Supplementary Information

A Silicon-containing Aryl/penta-1,4-dien-3-one/amine Hybrid Exhibits Antiproliferative Effects on Breast Cancer Cells by Targeting HSP90 C-terminus without Inducing the Heat-shock Response

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[¶] These authors were contributed equally.

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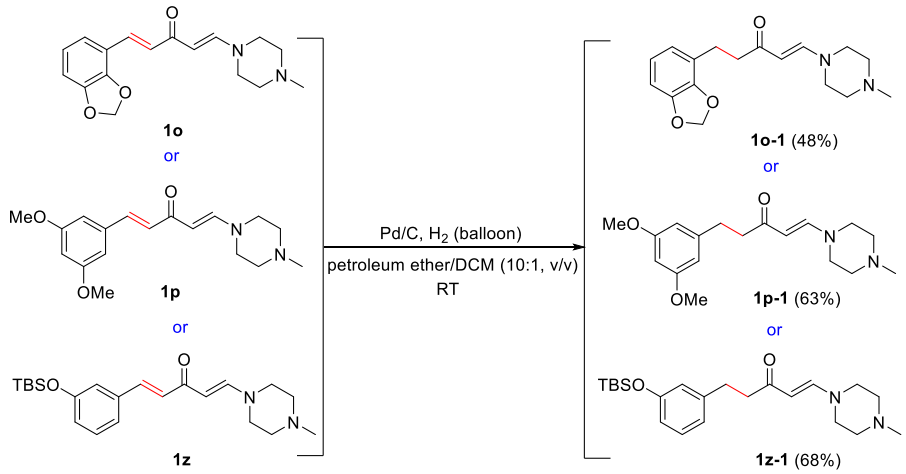
1. General procedure for synthesis of 1o-1, 1p-1, and 1z-1



Scheme S1. Synthesis of **1o-1, 1p-1**, and **1z-1**.

A solution of hybrids **1o** or **1p** or **1z** (0.1 mmol) and Pd/C (2 mg, 10% on activated carbon) in petroleum ether/DCM (2 mL, 10:1, v/v) was stirred under H_2 (balloon) atmosphere at room temperature for 1 h. The reaction mixture was passed through a short pad of Celite® and washed by DCM (2 mL × 5). The combined organic layer was concentrated in vacuo. The residue was purified by silica gel column chromatography (DCM/MeOH/diethylamine = 100:1:1, v/v) to afford hybrids **1o-1** or **1p-1** or**1z-1**.

(*E*)-5-(benzo[d][1,3]dioxol-4-yl)-1-(4-methylpiperazin-1-yl)pent-1-en-3-one (**1o-1**) (48% yield); yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 12.9 Hz, 1H), 6.75 – 6.63 (m, 3H), 5.91 (s, 2H), 5.14 (d, J = 12.9 Hz, 1H), 3.27 (brs, 4H), 2.93 – 2.81 (m, 2H), 2.71 – 2.59 (m, 2H), 2.44 – 2.36 (m, 4H), 2.30 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.2, 151.1, 147.0, 145.5, 123.5, 122.7, 121.5, 106.6, 100.6, 95.7, 54.4 (×4), 46.2, 41.2, 25.5. HRESI-MS calculated for $[M+H]^+$ $C_{17}H_{23}N_2O_3^+$ (*m/z*): 303.1703; found: 303.1708. HPLC purity: >99.9% (t_R = 4.45 min).

(*E*)-5-(3,5-dimethoxyphenyl)-1-(4-methylpiperazin-1-yl)pent-1-en-3-one (**1p-1**) (63% yield); yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 12.9 Hz, 1H), 6.37 (d, J = 2.2 Hz, 2H), 6.28 (t, J = 2.2 Hz, 1H), 5.14 (d, J = 12.9 Hz, 1H), 3.76 (s, 6H), 3.28 (s, 4H), 2.89 – 2.84 (m, 2H), 2.64 (dd, J = 9.1, 7.0 Hz, 2H), 2.44 – 2.38 (m, 4H), 2.31 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.2, 160.9 (×2), 151.1, 144.7, 106.6 (×2), 98.2, 96.0, 55.4, 54.4 (×4), 46.2, 43.3, 32.0, 29.8. *m/z*: $[M+H]^+$ calcd for $C_{18}H_{27}N_2O_3^+$,

319.2016, found, 319.2018. HPLC purity: >99.9% (t_R = 4.30 min).

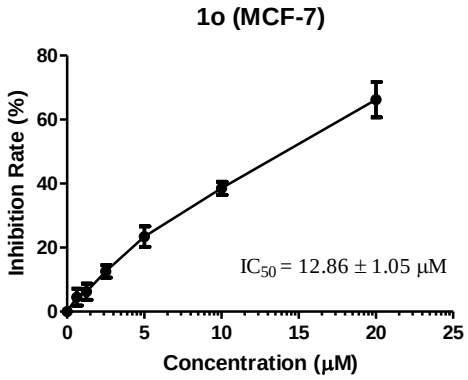
(*E*)-5-(3-((tert-butyldimethylsilyl)oxy)phenyl)-1-(4-methylpiperazin-1-yl)pent-1-en-3-one(**1z-1**) **(**68% yield); yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 12.9 Hz, 1H), 7.11 (t, J = 7.8 Hz, 1H), 6.80 (d, J = 7.6 Hz, 1H), 6.69 (s, 1H), 6.65 (dd, J = 8.0, 2.1 Hz, 1H), 5.14 (d, J = 12.9 Hz, 1H), 3.29 (s, 4H), 2.94 – 2.82 (m, 2H), 2.68 – 2.58 (m, 2H), 2.46 – 2.38 (m, 4H), 2.31 (s, 3H), 0.98 (d, J = 2.7 Hz, 9H), 0.18 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.4, 155.8, 151.2, 143.7, 129.3, 121.5, 120.3, 117.6, 96.0, 54.4 (×4), 46.2, 43.4, 31.7, 25.9 (×3), 18.3, -4.2 (×2). *m/z*: $[M+H]^+$ calcd for $C_{22}H_{37}N_2O_2Si^+$, 389.2619, found, 389.2620. HPLC purity: 99.5% (t_R = 14.06 min).

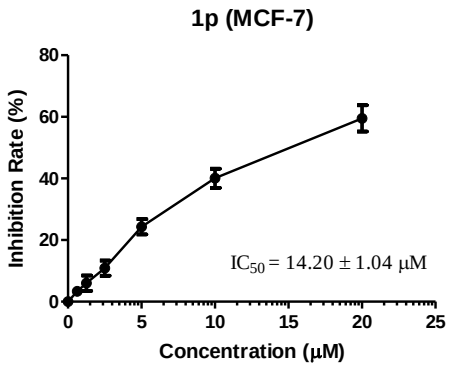
2. Cellular assays

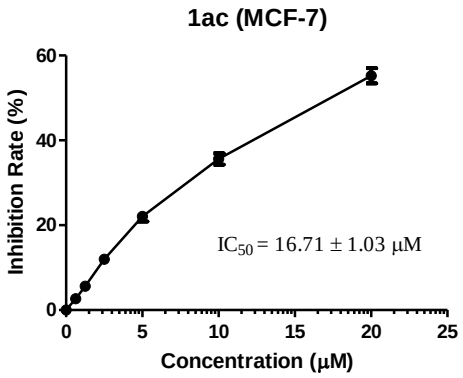
2.1 Determination the IC50s of the hybrids 1.

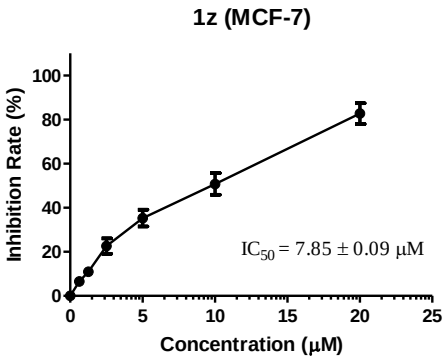
MCF-7, HCC1806, MDA-MB-231, MDA-MB-468, SKBr3, and BT474 were purchased Cell Bank, Type Culture Collection, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in MEM (Gibco, Beijing, China) with 10% fetal bovine serum (Gibco, Grand Island), supplemented with 1% Glutamax, 1% Non-essential Amino Acids, 1% Sodium Pyruvate 100 mM Solution (Gibco, Beijing, China) and 0.1% human recombinant insulin (VivaCell, Shanghai, China) and maintained in humidified atmosphere of 5% CO_2 at 37℃.

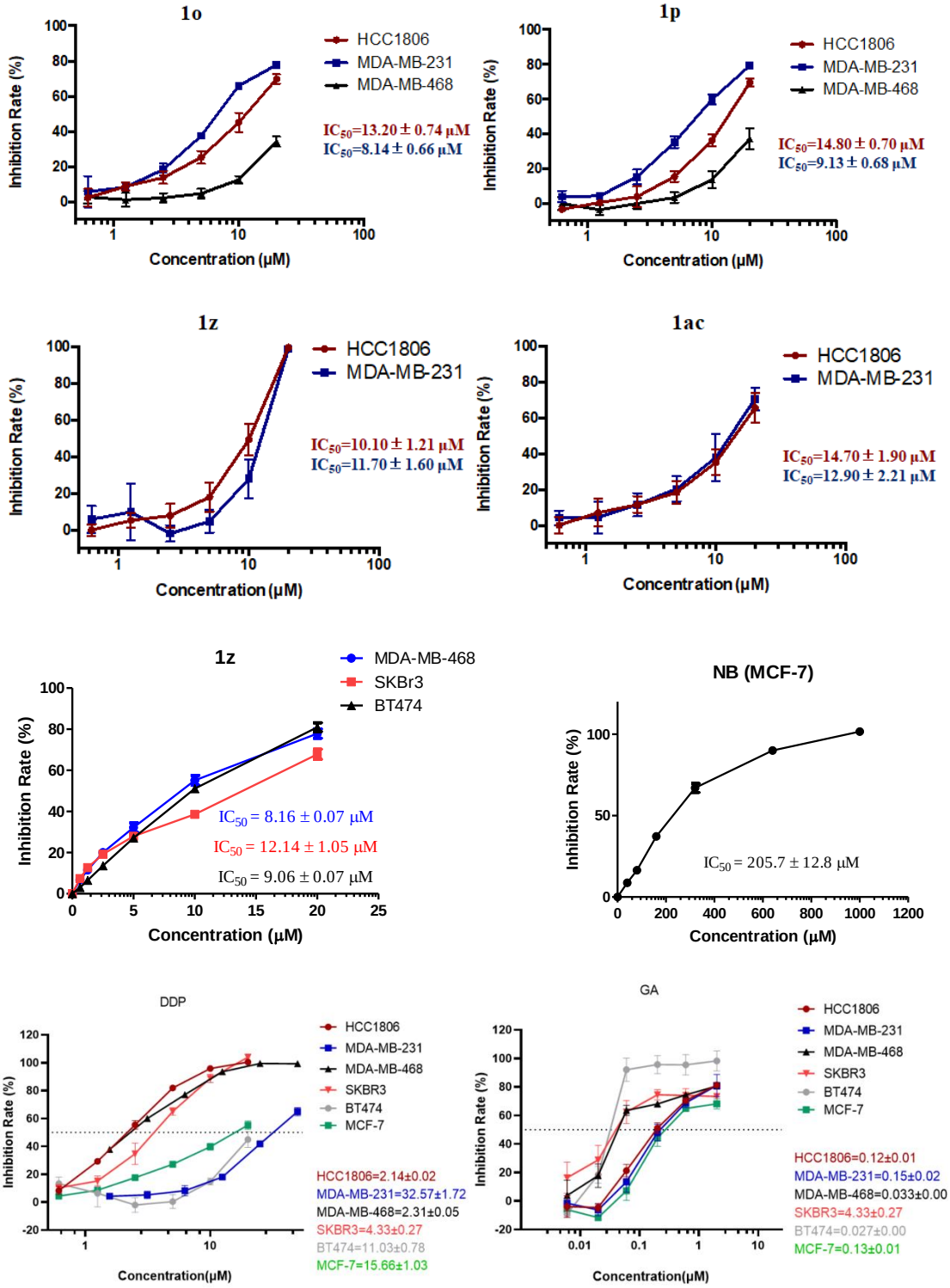
Cell viability after treatments of tested compounds (20 μM) was evaluated by MTT assay according to our previous work.[1] Cells were treated with the indicated compounds at concentrations of 0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 μM in 96-well plates. After 48 h, MTT solution was added to each well, for a final concentration of 20%. Cells were then incubated at 37 °C for 4 h and the absorbance was measured at 570 nm by spectrophotometry. The IC_{50} values were determined by non-linear regression analysis using GraphPad Prism 5 software (GraphPad, Inc., San Diego, CA).










1o
HCC1806
MDA-MB-231
MDA-MB-468
IC50=13.20 ± 0.74 μM
IC50=8.14 ± 0.66 μM
Inhibition Rate (%)
Concentration (μM)
1p
HCC1806
MDA-MB-231
MDA-MB-468
IC50=14.80 ± 0.70 μM
IC50=9.13 ± 0.68 μM
Inhibition Rate (%)
Concentration (μM)
1z
HCC1806
MDA-MB-231
IC50=10.10 ± 1.21 μM
IC50=11.70 ± 1.60 μM
Inhibition Rate (%)
Concentration (μM)
1ac
HCC1806
MDA-MB-231
IC50=14.70 ± 1.90 μM
IC50=12.90 ± 2.21 μM
Inhibition Rate (%)
Concentration (μM)
1z
MDA-MB-468
SKBr3
BT474
IC50 = 8.16 ± 0.07 μM
IC50 = 12.14 ± 1.05 μM
IC50 = 9.06 ± 0.07 μM
Inhibition Rate (%)
Concentration (μM)
NB (MCF-7)
IC50 = 205.7 ± 12.8 μM
Inhibition Rate (%)
Concentration (μM)
DDP
HCC1806
MDA-MB-231
MDA-MB-468
SKBR3
BT474
MCF-7
HCC1806=2.14±0.02
MDA-MB-231=32.57±1.72
MDA-MB-468=2.31±0.05
SKBR3=4.33±0.27
BT474=11.03±0.78
MCF-7=15.66±1.03
Inhibition Rate (%)
Concentration(μM)
GA
HCC1806
MDA-MB-231
MDA-MB-468
SKBR3
BT474
MCF-7
HCC1806=0.12±0.01
MDA-MB-231=0.15±0.02
MDA-MB-468=0.033±0.00
SKBR3=4.33±0.27
BT474=0.027±0.00
MCF-7=0.13±0.01
Inhibition Rate (%)
Concentration(μM)

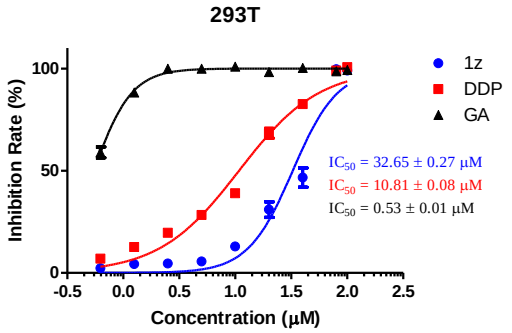
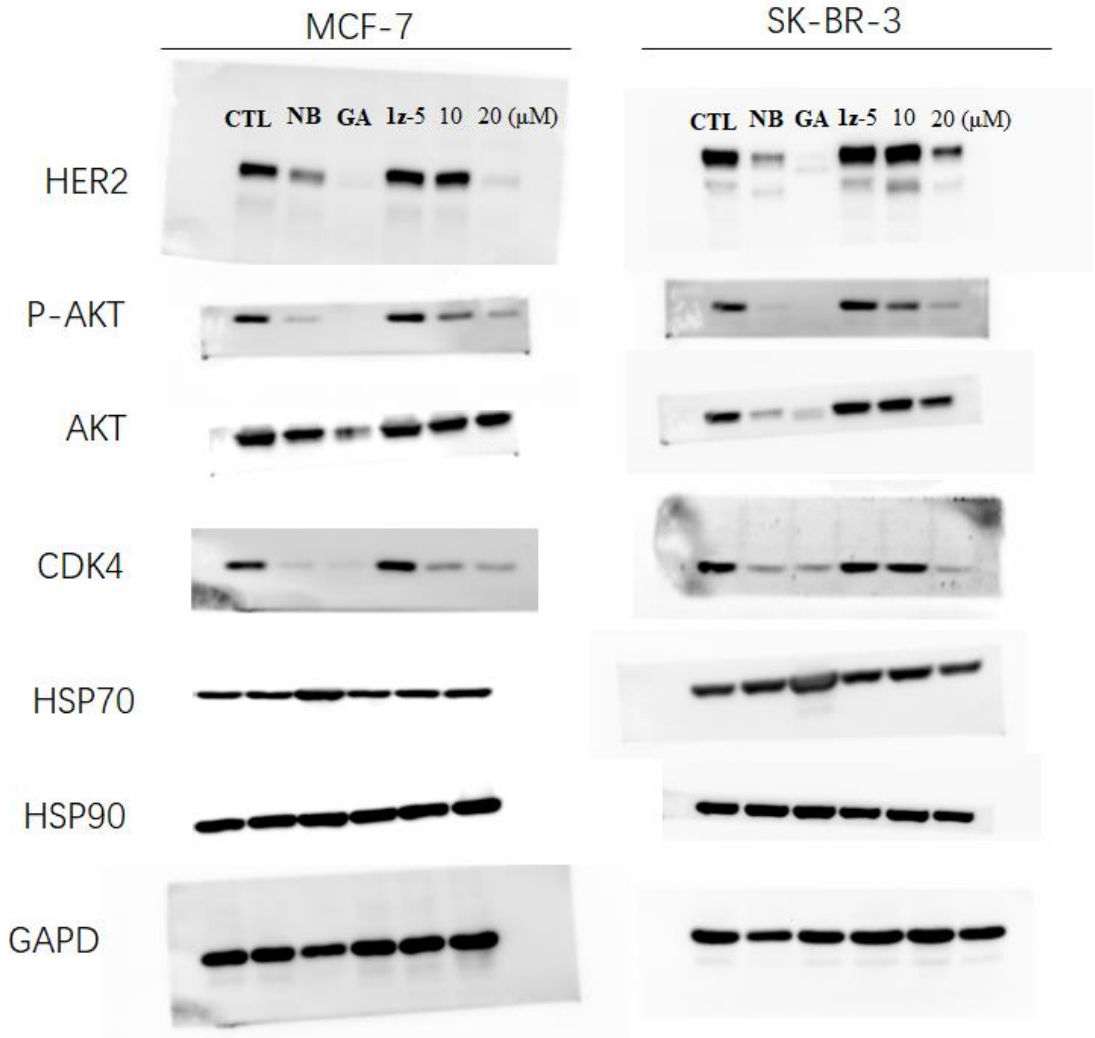
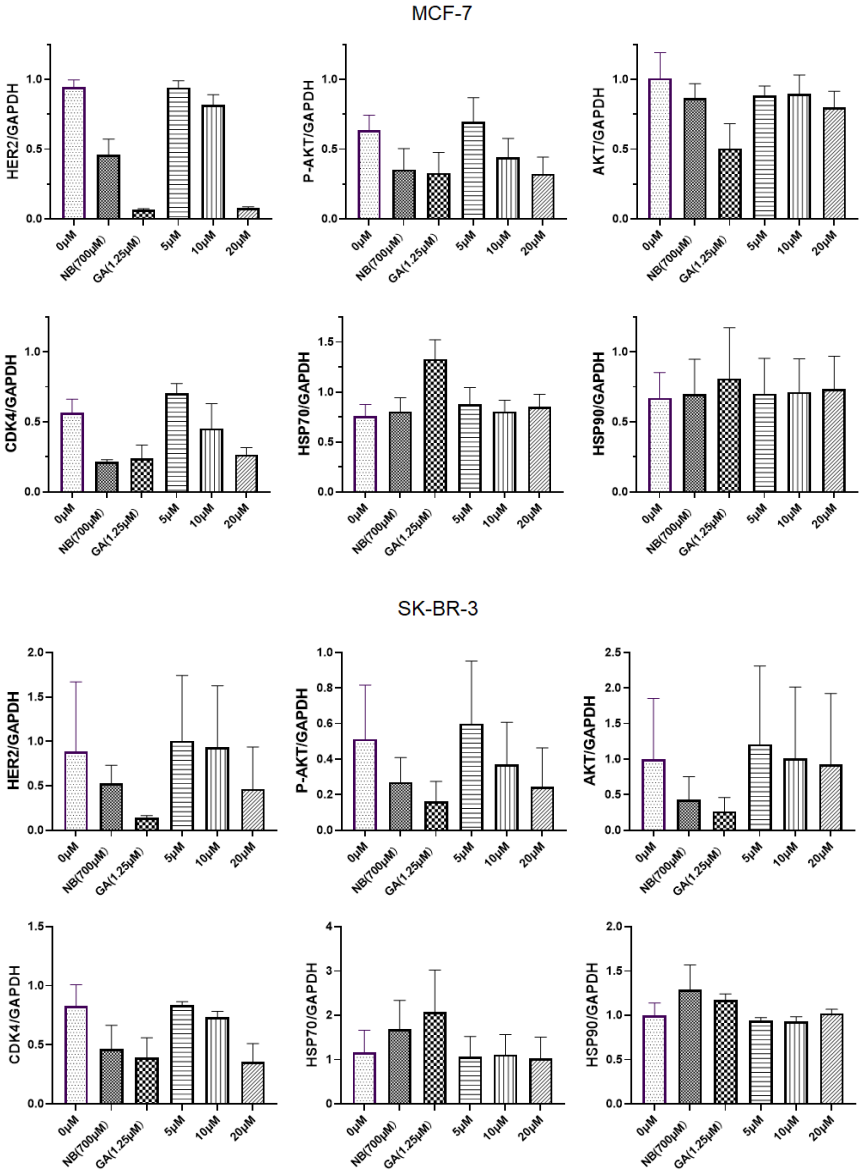


Fig. S1 IC_{50} curves of tested compounds.

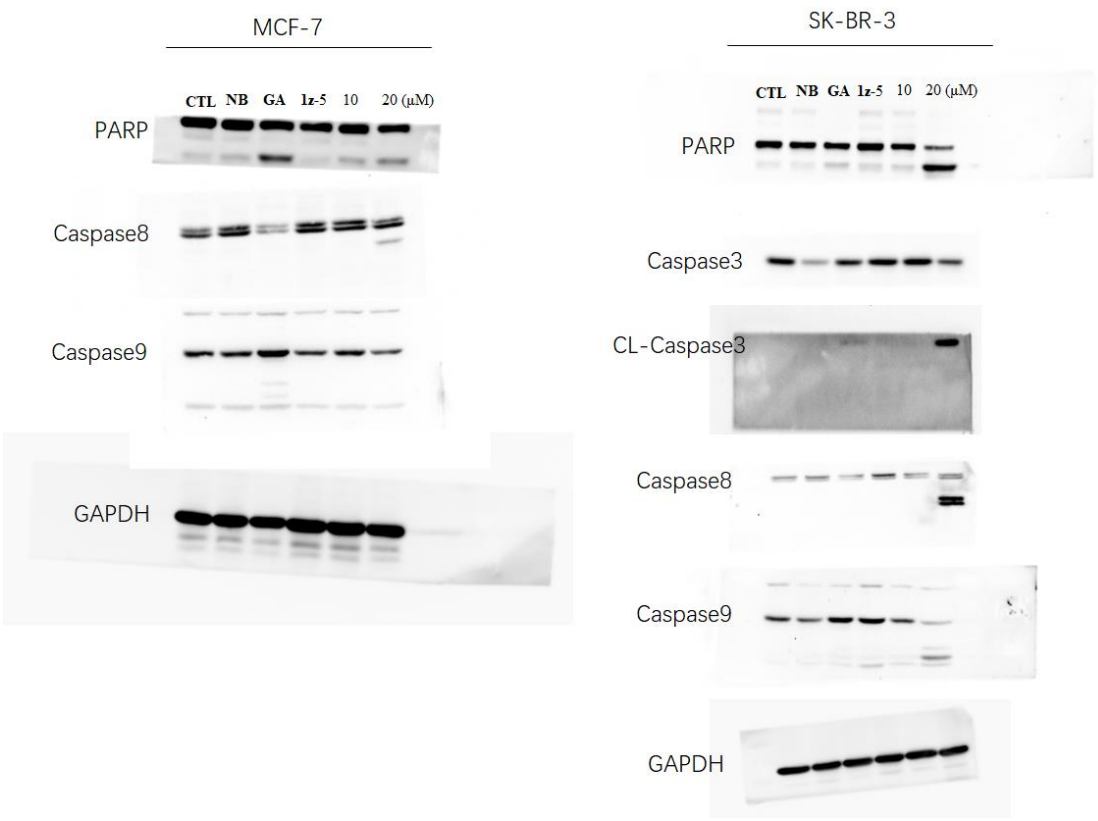
2.2 Western blot analysis

Antibodies: Primary antibodies targeted HER2, HSP70, HSP90, CDK4, AKT, pAKT, Caspase3, CL-Casepase3, PARP, Caspase8 (Cell Signaling, CA, USA), EGFR, and GAPDH (Proteintech Group, Chicago, USA). Secondary antibodies were HRP-linked anti-rat (Cell Signaling, CA, USA), and HRP-conjugated Affinipure Goat Anti-rabbit IgG and FITC-conjugated Affinipure Goat Anti-Rat lgG (Proteintech Group, Chicago, USA). The raw, unprocessed, representative western blot data as follow:





Analysis of the density ratios of HER2, P-AKT, AKT, CDK4, HSP70, HSP90 to GAPDH by Image J. Data are represented as the mean ± SD of three independent experiments.


MCF-7
CTL NB GA Iz-5 10 20 (μM)
PARP
Caspase8
Caspase9
GAPDH
SK-BR-3
CTL NB GA Iz-5 10 20 (μM)
PARP
Caspase3
CL-Caspase3
Caspase8
Caspase9
GAPDH

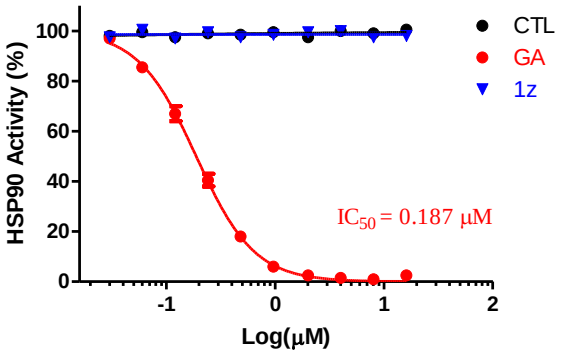
3 Enzyme-based assays

3.1 Fluorescence Polarization

The tested compounds were dissolved in DMSO and then appropriately diluted to obtain a gradient concentration (0.03 – 16.0 μM) for addition to the final reaction system. The HSP90 reaction system consisted of HSP90α (10 nM), BSA (1%), FITC-GA (5 nM), and reaction buffer.[2] After thoroughly mixing of all components (DMSO < 1%), the reaction system was incubated at room temperature for 3 h. Subsequently, the fluorescence was measured at 485 nm excitation wavelength and 530 nm emission wavelength using SpectraMax M5 plate reader. HSP90 NTD Activity% = $(FP_{drug}–FP_{backgroud})/(FP_{enzyme}–FP_{backgroud})\times100\%$. Curve fitting was performed using Graphpad Prism 5.

Table S1. The HSP90 NTD Activity (%)

Concentration (μM)	CTL		GA		**1z**	
0	98	100	99	97	99	101
0.030	97	99	96	98	98	97
0.060	101	98	84	87	99	102
0.12	98	97	64	70	96	98
0.24	101	97	43	38	100	99
0.48	99	98	20	16	97	98
0.96	100	99	4	8	99	98
2.00	98	97	1	4	100	99
4.00	101	99	2	1	99	101
8.00	100	98	1	1	98	97
16.00	102	99	3	2	100	96



3.2 Alphascreen-Based HSP90 C-Terminal Inhibition.

The HSP90 C-terminal inhibitory activity was determined by a modified Alphascreen assay system (PerkinElmer, Waltham, MA) according to our previous work.[1] Glutathione acceptor beads were added to the mixture, which was then incubated for another 30 min. Streptavidin-coated donor beads were added and mixed gently. Compound **1z** (0 – 1000 μM), GA (0 – 1000 μM), and NB (0 – 1000 μM) in DMSO were added into the final reaction system. The system was incubated for 10 min, and the fluorescence of each well was measured in a 2390 EnSpire Alpha Reader with excitation at 680 nm and emission in the range of 520−620 nm. HSP90 CTD Activity% = $(A_{drug}–A_{backgroud})/(A_{enzyme}–$

$A_{backgroud}$)×100%.

Table S2. The HSP90 CTD Activity (%) for tested compounds (100 μM)

CTL	NB	**1z**
102.1	43.5	38.1
99.9	48.2	32.6
102.3	38.7	28.4

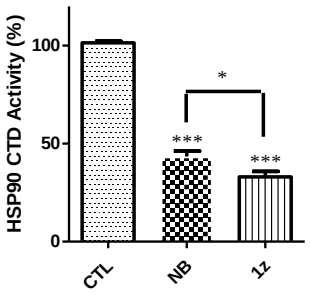
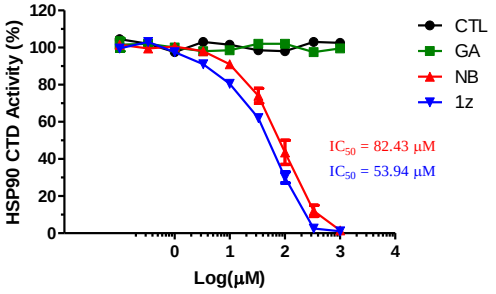
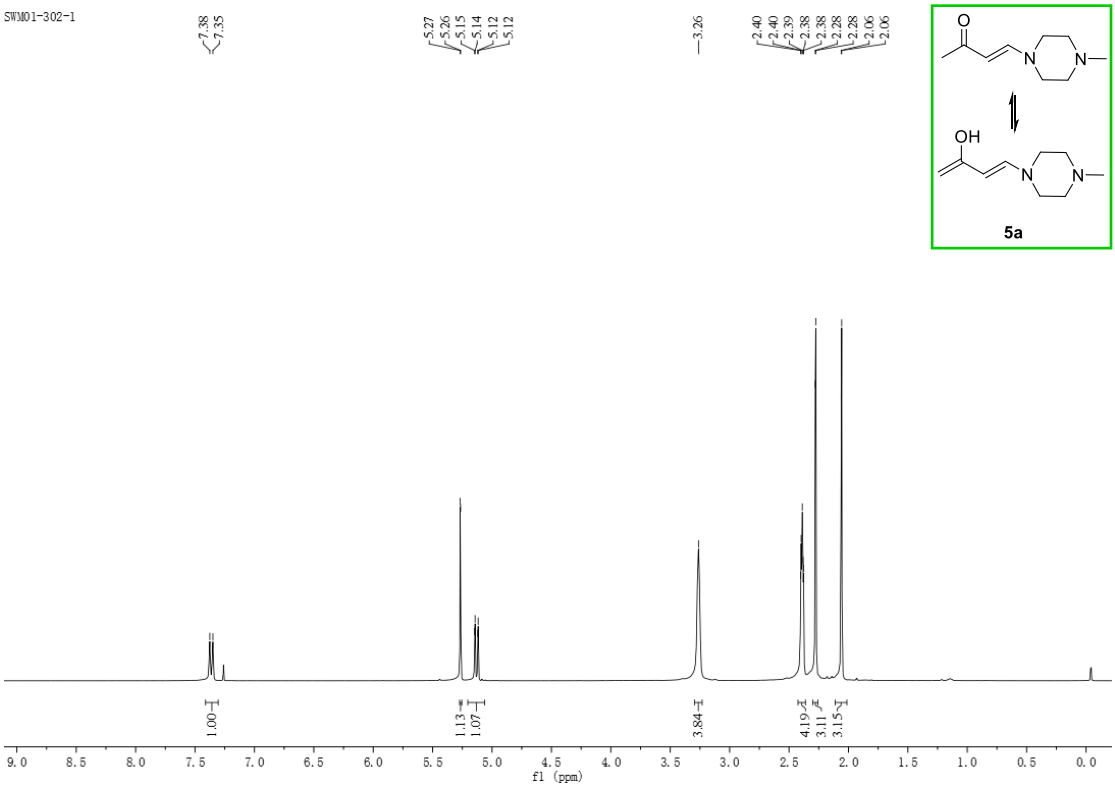


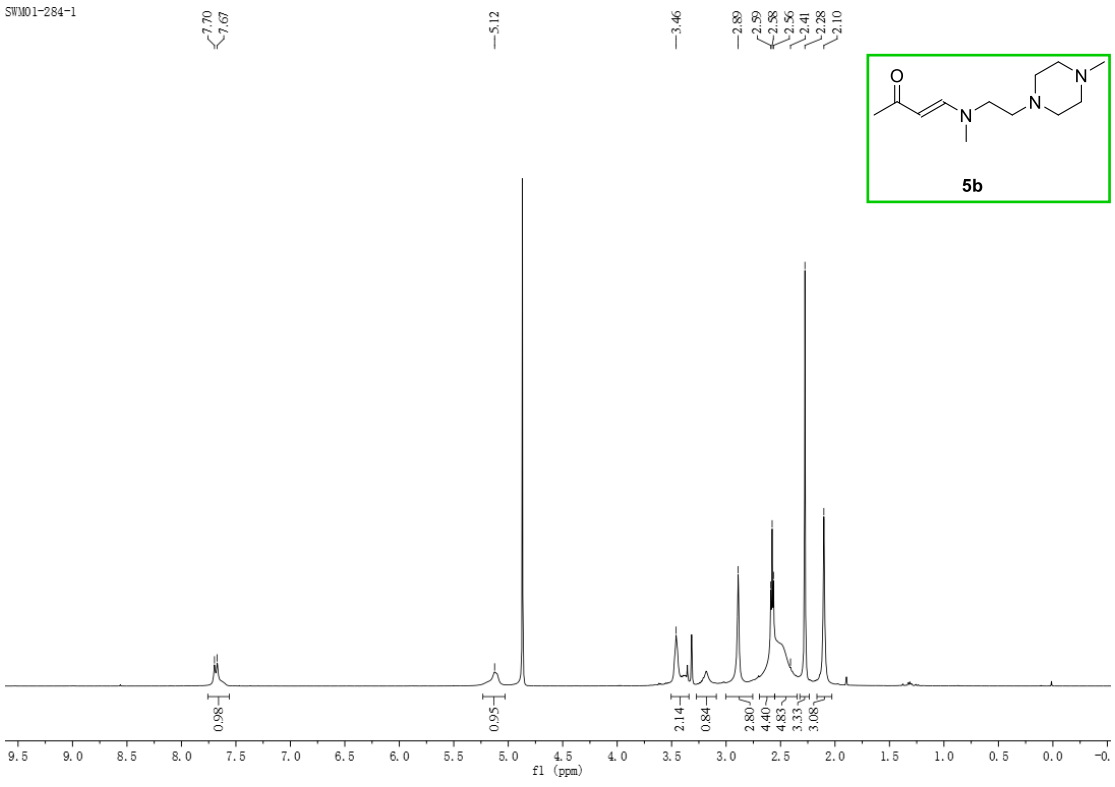
Table S3. The HSP90 CTD Activity (%) for tested compounds (0 - 1000 μM)

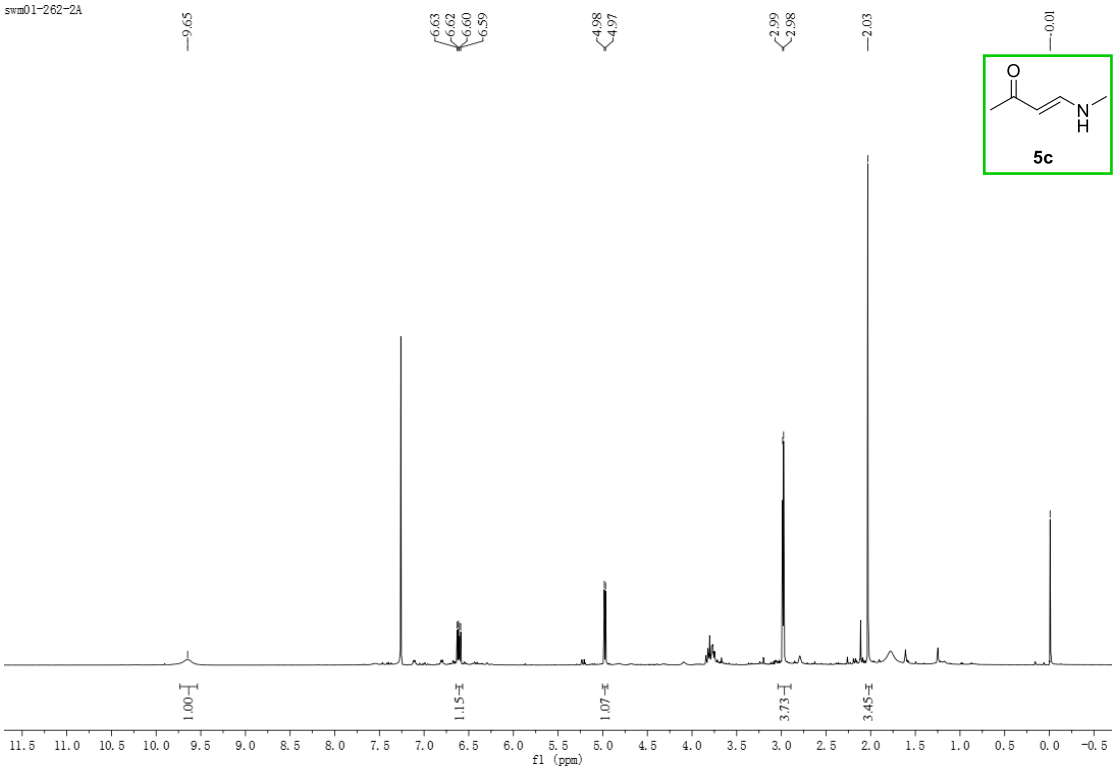
Concentration (μM)	CTL		GA		NB		1z	
0	102	99	103	97	102	101	102	104
0.10	104	105	105	98	100	103	99	100
0.33	101	103	101	103	99	100	102	104
1.0	99	96	99	101	102	99	98	97
3.3	101	105	97	99	96	100	90	92
10.0	103	100	99	98	93	89	82	79
33.0	100	97	102	102	78	70	64	60
100	97	99	104	100	50	37	33	27
333	101	105	99	96	15	9	3	2
1000	103	102	100	99	1	2	1	1



4. The ^{1}H and ^{13}C NMR spectra of synthetic compounds



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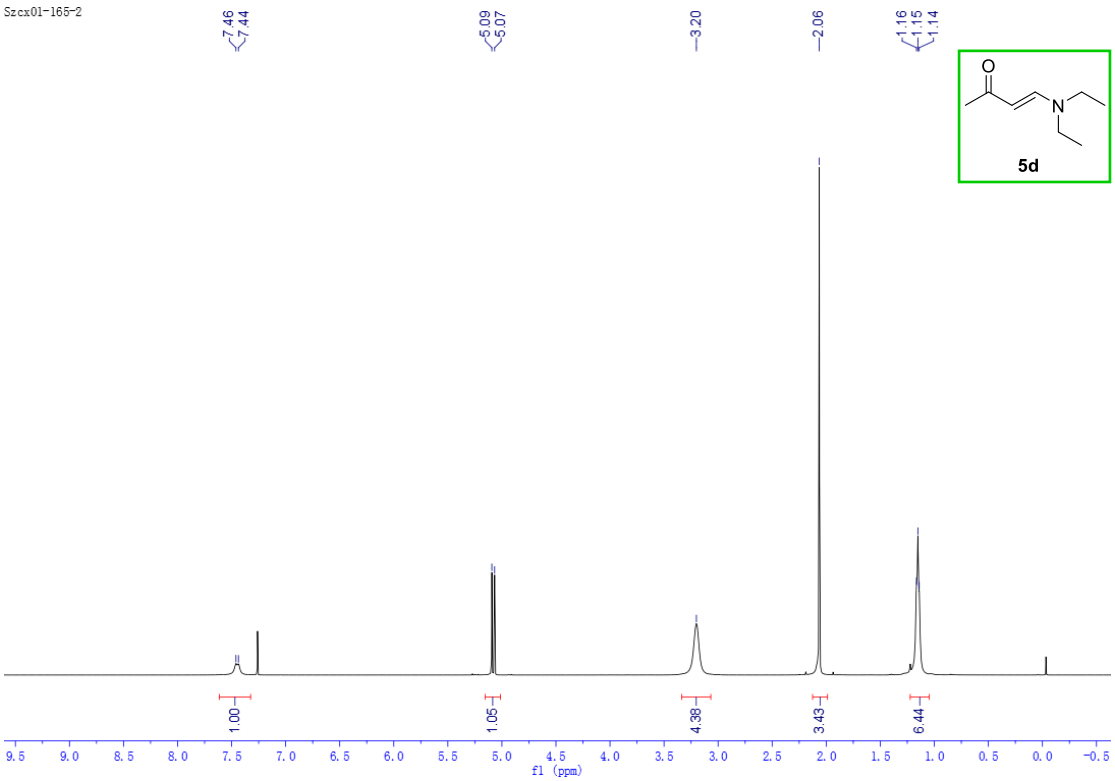
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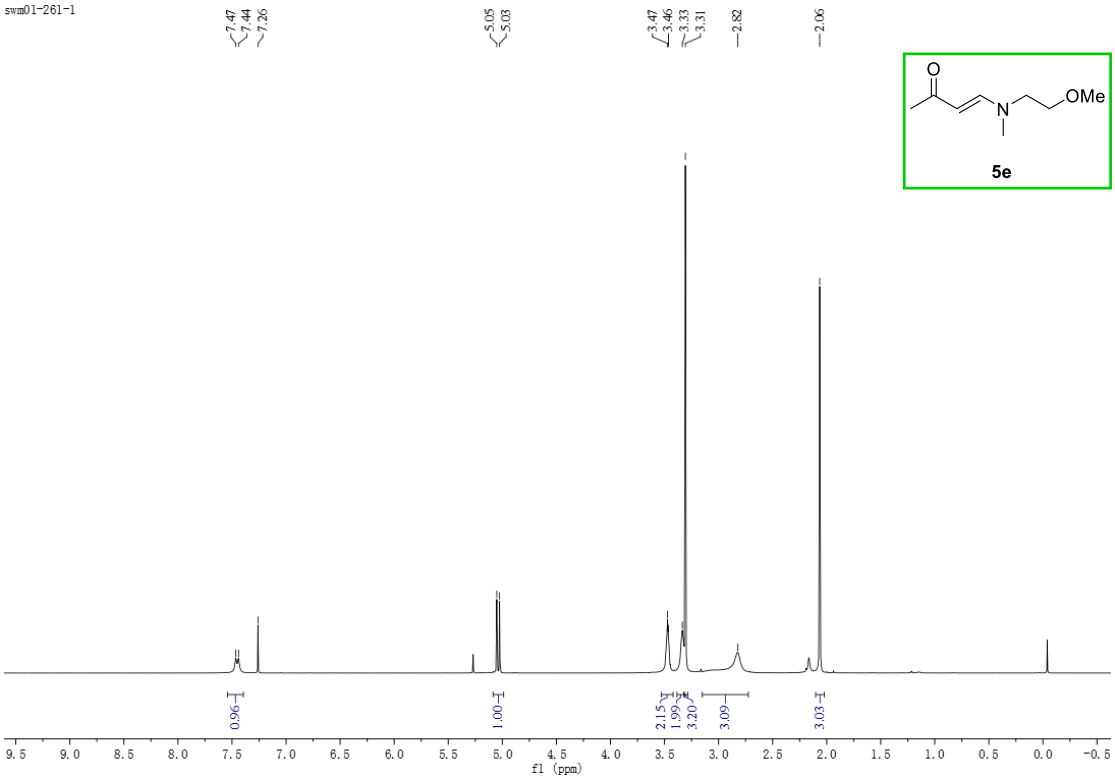
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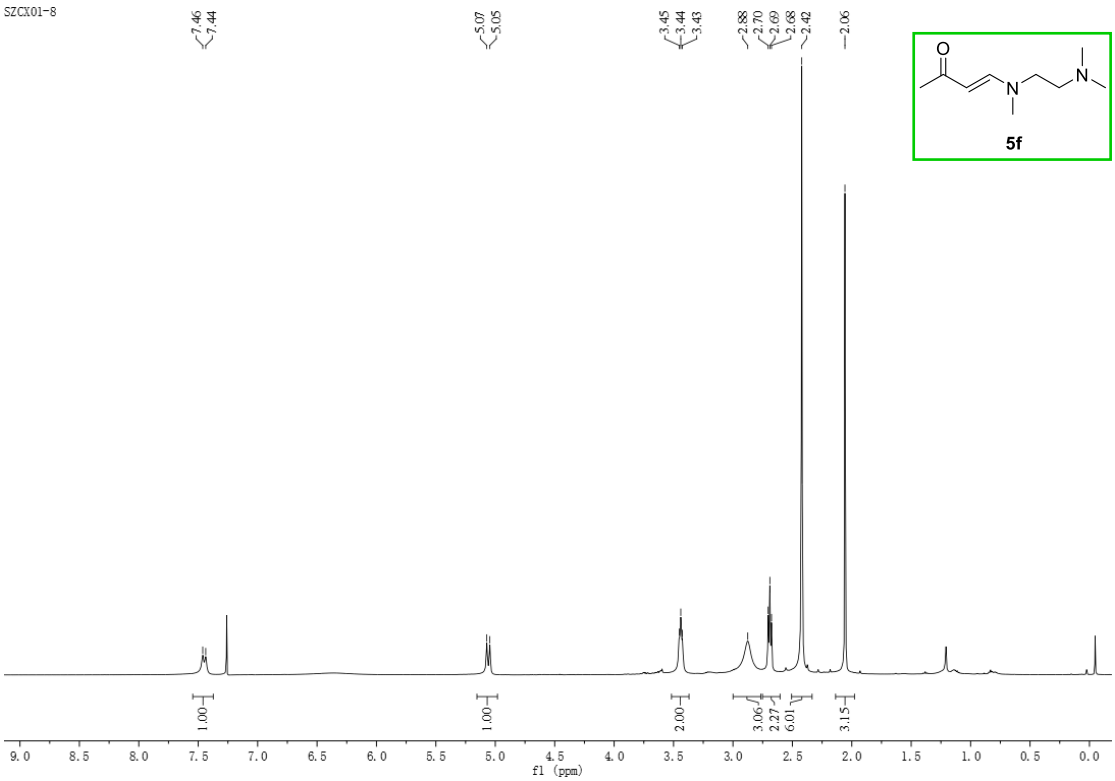
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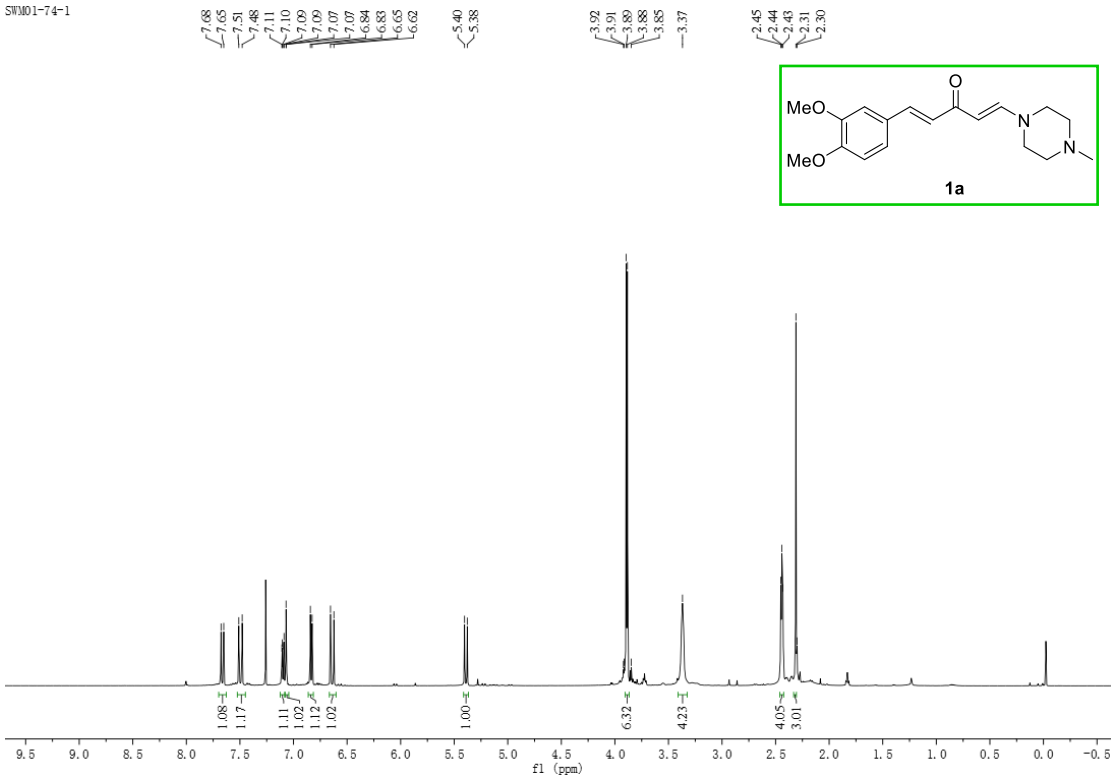
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2.44
2.43
2.31
2.30
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MeO
O
N
N
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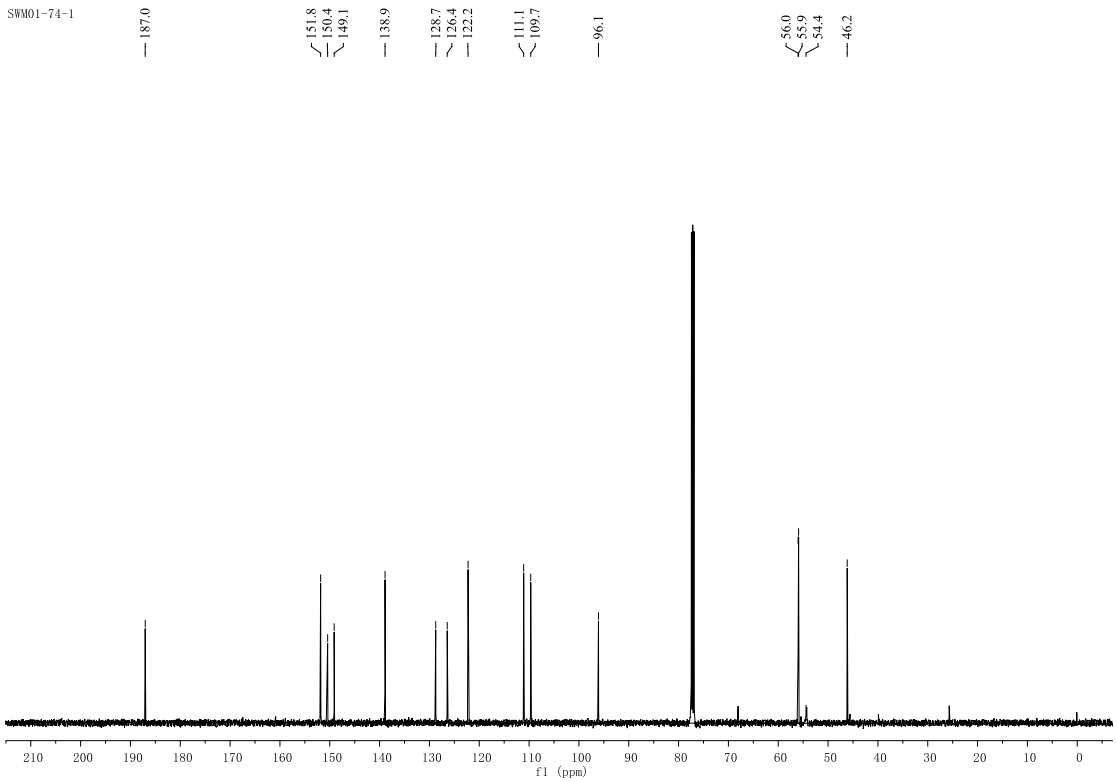
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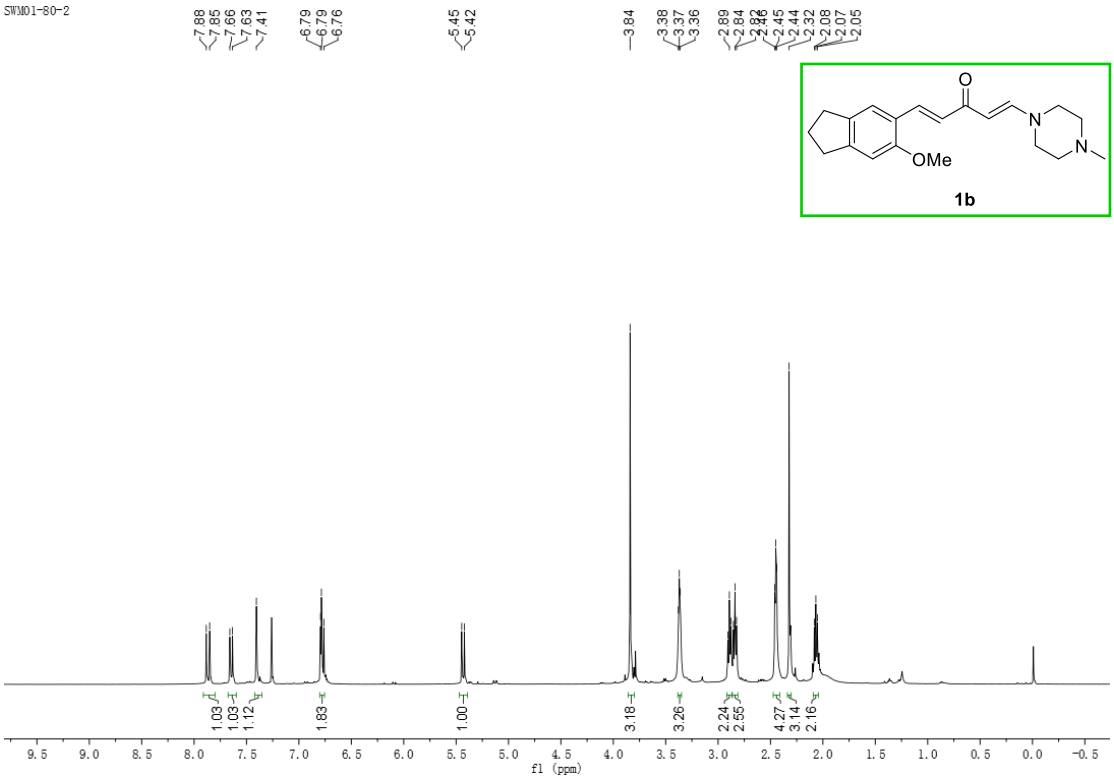
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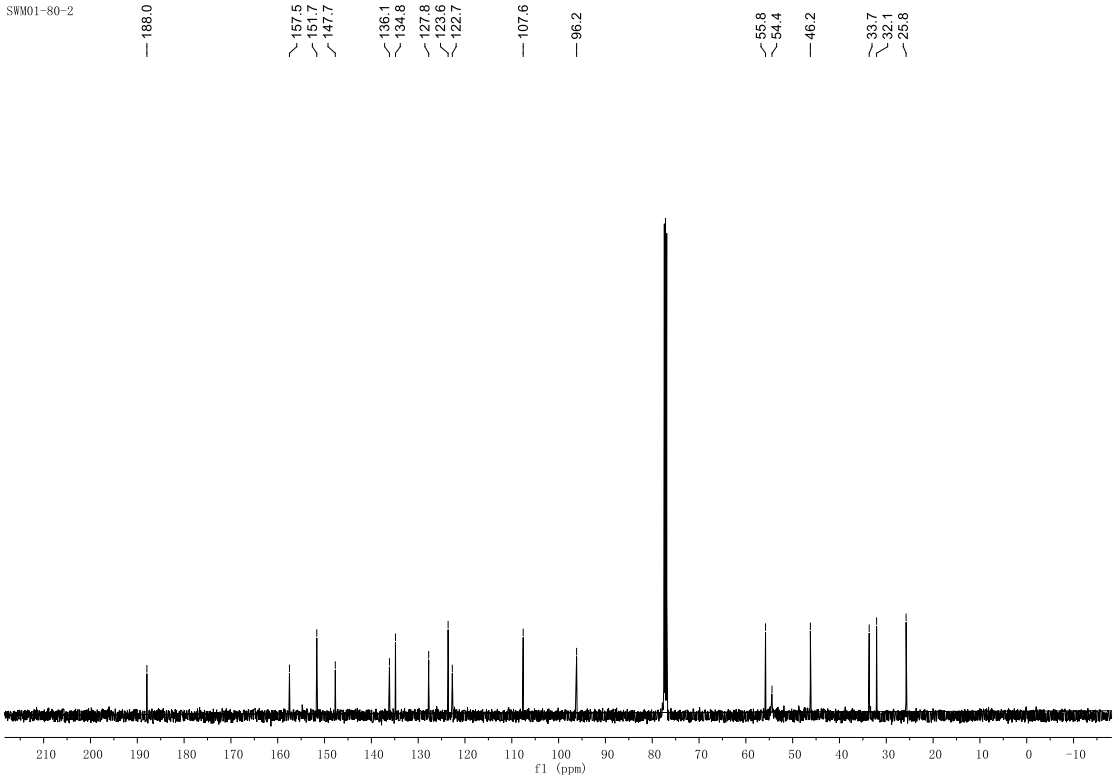
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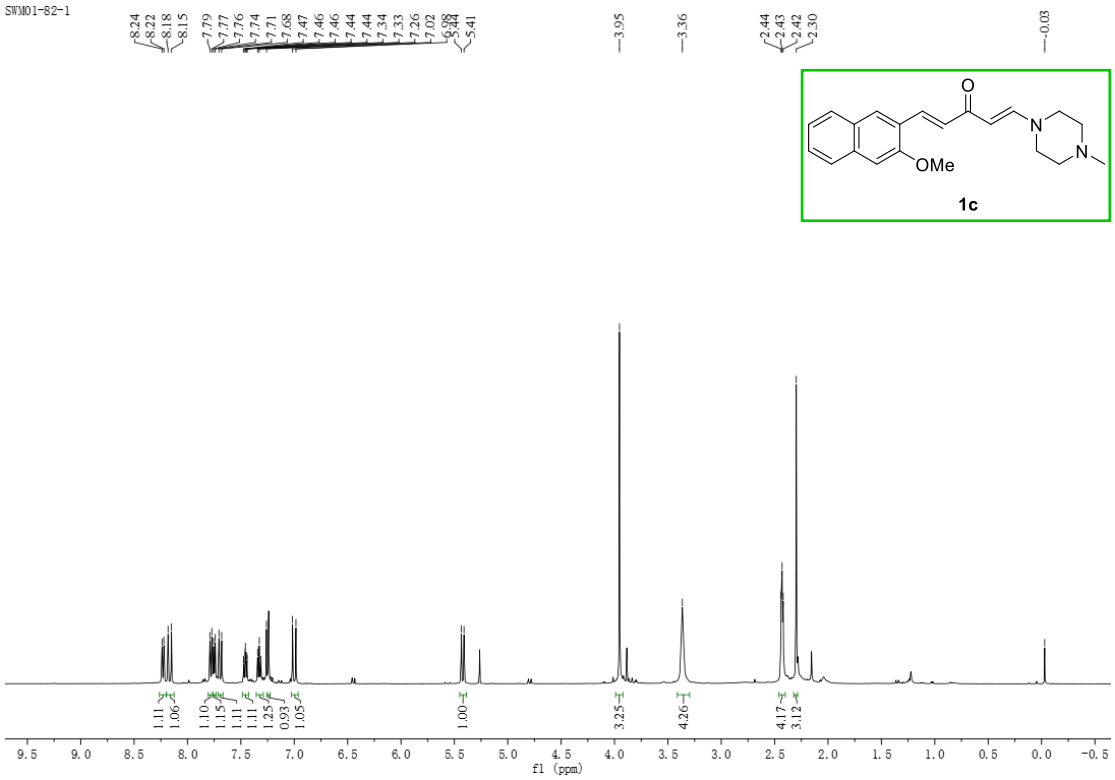
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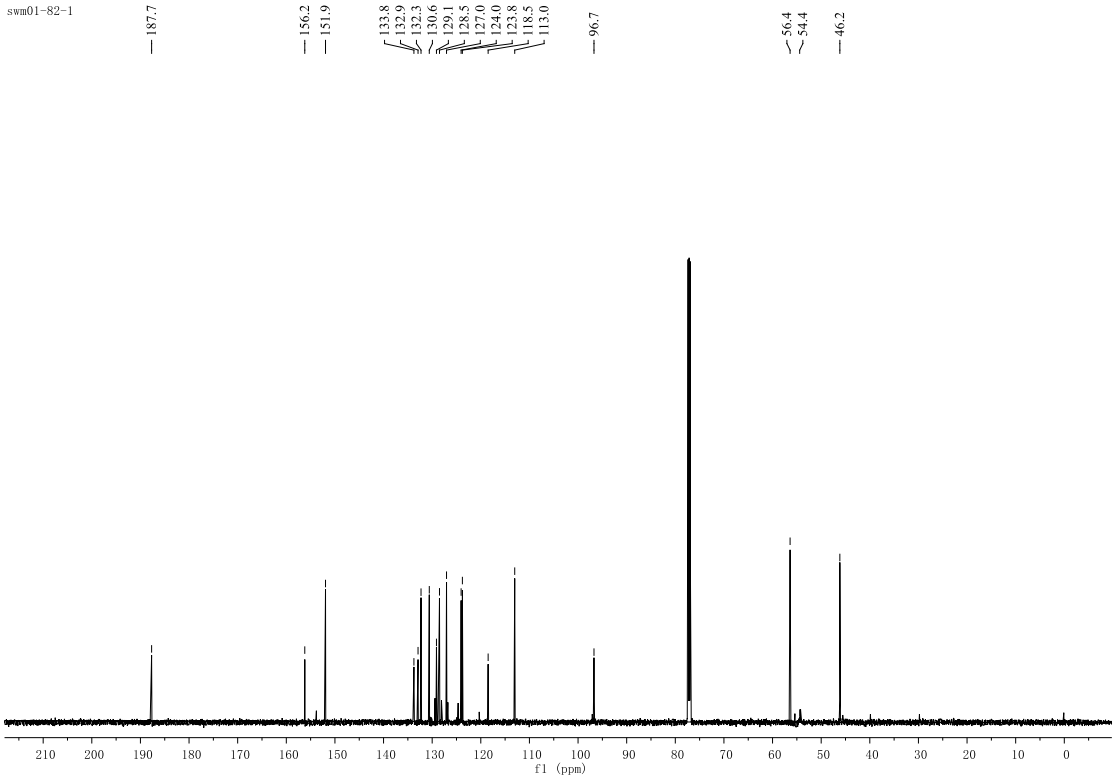
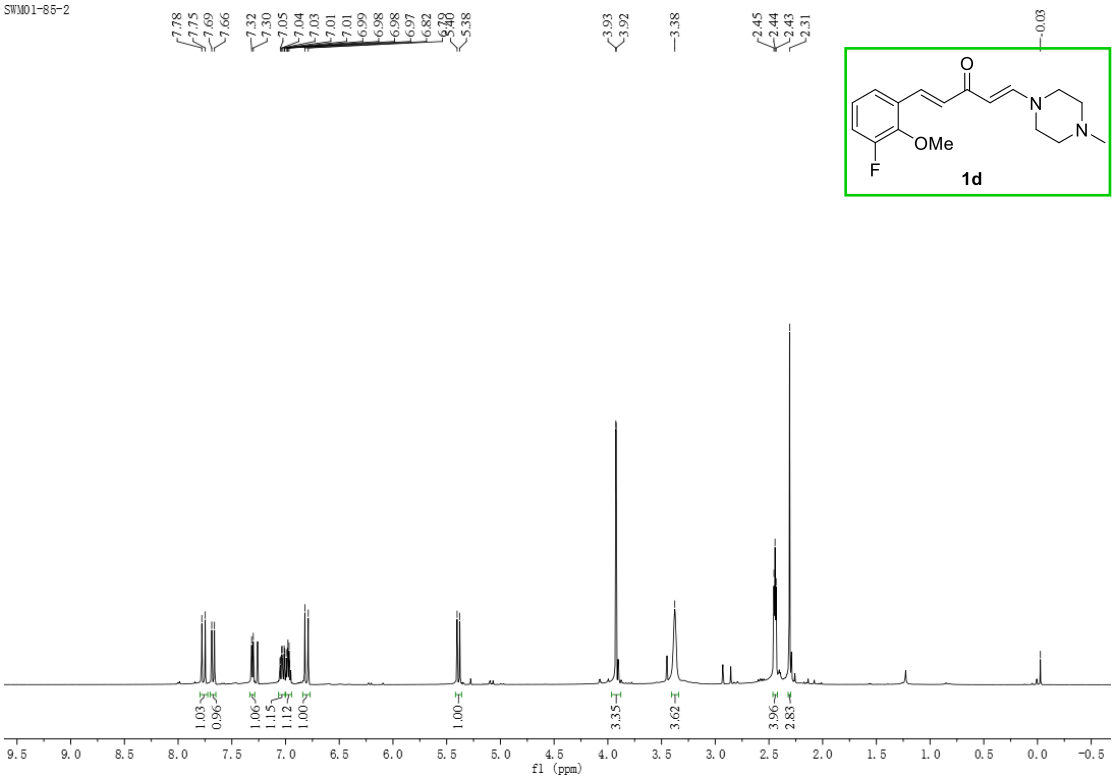
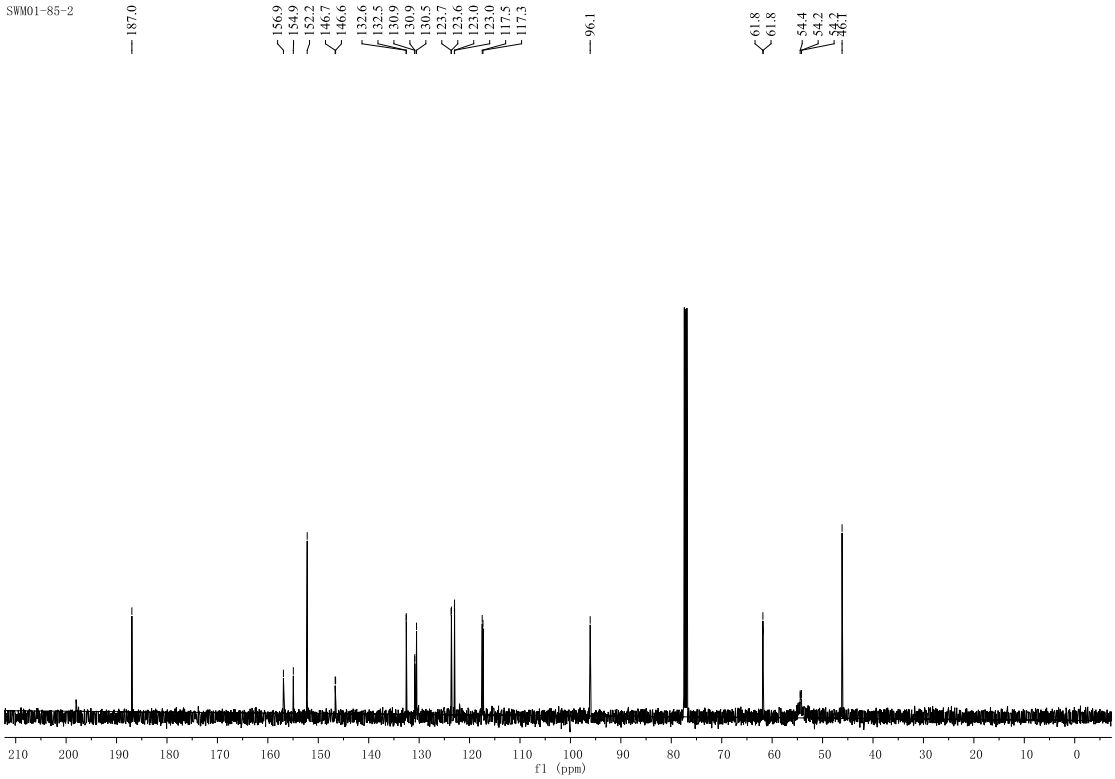
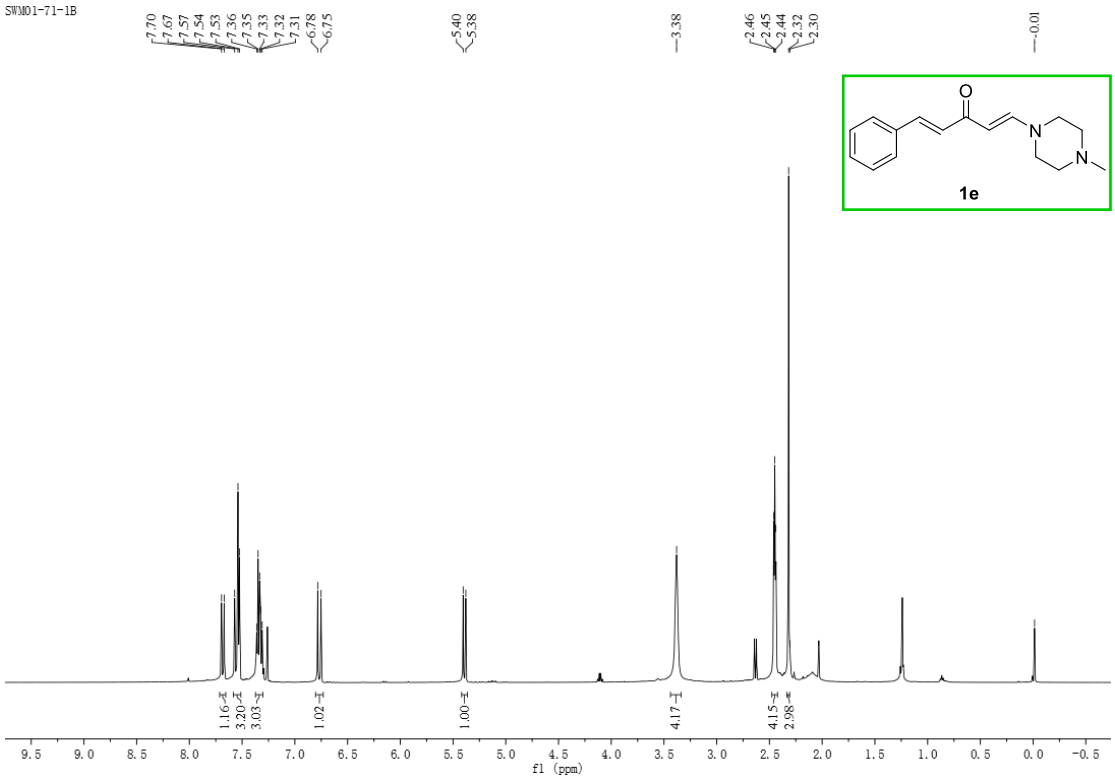
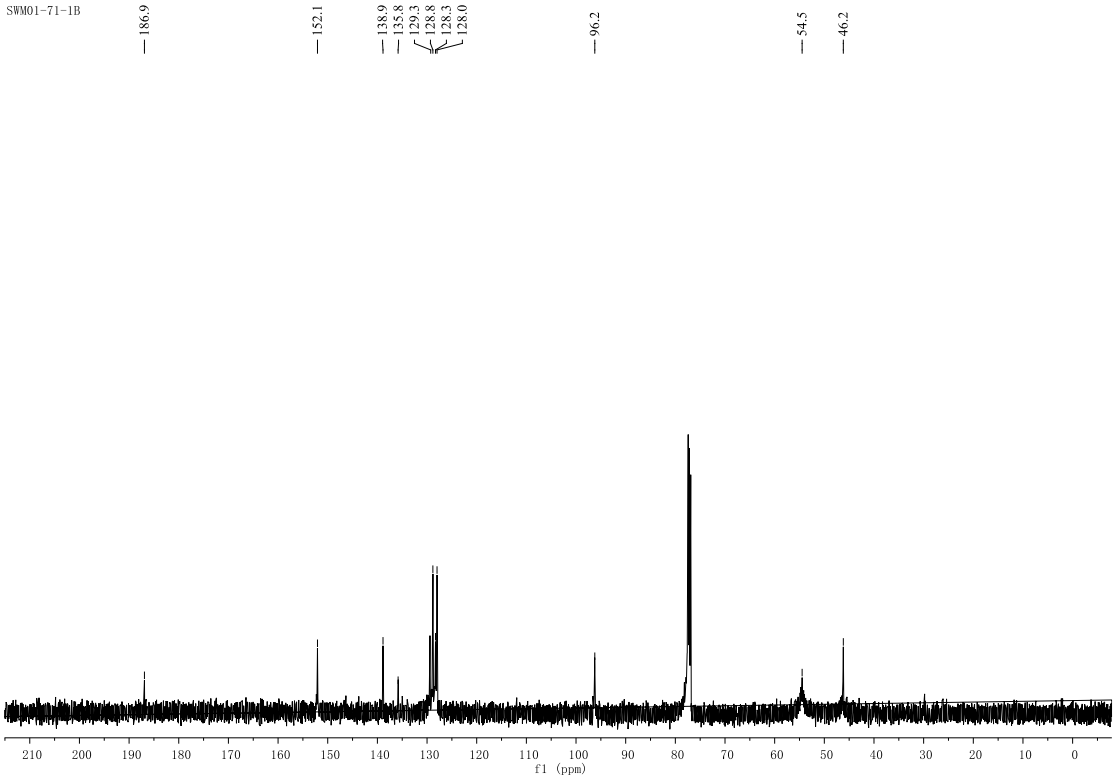
4.17
3.12
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

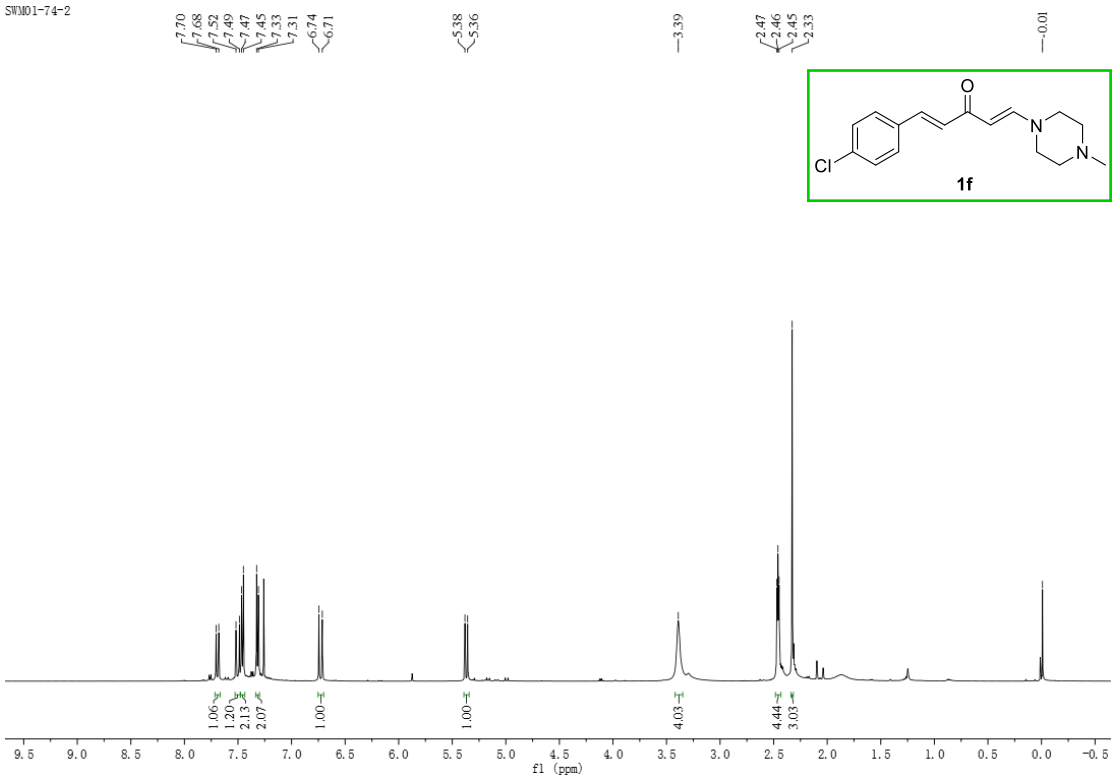

swm01-82-1
187.7
156.2
151.9
133.8
132.9
132.3
130.6
129.1
126.5
127.0
124.0
123.8
118.5
113.0
96.7
56.4
54.4
46.2
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

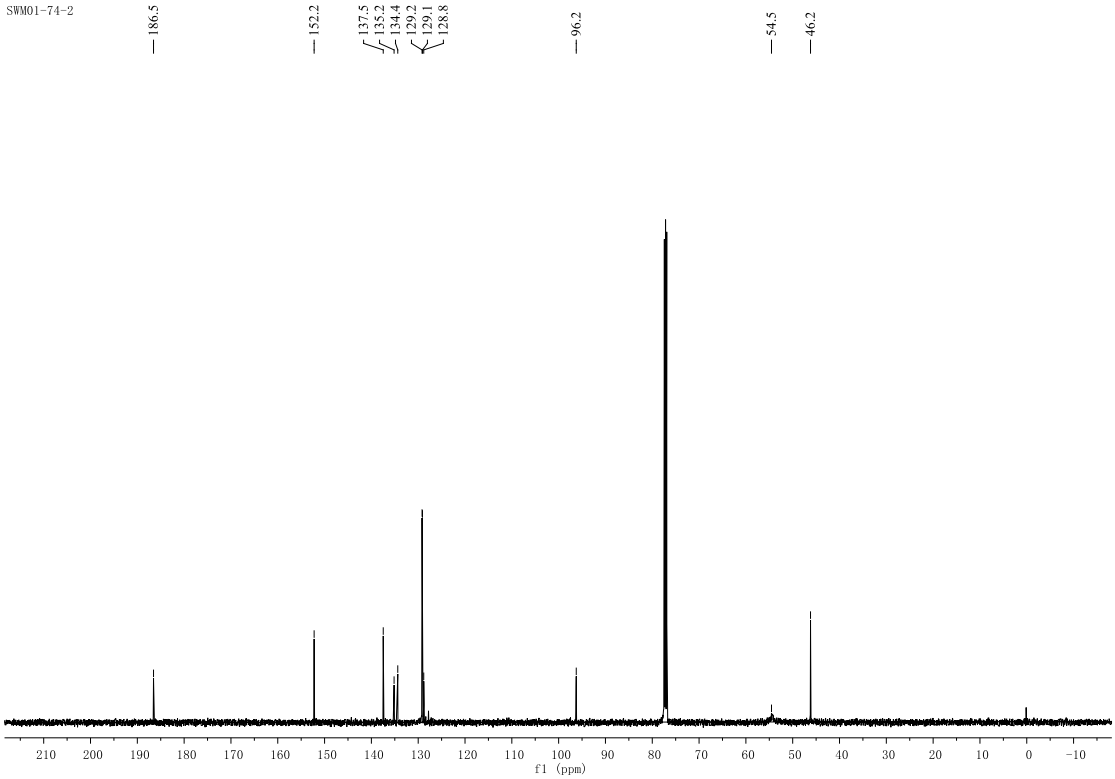

SWM01-85-2
7.78
7.75
7.69
7.66
7.32
7.30
7.05
7.04
7.03
7.01
7.01
6.99
6.98
6.98
6.97
6.82
6.40
6.40
5.38
3.93
3.92
3.38
2.45
2.44
2.43
2.31
0.03
O
N
N
OMe
F
1d
1.03
0.96
1.06
1.15
1.12
1.00
1.00
3.35
3.62
3.96
2.83
f1 (ppm)

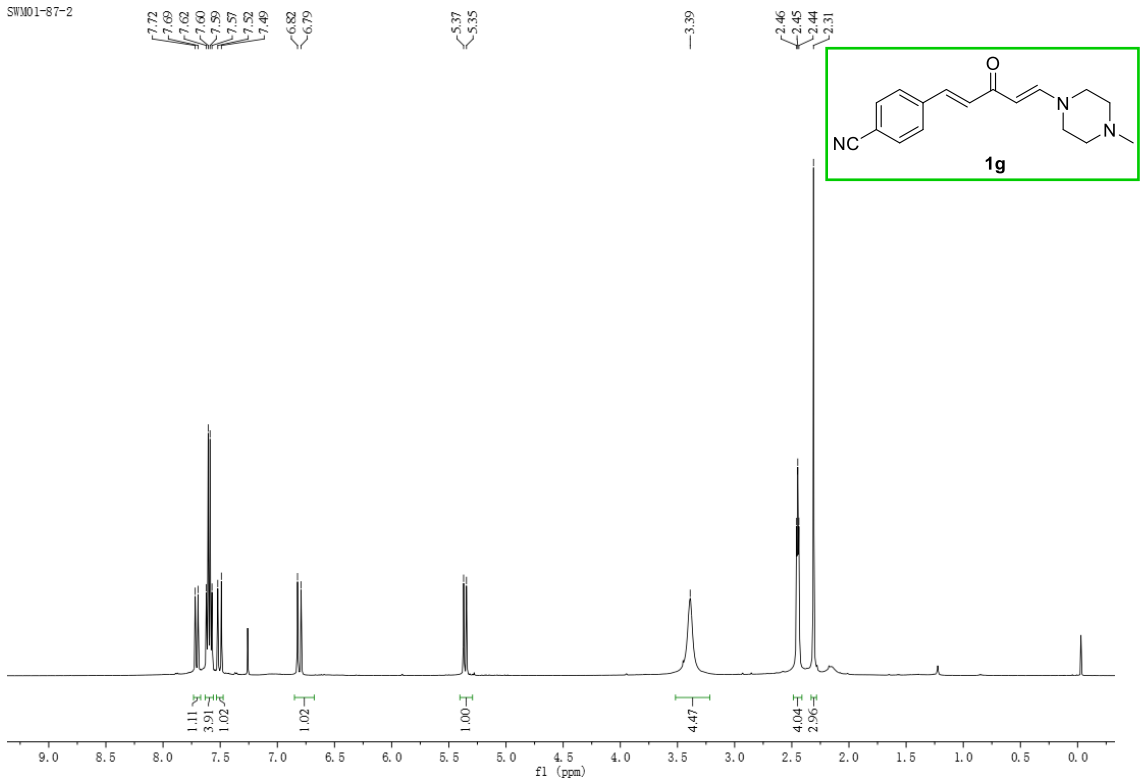

SWM01-85-2
187.0
156.9
154.9
152.2
146.7
146.6
132.6
132.5
130.9
130.9
130.5
123.7
123.6
123.0
123.0
117.5
117.3
96.1
61.8
61.8
54.4
54.2
54.2
46.1
f1 (ppm)

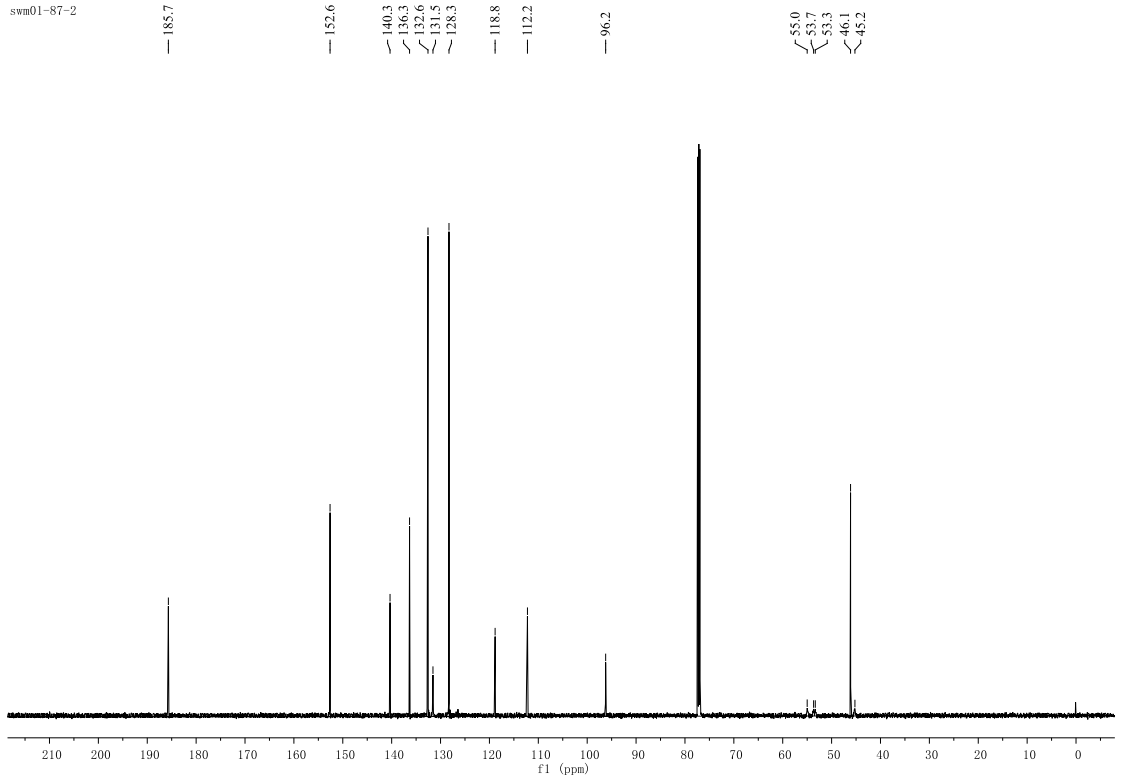

SWM01-71-1B
7.70
7.67
7.57
7.54
7.53
7.36
7.35
7.33
7.32
7.31
6.78
6.75
5.40
5.38
3.38
2.46
2.45
2.44
2.32
2.30
0.01
O
N
N
1e
1.16
3.20
3.03
1.02
1.00
4.17
4.15
2.98
f1 (ppm)

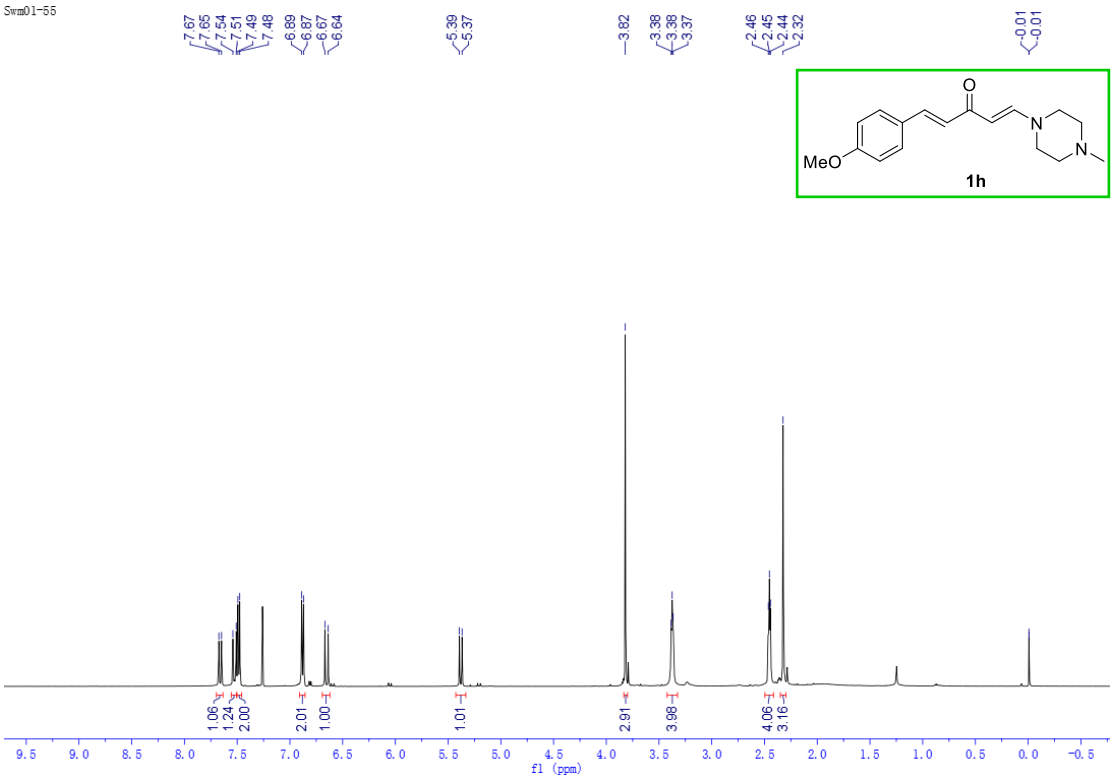

SWM01-71-1B
186.9
152.1
138.9
135.8
129.3
128.8
128.3
128.0
96.2
54.5
46.2
f1 (ppm)

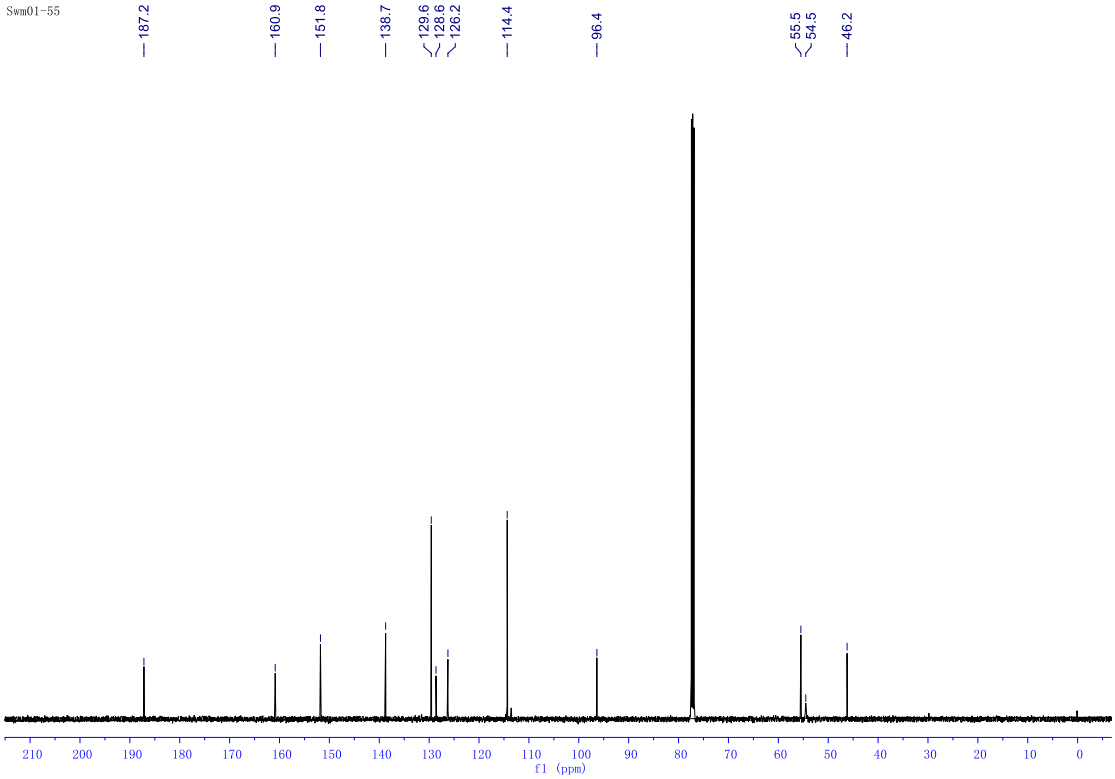

SWM01-74-2
7.70
7.68
7.52
7.49
7.47
7.45
7.33
7.31
6.74
6.71
5.38
5.36
3.39
2.47
2.46
2.45
2.33
0.01
O
N
N
Cl
1f
1.06
1.20
2.13
2.07
1.00
1.00
4.03
4.44
3.03
f1 (ppm)

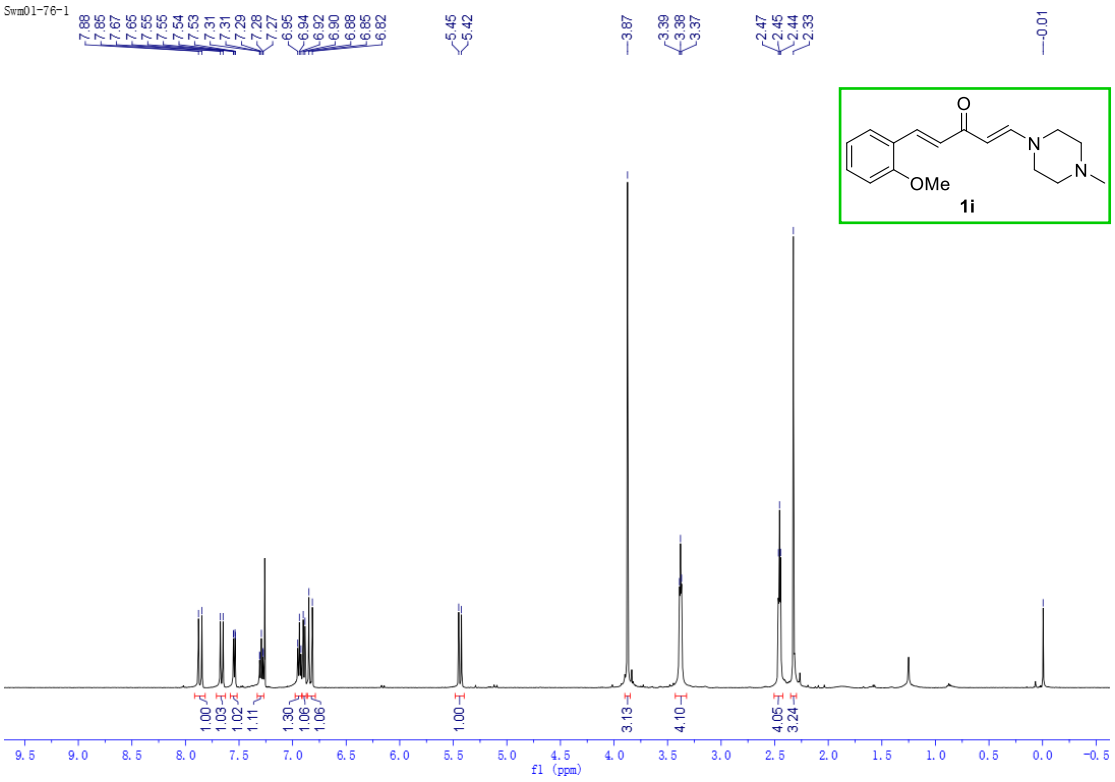

SWM01-74-2
186.5
152.2
137.5
135.2
134.4
129.2
129.1
128.8
96.2
54.5
46.2
f1 (ppm)

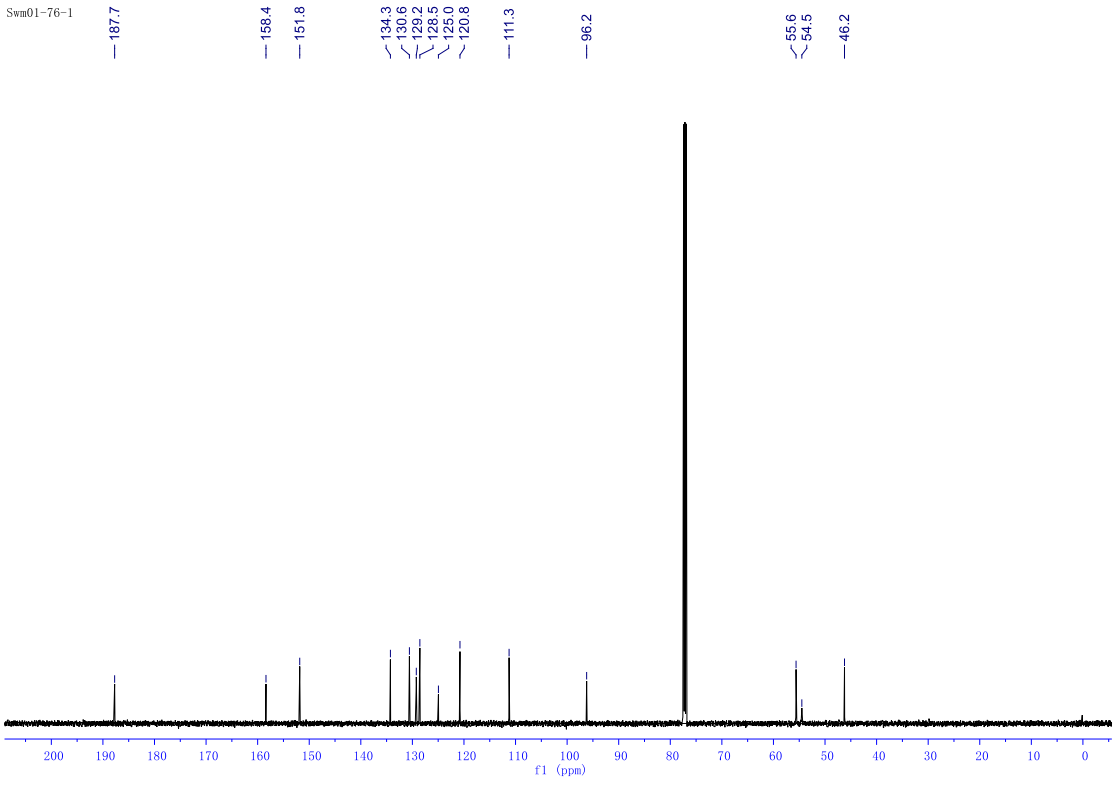

SWM01-87-2
7.72
7.69
7.62
7.60
7.59
7.57
7.52
7.49
6.82
6.79
5.37
5.35
3.39
2.46
2.45
2.44
2.31
O
N
N
NC
1g
1.11
3.91
1.02
1.02
1.00
4.47
4.04
2.96
f1 (ppm)

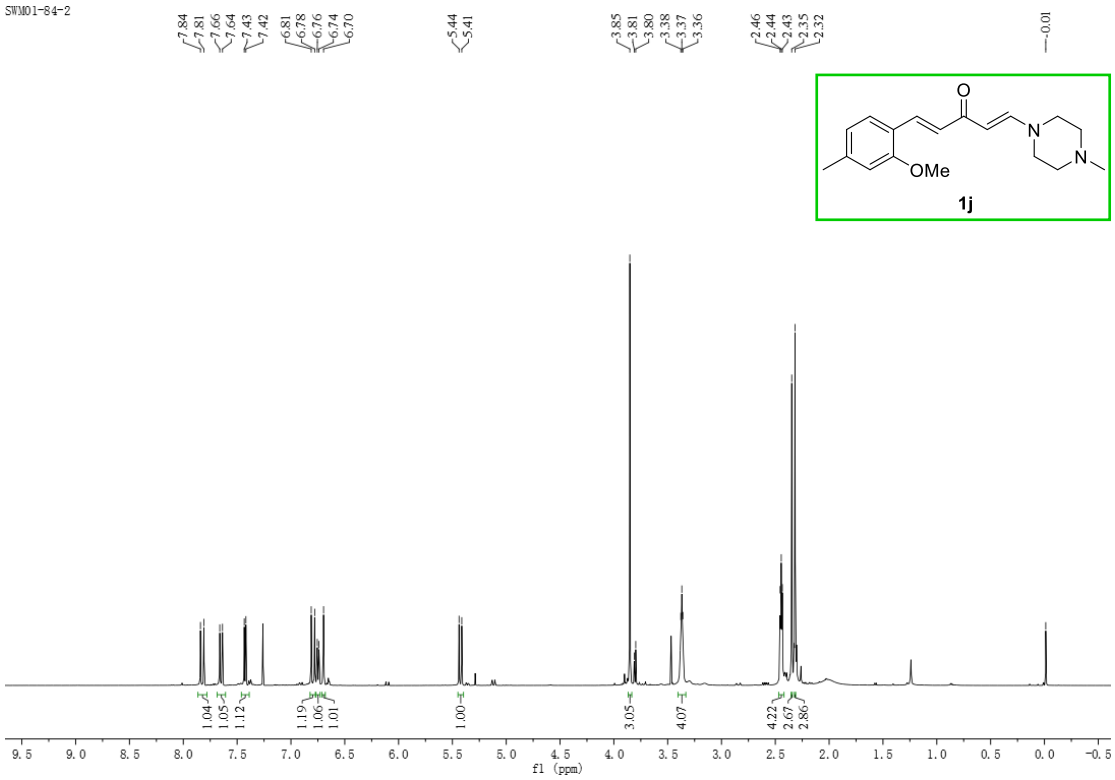

swm01-87-2
185.7
152.6
140.3
136.3
132.6
131.5
128.3
118.8
112.2
96.2
55.0
53.7
53.3
46.1
45.2
f1 (ppm)

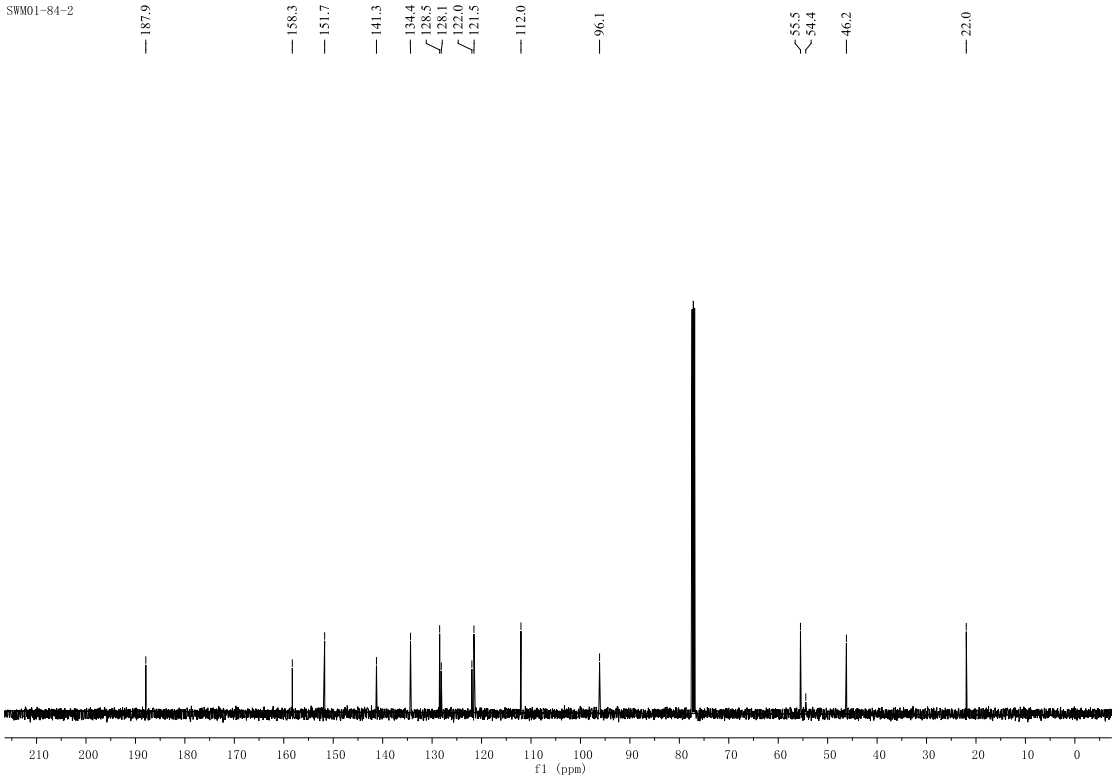

Swm01-55
7.67
7.65
7.54
7.51
7.49
7.48
6.89
6.87
6.67
6.64
5.39
5.37
3.82
3.38
3.38
3.37
2.46
2.45
2.44
2.32
0.01
0.01
O
N
N
MeO
1h
1.06
1.24
2.00
2.01
1.00
1.01
2.91
3.98
4.06
3.16
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

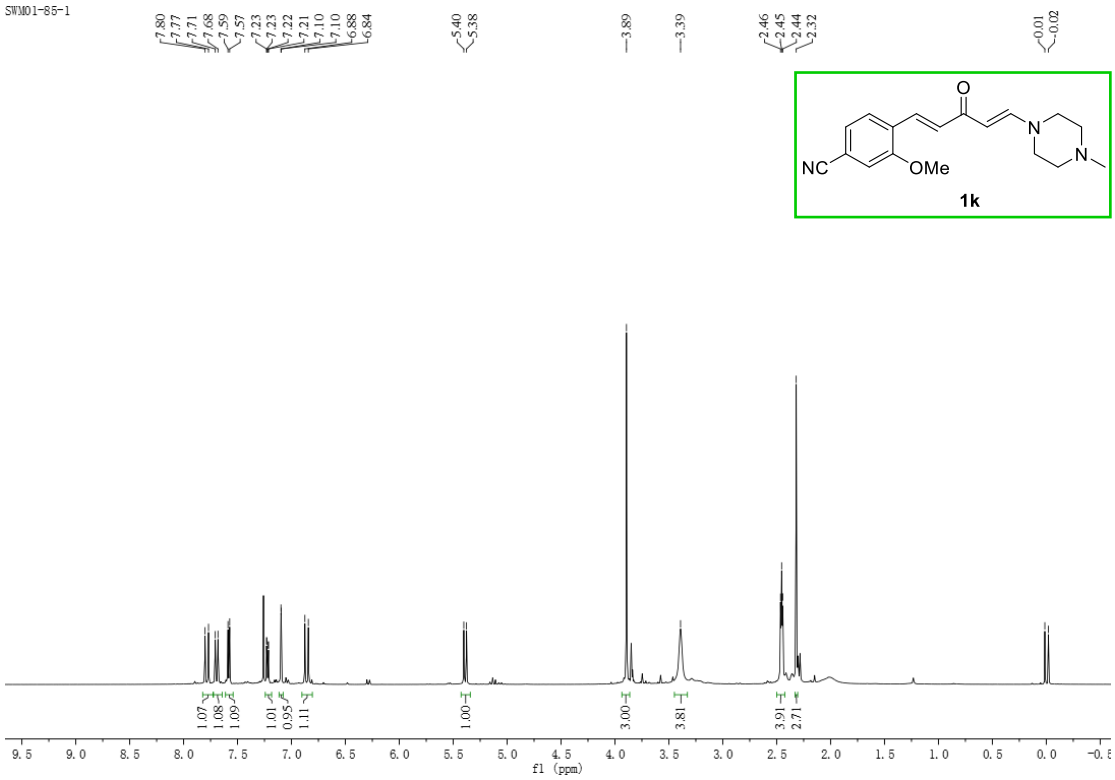

Swm01-55
187.2
160.9
151.8
138.7
129.6
128.6
126.2
114.4
96.4
55.5
54.5
46.2
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

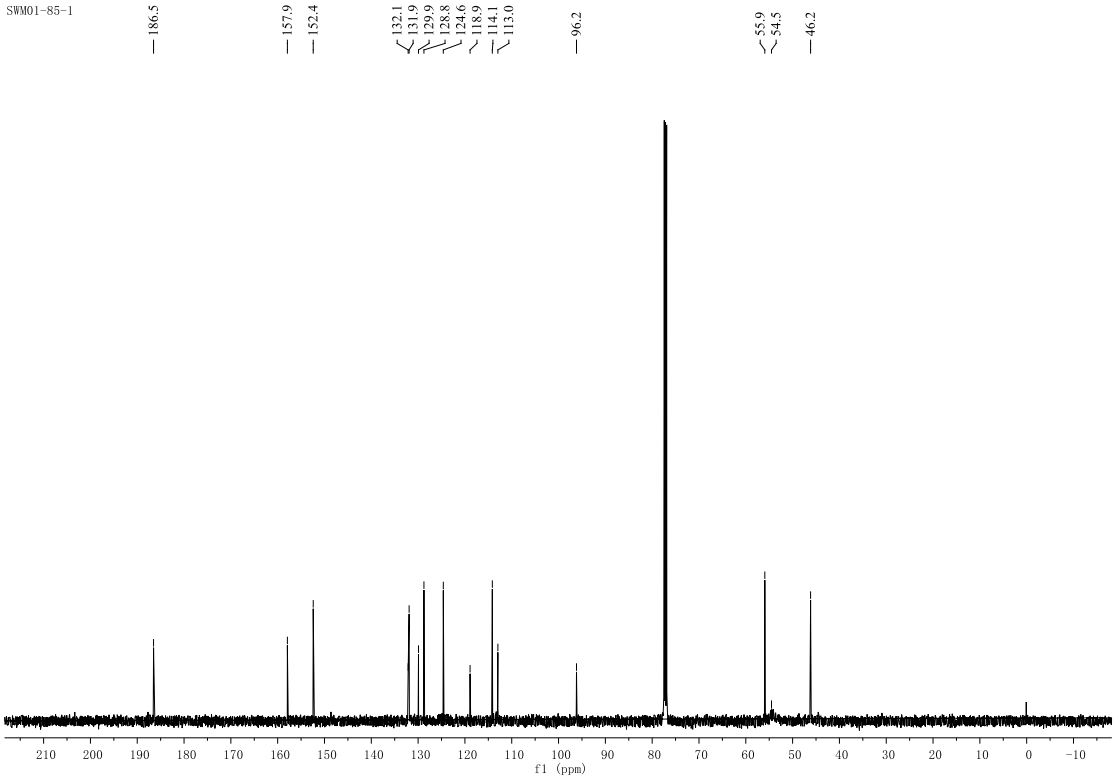

Swm01-76-1
7.88
7.85
7.67
7.65
7.55
7.55
7.54
7.53
7.31
7.29
7.28
7.27
6.95
6.94
6.92
6.90
6.88
6.85
6.82
5.45
5.42
3.87
3.39
3.38
3.37
2.47
2.45
2.44
2.33
-0.01
O
N
N
OMe
1i
1.00
1.03
1.02
1.11
1.30
1.06
1.06
1.00
3.13
4.10
4.05
3.24
f1 (ppm)

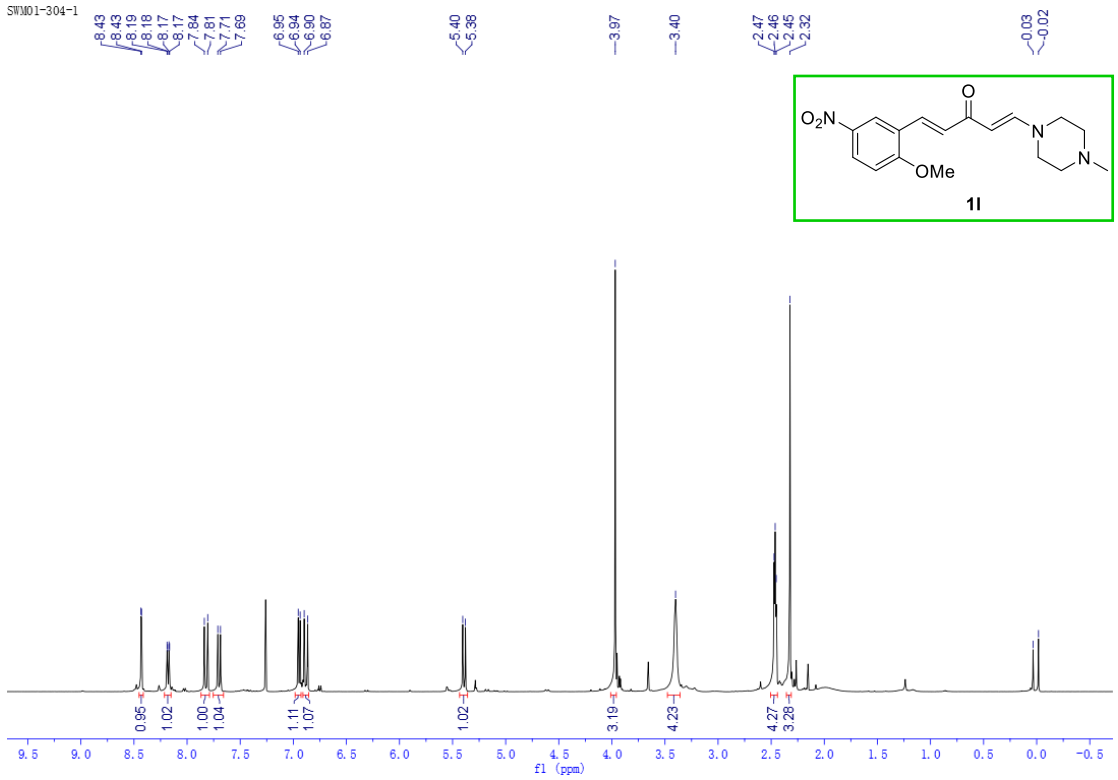

Swm01-76-1
187.7
158.4
151.8
134.3
130.6
129.2
128.5
125.0
120.8
111.3
96.2
55.6
54.5
46.2
f1 (ppm)

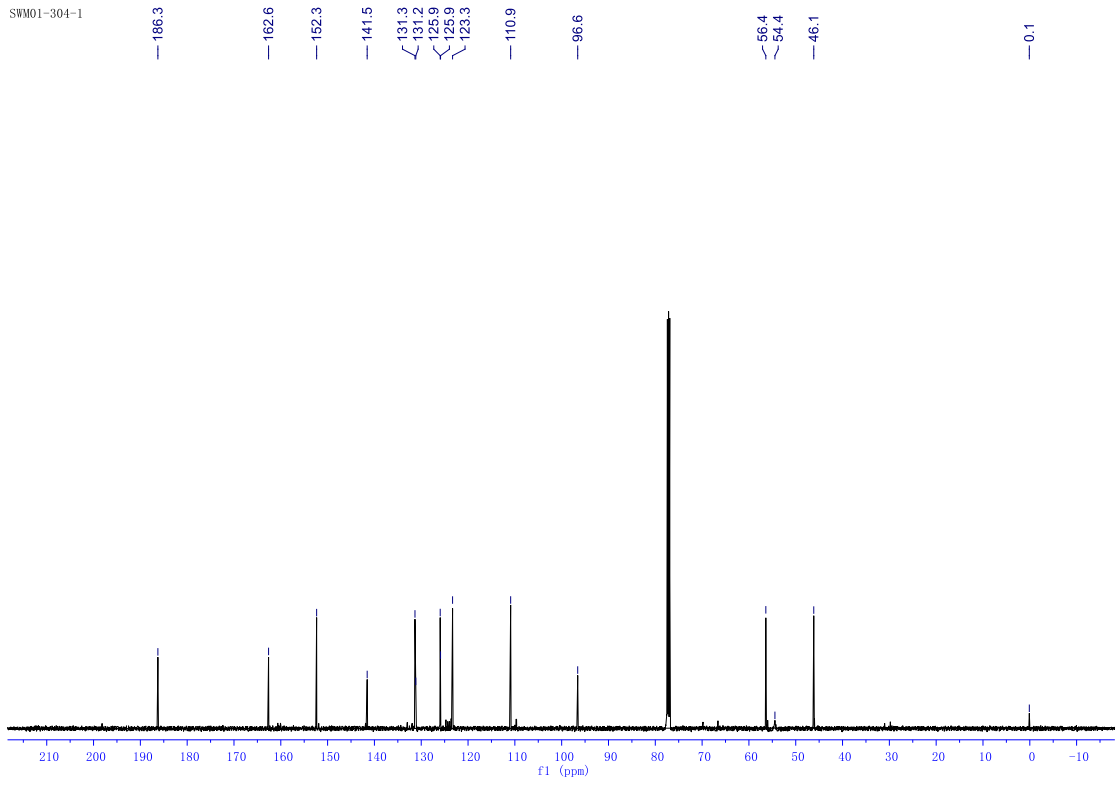

SWM01-84-2
7.84
7.81
7.66
7.64
7.43
7.42
6.81
6.78
6.76
6.74
6.70
5.44
5.41
3.85
3.81
3.80
3.38
3.37
3.36
2.46
2.44
2.43
2.35
2.32
-0.01
O
N
N
OMe
1j
1.04
1.05
1.12
1.19
1.06
1.01
1.00
3.05
4.07
4.22
2.67
2.86
f1 (ppm)

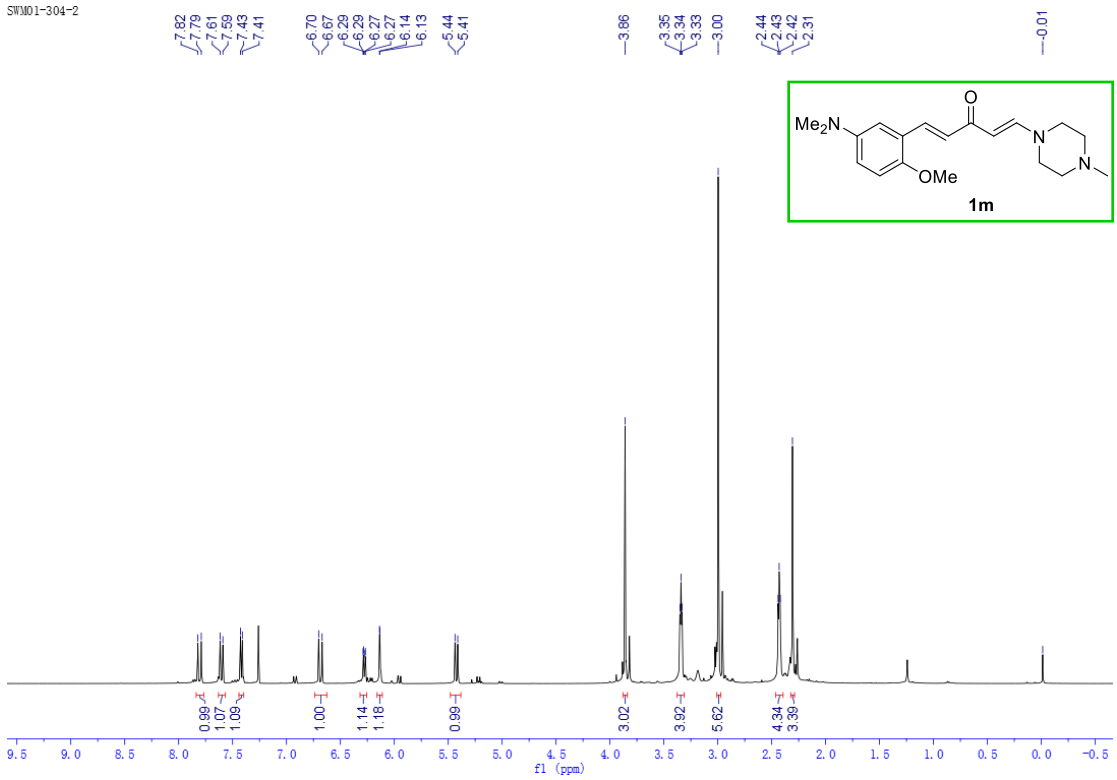

SWM01-84-2
187.9
158.3
151.7
141.3
134.4
128.5
128.1
122.0
121.5
112.0
96.1
55.5
54.4
46.2
22.0
f1 (ppm)

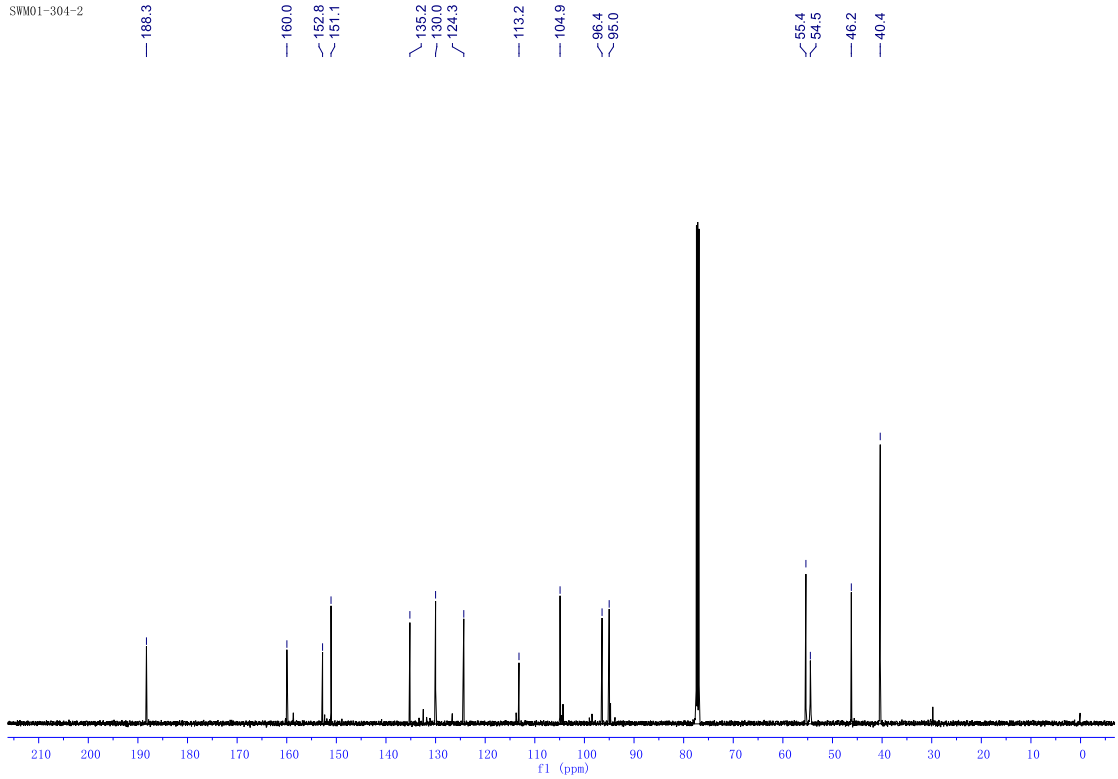

SWM01-85-1
7.80
7.77
7.71
7.68
7.59
7.57
7.23
7.23
7.22
7.21
7.10
7.10
6.88
6.84
5.40
5.38
3.89
3.39
2.46
2.45
2.44
2.32
0.01
-0.02
O
N
N
NC
OMe
1k
1.07
1.08
1.09
1.01
0.95
1.11
1.00
3.00
3.81
3.91
2.71
f1 (ppm)

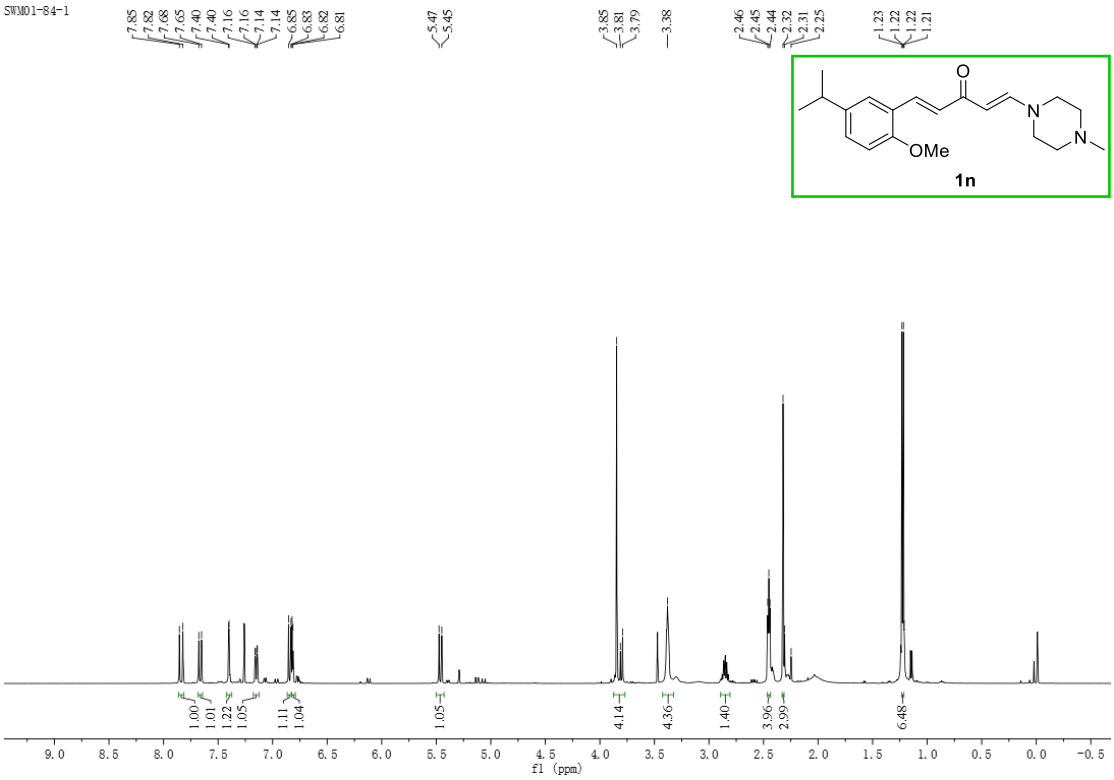

SWM01-85-1
186.5
157.9
152.4
132.1
131.9
129.9
128.8
124.6
118.9
114.1
113.0
96.2
55.9
54.5
46.2
f1 (ppm)

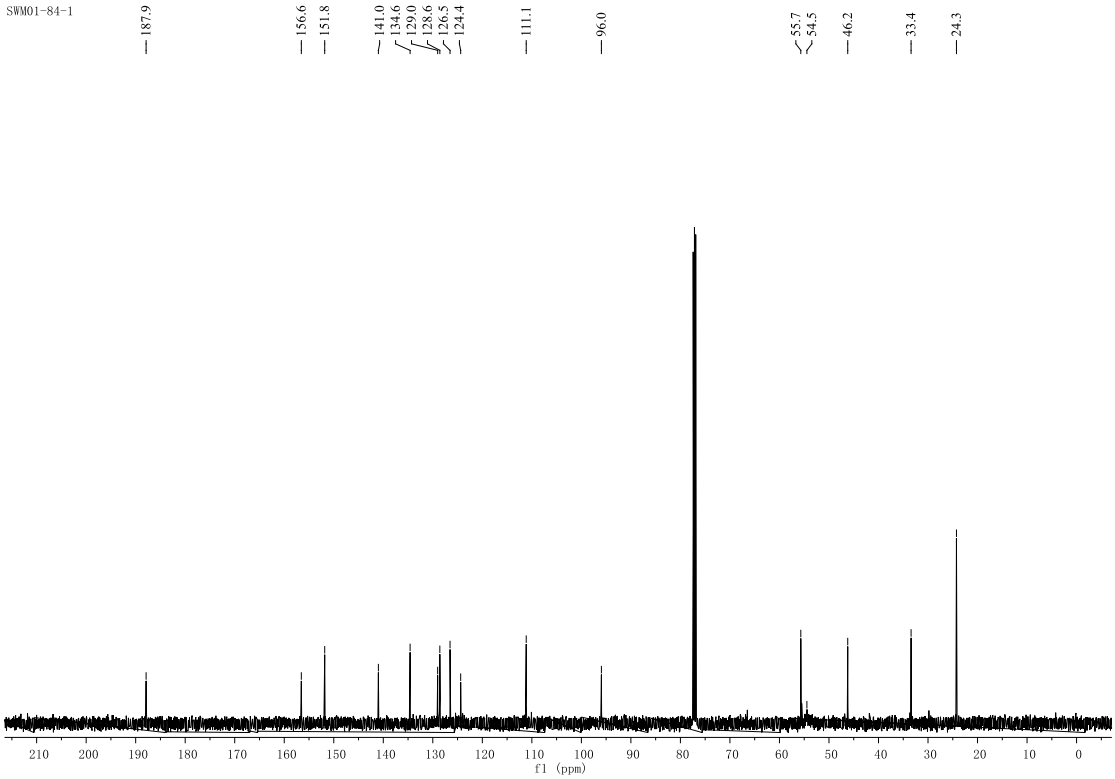

SWM01-304-1
8.43
8.43
8.19
8.18
8.17
8.17
7.84
7.81
7.71
7.69
6.95
6.94
6.90
6.87
5.40
5.38
3.97
3.40
2.47
2.46
2.45
2.32
0.03
-0.02
O2N
O
N
N
OMe
1l
0.95
1.02
1.00
1.04
1.11
1.07
1.02
3.19
4.23
4.27
3.28
f1 (ppm)

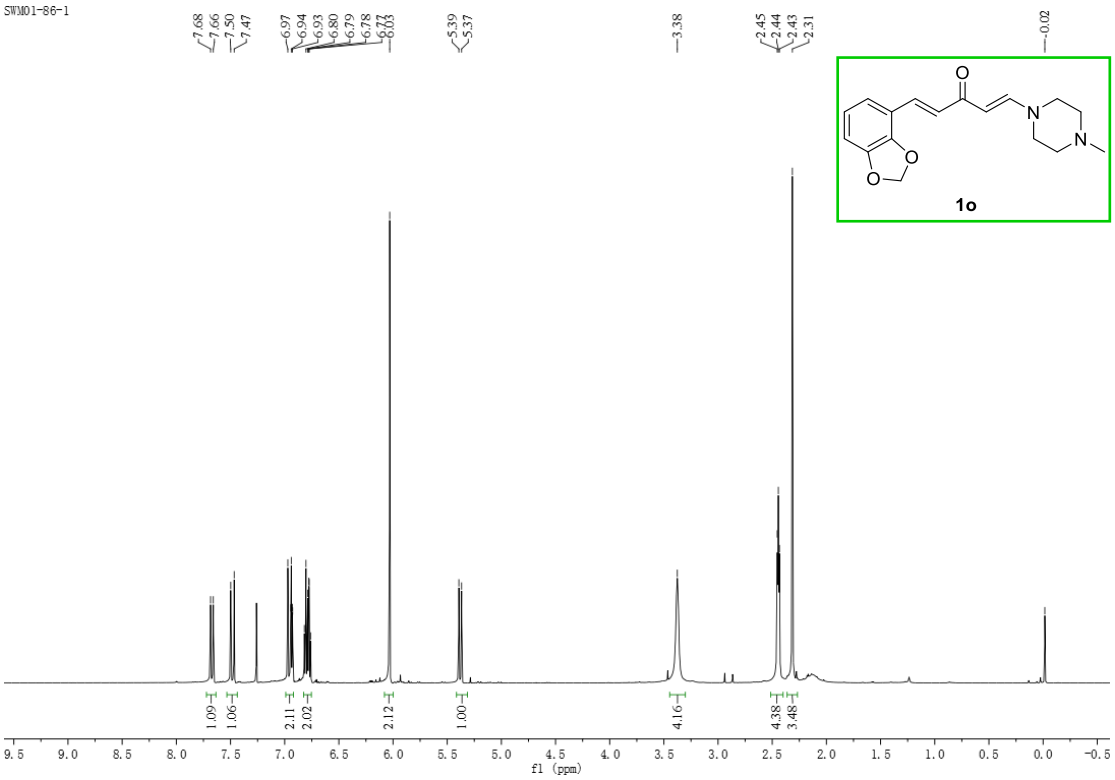

SWM01-304-1
186.3
162.6
152.3
141.5
131.3
131.2
125.9
125.9
123.3
110.9
96.6
56.4
54.4
46.1
0.1
f1 (ppm)

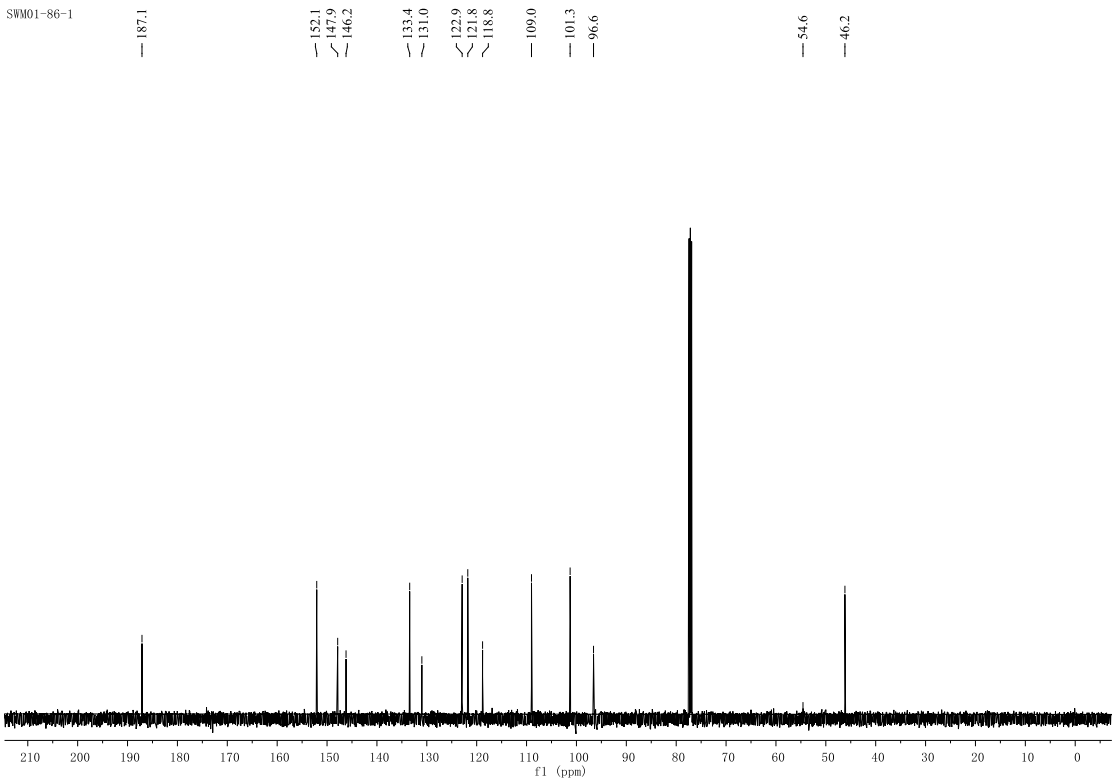

SWM01-304-2
7.82
7.79
7.61
7.59
7.43
7.41
6.70
6.67
6.29
6.29
6.27
6.27
6.14
6.13
5.44
5.41
3.86
3.35
3.34
3.33
3.00
2.44
2.43
2.42
2.31
-0.01
Me2N
O
N
N
OMe
1m
0.99
1.07
1.09
1.00
1.14
1.18
0.99
3.02
3.92
5.62
4.34
3.39
f1 (ppm)

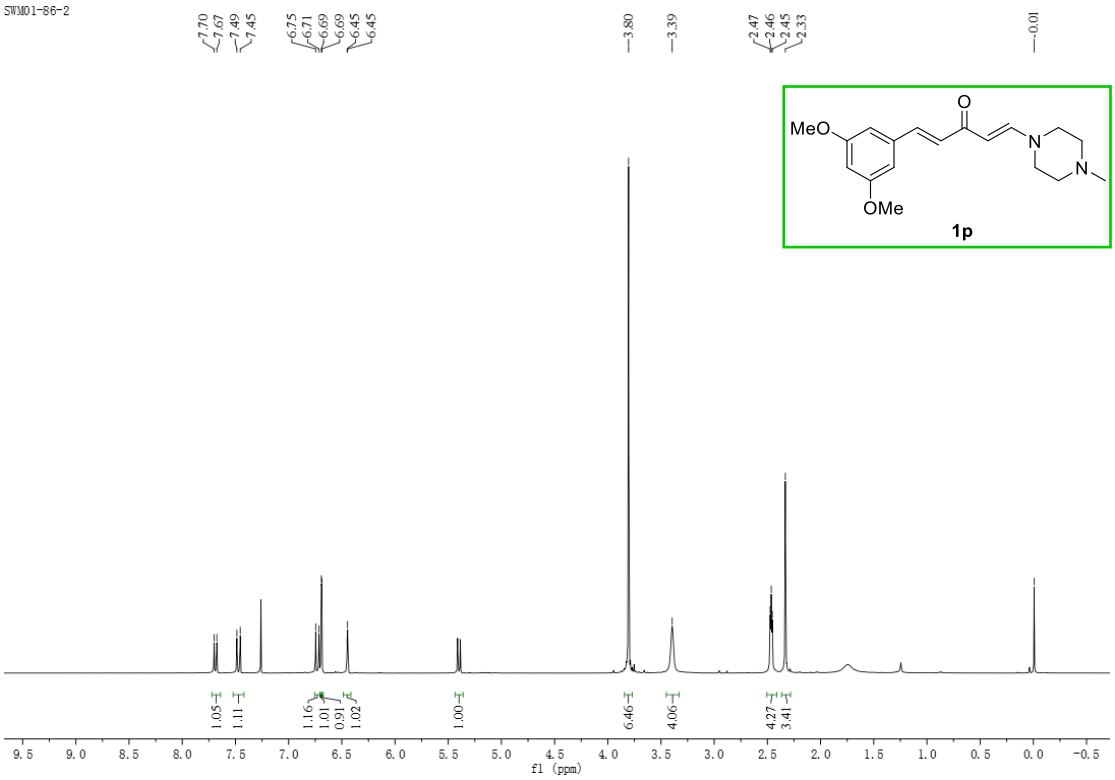

SWM01-304-2
188.3
160.0
152.8
151.1
135.2
130.0
124.3
113.2
104.9
96.4
95.0
55.4
54.5
46.2
40.4
f1 (ppm)

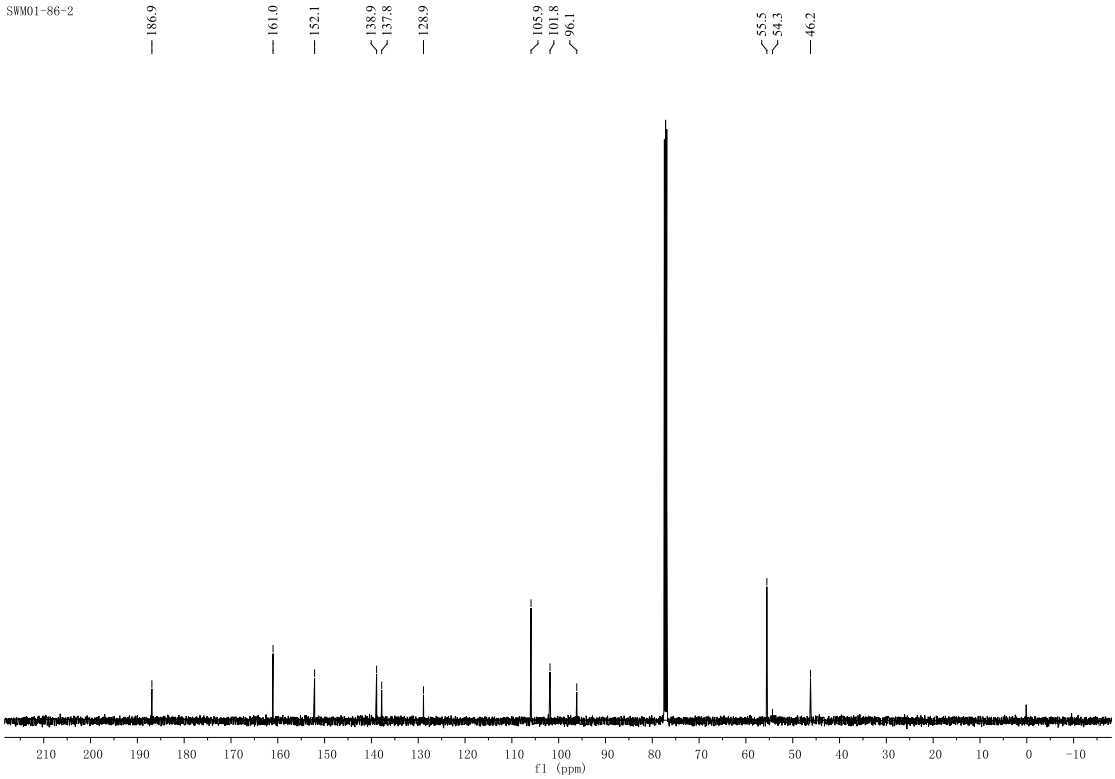

SWM01-84-1
7.85
7.82
7.68
7.65
7.40
7.40
7.16
7.16
7.14
7.14
6.85
6.83
6.82
6.81
5.47
5.45
3.85
3.81
3.79
3.38
2.46
2.45
2.44
2.32
2.31
2.25
1.23
1.22
1.22
1.21
O
N
N
OMe
1n
1.00
1.01
1.22
1.05
1.11
1.04
1.05
4.14
4.36
1.40
3.96
2.99
6.48
f1 (ppm)

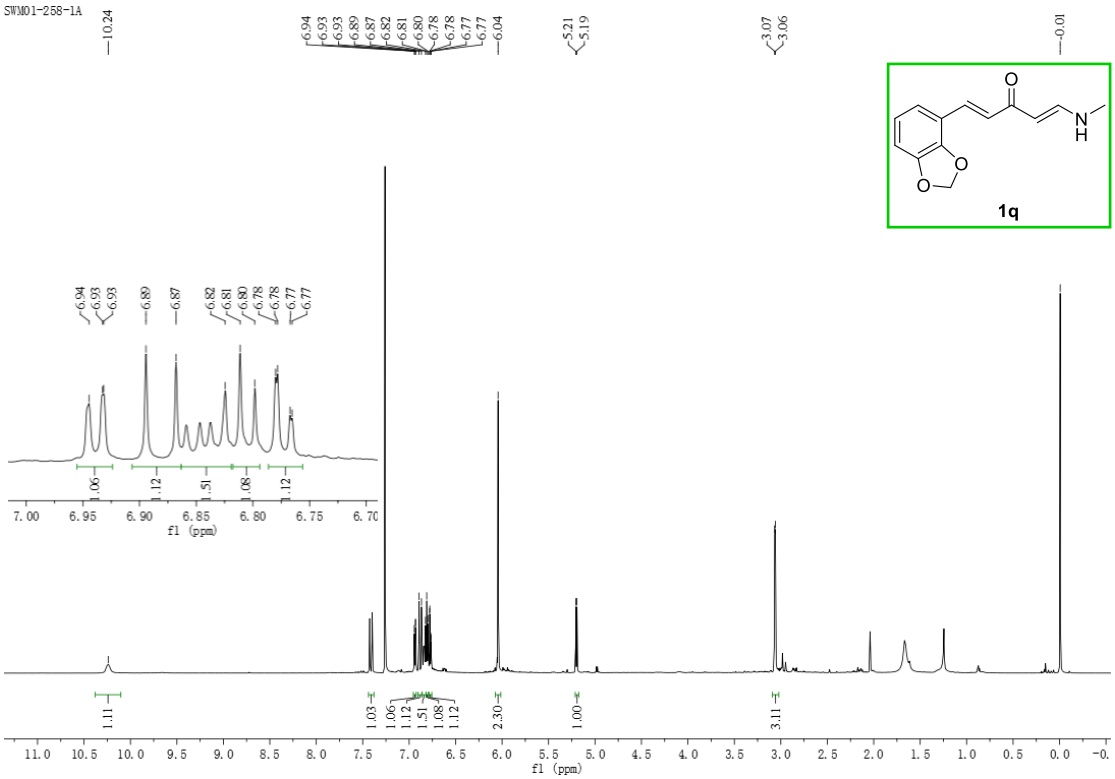

SWM01-84-1
187.9
156.6
151.8
141.0
134.6
129.0
128.6
126.5
124.4
111.1
96.0
55.7
54.5
46.2
33.4
24.3
f1 (ppm)

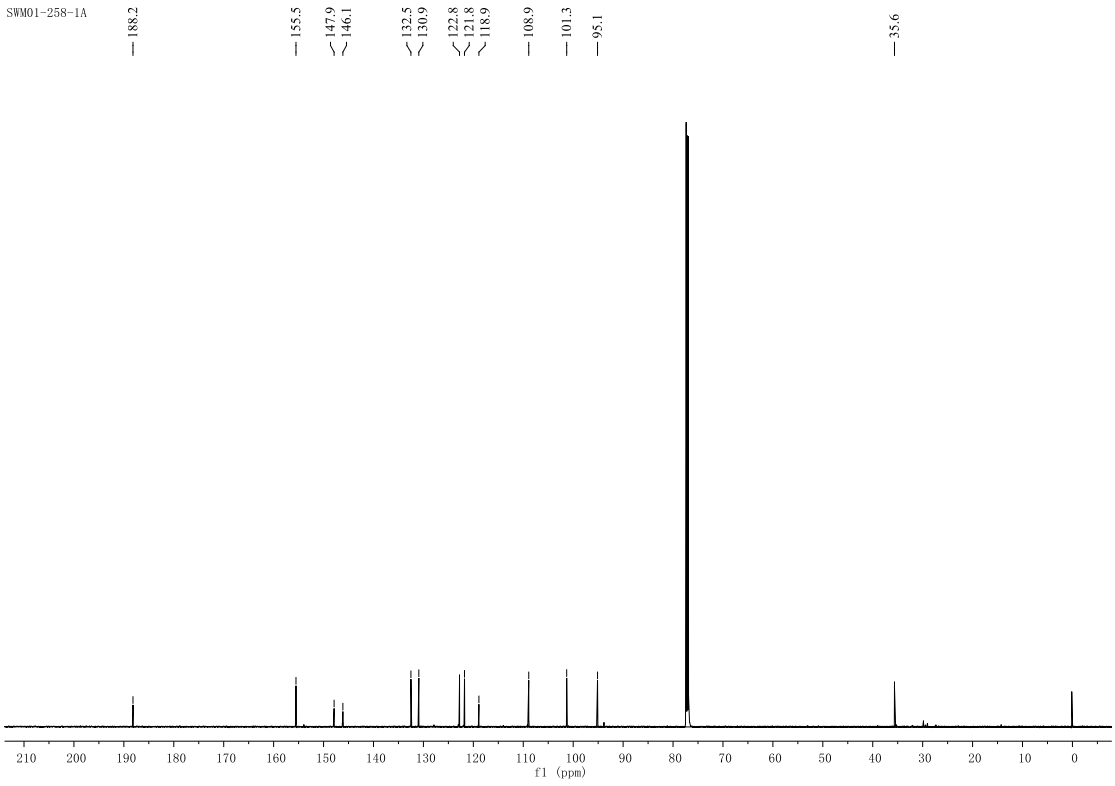

SWM01-86-1
7.68
7.66
7.50
7.47
6.97
6.94
6.93
6.80
6.79
6.78
6.77
6.03
5.39
5.37
3.38
2.45
2.44
2.43
2.31
-0.02
1.09
1.06
2.11
2.02
2.12
1.00
4.16
4.38
3.48
f1 (ppm)
1o

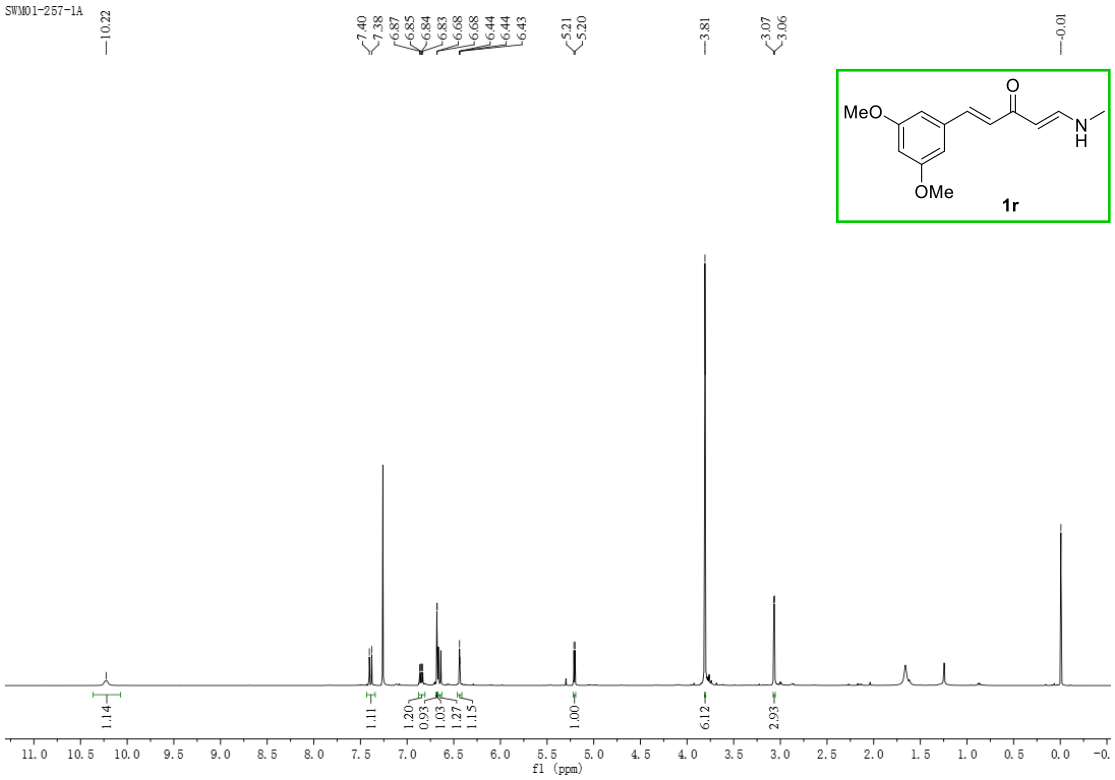

SWM01-86-1
187.1
152.1
147.9
146.2
133.4
131.0
122.9
121.8
118.8
109.0
101.3
96.6
54.6
46.2
f1 (ppm)

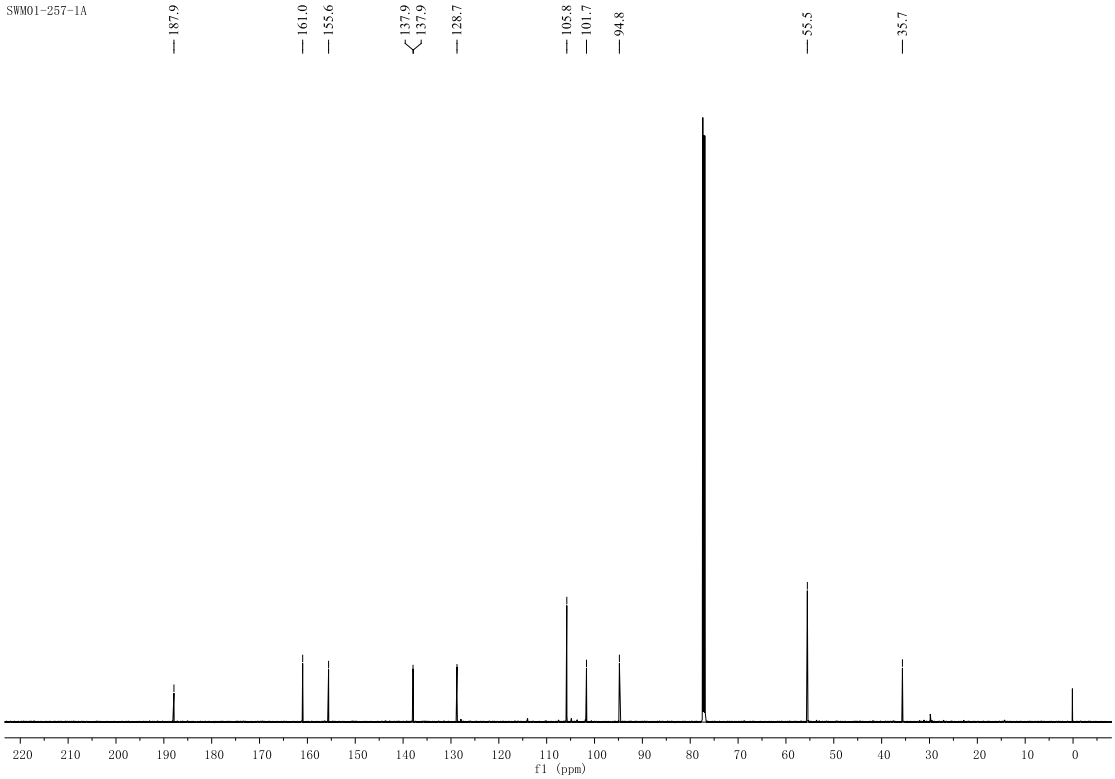

SWM01-86-2
7.70
7.67
7.49
7.45
6.75
6.71
6.69
6.69
6.45
6.45
3.80
3.39
2.47
2.46
2.45
2.33
0.01
MeO
OMe
O
N
N
1p
1.05
1.11
1.16
1.01
0.91
1.02
1.00
6.46
4.06
4.27
3.41
f1 (ppm)

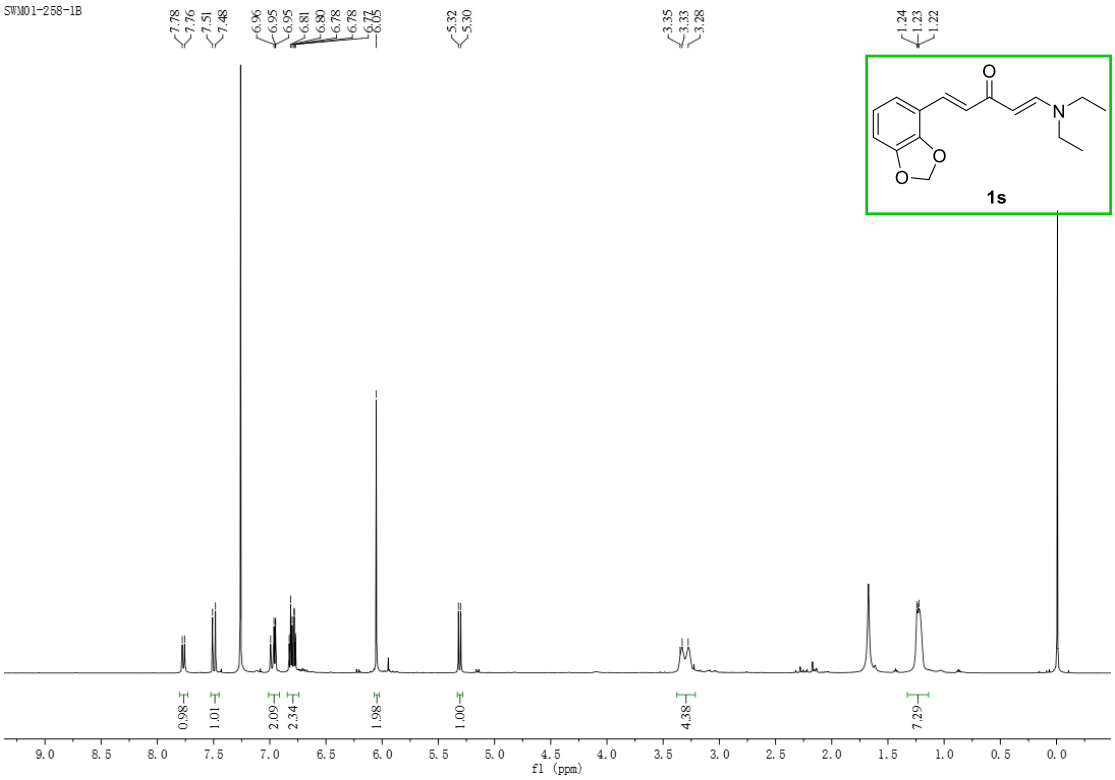

SWM01-86-2
186.9
161.0
152.1
138.9
137.8
128.9
105.9
101.8
96.1
55.5
54.3
46.2
f1 (ppm)

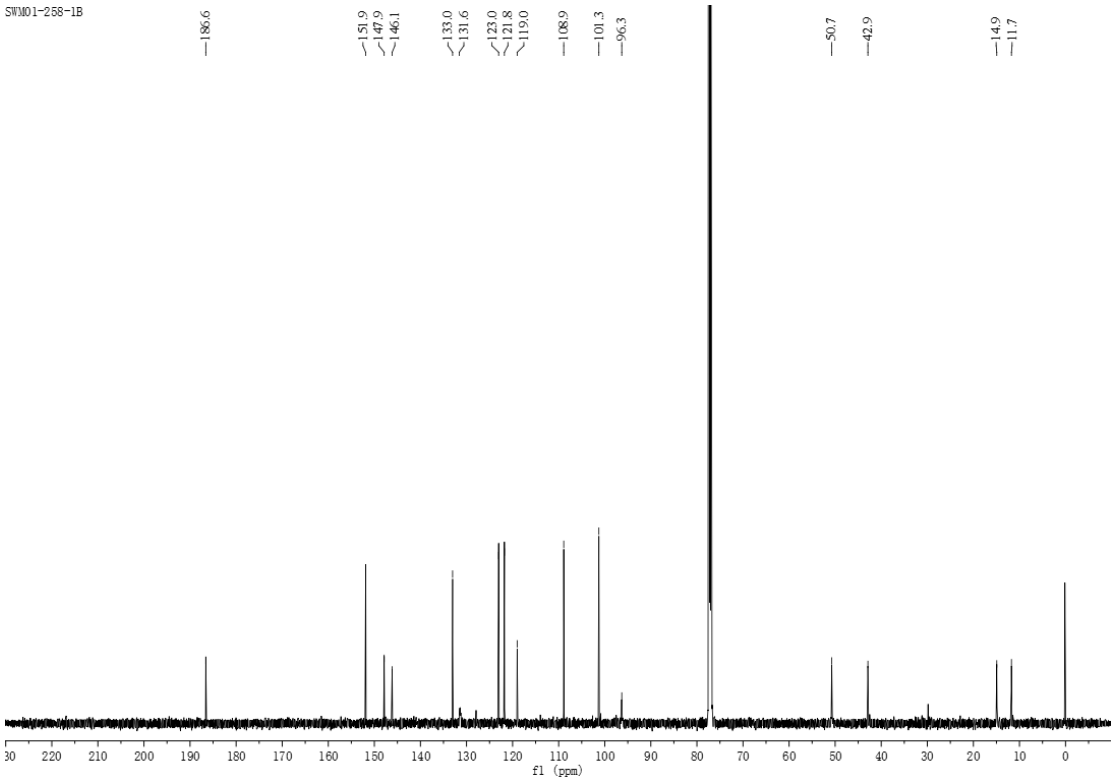

SWM01-258-1A
10.24
6.94
6.93
6.93
6.89
6.87
6.82
6.81
6.80
6.78
6.78
6.77
6.77
6.04
5.21
5.19
3.07
3.06
0.01
O
N
H
O
O
1q
1.06
1.12
1.51
1.08
1.12
f1 (ppm)
1.11
1.03
1.06
1.12
1.51
1.08
1.12
2.30
1.00
3.11
f1 (ppm)

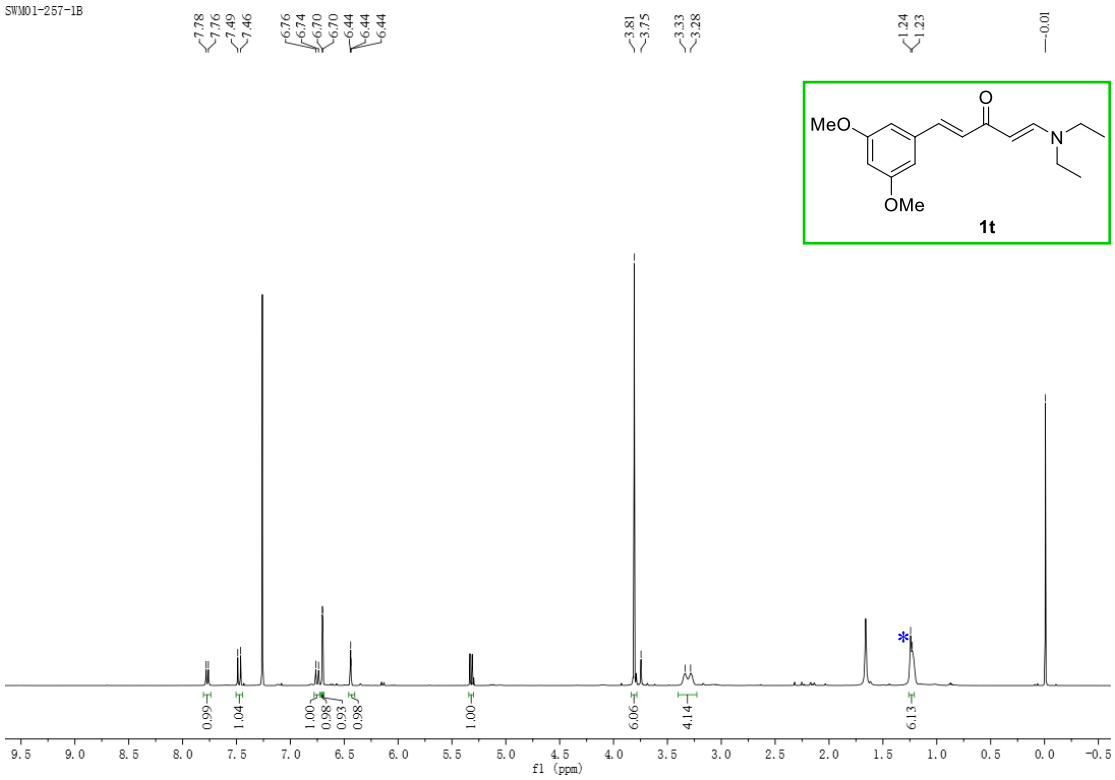

SWM01-258-1A
188.2
155.5
147.9
146.1
132.5
130.9
122.8
121.8
118.9
108.9
101.3
95.1
35.6
f1 (ppm)


SWM01-257-1A
10.22
7.40
7.38
6.87
6.85
6.84
6.83
6.68
6.68
6.68
6.44
6.44
6.43
5.21
5.20
3.81
3.07
3.06
0.01
MeO
O
N
H
OMe
1r
1.14
1.11
1.20
0.93
1.03
1.27
1.15
1.00
6.12
2.93
f1 (ppm)

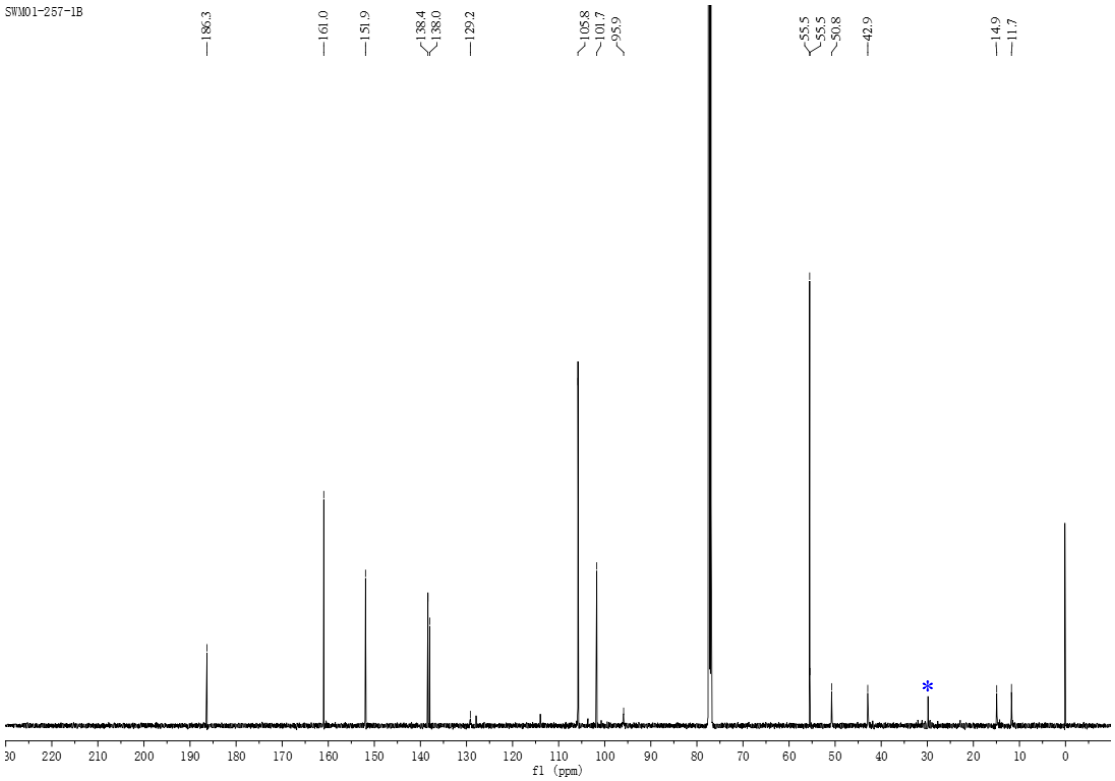

SWM01-257-1A
187.9
161.0
155.6
137.9
137.9
128.7
105.8
101.7
94.8
55.5
35.7
f1 (ppm)

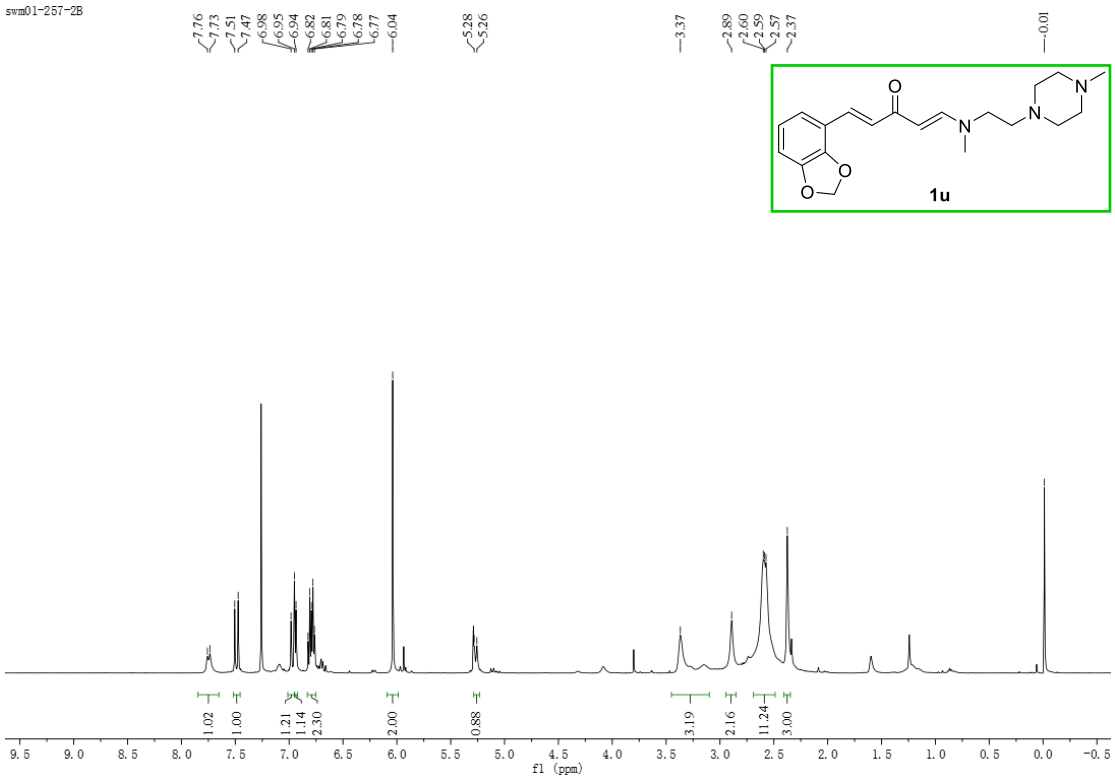

SWM01-258-1B
7.78
7.76
7.51
7.48
6.96
6.95
6.95
6.81
6.80
6.78
6.78
6.77
6.05
5.32
5.30
3.35
3.33
3.28
1.24
1.23
1.22
O
N
O
O
1s
0.98
1.01
2.09
2.34
1.98
1.00
4.38
7.29
f1 (ppm)

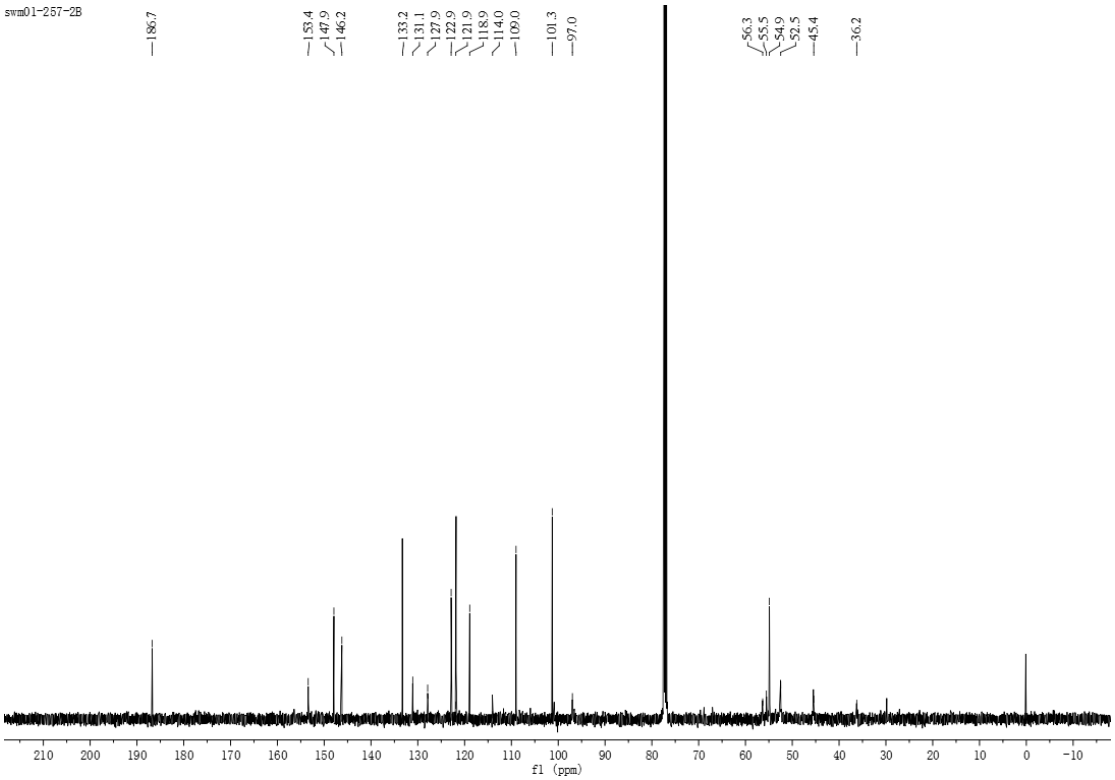

SWM01-258-1B
186.6
151.9
147.9
146.1
133.0
131.6
123.0
121.8
119.0
108.9
101.3
96.3
50.7
42.9
14.9
11.7
f1 (ppm)

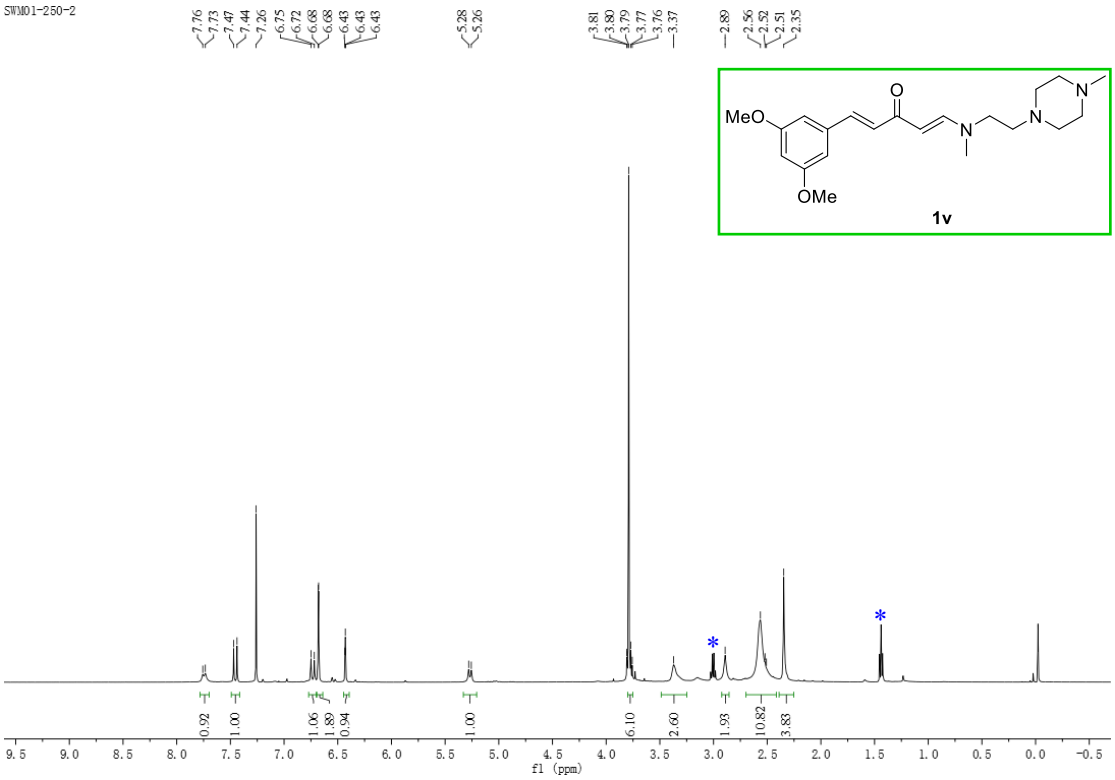

SWM01-257-1B
7.78
7.76
7.49
7.46
6.76
6.74
6.70
6.70
6.44
6.44
6.44
3.81
3.75
3.33
3.28
1.24
1.23
0.01
MeO
OMe
O
N
1t
*
0.99
1.04
1.00
0.98
0.93
0.98
1.00
6.06
4.14
6.13
f1 (ppm)

* grease

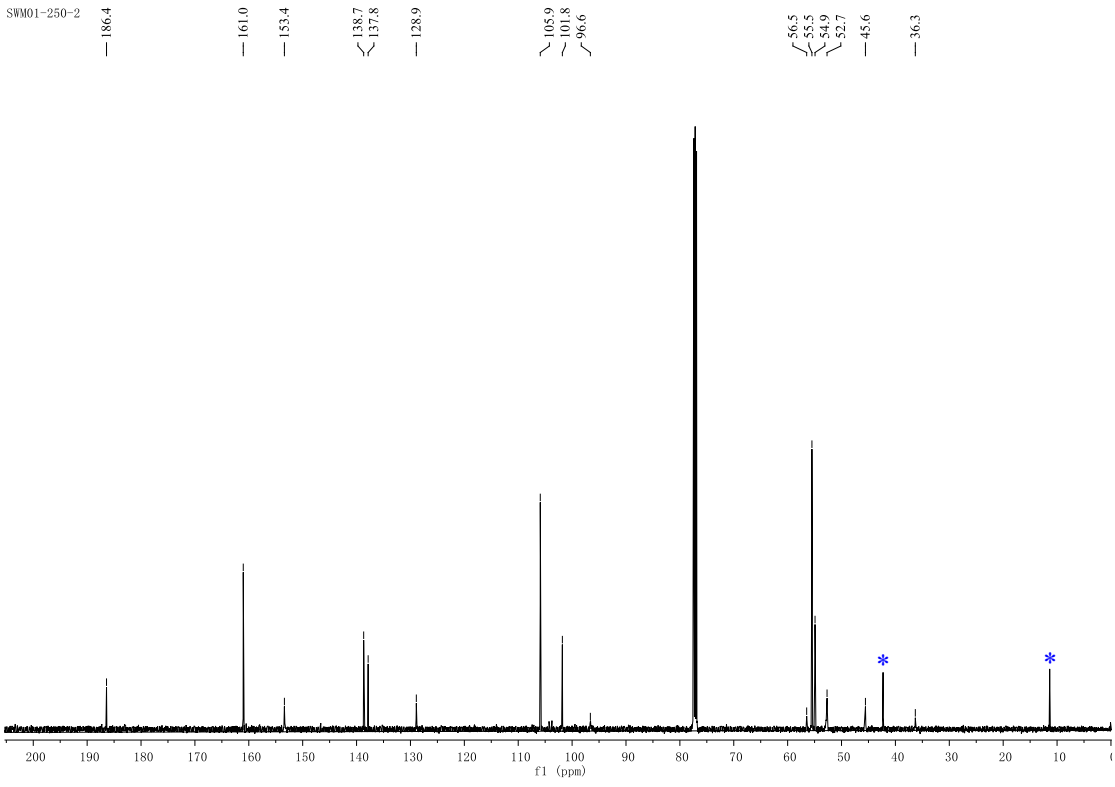

SWM01-257-1B
186.3
161.0
151.9
138.4
138.0
129.2
105.8
101.7
95.9
55.5
55.5
50.8
42.9
14.9
11.7
*
f1 (ppm)

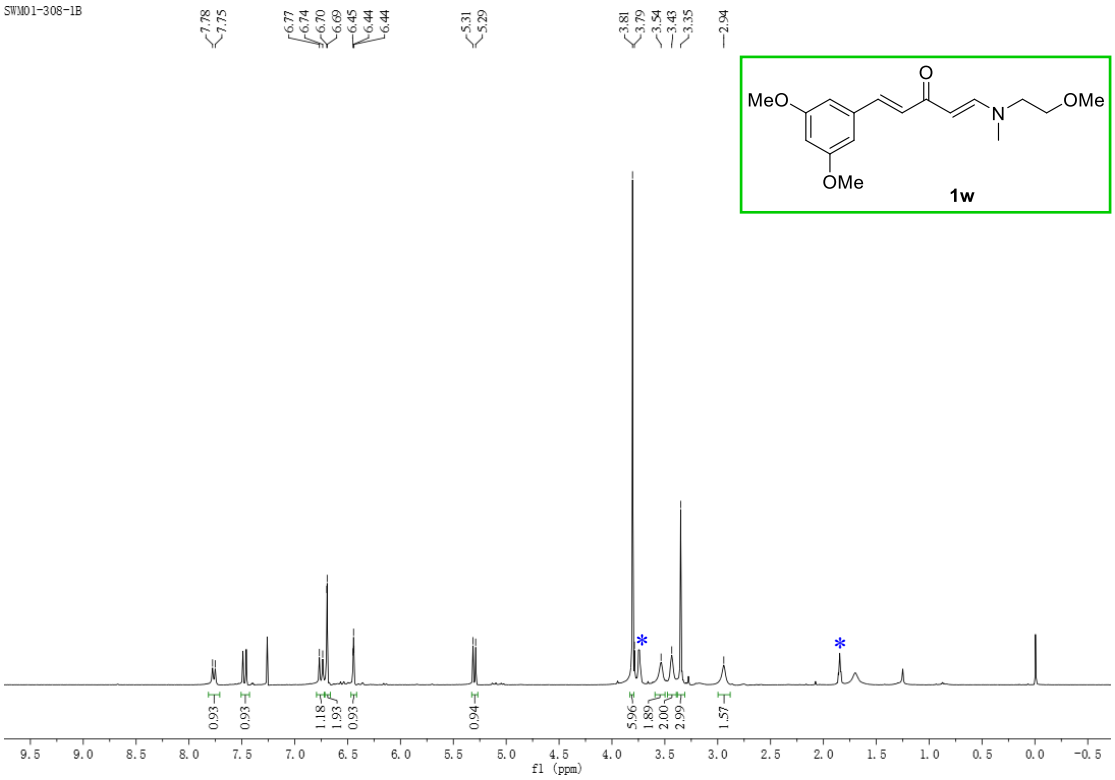

swm01-257-2B
7.76
7.73
7.51
7.47
6.98
6.95
6.94
6.82
6.81
6.79
6.78
6.77
6.04
5.28
5.26
3.37
2.89
2.60
2.59
2.57
2.37
0.01
1u
1.02
1.00
1.21
1.14
2.30
2.00
0.88
3.19
2.16
11.24
3.00
f1 (ppm)


swm01-257-2B
186.7
153.4
147.9
146.2
133.2
131.1
127.9
122.9
121.9
118.9
114.0
109.0
101.3
97.0
56.3
55.5
54.9
52.5
45.4
36.2
f1 (ppm)

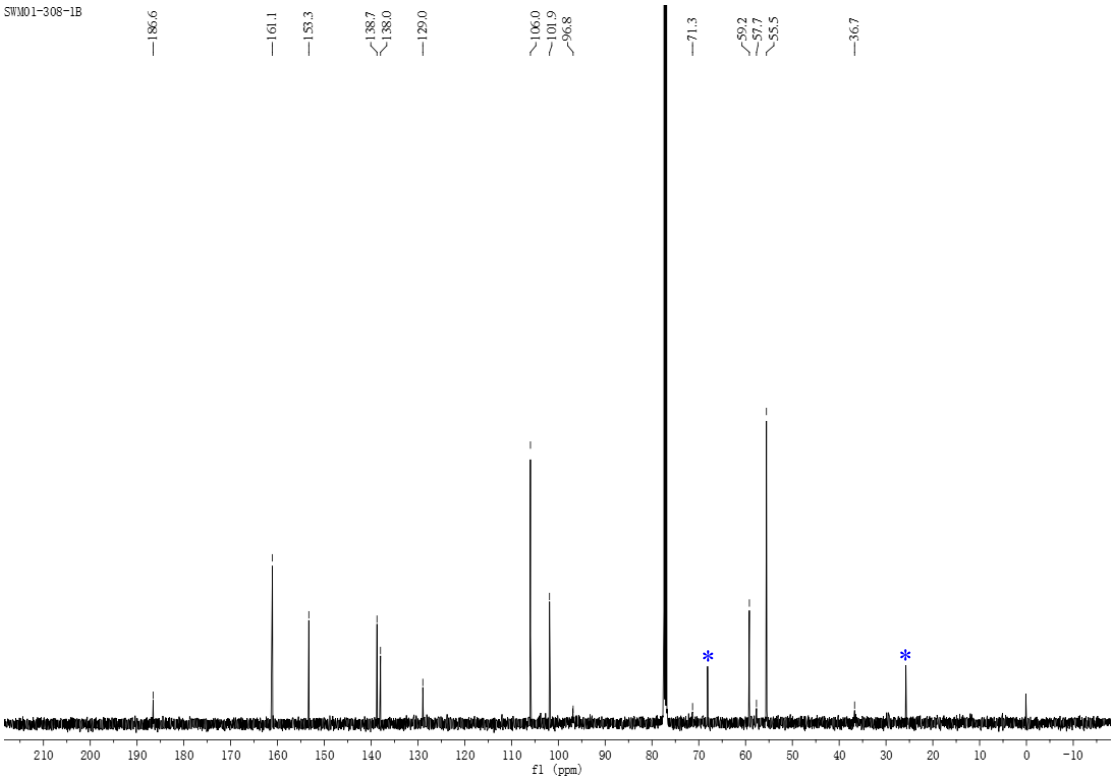


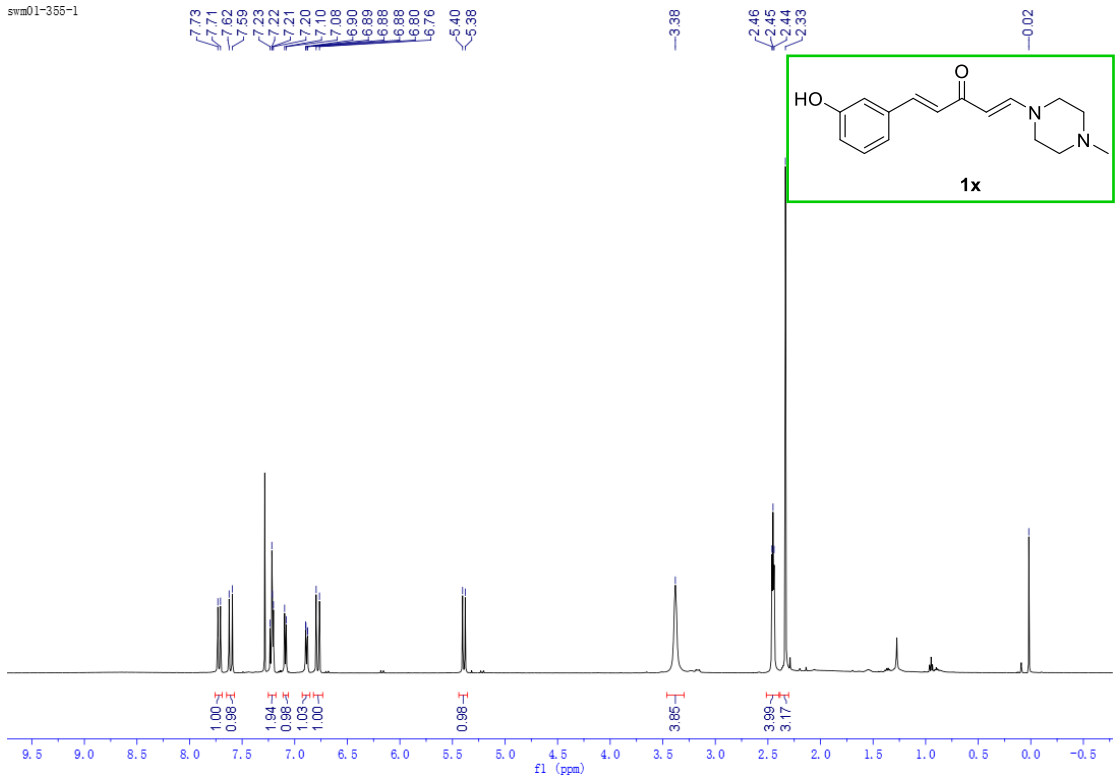
* $HNEt_2$

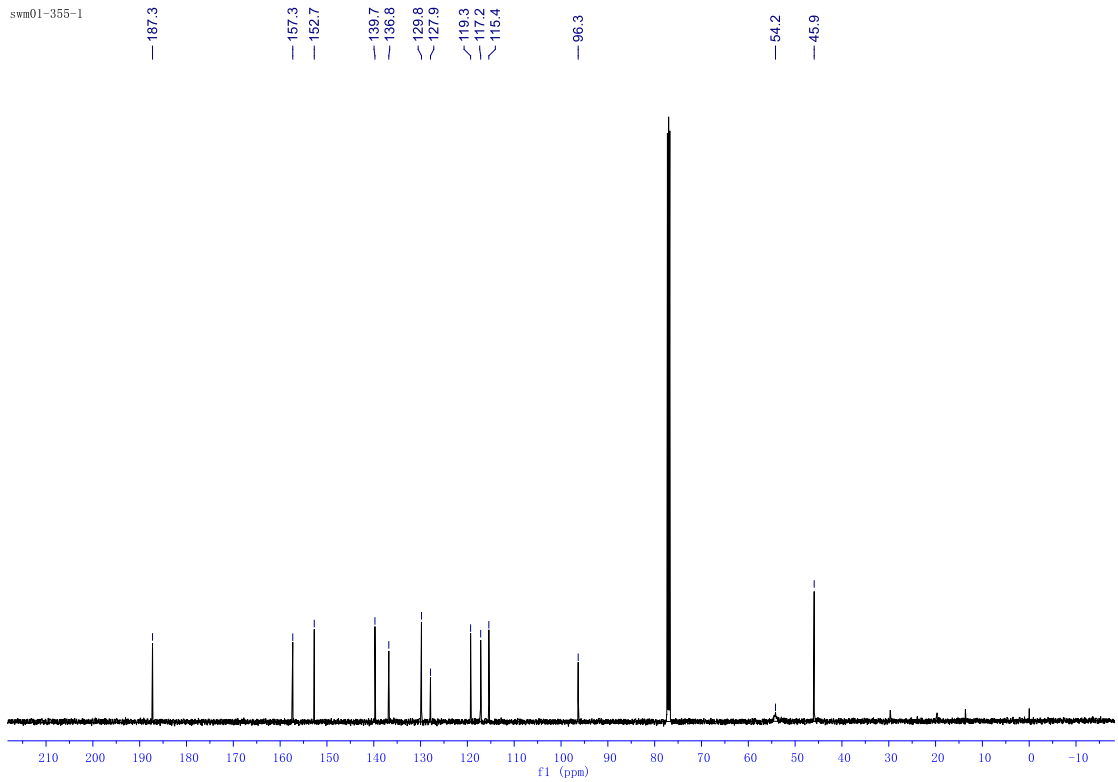


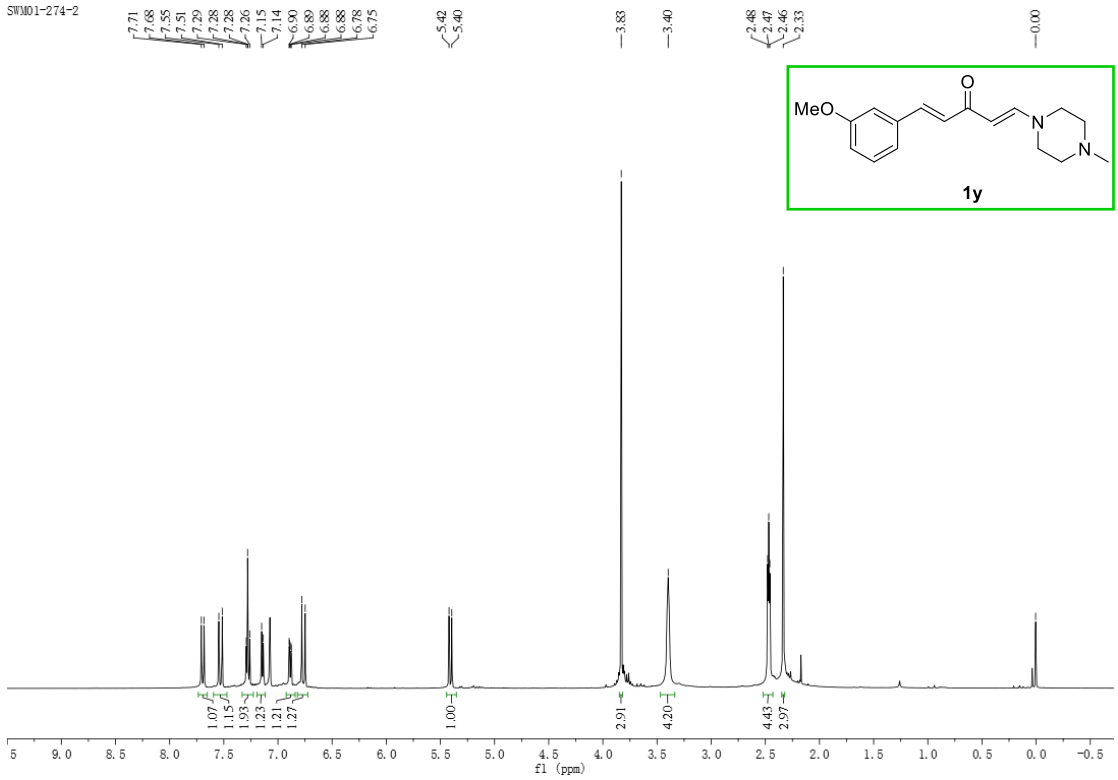

SWM01-308-1B
7.78
7.75
6.77
6.74
6.70
6.69
6.45
6.44
6.44
5.31
5.29
3.81
3.79
3.54
3.43
3.35
2.94
MeO
OMe
OMe
O
N
1w
0.93
0.93
1.18
1.93
0.93
0.94
5.96
1.89
2.00
2.99
1.57
f1 (ppm)

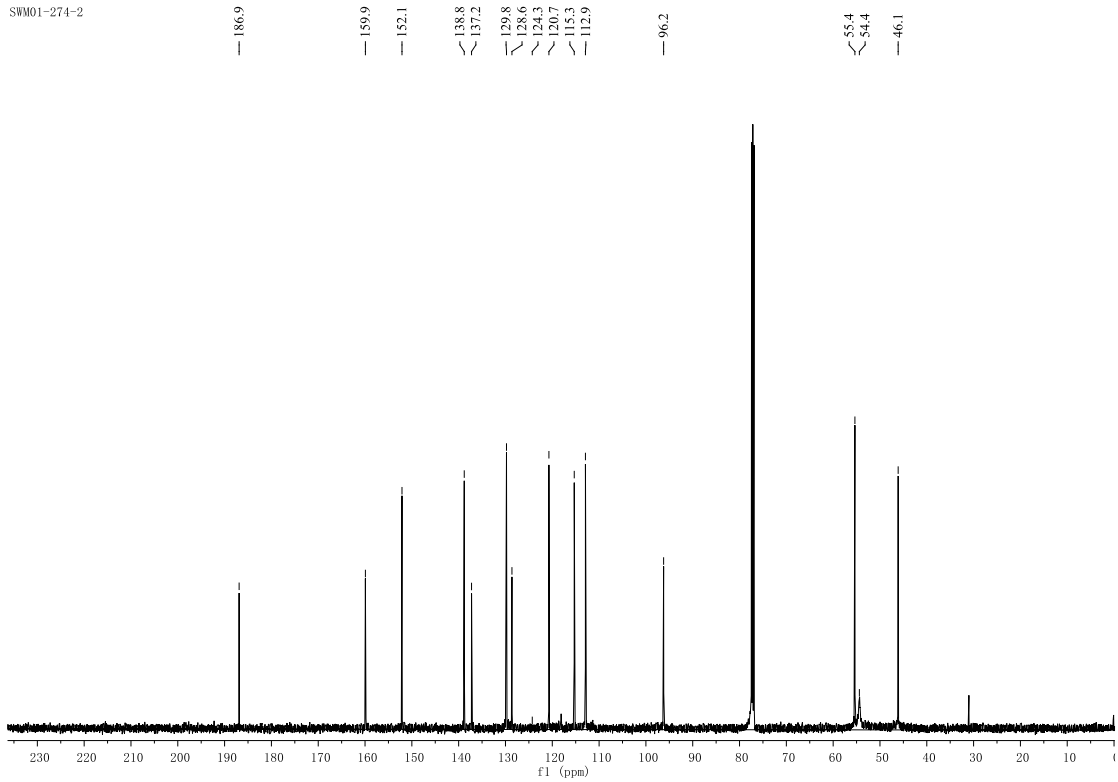
* THF

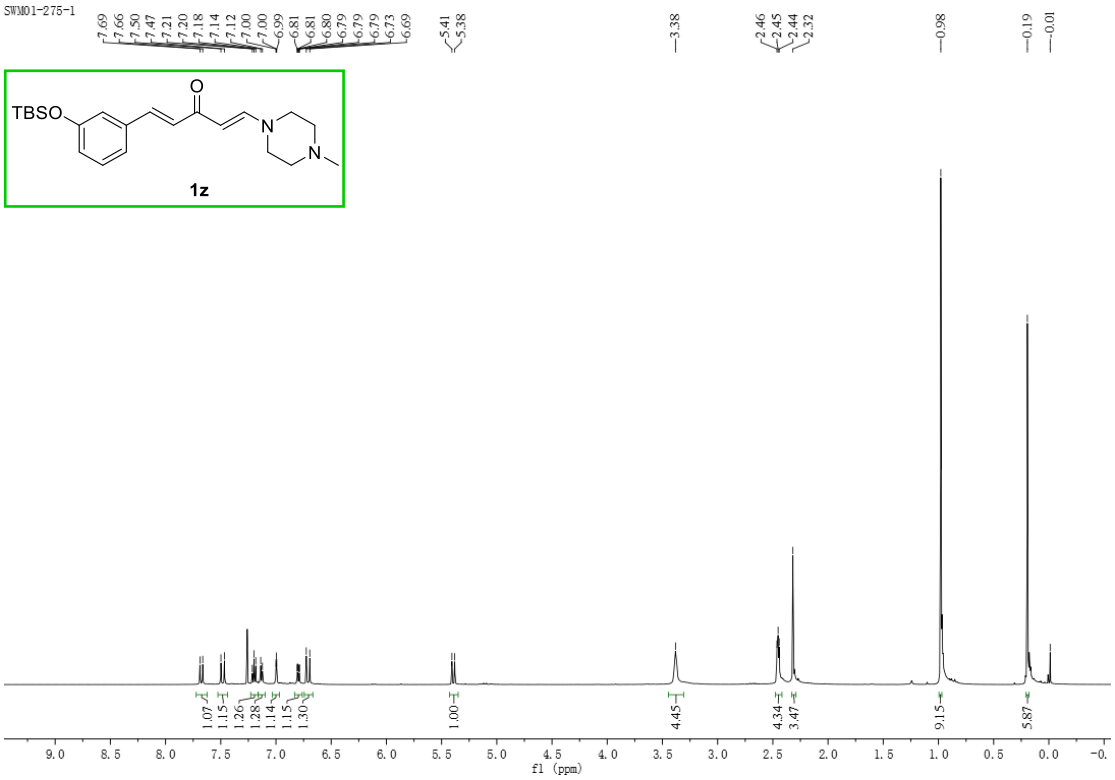

SWM01-308-1B
186.6
161.1
153.3
138.7
138.0
129.0
106.0
101.9
96.8
71.3
59.2
57.7
55.5
36.7
f1 (ppm)

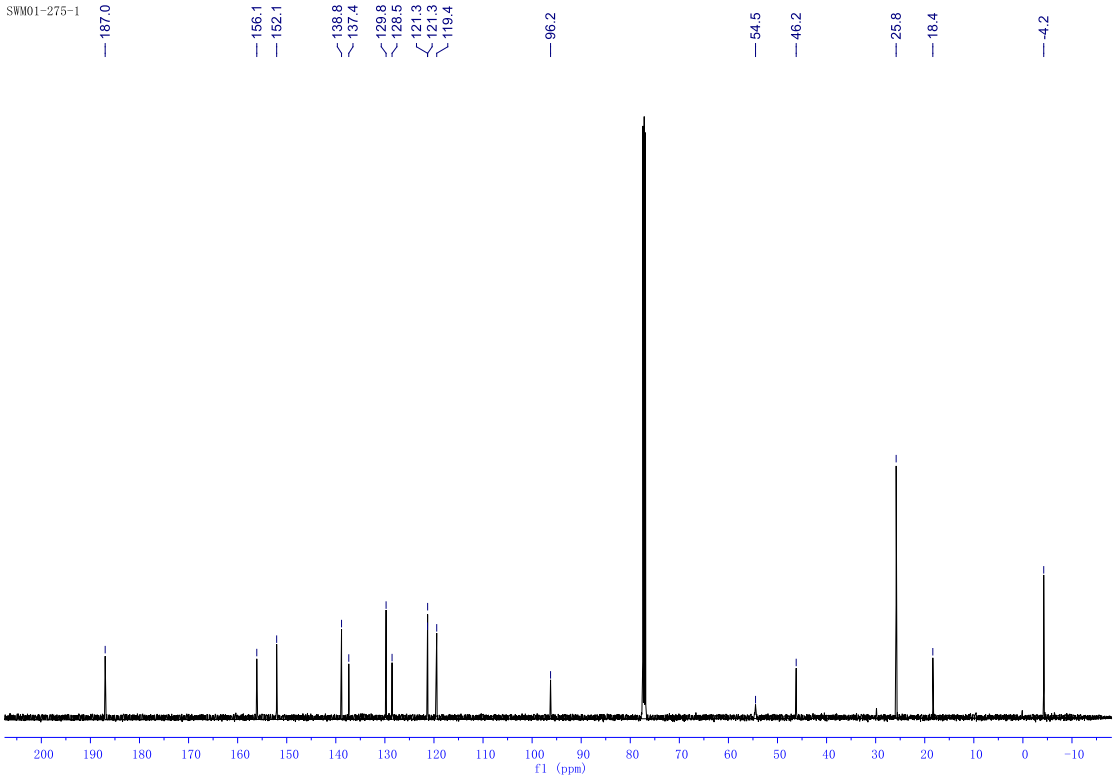

swm01-355-1
7.73
7.71
7.62
7.59
7.23
7.22
7.21
7.20
7.10
7.08
6.90
6.89
6.88
6.88
6.80
6.76
5.40
5.38
3.38
2.46
2.45
2.44
2.33
0.02
HO
O
N
N
1x
1.00
0.98
1.94
0.98
1.03
1.00
0.98
3.85
3.99
3.17
f1 (ppm)

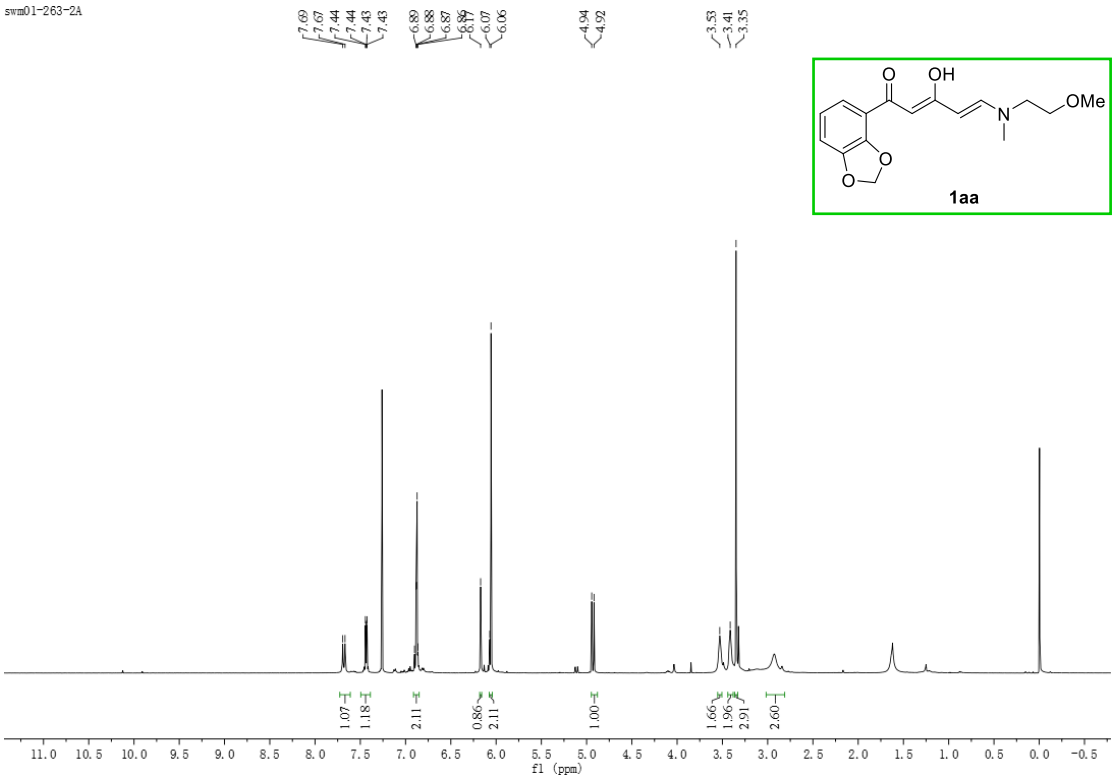

swm01-355-1
187.3
157.3
152.7
139.7
136.8
129.8
127.9
119.3
117.2
115.4
96.3
54.2
45.9
f1 (ppm)

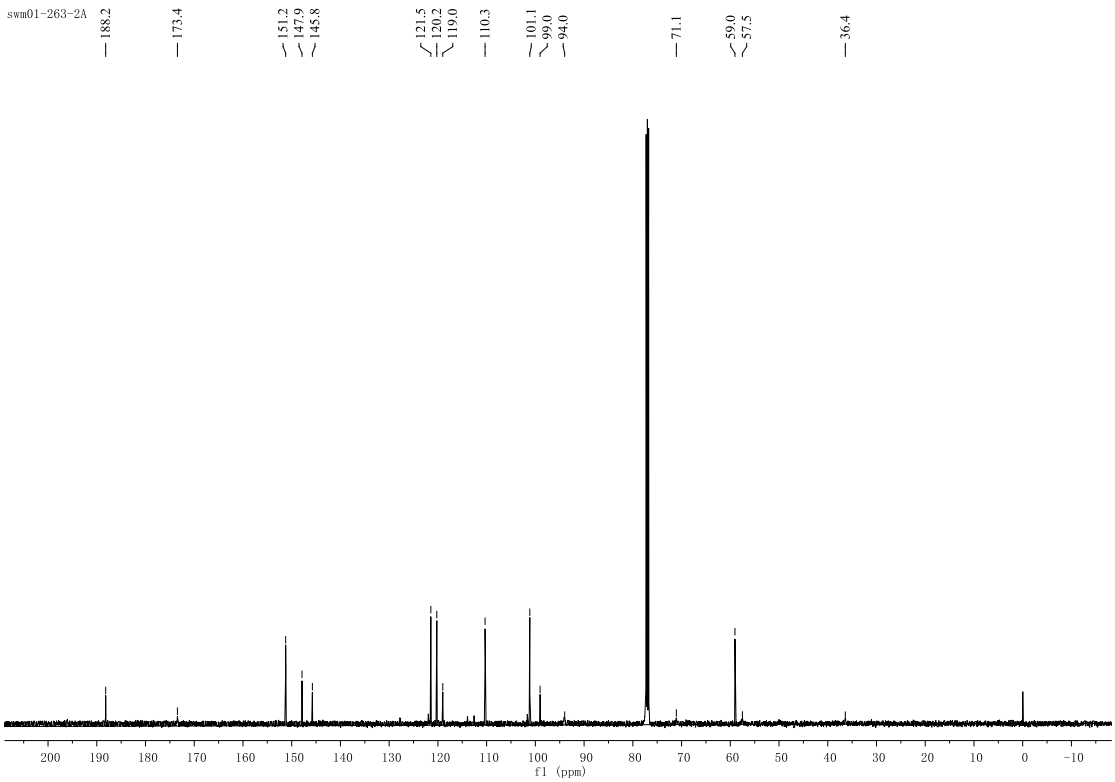

SWM01-274-2
7.71
7.68
7.55
7.51
7.29
7.28
7.28
7.26
7.15
7.14
6.90
6.89
6.88
6.88
6.78
6.75
5.42
5.40
3.83
3.40
2.48
2.47
2.46
2.33
0.00
MeO
O
N
N
1y
1.07
1.15
1.93
1.23
1.21
1.27
1.00
2.91
4.20
4.43
2.97
f1 (ppm)

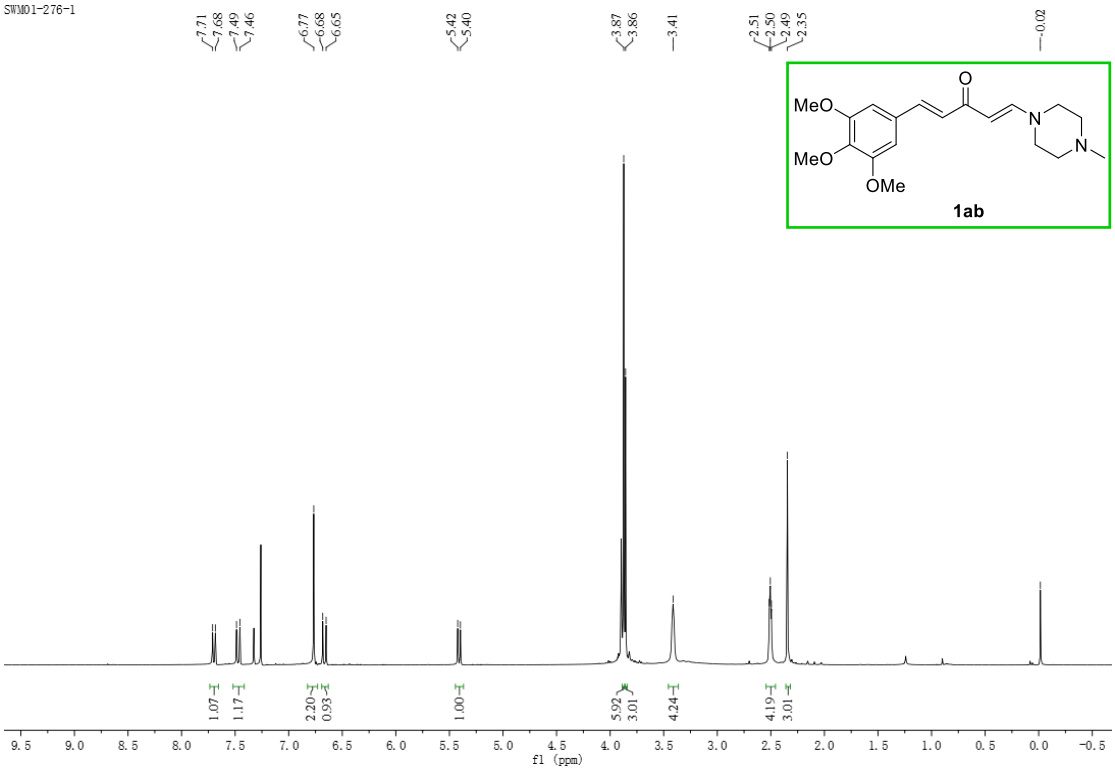

SWM01-274-2
186.9
159.9
152.1
138.8
137.2
129.8
128.6
124.3
120.7
115.3
112.9
96.2
55.4
54.4
46.1
f1 (ppm)

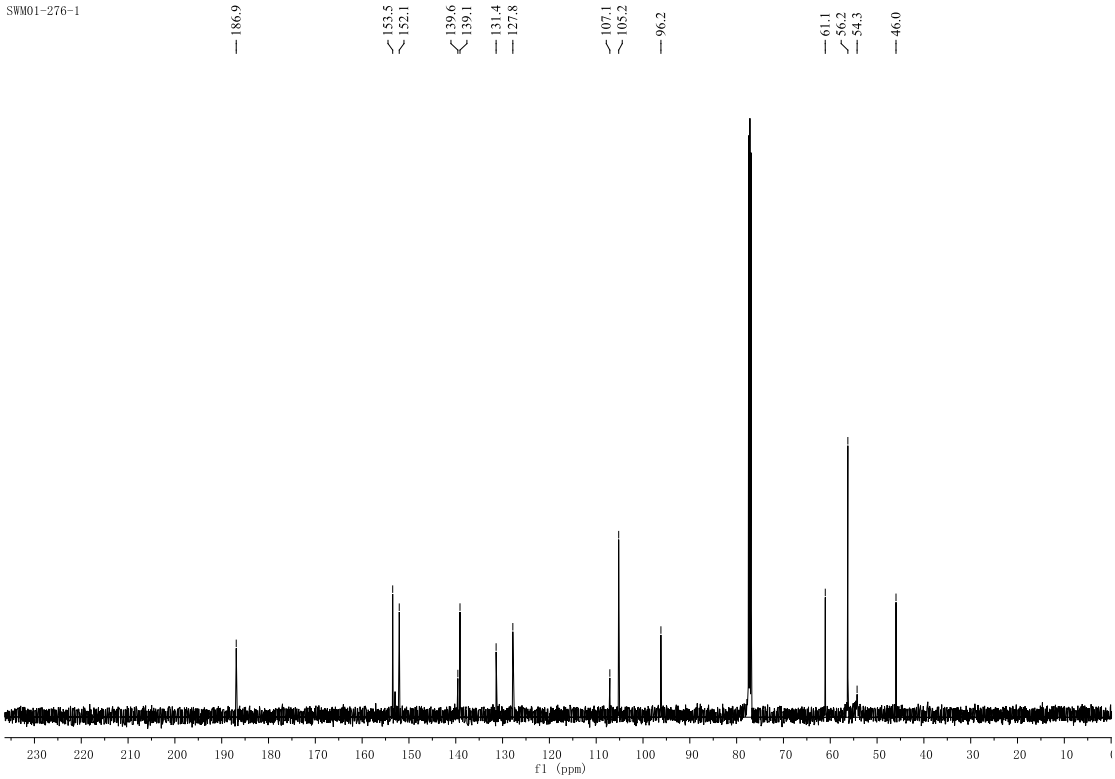

SWM01-275-1
TBSO
O
N
N
1z
f1 (ppm)

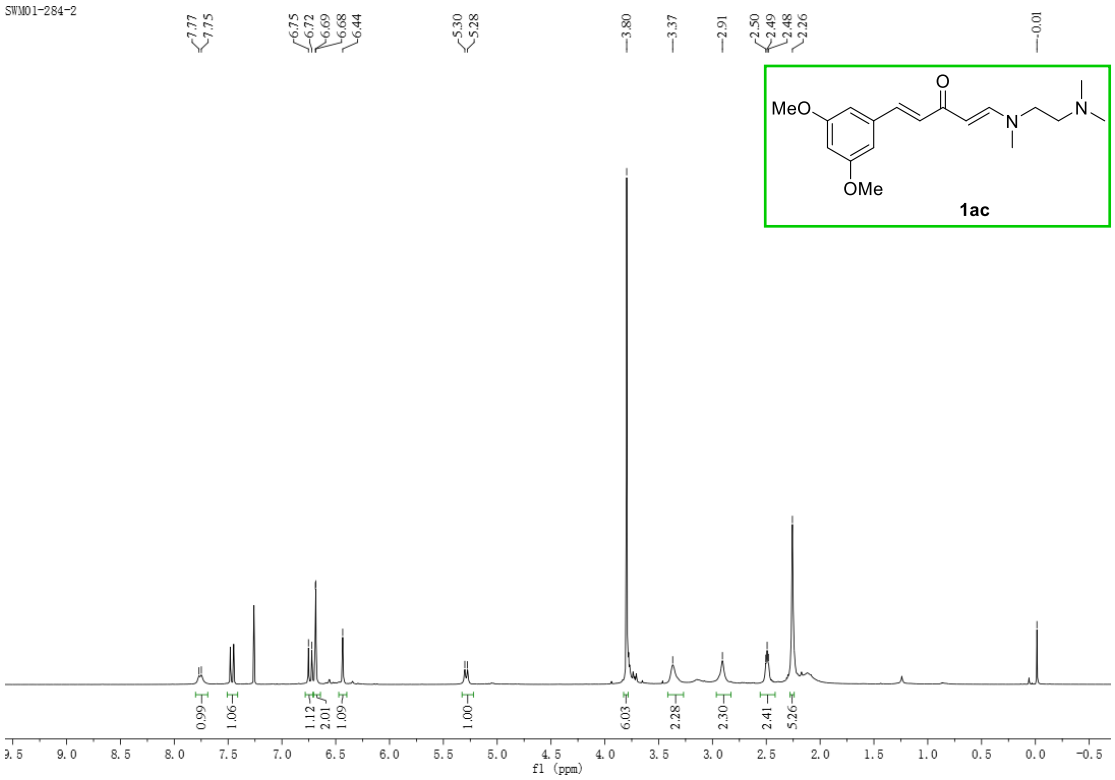

SWM01-275-1
f1 (ppm)

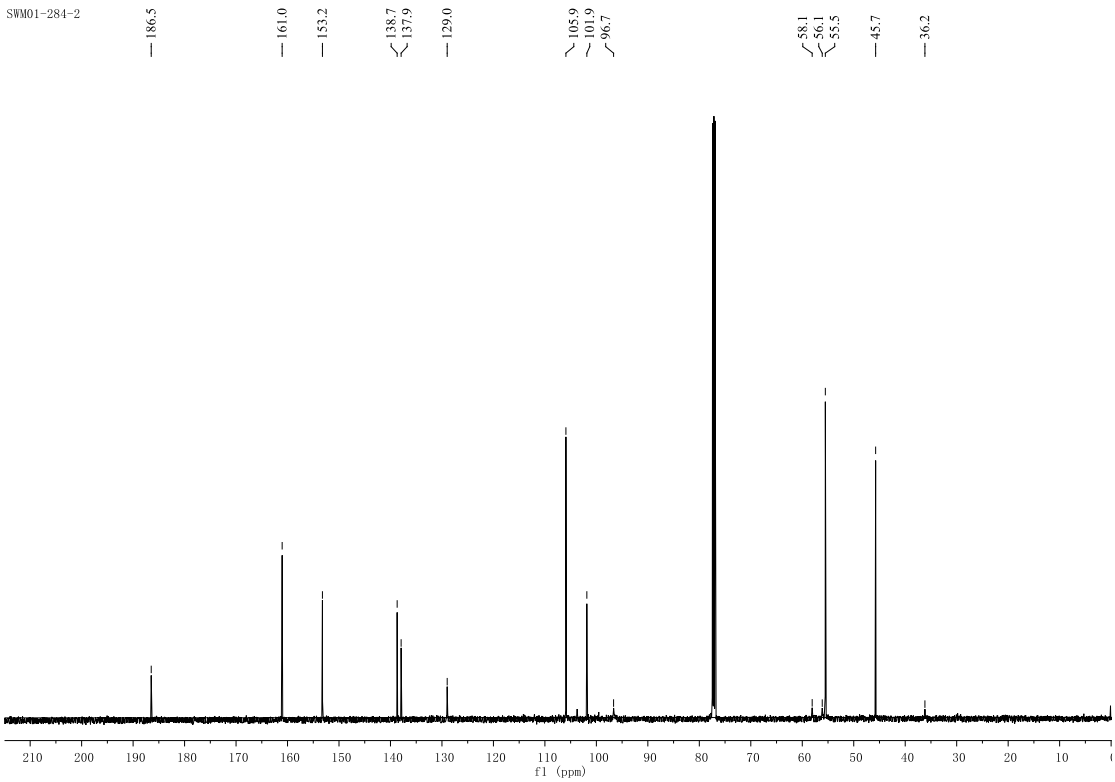

swm01-263-2A
7.69
7.67
7.44
7.44
7.43
7.43
6.89
6.88
6.87
6.86
6.07
6.06
4.94
4.92
3.53
3.41
3.35
O
OH
N
OMe
O
O
1aa
1.07
1.18
2.11
0.86
2.11
1.00
1.66
1.96
2.91
2.60
f1 (ppm)

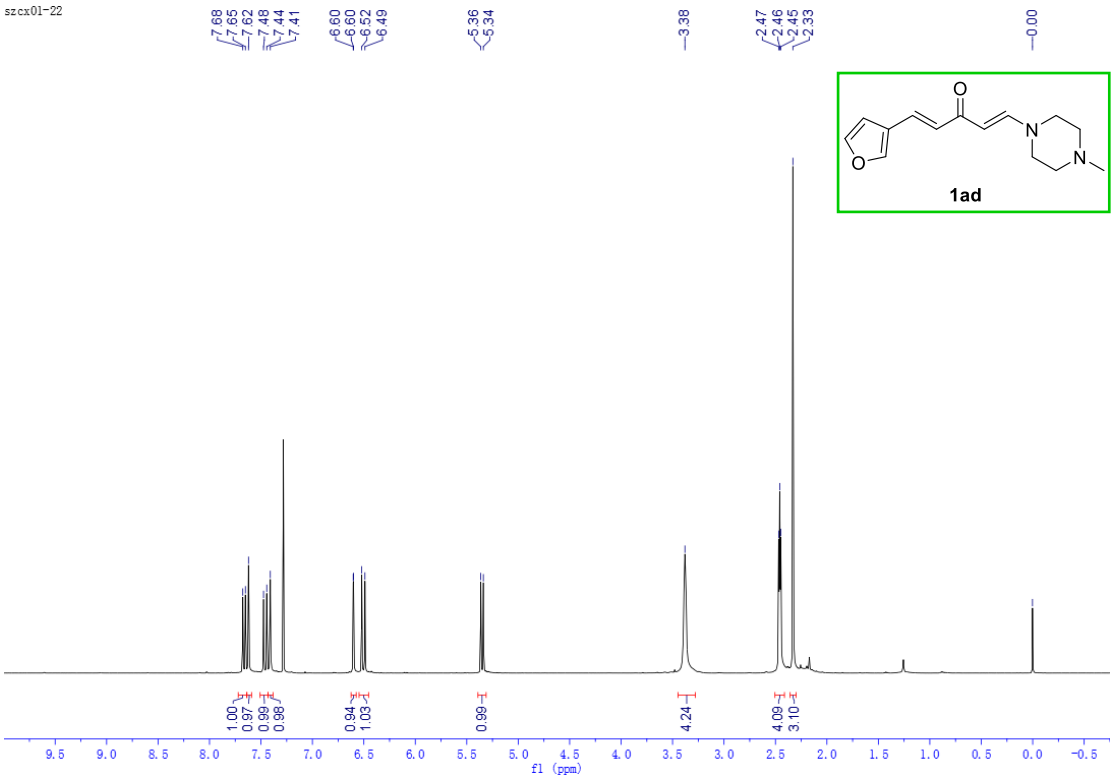

swm01-263-2A
188.2
173.4
151.2
147.9
145.8
121.5
120.2
119.0
110.3
101.1
99.0
94.0
71.1
59.0
57.5
36.4
f1 (ppm)

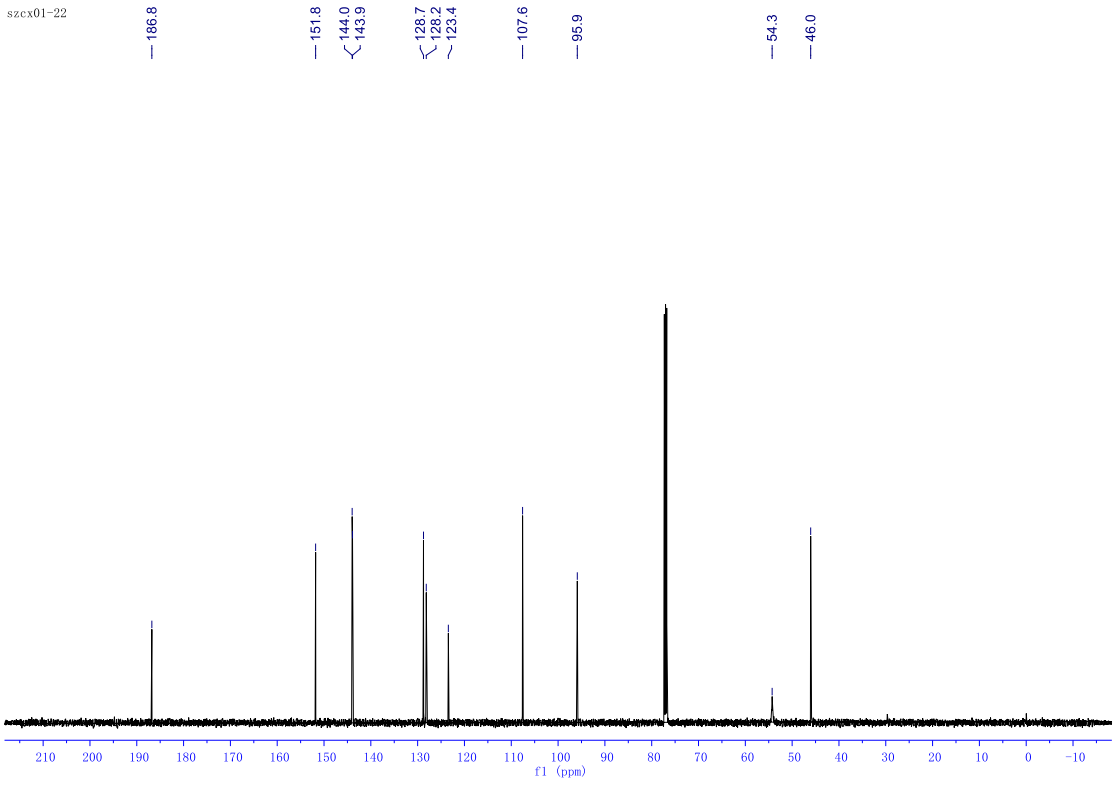

SWM01-276-1
7.71
7.68
7.49
7.46
6.77
6.68
6.65
5.42
5.40
3.87
3.86
3.41
2.51
2.50
2.49
2.35
-0.02
MeO
MeO
OMe
N
N
O
1ab
1.07
1.17
2.20
0.93
1.00
5.92
3.01
4.24
4.19
3.01
f1 (ppm)

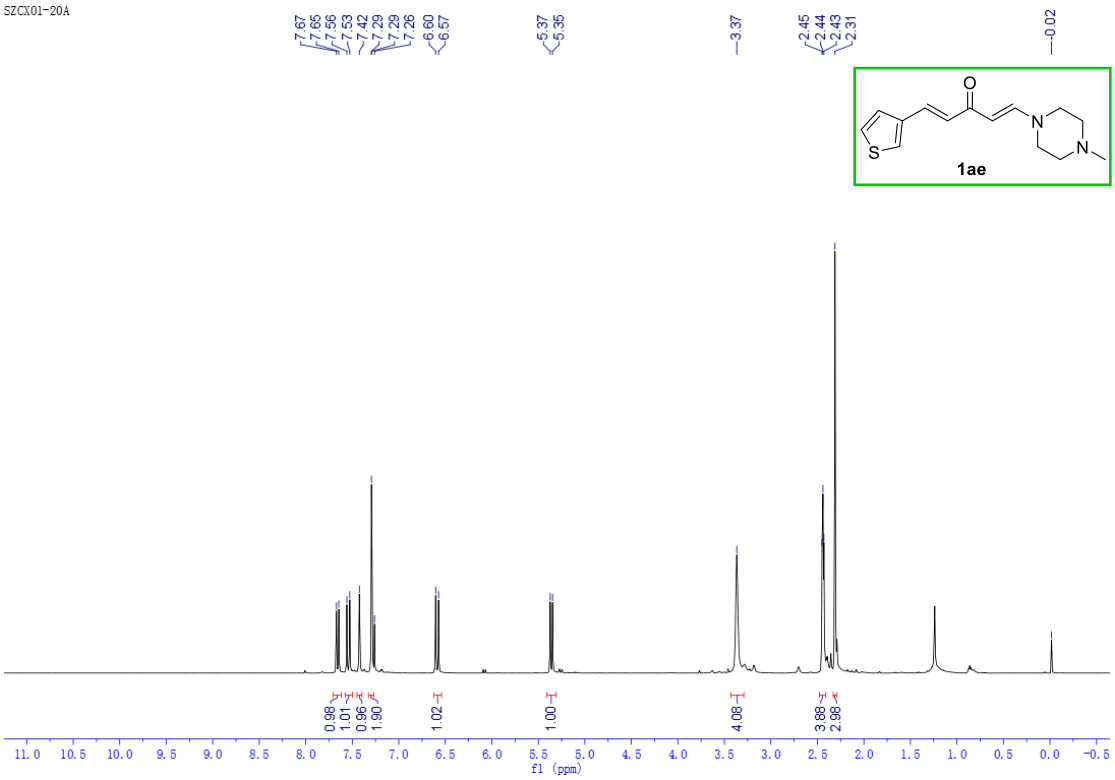

SWM01-276-1
186.9
153.5
152.1
139.6
139.1
131.4
127.8
107.1
105.2
96.2
61.1
56.2
54.3
46.0
f1 (ppm)

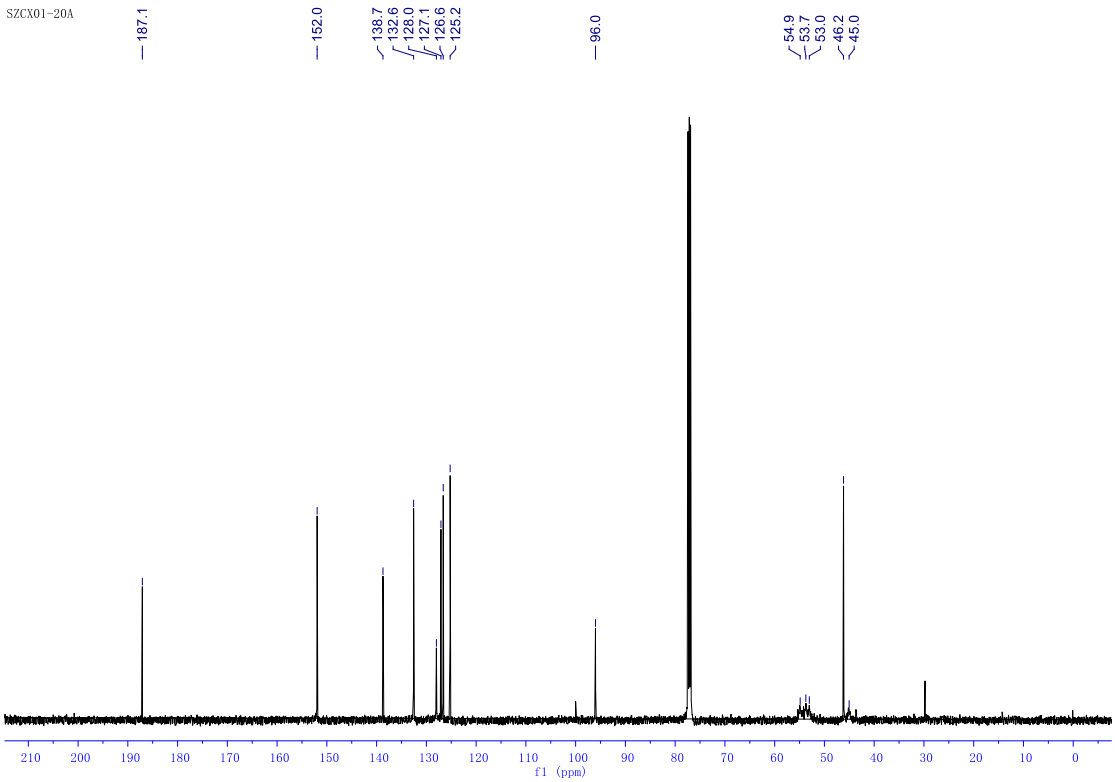

SWM01-284-2
7.77
7.75
6.75
6.72
6.69
6.68
6.44
5.30
5.28
3.80
3.37
2.91
2.50
2.49
2.48
2.26
0.01
MeO
OMe
O
N
N
1ac
0.99
1.06
1.12
2.01
1.09
1.00
6.03
2.28
2.30
2.41
5.26
f1 (ppm)


SWM01-284-2
186.5
161.0
153.2
138.7
137.9
129.0
105.9
101.9
96.7
58.1
56.1
55.5
45.7
36.2
f1 (ppm)


szcx01-22
7.68
7.65
7.62
7.48
7.44
7.41
6.60
6.60
6.52
6.49
5.36
5.34
3.38
2.47
2.46
2.45
2.33
0.00
O
N
O
N
1ad
1.00
0.97
0.99
0.98
0.94
1.03
0.99
4.24
4.09
3.10
f1 (ppm)

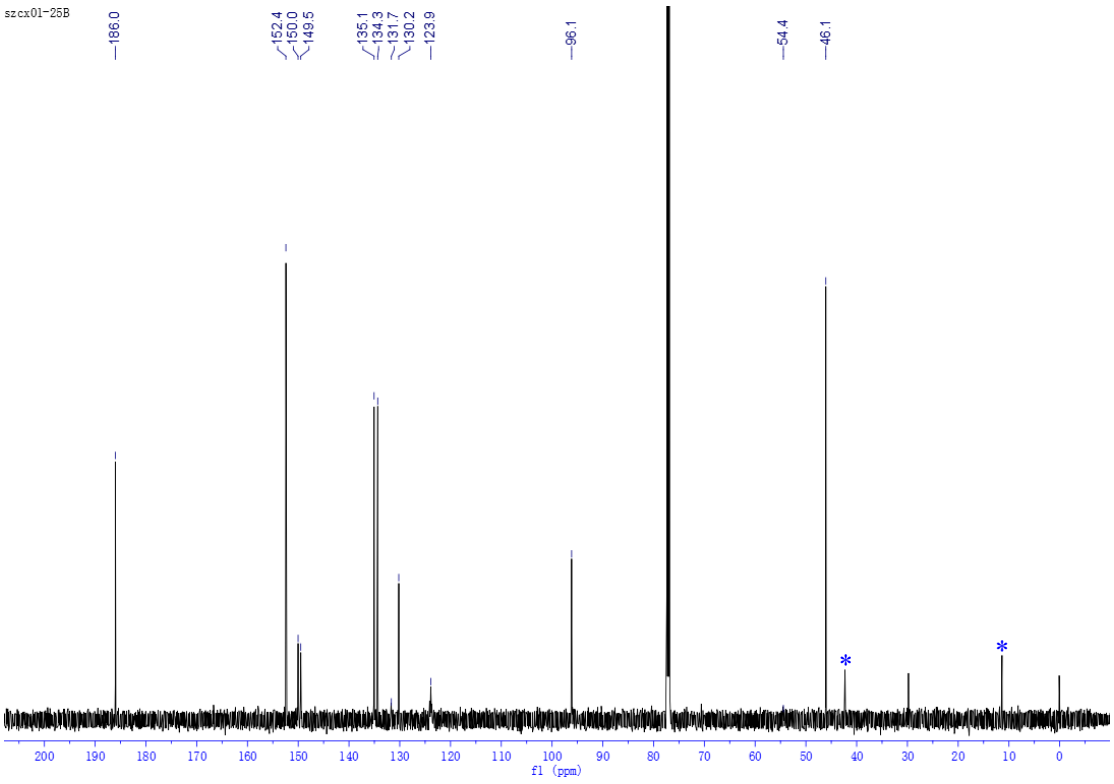

szcx01-22
186.8
151.8
144.0
143.9
128.7
128.2
123.4
107.6
95.9
54.3
46.0
f1 (ppm)

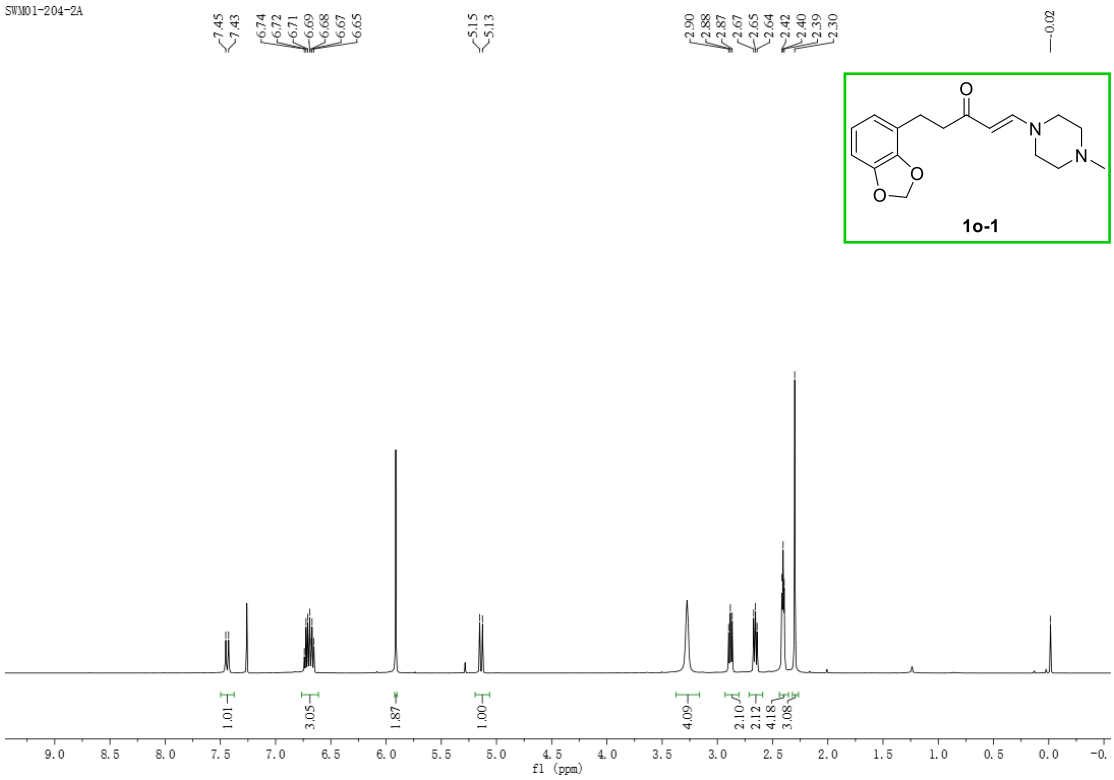

SZCX01-20A
7.67
7.65
7.56
7.53
7.42
7.29
7.29
7.26
6.60
6.57
5.37
5.35
3.37
2.45
2.44
2.43
2.31
0.02
O
N
N
S
1ae
0.98
1.01
0.96
1.90
1.02
1.00
4.08
3.88
2.98
f1 (ppm)

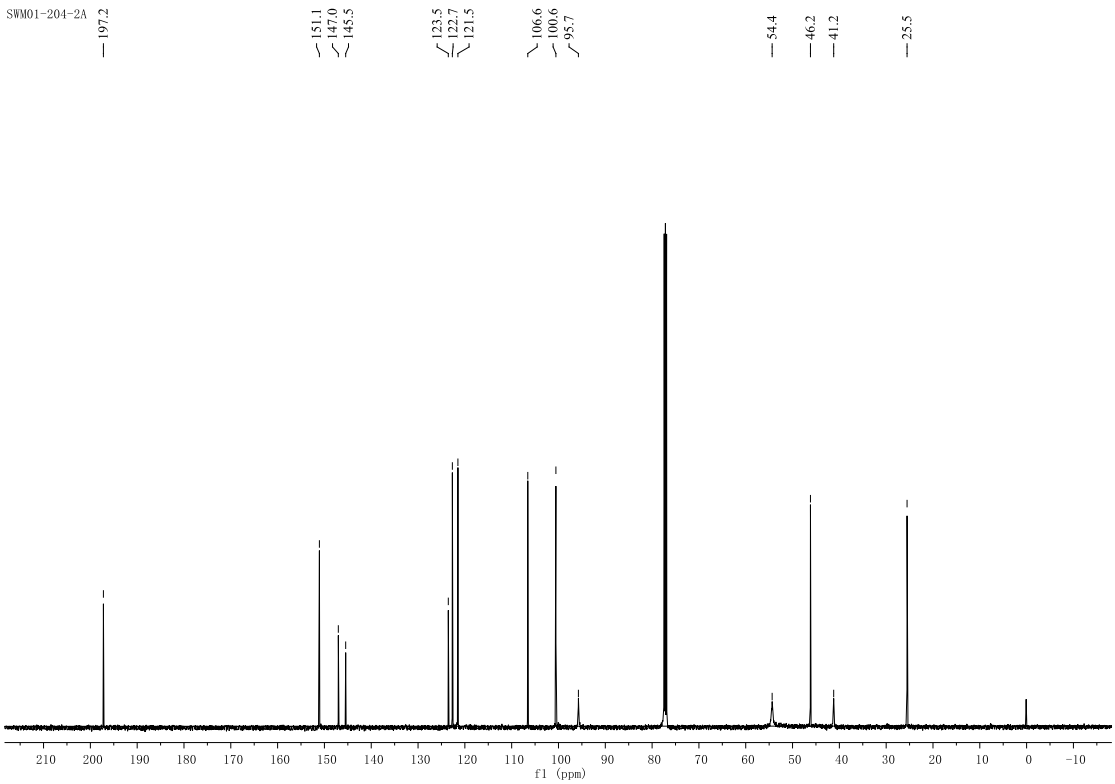

SZCX01-20A
187.1
152.0
138.7
132.6
128.0
127.1
126.6
125.2
96.0
54.9
53.7
53.0
46.2
45.0
f1 (ppm)

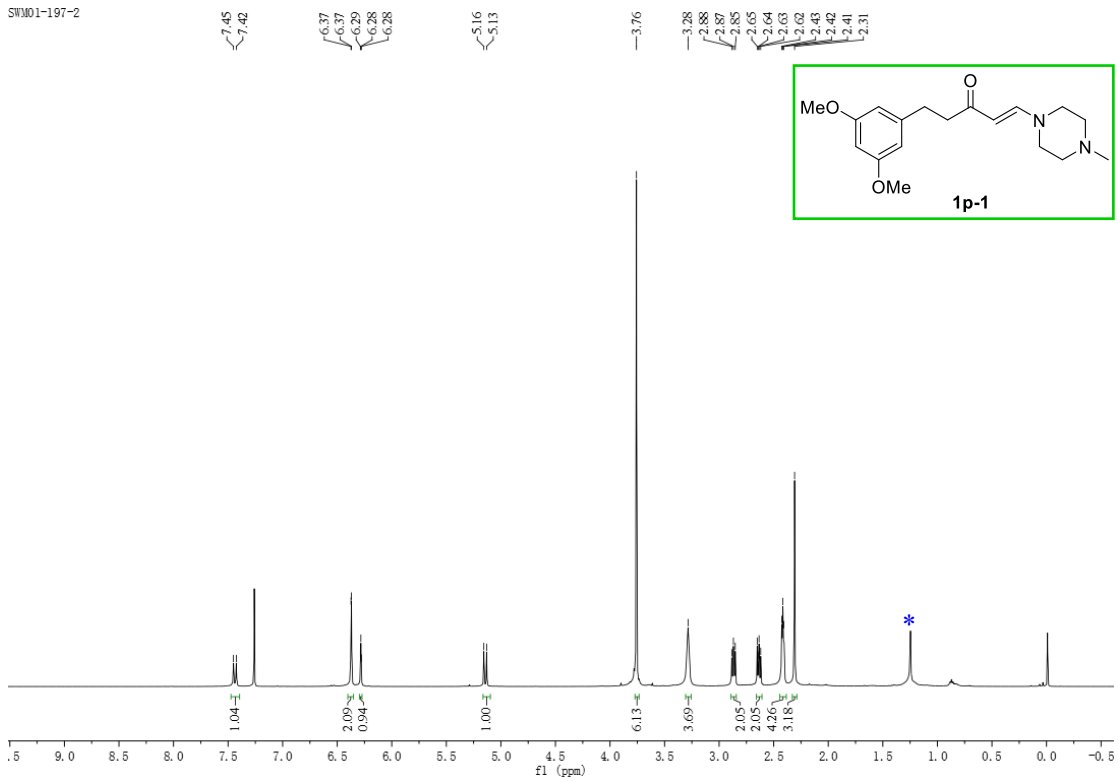

SZCX01-25B
8.76
8.54
7.82
7.80
7.71
7.69
7.54
7.50
7.28
6.83
6.79
5.39
5.36
3.41
2.49
2.48
2.47
2.34
0.02
O
N
N
N
1af
0.86
0.99
1.01
1.02
1.01
0.78
1.00
0.99
3.83
4.19
3.37
*
*
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

* $HNEt_2$

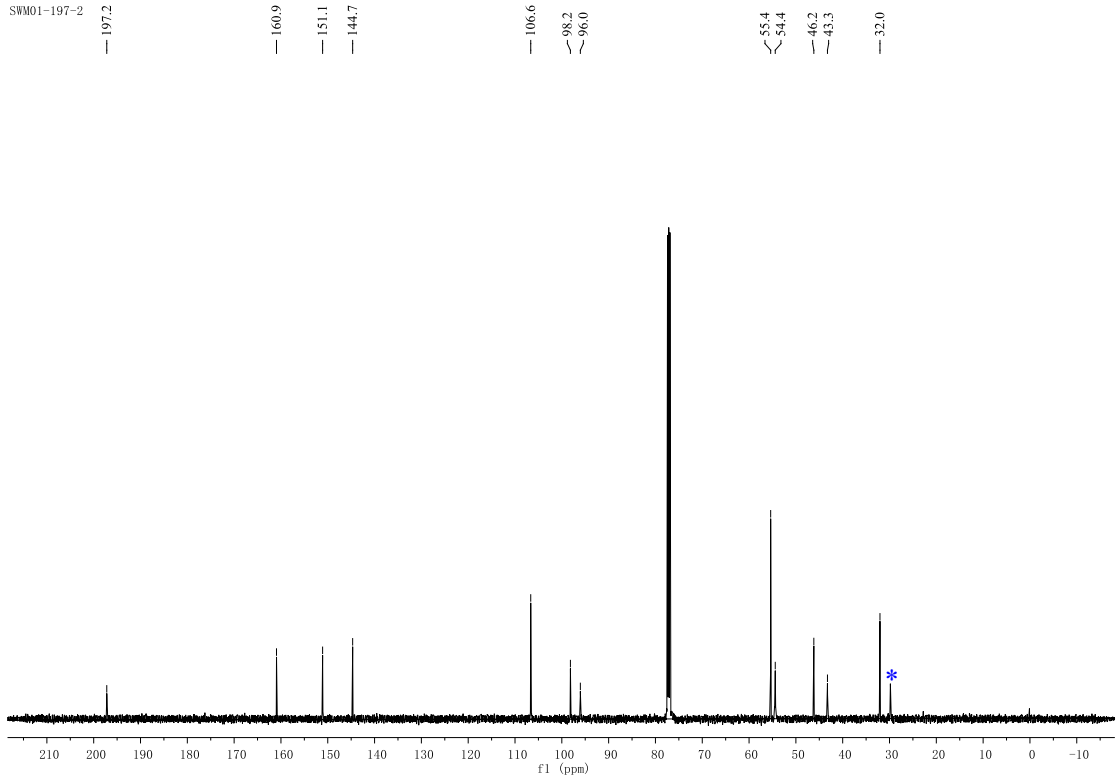

szcx01-25B
186.0
152.4
150.0
149.5
135.1
134.3
131.7
130.2
123.9
96.1
54.4
46.1
*
*
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

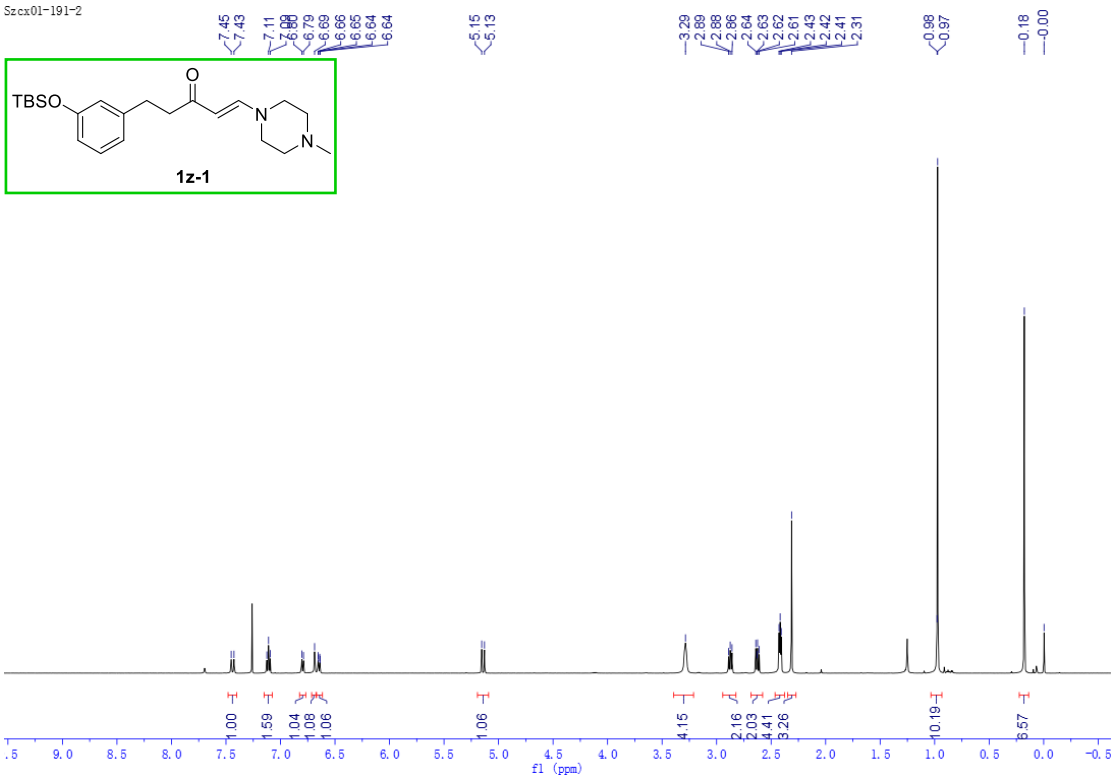

SWM01-204-2A
7.45
7.43
6.74
6.72
6.71
6.69
6.68
6.67
6.65
5.15
5.13
2.90
2.88
2.87
2.67
2.65
2.64
2.42
2.40
2.39
2.30
0.02
O
N
N
O
O
1o-1
1.01
3.05
1.87
1.00
4.09
2.10
2.12
4.18
3.08
f1 (ppm)

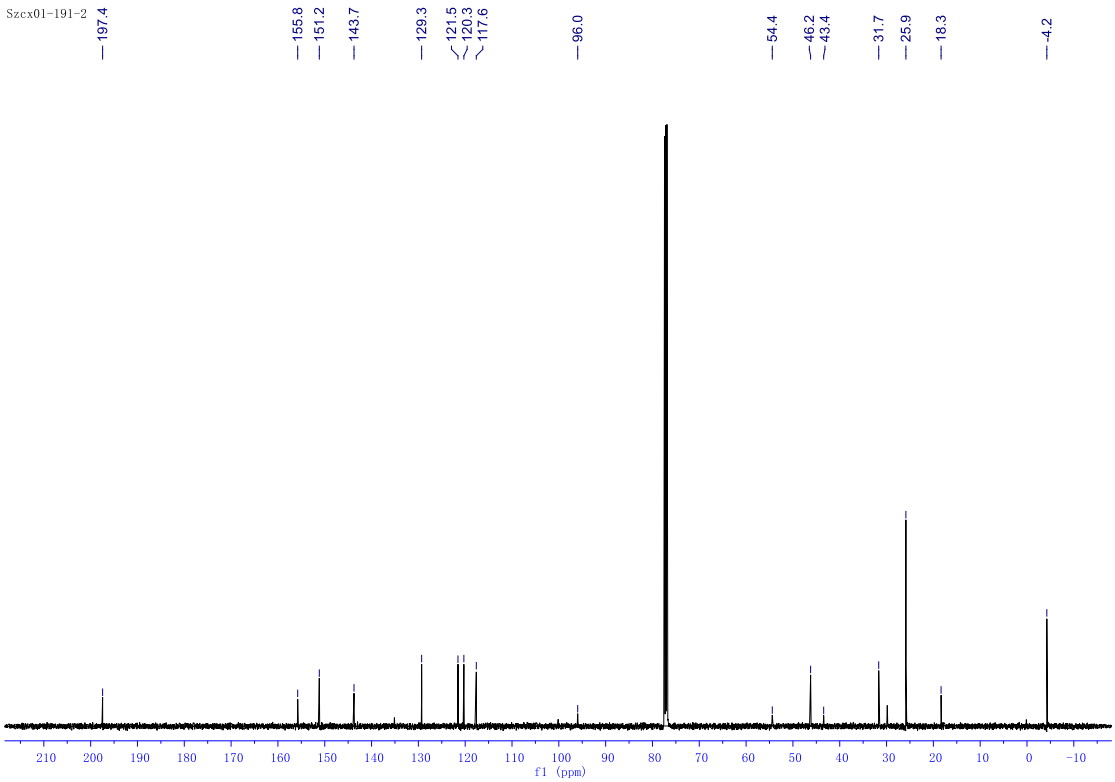

SWM01-204-2A
197.2
151.1
147.0
145.5
123.5
122.7
121.5
106.6
100.6
95.7
54.4
46.2
41.2
25.5
f1 (ppm)


SWM01-197-2
7.45
7.42
6.37
6.37
6.29
6.28
6.28
5.16
5.13
3.76
3.28
2.88
2.87
2.85
2.65
2.64
2.63
2.62
2.43
2.42
2.41
2.31
MeO
OMe
O
N
N
1p-1
*
1.04
2.09
0.94
1.00
6.13
3.69
2.05
2.05
4.26
3.18
f1 (ppm)

* grease


SWM01-197-2
197.2
160.9
151.1
144.7
106.6
98.2
96.0
55.4
54.4
46.2
43.3
32.0
*
f1 (ppm)


Szcx01-191-2
TBSO
O
N
N
1z-1
f1 (ppm)


Szcx01-191-2
f1 (ppm)

5. HPLC traces of synthetic compounds for biological evaluation

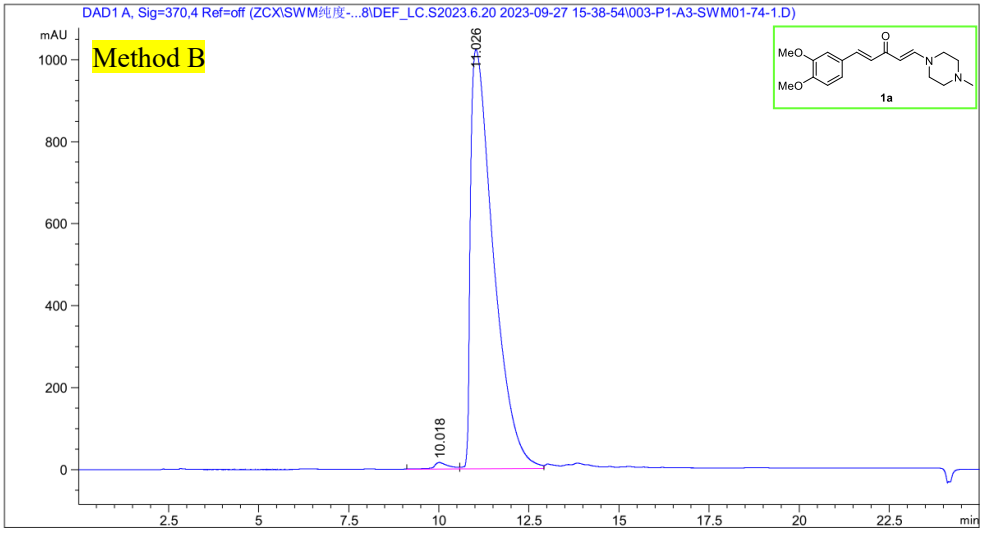
<Chromatogram>

Analytical column: Eclipse XDB-C18 5um (4.6×250 mm); **Velocity of flow**: 1mL/min

Method A: 0.00-2.00min: 70% MeOH/30% H_2O; 2.01-15.00min: form 70% MeOH/30% H_2O to 100% MeOH; 15.00-20.00min: 100% MeOH; 20.01-25.00min: 70% MeOH/30% H_2O

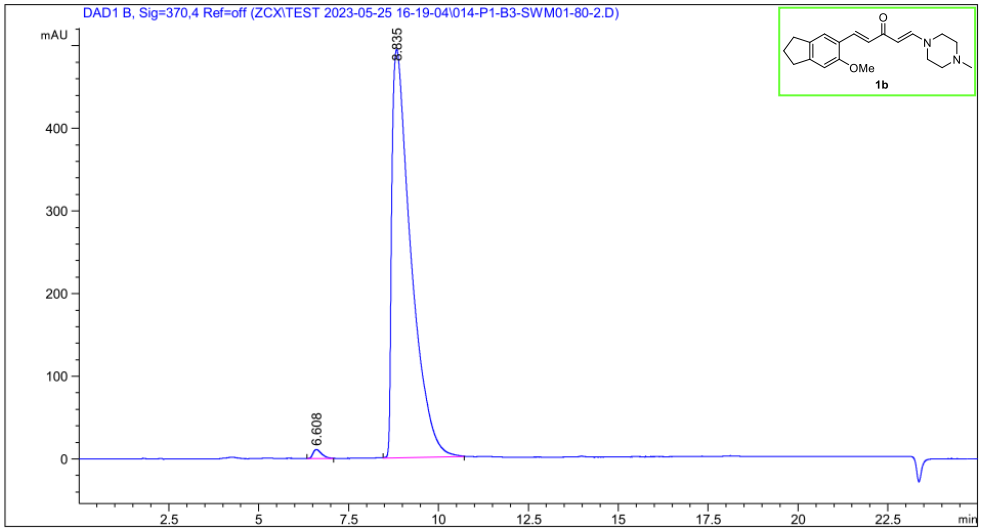
Method B: 0.00-2.00min: 45% MeOH/55% H_2O; 2.01-15.00min: form 45% MeOH/55% H_2O to 100% MeOH; 15.00-20.00min: 100% MeOH; 20.01-25.00min: 45% MeOH/55% H_2O

Unless otherwise noted, method A was used for HPLC analysis



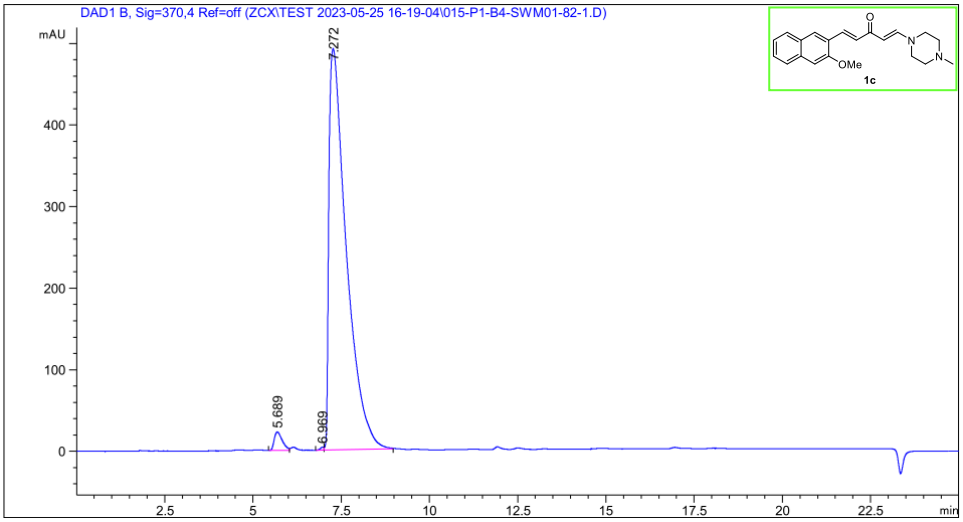
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.018	457.96048	16.10763	1.0039
2	11.026	4.51609e4	1023.83093	98.9961
Total		4.56189e4	1039.93856	100%



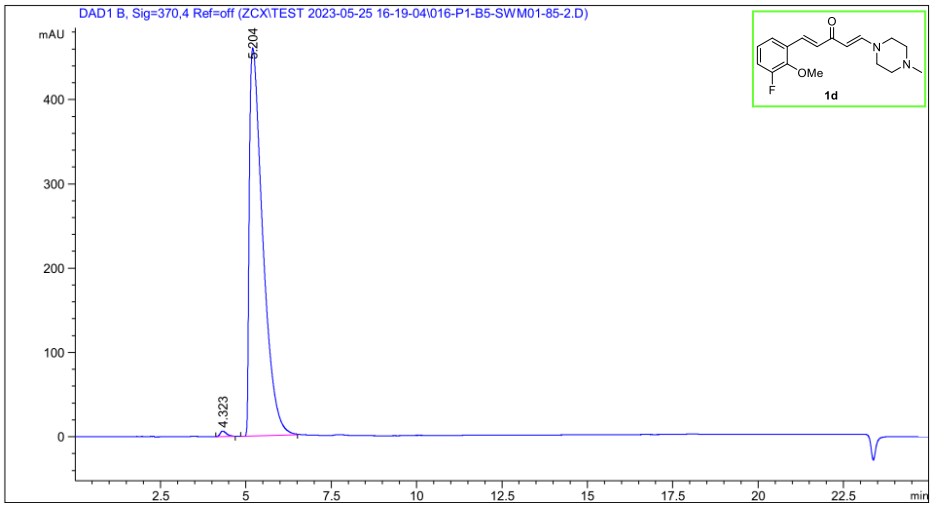
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.608	184.07951	10.84945	1.0015
2	8.835	1.81970e4	494.08472	98.9985
Total		1.83811e4	504.93417	100%



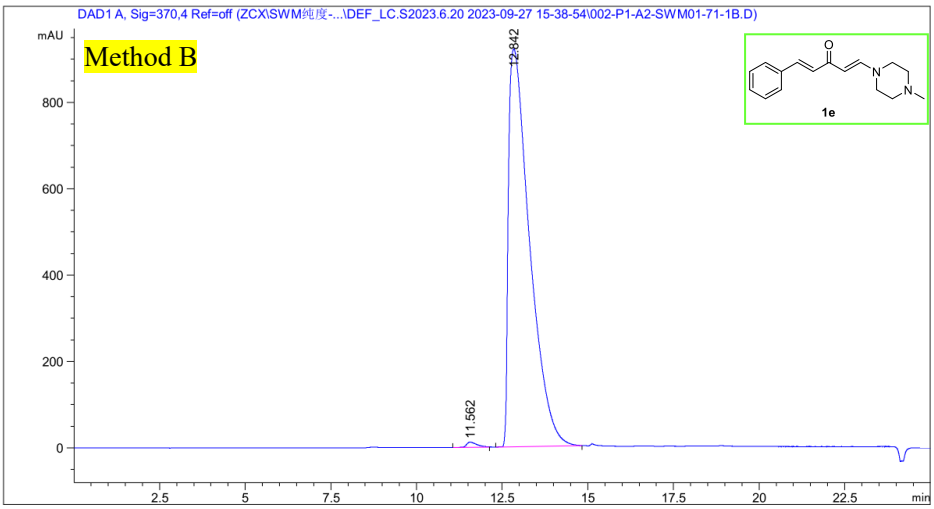
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.689	360.50446	22.55700	2.0912
2	6.969	24.786872	3.57796	0.1438
3	7.272	1.68536e4	491.97061	97.7650
Total		1.72389e4	518.10557	100%



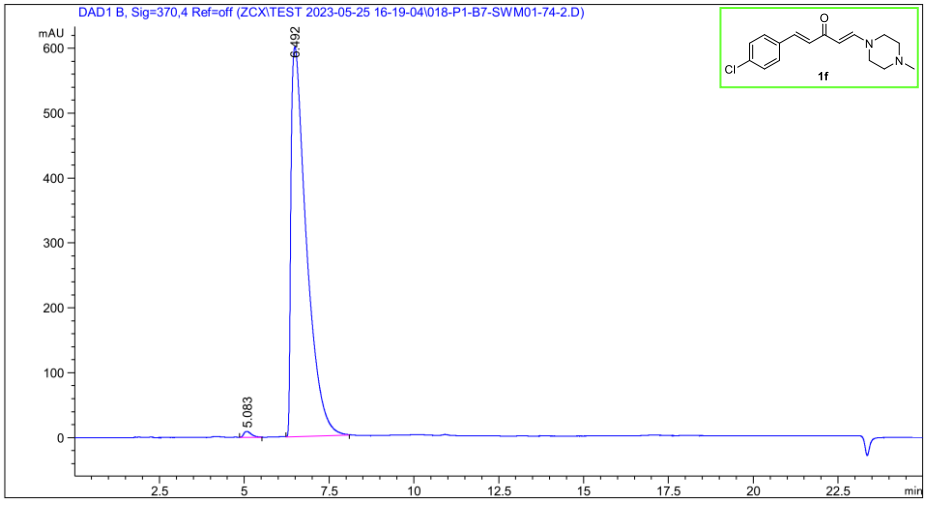
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.323	85.77954	6.47273	0.6780
2	5.204	1.25657e4	460.20627	99.3220
Total		1.26514e44	466.67900	100%



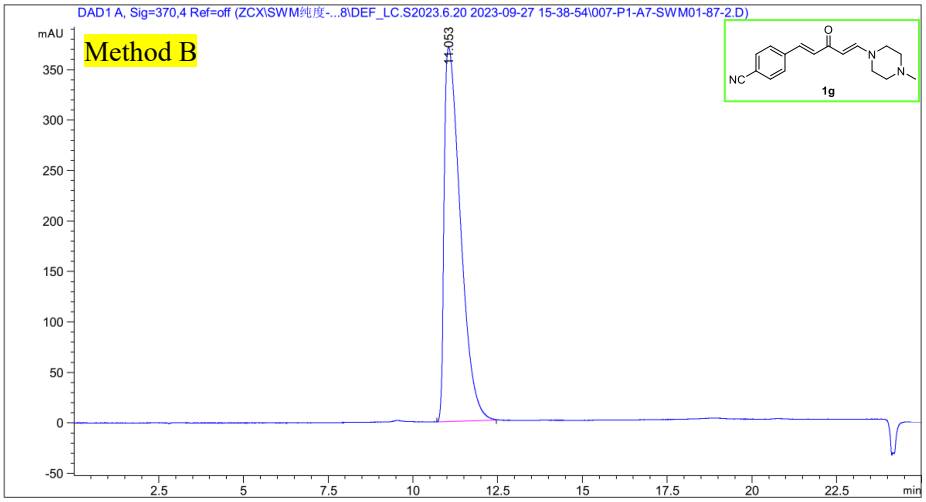
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.562	259.51498	12.18440	0.6543
2	12.842	3.94017e4	920.89319	99.3457
Total		3.96612e4	933.07759	100%



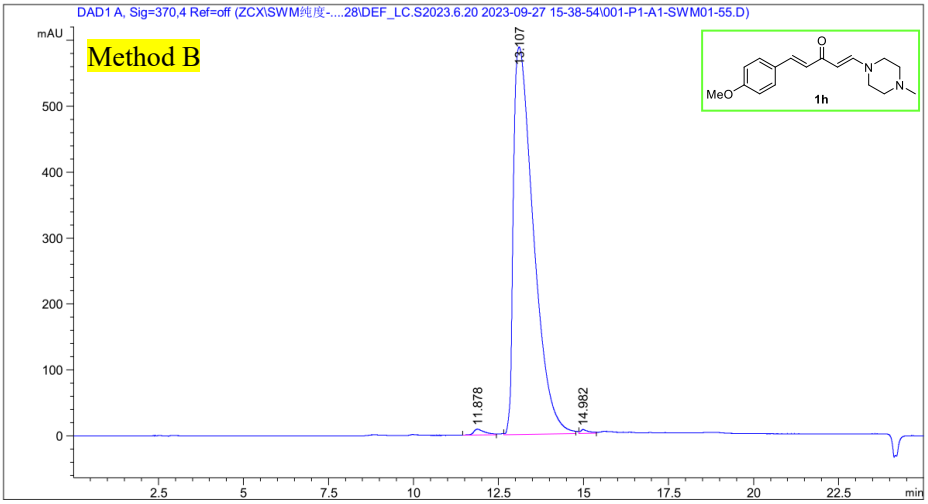
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.083	139.15068	9.09592	0.7284
2	6.492	1.89638e4	600.68518	99.2716
Total		1.91030e4	609.78110	100%



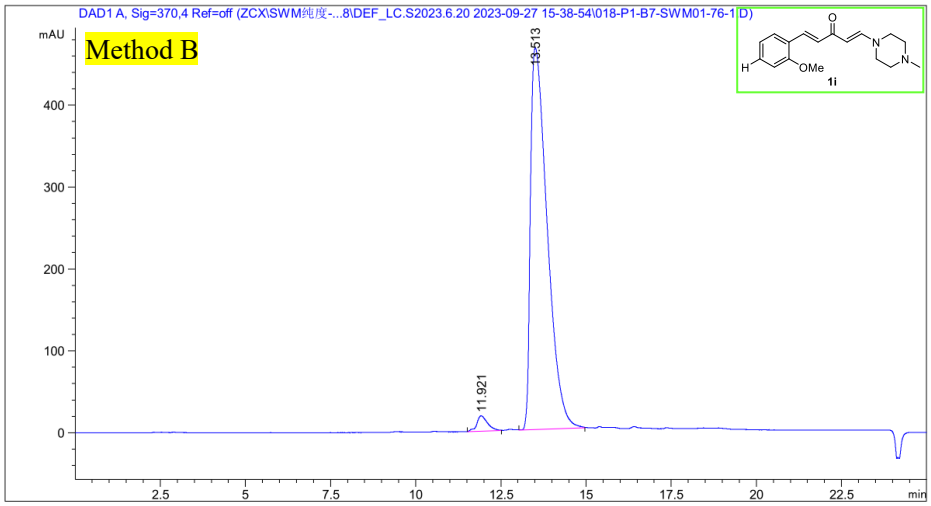
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.053	1.23426e4	370.79663	100.0000
Total		1.23426e4	370.79663	100%



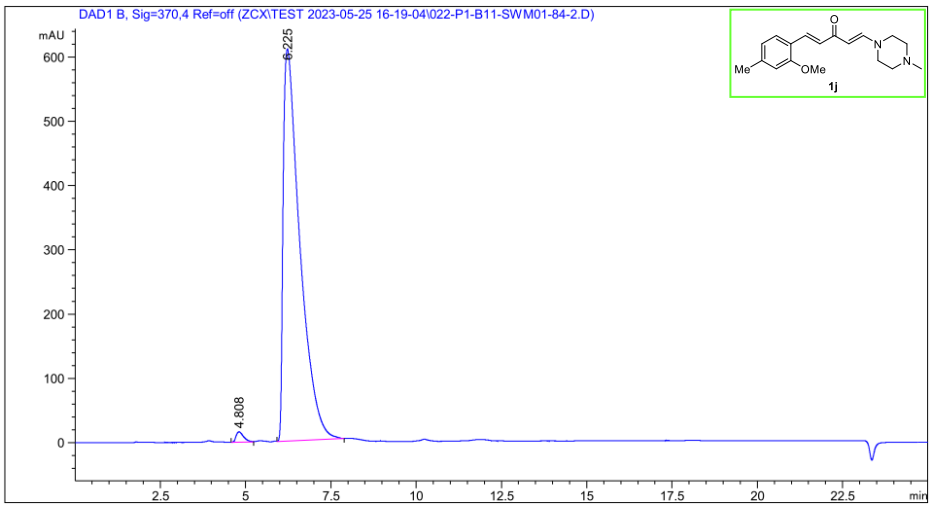
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.878	200.08829	8.86525	0.8195
2	13.107	2.41092e4	587.59375	98.7472
3	14.982	105.78890	6.23174	0.4333
Total		2.44151e4	602.69075	100%



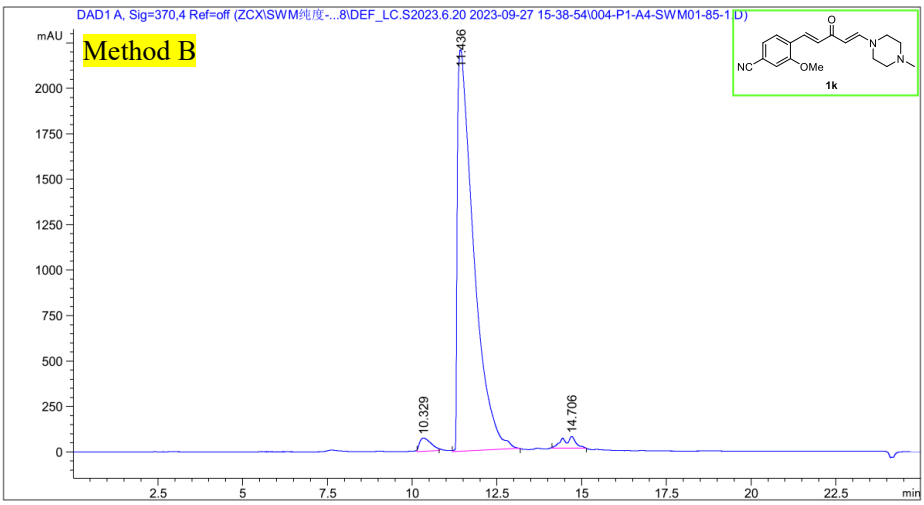
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.921	413.65088	18.79803	2.5344
2	13.513	1.59077e4	465.74844	97.4656
Total		1.63213e4	484.54648	100%



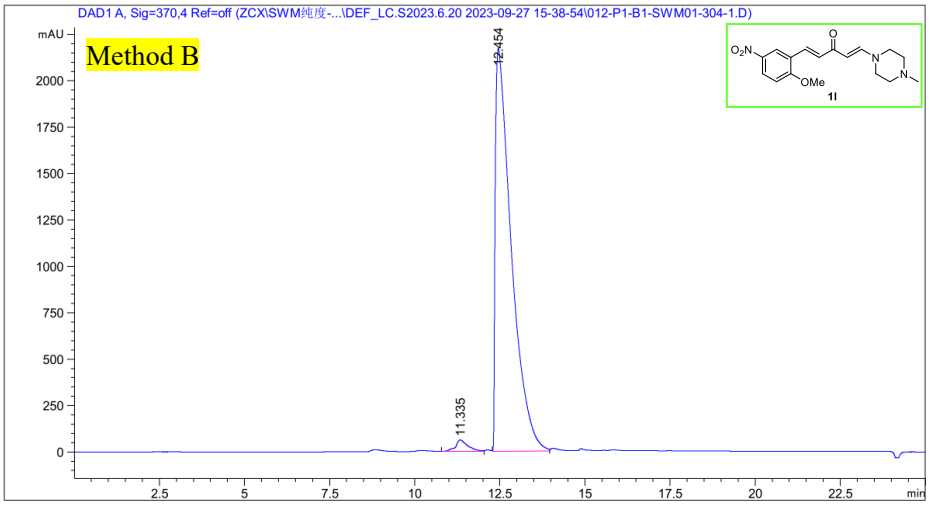
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.808	248.42706	16.12818	1.1843
2	6.225	2.07290e4	610.08374	98.8157
Total		2.09774e4	626.21192	100%



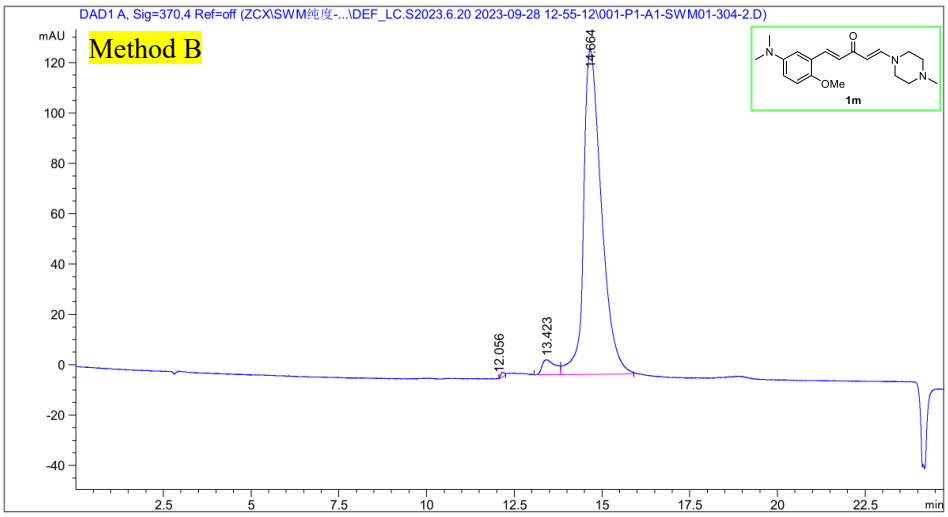
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.329	1729.49768	73.18595	2.2357
2	11.436	7.39151e4	2211.71118	95.5481
3	14.706	1714.41492	64.30698	2.2162
Total		7.73590e4	2349.20412	100%



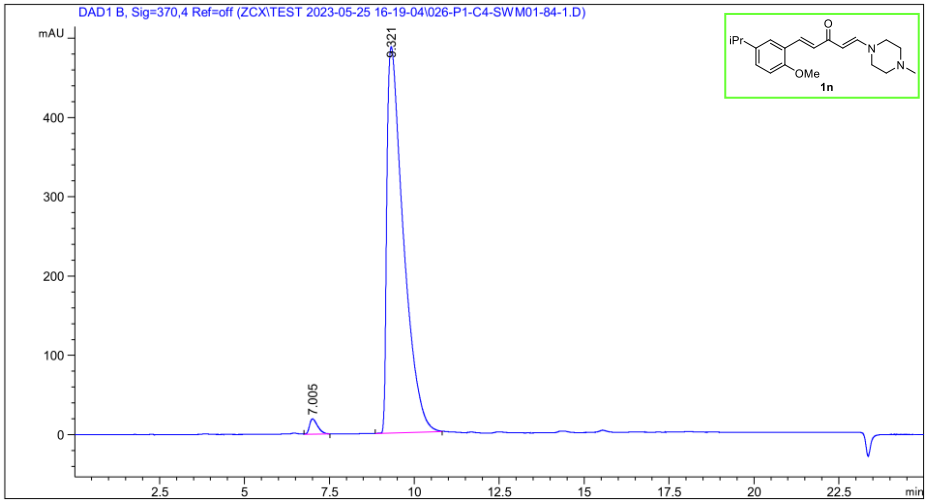
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.335	1557.74512	62.00557	2.1504
2	12.454	7.08804e4	2173.55908	97.8496
Total		7.24382e4	2235.56465	100%



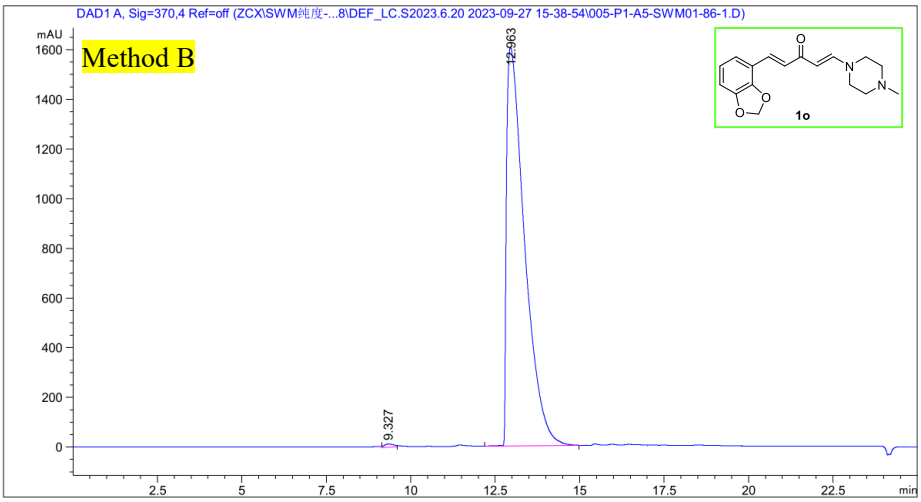
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.056	13.48341	1.91061e-1	0.2851
2	13.423	155.04950	5.89483	3.2783
3	14.664	4560.98682	128.80792	96.4366
Total		4729.51973	134.89381	100%



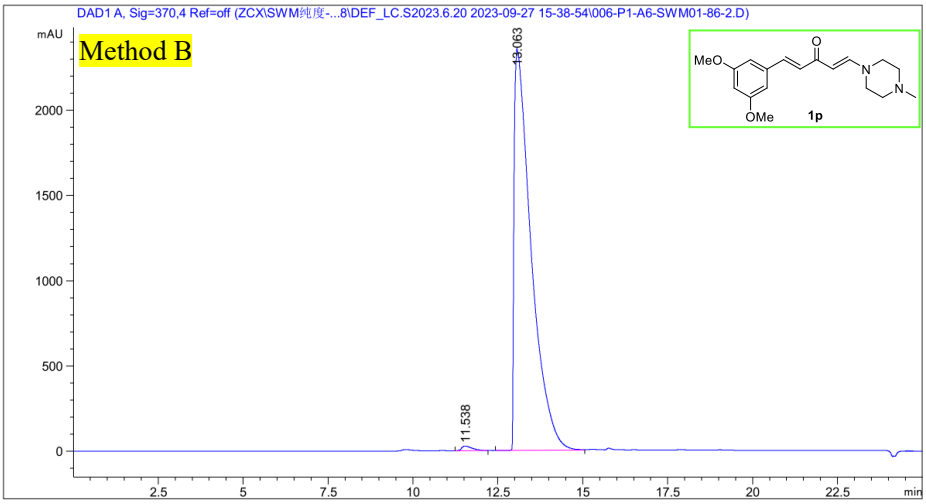
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.005	315.83197	19.12069	1.8811
2	9.321	1.64743e4	487.15234	98.1189
Total		1.67901e4	506.27304	100%



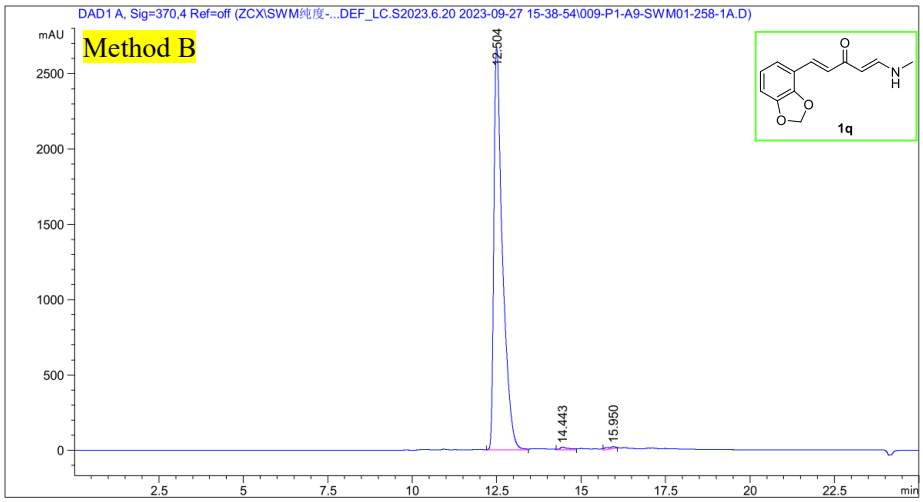
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.327	259.02371	13.61027	0.4321
2	12.963	5.96903e4	1603.10754	99.5679
Total		5.99493e4	1616.71781	100%



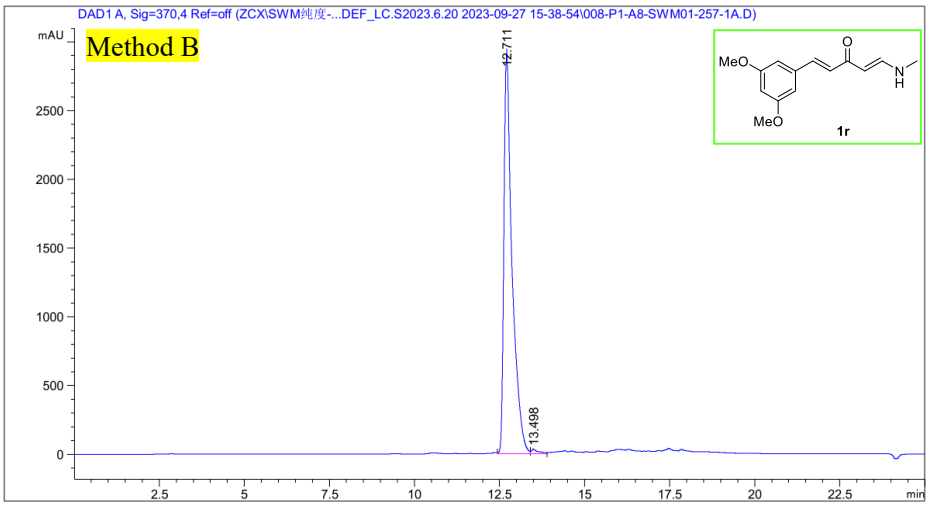
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.538	605.35199	27.41299	0.7318
2	13.063	8.21115e4	2363.67554	99.2682
Total		8.27169e4	2391.08853	100%



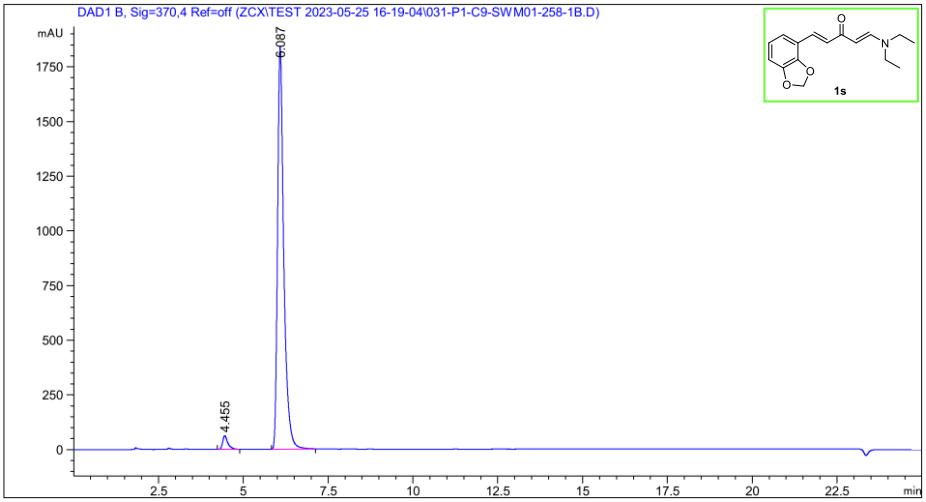
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.504	4.76747e4	2665.09058	98.6909
2	14.443	332.30191	18.48947	0.6879
3	15.950	300.08521	14.46169	0.6212
Total		4.83071e4	2698.04173	100%



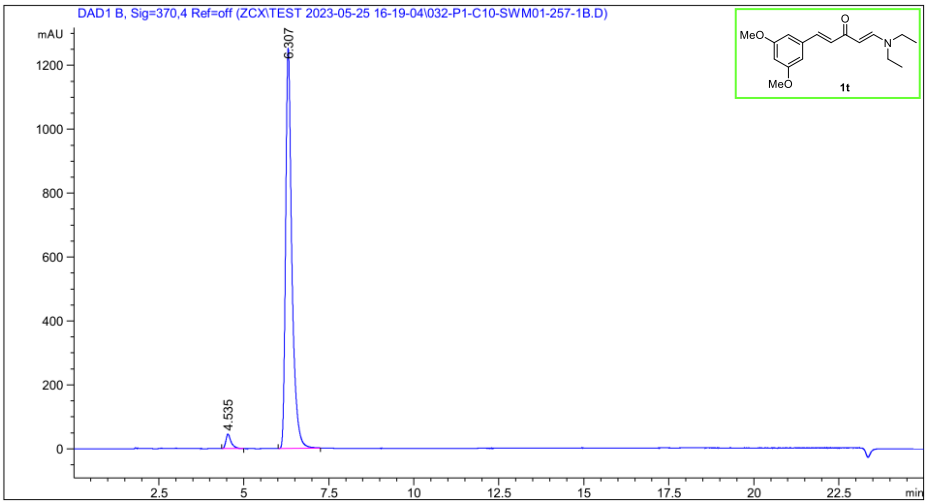
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.711	4.89096e4	2946.58740	99.0220
2	13.498	483.06485	32.63512	0.9780
Total		4.93927e4	2979.22253	100%



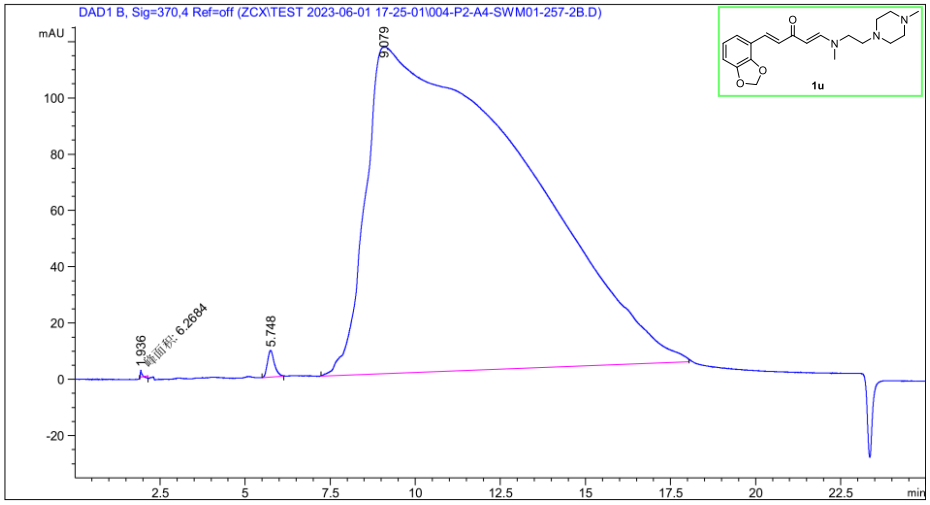
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.455	663.22437	62.63724	2.7396
2	6.087	2.35460e4	1839.85803	97.2604
Total		2.42092e4	1902.49527	100%



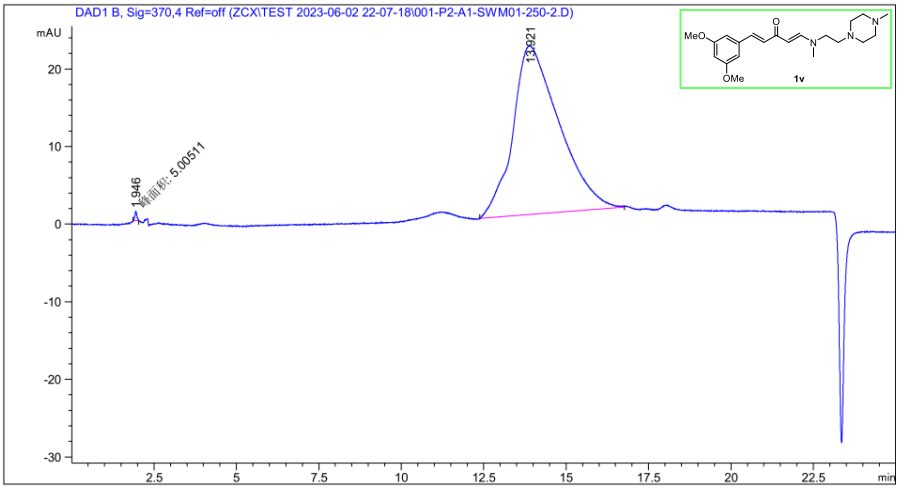
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.535	493.78650	45.76043	2.9839
2	6.307	1.60547e4	1253.5633	97.0161
Total		1.65485e4	1299.32378	100%



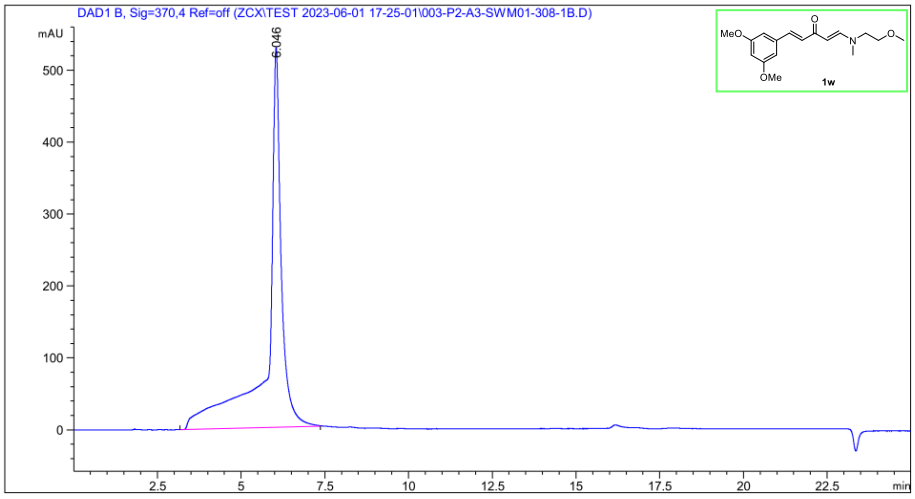
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.936	6.26840	2.42329	0.0166
2	5.748	132.82443	9.44076	0.3514
3	9.079	3.76591e4	116.08568	99.6320
Total		3.77982e4	127.94973	100%



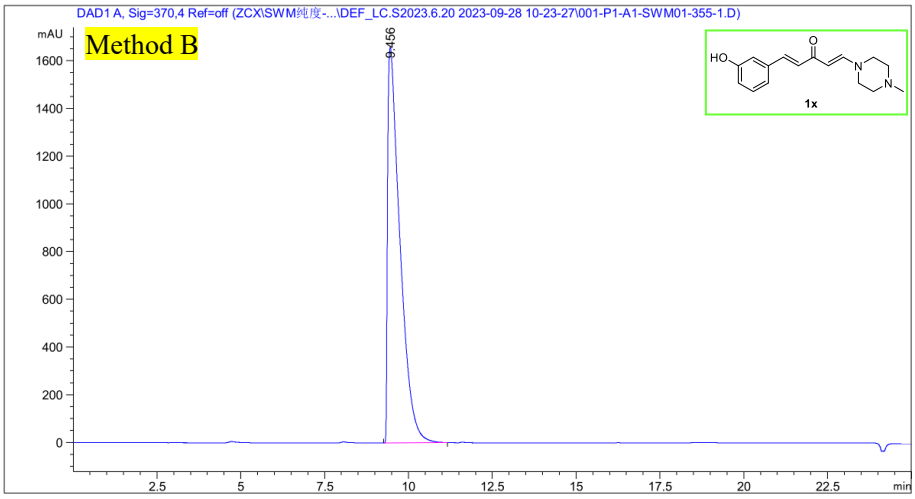
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.946	5.00511	1.20556	0.2363
2	13.921	2112.78589	21.69192	99.7637
Total		2117.79100	22.89748	100%



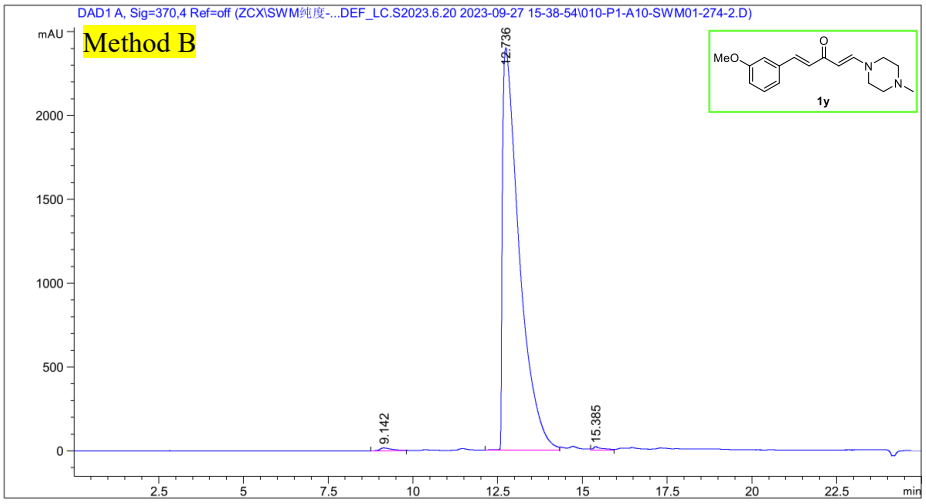
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.046	1.51852e4	528.68500	100.0000
Total		1.51852e4	528.68500	100%



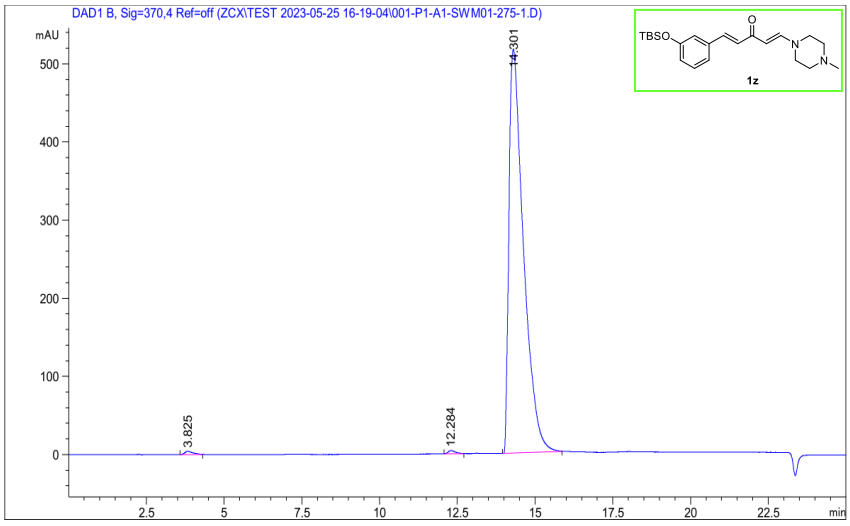
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.456	4.24587e4	1657.81506	100.0000
Total		4.24587e4	1657.81506	100%



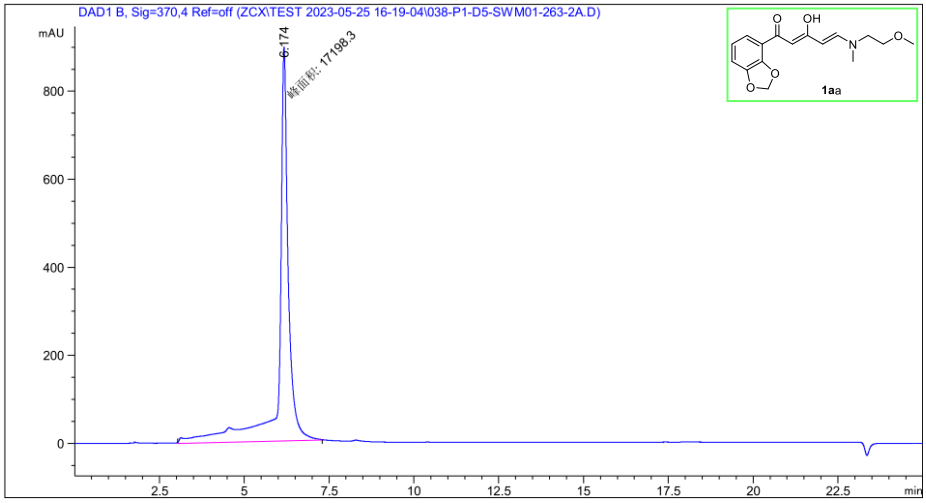
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.142	357.65002	15.96362	0.4280
2	12.736	8.28006e4	2401.56860	99.0839
3	15.385	407.93777	21.25139	0.4882
Total		8.35662e4	2438.78361	100%



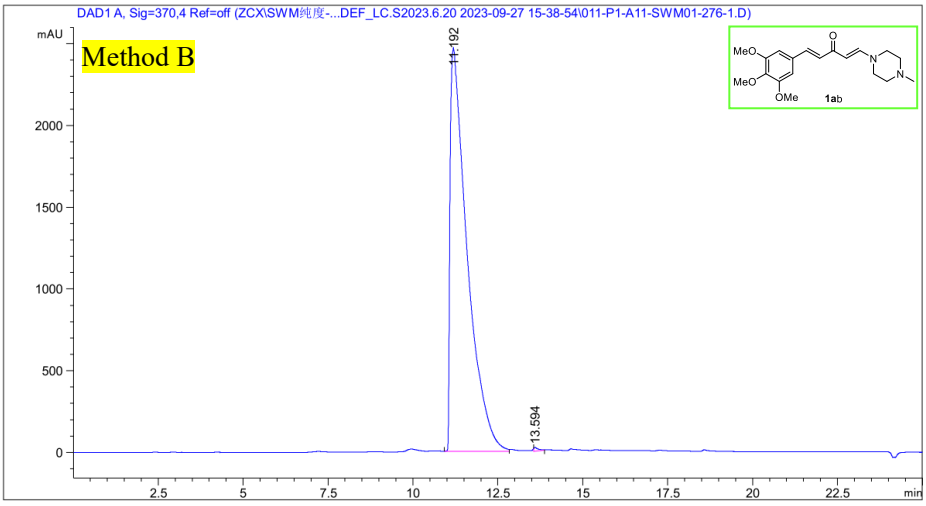
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.825	78.59293	4.09885	0.4648
2	12.284	64.59892	4.07131	0.3820
3	14.301	1.67658e4	516.88727	99.1532
Total		1.69090e4	525.05743	100%



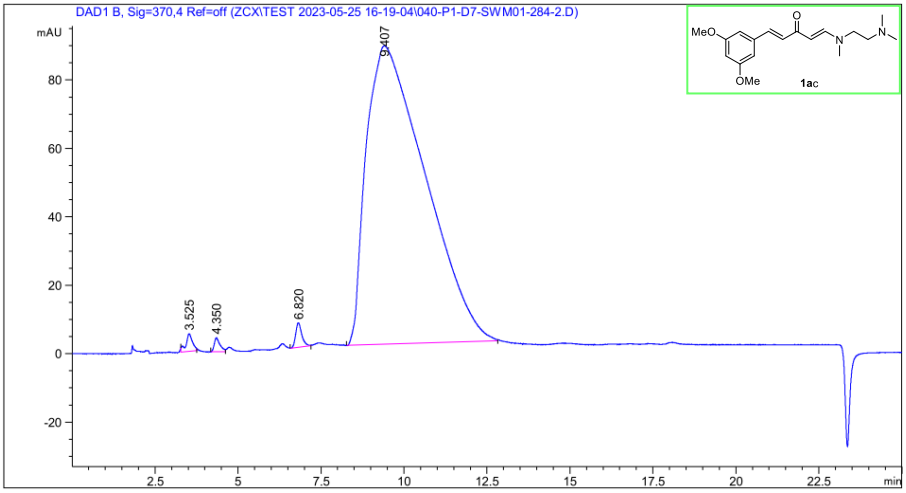
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.174	1.71983e4	895.33038	100.0000
Total		1.71983e4	895.33038	100%



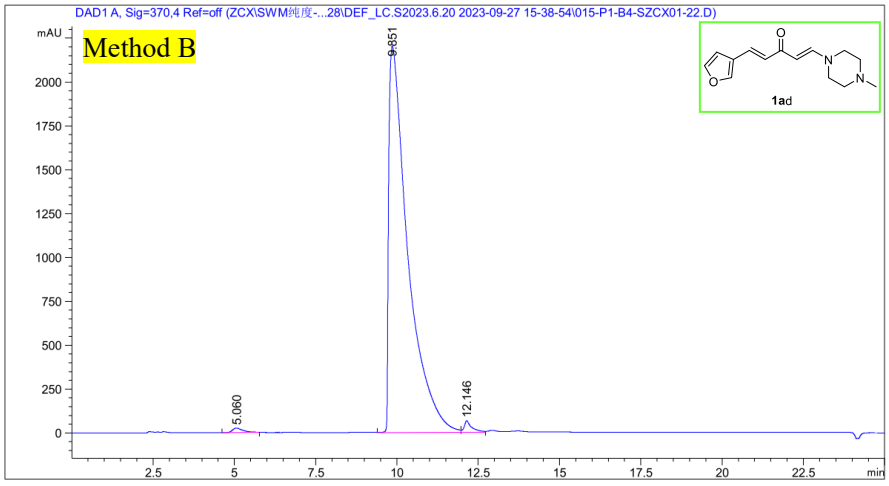
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.192	8.58173e4	2467.00391	99.7307
2	13.594	231.75595	23.57927	0.2693
Total		8.60490e4	2490.58317	100%



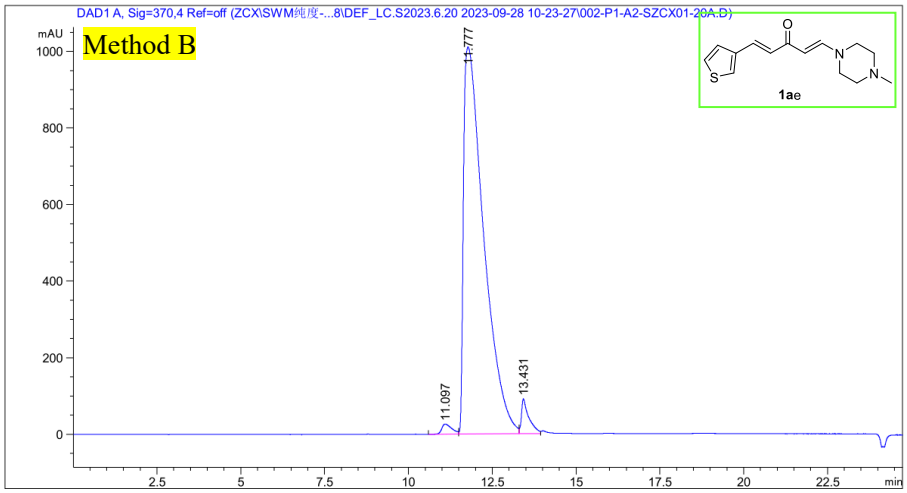
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.525	70.43506	5.16814	0.6409
2	4.350	49.31139	4.09085	0.4487
3	6.820	92.20303	7.16729	0.8390
4	9.407	1.07782e4	87.23535	98.0715
Total		1.09901e4	103.66163	100%



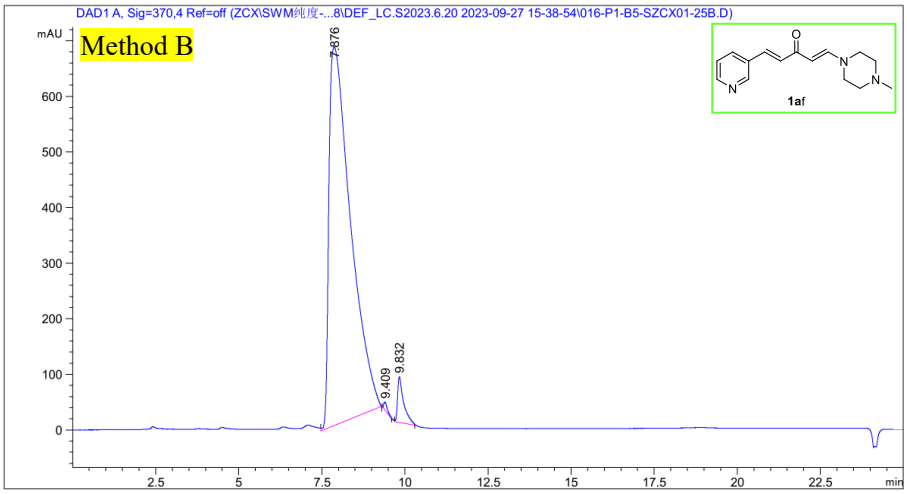
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.060	600.08527	26.09184	0.6584
2	9.851	8.94771e4	2202.93823	98.1703
3	12.146	1067.59460	66.21528	1.1713
Total		9.11448e4	2295.24535	100%



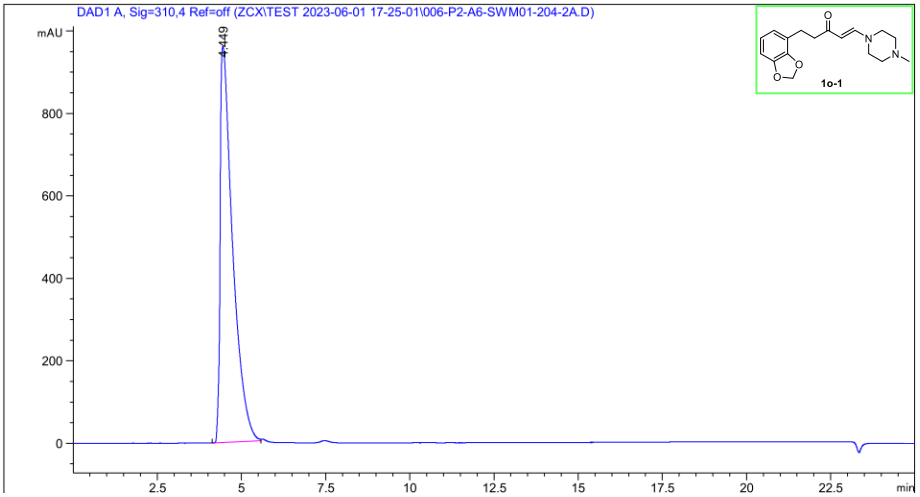
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.097	553.53833	26.41023	1.2524
2	11.777	4.22460e4	1011.09528	95.5810
3	13.431	1399.60962	90.61752	3.1666
Total		4.41992e4	1128.12303	100%



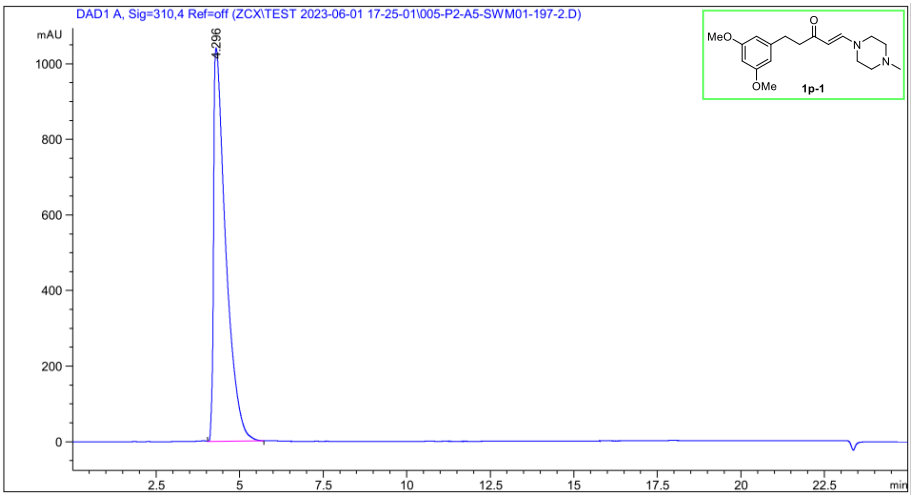
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.876	3.07036e4	681.11041	96.3592
2	9.409	115.73919	14.94840	0.3632
3	9.832	1044.35144	82.30061	3.2776
Total		3.18637e4	778.35943	100%



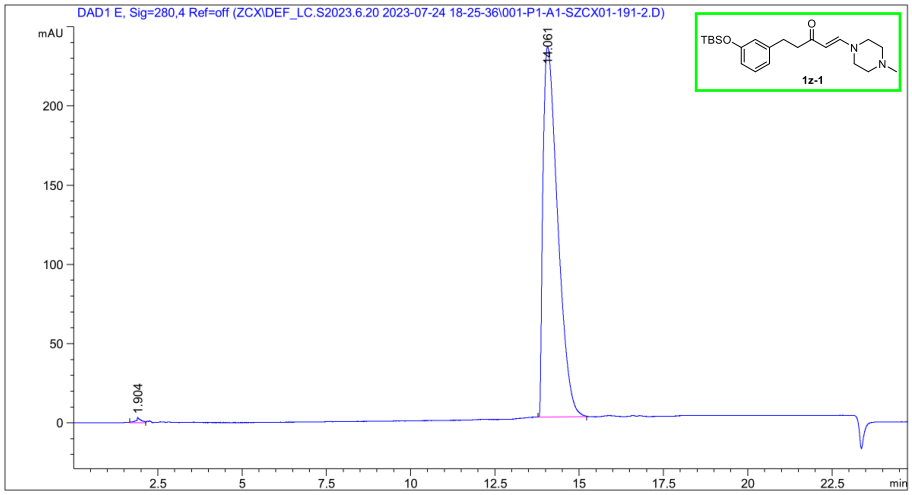
<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.449	2.47348e4	960.67657	100.0000
Total		2.47348e4	960.67657	100%



<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.296	2.64416e4	1040.63379	100.0000
Total		2.64416e4	1040.63379	100%



<Peak Table>

Peak	Ret.time [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.904	35.51863	3.29083	0.4995
2	14.061	7075.33838	233.84167	99.5005
Total		7110.85701	237.13250	100%

6. References

[1] Shao, L.-D.; Su, J.; Ye, B.; Liu, J.-X.; Zuo, Z.-L.; Li, Y.; Wang, Y.-Y.; Xia, C.; Zhao, Q.-S., Design, Synthesis, and Biological Activities of Vibsanin B Derivatives: A New Class of HSP90 C-Terminal Inhibitors. *Journal of Medicinal Chemistry* **2017,** *60* (21), 9053-9066.

[2] Khandelwal, A.; Kent, C. N.; Balch, M.; Peng, S.; Mishra, S. J.; Deng, J.; Day, V. W.; Liu, W.; Subramanian, C.; Cohen, M.; Holzbeierlein, J. M.; Matts, R.; Blagg, B. S. J., Structure-guided design of an Hsp90β N-terminal isoform-selective inhibitor. *Nature Communications* **2018,** *9* (1), 425.