Supporting Information

Advent of Imidazo[1,2-a]pyridine-3-carboxamides with Potent Multi- and Extended Drug Resistant Antituberculosis Activity

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Contents of Supporting Information

Experimental Section

All anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased from either Aldrich Chemical Co. (Milwaulkee, WI) or Fisher Scientific (Suwanee, GA). Water was distilled and purified through a Milli-Q water system (Millipore Corp., Bedford, MA). General methods of purification of compounds involved the use of silica cartridges purchased from AnaLogix, Inc. (Burlington, WI; www.ana-logix.com) and/or recrystallization. The reactions were monitored by TLC on precoated Merck 60 F_{254} silica gel plates and visualized using UV light (254 nm). All compounds were analyzed for purity by HPLC and characterized by ^{1}H and ^{13}C NMR using Varian 300MHz NMR and/or Varian 500 MHz NMR spectrometers. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra; chloroform δ 7.26 and δ 77.23, methanol δ 3.31 and δ 49.00 and coupling constants (*J*) are reported in hertz (Hz) (where, s = singlet, bs = broad singlet, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of dublet, t = triplet, tt – triple triplet, q = quartet, m = multiplet) and analyzed using MestReC NMR data processing. Mass spectra values are reported as *m/z*. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The HPLC HRMS analyses were carried out on a Dionex RSLC (UPLC) System with a Bruker MicroOTOF-Q II, using a Dionex Acclaim RSLC 120 C_{18}, 2.2 um, 120 Å, 2.1 x 100

mm column run at 40°C. Mobile phases: (A) Millipore purified water with 0.1% formic acid at a flow rate of 0.5 mL min^{-1} and UV dection at 254 nm. (B) HPLC grade acetonitrile with 0.1% formic acid at a flow rate of 0.5 mL min^{-1} and UV dection at 254 nm. A typical run time was 10 min with a linear gradient of 70% A to 30% B to start (time = 0 mins) and then 100% B at 6 min, 100% B at 8 min, 70% A to 30% B at 8.1 min and finally 70% A to 30% B at 10 min. All reactions were conducted under argon unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator. The 2,6-dimethylimidazo[1,2-a]pyrimidine-3-carboxylic acid was purchased from PracticaChem LLC (http://www.practicachem.com/, item PrC001522). Abbreviations: DCM = dichloromethane; DMF = dimethylformamide; ACN = acetonitrile; EtOAc = ethyl acetate; HOAc = acetic acid; EDC = *N*-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride. All compounds are >98% pure by HPLC analysis and MIC values reported are the average of three individual measurements. Additionally, all animal experiments were conducted in accordance with the animal care and use committee of Jubilant Biosciences Laboratories.

2,7-dimethylimidazo[1,2-*a*]pyridine-3-carboxylic acid

2-Amino-4-picoline (10.0 g, 91.5 mmol) and ethyl-2-chloroacetoacetate (7.93 g, 45.8 mmol) were dissolved in 92 mL of 1,2-dimethoxyethane (DME) and heated for 36 h at reflux. The reaction mixture was filtered and (2-amino-4-picoline hydrochloride salt) solids were collected and washed with hexanes. The filtrate was concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2 and washed with 5% acetic acid solution (2x) and brine. The organic phase was collected, dried over sodium sulfate (Na_2SO_4), filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column

chromatography with a 20% ethyl acetate : CH_2Cl_2 solvent system to give 7.8 g (78%) of ethyl 2,7-dimethylimidazo[1,2-a]pyridine-3-carboxylate as a tan solid. Mp 59 - 61°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.14 (d, *J* = 7.1 Hz, 1H), 7.34 (s, 1H), 6.78 (dd, *J* = 7.1, 1.7 Hz, 1H), 4.40 (q, *J* = 7.1, 7.1, 7.1 Hz, 2H), 2.66 (s, 3H), 2.42 (s, 3H), 1.42 (t, *J* = 7.1, 7.1 Hz, 3H). HRMS (EI), M+1 calcd. for $C_{12}H_{15}N_2O_2$, 219.1155; found 219.1128. HPLC t_R = 1.4 min.

Ethyl 2,7-dimethylimidazo[1,2-a]pyridine-3-carboxylate (6.64 g, 30.4 g) was dissolved in of 75 mL ethanol (95:5) and 61 mL of 1 N LiOH solution was added (61 mmol). The reaction was stirred at reflux for 36 h. Once complete, the reaction was concentrated *in vacuo* to near dryness and the pH was adjusted to 3 with slow addition of 4 *N* HCl while cooling in an ice bath. The resulting solids were collected by filtration and were dried under vacuum overnight to give 3.86 g (67%) of 2,7-dimethylimidazo[1,2-a]pyridine-3-carboxylic acid as an off white solid. Mp 180 - 183°C; ^{1}H NMR (300 MHz, CD_3OD) δ 9.52 (d, *J* = 7.1 Hz, 1H), 7.73 (td, *J* = 1.8, 0.9, 0.9 Hz, 1H), 7.48 (dd, *J* = 7.1, 1.3 Hz, 1H), 2.81 (s, 3H), 2.63 (s, 3H). HRMS (EI), M+1 calcd. for $C_{10}H_{11}N_2O_2$, 191.0815; found 191.0837. HPLC t_R = 0.6 min.

_N_-Benzyl-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (1)

2,7-Dimethylimidazo[1,2-a]pyridine-3-carboxylic acid (50 mg, 0.25 mmol) and benzyl amine (33 μL, 0.3 mmol) were dissolved in 2 mL of acetonitrile (ACN) and treated with *N*-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 96 mg, 0.5 mmol) and 4-dimethylaminopyridine (DMAP, 92 mg, 0.75 mmol). The resulting solution was stirred for 12 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with 10% aqueous $NaHCO_3$ solution (2x), water, and 5% acetic acid solution (2x). The organic phase

was collected, dried over sodium sulfate (Na_2SO_4), filtered and then concentrated *in vacuo*. Crude material obtained was purified by silica gel column chromatography with a 20% ethyl acetate : CH_2Cl_2 solvent system to give 49 mg (70%) of **1** as a white solid. Mp 166 - 167°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (d, *J* = 7.1 Hz, 1H), 7.39-7.28 (m, 5H), 7.25 (s, 1H), 6.75 (dd, *J* = 7.2, 1.8 Hz, 1H), 6.05 (bs, 1H, NH), 4.69 (d, *J* = 5.7 Hz, 2H), 2.65 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.73, 146.71, 145.56, 138.51, 138.46, 129.02, 127.79, 127.47, 115.89, 115.22, 115.02, 43.59, 21.52, 16.95. HRMS (EI), M+1 calcd. for $C_{17}H_{18}N_3O$, 280.1444; found 280.1480. HPLC t_R = 0.9 min.

***N*-(2-Methoxybenzyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (2)**

This compound was prepared using a similar coupling procedure as described for the preparation of amide **1**. The product was obtained as a white solid. Mp 160 - 161°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.30 (d, *J* = 7.1 Hz, 1H), 7.39-7.26 (m, 3H), 7.00-6.89 (m, 2H), 6.74 (dd, *J* = 7.2, 1.8 Hz, 1H), 6.05 (bs, 1H, NH), 4.67 (d, *J* = 5.7 Hz, 2H), 3.92 (s, 3H), 2.65 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.40, 157.77, 149.88, 146.52, 145.22, 138.19, 130.06, 129.20, 127.49, 126.46, 121.02, 115.71, 115.12, 110.49, 55.45, 39.83, 21.49, 16.60. HRMS (EI), M+1 calcd. for $C_{18}H_{20}N_3O_2$, 310.1550; found 310.1563. HPLC t_R = 1.6 min.

***N*-(3-Methoxybenzyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (3)**

This compound was prepared using a similar coupling procedure as described for the preparation of amide **1** to give the product as a white solid. Mp 174 - 175°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.31 (d, *J* = 7.1 Hz, 1H), 7.33-7.26 (m, 2H), 6.99-6.81 (m, 3H), 6.75 (dd, *J* =

7.2, 1.8 Hz, 1H), 6.06 (bs, 1H, NH), 4.67 (d, *J* = 5.7 Hz, 2H), 3.81 (s, 3H), 2.66 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.72, 160.17, 146.75, 145.59, 140.11, 138.51, 130.11, 127.51, 119.99, 115.93, 115.26, 115.02, 113.50, 113.12, 55.44, 43.57, 21.55, 17.02. HRMS (EI), M+1 calcd. for $C_{18}H_{20}N_3O_2$, 310.1550; found 310.1587. HPLC t_R = 1.2 min.

***N*-(4-Methoxybenzyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (4)**

This compound was prepared using a similar coupling procedure as described for the preparation of amide **1** to give the product as a white solid. Mp 150 - 151°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.30 (d, *J* = 6.7 Hz, 1H), 7.32-7.28 (m, 4H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 7.2 Hz, 1H), 6.04 (bs, 1H, NH), 4.66 (d, *J* = 5.4 Hz, 2H), 3.80 (s, 3H), 2.65 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.64, 159.25, 146.65, 145.48, 138.39, 130.52, 129.19, 127.44, 115.84, 115.19, 115.05, 114.37, 55.47, 43.11, 21.51, 16.91. HRMS (EI), M+1 calcd. for $C_{18}H_{20}N_3O_2$, 310.1550; found 310.1583. HPLC t_R = 1.1 min.

***N*-(4-Methylbenzyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (5)**

This compound was prepared using a similar coupling procedure as described for the preparation of amide **1** to give the product as a white solid. Mp 175 - 176°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.31 (d, *J* = 7.1 Hz, 1H), 7.33-7.27 (m, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 6.75 (dd, *J* = 7.2, 1.8 Hz, 1H), 6.05 (bs, 1H, NH), 4.65 (d, *J* = 5.6 Hz, 2H), 2.65 (s, 3H), 2.41 (s, 3H), 2.35 (s, 3H).). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.71, 146.72, 145.49, 138.52, 137.60, 135.44, 129.75, 127.88, 127.55, 115.94, 115.26, 115.04, 43.46, 21.57, 21.34, 16.99. HRMS (EI), M+1 calcd. for $C_{18}H_{20}N_3O$, 294.1601; found 294.161. HPLC t_R = 1.9 min.

***N*-(4-Chlorobenzyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (6)**

This compound was prepared using a similar coupling procedure as described for the preparation of amide **1** to give the product as a white solid. Mp 198 - 199°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.30 (d, *J* = 7.1 Hz, 1H), 7.36-7.25 (m, 5H), 6.76 (dd, *J* = 7.2, 1.8 Hz, 1H), 6.05 (bs, 1H, NH), 4.66 (d, *J* = 5.9 Hz, 2H), 2.66 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.73, 146.72, 145.68, 138.57, 137.14, 133.48, 129.09, 127.38, 115.92, 115.20, 114.87, 42.84, 21.50, 16.95. HRMS (EI), M+1 calcd. for $C_{17}H_{17}ClN_3O$, 314.1084; found 314.1055. HPLC t_R = 2.1 min

***N*-(3,4-Dichlorobenzyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (7)**

This compound was prepared using a similar coupling procedure as described for the preparation of amide **1** to give the product as a white solid. Mp 191 - 192°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.30 (d, *J* = 7.1 Hz, 1H), 7.45 (m, 2H), 7.33 (s, 1H), 7.23 (d, *J* = 1.73 Hz, 1H), 6.74 (dd, *J* = 7.0, 1.1 Hz, 1H), 6.04 (bs, 1H, NH), 4.64 (d, *J* = 5.7 Hz, 2H), 2.64 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.79, 146.88, 145.87, 139.06, 138.85, 133.02, 131.77, 130.93, 129.70, 127.52, 127.07, 116.13, 115.31, 114.76, 42.51, 21.62, 17.15. HRMS (EI), M+1 calcd. for $C_{17}H_{16}Cl_2N_3O$, 348.0665; found 348.0631. HPLC t_R = 5.4 min.

***N*-(3-Fluorobenzyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (8)**

This compound was prepared using a similar coupling procedure as described for the preparation of amide **1** to give the product as a white solid. Mp 154 - 155°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.30 (d, *J* = 7.1 Hz, 1H), 7.39-7.28 (m, 2H), 7.19-7.05 (m, 2H), 6.98 (d, *J* = 2.3 Hz, 1H), 6.74 (dd, *J* = 7.2, 1.8 Hz, 1H), 6.05 (bs, 1H, NH), 4.68 (d, *J* = 5.9 Hz, 2H), 2.68

(s, 3H), 2.42 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.24, 162.28, 161. 63, 146.44, 145.19, 141.20 (d, Jc_F = 6.9 Hz, 1C), 139.15, 130.56 (d, Jc_F = 8.3 Hz, 1C), 127.57, 123.27 (d, Jc_F = 2.9 Hz, 1C), 116.27, 115.07, 114.79, 114.62, 43.08, 21.58, 16.84. HRMS (EI), M+1 calcd. for $C_{17}H_{17}FN_3O$, 298.1350; found 298.1380. HPLC t_R = 1.4 min.

***N*-(Phenyl)-2,7-dimethylimidazo[1,2-a]pyridine-3-carboxamide (9)**

This compound was prepared using a similar coupling procedure as described for the preparation of amide **1** to give the product as a tan solid. Mp >295°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.27 (d, *J* = 7.1 Hz, 1H), 7.60 (m, 2H), 7.49 (bs, 1H, NH), 7.37 (m, 3H), 7.16 (m, 1H), 6.78 (dd, *J* = 7.1, 1.7 Hz, 1H), 2.78 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.76, 147.05, 145.92, 138.94, 137.81, 129.36, 127.44, 124.66, 120.42, 116.17, 115.37, 115.30, 21.55, 16.99. HRMS (EI), M+1 calcd. for $C_{16}H_{16}N_3O$, 266.1288; found 266.1267. HPLC t_R = 1.2 min.

***N*-(3-Methoxybenzyl)-2,6-dimethylimidazo[1,2-a]pyrimidine-3-carboxamide (10)**

2,6-Dimethylimidazo[1,2-a]pyrimidine-3-carboxylic acid (purchased from PracticaChem LLC (http://www.practicachem.com/; 50 mg, 0.25 mmol) and 3-methoxybenzylamine (39 μL, 0.3 mmol) were dissolved in 2 mL of acetonitrile (ACN) and treated with *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, 96 mg, 0.5 mmol) and 4-dimethylaminopyridine (DMAP, 92 mg, 0.75 mmol). The resulting solution was stirred for 12 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with 10% aqueous $NaHCO_3$ solution (2x), water and 5% acetic acid solution (2x). The organic phase was collected, dried over Na_2SO_4, filtered and then concentrated *in vacuo*. Crude material

obtained was purified by silica gel column chromatography with a 20% ethyl acetate : CH_2Cl_2 solvent system to give 38 mg (49%) of **10** as a white solid. Mp 148 - 149°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (qd, *J* = 2.1, 1.0, 1.0, 1.0 Hz, 1H), 8.49 (d, *J* = 2.5 Hz, 1H), 7.29 (t, *J* = 7.9, 7.9 Hz, 1H), 6.89 (m, 3H), 6.15 (m, 1H, NH), 4.67 (d, *J* = 5.7 Hz, 2H), 3.81 (s, 3H), 2.72 (s, 3H), 2.39 (s, 3H).). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.32, 158.50, 154.03, 148.38, 147.17, 139.73, 133.93, 130.14, 119.70, 119.36, 115.50, 114.84, 113.50, 70.00, 43.63, 22.21, 17.10. HRMS (EI), M+1 calcd. for $C_{17}H_{19}N_4O_2$, 311.1503; found 311.1495. HPLC t_R = 3.9 min.

Description of TB (GAS[1], GAST[2], 7H12[1]) by Microplate Alamar Blue assay (MABA) to determine MIC_{90} values against replicating TB:

The test compound MICs against *Mtb* H_{37}Rv (ATCC# 27294) were assessed by the MABA using rifampin and PA-824 as positive controls. Compound stock solutions were prepared in DMSO at a concentration of 128 μM, and the final test concentrations ranged from 128 μM to 0.5 μM. Two fold dilutions of compounds were prepared in glycerol-alanine-salt media in a volume of 100 μL in 96-well microplates (BD Optilux™, 96-well Microplates, black/clear flat bottom) for the GAS assay, in an iron deficient glycerol-alanine-salt media with 0.05% Tween 80 added in the GAST assay and in Middlebrook 7H12 medium (7H9 broth containing 0.1% w/v casitone, 5.6 μg/mL palmitic acid, 5 mg/mL bovine serum albumin, 4 mg/mL catalase) in a volume of 100 μL in 96-well microplates (BD Optilux™, 96-well Microplates, black/clear flat bottom) for the 7H12 assay. The TB cultures (100 μL inoculums of 2×10^5 cfu/mL) were added to the media, yielding a final testing volume of 200 μL. The plates were incubated at 37°C. On the seventh day of incubation, 12.5 μL of 20%

Tween 80, and 20 μL of Alamar Blue (Invitrogen BioSource™) were added to the wells of test plate. After incubation at 37 °C for 16-24 h, fluorescence of the wells was measured at 530 nm (excitation) and 590 nm (emmision). The MICs are defined as the lowest concentration effecting a reduction in fluorescence of ≥ 90% relative to the mean of replicate bacteria-only controls. For clinical strains, MIC determination was performed in 7H9 Middlebrook media supplemented with 0.2% glycerol/ 0.2 % glucose/ 0.5% BSA/ 0.08% NaCl/0.05% Tween 80 in a 2-fold dilution of compound series as above. The 96-well microplates contained a final volume of 100 μL at 2 $\times 10^4$ cfu/well and were scored for growth after 1-week incubation at 37 °C. The MIC was determined to be the concentration of compound that completely inhibited all growth of cells.

Description of low-oxygen recovery assay (LORA[3]) to determine activity against non-replicating *M. tuberculosis*:

A low-oxygen adapted culture of recombinant $H_{37}Rv$ (pFCA-luxAB), expressing a *Vibrio harveyii* luciferase gene with an acetamidase promoter, was grown in a BiostatQ fermentor. Cells were collected on ice, washed in PBS, and stored at –80°C. Circa 10^5 cfu/mL of thawed NRP cells were exposed to 2-fold serial dilutions of test compound in 7H12 broth in black 96-well plates, which were incubated anaerobically at 37°C for 10 days. Luminescence readings were obtained following a 28 h recovery in an aerobic environment (5% CO_2). The data were analyzed graphically, and the lowest concentration of test compound preventing metabolic recovery (90% reduction relative to untreated cultures) was determined.

Description of VERO[4] cytotoxicity assay to determine IC_{50} values:

Samples were dissolved at 12.8 mM in DMSO. Geometric three-fold dilutions were performed in growth medium MEM (Gibco, Grand Island, NY), containing 10% S5 fetal bovine serum (HyClone, Logan, UT), 25 mM *N*-(2-hydroxyethyl)-piperazine-*N*'-2-ethanesulfonic acid (HEPES, Gibco), 0.2% $NaHCO_3$ (Gibco), and 2 mM glutamine (Irvine Scientific, Santa Ana, CA). Final DMSO concentrations did not exceed 1% v/v. Drug dilutions were distributed in duplicate in 96-well tissue culture plates (Becton Dickinson Labware, Lincoln Park, NJ) at a volume of 50 μL per well. An equal volume containing 5×10^5 log phase VERO cells (Green African Monkey kidney cells, CCL-81; American Type Culture Collection, Rockville, MD) was added to each well and the cultures were incubated at 37 °C in an atmosphere containing 5% of CO_2. After 72 h, cell viability was measured using the CellTiter 96 aqueous non-radioactive cell proliferation assay (Promega Corp., Madison, WI) according to the manufacturer's instructions. Then absorbance at 490 nm was read in a Victor multilabel reader (PerkinElmer). The IC_{50} values (inhibition concentration at 50%) were determined using a curve-fitting program.

Spectrum of activity assays:

MICs against all other microorganisms were determined at an equal weight/volume basis using a standard microbroth dilution assay,[5] and the data were expressed in μM.

Description of microsomal assay for compounds 1, 3, 4, and 6:

Compounds (1 mM in DMSO) were mixed with NADPH (10 mM) in potassium phosphate buffer (66.7 mM, pH 7.4) in a total volume of 1 mL and preincubated at 37^{o} C for 5 min. The reaction was started by adding pooled male rat liver and human hepatic microsomes (Fischer

344 and lot 18888, respectively) purchased from BD Gentest (Woburn, MA). After a 30-min incubation, the reaction was quenched with 0.5% formic acid in acetonitrile containing internal standard (phenacetin) and centrifuged to remove protein. A second incubation was terminated at time 0. An aliquot of the supernatant was analyzed by LC/MS (Applied Biosystem API 4000) with UV detection at 254 nm. The peak area ratio relative to internal standard was measured and compared relative to that at time 0 and reported as percent metabolized and half-life calculated.

Microsomal results for compound 1, 3, 4, and 6:

Compound code	% metabolized ($t_{1/2}$, min)	
	RLM	HLM
1	71(17)	59(24)
3	80(13)	47(35)
4	69(19)	30(58)
6	79(13)	82(12)

Microsomal stability experiment has been performed in Human and Rat Liver Microsomes (HLM and RLM). Verapamil was used as control and it was in acceptable range (85 ± 7).

Protein binding assay description for compounds 1 and 10:

MICs were compared by MABA using Middlebrook 7H12 medium against *M. tuberculosis* H_{37}Rv in the presence and absence of 10% fetal bovine serum (FBS) and in the presence of 4% w/v bovine serum albumin (BSA). The latter is more stringent with regard to protein binding while the former should detect other serum-mediated abrogation of anti-TB activity.

The luminescent measurement of intracellular ATP was used an alternative readout for protein binding assay. The plates were incubated at 37 °C. On the seventh day of incubation, Cell Lysis agent and Luciferase Reagent (Roche, ATP Bioluminescence Assay Kit HS II) were added to the wells of test

plate. Luminescence of the wells was measured and the MICs were defined as the lowest concentration effecting a reduction in Luminescence of ≥ 90% relative to the mean of replicate bacteria-only controls.

Compound ID	7H12		4%BSA			10%FBS		
	ATP	MABA	ATP	MABA	MABA2	ATP	MABA	MABA2
1	< 0.195	0.38	< 0.195	0.28	0.23	0.21	0.91	0.76
10	2.24	4.95	2.32	4.02	2.39	2.45	> 5	> 5
RMP		0.06		0.11			0.21	
INH		0.38		0.49			0.25	

***In vivo* PK information for compounds 1, 3, 4, and 6:**

Discussion of *in vivo* PK for compound **1**.

The pharmacokinetics of compound **1** was evaluated in Sprague-Dawley (SD) rats following a single 10 mg/kg dose administration by oral route and 1 mg/kg dose by IV. Following oral administration maximum plasma concentration (C_{max}) was achieved at ~0.25 h (t_{max}). Following IV administration, elimination half life ($t_{1/2}$) was found to be ~0.35 h and clearance was ~91 mL/min/kg. The absolute bioavailability was ~76%.

Table 1: Pharmacokinetic parameters of compound **1** after an oral dose of 10 mg/kg.

PK parameter	Animal no.				Mean	S.D.	CV%
	1	2	3	4			
$t_{1/2ß}$ (h)	0.45	0.66	0.59	0.55	0.56	0.08	15.0
AUC_{0-t} (ng·h/mL)	1342	1404	1739	1234	1430	218	15.2
$AUC_{0-\infty}$ (ng·h/mL)	1344	1410	1746	1237	1434	219	15.3
C_{max} (ng/mL)	2630	3068	3653	2696	3012	469	15.6
t_{max} (h)	0.25	0.25	0.25	0.25	0.25	0.00	0.00
F (%)	75.9						
Time points considered in $t_{1/2ß}$ calculation	1 – 4 h	1 – 4 h	1 – 4 h	1 – 4 h			

Table 2: Pharmacokinetic parameters of compound **1** after an IV dose of 1 mg/kg.

PK parameter	Animal no.			Mean	S.D.	CV%
	1	2	3			
$t_{1/2ß}$ (h)	0.41	0.34	0.30	0.35	0.06	16.4
C_{max} (ng/mL)	539	460	367	455	86.0	18.9
C_0 (ng/mL)	776	703	621	700	77.5	11.1
AUC_{0-t} (ng·h/mL)	225	190	146	187	39.6	21.2

$AUC_{0-\infty}$ (ng·h/mL)	229	192	146	189	41.5	22.0
CL (mL/min/kg)	72.7	86.9	114	91.2	21.0	23.0
V_d (L/kg)	2.60	2.57	2.95	2.71	0.21	7.93
V_{dss} (L/kg)	1.70	1.60	1.87	1.72	0.14	8.07
Time points considered in $t_{1/2ß}$ calculation	0.5 – 2 h	0.5 – 2 h	0.5 – 2 h			
C_0 calculated manually considering initial 3 time points.						

$t_{1/2ß}$: terminal half life; AUC_{0-t}: the area under the plasma concentration-time curve from 0 to last measurable time point; $AUC_{0-\infty}$: area under the plasma concentration-time curve from time zero to infinity; C_{max}: maximum observed plasma concentration; t_{max}: time to the maximum observed plasma concentration; V_d: volume of distribution; $V_{dss:}$ volume of distribution at steady state; NA: not applicable; F: oral bioavailability.

Discussion of *in vivo* PK for compound **3**.

The pharmacokinetics of compound **3** was evaluated in SD rats following a single 10 mg/kg dose administration by oral route and 1 mg/kg dose by IV. Following oral administration maximum plasma concentration (C_{max}) was achieved at ~0.25 h (t_{max}). Following IV administration, elimination half life ($t_{1/2}$) was found to be ~0.33 h and clearance was ~43 mL/min/kg. The absolute bioavailability was ~43%.

Table 1: Pharmacokinetic parameters of compound **3** after an oral dose of 10 mg/kg.

PK parameter	**Animal no.**			**Mean**	**S.D.**	**CV%**
	2	**3**	**4**			
$t_{1/2ß}$ (h)	0.41	0.40	0.40	0.40	0.01	2.02
AUC_{0-t} (ng·h/mL)	1445	1678	1895	1672	225	13.5
$AUC_{0-\infty}$ (ng·h/mL)	1446	1679	1896	1673	225	13.5
C_{max} (ng/mL)	2635	3050	3734	3140	555	17.7
t_{max} (h)	0.25	0.25	0.25	0.25	0.00	0.00
F (%)	43.1					
Time points considered in $t_{1/2ß}$ calculation	1 – 4 h	1 – 4 h	1 – 4 h			

Table 2: Pharmacokinetic parameters of compound **3** after an IV dose of 1 mg/kg.

PK parameter	**Animal no.**			**Mean**	**S.D.**	**CV%**
	2	**3**	**4**			
$t_{1/2ß}$ (h)	0.53	0.24	0.21	0.33	0.18	54.0

C_{max} (ng/mL)	501	1073	926	833	297	35.7
C_0 (ng/mL)	604	1677	1555	1279	587	45.9
AUC_{0-t} (ng·h/mL)	353	422	353	376	39.7	10.6
$AUC_{0-\infty}$ (ng·h/mL)	378	422	364	388	30.7	7.90
CL (mL/min/kg)	44.1	39.5	45.8	43.1	3.30	7.65
V_d (L/kg)	2.02	0.81	0.84	1.22	0.69	56.3
V_{dss} (L/kg)	1.74	0.60	0.63	0.99	0.65	65.4
Time points considered in $t_{1/2ß}$ calculation	0.5 – 2 h	0.5 – 2 h	0.25 – 1 h			
C_0 calculated manually considering initial 3 time points.						

Discussion of *in vivo* PK for compound **4**.

The pharmacokinetics of compound **4** was evaluated in SD rats following a single 10 mg/kg dose administration by oral route and 1 mg/kg dose by IV. Following oral administration maximum plasma concentration (C_{max}) was achieved at ~0.25 h (t_{max}). Following IV administration, elimination half life ($t_{1/2}$) was found to be ~0.28 h and clearance was ~28 mL/min/kg. The absolute bioavailability was ~50%.

Table 1: Pharmacokinetic parameters of compound **4** after an oral dose of 10 mg/kg.

PK parameter	**Animal no.**				**Mean**	**S.D.**	**CV%**
	1	**2**	**3**	**4**			
$t_{1/2ß}$ (h)	0.36	0.52	0.48	0.63	0.50	0.11	22.5
AUC_{0-t} (ng·h/mL)	3361	3254	2768	2629	3003	359	11.9
$AUC_{0-\infty}$ (ng·h/mL)	3363	3261	2773	2648	3011	353	11.7
C_{max} (ng/mL)	5787	6568	5729	4881	5741	689	12.0
t_{max} (h)	0.25	0.25	0.25	0.25	0.25	0.00	0.00
F (%)	49.8						
Time points considered in $t_{1/2ß}$ calculation	1 – 4 h	1 – 4 h	1 – 4 h	1 – 4 h			

Table 2: Pharmacokinetic parameters of compound **4** after an IV dose of 1 mg/kg.

PK parameter	**Animal no.**				**Mean**	**S.D.**	**CV%**
	1	**2**	**3**	**4**			
$t_{1/2ß}$ (h)	0.25	0.32	0.30	0.27	0.28	0.03	11.4
C_{max} (ng/mL)	1337	1424	1594	1240	1399	150	10.8

C_0 (ng/mL)	2426	2362	2292	2068	2287	156	6.82
AUC_{0-t} (ng·h/mL)	564	676	657	510	602	78.6	13.1
$AUC_{0-\infty}$ (ng·h/mL)	565	682	662	511	605	80.5	13.3
CL (mL/min/kg)	29.5	24.5	25.2	32.6	27.9	3.82	13.7
V_d (L/kg)	0.64	0.68	0.66	0.75	0.68	0.05	7.52
V_{dss} (L/kg)	0.43	0.48	0.49	0.52	0.48	0.04	8.00
Time points considered in $t_{1/2ß}$ calculation	0.5 – 2 h	0.5 – 2 h	0.5 – 2 h	0.5 – 2 h			
C_0 calculated manually considering initial 3 time points.							

Discussion of *in vivo* PK for compound **6**.

The pharmacokinetics of compound **6** was evaluated in SD rats following a single 10 mg/kg dose administration by oral route and 1 mg/kg dose by IV. Following oral administration maximum plasma concentration (C_{max}) was achieved at ~0.31 h (t_{max}). Following IV administration, elimination half life ($t_{1/2}$) was found to be ~0.40 h and clearance was ~51 mL/min/kg. The absolute bioavailability was ~49%.

Table 1: Pharmacokinetic parameters of compound **6** after an oral dose of 10 mg/kg.

PK parameter	**Animal no.**				**Mean**	**S.D.**	**CV%**
	1	**2**	**3**	**4**			
$t_{1/2ß}$ (h)	1.37	1.85	1.64	0.60	1.36	0.54	39.8
AUC_{0-t} (ng·h/mL)	1229	1997	1339	1757	1580	359	22.7
$AUC_{0-\infty}$ (ng·h/mL)	1234	2021	1348	1769	1593	367	23.0
C_{max} (ng/mL)	1472	2347	1424	2737	1995	652	32.7
t_{max} (h)	0.25	0.50	0.25	0.25	0.31	0.13	40.0
F (%)	48.6						
Time points considered in $t_{1/2ß}$ calculation	2 – 8 h	2 – 8 h	2 – 8 h	1 – 4 h			

Table 2: Pharmacokinetic parameters of compound **6** after an IV dose of 1 mg/kg.

PK parameter	**Animal no.**				**Mean**	**S.D.**	**CV%**
	1	**2**	**3**	**4**			
$t_{1/2ß}$ (h)	0.35	0.32	0.41	0.51	0.40	0.08	20.6
C_{max} (ng/mL)	744	660	753	755	728	45.6	6.27
C_0 (ng/mL)	1067	877	1037	1213	1049	138	13.1
AUC_{0-t} (ng·h/mL)	310	294	317	364	321	29.9	9.32

$AUC_{0-\infty}$ (ng·h/mL)	313	297	324	378	328	34.9	10.6
CL (mL/min/kg)	53.2	56.1	51.5	44.1	51.2	5.10	10.0
V_d (L/kg)	1.60	1.57	1.83	1.94	1.74	0.18	10.3
V_{dss} (L/kg)	1.05	1.18	1.12	1.18	1.13	0.06	5.29
Time points considered in $t_{1/2ß}$ calculation	0.5 – 2 h	0.5 – 2 h	0.5 – 2 h	0.5 – 2 h			
C_0 calculated manually considering initial 3 time points.							

Transcriptional profiling experiments of *M. tuberculosis* treated with compound 1:

Mycobacterium tuberculosis growing in 7H9 Middlebrook broth base supplemented with 0.5% BSA, 0.085% sodium chloride, 0.05% Tween 80, 2 g/L glycerol and 2 g/L glucose at an OD_{650nm} of 0.2 was treated with 0.1 or 10 μg/mL of compound for 6 hours prior to RNA extraction as previously described[6,7]. Control cultures were treated with an equivalent amount of vehicle control (DMSO). RNA was labeled and hybridized to Agilent 4 X 44K arrays containing the MTb genome oligo set as previously described[6,7]. See excel file "transcriptional-profiling-supporting-information.xlsx" for complete gene expressions of *M. tuberculosis* treated with compound **1**.

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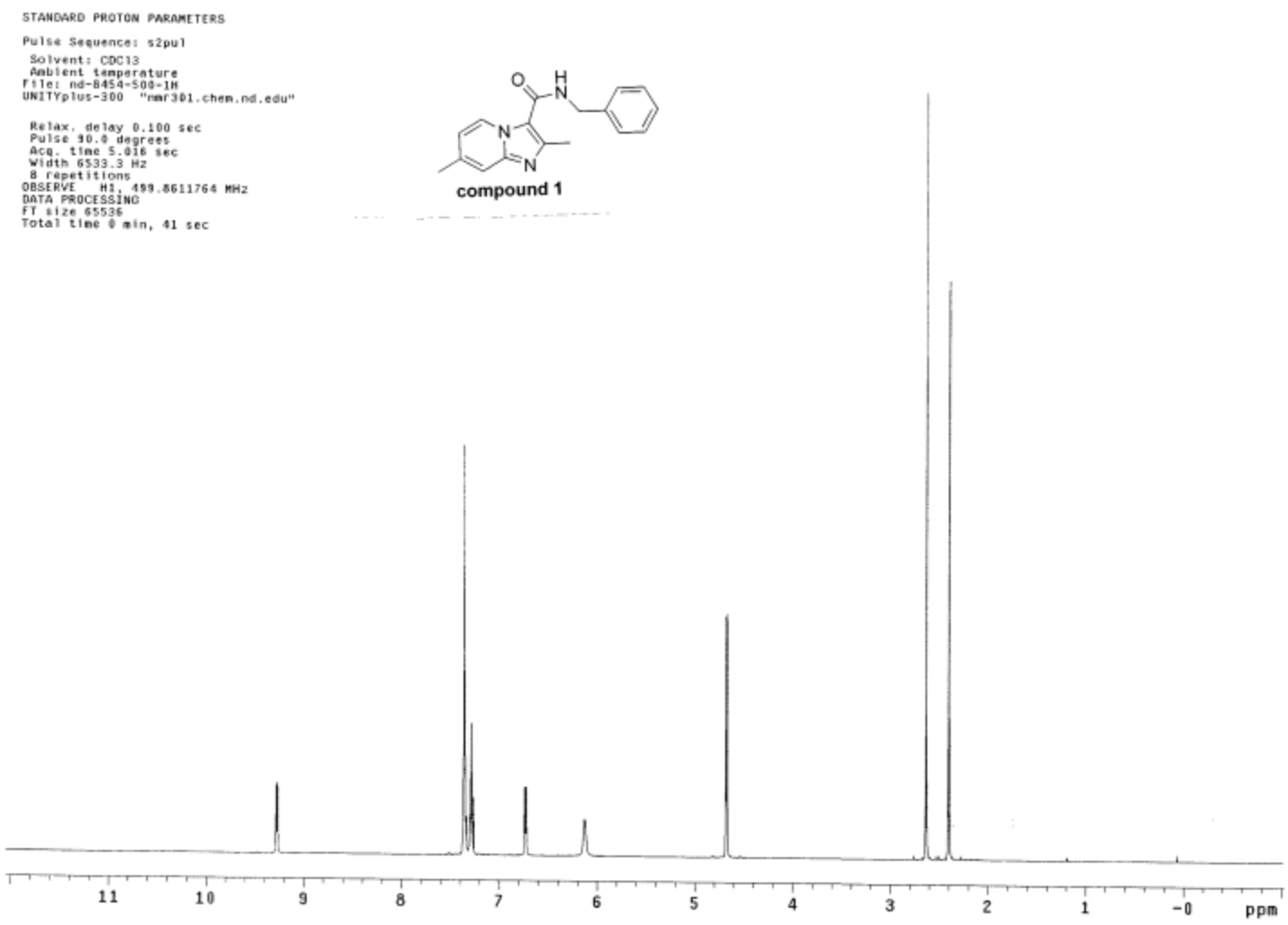
^{1}H NMR's and ^{13}C NMR's for compounds 1, 3, 4, and 6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N
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9
8
7
6
5
4
3
2
1
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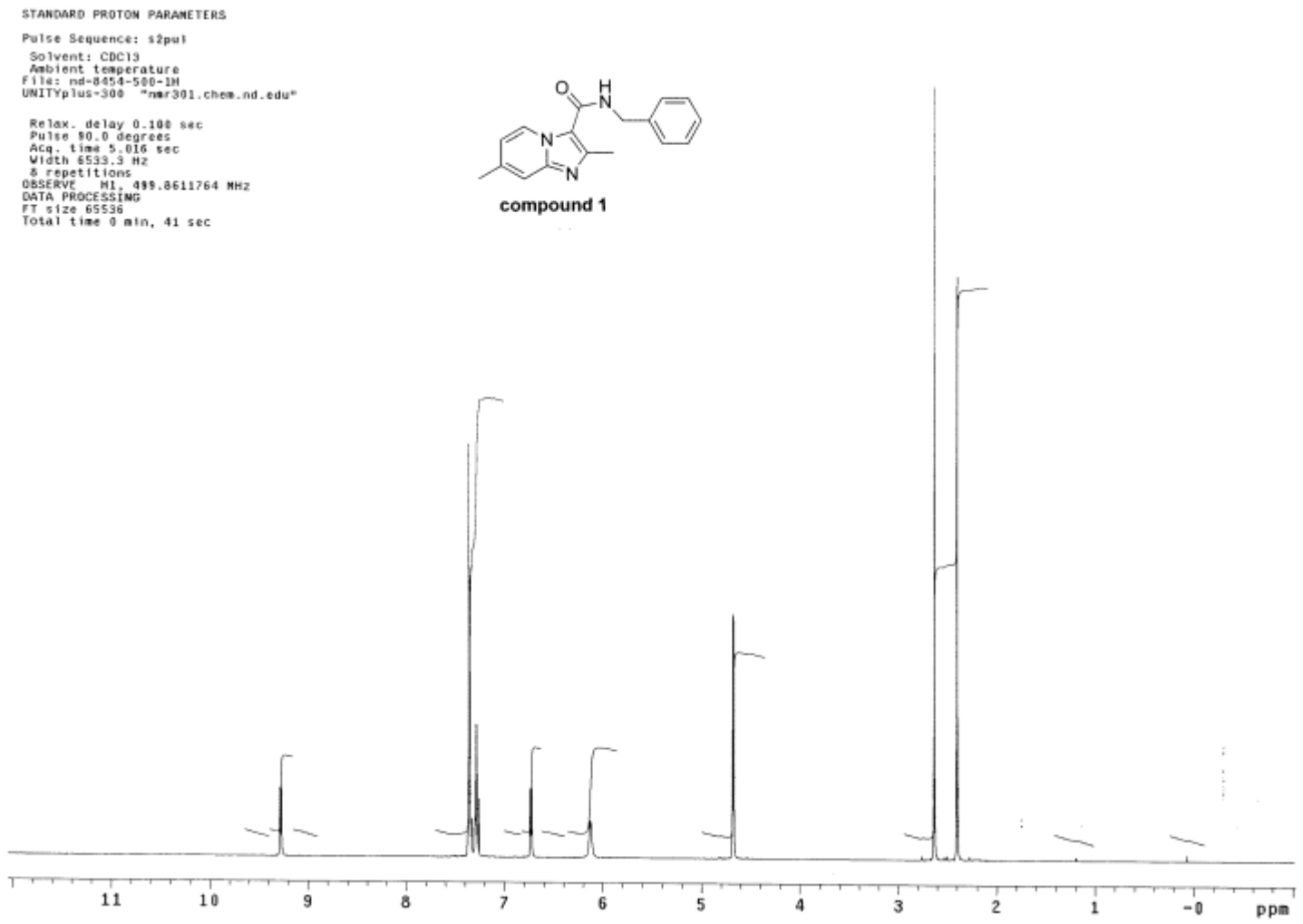
ppm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6
5
4
3
2
1
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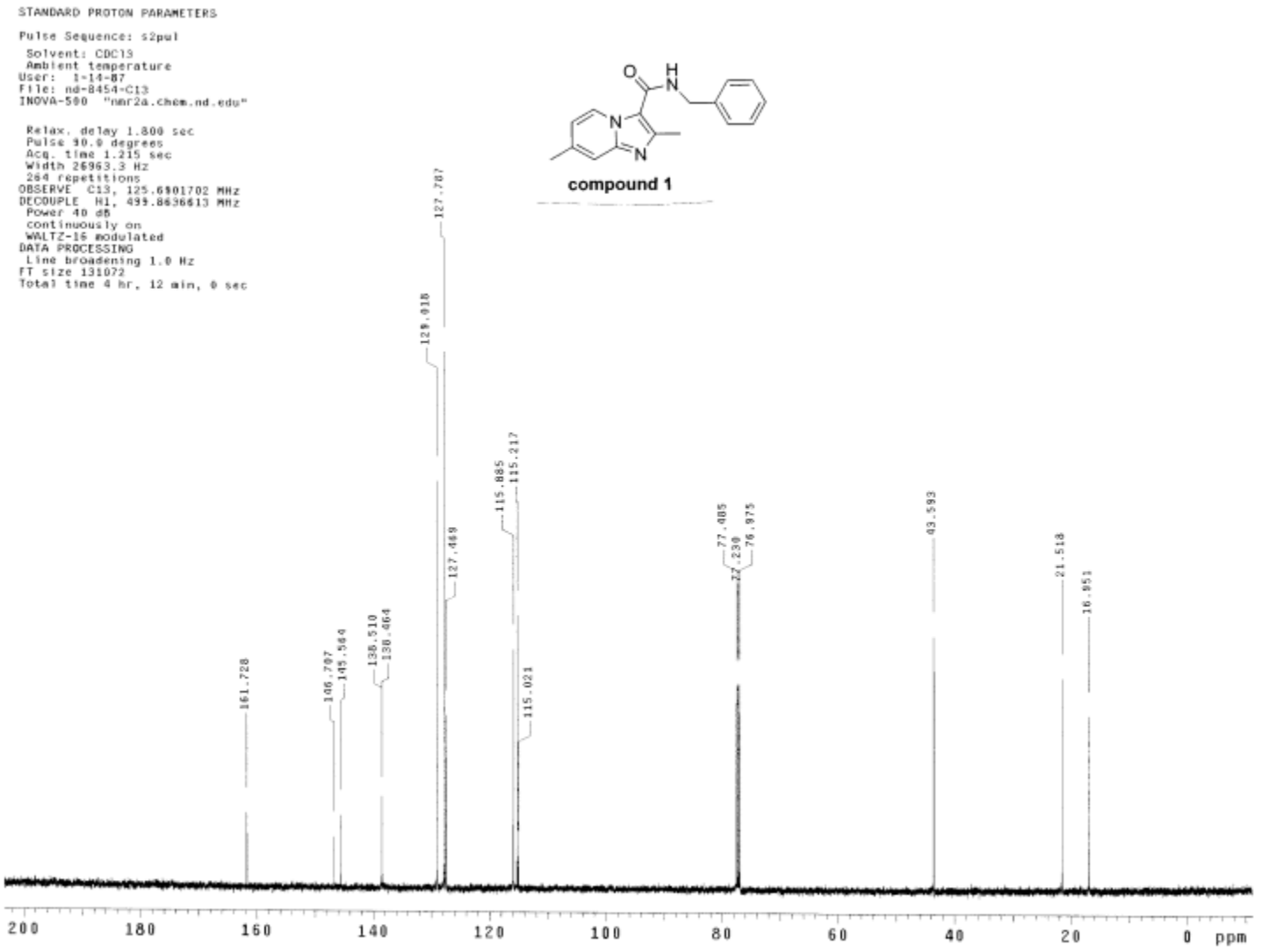
ppm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WALTZ-16 modulated
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145.584
138.510
130.464
129.018
127.787
127.469
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115.217
115.021
77.485
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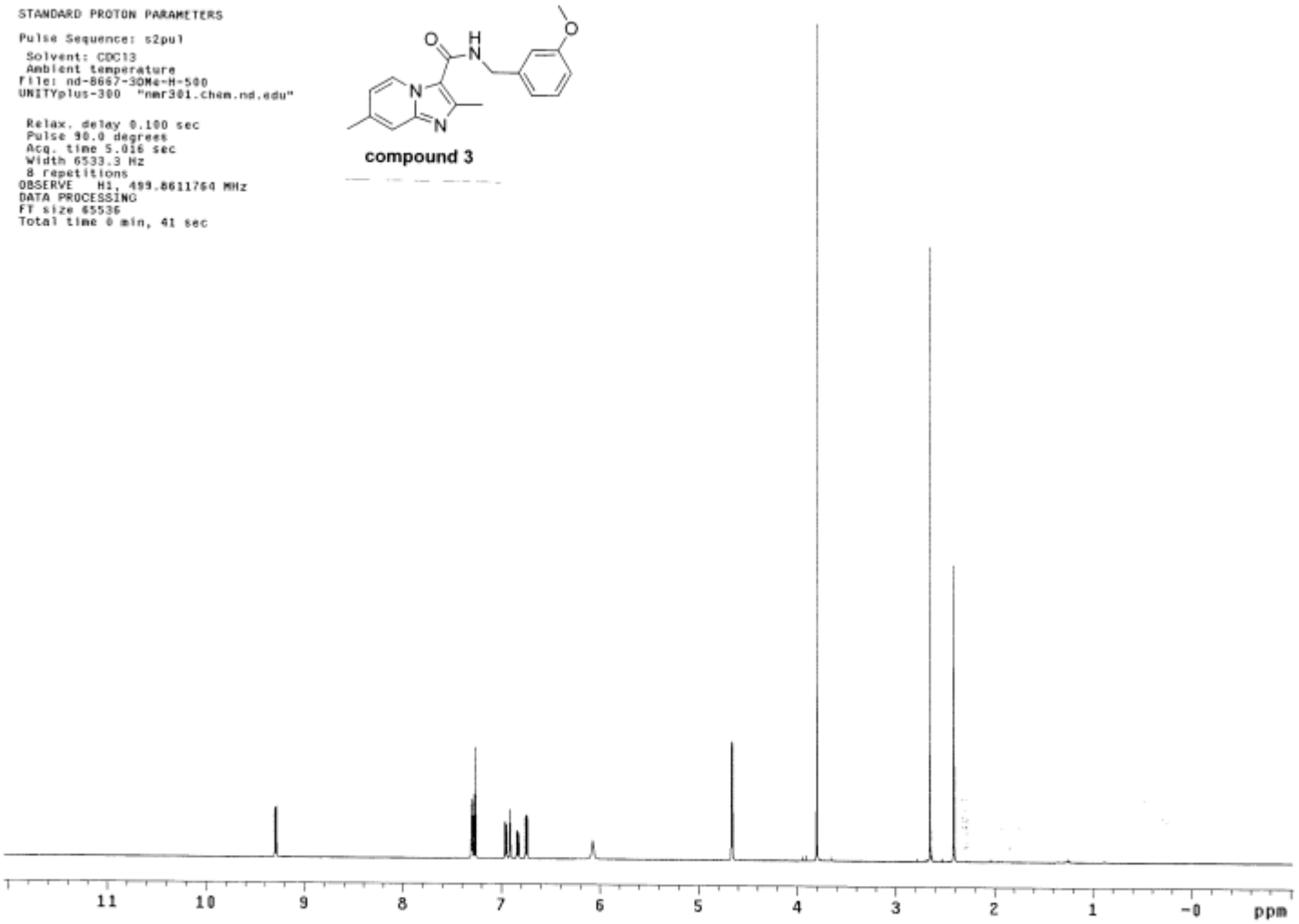
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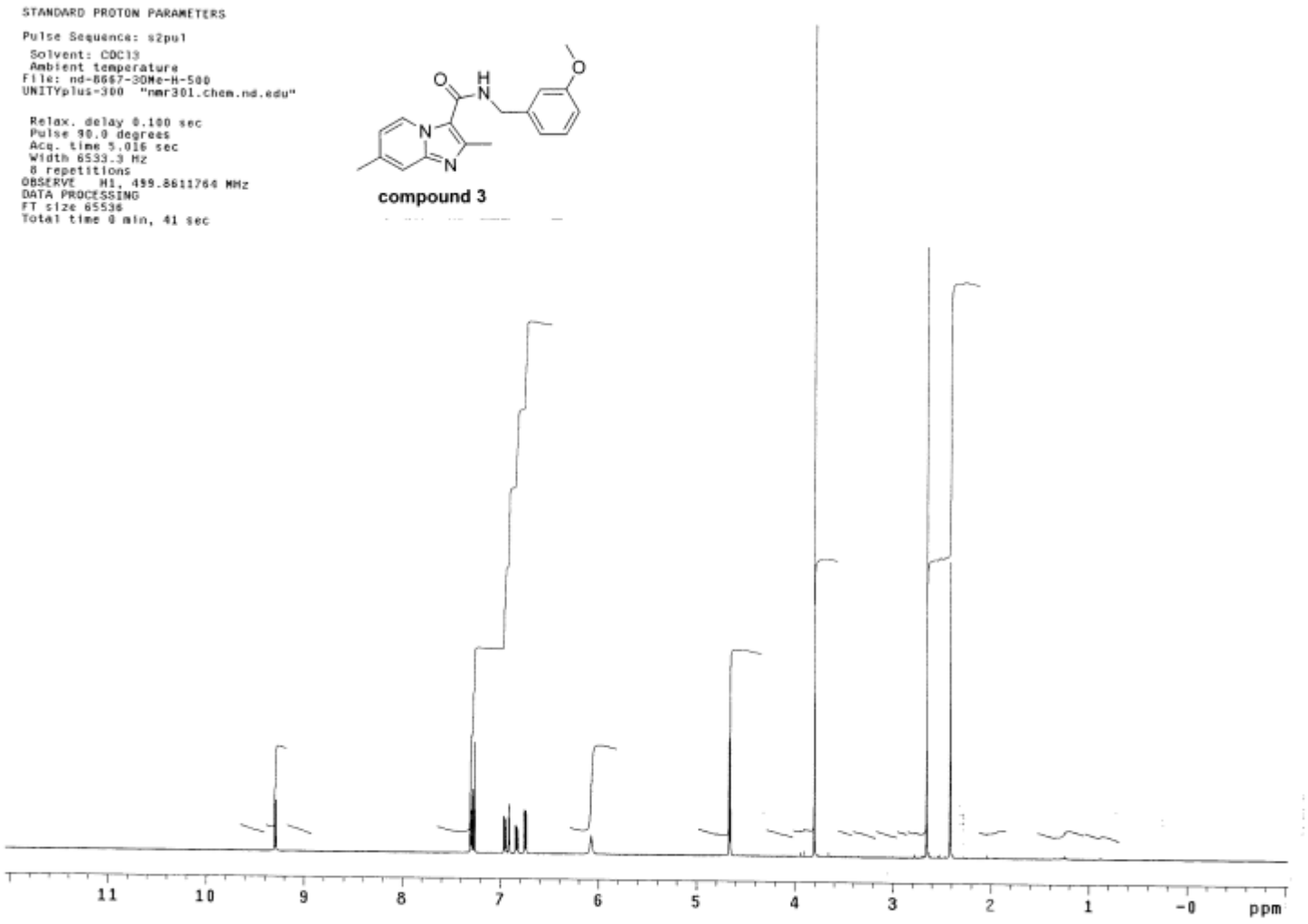
9
8
7
6
5
4
3
2
1
-0
ppm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11
10
9
8
7
6
5
4
3
2
1
-0
ppm

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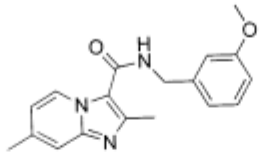

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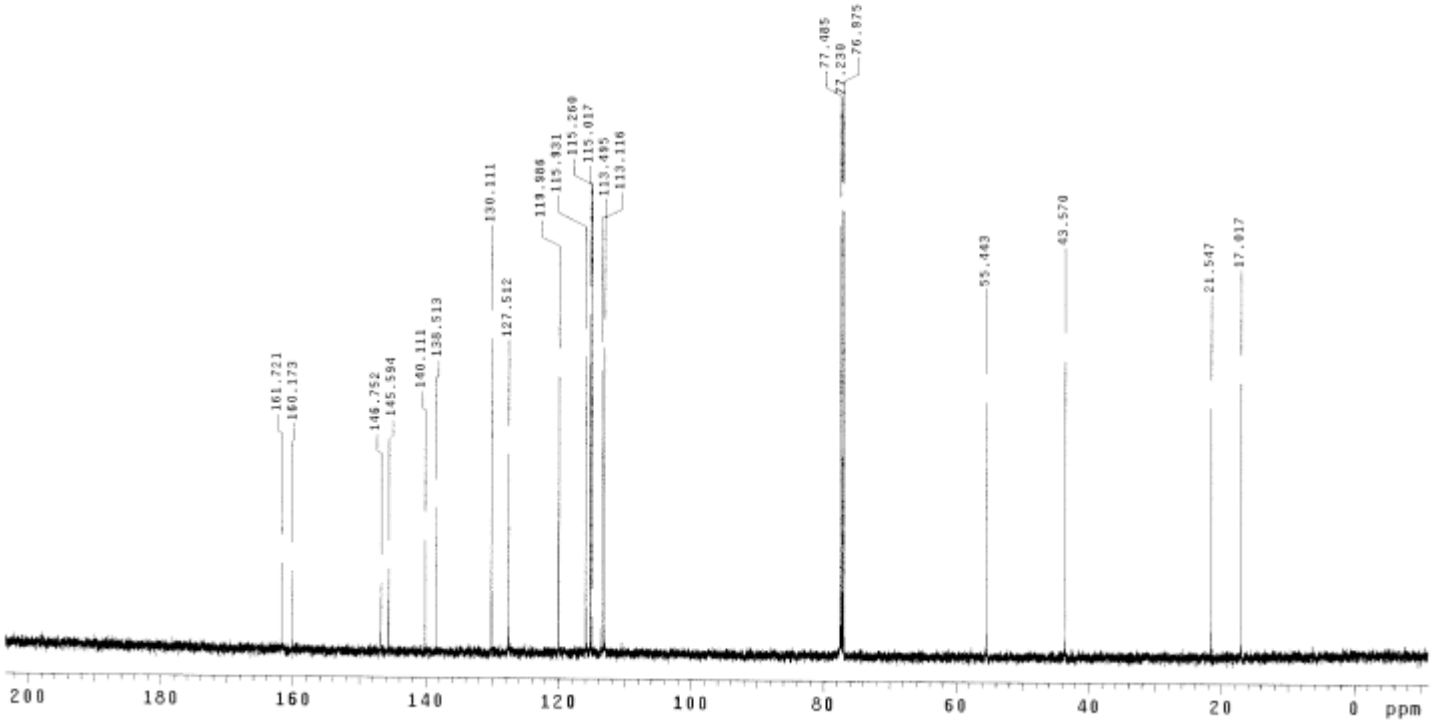

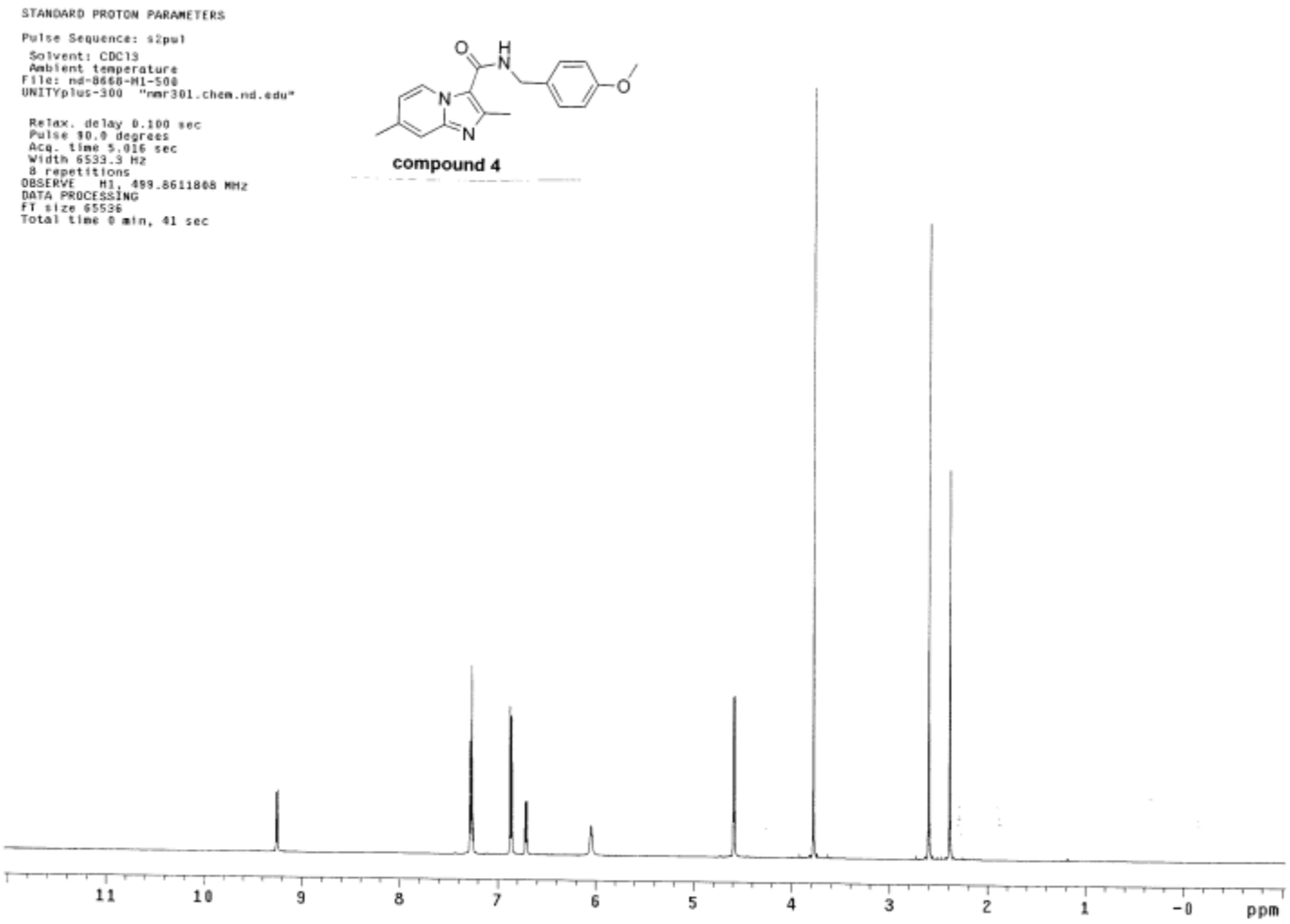
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5
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2
1
-0
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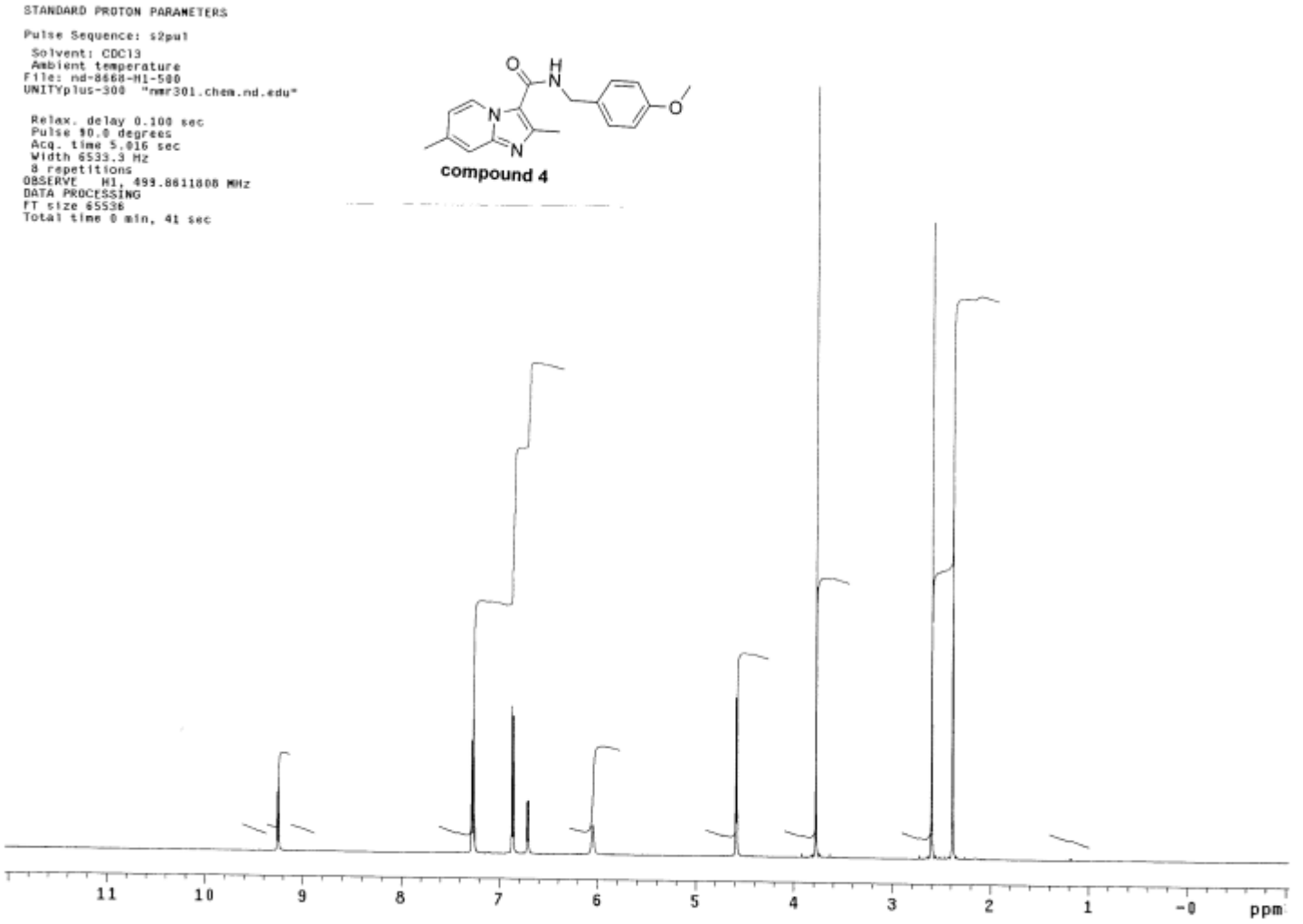
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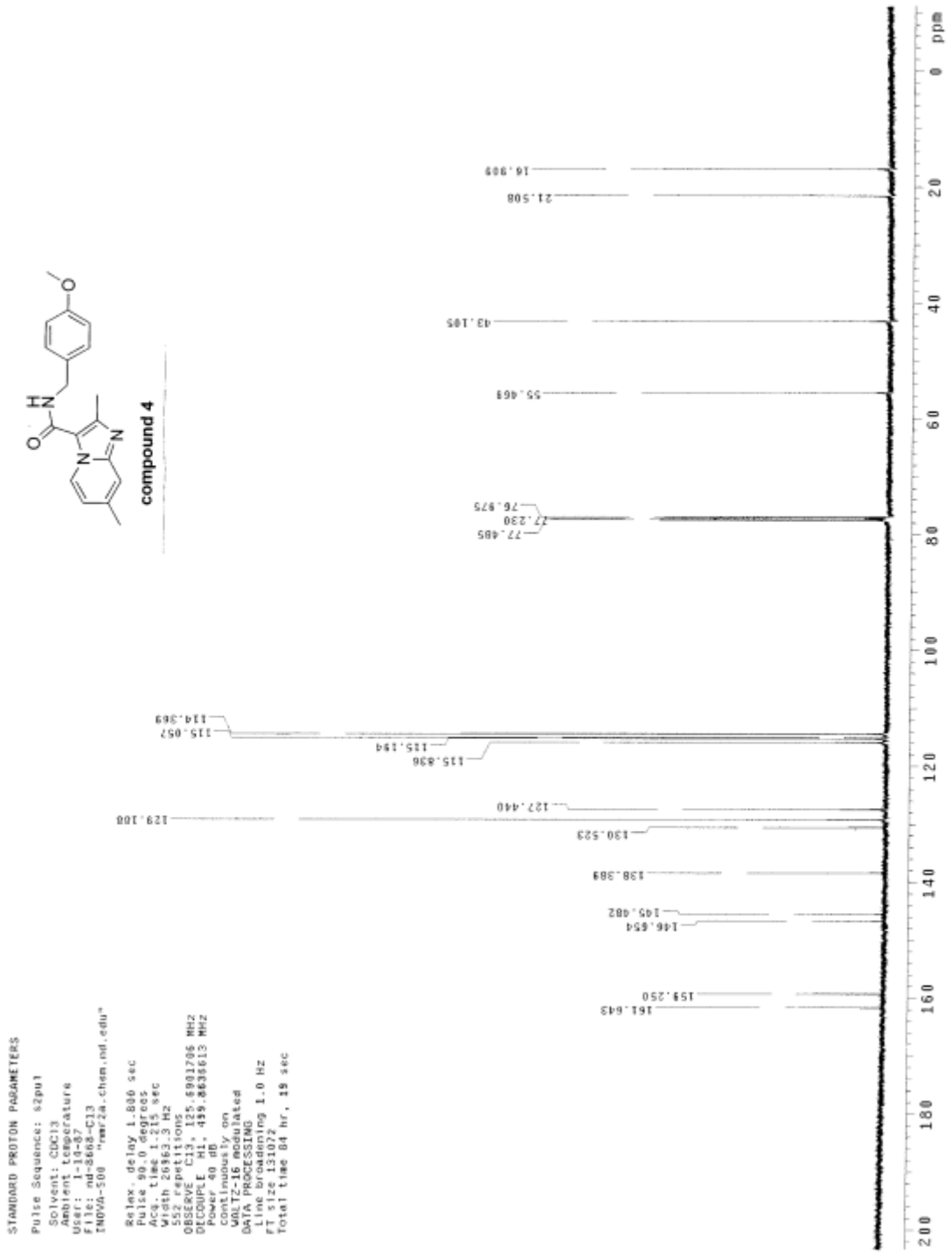
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77.485
114.369
115.057
115.194
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129.188
130.523
138.389
145.402
146.654
159.250
161.643
200
180
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140
120
100
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60
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20
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STANDARD PROTON PARAMETERS

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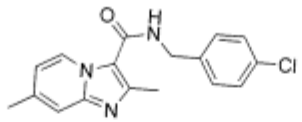
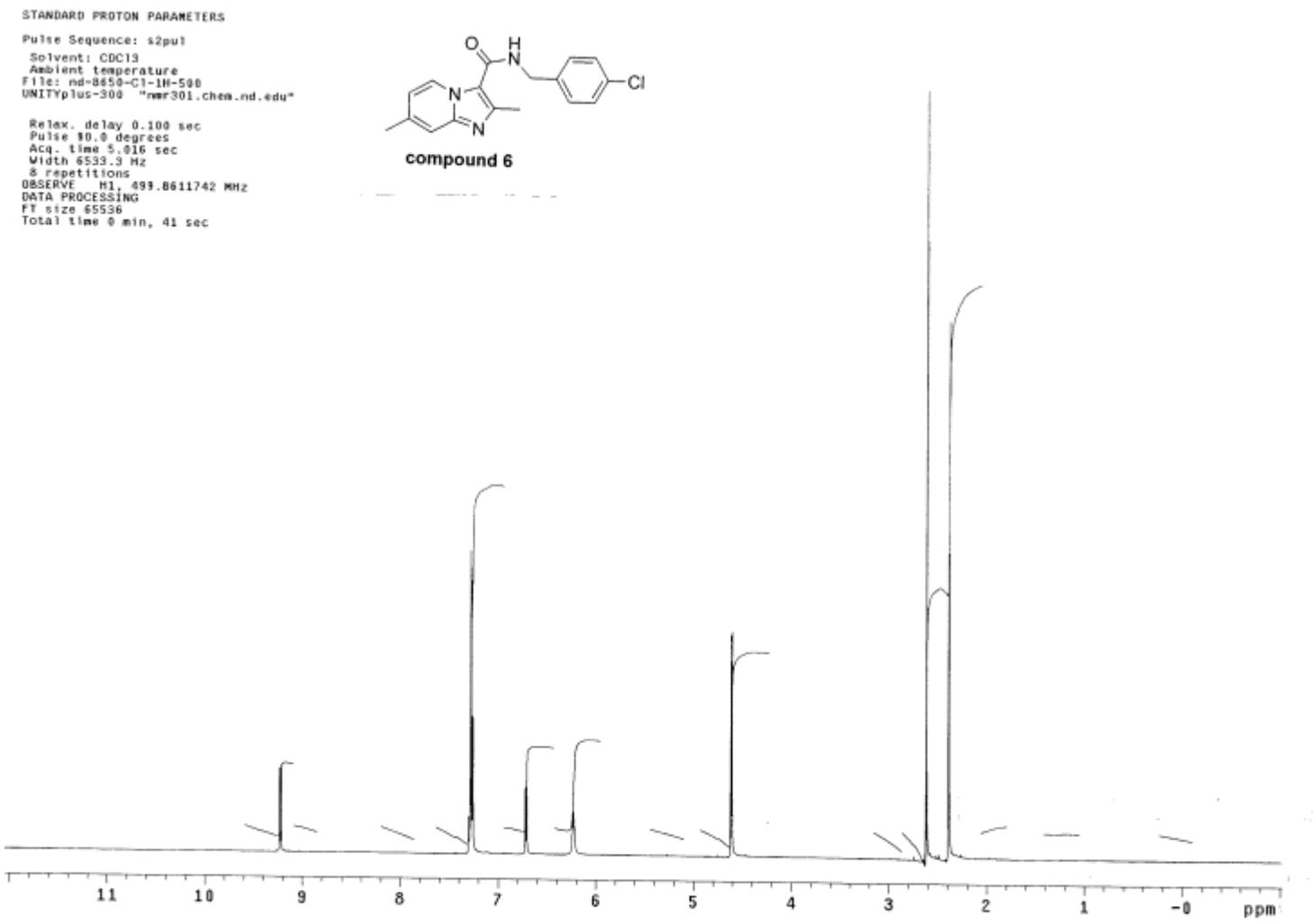


compound 6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O
H
N
N
N
Cl
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11
10
9
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7
6
5
4
3
2
1
-0
ppm

STANDARD PROTON PARAMETERS

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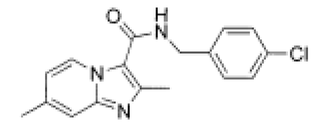

compound 6