Supporting Information for -

Driving Factors in Amiloride Recognition of HIV RNA Targets

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Section A. Tat peptide displacement assays with amiloride derivatives

a. RNA Preparation: RNA with the following sequences were purchased from Integrated DNA Technologies, and dissolved in nuclease free water.

RNA Name	Sequence
HIV-1-TAR	5'-GGCAGAUCUGAGCCUGGGAGCUCUCUGCC-3'
HIV-2-TAR	5'-GGCAGAUUGAGCCCUGGGAGGUUCUCUGCC-3'
RRE-IIB	5'-GGUCUGGGCGCAGCGCAAGCUGACGGUACAGGCC-3'
HIV-1-FSS	5'-CUGGCCUUCCCACAAGGGAAGGCCAG-3'

HIV-I-ESSV RNA was a gift from Prof. Blanton S. Tolbert, Department of Chemistry, Case Western Reserve University, Cleveland, OH and had the following sequence –

HIV-1-ESSV	5'-GGUCUGCUAUAAGAAAGGCCUUAUUAGGACAU AUAGUUAGCCCUAGGUGUGUGAAUAUCAAGCAGGCC-3'

HIV-1-TAR, HIV-2-TAR, RRE-IIB, and ESSV were diluted to 50 μM concentration, and annealed by heating to heating to 95 ºC and cooling on ice for 30 min. HIV-1-FSS RNA was annealed according to the procedure outlined by Miller and co-workers.[1] Purity of RNA samples was monitored periodically by gel electrophoresis. Concentrations of RNA were determined using Beer's law with the following extinction coefficients $(\varepsilon)_{260}$ of HIV-1-TAR: 268900 M^{-1} cm^{-1}; HIV-2-TAR: 283900 M^{-1} cm^{-1}; RRE-IIB: 322900 M^{-1} $cm^{-1;}$ HIV-1-FSS 246000 M^{-1} cm^{-1}; HIV-1-ESSV 677100 M^{-1} cm^{-1}.

b. Amiloride sample preparation: Amiloride stock solutions (50 mM) were prepared by dissolving them in DMSO and diluted to appropriate stock concentrations in the assay buffer containing 15% DMSO. Sample purity was periodically monitored using HPLC analysis as indicated in the synthesis section below.

c. ***Binding assays for Tat peptide to different RNA:*** Binding constants for HIV-1-TAR, HIV-2-TAR, and RRE have been reported in our previous study.[2] Binding of HIV-1-FSS and HIV-1-ESSV was measured using the same procedure. Briefly, Tat peptide [N-(5-FAM)-AAARKKRRQRRRAAAK(TAMRA)- C] was purchased from Lifetein and dissolved at a concentration of 100 nM in the assay buffer containing 50 mM Tris, 50 mM KCl, 0.01% Triton-X-100, pH = 7.4. RNA stock solutions were serially diluted in assay buffer from 0 - 1 μM concentrations. The Tat peptide solution (10 μL) and RNA solution (10 μL) were combined in a Corning™ low volume round-bottom black 384 well-plates, shaken on a platform stirrer for 5 min, centrifuged at 4000 rpm for 1 min, and allowed to incubate for 30 min (Final Tat concentration = 50 nM, final RNA concentrations = 0 - 500 nM). The 384 well-plates were then read on a Clariostar™ monochromator (BMG Labtech) microplate reader using the excitation and emission wavelengths of 485 nm (FAM) and 590 nm (TAMRA) respectively. The fluorescence intensities at each RNA concentrations were normalized to the Tat-only control wells. Dissociation constants (K_d) were calculated by fitting the observed relative fluorescence intensity values at each concentration to equation (S1) using the GraphPad Prism curve fitting software. Assays were conducted in triplicates and each replicate contained three technical replicates.

$$\mathrm{Y} = \mathrm{Bmax} * \mathrm{X}/(K\mathrm{d} + \mathrm{X}) \; + \; \mathrm{NS} * \mathrm{X} \; + \; \mathrm{Background} \quad \text{.....Equation S1}$$

Where, Y = Total binding; X = Conc. of added ligand; Bmax = Maximum binding; K_d = Equilibrium dissociation constant; NS: Slope of Nonlinear regression; Background: Measured binding without the ligand.

The K_d for each RNA are shown in Table S1 below –

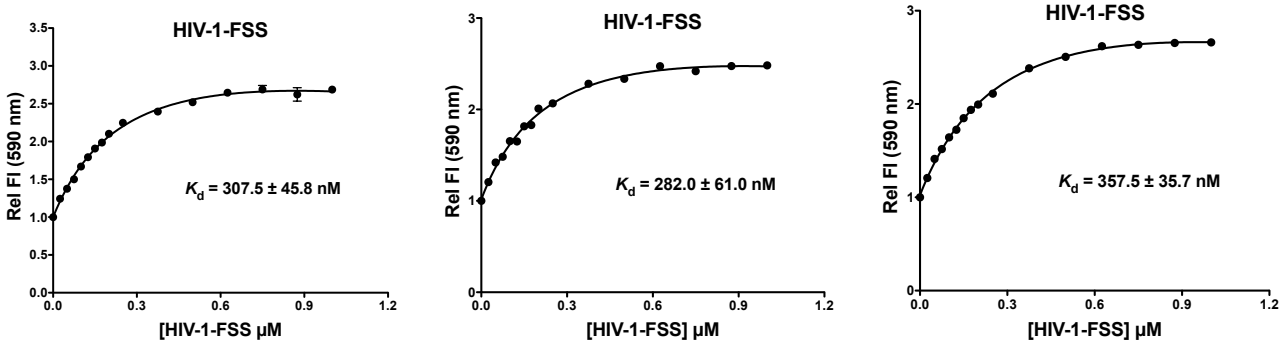
Table S1: Observed binding constants (K_d) for each RNA Target studied.

RNA	Kd (nM)
HIV-1-TAR	22.1 ± 6.9 [a]
HIV-2-TAR	24.9 ± 8.1 [a]
RRE-IIB	32.7 ± 6.6 [a]

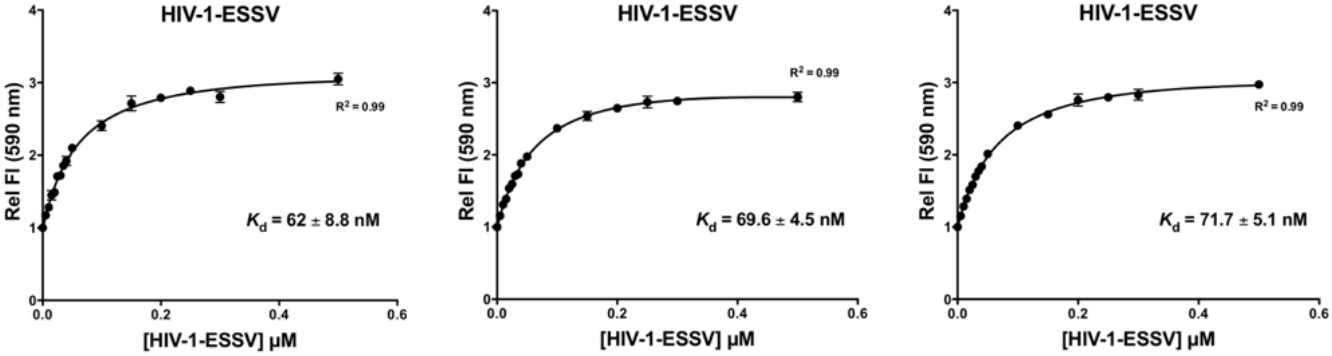
HIV-1-FSS	315.5 ± 47.5
HIV-1-ESSV	67.8 ± 6.1

[a]: Previously reported.[2]

d. Binding Curves for HIV-1-FSS and HIV-1-ESSV binding to the indicator Tat Peptide



Average K_d = 315.5 ± 47.5 nM



Average K_d = 67.8 ± 6.1 nM

e. Screening experiments with amiloride derivative library:

Screening of 25 new amiloride derivatives along with 10 previously developed compounds against these 5 RNA targets was performed as described previously.[2] Briefly, Tat peptide was diluted at a concentration of 150 nM in the assay buffer. RNA solutions were prepared by diluting stocks in the assay buffer at the following concentrations - HIV-1-TAR: 120 nM, HIV-2-TAR: 120 nM, RRE-IIB: 225 nM, HIV-1-FSS: 750 nM, HIV-1-ESSV: 210 nM. These concentrations were chosen to provide best fluorescence intensities possible in the bound state [at these concentrations]. Small molecules were diluted to concentrations of 15 µM, 30 µM, 75 µM, and 150 µM. We note that all small molecules were in the HCl salt form except DMA-207 which degraded (loss of the methyl indole moiety) under conditions used to form the HCl salt. Tat peptide (6 µL), RNA (6 µL), and

small molecule solution (6 µL) were combined in a 384 well-plate. Final concentrations in each assay well were as follows- Tat peptide: 50 nM; RNA conc: 40 nM (HIV-1 and 2-TAR), 75 nM (RRE-IIB), 250 nM (HIV-1-FSS), and 70 nM (HIV-1-ESSV); small molecule: 0 µM, 5 µM, 10 µM, and 25 µM. The well-plate was shaken on a platform stirrer for 5 min, centrifuged at 4000 rpm for 1 min, and allowed to incubate in dark for 30 min and read using the excitation and emission wavelengths of 485 nm (FAM) and 590 nm (TAMRA) respectively. % Displacement of Tat peptide from RNA was calculated using the equation shown below. Each screening experiment was performed in triplicates and each replicate contains a technical triplicate.

$$\%\mathrm{FID} = 100 - \left(100 \times \frac{F}{F0}\right)$$

$$F = F_{(\text{ligand + RNA + Tat})} - F_{(\text{RNA+ Ligand+ Buffer})}$$

$$F_0 = F_{(\text{RNA+ Tat+ Buffer})}$$

Molecules that show >25% FID at tested concentrations of 5 µM, 10 µM, or 25 µM, were labeled as hits of the screening assay at that concentration.

f. Z' Scores: Z' Scores for each RNA: Tat system were calculated using the equation below, using 48 data points for each RNA:Tat complex –

$$Z' = 1 - \left(\frac{3\,(\sigma_{(\text{positive})} + \sigma_{(\text{negative})})}{|\,\mu_{(\text{positive})} - \mu_{(\text{negative})}|}\right)$$

$\mu_{(positive)}$ = Mean Fluorescence Intensity for Tat + RNA
$\mu_{(negative)}$ = Mean Fluorescence Intensity for Tat alone
$\sigma_{(positive)}$ = Standard deviation for positive
$\sigma_{(negative)}$ = Standard deviation for negative

Table S2: Fraction of Tat peptide bound and Z' score for each RNA studied at the indicated concentration

RNA	K_d (nM)	RNA Conc nM	Fb_0	Z' Score
HIV-1-TAR	23.6 ± 3.6	40[a]	0.48[a]	0.54
HIV-2-TAR	19.5 ± 4.0	40[a]	0.46[a]	0.65
RRE-IIB	35.1 ± 5.2	75[a]	0.56[a]	0.48
HIV-1-FSS	315.5 ± 47.5	250	0.40	0.33
HIV-1-ESSV	67.8 ± 6.1	70	0.42	0.40

[a]: Previously reported.[2]

g. Raw data for screening of amilorides against each RNA target at the studied concentration – Hit molecules indicated by pink shaded cells.

i. Raw data for screening at 5 µM SM conc.

Comp	HIV-1-TAR	Stdev	HIV-2-TAR	Stdev	RRE-IIB	Stdev	HIV-1-FSS	Stdev	ESSV	Stdev
Neomycin	35	1	50	4	38	5	37	3	38	4
1	0	8	2	7	0	6	6	3	2	12

19	2	4	2	5	1	5	9	2	0	5
96	5	11	9	4	8	6	12	8	3	7
97	6	13	3	4	2	5	4	4	0	6
101	6	6	8	5	2	3	6	6	8	6
118	2	1	7	4	9	4	4	4	4	4
132	1	5	-5	6	0	5	-3	10	0	5
155	3	3	6	2	4	3	11	6	13	5
164	9	8	2	1	10	5	5	2	5	2
169	37	4	21	9	8	5	3	3	2	3
176	5	7	4	8	5	2	-1	4	10	9
177	3	1	4	4	8	5	4	8	11	3
178	14	6	7	5	10	6	-1	3	9	2
179	11	10	9	7	9	9	5	5	13	1
180	23	0	12	5	8	6	1	3	3	10
181	8	3	3	1	4	6	7	3	6	4
182	37	3	13	5	9	6	6	2	5	9
183	9	9	6	4	8	8	2	14	1	7
184	9	7	6	2	8	16	6	7	7	8
185	11	7	5	4	2	9	6	11	9	3
186	-3	5	1	2	12	3	4	6	7	12
187	27	5	15	4	7	3	-5	12	13	2
188	52	6	23	3	5	3	2	7	11	5
190	6	3	11	4	11	1	11	11	19	2
191	13	6	3	2	9	2	8	9	23	7
192	-1	6	5	5	7	4	4	7	15	3
193	22	6	19	4	27	4	15	4	45	2
194	11	6	11	6	13	4	13	8	34	1
195	6	4	2	4	2	4	-4	8	9	5
201	0	3	-1	3	3	1	0	4	0	3
202	2	3	1	2	4	1	2	1	0	1
203	3	5	3	1	4	1	2	4	2	2
204	2	2	3	1	-2	2	-2	4	1	2
205	-1	2	-1	0	-3	2	-4	4	1	0
206	8	0	4	1	1	2	2	7	6	1

ii. Raw data for screening at 10 μM SM conc.

Comp	HIV-1-TAR	Stdev	HIV-2-TAR	Stdev	RRE-IIB	Stdev	HIV-1-FSS	Stdev	ESSV	Stdev
Neomycin	43	3	54	3	38	7	38	5	42	2
1	-3	3	-1	6	12	8	-2	9	4	1
19	8	9	1	4	2	5	0	13	8	6
96	6	9	5	5	0	6	-2	7	3	5
97	4	3	5	2	-1	7	0	10	1	8
101	1	6	10	6	7	3	8	3	13	0
118	7	8	15	7	11	6	8	7	3	7
132	3	5	7	5	-3	1	-8	17	9	5
155	10	6	10	3	3	7	10	10	14	7
164	5	5	6	2	2	10	5	7	4	2
169	44	5	32	2	-1	9	2	2	15	5
176	1	8	4	5	0	13	0	2	12	2
177	11	8	-2	8	4	3	2	7	23	7
178	12	3	10	4	9	3	0	5	15	2
179	18	9	16	3	11	3	4	9	21	4
180	33	5	21	4	7	2	0	2	6	6
181	6	1	-1	7	5	3	2	6	8	2
182	44	2	20	5	15	6	4	2	8	6
183	8	4	-1	7	2	5	0	7	6	4
184	13	5	8	11	2	1	3	6	8	9
185	9	3	5	2	7	3	-2	8	13	1
186	12	3	6	4	11	6	5	5	9	5
187	32	2	27	1	10	1	1	6	19	6
188	59	3	40	6	8	3	9	5	12	5
190	20	7	18	5	12	7	11	3	26	3
191	13	3	12	5	12	4	8	9	30	4
192	15	4	15	2	13	8	12	6	50	12
193	41	5	30	8	40	7	29	2	69	3
194	23	3	14	3	19	4	11	4	56	3
195	7	5	11	6	13	5	6	1	55	17
201	1	3	1	1	4	0	3	5	1	1
202	3	2	1	2	5	1	0	2	2	1
203	6	4	6	3	6	2	3	4	5	4
204	4	2	4	1	1	3	1	3	4	1

205	-1	1	0	1	-4	2	-7	2	2	3
206	12	1	8	0	4	1	5	7	10	2

iii. Raw data for screening at 25 μM SM Conc.

Comp	HIV-1-TAR	Stdev	HIV-2-TAR	Stdev	RRE-IIB	Stdev	HIV-1-FSS	Stdev	ESSV	Stdev
Neomycin	44	6	56	4	43	8	43	7	44	2
1	6	3	2	4	11	9	0	3	8	4
19	8	3	11	3	9	3	7	7	8	1
96	4	7	13	3	13	8	5	8	8	3
97	9	10	4	12	11	2	6	1	6	2
101	9	9	17	6	10	7	4	2	24	4
118	10	4	14	9	18	6	3	6	12	2
132	-5	4	-2	7	4	8	2	3	0	5
155	23	4	18	4	16	5	14	8	57	3
164	12	11	18	7	21	7	6	4	14	7
169	65	2	52	5	10	5	4	5	29	5
176	28	13	30	10	31	6	13	9	69	3
177	50	4	39	2	46	3	20	9	75	1
178	29	1	18	5	10	4	4	4	28	6
179	36	2	32	7	18	4	12	6	68	1
180	49	1	35	8	7	2	1	3	17	1
181	3	6	7	4	8	9	7	3	13	2
182	56	3	37	6	7	2	3	4	18	6
183	19	8	11	8	7	6	-3	10	1	9
184	7	2	19	4	6	24	2	8	15	7
185	21	4	18	8	20	7	20	5	67	8
186	4	8	11	5	17	2	7	4	21	8
187	45	3	46	4	25	5	8	3	29	1
188	58	6	45	4	14	10	-9	5	29	2
190	27	1	22	8	21	1	19	7	47	4
191	29	7	23	2	27	4	12	5	56	8
192	49	5	56	5	44	3	19	5	60	5
193	81	5	83	3	80	3	60	6	83	1
194	68	5	62	7	66	6	54	4	85	1
195	63	11	65	9	57	8	49	4	74	1
201	3	2	3	1	6	2	1	1	2	1
202	4	3	4	1	5	0	-3	1	1	3
203	12	4	11	0	13	4	8	1	14	2
204	13	7	8	1	5	2	5	3	10	1
205	26	11	24	20	26	9	-7	2	60	4
206	25	1	17	2	12	1	10	7	24	3

h. Bar chart representations of the screening data at 25 μM SM concentration.

Figure S1: Screening data for amiloride derivatives vs RNA targets at 25 μM concentration.

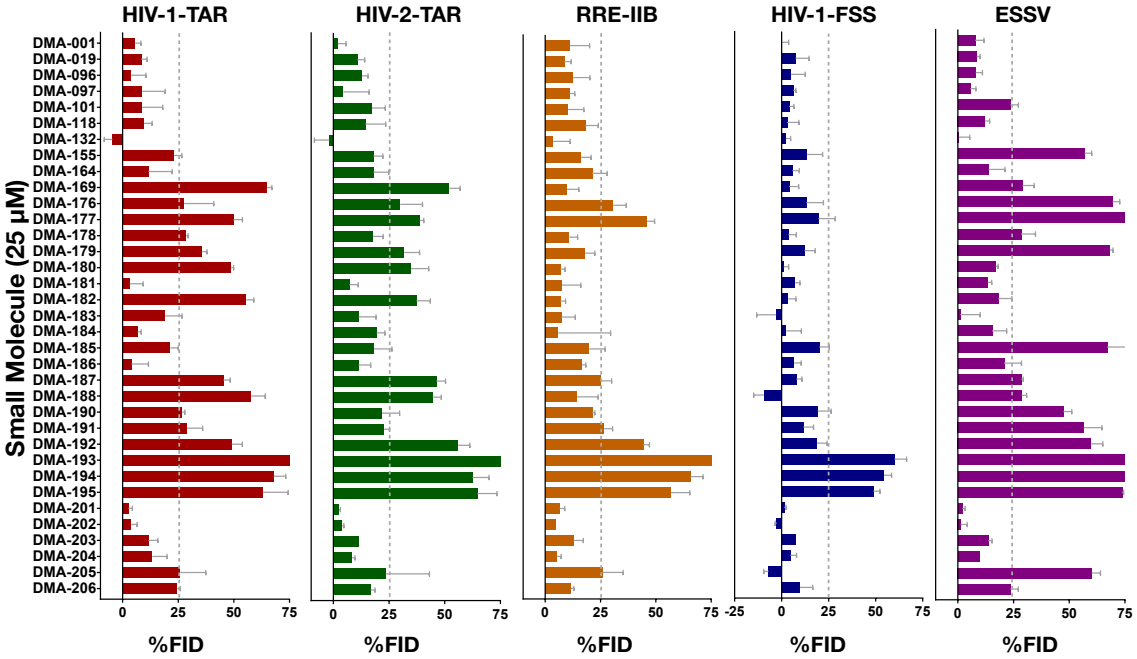
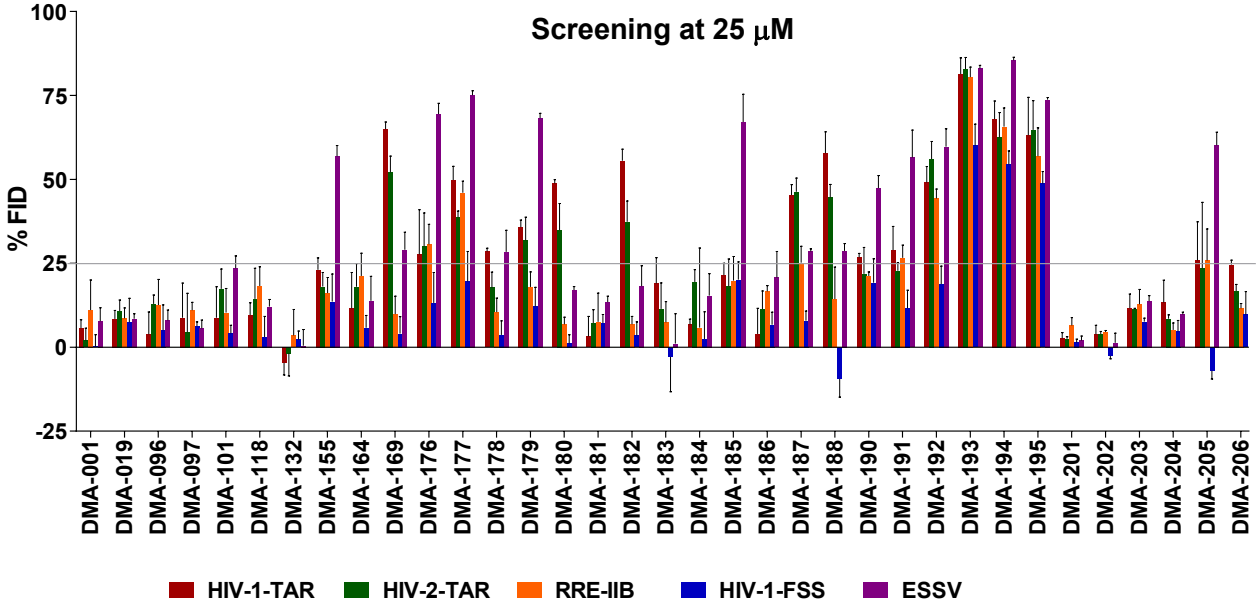


Figure S2: Screening data for amiloride derivatives vs RNA targets at 25 μM concentration.



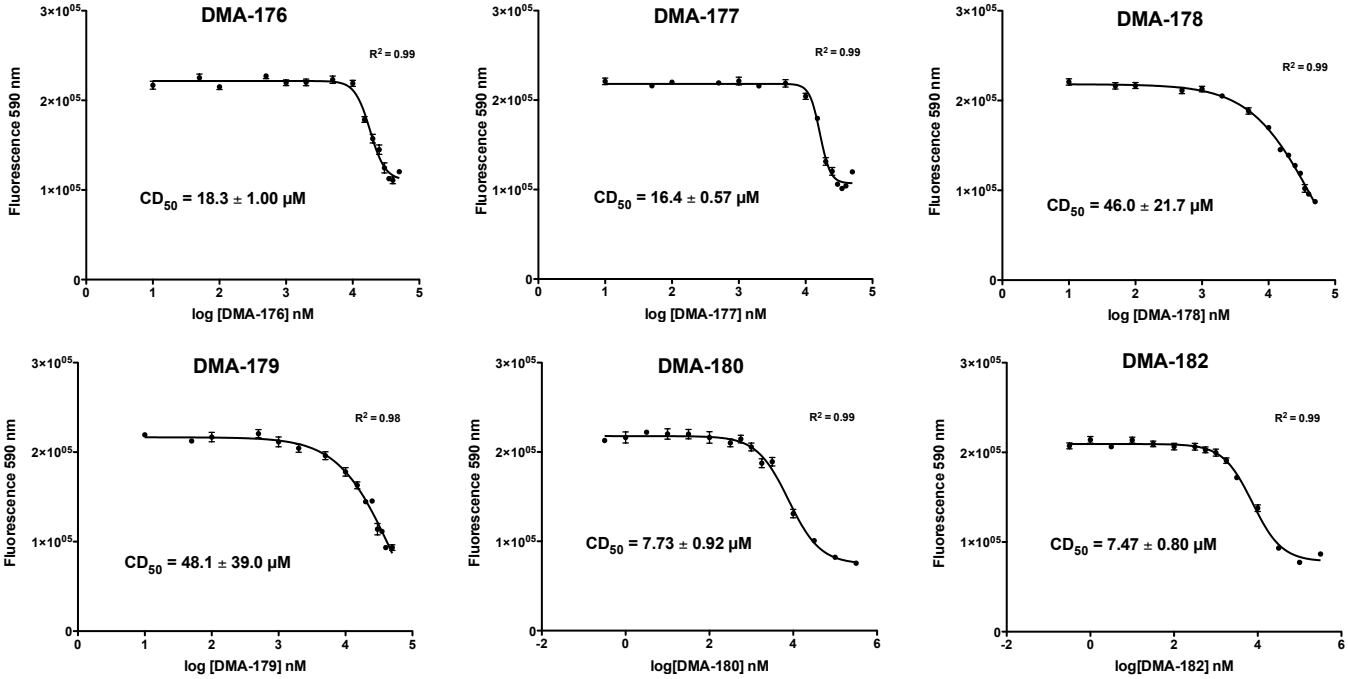
i. ***Tat peptide displacement assay titrations to measure the competitive displacement at 50% peptide from each RNA with small molecule hits:*** Displacement assay titrations to measure the activity of small molecule against each RNA target was measured as previously reported.[2] Briefly, small molecules were diluted in the assay buffer to achieve final assay concentrations between 0-334 μM. Tat peptide (6 μL), RNA (6 μL), and small molecule solution (6 μL) were combined in a 384 well-plate, shaken on a platform stirrer for 5 min, centrifuged at 4000 rpm for 1 min, and allowed to incubate for 30 min before being read using the excitation and emission wavelengths of 485 nm (FAM) and 590 nm (TAMRA) respectively. Observed fluorescence at each small molecule concentration was normalized to the fluorescence of Tat peptide: RNA complex. Competitive displacement of 50% of peptide from RNA was calculated by fitting the normalized data to the equations S2 and S3 below using the GraphPad Prism curve fitting software. Assays were conducted in triplicate and each replicate contained three technical replicates. Errors presented are the standard errors of mean.

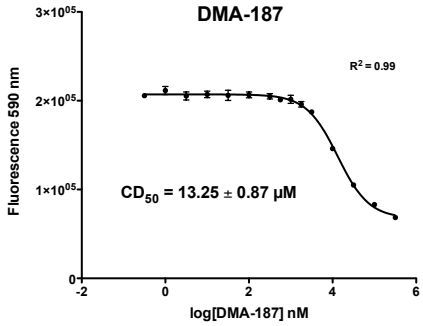
$$y = A1 + \frac{A2-A1}{1+10^{(Logx0-x)p}} \text{…….. Equation S2}$$

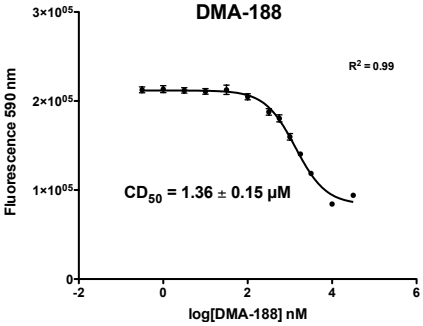
$$CD_{50} = 10^{Logx0} \text{…….. Equation S3}$$

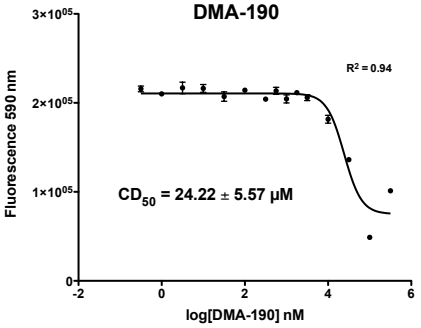
Displacement assay curves for small molecules against each RNA Target.

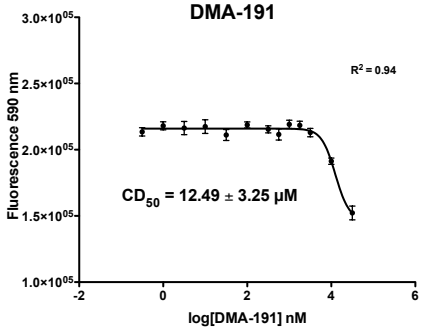
HIV-1-TAR

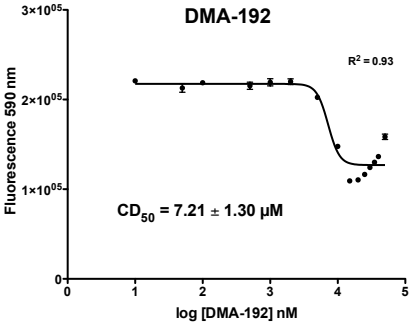


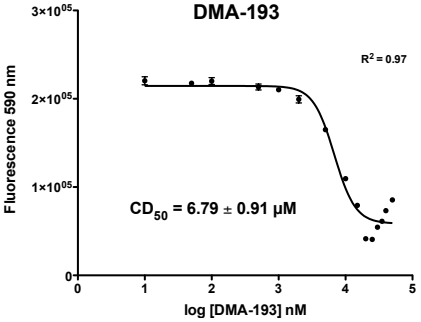

DMA-187
R² = 0.99
CD50 = 13.25 ± 0.87 μM
Fluorescence 590 nm
log[DMA-187] nM

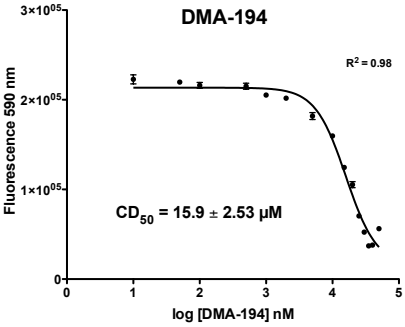

DMA-188
R² = 0.99
CD50 = 1.36 ± 0.15 μM
Fluorescence 590 nm
log[DMA-188] nM

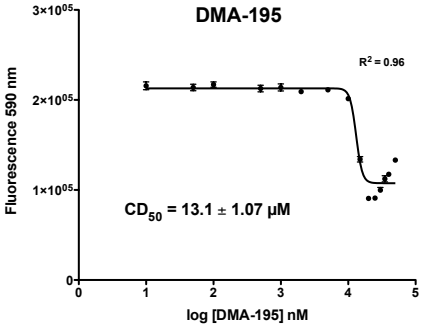

DMA-190
R² = 0.94
CD50 = 24.22 ± 5.57 μM
Fluorescence 590 nm
log[DMA-190] nM

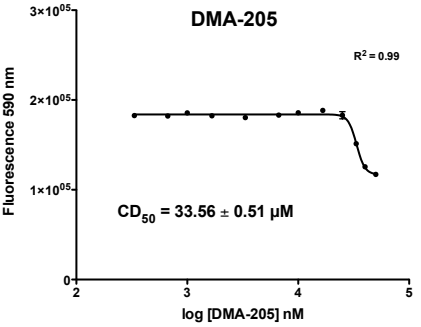

DMA-191
R² = 0.94
CD50 = 12.49 ± 3.25 μM
Fluorescence 590 nm
log[DMA-191] nM


DMA-192
R² = 0.93
CD50 = 7.21 ± 1.30 μM
Fluorescence 590 nm
log [DMA-192] nM

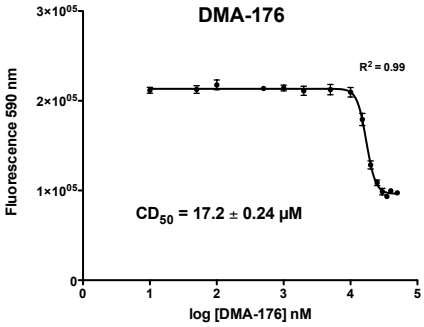

DMA-193
R² = 0.97
CD50 = 6.79 ± 0.91 μM
Fluorescence 590 nm
log [DMA-193] nM

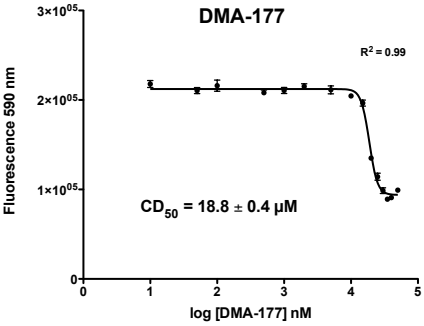

DMA-194
R² = 0.98
CD50 = 15.9 ± 2.53 μM
Fluorescence 590 nm
log [DMA-194] nM

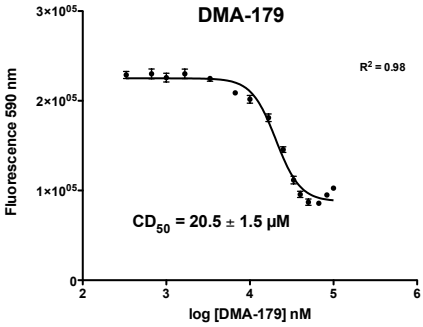

DMA-195
R² = 0.96
CD50 = 13.1 ± 1.07 μM
Fluorescence 590 nm
log [DMA-195] nM

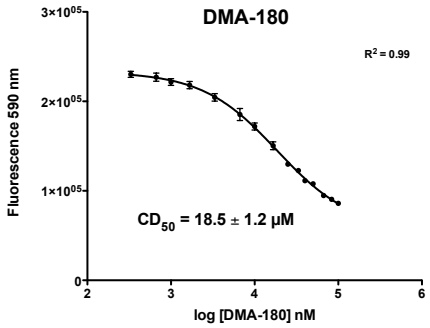

DMA-205
R² = 0.99
CD50 = 33.56 ± 0.51 μM
Fluorescence 590 nm
log [DMA-205] nM

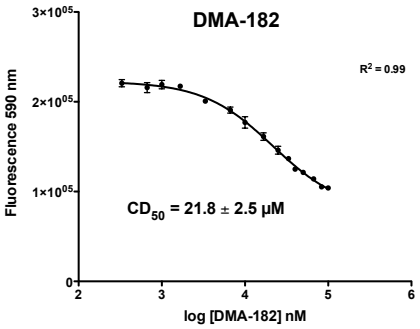
HIV-2-TAR

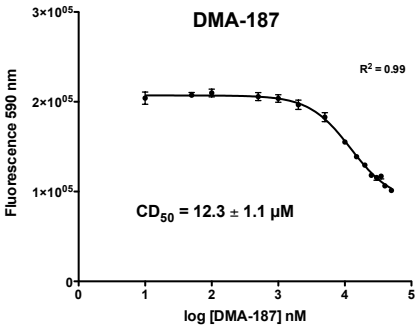

DMA-176
R² = 0.99
CD50 = 17.2 ± 0.24 μM
Fluorescence 590 nm
log [DMA-176] nM

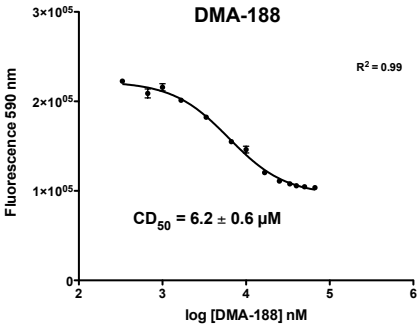

DMA-177
R² = 0.99
CD50 = 18.8 ± 0.4 μM
Fluorescence 590 nm
log [DMA-177] nM

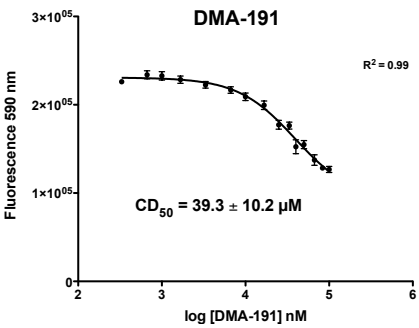

DMA-179
R² = 0.98
CD50 = 20.5 ± 1.5 μM
Fluorescence 590 nm
log [DMA-179] nM

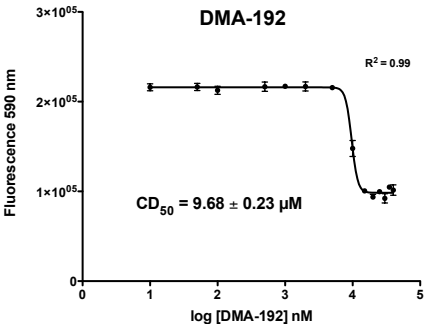

DMA-180
R² = 0.99
CD50 = 18.5 ± 1.2 μM
Fluorescence 590 nm
log [DMA-180] nM

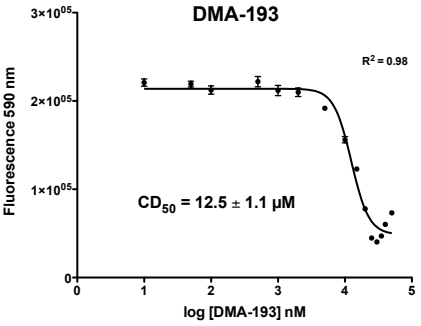

DMA-182
R² = 0.99
CD50 = 21.8 ± 2.5 μM
Fluorescence 590 nm
log [DMA-182] nM

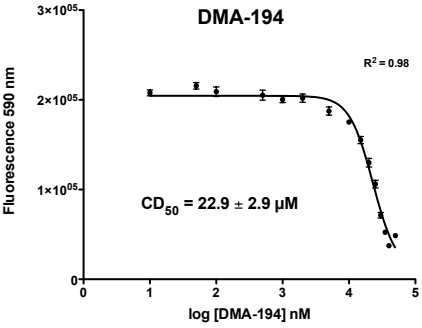

DMA-187
R² = 0.99
CD50 = 12.3 ± 1.1 μM
Fluorescence 590 nm
log [DMA-187] nM

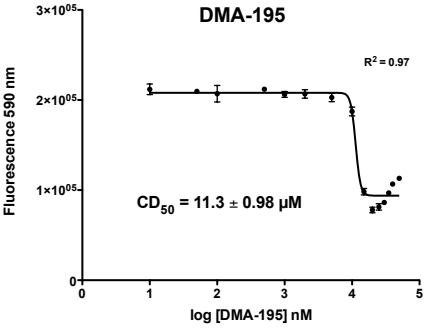

DMA-188
R² = 0.99
CD50 = 6.2 ± 0.6 μM
Fluorescence 590 nm
log [DMA-188] nM

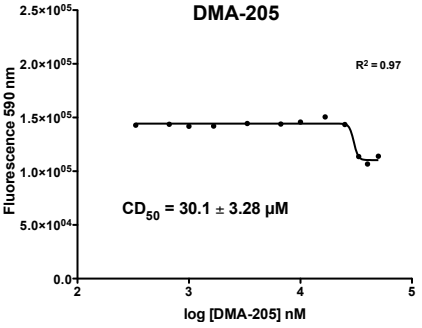

DMA-191
R² = 0.99
CD50 = 39.3 ± 10.2 μM
Fluorescence 590 nm
log [DMA-191] nM


DMA-192
R² = 0.99
CD50 = 9.68 ± 0.23 μM
Fluorescence 590 nm
log [DMA-192] nM

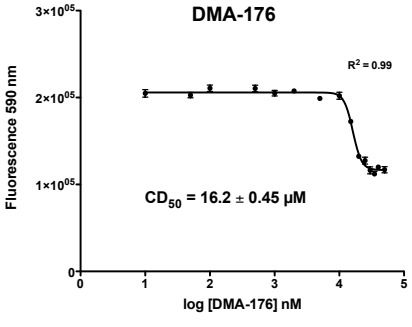

DMA-193
R² = 0.98
CD50 = 12.5 ± 1.1 μM
Fluorescence 590 nm
log [DMA-193] nM

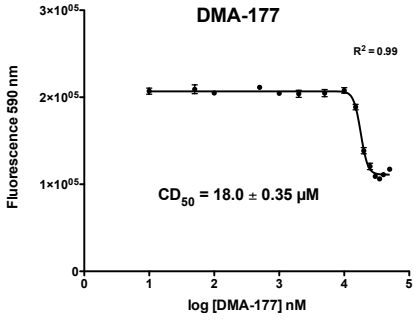

DMA-194
R² = 0.98
CD50 = 22.9 ± 2.9 μM
Fluorescence 590 nm
log [DMA-194] nM

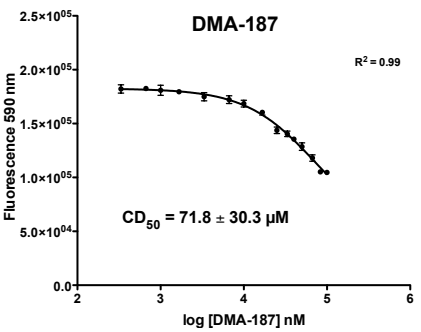

DMA-195
R² = 0.97
CD50 = 11.3 ± 0.98 μM
Fluorescence 590 nm
log [DMA-195] nM

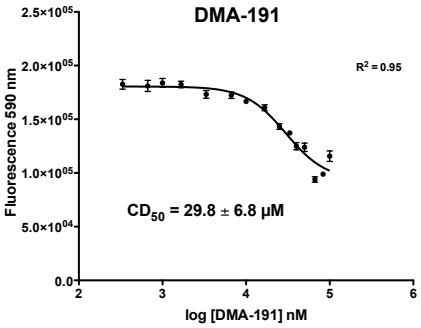

DMA-205
R² = 0.97
CD50 = 30.1 ± 3.28 μM
Fluorescence 590 nm
log [DMA-205] nM

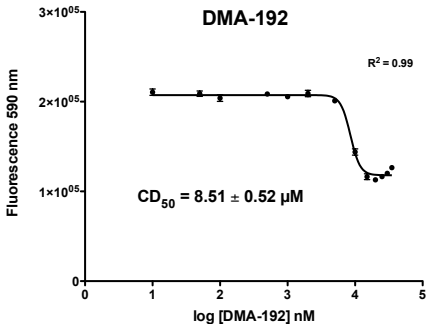
RRE-IIB

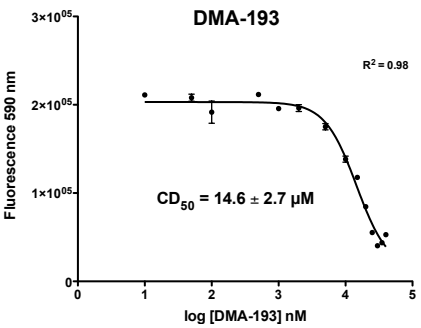

DMA-176
R² = 0.99
CD50 = 16.2 ± 0.45 μM
Fluorescence 590 nm
log [DMA-176] nM

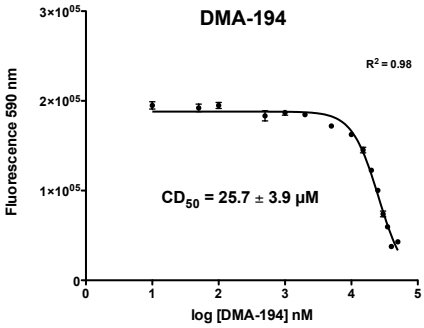

DMA-177
R² = 0.99
CD50 = 18.0 ± 0.35 μM
Fluorescence 590 nm
log [DMA-177] nM

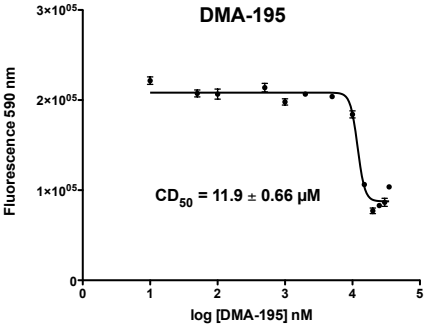

DMA-187
R² = 0.99
CD50 = 71.8 ± 30.3 μM
Fluorescence 590 nm
log [DMA-187] nM

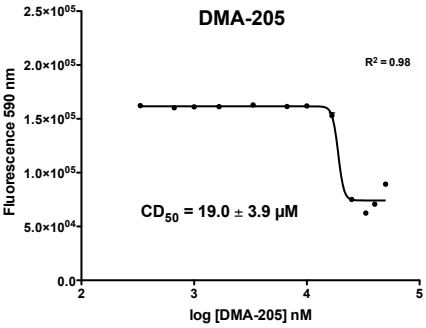

DMA-191
R² = 0.95
CD50 = 29.8 ± 6.8 µM
Fluorescence 590 nm
log [DMA-191] nM


DMA-192
R² = 0.99
CD50 = 8.51 ± 0.52 µM
Fluorescence 590 nm
log [DMA-192] nM

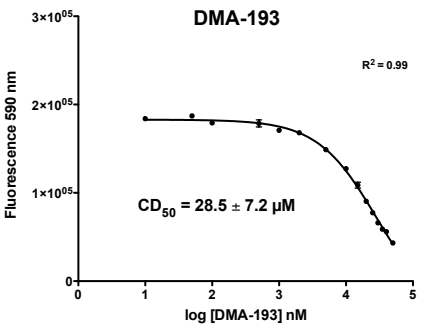

DMA-193
R² = 0.98
CD50 = 14.6 ± 2.7 µM
Fluorescence 590 nm
log [DMA-193] nM

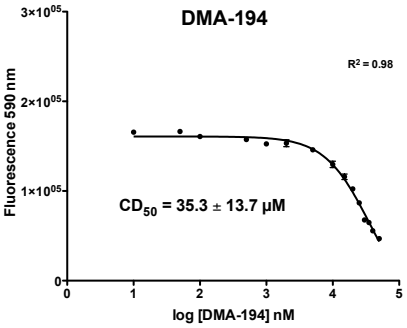

DMA-194
R² = 0.98
CD50 = 25.7 ± 3.9 µM
Fluorescence 590 nm
log [DMA-194] nM

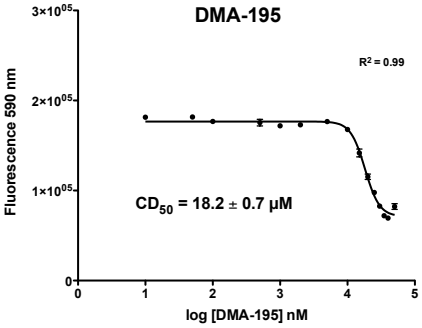

DMA-195
CD50 = 11.9 ± 0.66 µM
Fluorescence 590 nm
log [DMA-195] nM


DMA-205
R² = 0.98
CD50 = 19.0 ± 3.9 µM
Fluorescence 590 nm
log [DMA-205] nM

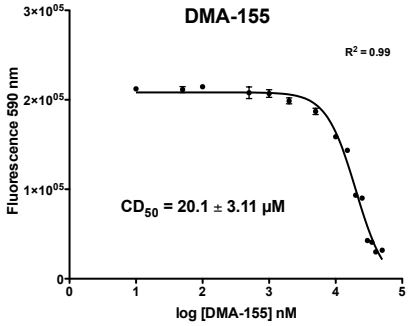
HIV-1-FSS

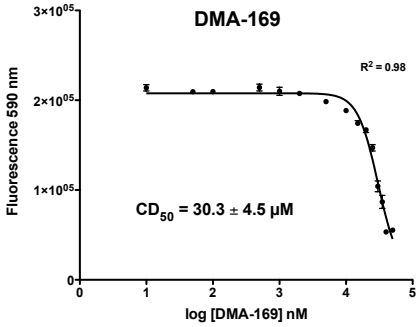

DMA-193
R² = 0.99
CD50 = 28.5 ± 7.2 µM
Fluorescence 590 nm
log [DMA-193] nM

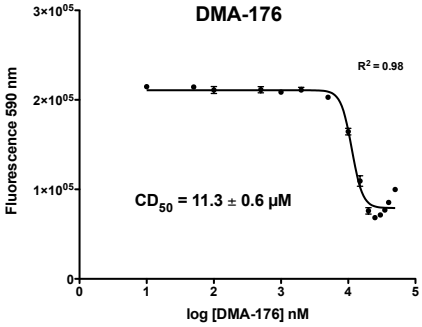

DMA-194
R² = 0.98
CD50 = 35.3 ± 13.7 µM
Fluorescence 590 nm
log [DMA-194] nM

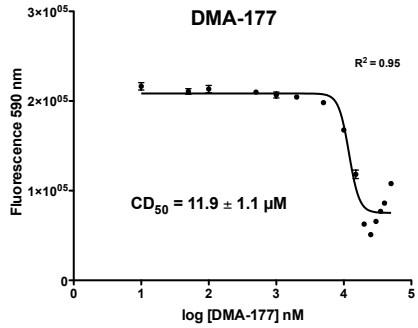

DMA-195
R² = 0.99
CD50 = 18.2 ± 0.7 µM
Fluorescence 590 nm
log [DMA-195] nM

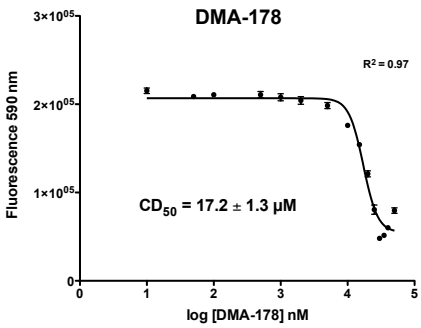
HIV-1-ESSV

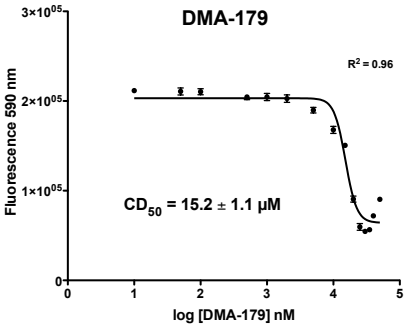

DMA-155
R² = 0.99
CD50 = 20.1 ± 3.11 µM
Fluorescence 590 nm
log [DMA-155] nM

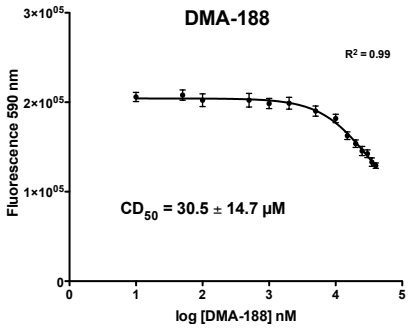

DMA-169
R² = 0.98
CD50 = 30.3 ± 4.5 µM
Fluorescence 590 nm
log [DMA-169] nM

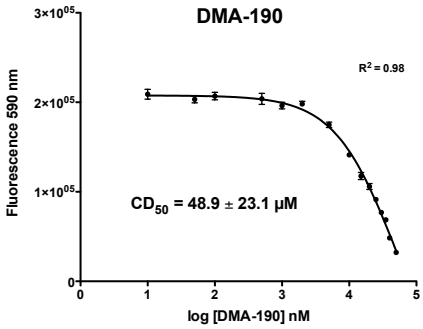

DMA-176
R² = 0.98
CD50 = 11.3 ± 0.6 µM
Fluorescence 590 nm
log [DMA-176] nM

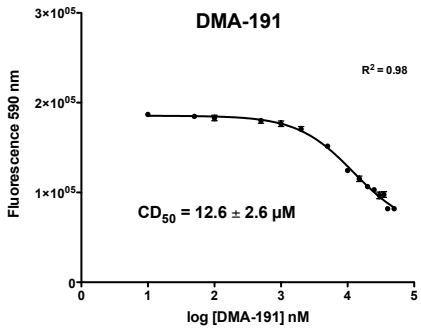

DMA-177
R² = 0.95
CD50 = 11.9 ± 1.1 µM
Fluorescence 590 nm
log [DMA-177] nM

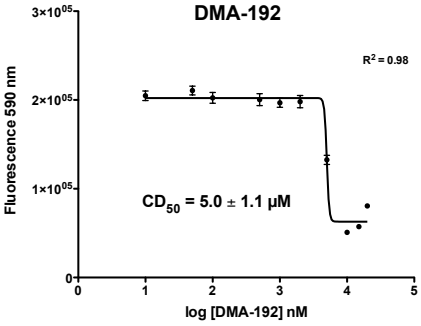

DMA-178
R² = 0.97
CD50 = 17.2 ± 1.3 µM
Fluorescence 590 nm
log [DMA-178] nM

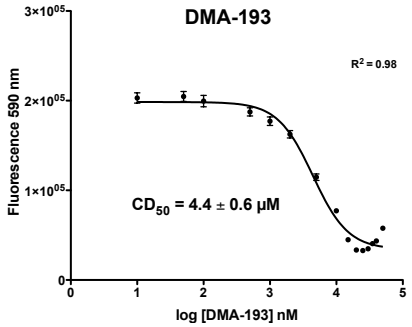

DMA-179
R² = 0.96
CD50 = 15.2 ± 1.1 µM
Fluorescence 590 nm
log [DMA-179] nM

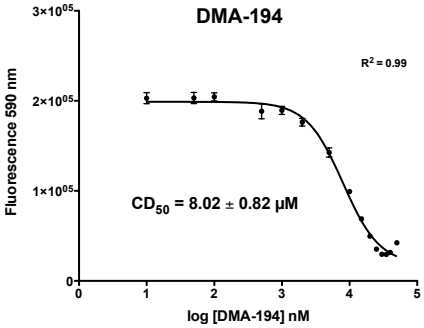

DMA-188
R² = 0.99
CD50 = 30.5 ± 14.7 µM
Fluorescence 590 nm
log [DMA-188] nM

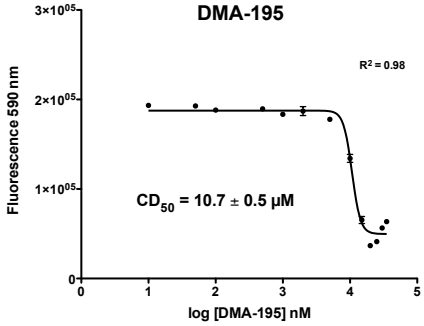

DMA-190
R² = 0.98
CD50 = 48.9 ± 23.1 µM
Fluorescence 590 nm
log [DMA-190] nM

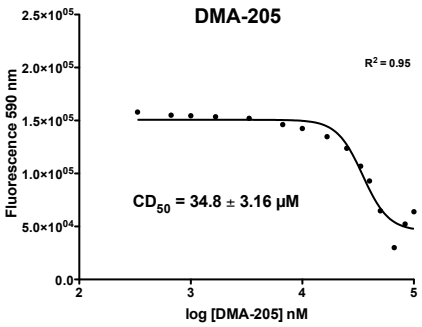

DMA-191
R² = 0.98
CD50 = 12.6 ± 2.6 µM
Fluorescence 590 nm
log [DMA-191] nM


DMA-192
R² = 0.98
CD50 = 5.0 ± 1.1 µM
Fluorescence 590 nm
log [DMA-192] nM


DMA-193
R² = 0.98
CD50 = 4.4 ± 0.6 µM
Fluorescence 590 nm
log [DMA-193] nM


DMA-194
R² = 0.99
CD50 = 8.02 ± 0.82 µM
Fluorescence 590 nm
log [DMA-194] nM


DMA-195
R² = 0.98
CD50 = 10.7 ± 0.5 µM
Fluorescence 590 nm
log [DMA-195] nM


DMA-205
R² = 0.95
CD50 = 34.8 ± 3.16 µM
Fluorescence 590 nm
log [DMA-205] nM

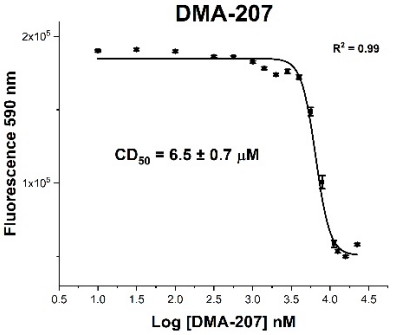

DMA-207
R² = 0.99
CD50 = 6.5 ± 0.7 µM
Fluorescence 590 nm
Log [DMA-207] nM

Table S3: Relative selectivities of DMA-169 derivatives (CD_{50}(ESSV or HIV-2-TAR) / CD_{50}(HIV-1-TAR)).[2]

	HIV-1-TAR CD_{50} (μM)	HIV-2-TAR CD_{50} (μM)	ESSV CD_{50} (μM)	Selectivity for HIV-1-TAR vs. ESSV	Selectivity for HIV-1-TAR vs HIV-2-TAR
DMA-169	3.9 ± 0.7	9.3 ± 1.3	30.3 ± 4.5	7.8	2.4
DMA-188	1.36 ± 0.15	6.2 ± 0.6	30.5 ± 14.7	22.4	4.6

Section B: Cheminformatics analysis.

Agglomerative Hierarchical Clustering (AHC) Analysis:

AHC plots of the screening data at all three concentrations of 5 μM, 10 μM, and 25 μM concentrations were constructed using the Agglomerative Clustering Analysis plugin from XLSTAT.

Data clustering by class:

Class	1	2	3
Objects	**19**	**15**	**1**
Sum of weights	**19**	**15**	**1**
Within-class variance	**538.327**	**2710.169**	**0.000**
Minimum distance to centroid	**6.417**	**23.461**	**0.000**
Average distance to centroid	**20.118**	**47.509**	**0.000**
Maximum distance to centroid	**56.587**	**83.207**	**0.000**
Molecules in each class	DMA-001	DMA-155	DMA-193
	DMA-019	DMA-169	
	DMA-096	DMA-176	
	DMA-097	DMA-177	
	DMA-101	DMA-179	
	DMA-118	DMA-180	
	DMA-132	DMA-182	
	DMA-164	DMA-185	
	DMA-178	DMA-187	
	DMA-181	DMA-188	
	DMA-183	DMA-190	
	DMA-184	DMA-191	
	DMA-186	DMA-192	
	DMA-201	DMA-194	
	DMA-202	DMA-195	
	DMA-203		
	DMA-204		
	DMA-205		
	DMA-206		

Data clustering by molecules:

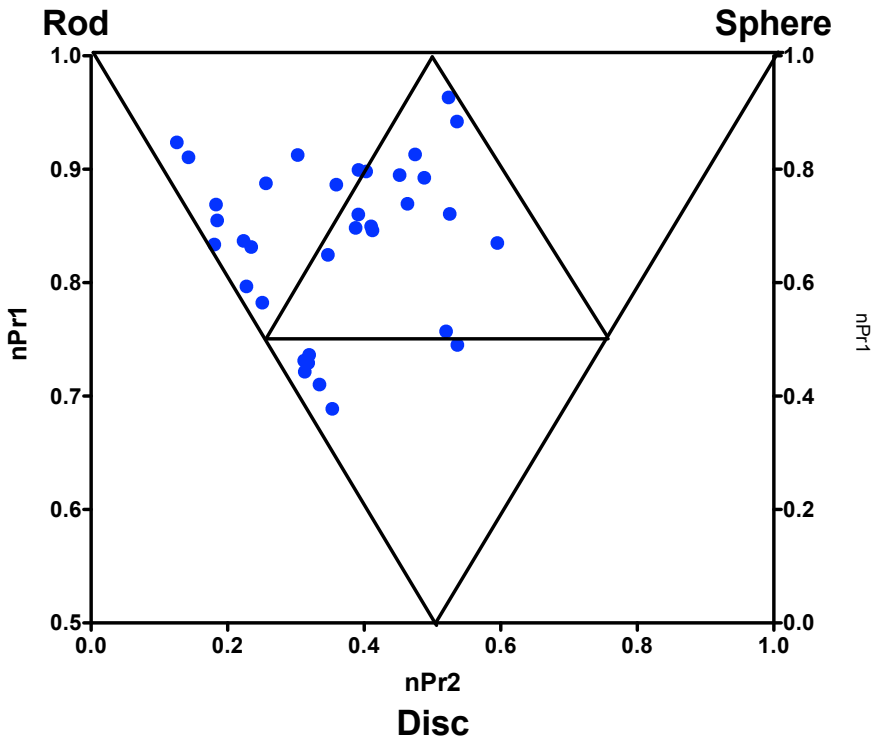
Molecule	Class
DMA-001	1
DMA-019	1
DMA-096	1
DMA-097	1
DMA-101	1
DMA-118	1
DMA-132	1
DMA-155	2
DMA-164	1
DMA-169	2
DMA-176	2
DMA-177	2
DMA-178	1
DMA-179	2
DMA-180	2
DMA-181	1
DMA-182	2

DMA-183	1
DMA-184	1
DMA-185	2
DMA-186	1
DMA-187	2
DMA-188	2
DMA-190	2
DMA-191	2
DMA-192	2
DMA-193	3
DMA-194	2
DMA-195	2
DMA-201	1
DMA-202	1
DMA-203	1
DMA-204	1
DMA-205	1
DMA-206	1

Principal Moments of Inertia Calculations

Principal Moments of Inertia calculations were performed using the Molecular Operating Environment (MOE, v2018.01) software package. Details can be found in the following reference as well as information on Boltzmann averaging, cell-based partitioning, and triangular and cumulative distribution plots.[3, 4]

Figure S3: Triangular Plot for calculated Principal Moments of Inertia of the 35 amiloride derivatives included in this study.



Preliminary Cheminformatics and LDA Analysis

Twenty cheminformatics parameters were calculated as previously reported.[4] SMILES strings for amilorides were batch processed. Using the ChemAxon Calculator Plugins, all structures were corrected to their major protonation and tautomeric states (pH = 7.4), and then the cheminformatic descriptors were evaluated using the ChemAxon Chemical Terms Evaluator (Marvin 16.4.11.0, 2016, http://www.chemaxon.com). Excel files with all calculated parameters available upon request.

Table S4: Cheminformatic descriptors

Category	Type	Parameter	Description	Chemical Terms Evaluator Expression
Established Medicinal Chemistry Descriptors	Lipinski's Rules	MW	Molecular Weight	mass()
		HBA	Number of Hydrogen Bond Acceptors	acceptorCount()
		HBD	Number of Hydrogen Bond Donors	donorCount()
		LogP	n-Octanol/Water Partition Coefficient	logPKLOP()
	Veber's Rules	RotB	Number of Rotatable Bonds	rotatableBondCount()
		tPSA	Topological Polar Surface Area	PSA()
	Oral Availability	LogD	n-Octanol/Water Distribution Coefficient	logD('7.4')
Additional Descriptors	Structural	N	Number of Nitrogen Atoms	atomCount('7')
		O	Number of Oxygen Atoms	atomCount('8')
		Rings	Number of Rings	ringCount()
		ArRings	Number of Aromatic Rings	aromaticRingCount()
		HetRings	Number of Heteroatom-containing Rings	heteroRingCount()
		SysRings	Number of Ring Systems	ringSystemCount()
		SysRR	Ring Complexity	ringCount()/ringSystemCount()
	Molecular Complexity	Fsp^3	Fraction of sp^3 Hybridized Carbons	count(filter('atno()==6&&connections()==4'))/atom Count('6')
		nStereo	Number of Stereocenters	chiralCenterCount()
	Molecular Recognition	ASA	Accessible Surface Area	ASA()
		relPSA	Relative Polar Surface Area	PSA()/vanDerWaalsSurfaceArea()
		TC	Total Charge	totalCharge()
		VWSA	Van der Waals Surface Area	vanDerWaalsSurfaceArea()

Before using these parameters for LDA analysis, we removed total charge (TC) and number of stereocenters (nStereo) as they were constant for all amilorides (n=1 and n=0, respectively).

LDA plots of cheminformatic data were constructed using the Discriminatory Analysis plugin from XLSTAT as previous described.[5]

Table S5. Means by Class

Class \ Variable	MW	HBA	HBD	LogPKLOP	RotB	tPSA	logD	N	O
ESSV	428.50	7.43	4.43	2.02	5.57	161.21	2.84	8.14	1.00
None	317.99	7.00	3.50	0.11	3.00	143.14	0.78	7.13	1.38
Promisc	545.38	10.75	5.75	1.31	9.00	208.56	2.15	12.25	1.00
TAR	402.47	7.80	4.60	0.79	6.00	163.83	1.51	8.60	1.00
Weak	378.16	7.73	4.09	0.45	4.64	157.05	1.15	8.36	1.09

Class \ Variable	Rings	ArRings	HetRings	SysRings	SysRR*	Fsp3	ASA	relPSA	VWSA
ESSV	4.14	4.00	2.14	3.29	1.27	0.11	602.09	0.28	585.93
None	2.25	1.50	1.75	2.25	1.00	0.31	482.99	0.34	426.44
Promisc	5.50	5.00	4.25	4.25	1.30	0.20	727.85	0.28	744.81
TAR	3.60	3.40	2.00	3.00	1.20	0.16	592.38	0.30	555.23
Weak	3.09	2.45	2.27	2.82	1.12	0.24	557.91	0.31	507.17

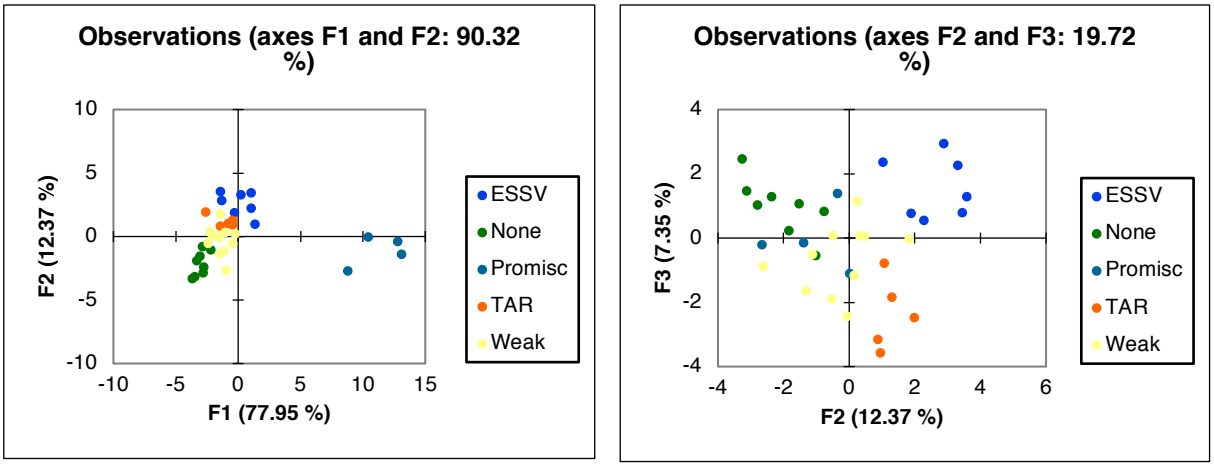


Figure S4. LDA analysis of ESSV-binding, TAR-binding, promiscuous, weak and non-binders for the first three factors. **3D plot in main text.**

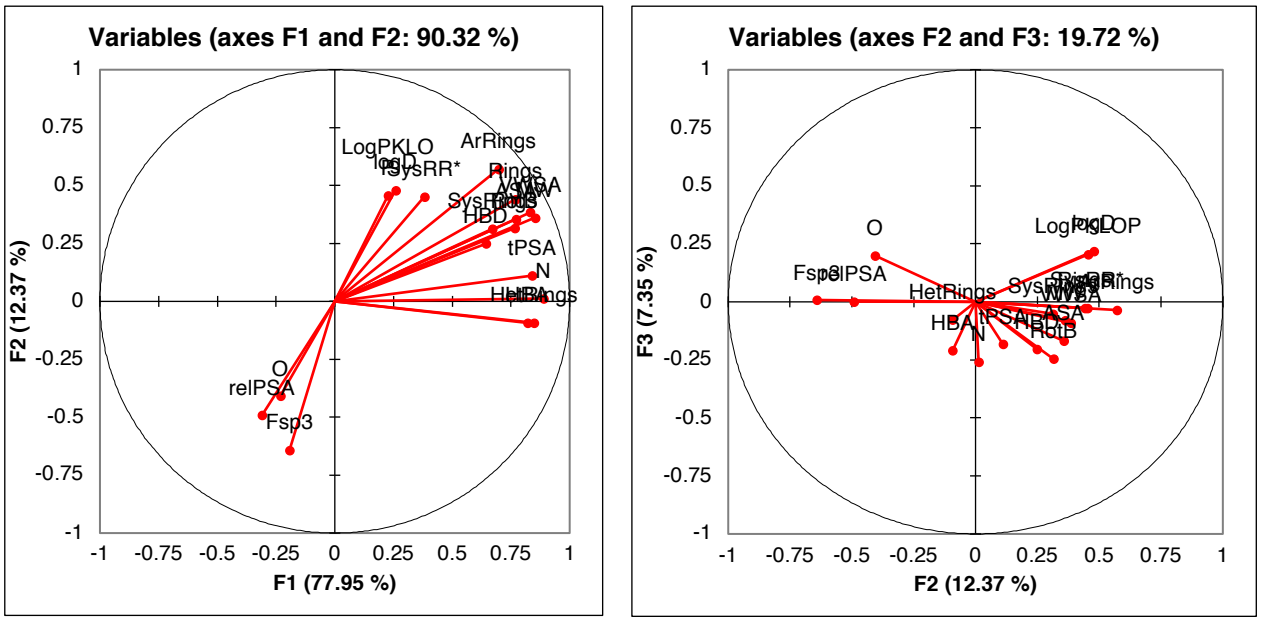


Figure S5. Loading plots from LDA analysis for first three factors.

Table S6. Confusion matrix for training sample.

from \ to	ESSV	None	Promisc	TAR	Weak	Total	% correct
ESSV	7	0	0	0	0	7	100.00%
None	0	8	0	0	0	8	100.00%
Promisc	0	0	4	0	0	4	100.00%
TAR	0	0	0	5	0	5	100.00%
Weak	0	0	0	0	11	11	100.00%
Total	7	8	4	5	11	35	100.00%

Table S7. Confusion matrix for cross-validation

from \ to	ESSV	None	Promisc	TAR	Weak	Total	% correct
ESSV	2	1	1	1	2	7	28.57%
None	0	5	0	0	3	8	62.50%
Promisc	0	0	4	0	0	4	100.00%
TAR	0	0	0	1	4	5	20.00%
Weak	4	3	0	3	1	11	9.09%
Total	6	9	5	5	10	35	37.14%

Table. S8. Variables/Factors correlations

	F1	F2	F3	F4
MW	0.85	0.36	-0.08	0.05
HBA	0.82	-0.09	-0.21	0.08
HBD	0.64	0.25	-0.20	-0.05
LogPKLOP	0.23	0.46	0.20	-0.05
RotB	0.76	0.32	-0.25	-0.06
tPSA	0.84	0.11	-0.18	0.01
logD	0.26	0.48	0.22	-0.05
N	0.89	0.01	-0.26	0.11
O	-0.23	-0.41	0.20	-0.16
Rings	0.77	0.44	-0.03	-0.01
ArRings	0.70	0.57	-0.04	-0.10
HetRings	0.85	-0.09	-0.08	0.22
SysRings	0.67	0.31	-0.05	0.07
SysRR*	0.38	0.45	-0.03	0.01
Fsp3	-0.19	-0.64	0.01	0.12
ASA	0.77	0.36	-0.17	0.06
relPSA	-0.31	-0.49	0.00	0.00
VWSA	0.83	0.38	-0.09	-0.01

Quantitative structure-activity relationship study

1. Descriptor calculation

Before calculation, all the ligands were tuned to the correct protonation and tautomerization states using Molecular Operating Environment (MOE, Chemical Computing Group, 2018.01). Each protonation and tautomerization state were sent to conformational search individually to account for the flexibility of the ligand. Low energy conformations of each molecule were calculated using the Conformation Search algorithm in MOE. The Conformation Search function was performed using the stochastic method with the MMFF94 force field and generalized Born solvation model. The input for each parameter is listed in SI Table S8, and the following options were checked: hydrogens.

Table S8: Parameters used for conformation search

Parameter	**Input**
Rejection limit	100
Iteration limit	10000
RMS gradient	0.005
MM iteration limit	500
RMSD limit	0.15
Energy window	3
Conformation limit	10000

The 3 kcal/mol energy window was selected to survey biologically relevant conformation space and to obtain a representative population of conformers at equilibrium (> 99%) as described by Equation (S4).

$$\frac{N_1}{N_0} = e^{-\Delta E/RT} \qquad \text{Equation S4}$$

where N_1/N_0 is the ratio of the number of molecules in the relative energy states, ΔE is the energy difference between N_0 and N_1 (3 kcal/mol), R is the ideal gas constant (0.00198588 kcal/K mol), and T is the temperature (298 K). After the conformation search was complete, the 435 descriptors, ranging from electrostatic properties to topological terms, were calculated for each conformation and averaged using Boltzmann weighted equation (Equation S5).

$$\mathrm{A} = \frac{\sum_i A_i e^{-\frac{E_i}{k_B T}}}{\sum_i e^{-\frac{E_i}{k_B T}}} \qquad \text{Equation S5}$$

The final descriptor set of each molecule was obtained by further averaged based on the distribution of the protonation and tautomerization states.

2. Multivariate linear regression

The multivariate linear regression was performed on Matlab (R2019a, MathWorks). The generalized model has the structure as follow, containing a constant term, first-ordered descriptor term (D_i) and its coefficients (β_i):

$$\ln CD_{50} = Const. + \sum_i \beta_i D_i$$

The model predicts the lnCD_{50} values and will be plotted with measured lnCD_{50} values to evaluate the performance of the model. The linear regression of the measured lnCD_{50} values vs. predicted lnCD_{50} values will have the slope the same as the R^2 from model fitting, the intercept will be the variance of the experimental results that the model could not account for.[6]

The descriptor values were normalized before modeling, in case the exact numbers have huge discrepancies in numeric scale and affect the modeling. The normalization was performed by first subtracting the mean value (μ) and then divided by the standard deviation (σ):

$$D_{norm} = \frac{D - \mu_D}{\sigma_D}$$

Preliminary model (two-parameter linear model) searching was performed using the method of exhaustion, namely all the combinations of descriptors ($434 \times 435 \div 2 = 94395$) were set for fitting and the basic fitting statistics (R^2) were extracted for each model, then ranked the models based on R^2.

For HIV-1 TAR, the two-parameter linear models have moderate R^2 values, only 3 models have $R^2 > 0.6$. The best model is summarized in the main text, and the fitting statistics is shown here:

```
>> mdl=fitlm([x(:,179), x(:,331)], y,'linear')

mdl =


Linear regression model:
    y ~ 1 + x1 + x2

Estimated Coefficients:
                   Estimate      SE        tStat       pValue
                   ________    _______    _______    __________

    (Intercept)      2.5109    0.13296     18.885    7.8219e-11
    x1               1.1884    0.21139      5.622    8.3121e-05
    x2              -0.95462   0.21139     -4.5159   0.00058015


Number of observations: 16, Error degrees of freedom: 13
Root Mean Squared Error: 0.532
R-squared: 0.709,  Adjusted R-Squared: 0.665
F-statistic vs. constant model: 15.9, p-value = 0.000324
```

Figure S6. Fitting statistics of the best HIV-1 TAR QSAR model

For HIV-2 TAR, similarly, only 3 models have $R^2 > 0.6$. The best model contains two descriptors: *SlogP_VSA8* (#326, *sum of van der Waals surface area of atoms whose contribution to logP(o/w) is in (0.30,0.40]*) and *vsurf_EDmin1* (#380, *lowest hydrophobic energy*). The fitting statistics as well as the model description/linear fitting/LOOCV are shown below:

```
>> mdl=fitlm([x(:,326), x(:,380)], y,'linear')

mdl =


Linear regression model:
    y ~ 1 + x1 + x2

Estimated Coefficients:
                   Estimate      SE       tStat       pValue
                   ________    ________    ______    __________

    (Intercept)     2.7708      0.08673    31.947    3.3628e-12
    x1             0.35971      0.098366   3.6569    0.0037752
    x2             0.37298      0.098366   3.7918    0.0029854


Number of observations: 14, Error degrees of freedom: 11
Root Mean Squared Error: 0.325
R-squared: 0.643,  Adjusted R-Squared: 0.578
F-statistic vs. constant model: 9.89, p-value = 0.00348
```

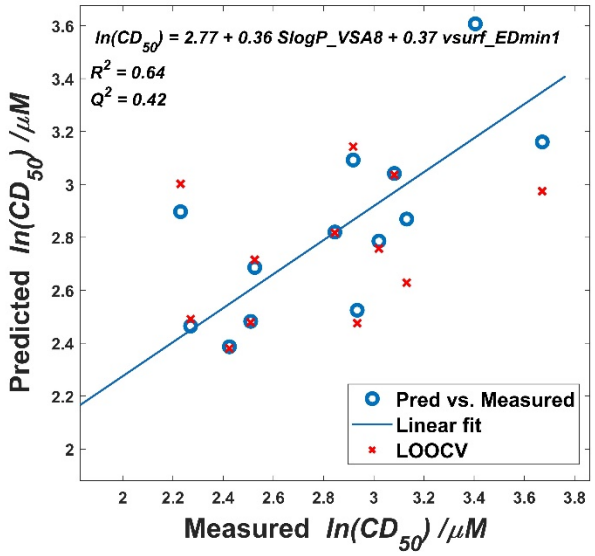


Figure S7. Fitting statistics and model description of the best HIV-2 TAR QSAR model

```
Linear regression model:
    y ~ 1 + x1 + x2

Estimated Coefficients:
                   Estimate      SE       tStat       pValue
                   ________    ________    ______    __________

    (Intercept)     2.7375      0.067441   40.592    3.2329e-14
    x1             -0.6833      0.07223    -9.46    6.5023e-07
    x2              0.31383     0.07223    4.3448    0.00095355


Number of observations: 15, Error degrees of freedom: 12
Root Mean Squared Error: 0.261
R-squared: 0.886,  Adjusted R-Squared: 0.867
F-statistic vs. constant model: 46.7, p-value = 2.18e-06
```

Figure S8. Fitting statistics and model description of the best ESSV QSAR model

For ESSV, the models have much higher R^2 values, with 93 models have R^2 >0.8 and 1170 models have R^2 >0.7. Therefore, the number of best models is sufficient to perform statistical occurrence analysis shown in later.

3. **Occurrence histogram**

The number of occurrence of descriptors was counted and summarized into the histogram using Matlab. The x-axis stands for the ID of the descriptor, and the y-axis is the number of occurrences. For some descriptors, the occurrence time were zero since these descriptors have zero values for all DMAs, such as “number of Si atoms”, “number of B atoms” etc. These descriptors were not considered in the results of modeling.

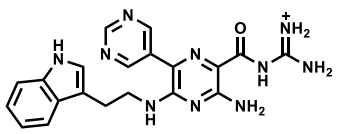
4. **Leave-one-out cross validation (LOOCV) and prediction**

LOOCV of the hit models were performed using Matlab. For each LOOCV, the whole dataset was first divided into training set and test set. Specifically, for HIV-1 TAR, the dataset containing 16 data points was divided into 16 indices, each index has one data point. The LOOCV took one index out each time as the test set, using the remaining 15 indices as training set to obtain the model and predict the test set data point. The modeling process utilized the two descriptors obtained from previous study, but the coefficients changed every time. Overall, for HIV-1 TAR, 16 times LOOCV were performed independently for HIV-1 TAR. The predicted results were scattered with measured results to get the LOOCV efficiency Q^2. Similarly, for HIV-2 TAR and ESSV, the above LOOCV was performed but 14/15 indices were generated instead.

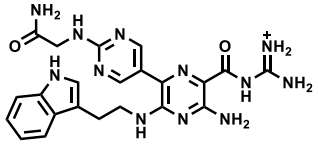
The prediction of CD_{50} value of DMA-205 vs. ESSV was conducted based on the ESSV QSAR model. Firstly, the descriptor values of DMA-205 were calculated based on the procedure mentioned in above descriptor calculation section. Then these values were normalized based on the mean and standard deviation of data set consisting of the parent 15 data points. By plugging in the normalized value of descriptors of interest, the prediction could be made.

5. **Identification of new ESSV ligand by applying QSAR model**

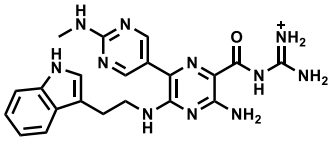
Fifteen DMAs were designed virtually based on the synthesis feasibility. After conformational search and protonation/tautomerization adjustment, the molecular descriptors of interest, namely *pmi1* and *SMR_VSA0* were calculated and plugged into the ESSV QSAR model. The predicted CD_{50} values along with corresponding chemical structures are shown below:



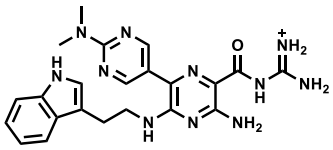
ligand 1, CD_{50} = 22.2



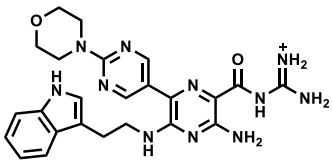
Ligand 2, CD_{50} = 712.7
(out of 3-sigma applicability domain)



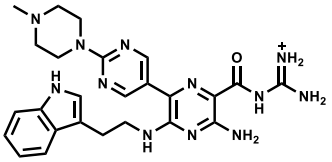
Ligand 3, CD_{50} = 703.7
(out of 3-sigma applicability domain)



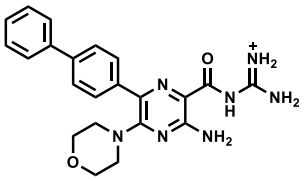
Ligand 4, CD_{50} = 7.3



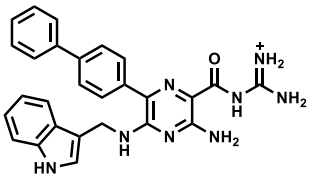
Ligand 5, CD_{50} = 22.9



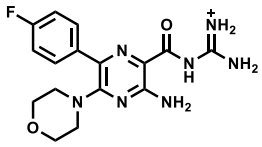
Ligand 6, CD_{50} = 4.1



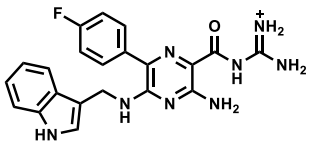
Ligand 7, CD_{50} = 94.4
(out of 3-sigma applicability domain)



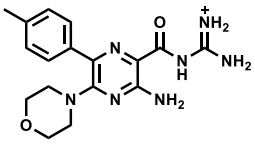
Ligand 8 (DMA-207), CD_{50} = 2.3



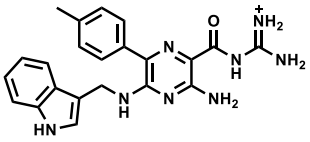
Ligand 9, CD_{50} = 250.0
(out of 3-sigma applicability domain)



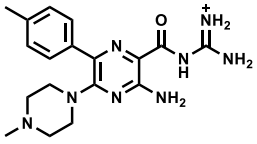
Ligand 10, CD_{50} = 14.6



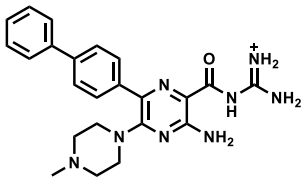
Ligand 11, CD_{50} = 261.4
(out of 3-sigma applicability domain)



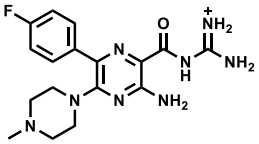
Ligand 12, CD_{50} = 16.1



Ligand 13, CD_{50} = 36.3



Ligand 14, CD_{50} = 8.4



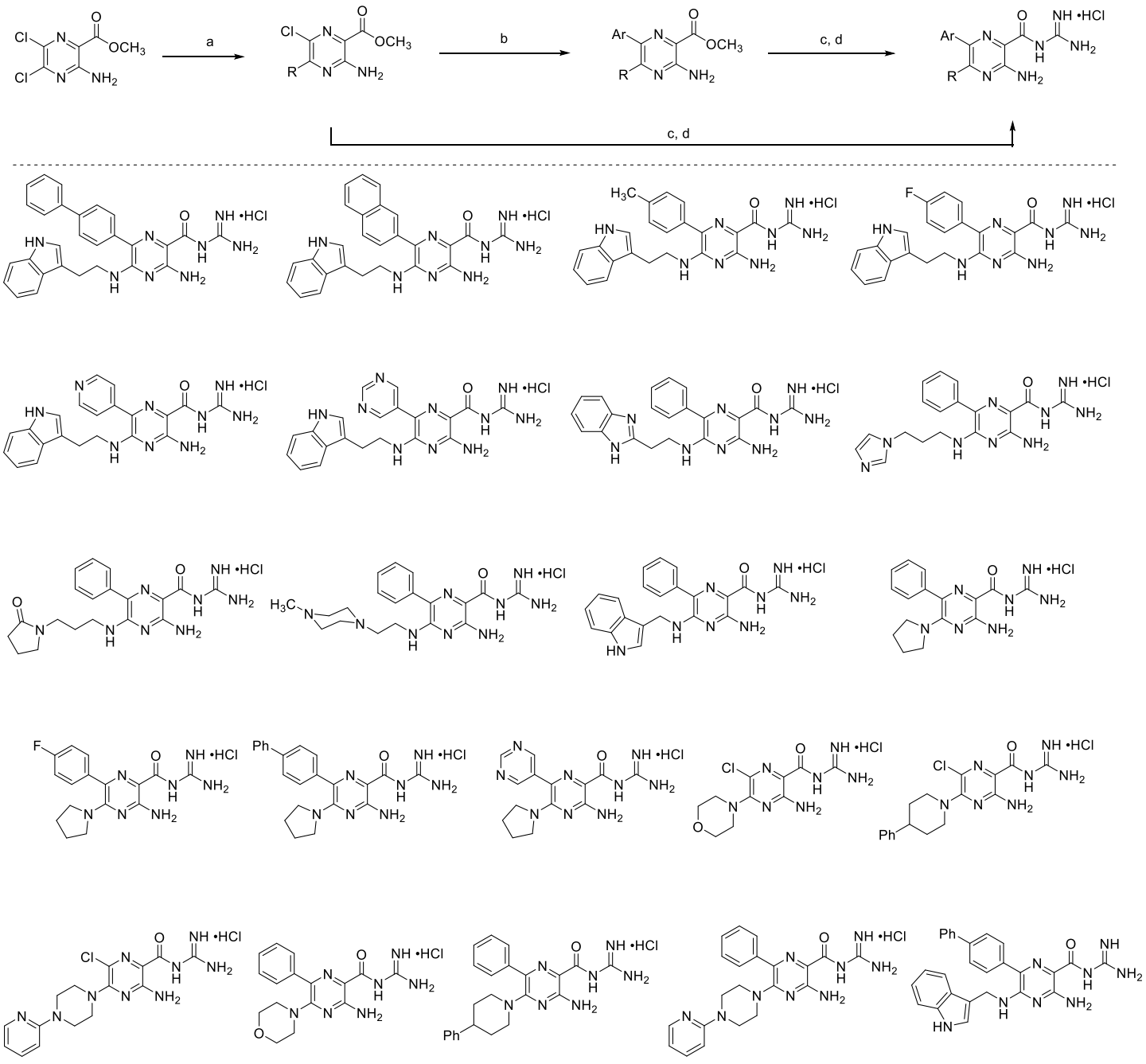
Ligand 15, CD_{50} = 35.1

Figure S9. Virtual library designed for ESSV QSAR model prediction

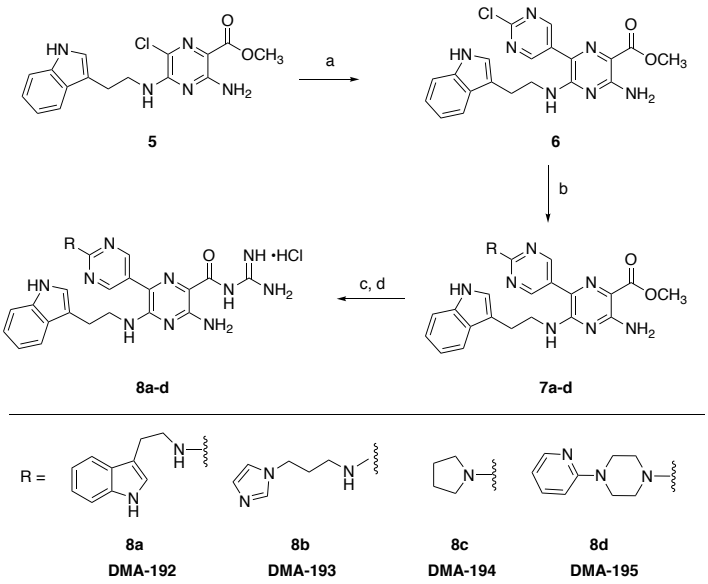
The “out of 3-sigma applicability domain” indicates either the pmi1 or SMR_VSA0 descriptor values were deviated from the original 15-data training set, which might cause imprecise predictions. Out of the 15 DMAs, the ligand 8 (DMA-207) was synthesized. The CD_{50} value were measured using the Tat displacement assay as described above.

Section D: Chemistry

1. Synthetic of Amiloride derivatives:



Scheme S1: Synthesis of different C(6) aryl subunits of DMA-169. Reagents: a: Ar-$B(OH)_2$ (1.1 equiv), Na_2CO_3 (5 equiv), $Pd(PPh_3)_4$ (5 mol%), THF:H_2O (5:1); b: Guanidine (1M in MeOH, 3 equiv), THF: MeOH (5:1); c: HCl in Et_2O. Compound 4v was not converted into an HCl salt because the procedure led to loss of the methyl indole moiety.



Scheme S2: Synthesis of C6 amino substituted derivatives of **DMA-169**. Reagents: a: 2-Chloropyrimidin-5yl-boronic acid (1.1 equiv), Na_2CO_3 (5 equiv), $Pd(PPh_3)_4$ (5 mol%), THF:H_2O (5:1); b: Amine (1.2 equiv), DIEA (5 equiv), DMF; c: Guanidine (1M in MeOH, 3 equiv), THF: MeOH (5:1); d: HCl in Et_2O.

2. Synthetic procedures and characterization data:

General Procedures: All reactions were conducted in oven-dried glassware under an inert atmosphere of nitrogen or argon using magnetic stirring. Dry solvents were obtained using the PureSolv™ solvent purification system prior to use. All chemical reagents were purchased from commercial sources and were used without further purification. Thin layer chromatography (TLC) was performed on aluminum backed silica gel plates purchased from Sigma. Column chromatography was performed on flash grade silica gel (40-63 μm) purchased from Silicycle Inc. ^{1}H NMR spectra were recorded on a Bruker 500 MHz or Varian Inova 400 MHz spectrometers; the corresponding ^{13}C NMR resonant frequencies were 126 and 101 MHz, respectively. Chemical shifts are reported in ppm from tetramethylsilane (TMS) with the solvent resonance as an internal standard (Ex: $CDCl_3$: 7.26 ppm). ^{1}H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), and integration. Broad singlet peaks, speculated to arise from NH protons, are also noted. In case of ^{13}C NMR, chemical shifts are reported in ppm with the solvent resonance as the internal standard (Ex. $CDCl_3$: 77.16 ppm). Extra peaks from solvents and other known impurities are marked as X on the spectra and not integrated in ^{1}H NMR or picked in ^{13}C-NMR. High-resolution mass spectroscopy (HRMS) was performed on an Agilent LCMS time-of-flight (TOF) mass spectrometer using either electrospray ionization (ESI) or atmospheric pressure chemical ionization

(APCI). HPLC analyses were performed on Shimadzu LC system using a Phenomex-C_{18} reverse phase column using water (with 0.1% v/v of TFA) and acetonitrile as eluents. All compounds tested in binding or activity assays are >95% pure by ^{1}H NMR and HPLC analyses unless noted otherwise.

Synthetic procedures: SNAr reactions of the dichloropyrazine starting material 4 with different amines, Suzuki-Miyura coupling reactions, and guanidinylation reactions are followed as previously reported.[5]

Characterization data for intermediates and final amiloride derivatives.

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-aminopyrazine-2-carboxylate (**3a**): 96% yield, Rf: 0.5 (1:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.1 (s, 1H), 7.6 (m, 3H), 7.5 (m, 4H), 7.4 (m, 4H), 7.2 (m, 2H), 6.9 (m, 1H), 5.5 (s, 1H), 3.8 (m, 3H), 3.7 (m, 2H), 3.0 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.6, 141.1, 140.5, 136.4, 131.8, 128.8, 127.6, 127.5, 127.2, 127.0, 122.2, 122.1, 119.5, 118.7, 112.7, 111.4, 111.2, 51.9, 41.3, 24.7. HRMS (ESI+): Calculated for $C_{28}H_{25}N_5O_2$ [M+H]: 464.2081, Found: 464.208 (± 0.2 ppm).

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(naphthalen-2-yl)pyrazine-2-carboxylate (3b): 34% yield, Rf: 0.35 (3:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.1 (s, 2H), 7.8 (m, 3H), 7.7 (m, 1H), 7.6 (m, 2H), 7.5 (m, 4H), 7.4 (m, 5H), 7.2 (m, 2H), 7.0 (m, 4H), 3.7 (s, 3H), 3.6 (m, 2H), 3.0 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 156.1, 153.4, 136.7, 134.5, 133.2, 132.9, 131.0, 130.4, 128.7, 128.5, 127.8, 127.4, 127.2, 126.6, 123.2, 121.4, 118.9, 118.7, 112.4, 111.8, 110.3, 51.4, 41.8, 24.7. HRMS (ESI+): Calculated for $C_{26}H_{23}N_5O_2$ [M+H]: 438.1925, Found: 438.1920 (± 1.0 ppm).

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(4-fluorophenyl)pyrazine-2-carboxylate (3c): 95% yield, Rf: 0.35 (4:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, DMSO) δ 7.6 (m, 1H), 7.4 (m, 2H), 7.3, (m, 1H), 7.25 (m, 4H), 7.1 (m, 1H) 6.9 (m, 1H), 6.7 (m, 1H), 3.7 (s, 3H), 3.6 (m, 2H), 3.0 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ167.4, 161.2, 156.1, 153.2, 136.7, 133.6, 131.0, 130.2, 127.7, 123.2, 121.3, 118.9, 118.7, 115.9, 115.7, 112.3, 111.8, 110.0, 51.4, 41.8, 24.8. HRMS (ESI+): Calculated for $C_{22}H_{20}FN_5O_2$ [M+H]: 406.1674, Found: 406.1672 (± 0.5 ppm).

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(*p*-tolyl)pyrazine-2-carboxylate (3d): 87% yield, Rf: 0.25 (5:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, DMSO) δ 7.6 (m, 1H), 7.4-7.2 (m, 7H), 7.1 (m, 1H), 6.9 (m, 1H), 6.7 (m, 1H), 3.7 (m, 3H), 3.6 (m, 2H), 3.0 (m, 2H), 2.4 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 167.5, 155.9, 153.2, 137.5, 136.7, 134.2, 131.2, 129.5, 128.6, 127.7, 123.2, 121.3, 118.9, 118.6, 112.3, 111.7, 109.9, 51.4, 41.7, 24.8, 21.3. HRMS (ESI+): Calculated for $C_{23}H_{23}N_5O_2$ [M+H]: 402.1925, Found: 402.1924 (± 0.2 ppm).

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(pyridin-4-yl)pyrazine-2-carboxylate (3e): 83% yield, Rf: 0.35 (1:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.1 (s, 1H), 7.57 (d, J = 7.9 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.2 (m, 2H), 7.1 (m, 1H), 6.9 (m, 1H), 5.5 (m, 1H), 3.7 (s, 3H), 3.6 (m, 2H), 3.0 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6, 155.7, 151.5, 136.5, 129.0, 128.5, 128.4, 127.2, 122.4, 122.1, 121.6, 119.6, 118.7, 112.6, 111.4, 110.2, 52.0, 41.5, 25.0; HRMS (ESI+): Calculated for $C_{21}H_{20}N_6O_2$ [M+H]: 389.1721, Found: 389.1726 (± 1.2 ppm).

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(pyrimidin-5-yl)pyrazine-2-carboxylate (3f): 69% yield, Rf: 0.35 (1:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, DMSO) δ 10.8 (s, 1H), 9.2 (s, 1H), 8.7 (s, 2H), 7.6 (m, 1H), 7.4-7.2 (m, 3H), 7.2 (s, 1H), 7.06 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 7.4 Hz, 1H), 3.7 (s, 3H), 3.60 (q, J = 6.7 Hz, 2H), 2.98 (t, J = 7.8 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 167.3, 157.7, 156.9, 156.5, 153.7, 136.7, 131.6, 127.8, 125.3, 123.2, 121.4, 118.8, 118.7, 112.5, 111.9, 111.1, 108.8, 51.6, 42.0, 24.8; HRMS (ESI+): Calculated for $C_{20}H_{19}N_7O_2$ [M+H]: 390.1673, Found: 390.1677 (± 0.9 ppm).

Methyl 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3g): 94% yield, Rf: 0.25 (95:5 - DCM: MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, J = 7.1 Hz, 2H), 7.53 (ddd, J = 12.2, 8.1, 1.4 Hz, 2H), 7.4 (m, 2H), 7.35 (td, J = 7.7, 2.9 Hz, 2H), 7.2 (m, 1H), 7.1 (m, 5H), 5.8 (m, 1H), 3.8-3.6 (m, 5H), 3.05 (t, J = 6.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.3, 155.5, 152.9, 152.3, 135.8, 132.4, 132.0, 129.0, 128.8, 128.7, 128.6, 128.5, 127.4, 122.8, 111.5, 51.9, 38.8, 28.3; HRMS (ESI+): Calculated for $C_{21}H_{20}N_6O_2$ [M+H]: 389.1721, Found: 389.1727 (± 1.8 ppm).

Methyl 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3h): 76% yield, Rf: 0.25 (95:5 - DCM: MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.9 (s, 1H), 7.4 (m, 3H), 7.35 (m, 2H), 7.3 (m, 2H), 7.0 (s, 1H), 6.7 (m, 1H), 5.3 (m, 2H), 3.89 (t, J = 6.8 Hz, 2H), 3.80 (s, 3H), 3.32 (q, J = 6.7 Hz, 2H), 1.97 (p, J =

6.9 Hz, 2H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.6, 155.4, 153.0, 136.9, 136.4, 133.6, 131.8, 129.3, 128.7, 128.6, 119.0, 112.0, 52.0, 44.7, 38.0, 30.6; HRMS (ESI+): Calculated for $C_{18}H_{20}N_6O_2$ [M+H]: 353.1721, Found: 353.1730 (± 2.5 ppm).

Methyl 3-amino-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)-6-phenylpyrazine-2-carboxylate (3i): 82% yield, Rf: 0.25 (95:5 - DCM: MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.7 (m, 1H), 7.6 (m, 3H), 7.4 (m, 3H), 7.3 (m, 1H), 5.9 (m, 1H), 3.8 (s, 3H), 3.4-3.2 (m, 6H), 2.3 (t, 2H), 2.0 (m, 2H), 1.8 (p, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.6, 167.7, 155.6, 153.1, 136.5, 134.0, 129.1, 128.6, 128.6, 127.7, 111.4, 51.9, 47.3, 40.1, 38.0, 30.9, 26.5; HRMS (ESI+): Calculated for $C_{19}H_{23}N_5O_3$ [M+H]: 369.1874, Found: 369.1881 (± 2.1 ppm).

Methyl 3-amino-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)-6-phenylpyrazine-2-carboxylate (3j): 93% yield, Rf: 0.25 (95:5 - DCM: MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (d, J = 6.8 Hz, 2H), 7.37 (t, J = 7.6 Hz, 2H), 7.30 (dd, J = 8.6, 6.4 Hz, 1H), 6.1 (s, 1H), 3.81 (s, 3H), 3.37 (q, J = 5.4 Hz, 2H), 3.0-1.5 (m, 13H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 155.8, 153.1, 136.8, 132.3, 129.0, 128.6, 128.4, 111.3, 55.2, 52.3, 51.9, 46.0, 37.4; HRMS (ESI+): Calculated for $C_{19}H_{26}N_6O_2$ [M+H]: 371.2190, Found: 371.2197 (± 1.9 ppm).

Methyl 5-(((1*H*-indol-3-yl)methyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3k): 60% yield, Rf: 0.2 (4:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, DMSO) δ 7.74 (d, J = 7.8 Hz, 1H), 7.5 (m, 4H), 7.4 (m, 3H), 7.3 (bs, 2H), 7.12 (ddd, J = 8.0, 7.0, 1.2 Hz, 1H), 7.08 – 7.01 (m, 2H), 5.7 (s, 1H), 4.75 (d, 2H), 3.7 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 167.5, 156.0, 153.0, 137.3, 136.6, 131.1, 129.1, 128.8, 128.3, 127.0, 125.1, 121.4, 119.2, 118.9, 112.5, 111.8, 110.1, 51.4, 36.2; HRMS (ESI+): Calculated for $C_{21}H_{18}N_5O_2Na$ [M+Na]: 396.1431, Found: 396.1431 (± 0.1 ppm).

Methyl 3-amino-6-phenyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3l): 91% yield, Rf: 0.25 (5:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, CD_3OD) δ 7.4 (m, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.2 (m, 1H), 3.8 (s, 3H), 3.1 (m, 4H), 1.4 (m, 4H); ^{13}C NMR (126 MHz, CD_3OD) δ 167.6, 154.1, 153.1, 140.1, 132.0, 128.8, 128.1, 127.4, 111.9, 51.9, 50.1, 25.5; HRMS (ESI+): Calculated for $C_{16}H_{18}N_4O_2$ [M+H]: 299.1503, Found: 299.1512 (± 3.3 ppm).

Methyl 3-amino-6-(4-fluorophenyl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3m): 77% yield, Rf: 0.25 (5:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.3 (m, 2H), 7.0 (m, 2H), 3.81 (s, 3H), 3.10 (d, J = 6.6 Hz, 4H), 1.6 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.5, 163.2, 161.2, 154.1, 153.1, 136.2, 130.9, 130.5, 130.4,

115.1, 112.0, 51.9, 50.1, 25.5; HRMS (ESI+): Calculated for $C_{16}H_{17}FN_4O_2$ [M+H]: 317.1408, Found: 317.1413 (± 1.6 ppm).

Methyl 6-([1,1'-biphenyl]-4-yl)-3-amino-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3n): 81% yield, Rf: 0.35 (5:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.6 (m, 4H), 7.4 (m, 2H), 7.36 (t, J = 7.7 Hz, 2H), 7.2 (m, 2H), 3.8 (s, 3H), 3.16 (d, J = 6.6 Hz, 4H), 1.80 – 1.64 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.1, 153.2, 140.8, 140.1, 139.1, 131.6, 129.1, 128.8, 127.3, 127.0, 126.8, 112.1, 51.9, 50.2, 25.6; HRMS (ESI+): Calculated for $C_{22}H_{22}N_4O_2$ [M+H]: 375.1816, Found: 375.1825 (± 2.5 ppm).

Methyl 3-amino-6-(pyrimidin-5-yl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3o): 48% yield, Rf: 0.35 (95:5 - DCM: MeOH); Sample contains triphenylphosphine contaminant that could not be completely removed by chromatography or trituration. Taken forward as is to next step guanidinylation. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.1 (s, 1H), 8.7 (s, 2H), 3.8 (s, 3H), 3.1 (m, 4H), 1.6 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.1, 157.0, 156.1, 154.4, 153.1, 128.4, 124.4, 113.8, 52.1, 50.8, 25.6; HRMS (ESI+): Calculated for $C_{14}H_{16}N_6O_2$ [M+H]: 301.1408, Found: 301.2409 (± 0.5 ppm).

Methyl 3-amino-6-chloro-5-morpholinopyrazine-2-carboxylate (3p): 93% Yield, Rf: 0.35 (3:2 Hexanes: EtOAc); ^{1}H NMR (500 MHz, Chloroform-d) δ 3.92 (s, 3H), 3.83 – 3.75 (m, 4H), 3.65 (m, 4H).; ^{13}C NMR (126 MHz, CDCl3) δ 166.4, 154.7, 154.0, 123.9, 113.9, 66.7, 52.5, 48.6; HRMS (ESI+): Calculated for $C_{10}H_{13}N_4O_3Cl$ [M+H]: 273.0749, Found: 273.0751 (± 0.9 ppm).

Methyl 3-amino-6-chloro-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxylate (3q): 98% Yield, Rf: 0.35 (3:2 Hexanes: EtOAc); ^{1}H NMR (500 MHz, Chloroform-d) δ 7.31 (t, J = 7.6 Hz, 2H), 7.21 (dd, J = 12.6, 7.2 Hz, 4H), 4.47 (d, J = 13.0 Hz, 2H), 3.90 (s, 3H), 2.98 (td, J = 12.7, 2.6 Hz, 2H), 2.77 (tt, J = 12.3, 3.9 Hz, 1H), 2.03 – 1.90 (m, 2H), 1.84 (qd, J = 12.6, 3.9 Hz, 2H); ^{13}C NMR (126 MHz, CDCl3) δ 166.3, 154.9, 154.0, 145.4, 128.6, 126.8, 126.5, 123.9, 113.1, 52.2, 48.9, 42.6, 33.2; HRMS (ESI+): Calculated for $C_{17}H_{19}N_4O_2Cl$ [M+H]: - 347.1269, Found: 347.1272 (± 0.6 ppm).

Methyl 3-amino-6-chloro-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxylate (3r): 98% Yield, Rf: 0.35 (3:2 Hexanes: EtOAc); ^{1}H NMR (500 MHz, Chloroform-d) δ 8.21 (t, J = 3.5 Hz, 1H), 7.61 – 7.38 (m, 1H), 6.74 – 6.57 (m, 2H), 3.91 (s, 3H), 3.78 (t, J = 5.3 Hz, 4H), 3.67 (t, J = 5.3 Hz, 4H); ^{13}C NMR (126 MHz, CDCl3) δ

166.4, 159.4, 154.8, 154.0, 148.1, 137.7, 123.8, 114.0, 113.7, 107.2, 52.4, 47.8, 45.1; HRMS (ESI+): Calculated for $C_{15}H_{17}N_6O_2Cl$ [M+H]: 349.1174, Found: 349.1180 (± 1.6 ppm).

Methyl 3-amino-5-morpholino-6-phenylpyrazine-2-carboxylate (3s): 59% yield, Rf: 0.4 (7:3 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, Chloroform-d) δ 7.7 (m, 2H), 7.4 (m, 2H), 7.33 (dd, 1H), 3.91 (s, 3H), 3.6 (m, 4H), 3.25 (m, 4H); ^{13}C NMR (126 MHz, CDCl3) δ 167.4, 155.9, 153.8, 139.5, 133.5, 128.8, 128.1, 127.6, 114.6, 66.5, 52.3, 48.4; HRMS (ESI+): Calculated for $C_{16}H_{18}N_4O_3$ [M+H]: 315.1452, Found: 315.1458 (± 2.0 ppm).

Methyl 3-amino-6-phenyl-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxylate (3t): 61% yield, Rf: 0.6 (7:3 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, Chloroform-d) δ 7.75 – 7.69 (m, 2H), 7.40 (t, J = 7.7 Hz, 2H), 7.30 (q, J = 7.7 Hz, 3H), 7.23 – 7.16 (m, 3H), 4.02 (dp, J = 13.6, 2.0 Hz, 2H), 3.92 (s, 3H), 2.80 (td, J = 12.9, 2.5 Hz, 2H), 2.66 (tt, J = 12.2, 3.8 Hz, 1H), 1.81 – 1.72 (m, 2H), 1.67 (tt, J = 12.6, 6.3 Hz, 2H); ^{13}C NMR (126 MHz, CDCl3) δ 167.5, 156.2, 153.9, 145.7, 140.0, 133.7, 128.8, 128.6, 127.9, 127.4, 126.8, 126.5, 114.0, 52.2, 48.9, 42.5, 32.9; HRMS (ESI+): Calculated for $C_{23}H_{24}N_4O_2$ [M+H]: 389.1972, Found: 389.1976 (± 1.0 ppm).

Methyl 3-amino-6-phenyl-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxylate (3u): 79% Yield, Rf: 0.35 (3:2 Hexanes: EtOAc); ^{1}H NMR (500 MHz, Chloroform-d) δ 8.17 (dd, J = 5.0, 1.9 Hz, 1H), 7.76 – 7.65 (m, 2H), 7.47 (ddd, J = 8.8, 7.2, 1.9 Hz, 1H), 7.40 (t, J = 7.6 Hz, 2H), 7.33 – 7.27 (m, 1H), 6.64 (dd, J = 7.1, 4.9 Hz, 1H), 6.60 (d, J = 8.6 Hz, 1H), 3.91 (s, 3H), 3.52 – 3.37 (m, 8H); ^{13}C NMR (126 MHz, CDCl3) δ 167.5, 159.5, 156.1, 153.8, 148.1, 139.6, 137.7, 133.7, 128.9, 128.1, 127.7, 114.6, 113.9, 107.4, 52.3, 47.7, 44.9; HRMS (ESI+): Calculated for $C_{21}H_{22}N_6O_2$ [M+H]: 391.1877, Found: 391.1886 (± 2.2 ppm).

5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (4a) (DMA-176): 48% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.6 (m, 4H), 7.44 (d, J = 7.9 Hz, 1H), 7.37 (t, J = 7.7 Hz, 2H), 7.2 (m, 4H), 6.99 (m, 2H), 6.88 (t, J = 7.4 Hz, 1H), 3.71 (t, J = 6.9 Hz, 2H), 3.00 (t, J = 6.9 Hz, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.4, 156.0, 155.6, 154.1, 141.4, 140.2, 136.9, 134.5, 132.2, 128.7, 128.6, 127.4, 127.4, 127.0, 126.6, 122.4, 121.1, 118.4, 118.0, 111.6, 110.9, 108.9, 41.5, 23.9; HRMS (ESI+): Calculated for $C_{28}H_{27}N_8O$ [M+H]: 491.2302, Found: 491.2308 (± 1.1 ppm). HPLC Analysis: Retention time = 9.763 min, Purity = 97.817%.

5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(naphthalen-2-yl)pyrazine-2-carboxamide hydrochloride (4b) (DMA-177): 55% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.76 (dd, J = 7.8, 2.4 Hz, 2H), 7.58 (dd, J = 8.0, 1.8 Hz, 2H), 7.45 – 7.33 (m, 4H), 7.27 (d, J = 8.2 Hz, 1H), 7.02 – 6.95 (m, 2H), 3.71 (t, J = 6.7 Hz, 2H), 3.00 (t, J = 6.7 Hz, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.3, 155.6, 155.5, 153.8, 136.8, 133.3, 133.1, 132.7, 132.5, 128.4, 128.0, 127.5, 127.4, 127.3, 126.4, 126.1, 125.9, 122.5, 121.1, 118.4, 117.9, 111.6, 111.0, 109.0, 41.8, 23.8; HRMS (ESI+): Calculated for $C_{26}H_{25}N_8O$ [M+H]: 465.2149, Found: 465.2147 (± 0.1 ppm). HPLC Analysis: Retention time = 9.387 min, Purity = 96.746%.

5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(*p*-tolyl)pyrazine-2-carboxamide hydrochloride (4c) (DMA-178): 50% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.41 (d, J = 7.9 Hz, 1H), 7.25 (d, J = 8.1 Hz, 1H), 7.08 (s, 4H), 7.03 – 6.95 (m, 2H), 6.92 – 6.85 (m, 1H), 3.69 (t, J = 6.8 Hz, 2H), 2.98 (t, J = 6.8 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.2, 162.0, 155.5, 154.7, 153.1, 136.8, 131.6, 131.4, 130.5, 127.4, 122.6, 121.1, 118.4, 117.9, 115.5, 115.5, 111.3, 111.0, 108.8, 41.9, 23.8; HRMS (ESI+): Calculated for $C_{23}H_{25}N_8O$ [M+H]: 429.2146, Found: 429.2150 (± 0.9 ppm). HPLC Analysis: Retention time = 9.117 min, Purity = 99.810%.

5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(4-fluorophenyl)pyrazine-2-carboxamide hydrochloride (4d) (DMA-179): 54% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.40 (d, J = 7.9 Hz, 1H), 7.26 (d, J = 8.1 Hz, 1H), 7.23 – 7.18 (m, 2H), 7.03 – 6.95 (m, 4H), 6.91 – 6.85 (m, 1H), 3.70 (t, J = 6.8 Hz, 2H), 3.00 (t, J = 6.8 Hz, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.3, 155.6, 153.4, 138.8, 136.8, 132.8, 132.3, 130.9, 129.2, 128.1, 127.3, 122.5, 121.1, 118.4, 117.9, 111.3, 110.9, 108.7, 41.7, 23.9, 19.9; HRMS (ESI+): Calculated for $C_{22}H_{22}FN_8O$ [M+H]: 433.1895, Found: 433.1893 (± 0.7 ppm). HPLC Analysis: Retention time = 8.858 min, Purity = 98.578%.

5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(pyridin-4-yl)pyrazine-2-carboxamide hydrochloride (4e) (DMA-190): 73% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 8.54 – 8.45 (m, 2H), 8.07 – 7.98 (m, 2H), 7.44 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.06 (s, 1H), 7.00 (t, J = 7.6 Hz, 1H), 6.88 (t, J = 7.4 Hz, 1H), 3.75 (t, J = 7.0 Hz, 2H), 3.06 (t, J = 7.0 Hz, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.0, 156.4, 155.5, 154.3, 153.5, 140.7, 136.7, 127.6, 125.2, 123.8, 122.5, 121.1,

118.4, 118.0, 112.5, 112.0, 111.1, 41.8, 23.8 ; HRMS (ESI+): Calculated for $C_{21}H_{22}N_9O$ [M+H]: 416.1942, Found: 416.1946 (± 1.0 ppm). HPLC Analysis: Retention time = 7.074 min, Purity = 99.393%.

5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (4f) (DMA-191): 47% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 9.04 (s, 1H), 8.68 (s, 2H), 7.38 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H), 7.02 – 6.93 (m, 2H), 6.86 (t, J = 7.5 Hz, 1H), 3.66 (t, J = 7.0 Hz, 2H), 3.01 (t, J = 7.0 Hz, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.2, 156.8, 156.6, 154.7, 136.8, 127.6, 125.4, 122.2, 120.9, 118.2, 117.9, 112.1, 111.0, 110.2, 41.8, 23.9; HRMS (ESI+): Calculated for $C_{20}H_{21}N_{10}O$ [M+H]: 417.1894, Found: 417.1903 (± 2.1 ppm). HPLC Analysis: Retention time = 7.835 min, Purity = 96.171%.

5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4g) (DMA-180): 41% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.64 (td, J = 6.3, 2.9 Hz, 2H), 7.47 (dq, J = 6.6, 3.6 Hz, 2H), 7.41 (dq, J = 4.4, 2.5 Hz, 2H), 7.35 – 7.25 (m, 3H), 3.89 (t, J = 6.3 Hz, 2H), 3.41 (t, J = 6.3 Hz, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 166.4, 155.8, 155.5, 154.3, 152.2, 135.6, 132.2, 131.0, 128.6, 128.5, 128.4, 126.0, 113.3, 109.6, 38.9, 26.7; HRMS (ESI+): Calculated for $C_{21}H_{21}N_9O$ [M+H]: 416.1942, Found: 416.1945 (± 0.7 ppm). HPLC Analysis: Retention time = 2.026 min, Purity = 93.194%.

5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4h) (DMA-182): 74% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 8.89 (d, J = 1.6 Hz, 1H), 7.60 (q, J = 2.1, 1.7 Hz, 1H), 7.53 – 7.46 (m, 4H), 7.43 (dd, J = 8.2, 6.5 Hz, 2H), 7.40 – 7.35 (m, 1H), 4.25 (t, J = 7.2 Hz, 2H), 3.46 (t, J = 6.6 Hz, 2H), 2.16 (p, J = 6.9 Hz, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.4, 155.9, 155.6, 154.4, 135.8, 135.1, 132.5, 128.7, 128.6, 128.5, 121.9, 119.8, 109.3, 47.2, 37.6, 29.3; HRMS (ESI+): Calculated for $C_{18}H_{21}N_9O$ [M+H]: 380.1942, Found: 380.1942 (± 0.1 ppm). HPLC Analysis: Retention time = 1.986 min, Purity = 97.655%.

3-amino-*N*-carbamimidoyl-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)-6-phenylpyrazine-2-carboxamide hydrochloride (4i) (DMA-183): 48% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.61 – 7.53 (m, 2H), 7.49 – 7.35 (m, 3H), 3.38 (dq, J = 21.7, 7.9, 7.3 Hz, 4H), 3.25 (t, J = 6.9 Hz,

2H), 2.25 (dd, J = 10.0, 6.2 Hz, 2H), 1.94 (p, J = 7.6 Hz, 2H), 1.84 – 1.70 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 158.8, 154.7, 151.8, 147.5, 143.1, 134.9, 129.6, 128.8, 128.3, 114.5, 47.1, 39.8, 38.4, 30.5, 25.7, 17.5; HRMS (ESI+): Calculated for $C_{19}H_{24}N_8O_2$ [M+H]: 397.2095, Found: 397.2097 (± 0.4 ppm). HPLC Analysis: Retention time = 6.474 min, Purity = 91.680%.

3-amino-*N*-carbamimidoyl-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)-6-phenylpyrazine-2-carboxamide hydrochloride (4j) (DMA-187): 73% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.7 (m, 2H), 7.6-7.4 (m, 3H), 4.3-3.3 (m, 12H), 3.0 (s, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.5, 156.0, 154.8, 135.7, 132.6, 128.8, 128.6, 128.5, 109.9, 56.0, 50.0, 49.0, 35.5; HRMS (ESI+): Calculated for $C_{19}H_{28}N_9O$ [M+H]: 398.2411, Found: 398.2411 (± 0.2 ppm). HPLC Analysis: Retention time = 5.776 min, Purity = 94.541%.

5-(((1*H*-indol-3-yl)methyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4k) (DMA-188): 76% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.55 – 7.48 (m, 2H), 7.43 – 7.38 (m, 3H), 7.37 – 7.27 (m, 4H), 7.25 (d, J = 8.1 Hz, 1H), 7.15 (s, 1H), 7.03 – 6.98 (m, 1H), 6.91 (t, J = 7.5 Hz, 1H), 4.71 (s, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.5, 156.3, 155.6, 154.3, 136.8, 135.9, 132.5, 128.6, 128.3, 128.2, 126.5, 123.7, 121.2, 118.1, 111.5, 111.0, 108.9, 36.1; HRMS (ESI+): Calculated for $C_{21}H_{21}N_8O$ [M+H]: 401.1833, Found: 401.1840 (± 1.9 ppm). HPLC Analysis: Retention time = 6.636 min, Purity = 95.354%

3-amino-*N*-carbamimidoyl-6-phenyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4l) (DMA-181): 65% yield, Rf: 0.15 (90: 9: 1 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.5-7.3 (m, 5H), 3.2 (m, 4H), , 1.7 (m, 4H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.3, 155.6, 155.0, 153.9, 139.6, 132.1, 128.7, 127.8, 127.6, 109.4, 50.1, 25.0; HRMS (ESI+): Calculated for $C_{16}H_{20}N_7O$ [M+H]: 326.1724, Found: 326.1727 (± 0.9 ppm). HPLC Analysis: Retention time = 8.336 min, Purity = 97.267%

3-amino-*N*-carbamimidoyl-6-(4-fluorophenyl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4m) (DMA-184): 70% yield, Rf: 0.15 (90: 9: 1 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.4 (m, 2H), 7.2 (m, 2H), 3.3 (m, 4H) Overlaps with solvent peak, 1.7 (m, 4H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.0, 163.7, 161.7, 155.5, 151.5, 135.0, 131.2, 130.9, 130.8, 114.8, 114.6, 109.4, 50.9, 24.9; HRMS (ESI+):

Calculated for $C_{16}H_{20}FN_7O$ [M+H]: 344.1630, Found: 344.1633 (± 0.9 ppm). HPLC Analysis: Retention time = 8.488 min, Purity = 98.511%.

6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-carbamimidoyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4n) (DMA-185): 38% yield, Rf: 0.15 (90: 9: 1 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 7.65 – 7.61 (m, 2H), 7.60 – 7.56 (m, 2H), 7.50 – 7.45 (m, 2H), 7.37 (t, J = 7.6 Hz, 2H), 7.27 (t, J = 7.4 Hz, 1H), 3.3 (m, 4H) Overlaps with solvent peak, 1.79 – 1.69 (m, 4H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.3, 155.6, 154.5, 153.4, 140.7, 140.3, 138.3, 131.8, 129.2, 128.6, 127.3, 126.5, 126.3, 109.6, 50.4, 25.0; HRMS (ESI+): Calculated for $C_{22}H_{24}N_7O$ [M+H]: 402.2037, Found: 402.2048 (± 2.7 ppm). HPLC Analysis: Retention time = 9.570, Purity = 95.456%.

3-amino-*N*-carbamimidoyl-6-(pyrimidin-5-yl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4o) (DMA-186): 39% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 9.25 (s, 1H), 9.15 (s, 2H), 3.3 (m, 4H) Overlaps with solvent peak, 1.7 (m, 4H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.1, 155.9, 155.5, 154.8, 153.4, 152.7, 123.1, 122.4, 112.3, 51.1, 25.1; HRMS (ESI+): Calculated for $C_{14}H_{18}N_9O$ [M+H]: 328.1629, Found: 328.1635 (± 1.8 ppm). HPLC Analysis: Retention time = 6.770, Purity = 98.858%.

3-Amino-N-carbamimidoyl-6-chloro-5-morpholinopyrazine-2-carboxamide (4p) (DMA-201): 75% Yield, Rf: 0.35 (85:13:2 - DCM:MeOH:NH_4OH); ^1H NMR (500 MHz, Methanol-d4) δ 3.82 – 3.76 (m, 4H), 3.76 – 3.68 (m, 4H); ^{13}C NMR (126 MHz, MeOD) δ 167.4, 157.3, 156.6, 155.8, 123.5, 113.5, 67.6, 49.7; HRMS (ESI+): Calculated for $C_{10}H_{14}N_7O_2Cl$ [M+H]: 300.0970, Found: 300.0974 (± 1.1 ppm). HPLC Analysis: Retention time = 6.737 min, Purity = 97.708%.

3-Amino-N-carbamimidoyl-6-chloro-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxamide (4q) (DMA-202): 68% Yield, Rf: 0.25 (85:13:2 - DCM:MeOH:NH_4OH); ^1H NMR (500 MHz, Methanol-d4) δ 7.31 – 7.21 (m, 4H), 7.21 – 7.14 (m, 1H), 4.63 (dp, J = 13.6, 1.9 Hz, 2H), 3.12 (td, J = 12.9, 2.4 Hz, 2H), 2.87 (tq, J = 12.2, 4.2 Hz, 1H), 1.95 (dd, J = 13.9, 3.6 Hz, 2H), 1.84 (qd, J = 12.6, 3.9 Hz, 2H); ^{13}C NMR (126 MHz, MeOD) δ 166.9, 156.89, 156.0, 146.8, 129.6, 127.8, 127.4, 123.6, 112.4, 50.1, 43.7, 34.5; HRMS (ESI+): Calculated for $C_{17}H_{20}N_7OCl$ [M+H]: 374.1491, Found: 374.1494 (± 0.8 ppm). HPLC Analysis: Retention time = 9.168 min, Purity = 94.865%.

3-Amino-N-carbamimidoyl-6-chloro-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxamide (4r) (DMA-203): 77% Yield, Rf: 0.15 (85:13:2 - DCM:MeOH:NH_4OH); ^1H NMR (500 MHz, Methanol-d4) δ 8.10 (s, 1H), 7.69 (ddd, J = 8.9, 7.1, 1.9 Hz, 1H), 6.97 (d, J = 8.8 Hz, 1H), 6.78 (dd, J = 7.1, 5.2 Hz, 1H), 3.97 – 3.89 (m, 4H), 3.76 – 3.67 (m, 4H); ^{13}C NMR (126 MHz, MeOD) δ 166.9, 159.5, 156.9, 156.7, 155.9, 146.5, 140.4, 123.4, 114.9, 112.9, 110.0, 46.3; HRMS (ESI+): Calculated for $C_{15}H_{18}N_9OCl$ [M+H]: 376.1396, Found: 376.1398 (± 0.7 ppm). HPLC Analysis: Retention time = 5.945 min, Purity = 96.500%.

3-Amino-N-carbamimidoyl-5-morpholino-6-phenylpyrazine-2-carboxamide (4s) (DMA-204): 73% yield, Rf: 0.25 (85:13:2 - DCM:MeOH:NH_4OH); ^1H NMR (500 MHz, Methanol-d4) δ 7.59 (d, J = 7.6 Hz, 2H), 7.38 (t, J = 7.6 Hz, 2H), 7.29 (t, J = 7.4 Hz, 1H), 3.53 – 3.46 (m, 4H), 3.26 – 3.19 (m, 5H); ^{13}C NMR (126 MHz, MeOD) δ 167.8, 158.3, 156.8, 155.9, 140.3, 134.2, 129.9, 129.4, 128.8, 113.1, 67.3, 49.5; HRMS (ESI+): Calculated for $C_{16}H_{19}N_7O_2$ [M+H]: 342.1673, Found: 342.1677 (± 1.1 ppm). HPLC Analysis: Retention time = 7.714 min, Purity = 92.874%.

3-Amino-N-carbamimidoyl-6-phenyl-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxamide (4t) (DMA-205): 32% yield, Rf: 0.28 (85:13:2 - DCM:MeOH:NH_4OH); ^1H NMR (500 MHz, Methanol-d4) δ 7.73 – 7.67 (m, 2H), 7.49 (t, J = 7.7 Hz, 2H), 7.39 (t, J = 7.4 Hz, 1H), 7.26 (t, J = 7.6 Hz, 2H), 7.22 – 7.13 (m, 3H), 4.10 (dq, J = 13.5, 2.3 Hz, 2H), 2.88 (td, J = 12.7, 3.0 Hz, 2H), 2.72 (tt, J = 11.7, 4.3 Hz, 1H), 1.76 – 1.59 (m, 4H); ^{13}C NMR (126 MHz, MeOD) δ 166.4, 157.1, 155.5, 154.7, 145.5, 139.5, 133.0, 128.5, 128.1, 127.8, 127.2, 126.3, 126.0, 111.1, 48.5, 42.2, 32.7; HRMS (ESI+): Calculated for $C_{23}H_{25}N_7O$ [M+H]: 416.2193, Found: 416.2193 (± 0.2 ppm). HPLC Analysis: Retention time = 9.856 min, Purity = 98.513%.

3-Amino-N-carbamimidoyl-6-phenyl-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxamide (4u) (DMA-206): 20% yield, Rf: 0.25 (85:13:2 - DCM:MeOH:NH_4OH); ^1H NMR (500 MHz, Methanol-d4) δ 8.01 (ddd, J = 9.1, 7.0, 1.8 Hz, 1H), 7.96 (dd, J = 6.2, 1.7 Hz, 1H), 7.77 – 7.71 (m, 2H), 7.52 (t, J = 7.7 Hz, 2H), 7.45 – 7.40 (m, 1H), 7.32 (d, J = 9.2 Hz, 1H), 7.00 (t, J = 6.7 Hz, 1H), 3.73 (dd, J = 6.7, 4.0 Hz, 4H), 3.63 – 3.56 (m, 4H); ^{13}C NMR (126 MHz, MeOD) δ 167.9, 158.0, 156.9, 155.9, 154.1, 145.2, 140.2, 138.0, 134.0, 130.0, 129.5, 129.1, 114.4, 113.8, 113.6, 47.7, 46.2. HRMS (ESI+): Calculated for $C_{21}H_{23}N_9O$ [M+H]: 418.2098, Found: 418.2098. HPLC Analysis: Retention time = 6.573 min, Purity = 93.253%.

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-chloropyrimidin-5-yl)pyrazine-2-carboxylate (6): 45% yield, Rf: 0.45 (1:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (s, 2H), 8.17 (s, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H), 7.11 (t, J = 7.6 Hz, 1H), 6.99 (t, J = 7.6 Hz, 1H), 6.90 (d, J = 2.8 Hz, 1H), 5.06 (t, J = 5.2 Hz, 1H), 3.83 (s, 3H), 3.68 (q, J = 6.1 Hz, 2H), 3.01 (t, J = 6.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.0, 160.2, 159.0, 156.1, 153.2, 136.4, 129.4, 127.0, 123.6, 122.5, 119.7, 118.4, 113.1, 112.6, 111.6, 52.1, 41.5, 24.5; HRMS (ESI+): Calculated for $C_{20}H_{18}N_7O_2Cl$ [M+H]: 424.1283, Found: 424.1286 (± 0.6 ppm).

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-(2-((2-(1*H*-indol-3-yl)ethyl)amino)pyrimidin-5-yl)-3-aminopyrazine-2-carboxylate (7a): 38% yield, Rf: 0.15 (1:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.3 (s, 1H), 8.2-8.0 (m, 2H), 7.6 (m, 1H), 7.4 (m, 1H), 7.2 (m, 2H), 7.1 (m, 1H), 5.1 (s, 1H), 7.1-6.9 (m, 5H), 6.9 (m, 1H), 6.7 (m, 1H), 5.6 (m, 1H), 5.1 (m, 1H), 3.7 (s, 3H), 3.6-3.5 (m, 4H), 3.0 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.5, 161.5, 161.2, 158.0, 155.9, 153.7, 136.4, 127.5, 127.4, 127.1, 122.3, 122.2, 122.1, 119.6, 119.5, 119.4, 119.2, 118.8, 118.6, 113.0, 112.7, 111.7, 111.4, 111.3, 52.0, 41.7, 41.3, 25.2, 24.7; HRMS (ESI+): Calculated for $C_{30}H_{30}N_9O_2$ [M+H]: 548.2517, Found: 548.2519 (± 0.4 ppm).

Methyl 6-(2-((3-(1*H*-imidazol-1-yl)propyl)amino)pyrimidin-5-yl)-5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-aminopyrazine-2-carboxylate (7b): 48% yield, Rf: 0.15 (1:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, DMSO) δ 8.29 (s, 2H), 7.66 (s, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.26 – 7.14 (m, 3H), 7.10 – 7.03 (m, 1H), 7.01 – 6.95 (m, 1H), 6.90 (s, 1H), 4.05 (t, J = 6.9 Hz, 2H), 3.73 (s, 3H), 3.60 (dt, J = 14.7, 7.0 Hz, 2H), 3.28 (q, J = 7.6, 6.7 Hz, 2H), 2.97 (t, J = 7.5 Hz, 2H), 1.99 (h, J = 6.9, 6.3 Hz, 2H).; ^{13}C NMR (126 MHz, DMSO) δ 167.5, 161.9, 156.1, 153.8, 137.8, 136.7, 136.5, 128.9, 127.8, 127.4, 123.1, 122.9, 121.4, 119.9, 118.9, 118.7, 112.6, 111.8, 110.1, 51.4, 44.2, 41.9, 38.3, 31.0, 24.8; HRMS (ESI+): Calculated for $C_{26}H_{29}N_{10}O_2$ [M+H]: 513.2470, Found: 513.2476 (± 1.2 ppm).

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-(pyrrolidin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxylate (7c): 33% yield, Rf: 0.15 (1:1 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.83 (d, J = 2.3 Hz, 1H), 8.36 (s, 2H), 7.67 – 7.60 (m, 1H), 7.59 – 7.51 (m, 2H), 7.34 (d, J = 7.9 Hz, 1H), 7.17 (t, J = 4.5 Hz, 2H), 7.06 (ddd, J = 8.2, 6.9, 1.2 Hz, 1H), 6.98 (ddd, J = 7.9, 6.9, 1.1 Hz, 1H), 3.73 (s, 3H), 3.63 – 3.57 (m, 2H), 3.55 – 3.49 (m, 4H), 2.99 (t, J = 7.6 Hz, 2H), 2.00 – 1.89 (m, 4H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.6, 159.3,

157.7, 155.8, 154.0, 136.4, 132.1, 128.6, 127.9, 127.3, 122.1, 119.3, 118.6, 117.8, 113.0, 111.7, 111.3, 51.9, 46.7, 41.7, 25.5, 24.8; HRMS (ESI+): Calculated for $C_{24}H_{27}N_8O_2$ [M+H]: 459.2252, Found: 459.2253 (± 0.3 ppm).

Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxylate (7d): 29% yield, Rf: 0.25 (1:2 - Hexanes: EtOAc); ^{1}H NMR (500 MHz, DMSO) δ 8.42 (s, 2H), 8.15 (dd, J = 5.3, 1.9 Hz, 1H), 7.66 – 7.60 (m, 1H), 7.59 – 7.50 (m, 3H), 7.34 (d, J = 8.1 Hz, 1H), 7.24 (t, J = 5.7 Hz, 1H), 7.17 (d, J = 2.3 Hz, 1H), 7.06 (ddd, J = 8.0, 7.0, 1.2 Hz, 1H), 6.98 (ddd, J = 7.9, 6.9, 1.1 Hz, 1H), 6.89 (d, J = 8.6 Hz, 1H), 6.67 (dd, J = 7.1, 4.9 Hz, 1H), 3.97 – 3.87 (m, 4H), 3.73 (s, 3H), 3.65 – 3.54 (m, 6H), 2.98 (t, J = 7.5 Hz, 2H).; ^{13}C NMR (126 MHz, DMSO) δ 167.5, 161.0, 159.4, 157.7, 155.8, 153.7, 148.0, 137.6, 136.4, 132.2, 128.5, 127.2, 122.3, 122.0, 119.5, 119.0, 118.6, 113.7, 112.9. 112.0, 111.3, 107.3, 52.0, 45.1, 43.7, 41.6, 24.9; HRMS (ESI+): Calculated for $C_{29}H_{31}N_{10}O_2$ [M+H]: 551.2626, Found: 551.2624 (± 0.3 ppm).

5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-(2-((2-(1*H*-indol-3-yl)ethyl)amino)pyrimidin-5-yl)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (8a) (DMA-192): 45% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.12 (s, 2H), 7.50 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.22 (dd, J = 13.8, 8.1 Hz, 2H), 7.01 – 6.82 (m, 7H), 3.61 (dt, J = 17.7, 7.2 Hz, 4H), 2.96 (dt, J = 9.7, 7.1 Hz, 4H).; ^{13}C NMR (126 MHz, CD_3OD) δ 161.5, 157.8, 156.4, 156.1, 154.9, 136.8, 136.7, 127.6, 127.5, 127.4, 122.1, 122.1, 120.9, 118.7, 118.2, 118.0, 117.9, 112.1, 112.0, 110.9, 110.8, 41.8, 41.8, 24.9, 24.0; HRMS (ESI+): Calculated for $C_{30}H_{31}N_{12}O$ [M+H]: 575.2738, Found: 575.2744 (± 1.0 ppm). HPLC Analysis: Retention time = 9.091 min, Purity = 94.314%.

6-(2-((3-(1*H*-imidazol-1-yl)propyl)amino)pyrimidin-5-yl)-5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (8b) (DMA-193): 46% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (s, 2H), 7.71 (s, 1H), 7.39 (d, J = 7.9 Hz, 1H), 7.22 (d, J = 8.1 Hz, 1H), 7.12 (s, 1H), 6.99 – 6.91 (m, 3H), 6.86 (td, J = 7.5, 7.0, 1.0 Hz, 1H), 4.05 (t, J = 6.9 Hz, 2H), 3.65 (t, J = 7.1 Hz, 2H), 3.31 (t, J = 6.7 Hz, 2H), 2.98 (t, J = 7.0 Hz, 2H), 2.03 (p, J = 6.8 Hz, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 166.3, 161.7, 157.9, 156.5, 155.6, 155.1, 136.7, 127.8, 127.6, 122.1, 120.9, 119.0,

118.2, 117.9, 112.1, 110.9, 109.2, 44.5, 41.8, 37.9, 30.3, 24.0; HRMS (ESI+): Calculated for $C_{26}H_{30}N_{13}O$ [M+H]: 540.2691, Found: 540.2699 (± 1.5 ppm). HPLC Analysis: Retention time = 1.762 min, Purity = 99.549%

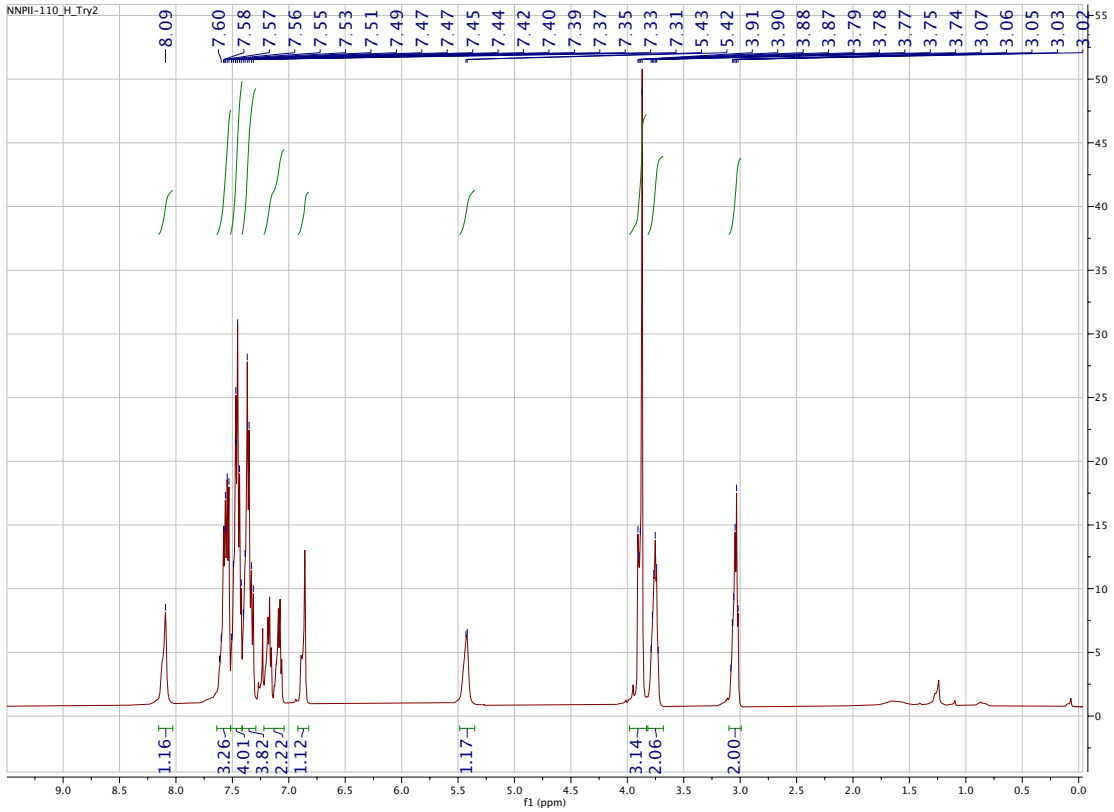
5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(2-(pyrrolidin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (8c) (DMA-194): 53% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 8.41 (s, 2H), 7.38 (d, J = 7.9 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.00 (s, 1H), 6.96 (t, J = 7.6 Hz, 1H), 6.85 (t, J = 7.5 Hz, 1H), 3.65 (t, J = 7.1 Hz, 2H), 3.58 (d, J = 6.8 Hz, 4H), 2.99 (t, J = 7.0 Hz, 2H), 2.01 (dq, J = 9.7, 6.1, 4.9 Hz, 4H); ^{13}C NMR (126 MHz, CD_3OD) δ 166.2, 156.5, 155.6, 154.8, 136.7, 127.6, 124.9, 122.2, 120.9, 118.2, 117.9, 117.8, 112.0, 111.0, 109.7, 47.5, 41.9, 24.8, 24.0; HRMS (ESI+): Calculated for $C_{24}H_{28}N_{11}O$ [M+H]: 486.2473, Found: 486.2481 (± 1.8 ppm). HPLC Analysis: Retention time = 8.187 min, Purity = 95.491%.

5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (8d) (DMA-195): 29% yield, Rf: 0.15 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, CD_3OD) δ 8.27 (s, 2H), 8.07 – 7.98 (m, 1H), 7.50 (ddd, J = 8.9, 7.1, 2.0 Hz, 1H), 7.40 (d, J = 7.9 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.00 – 6.93 (m, 2H), 6.89 – 6.84 (m, 1H), 6.80 (d, J = 8.7 Hz, 1H), 6.62 (dd, J = 7.1, 5.0 Hz, 1H), 3.95 – 3.85 (m, 4H), 3.65 (t, J = 7.1 Hz, 2H), 3.56 – 3.46 (m, 4H), 2.99 (t, J = 7.1 Hz, 2H).; ^{13}C NMR (126 MHz, CD_3OD) δ 166.3, 159.5, 157.6, 148.5, 147.0, 138.0, 136.8, 122.1, 120.9, 118.7, 118.2, 117.9, 113.5, 112.0, 110.9, 108.0, 45.0, 43.4, 41.8, 24.0 ; HRMS (ESI+): Calculated for $C_{29}H_{32}N_{13}O$ [M+H]: 578.2847, Found: 578.2846 (± 0.3 ppm). HPLC Analysis: Retention time = 1.709 min, Purity = 99.898%.

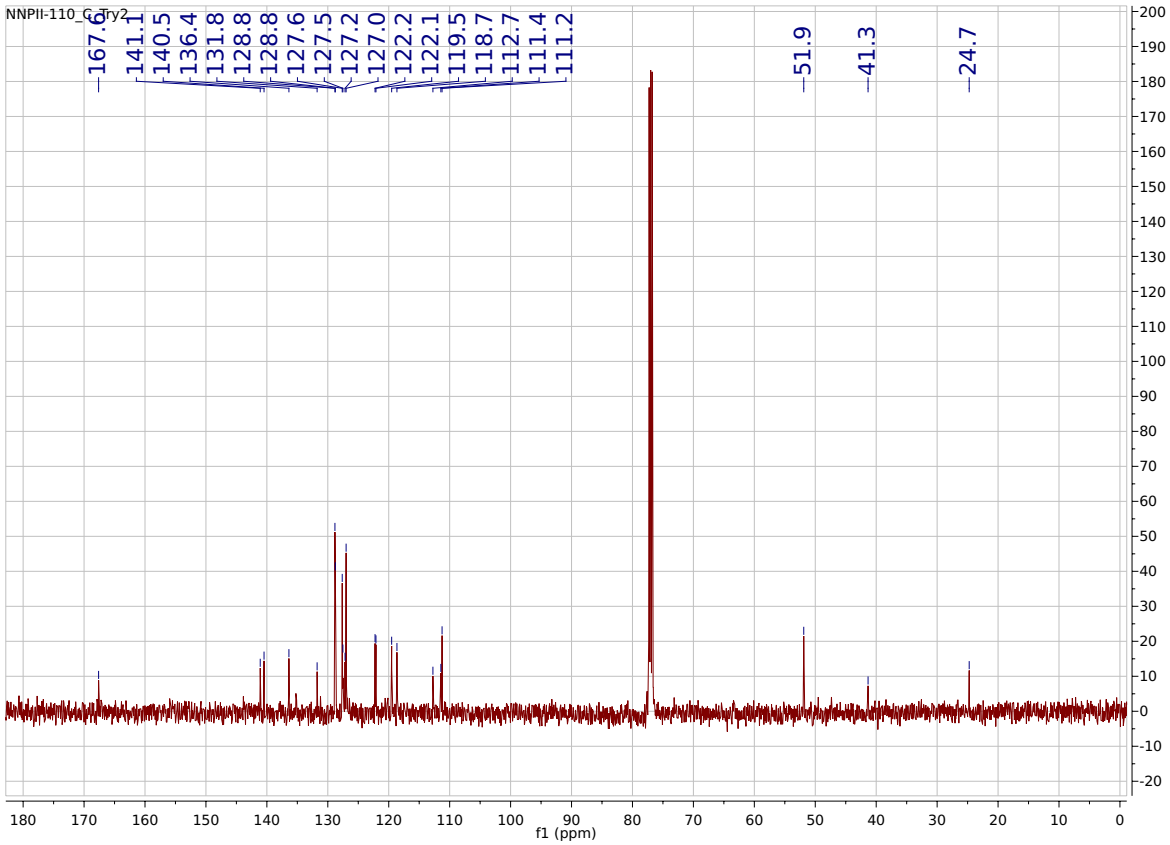
5-(((1H-indol-3-yl)methyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-amino-N-carbamimidoylpyrazine-2-carboxamide (4v) (DMA-207): 11% yield, Rf: 0.16 (85:13:2 – DCM: MeOH: NH_4OH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.75 – 7.64 (m, 5H), 7.61 (d, *J* = 8.1 Hz, 2H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.39 – 7.30 (m, 3H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 4.71 (s, 2H).; ^{13}C NMR (126 MHz, DMSO) δ 155.78, 140.37, 139.97, 136.39, 135.49, 129.58, 128.19, 127.36, 127.03, 126.93, 125.09, 121.61, 119.12, 112.26, 111.88, 36.20. HRMS (ESI+): Calculated for $C_{27}H_{24}N_8O$ [M+H]: 477.2146, Found: 477.2156 (± 2.2 ppm). HPLC Analysis: Retention time = 9.112 min, Purity = 95.271%.

NMR spectra for intermediates and final compounds -

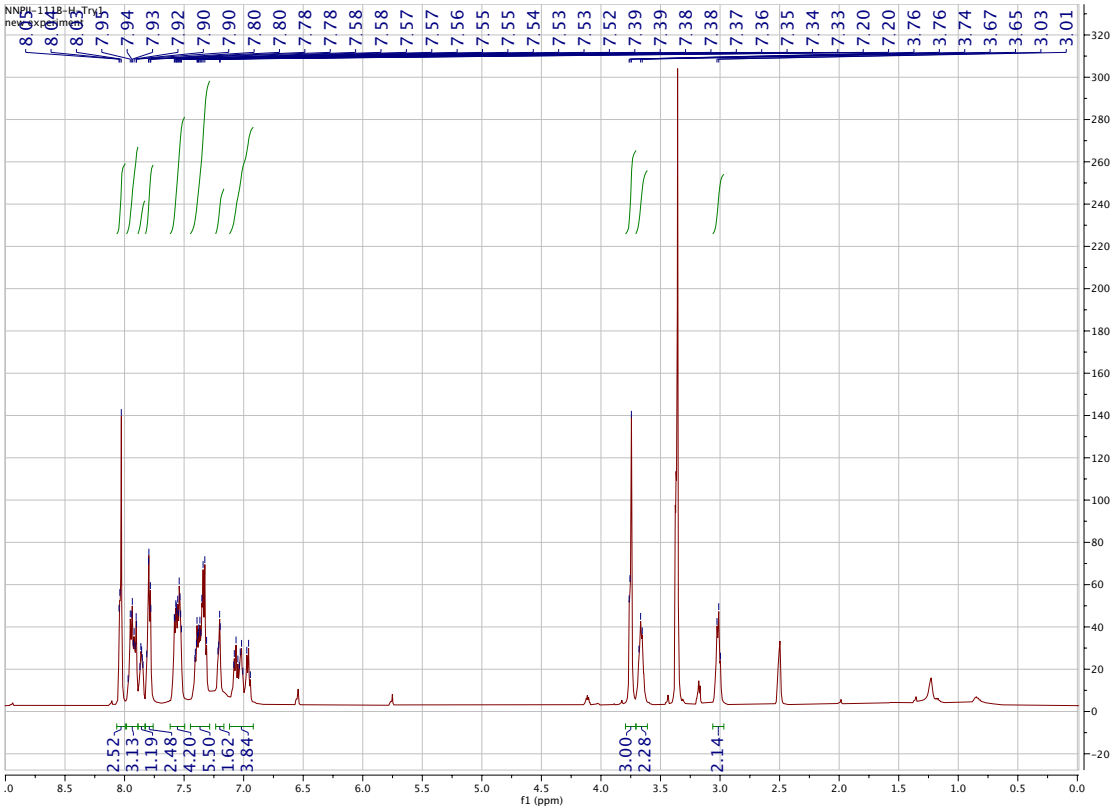
^{1}H – NMR of Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-aminopyrazine-2-carboxylate (3a):



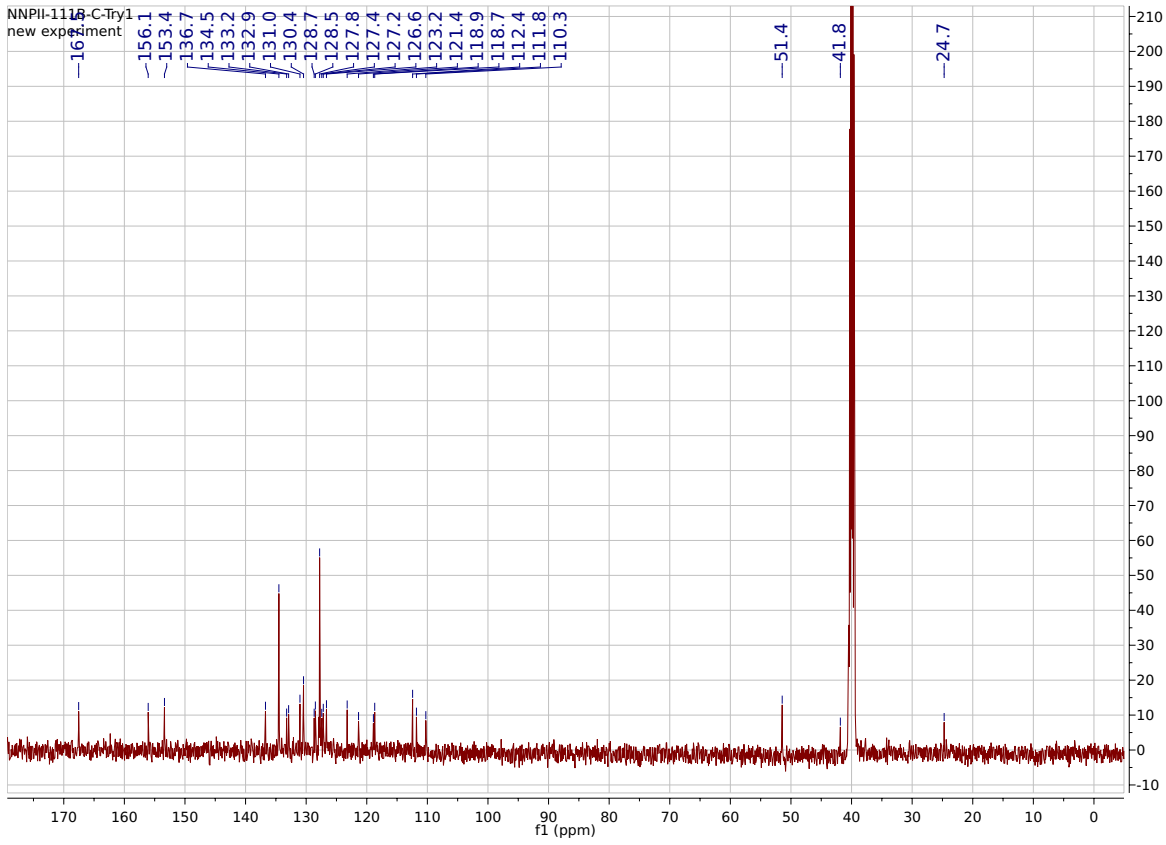
^{13}C – NMR of Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-aminopyrazine-2-carboxylate (3a):



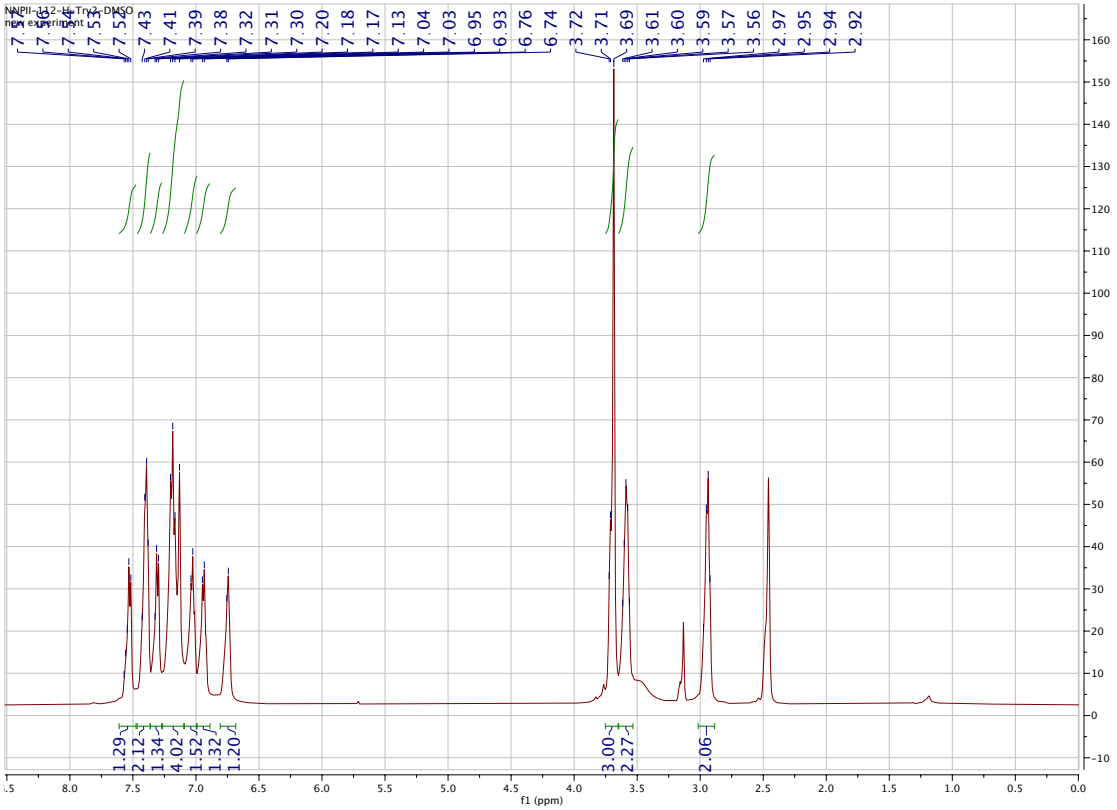
^{1}H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(naphthalen-2-yl)pyrazine-2-carboxylate (3b)



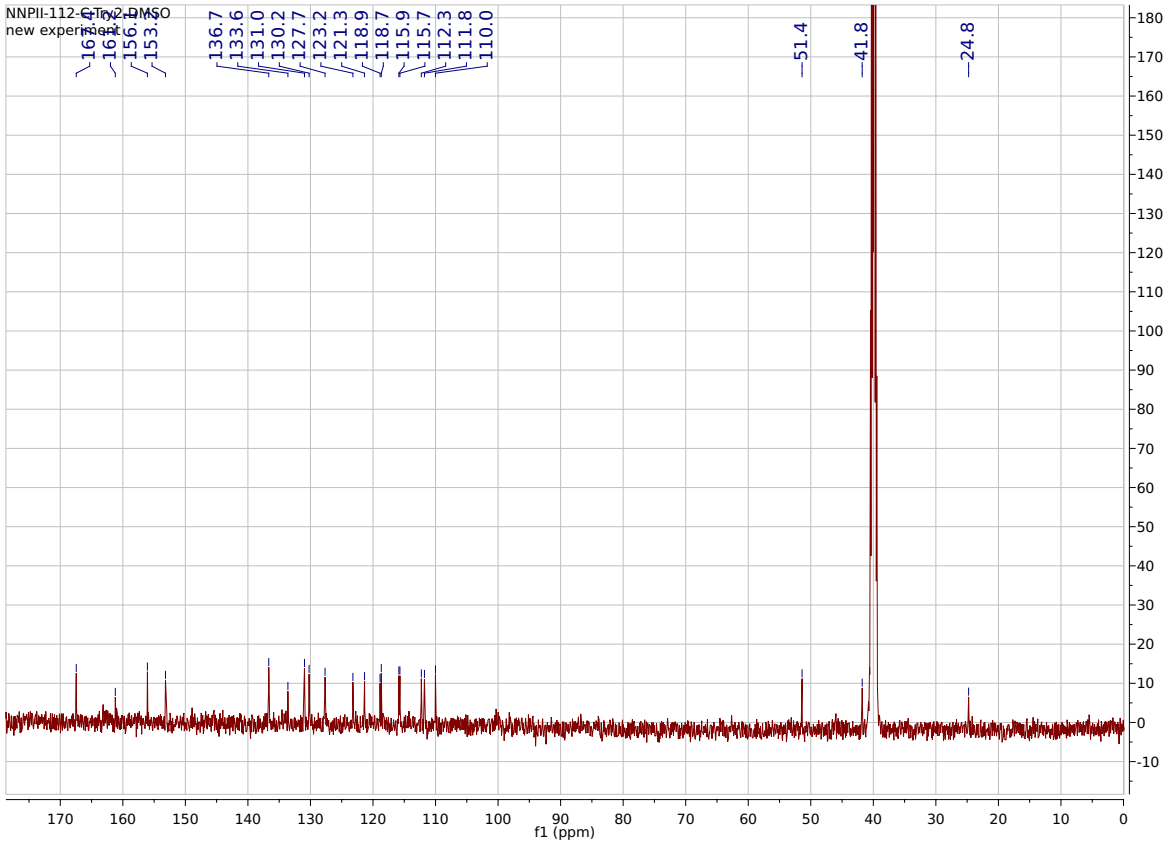
^{13}C NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(naphthalen-2-yl)pyrazine-2-carboxylate (3b)



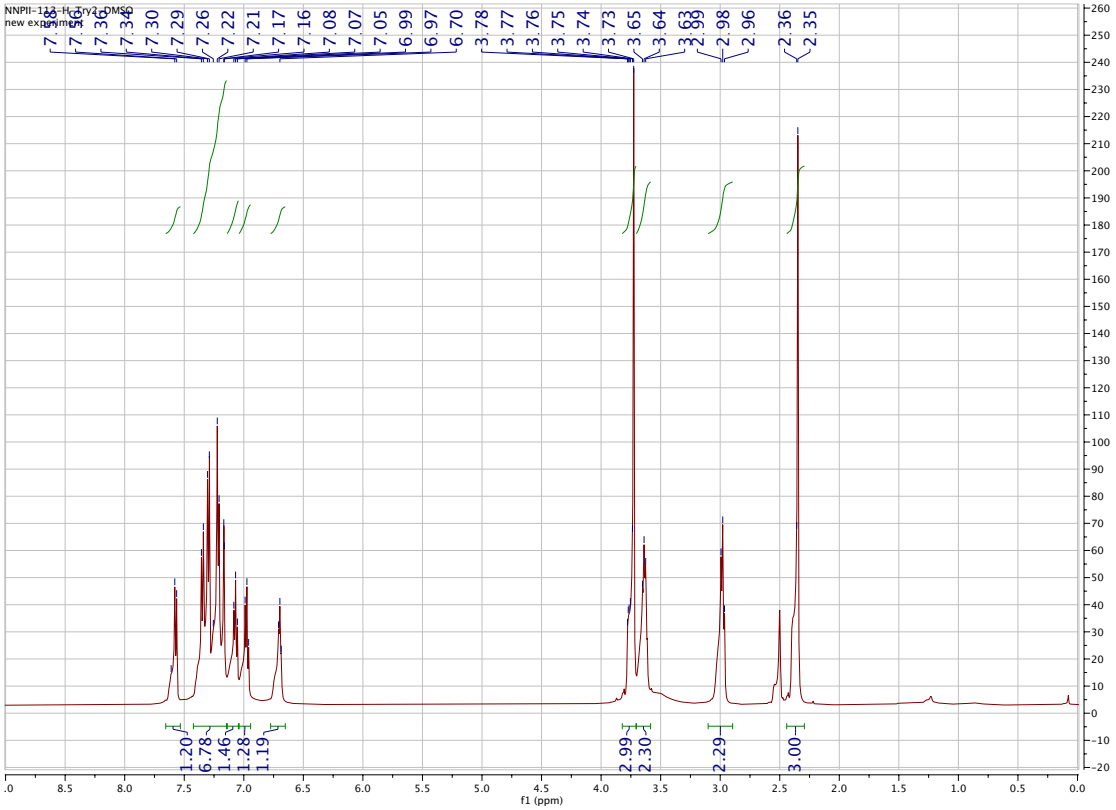
^{1}H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(4-fluorophenyl)pyrazine-2-carboxylate (3c):



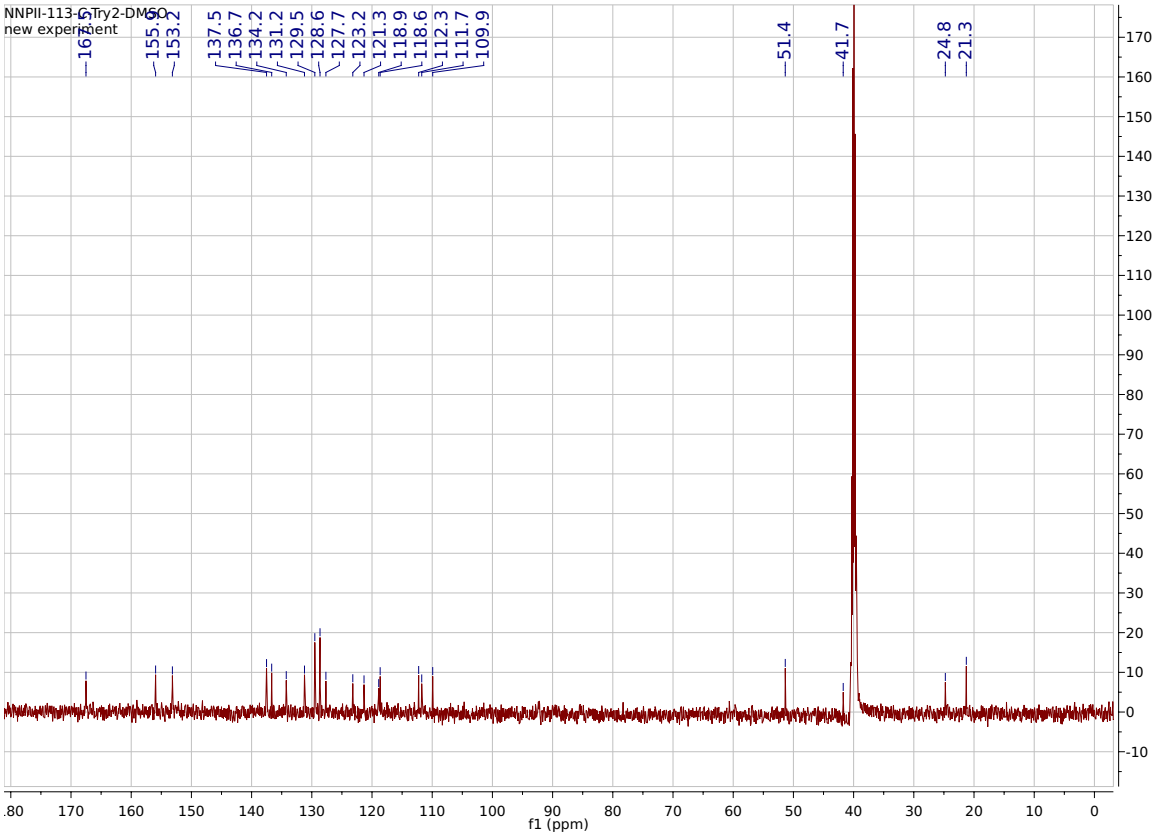
^{13}C NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(4-fluorophenyl)pyrazine-2-carboxylate (3c):



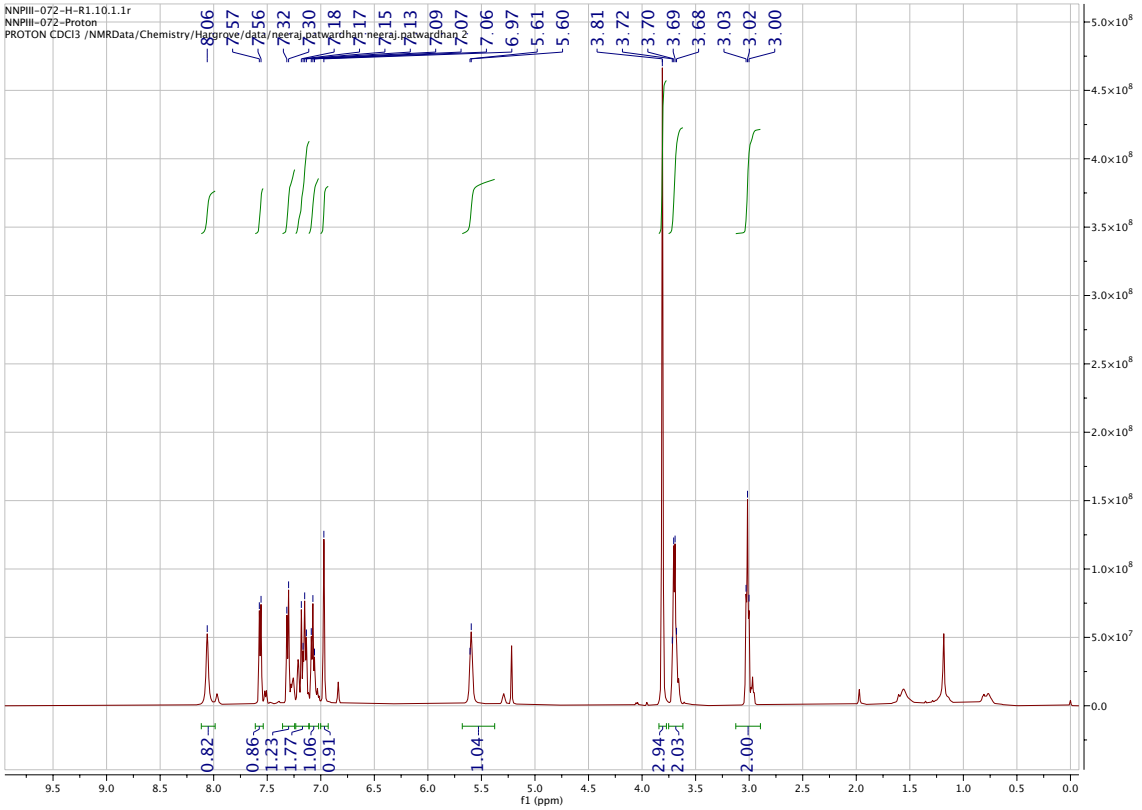
^{1}H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(*p*-tolyl)pyrazine-2-carboxylate (3d):



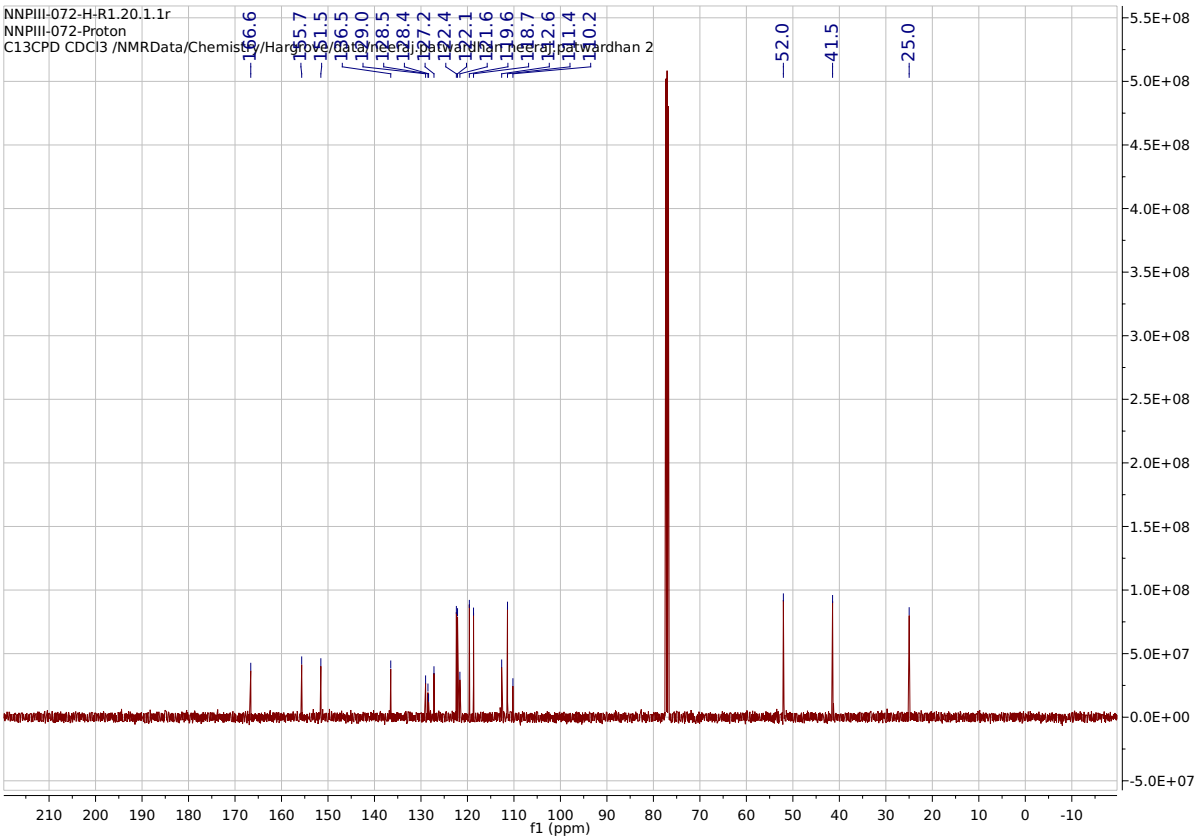
^{13}C NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(*p*-tolyl)pyrazine-2-carboxylate (3d):



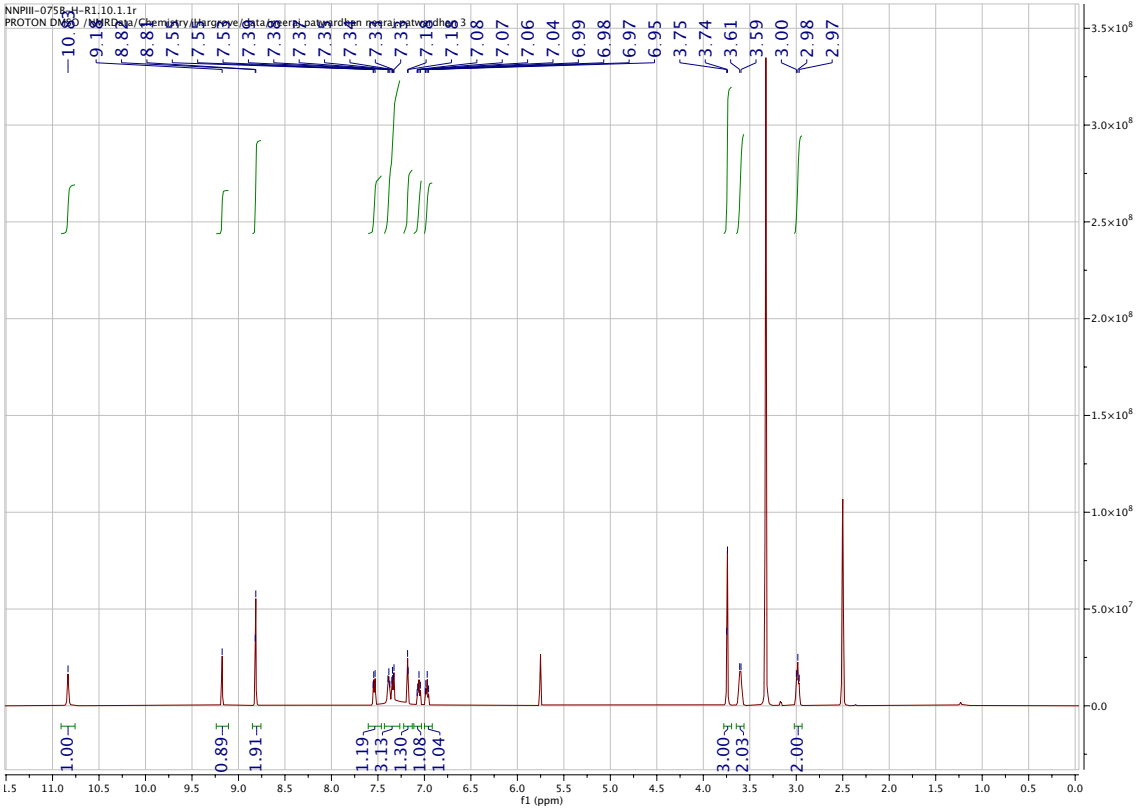
1H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(pyridin-4-yl)pyrazine-2-carboxylate (3e)



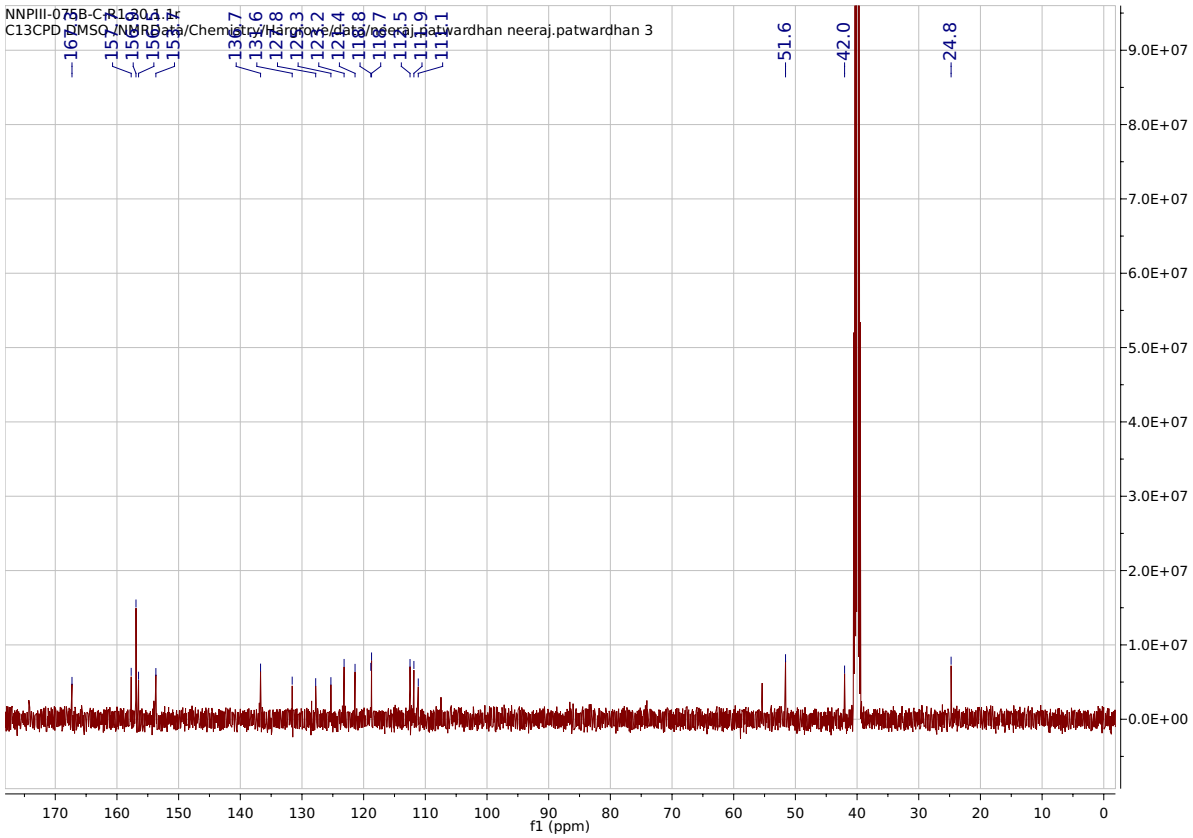
13C NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(pyridin-4-yl)pyrazine-2-carboxylate (3e)



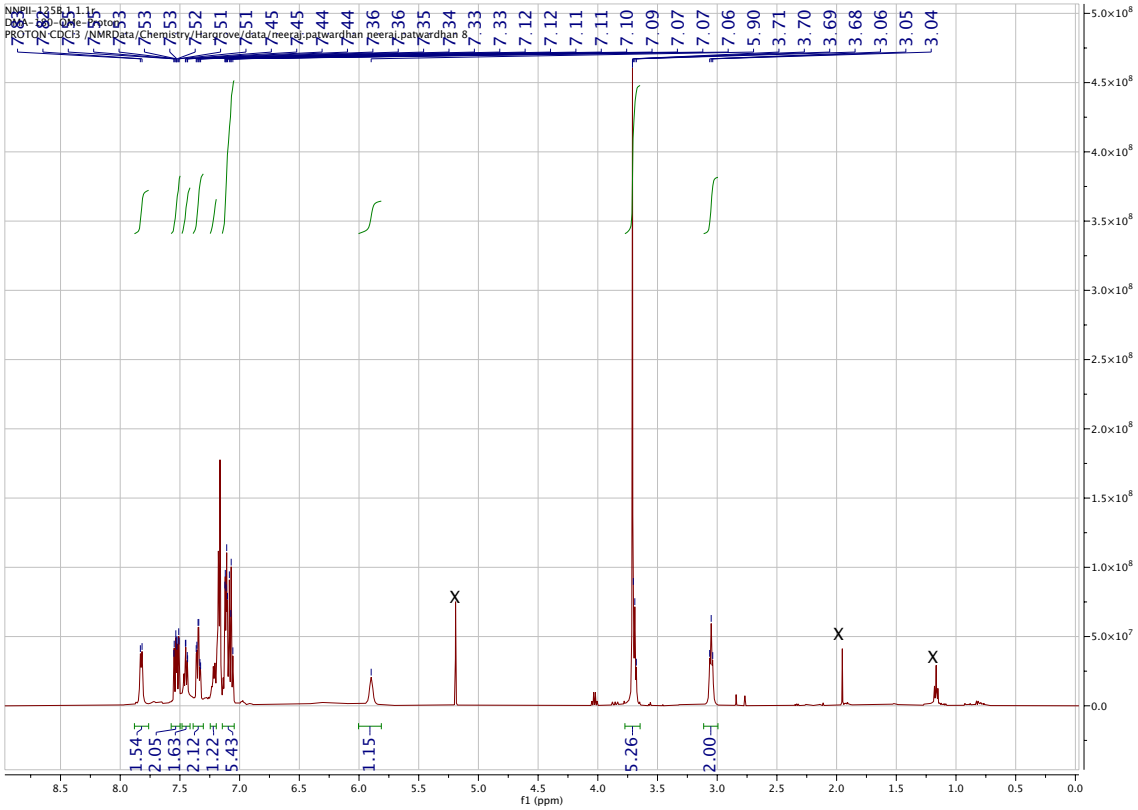
^{1}H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(pyrimidin-5-yl)pyrazine-2-carboxylate (3f)



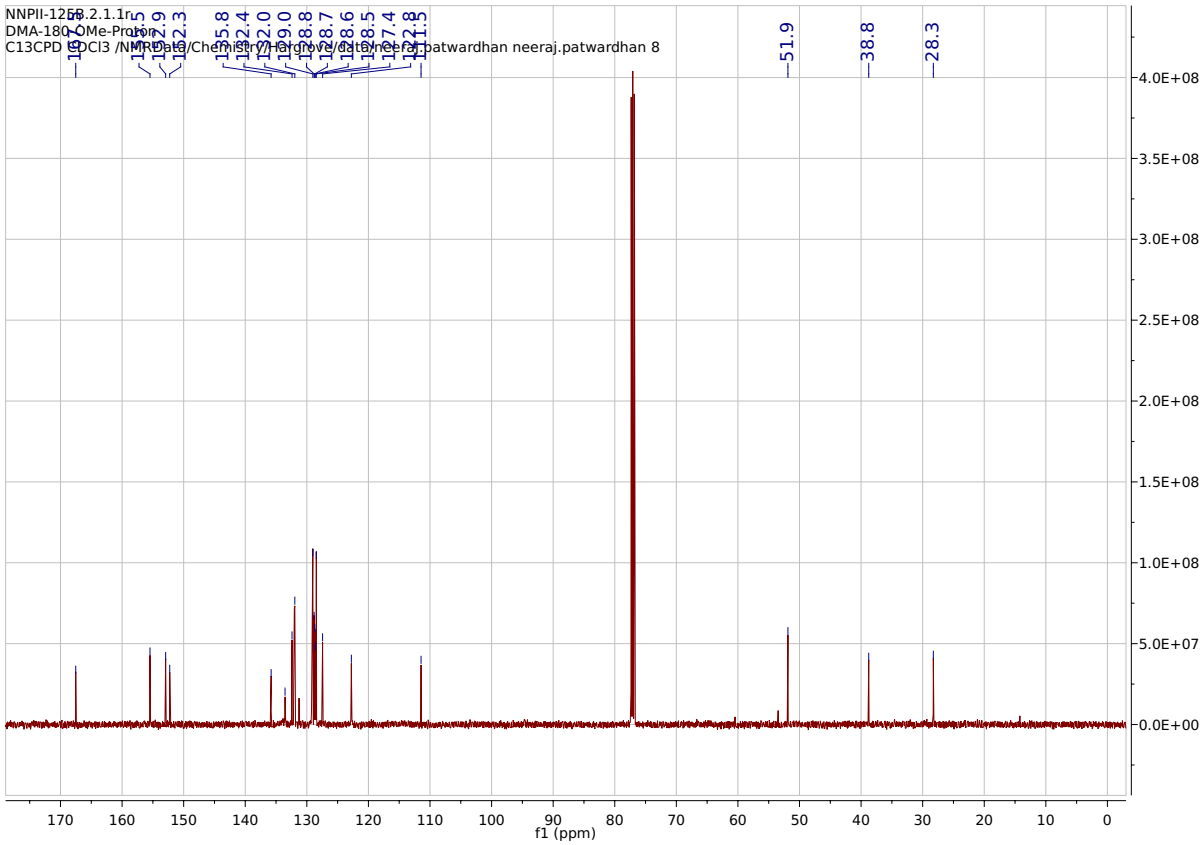
^{13}C NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(pyrimidin-5-yl)pyrazine-2-carboxylate (3f)



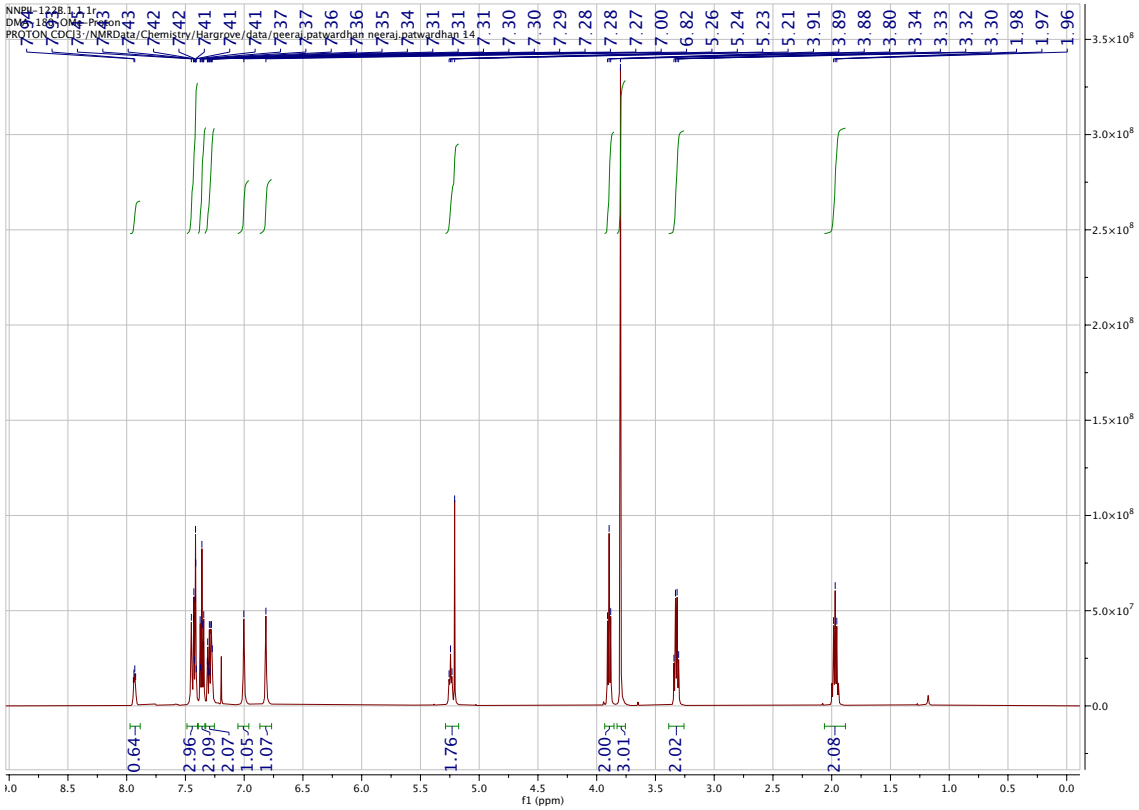
^{1}H NMR for Methyl 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3g):



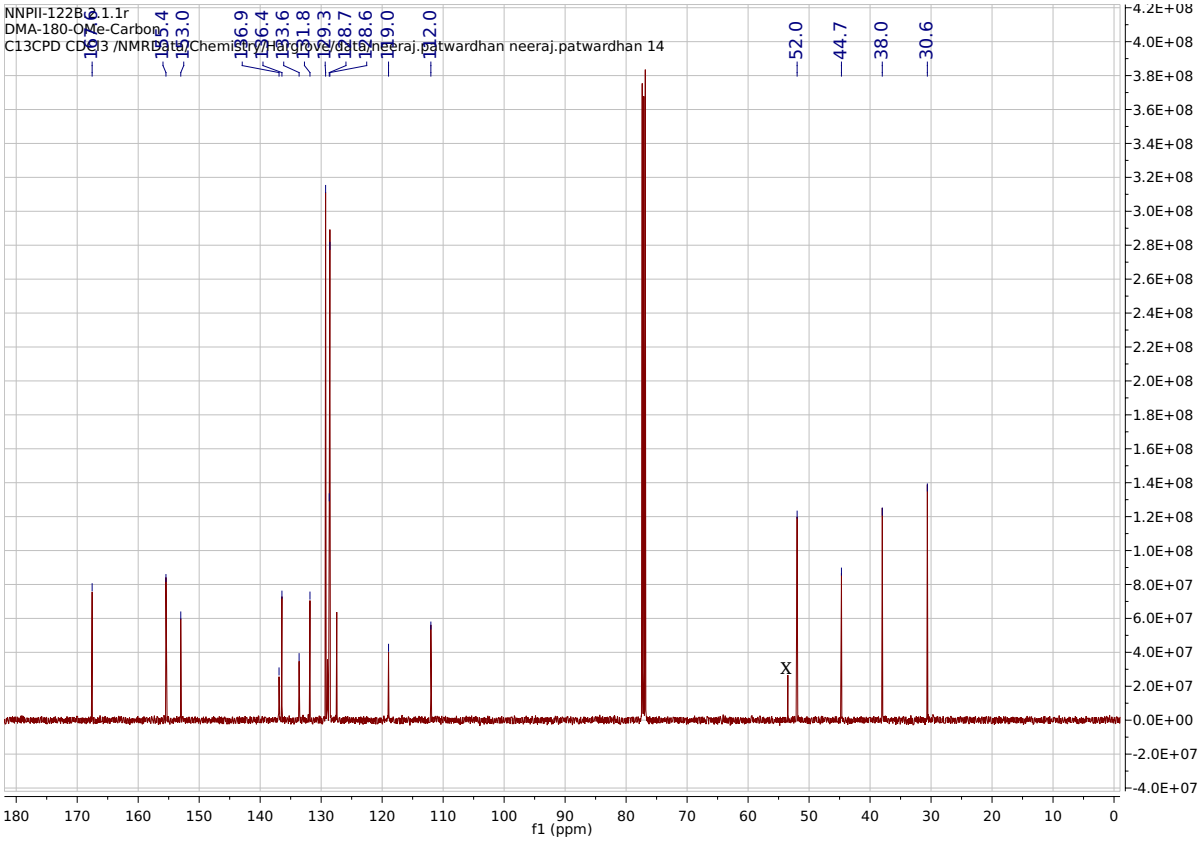
^{13}C NMR for Methyl 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3g):



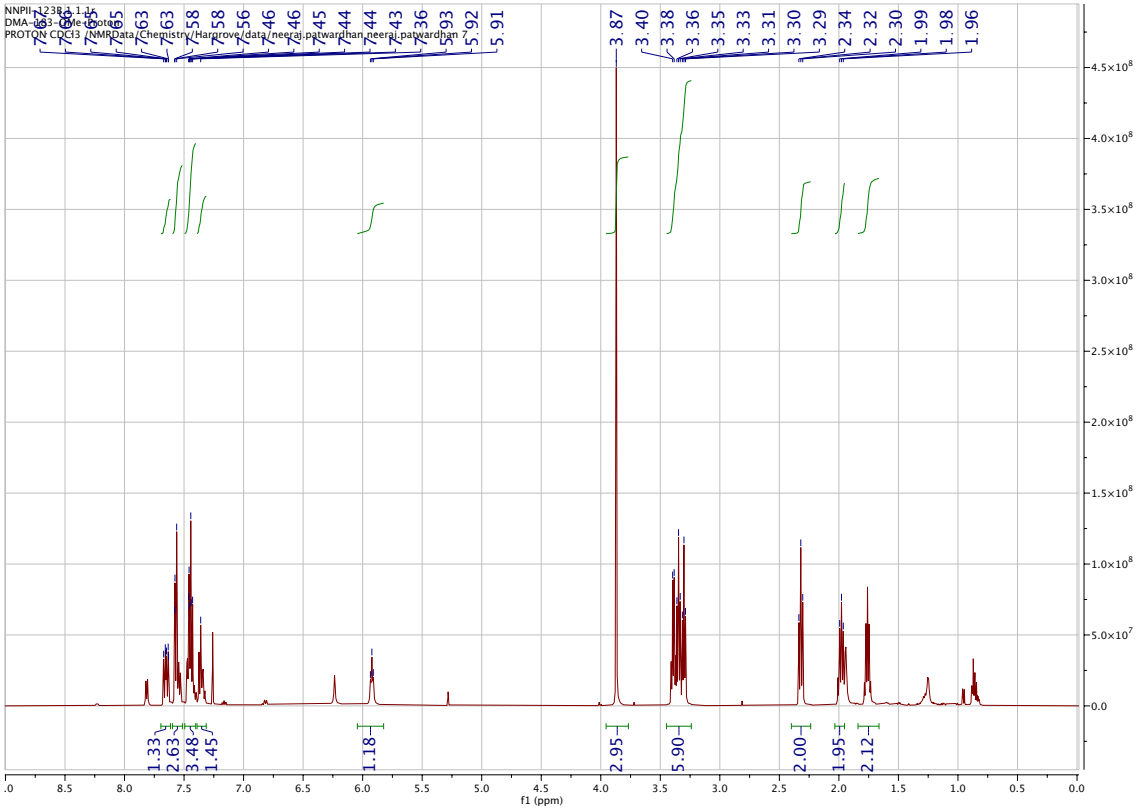
^{1}H NMR for Methyl 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3h)



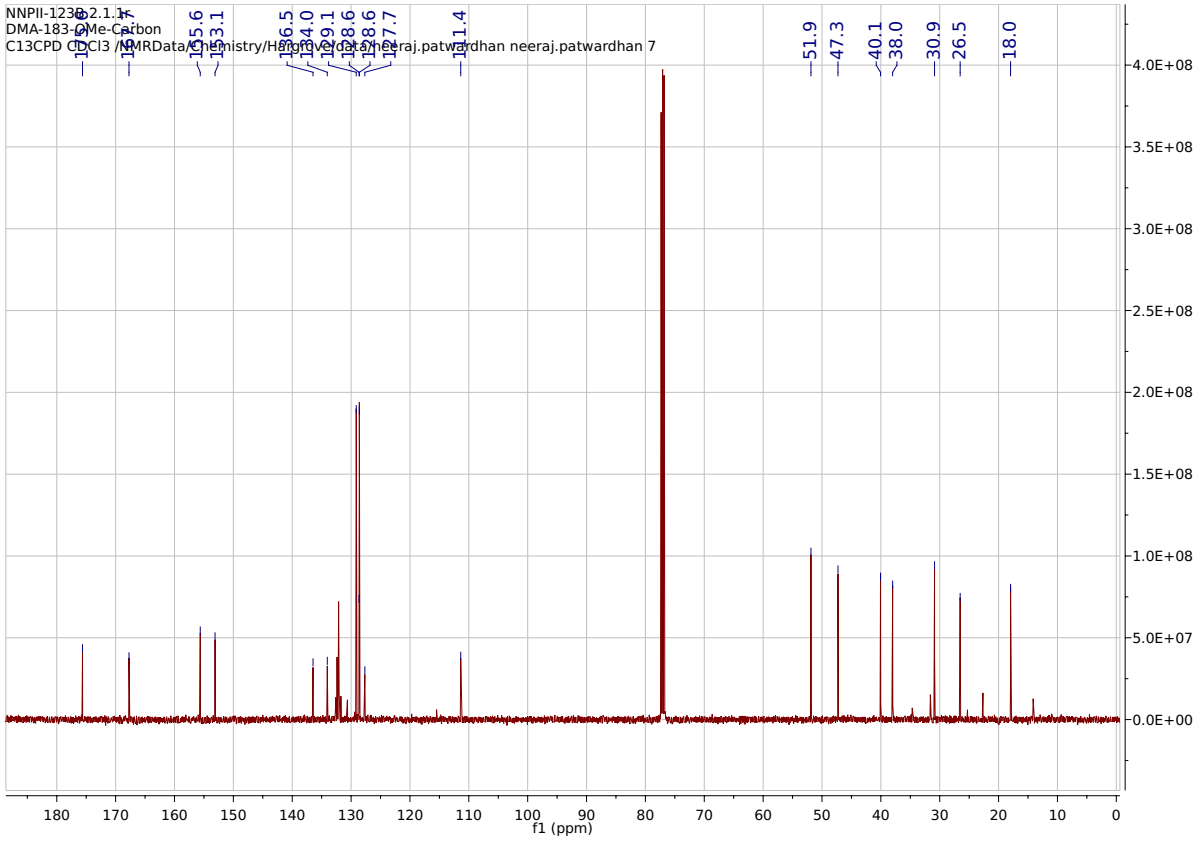
^{13}C NMR for Methyl 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3h)



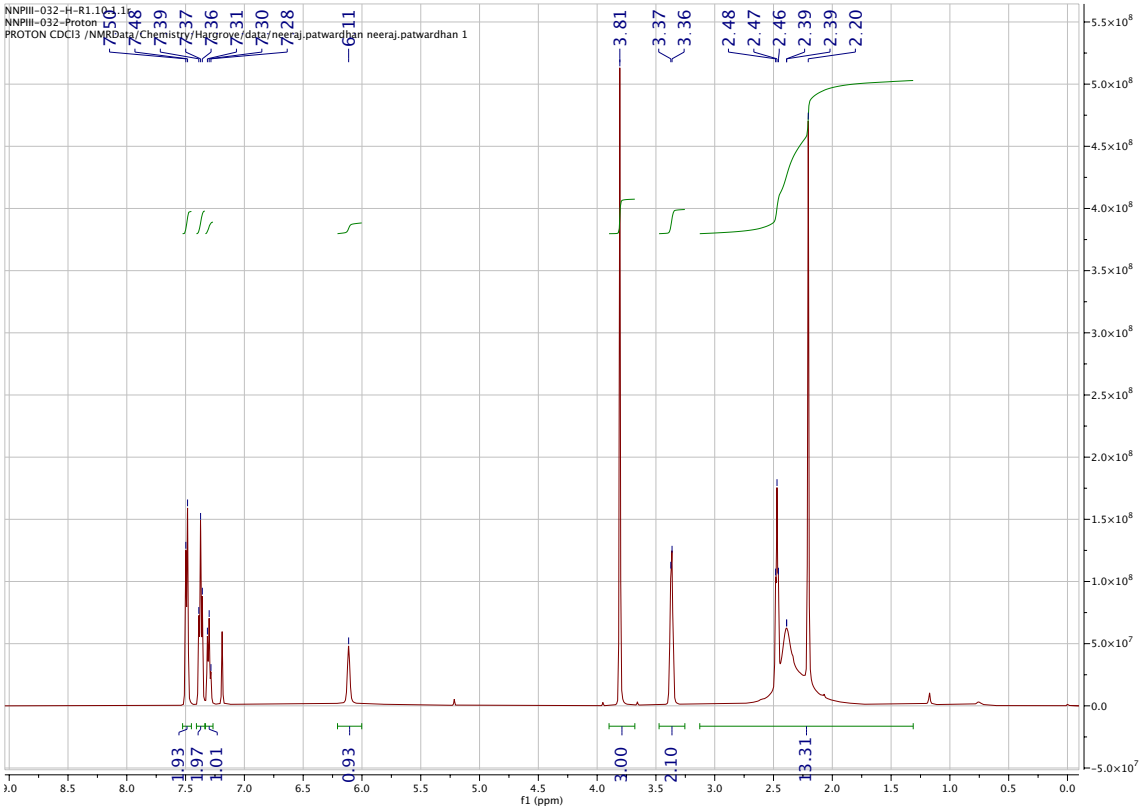
^{1}H NMR for Methyl 3-amino-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)-6-phenylpyrazine-2-carboxylate (3i)



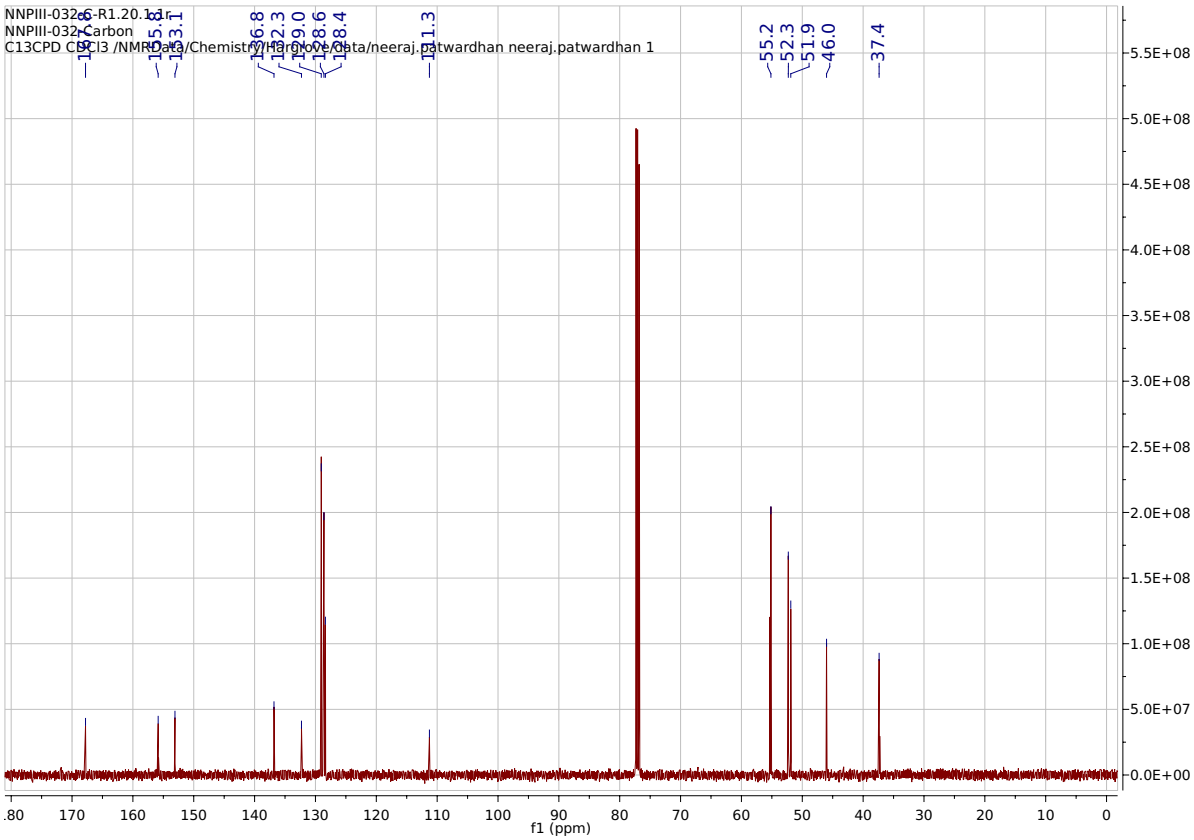
^{13}C NMR for Methyl 3-amino-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)-6-phenylpyrazine-2-carboxylate (3i)



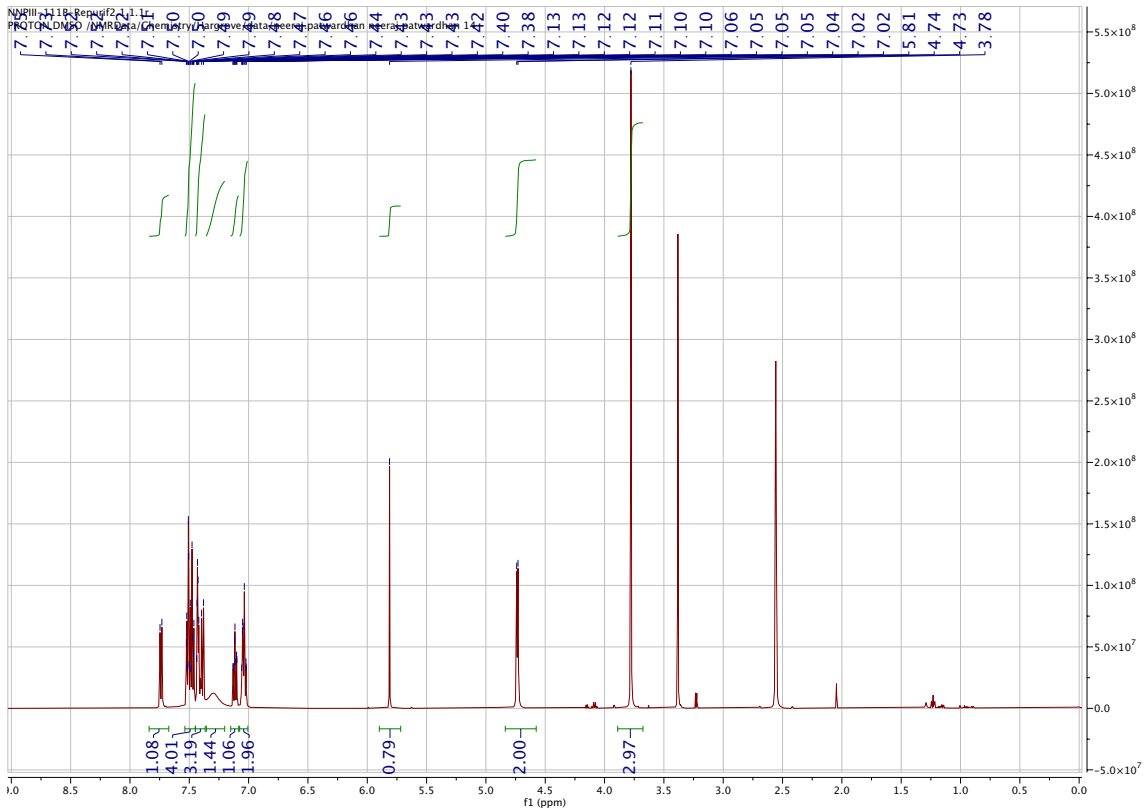
^{1}H NMR for Methyl 3-amino-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)-6-phenylpyrazine-2-carboxylate (3j)



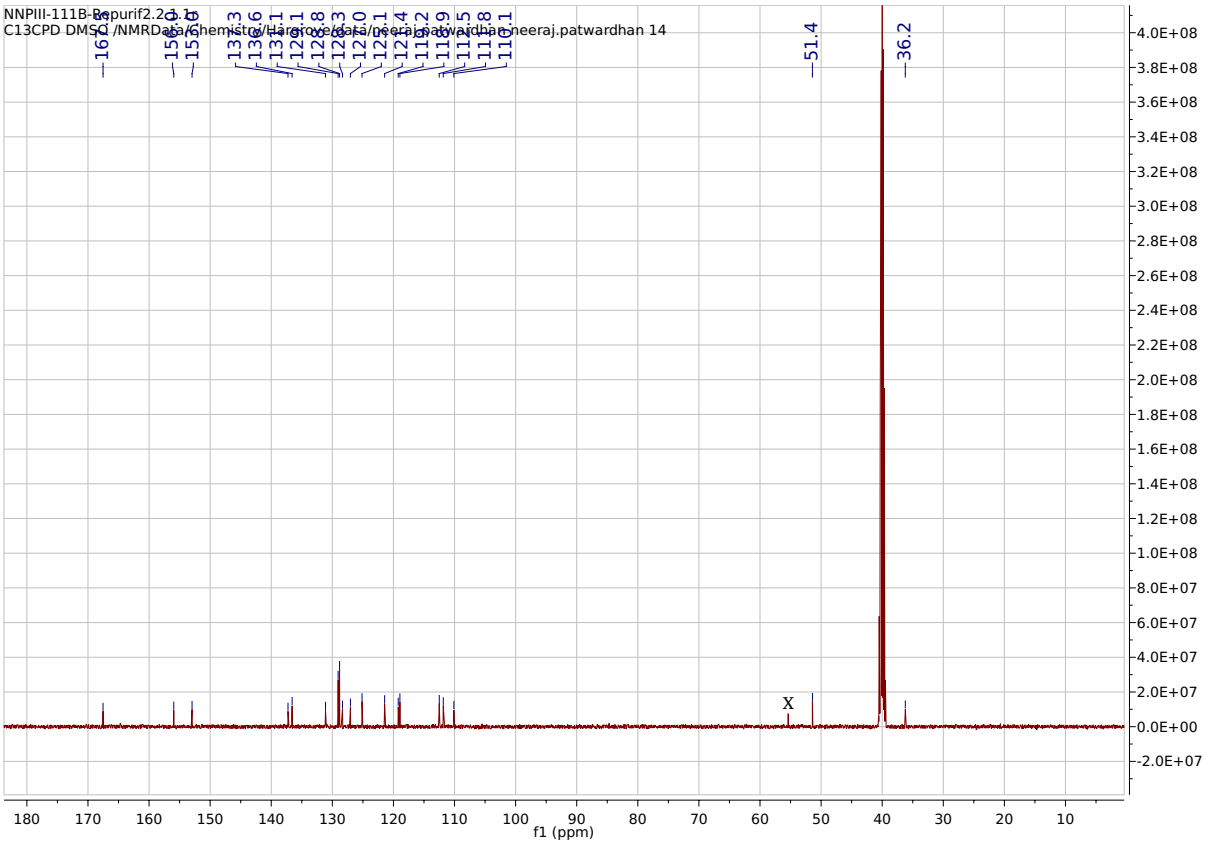
^{13}C NMR for Methyl 3-amino-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)-6-phenylpyrazine-2-carboxylate (3j)



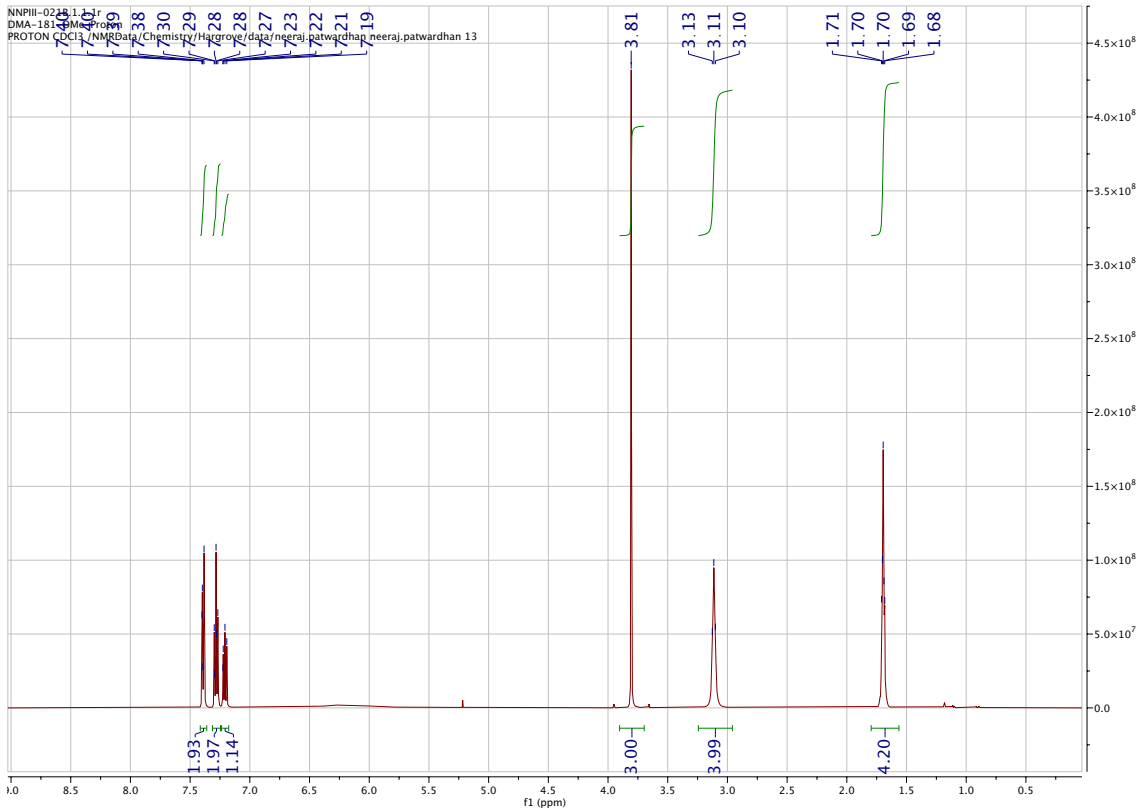
^{1}H NMR for Methyl 5-(((1*H*-indol-3-yl)methyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3k)



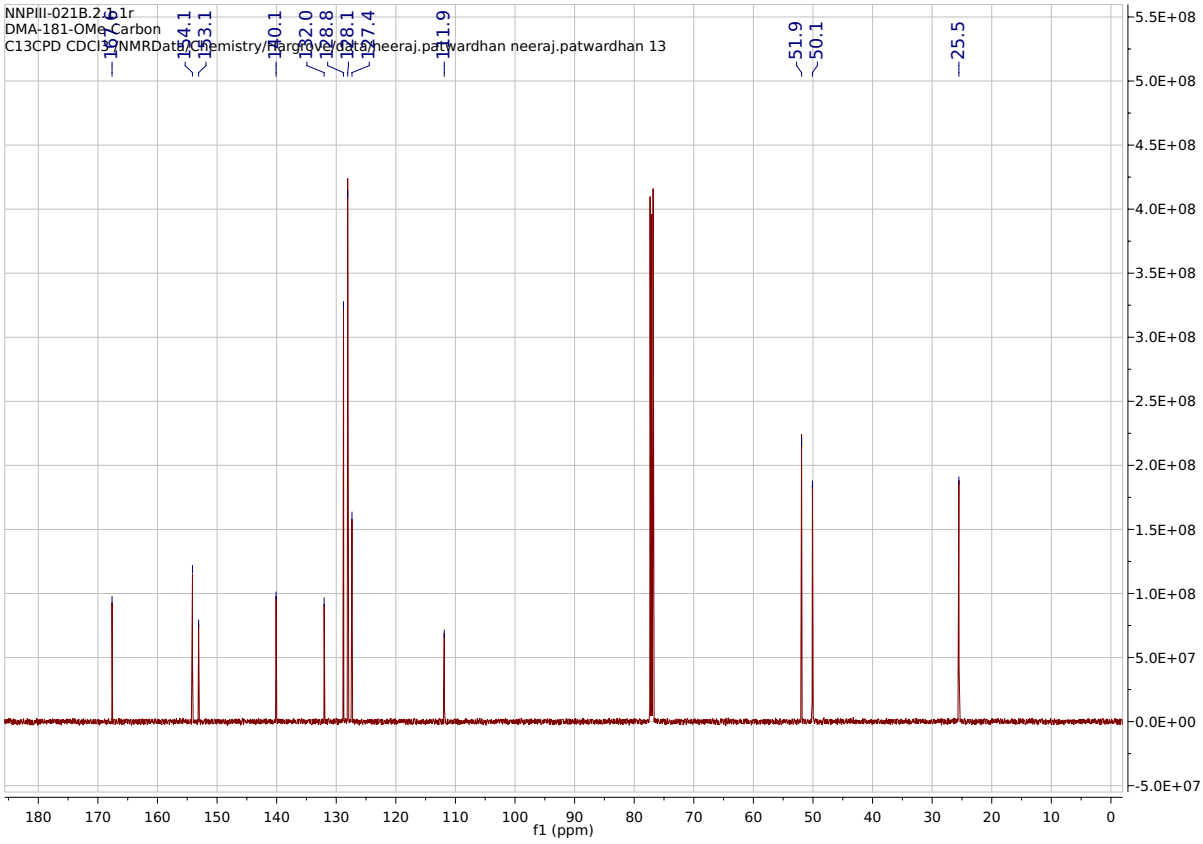
^{13}C NMR for Methyl 5-(((1*H*-indol-3-yl)methyl)amino)-3-amino-6-phenylpyrazine-2-carboxylate (3k)



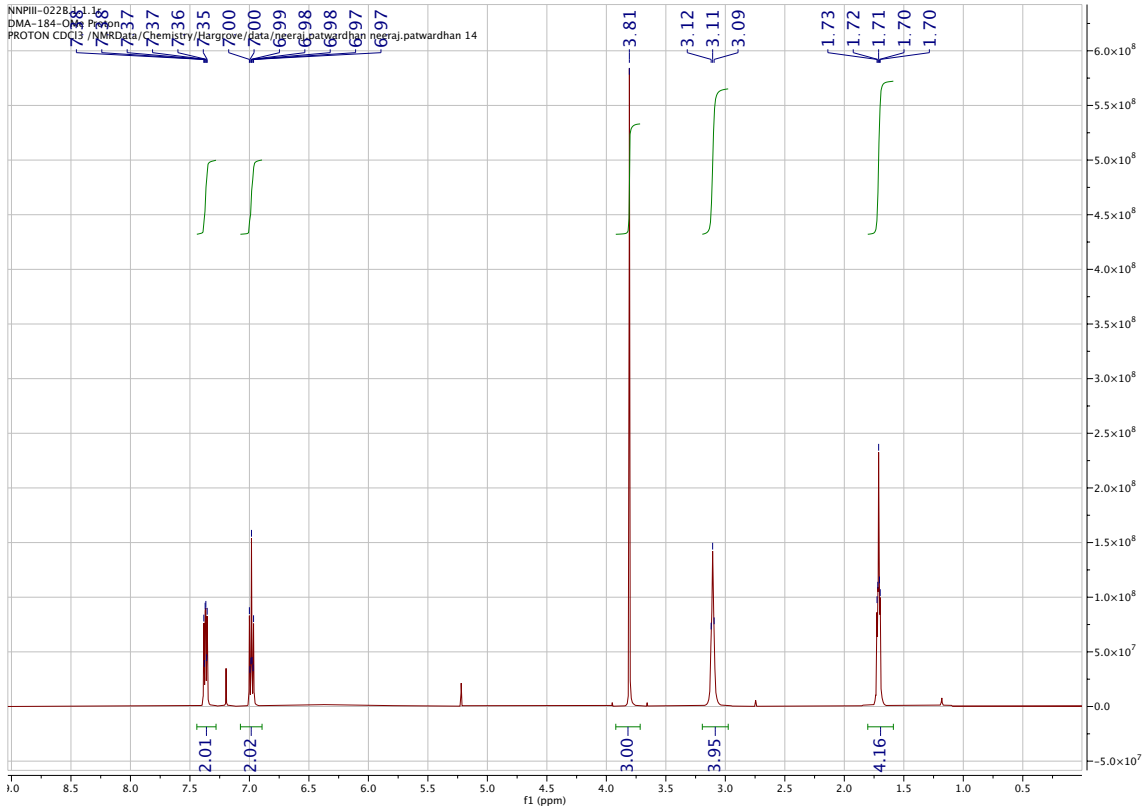
^{1}H NMR for Methyl 3-amino-6-phenyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3l)



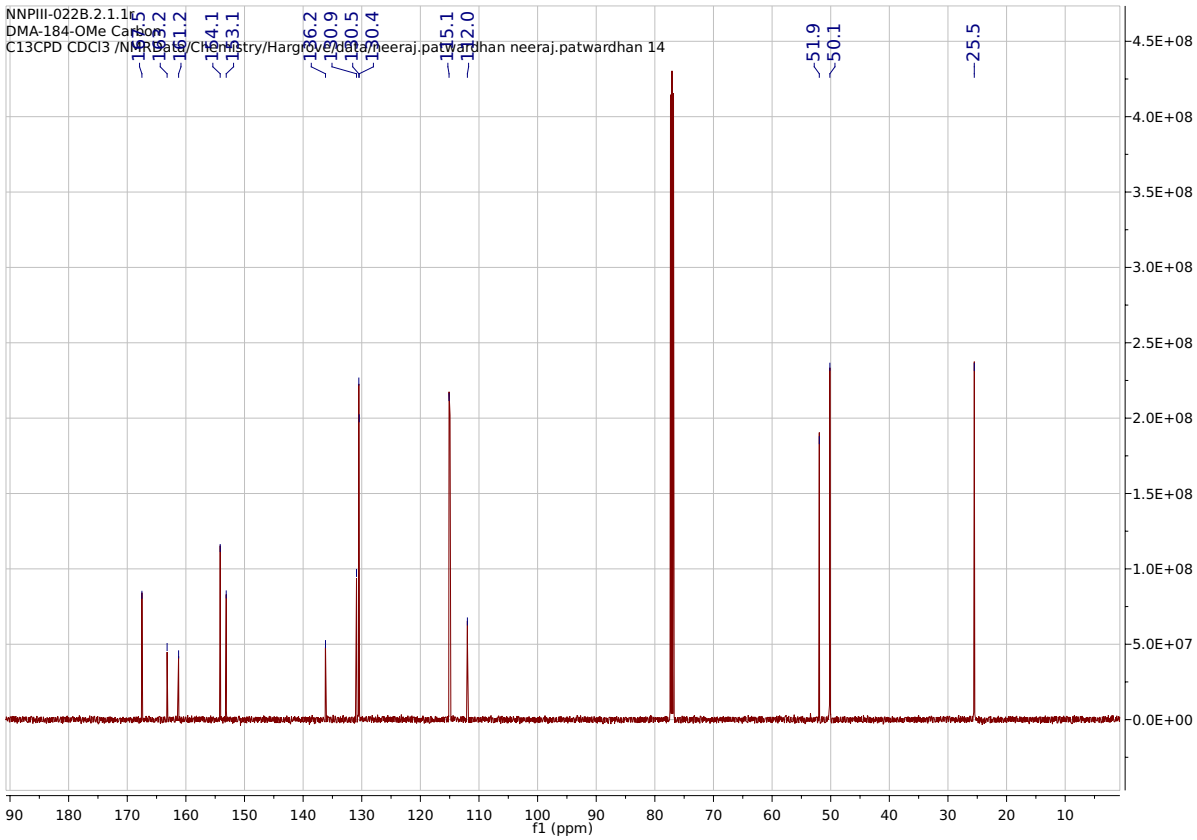
^{13}C NMR for Methyl 3-amino-6-phenyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3l)



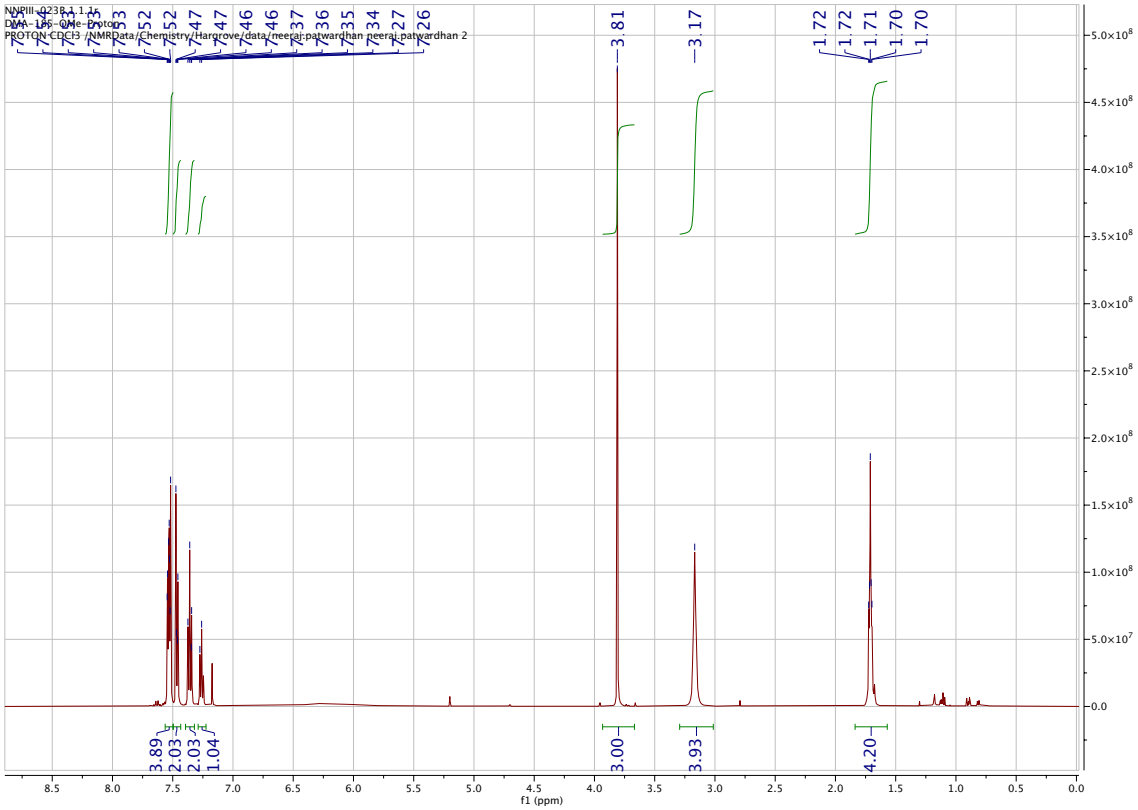
^{1}H NMR for Methyl 3-amino-6-(4-fluorophenyl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3m)



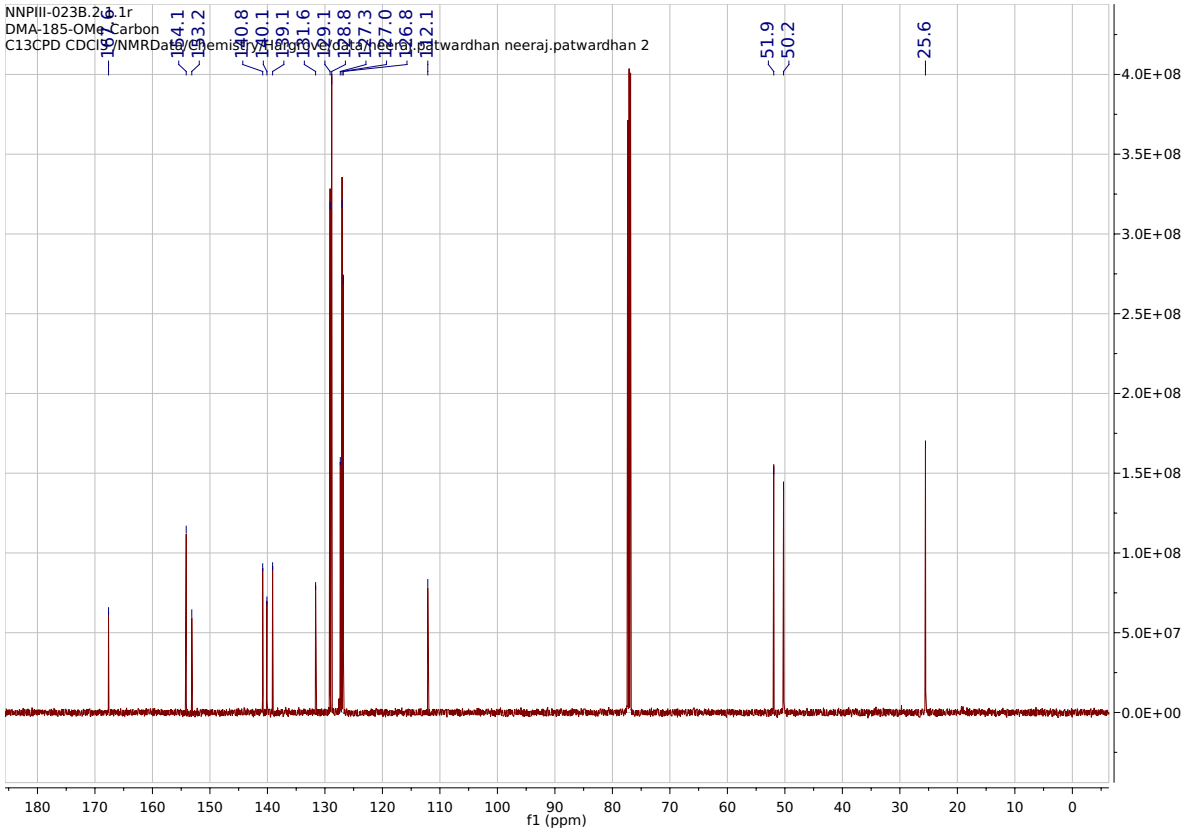
^{13}C NMR for Methyl 3-amino-6-(4-fluorophenyl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3m)



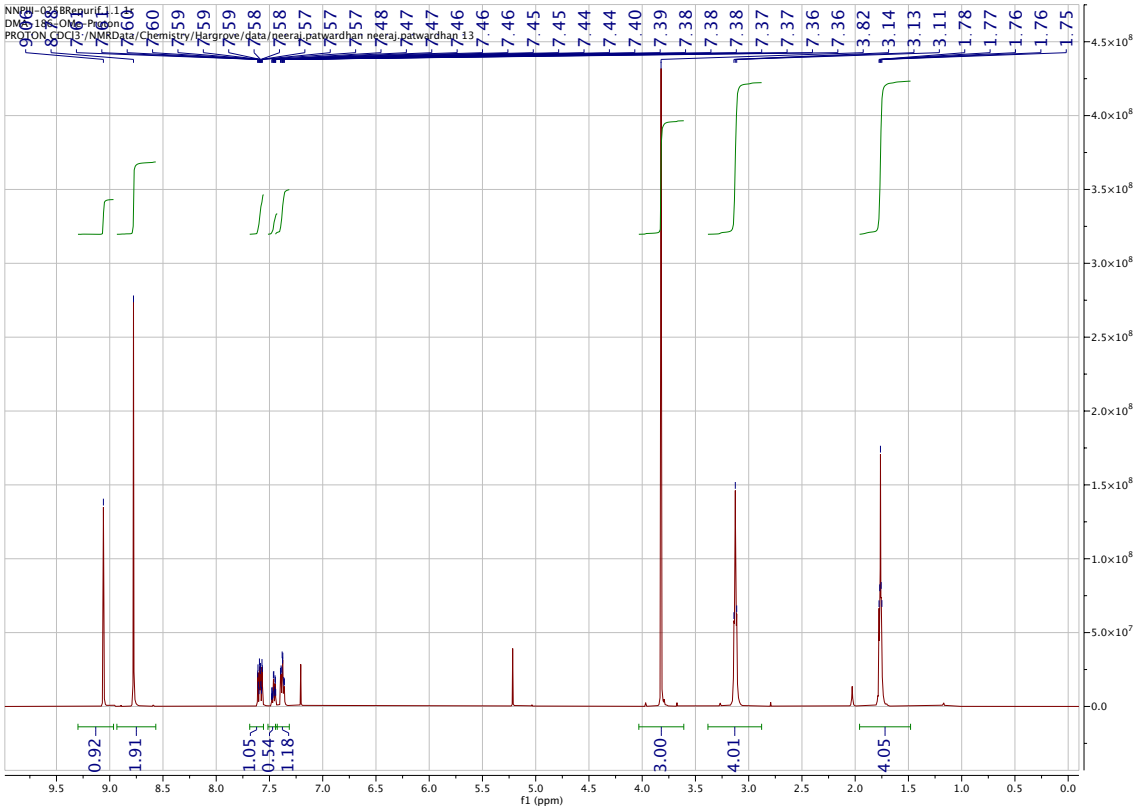
^{1}H NMR for Methyl 6-([1,1'-biphenyl]-4-yl)-3-amino-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3n)



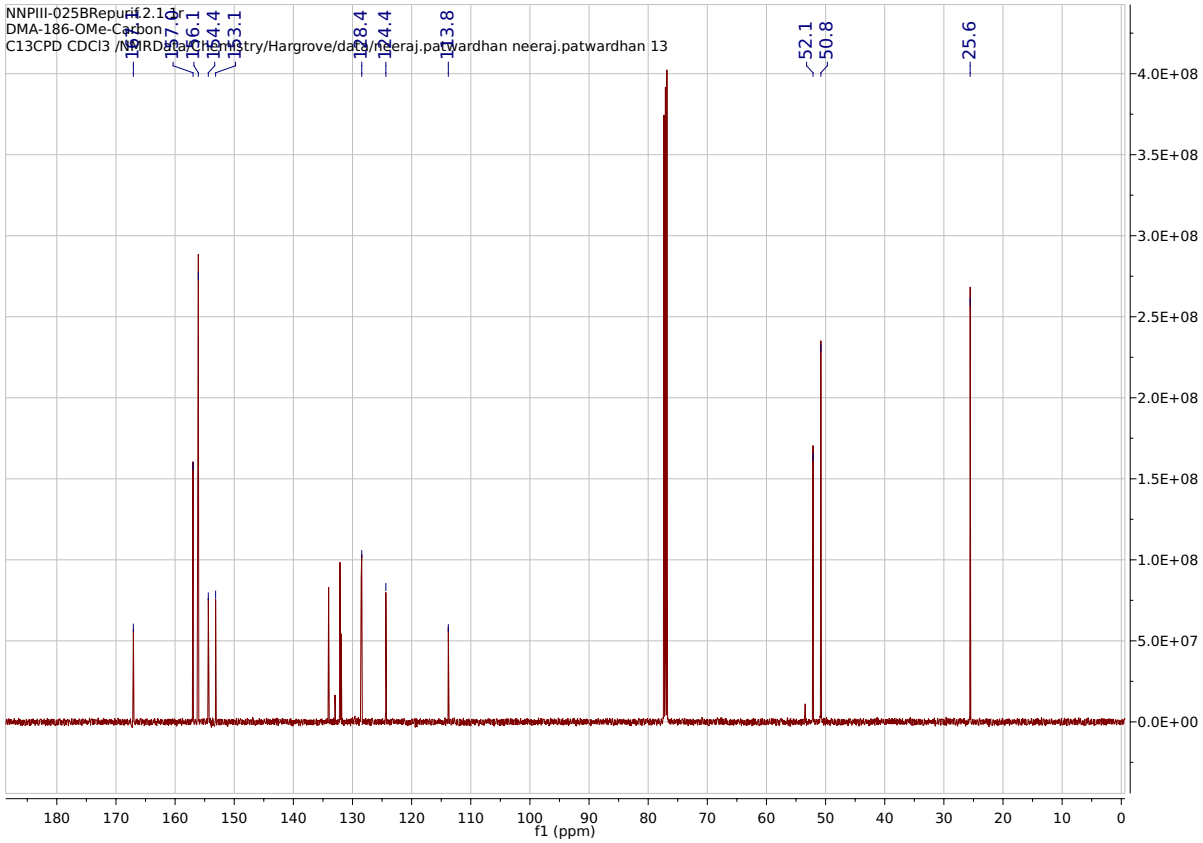
^{13}C NMR for Methyl 6-([1,1'-biphenyl]-4-yl)-3-amino-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3n)



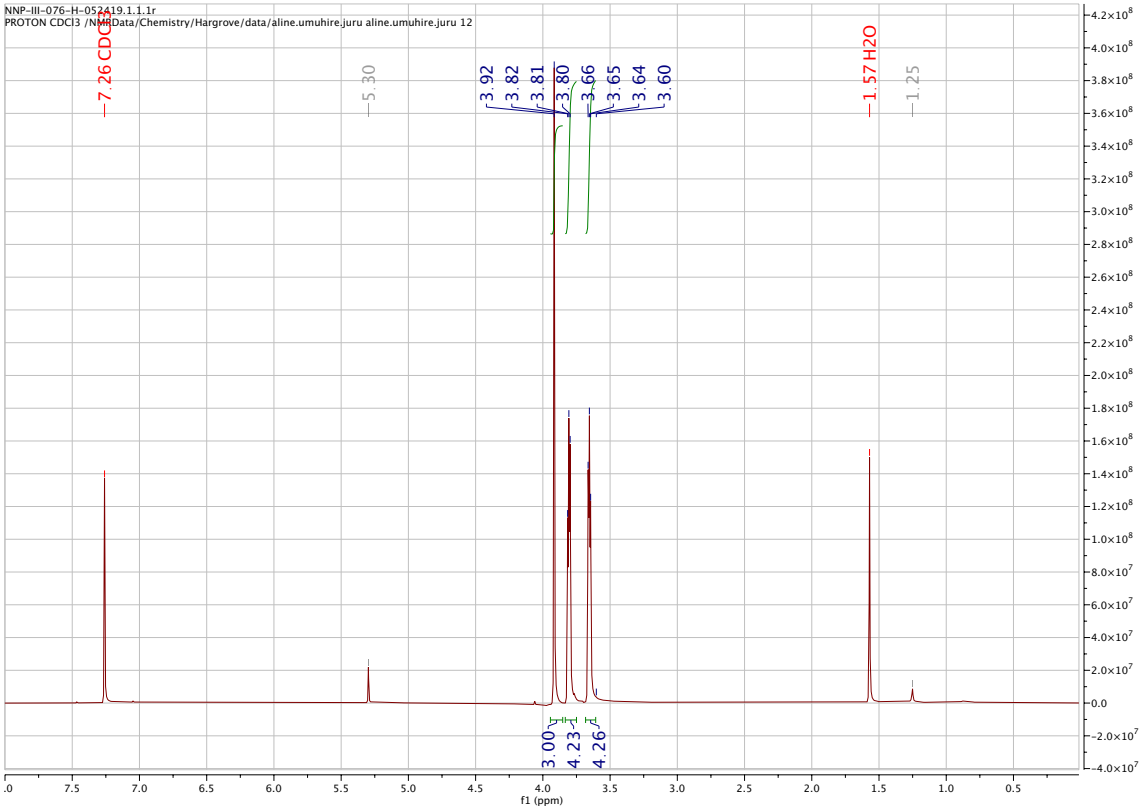
^{1}H NMR for Methyl 3-amino-6-(pyrimidin-5-yl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3o):



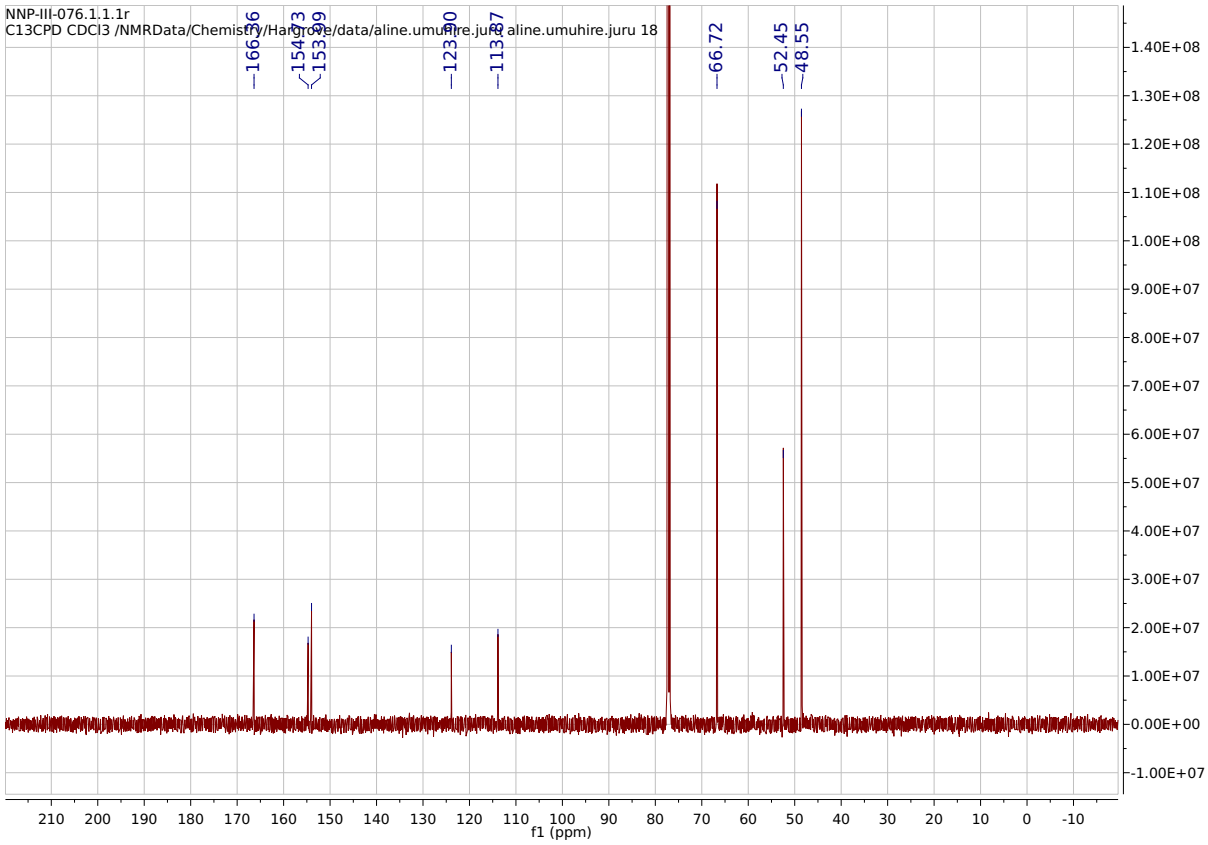
^{13}C NMR for Methyl 3-amino-6-(pyrimidin-5-yl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxylate (3o):



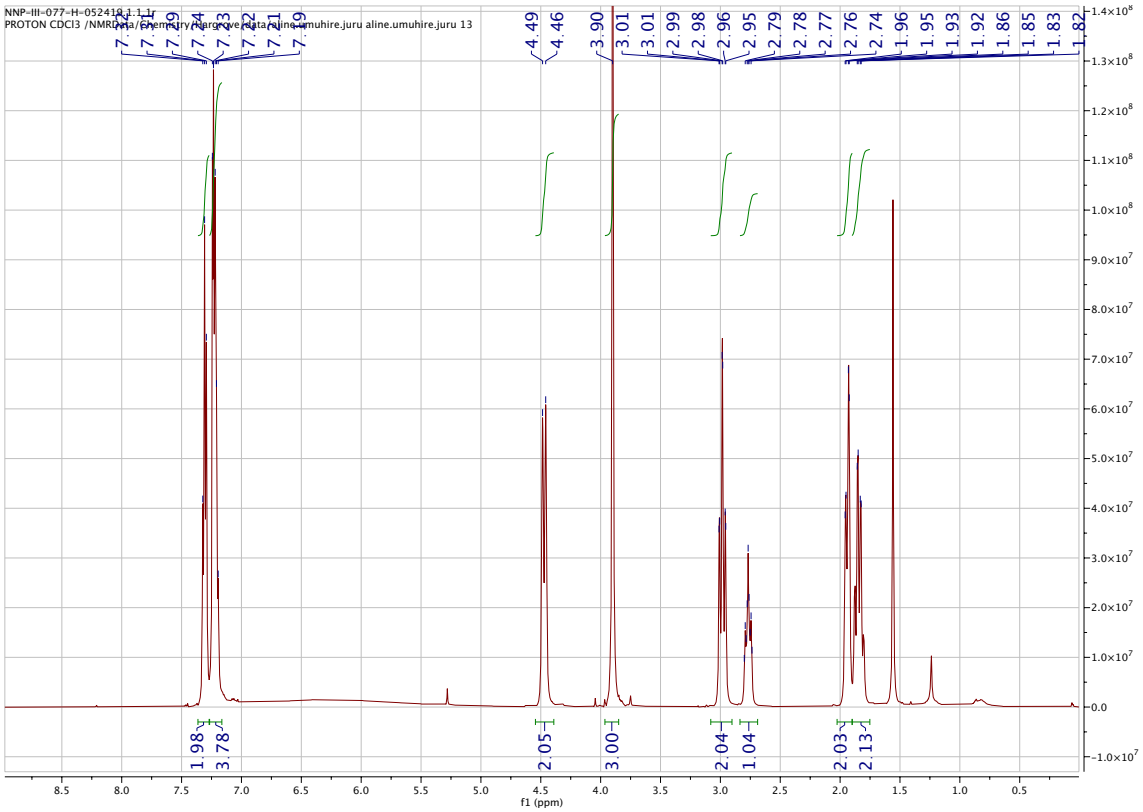
^{1}H NMR Spectrum for Methyl 3-amino-6-chloro-5-morpholinopyrazine-2-carboxylate (3p):



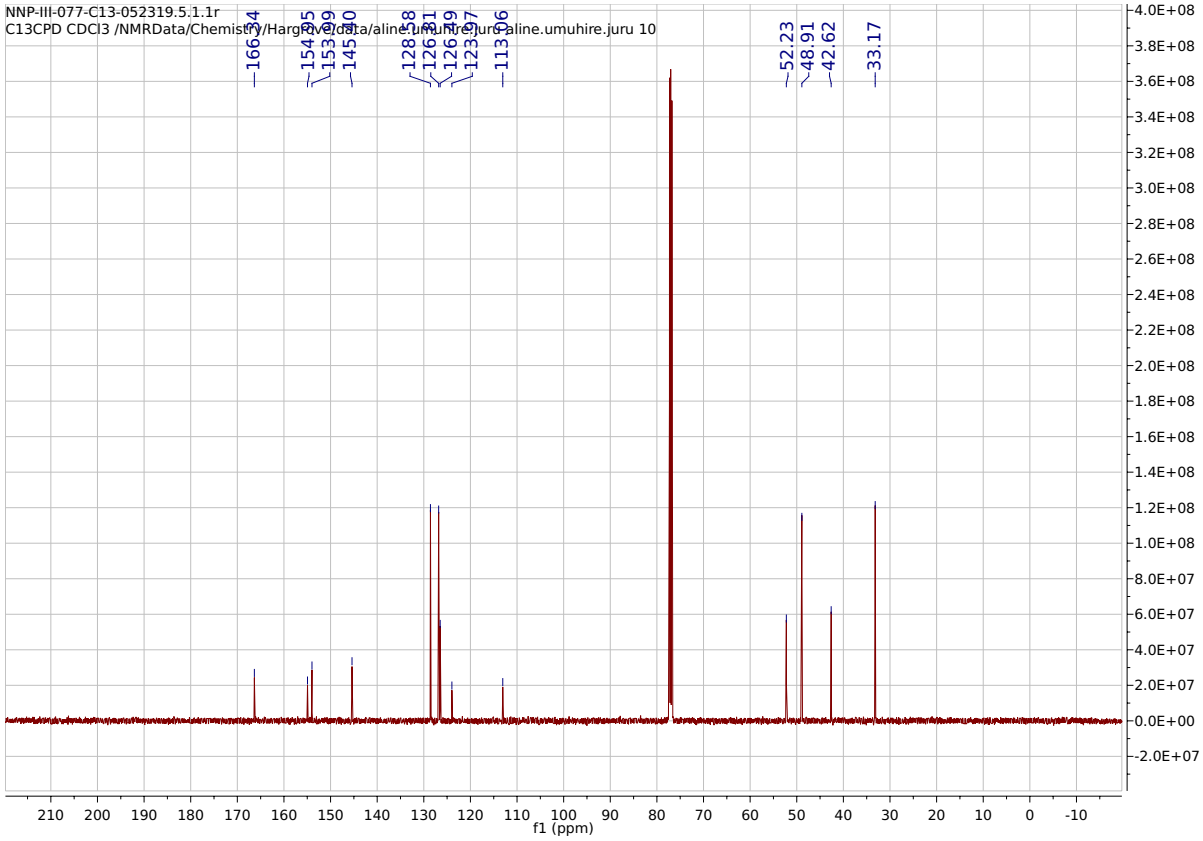
^{13}C NMR Spectrum for Methyl 3-amino-6-chloro-5-morpholinopyrazine-2-carboxylate (3p):



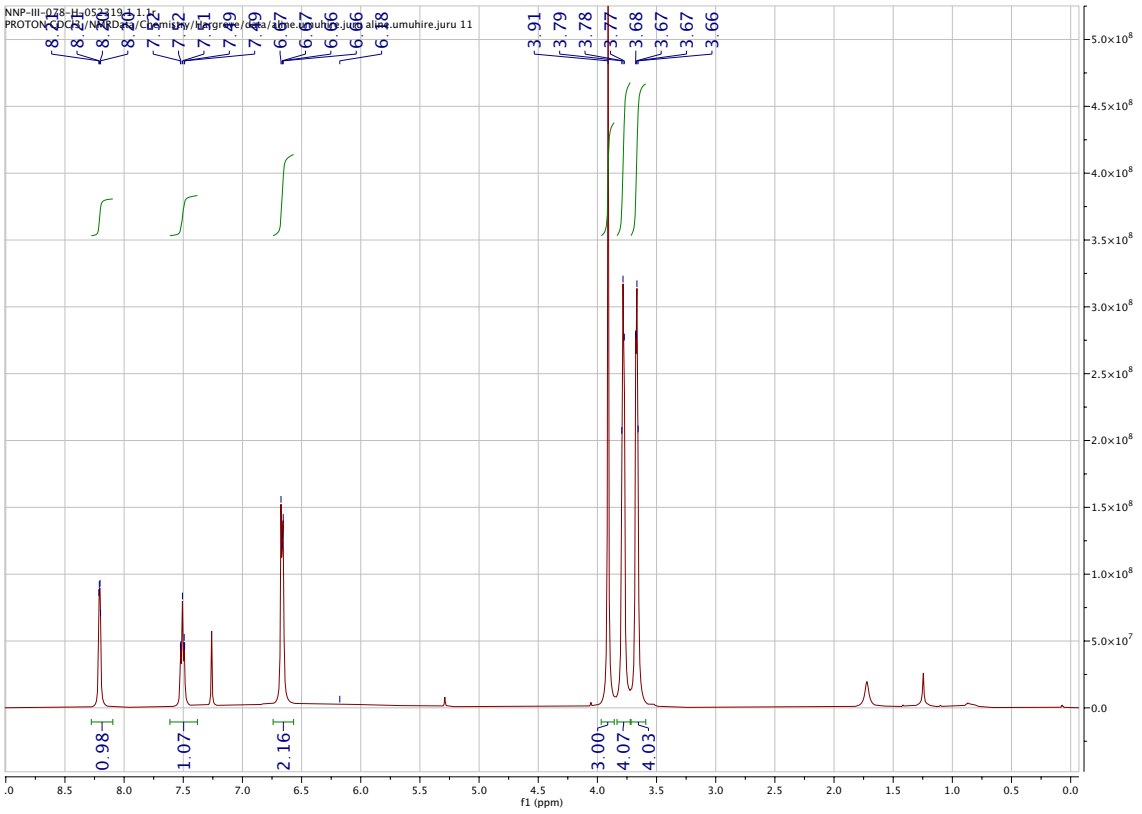
^{1}H NMR Spectrum for Methyl 3-amino-6-chloro-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxylate (3q):



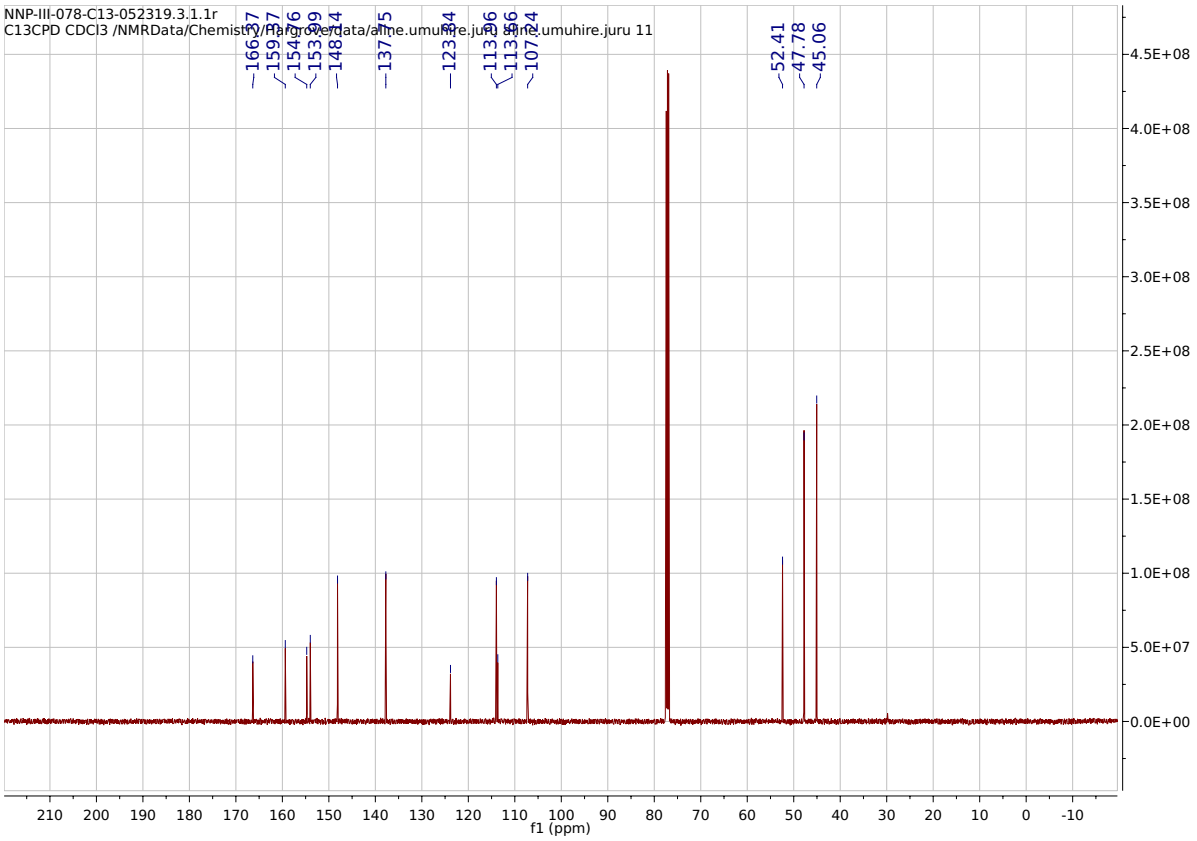
^{13}C NMR Spectrum Methyl 3-amino-6-chloro-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxylate (3q):



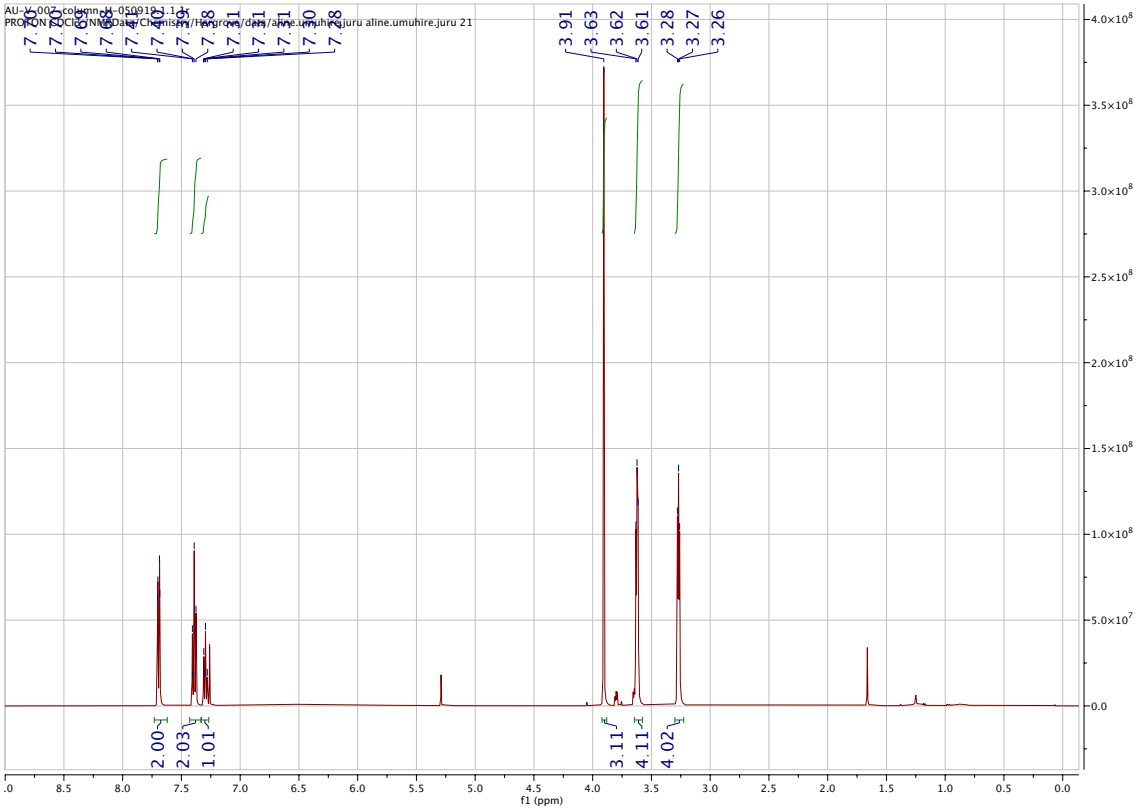
^{1}H NMR Spectrum for Methyl 3-amino-6-chloro-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxylate (3r):



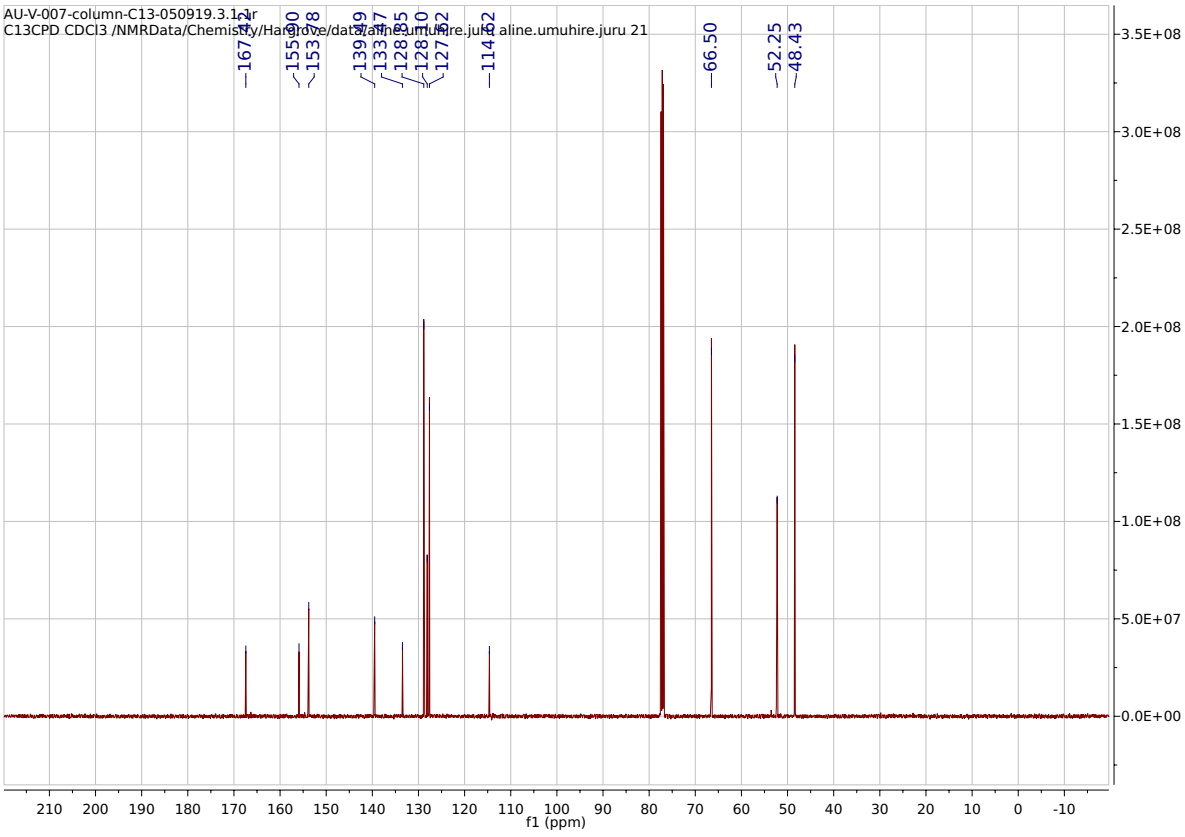
^{13}C NMR Spectrum for Methyl 3-amino-6-chloro-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxylate (3r):



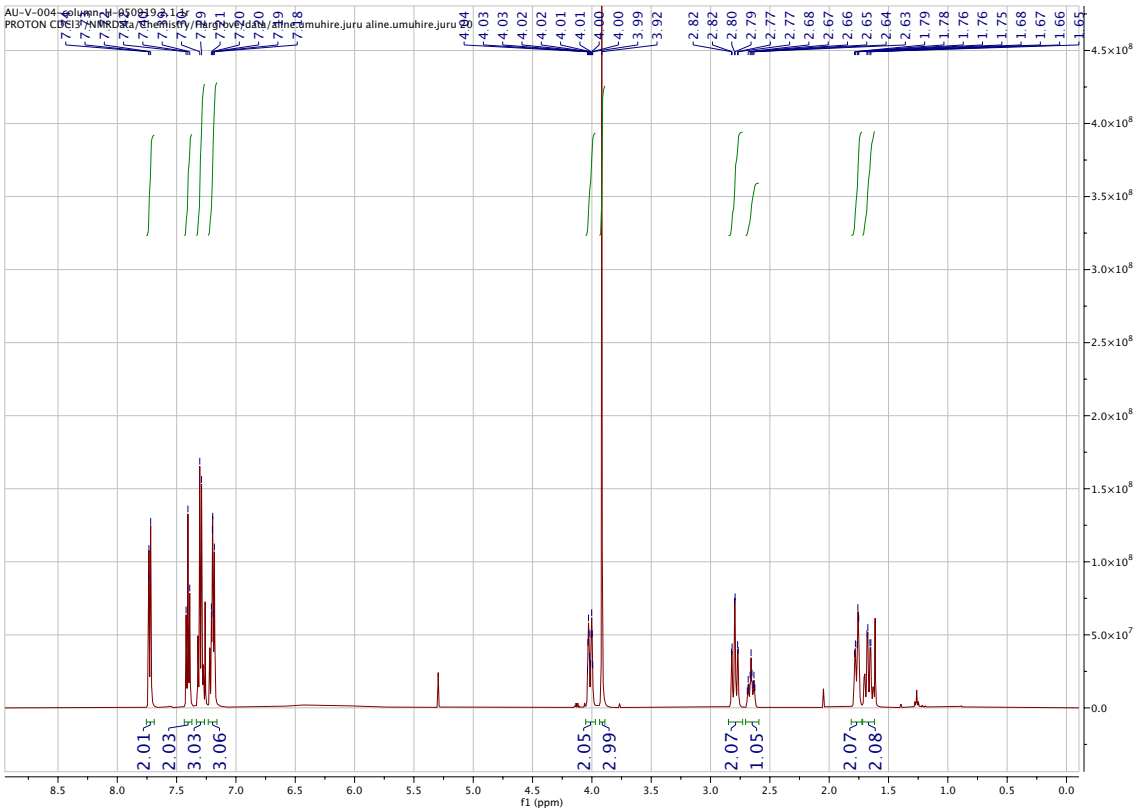
^{1}H NMR Spectrum for Methyl 3-amino-5-morpholino-6-phenylpyrazine-2-carboxylate (3s):



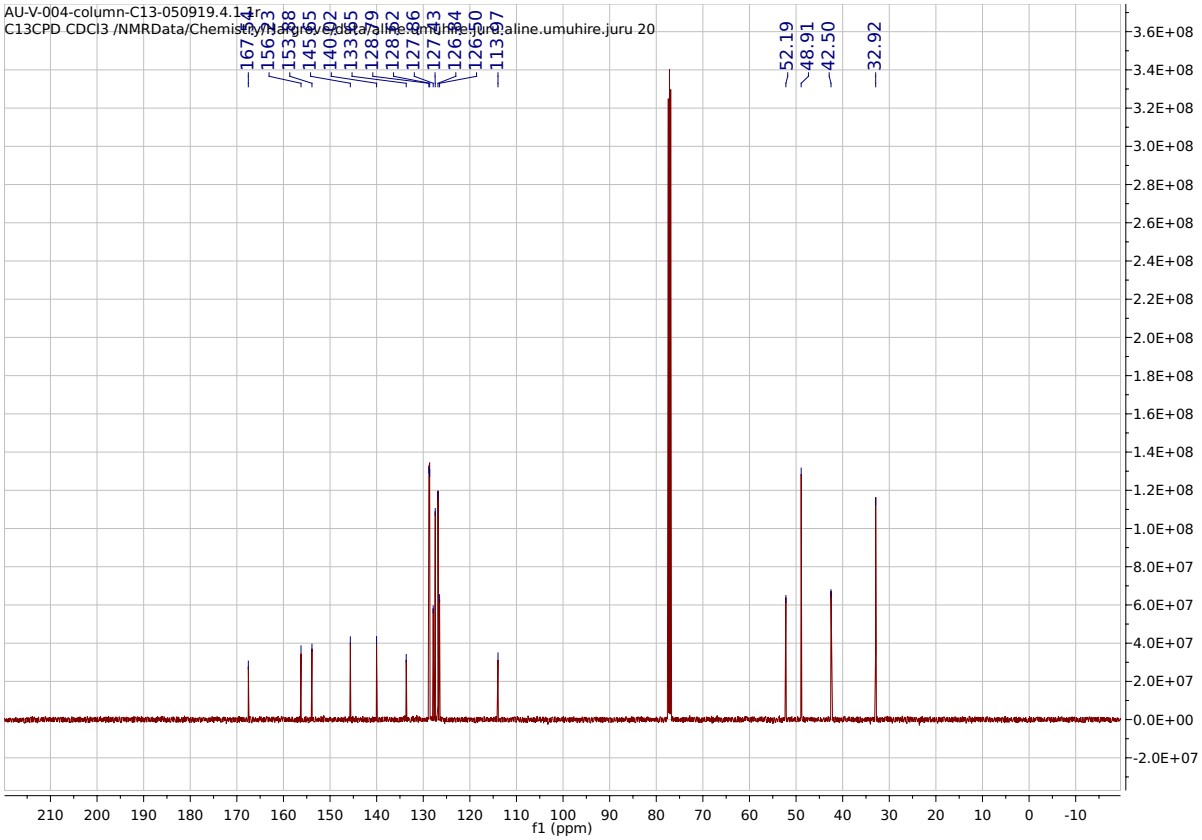
^{13}C NMR Spectrum for Methyl 3-amino-5-morpholino-6-phenylpyrazine-2-carboxylate (3s):



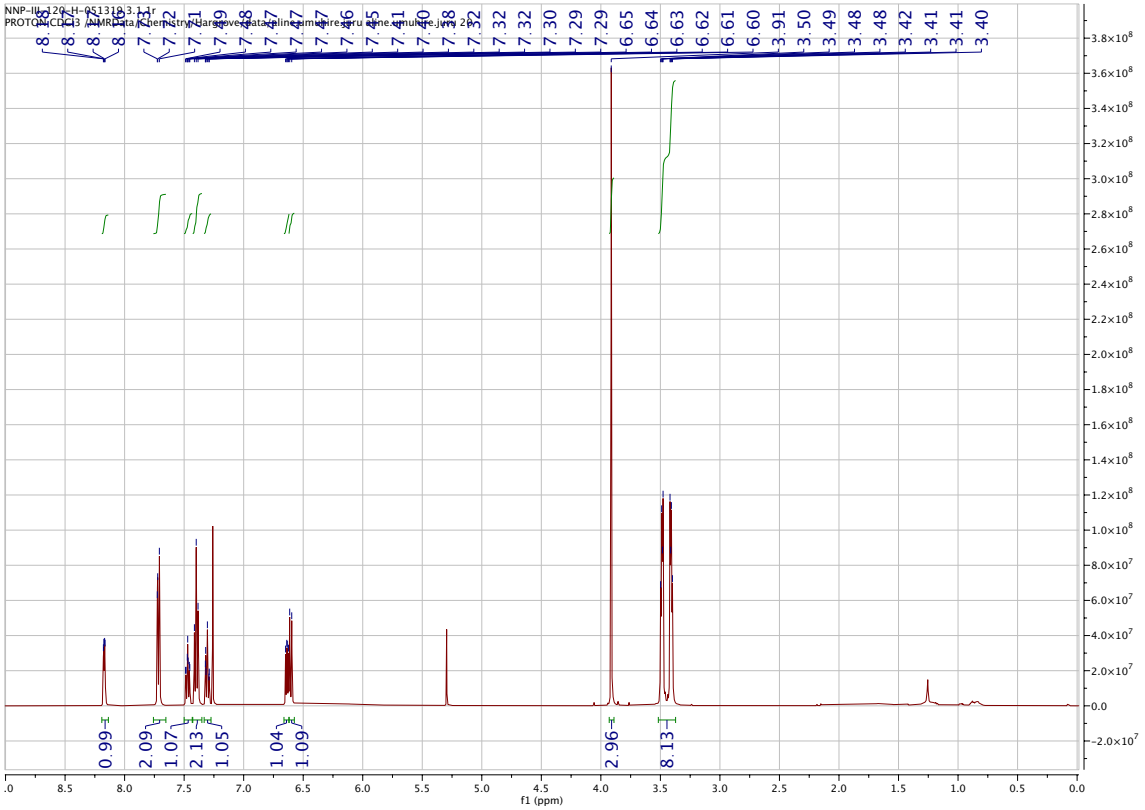
^{1}H NMR Spectrum for Methyl 3-amino-6-phenyl-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxylate (3t):



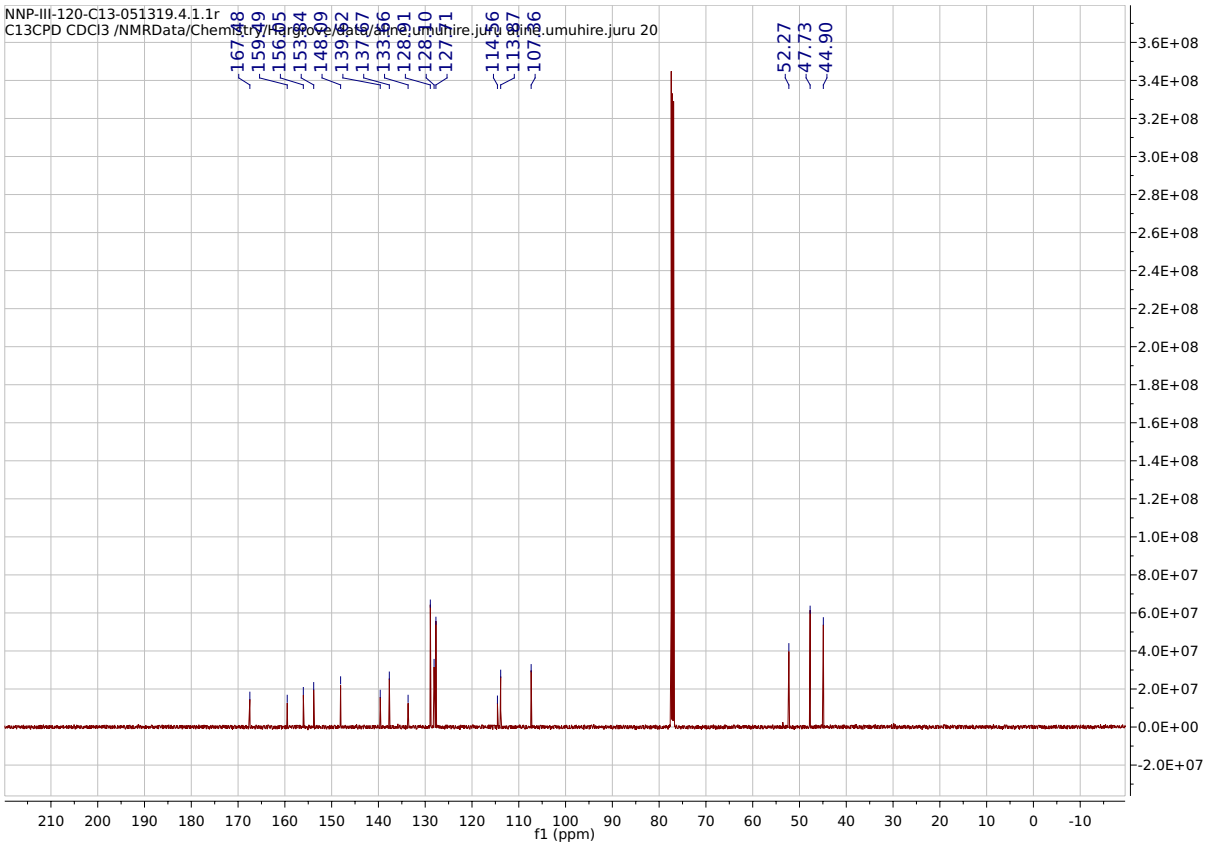
^{13}C NMR Spectrum for Methyl 3-amino-6-phenyl-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxylate (3t):



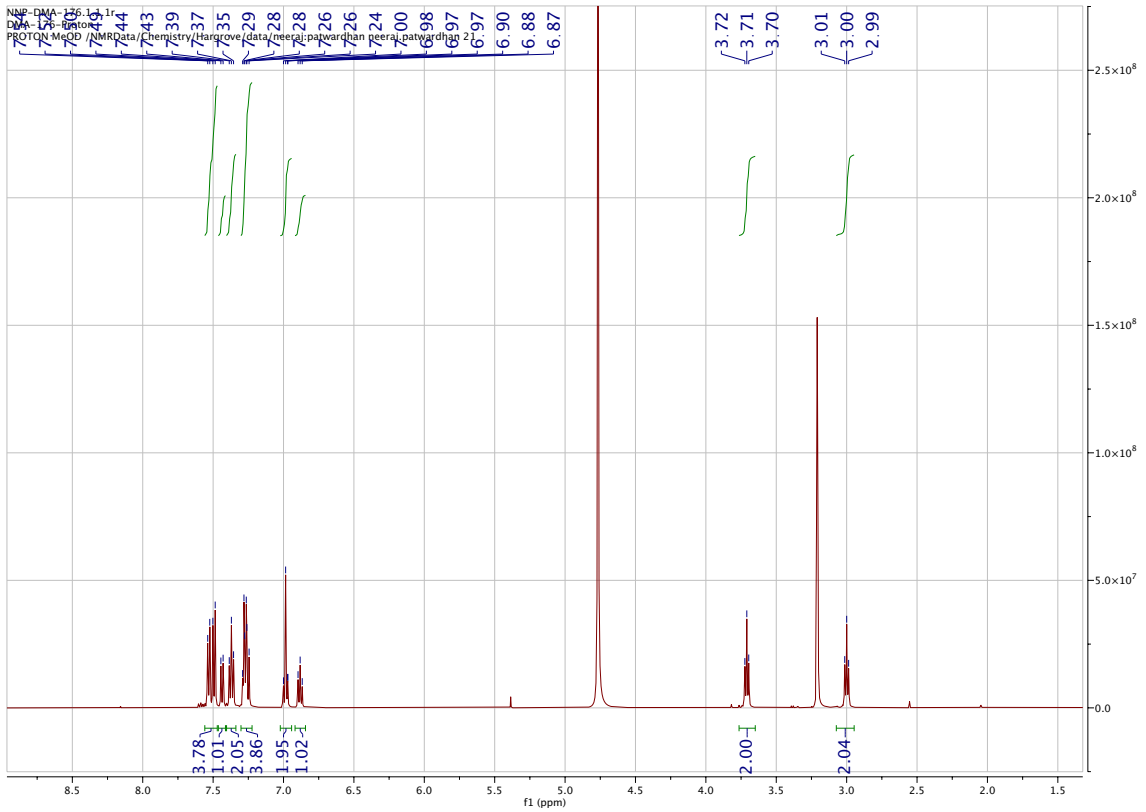
^{1}H NMR Spectrum for Methyl 3-amino-6-phenyl-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxylate (3u):



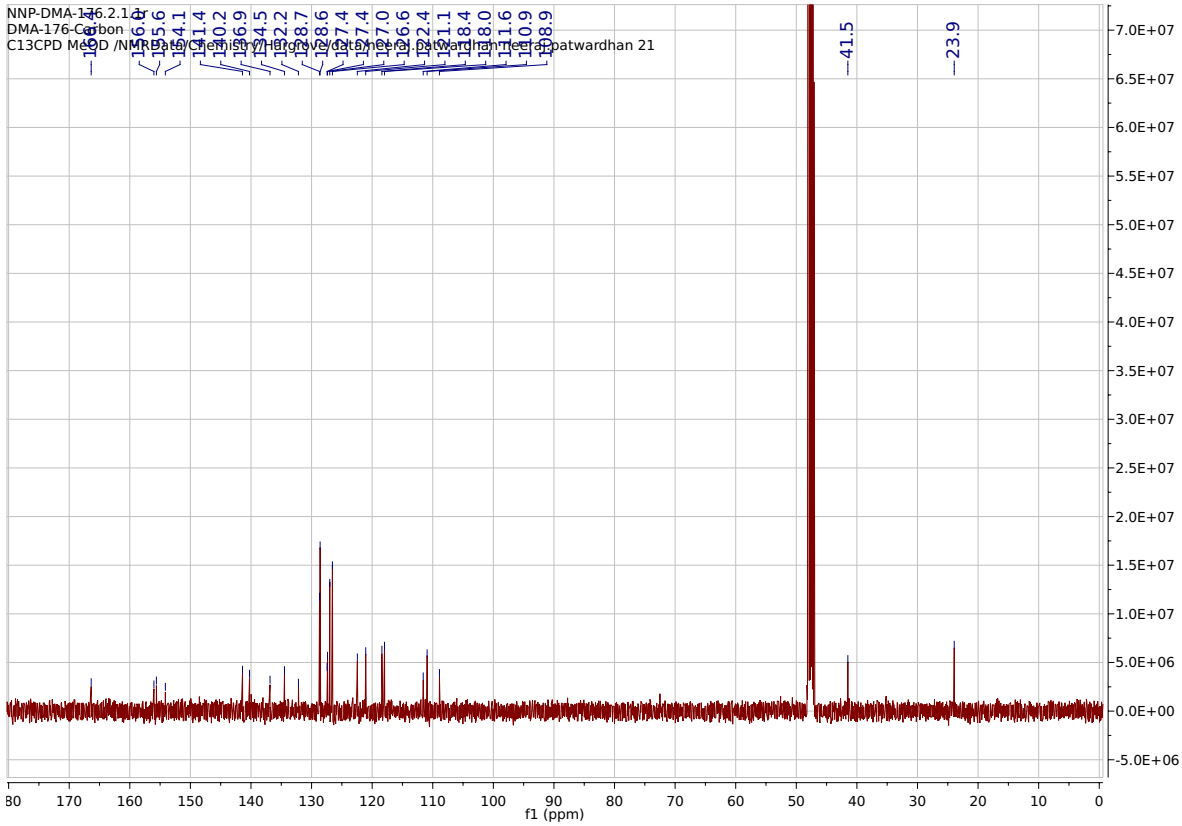
^{13}C NMR Spectrum for Methyl 3-amino-6-phenyl-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxylate (3u):



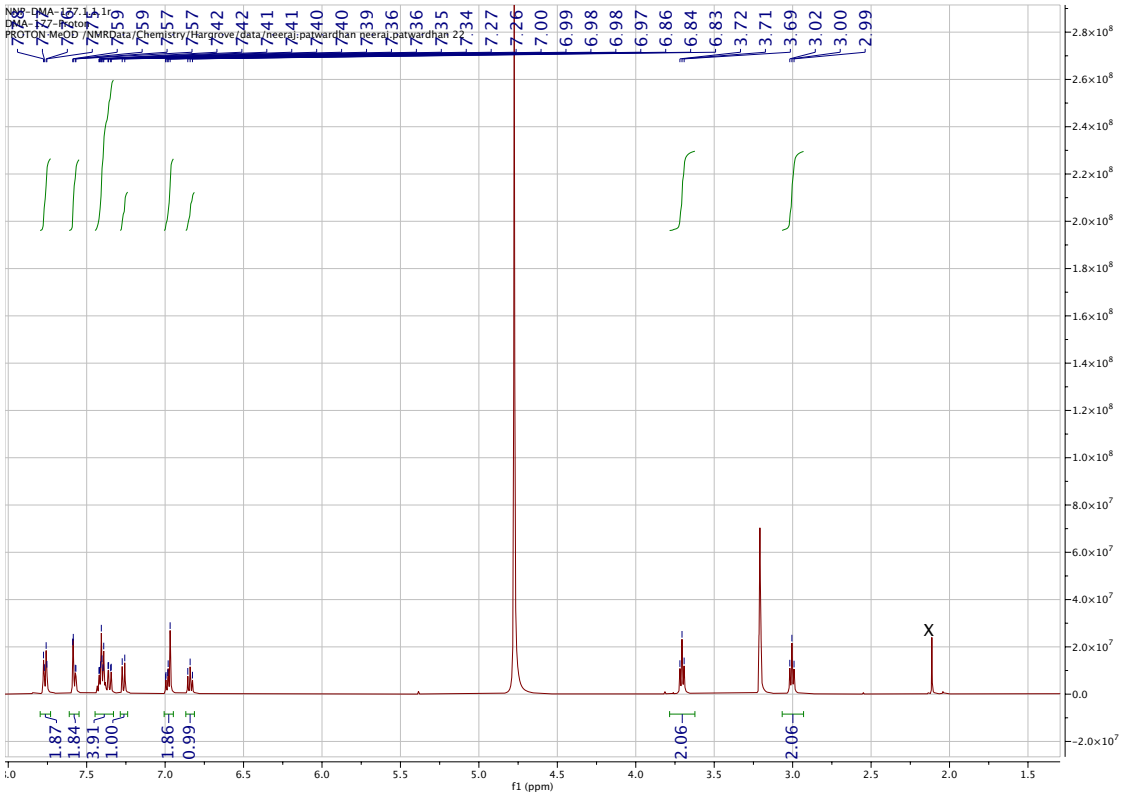
^{1}H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (4a) (DMA-176)



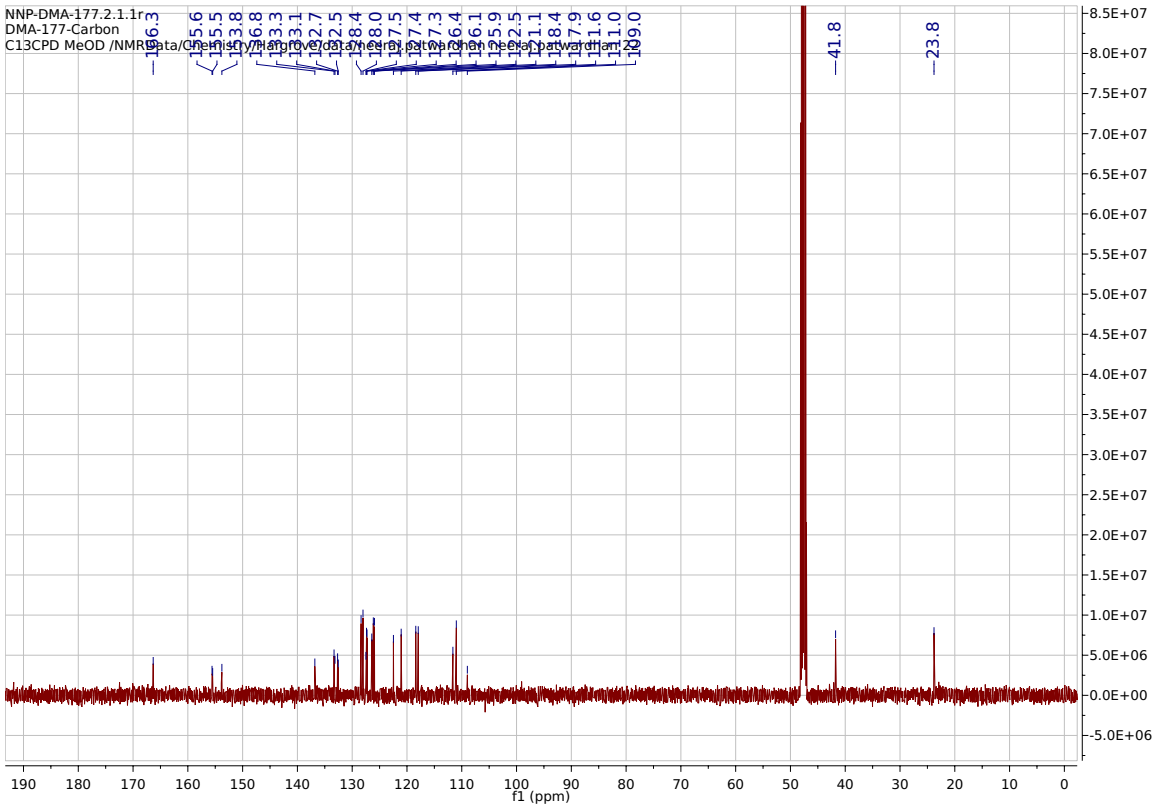
^{13}C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (4a) (DMA-176)



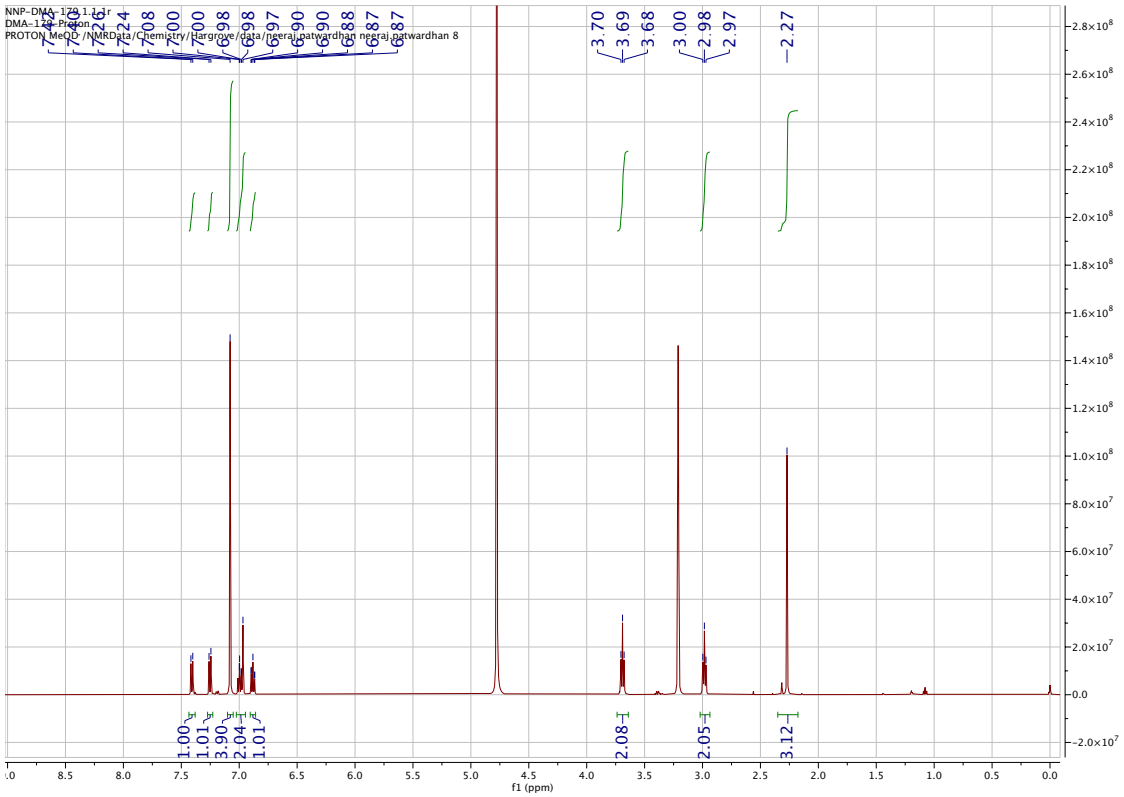
^{1}H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(naphthalen-2-yl)pyrazine-2-carboxamide hydrochloride (4b) (DMA-177):



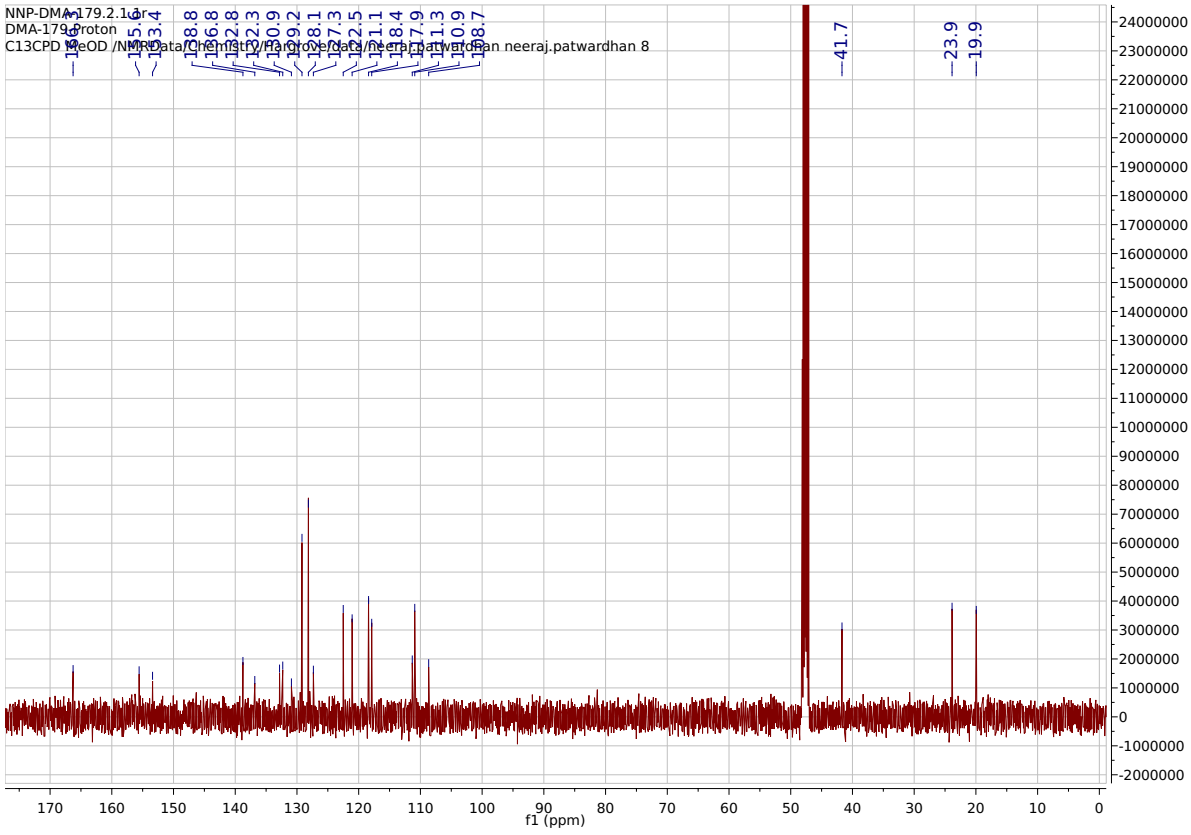
^{13}C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(naphthalen-2-yl)pyrazine-2-carboxamide hydrochloride (4b) (DMA-177):



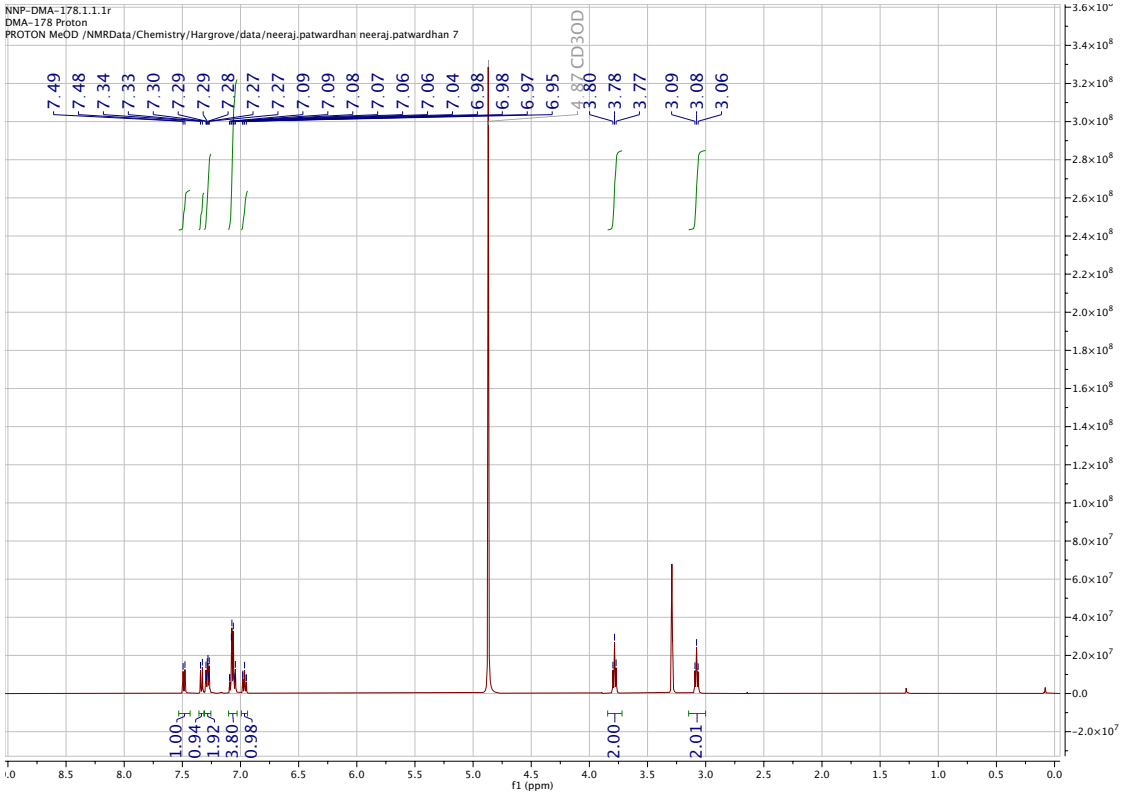
^{1}H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(*p*-tolyl)pyrazine-2-carboxamide hydrochloride (4c) (DMA-178):



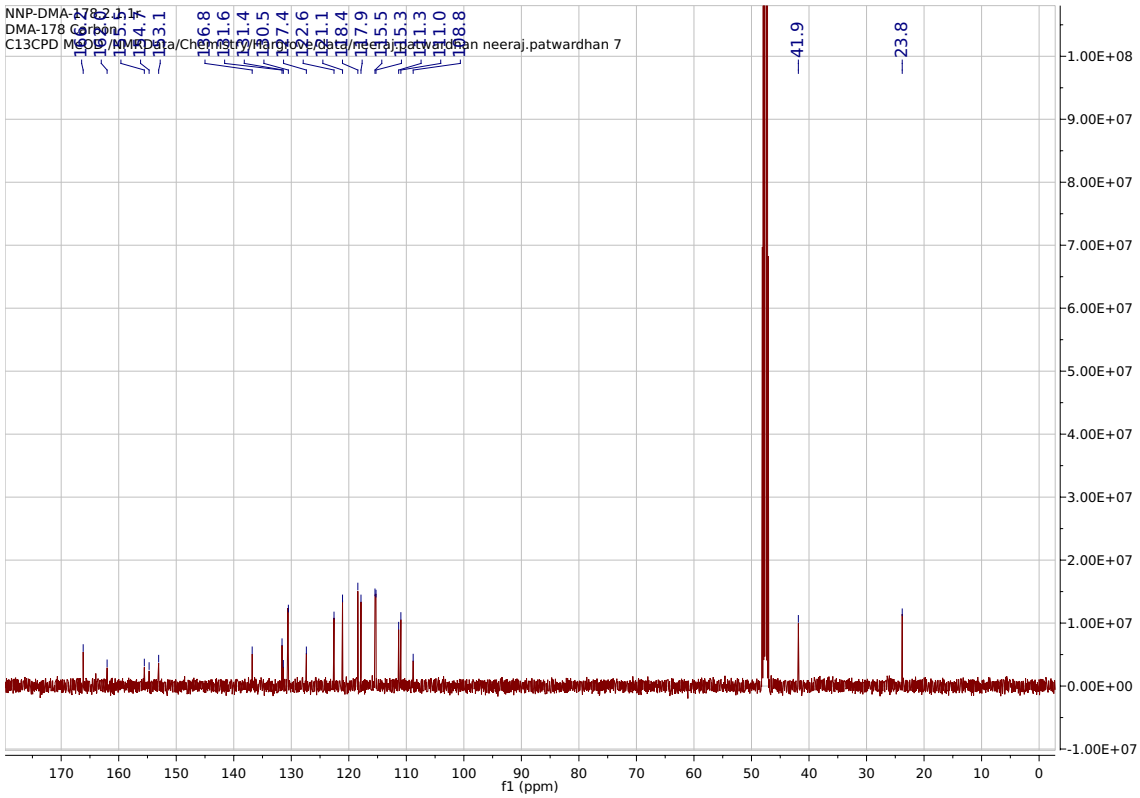
^{13}C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(*p*-tolyl)pyrazine-2-carboxamide hydrochloride (4c) (DMA-178):



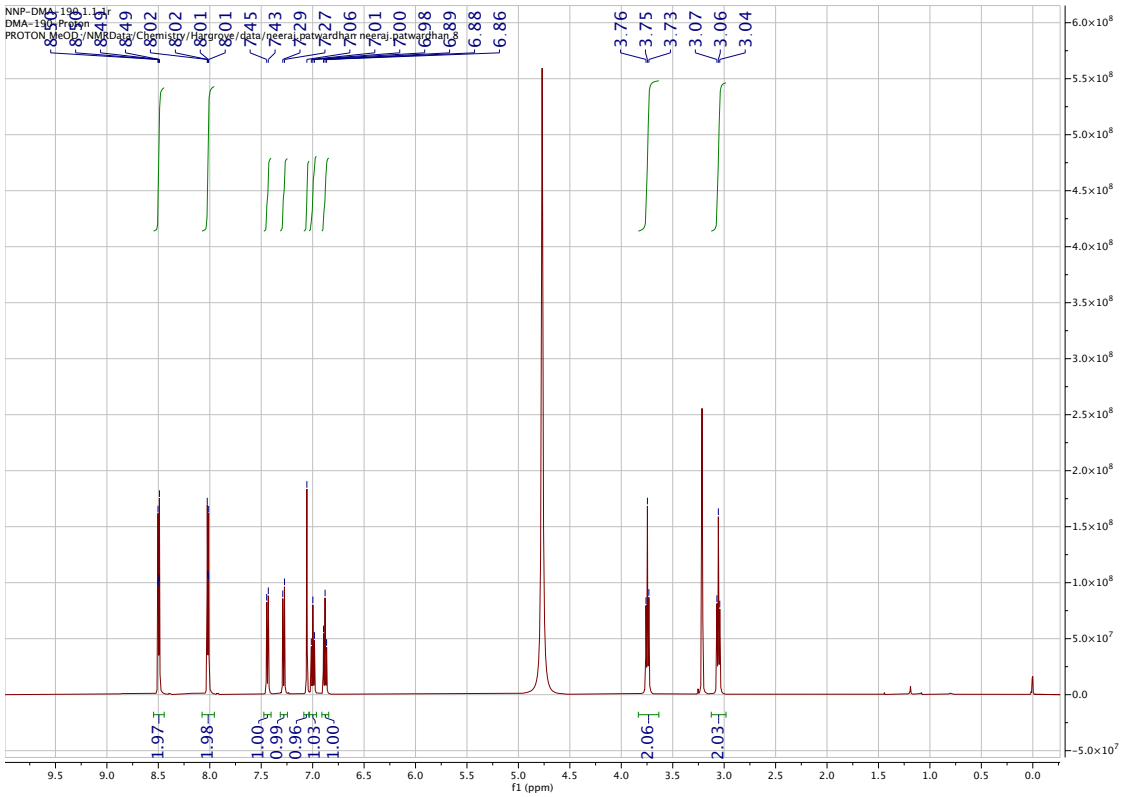
^{1}H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(4-fluorophenyl)pyrazine-2-carboxamide hydrochloride (4d) (DMA-179):



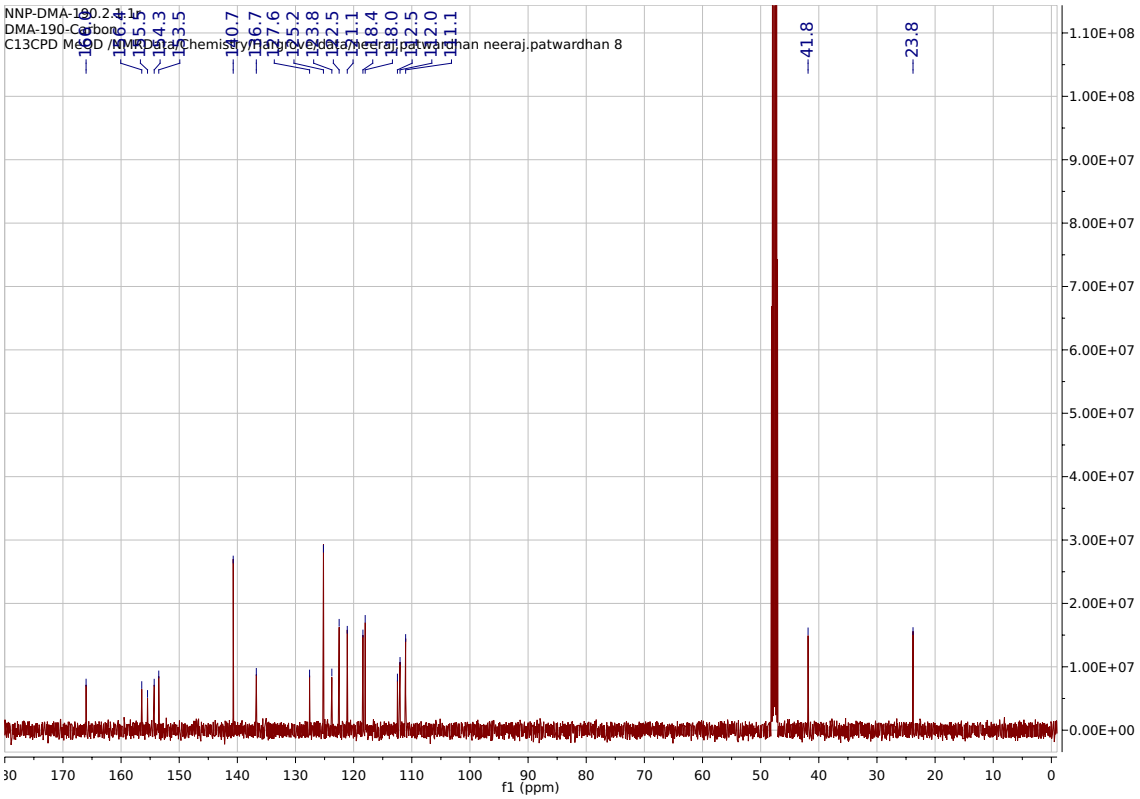
^{13}C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(4-fluorophenyl)pyrazine-2-carboxamide hydrochloride (4d) (DMA-179):



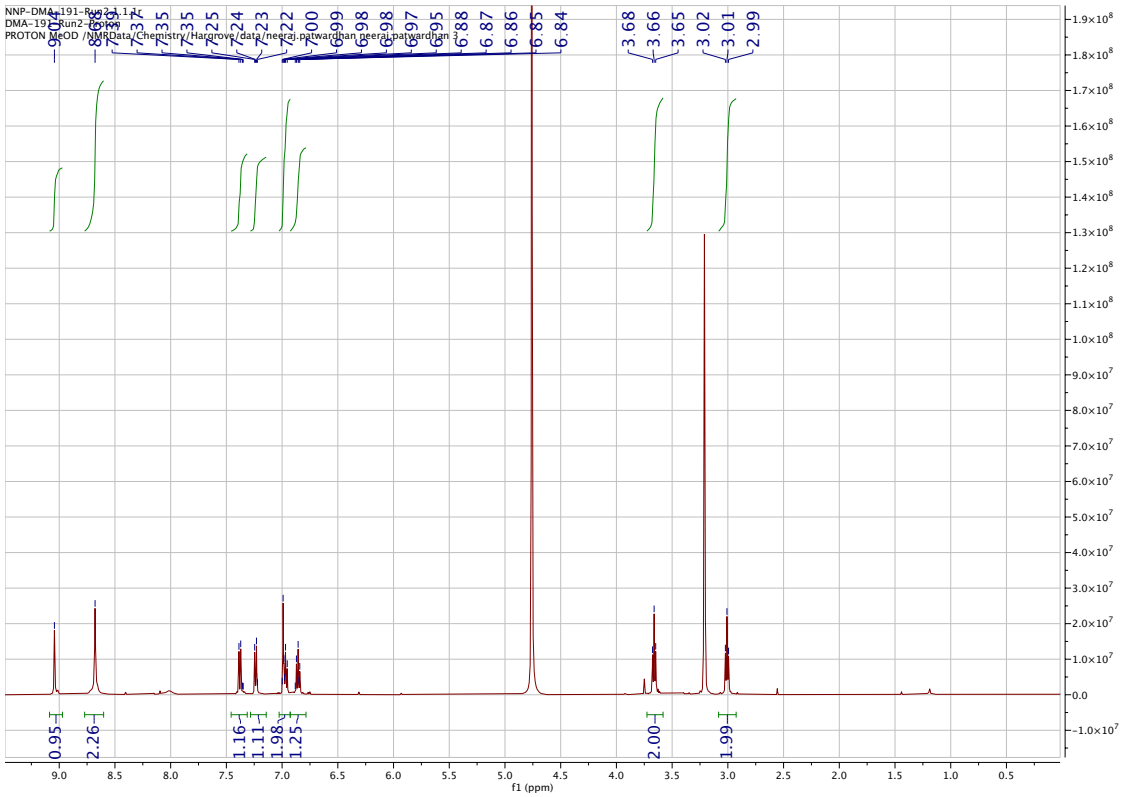
^{1}H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(pyridin-4-yl)pyrazine-2-carboxamide hydrochloride (4e) (DMA-190):



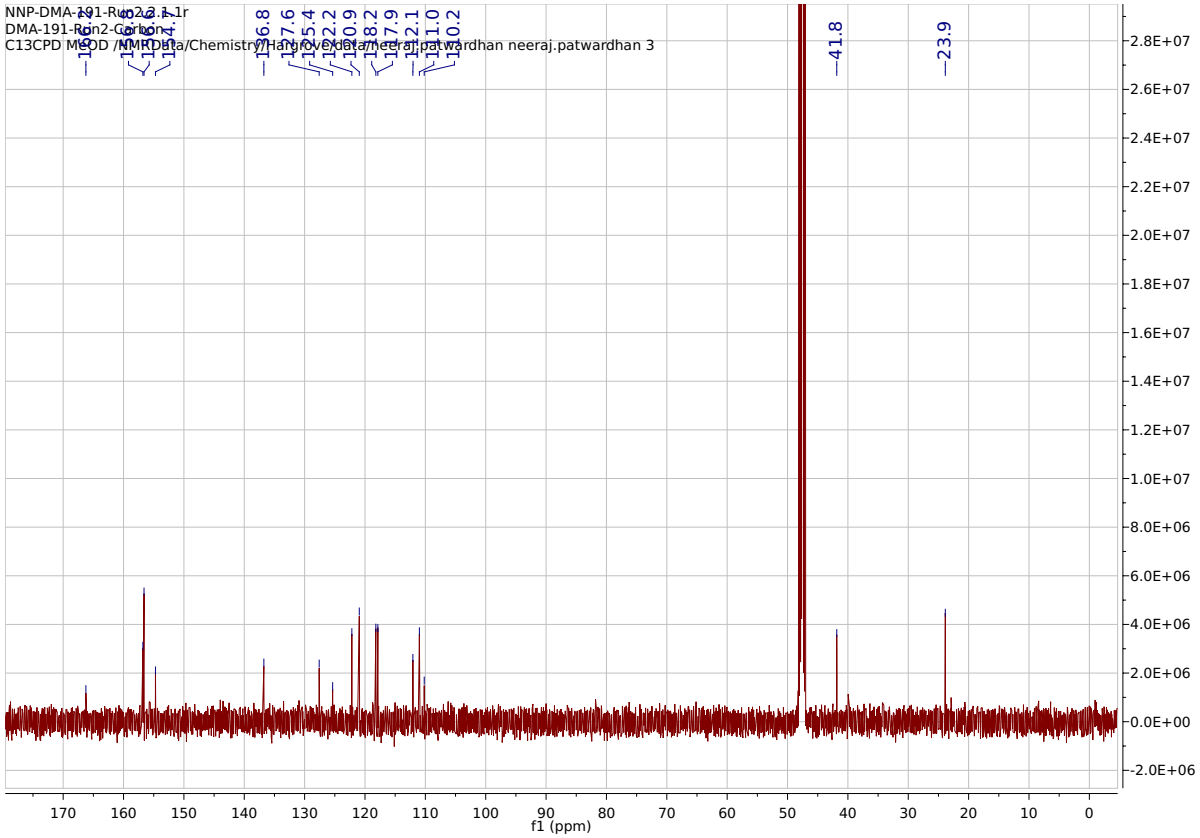
^{13}C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(pyridin-4-yl)pyrazine-2-carboxamide hydrochloride (4e) (DMA-190):



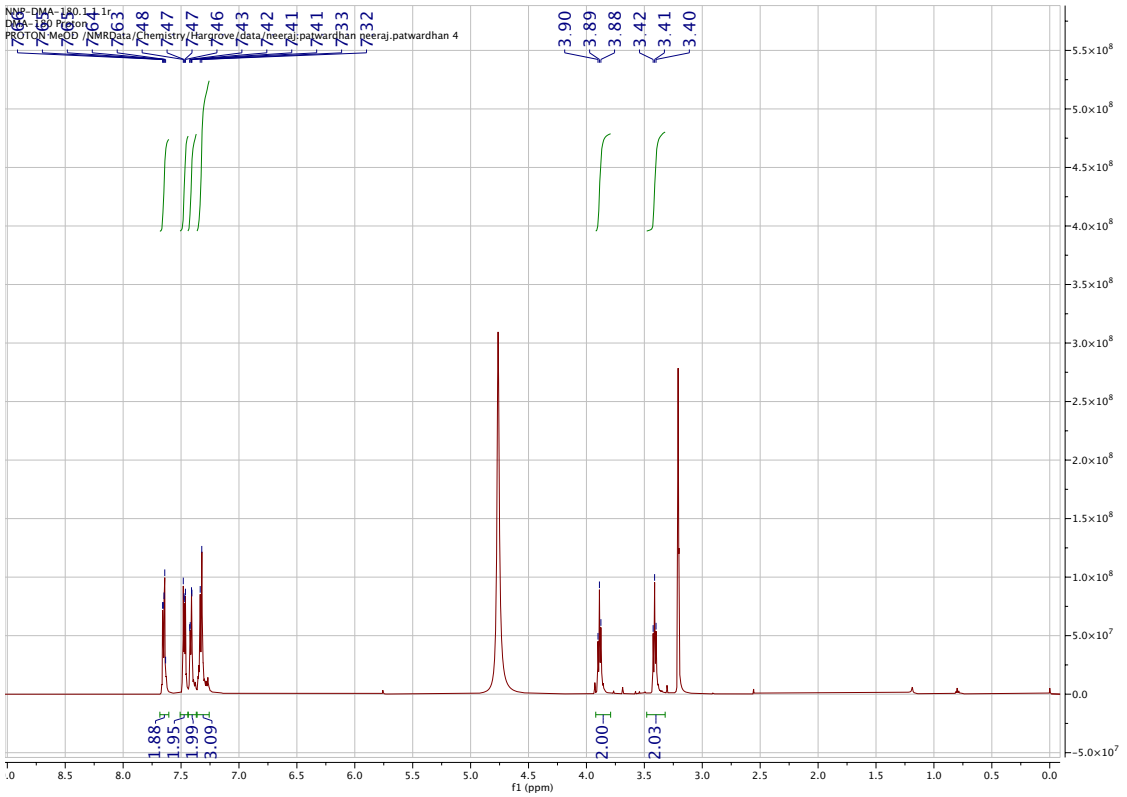
^{1}H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (4f) (DMA-191):



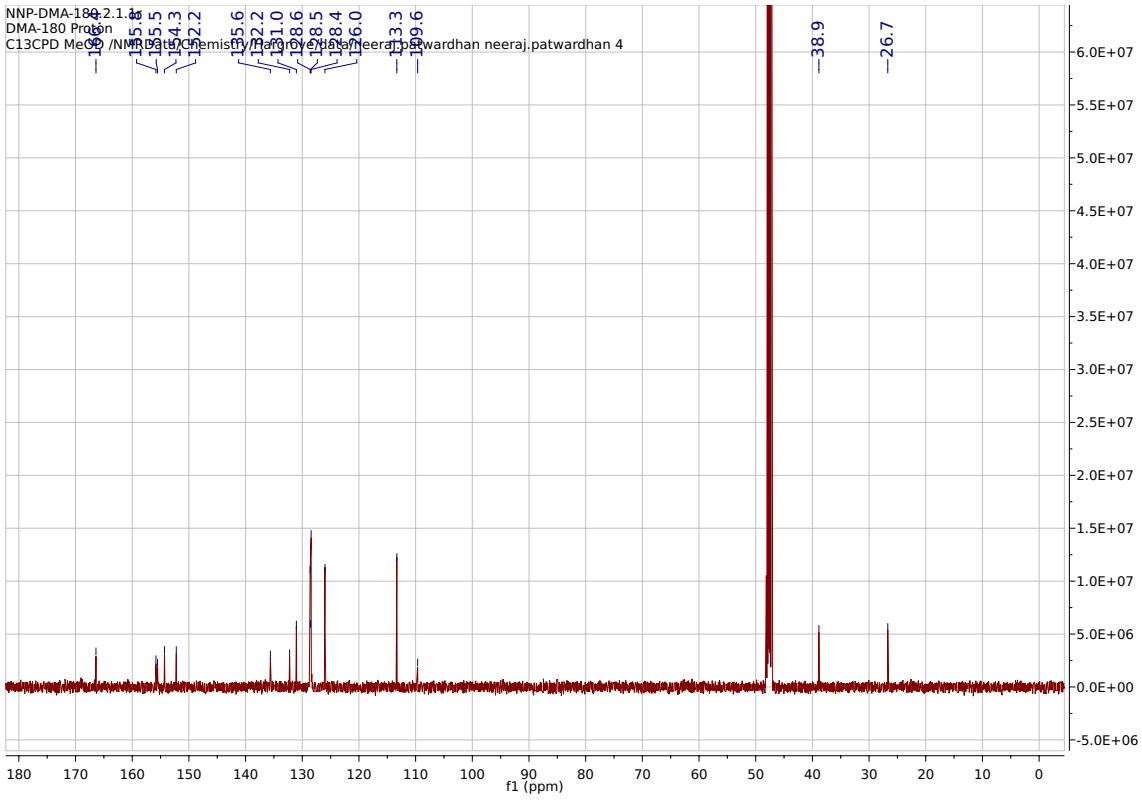
^{13}C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (4f) (DMA-191):



^{1}H NMR for 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4g) (DMA-180):

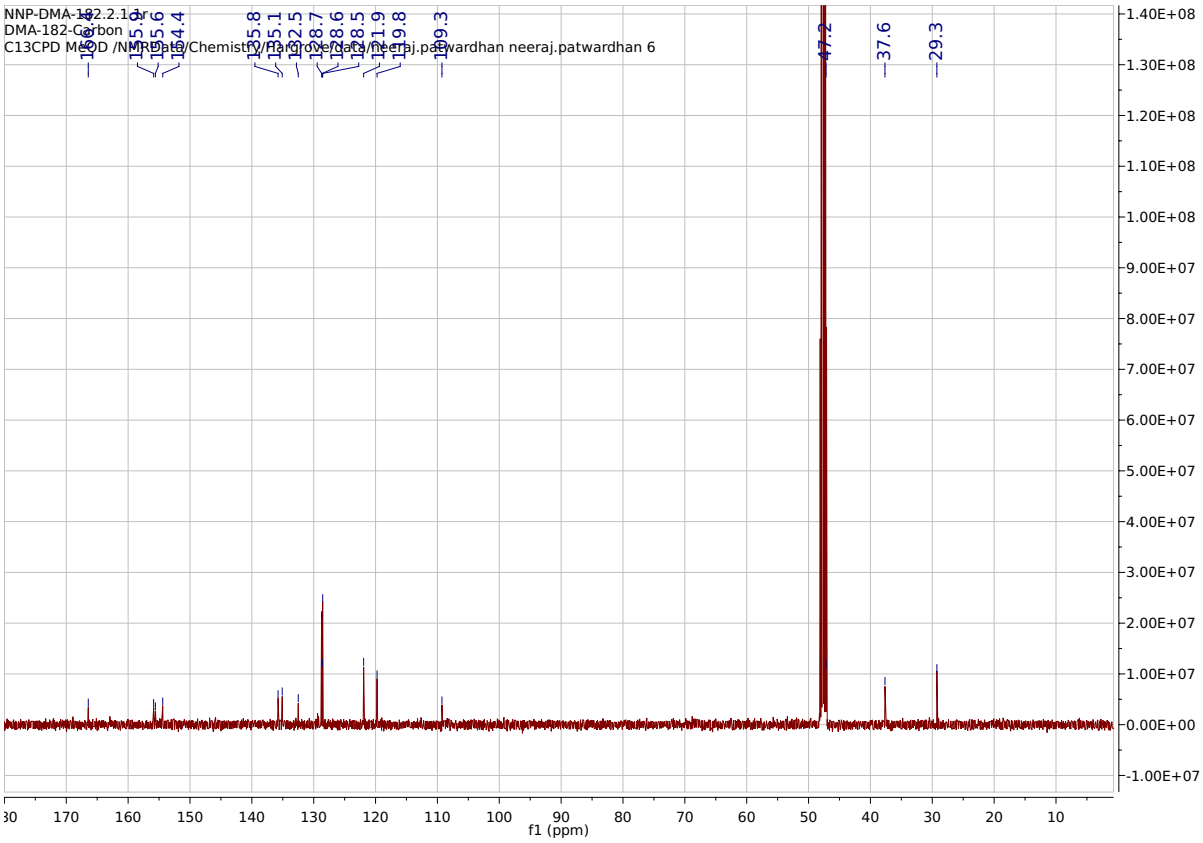


^{13}C NMR for 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4g) (DMA-180):

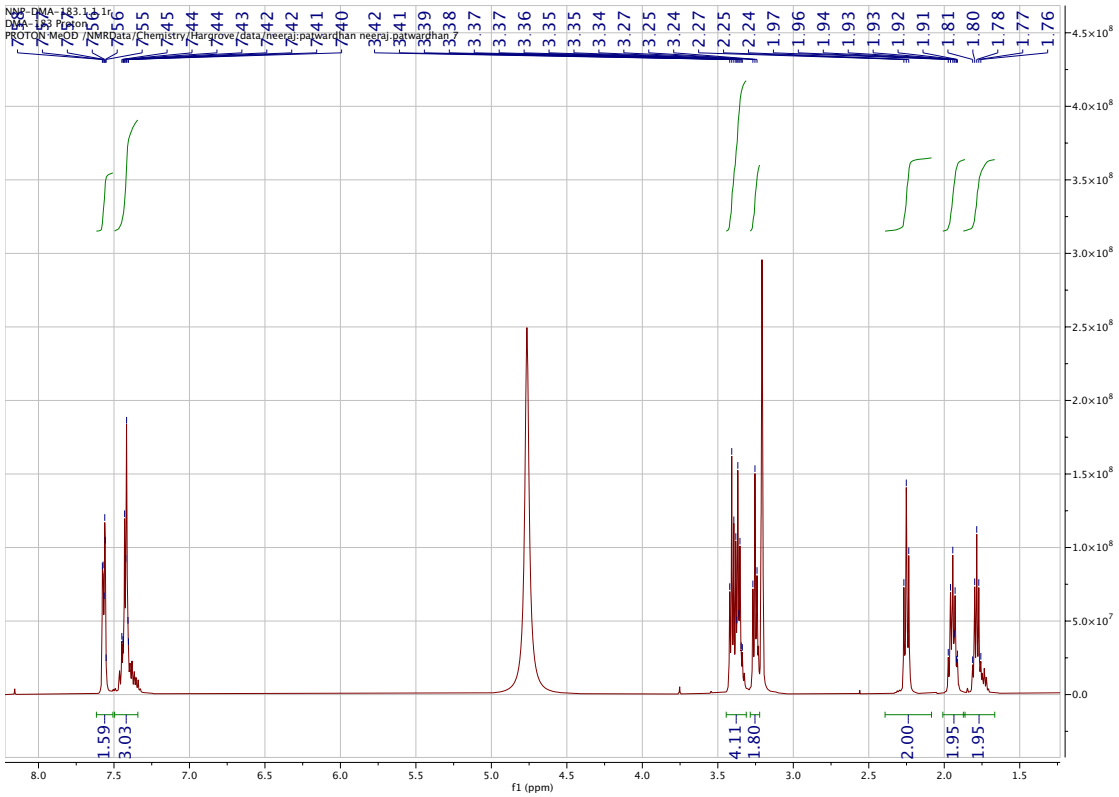


^{1}H NMR for 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4h) (DMA-182):

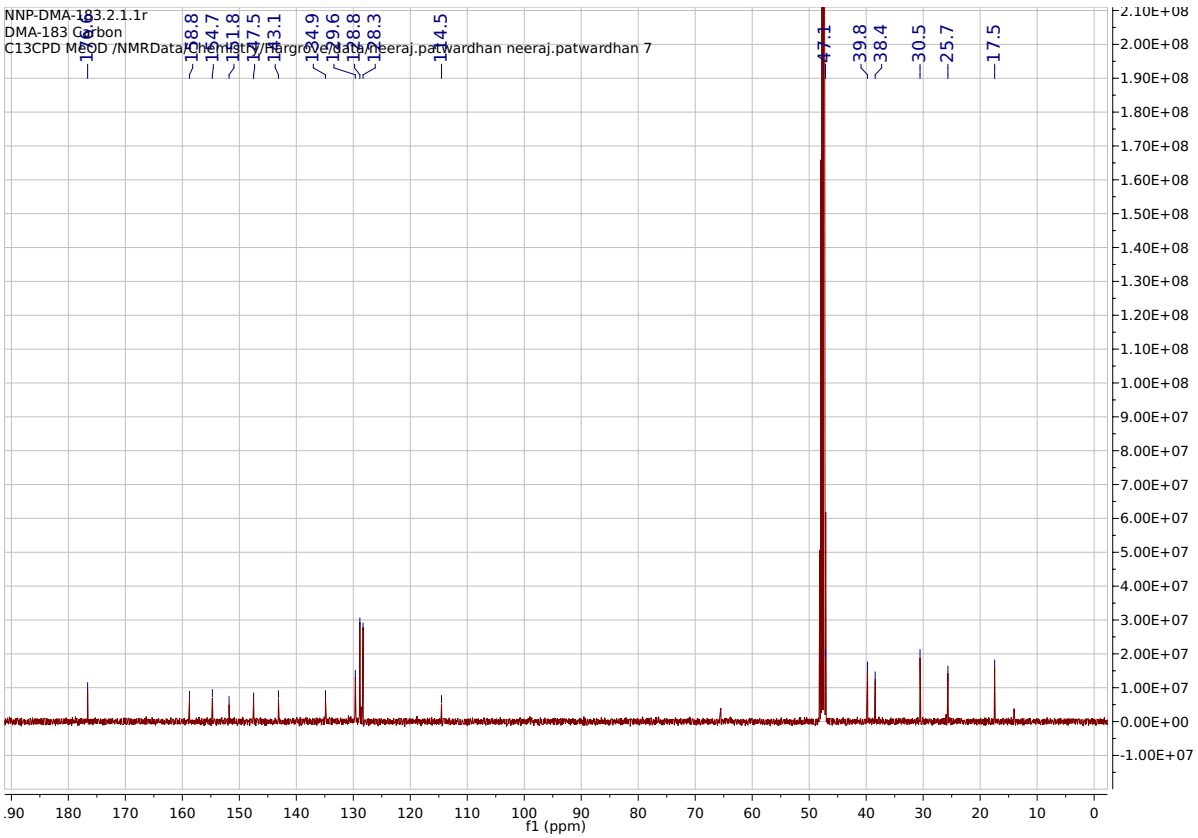
^{13}C NMR for 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4h) (DMA-182):



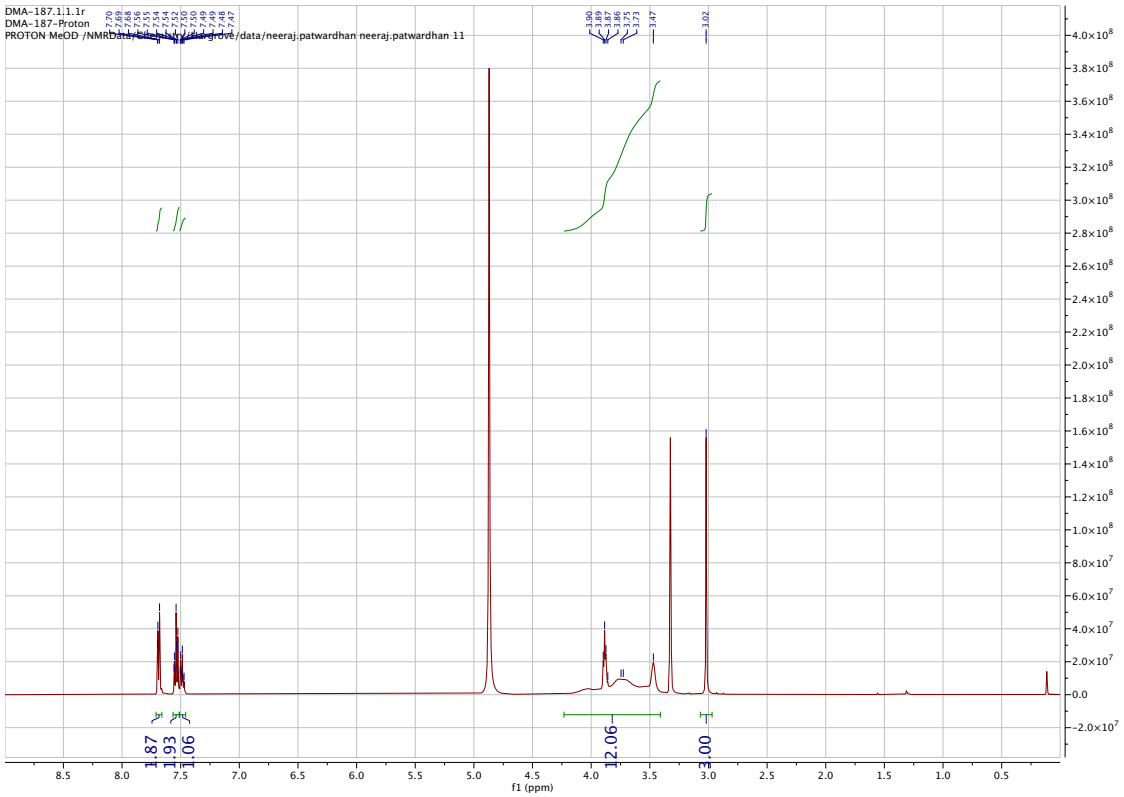
^{1}H NMR for 3-amino-*N*-carbamimidoyl-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)-6-phenylpyrazine-2-carboxamide hydrochloride (4i) (DMA-183):



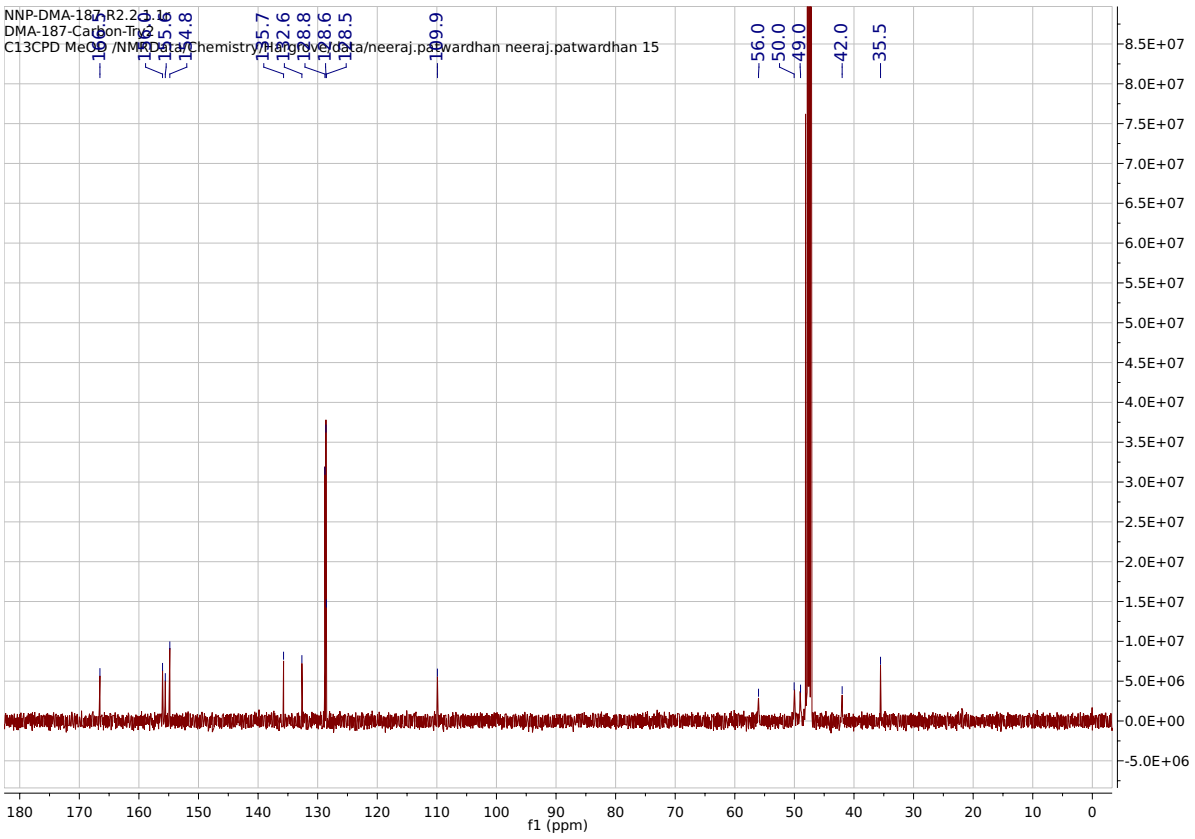
^{13}C NMR for 3-amino-*N*-carbamimidoyl-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)-6-phenylpyrazine-2-carboxamide hydrochloride (4i) (DMA-183):



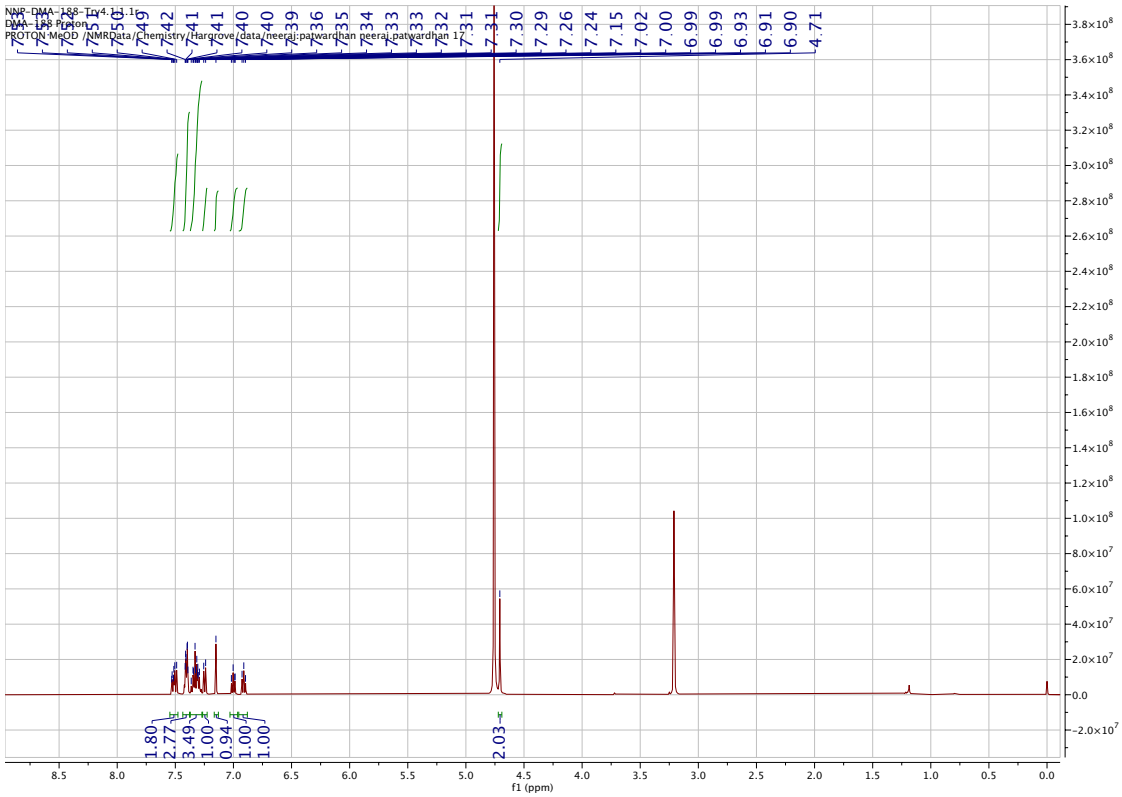
^{1}H NMR for 3-amino-*N*-carbamimidoyl-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)-6-phenylpyrazine-2-carboxamide hydrochloride (4j) (DMA-187):



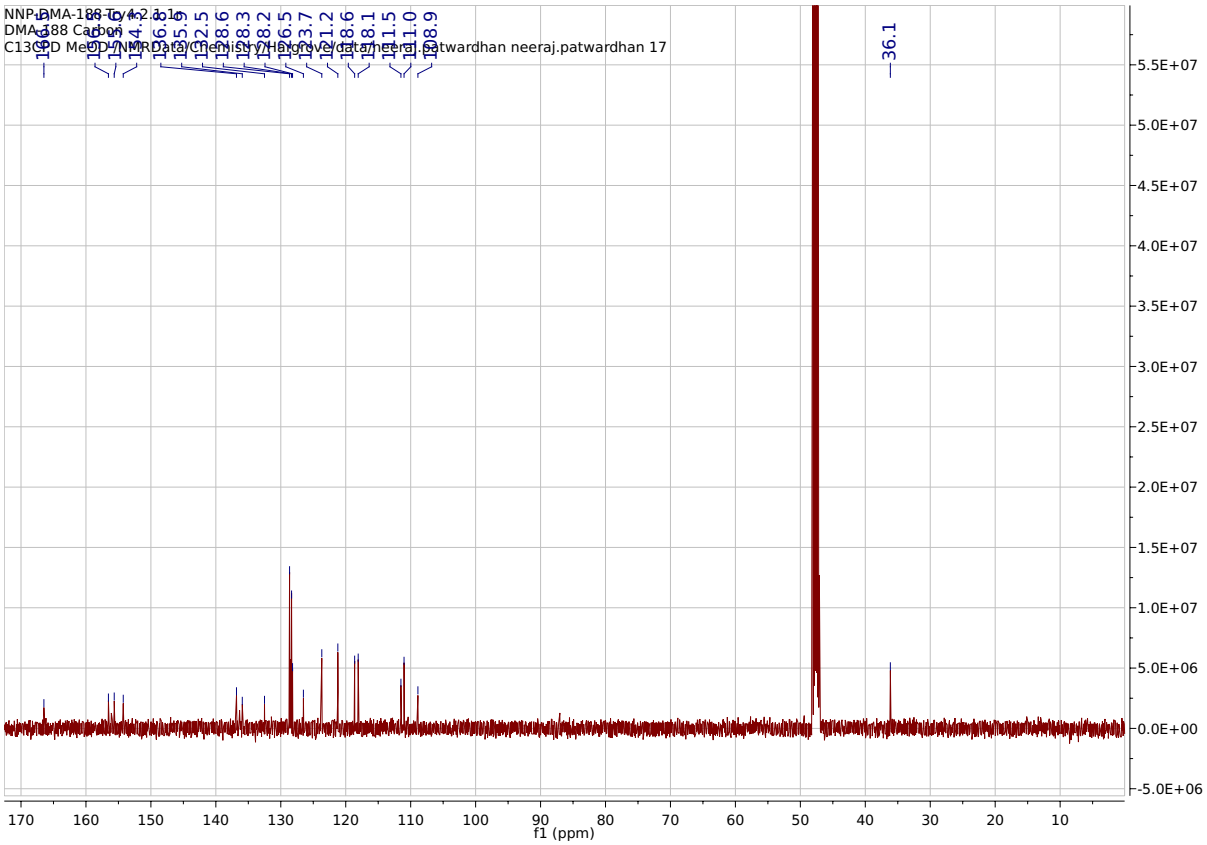
^{13}C NMR for 3-amino-*N*-carbamimidoyl-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)-6-phenylpyrazine-2-carboxamide hydrochloride (4j) (DMA-187):



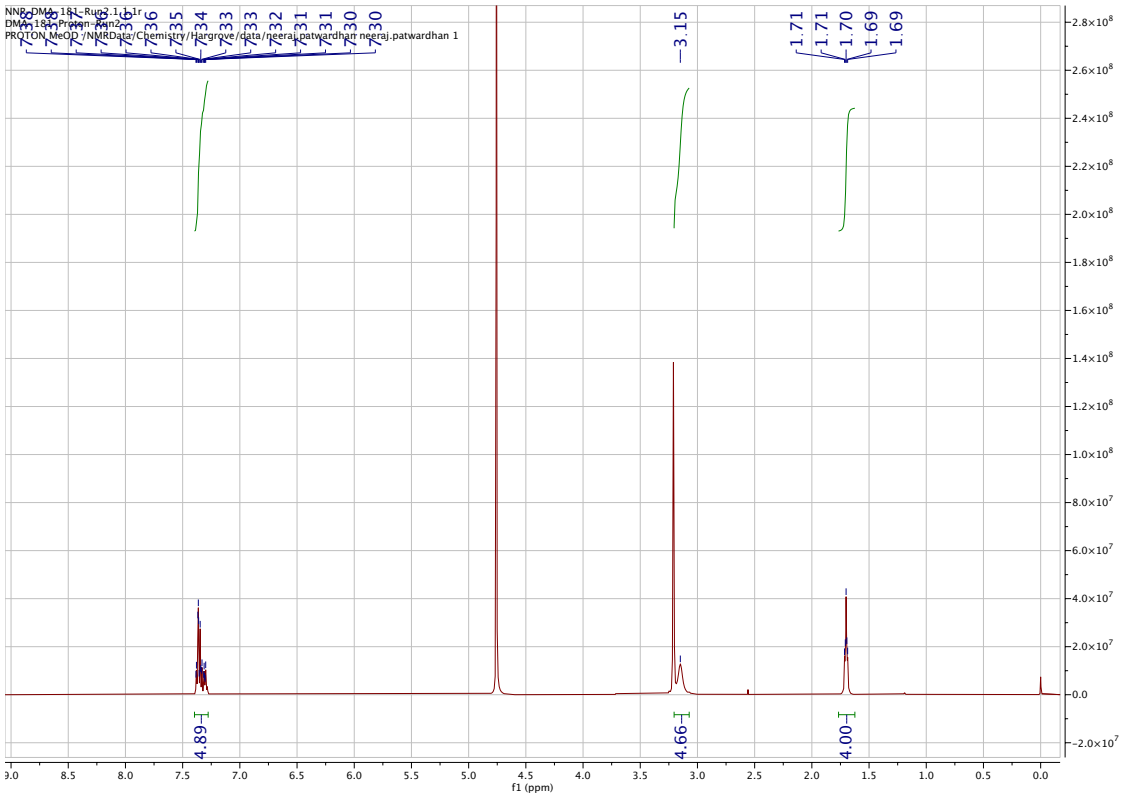
^{1}H NMR for 5-(((1*H*-indol-3-yl)methyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4k) (DMA-188):



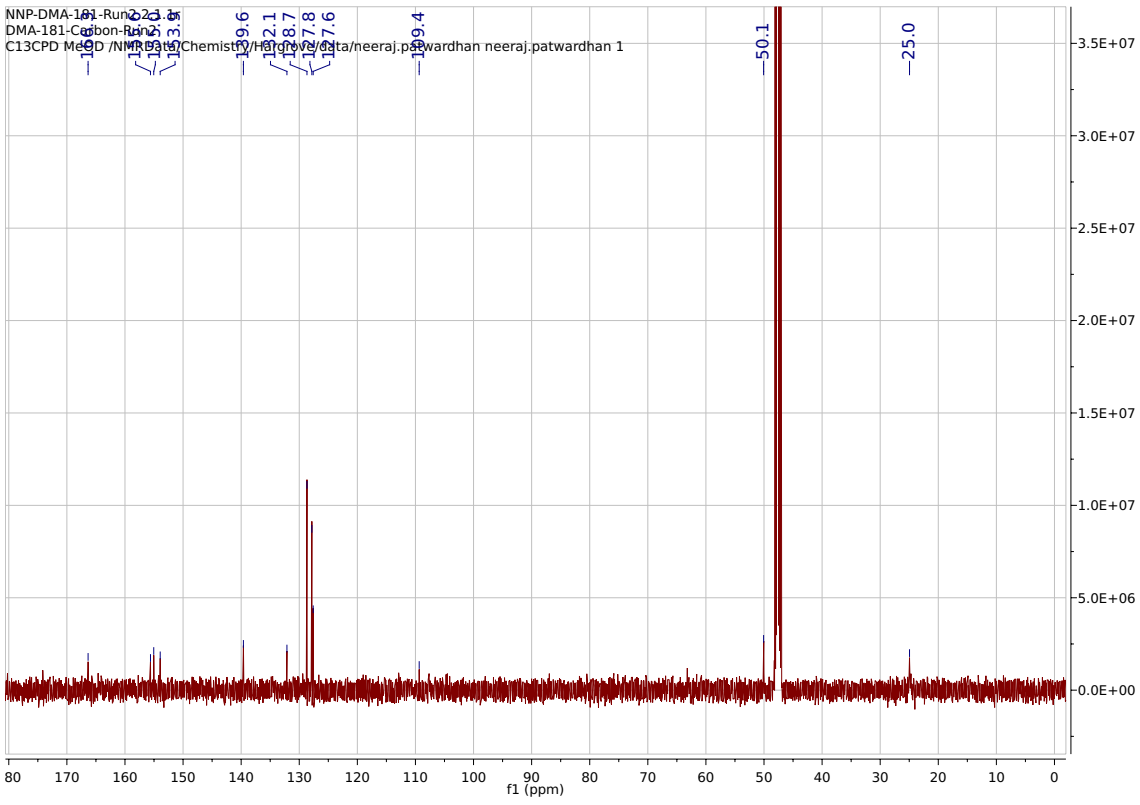
^{13}C NMR for 5-(((1*H*-indol-3-yl)methyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride (4k) (DMA-188):



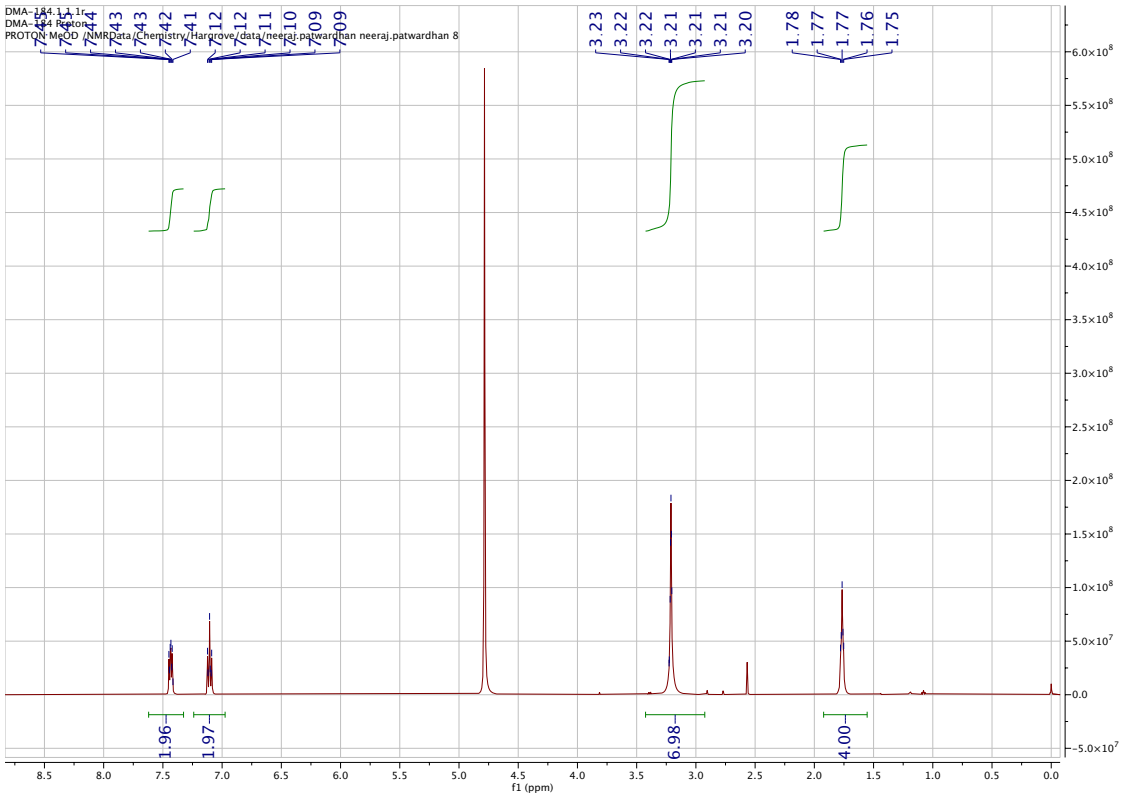
^{1}H NMR for 3-amino-*N*-carbamimidoyl-6-phenyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4l) (DMA-181):



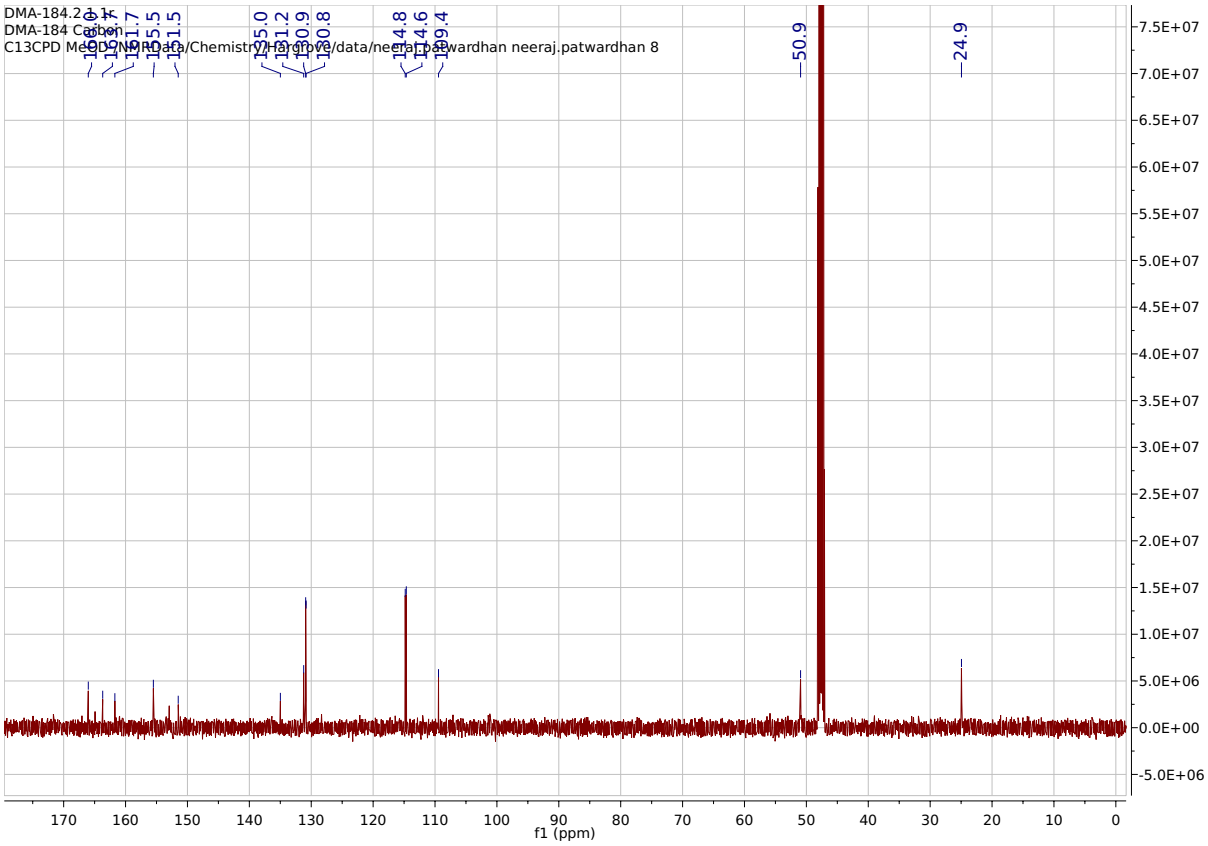
^{13}C NMR for 3-amino-*N*-carbamimidoyl-6-phenyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4l) (DMA-181):



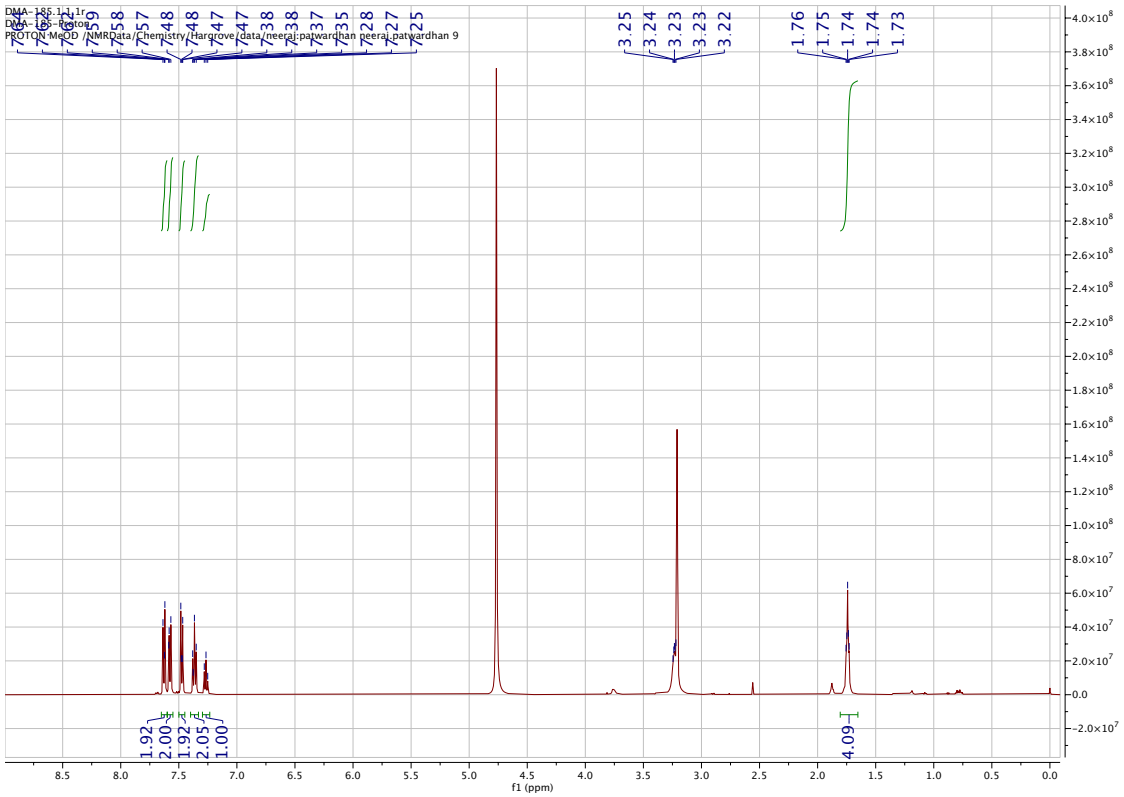
^{1}H NMR for 3-amino-*N*-carbamimidoyl-6-(4-fluorophenyl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4m) (DMA-184):



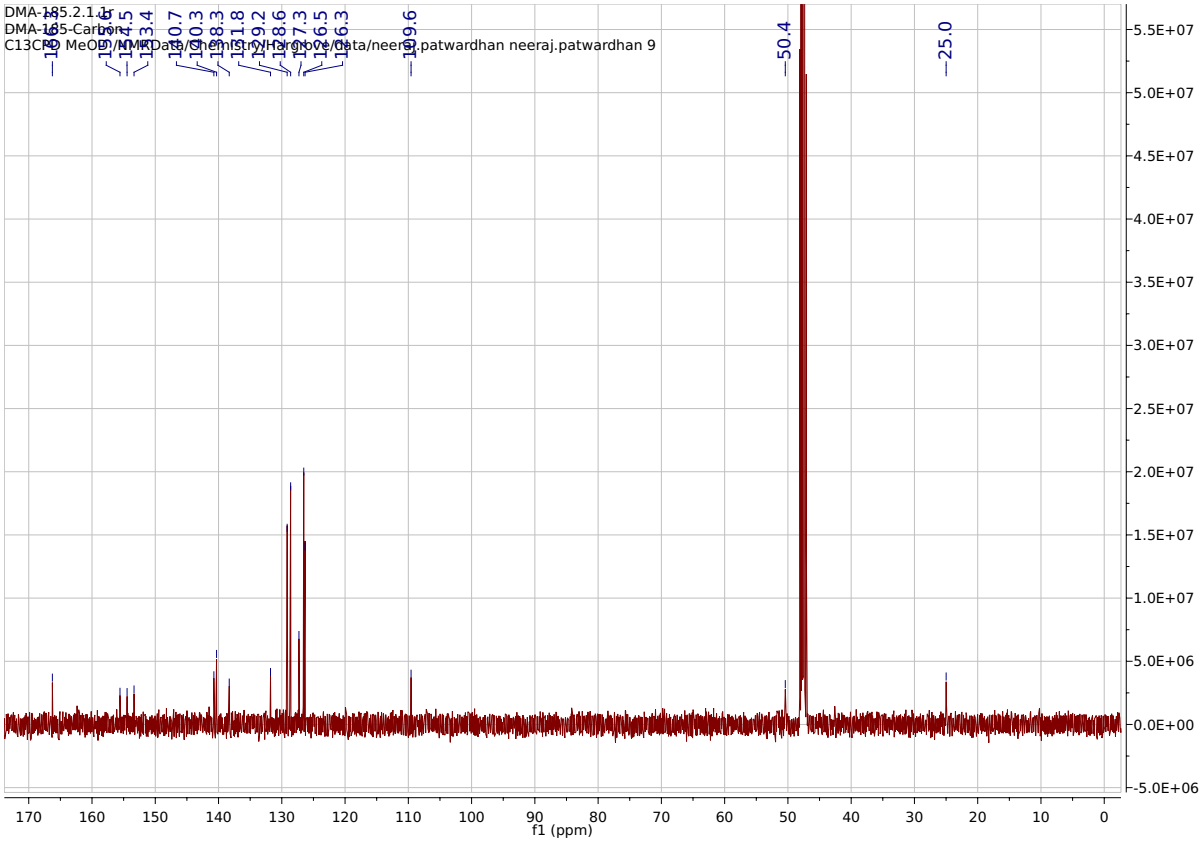
^{13}C NMR for 3-amino-*N*-carbamimidoyl-6-(4-fluorophenyl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4m) (DMA-184):



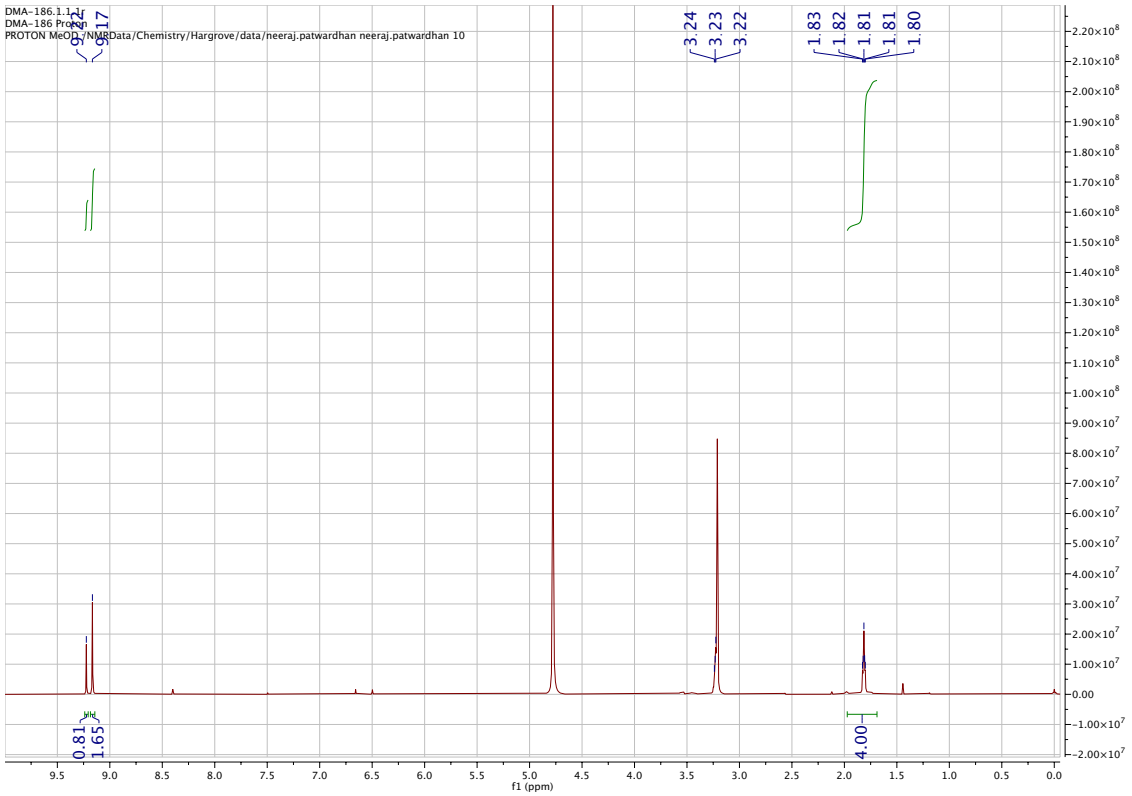
^{1}H NMR for 6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-carbamimidoyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4n) (DMA-185):



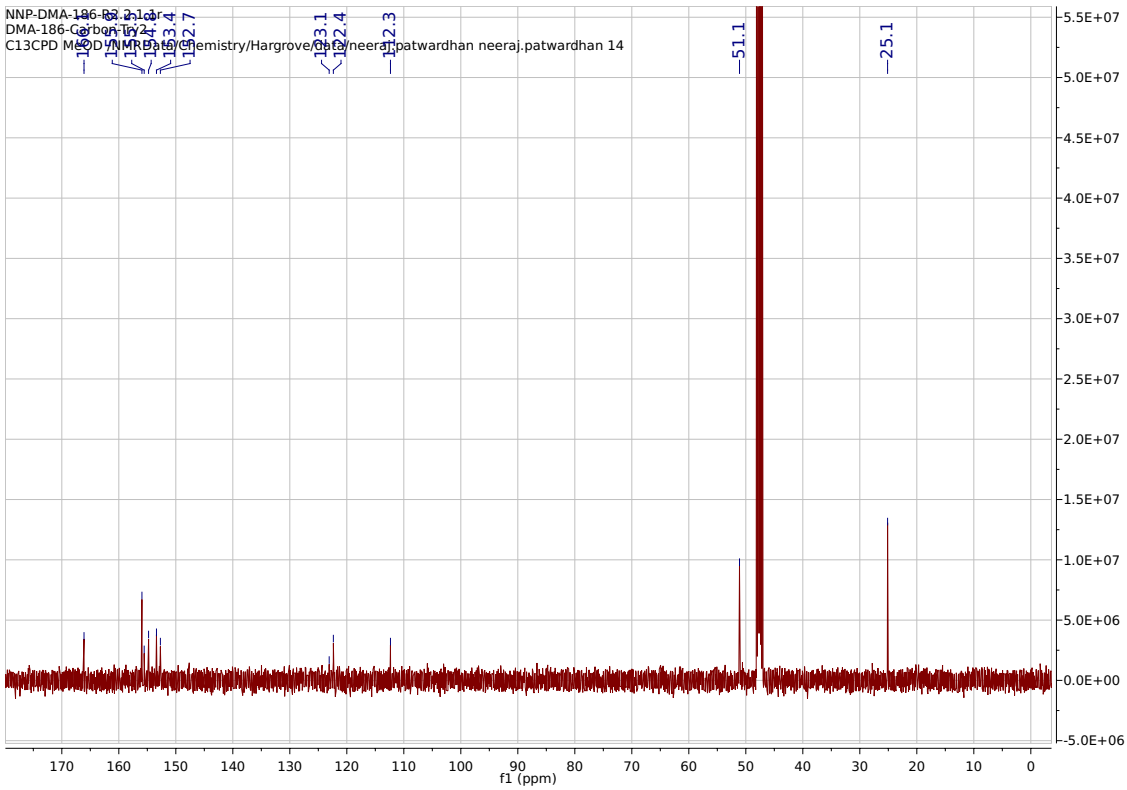
^{13}C NMR for 6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-carbamimidoyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4n) (DMA-185):



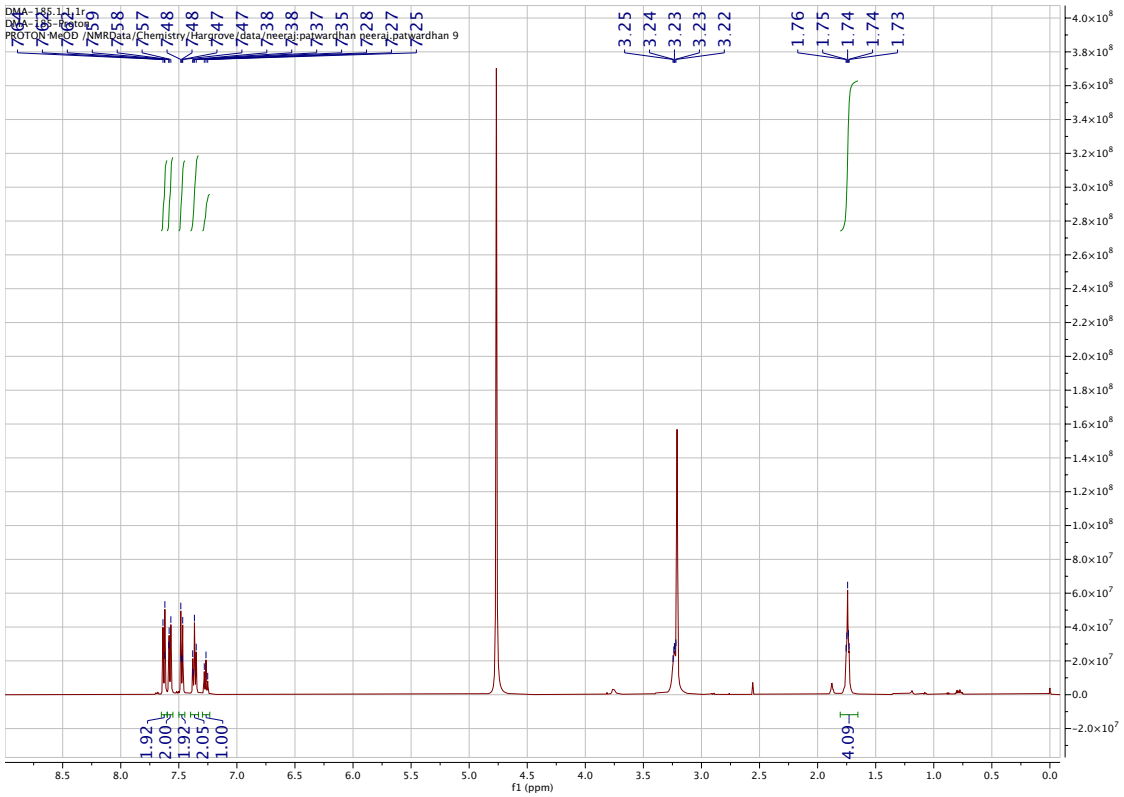
^{1}H NMR for 3-amino-*N*-carbamimidoyl-6-(pyrimidin-5-yl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4o) (DMA-186):



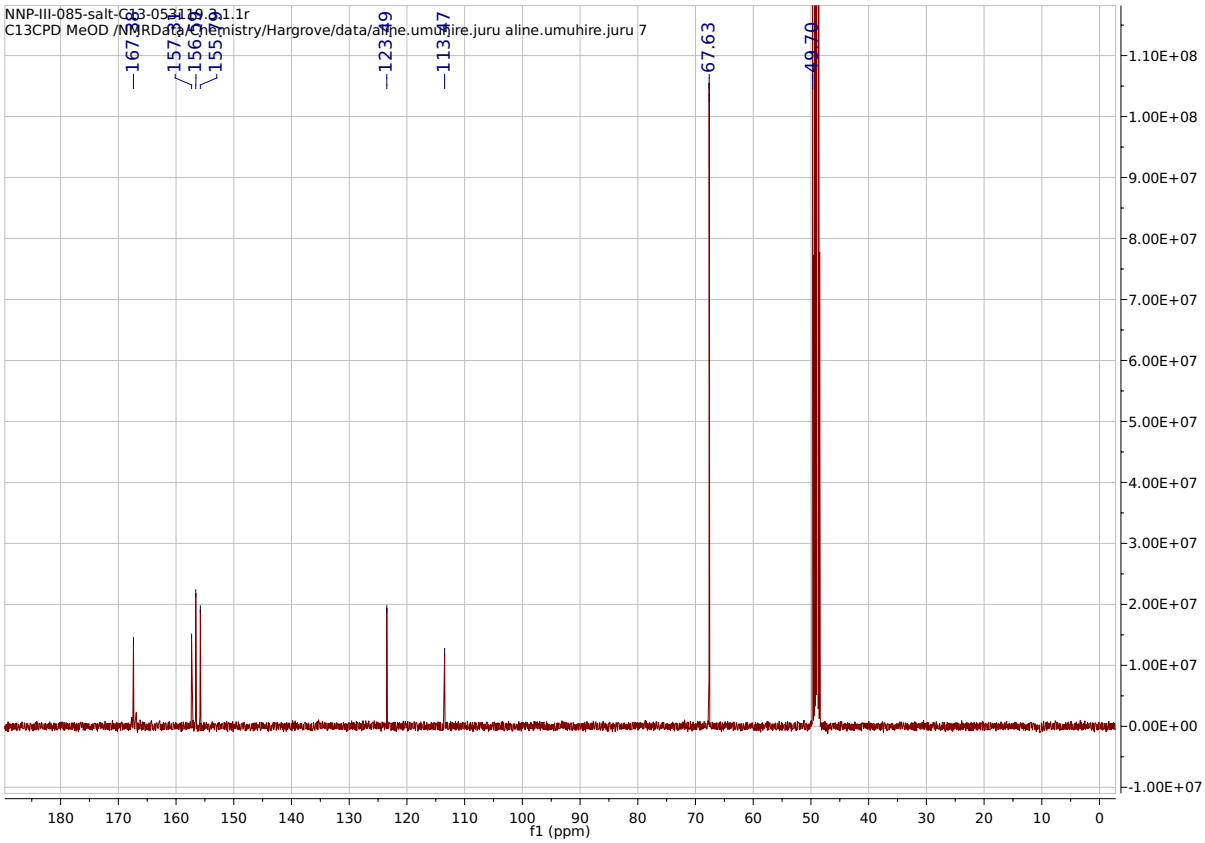
^{13}CNMR for 3-amino-*N*-carbamimidoyl-6-(pyrimidin-5-yl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride (4o) (DMA-186):



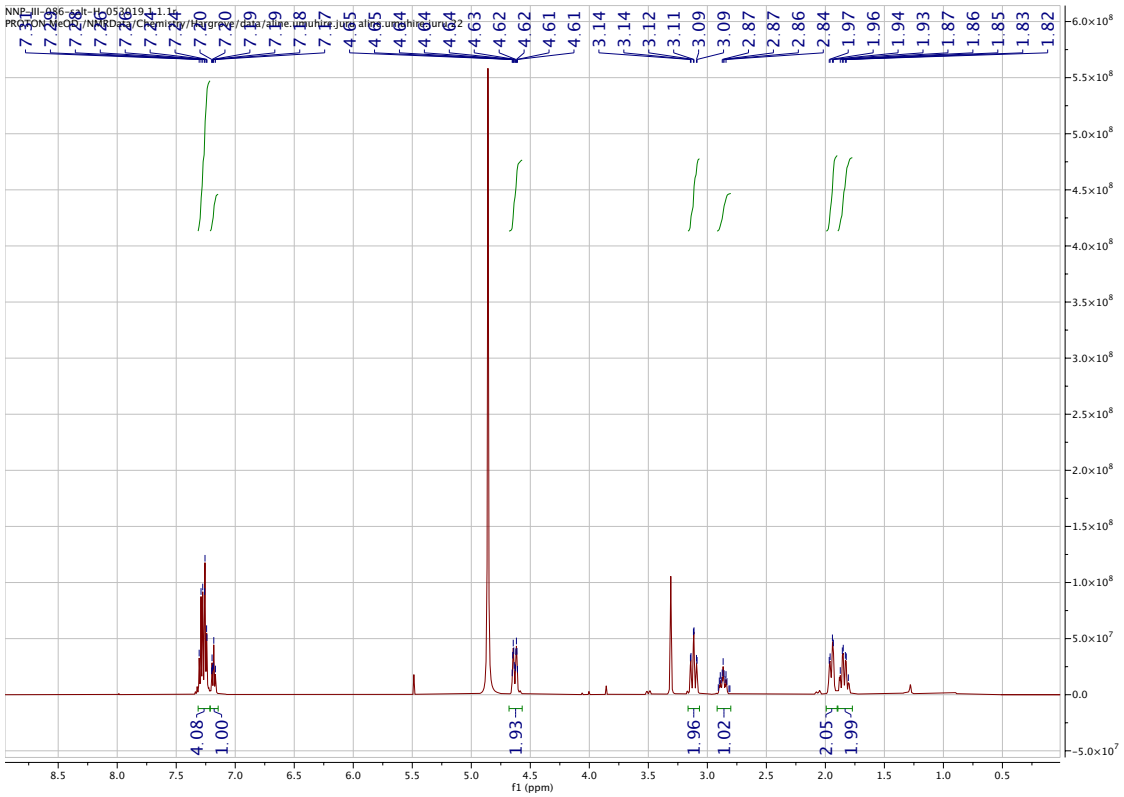
^{1}H NMR Spectrum for 3-Amino-N-carbamimidoyl-6-chloro-5-morpholinopyrazine-2-carboxamide (4p) (DMA-201):



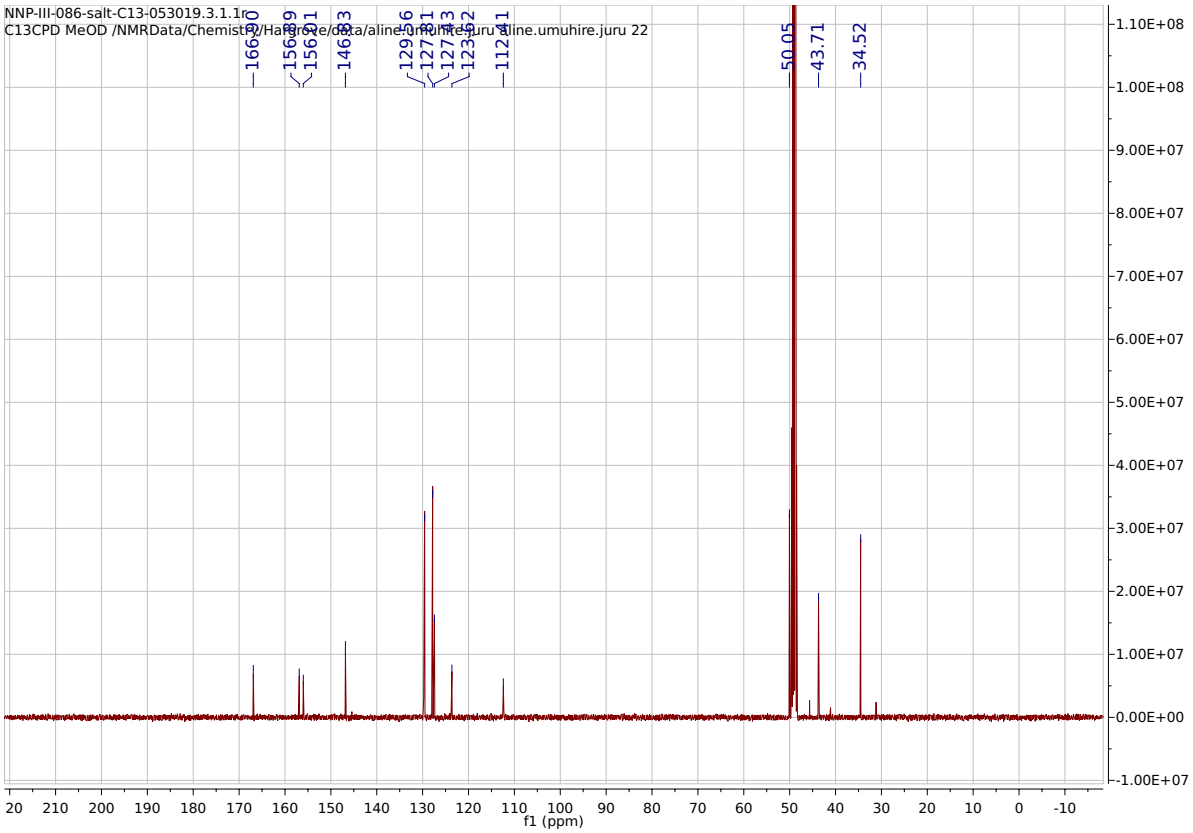
^{13}C NMR Spectrum for 3-Amino-N-carbamimidoyl-6-chloro-5-morpholinopyrazine-2-carboxamide (4p) (DMA-201):



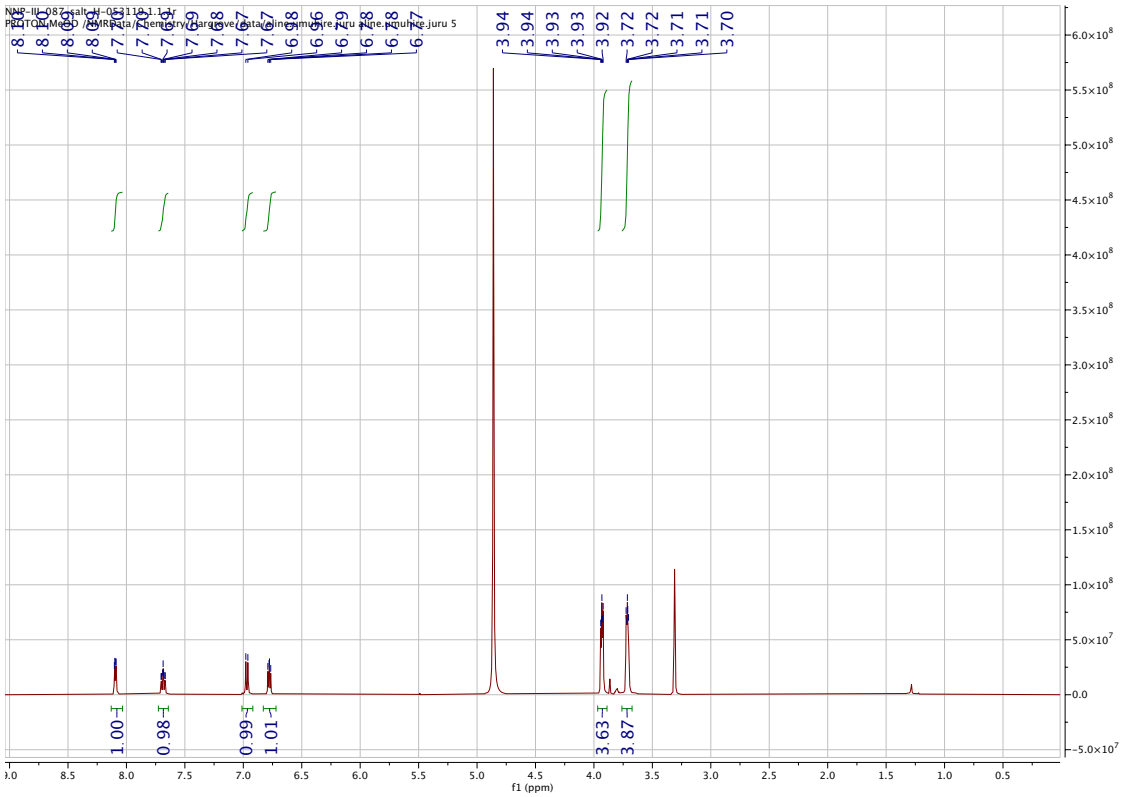
^{1}H NMR Spectrum for 3-Amino-N-carbamimidoyl-6-chloro-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxamide (4q) (DMA-202):



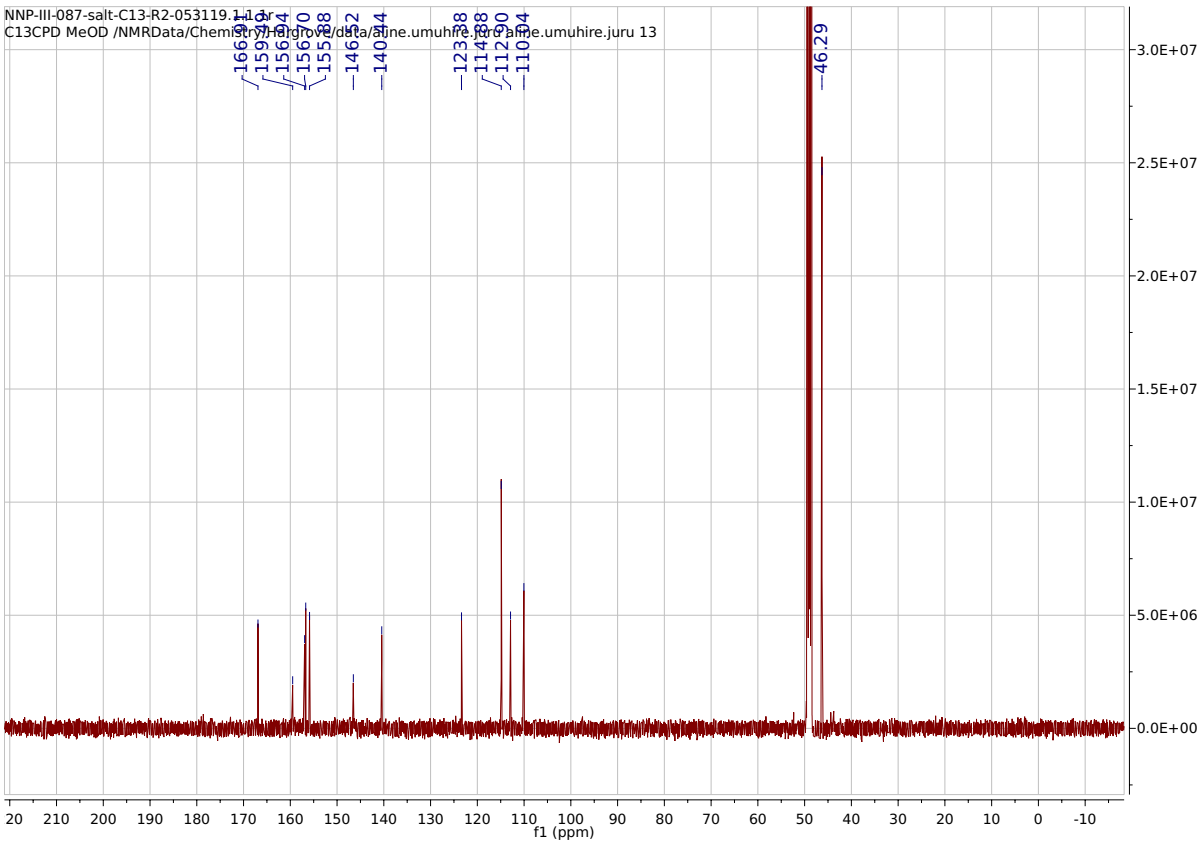
^{13}C NMR Spectrum for 3-Amino-N-carbamimidoyl-6-chloro-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxamide (4q) (DMA-202):



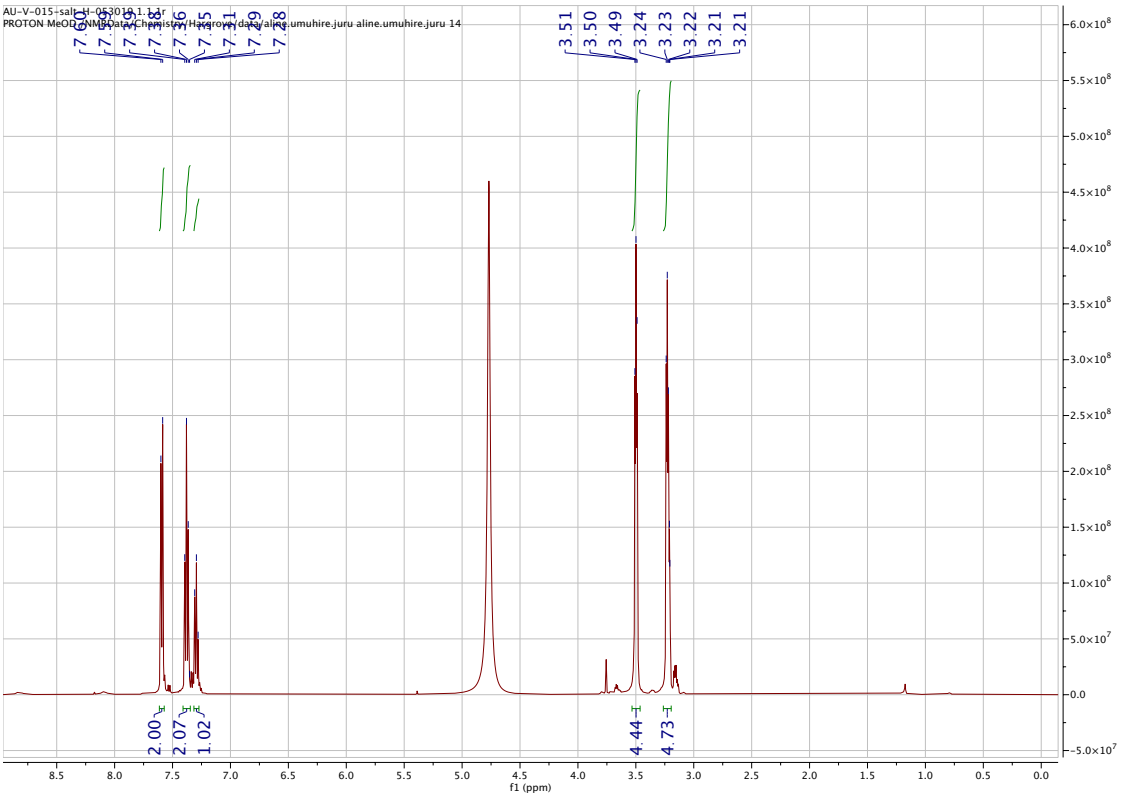
^{1}H NMR Spectrum for 3-Amino-N-carbamimidoyl-6-chloro-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxamide (4r) (DMA-203):



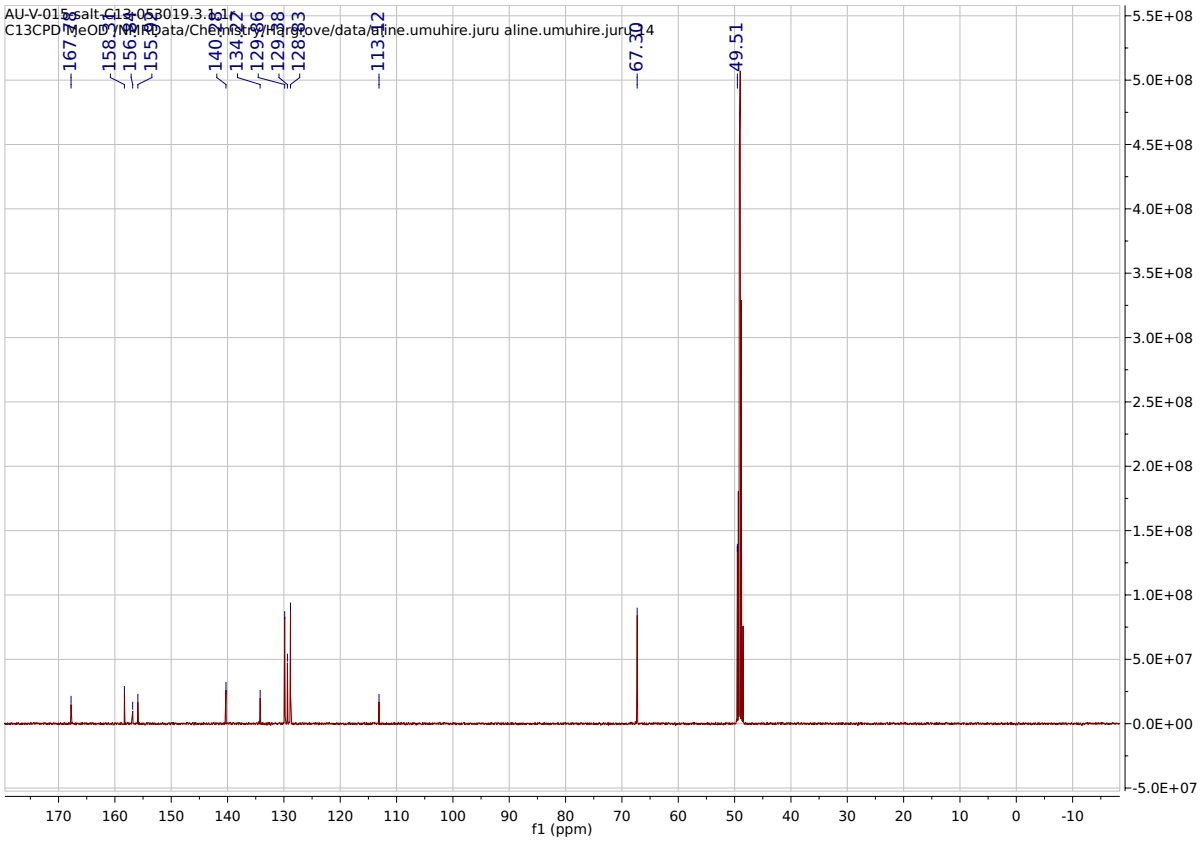
^{13}C NMR Spectrum for 3-Amino-N-carbamimidoyl-6-chloro-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxamide (4r) (DMA-203):



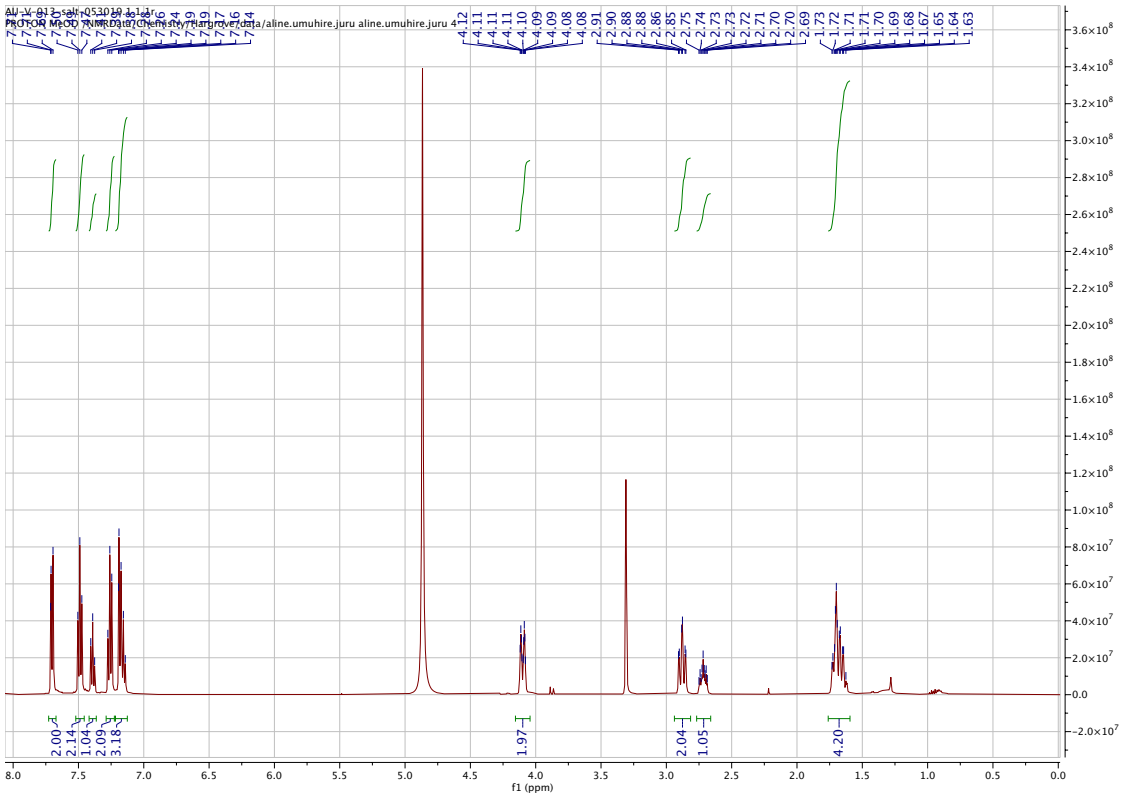
^{1}H NMR Spectrum for 3-Amino-N-carbamimidoyl-5-morpholino-6-phenylpyrazine-2-carboxamide (4s) (DMA-204):



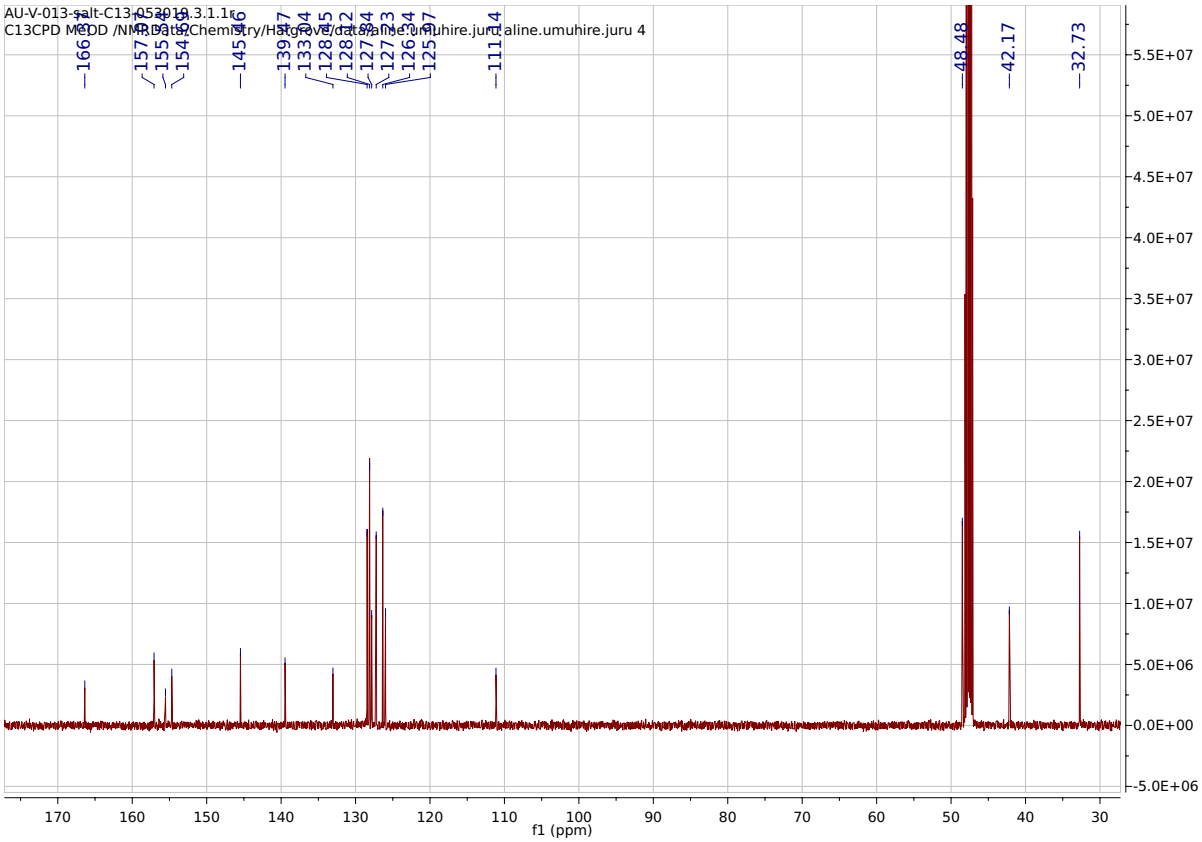
^{13}C NMR Spectrum for 3-Amino-N-carbamimidoyl-5-morpholino-6-phenylpyrazine-2-carboxamide (4s) (DMA-204):



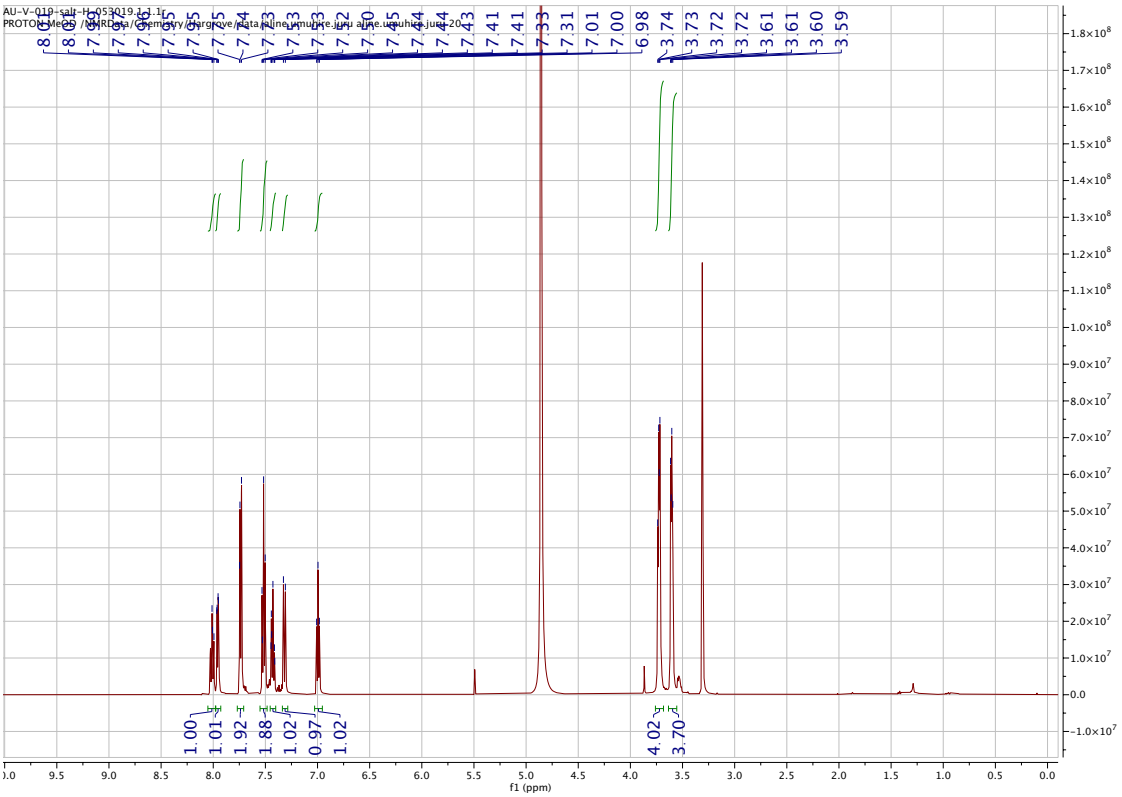
^{1}H NMR Spectrum for 3-Amino-N-carbamimidoyl-6-phenyl-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxamide (4t) (DMA-205):



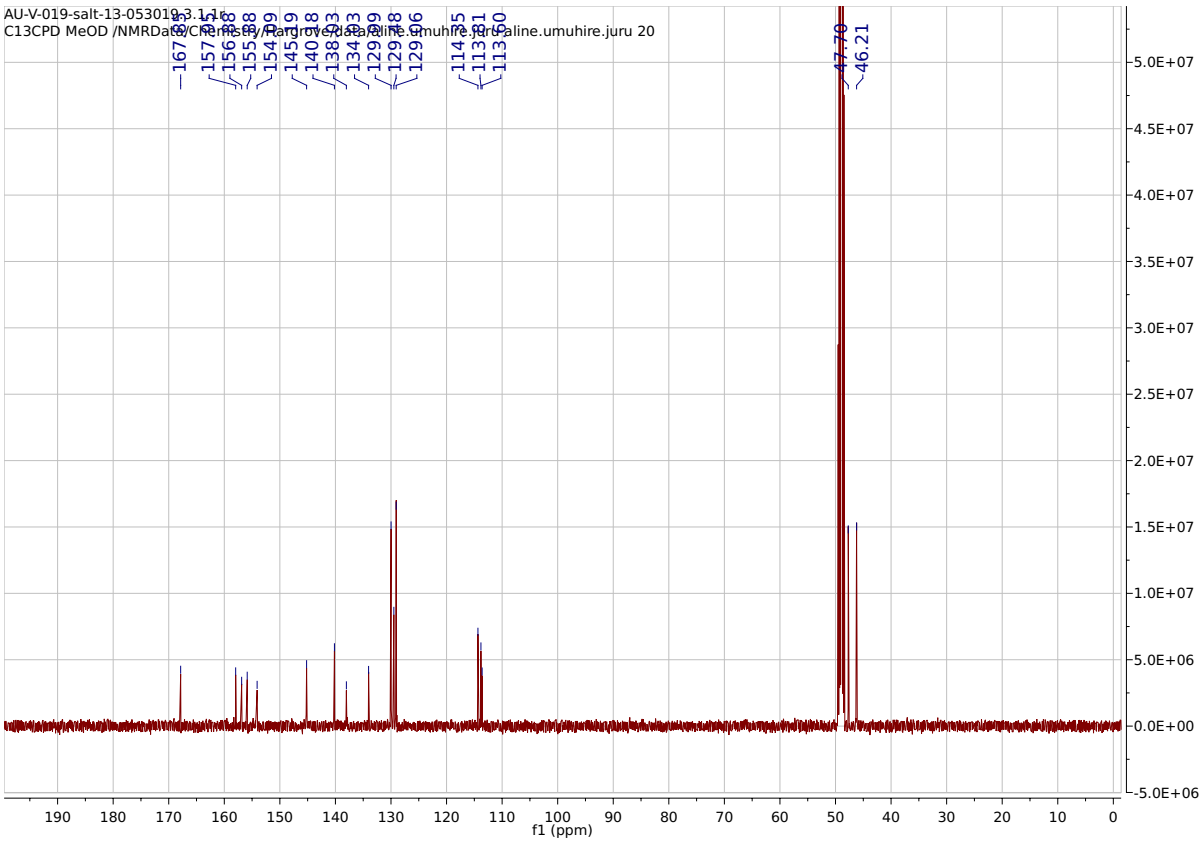
^{13}C NMR Spectrum for 3-Amino-N-carbamimidoyl-6-phenyl-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxamide (4t) (DMA-205):



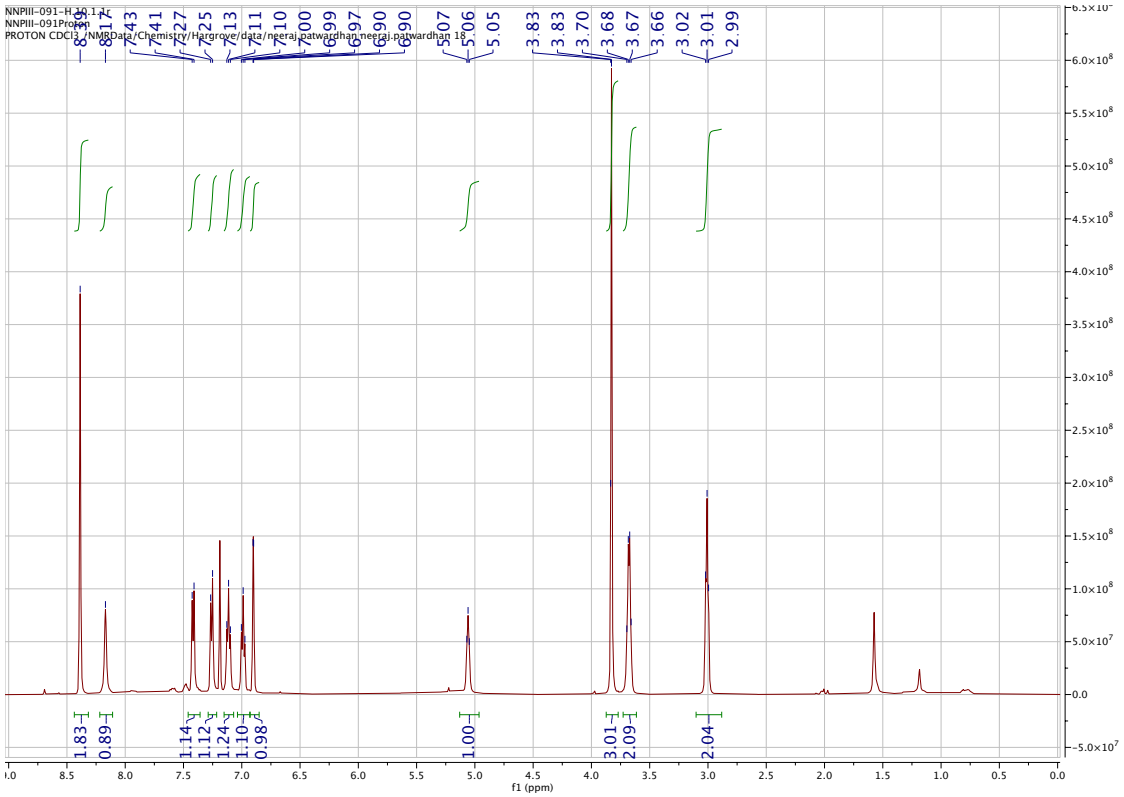
^{1}H NMR Spectrum for 3-Amino-N-carbamimidoyl-6-phenyl-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxamide (4u) (DMA-206):



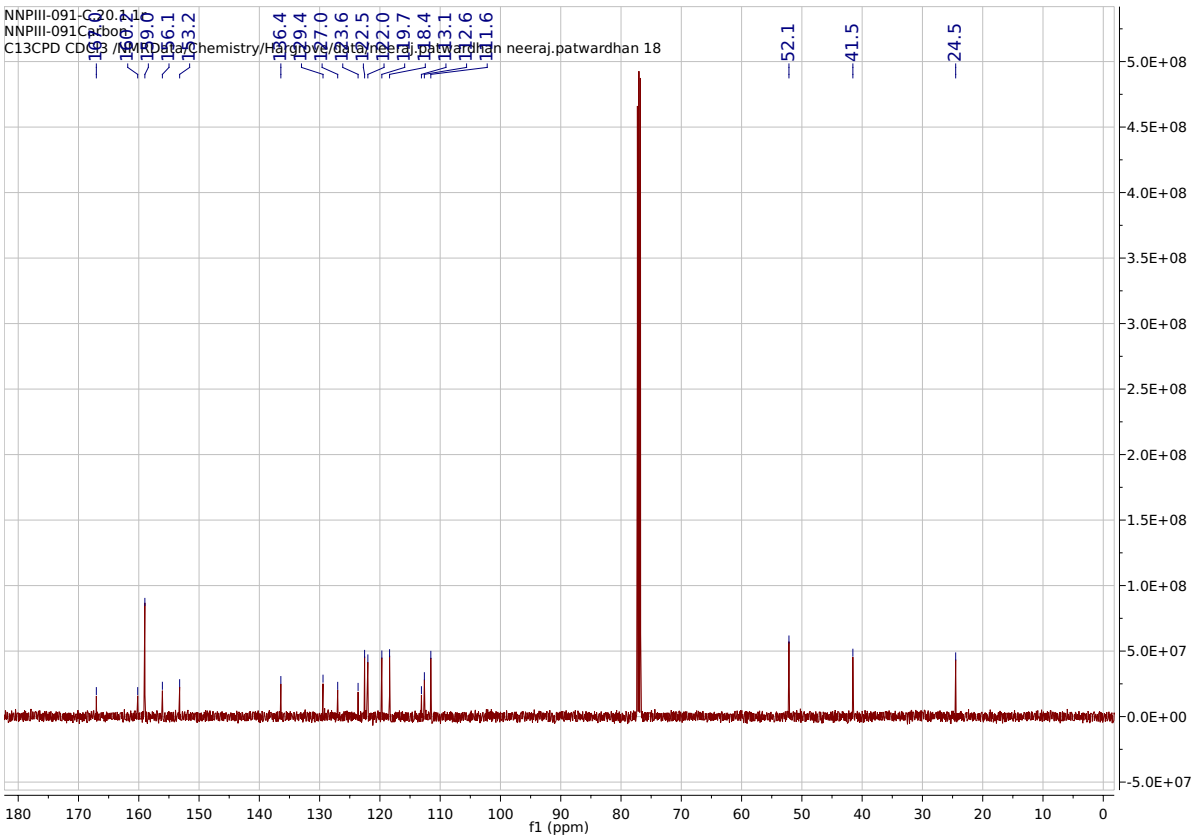
^{13}C NMR Spectrum for 3-Amino-N-carbamimidoyl-6-phenyl-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxamide (4u) (DMA-206):



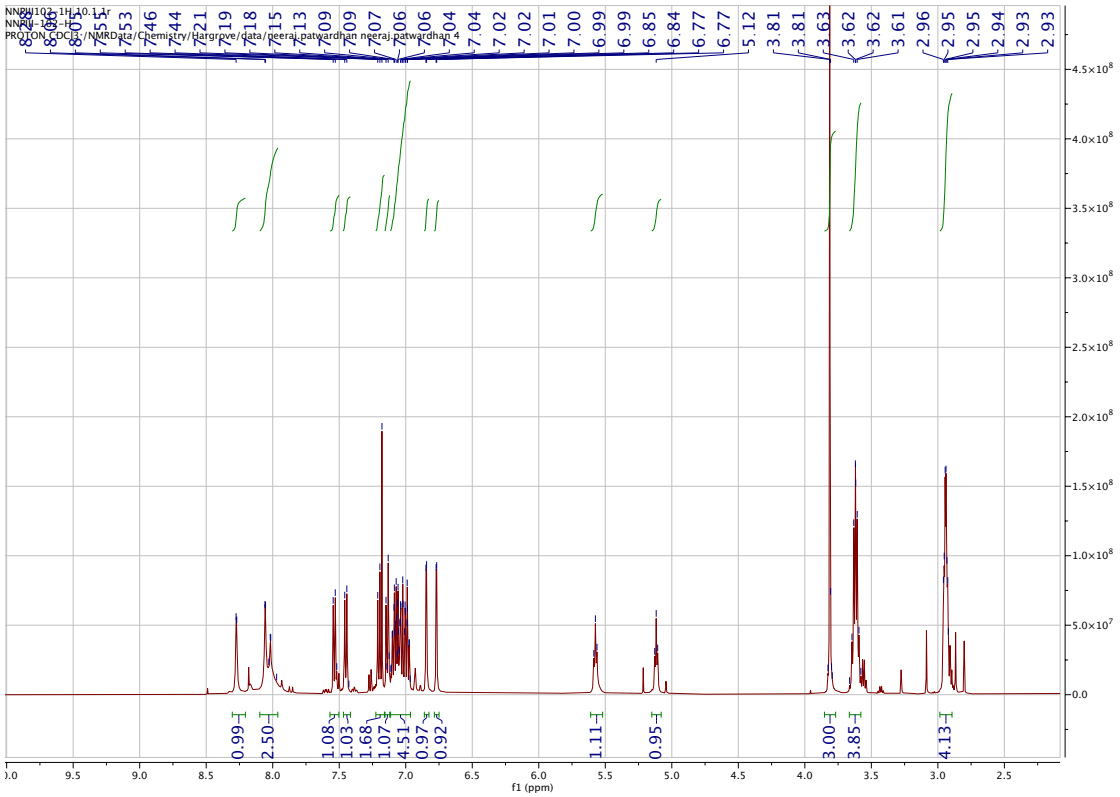
^{1}H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-chloropyrimidin-5-yl)pyrazine-2-carboxylate (6):



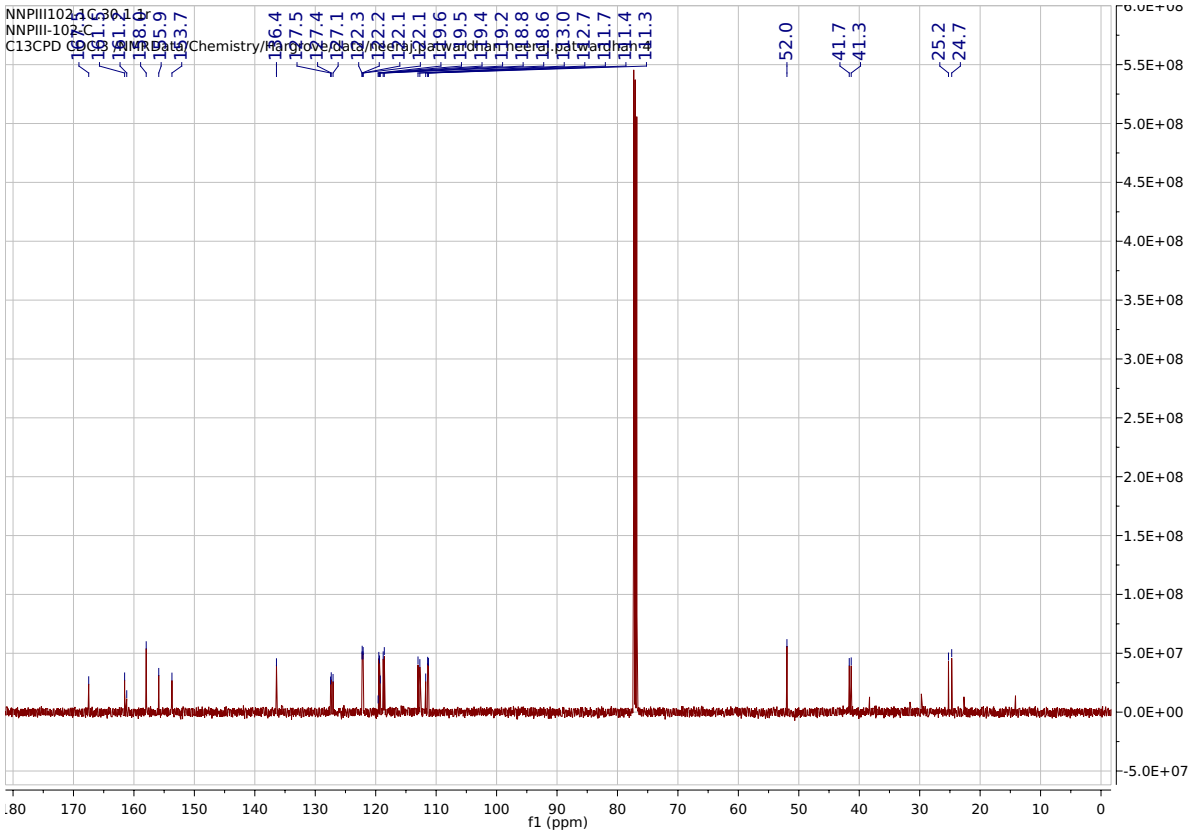
^{13}CNMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-chloropyrimidin-5-yl)pyrazine-2-carboxylate (6):



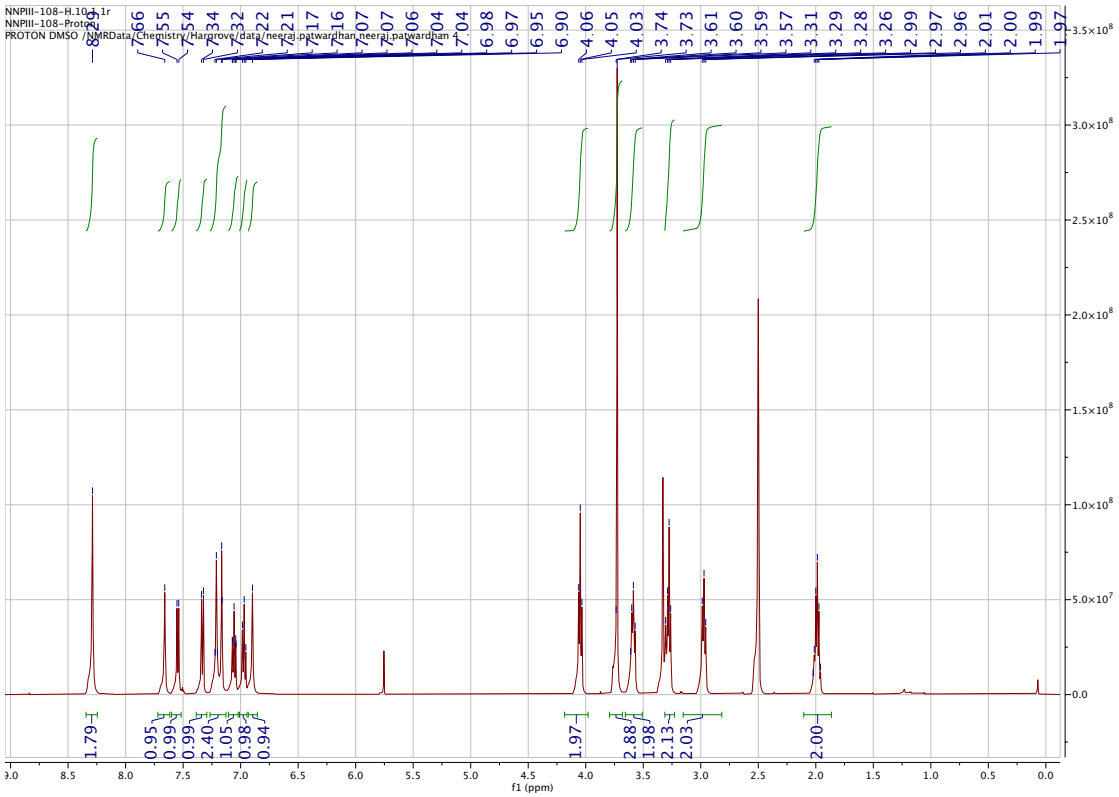
^{1}H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-(2-((2-(1*H*-indol-3-yl)ethyl)amino)pyrimidin-5-yl)-3-aminopyrazine-2-carboxylate (7a):



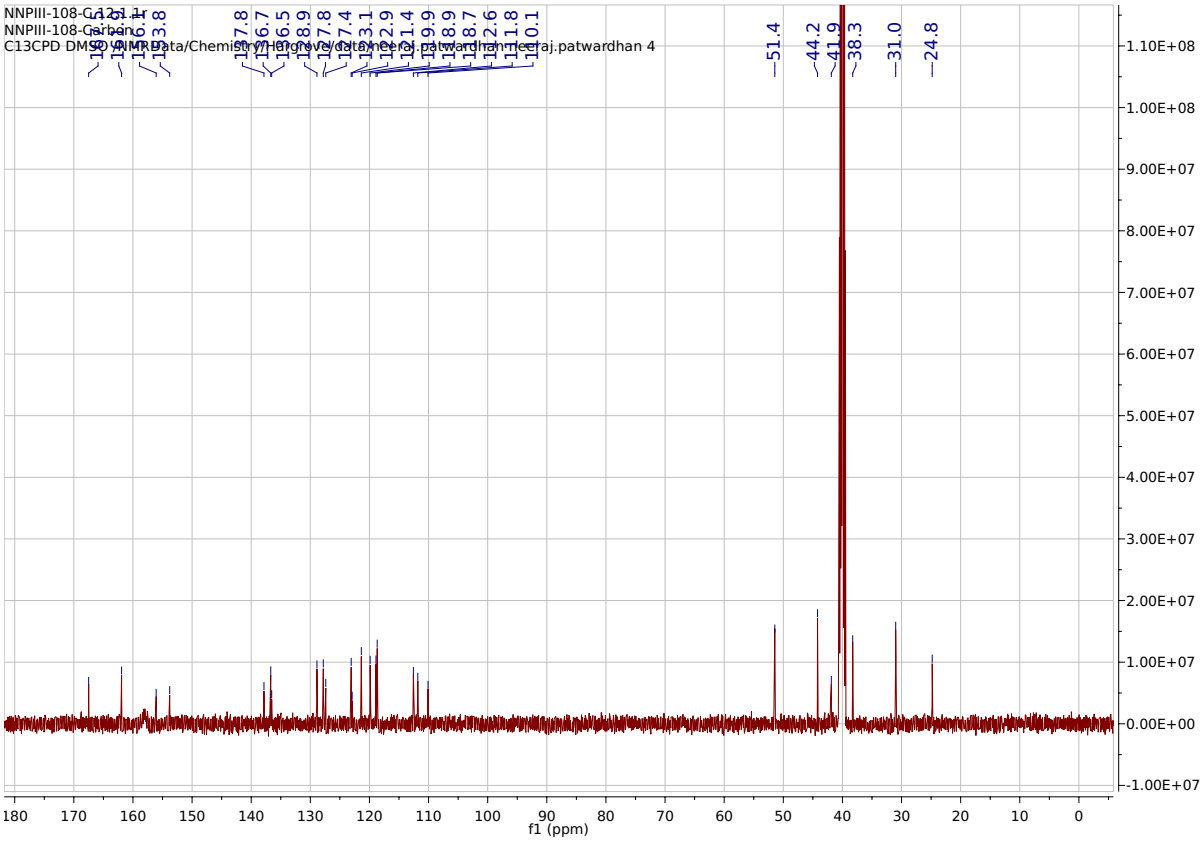
^{13}C NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-(2-((2-(1*H*-indol-3-yl)ethyl)amino)pyrimidin-5-yl)-3-aminopyrazine-2-carboxylate (7a):



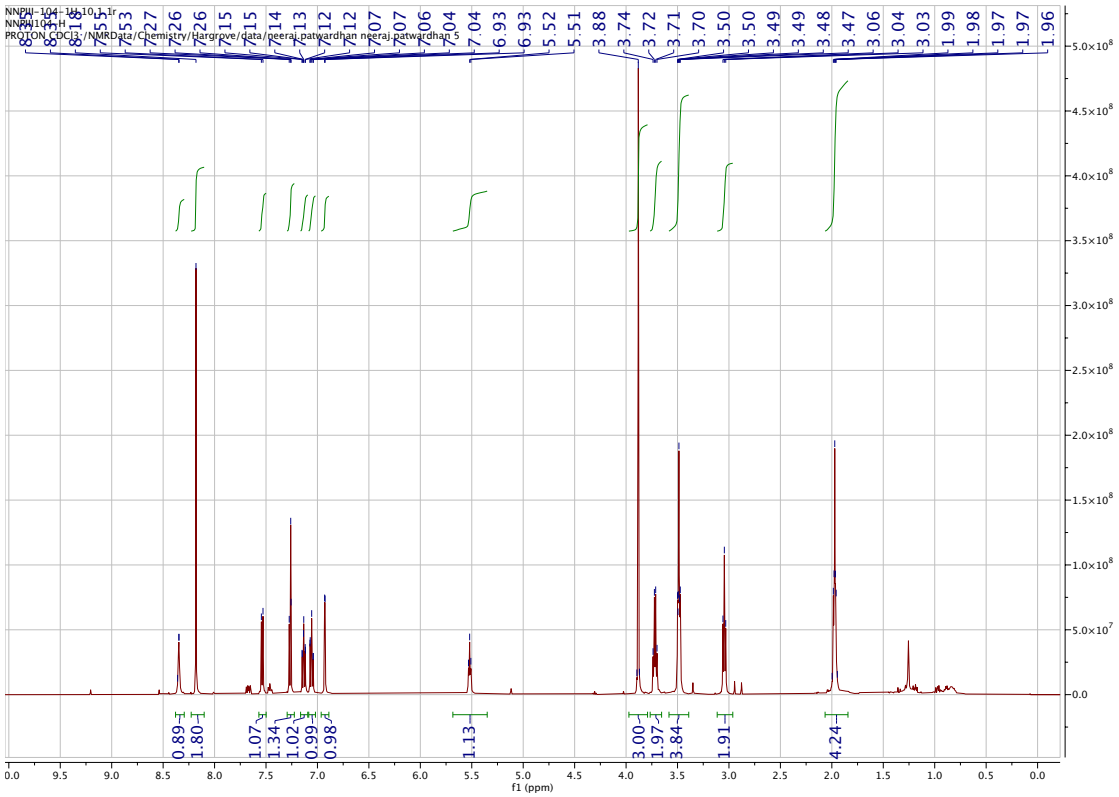
^{1}H NMR for Methyl 6-(2-((3-(1*H*-imidazol-1-yl)propyl)amino)pyrimidin-5-yl)-5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-aminopyrazine-2-carboxylate (7b):



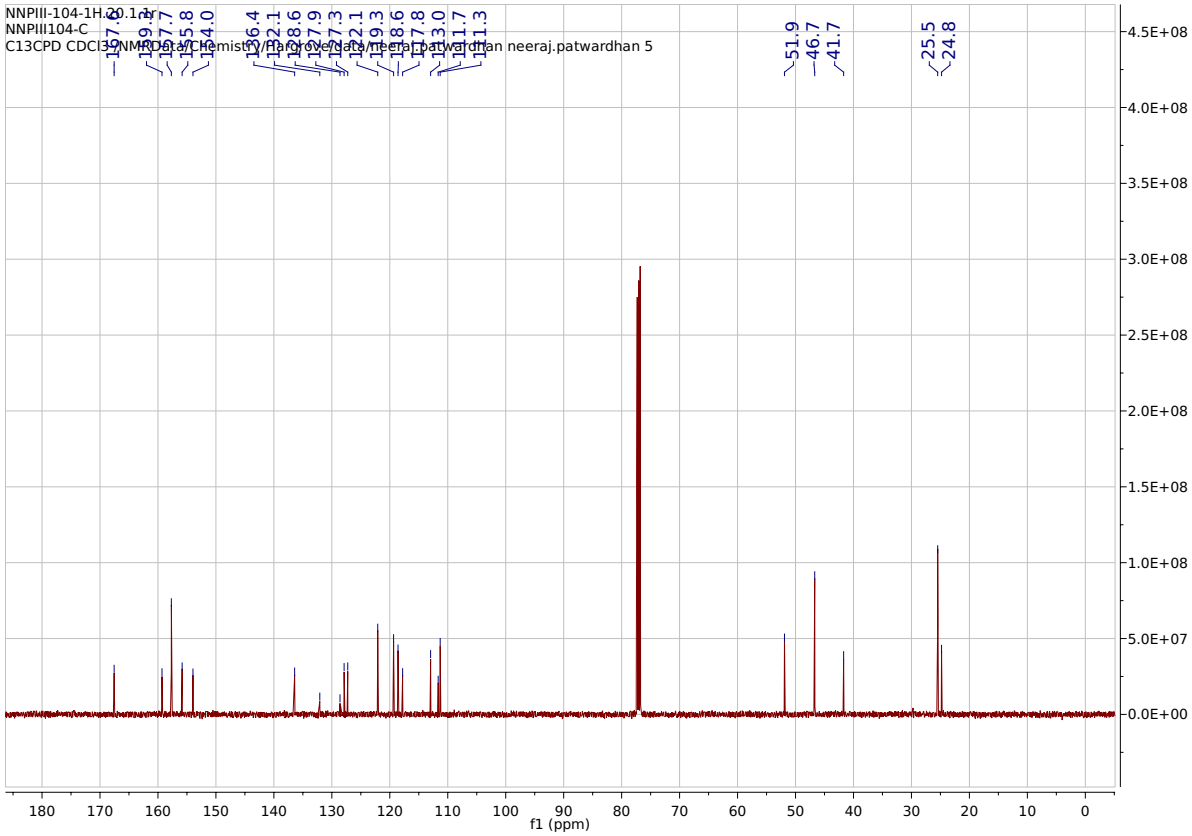
^{13}CNMR for Methyl 6-(2-((3-(1*H*-imidazol-1-yl)propyl)amino)pyrimidin-5-yl)-5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-aminopyrazine-2-carboxylate (7b):



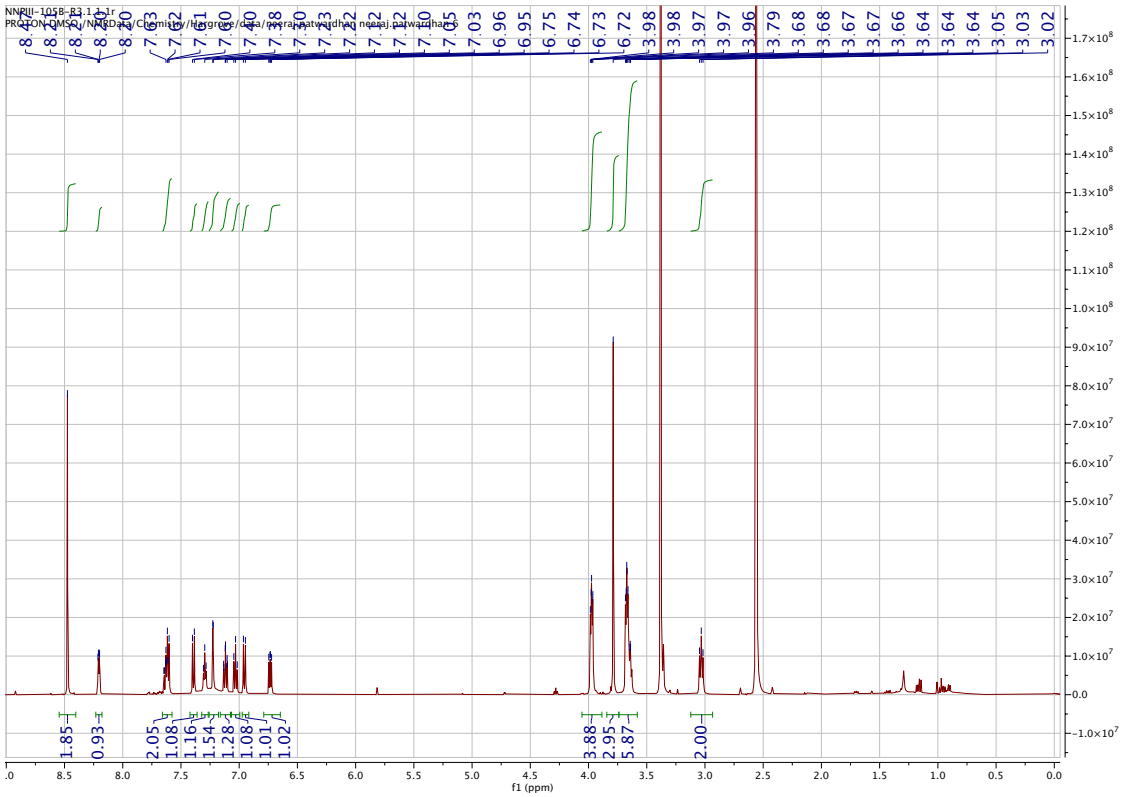
^{1}H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-(pyrrolidin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxylate (7c):



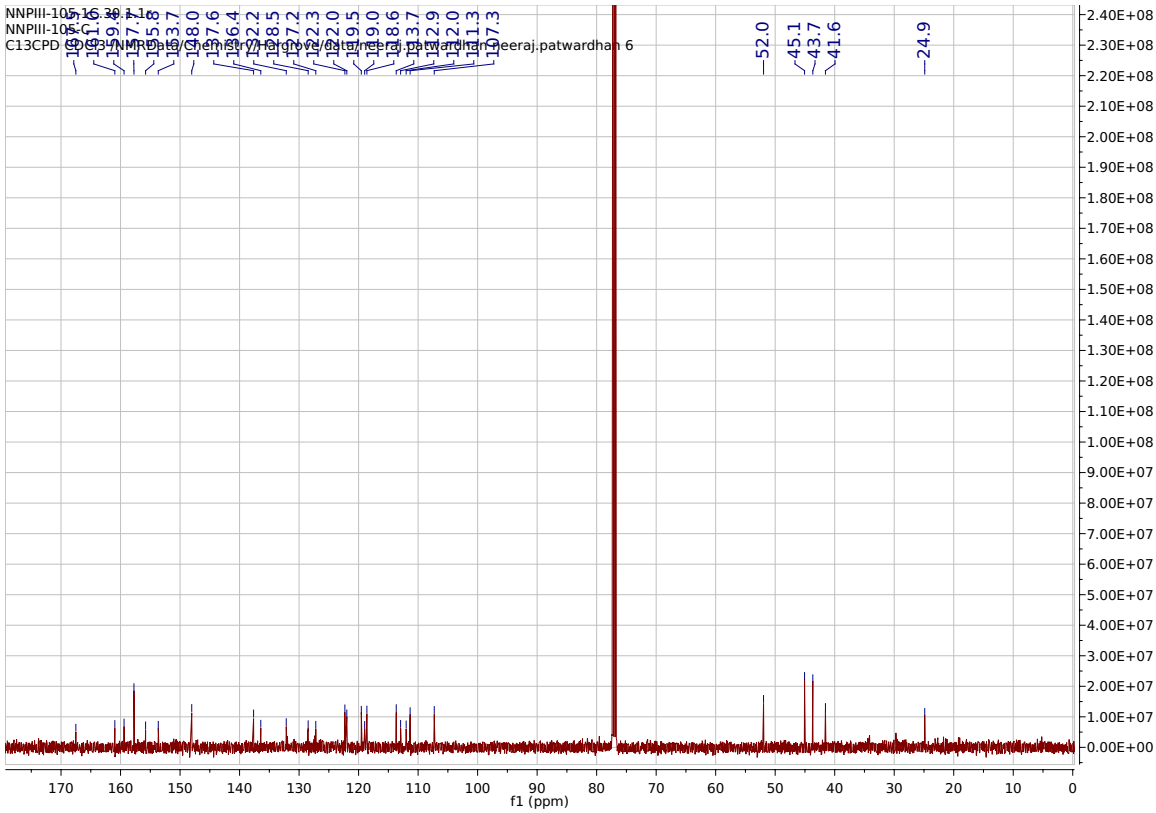
^{13}C NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-(pyrrolidin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxylate (7c):



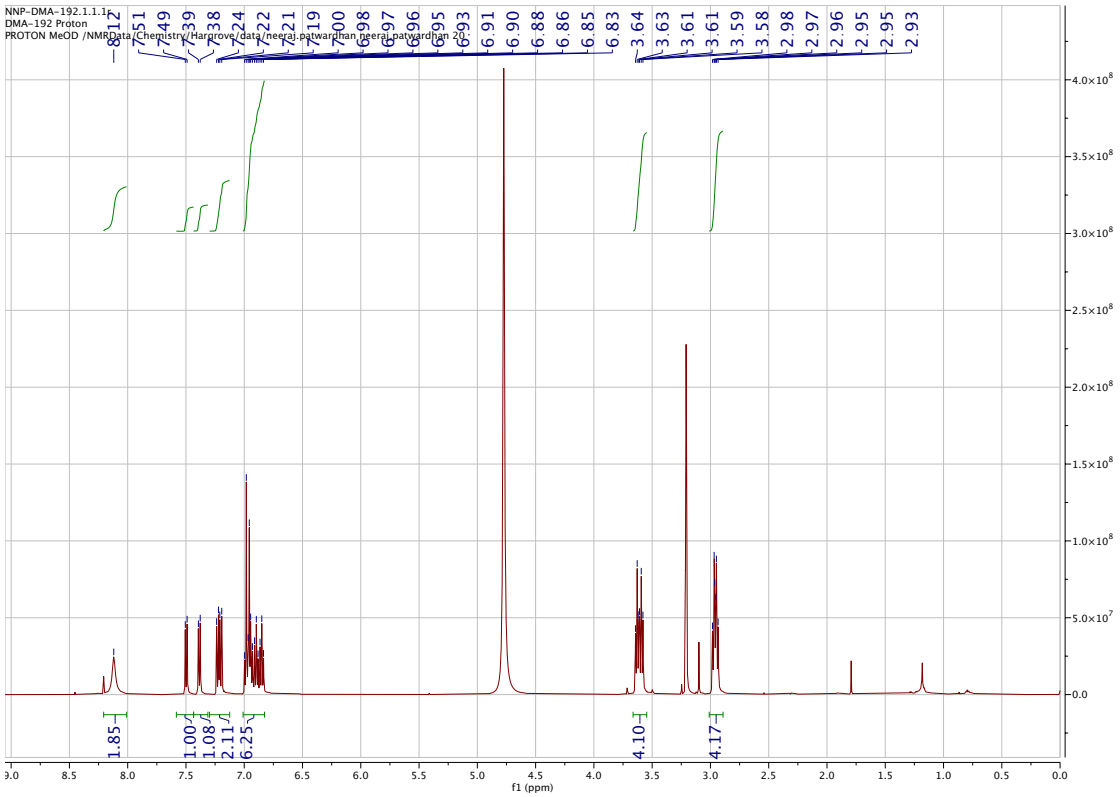
^{1}H NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxylate (7d):



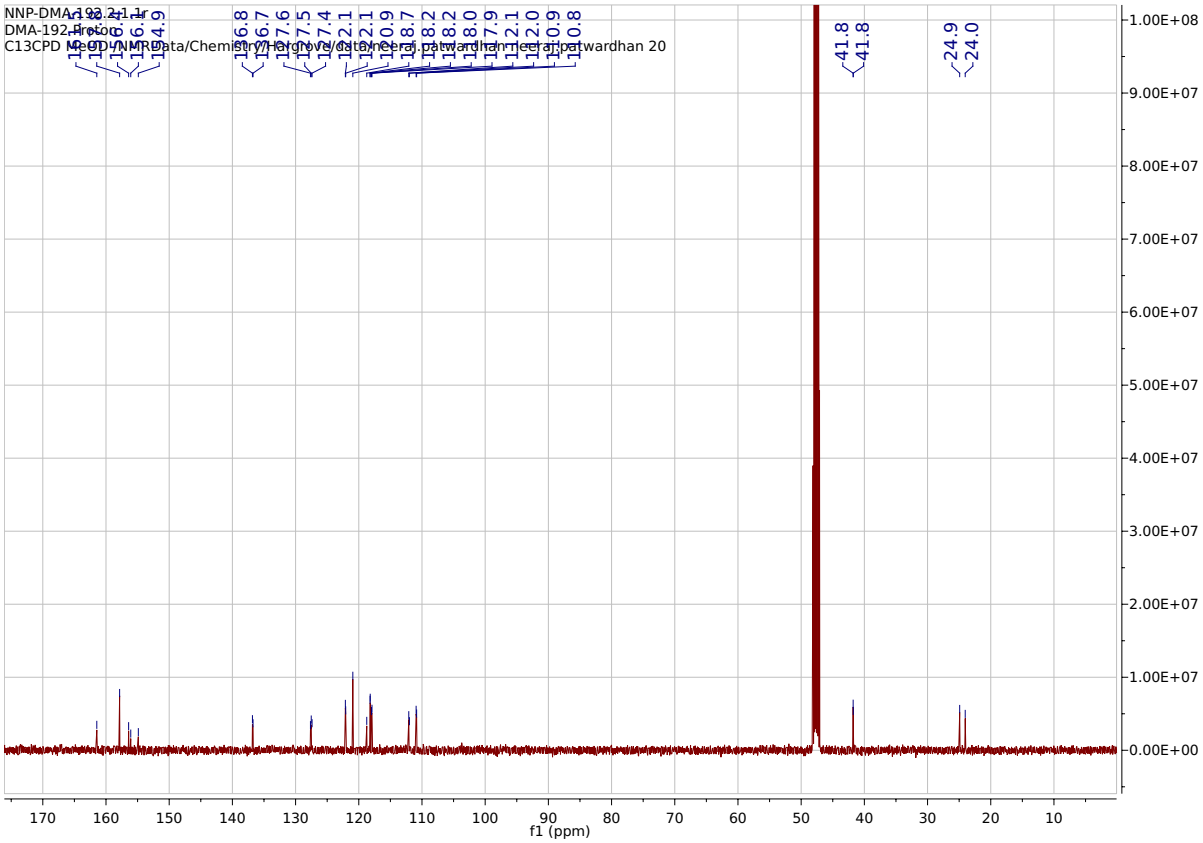
^{13}C NMR for Methyl 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-6-(2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxylate (7d):



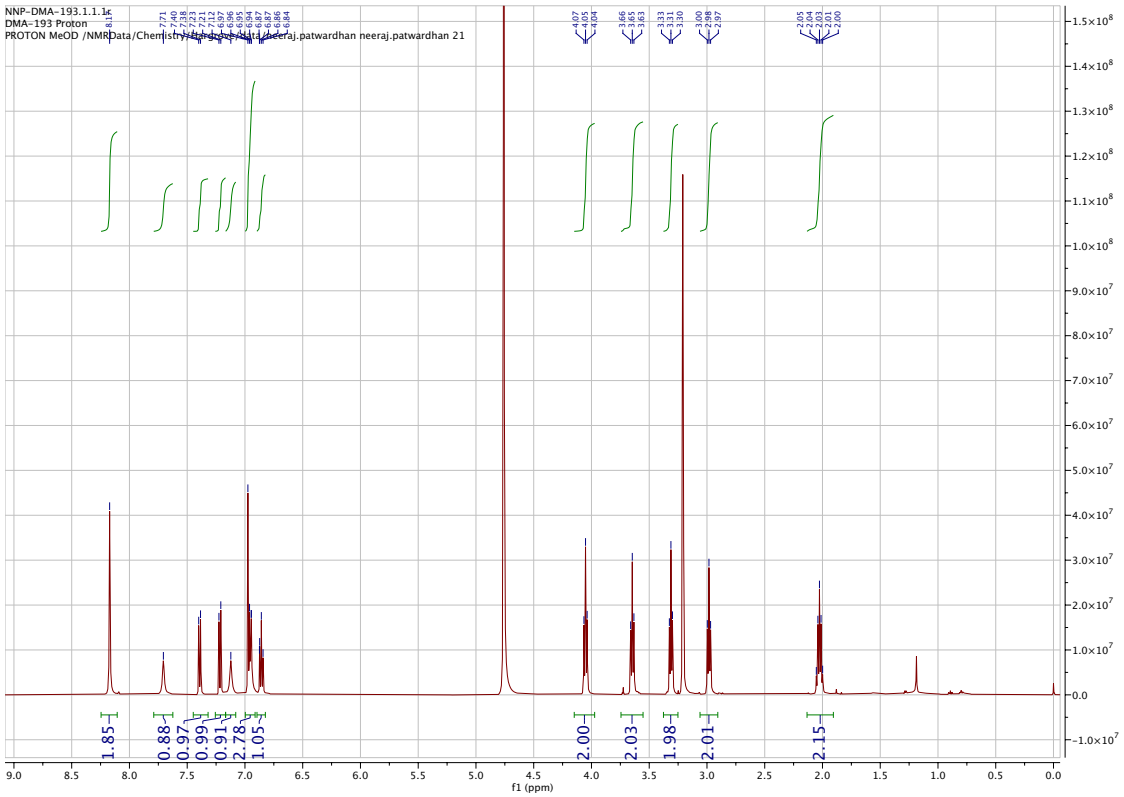
1H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-(2-((2-(1*H*-indol-3-yl)ethyl)amino)pyrimidin-5-yl)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (8a) (DMA-192):



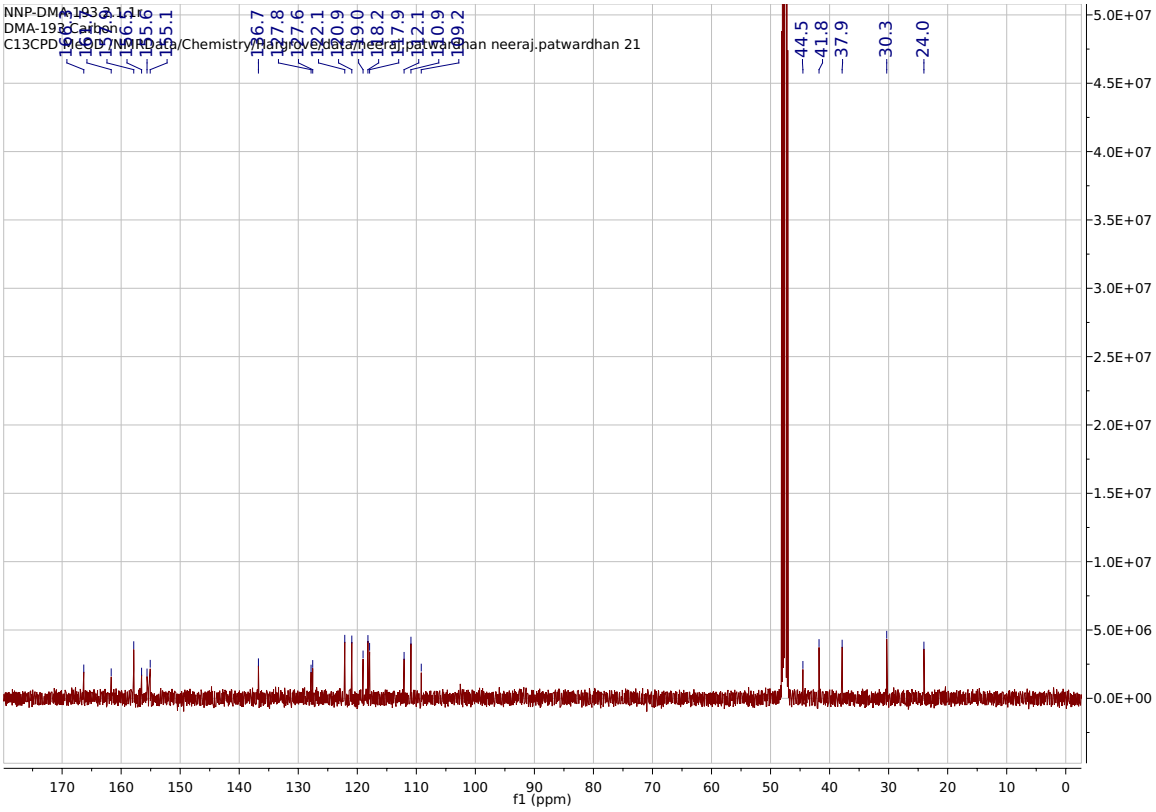
13C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-(2-((2-(1*H*-indol-3-yl)ethyl)amino)pyrimidin-5-yl)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (8a) (DMA-192):



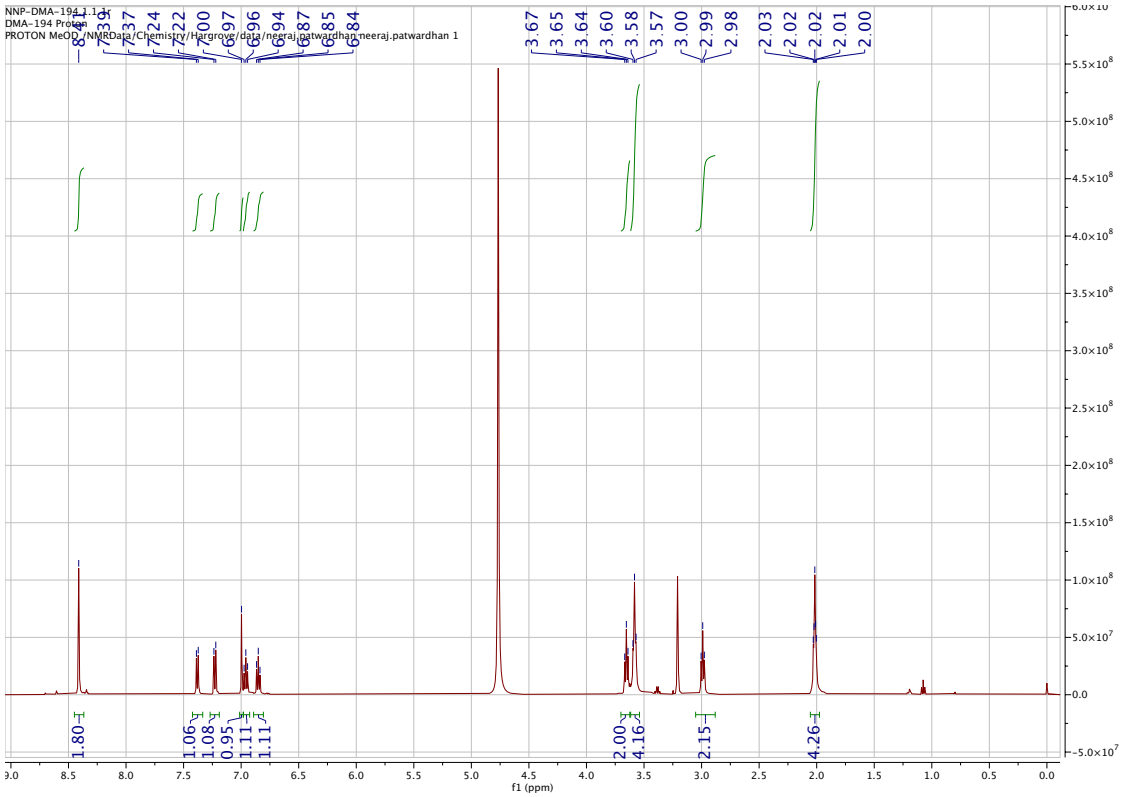
^{1}H NMR for 6-(2-((3-(1*H*-imidazol-1-yl)propyl)amino)pyrimidin-5-yl)-5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (8b) (DMA-193):



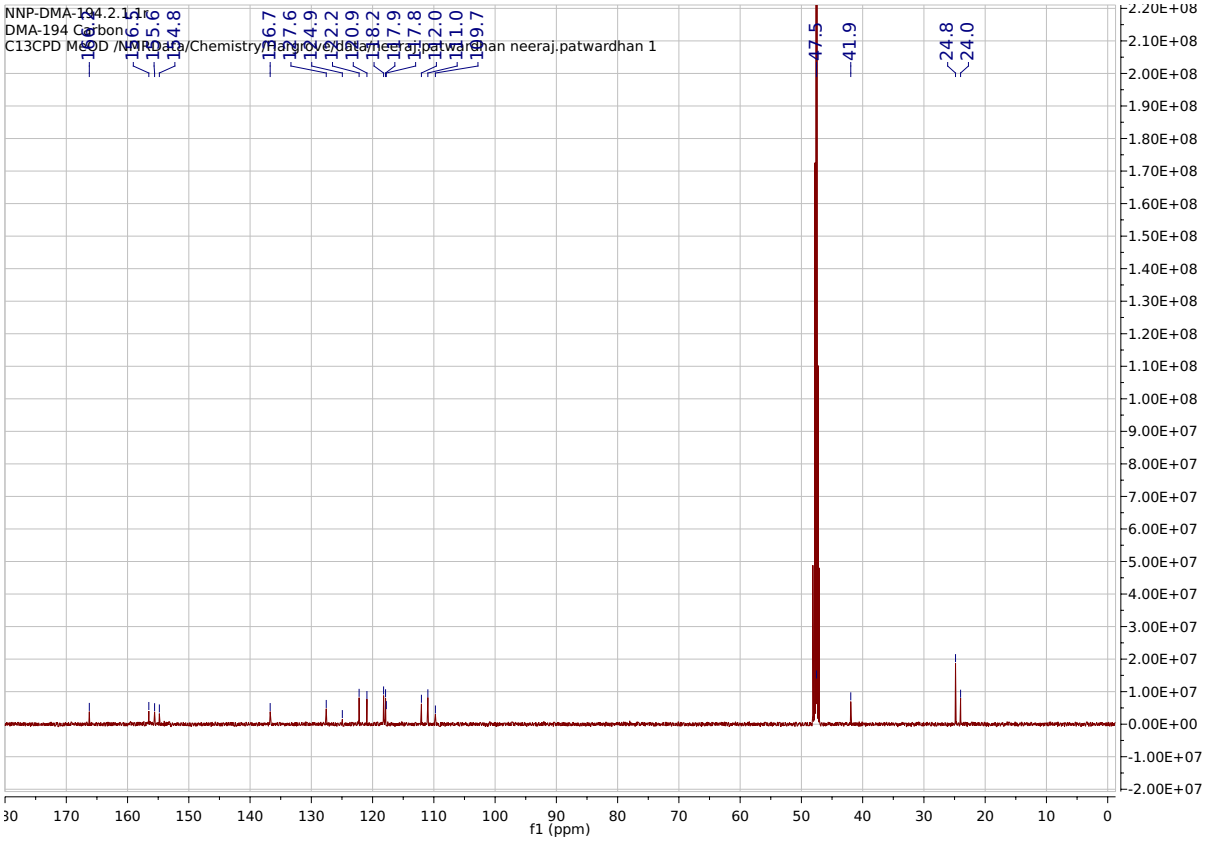
^{13}C NMR for 6-(2-((3-(1*H*-imidazol-1-yl)propyl)amino)pyrimidin-5-yl)-5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride (8b) (DMA-193):



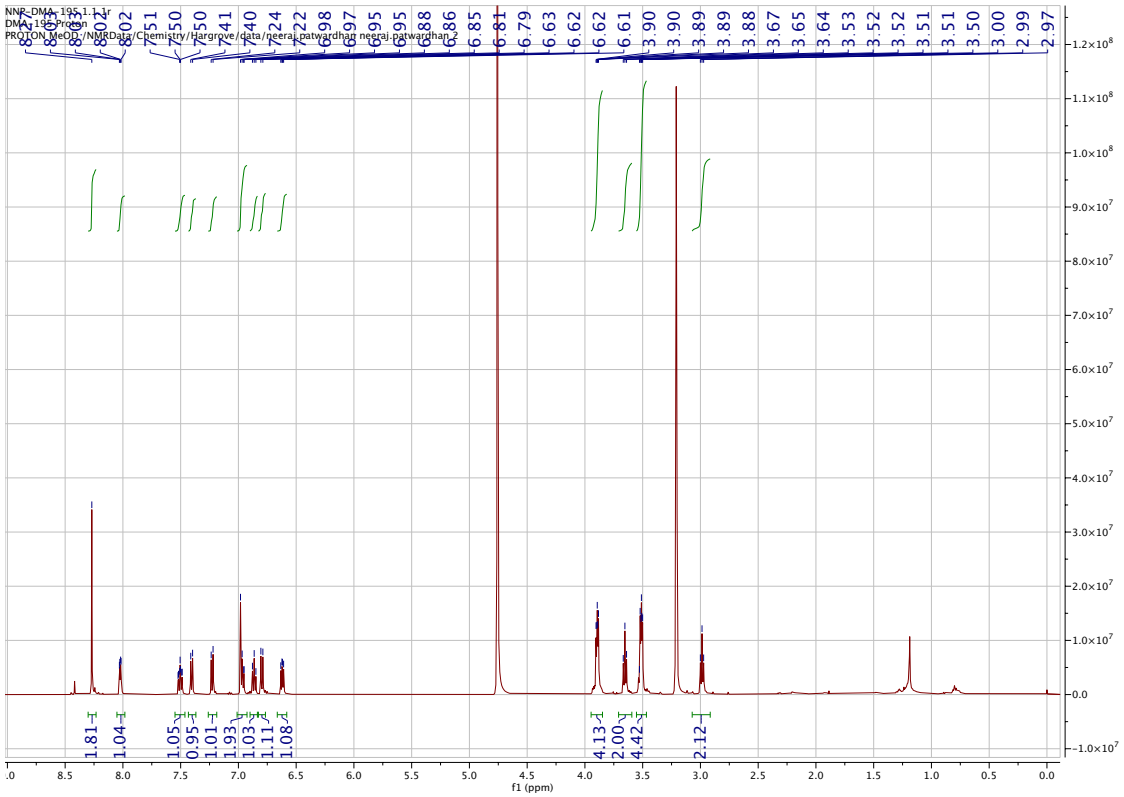
^{1}H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(2-(pyrrolidin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (8c) (DMA-194):



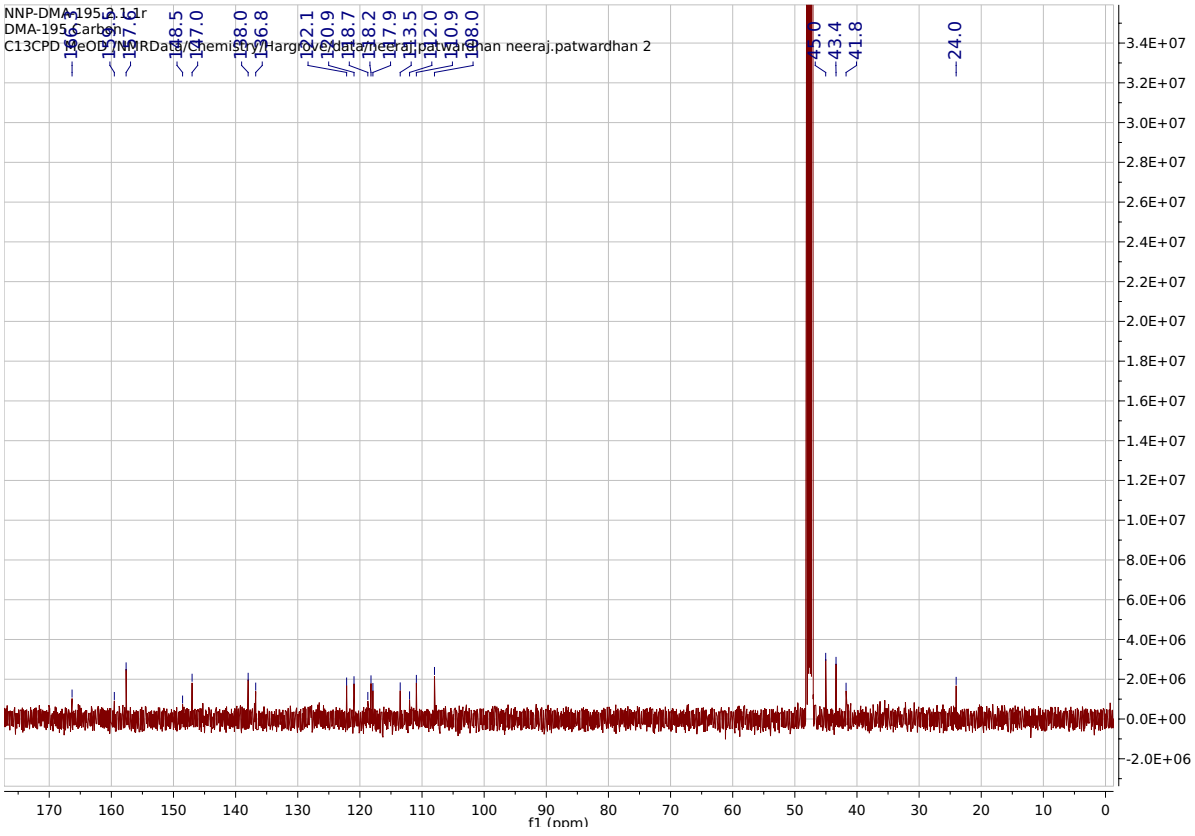
^{13}C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(2-(pyrrolidin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (8c) (DMA-194):



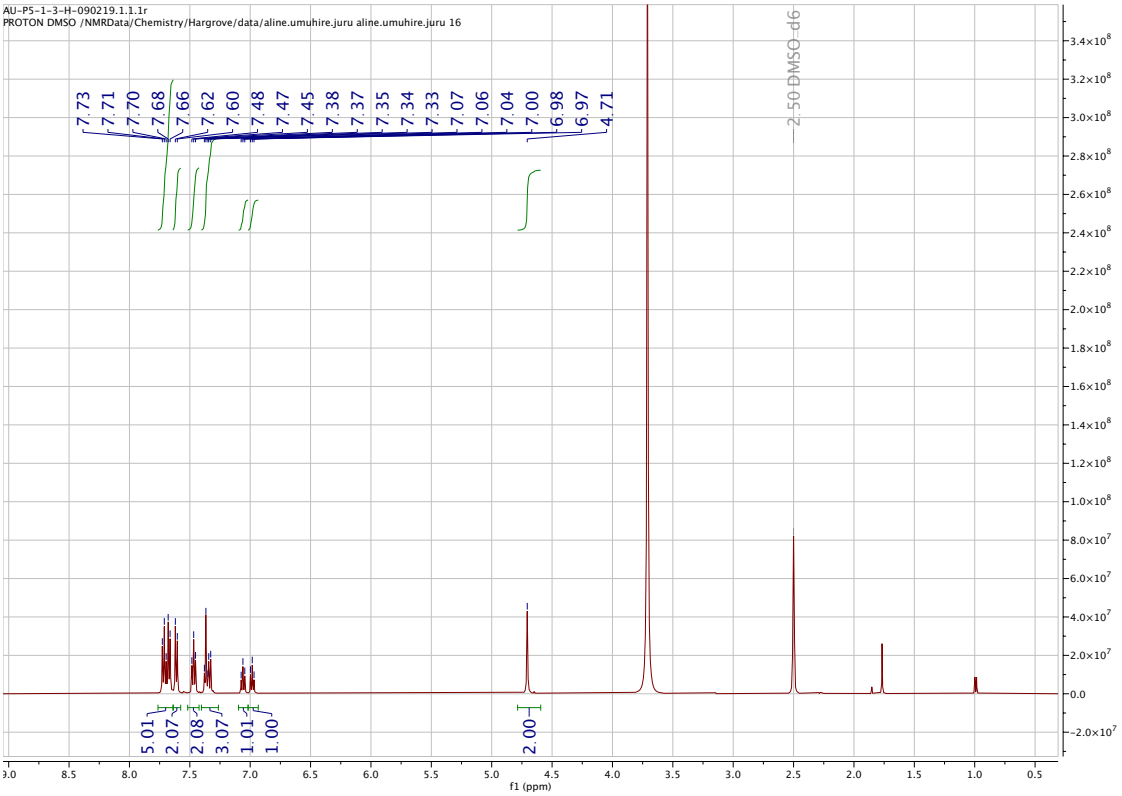
^{1}H NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (8d) (DMA-195):



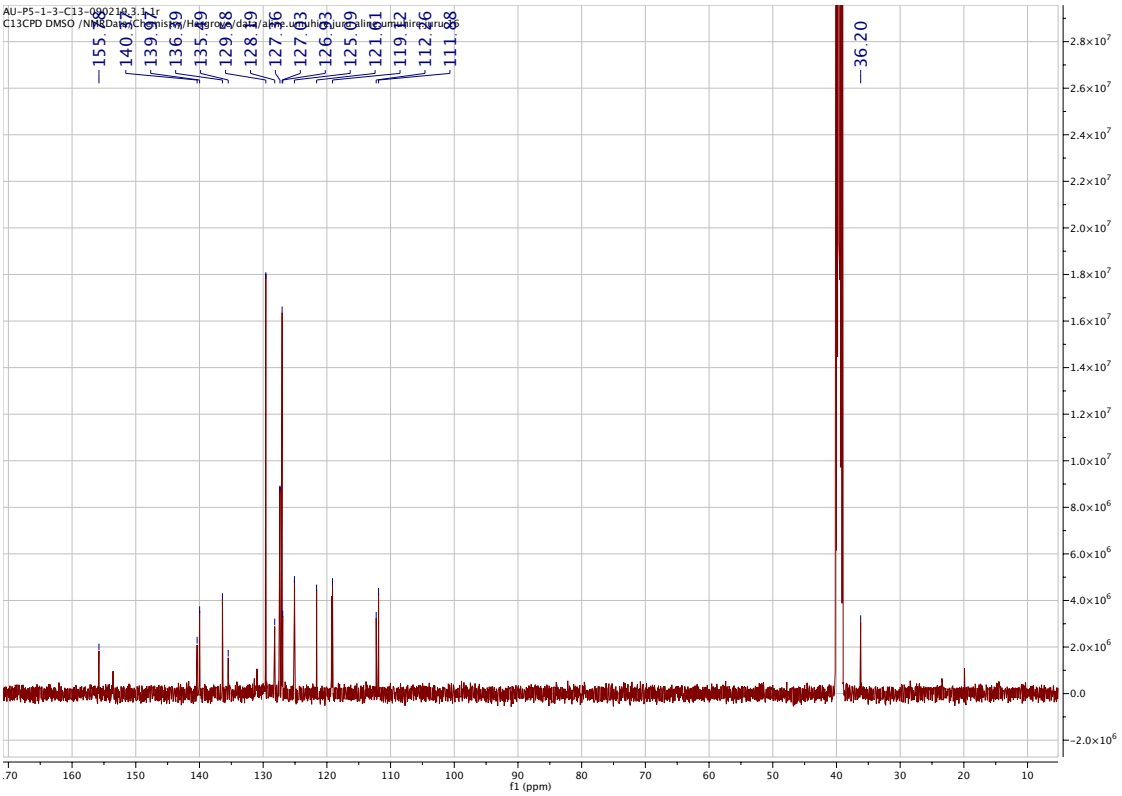
^{13}C NMR for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride (8d) (DMA-195):



^{1}H NMR for 5-(((1H-indol-3-yl)methyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-amino-N-carbamimidoylpyrazine-2-carboxamide (4v) (DMA-207):



^{13}C NMR for 5-(((1H-indol-3-yl)methyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-amino-N-carbamimidoylpyrazine-2-carboxamide (4v) (DMA-207):



HPLC chromatograms for amiloride derivatives:

HPLC chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride **(4a) (DMA-176):**

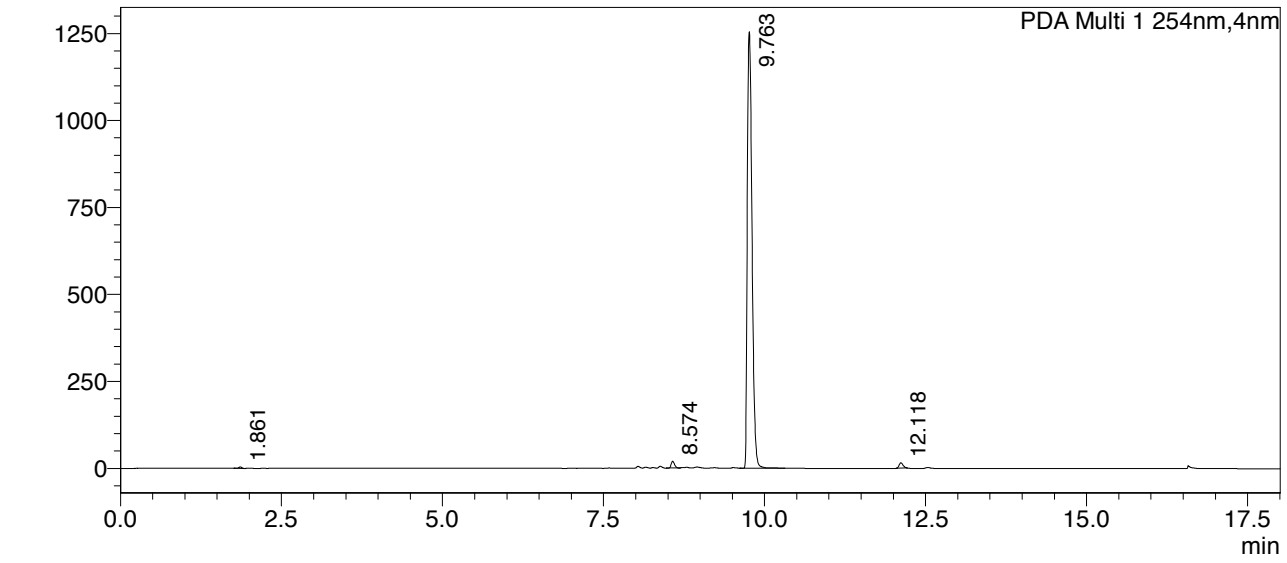
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-120-R3		
Sample ID	: NNPII-120-R3		
Data Filename	: NNPII-120-R3.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_1_13_17_R1.lcb		
Vial #	: 1-9	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 1/13/2017 3:03:15 PM	Acquired by	: chemist
Date Processed	: 1/13/2017 3:41:42 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	1.861	13058	0.198
2	8.574	66516	1.009
3	9.763	6446590	97.817
4	12.118	64271	0.975
Total		6590436	100.000

HPLC Chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(naphthalen-2-yl)pyrazine-2-carboxamide hydrochloride **(4b) (DMA-177):**

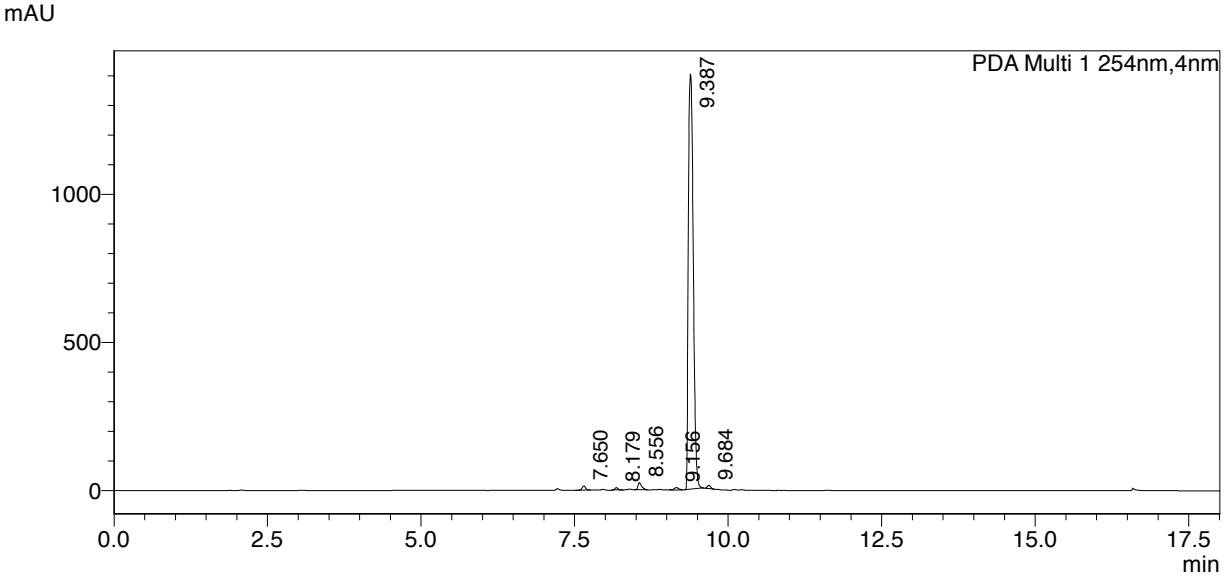
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-121-R3		
Sample ID	: NNPII-121-R3		
Data Filename	: NNPII-121-R3.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_1_13_17_R1.lcb		
Vial #	: 1-10	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 1/13/2017 3:21:47 PM	Acquired by	: chemist
Date Processed	: 1/13/2017 3:44:25 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	7.650	55611	0.690
2	8.179	21785	0.270
3	8.556	98081	1.218
4	9.156	47555	0.590
5	9.387	7793700	96.746
6	9.684	39069	0.485
Total		8055800	100.000

HPLC Chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(*p*-tolyl)pyrazine-2-carboxamide hydrochloride **(4c) (DMA-178):**

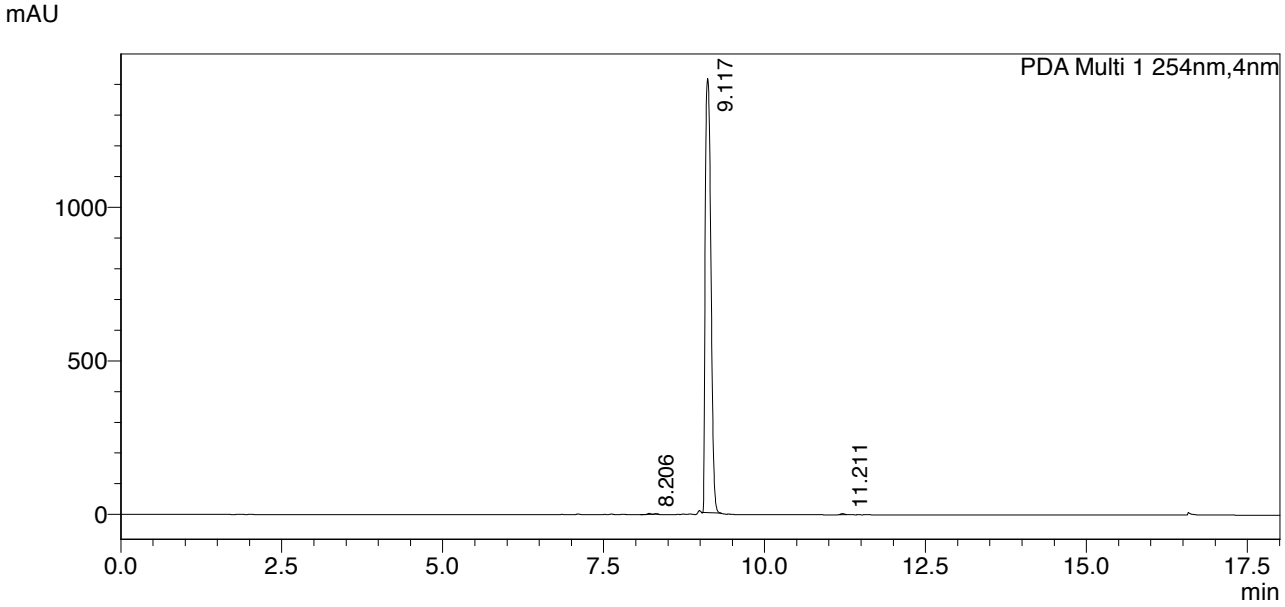
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-119-R3		
Sample ID	: NNPII-119-R3		
Data Filename	: NNPII-119-R3.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_1_13_17_R1.lcb		
Vial #	: 1-8	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 1/13/2017 2:44:43 PM	Acquired by	: chemist
Date Processed	: 1/13/2017 3:27:11 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	8.206	5808	0.067
2	9.117	8610225	99.810
3	11.211	10559	0.122
Total		8626592	100.000

HPLC Chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(4-fluorophenyl)pyrazine-2-carboxamide hydrochloride **(4d) (DMA-179):**

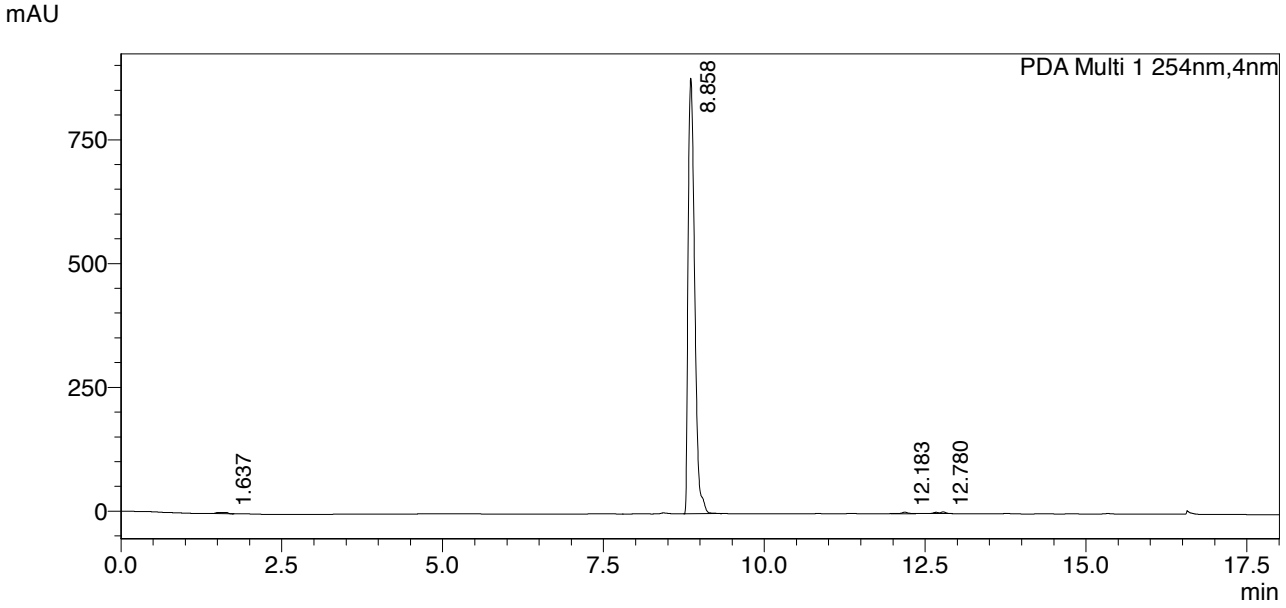
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-118-R2		
Sample ID	: NNPII-118-R2		
Data Filename	: NNPII-118-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP-01_03_17_R2.lcb		
Vial #	: 1-11	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 1/3/2017 4:32:17 PM	Acquired by	: chemist
Date Processed	: 1/5/2017 10:02:41 AM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	1.637	30732	0.488
2	8.858	6211891	98.578
3	12.183	29561	0.469
4	12.780	29303	0.465
Total		6301487	100.000

HPLC Chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(pyridin-4-yl)pyrazine-2-carboxamide hydrochloride **(4e) (DMA-190):**

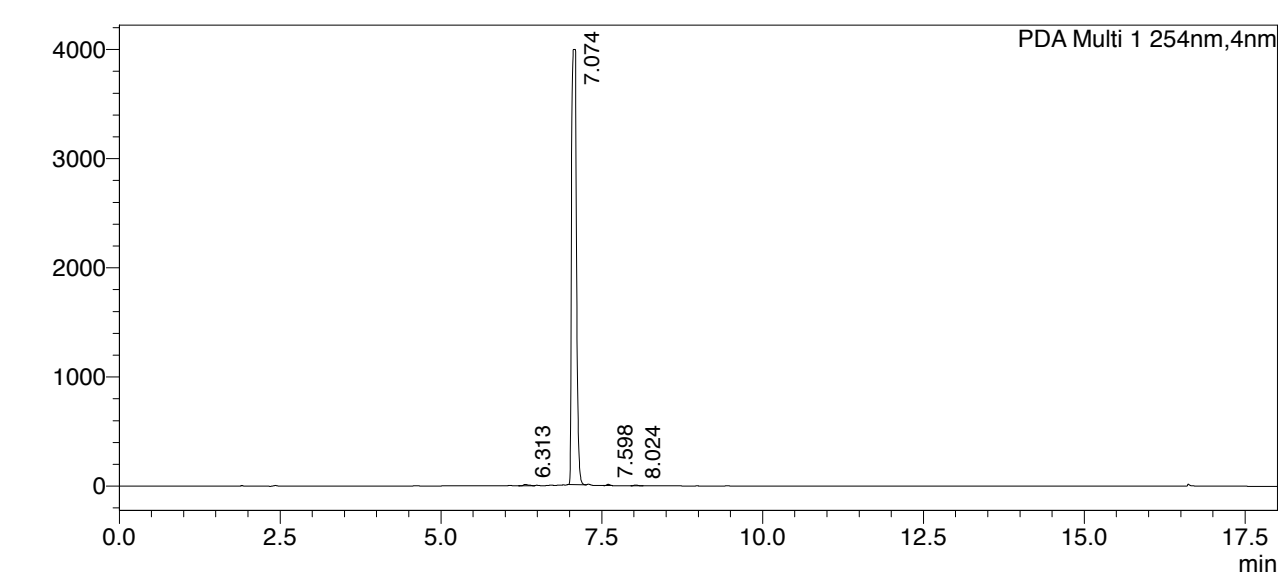
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPIII-096B-R1		
Sample ID	: NNPIII-096B-R1		
Data Filename	: NNPIII-096B-R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNPIII_12_14_R1.lcb		
Vial #	: 1-96	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 12/14/2018 11:45:06 AM	Acquired by	: chemist
Date Processed	: 12/14/2018 12:10:33 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	6.313	63819	0.303
2	7.074	20913450	99.393
3	7.598	35128	0.167
4	8.024	28784	0.137
Total		21041180	100.000

HPLC Chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride **(4f) (DMA-191):**

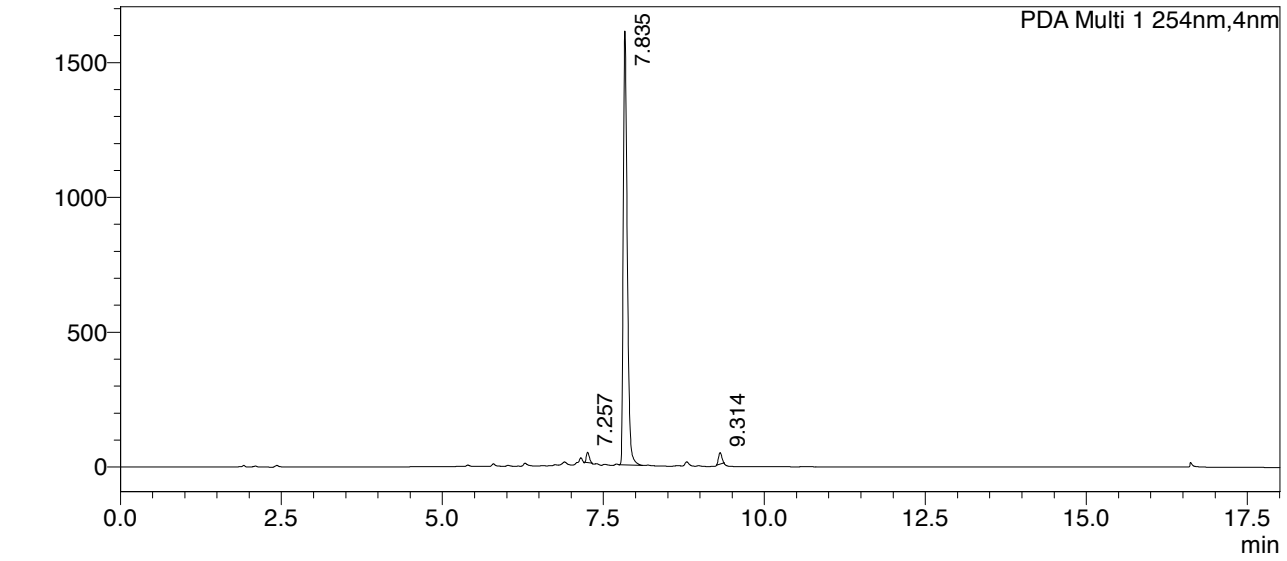
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPIII-097-R2		
Sample ID	: NNPIII-097-R1		
Data Filename	: NNPIII-097-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNPIII_12_14_R1.lcb		
Vial #	: 1-97	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 12/14/2018 2:26:31 PM	Acquired by	: chemist
Date Processed	: 12/18/2018 11:41:02 AM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	7.257	122365	1.717
2	7.835	6853210	96.171
3	9.314	150518	2.112
Total		7126093	100.000

HPLC Chromatogram for 5-((2-(1*H*-benzo[*d*]imidazol-2-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride **(4g) (DMA-180):**

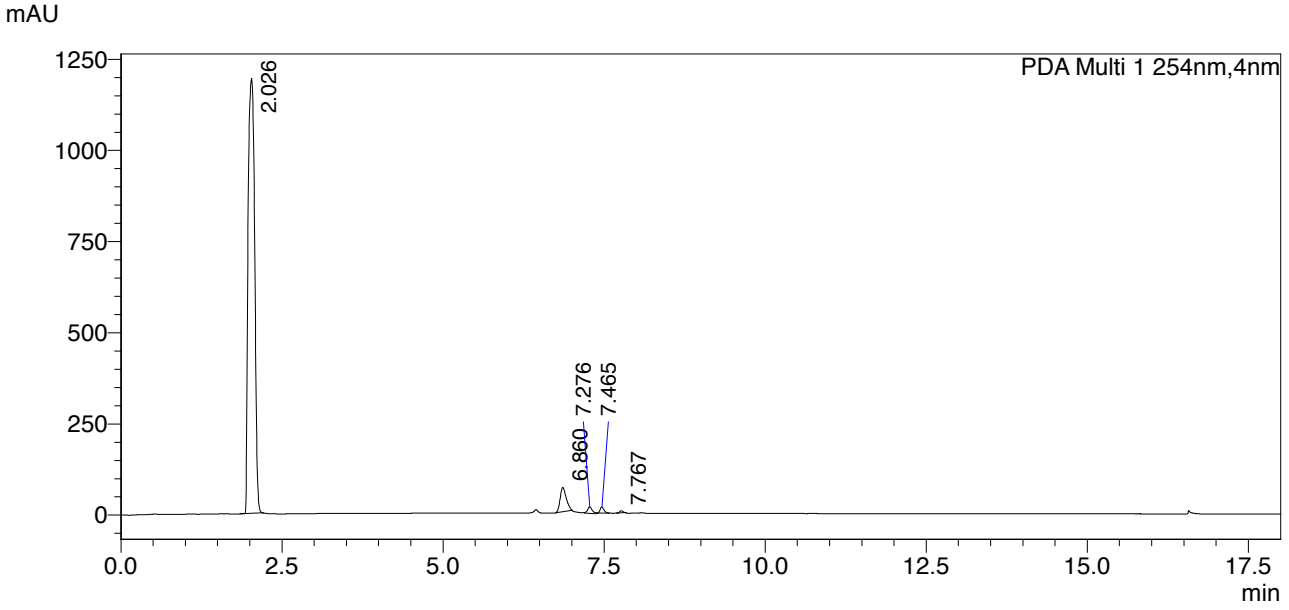
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII130-R5		
Sample ID	: NNPII130-R5		
Data Filename	: NNPII130-R5.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNPII-1_15_17_R1.lcb		
Vial #	: 1-27	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 1/15/2017 3:01:58 PM	Acquired by	: chemist
Date Processed	: 1/15/2017 3:20:00 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	2.026	8441755	93.194
2	6.860	439301	4.850
3	7.276	90107	0.995
4	7.465	70076	0.774
5	7.767	17001	0.188
Total		9058240	100.000

HPLC chromatogram for 5-((3-(1*H*-imidazol-1-yl)propyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride **(4h) (DMA-182):**

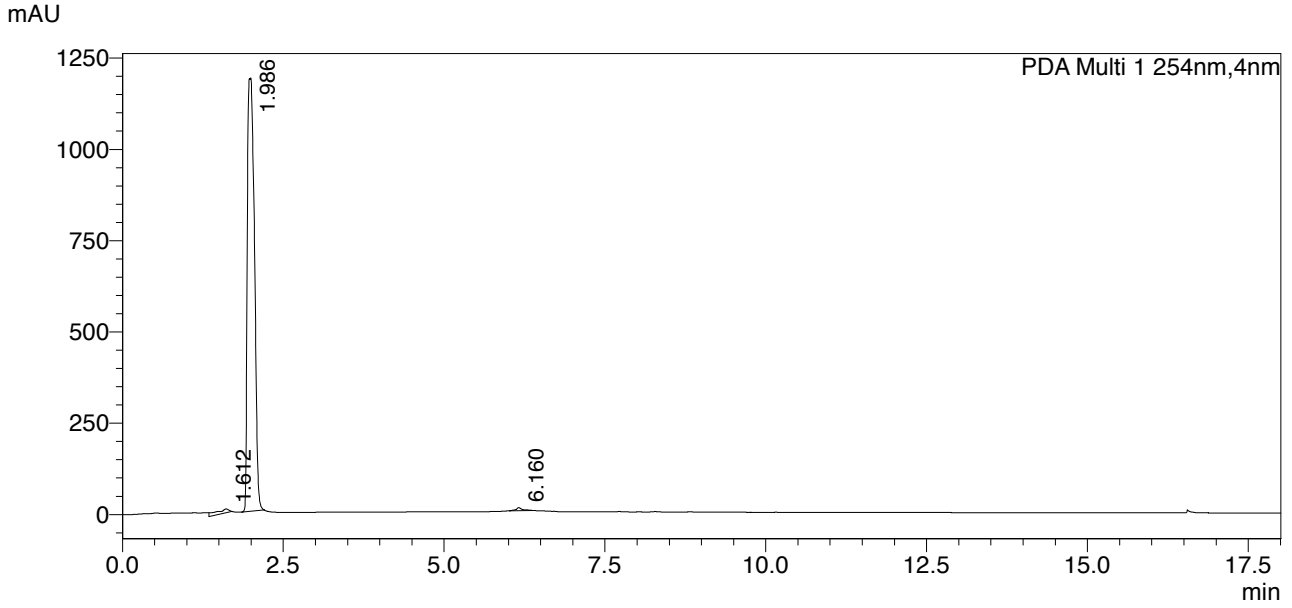
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII128-R5		
Sample ID	: NNPII128-R5		
Data Filename	: NNPII128-R5.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNPII-1_15_17_R1.lcb		
Vial #	: 1-25	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 1/15/2017 2:29:09 PM	Acquired by	: chemist
Date Processed	: 1/15/2017 3:24:51 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	1.612	161618	1.739
2	1.986	9073366	97.655
3	6.160	56305	0.606
Total		9291289	100.000

HPLC Chromatogram for 3-amino-*N*-carbamimidoyl-5-((3-(2-oxopyrrolidin-1-yl)propyl)amino)-6-phenylpyrazine-2-carboxamide hydrochloride **(4i) (DMA-183)**:

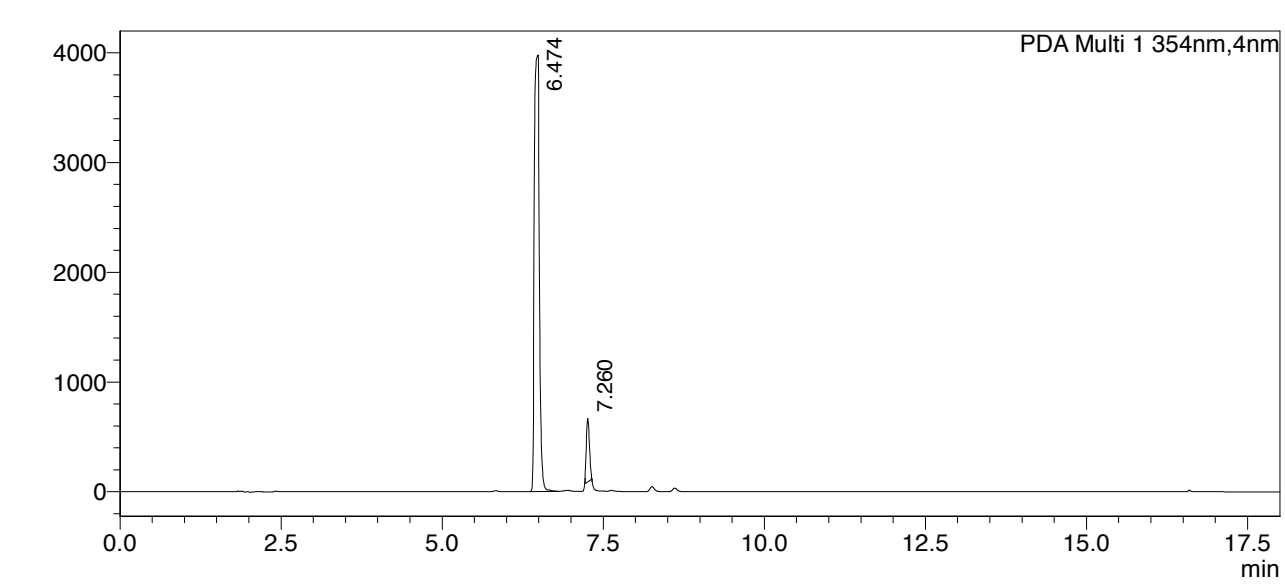
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: DMA-183B2_RepurifR1		
Sample ID	: DMA-183B2_RepurifR1		
Data Filename	: DMA-183B2_RepurifR1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_11_13_17_R1.lcb		
Vial #	: 1-53	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 11/13/2017 2:19:33 PM	Acquired by	: chemist
Date Processed	: 6/3/2019 5:20:47 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 354nm

Peak#	Ret. Time	Area	Area%
1	6.474	21680848	91.680
2	7.260	1967469	8.320
Total		23648317	100.000

HPLC Chromatogram for 3-amino-*N*-carbamimidoyl-5-((2-(4-methylpiperazin-1-yl)ethyl)amino)-6-phenylpyrazine-2-carboxamide hydrochloride **(4j) (DMA-187):**

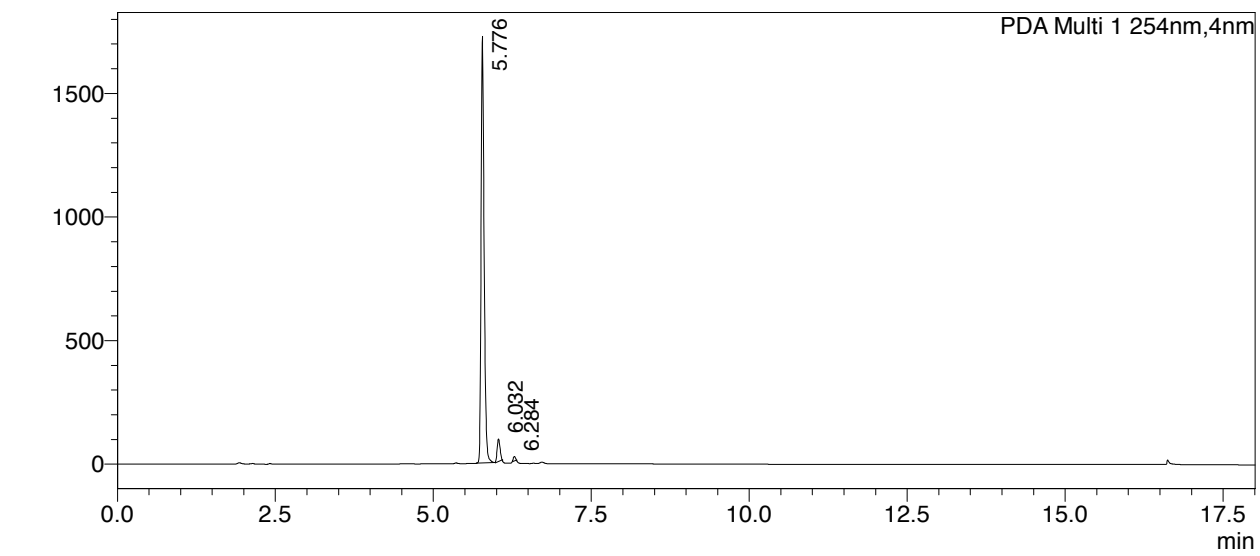
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPIII-095S2-R2		
Sample ID	: NNPIII-095S2-R2		
Data Filename	: NNPIII-095S2-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNPIII_12_18_18_R1.lcb		
Vial #	: 1-2	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 12/18/2018 2:13:01 PM	Acquired by	: chemist
Date Processed	: 3/13/2019 1:46:07 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	5.776	5692576	94.541
2	6.032	289011	4.800
3	6.284	39701	0.659
Total		6021288	100.000

HPLC Chromatogram for5-(((1*H*-indol-3-yl)methyl)amino)-3-amino-*N*-carbamimidoyl-6-phenylpyrazine-2-carboxamide hydrochloride **(4k) (DMA-188):**

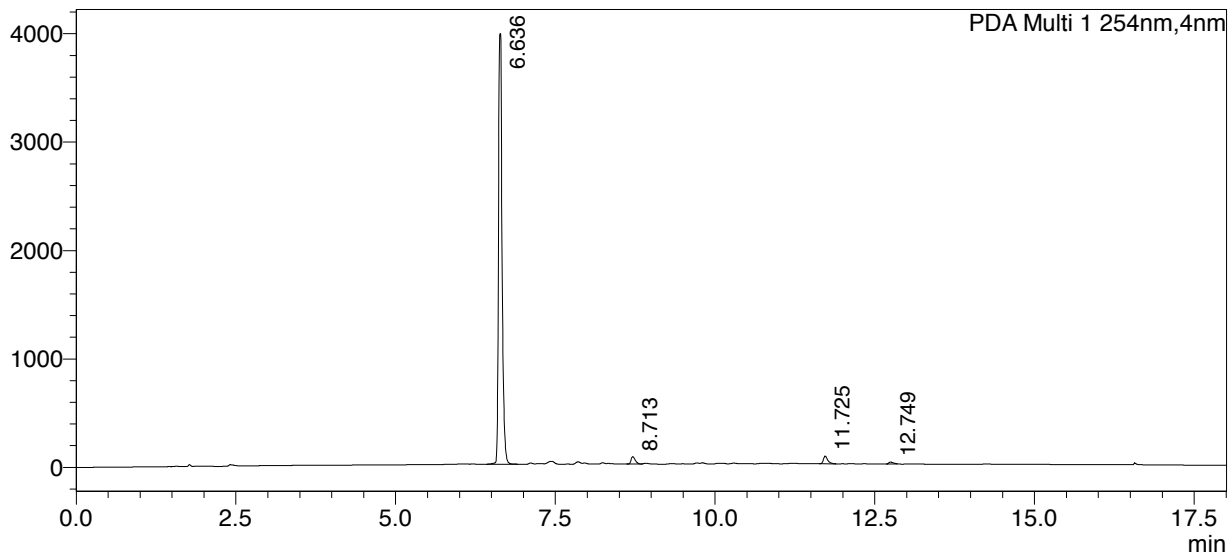
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPIII112-R2		
Sample ID	: NNPIII112-R2		
Data Filename	: NNPIII112-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_02_18_19_R1.lcb		
Vial #	: 1-2	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 2/18/2019 2:12:13 PM	Acquired by	: chemist
Date Processed	: 2/18/2019 2:32:50 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	6.636	15327412	95.394
2	8.713	306902	1.910
3	11.725	343075	2.135
4	12.749	90171	0.561
Total		16067560	100.000

HPLC chromatogram for 3-amino-*N*-carbamimidoyl-6-phenyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride **(4l) (DMA-181):**

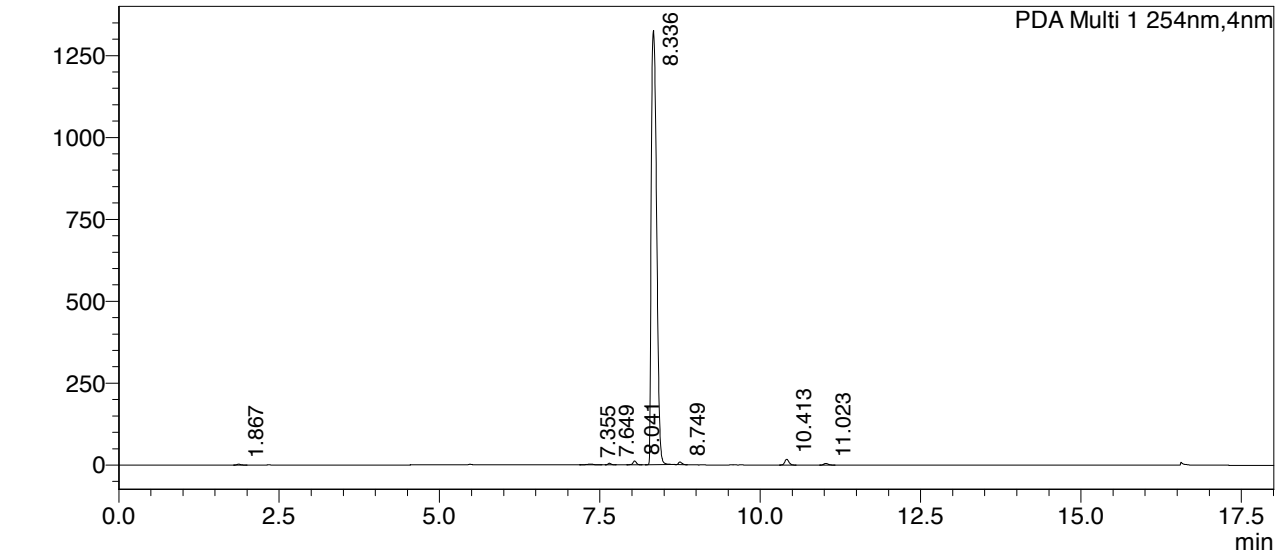
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPII-127-R3		
Sample ID	: NNPII-127-R3		
Data Filename	: NNPII-127-R3.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA.lcm		
Batch Filename	: NNP_1_13_17_R1.lcb		
Vial #	: 1-11	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 1/13/2017 3:40:21 PM	Acquired by	: chemist
Date Processed	: 1/13/2017 4:01:25 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	1.867	9610	0.122
2	7.355	16806	0.213
3	7.649	16310	0.207
4	8.041	42500	0.540
5	8.336	7660772	97.267
6	8.749	28207	0.358
7	10.413	78766	1.000
8	11.023	23056	0.293
Total		7876028	100.000

HPLC chromatogram for 3-amino-*N*-carbamimidoyl-6-(4-fluorophenyl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride **(4m) (DMA-184):**

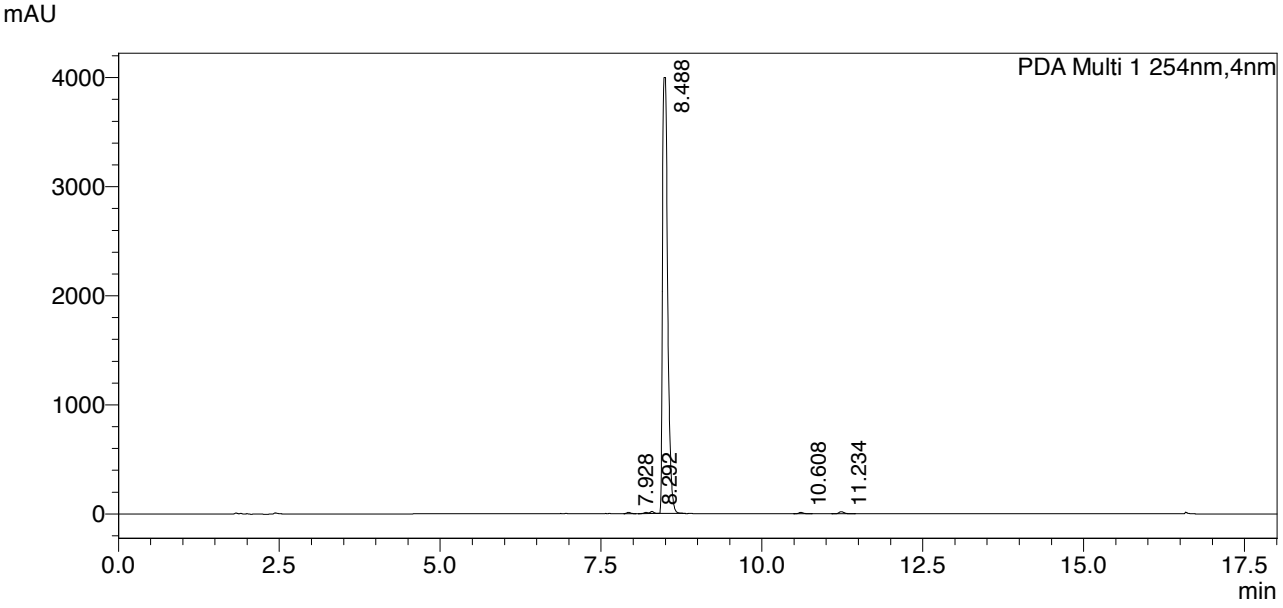
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPIII-022_R1		
Sample ID	: NNPIII-022_R1		
Data Filename	: NNPIII-022_R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_11_13_17_R1.lcb		
Vial #	: 1-54	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 11/13/2017 2:38:05 PM	Acquired by	: chemist
Date Processed	: 11/13/2017 3:28:54 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	7.928	46917	0.207
2	8.292	113541	0.501
3	8.488	22338454	98.511
4	10.608	66041	0.291
5	11.234	111068	0.490
Total		22676020	100.000

HPLC Chromatogram for 6-([1,1'-biphenyl]-4-yl)-3-amino-*N*-carbamimidoyl-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride **(4n) (DMA-185):**

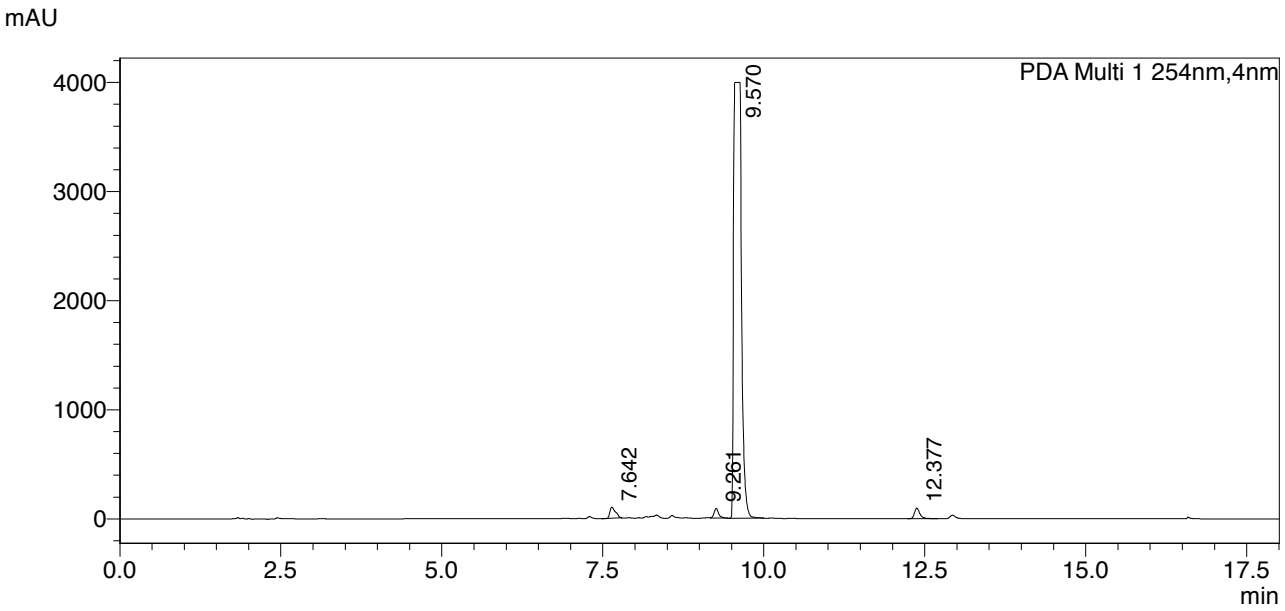
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPIII-023_R1		
Sample ID	: NNPIII-023_R1		
Data Filename	: NNPIII-023_R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_11_13_17_R1.lcb		
Vial #	: 1-55	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 11/13/2017 2:56:37 PM	Acquired by	: chemist
Date Processed	: 11/13/2017 3:35:11 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	7.642	613396	1.748
2	9.261	420931	1.200
3	9.570	33496924	95.456
4	12.377	560151	1.596
Total		35091402	100.000

HPLC Chromatogram for 3-amino-*N*-carbamimidoyl-6-(pyrimidin-5-yl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide hydrochloride **(4o) (DMA-186):**

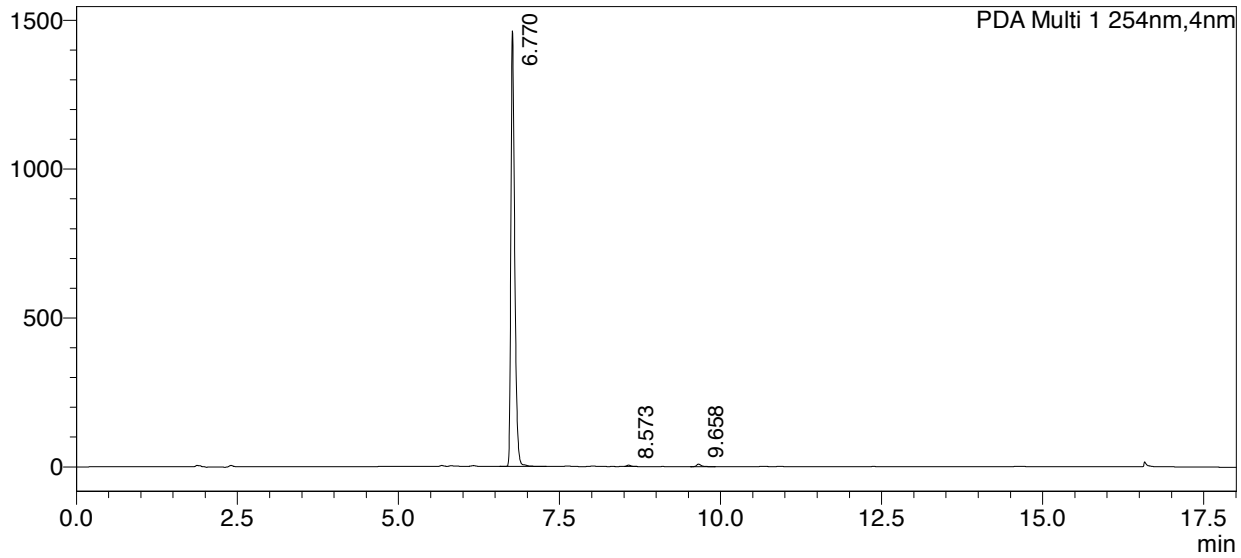
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPIII-029_R1		
Sample ID	: NNPIII-029_R1		
Data Filename	: NNPIII-029_R1.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_11_13_17_R1.lcb		
Vial #	: 1-56	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 11/13/2017 3:15:08 PM	Acquired by	: chemist
Date Processed	: 11/13/2017 3:36:03 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	6.770	5964638	98.858
2	8.573	19745	0.327
3	9.658	49150	0.815
Total		6033533	100.000

HPLC Chromatogram for 3-Amino-N-carbamimidoyl-6-chloro-5-morpholinopyrazine-2-carboxamide **(4p) (DMA-201)**:

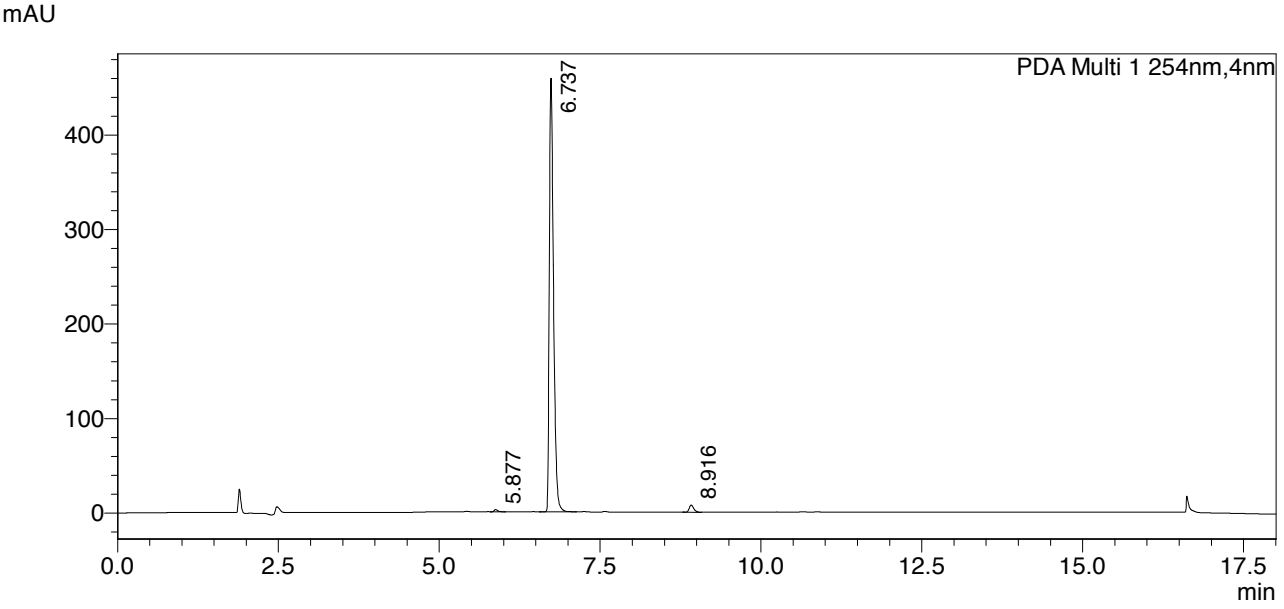
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNP-III-085--salt-052219		
Sample ID	: NNP-III-085--salt-052219		
Data Filename	: NNP-III-085--salt-052219.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU052219-R2.lcb		
Vial #	: 1-97	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/22/2019 3:05:23 PM	Acquired by	: chemist
Date Processed	: 5/22/2019 3:23:26 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	5.877	10583	2337	0.492
2	6.737	2101593	440758	97.708
3	8.916	38718	7467	1.800
Total		2150894	450562	100.000

HPLC Chromatogram for 3-Amino-N-carbamimidoyl-6-chloro-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxamide **(4q) (DMA-202)**:

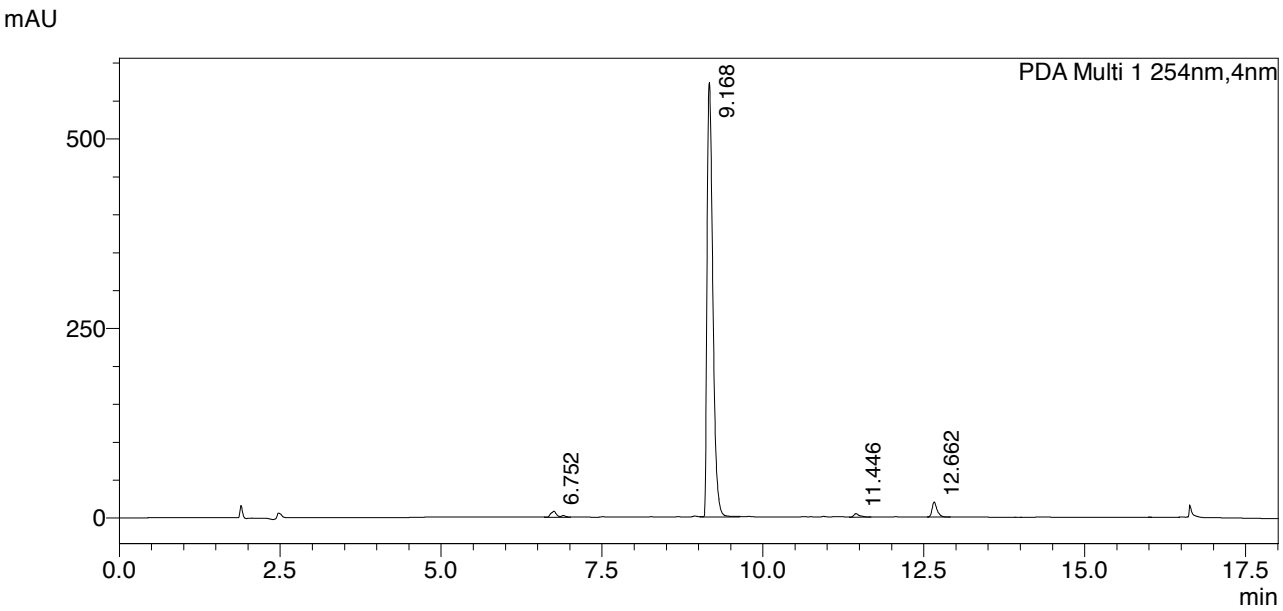
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNP-III-086-salt-052219		
Sample ID	: NNP-III-086-salt-052219		
Data Filename	: NNP-III-086-salt-052219.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU052219.lcb		
Vial #	: 1-95	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/22/2019 1:38:49 PM	Acquired by	: chemist
Date Processed	: 5/22/2019 1:56:51 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.752	58165	7727	1.546
2	9.168	3568588	565288	94.865
3	11.446	29965	4600	0.797
4	12.662	105018	19345	2.792
Total		3761736	596959	100.000

HPLC Chromatogram for 3-Amino-N-carbamimidoyl-6-chloro-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxamide **(4r) (DMA-203)**:

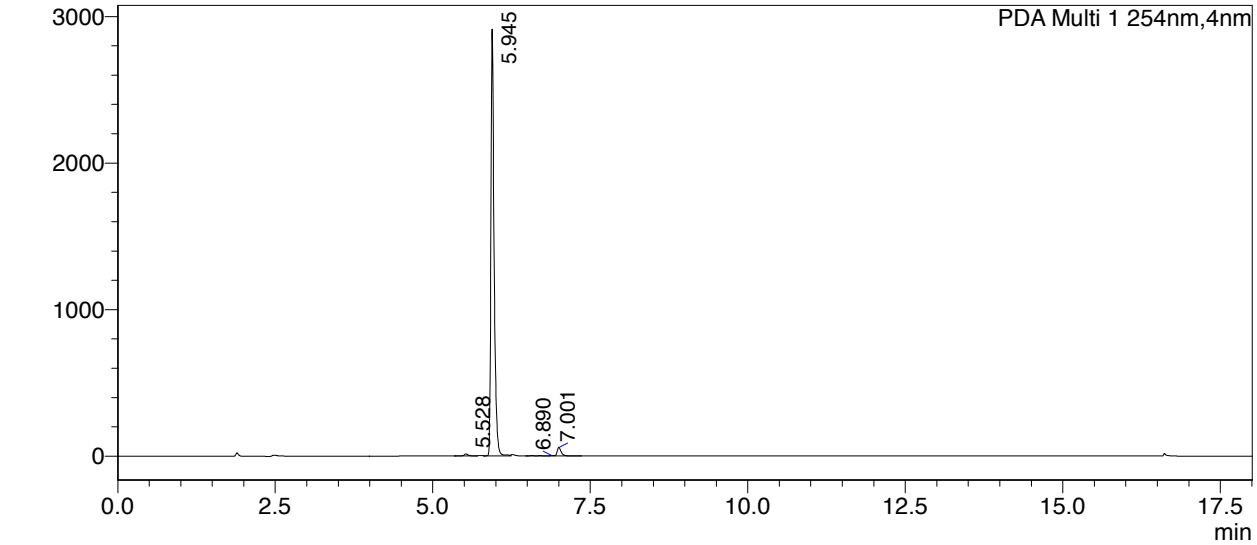
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNP-III-087-salt-052219		
Sample ID	: NNP-III-087-salt-052219		
Data Filename	: NNP-III-087-salt-052219.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU052219.lcb		
Vial #	: 1-96	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/22/2019 2:15:51 PM	Acquired by	: chemist
Date Processed	: 5/22/2019 2:33:53 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	5.528	64675	13799	0.617
2	5.945	10115261	2819727	96.500
3	6.890	34634	2755	0.330
4	7.001	267537	57941	2.552
Total		10482107	2894223	100.000

HPLC Chromatogram for 3-Amino-N-carbamimidoyl-5-morpholino-6-phenylpyrazine-2-carboxamide **(4s) (DMA-204)**:

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: AU-V-015-salt-052219		
Sample ID	: AU-V-015-salt-052219		
Data Filename	: AU-V-015-salt-052219.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU052219.lcb		
Vial #	: 1-93	Sample Type	: Unknown
Injection Volume	: 1 uL		
Date Acquired	: 5/22/2019 12:24:45 PM	Acquired by	: chemist
Date Processed	: 5/22/2019 12:42:47 PM	Processed by	: chemist

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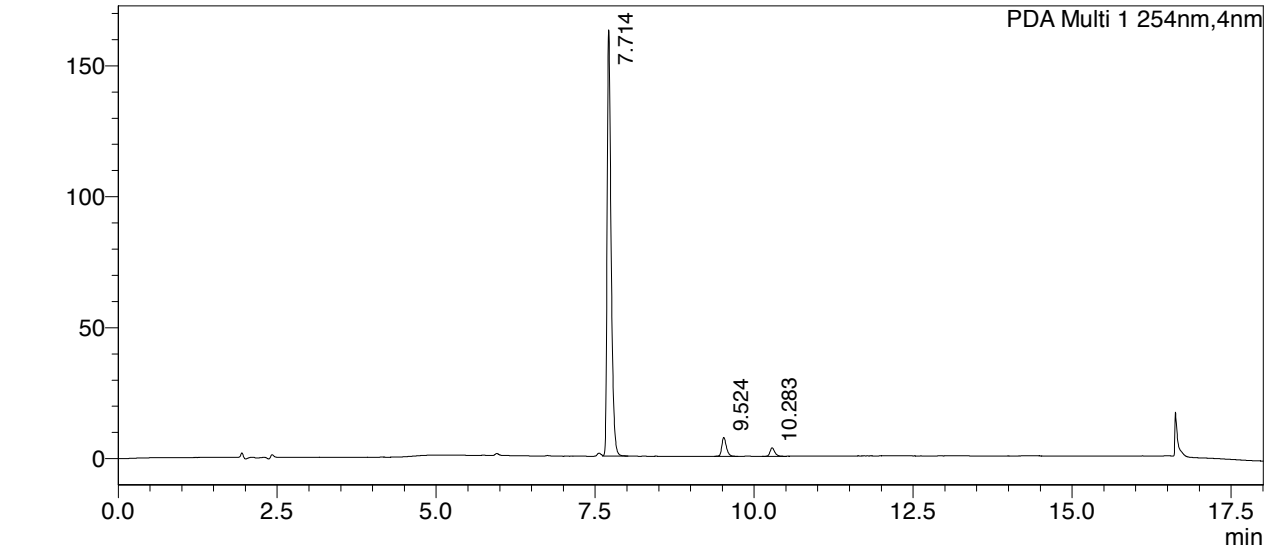


<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.714	709125	154147	92.874
2	9.524	37049	7035	4.852
3	10.283	17358	3235	2.273
Total		763533	164417	100.000

HPLC Chromatogram for 3-Amino-N-carbamimidoyl-6-phenyl-5-(4-phenylpiperidin-1-yl)pyrazine-2-carboxamide **(4t) (DMA-205)**:

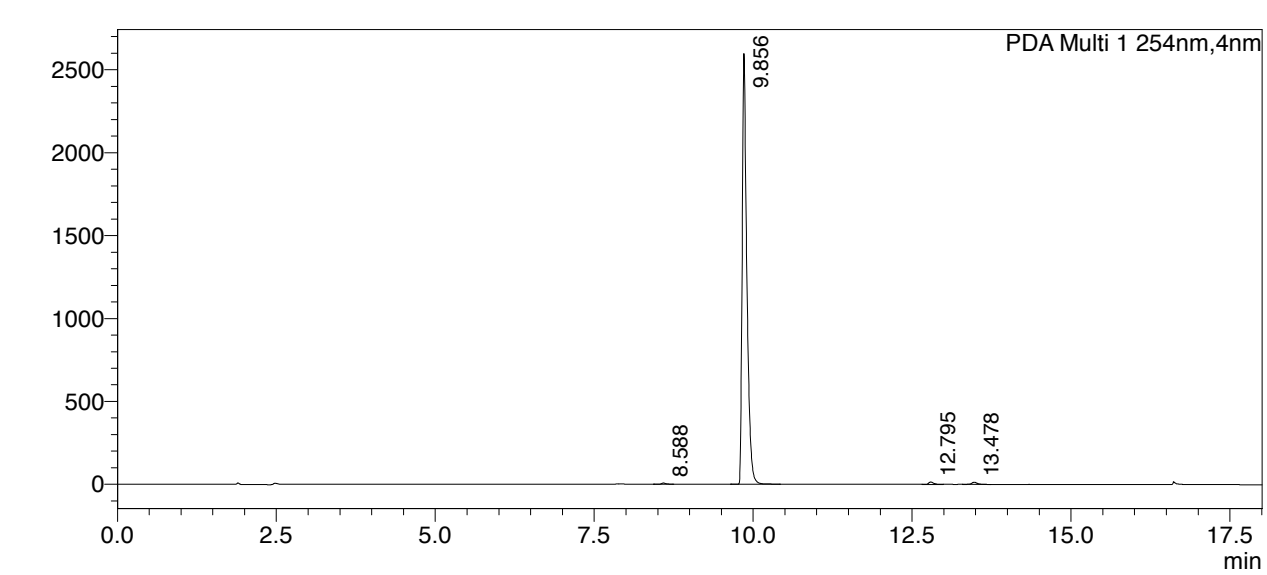
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: AU-V-013-salt-052219		
Sample ID	: AU-V-013-salt-052219		
Data Filename	: AU-V-013-salt-052219.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU052219.lcb		
Vial #	: 1-92	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/22/2019 11:47:42 AM	Acquired by	: chemist
Date Processed	: 5/22/2019 12:05:45 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	8.588	46041	7902	0.344
2	9.856	13173189	2498340	98.513
3	12.795	78101	14041	0.584
4	13.478	74719	12918	0.559
Total		13372051	2533202	100.000

HPLC Chromatogram for 3-Amino-N-carbamimidoyl-6-phenyl-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrazine-2-carboxamide **(4u) (DMA-206)**:

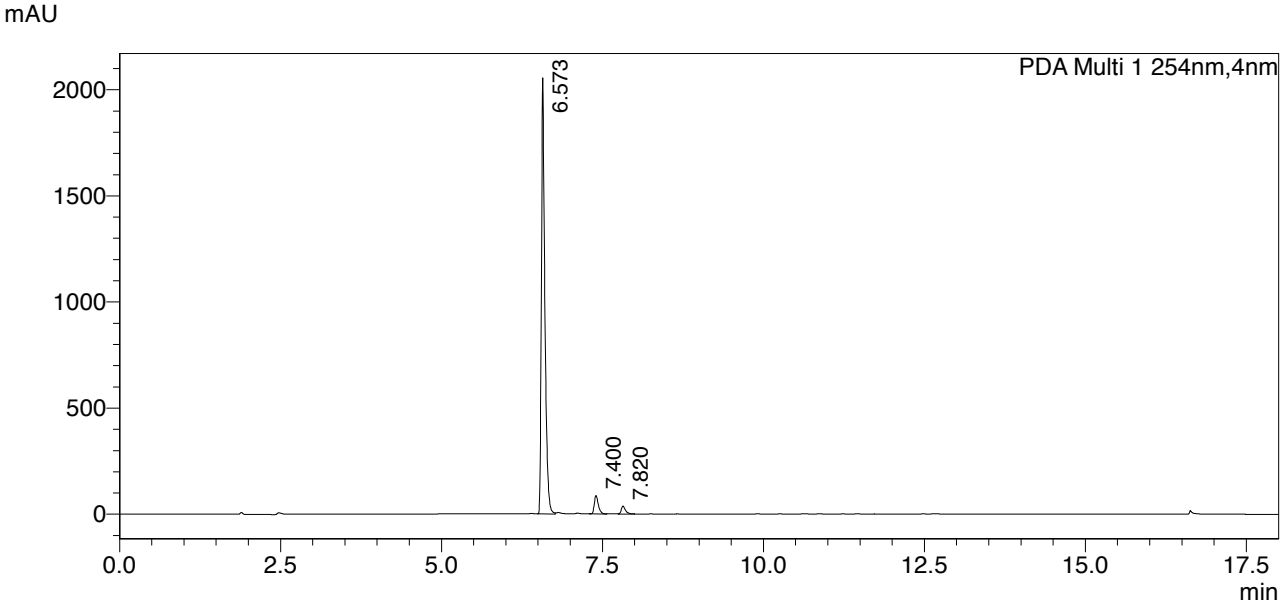
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: AU-V-019-salt-052219		
Sample ID	: AU-V-019-salt-052219		
Data Filename	: AU-V-019-salt-052219.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU052219.lcb		
Vial #	: 1-94	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 5/22/2019 1:01:48 PM	Acquired by	: chemist
Date Processed	: 5/22/2019 1:19:51 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	6.573	7638678	1965439	93.253
2	7.400	376726	84673	4.599
3	7.820	175966	37068	2.148
Total		8191371	2087181	100.000

HPLC Chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-6-(2-((2-(1*H*-indol-3-yl)ethyl)amino)pyrimidin-5-yl)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride **(8a) (DMA-192):**

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: DMA-192-R2		
Sample ID	: DMA-192-R2		
Data Filename	: DMA-192-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_06_02_19_R1.lcb		
Vial #	: 1-2	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 6/3/2019 4:08:26 PM	Acquired by	: chemist
Date Processed	: 6/3/2019 4:40:37 PM	Processed by	: chemist

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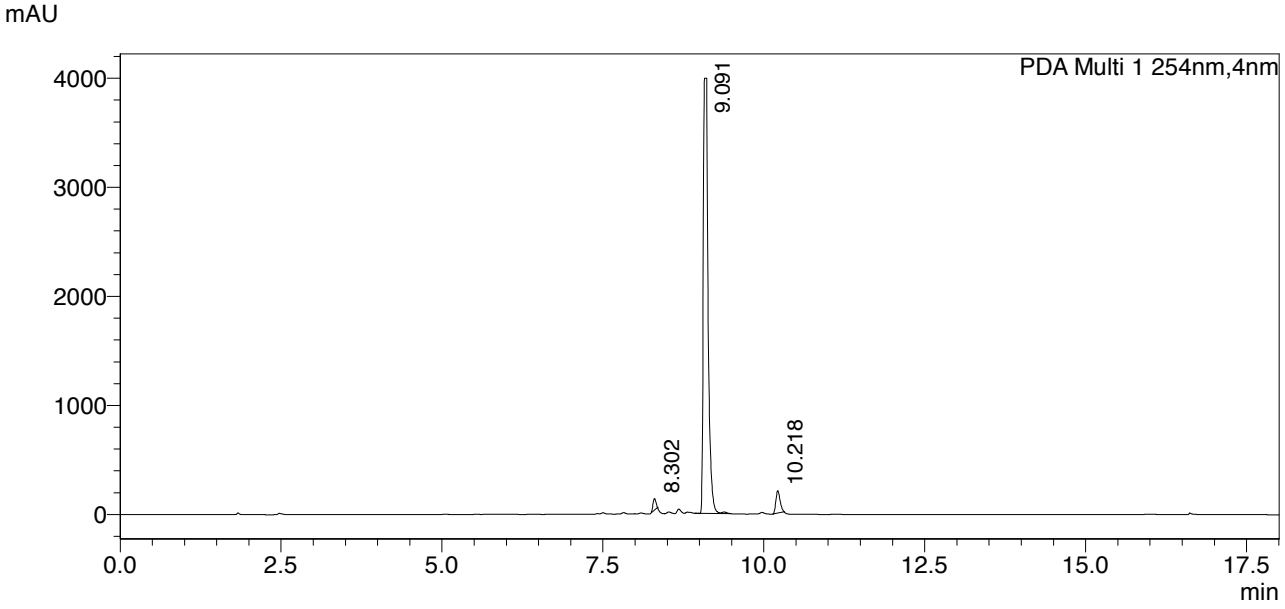


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	8.302	338144	1.526
2	9.091	20894506	94.314
3	10.218	921455	4.159
Total		22154105	100.000

HPLC Chromatogram for 6-(2-((3-(1*H*-imidazol-1-yl)propyl)amino)pyrimidin-5-yl)-5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoylpyrazine-2-carboxamide hydrochloride **(8b) (DMA-193):**

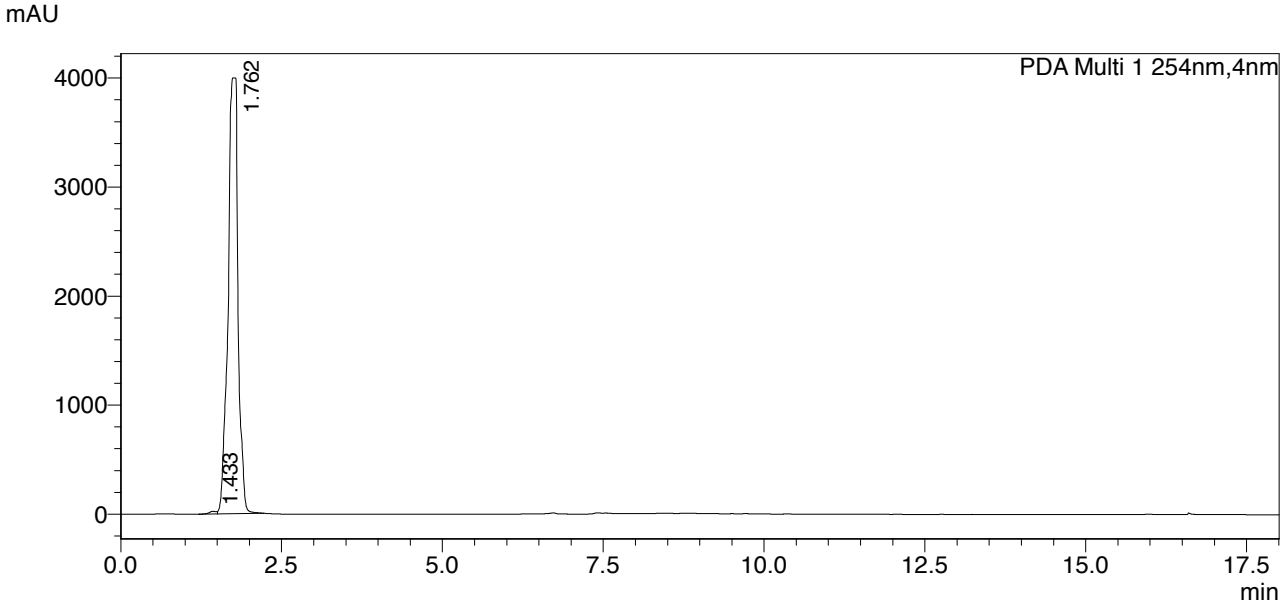
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: DMA-193-R5		
Sample ID	: DMA-193-R5		
Data Filename	: DMA-193-R5.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_06_04_19_R1.lcb		
Vial #	: 1-2	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 6/4/2019 3:26:09 PM	Acquired by	: chemist
Date Processed	: 6/4/2019 3:44:12 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	1.433	196826	0.451
2	1.762	43463723	99.549
Total		43660549	100.000

HPLC Chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(2-(pyrrolidin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride **(8c) (DMA-194):**

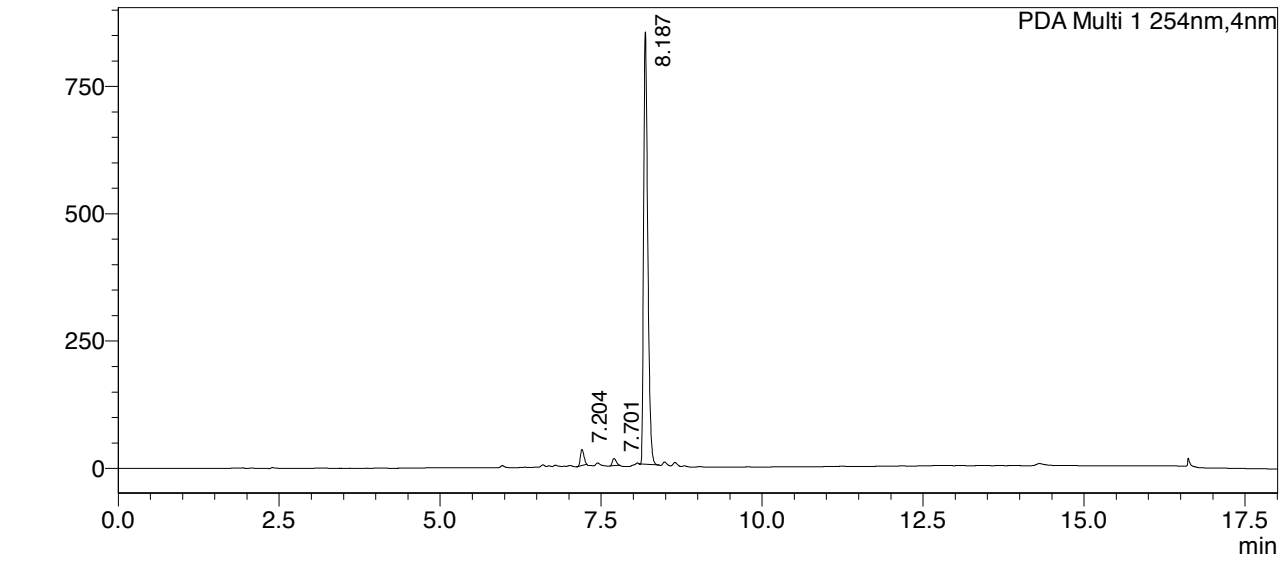
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: NNPIII-117-purif3-R2		
Sample ID	: NNPIII-117-purif3-R2		
Data Filename	: NNPIII-117-purif3-R2.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_03_13_19_R1.lcb		
Vial #	: 1-5	Sample Type	: Unknown
Injection Volume	: 5 uL		
Date Acquired	: 3/13/2019 5:23:49 PM	Acquired by	: chemist
Date Processed	: 3/14/2019 10:23:49 AM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	7.204	119206	3.015
2	7.701	59042	1.493
3	8.187	3775055	95.491
Total		3953304	100.000

HPLC Chromatogram for 5-((2-(1*H*-indol-3-yl)ethyl)amino)-3-amino-*N*-carbamimidoyl-6-(2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidin-5-yl)pyrazine-2-carboxamide hydrochloride **(8d) (DMA-195):**

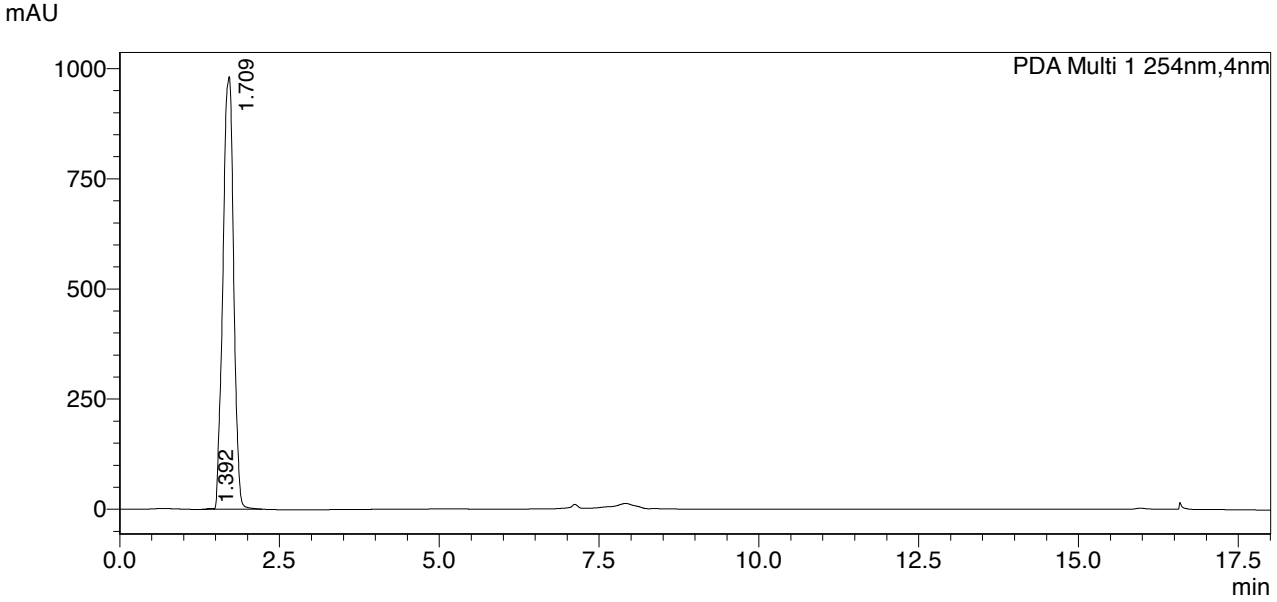
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: DMA-195-R5		
Sample ID	: DMA-195-R5		
Data Filename	: DMA-195-R5.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: NNP_06_04_19_R1.lcb		
Vial #	: 1-3	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 6/4/2019 3:55:19 PM	Acquired by	: chemist
Date Processed	: 6/4/2019 4:13:22 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	1.392	11650	0.102
2	1.709	11464600	99.898
Total		11476250	100.000

HPLC Chromatogram for 5-(((1H-indol-3-yl)methyl)amino)-6-([1,1'-biphenyl]-4-yl)-3-amino-N-carbamimidoylpyrazine-2-carboxamide **(4v) (DMA-207):**

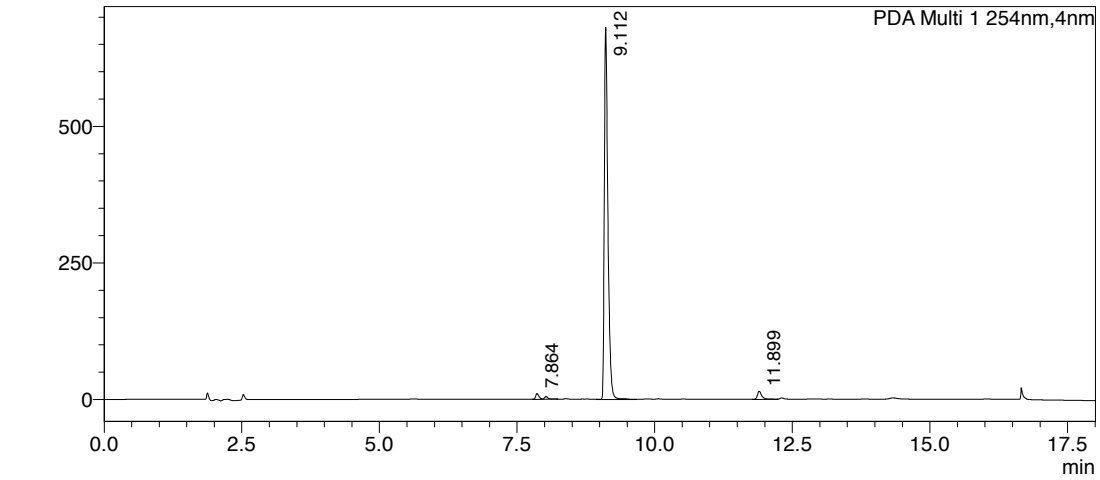
SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: AU-P5-1-3-083019		
Sample ID	: AU-P5-1-3-083019		
Data Filename	: AU-P5-1-3-083019.lcd		
Method Filename	: NNP-Grd10-90_Slow_PDA_D2only.lcm		
Batch Filename	: AU083019.lcb		
Vial #	: 1-77	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 8/30/2019 2:57:02 PM	Acquired by	: chemist
Date Processed	: 8/30/2019 3:15:05 PM	Processed by	: chemist

<Chromatogram>



<Peak Table>

RF-20A Ex:350nm,Em:450nm

Peak#	Ret. Time	Area	Height	Area%
Total				

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	7.864	66929	10280	2.053
2	9.112	3105457	668099	95.271
3	11.899	87204	14810	2.675
Total		3259591	693189	100.000

References:

[1] Palde, P. B., Ofori, L. O., Gareiss, P. C., Lerea, J., and Miller, B. L. (2010) Strategies for Recognition of Stem−Loop RNA Structures by Synthetic Ligands: Application to the HIV-1 Frameshift Stimulatory Sequence, *J. Med. Chem. 53*, 6018-6027.

[2] Patwardhan, N. N., Cai, Z., Newson, C. N., and Hargrove, A. E. (2019) Fluorescent peptide displacement as a general assay for screening small molecule libraries against RNA, *Org. Biomol. Chem. 17*, 1778-1786.

[3] Donlic, A., Morgan, B. S., Xu, J. L., Liu, A., Roble Jr, C., and Hargrove, A. E. (2018) Discovery of Small Molecule Ligands for MALAT1 by Tuning an RNA-Binding Scaffold, *Angew. Chem. Int. Ed. 57*, 13242-13247.

[4] Morgan, B. S., Forte, J. E., Culver, R. N., Zhang, Y., and Hargrove, A. E. (2017) Discovery of Key Physicochemical, Structural, and Spatial Properties of RNA-Targeted Bioactive Ligands, *Angew. Chem. 129*, 13683-13687.

[5] Patwardhan, N. N., Ganser, L. R., Kapral, G. J., Eubanks, C. S., Lee, J., Sathyamoorthy, B., Al-Hashimi, H. M., and Hargrove, A. E. (2017) Amiloride as a new RNA-binding scaffold with activity against HIV-1 TAR, *MedChemComm 8*, 1022-1036.

[6] Guo, J.-Y., Minko, Y., Santiago, C. B., and Sigman, M. S. (2017) Developing Comprehensive Computational Parameter Sets To Describe the Performance of Pyridine-Oxazoline and Related Ligands, *ACS Catalysis 7*, 4144-4151.