Supporting information

for

Fluorescence enhancement, cellular imaging and biological investigation of chiral pyrrolidinols modified naphthalimide derivatives

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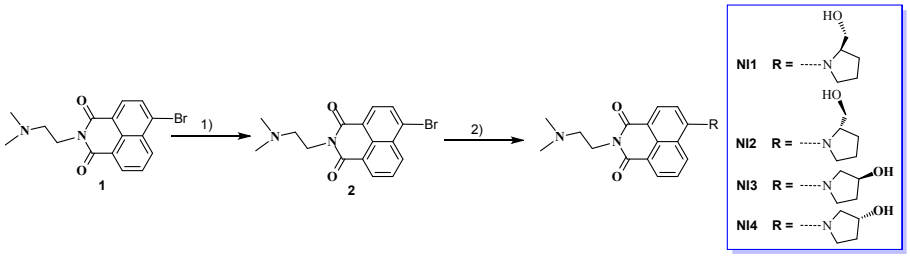

Scheme 1. 1) *N*,*N*-dimethylethylenediamine, EtOH; 2) 2-methoxyethanol, DIPEA, amino alcohols (L-prolinol, D-prolinol, R-3-hydroxypyrrolidine and S-3- hydroxypyrrolidine).

1. Experimental part

1.1 Measurements

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 600 spectrometer. HRMS analysis was performed on an Apex Ultra 7.0T FT-MS (Bruker Dalonik Company). UV−Vis spectra were

recorded in a quartz cell (light path 10 mm or 5 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a S-1700 temperature controller. Fluorescence spectra were performed on F-7000 (Hitachi Instruments). The fluorescence images were obtained using Olympus confocal laser scanning microscopy (Olympus Fluoview FV1000).

1.2 MTT assay

The compounds **NI1-4** were dissolved in phosphate buffered saline (PBS) and diluted to the required concentration with culture medium. The cytotoxicity was evaluated by MTT assay. Briefly, cells were plated in 96-well microassay culture plates (10^4 cells per well) and grown overnight at 37 °C in a 5% CO_2 incubator. The compounds **NI1-4** were then added to the wells to achieve final concentrations ranging from 10^{-7} to 10^{-4} M. Wells containing culture medium without cells were used as control blanks; wells containing culture medium and cisplatin or amonofide was used as positive control. The plates were incubated at 37 °C in a 5% CO_2 incubator for 48 h. Upon completion of the incubation, stock MTT (Sigma) dye solution (20 μL, 5 mg/μL) was added to each well. After 4 h incubation, 2-propanol (100 μL) was added to solubilize the MTT formazan. The optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} value was determined from the plot of % viability against dose of complexes added.

1.3 Confocal microscopy study

The A549 cells were seeded 1 day before experiments in a 6-well plate at 4.0×10^5 cells/well. Then cells were incubated with **NI1** (5.0 μM), **NI2** (4.0 μM), **NI3** (2.0 μM) and **NI4** (1.0 μM) at 37 °C for 12 h. After incubation, the unbound molecules were washed third with PBS buffer.

1.4 Synthesis procedures and analytical data

General synthesis of **NI1-4**: In a 50-mL flask, 500 mg (1.44 mmol) of compound **M-1** was dissolved in 10 mL of 2-methoxyethanol. Then, 2.88 mmol of amino alcohols (L-prolinol, D-prolinol, R-3-hydroxypyrrolidine and S-3- hydroxypyrrolidine) and 3 mL of DIPEA were added, and the mixture was heated under an N_2 atmosphere at 120°C for 24 h or 48 h. After the mixture was cooled to room temperature, the solvent was removed *in vacuo*. The solid obtained was purified by column chromatography to afford pure **NI1-4** with the yields of 62.6%, 58.8%, 50.8% and 39.1%.

NI1: m. p. 92.4-94.2 °C ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.82 (m, 1H), 2.09 (m, 1H), 2.16 (m, 1H),

2.31 (m, 1H), 2.72 (m, 6H), 3.11 (t, 2H, J = 6.0 Hz), 3.62 (t, 1H, J = 8.4 Hz), 3.75 (dd, 1H, J = 3.0 Hz, 11.4 Hz), 3.82 (dd, 1H, J = 4.8 Hz, 11.4 Hz), 4.13 (m, 1H), 4.31 (m, 1H), 4.38 (m, 2H), 7.06 (d, 1H, J = 8.4 Hz, Ar-H), 7.52 (t, 1H, J = 7.8 Hz, Ar-H), 8.28 (d, 1H, J = 8.4 Hz, Ar-H), 8.43 (d, 1H, J = 7.2 Hz, Ar-H), 8.48 (d, 1H, J = 8.4 Hz, Ar-H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 25.64, 28.47, 36.33, 44.64, 56.19, 57.45, 61.26, 62.23, 110.81, 111.80, 122.08, 123.73, 124.34, 130.74, 130.78, 131.28, 132.13, 133.00, 153.47, 163.75, 164.51; HRMS (ESI): calcd. for $C_{21}H_{26}N_3O_3$: 368.1974, found 368.1969.

NI2: m.p. 89.2-90.6 °C ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.84 (m, 1H), 2.10 (m, 1H), 2.16 (m, 1H), 2.32 (m, 1H), 2.77 (m, 6H), 3.17 (t, 2H, J = 6.0 Hz, -CH_2), 3.64 (t, 1H, J = 8.4 Hz), 3.77 (dd, 1H, J = 3.0 Hz, 11.4 Hz), 3.82 (dd, 1H, J = 4.8 Hz, 11.4 Hz), 4.13 (m, 1H), 4.32 (m, 1H), 4.43 (m, 2H), 7.08 (d, 1H, J = 8.4 Hz, Ar-H), 7.54 (t, 1H, J = 7.8 Hz,), 8.33 (d, 1H, J = 8.4 Hz, Ar-H), 8.47 (d, 1H, J = 7.2 Hz, Ar-H), 8.49 (d, 1H, J = 8.4 Hz, Ar-H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 25.63, 28.54, 35.62, 44.18, 55.97, 57.42, 61.32, 62.39, 111.01, 123.86, 124.48, 130.91, 131.45, 132.32, 133.18, 153.65, 163.86, 164.71; HRMS (ESI): calcd. for $C_{21}H_{26}N_3O_3$: 368.1974, found 368.1963.

NI3: m.p. 102.3-104.6 °C. ^{1}H NMR (CD_3OD, 600 MHz): δ 2.17 (m, 1H), 2.22 (m, 1H), 2.57 (s, 6H), 2.93 (m, 2H), 3.67 (d, 1H, J = 10.8 Hz), 3.77 (t, 1H, J = 7.8 Hz), 4.10 (m, 2H), 4.34 (t, 2H, J = 7.2 Hz, -CH_2), 4.62 (m, 1H), 6.88 (d, 1H, J = 8.4 Hz, Ar-H), 7.59 (t, 1H, J = 7.8 Hz, Ar-H), 8.30 (d, 1H, J = 9.0 Hz, Ar-H), 8.48 (d, 1H, J = 7.2 Hz, Ar-H), 8.72 (d, 1H, J = 9.0 Hz, Ar-H); ^{13}C NMR (CD_3OD, 150 MHz): δ 37.23, 40.24, 47.71, 54.29, 60.45, 64.61, 73.70, 112.39, 113.03, 125.58, 126.22, 126.81, 134.86, 135.15, 136.71, 137.33, 157.23, 168.18, 169.02; HRMS (ESI): calcd. for $C_{20}H_{24}N_3O_3$: 354.1818, found 354.1813.

NI4: m.p. 110.4-112.8 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.19 (m, 2H), 2.39 (s, 6H), 2.69 (m, 2H), 3.61 (m, 2H), 3.73 (m, 1H), 3.99 (m, 1H), 4.06 (m, 1H), 4.33 (t, 2H, J = 7.2 Hz), 4.68 (s, 1H), 6.81 (d, 1H, J = 8.4 Hz, Ar-H), 7.55 (t, 1H, J = 7.2 Hz, Ar-H), 8.41 (d, 1H, J = 9.0 Hz, Ar-H), 8.54 (d, 1H, J = 8.4 Hz, Ar-H), 8.57 (d, 1H, J = 7.2 Hz, Ar-H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 34.05, 37.59, 45.45, 50.56, 56.77, 60.95, 108.81, 123.28, 131.17, 131.89, 133.45, 152.72, 164.12, 164.92; HRMS (ESI): calcd. for $C_{20}H_{24}N_3O_3$: 354.1818, found 354.1809.

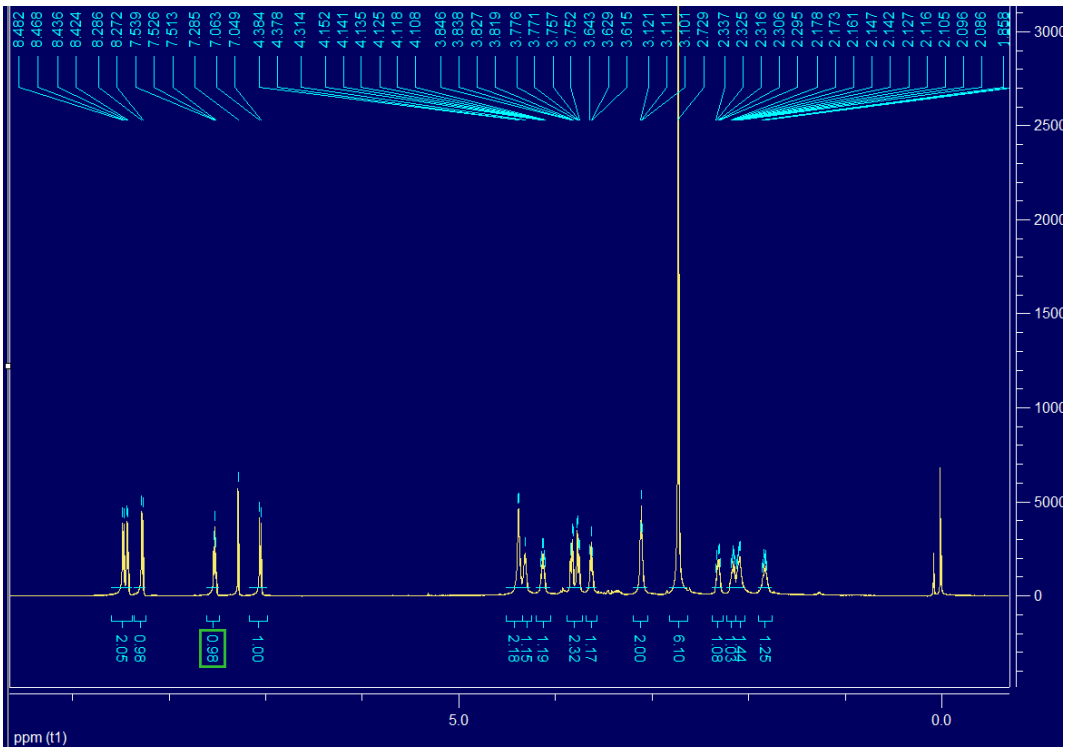

Fig. S1 ^{1}H NMR of compound **NI1** (600 MHz, $CDCl_3$).

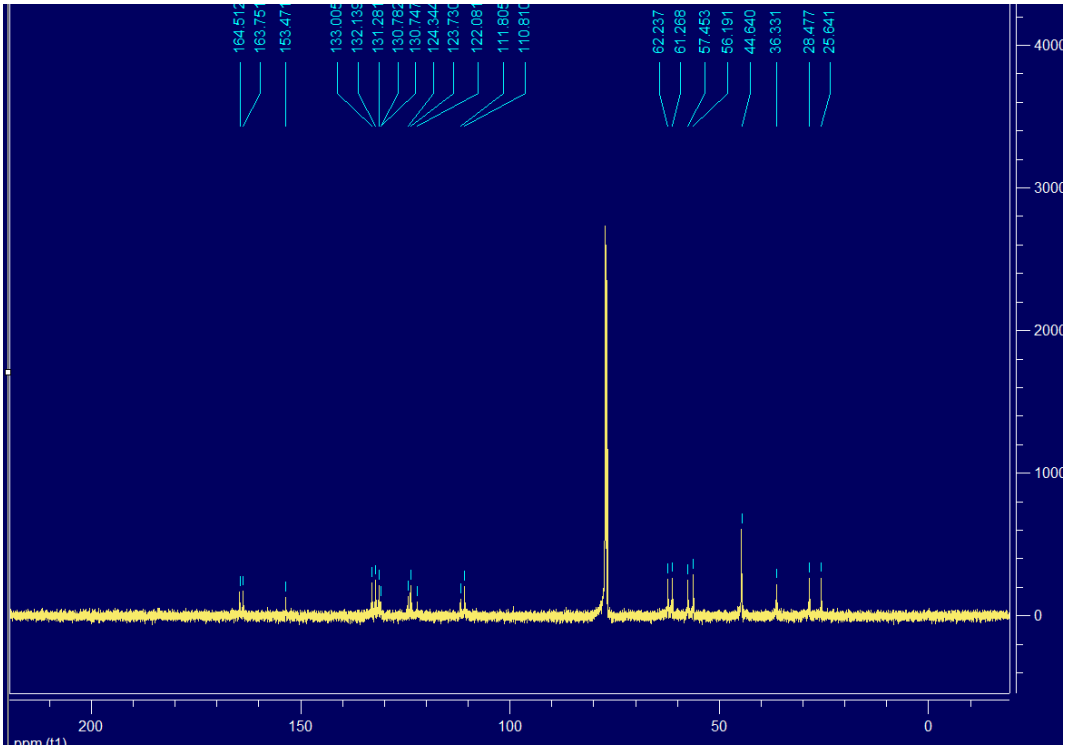

Fig. S2 ^{13}C NMR of compound **NI1** (150 MHz, $CDCl_3$).

Mass Spectrum SmartFormula Report

Analysis Info

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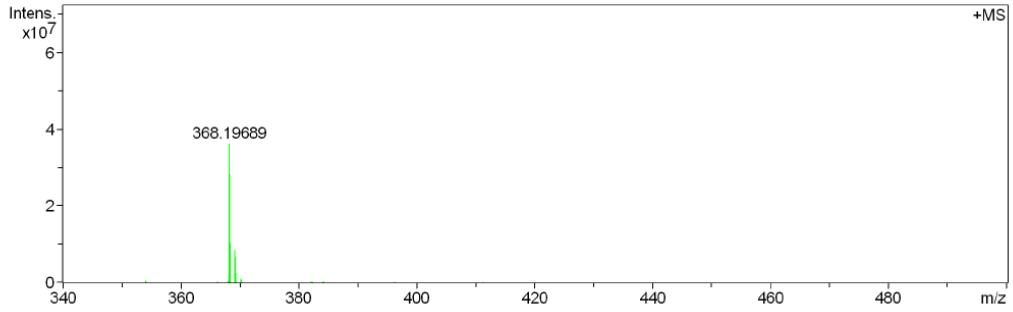
Method	LC_MS_SmallMolecular	Operator	ChuanqiZhou@163.com
Sample Name		Instrument	apex-Ultra

Acquisition Parameter

Polarity	Positive	Source	APCI	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	1500.0 V	MALDI Plate	300.0 V
Broadband High Mass	1000.0 m/z	Capillary Entrance	2000.0 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	Sat Apr 6 08:55:38 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
368.19689	1	C 21 H 26 N 3 O 3	100.00	368.19687	-0.0	-0.1	3.1	10.5	even	ok

Fig. S3 HRMS (ESI) of compound **NI1**.

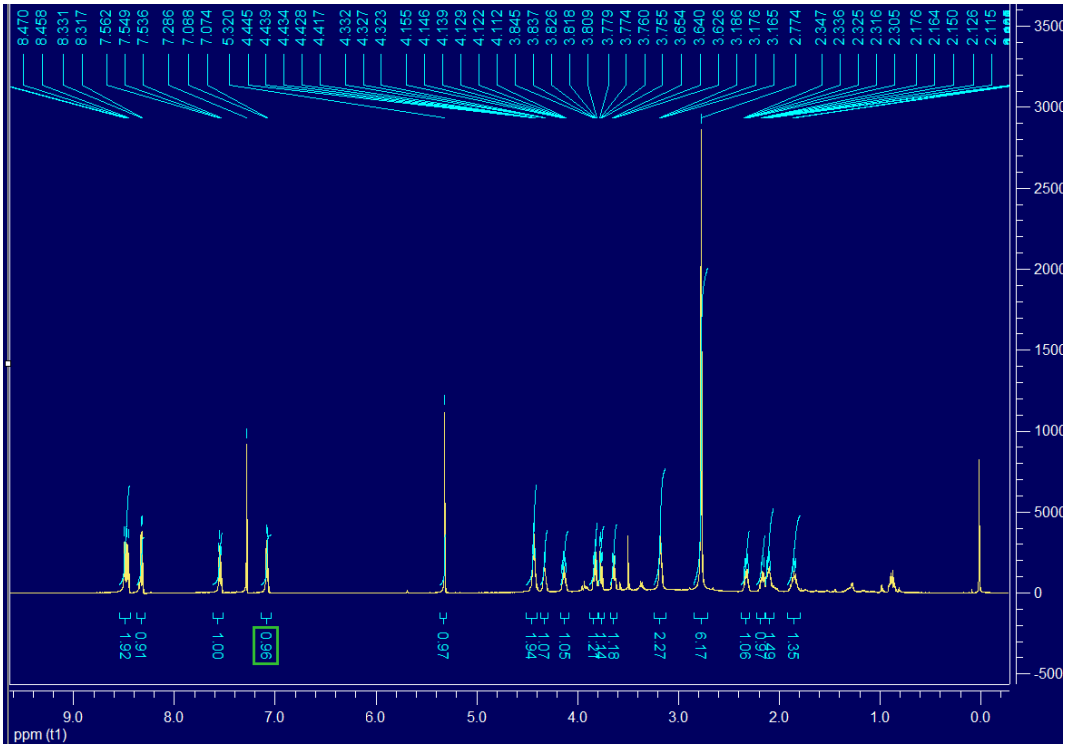


Fig. S4 ^{1}H NMR of compound **NI2** (600 MHz, $CDCl_3$).

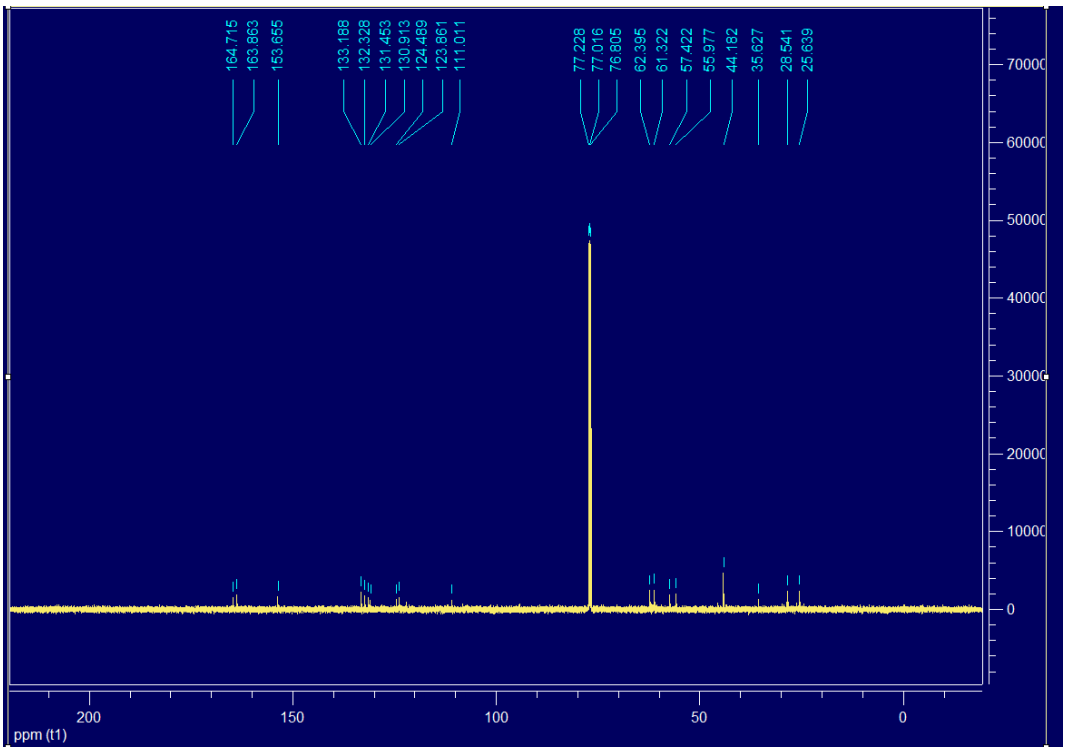


Fig. S5 ^{13}C NMR of compound **NI2** (150 MHz, $CDCl_3$).

Mass Spectrum SmartFormula Report

Analysis Info

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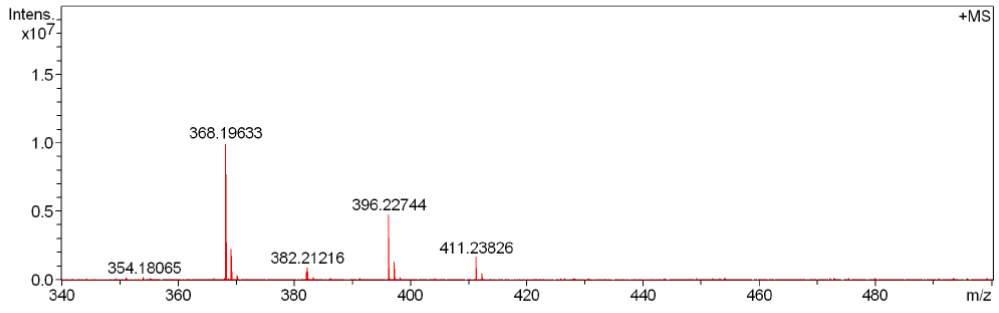
Method	LC_MS_SmallMolecular	Operator	ChuanqiZhou@163.com
Sample Name		Instrument	apex-Ultra

Acquisition Parameter

Polarity	Positive	Source	APCI	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	1500.0 V	MALDI Plate	300.0 V
Broadband High Mass	1000.0 m/z	Capillary Entrance	2000.0 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	Sat Apr 6 08:55:38 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
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Fig. S6 HRMS (ESI) of compound **NI2**.

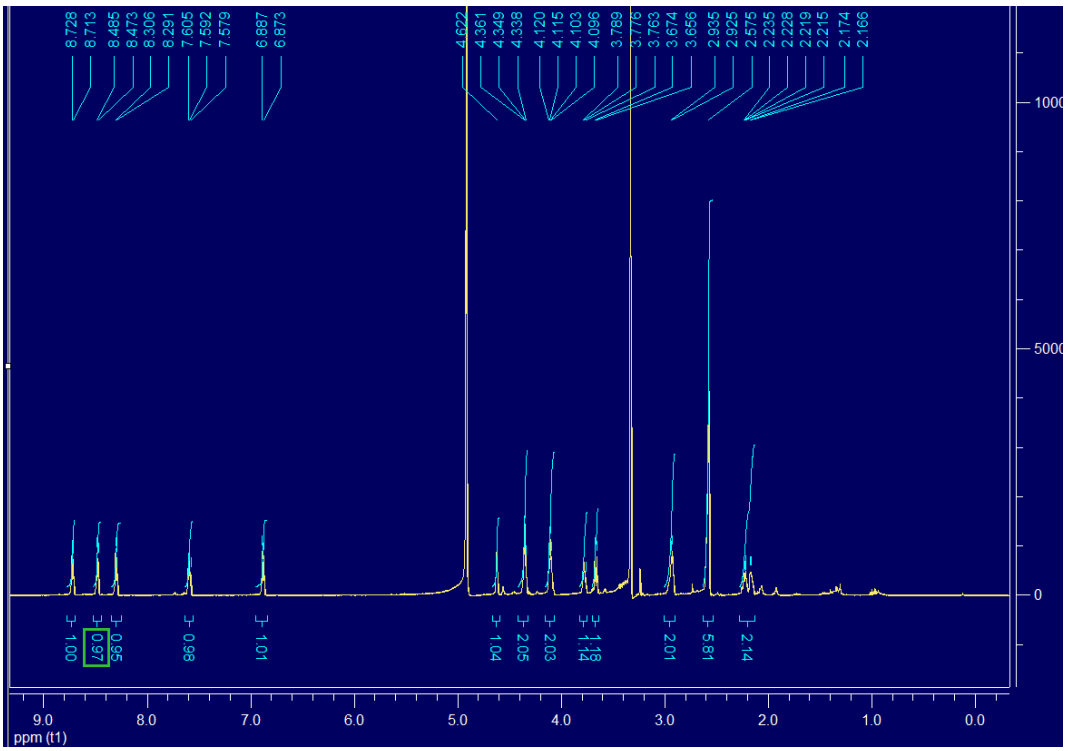

Fig. S7 ^{1}H NMR of compound **NI3** (600 MHz, CD_3OD).

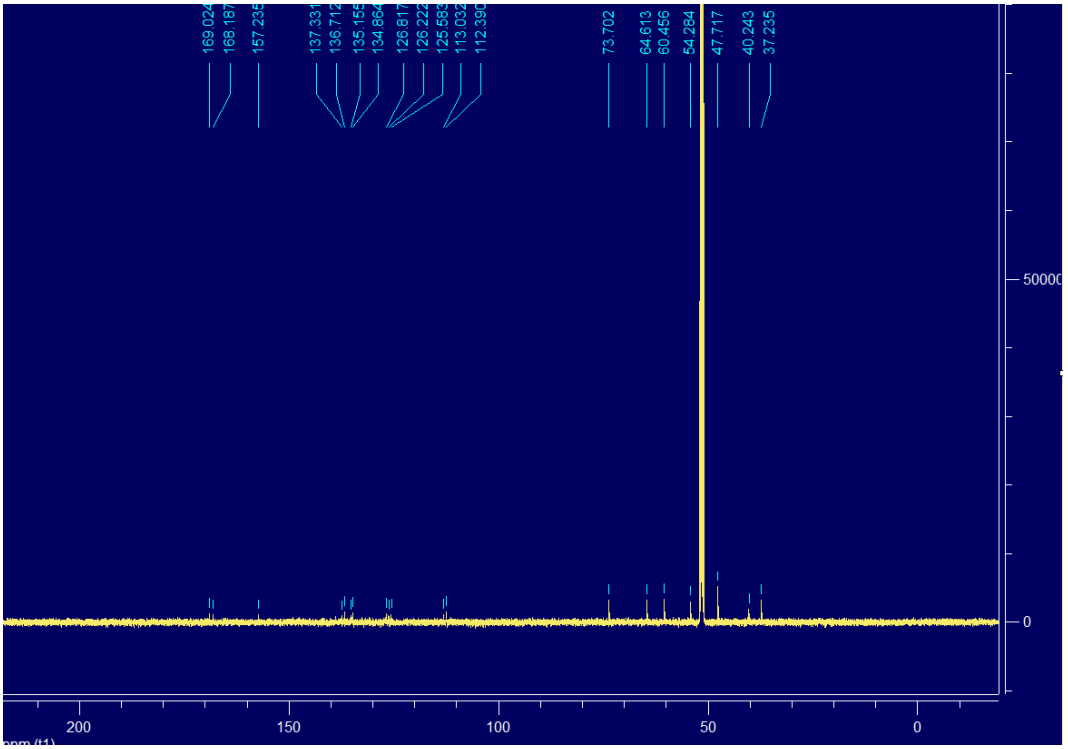

Fig. S8 ^{13}C NMR of compound **NI3** (150 MHz, CD_3OD).

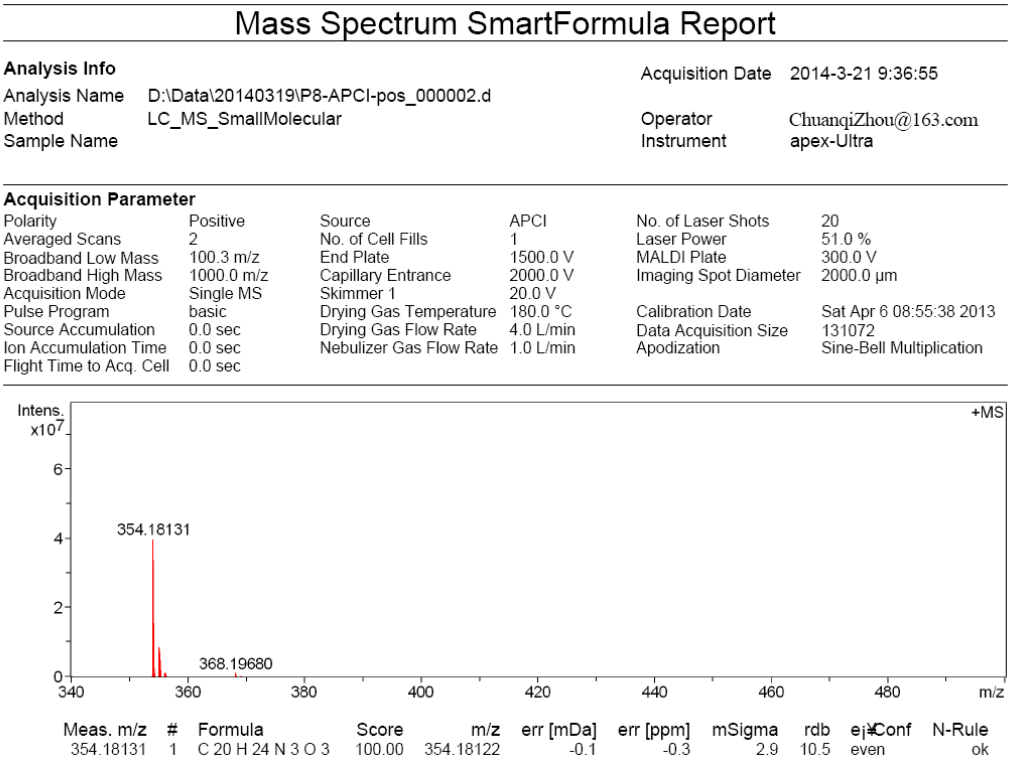

Fig. S9 HRMS (ESI) of compound **NI3**.

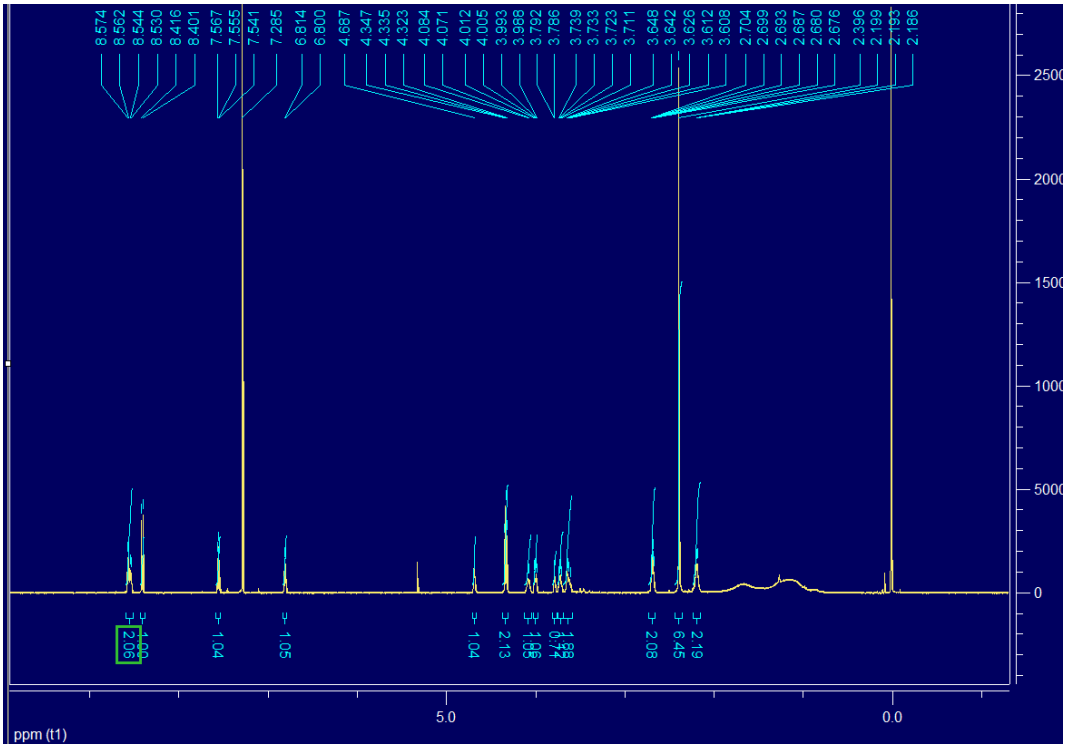

Fig. S10 ^{1}H NMR of compound **NI4** (600 MHz, $CDCl_3$).

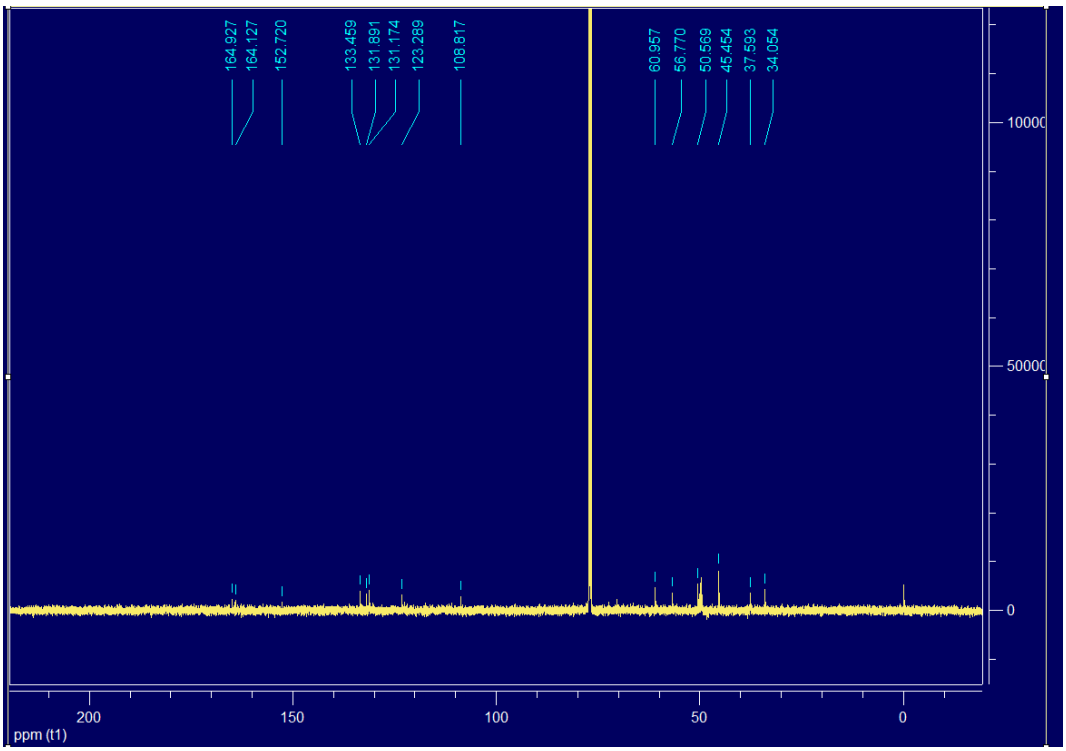

Fig. S11. ^{13}C NMR of compound NI4 (150 MHz, $CDCl_3$).

Mass Spectrum SmartFormula Report

Analysis Info

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Method	LC_MS_SmallMolecular	Operator	ChuanqiZhou@163.com
Sample Name		Instrument	apex-Ultra

Acquisition Parameter

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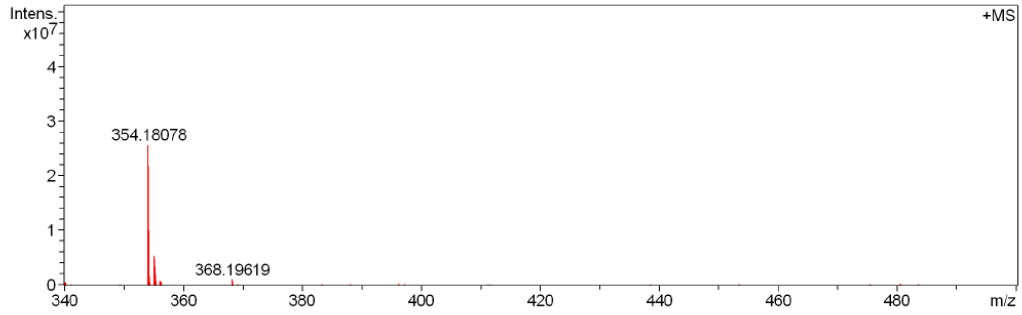
Averaged Scans	2	No. of Cell Fills	1	Laser Power	51.0 %
Broadband Low Mass	100.3 m/z	End Plate	1500.0 V	MALDI Plate	300.0 V
Broadband High Mass	1000.0 m/z	Capillary Entrance	2000.0 V	Imaging Spot Diameter	2000.0 µm
Acquisition Mode	Single MS	Skimmer 1	20.0 V		
Pulse Program	basic	Drying Gas Temperature	180.0 °C	Calibration Date	Sat Apr 6 08:55:38 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
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Fig. S12 HRMS (ESI) of compound **NI4**.

Table S1. Cytotoxicity data for compounds **NI1-4** (IC_{50}, μM)

compounds	IC_{50} (μM)			
	Hela	MCF-7	SGC-7901	A549
NI1	15.209±0.053	42.704±0.119	28.389±0.0499	5.4211±0.056
NI2	21.343±0.021	99.662±0.098	51.597±0.117	3.654±0.023
NI3	3.845±0.183	3.531±0.039	3.404±0.274	1.961±0.042
NI4	3.249±0.312	3.686±0.099	2.546±0.307	0.874±0.023
Amonofide	4.365±0.135	8.022±0.038	5.327±0.200	1.595±0.072
Cisplatin	13.413±0.062	7.73±0.094	15.057±0.102	4.776±0.048

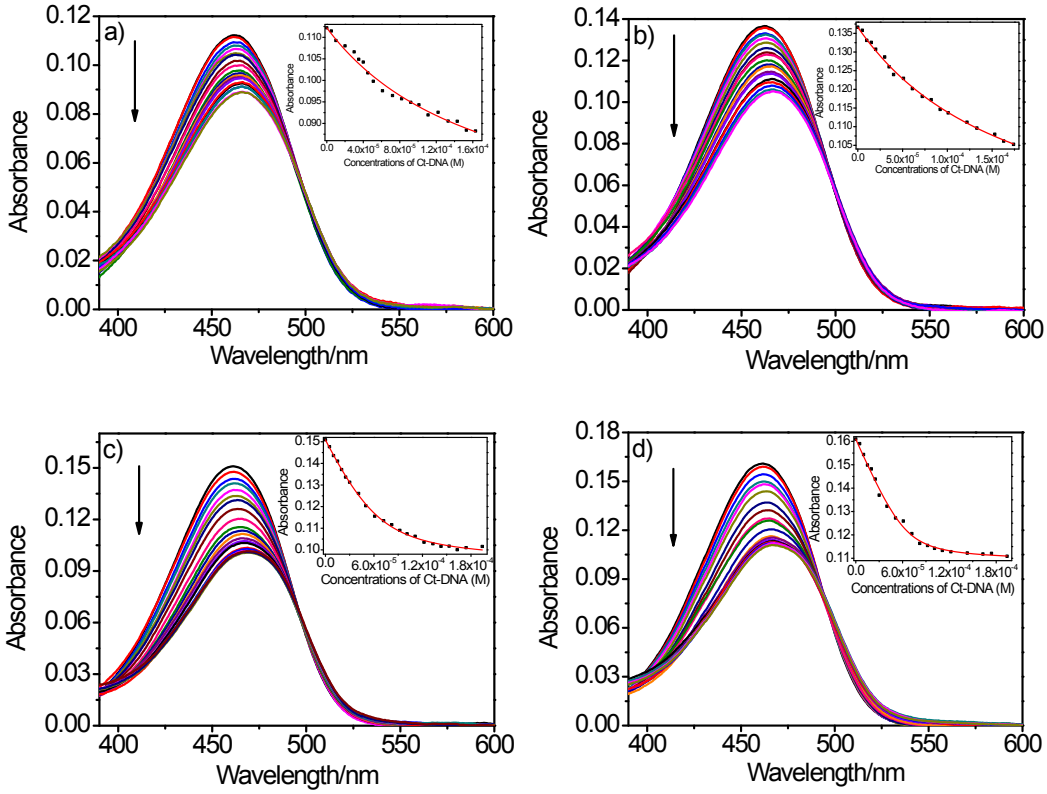

Fig. S13 UV-Vis spectra of compounds **NI1-4** (1×10^{-5} M) binding with Ct-DNA in phosphate buffer (10 mM, pH 7.4, 50 mM NaCl); Inset: the fitting curves at the maximum absorption band.

Table S2. Average T_m and ΔT_m for Ct-DNA in the absence and presence of **NI1-4**.

Compounds	T_m (°C)	ΔT_m (°C)
Ct-DNA	69.8	0
NI1	71.7	1.9
NI2	71.6	1.8
NI3	74.1	4.3
NI4	73.4	3.6

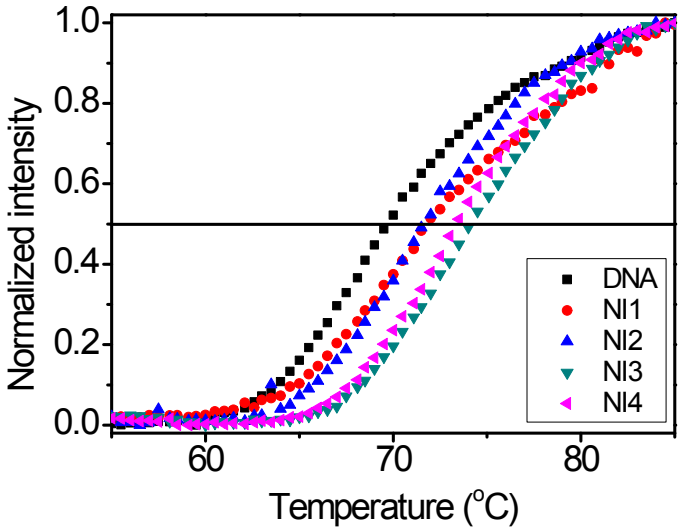

Fig. S14. The T_m curves of compounds **NI1-4** (5.0×10^{-6} M) binding with Ct-DNA (5.0×10^{-5} M) (69.8, 71.8, 71.7, 73.4, 74.0) in phosphate buffer (1 mM, pH 7.4, 5 mM NaCl).