Electronic supplementary materials *Mendeleev Commun.*, 2021, **31**, 210–212

Renaissance of 4-(5-nitrofuran-2-yl)-5-arylamino substituted pyrimidines: microwave-assisted synthesis and antitubercular activity

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Experimental Section

General Information. All reagents and solvents were obtained from commercial sources and dried by using the standard procedures before use. 5-Bromo-4-(5-nitrofuran-2-yl)-pyrimidine was synthesized as described previously.[1] 1,4-Dioxane for the Buchwald–Hartwig cross-coupling reaction was deoxygenated by bubbling argon for 1 h.

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE-500 instrument using Me_4Si as an internal standard. Elemental analysis was carried on a Eurovector EA 3000 automated analyzer. Melting points were determined on Boetius combined heating stages and were not corrected.

The GC-MS analysis of all samples was carried out using an Agilent GC 7890A MS 5975C Inert XL EI/CI GC-MS spectrometer with a quadrupole mass-spectrometric detector with electron ionization (70 eV) and scan over the total ionic current in the range m/z 20–1000 and a quartz capillary column HP-5MS (30 m×0.25 mm, film thickness 0.25 mm). Helium served as a carrier gas, the split ratio of the flow was 1:50, and the consumption through the column was 1.0 mL min^{-1}; the initial temperature of the column was 40 °C (keeping 3 min), programming rate was 10 K min^{-1} to 290 °C (keeping 20 min), the temperature of the injector was 250 °C, the temperature of the source was 230 °C, the temperature of the quadrupole was 150 °C, and the temperature of the transition chamber was 280 °C. Solutions of the samples with a concentration of 3-4 mg ml^{-1} were prepared in THF. Samples of 1 ml of the obtained solutions were analyzed.

Column chromatography was carried out using Alfa Aesar silica gel 0.040-0.063 mm (230–400 mesh). The progress of reactions and the purity of compounds were checked by TLC on Sorbfil plates (Russia), in which the spots were visualized with UV light (λ 254 or 365 nm).

Microwave experiments were carried out in a Discover SP unimodal microwave system (CEM, USA) with a working frequency of 2.45 GHz and the power of microwave radiation

[1]Verbitskiy, E. V.; Baskakova, S. A.; Gerasimova, N. A.; Evstigneeva, N. P.; Zil'berberg, N. V.; Kungurov, N. V.; Kravchenko, M. A.; Skornyakov, S. N.; Pervova, M. G.; Rusinov, G. L.; Chupakhin, O. N.; Charushin, V. N. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 3003.

ranged from 0 to 300 W. The reactions were carried out in a 35 ml reaction tube with the hermetic Teflon cork. The reaction temperature was monitored, using an inserted IR sensor by the external surface of the reaction vessel.

***N*-Aryl-4-(5-nitrofuran-2-yl)-pyrimidin-5-amines 3a-l (general procedure).** A mixture of 5-bromo-4-(5-nitrofuran-2-yl)pyrimidine **1** (270 mg, 1.0 mmol), the corresponding aniline (1.2 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos, 116 mg, 0.2 mmol), $Pd(OAc)_2$ (22 mg, 10 mol%) and K_3PO_4 (531 mg, 2.5 mmol) in degassed 1,4-dioxane (15 ml) was irradiated in a microwave apparatus at 150 °C (250 W) for 10 min. The reaction mixture was cooled, filtered, treated with a mixture of AcOEt and water 1:1 (50 ml), and the organic layer was separated. The aqueous layer was extracted with AcOEt (2×25 ml). The combined organic extracts were dried with $MgSO_4$ and the solvents evaporated. The residue was purified by flash column chromatography (hexane/ethyl acetate, 1:3) to afford products **3a-l**. Analytical data (NMR spectra and elemental analysis data) for pyrimidines **3a,c,d,f-l** have been authentic to those previously obtained[2], and their yields are shown in Scheme 2.

4-(5-Nitrofuran-2-yl)-*N*-(*o*-tolyl)pyrimidin-5-amine 3b. Yield 193 mg (65%), dark red crystals, mp 127-129 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.46 (s, 1H), 7.82 (d, J = 4.0 Hz, 1H), 7.75 (s, 1H), 7.45 (d, J = 4.0 Hz, 1H), 7.30–7.26 (m, 1H), 7.11 (td, J = 7.6, 1.6 Hz, 1H), 7.03–6.97 (m, 2H), 2.29 (s, 3H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.4, 151.5, 149.8, 148.7, 139.2, 139.1, 135.8, 131.0, 130.1, 126.8, 123.7, 120.0, 115.4, 114.1, 17.7 ppm. GC t_R 26.73 min; MS m/z (rel intensity) 296 (M^+, 100). Anal. Calcd for $C_{15}H_{12}N_4O_3$ (296.29): C 60.81, H 4.08, N 18.91. Found: C 60.90, H 4.16, N 19.00. HRMS (APCI): m/z calcd for $C_{15}H_{13}N_4O_3$: 297.0982 $[M+H]^+$; found: 297.0986.

***N*-(2-Methoxyphenyl)-4-(5-nitrofuran-2-yl)pyrimidin-5-amine 3e**. Yield 209 mg (67%), dark red powder, mp 190-191 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.56 (s, 1H), 7.89 (s, 1H), 7.85 (d, J = 4.0 Hz, 1H), 7.48 (d, J = 4.0 Hz, 1H), 7.11 (ddd, J = 9.6, 7.9, 1.5 Hz, 2H), 7.06 (td, J = 8.2, 7.7, 1.6 Hz, 1H), 6.89 (td, J = 7.5, 1.5 Hz, 1H), 3.85 (s, 3H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.6, 151.6, 150.4, 149.8, 148.7, 138.8, 134.9, 129.2, 123.7, 120.8, 119.1, 115.3, 114.2, 111.9, 55.6 ppm. GC t_R 27.78 min; MS m/z (rel intensity) 312 (M^+, 100). Anal. Calcd for $C_{15}H_{12}N_4O_4$ (312.09): C 57.69, H 3.87, N 17.94. Found: C 57.53, H 4.00, N 17.98. HRMS (APCI): m/z calcd for $C_{15}H_{13}N_4O_4$: 313.0931 $[M+H]^+$; found: 313.0927.

[2]E.V. Verbitskiy, S.A. Baskakova, N.A. Gerasimova, N.P. Evstigneeva, N.V. Zil'berberg, N.V. Kungurov, M.A. Kravchenko, G.L. Rusinov, O.N. Chupakhin, V.N. Charushin. *Mendeleev Communications* **2018**, *28*, 393-395.

Brominated *N*-aryl-4-(5-nitrofuran-2-yl)pyrimidin-5-amine 4a-c (general procedure). *N*-Bromosuccinimide (178 mg, 1.0 mmol; 356 mg, 2.0 mmol or 534 mg, 3.0) was added to a solution of 4-(5-nitrofuran-2-yl)-*N*-phenylpyrimidin-5-amine **3a** (282 mg, 1.0 mmol) in DMF (10 ml). The obtained solution was stirred overnight at room temperature. The reaction mixture was diluted with water. The formed precipitate was filtered off, washed with water, dried, and purified by silica gel column chromatography with EtOAc/hexane (1:3) as an eluent to afford the desired bromo-substituted products.

***N*-(4-Bromophenyl)-4-(5-nitrofuran-2-yl)pyrimidin-5-amine (4a)**. Yield 289 mg (80%), orange solid, mp 244-245 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.86 (s, 1H), 8.44 (s, 1H), 7.83 (d, J = 4.0 Hz, 1H), 7.46 (d, J = 4.0 Hz, 1H), 7.43–7.34 (m, 2H), 7.02–6.92 (m, 2H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.9, 151.8, 151.7, 151.4, 142.7, 141.8, 134.2, 132.0, 119.0, 116.5, 114.1, 112.3. GC t_R 29.40 min; MS m/z (rel intensity) 360 (M^+, 100) for ^{79}Br, 362 (M^+, 97) for ^{81}Br. Anal. Calcd for $C_{14}H_9BrN_4O_3$ (361.16): C 46.56, H 2.51, N 15.51. Found: C 46.15, H 2.54, N 15.18. HRMS (APCI): m/z calcd for $C_{14}H_{10}BrN_4O_3$: 360.9931 $[M+H]^+$; found: 360.9934.

***N*-(2,4-Dibromophenyl)-4-(5-nitrofuran-2-yl)pyrimidin-5-amine (4b)**. Yield 75 mg (17%), yellow-orange solid, mp 245-244 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.71 (s, 1H), 8.05 (s, 1H), 7.87 (d, J = 2.3 Hz, 1H), 7.82 (d, J = 3.9 Hz, 1H), 7.41 (d, J = 4.0 Hz, 1H), 7.37 (dd, J = 8.7, 2.3 Hz, 1H), 6.87 (d, J = 8.7 Hz, 1H) ppm. The ^{13}C NMR spectra of **4b** could not be obtained due to the poor solubility of this compound in deuteriated solvents. GC t_R 31.15 min; MS m/z (rel intensity) 438 (M^+, 52) for ^{79}Br, 440 (M^+, 100) for ^{81}Br. Anal. Calcd for $C_{14}H_8Br_2N_4O_3$ (440.05): C 38.21, H 1.83, N 12.73. Found: C 38.22, H 1.91, N 12.14. HRMS (APCI): m/z calcd for $C_{14}H_9Br_2N_4O_3$: 438.9036 $[M+H]^+$; found: 438.9039.

4-(5-Nitrofuran-2-yl)-*N*-(2,4,6-tribromophenyl)pyrimidin-5-amine (4c). Yield 78 mg (15%), yellow-brown solid, mp 248-250 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.10 (s, 2H), 8.01 (s, 1H), 7.95 (s, 1H), 7.92 (d, J = 4.0 Hz, 1H), 7.61 (d, J = 4.0 Hz, 1H) ppm. The ^{13}C NMR spectra of **4c** could not be obtained due to the poor solubility of this compound in deuteriated solvents. GC t_R 33.04 min; MS m/z (rel intensity) 518 (M^+, 100) for ^{79}Br, 520 (M^+, 96) for ^{81}Br. Anal. Calcd for $C_{14}H_7Br_3N_4O_3$ (518.95): C 32.40, H 1.36, N 10.80. Found: C 32.59, H 1.39, N 10.76. HRMS (APCI): m/z calcd for $C_{14}H_8Br_3N_4O_3$: 516.8141 $[M+H]^+$; found: 516.8140.

Table S1 Bromination of 4-(5-nitrofuran-2-yl)-*N*-phenylpyrimidin-5-amine **3a** with various amounts of NBS (DMF, room temperature, 24 h).

Entry	NBS	Product mixtures, GC-MS (%)	Product (isolated yields, %)
1	1 equiv.	**4a** (98), Impurities(2)	**4a** (80)
2	2 equiv.	**4a** (46), **4b** (12), impurities (42)	**4a** (31) **4b** (11)
3	3 equiv.	**4b** (33), **4c** – (31), impurities – 36.0	**4b** (17) **4c** (15)

Antimycobacterial assay.

The study of the tuberculostatic activity of the compounds was carried out on the basis of the REMA procedure.[3]

Preparation of MBT suspension.

Suspension of MBT with 1.0 McFarland turbidity was prepared (using saline) from a culture of *Mycobacterium tuberculosis* $H_{37}Rv$ in the logarithmic phase of growth on the Löwenstein–Jensen medium. The resulting suspension (50 μl) was transferred into a tube with Middlebrook 7H9 nutrient broth and OADC growth supplement. In the wells of the plates, the resulting suspension (100 μl) was added. Preparation of dilutions of the test compounds. Dilutions of the test compounds were prepared using DMSO and sterile distilled water (Isoniazid was dissolved only in water). Weighed portions of testing compounds were dissolved in the calculated volume of DMSO in such a way as to obtain a stock solution with a concentration of 10000 μg ml^{-1}. Further, the dilution was carried out using pure DMSO.

Evaluating procedure:

For testing compounds, culture medium (97 μl) and solution of the test compounds (3 μl) prepared as described above were added to the wells of a 96-well plate. Then, MBT suspension (100 μl) was added to the wells of the plates. Thus, the required concentration of test compounds was obtained in the wells of the plate. The DMSO concentration in all wells is 1.5 vol.%. As a positive control, an MBT culture was used without adding compounds and with the addition of DMSO (final concentration 1.5%). Isoniazid was used as a reference drug. The plates were incubated at 37 °C for 7 days. After the incubation time, 30 μl of Resazurin solution (with the addition of Tween 80) was added to the wells, and the incubation was continued at 37 °C. The result was taken into account after 24, 48, and 72 hours. The MIC was taken as the minimum concentration of the test compound that prevents the color change of Resazurin.

[3](*a*) J. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swings, F. Portaels, Resazurin Microtiter Assay Plate: Simple and Inexpensive Method for Detection of Drug Resistance in *Mycobacterium tuberculosis* / // *Antimicrob. Agents Chemother*. - **2002**. - Vol. 46. - N 8. - P. 2720–2722; (*b*) N. Taneja, J. Tyagi, Resazurin reduction assays for screening of anti-tubercular compounds against dormant and actively growing *Mycobacterium tuberculosis*, *Mycobacterium bovis* BCG and *Mycobacterium smegmatis* / // *J. Antimicrob. Chemother*. - 2007. - Vol. 60. - P. 288-293.

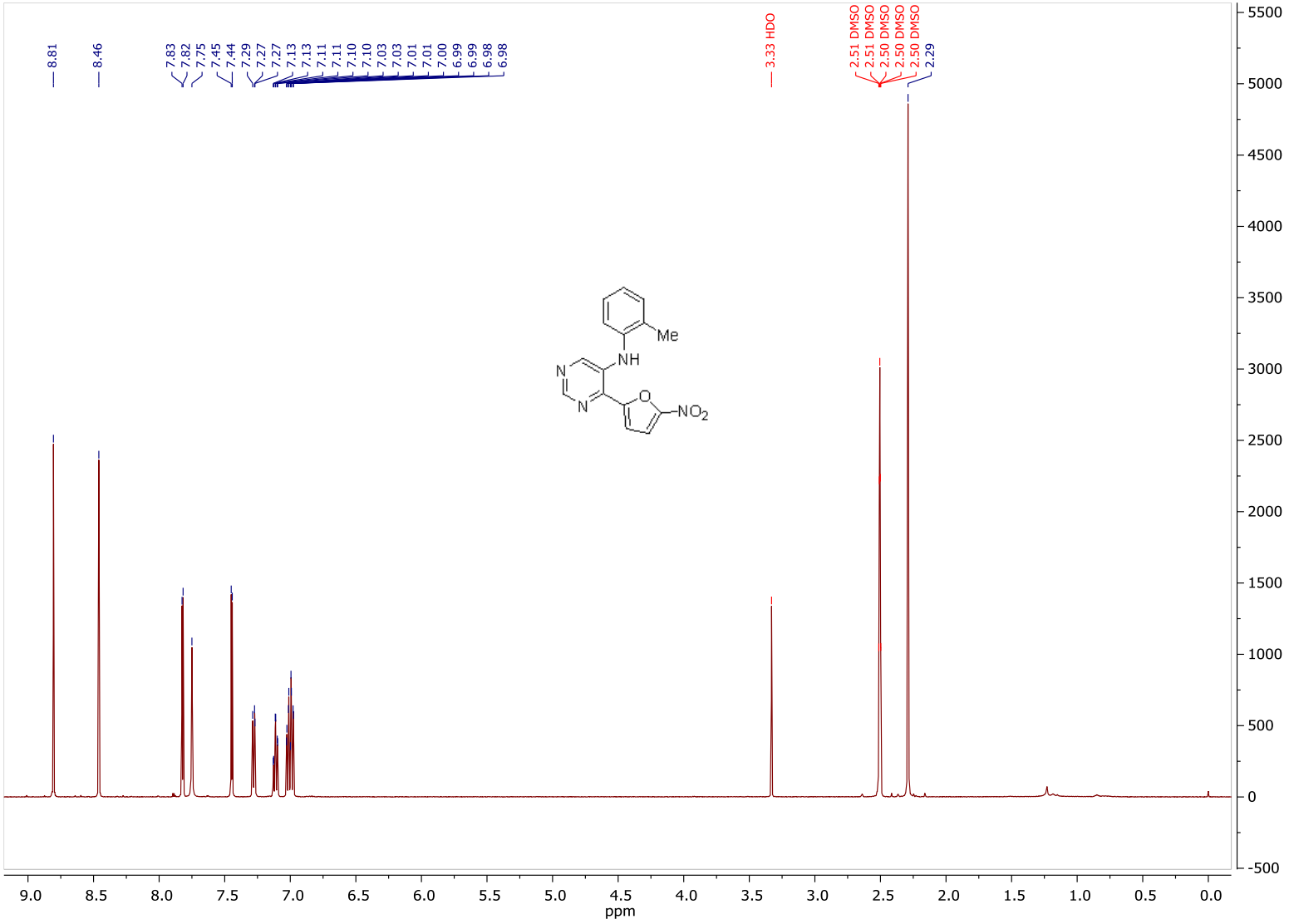

Figure S1. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **3b**.

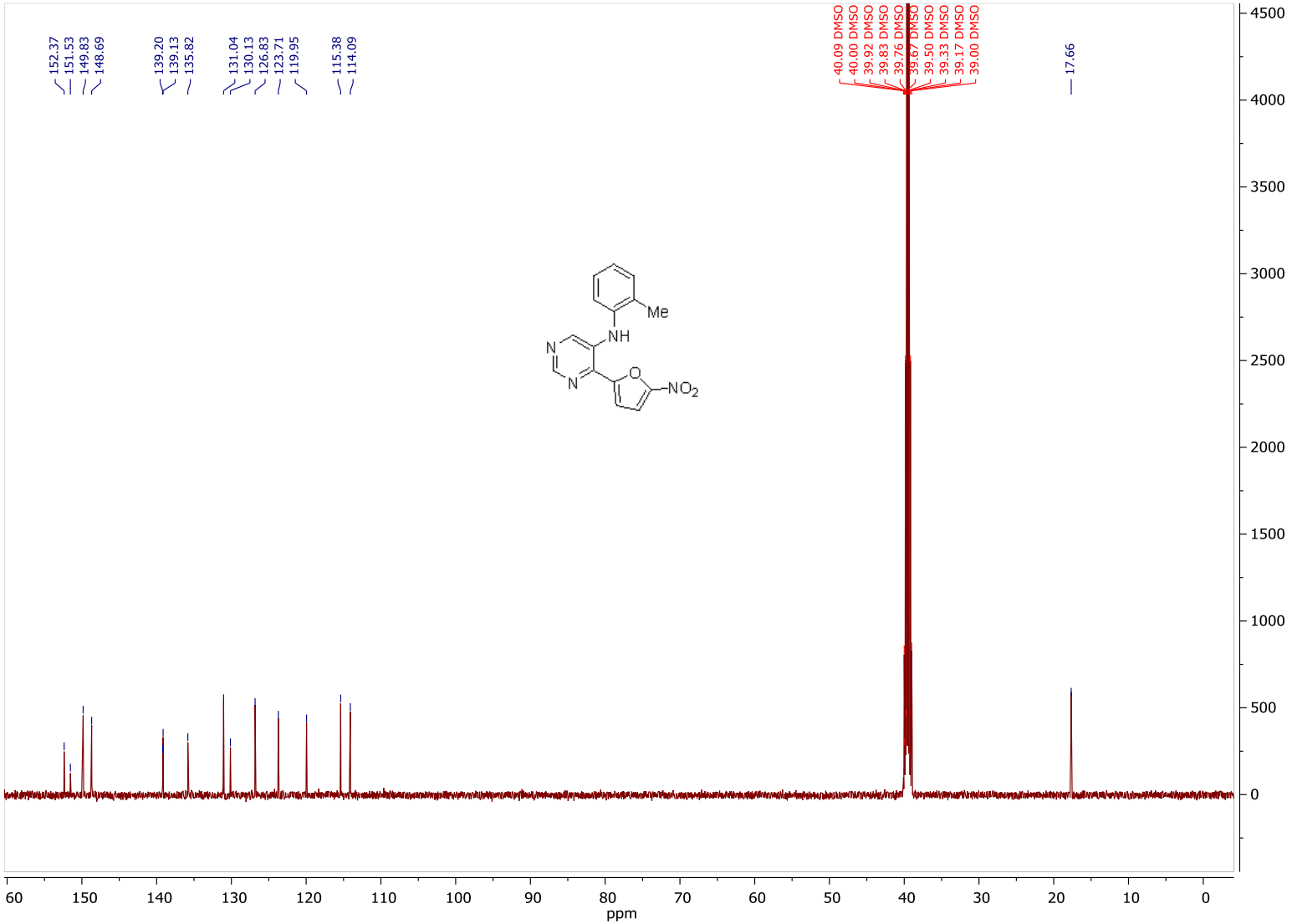

Figure S2. ^{13}C NMR (126 MHz, DMSO-d_6) spectrum of **3b**.

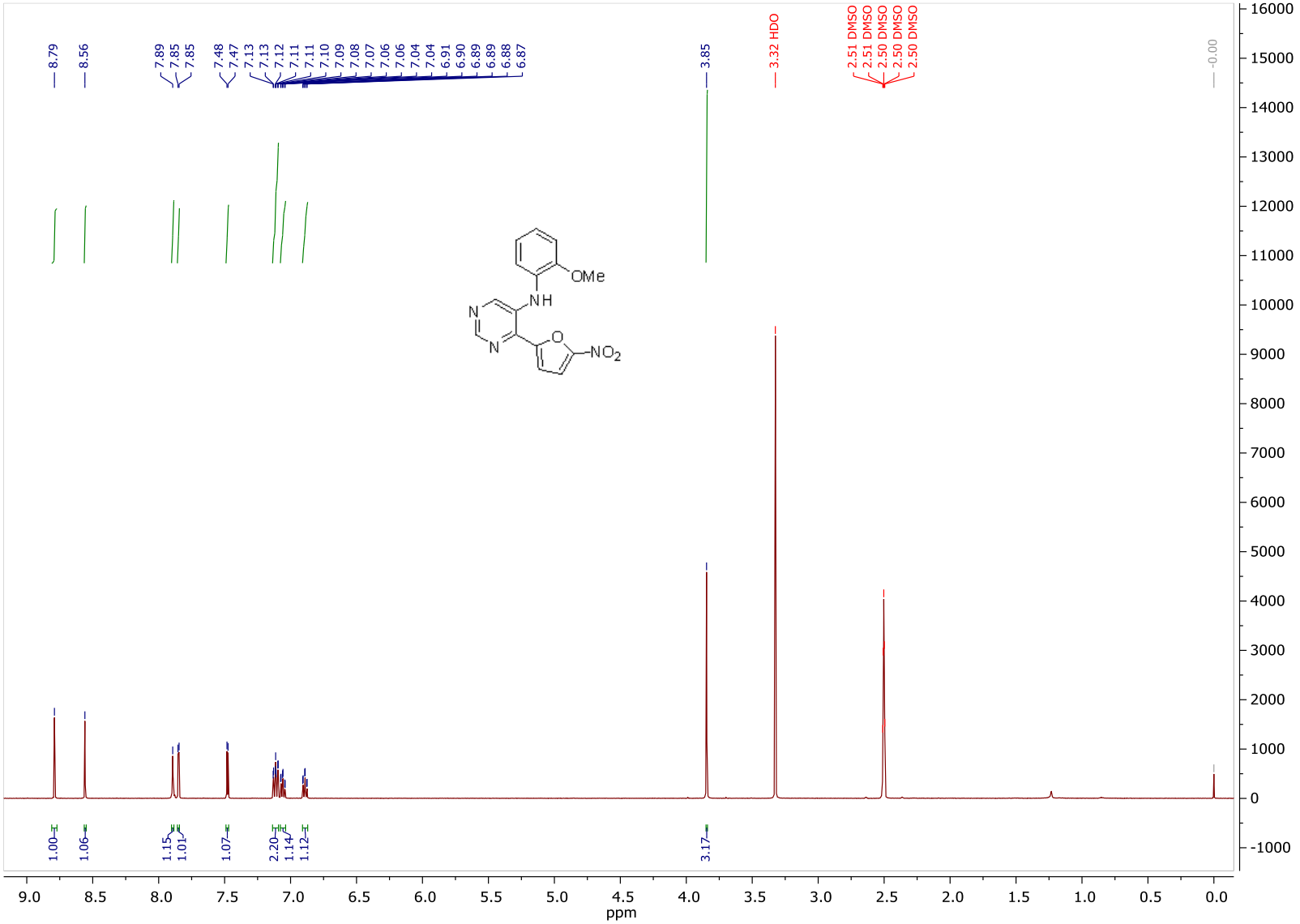

Figure S3. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **3e**.

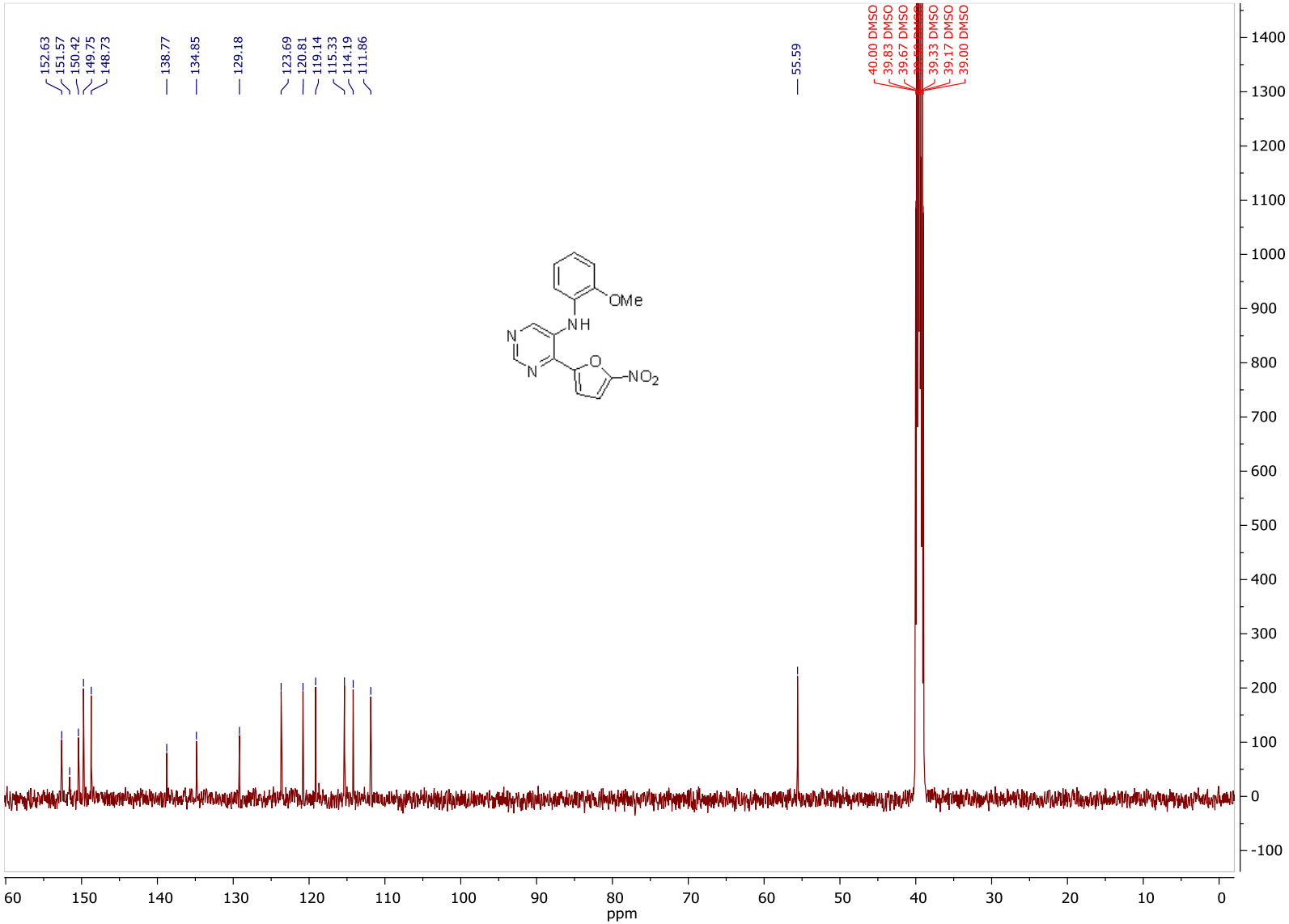

Figure S4. ^{13}C NMR (126 MHz, DMSO-d_6) spectrum of **3e**.

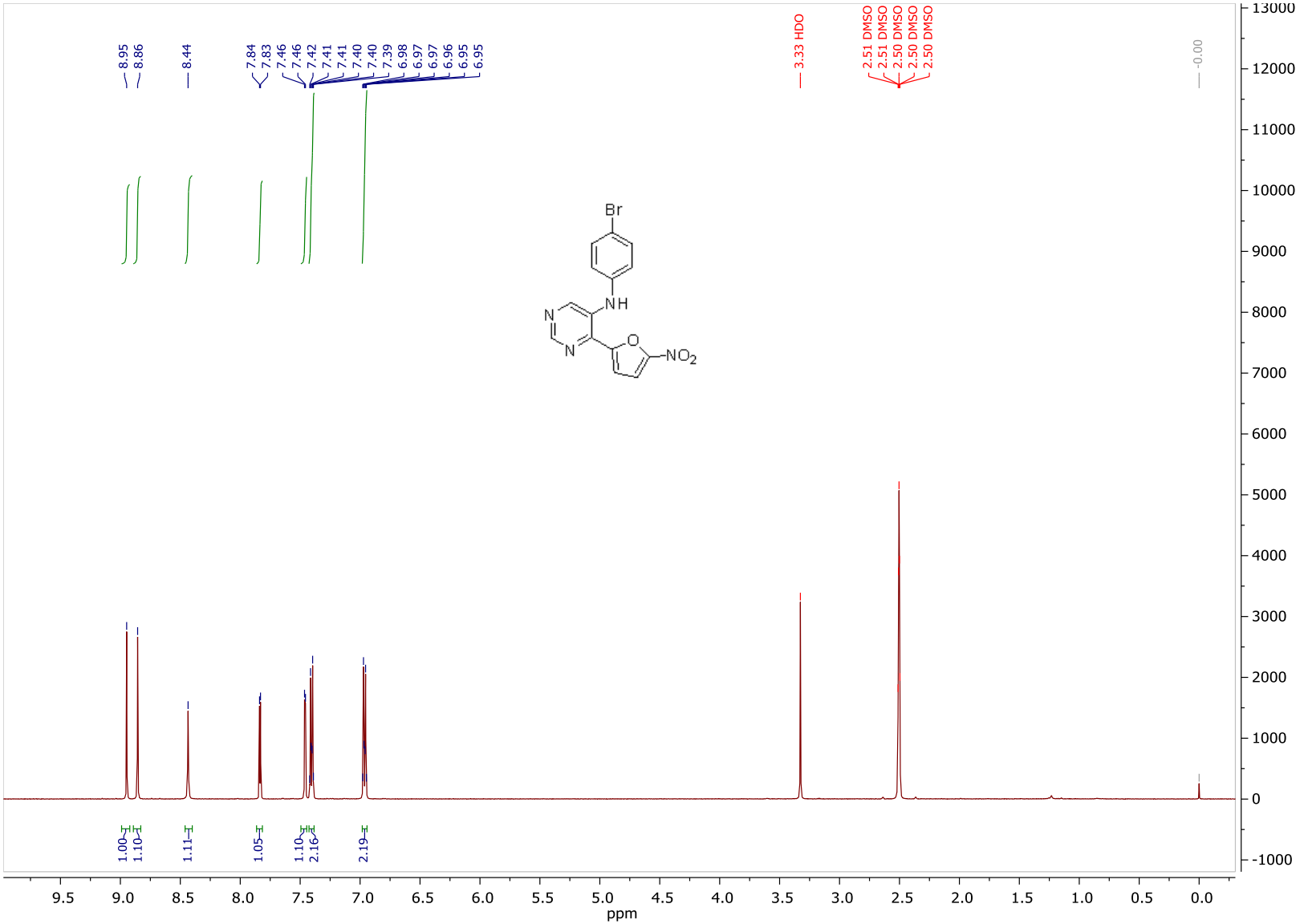

Figure S5. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **4a**.

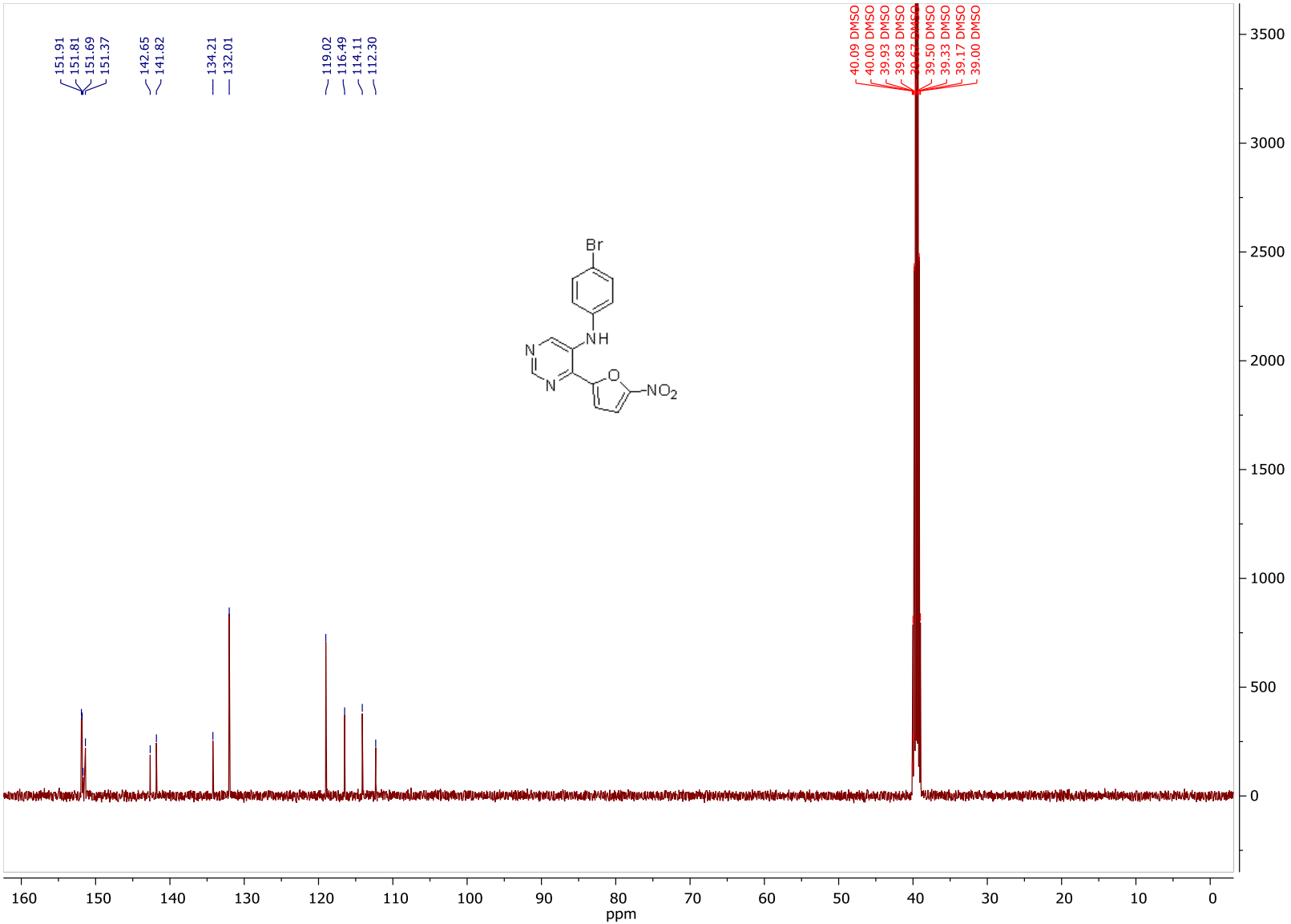

Figure S6. ^{13}C NMR (126 MHz, DMSO-d_6) spectrum of **4a**.

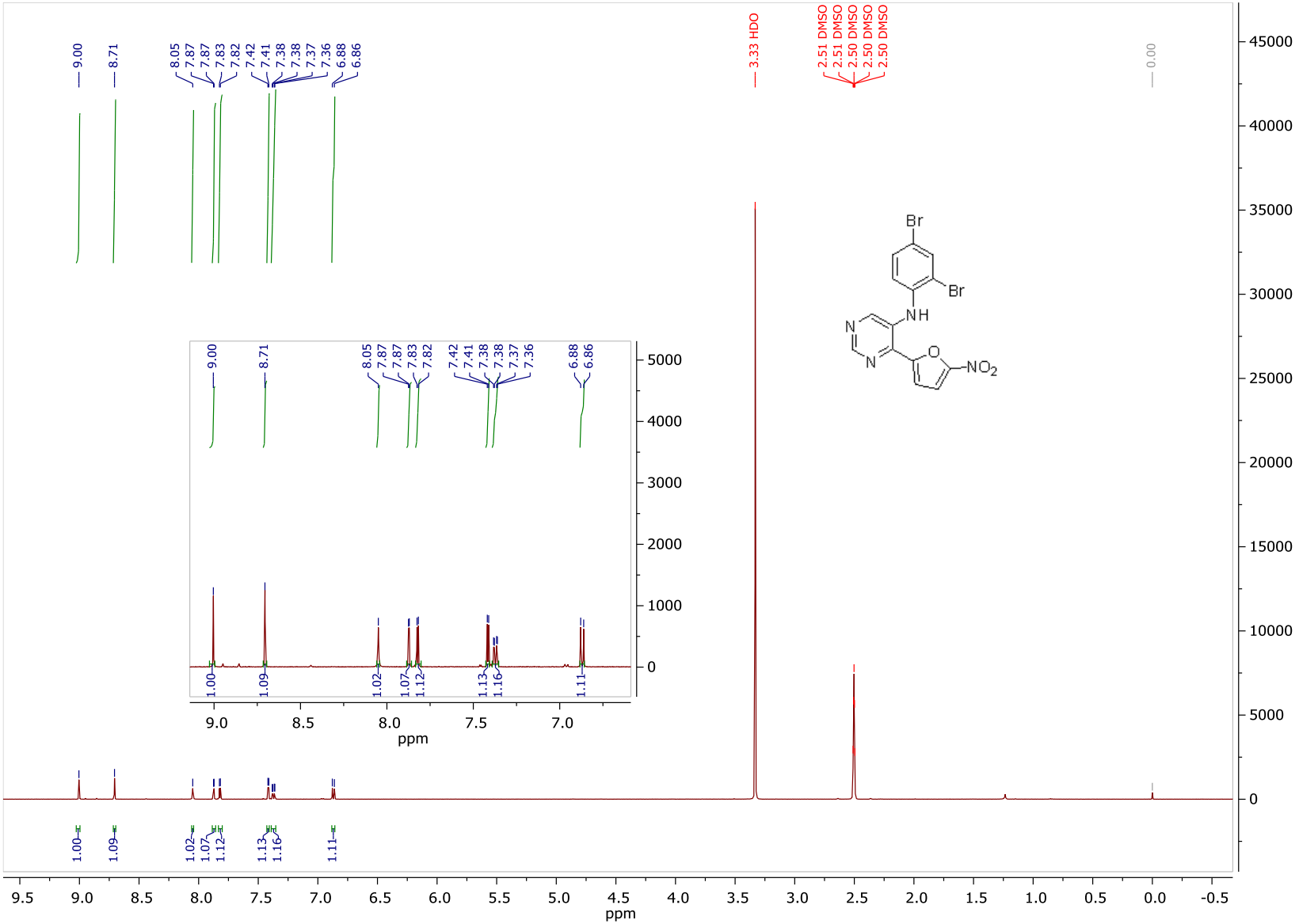

Figure S7. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **4b**.

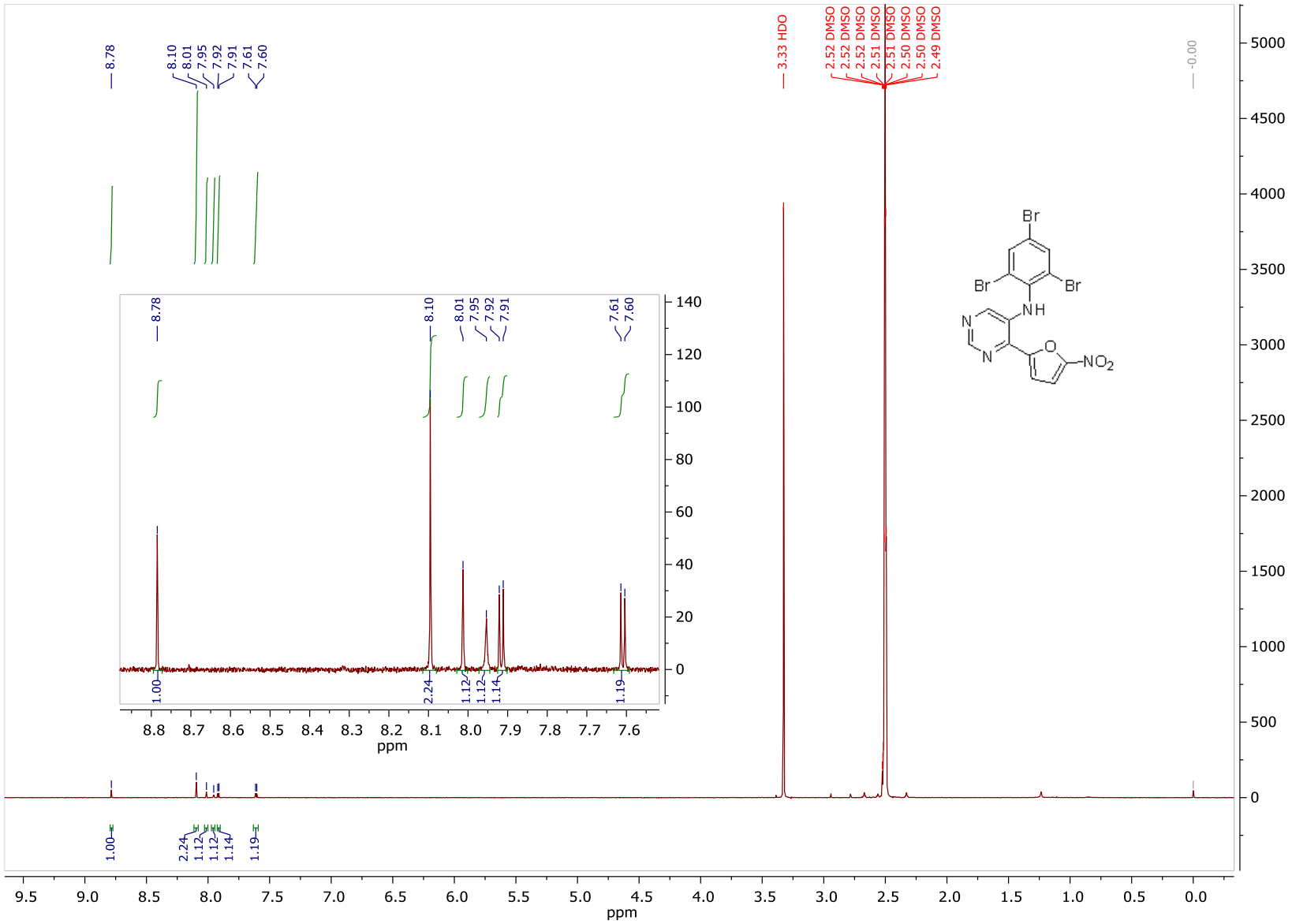

Figure S8. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **4c**.