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Synthesis and cytotoxic activity of novel 4-amino-5-cyano-2-sulfonylpyrimidines

Dmitry A. Khochenkov, Yulia A. Khochenkova, Yulia S. Machkova, Rovshan E. Gasanov, Eugenia V. Stepanova and Alexander S. Bunev

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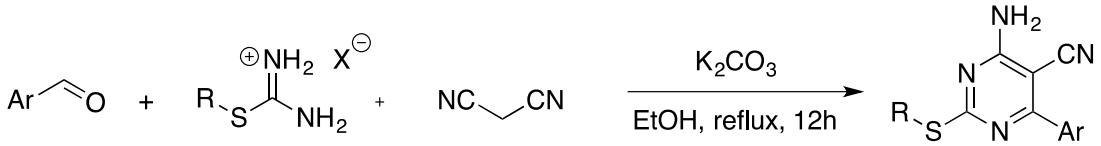
Chemistry experimental

General methods

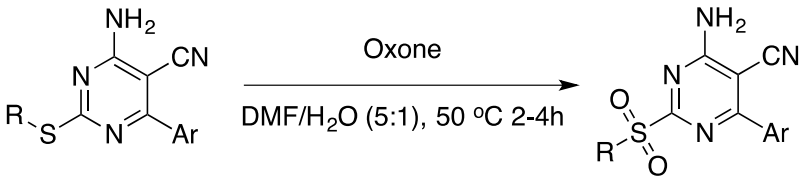
All reagents and solvents were used as purchased. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance spectrometer (600 and 150 MHz, respectively) in DMSO-d_6, internal standard was TMS. ^{19}F NMR spectra were recorded on the Bruker Avance III 500 spectrometer, operating at 471 MHz. ^{19}F chemical shifts was measured relative to $CFCl_3$ as an external standard. Elemental analysis was performed on a vario EL cube analyzer. Melting points were determined on a Boetius hot stage and were not corrected. All compounds reported in this communication are at least 95+% pure judged by HPLC UV detection (Agilent Technologies 1220 Infinity LC) and optionally NMR.

Synthesis of 5-cyanopyrimidines, structure type I

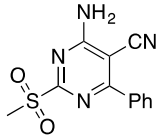


Synthesis of 2-alkylthio-4-amino-6-arylpyrimidine-5-carbonitriles (typical procedure)

A mixture of aromatic aldehyde (10 mmol), *S*-alkylisothiourea salt (hydrochloride, hydroiodide, hydrosulfate or mesylate, 10 mmol), malononitrile (10 mmol), potassium carbonate (20 mmol), and ethanol (100 ml) was refluxed for approximately 12 h. When the reaction was complete (TLC monitoring, hexane/EtOAc 7:3), the mixture was poured into water (400 ml), the solid was filtered off and washed thoroughly with water. The product was dried and recrystallized from 95% ethanol.

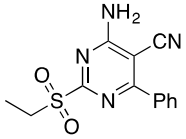


Synthesis of 2-alkylsulfonyl-4-amino-6-arylpyrimidine-5-carbonitriles ***4a-k*** *(typical procedure).* To a solution of 2-alkylthio-4-amino-6-arylpyrimidine-5-carbonitrile (5 mmol) in DMF/H_2O mixture (5:1, 30 ml), Oxone (15 mmol) was added, and the mixture was stirred and heated to 50 °C for 2-4 h (HPLC monitoring, MeCN/H_2O, 80:20, C-18). When the reaction was complete, the mixture was poured into water (150 ml). The product was isolated by filtration and dried.



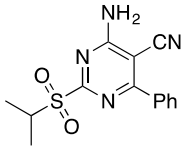
4-Amino-2-methylsulfonyl-6-phenylpyrimidine-5-carbonitrile (**1a**) was prepared using *S*-methylisothiouronium iodide [S1].

Yield 73%, mp 175-176 °C. Found, %: C 52.63; H 3.72; N 20.47. $C_{12}H_{10}N_4O_2S$. Calculated, %: C 52.55; H 3.67; N 20.43. ^{1}H NMR (600 MHz, DMSO-d_6): 8.69 (s, 2H, NH_2), 7.99 – 7.90 (m, 2H), 7.72 – 7.52 (m, 3H), 3.37 (s, 3H, SO_2Me). ^{13}C NMR (150 MHz, DMSO-d_6): 169.4, 168.9, 166.5, 165.3, 135.5, 132.2, 129.2, 115.5, 89.1, 39.1.



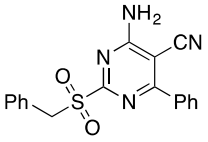
4-Amino-2-ethylsulfonyl-6-phenylpyrimidine-5-carbonitrile (**1b**) was prepared using *S*-ethylisothiouronium bromide [S2]

Yield 74%, mp 185-187 °C. Found, %: C 54.22; H 4.30; N 19.48. $C_{13}H_{12}N_4O_2S$. Calculated, %: C 54.16; H 4.20; N 19.43. ^{1}H NMR (600 MHz, DMSO-d_6): 8.69 (s, 2H, NH_2), 7.97 – 7.90 (m, 2H), 7.68 – 7.56 (m, 3H), 3.56 (q, J = 7.4 Hz, 2H, CH_2), 1.29 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): 169.4, 165.8, 165.3, 135.5, 132.2, 129.3, 129.1, 115.5, 89.2, 45.3, 7.2.



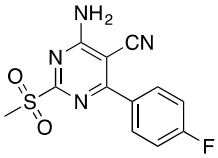
4-Amino-2-isopropylsulfonyl-6-phenylpyrimidine-5-carbonitrile (**1c**) was prepared using *S*-isopropylisothiouronium iodide [S3]

Yield 89%, mp 198-200 °C. Found, %: C 55.74; H 4.73; N 18.57. $C_{14}H_{14}N_4O_2S$. Calculated, %: C 55.62; H 4.67; N 18.53. ^{1}H NMR (600 MHz, DMSO-d_6): 8.69 (s, 2H, NH_2), 7.96 – 7.89 (m, 2H), 7.67 – 7.56 (m, 3H), 3.93 (sept, J = 6.8 Hz, 1H, CH), 1.30 (d, J = 6.9 Hz, 6H, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): 169.4, 165.3, 165.3, 135.5, 132.2, 129.2, 129.1, 115.5, 89.2, 50.6, 15.1.



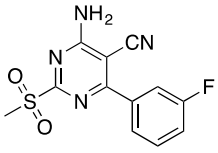
4-Amino-2-benzylsulfonyl-6-phenylpyrimidine-5-carbonitrile (**1d**) was prepared using *S*-benzylisothiouronium chloride [S4]

Yield 91%, mp 204-205 °C. Found, %: C 61.78; H 4.09; N 16.05. $C_{12}H_{10}N_4O_2S$. Calculated, %: C 61.70; H 4.03; N 15.99. ^{1}H NMR (600 MHz, DMSO-d_6): 8.83 (s, 2H, NH_2), 7.96 (dt, J = 7.1, 1.6 Hz, 2H), 7.70 – 7.58 (m, 3H), 7.39 (dq, J = 16.8, 3.3, 2.8 Hz, 5H), 4.95 (s, 2H, CH_2). ^{13}C NMR (150 MHz, DMSO-d_6): 169.4, 166.0, 165.2, 135.4, 132.3, 132.0, 129.3, 129.2, 129.0, 128.9, 115.4, 89.1, 56.8.



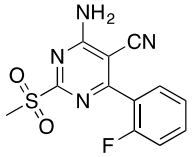
4-Amino-6-(4-fluorophenyl)-2-(methylsulfonyl)pyrimidine-5-carbonitrile (**1e**) was prepared using *S*-methylisothiouronium iodide [S1].

Yield 80%, mp 171-172 °C. Found, %: C 49.36; H 3.19; N 19.25. $C_{12}H_9FN_4O_2S$. Calculated, %: C 49.31; H 3.10; N 19.17. ^{1}H NMR (600 MHz, DMSO-d_6): 8.69 (s, 2H, NH_2), 8.09 – 7.98 (m, 2H), 7.45 (t, J = 8.9 Hz, 2H), 3.37 (s, 3H, SO_2Me). ^{13}C NMR (150 MHz, DMSO-d_6): 168.2, 166.5, 165.3, 163.6 (d, J = 247.6 Hz), 132.1, 132.0, 132.0, 131.9, 116.3 (d, J = 22.1 Hz), 89.0, 39.1. ^{19}F NMR (471 MHz, DMSO-d_6): -107.98.



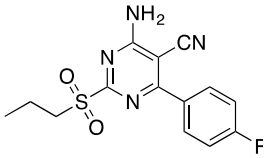
4-Amino-6-(3-fluorophenyl)-2-(methylsulfonyl)pyrimidine-5-carbonitrile (**1f)** was prepared using *S*-methylisothiouronium iodide [S1]

Yield 73%, mp 188-189 °C. Found, %: C 49.42; H 3.16; N 19.21. $C_{12}H_9FN_4O_2S$. Calculated, %: C 49.31; H 3.10; N 19.17. ^{1}H NMR (600 MHz, DMSO-d_6): 8.74 (s, 2H, NH_2), 7.85 – 7.43 (m, 4H), 3.38 (s, 3H, SO_2Me). ^{13}C NMR (150 MHz, DMSO-d_6): 168.0, 166.5, 165.2, 163.3 (d, J = 244.5 Hz), 161.3, 137.7, 131.4 (d, J = 8.3 Hz), 125.5 (d, J = 2.9 Hz), 119.1 (d, J = 21.1 Hz), 116.1 (d, J = 23.5 Hz), 115.2, 89.5, 39.1. ^{19}F NMR (471 MHz, DMSO-d_6): -112.10.



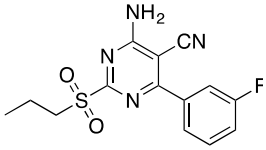
4-Amino-6-(2-fluorophenyl)-2-(methylsulfonyl)pyrimidine-5-carbonitrile (**1g**) was prepared using *S*-methylisothiouronium iodide [S1]

Yield 87%, mp 182-184 °C. Found, %: C 49.39; H 3.17; N 19.22. $C_{12}H_9FN_4O_2S$. Calculated, %: C 49.31; H 3.10; N 19.17. ^{1}H NMR (600 MHz, DMSO-d_6): 8.80 (s, 2H, NH_2), 7.73 – 7.65 (m, 2H), 7.51 – 7.38 (m, 2H), 3.34 (s, 3H, SO_2Me). ^{13}C NMR (150 MHz, DMSO-d_6): 166.7, 166.2, 164.4, 160.3 (d, J = 248.2 Hz), 158.3, 134.0 (d, J = 8.5 Hz), 131.7, 125.5, 124.0, 123.9, 116.7 (d, J = 21.2 Hz), 114.4, 92.1, 39.2. ^{19}F NMR (471 MHz, DMSO-d_6): -113.41.



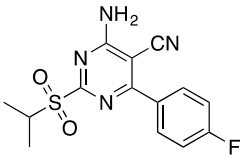
4-Amino-6-(4-fluorophenyl)-2-(propylsulfonyl)pyrimidine-5-carbonitrile (**1h**) was prepared using *S*-propylisothiouronium chloride [S5]

Yield 86%, mp 170-171 °C. Found, %: C 52.58; H 4.15; N 17.55. $C_{14}H_{13}FN_4O_2S$. Calculated, %: C 52.49; H 4.09; N 17.49. ^{1}H NMR (600 MHz, DMSO-*d*$_6$): 8.70 (s, 2H, NH_2), 8.06 – 7.98 (m, 2H), 7.46 (t, *J* = 8.8 Hz, 2H), 3.57 – 3.49 (m, 2H, CH_2), 1.77 (h, *J* = 7.5 Hz, 2H, CH_2), 1.01 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-*d*$_6$): 168.2, 166.0, 165.5, 165.3, 163.5 (d, *J* = 247.7 Hz), 132.0 (d, *J* = 9.5 Hz), 116.3 (d, *J* = 22.0 Hz), 115.4, 89.0, 52.1, 16.1, 13.3. ^{19}F NMR (471 MHz, DMSO-*d*$_6$): -108.03.



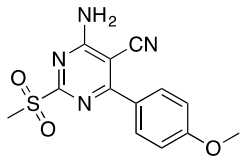
4-Amino-6-(3-fluorophenyl)-2-(propylsulfonyl)pyrimidine-5-carbonitrile (**1i**) was prepared using *S*-propylisothiouronium chloride [S5].

Yield 68%, mp 168-170 °C. Found, %: C 52.57; H 4.14; N 17.54. $C_{12}H_{10}N_4O_2S$. Calculated, %: C 52.49; H 4.09; N 17.49. ^{1}H NMR (600 MHz, DMSO-*d*$_6$): 8.75 (s, 2H, NH_2), 7.80 – 7.76 (m, 1H), 7.75 – 7.71 (m, 1H), 7.70 – 7.63 (m, 1H), 7.53 – 7.47 (m, 1H), 3.61 – 3.49 (m, 2H, CH_2), 1.85 – 1.70 (m, 2H, CH_2), 1.02 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-*d*$_6$): 168.0, 166.1, 165.2, 162.3 (d, *J* = 241.2 Hz), 137.7, 131.4 (d, *J* = 8.2 Hz), 125.5 (d, *J* = 2.7 Hz), 119.0 (d, *J* = 21.0 Hz), 116.1 (d, *J* = 23.5 Hz), 115.2, 89.6, 52.1, 16.1, 13.3. ^{19}F NMR (471 MHz, DMSO-*d*$_6$): -112.15.



4-Amino-6-(4-fluorophenyl)-2-(isopropylsulfonyl)pyrimidine-5-carbonitrile (**1j**) was prepared using *S*-isopropylisothiouronium iodide [S3]

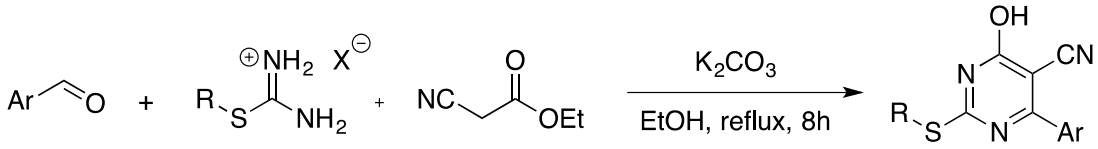
Yield 85%, mp 225-227 °C. Found, %: C 52.61; H 4.16; N 17.58. $C_{14}H_{13}FN_4O_2$. Calculated, %: C 52.49; H 4.09; N 17.49. ^{1}H NMR (600 MHz, DMSO-*d*$_6$): 8.70 (s, 2H, NH_2), 8.07 – 7.95 (m, 2H), 7.51 – 7.38 (m, 2H), 3.92 (p, *J* = 6.9 Hz, 1H, CH), 1.29 (d, *J* = 6.9 Hz, 6H, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-*d*$_6$): 168.2, 165.3, 163.5 (d, *J* = 247.7 Hz), 132.0 (d, *J* = 9.2 Hz), 116.3 (d, *J* = 22.1 Hz), 89.1, 50.6, 15.0.



4-Amino-6-(4-methoxyphenyl)-2-(methylsulfonyl)pyrimidine-5-carbonitrile (**1k**) was prepared using *S*-methylisothiouronium iodide [S1]

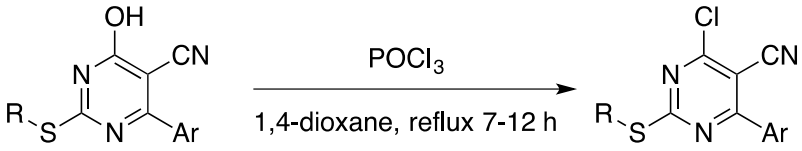
Yield 66%, mp 146-147 °C. Found, %: C 51.37; H 4.07; N 18.46. $C_{13}H_{12}N_4O_3S$. Calculated, %: C 51.31; H 3.97; N 18.41. ^{1}H NMR (600 MHz, DMSO-*d*$_6$): 8.58 (s, 2H, NH_2), 8.00 (d, *J* = 8.9 Hz, 2H), 7.15 (d, *J* = 8.9 Hz, 2H), 3.87 (s, 3H, OMe), 3.37 (s, 3H, SO_2Me). ^{13}C NMR (150 MHz, DMSO-*d*$_6$): 168.4, 166.4, 165.5, 162.7, 131.3, 127.5, 115.9, 114.6, 87.6, 56.0, 39.1.

Synthesis of 5-cyanopyrimidines, structure type II



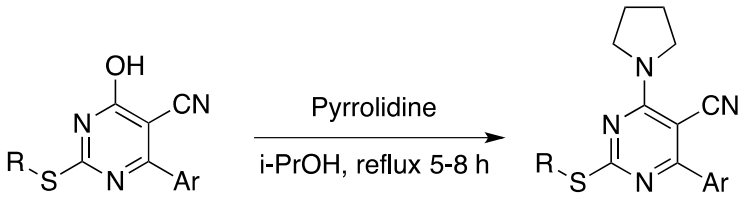
Synthesis of 2-alkylthio-6-aryl-4-hydroxypyrimidine-5-carbonitriles (typical procedure)

A mixture of aromatic aldehyde (10 mmol), *S*-alkylisothiourea salt (hydrochloride, hydroiodide, hydrosulfate or mesylate, 10 mmol), ethyl cyanoacetate (10 mmol), potassium carbonate (20 mmol), and ethanol (100 m) was refluxed for approximately 8 h. When the reaction was complete (TLC monitoring, hexane/EtOAc 7:3), the mixture was poured into water (400 ml), acidified (5% solution HCl). The product was isolated by filtration, dried, and recrystallized from 95 % ethanol.

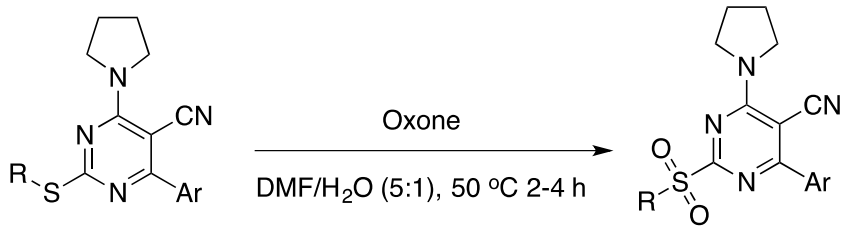


Synthesis of 2-alkylthio-6-aryl-4-chloropyrimidine -5-carbonitriles (typical procedure)

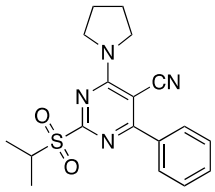
A mixture of 2-alkylthio-6-aryl-4-hydroxypyrimidine-5-carbonitrile (5 mmol), 1,4-dioxane (70 ml) and $POCl_3$ (50 mmol) was refluxed for 7-12 h (TLC monitoring, hexane/EtOAc 7:3) and the concentrated under vacuum. The residue was dissolved in EtOAc (100 ml) and washed with cold water, saturated $NaHCO_3$ solution, water, and brine. The solution was dried over $MgSO_4$ and concentrated under vacuum. The residue was recrystallized from 95% ethanol.



Synthesis of 2-alkylthio-4-aryl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitriles (typical procedure). A mixture of 2-alkylthio-6-aryl-4-chloropyrimidine-5-carbonitriles (3 mmol), pyrrolidine (6 mmol) and Pr^iOH (50 ml) was refluxed for 5-8 h (TLC monitoring, hexane/EtOAc 7:3). When the reaction was complete, the reaction mixture was poured into water (200 ml). The product was isolated by filtration, dried, and recrystallized from 95% ethanol.

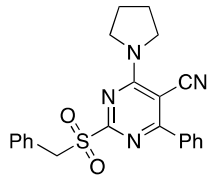


Synthesis of 2-alkylsulfonyl-4-aryl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitriles (typical procedure). To a solution of 2-alkylthio-4-aryl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (2 mmol) in DMF/H_2O mixture (5:1, 30 ml), Oxone (6 mmol) was added, and the mixture was stirred and heated to 50 °C for 2-4 h (HPLC monitoring, MeCN/H_2O, 80:20, C-18). When the reaction was complete, the mixture was poured into water (120 ml). The product was isolated by filtration and dried.



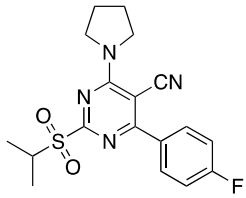
2-Isopropylsulfonyl-4-phenyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2a**) was prepared using *S*-isopropylisothiouronium iodide.

Yield 55%, mp 137-139 °C. Found, %: C 60.73; H 5.72; N 15.80. $C_{18}H_{20}N_4O_2S$. Calculated, %: C 60.65; H 5.66; N 15.72. ^{1}H NMR (600 MHz, DMSO-d_6): 7.86 (td, J = 7.9, 1.5 Hz, 2H), 7.67 – 7.52 (m, 3H), 3.99 (p, J = 6.9 Hz, 1H, CH), 3.71 (s, 4H), 1.35 – 1.30 (m, 10H). ^{13}C NMR (150 MHz, DMSO-d_6): 171.5, 170.7, 164.1, 159.6, 136.0, 132.0, 131.8, 129.7, 129.0, 128.9, 117.5, 89.2, 52.9, 50.6, 17.6, 15.2, 14.0.



2-Benzylsulfonyl- 4-phenyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2b**) was prepared using *S*-benzylisothiouronium chloride.

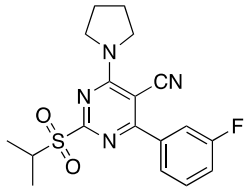
Yield 85%, mp 205-207 °C. Found, %: C 65.41; H 5.07; N 13.93. $C_{22}H_{20}N_4O_2S$. Calculated, %: C 65.33; H 4.98; N 13.85. ^{1}H NMR (600 MHz, DMSO-d_6): 7.98 – 7.13 (m, 10H), 4.97 (s, 2H, CH_2), 4.14 – 3.65 (m, 4H), 2.12 – 1.76 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6): 172.9, 171.4, 170.8, 164.8, 159.4, 136.3, 135.9, 132.0, 131.8, 131.1, 130.7, 129.1, 128.0, 117.8, 89.1, 59.3, 56.4, 26.5, 24.0.



4-(4-Fluorophenyl)-2-isopropylsulfonyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2c**) was prepared using *S*-isopropylisothiouronium iodide.

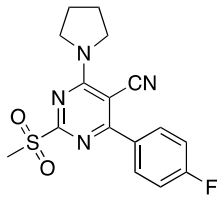
Yield 77%, mp 105-107 °C. Found, %: C 57.85; H 5.18; N 15.05. $C_{18}H_{19}FN_4O_2S$. Calculated, %: C 57.74; H 5.11; N 14.96. ^{1}H NMR (600 MHz, DMSO-d_6): 7.98 – 7.93 (m, 2H), 7.49 – 7.40 (m, 2H), 3.99 (p, J = 6.9 Hz, 1H, CH), 3.79 – 3.64 (m, 4H), 2.11 – 1.88 (m, 4H), 1.32 (d, J =

6.9 Hz, 6H, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): 170.3, 165.4, 164.1, 163.5 (d, J = 248.1 Hz), 159.5, 132.4 (d, J = 9.2 Hz), 117.4, 116.1, 89.1, 50.6, 49.6, 45.3, 26.5, 24.0, 15.2. ^{19}F NMR (471 MHz, DMSO-d_6): -108.31.



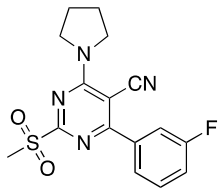
4-(3-Fluorophenyl)-2-isopropylsulfonyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2d**) was prepared using *S*-isopropylisothiouronium iodide.

Yield 56%, mp 138-140 °C. Found, %: C 57.80; H 5.16; N 15.03. $C_{18}H_{19}FN_4O_2S$. Calculated, %: C 57.74; H 5.11; N 14.96. ^1H NMR (600 MHz, DMSO-d_6): 7.75 – 7.43 (m, 3H), 3.99 (p, J = 6.9 Hz, 1H, CH), 3.79 – 3.64 (m, 4H), 2.11 – 1.88 (m, 4H), 1.32 (d, J = 6.9 Hz, 6H, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): 170.0, 169.3, 164.1, 163.1 (d, J = 244.5 Hz), 161.2, 131.4, 131.2, 125.9, 118.9, 117.7, 89.6, 52.9, 50.6, 26.5, 23.9, 17.7, 15.2. ^{19}F NMR (471 MHz, DMSO-d_6): -112.35.



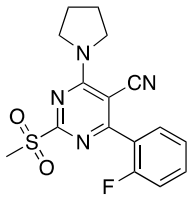
4-(4-Fluorophenyl)-2-methylsulfonyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2e**) was prepared using *S*-methylisothiouronium iodide.

Yield 78%, mp 95-97 °C. Found, %: C 55.58; H 4.47; N 16.23. $C_{16}H_{15}FN_4O_2S$. Calculated, %: C 55.48; H 4.37; N 16.18. ^1H NMR (600 MHz, DMSO-d_6): 8.05 – 7.92 (m, 2H), 7.51 – 7.40 (m, 2H), 4.17 – 3.60 (m, 4H), 3.39 (s, 3H, SO_2Me), 2.20 – 1.76 (m, 4H). ^{19}F NMR (471 MHz, DMSO-d_6): -108.27.



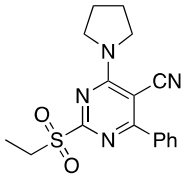
4-(3-Fluorophenyl)-2-methylsulfonyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2f**) was prepared using *S*-methylisothiouronium iodide.

Yield 85%, mp 148-150 °C. Found, %: C 55.48; H 5.45; N 16.25. $C_{16}H_{15}FN_4O_2S$. Calculated, %: C 55.48; H 4.37; N 16.18. ^1H NMR (600 MHz, DMSO-d_6): 7.78 – 7.59 (m, 3H), 7.56 – 7.41 (m, 1H), 4.11 – 3.64 (m, 4H), 3.40 (s, 3H, SO_2Me), 2.12 – 1.86 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6): 174.6, 165.3, 163.2 (d, J = 244.5Hz), 161.2,159.4, 131.1, 125.9, 117.2, 116.4, 89.5, 87.7, 39.9, 26.5, 24.0. ^{19}F NMR (471 MHz, DMSO-d_6): -112.36.



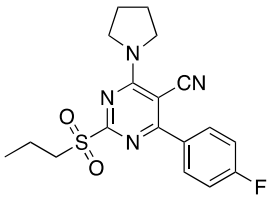
4-(2-Fluorophenyl)-2-methylsulfonyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2g**) was prepared using *S*-methylisothiouronium iodide.

Yield 98%, mp 149-151 °C. Found, %: C 55.57; H 4.45; N 16.29. $C_{12}H_{10}N_4O_2S$. Calculated, %: C 55.48; H 4.37; N 16.18. ^{1}H NMR (600 MHz, DMSO-d_6): 7.71 – 7.62 (m, 2H), 7.50 – 7.39 (m, 2H), 4.05 – 3.67 (m, 4H), 3.38 (s, 3H, SO_2Me), 2.11 – 1.89 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6): 168.0, 165.5, 160.4 (d, J = 248.3 Hz),, 158.5, 133.8 (d, J = 8.5 Hz), 131.7, 125.4, 124.5, 116.6 (d, J = 21.1 Hz), 116.4, 91.8, 50.4, 49.3, 39.1, 26.4, 23.9. ^{19}F NMR (471 MHz, DMSO-d_6): -113.86.



2-Ethylsulfonyl-4-phenyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2h**) was prepared using *S*-ethylisothiouronium bromide.

Yield 73%, mp 158-160 °C. Found, %: C 59.69; H 5.36; N 16.42. $C_{17}H_{18}N_4O_2S$. Calculated, %: C 59.63; H 5.30; N 16.36. ^{1}H NMR (600 MHz, DMSO-d_6): 7.90 – 7.85 (m, 2H), 7.68 – 7.56 (m, 3H), 4.07-3.67 (m, 4H), 3.59 (q, J = 7.3 Hz, 2H, CH_2), 2.12 – 1.85 (m, 4H), 1.31 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): 171.5, 164.5, 159.6, 136.0, 132.0, 129.7, 129.0, 117.4, 89.1, 50.6, 49.5, 45.3, 26.5, 24.0, 7.4.



4-(4-Fluorophenyl)-2-propylsulfonyl-6-(pyrrolidin-1-yl)pyrimidine-5-carbonitrile (**2i**) was prepared using *S*-propylisothiouronium chloride.

Yield 70%, mp 101-103 °C. Found, %: C 57.81; H 5.21; N 15.04. $C_{18}H_{19}FN_4O_2S$. Calculated, %: C 57.74; H 5.11; N 14.96. ^{1}H NMR (600 MHz, DMSO-d_6): 8.00 – 7.91 (m, 1H), 7.46 – 7.40 (m, 1H), 4.10 – 3.63 (m, 4H), 3.57 – 3.49 (m, 2H, CH_2), 2.10 – 1.87 (m, 4H), 1.77 (h, J = 7.5 Hz, 2H, CH_2), 1.01 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): 173.9, 169.7, 165.3, 163.3 (d, J = 248.3 Hz), 159.4, 132.3, 131.9, 118.7, 117.9, 116.3, 87.1, 54.6, 52.0, 49.4, 32.7, 22.9, 16.0, 13.4. ^{19}F NMR (471 MHz, DMSO-d_6): -108.7.

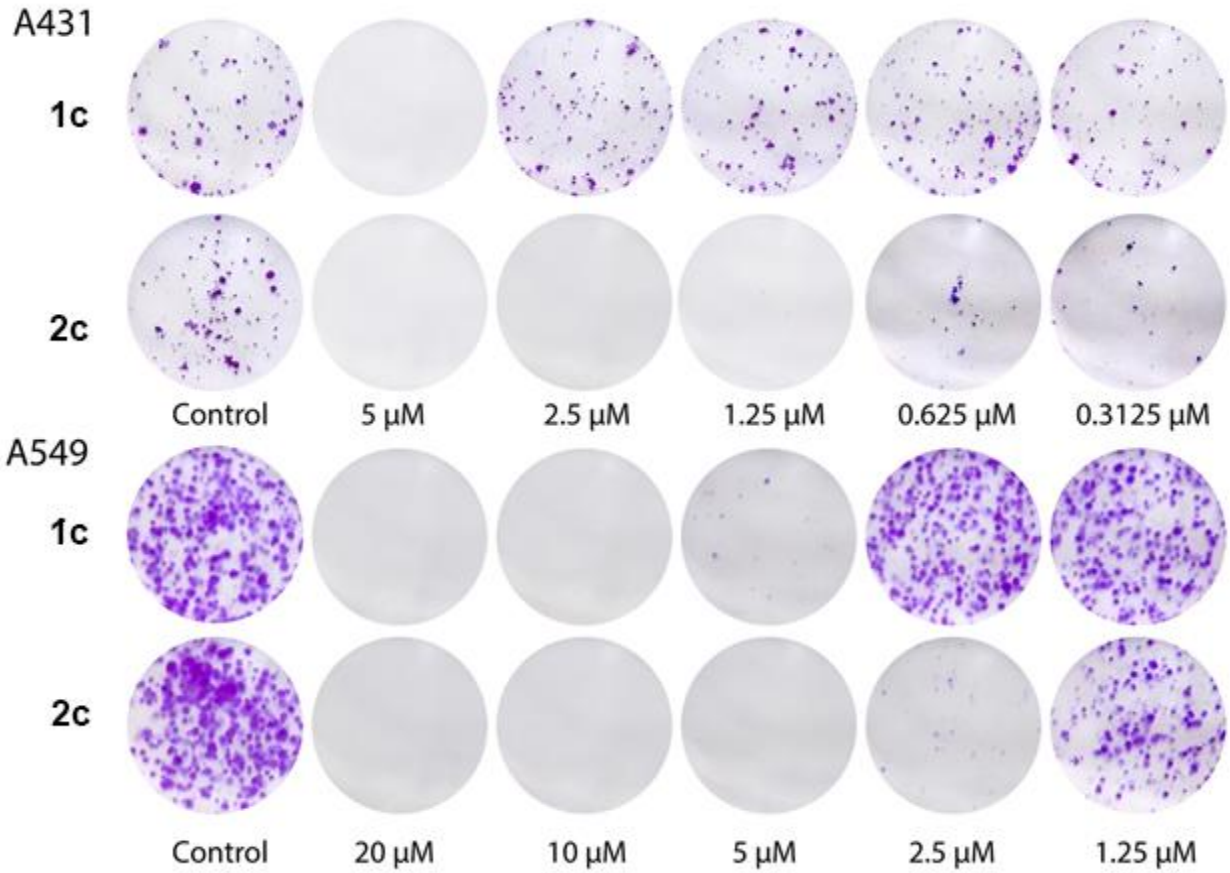
Biological assays

Cell Proliferation Assay [S6]. The human lung adenocarcinoma cell line A549, human epidermoid carcinoma cell line A431, human melanoma cell line A375, human colorectal cancer cell lines HCT-116, human breast cancer cell line MCF-7, human prostate cancer cells LNCaP, human neuroblastoma cell lines SH-SY5Y were used to evaluate the potency of synthesized analogues in cell-based level. All cell lines were purchased from American type culture collection (ATCC). A431, A375, MCF-7 cell line was cultured in DMEM (Gibco), A549, HCT-116 and LnCaP were cultured with RPMI 1640 (Gibco), SH-SY5Y cell line was cultured in

DMEM/F12 medium (Gibco). All culture mediums were supplemented with *L*-glutamine, penicillin/streptomycin (Invitrogen) and 10% fetal bovine serum (Gibco).

All cells were seeded in 96-well tissue culture plates (Eppendorf) in density of 7×10^3 cells/well in 200 µl of complete growth media for 24 hours. Triplicate wells were treated with test compounds starting at 500.0 µM concentration and diluted 12 times for 48 hours at various concentrations or DMSO (Sigma) as control with final concentration 0.1%. Plates were incubated at 37°C in 5% CO_2 atmosphere. Following treatment, 40 µL of MTT (5 mg ml^{-1}) reagent was incubated in each well for 4 hr. After 4 h, medium/MTT mixtures were replaced with DMSO (200 µl per well), and optical density at a wavelength of 570 nm was measured with a microplate reader (GloMax Multi+, Promega).

Clonogenic Survival Assay [S7]. A549 and A431 cells were placed in 6-well plates at 1000 cells per well. Twenty-four hours later cells were treated with varying concentrations of 5-cyanopyrimidine derivates and then monitored microscopically for colony growth. Ten days later the cells were fixed with 4% paraformaldehyde solution. Following fixation for 15 min, the plates were washed and stained with a 0.4% solution of crystal violet in 20% methanol for 30 min, washed with PBS, and air-dried. Colonies consist more than 50 cells were counted as positive. Results are normalized to percent clonogenic survival from untreated control cells (Figure S1)



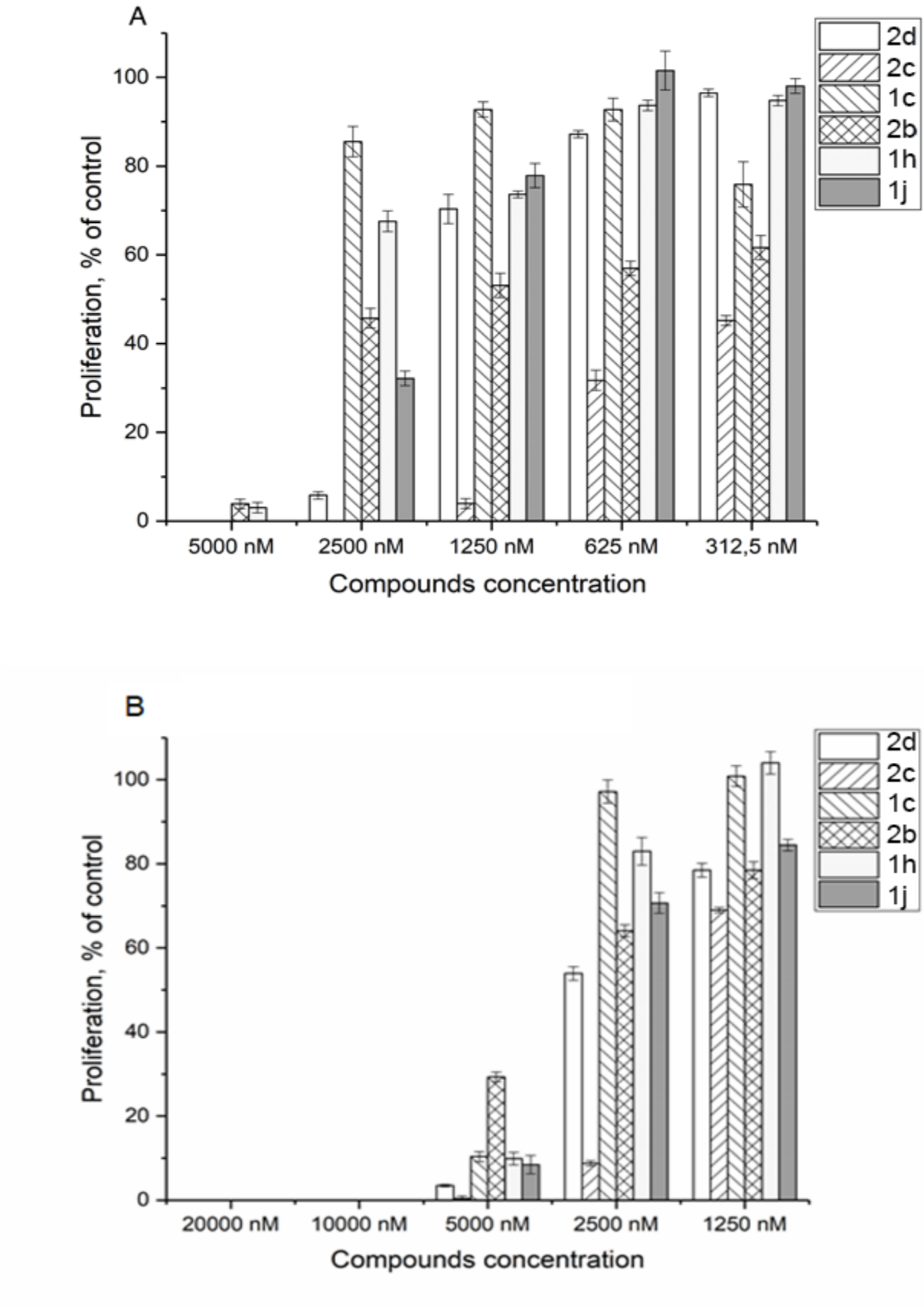


Figure S1. Clonogenic survival assay. (*A*) A431 cell line, (*B*) A549 cell line

Apoptosis Assay [S8] The induction of apoptosis A549 and A431 cancer cells by 5-cyanopyrimidine derivates was assayed by staining with Annexin V and propidium iodide (PI) using the AnnexinV-FITC kit (Invitrogen, USA) in accordance with the manufacturer's protocol. After incubation cells with the 5-cyanopyrimidine derivates for 24 h, they were centrifuged, the pellet was resuspended in 100 µl of binding buffer and a solution containing PI and annexin V was added. The cells were incubated at room temperature in the dark for 15 minutes, then 400 µl of binding buffer was added to stop the reaction. Data analysis (at least 2×10^4 events) was performed on a NovoCyte 2000R flow cytometer (ACEA Biosciences, San Diego, CA, USA) using NovoExpress v.1.2.4 software.

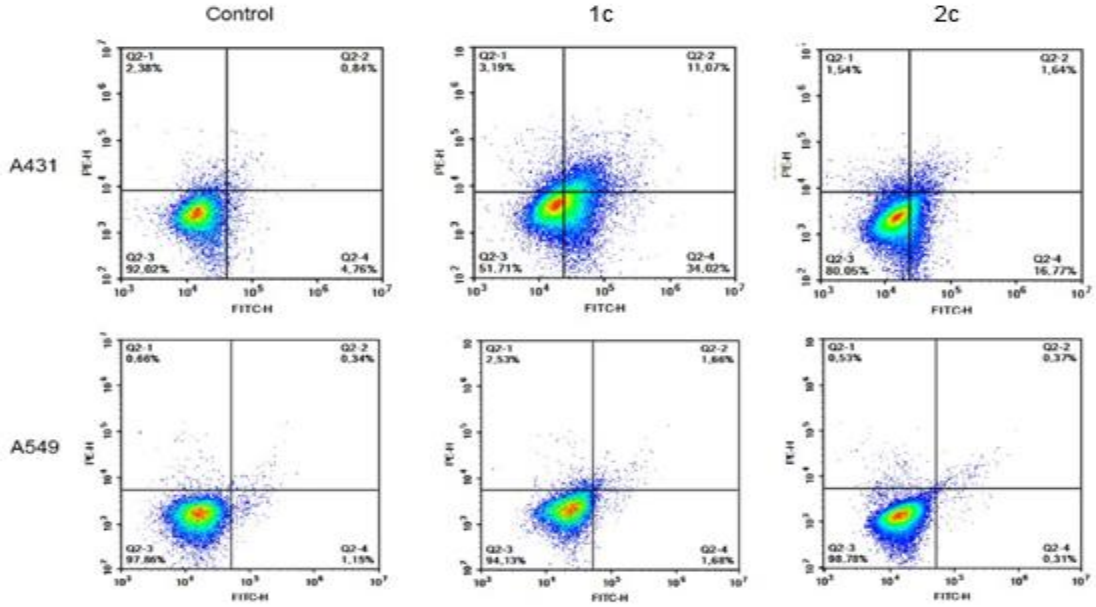


Figure S2. Apoptosis assay on A431 and A549 cell lines

Cell cycle analysis [S9]. A549 and A431 cancer cells were placed in 6-well cultures plates (Nunc, Denmark) at 2×10^5 cells per well and cultivated overnight in complete grow RPMI 1640 media. After media was replaced on RPMI 1640 with 2% FBS and incubate for 24 h.

Tested compound in noncytotoxic concentration were added to A549 cells in RPMI 1640 media with 2% FBS and 20 ng/ml recombinant human EGF (Invitrogen, USA). After 24 h of treatment, the cells were harvested, washed with PBS. The cell pellets were resuspended in 500 µl of 50 µg/ml solution of propidium iodide (PI) in buffer (BD Biosciences, Franklin Lakes, NJ, USA) and incubated in the dark at room temperature for 15 min. The PI fluorescence signal was assessed on a NovoCyte 2000R flow cytometer (ACEA Biosciences, San Diego, CA, USA) and the cell cycle distribution was analyzed using NovoExpress v.1.2.4 software.

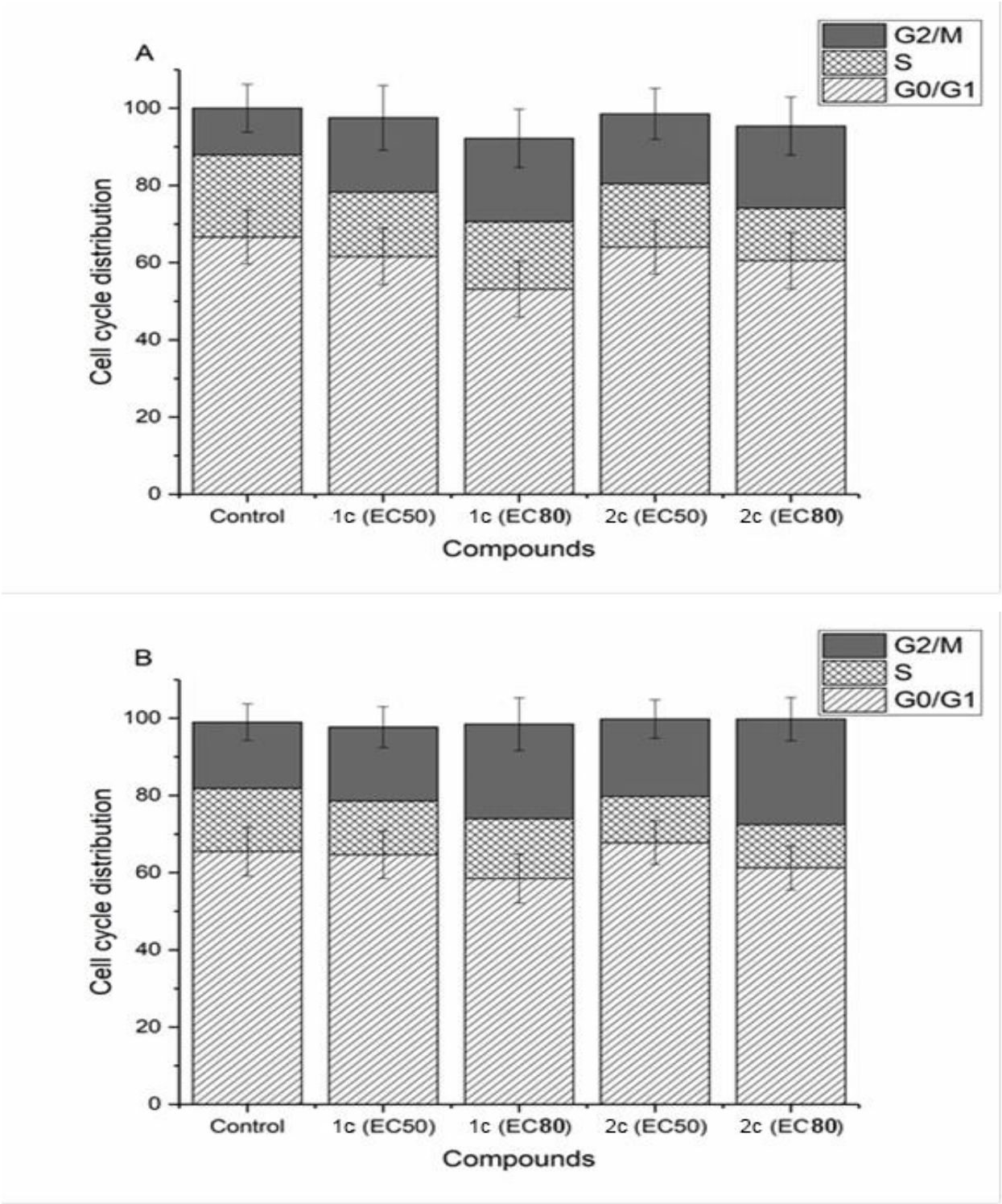


Figure S3. Cell cycle. (*A*) A549 cell line, (*B*) A431 cell line

Cell migration assay [S10]. The wound healing assay mimics tumor cell migration *in vivo*. A549 cancer cells were seeded in 24-well cell culture plates and cultured to a confluent monolayer. A 200 μl pipette tip was used to scratch a wound on the midline of the culture well, and cell layer was then washed twice with RPMI 1640 and were treated with the control RPMI 1640 medium or medium containing 20 ng ml^{-1} EGF and 5-cyanopyrimidines derivate for 48 h. At 0 and 48 h,

images were captured on a microscope Leica DMIL (Leica, Mannheim, Germany) with Canon 60D camera (Canon Inc., Tokyo, Japan) at a magnification of ×100. The migration ability of the cells was assessed by counting the wound closure rate (Figure S4).

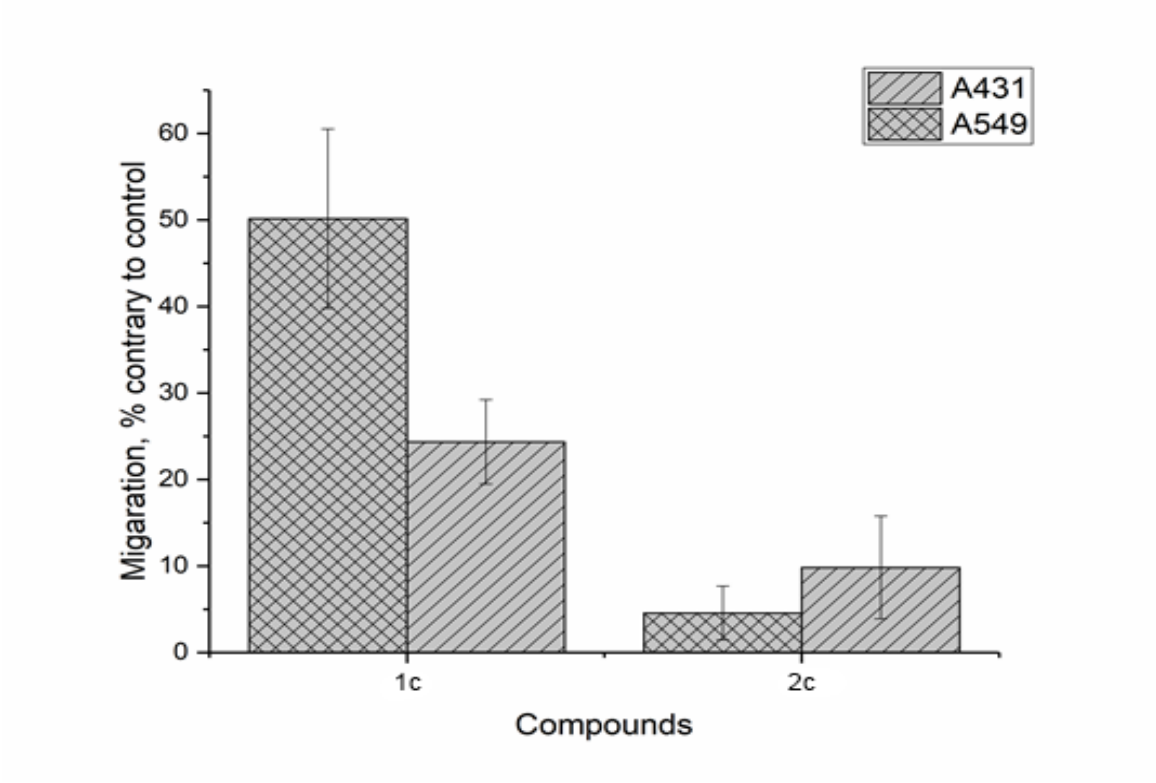


Figure S4. Migration assay at A431 and A549 cell line

Colorimetric detection of EGFR, Akt and ERK1/2 levels. To evaluate EGFR, Akt, ERK1/2 and its phosphorylated forms expression we performed colorimetric detection using Pierce EGFR Colorimetric In-Cell ELISA Kits for EGFR, Akt, ERK1/2 (Thermo scientific). Briefly, A431 cells were placed in 96-well plate at 10,000 cells per well and incubated overnight at 37°C in 5% CO_2. After cells were treated with selected 5-cyanopyrimidine derivates at IC50 concentration in complete DMED containing 20 ng ml^{-1} EGF for 24 h. Cells were fixed with 4% formaldehyde for 15 minutes and washed twice with 100µl per well of 1X TBS. Cells were treated with 100µL/well of 1X Permeabilization buffer for 15 minutes and washed once with 1X TBS. Cells were treated of Quenching solution and incubate at room temperature for 20 min, washed once with 1X TBS and blocked with 100 µl per well of Blocking buffer at room temperature for 30 min. Cells were incubated with 50 µl per well of primary antibody overnight at 4°C. Later in well was added diluted HRP conjugate and TMB substrate. After stopping reaction absorbance was measured at 450nm within 30 minutes of stopping the reaction.

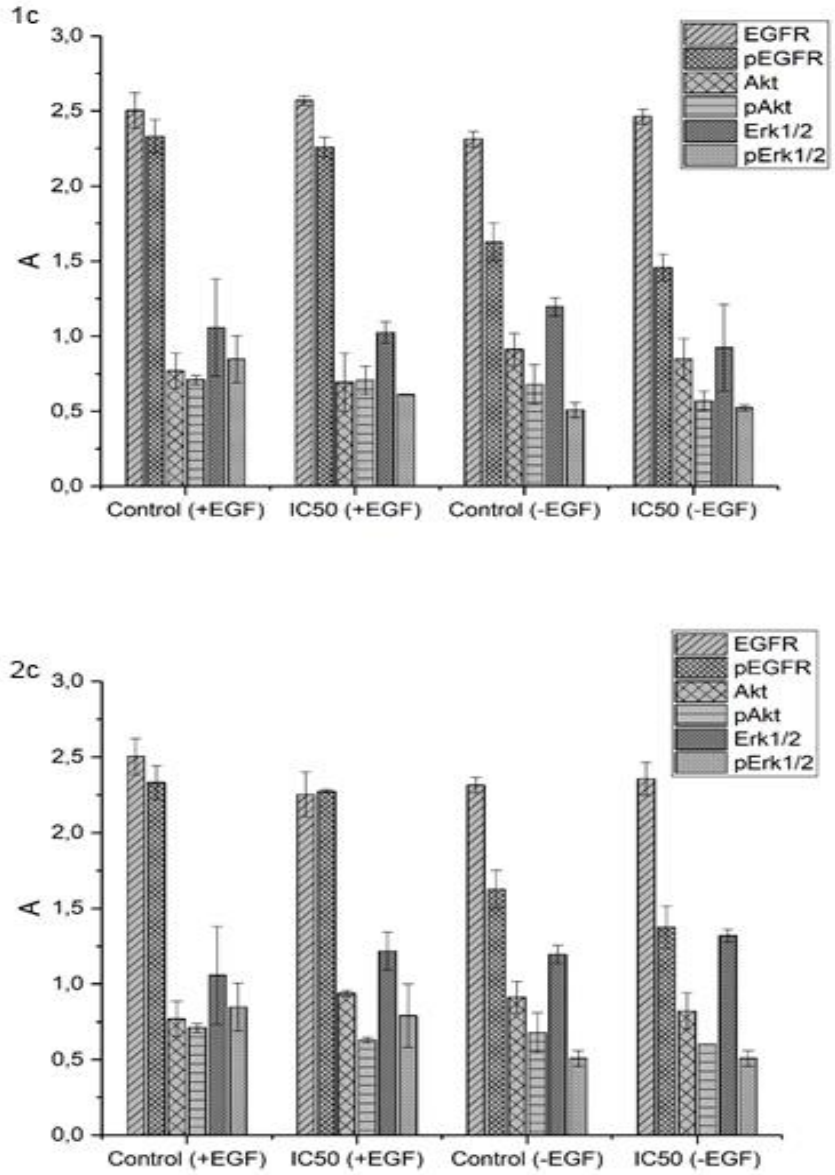


Figure S5. In-Cell ELISA assay for determining EGFR, pEGFR, Akt, pAkt, ERK1/2 and pERK1/2 levels

Generation of Spheroids. Spheroids were generated by plating A549 cancer cells at 1×10^4 cells per well into U-bottom Ultra Low Adherence (ULA) 96-well plates (Corning, USA) at 200 μl per well for formation of a single spheroid. After the cells were introduced into the plate, compounds **1c** and **2c** were added in a non-cytotoxic concentration and incubated for 72 hours at 37°C, 5% CO_2. Images were captured InCell Analyzer 6000 at magnification 4x and InCell Investigator software (GE Healthcare).

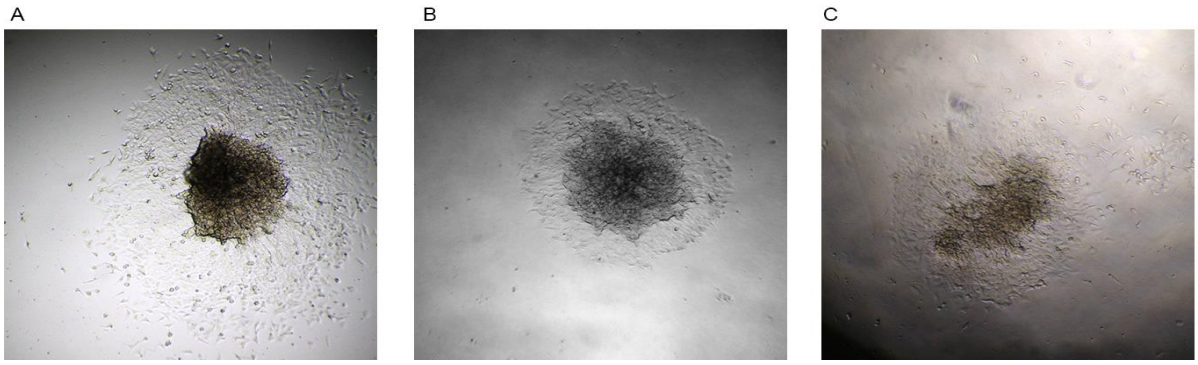


Figure S6 Generation of tumor spheroids in culture (*A* – Control, *B* – **1c**, *C* – **2c**)

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NMR spectra

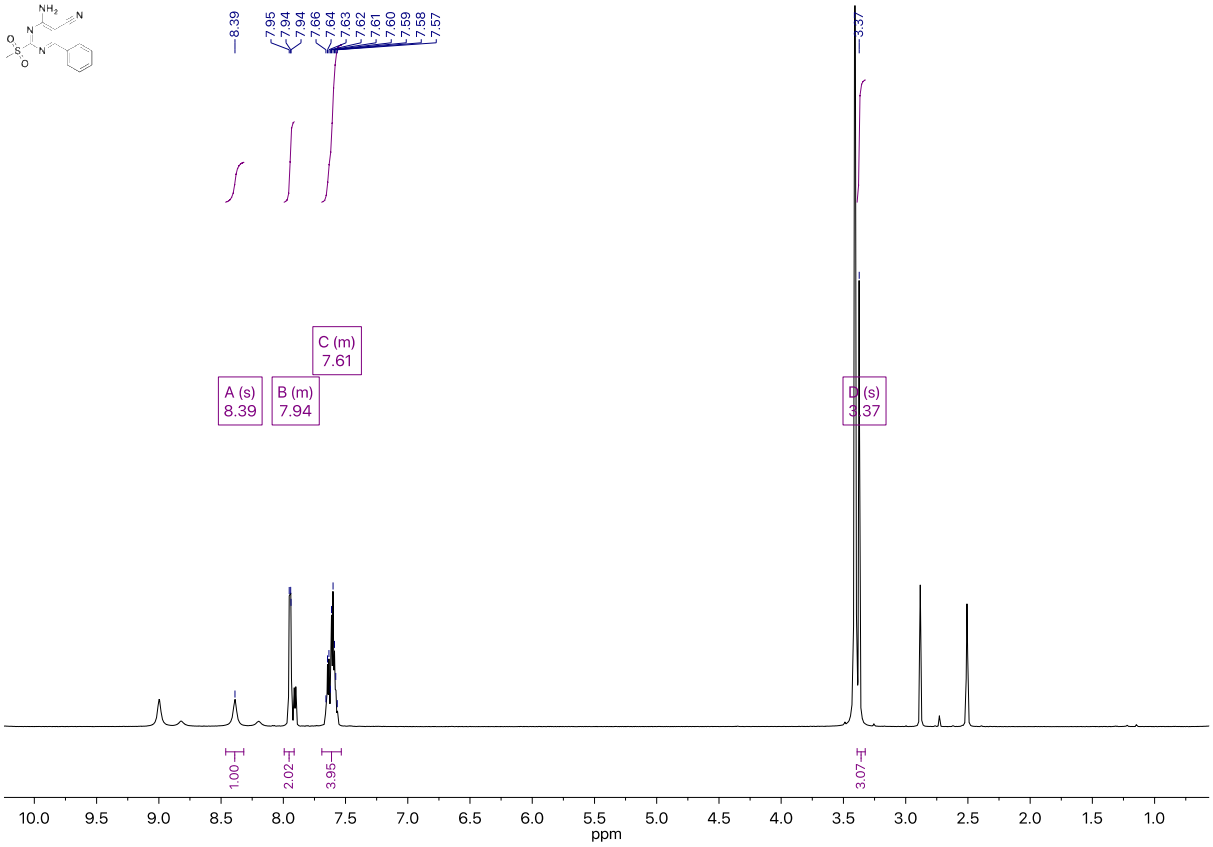

Figure S7. The ^{1}H NMR spectrum of **1a**.

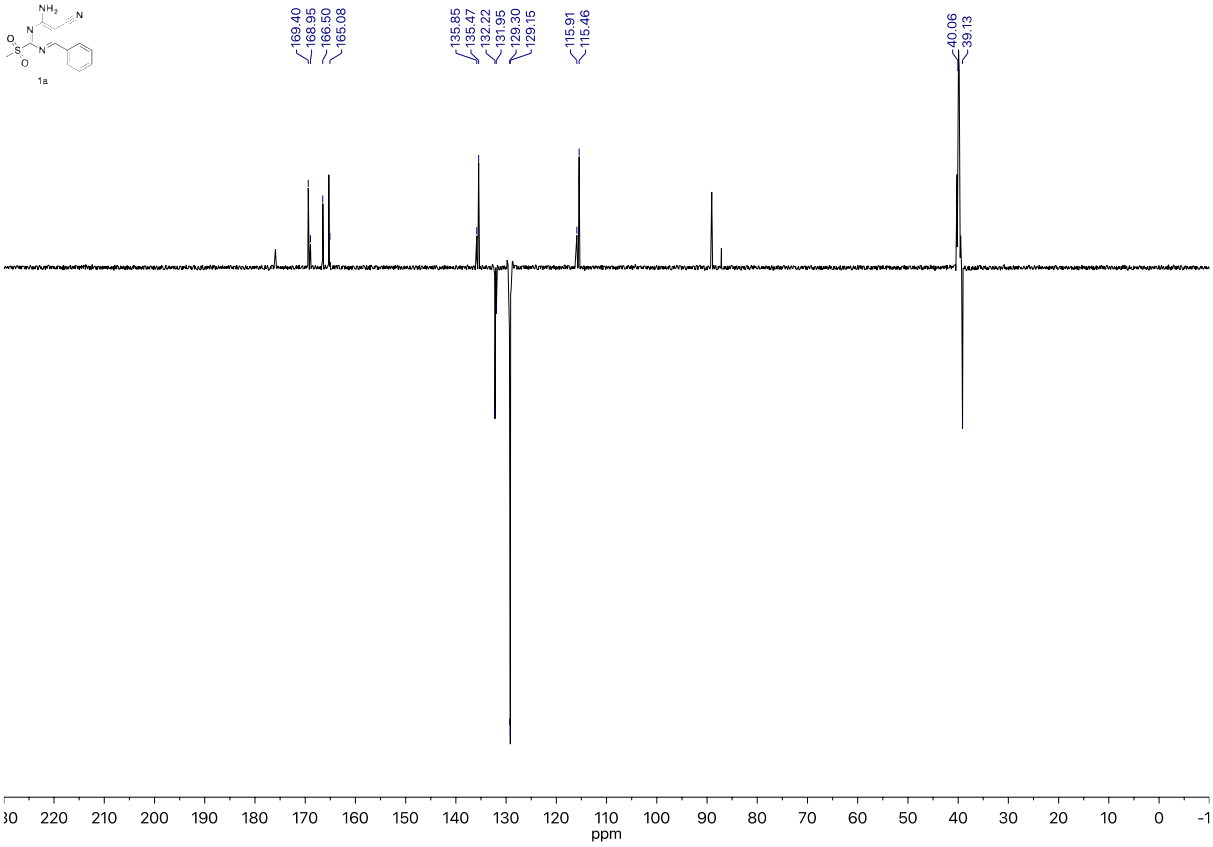

Figure S8. The ^{13}C NMR spectrum of **1a**

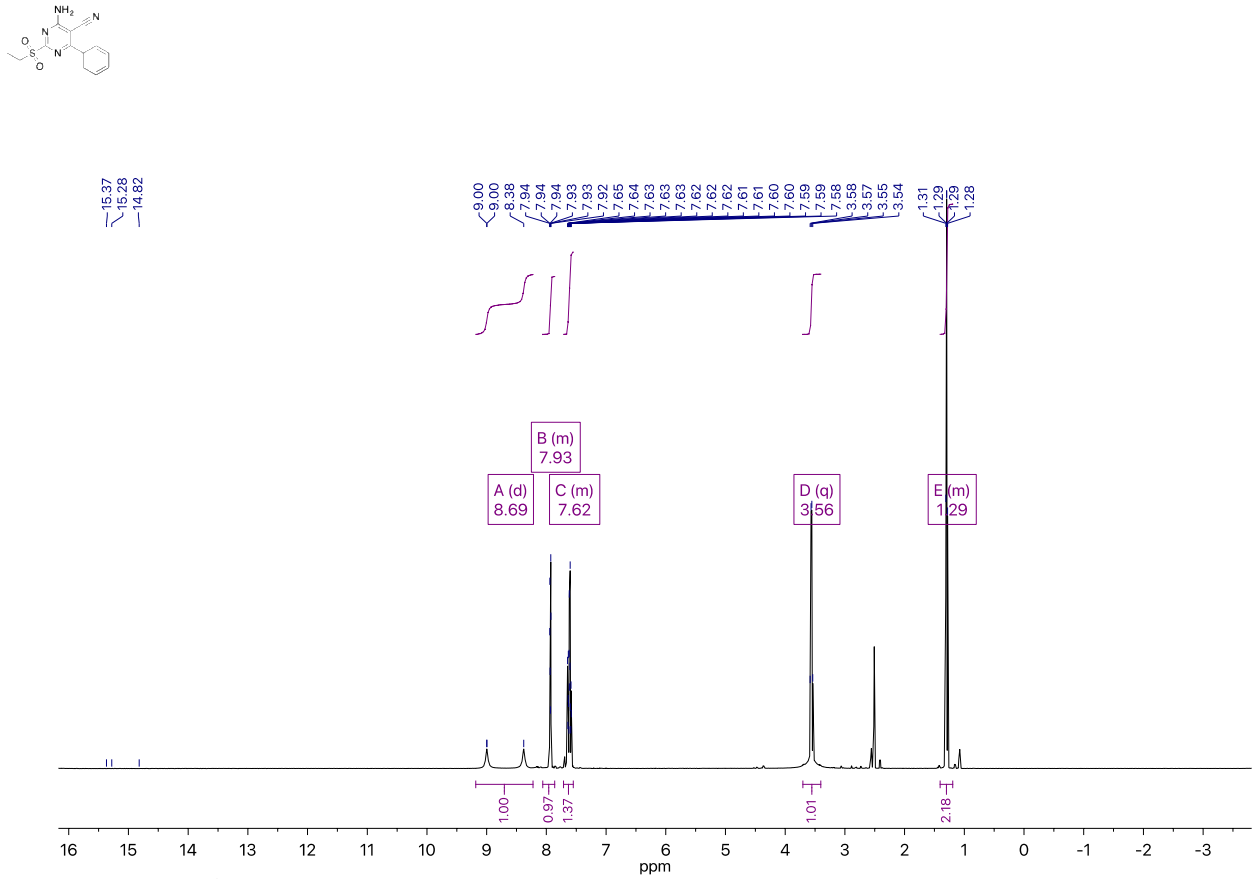

Figure S9. The ^{1}H NMR spectrum of **1b**.

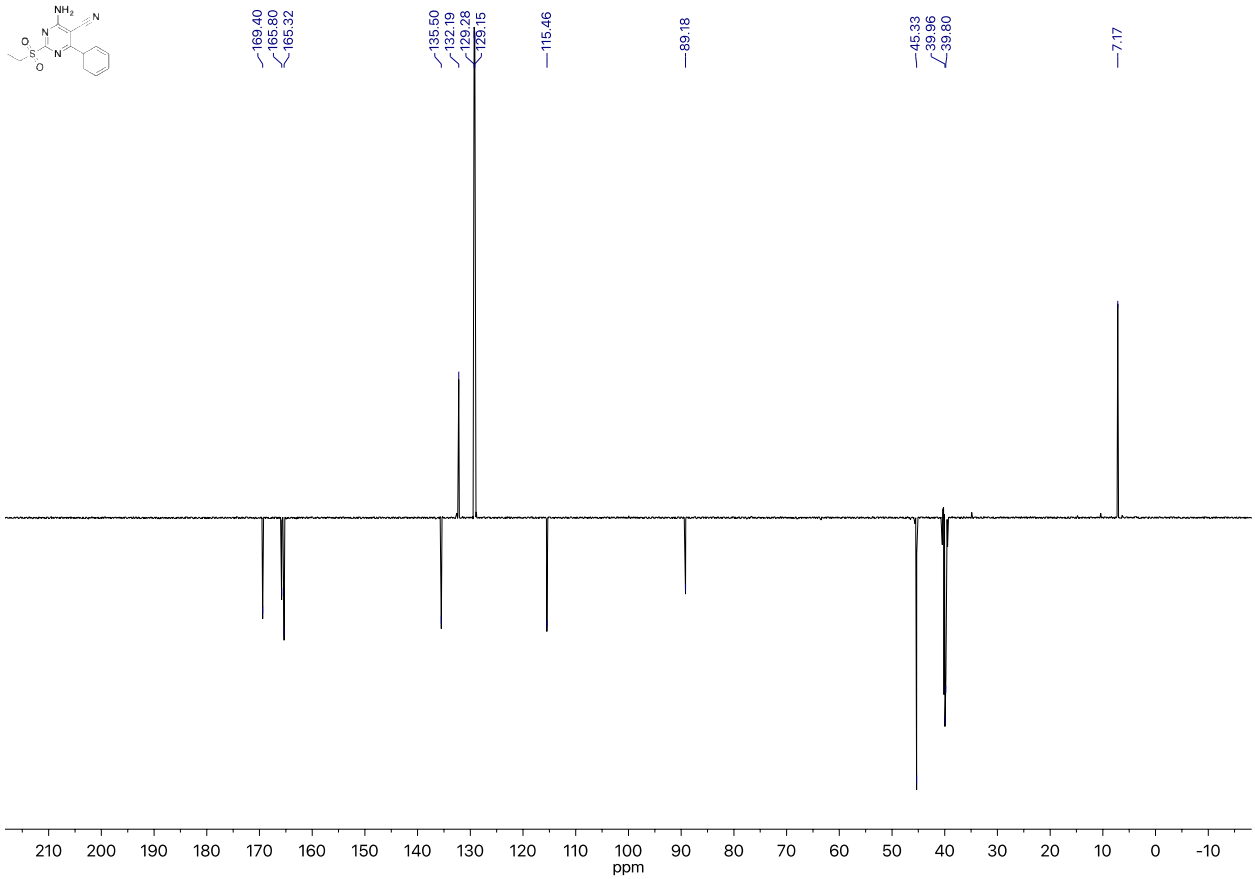

Figure S10. The ^{13}C NMR spectrum of **1b**.

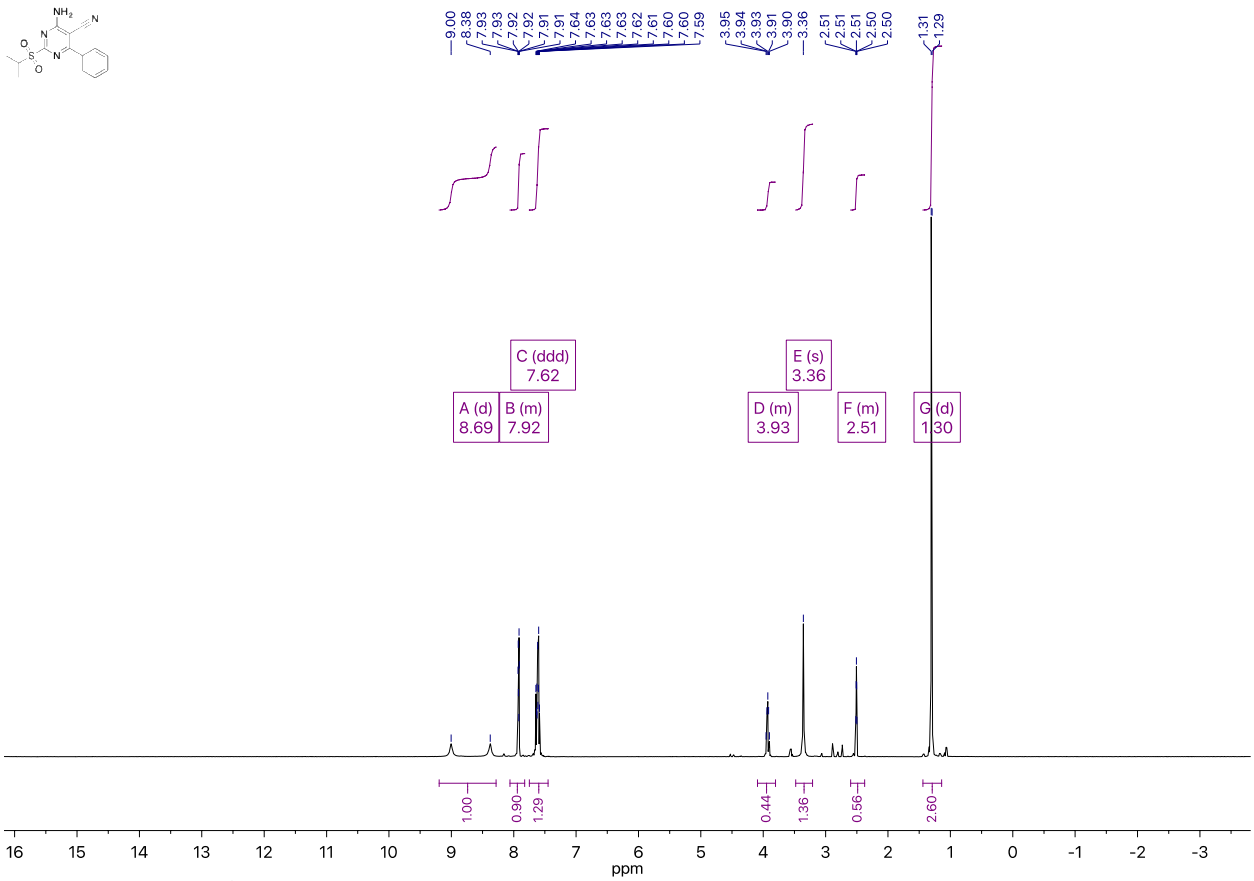

Figure S11. The ^{1}H NMR spectrum of **1c**.

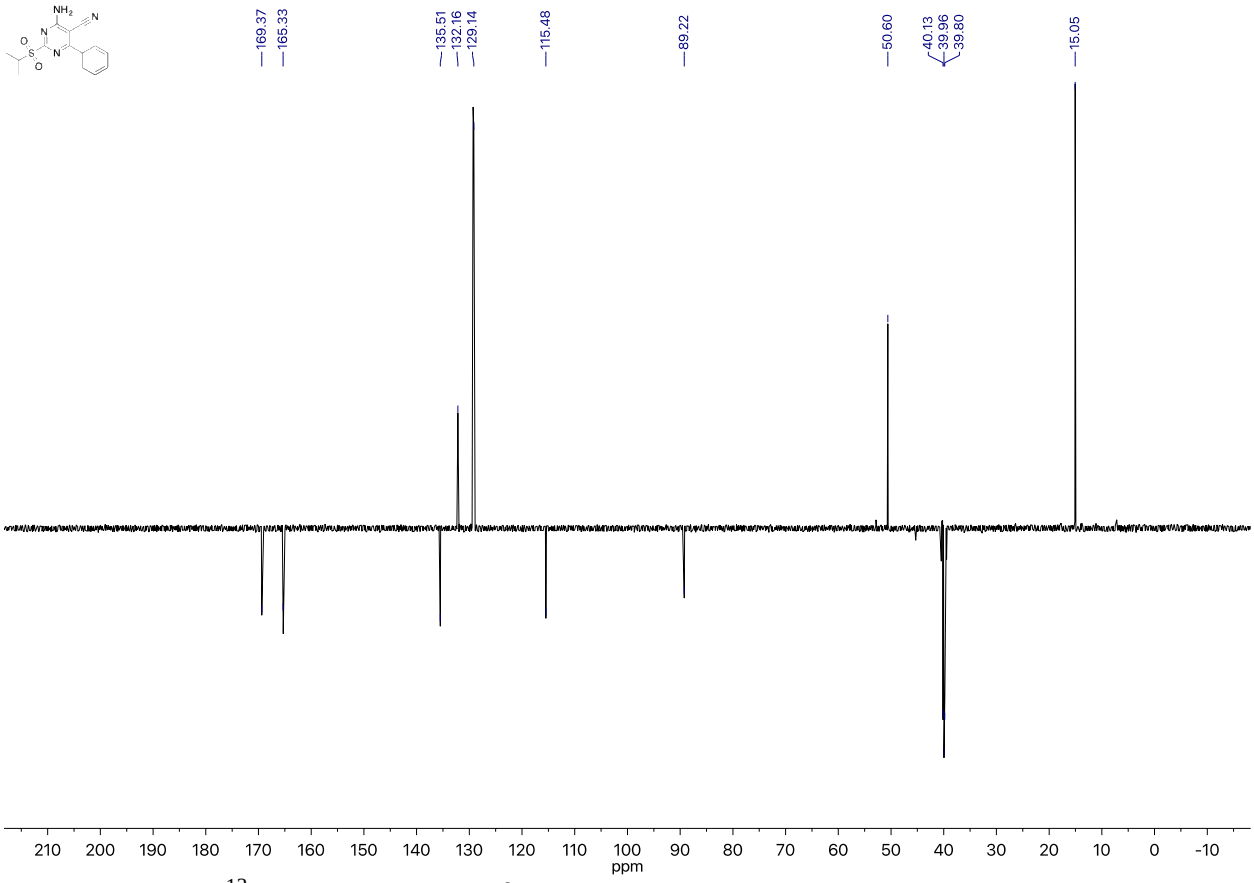

Figure S12. The ^{13}C NMR spectrum of **1c**.

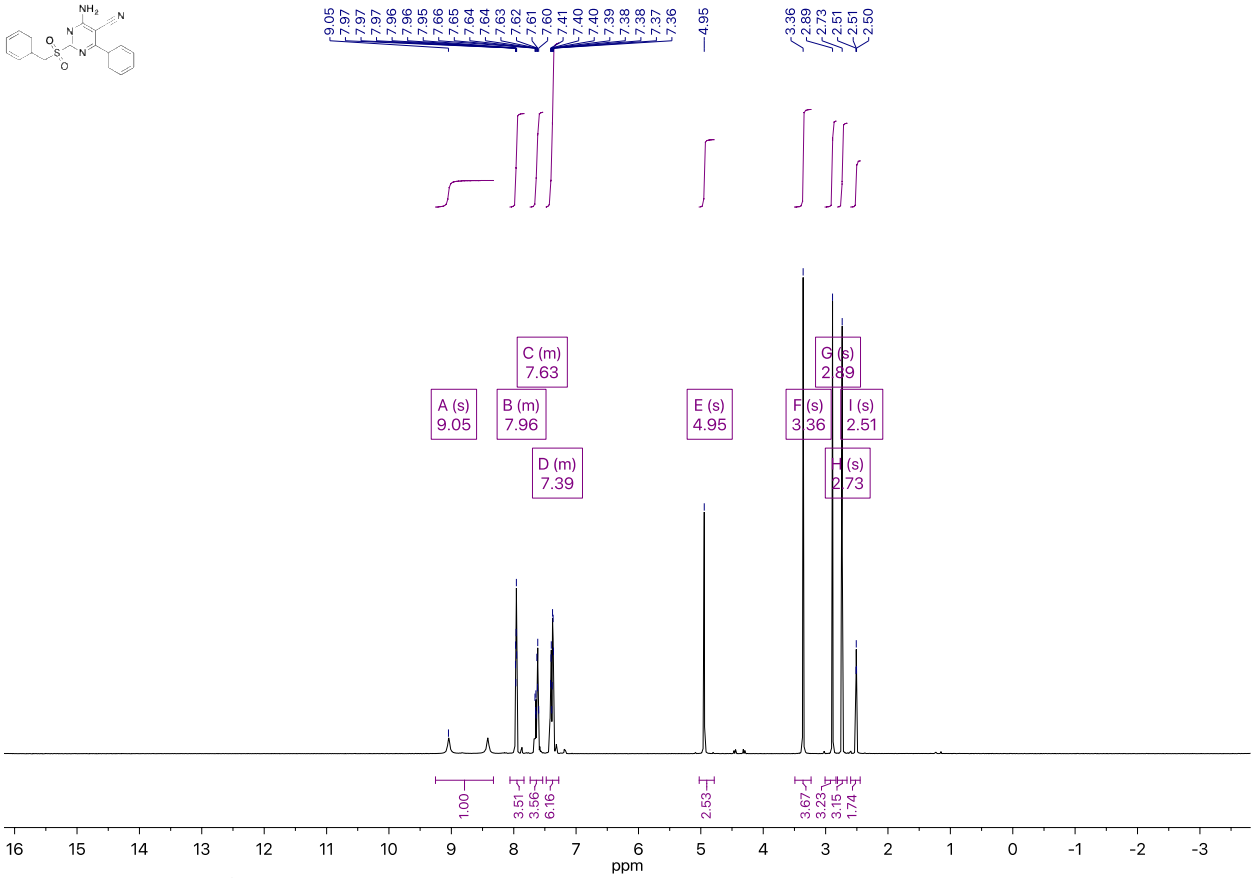

Figure S13. The ^{1}H NMR spectrum of **1d**.

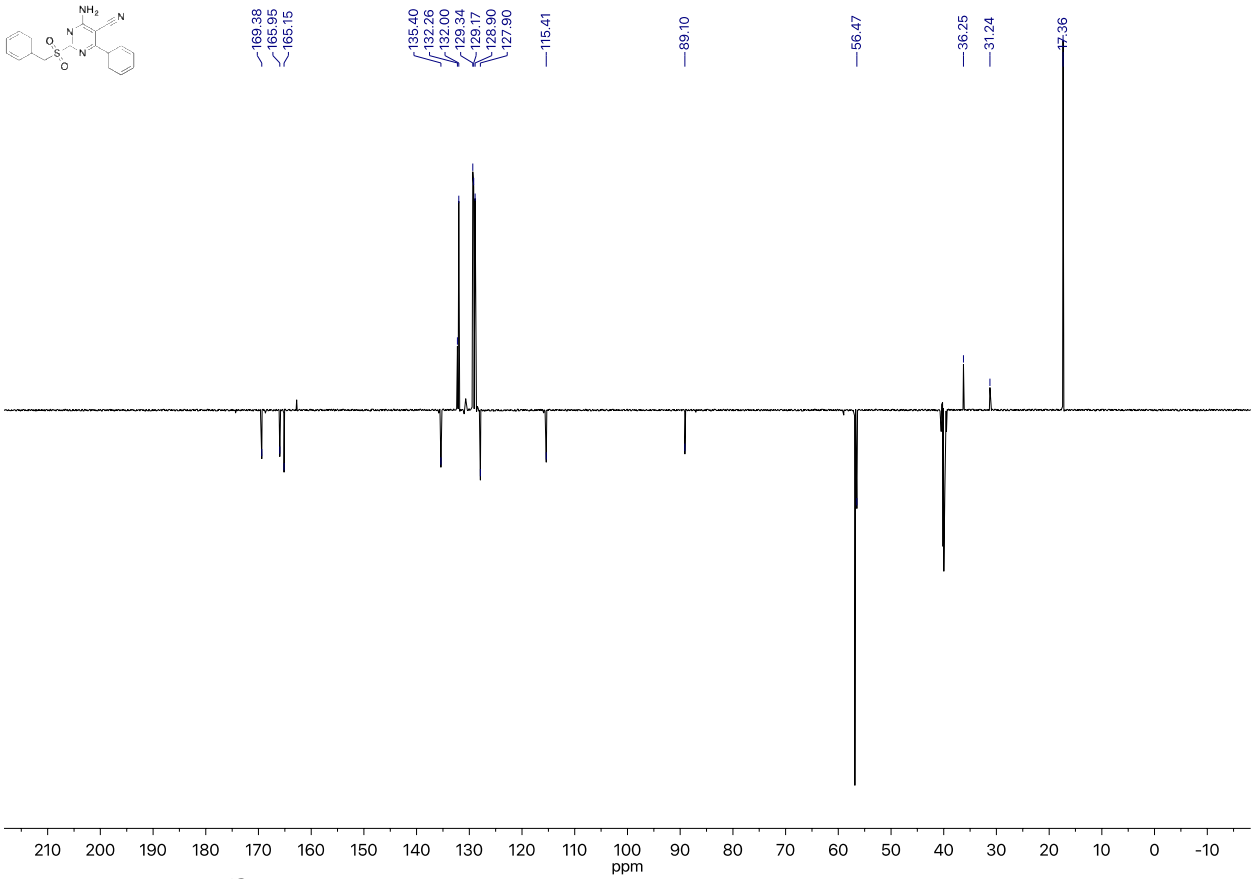

Figure S14. The ^{13}C NMR spectrum of **1d**.

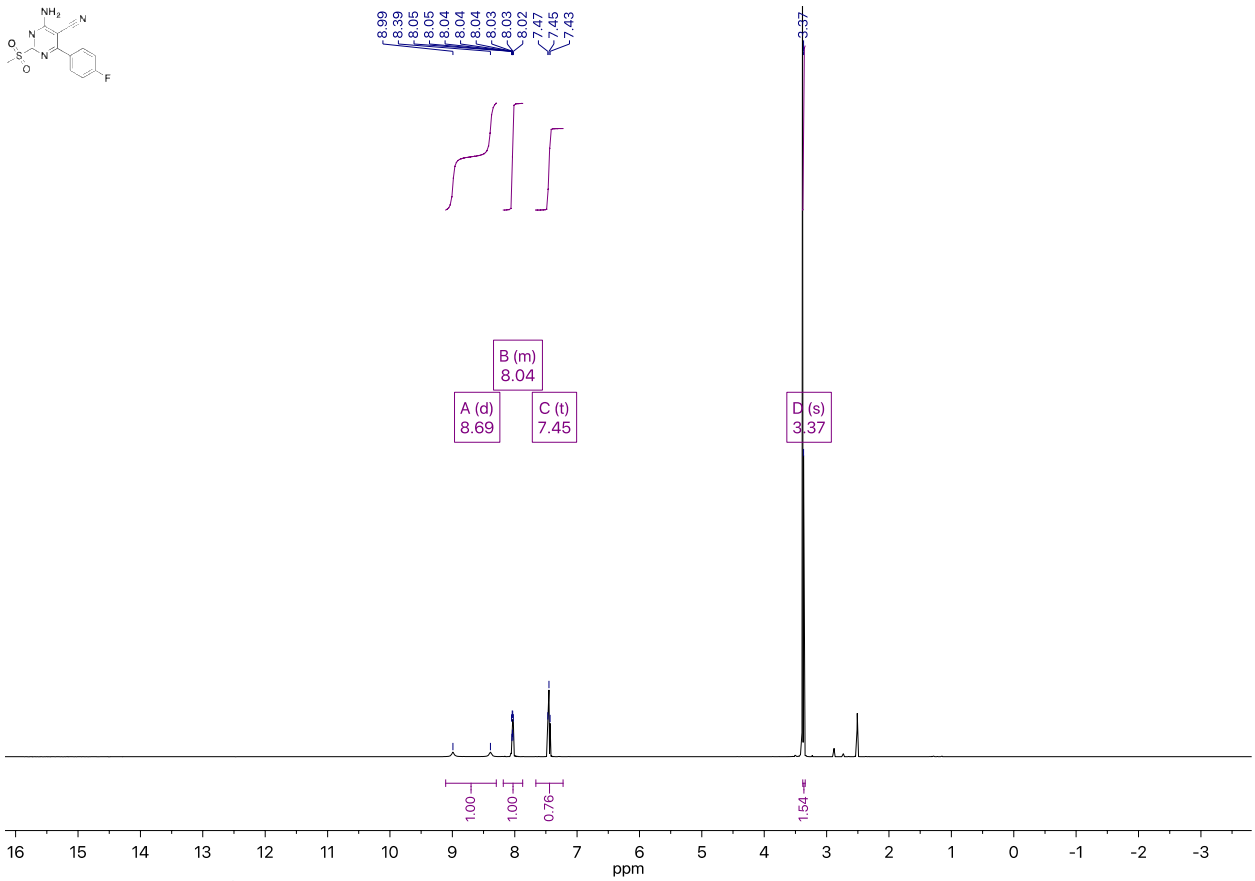

Figure S15. The ^{1}H NMR spectrum of **1e**.

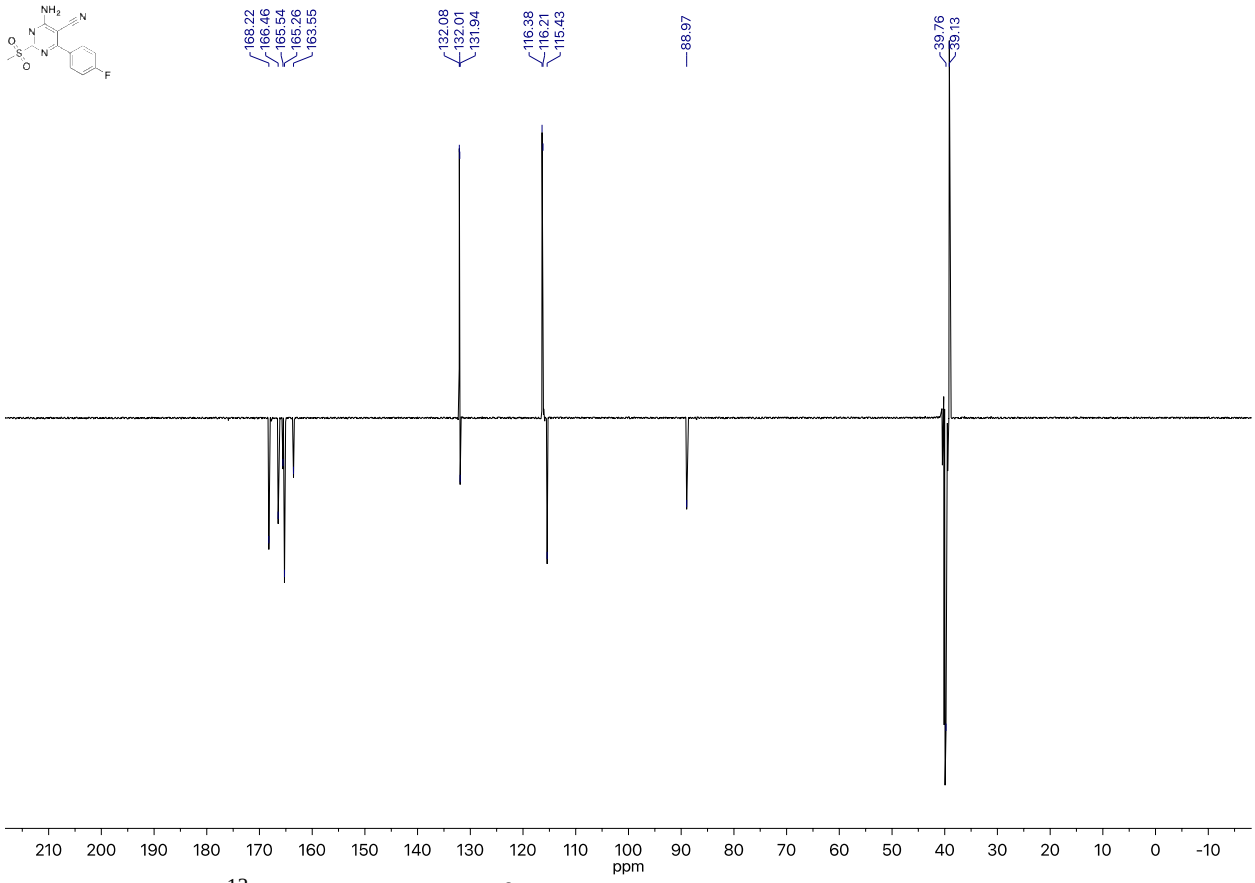

Figure S16. The ^{13}C NMR spectrum of **1e**.

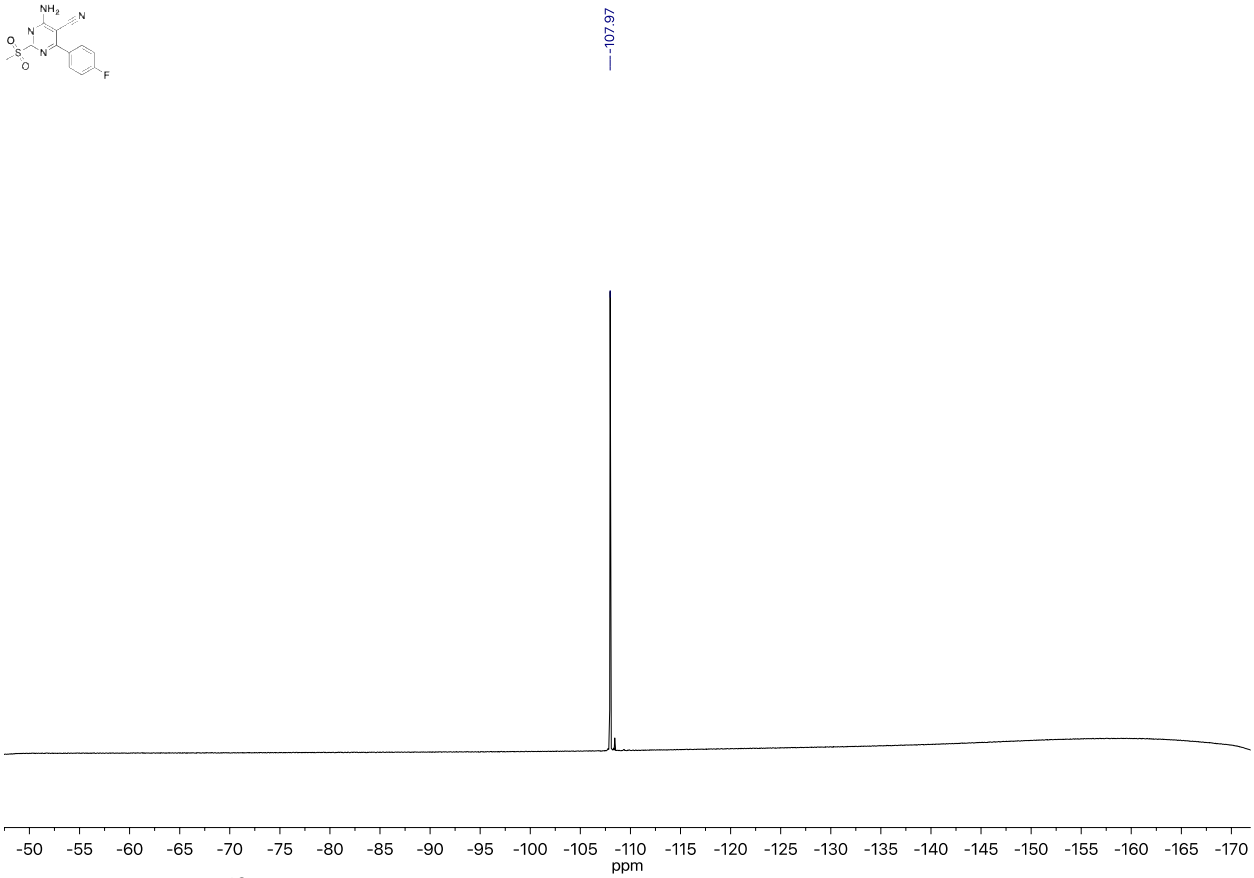

Figure S17. The ^{19}F NMR spectrum of **1e**.

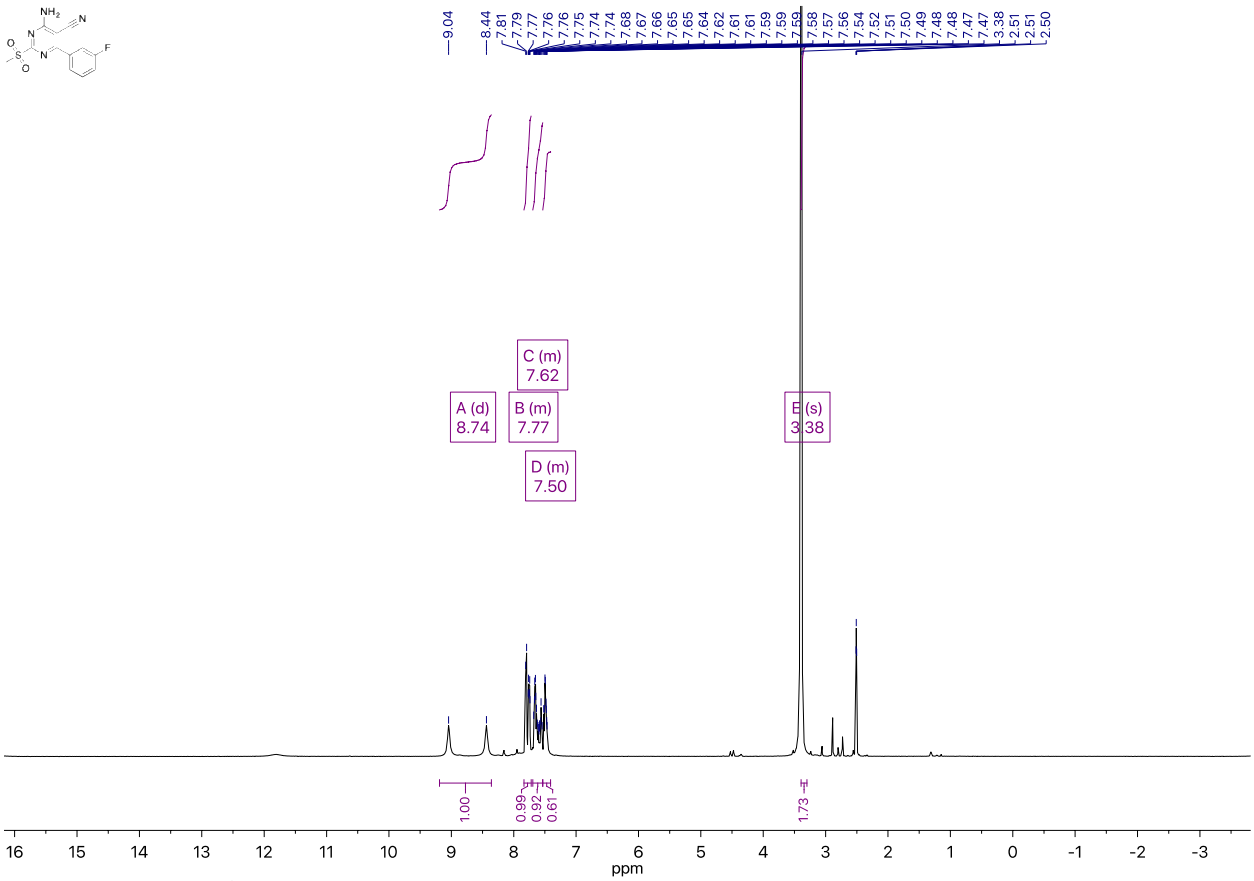

Figure S18. The ^{1}H NMR spectrum of **1f**.

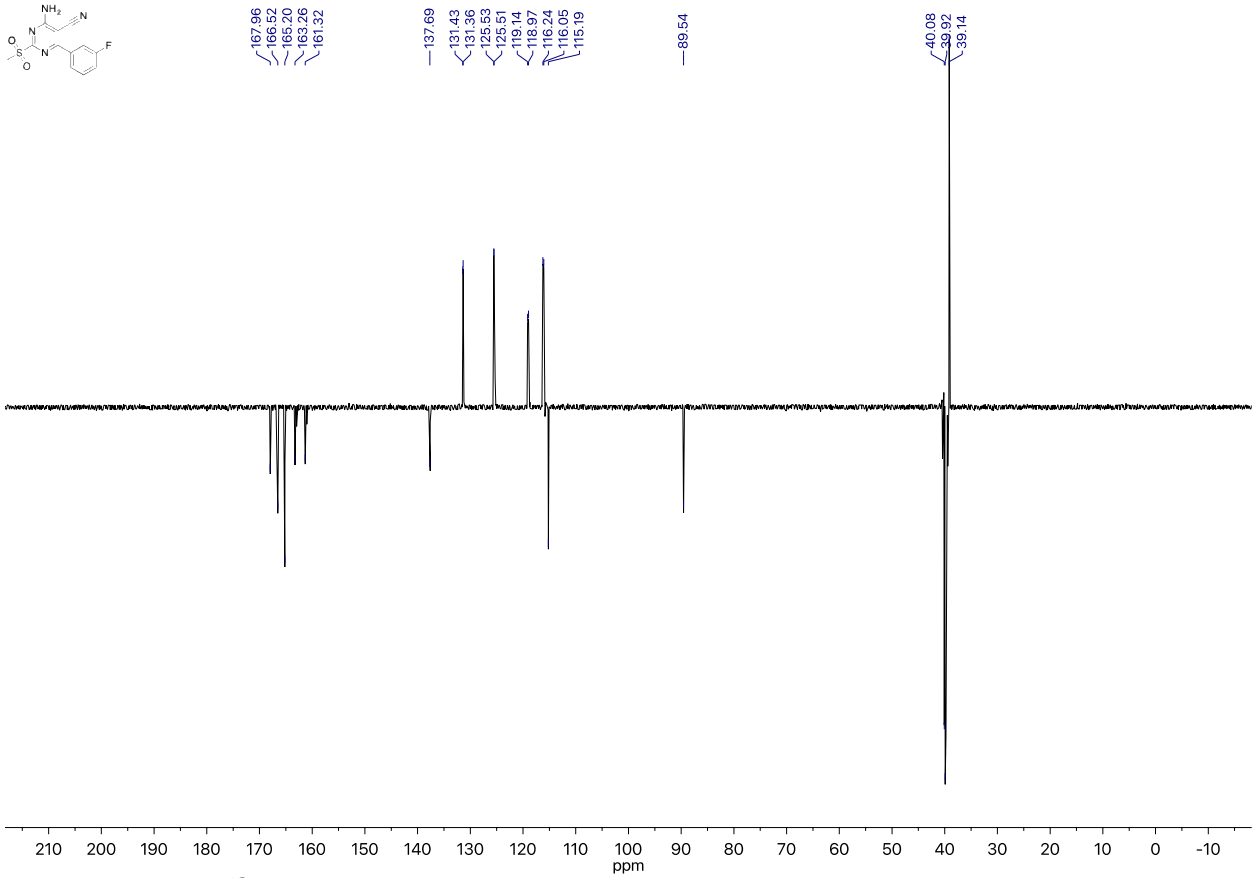

Figure S19. The ^{13}C NMR spectrum of **1f**.

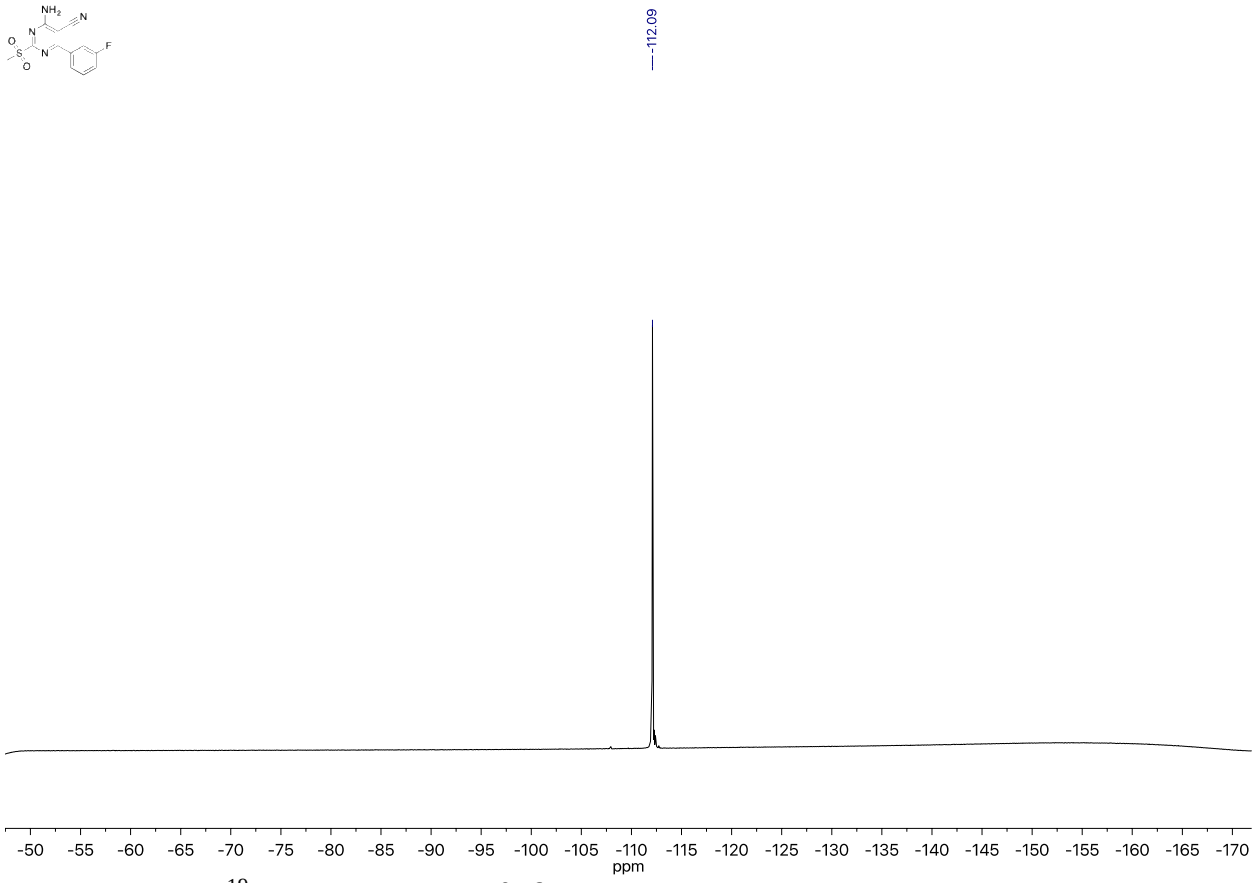

Figure S20. The ^{19}F NMR spectrum of **1f**.

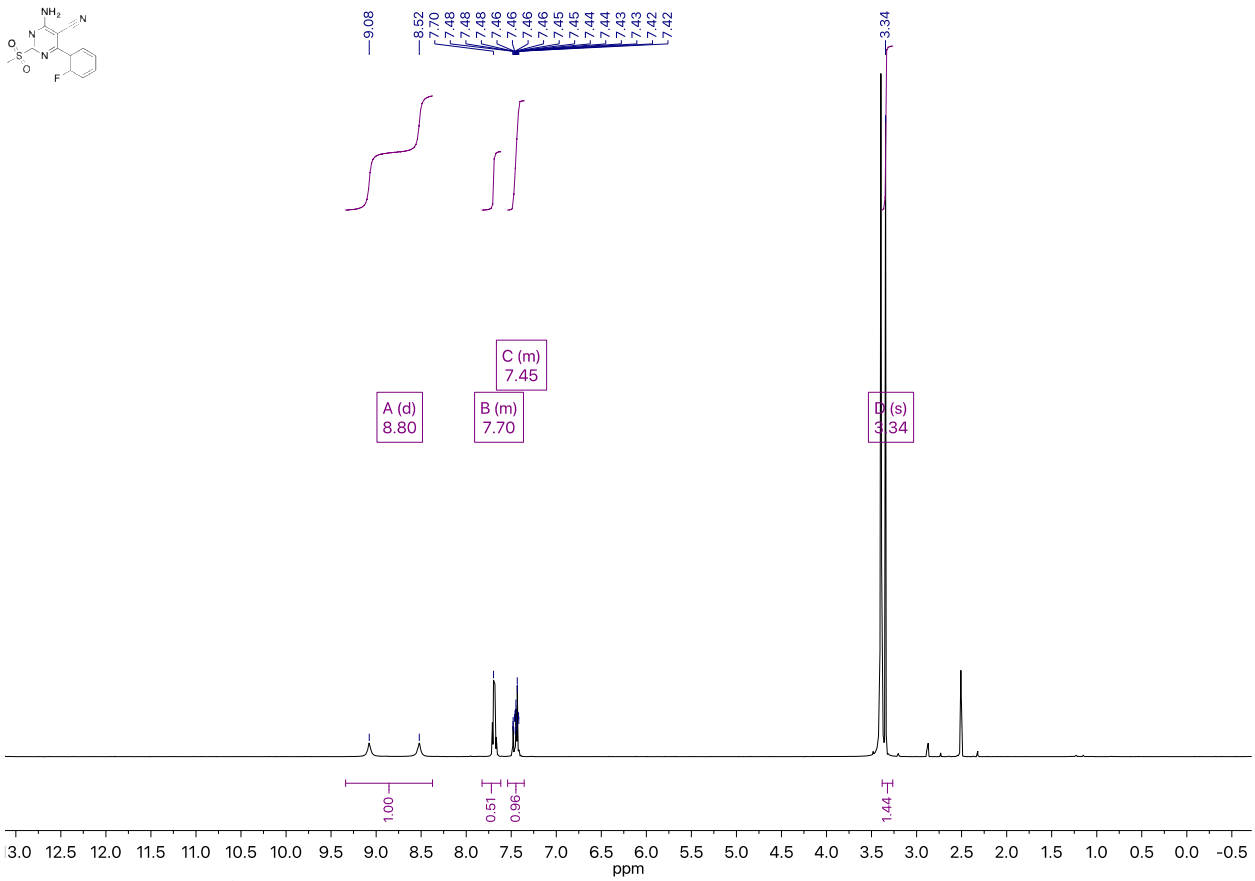

Figure S21. The ^{1}H NMR spectrum of **1g**.

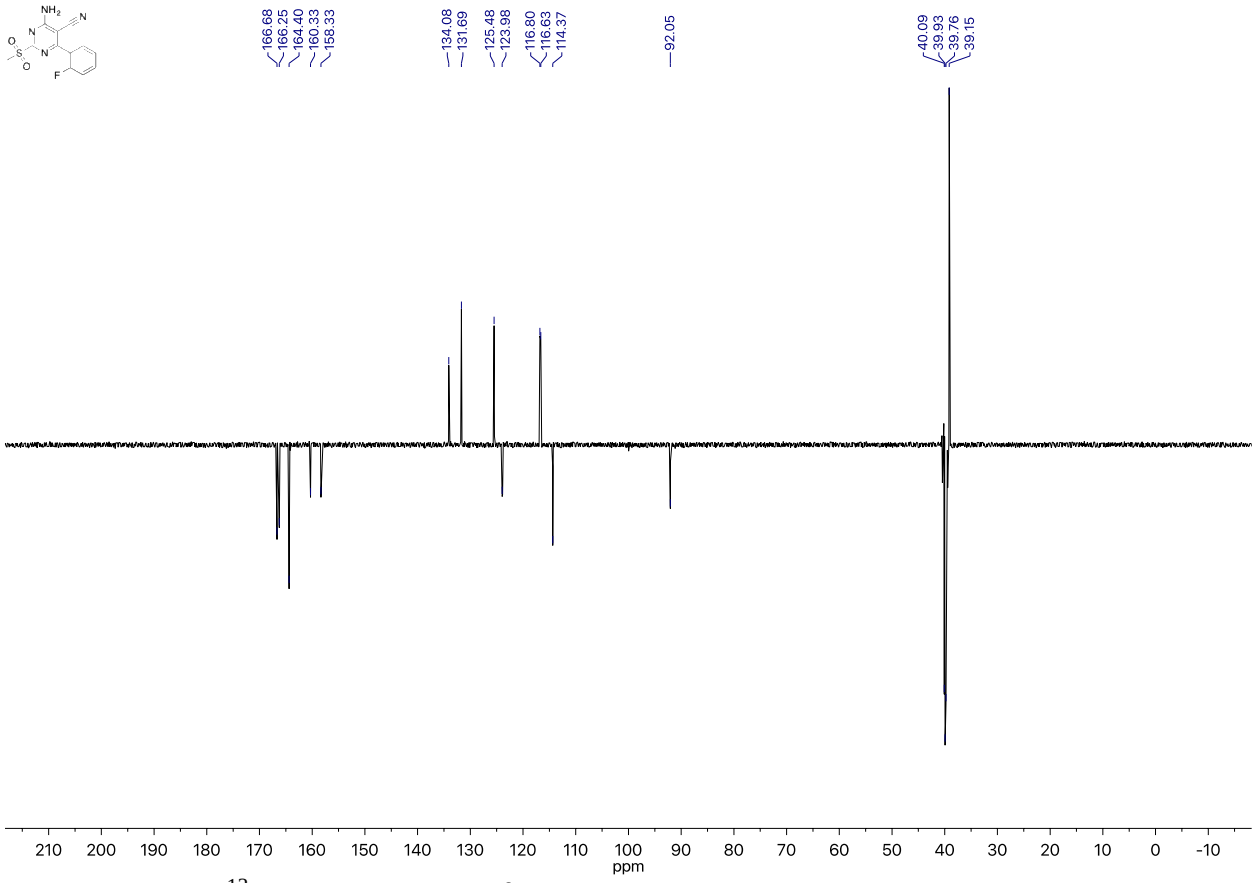

Figure S22. The ^{13}C NMR spectrum of **1g**.

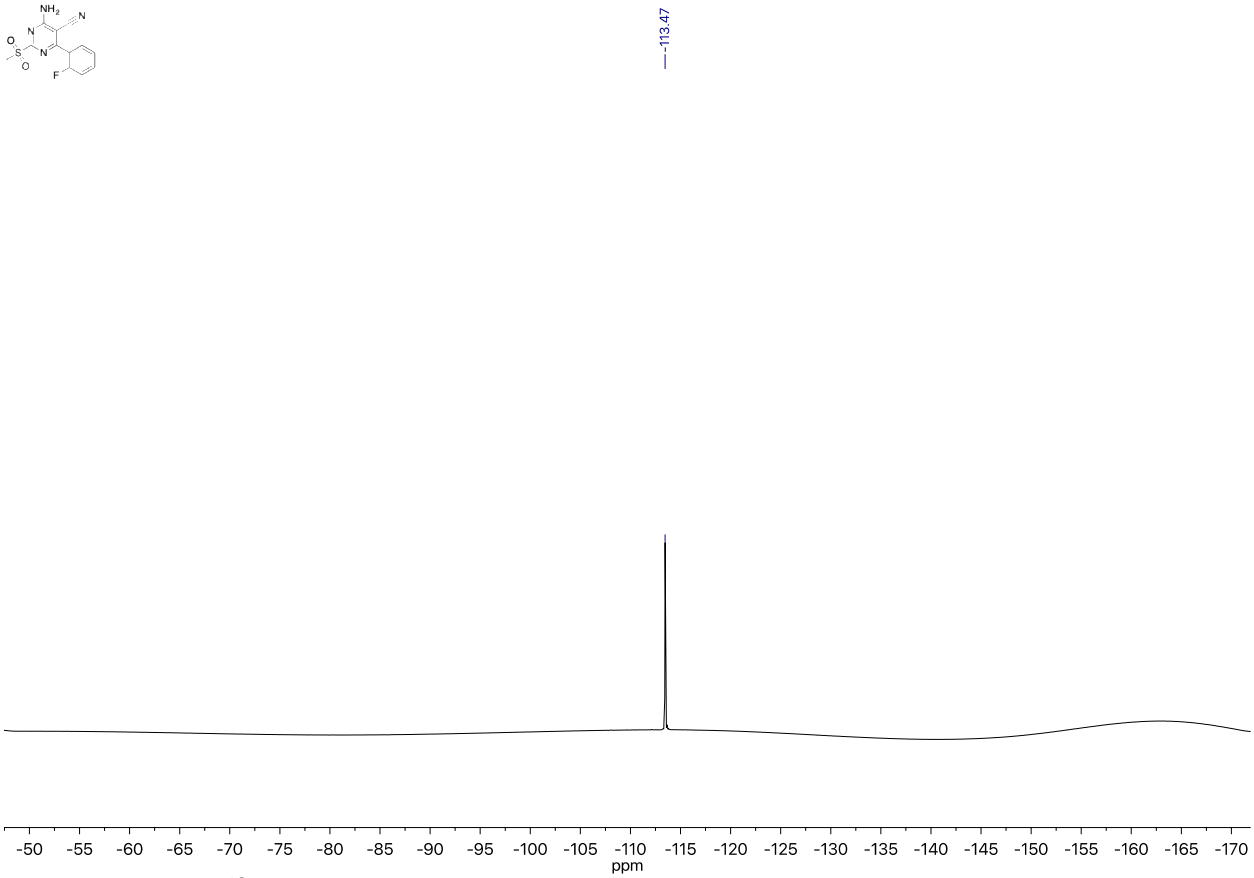

Figure S23. The ^{19}F NMR spectrum of **1g**.

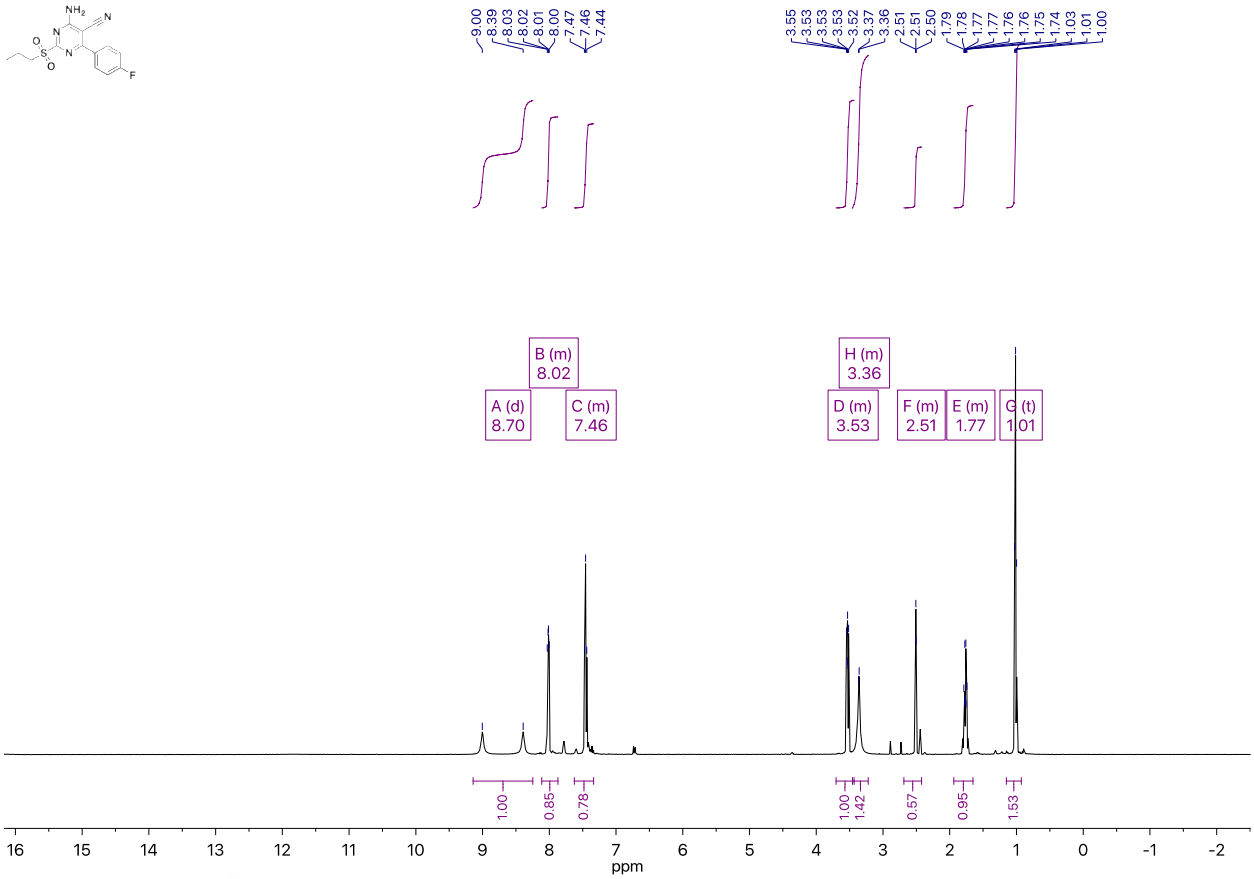

Figure S24. The ^{1}H NMR spectrum of **1h**.

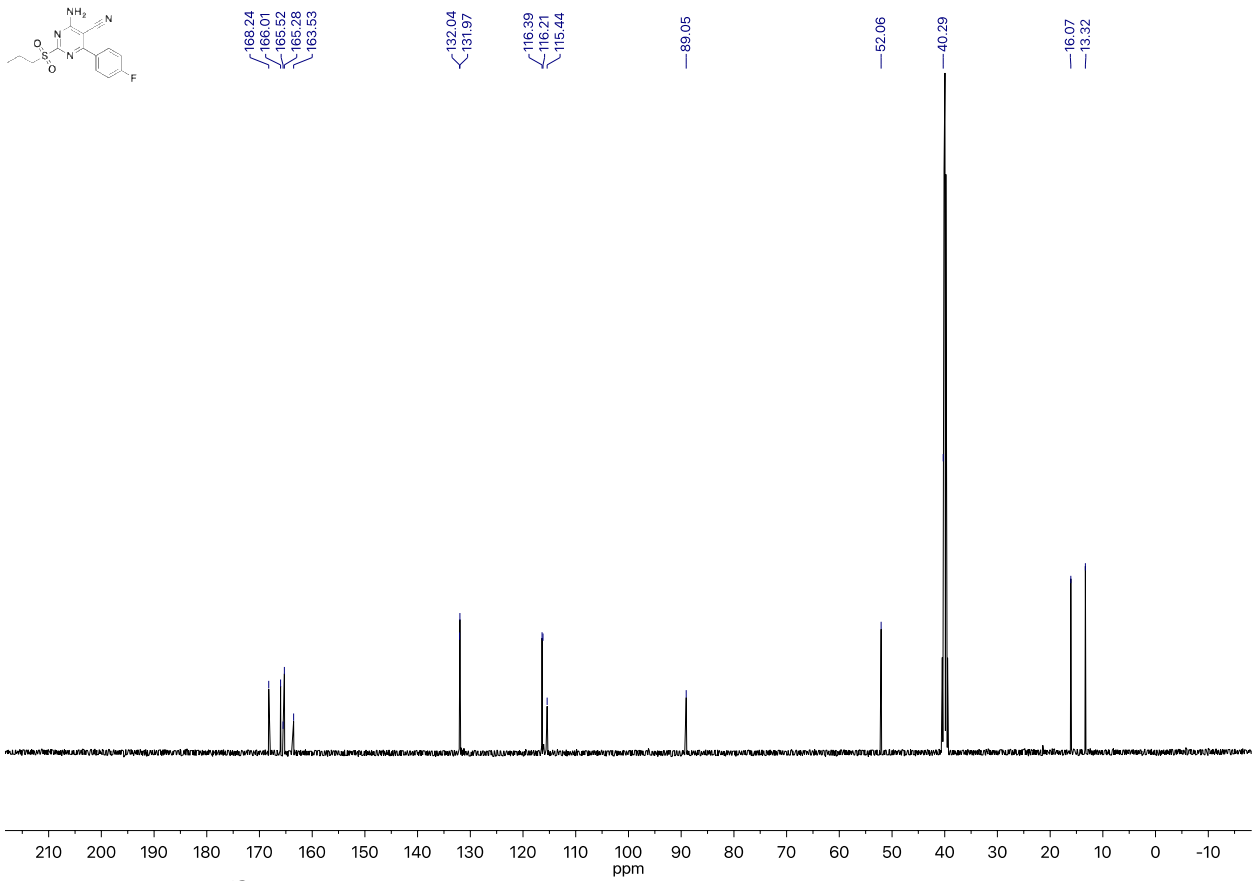

Figure S25. The ^{13}C NMR spectrum of **1h**.

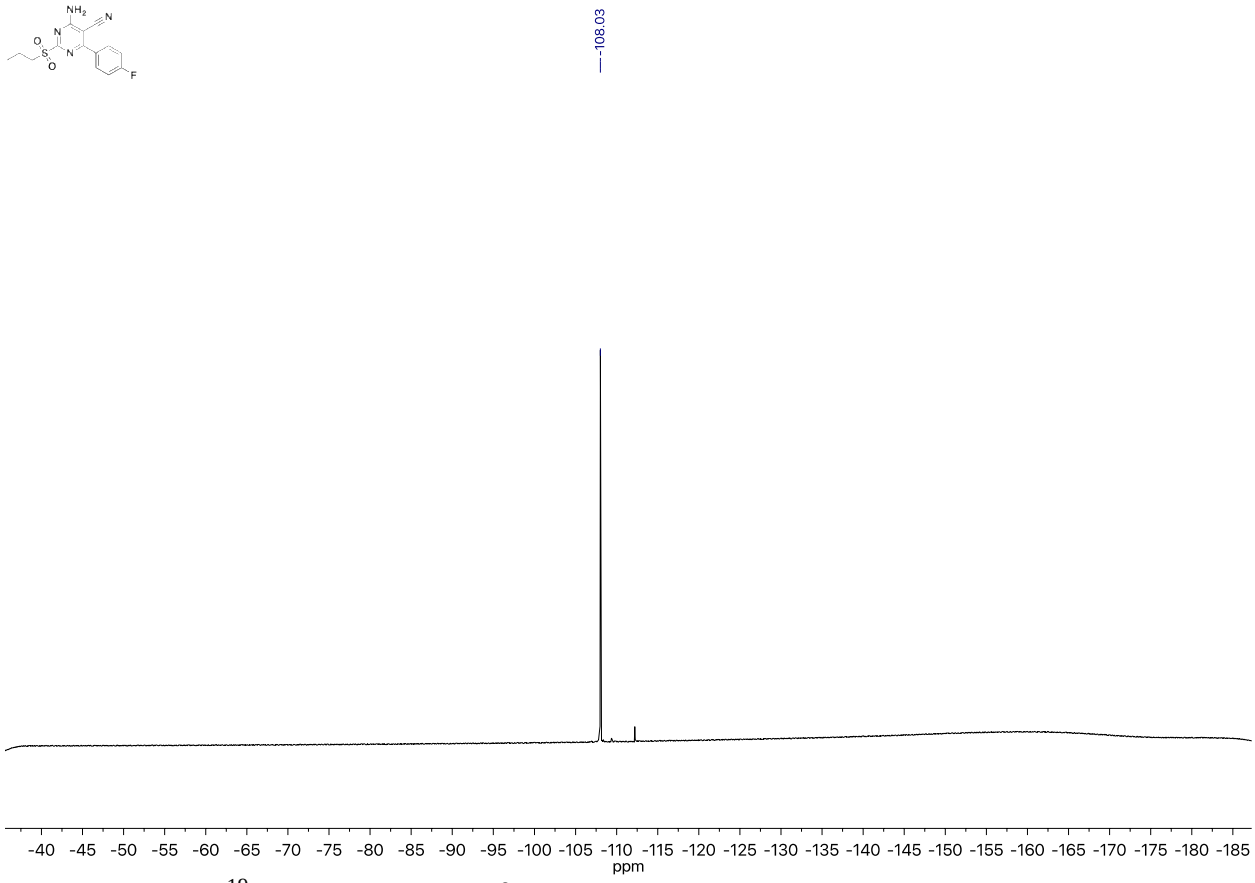

Figure S26. The ^{19}F NMR spectrum of **1h**.

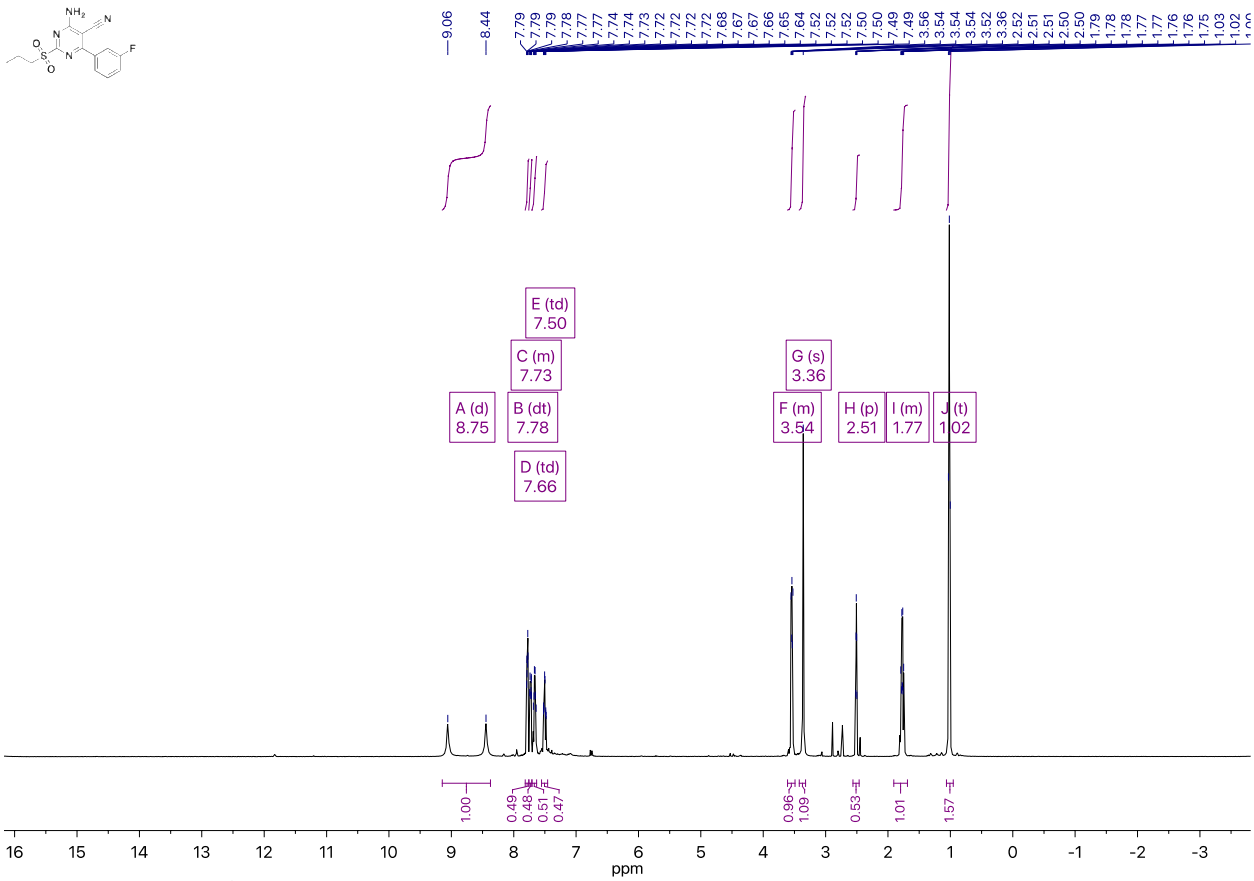

Figure S27. The ^{1}H NMR spectrum of **1i**.

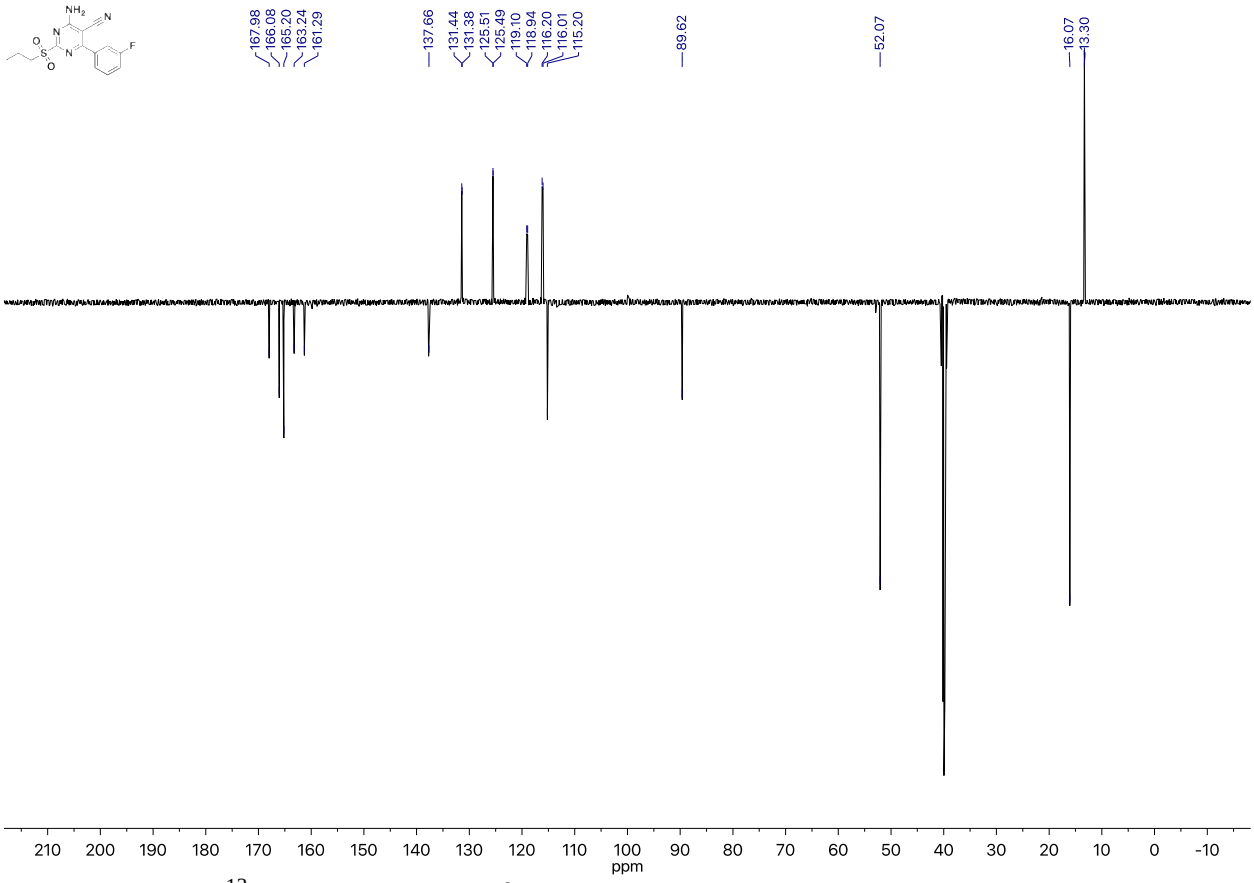

Figure S28. The ^{13}C NMR spectrum of **1i**.

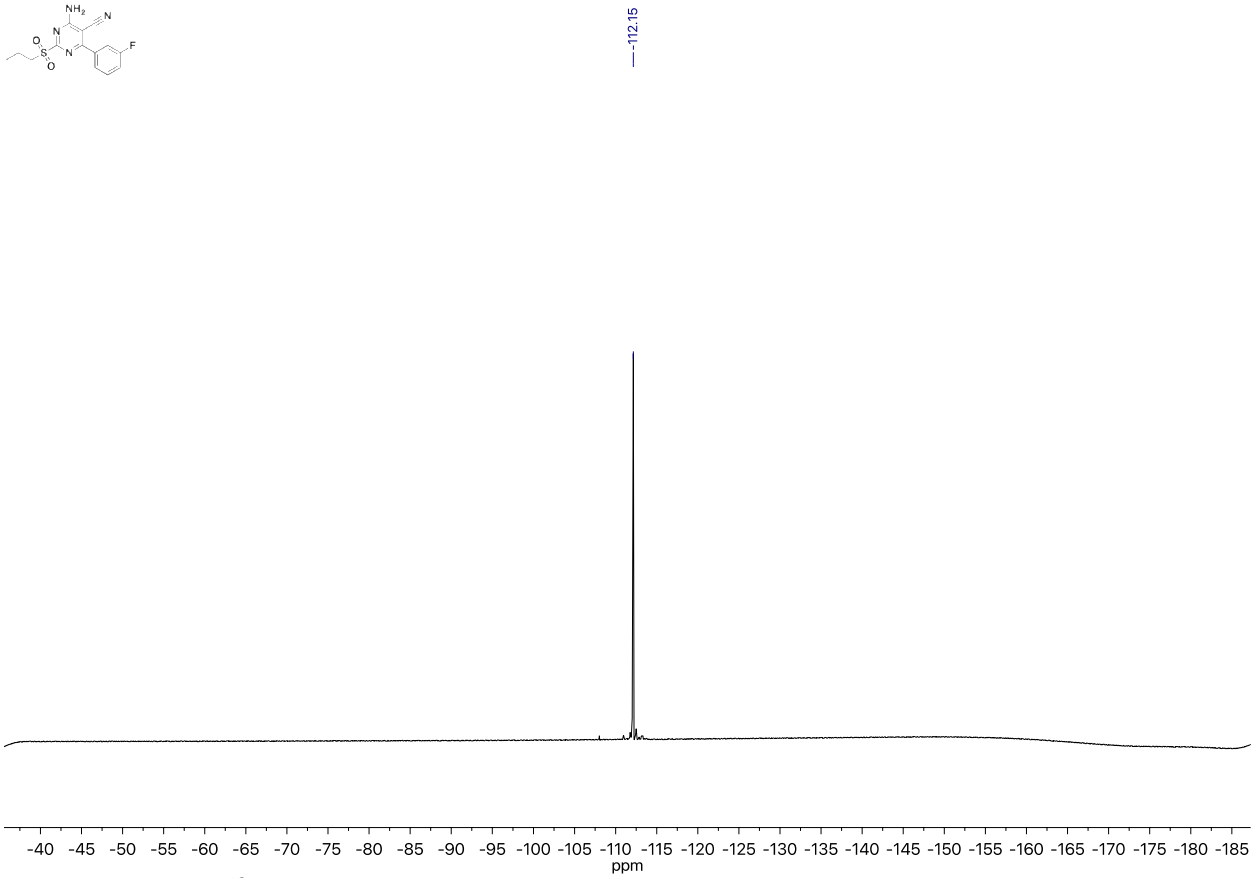

Figure S29. The ^{19}F NMR spectrum of **1i**.

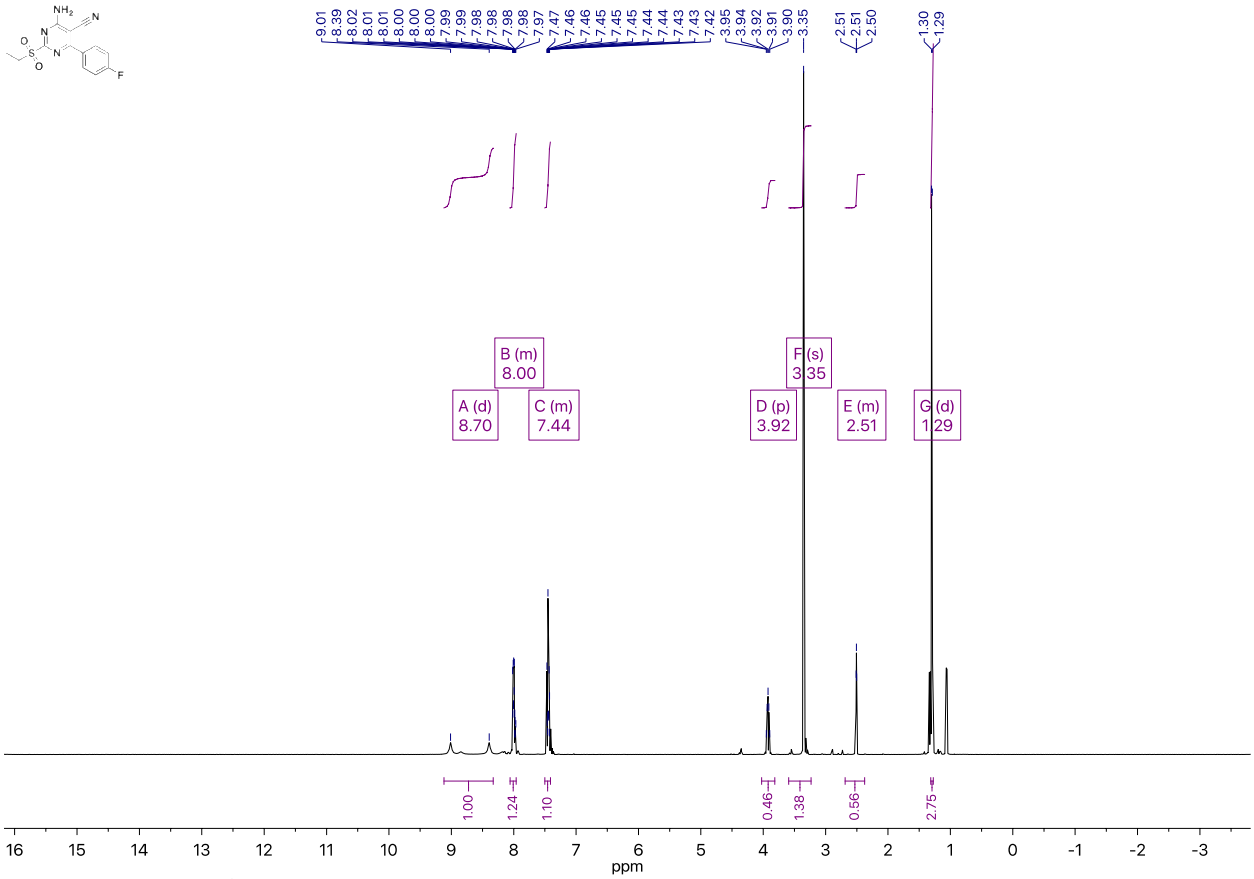

Figure S30. The ^{1}H NMR spectrum of **1j**.

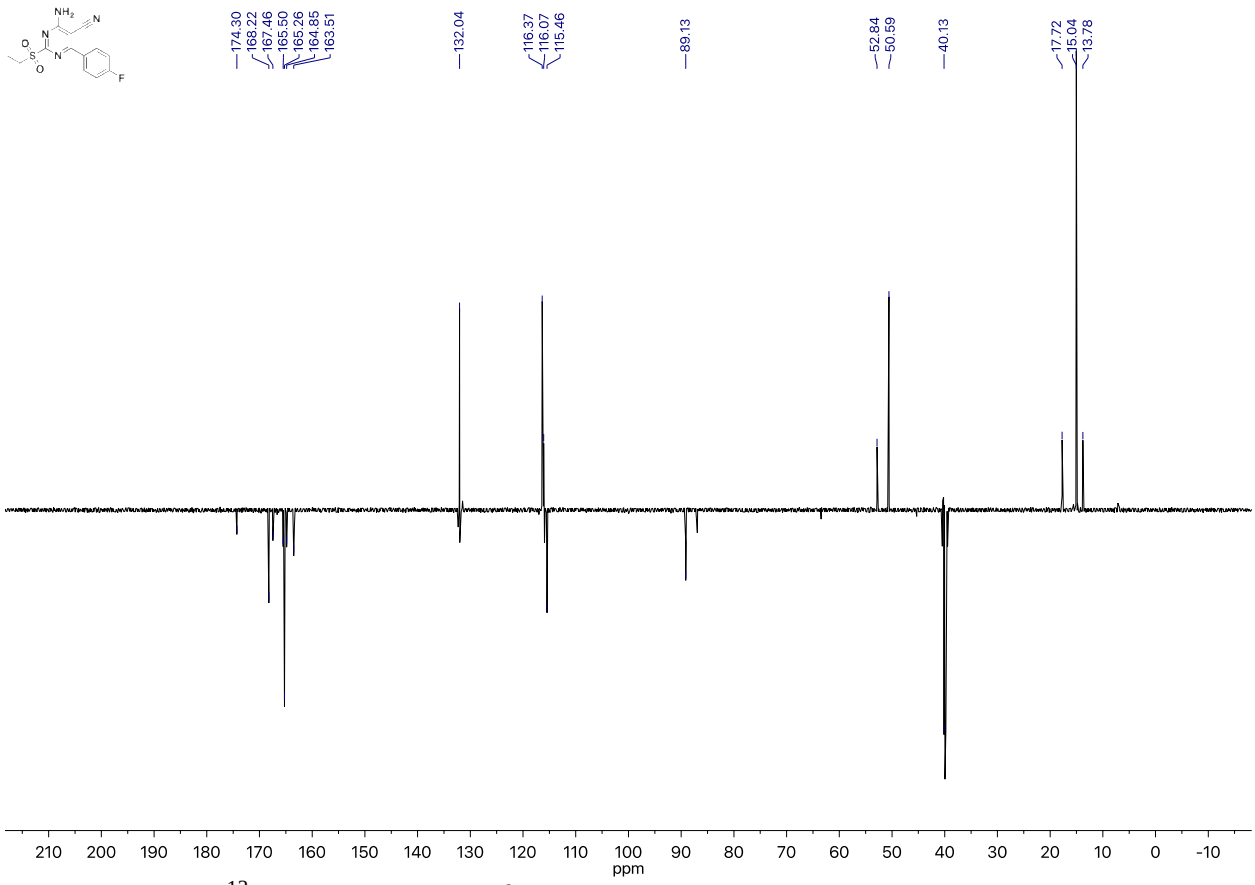

Figure S31. The ^{13}C NMR spectrum of **1j**.

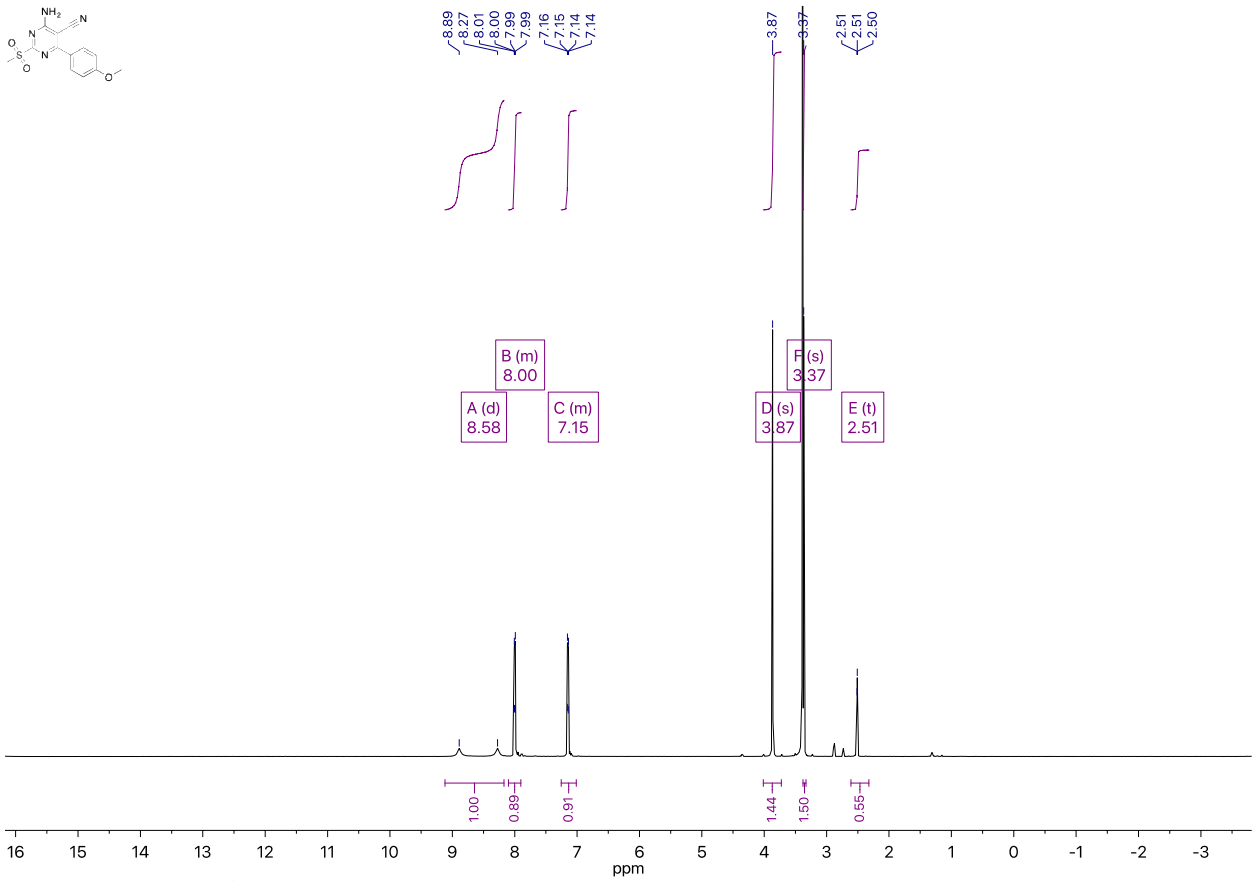

Figure S32. The ^{1}H NMR spectrum of **1k**.

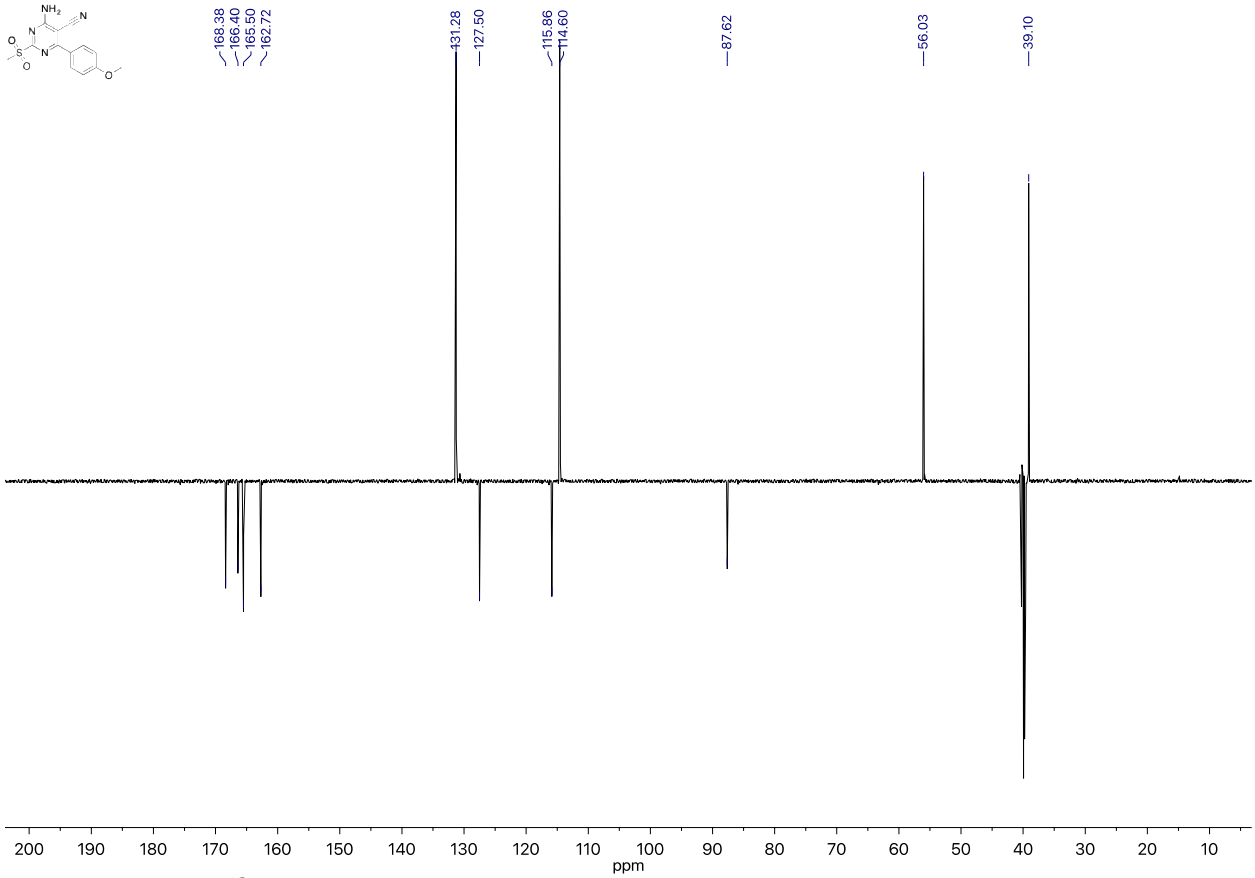

Figure S33. The ^{13}C NMR spectrum of **1k**.

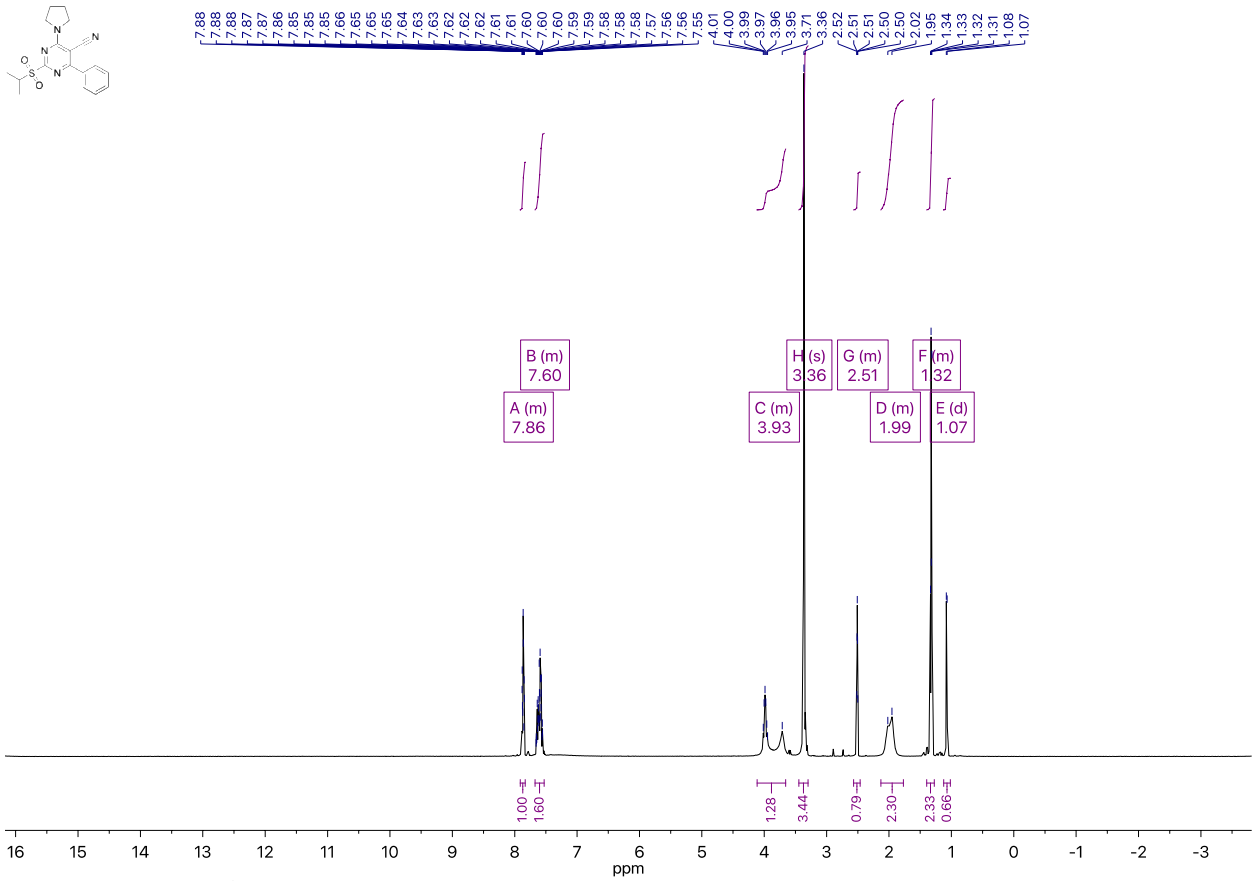

Figure S34. The ^{1}H NMR spectrum of **2a**.

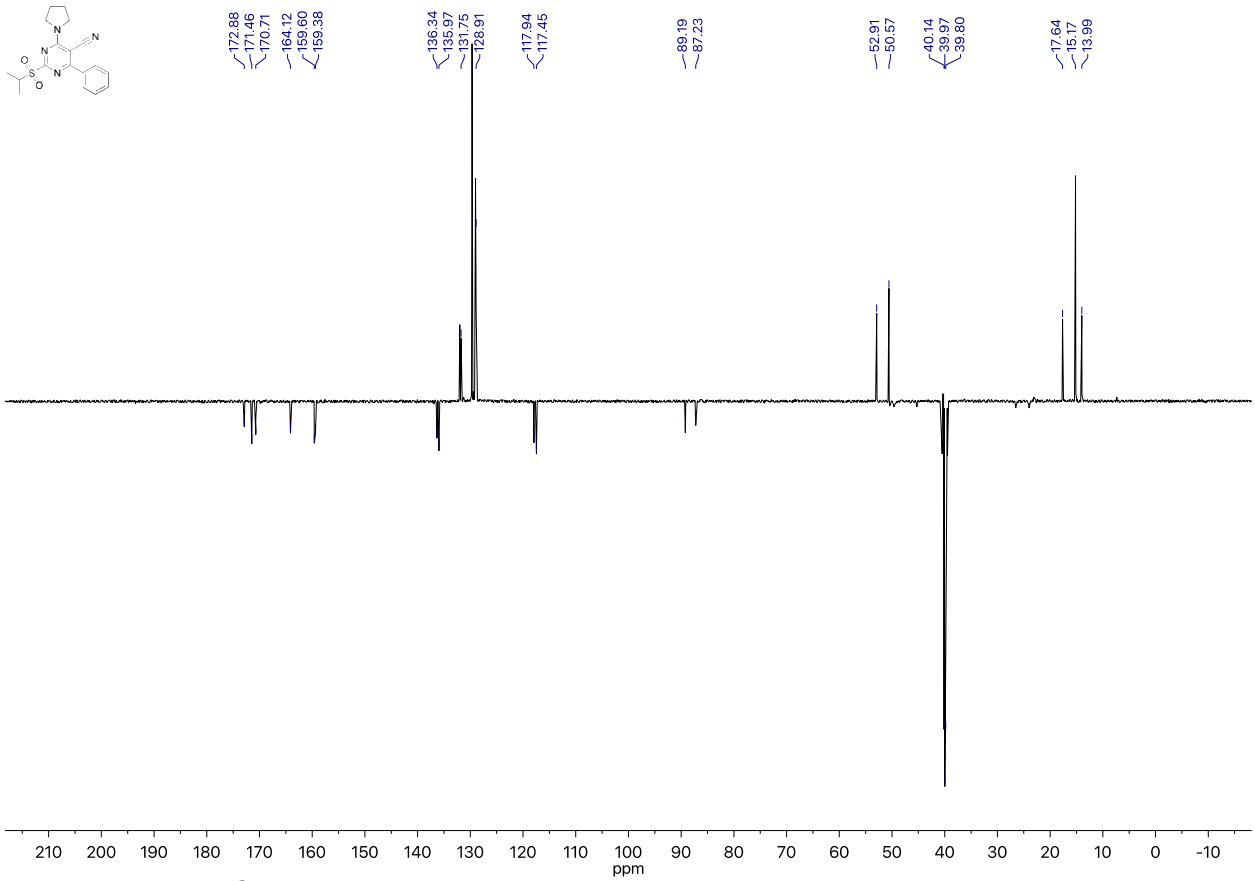

Figure S35. The ^{13}C NMR spectrum of **2a**.

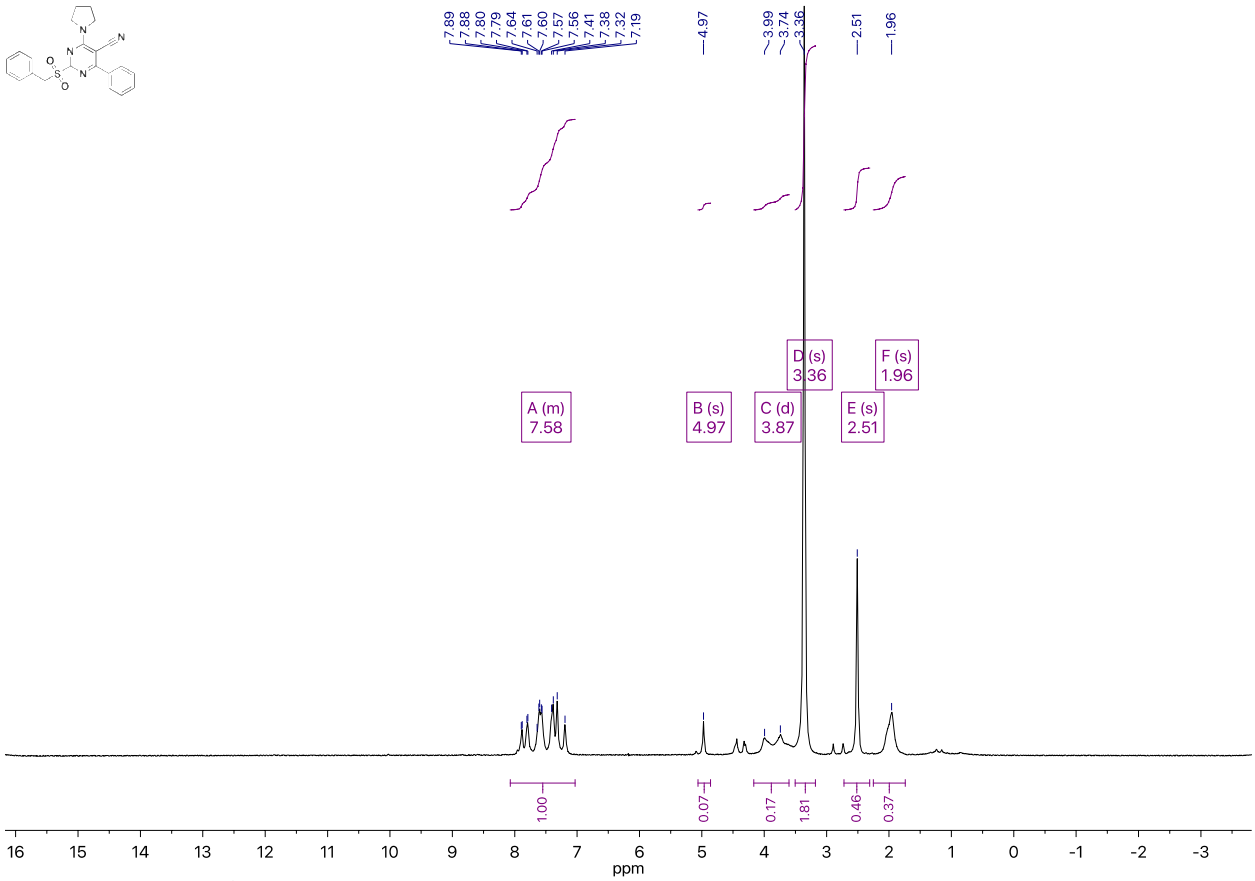

Figure S36. The ^{1}H NMR spectrum of **2b**.

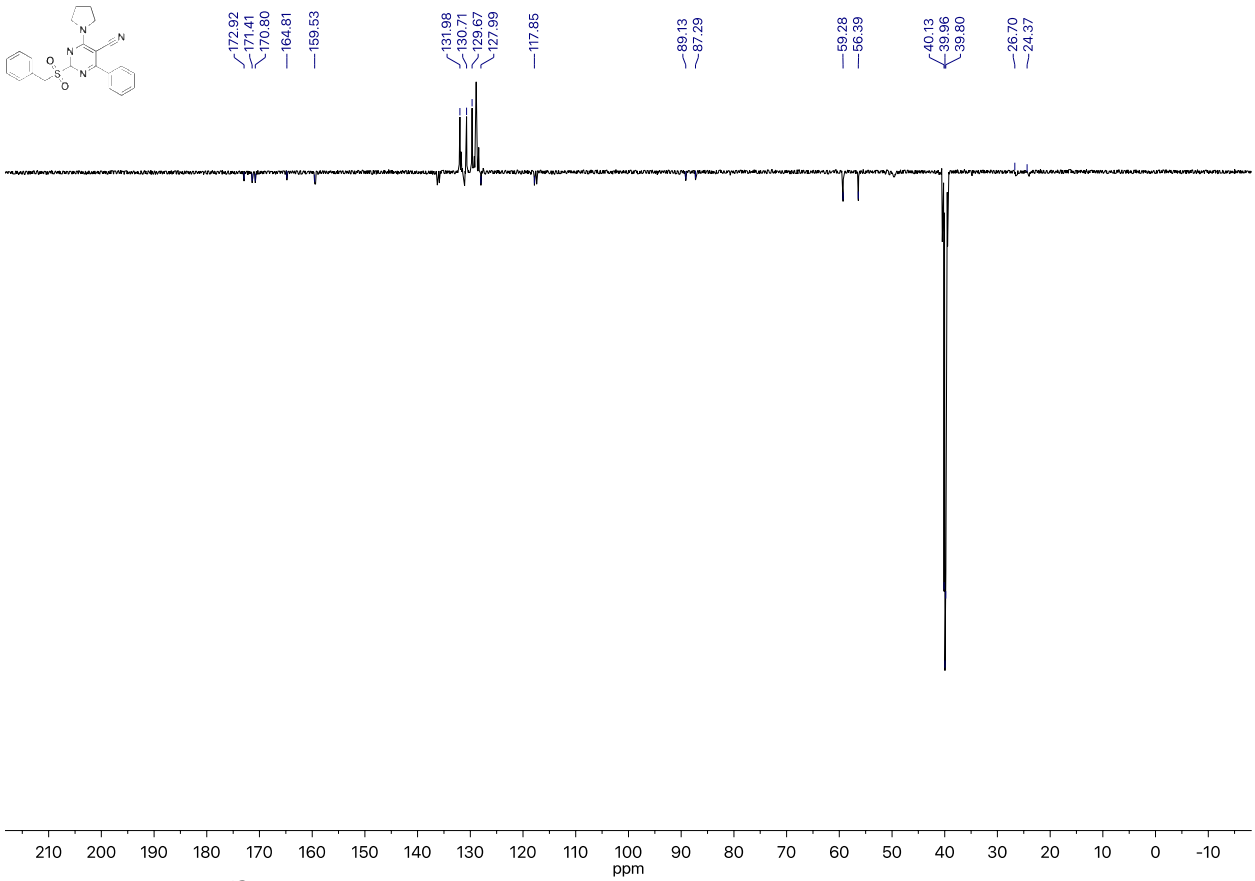

Figure S37. The ^{13}C NMR spectrum of **2b**.

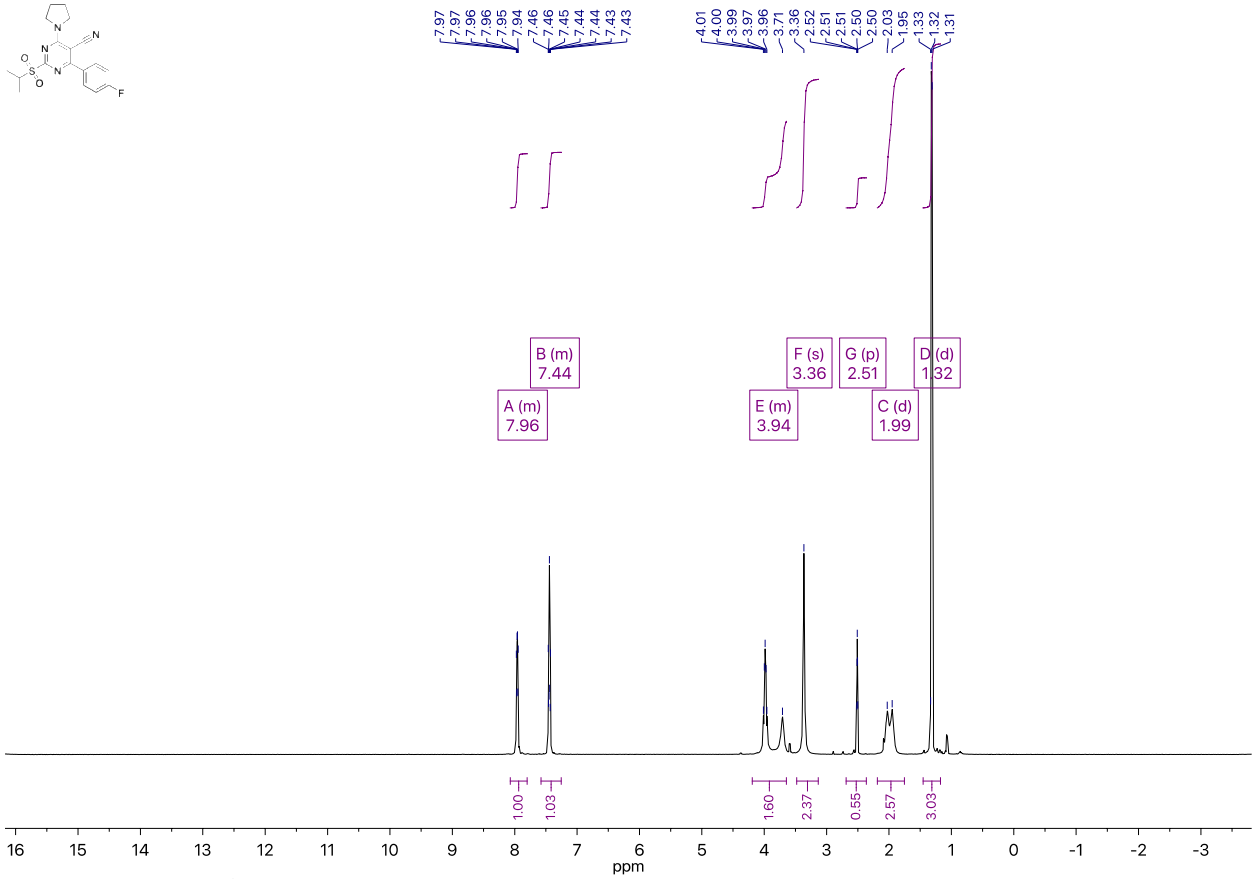

Figure S38. The ^{1}H NMR spectrum of **2c**.

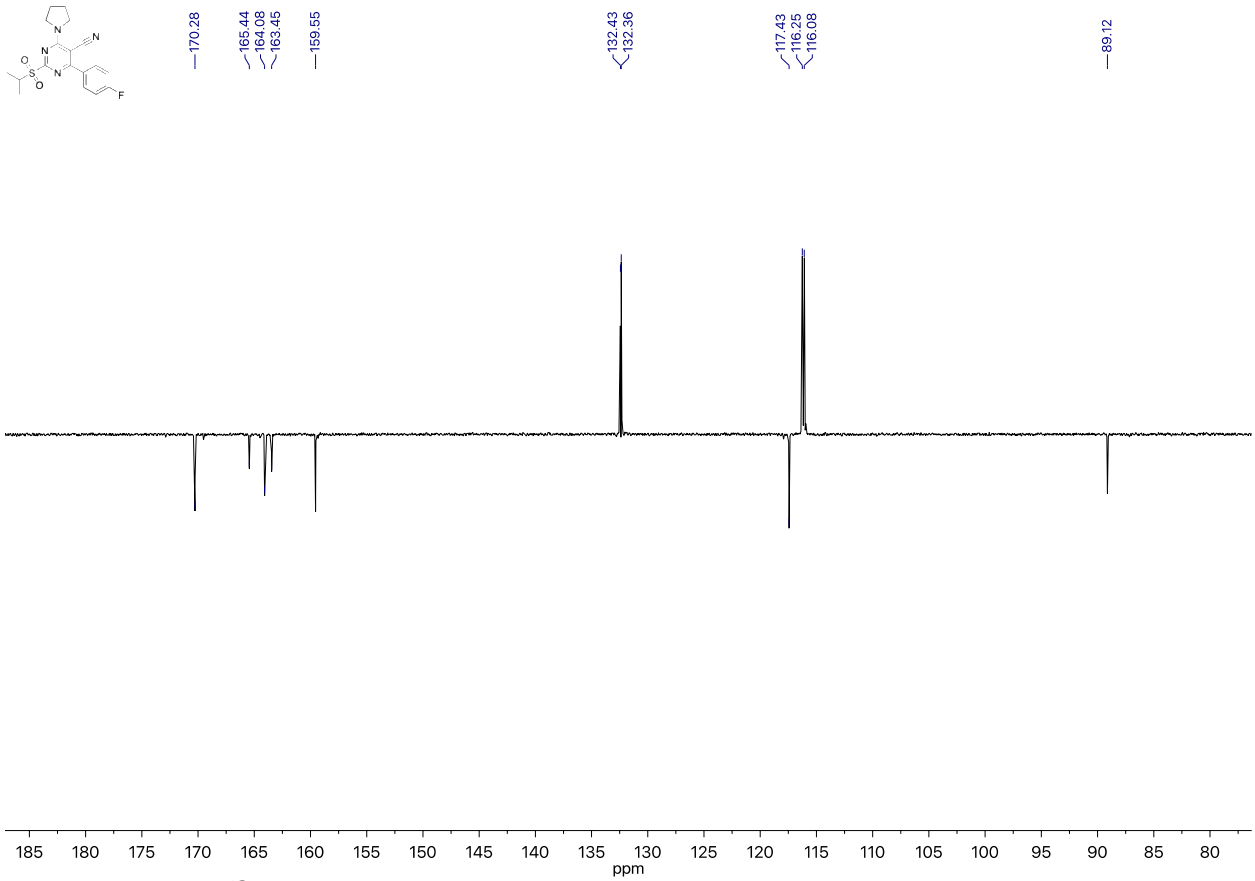

Figure S39. The ^{13}C NMR spectrum of **2c**.

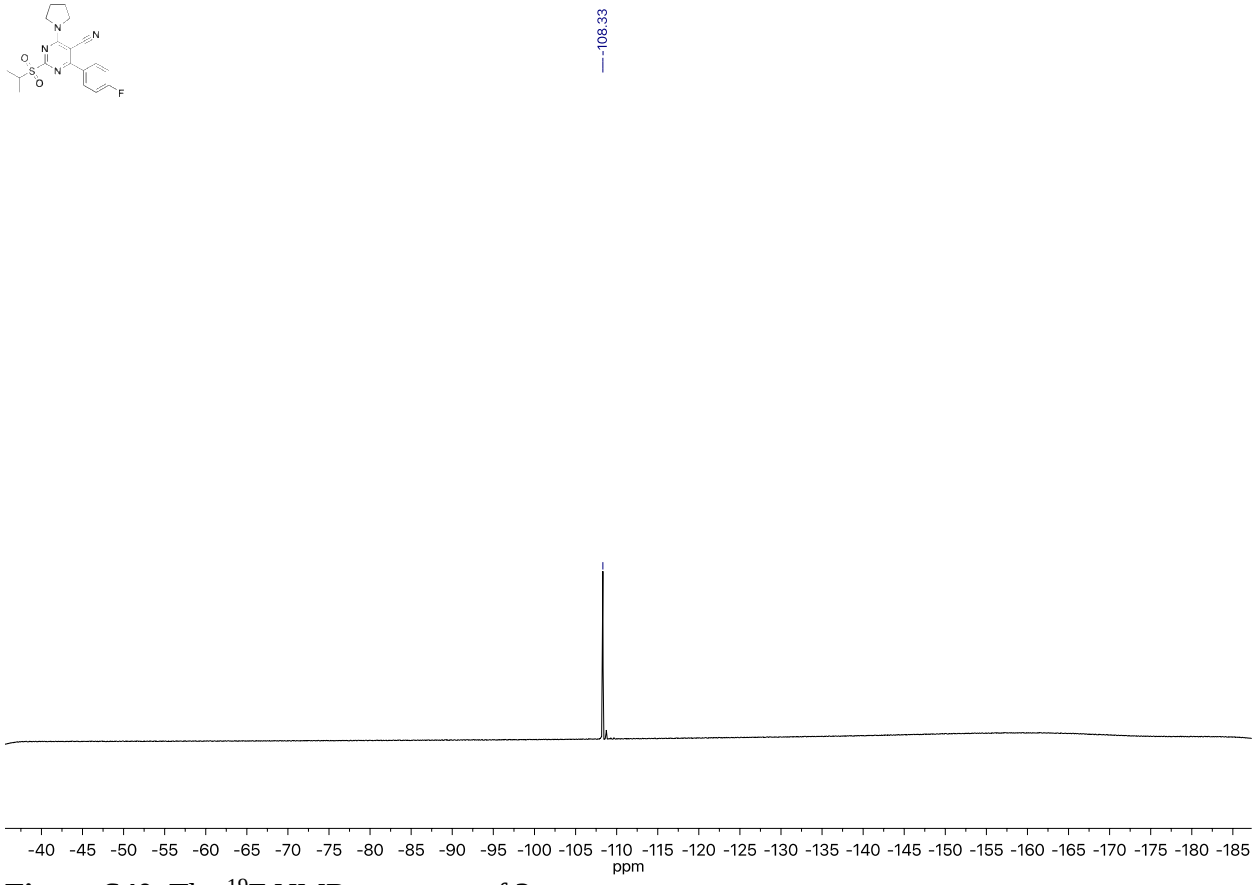

Figure S40. The ^{19}F NMR spectrum of **2c**.

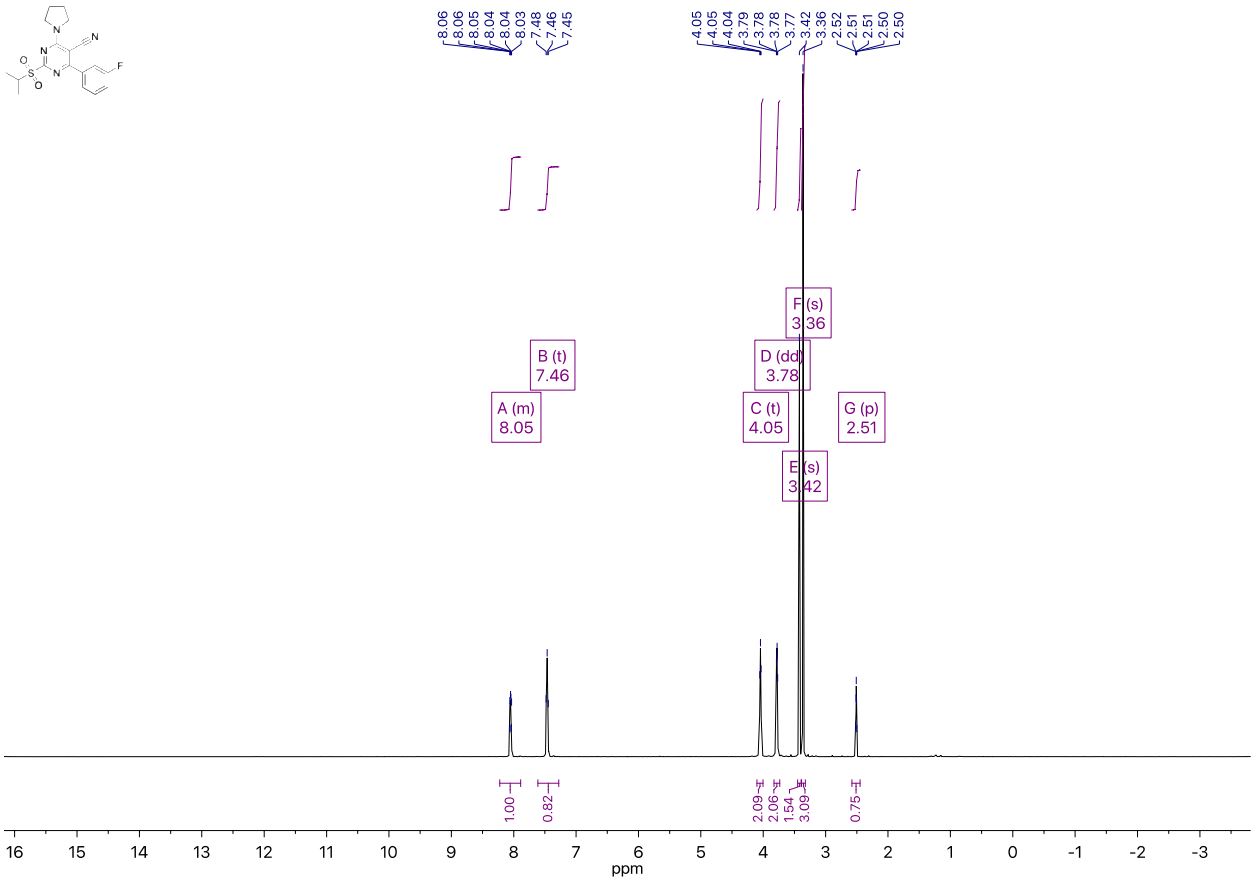

Figure S41. The ^{1}H NMR spectrum of **2d**.

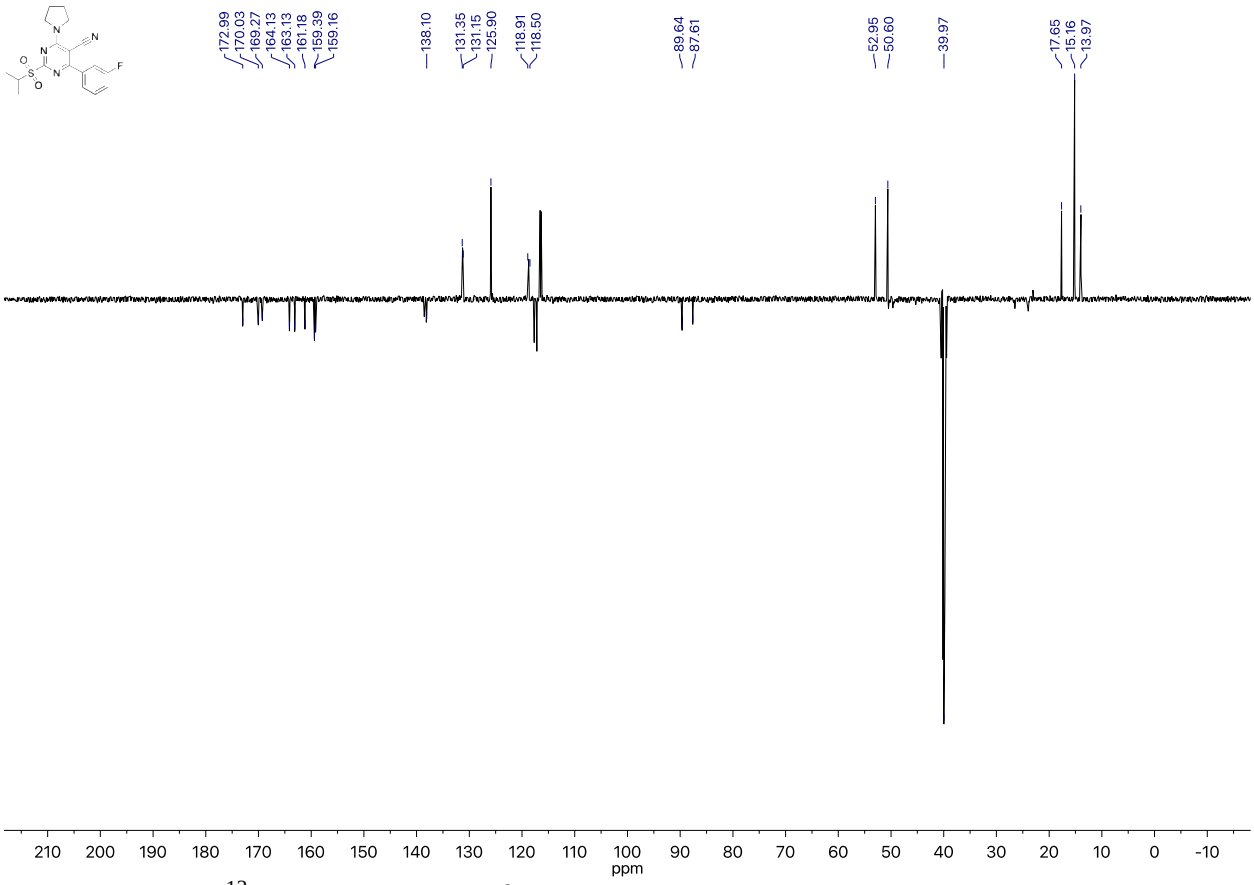

Figure S42. The ^{13}C NMR spectrum of **2d**.

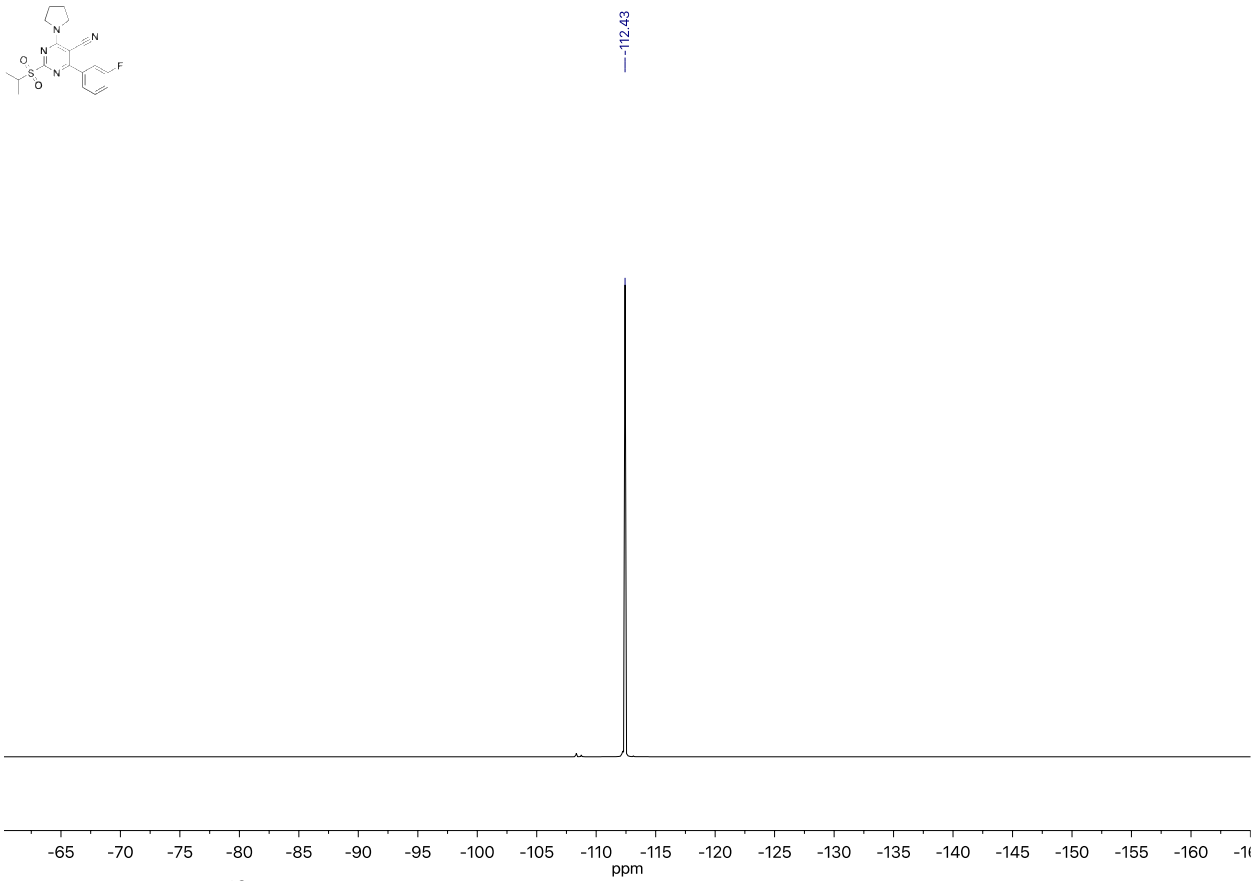

Figure S43. The ^{19}F NMR spectrum of **2d**.

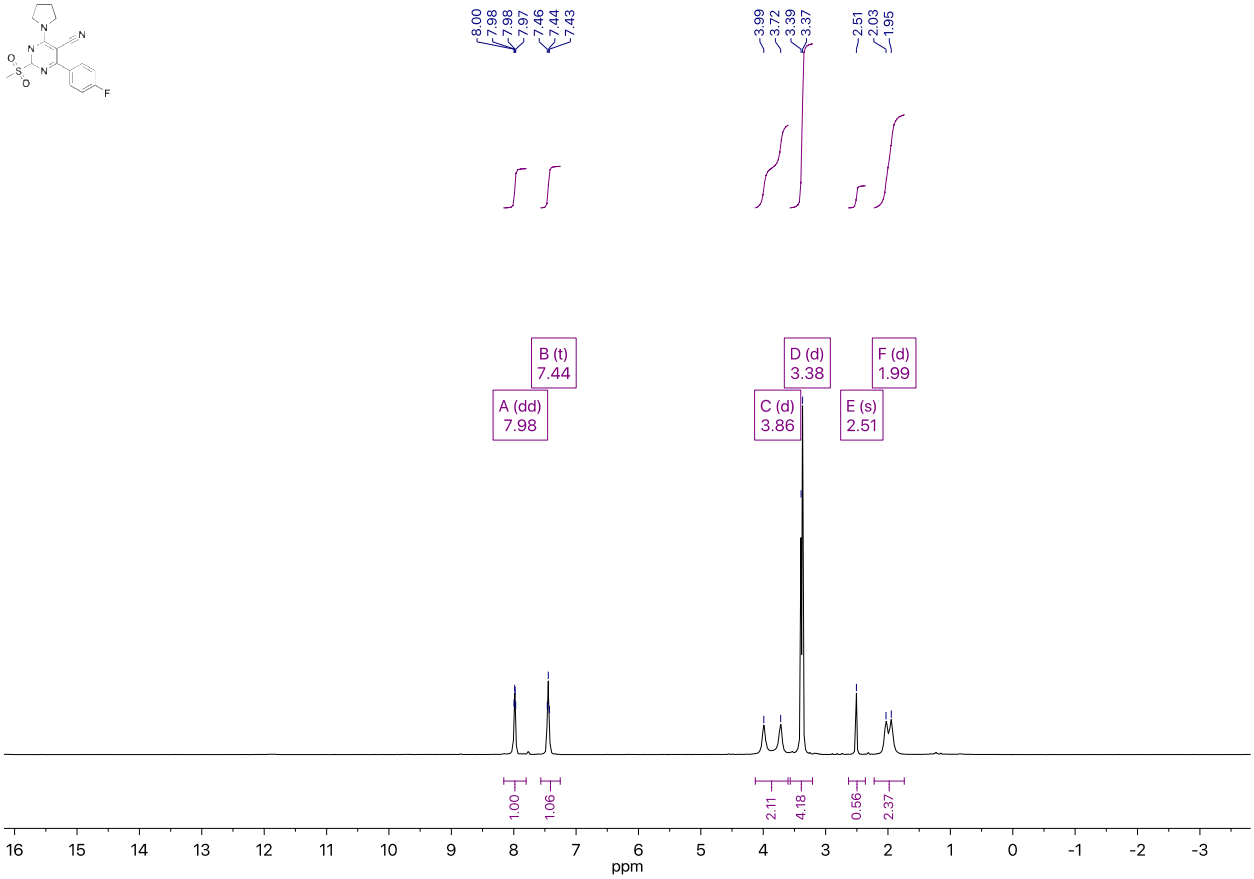

Figure S44. The ^{1}H NMR spectrum of **2e**.

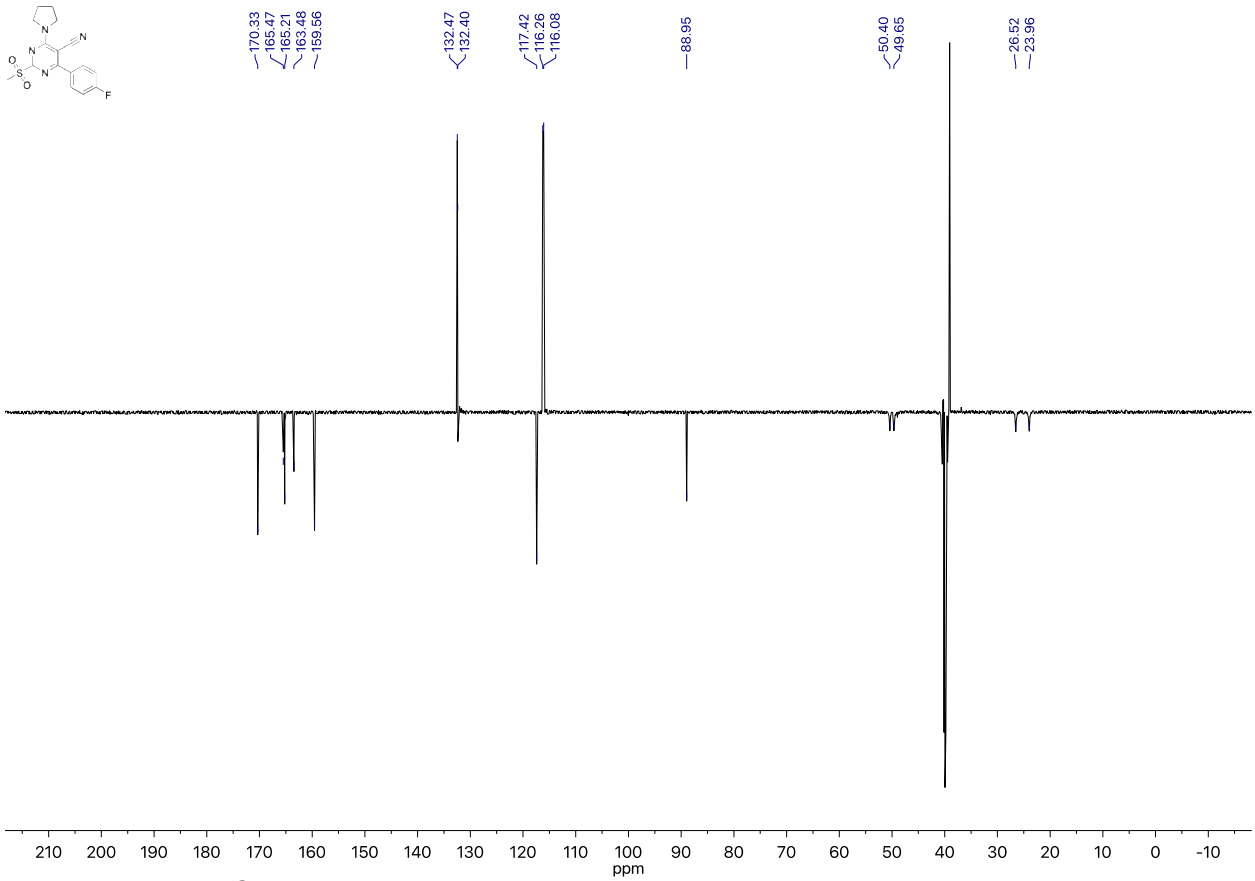

Figure S45. The ^{13}C NMR spectrum of **2e**.

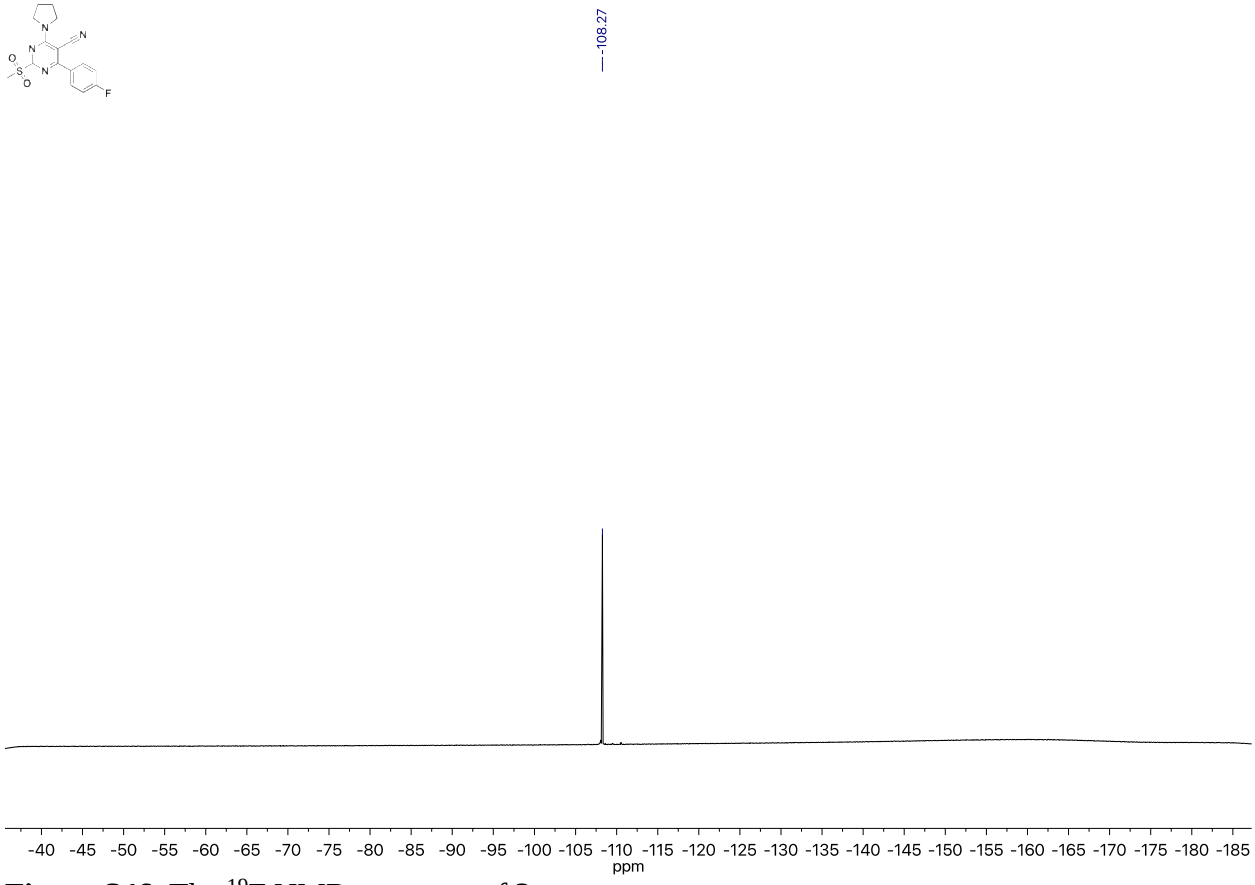

Figure S46. The ^{19}F NMR spectrum of **2e**.

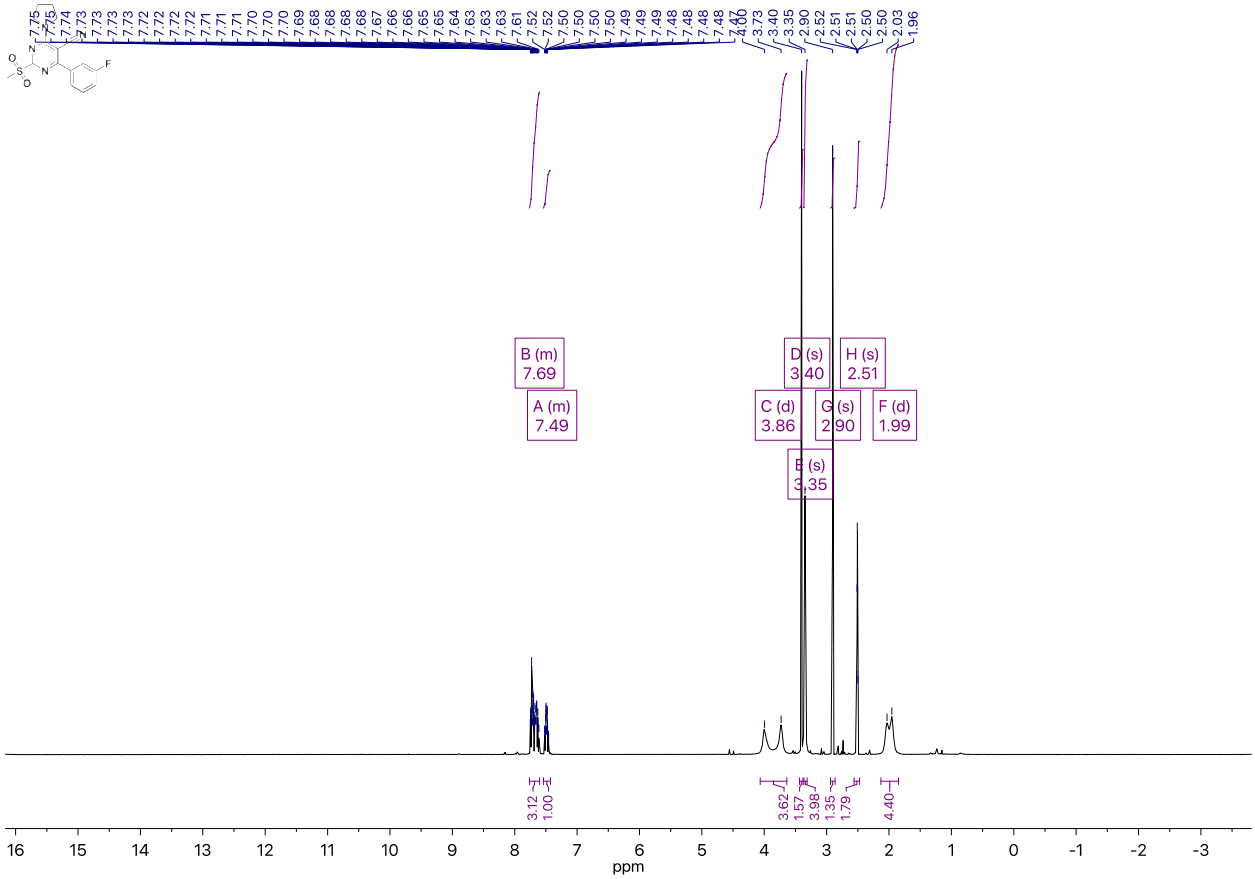

Figure S48. The ^{1}H NMR spectrum of **2f**.

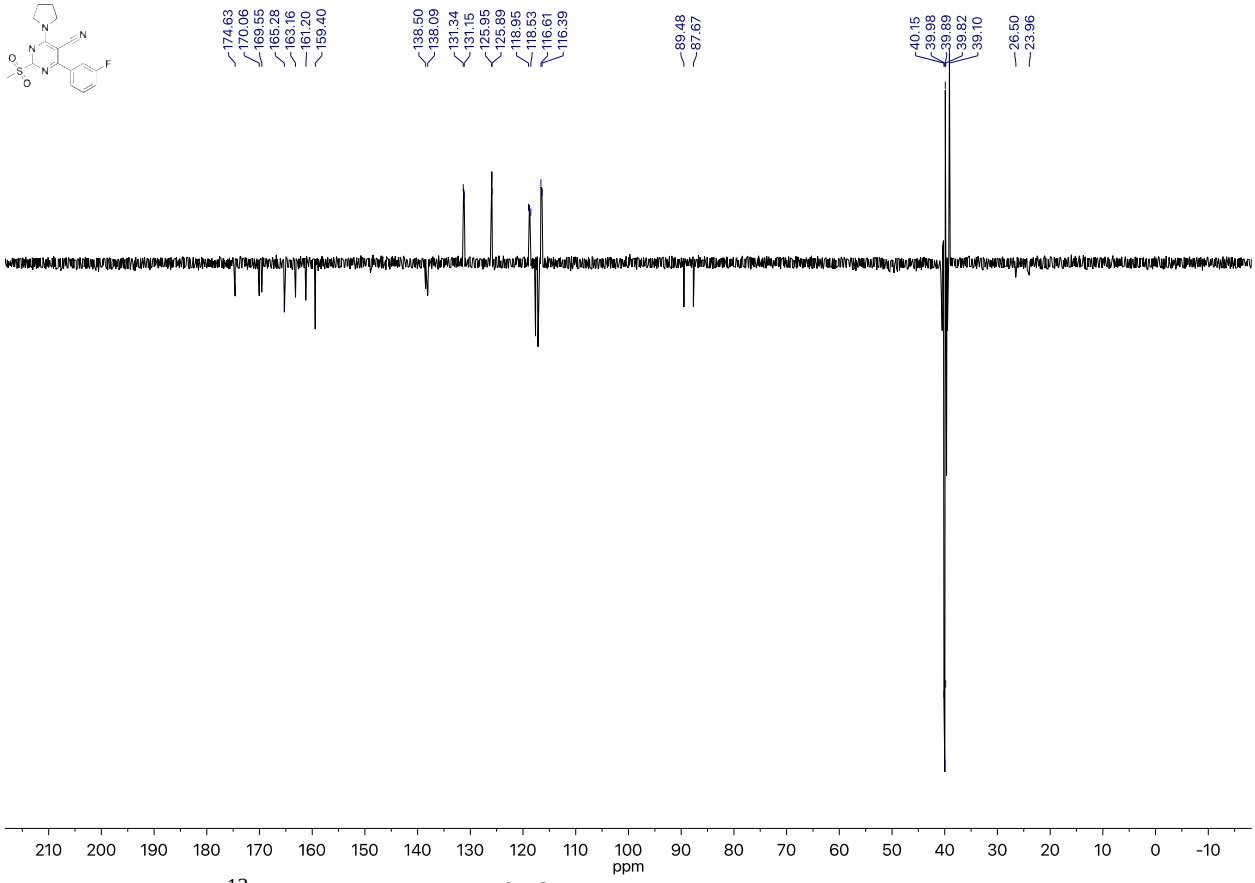

Figure S49. The ^{13}C NMR spectrum of **2f**.

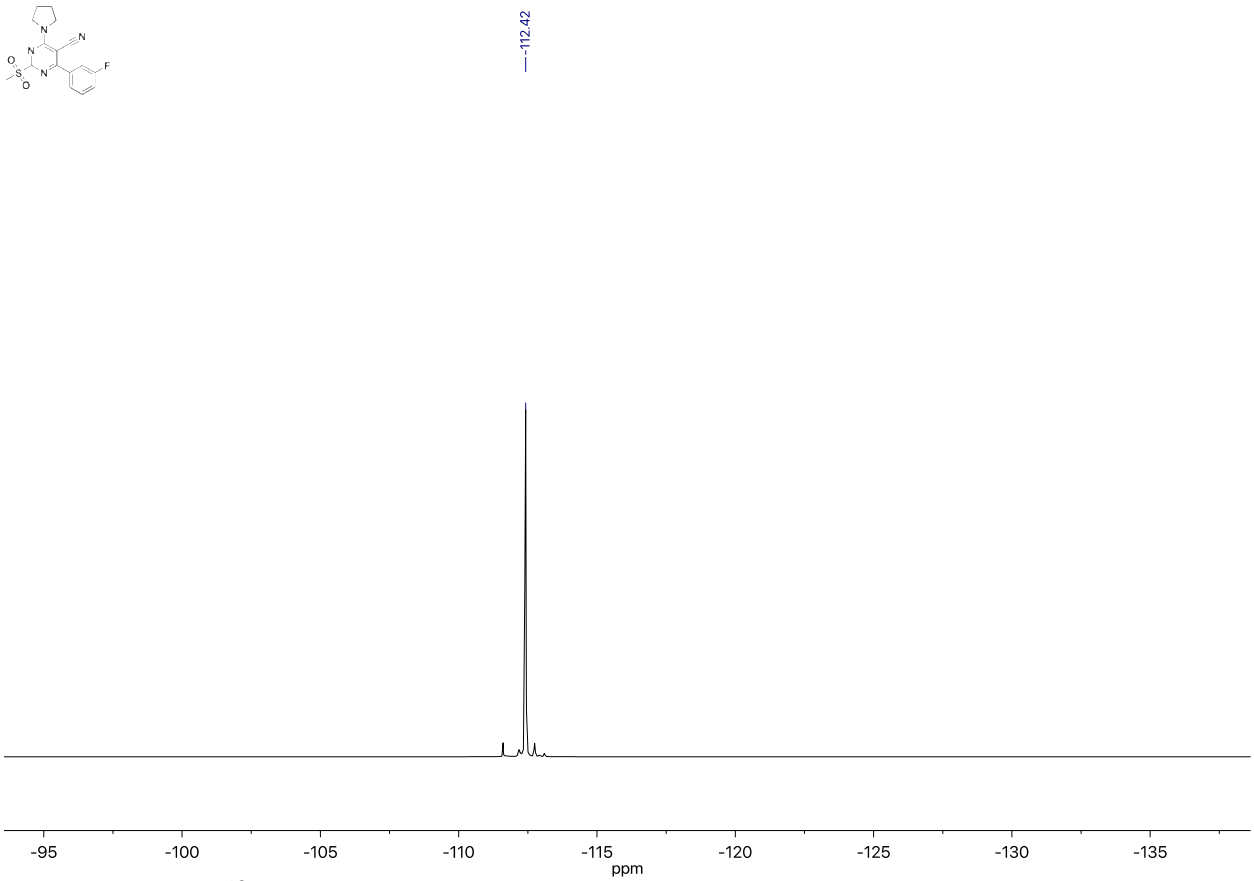

Figure S50. The ^{19}F NMR spectrum of **2f**.

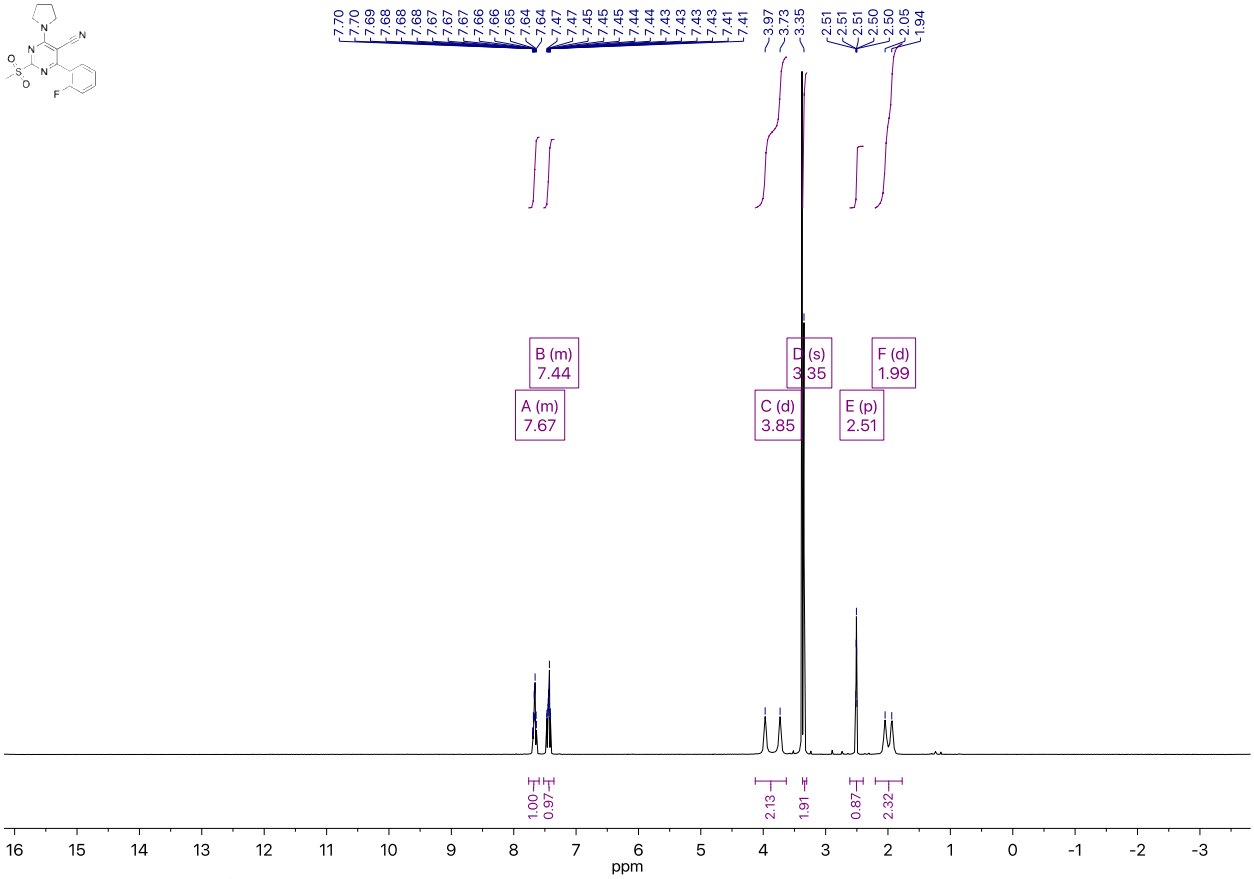

Figure S51. The ^{1}H NMR spectrum of **2g**.

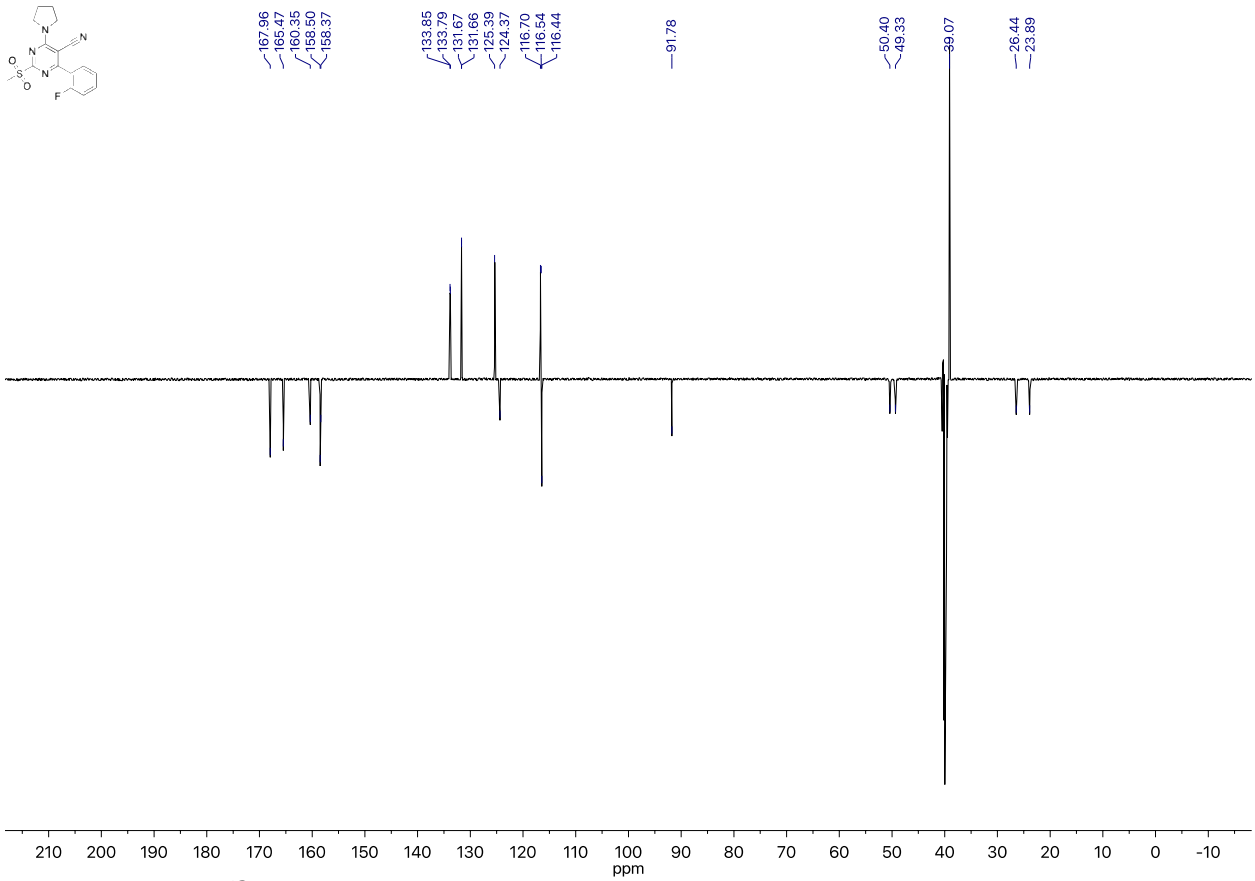

Figure S52. The ^{13}C NMR spectrum of **2g**.

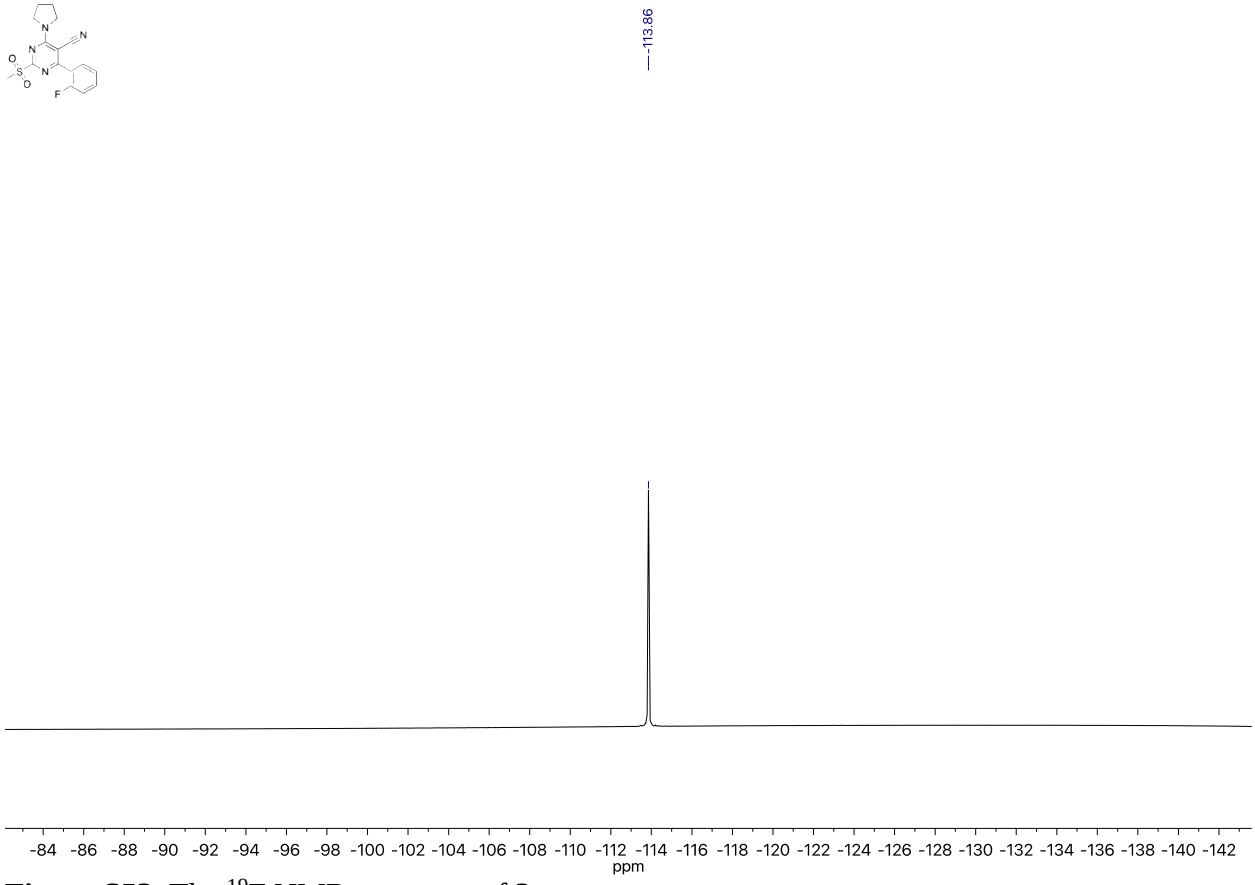

Figure S53. The ^{19}F NMR spectrum of **2g**.

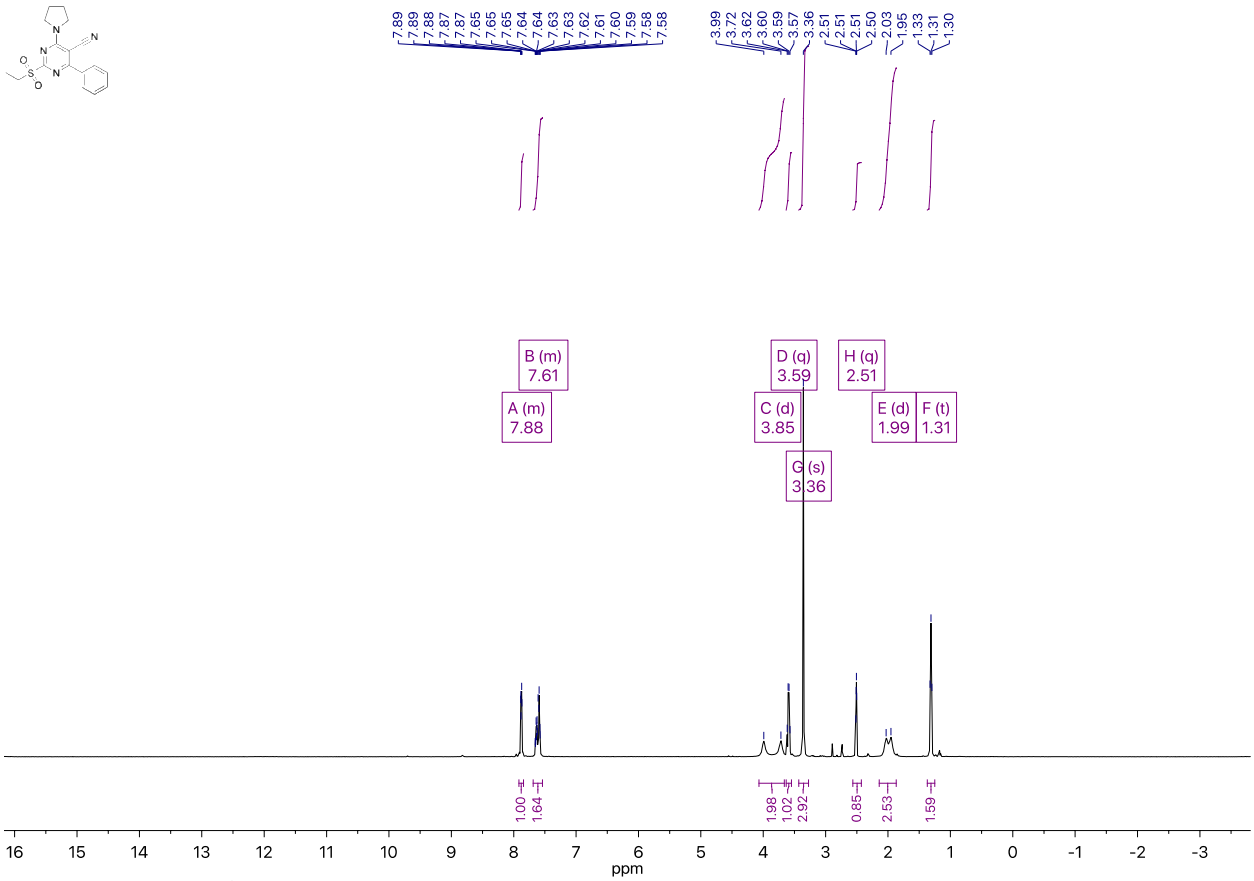

Figure S54. The ^{1}H NMR spectrum of **2h**.

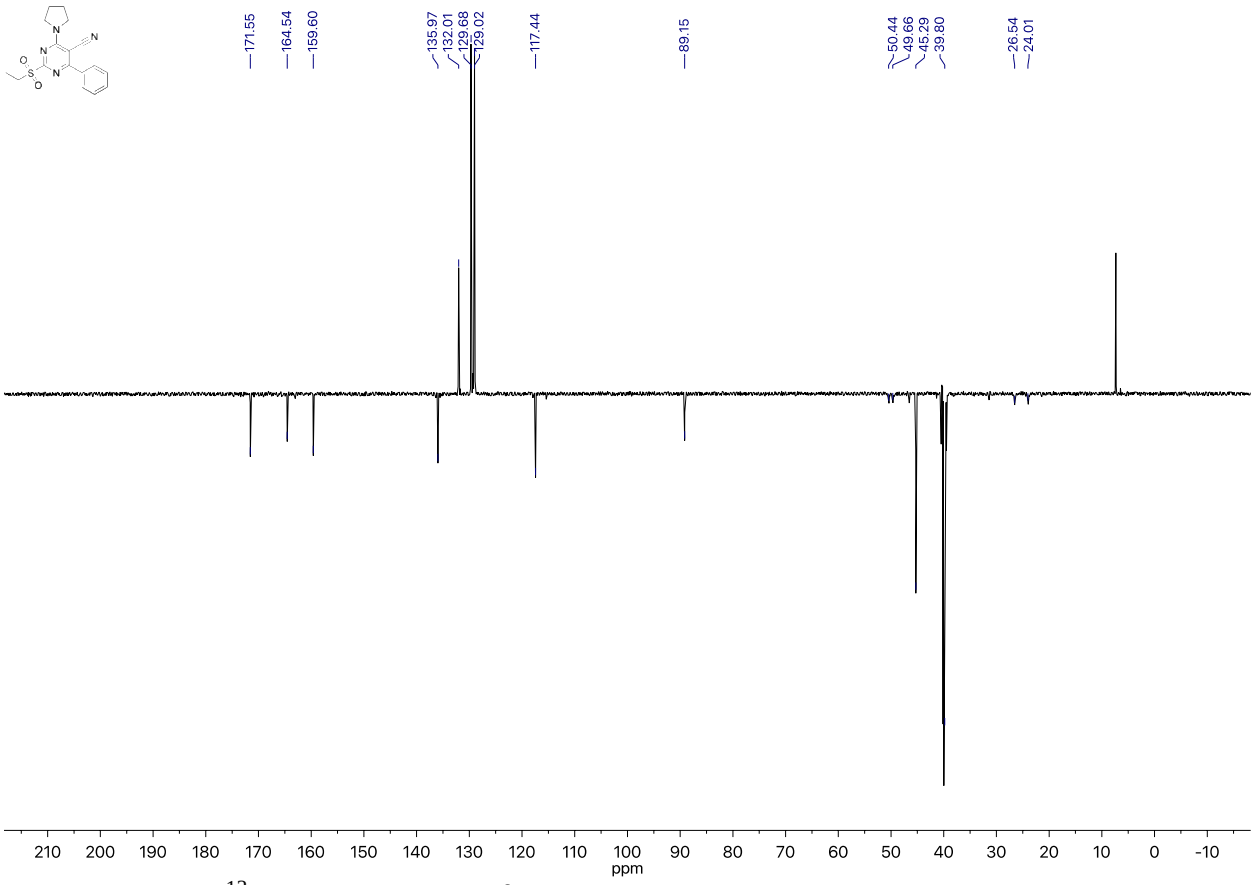

Figure S55. The ^{13}C NMR spectrum of **2h**.