Supplementary Information

Contents

Figure S1. IC_{50} values (μg/mL) of celastrol and 1 analogues on BGC823.

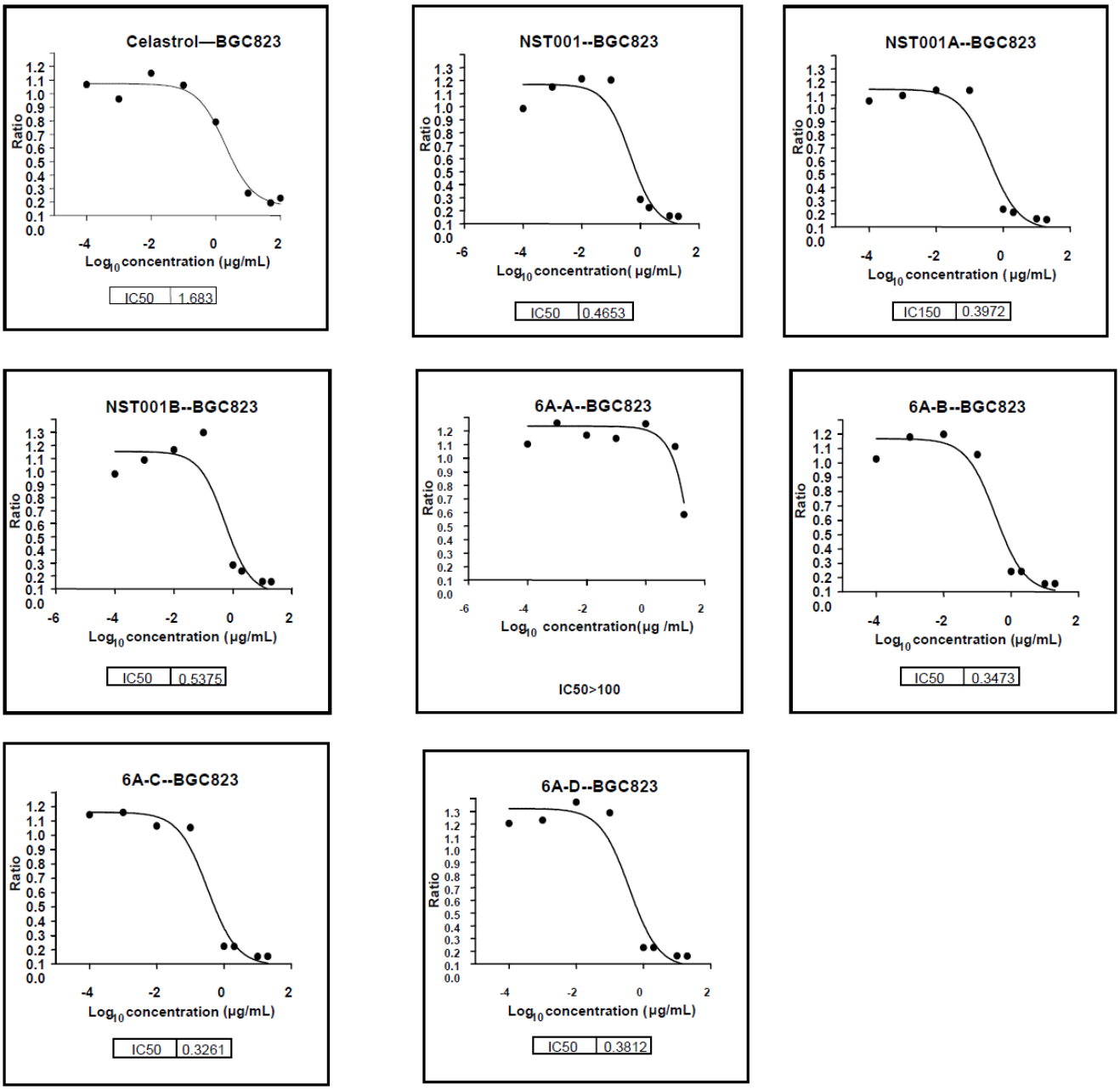


Figure S2. IC_{50} values (μg/mL) of celastrol and 1 analogues on H4.

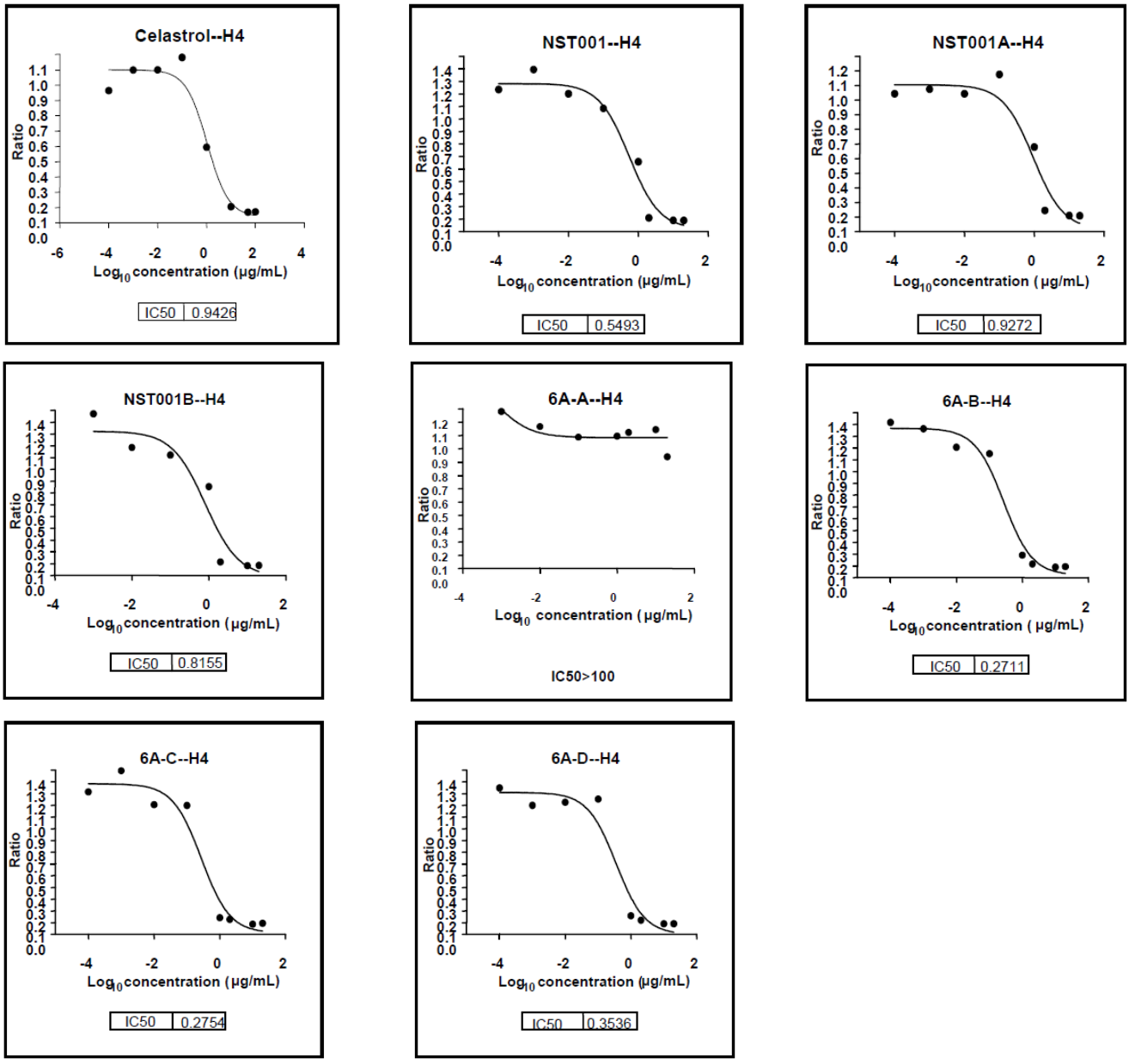


Figure S3. IC_{50} values (μg/mL) of celastrol and 1 analogues on Bel7402.

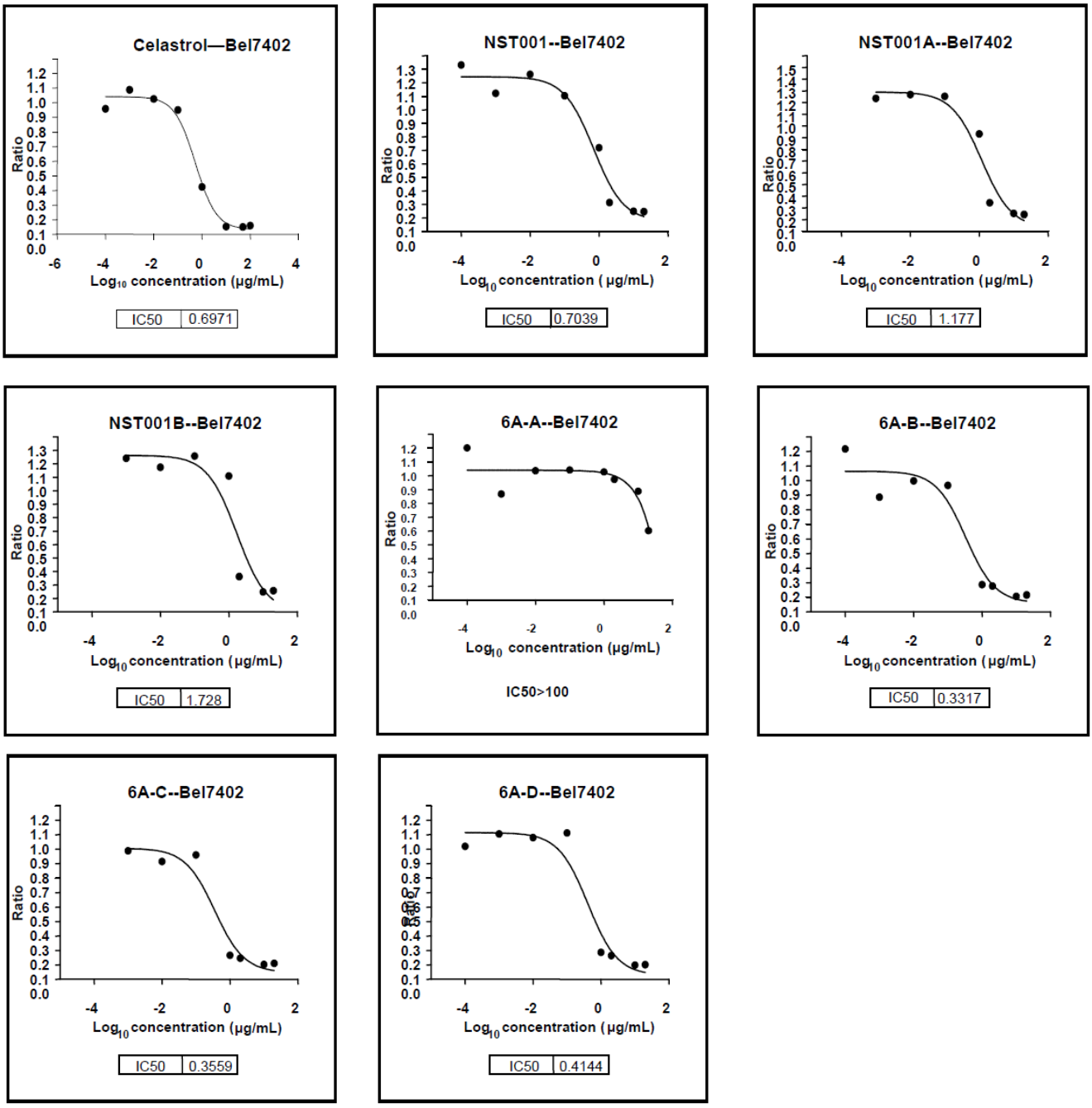


Figure S4. IC_{50} values (μg/mL) of NST001A on human cancer cell lines.

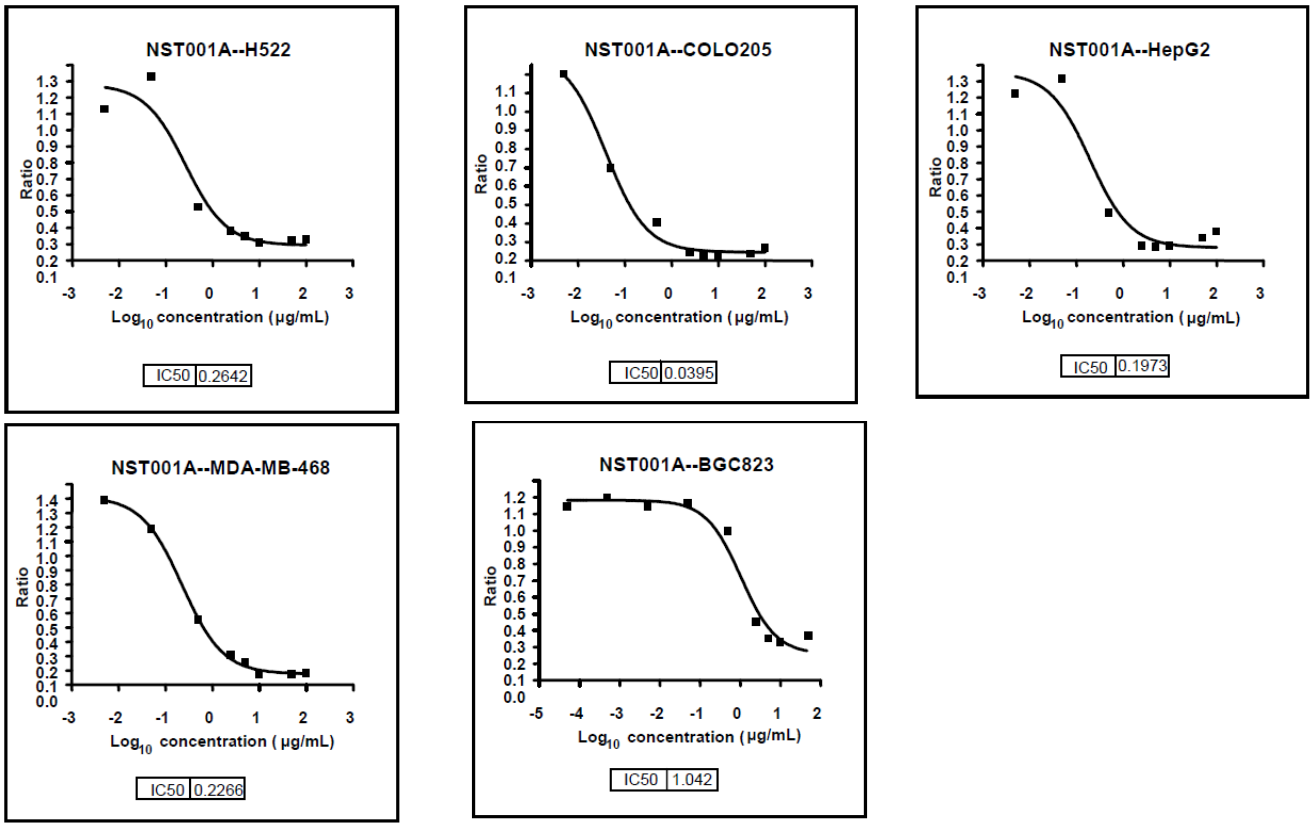
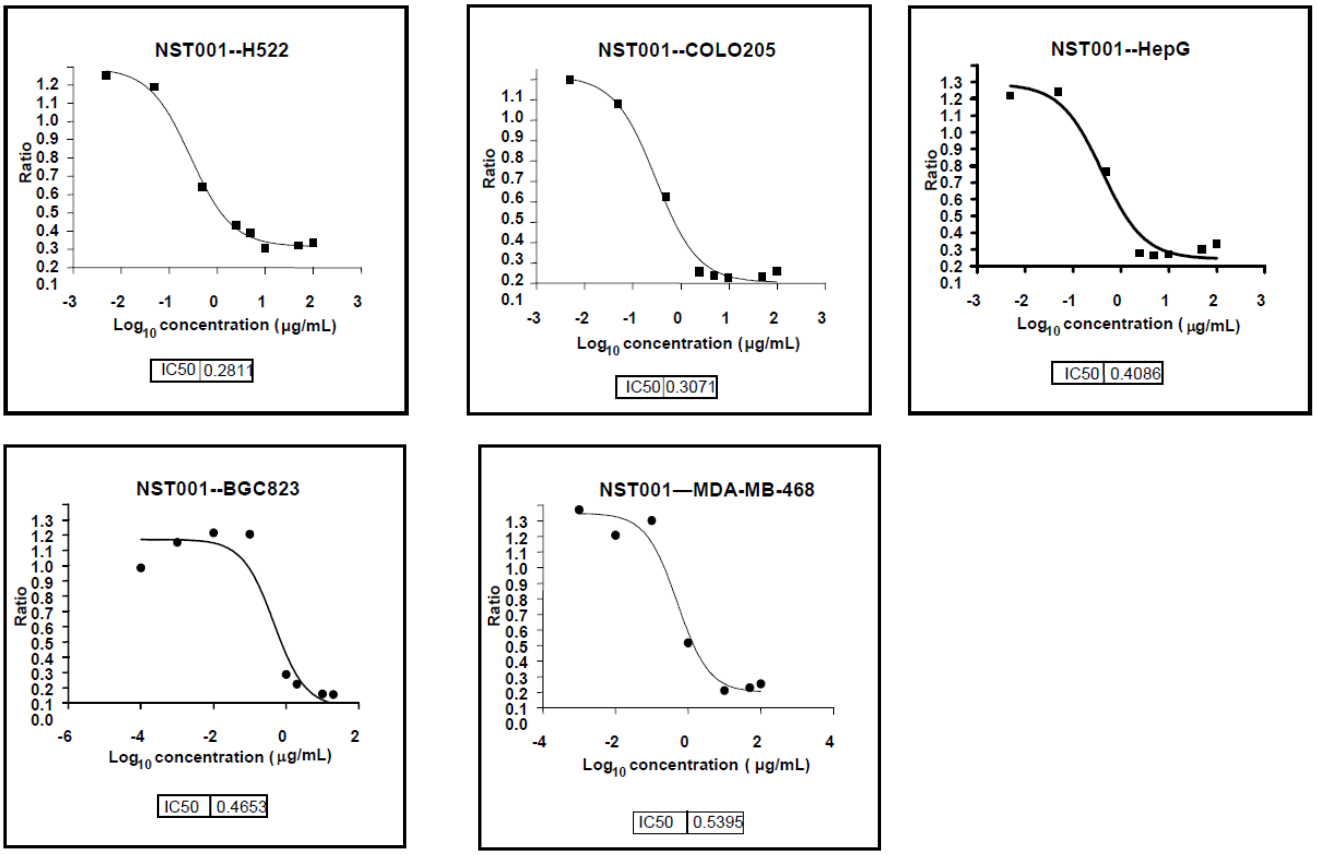


Figure S5. IC_{50} values (μg/mL) of NST001 on human cancer cell lines.



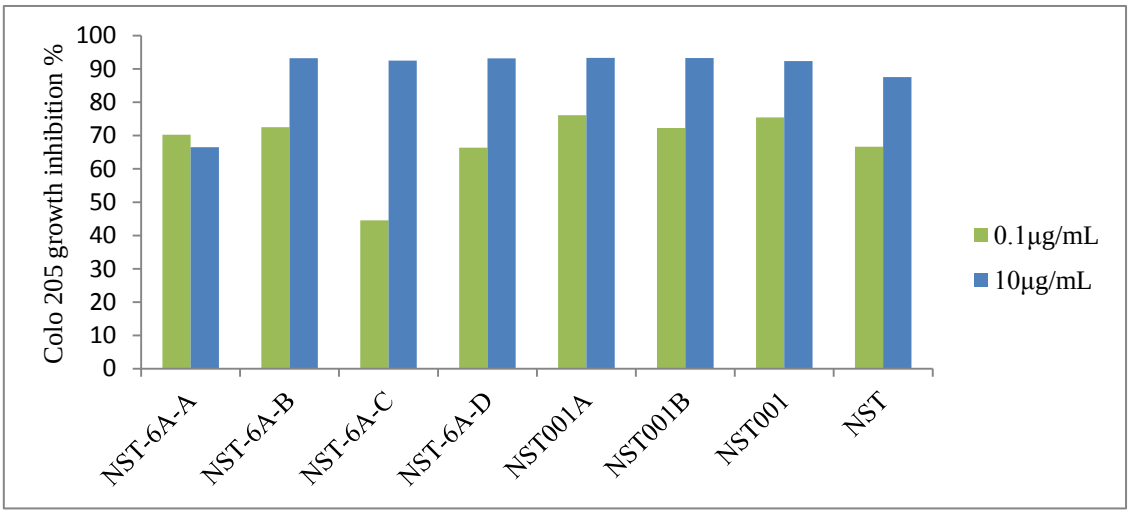
The IC_{50} values (μM) in the article was calculated according to the following formula: IC_{50} (μM) = IC_{50} (μg/mL)/Mw × 1000, the molecular weight (Mw) was list in the table.

Table S1. The Mw of the Compounds (g/mol).

NST	NST001	NST001B	NST001A	NST-6A-B	NST-6A-C	NST-6A-D
450.61	603.67	726.7	678.83	733.87	778.09	764.06

Figure S6. The inhibitory rate (at concentrations of 0.1, 10 μg/mL for 72 h) on Colo 205 growth. The results are expressed as the percentage of that of the treat cells.

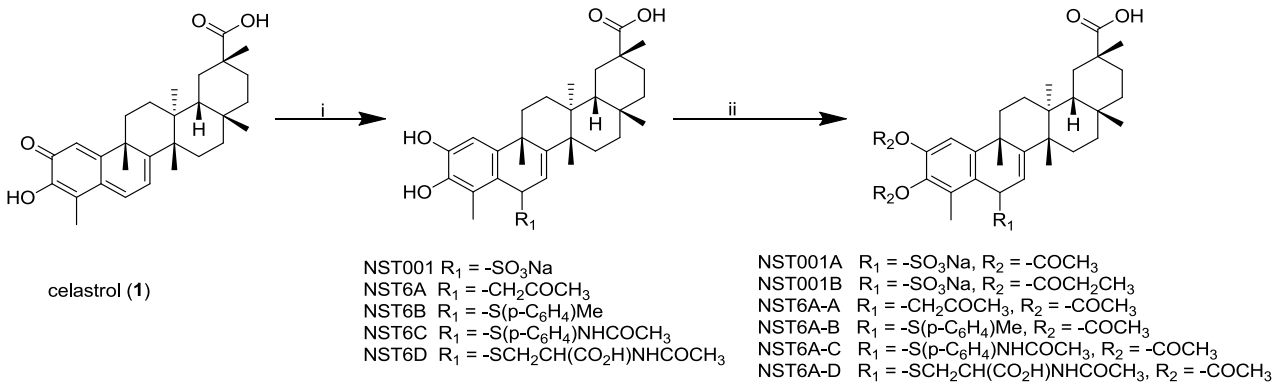
	NST-6A-A	NST-6A-B	NST-6A-C	NST-6A-D	NST001A	NST001B	NST001	NST
10 μg/mL	66.51%	93.24%	92.50%	93.16%	93.32%	93.27%	92.34%	87.54%
0.1 μg/mL	70.26%	72.52%	44.55%	66.35%	76.12%	72.29%	75.45%	66.65%



As **NST001** and **NST001A** showed most potent inhibition at both concentrations, these two compounds were selected to identify precise IC_{50} values on human non-small cell lung cell line H522, human colon cancer cell line Colo 205, human hepatocellular liver carcinoma cell line HepG2, human breast adenocarcinoma cell line MDA-MB-468 and human gastric cancer cell line BGC823. As shown in Figure S4, **NST001A** was more sensitive to all human cancer cells, especially Colo 205 cell. Therefore, **NST001A** was selected to conduct further *in vivo* assays.

A possible reaction mechanism for the synthesis of the intermediate compounds NST001 and NST6A~D was outlined in Scheme S1.

Scheme S1. Synthetic scheme of the designed compounds.



Reagents and conditions: (i) For **NST001**: $NaHSO_3$, MeOH, RT, 1.5 h, N_2; For **NST6A**: acetone, 1 N HCl (cat.), RT, N_2; For **NST6A~D**: RSH, MeOH, RT, N_2; (ii) Ac_2O, Py, RT, N_2; or $(CH_3CH_2CO)_2O$, Py, RT, N_2.

Possible mechanism of the synthesis of NST001and NST6A~D:

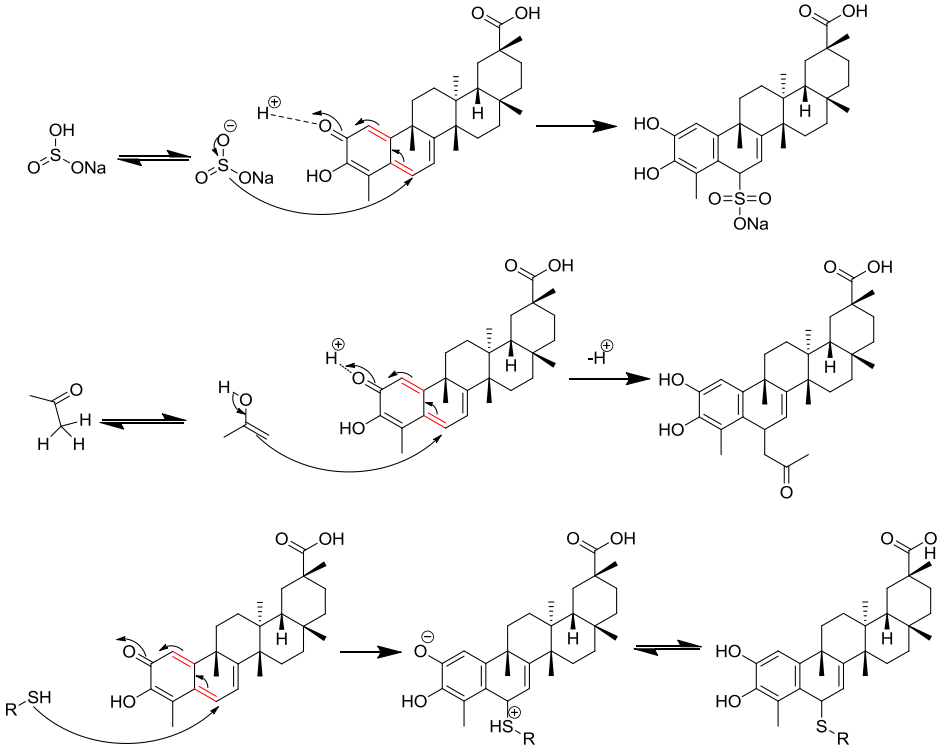
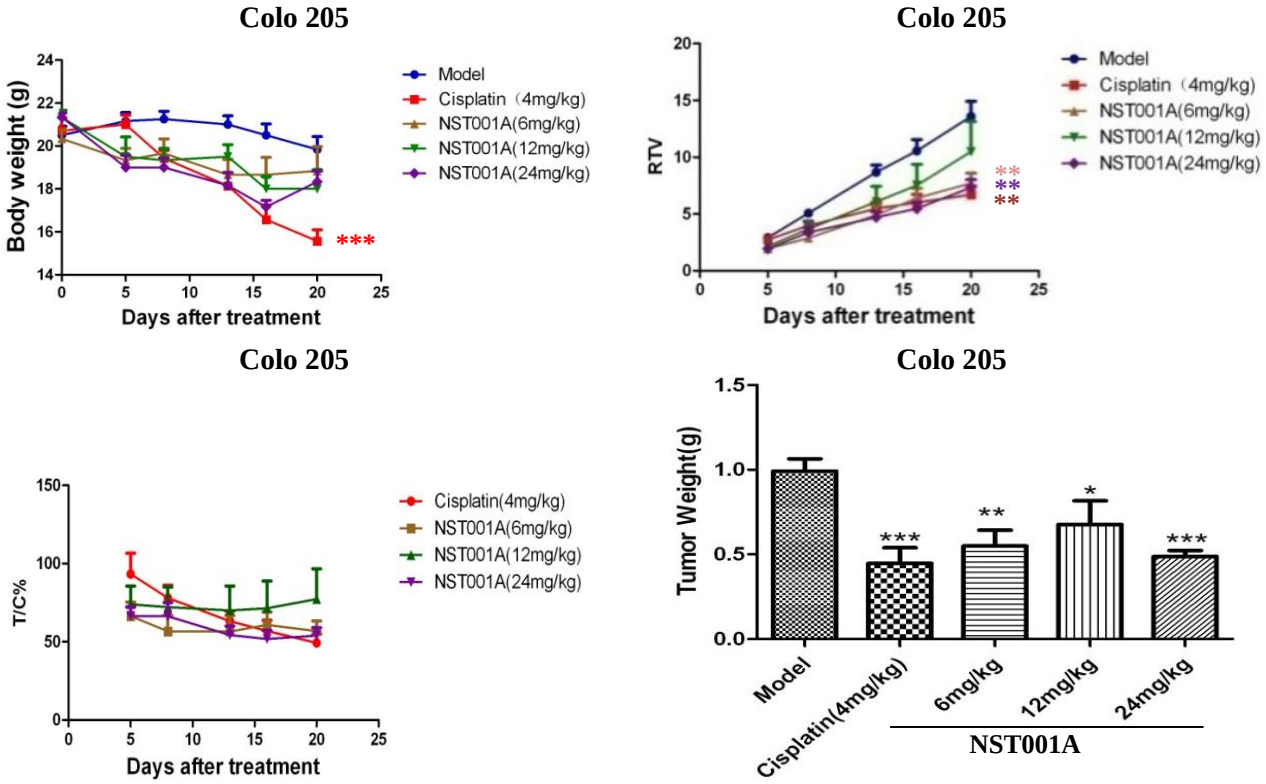


Table S2. The change of the mice body weight and the inhibitory rate of tumor(T/C) of different drug loaded system against Colo 205 tumor in nude mice (mean ± SD).

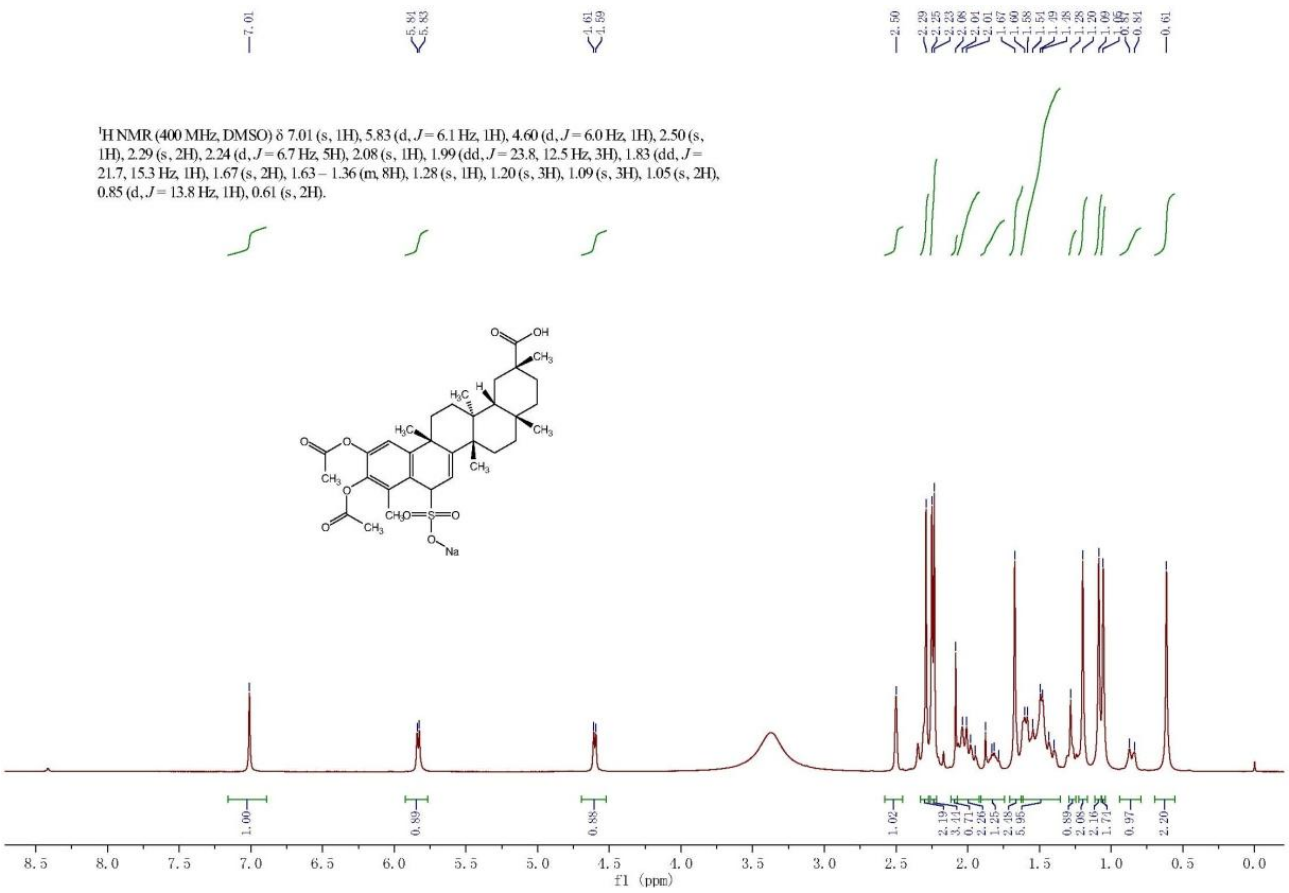
Group	N.O. of Mice		Body Weight (g)		Tumer Volume (TV, mm^3)		T/C/%	Tumer Weight (g)	IRT/%
	Initial	Final	Initial	Final	Initial	Final			
model	12	12	20.50 ± 1.45	19.83 ± 2.08	118.13 ± 36.46	1528.59 ± 415.00	--	0.99 ± 0.25	--
Cisplatin (4mg/kg)	7	7	20.71 ± 1.50	15.57 ± 1.40 ***	122.11 ± 47.85	793.58 ± 379.56 **	49.32	0.45 ± 0.25 ***	55.00
NST001A (6mg/kg)	6	6	20.33 ± 1.03	18.83 ± 2.79	119.97 ± 27.66	910.83 ± 301.60 **	56.75	0.55 ± 0.23 **	44.66
NST001A (12mg/kg)	6	6	21.33 ± 0.82	18.00 ± 2.19	114.01 ± 26.15	1104.71 ± 494.57	77.25	0.68 ± 0.35 *	31.82
NST001A (24mg/kg)	6	6	21.33 ± 0.52	18.33 ± 1.21	118.05 ± 39.37	818.65 ± 162.53 **	54.00	0.49 ± 0.09 ***	50.96

All date are means ± SD. --: Not detect, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared with model.

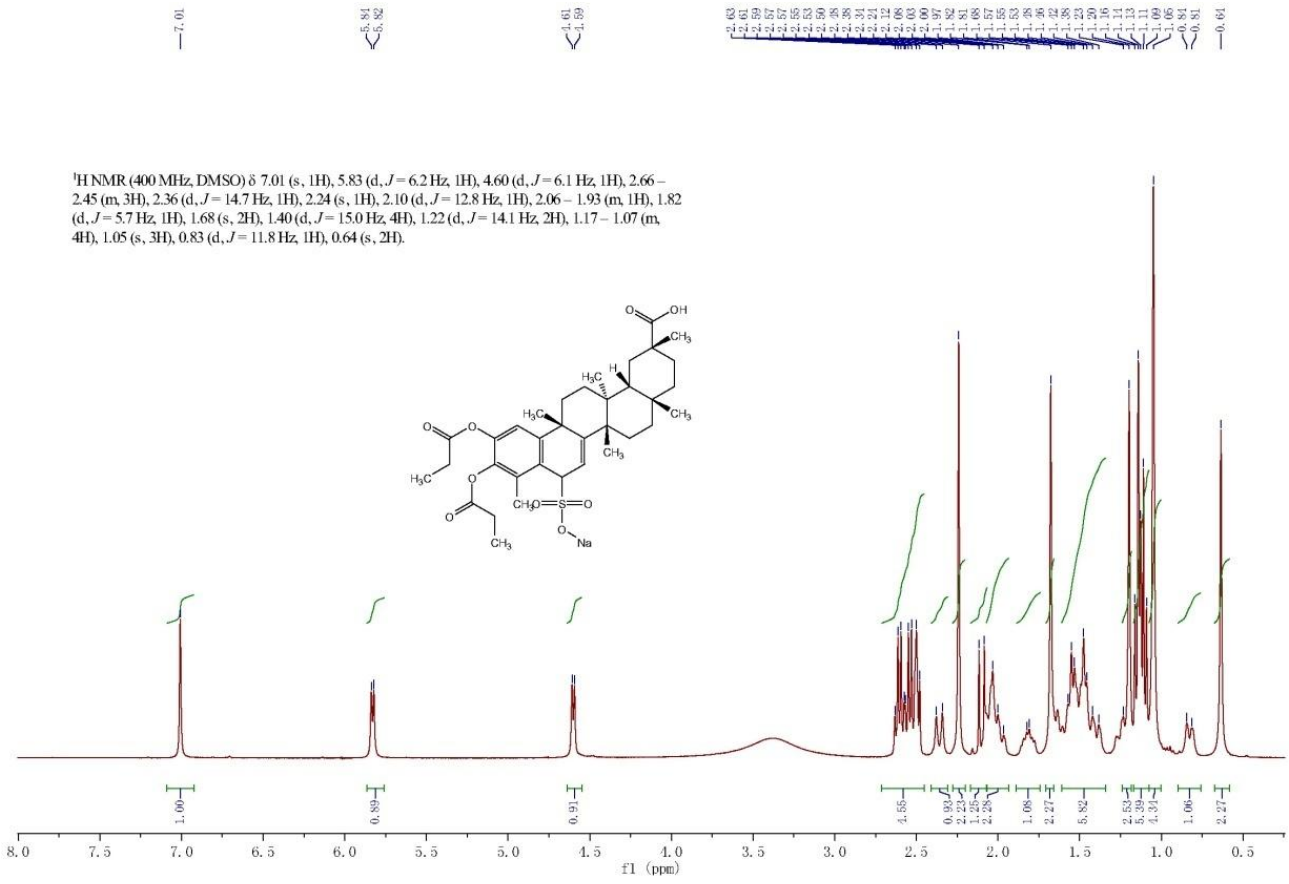
Figure S7. Effects of NST001A on the growth of Colo 205 xenografts in nude mice.



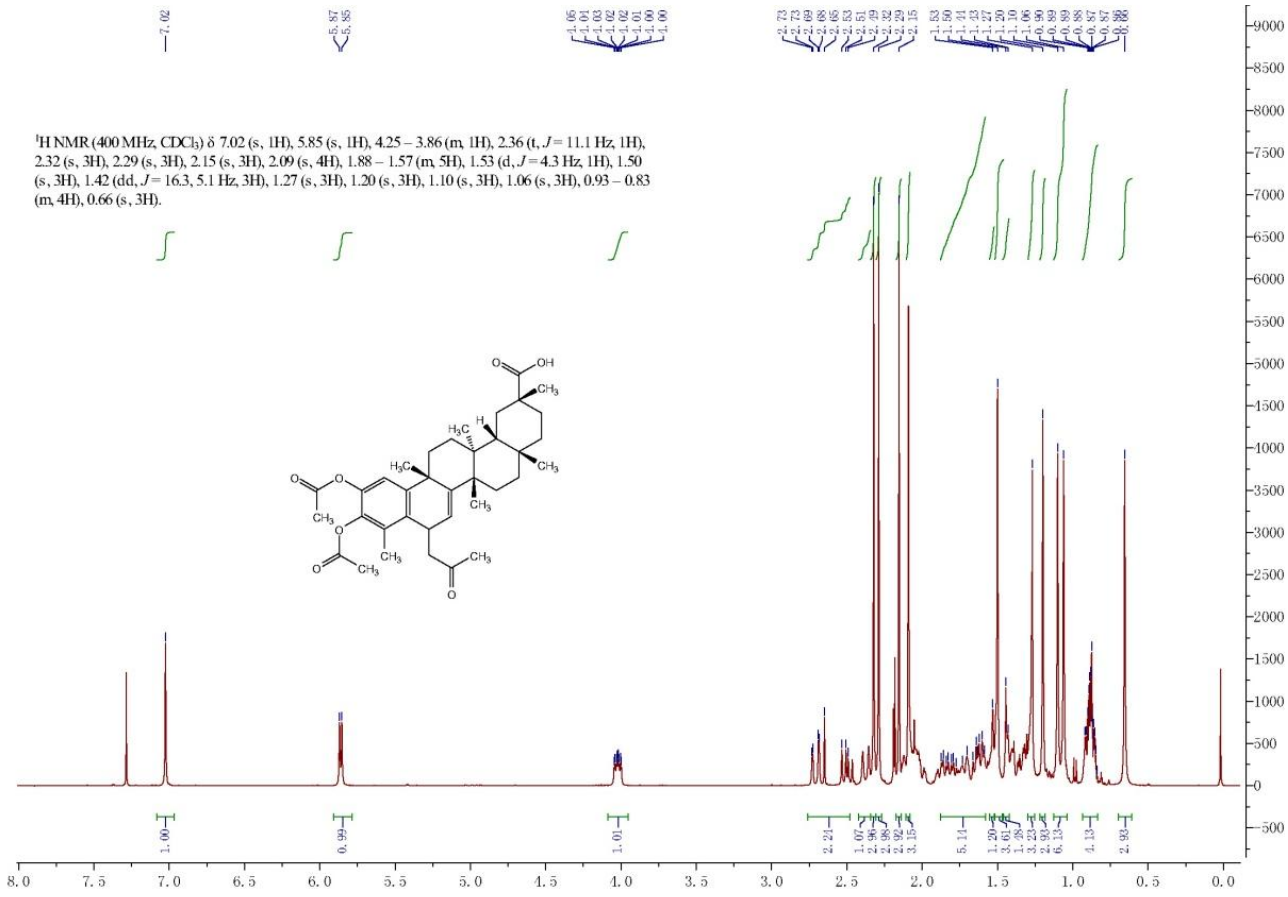
All date are means ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared with model.



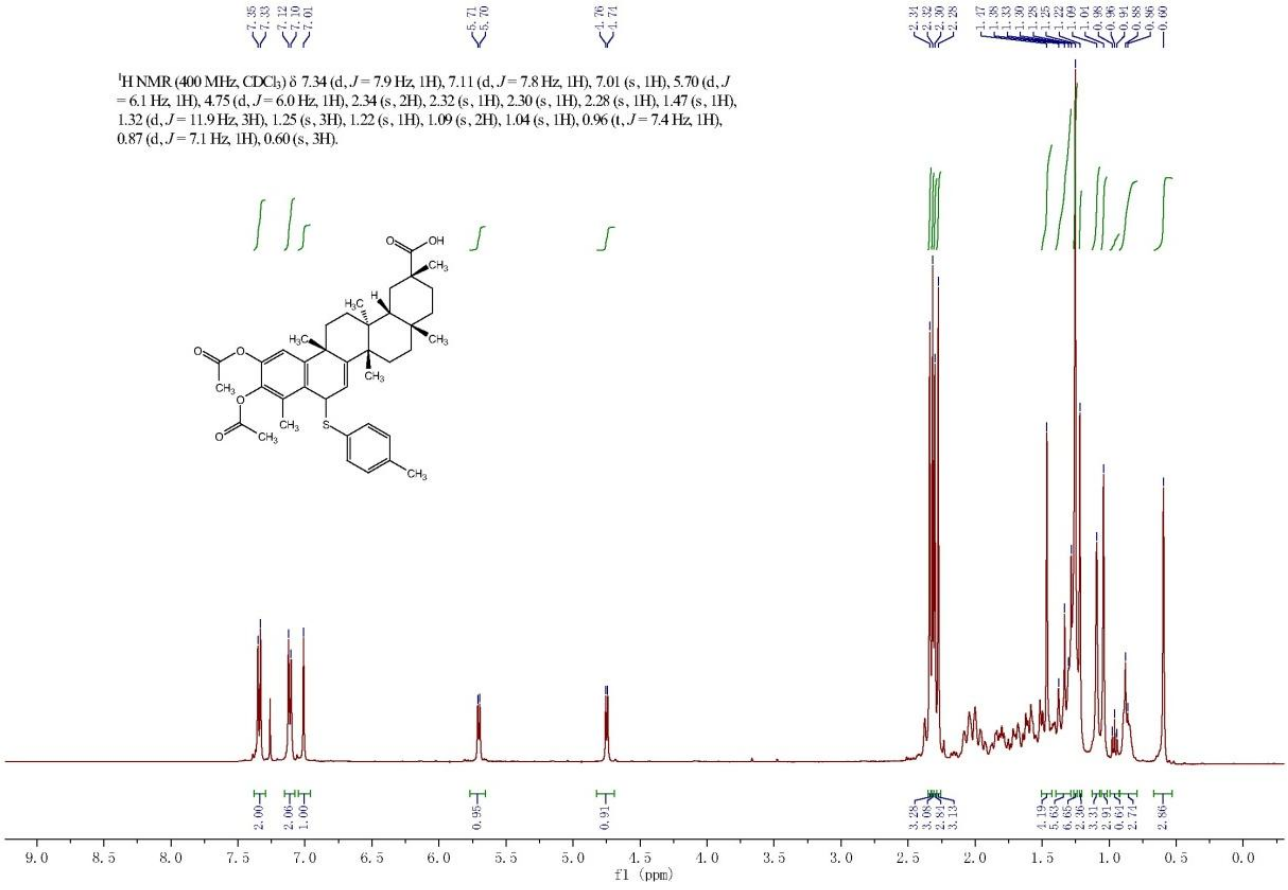
^{1}H-NMR (NST001A)



^{1}H-NMR (NST001B)

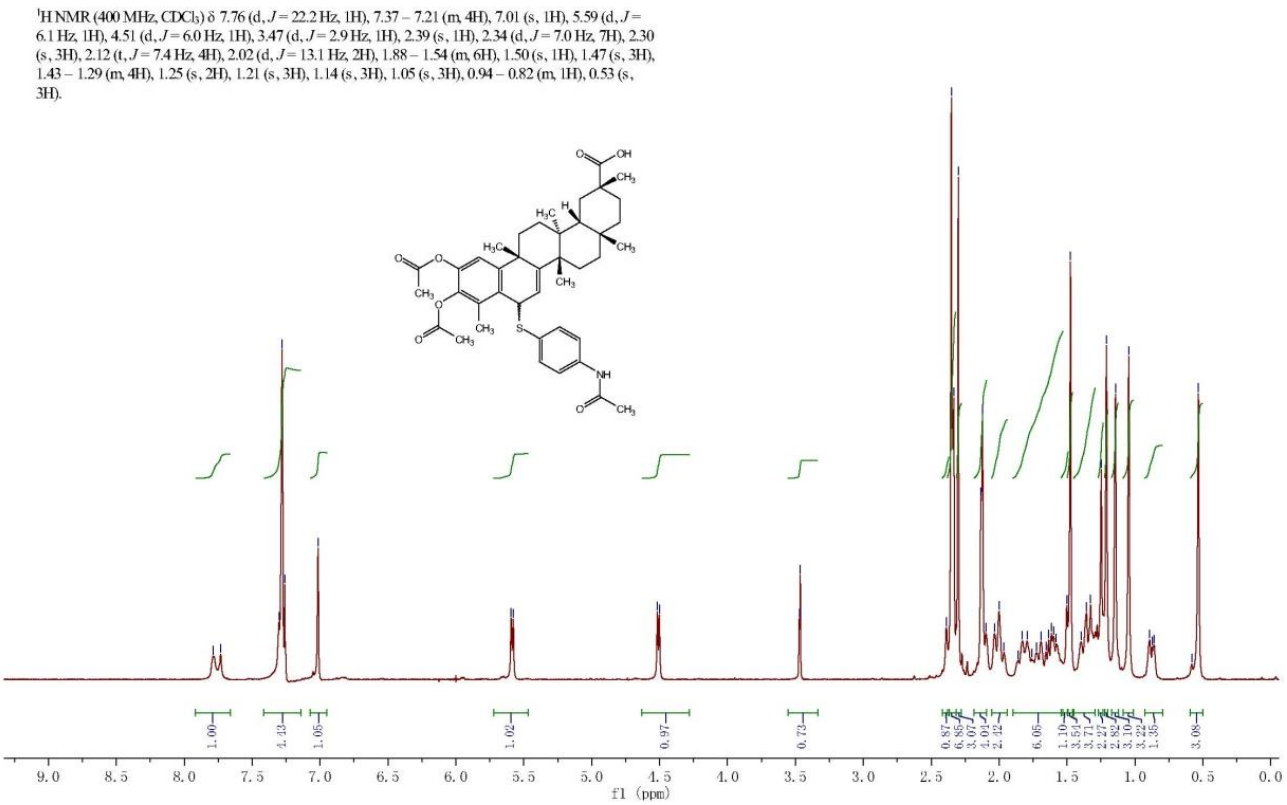


^{1}H-NMR (NST6A-A)



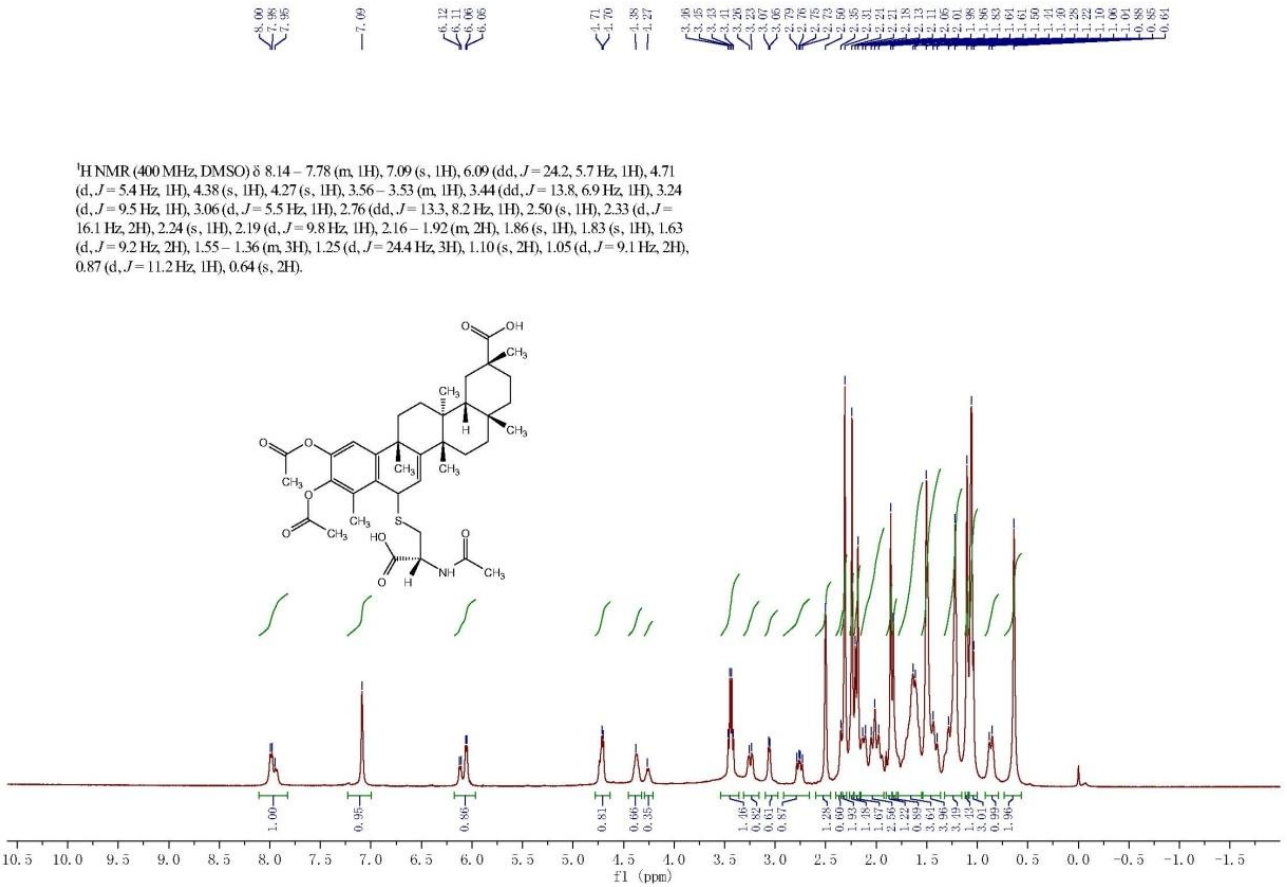
^{1}H-NMR (NST6A-B)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 22.2 Hz, 1H), 7.37 – 7.21 (m, 4H), 7.01 (s, 1H), 5.59 (d, J = 6.1 Hz, 1H), 4.51 (d, J = 6.0 Hz, 1H), 3.47 (d, J = 2.9 Hz, 1H), 2.39 (s, 1H), 2.34 (d, J = 7.0 Hz, 7H), 2.30 (s, 3H), 2.12 (t, J = 7.4 Hz, 4H), 2.02 (d, J = 13.1 Hz, 2H), 1.88 – 1.54 (m, 6H), 1.50 (s, 1H), 1.47 (s, 3H), 1.43 – 1.29 (m, 4H), 1.25 (s, 2H), 1.21 (s, 3H), 1.14 (s, 3H), 1.05 (s, 3H), 0.94 – 0.82 (m, 1H), 0.53 (s, 3H).

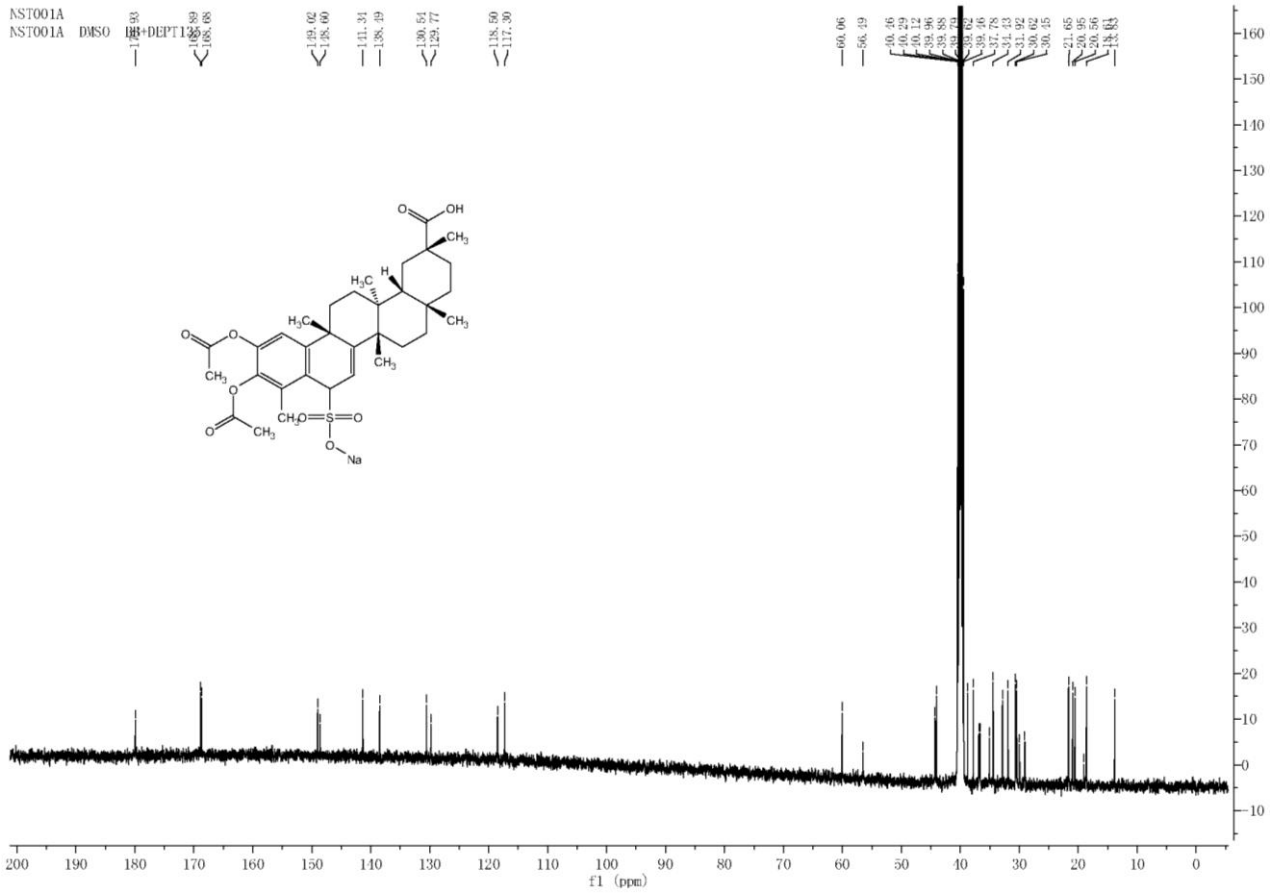


^{1}H-NMR (NST6A-C)

^{1}H NMR (400 MHz, DMSO) δ 8.14 – 7.78 (m, 1H), 7.09 (s, 1H), 6.09 (dd, J = 24.2, 5.7 Hz, 1H), 4.71 (d, J = 5.4 Hz, 1H), 4.38 (s, 1H), 4.27 (s, 1H), 3.56 – 3.53 (m, 1H), 3.44 (dd, J = 13.8, 6.9 Hz, 1H), 3.24 (d, J = 9.5 Hz, 1H), 3.06 (d, J = 5.5 Hz, 1H), 2.76 (dd, J = 13.3, 8.2 Hz, 1H), 2.50 (s, 1H), 2.33 (d, J = 16.1 Hz, 2H), 2.24 (s, 1H), 2.19 (d, J = 9.8 Hz, 1H), 2.16 – 1.92 (m, 2H), 1.86 (s, 1H), 1.83 (s, 1H), 1.63 (d, J = 9.2 Hz, 2H), 1.55 – 1.36 (m, 3H), 1.25 (d, J = 24.4 Hz, 3H), 1.10 (s, 2H), 1.05 (d, J = 9.1 Hz, 2H), 0.87 (d, J = 11.2 Hz, 1H), 0.64 (s, 2H).



^{1}H-NMR (NST6A-D)



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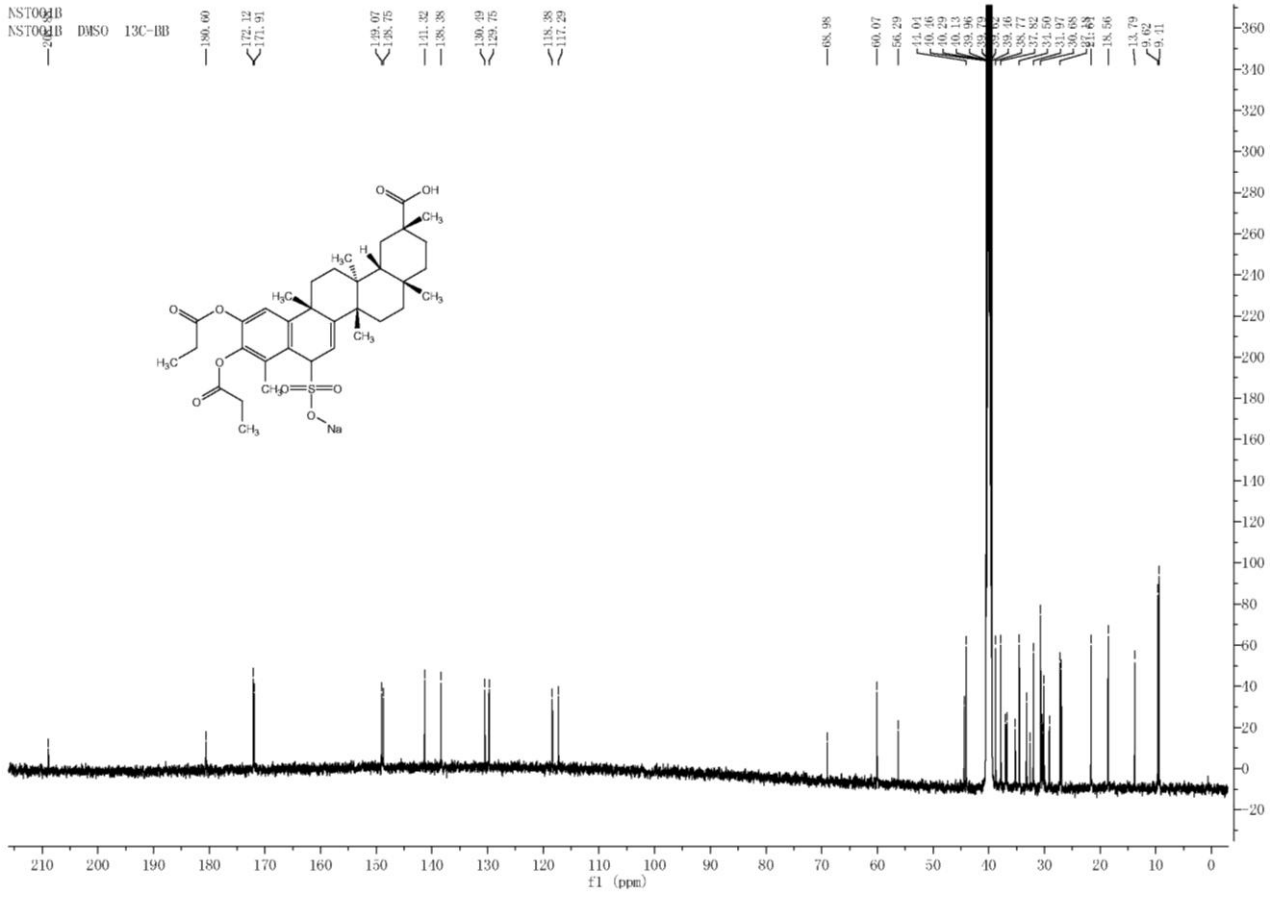
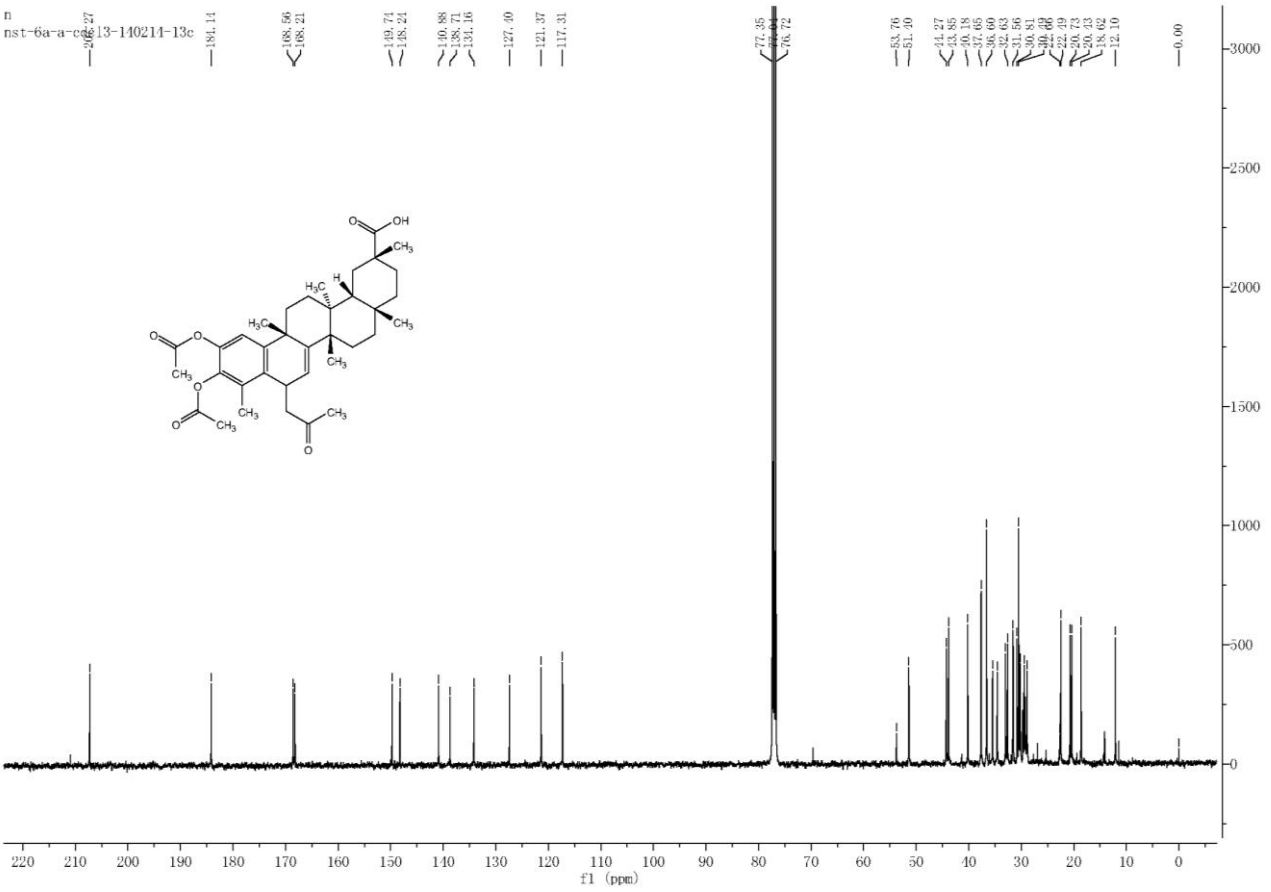


^{13}C-NMR (NST00B)



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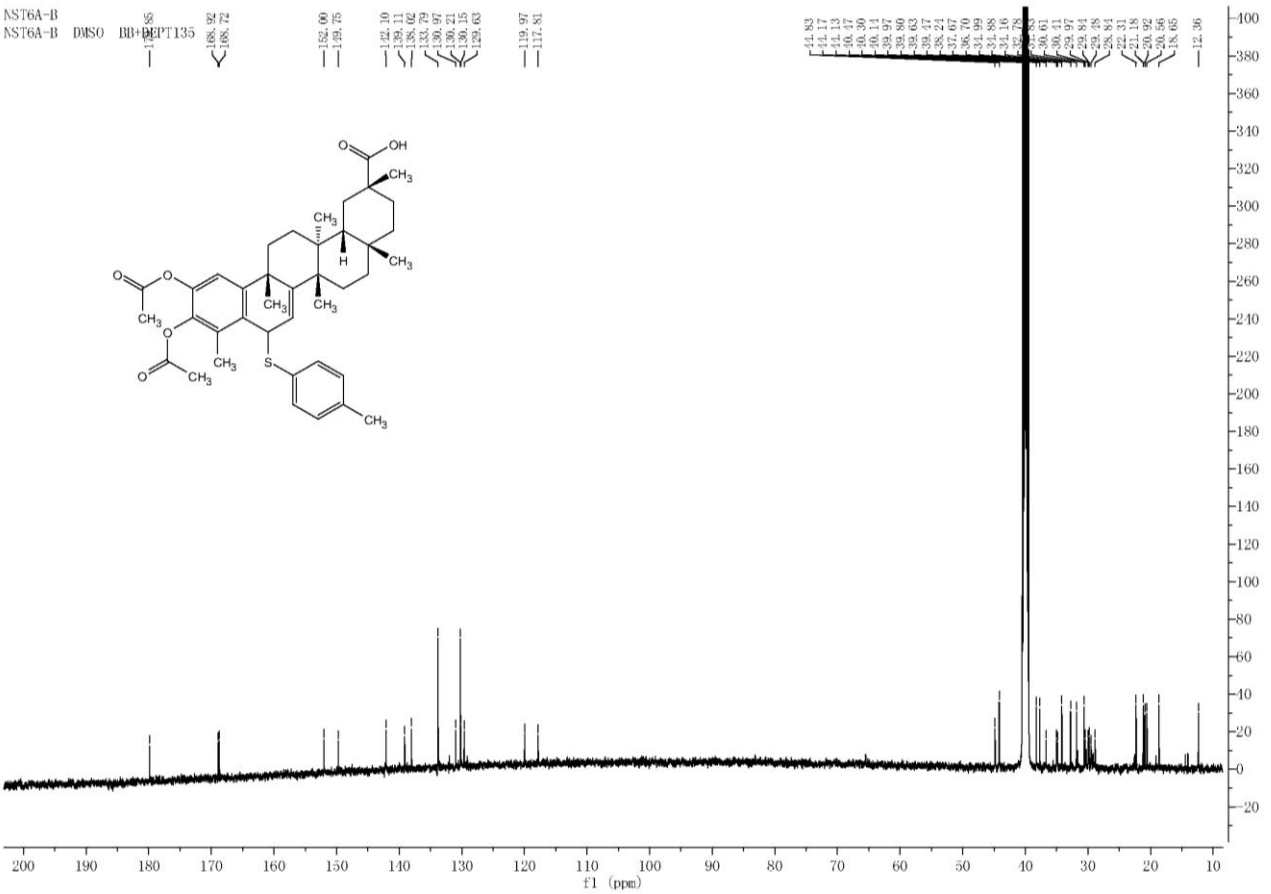

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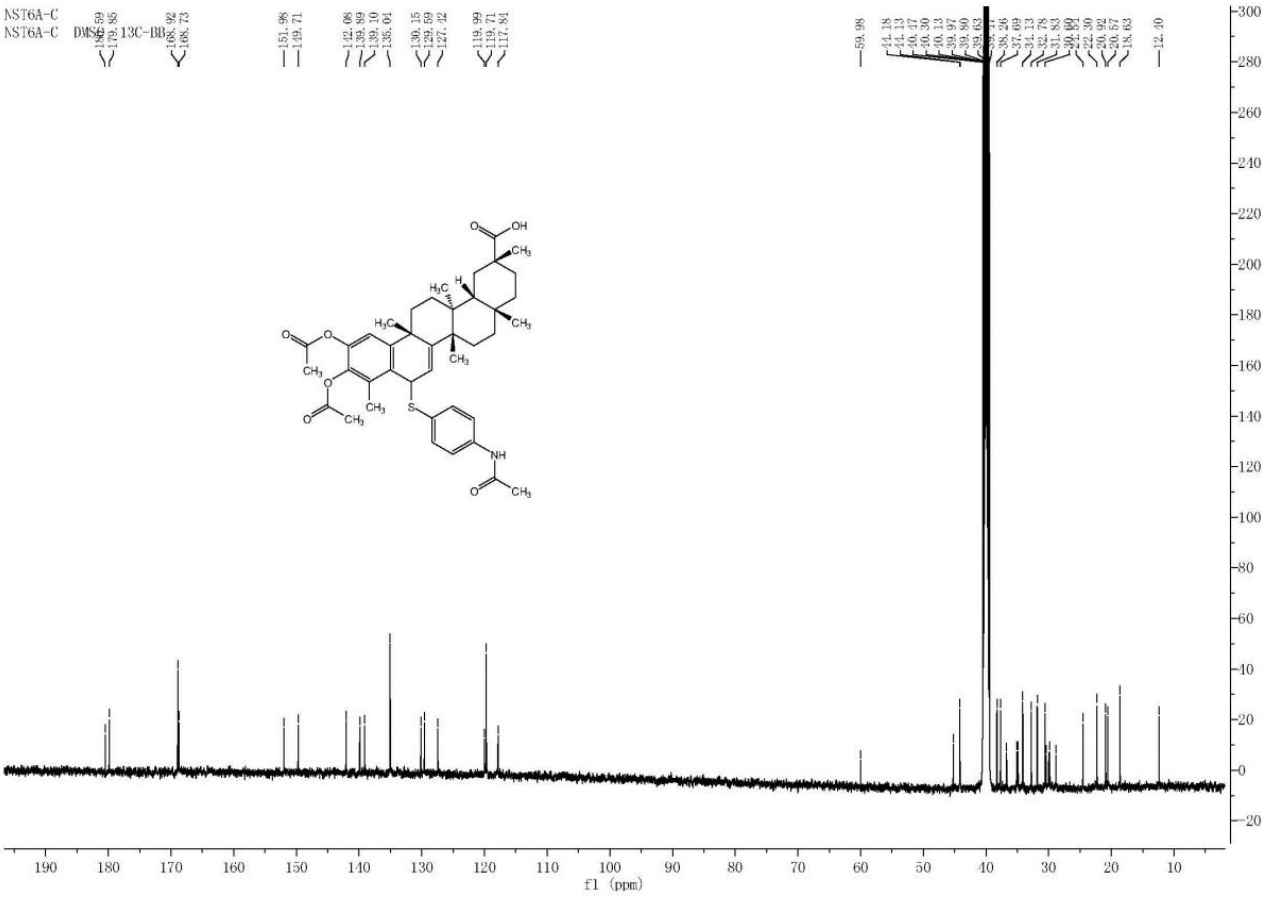

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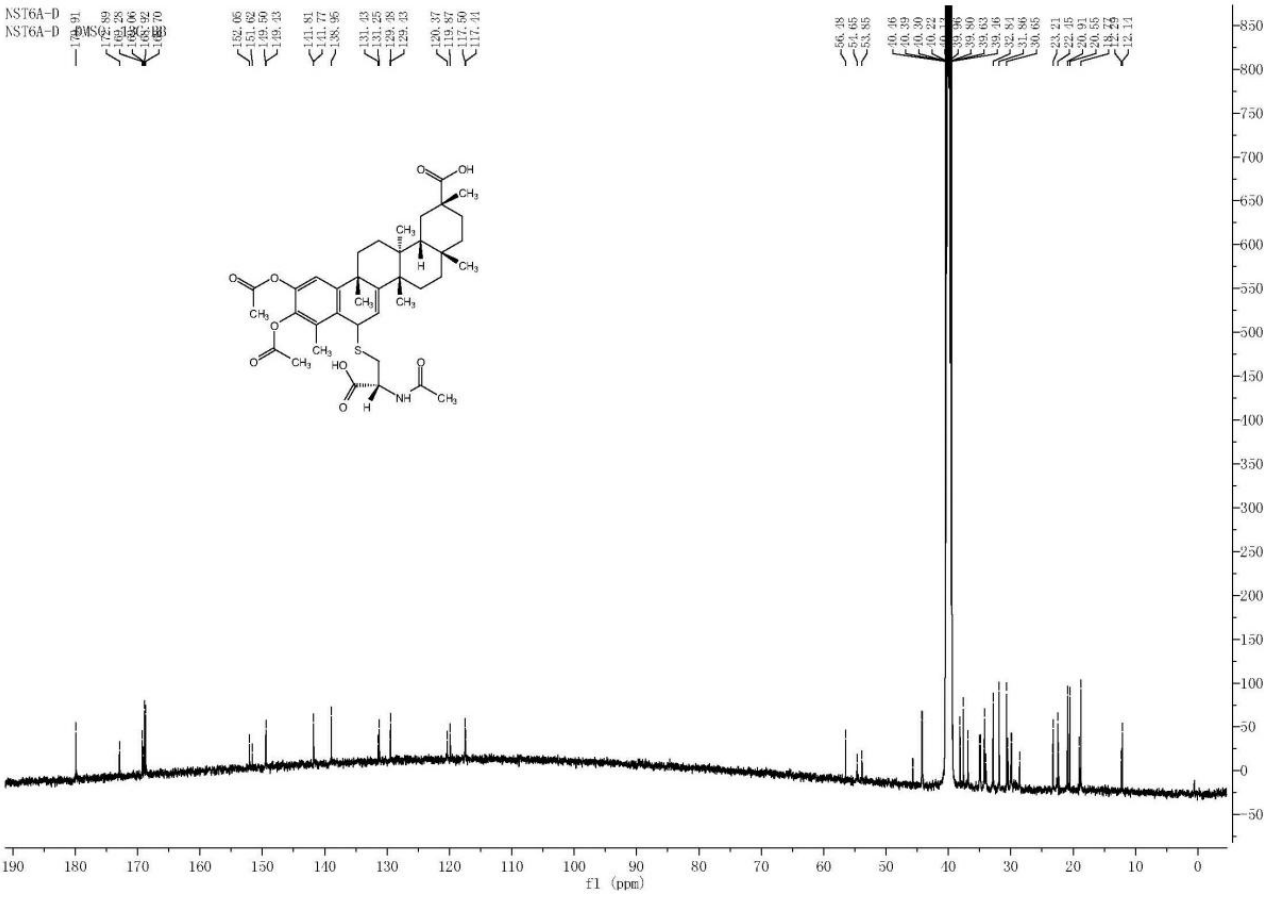

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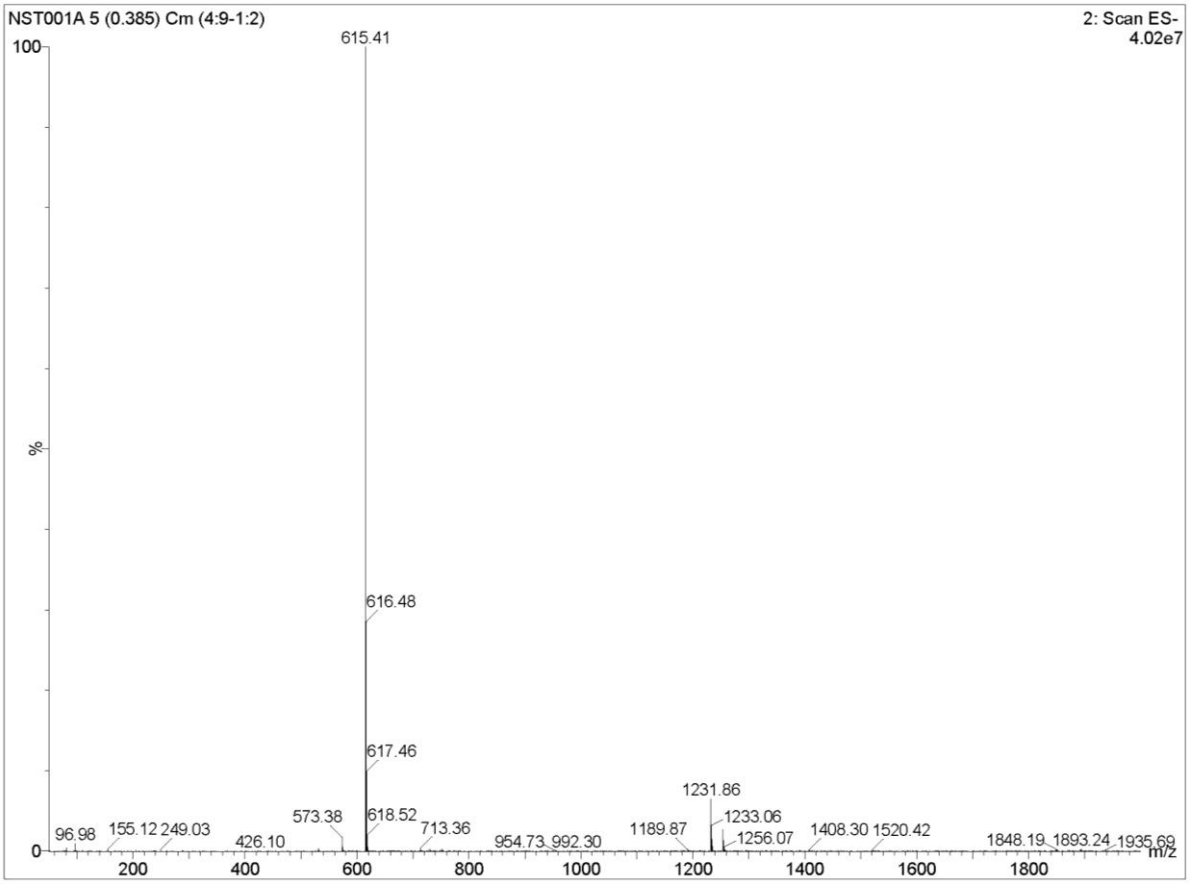

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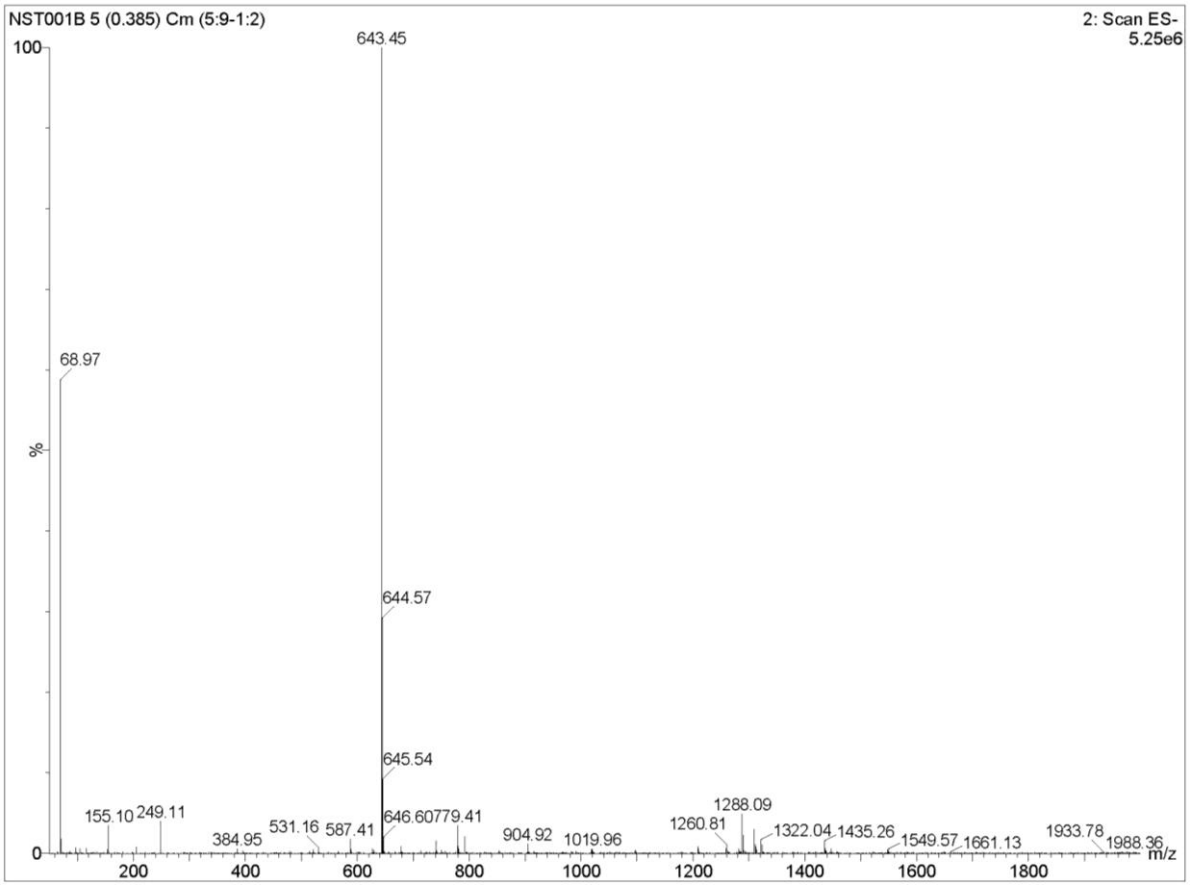

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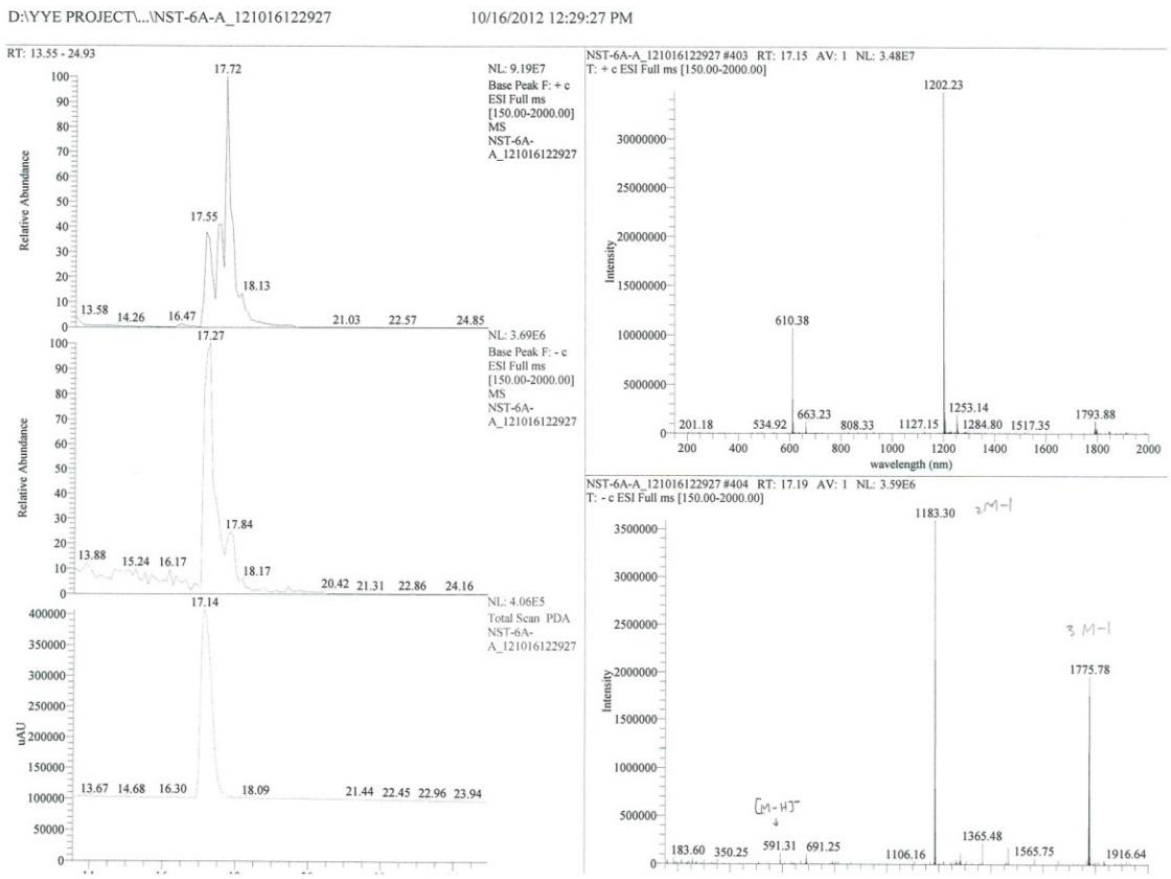

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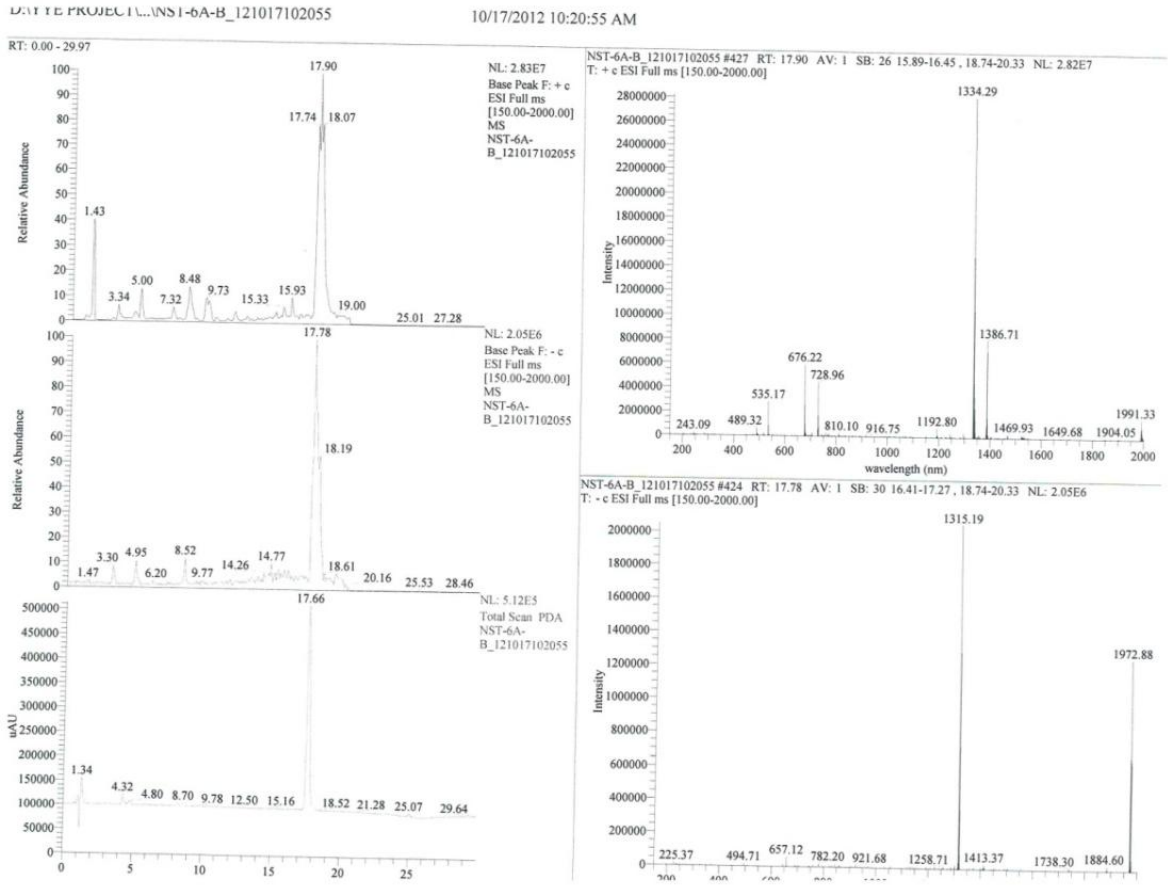

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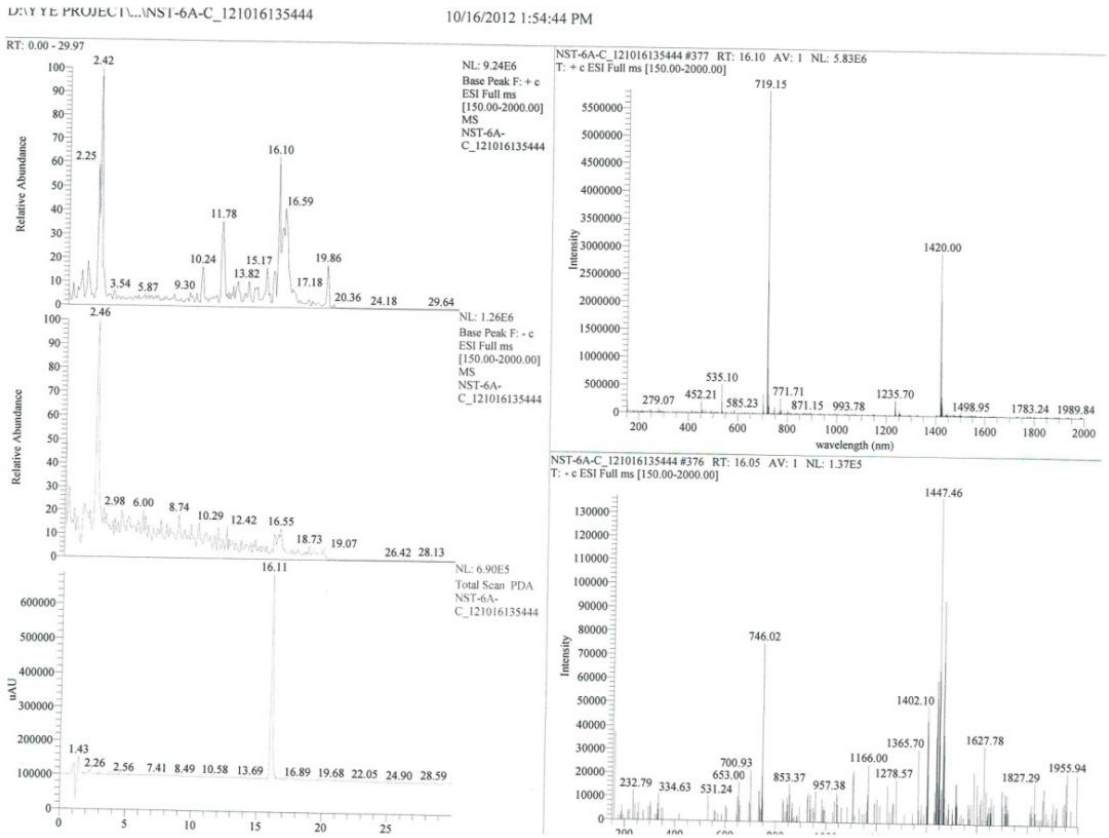

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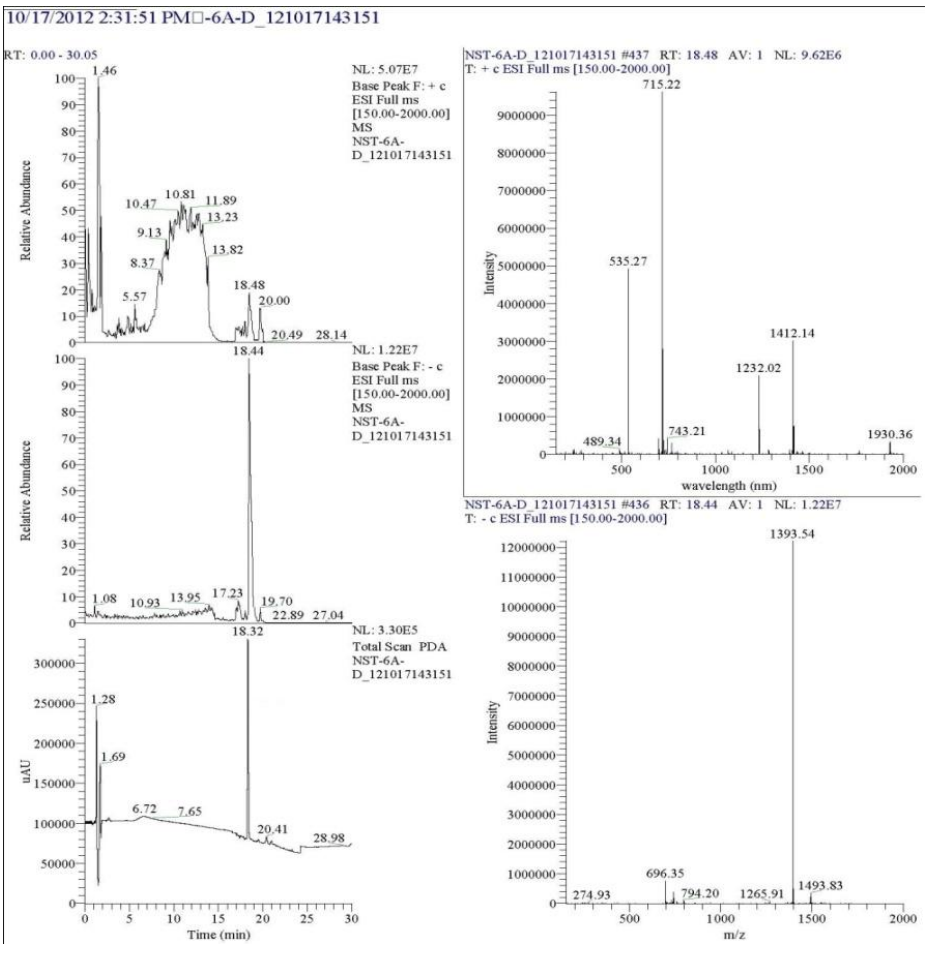

LC-ESI-MS(−) (NST6A-D)

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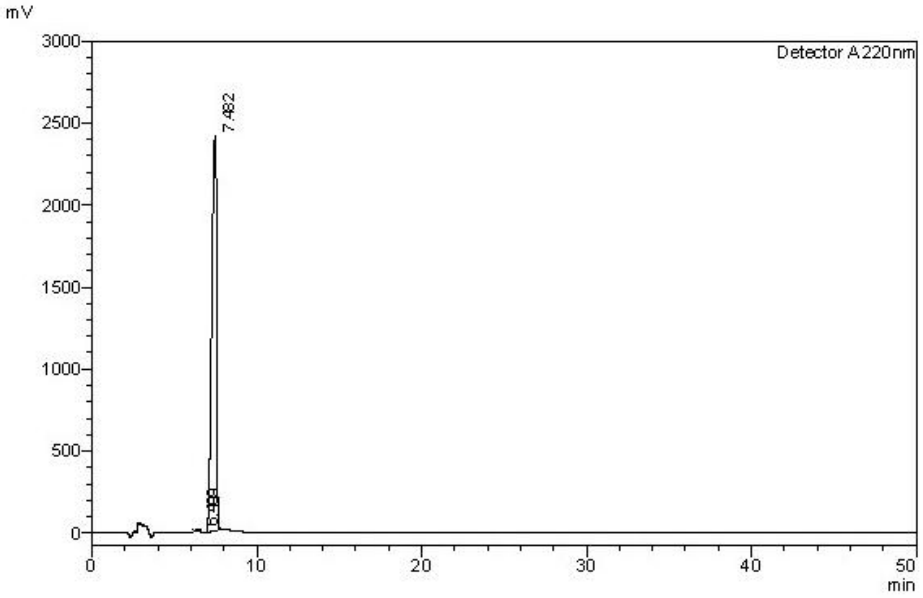

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HPLC purity (NST001)

SHIMADZU LabSolutions **Analysis Report**

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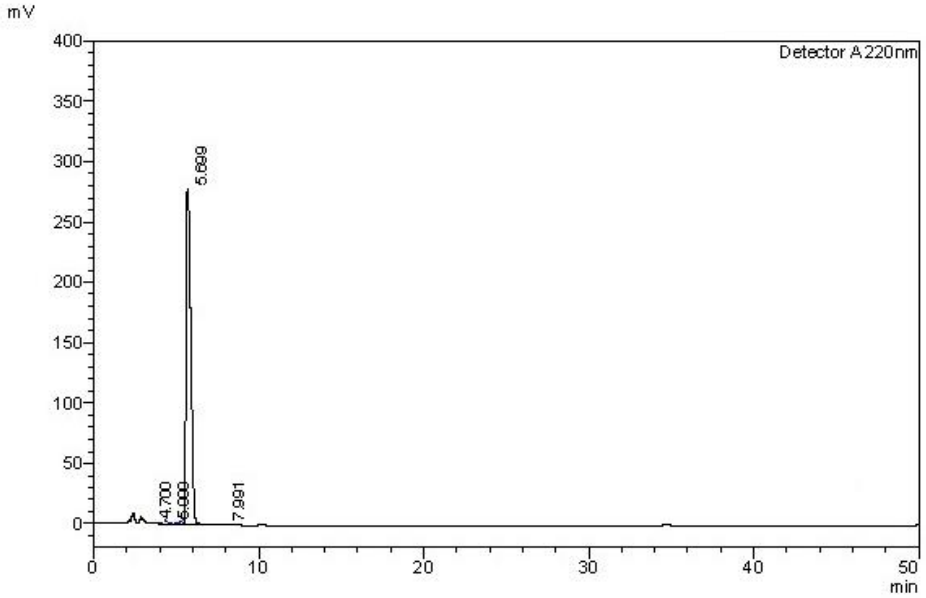

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4	7.991	4354	0.078	483	98610
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HPLC purity (NST001A)

SHIMADZU LabSolutions **Analysis Report**

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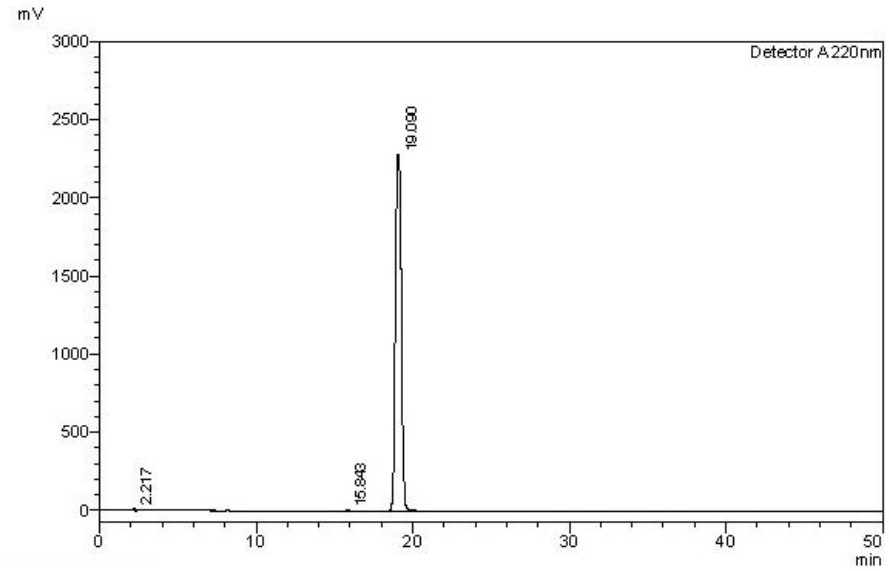

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HPLC purity (NST001B)

SHIMADZU LabSolutions **Analysis Report**

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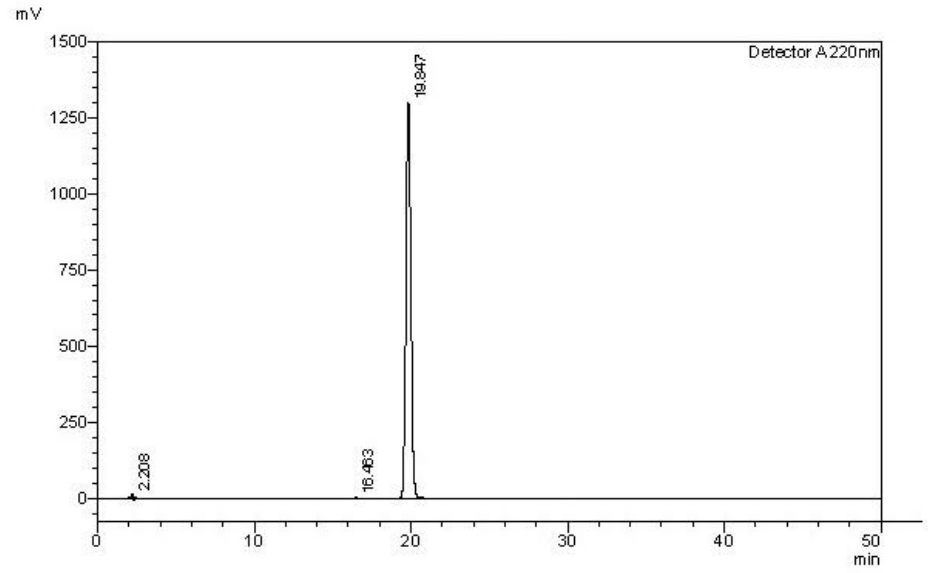

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2	16.463	21454	0.075	1241	132149
3	19.847	28523582	99.757	1296761	124165
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HPLC purity (NST6A-A)

SHIMADZU LabSolutions **Analysis Report**

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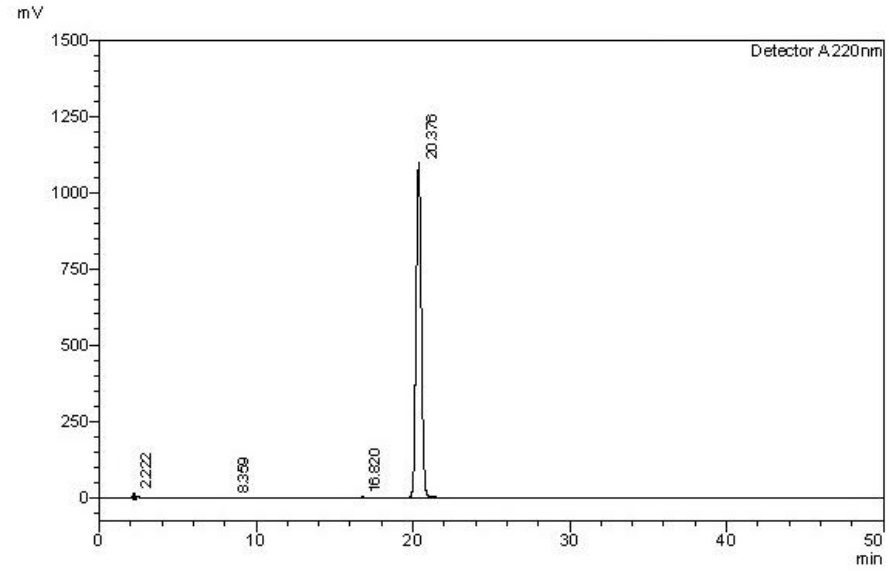

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HPLC purity (NST6A-B)

SHIMADZU LabSolutions **Analysis Report**

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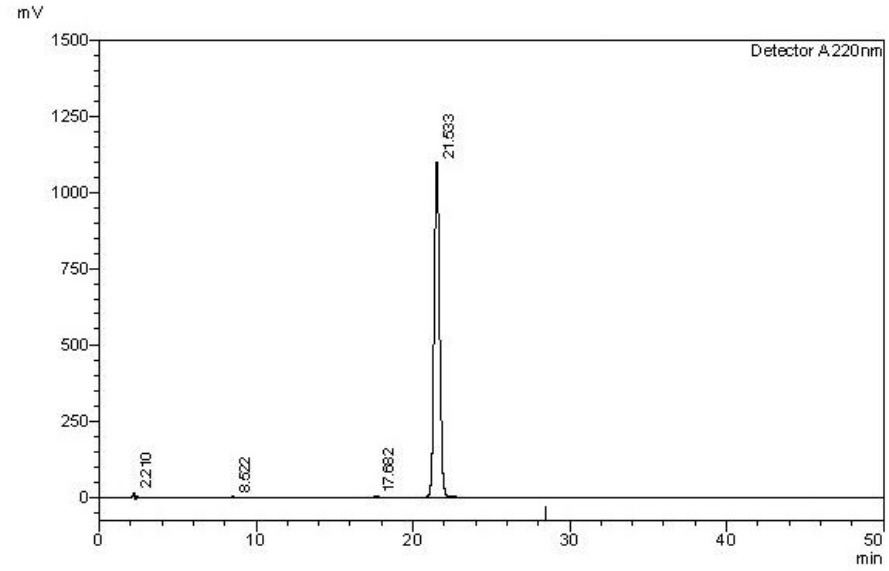

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3	17.682	19648	0.073	1065	142391
4	21.533	26734204	99.744	1097037	119743
Total		26802838	100.000	1112520	

G:\220nm\NST17.lcd

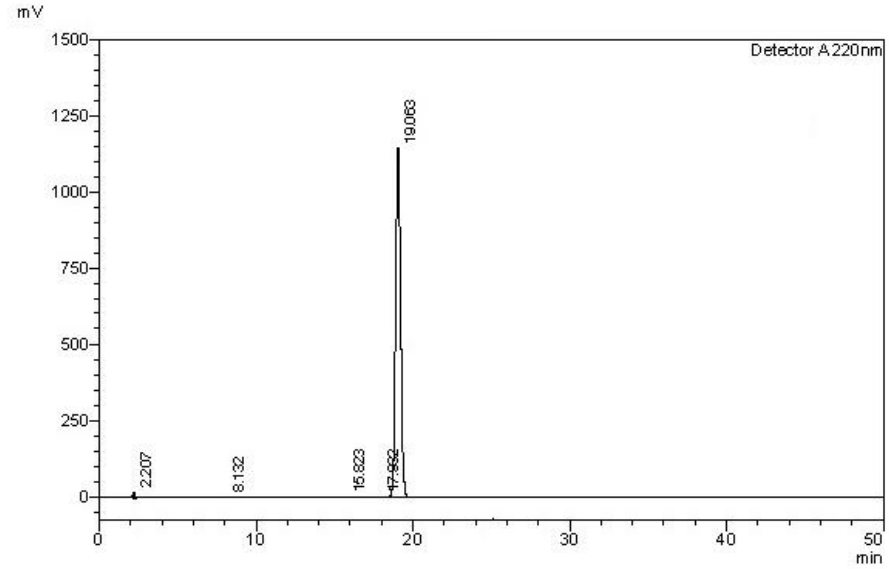
HPLC purity (NST6A-C)

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: NST6A-D		
Data Filename	: NST6A-D		
Method Filename	: NST analog.lcm		
Vial #	:		
Injection Volume	: 20 uL	Level	: 1
Date Acquired	: 6/12/2014 10:04:20 PM	Acquired by	: Administrator
Date Processed	: 6/16/2014 3:19:27 PM	Processed by	: System Administrator

<Chromatogram>



<Peak Table>

Detector A 220nm

Peak#	Ret. Time	Area	Area%	Height	tical Plates/mete
1	2.207	126461	0.510	19650	19741
2	8.132	3415	0.014	351	132585
3	15.823	15027	0.061	1006	131581
4	17.932	3526	0.014	205	95991
5	19.063	24643514	99.401	1144638	119765
Total		24791942	100.000	1165852	

G:\220nm\NST15.lcd

HPLC purity (NST6A-D)