Supporting Information

Discovery of Imidazo[1,2-α][1,8]naphthyridine Derivatives as Potential HCV Entry Inhibitor

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1. General Information

Experimental details

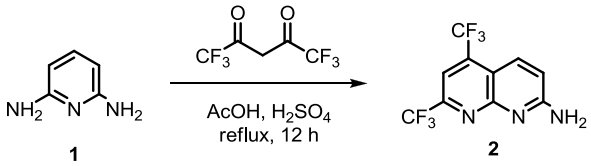
Unless otherwise mentioned, all reactions were carried out under a nitrogen atmosphere and anhydrous conditions, and all reagents were purchased from commercial suppliers without further purification. Solvent purification was conducted according to Purification of Laboratory Chemicals (Peerrin, D. D.; Armarego, W. L. and Perrins, D. R., Pergamon Press: Oxford, 1980). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials.

Reactions were monitored by Thin Layer Chromatography on plates (GF254) supplied by Yantai Chemicals (China) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate, and heat as developing agents. If not specially mentioned, flash column chromatography uses silica gel (200-300 mesh) supplied by Tsingtao Haiyang Chemicals (China).

NMR spectra were recorded on Bruker AV400 instrument. TMS was used as internal standard for ^{1}H NMR (0 ppm), and solvent signal was used as reference for ^{13}C NMR ($CDCl_3$, 77.16 ppm; CD_3OD-d_4, 49.00 ppm; Acetone-d_6, 29.84 ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-Q/TOF MS, Low-resolution mass spectral analyses were performed with a Waters AQUITY UPLCTM/MS.

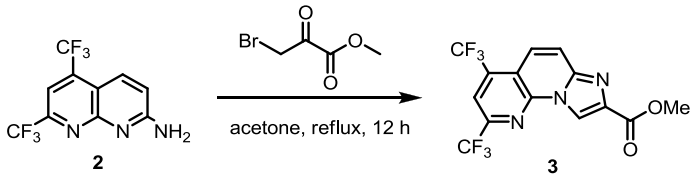
2. General Procedure for the Synthesis of 2, 3, 4 and 5a-w



1 2

5, 7-bis (trifluoromethyl)-1, 8-naphthyridin-2-amine (**2**)

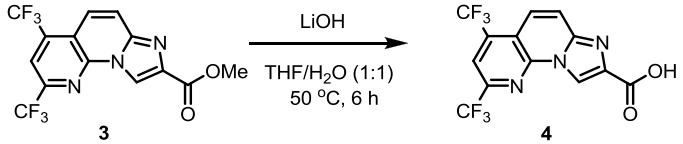
A mixture of pyridine-2,6-diamine (**1**) (3.65 g, 33.5 mmol) and 1, 1, 1, 5, 5, 5-hexafluoropentane-2,4-dione (5.2 mL, 36.7 mmol) dissolved in acetic acid (20 mL) and sulfuric acid (1.1 mL) was refluxed at 120℃ under nitrogen for 12 h. After cooling to room temperature, the reaction mixture was added dropwise in saturated sodium hydroxide solution (keeping the pH > 8). The gray solid was filtered under reduced pressure and the residue was washed with water (20 mL×5). Then the solid was dissolved in dry acetone and dried over Na_2SO_4, filtered and concentrated in vacuo to give 5, 7-bis (trifluoromethyl)-1, 8-naphthyridin-2-amine (**2**) as gray solid (5.94 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.23 (dd, *J* = 9.2 Hz, *J* = 1.6 Hz, 1H), 7.77 (s, 1H), 7.02 (d, *J* = 9.2 Hz, 1H), 5.68 (s, 2H).



2 3

Methyl 2, 4-bis (trifluoromethyl)-imidazo-[1, 2-α] [1, 8]-naphthyridine-8-carboxylate (**3**)

To a suspension of 5,7-bis (trifluoromethyl)-1,8-naphthyridin-2-amine (**2**) (2.81 g, 10 mmol) in acetone (40 mL) was added methyl bromopyruvate (3.61g, 20 mL), and the reaction was heated at 78℃ under nitrogen for 12 h. The mixture was cooled to room temperature, and the resulting solid was filtered. The crude product was washed with EtOH (3 mL×5) to give ethyl 2,4-bis (trifluoromethyl)-imidazo-[1, 2-α] [1, 8]-naphthyridine-8-carboxylate (**3**) as a yellow brown solid (1.30 g, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.15 (s, 1H), 8.12 (s, 1H), 7.90 (s, 2H), 4.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.8, 146.7 （q, *J* = 49.0 Hz）, 144.0, 143.7, 138.1 （q, *J* = 39.0 Hz）, 122.1 （q, *J* = 276.0 Hz）, 123.2, 120.3 （q, *J* = 276.0 Hz）, 121.1, 118.0, 117.5, 114.7, 52.3.

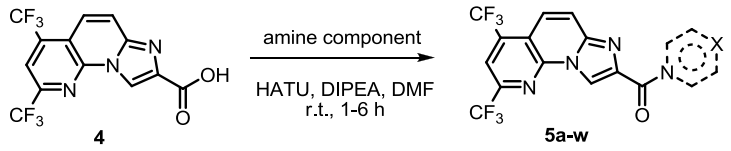


3 4

2, 4-bis (trifluoromethyl) imidazo [1, 2-α] [1, 8] naphthyridine-8-carboxylic acid (**4**)

To a solution of ethyl 2,4-bis(trifluoromethyl)-imidazo [1,2-α] [1, 8] naphthyridine-8-carboxylate (**3**) (1.09 g, 3.0 mmol) in THF/H_2O (*v*/*v*: 1:1, 10 mL) was added lithium hydroxide (144 mg, 6.0 mmol). The reaction mixture was stirred at 50℃ for 6 h, then added dropwise 1 M dilute hydrochloric acid to adjust the pH = 3. The mixture was extracted with ethyl acetate (20 mL×2). Combined the organic layer and dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (MeOH: DCM=1: 8)

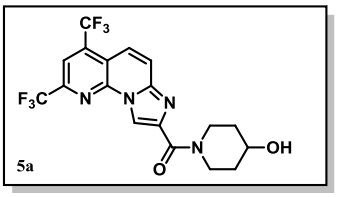
to get off-white solid **4** (942 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.88 (s, 1H), 8.46 (s, 1H), 8.02-8.04 (m, 1H), 7.90-7.92 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.37, 144.7 （q, J = 42.0 Hz）, 143.71, 143.39, 138.07, 136.2 （q, J = 50.0 Hz）, 123.33, 122.2（q, J = 273.0 Hz）, 120.6（q, J = 273.0 Hz）, 120.75, 117.52, 116.74, 115.29.



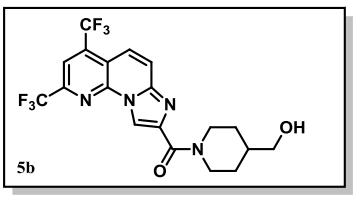
General procedure for the synthesis of **5a-w**:

A suspension of 2,4-bis (trifluoromethyl)-imidazo-[1,2-α][1,8]-naphthyridine-8-carboxylic acid (**4**) (34.9 mg, 0.1 mmol) and the corresponding amine component (0.15 mmol) in DMF (0.2 mL) was added DIPEA (25.8 mg, 0.2 mmol) and HATU (57 mg, 0.15 mmol) at room temperature, and the mixture was stirred at the same temperature for 1-6 h. The reaction was quenched with water (1 mL) and the aqueous layer was extracted with DCM (3 mL×5). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (MeOH: DCM=1: 8) to get off-white solid **5a-w**.

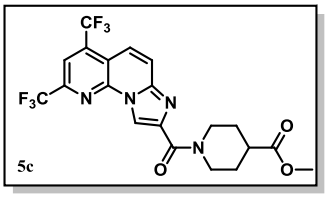
3. Analysis Data of 5a-w:



(2,4-bis (trifluoromethyl) imidazo-[1,2-α][1, 8]-naphthyridin-8-yl) (4-hydroxypiperidin-1-yl) methanone (**5a**): off-white solid; yield: 51%. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ: 9.04 (s, 1H), 8.09 (s, 1H), 7.84-7.85 (s, 2H), 4.62 (s, 1H), 4.30 (s, 1H), 4.02-4.04 (m, 1H), 3.72-3.74 (s, 1H), 3.45-3.48 (s, 1H), 2.01-2.04 (m, 2H), 1.65-1.67 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 162.9, 145.7 (q, J = 37.0 Hz), 143.9, 143.0, 140.1, 137.6 (q, J = 33.0 Hz), 122.4 (q, J = 273.0 Hz), 121.0, 120.1 (q, J = 273.0 Hz), 117.3, 115.9, 114.7, 66,4, 44.2, 34.3, 33.5. HRMS(EI+) m/z: Calcd $C_{18}H_{15}F_6N_4O_2$ (M+H) 433.1099, found 433.1101 (M+1).

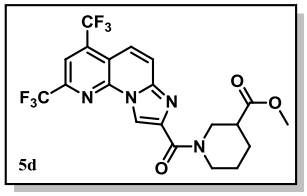


(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridin-8-yl)(4-(hydroxymethyl)piperidin-1-yl)methanone (**5b**): off-white solid; yield: 37%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.01 (s, 1H), 8.09 (s, 1H), 7.84 (s, 2H), 4.81-4.99 (m, 2H), 3.55 (s, 2H), 3.21-3.22 (m, 1H), 2.84-2.87 (m, 1H), 1.86 (s, 2H), ; 1.68 (s, 1H), 1.25-1.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.36, 146.6（q, J = 41.0 Hz）, 143.8, 142.5, 141. 7, 137.8 （q, J = 31.0 Hz）, 122.7, 122.1 （q, J = 281.0 Hz）, 120.4 （q, J = 264.0 Hz）, 120.2, 117.1, 116.9, 114.4, 67. 5, 47.0, 42.9, 38.9, 29.8, 28.5. HRMS(EI+) m/z: Calcd $C_{19}H_{17}F_6N_4O_2$ (M+H) 447.1256, found 447.1255 (M+1).

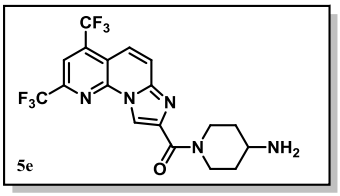


Methyl 1-(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-ca rbonyl)piperidine-4-carboxylate (**5c**): white solid; yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 8.11 (s, 1H), 7.86 (s,

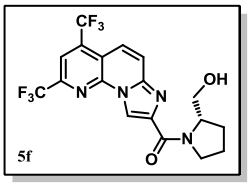
2H), 4.92 (s, 1H), 4.61 (s, 1H), 3.73 (s, 3H), 3.46 (s, 1H), 3.10 (s, 1H), 2.65-2.70 (m, 1H), 1.82-2.05 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.58, 162.43, 146.6（q, *J* = 39.0 Hz）, 143.78, 142.37, 141.51, 137.8（q, *J* = 31.0 Hz）, 122.1 （q, *J* = 273.0 Hz）, 122.97, 120.3 （q, *J* = 274.0 Hz）, 120.18, 117.38, 117.20, 114.33, 51.75, 46.45, 42.01, 40.96, 29.03, 27.97. HRMS(EI+) m/z: Calcd $C_{20}H_{16}F_6N_4O_3$ (M+H) 475.1199, found 475.1197 (M+1)



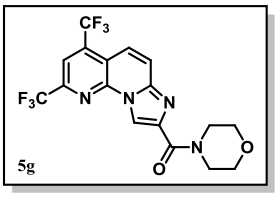
Methyl 1-(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-carbonyl)piperidine-2-carboxylate (**5d**): off-white solid; yield: 49%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.06 (s, 1H), 8.11 (s, 1H), 7.87 (s, 2H), 4.48-4.95 (m, 2H), 3.71 (s, 3H), 3.11-3.33 (m, 2H), 2.66-2.71 (m, 1H), 2.17-2.20 (m, 1H), 1.69-1.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.7, 162.5, 146.4（q, *J* = 41.0 Hz）, 143.7, 142.3, 141.5, 137.6（q, *J* = 38.0 Hz）, 122.9, 122.1 （q, *J* = 276.0 Hz）, 120.4 （q, *J* = 275.0 Hz）, 120.2, 117.2, 117.1, 114.4, 51.7, 48.7, 47.4, 41.3, 27.6, 24.3. HRMS(EI+) m/z: Calcd $C_{20}H_{16}F_6N_4O_3$ (M+H) 475.1199, found 475.1195 (M+1).



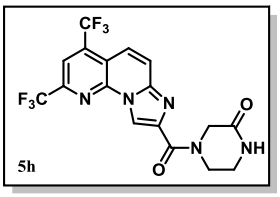
(4-aminopiperidin-1-yl)(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridin-8-yl)methanone (**5e**): off-white solid; yield: 42%. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ: 8.92 (s, 1H), 8.32 (s, 1H), 7.91-8.03 (m, 2H), 4.74 (s, 2H), 3.33-3.93 (m, 2H), 3.00 (s, 1H), 2.11 (s, 2H), 1.69 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 163.1, 146.1（q, *J* = 38.0 Hz）, 144.0, 143.2, 139.7, 137.7（q, *J* = 33.0 Hz）, 122.4（q, *J* = 271.0 Hz）, 127.1, 121.2, 120.7 （q, *J* = 272.0 Hz）, 117.2, 116.0, 114.8, 45.1, 41.0, 32.0, 30.7, 28.1. HRMS (EI+) m/z: Calcd $C_{18}H_{15}F_6N_5O$ (M+H) 432.1254, found 432.1254 (M+1).



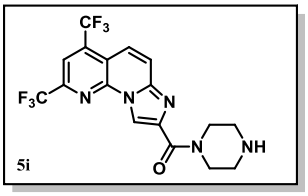
(*S*)-(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridin-8-yl)(2-(hydroxymethyl)pyrrolidin-1-yl)methanone (**5m**): off-white solid; yield: 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.18 (s, 0.5H), 9.14 (s, 1H), 8.12 (s, 0.5H), 8.10 (s, 1H), 7.82-7.89 (m, 2H), 5.05 (s, 1H), 4.40-4.45 (m, 1H), 4.02-4.08 (m, 1H), 3.67-3.8 (m, 2H), 1.70-2.19 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.0, 161.8, 146.7 (q, *J* = 44.0 Hz), 143.8, 142.6, 142.0, 141.5, 137.8 (q, *J* = 34.0 Hz), 122.8, 122.1, 122.0 (q, *J* = 270.0 Hz), 121.0, 120.4 (q, *J* = 297.0 Hz), 120.2, 118.1, 117.7, 117.3, 114.7, 114.4, 67.5, 65.7, 62.4, 59.1, 50.0, 46.2, 29.2, 27.9, 25.1, 21.1. HRMS(EI+) m/z: Calcd $C_{18}H_{14}F_6N_4O_2$ (M+H) 432.29, found 433.44 (M+1).



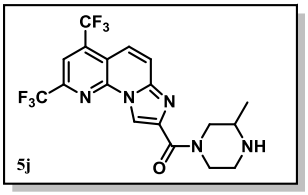
(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridin-8-yl)(morpholino)methanone (**5g**): white solid; yield: 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.12 (s, 1H), 8.13(s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 4.36 (s, 2H), 3.84 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.1, 146.7 （q, *J* = 34.0 Hz）, 143.7, 142.4, 141.2, 138.0 （q, *J* = 33.0 Hz）, 122.1 (q, *J* = 278.0 Hz), 122.8, 120.4 （q, *J* = 293.0 Hz）, 120.4, 117.9, 117.2, 114.4, 67.3, 47.9, 43.2. HRMS(EI+) m/z: Calcd $C_{17}H_{13}F_6N_4O_2$ (M+H) 419.0945, found 419.0943 (M+1).



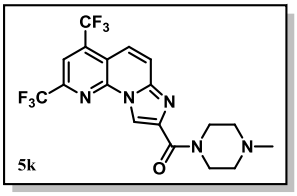
4-(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-car-bonyl) piperazin-2-one (**5h**): off-white solid; yield: 49%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.15 (s, 1H), 8.12 (s, 1H), 7.89 (d, *J* = 9.2 Hz, 1H), 7.83 (d, J = 9.2 Hz, 1H), 6.65 (s, 0.5H), 6.50 (s, 0.5H), 5.10 (s, 1H), 4.62 (s, 1H), 4.49 (s, 1H), 4.06 (s, 1H), 3.57 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.8, 162.1, 146.9 （q, *J* = 37.0 Hz）, 143.9, 142.6, 138.2 （q, *J* = 33.3 Hz）, 123.0, 122.2 （q, *J* = 274.0 Hz）, 120.8, 120.5 （q, *J* = 273.0 Hz）, 118.6, 117.4, 114.8, 43.5, 42.1, 40.9, 39.8. HRMS (EI+) m/z: Calcd $C_{17}H_{11}F_6N_5O_2$ (M+H) 432.0890, found 432.0892 (M+1).



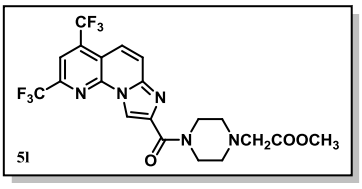
(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridin-8-yl)(pipe-razin-1-yl)methanone (**5i**): light-yellow solid; yield: 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.04 (s, 1H), 8.09 (s, 1H), 7.84 (s, 2H), 4.20 (s, 2H), 3.82 (s, 2H), 2.98 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.2, 146.6 （q, *J* = 35.0 Hz）, 143.7, 142.2, 141.4, 137.9 （q, *J* = 41.0 Hz）, 122.8, 122.1 （q, *J* = 261.0 Hz）, 120.8 (q, *J* = 279.0 Hz) 120.2, 117.4, 117.1, 114.3, 48.3, 46.8, 46.1, 43.9. HRMS (EI+) m/z: Calcd $C_{17}H_{13}F_6N_5O$ (M+H) 418.1097, found 418.21099 (M+1).



(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridin-8-yl)(3-methylpiperazin-1-yl)methanone (**5j**): off-white solid; yield: 56%. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ: 8.93 (s, 1H), 8.28 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 5.18 (s, 1H), 4.55 (s, 1H), 3.44 (s, 2H), 3.24-3.26 (m, 3H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 161.4, 144.5（q, *J* = 37.0 Hz）, 142.3, 141.6, 137.6, 136.4（q, *J* = 40.0 Hz）, 120.8 （q, *J* = 273.0 Hz）, 120.6, 119.8, 119.2 （q, *J* = 275.0 Hz）, 116.0, 115.7, 113.3, 55.4, 49.9, 41.9, 41.5, 13.3. HRMS (EI+) m/z: Calcd $C_{18}H_{15}F_6N_5O$ (M+H) 432.1254, found 432.1251 (M+1).

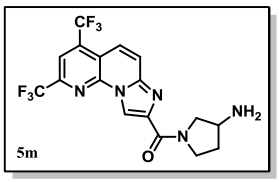


(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridin-8-yl)(4-methylpiperazin-1-yl)methanone (**5k**)**: o**ff-white solid. Yield: 55%. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.91 (s, 1H), 8.41 (s, 1H), 8.01 (s, 2H), 4.30 (s, 2H), 3.78 (s, 2H), 2.51 (s, 4H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, Acetone-d_6) δ: 161.3, 145.6 （q, *J* = 37.0 Hz）, 144.2, 141.7, 137.3 （q, *J* = 37.0 Hz）, 123.1, 122.5 (q, *J* = 279.0 Hz), 120.9 (q, *J* = 268.0 Hz), 120.5, 119.2, 117.6, 116.8, 116.7, 55.5, 54.7, 46.2, 45.3, 42.1. HRMS(EI+) m/z: Calcd $C_{18}H_{16}F_6N_5O$ (M+H) 433.1099, found 433.1098 (M+1).

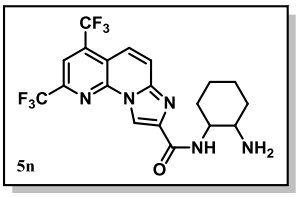


Methyl 2-(4-(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]nap-hthyridine-8-carbonyl)piperazin-1-yl)acetate (**5l**): white solid, yield: 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.08 (s, 1H), 8.11 (s, 1H), 7.83-7.89 (m, 2H), 4.30 (s, 2H), 3.93 (s, 2H), 3.76 (s, 3H), 3.31 (s, 2H), 2.72 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.8, 170.6, 162.1, 146.6（q, *J* = 37.0 Hz）, 143.8, 142.3, 141.2, 137.9 （q, *J* = 45.0 Hz）, 122.9, 122.1 （q, *J* = 283.0 Hz）, 120.3 （q, *J* = 277.0 Hz）, 120.2, 117.6, 117.1, 114.4, 59.2, 53.4, 52.6, 51.6,

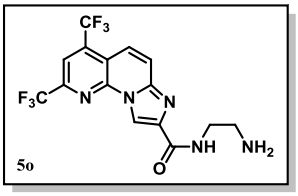
46.7, 42.6. HRMS(EI+) m/z: Calcd $C_{20}H_{17}F_6N_5O_3$ (M+H) 490.1308, found 490.1313 (M+1).



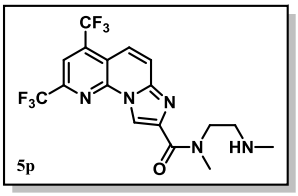
(2-aminopyrrolidin-1-yl)(2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridin-8-yl)methanone (**5f**): off-white solid; yield: 46%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.12 (s, 1H), 8.09 (s, 1H), 7.84 (s, 2H), 4.19-4.38 (m, 1.5H), 3.71-3.94 (m, 3H), 3.52-3.54 (m, 0.5H), 2.13-2.26 (m, 1H), 1.71-1.90 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.8, 146.6 （q, *J* = 74.0 Hz）, 144.2, 142.6, 141.8, 137.8（q, *J* = 60.0 Hz）, 122.3（q, *J* = 259.0 Hz）, 123.0, 117.7（q, *J* = 280.0 Hz）, 120.1, 117.2, 114.4, 57.0, 55.5, 52.1, 49.5, 47.1, 45.3, 35.4, 32.9. HRMS(EI+) m/z: Calcd $C_{17}H_{13}F_6N_5O_2$(M+H) 418.1097, found 418.1096 (M+1).



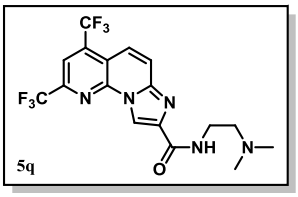
N-(2-aminocyclohexyl)-2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-carboxamide (**5n**): light-yellow solid, yield: 41%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.04 (s, 1H), 8.08 (s, 1H), 7.79-7.89 (m, 2H), 4.77 (s, 2H), 4.40 (s, 1H), 3.48 (s, 1H), 1.52-1.89 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.9, 146.6 （q, *J* = 37.0 Hz）, 144.0, 142.7, 140.5, 137.8（q, *J* = 31.0 Hz）, 122.7, 122.1（q, *J* = 279.0 Hz）, 120.4（q, *J* = 271.0 Hz）, 120.4, 117.0, 115.2, 114.4, 51.0, 48.8, 29.3, 27.8, 22.6, 20.8. HRMS (EI+) m/z: Calcd $C_{19}H_{19}F_6N_5O$ (M+H) 446.1336, found 446.1436 (M+1).



N-(2-aminoethyl)-2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-carboxamide (**5o**): light-yellow solid, yield: 36%. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ: 9.05 (s, 1H), 8.32 (s, 1H), 8.02 (d, *J* = 9.8 Hz, 1H), 7.93 (d, *J* = 9.8 Hz 1H), 3.74 (t, *J* = 5.6 Hz, 2H), 3.22 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 165.4, 147.5 (q, *J* = 36.0 Hz), 145.4, 145.0, 140.4, 139.1 (q, *J* = 33.0 Hz), 123.7 (q, *J* = 273.0 Hz), 122.0 (q, *J* = 272.0 Hz), 122.3, 120.7, 117.9, 116.2, 116.1, 40.0, 22.6. HRMS (EI+) m/z: Calcd $C_{15}H_{12}F_6N_5O$ (M+H) 392.0946, found 392.0955 (M+1).

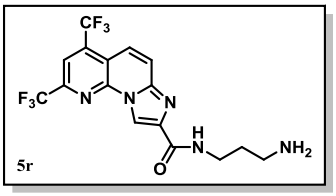


N-methyl-N-(2-(methylamino)ethyl)-2,4bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-carboxamide (**5p**): obtained as a pair of rotamers; light-yellow solid; yield: 47%. ^{1}H NMR (400 MHz, CD_3OD-d_4,) δ: 8.95 (s, 1H), 8.32 (s, 1H), 8.11 (d, *J* = 10.0 Hz, 0.5H), 8.07 (d, *J* = 10.0 Hz, 1H), 8.00 (d, *J* = 10.0 Hz, 0.5H), 4.59 (s, 1H), 4.25 (s, 1H), 3.96 (s, 1H), 3.54 (s, 3H), 3.41 (s, 1H), 2.91 (s, 1.5H), 2.82 (s, 1.5H); ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 167.3, 166.0, 147.6 (q, *J* = 37.0 Hz), 145.2, 144.3, 140.6, 139.5 (q, *J* = 33.0 Hz), 123.9, 123.7 (q, *J* = 273.0 Hz), 123.4, 122.8, 122.0 (q, *J* = 272.0 Hz), 118.9, 118.6, 117.8, 116.4, 46.6, 40.4, 38.0, 34.5, 34.2, 30.7, 30.7; HRMS(EI+) m/z: Calcd $C_{17}H_{16}F_6N_5O$ (M+H) 420.1259, found 420.1255 (M+1).

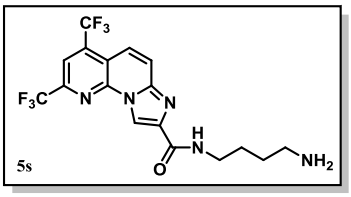


N-(2-(dimethylamino)ethyl)-2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-carboxamide (**5q**): light-yellow solid; yield: 44%. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ: 9.02 (s, 1H), 8.32 (s, 1H), 7.97 (d, *J* = 9.8 Hz, 1H), 7.91 (d, *J* = 9.8 Hz, 1H), 3.76 (t, *J* = 6.0 Hz,

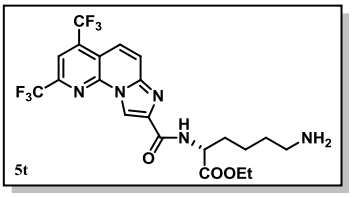
2H), 3.13 (t, *J* = 6.0 Hz, 2H), 2.75 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 164.9, 147.5 (q, *J* = 37.0 Hz), 145.3, 145.0, 140.5, 139.1 (q, *J* = 34.0 Hz), 123.7 (q, *J* = 273.0 Hz), 122.0 (q, *J* = 272.0 Hz), 123.8, 122.7, 116.2, 115.9, 58.7, 44.5, 44.5, 36.4. HRMS (EI+) m/z: Calcd $C_{17}H_{15}F_6N_5O$ (M+H) 420.1259, found 420.1258 (M+1).



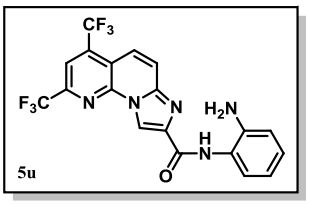
N-(3-aminopropyl)-2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]na-phthyridine-8-carboxamide (**5r**): yellow solid, yield: 48%. ^{1}H NMR (400 MHz CD_3OD-d_4) δ (ppm): 9.02 (s, 1H), 8.49 (s, 1H), 8.07-8.06 (d, *J* = 10.0 Hz, 1H), 7.96-7.93 (d, *J* = 10.0 Hz, 1H), 3.57 (t, *J* = 6.5 Hz, 2H), 3.05 (t, *J* = 6.5 Hz, 2H), 2.03 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 165.0, 147.5, 145.5 (q, *J* = 37.0 Hz), 145.1, 140.7, 139.1 (q, *J* = 33.0 Hz), 123.7 (q, *J* = 273.0 Hz), 123.7, 122.7, 122.1 (q, *J* = 273.0 Hz), 118.8, 116.2, 116.0, 38.4, 37.0, 29.0. HRMS (EI+) m/z: Calcd $C_{16}H_{13}F_6N_5O$ (M+H) 406.1098, found 406.1103 (M+1).



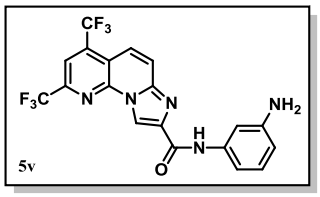
N-(4-aminobutyl)-2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8] naphthyridine-8-carboxamide (**5s**): light yellow solid; yield: 48%. ^{1}H NMR (400 MHz CD_3OD-d_4) δ (ppm): 9.02 (s, 1H), 8.33 (s, 1H), 8.03-8.00 (d, *J* = 9.6 Hz, 1H), 7.94-7.91 (d, *J* = 9.6 Hz, 1H), 3.53-3.51 (t, *J* = 6.4 Hz, 2H), 3.05-3.02 (t, *J* = 6.4 Hz, 2H), 1.78(m, 4H). ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 164.4, 147.6 (q, *J* = 37.0 Hz), 145.4, 145.0, 140.9, 139.6 (q, *J* = 33.0 Hz), 123.7 (q, *J* = 273.0 Hz), 122.6, 122.6, 122.1 (q, *J* = 273.0 Hz), 118.0, 116.2, 115.8, 40.4, 39.5, 27.6, 25.9. HRMS (EI+) m/z: Calcd $C_{17}H_{15}F_6N_5O$ (M+H) 420.1247, found 420.1259 (M+1).



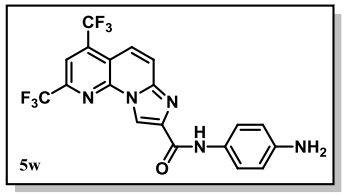
Ethyl (2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine -8-carbonyl)-D-lysinate (**5t**): yield: 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.14 (s, 1H), 8.12 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 4.21 (q, *J* = 7.2 Hz, 2H), 3.55 (m, 3H), 2.76 (s, 2H), 1.89-1.56 (m, 7H), 1.30 (t, *J* = 7.2 Hz, 3H), 1.24 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD-d_4) δ: 164.2, 147.5 (q, *J* = 37.0 Hz), 145.5, 145.0, 141.1, 139.0 (q, *J* = 33.0 Hz), 123.8 (q, *J* = 273.0 Hz), 123.6, 122.6, 122.1 (q, *J* = 272.0 Hz), 118.8, 116.2, 115.8, 64.2, 62.2, 54.9, 40.0, 30.7, 30.3, 23.8, 14.5. HRMS (EI+) m/z: Calcd $C_{21}H_{21}F_6N_5O_3$ (M+H) 506.1617, found 506.1627 (M+1).



N-(2-aminophenyl)-2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]nap ht-hyridine-8-carboxamide (**5u**): light-yellow solid; yield: 53%. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ (ppm): 9.18 (s, 1H), 8.37 (s, 1H), 8.07 (d, *J* = 9.6 Hz, 1H), 8.02 (d, *J* = 9.6 Hz, 1H), 7.43 (d, *J* = 7.6 Hz, 1H), 7.12-7.08 (t, *J* = 7.2 Hz, 1H), 6.96-6.94 (d, *J* = 7.6 Hz, 1H), 6.84-6.80 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.6, 144.7 (q, *J* = 36.0 Hz), 143.9, 143.0, 142.1, 140.1, 136.4 (q, *J* = 33.0 Hz), 126.1, 125.3, 123.6, 123.0, 120.9, 120.7 (q, *J* = 273.0 Hz), 116.7 (q, *J* = 280.0 Hz), 117.4, 116.8, 116.7, 115.3, 114.7. HRMS(EI+) m/z: Calcd $C_{19}H_{11}F_6N_5O$ (M+H) 440.0942, found 440.0946 (M+1).

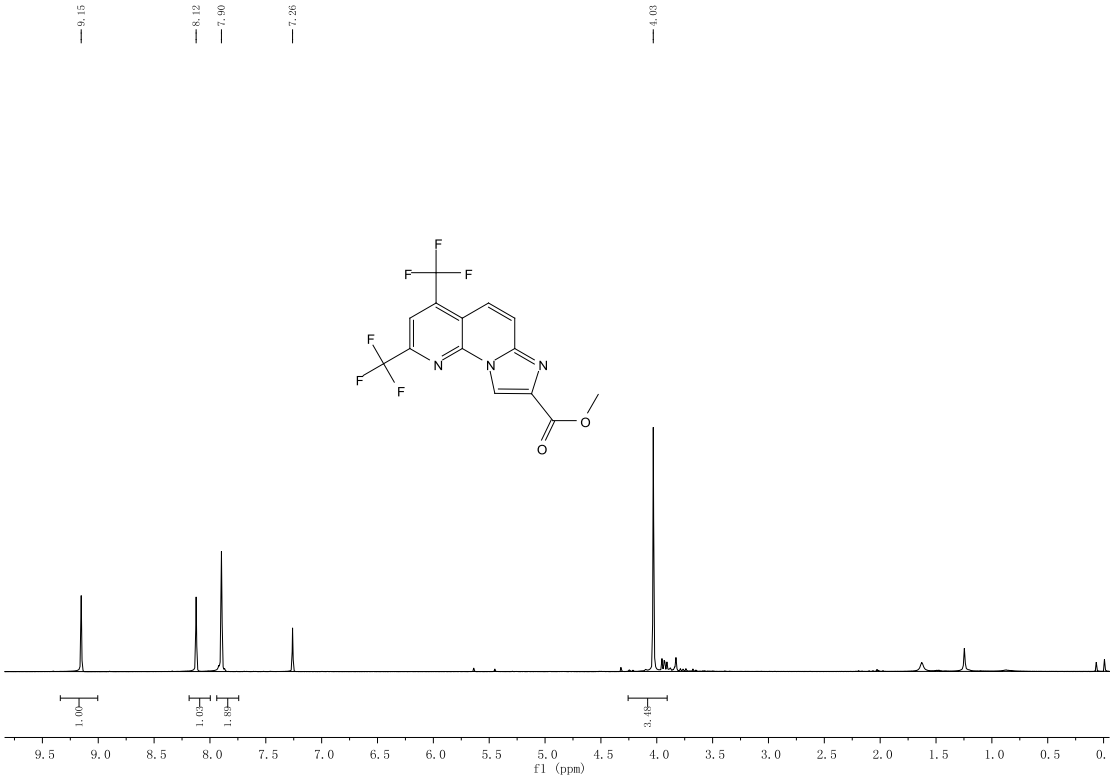


N-(3-aminophenyl)-2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-carboxamide (**5v**): light-yellow solid; yield: 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.04 (s, 1H), 9.07 (s, 1H), 8.50 (s, 1H), 8.09-8.07 (d, J = 9.6 Hz, 1H), 7.96-7.94 (d, J = 9.6 Hz, 1H), 7.52 (s, 1H), 7.00-6.92 (m, 2H), 6.34-6.32 (d, J = 7.6 Hz, 1H), 5.16 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.9, 148.5, 144.2 (q, J = 28.0 Hz), 143.3, 142.5, 139.7, 138.6, 136.0 (q, J = 29.0 Hz), 128.4, 122.5, 121.7 (q, J = 280.0 Hz), 120.5, 120.1 (q, J = 268.0 Hz), 117.0, 114.9, 114.3, 109.5, 107.7, 105.3. HRMS(EI+) m/z: Calcd $C_{19}H_{11}F_6N_5O$ (M+H) 440.0948, found 440.0946 (M+1).

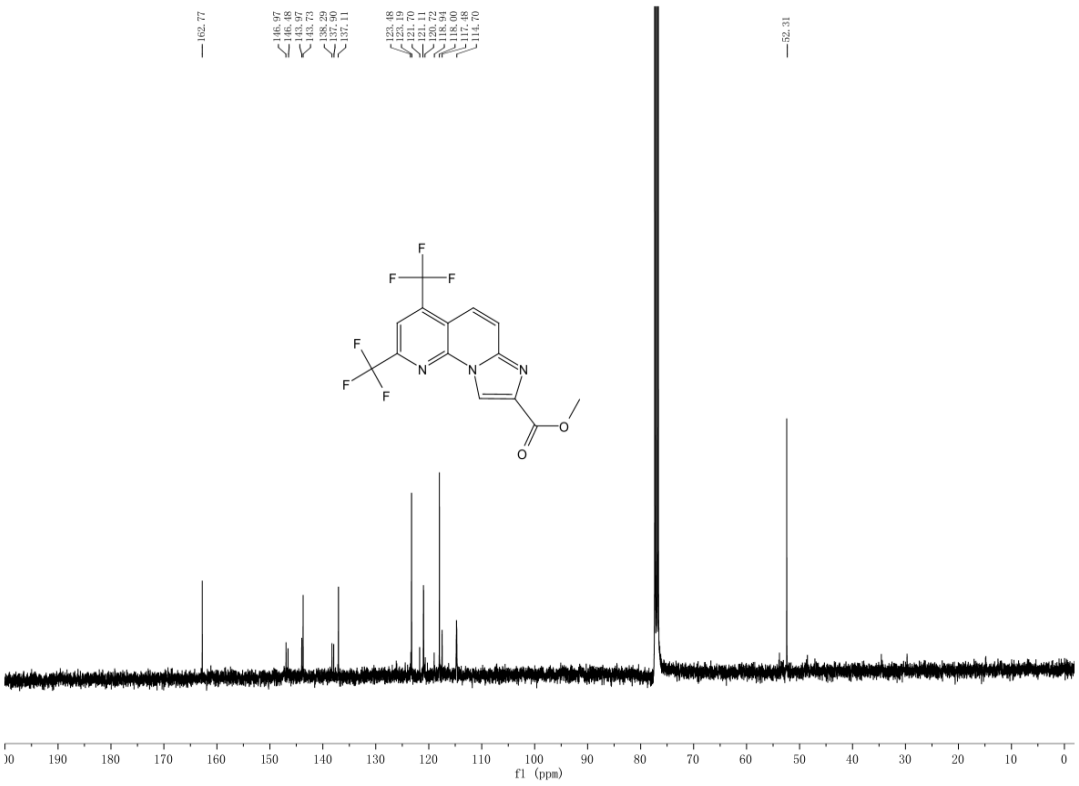


N-(4-aminophenyl)-2,4-bis(trifluoromethyl)imidazo[1,2-α][1,8]naphthyridine-8-carboxamide (**5w**): light-yellow solid, yield: 15% . ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.05 (s, 1H), 9.02 (s, 1H), 8.49 (s, 1H), 8.07-8.06 (d, J = 10.0 Hz, 1H), 7.96-7.93 (d, J = 10.0 Hz, 1H), 7.52-7.50 (d, J = 5.6 Hz, 2H), 6.56-6.54 (d, J = 5.6 Hz, 2H), 4.96 (s, 2H); ^{1}3C NMR (100 MHz, DMSO-d_6) δ: 158.9, 145.3, 144.7 （q, J = 33.0 Hz）, 143.7, 142.9, 140.6, 136.4 （q, J = 36.0 Hz）, 127.6, 122.9, 122.3 (q, J = 275.0 Hz), 121.8, 120.9, 120.6 (q, J = 273.0 Hz), 117.4, 115.3, 114.3, 113.6. HRMS(EI+) m/z: Calcd $C_{19}H_{11}F_6N_5O$ (M+H) 440.0942, found 440.0946 (M+1).

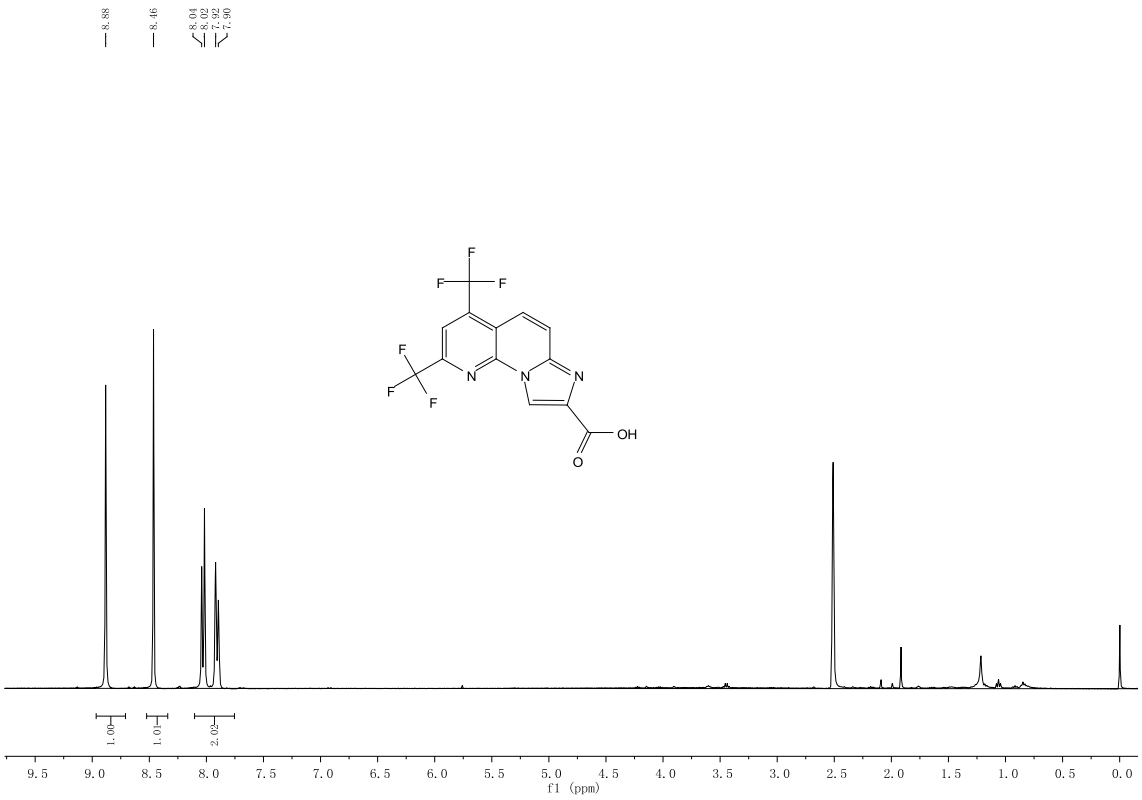
4. NMR Spectra of 3, 4 and 5a-w:



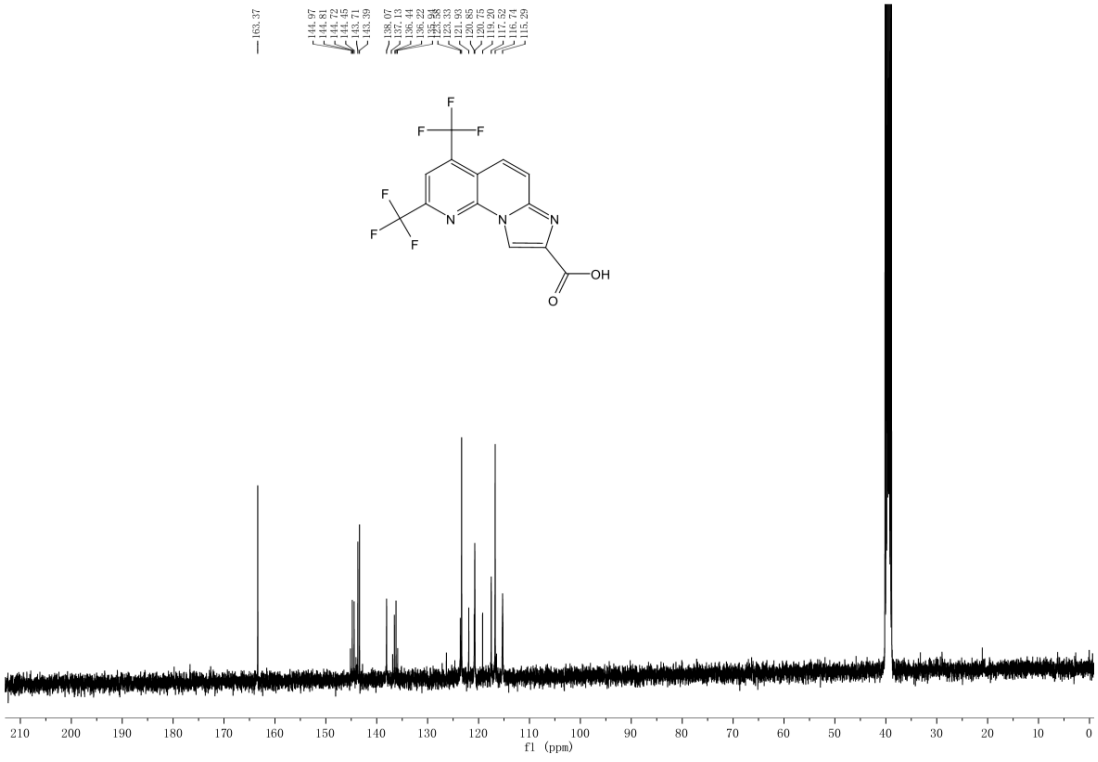
^{1}H NMR Spectrum for **3** ($CDCl_3$, 400 MHz)



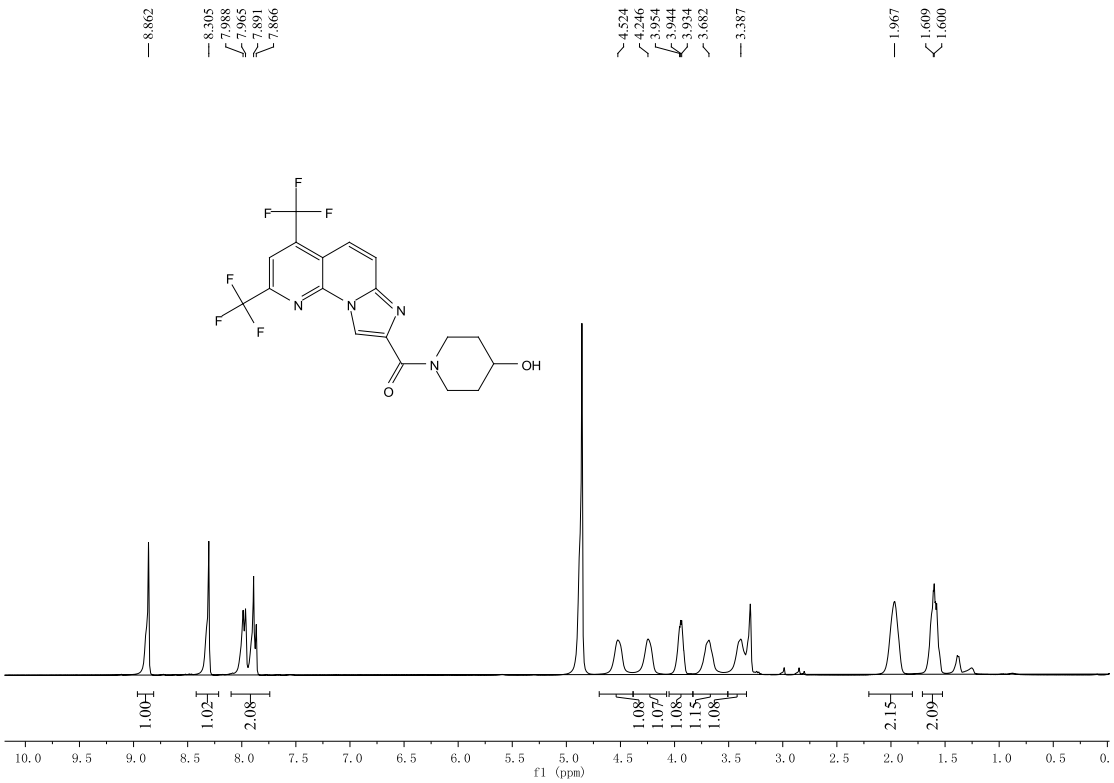
^{13}C NMR Spectrum for **3** ($CDCl_3$, 100 MHz)



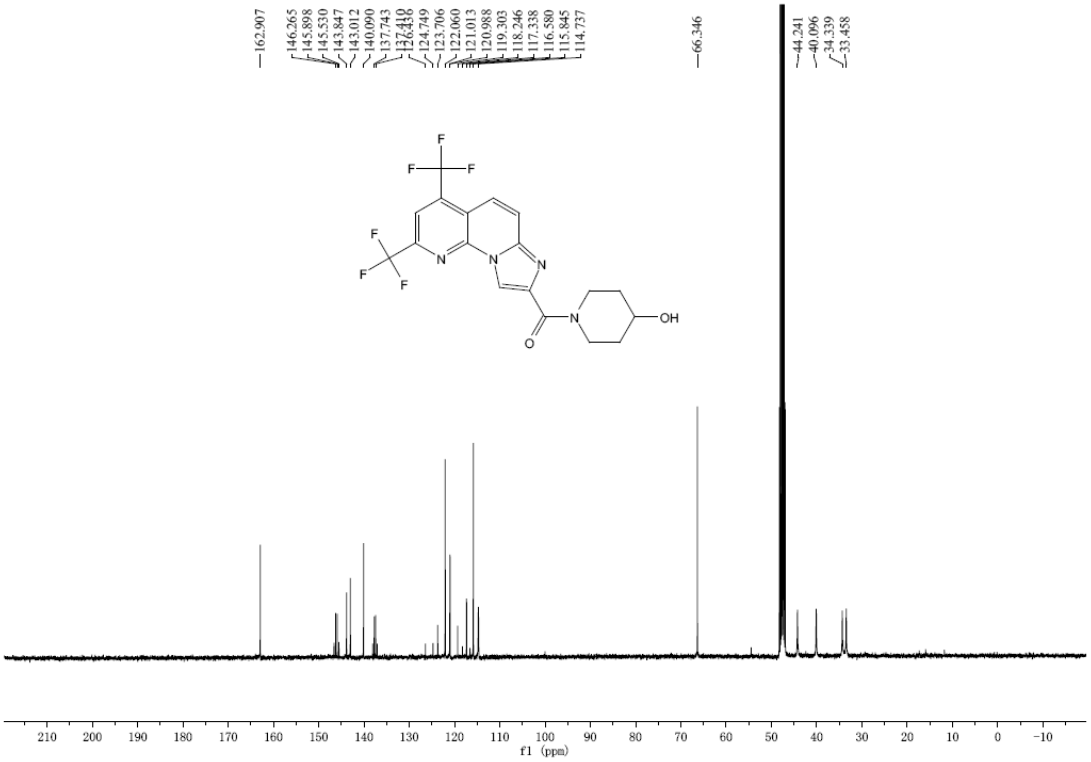
^{1}H NMR Spectrum for **4** (DMSO-d_6, 400 MHz)



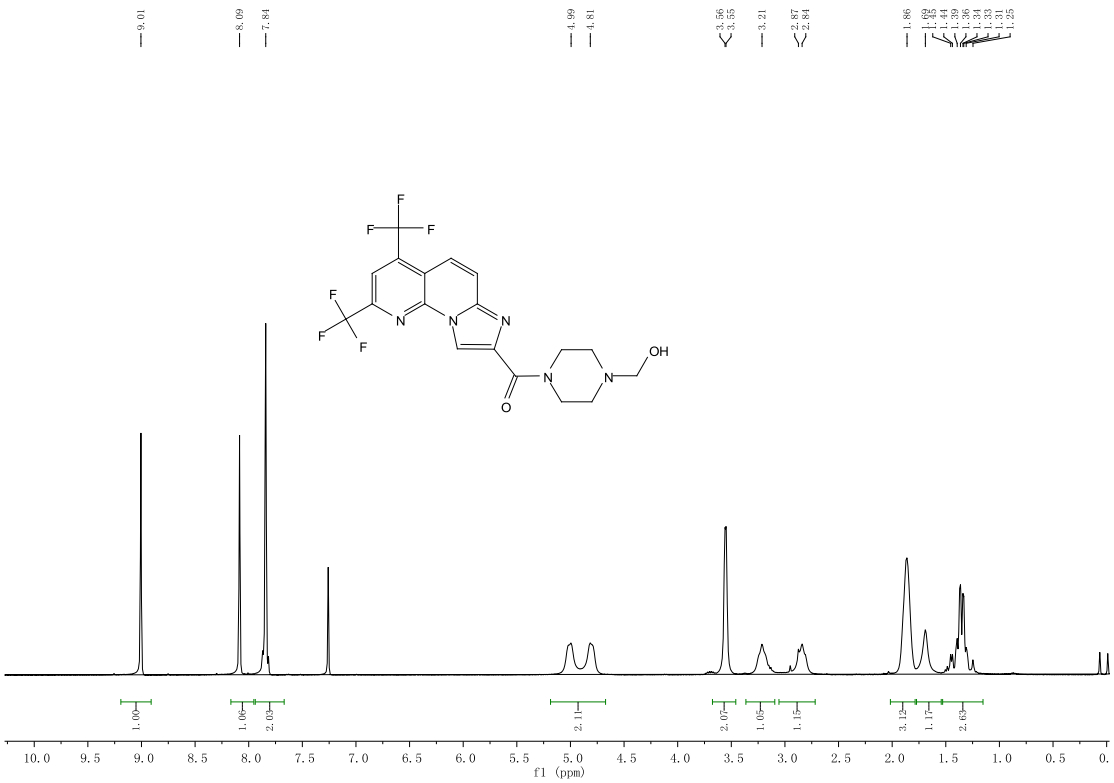
^{13}C NMR Spectrum for **4** (DMSO-d_6, 100 MHz)



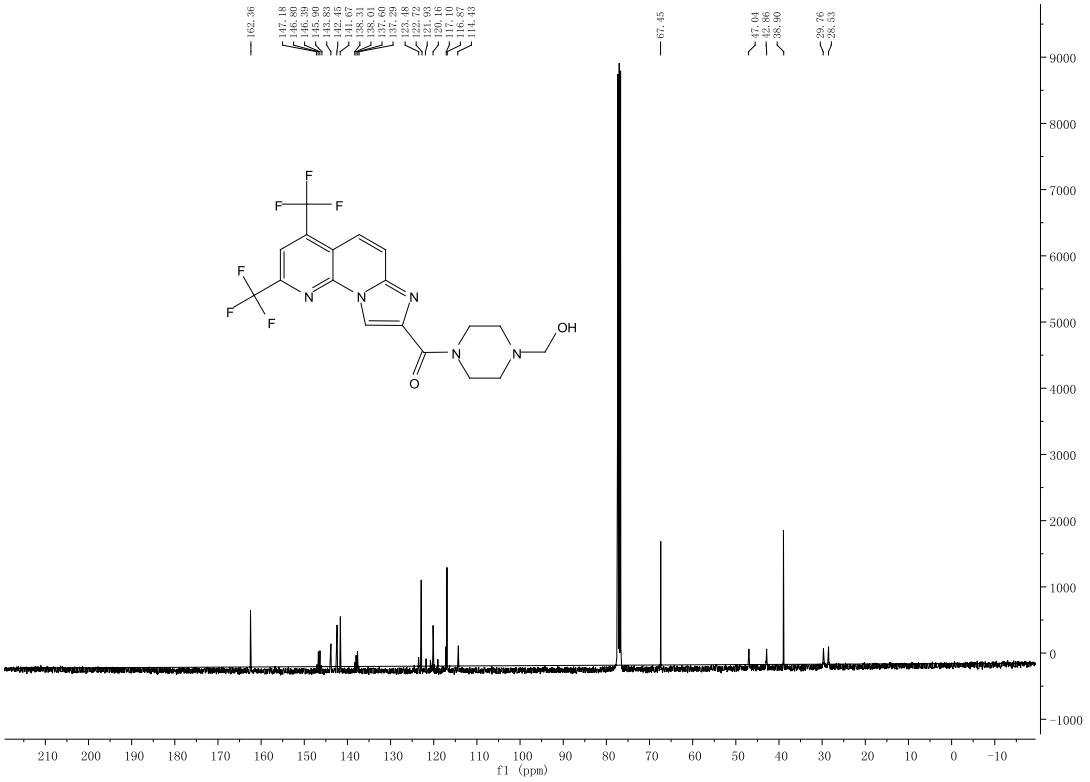
^{1}H NMR Spectrum for **5a** (CD_3OD-d_4, 400 MHz)



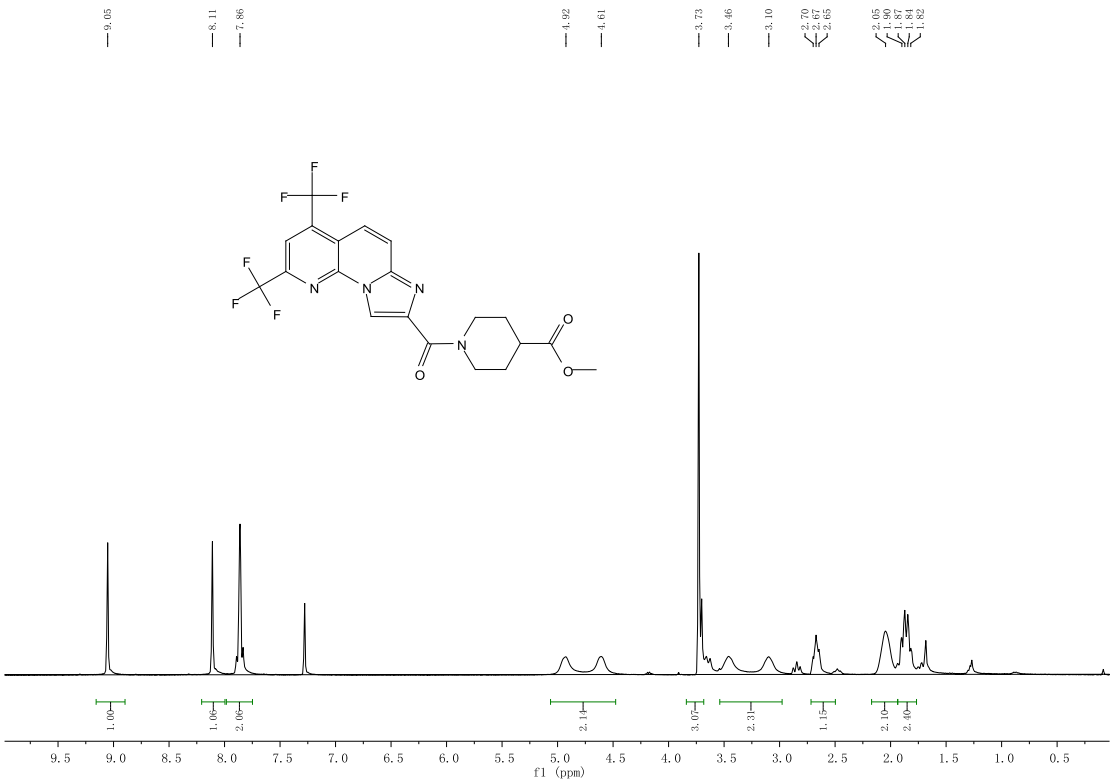
^{13}C NMR Spectrum for **5a** (CD_3OD-d_4, 100 MHz)



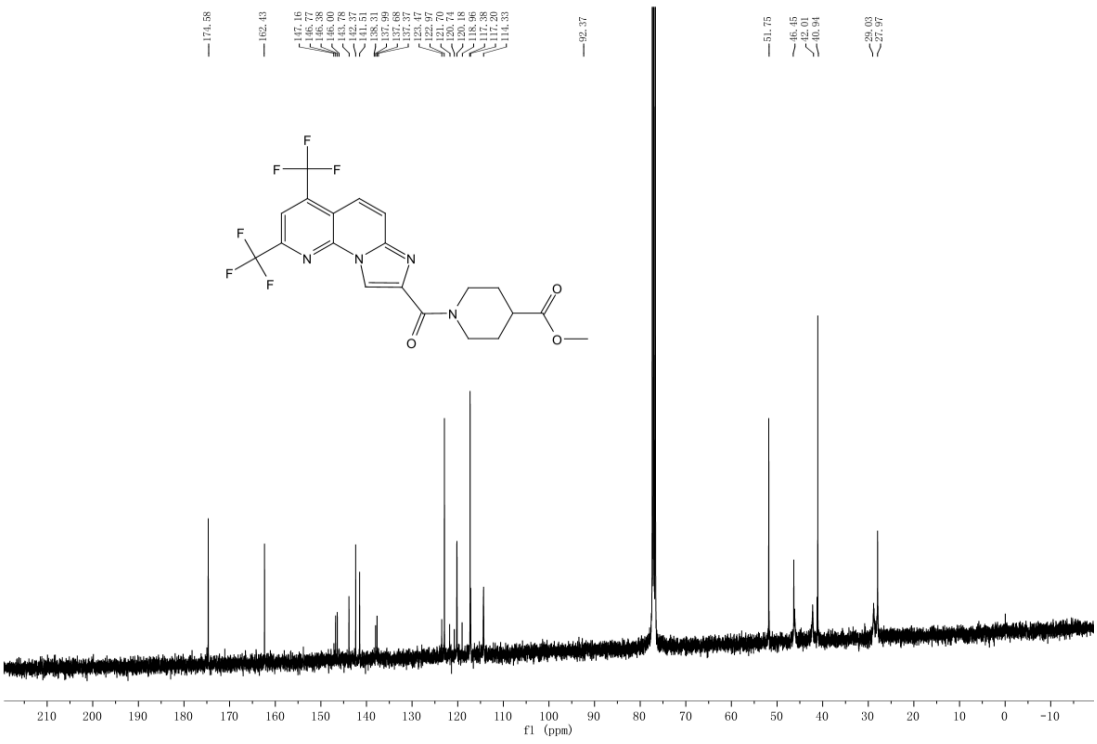
^{1}H NMR Spectrum for **5b** ($CDCl_3$, 400 MHz)



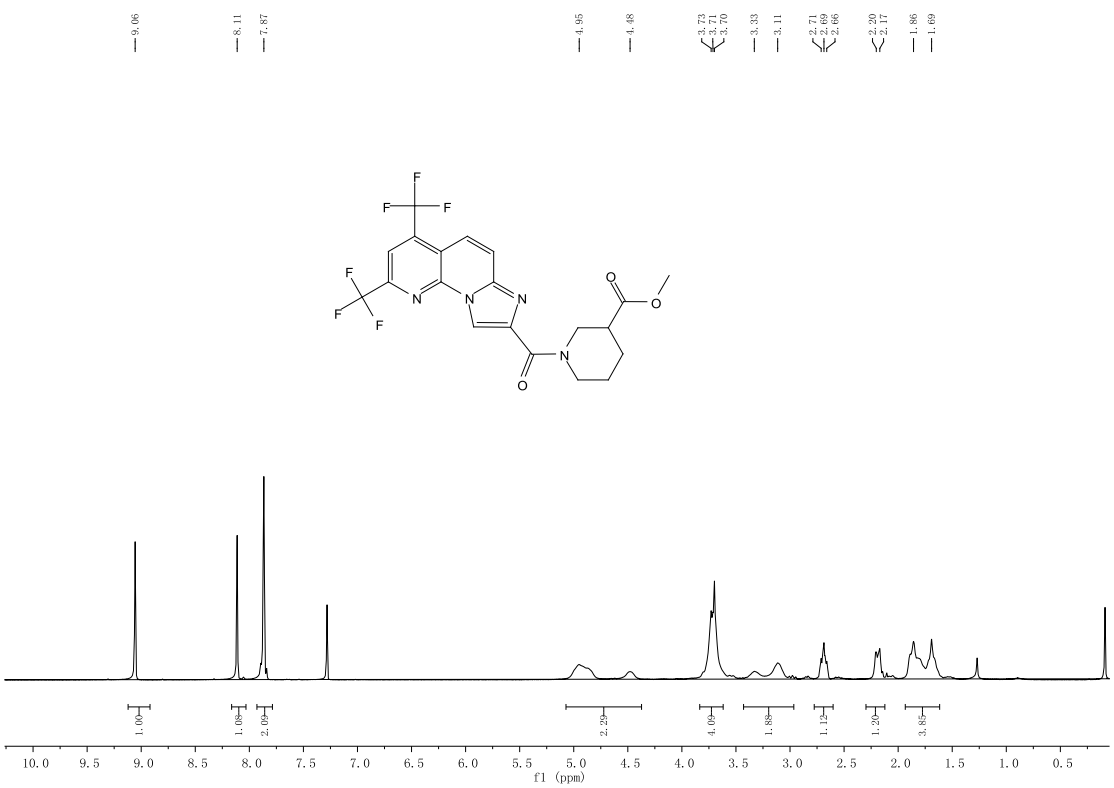
^{13}C NMR Spectrum for **5b** ($CDCl_3$, 100 MHz)



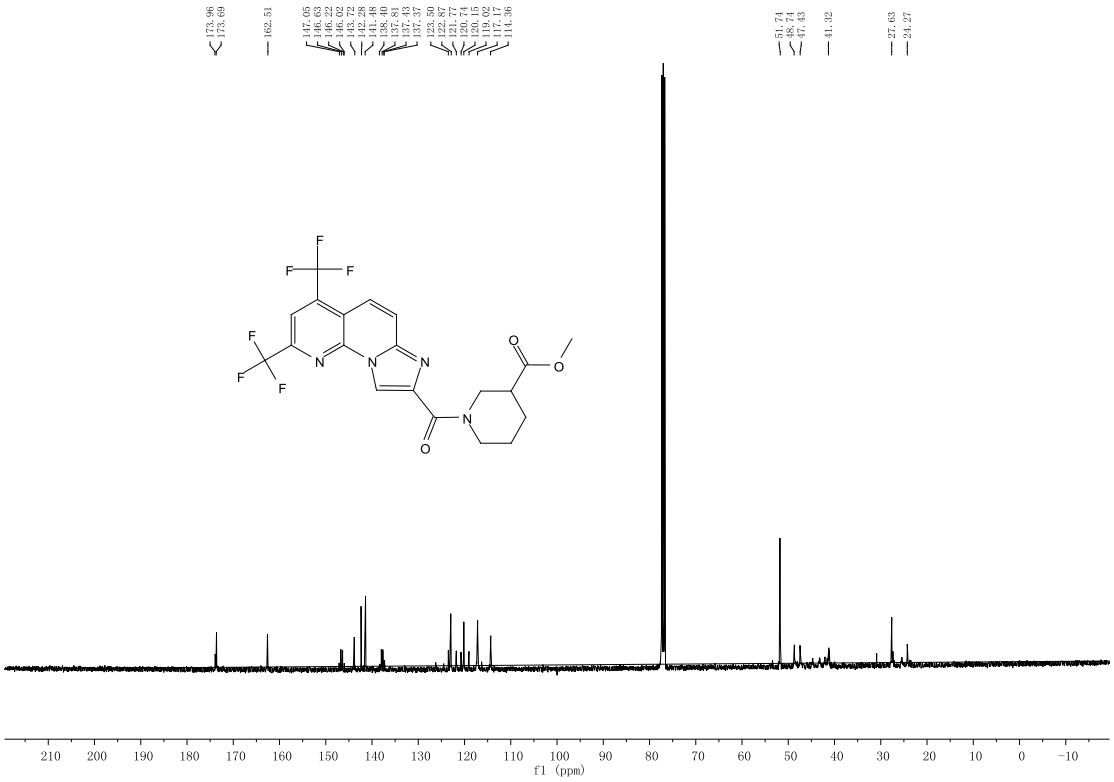
^{1}H NMR Spectrum for **5c** ($CDCl_3$, 400 MHz)



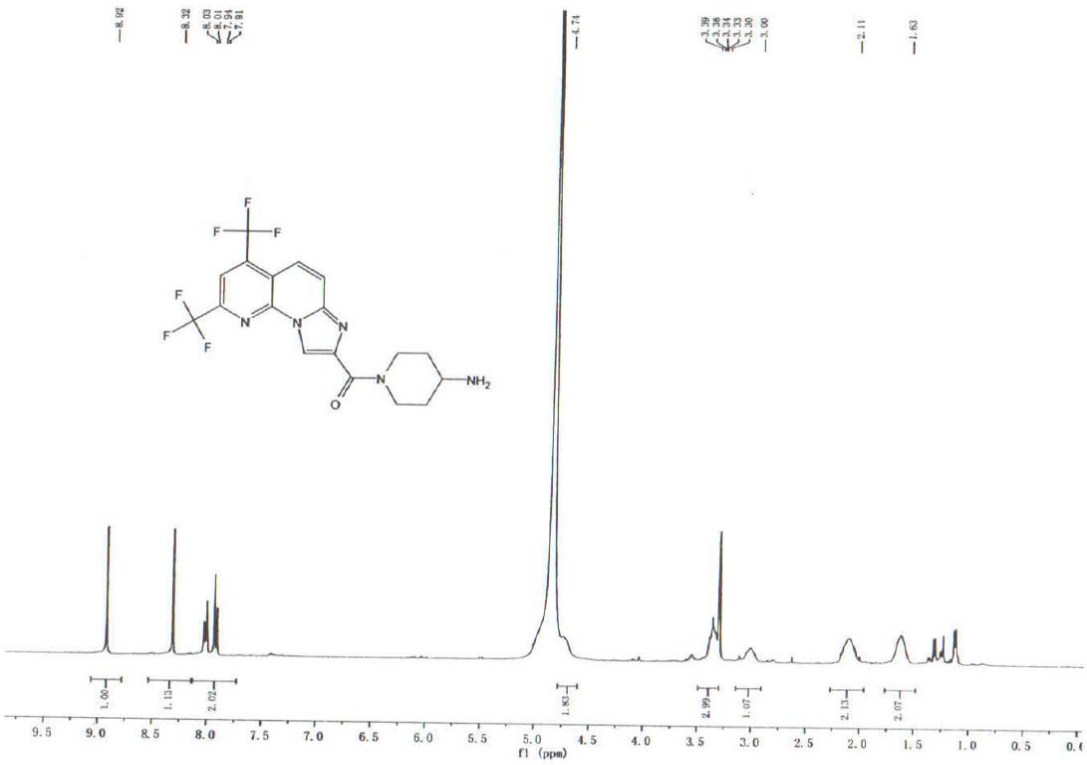
^{13}C NMR Spectrum for **5c** ($CDCl_3$, 100 MHz)



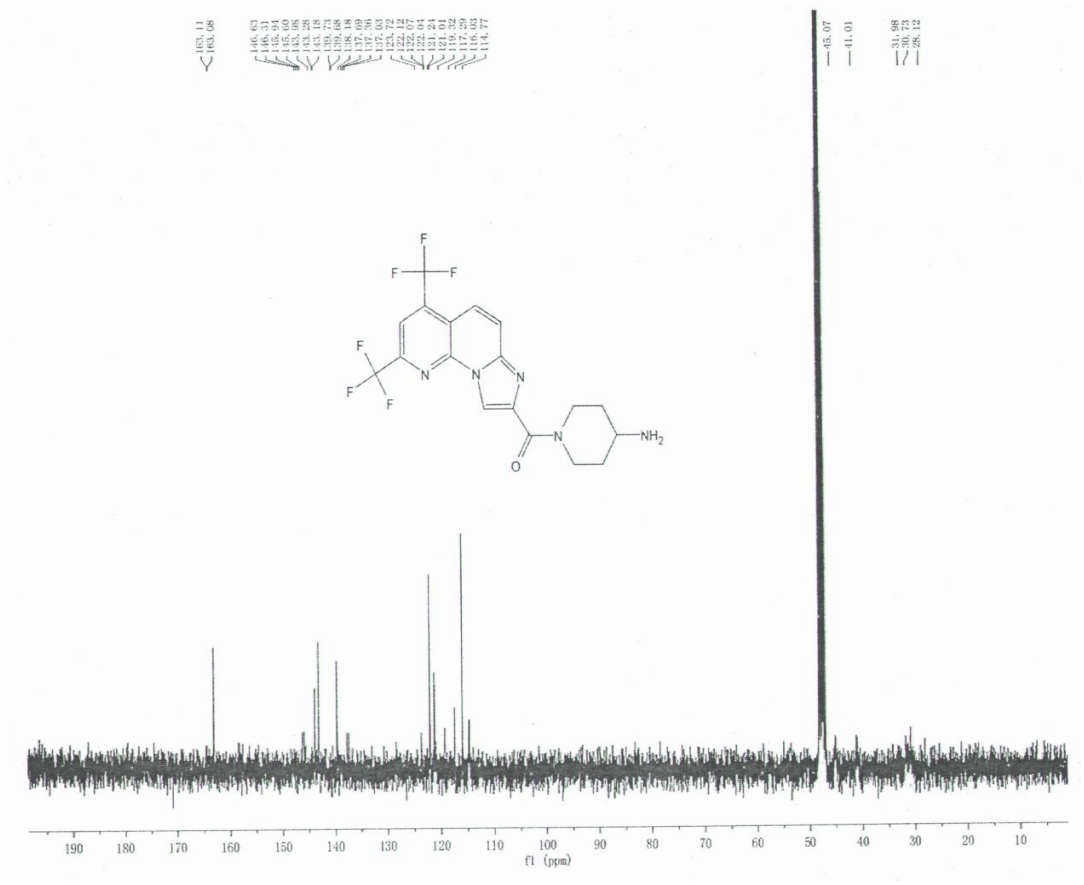
^{1}H NMR Spectrum for **5d** ($CDCl_3$, 400 MHz)



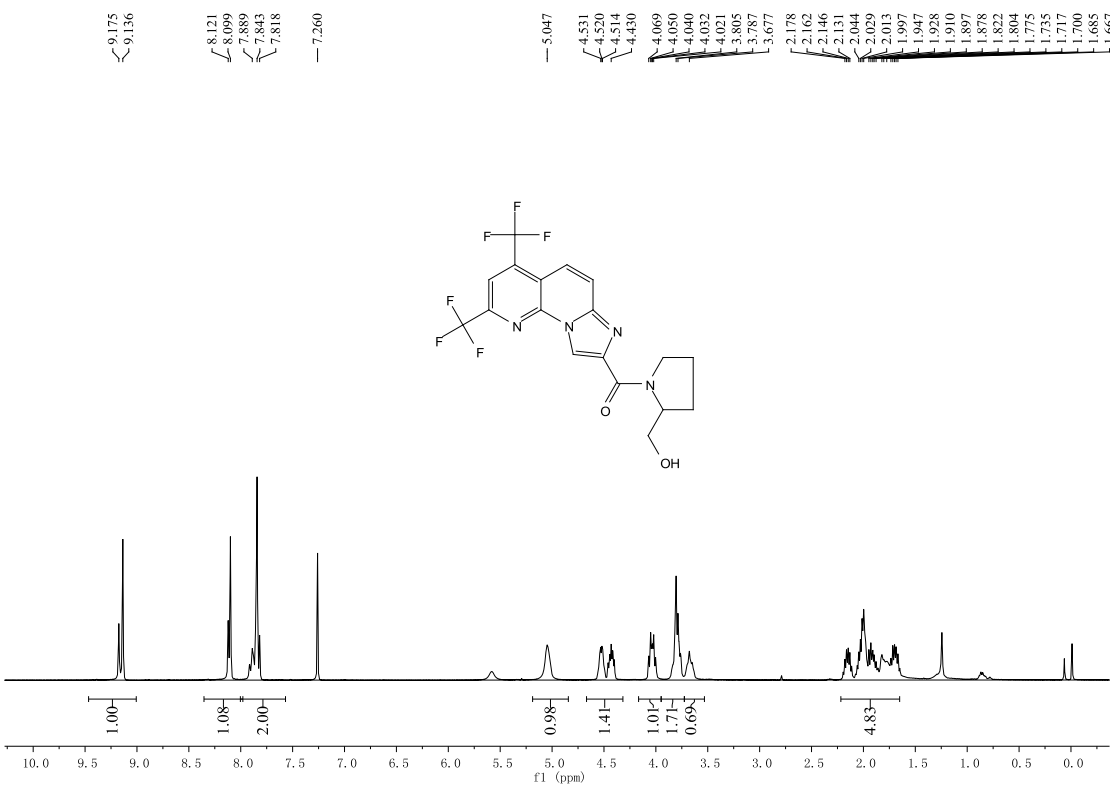
^{13}C NMR Spectrum for **5d** ($CDCl_3$, 100 MHz)



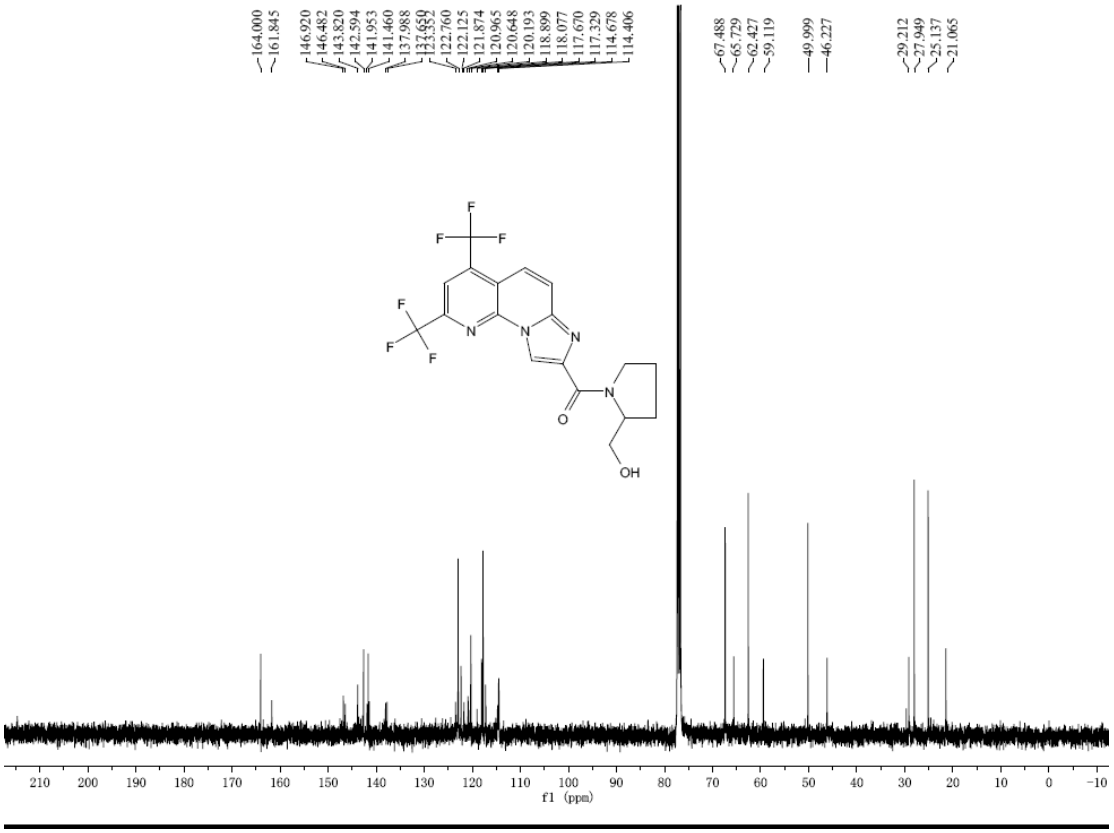
^{1}H NMR Spectrum for **5e** (CD_3OD-d_4, 400 MHz)



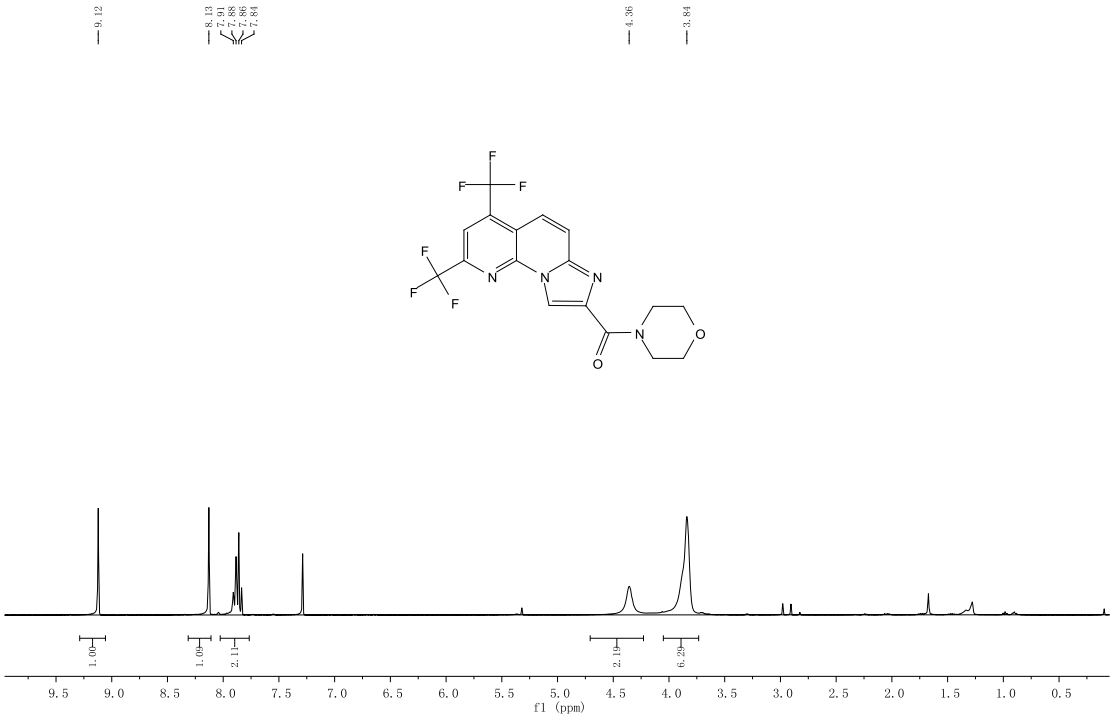
^{13}C NMR Spectrum for **5e** (CD_3OD-d_4, 100 MHz)



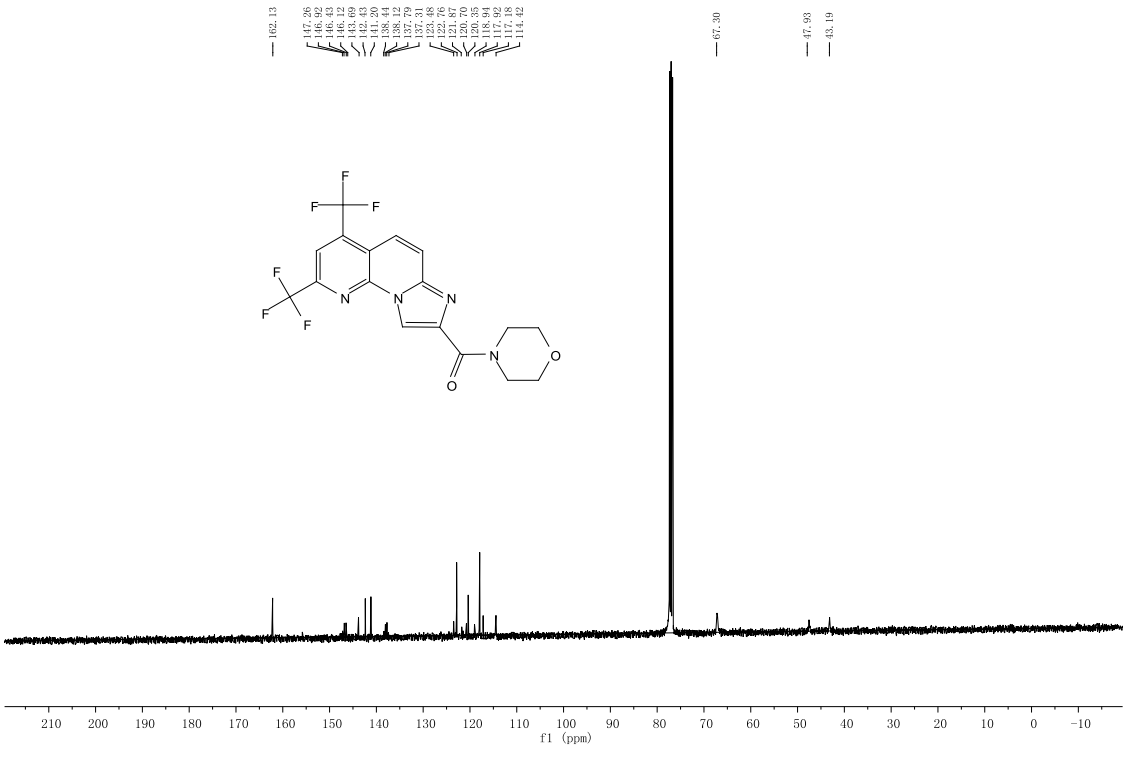
^{1}H NMR Spectrum for **5f** ($CDCl_3$, 400 MHz)



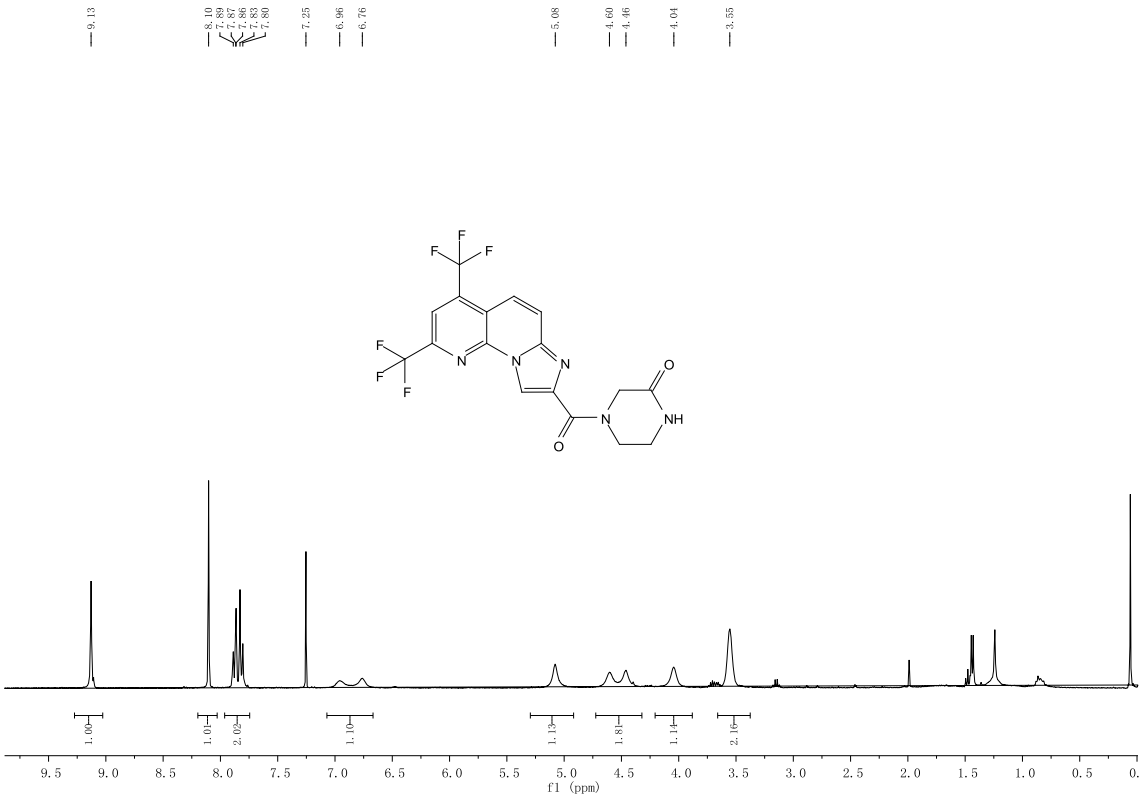
^{13}C NMR Spectrum for **5f** ($CDCl_3$, 100 MHz)



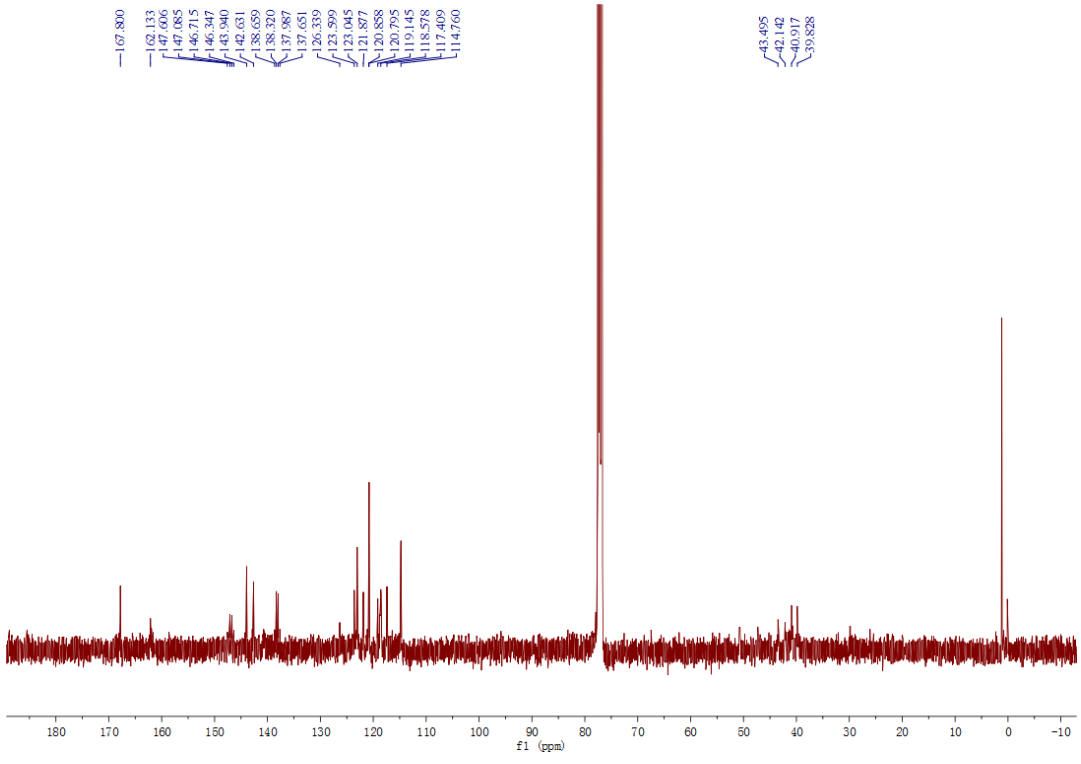
^{1}H NMR Spectrum for **5g** ($CDCl_3$, 400 MHz)



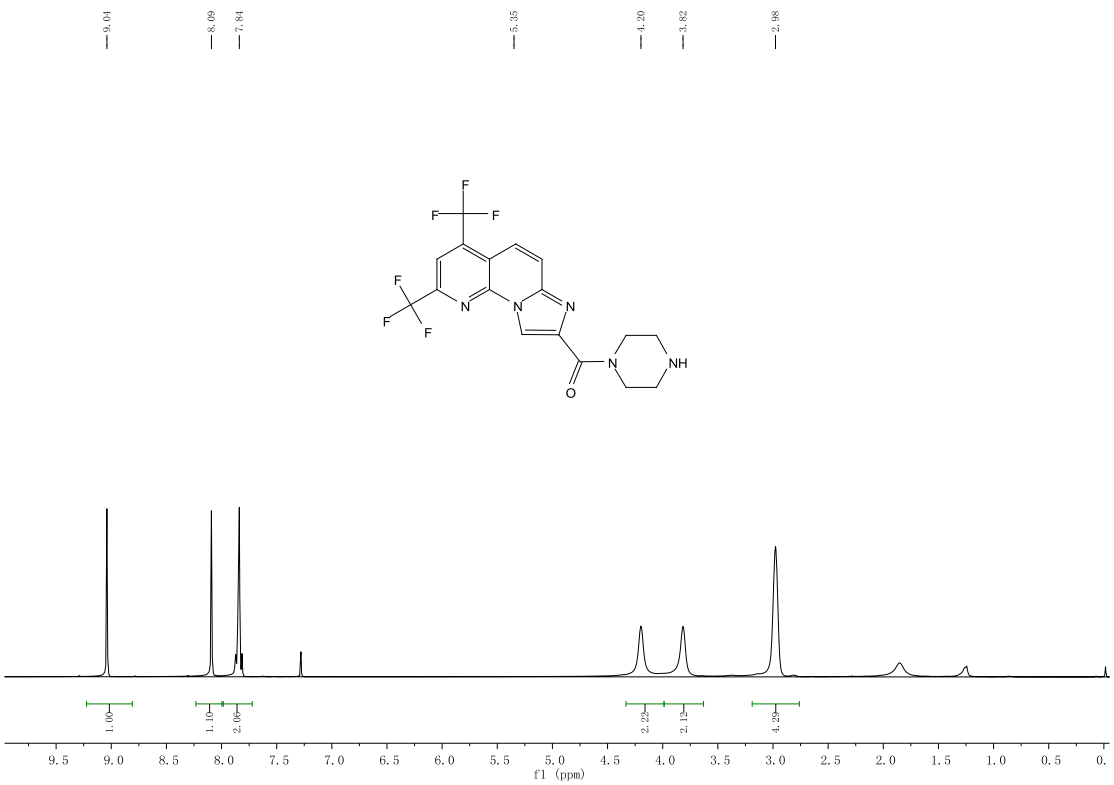
^{13}C NMR Spectrum for **5g** ($CDCl_3$, 100 MHz)



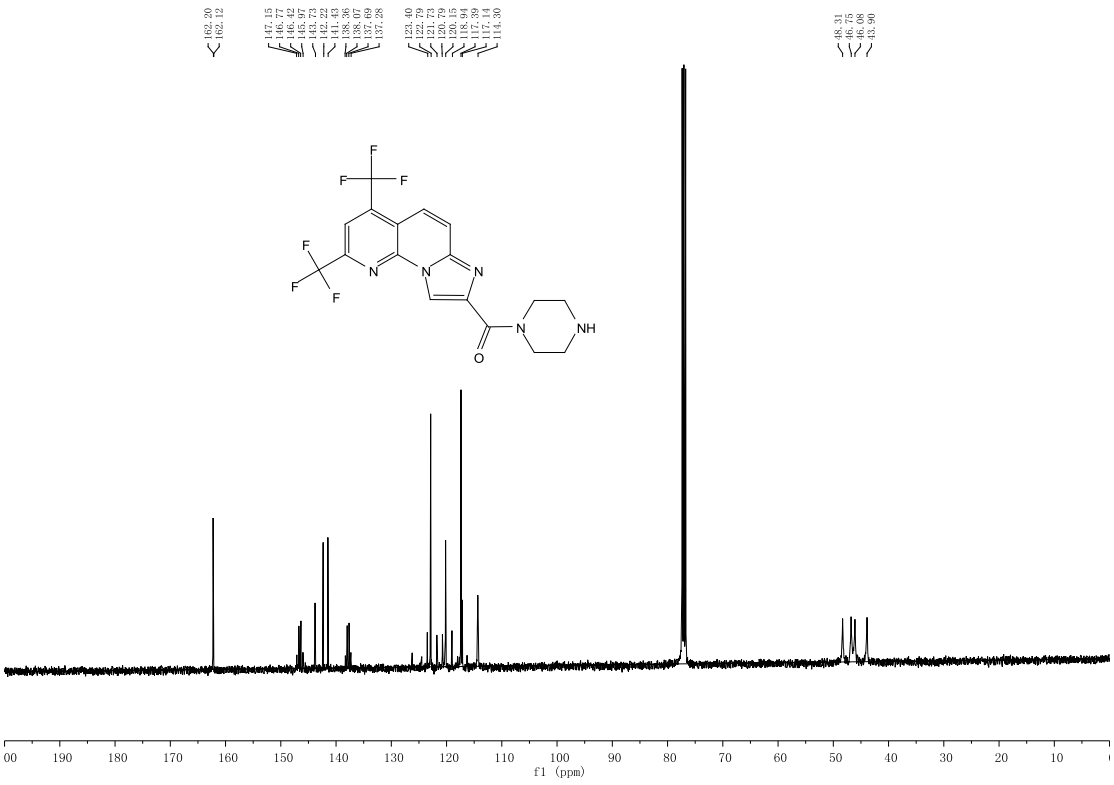
^{1}H NMR Spectrum for **5h** ($CDCl_3$, 400 MHz)



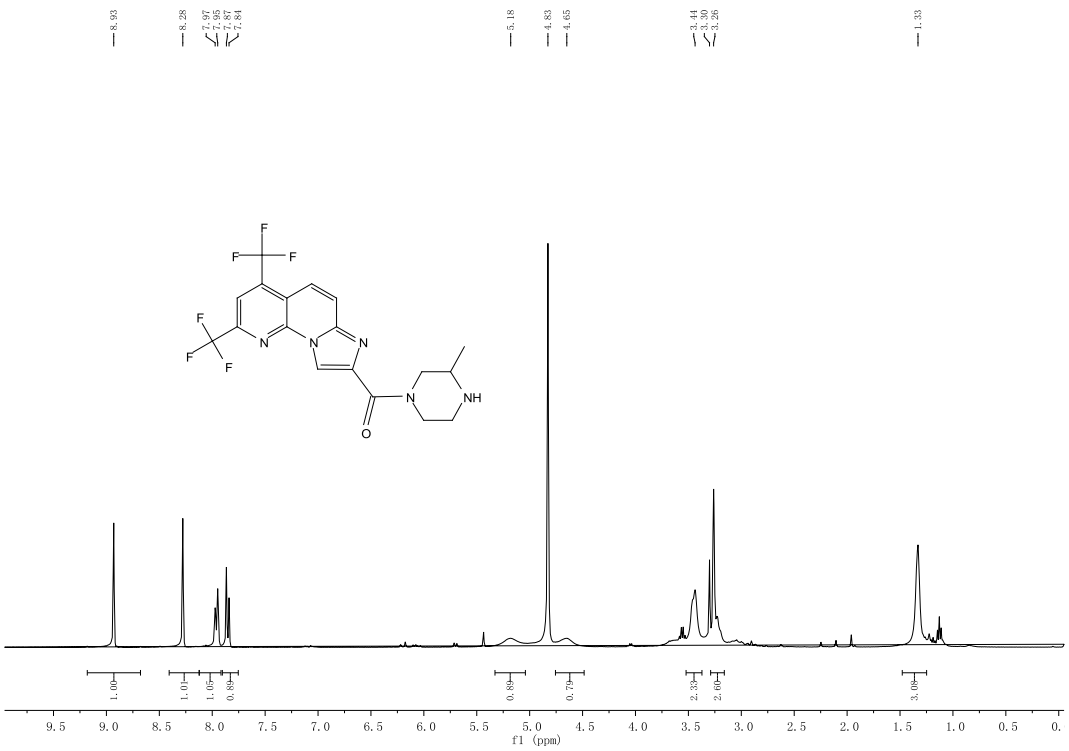
^{13}C NMR Spectrum for **5h** ($CDCl_3$, 100 MHz)



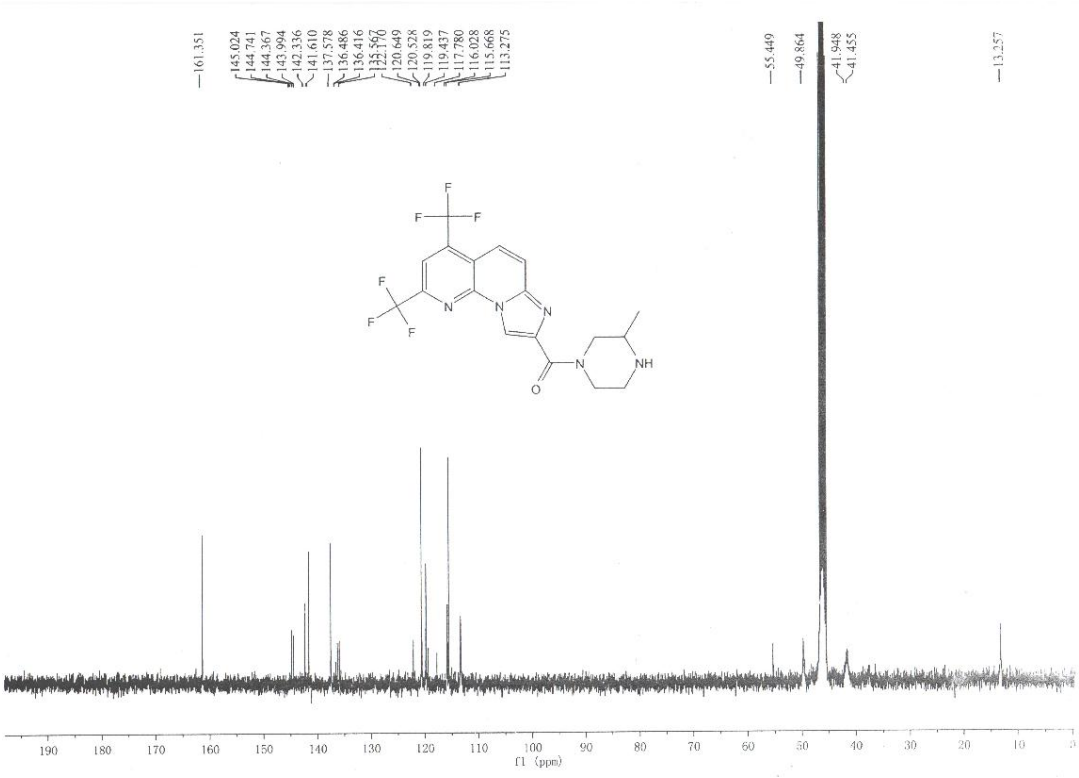
^{1}H NMR Spectrum for **5i** ($CDCl_3$, 400 MHz)



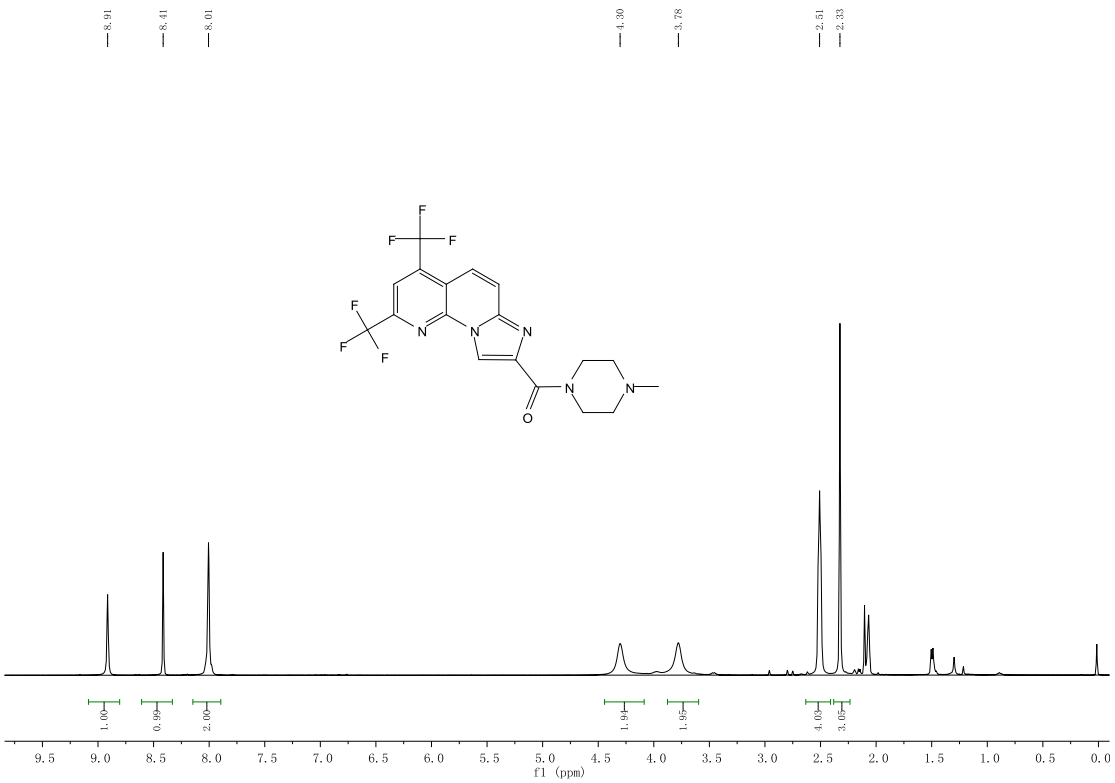
^{13}C NMR Spectrum for **5i** ($CDCl_3$, 100 MHz)



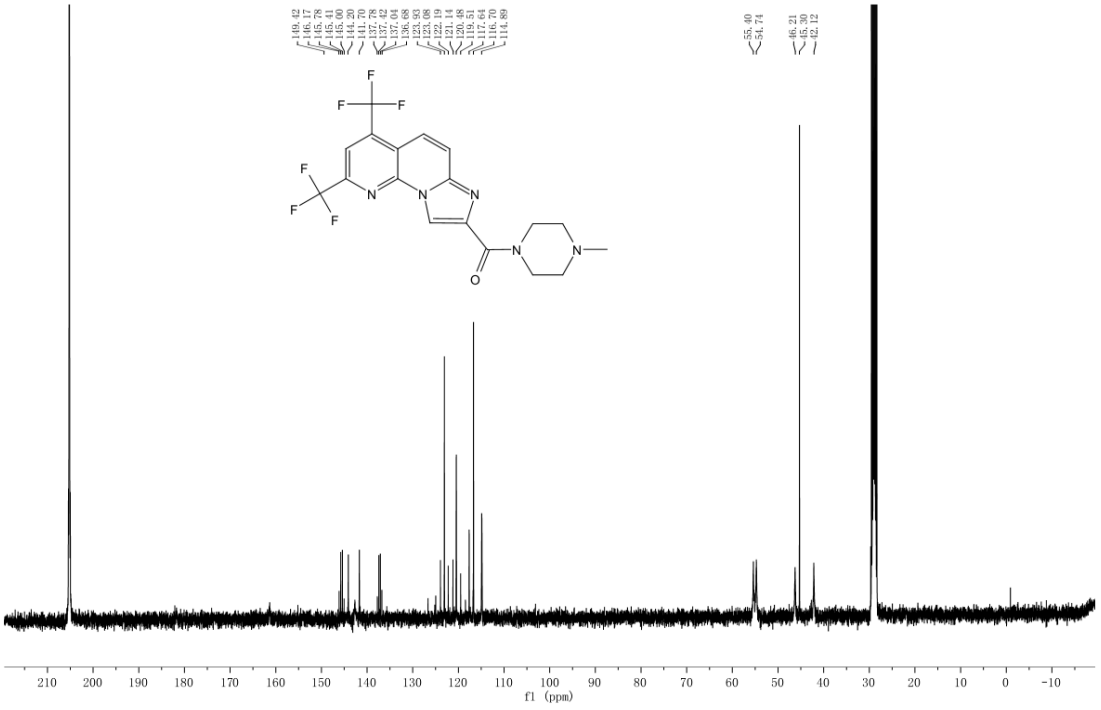
^{1}H NMR Spectrum for **5j** (CD_3OD-d_4, 400 MHz)



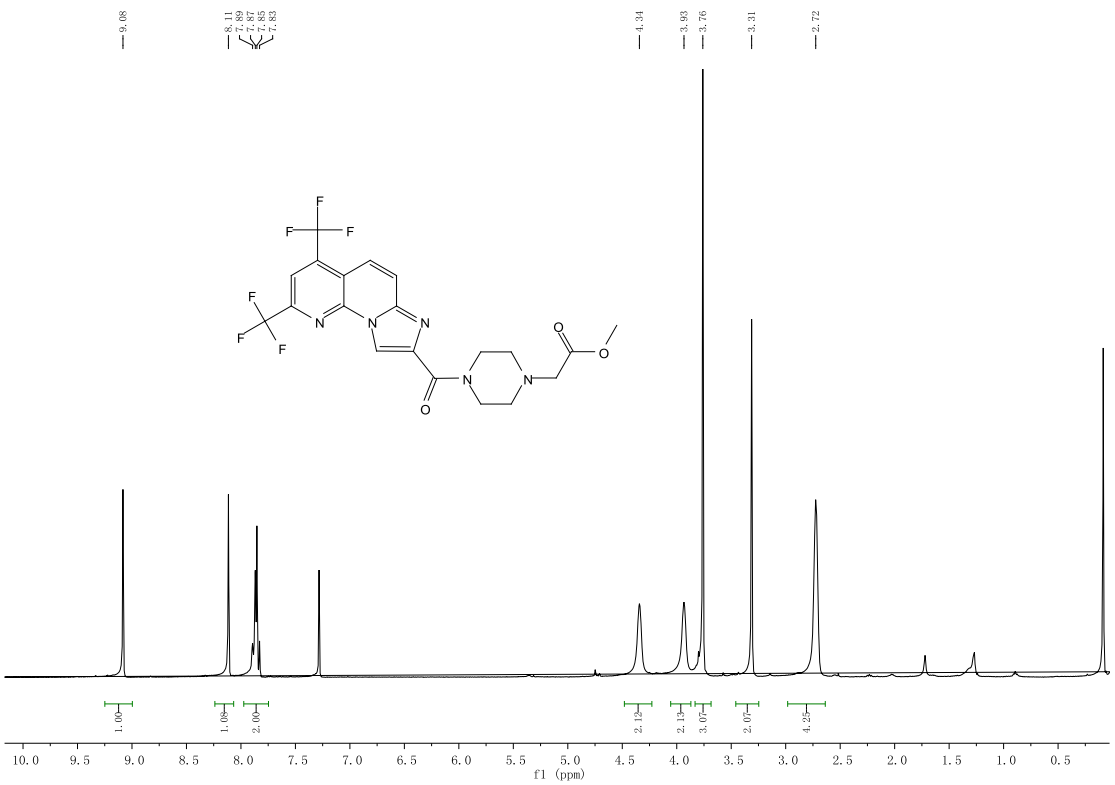
^{13}C NMR Spectrum for **5j** (CD_3OD-d_4, 100 MHz)



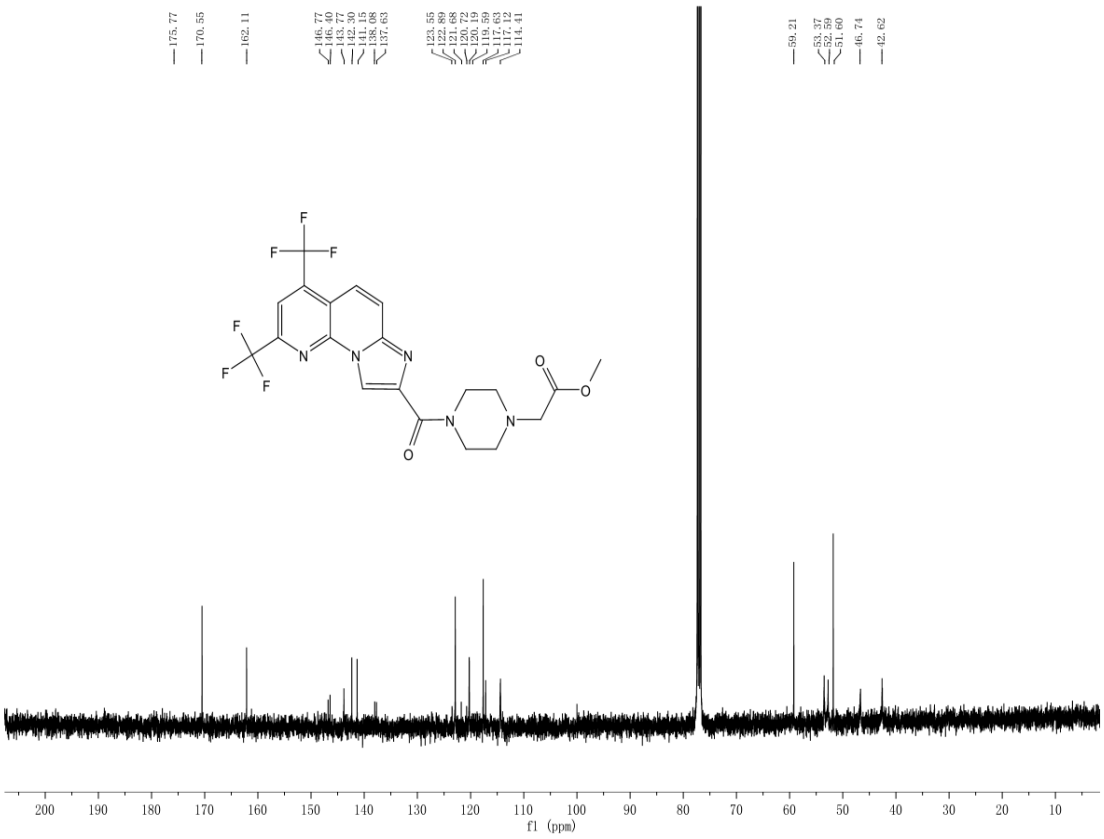
^{1}H NMR Spectrum for **5k** (Acetone-d_6, 400 MHz)



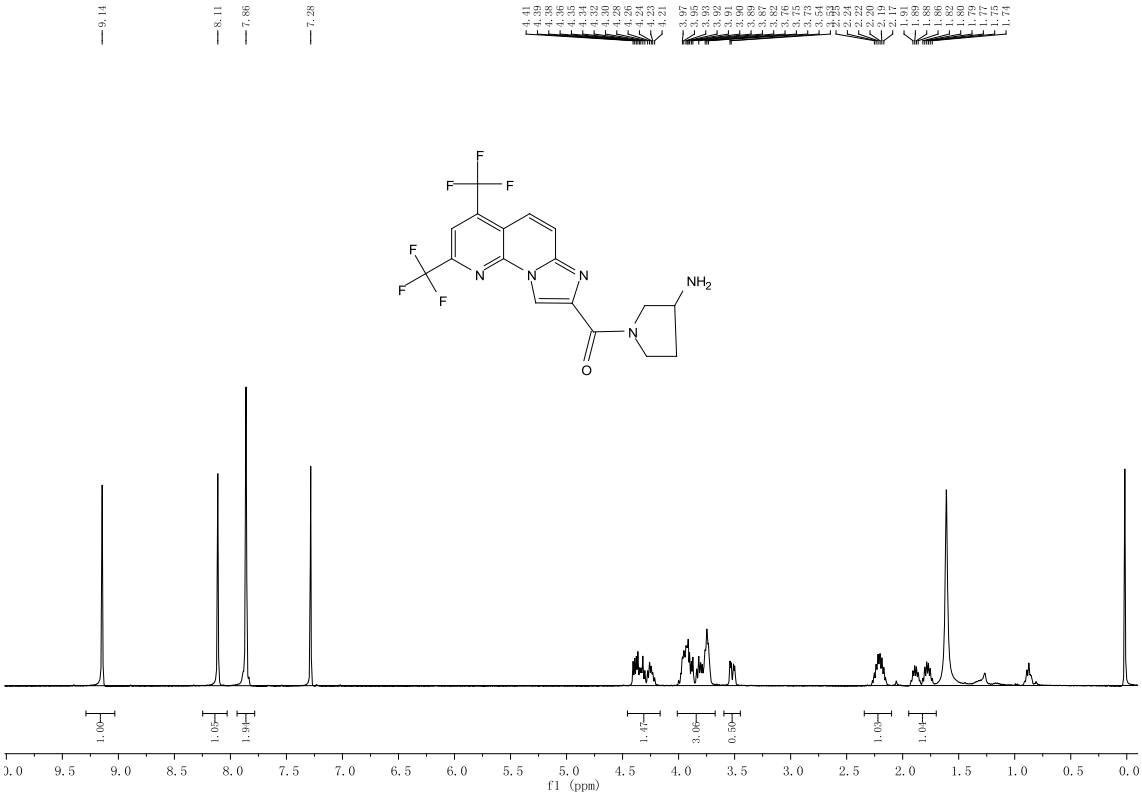
^{13}C NMR Spectrum for **5k** (Acetone-d_6, 100 MHz)



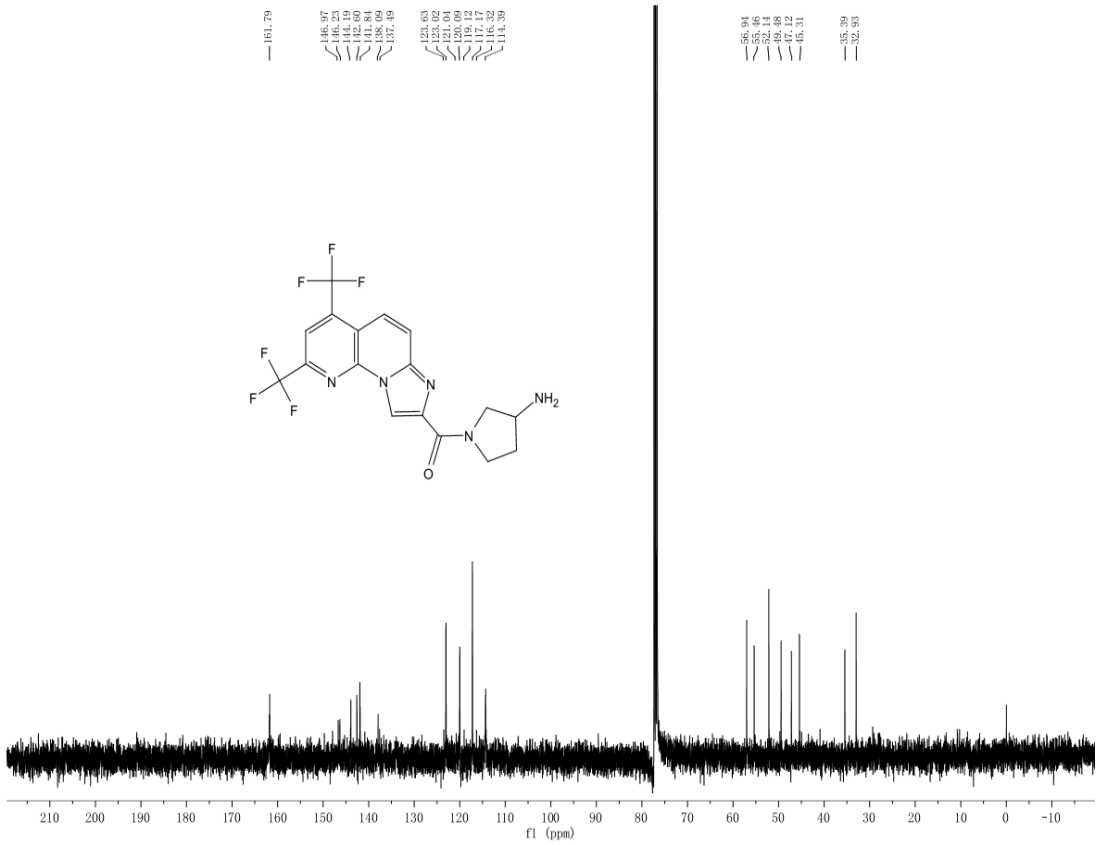
^{1}H NMR Spectrum for **5l** ($CDCl_3$, 400 MHz)



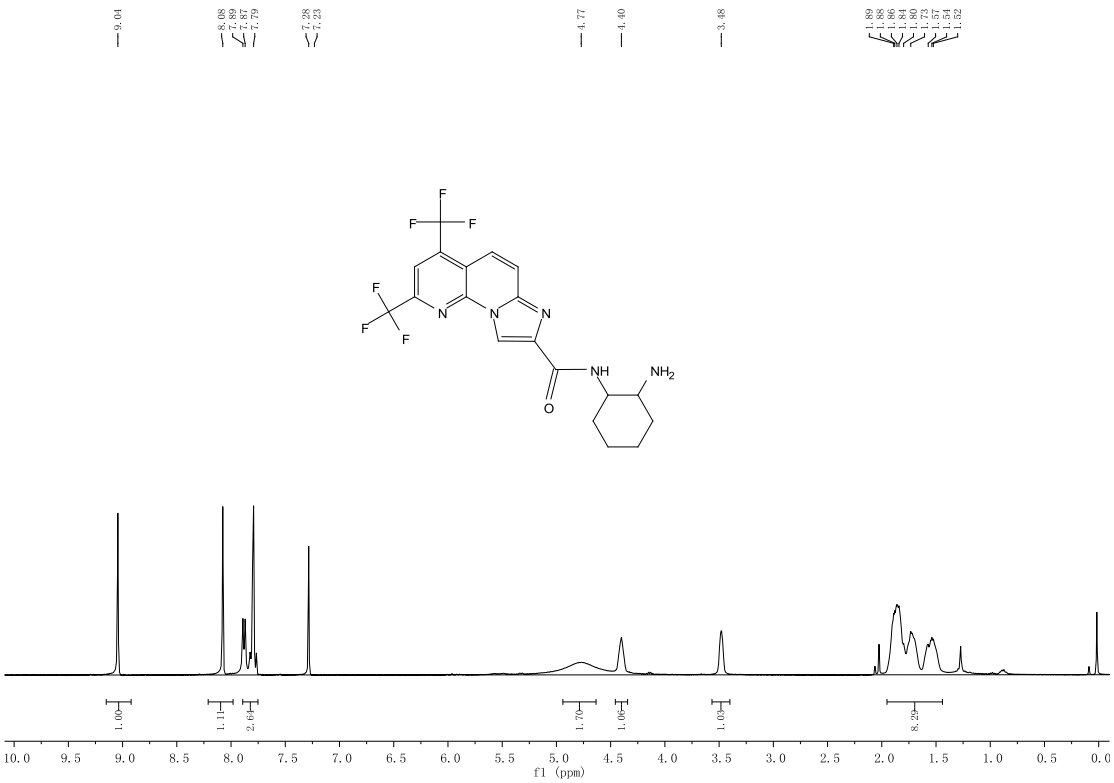
^{13}C NMR Spectrum for **5l** ($CDCl_3$, 100 MHz)



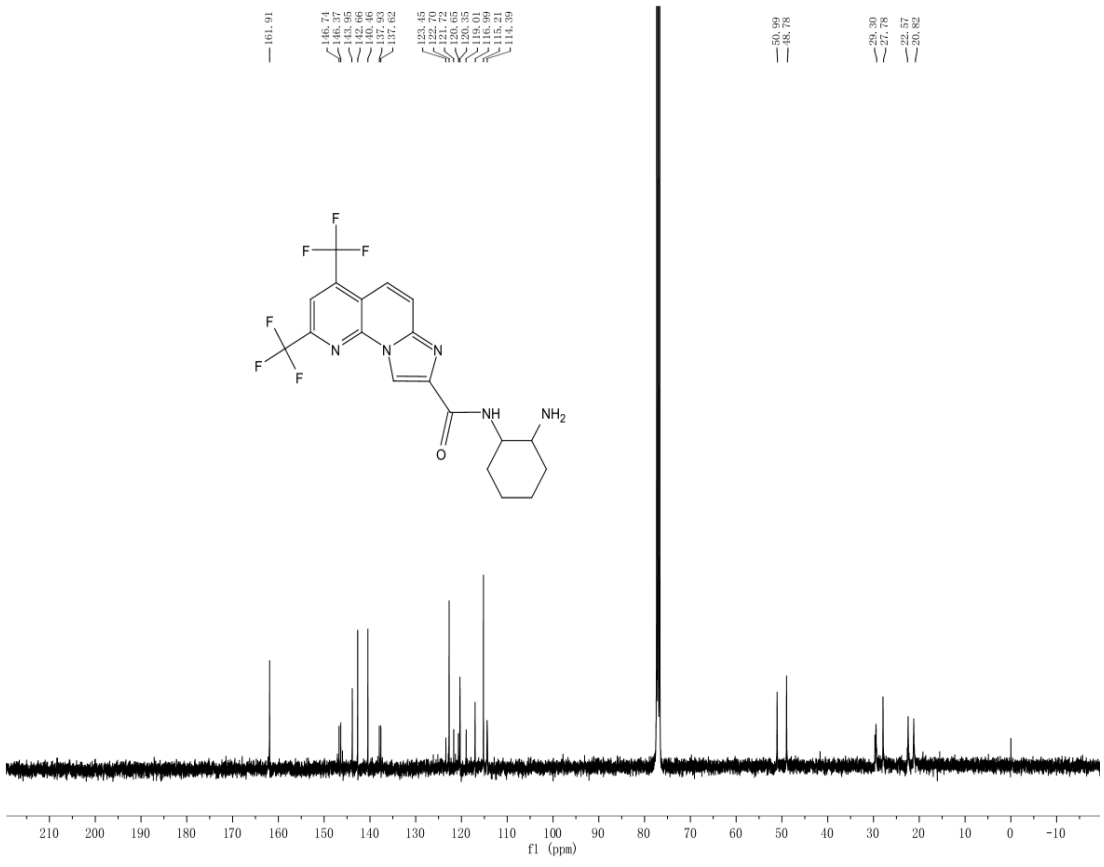
^{1}H NMR Spectrum for **5m** ($CDCl_3$, 400 MHz)



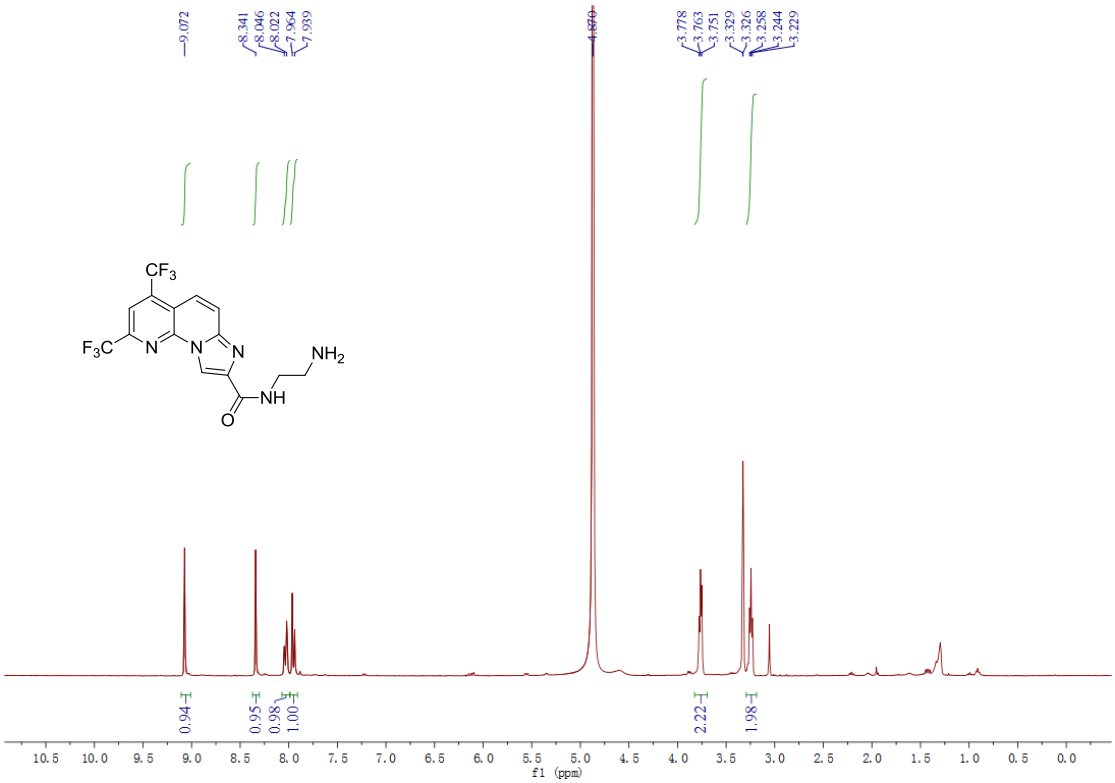
^{13}C NMR Spectrum for **5m** ($CDCl_3$, 100 MHz)



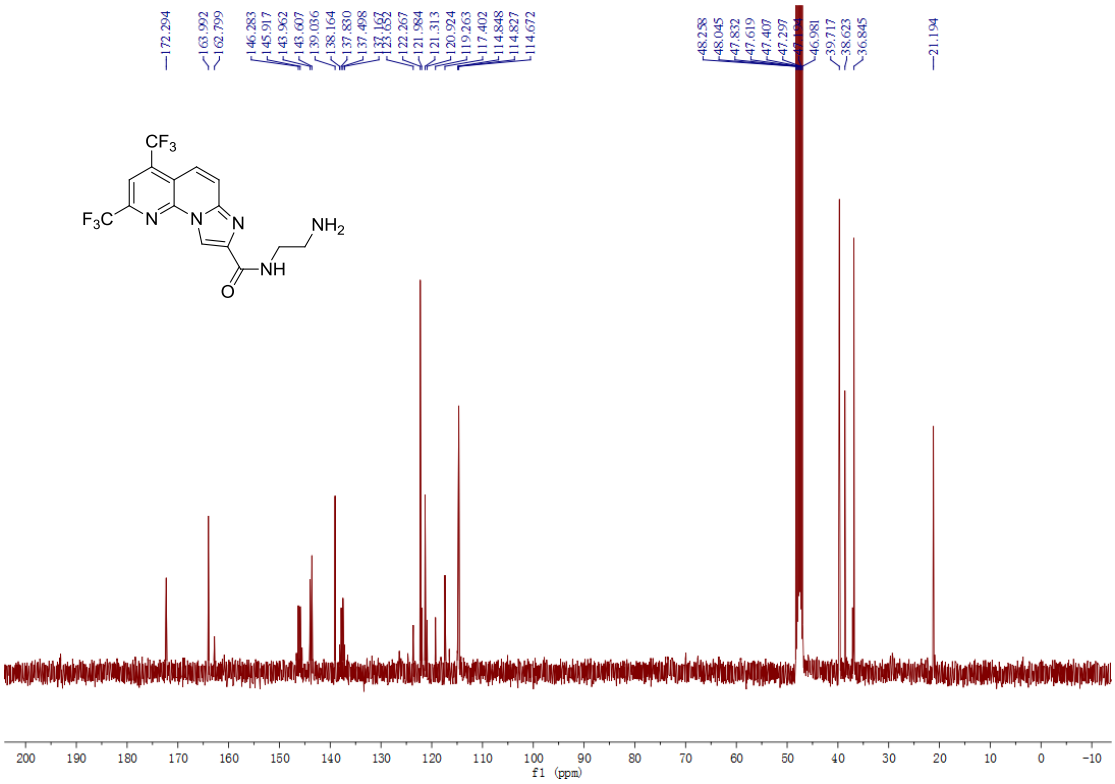
^{1}H NMR Spectrum for **5n** ($CDCl_3$, 400 MHz)



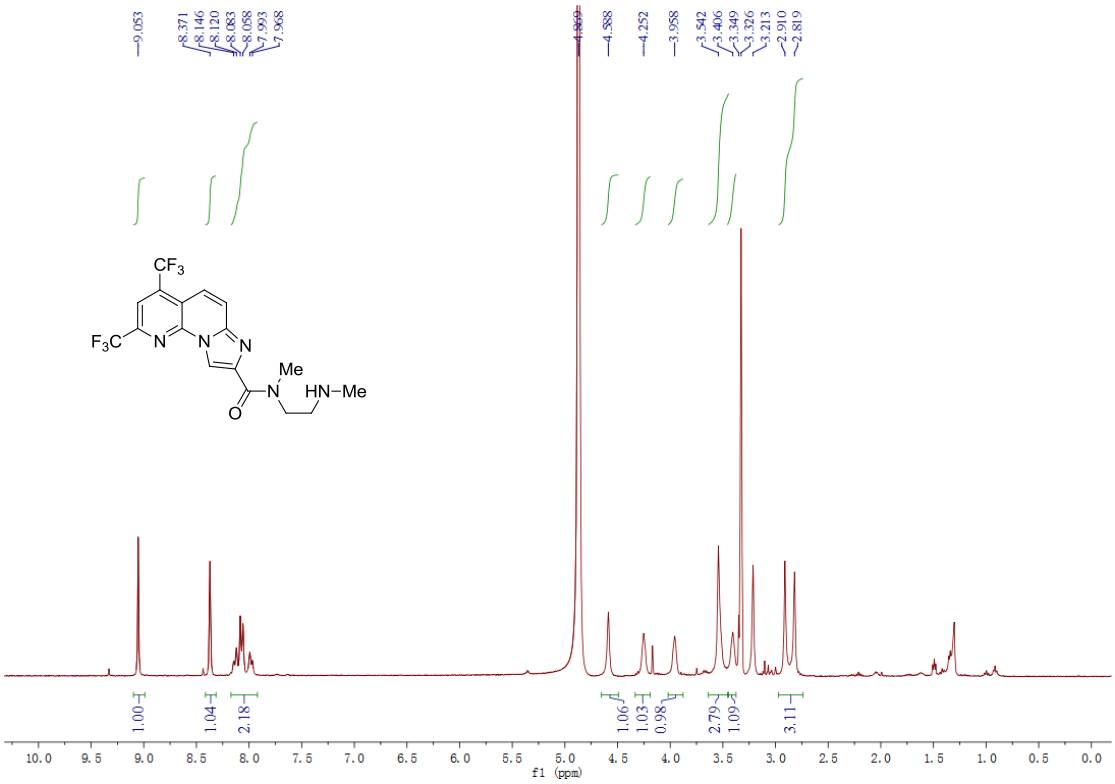
^{13}C NMR Spectrum for **5n** ($CDCl_3$, 100 MHz)



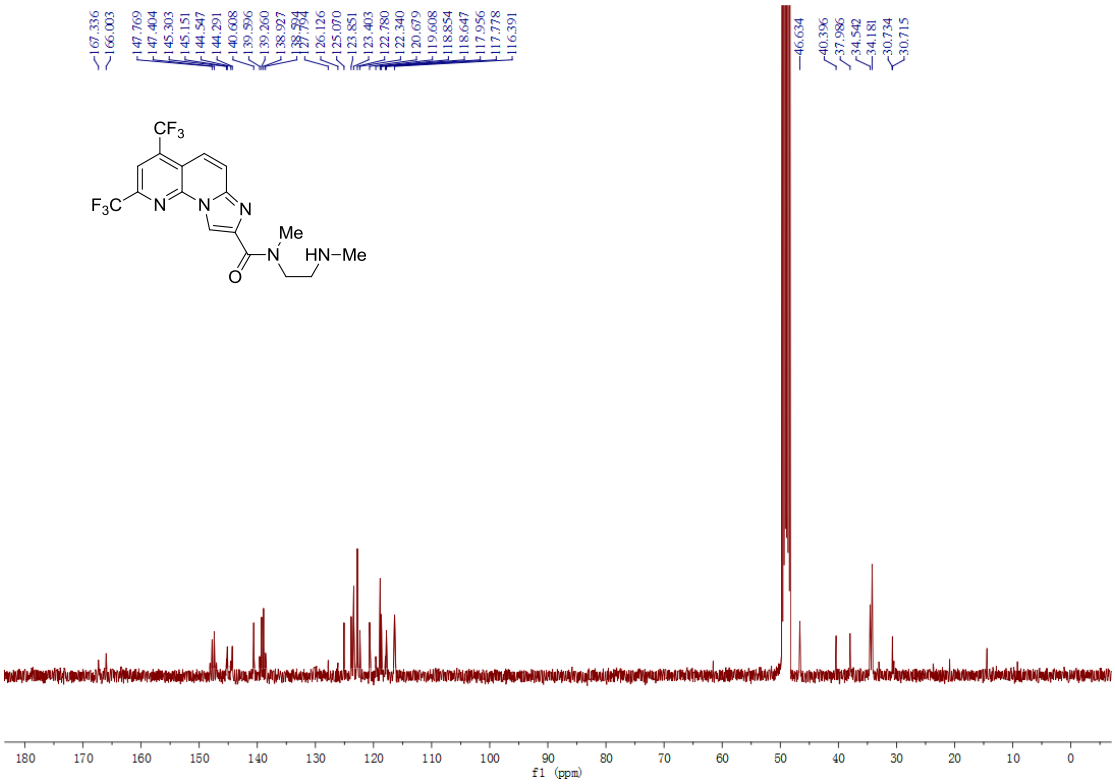
^{1}H NMR Spectrum for **5o** (CD_3OD-d_4, 400 MHz)



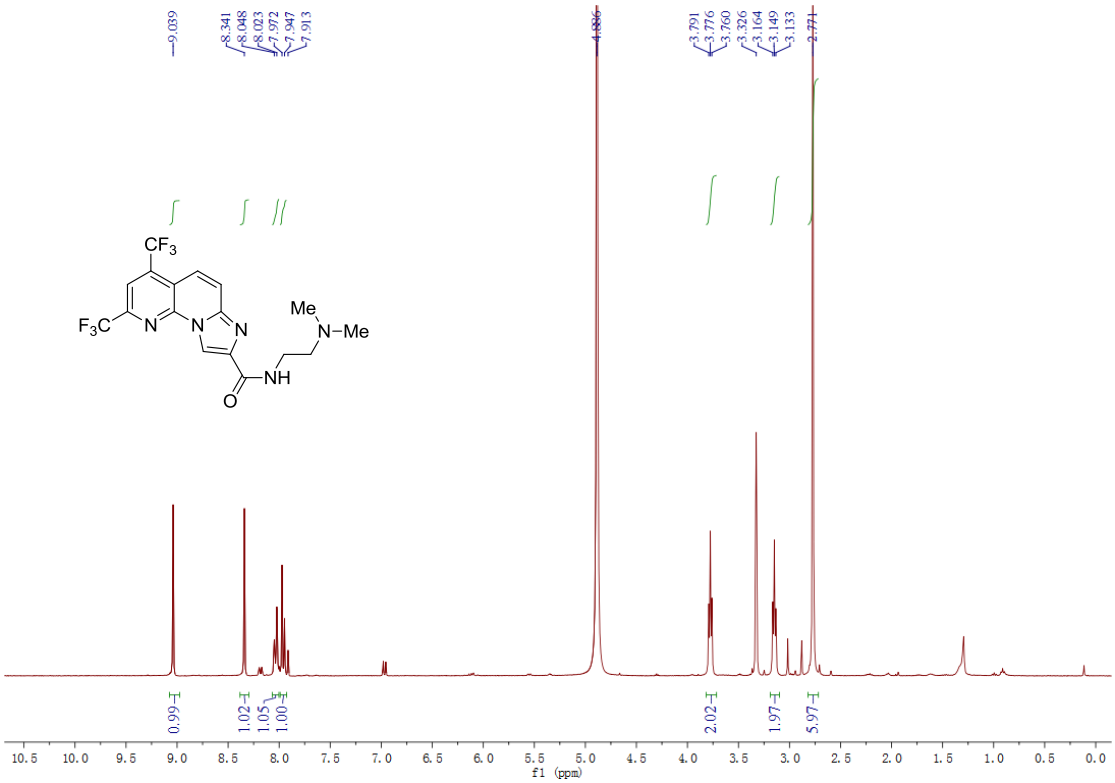
^{13}C NMR Spectrum for **5o** (CD_3OD-d_4, 100 MHz)



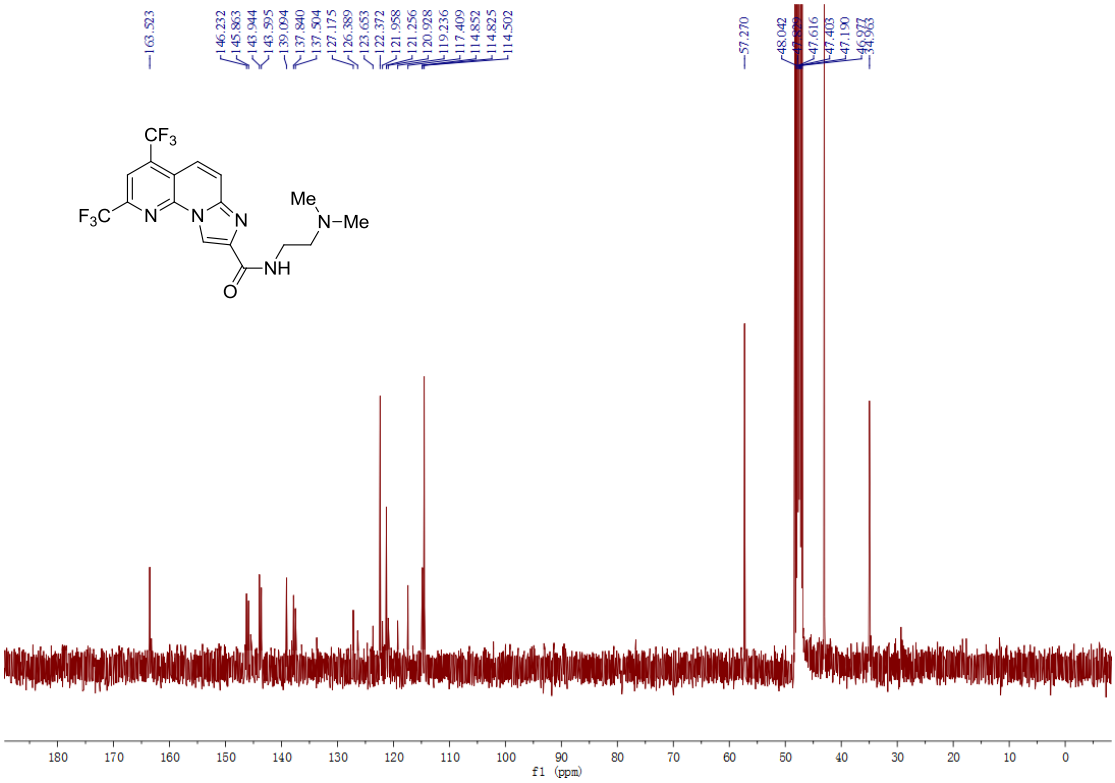
^{1}H NMR Spectrum for **5p** (CD_3OD-d_4, 400 MHz)



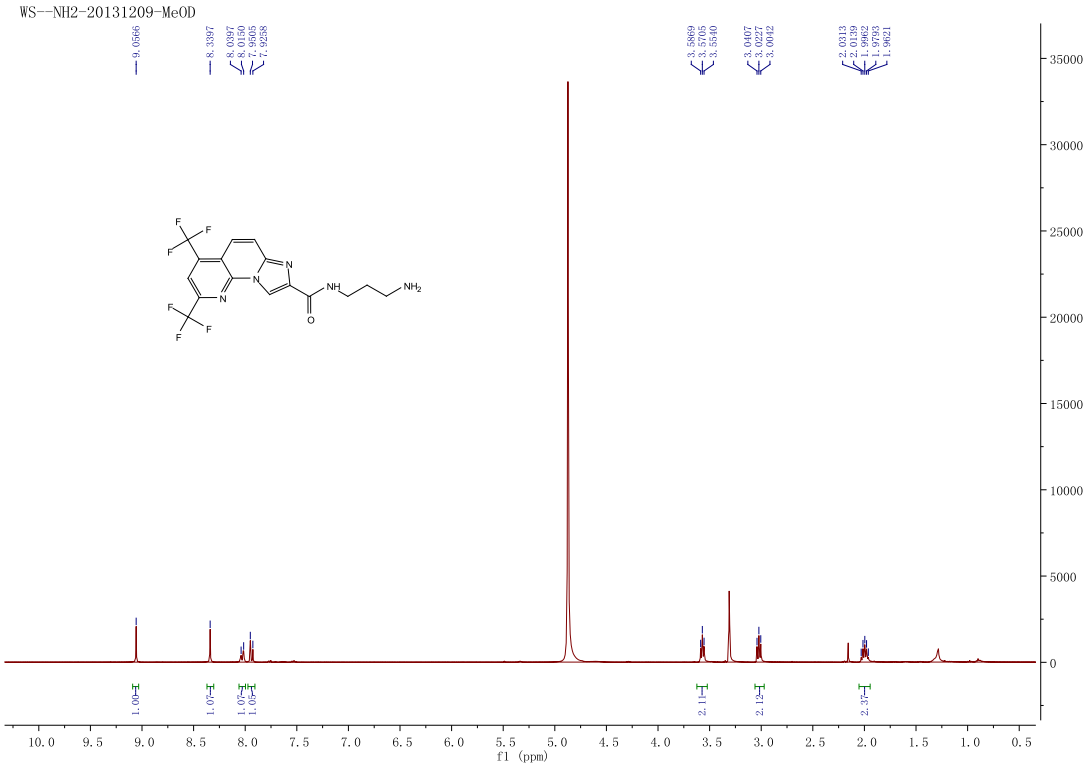
^{13}C NMR Spectrum for **5p** (CD_3OD-d_4, 100 MHz)



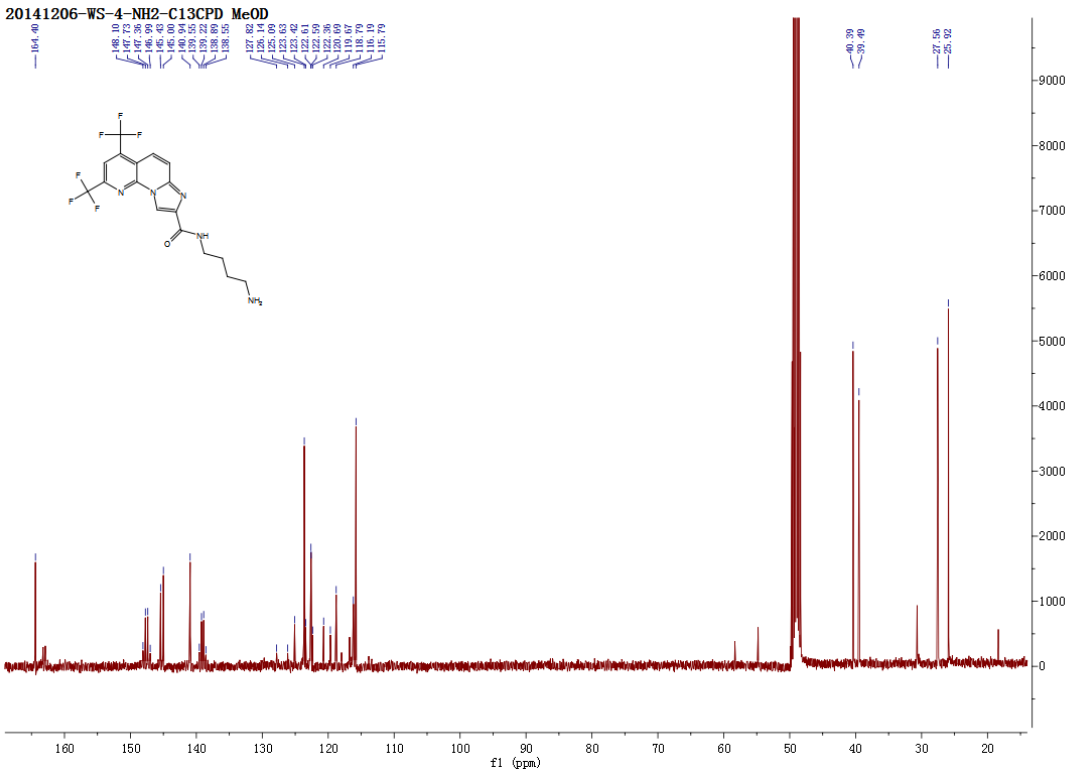
^{1}H NMR Spectrum for **5q** (CD_3OD-d_4, 400 MHz)



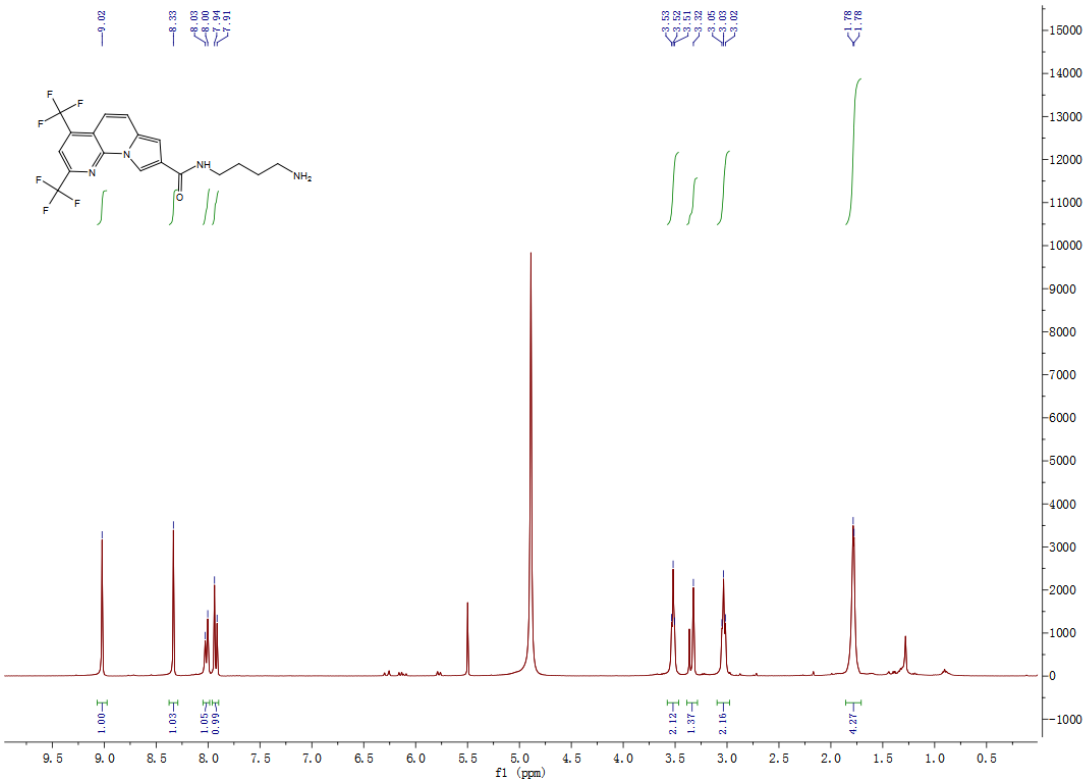
^{13}C NMR Spectrum for **5q** (CD_3OD-d_4, 100 MHz)



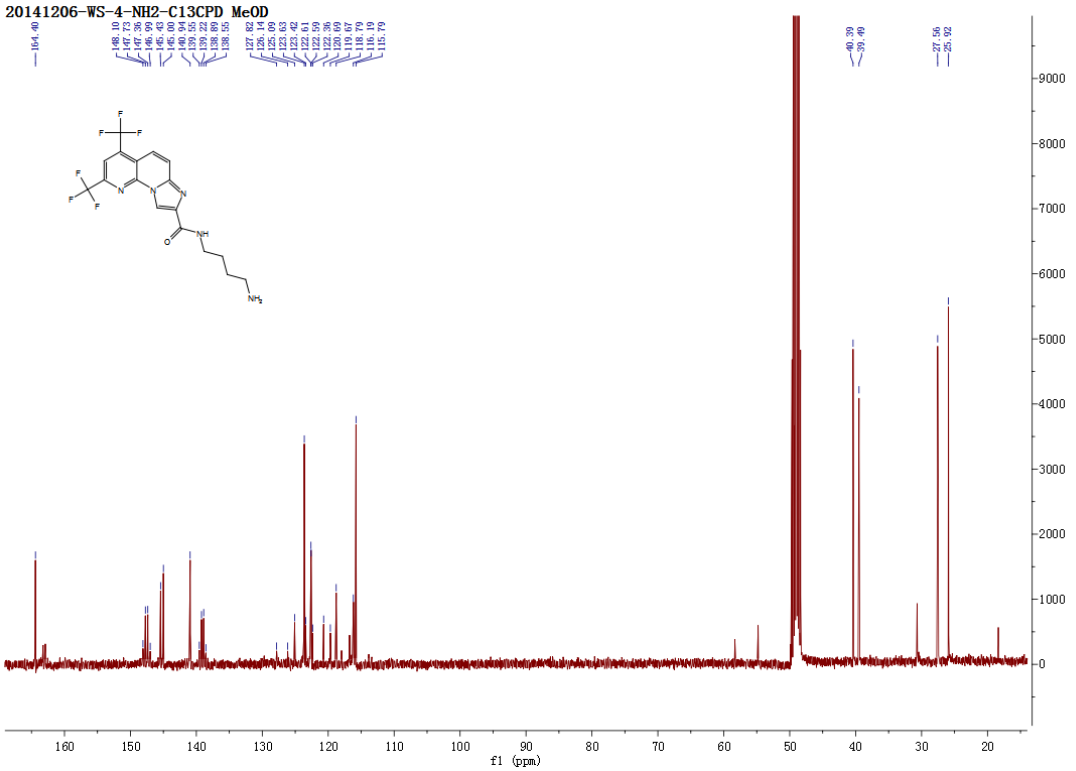
^{1}H NMR Spectrum for **5r** (CD_3OD-d_4, 400 MHz)



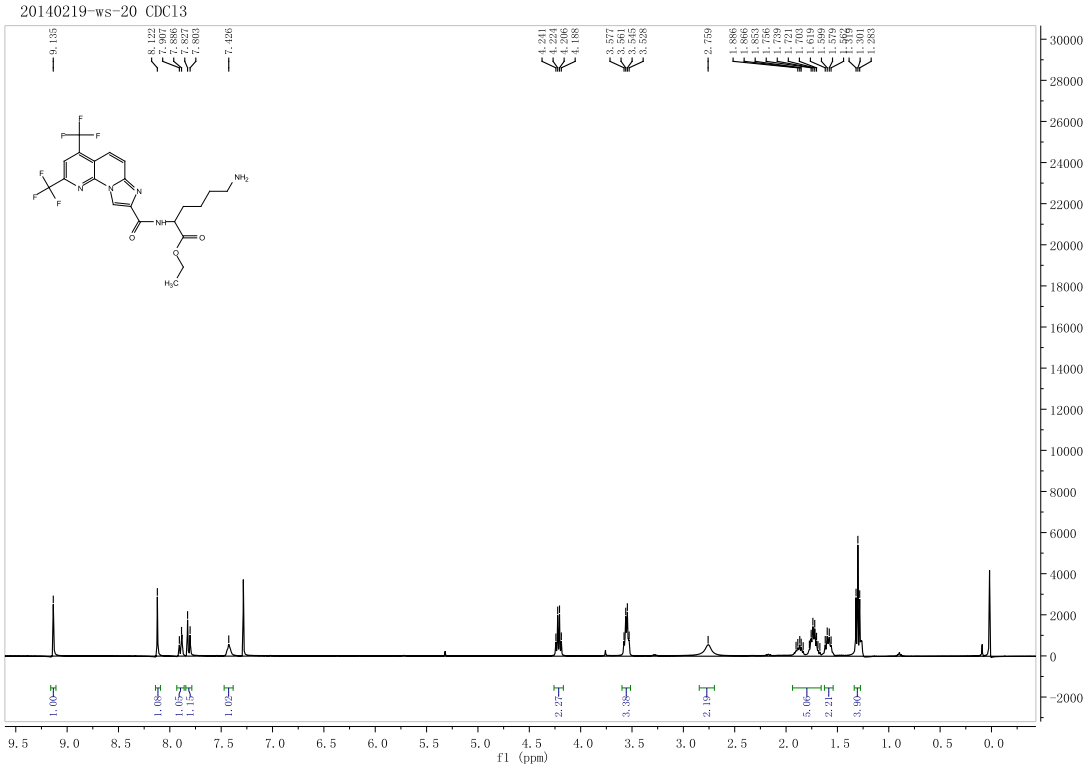
^{13}C NMR Spectrum for **5r** (CD_3OD-d_4, 100 MHz)



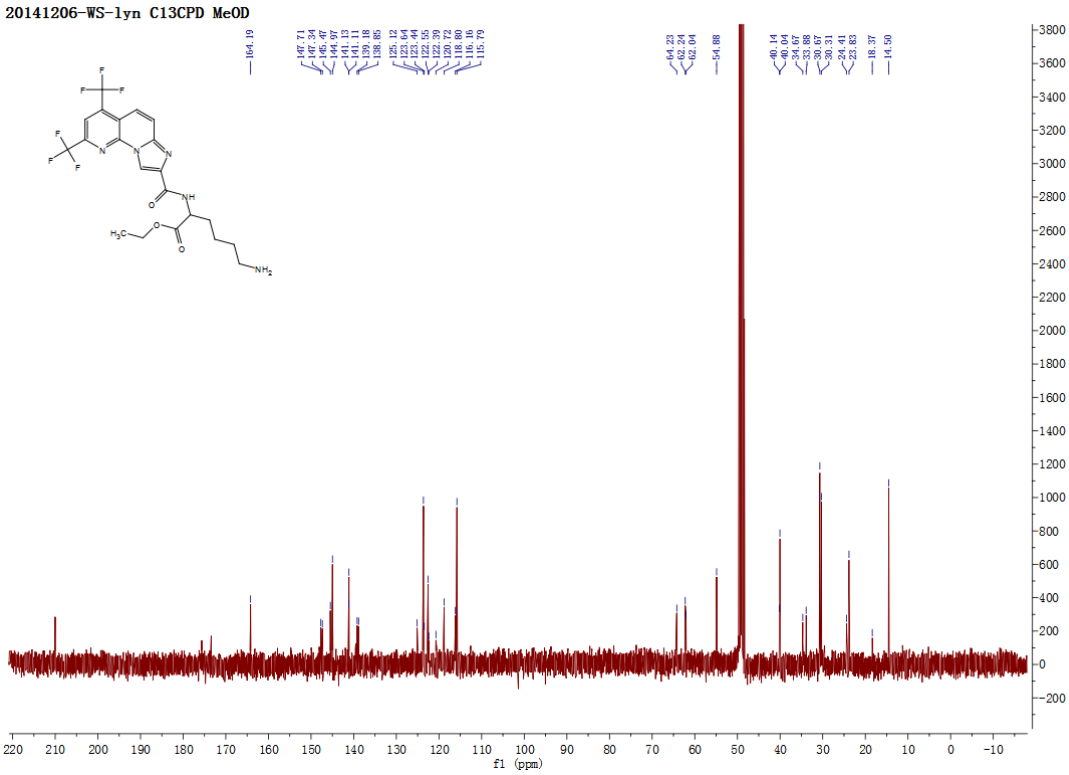
^{1}H NMR Spectrum for **5s** (CD_3OD-d_4, 400 MHz)



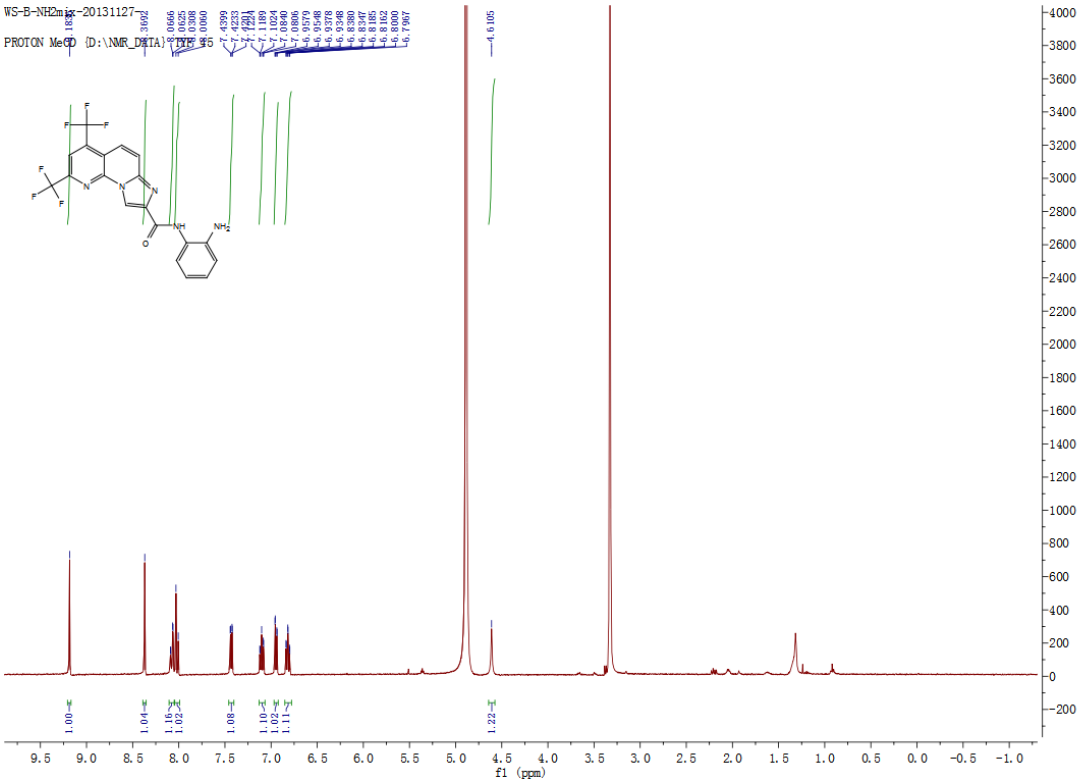
^{13}C NMR Spectrum for **5s** (CD_3OD-d_4, 100 MHz)



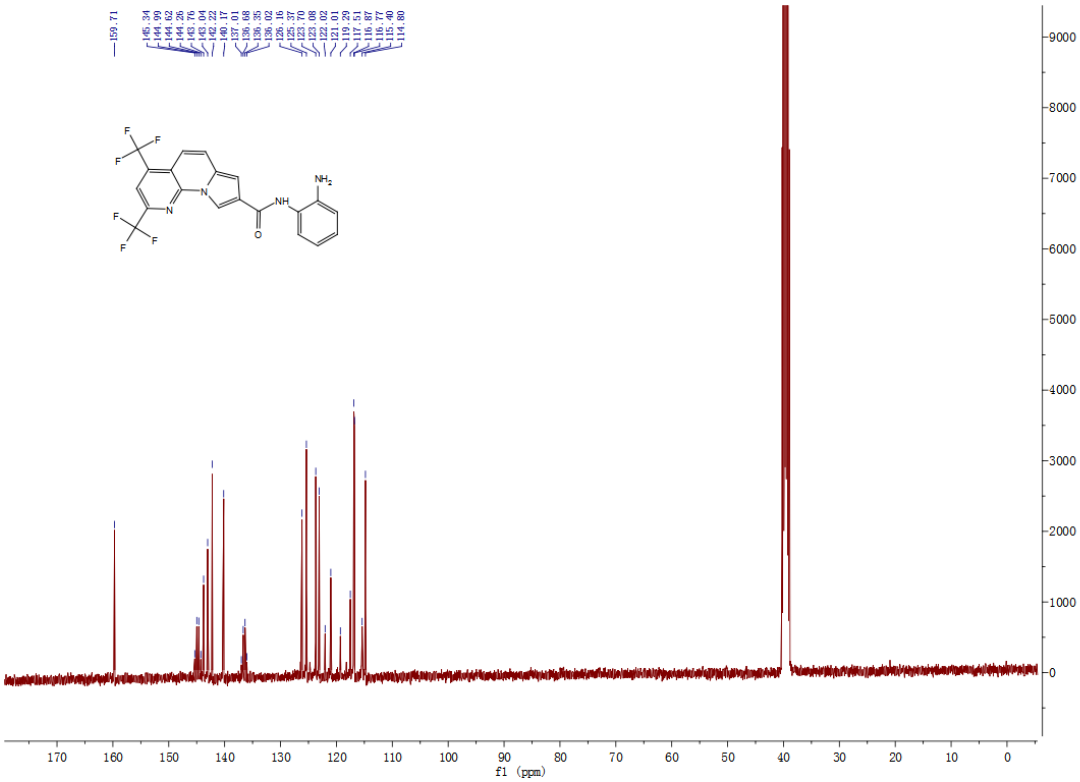
^{1}H NMR Spectrum for **5t** ($CDCl_3$, 400 MHz)



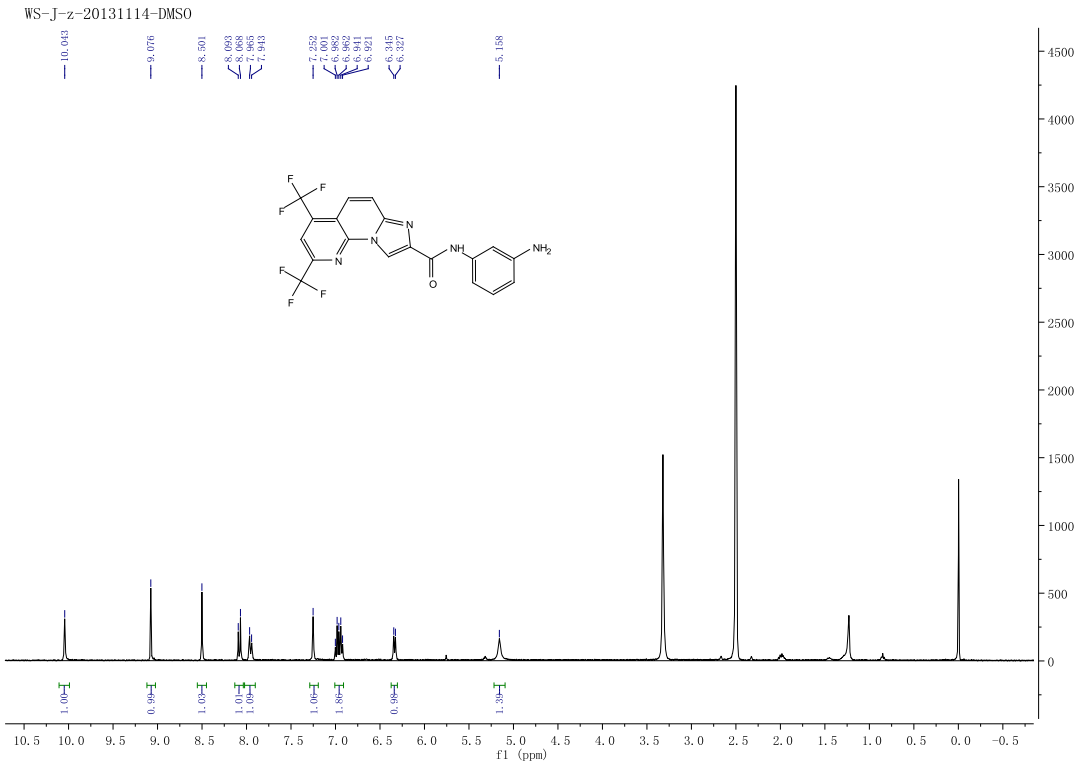
^{13}C NMR Spectrum for **5t** (CD_3OD-d_4, 100 MHz)



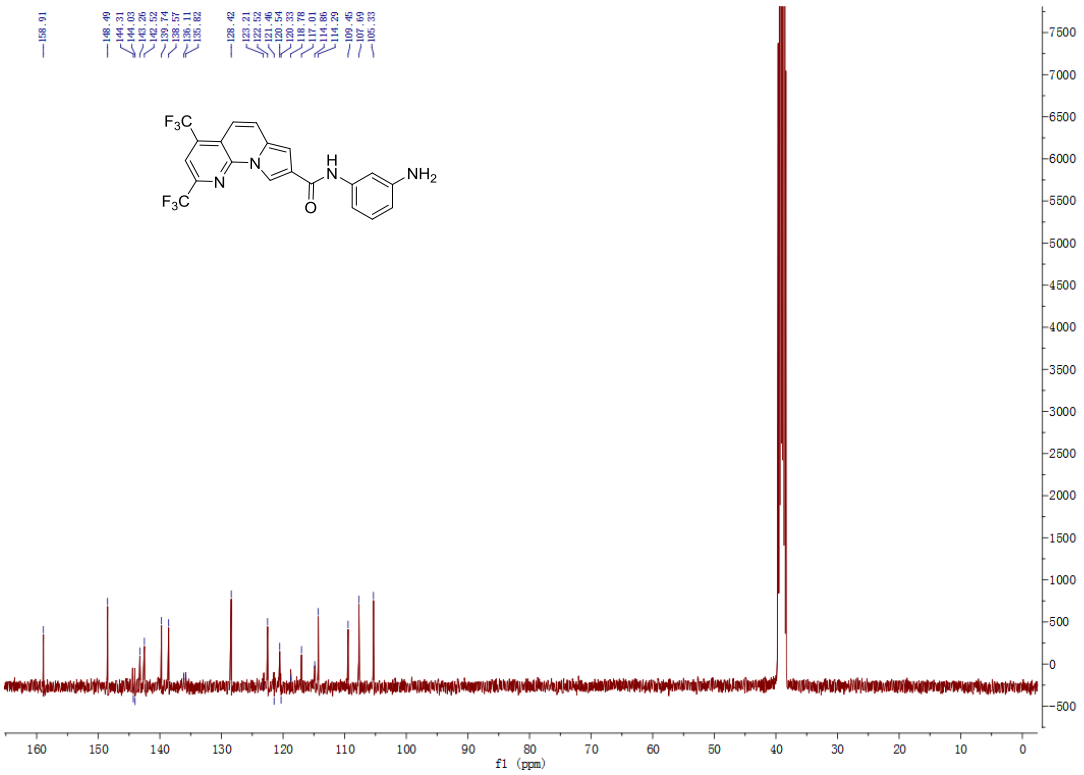
^{1}H NMR Spectrum for **5u** (CD_3OD-d_4, 400 MHz)



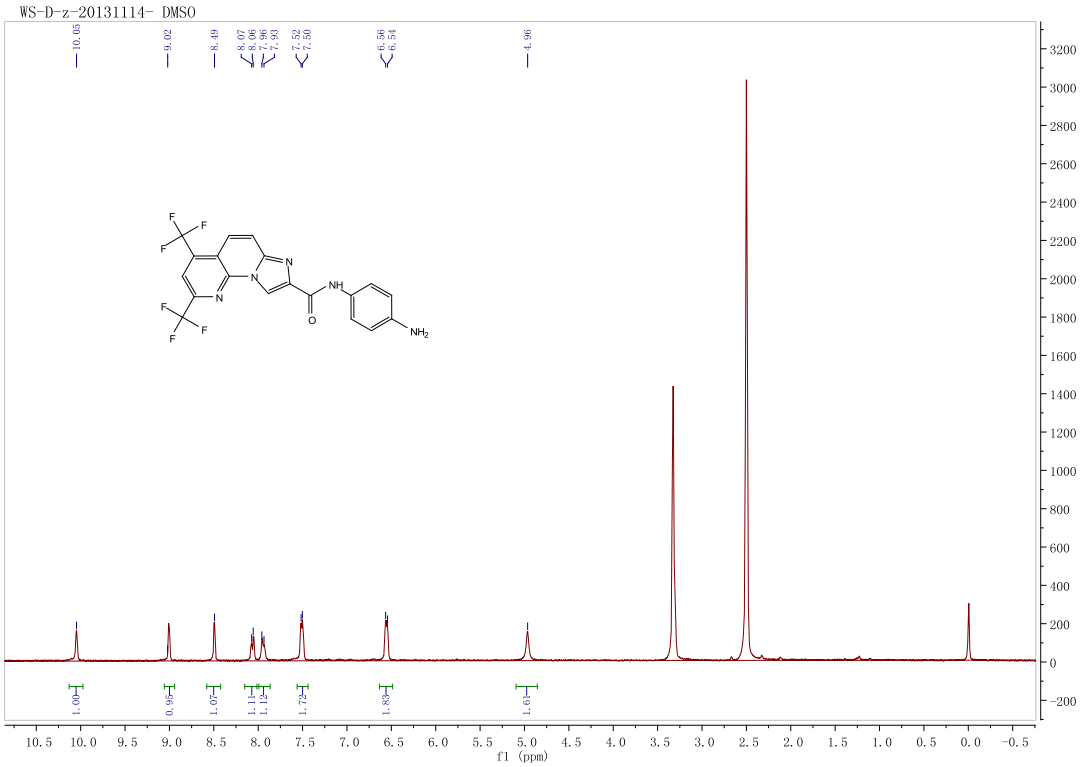
^{13}C NMR Spectrum for **5u** (DMSO-d_6, 100 MHz)



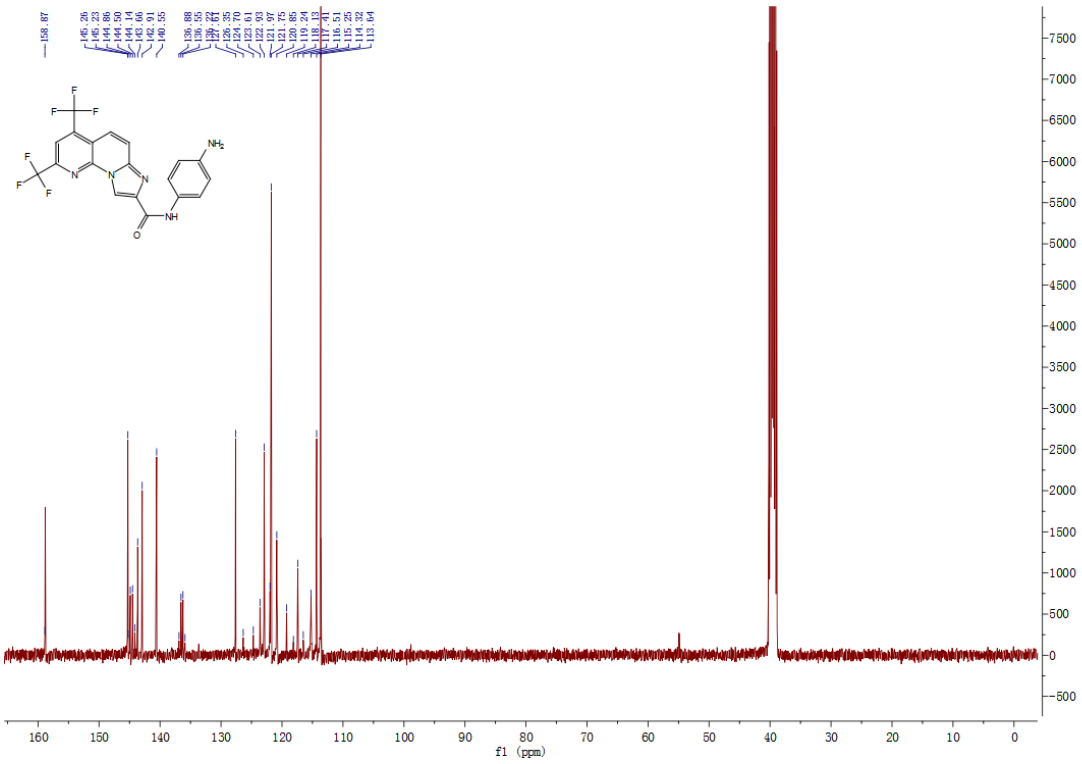
^{1}H NMR Spectrum for **5v** (DMSO-d_6, 400 MHz)



^{13}C NMR Spectrum for **5v** (DMSO-d_6, 100 MHz)



^{1}H NMR Spectrum for **5w** (DMSO-d_6, 400 MHz)



^{13}C NMR Spectrum for **5w** (DMSO-d_6, 100 MHz)

5. Materials and Methods for Biological Assays:

5.1 Preparation of HCV Cell Culture (HCVcc-hRluc-JFH1) System

The fully replicating cell culture-derived HCV system (HCVcc) was generated as previously reported.[1-2] Briefly, the plasmid of pRluc-JFH-1, a gift from Apath, L.L.C., was used as a template for RNA transcription. And the plasmid was constructed through digestion with Xbal restrict ion enzyme. A humanized Renilla luciferase reporter gene was introduced to the plasmid of pJFH-1, and was separated by a FMDV cleavage site between the 5'untranslated regions and HCV open reading frame (ORM).Virus RNA was prepared using the Ambion MEGA script Kits in vitro. And 10 μg RNA was transfected by electroporation into 400 ml Huh7.5.1 cells at a concentration of 1×10^7 cells/ml.[3-4] Contained virus transcripts, Huh7.5.1 cells were then seeded in a 10 cm dish. Media supernatant containing virus was collected, and filtered by low speed centrifugation at -20℃ after Huh7.5.1 cells were incubated for 3 days at 37℃. To generate high titer stock solution, virus preparations required several passages, and the solution was stored at -80℃. HCVcc titers were determined by diluting the virus stock solution at a gradient of 1:10, and incubated with Huh7.5.1 cells at 37℃. 48 hours later, the cells were harvested and measured luciferase activity using the Renilla-Glo™ Luciferase Assay System（Promega）as described by the manufacturer.

5.2 HCVcc inhibition assay

Synthetic compounds were dissolved in dimethyl sulfoxide (DMSO) at the stock solution of 10 mM, and were serially diluted 5-fold at concentrations ranging from 25 μM to 8 nM. For compound screening, Huh7.5.1 cells were seeded in 96-well plates at the density of 2×10^4 cells per well at 37℃. 24 hours later, serial diluted compounds were mixed with a certain titer of HCVcc-hRluc-JFH1 virus and added to Huh 7.5.1 cells. The final concentration of HCV JFH1 virus was diluted to the number ranging from 20,000 to 50,000 relative luminescence units (RLU). The Huh7.5.1 cells were incubated for 2 days at 37℃ and then harvested. The luminescence which reflected the degree of entry of the HCVcc JFH1 virus into Huh 7.5.1 cells was tested as protocol of Renilla-Glo™ Luciferase Assay System (Promega). The positive control was RO8191, blank control was wells containing 0.25% DMSO without inhibitors and background were derived from uninfected wells. To determine each tested compound percent activity, mean value of 3 paralleled wells was divided by the averaged blank control values and subtracted by 1, the corresponding inhibition values were calculated by multiplying 100%. The results are mean of triplicate assays and EC_{50} (compound concentration at which 50% inhibition of HCV luminescence level in the Huh7.5.1 cells was achieved) average values were calculated by the GraphPad Prism 5 software.

5.3 HCV Replicon Assay

Transient assay of genotype 1b (Con-1) and 2a (JFH-1) subgenomic reporter replicons have been reported previously. Subgenomic reporter replicon RNAs were synthesized by in vitro transcription with linearized template DNA plasmid, and 2 μg of RNA was transfected into Huh7 cells seeded in 6-well plate at a density of 1×10^5/ml 16 h before transfection. 4 h after transfection, cells in a portion of the plates were harvested as control for transfection efficiency, and a remaining portion of the cells in the plates received compounds. After compound administration, cells were harvested at 72 h after transfection for luciferase measurement. Replication efficiency was calculated as ratio of luciferase activities of 72 h over 4 h.

5.4 Time-course of inhibition

Huh7.5.1 cells were seeded in 48-well plates at a density of 1.0×10^5 cells per well. 24 hours later, the cell culture supernatant was removed and replaced with HCVcc inoculum in a volume of 100 ml/well. The plates were chilled to 4℃ for 1.5 h, and shook every 15 minutes. The HCVcc inoculum was removed and unbound virus was washed by phosphate buffered saline (PBS) for 2 times. Fresh DMEM medium with 10% FBS was added and the plates were shifted to a 37℃ incubator. At specific time points during infection, 200 mg/ml hog intestine heparin (TCI), 10 nM bafilomycin A1 (Meilun Biotech.), or 0.2 uM **5o** were added to the plates. After 3 days infection, renilla luciferase activity was quantified by Renilla-Glo™ Luciferase Assay System (Promega) according to the manufacturer's protocol. Experiments were done in triplicate with each data point has four parallel test wells, and the mean % inhibition was calculated relative to control infections without inhibitor.

5.5 Inhibitor effects on attachment and post-attachment stages

Huh7.5.1 cells were infected by HCVcc (JFH-1) at 4℃ and incubated for 1.5 h. Heparin (200 mg/ml), **5o** (0.2 uM), or bafilomycin A1 (10 nM) were present in the media either continuously, during the 4℃ incubation only (attachment), or during the 37℃ incubation phase only (post-attachment). After 3 days infection, renilla luciferase activity was quantified by Renilla-Glo™ Luciferase Assay System (Promega) according to the manufacturer's protocol. Inhibition was calculated as % relative to control infections lacking inhibitor. Results represented are from 3 independent experiments.

5.6 Transfection of ISRE reporter gene

ISRE reporter gene was transiently transfected into HepG2 cells, Huh-7 cells, Vero cells and Huh7.5.1 cells. 24h later, the cells were treated with **5o**, RO8191, or IFN-α. After an additional 24 h, luciferase activity was measured (Figure S1). HepG2 cells and Huh-7 cells are belonged to human hepatocellular carcinoma (HCC) cell lines. Huh7.5.1 cells are derived from Huh7, with a mutation in the RIG-I gene as described previously,[5-6] which impairs interferon signaling, leading to that IFN-α showed no effects on ISRE expression in Huh7.5.1 cells, while RO8191 effects significantly. Vero cells were from green monkey kidney epithelium, which are interferon-deficient, they do not secrete interferon when infected by viruses but have interferon receptor and respond normally to exotic.[7-9] RO8191 induced the ISRE expression better than IFN-α, indicating that RO8191 acts independently of inducing IFN.

6. Materials and Methods for Pharmacokinetics Study

Specific pathogen free grade male Wistar rats (200±10 g) were supplied by laboratory animal center of Tsinghua university (Beijing, China). The rats were housed under controlled conditions of temperature (22 ± 2℃) and relative humidity (50 ± 20%) with a 12 h light/dark cycle with water and food freely available. All the studies were approved by the Institutional Animal Care and Use Committee of Tsinghua University.

All analytical procedures were performed using a Agilent HPLC-ELSD system (Agilent Technologies, USA), and the column used was phenomenex synergi hydro-RP (150×2.00 mm, 4μm)(Phenomenex, USA). The mobile phase A was 0.1% formic acid in water and the mobile phase B was methanol, the flow rate was 0.5 mL/min, and injection volume was 20μL. The ELSD parameters were as follows: evaporation and nebulization temperature were 80 ℃, and the gas

flow rate was 1.5 standard liters per minute (SLM); gain factor was 1.

For pharmacokinetic study,[10] the rats were acclimated to the housing environment for 7 days and were fasted over night before use with free access to water. After that, five male rats were administrated by oral gavage at a dose of 100 mg/kg. Blood samples (0.3 mL) were collected via the posterior orbital venous plexus at times of 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after administration, and immediately transferred into a heparinized EP tube. The plasma was prepared by centrifuging the blood samples at 14,000 rpm for 10 min, and the resulting plasma layers were collected and stored at -80℃ until analysis.

An aliquot (100 μL) of rat plasma was transferred into a 1.5 mL tube, and was added 4-fold volume of methanol. After vortex-mixed for 30s and centrifuging at 14,000 rpm for 10 min, the supernatant was transferred into another EP tube and dried by Vacuum Dryer. The residue was reconstituted with 100 μL Water and vortex-mixed. After centrifuging at 14,000 rpm for 10 min, 20 μL aliquot was injected into HPLC for analysis. The pharmacokinetic parameters were obtained by fitting the raw data using a noncompartmental model with WinNonlin (Pharsight, Princeton, NJ, USA).

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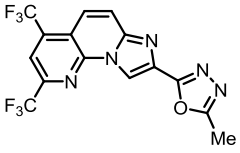
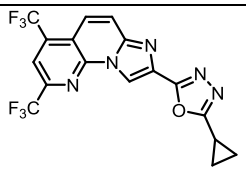
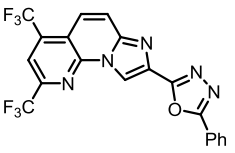
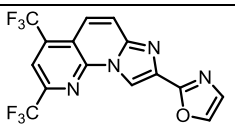
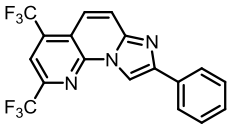
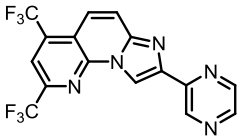
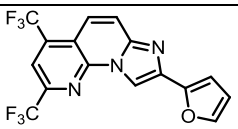
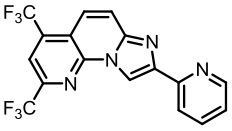
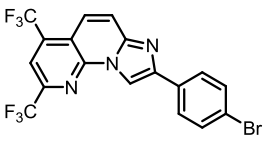
7. Supplementary Table and Fugures

Table S-1: Anti-HCV activity of some unpublished analogs

Compound	Structure	EC_{50} (μM)	CC_{50} (μM)
S-1		3.3	>25
S-2		11.2	>25
S-3		>20	>25
S-4		3.4	>25
S-5		14.4	>25
S-6		13.5	>25
S-7		4.0	>25
S-8		>20	>25
S-9		14.4	>25

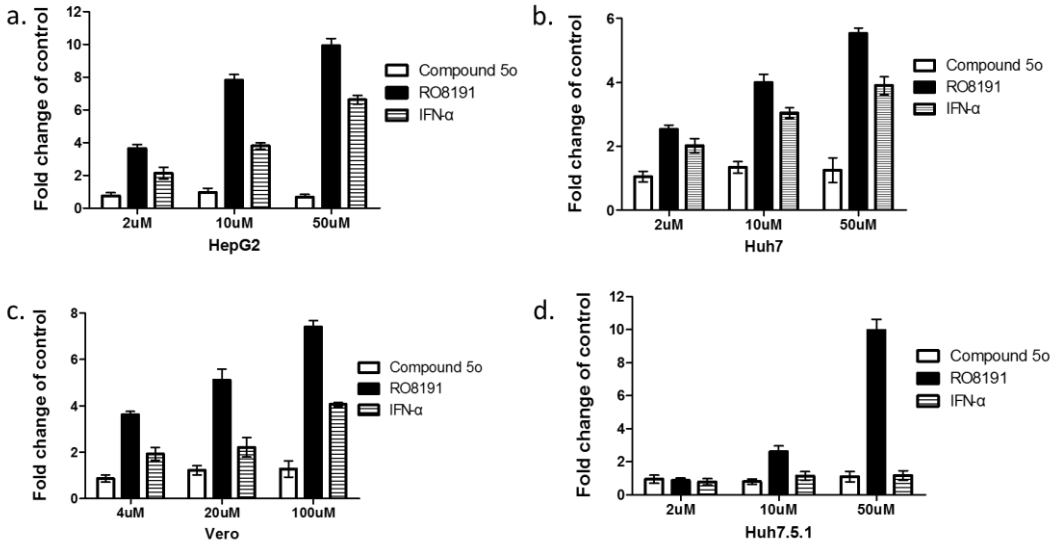


Figure S1. ISRE reporter gene activation by **5o**, RO8191, or IFN-α treatment.

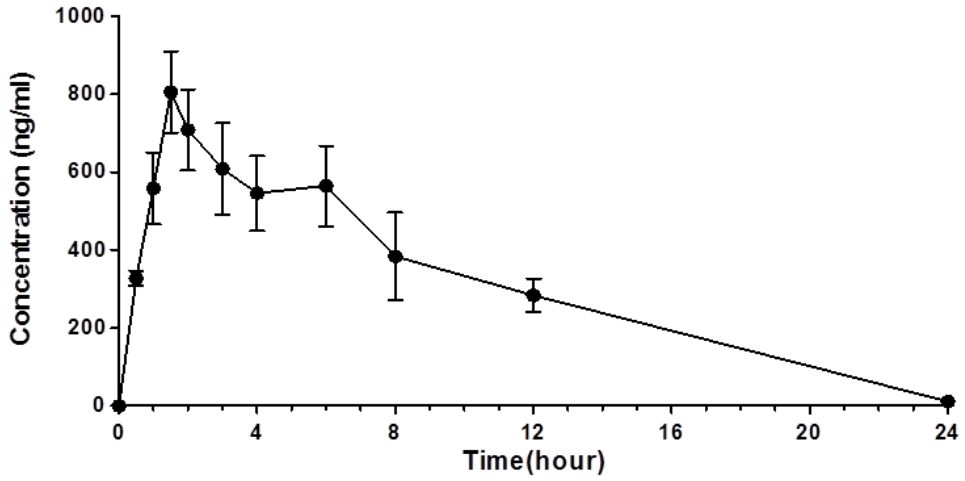


Figure S2. Pharmacokinetic profiles of 5**o** after oral (100 mg/kg)) administration in rats. Shown here are blood **5o** concentration–time profiles. The rats (n = 5 per group) were given an oral dose of **5o** (100 mg/kg). Data represent mean ± SD (n=5).