Supporting Information

Synthesis and Biological Evaluation of 1-Arylsulfonyl-5-(N-hydroxyacrylamide)indoles as Potent Histone Deacetylase Inhibitors with Antitumor Activity in vivo

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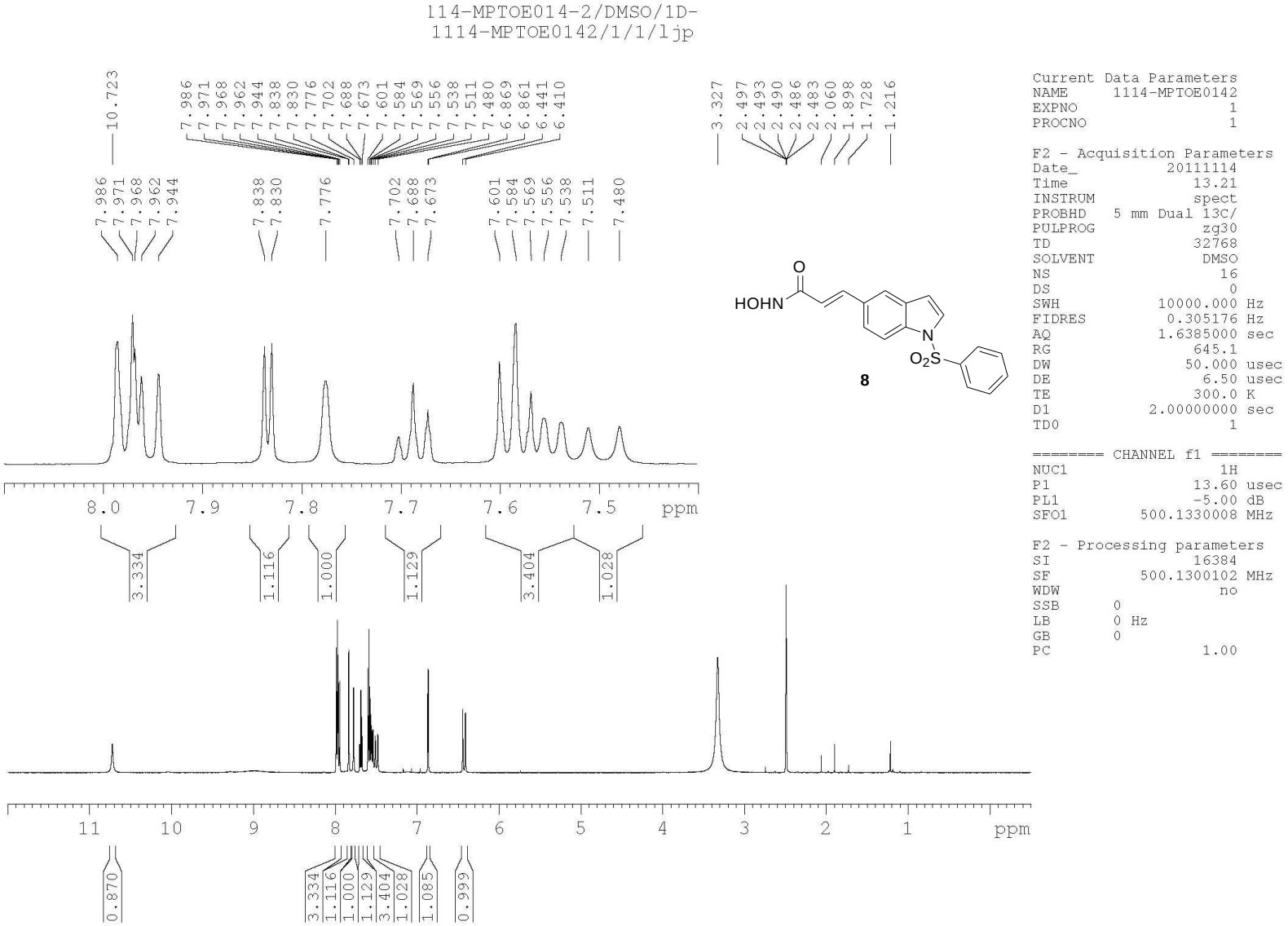
Contents:

1. ^{1}H-NMR Spectrum of compounds **8**

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PROCNO                 1

F2 - Acquisition Parameters
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INSTRUM            spect
PROBHD   5 mm Dual 13C/
PULPROG             zg30
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AQ             1.6385000 sec
RG                 645.1
DW                50.000 usec
DE                 6.50 usec
TE                 300.0 K
D1             2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
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SFO1         500.1330008 MHz

F2 - Processing parameters
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SF           500.1300102 MHz
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GB        0
PC                 1.00
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2. HPLC purity determination:

Instrument & column: HPLC purity were determined using an Hitachi 2000 series HPLC system using C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm. 4.6 mm × 150 mm).
Solvent system: Elution conditions: Mobile phase A-Acetonitrile; Mobile phase B-Water containing 0.1% formic acid + 10 mmol NH_4OAc. The flow-rate was 0.5 ml/min and the injection volume was 5 μl. The system operated at 25 °C. Peaks were detected at 210 nm.

Table 6. Elution condition

Time (min)	Mobile Phase A (ratio)	Mobile Phase B (ratio)
0	10	90
45	90	10
50	10	90
60	10	90

Table 7. Purity of compounds **6-21**

Compounds	Retention time (min)	% Purity
6	23.8	96.8
7	26.1	97.1
8	24.2	98.5
9	24.5	98.6
10	24.5	98.6
11	25.1	97.5
12	23.8	96.8
13	25.0	95.2
14	25.0	96.8
15	22.9	97.9
16	23.9	98.7
17	25.6	97.7
18	23.0	99.3
19	12.4	98.6
20	23.9	98.7
21	16.7	95.8

3. The spectrum of HPLC purity of representative compounds **8**, **11-13**

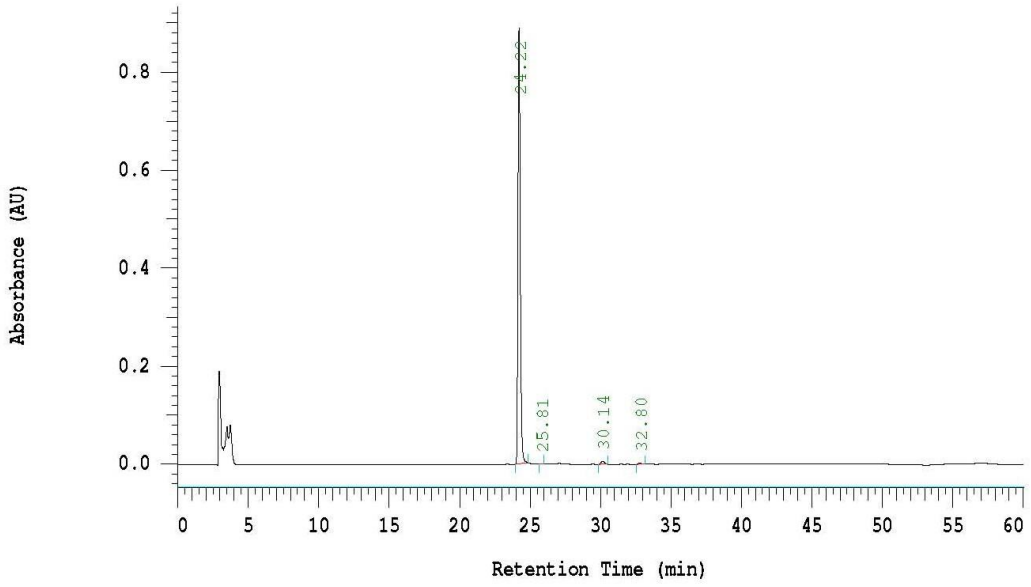
HPLC spectrum of compound 8

D-2000: Samples Series: 0155_001 Report Name: modified System: Sys 1

Hitachi D-2000 Elite HPLC System Manager Report

Analyzed Date and Time: 2011/11/23 11:06 上午
Reported Date and Time: 2011/11/23 02:55 下午
Data Path: C:\Win32app\D2000HSM\samples\DATA\0155_001\
Processing Method: Purity 2007/12/24
Sample Name: MPTOE014-50 Vial Number: 67
Injection from this vial: 1 of 1 Volume: 20.0 ul
Sample Description:

Chrom Type: Fixed WL Chromatogram, 254 nm



Processing Method: Purity 2007/12/24
Column Type: Column Method Developer: Bob
Method Description:

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Height	Conc 1
1	24.22	4861170	444133	98.536
2	25.81	5793	623	0.117
3	30.14	48034	3174	0.974
4	32.80	18390	1430	0.373
		4933387	449360	100.000

Peak rejection level: 0

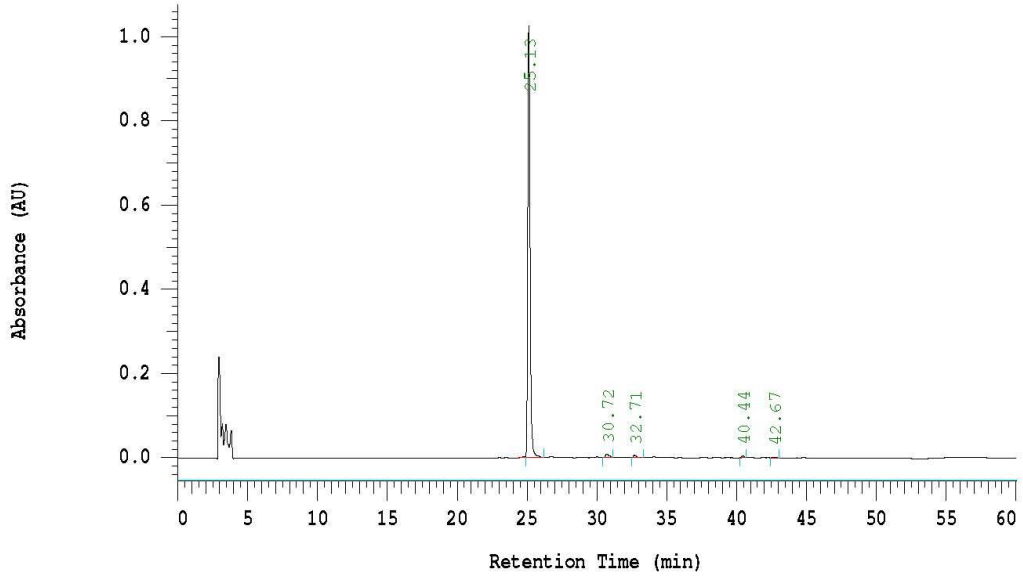
HPLC spectrum of compound 11

D-2000: Samples Series: 1907 Report Name: modified System: Sys 1

Hitachi D-2000 Elite HPLC System Manager Report

Analyzed Date and Time: 2011/07/27 08:28 上午
Reported Date and Time: 2011/07/28 10:25 上午
Data Path: C:\Win32app\D2000HSM\samples\DATA\1907\
Processing Method: Purity 2007/12/24
Sample Name: 19-826-2B-50 Vial Number: 12
Injection from this vial: 1 of 1 Volume: 20.0 ul
Sample Description:

Chrom Type: Fixed WL Chromatogram, 254 nm



Processing Method: Purity 2007/12/24
Column Type: Column Method Developer: Bob
Method Description:

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Height	Conc 1
1	25.13	5708618	512114	97.542
2	30.72	72015	4305	1.231
3	32.71	35595	2502	0.608
4	40.44	21306	2045	0.364
5	42.67	14926	1221	0.255
		5852460	522187	100.000

Peak rejection level: 0

HPLC spectrum of compound 12

D-2000: Samples Series: 1572 Report Name: modified System: Sys 1

Hitachi D-2000 Elite HPLC System Manager Report

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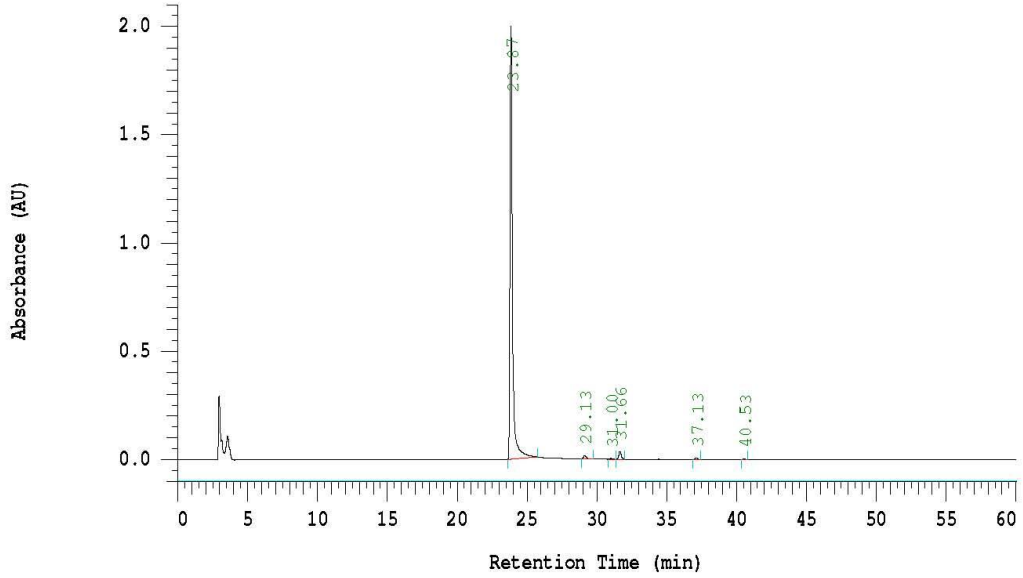
Data Path: C:\Win32app\D2000HSM\samples\DATA\1572\
Processing Method: Purity 2007/12/24

Sample Name: 7E-68-R-50 Vial Number: 56
Injection from this vial: 1 of 1 Volume: 20.0 ul
Sample Description:

Chrom Type: Fixed WL Chromatogram, 254 nm



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Column Type: Column Method Developer: Bob
Method Description:

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Height	Conc 1
1	23.87	11959128	998134	96.877
2	29.13	107579	8231	0.871
3	31.00	19199	1682	0.156
4	31.66	198715	17867	1.610
5	37.13	45141	3936	0.366
6	40.53	14838	1371	0.120
		12344600	1031221	100.000

Peak rejection level: 0

HPLC spectrum of compound 13

D-2000: Samples Series: 0013 Report Name: modified System: Sys 1

Hitachi D-2000 Elite HPLC System Manager Report

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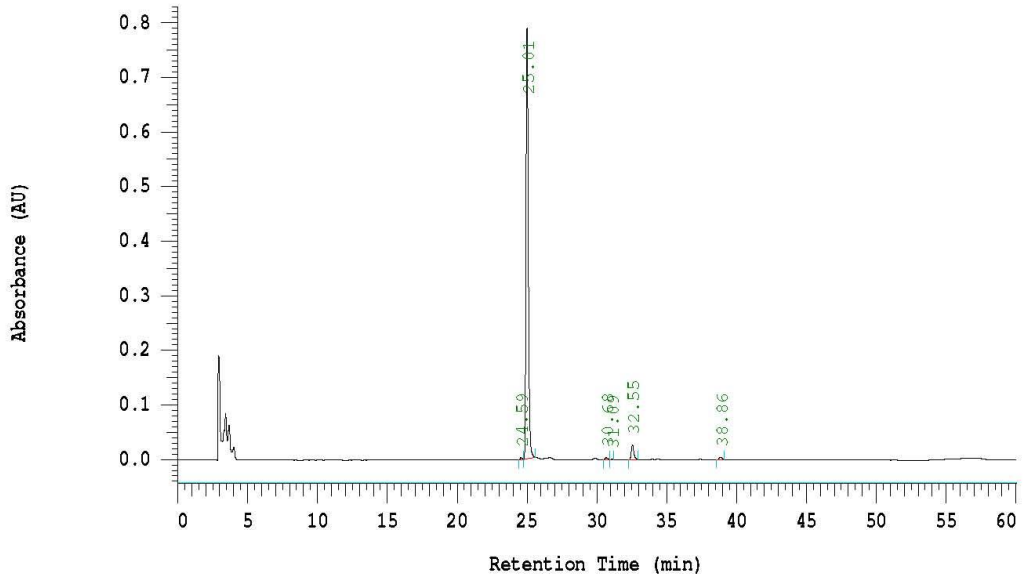
Reported Date and Time: 2011/09/06 03:24 下午
Data Path: C:\Win32app\D2000HSM\samples\DATA\0013\
Processing Method: Purity 2007/12/24
Sample Name: 19-829-2H-50 Vial Number: 4
Injection from this vial: 1 of 1 Volume: 20.0 ul
Sample Description:

Chrom Type: Fixed WL Chromatogram, 254 nm



Processing Method: Purity 2007/12/24
Column Type: Column Method Developer: Bob
Method Description:

Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Height	Conc 1
1	24.59	15303	1541	0.336
2	25.01	4337104	394065	95.245
3	30.68	19546	1772	0.429
4	31.09	2925	377	0.064
5	32.55	158377	13337	3.478
6	38.86	20370	1712	0.447
		4553625	412804	100.000

Peak rejection level: 0

4. Individual animal body weight change of compounds **8** and **1** treatment *in vivo*

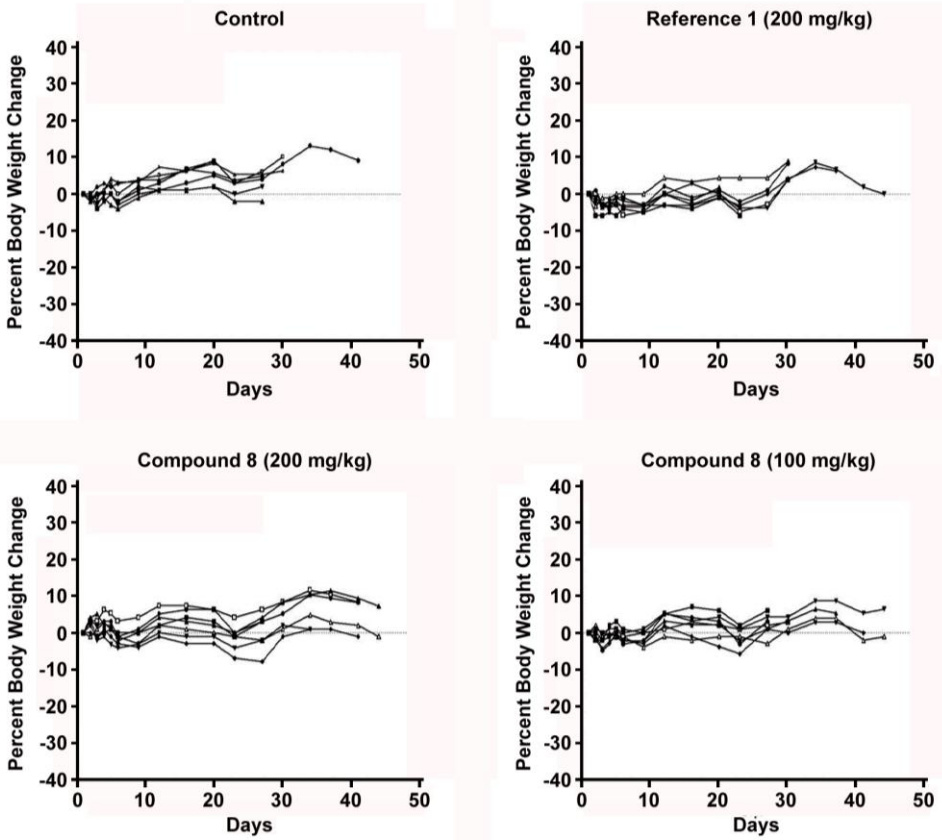
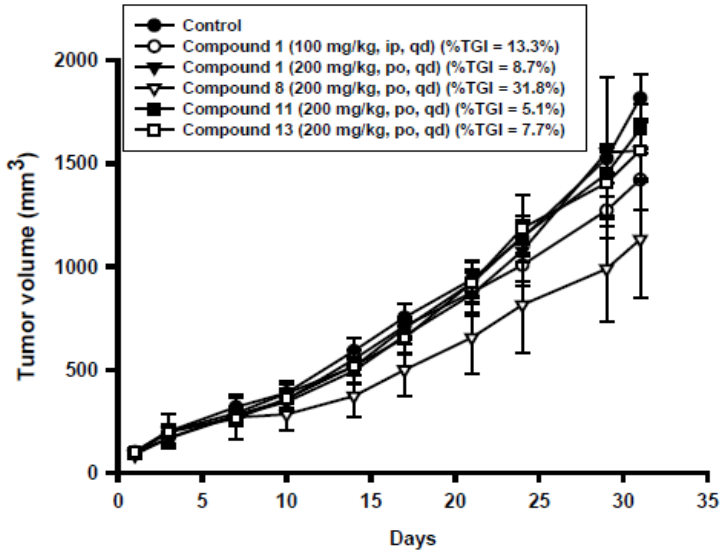


Figure 5. Individual animal body weight change of compounds **8** and **1** treatment *in vivo*. Animals were weight daily for the first seven days, then twice weekly until the completion of the study. The individual animal body weight change for each treatment group.

5. Evaluation of compounds **8**, **11**, **13**, and **1** on human A549 lung cancer xenograft growth in nude mice.

A



B

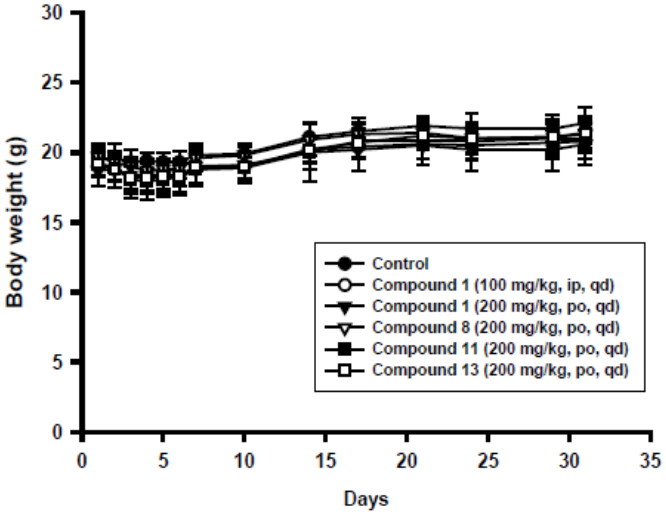


Figure 6. Evaluation of compounds **1**, **8**, **11**, and **13** on human A549 lung cancer xenograft model in nude mice. All test compounds were suspended in the 0.5% carboxymethyl cellulose for oral administration (p.o.). In addition, compound 1 (100 mg/kg) was administered intraperitoneally (i.p.) in a 5% ethanol/5% Cremophor/90% D5W solution. The study utilized six groups of mice (n = 5-6) bearing established human A549 lung cancer cells. The percentage of tumor growth inhibition (%TGI) showed inhibition of test compounds treatment comparison with control group. (A) % of Tumor growth inhibition (%TGI) : control (●); 100 mg/kg compound 1 i.p. daily (qd) (○); 200 mg/kg compound 1 p.o. daily (▼); 200 mg/kg compound 8 p.o. daily (▽); 200 mg/kg compound 11 p.o. daily (■); and 200 mg/kg compound 13 p.o. daily (□). (B) Body weight: Animals were weighed daily for the first seven days, then twice weekly until the completion of the study. There were no significant changes in body weight during the study.

6. Activities of compounds **8**, **11**-**14**, **17**, and **1** against HDAC 8.

Table 8. Activities of compounds against HDAC 8.

Compd	HDAC8
	IC_{50} (nM ± SD[a])
8	1819.2 ± 201.8
11	1317.1 ± 110.5
12	1366.3 ± 55.4
13	1208.3 ± 56.8
14	1216.0 ± 202.9
17	1504.0 ± 74.3
1	2898.9 ± 128.7

[a]SD: standard deviation. All experiments were independently performed at least three times.

7. CYP Inhibition and Solubility of compound **11-14**

Table 9. CYP Inhibition and Solubility of compounds **11-14**

	% CYP inhibition[a] at 10 μM							sol (μg/mL)	sol (μg/mL)
Compd	1A2	2C19	2C8	2C9	2D6	2E1	3A4	H_2O	EtOH
11	68	91	84	21	23	37	82	< 1	1002
12	72	95	91	29	10	33	89	40.8	1278
13	23	84	90	35	17	16	86	2.4	812
14	21	66	75	12	21	31	76	< 1	646

[a]The studies were performed by Ricerca Biosciences

8. *In vitro* metabolic stability in rat microsomes

The incubation mixture, in potassium phosphate buffer (pH 7.4), contained: microsomal proteins; NADPH, $MgCl_2$, test compound. Incubation was carried out, in triplicate, aerobically at 37 ℃ with constant shaking on a temperature-controlled heating block. Reaction was started by the addition of NADPH after pre-incubating the reaction mixture (without NADPH) for 10 min at 37 ℃. Control incubation without NADPH was performed as described above. At 0, and 30 min after the start of reaction, an aliquot (30 μL) of the incubation mixture was taken from each incubation, mixed with 100 μL of ice-cold acetonitrile to terminate the reaction. Before analysis, the sample was precipitated by centrifugation at room temperature. The remaining supernatant was analyzed for the concentration for each compound and the percentage of remaining was determined.

Table 10. Metabolic stability in rat microsomes.

Compounds	% of remaining after 30 min
8	47.4
11	51.8
12	49.9
13	44.9
14	46.9

9. The Correlation between inhibition of HDACs activity of HeLa cells and growth inhibition of compounds **6**-**21** and **1**

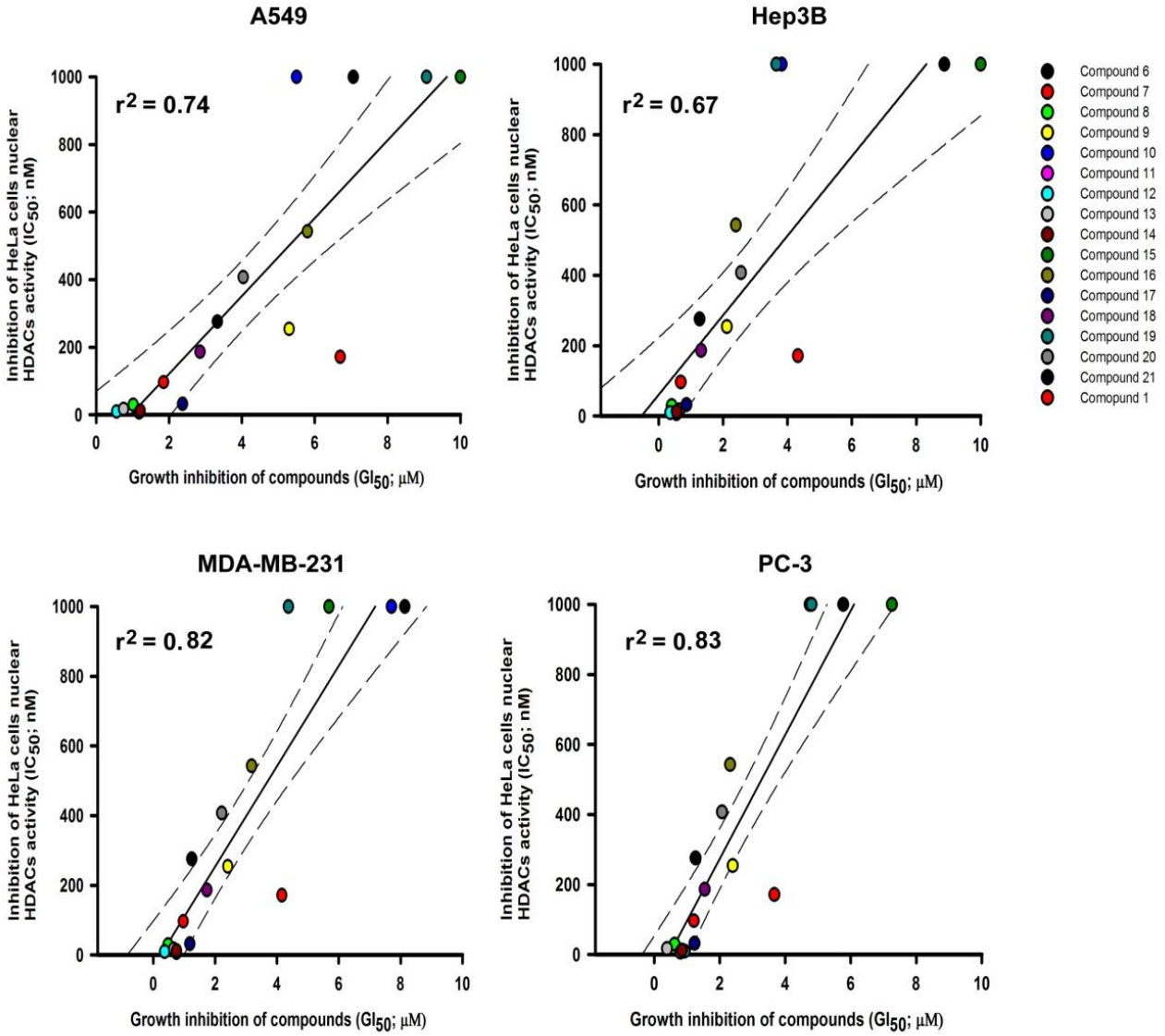


Figure 7. The correlation between inhibition of HDACs activity of HeLa cells and growth inhibition of compounds **6**-**21** and **1**.