Supporting Information

Discovery of Highly Potent Adenosine A_1 Receptor Agonists: Targeting Positron Emission Tomography Probes

Min Guo,[†‡] Abolghasem Bakhoda,[†‡] Zhan-Guo Gao,[^] Joseph M. Ramsey,[†] Yang Li,[†] Kelly A O'Conor,[†] Andrew C. Kelleher,[†] Seth M. Eisenberg,[†] Yeona Kang,[†§] Xuefeng Yan,[#] Cameron Javdan,[†] Joanna S. Fowler,[†] Kenner C. Rice,[‖] Jacob M. Hooker,[¶] Kenneth A. Jacobson,[^] Sung Won Kim,[†]* and Nora D. Volkow[†▽]*

Email: sunny.kim@nih.gov

Email: nvolkow@nida.nih.gov

[†]Laboratory of Neuroimaging, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland 20892-1013, United States

[^]Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0810, United States

[§]Department of Mathematics, Howard University, Washington, DC 20059, United States

[#]Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892, United States

[‖]Drug Design and Synthesis Section, National Institute on Drug Abuse, National Institutes of Health, Rockville, Maryland 20850, United States

[¶] Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital,

Harvard Medical School, Charlestown, Massachusetts 02129, United States

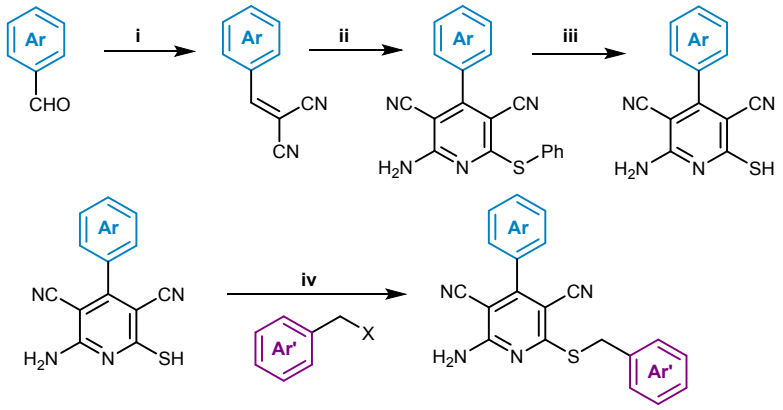
[▽]National Institute on Drug Abuse, National Institutes of Health, Bethesda, Maryland 20892-1013, United States

Table of Contents

1. Methods and Materials

All solvents and most reagents were purchased from Sigma–Aldrich, Alfa Aesar, TCI America, or VWR and used without purification unless otherwise noted. Absolute ethanol was used for HPLC eluent. 2–(Bromomethyl)–1H–imidazole HBr salt (CAS Registry No. 735273–40–2) was purchased from AEchem Scientific Corporation. 4–(Chloromethyl)–1H–imidazole HCl salt (CAS Registry No. 38585–61–4) and 2–Chloromethyl–thiazole (CAS Registry No. 3364–78–1) were obtained from Combi–Blocks, Inc. NMR spectra were recorded with a Varian 400 MHz spectrometer at 25 °C with temperature control. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. and were dried over activated 4A molecular sieves. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Mass spectra (HRMS) were recorded on a VG 7070E spectrometer (VG Analytical Ltd., Altrincham, Cheshire, England, UK) or a SX102a mass spectrometer (JEOL, Tokyo, Japan). Flash chromatography was performed on a Teledyne Isco CombiFlash Rf system with disposable silica columns (RediSep Rf, Teledyne Isco). Analytical HPLC analysis was performed for quality control on an Agilent 1200 system equipped with reverse phase column Chromolith® SpeedROD RP-18 endcapped 50-4.6 HPLC column. The radioactivity was tracked using a flow count radioactivity detector (Berthold LB 514 with BGO-X 30 µL flow cell). GE Healthcare TRACERlab FX–MeI and FX–M auto synthesizers were used for performing radiosynthesis. The purities of all tested compounds were determined by analytical HPLC, and the purity of each compound was confirmed ≥95%.

2. Synthetic Procedures



Scheme S1. Synthetic procedure for the synthesis of compounds **10–29**. Reagents and conditions: (i) malononitrile, piperidine, EtOH, reflux, 2 h; (ii) malononitrile, PhSH, Et_3N, EtOH, reflux, 4 h; (iii) Na_2S, DMF, 80 °C; (iv) Et_3N, MeCN, 50 °C, 4-16 h.

Preparation of compounds 10-29. The synthesis of compounds **10-29** was performed using the know literature procedures with slight changes.[1-4] The purity of each and every intermediate was checked against the reported data (^1H and ^{13}C NMR spectroscopy)[1-4] and they were used without any further purification. If needed, all intermediates could be purified via SiO_2 flash chromatography using $CHCl_3$/MeOH (0-10% gradient of MeOH).

2–benzylidenemalononitriles (**7**)

Anhydrous piperidine (25 μL) was added to a solution of substituted aldehyde (**6**, 10 mmol) and malononitrile (1.05 equiv) in of anhydrous EtOH (10 mL). The resulting solution was refluxed for 2 h until yellow solid forms. The mixture was then allowed to cool down to room temperature and the majority of EtOH was removed under dynamic vacuum. The residue was washed with a cold mixture of EtOH and hexanes (1:4, v/v) before vacuum filtration. The filter cake was further washed with more EtOH : hexanes (1:4 v/v) until the filtrate was colorless. The crude product was

then dried under dynamic vacuum to obtain the functionalized 2–benzylidenemalononitrile products (**7**) and was used without any further purification (crude yield, 80~95%).

4–aryl-6-(phenylthiol)-2-amino-3,5-dicyanopyrindines **(8)**

Anhydrous NEt_3 (50 μL) was added to a solution of functionalized 2–benzylidenemalononitriles (**7**, 1 mmol), malononitrile (1 mmol, 66.1 mg), and thiophenol (1 equiv, 103 μL) in ethanol (4 mL). The mixture was then brought to reflux and continued for an additional 4 h. A yellowish precipitate was formed over the course of the reaction. The reaction mixture was cooled to room temperature, and all volatiles were removed under vacuum. The residue was washed with a cold mixture of EtOH:hexanes = 1:4 (v/v). The suspension was filtered, and the filter cake was washed with more EtOH:hexanes = 1:4 (v/v) until the filtrate was colorless. The crude product was then dried under vacuum and the crude product was directly taken into the next step without further purification (crude yield 40−75%).

4–aryl-6-mercapto-2-amino-3,5-dicyanopyrindines (**9**)

To a solution of 2-amino-4-aryl-6-(phenylthio)pyridine-3,5-dicarbonitrile (**8**, 0.5 mmol) in anhydrous DMF (1.5 mL) was added anhydrous Na_2S (1.75 mmol, 137 mg) . The suspension was sonicated for 2 min and then heated at 80 °C for 2 h. The reaction mixture was cooled to room temperature afterwards, and the majority of the DMF was removed in high vacuum. Pre-cooled aqueous hydrochloric acid (1 M) was slowly added to the residual to adjust the pH to ~ 6. Yellow solid precipitated out that was filtered. The yellow filter cake was washed with more water, followed by a mixed solvent of EtOH:hexanes = 1:4 (v/ v). The crude thiol was dried under vacuum and was taken into next step without further purification (crude yield 80%−quantitative yield).

Free thiol and benzylic halide coupling reaction

To a suspension of the free thiol (1 mmol) and corresponding benzylic halide (1.05 mmol) in anhydrous acetonitrile (5 mL) was added Et_3N (2 mmol), where the mixture turned to darker solution in most cases. The resulting solution was then stirred at room temperature overnight. All volatiles were then removed under vacuum and the residue was triturated with a small amount of methanol. Water was added to induce crystallization and solidified product was filtered, washed with more water and dried under vacuum. This purification step can be repeated if needed or flash silica chromatography can be performed for further purification (0−10% MeOH in $CHCl_3$ gradient with 0.1% NH_4OH).

2-amino-4-(benzo[d][1,3]dioxol-5-yl)-6-(((2-(4-methoxyphenyl)thiazol-4-yl)methyl)thio)pyridine-3,5-dicarbonitrile (**30**)

This compound was synthesized following the literature method reported by Louvel and co-workers.[5] ^{1}H and ^{13}C NMR as well as HRMS was used to confirm the formation and purity of the synthesized compound.

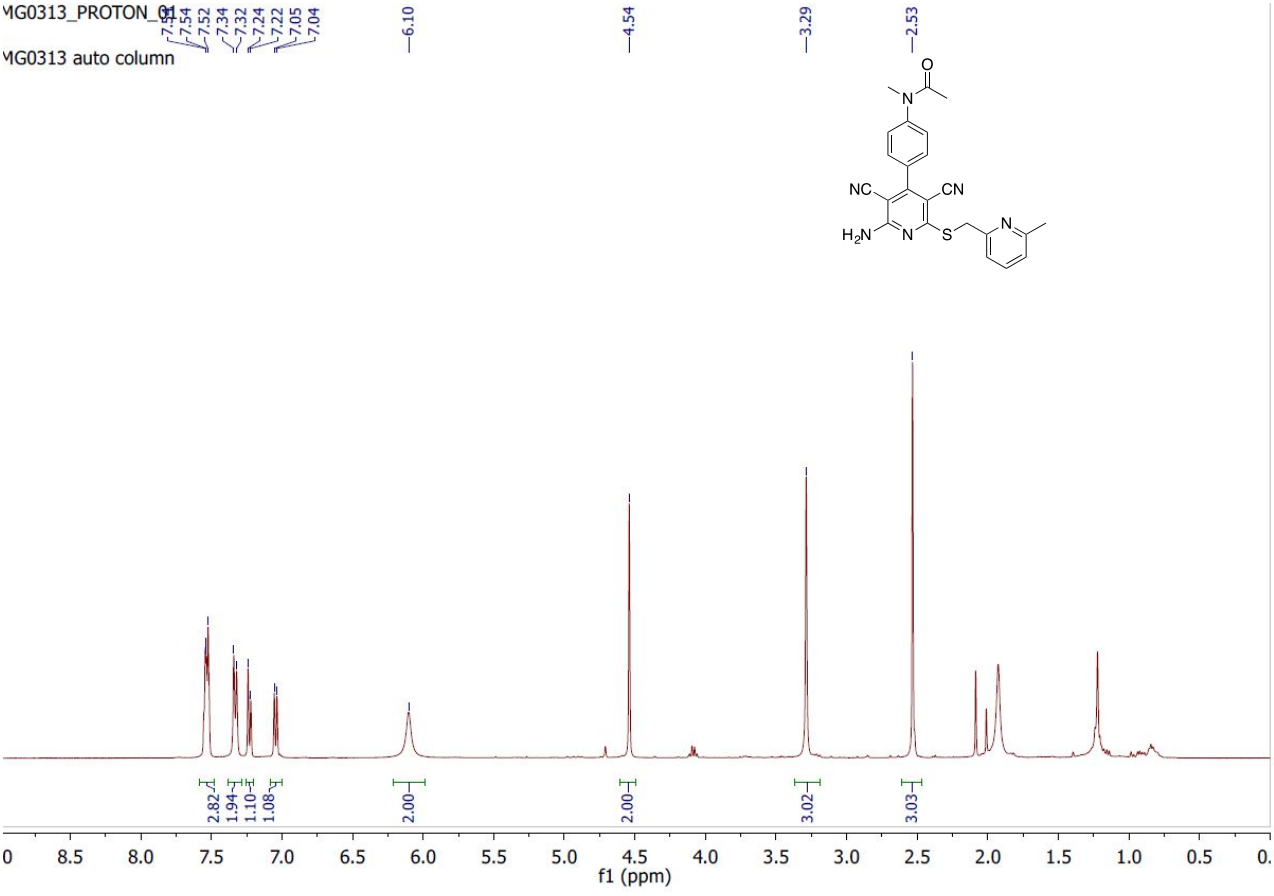
Representative demethylation procedure to prepare precursors of [^{11}C]27 and [^{11}C]29.

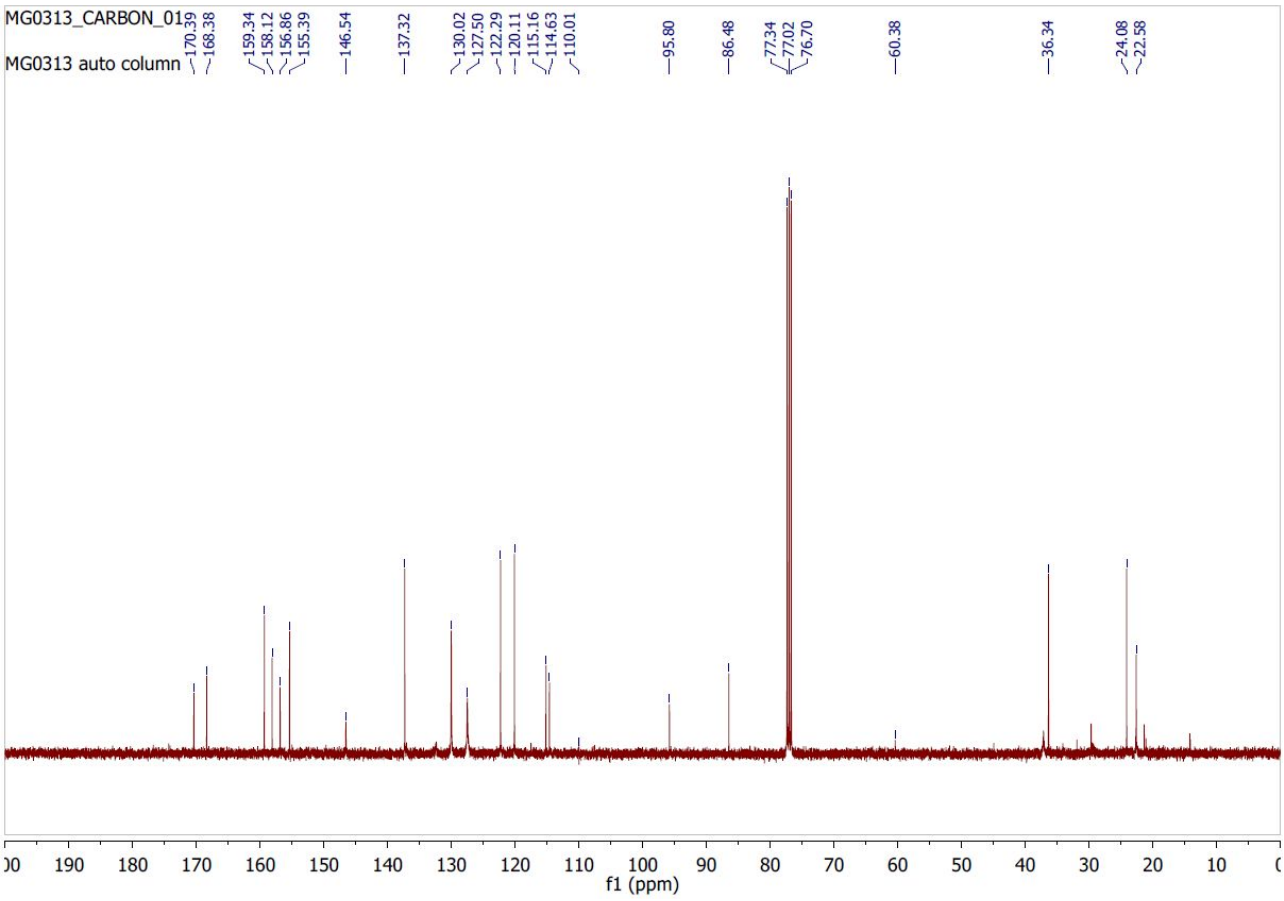
To a precooled (0 °C) suspension of corresponding methoxy compound (**27** or **29**) in anhydrous dichloromethane (5 mL) was added excess BBr_3 (1.0 M in dichloromethane, 10 equiv) dropwise at 0 °C under a nitrogen atmosphere. The reaction mixture was allowed to react and brought to room temperature gradually and then stirred at room temperature overnight. The resulting dark brown suspension was diluted with more anhydrous DCM and quenched by 200 μL of 2-propanol at 0 °C. The mixture turned orange before saturated $NaHCO_3$ and dichloromethane were added to the reaction mixture. The yellow colored organic layer was separated, and the aqueous layer was

extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and purified by flash SiO_2 chromatography (0−10% MeOH in $CHCl_3$ gradient with 0.1% NH_4OH).

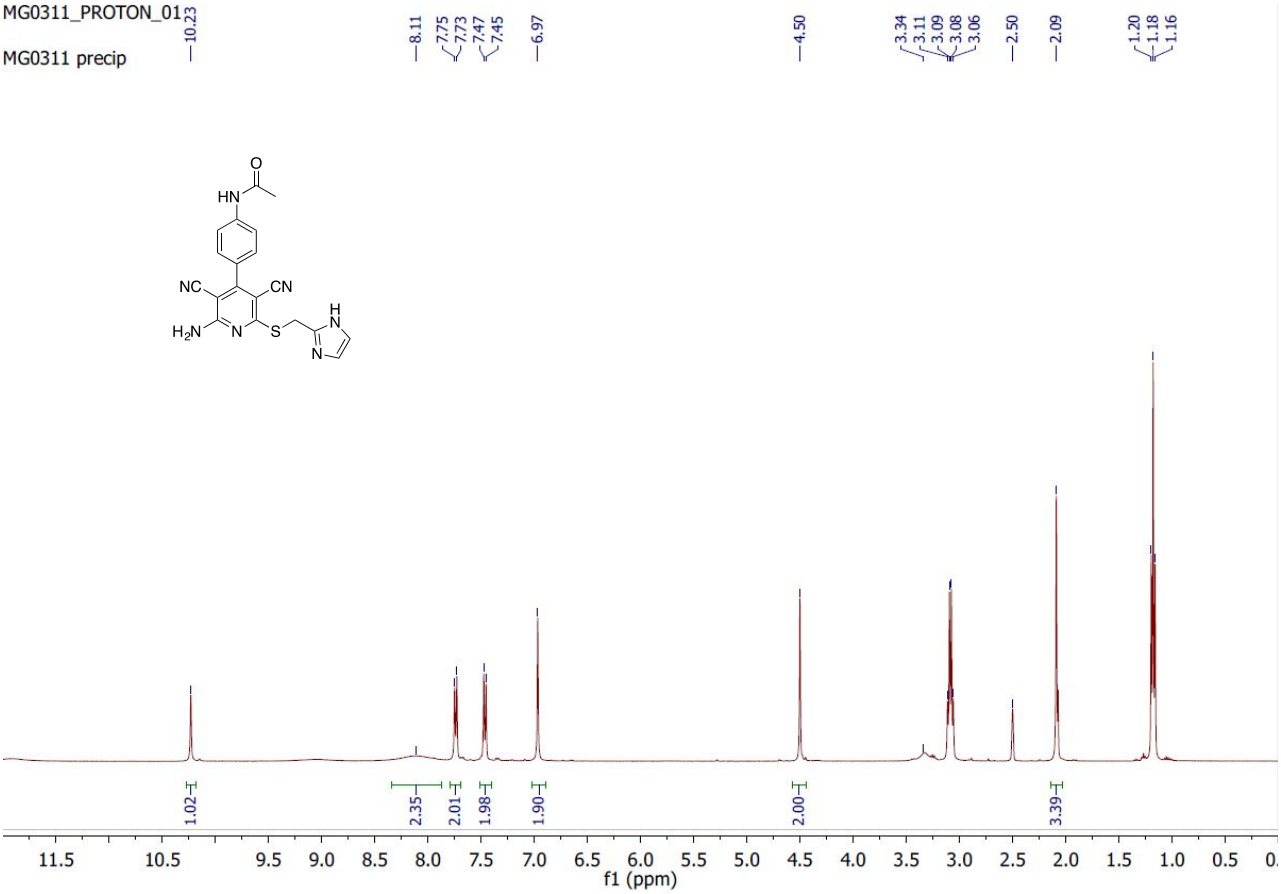
NMR Spectra

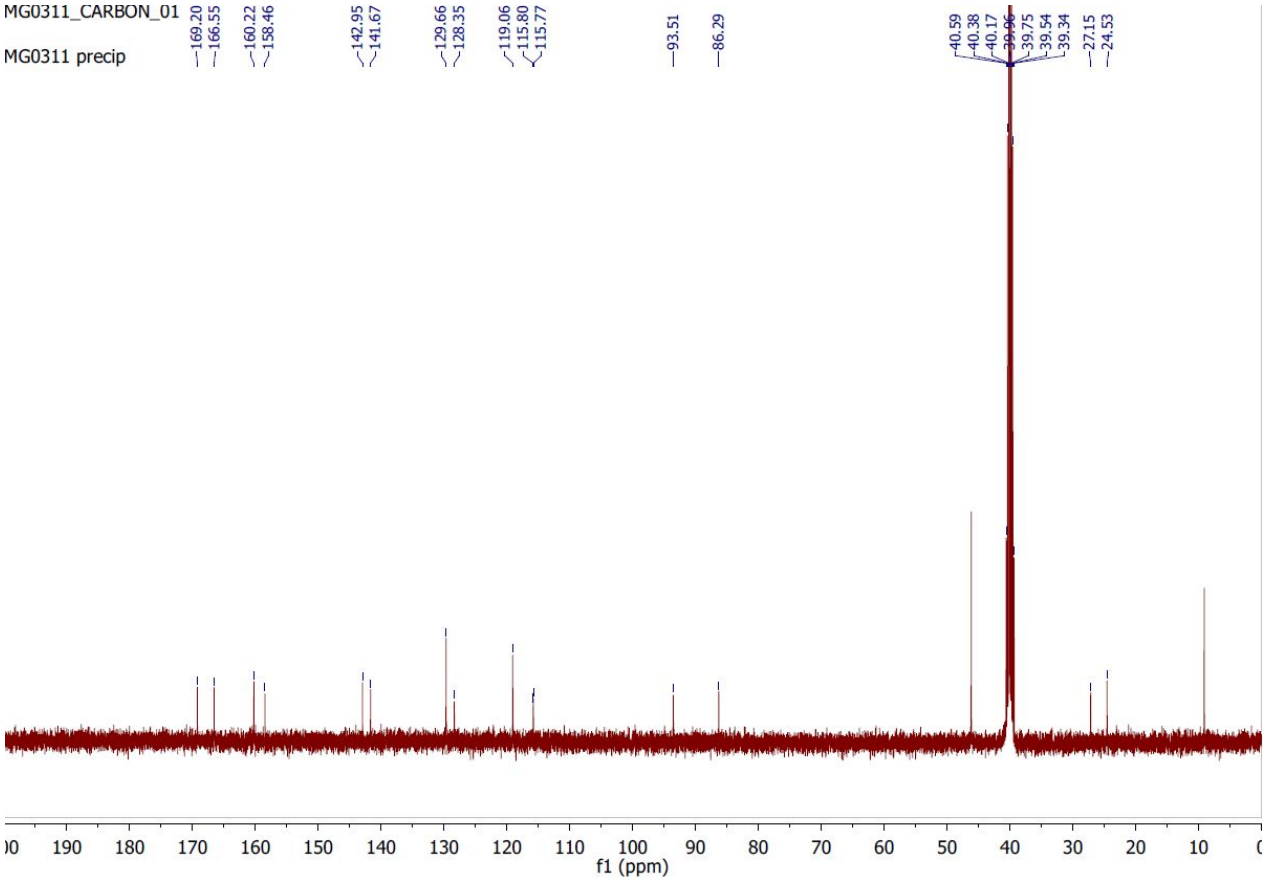
Compound 11. ^{1}H NMR (400 MHz, $CDCl_3$), 7.58-7.48 (m, 3H), 7.37-7.29 (m, 2H), 7.26-7.19 (m, 1H), 7.04 (d, J = 7.7 Hz, 1H), 6.10 (brs, 2H), 4.54 (s, 2H), 3.29 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 170.4, 168.4, 159.3, 158.1, 156.9, 155.4, 146.5, 137.3, 130.0, 127.5, 122.3, 120.1, 115.2, 114.6, 110.0, 95.8, 86.5, 60.4, 36.3, 24.1, 22.6. MS (ESI+): *m/z* calcd. for $C_{23}H_{21}N_6OS$ $[M+1]^+$ 429.1419, found 429.1422.



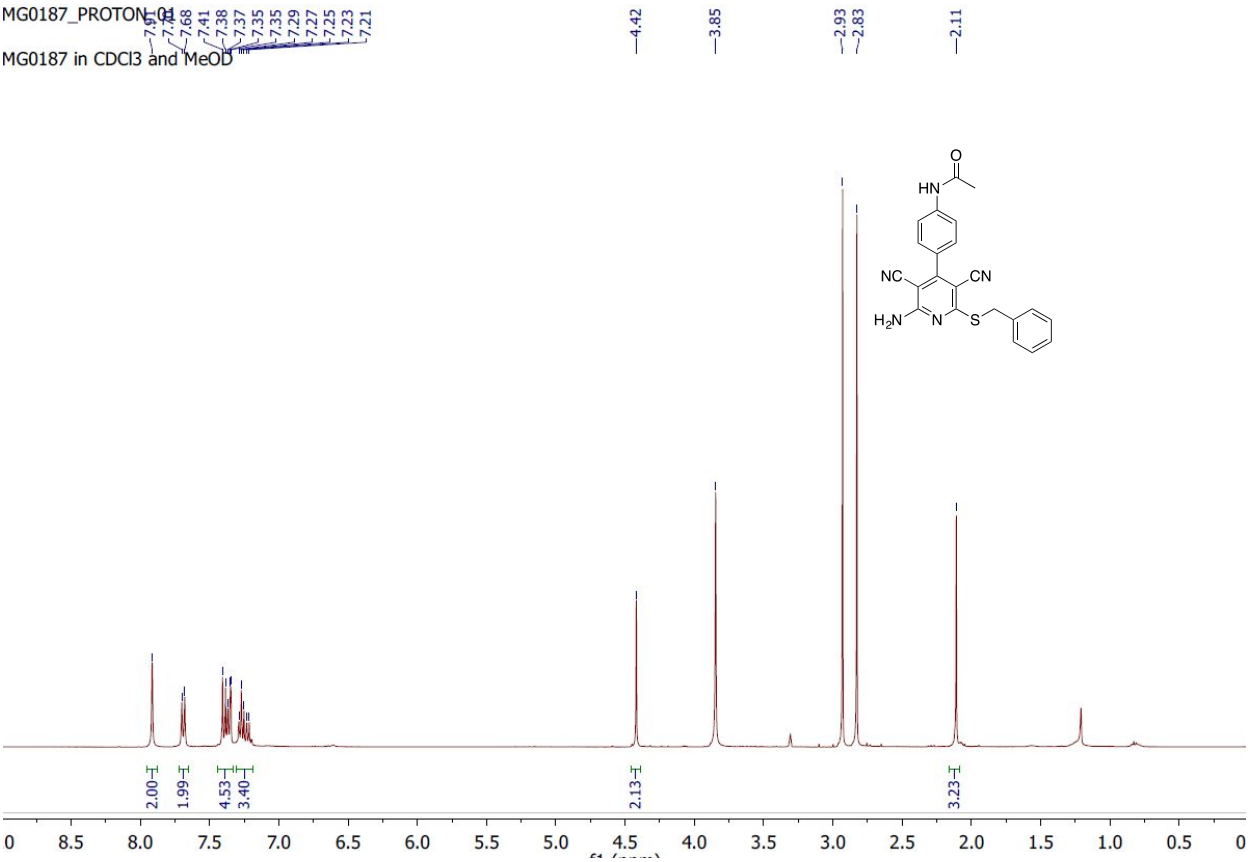

MG0313_CARBON_01
MG0313 auto column
170.39
168.38
159.34
158.12
156.86
155.39
146.54
137.32
130.02
127.50
122.29
120.11
115.16
114.63
110.01
95.80
86.48
77.34
77.02
76.70
60.38
36.34
24.08
22.58
f1 (ppm)

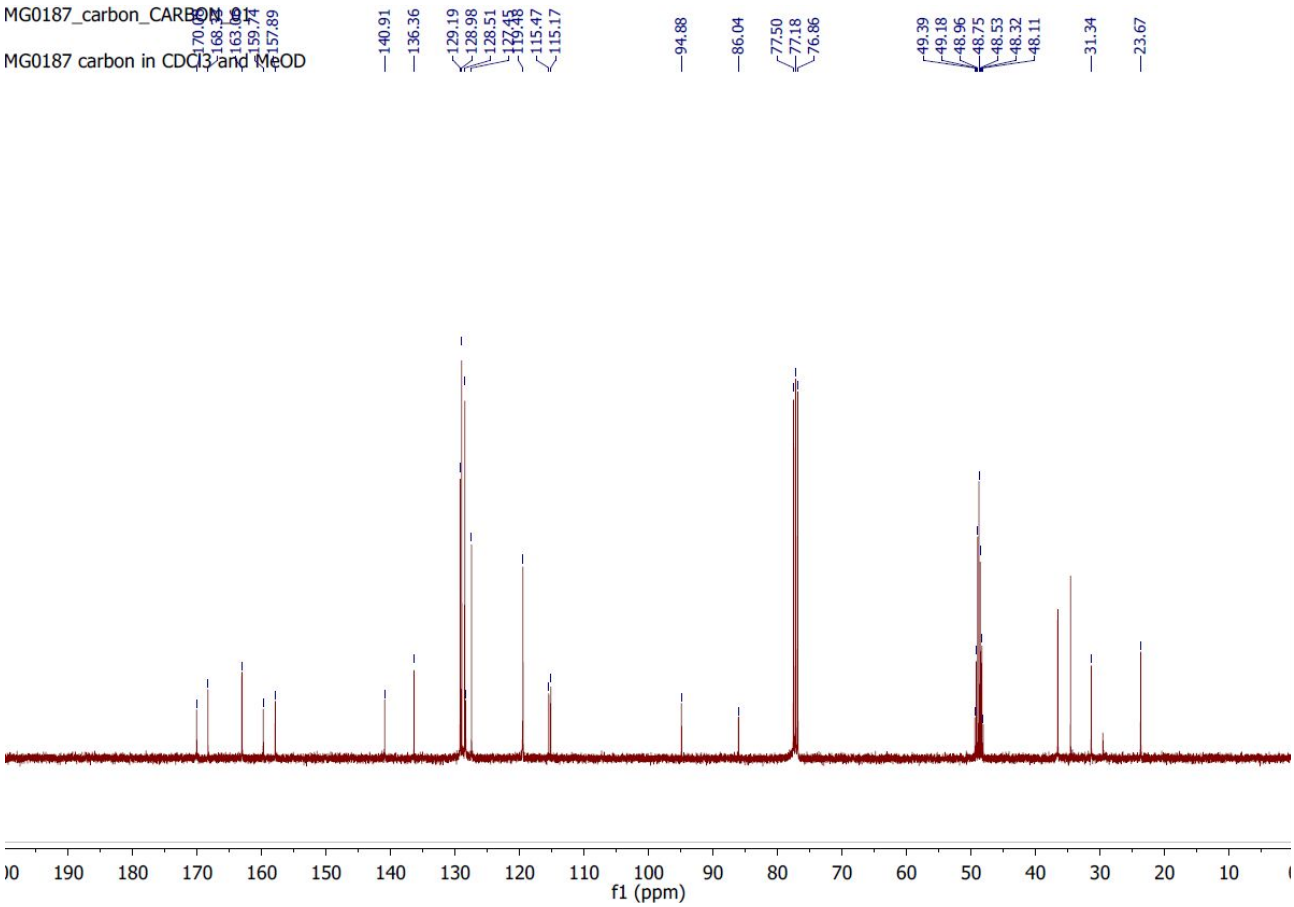
Compound 12. ^{1}H NMR (400 MHz, d_6-DMSO): 10.23 (s, 1H), 8.11 (brs, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 6.97 (s, 2H), 4.50 (s, 2H), 2.09 (s, 3H). ^{13}C NMR (125 MHz, d_6-DMSO): 169.2, 166.6, 160.2, 158.5, 142.9, 141.7, 129.7, 128.4, 119.1, 115.8, 115.7, 93.5, 86.3, 27.2, 24.5. MS (ESI+): m/z calcd. for $C_{19}H_{16}N_7OS$ $[M+1]^+$ 390.1059, found 390.1061.



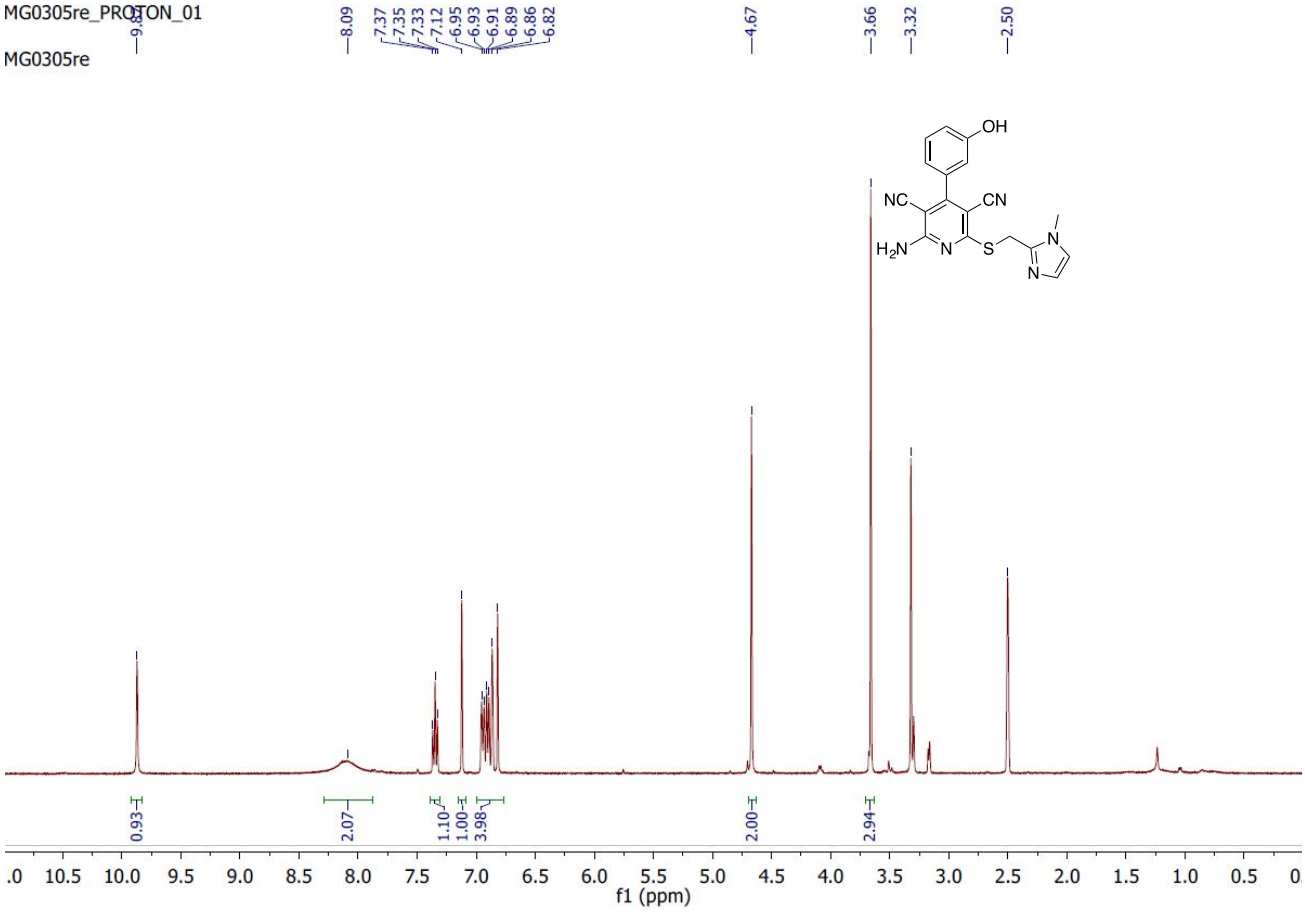

MG0311_CARBON_01
MG0311 precip
169.20
166.55
160.22
158.46
142.95
141.67
129.66
128.35
119.06
115.80
115.77
93.51
86.29
40.59
40.38
40.17
39.96
39.75
39.54
39.34
27.15
24.53
f1 (ppm)

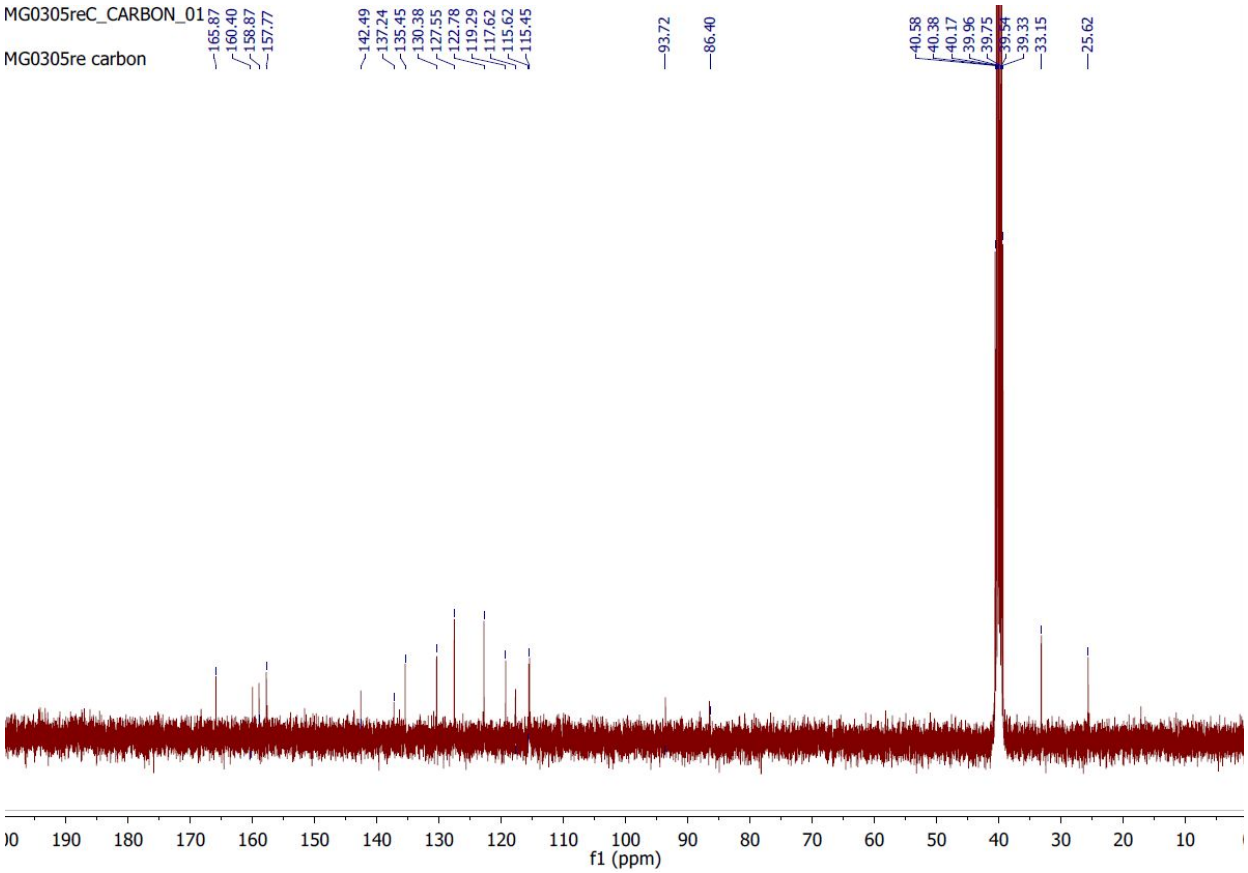
Compound 13. ^{1}H NMR (400 MHz, $CDCl_3$/d_4-MeOD): 7.91 (s, 2H), 7.69 (d, J = 8.6 Hz, 2H), 7.43-7.33 (m, 4H), 7.31-7.19 (m, 3H), 4.42 (s, 2H), 2.11 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$/d_4-MeOD): 170.1, 168.3, 163.1, 159.7, 157.9, 140.9, 136.4, 129.2, 129.0, 128.5, 128.4, 127.4, 119.5, 115.5, 115.2, 94.9, 86.0, 31.3, 23.7. MS (ESI+): m/z calcd. for $C_{22}H_{18}N_5OS$ $[M+1]^+$ 400.1154, found 400.1155.



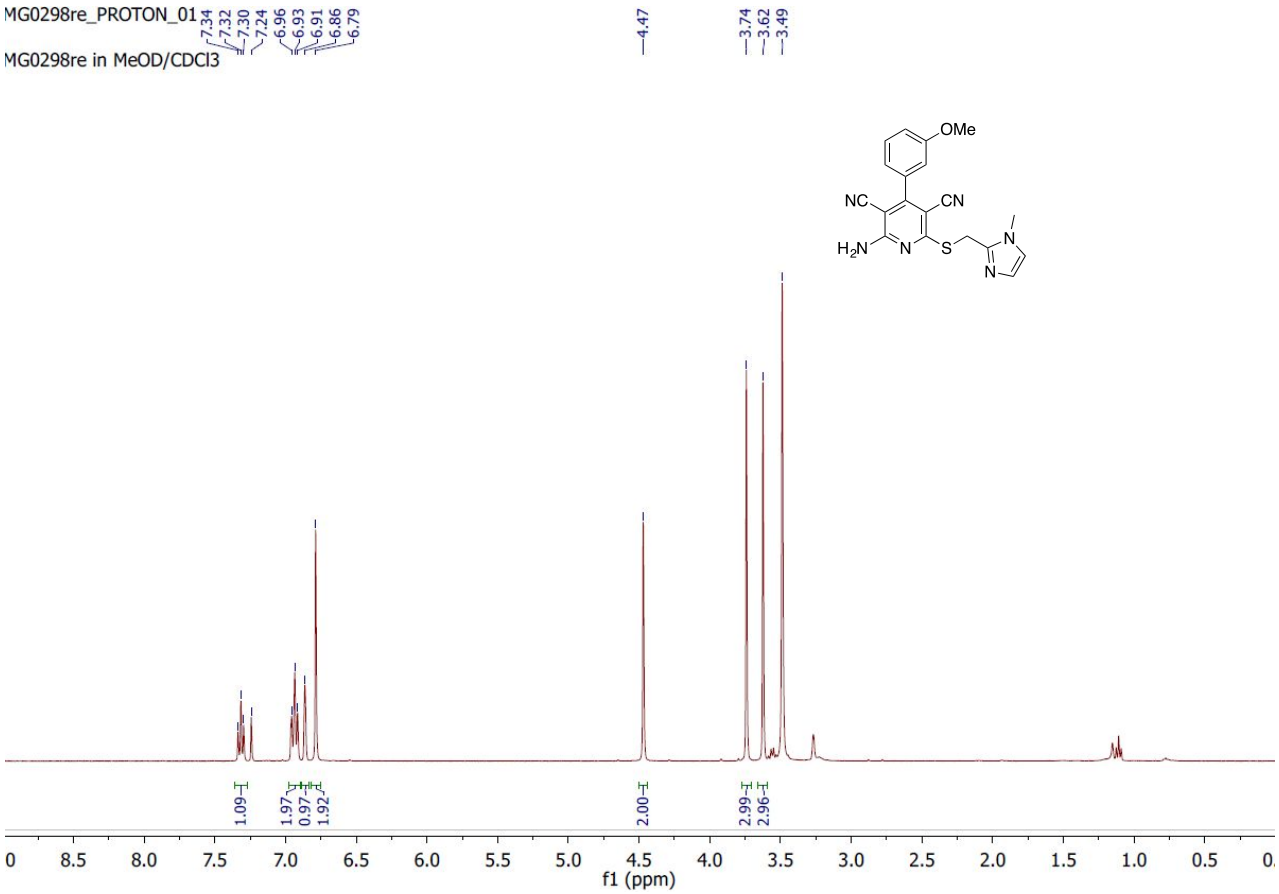

MG0187_carbon_CARBON_01
MG0187 carbon in CDCl3 and MeOD
170.04
168.31
163.44
159.74
157.89
140.91
136.36
129.19
128.98
128.51
127.45
119.48
115.47
115.17
94.88
86.04
77.50
77.18
76.86
49.39
49.18
48.96
48.75
48.53
48.32
48.11
31.34
23.67
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

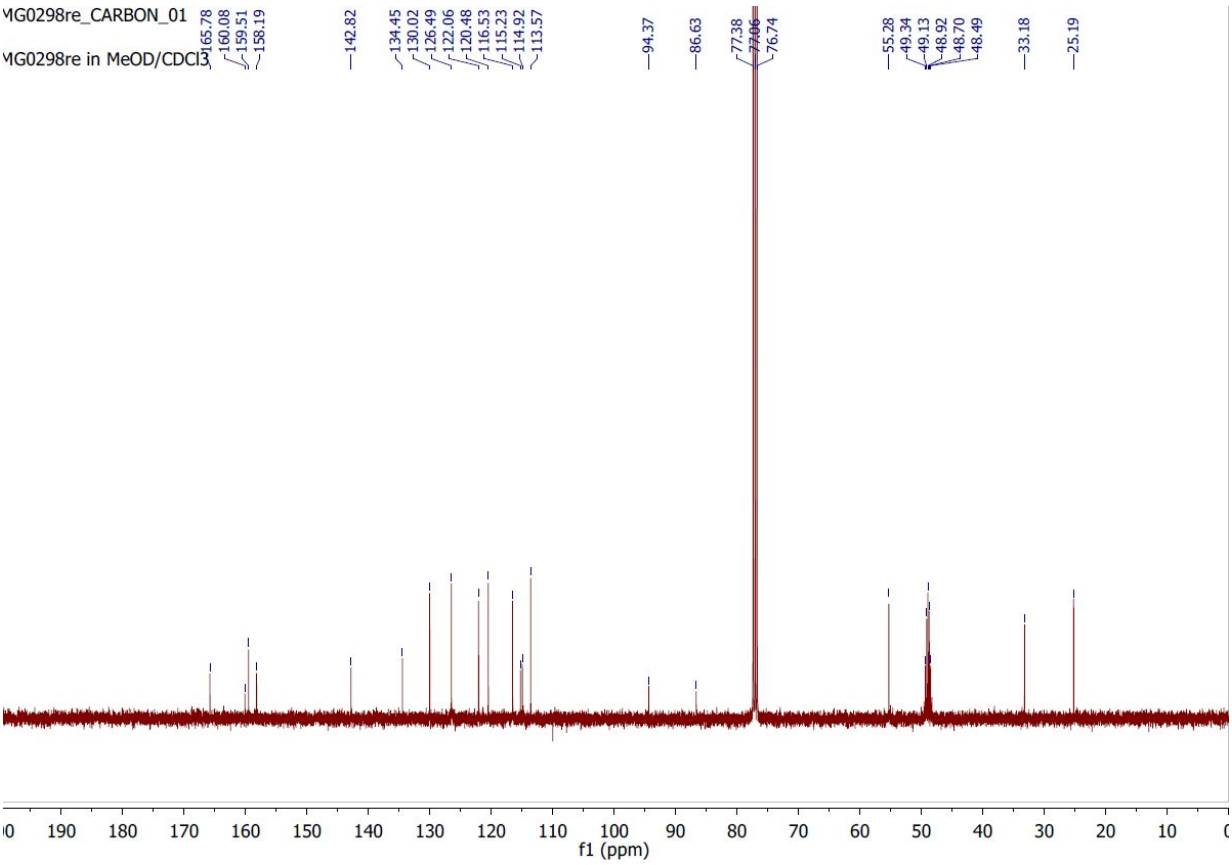
Compound 16. ^{1}H NMR (400 MHz, d_6-DMSO): 9.87 (s, 1H), 8.09 (brs, 2H), 7.39-7.31 (m, 1H), 7.12 (s, 1H), 6.98-6.78 (m, 4H), 4.67 (s, 2H), 3.66 (s, 3H). ^{13}C NMR (125 MHz, d_6-DMSO): 165.9, 160.4, 158.9, 157.8, 142.5, 137.2, 135.4, 130.4, 127.6, 122.8, 119.3, 117.6, 115.6, 115.4, 93.7, 86.4, 33.2, 25.6. MS (ESI+): m/z calcd. for $C_{18}H_{15}N_6OS$ $[M+1]^+$ 363.0950, found 363.0955.



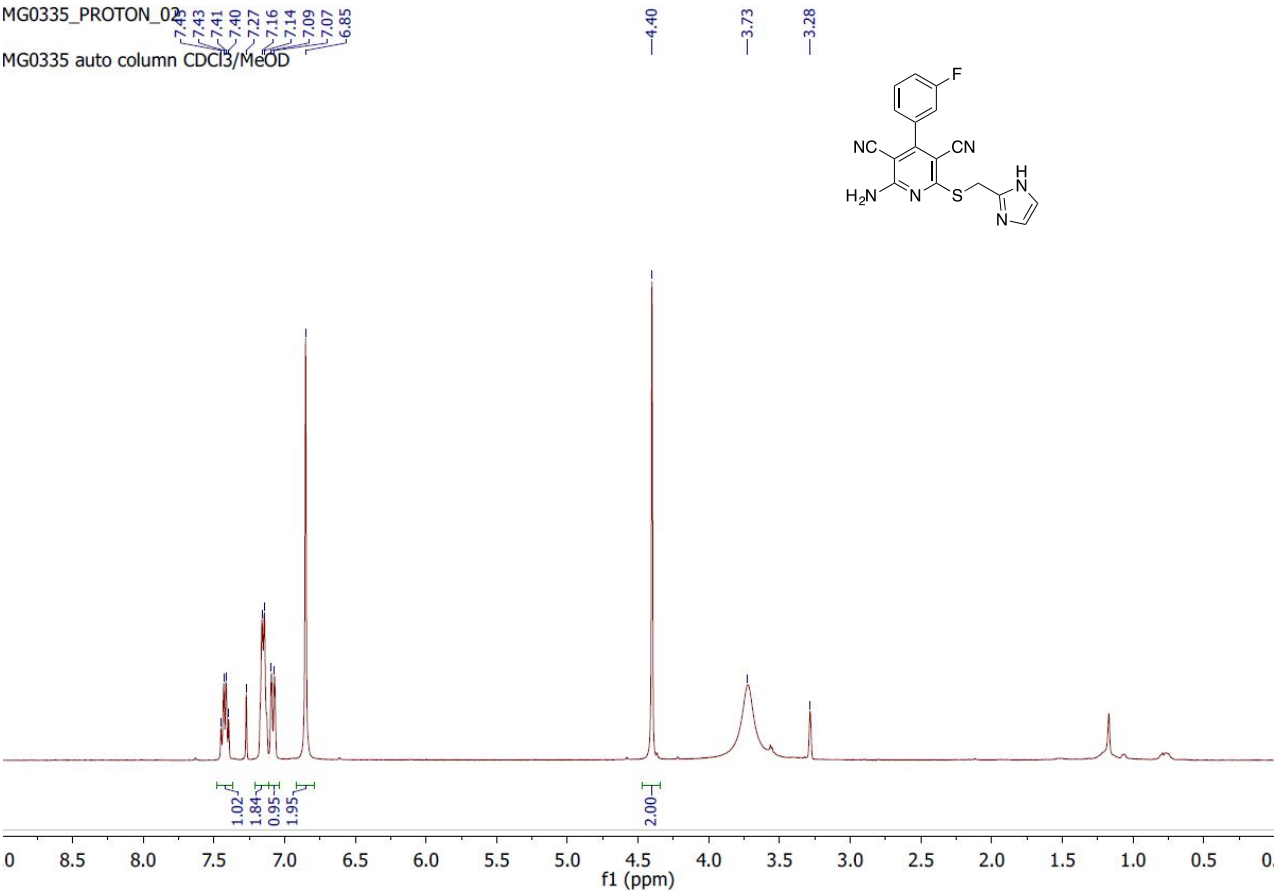

MG0305reC_CARBON_01
MG0305re carbon
165.87
160.40
158.87
157.77
142.49
137.24
135.45
130.38
127.55
122.78
119.29
117.62
115.62
115.45
93.72
86.40
40.58
40.38
40.17
39.96
39.75
39.54
39.33
33.15
25.62
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

Compound 17. ^{1}H NMR (400 MHz, $CDCl_3$/d_4-MeOD): 7.35-7.27 (m, 1H), 6.98-6.90 (m, 2H), 6.86 (s, 1H), 6.79 (s, 2H), 4.47 (s, 2H), 3.74 (s, 3H), 3.62 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$/d_4-MeOD): 165.8, 160.1, 159.5, 158.2, 142.8, 134.4, 130.0, 126.5, 122.1, 120.5, 116.5, 115.2, 114.9, 113.6, 94.4, 86.6, 55.3, 33.2, 25.2. MS (ESI+): *m*/*z* calcd. for $C_{19}H_{17}N_6OS$ $[M+1]^+$ 377.1106, found 377.1109.



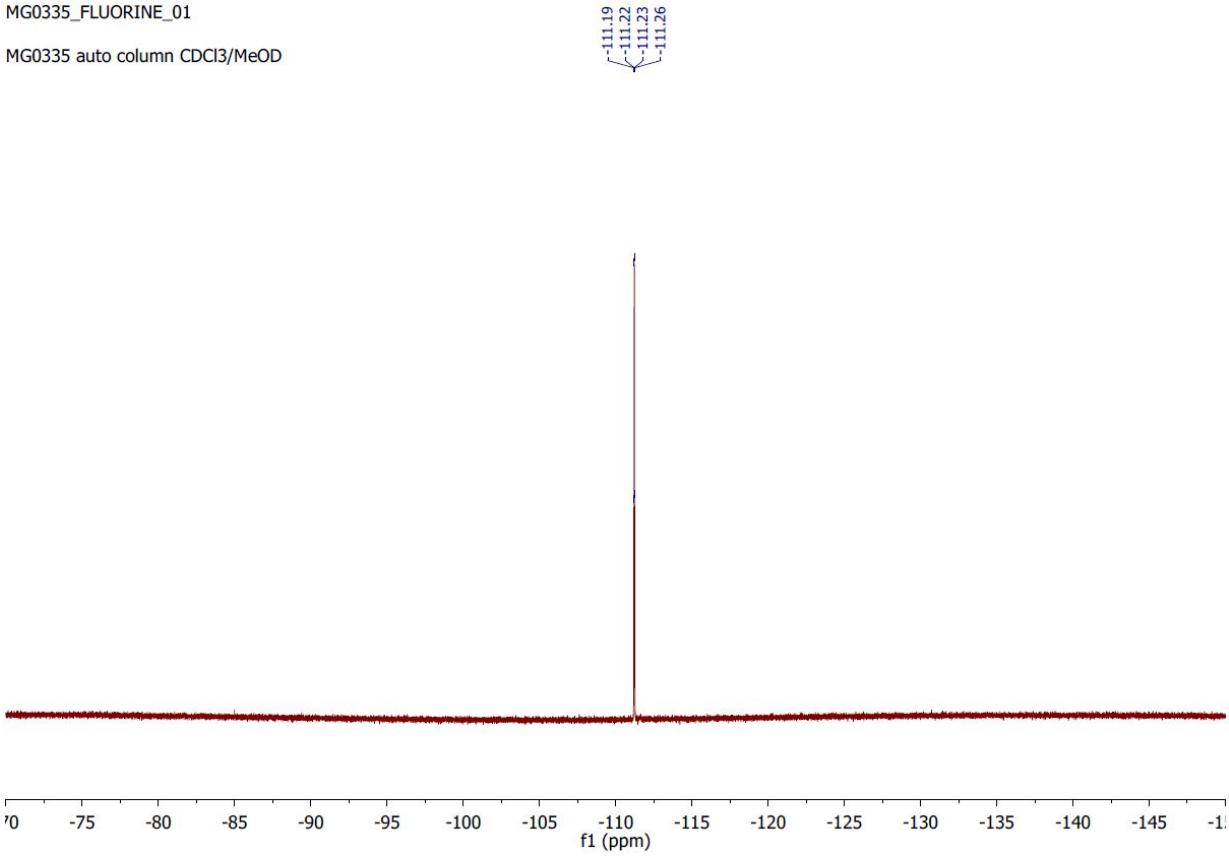

MG0298re_CARBON_01
MG0298re in MeOD/CDCl3
165.78
160.08
159.51
158.19
142.82
134.45
130.02
126.49
122.06
120.48
116.53
115.23
114.92
113.57
94.37
86.63
77.38
77.06
76.74
55.28
49.34
49.13
48.92
48.70
48.49
33.18
25.19
f1 (ppm)

Compound 18. ^{1}H NMR (400 MHz, $CDCl_3$/d_4-MeOD): 7.47-7.37 (m, 1H), 7.20-7.11 (m, 2H), 7.11-7.04 (m, 1H), 6.85 (s, 2H), 4.40 (s, 2H). ^{19}F NMR (376 MHz, $CDCl_3$/d_4-MeOD): -111.2 (dd, J = 14.6, 8.7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$/d_4-MeOD): 167.0, 163.3, 161.2, 160.0, 156.8, 143.8, 135.2 (d, J = 7.9 Hz), 130.8 (d, J = 8.3 Hz), 124.1 (d, J = 3.3 Hz), 117.7 (d, J = 21.0 Hz), 115.5 (d, J = 23.5 Hz), 114.8 (d, J = 20.3 Hz), 94.2, 86.4, 26.5. MS (ESI+): m/z calcd. for $C_{17}H_{11}FN_6S$ $[M+1]^+$ 351.0750, found 351.0753.



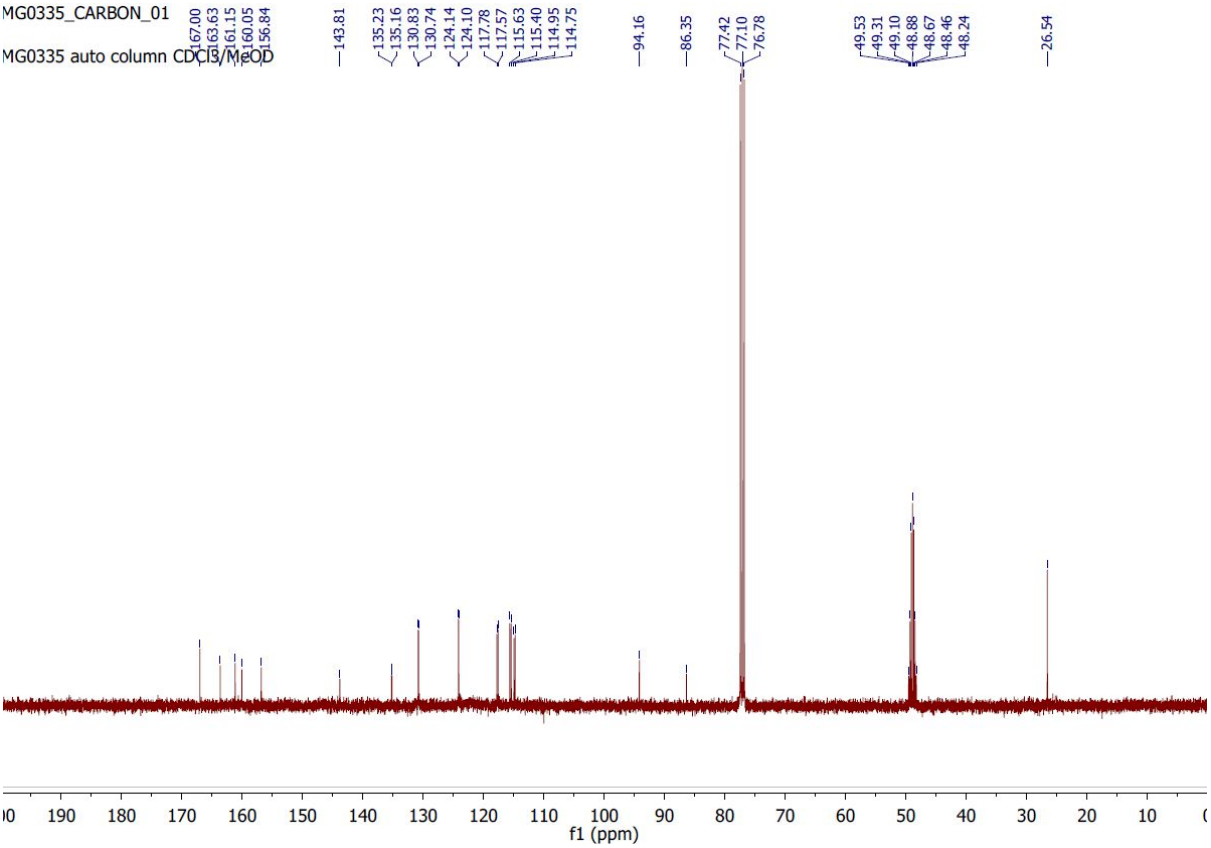
MG0335_FLUORINE_01

MG0335 auto column CDCl3/MeOD

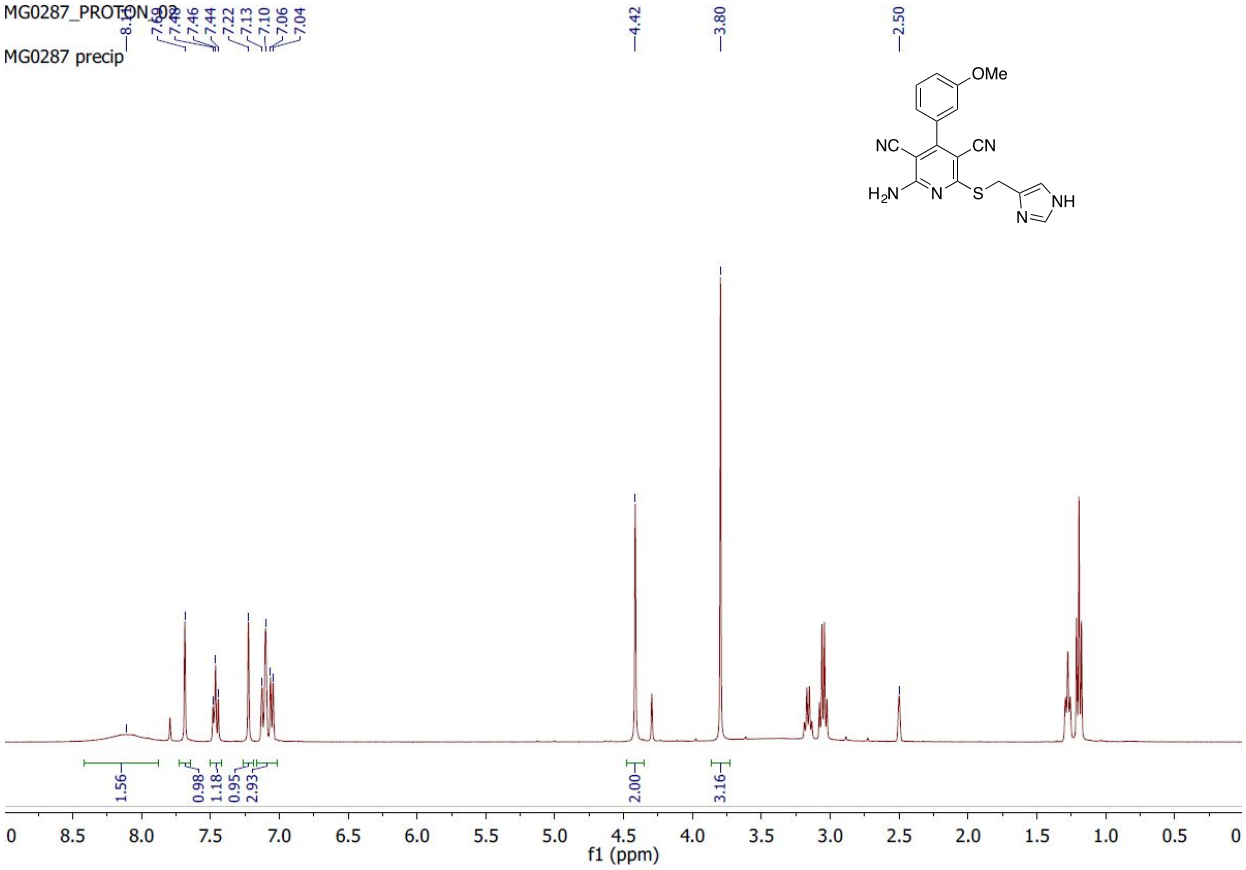


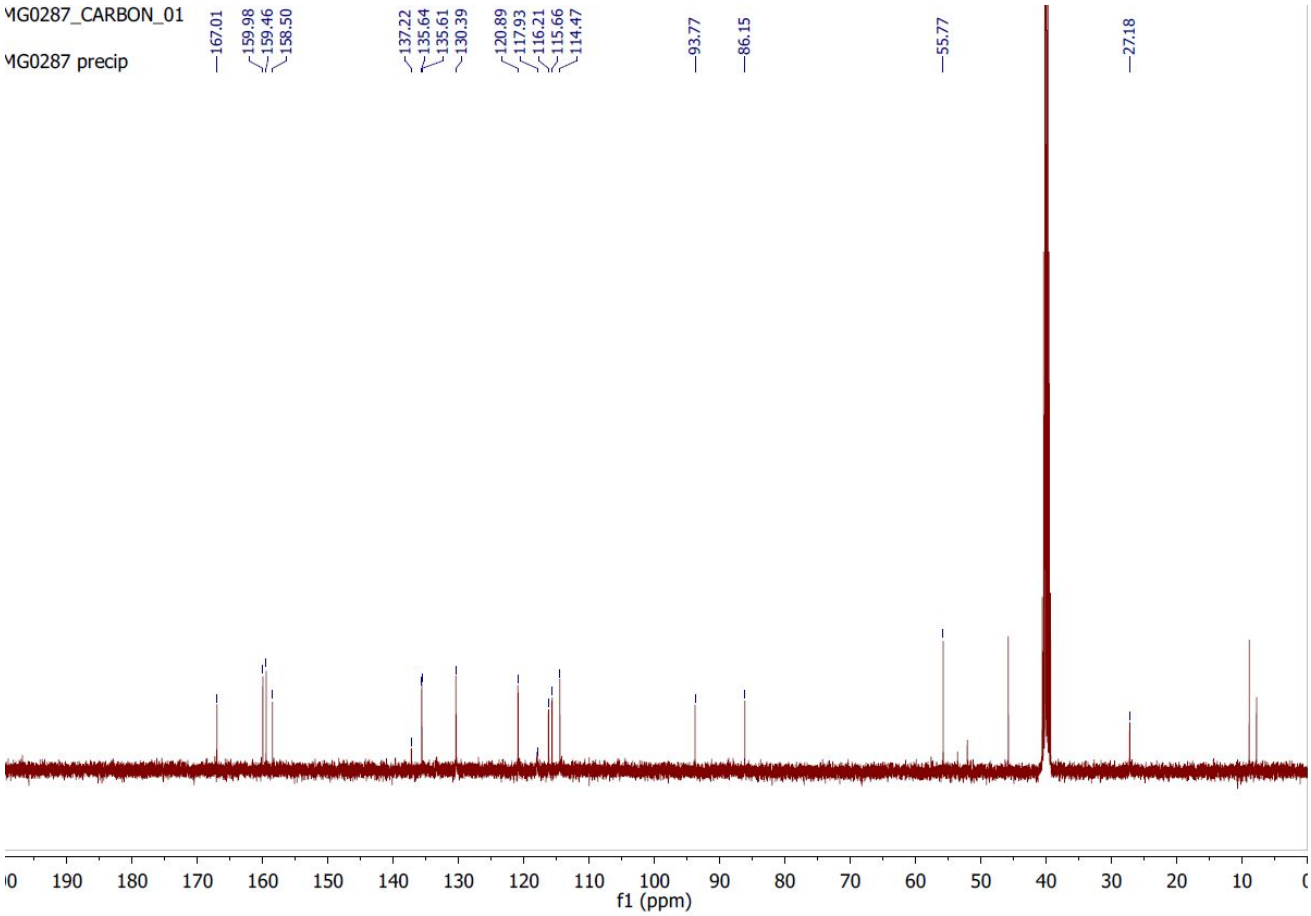
MG0335_CARBON_01

MG0335 auto column CDCl3/MeOD

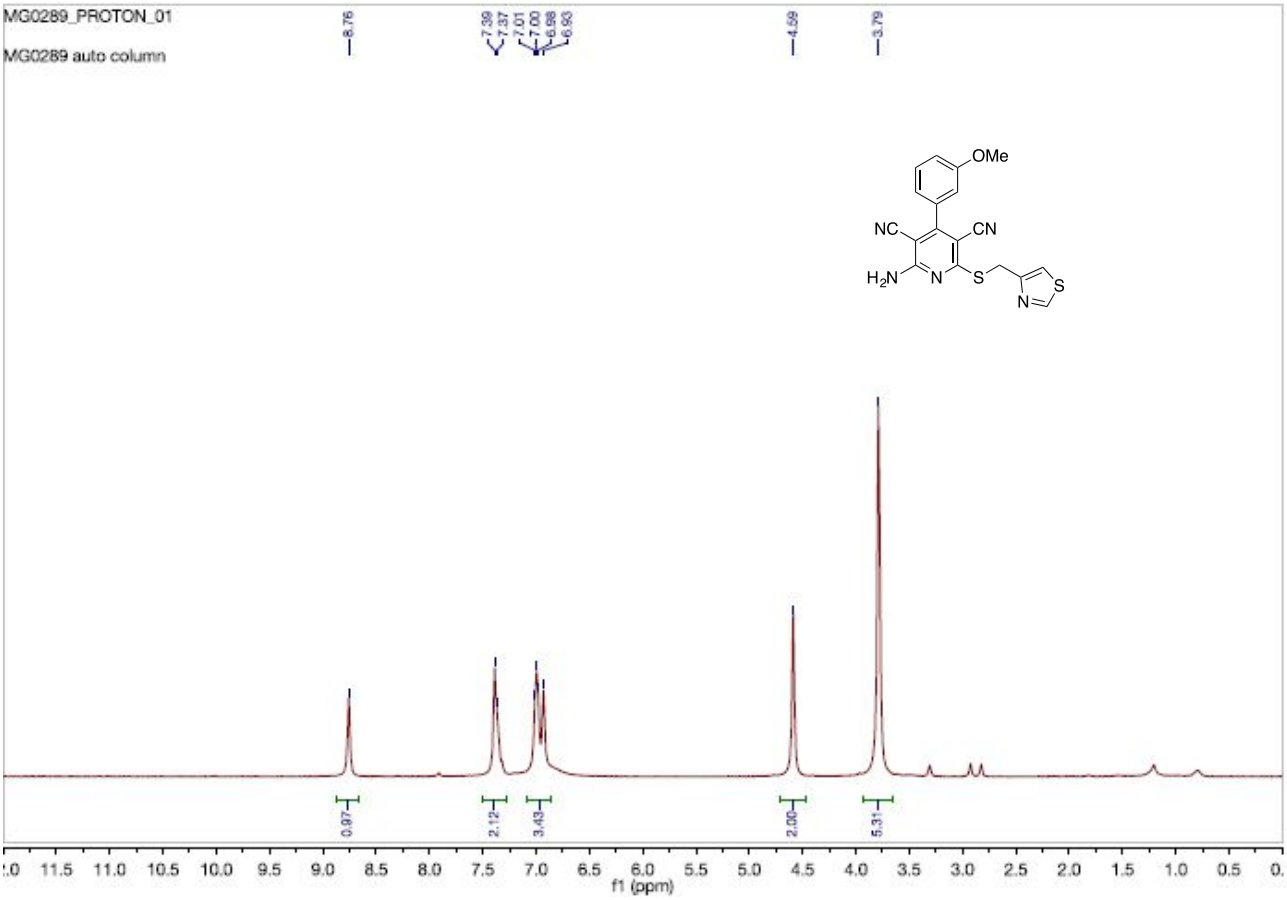


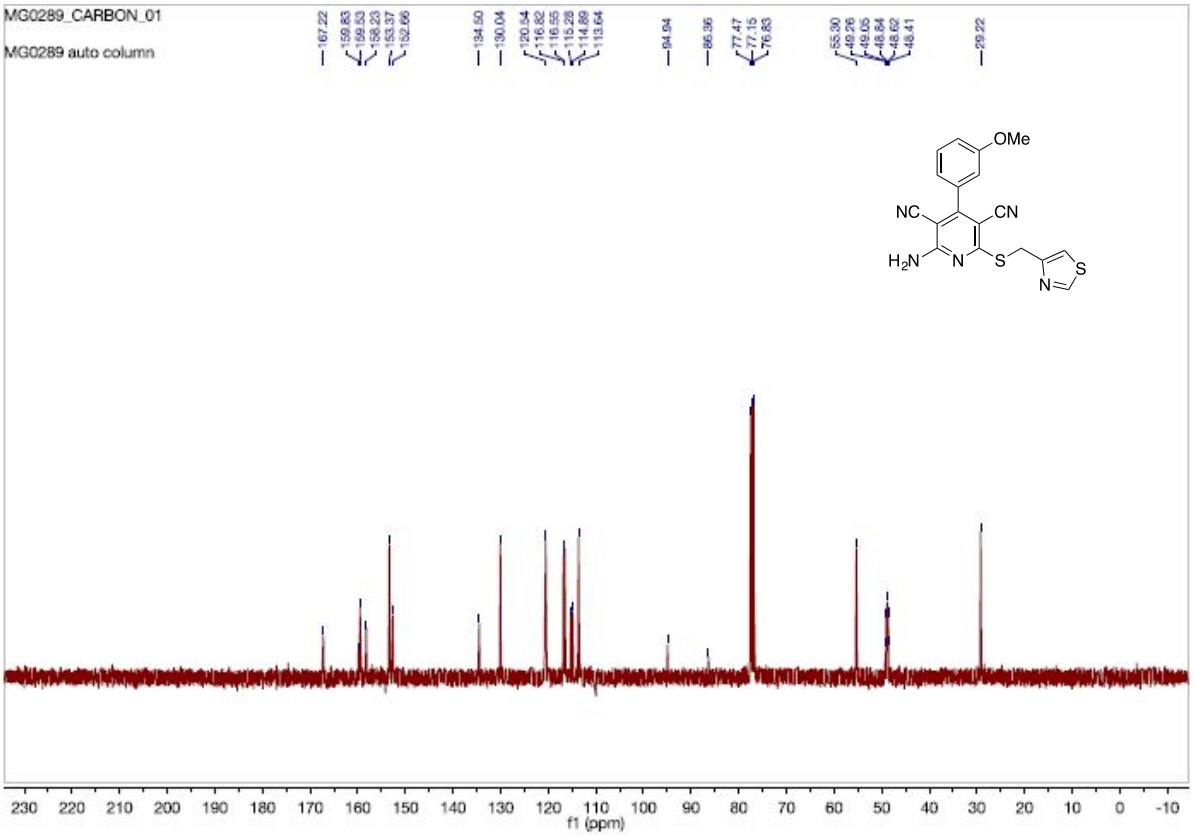
Compound 19. ^{1}H NMR (400 MHz, d_6-DMSO): 8.11 (brs, 2H), 7.69 (s, 1H), 7.50-7.41 (m, 1H), 7.22 (s, 1H), 7.16-7.02 (m, 3H), 4.42 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (125 MHz, d_6-DMSO): 167.0, 160.0, 159.5, 158.5, 137.2, 135.7, 135.6, 130.4, 120.9, 117.9, 116.2, 115.7, 114.5, 93.8, 86.2, 55.8, 27.2. MS (ESI+): *m*/*z* calcd. for $C_{18}H_{15}N_6OS$ $[M+1]^+$ 363.0950, found 363.0952.




MG0287_CARBON_01
MG0287 precip
167.01
159.98
159.46
158.50
137.22
135.64
135.61
130.39
120.89
117.93
116.21
115.66
114.47
93.77
86.15
55.77
27.18
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

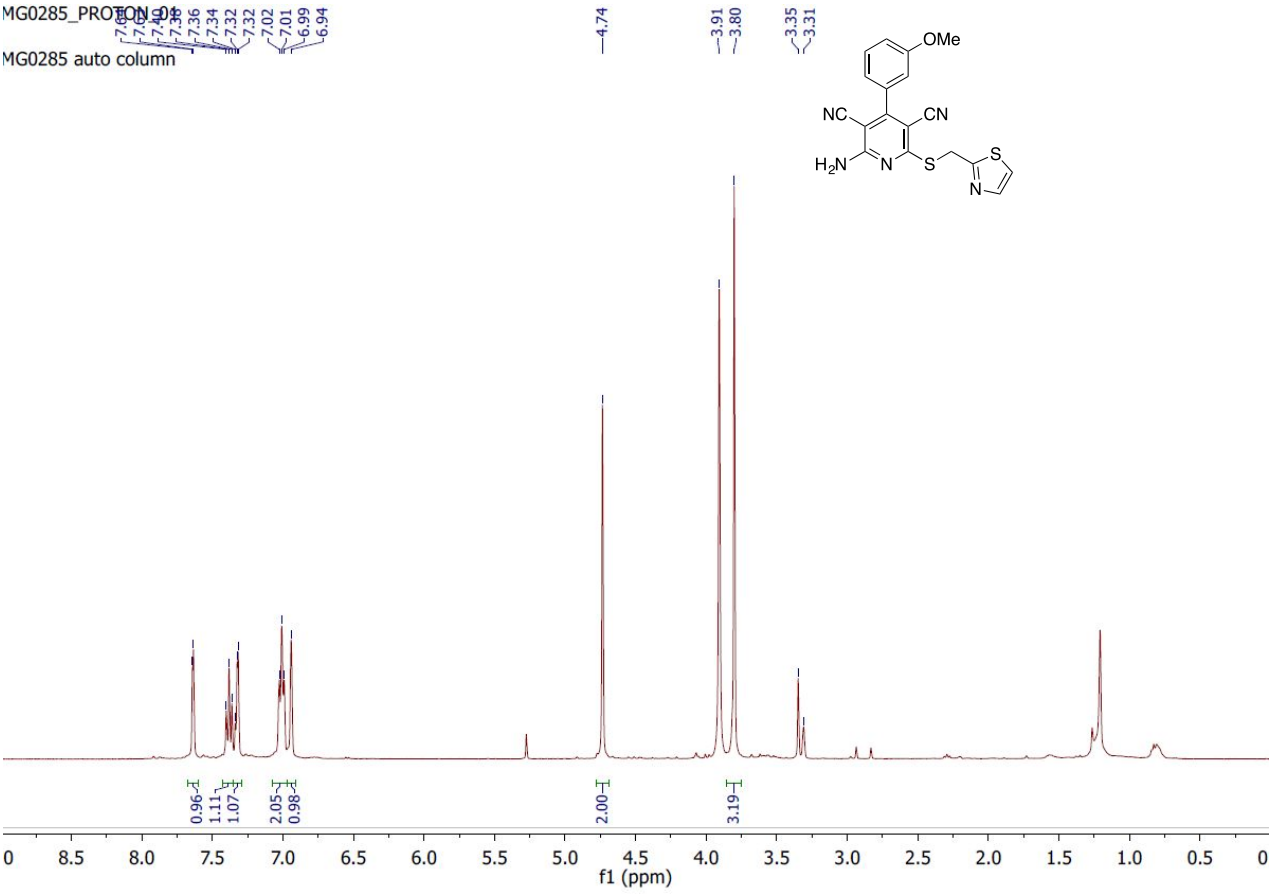
Compound 20. ^{1}H NMR (400 MHz, $CDCl_3$/d_4-MeOD): 8.76 (s, 1H), 7.49-7.27 (m, 2H), 7.12-6.76 (m, 3H), 4.59 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$/d_4-MeOD): 167.2, 159.8, 159.5, 158.2, 153.4, 152.7, 134.5, 130.0, 120.5, 116.8, 116.6, 115.3, 114.9, 113.6, 94.9, 86.4, 55.3, 29.2. MS (M+1): calc. MS (ESI+): *m*/*z* calcd. for $C_{18}H_{14}N_5OS_2$ $[M+1]^+$ 380.0562, found 380.0566.

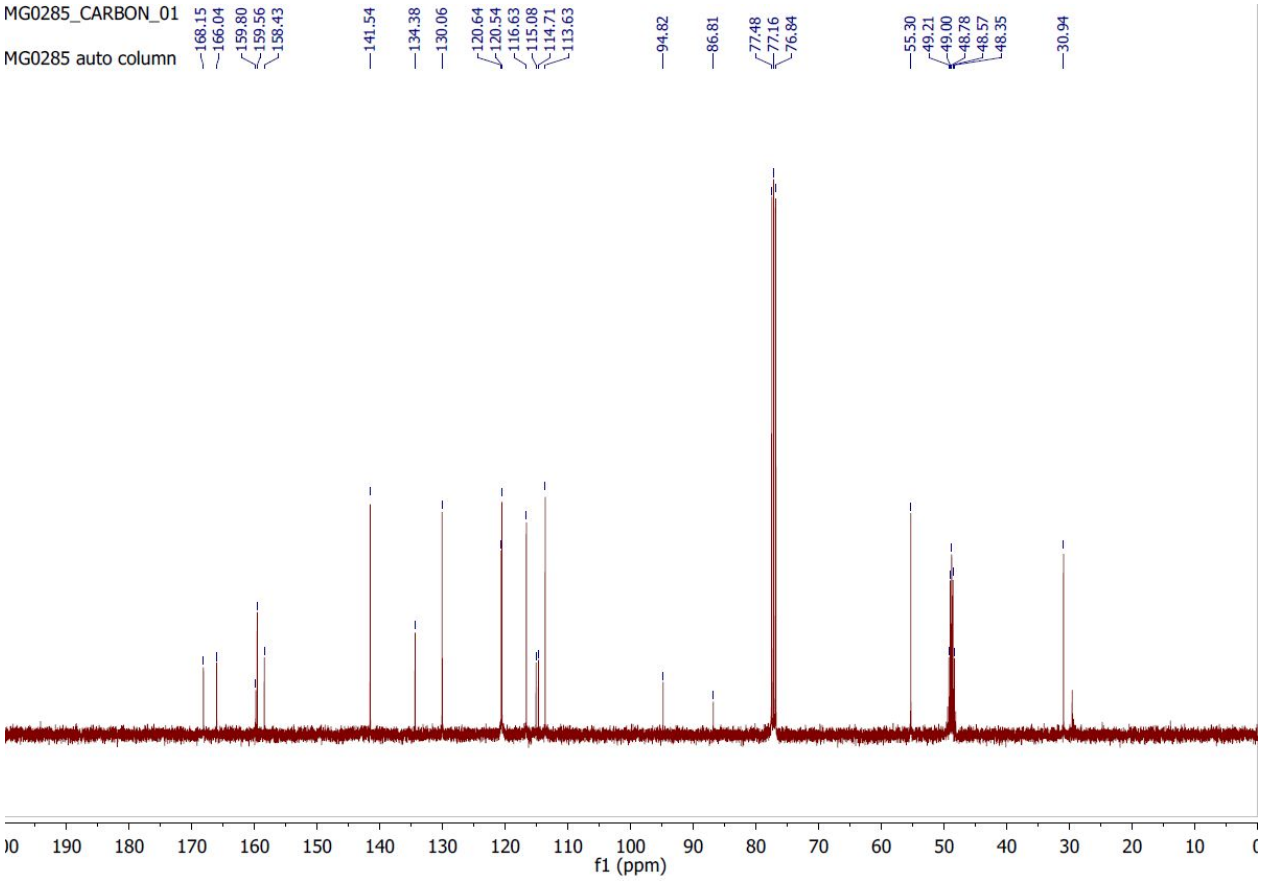




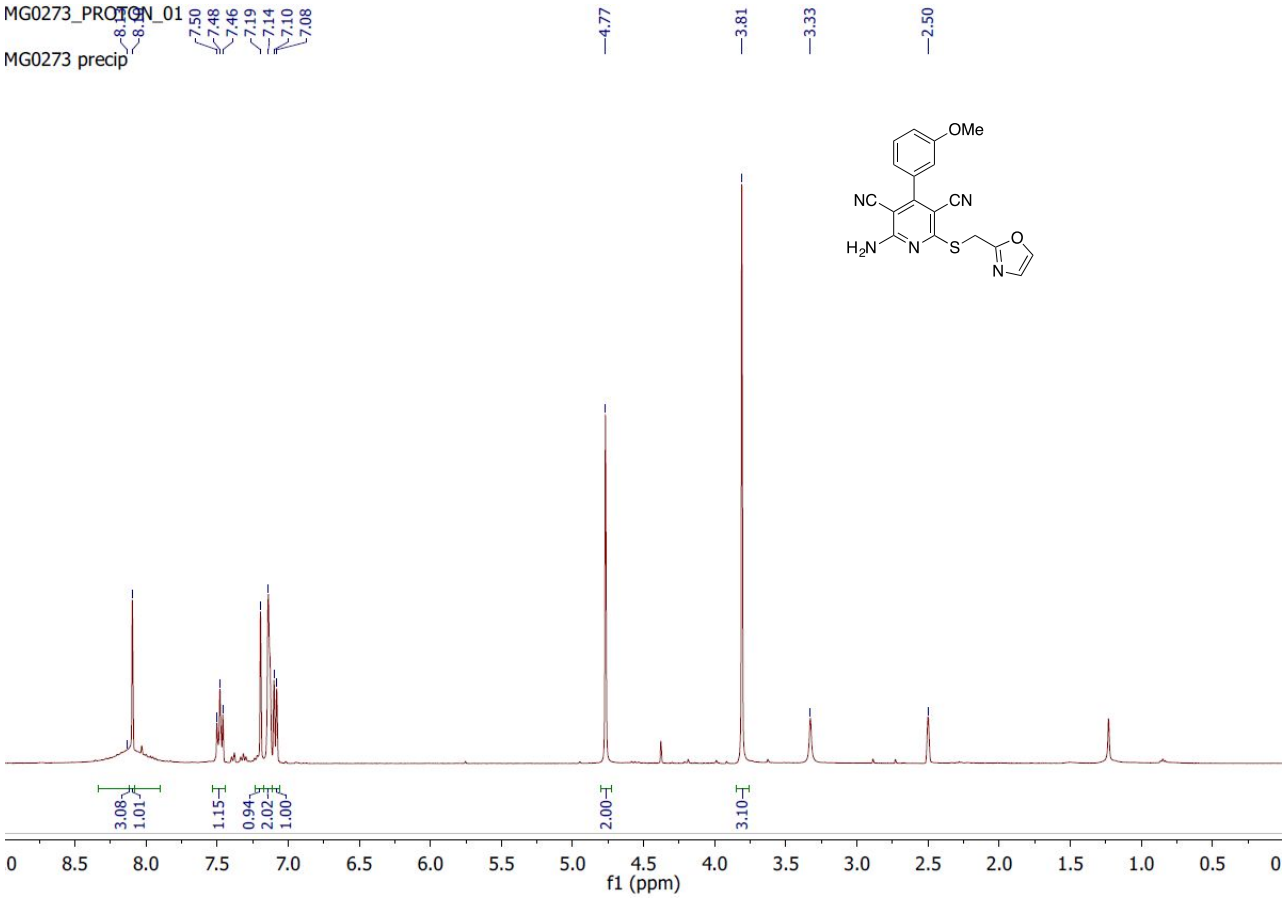
Compound 21. ^{1}H NMR (400 MHz, $CDCl_3$/d_4-MeOD): 7.67-7.60 (m, 1H), 7.43-7.35 (m, 1H), 7.35-7.29 (m, 1H), 7.07-6.97 (m, 2H), 6.94 (s, 1H), 4.74 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$/d_4-MeOD):

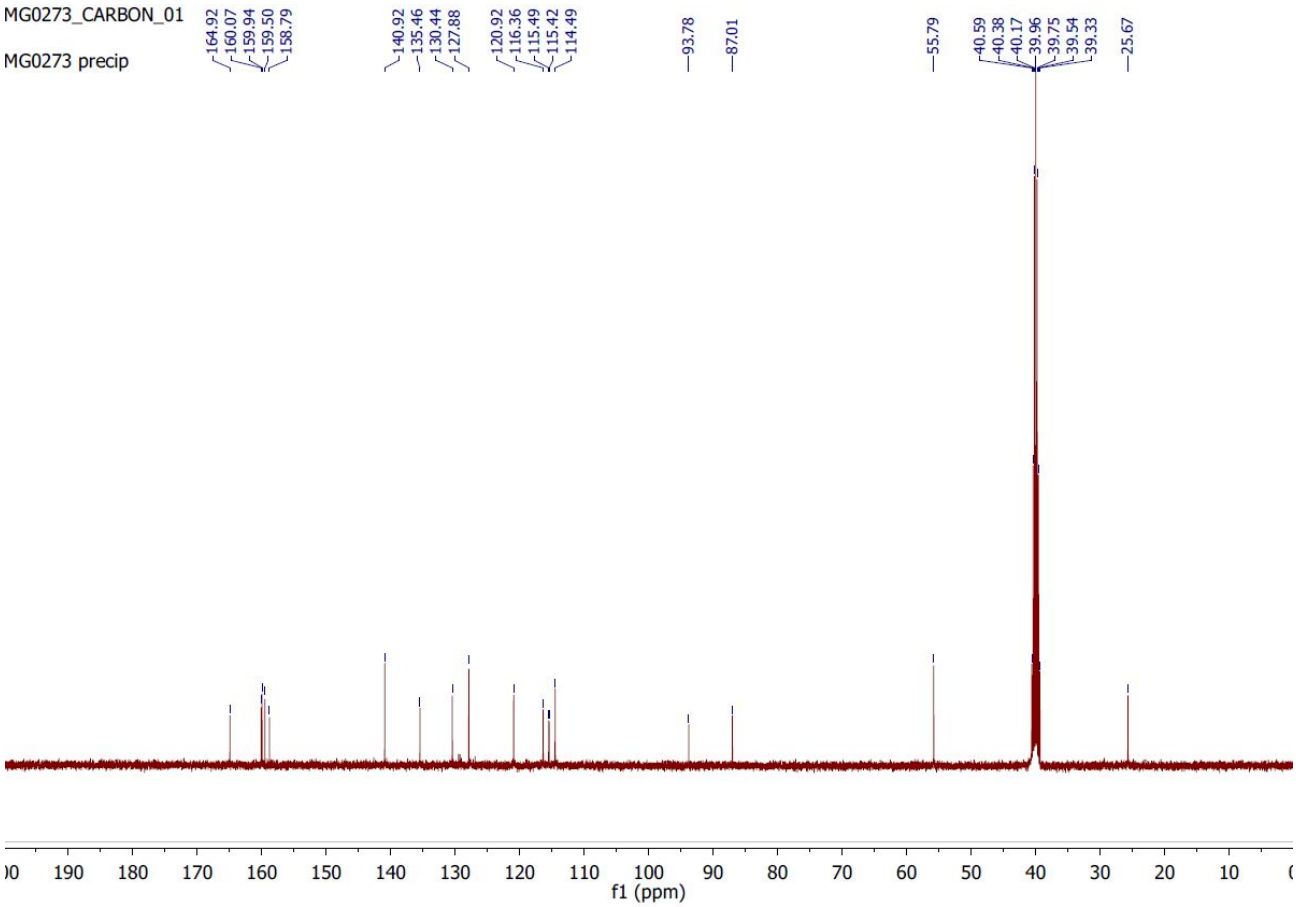
168.2, 166.0, 159.8, 159.6, 158.4, 141.5, 134.4, 130.1, 120.6, 120.5, 116.6, 115.1, 114.7, 113.6, 94.8, 86.8, 55.3, 30.9. MS (ESI+): *m/z* calcd. for $C_{18}H_{14}N_5OS_2$ $[M+1]^+$ 380.0562, found 380.0569.



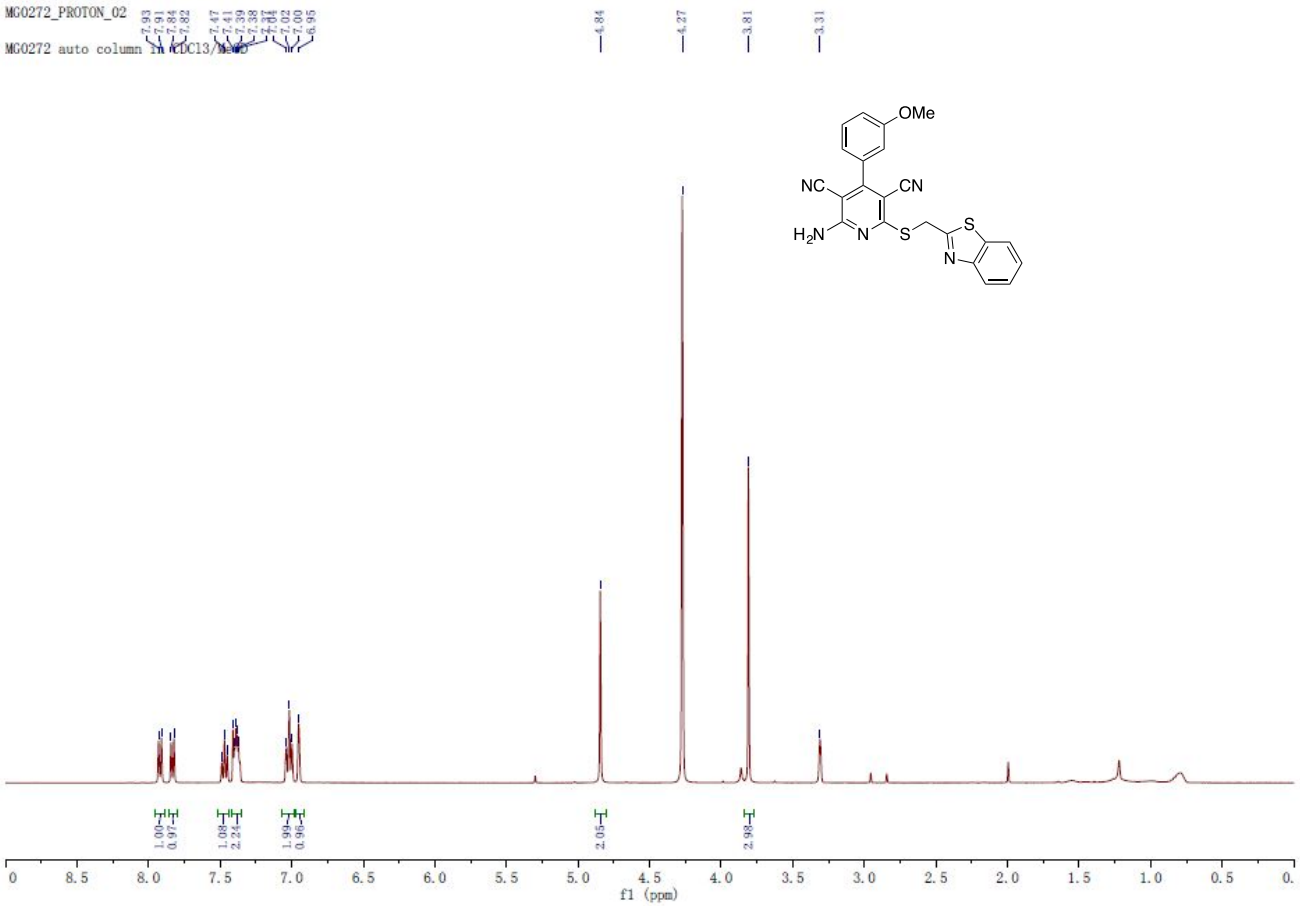

MG0285_CARBON_01
MG0285 auto column
168.15
166.04
159.80
159.56
158.43
141.54
134.38
130.06
120.64
120.54
116.63
115.08
114.71
113.63
94.82
86.81
77.48
77.16
76.84
55.30
49.21
49.00
48.78
48.57
48.35
30.94
f1 (ppm)

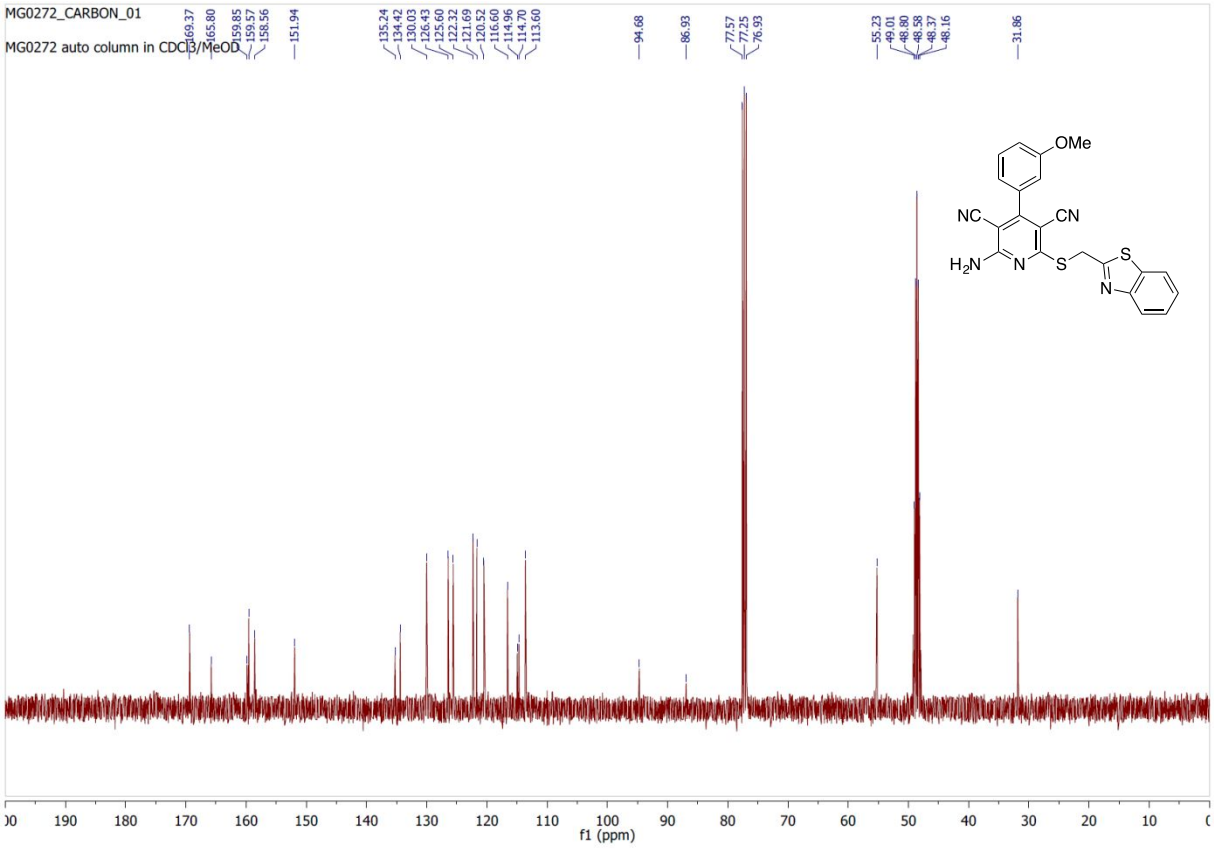
Compound 22.[1]H NMR (400 MHz, d_6-DMSO): 8.13 (brs, 2H), 8.10 (s, 1H), 7.52-7.44 (m, 1H), 7.19 (s, 1H), 7.16-7.11 (m, 2H), 7.11-7.06 (m, 1H), 4.77 (s, 2H), 3.81 (s, 3H). [13]C NMR (125 MHz, d_6-DMSO): 164.9, 160.1, 159.9, 159.5, 158.8, 140.9, 135.5, 130.4, 127.9, 120.9, 116.4, 115.5, 115.4, 114.5, 93.8, 87.0, 55.8, 25.7. MS (ESI+): *m*/*z* calcd. for $C_{18}H_{14}N_5O_2S$ $[M+1]^+$ 364.0790, found 364.0793.




MG0273_CARBON_01
MG0273 precip
164.92
160.07
159.94
159.50
158.79
140.92
135.46
130.44
127.88
120.92
116.36
115.49
115.42
114.49
93.78
87.01
55.79
40.59
40.38
40.17
39.96
39.75
39.54
39.33
25.67
f1 (ppm)

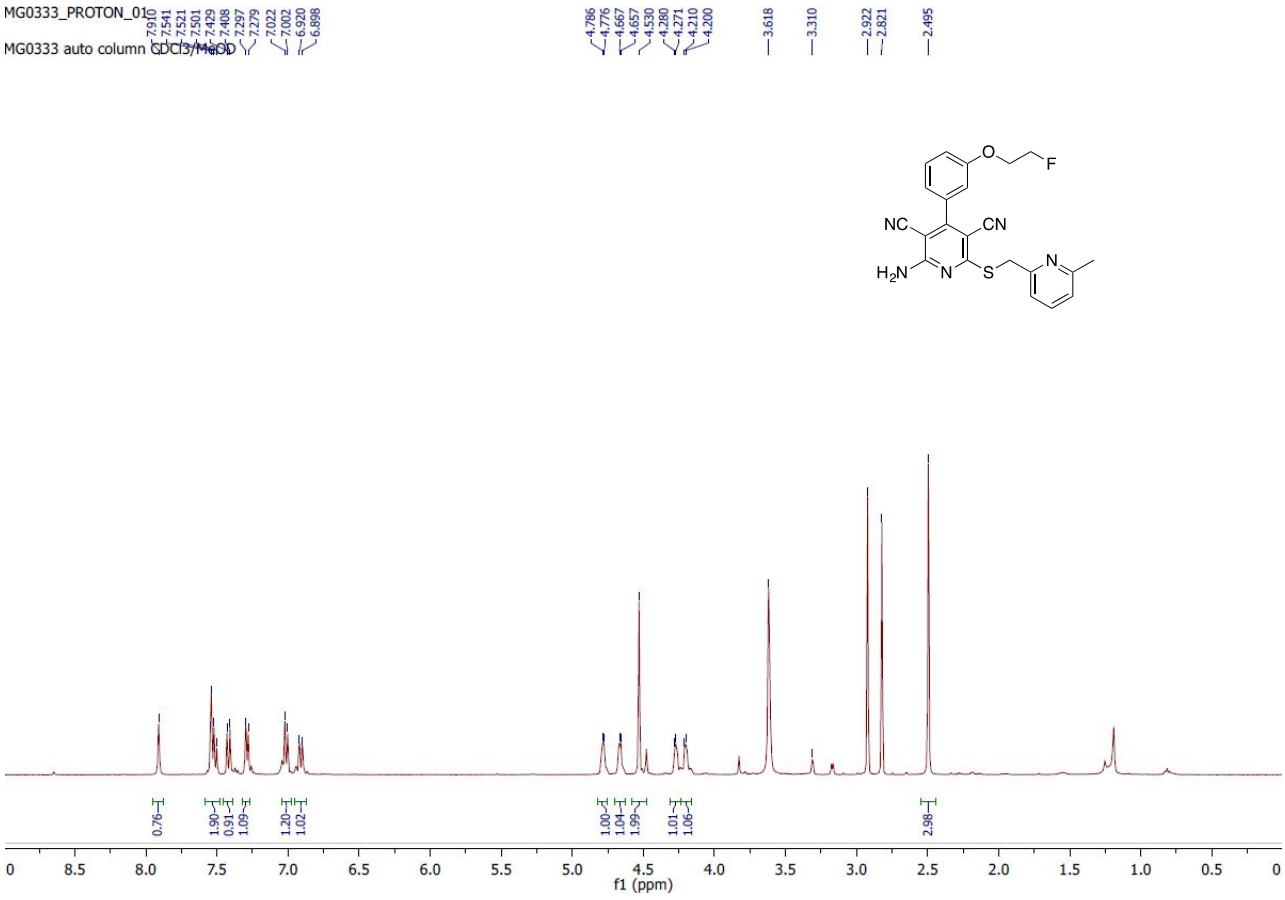
Compound 23. ^{1}H NMR (400 MHz, $CDCl_3$/d_4-MeOD): 7.92 (d, J = 8.1 Hz, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.42-7.34 (m, 2H), 7.02 (t, J = 7.7 Hz, 2H), 6.96 (s, 1H), 4.84 (s, 2H), 3.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$/d_4-MeOD): 169.4, 165.8, 159.8, 159.6, 158.6, 151.9, 135.2, 134.4, 130.0, 126.4, 125.6, 122.3, 121.7, 120.5, 116.6, 115.0, 114.7, 113.6, 94.7, 86.9, 55.2, 31.9. MS (ESI+): m/z calcd. for $C_{22}H_{16}N_5OS_2$ $[M+1]^+$ 430.0718, found 430.0722.

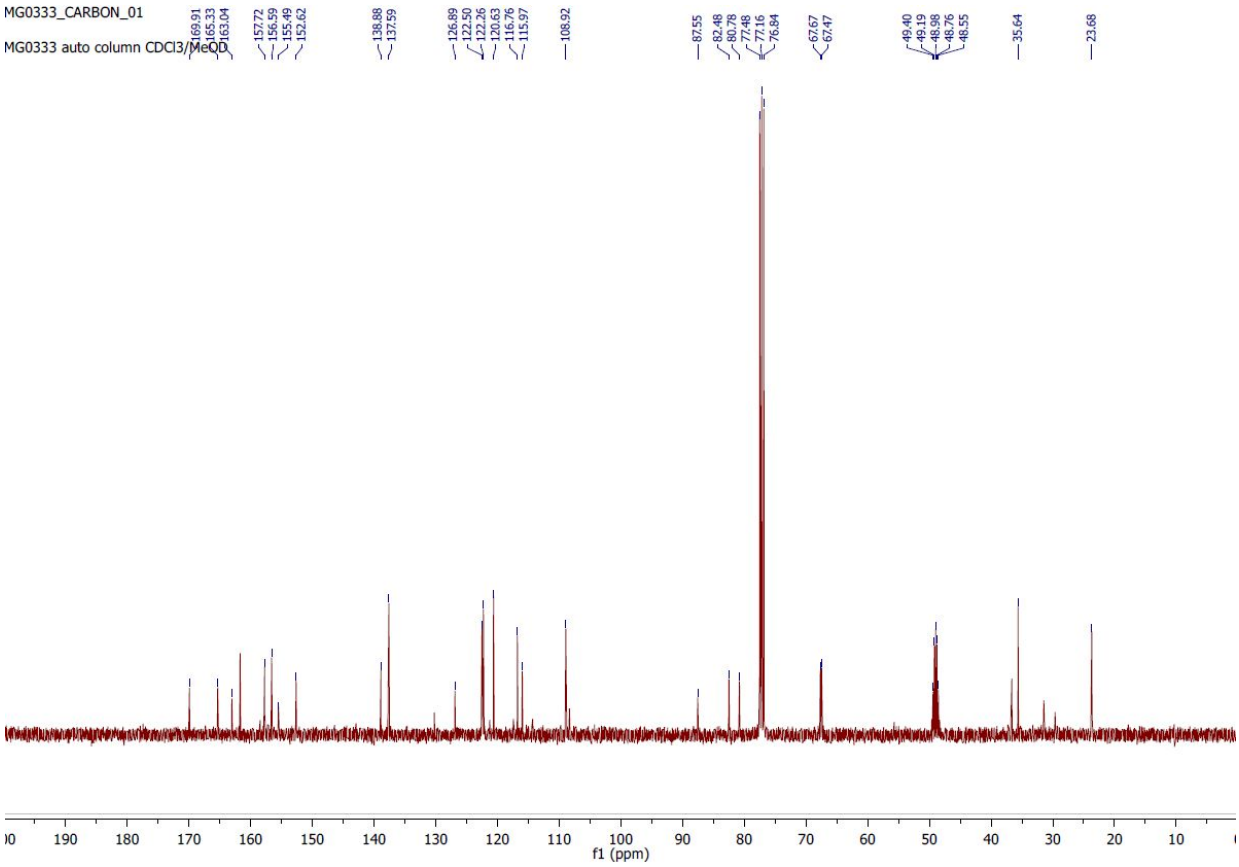




Compound 25. ^{1}H NMR (400 MHz, $CDCl_3$/d_4-MeOD): 7.91 (s, 1H), 7.58-7.48 (m, 2H), 7.45-7.38 (m, 1H), 7.32-7.26 (m, 1H), 7.04-6.98 (m, 1H), 6.94-6.86 (m, 1H), 4.82-4.74 (m, 1H), 4.71-4.62 (m, 1H), 4.53 (m,

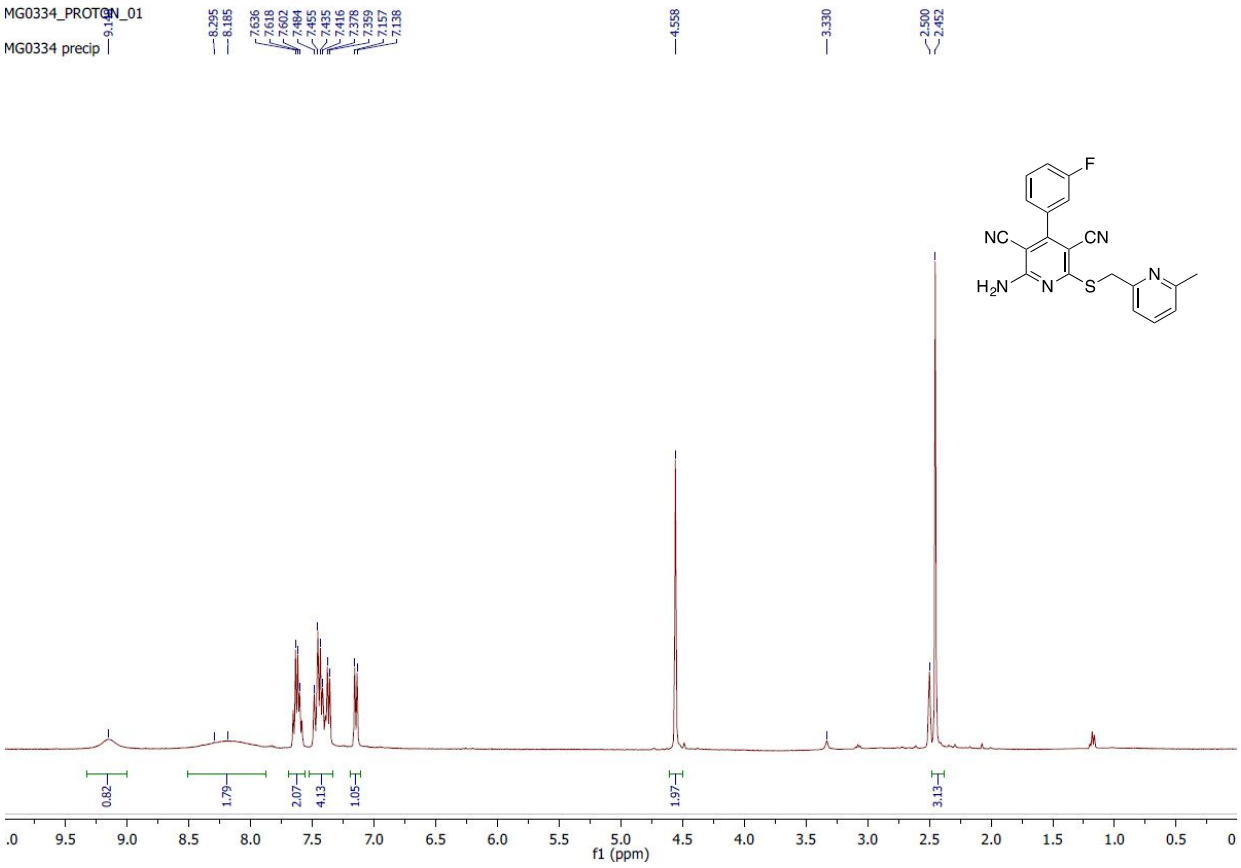
2H), 4.31-4.24 (m, 1H), 4.24-4.16 (m, 1H), 2.49 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$/d_4-MeOD): 169.9, 165.3, 163.0, 157.7, 156.6, 155.5, 152.6, 138.9, 137.6, 126.9, 122.4, 122.2, 120.6, 116.8, 116.0, 108.9, 87.6, 82.5, 80.8, 67.5 (d, J = 20.3 Hz), 35.6, 23.6. MS (ESI+): m/z calcd. for $C_{22}H_{19}FN_5OS$ $[M+1]^+$ 420.1216, found 420.1221.





Compound 26. ^{1}H NMR (400 MHz, d_6-DMSO): 9.15 (brs, 1H), 8.18 (brs, 2H), 7.67-7.56 (m, 2H), 7.51-7.34 (m, 4H), 7.18-7.10 (m, 1H), 4,56 (s, 2H), 2.45 (s, 3H). ^{19}F NMR (376 MHz, d_6-DMSO): -112.2 (dd, *J*

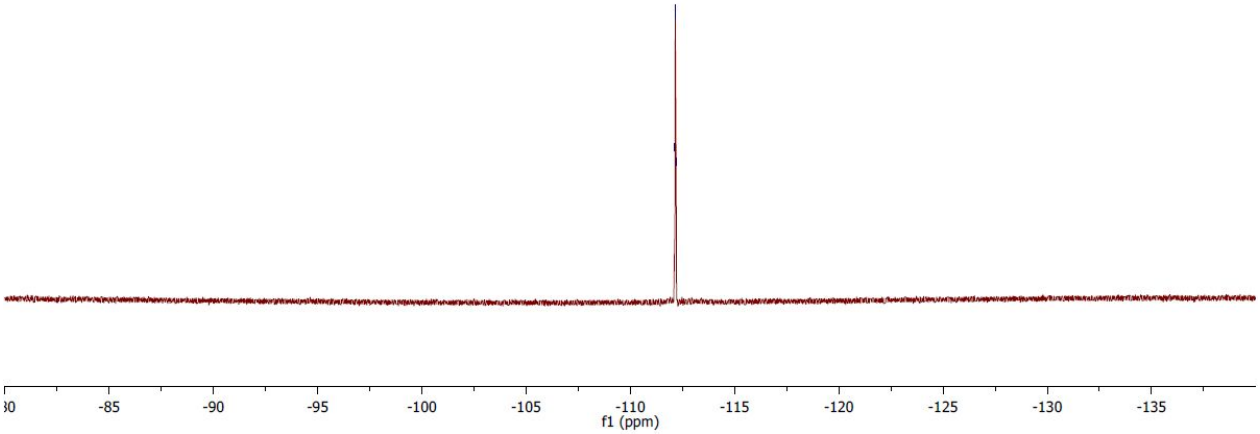
= 15.4, 9.1 Hz). ^{13}C NMR (125 MHz, d_6-DMSO): 166.6, 163.3, 160.1, 159.8, 158.2, 157.4, 156.1, 137.5, 136.5 (d, J = 8.5 Hz), 131.4 (d, J = 8.4 Hz), 125.2, 122.3, 121.2, 117.6 (d, J = 20.7 Hz), 116.0 (d, J = 23.5 Hz), 115.4 (d, J = 8.9 Hz), 93.6, 86.4, 35.8, 24.4. MS (ESI+): m/z calcd. for $C_{20}H_{15}FN_5S$ $[M+1]^+$ 376.0954, found 376.0957.



MG0334_FLUORINE_01

MG0334 precip

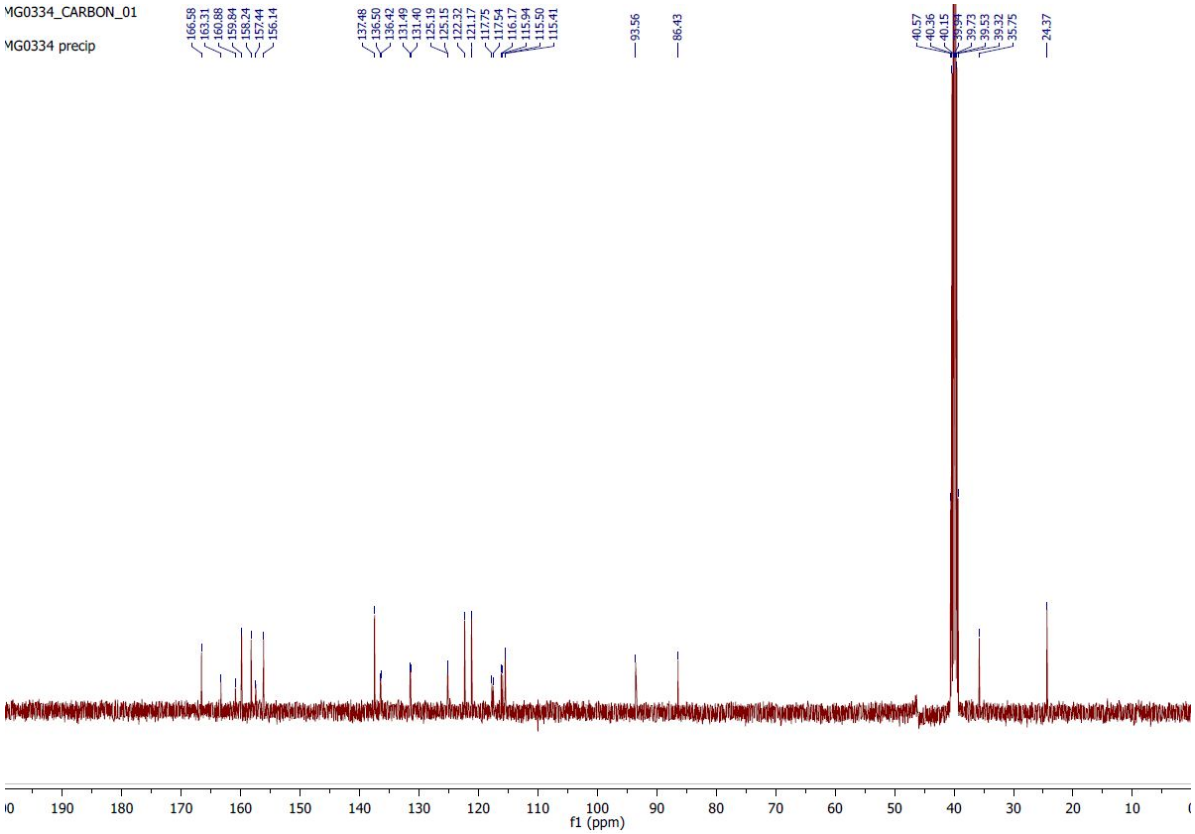
112.13
112.16
112.17
112.20



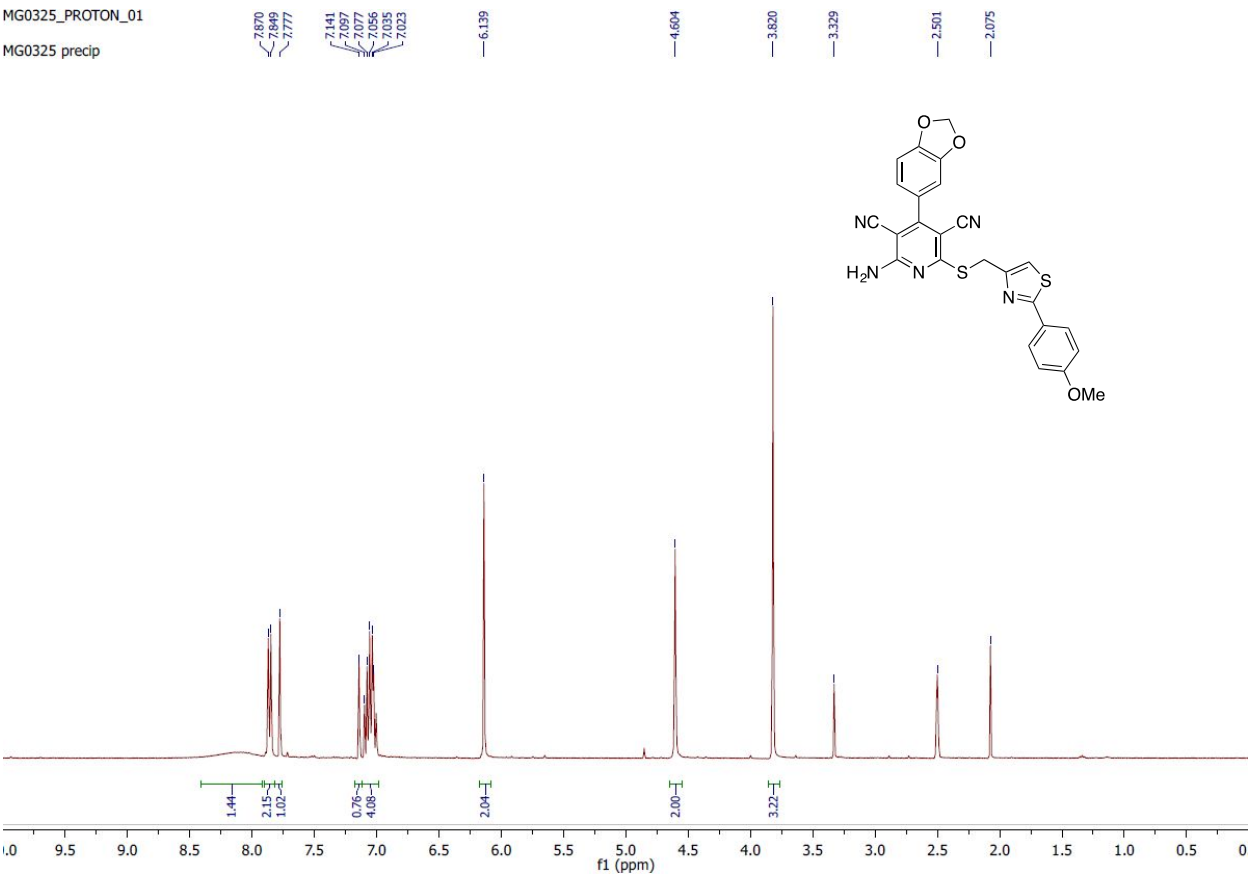
MG0334_CARBON_01

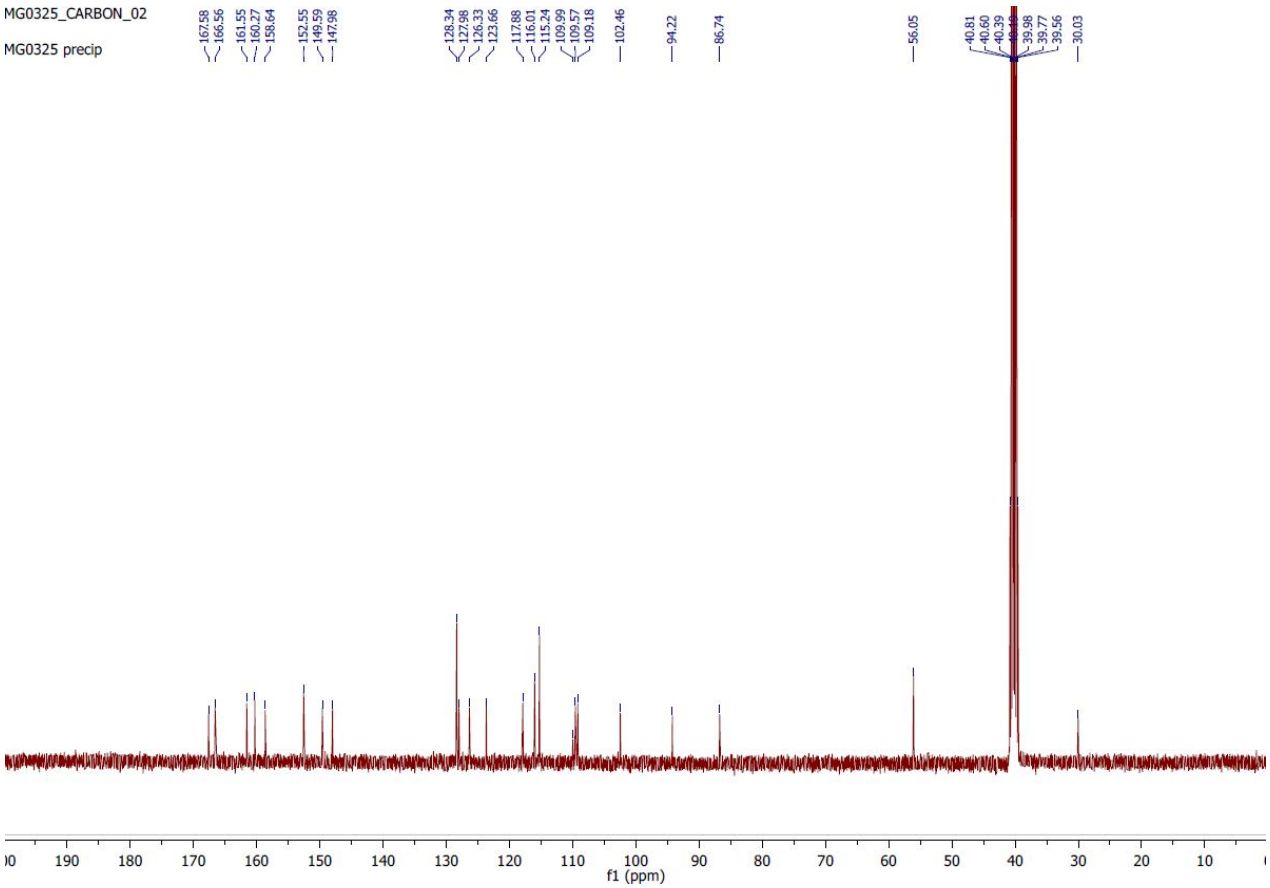
MG0334 precip

166.58
163.31
160.88
159.84
158.24
157.44
156.14
137.48
136.50
136.42
131.49
131.40
125.19
125.15
122.32
121.17
117.75
117.54
116.17
115.94
115.50
115.41
93.56
86.43
40.57
40.36
40.15
39.94
39.73
39.53
39.32
35.75
24.37

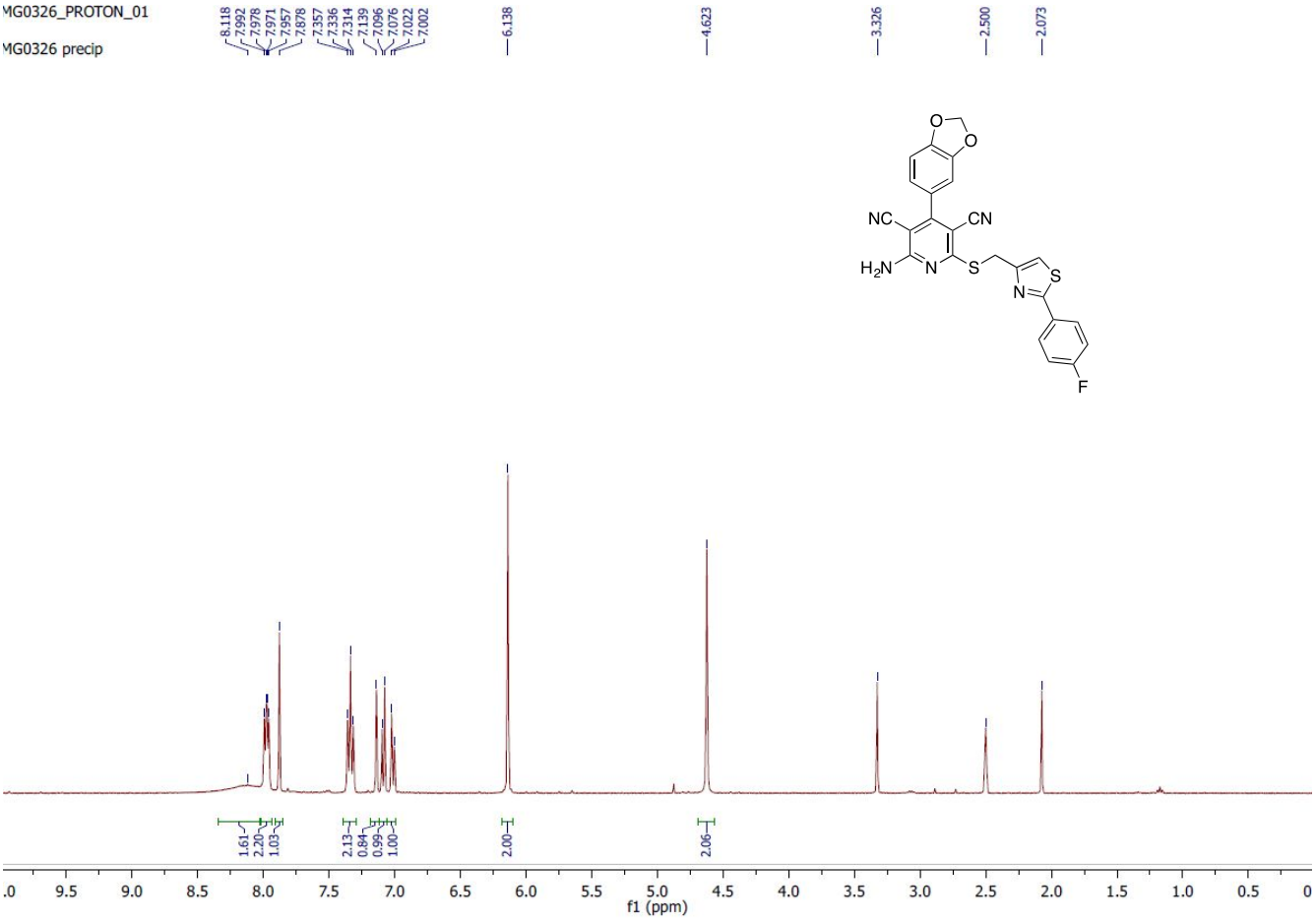


Compound 27. ^{1}H NMR (400 MHz, d_6-DMSO): 8.13 (brs, 2H), 7.90-7.82 (m, 2H), 7.78 (s, 1H), 7.14 (s, 1H), 7.12-6.99 (m, 4H), 6.14 (s, 2H), 4.60 (s, 2H), 3.82 (s, 3H). ^{13}C NMR (125 MHz, d_6-DMSO): 167.6, 166.6, 161.6, 160.3, 158.6, 152.6, 149.6, 148.0, 128.3, 128.0, 126.3, 123.7, 117.9, 116.0, 115.2, 110.0, 109.6, 109.2, 102.5, 94.2, 86.7, 56.0, 30.0. MS (ESI+): *m/z* calcd. for $C_{25}H_{18}N_5O_3S_2$ $[M+1]^+$ 500.0773, found 500.0778.



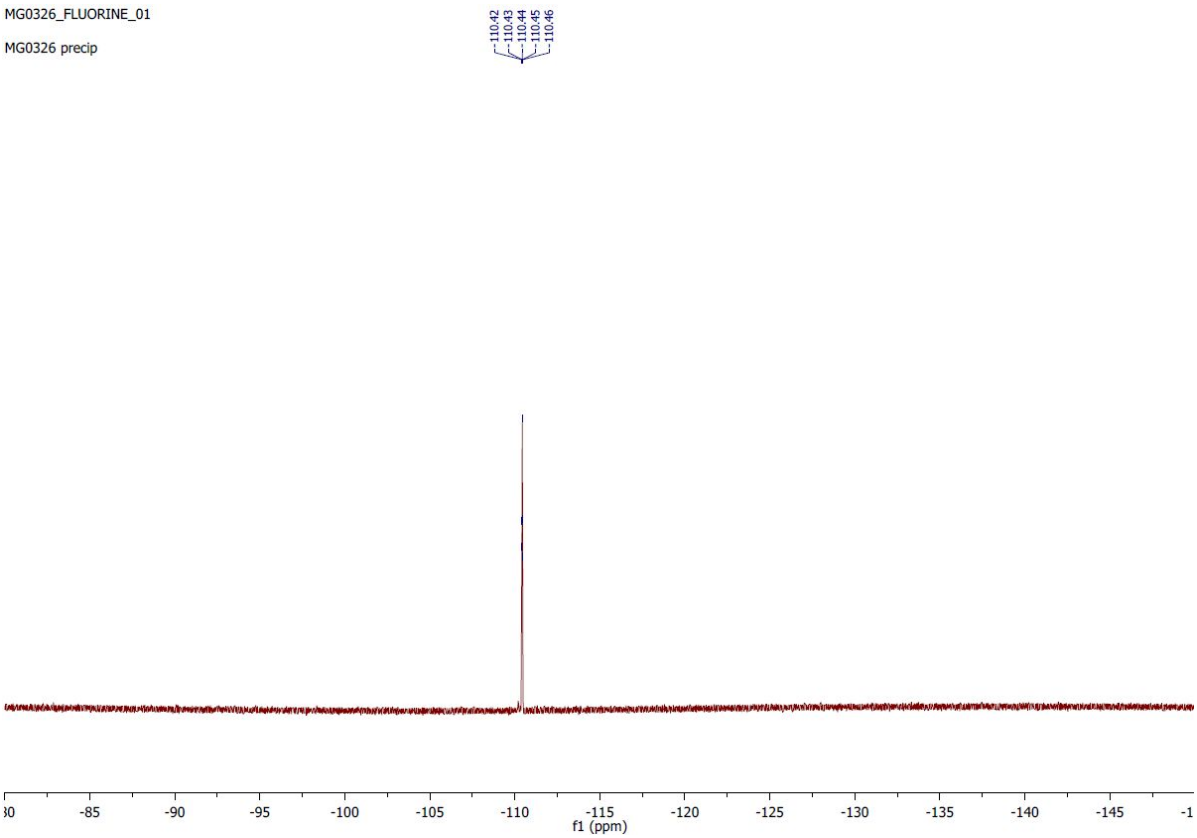

MG0325_CARBON_02
MG0325 precip
167.58
166.56
161.55
160.27
158.64
152.55
149.59
147.98
128.34
127.98
126.33
123.66
117.88
116.01
115.24
109.99
109.57
109.18
102.46
94.22
86.74
56.05
40.81
40.60
40.39
39.98
39.77
39.56
30.03
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

Compound 28. ^{1}H NMR (400 MHz, d_6-DMSO): 8.12 (brs, 2H), 8.01-7.93 (m, 2H), 7.88 (s, 1H), 7.38-7.29 (m, 2H), 7.14 (s, 1H), 7.11-7.06 (m, 1H), 7.04-6.98 (m, 1H), 6.14 (s, 2H), 4.62 (s, 2H). ^{19}F NMR (376 MHz, d_6-DMSO): -110.4 (m). ^{13}C NMR (125 MHz, d_6-DMSO): 166.4, 165.0, 162.6, 160.2, 158.6, 152.9, 149.6, 147.9, 130.2, 129.0 (d, J = 8.6 Hz), 127.9, 123.6, 119.1, 116.9 (d, J = 22.1 Hz), 116.0, 109.5, 109.1, 102.4, 94.2, 86.7, 29.9. MS (ESI+): m/z calcd. for $C_{24}H_{15}FN_5O_2S_2$ $[M+1]^+$ 488.0573, found 488.0578.



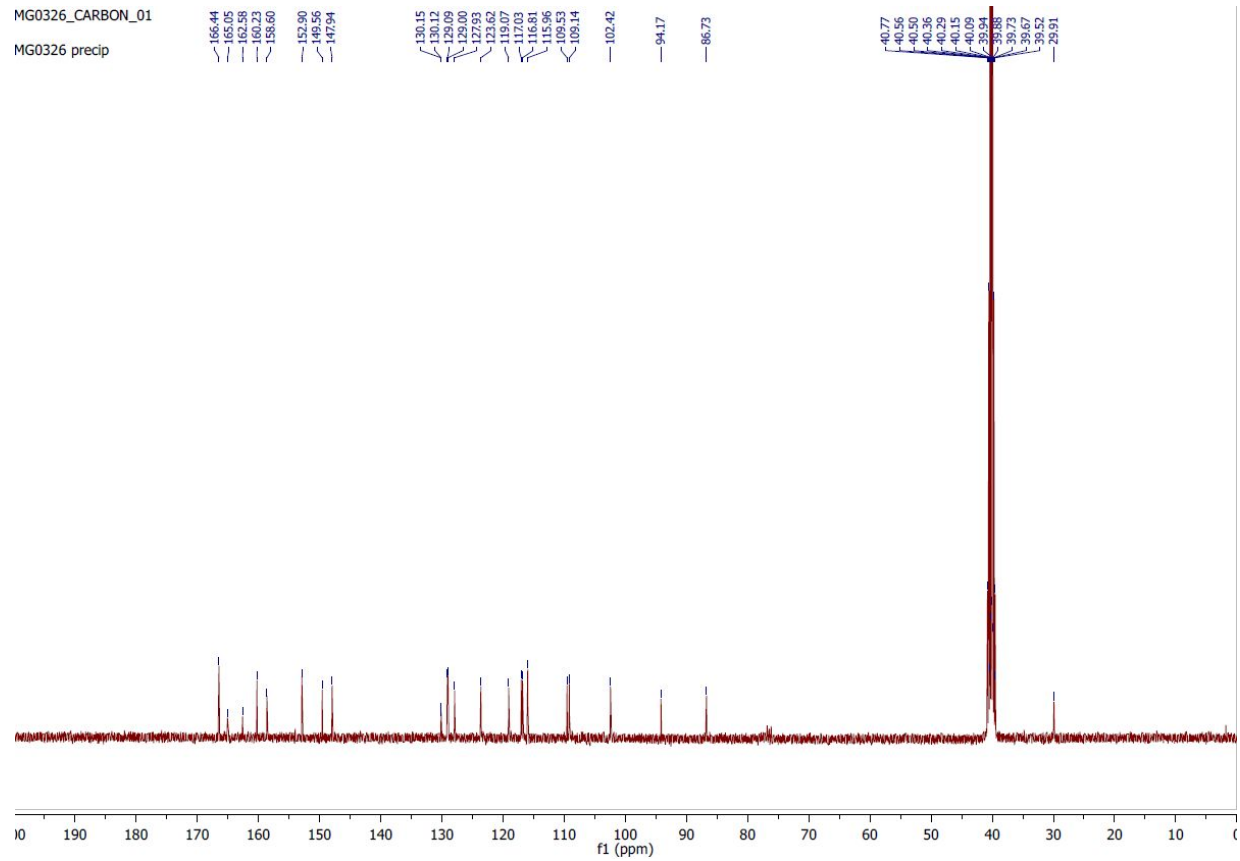
MG0326_FLUORINE_01

MG0326 precip

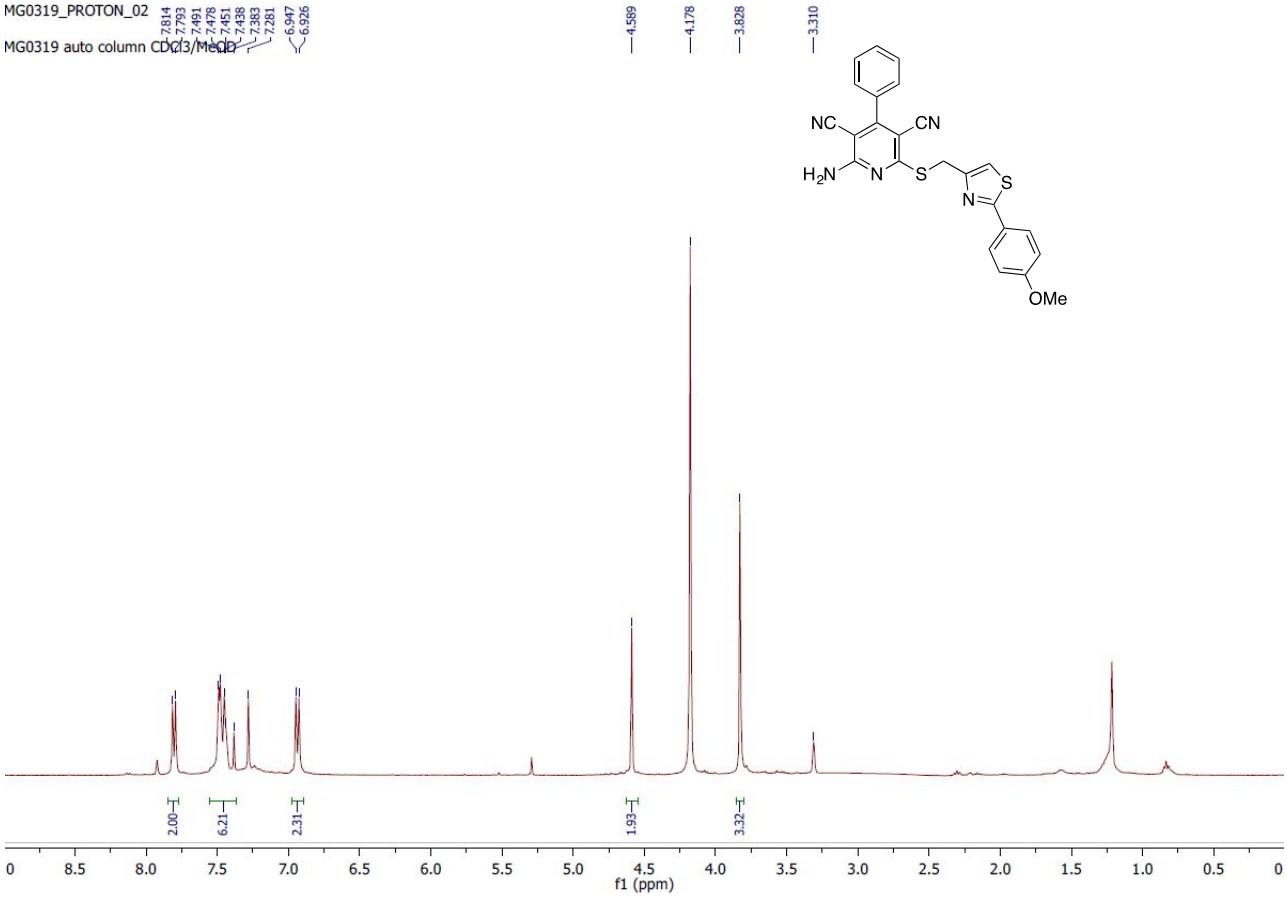


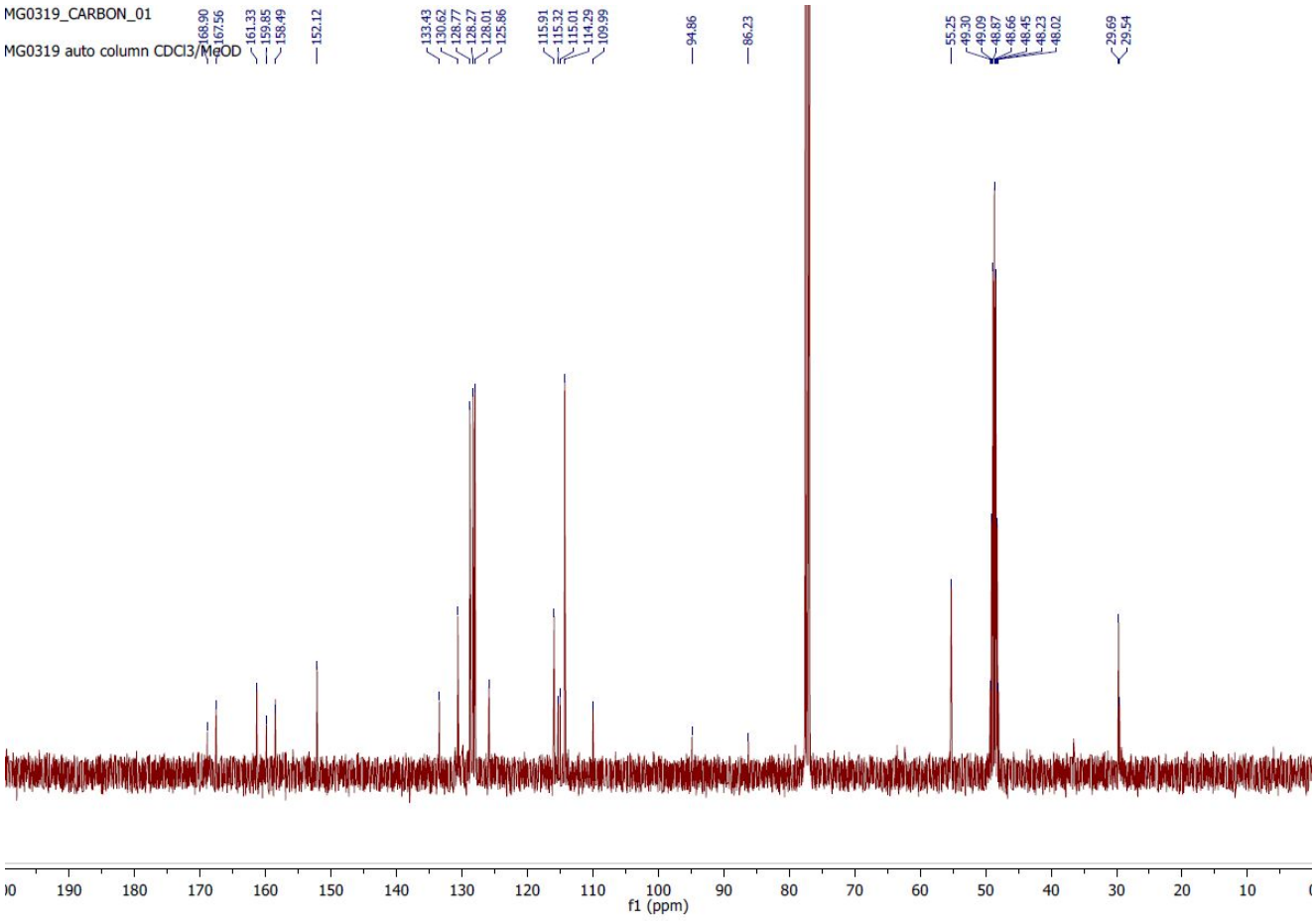
MG0326_CARBON_01

MG0326 precip



Compound 29. ^{1}H NMR (400 MHz, $CDCl_3$/d_4-MeOD): 7.80 (d, J = 8.6 Hz, 2H), 7.59-7.34 (m, 6H), 6.94 (d, J = 8.6 Hz, 2H), 4.59 (s, 2H), 3.83 (s, 3H).). ^{13}C NMR (125 MHz, $CDCl_3$/d_4-MeOD): 168.9, 167.6, 161.3, 159.8, 158.5, 152.1, 133.4, 130.6, 128.8, 128.3, 128.0, 125.9, 115.9, 115.3, 115.0, 114.3, 110.0, 94.9, 86.2, 55.2, 29.7. MS (ESI+): m/z calcd. for $C_{24}H_{18}N_5OS_2$ $[M+1]^+$ 456.0875, found 456.0878.




MG0319_CARBON_01
MG0319 auto column CDCl3/MeOD
168.90
167.56
161.33
159.85
158.49
152.12
133.43
130.62
128.77
128.27
128.01
125.86
115.91
115.32
115.01
114.29
109.99
94.86
86.23
55.25
49.30
49.09
48.87
48.66
48.45
48.23
48.02
29.69
29.54
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

Radiochemistry

No-carrier-added (n.c.a.) [^{11}C]CO_2 was generated by bombarding a N_2/1% O_2 gas target with a proton beam (16 MeV, 45 μA × 45 min or 45 μA × 45 min) from a cyclotron (PETrace, GE) via nuclear reaction $^{14}N(p,\alpha)^{11}C$. All radiochemical reactions including conversion of [^{11}C]CO_2 to [^{11}C]CH_3I, and [^{11}C]methylation were performed in a lead-shielded hot cell by using FX-MeI and FX-M automated synthesizers (GE Healthcare, USA), respectively, controlled by TRACERlab software.

Preparation of [^{11}C]27 and [^{11}C]29. A solution of precursor (0.8-1.0 mg ± 0.1 mg) was suspended in 200 μL anhydrous MeCN and 2.5 μL of tetrabutylammonium hydroxide (1 M in methanol) in was added through?? the reaction flask wall. The mixture was then vortexed for 1 min that led to formation of a clear yellow solution. The mixture was allowed to react with [^{11}C]CH_3OTf (trapped at 0 °C) in a stream of helium at 80 °C for 3 min before being injected into a semi-preparative HPLC column (Phenomenex Onyx™ Monolithic Semi-PREP C18, LC Column 100 x 10 mm). The mixture was eluted at 5 mL/min with an isocratic mixture of 60% solvent A (90% 0.01M phosphate buffer, 10% EtOH pH = 7.2-7.4) and 40% solvent B (100% EtOH) and monitored for absorbance at 280 nm and radioactivity using the flow count detector (NaI(Tl)) built into the FX-M. The product [^{11}C]**27** was collected between 9.8 and 10.2 min ([^{11}C]**27**), while the product [^{11}C]**29** was collected between 11.6 and 12.3 min (Figure S2), and radioactivity was measured by a dose calibrator (Capintec, CRC 712M) to determine radiochemical yield (RCY). RCY for [^{11}C]**27** 22.2 ± 5.8 %, n = 6) and molar activity (832 ± 411 GBq/μmol @ EOB, n = 6). The collected product solution was formulated with 2.5 mL of sterile water (pH ≈ 7.2) for rodent PET studies. RCY for [^{11}C]**29** 24.3 ± 6.7 %, n = 5) and molar activity (1195 ± 492 GBq/μmol @ EOB, n = 5). The collected product (1 mL) solution was formulated with 3.0 mL of sterile water (pH ≈ 7.2; final

ethanol content ≤ 10%) for rodent PET studies. Radiochemical synthesis data obtained from the Tracerlab FXM for compounds **[^{11}C]27** and **[^{11}C]29** are shown in Figures S1–S2.

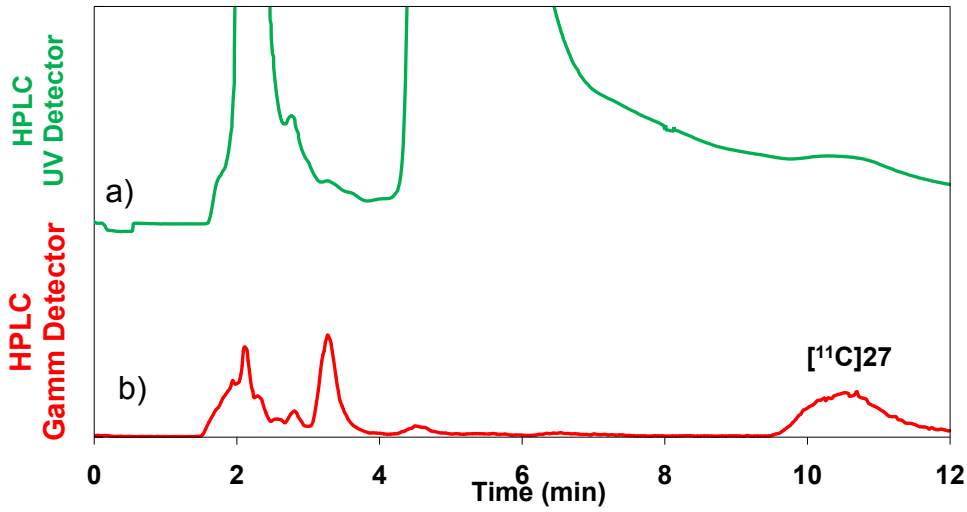


Figure S1. Semi-preparative HPLC profile of **[^{11}C]27**. (Top: radio chromatogram, bottom: UV chromatogram recorded at 280 nm).

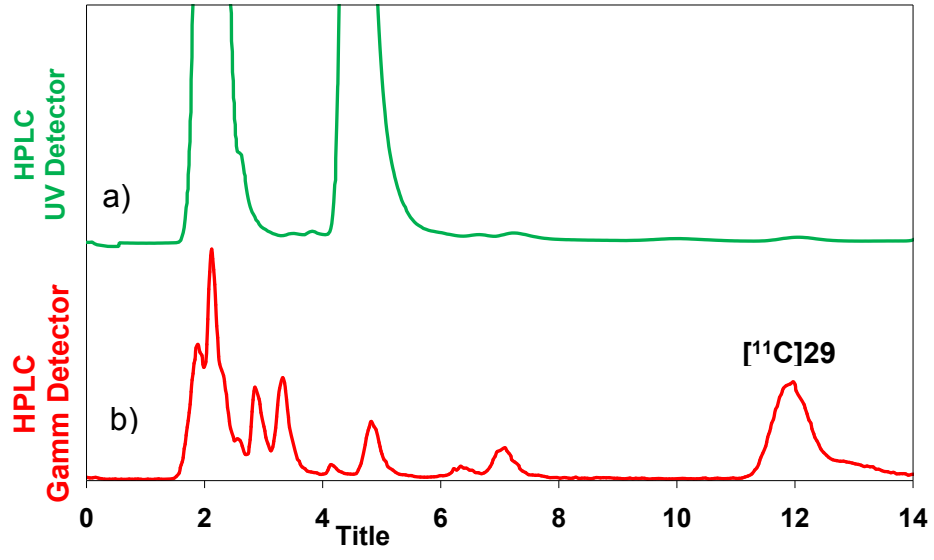


Figure S2. Semi-preparative HPLC profile of **[^{11}C]29**. (Top: radio chromatogram, bottom: UV chromatogram recorded at 280 nm).

Analysis of [^{11}C]27 with Analytical HPLC. Analytical HPLC analysis was performed for quality control on an Agilent 1200 system monitoring for absorbance at 280 nm and radioactivity using a flow count radioactivity detector (Berthold LB 514 with BGO-X 30 µL flow cell). A fraction of **[^{11}C]27** was co-injected with cold reference compound to a reverse phase column (Chromolith® SpeedROD RP-18 endcapped 50-4.6 HPLC column) and eluted at a flow rate of 1.0 mL/min with an isocratic mixture of 60% solvent A (90% 0.01M phosphate buffer, 10% EtOH pH = 7.2-7.4) and 40% solvent B (100% EtOH). The HPLC profile (Figure S3) showed high radiochemical purity (>99%).

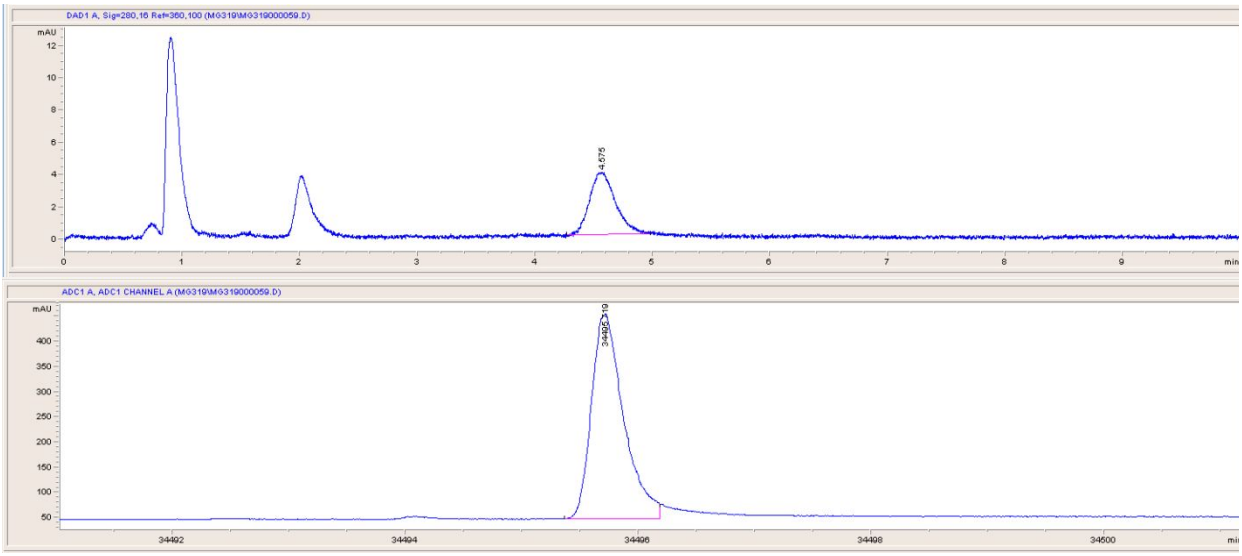


Figure S3. Analytical HPLC profile of **[^{11}C]27**. (Top: UV chromatogram recorded at 280 nm, retention time: 4.575 min; bottom: Radio chromatogram).

Analysis of [^{11}C]29 with Analytical HPLC. Analytical HPLC analysis was performed for quality control on an Agilent 1200 system monitoring for absorbance at 280 nm and radioactivity using a flow count radioactivity detector (Berthold LB 514 with BGO-X 30 µL flow cell). A fraction of **[^{11}C]29** was co-injected with cold reference compound to a reverse phase column (Chromolith® SpeedROD RP-18 endcapped 50-4.6 HPLC column) and eluted at a flow rate of 1.0 mL/min with

an isocratic mixture of 62% solvent A (90% 0.01M phosphate buffer, 10% EtOH pH = 7.2-7.4) and 38% solvent B (100% EtOH). The HPLC profile (Figure S4) showed high radiochemical purity (>99%).

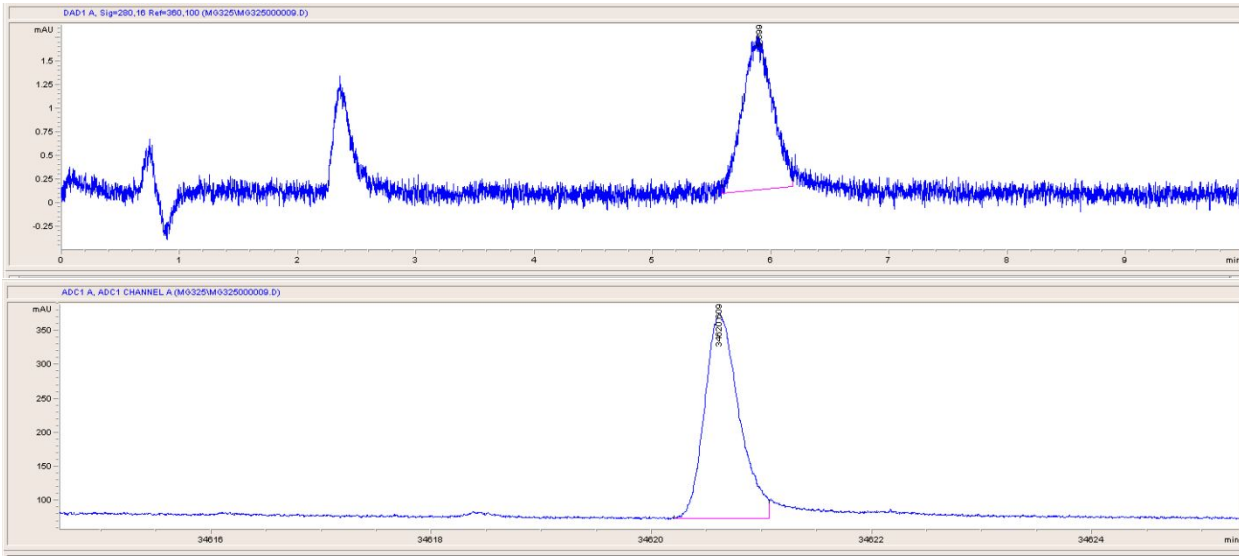


Figure S4. Analytical HPLC profile of **[^{11}C]29**. (Top: UV chromatogram recorded at 280 nm, retention time: 5.889 min; bottom: Radio chromatogram).

5. Small Animal PET Studies

Table 1. Whole Brain biodistribution data from KO and control mice 15 min post IV injection of [^{11}C]**27**.

	tKO (n=3)	MDR1a/1a-KO (n=2)	BCPR-KO (n=2)	MRP1-KO (n=3)	Control (n=3)
SUV (avg±SD)	0.165±0.026	0.289±0.329	0.078±0.013	0.225±0.291	0.037±0.006

All animal handling and experiments were approved by the Animal Care & Use Committee at the National Institutes of Health. Male Wistar rats (n=4, 250-400g) and FVB/NTac Swiss mice (n=13, 30-40 g) were purchased from Charles River Laboratories (Frederick, MD) and Taconic Biosciences, Inc. (Derwood, MD), respectively. Four mouse models with targeted knockout (KO) mutations of the multi-drug resistance genes were bred in house: MDR1a/1a-KO (n=2), BCPR-KO (n=2), MRP1-KO (n=3), and triple-KO (tKO) of p-glycoprotein 3, p-glycoprotein 1, and BCRP/ABCG2 (n=3). Animals were housed with wood chip bedding under a reverse 12 h/12 h light/dark cycle. Food and water were provided ad libitum.

For PET studies, rats were anesthetized under isoflurane (Forane, 99.9%; 5.0% induction for 5 min, 1.0-2.5% maintenance) prior to catheter placement and for the duration of scanning. A catheter was inserted into the penile vein for radiotracer injection. Subjects were placed prone position side-by-side into a Siemens microPET Focus 220 scanner. Vitals (heart rate, respiratory rate, spO2, temperature) were monitored using a PhysioSuite (Kent Scientific #: PS-04). Temperature was maintained close to 36 °C with a homeothermic blanket with negative feedback control (Harvard Apparatus #: 507222F). A 10 min transmission scan with a Co-57 point source was collected for attenuation correction prior to 90 min emission scans. Radiotracer was injected as a bolus over 1 minute using a syringe pump (Harvard Apparatus #: HA1100WD) and

immediately flushed with 250 uL heparinized saline. Injected dose was 16.6 ± 3.2 MBq. For the [^{11}C]29 blocking study, an intraperitoneal injection of DPCPX (2.0 mg/kg, 550μL), a selective A1R agonist, was administered 5 mins prior to radiotracer injection. PET data was collected in list mode and reconstructed into 23 frames (6 x 20 sec, 5 x 60 sec, 4 x 120 sec, 3 x 300 sec, 3 x 600 sec, and 2 x 1200 sec), and sinogram reconstruction was performed using 2D Filtered Back Projection. Time-activity curves were generated in PMOD (version 3.807) and normalized to subject mass and injected activity and represented as standard uptake value (SUV).

For *ex vivo* studies, all mice were placed under anesthesia as described above. Catheters were constructed using 20cm BPTE-10 Polyethlyene tubing (Instech Las©), Sharp Tip Needles (27 GA, Becton Dickinson©), and Blunt Tip Needles (30 GA, Component Supply©). Catheters were flushed with heparinized saline (0.6% Heparin, 0.9% HCl Saline) and placed in the tail vein. [^{11}C]27 (2.63 ± 1.54 MBq, 100μL) was administered intravenously and immediately flushed with heparinized saline (100 μL). Animals were euthanized 15 minutes post injection via decapitation. Whole brain tissue and ventricular whole blood were extracted, stored in pre-weighed glass vials, and placed in an automatic well-type gamma counter (Wallac Wizard 3″; Perkin Elmer) to measure radioactivity. Vials were post-weighed to obtain tissue mass, and SUV was generated using total injected activity and subject weight.

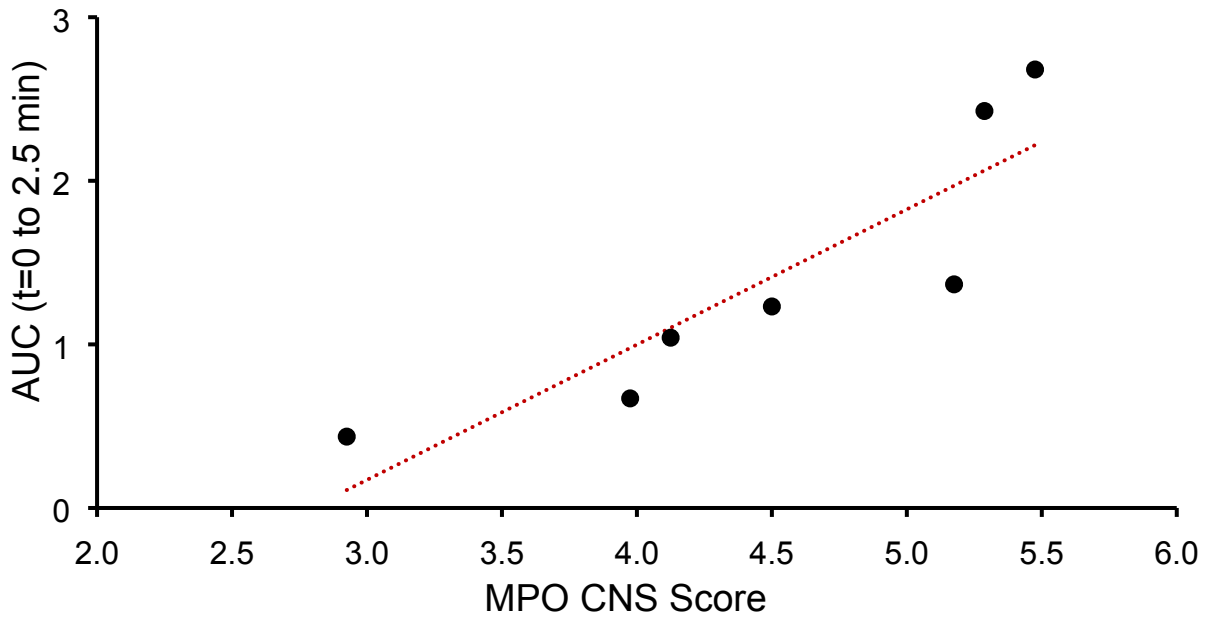


Figure S5. Correlation of whole brain uptake area under the curve (AUC) vs. MPO CNS score of [^{11}C] labeled 3,5-dicyanopyridine derivatives synthesized in our laboratory. AUC was calculated from time-activity curves presented as standard uptake value (SUV) using the trapezoidal sum from 0-2.5 min. Linear regression calculated using sum of least squares residuals ($R^2 = 0.780$).

6. References

1) Beukers, M. W., Chang, L. C. W., von Frijtag, J. K., Künzel, D., Mulder-Krieger, T., Spanjersberg, R. F., Brussee, J., IJzerman, A. P. New, Non-Adenosine, High-Potency Agonists for the Human Adenosine A_2B Receptor with an Improved Selectivity Profile Compared to the Reference Agonist *N*-Ethylcarboxamidoadenosine. *J. Med. Chem*. **2004**, *47*, 3707.

2) Chang, L. C. W., von Frijtag Drabbe Künzel, J. K., Mulder-Krieger, T., Spanjersberg, R. F., Roerink, S. F., van den Hout, G., Beukers, M. W., Brussee, J., IJzerman, A. P. A Series of Ligands Displaying a Remarkable Agonistic−Antagonistic Profile at the Adenosine A1 Receptor. *J. Med. Chem.* **2005**, *48*, 2045.

3) Rosentreter, U., Kraemer, T., Shimada, M., Huebsch, W., Diedrichs, N., Krahn, T., Henninger, K., Stasch, J. P., Wischnat, R. Substituted 2-thio-3,5-dicyano-4-phenyl-6-aminopyridines and the use of the same. EP-1455785-B1, **2009**.

4) Guo, M., Gao, Z.-G., Tyler, R., Stodden, T., Li, Y., Ramsey, J., Zhao, W.-J., Wang, G.-J., Wiers, C. E., Fowler, J. S., Rice, K. C., Jacobson, K. A., Kim, S. W., Volkow, N. D. Preclinical Evaluation of the First Adenosine A 1 Receptor Partial Agonist Radioligand for Positron Emission Tomography Imaging. *J. Med. Chem.* **2018**, *61*, 9966.

5) Louvel, J., Guo, D., Agliardi, M., Mocking, T. A. M., Kars, R., Phát Pham, T., Xia, L., de Vries, H., Brussee, J., Heitman, L. H., IJzerman, A. P. Agonists for the Adenosine A1 Receptor with Tunable Residence Time. A Case for Nonribose 4-Amino-6-aryl-5-cyano-2-thiopyrimidines. *J. Med. Chem.* **2014**, *57*, 3213.