Synthesis and Biological Evaluation of 1,2,4-Oxadiazole linked 1,3,4-Oxadiazole Derivatives as Tubulin Binding Agents

Ravikumar Polothi[1,2], Gajendra Sinh B. Raolji,[2] Venkata Sastry K[3], Kalidasu Sheelam[1,2], Balaaraju Tuniki[4], Prashanth Thodupunuri[5]

[1]Department of Chemistry, JNT University, Hyderabad, Telangana 500082, India
[2]GVK Biosciences Private Limited, Nacharam, IDA Mallapur, Hyderabad, Telangana 500076,India
[3]C.M.R. College of Pharmacy, Medchal, Hyderabad, Telangana 501401, India
[4]Department of Chemistry, School of Science, Gitam University, Hyderabad Campus, India - 502329
[5]Organic and Bimolecular Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

(*Email:* ravipolothi@gmail.com)

SUPPORTING INFORMATION

CONTENTS

GENERAL PROCEDURES

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. ^{1}H and ^{13}C NMR spectra were recorded on Bruker, Bruker UXNMR/XWIN-NMR (400 MHz, 300 MHz) instrument. Chemical shifts (d) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an electrothermal melting point apparatus, and are uncorrected.

N'1-(3,4,5-Trimethoxybenzoyl)-4-cyano-1-benzenecarbohydrazide (7)

A mixture of 4-cyanobenzohydrazide (**5**) (10 g, 62 mmol) and 3,4,5-trimethoxybenzoyl chloride (**6**) (14.3 g, 62 mmol), in dry pyridine (50 mL), was heated under reflux for 30 minutes. On cooling, the mixture was poured onto cold water (150 mL) and stirred for 10 minutes. The separated solid was filtered, washed thoroughly with cold water and dried to afford pure compound **7**, 20.2 g with 92% yield. The products were pure enough to be used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d6): δ 3.84 (s, 3H), 3.89 (s, 6H), 7.34 (s, 2H), 7.56 (d, 2H, J = 8.22 Hz), 7.66 (d, 2H, J = 8.22 Hz), 10.23 (d, 1H, J = 9.01 Hz), 10.42 (d, 1H, J = 9.32 Hz); HRMS (ESI): *m/z* calculated for $C_{18}H_{17}N_3O_5Na$ $[M+Na]^+$ 378.1066, found 378.1057.

4-[5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl]benzonitrile (8)

Compound **7** (9 g, 25.3 mmol) was added to $POCl_3$ (30 mL) and the mixture was heated under reflux for 1 hour. On cooling, crushed ice was added cautiously and the mixture was

stirred for 30 minutes. The separated crude product was filtered, washed with water then with saturated $NaHCO_3$ solution and finally with water, dried and recrystallized from ethanol to afford compound **8,** 7.3 g, with 85% yield. ^{1}H NMR (400 MHz, DMSO-d6): δ 3.84 (s, 3H), 3.89 (s, 6H), 7.59 (s, 2H), 7.63 (d, 2H, *J* = 8.34 Hz), 8.20 (d, 2H, *J* = 8.34 Hz); HRMS (ESI): *m/z* calculated for $C_{18}H_{15}N_3O_4Na$ $[M+Na]^+$ 360.0960, found 360.0949.

(Z)-N'-Hydroxy-4-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)benzamidine (9)

A solution of 4-[5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl]benzonitrile **(8)** (6.5 g, 19.28 mmol) hydroxylamine hydrochloride (1.98 g, 28.9 mmol), aqsodium hydroxide (5 mL) and ethanol (20 mL) was stirred at room temperature for 20 minutes and the solution was concentrated. The product was suspended in hexane, refluxed for 5 minutes, cooled to room temperature, and filtered to afford pure compound **9**, 6.2 g, with 87% yield. ^{1}H NMR (400 MHz, DMSO-d6): δ3.84 (s, 3H), 3.89 (s, 6H), 5.67 (s, 2H), 7.66-7.72 (m, 4H), 8.17 (d, 2H, *J* = 8.30 Hz), 9.32 (s, 1H); HRMS (ESI): *m/z* calculated for $C_{18}H_{18}N_4O_5Na$ $[M+Na]^+$ 393.1175, found 393.1181.

2-(3,4,5-Trimethoxyphenyl)-5-(4-(5-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazol-3-yl)phenyl)-1,3,4-oxadiazole (11b)

196 mg (66% yield). Mp: 208-210 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H), 3.90 (s, 6H), 3.94 (s, 6H), 3.96 (s, 3H), 7.64 (s, 2H), 7.66 (s, 2H), 7.70 (d, 2H, *J* = 8.32 Hz), 8.23 (d, 2H, *J* = 8.32 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 57.5, 61.6, 63.7, 64.8, 105.5, 106.8, 127.6, 130.5, 130.8, 132.5, 134.6, 135.7, 142.3, 142.8, 155.4, 156.4, 156.6, 157.6, 158.5, 161.7; HRMS (ESI): *m/z* calculated for $C_{28}H_{26}N_4O_8Na$ $[M+Na]^+$ 569.1648, found 569.1653.

2-(3,4,5-Trimethoxyphenyl)-5-(4-(5-(4-methoxyphenyl)-1,2,4-oxadiazol-3-yl)phenyl)-1,3,4-oxadiazole (11c)

181 mg (69% yield). Mp: 201-203 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H), 3.91 (s, 6H), 3.94 (s, 3H), 6.86 (d, 2H, *J* = 8.12 Hz), 7.64 (s, 2H), 7.70 (d, 2H, *J* = 8.23 Hz), 7.72 (d, 2H, *J* = 8.33 Hz), 8.23 (d, 2H, *J* = 8.33 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 56.7, 58.4, 61.4, 105.6, 118.6, 119.6, 127.4, 129.4, 130.4, 132.5, 134.5, 135.6, 142.8, 155.7, 156.4, 156.7, 156.9, 160.4, 161.4; HRMS (ESI): *m/z* calculated for $C_{26}H_{22}N_4O_6Na$ $[M+Na]^+$ 509.1437, found 509.1443.

2-(4-(5-(4-Chlorophenyl)-1,2,4-oxadiazol-3-yl)phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (11d)

210 mg (80% yield). Mp: 216-218 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H), 3.91 (s, 6H), 7.54 (d, 2H, *J* = 8.36 Hz), 7.65-7.70 (m, 3H), 7.72 (d, 2H, *J* = 8.37 Hz), 8.24 (d, 2H, *J* = 8.37 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 57.4, 61.3, 105.4, 127.5, 129.5, 130.4, 130.8, 132.4, 133.5, 134.2, 134.8, 135.6, 142.5, 149.6, 150.5, 155.6, 156.7, 161.8; MS (ESI): 491 $[M+H]^+$. HRMS (ESI): *m/z* calculated for $C_{25}H_{19}N_4O_5NaCl$ $[M+Na]^+$ 513.0942, found 513.0938.

2-(4-(5-(4-Bromophenyl)-1,2,4-oxadiazol-3-yl)phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (11e)

221 mg (76% yield). Mp: 222-224 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H), 3.91 (s, 6H), 7.55 (d, 2H, *J* = 8.36 Hz), 7.65 (s, 2H), 7.69 (d, 2H, *J* = 8.36 Hz), 7.73 (d, 2H, *J* = 8.38 Hz), 8.25 (d, 2H, *J* = 8.38 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 57.6, 61.7, 105.6, 126.4, 127.4, 127.9, 130.5, 132.5, 133.4, 134.3, 134.7, 135.5, 142.4, 149.6, 150.6, 156.5, 156.9, 161.8; HRMS (ESI): *m/z* calculated for $C_{25}H_{19}N_4O_5NaBr$ $[M+Na]^+$ 557.0437, found 557.0432.

2-(4-(5-(4-Fluorophenyl)-1,2,4-oxadiazol-3-yl)phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (11f)

201 mg (79% yield). Mp: 199-201 ºC, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H), 3.90 (s, 6H), 7.45 (d, 2H, *J* = 8.27 Hz), 7.64 (s, 2H), 7.70 (d, 2H, *J* = 8.35 Hz), 7.84 (d, 2H, *J* = 8.27 Hz), 8.24 (d, 2H, *J* = 8.35 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 57.6, 61.7, 105.4, 126.5, 127.5, 127.8, 130.5, 132.6, 132.8, 133.5, 134.4, 134.9, 135.6, 142.7, 150.6, 153.7, 156.4, 157.8, 161.8; HRMS (ESI): *m/z* calculated for $C_{25}H_{19}N_4O_5FNa$ $[M+Na]^+$ 497.1237, found 497.1241.

2-(3,4,5-Trimethoxyphenyl)-5-(4-(5-(4-nitrophenyl)-1,2,4-oxadiazol-3-yl)phenyl)-1,3,4-oxadiazole (11g)

230 mg (85% yield). Mp: 230-232 ºC, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H), 3.91 (s, 6H), 7.64 (s, 2H), 7.71 (d, 2H, *J* = 8.37 Hz), 7.77 (d, 2H, *J* = 8.38 Hz), 7.82 (d, 2H, *J* = 8.38 Hz), 8.25 (d, 2H, *J* = 8.37 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 57.7, 61.9, 105.7, 126.7, 127.6, 127.8, 130.4, 132.4, 134.5, 135.6, 139.5, 142.3, 149.6, 150.5, 154.7, 156.5, 157.8, 161.9; HRMS (ESI): *m/z* calculated for $C_{25}H_{19}N_5O_7Na$ $[M+Na]^+$ 524.1182, found 524.1177.

2-(3,4,5-Trimethoxyphenyl)-5-(4-(5-(3-nitrophenyl)-1,2,4-oxadiazol-3-yl)phenyl)-1,3,4-oxadiazole (11h)

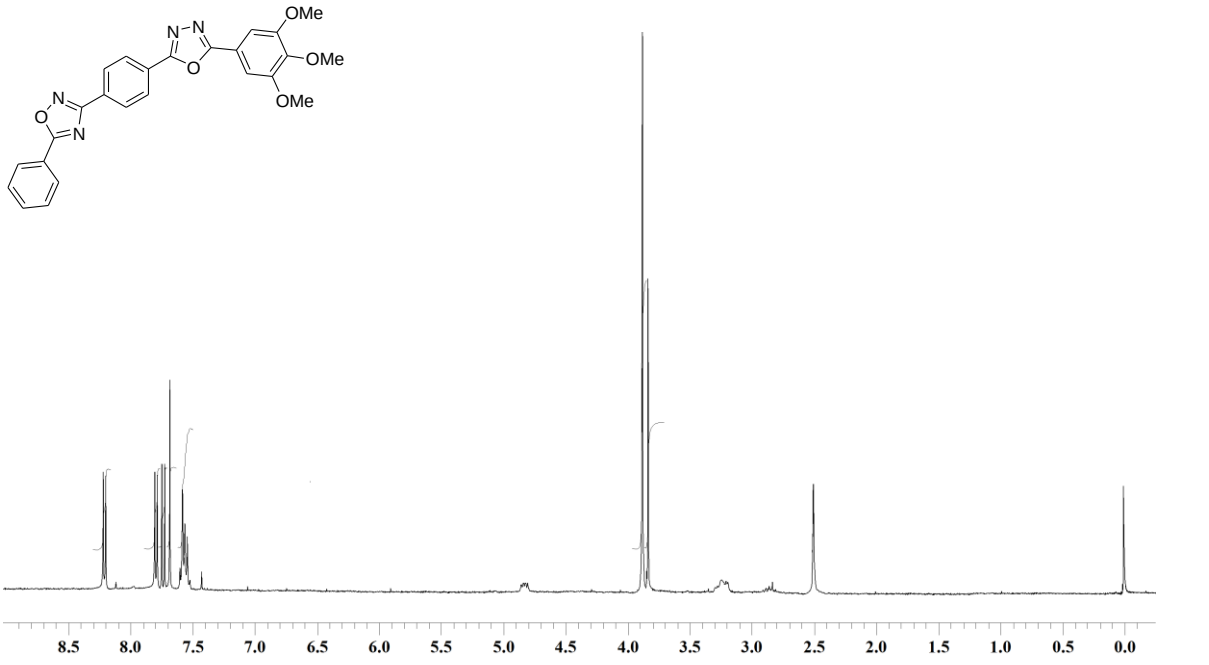
238 mg (88% yield). Mp: 234-236 ºC, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H), 3.91 (s, 6H), 7.64 (s, 2H), 7.71-7.80 (m, 4H), 7.83 (d, 1H, *J* = 8.36 Hz), 8.25-8.28 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d6): δ 57.6, 61.8, 105.8, 122.5, 127.6, 127.9, 130.5, 132.5, 132.8, 134.5, 134.8, 135.5, 136.6, 142.4, 150.5, 154.8, 156.4, 158.6, 161.9; MS (ESI): 502 $[M+H]^+$.

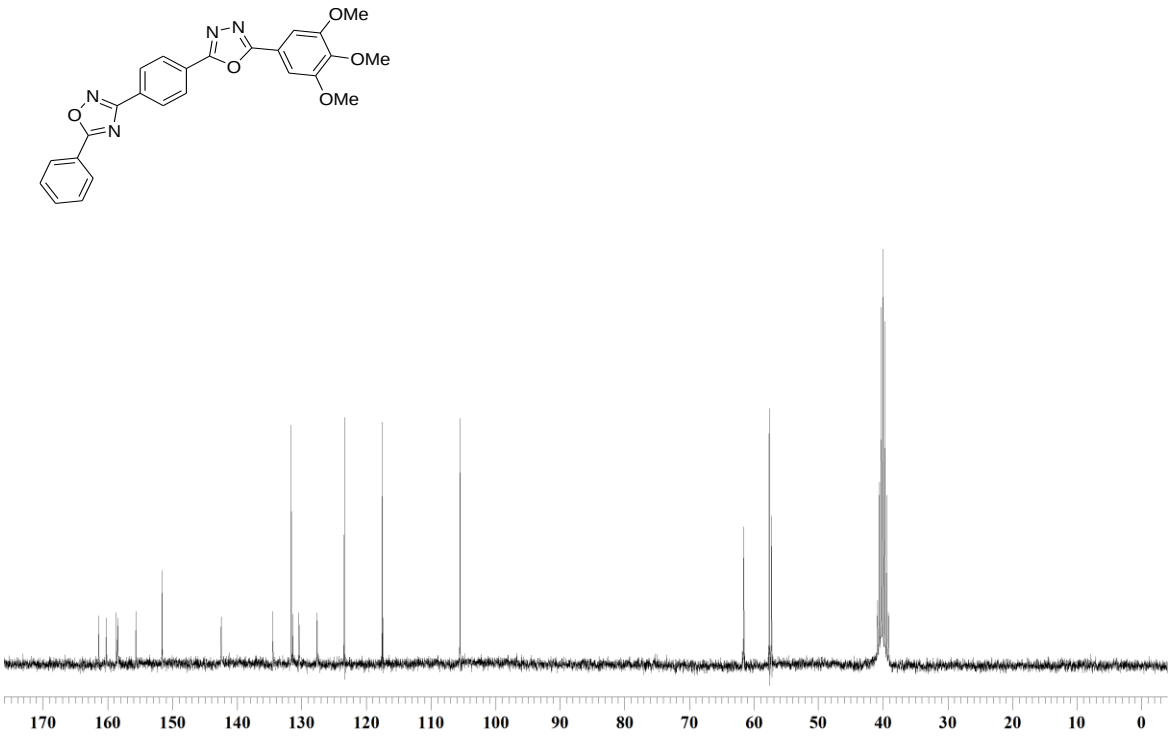
4-(3-(4-(5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)phenyl)-1,2,4-oxadiazol-5-yl)benzonitrile (11i)

232 mg (89% yield). Mp: 237-239 ºC, ^{1}H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H), 3.91 (s, 6H), 7.65 (s, 2H), 7.68 (d, 2H, *J* = 8.34 Hz), 7.70 (d, 2H, *J* = 8.34 Hz), 7.73 (d, 2H, *J* = 8.38 Hz), 8.25 (d, 2H, *J* = 8.38 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 57.6, 61.9, 105.6, 115.6, 119.6, 127.4, 130.5, 132.5, 134.4, 134.9, 135.4, 138.9, 142.3, 150.6, 154.6, 156.6, 158.6, 161.8; HRMS (ESI): *m/z* calculated for $C_{26}H_{19}N_5O_5Na$ $[M+Na]^+$ 504.1284, found 504.1281.

2-(4-(5-(4-(Trifluoromethyl)phenyl)-1,2,4-oxadiazol-3-yl)phenyl)-5-(3,4,5-trimethoxy-phenyl)-1,3,4-oxadiazole (11j)

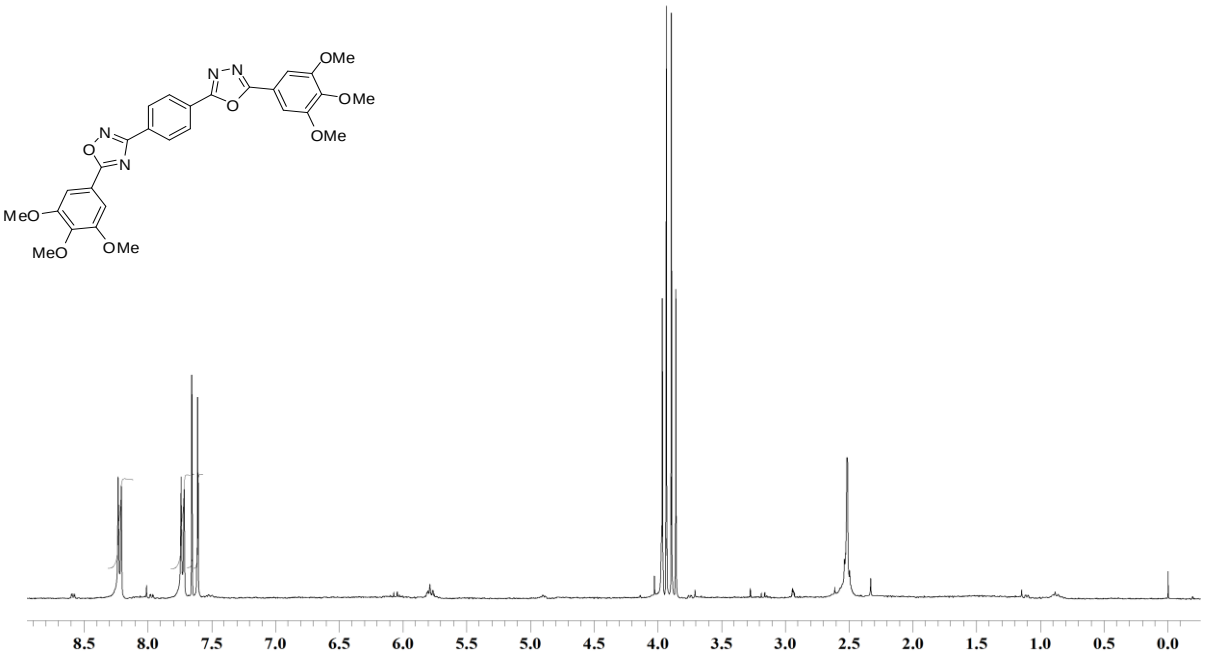
198 mg (70% yield). Mp: 209-211 ºC, ^{1}H NMR (400 MHz, DMSO-d6):δ 3.85 (s, 3H), 3.91 (s, 6H), 7.64 (s, 2H), 7.55 (d, 2H, *J* = 8.17 Hz), 7.71 (d, 2H, *J* = 8.35 Hz), 7.74 (d, 2H, *J* = 8.17 Hz), 8.23 (d, 2H, *J* = 8.35 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 57.6, 61.8, 105.6, 121.4, 127.5, 128.4, 130.5, 130.9, 131.7, 132.5, 134.5, 135.5, 138.6, 142.5, 149.6, 150.6, 156.5, 156.9, 161.5; HRMS (ESI): *m/z* calculated for $C_{26}H_{19}N_5O_5F_3Na$ $[M+Na]^+$ 547.1205, found 547.1208.

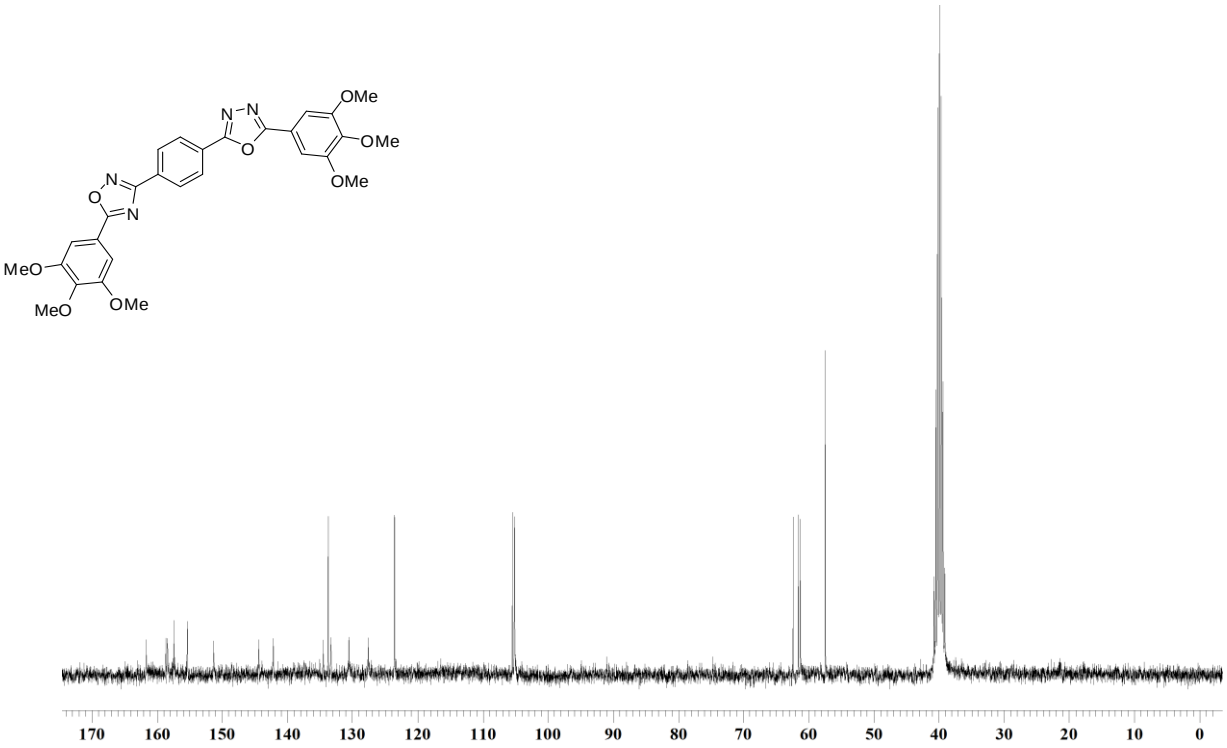




Spectrum 1: ^{1}H NMR Spectrum of **11a** (400 MHz, DMSO-*d6*).

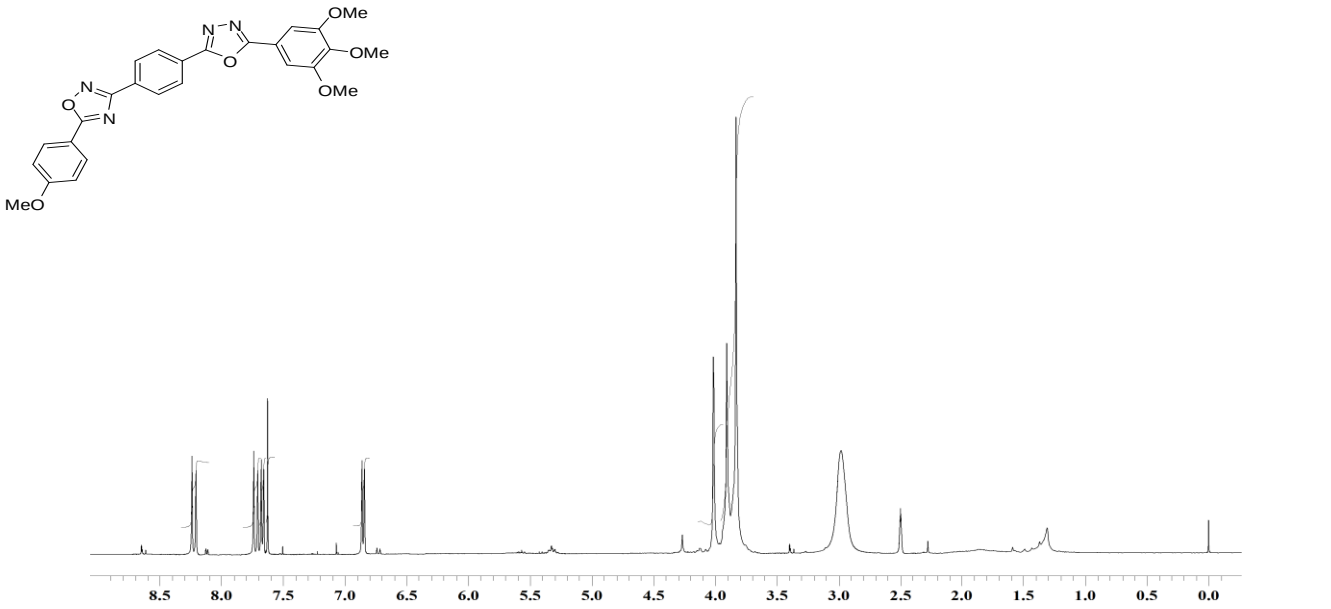
^{13}C NMR Spectrum of **11a** (100 MHz, DMSO-*d6*)

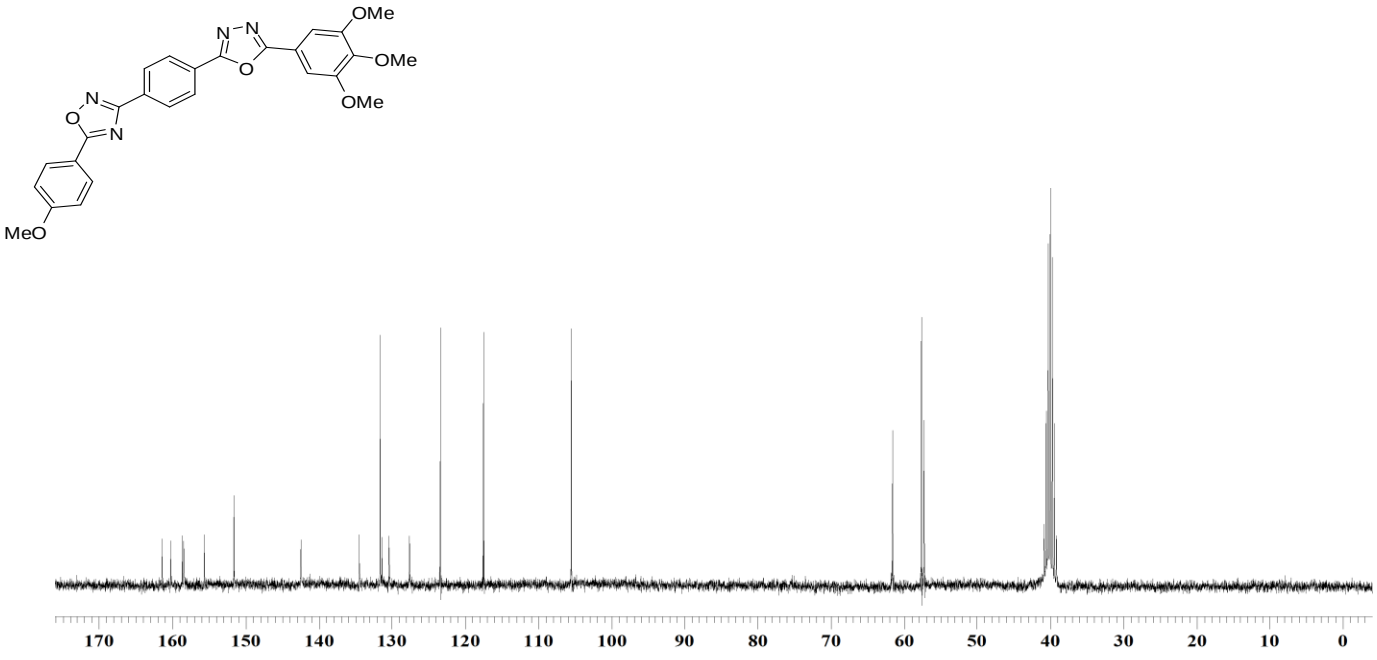




Spectrum 2: ^{1}H NMR Spectrum of **11b** (400 MHz, DMSO-*d6*)

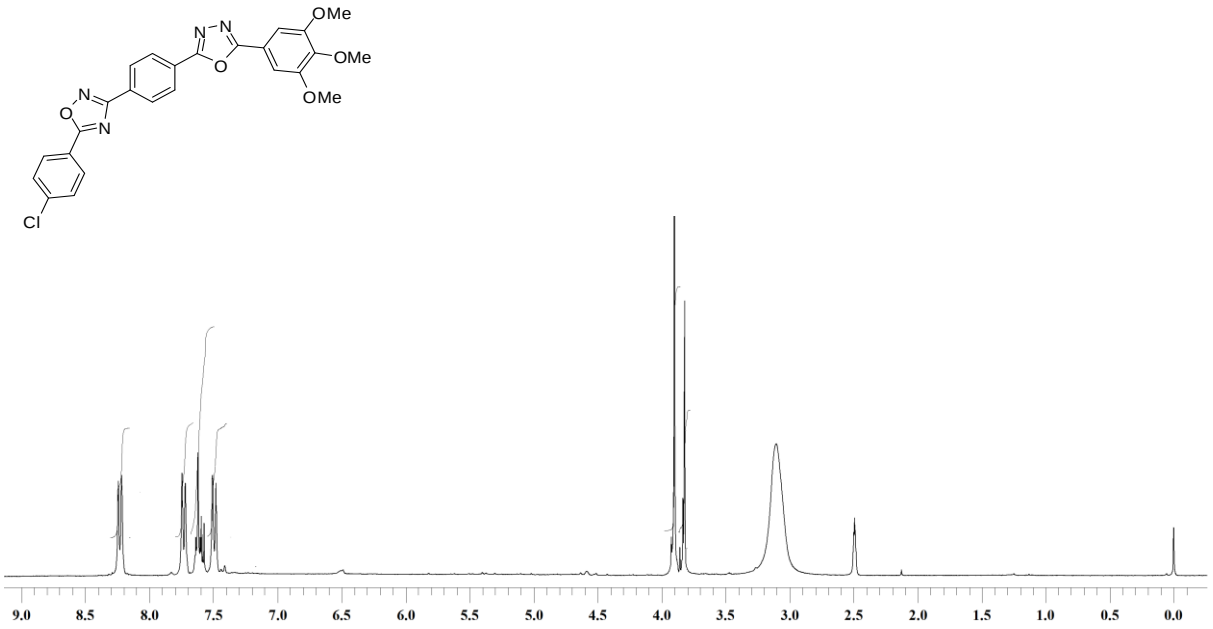
^{13}C NMR Spectrum of **11b** (100 MHz, DMSO-*d6*)

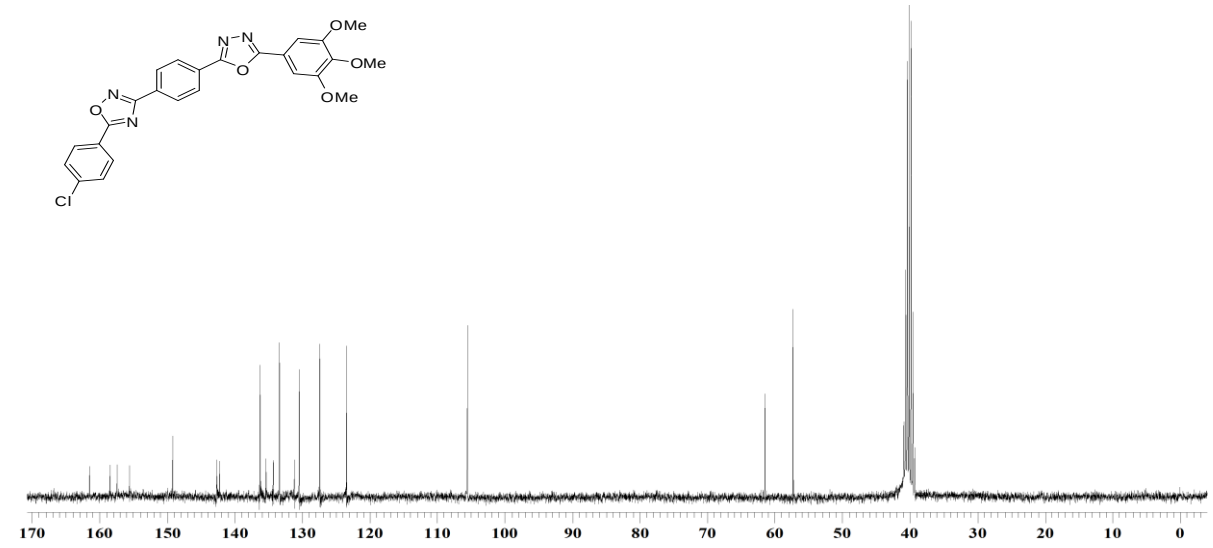




Spectrum 3: ^{1}H NMR Spectrum of **11c** (400 MHz, DMSO-*d6*)

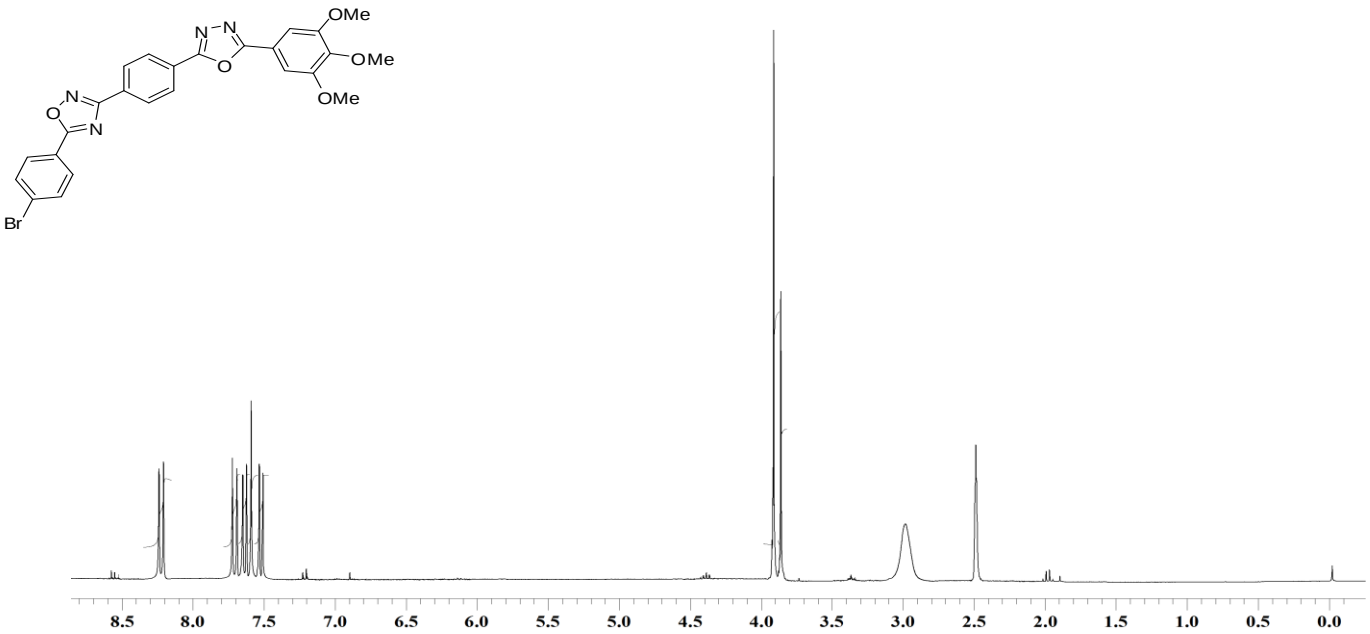
^{13}C NMR Spectrum of **11c** (100 MHz, DMSO-*d6*)

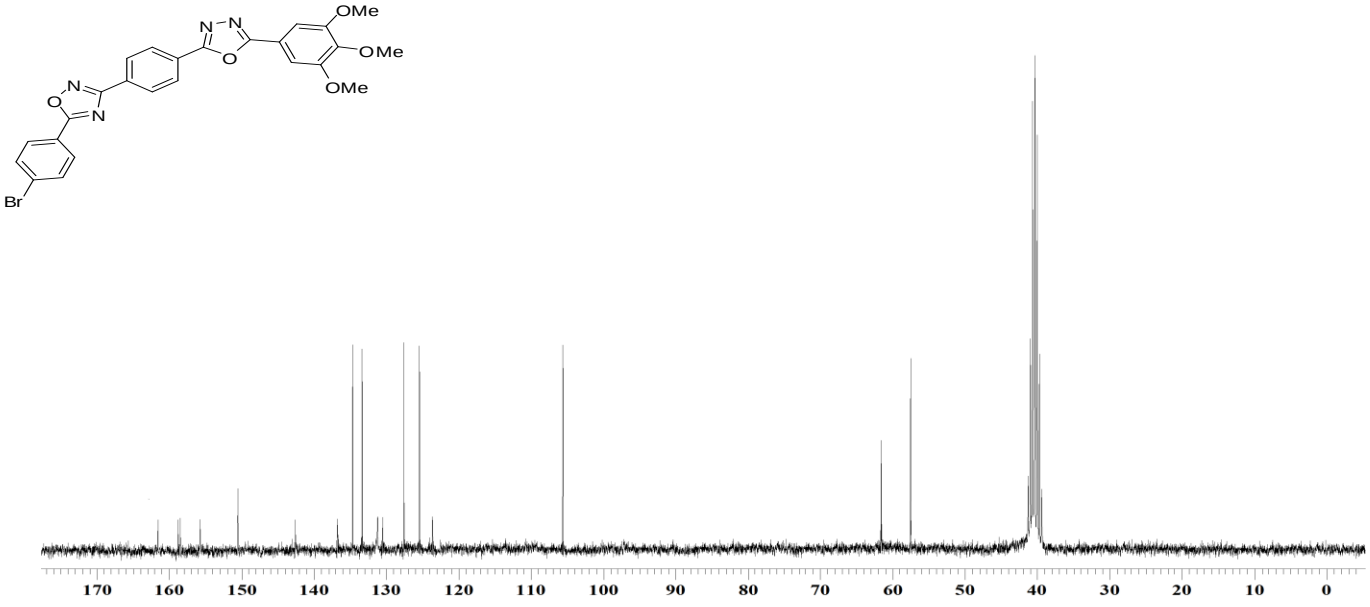




Spectrum 4: ^{1}H NMR Spectrum of **11d** (400 MHz, DMSO-*d6*)

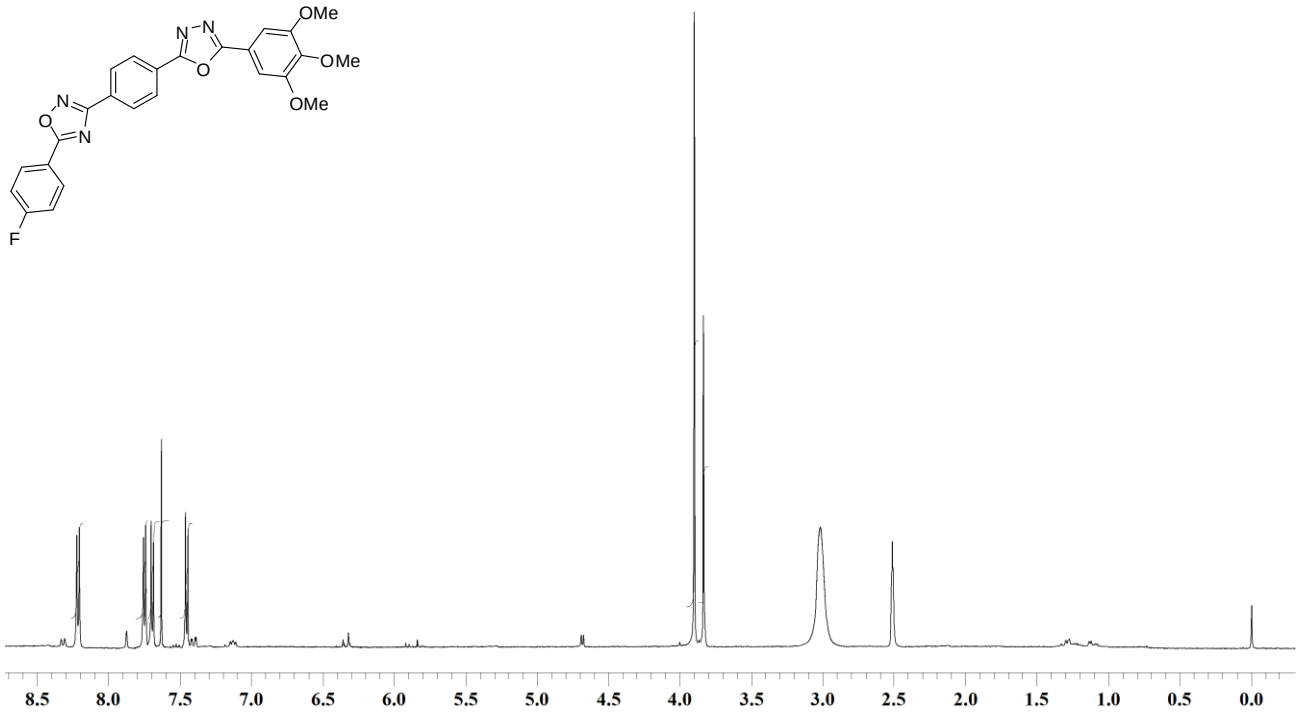
^{13}C NMR Spectrum of **11d** (100 MHz, DMSO-*d6*)

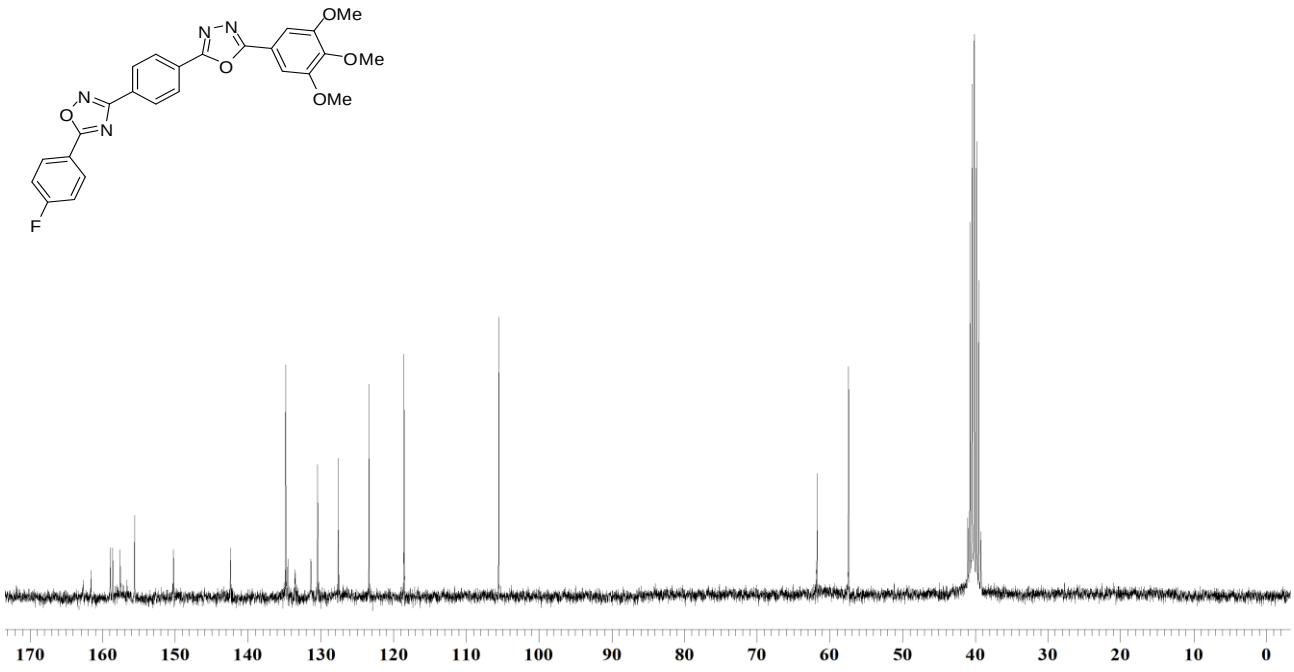




Spectrum 5: ^{1}H NMR Spectrum of **11e** (400 MHz, DMSO-*d6*)

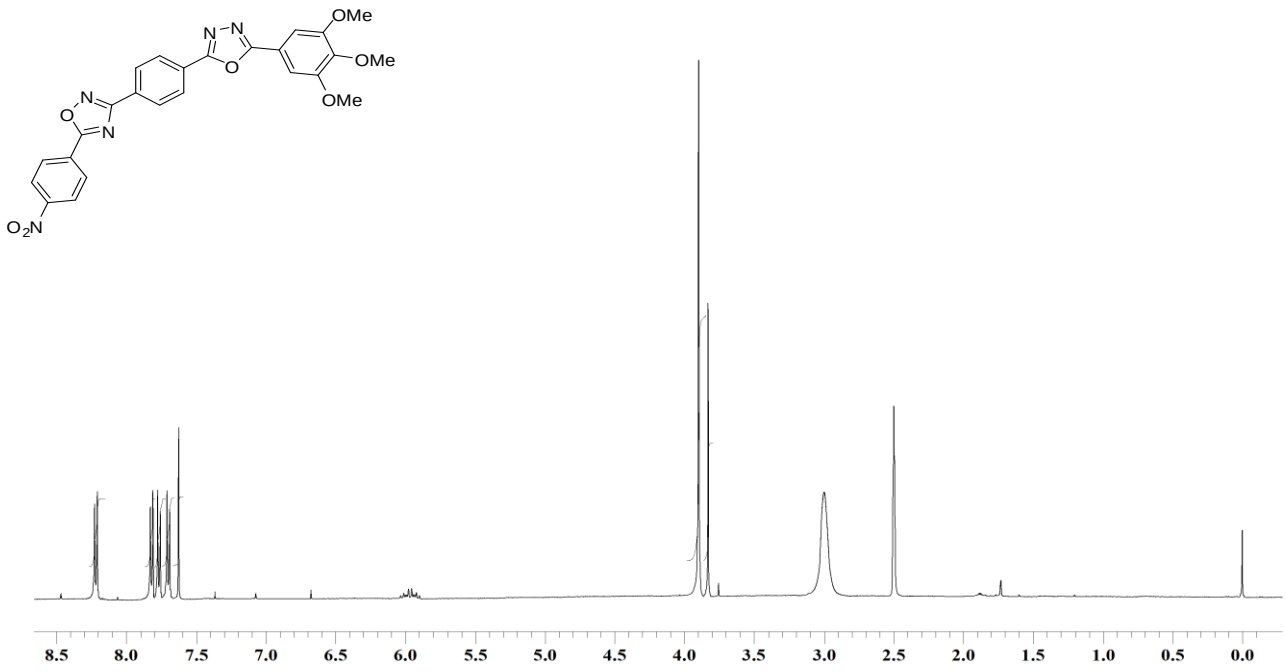
^{13}C NMR Spectrum of **11e** (100 MHz, DMSO-*d6*)

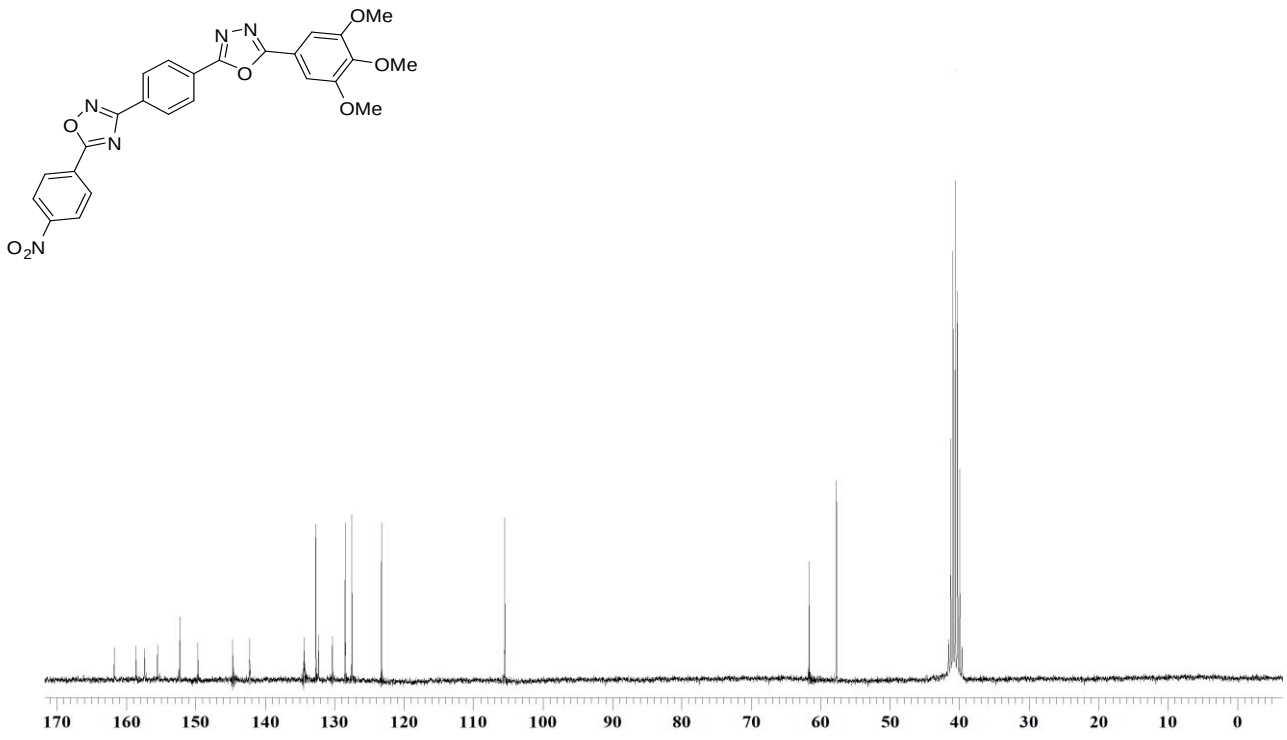




Spectrum 6: ^{1}H NMR Spectrum of **11f** (400 MHz, DMSO-*d6*)

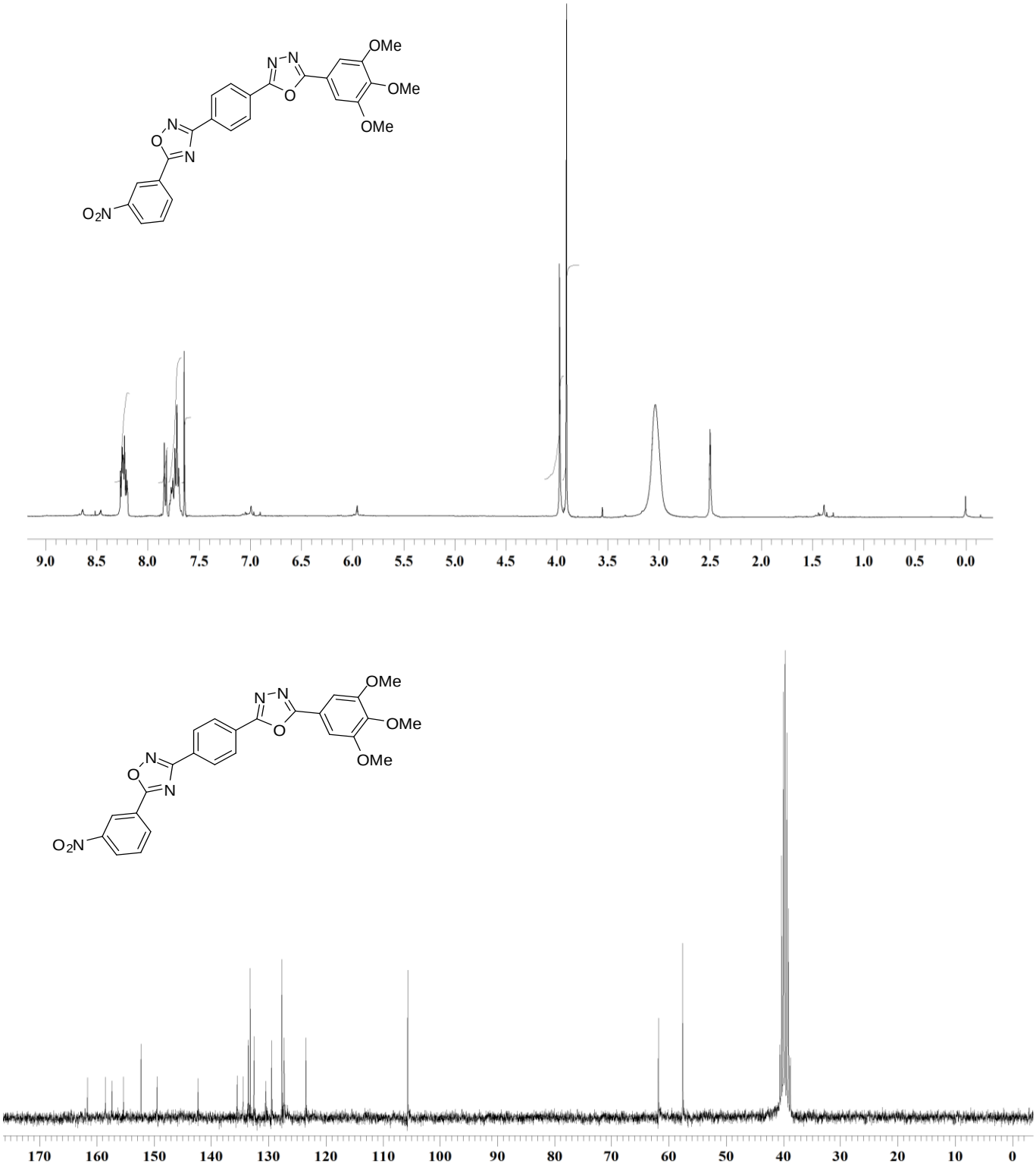
^{13}C NMR Spectrum of **11f** (100 MHz, DMSO-*d6*)





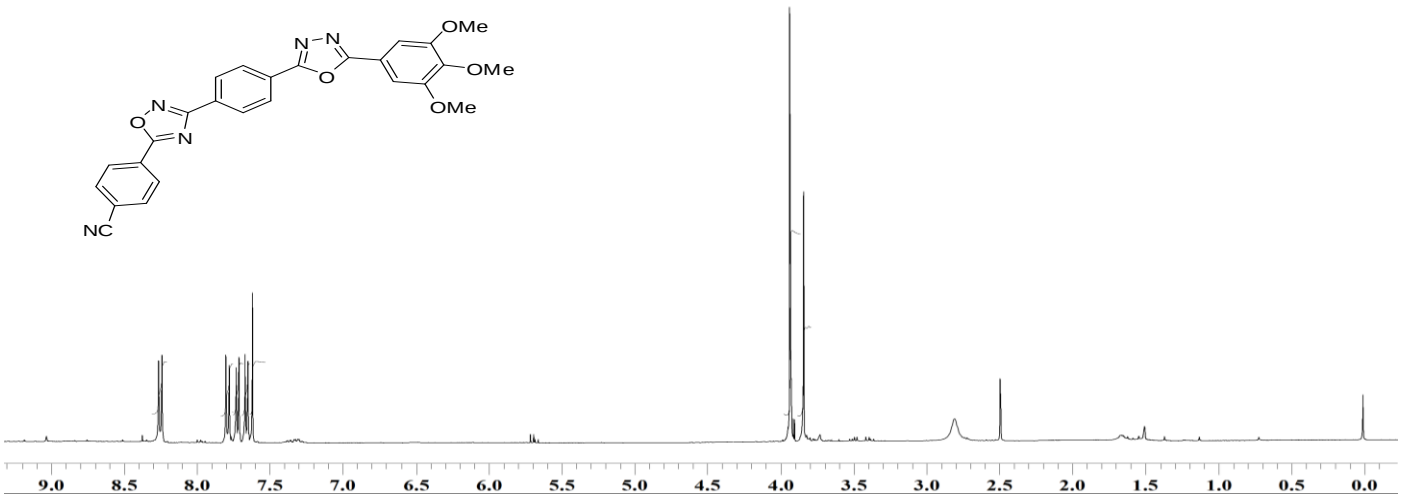
Spectrum 7: ^{1}H NMR Spectrum of **11g** (400 MHz, DMSO-*d6*)

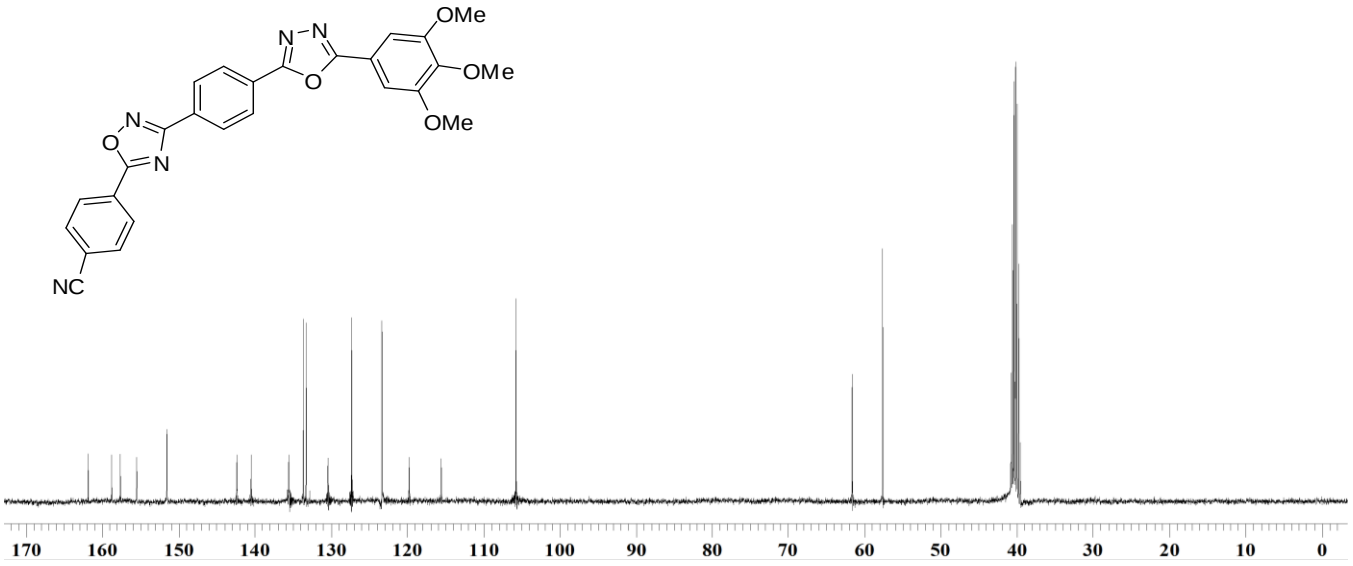
^{13}C NMR Spectrum of **11g** (100 MHz, DMSO-*d6*)



Spectrum 8: ^{1}H NMR Spectrum of **11h** (400 MHz, DMSO-*d6*)

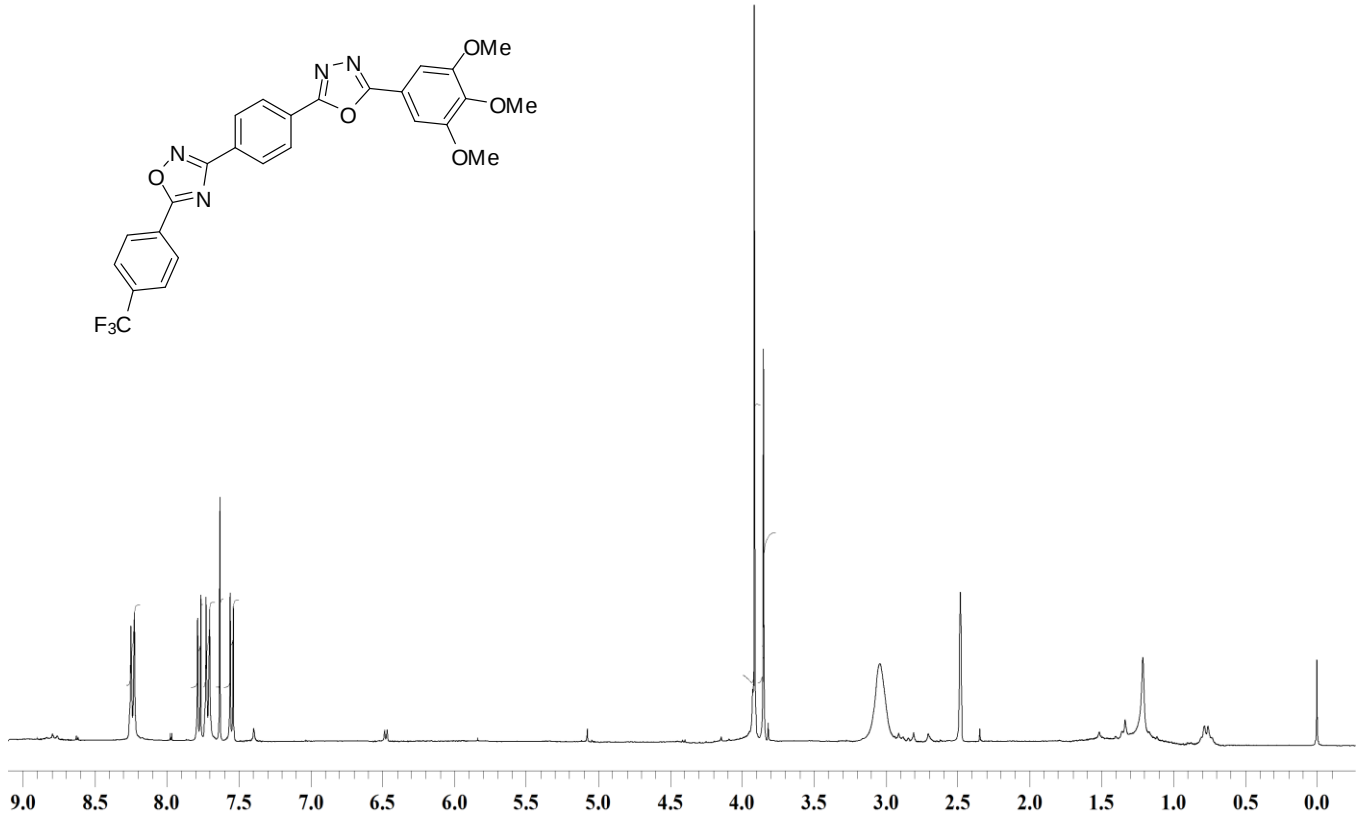
^{13}C NMR Spectrum of **11h** (100 MHz, DMSO-*d6*)

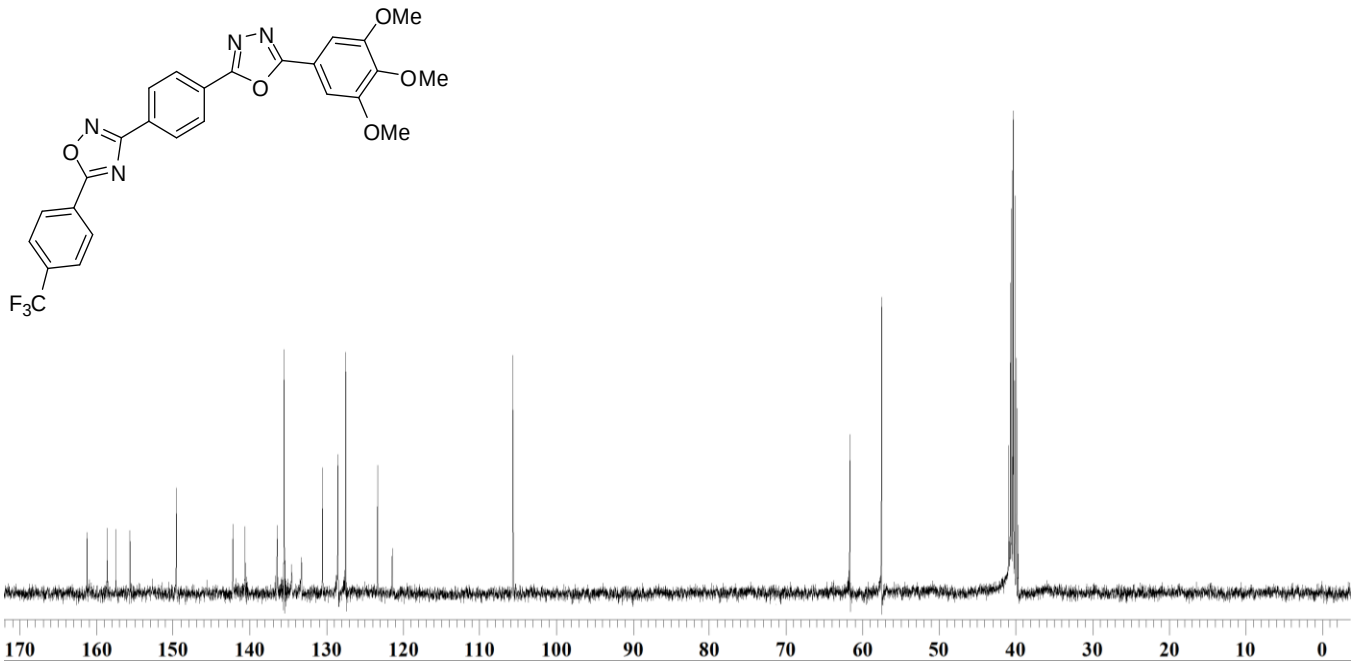




Spectrum 9: ^{1}H NMR Spectrum of **11i** (400 MHz, DMSO-*d6*)

^{13}C NMR Spectrum of **11i** (100 MHz, DMSO-*d6*)





Spectrum 10: ^{1}H NMR Spectrum of **11j** (400 MHz, DMSO-*d6*)

^{13}C NMR Spectrum of **11j** (100 MHz, DMSO-*d6*)