

Rational Design of Suprastat, a Novel Selective Histone Deacetylase 6 Inhibitor with the Ability to Potentiate Immunotherapy in Melanoma Models

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1. Supplementary Figures

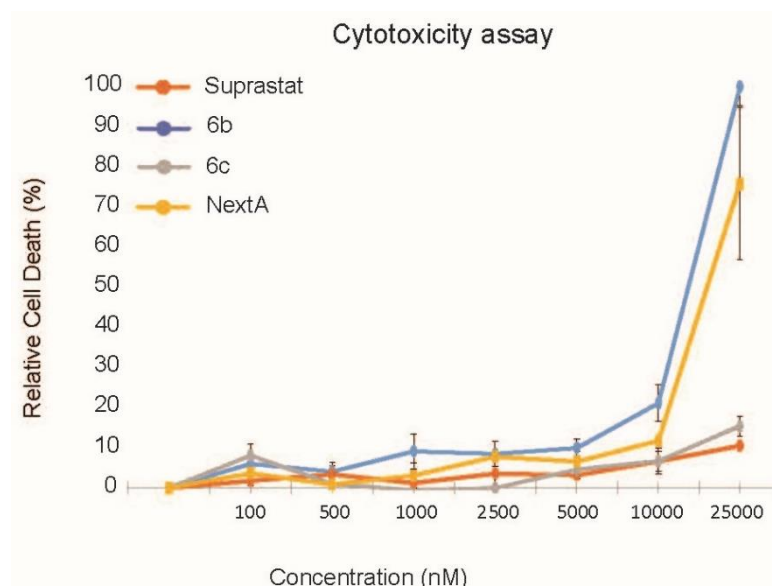


Figure S1. Suprastat induces low cytotoxicity compared to other HDAC6 inhibitors.

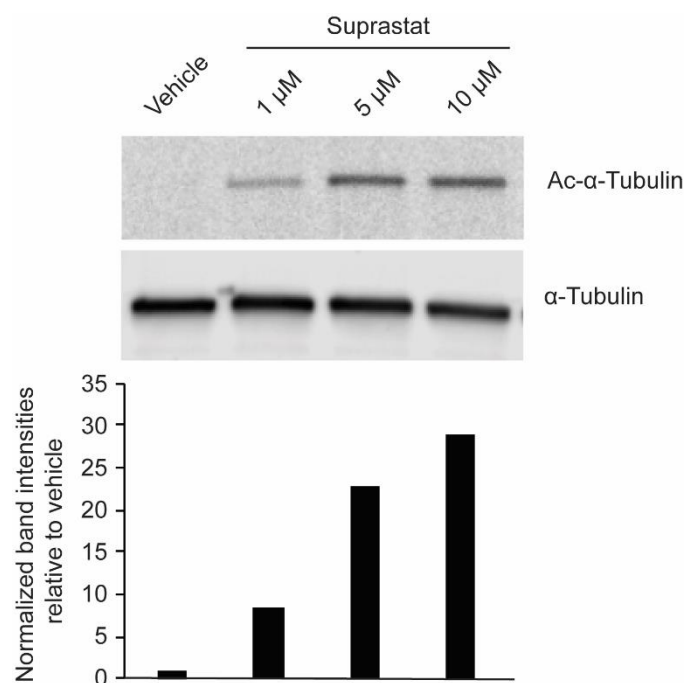


Figure S2. RAW 264.7 macrophages treated with increasing concentration of Suprastat. Band intensities quantified and represented as fold change relative to vehicle indicates a dose-dependent effect of Suprastat on α -tubulin acetylation.

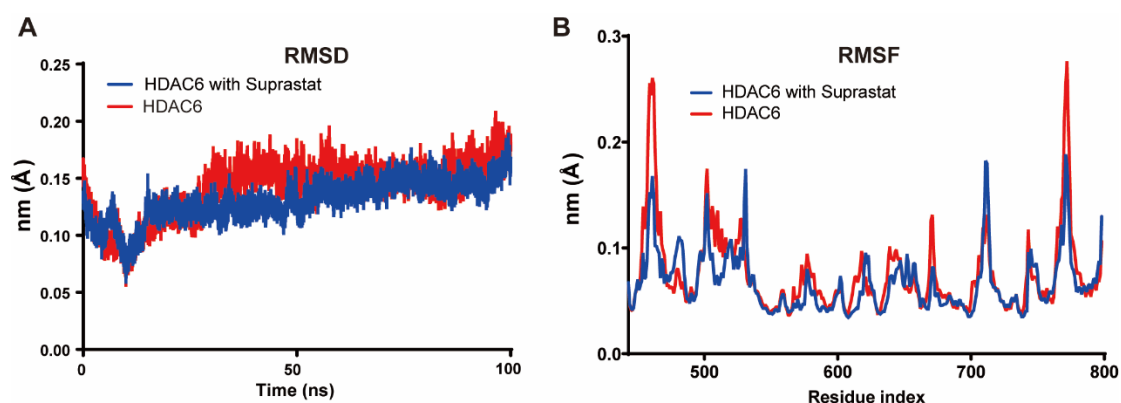


Figure S3. Variation of RMSD (A) and RMSF (B) values for HDAC6 and the HDAC6-Suprastat complex along the MD simulations. Values were calculated for the protein backbone.

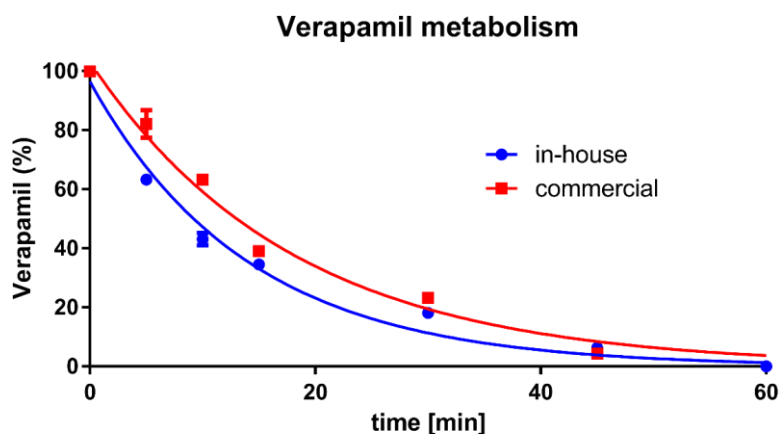


Figure S4. Comparison of commercial and in-house microsomal preparations. Both microsomal preparations were diluted to the final concentration of 0.5 mg/mL (total protein), Verapamil was added to the final concentration of 10 μ M and the reaction started by the addition of NADPH solution. Aliquots were collected at defined time points and samples processed and analyzed by LC-MS. The metabolic activity of our in-house rat liver microsomes (blue) is virtually identical to commercially available preparations (red; rat liver microsomes, RTCPL, Life Technologies, CA, USA).

2. Supplementary Tables

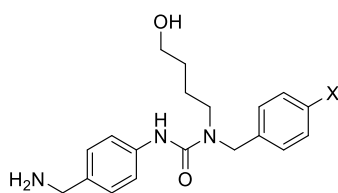


Table S1. Inhibitory potency of NextA and its analogues **6d-f** against HDAC 1 and HDAC6

Compound	X	HDAC6 (IC ₅₀ , nM)	HDAC1 (IC ₅₀ , nM)	HDAC1/6
Suprastat (6a)		0.4 ± 0.0	117 ± 10	293
6d		16,755	N.D. ^g	N.D.
6e		>30,000	N.D.	N.D.
6f		>30,000	N.D.	N.D.
Vorinostat	-	6.7 ± 1.0	31 ± 12	5

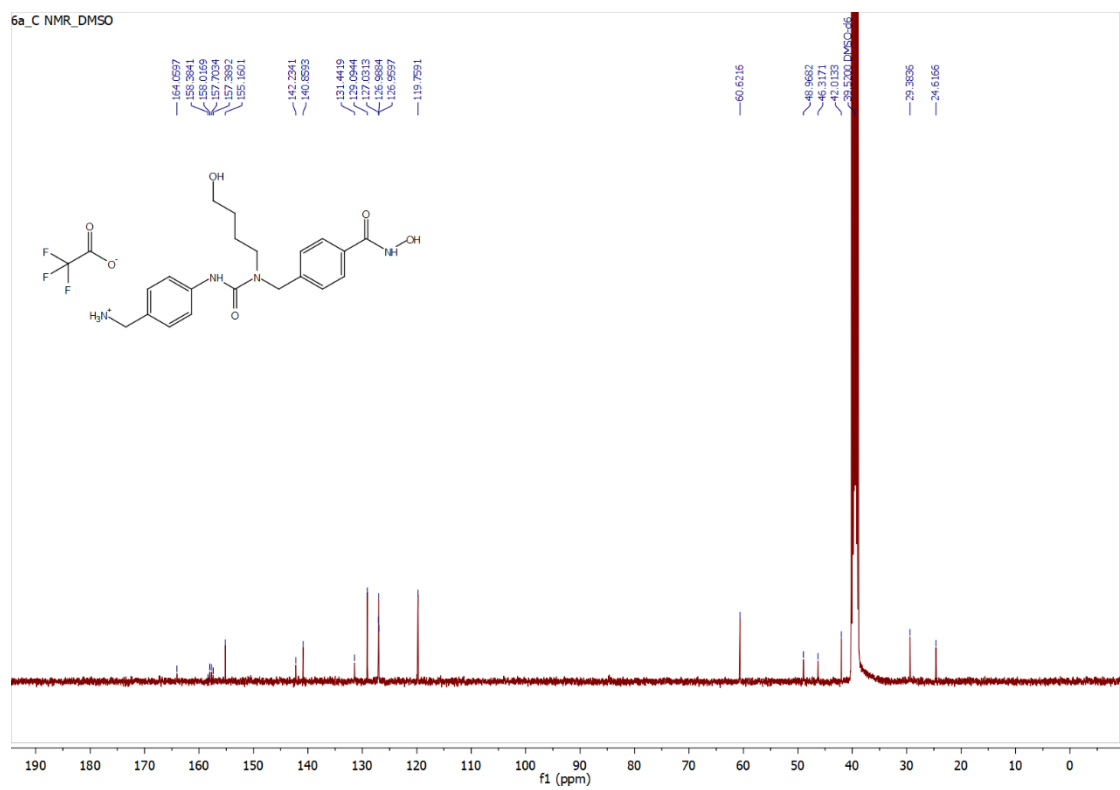
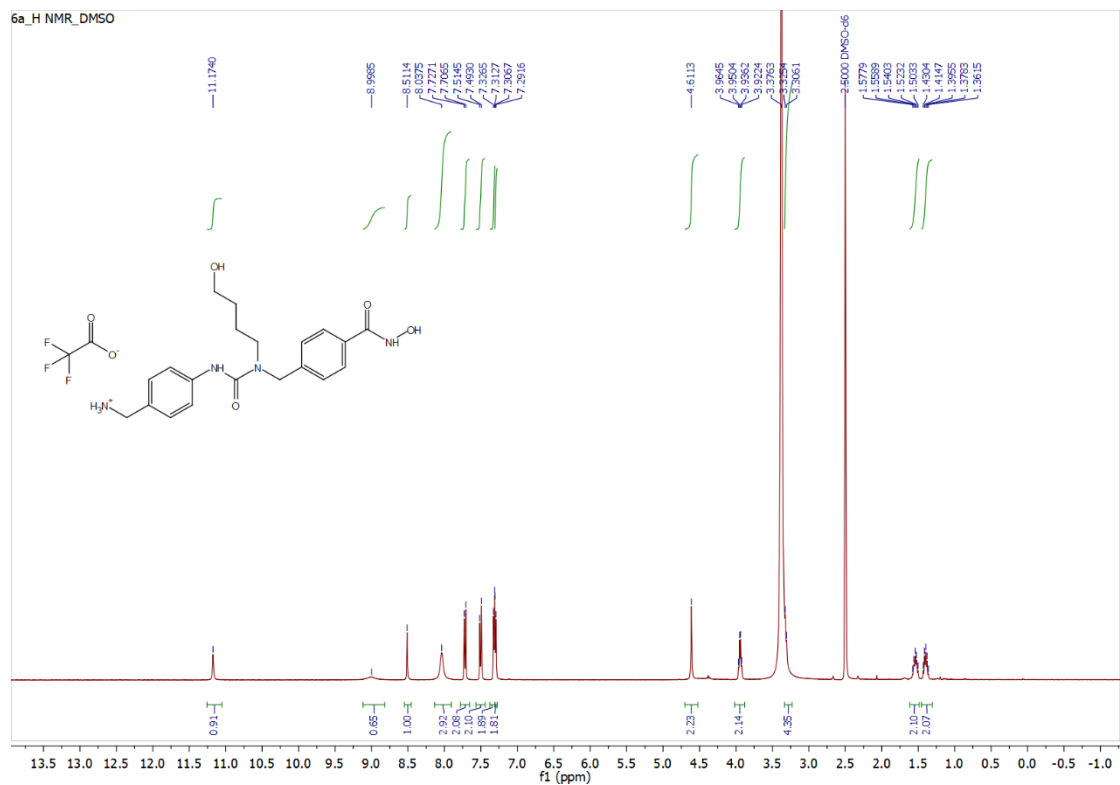
Table S2. Crystal data collection and refinement statistics

X-ray source	BL 14.2, Bessy II
Detector	Pilatus3 2M
Wavelength (Å)	0.9184
Crystal-to-detector distance (mm)	207.4
No. of frames	3600
Exposure per degree (s)	2
Oscillation angle (°)	0.1
Space group	P 1 2 ₁ 1
Unit-cell parameters	A = 54.809 Å, b = 83.811 Å, c = 86.092 Å $\alpha = 90.0^\circ$, $\beta = 98.0^\circ$, $\gamma = 90.000^\circ$
Resolution (Å)	85.3 – 1.6 (1.63 – 1.6)
No. of measured reflections	641645 (32445)
No. of unique reflections	100361 (5038)
Average multiplicity	6.7 (6.4)
Completeness (%)	98.8 (99.6)
Average $I/\sigma(I)$	6.5 (1.3)
$CC_{1/2}$	0.994 (0.612)
R_{merge}	0.16 (1.164)
R_{pim}	0.07 (0.494)
R_{work}	0.191
R_{free}	0.230
R_{all}	0.192
Average ADP (Å ²)	15.0
R.m.s.d, bond lengths (Å)	0.012
R.m.s.d., bond angles (°)	1.7
Solvent content (%)	49.5
Matthews coefficient (Å ³ · Da ⁻¹)	2.433

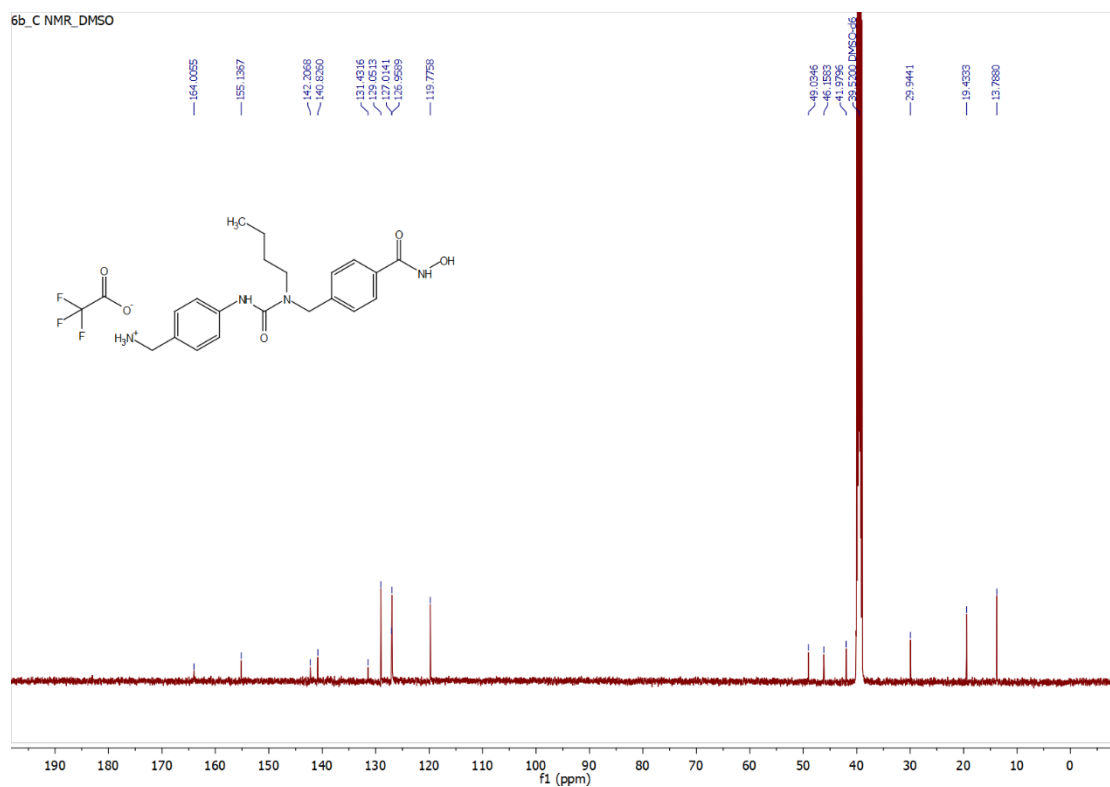
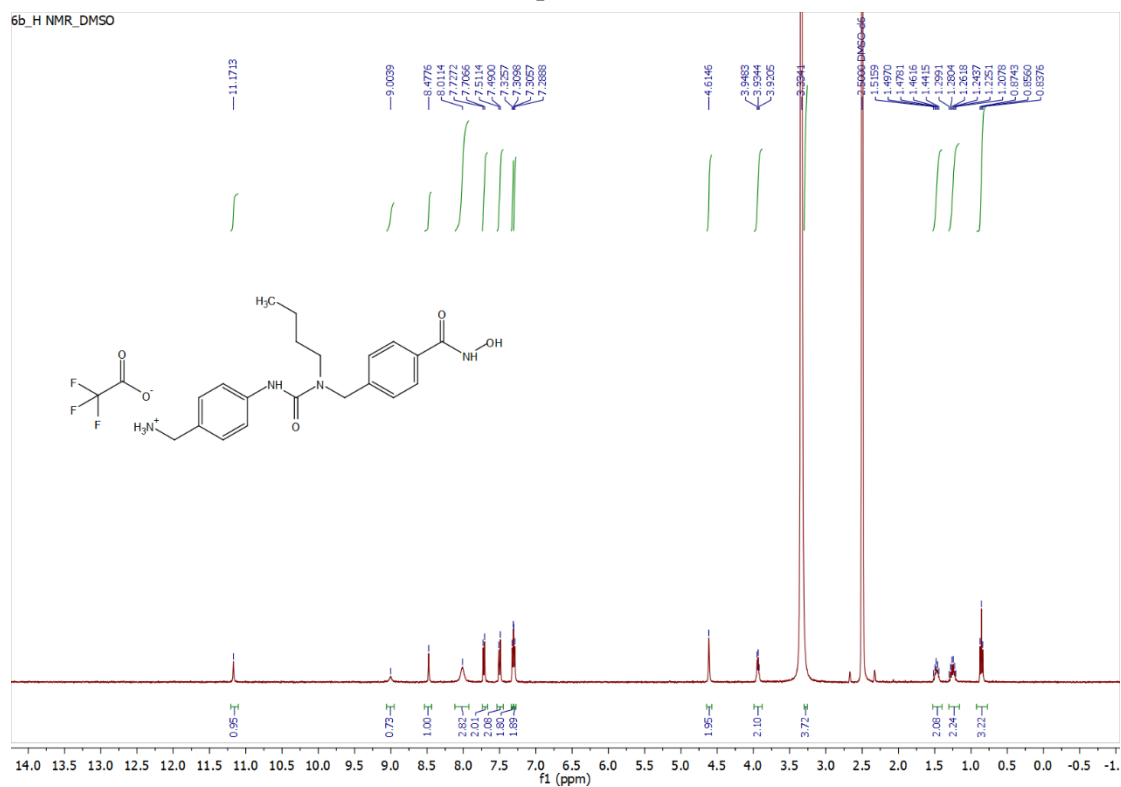
No. of non-H atoms	6560
No. of monomers in asymmetric unit	2
No. of localized amino acids	2×356
No. of water molecules	762
Localized ions	$2 \times \text{Zn}^{2+}$, $4 \times \text{K}^+$, $1 \times \text{Cl}^-$
Ligands	$2 \times \text{Suprastat}$, $7 \times 1,2\text{-ethanediol}$, $2 \times \text{PEG residue}$, $1 \times \text{DMSO}$, $1 \times \text{glycerol}$

3. ^1H NMR and ^{13}C NMR spectra for compounds 6a-c

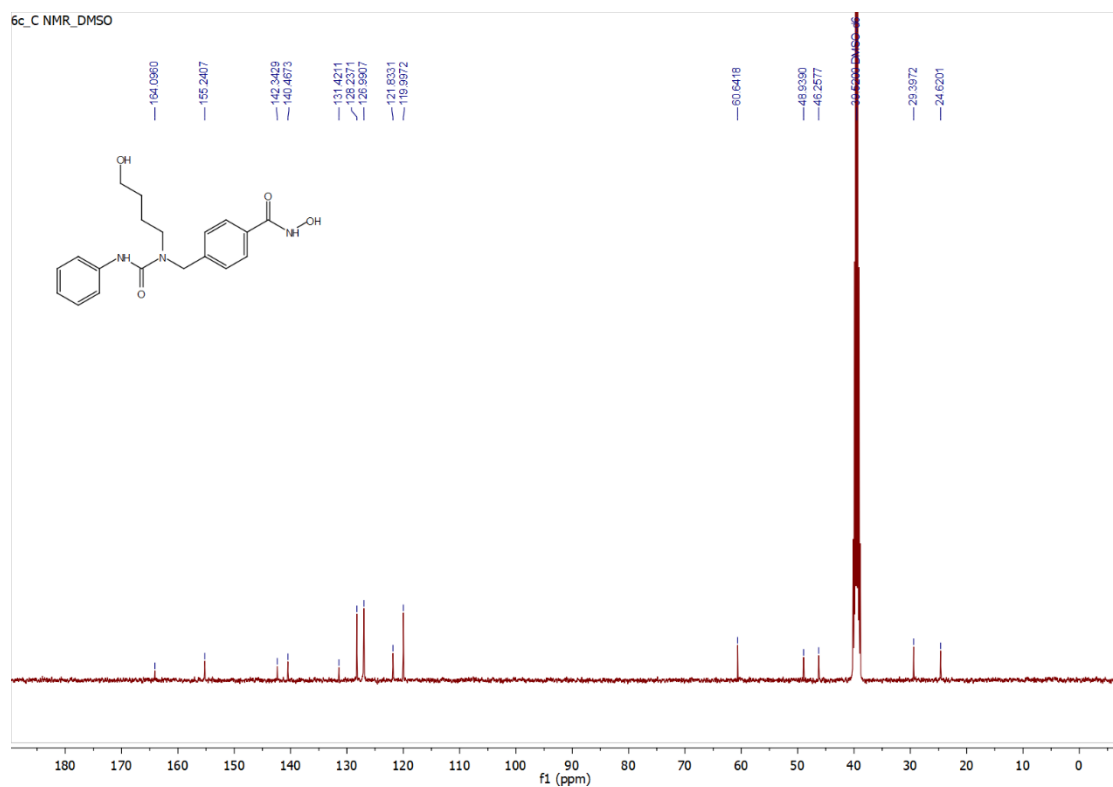
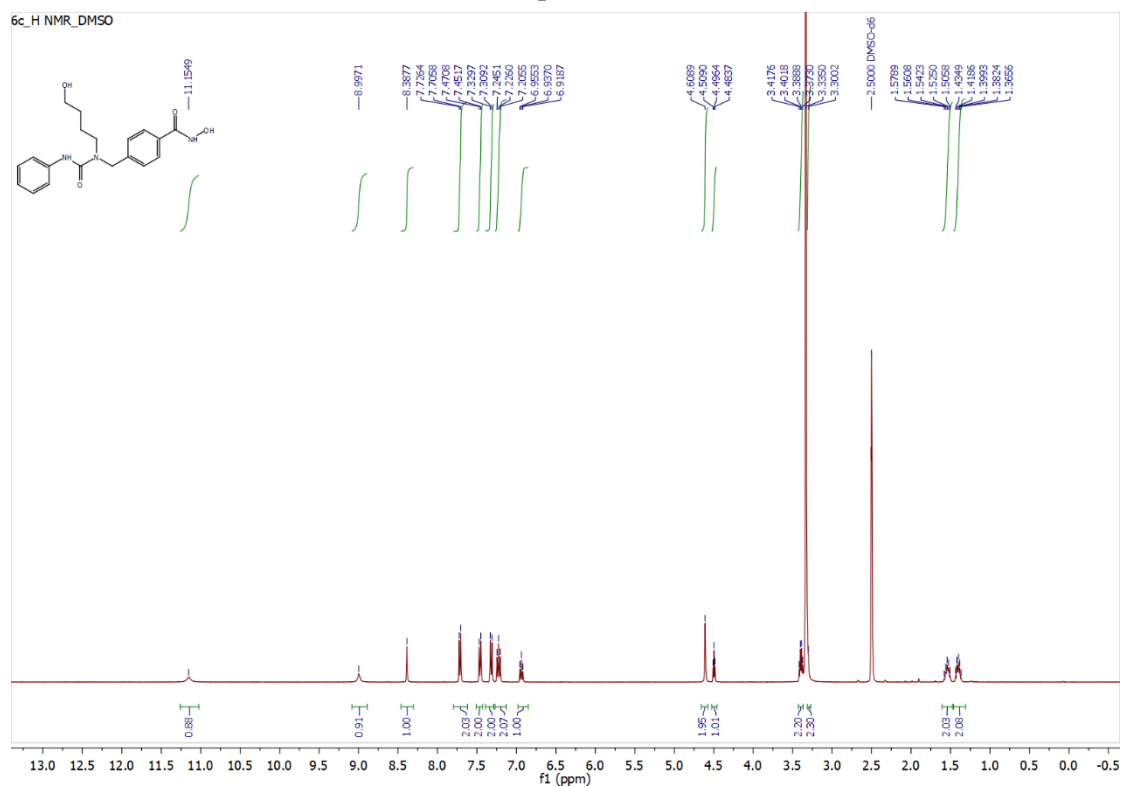
Compound 6a (Suprastat)



Compound 6b



Compound 6c



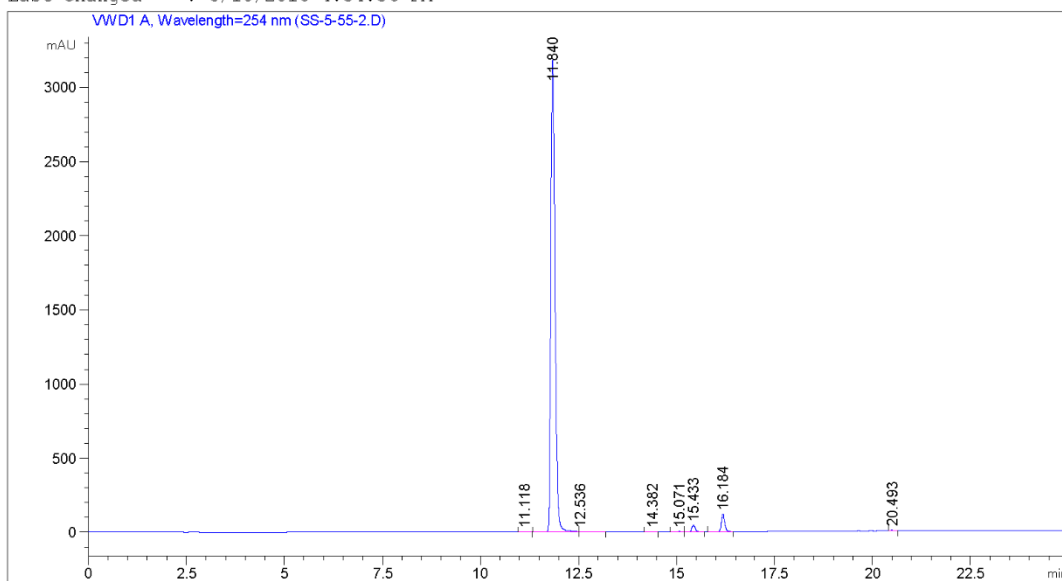
4. HPLC purity reports for compounds 6a-c

Compound 6a (Suprastat)

Data File C:\CHEM32\1\DATA\DEF_LC 2016-08-30 15-00-19\SS-5-55-2.D

Sample Name: SS-5-55

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Acq. Operator   :                               Seq. Line :    5
Acq. Instrument : Instrument 1                   Location  : Vial 73
Injection Date  : 8/30/2016 4:57:35 PM           Inj       :    1
                                           Inj Volume : 5.000 µl
Sequence File   : C:\Chem32\1\DATA\DEF_LC 2016-08-30 15-00-19\DEF_LC.S
Method          : C:\CHEM32\1\DATA\DEF_LC 2016-08-30 15-00-19\METHOD 3_254.M (Sequence
                  Method)
Last changed    : 8/10/2016 4:34:36 PM
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Area Percent Report

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Sorted By      :      Signal
Multiplier:    :      1.0000
Dilution:      :      1.0000
Use Multiplier & Dilution Factor with ISTDs
=====
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Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.118	BB	0.1092	29.67868	4.15943	0.1183
2	11.840	BV	0.1188	2.39479e4	3182.66357	95.4289
3	12.536	VV	0.2583	82.24677	3.99242	0.3277
4	14.382	VB	0.0913	18.88880	3.18013	0.0753
5	15.071	BV	0.0975	21.03066	3.25171	0.0838
6	15.433	VB	0.0948	275.78290	44.17424	1.0990
7	16.184	BV	0.0895	687.93182	117.16744	2.7413
8	20.493	VB	0.0777	31.54695	6.26656	0.1257

Data File C:\CHEM32\1\DATA\DEF_LC 2016-08-30 15-00-19\SS-5-55-2.D
Sample Name: SS-5-55

Totals : 2.50950e4 3364.85551

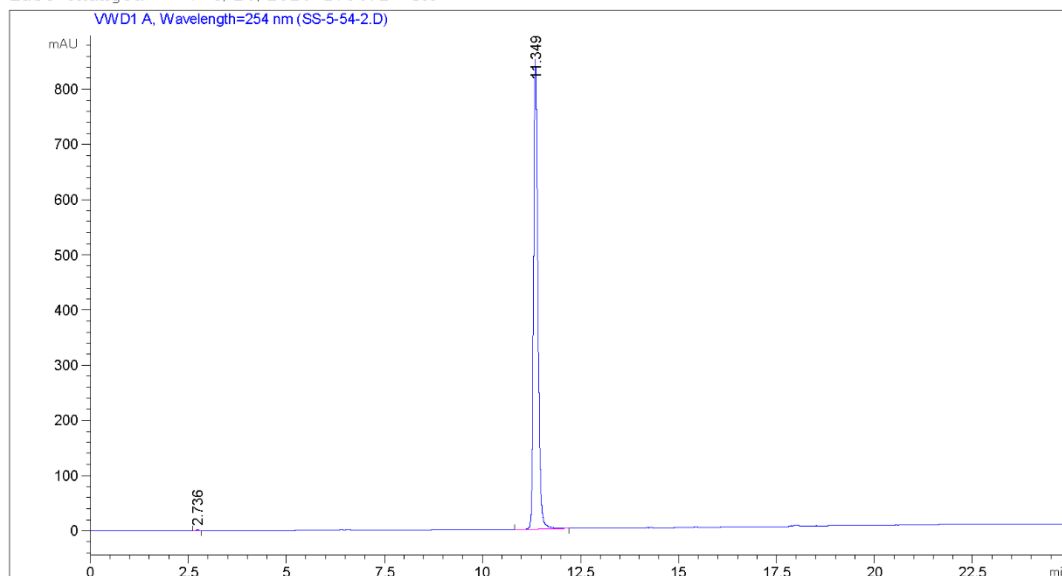
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*** End of Report ***

Compound 6b

Data File C:\CHEM32\1\DATA\DEF_LC 2016-08-30 15-00-19\SS-5-54-2.D

Sample Name: SS-5-54

```
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Acq. Operator   :                               Seq. Line :    4
Acq. Instrument : Instrument 1                   Location  : Vial 72
Injection Date  : 8/30/2016 4:28:49 PM           Inj       :    1
                                                Inj Volume: 5.000 µl
Different Inj Volume from Sequence !      Actual Inj Volume: 10.000 µl
Sequence File   : C:\Chem32\1\DATA\DEF_LC 2016-08-30 15-00-19\DEF_LC.S
Method          : C:\CHEM32\1\DATA\DEF_LC 2016-08-30 15-00-19\METHOD 2_254.M (Sequence
                  Method)
Last changed    : 4/26/2016 1:06:17 PM
=====
```



Area Percent Report

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=====
Sorted By      :      Signal
Multiplier:    :      1.0000
Dilution:      :      1.0000
Use Multiplier & Dilution Factor with ISTDs
=====
```

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.736	BB	0.0768	10.45858	1.97587	0.1582
2	11.349	BB	0.1184	6601.07422	851.90906	99.8418

Totals : 6611.53280 853.88493

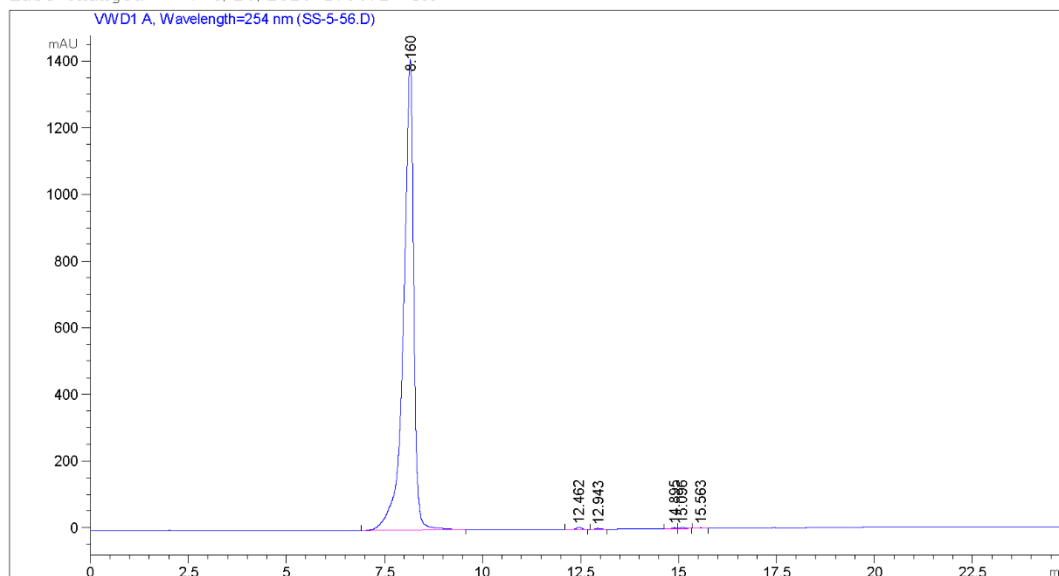
*** End of Report ***

Compound 6c

Data File C:\CHEM32\1\DATA\DEF_LC 2016-08-29 15-08-34\SS-5-56.D

Sample Name: SS-5-56

```
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Acq. Operator   :                               Seq. Line :    2
Acq. Instrument : Instrument 1                   Location  : Vial 61
Injection Date  : 8/29/2016 3:22:15 PM           Inj       :    1
                                                Inj Volume: 5.000 µl
Different Inj Volume from Sequence !      Actual Inj Volume: 10.000 µl
Sequence File   : C:\Chem32\1\DATA\DEF_LC 2016-08-29 15-08-34\DEF_LC.S
Method          : C:\CHEM32\1\DATA\DEF_LC 2016-08-29 15-08-34\METHOD 2_254.M (Sequence
                  Method)
Last changed    : 4/26/2016 1:06:17 PM
=====
```



Area Percent Report

```
=====
Sorted By      :      Signal
Multiplier:    :      1.0000
Dilution:      :      1.0000
Use Multiplier & Dilution Factor with ISTDs
=====
```

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.160	BB	0.2545	2.54958e4	1412.95740	99.5091
2	12.462	BB	0.1199	45.00331	5.71247	0.1756
3	12.943	BB	0.1211	14.73167	1.82691	0.0575
4	14.895	EV	0.0918	18.45063	3.08268	0.0720
5	15.096	VB	0.1218	30.43163	3.74408	0.1188
6	15.563	BB	0.0964	17.15232	2.72644	0.0669

Totals : 2.56215e4 1430.04998

Instrument 1 8/29/2016 3:47:18 PM

Page 1 of 2

Data File C:\CHEM32\1\DATA\DEF_LC 2016-08-29 15-08-34\SS-5-56.D
Sample Name: SS-5-56

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*** End of Report ***