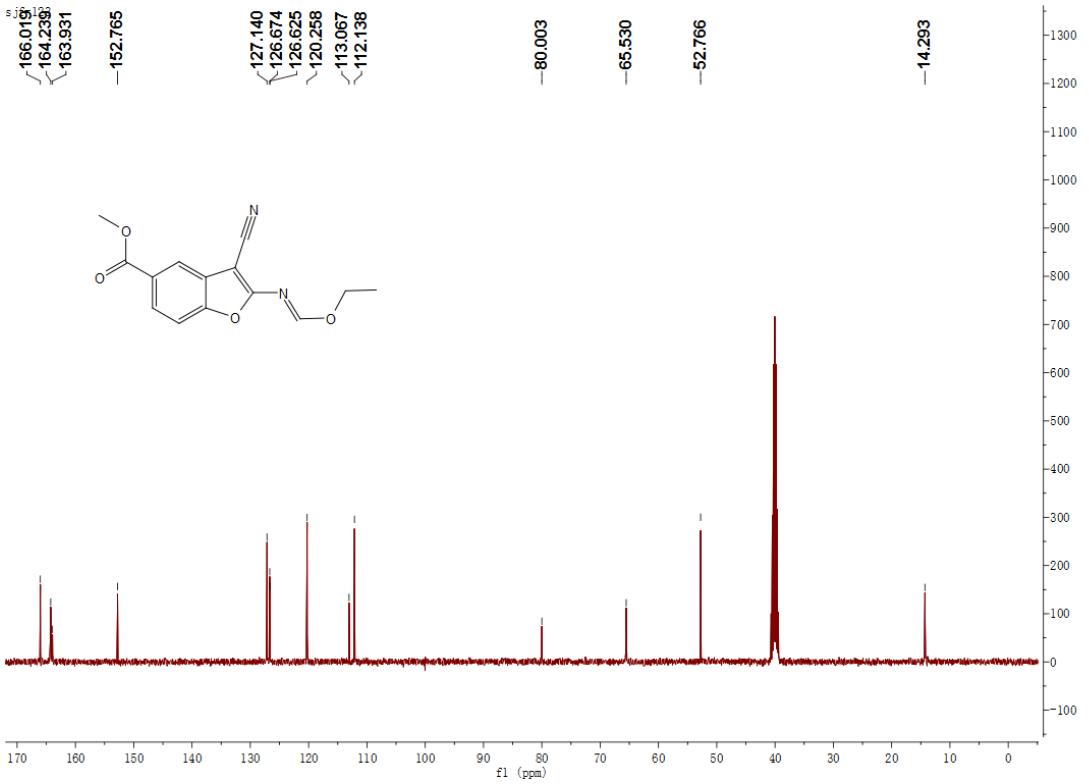
Fig. S1 X-ray single crystal structure of 5a and the possible regio-isomer of 5a.

Empirical formula	$C_{19}H_{15}N_3O_4$
Formula weight	349.35
Temperature/K	100
Crystal system	triclinic
Space group	P-1
a/Å	7.7171(4)
b/Å	10.6392(6)
c/Å	11.0090(4)
α/°	110.700(5)
β/°	100.085(4)
γ/°	101.913(5)
Volume/Å^3	796.35(8)
Z	2
ρ_{calc}g/cm^3	1.4568
μ/mm^{-1}	0.867
F(000)	365.3
Crystal size/mm^3	0.3 × 0.2 × 0.1
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	9.94 to 134.14
Index ranges	$-8 \le h \le 9$, $-13 \le k \le 12$, $-13 \le l \le 9$
Reflections collected	6140
Independent reflections	2817 [R_{int} = 0.0252, R_{sigma} = 0.0270]
Data/restraints/parameters	2817/0/236
Goodness-of-fit on F^2	1.049
Final R indexes [I>=2σ (I)]	R_1 = 0.0385, wR_2 = 0.1025
Final R indexes [all data]	R_1 = 0.0412, wR_2 = 0.1054
Largest diff. peak/hole / e Å^{-3}	0.42/-0.49

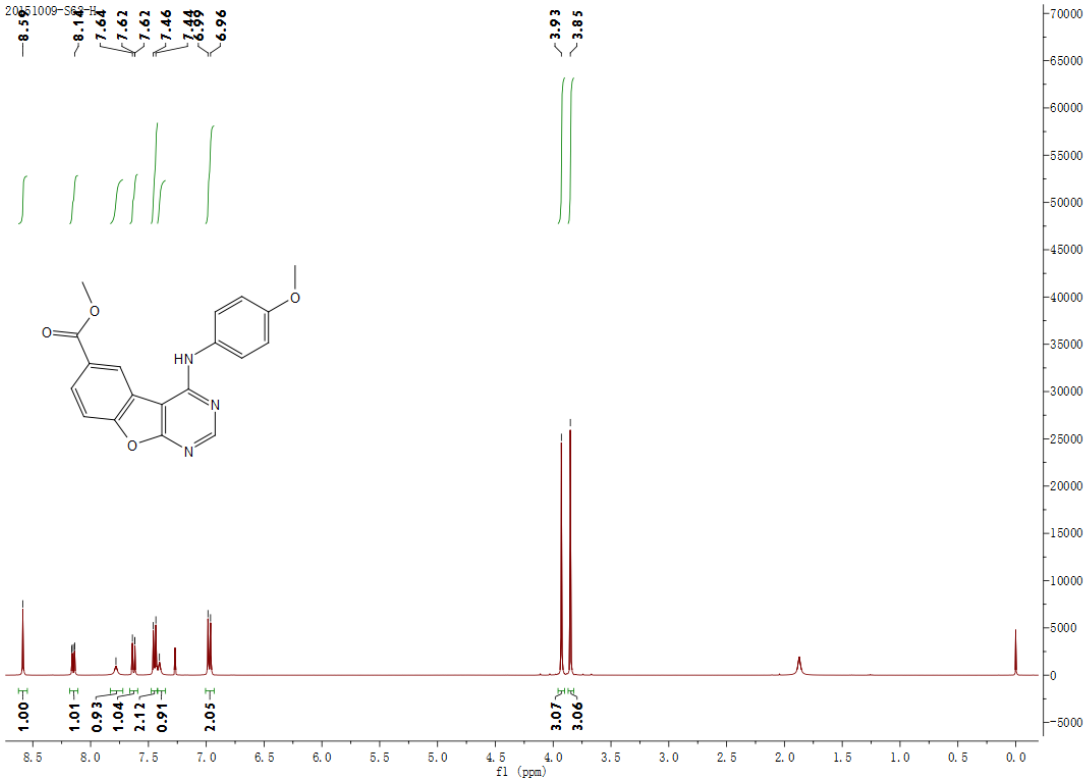
9. Copies of ^{1}H- and ^{13}C-NMR

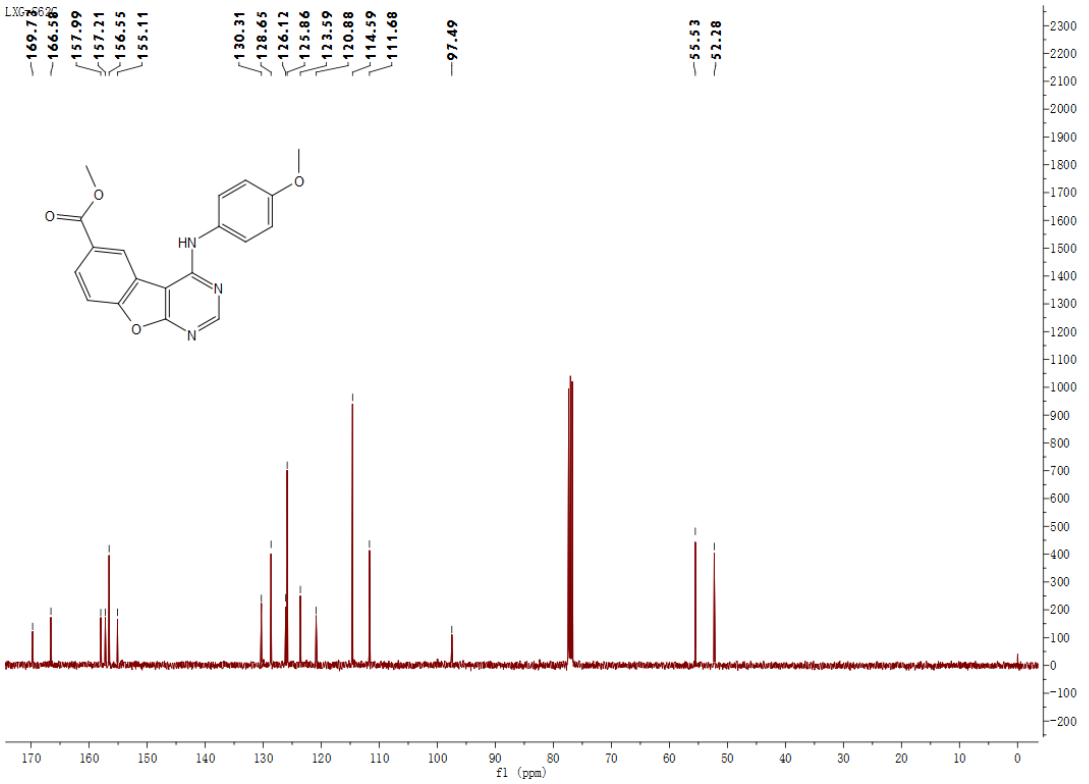
^{1}H and ^{13}C spectra of Intermediate A



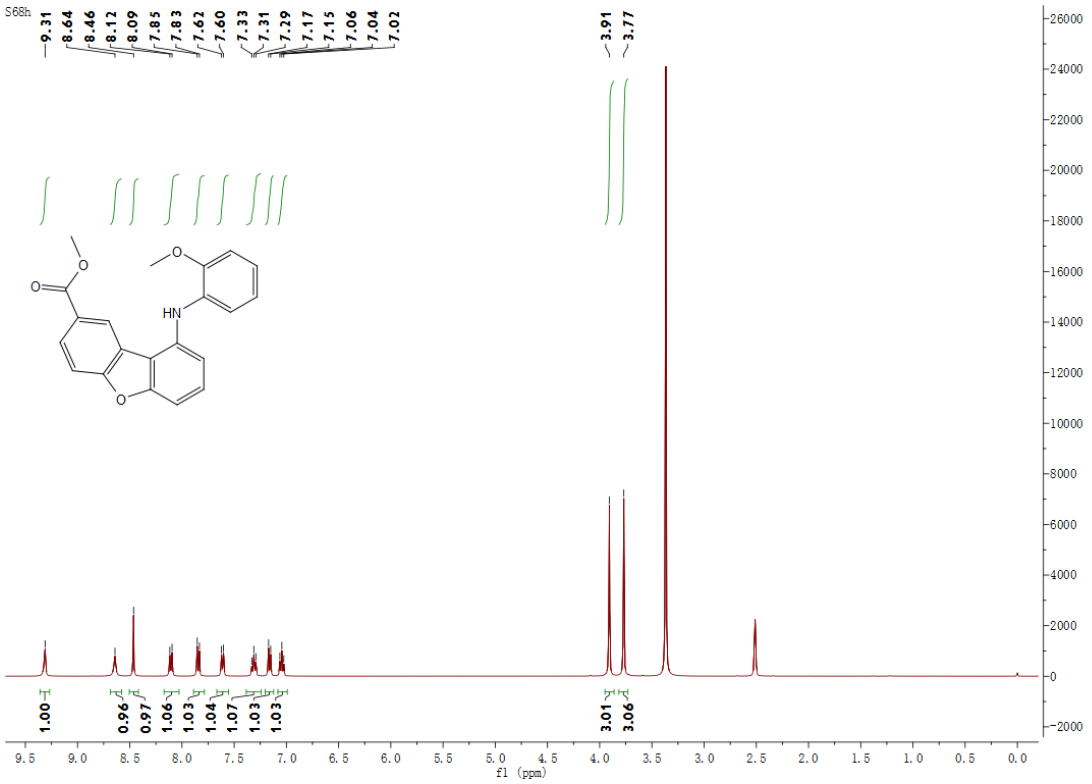


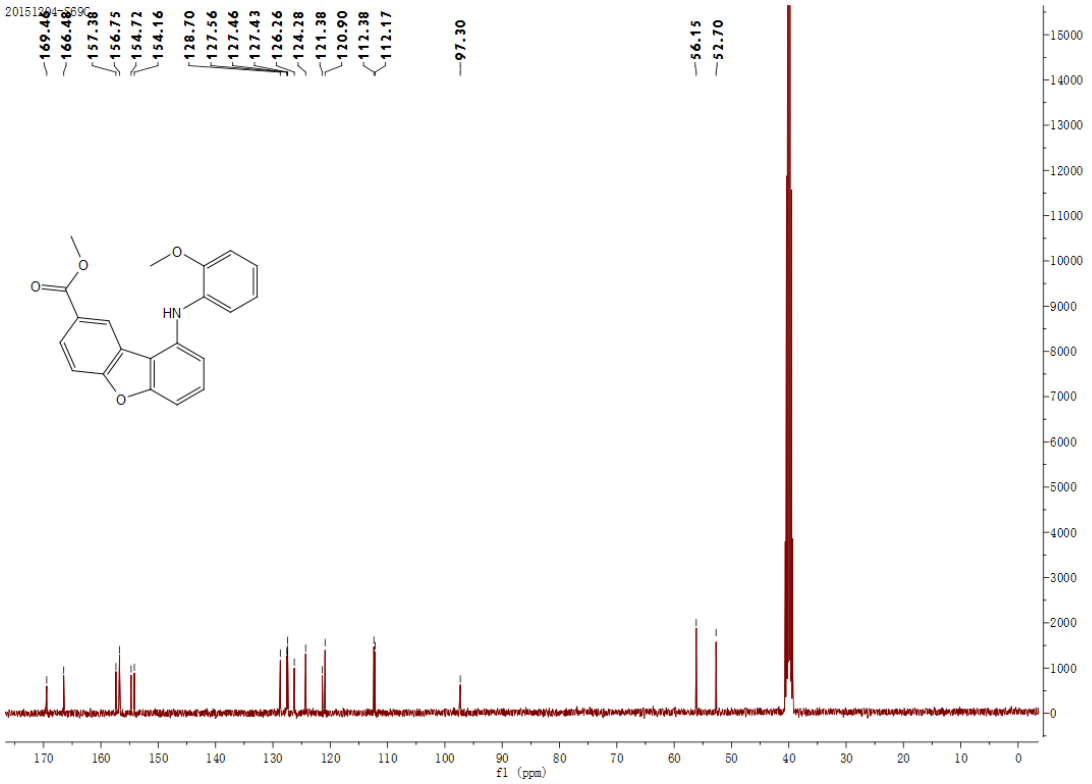
^{1}H and ^{13}C spectra of 5a



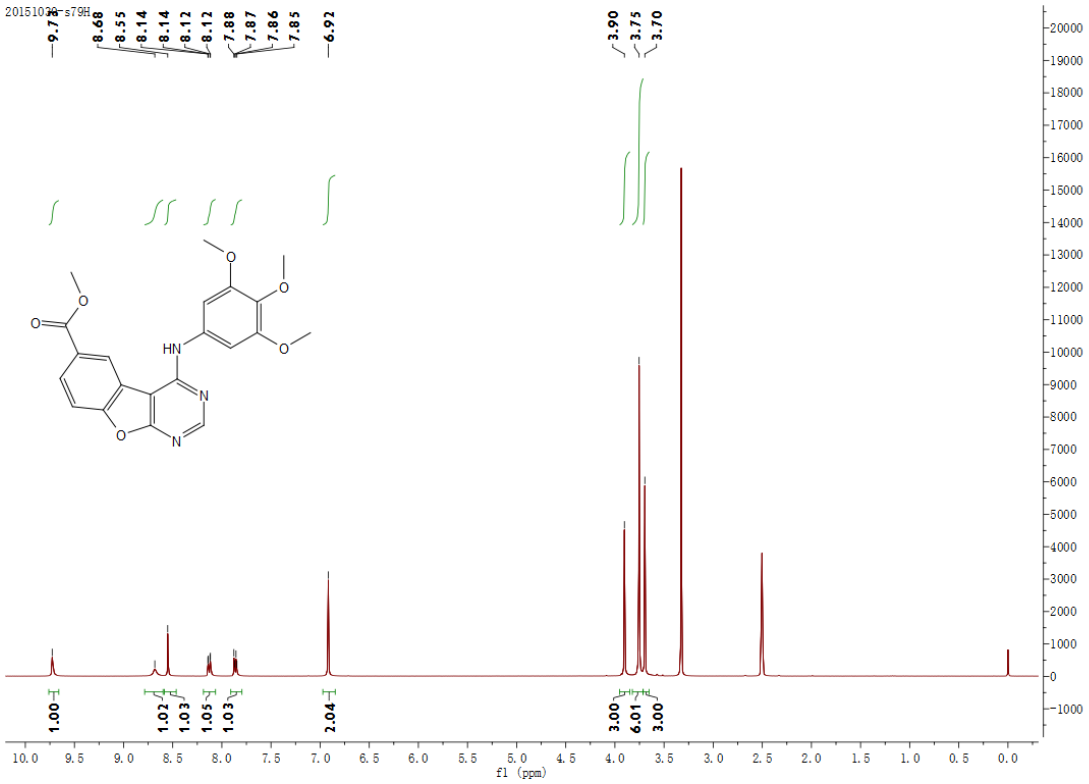


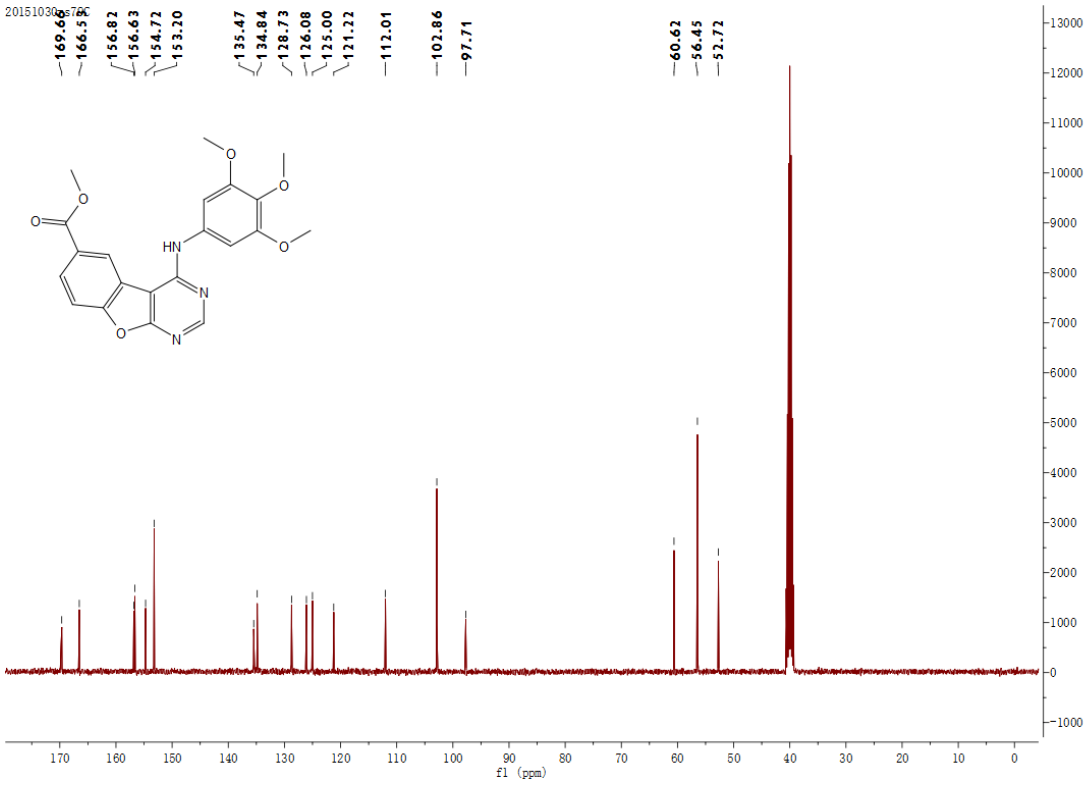
^{1}H and ^{13}C spectra of 5b



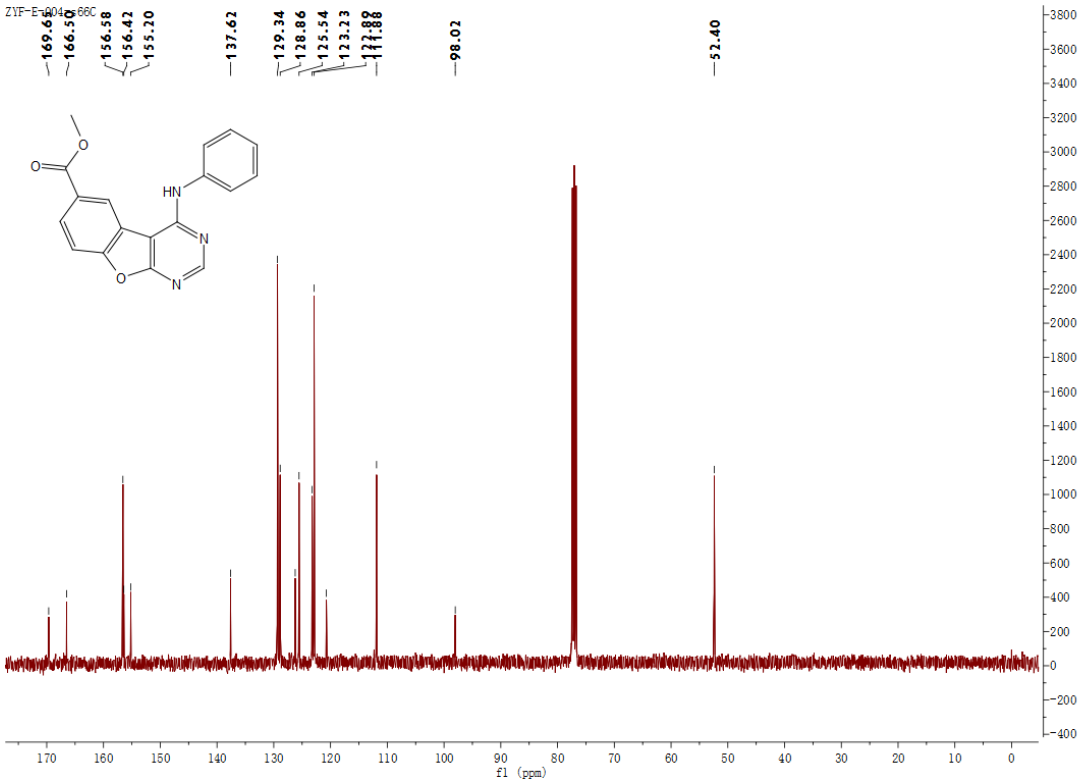


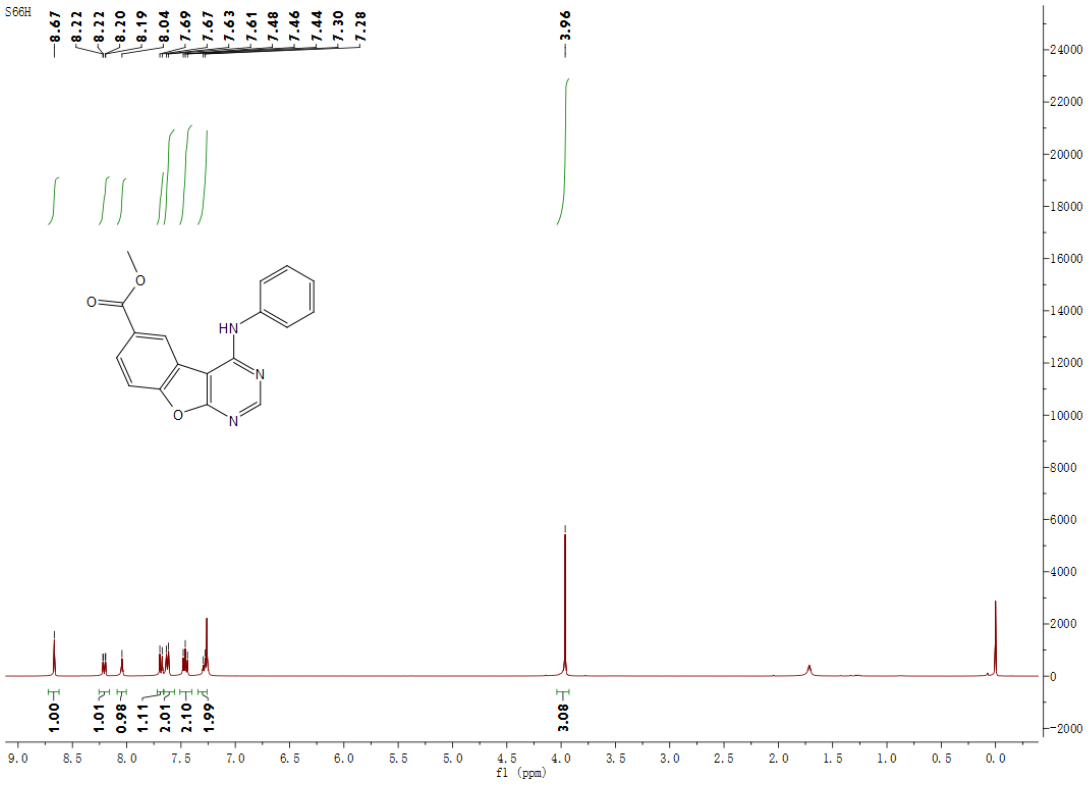
^{1}H and ^{13}C spectra of 5c


9.73
8.68
8.55
8.14
8.14
8.12
8.12
7.88
7.87
7.86
7.85
6.92
3.90
3.75
3.70
1.00
1.02
1.03
1.05
1.03
2.04
3.00
6.01
3.00
f1 (ppm)

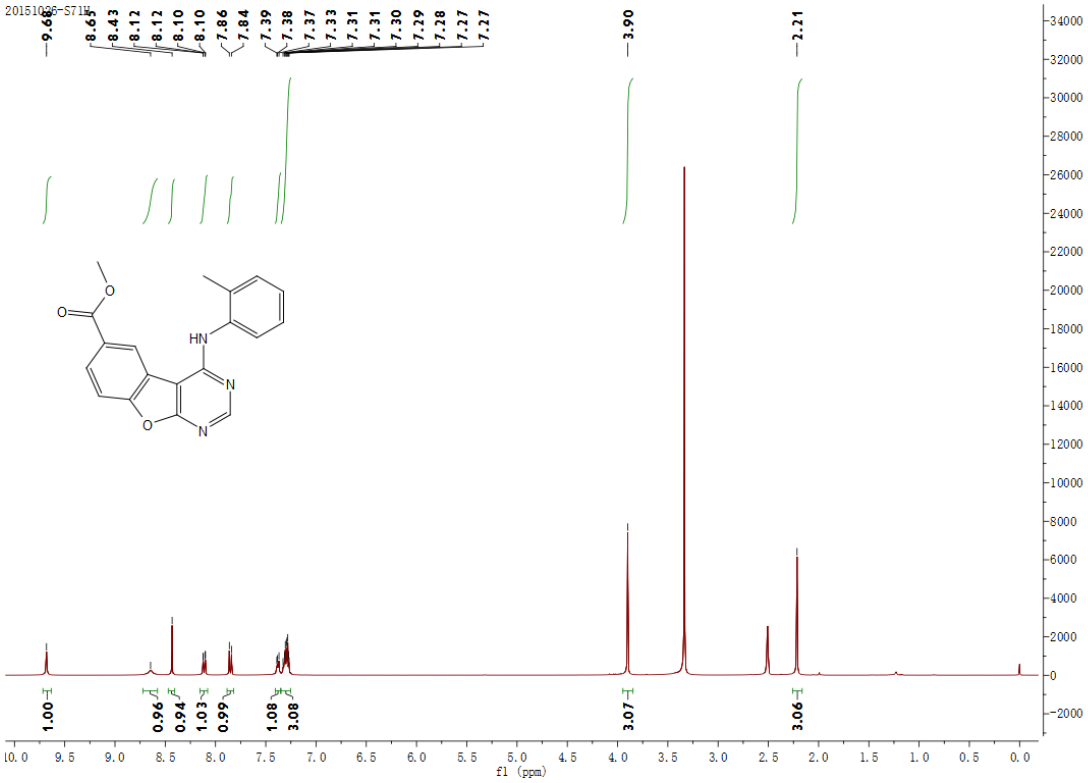

169.66
166.59
156.82
156.63
154.72
153.20
135.47
134.84
128.73
126.08
125.00
121.22
112.01
102.86
97.71
60.62
56.45
52.72
f1 (ppm)

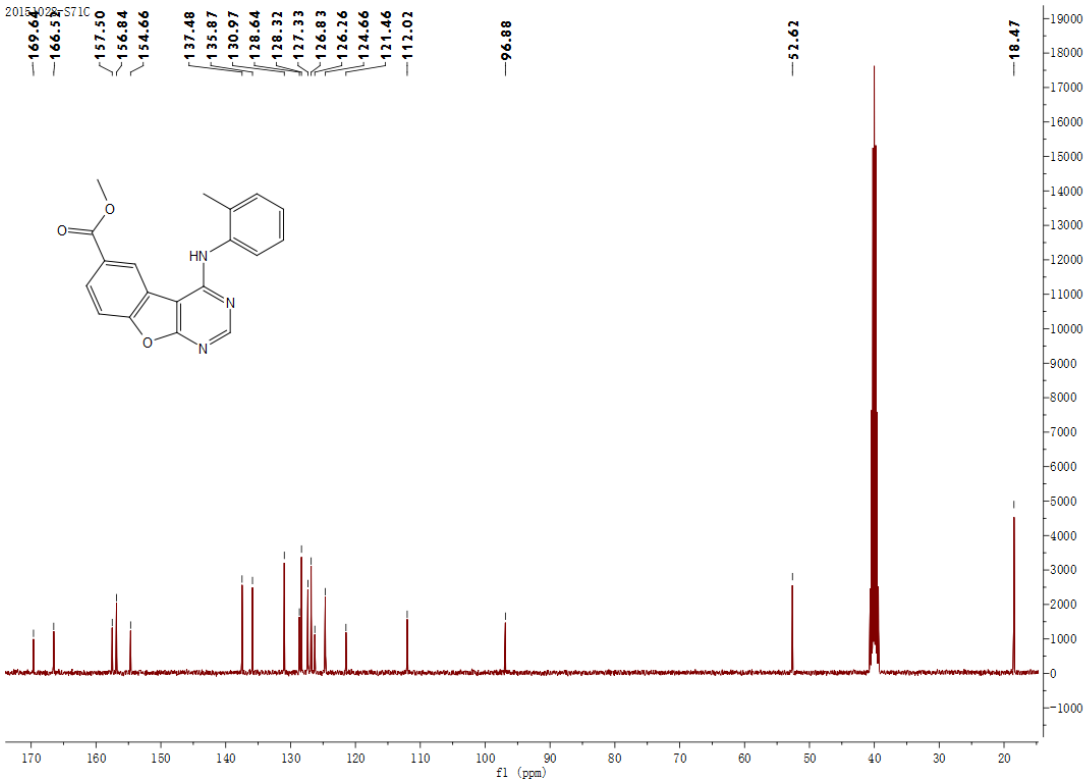
^{1}H and ^{13}C spectra of 5d



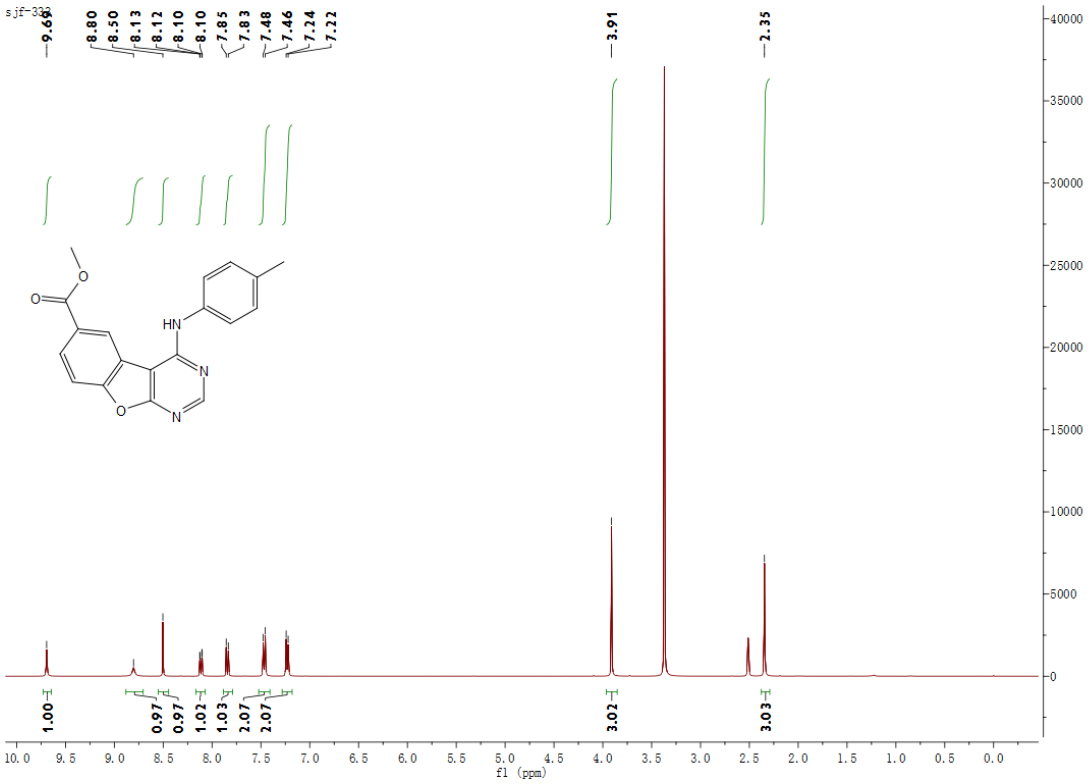


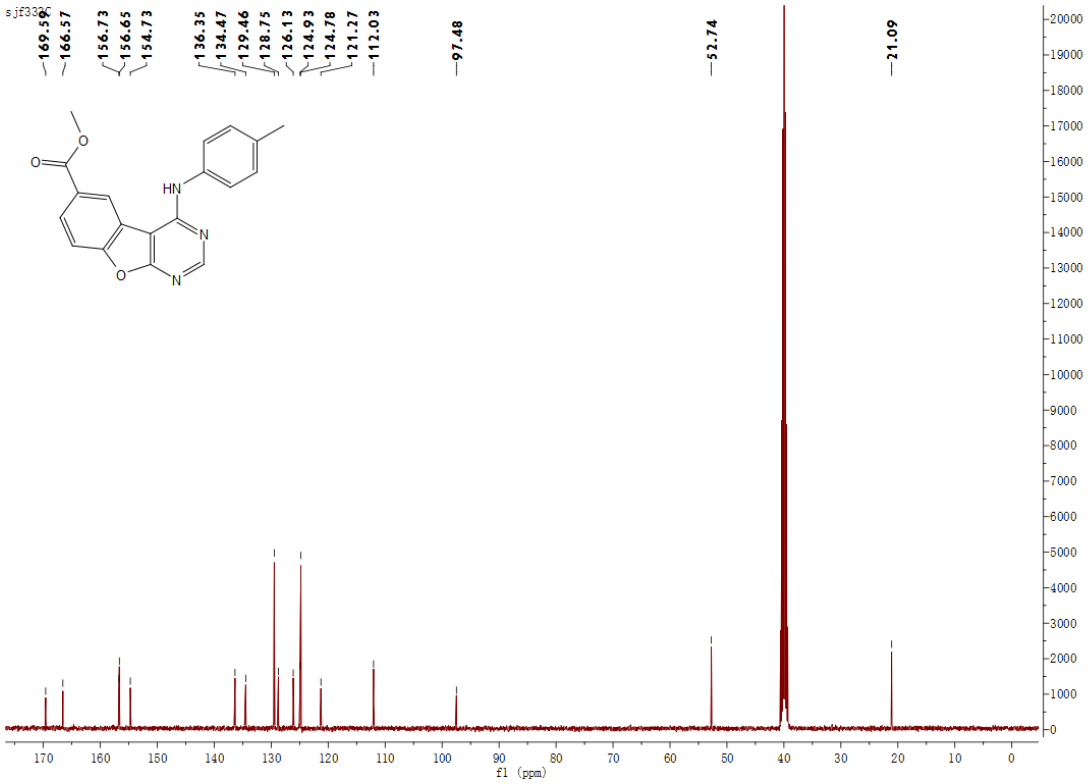
^{1}H and ^{13}C spectra of 5e



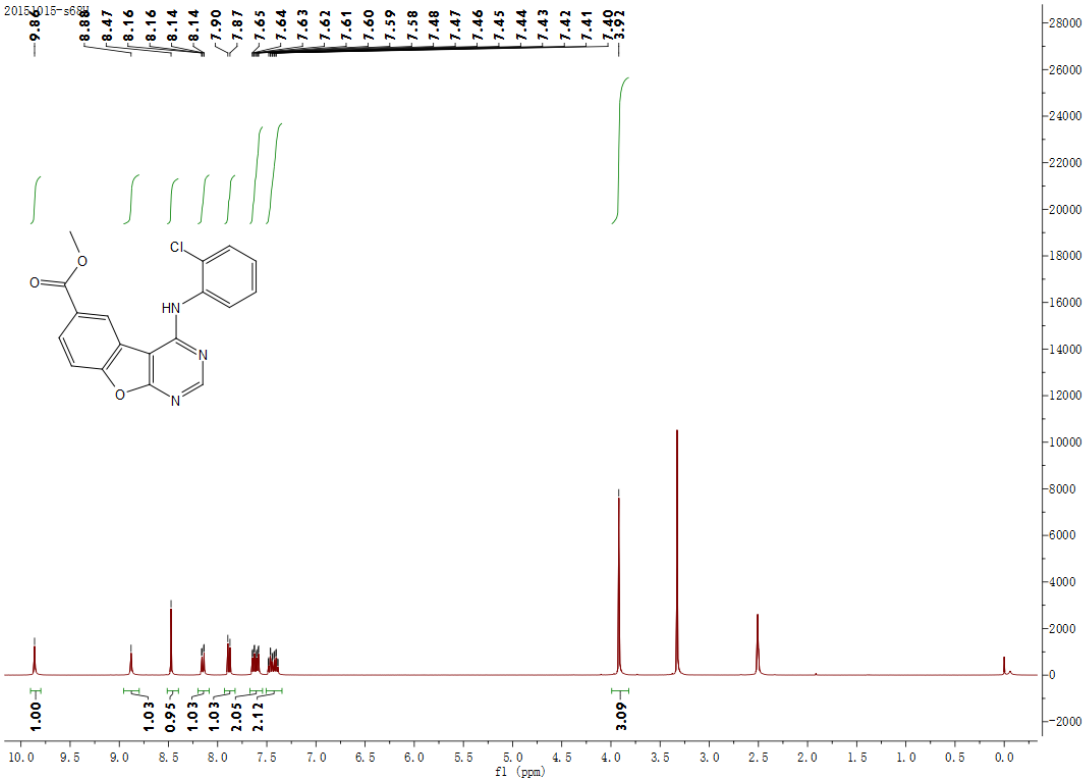


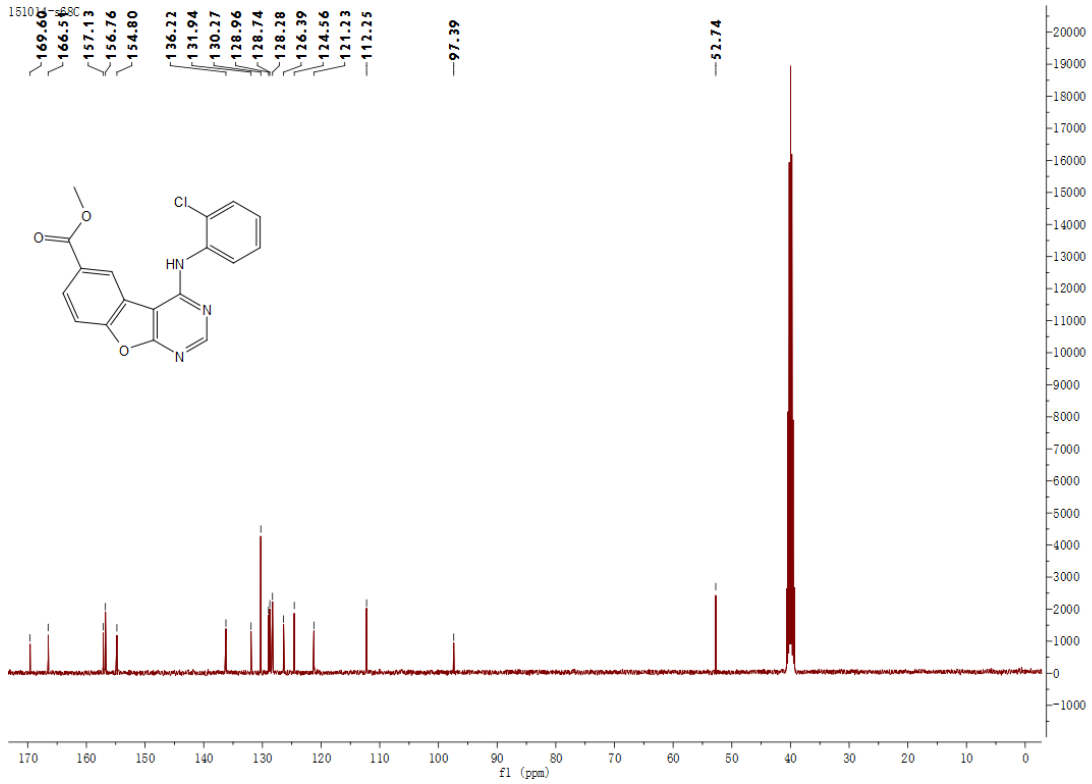
^{1}H and ^{13}C spectra of 4f



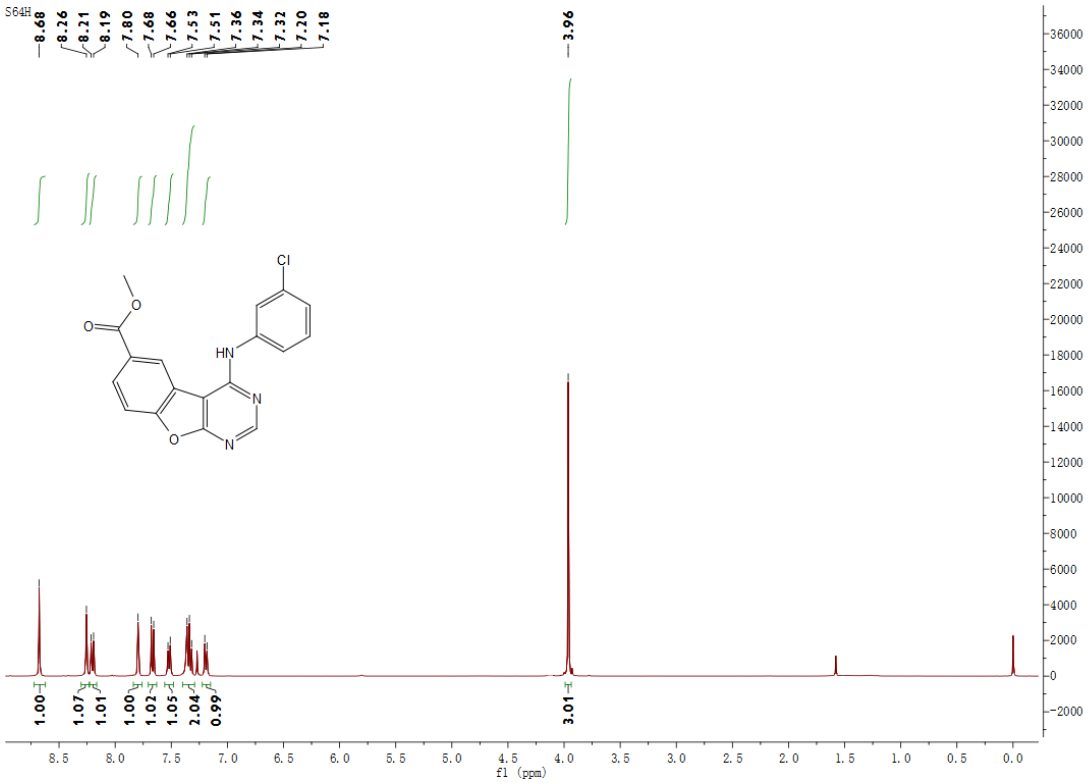


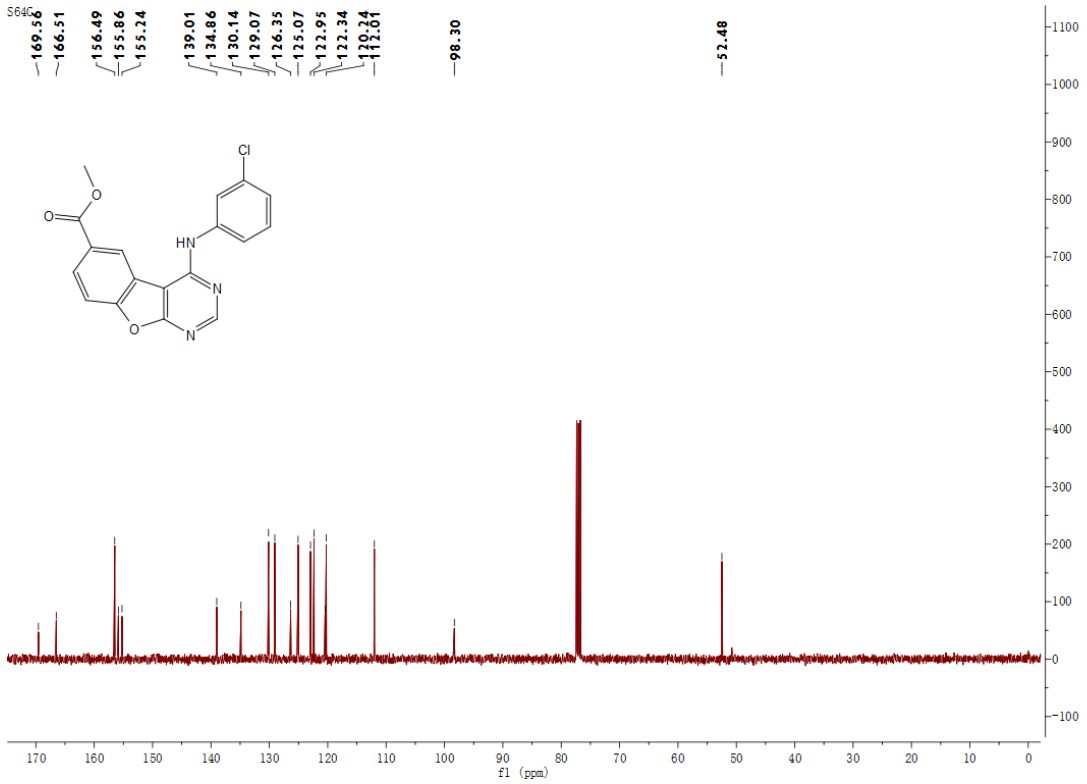
^{1}H and ^{13}C spectra of 5g



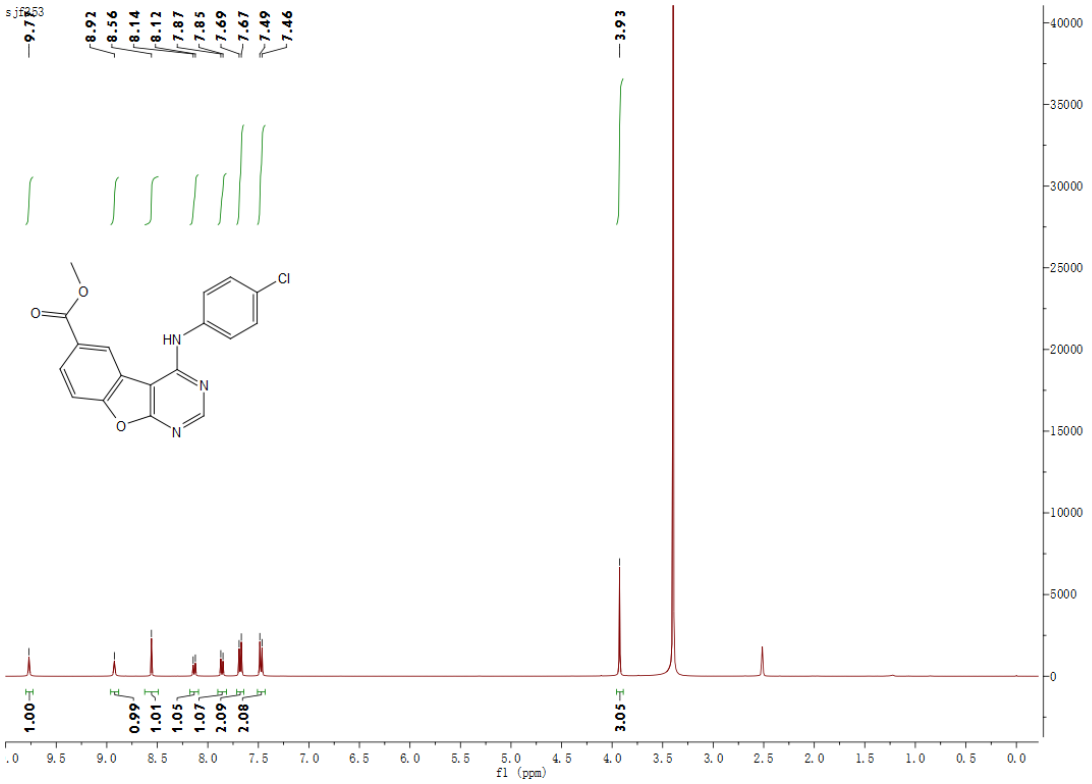


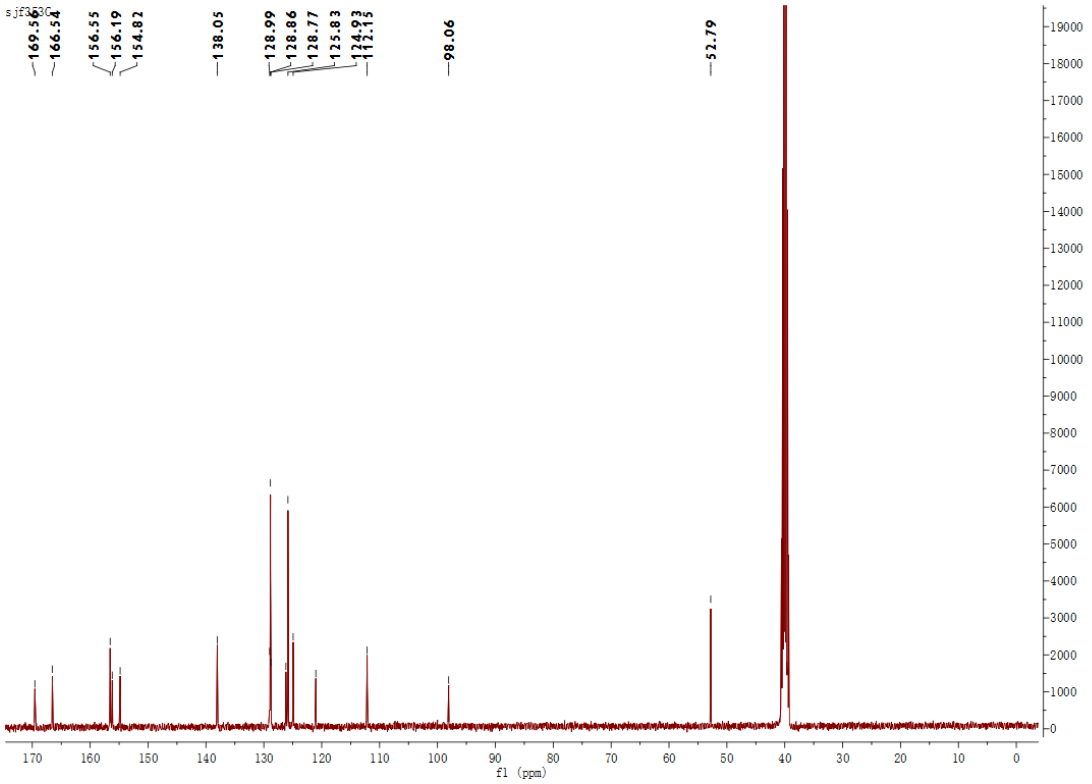
^{1}H and ^{13}C spectra of 5h



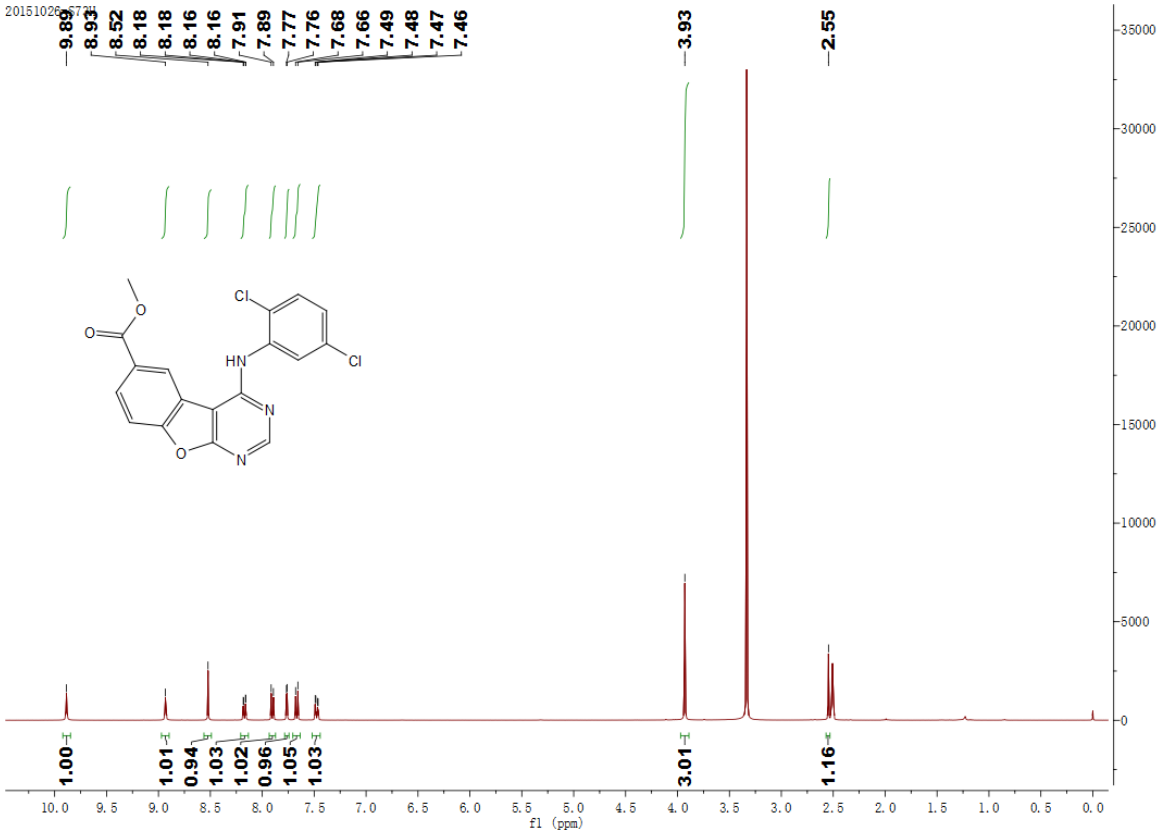


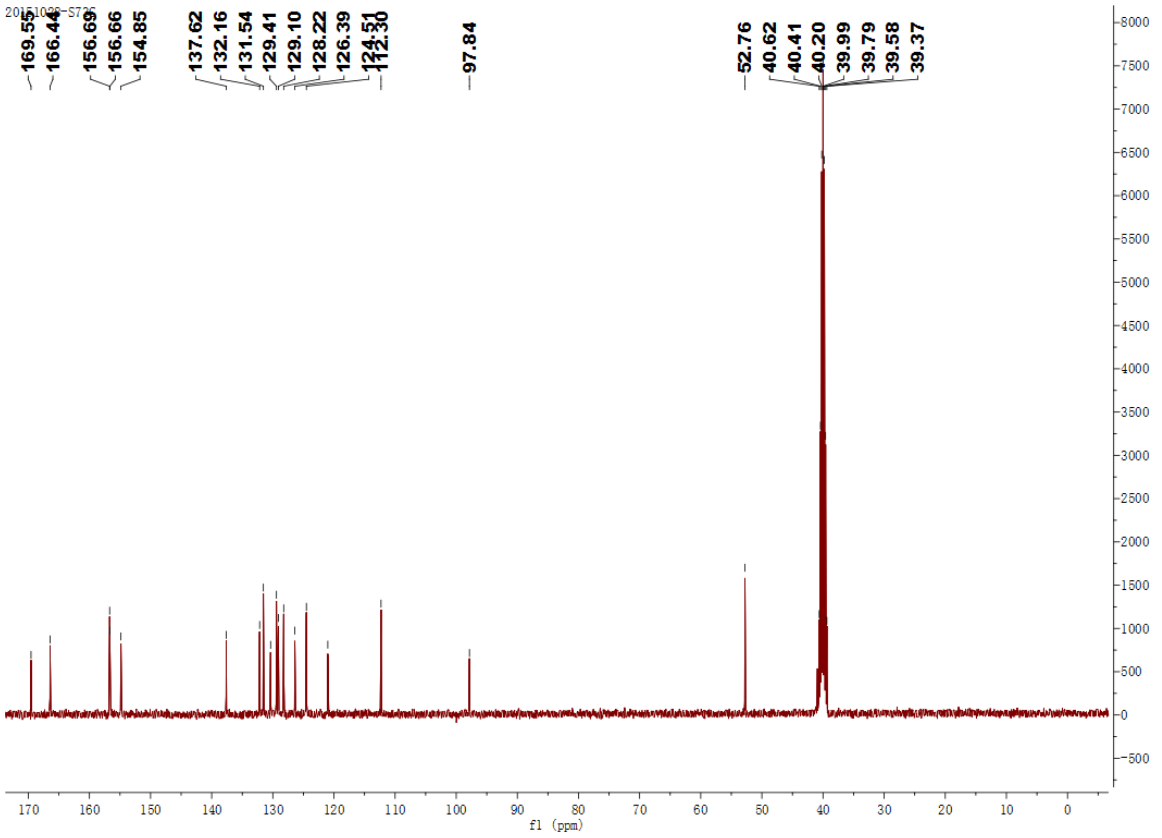
^{1}H and ^{13}C spectra of 5i



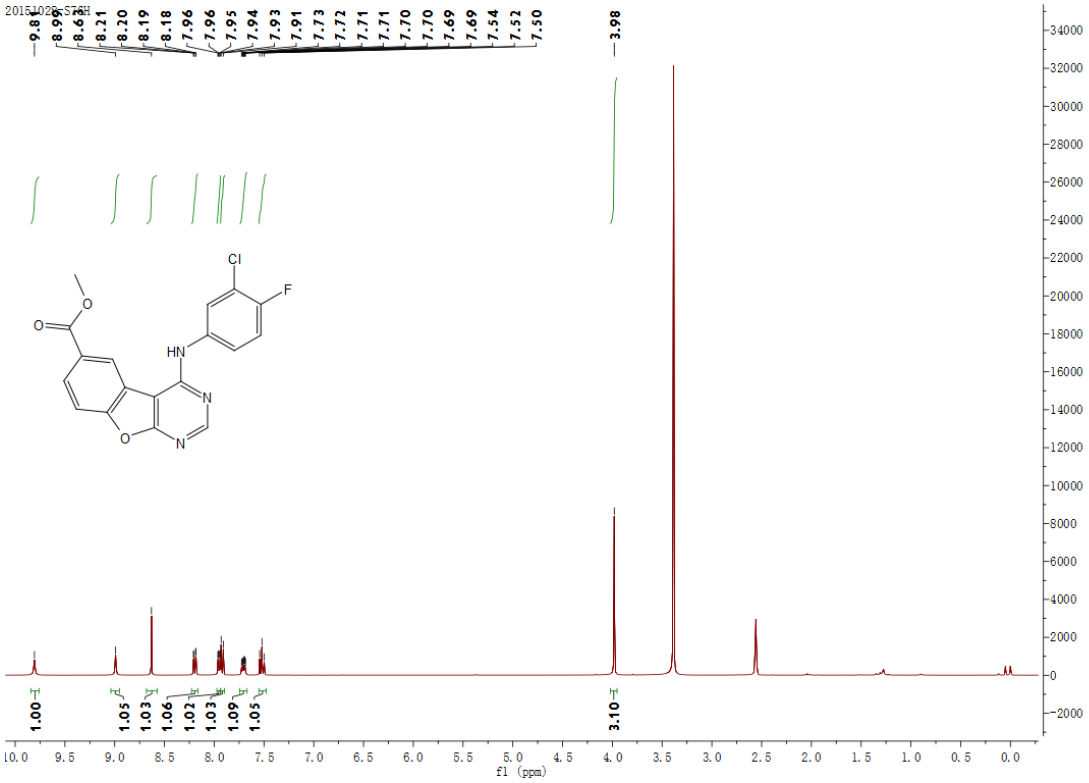


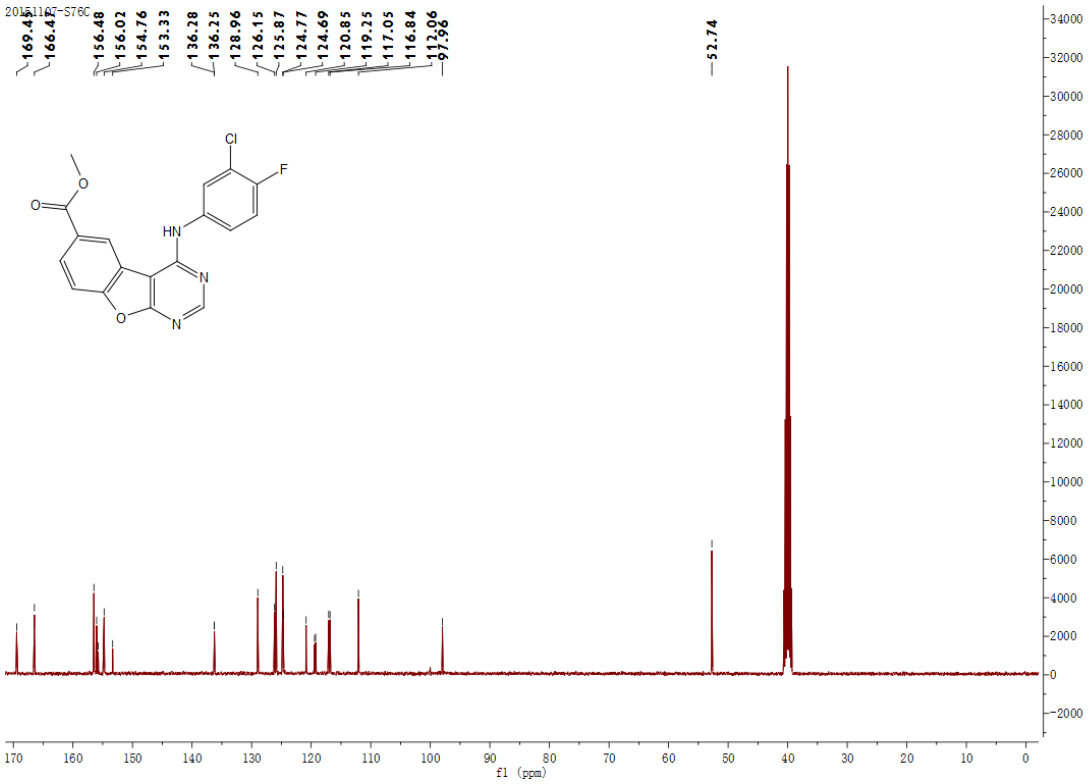
^{1}H and ^{13}C spectra of 5j



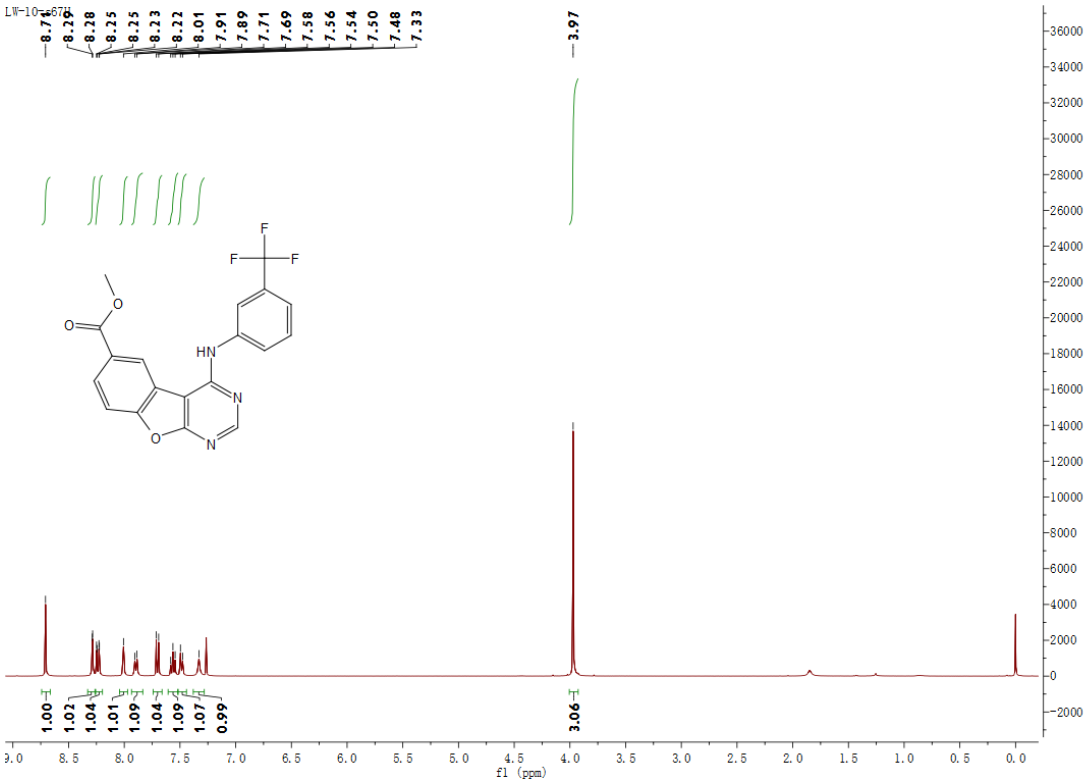


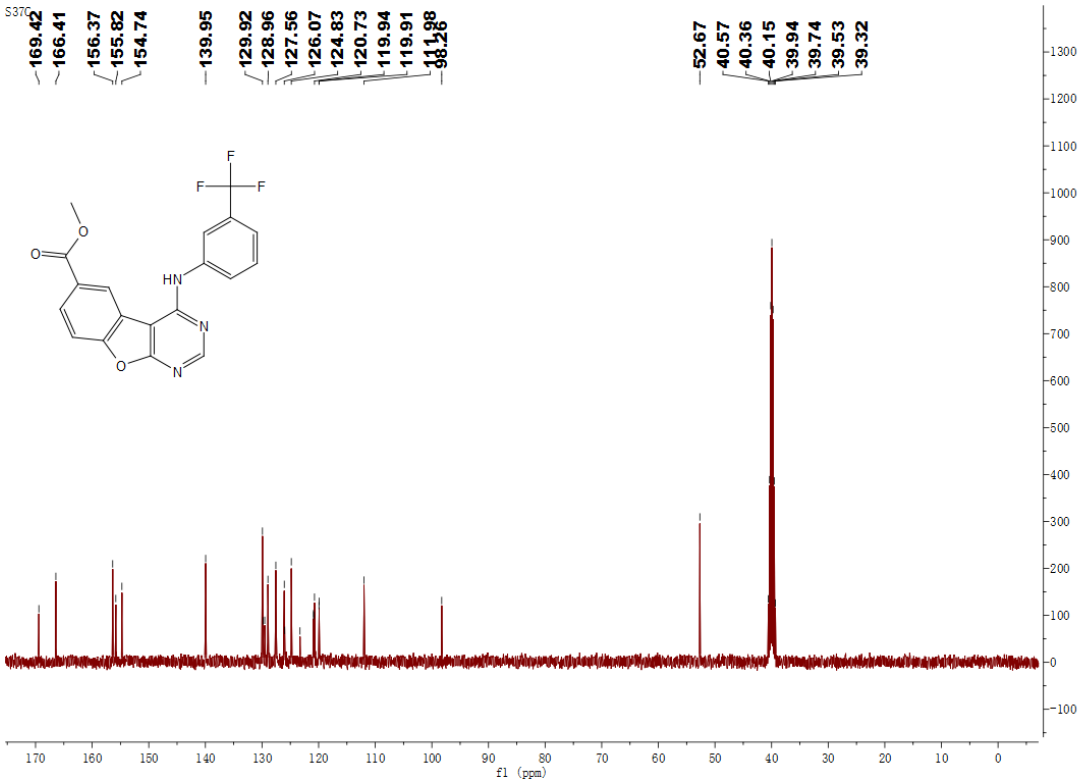
^{1}H and ^{13}C spectra of 5k



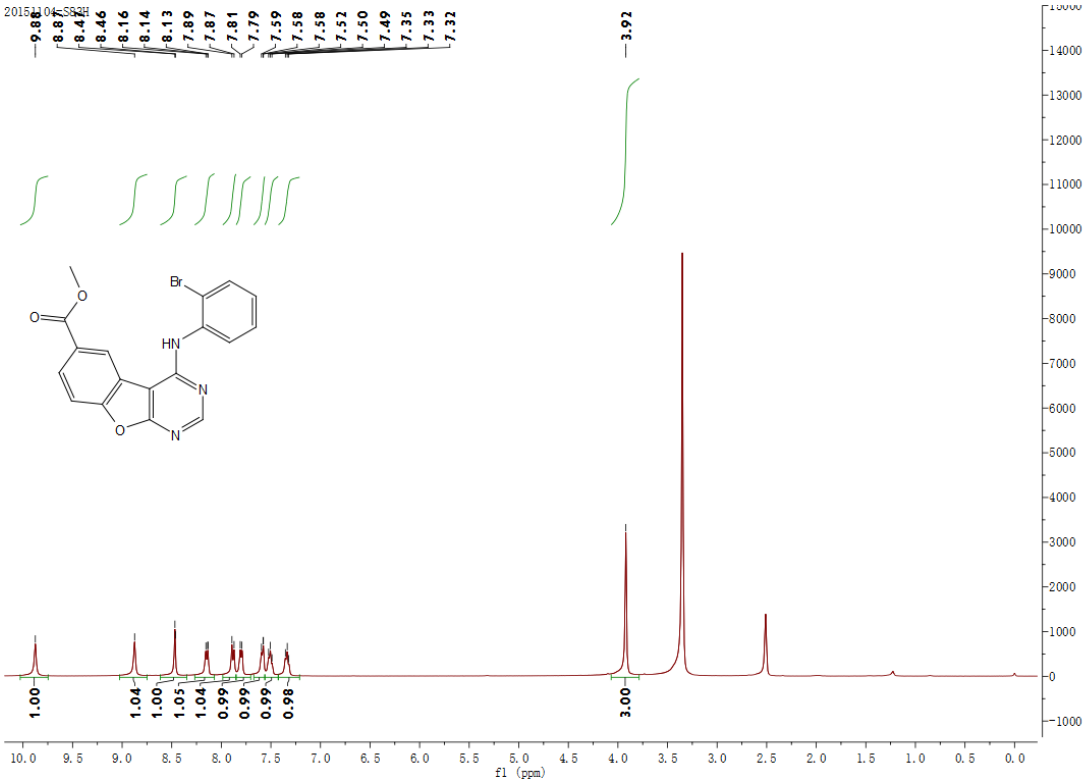


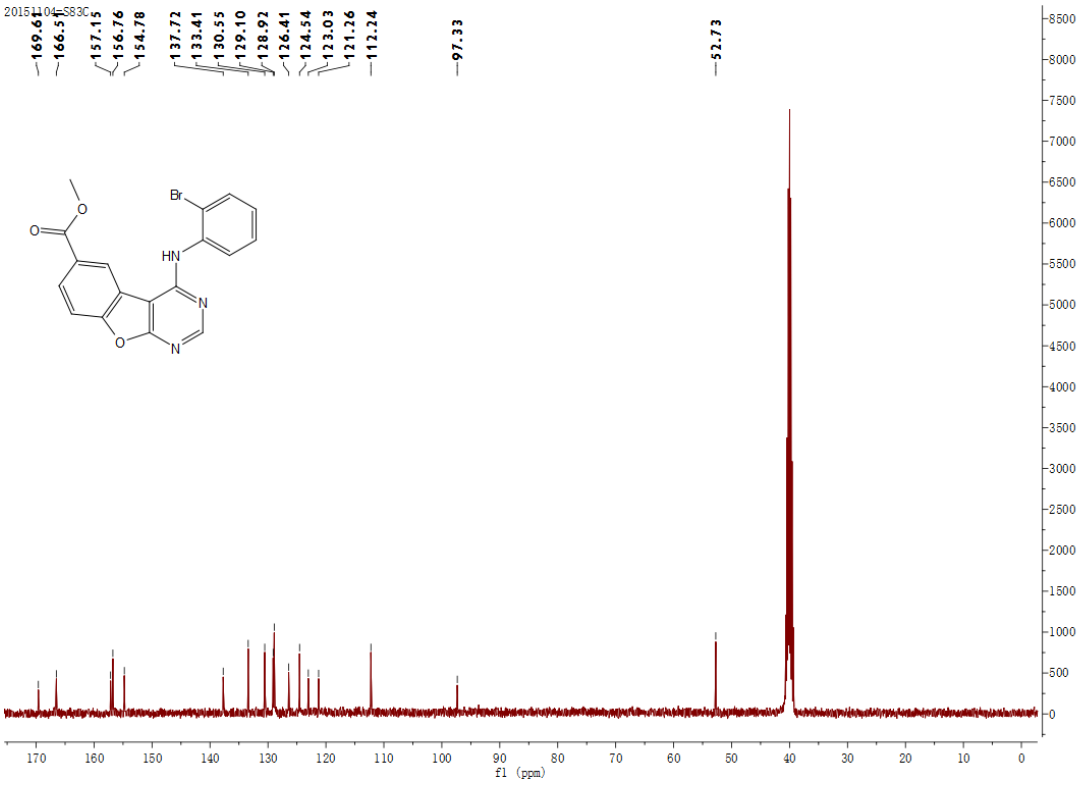
^{1}H and ^{13}C spectra of 5l



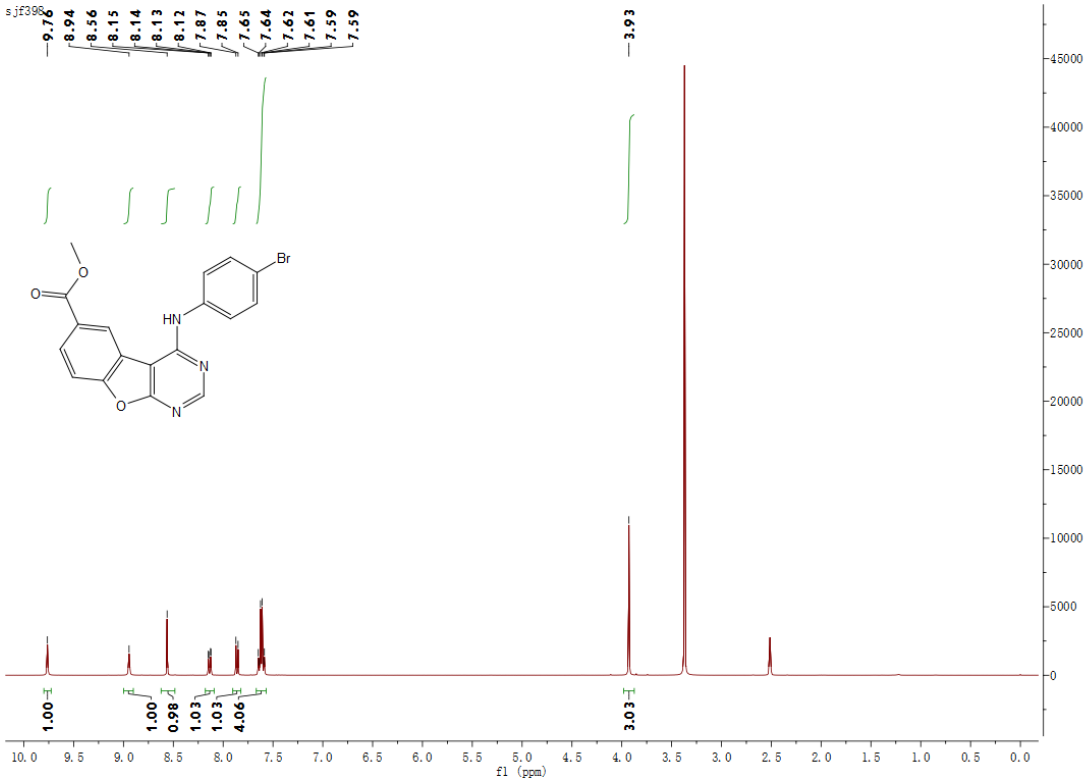


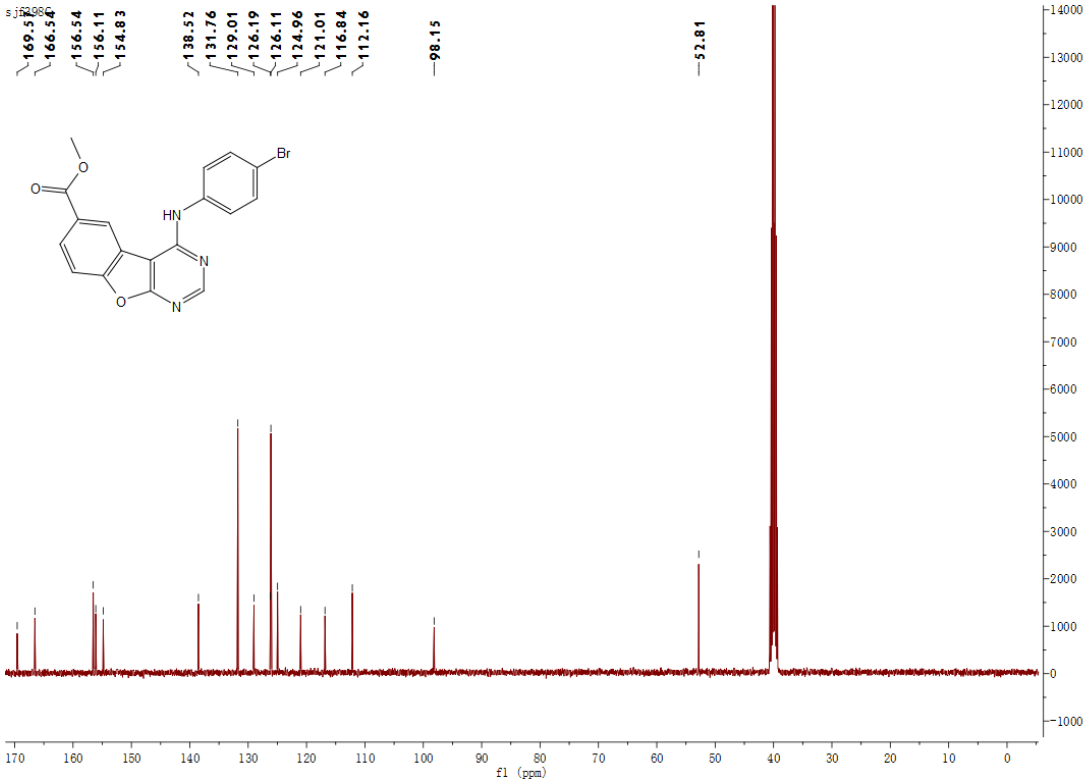
^{1}H and ^{13}C spectra of 5m



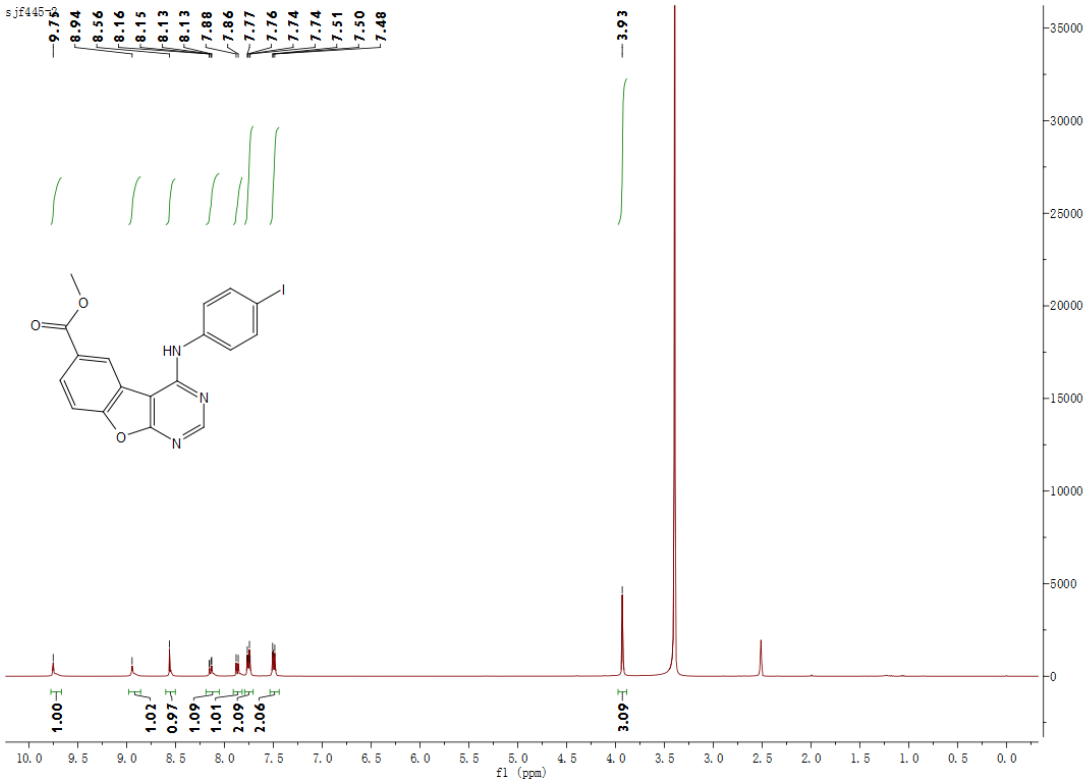


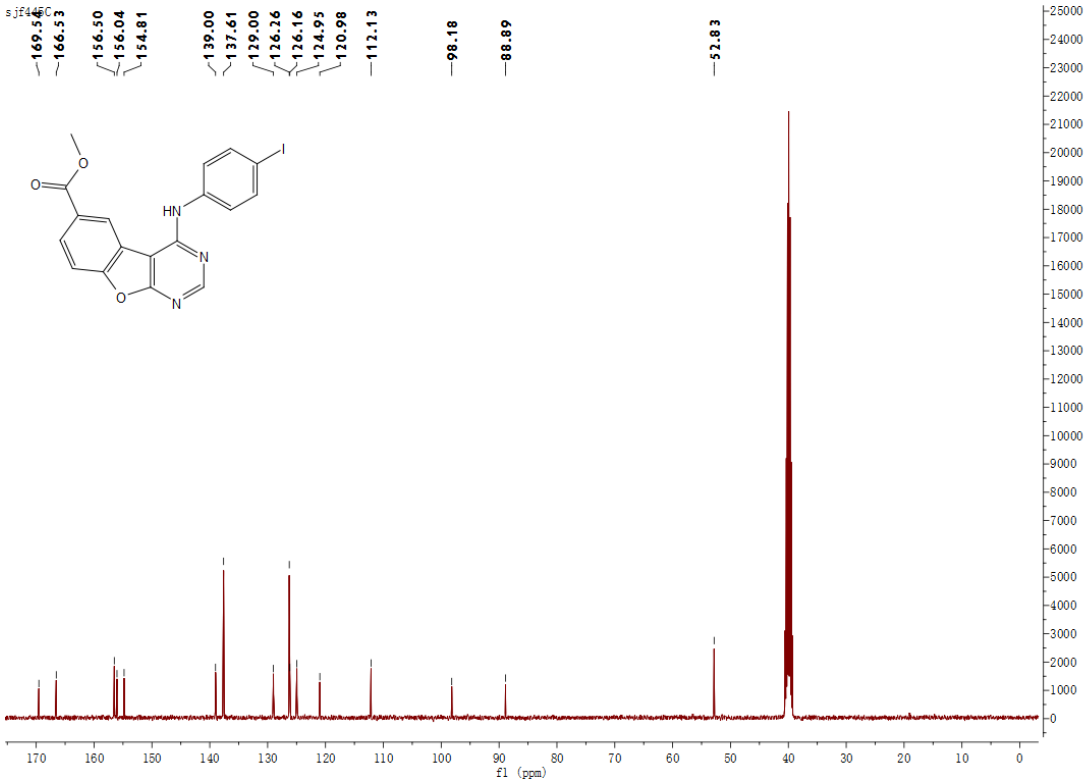
^{1}H and ^{13}C spectra of 5n



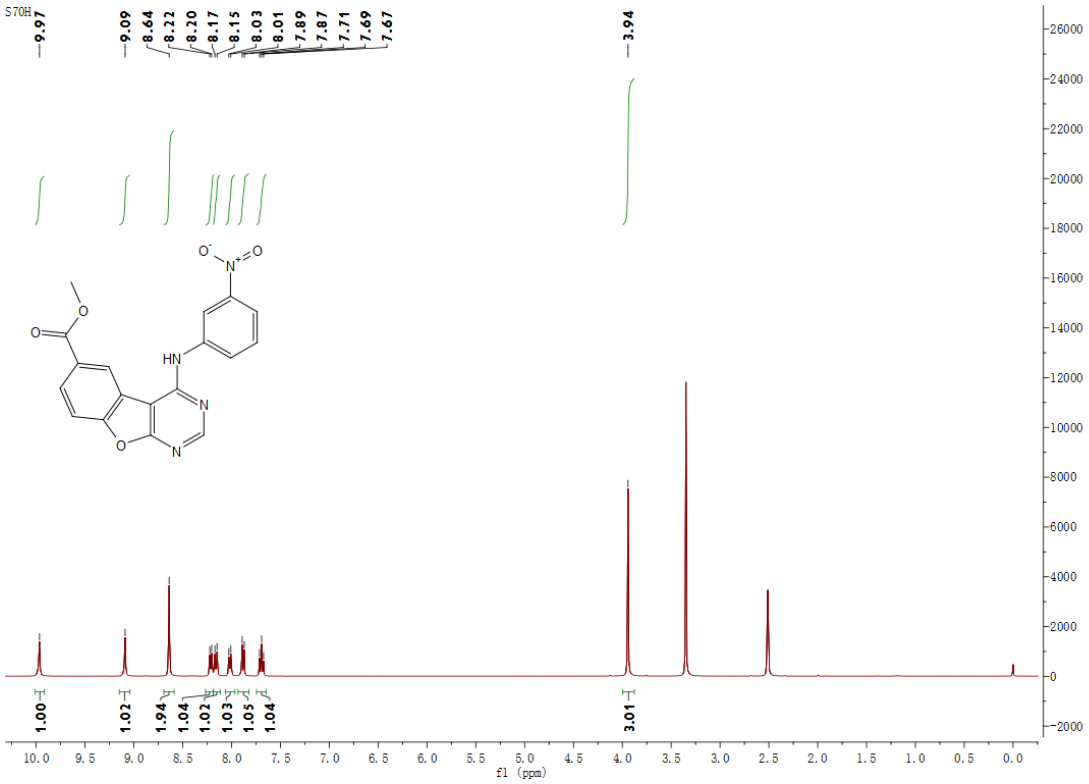


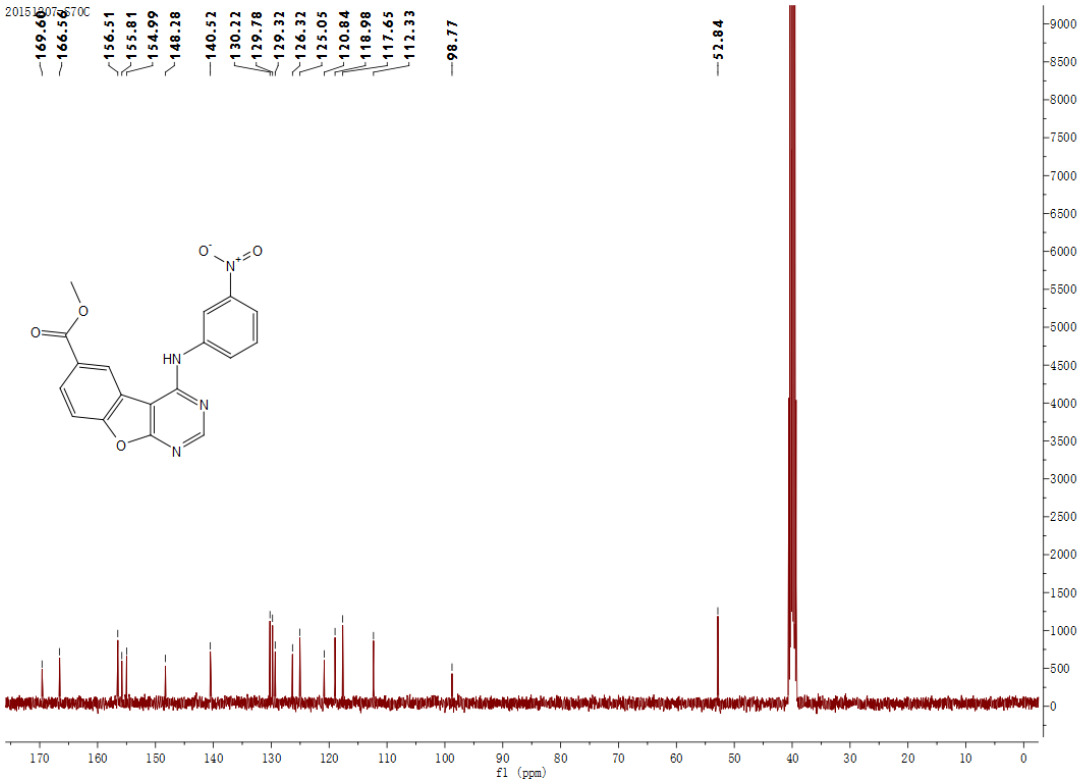
^{1}H and ^{13}C spectra of 5o



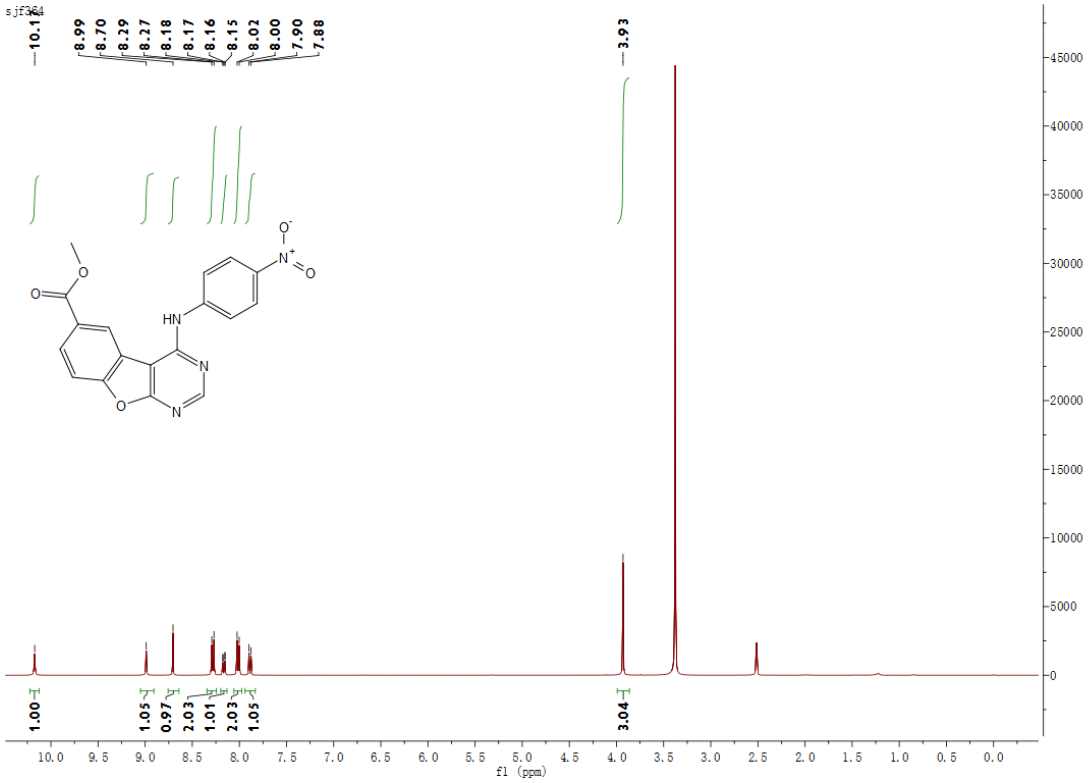


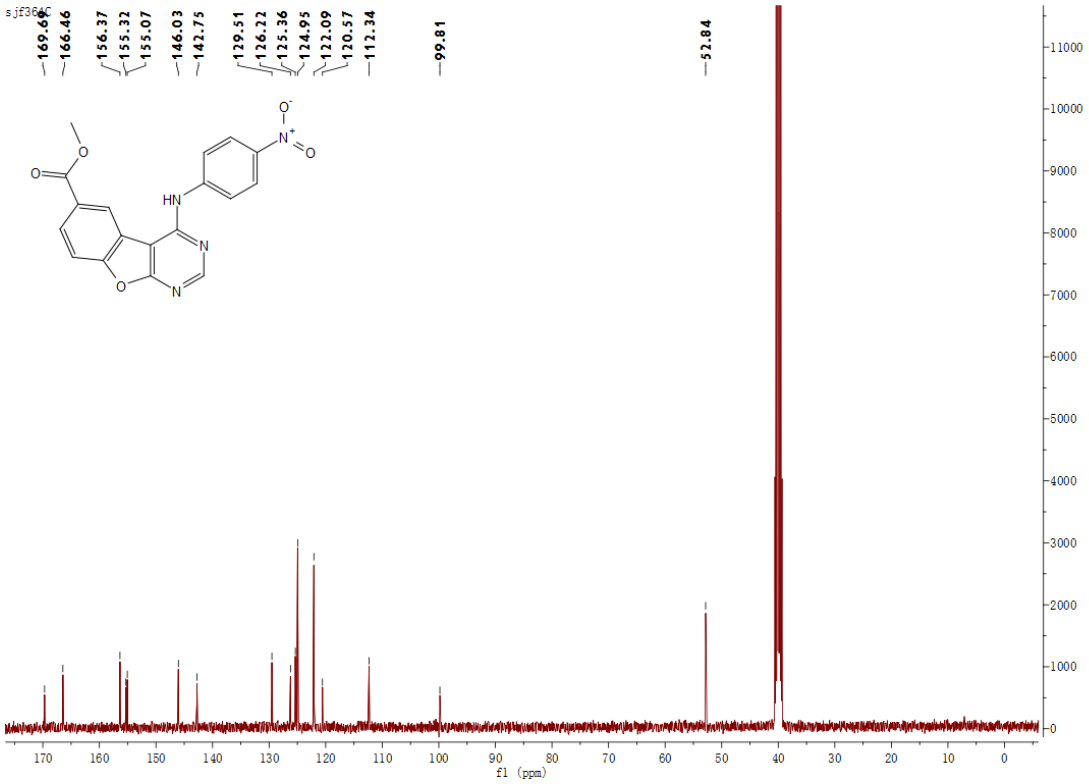
^{1}H and ^{13}C spectra of 5p



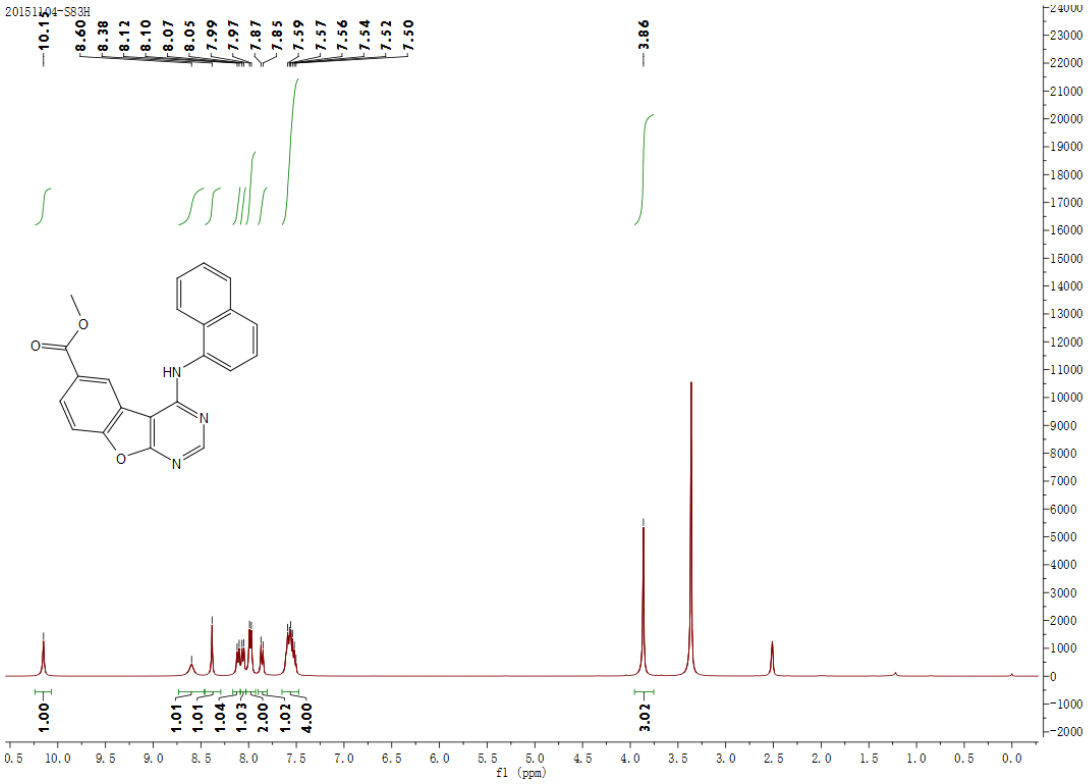


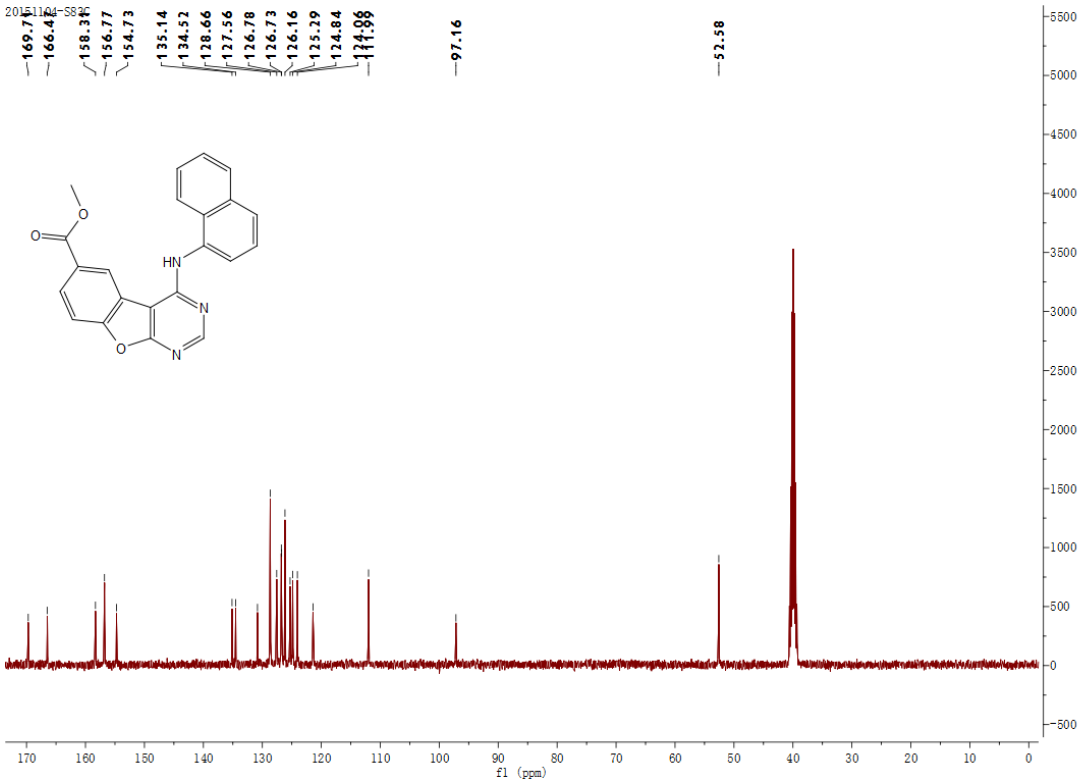
^{1}H and ^{13}C spectra of 5q



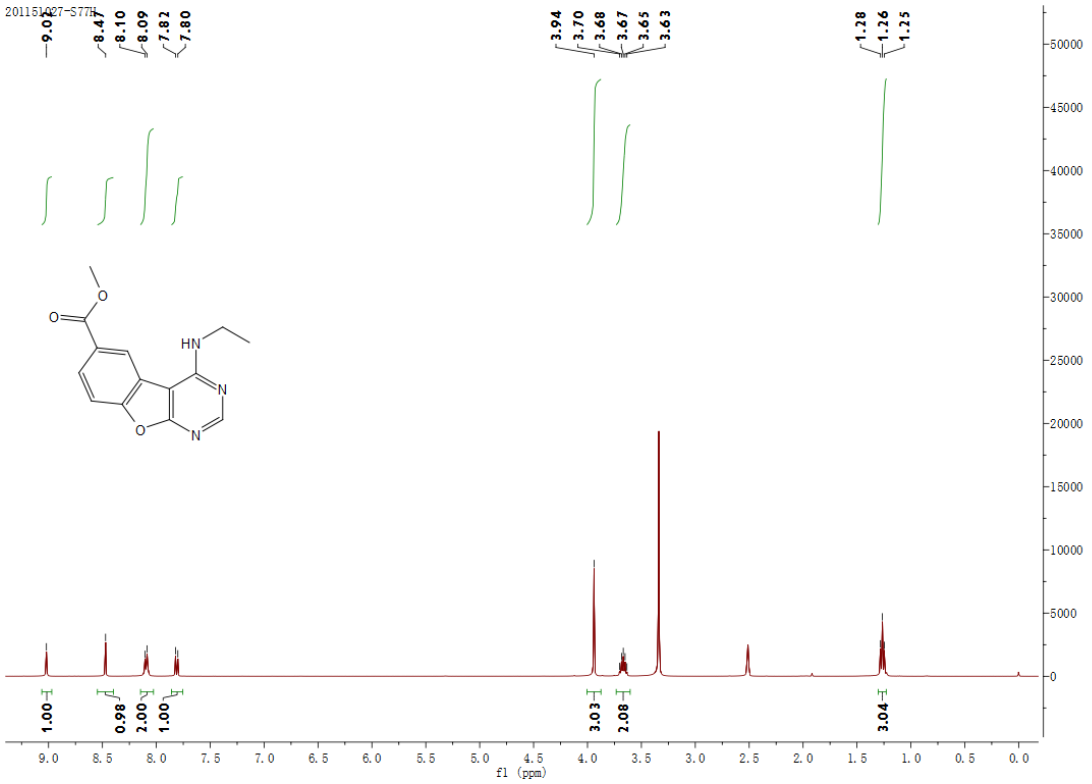


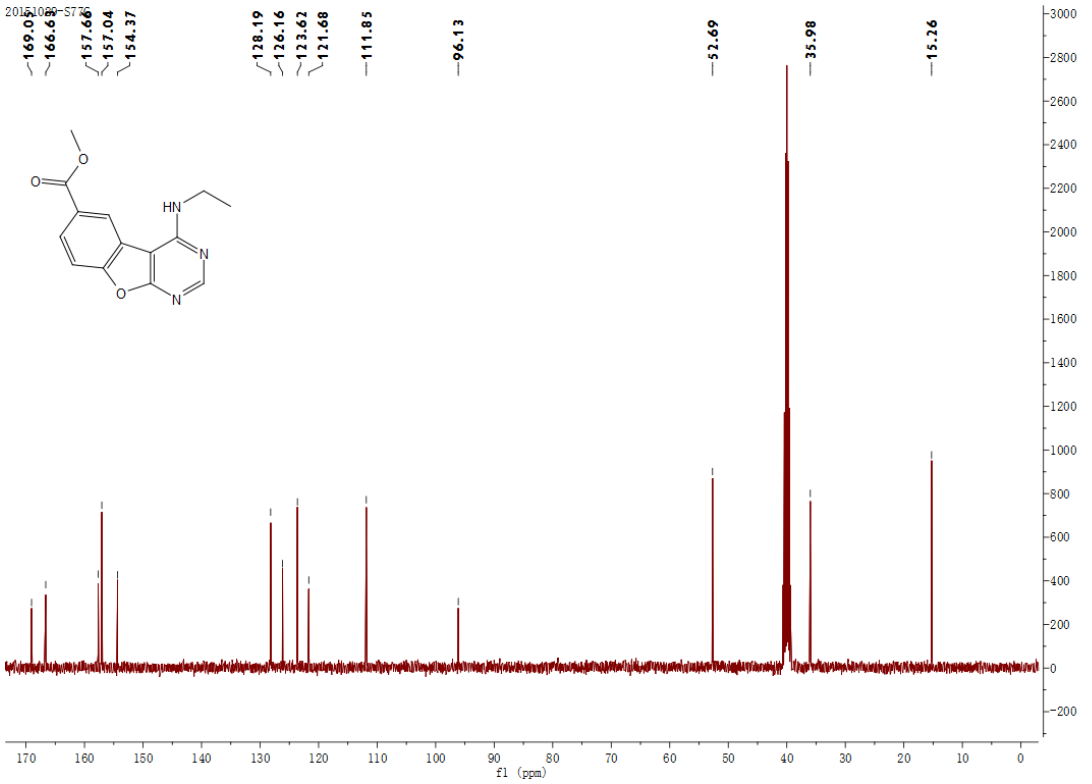
^{1}H and ^{13}C spectra of 5r



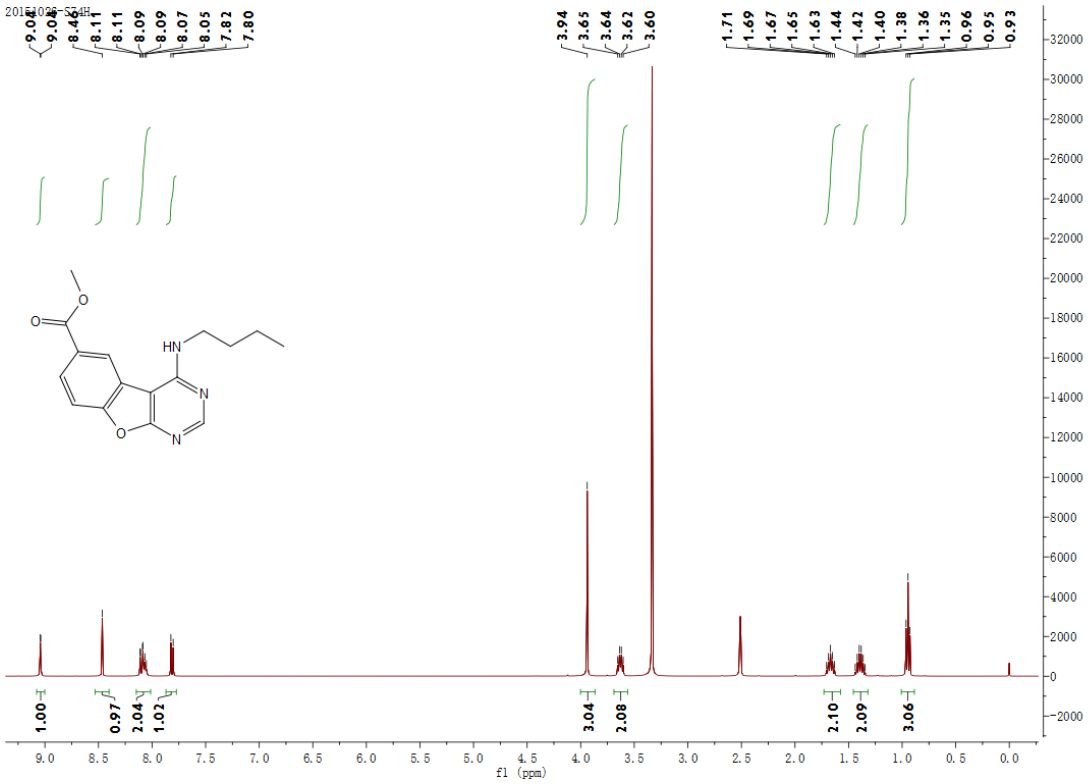


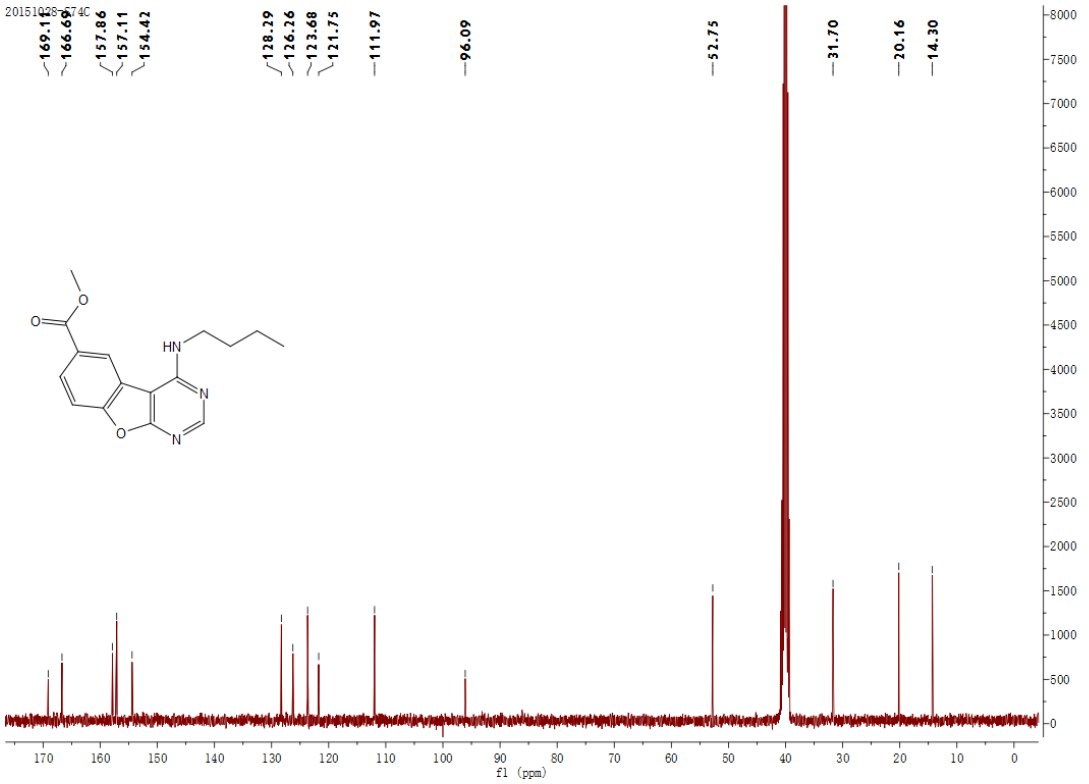
^{1}H and ^{13}C spectra of 7a



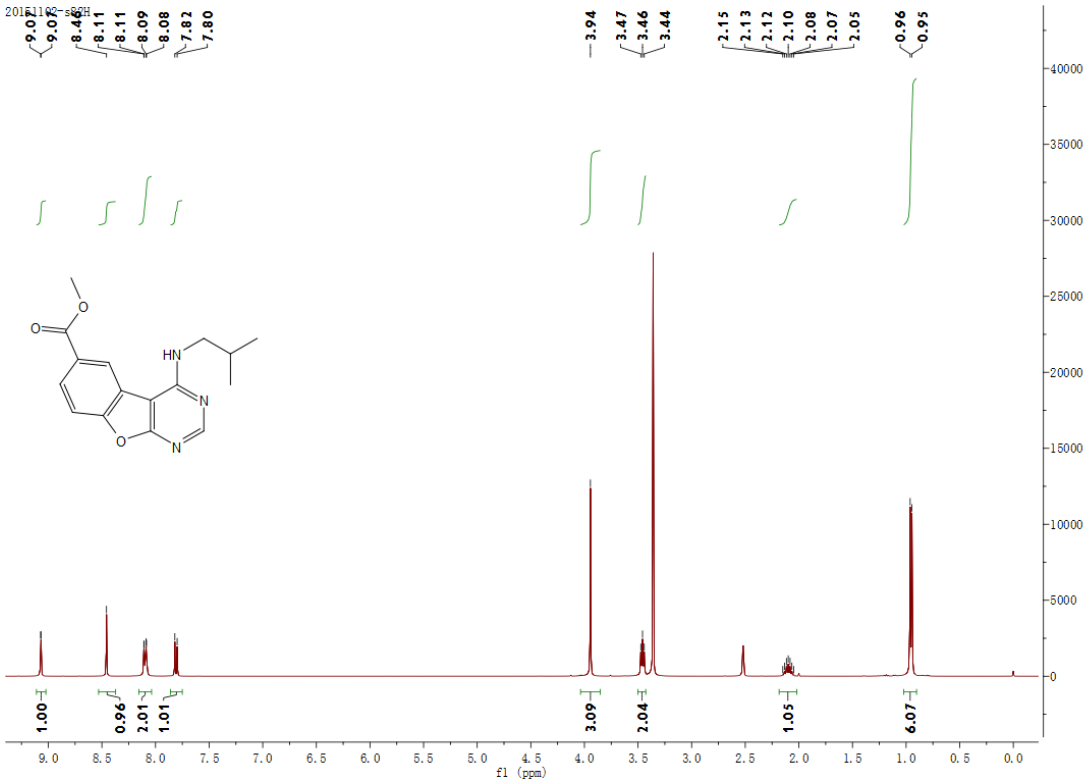


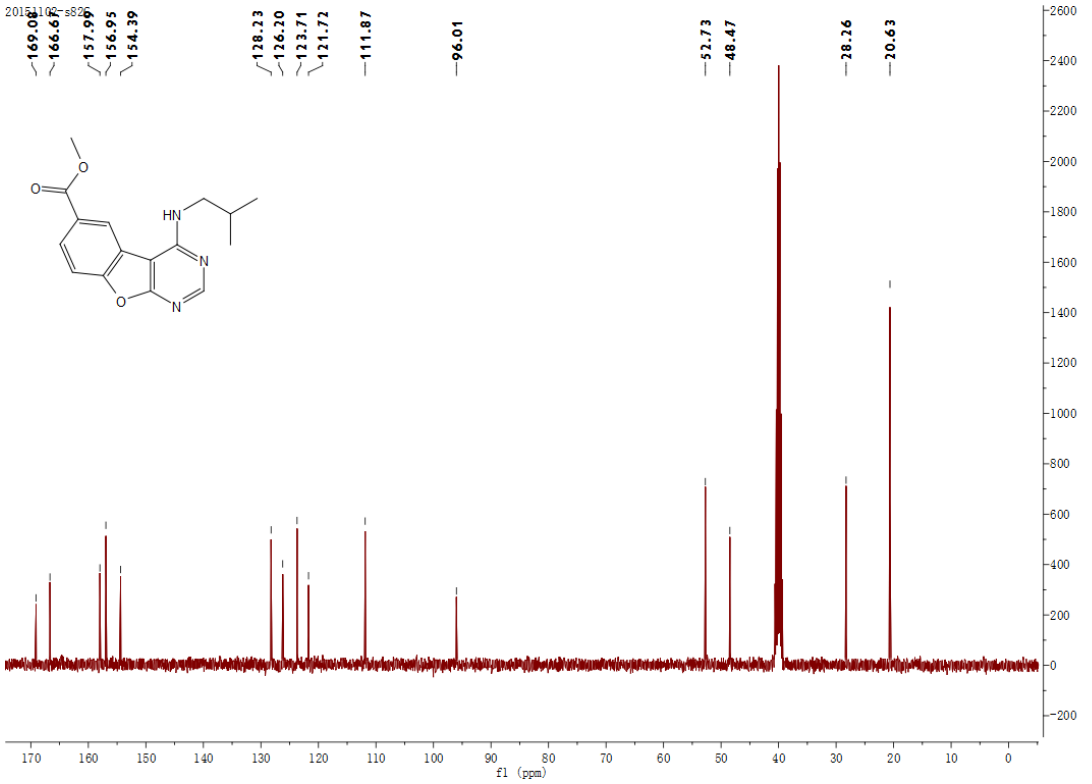
^{1}H and ^{13}C spectra of 7b



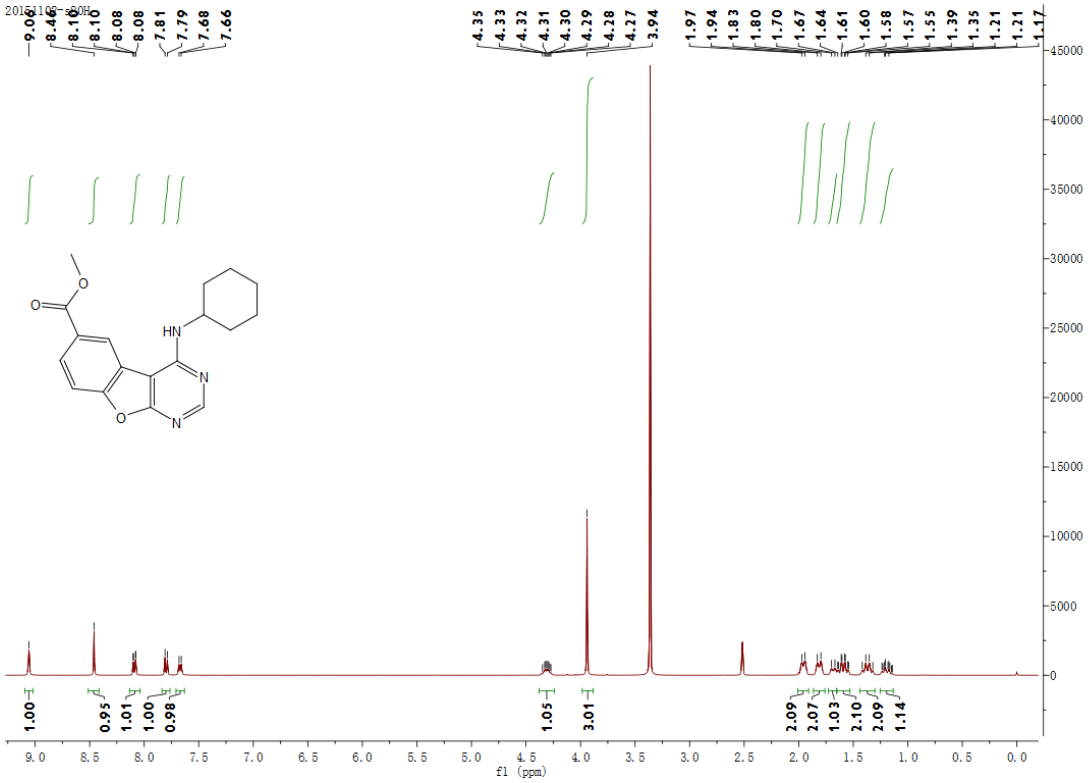


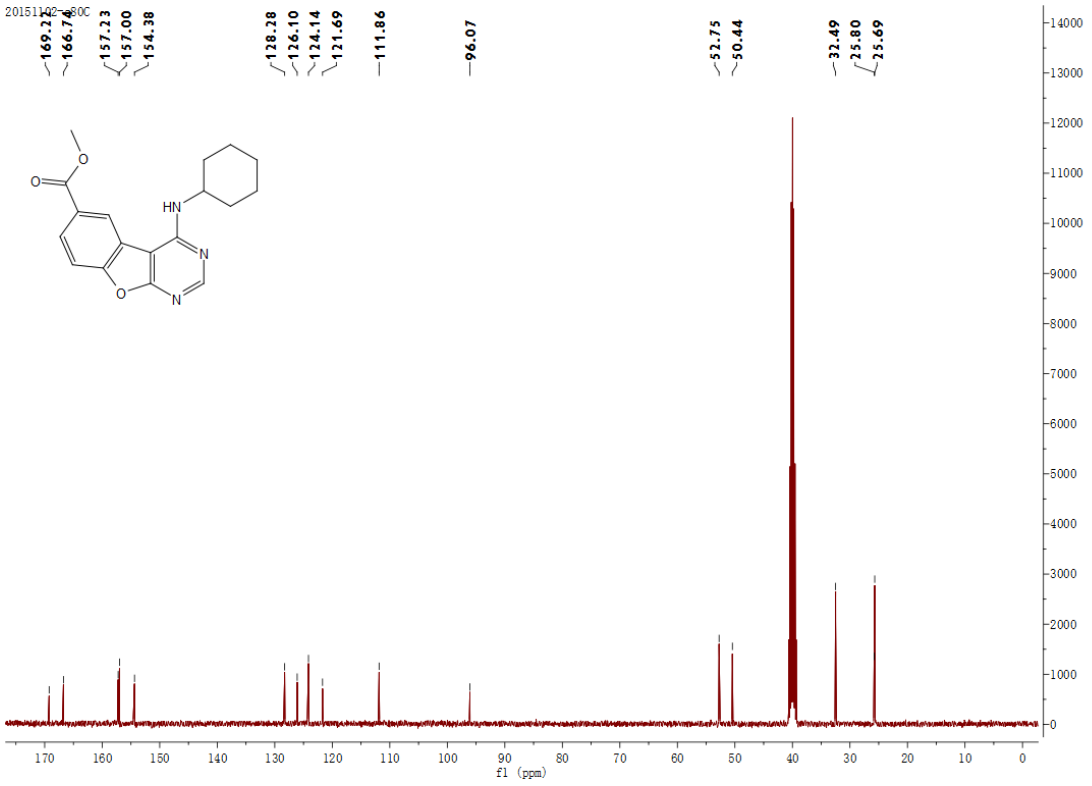
^{1}H and ^{13}C spectra of 7c



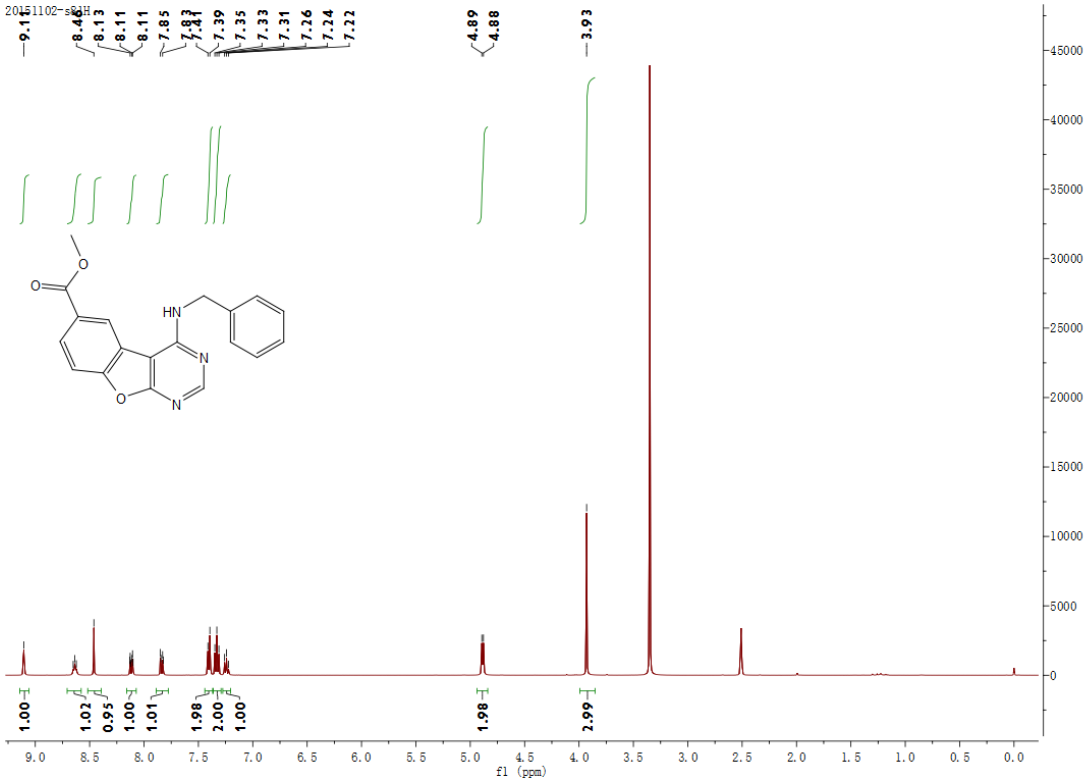


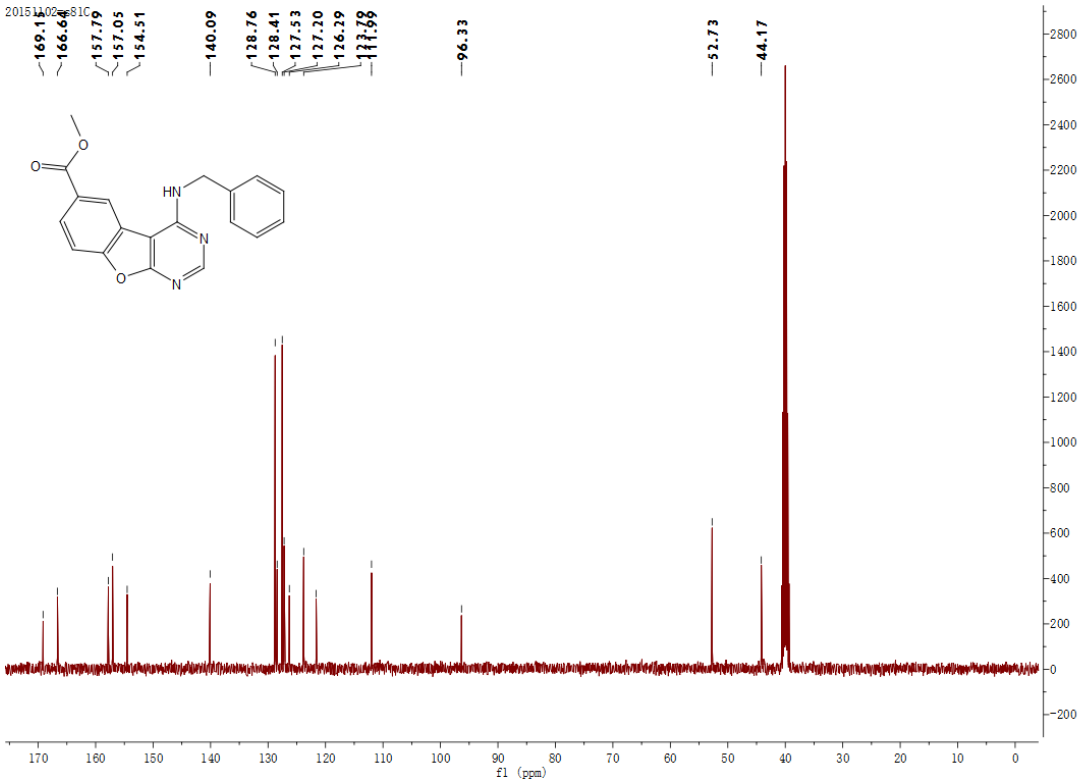
^{1}H and ^{13}C spectra of 7d



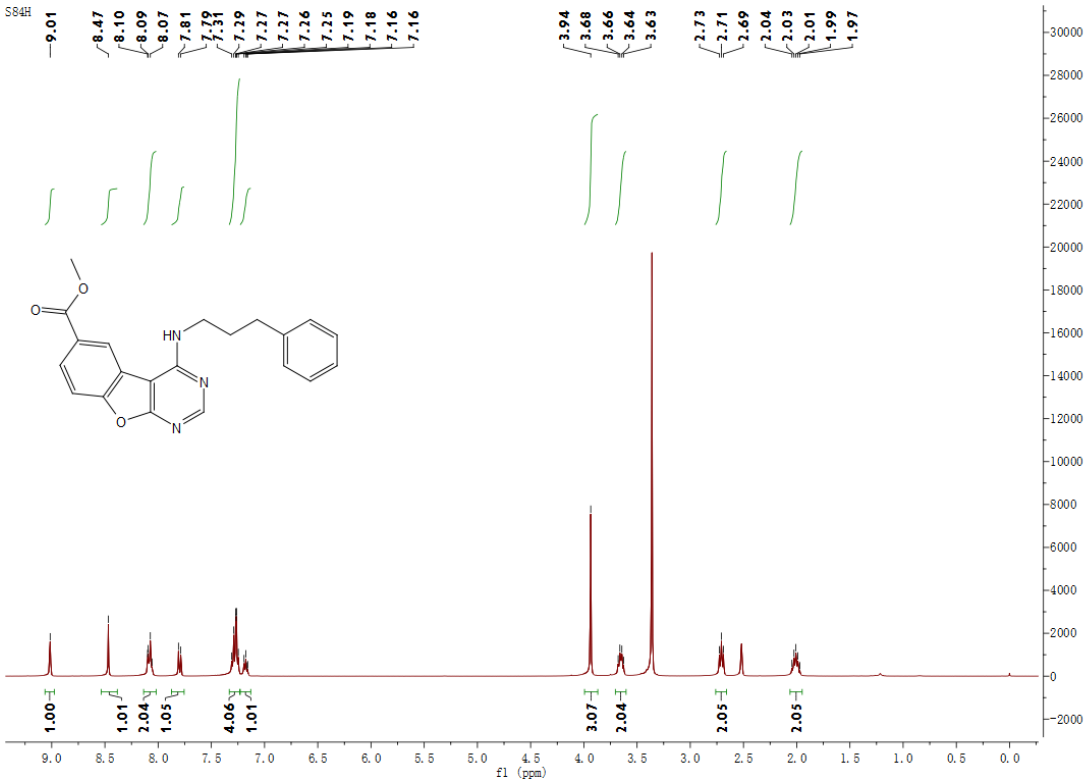


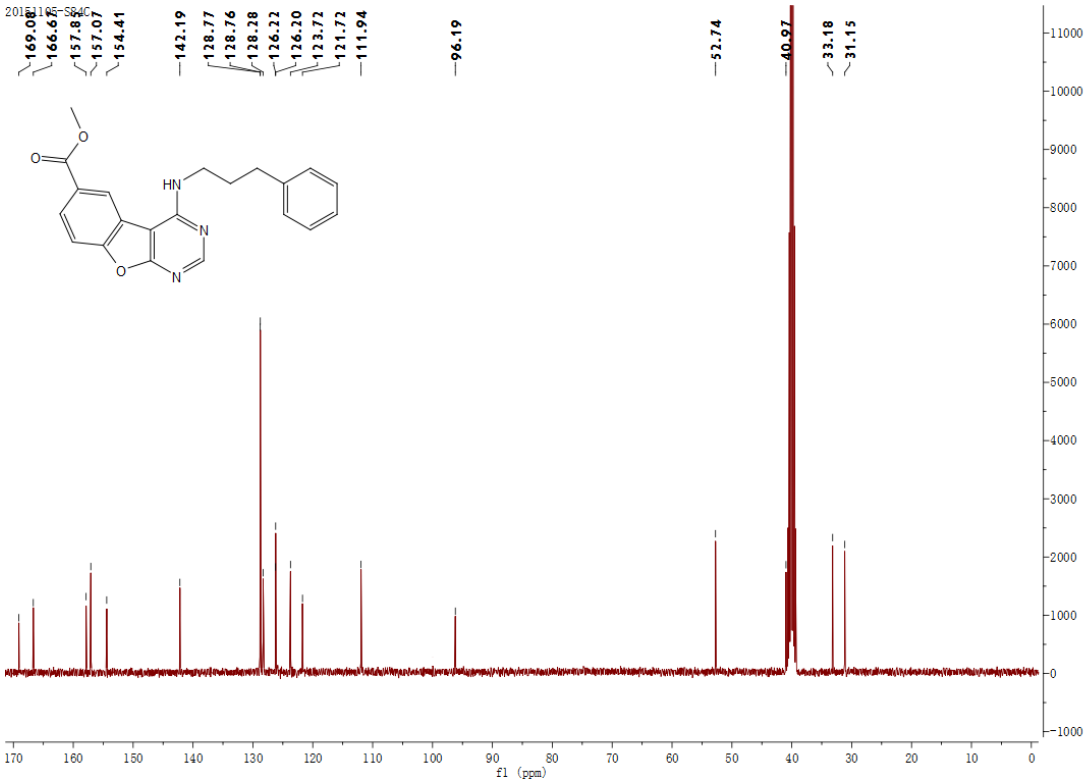
^{1}H and ^{13}C spectra of 7e





^{1}H and ^{13}C spectra of 7f





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