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New hybrid furoxan structures with antiaggregant activity

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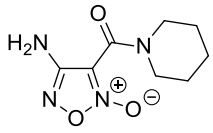
Contents

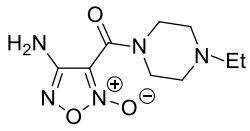
General remarks

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300.13 and 75.47 MHz, respectively) and Bruker AC-200 (200.13 and 50.32 MHz, respectively) spectrometers and referenced to residual solvent peak. The chemical shifts are reported in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Coupling constants, *J*, are reported in Hertz. The IR spectra were recorded on a Bruker "Alpha" spectrometer in the range 400-4000 cm^{-1} (resolution 2 cm^{-1}) as pellets with KBr or as a thin layer. The melting points were determined on Stuart SMP20 apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was carried out on Merck 25 TLC silica gel 60 F_{254} aluminum sheets. The visualization of the TLC plates was accomplished with a UV light. Flash chromatography was performed on silica gel 60 A (0.060-0.200 mm, Acros Organics). High resolution mass spectra were recorded on a Bruker microTOF spectrometer with electrospray ionization (ESI). 4-Amino-3-azidocarbonylfuroxan **7** [S1], 4-amino-3-phenylfuroxan **8** [S2], CAS-1609 [S3] were prepared according to published procedures.

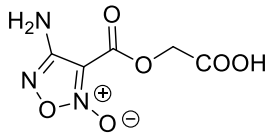
Characteristics of the synthesized compounds



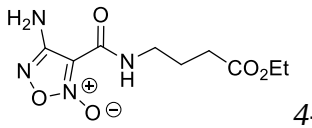
4-Amino-3-(piperidine-1-carbonyl)-1,2,5-oxadiazole 2-oxide **A**. White solid, yield 1.2 g (86%), mp 115.5-116.5 ºC (lit. [S4] mp 115-116 °C).



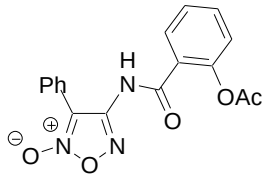
4-Amino-3-(4-ethylpiperazine-1-carbonyl)-1,2,5-oxadiazole 2-oxide **2.** White solid, yield 0.23 g (55%). Mp. 144-145 °C. R_f 0.15 ($CHCl_3$-EtOAc, 5:1). IR (KBr): 3415, 3302, 3221, 1654, 1625, 1586, 1501, 1458, 1380, 1323, 1286, 1229, 1163, 1128, 981, 870, 839 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ_H: 1.01 (3H, t, ^3J 7.0 Hz, CH_3), 2.23-2.65 (4H, m, 2 CH_2 piperazine), 3.21-3.51 (4H, m, 2 CH_2 piperazine) 3.57 (2H, d $\underline{CH_2}CH_3$), 6.46 (2H, s, NH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ_C: 11.7, 41.9, 45.9, 51.2, 51.4, 52.5, 106.0, 153.9, 155.9. HRMS (ESI) m/z for $C_9H_{16}N_5O_3$ $(M+H)^+$: calcd 242.1248, found 242.1256.



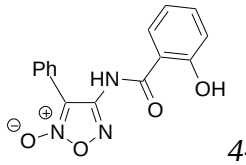
4-Amino-3-(carboxymethoxy)carbonyl-1,2,5-oxadiazole 2-oxide **3**. Light yellow solid. Yield 0.26 g (56%). Mp. 156-157 °C. R_f 0.15 ($CHCl_3$-EtOAc, 10:1). IR (KBr): 3254, 3226, 1754, 1689, 1595, 1543, 1510, 1455, 1436, 1372, 1306, 1256, 1188, 917, 760 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ_H: 4.83 (2H, s, CH_2), 6.59 (2H, s, NH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ_C: 61.6, 102.9, 155.8, 155.9, 168.2. HRMS (ESI) m/z for $C_5H_6N_3O_6$ $(M+H)^+$: calcd 204.0251, found 204.0260.



4-Amino-3-[N-(4-ethoxy-4-oxobutyl)carbamoyl])-1,2,5-oxadiazole 2-oxide **4.** White solid. Yield 0.45 g (65%). Mp. 102-103 °C. R_f 0.15 ($CHCl_3$-EtOAc, 10:1). IR (KBr): 3406, 3358, 3310, 2982, 1727, 1672, 1620, 1588, 1536, 1408, 1346, 1326, 1267, 1214, 1201, 1027, 989, 856 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ_H: 1.16 (3H, t, ^3J 7.1 Hz, CH_3), 1.76 (2H, m, CH_2), 2.31 (2H, t, ^3J 7.1 Hz ,CH_2), 3.31 (2H, m, CH_2), 4.31 (2H, q, ^3J 7.1 Hz, $\underline{CH_2}CH_3$), 6.54 (2H, s, NH_2), 8.35 (1H, br s, NH). ^{13}C NMR (50.3 MHz, DMSO-d_6) δ_C: 14.0, 24.1, 30.8, 38.0, 59.8, 104.5, 155.8, 157.1, 172.5. HRMS (ESI) m/z for $C_9H_{15}N_4O_5$ $(M+H)^+$: calcd 259.1037, found 259.1049.



4-(2-Acetoxybenzamido)-3-phenyl-1,2,5-oxadiazole 2-oxide **5.** White solid. Yield 0.54 g (39%). Mp. 122-123 °C. R_f 0.21 ($CHCl_3$-EtOAc, 10:1). IR (KBr): 3468, 3351, 1749, 1721, 1610, 1548, 1459, 1427, 1281, 1220, 1050, 939, 831 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ_H: 2.23 (3H, s, CH_3), 7.19 (2H, d, ^3J 7.8 Hz, H Ar), 7.37 (3H, t, ^3J 7.8 Hz, H Ar), 7.51-7.67 (2H, m, H Ar), 7.70-7.77 (1H, m, H Ar), 7.88 (1H, d, ^3J 7.8 Hz, H Ar), 10.85 (1H, s, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ_C: 22.6, 111.9, 122.3, 123.7, 125.9, 127.0, 128.8, 130.5, 131.2, 133.6, 150.8, 165.5. HRMS (ESI) m/z for $C_{17}H_{14}N_3O_5$ $(M+H)^+$: calcd 340.0927, found 340.0918.



4-(2-Hydroxybenzamido)-3-phenyl-1,2,5-oxadiazole 2-oxide **6**. White solid. Yield 0.15 g (58%). Mp. 162-163 °C. R_f 0.18 ($CHCl_3$-EtOAc, 10:1). IR (KBr): 3278, 1698, 1604, 1509, 1450, 1420, 1250, 1231, 1152, 1077, 982, 858, 773 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ_H: 7.49-7.81 (9H, m, H Ar), 10.89 (1H, s, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ_C: 111.9, 123.3, 124.7, 125.0, 127.1, 128.8, 129.0, 130.4, 132.3, 133.5, 136.0, 151.2, 169.8. HRMS (ESI) m/z for $C_{15}H_{12}N_3O_4$ $(M+H)^+$: calcd 298.0822, found 298.0813.

Study of cytotoxic, antiaggregant and NO-donor activity

MTT-test. Human mononuclear cells (MNC) were isolated from the peripheral vein blood of healthy donor as described previously [S5]. To a suspension of LnCap cells (ATCC, 400 μl) or MNC (10^6 per ml) in RPMI-1640 medium (Paneco, Russia) with 10% of fetal bovine serum, furoxan solution in 0.9% $NaCl_{aq}$ (0.5 *M*, 40 μl) was added. A portion (100 μl) of this mixture was put in 96-well plate in triplicate and incubated at 37 °C. A solution of $NaCl_{aq}$ (0.9%) was used as a control. After 24 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 1 g dm^{-3} solution, 50 μl) was added to each well, and the plate was incubated at 37 °C for 2 h. Then the medium was aspirated and the formazan crystals were dissolved using DMSO (100 μl). After 15 min, absorbance was measured at 590 nm using a plate-reader. The ratio between optical density in experiment and in a control was calculated. The value ~ 1 was considered as a lack of cell death.

Antiaggregant activity. The test was performed using Biola platelet aggregation analyzer (Biola Ltd, Russia) according to the established procedure. ADP, adrenaline, collagen, ristocetin and arachidonic acid (Helena, GB) kits were used for the experiments. Blood with citrate buffer (9:1) was centrifuged at 100 *g* for 10 min, and platelets rich plasma (PRP) was collected. To heated at 37 °C PRP (300 μl), water solution of a tested sample (1.25 m*M*, 10 μl) was added, and the mixture was incubated at 37 °C for 2 min under stirring. Then a solution of an inducer (30 μl) was added, and light transmission was measured for 6-10 min at 37 °C. Purified water was used in control experiments. If aggregation did not occur, another inducer of aggregation was added and light transmission was measured for 5 min at 37 °C. The results of antiaggregant activity of furoxans **1-6** are presented on Figures S1,S2.

NO release assay. The test molecule (0.1 mmol) was dissolved in DMSO (50 ml). A 20 μl aliquot of the solution was diluted with phosphate buffer solution (180 μl, pH 7.4, containing 2 μmol L-cysteine). The final concentration of the furoxan derivative was $2 \cdot 10^{-4}$ *M*. The mixture was incubated at 37 °C for 1 h. A 50 μl aliquot of the Griess reagent (prepared by mixing sulfanilamide (4 g), *N*-naphthylethylenediamine dihydrochloride (0.2 g) and 85% H_3PO_4 (10 ml) in distilled and deionized water (final volume 100 ml) was added and incubated for 10 min at 37 °C. UV absorbance at 540 nm was measured using a Multiskan GO Microplate Photometer and calibrated using a standard curve prepared from standard solutions of $NaNO_2$ to give the nitrite concentration. All measurements were made in triplicate. No significant NO release was measured at the absence of L-cysteine.

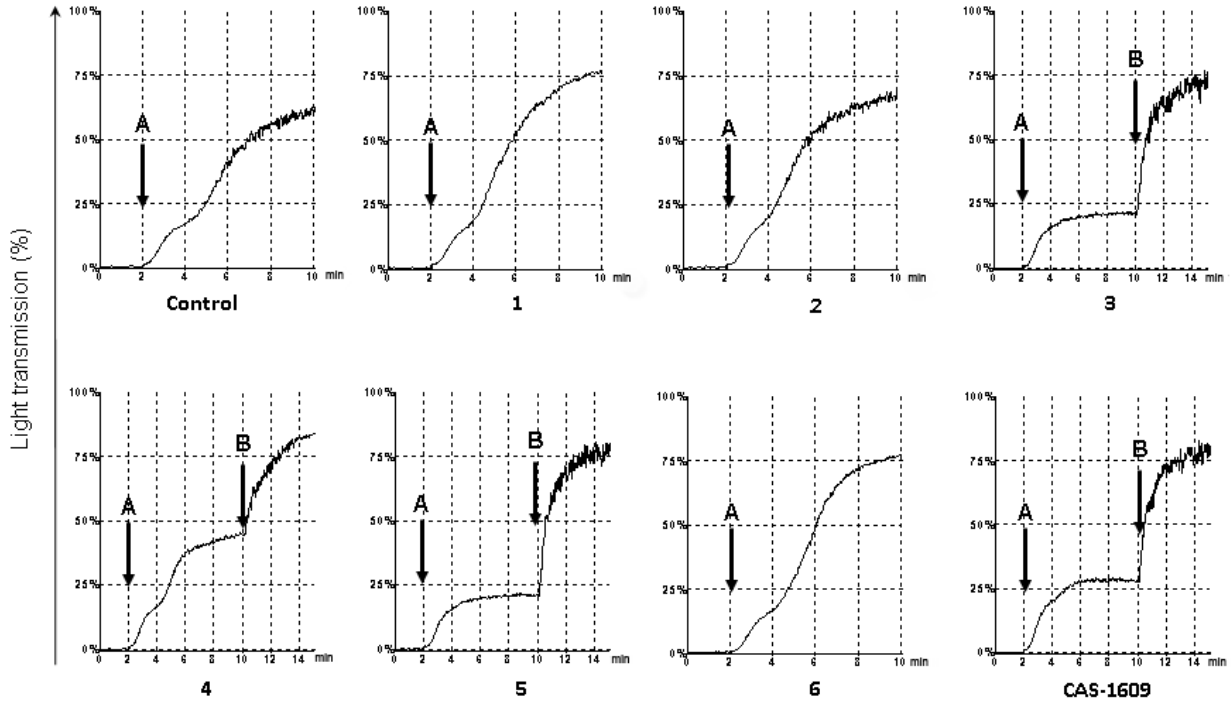


Figure S1 Influence of furoxans **1-6** on platelets aggregation *in vitro.* **A** – adrenaline, **B** – ristocetin.

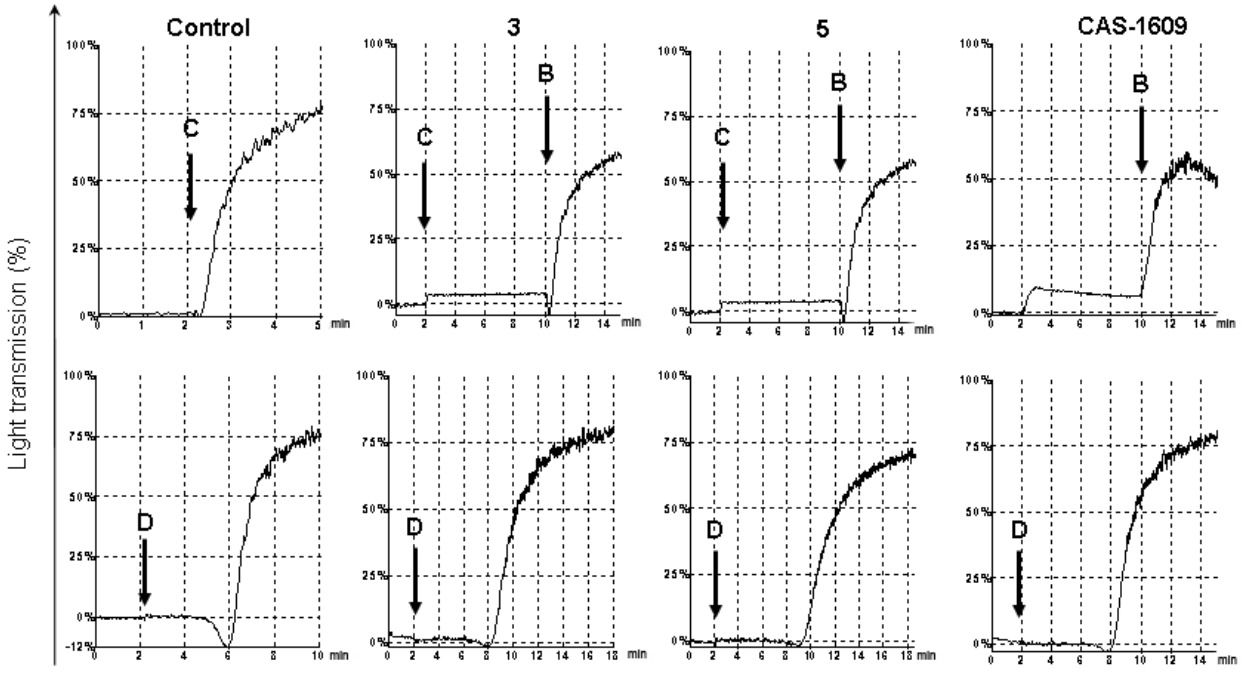


Figure S2. Influence of furoxans **3** and **5** on platelets aggregation *in vitro.* **B** – ristocetin, **C** – ADP, **D** - collagen.

References

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[S2] A. R. Gagneux and R. Meier, *Helv. Chim. Acta*, 1970, **53**, 1883.

[S3] H. Bohn, J. Brendel, P. A. Martorana and K. Schönafinger, *Br. J. Pharmacol.*, 1995, **114**, 1605.

[S4] I. V. Ovchinnikov, A. S. Kulikov, N. N. Makhova, P. Tosco, A. Di Stilo, R. Fruttero and A. Gasco, *Il Farmaco*, 2003, **58**, 677.

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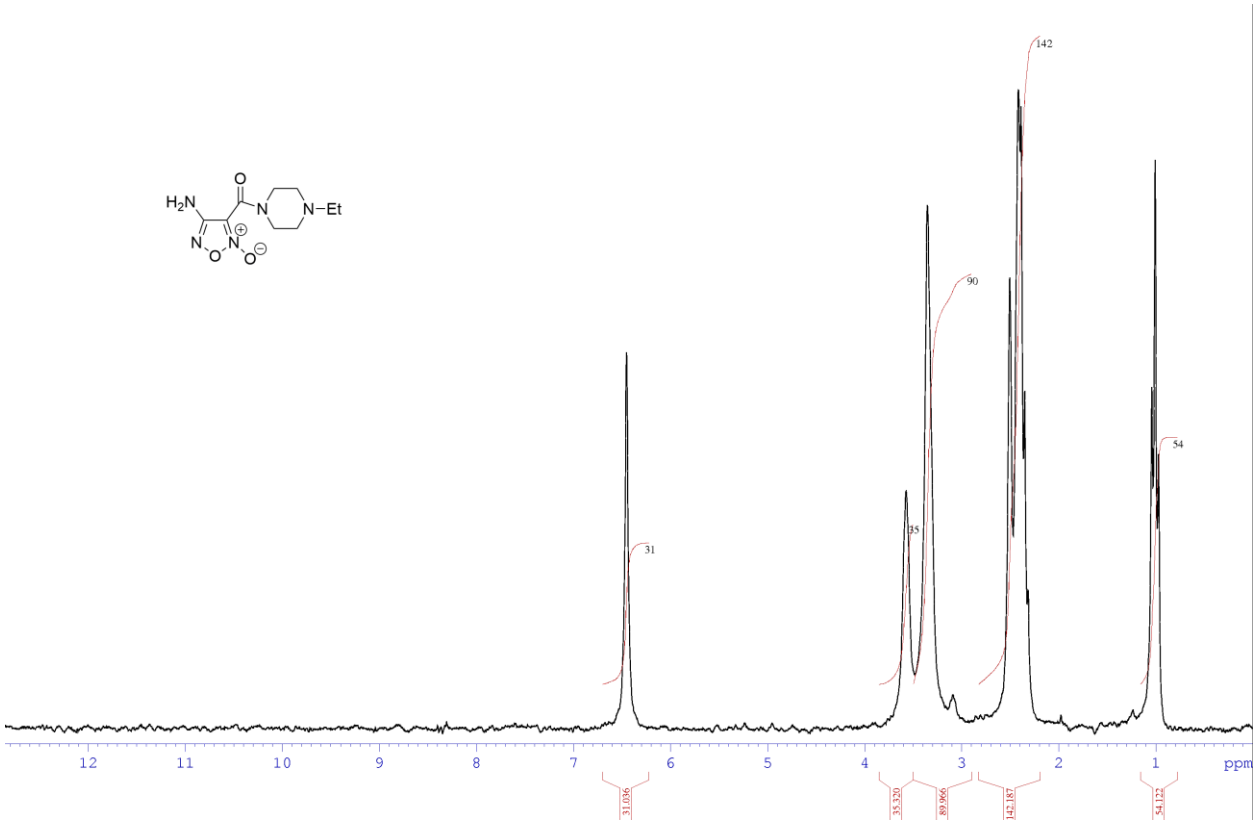
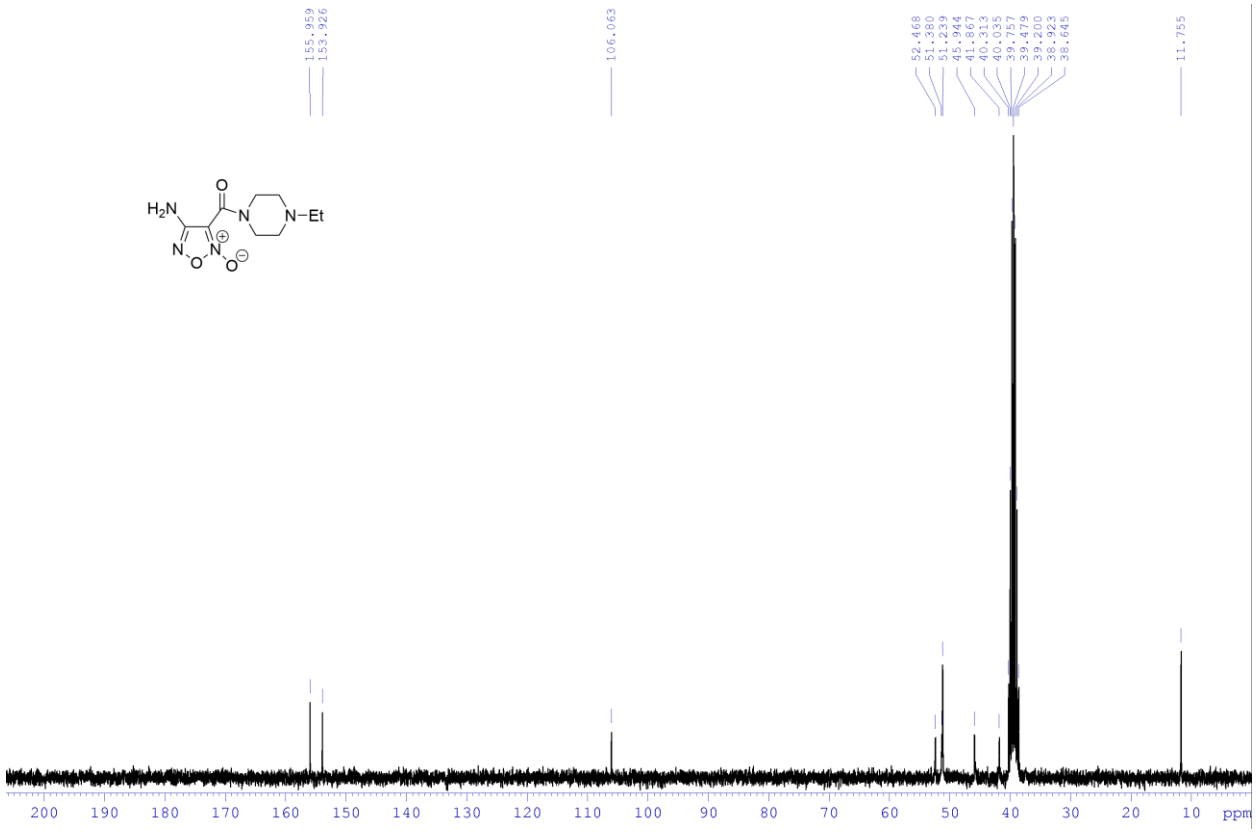
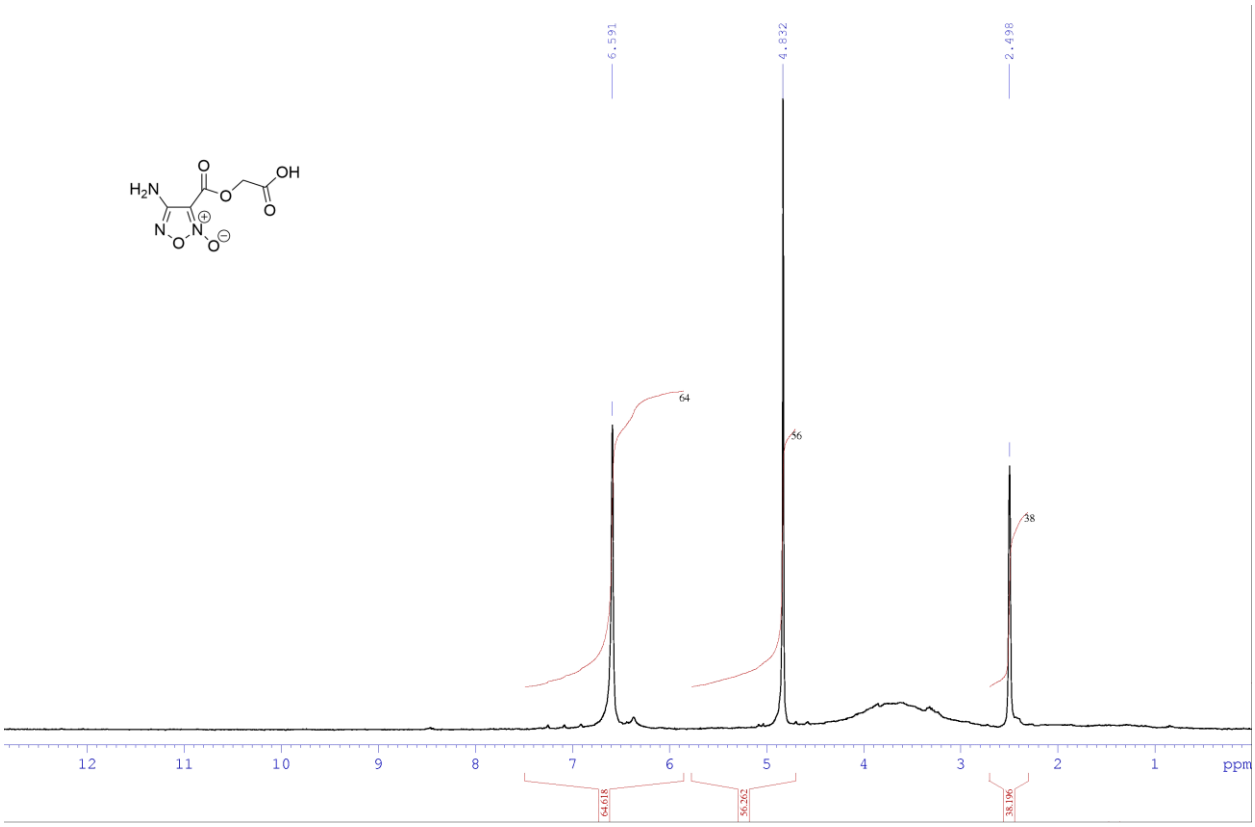
Copies of ^{1}H and ^{13}C NMR spectra



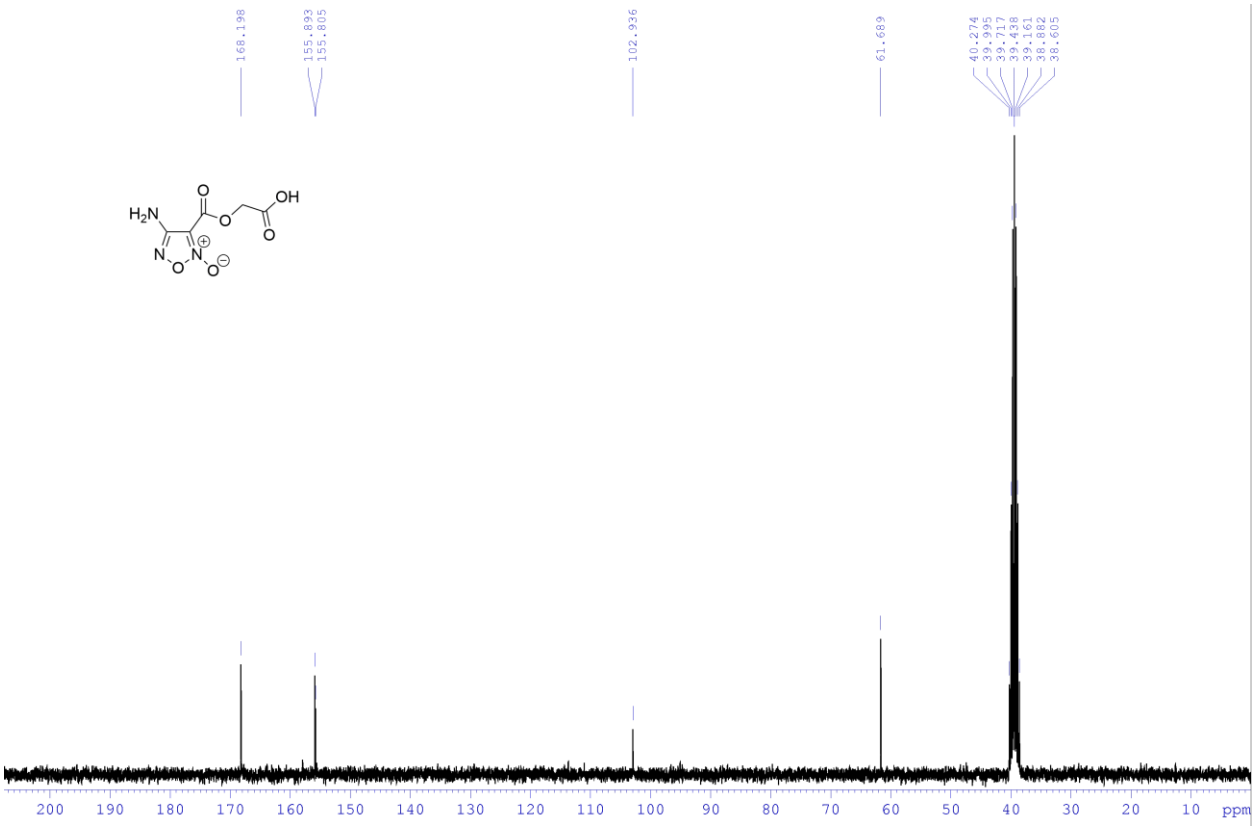
^{1}H NMR (200 MHz, DMSO) of compound **2**



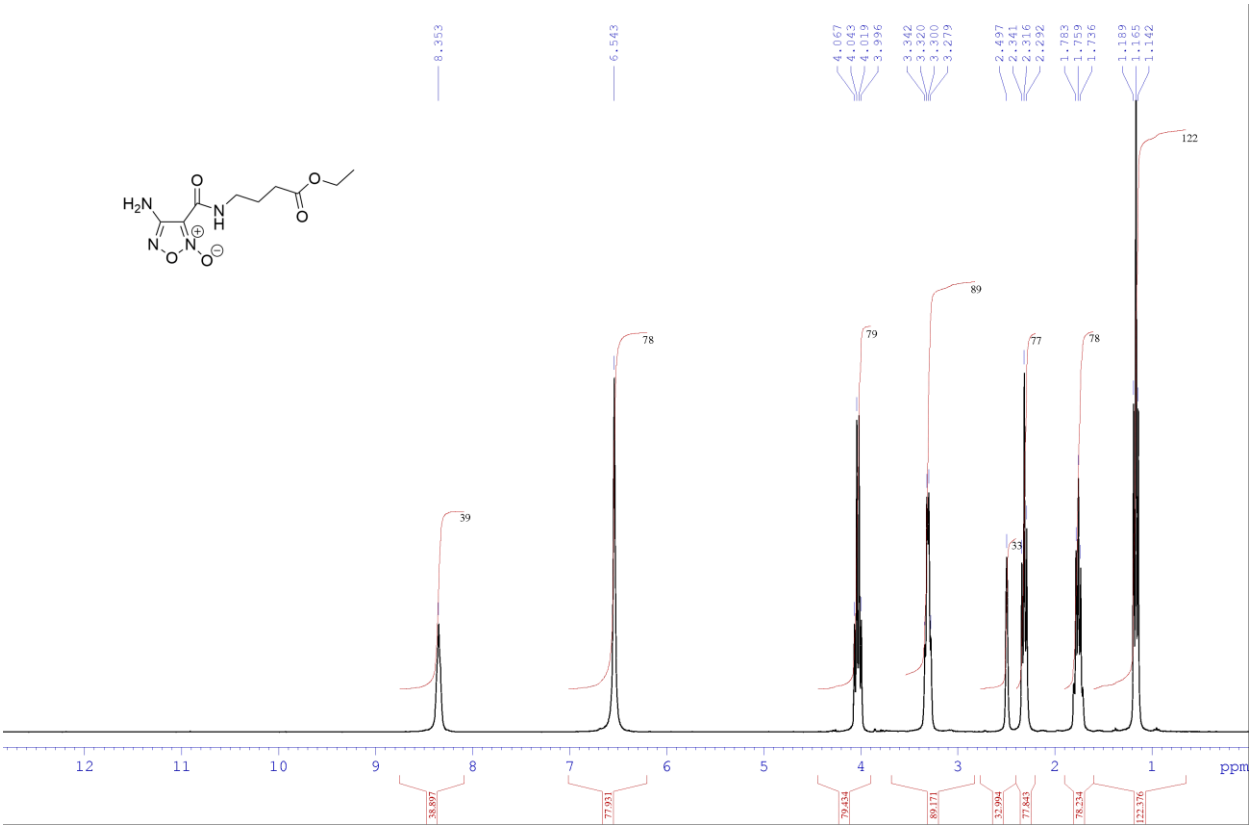
^{13}C NMR (75.5 MHz, DMSO) of compound **2**



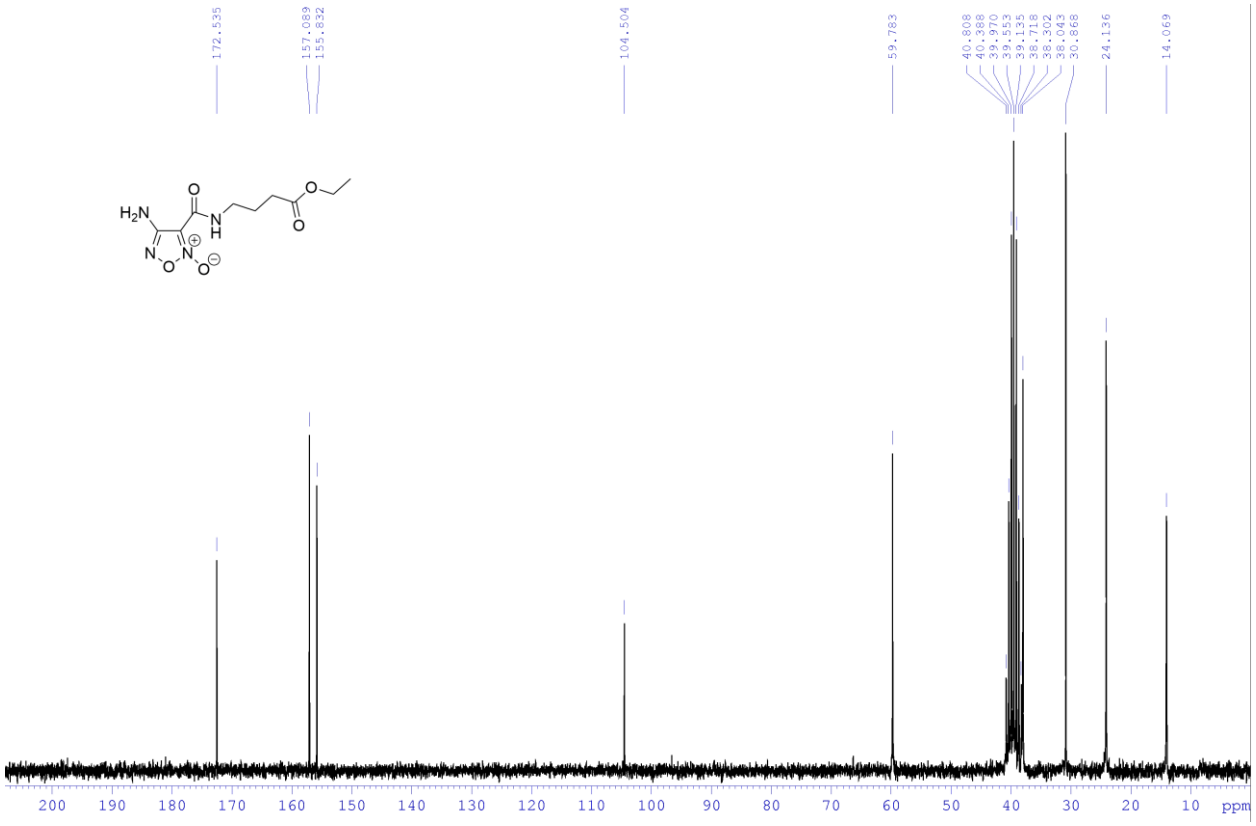
^{1}H NMR (300 MHz, DMSO) of compound **3**



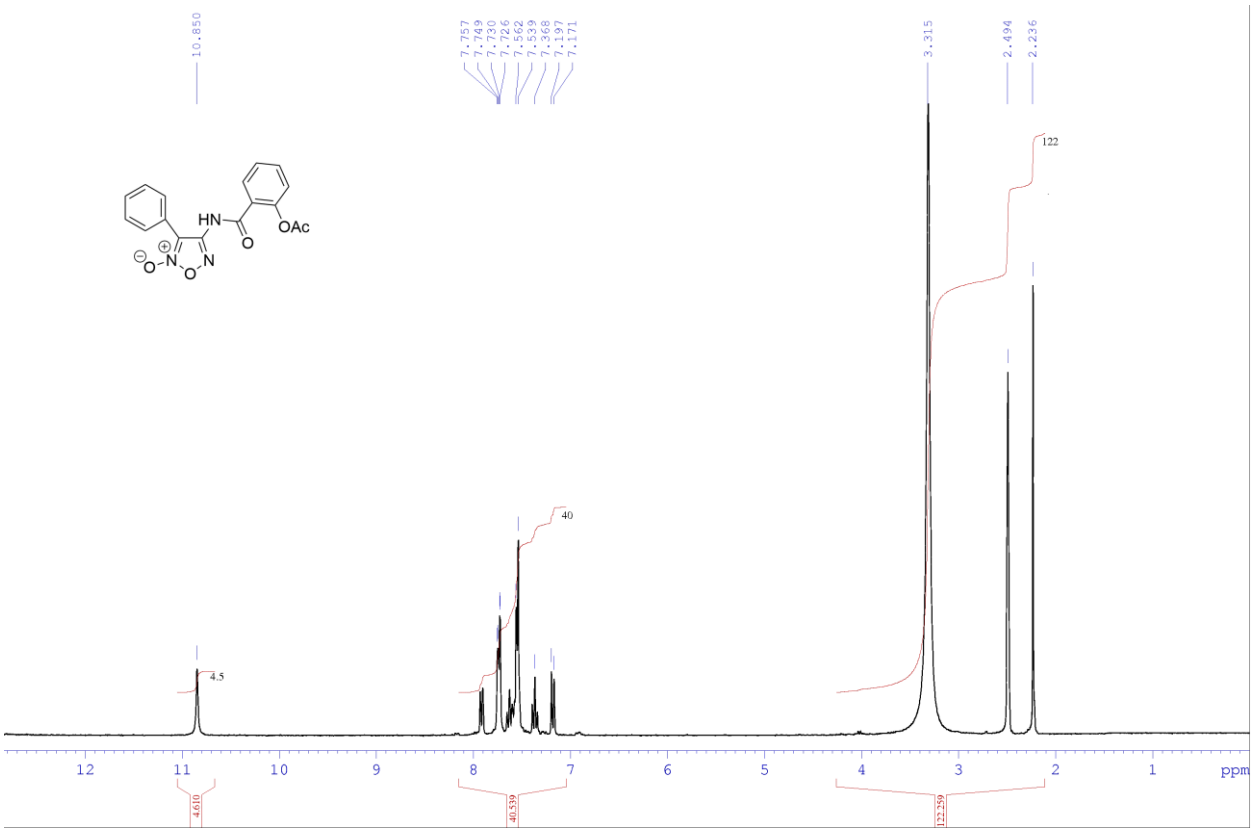
^{13}C NMR (75.5 MHz, DMSO) of compound **3**



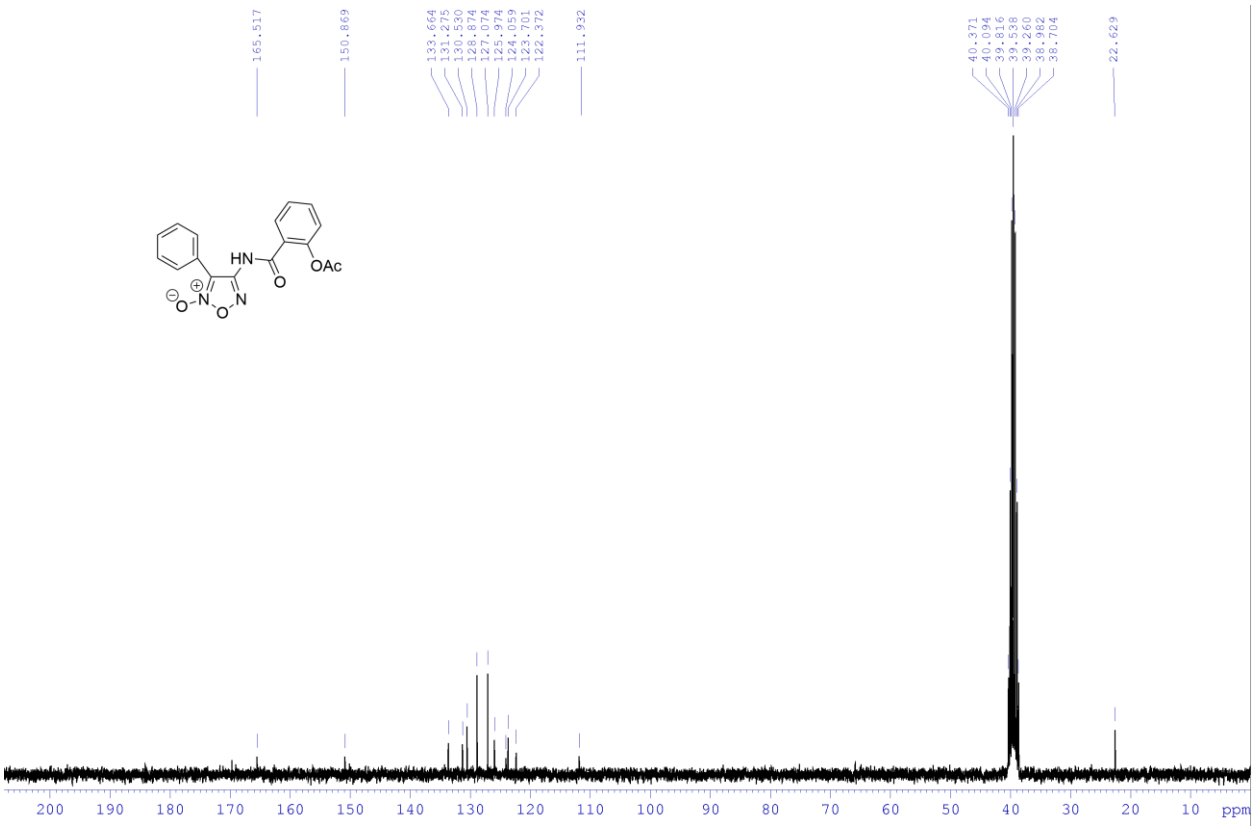
^{1}H NMR (300 MHz, DMSO) of compound **4**



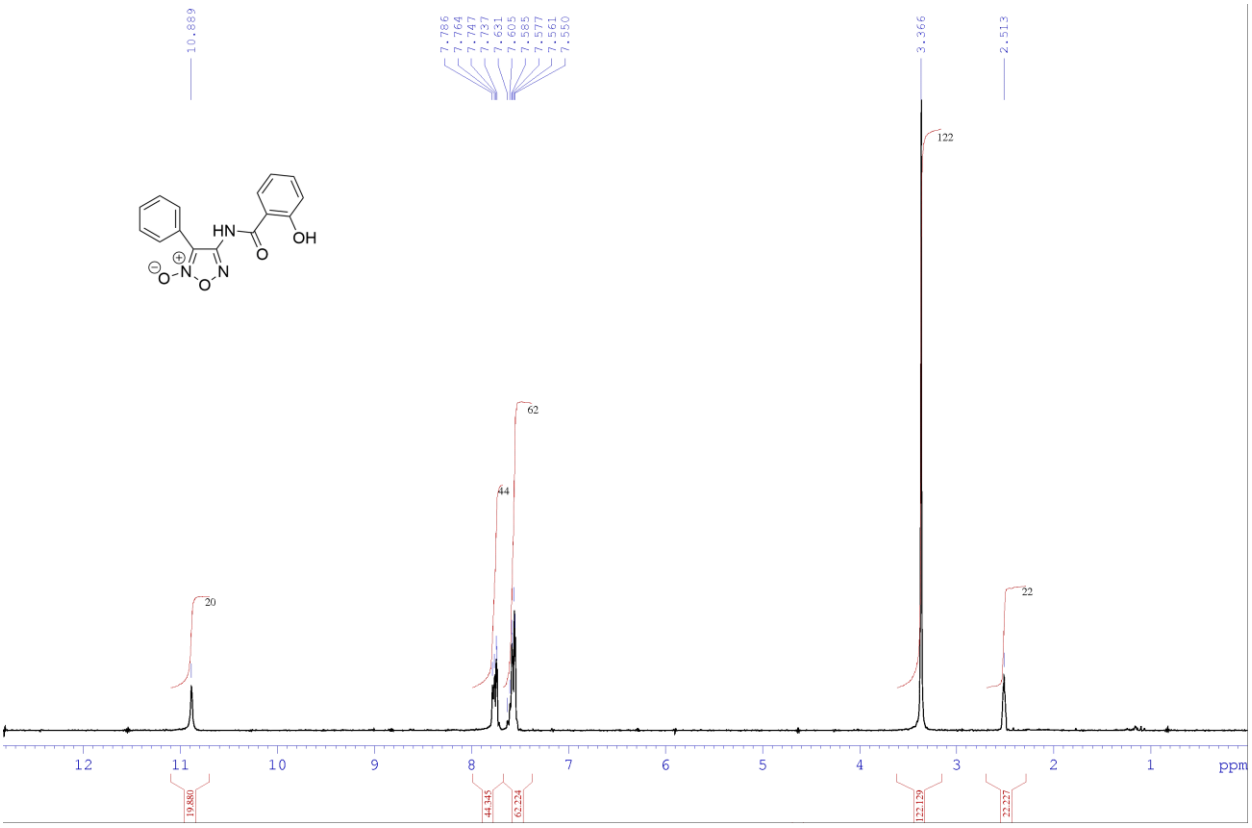
^{13}C NMR (50.3 MHz, DMSO) of compound **4**



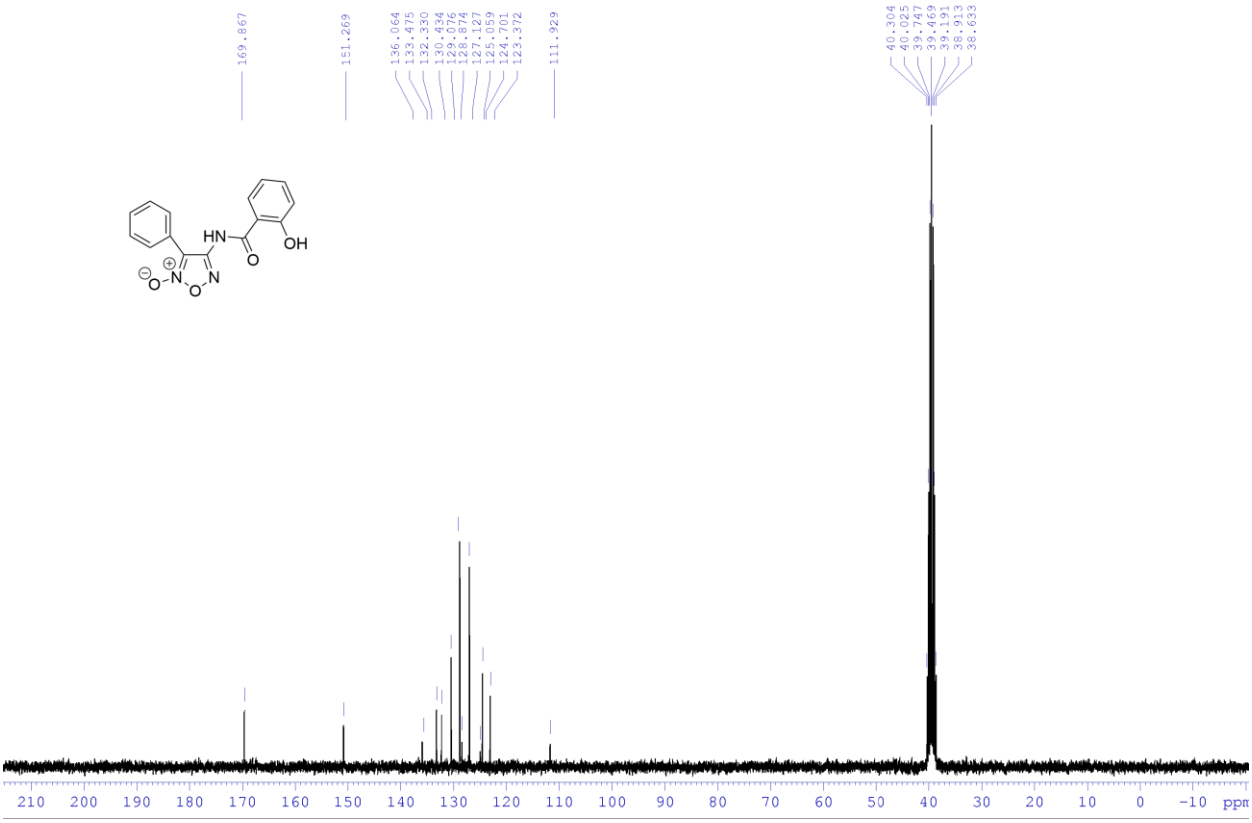
^{1}H NMR (300 MHz, DMSO) of compound **5**



^{13}C NMR (75.5 MHz, DMSO) of compound **5**



^{1}H NMR (200 MHz, DMSO) of compound **6**



^{13}C NMR (75.5 MHz, DMSO) of compound **6**