Supporting Information for

Brain Penetrable Histone Deacetylase 6 Inhibitor SW-100 Ameliorates Memory and Learning Impairments in a Mouse Model of Fragile X Syndrome

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Supplementary tables and figures.

Table S1. Physicochemical properties of analogs **6-12**, SW-100**,** and TubA[a]

Compound	MW	clogP	clogD (7.4)	pK_a (basic)	tPSA	HBD	CNS MPO[b]
6	217.23 (1)	0.97 (1)	0.97 (1)	3.46 (1)	64.93 (1)	2 (0.5)	5.50
7	270.33 (1)	3.02 (0.995)	3.01 (0.495)	5.12 (1)	52.57 (1)	2 (0.5)	4.99
8	299.31 (1)	2.32 (1)	2.30 (0.85)	5.95 (1)	64.93 (1)	2 (0.5)	5.35
9	299.31 (1)	2.32 (1)	2.30 (0.85)	5.86 (1)	64.93 (1)	2 (0.5)	5.35
10	302.76 (1)	3.30 (0.85)	3.29 (0.355)	0.99 (1)	52.57 (1)	2 (0.5)	4.71
11	286.31 (1)	2.84 (1)	2.83 (0.585)	0.84 (1)	52.57 (1)	2 (0.5)	5.09
12	296.33 (1)	2.21 (1)	2.15 (0.925)	6.55 (1)	76.96 (1)	3 (0.1)	4.19
SW-100	316.78 (1)	3.74 (0.63)	3.73 (0.135)	2.91 (1)	52.57 (1)	2 (0.5)	4.27
TubA	335.41 (1)	2.54 (1)	2.34 (0.83)	7.14 (1)	55.81 (1)	2 (0.5)	5.33

[a]All the values were obtained by MarvinSketch version 18.5. [b]CNS MPO values were calculated according to the equation in the article by Wager, T. T. et al., *ACS Chem. Neurosci*. **2016**, *7*, 767-775.

Table S2. Brain/plasma pilot PK studies of TubA.[a]

route	dose (mg/kg)	time (h)	brain concentration (ng/mL)	plasma concentration (ng/mL)	brain/plasma ratio
IV	3	0.133	40.5 ± 3.4	273.5 ± 30.4	0.15
IV	3	1	17.5 ± 2.6	20.3 ± 0.64	0.86

[a]TubA was administered to C57BL/6 male mice by IV administration at the dose of 3 mg/kg. Blood and brain samples were collected at 8 min and 60 min time-points. Brain tissues were homogenized at a 1:4 ratio of tissue weight (g) to volume of PBS (mL). The data found (ng/mL) was multiplied by 5 to obtain the concentration (ng/mL) in brain tissues. PK studies were performed by Pharmaron, Inc. (Irvine, CA).

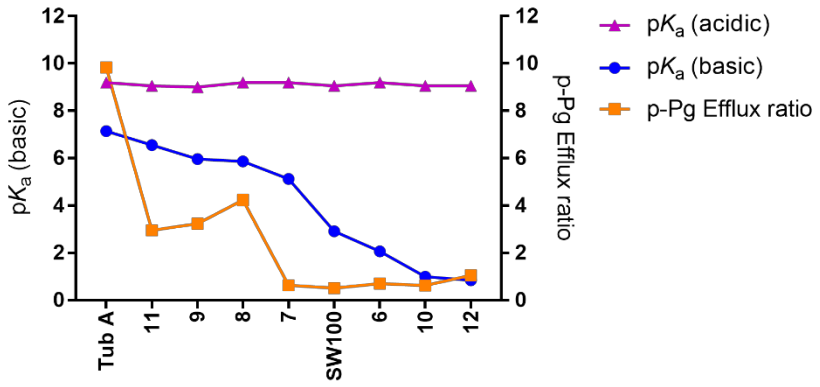
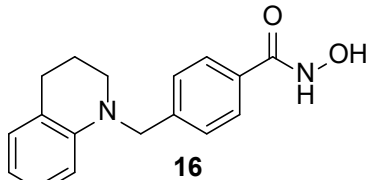


Figure S1. The comparison of *p*K$_a$ values (basic and acidic) and P-gp efflux ratios.



16

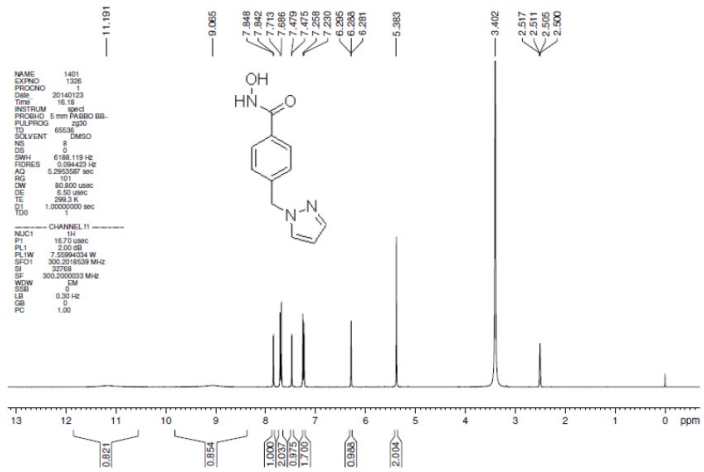
IC_{50} (HDAC6) = 15 nM

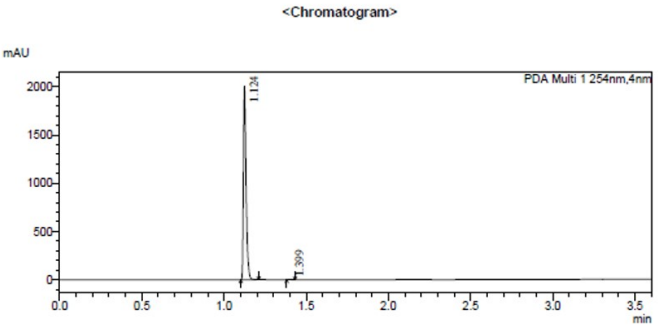
IC_{50} (HDAC1) = 11 μM

Figure S2. The structure of compound **16**.

^{1}H NMR spectra and LC-MS purity reports for compounds 6-12, SW-100, TubA, and NexA

Compound 6

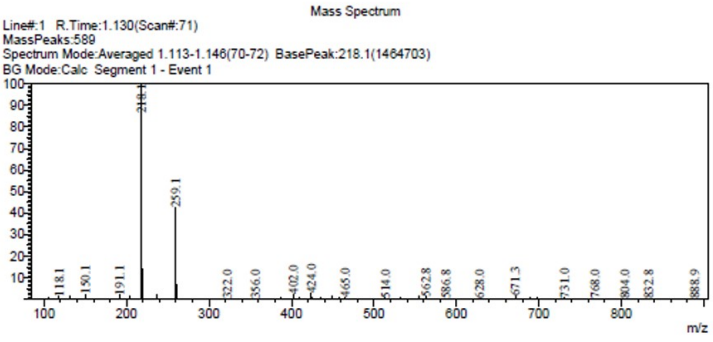


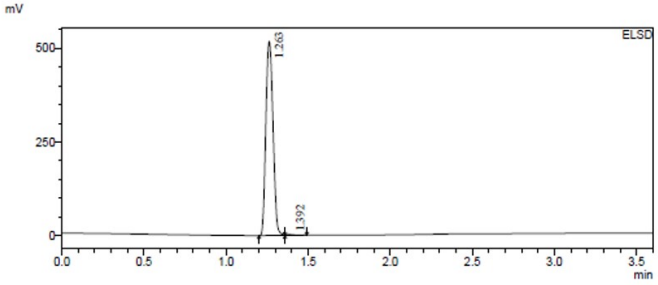


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.124	2011322	99.851	2243192	99.855
2	1.399	3003	0.149	3247	0.145
Total		2014325	100.000	2246439	100.000



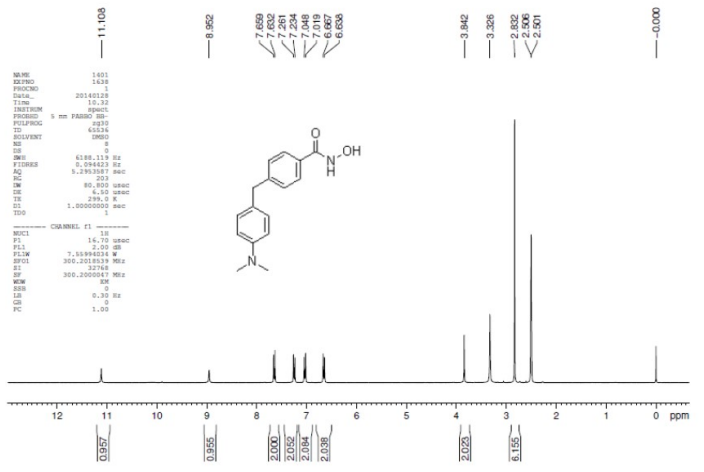


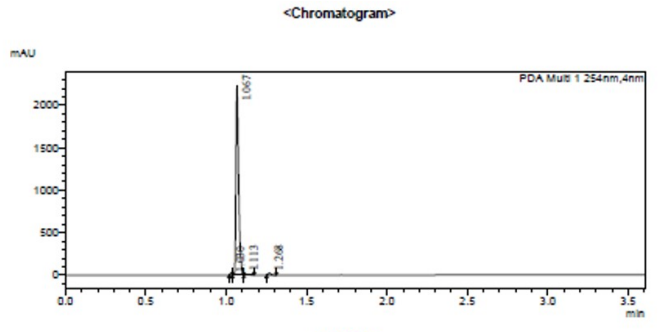
Peak Table

ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.263	514409	99.536	1567146	99.448
2	1.392	2397	0.464	8697	0.552
Total		516805	100.000	1575843	100.000

Compound 7

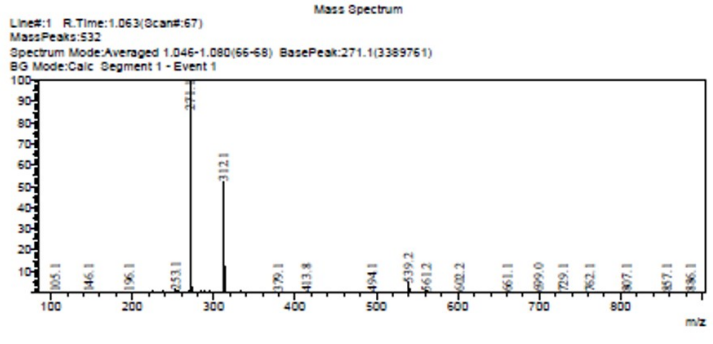


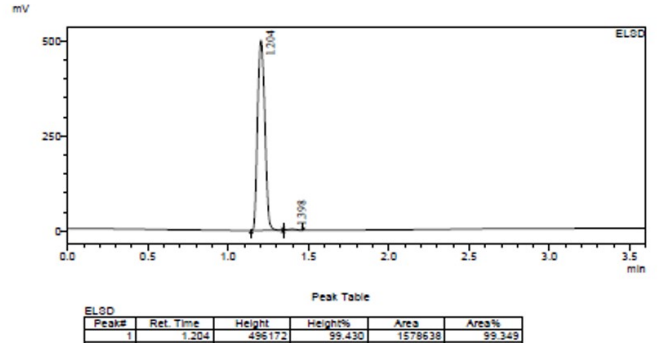


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.030	802	0.035	576	0.022
2	1.067	2236401	98.564	2544164	98.660
3	1.113	6772	0.298	8087	0.314
4	1.268	25017	1.103	25896	1.004
Total		2268992	100.000	2578722	100.000



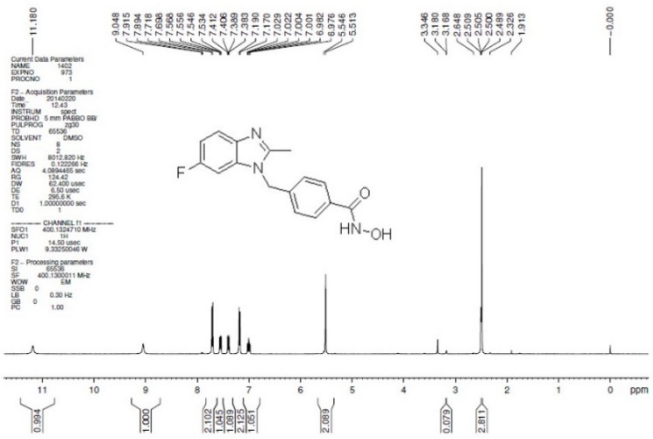


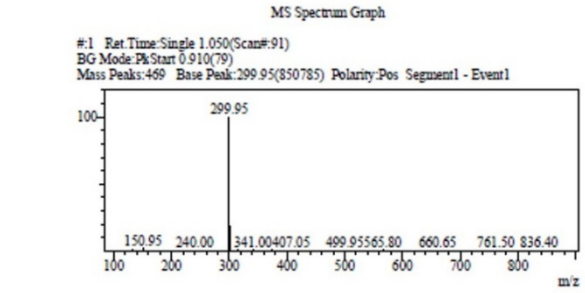
Peak Table

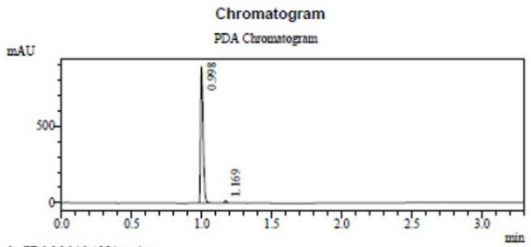
ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.204	496172	99.430	1578638	99.349
2	1.398	2845	0.570	10338	0.651
Total		499017	100.000	1588977	100.000

Compound 8



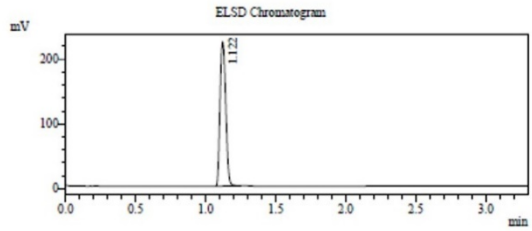




PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Height	Height %	Area	Area %
1	0.998	888498	98.329	1102142	98.492
2	1.169	15100	1.671	16879	1.508
Total		903598	100.000	1119021	100.000

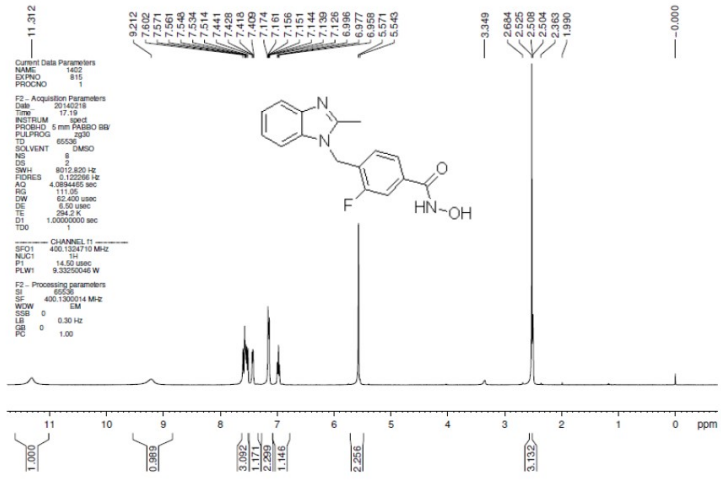


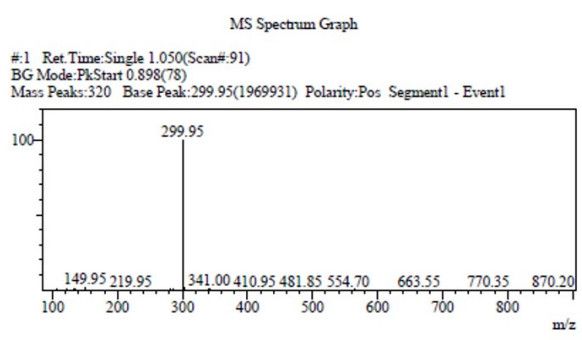
PeakTable

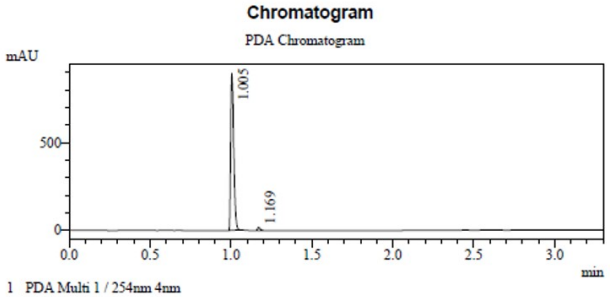
ELSD Ch1

Peak#	Ret. Time	Height	Height %	Area	Area %
1	1.122	222429	100.000	631034	100.000
Total		222429	100.000	631034	100.000

Compound 9



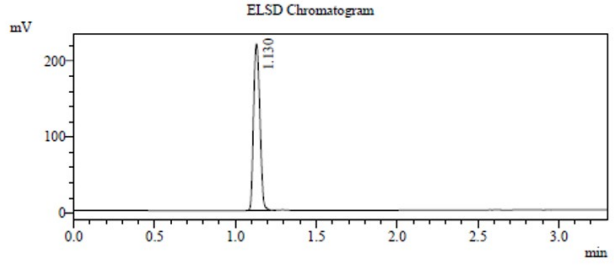




PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Height	Height %	Area	Area %
1	1.005	895953	97.979	1153107	98.165
2	1.169	18481	2.021	21549	1.835
Total		914434	100.000	1174656	100.000

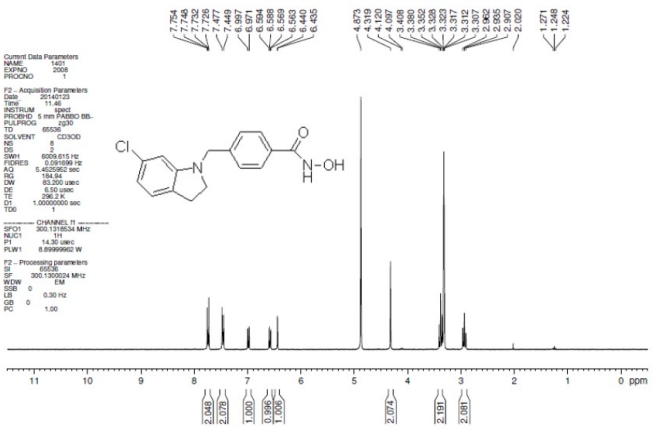


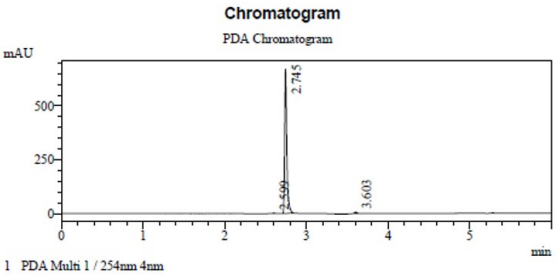
PeakTable

ELSD Ch1

Peak#	Ret. Time	Height	Height %	Area	Area %
1	1.130	216331	100.000	592521	100.000
Total		216331	100.000	592521	100.000

Compound 10





PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Height	Height %	Area	Area %
1	2.599	2748	0.404	4849	0.399
2	2.745	670731	98.562	1197410	98.495
3	3.603	7038	1.034	13443	1.106
Total		680517	100.000	1215702	100.000

MS Spectrum Graph

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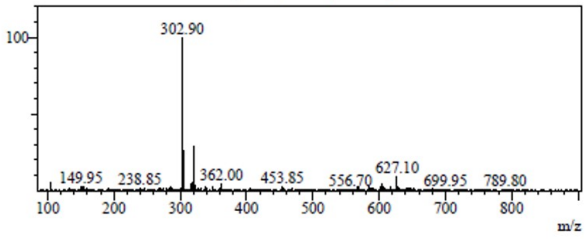
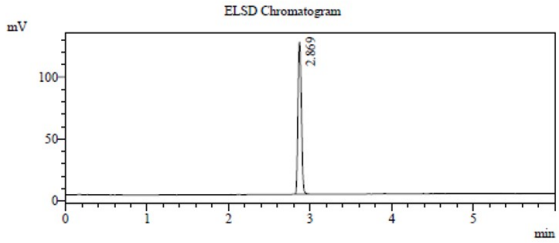
BG Mode:PkStart 2.765(238)
Mass Peaks:557 Base Peak:302.90(83826) Polarity:Pos Segment1 - Event1



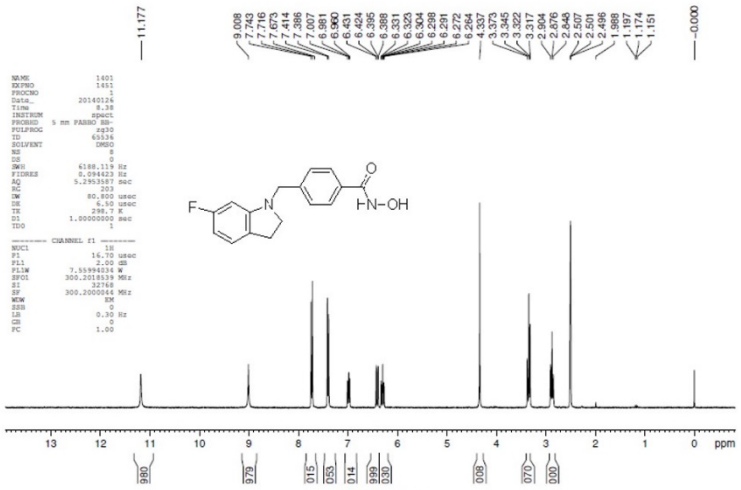


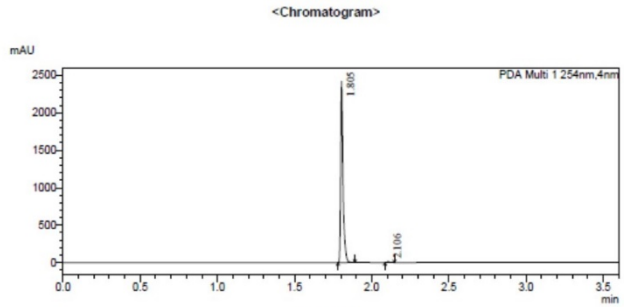
PeakTable

AD2 Ch1

Peak#	Ret. Time	Height	Height %	Area	Area %
1	2.869	122053	100.000	358435	100.000
Total		122053	100.000	358435	100.000

Compound 11





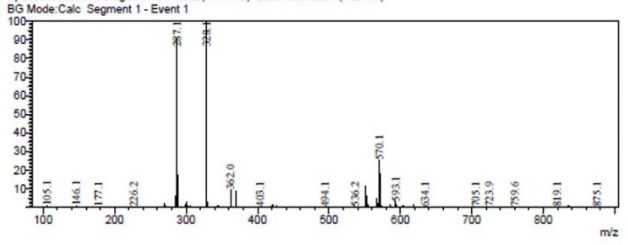
Peak Table

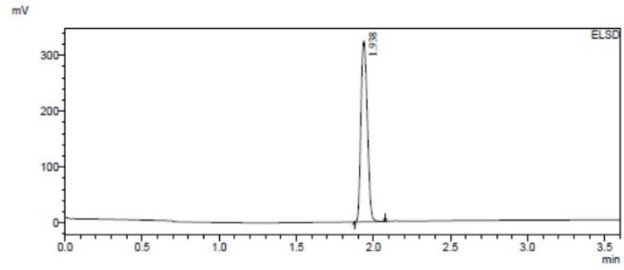
PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.805	2425960	99.448	2511464	99.437
2	2.106	13464	0.552	14213	0.563
Total		2439424	100.000	2525676	100.000

Mass Spectrum

Line#:1 R.Time:1.813(Scan#:112)
MassPeaks:528
Spectrum Mode:Averaged 1.796-1.830(111-113) BasePeak:328.1(762739)
BG Mode:Calc Segment 1 - Event 1



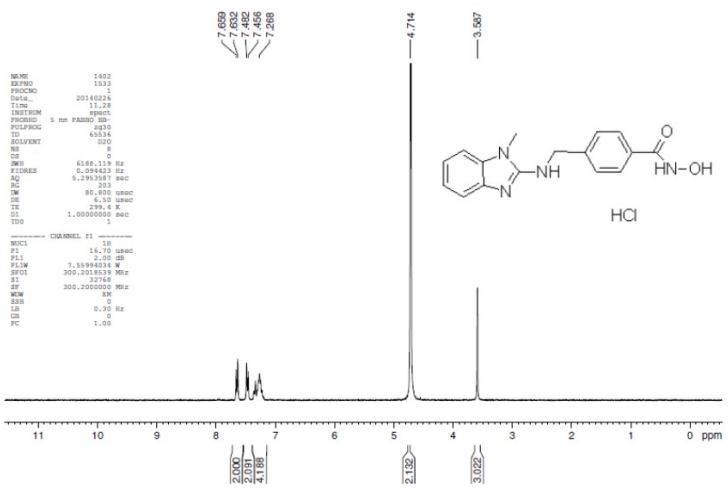


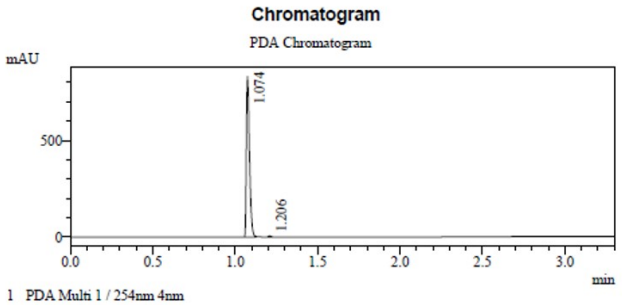
Peak Table

ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.938	321499	100.000	937396	100.000
Total		321499	100.000	937396	100.000

Compound 12



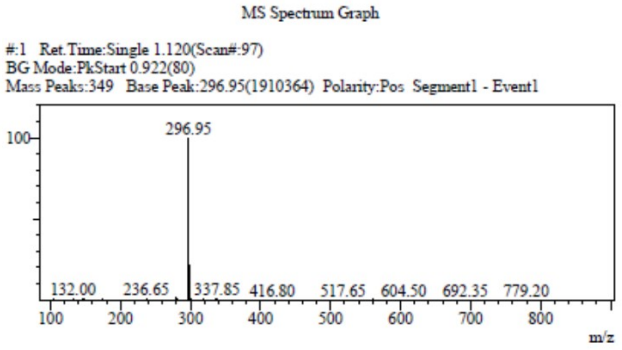


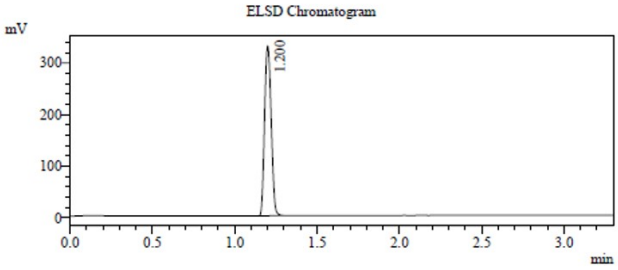
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Height	Height %	Area	Area %
1	1.074	831308	99.211	1087782	99.303
2	1.206	6607	0.789	7637	0.697
Total		837915	100.000	1095420	100.000



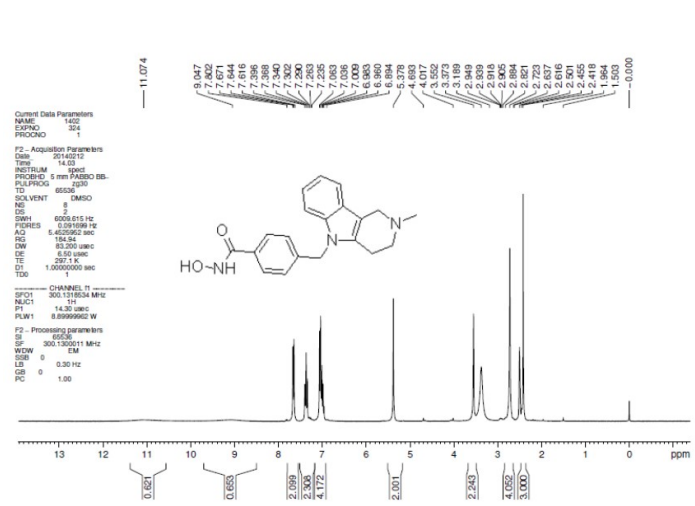


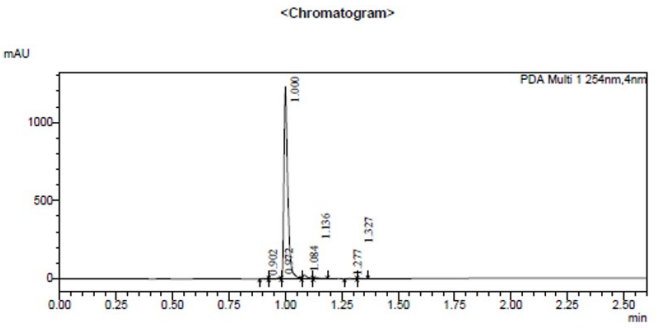
PeakTable

ELSD Ch1

Peak#	Ret. Time	Height	Height %	Area	Area %
1	1.200	326692	100.000	918862	100.000
Total		326692	100.000	918862	100.000

Tubastatin A

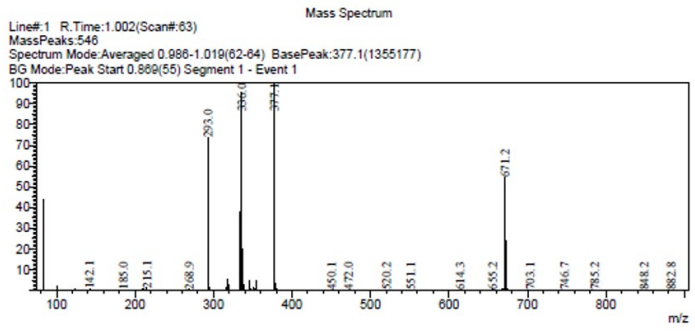


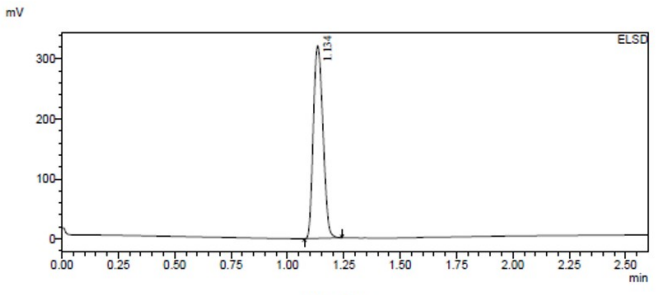


Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.902	3203	0.250	3469	0.234
2	0.972	7831	0.611	9300	0.626
3	1.000	1233450	96.282	1417452	95.488
4	1.084	23598	1.842	33350	2.247
5	1.136	6401	0.500	10365	0.698
6	1.277	2734	0.213	5892	0.397
7	1.327	3861	0.301	4595	0.310
Total		1281077	100.000	1484423	100.000



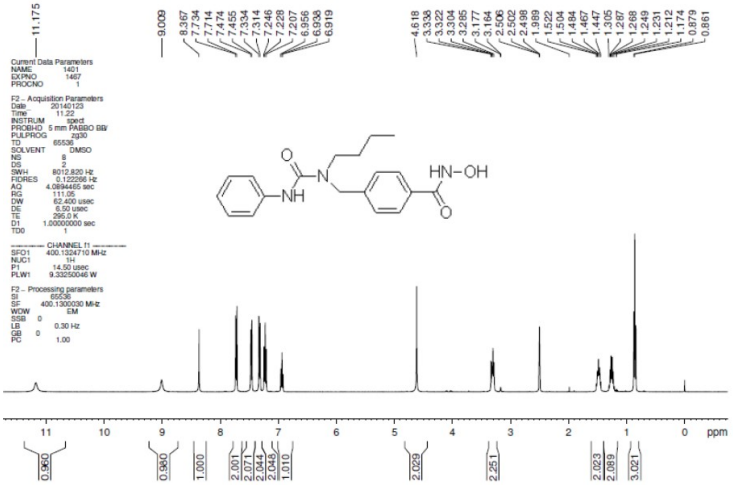


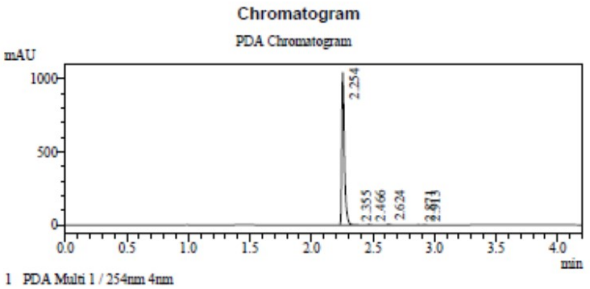
Peak Table

ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.134	313863	100.000	954808	100.000
Total		313863	100.000	954808	100.000

Nexturastat A





1 PDA Multi 1 / 254nm 4nm

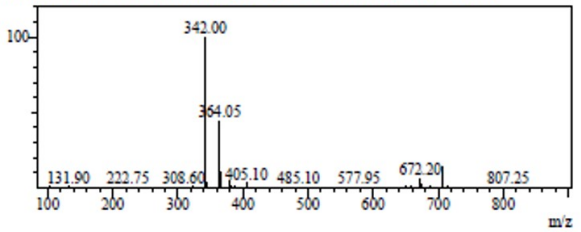
PeakTable

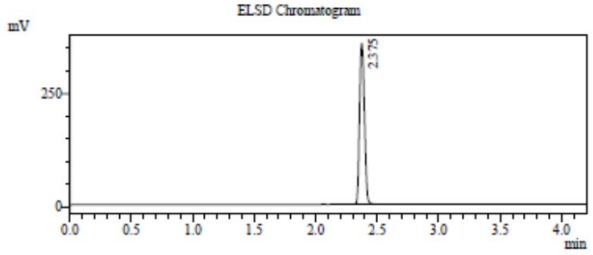
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Height	Height %	Area	Area %
1	2.254	1041926	98.603	1549967	98.630
2	2.355	1311	0.124	2250	0.143
3	2.466	3767	0.356	5128	0.326
4	2.624	5766	0.546	7498	0.477
5	2.871	1006	0.095	2469	0.157
6	2.913	2913	0.276	4180	0.266
Total		1056688	100.000	1571492	100.000

MS Spectrum Graph

#:1 Ret.Time:Single 2.298(Scan#:198)
BG Mode:None
Mass Peaks:478 Base Peak:342.00(964326) Polarity:Pos Segment1 - Event1



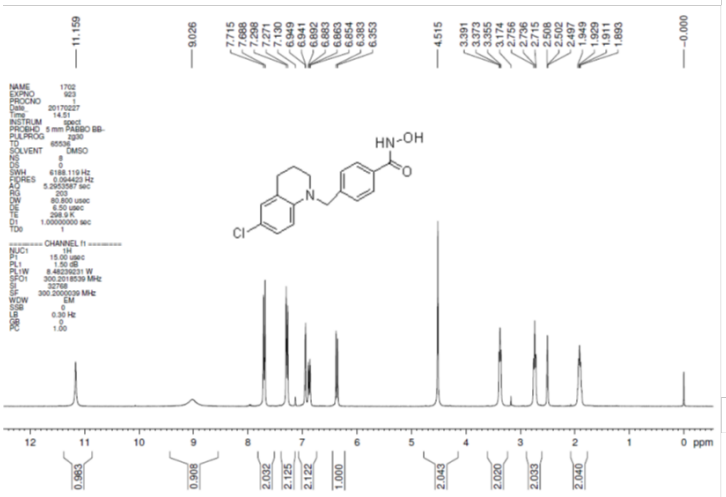


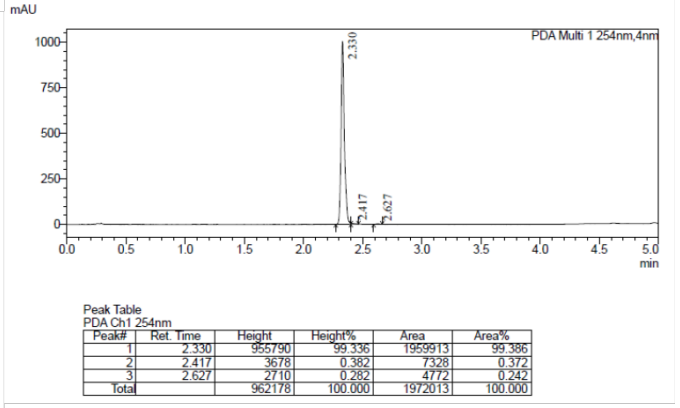
PeakTable

AD2 Ch1

Peak#	Ret. Time	Height	Height %	Area	Area %
1	2.375	351533	100.000	985545	100.000
Total		351533	100.000	985545	100.000

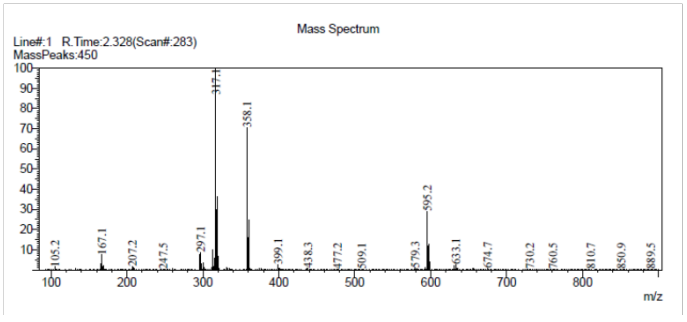
SW-100

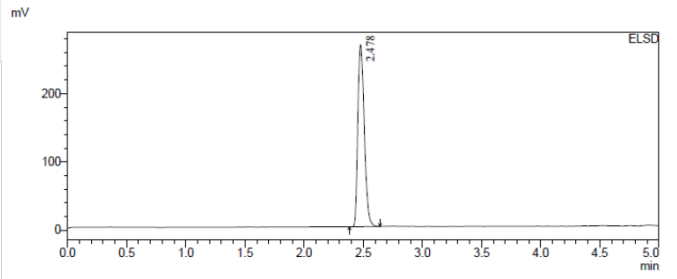




Peak Table
PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	2.330	955790	99.336	1959913	99.386
2	2.417	3678	0.382	7328	0.372
3	2.627	2710	0.282	4772	0.242
Total		962178	100.000	1972013	100.000





Peak Table
ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	2.478	264302	100.000	1014729	100.000
Total		264302	100.000	1014729	100.000