SUPPLEMENTARY MATERIAL

Identification of a BAZ2A-bromodomain hit compound by fragment-joining

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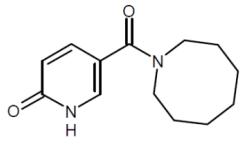
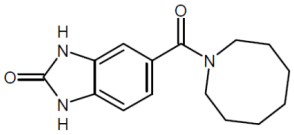
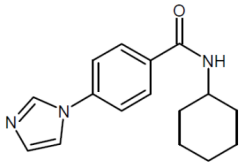
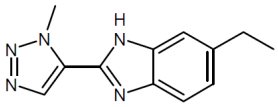
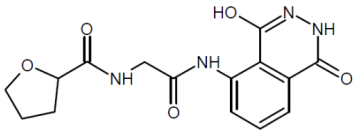
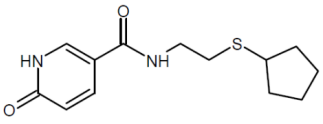
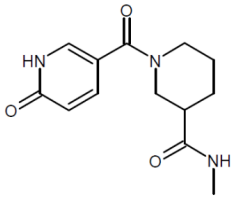
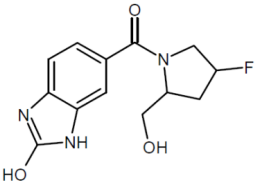
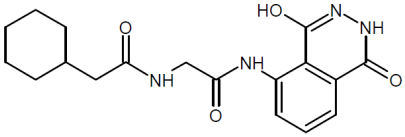
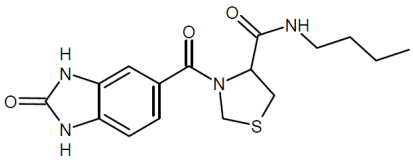
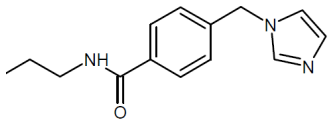
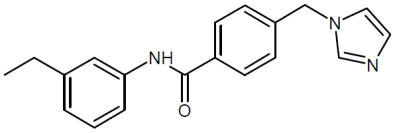
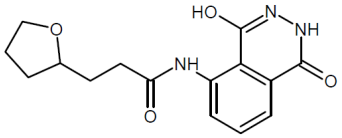
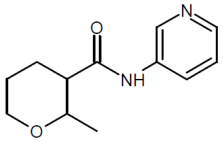
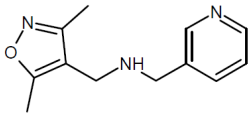
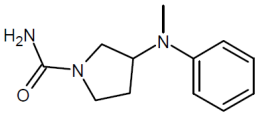
Table S1. Residual BAZ2 binding to the acetylated peptide for selected fragments

Compound	2D structure	% residual binding BAZ2A	% residual binding BAZ2B
1		95	100
2		83	82
3		100	100
4		3	4
5		92	100
6		78	85
7		100	100
8		100	100
9		82	88
10		99	100

11		84	100
12		100	100
13		82	87
14		87	100
15		92	100
16		81	80

Each compound was assayed in AlphaScreen at 500 μM in duplicate.

Table S2. SEED energies for the 34 selected compounds

Name	Tot (kcal/mol)	ElinW (kcal/mol)	rec_des (kcal/mol)	frg_des (kcal/mol)	vdW (kcal/mol)	DElec (kcal/mol)	DG_hydr (kcal/mol)	Tot_eff (kcal/mol HAC)	vdW_eff (kcal/mol HAC)	Elec_eff (kcal/mol HAC)	HAC	MW (g/mol)
PART A												
ZINC000003621142_tauto_1$31	-20.6	-14.2	4.9	5.9	-17.2	-9.0	-5.2	-1.3	-1.0	-0.9	16	219
ZINC000006552654_tauto_1	-22.4	-13.9	7.4	5.3	-21.2	-8.8	-5.1	-1.5	-1.4	-0.9	15	218
ZINC000012535522_tauto_1	-22.4	-13.6	7.0	4.2	-20.2	-8.2	-5.4	-1.1	-1.0	-0.7	20	269
ZINC000013020625_tauto_1$2	-18.0	-17.3	7.8	9.1	-17.6	-8.9	-8.4	-1.0	-1.0	-1.0	17	234
ZINC000014030294_tauto_1$3	-20.5	-21.9	8.1	11.4	-18.2	-10.2	-11.6	-1.0	-1.0	-1.1	19	263
ZINC000014094150_tauto_1	-23.3	-14.5	8.4	6.9	-24.0	-7.9	-6.6	-0.9	-0.9	-0.6	26	352
ZINC000017122432_tauto_1	-19.9	-14.4	7.2	6.3	-19.0	-8.7	-5.7	-1.0	-0.9	-0.7	20	278
ZINC000020253306_tauto_2	-14.6	-14.6	8.4	6.4	-15.0	-8.0	-6.5	-0.9	-0.9	-0.9	16	217
ZINC000030938279_tauto_1	-21.8	-16.3	11.0	6.9	-23.4	-9.5	-6.9	-0.9	-0.9	-0.6	25	333
ZINC000032824806_tauto_1	-15.5	-19.5	10.4	10.1	-16.5	-9.9	-9.6	-0.9	-0.9	-1.0	18	266
ZINC000032913031_tauto_1$2	-20.0	-15.9	10.7	6.8	-21.6	-8.8	-7.0	-0.9	-0.9	-0.7	23	305
ZINC000035186970_tauto_1	-17.5	-20.3	8.0	9.9	-15.1	-10.0	-10.3	-1.0	-0.9	-1.3	16	222
ZINC000035186971_tauto_1	-19.0	-21.4	8.0	9.6	-15.2	-10.8	-10.6	-1.2	-0.9	-1.3	16	222
ZINC000035218339_tauto_1	-17.0	-18.9	8.3	10.2	-16.5	-8.4	-10.5	-1.0	-1.0	-1.1	17	236
ZINC000038857516_tauto_1	-16.4	-14.6	10.3	6.6	-18.7	-8.4	-6.2	-0.9	-1.0	-0.8	18	243
ZINC000046125885_tauto_1	-19.0	-17.2	8.9	8.6	-19.2	-8.0	-9.2	-0.9	-1.0	-0.9	20	273
ZINC000051884303_tauto_1	-17.6	-18.4	7.6	10.1	-16.9	-8.3	-10.1	-1.1	-1.0	-1.1	16	222
ZINC000051884304_tauto_1	-17.8	-20.3	7.4	10.0	-15.0	-10.0	-10.3	-1.1	-0.9	-1.3	16	222
ZINC000071376083_tauto_1	-18.9	-19.3	6.3	10.4	-16.3	-8.0	-11.2	-1.2	-1.0	-1.2	16	218
ZINC000095443934_tauto_1	-18.43	-15.8	6.3	6.9	-15.8	-9.0	-6.8	-1.0	-0.9	-0.9	17	227
ZINC000133157645_tauto_1	-18.2	-18.7	7.6	7.1	-14.2	-10.1	-8.6	-1.2	-0.9	-1.2	15	206
ZINC000190309945_tauto_1	-16.6	-14.9	7.8	5.3	-14.7	-8.0	-6.9	-1.0	-0.9	-0.9	16	220
ZINC000195385560_tauto_1	-22.8	-19.4	8.6	10.3	-22.4	-8.3	-11.0	-0.9	-0.9	-0.8	24	348
ZINC000334358972_tauto_1	-20.6	-20.1	8.3	11.1	-20.0	-8.4	-11.8	-1.0	-1.0	-1.0	20	279
ZINC000534647291_tauto_1$2	-22.8	-19.1	9.3	8.7	-21.8	-9.5	-9.6	-1.1	-1.0	-0.9	21	314
PART B												
ZINC000031807840_tauto_2	-23.9	-20.7	8.1	15.9	-27.3	-2.9	-17.7	-0.8	-0.9	-0.7	30	419
ZINC000048087838_tauto_1	-21.1	-19.2	8.9	14.2	-25.0	-0.6	-18.6	-0.8	-1.0	-0.8	25	342
ZINC000058278898_tauto_1	-20.6	-19.4	8.1	17.2	-26.5	-0.0	-19.3	-0.9	-1.1	-0.8	24	332
ZINC000058370869_tauto_1	-21.3	-19.6	8.1	13.8	-23.7	-3.0	-16.5	-0.8	-0.9	-0.7	26	358
ZINC000068590748_tauto_1$2	-11.5	-24.7	7.1	20.1	-14.0	12.1	-36.8	-0.8	-0.9	-1.6	15	207
ZINC000078914792_tauto_1	-23.4	-20.8	8.0	15.5	-26.1	-5.1	-15.7	-1.0	-1.2	-0.9	22	303
ZINC000091452492_tauto_1	-17.8	-24.2	8.1	24.7	-26.3	17.8	-42.0	-0.8	-1.2	-1.1	21	292
ZINC000219329883_tauto_1	-20.2	-20.4	8.4	21.6	-29.9	1.6	-22.0	-0.8	-1.2	-0.8	25	343
ZINC000221227641_tauto_1	-19.4	-20.1	8.2	21.5	-29.1	2.4	-22.5	-0.8	-1.2	-0.8	24	328

Table S3. Data Collection and Refinement Statistics

	Cmp **4**	Cmps **4** + **18**	Cmp **19**	Cmp **20**	Cmp **21**
Data Collection					
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P3_121$
Unit-cell parameters (Å, °)	a = 37.12 b = 35.16 c = 37.53 β = 92.48	a = 37.42 b = 35.30 c = 37.80 β = 92.66	a = 37.35 b = 35.05 c = 37.69 β = 92.05	a = 37.40 b = 34.77 c = 37.58 β = 92.28	a = 94.60 b = 94.60 c = 32.74
Wavelength (Å)	1.00	1.00	0.97	0.97	0.97
Resolution (Å)	37.50-1.40 (1.42-1.40)	37.77-1.43 (1.45-1.43)	37.68-1.15 (1.17-1.15)	37.56-1.25 (1.27-1.25)	81.93-2.00 (2.05-2.00)
R_{merge} (%)	4.9 (83.8)	6.9 (20.0)	5.0 (43.6)	6.6 (74.8)	9.5 (146.9)
R_{meas} (%)	5.6 (95.6)	7.5 (22.1)	5.5 (52.7)	7.2 (83.2)	9.7 (151.8)
R_{pim} (%)	2.6 (45.2)	2.9 (9.2)	2.2 (28.6)	2.9 (35.8)	2.2 (38.0)
$\langle I/\sigma(I)\rangle$	16.8 (1.9)	17.8 (6.6)	16.2 (2.6)	11.6 (1.9)	22.8 (2.0)
$CC^{1/2}$	0.999 (0.805)	0.997 (0.977)	0.998 (0.789)	0.998 (0.802)	0.969 (0.588)
Completeness (%)	98.7 (97.8)	98.3 (95.0)	94.4 (57.7)	98.0 (94.3)	100.0 (100.0)
Multiplicity	4.3 (4.3)	6.4 (5.6)	5.7 (3.1)	6.2 (5.2)	18.8 (15.7)
Refinement					
Resolution (Å)	37.50-1.40	37.77-1.43	37.68-1.15	37.56-1.25	47.31-2.00
R_{work}/R_{free} (%)	14.4/18.2	13.4/16.1	13.9/16.3	15.1-18.0	16.8/18.6
R.m.s. deviations					
Bond lengths (Å)	0.007	0.008	0.007	0.007	0.006
Bond angles (°)	0.88	0.94	0.96	0.97	0.85
PDB entry	7B7B	7B7G	7B7I	7B82	7BC2

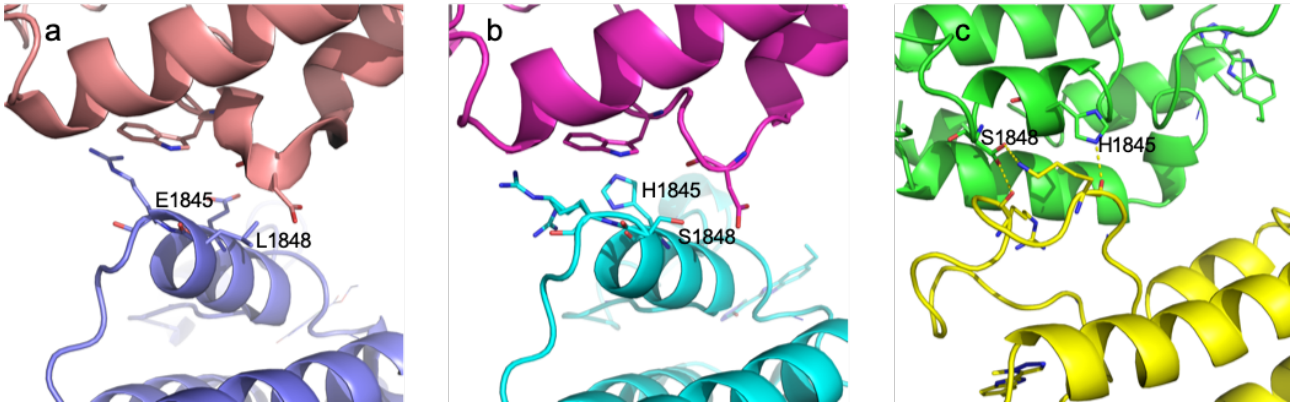


Figure S1. Effect of E1845H/L1848S mutations on BAZ2A crystallographic packing. a) WT-BAZ2A trigonal crystal form; b) BAZ2A-DM trigonal crystal form; c) BAZ2A-DM monoclinic crystal form (PDB 7B7B).

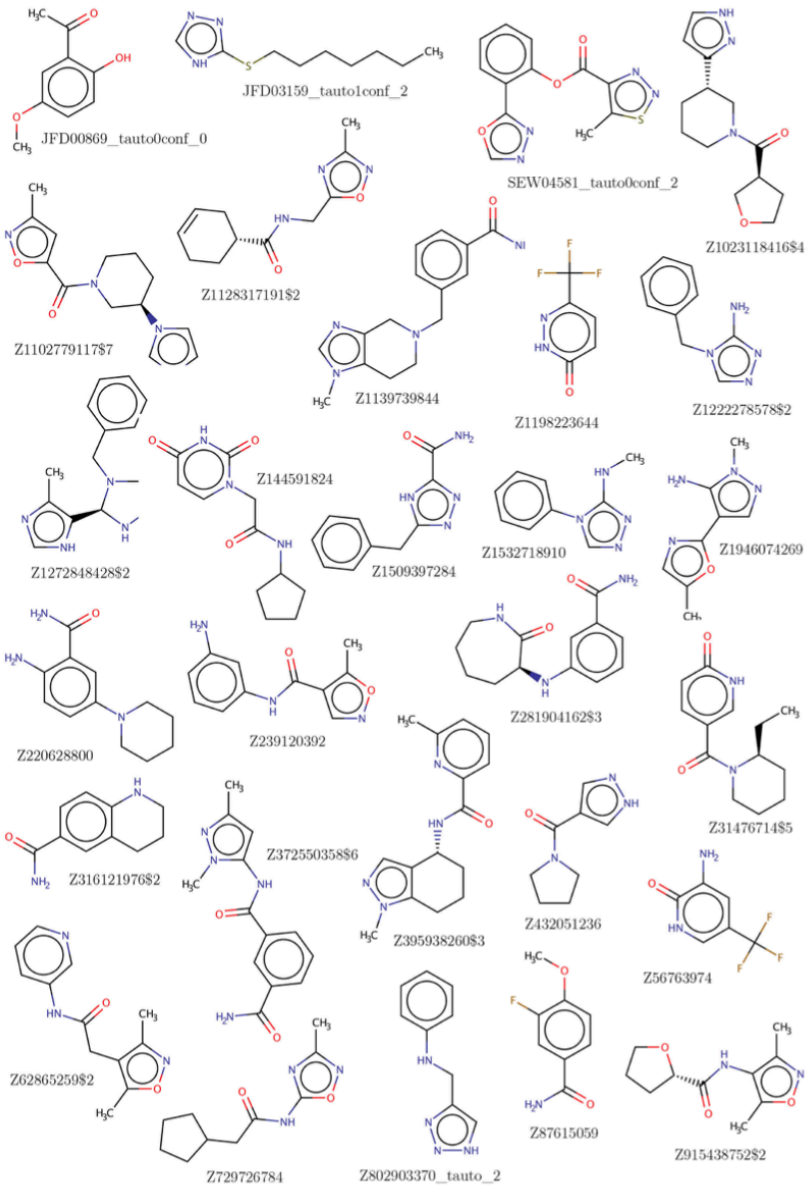


Figure S2. 2D chemical structures of the 28 compounds selected from the first docking cycle identified by their commercial catalogue numbers.

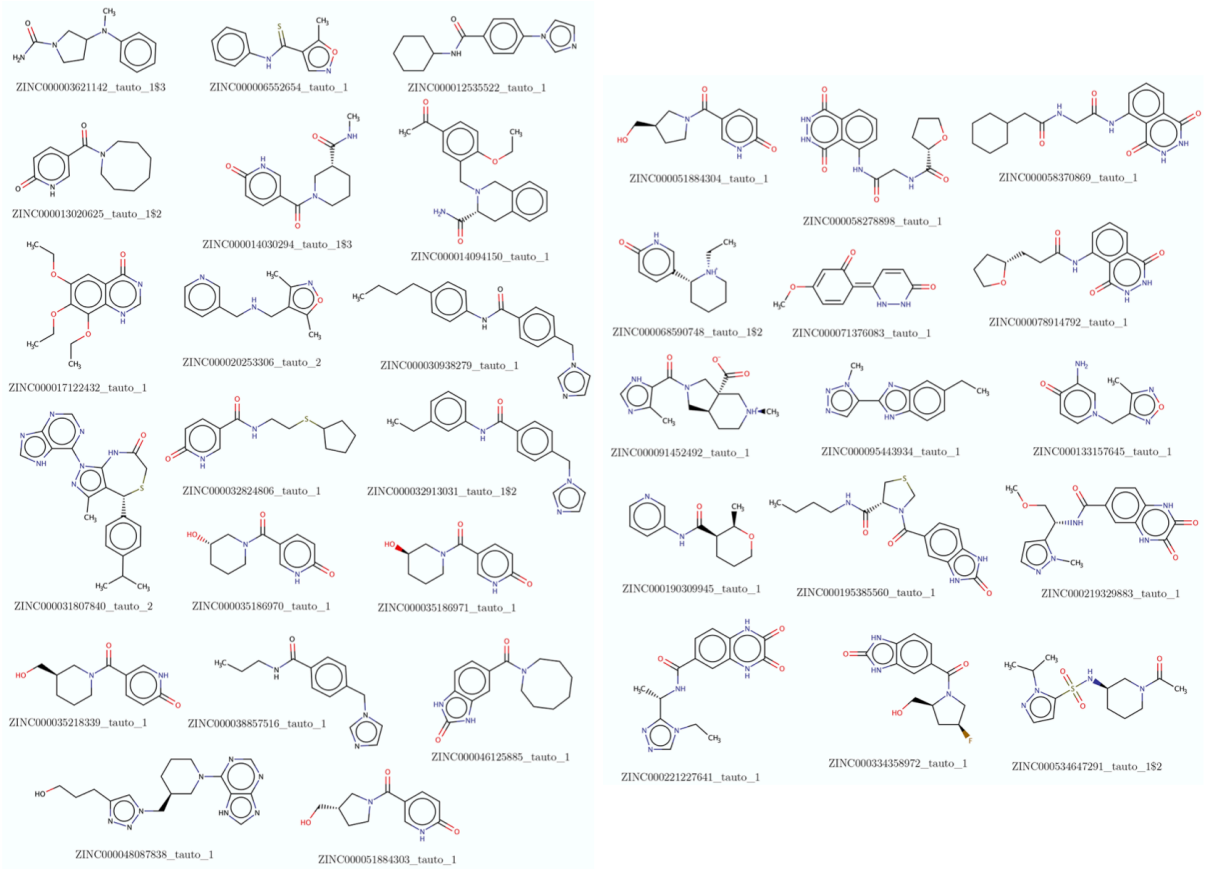


Figure S3. 2D chemical structures of the 34 compounds selected from the second docking cycle identified by their ZINC15 numbers.

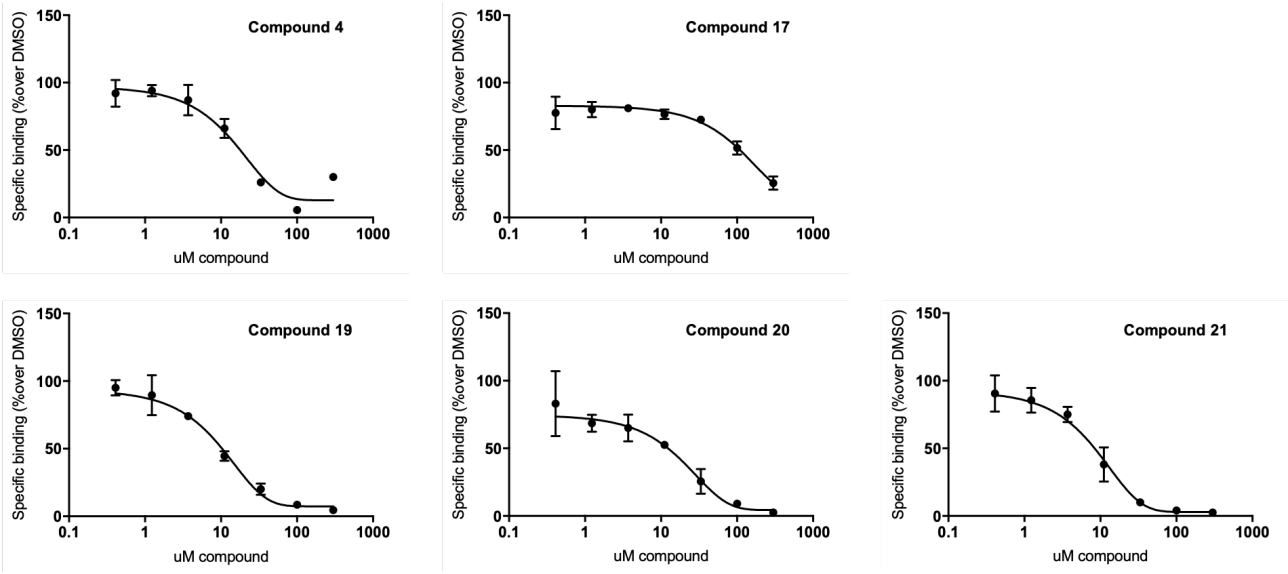


Figure S4. AlphaScreen competition binding assay. The specific binding to the acetylated peptide relative to the control DMSO (y-axis) is plotted against the corresponding compound concentration in μM in log10 scale (x-axis).

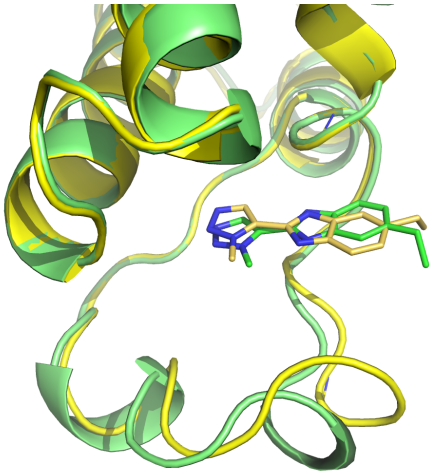


Figure S5. Superposition of experimental (yellow, PDB 7B7B) and docked (green) poses for compound **4**. The BAZ2A structure used for the molecular docking procedure carried the ZA loop in the open conformation.

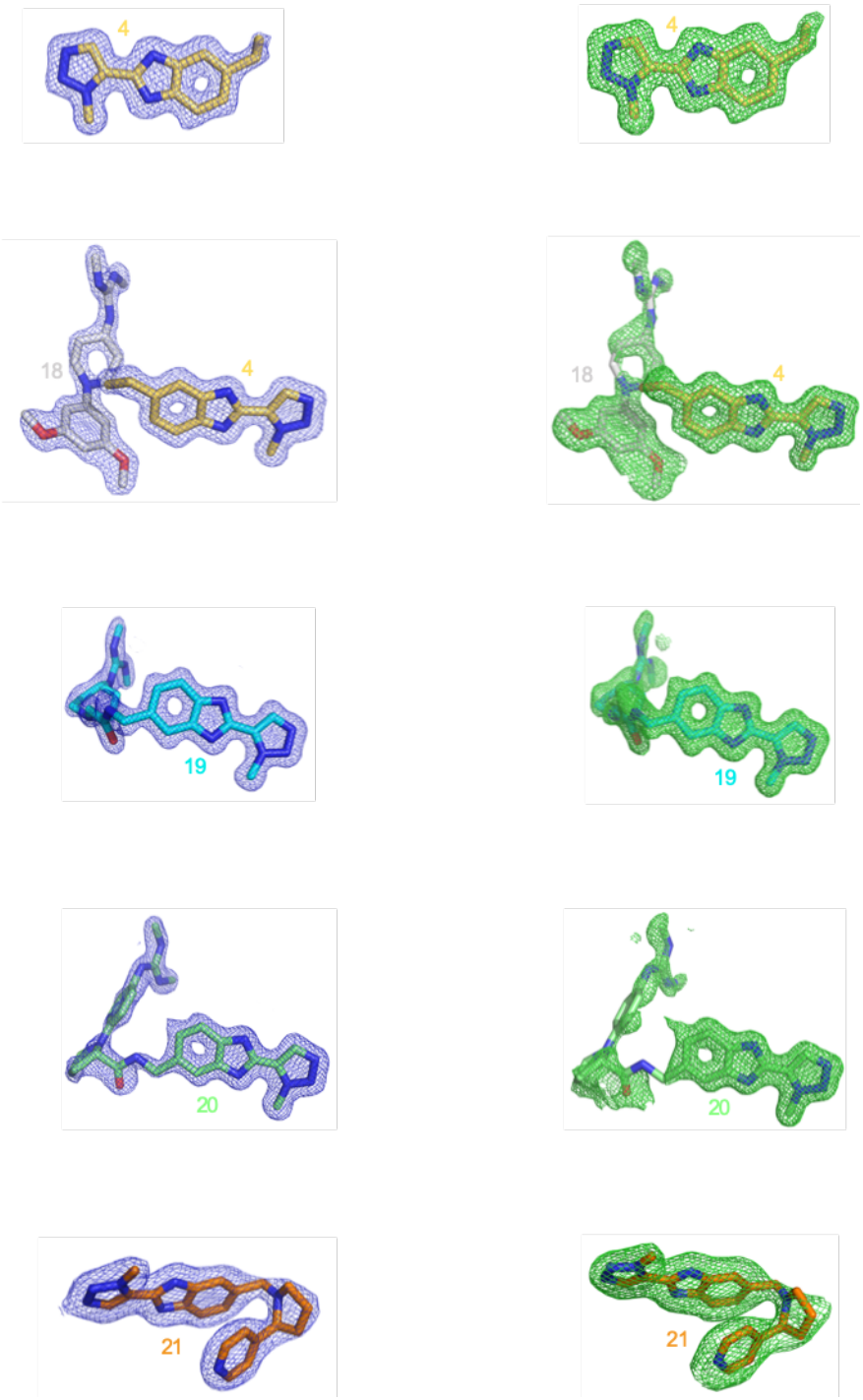
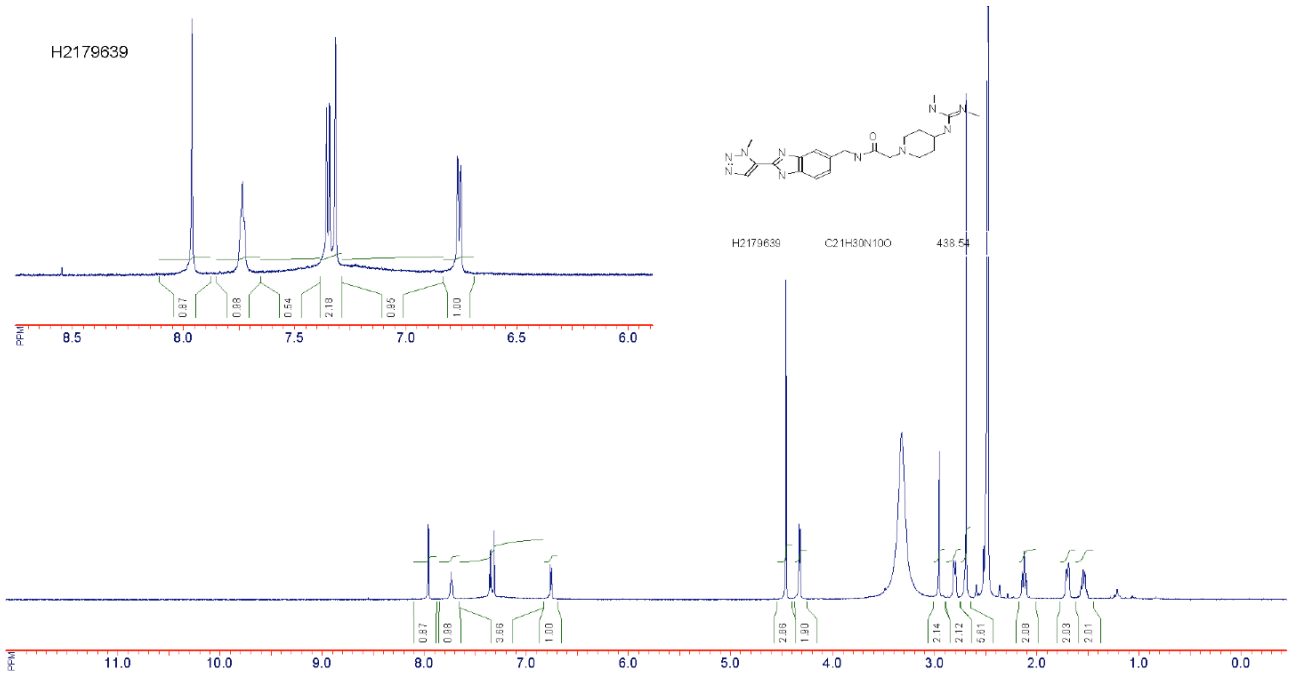
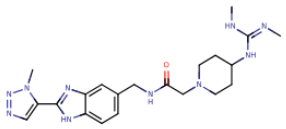


Figure S6. Electron densities for the analyzed compounds. $2F_o$-F_c map (blue) is contoured at 1σ for compounds **4** (yellow, PDB 7B7B), **18** (white, PDB 7B7G), **19** (cyan, PDB 7B7I), **20** (green, PDB 7B82), and **21** (orange, PDB 7BC2); F_o-F_c polder OMIT map (green) is contoured at 3σ.



^{1}H NMR (600 MHz, dmso) δ 7.96 (s, 1H), 7.73 (t, *J* = 5.9, 5.9 Hz, 1H), 7.52 (br s, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.32 (s, 1H), 7.12 (br s, 1H), 6.76 (d, *J* = 8.1 Hz, 1H), 4.46 (s, 3H), 4.32 (d, *J* = 5.5 Hz, 2H), 2.96 (s, 2H), 2.81 (d, *J* = 11.4 Hz, 2H), 2.69 (s, 6H), 2.58 – 2.52 (m, 1H), 2.13 (t, *J* = 11.8, 11.8 Hz, 2H), 1.70 (d, *J* = 10.9 Hz, 2H), 1.58 – 1.50 (m, 2H).

Ret_Time: 0.675 min



Mol Wt **438.53**

Exact Mass **438.29**

#	Time	Area%
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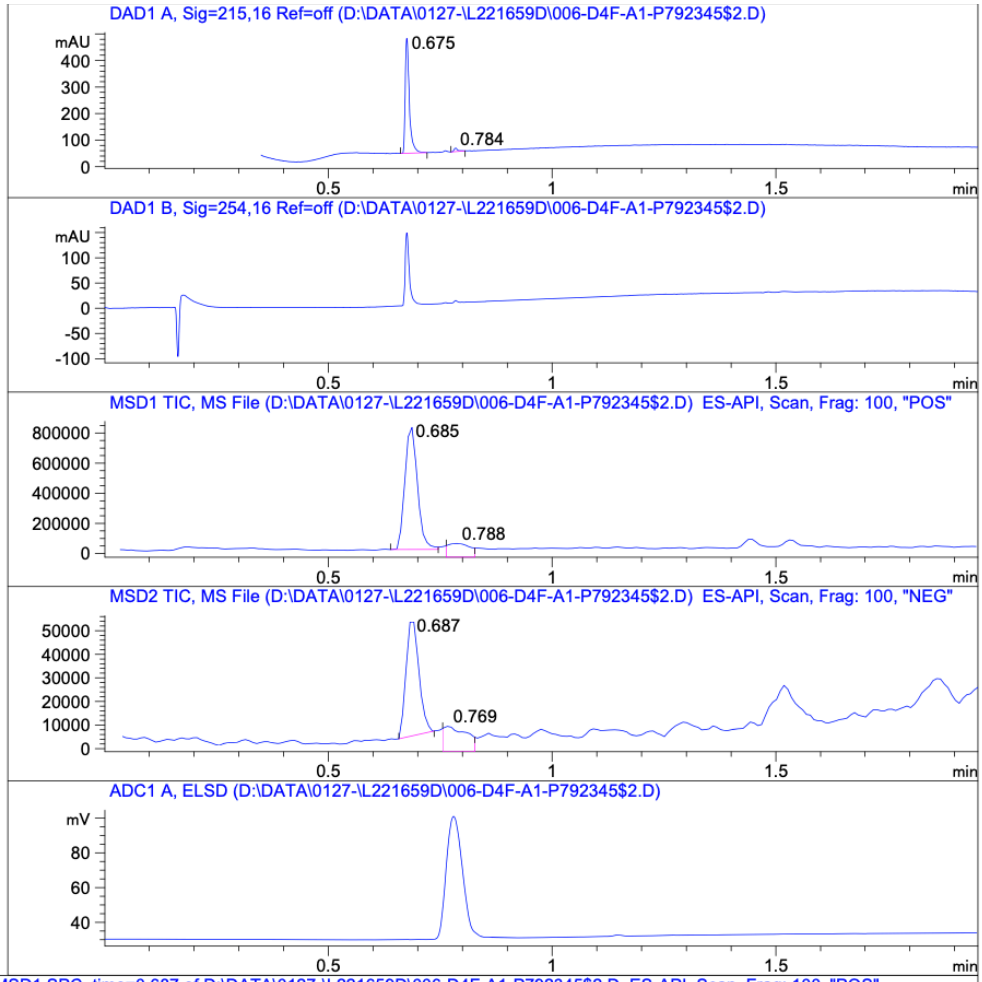
2	0.784	2.61



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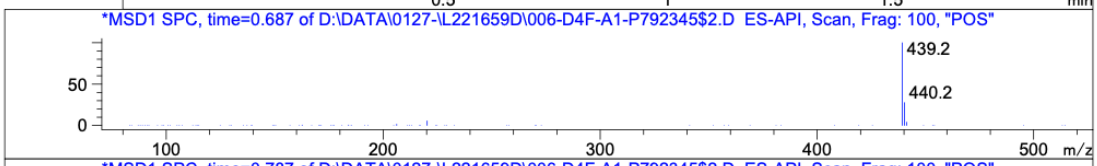
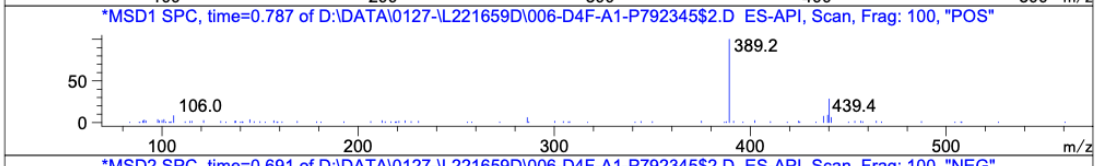


RT 0.788



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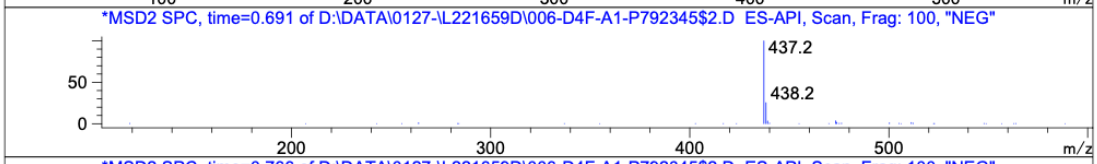
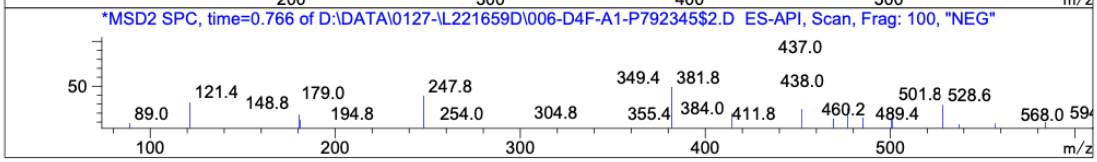
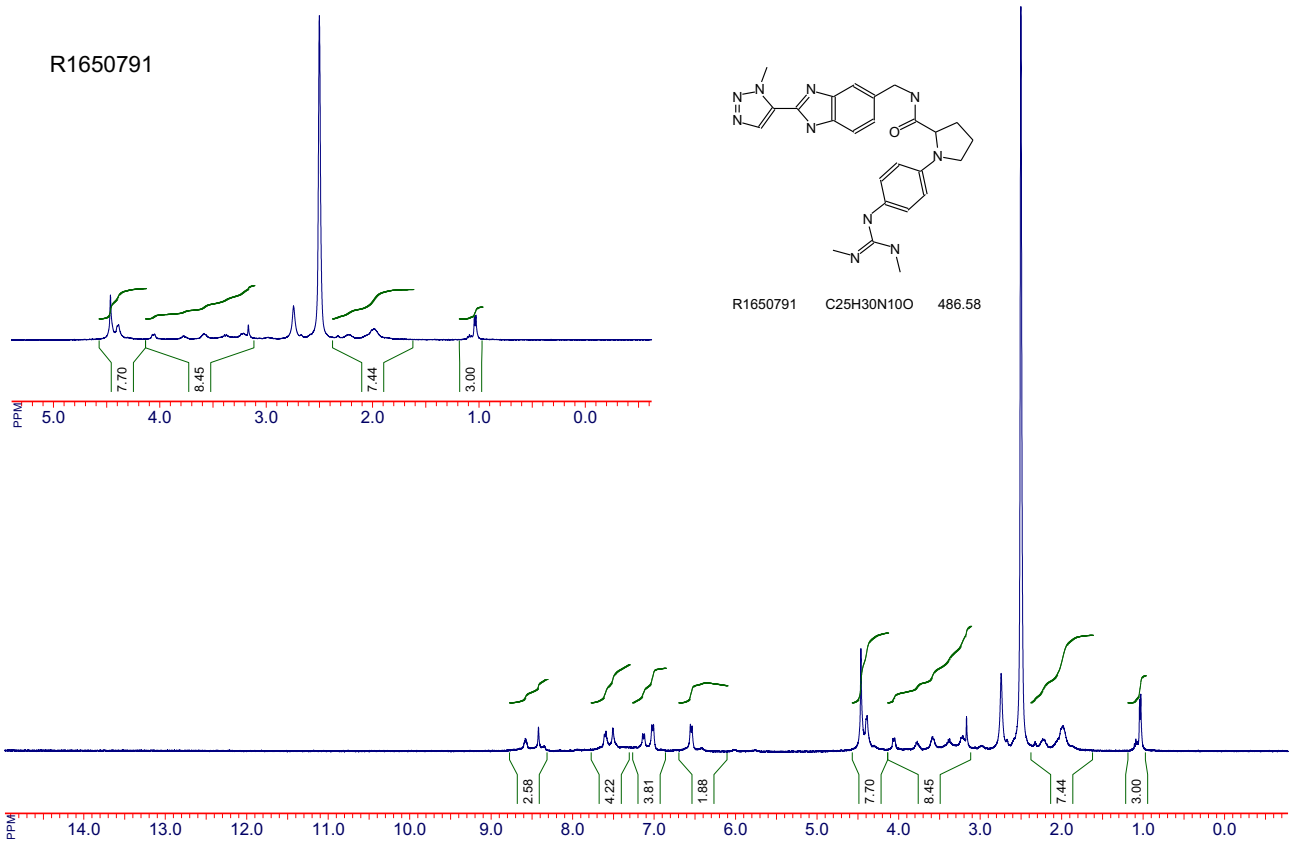


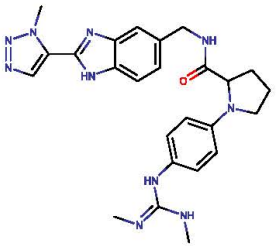
RT 0.769





^{1}H NMR spectrum (400MHz, DMSO-d_6), δ: 8.58 (br. s, 1NH); 8.42(s, 1NH); 7.59.-6.54 (m, 8H, Ar); 4.46 (s, 3H); 4.39 (d, J = 7.88 Hz, 2H); 4.06 (m, 1H); 3.76 (m, 1H); 3.59 (m, 1H); 3.37 (m, 1H); 3.17 (m, 1H); 2.74 (s, 3H); 2.21 (m, 1H); 1.98 (m, 2H); 1.04 (br. s, 3H).

Ret_Time: 0.889 min



Mol Wt **486.57**

Exact Mass **486.29**

#	Time	Area%
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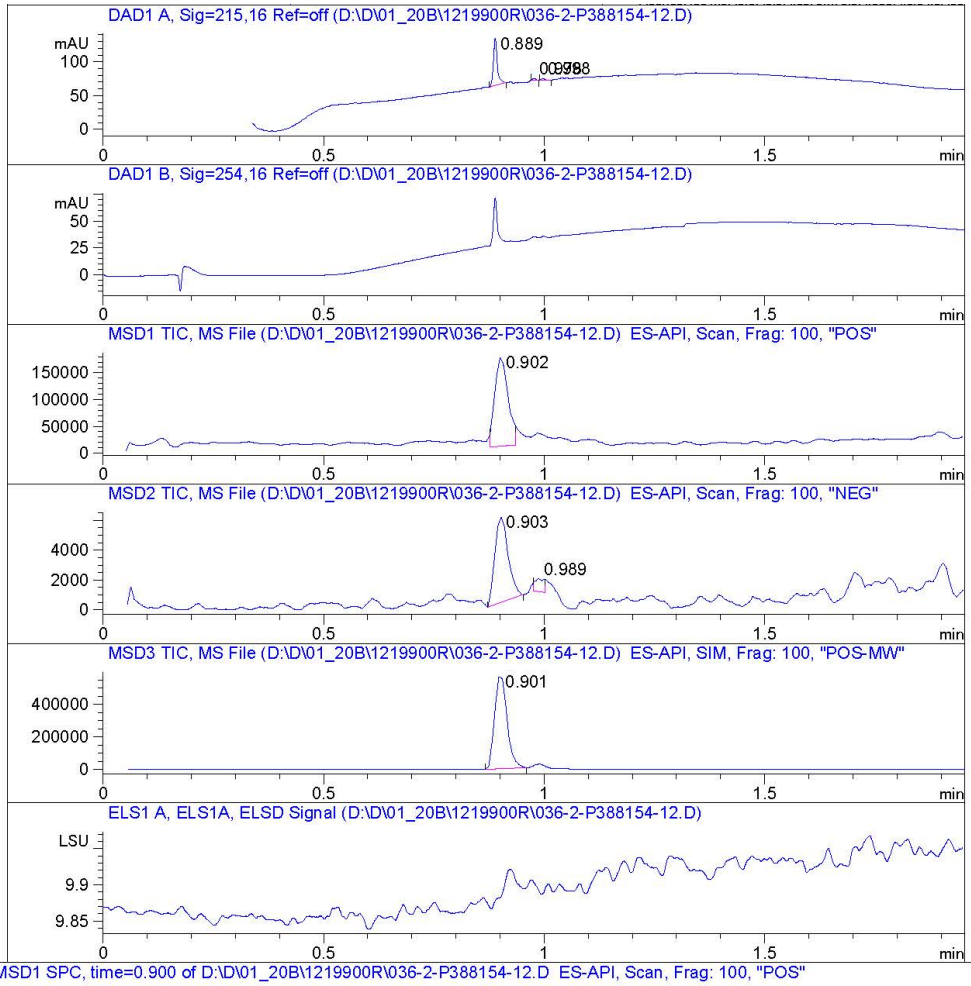
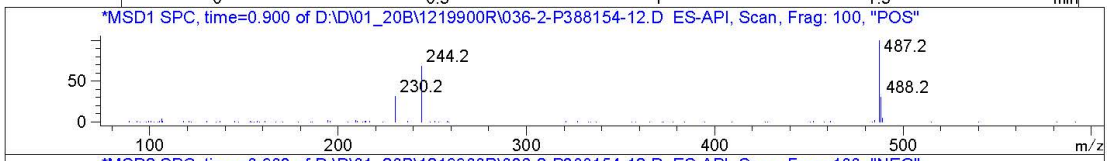
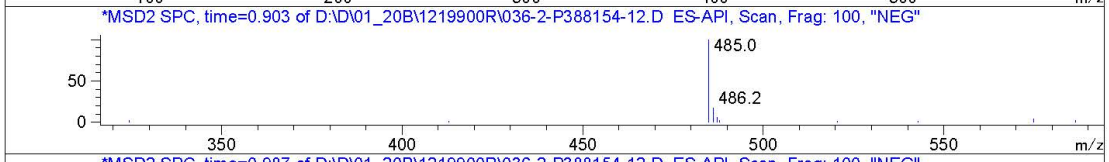
2	0.978	3.30
3	0.998	2.77



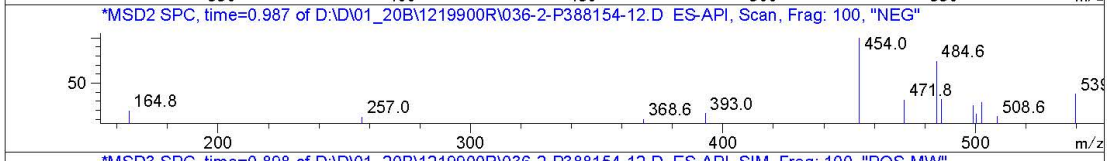
RT 0.902



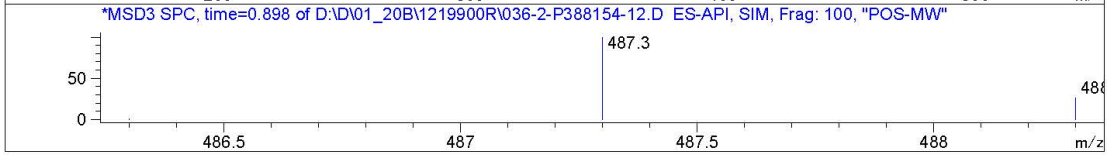
RT 0.903

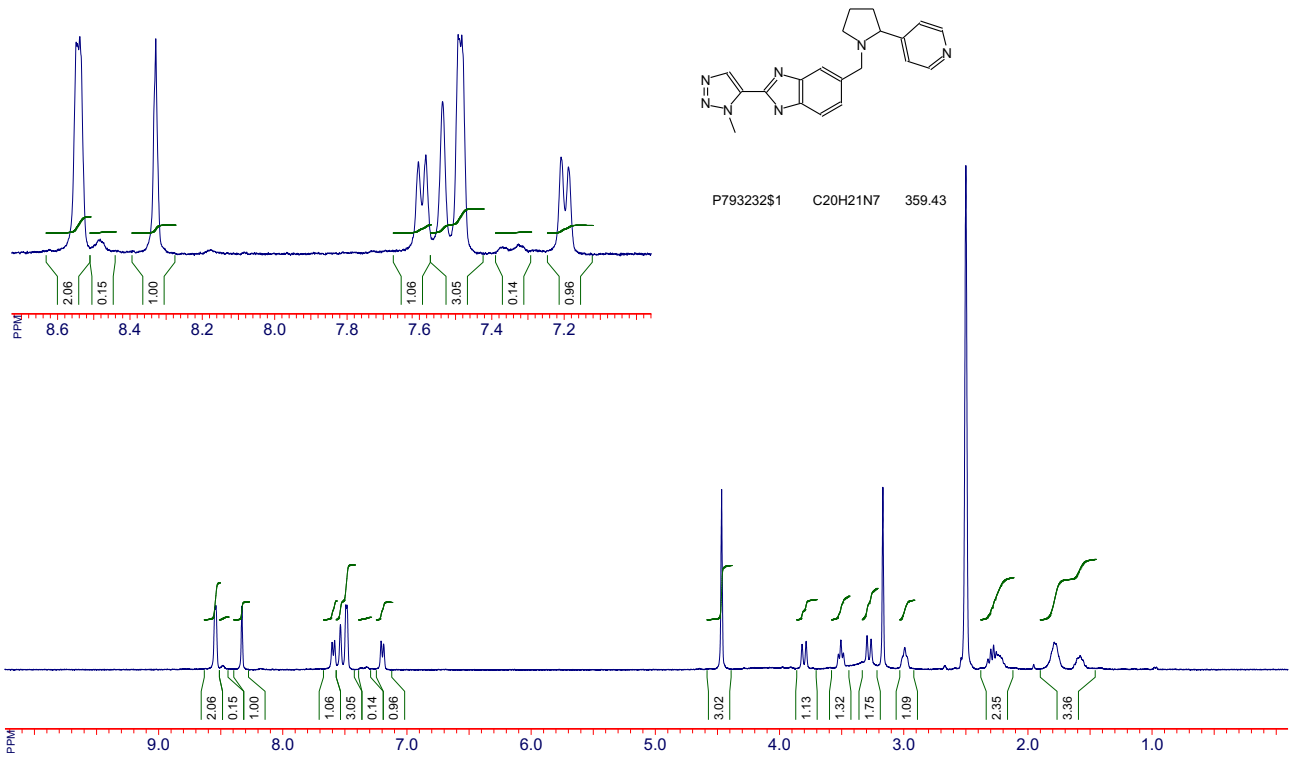


RT 0.989



RT 0.901





^{1}H NMR spectrum (400MHz, DMSO-d_6), δ: 8.5-7.2 (m, 8H, Ar); 4.47 (s, 3H, NCH_3); 3.8 (d, J = 12.99 Hz, 2H), 3.51 (t, J = 8.04 Hz, 1H); 3.28 (d, J = 12.99 Hz, 2H); 2.99 (m, 1H); 2.29 (m, 2H); 1.79 (m, 2H); 1.58 (m, 1H).

Mol Wt **359.43**

Exact Mass **359.21**

#	Time	Area%
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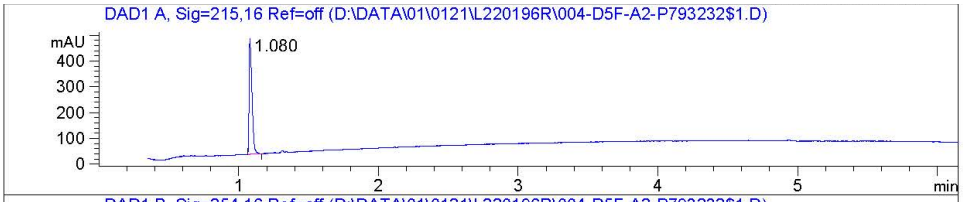
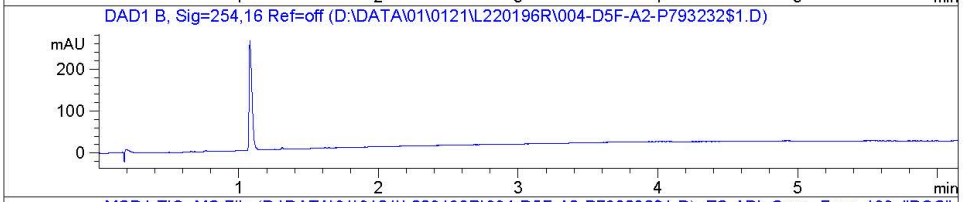
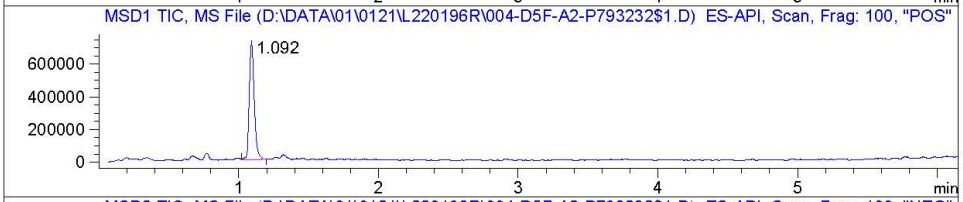
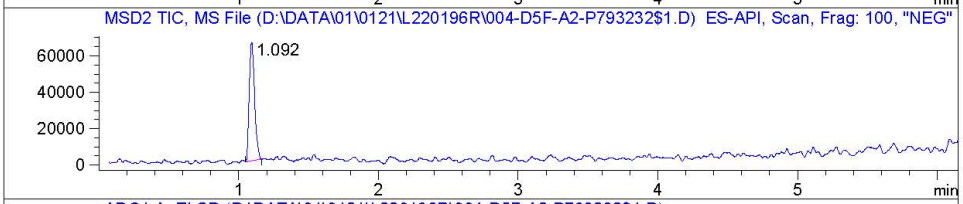
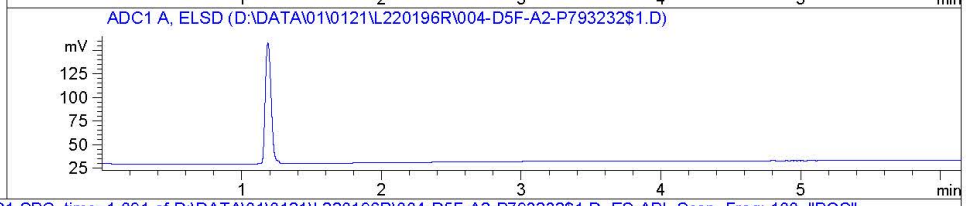


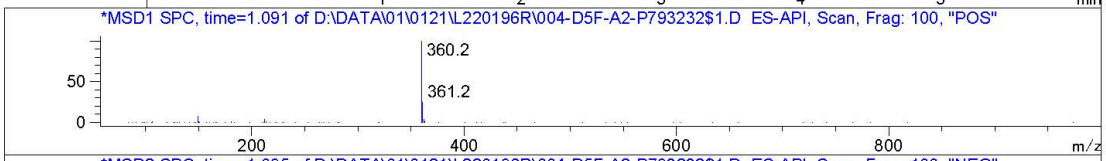








RT 1.092



RT 1.092