Electronic Supplementary Information

A novel NBD-based pH "on-off" fluorescent probe equipped with the N-phenylpiperazine group for lysosome Imaging

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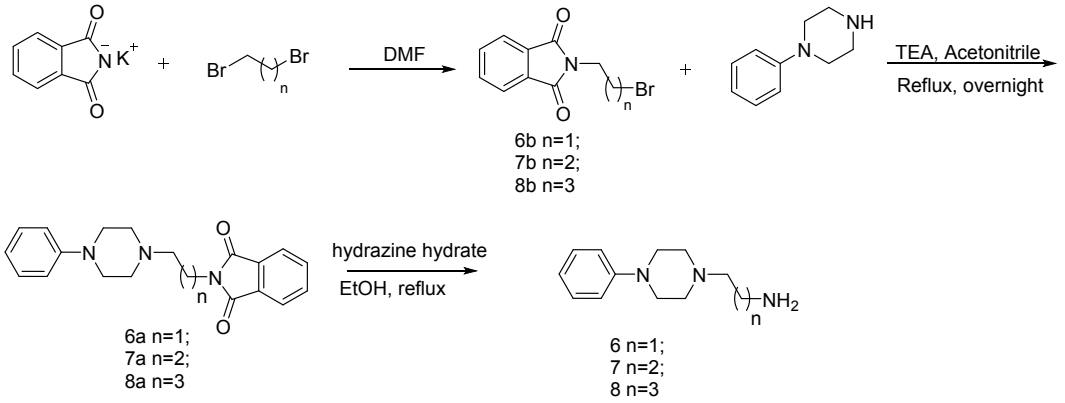
1. Materials and instruments

General chemicals used in the synthesis were purchased from J&K, Accela and Aladdin. Buffer reagents purchased from Sigma-Aldrich and Acroswere used without purification. Water used in activity studies was doubly distilled and further purified with a Mill-Q filtration system. Lyso Tracker Red, Mito Tracker Red and Golgi Tracker Red were available commercially from the Beyotime Institute of Biotechnology (Shanghai, China). Absorption spectra, fluorescence spectra and quantum yield were recorded using Varioskan microplate reader (Thermo Electron Corporation). Melting points were determined on an electrothermal melting point apparatus and were uncorrected.^{1}HNMR and ^{13}CNMR were recorded on Bruker 400 NMR spectrometers. Mass spectra were obtained using the analytical and mass spectrometry facilities at Shandong University. Confocal microscopy was recorded using a Zeiss LSM 700 or LSM 780 at the Microscopy Characterization Facility, Shandong University.

2. Synthesis



Scheme S1 Synthesis of intermediate compounds **6**, **7** and **8.**

2-(2-bromoethyl)isoindoline-1,3-dione (6b)

To the mixture of potassium phthalimide (2.0 g, 10.8 mmol) in 10 mL DMF was added 1,2-dibromoethane (6.1 g, 32.4 mmol) rapidly. The mixture was stirred at r.t. for 24 h, and then the solvent was evaporated in vacuum. The residue was dissolved with EtOAc (200 mL) and water (50 mL). The organic phase was collected, and washed with saturated ammonium chloride solution (100 mL × 2) and brine (100 mL × 2), and then dried with anhydrous Na_2SO_4 overnight. The solvent was concentrated into oil, and then 30 mL methanol was added to produce much white solid as compound 6b. Yield: 2.24 g, 82.3%. M.p. 77-79 °C。^{1}HNMR (400MHz, $CDCl_3$): δ 7.82-7.79 (m, 2 H), 7.70-7.67 (m, 2 H), 4.06 (t, J = 6.8 Hz, 2 H), 3.57 (t, J = 6.8 Hz, 2 H).

2-(3-bromopropyl)isoindoline-1,3-dione (7b)

Compound **7b** was synthesized synthesized as described for compound **6b**, except for the use of 1, 3-dibromopropane (6.5 g, 32.4 mmol). Yield: 2.52 g, 87.2%; M.p. 64-66 °C。^{1}HNMR (400MHz, $CDCl_3$): δ 7.88-7.86 (m, 2 H), 7.76-7.73 (m, 2 H), 3.88 (t, J = 6.8 Hz, 2 H), 3.45 (t, J =

6.8 Hz, 2 H), 2.32-2.25(m, 2 H).

2-(4-bromobutyl)isoindoline-1,3-dione (8b)

Compound **8b** was synthesized synthesized as described for compound **6b**, except for the use of 1, 4-dibromobutane (7.0 g, 32.4 mmol). Yield: 1.46 g, 48.0%; M.p. 79-80 °C。^{1}HNMR (400MHz, $CDCl_3$): δ 7.79-7.70 (m, 2 H), 7.66-7.64 (m, 2 H), 3.68 (t, J = 6.4 Hz, 2 H), 3.39 (t, J = 6.0 Hz, 2 H), 1.86-1.77(m, 4 H).

2-(2-(4-phenylpiperazin-1-yl)ethyl)isoindoline-1,3-dione (6a)

The solution of N-phenylpiperazine (697 mg, 4.3mmol), **6b** (913 mg, 3.6mmol), triethylamine (1.6 mL, 10.8mmol) in 20 mL acetonitrile was refluxed overnight. After the mixture was allowed to cool to ambient temperature, the acetonitrile was evaporated under the vacuum condition. The residue was recrystallized from ethanol to afford yellowish solid as compound **6a**. Yield: 700 mg, 58.0%; M.p. 151-153°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ7.85 (2 H, m), 7.72(2 H, m), 7.25 (2 H, td, J_1 = 6.8 Hz, J_2= 2.0 Hz),6.91 (2 H, d, J = 8.0 Hz), 6.85 (1 H, t, J = 7.2 Hz),3.88(2 H, t, J = 6.8 Hz), 3.15 (4 H, t, J = 4.8 Hz), 2.72 (6 H, m); ESI-MS: m/z [M+H$^+$] calcd for $C_{20}H_{22}N_3O_2^+$336.2,found 336.2

2-(3-(4-phenylpiperazin-1-yl)propyl)isoindoline-1,3-dione (7a)

The solution of N-phenylpiperazine (486.6 mg, 3 mmol), **7b** (665.2 mg, 2.5 mmol), triethylamine (1.1 mL, 7.5 mmol) in 20 mL acetonitrile was refluxed overnight. After the mixture was allowed to cool to ambient temperature, the acetonitrile was evaporated under the vacuum condition. And thenthe residue was purified by silica gel column chromatography (EA : PE=1 : 2~ EA) to afford white solid as compound **7a**. Yield: 620 mg, 71.0%; M.p. 125-127;^{1}HNMR (400 MHz, $CDCl_3$): δ 7.85 (2 H, m), 7.70 (2 H, m), 7.25 (2 H, td, J_1 = 6.8 Hz, J_2= 1.6 Hz), 6.89 (3 H, m), 3.81 (2 H, t, J = 6.8 Hz), 3.06 (4 H, t, J = 4.8 Hz), 2.56(4 H, t, J = 5.2 Hz), 2.50 (2 H, t, J = 7.2 Hz), 1.94 (2 H, m);ESI-MS: m/z [M+H$^+$] calcd for $C_{21}H_{24}N_3O_2^+$350.2, found 350.5.

2-(4-(4-phenylpiperazin-1-yl)butyl)isoindoline-1,3-dione (8a)

The solution of N-phenylpiperazine (697 mg, 4.3 mmol), **8b** (1010 mg, 3.6 mmol), triethylamine (1.6 mL, 10.8 mmol) in 20 mL acetonitrile was refluxed overnight. After the mixture was allowed to cool to ambient temperature, the acetonitrile was evaporated under the vacuum condition. The residue was recrystallized from ethanol to afford yellowish solid as compound **8a**. Yield: 900 mg, 68.8%; M.p. 135-137;^{1}HNMR (400 MHz, $CDCl_3$): δ 7.84 (2 H, m), 7.74 (2 H, m), 7.28 (2 H, m), 6.93 (2 H, d, J = 8.0 Hz), 6.86 (1 H, t, J = 7.2 Hz), 3.75 (2 H, t, J = 7.2 Hz), 3.20 (4 H, t, J = 4.8 Hz), 2.61 (4 H, t, J = 5.2 Hz), 2.45 (2 H, t, J = 7.6 Hz), 1.78 (2 H, m) 1.62 (2 H, m); ESI-MS: m/z [M+H$^+$] calcd for $C_{22}H_{26}N_3O_2^+$364.2, found 364.5.

2-(4-phenylpiperazin-1-yl)ethan-1-amine (6)

To the solution of N-phthaloyl derivatives compound **6a** (600 mg, 1.79mmol) dissolved in 30 mL heated ethanol was added the hydrazine monohydrate (343 mg, 6.87 mmol). After refluxed for 4 h, the mixture was allowed to cool to ambient temperature. The precipitate was filtered and the filtrate was evaporated under the vacuum condition to afford white solid as **6**. Yield: 350 mg, 94.9%; M.p. 104-107°C; ^{1}HNMR (400 MHz, CD_3OD): δ 7.36 (2, t, J = 8.4 Hz), 7.08 (2 H, d, J = 8.0 Hz), 6.96 (1 H, t, J = 7.2 Hz), 3.31 (4 H, t, J = 4.8 Hz), 3.03 (2 H, t, J = 6.4 Hz), 2.78 (4 H, t, J = 5.2 Hz), 2.69 (2 H, t, J = 6.4 Hz).ESI-MS: m/z [M+H$^+$] calcd for $C_{12}H_{20}N_3^+$ 206.2, found 206.3.

3-(4-phenylpiperazin-1-yl)propan-1-amine (7)

Compound 7 was synthesized synthesized as described for compound **6**, except for the use of 7a

(600 mg, 1.72mmol).Yield: 300 mg, 79.3%; M.p. 76-79°C;[1]HNMR (400 MHz, CD_3OD): δ 7.24 (2, t, J = 8.8 Hz), 6.97 (2 H, d, J = 8.0 Hz), 6.85 (1 H, t, J = 7.2 Hz), 3.19 (4 H, t, J = 4.8 Hz), 2.84 (2 H, t, J = 6.8 Hz), 2.66 (4 H, t, J = 4.8 Hz), 2.52 (2 H, t, J = 7.2 Hz), 1.79 (2 H, m);ESI-MS: m/z [M+H$^+$] calcd for $C_{13}H_{22}N_3^+$220.2, found 220.4

4-(4-phenylpiperazin-1-yl)butan-1-amine (8)

Compound **8** was synthesized synthesized as described for compound **6**, except for the use of **8a** (600 mg, 1.65mmol).Yield: 350 mg, 90.6%; M.p. 104-106°C;[1]HNMR (400 MHz, CD_3OD): δ 7.36 (2, t, J = 8.4 Hz), 7.09 (2 H, d, J = 8.0 Hz), 6.97 (1 H, t, J = 7.2 Hz), 3.12 (4 H, t, J = 4.8 Hz), 2.94 (2 H, t, J = 6.4 Hz), 2.78 (4 H, t, J = 5.2 Hz), 2.58 (2 H, t, J = 6.8 Hz), 1.74 (4 H, m); ESI-MS: m/z [M+H$^+$] calcd for $C_{14}H_{24}N_3^+$ 234.2, found 234.4.

***N*^{1},*N*^{1}-dimethyl-*N*^{2}-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)ethane-1,2-diamine (LN1)**

To the solution of **1**(200 mg, 1.0 mmol) in 5 mL acetontrile was added dropwise **2**(460 mg, 5.0 mmol) in 5 mL acetontrile. After stirred for 2 h at room temperature, the solution was diluted by ethyl acetate (200 mL), washed by saturated sodium bicarbonate solution (50 mL×2), water (50 mL × 2) and brine (50 mL × 2), and then dried by anhydrous $MgSO_4$for 6h. The solvent was evaporated under the vacuum condition and the residue was purified by silica gel column chromatography (EtOAc:MeOH=200:1) to afford **LN1** as reddish brown solid. Yield: 70 mg, 27.9%; M.p. 134-136°C; [1]HNMR (400 MHz, $CDCl_3$): δ 8.49 (d, J = 8.8 Hz, 1 H), 6.14 (d, J = 8.4 Hz, 1 H), 3.50 (s, 2 H), 2.74 (t, J = 5.6 Hz, 2 H), 2.33 (s, 6 H); [13]CNMR (100 MHz, $CDCl_3$): δ 144.2, 144.0, 144.0, 136.5, 123.7, 98.7, 56.1, 44.9, 40.0. ESI-HRMS: m/z [M+H$^+$] calcd for $C_{10}H_{14}N_5O_3^+$ 252.1091, found 252.1090.

***N*^{1},*N*^{1}-dimethyl-*N*^{3}-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)propa-ne-1,3-diamine (LN2)**

LN2 was synthesized as described for **LN1**, except for the use of **3** (510 mg, 5.0 mmol). Yield: 80 mg, 30.2%; M.p. 140-142°C; [1]HNMR (400 MHz, $CDCl_3$): δ 8.45 (d, J = 8.4 Hz, 1 H), 6.01 (d, J = 8.0 Hz, 1 H), 3.60 (s, 2 H), 2.66 (s, 2 H), 2.38 (s, 6 H), 1.94 (s, 2 H); [13]CNMR (100 MHz, $CDCl_3$): δ144.9, 144.6, 144.1, 136.9, 122.5, 97.5, 59.0, 45.1, 45.0, 23.4. ESI-HRMS: m/z [M+H$^+$] calcd for $C_{11}H_{16}N_5O_3^+$ 266.1248, found 266.1248.

***N*-(2-morpholinoethyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine (LN3)**

To the solution of**4** (130.2 mg, 1.0 mmol) and triethylamine (415 μL, 3.0 mmol) in 15 mL acetontrile was added dropwise the solution of **1** (200 mg, 1.0 mmol) in 5 mL acetontrile. After stirred for 4 h at room temperature, the solution was diluted by dichloromethane (200 mL), washed by water (50 mL × 2) and brine (50 mL × 2), and then dried by anhydrous $MgSO_4$for 6h. The solvent was evaporated under the vacuum condition and the residue was purified by silica gel column chromatography (EtOAc :PE=1:2-2:1) to afford **LN3** as brown solid. Yield: 100 mg, 34.1%; M.p. 238-240°C; [1]HNMR (400 MHz, $CDCl_3$): δ 8.46 (d, J = 8.8 Hz, 1 H), 6.14 (d, J = 8.4 Hz, 1 H), 3.80 (t, J = 4.4 Hz, 4 H), 3.55 (s, 2 H), 2.84 (t, J = 6.0 Hz, 2 H), 2.59 (t, J = 4.0 Hz, 4 H); [13]CNMR (100 MHz, $CDCl_3$): δ 144.2, 143.9, 143.8, 136.4, 123.9, 98.8, 66.9, 55.4, 53.2, 39.4; ESI-HRMS: m/z [M+H$^+$] calcd for $C_{12}H_{16}N_5O_4^+$ 294.1197, found 294.1202.

***N*-(3-morpholinopropyl)-7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ami-ne (LN4)**

To the solution of **5** (144.2 mg, 1.0 mmol) and triethylamine (415 μL, 3.0 mmol) in 15 mL acetontrile was added dropwise the solution of **1** (200 mg, 1.0 mmol) in 5 mL acetontrile. After stirred for 2 h at room temperature, the precipitate was filtered, washed with acetonitrile (10 mL) and methanol (5 mL), to afford brownish red solid as **LN4**. Yield: 190 mg, 61.9%; M.p. 141-144°C; [1]HNMR (400 MHz, DMSO-d_6): δ 9.68 (s, 1 H), 8.54 (d, J = 8.4 Hz, 1 H), 6.44 (d, J = 8.8

Hz, 1 H), 3.60 (m, 6 H), 2.42 (m, 6 H), 1.86 (t, *J* = 6.4 Hz, 2 H); ^{13}CNMR (100 MHz, DMSO-d_6): δ145.7, 145.0, 144.7, 138.5, 121.1, 99.6, 66.6, 56.2, 53.7, 42.6, 24.6. ESI-HRMS: m/z [M+H$^+$] calcd for $C_{13}H_{18}N_5O_4^+$ 308.1353, found 308.1348.

7-nitro-N-(2-(4-phenylpiperazin-1-yl)ethyl)benzo[*c*][1,2,5]oxadi-azol-4-amine (LN5)

LN5 was synthesized as described for **LN3**, except for the use of **6** (103 mg, 0.5 mmol).Yield: 32 mg, 17.4%; M.p. 207-210°C;^{1}HNMR (100 MHz, DMSO-d_6):δ9.40 (s, 1H), 8.53 (d, *J* = 8.4 Hz, 1 H), 7.22 (t, *J* = 8.4 Hz, 2 H), 6.94 (d, *J* = 8.0 Hz, 2 H), 6.79 (t, *J* = 7.2 Hz, 1 H), 6.49 (d, *J* = 8.8 Hz, 2 H),3.65 (s, 2 H), 3.13 (s, 4 H), 2.73 (t, *J* = 6.4 Hz, 2 H), 2.64 (t, *J* = 4.8 Hz, 2 H);^{13}CNMR (100 MHz, DMSO-d_6):δ151.5, 145.6, 144.9, 144.7, 138.4, 129.2, 121.2, 119.3, 115.8, 99.8, 55.9, 53.1, 48.7, 41.5.ESI-HRMS: m/z [M+H$^+$] calcd for $C_{18}H_{21}N_6O_3^+$369.1670, found 369.1670.

7-nitro-*N*-(3-(4-phenylpiperazin-1-yl)propyl)benzo[*c*][1,2,5]oxa-diazol-4-amine (LN6)

LN6 was synthesized as described for **LN3**, except for the use of **7** (109 mg, 0.5 mmol). Yield: 30 mg, 15.7%; M.p. 187-189°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ9.83 (s, 1H),8.42 (d, *J* = 8.4 Hz, 1 H),7.33 (t, *J* = 7.6 Hz, 2 H), 7.01 (d, *J* = 7.6 Hz, 2 H), 6.93 (t, *J* = 7.2 Hz, 1 H), 6.01 (d, *J* = 8.8 Hz, 2 H),3.60 (s, 2 H), 3.42 (t, *J* = 4.8 Hz, 4 H), 2.78-2.75 (m, 6 H), 2.04-1.99(m, 2 H);^{13}CNMR (100 MHz, $CDCl_3$): δ151.2, 144.6, 144.4, 144.0, 136.7, 129.2, 122.8, 120.1, 116.5, 97.7, 58.2, 53.5, 49.0, 45.2, 22.5.ESI-HRMS: m/z [M+H$^+$] calcd for $C_{19}H_{23}N_6O_3^+$383.1826, found383.1833.

7-nitro-*N*-(4-(4-phenylpiperazin-1-yl)butyl)benzo[*c*][1,2,5]oxad-iazol-4-amine (LN7)

LN7 was synthesized as described for **LN3**, except for the use of **8** (116 mg, 0.5 mmol).Yield: 34 mg, 17.2%; M.p. 112-114°C;^{1}HNMR (400 MHz, $CDCl_3$): δ 8.45 (d, *J* = 8.4 Hz, 1 H), 7.79 (s, 1H), 7.33 (t, *J* = 8.0 Hz, 2 H), 6.97 (d, *J* = 8.0 Hz, 2 H), 6.89 (t, *J* = 7.2 Hz, 1 H), 6.14 (d, *J* = 8.8 Hz, 2 H),3.52 (t, 2 H), 3.34 (t, *J* = 4.8 Hz, 4 H), 2.70 (t, *J* = 4.8 Hz, 4 H), 2.55 (t, *J* = 6.8 Hz, 2 H), 1.96-1.91 (m, 2 H), 1.83-1.78 (m, 2 H);^{13}CNMR (100 MHz, $CDCl_3$): δ151.3, 144.4, 144.2, 144.1, 136.7, 129.3, 123.6, 120.1, 116.3, 98.5, 56.9, 53.3, 51.0 (methaol), 49.1, 44.2, 25.8, 24.6. ESI-HRMS: m/z [M+H$^+$] calcd for $C_{20}H_{25}N_6O_3^+$397.1983, found397.1983

3. Measurement of optical properties

All samples were prepared at room temperature, and before measurement, each solution was incubated for 5 min. All of the compounds (**LN1-7**) were dissolved with DMSO to prepare the stock solutions (1 mM). The absorption and fluorescence spectra were in 10 mM PBS solution (pH = 7.40). The relative quantum yield for each sample in 10 mM PBS was calculated according the equation below:

$$\Phi_x = \Phi_s(A_s/A_x)\,(FA_x/FA_s)\,(\eta_x/\eta_s)^2$$

Where Φ is the quantum yield, A is the absorbance at the excitation wavelength. FA is the area under corrected emission curve, n is the refractive index of the solvent. X refers to the sample, and S refers the standard. Fluorescien was chosen as the standard, which has the fluorescence quantum yield of 0.92 in 0.1 M NaOH solution.

The pH response of compounds was tested in the Britton-Robinson buffer solutions (40 mM acetic acid, boric acid, and phosphoric acid) with various pH values (3.2-10.0), and the pKa values were calculated according to the literature.[1]

In the case of the investigation of photostability, the prepared solutions (30 μM) of **LN6** dissolved in 40 mM B-R buffers (pH=4.0) were illuminated continuously by ultraviolet light at

365 nm and their fluorescence intensity were recorded at different time intervals.

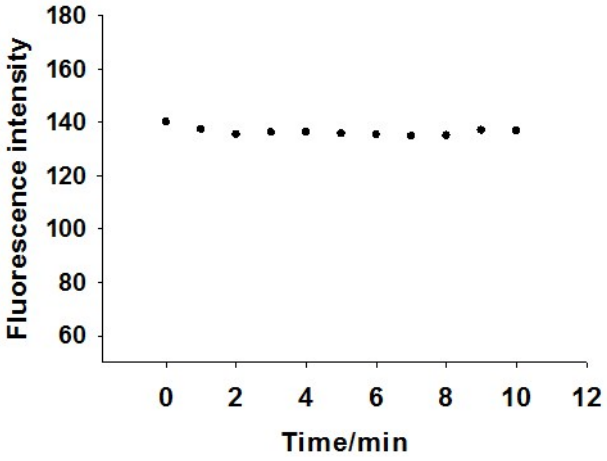


Fig. S1 Fluorescence intensity of **LN6** (10 μM) in B-R buffers (pH = 4.0) solution illuminated continuously by ultraviolet light at 365 nm. λ_{ex} = 470 nm, λ_{em} = 545 nm.

4. Cell culture, MTT assay and cell imaging

HeLa cells were grown in DMEM medium supplemented with 10% (v/v) fetal bovine serum in an atmosphere of 5% CO2 and 95% air at 37°C. Cells with density of 5×10^4/mL were seeded noto confocal dishes in 2 mL medium, incubated for 24 h, and then washed with cell culture medium once. For imaging research, the samples were prepared as the procedure in legend of each figure. The compounds **LN1-7** were used at the concentration of 50 μM, and the three commercial trackers were used according their instructions for use. The cell imaging was performed on the LSM700 or LSM780 confocal microscope under a 63× immersion objective. All images were adjusted by ImageJ software, and the colocalization coefficient was calculated using this software as well.

The cell viability of HeLa cells exposed to compounds **LN1-7** was evaluated using a 24 h MTT assay on 96-well plates. The absorbance values were recorded at 590 nm to avoid the interference from absorption of **LN1-7**.

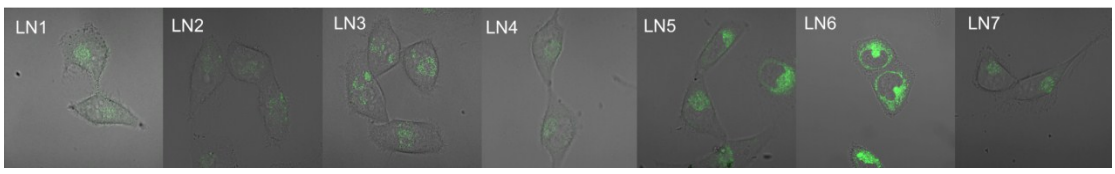
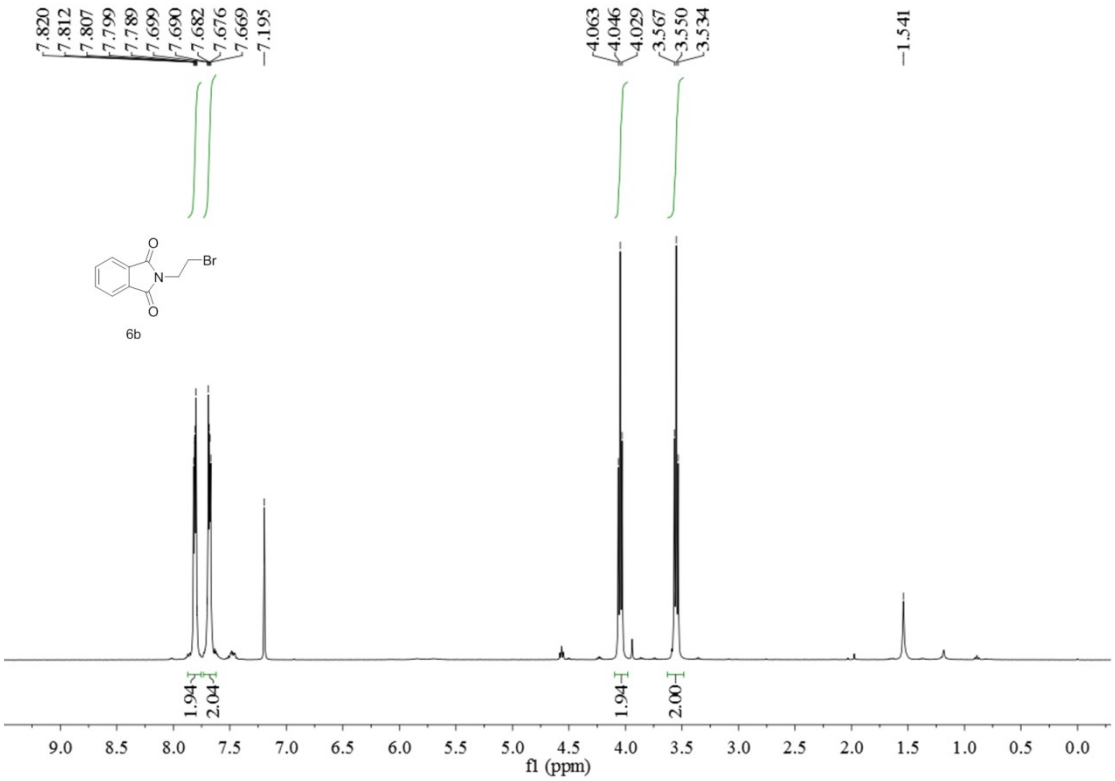


Fig. S2 Confocal images of Hela cells treated by compounds **LN1**, **LN2**, **LN3**, **LN4**, **LN5**, **LN6** or **LN7,** respectively. Cells were incubated with compounds (50 μM) for 1 h at 37 °C and then the probe solutions were discarded followed by washing with PBS buffer once. Then the cell imaging were carried out on the LSM 700 or 780 microscope (λ_{ex}=488 nm). Objective lens, 63×.

5. NMR and MS data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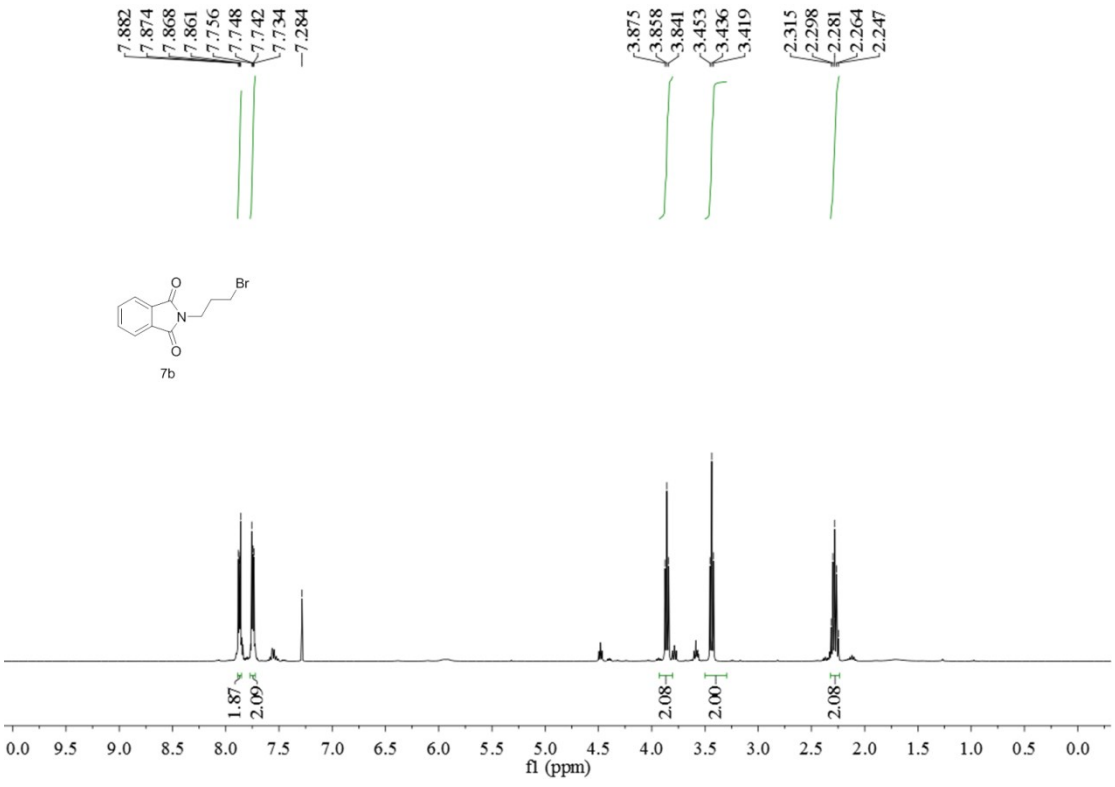
fl (ppm)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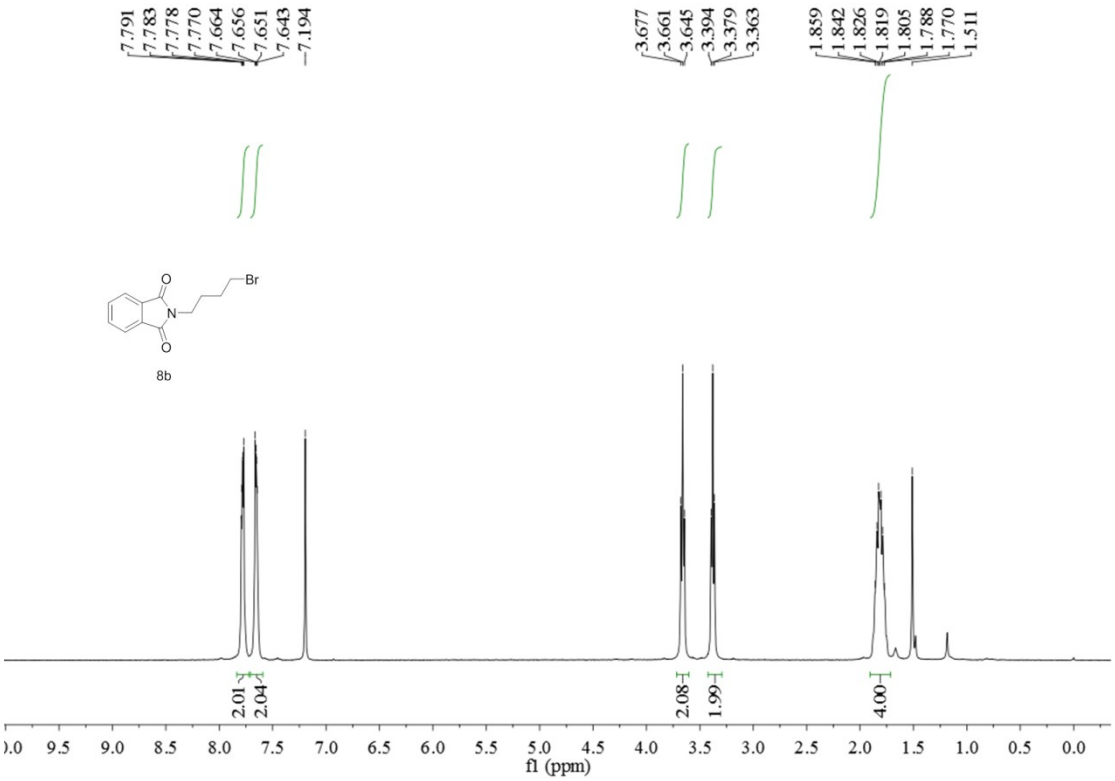
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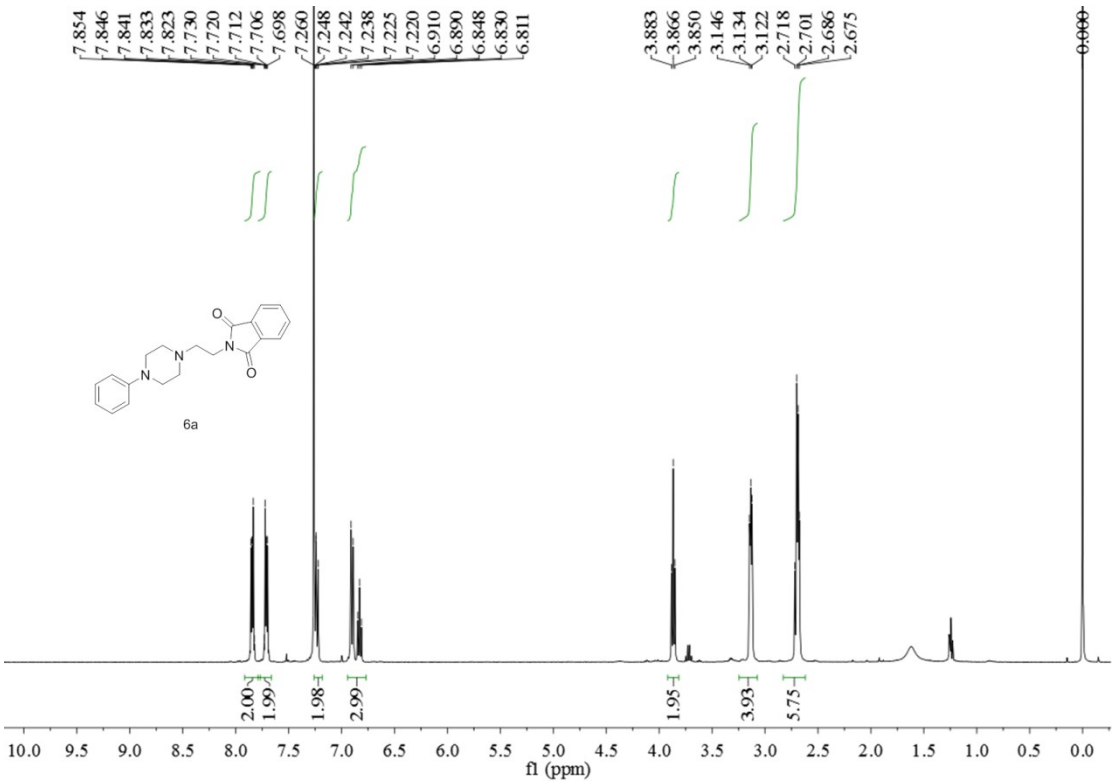
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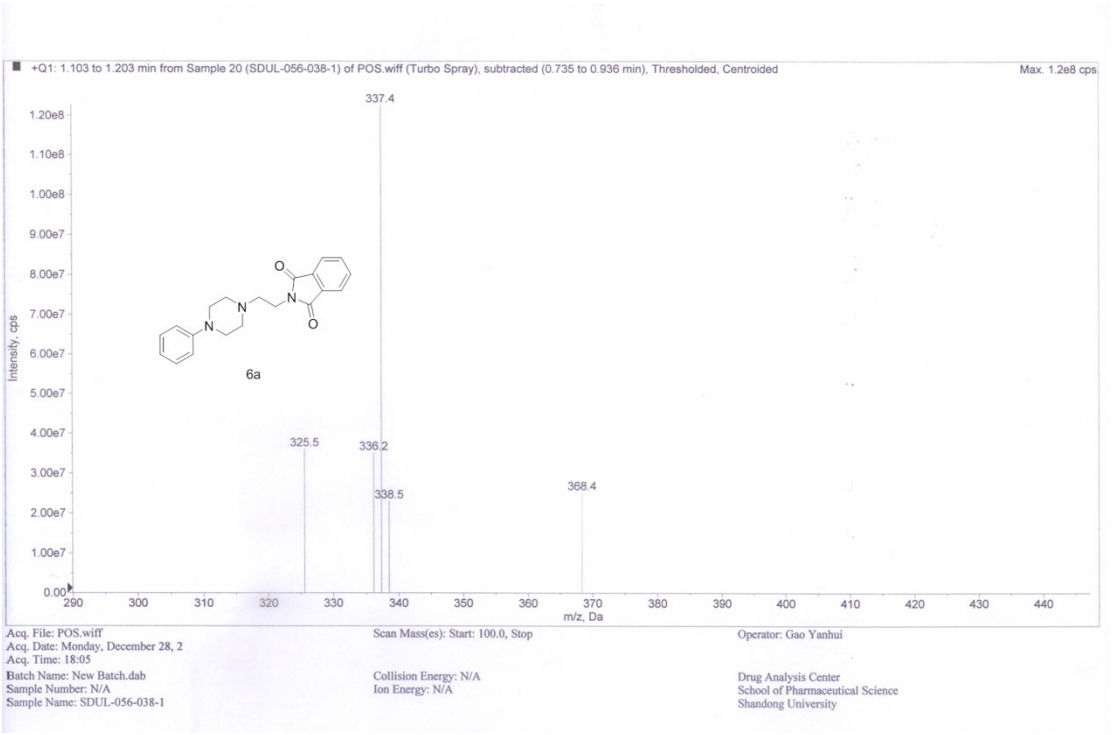
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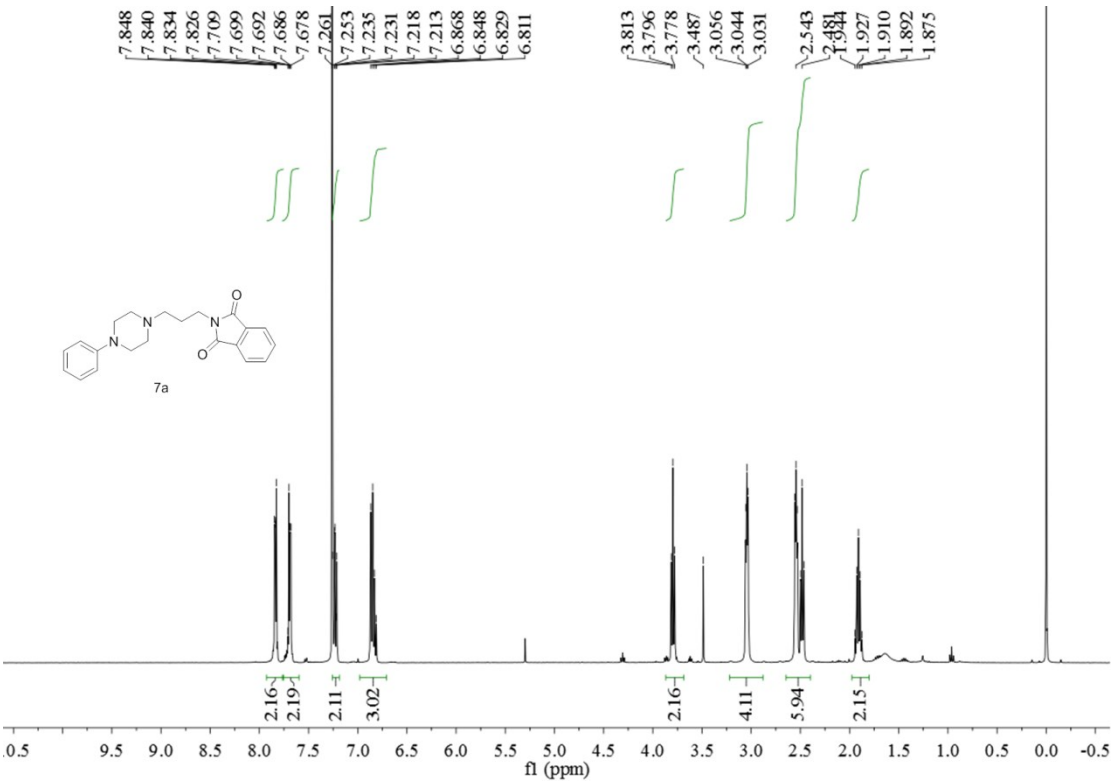
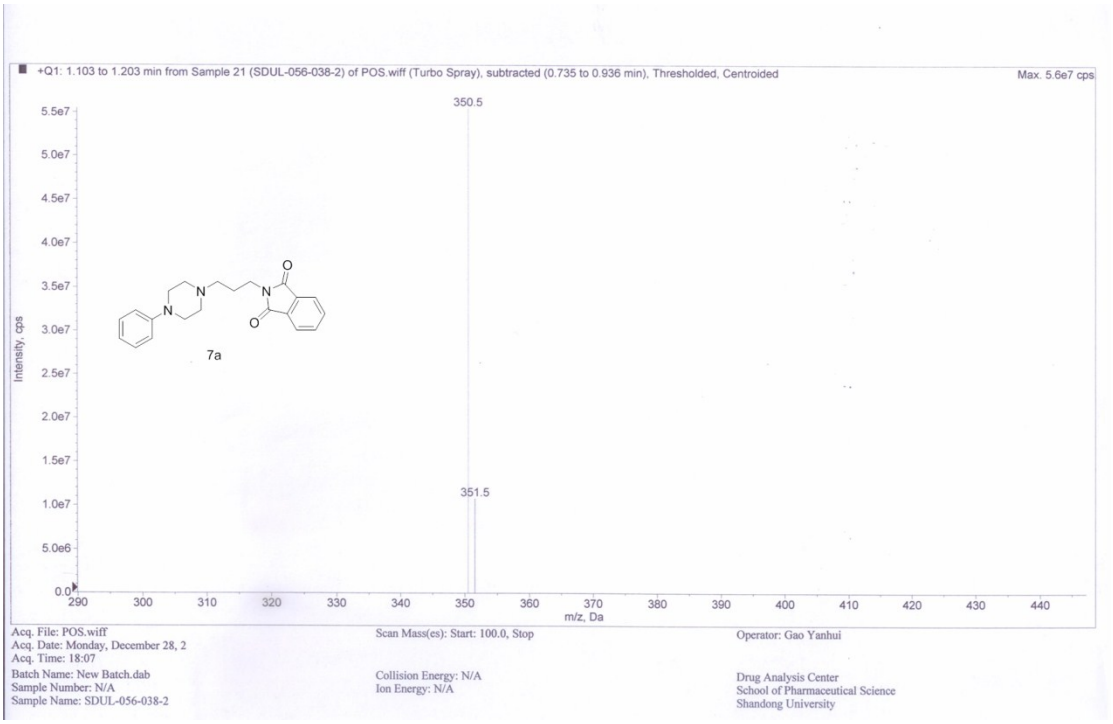
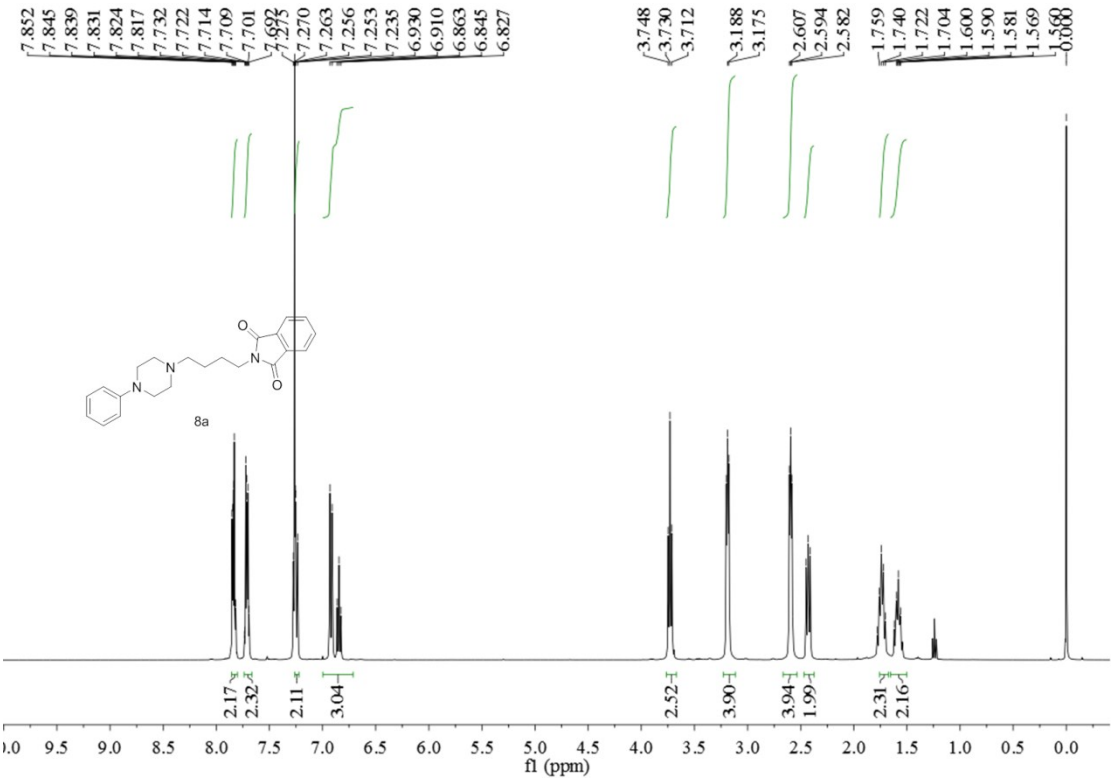
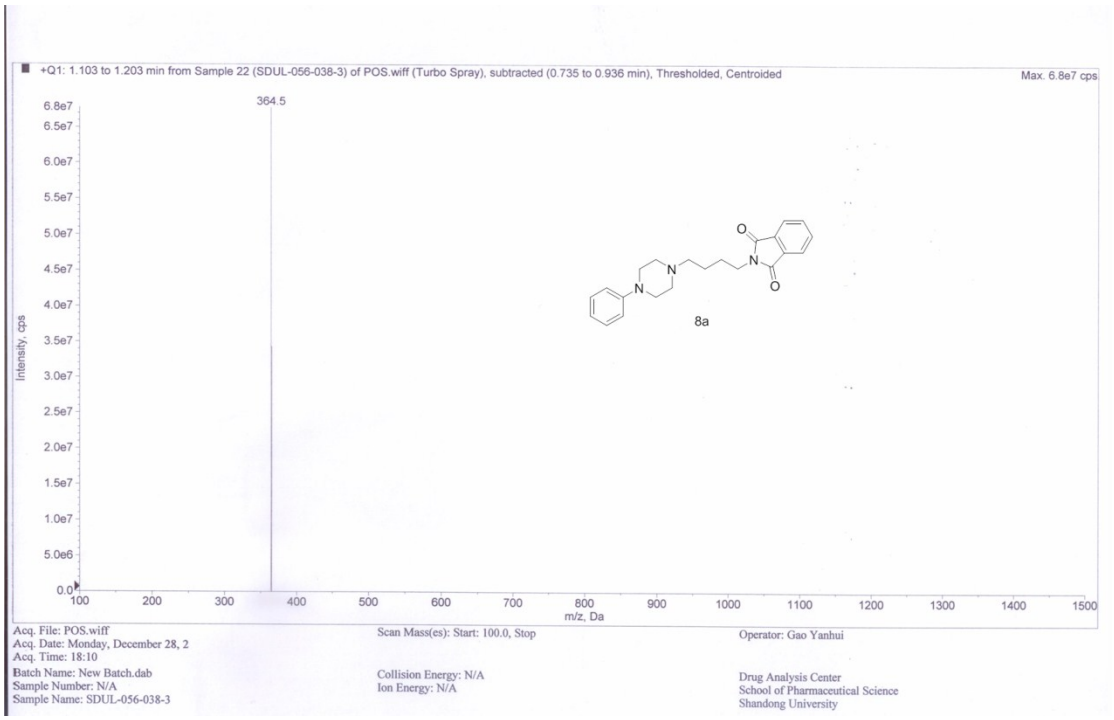
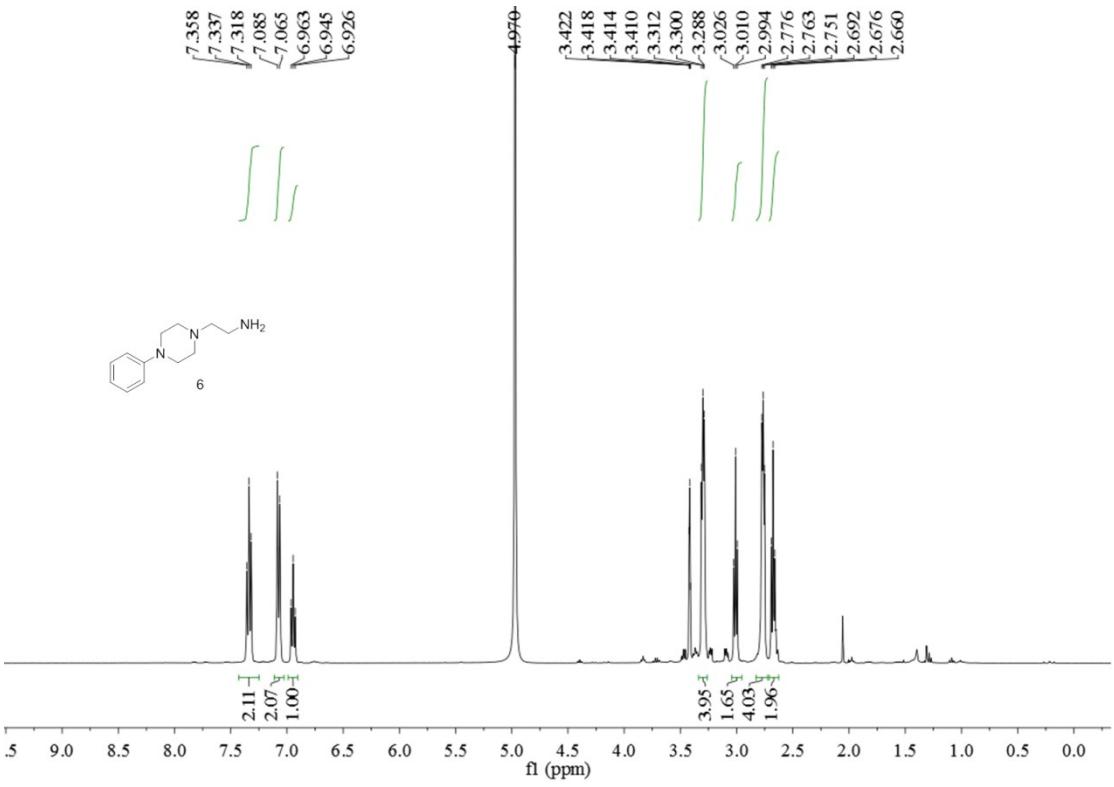
337.4
325.5
336.2
338.5
368.4
Intensity, cps
m/z, Da
6a
Acq. File: POS.wiff
Acq. Date: Monday, December 28, 2
Acq. Time: 18:05
Batch Name: New Batch.dab
Sample Number: N/A
Sample Name: SDUL-056-038-1
Scan Mass(es): Start: 100.0, Stop
Collision Energy: N/A
Ion Energy: N/A
Operator: Gao Yanhui
Drug Analysis Center
School of Pharmaceutical Science
Shandong University

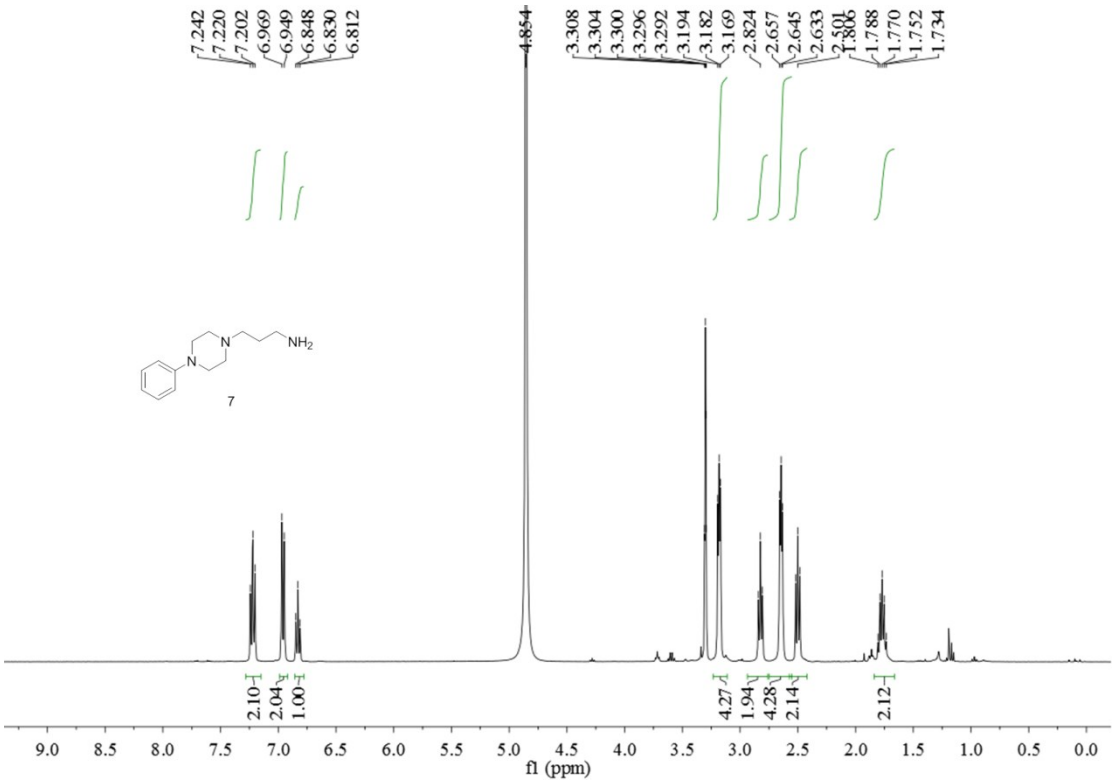

7.848
7.840
7.834
7.826
7.709
7.699
7.692
7.686
7.678
7.261
7.253
7.235
7.231
7.218
7.213
6.868
6.848
6.829
6.811
3.813
3.796
3.778
3.487
3.056
3.044
3.031
2.543
1.927
1.910
1.892
1.875
7a
2.16
2.19
2.11
3.02
2.16
4.11
5.94
2.15
f1 (ppm)

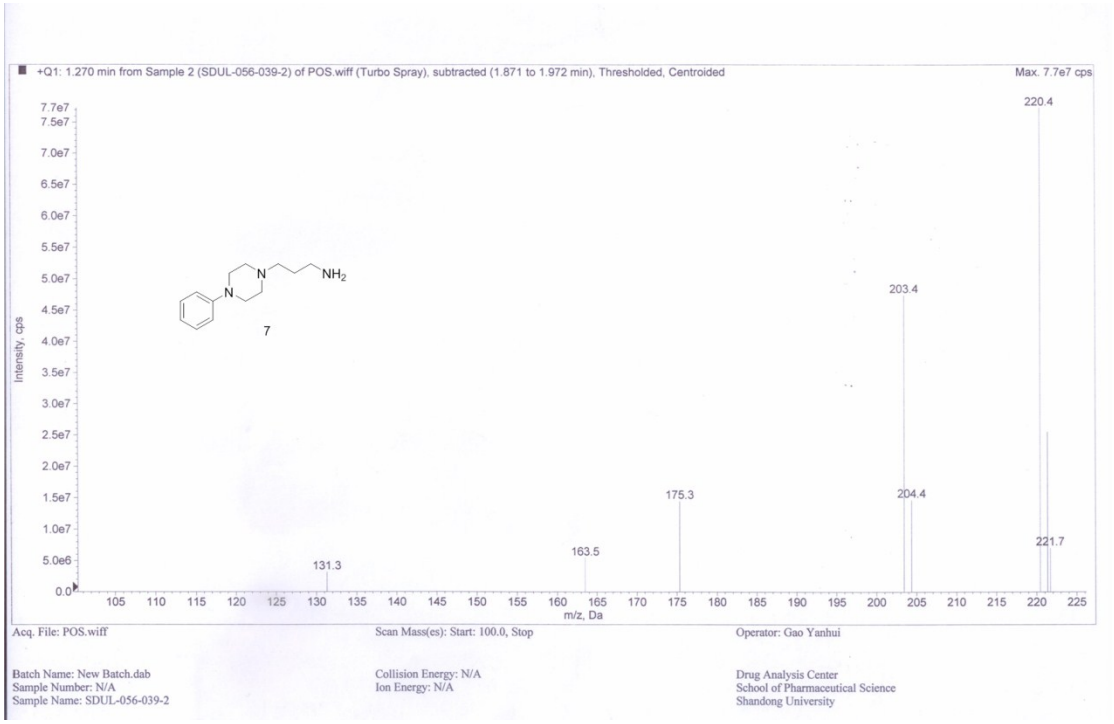

+Q1: 1.103 to 1.203 min from Sample 21 (SDUL-056-038-2) of POS.wiff (Turbo Spray), subtracted (0.735 to 0.936 min), Thresholded, Centroided
Max. 5.6e7 cps.
350.5
351.5
Intensity, cps
m/z, Da
7a
Acq. File: POS.wiff
Acq. Date: Monday, December 28, 2
Acq. Time: 18:07
Batch Name: New Batch.dab
Sample Number: N/A
Sample Name: SDUL-056-038-2
Scan Mass(es): Start: 100.0, Stop
Collision Energy: N/A
Ion Energy: N/A
Operator: Gao Yanhui
Drug Analysis Center
School of Pharmaceutical Science
Shandong University

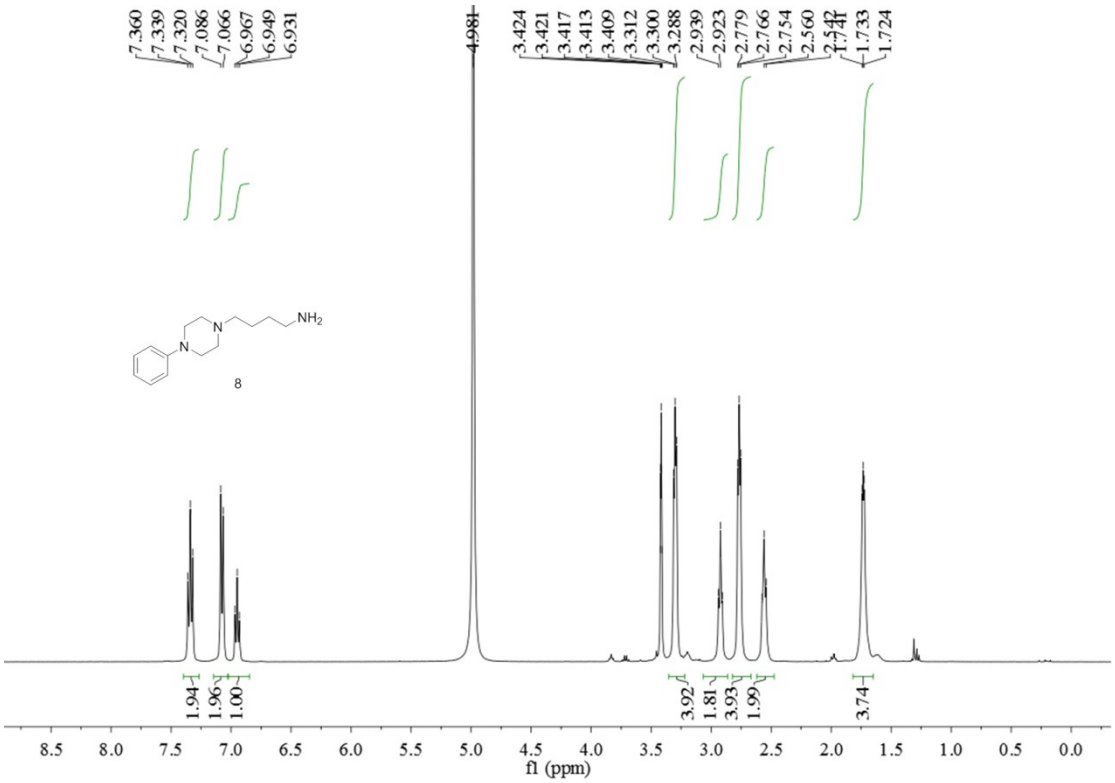

8a
f1 (ppm)

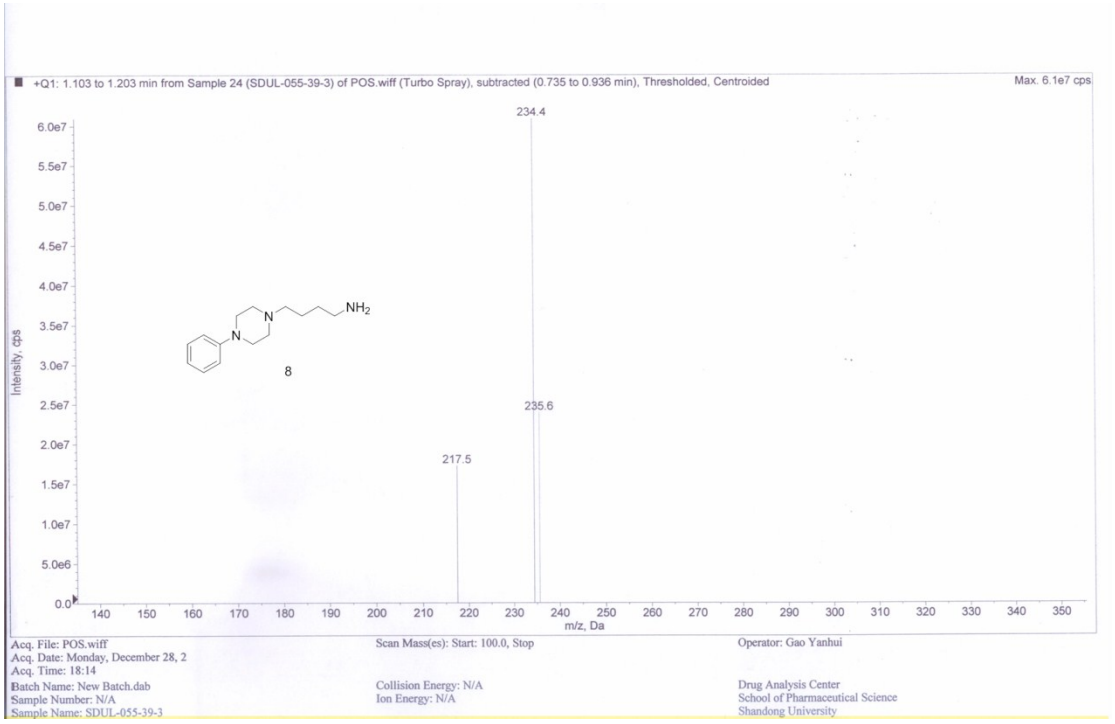

+Q1: 1.103 to 1.203 min from Sample 22 (SDUL-056-038-3) of POS.wiff (Turbo Spray), subtracted (0.735 to 0.936 min), Thresholded, Centroided
Max. 6.8e7 cps
364.5
Intensity, cps
m/z, Da
8a
Acq. File: POS.wiff
Acq. Date: Monday, December 28, 2
Acq. Time: 18:10
Batch Name: New Batch.dab
Sample Number: N/A
Sample Name: SDUL-056-038-3
Scan Mass(es): Start: 100.0, Stop
Collision Energy: N/A
Ion Energy: N/A
Operator: Gao Yanhui
Drug Analysis Center
School of Pharmaceutical Science
Shandong University

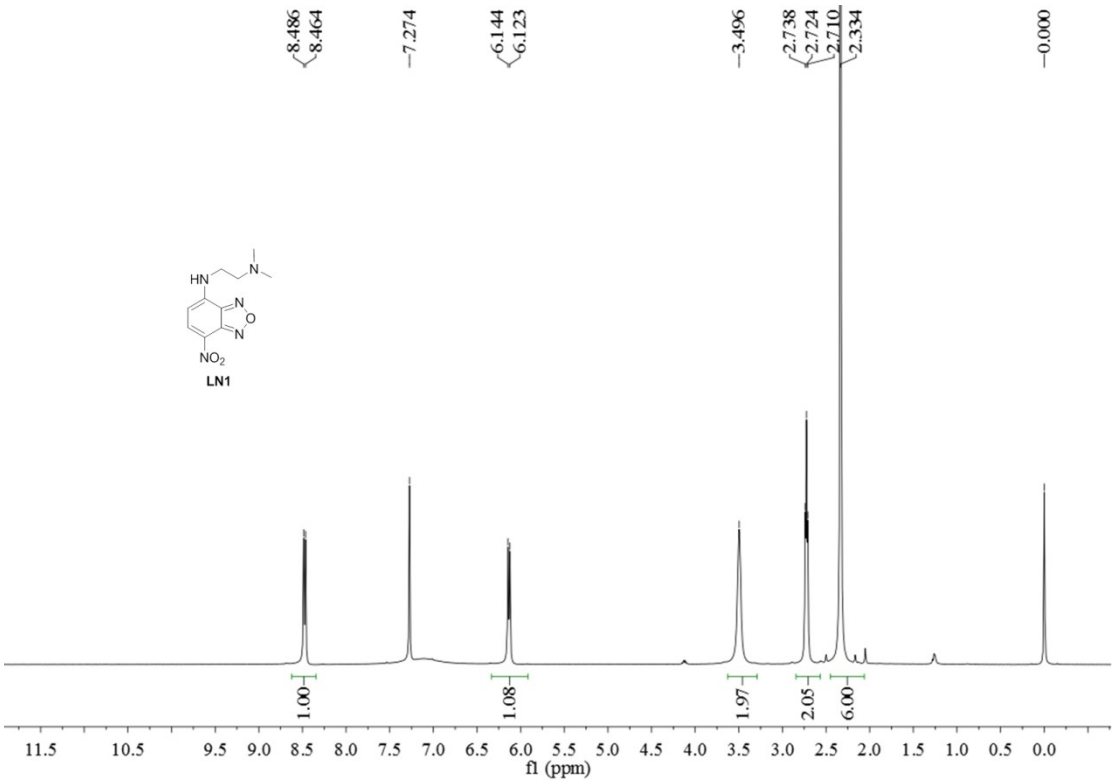

7.358
7.337
7.318
7.085
7.065
6.963
6.945
6.926
4.970
3.422
3.418
3.414
3.410
3.312
3.300
3.288
3.026
3.010
2.994
2.776
2.763
2.751
2.692
2.676
2.660
NH2
6
2.11
2.07
1.00
3.95
1.65
4.03
1.96
f1 (ppm)

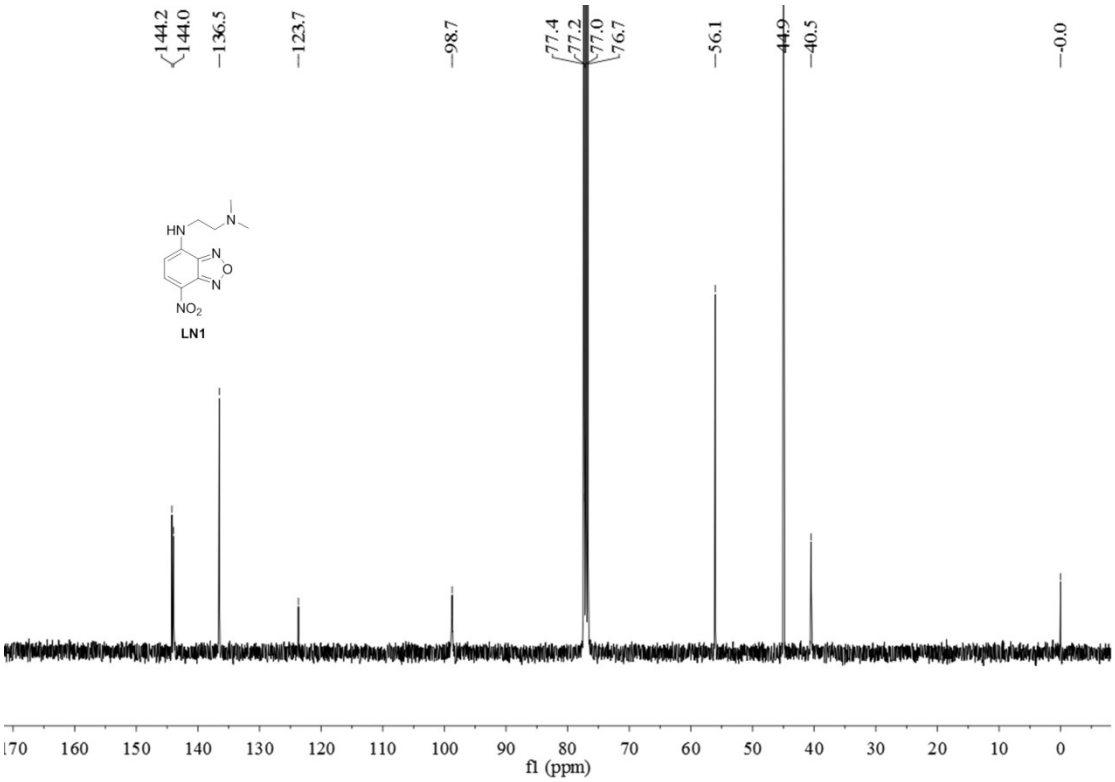

7.242
7.220
7.202
6.969
6.949
6.848
6.830
6.812
4.854
3.308
3.304
3.300
3.296
3.292
3.194
3.182
3.169
2.824
2.657
2.645
2.633
2.501
1.806
1.788
1.770
1.752
1.734
NH2
N
N
7
2.10
2.04
1.00
4.27
1.94
4.28
2.14
2.12
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

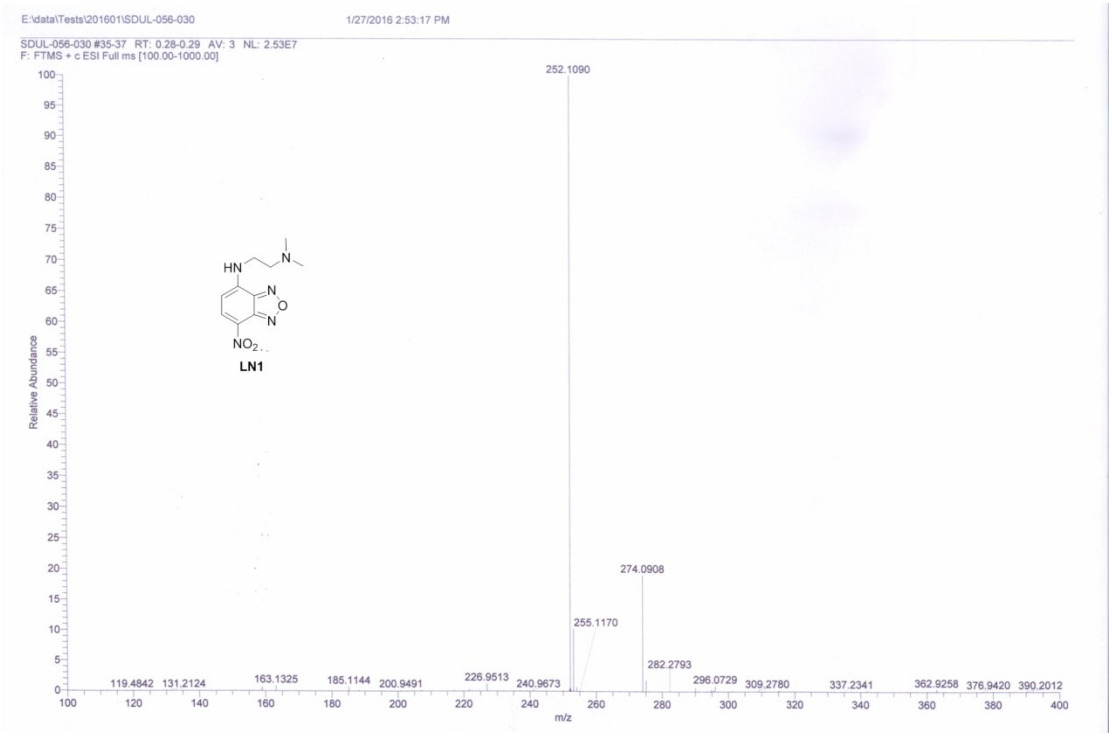

+Q1: 1.270 min from Sample 2 (SDUL-056-039-2) of POS.wiff (Turbo Spray), subtracted (1.871 to 1.972 min), Thresholded, Centroided
Max. 7.7e7 cps
Intensity, cps
7.7e7
7.5e7
7.0e7
6.5e7
6.0e7
5.5e7
5.0e7
4.5e7
4.0e7
3.5e7
3.0e7
2.5e7
2.0e7
1.5e7
1.0e7
5.0e6
0.0
220.4
203.4
204.4
175.3
163.5
131.3
221.7
NH2
N
N
7
105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225
m/z, Da
Acq. File: POS.wiff
Scan Mass(es): Start: 100.0, Stop
Operator: Gao Yanhui
Batch Name: New Batch.dab
Sample Number: N/A
Sample Name: SDUL-056-039-2
Collision Energy: N/A
Ion Energy: N/A
Drug Analysis Center
School of Pharmaceutical Science
Shandong University

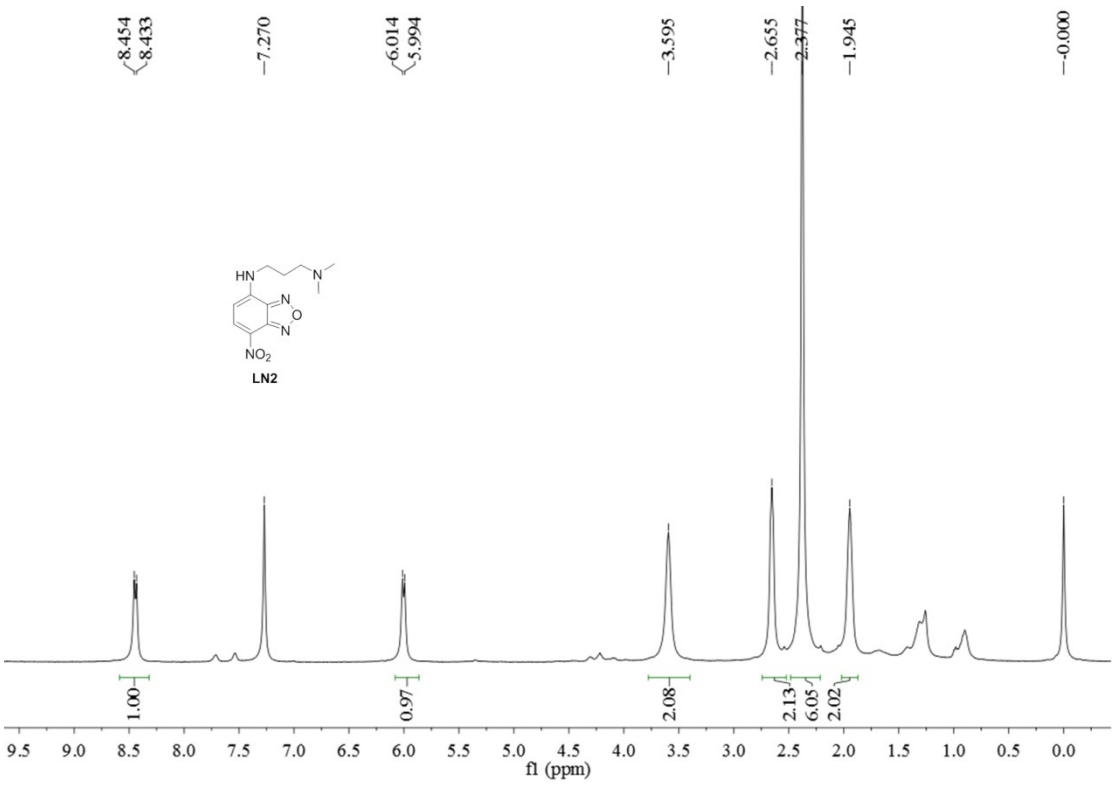

7.360
7.339
7.320
7.086
7.066
6.967
6.949
6.931
4.981
3.424
3.421
3.417
3.413
3.409
3.312
3.300
3.288
2.939
2.923
2.779
2.766
2.754
2.560
1.733
1.724
NH2
N
N
8
1.94
1.96
1.00
3.92
1.81
3.93
1.99
3.74
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

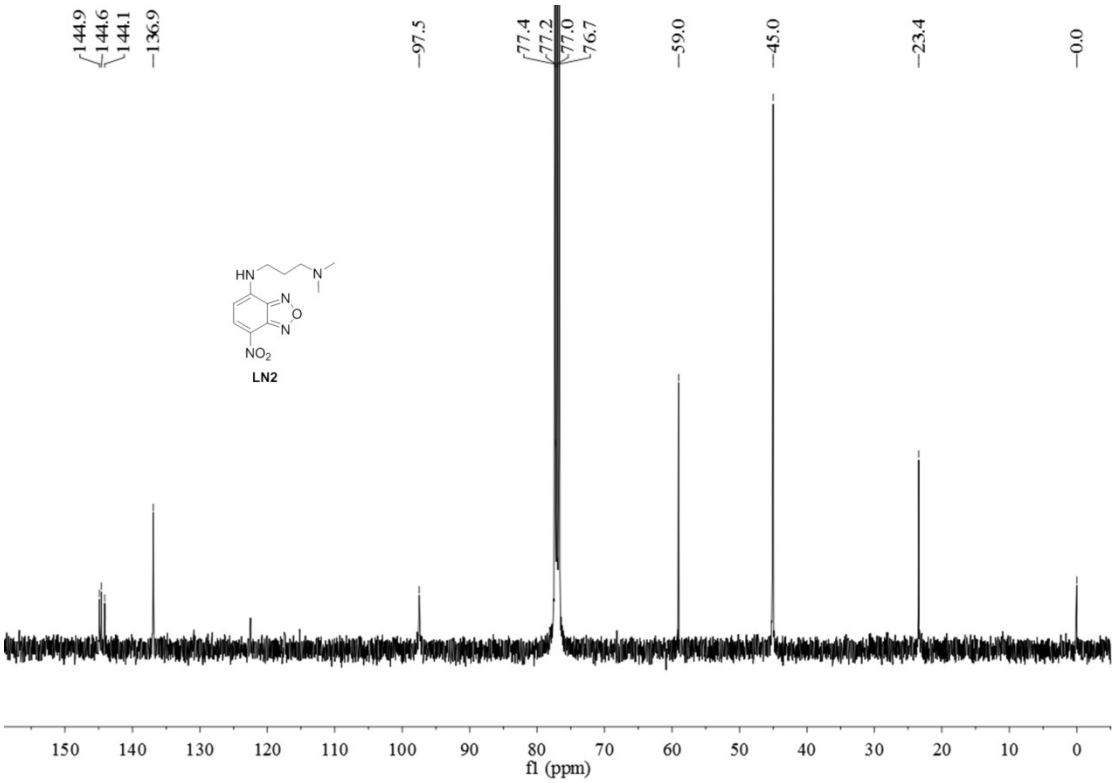

+Q1: 1.103 to 1.203 min from Sample 24 (SDUL-055-39-3) of POS.wiff (Turbo Spray), subtracted (0.735 to 0.936 min), Thresholded, Centroided
Max. 6.1e7 cps
234.4
235.6
217.5
NH2
N
N
8
Intensity, cps
6.0e7
5.5e7
5.0e7
4.5e7
4.0e7
3.5e7
3.0e7
2.5e7
2.0e7
1.5e7
1.0e7
5.0e6
0.0
140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350
m/z, Da
Acq. File: POS.wiff
Acq. Date: Monday, December 28, 2
Acq. Time: 18:14
Batch Name: New Batch.dab
Sample Number: N/A
Sample Name: SDUL-055-39-3
Scan Mass(es): Start: 100.0, Stop
Collision Energy: N/A
Ion Energy: N/A
Operator: Gao Yanhui
Drug Analysis Center
School of Pharmaceutical Science
Shandong University

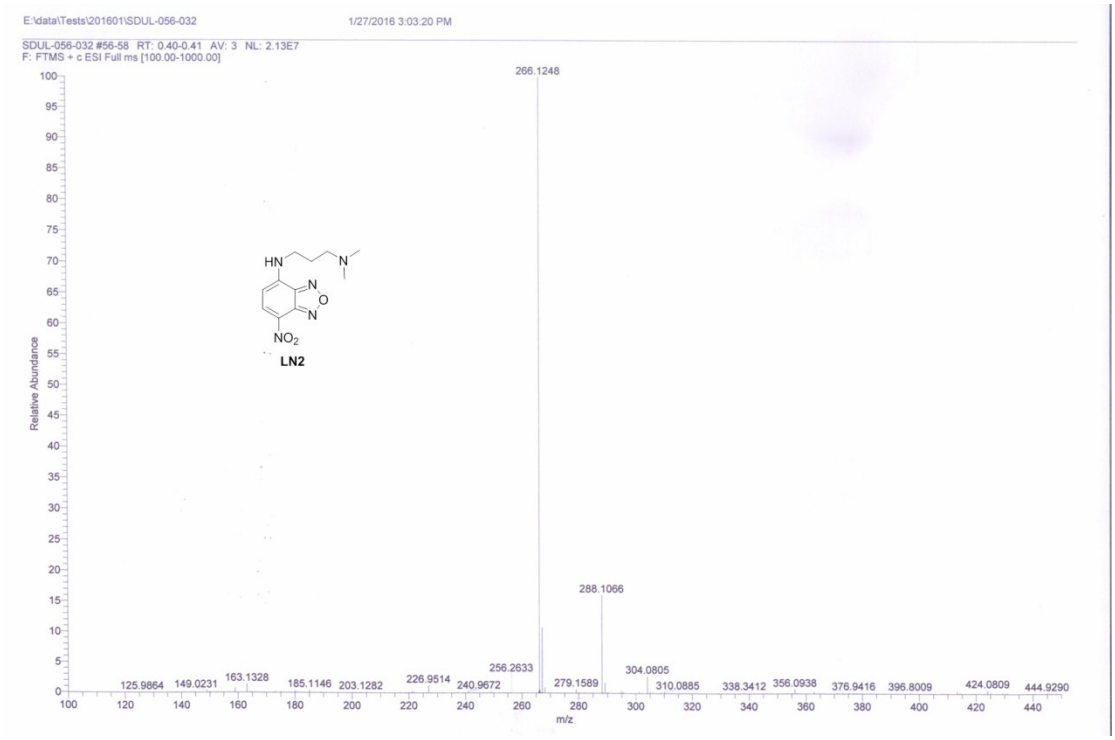

8.486
8.464
7.274
6.144
6.123
3.496
2.738
2.724
2.710
2.334
0.000
HN
N
N
O
N
NO2
LN1
1.00
1.08
1.97
2.05
6.00
11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

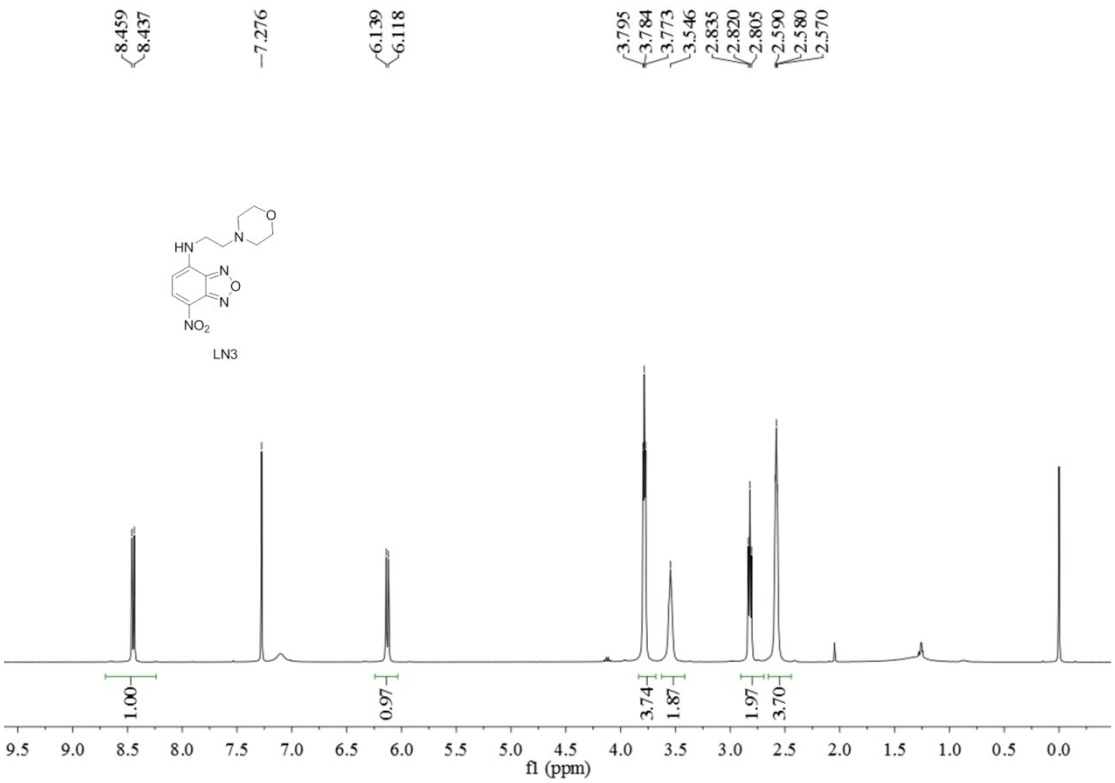

144.2
144.0
136.5
123.7
98.7
77.4
77.2
77.0
76.7
56.1
44.9
40.5
0.0
HN
N
N
O
N
NO2
LN1
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

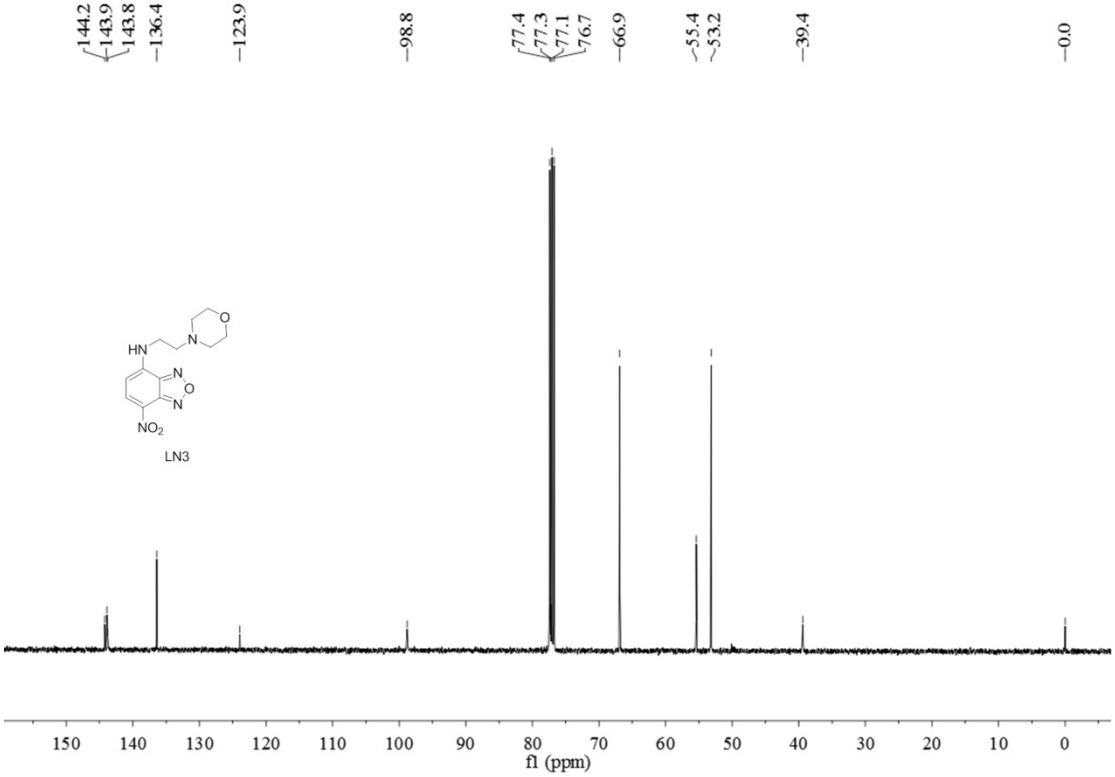

E:\data\Tests\201601\SDUL-056-030
1/27/2016 2:53:17 PM
SDUL-056-030 #35-37 RT: 0.28-0.29 AV: 3 NL: 2.53E7
F: FTMS + c ESI Full ms [100.00-1000.00]
252.1090
274.0908
255.1170
282.2793
119.4842
131.2124
163.1325
185.1144
200.9491
226.9513
240.9673
296.0729
309.2780
337.2341
362.9258
376.9420
390.2012
Relative Abundance
m/z
LN1

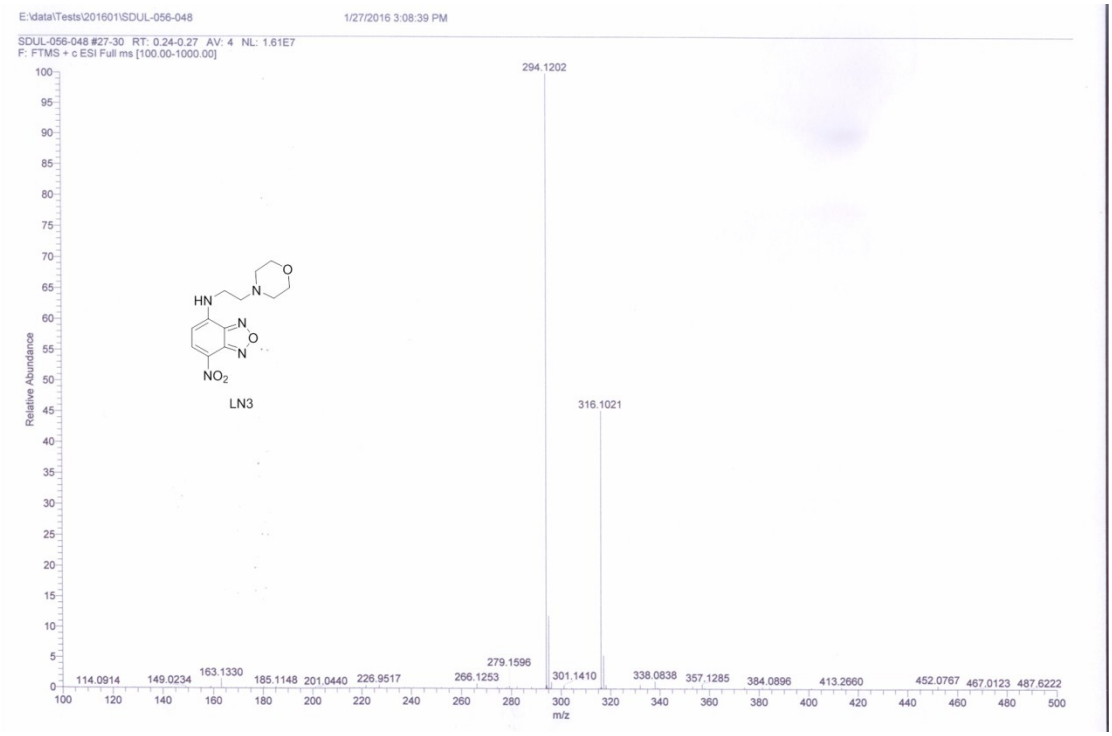

8.454
8.433
7.270
6.014
5.994
3.595
2.655
2.377
1.945
0.000
LN2
1.00
0.97
2.08
2.13
6.05
2.02
f1 (ppm)

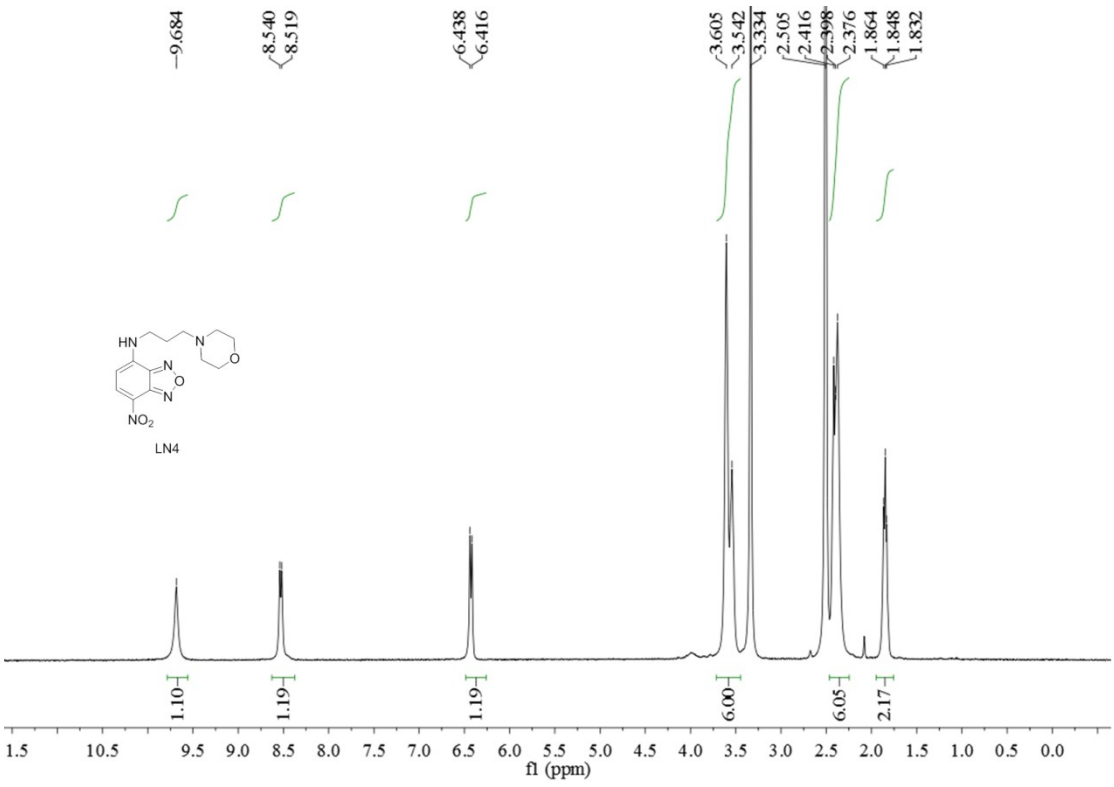

144.9
144.6
144.1
136.9
97.5
77.4
77.2
77.0
76.7
59.0
45.0
23.4
0.0
HN
N
N
O
N
NO2
LN2
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

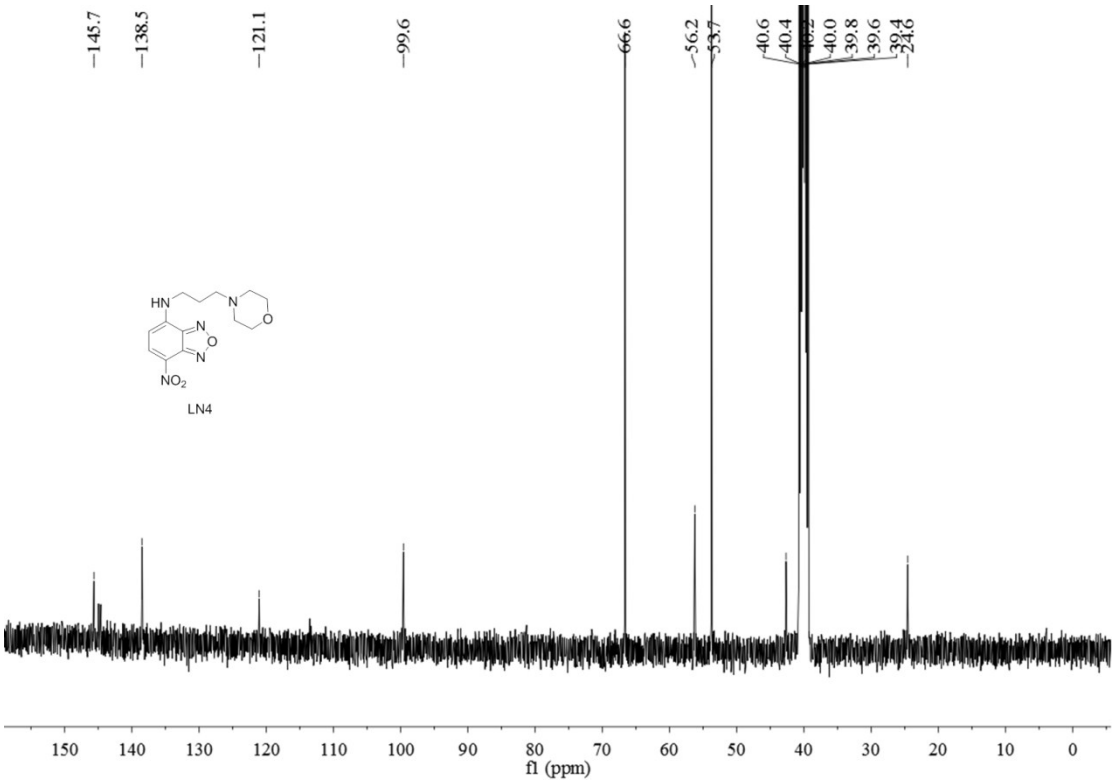

E:\data\Tests\201601\SDUL-056-032
1/27/2016 3:03:20 PM
SDUL-056-032 #56-58 RT: 0.40-0.41 AV: 3 NL: 2.13E7
F: FTMS + c ESI Full ms [100.00-1000.00]
266.1248
288.1066
256.2633
304.0805
125.9864
149.0231
163.1328
185.1146
203.1282
226.9514
240.9672
279.1589
310.0885
338.3412
356.0938
376.9416
396.8009
424.0809
444.9290
Relative Abundance
m/z
HN
N
N
O
N
NO2
LN2

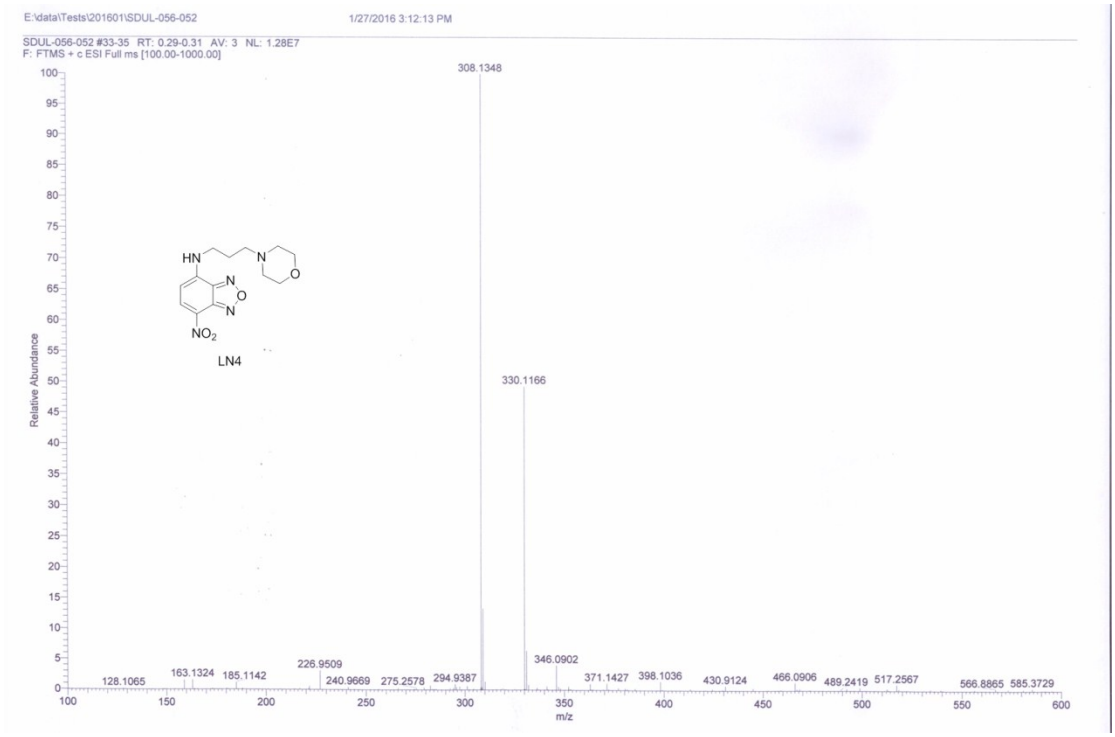

8.459
8.437
7.276
6.139
6.118
3.795
3.784
3.773
3.546
2.835
2.820
2.805
2.590
2.580
2.570
HN
N
O
N
O
N
NO2
LN3
1.00
0.97
3.74
1.87
1.97
3.70
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

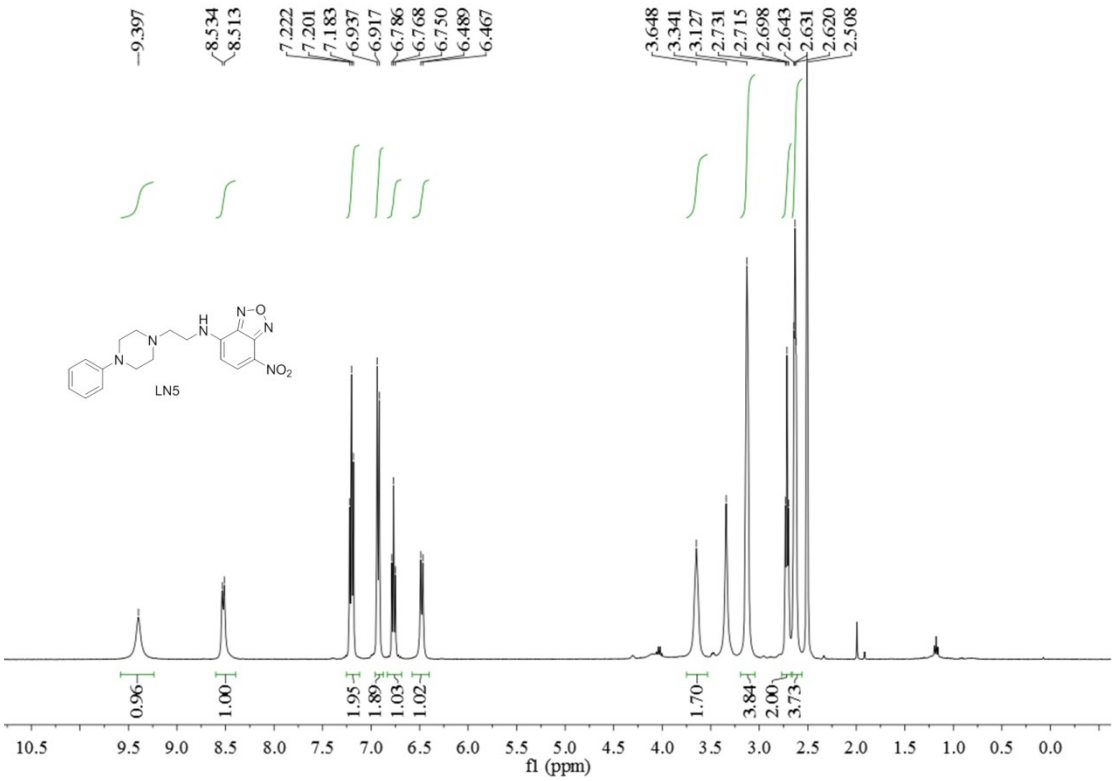

144.2
143.9
143.8
136.4
123.9
98.8
77.4
77.3
77.1
76.7
66.9
55.4
53.2
39.4
0.0
HN
N
O
N
O
N
NO2
LN3
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

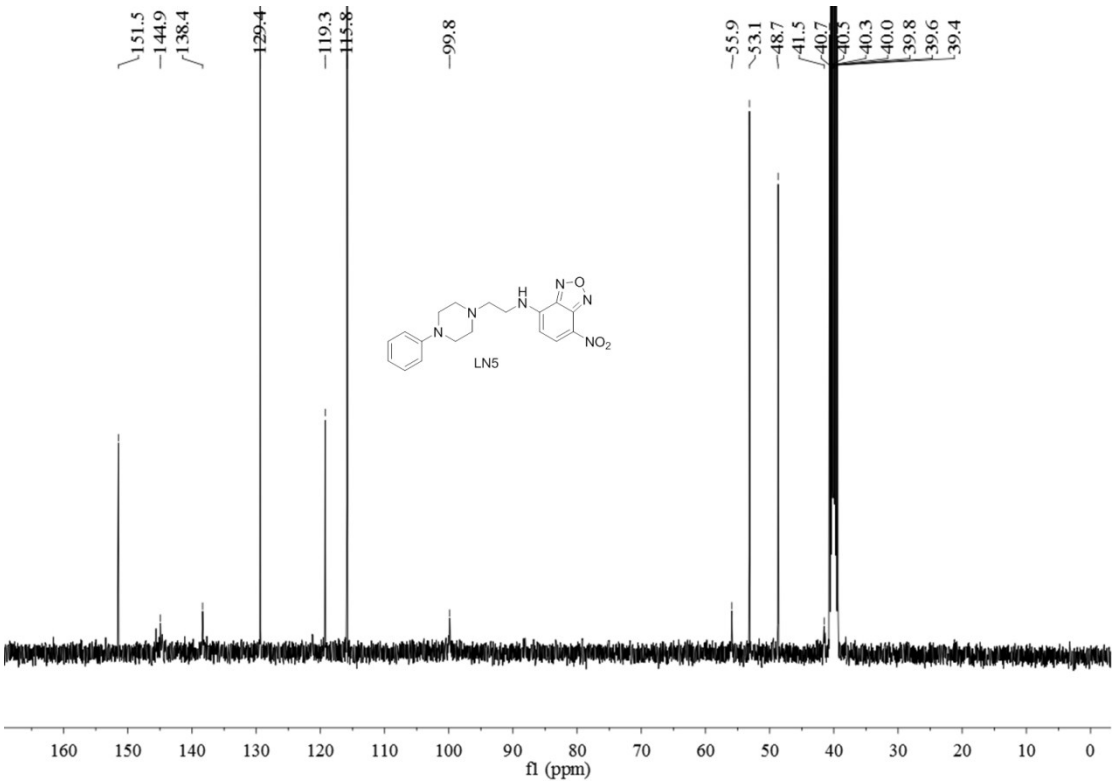

E:\data\Tests\201601\SDUL-056-048
1/27/2016 3:08:39 PM
SDUL-056-048 #27-30 RT: 0.24-0.27 AV: 4 NL: 1.61E7
F: FTMS + c ESI Full ms [100.00-1000.00]
294.1202
316.1021
279.1596
Relative Abundance
m/z
LN3

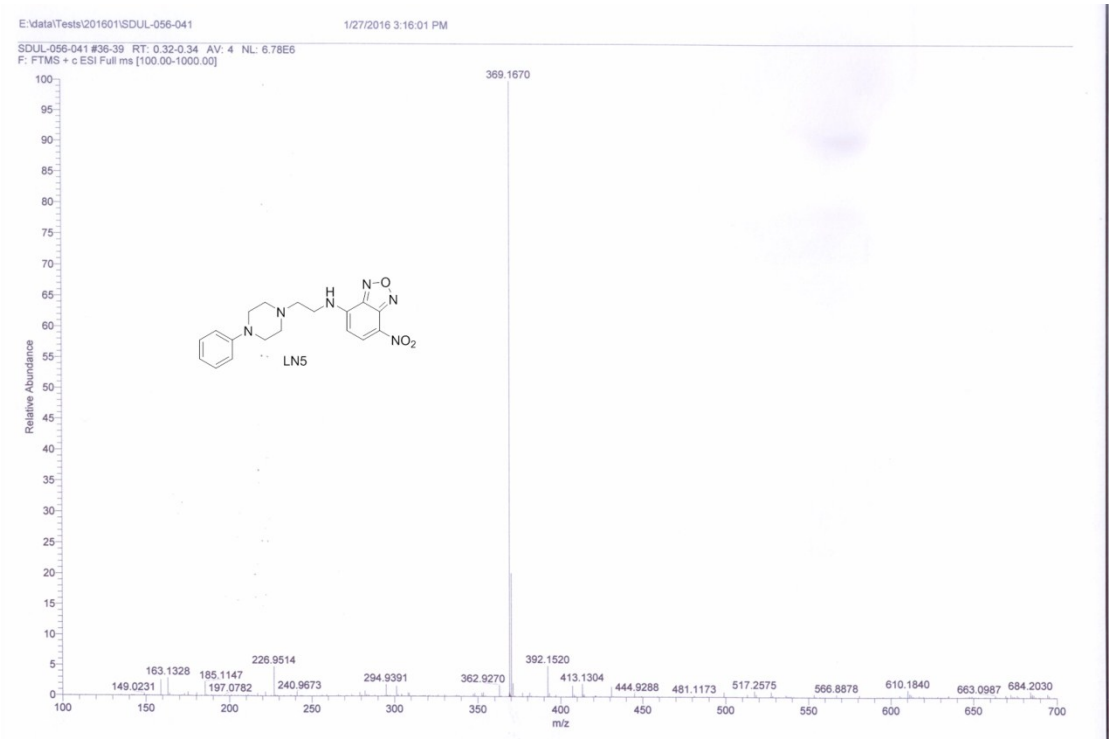

9.684
8.540
8.519
6.438
6.416
3.605
3.542
3.334
2.505
2.416
2.398
2.376
1.864
1.848
1.832
1.10
1.19
1.19
6.00
6.05
2.17
f1 (ppm)
LN4

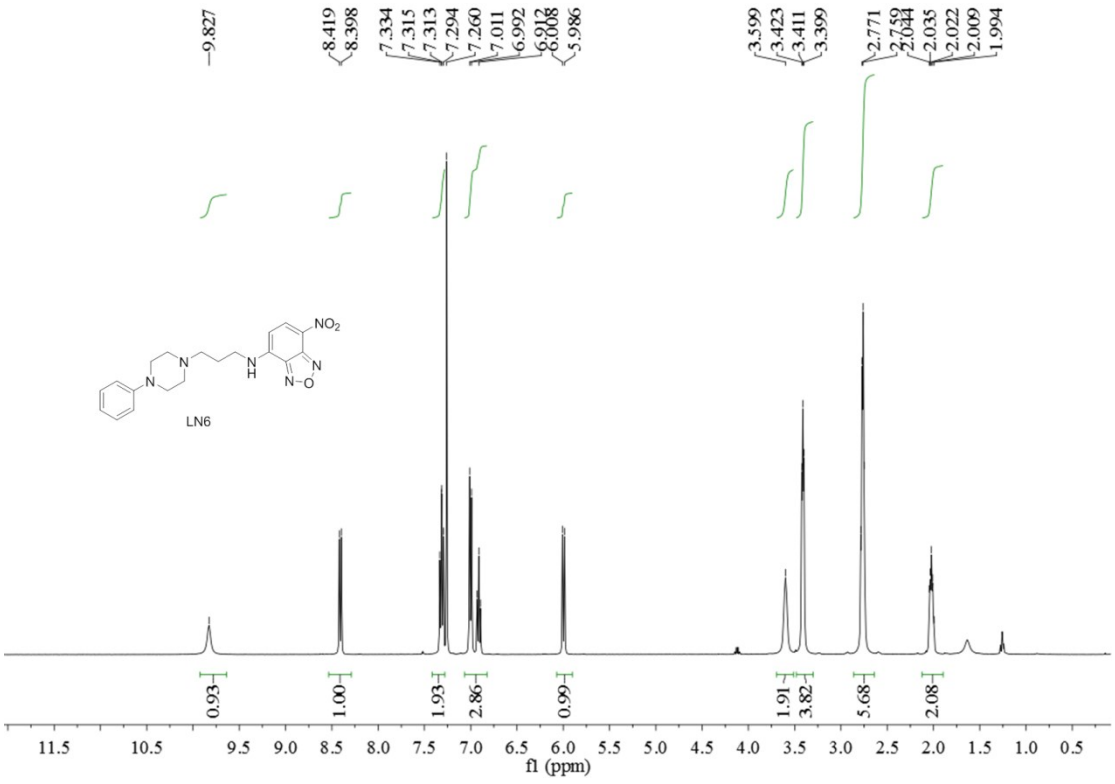

145.7
138.5
121.1
99.6
66.6
56.2
53.7
40.6
40.4
40.2
40.0
39.8
39.6
39.4
24.6
HN
N
O
N
O
N
NO2
LN4
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

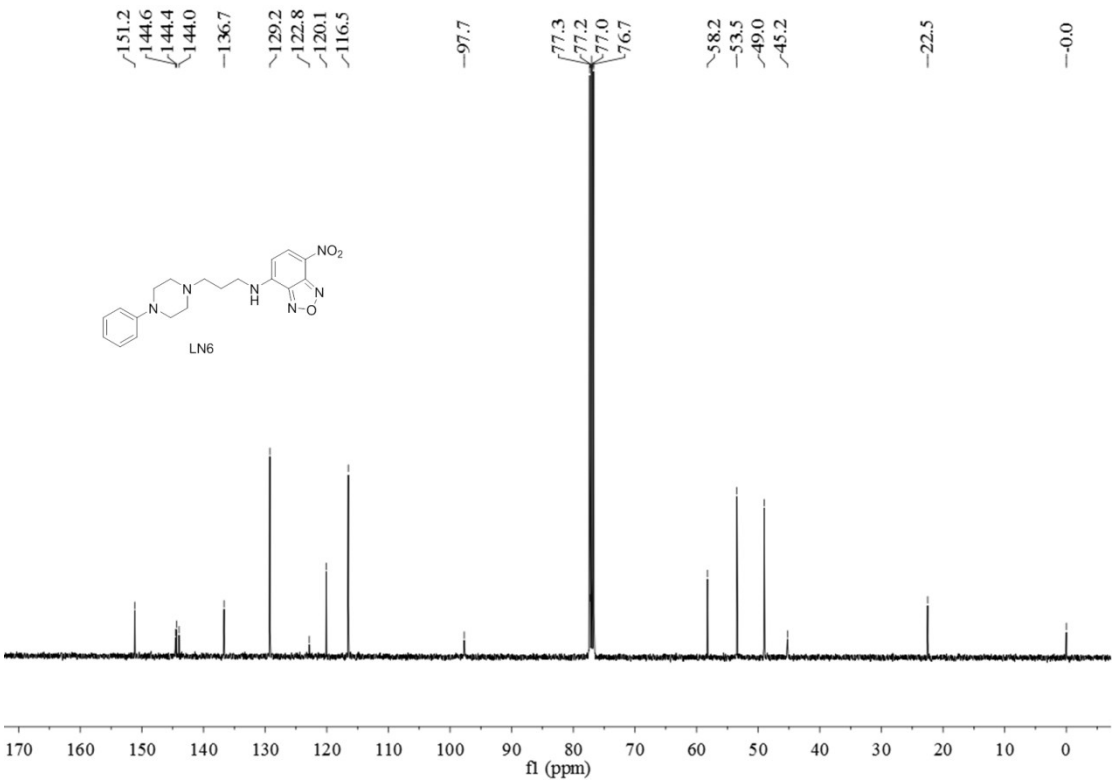

E:\data\Tests\201601\SDUL-056-052
1/27/2016 3:12:13 PM
SDUL-056-052 #33-35 RT: 0.29-0.31 AV: 3 NL: 1.28E7
F: FTMS + c ESI Full ms [100.00-1000.00]
308.1348
330.1166
346.0902
226.9509
128.1065
163.1324
185.1142
240.9669
275.2578
294.9387
371.1427
398.1036
430.9124
466.0906
489.2419
517.2567
566.8865
585.3729
HN
N
O
N
O
N
NO2
LN4
Relative Abundance
m/z

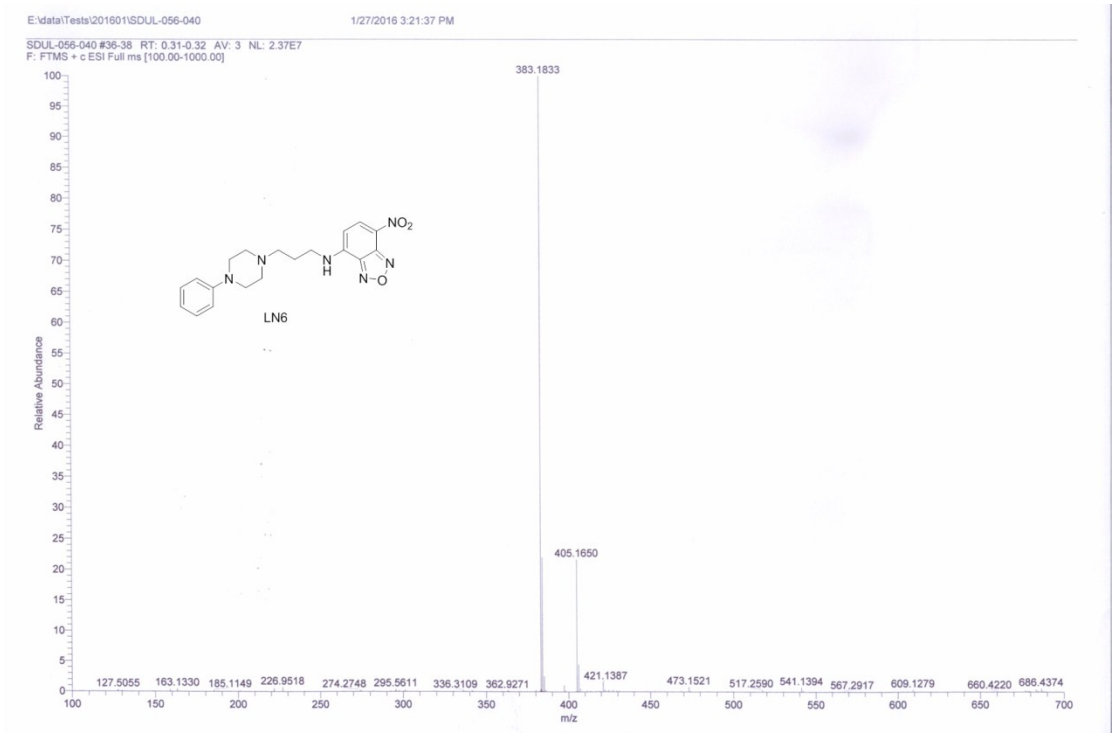

9.397
8.534
8.513
7.222
7.201
7.183
6.937
6.917
6.786
6.768
6.750
6.489
6.467
3.648
3.341
3.127
2.731
2.715
2.698
2.643
2.631
2.620
2.508
LN5
0.96
1.00
1.95
1.89
1.03
1.02
1.70
3.84
2.00
3.73
f1 (ppm)

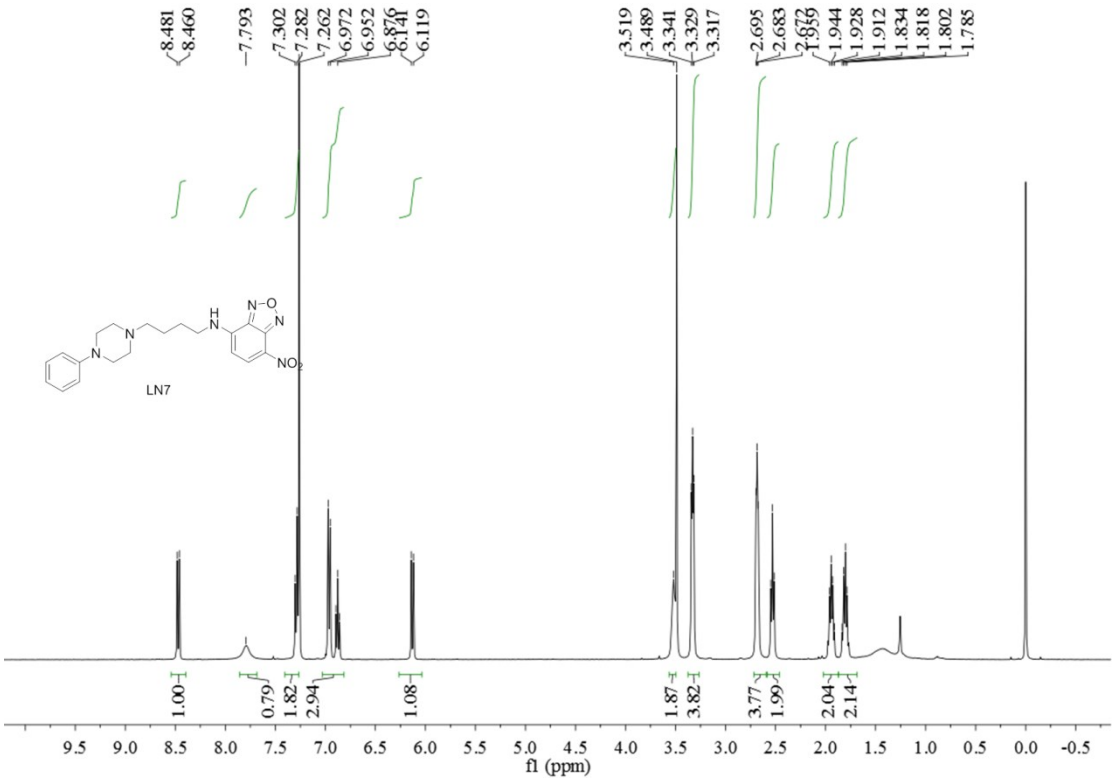

151.5
144.9
138.4
129.4
119.3
115.8
99.8
55.9
53.1
48.7
41.5
40.7
40.5
40.3
40.0
39.8
39.6
39.4
LN5
f1 (ppm)

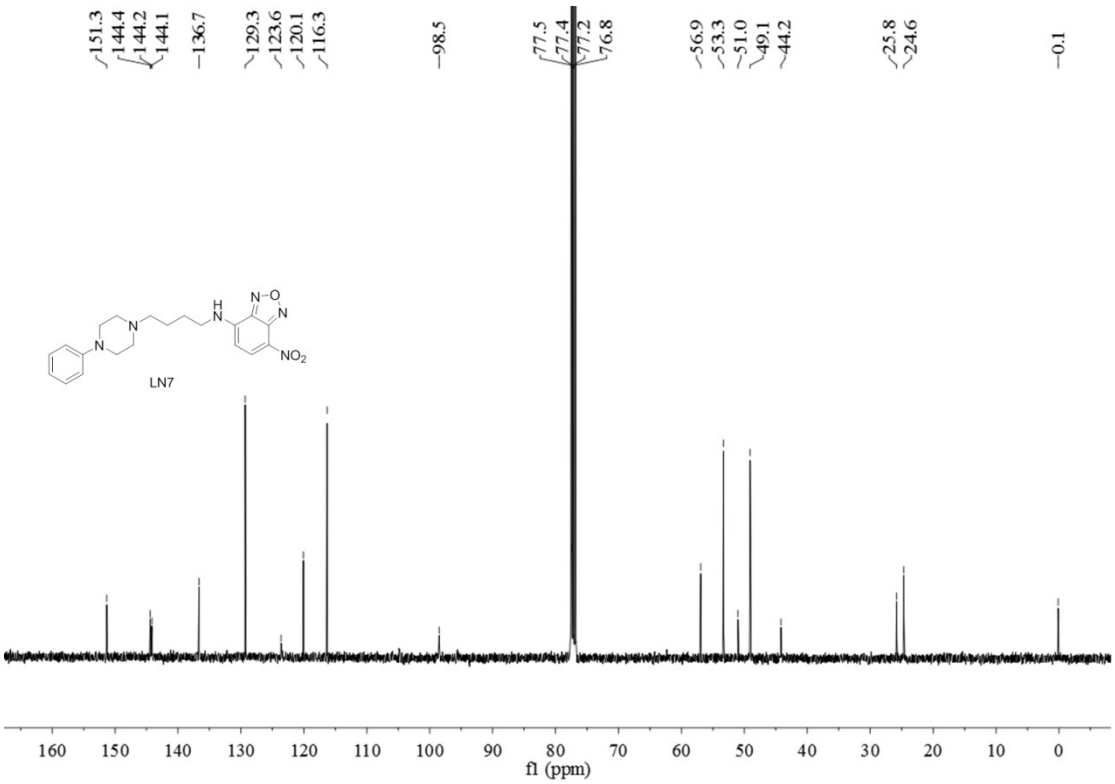

E:\data\Tests\201601\SDUL-056-041
1/27/2016 3:16:01 PM
SDUL-056-041 #36-39 RT: 0.32-0.34 AV: 4 NL: 6.78E6
F: FTMS + c ESI Full ms [100.00-1000.00]
369.1670
LN5
Relative Abundance
m/z

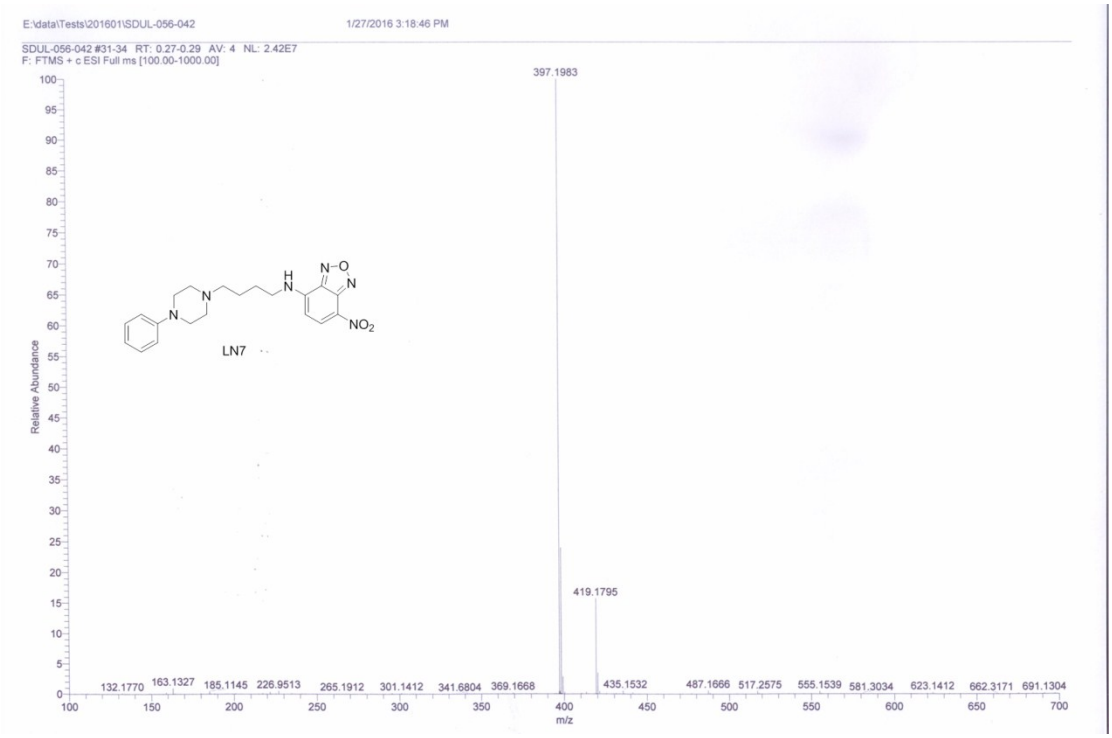

LN6
fl (ppm)


151.2
144.6
144.4
144.0
136.7
129.2
122.8
120.1
116.5
97.7
77.3
77.2
77.0
76.7
58.2
53.5
49.0
45.2
22.5
0.0
NO2
N
H
N
N-O
LN6
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


E:\data\Tests\201601\SDUL-056-040
1/27/2016 3:21:37 PM
SDUL-056-040 #36-38 RT: 0.31-0.32 AV: 3 NL: 2.37E7
F: FTMS + c ESI Full ms [100.00-1000.00]
383.1833
405.1650
421.1387
127.5055
163.1330
185.1149
226.9518
274.2748
295.5611
336.3109
362.9271
473.1521
517.2590
541.1394
567.2917
609.1279
660.4220
686.4374
NO2
N
H
N
N-O
LN6
Relative Abundance
m/z


8.481
8.460
7.793
7.302
7.282
7.262
6.972
6.952
6.876
6.141
6.119
3.519
3.489
3.341
3.329
3.317
2.695
2.683
1.944
1.928
1.912
1.834
1.818
1.802
1.785
LN7
1.00
0.79
1.82
2.94
1.08
1.87
3.82
3.77
1.99
2.04
2.14
fl (ppm)


151.3
144.4
144.2
144.1
136.7
129.3
123.6
120.1
116.3
98.5
77.5
77.4
77.2
76.8
56.9
53.3
51.0
49.1
44.2
25.8
24.6
0.1
LN7
fl (ppm)



Reference

1. M. I. Burguete, F. Galindo, M. Izquierdo, J. E. O'Connor, G. Herrera, S. V. Luis and L. Vigara, *Eur. J. Org. Chem*, 2010, **2010**, 5967-5979.