Structure-property studies of an imidazoquinoline chemotype with antitrypanosomal activity

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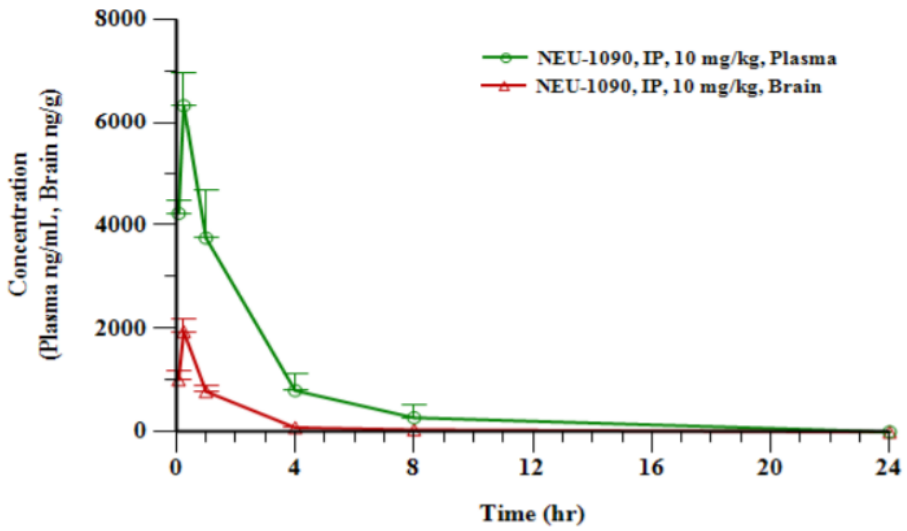


Figure S1. Peripheral blood levels of **NEU-1090** (**2**) after IP administration to female Balb/C mice (n=18) at a target dose 10 mg/kg in 10% DMA/5% solutol/30% PEG400/55% of a 20% HPβ-CD in water. Individual values for each time point are represented in the plot.

PK protocol for 2

Blood samples (approximately 60 µL) were collected from retro-orbital plexus of three mice at 0.08, 0.25, 1, 4, 8 and 24 h (IP). Samples were collected into labeled micro-tubes, containing K_2EDTA solution (20% K_2EDTA solution) as an anticoagulant. Plasma was immediately harvested from the blood by centrifugation at 4000 rpm for 10 min at 4 ± 2 °C and stored below -70 °C until bioanalysis. Immediately after collection of blood, animals were euthanized brain samples were isolated at 0.08, 0.25, 1, 4, 8 and 24 hr (i.p.). The tissue samples (brain) were homogenized using ice-cold phosphate buffer saline (pH 7.4) and homogenates were stored below -70 °C until analysis. Total homogenate volume was three times the brain weight.

Concentrations of **NEU-1090** (**2**) in mouse plasma and brain samples were determined by fit-for-purpose LC-MS/MS method. Non-Compartmental-Analysis module in Phoenix WinNonlin® (Version 6.3) was used to assess the pharmacokinetic parameters.

β-D-Galactosidase Transgenic *T. cruzi* Assay

A Thermo Scientific Multidrop Combi dispenser (MTX Lab Systems, Vienna, VA) was used to dispense 90 μL of *T. cruzi* amastigote–infected L6 cell culture (4×10^3 infected L6 cells per well) into 96-well Corning assay plates (Corning Inc., Corning, NY) already containing 10 μL of the compounds to be screened and controls. The plates were incubated at 37 °C for 96 h. Then, 30 μL of 100 μM CPRG and 0.1% NP40 diluted with PBS were added to each well, and the plates were incubated for 4 h at 37 °C in the dark. Absorbance at 585 nm was measured in a Vmax kinetic microplate reader (Molecular Probes). Compound activities were normalized using the in-plate negative (benznidazole at 10 μg/mL) and positive (0.2% DMSO) growth controls.

Resazurin-Based L6 Assay

One hundred microliters (100 μL) per well of culture medium containing the compounds and controls were added to L6 cells previously cultured (4×10^3 L6 cells per well). After 72 h at 37 °C the medium was exchanged, and the viable cell number was determined by resazurin (Sigma–Aldrich) reduction. 20 μl of resazurin (1.1 mg/ml) was added to each well and incubated in the dark for 2 h at 37 ºC. Cell viability was estimated by measuring the final fluorescence at 570-590 nm in an Infinite F200 plate reader (Tecan).

Cytotoxicity assay in THP-1

Cellular toxicity of all compounds was determined using the colorimetric MTT-based assay after incubation at 37 °C for 72 h in the presence of increasing concentrations of compounds (final maximal concentration was 50 μM in 0.5% DMSO per well).[1] The results are expressed as EC_{50} values, the concentration of compound that reduces cell growth by 50% versus untreated control cells. Assays were performed in duplicate at least twice to achieve a minimal n=3 per dose response.

Determination of EC_{50} in *L. donovani*

Macrophage-differentiated THP-1 cells were infected at a macrophage/parasite ratio of 1/10 with stationary *L. donovani* promastigotes for 24 h at 35 °C and 5% CO_2, and extracellular parasites were removed by washing with PBS. Infected cell cultures were then incubated with different compounds concentrations at 37 °C for 72 h. Luminescence was measured using the Promega kit luciferase assay system (Promega, Madison, WI). Assays were performed in duplicate at least twice, to achieve a minimal n=3 per dose response.

Table S1. *T. cruzi*, *L. donovani* and host cell line toxicity for all analogues presented in the manuscript.

ID	Molecule Name	*T cruzi* pEC_{50}	*L donovani* pEC_{50}	MRC5 pTC_{50}	L6 pTC_{50}	THP-1 pTC_{50}
5a	NEU-0004470	nt[a]	nt[e]	< 4.3 ± 0.0	> 6.2 ± 0.0	5.6 ± 0.08
5b	NEU-0004783	nt[a]	< 5.3 ± 0.0	< 4.3 ± 0.0	5.8 ± 0.32	4.3 ± 0.0
5c	NEU-0004791	nt[a]	6.6 ± 0.22	nd	> 6.2 ± 0.0	5.2 ± 0.0
5d	NEU-0006054	6.9 ± 0.03	6.4 ± 0.12[b,c]	< 4.3 ± 0.0	> 6.2 ± 0.0	< 4.3 ± 0.0
6	NEU-0004790	nd	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	nd
7a	NEU-0004914	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0
7b	NEU-0005815	4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0
7c	NEU-0005293	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	nt	< 4.3 ± 0.0
7d	NEU-0005294	5.3 ± 0.02	< 5.3 ± 0.0	< 4.3 ± 0.0	4.5 ± 0.03	< 4.3 ± 0.0
7e	NEU-0005316	< 4.7 ± 0.0	nd	< 4.3 ± 0.0	4.7 ± 0.12	< 4.3 ± 0.0
8a	NEU-0005876	< 5.0 ± 0.0	< 5.3 ± 0.0	< 4.6 ± 0.0	< 4.6 ± 0.0	< 4.6 ± 0.0
9a	NEU-0006050	5.6 ± 0.01	5.2 ± 0.23	4.5 ± 0.02	4.6 ± 0.03	nd
9b	NEU-0005997	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.9 ± 0.0	< 4.9 ± 0.0	< 4.9 ± 0.0
10a	NEU-0006051	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0
10b	NEU-0005998	< 4.7 ± 0.0	< 5.3 ± 0.0	4.4 ± 0.04	4.3 ± 0.06	< 4.3 ± 0.0
11a	NEU-0005118	6.0 ± 0.1	< 5.3 ± 0.0	< 4.3 ± 0.0	> 6.2 ± 0.0	< 4.3 ± 0.0
11b	NEU-0005368	nt[a]	6.6 ± 0.17	< 4.3 ± 0.0	> 6.2 ± 0.0	< 4.3 ± 0.0
12a	NEU-0005370	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0
12b	NEU-0005369	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0
13	NEU-0005379	5.8 ± 0.03	< 5.3 ± 0.0	< 4.3 ± 0.0	> 5.3 ± 0.0	5.5 ± 0.08
14a	NEU-0004965	6.0 ± 0.04	< 5.3 ± 0.0	< 5.3 ± 0.0	5.6 ± 0.04	5.3 ± 0.27
14c	NEU-0005121	6.8 ± 0.05	< 5.3 ± 0.0	< 4.3 ± 0.0	> 6.2 ± 0.0	< 4.3 ± 0.0
15a	NEU-0005120	6.8 ± 0.07	6.3 ± 0.2[d]	< 4.3 ± 0.0	> 6.2 ± 0.0	< 4.3 ± 0.0
15b	NEU-0006481	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	4.7 ± 0.06
15c	NEU-0006485	4.9 ± 0.06	< 5.3 ± 0.0	< 4.3 ± 0.0	4.9 ± 0.0	nd
15d	NEU-0005511	5.2 ± 0.01	< 5.3 ± 0.0	< 4.3 ± 0.0	4.7 ± 0.02	< 4.3 ± 0.0
15e	NEU-0006777	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0
15f	NEU-0006778	< 4.7 ± 0.0	< 5.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0	< 4.3 ± 0.0

nt=not tested
nd=no data
[a]Not progressed due to toxicity
[b]Top concentrations showed only partial inhibition of parasites
[c]Solubility issues
[d]Top concentration doesn't achieve 100% inhibition of parasites
[e]Toxic against host cells

Table S2. ADME data of all compounds presented in the manuscript.

ID	Molecule Name	Aqueous Solubility (µM)	Human PPB (%)	HLM CL_{int} (µL/min/mg protein)	Rat Hepatocyte CL_{int} (µL/min/10^6 cells)	$LogD_{7.4}$
5a	NEU-0004470	1.6	93	38	7.9	3.0
5b	NEU-0004783	4	98	28	11	3.1
5c	NEU-0004791	2	94	28	18	3
5d	NEU-0006054	14	8.4	22	21	2.8
6	NEU-0004790	9	92	130	10	2.2
7a	NEU-0004914	> 1000	nd	8	8	1
7b	NEU-0005815	960	63	45	10	1.8
7c	NEU-0005293	10[b]	94	66	65	3.5
7d	NEU-0005294	0.6[b]	99	95	70	4.1[e]
7e	NEU-0005316	4	98	84	43	3.2[b]
8a	NEU-0005876	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]
9a	NEU-0006050	8	98	> 300[d]	46	4.1
9b	NEU-0005997	7	99	> 300[d]	110	4.2
10a	NEU-0006051	690	46	< 3	11	0.4
10b	NEU-0005998	940	44	< 3	18	0.3
11a	NEU-0005118	0.3	94	36	11	3.7
11b	NEU-0005368	0.4	90	95	27	3.5
12a	NEU-0005370	130	nd[c]	3	1.9	0.2
12b	NEU-0005369	650	65	3	1	0.1
13	NEU-0005379	4	nd[c]	28	7.3	2
14a	NEU-0004965	4	94	12	38	3.3
14c	NEU-0005121	3	96	32	16	3.4
15a	NEU-0005120	0.9	98	48	13	3.4
15b	NEU-0006481	0.5	> 99	15	3.3	3.9
15c	NEU-0006485	0.3	> 99	23	18	4
15d	NEU-0005511	1.5	98	22	7.6	4
15e	NEU-0006777	4	99	31	150	3.7
15f	NEU-0006778	> 1000	33	nd	30	1.3

nd=no data

[a]Not soluble in DMSO, insoluble in DMSO

[b]Non-linear response

[c]Low recovery

[d]Compound detected only in first two samples

[e]Poor MS response

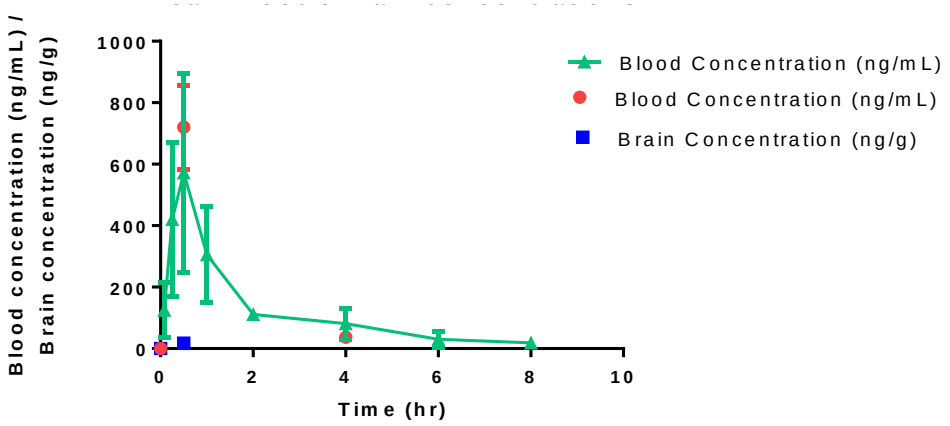


Figure S2. Peripheral blood levels of **5a** after IP administration to female NMRI mice (n=3) at a target dose 10 mg/kg in 1% DMSO:99%, 20% Captisol® in water. Individual values for each time point are represented in the plot. Y-axis is represented in logarithmic scale.

Table S3. Rate of action for representative compounds from the series.

ID	Molecule Name	pEC_{50} 6h ± SD	pEC_{50} 12h ± SD	pEC_{50} 18h ± SD	pEC_{50} 24h ± SD
5a	NEU-0004470	5.9 ± 0.05	6.6 ± 0.26	7.7 ± 0.28	8.3 ± 0.18
5b	NEU-0004783	4.4 ± 0.00	5.0 ± 0.08	5.4 ± 0.15	5.6 ± 0.13
5c	NEU-0004791	5.4 ± 0.07	6.5 ± 0.06	7.1 ± 0.30	7.1 ± 0.10
5d	NEU-0006054	4.4 ± 0.00	4.4 ± 0.00	6.0 ± 0.32	6.6 ± 0.22
7a	NEU-0004914	4.4 ± 0.00	4.4 ± 0.00	4.5 ± 0.03	4.5 ± 0.04
7e	NEU-0005316	4.4 ± 0.00	4.7 ± 0.23	5.7 ± 0.12	6.0 ± 0.19
13	NEU-0005379	5.6 ± 0.11	6.2 ± 0.23	7.1 ± 0.22	7.1 ± 0.15
14a	NEU-0004965	5.2 ± 0.08	5.9 ± 0.09	7.0 ± 0.34	7.4 ± 0.10
14b	NEU-0005121	5.8 ± 0.04	6.5 ± 0.14	6.7 ± 0.25	6.6 ± 0.19
15a	NEU-0005120	5.5 ± 0.14	6.5 ± 0.06	6.6 ± 0.37	6.8 ± 0.29

Table S4. Cidality assay for representative compounds. The pEC_{99} was determined at 72 h, a $pEC_{99} \geq 6$ signifies a cidal compound.

ID	Molecule Name	*Tbb* pEC_{50} ± SD	*Tbb* pEC_{99} ± SD
5a	NEU-0004470	8.4 ± 0.04	7.1 ± 0.49
5b	NEU-0004783	6.2 ± 0.18	4.1 ± 0.50
5c	NEU-0004791	7.4 ± 0.06	6.7 ± 0.17
5d	NEU-0006054	6.6 ± 0.14	5.5 ± 0.27
7a	NEU-0004914	4.7 ± 0.14	4.5 ± 0.14
7e	NEU-0005316	5.5 ± 0.08	4.3 ± 0.31
13	NEU-0005379	7.1 ± 0.05	6.1 ± 0.48
14a	NEU-0004965	7.4 ± 0.09	5.9 ± 0.40
14b	NEU-0005121	6.8 ± 0.02	6.6 ± 0.13
15a	NEU-0005120	7.0 ± 0.12	6.0 ± 0.27

General Chemistry Experimental

All compounds tested had a purity of > 95% as measured by LCMS, unless otherwise noted.

Reagents purchased were used as received, unless otherwise noted. Purification of intermediates was performed using silica gel chromatography using the Biotage® Isolera™One flash purification system. LCMS analysis was performed using a Waters Alliance reverse phase HPLC using a multi-wavelength photodiode array detector from 210 nm to 600 nm.

Preparative HPLC was conducted for final compounds on Waters FractionLynx system using acetonitrile/water and 0.1% formic acid gradient and collected based on UV monitoring at 254 nm.

^{1}H NMR spectra were obtained with Varian NMR systems, operating at either 400 or 500 MHz at room temperature, using solvents from Cambridge Isotope Laboratories. Chemical shifts (δ, ppm) are reported relative to the solvent peak ($CDCl_3$: 7.26 [^{1}H]; or DMSO-d_6: 2.50 [^{1}H]). Data for ^{1}H NMR spectra are reported as follows: chemical shift (ppm), multiplicity (s for singlet, d for doublet, t for triplet, dd for doublet of doublet, m for multiplet), coupling constant (Hz), and integration.

General Procedure A – Suzuki coupling.

A microwave reaction vessel was loaded with iodoquinoline, boronic acid or ester (4.0 equiv.), potassium carbonate (3.0 equiv.), and tetrakis(triphenylphosphine)palladium(0) (5 mol%). To the reaction tube was added a 4:2:1 (v/v, 0.04M) mixture of dimethoxyethane, ethanol, and deionized water, and Teflon coated

magnetic stir bar. The reaction was sealed, sparged with nitrogen for 5 minutes then heated to 175 °C for 15 minutes in a microwave reactor. Once cooled to ambient temperature, the reaction was diluted with ethyl acetate, filtered through Celite and partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, until no product remained in the aqueous layer by TLC. Combined organic extracts were dried with anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude residue was adsorbed to silica and purified by flash column chromatography.

General Procedure B – Buchwald-Hartwig coupling.

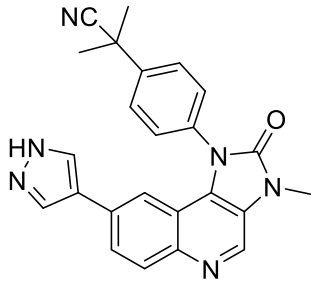
To a 4-mL vial was added halogen-quinoline, amine (1.2 equiv.), cesium carbonate (1.2 equiv.), and *tert*-butanol (0.2 M). The reaction mixture was sparged with nitrogen for two minutes before the addition of tris(dibenzylideneacetone)dipalladium(0) (2 mol%) and Xantphos (4 mol%); the reaction mixture was sealed and then sparged for an additional two minutes. The reaction was then heated to 100 °C overnight on a shaker plate. After cooling to ambient temperature, the reaction mixture was diluted with dichloromethane or ethyl acetate and filtered through Celite; the filtrate was concentrated under reduced pressure. The crude residue was adsorbed to silica and purified by flash column chromatography.

General Procedure C – Transfer hydrogenation.

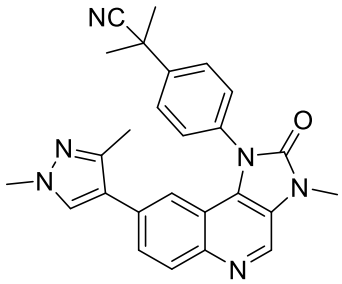
To an 8 mL vial was added alkene, ethanol (0.05 M) ammonium formate (2.5 equiv.) and 10% wt Pd/C (4 mol%). The vial was then sealed and heated on a heater shaker at 85 °C. Once LCMS indicated consumption of the starting material the reaction was cooled to ambient temperature, diluted with dichloromethane or ethyl acetate, and filtered through Celite; the filtrate was concentrated under reduced pressure. The crude residue was adsorbed to silica and purified by flash column chromatography.

General Procedure D – Boc deprotection.

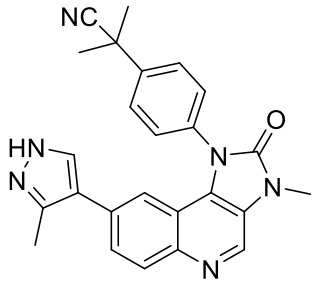
In a 4 mL vial was added Boc-amine, 4M HCl in dioxane (18.5 equiv.) and a Teflon coated magnetic stir bar. The reaction was then sealed and stirred at ambient until complete. The solvent was then removed under reduced pressure. The resultant orange residue was dissolved in methanol (0.1 M) and Si-Carbonate was added, the suspension was then stirred overnight at ambient. Si-Carbonate was removed by filtration, the filtrate adsorbed onto silica and purified by flash column chromatography.



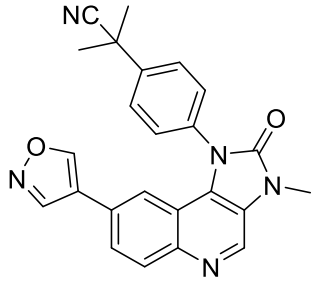
2-Methyl-2-(4-(3-methyl-2-oxo-8-(1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (5a): The title compound was prepared according to general procedure A on a 50-mg scale using 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole. The crude residue was purified by flash chromatography, eluting with 0-10% methanol in methylene chloride, with a constant 20% ethyl acetate additive to afford an off-white amorphous solid (28.8 mg, 66%). LCMS $[M+H]^+$ 409.0 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.86 (s, 6 H) 3.59 (s, 3 H) 6.97 (d, *J* = 1.5 Hz, 1 H) 7.29 (br. s., 1 H) 7.74 (d, *J* = 8.8 Hz, 2 H) 7.82 (dd, *J* = 8.8, 2.2 Hz, 1 H) 7.86 - 7.93 (m, 3 H) 7.99 (d, *J* = 8.8 Hz, 1 H) 8.92 (s, 1 H).



2-(4-(8-(1,3-Dimethyl-1*H*-pyrazol-4-yl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (5b). The title compound was prepared according to general procedure A on a 20-mg scale using 1,3-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole. The crude residue was purified by flash chromatography over silica, eluting with 0-10% methanol in methylene chloride, with a constant 20% ethyl acetate additive to afford a tan amorphous solid (14.8 mg, 79%). LCMS $[M+H]^+$ 437.1 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.74 (s, 3 H) 1.81 (s, 6 H) 3.59 (s, 3 H) 3.74 (s, 3 H) 7.15 (d, *J* = 2.0 Hz, 1 H) 7.67 - 7.73 (m, 3 H) 7.81 - 7.86 (m, 2 H) 7.89 (s, 1 H) 8.02 (d, *J* = 8.8 Hz, 1 H) 8.96 (s, 1 H).

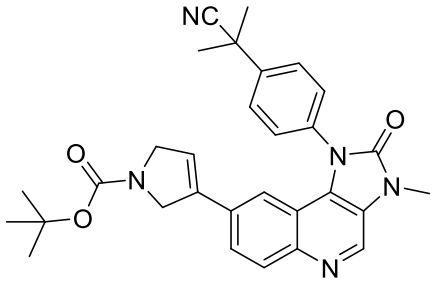


2-Methyl-2-(4-(3-methyl-8-(3-methyl-1*H*-pyrazol-4-yl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (5c). The title compound was prepared according to general procedure A on a 20-mg scale using 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole. The crude residue was purified by flash chromatography over silica, eluting with 0-10% methanol in methylene chloride, with a constant 20% ethyl acetate additive. Fractions containing product by TLC were combined and re-purified by prep HPLC, eluting with 5-95% acetonitrile in water to afford a tan amorphous solid (4.8 mg, 27%). LCMS $[M+H]^+$ 423.2 *m*/*z*; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 1.85 (s, 6 H) 2.00 (s, 3 H) 3.67 (s, 3 H) 7.21 (d, *J* = 1.5 Hz, 1 H) 7.60 - 7.68 (m, 3 H) 7.76 (dd, *J* = 8.8, 2.0 Hz, 1 H) 7.85 - 7.89 (m, 2 H) 8.04 (d, *J* = 8.8 Hz, 1 H) 8.84 (s, 1 H).



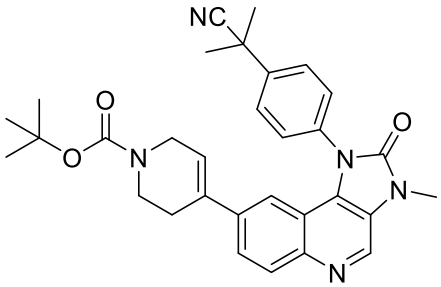
2-(4-(8-(Isoxazol-4-yl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (**5d).** A round bottom flask was loaded with 2-(4-(8-iodo-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (50 mg, 0.107 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole (25 mg, 0.128 mmol), potassium fluoride (18.6 mg, 0.320 μmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (8.7 mg, 0.011 mmol). To this was added dimethylformamide (2.0 mL, 0.05 M) and deionized water (0.77 mL). The reaction was sealed, degassed for five minutes with nitrogen, then evacuated and backfilled with nitrogen three times. The reaction was heated to 50 °C for 1 hour. Once cooled to room temperature, the reaction mixture was diluted with dichloromethane, filtered through Celite, then partitioned between dichloromethane and water. The aqueous layer was extracted twice with dichloromethane. Combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude residue was adsorbed to silica and purified by flash chromatography eluting with 1-3% methanol in dichloromethane to afford the title compound as a buff solid (7.4 mg, 17%). LCMS $[M+H]^+$ 410.1 *m*/*z*; ^{1}H

NMR (500 MHz, DMSO-d_6) δ ppm 1.85 (s, 6 H), 3.61 (s, 3 H), 7.02 (d, *J* = 1.5 Hz, 1 H), 7.74 (d, *J* = 8.8 Hz, 2 H), 7.86 (dd, *J* = 8.8, 2.0 Hz, 1 H), 7.89 (d, *J* = 8.8 Hz, 2 H), 8.10 (d, *J* = 8.8 Hz, 1 H), 8.35 (s, 1 H), 9.01 (s, 1 H), 9.17 (s, 1 H).



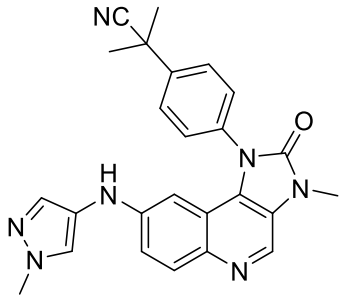
***tert*-Butyl 3-(1-(4-(2-cyanopropan-2-yl)phenyl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl)-2,5-dihydro-1*H*-pyrrole-1-carboxylate (8a).** The title compound was prepared according to general procedure A on a 70-mg scale using tert-butyl 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The crude residue was purified by flash chromatography over silica, eluting with 8 % methanol (modified with 5% NH_4OH) in ethyl acetate to afford an amorphous solid (57 mg, 75%). LCMS $[M+H]^+$ 510.2 *m/z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*)[a] δ ppm 1.43 - 1.53 (m, 9 H) 1.83 - 1.95 (m, 6 H) 3.69 (s, 3 H) 4.06 (br. s., 1 H) 4.20 - 4.29 (m, 2 H) 6.04 (br. s., 1 H) 7.00 (s, 1 H) 7.51 - 7.61 (m, 2 H) 7.68 (dd, *J* = 8.8, 1.5 Hz, 1 H) 7.74 - 7.87 (m, 2 H) 8.07 (d, *J* = 8.8 Hz, 1 H) 8.74 - 8.83 (m, 1 H)**.**

a) Peaks in 1 H-NMR spectrum broad and split due to the presence of N-Boc rotamers

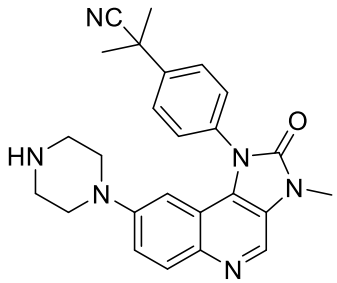


***tert*-Butyl 4-(1-(4-(2-cyanopropan-2-yl)phenyl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (8b).** The title compound was prepared according to general procedure A on a 100-mg scale using tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate. The crude residue was purified by flash chromatography over silica, eluting with 3 % methanol (modified with 5% NH_4OH) in ethyl acetate to afford a colorless solid (82 mg, 75%). LCMS $[M+H]^+$ 523.4 *m/z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 1.49 (s, 9 H), 1.86 (s, 6 H), 2.08 (br. s., 2 H), 3.54 (t, *J* = 5.6 Hz, 2 H), 3.69 (s, 3 H), 4.05 (br. s.,

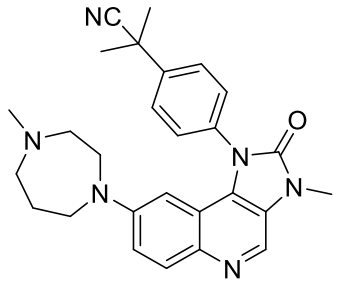
2 H), 5.93 - 6.22 (m, 1 H), 7.00 (br. s., 1 H), 7.59 (d, *J* = 8.8 Hz, 2 H), 7.68 (d, *J* = 8.8 Hz, 1 H), 7.78 (d, *J* = 8.3 Hz, 2 H), 8.06 (d, *J* = 8.8 Hz, 1 H), 8.76 (s, 1 H).



2-Methyl-2-(4-(3-methyl-8-((1-methyl-1*H*-pyrazol-4-yl)amino)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (**6**). The title compound was prepared according to general procedure B on a 25-mg scale using 1-methyl-1*H*-pyrazol-4-amine. Crude material was purified by flash chromatography, eluting with 0-10% methanol in methylene chloride. Fractions containing product were combined, concentrated, and re-purified by reversed phase prep HPLC, eluting with 5-95% acetonitrile in water to afford a yellow amorphous solid (11 mg, 46%). LCMS $[M+H]^+$ 438.2 *m*/*z*; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 1.85 (s, 6 H) 3.65 (s, 3 H) 3.86 (s, 3 H) 5.51 (s, 1 H) 6.49 (d, *J* = 2.9 Hz, 1 H) 7.05 (d, *J* = 1.0 Hz, 1 H) 7.16 (dd, *J* = 9.3, 2.4 Hz, 1 H) 7.27 (s, 1 H) 7.55 (d, *J* = 8.8 Hz, 2 H) 7.76 (d, *J* = 8.8 Hz, 2 H) 7.83 (d, *J* = 9.3 Hz, 1 H) 8.59 (s, 1 H).



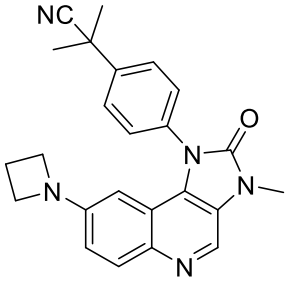
2-Methyl-2-(4-(3-methyl-2-oxo-8-(piperazin-1-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (**7a**)**.** The title compound was prepared according to general procedure B on a 40-mg scale using 1-Boc piperazine. The resultant intermediate material was then deprotected using general procedure D. The crude material was then reversed phase prep HPLC, eluting with 5-95% acetonitrile in water to afford a yellow amorphous solid (5.3 mg, 22% *over two steps*). $[M+H]^+$ 427.2 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.79 (s, 6 H) 2.64 - 2.77 (m, 8 H) 3.55 (s, 2 H) 6.04 (d, *J* = 2.4 Hz, 1 H) 7.38 (dd, *J* = 9.3, 2.4 Hz, 1 H) 7.66 (d, *J* = 8.3 Hz, 2 H) 7.79 - 7.85 (m, 3 H) 8.72 (s, 1 H).



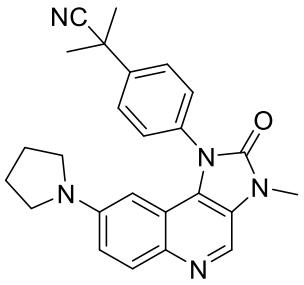
2-Methyl-2-(4-(3-methyl-8-(4-methyl-1,4-diazepan-1-yl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (**7b**)**.**

The title compound was prepared according to general procedure B on a 14.6-mg scale using 1-methylhomopiperazine. Crude material was purified by column chromatography, eluting with 5% methanol (modified with 5% NH_4OH) in dichloromethane to afford a beige solid (6.4 mg, 13%).

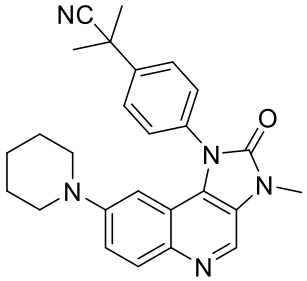
LCMS $[M+H]^+$ 455.2 *m*/*z*; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 1.81 - 1.87 (m, 8 H) 2.34 (s, 3 H) 2.49 (dd, *J* = 5.4 Hz, 2 H) 2.57 (dd, *J* = 4.4, 4.4 Hz, 2 H) 3.24 (t, *J* = 6.3 Hz, 2 H) 3.37 (dd, *J* = 5.4, 4.4 Hz, 2 H) 3.64 (s, 3 H) 6.05 (d, *J*=2.4 Hz, 1 H) 7.29 (dd, *J* = 9.8, 2.9 Hz, 1 H) 7.66 (d, *J* = 8.3 Hz, 2 H) 7.79 - 7.93 (m, 3 H) 8.57 (s, 1 H)



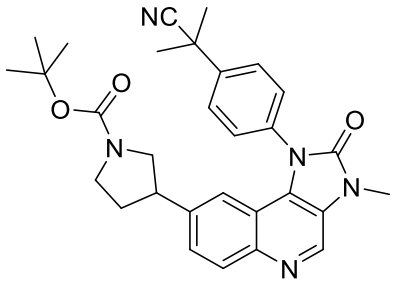
2-(4-(8-(Azetidin-1-yl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (**7c**)**.** The title compound was prepared according to general procedure B on a 50-mg scale using azetidine hydrochloride and additional equivalent of cesium carbonate. Crude material was purified by column chromatography, eluting with 50-100% acetone in hexanes to afford a yellow amorphous solid (11 mg, 26%). LCMS $[M+H]^+$ 398.2 *m*/*z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 1.81 (s, 6 H) 2.31 (quin, *J* = 7.2 Hz, 2 H) 3.64 (s, 3H), 3.67 (t, *J* = 7.2 Hz, 4 H) 5.69 (d, *J* = 2.4 Hz, 1 H) 6.82 (dd, *J* = 8.8, 2.4 Hz, 1 H) 7.56 (d, *J* = 8.3 Hz, 2 H) 7.71 (d, *J* = 8.3 Hz, 2 H) 7.94 (d, *J* = 9.3 Hz, 1 H) 8.54 (s, 1 H).



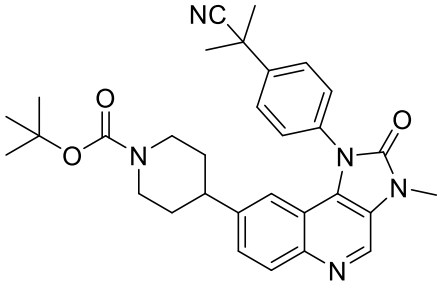
2-Methyl-2-(4-(3-methyl-2-oxo-8-(pyrrolidin-1-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (7d). The title compound was prepared according to general procedure B on a 50-mg scale using pyrrolidine. Crude material was purified by flash chromatography, eluting with 0-20% methanol with 5% ammonium hydroxide in methylene chloride. Fractions containing product were combined, concentrated, and re-purified by reversed phase preparative HPLC, eluting with 5-70% acetonitrile in water with 0.01% formic acid to afford a yellow amorphous solid (6 mg, 13%). LCMS $[M+H]^+$ 412.3 *m/z*; ^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 1.81 (s, 6 H) 1.95 (dt, *J* = 6.1, 3.3 Hz, 4 H) 3.01 (br. s, 4 H) 3.66 (s, 3 H) 5.72 (d, *J* = 2.4 Hz, 1 H) 7.15 (dd, *J* = 9.3, 2.4 Hz, 1 H) 7.59 (d, *J* = 8.3 Hz, 2 H) 7.77 (d, *J* = 7.83 Hz, 2 H) 8.35 (d, *J* = 9.3 Hz, 1 H) 8.55 (s, 1 H).



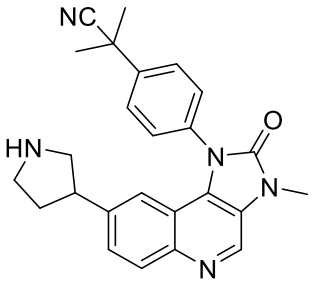
2-Methyl-2-(4-(3-methyl-2-oxo-8-(piperidin-1-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (**7e**)**.** The title compound was prepared according to general procedure B on a 100-mg scale using piperidine. The crude material was purified by flash chromatography, eluting with 50-70% acetone in hexanes, to afford a yellow amorphous solid (21 mg, 47%). LCMS $[M+H]^+$ 426.4 *m/z*; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 1.55 (br. s., 6 H) 1.84 (s, 6 H) 2.90 (m, 4 H) 3.64 (s, 3 H) 6.23 (d, *J* = 2.6 Hz, 1 H) 7.40 (dd, *J* = 9.5, 2.6 Hz, 1 H) 7.64 (d, *J* = 8.3 Hz, 2 H) 7.82 - 7.88 (m, 3 H) 8.63 (s, 1 H).



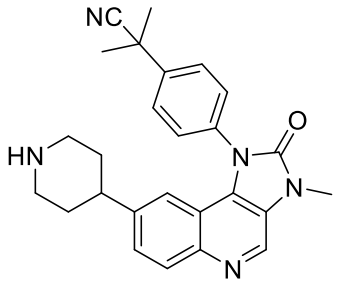
***tert*-Butyl 3-(1-(4-(2-cyanopropan-2-yl)phenyl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl)pyrrolidine-1-carboxylate (9a).** The title compound was prepared according to general procedure C on a 65 mg-scale using *tert*-Butyl 3-(1-(4-(2-cyanopropan-2-yl)phenyl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl)-2,5-dihydro-1*H*-pyrrole-1-carboxylate (**8a** NEU-5876). The crude residue was purified by flash chromatography over silica, eluting with 1 – 3 % methanol in ethyl acetate to afford a colorless solid (41 mg, 63%). LCMS [M+H]+ 512.2 *m*/*z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 1.46 (br. s., 9 H), 1.61 - 1.73 (m, 1 H), 1.79 - 1.90 (m, 6 H), 1.94 - 2.13 (m, 1 H), 2.92 - 3.05 (m, 1 H), 3.16 - 3.38 (m, 2 H), 3.39 - 3.53 (m, 1 H), 3.68 (s, 3 H), 3.71 - 3.80 (m, 1 H), 6.92 (s, 1 H), 7.42 (d, *J* = 8.8 Hz, 1 H), 7.56 (d, *J* = 8.3 Hz, 1 H), 7.72 - 7.82 (m, 2 H), 8.07 (d, *J* = 8.8 Hz, 1 H), 8.77 (s, 1 H).



***tert*-Butyl 4-(1-(4-(2-cyanopropan-2-yl)phenyl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl)piperidine-1-carboxylate (9b)**. The title compound was prepared according to general procedure C on a 64 mg-scale using *tert*-Butyl 4-(1-(4-(2-cyanopropan-2-yl)phenyl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (**8b**). The crude residue was purified by flash chromatography over silica, eluting with 5% methanol (modified with 5% NH_4OH) in dichloromethane to afford a faint brown solid (23.4 mg, 37%). LCMS $[M+H]^+$ 526.3 *m*/*z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 1.13 - 1.27 (m, 2 H) 1.47 (s, 9 H) 1.70 (d, *J* = 12.7 Hz, 2 H) 1.84 (s, 6 H) 2.47 - 2.60 (tt, *J* = 11.7, 2.9 Hz, 1 H) 2.64 - 2.81 (t, *J* = 11.7 Hz, 2 H) 3.68 (s, 9 H) 4.06 - 4.23 (m, 2 H) 6.82 (s, 1 H) 7.41 (dd, *J* = 8.8, 2.0 Hz, 1 H) 7.56 (d, *J* = 8.8 Hz, 2 H) 7.76 (d, *J* = 8.8 Hz, 2 H) 8.05 (d, *J* = 8.8 Hz, 1 H) 8.76 (s, 1 H)

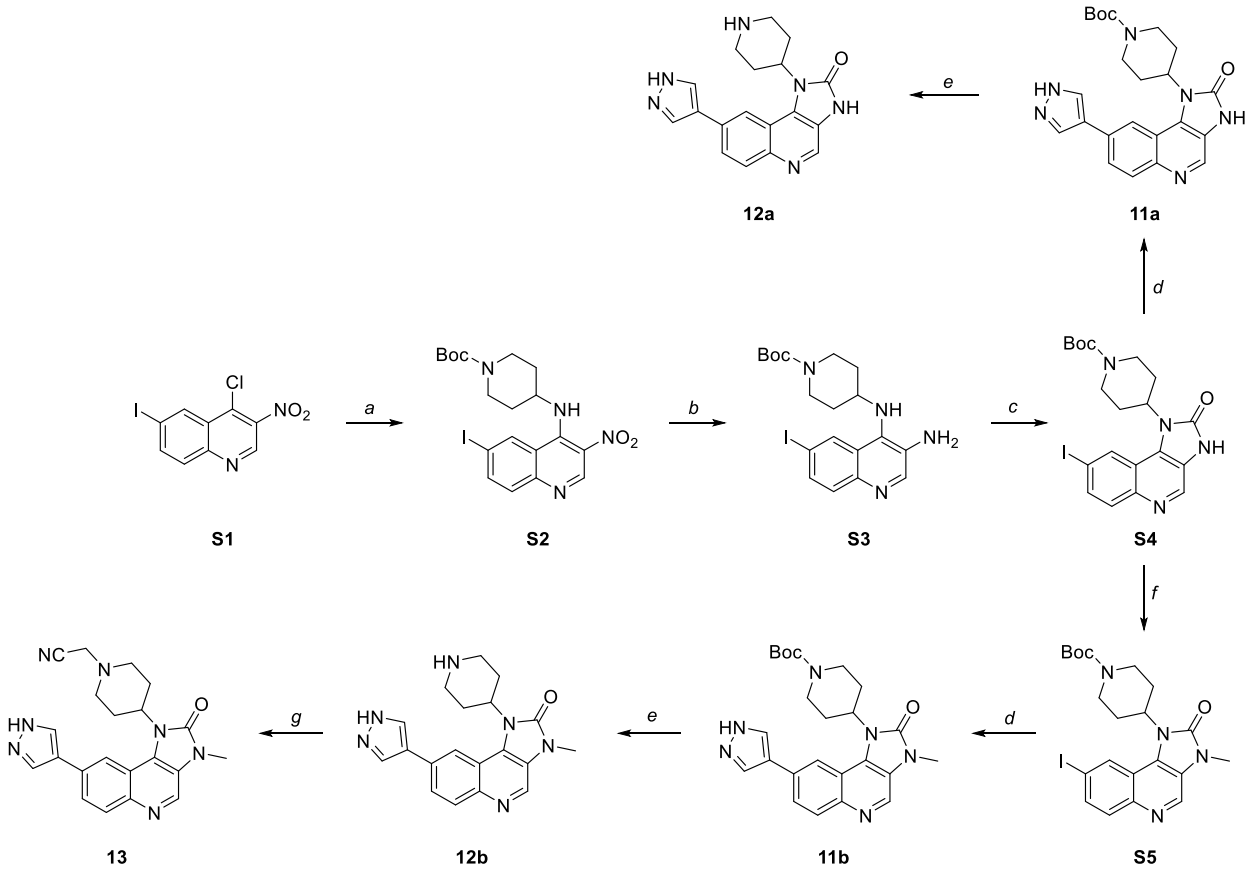


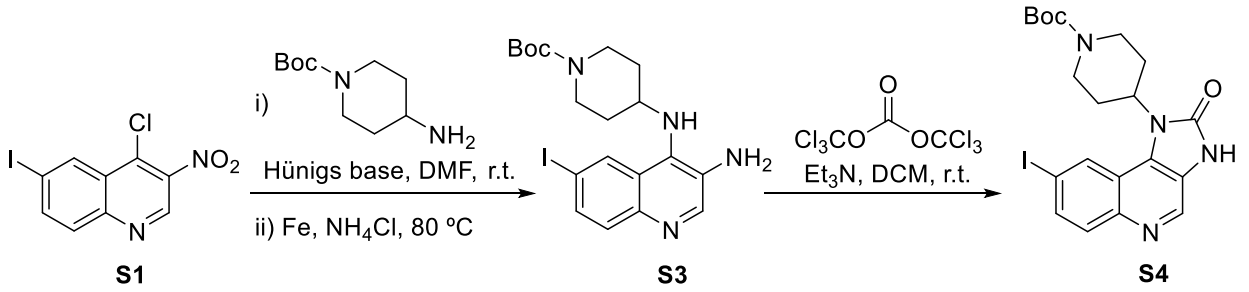
2-Methyl-2-(4-(3-methyl-2-oxo-8-(pyrrolidin-3-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (**10a**)**.** The title compound was prepared according to general procedure D on a 33 mg-scale using *tert*-Butyl 3-(1-(4-(2-cyanopropan-2-yl)phenyl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl)pyrrolidine-1-carboxylate (**9a** NEU-6050)**.** The crude residue was purified by flash chromatography over silica, eluting with 1 – 18 % methanol (modified with 5% NH_4OH) in dichloromethane to afford a faint brown solid (15.9 mg, 60%). LCMS $[M+H]^+$ 412.2 *m*/*z*; ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 1.75 (quin., *J* = 9.8 Hz, 1 H) 1.86 (s, 6 H) 2.18 – 2.26 (m, 1 H) 2.87 (t, *J* = 10.5 Hz, 1 H) 3.22 - 3.30 (m, 1 H) 3.31 - 3.42 (m, 2 H) 3.52 - 3.59 (m, 1 H) 3.68 (s, 3 H) 6.90 (s, 1 H) 7.43 (dd, *J* = 8.8, 1.5 Hz 2 H) 7.57 (t, *J* = 6.6 Hz, 2 H) 7.76 – 7.80 (m, 2 H) 8.08 (d, *J* = 8.8 Hz, 1 H) 8.79 (s, 1 H).



2-Methyl-2-(4-(3-methyl-2-oxo-8-(piperidin-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (**10b).** The title compound was prepared according to general procedure D on a 24.8 mg-scale using *tert*-Butyl 4-(1-(4-(2-cyanopropan-2-yl)phenyl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl)piperidine-1-carboxylate (**9b** NEU-5997). The crude residue was purified by flash chromatography over silica, eluting with 40 % methanol (modified with 5% NH_4OH) in ethyl acetate to afford a colorless solid (13.1 mg, 66%). LCMS $[M+H]^+$ 426.3 *m*/*z*; ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 1.24 (qd, *J*=13.0, 3.4 Hz, 3 H) 1.70 (d, *J*=13.0 Hz, 3 H) 1.85 (s, 6 H) 2.54 (tt, *J*=11.7, 3.4 Hz, 1 H) 2.67 (td, *J*=12.1, 1.7 Hz, 4 H) 3.10 (d, *J*=12.2 Hz, 2 H) 3.68 (s, 3 H) 6.85 (s, 2 H) 7.43 (dd, *J*=8.8, 2.0 Hz, 2 H) 7.57 (d, *J*=8.8 Hz, 5 H) 7.57 (d, *J*=8.8 Hz, 2 H) 7.76 (d, *J*=8.3 Hz, 4 H) 8.04 (d, *J*=8.8 Hz, 2 H) 8.75 (s, 2 H)

Scheme S1. Synthesis of saturated headgroups. *Reagents and reaction conditions*: *a*) Hünigs base, DMF, 16 h. 54% yield *b*) Fe, NH_4Cl, ethanol, 80 ºC, 16h. 38% yield *c*) Triphosgene, CH_2Cl_2, 0 – rt, 2 h. 8% yield *d*) 5 mol% $Pd(PPh_3)_4$, K_2CO_3, 4:2:1 DME, EtOH, H_2O 175 ºC µwave, 20 min. 22%-56% yield *e*) HCl, dioxane. 95%-96% yield *f*) Bu_4NBr, MeI, NaOH, 1:1 CH_2Cl_2:H_2O. 83% yield *g*) Hünigs base, DMF, 16 h. 48% yield.





***tert*-butyl 4-((6-iodo-3-nitroquinolin-4-yl)amino)piperidine-1-carboxylate**

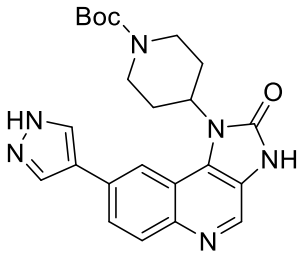
To a 25 mL RBF was added 4-chloro-6-iodo-3-nitroquinoline (**S1**)[2] (446 mg, 1.33 mmol, 1 equiv.), 1-boc-4-amino-piperidine (269 mg, 1.33 mmol, 1 equiv.), Hünigs base (279 μL, 1.6 mmol, 1.2 equiv.) and dimethylformamide (8mL, 0.17M). The reaction was then stirred overnight at ambient temperature before the reaction mixture was diluted with water (40 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organics were then washed with brine and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography over silica, eluting with 1 – 10 % methanol in dichloromethane to afford a yellow solid (360 mg, 54%). LCMS $[M+H]^+$ 499.0 *m*/*z*; ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 1.41 (s, 9 H) 1.63 (dddd, *J* = 12.5, 4.4 Hz, 2 H) 1.93 (d, *J* = 12.5 Hz, 2 H) 2.82 (br. s., 2 H) 3.58 - 3.67 (m, 1 H) 3.94 (d, *J* = 12.5 Hz, 2 H) 7.66 (d, *J* = 8.8 Hz, 1 H) 8.09 (dd, *J* = 8.8, 1.5 Hz, 1 H) 8.13 (d, *J* = 8.8 Hz, 1 H) 8.86 (s, 1 H) 8.97 (s, 1 H)

***tert*-butyl 4-((3-amino-6-iodoquinolin-4-yl)amino)piperidine-1-carboxylate** (**S3**)

To a 45 mL vial was added *tert*-butyl 4-((6-iodo-3-nitroquinolin-4-yl)amino)piperidine-1-carboxylate (310 mg, 622.1 μmol, 1 equiv.) iron (347 mg, 6.22 mmol, 10 equiv.), ammonium chloride (333 mg, 6.22 mmol, 10 equiv.) and ethanol (31 mL, 0.02M). The vial was sealed with a Teflon lined lid and heated to 80 ºC on a shaker plate. Once LCMS analysis confirmed the reaction was complete the reaction mixture was cooled to ambient and filtered through Celite. Ethanol was removed under reduced pressure and the residue partitioned between dichloromethane (25 mL) and water (25 mL). The aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organics were then washed with brine, dried over sodium sulfate, filtered, and concentrated on the rotovap. The crude residue was purified by flash chromatography over silica, eluting with 1 – 10 % methanol (modified with 5% NH_4OH) in dichloromethane to afford a yellow solid (110 mg, 38%). LCMS $[M+H]^+$ 469.0 *m*/*z*; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 9 H) 1.42 - 1.48 (m, 2 H) 1.72 (d, *J* = 11.7 Hz, 1 H) 2.67 (br. s., 1 H) 3.20 - 3.32 (m, 1 H) 3.92 (br. s., 2 H) 4.67 (d, *J* = 10.3 Hz, 1 H) 5.22 (br. s, 2 H) 7.50 (d, *J* = 8.1 Hz, 1 H) 7.56 (dd, *J* = 8.1, 1.5 Hz, 1 H) 8.41 (s, 1 H) 8.42 (d, *J* = 1.5 Hz, 1 H)

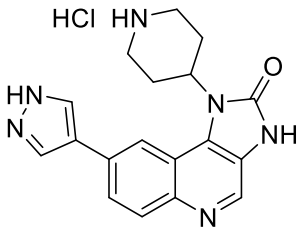
***tert*-butyl 4-(8-iodo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (S4)**

To a dry, 0 ºC solution of *tert*-butyl 4-((3-amino-6-iodoquinolin-4-yl)amino)piperidine-1-carboxylate (**S3**) (300 mg, 641 μmol, 1 equiv.) and triethyl amine (107.2 μL, 768.7 μmol, 1.2 equiv.) in dichloromethane (12.9 mL, 0.05M) was added dropwise a solution of triphosgene (209 mg, 704.6 μmol, 1.1 equiv.) in dichloromethane (5.1 mL, 0.14M). The reaction was then allowed to warm to ambient temperature over 2 h. The reaction was then quenched with saturated sodium bicarbonate (20 mL). The biphasic mixture was then separated, and the aqueous layer was extracted with dichloromethane (2 x 25 mL). The combined organics were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography over silica, eluting with 1 – 20 % methanol in dichloromethane to afford the titled compound as light orange solid (24 mg, 8%). LCMS $[M+H]^+$ 493.1 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.45 (s, 9 H) 1.91 (d, *J* = 11.7 Hz, 2 H) 2.81 - 3.16 (m, 2 H) 3.86 - 4.38 (m, 2 H) 4.84 (t, *J* = 11.7 Hz, 1 H) 7.79 (d, *J* = 8.8 Hz, 1 H) 7.88 (d, *J* = 8.8 Hz, 1 H) 8.55 (s, 1 H) 8.66 (s, 1 H) 11.61 (br.s, 1 H). *Note one 2H signal is absent. Signal overlaps with DMSO-d5*.



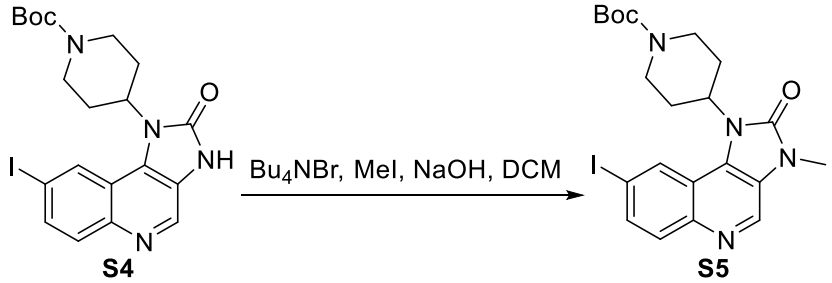
***tert*-butyl 4-(2-oxo-8-(1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (11a)**

The title compound was prepared according to general procedure A on a 100 mg-scale using *tert*-butyl 4-(8-iodo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (**S3**) and pyrazole-4-boronic acid pinacol ester. The crude residue was purified by flash chromatography over silica, eluting with 1 – 20 % methanol in dichloromethane to afford a colorless solid (20 mg, 22%). LCMS $[M+H]^+$ 435.2 *m*/*z*; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.45 (s, 9 H) 1.96 (d, *J* = 11.7 Hz, 2 H) 2.52 – 2.61 (m, 2 H) 2.98 - 3.15 (m, 2 H) 4.17 (br. s., 2 H) 4.98 (t, *J* = 11.7 Hz, 1 H) 7.90 (d, *J* = 8.8 Hz, 1 H) 8.00 (d, *J* = 8.8 Hz, 1 H) 8.07 (br. s., 1 H) 8.27 (s, 1 H) 8.37 (br. s., 1 H) 8.57 (s, 1 H) 11.48 (br. s., 1 H) 13.09 (br. s., 1 H)



1-(piperidin-4-yl)-8-(1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one hydrochloride (12a)

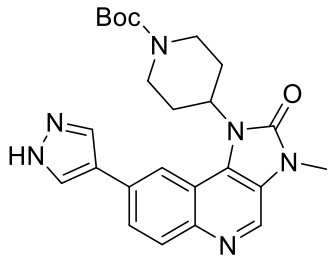
The title compound was prepared according to general procedure D on a 9 mg-scale *tert*-butyl 4-(2-oxo-8-(1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (**10** NEU-5118). The crude residue was purified *via* trituration from ethyl acetate (8.1 mg, 95%). LCMS $[M+H]^+$ 335.2 *m*/*z*; ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 2.41 (d, *J* = 13.4 Hz, 1 H) 3.17 (dddd, *J* = 13.4, 11.7, 4.0 Hz, 2 H) 3.44 (ddd, *J* = 13.4, 2.9 Hz, 2 H) 3.66 (d, *J* = 13.4 Hz, 2 H) 5.40 (tt, *J* = 11.7, 3.9 Hz, 1 H) 8.20 (d, *J* = 9.2 Hz, 1 H) 8.33 (dd, *J*=8.8, 1.5 Hz, 1 H) 8.35 – 8.37 (m, 2 H) 8.56 (d, *J* = 1.5 Hz, 1 H) 8.85 (s, 1 H)



***tert*-butyl 4-(8-iodo-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (S5)**

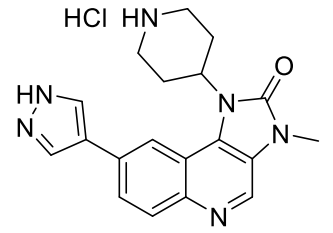
To a stirred solution of *tert*-butyl 4-(8-iodo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (**S4**) (122.4 mg, 0.248 mmol, 1 equiv.), tetrabutylammonium bromide (7.98 mg, 0.025 mmol, 0.1 equiv.) and methyl iodide (23.1 μL, 0.371 mmol, 3 equiv.), in dichloromethane (5 mL, 0.05M), was added a 0.15 M aqueous solution of NaOH (2.5 mL, 0.375 mmol, 3 equiv.). The solution was stirred overnight before the biphasic reaction was separated and the aqueous layer extracted with dichloromethane (2 x 20 mL). The combined organics were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude residue was purified by flash chromatography over silica, eluting with 2 – 7 % methanol in dichloromethane to afford a faint beige solid (104 mg, 83%). LCMS $[M+H]^+$ 495.0 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.45 (s, 9 H) 1.91 (d, *J* = 12.2 Hz, 2 H) 2.85 -

3.16 (m, 2 H) 3.48 (s, 3 H) 4.04 - 4.25 (m, 2 H) 4.89 (t, *J* = 12.2 Hz, 1 H) 7.82 (d, *J* = 8.8 Hz, 1 H) 7.90 (dd, *J* = 8.8, 1.5 Hz, 1 H) 8.57 (s, 1 H) 8.91 (s, 1 H)



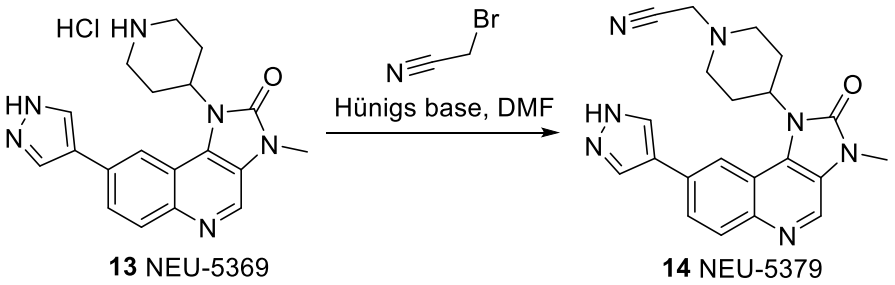
***tert*-butyl 4-(3-methyl-2-oxo-8-(1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (11b)**

The title compound was prepared according to general procedure A on a 60 mg-scale using *tert*-butyl 4-(8-iodo-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (**S5**) and pyrazole-4-boronic acid pinacol ester. The crude residue was purified by flash chromatography over silica, eluting with 5 % methanol in dichloromethane to afford a colorless solid (29.7 mg, 56%). LCMS $[M+H]^+$ 449.1 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.45 (s, 9 H) 1.97 (dd, *J* = 11.2, 2.4 Hz, 2 H) 2.52 - 2.65 (m, 2 H) 2.93 - 3.26 (m, 2 H) 3.48 (s, 3 H) 4.06 - 4.28 (m, 2 H) 5.03 (t, *J* = 11.2 Hz, 1 H) 7.92 (dd, *J* = 8.8, 1.5 Hz, 1 H) 8.04 (d, *J* = 8.8 Hz, 1 H) 8.09 (s, 1 H) 8.29 (s, 1 H) 8.38 (s, 1 H) 8.81 (s, 1 H)



3-methyl-1-(piperidin-4-yl)-8-(1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one hydrochloride (12b)

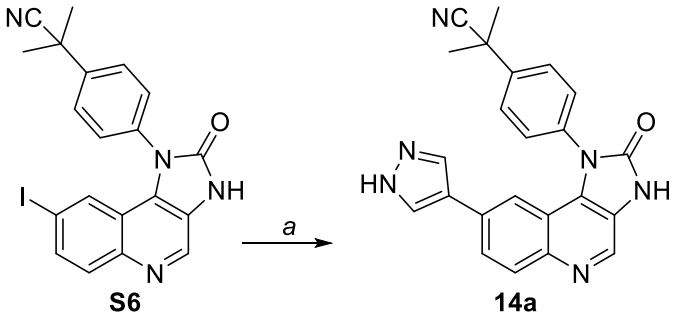
The title compound was prepared according to general procedure D on a 60 mg-scale using *tert*-butyl 4-(3-methyl-2-oxo-8-(1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidine-1-carboxylate (**11b**). The crude residue was purified *via* trituration from ethyl acetate (12.3 mg, 96%). LCMS $[M+H]^+$ 349.2 *m*/*z*; ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 2.41 (d, *J* = 13.6 Hz, 2 H) 3.17 (dddd, *J* = 13.6, 12.6, 4.4 Hz, 2 H) 3.47 (ddd, *J* = 13.6, 2.4 Hz, 3 H) 3.63 (s, 3 H) 3.66 (s, 2 H) 5.45 (tt, *J* = 12.6, 4.4 Hz, 1 H) 8.23 (d, *J* = 8.8 Hz, 1 H) 8.35 (d, *J* = 8.8 Hz, 1 H) 8.47 (s, 2 H) 8.60 (s, 1 H) 9.15 (s, 1 H)



13 NEU-5369 **14** NEU-5379

2-(4-(3-methyl-2-oxo-8-(1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)piperidin-1-yl)acetonitrile (**13**)

To a stirred 0 °C solution of 3-methyl-1-(piperidin-4-yl)-8-(1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one hydrochloride (**12b**) (13 mg, 31.3 µmol, 1 equiv.), Hünigs base (13 µL, 74.6 µmol, 2.0 equiv.) in DMF (1.0 mL, 0.03M), was added bromoacetonitrile (3.1 µL, 44.8 µmol). The reaction mixture was slowly warmed to ambient over 2 h. The reaction was quenched with water (5 mL) and the aqueous layer extracted with ethyl acetate (3 x 10 mL). The combined organics were washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica, eluting with 1 – 10 % methanol in dichloromethane to afford a colorless solid (6.9 mg, 48%). LCMS $[M+H]^+$ 388.2 *m/z*; ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 1.98 (d, *J* = 11.2 Hz, 2 H) 2.47 (d, *J* = 12.6 Hz, 2 H) 2.74 (dddd, *J* = 12.6, 12.6, 12.6, 4.9 Hz, 2 H) 3.07 (d, *J* = 11.2 Hz, 2 H) 3.50 (s, 3 H) 3.88 (s, 1 H) 4.86 (tt, *J* = 12.6, 4.9 Hz, 1 H) 7.93 (d, *J* = 8.8 Hz, 1 H) 8.04 (d, *J* = 8.8 Hz, 1 H) 8.07 (br. s., 1 H) 8.35 (br. s., 1 H) 8.43 (br. s., 1 H) 8.82 (s, 1 H) 13.14 (br. s, 1 H)

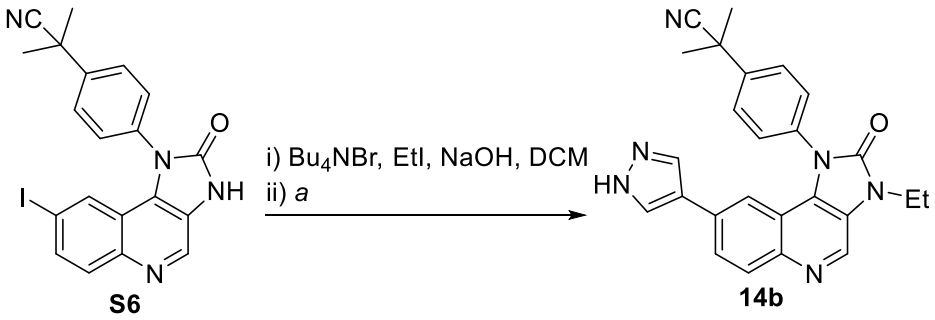


S6 **14a**

2-methyl-2-(4-(2-oxo-8-(1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)propanenitrile (**14a**)

The title compound was prepared according to general procedure A on a 100 mg-scale using 2-(4-(8-iodo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (**S6**)[2] and pyrazole-4-

boronic acid pinacol ester. The crude residue was purified by flash chromatography over silica, eluting with 0 – 6 % methanol in dichloromethane modified with 20% ethyl acetate to afford a yellow solid (26.4 mg, 54%). LCMS $[M+H]^+$ 395.1 *m/z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.86 (s, 6 H) 6.97 (d, *J* = 2.0 Hz, 1 H) 7.23 - 7.37 (m, 1 H) 7.73 (d, *J* = 8.8 Hz, 2 H) 7.80 (dd, *J* = 8.8, 2.0 Hz, 1 H) 7.86 (br. s, 1 H) 7.89 (d, *J* = 8.8 Hz, 2 H) 7.96 (d, *J* = 8.8 Hz, 1 H) 8.68 (s, 1 H) 11.71 (br. s, 1 H) 13.03 (br. s, 1 H)

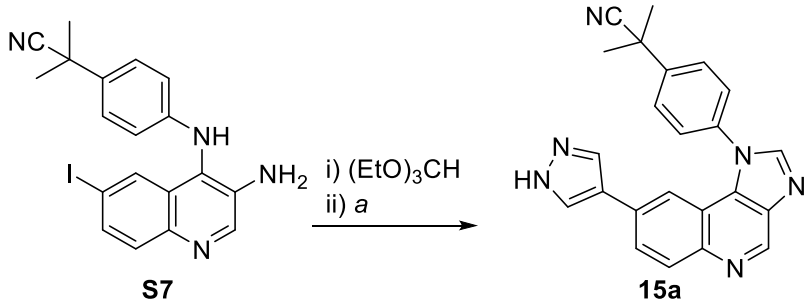


2-(4-(3-ethyl-2-oxo-8-(1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (**14b**)

To a 20 mL vial was added tetrabutylammonium bromide (7.1 mg, 10 mol %), iodoethane (26.6 µL, 330 µmol, 1.1 equiv.), 2-(4-(8-iodo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (**S6**; 100 mg, 220 µmol, 1.0 equiv.) and dichloromethane (5 mL, 0.044M). To this solution was added an aqueous solution of sodium hydroxide (2.20 mL, 0.15 M, 1.1 equiv.). The reaction was sealed and allowed to stir overnight at ambient temperature. After which an additional 1.00 eq. iodoethane (17.7 microliters) and 1.5 mL 0.15 M NaOH were added to the vial; stirring was continued for an additional 16 h. The biphasic reaction mixture was then separated, and the aqueous layer was extracted with dichloromethane (3 x 15 mL). The combined organic layers were washed with brine, dried with sodium sulfate, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography over silica, eluting with 0 – 7 % methanol (modified with 5% NH_4OH) in dichloromethane to afford 2-(4-(3-ethyl-8-iodo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile as a yellow solid (102.2 mg, 96%). LCMS $[M+H]^+$ 483.0 *m/z*; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.37 (t, *J* = 7.1 Hz, 3 H) 1.82 (s, 6 H) 4.13 (q, *J* = 7.1 Hz, 3 H) 7.15 (s, 1 H) 7.71 (d, *J* = 8.4 Hz, 2 H) 7.75 - 7.81 (m, 2 H) 7.86 (d, *J* = 8.4 Hz, 2 H) 9.08 (s, 1 H)

The title compound was prepared according to general procedure A on a 100 mg-scale using 2-(4-(3-ethyl-8-iodo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile and pyrazole-4-boronic acid pinacol ester. The crude residue was purified by flash chromatography over silica, eluting

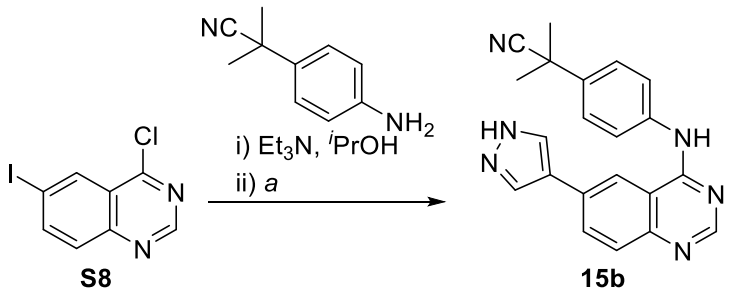
with 0 – 10 % methanol in dichloromethane modified with 20% ethyl acetate to afford a faint brown solid (52.1 mg, 59%). LCMS $[M+H]^+$ 423.1 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.39 (t, *J* = 7.2 Hz, 3 H) 1.86 (s, 6 H) 4.13 (q, *J* = 7.2 Hz, 2 H) 6.97 (d, *J* = 2.0 Hz, 1 H) 7.29 (br. s., 1 H) 7.75 (d, *J* = 8.5 Hz, 2 H) 7.82 (dd, *J* = 8.8, 2.0 Hz, 1 H) 7.87 (br. s., 1 H) 7.90 (d, *J* = 8.5 Hz, 2 H) 7.99 (d, *J* = 8.8 Hz, 1 H) 8.97 (s, 1 H) 13.03 (br. s., 1 H)



2-(4-(8-(1*H*-pyrazol-4-yl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (**15a**)

To a 5 mL RBF was added 2-(4-((3-amino-6-iodoquinolin-4-yl)amino)phenyl)-2-methylpropanenitrile[2] (**S7**; 285 mg, 665 μmol, 1.0 equiv.) and triethyl orthoformate (2.75 mL, 16.6 mmol, 100 equiv.). The solution was then heated at 115 °C for 4h. The solvent was then removed under reduced pressure and the residue purified by column chromatography eluting with 1 – 10% MeOH in dichloromethane to provide 2-(4-(8-iodo-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile (170 mg, 58%). LCMS $[M+H]^+$ 439.0 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.84 (s, 6 H) 7.56 (s, 1 H) 7.81 - 7.97 (m, 6 H) 8.64 (d, *J* = 1.5 Hz, 1 H) 9.35 (d, *J* = 1.5 Hz, 1 H).

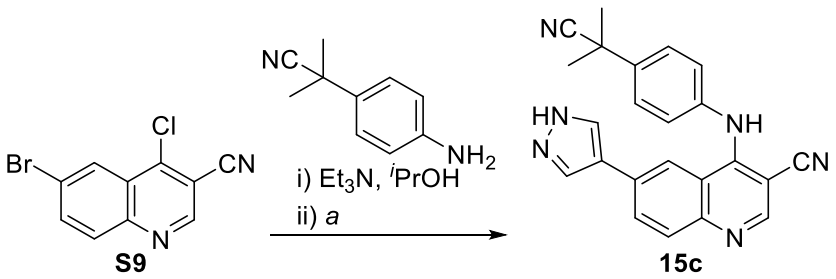
The title compound was prepared according to general procedure A on a 50 mg-scale using 2-(4-(8-iodo-1*H*-imidazo[4,5-*c*]quinolin-1-yl)phenyl)-2-methylpropanenitrile and pyrazole-4-boronic acid pinacol ester. The crude residue was purified by flash chromatography over silica, eluting with 0 – 10 % methanol in dichloromethane to afford the titled compound as a colorless solid (35 mg, 81%). LCMS $[M+H]^+$ 379.1 *m*/*z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.87 (s, 6 H) 7.34 (d, *J* = 1.7 Hz, 1 H) 7.41 (d, *J* = 1.7 Hz, 1 H) 7.86 - 7.90 (m, 2 H) 7.91 - 7.98 (m, 4 H) 8.12 (d, *J* = 8.8 Hz, 1 H) 8.61 (s, 1 H) 9.25 (s, 1 H) 12.96 - 13.14 (m, 1 H).



2-(4-((6-(1*H*-pyrazol-4-yl)quinazolin-4-yl)amino)phenyl)-2-methylpropanenitrile (**15b**)

To an 8 mL vial was added 4-chloro-6-iodoquinazoline[3] (**S8**) (100 mg, 344 µmol, 1 equiv.), 2-(4-aminophenyl)-2-methylpropanenitrile (72 mg, 449 µmol, 1.4 equiv.), triethylamine (170 µL, 1.22 mmol, 3.54 equiv.) and *iso*-propanol (3.7 mL, 0.1M). The suspension was then heated at 80 ºC for 16 h before the solvent was removed under reduced pressure. The residue was then partitioned between ethyl acetate (10 mL) and an aqueous solution of sodium hydroxide (10 mL, 1 M), the aqueous was then extracted with ethyl acetate (2 x 15 mL). The combined organics were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure and the residue purified by column chromatography eluting with 20 – 50% ethyl acetate in hexanes to provide 2-(4-((6-chloroquinazolin-4-yl)amino)phenyl)-2-methylpropanenitrile as a beige solid (90 mg, 63%). LCMS [M+H]+ 415.1 *m/z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.80 (s, 1 H) 8.27 (s, 1 H) 8.07 (d, *J* = 8.8 Hz, 1 H) 7.79 (d, *J* = 8.3 Hz, 2 H) 7.68 (d, *J* = 8.8 Hz, 1 H) 7.55 (d, *J* = 8.3 Hz, 2 H) 7.41 (s, 1 H) 1.77 (s, 6 H).

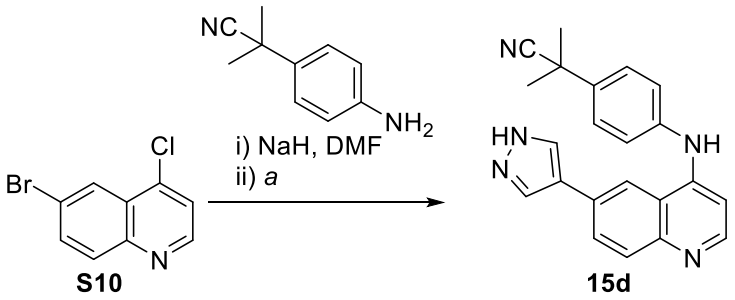
The titled compound was prepared according to general procedure A on a 90 mg-scale using 2-(4-((6-chloroquinazolin-4-yl)amino)phenyl)-2-methylpropanenitrile and pyrazole-4-boronic acid pinacol ester. The crude residue was purified by flash chromatography over silica, eluting with 0 – 10 % methanol in dichloromethane to afford the titled compound as a colorless solid (55 mg, 72%). LCMS $[M+H]^+$ 355.2 *m/z*; ^{1}H NMR (500 MHz, DMSO-*d*6) δ ppm 13.09 (br. s., 1 H) 9.78 (s, 1 H) 8.73 (s, 1 H) 8.54 (s, 1 H) 8.36 (br. s., 1 H) 8.10 - 8.20 (m, 2 H) 7.91 (d, *J* = 8.7 Hz, 2 H) 7.78 (d, *J* = 8.7 Hz, 1 H) 7.57 (d, *J* = 8.7 Hz, 2 H) 1.72 (s, 6 H).



4-((4-(2-cyanopropan-2-yl)phenyl)amino)-6-(1*H*-pyrazol-4-yl)quinoline-3-carbonitrile (**15c**)

To an 8 mL vial was added 4-chloro-6-bromo-3-cyanoquinoline[4] (**S9**; 100 mg, 344 µmol, 1 equiv.), 2-(4-aminophenyl)-2-methylpropanenitrile (72 mg, 449 µmol, 1.4 equiv.), triethylamine (170 µL, 1.22 mmol, 3.54 equiv.) and *iso*-propanol (3.7 mL, 0.1M). The suspension was then heated at 80 ºC for 16 h before the solvent was removed under reduced pressure. The residue was then partitioned between ethyl acetate (10 mL) and an aqueous solution of sodium hydroxide (10 mL, 1 M), the aqueous was then extracted with ethyl acetate (2 x 15 mL). The combined organics were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure and the residue purified by column chromatography eluting with 10 – 30% ethyl acetate in hexanes to provide 4-((4-(2-cyanopropan-2-yl)phenyl)amino)-6-iodoquinoline-3-carbonitrile as a yellow waxy solid (18 mg, 12%). LCMS $[M+H]^+$ 391.1 *m/z* (^{79}Br), 393.1 *m/z* (^{81}Br); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.73 (s, 1 H) 7.99 (d, *J* = 1.5 Hz, 1 H) 7.94 (d, *J* = 8.8 Hz, 1 H) 7.84 (dd, *J* = 8.8, 1.7 Hz, 1 H) 7.53 (d, *J* = 8.8 Hz, 2 H) 7.33 (s, 1 H) 7.22 (d, *J* = 8.8 Hz, 2 H) 1.77 (s, 6 H).

The titled compound was prepared according to general procedure A on a 40 mg-scale using 4-((4-(2-cyanopropan-2-yl)phenyl)amino)-6-iodoquinoline-3-carbonitrile and pyrazole-4-boronic acid pinacol ester. The crude residue was purified by flash chromatography over silica, eluting with 50 – 100% ethyl acetate in hexanes to afford the titled compound as a colorless solid (19 mg, 49). LCMS $[M+H]^+$ 379.2 *m/z*; ^{1}H NMR (500 MHz, DMSO-*d*6) δ ppm 13.09 (br. s., 1 H) 9.76 (s, 1 H) 8.65 (s, 1 H) 8.54 (s, 1 H) 8.35 (br. s., 1 H) 8.15 (d, *J* = 8.8 Hz, 1 H) 8.09 (br. s., 1 H) 7.93 (d, *J* = 8.8 Hz, 1 H) 7.57 (d, *J* = 8.8 Hz, 2 H) 7.38 (d, *J* = 8.8 Hz, 2 H) 1.71 (s, 6 H).

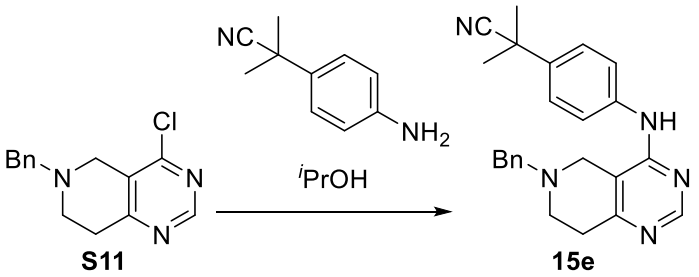


2-(4-((6-(1*H*-pyrazol-4-yl)quinolin-4-yl)amino)phenyl)-2-methylpropanenitrile (**15d**)

To a solution of 2-(4-aminophenyl)-2-methylpropanenitrile (124 mg, 773 µmol, 1.88 equiv.) in dry dimethyl formamide (1.6 mL, 0.2 M) was added sodium hydride (36 mg, 1.5 mmol, 3.64 equiv.). followed by the portion-wise addition of 4-chloro-6-bromoquinoline[5] (**S10**; 100 mg, 412 µmol, 1 equiv.), The

suspension was then heated at 50 ºC for 24 h before the solvent was removed under reduced pressure. The reaction was then quenched with the slow addition of water. The aqueous was then extracted with dichloromethane (3 x 15 mL). The combined organics were washed with lithium chloride (2 x 15 mL, 1.0 M), brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. Crude residue was then purified by column chromatography eluting with 20 – 50% ethyl acetate in hexanes to provide 2-(4-((6-bromoquinolin-4-yl)amino)phenyl)-2-methylpropanenitrile as a dark purple solid (59 mg, 39%). LCMS $[M+H]^+$ 648.0 *m/z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.71 (s, 6 H) 7.03 (d, *J* = 5.4 Hz, 1 H) 7.42 (d, *J* = 8.6 Hz, 2 H) 7.56 (d, *J* = 8.6 Hz, 2 H) 7.82 (s, 2 H) 8.50 (d, *J* = 5.4 Hz, 1 H) 8.66 (s, 1 H) 9.11 (s, 1 H)

The titled compound was prepared according to general procedure A on a 18 mg-scale using 2-(4-((6-bromoquinolin-4-yl)amino)phenyl)-2-methylpropanenitrile and pyrazole-4-boronic acid pinacol ester. The crude residue was purified by flash chromatography over silica, eluting with 50 – 100% ethyl acetate in hexanes to afford the titled compound as a faint yellow solid (9 mg, 51%). LCMS $[M+H]^+$ 354.1 *m/z*; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.72 (s, 6 H) 6.97 (d, *J* = 5.2 Hz, 1 H) 7.45 (d, *J* = 8.8 Hz, 2 H) 7.58 (d, *J* = 8.8 Hz, 2 H) 7.85 (d, *J* = 8.8 Hz, 1 H) 7.98 (dd, *J* = 8.8, 1.6 Hz, 1 H) 8.11 (s, 1 H) 8.33 (s, 1 H) 8.41 (d, *J* = 5.2 Hz, 1 H) 8.57 (s, 1 H) 8.95 (s, 1 H) 13.05 (br. s, 1 H)

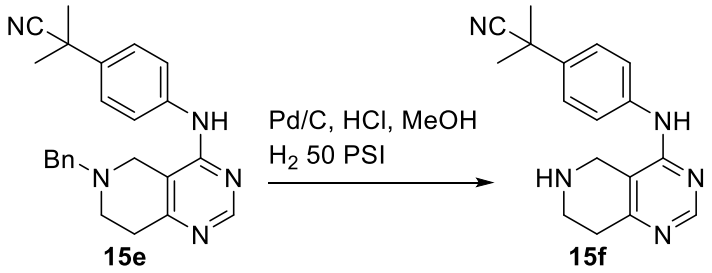


S11 **15e**

2-(4-((6-benzyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)phenyl)-2-methylpropanenitrile (**15e**)

To a stirred reaction of 6-benzyl-4-chloro-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidine (2.200 g, 8.47 mmol, 1.0 equiv.) in *iso*-propanol (35 mL, 0.24M) was added 2-(4-aminophenyl)-2-methylpropanenitrile (3.120 g, 19.48 mmol, 2.3 equiv.) in one portion. The reaction was then heated to a gentle reflux under a nitrogen atmosphere for 22 h, resulting in a dark red solution suspending a white precipitate. The reaction was then concentrated under reduced pressure, and the residue partitioned between brine (50 mL) and dichloromethane (50 mL), the pH of the aqueous layer was then adjusted to ~ 9 with sodium hydroxide (1.0M). The layers were separated and the aqueous was extracted with dichloromethane (3 x 50 mL),

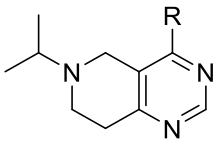
washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure to a dark brown solid. The crude dark brown solid was then purified via trituration from 60 mL of *iso*-propanol. The precipitate was filtered, washed with Et_2O and dried under reduced pressure to provide the titled compound as a faint beige solid (2.3 g, 71%). LCMS $[M+H]^+$ 384.6 *m/z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 1.73 (s, 6 H) 2.64 (t, *J* = 5.6 Hz, 2 H) 2.87 (t, *J* = 5.6 Hz, 2 H) 3.62 (s, 2 H) 3.75 (s, 2 H) 6.32 (s, 3 H) 7.28 - 7.41 (m, 18 H) 7.47 (d, *J* = 8.8 Hz, 8 H) 7.62 (d, *J* = 8.8 Hz, 2 H) 8.54 (s, 1 H)

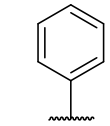
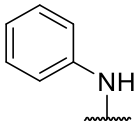


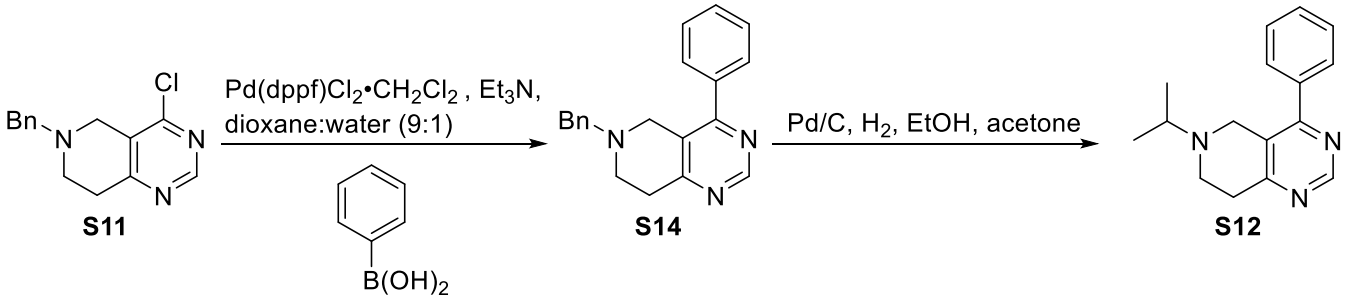
2-methyl-2-(4-((5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)phenyl)propanenitrile (**15f**)

To a 45 mL vial was added 2-(4-((6-benzyl-5,6,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-yl)amino)phenyl)-2-methylpropanenitrile (**15e**; 1.00 g, 2.61 mmol, 1 equiv.), 10% wt.%, 50 wt.% water palladium/carbon (195 mg, 14 mol%), concentrated hydrochloric acid (550 µL, 6.52 mmol, 2.5 equiv.) and ethanol (10.5 mL, 0.25 M). The vial was then transferred to a Parr reactor vessel and the atmosphere was evacuated and replaced with hydrogen (50 psi). The reaction was then stirred at ambient temperature for 16 h, the reaction mixture was then filtered through Celite and concentrated to a brown residue. The residue was then partitioned between brine and ethyl acetate, the pH adjusted to 8-9 with saturated sodium hydrogen carbonate. The aqueous layer was then extracted with ethyl acetate (2 x 30 mL), the combined organics were washed with brine, dried over sodium sulfate, filtered and concentrated to a residue. The crude residue was purified by flash chromatography over silica, eluting with 1 – 10 % methanol (modified with 5% NH_4OH) in dichloromethane to afford a colorless solid (628 mg, 82%). LCMS $[M+H]^+$ 294.6 *m/z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 1.73 (s, 6 H) 2.56 (t, *J* = 5.6 Hz, 2 H) 3.27 (t, *J* = 5.6 Hz, 2 H) 3.97 (s, 2 H) 6.38 (s, 1 H) 7.47 (d, *J* = 8.3 Hz, 2 H) 7.63 (d, *J* = 8.3 Hz, 2 H) 8.55 (s, 1 H).

Table S5. Additional tetrahydropyrido[4,3-*d*]pyrimidine compounds not presented in the manuscript.



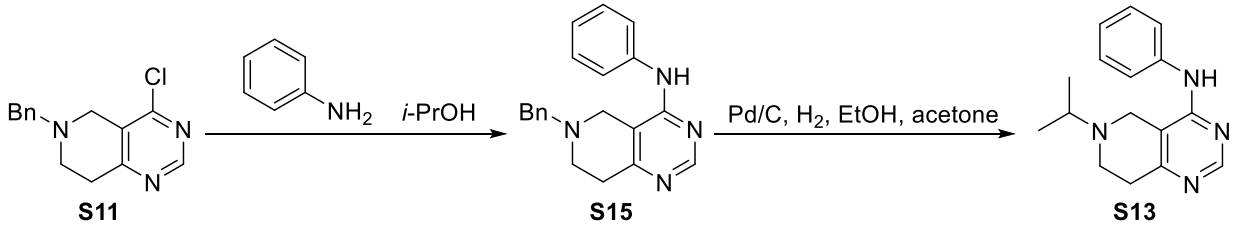
NEU-	R	*Tbb* pEC_{50}	MRC5 pTC_{50}	Aq. Sol. (μM)	MPO Score
S12		< 4.4 ± 0.00	< 4.3 ± 0.00	810	5.4
S13		< 4.4 ± 0.00	< 4.3 ± 0.00	770	5.5



6-isopropyl-4-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**S12**)

To an 8 mL vial equipped with a stir bar was added 6-benzyl-4-chloro-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**S11**; 50 mg, 0.19 mmol), phenylboronic acid (31 mg, 0.25 mmol), $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (2.2 mg, 9.6 μmol), 1,4-dioxane (1.8 mL), H2O (0.2 mL), and triethylamine (0.08 mL, 0.58 mmol). The vial was sealed with a septum cap and the contents were sparged with N_2 for 10 minutes and heated to reflux for 15 h. The mixture was cooled to room temperature, filtered through diatomaceous earth, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on SiO_2 using a gradient of 50-100% EtOAc in hexanes to give 6-benzyl-4-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine as an orange/brown semi-solid in 52% yield (30 mg, 0.10 mmol). LCMS $[M+H]^+$ 302.2 *m/z*; ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 9.05 (s, 1 H), 7.51 - 7.58 (m, 2 H), 7.41 - 7.50 (m, 3 H), 7.26 (br. s., 5 H), 3.72 (s, 2 H), 3.67 (s, 2 H), 3.05 (t, *J* = 6.1 Hz, 2 H), 2.82 (t, *J* = 6.1 Hz, 2 H)

A 100 mL round bottom flask equipped with a stir bar was charged with 6-benzyl-4-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**S14**; 193 mg, 0.64 mmol) and Pd/C (10 wt%, 68 mg, 64 µmol). The flask was sealed with a septum and vacuum purged with N_2 (3x). EtOH (13 mL) was added via syringe and the mixture was stirred vigorously at room temperature under an atmosphere of H_2. Acetone (5 mL) was added to the mixture and stirring was continued under an atmosphere of H_2 overnight. The flask was purged with N_2 and the contents were filtered through a plug of diatomaceous earth. The filtrate was concentrated and purified by flash column chromatography using a gradient of 1-15% methanol (modified with 5% NH_4OH) in dichloromethane to give the desired product as a brown solid in 28% yield (45 mg, 0.18 mmol). LCMS $[M+H]^+$ 254.1 *m/z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.55 (s, 1 H), 7.55 (d, *J* = 7.8 Hz, 2 H), 7.36 (t, *J* = 7.8 Hz, 2 H), 7.12 (t, *J* = 7.8 Hz, 1 H), 6.11 (br. s., 1 H), 3.55 (s, 2 H), 3.04 (spt, J=6.6 Hz, 1 H), 2.91 (t, *J* = 5.4 Hz, 2 H), 2.84 (t, *J* = 5.4 Hz, 2 H), 1.18 (d, *J* = 6.6 Hz, 6 H)



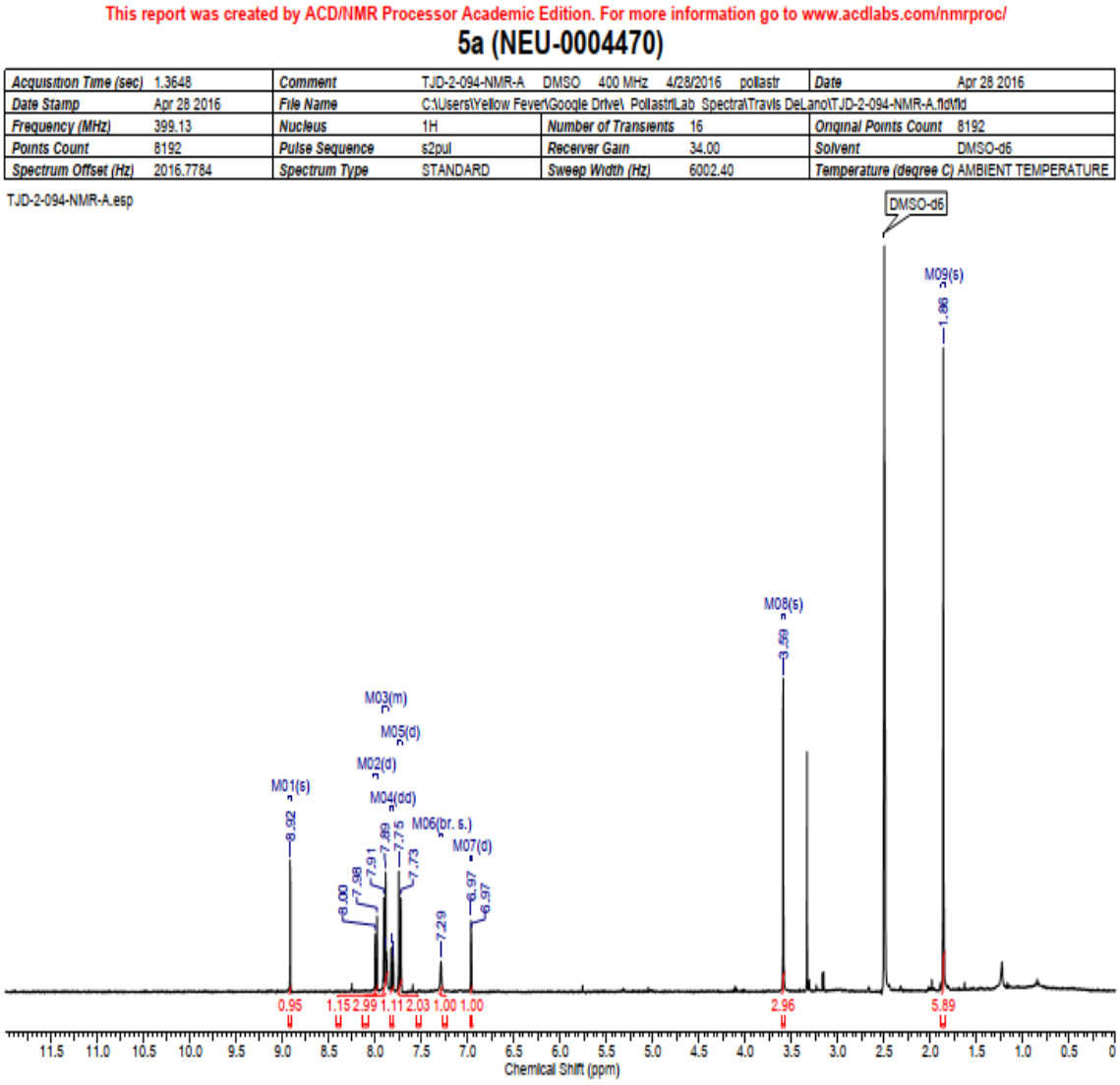
S11 **S15** **S13**

6-isopropyl-*N*-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (**S13**)

To a mixture of 6-benzyl-4-chloro-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**S11**; 250 mg, 0.96 mmol) in *i*-PrOH (4 mL) was added aniline (0.18 mL, 1.9 mmol). The mixture was refluxed with stirring overnight, then cooled to room temperature, poured over water (20 mL) and sat. aq. $NaHCO_3$ (20 mL), and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine (30 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on SiO_2 using ethyl acetate, then 5% methanol in dichloromethane to give 6-benzyl-*N*-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine as a tan colored solid in 81% yield (247 mg, 0.78 mmol). LCMS $[M+H]^+$ 317.1 *m/z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.55 (s, 1 H), 7.50 (d, J=7.8 Hz, 2 H), 7.27 - 7.42 (m, 7 H), 7.11 (t, J=7.6 Hz, 1 H), 6.08 (s, 1 H), 3.79 (s, 2 H), 3.46 (s, 2 H), 2.91 (t, J=5.4 Hz, 2 H), 2.85 (t, J=5.4 Hz, 2 H)

A 100 mL round bottom flask equipped with a stir bar was charged with 6-benzyl-*N*-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (**S15**; 218 mg, 0.69 mmol) and Pd/C (10 wt.%, 73 mg, 69 µmol). The flask was sealed with a septum and vacuum purged with N_2 (3x). Ethanol (14 mL) was added via syringe and the mixture was stirred vigorously at room temperature under an atmosphere of H_2. Acetone (5 mL) was added to the mixture and stirring was continued under an atmosphere of H_2 overnight. The flask was purged with N_2 and the contents were filtered through a plug of diatomaceous earth. The filtrate was concentrated and purified by flash column chromatography using a gradient of 1-15% methanol (modified with 5% NH_4OH) in dichloromethane to give the desired product as a yellow solid in 57% yield (106 mg, 0.40 mmol). LCMS $[M+H]^+$ 269.1 *m*/*z*; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.55 (s, 1 H), 7.55 (d, *J* = 7.8 Hz, 2 H), 7.36 (t, *J* = 7.8 Hz, 2 H), 7.12 (t, *J* = 7.8 Hz, 1 H), 6.11 (br. s., 1 H), 3.55 (s, 2 H), 3.04 (spt, J=6.6 Hz, 1 H), 2.91 (t, *J* = 5.4 Hz, 2 H), 2.84 (t, *J* = 5.4 Hz, 2 H), 1.18 (d, *J* = 6.6 Hz, 6 H).

HNMR Spectra for all novel compounds presented



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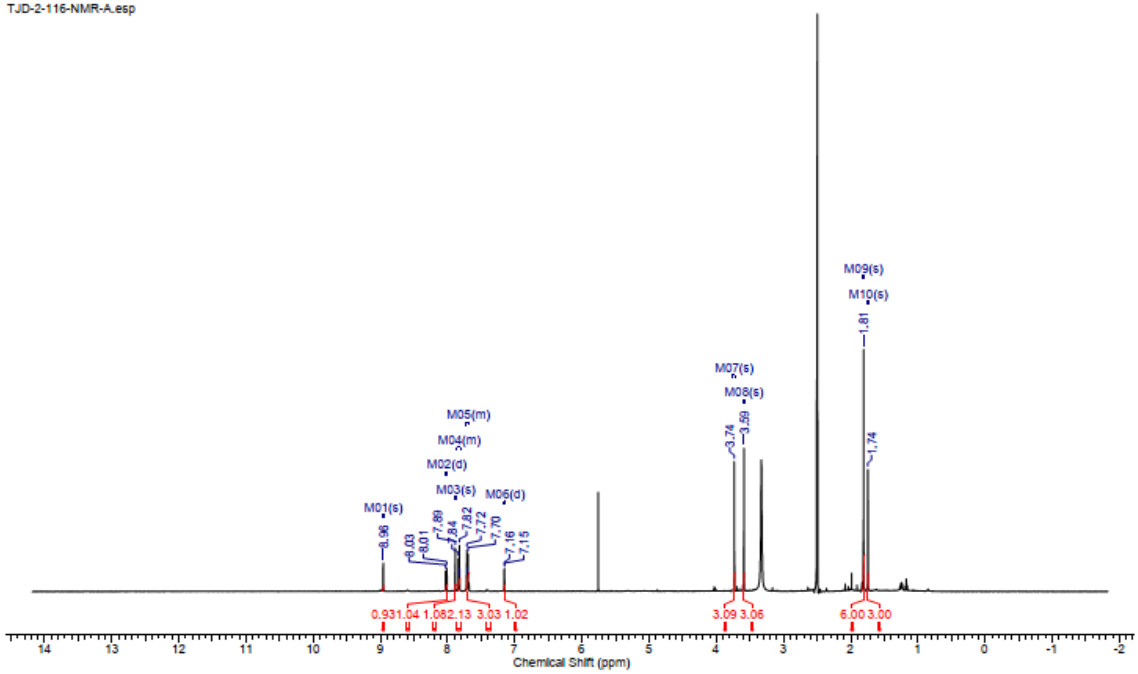

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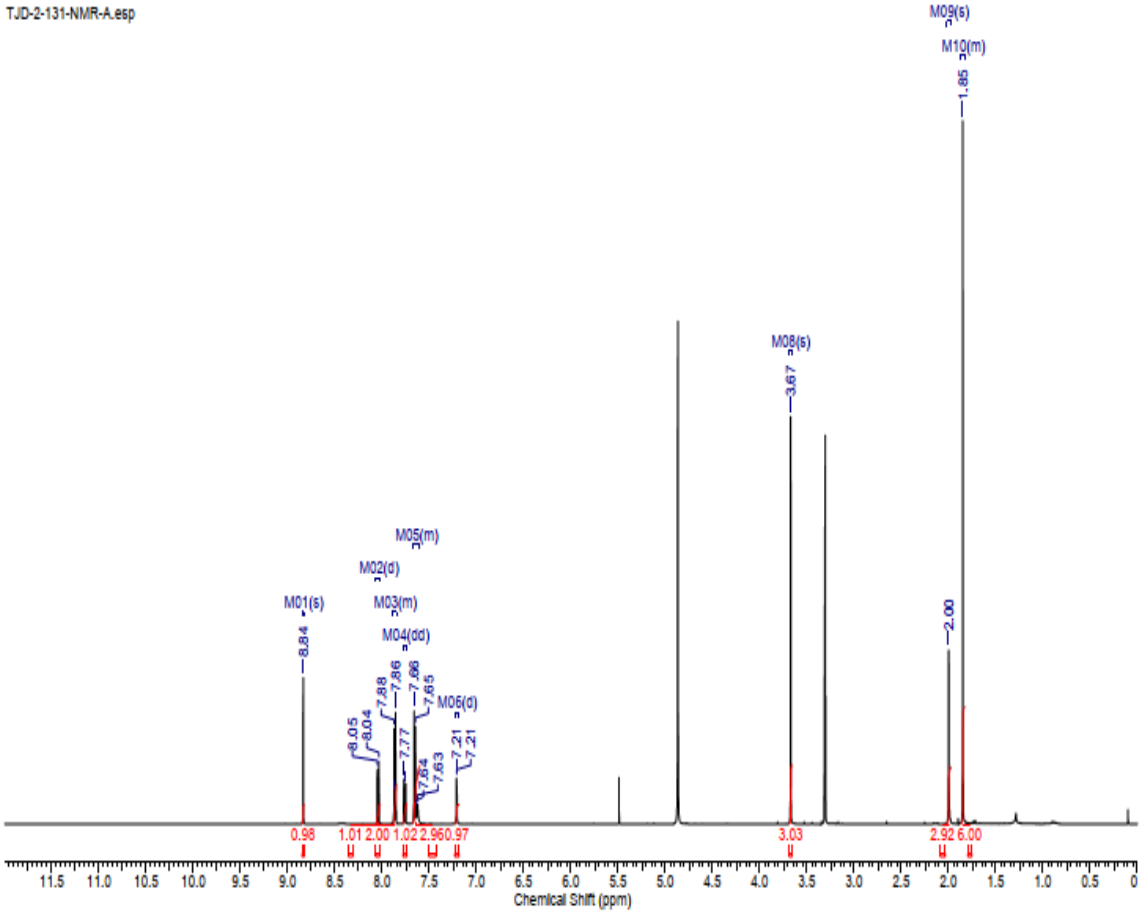

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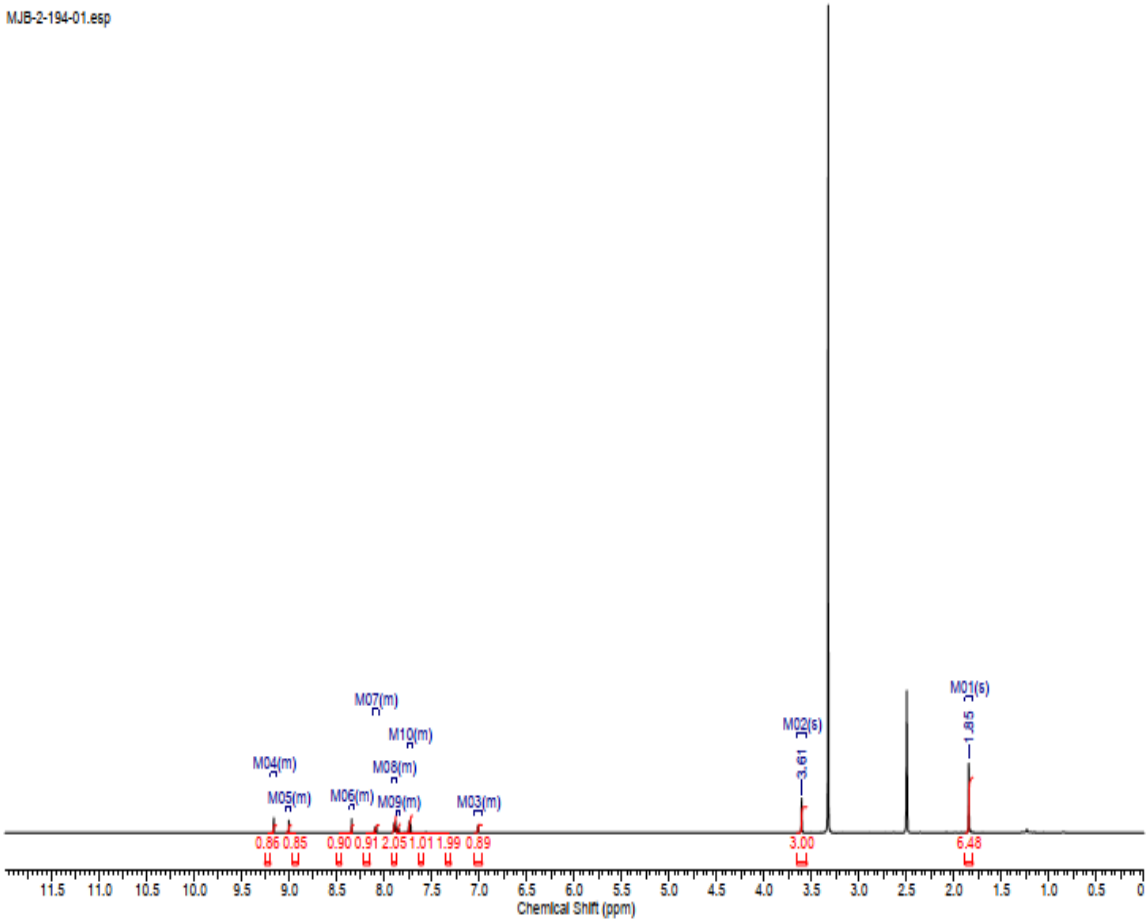

6 (NEU-0004790)

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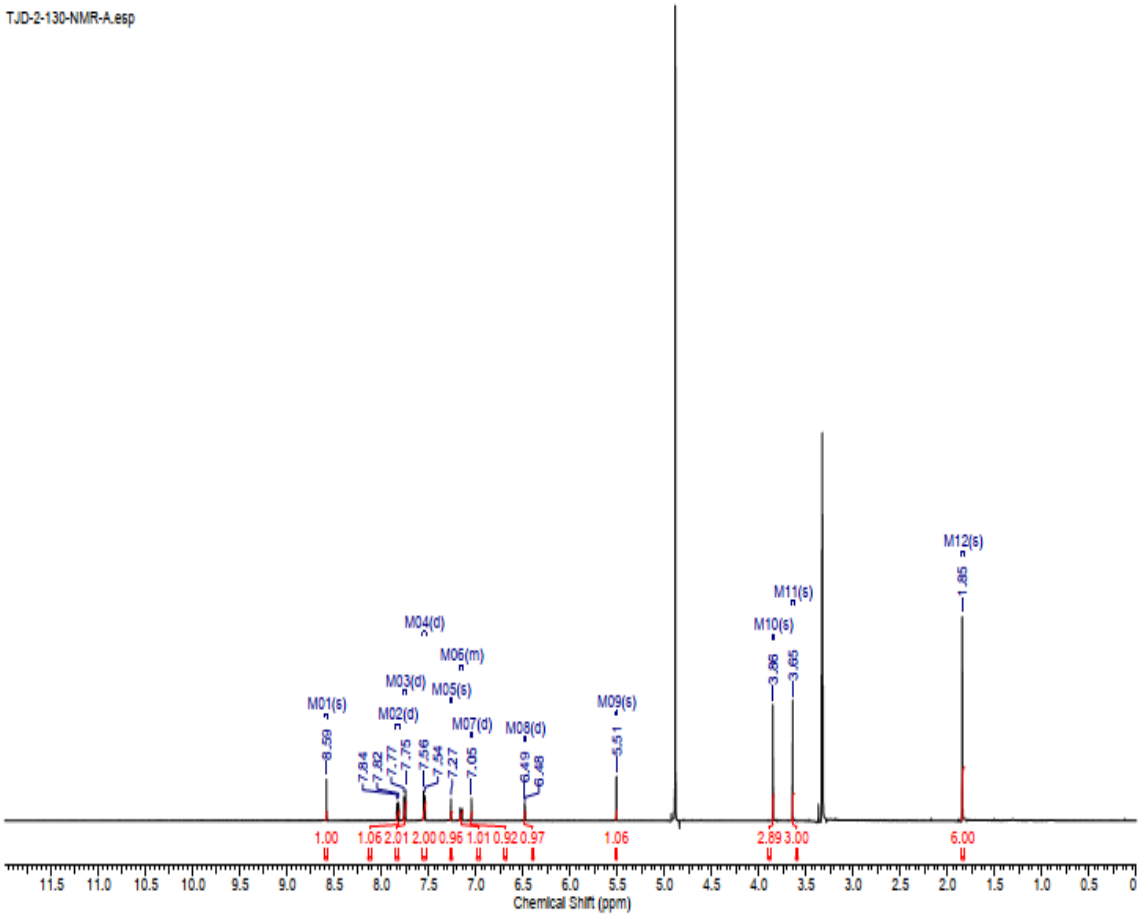

7a (NEU-0004914)

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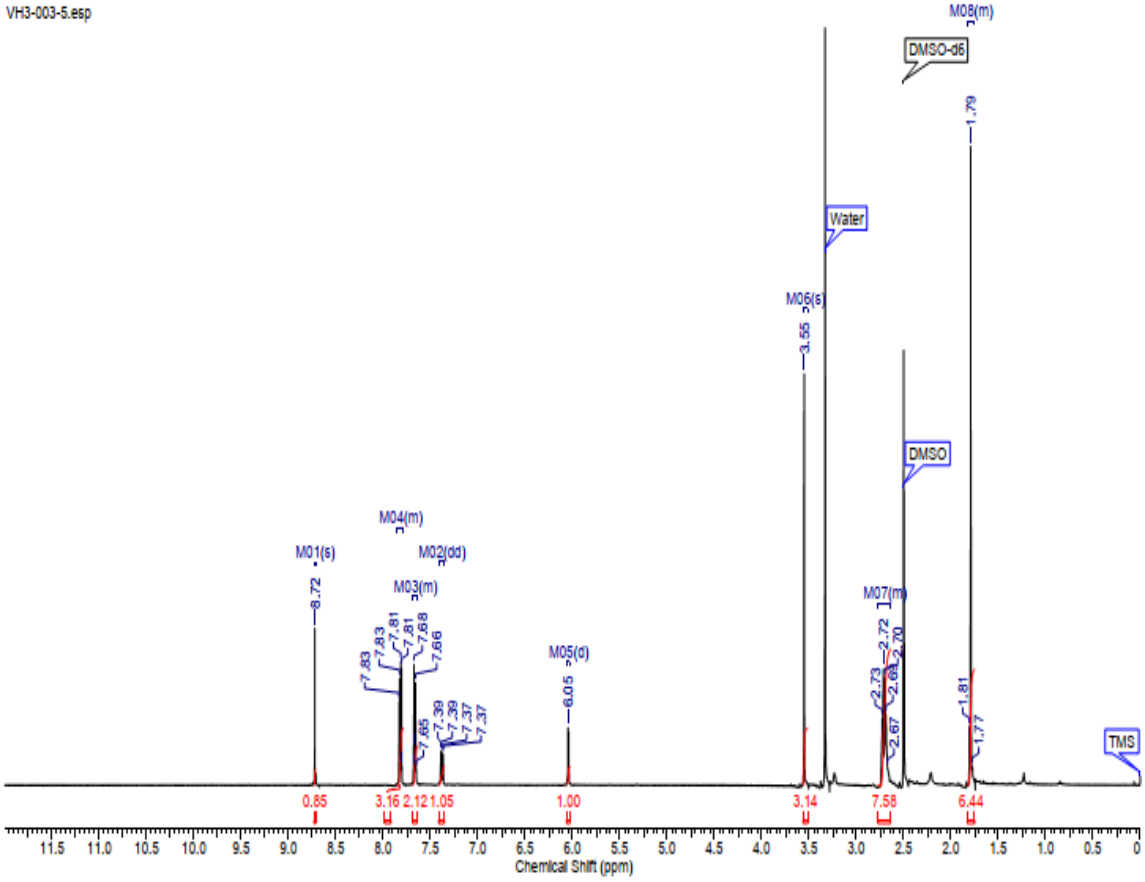
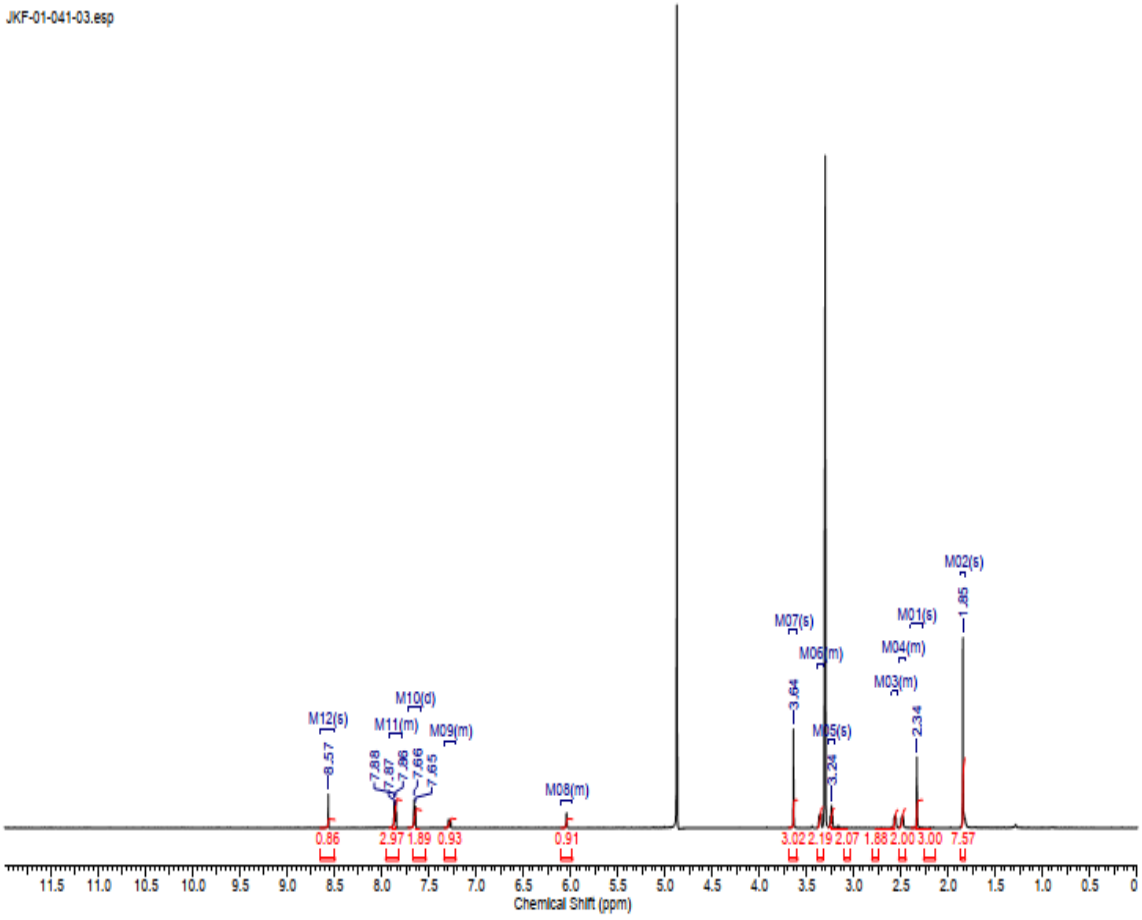
VH3-003-5.esp



7b (NEU-0005815)

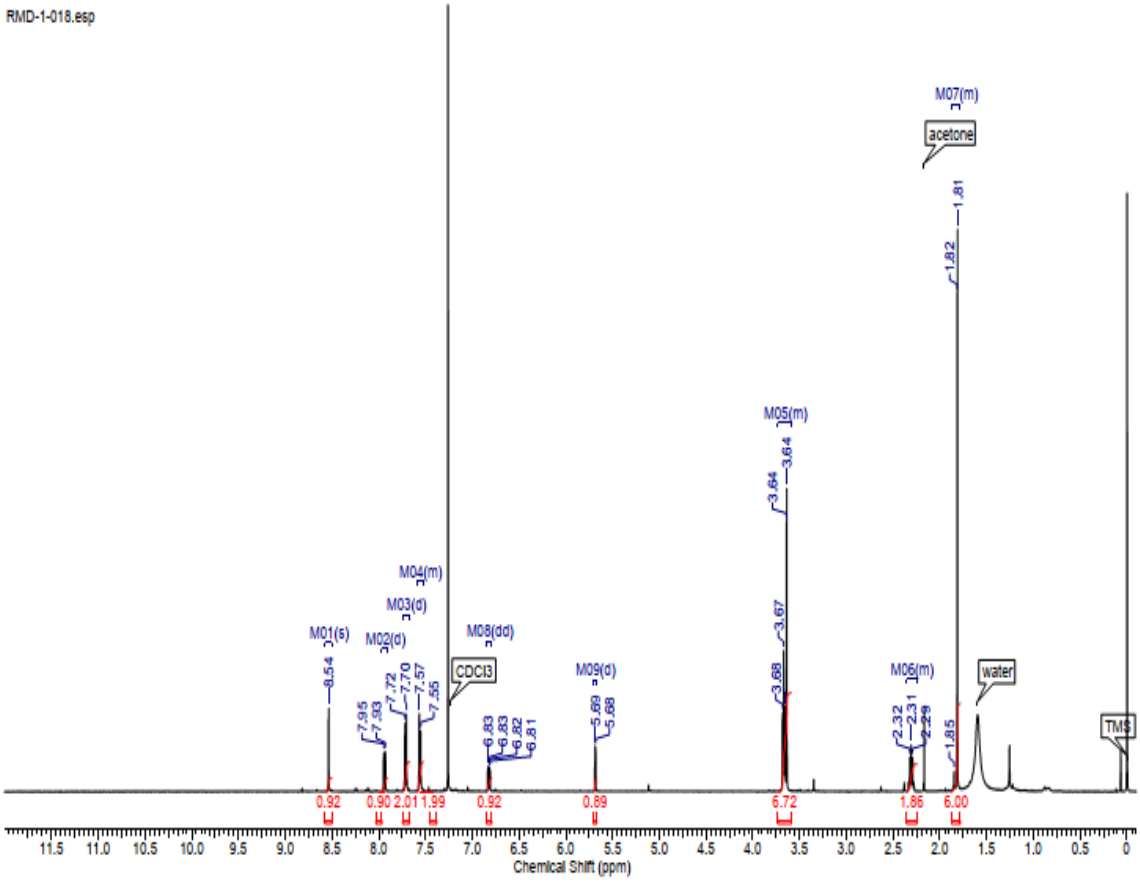
Acquisition Time (sec)	2.0494	*Date*	Jan 16 2018	*Date Stamp*	Jan 16 2018		
File Name	C:\Users\mjbus\Application Data\SSH\temp\JKF-01-041-03.fid\fid					*Frequency (MHz)*	499.67
Nucleus	1H	*Number of Transients*	16	*Original Points Count*	16384	*Points Count*	16384
Pulse Sequence	s2pul	*Receiver Gain*	54.00	*Solvent*	METHANOL-d4		
Spectrum Offset (Hz)	2504.6985	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000



7c (NEU-0005293)

Formula $C_{24}H_{12}N_2O_3$	FW	397.4723

Acquisition Time (sec)	2.0480	Comment	RMD-1-018 6-13-17 CDCl3 tear:500			Date	Jun 13 2017
Date Stamp	Jun 13 2017	File Name	C:\Users\UCHS T410\Google Drive\ Pollastri\Lab Spectra\Raeann Dalton\RMD-1-018.fid\fid				
Frequency (MHz)	499.67	Nucleus	1H	Number of Transients	20	Original Points Count	16384
Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	58.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	3092.6426	Spectrum Type	STANDARD	Sweep Width (Hz)	8000.00	Temperature (degree C)	AMBIENT TEMPERATURE

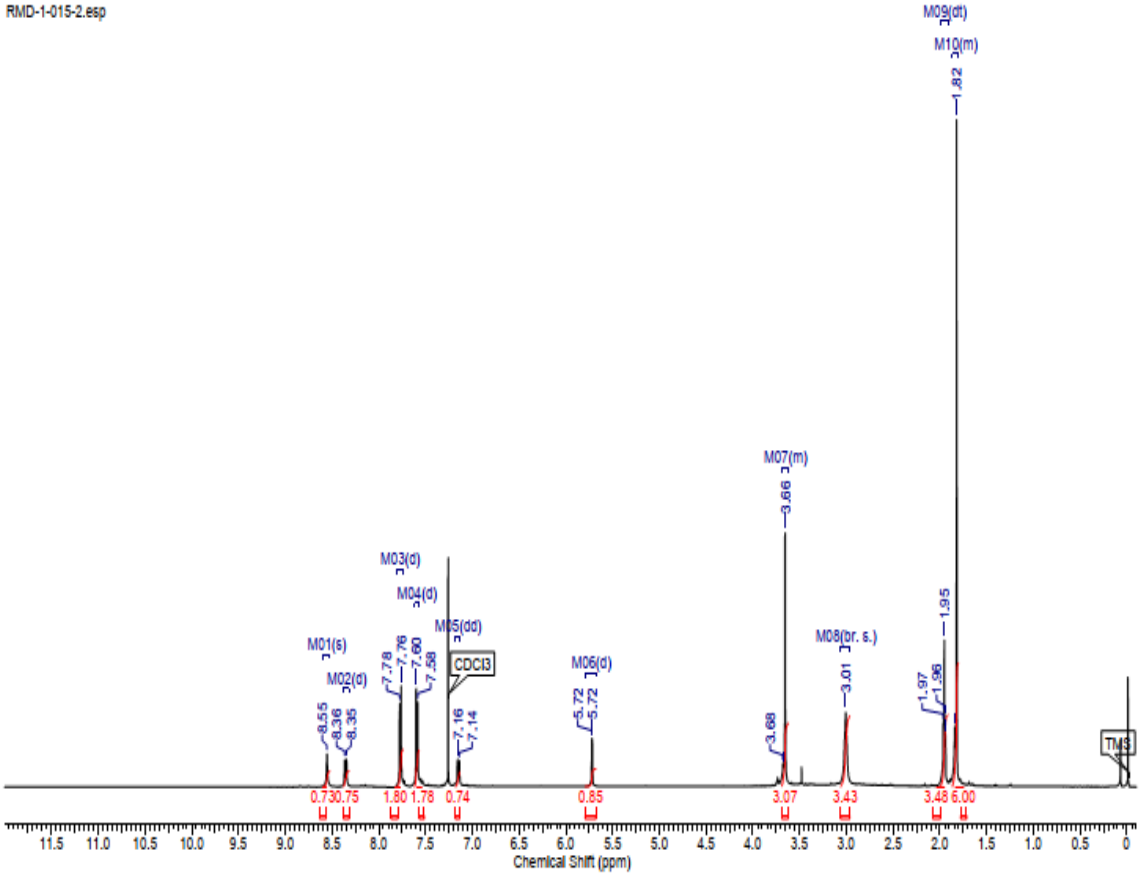


7d (NEU-0005294)

Formula $C_{23}H_{19}NO_3$	FW	411.4989

Acquisition Time (sec)	2.0480	Comment	RMD-1-015-2 6-14-17 CDCl3 tear:500			Date	Jun 14 2017
Date Stamp	Jun 14 2017	File Name	C:\Users\UCHS T410\Downloads\drive-download-20170614T144528Z-001\RMD-1-015-2.fid\fid				
Frequency (MHz)	499.67	Nucleus	1H	Number of Transients	14	Original Points Count	16384
Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	52.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	3092.6426	Spectrum Type	STANDARD	Sweep Width (Hz)	8000.00	Temperature (degree C)	AMBIENT TEMPERATURE

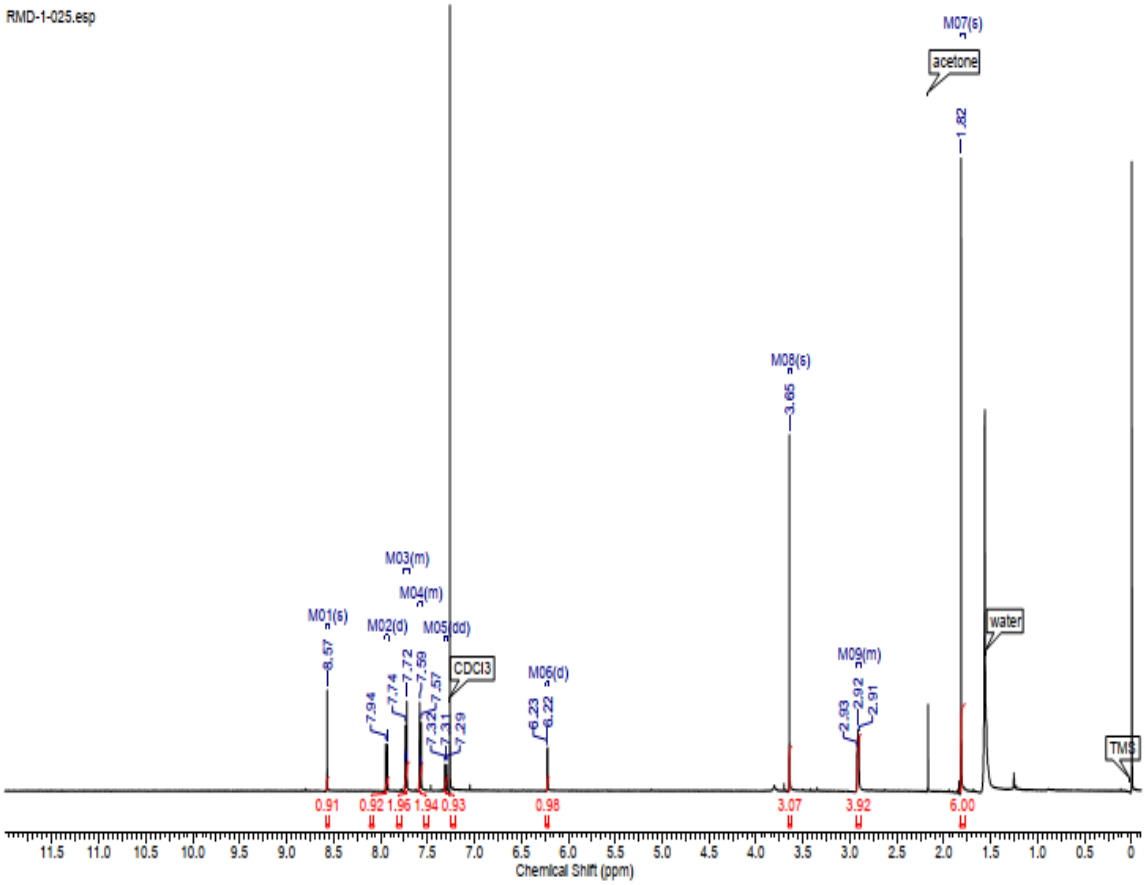
RMD-1-015-2.esp



7e (NEU-0005316)

Formula $C_{18}H_{19}NO_3$	FW	425.5255

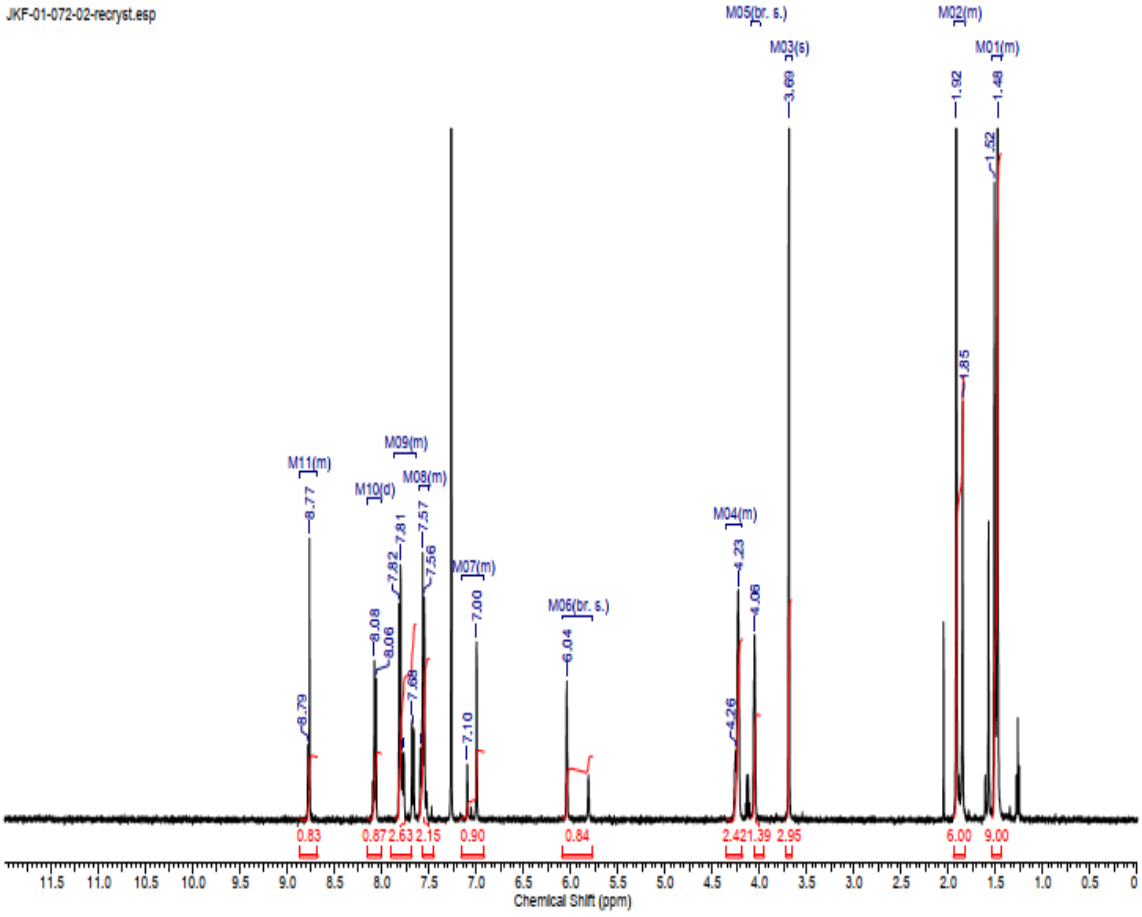
Acquisition Time (sec)	2.0480	Comment	RMD-1-025 6-23-17 CDCl3 tear:500			Date	Jun 23 2017
Date Stamp	Jun 23 2017	File Name	C:\Users\UCHS T410\Google Drive\ Pollastri\Lab Spectra\Raeann Dalton\RMD-1-025.fid\fid				
Frequency (MHz)	499.67	Nucleus	1H	Number of Transients	14	Original Points Count	16384
Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	58.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	3092.6426	Spectrum Type	STANDARD	Sweep Width (Hz)	8000.00	Temperature (degree C)	AMBIENT TEMPERATURE



8a (NEU-0005876)

Acquisition Time (sec)	2.0494	*Date*	Oct 25 2018	*Date Stamp*	Oct 25 2018		
File Name	C:\Users\Administrator\Google Drive\ Pollastri Lab Spectra\Previous lab members\Jack Fisher\NMR Spectra\JKF-01-072-02-recryst.fid\fid						
Frequency (MHz)	499.67	*Nucleus*	1H	*Number of Transients*	4	*Original Points Count*	16384
Points Count	16384	*Pulse Sequence*	s2pul	*Receiver Gain*	56.00	*Solvent*	CHLOROFORM-d
Spectrum Offset (Hz)	2502.7285	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000

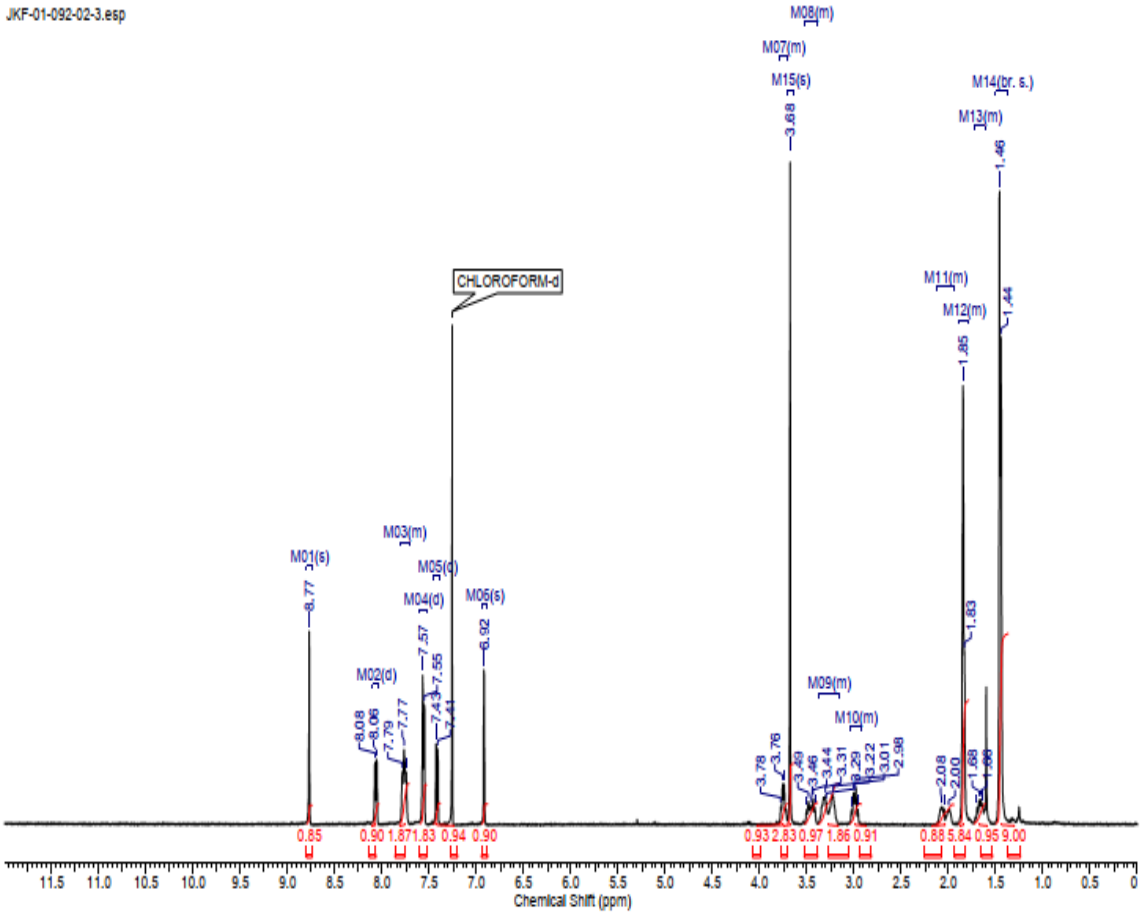
JKF-01-072-02-recryst.esp



9a (NEU-0006050)

Acquisition Time (sec)	2.0494	*Date*	Oct 31 2018	*Date Stamp*	Oct 31 2018		
File Name	G:\MY DRIVE\ POLLASTRILAB_SPECTRA\JACK FISHER\NMR SPECTRA\JKF-01-092-02-3.FID\FID					*Frequency (MHz)*	499.67
Nucleus	1H	*Number of Transients*	4	*Original Points Count*	16384	*Points Count*	16384
Pulse Sequence	s2pul	*Receiver Gain*	50.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2498.2200	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000

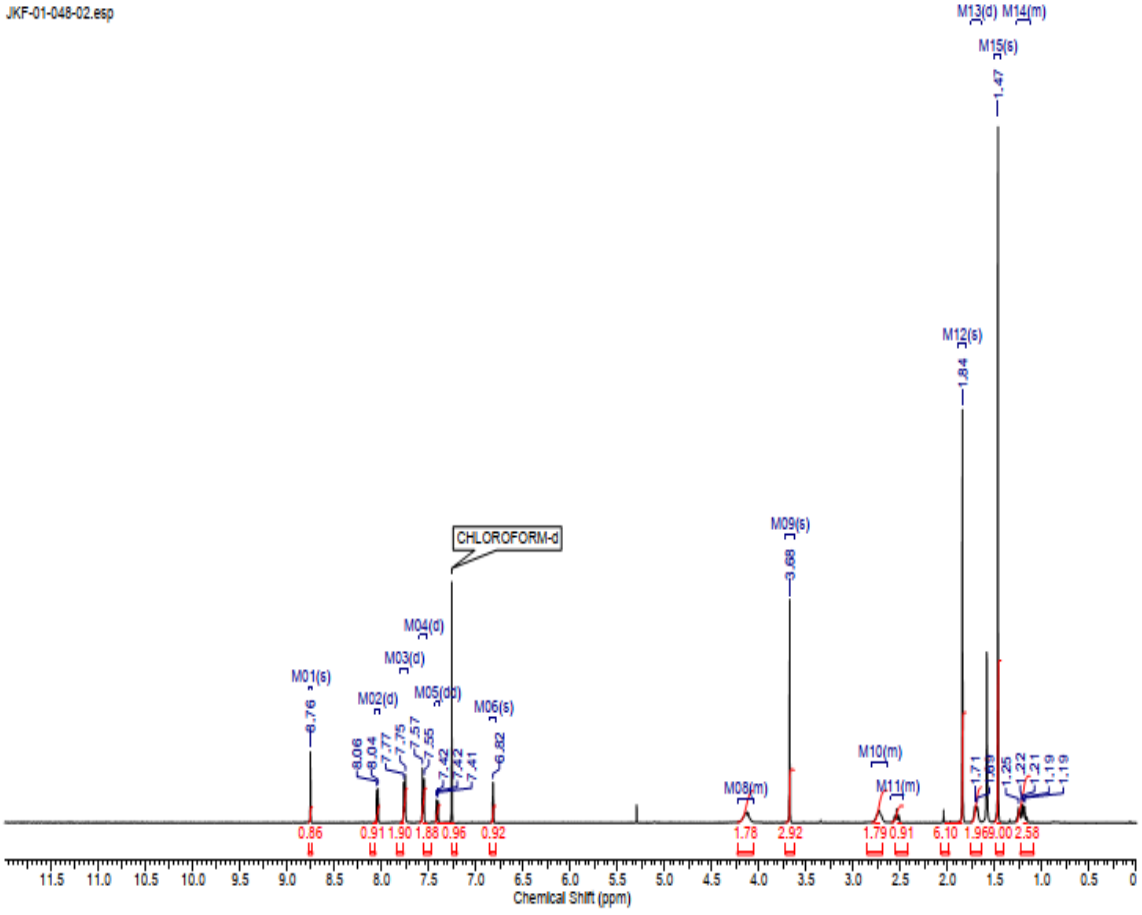
JKF-01-092-02-3.esp



9b (NEU-0005997)

Acquisition Time (sec)	2.0494	*Date*	Jul 18 2018	*Date Stamp*	Jul 18 2018		
File Name	G:\MY DRIVE\ POLLASTRILAB_SPECTRA\JACK FISHER\NMR SPECTRA\JKF-01-048-02.FID\FID					*Frequency (MHz)*	499.67
Nucleus	1H	*Number of Transients*	8	*Original Points Count*	16384	*Points Count*	16384
Pulse Sequence	s2pul	*Receiver Gain*	52.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2497.7319	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000

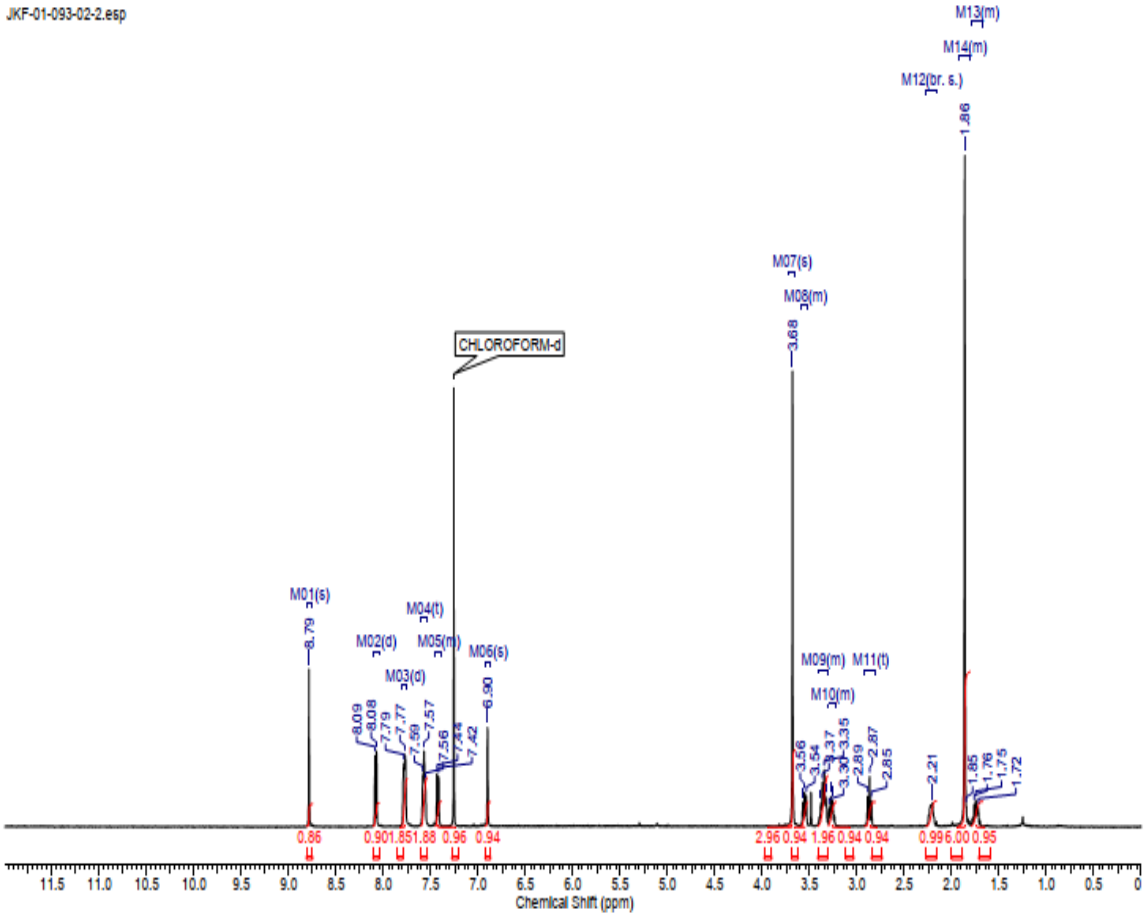
JKF-01-048-02.esp



10a (NEU-0006051)

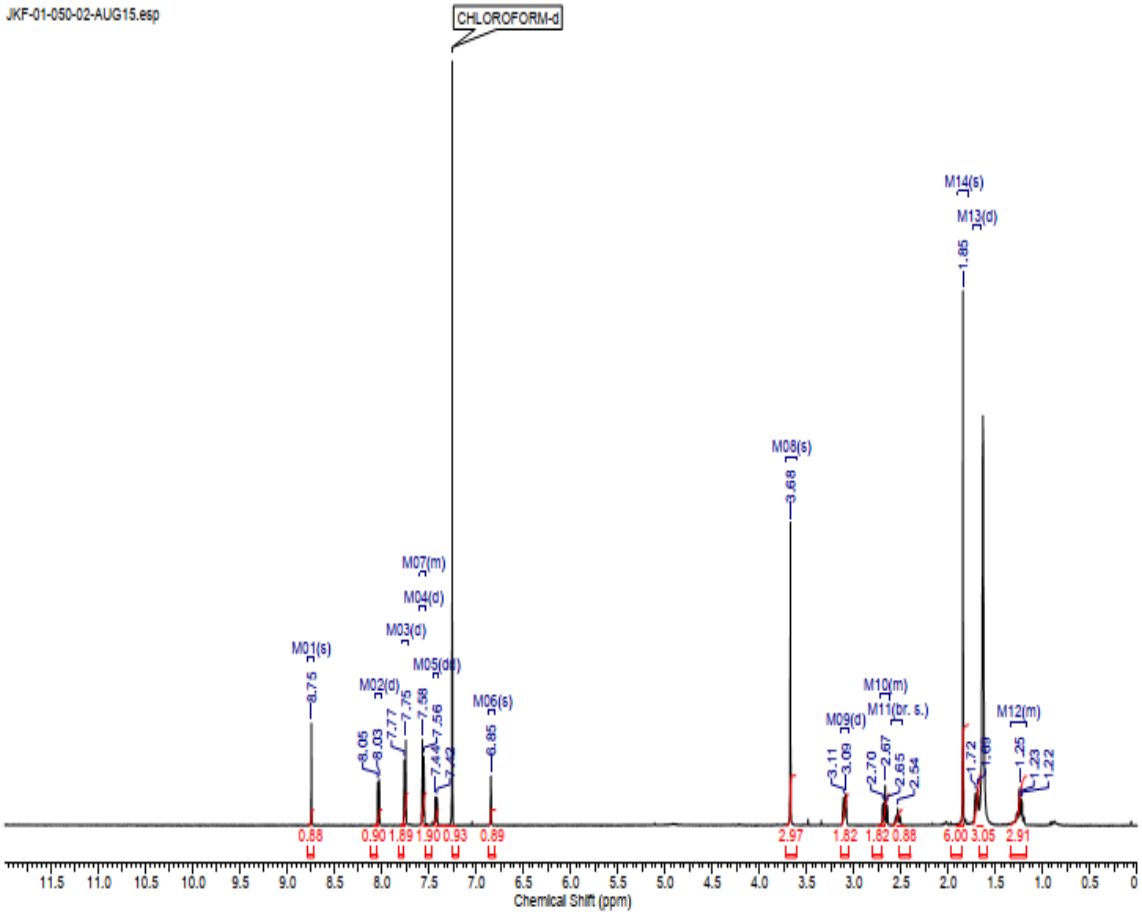
Acquisition Time (sec)	2.0494	*Date*	Nov 7 2018	*Date Stamp*	Nov 7 2018		
File Name	G:\MY DRIVE\ POLLASTRILAB SPECTRA\JACK FISHER\NMR SPECTRA\JKF-01-093-02-2.FID\FID					*Frequency (MHz)*	499.67
Nucleus	1H	*Number of Transients*	8	*Original Points Count*	16384	*Points Count*	16384
Pulse Sequence	s2pul	*Receiver Gain*	54.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2497.7319	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000

JKF-01-093-02-2.esp



10b (NEU-0005998)

Acquisition Time (sec)	2.0494	*Date*	Aug 15 2018	*Date Stamp*	Aug 15 2018		
File Name	G:\MY DRIVE\ POLLASTRILAB_SPECTRA\JACK FISHER\NMR SPECTRA\JKF-01-050-02-AUG15.FID\FID					*Frequency (MHz)*	499.67
Nucleus	1H	*Number of Transients*	8	*Original Points Count*	16384	*Points Count*	16384
Pulse Sequence	s2pul	*Receiver Gain*	60.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2497.7319	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000

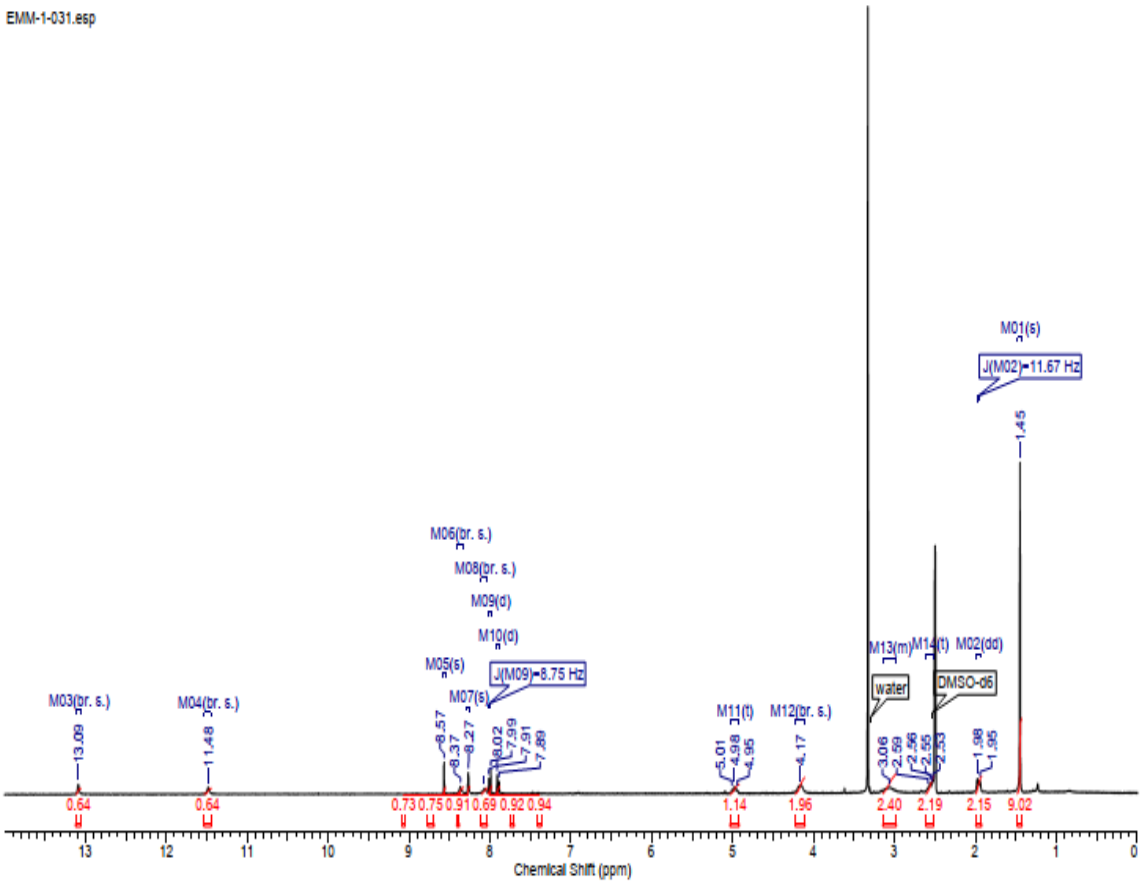


11a (NEU-0005118)

Formula $C_{13}H_{16}N_4O_3$	FW	434.4909

Acquisition Time (sec)	1.3622	Comment	EMM-1-031B 400MHz DMSO 05/01/2017			Date	May 1 2017
Date Stamp	May 1 2017	File Name	C:\Users\Abdul Shaikh\Documents\Efi\Volunt\Lab\NMR\EMM-1-031B.fid\fid				
Frequency (MHz)	399.13	Nucleus	1H	Number of Transients	32	Original Points Count	10854
Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	36.00	Solvent	DMSO-d6
Spectrum Offset (Hz)	2016.9929	Spectrum Type	STANDARD	Sweep Width (Hz)	7968.13	Temperature (degree C)	AMBIENT TEMPERATURE

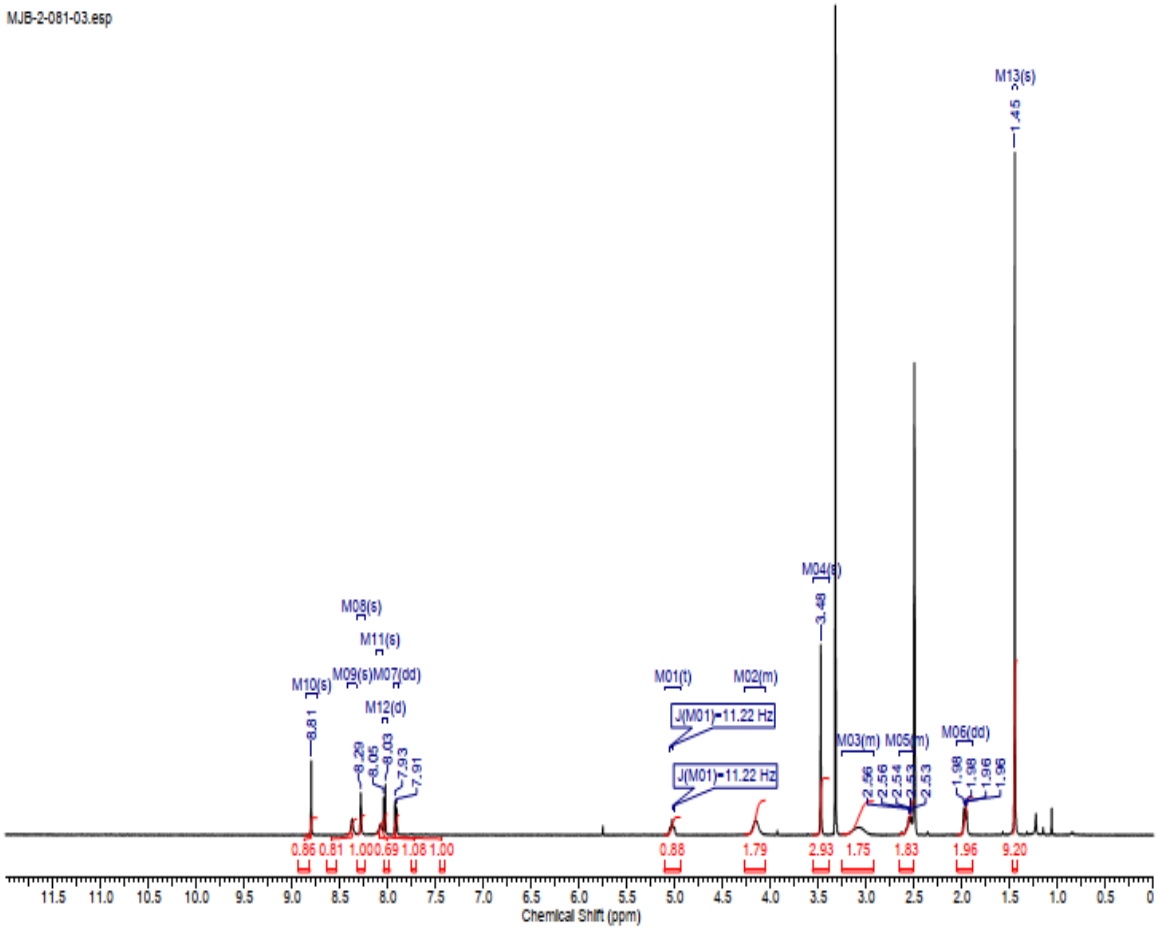
EMM-1-031.esp



11b (NEU-0005368)

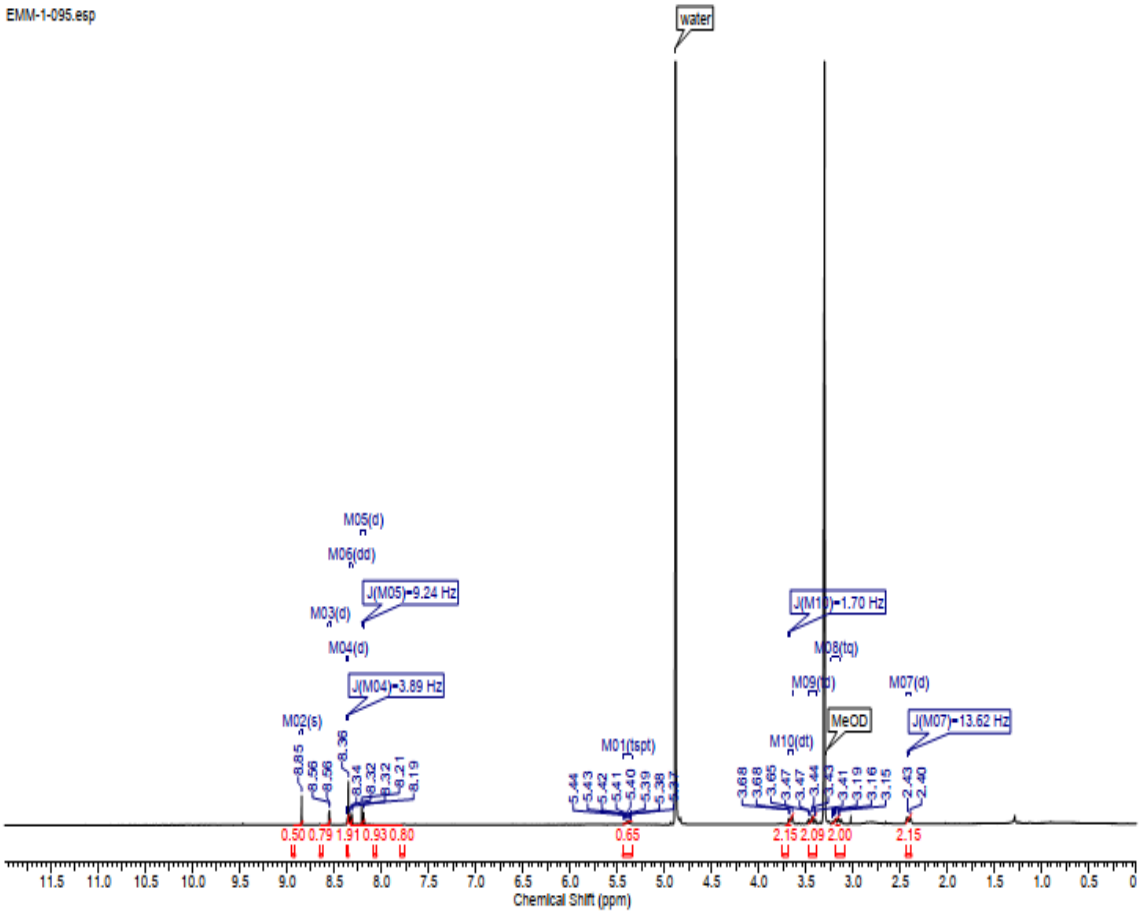
Acquisition Time (sec)	2.0494	*Date*	Feb 12 2018	*Date Stamp*	Feb 12 2018		
File Name	C:\Users\mjbus\Google Drive\Pollastri Lab\ Pollastri Lab Spectra\Melissa Buskes\NMR\MJB-2-081-03.fid\1r					*Frequency (MHz)*	499.68
Nucleus	1H	*Number of Transients*	8	*Original Points Count*	16384	*Points Count*	16384
Pulse Sequence	s2pul	*Receiver Gain*	54.00	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	2521.5686
Spectrum Type	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000		

MJB-2-081-03.esp



12a (NEU-0005370)

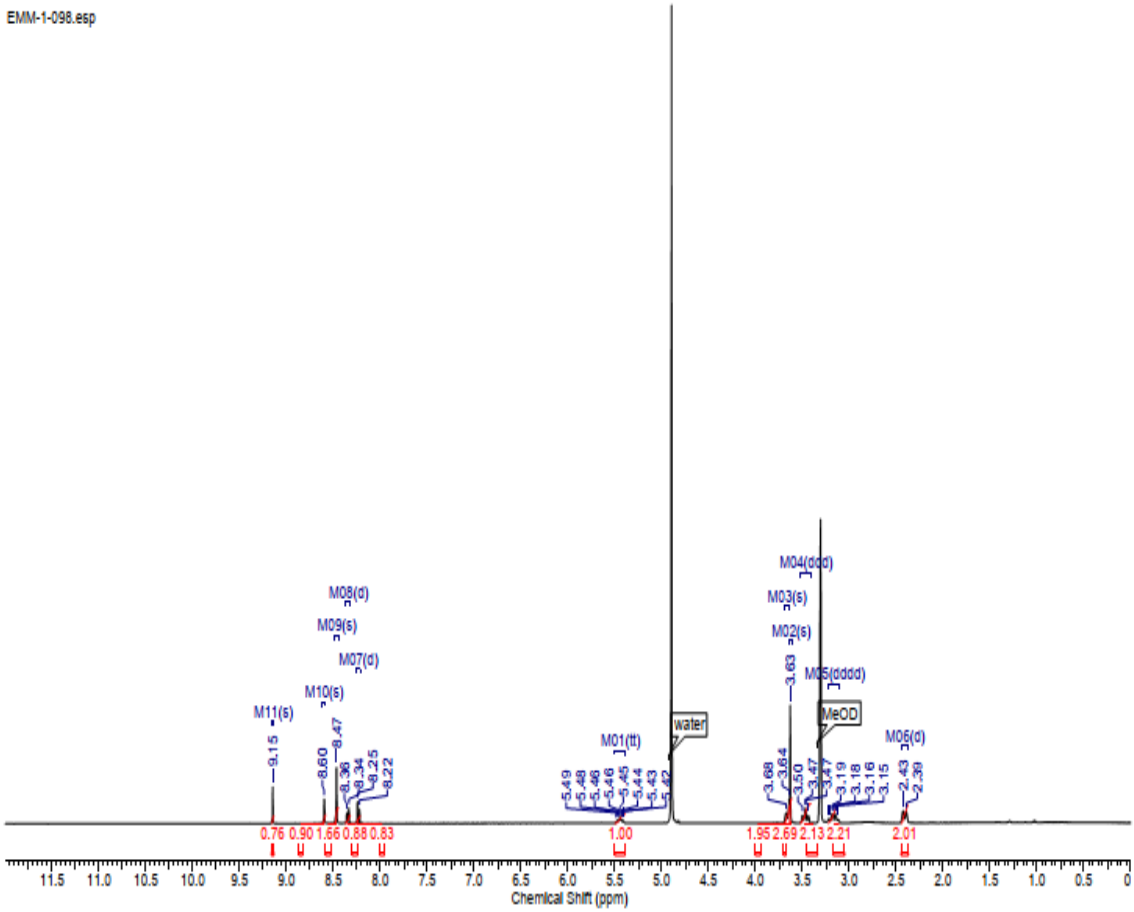
Acquisition Time (sec)	1.3662	*Comment*	EMM-1-095 MeOD			*Date*	Jul 14 2017
Date Stamp	Jul 14 2017	*File Name*	C:\Users\Abdul Shaikh\Documents\EfiVolunt\Lab\NMR\EMM-1-095 MeOD.fid\fid				
Frequency (MHz)	399.13	*Nucleus*	1H	*Number of Transients*	1388	*Original Points Count*	10886
Points Count	16384	*Pulse Sequence*	s2pul	*Receiver Gain*	36.00	*Solvent*	METHANOL-d4
Spectrum Offset (Hz)	2012.4731	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7968.13	*Temperature (degree C)*	AMBIENT TEMPERATURE



12b (NEU-0005369)

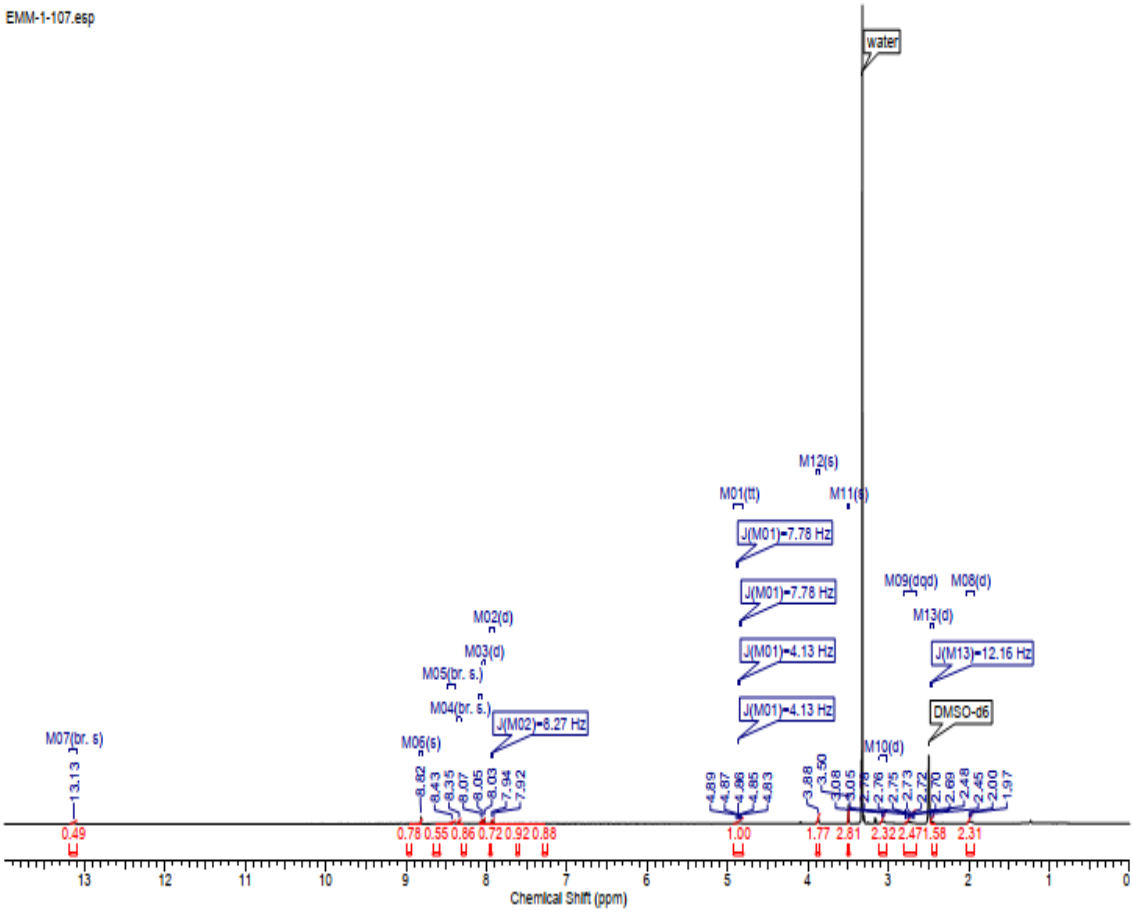
Acquisition Time (sec)	1.3662	*Comment*	EMM-1-098 400MHz MeOD 07/20/2017			*Date*	Jul 20 2017
Date Stamp	Jul 20 2017	*File Name*	C:\Users\Abdul Shaikh\Documents\Efflvolunt\Lab\NMR\EMM-1-098.fid\fid				
Frequency (MHz)	399.13	*Nucleus*	1H	*Number of Transients*	448	*Original Points Count*	10886
Points Count	16384	*Pulse Sequence*	s2pul	*Receiver Gain*	34.00	*Solvent*	METHANOL-d4
Spectrum Offset (Hz)	2011.9868	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7968.13	*Temperature (degree C)*	AMBIENT TEMPERATURE

EMM-1-098.esp



13 (NEU-0005379)

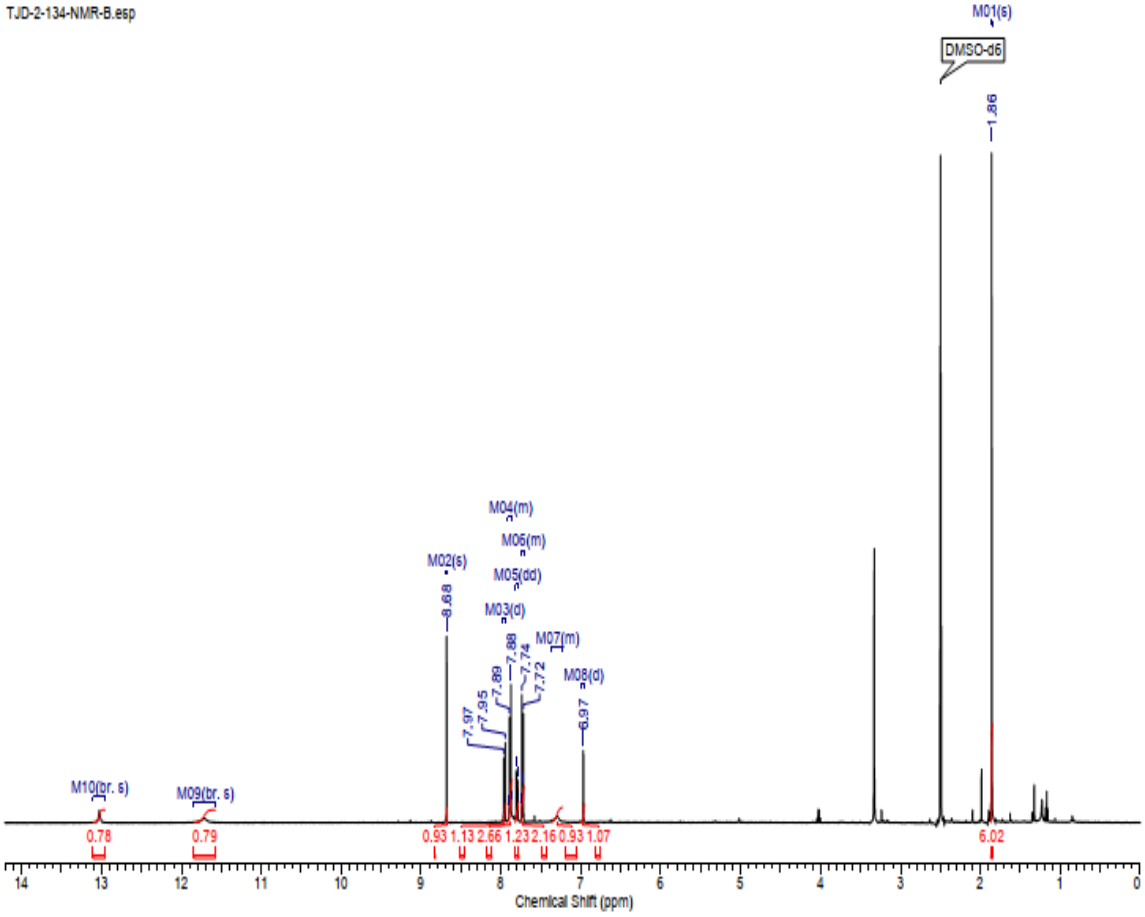
Acquisition Time (sec)	1.3662	*Comment*	EMM-1-107 400MHz 08/04/2017 DMSO			*Date*	Aug 4 2017
Date Stamp	Aug 4 2017	*File Name*	C:\Users\Abdul Shaikh\Documents\Efi\Volunt\Lab\NMR\EMM-1-107.fid\fid				
Frequency (MHz)	399.13	*Nucleus*	1H	*Number of Transients*	304	*Original Points Count*	10886
Points Count	16384	*Pulse Sequence*	s2pul	*Receiver Gain*	39.00	*Solvent*	DMSO-d6
Spectrum Offset (Hz)	2016.9929	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7968.13	*Temperature (degree C)*	AMBIENT TEMPERATURE



14a (NEU-0004965)

Acquisition Time (sec)	2.0480	*Comment*	STANDARD PROTON PARAMETERS			*Date*	Jan 19 2017
Date Stamp	Jan 19 2017	*File Name*	C:\Users\Yellow Fever\Google Drive\ Pollastri\Lab Spectra\Travis DeLano\TJD-2-134-NMR-B.fid\fid				
Frequency (MHz)	499.68	*Nucleus*	1H	*Number of Transients*	16	*Original Points Count*	16384
Points Count	16384	*Pulse Sequence*	s2pul	*Receiver Gain*	58.00	*Solvent*	DMSO-d6
Spectrum Offset (Hz)	3100.6211	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	8000.00	*Temperature (degree C)*	AMBIENT TEMPERATURE

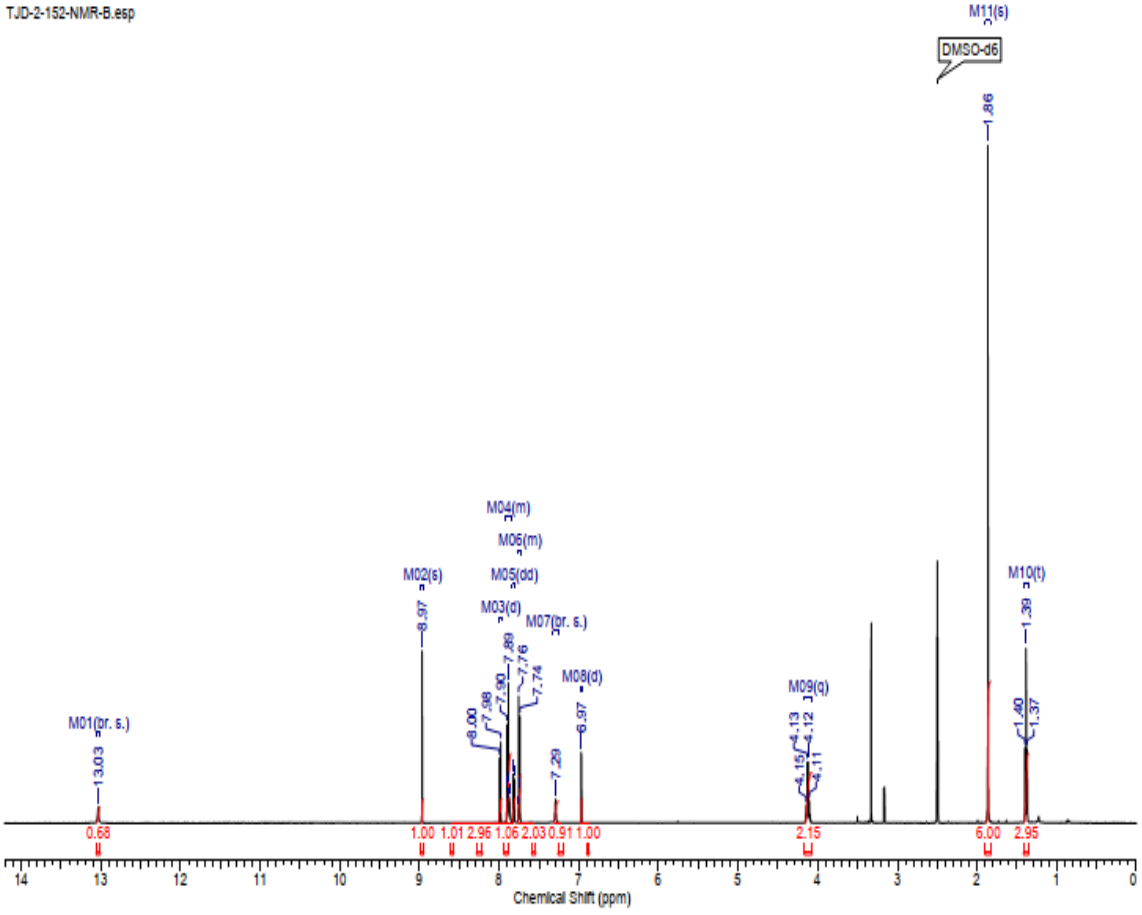
TJD-2-134-NMR-B.esp



14c (NEU-0005121)

Acquisition Time (sec)	3.7500	*Comment*	STANDARD PROTON PARAMETERS			*Date*	May 12 2017
Date Stamp	May 12 2017	*File Name*	C:\Users\Yellow Fever\Google Drive\ PollastriLab Spectra\Travis DeLanoi\TJD-2-152-NMR-B.fid\fid				
Frequency (MHz)	499.68	*Nucleus*	1H	*Number of Transients*	16	*Original Points Count*	30000
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	52.00	*Solvent*	DMSO-d6
Spectrum Offset (Hz)	3099.9541	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	8000.00	*Temperature (degree C)*	AMBIENT TEMPERATURE

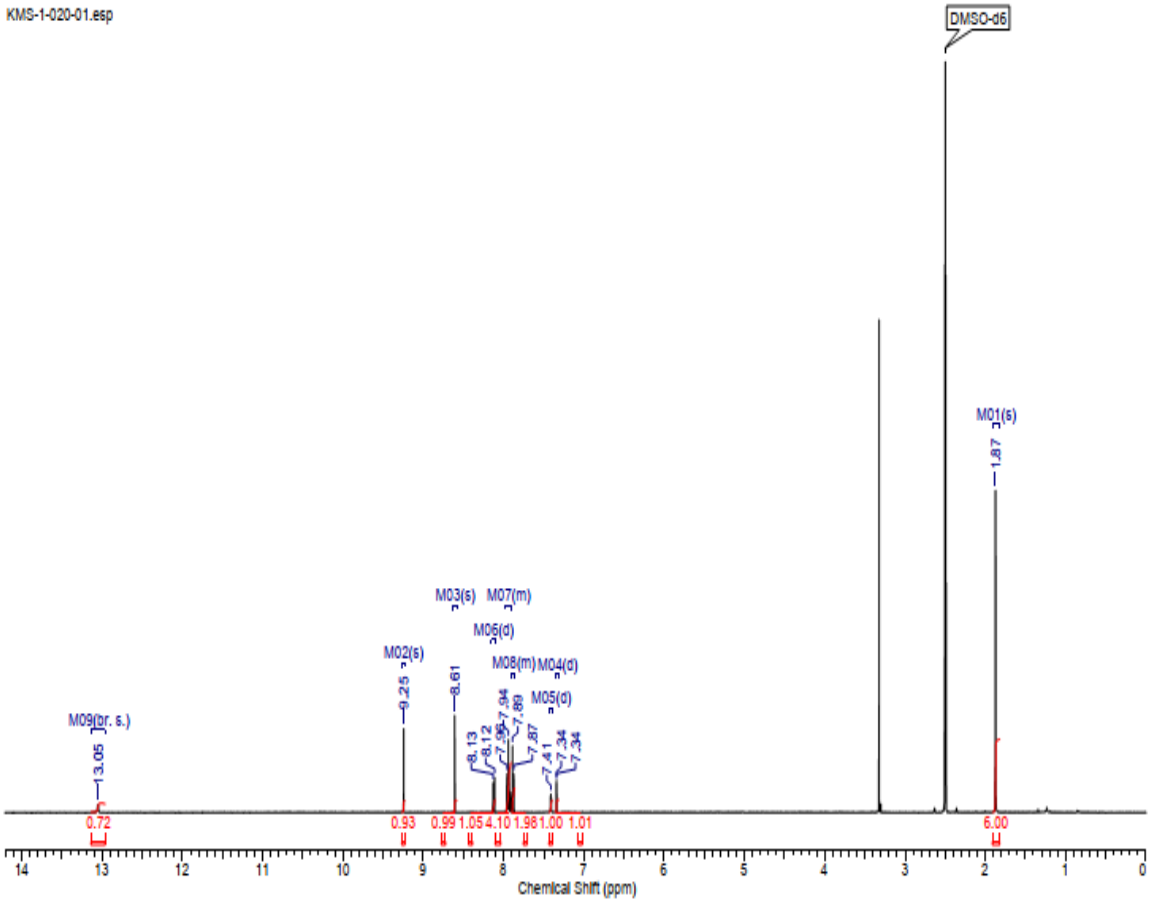
TJD-2-152-NMR-B.esp



15a (NEU-0005120)

Acquisition Time (sec)	3.7500	*Comment*	KMS-1-020-01 Lori Ferrins HNMR 2nd May, 2017 NMR500			*Date*	May 2 2017
Date Stamp	May 2 2017	*File Name*	C:\Users\PollastriLabAnalyt\Google Drive\ PollastriLab Spectra\Kate Schneider\KMS-1-020-01.fid\fid				
Frequency (MHz)	499.68	*Nucleus*	1H	*Number of Transients*	16	*Original Points Count*	30000
Points Count	32768	*Pulse Sequence*	s2pul	*Receiver Gain*	58.00	*Solvent*	DMSO-d6
Spectrum Offset (Hz)	3099.9541	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	8000.00	*Temperature (degree C)*	AMBIENT TEMPERATURE

KMS-1-020-01.esp

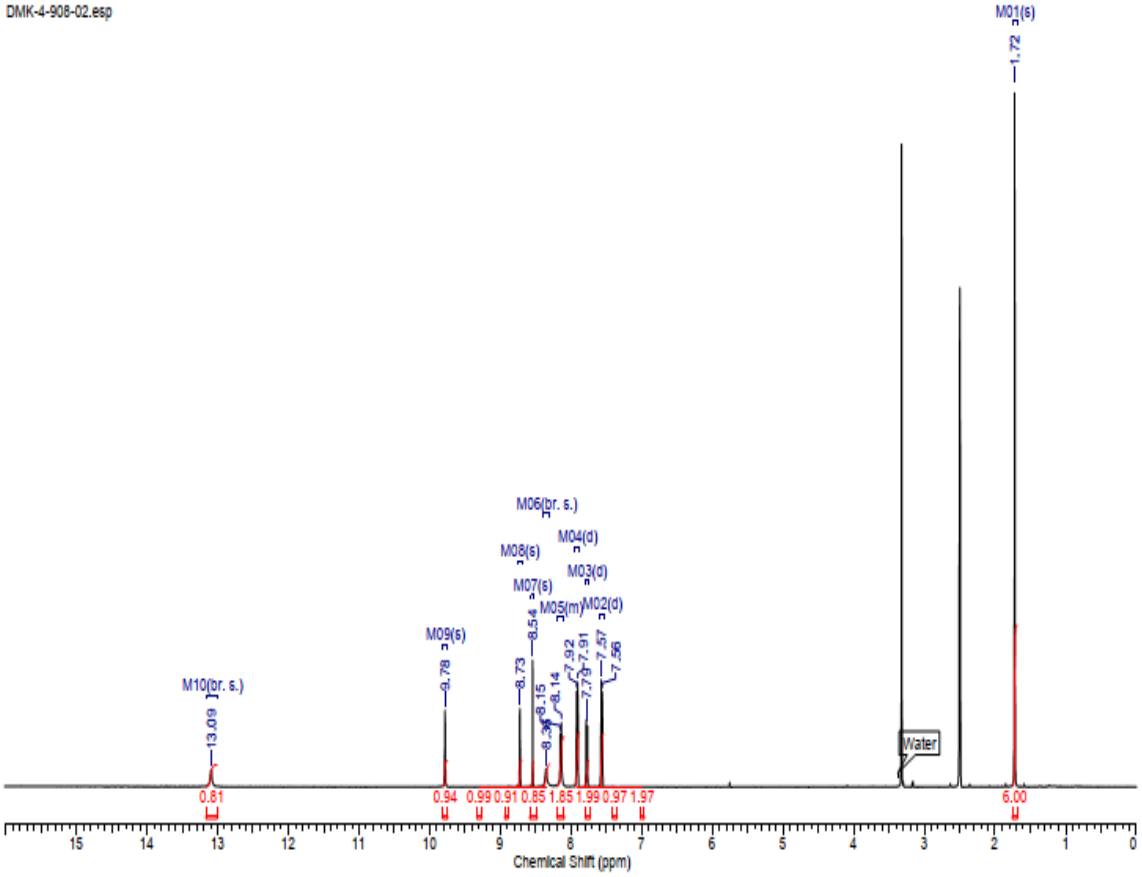


15b (NEU-0006481)

Formula $C_{21}H_{18}N_4$	FW 354.4078		

Acquisition Time (sec)	1.7258	Date	Jul 8 2019	Date Stamp	Jul 8 2019		
File Name	C:\Users\Dana\Desktop\NMR\DMK-4-908-02 20190708 03\PROTON 01.fid\fid					Frequency (MHz)	499.68
Nucleus	1H	Number of Transients	16	Original Points Count	16384	Points Count	16384
Pulse Sequence	s2pul	Receiver Gain	56.00	Solvent	DMSO-d6	Spectrum Offset (Hz)	3255.5706
Spectrum Type	STANDARD	Sweep Width (Hz)	9493.29	Temperature (degree C)	25.000		

DMK-4-908-02.esp

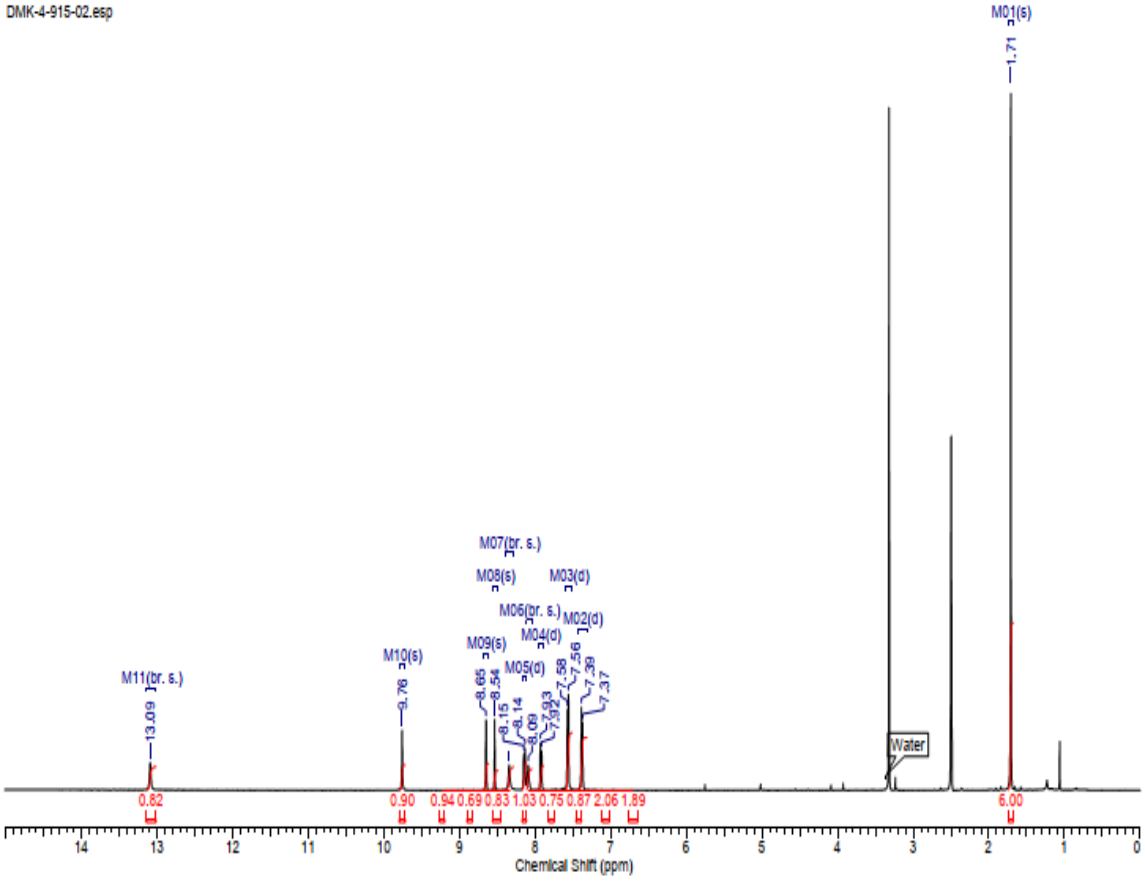


15c (NEU-0006485)

Formula $C_{13}H_{14}N_4$	FW	378.4292

Acquisition Time (sec)	1.8217	Date	Jul 16 2019	Date Stamp	Jul 16 2019		
File Name	C:\Users\Dana\Desktop\NMR\DMK-4-915-02_20190716_01\PROTON_01.fid\fid					Frequency (MHz)	499.68
Nucleus	1H	Number of Transients	16	Original Points Count	16384	Points Count	16384
Pulse Sequence	s2pul	Receiver Gain	54.00	Solvent	DMSO-d6	Spectrum Offset (Hz)	3006.1758
Spectrum Type	STANDARD	Sweep Width (Hz)	8993.82	Temperature (degree C)	25.000		

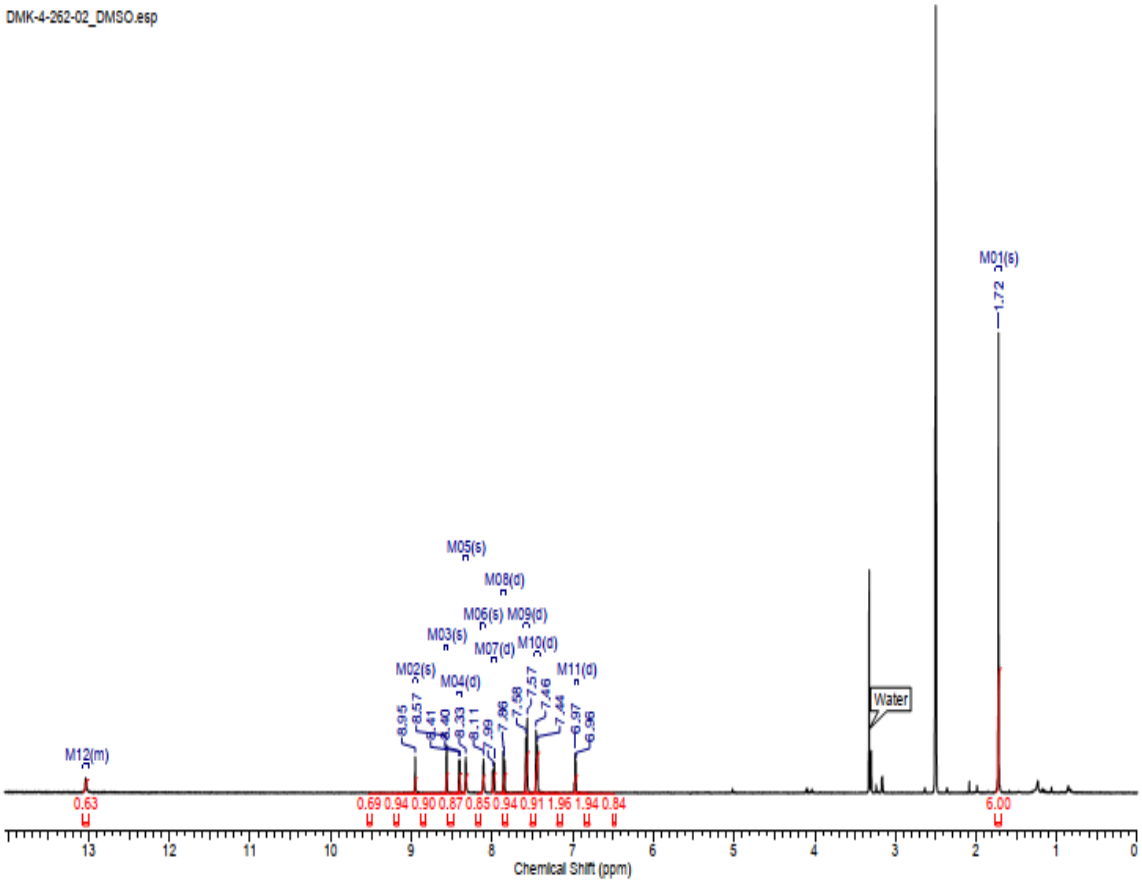
DMK-4-915-02.esp



15d (NEU-0005511)

Formula $C_{12}H_{19}N_3$	FW	353.4198

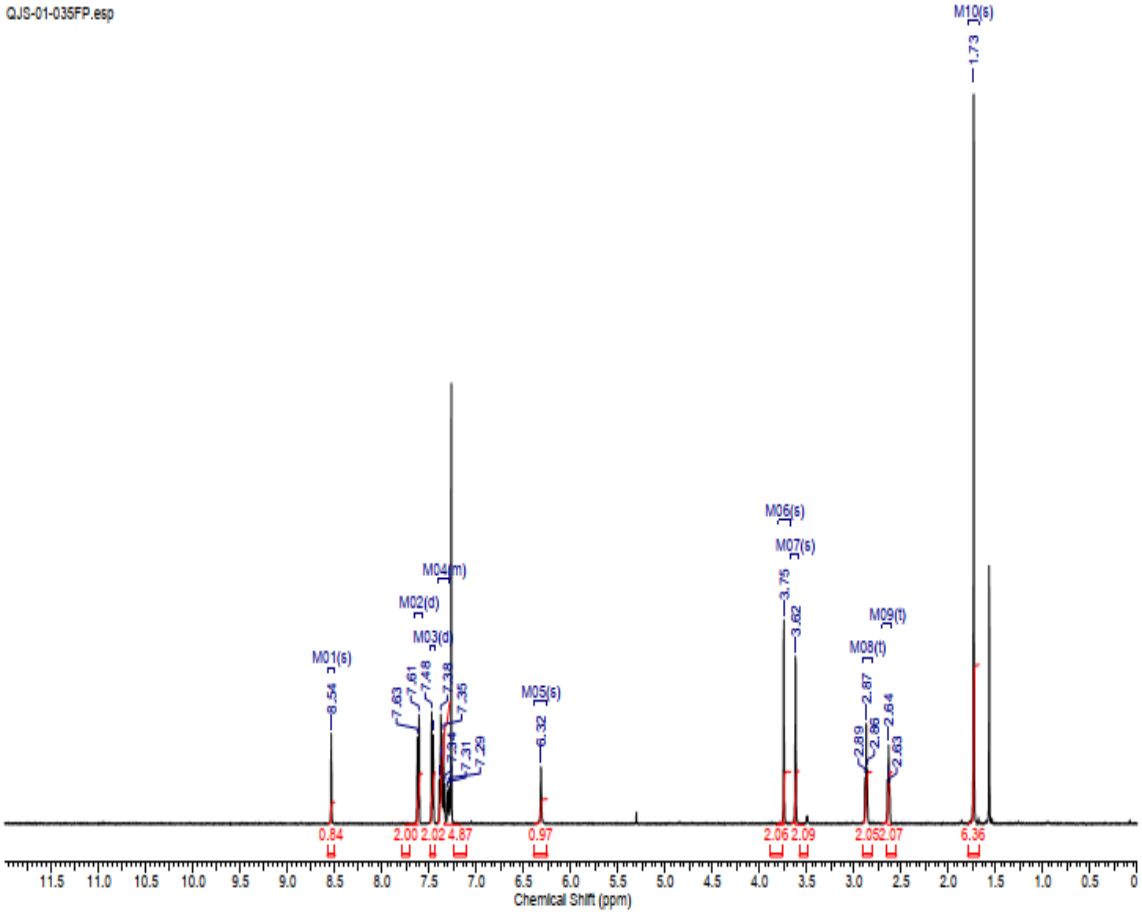
Acquisition Time (sec)	1.9288	Date	Nov 17 2017	Date Stamp	Nov 17 2017		
File Name	C:\Users\Dana\Desktop\NMR\DMK-4-262-02_DMSO_20171117_01\PROTON_02.fid\fid					Frequency (MHz)	499.68
Nucleus	1H	Number of Transients	8	Original Points Count	16384	Points Count	16384
Pulse Sequence	s2pul	Receiver Gain	60.00	Solvent	DMSO-d6	Spectrum Offset (Hz)	2770.1704
Spectrum Type	STANDARD	Sweep Width (Hz)	8494.37	Temperature (degree C)	25.000		



15e (NEU-0006777)

Acquisition Time (sec)	2.0494	*Date*	Nov 19 2019	*Date Stamp*	Nov 19 2019		
File Name	C:\Users\Administrator\Google Drive\ PollastriLab SpectralQuilion Simpson\NMR\QJS-01-035FP.fid\fid					*Frequency (MHz)*	499.66
Nucleus	1H	*Number of Transients*	4	*Original Points Count*	16384	*Points Count*	16384
Pulse Sequence	s2pul	*Receiver Gain*	54.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2493.3384	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000

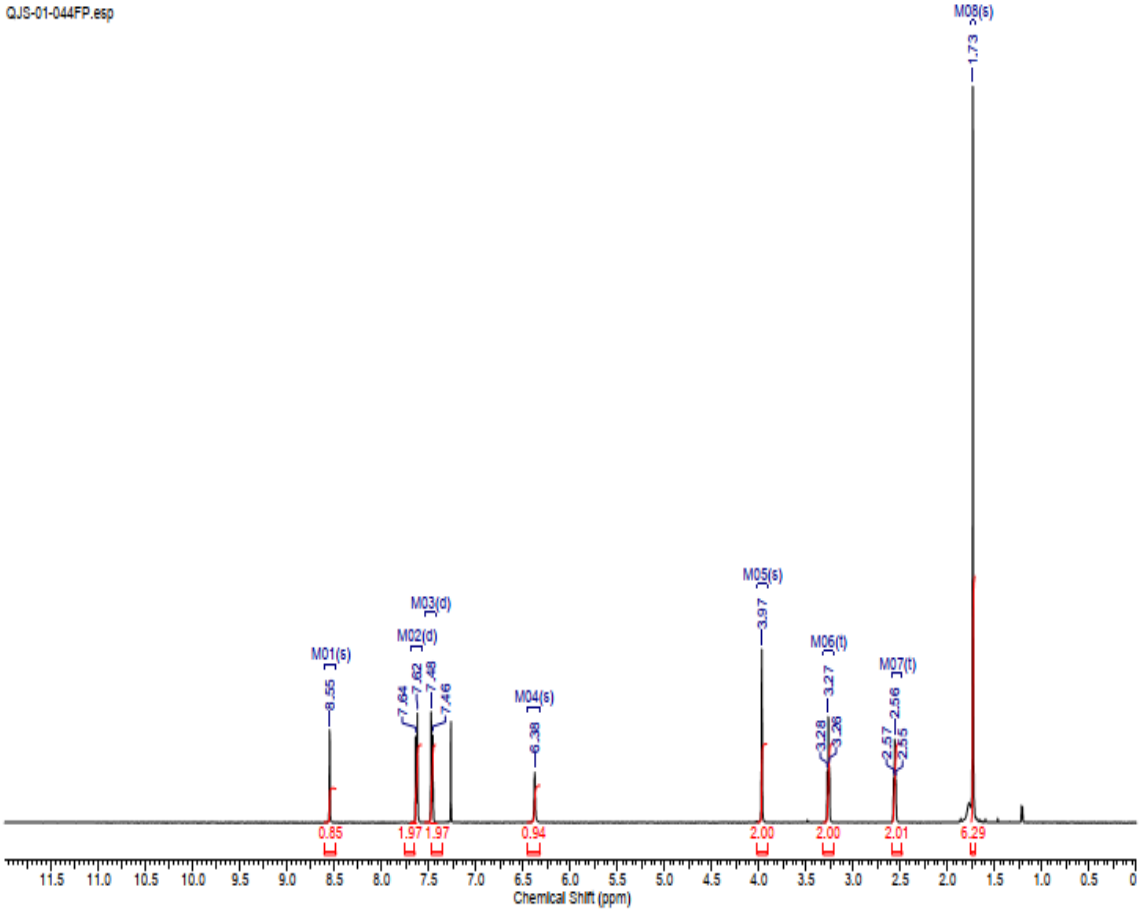
QJS-01-035FP.esp



15f (NEU-0006778)

Acquisition Time (sec)	2.0494	*Date*	Nov 19 2019	*Date Stamp*	Nov 19 2019		
File Name	C:\Users\Administrator\Google Drive_PollastriLab_Spectra\Quillon Simpson\NMR\QJS-01-044FP.fid\fid					*Frequency (MHz)*	499.66
Nucleus	1H	*Number of Transients*	4	*Original Points Count*	16384	*Points Count*	16384
Pulse Sequence	s2pul	*Receiver Gain*	48.00	*Solvent*	CHLOROFORM-d		
Spectrum Offset (Hz)	2493.3384	*Spectrum Type*	STANDARD	*Sweep Width (Hz)*	7994.40	*Temperature (degree C)*	25.000

QJS-01-044FP.esp

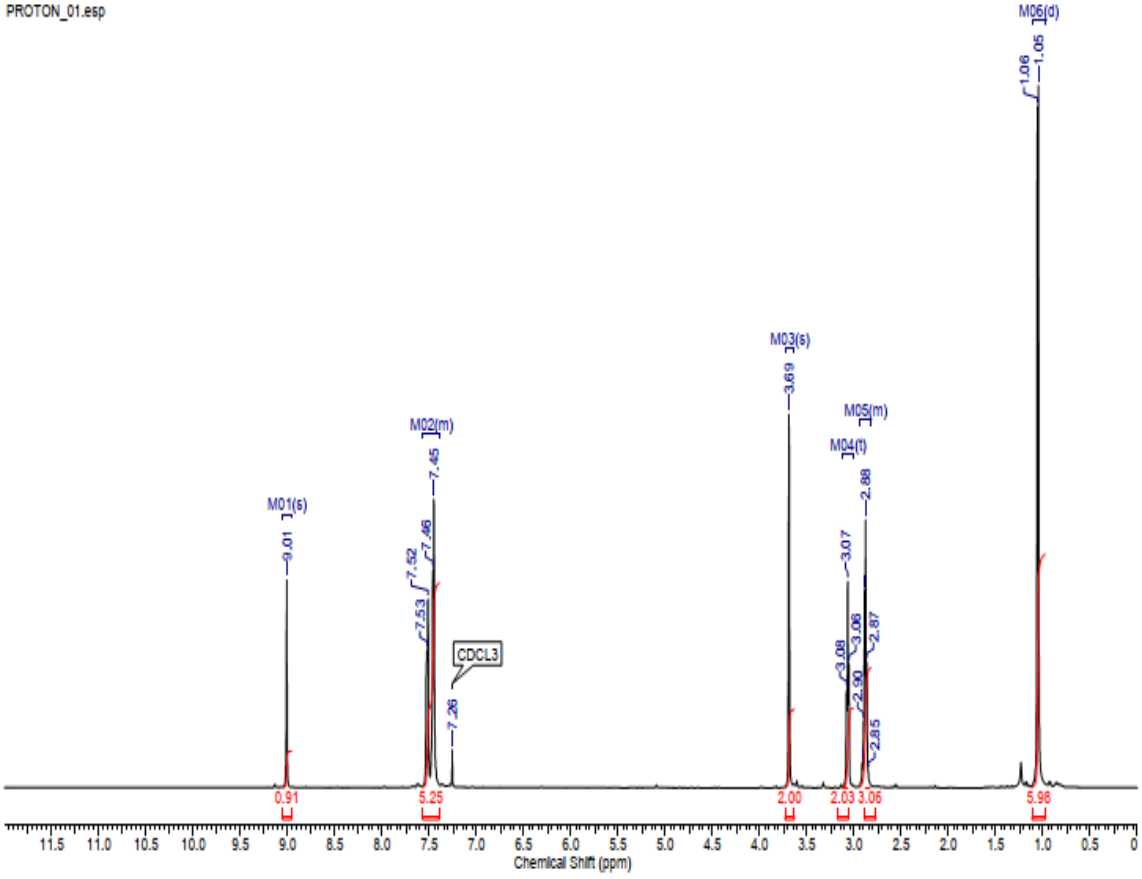


S12 (NEU-006437)

Formula $C_{18}H_{19}N_3$	FW	253.3422

Acquisition Time (sec)	2.0494	Comment	Column 2 Fractions 4-13			Date	Apr 5 2019
Date Stamp	Apr 5 2019	File Name	C:\Users\Polla\OneDrive\Documents\Devine NMR\WD1-193-3 20190405 01\PROTON 01.fid\fid				
Frequency (MHz)	499.67	Nucleus	1H	Number of Transients	16	Original Points Count	16384
Points Count	16384	Pulse Sequence	s2pul	Receiver Gain	38.00	Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2997.4163	Spectrum Type	STANDARD	Sweep Width (Hz)	7994.40	Temperature (degree C)	25.000

PROTON_01.esp



S13 (NEU-0006436)

Formula $C_{18}H_{20}N_4$	FW	268.3568

Acquisition Time (sec)	2.0494	Date	Apr 4 2019	Date Stamp	Apr 4 2019		
File Name	C:\Users\Polla\OneDrive\Documents\Devine NMR\WD1-191-2 20190404 01\PROTON 01.fid\fid					Frequency (MHz)	499.67
Nucleus	1H	Number of Transients	16	Original Points Count	16384	Points Count	16384
Pulse Sequence	s2pul	Receiver Gain	50.00	Solvent	CHLOROFORM-d		
Spectrum Offset (Hz)	2997.4163	Spectrum Type	STANDARD	Sweep Width (Hz)	7994.40	Temperature (degree C)	25.000

PROTON_01.esp

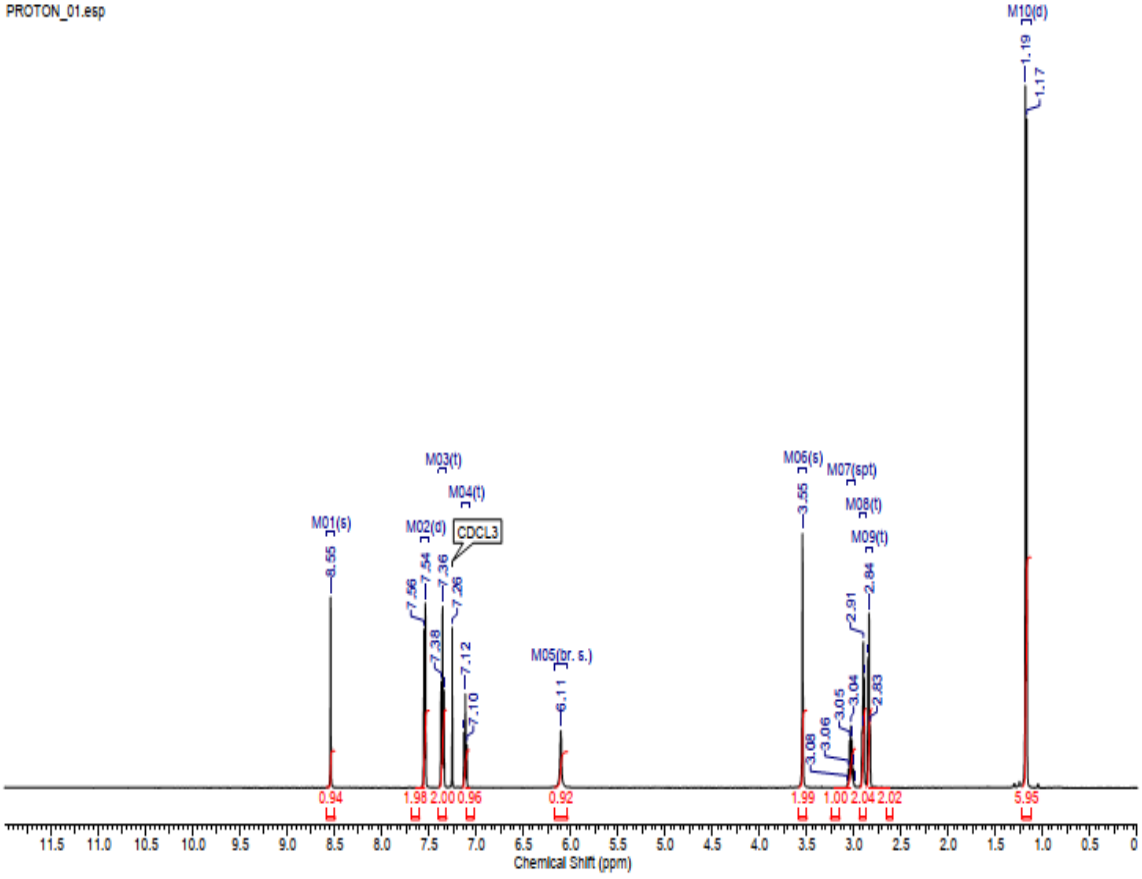


Table S6. SMILES strings and NEU numbers of all compounds presented in the manuscript.

ID	Molecule Name	SMILES
5a	NEU-0004470	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1=CNN=C1)C1=CC=C(C=C1)C(C)(C)C#N
5b	NEU-0004783	CN1C=C(C(C)=N1)C1=CC=C2N=CC3=C(N(C(=O)N3C)C3=CC=C(C=C3)C(C)(C)C#N)C2=C1
5c	NEU-0004791	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1=CNN=C1C)C1=CC=C(C=C1)C(C)(C)C#N
5d	NEU-0006054	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1=CON=C1)C1=CC=C(C=C1)C(C)(C)C#N
6	NEU-0004790	CN1C=C(NC2=CC=C3N=CC4=C(N(C(=O)N4C)C4=CC=C(C=C4)C(C)(C)C#N)C3=C2)C=N1
7a	NEU-0004914	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)N1CCNCC1)C1=CC=C(C=C1)C(C)(C)C#N
7b	NEU-0005815	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)N1CCCN(C)CC1)C1=CC=C(C=C1)C(C)(C)C#N
7c	NEU-0005293	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)N1CCC1)C1=CC=C(C=C1)C(C)(C)C#N
7d	NEU-0005294	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)N1CCCC1)C1=CC=C(C=C1)C(C)(C)C#N
7e	NEU-0005316	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)N1CCCCC1)C1=CC=C(C=C1)C(C)(C)C#N
8a	NEU-0005876	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1=CCN(C1)C(=O)OC(C)(C)C)C1=CC=C(C=C1)C(C)(C)C#N
9a	NEU-0006050	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1CCN(C1)C(=O)OC(C)(C)C)C1=CC=C(C=C1)C(C)(C)C#N
9b	NEU-0005997	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1CCN(CC1)C(=O)OC(C)(C)C)C1=CC=C(C=C1)C(C)(C)C#N
13	NEU-0005379	CN1C(=O)N(C2CCN(CC#N)CC2)C2=C1C=NC1=CC=C(C=C21)C1=CNN=C1
10a	NEU-0006051	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1CCNC1)C1=CC=C(C=C1)C(C)(C)C#N
10b	NEU-0005998	CN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1CCNCC1)C1=CC=C(C=C1)C(C)(C)C#N
11a	NEU-0005118	CC(C)(C)OC(=O)N1CCC(CC1)N1C(=O)NC2=C1C1=CC(=CC=C1N=C2)C1=CNN=C1
11b	NEU-0005368	CN1C(=O)N(C2CCN(CC2)C(=O)OC(C)(C)C)C2=C1C=NC1=CC=C(C=C21)C1=CNN=C1
12a	NEU-0005370	O=C1NC2=C(N1C1CCNCC1)C1=CC(=CC=C1N=C2)C1=CNN=C1
12b	NEU-0005369	CN1C(=O)N(C2CCNCC2)C2=C1C=NC1=CC=C(C=C21)C1=CNN=C1
14a	NEU-0004965	CC(C)(C#N)C1=CC=C(C=C1)N1C(=O)NC2=C1C1=CC(=CC=C1N=C2)C1=CNN=C1
14c	NEU-0005121	CCN1C(=O)N(C2=C1C=NC1=CC=C(C=C21)C1=CNN=C1)C1=CC=C(C=C1)C(C)(C)C#N

15a	NEU-0005120	CC(C)(C#N)C1=CC=C(C=C1)N1C=NC2=CN=C3C=CC(=CC3=C12)C1=CN N=C1
15b	NEU-0006481	CC(C)(C#N)C1=CC=C(NC2=NC=NC3=CC=C(C=C23)C2=CNN=C2)C=C1
15c	NEU-0006485	CC(C)(C#N)C1=CC=C(NC2=C(C=NC3=CC=C(C=C23)C2=CNN=C2)C#N) C=C1
15d	NEU-0005511	CC(C)(C#N)C1=CC=C(NC2=CC=NC3=CC=C(C=C23)C2=CNN=C2)C=C1
15e	NEU-0006777	CC(C)(C#N)C1=CC=C(NC2=NC=NC3=C2CN(CC2=CC=CC=C2)CC3)C=C 1
15f	NEU-0006778	CC(C)(C#N)C1=CC=C(NC2=C3CNCCC3=NC=N2)C=C1
S12	NEU-0006437	CC(C)N1CCC2=C(C1)C(=NC=N2)C1=CC=CC=C1
S13	NEU-0006436	CC(C)N1CCC2=C(C1)C(NC1=CC=CC=C1)=NC=N2

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