Supporting information

For

Development of novel bis-naphthalimide derivatives and their anticancer properties

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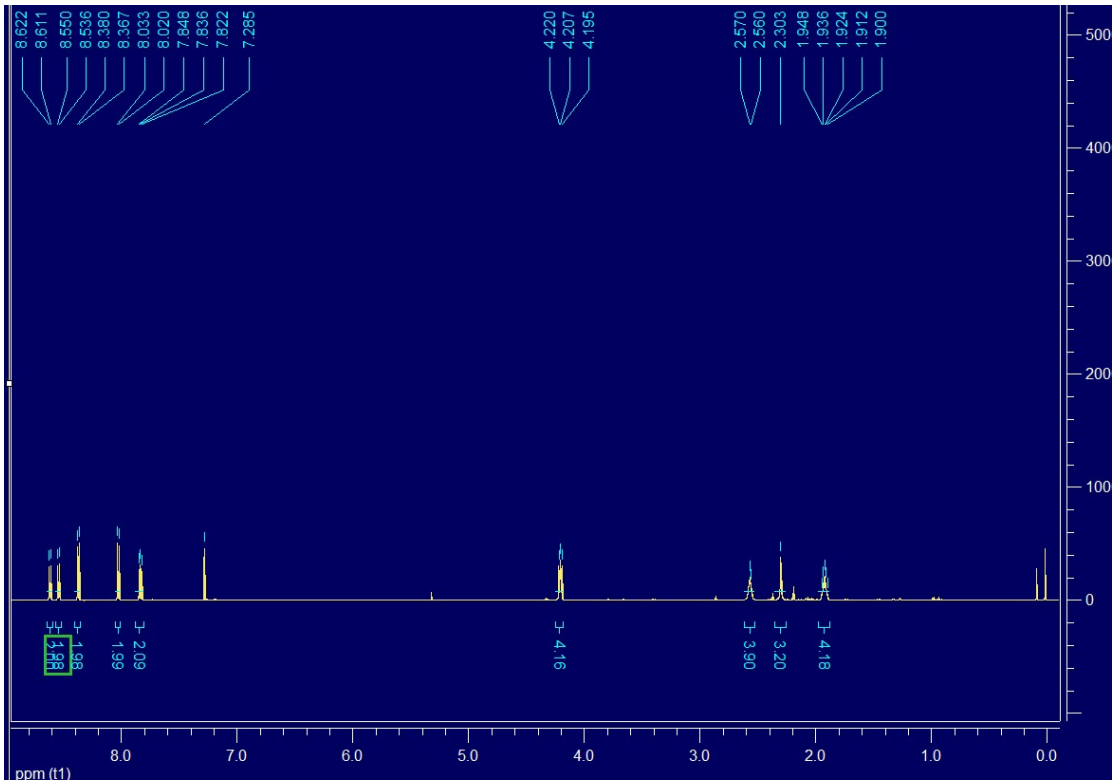

Fig. S1 ^{1}H NMR (600 MHz, $CDCl_3$) of compound **M1**.

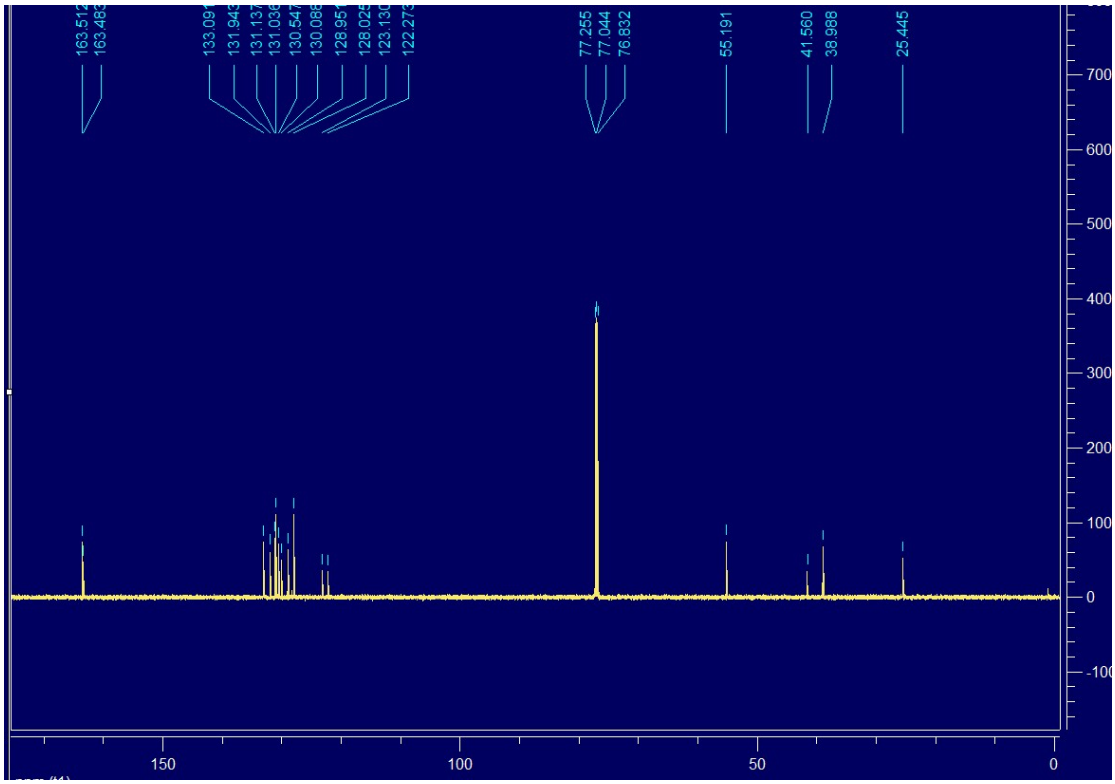

Fig. S2 ^{13}C NMR (150 MHz, $CDCl_3$) of compound **M1**.

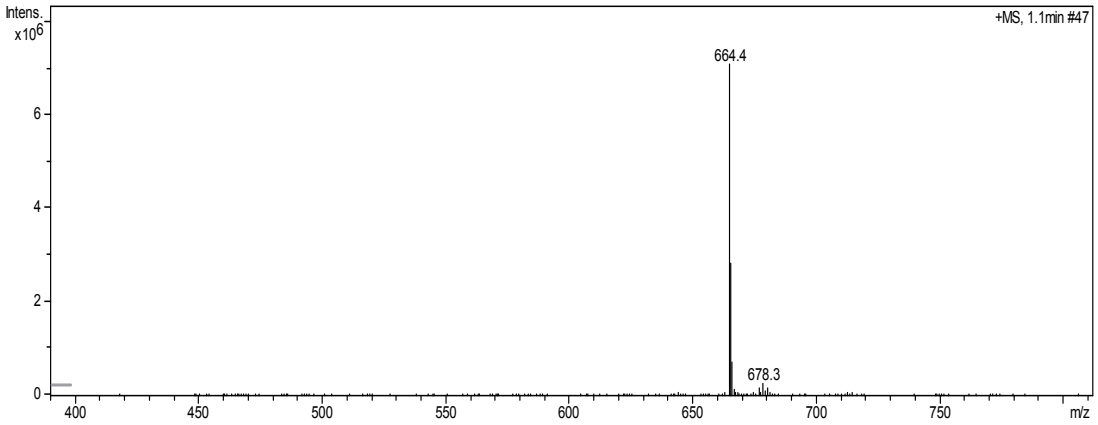

Fig. S3 MS (ESI) of compound **M1**.

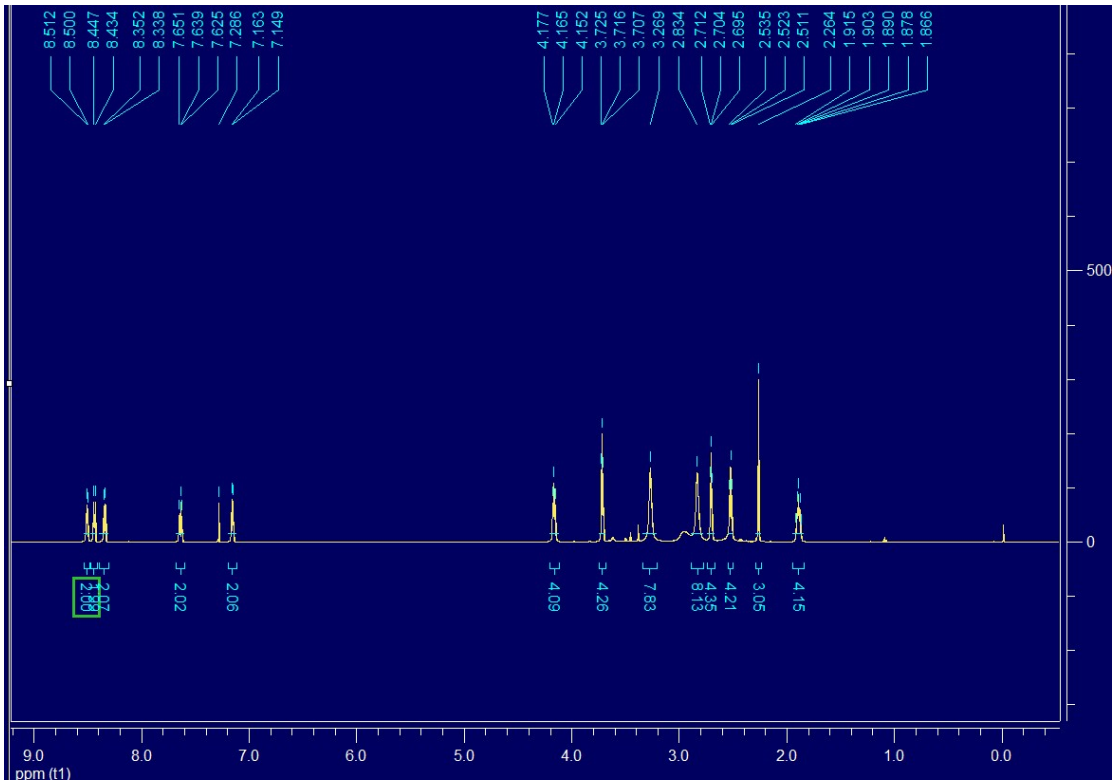

Fig. S4 ^{1}H NMR (600 MHz, $CDCl_3$) of compound **N1**.

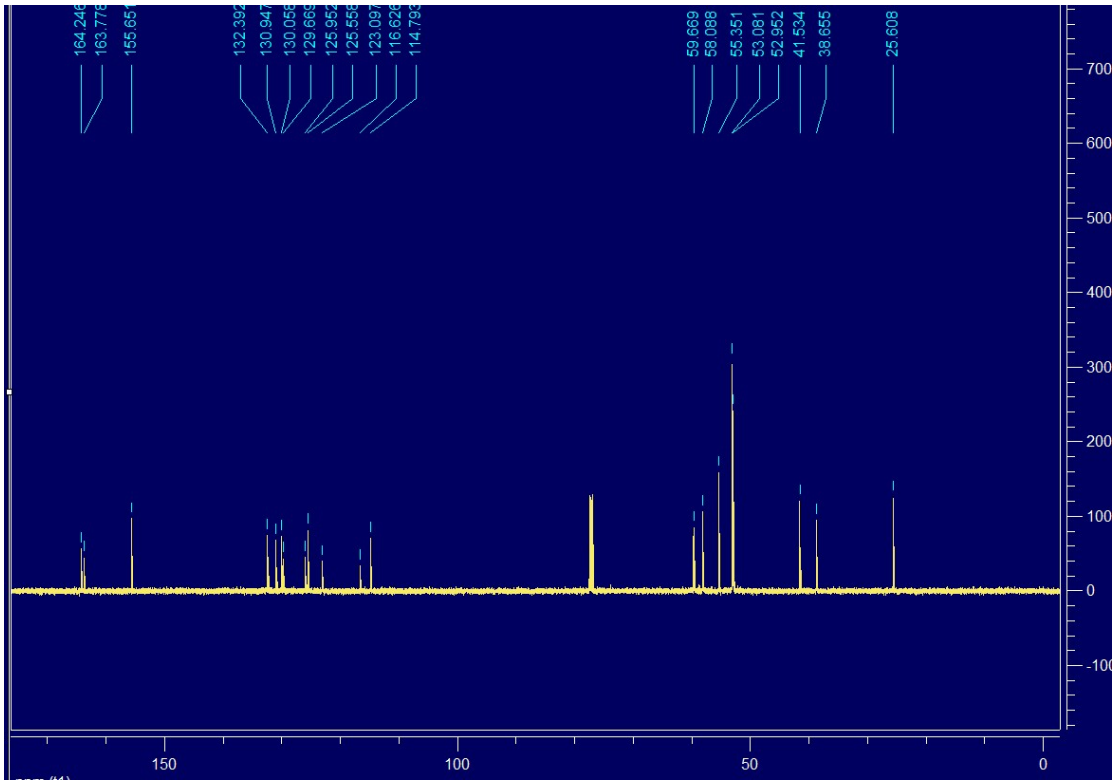

Fig. S5 ^{13}C NMR (150 MHz, $CDCl_3$) of compound **N1**.

Mass Spectrum SmartFormula Report

Analysis Info

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Method	20130927	Operator	
Sample Name		Instrument	apex-Ultra
Comment			

Acquisition Parameter

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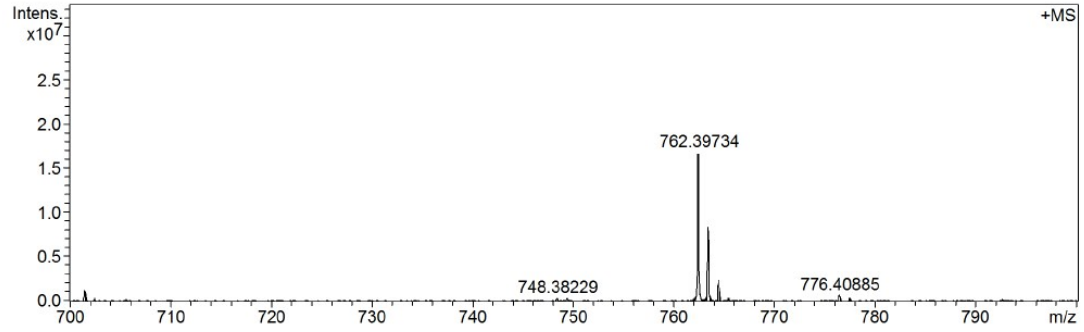
Averaged Scans	2	No. of Cell Fills	1	Laser Power	51.0 %
Broadband Low Mass	100.3 m/z	End Plate	3500.0 V	MALDI Plate	300.0 V
Broadband High Mass	800.0 m/z	Capillary Entrance	4000.0 V	Imaging Spot Diameter	2000.0 µm
Acquisition Mode	Single MS	Skimmer 1	20.0 V		
Pulse Program	basic	Drying Gas Temperature	180.0 °C	Calibration Date	Tue Oct 8 03:08:56 2013
Source Accumulation	0.0 sec	Drying Gas Flow Rate	4.0 L/min	Data Acquisition Size	131072
Ion Accumulation Time	0.0 sec	Nebulizer Gas Flow Rate	1.0 L/min	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.0 sec				



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
762.39734	1	C 43 H 52 N 7 O 6	100.00	762.39736	0.0	0.0	3.1	21.5	even	ok

Fig. S6 HRMS (ESI) of compound **N1**.

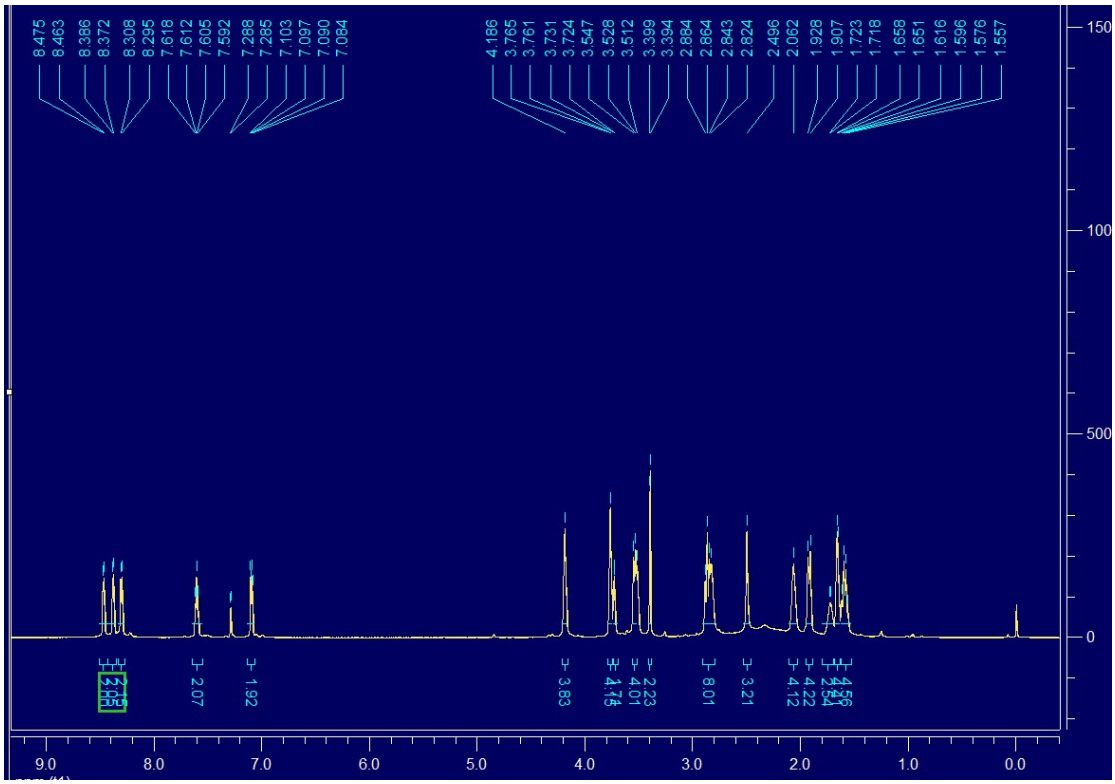


Fig. S7 ^{1}H NMR (600 MHz, $CDCl_3$) of compound **N2**.

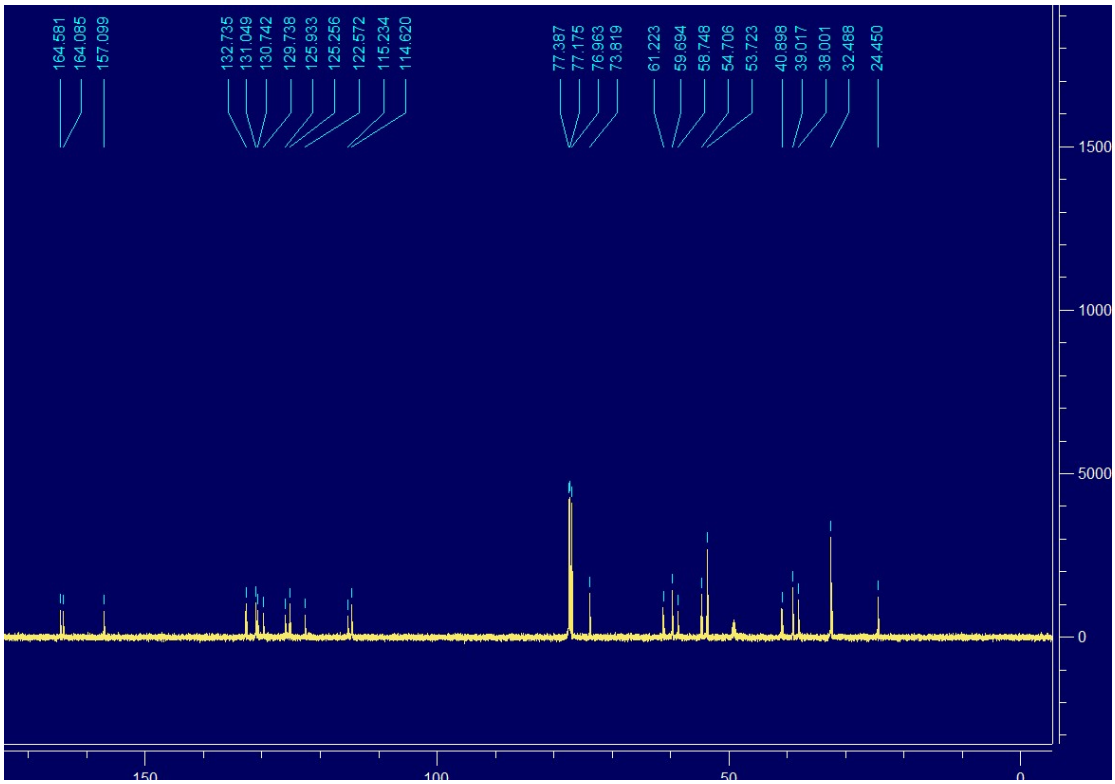


Fig. S8 ^{13}C NMR (150 MHz, $CDCl_3$) of compound **N2**.

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\20131012\Q6-ESI-POS-MSMS_000002.d	Acquisition Date	2013/10/12 15:25:14
Method	20130927	Operator	
Sample Name		Instrument	apex-Ultra
Comment			

Acquisition Parameter

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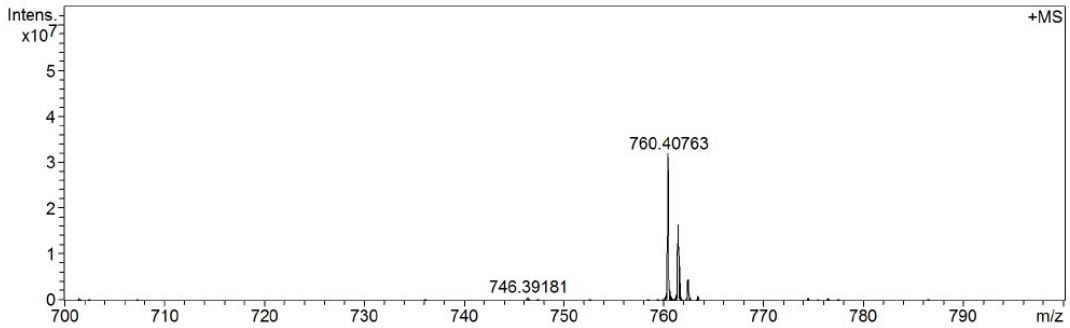
Averaged Scans	2	No. of Cell Fills	1	Laser Power	51.0 %
Broadband Low Mass	100.3 m/z	End Plate	3500.0 V	MALDI Plate	300.0 V
Broadband High Mass	800.0 m/z	Capillary Entrance	4000.0 V	Imaging Spot Diameter	2000.0 µm
Acquisition Mode	Single MS	Skimmer 1	20.0 V		
Pulse Program	basic	Drying Gas Temperature	180.0 °C	Calibration Date	Tue Oct 8 03:08:56 2013
Source Accumulation	0.0 sec	Drying Gas Flow Rate	4.0 L/min	Data Acquisition Size	131072
Ion Accumulation Time	0.0 sec	Nebulizer Gas Flow Rate	1.0 L/min	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.0 sec				



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¡¥Conf	N-Rule
760.40763	1	C 45 H 54 N 5 O 6	100.00	760.40686	-0.8	-1.0	5.4	21.5	even	ok

Fig. S9 HRMS (ESI) of compound **N2**.

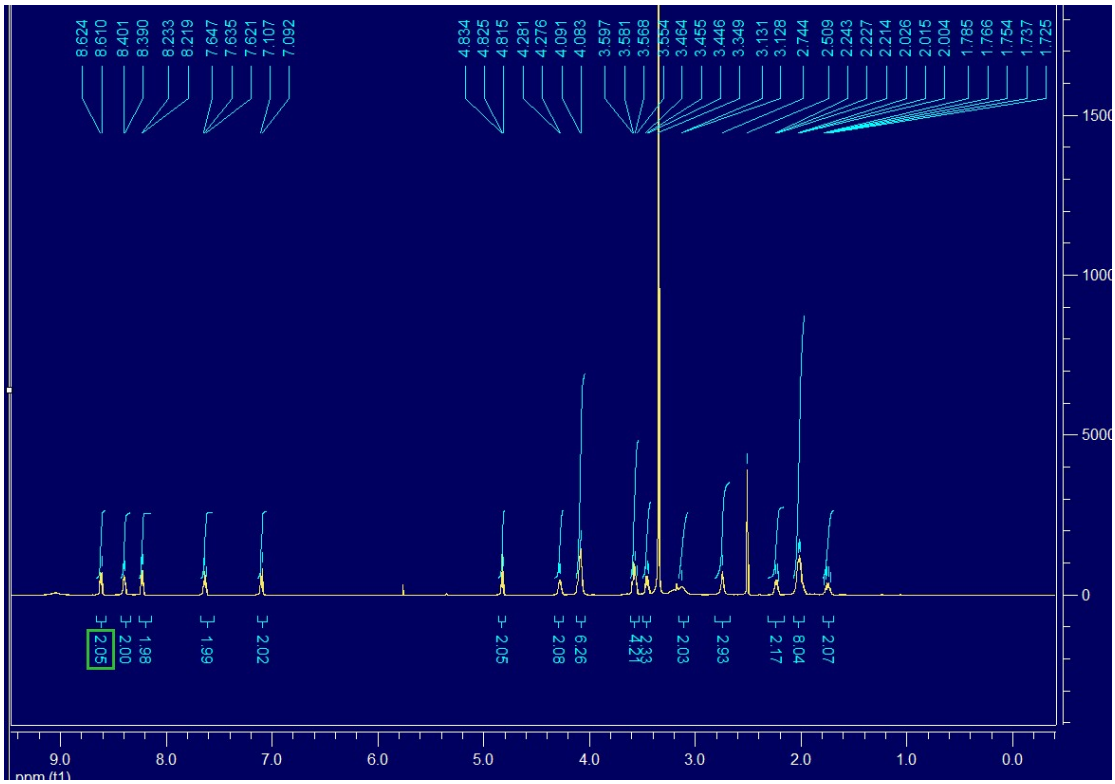


Fig. S10 ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O) of compound **N3**.

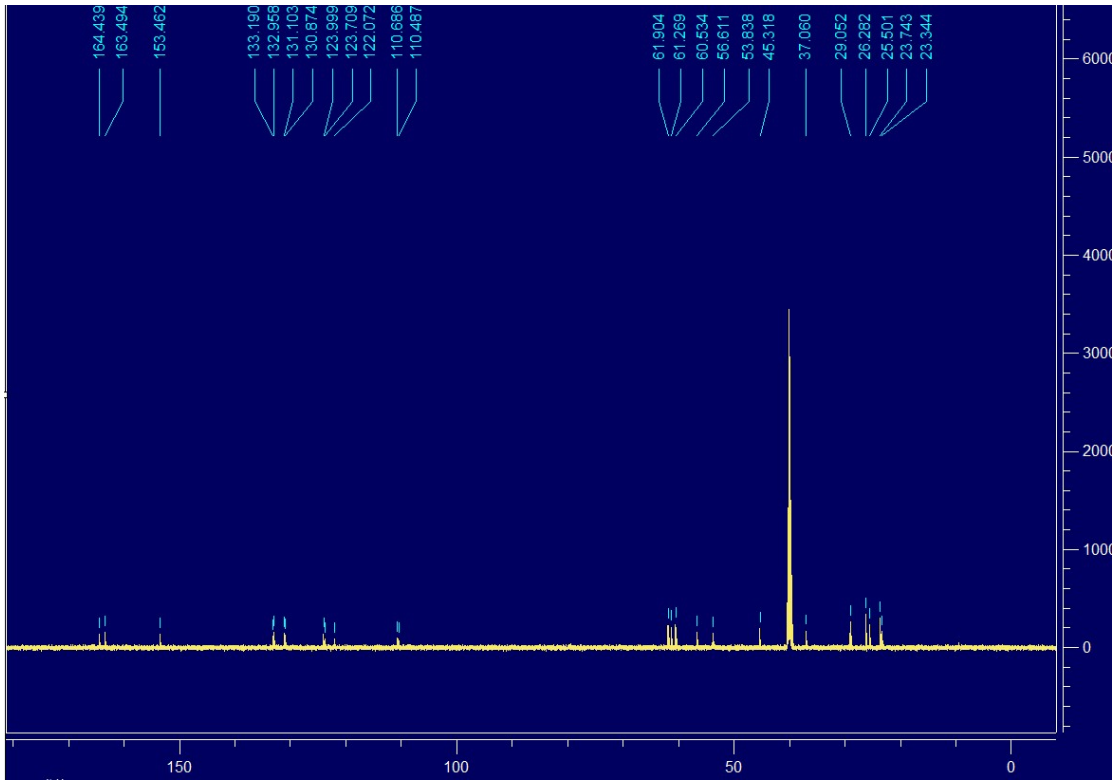


Fig. S11 ^{13}C NMR (150 MHz, DMSO-d_6 + D_2O) of compound **N3**.

Mass Spectrum SmartFormula Report

Analysis Info

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Method	20130927	Operator	
Sample Name		Instrument	apex-Ultra
Comment			

Acquisition Parameter

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Averaged Scans	2	No. of Cell Fills	1	Laser Power	51.0 %
Broadband Low Mass	100.3 m/z	End Plate	3500.0 V	MALDI Plate	300.0 V
Broadband High Mass	800.0 m/z	Capillary Entrance	4000.0 V	Imaging Spot Diameter	2000.0 µm
Acquisition Mode	Single MS	Skimmer 1	20.0 V		
Pulse Program	basic	Drying Gas Temperature	180.0 °C	Calibration Date	Tue Oct 8 03:08:56 2013
Source Accumulation	0.0 sec	Drying Gas Flow Rate	4.0 L/min	Data Acquisition Size	131072
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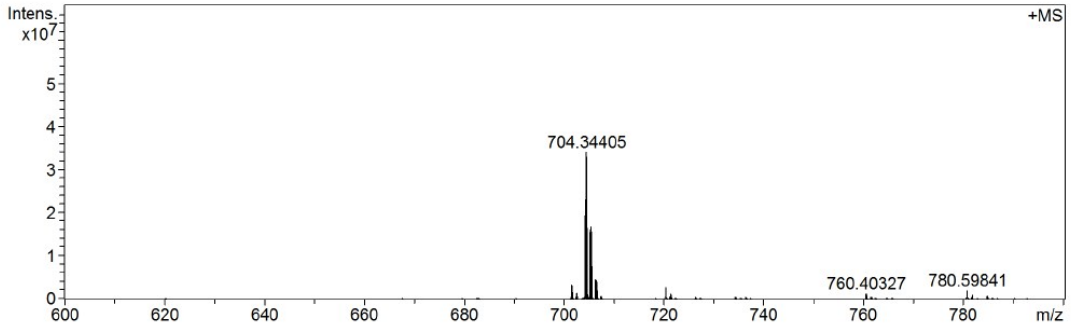
Flight Time to Acq. Cell	0.0 sec				



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704.34405	1	C 41 H 46 N 5 O 6	100.00	704.34426	0.2	0.3	15.8	21.5	even	ok

Fig. S12 HRMS (ESI) of compound **N3**.

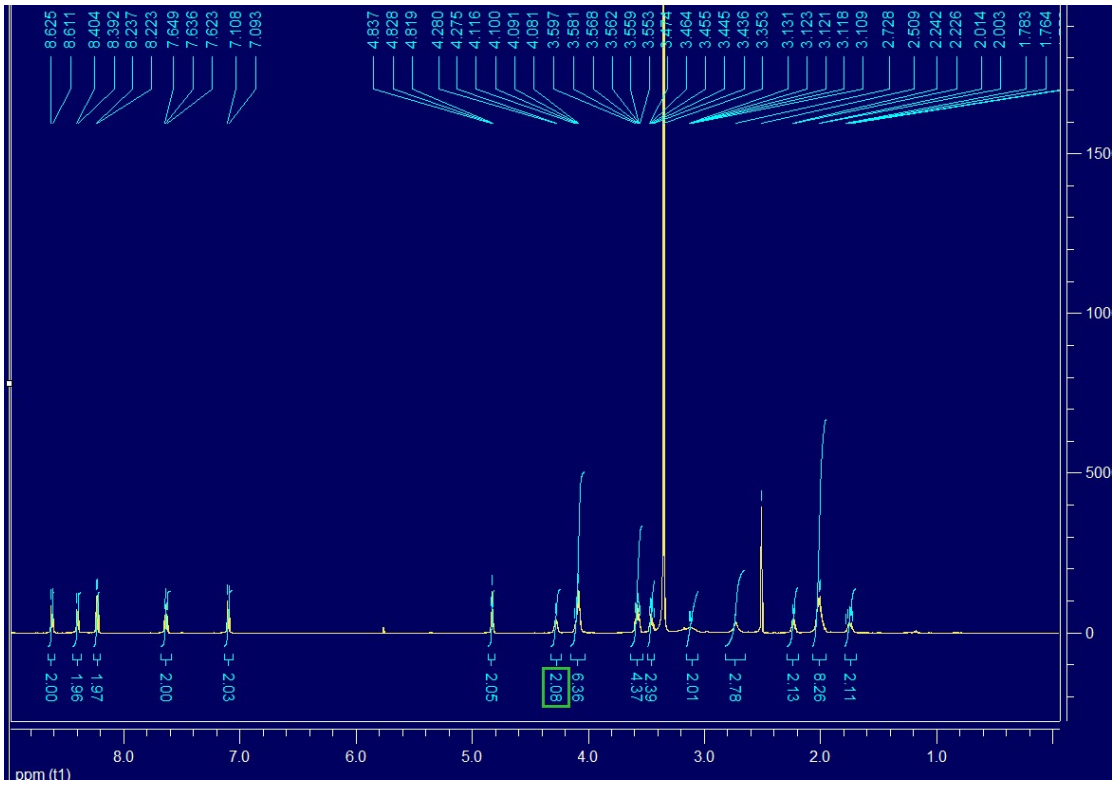

Fig. S13 ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O) of compound **N4**.

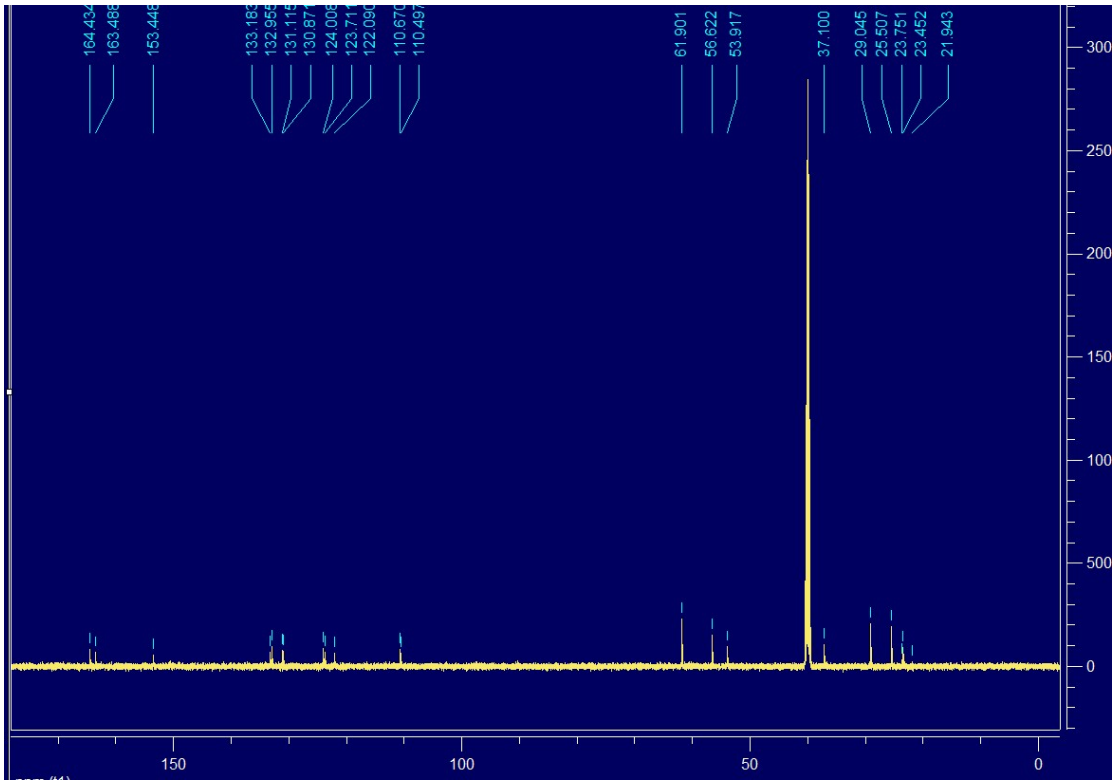

Fig. S14 ^{13}C NMR (150 MHz, DMSO-d_6 + D_2O) of compound **N4**.

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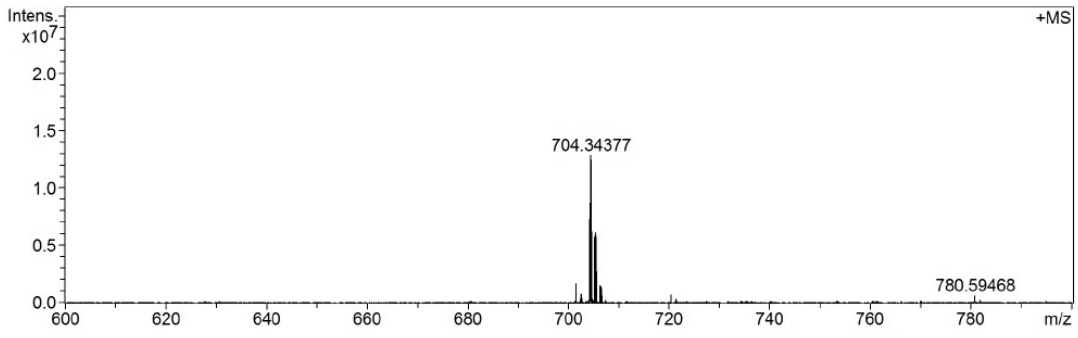
Analysis Info

Analysis Name	D:\Data\20131012\Q8-ESI-POS-MSMS_000002.d	Acquisition Date	2013/10/12 15:27:36
Method	20130927	Operator	
Sample Name		Instrument	apex-Ultra
Comment			

Acquisition Parameter

Polarity	Positive	Source	ESI	No. of Laser Shots	20
Averaged Scans	2	No. of Cell Fills	1	Laser Power	51.0 %
Broadband Low Mass	100.3 m/z	End Plate	3500.0 V	MALDI Plate	300.0 V
Broadband High Mass	800.0 m/z	Capillary Entrance	4000.0 V	Imaging Spot Diameter	2000.0 µm
Acquisition Mode	Single MS	Skimmer 1	20.0 V		
Pulse Program	basic	Drying Gas Temperature	180.0 °C	Calibration Date	Tue Oct 8 03:08:56 2013
Source Accumulation	0.0 sec	Drying Gas Flow Rate	4.0 L/min	Data Acquisition Size	131072
Ion Accumulation Time	0.0 sec	Nebulizer Gas Flow Rate	1.0 L/min	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.0 sec				



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
704.34377	1	C 41 H 46 N 5 O 6	100.00	704.34426	0.5	0.7	7.4	21.5	even	ok

Fig. S15 HRMS (ESI) of compound **N4**.

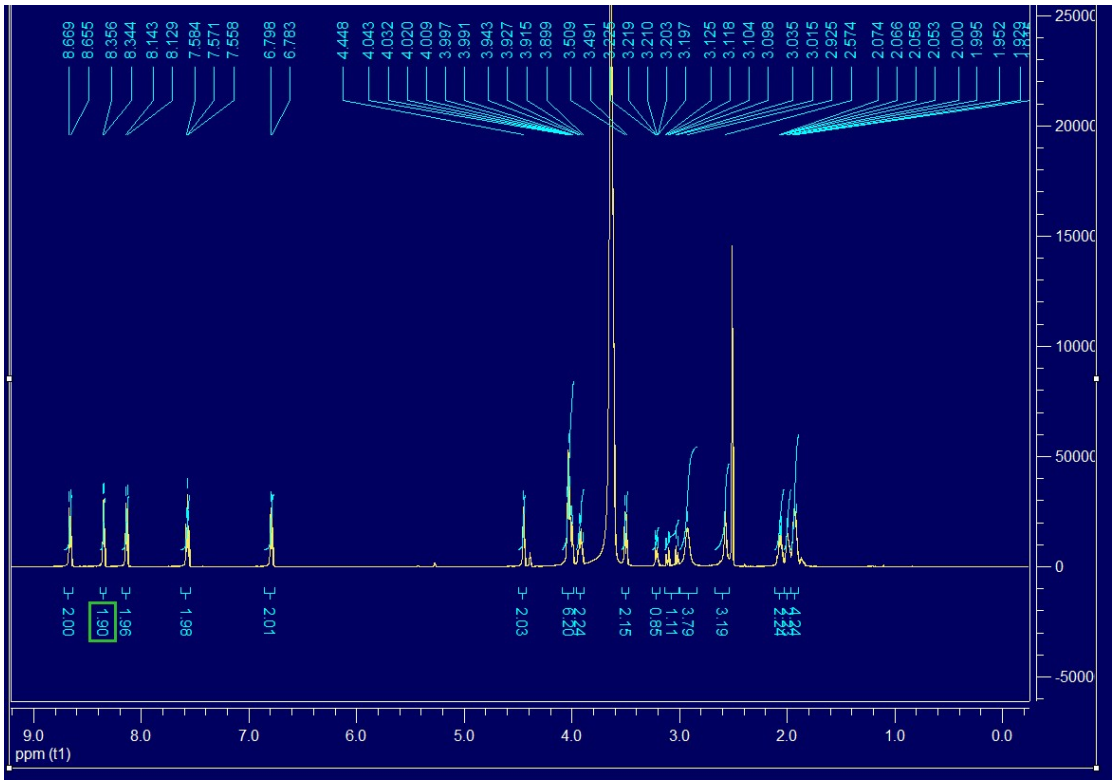


Fig. S16 ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O) of compound **N5**.

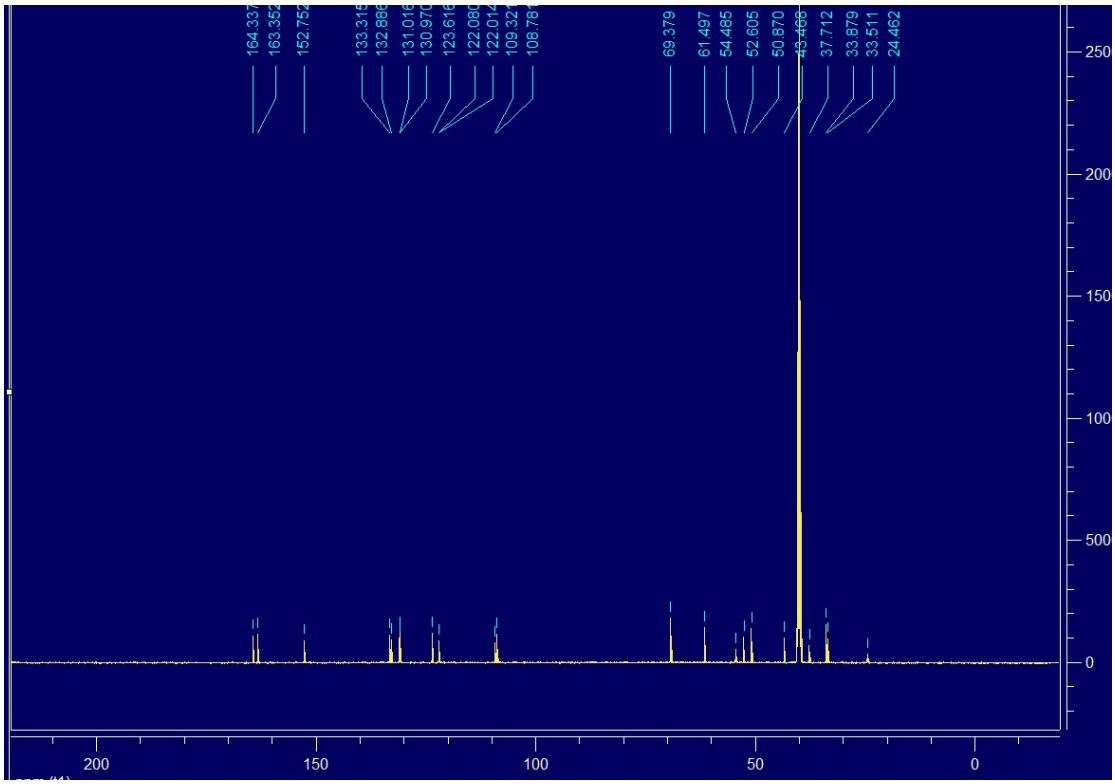


Fig. S17 ^{13}C NMR (150 MHz, DMSO-d_6 + D_2O) of compound **N5**.

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\20131012\Q10-ESI-POS-MSMS_000002.d	Acquisition Date	2013/10/12 15:29:51
Method	20130927	Operator	
Sample Name		Instrument	apex-Ultra
Comment			

Acquisition Parameter

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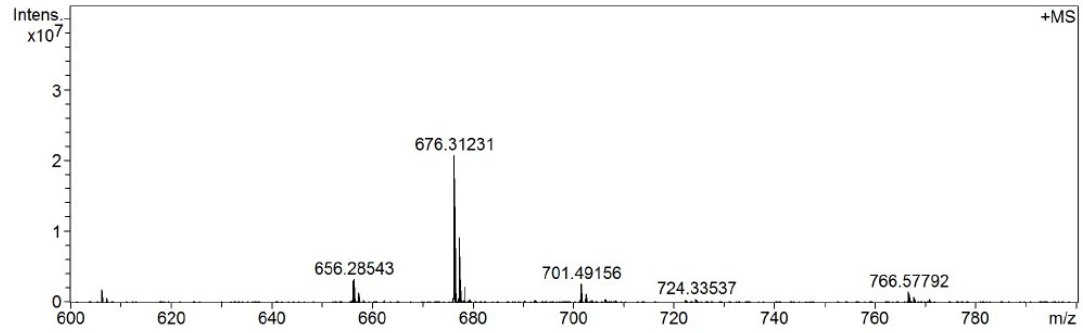
Averaged Scans	2	No. of Cell Fills	1	Laser Power	51.0 %
Broadband Low Mass	100.3 m/z	End Plate	3500.0 V	MALDI Plate	300.0 V
Broadband High Mass	800.0 m/z	Capillary Entrance	4000.0 V	Imaging Spot Diameter	2000.0 µm
Acquisition Mode	Single MS	Skimmer 1	20.0 V		
Pulse Program	basic	Drying Gas Temperature	180.0 °C	Calibration Date	Tue Oct 8 03:08:56 2013
Source Accumulation	0.0 sec	Drying Gas Flow Rate	4.0 L/min	Data Acquisition Size	131072
Ion Accumulation Time	0.0 sec	Nebulizer Gas Flow Rate	1.0 L/min	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.0 sec				



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676.31231	1	C 39 H 42 N 5 O 6	100.00	676.31296	0.7	1.0	4.1	21.5	even	ok

Fig. S18 HRMS (ESI) of compound **N5**.

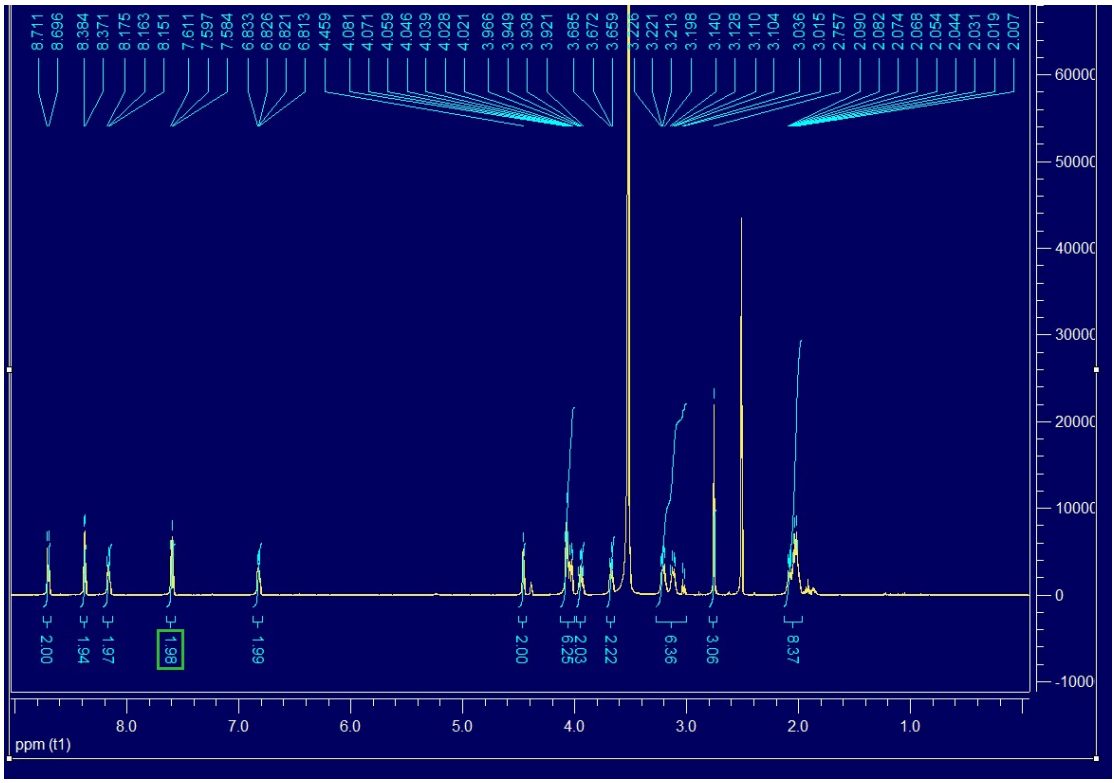

Fig. S19 ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O) of compound **N6**.

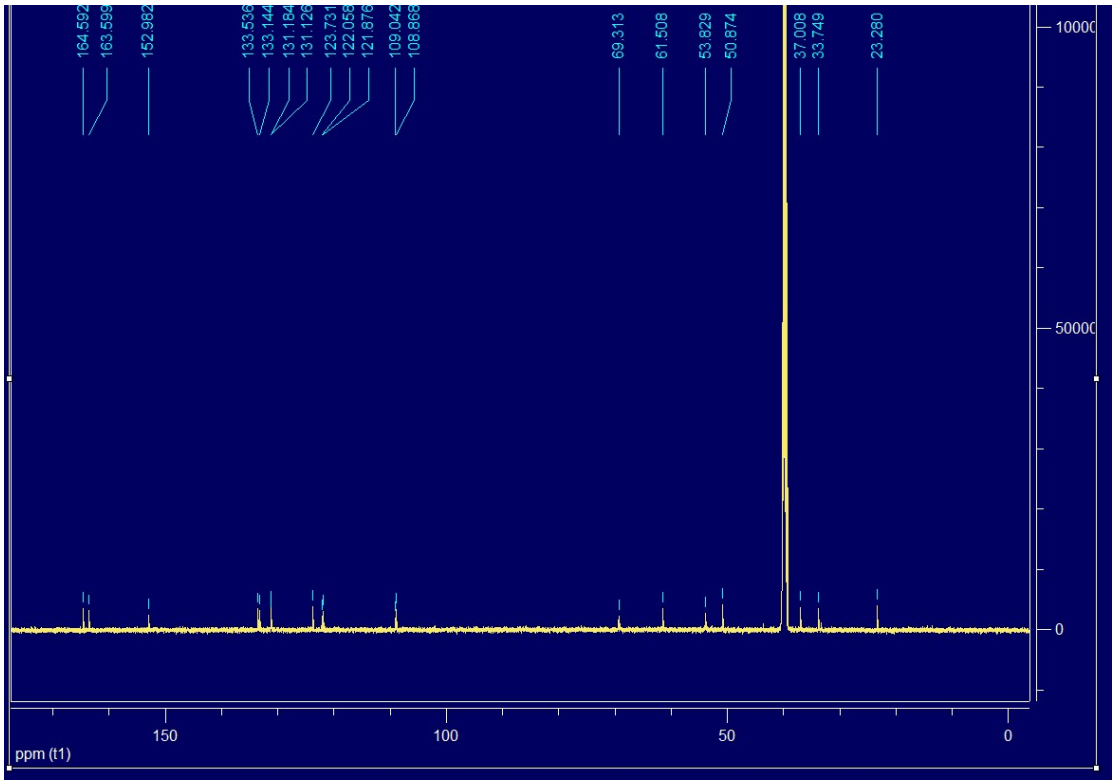

Fig. S20 ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O) of compound **N6**.

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\20131012\Q9-ESI-POS-MSMS_000002.d	Acquisition Date	2013/10/12 15:28:41
Method	20130927	Operator	
Sample Name		Instrument	apex-Ultra
Comment			

Acquisition Parameter

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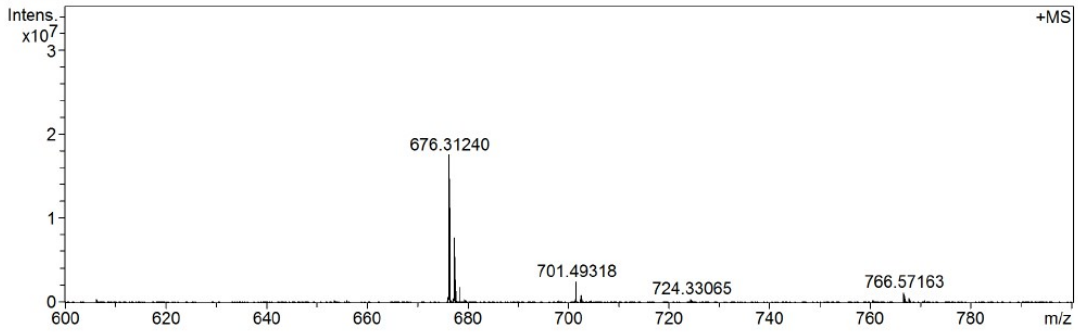
Broadband Low Mass	100.3 m/z	End Plate	3500.0 V	MALDI Plate	300.0 V
Broadband High Mass	800.0 m/z	Capillary Entrance	4000.0 V	Imaging Spot Diameter	2000.0 µm
Acquisition Mode	Single MS	Skimmer 1	20.0 V		
Pulse Program	basic	Drying Gas Temperature	180.0 °C	Calibration Date	Tue Oct 8 03:08:56 2013
Source Accumulation	0.0 sec	Drying Gas Flow Rate	4.0 L/min	Data Acquisition Size	131072
Ion Accumulation Time	0.0 sec	Nebulizer Gas Flow Rate	1.0 L/min	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.0 sec				



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¡¥Conf	N-Rule
676.31240	1	C 39 H 42 N 5 O 6	100.00	676.31296	0.6	0.8	3.6	21.5	even	ok

Fig. S21 HRMS (ESI) of compound **N6**.

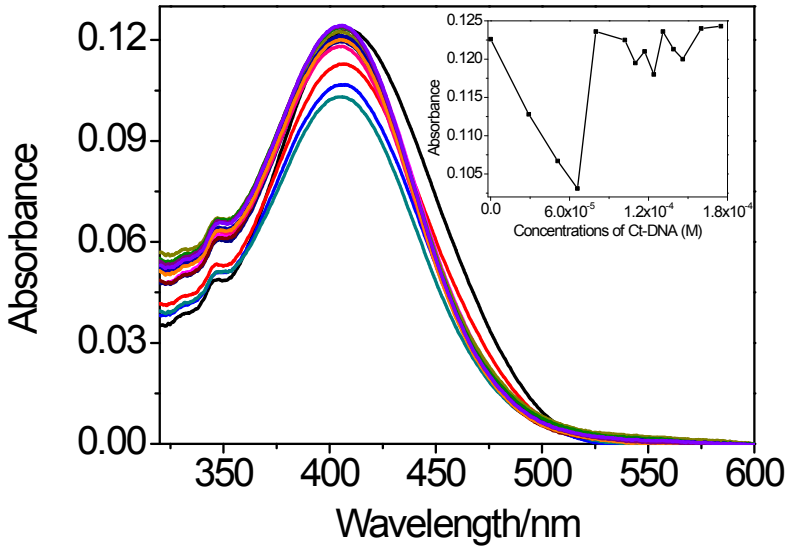


Fig. S22 UV-Vis spectra of compound NI1 (1× 10^{-5} M) binding with Ct-DNA in phosphate buffer (10 mM, pH 7.4, 50 mM NaCl).

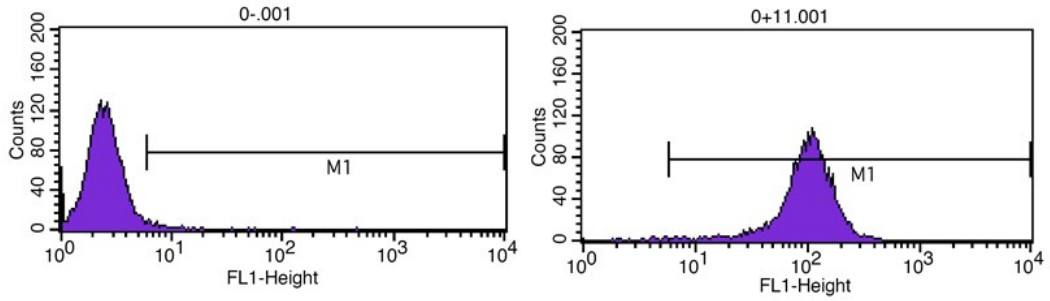


Fig. S23 Compound **NI1** induced lysosomal membrance permeabilization (LMP). Hela cells were stained with Annexin V-TITC. Numbers indicate the percentage of cells with Annexin V-TITC fluorescence.

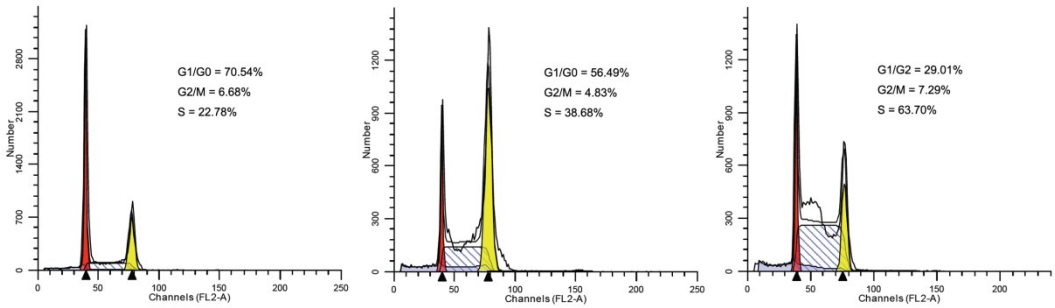


Fig. S24 Inhibition of cell cycle process in Hela cellstreated with Amonafide for 48 h. Cell cycle distribution was analyzed by flow cytometry.

Table S1. Quantitative determination of hematoxic effects of compound **NI1** on the numbers of platelets (PLT), red blood cells (RBC) and white blood cells (WBC) in healthy mice when administered i.v. with different doses (5 mg/kg, 10 mg/kg and 20 mg/kg). Analyses were performed at 24 h, 48 h and 96 h following administration.

	Doses (mg/kg)	PLT (10^9/µL)	RBC (10^{12}/µL)	WBC (10^9/µL)
Control		296.75±9.18	6.24±0.18	3.62±0.04
24 h	5	284.25±4.92*	6.34±0.05	3.48±0.24
	10	288.75±4.65	5.99±0.13	3.35±0.09
	20	299±6.48	5.8±0.46*	3.71±0.28
48 h	5	343.75±7.14*	6.1±0.25	3.71±0.28
	10	259.5±2.38*	6.19±0.96	2.03±0.09*
	20	407.75±23.16*	6.8±0.3	2.8±0.12*
96 h	5	343.5±6.56*	6.27±0.1	3.05±0.04*
	10	384.75±9.74*	6.2±1.4	3.14±0.17*
	20	364.5±6.61*	6.18±0.63	2.43±0.09*

*Statistical significance (control versus treatment) was assessed using the Mean-Whitney U-test: * = $p < 0.05$.*

Table S2. Quantitative determination of cardiotoxic effects of compound **NI1** on the levels of aspartate transaminase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH) in healthy mice when administered i.v. with different doses (5 mg/kg, 10 mg/kg and 20 mg/kg). Analyses were performed at 24 h, 48 h and 96 h following administration.

	Doses (mg/kg)	AST (U/L)	LDH (U/L)	CK (U/L)
Control		159.83±9.82	861.5±90.78	1342.5±60.64
24 h	5	175.13±5.91	958.80±9.92	1628.2±111.51
	10	311.9±6.87*	1778.2±72.29*	1832.0±109.12*
	20	356.87±38.79*	1805.9±87.32*	1799.9±305.68
48 h	5	123.97±7.20*	1109.3±59.21*	1292.1±60.21
	10	139.73±8.93*	985.3±78.63	1196.7±37.4
	20	146.8±9.44	947.63±44.87	2035.3±45.66*
96 h	5	146.33±3.22	1146.2±40.06*	1648.8±51.51*
	10	163.37±15.2	1213.5±104.36*	2155.3±63.85*
	20	166.5±11.21	999.13±70.76	1483.7±58.42*

*Statistical significance (control versus treatment) was assessed using the Mean-Whitney U-test: * = $p < 0.05$.*