Electronic supplementary materials *Mendeleev Commun.*, 2022, **32**, 452–453

Hetaryl- and heteroarylvinyl-substituted nitrofurans identified as non-cytotoxic selective antitubercular agents

Mikhail Krasavin, Anton Shetnev, Valeria Panova, Sergey Ivanovskyi, Stanislav Kalinin, Valentina Vinogradova, Vladimir Sharoyko and Piotr Yablonsky

Contents

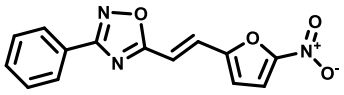
General All and reagents and solvents were obtained from commercial sources and used without purification. All reactions implemented in an open flask without any protection from CO_2 and H_2O. Reactions were monitored by analytical thin layer chromatography (TLC) Macherey-Nagel, TLC plates Polygram® Sil G/UV254. Visualization of the developed chromatograms was performed by fluorescence quenching at 254 nm. ^{1}H and ^{13}C NMR spectra were measured on Bruker AVANCE DPX 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C respectively). All chemical shifts (δ) are given in parts per million (ppm) with reference to solvent residues in DMSO-d_6 (2.50 for proton and 39.52 for carbon) and coupling constant (*J*) are reported in hertz (Hz). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Melting points were determined on Electrothermal IA 9300 series Digital Melting Point Apparatus. Mass spectra were recorded on microTOF spectrometers (ESI ionization).

3-(5-Nitrofuran-2-yl)propenoyl chloride 2 was prepared as reported [R. Pozas, J. Carballo, C. Castro and J. Rubio, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1417].

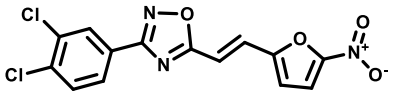
5-Nitrofuran-2-carbonyl chloride 2' was obtained as described [F. Mancuso, A. Di Fiore, L. De Luca, A. Angeli, G. De Simone, C. T. Supuran and R. Gitto, *Bioorg. Med. Chem.*, 2021, **44**, 116279].

General procedure for the synthesis of 1,2,4-oxadiazoles 4a-h and 5a-f

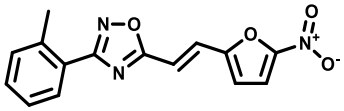
To a solution of amidoxime **3** (1 mmol) in dry dioxane (5 ml) was added acid chloride **2** or **2′** (1.05 mmol) and triethylamine (1.2 mmol) under ice-cooling, and the mixture was stirred at room temperature for 60 min and then refluxed for 6-12 h (TLC monitoring). Dioxane was removed *in vacuo*, and the residue was treated with water (10 ml), filtered and dried. The residue was purified by chromatography on silica gel using 0 → 1% MeOH/CH_2Cl_2 as the eluent.



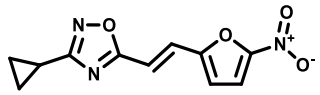
(*E*)-5-[2-(5-Nitrofuran-2-yl)vinyl]-3-phenyl-1,2,4-oxadiazole (**4a**)**:** Yield 24%, yellow powder, mp 161.1-162.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.05 (d, *J* = 6.8 Hz, 2H), 7.86 (d, *J* = 16.6 Hz, 1H), 7.82 (d, J = 3.9 Hz, 1H), 7.64 – 7.57 (m, 3H), 7.41 (d, J = 3.9 Hz, 1H), 7.37 (d, J = 16.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 174.19, 168.25, 152.31, 152.10, 131.71, 129.28 (2C), 127.71, 127.02 (2C), 125.95, 117.46, 114.73, 113.41. HRMS (ESI), Calc. for $C_{14}H_9N_3O_4$: $[M+H]^+$ 284.0666; found *m/z* 284.0689.



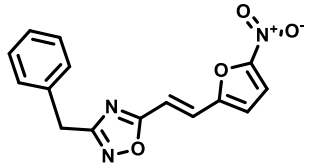
(*E*)-3-(3,4-Dichlorophenyl)-5-[2-(5-nitrofuran-2-yl)vinyl]-1,2,4-oxadiazole (4b): Yield 48%, light brown powder, mp 170,2-171,0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.17 (s, 1H), 7.99 (d, J = 9.0 Hz, 1H), 7.93 – 7.84 (m, 2H), 7.81 (d, J = 3.1 Hz, 1H), 7.41 (d, J = 3.3 Hz, 1H), 7.36 (d, J = 15.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 175.35, 167.48, 153.06, 152.92, 135.29, 133.02, 132.34, 129.45, 128.81, 127.77, 127.38, 117.95, 114.98, 113.96. HRMS (ESI), Calc. for $C_{14}H_7Cl_2N_3O_4$: $[M+H]^+$ 351.9892; found *m/z* 351.9889.



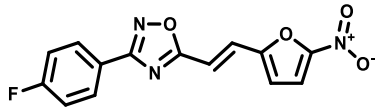
(*E*)-5-[2-(5-Nitrofuran-2-yl)vinyl]-3-(*o*-tolyl)-1,2,4-oxadiazole (**4c**): Yield 32%, light brown powder, mp 135.5-137.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.94 (d, *J* = 7.7, 1H), 7.85 - 7.79 (m, 2H), 7.48 - 7.34 (m, 5H), 2.58 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 173.09, 168.85, 152.33, 152.04, 137.53, 131.42, 130.93, 129.62, 127.54, 126.19, 125.27, 117.28, 114.67, 113.43, 21.51. HRMS (ESI), Calc. for $C_{15}H_{11}N_3O_4$: $[M+H]^+$ 298.0822; found *m/z* 298.0823.



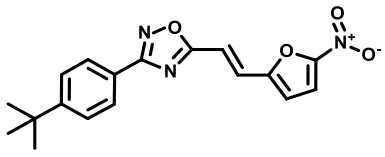
(*E*)-3-Cyclopropyl-5-[2-(5-nitrofuran-2-yl)vinyl]-1,2,4-oxadiazole (4d): Yield 30%, yellow powder, mp 137.6-139.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.79 (d, J = 3.9 Hz, 1H), 7.71 (d, J = 16.3 Hz, 1H), 7.35 (d, J = 3.9 Hz, 1H), 7.24 - 7.20 (d, J = 16.3 Hz, 1H), 2.20 - 2.13 (m, 1H), 1.12 - 1.08 (m, 2H), 0.95 - 0.91 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 173.37, 172.54, 152.33, 152.01, 127.18, 117.21, 114.67, 113.41, 7.56 (2C), 6.32. HRMS (ESI), Calc. for $C_{11}H_9N_3O_4$: $[M+H]^+$ 248.0666; found *m/z* 248.0673.



(*E*)-3-Benzyl-5-[2-(5-nitrofuran-2-yl)vinyl]-1,2,4-oxadiazole (4e): Yield 27%, orange powder, mp 118.2-119.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.78 - 7.72 (m, 2H), 7.33 (m, 5H), 7.27 - 7.23 (m, 2H), 4.13 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 173.80, 169.95, 152.29, 152.02, 135.60, 128.89 (2C), 128.52 (2C), 127.39, 126.86, 117.28, 114.63, 113.35, 31.31. HRMS (ESI), Calc. for $C_{15}H_{11}N_3O_4$: $[M+H]^+$ 298.0822; found *m/z* 298.0817.

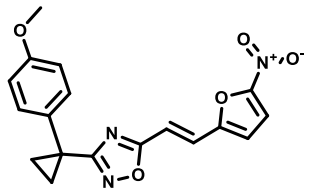


(*E*)-3-(4-Fluorophenyl)-5-[2-(5-nitrofuran-2-yl)vinyl]-1,2,4-oxadiazole (**4f**): Yield 49%, beige powder, mp 183.8-185.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.12 - 8.08 (m, 2H), 7.86 (d, J = 16.4 Hz, 1H), 7.82 - 7.81 (d, J = 3.9 Hz, 1H), 7.48 - 7.40 (m, 3H), 7.35(d, J= 16.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 174.19, 167.41, 163.99 (d, $J_{C\text{-}F}$ = 249.51 Hz), 152.23, 152.06, 129.52 (d, $J_{C\text{-}F}$ = 9.06 Hz), 127.71, 122.49 (d, $J_{C\text{-}F}$ = 3.12 Hz), 117.44, 116.40 (d, $J_{C\text{-}F}$ = 22.21 Hz), 114.64, 113.25. HRMS (ESI), Calc. for $C_{14}H_8FN_3O_4$: $[M+H]^+$ 302.0572; found *m/z* 302.0575.

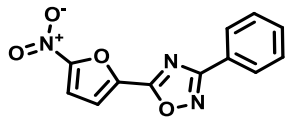


(*E*)-3-(4-*tert*-Butylphenyl)-5-[2-(5-nitrofuran-2-yl)vinyl]-1,2,4-oxadiazole (4g): Yield 40%, yellow powder, mp 172.9-173.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.97 (d, J = 8.5 Hz, 2H), 7.85 (d, J = 16.4 Hz, 1H), 7.80 (d, J = 3.9 Hz, 1H), 7.60 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 3.9 Hz, 1H), 7.37 (d, J = 16.4 Hz, 1H), 1.32 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 174.72, 168.85,

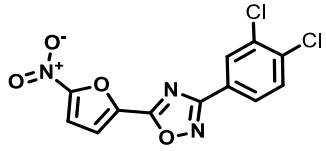
155.25, 153.04, 152.77, 128.28, 127.55 (2C), 126.75 (2C), 123.89, 118.11, 115.41, 114.12, 35.43, 31.54 (3C). HRMS (ESI), Calc. for $C_{18}H_{17}N_3O_4$: $[M+H]^+$ 340.1292; found *m/z* 340.1299.



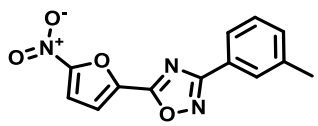
(*E*)-3-[1-(4-Methoxyphenyl)cyclopropyl]-5-[2-(5-nitrofuran-2-yl)vinyl]-1,2,4-oxadiazole (4h): Yield 43 %, yellow powder, mp 150.0-151.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.77 (d, *J* = 3.9 Hz, 1H), 7.70 (d, *J* = 16.4 Hz, 1H), 7.36 - 7.34 (m, 3H), 7.21 (d, *J* = 16.4 Hz, 1H), 6.92 - 6.90 (m, 2H), 3.76 (s, 3H), 1.51(m, 2H), 1.36 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 174.81, 173.44, 158.36, 152.32, 151.99, 131.45 (2C), 130.90, 127.22, 117.22, 114.65, 113.65 (2C), 113.42, 55.05 (2C), 22.11, 15.61. HRMS (ESI), Calc. for $C_{18}H_{15}N_3O_5$: $[M+H]^+$ 354.1084; found *m/z* 354.1089.



5-(5-Nitrofuran-2-yl)-3-phenyl-1,2,4-oxadiazole (5a): Yield 45%, pink powder, mp 166.2-171.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 88.12 – 8.09 (m, 1H), 8.09 – 8.07 (m, 1H), 7.97 (d, J = 4.03 Hz, 1H), 7.95 (d, J = 4.03 Hz, 1H), 7.69 – 7.57 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 168.38, 165.89, 152.99, 139.84, 132.06, 129.36 (2C), 127.21 (2C), 125.25, 119.21, 113.81. HRMS (ESI), Calc. for $C_{12}H_7N_3O_4$: $[M+H]^+$ 258.0509; found *m/z* 258.0515.

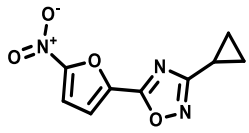


3-(3,4-Dichlorophenyl)-5-(5-nitrofuran-2-yl)-1,2,4]-oxadiazole (5b): Yield 68%, beige powder, mp 1558.-158.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.23 (d, J = 1.82 Hz, 1H), 8.05 (dd, J = 8.38, 1.73 Hz, 1H), 7.99 (s, 2H), 7.89 (d, J = 8.38 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 166.71, 166.17, 153.04, 139.52, 134.88, 132.29, 131.79, 128.75, 127.23, 125.71, 119.55, 113.77. HRMS (ESI), Calc. for $C_{12}H_5Cl_2N_3O_4$: $[M+H]^+$ 325.9730; found *m/z* 325.9735.



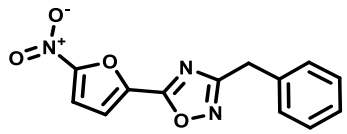
5-(5-Nitrofuran-2-yl)-3-(*m*-tolyl)-1,2,4-oxadiazole (5c): Yield 49%, beige powder, mp 148.1-149.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.01 - 7.94 (m, 3H), 7.54 - 7.40 (m, 3H), 2.60 (s,

3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 168.90, 164.90, 152.97, 139.88, 137.72, 131.49, 131.29, 129.81, 126.30, 124.60, 119.09, 113.77, 21.55. HRMS (ESI), Calc. for $C_{13}H_9N_3O_4$: $[M+H]^+$ 272.0668; found *m/z* 272.0673.

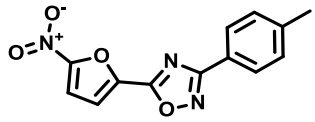


3-Cyclopropyl-5-(5-nitrofuran-2-yl)-1,2,4-oxadiazole (5d):

Yield 39%, yellow powder, mp 65.3-66.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.92 (d, *J* = 4.1, 1H), 7.82 (d, *J* = 4.1, 1H), 2.25 (m, 1H), 1.17 (m, 2H), 1.00 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ: 173.53, 165.88, 153.56, 140.59, 119.50, 114.44, 8.51 (2C), 6.97. HRMS (ESI), Calc. for $C_9H_7N_3O_4$: $[M+H]^+$ 221.0437; found *m/z* 221.0444.



3-Benzyl-5-(5-nitrofuran-2-yl)-1,2,4-oxadiazole (5e): Yield 45%, beige powder, mp 85.2-88.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.92 (d, *J* = 4.01 Hz, 1H), 7.85 (d, *J* = 4.01 Hz, 1H), 7.36 (d, *J* = 4.39 Hz, 4H), 7.32 – 7.25 (m, 1H), 4.22 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ: 170.19, 165.57, 152.90, 139.87, 135.24, 128.95 (2C), 128.60 (2C), 127.00, 118.93, 113.74, 31.14. HRMS (ESI), Calc. for $C_{13}H_9N_3O_4$: $[M+H]^+$ 272.0666; found *m/z* 272.0672.



5-(5-Nitrofuran-2-yl)-3-(*p*-tolyl)-1,2,4-oxadiazole (**5f**): Yield 37%, brown powder, mp 154.5-157.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.99 – 7.93 (m, 4H), 7.42 (d, J = 8.11 Hz, 2H), 2.40 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 168.32, 165.69, 152.92, 142.11, 139.86, 129.85 (2C), 127.11(2C), 122.43, 119.06, 113.77, 21.05. HRMS (ESI), Calc. for $C_{13}H_9N_3O_4$: $[M+H]^+$ 272.0666; found *m/z* 272.0667.

Antibacterial assay

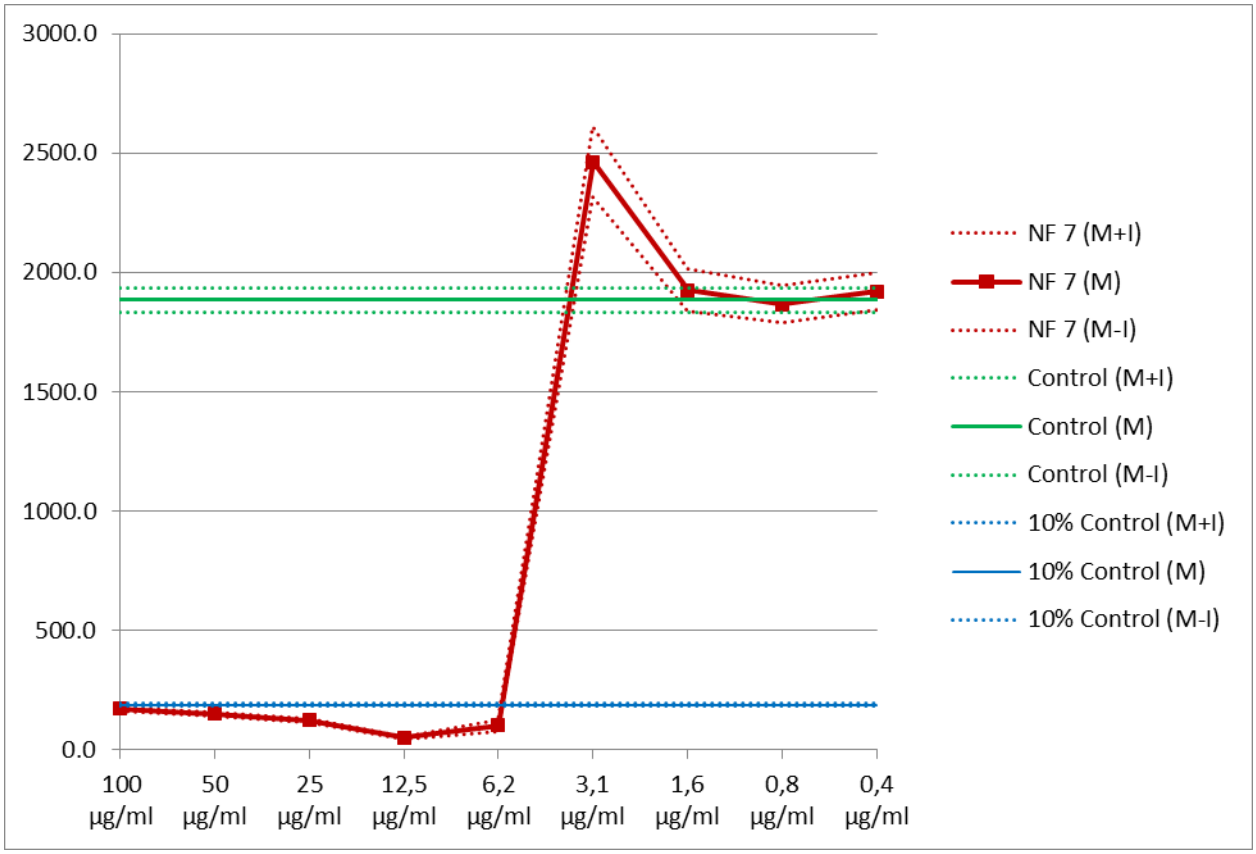
All the synthesized compounds **4a-h** and **5a-f** were evaluated for their *in vitro* antimicrobial activity against Staphylococcus aureus (ATCC 25923) and Bacillus subtillis (VCM V3142D) as examples of Gram-positive bacteria, and Escherichia coli (ATCC 25922) (P218) as an example of Gram-negative bacteria.

Overnight cultures were grown at 37 °C in Lysogeny broth (LB) and diluted to obtain an opacity equivalent to 0.5 on the McFarland scale. Screening vials were filled with solutions of the test compounds in 0.5% DMSO as prepared above with three replications for each treatment. API pefloxacin (0.5-256 µg/mL) and 0.5% DMSO served as positive and negative controls, respectively. The entire vial was incubated at 35 ± 2 °C for 18 h. After incubation, the antibacterial activity of the test compounds was determined by measuring the absorption of the solution with a spectrophotometer on 500 nm.

MIC measurement

The MICs of the most active compounds were measured using the twofold serial broth dilution method. The test organisms were grown in suitable broth for 18 h at 37 °C. Twofold serial dilutions of solutions of the test compounds were prepared at 256, 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 µg ml^{-1}. The tubes were then inoculated with the test microbe; each 5 mL received 0.1 ml of the above inoculums and were incubated at 37 °C. The vials were subsequently observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in Table 1.

ILLUSTRATIVE 96-WELL PLATES FOR THE MOST ACTIVE ANTIMYCOBACTERIAL COMPOUNDS



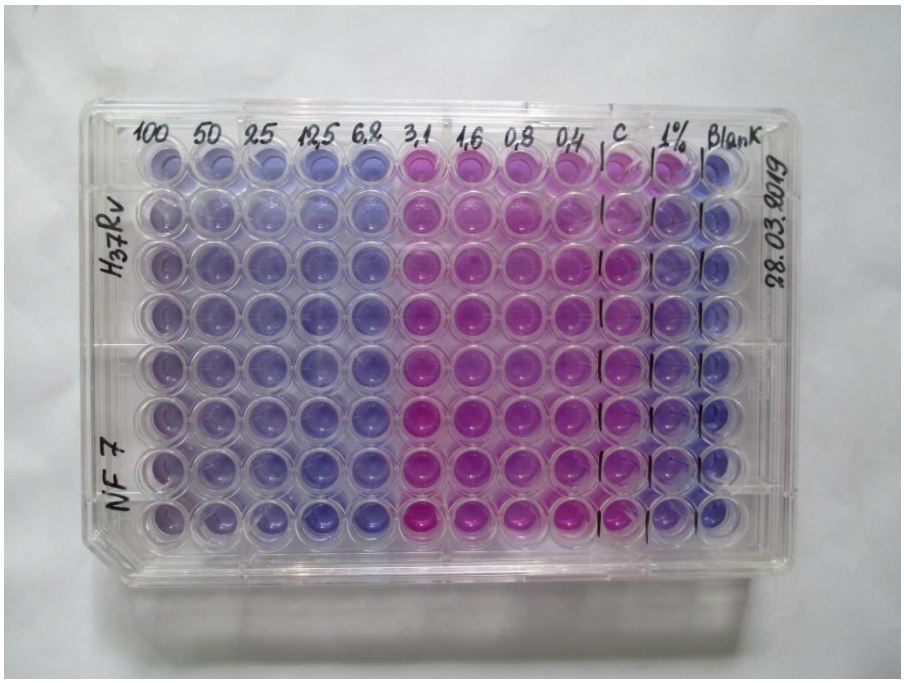
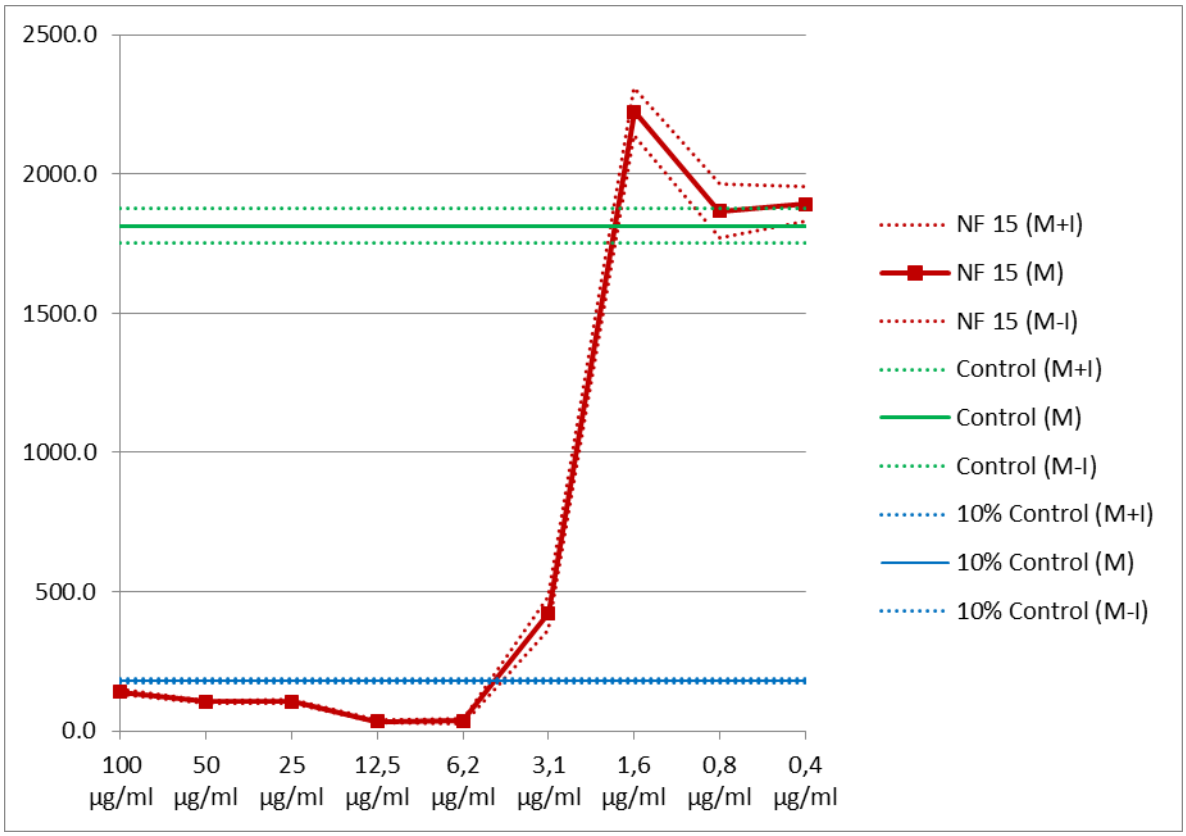


Figure S1. Compound **4e** tested for MIC against *MTb* H37Rv (MIC 6.2 µg ml^{-1})



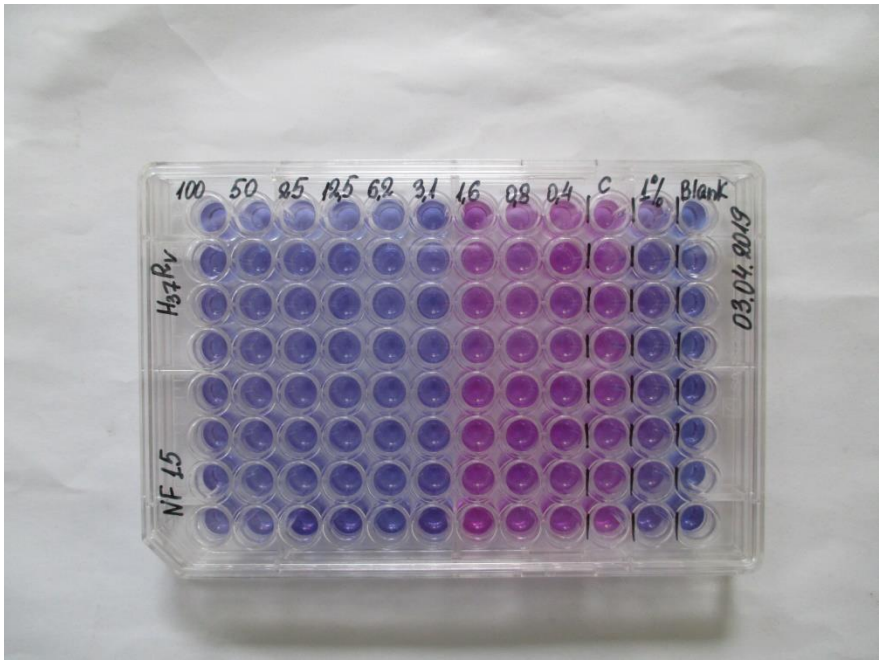


Figure S2. Compound **5d** tested for MIC against *MTb* H37Rv (MIC 6.2 µg ml^{-1}).

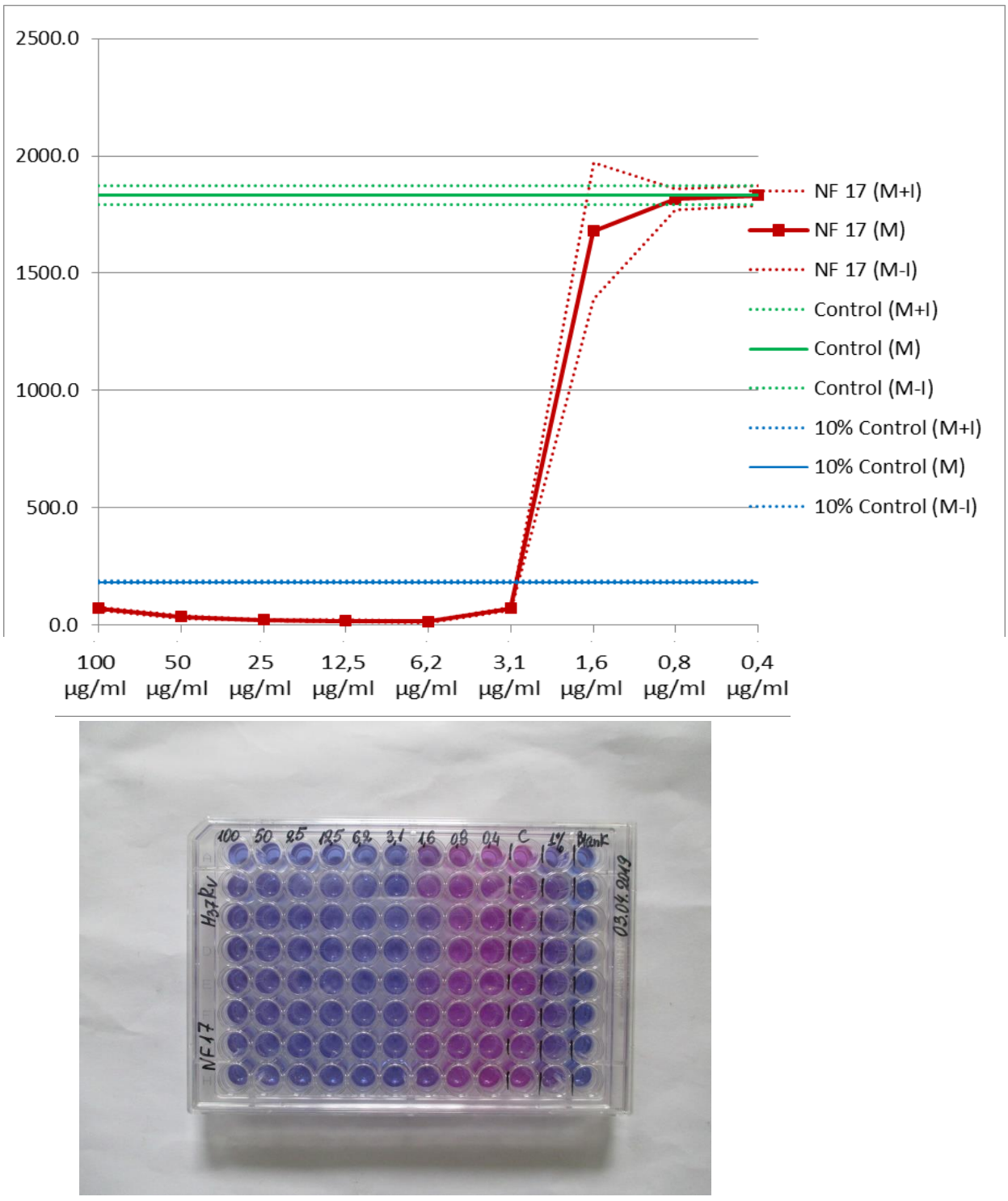
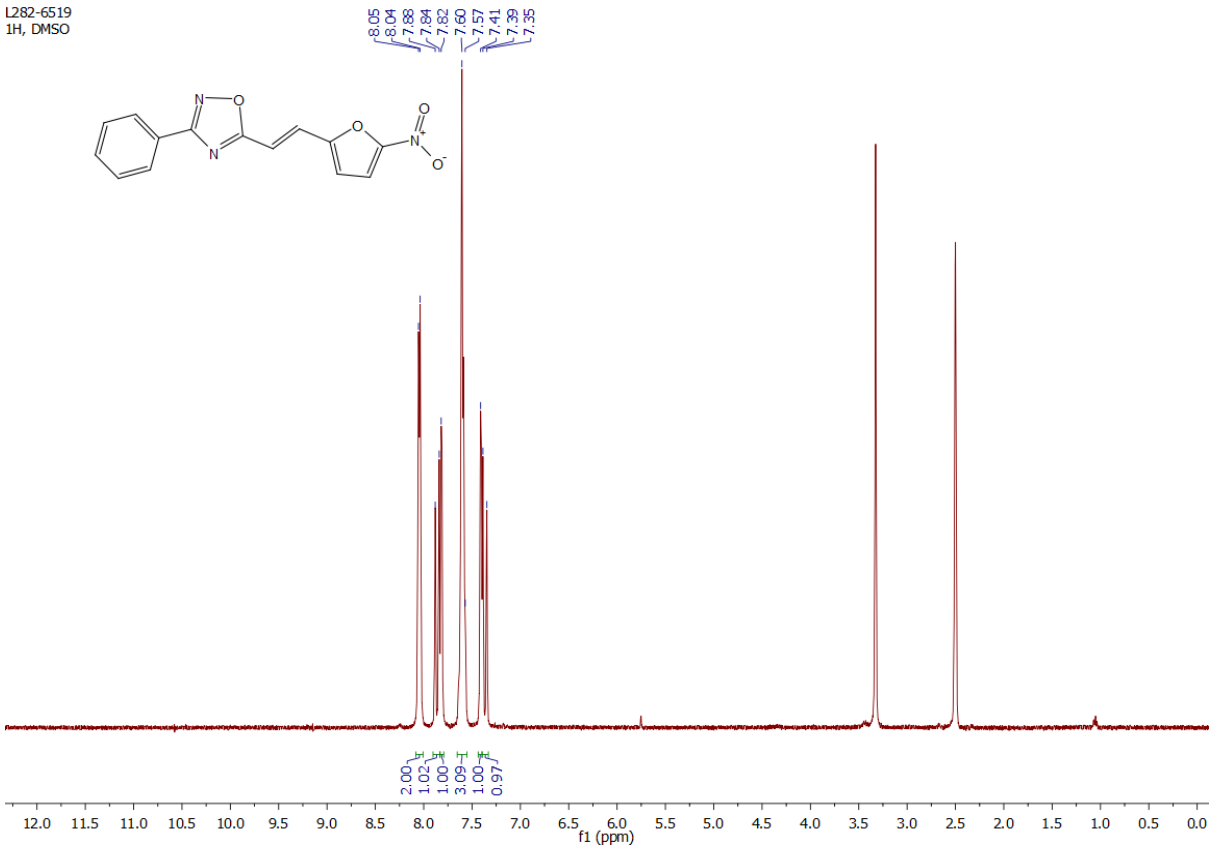
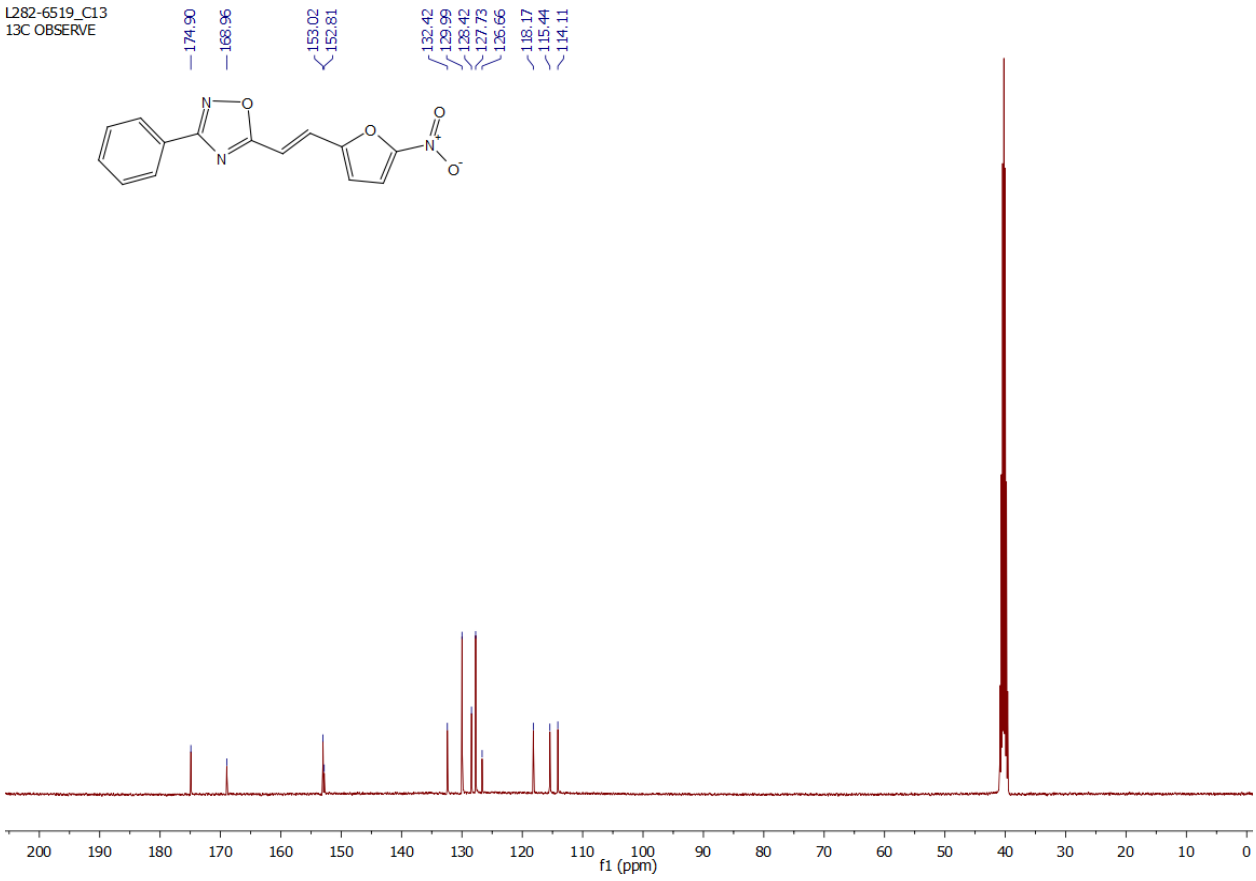


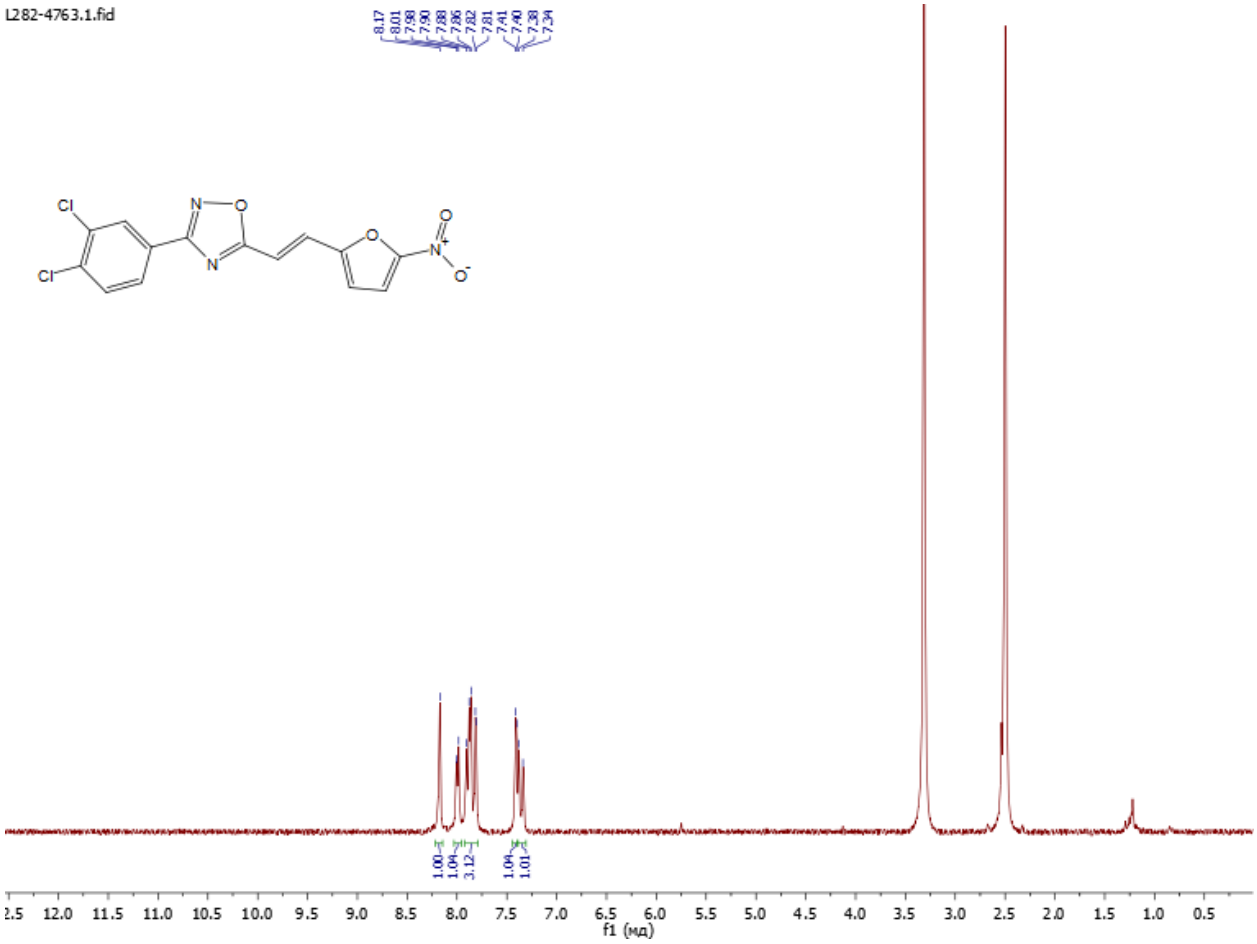
Figure S3. Compound **5e** tested for MIC against *MTb* H37Rv (MIC 3.1 µg ml^{-1}).

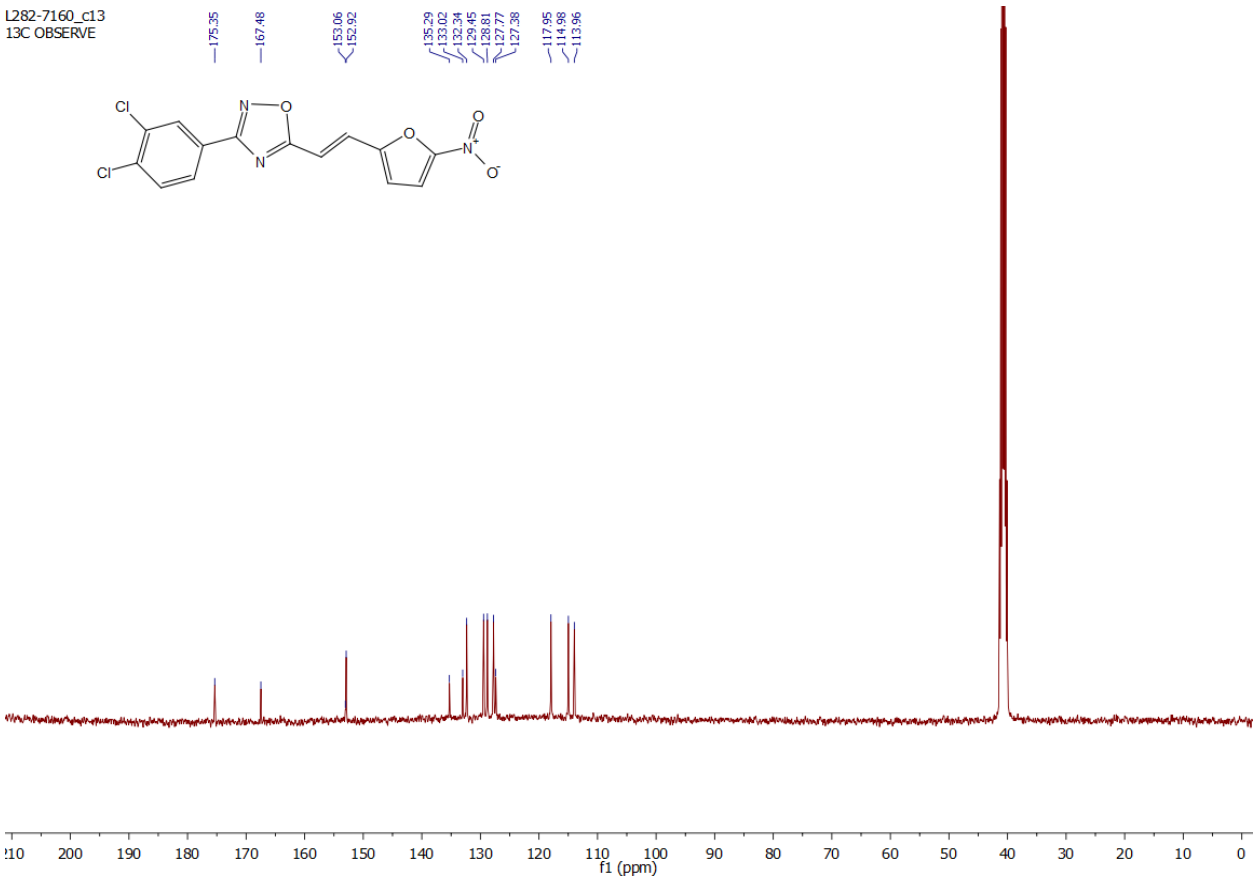
(*E*)-5-(2-(5-nitrofuran-2-yl)vinyl)-3-phenyl-[1,2,4]-oxadiazole (Compound **4a**)


L282-6519
1H, DMSO
8.05
8.04
7.88
7.84
7.82
7.60
7.57
7.41
7.39
7.35
2.00
1.02
1.00
3.09
1.00
0.97
f1 (ppm)

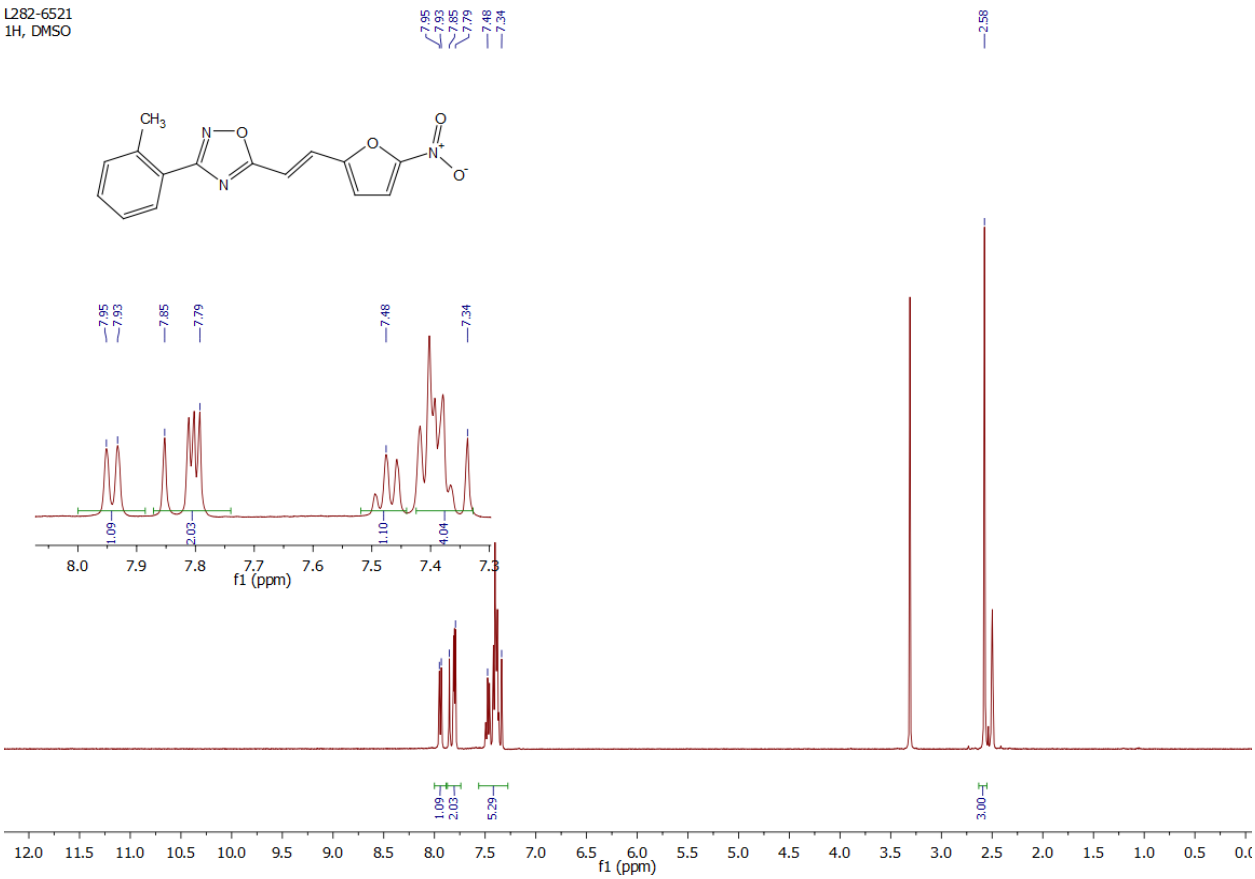

L282-6519_C13
13C OBSERVE
174.90
168.96
153.02
152.81
132.42
129.99
128.42
127.73
126.66
118.17
115.44
114.11
f1 (ppm)

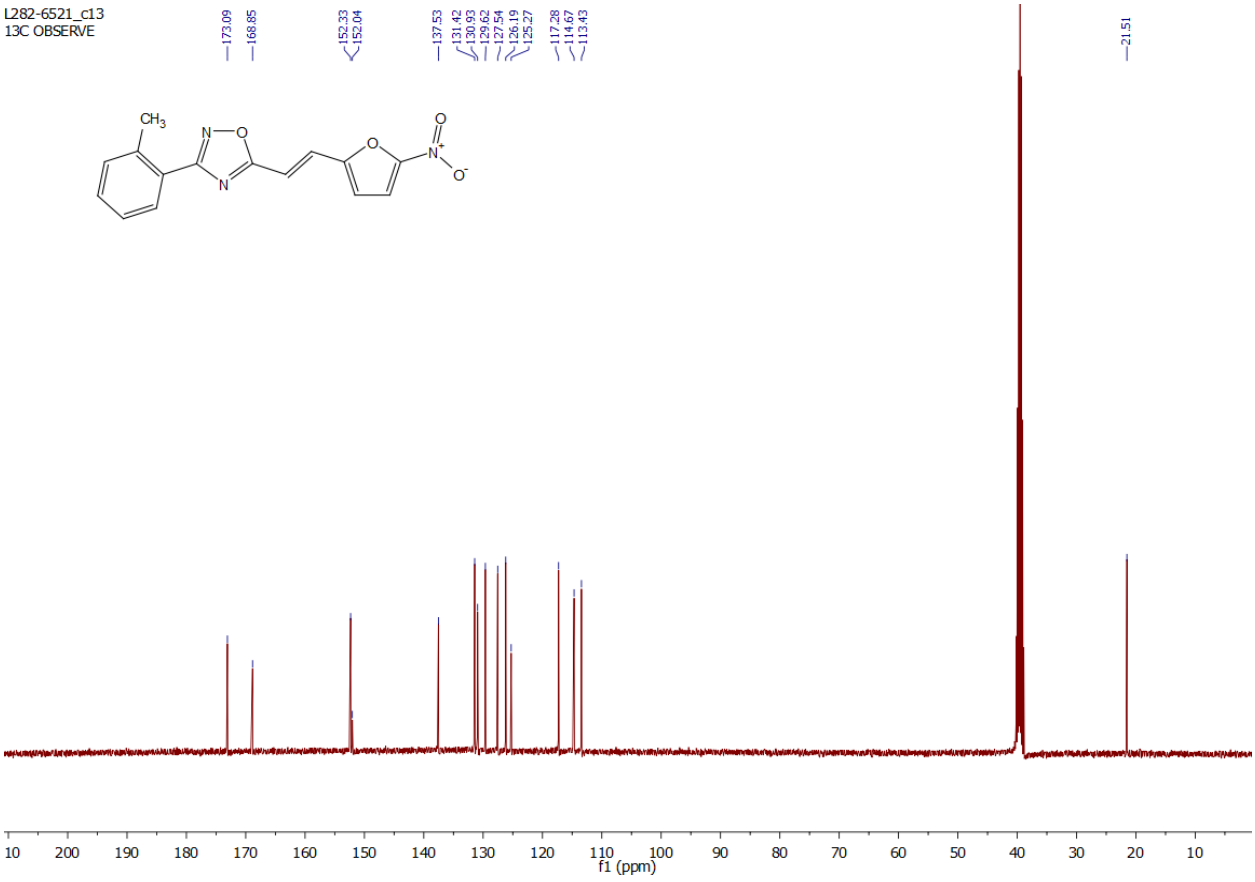
(*E*)-3-(3,4-dichlorophenyl)-5-[2-(5-nitrofuran-2-yl)vinyl]-[1,2,4]-oxadiazole (Compound **4b**)



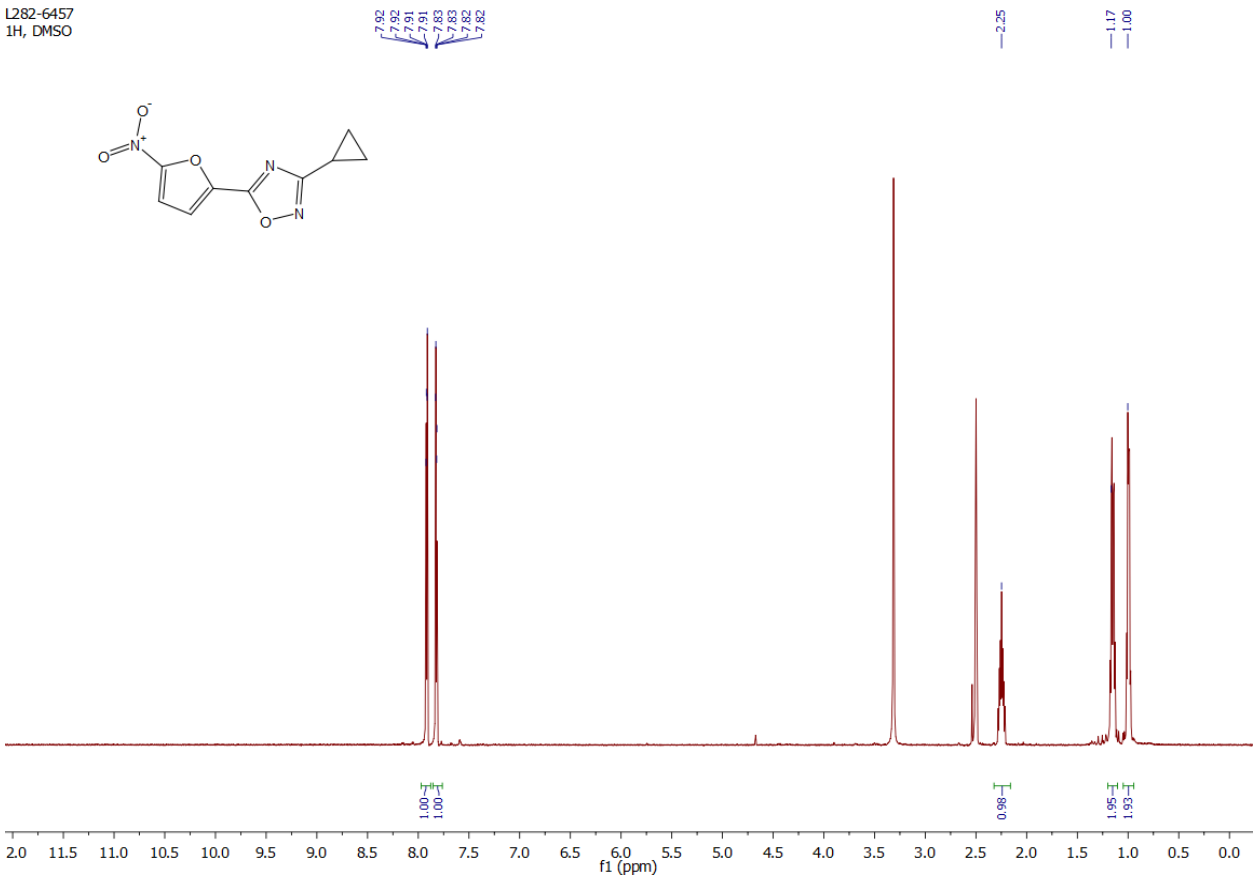

L282-7160_c13
13C OBSERVE
175.35
167.48
153.06
152.92
135.29
133.02
132.34
129.45
128.81
127.77
127.38
117.95
114.98
113.96
Cl
Cl
N
O
N
O
O
N+
O-
f1 (ppm)

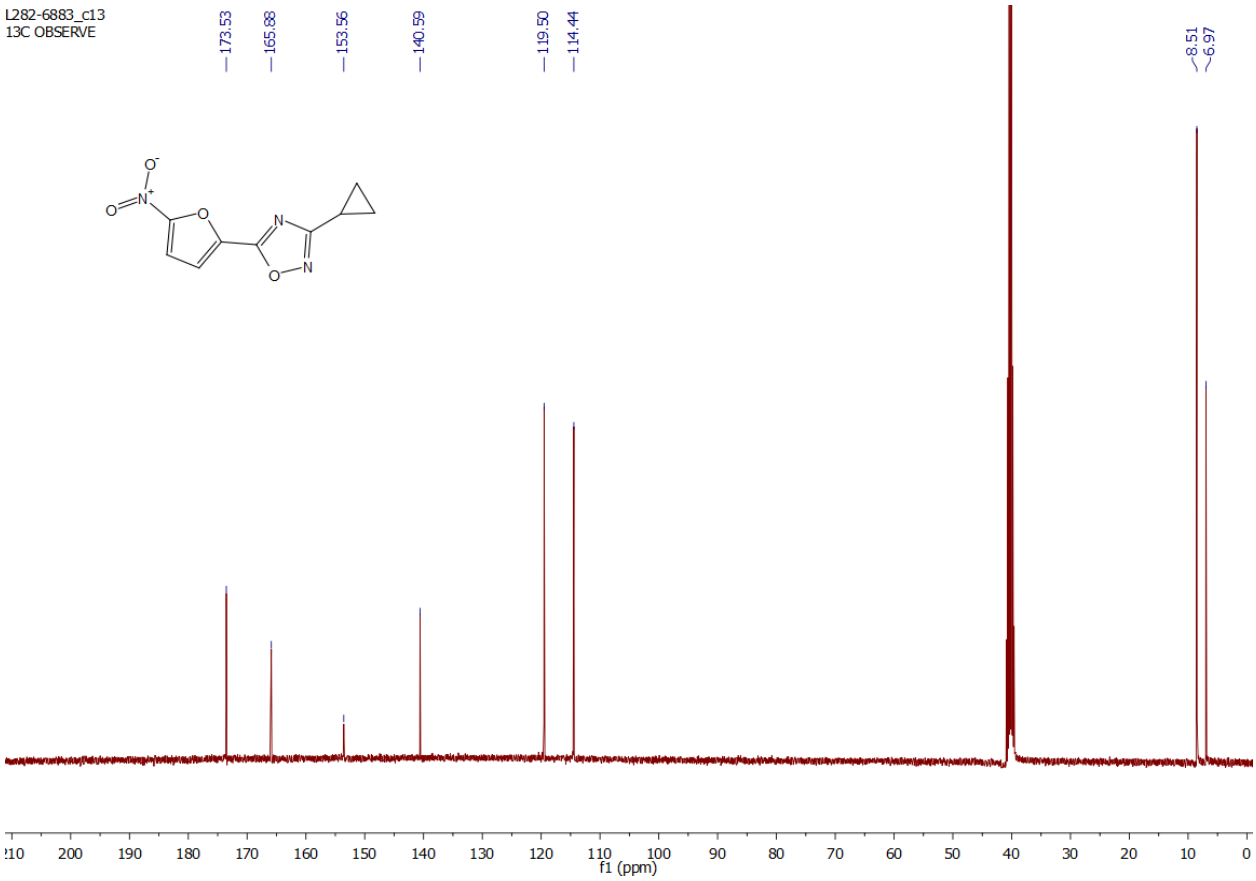
(*E*)-5-(2-(5-nitrofuran-2-yl)vinyl)-3-(o-tolyl)-[1,2,4]-oxadiazole (Compound **4c**)



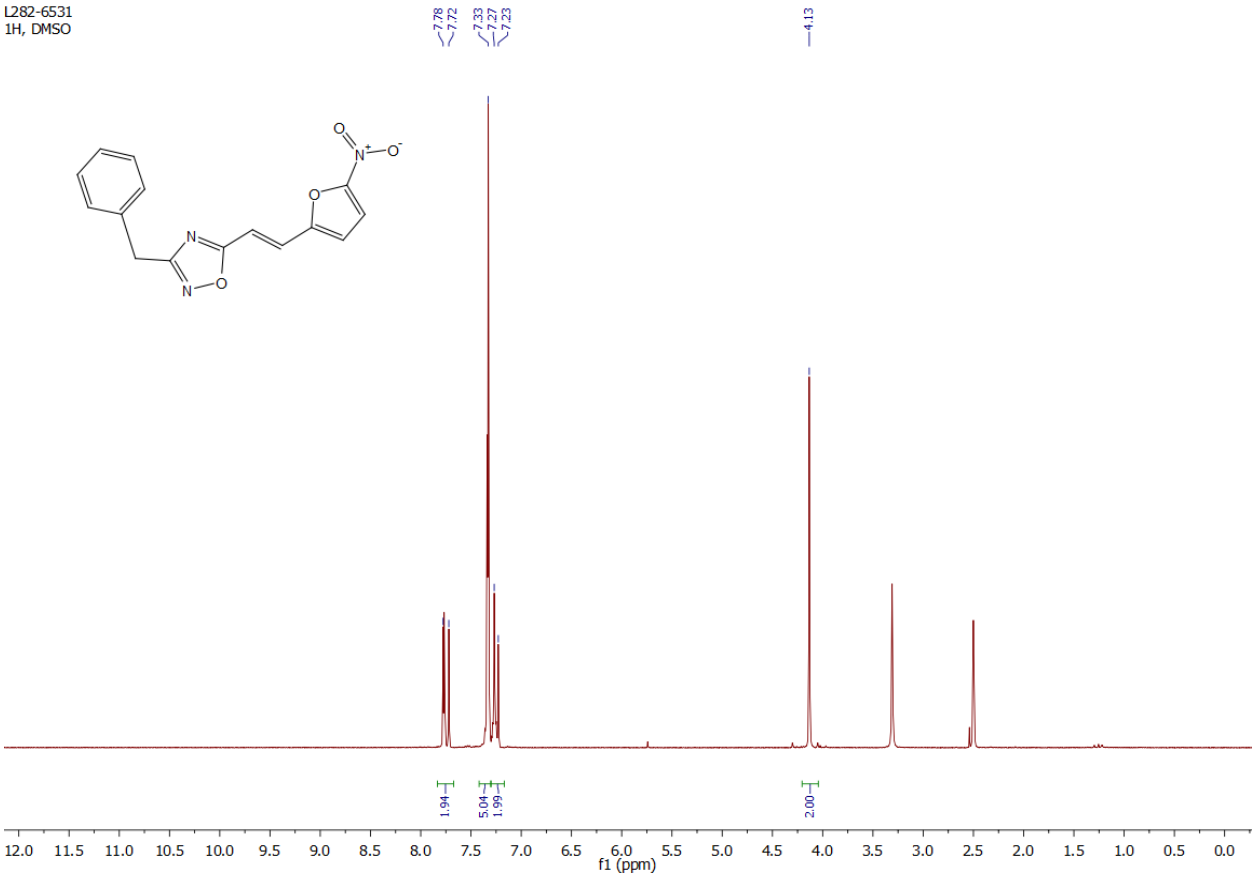


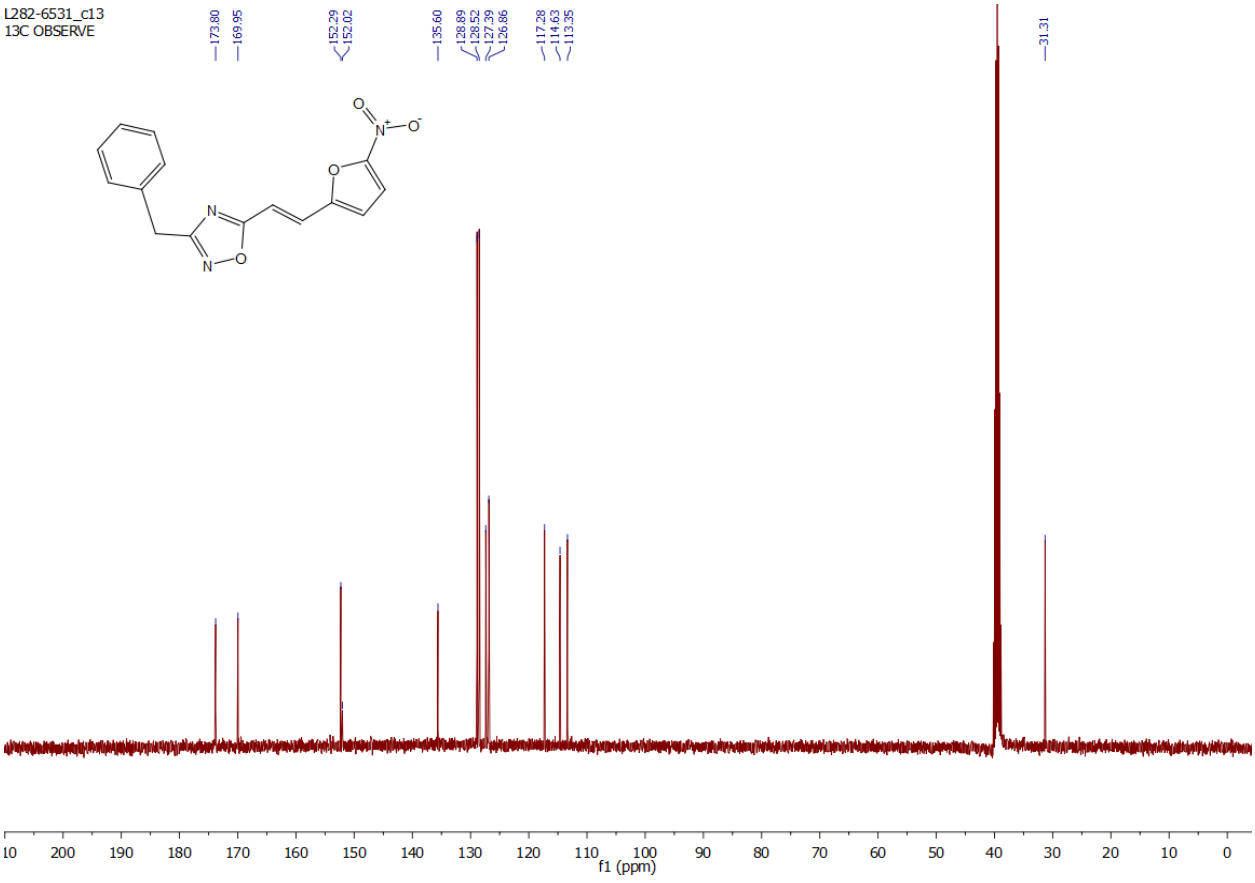
(*E*)-3-cyclopropyl-5-(2-(5-nitrofuran-2-yl)vinyl)-[1,2,4]-oxadiazole (Compound **4d**)



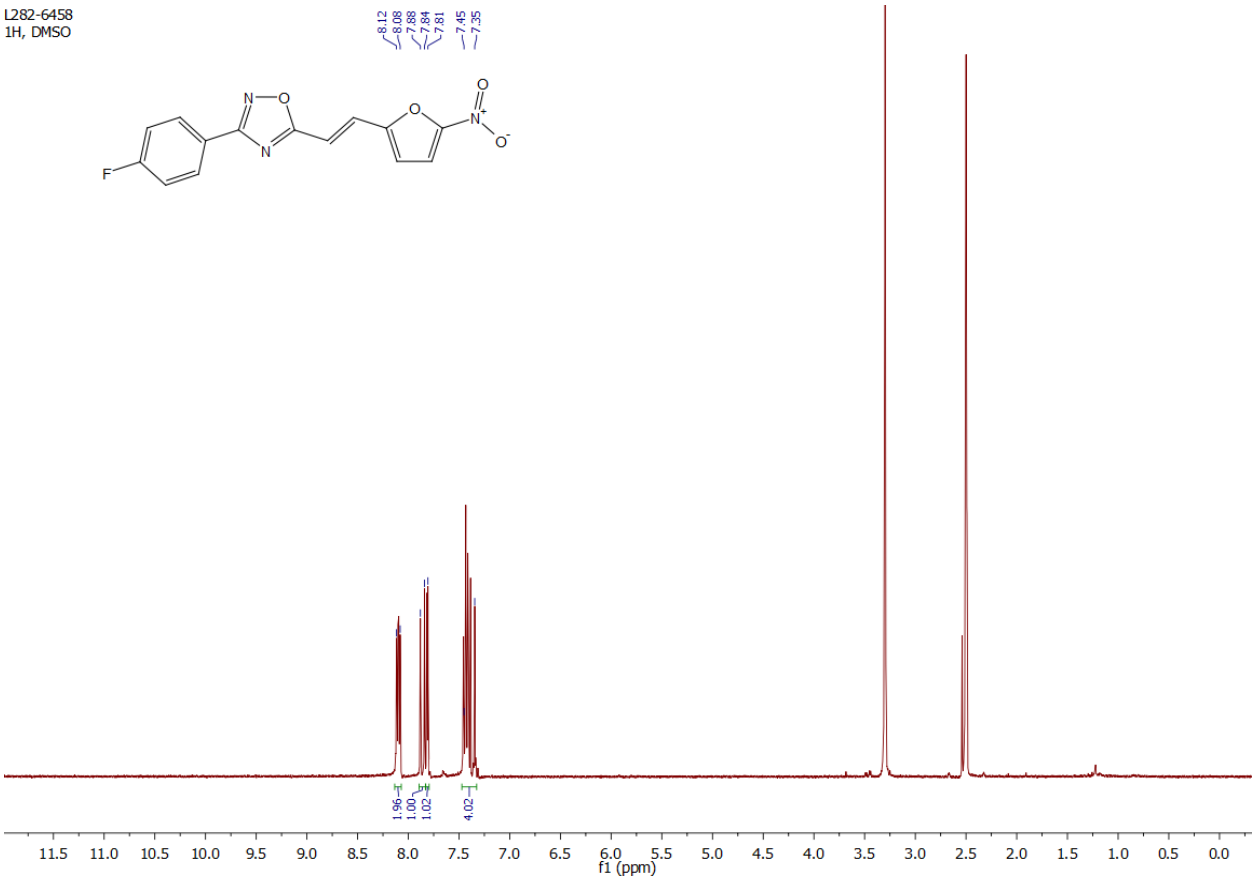


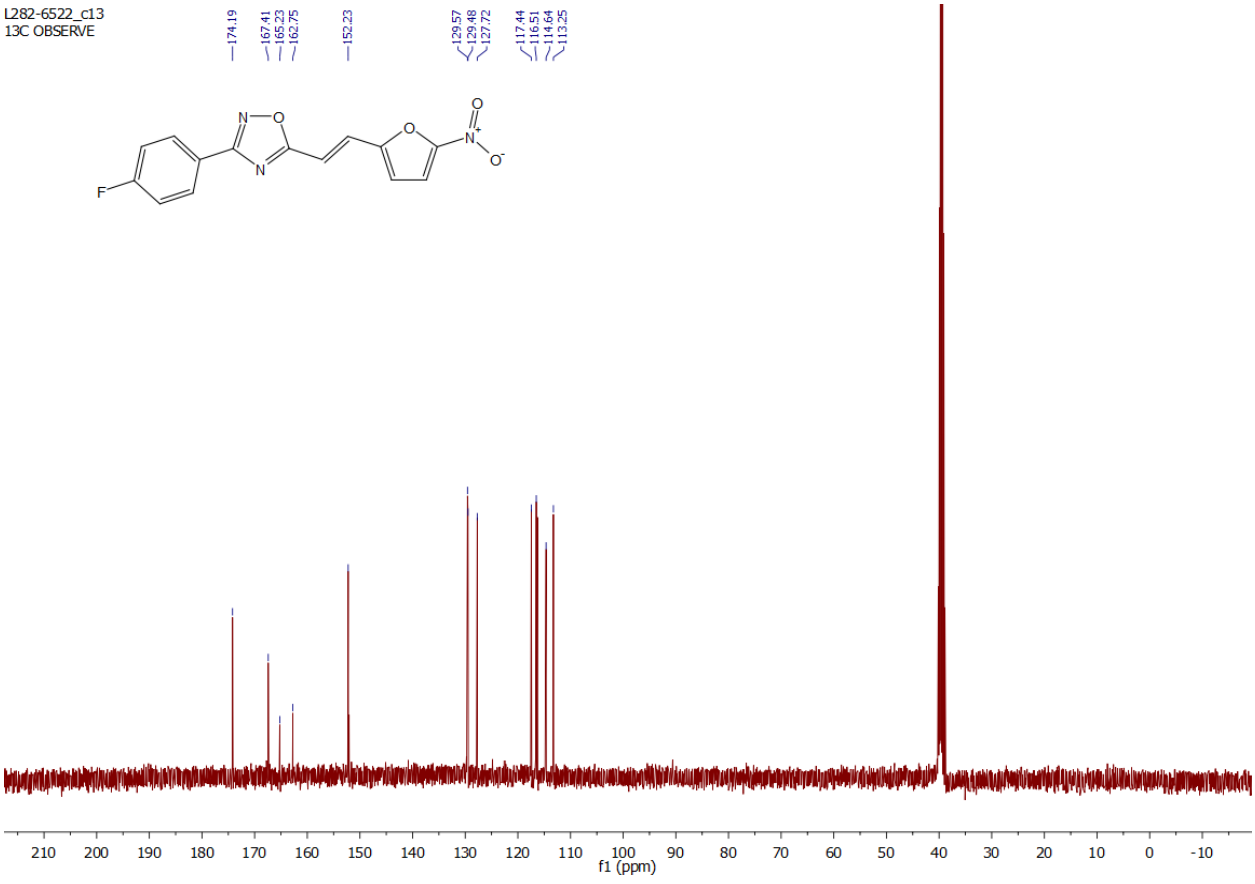
(*E*)-3-benzyl-5-(2-(5-nitrofuran-2-yl)vinyl)-[1,2,4]-oxadiazole (Compound **4e**)



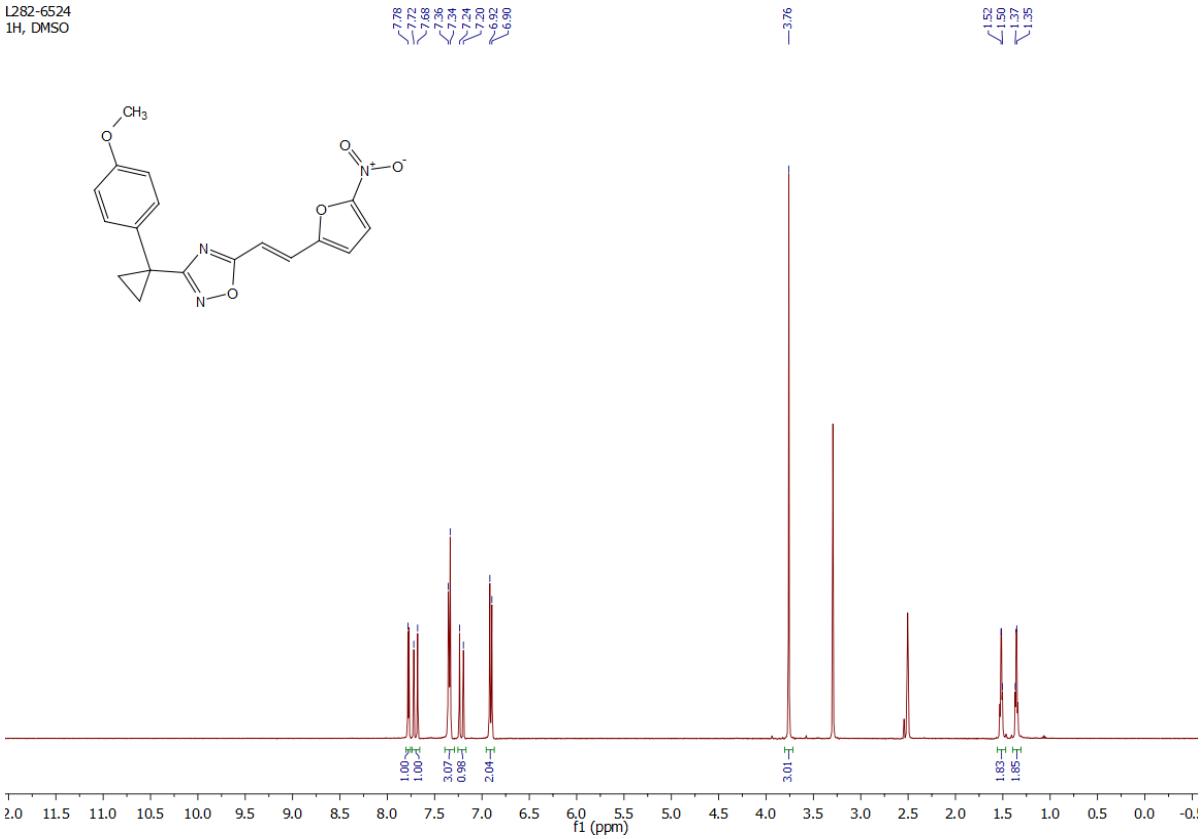


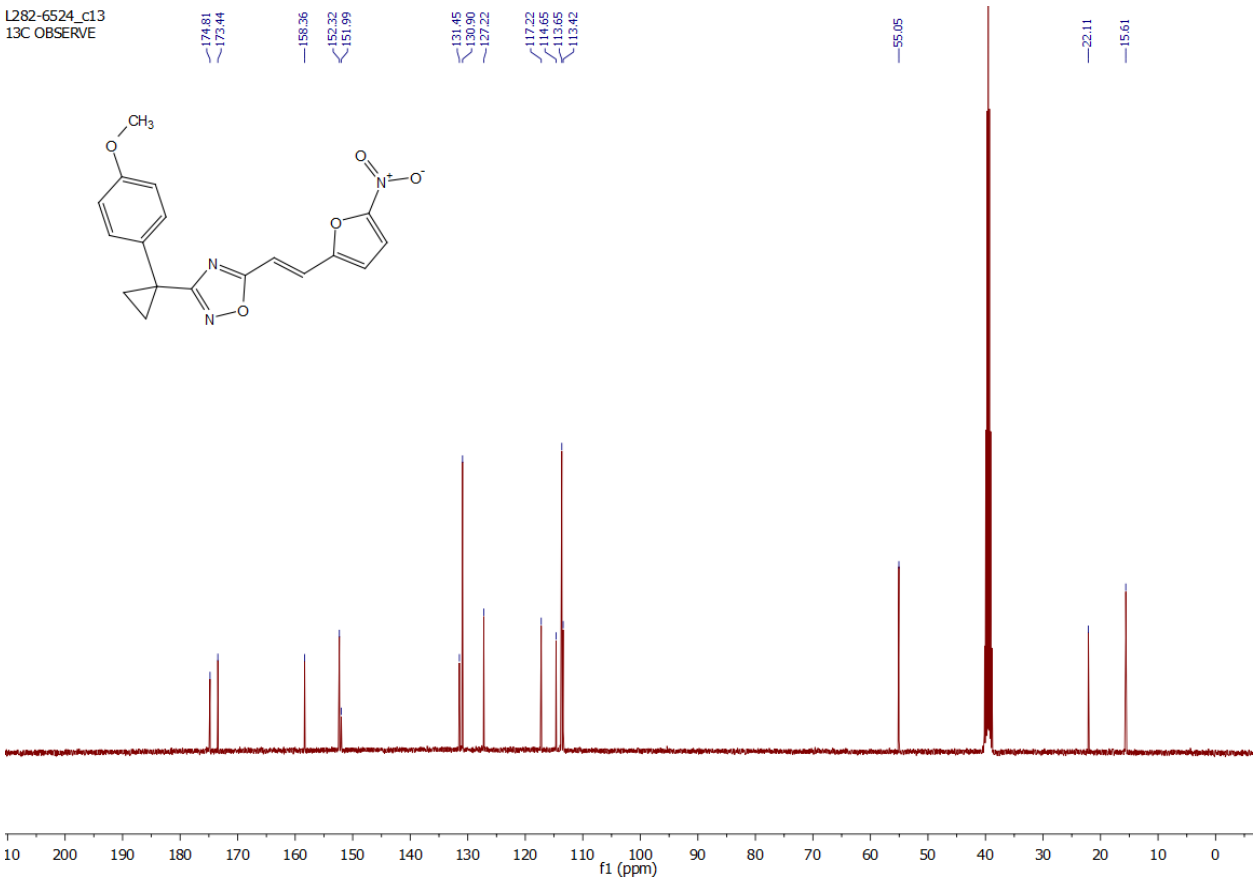
(*E*)-3-(4-fluorophenyl)-5-[2-(5-nitrofuran-2-yl)vinyl]-[1,2,4]-oxadiazole (Compound **4f**)



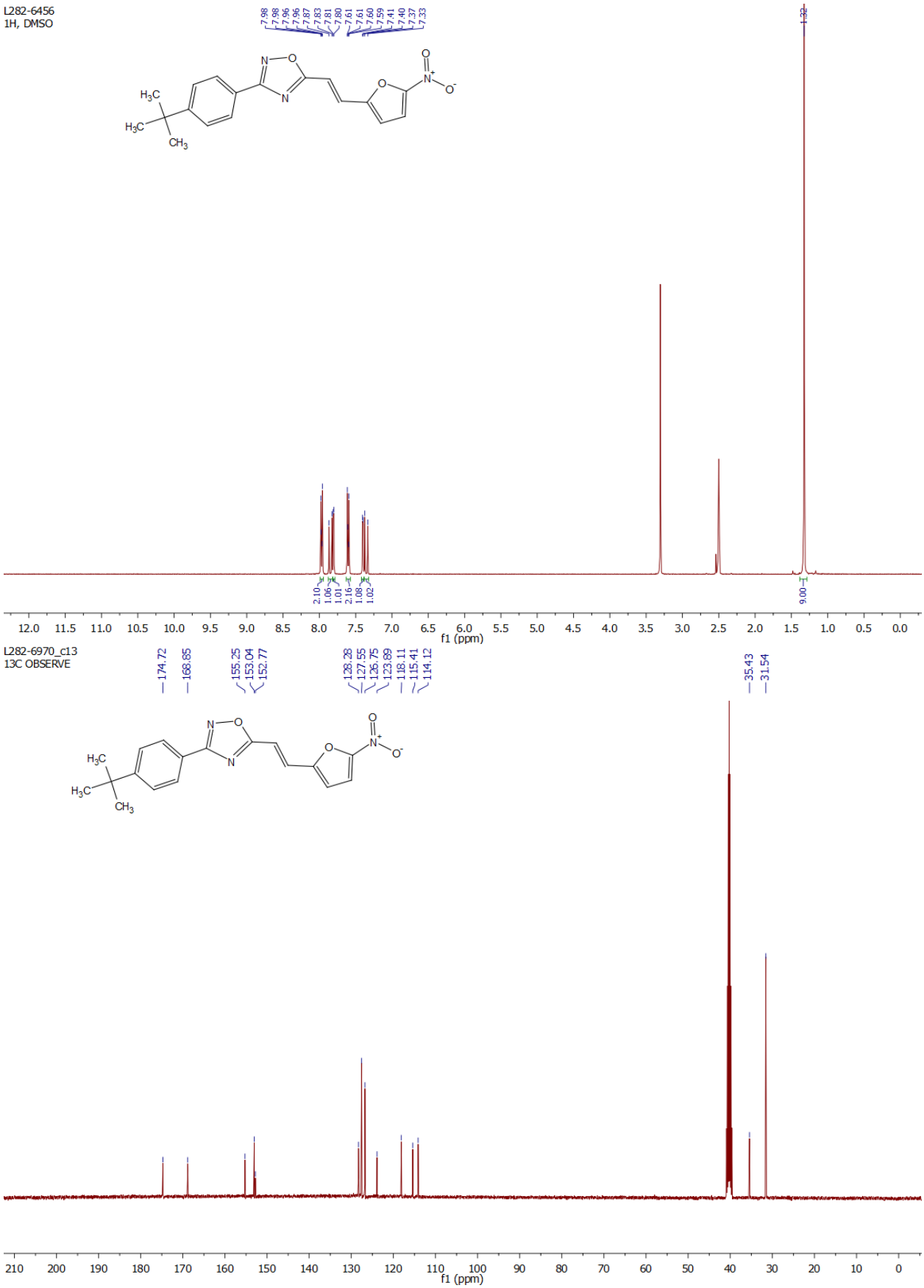


(*E*)-3-(1-(4-Methoxyphenyl)cyclopropyl)-5-[2-(5-nitrofuran-2-yl)vinyl]-4,5-dihydro-[1,2,4]-oxadiazole (Compound **4g**)

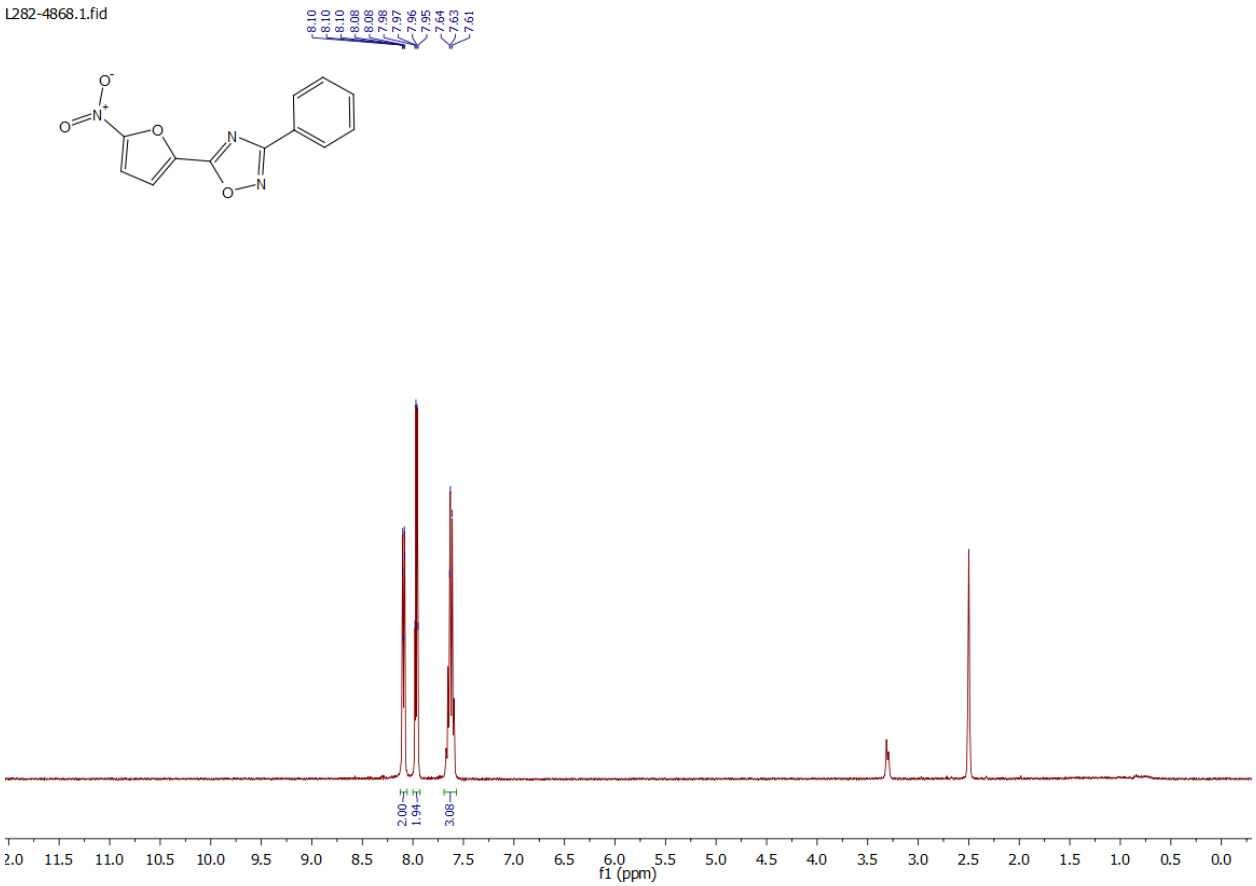


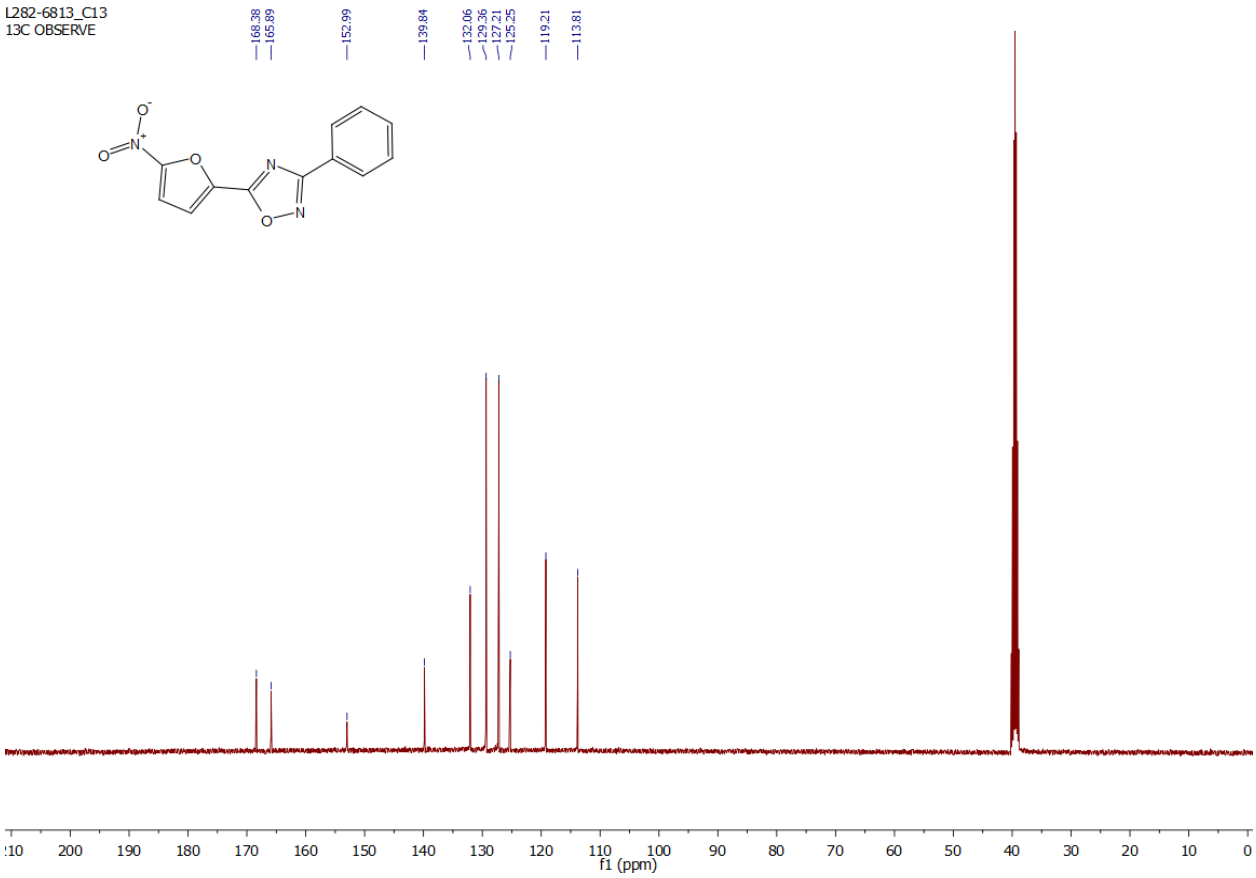


(*E*)-3-(4-(*tert*-Butyl)phenyl)-5-[2-(5-nitrofuran-2-yl)vinyl]-[1,2,4]-oxadiazole (Compound **4h**)

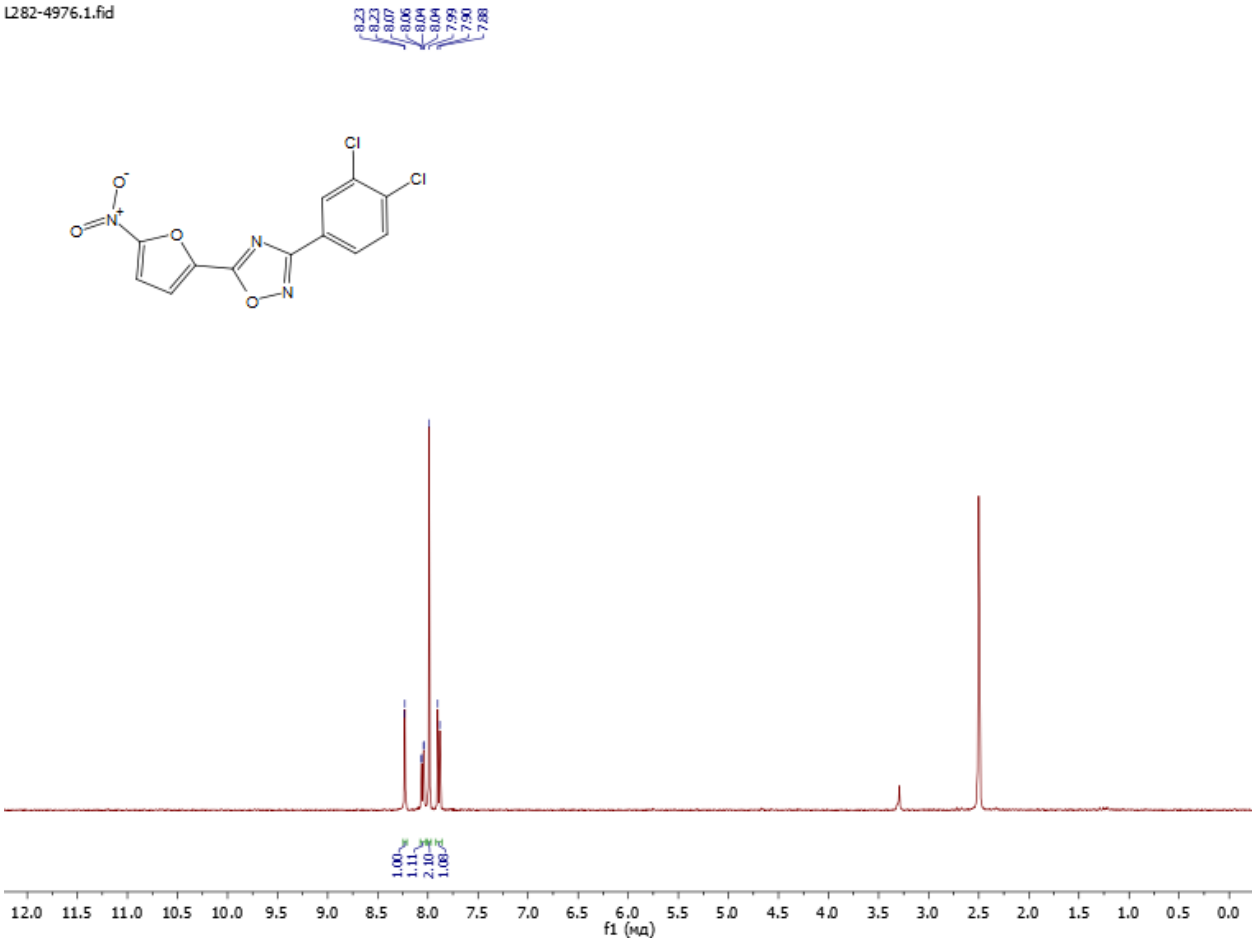


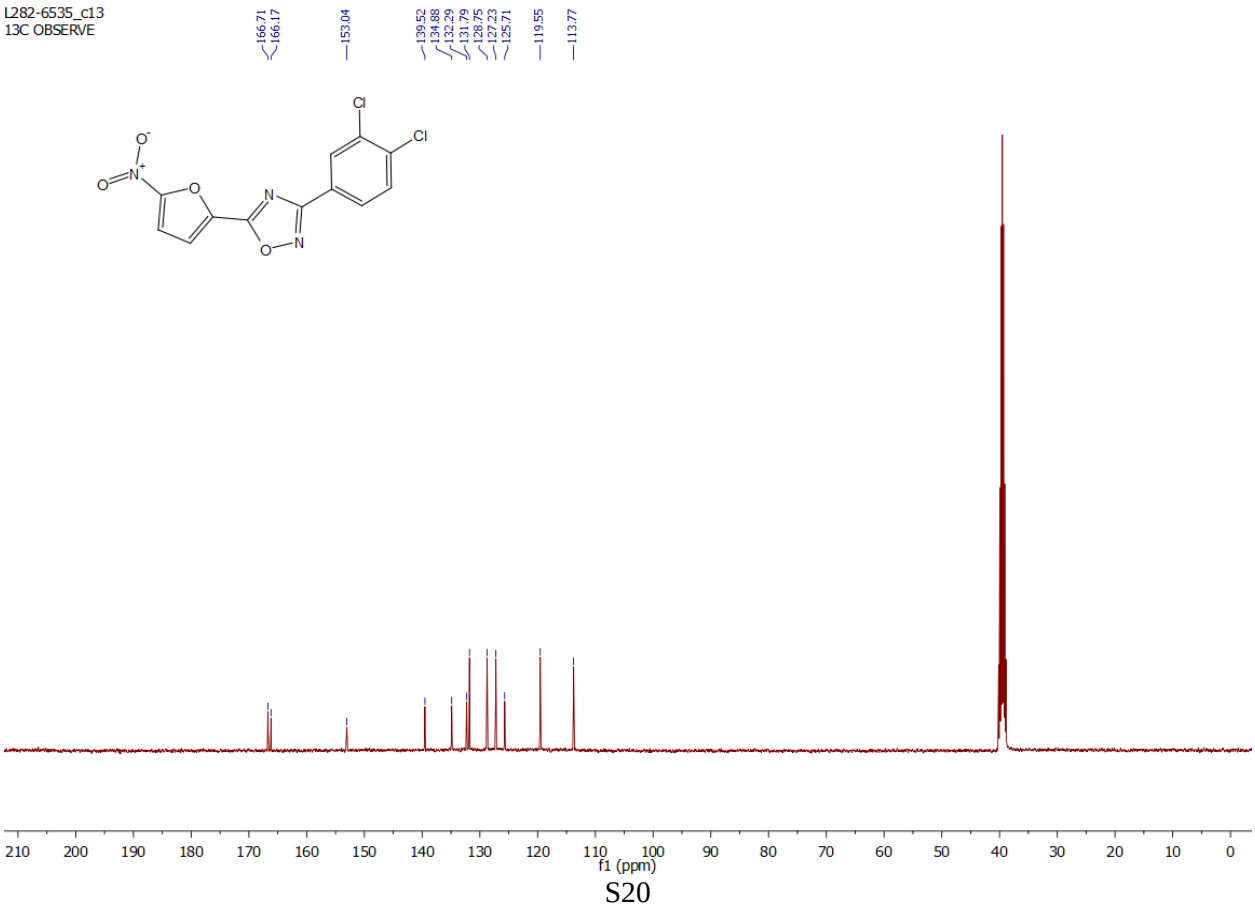
5-(5-Nitrofuran-2-yl)-3-phenyl-[1,2,4]-oxadiazole (Compound **5a**)



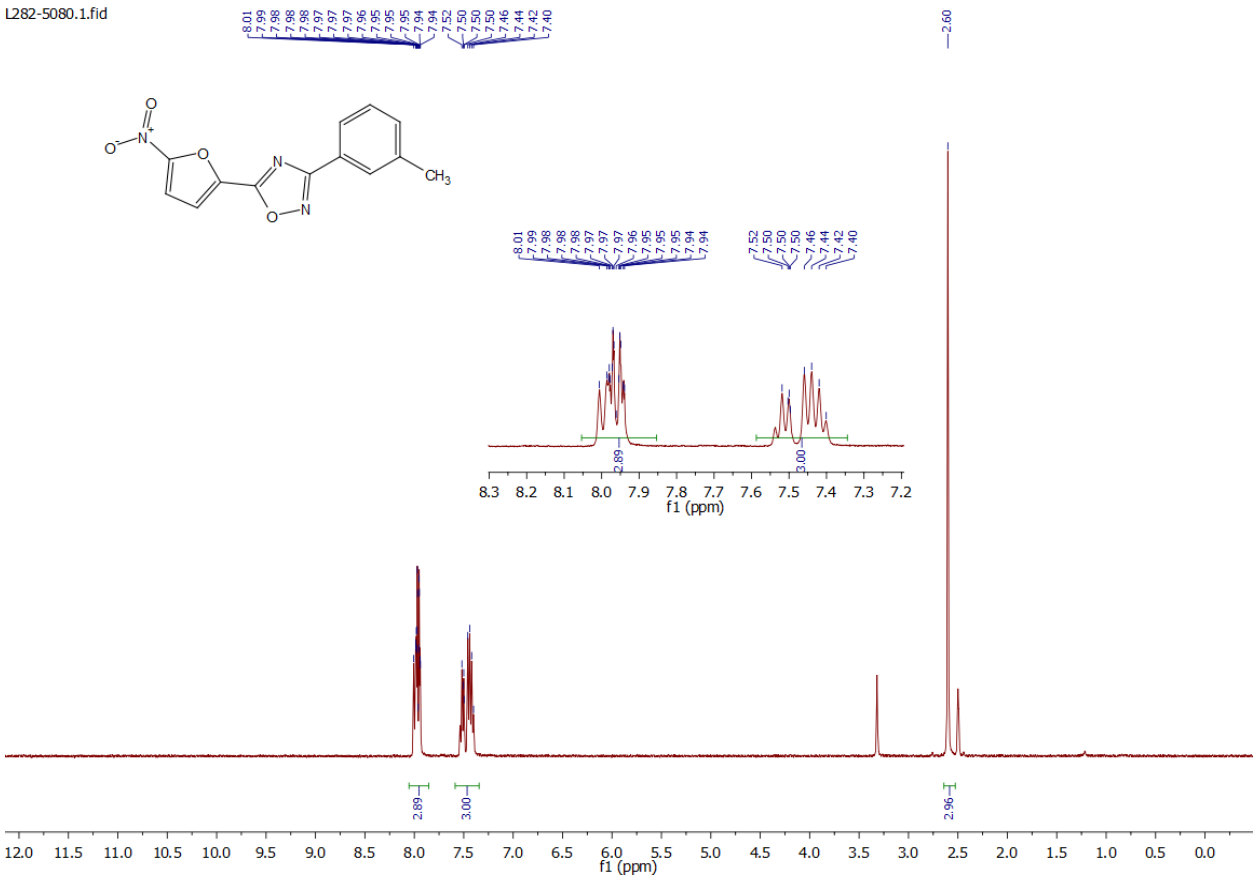


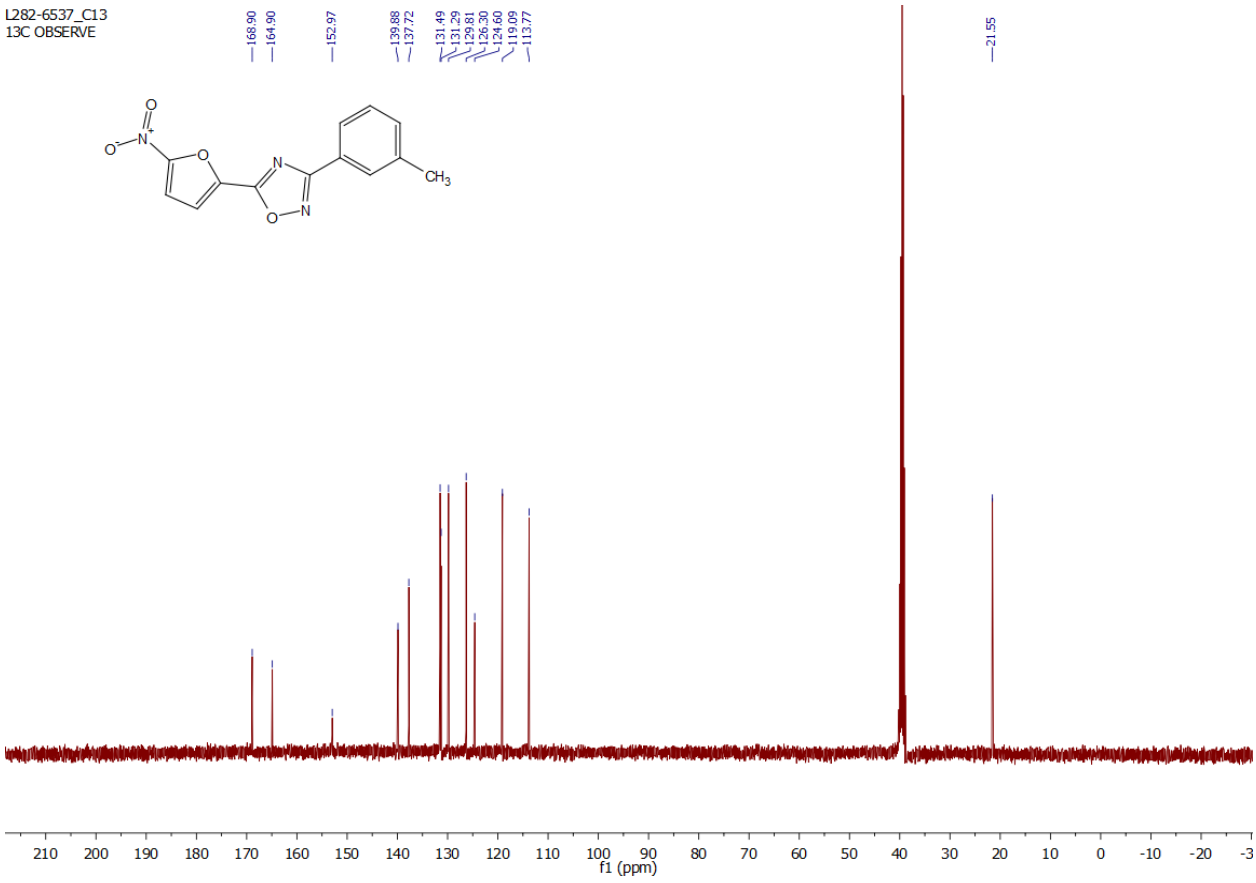
3-(3,4-Dichlorophenyl)-5-(5-nitrofuran-2-yl)-[1,2,4]-oxadiazole (compound **5b**)



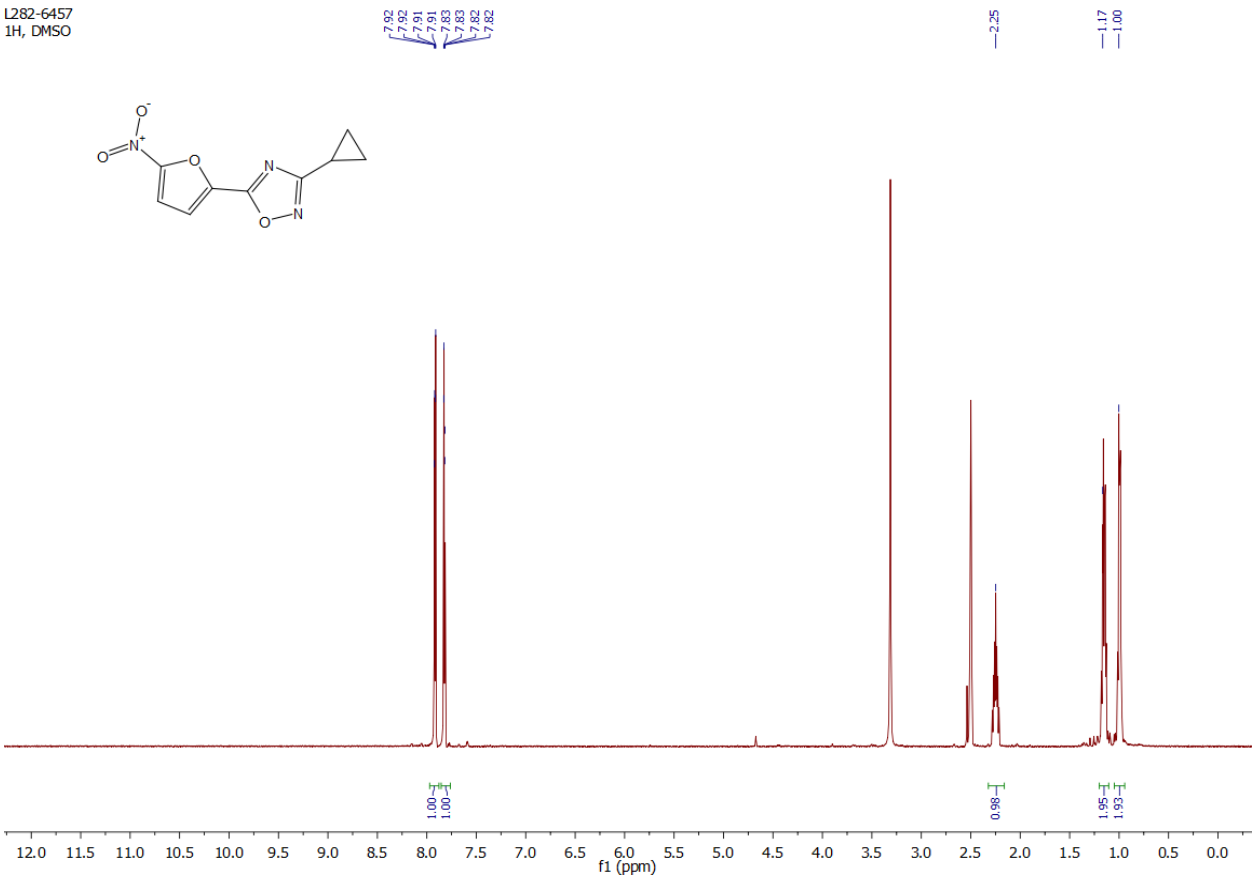


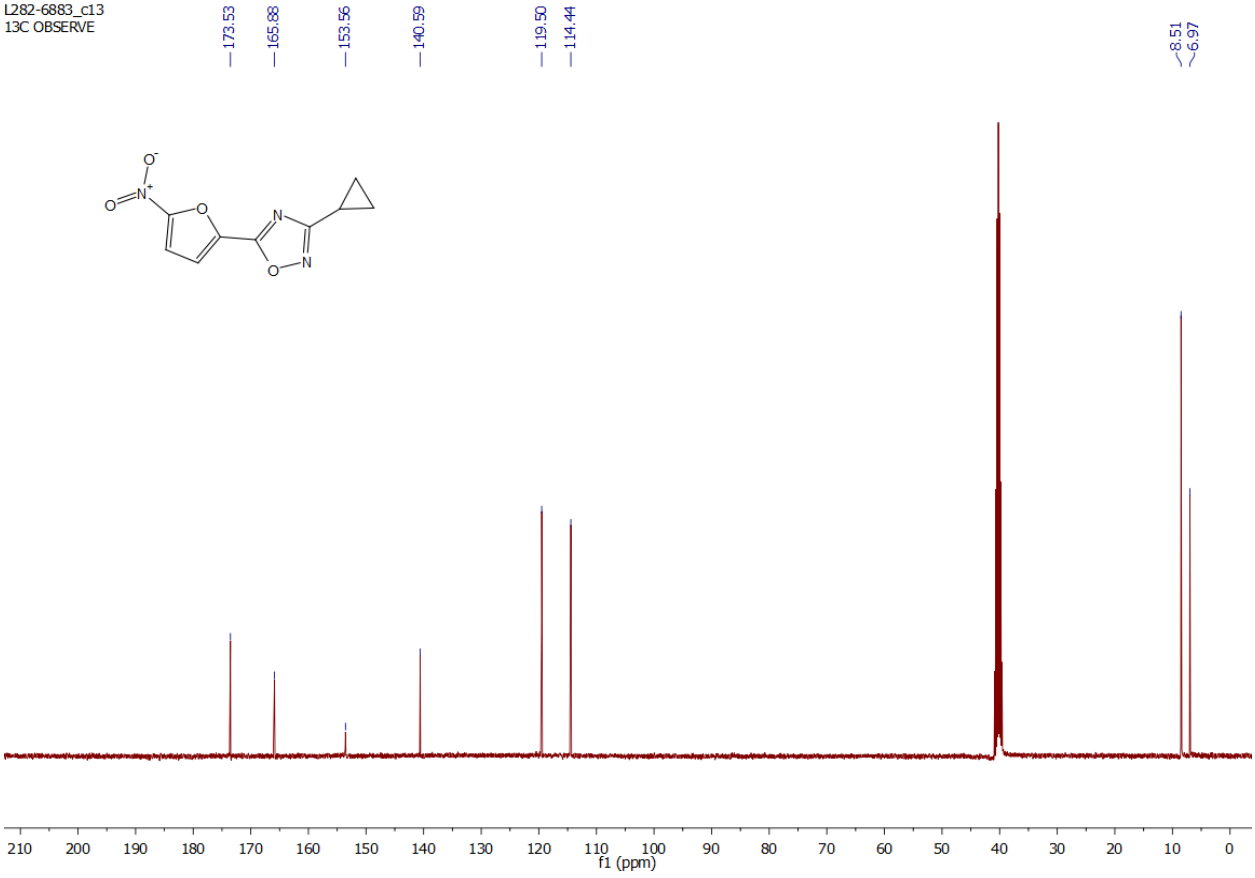
5-(5-Nitrofuran-2-yl)-3-(3-tolyl)-[1,2,4]-oxadiazole (Compound **5c**)



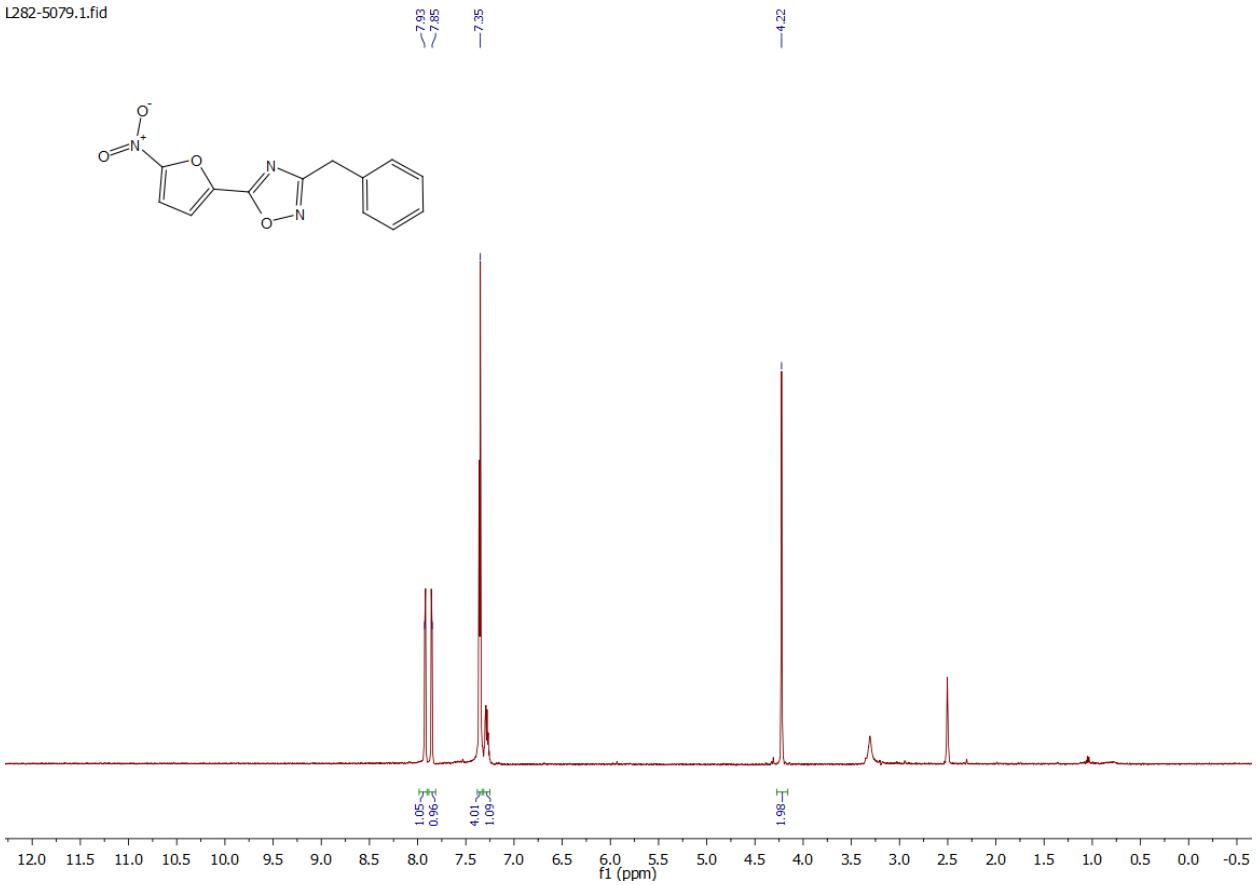


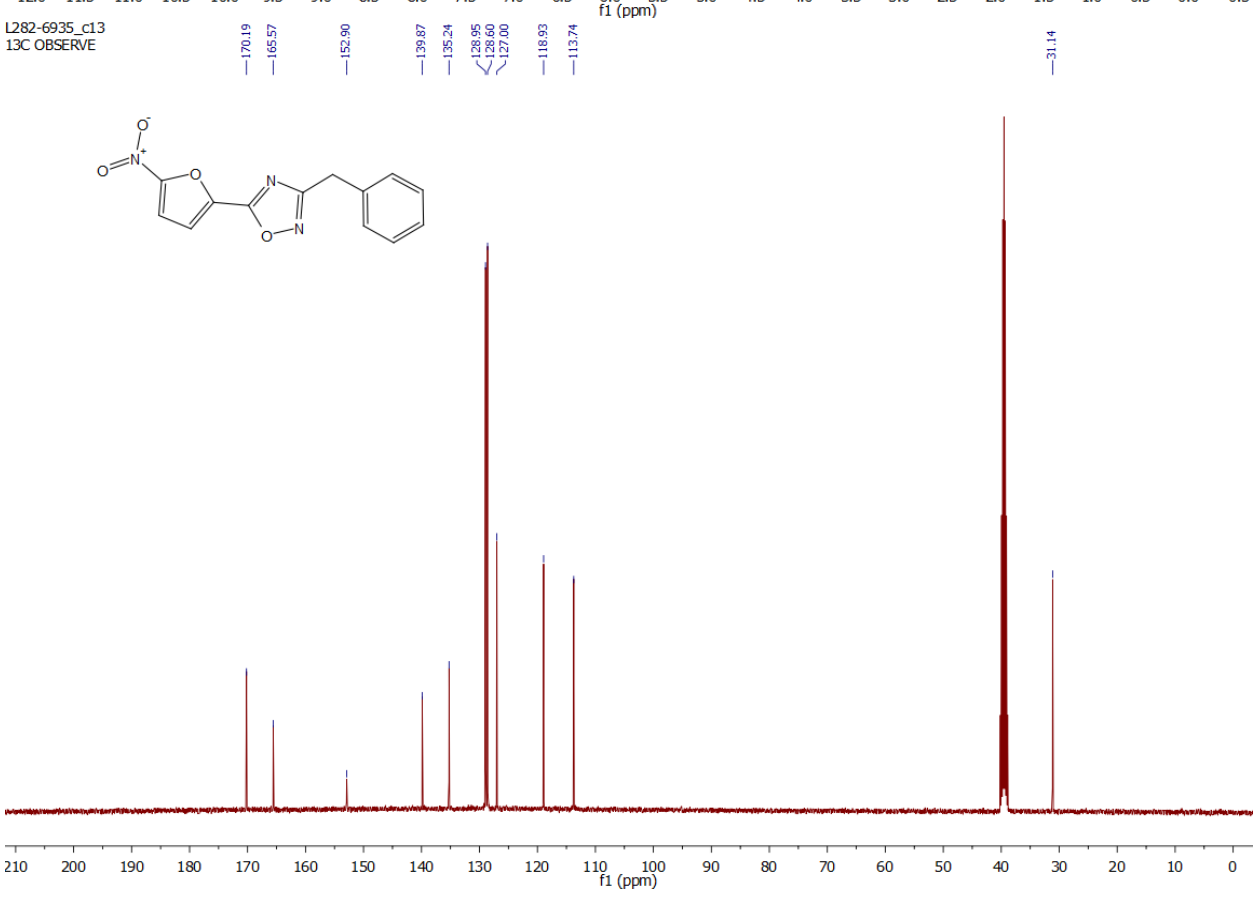
3-Cyclopropyl-5-(5-nitrofuran-2-yl)-[1,2,4]-oxadiazole (Compound **5d**)





3-Benzyl-5-(5-nitrofuran-2-yl)-[1,2,4]-oxadiazole (compound **5e**)





5-(5-Nitrofuran-2-yl)-3-(p-tolyl)-[1,2,4]-oxadiazole (Compound **5f**)