Supporting Information

The synthesis and evaluation of new benzophenone derivatives as tubulin polymerization inhibitors

Shun Zhang, Baijiao An, Jun Yan, Ling Huang, Xingshu Li*

[a]School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, 510006, China

SI1. Biological assay

Cell Culture. The human cancer cell lines (A549, HeLa, A2780, HCT116, MGC803) used in this study were cultivated in DMEM containing 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg /mL streptomycin, respectively. The cells were incubated at 37 ℃ under a 5% CO_2 and 90% relative humidity (RH) atmosphere.

MTT assay. The cells grown in the logarithmic phase were seeded into a 96-well plates (5×10^3 cells/well) for 24 h, then, they were exposed to different concentrations of the test compounds for 48 h. After attached cells were incubated with 5 mg/mL MTT (Sigma, USA) for another 4 h, the suspension was discarded and subsequently the dark blue crystals (formazan) were solubilized in dimethyl sulfoxied (DMSO). The solution was measured using a multifunction microplate reader (Molecular Devices, Flex Station 3) at the absorbance of 570 nm, and each experiment was performed at least in triplicate. IC_{50} values which represent the drug concentrations required to cause 50% cancer cell growth inhibition were used to express the cytotoxic effects of each compound, and were calculated with GraphPad Prism Software version 5.02 (GraphPad Inc., La Jolla, CA, USA).

Antiproliferative activity of compound 10a against human normal cells. The cytotoxic effects of the target compound on normal human cells, were examined by MTT (3-(4,5-dimethyl-2-thia- zolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assay. Briefly, when the cells reached the logarithmic phase, 5 _103 cells/well were harvested and plated into 96-well plates for 24 h, and then, the cells were exposed to different concentrations of the test compounds for 48 h; each experiment was performed in triplicate. Afterward, 20 ml of 5 mg/ml MTT (Sigma, USA) was added, and the cells were incubated for another 4 h. Then, the suspension was discarded and 150 ml of DMSO was added to each well. After the plates were shocked for 10 min to dissolve the dark blue crystals (formazan), the absorbance at 570 nm was measured using a multifunction microplate reader (Molecular Devices, Flex Station 3). The IC_{50} values were calculated with Grap Pad Prism version 5.0.

In vitro tubulin polymerisation assay. The tubulin polymerisation assay was performed by an increase in fluorescence intensity, which can be easily recorded due to the incorporation of a fluorescent reporter, DAPI (4',6-diamidino-2-phenylindole), a fluorophore already known as a DNA intercalator. In our experiment, a commercial kit (cytoskeleton, cat. #BK011P) purchased from Cytoskeleton (Danvers, MA, USA), was used for the tubulin polymerisation. The final buffer concentration used for tubulin polymerisation contained 80.0 mM piperazine-N, N′-bis (2-ethanesulfonic acid) sequisodium salt (pH 6.9), 2.0 mM $MgCl_2$, 0.5 mM EGTA, 1 mM GTP, and 10.2% glycerol. First, 5 μL of the tested compounds at the indicated concentrations was added and the mixture was warmed to 37 °C for 1 minute, then, the reaction was initiated by the addition of 55 μL of the tubulin solution. The fluorescence intensity enhancement was recorded every 60sec for 90 min in a multifunction microplate reader (Molecular Devices, Flex Station 3) (emission wavelength is 410 nm, excitation wavelength is 340 nm). The area under the curve was used to determine the concentration that inhibited tubulin polymerisation by 50% (IC50), and was calculated with GraphPad Prism Software version 5.02 (GraphPad Inc., La Jolla, CA, USA).

Immunofluorescence microscopy. In a 10 mm confocal culture dish, 3X104 cells were grown for 24 h, and then incubated in the presence / absence of compound 10a at the indicated concentrations for another 12 h. After washed with phosphate buffer solution (PBS) and fixed in 4% pre-warmed (37 °C) paraformaldehyde for 15 min, the cells were permeabilized with 0.5% Triton X-100 for 15 min and blocked for 30 min in 10% goat serum. Then, the cells were incubated with mouse anti-tubulin antibody (CST, USA) at 4 °C overnight, and were washed with PBS for three times and incubated with goat anti-mouse IgG/Alexa-Fluor 488 antibody (Invitrogen, USA) for 1 h. The samples were immediately visualized on a Zeiss LSM 570 laser scanning confocal microscope (Carl Zeiss, Germany) after the nuclei were stained with Hoechst 33342 (Sigma, USA) in the dark at room temperature for 30 min.

Cell cycle analysis. A549 cell were seeded in 6-well plates (3X105 cells/well) and incubated in the presence / absence of compound **10a** at the indicated concentrations for 24 h and then harvested by centrifugation, fixed in ice-cold 70% ethanol overnight. After the ethanol was removed in the next day, the cells were resuspended in the ice-cold PBS and treated with RNAse A (Keygen Biotech, China) at 37 °C for 30 min, followed by incubated with the DNA staining solution propidium iodide (PI, Keygen

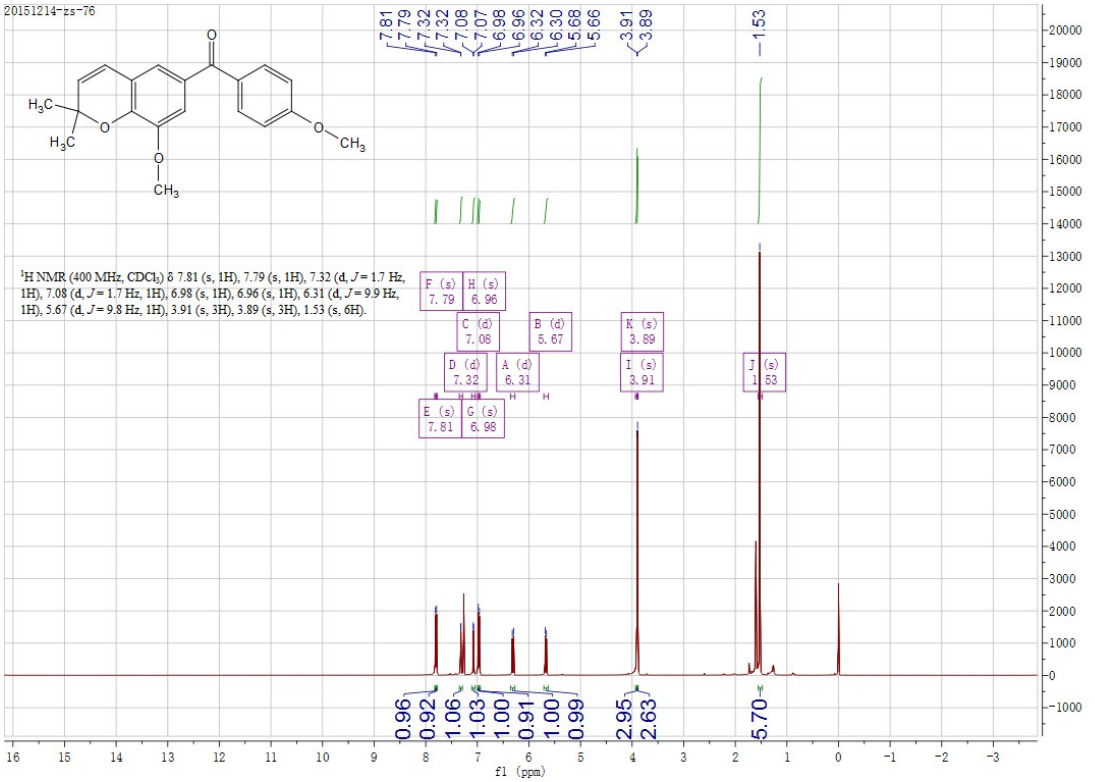
Biotech, China) at 4 °C for 30 min. About 10,000 events were detected by flow cytometry (Beckman Coulter, Epics XL) at 488 nm. The data regarding the number of cells in different phases of the cell cycle were analysed by EXPO32 ADC analysis software.

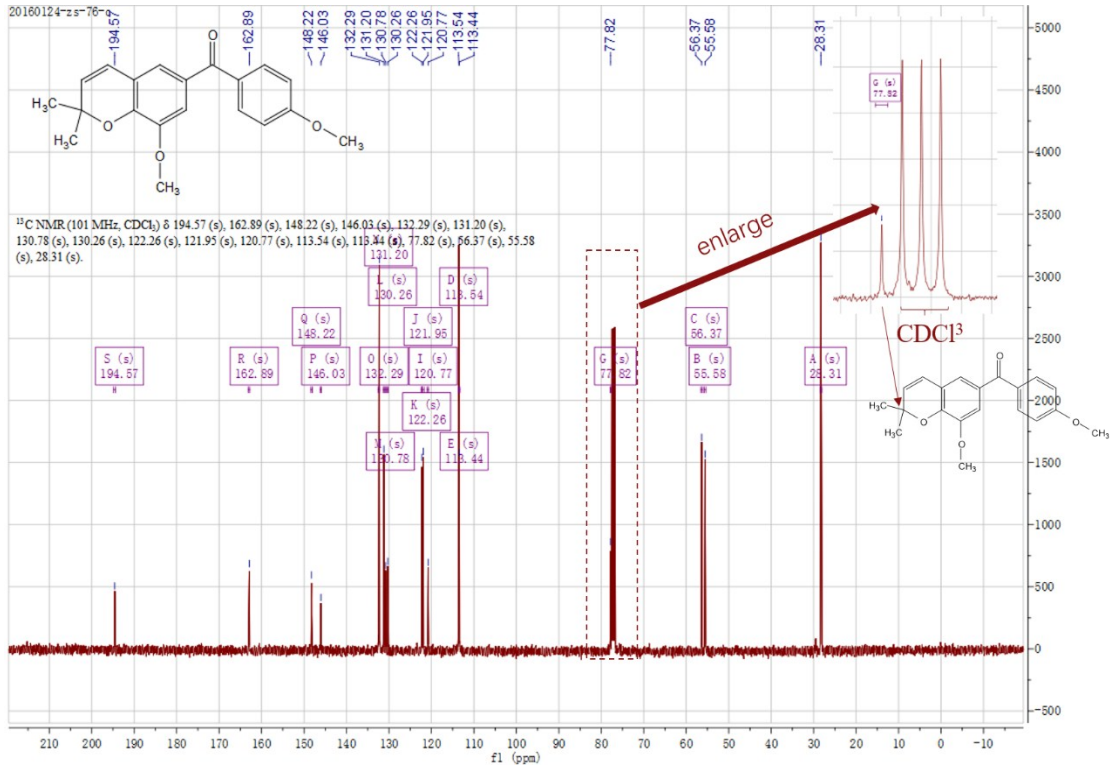
Apoptosis analysis. The preparation of A549 cell sample was the same as cell cycle analysis. After incubation, cells were harvested and incubated with 5 μL of Annexin-V/FITC (Keygen Biotech, China) in binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$ at pH 7.4) at room temperature for 15 min. PI solution was then added to the medium for another 10 min-incubation. Almost 10,000 events were collected for each sample and analysed by flow cytometry (Beckman Coulter, Epics XL). The percentage of apoptotic cells was calculated with EXPO32 ADC Analysis software.

Mitochondrial membrane potential assay. A lipophilic cationic dye, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazolcarbocyanine (JC-1, Beyotime, China) was used to monitor the level of MMP in the cells. At normal state, the MMP is high and JC-1 appears as aggregates, which indicated by red fluorescence. However, when apoptosis occurs, the MMP reduced and JC-1 displayed as monomers, which indicated by green fluorescence. We applied two methods which including flow cytometry and fluorescence microscopy to detected the MMP. For flow cytometry analysis, A549 cells were plated in 6-well plates (3X105 cells/well) and grown for 24 h, and treated with compound **10a** at the indicated concentrations for 48 h. Then the cells were harvested by centrifugation and incubated with JC-1 solution for 30 min. After briefly washing, the proportion of green and red fluorescence intensity were immediately detected and analysed by flow cytometry. For the fluorescence microscopy detection, A549 cells were plated in 6-well plates (3×105 cells/well) and grown for 24 h, and treated with compound **10a** at the indicated concentrations for another 48 h. Then the cells were stained with 2 μM JC-1 at 37 °C for 30 min, washed with PBS and then the cell nuclei were stained with Hoechst 33342 (Sigma, USA) for 10 min in the dark. The cell images were immediate detected by a fluorescence microscopy (EVOS FL Auto).

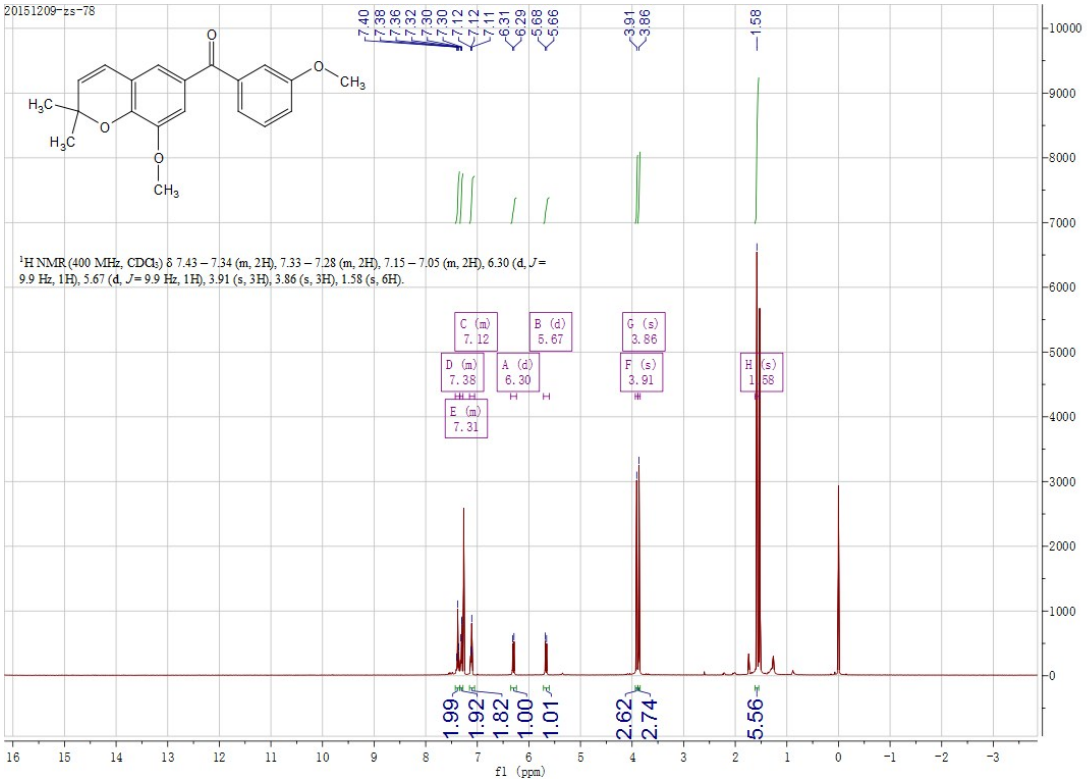
SI2. NMR spectrums of target compounds

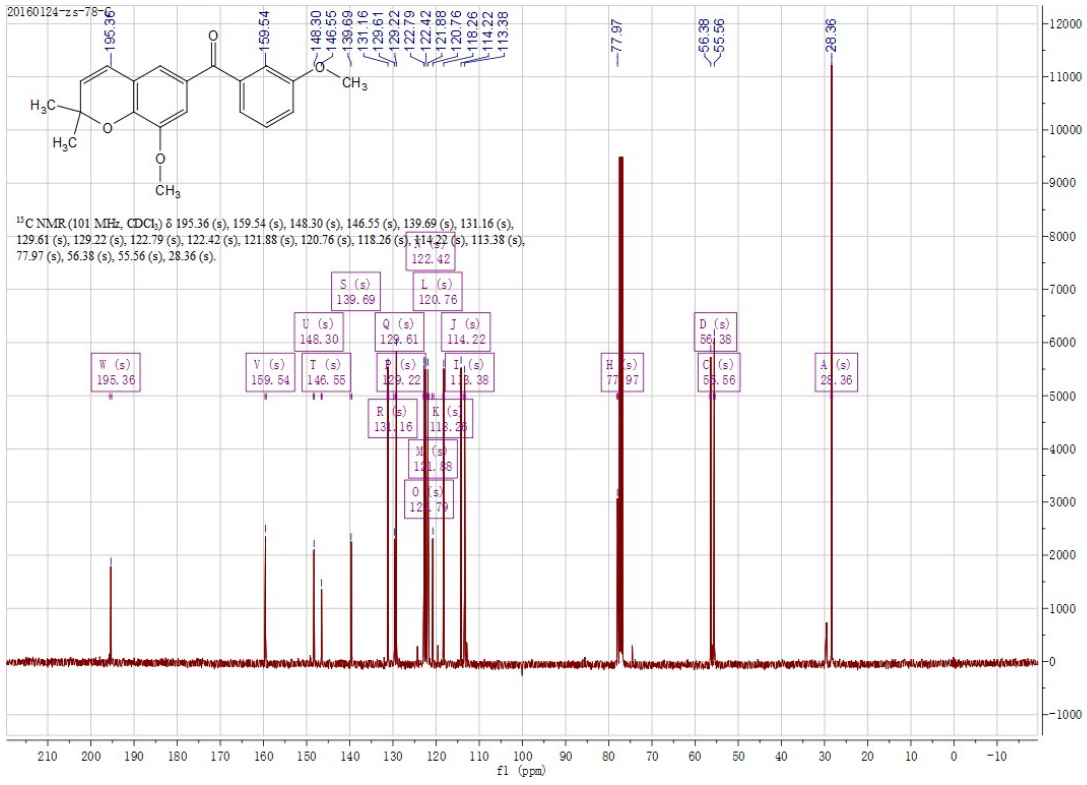
5a



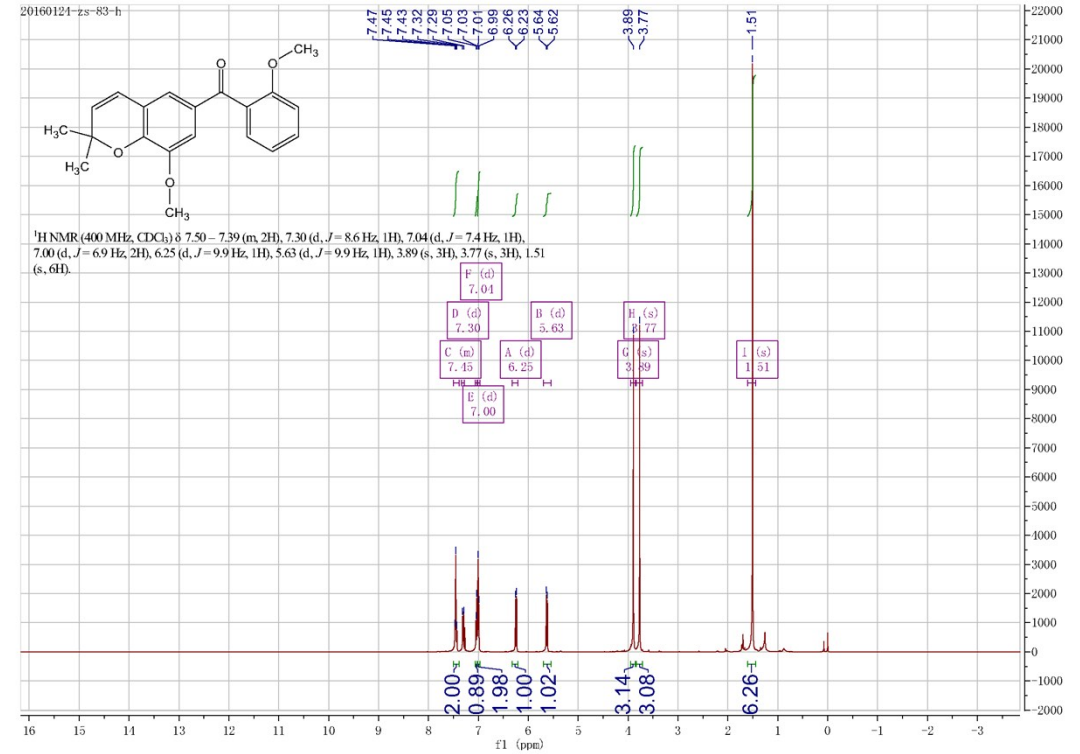


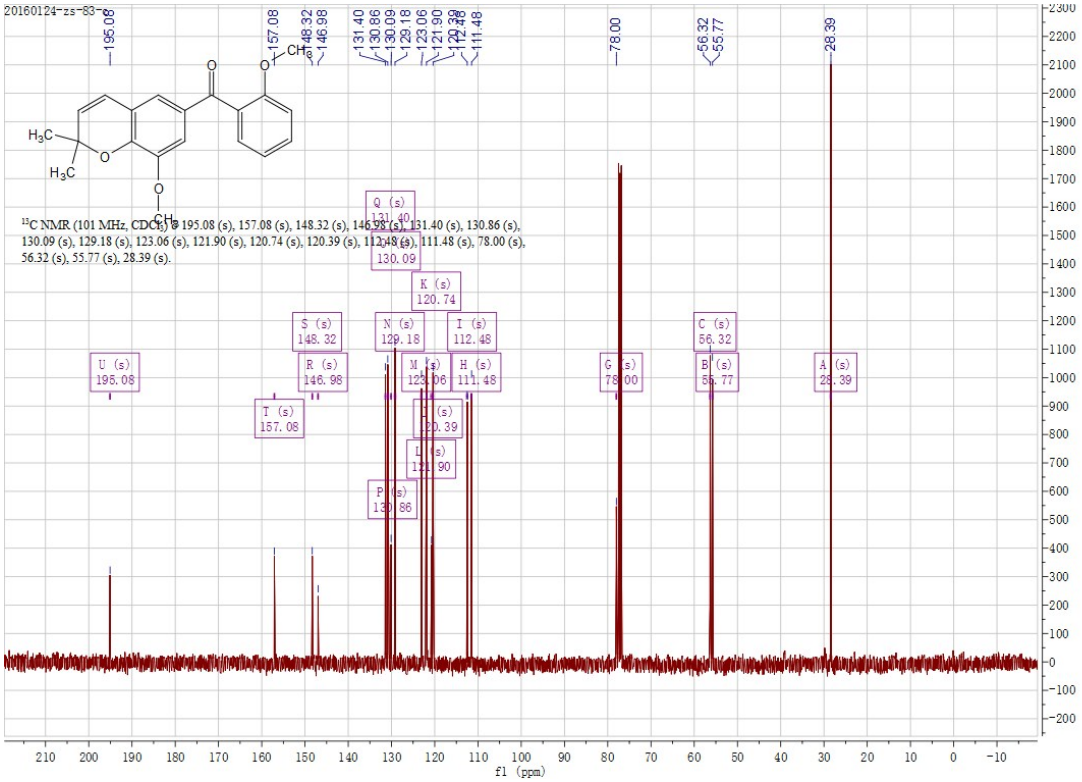
5b


20151209-zs-78
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.43 – 7.34 (m, 2H), 7.33 – 7.28 (m, 2H), 7.15 – 7.05 (m, 2H), 6.30 (d, J = 9.9 Hz, 1H), 5.67 (d, J = 9.9 Hz, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 1.58 (s, 6H).
f1 (ppm)

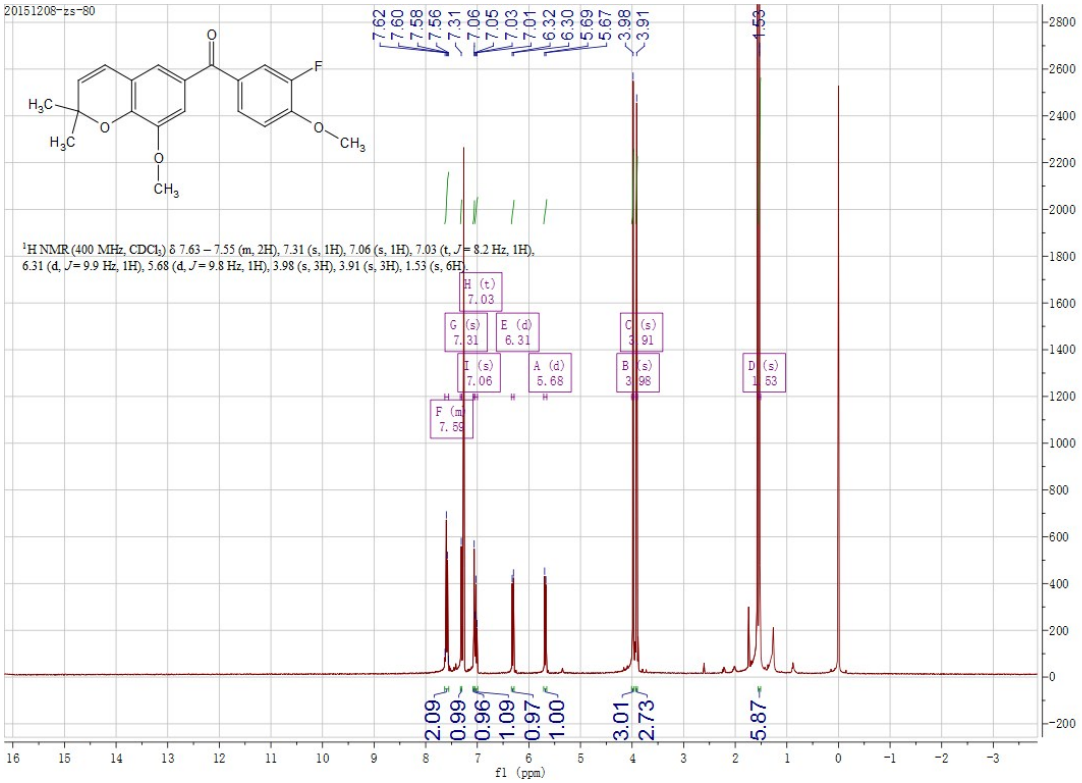

20160124-zs-78-c
^{13}C NMR (101 MHz, CDCl$_3$) δ 195.36 (s), 159.54 (s), 148.30 (s), 146.55 (s), 139.69 (s), 131.16 (s), 129.61 (s), 129.22 (s), 122.79 (s), 122.42 (s), 121.88 (s), 120.76 (s), 118.26 (s), 114.22 (s), 113.38 (s), 77.97 (s), 56.38 (s), 55.56 (s), 28.36 (s).
f1 (ppm)

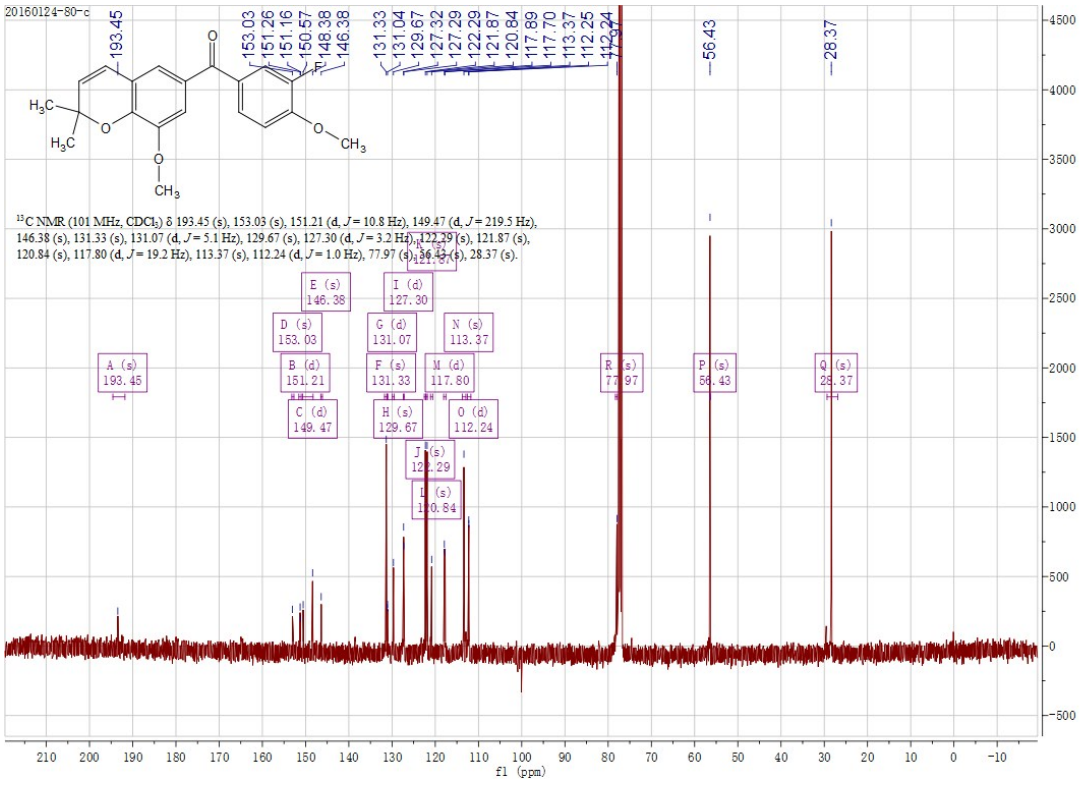
5c


20160124-zs-83-h
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.50 – 7.39 (m, 2H), 7.30 (d, J = 8.6 Hz, 1H), 7.04 (d, J = 7.4 Hz, 1H), 7.00 (d, J = 6.9 Hz, 2H), 6.25 (d, J = 9.9 Hz, 1H), 5.63 (d, J = 9.9 Hz, 1H), 3.89 (s, 3H), 3.77 (s, 3H), 1.51 (s, 6H).
f1 (ppm)

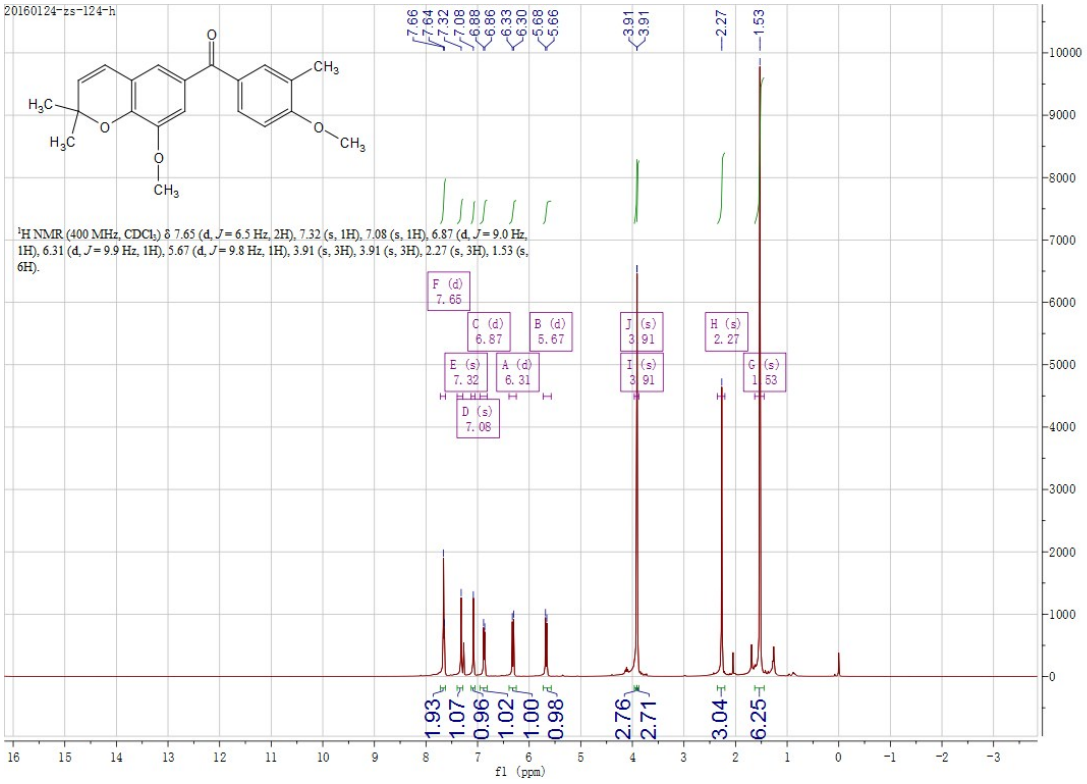

20160124-zs-83-c
^{13}C NMR (101 MHz, CDCl$_3$) δ 195.08 (s), 157.08 (s), 148.32 (s), 146.98 (s), 131.40 (s), 130.86 (s), 130.09 (s), 129.18 (s), 123.06 (s), 121.90 (s), 120.74 (s), 120.39 (s), 112.48 (s), 111.48 (s), 78.00 (s), 56.32 (s), 55.77 (s), 28.39 (s).
f1 (ppm)

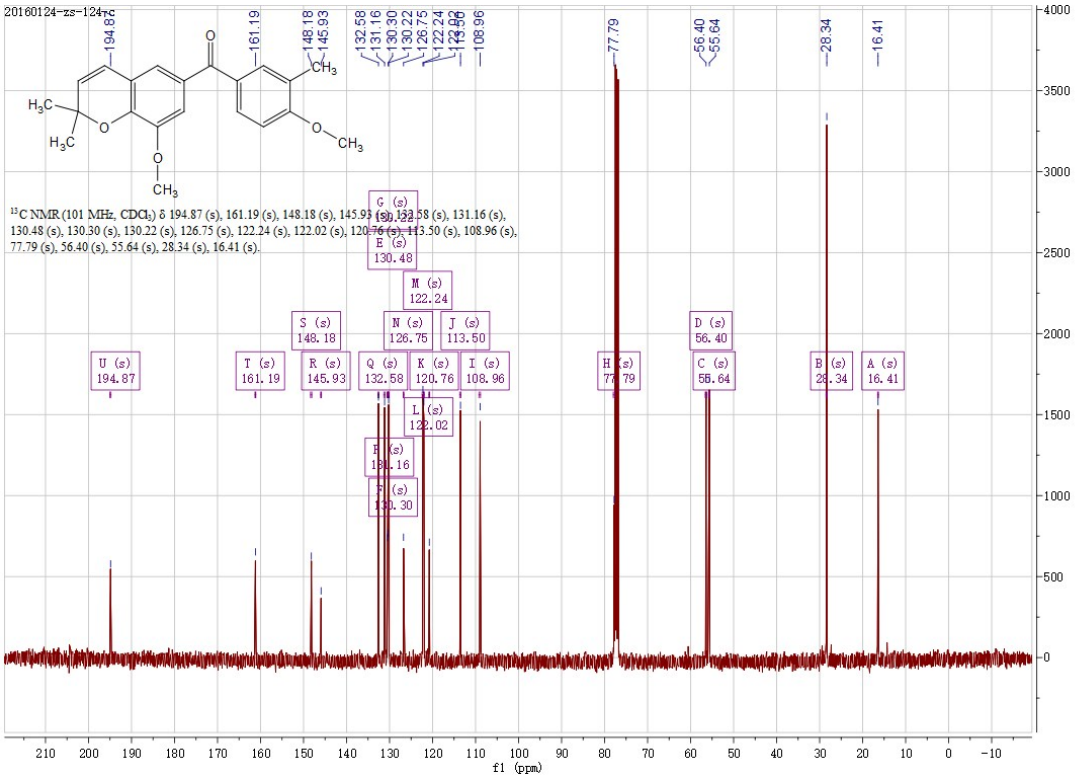
5d


20151208-zs-80
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.63 – 7.55 (m, 2H), 7.31 (s, 1H), 7.06 (s, 1H), 7.03 (t, J = 8.2 Hz, 1H), 6.31 (d, J = 9.9 Hz, 1H), 5.68 (d, J = 9.8 Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 1.53 (s, 6H).
7.62
7.60
7.58
7.56
7.31
7.06
7.05
7.03
7.01
6.32
6.30
5.69
5.67
3.98
3.91
1.53
2.09
0.99
0.96
1.09
0.97
1.00
3.01
2.73
5.87
f1 (ppm)

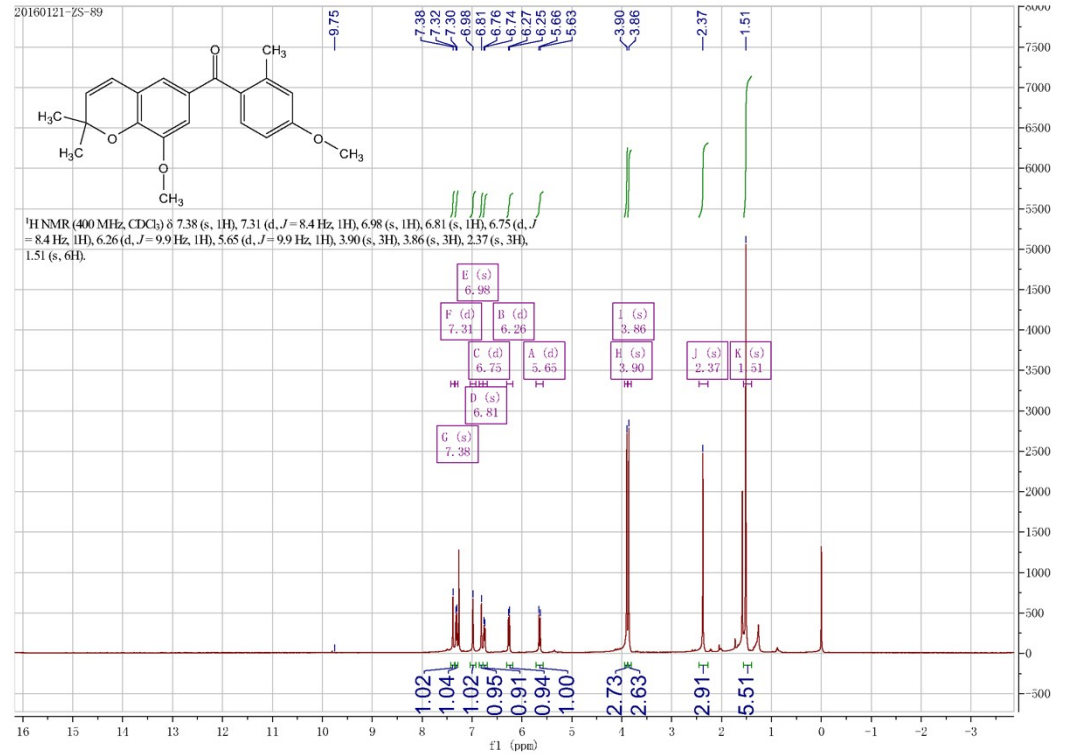

20160124-80-c
^{13}C NMR (101 MHz, CDCl$_3$) δ 193.45 (s), 153.03 (s), 151.21 (d, J = 10.8 Hz), 149.47 (d, J = 219.5 Hz), 146.38 (s), 131.33 (s), 131.07 (d, J = 5.1 Hz), 129.67 (s), 127.30 (d, J = 3.2 Hz), 122.29 (s), 121.87 (s), 120.84 (s), 117.80 (d, J = 19.2 Hz), 113.37 (s), 112.24 (d, J = 1.0 Hz), 77.97 (s), 56.43 (s), 28.37 (s).
193.45
153.03
151.26
151.16
150.57
148.38
146.38
131.33
131.04
129.67
127.32
127.29
122.29
121.87
120.84
117.89
117.70
113.37
112.25
112.24
77.97
56.43
28.37
f1 (ppm)

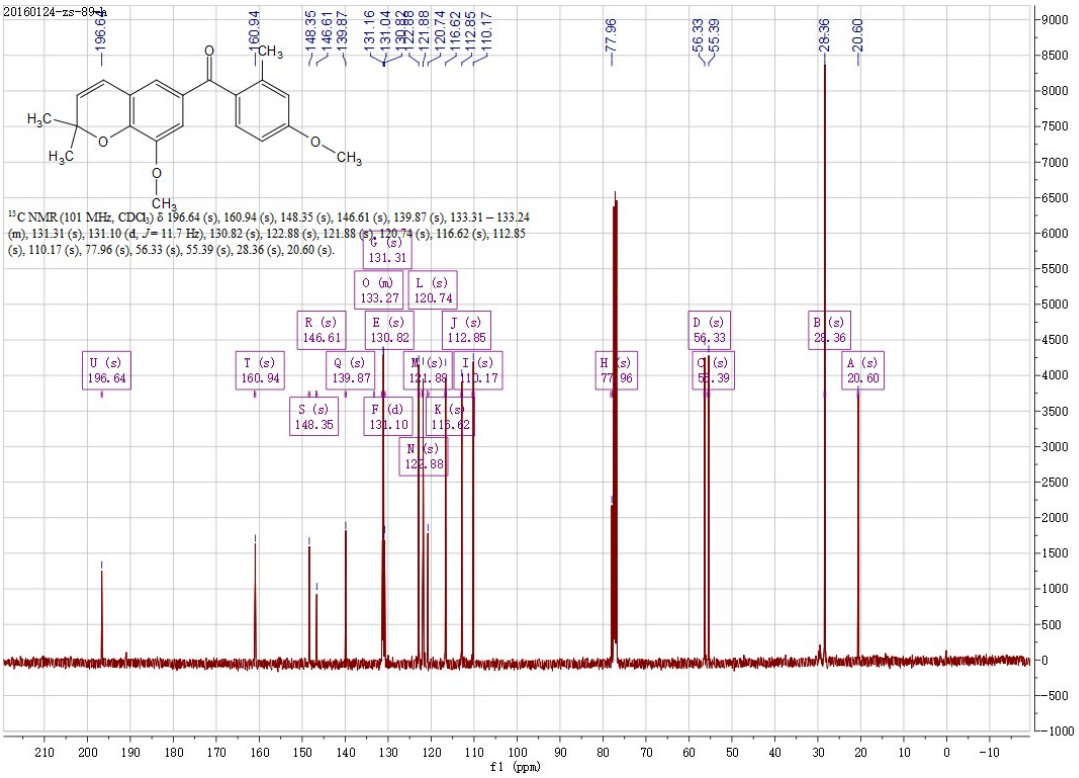
5e


20160124-zs-124-h
7.66
7.64
7.32
7.08
6.88
6.86
6.33
6.30
5.68
5.66
3.91
3.91
2.27
1.53
F (d) 7.65
C (d) 6.87
B (d) 5.67
J (s) 3.91
H (s) 2.27
E (s) 7.32
A (d) 6.31
I (s) 3.91
G (s) 1.53
D (s) 7.08
1.93
1.07
0.96
1.02
1.00
0.98
2.76
2.71
3.04
6.25
f1 (ppm)

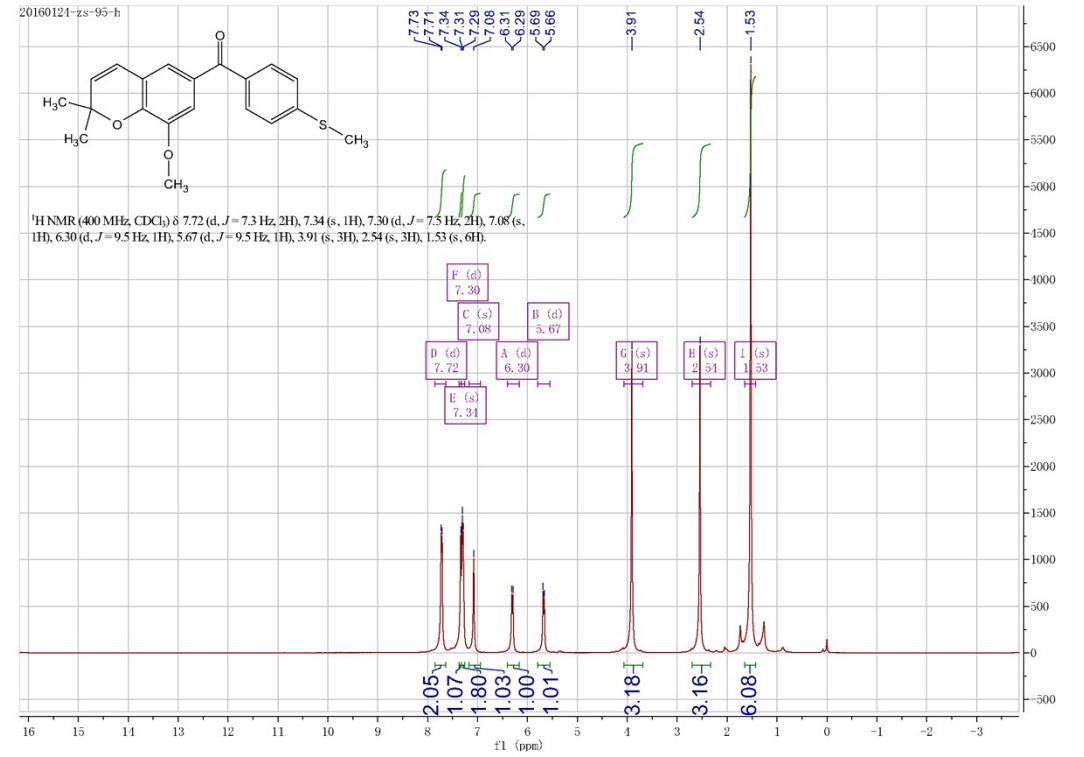

20160124-zs-124-c
194.87
161.19
148.18
145.93
132.58
131.16
130.30
130.22
126.75
122.24
122.02
120.76
113.50
108.96
77.79
56.40
55.64
28.34
16.41
G (s) 130.22
E (s) 130.48
M (s) 122.24
S (s) 148.18
N (s) 126.75
J (s) 113.50
D (s) 56.40
U (s) 194.87
T (s) 161.19
R (s) 145.93
Q (s) 132.58
K (s) 120.76
I (s) 108.96
H (s) 77.79
C (s) 55.64
B (s) 28.34
A (s) 16.41
L (s) 122.02
P (s) 131.16
O (s) 130.30
f1 (ppm)

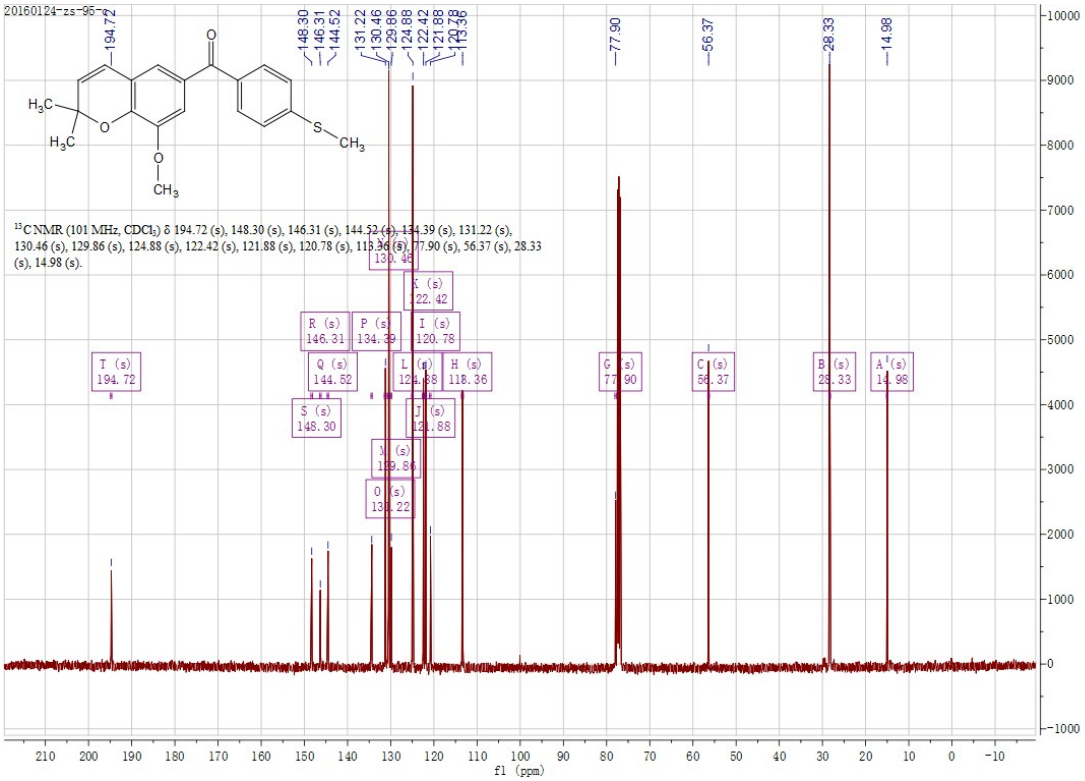
5f


20160121-ZS-89
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38 (s, 1H), 7.31 (d, J = 8.4 Hz, 1H), 6.98 (s, 1H), 6.81 (s, 1H), 6.75 (d, J = 8.4 Hz, 1H), 6.26 (d, J = 9.9 Hz, 1H), 5.65 (d, J = 9.9 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 2.37 (s, 3H), 1.51 (s, 6H).
f1 (ppm)

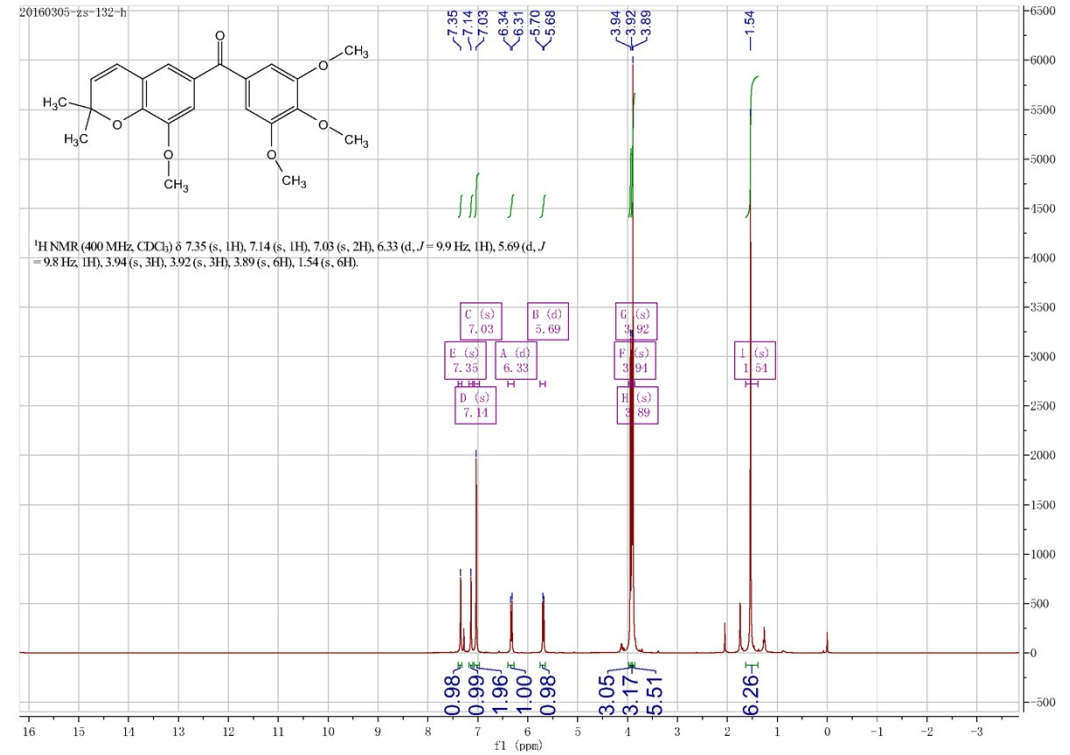

20160124-zs-89-h
^{13}C NMR (101 MHz, CDCl$_3$) δ 196.64 (s), 160.94 (s), 148.35 (s), 146.61 (s), 139.87 (s), 133.31 – 133.24 (m), 131.31 (s), 131.10 (d, J = 11.7 Hz), 130.82 (s), 122.88 (s), 121.88 (s), 120.74 (s), 116.62 (s), 112.85 (s), 110.17 (s), 77.96 (s), 56.33 (s), 55.39 (s), 28.36 (s), 20.60 (s).
f1 (ppm)

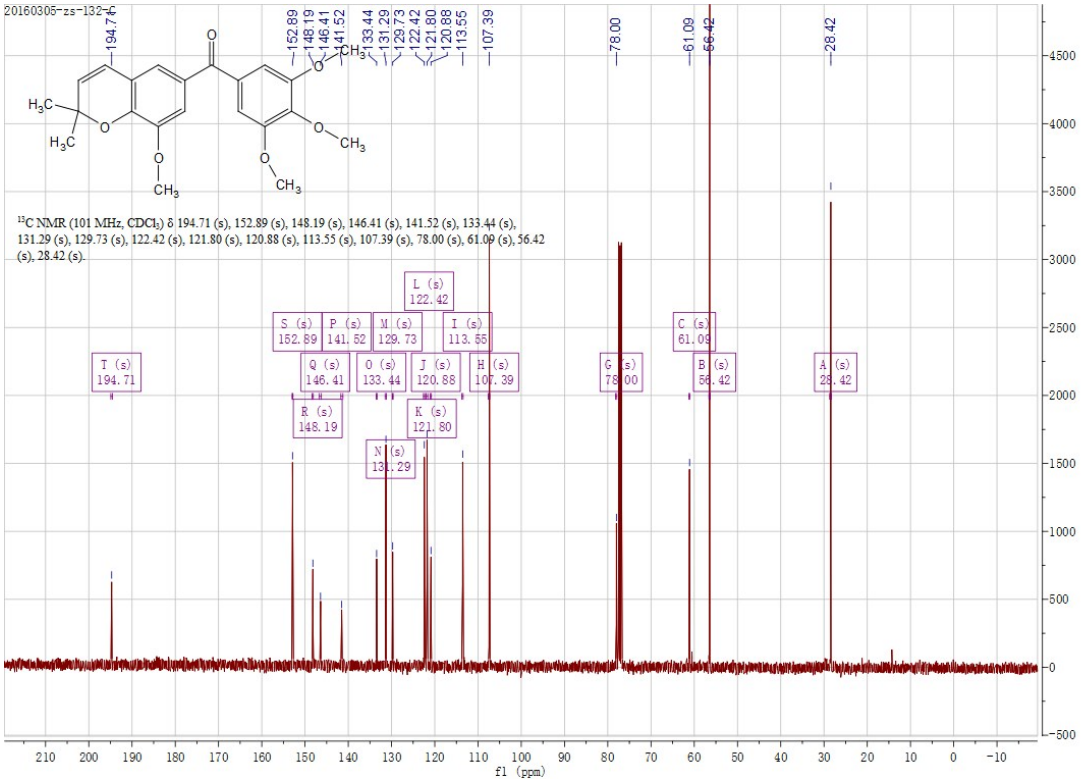
5g


20160121-zs-95-h
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.72 (d, J = 7.3 Hz, 2H), 7.34 (s, 1H), 7.30 (d, J = 7.5 Hz, 2H), 7.08 (s, 1H), 6.30 (d, J = 9.5 Hz, 1H), 5.67 (d, J = 9.5 Hz, 1H), 3.91 (s, 3H), 2.54 (s, 3H), 1.53 (s, 6H).
f1 (ppm)

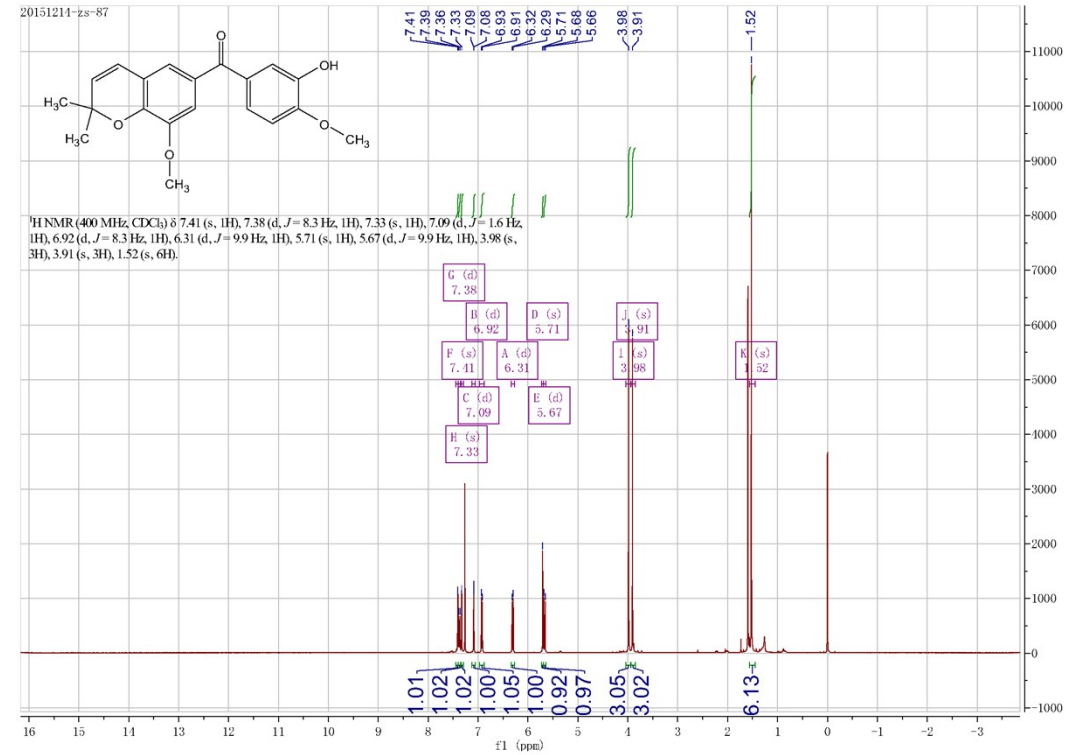

20160124-zs-95-c
^{13}C NMR (101 MHz, CDCl$_3$) δ 194.72 (s), 148.30 (s), 146.31 (s), 144.52 (s), 134.39 (s), 131.22 (s), 130.46 (s), 129.86 (s), 124.88 (s), 122.42 (s), 121.88 (s), 120.78 (s), 118.36 (s), 77.90 (s), 56.37 (s), 28.33 (s), 14.98 (s).
f1 (ppm)

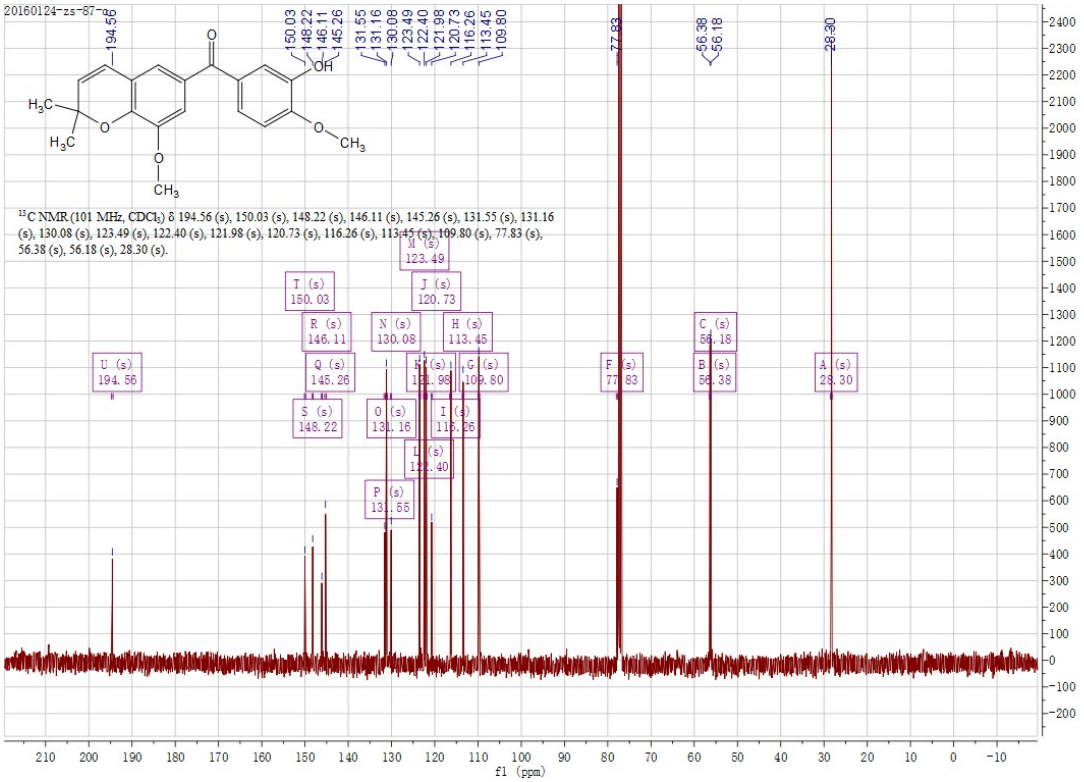
5h


20160305-zs-132-h
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.35 (s, 1H), 7.14 (s, 1H), 7.03 (s, 2H), 6.33 (d, J = 9.9 Hz, 1H), 5.69 (d, J = 9.8 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.89 (s, 6H), 1.54 (s, 6H).
f1 (ppm)

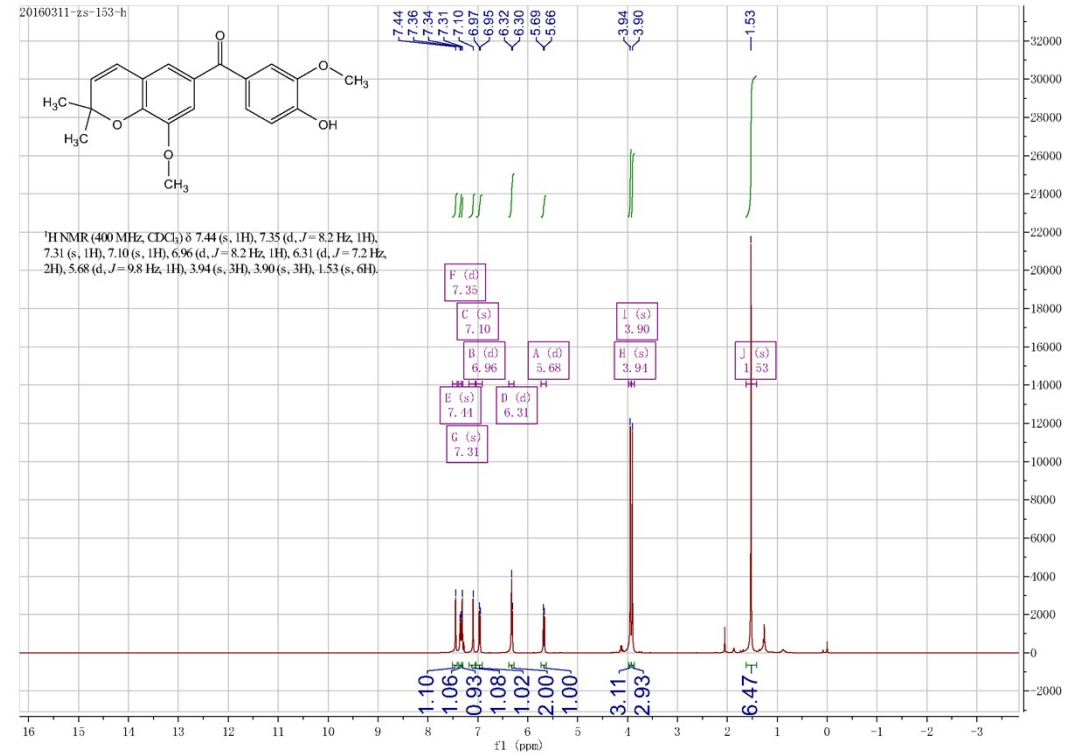

20160305-zs-132-C
^{13}C NMR (101 MHz, CDCl$_3$) δ 194.71 (s), 152.89 (s), 148.19 (s), 146.41 (s), 141.52 (s), 133.44 (s), 131.29 (s), 129.73 (s), 122.42 (s), 121.80 (s), 120.88 (s), 113.55 (s), 107.39 (s), 78.00 (s), 61.09 (s), 56.42 (s), 28.42 (s).
f1 (ppm)

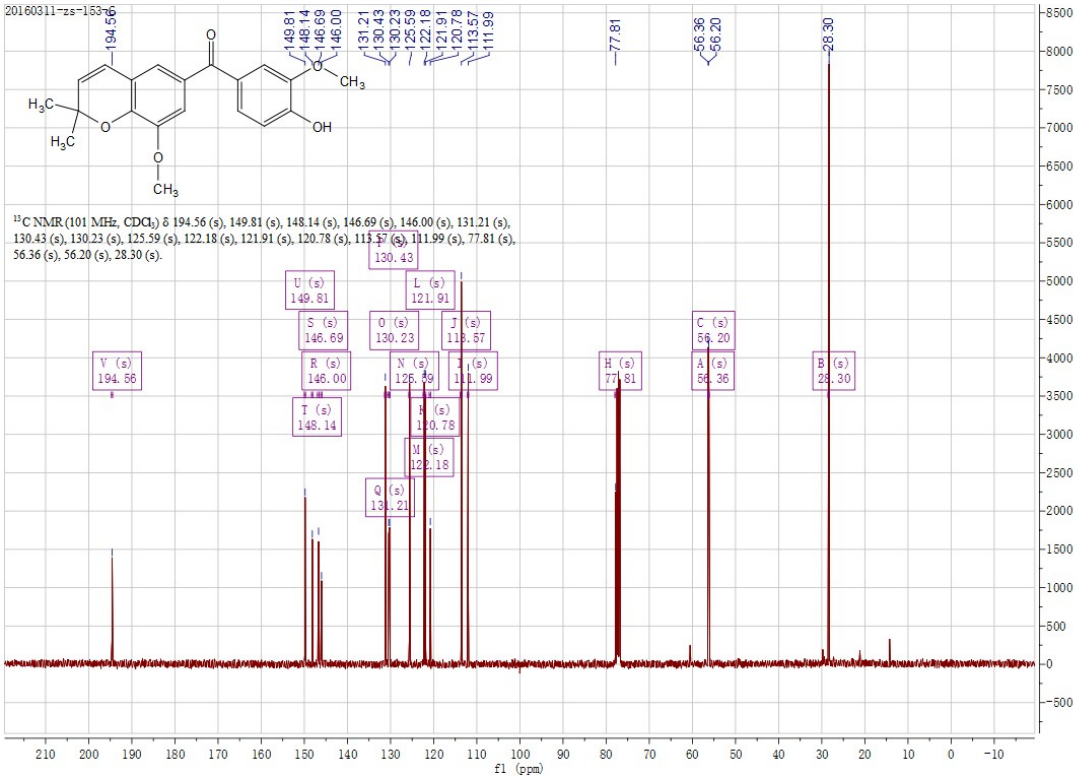
10a


20151214-zs-87
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.41 (s, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.33 (s, 1H), 7.09 (d, J = 1.6 Hz, 1H), 6.92 (d, J = 8.3 Hz, 1H), 6.31 (d, J = 9.9 Hz, 1H), 5.71 (s, 1H), 5.67 (d, J = 9.9 Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 1.52 (s, 6H).
f1 (ppm)

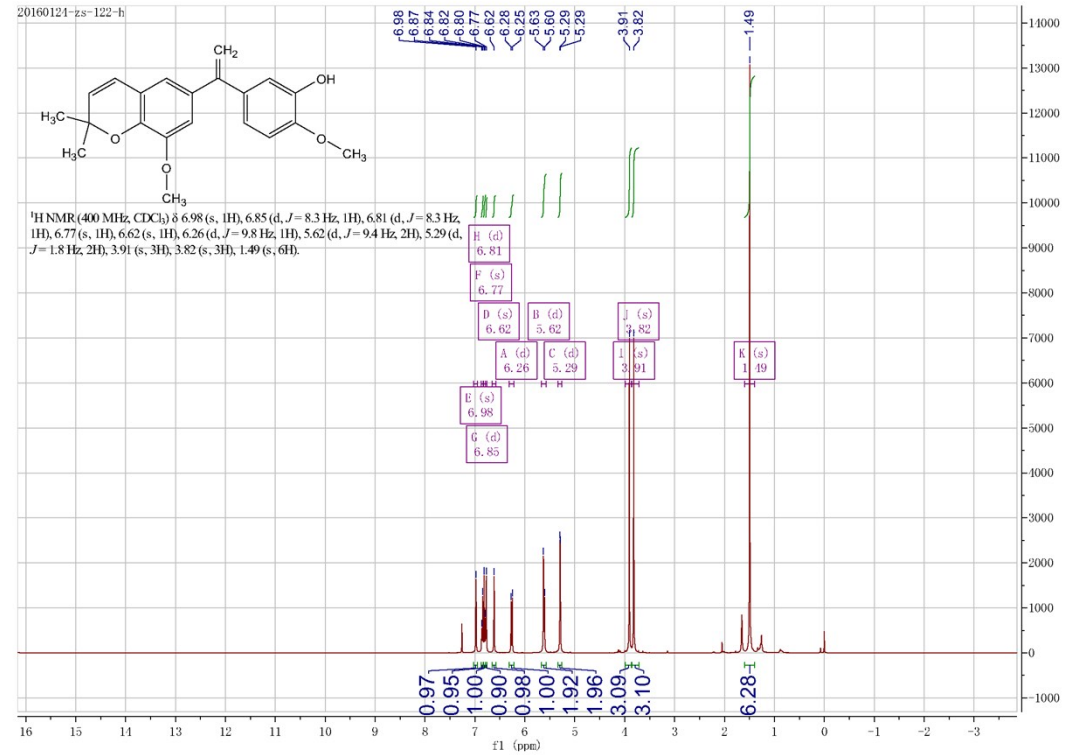

20160124-zs-87
^{13}C NMR (101 MHz, CDCl$_3$) δ 194.56 (s), 150.03 (s), 148.22 (s), 146.11 (s), 145.26 (s), 131.55 (s), 131.16 (s), 130.08 (s), 123.49 (s), 122.40 (s), 121.98 (s), 120.73 (s), 116.26 (s), 113.45 (s), 109.80 (s), 77.83 (s), 56.38 (s), 56.18 (s), 28.30 (s).
f1 (ppm)

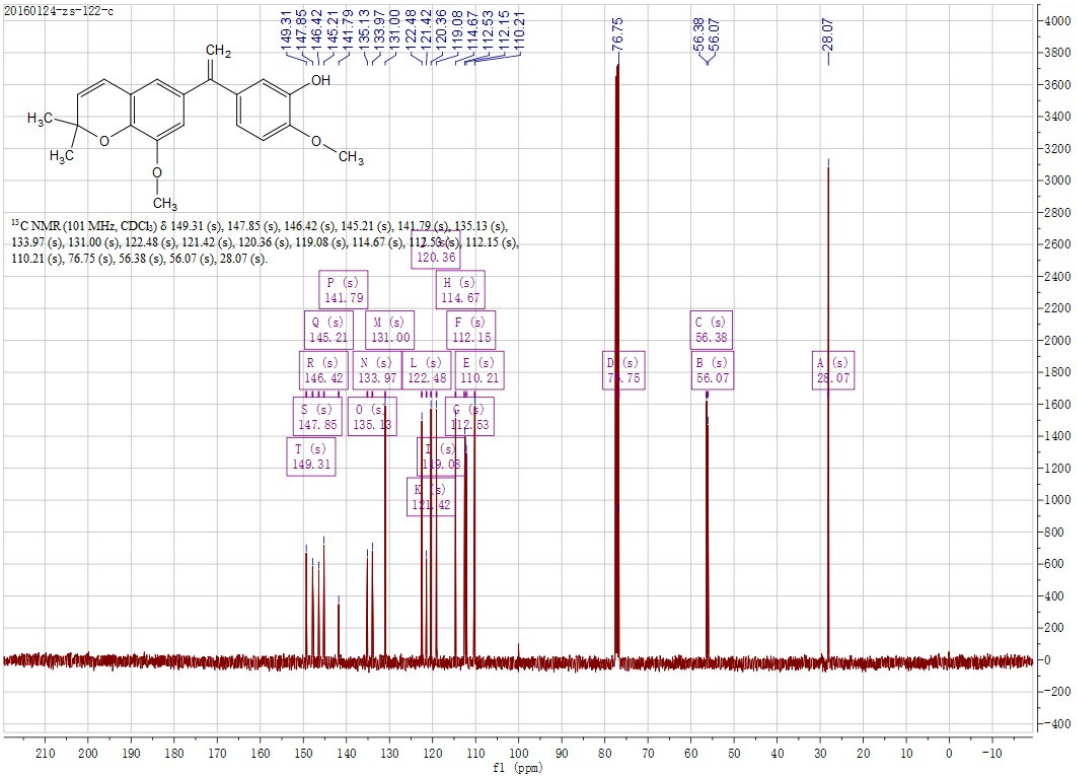
10b


20160311-zs-153-h
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.31 (s, 1H), 7.10 (s, 1H), 6.96 (d, J = 8.2 Hz, 1H), 6.31 (d, J = 7.2 Hz, 2H), 5.68 (d, J = 9.8 Hz, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 1.53 (s, 6H).
f1 (ppm)

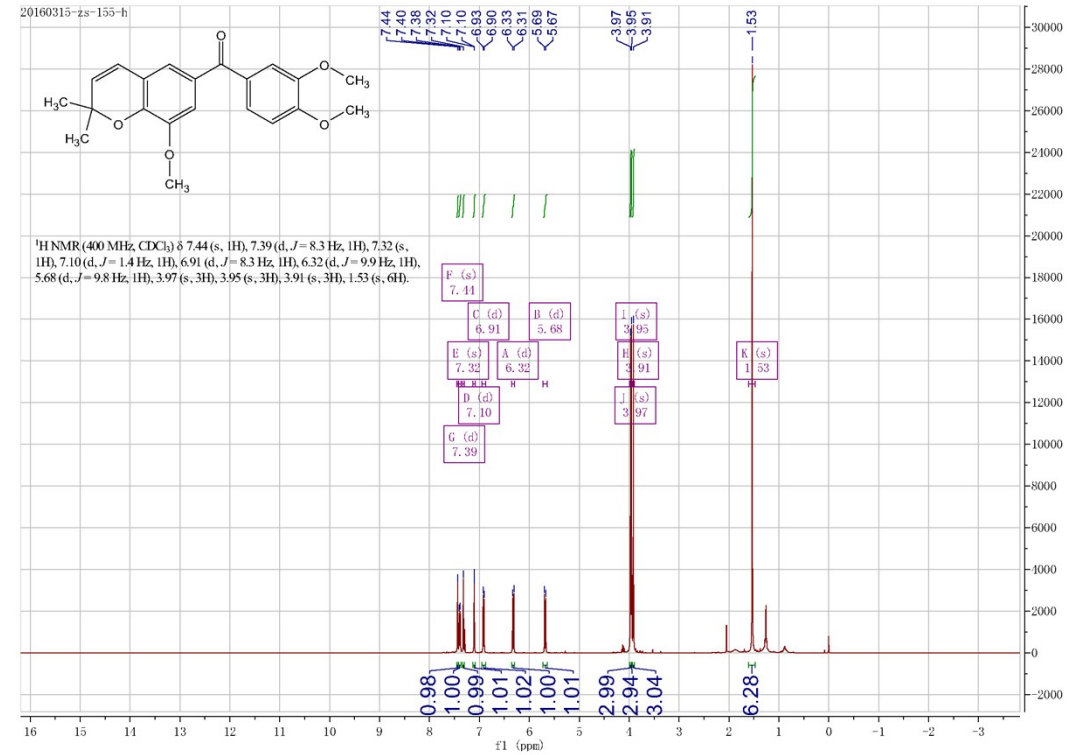

20160311-zs-153-c
^{13}C NMR (101 MHz, $CDCl_3$) δ 194.56 (s), 149.81 (s), 148.14 (s), 146.69 (s), 146.00 (s), 131.21 (s), 130.43 (s), 130.23 (s), 125.59 (s), 122.18 (s), 121.91 (s), 120.78 (s), 113.57 (s), 111.99 (s), 77.81 (s), 56.36 (s), 56.20 (s), 28.30 (s).
f1 (ppm)

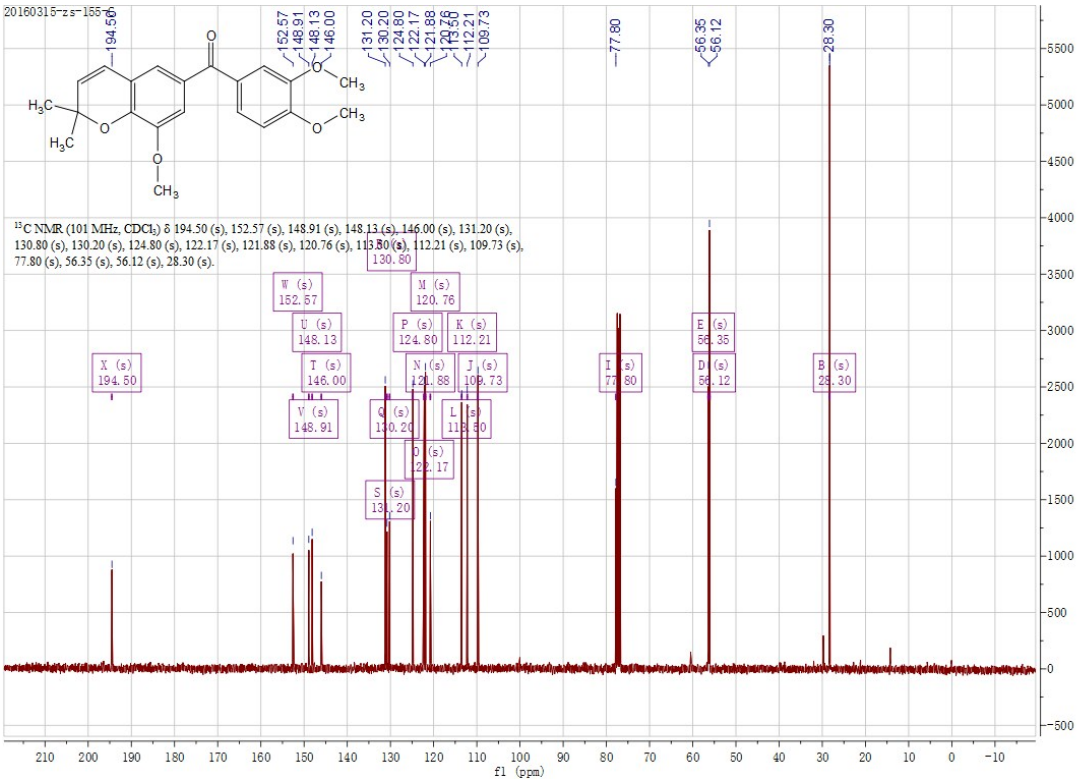
11


20160124-zs-122-h
¹H NMR (400 MHz, CDCl₃) δ 6.98 (s, 1H), 6.85 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.77 (s, 1H), 6.62 (s, 1H), 6.26 (d, J = 9.8 Hz, 1H), 5.62 (d, J = 9.4 Hz, 2H), 5.29 (d, J = 1.8 Hz, 2H), 3.91 (s, 3H), 3.82 (s, 3H), 1.49 (s, 6H).
f1 (ppm)


20160124-zs-122-c
¹³C NMR (101 MHz, CDCl₃) δ 149.31 (s), 147.85 (s), 146.42 (s), 145.21 (s), 141.79 (s), 135.13 (s), 133.97 (s), 131.00 (s), 122.48 (s), 121.42 (s), 120.36 (s), 119.08 (s), 114.67 (s), 112.53 (s), 112.15 (s), 110.21 (s), 76.75 (s), 56.38 (s), 56.07 (s), 28.07 (s).
f1 (ppm)

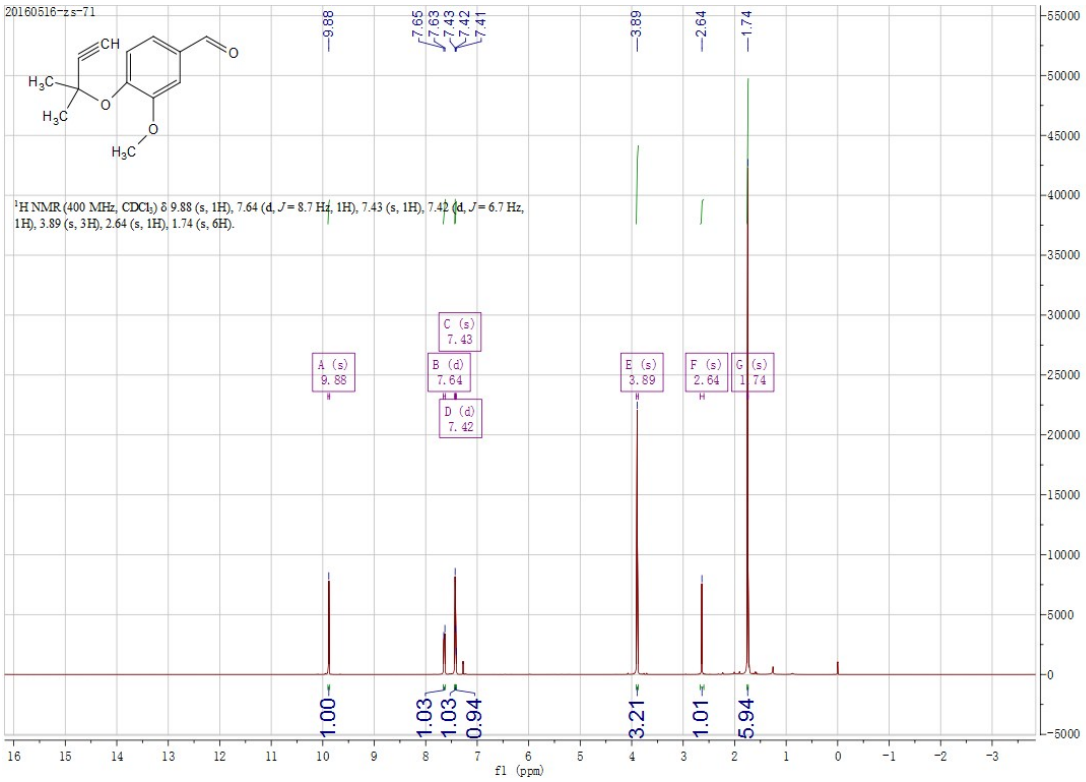
12


20160315-zs-155-h
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.39 (d, J = 8.3 Hz, 1H), 7.32 (s, 1H), 7.10 (d, J = 1.4 Hz, 1H), 6.91 (d, J = 8.3 Hz, 1H), 6.32 (d, J = 9.9 Hz, 1H), 5.68 (d, J = 9.8 Hz, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 3.91 (s, 3H), 1.53 (s, 6H).
f1 (ppm)

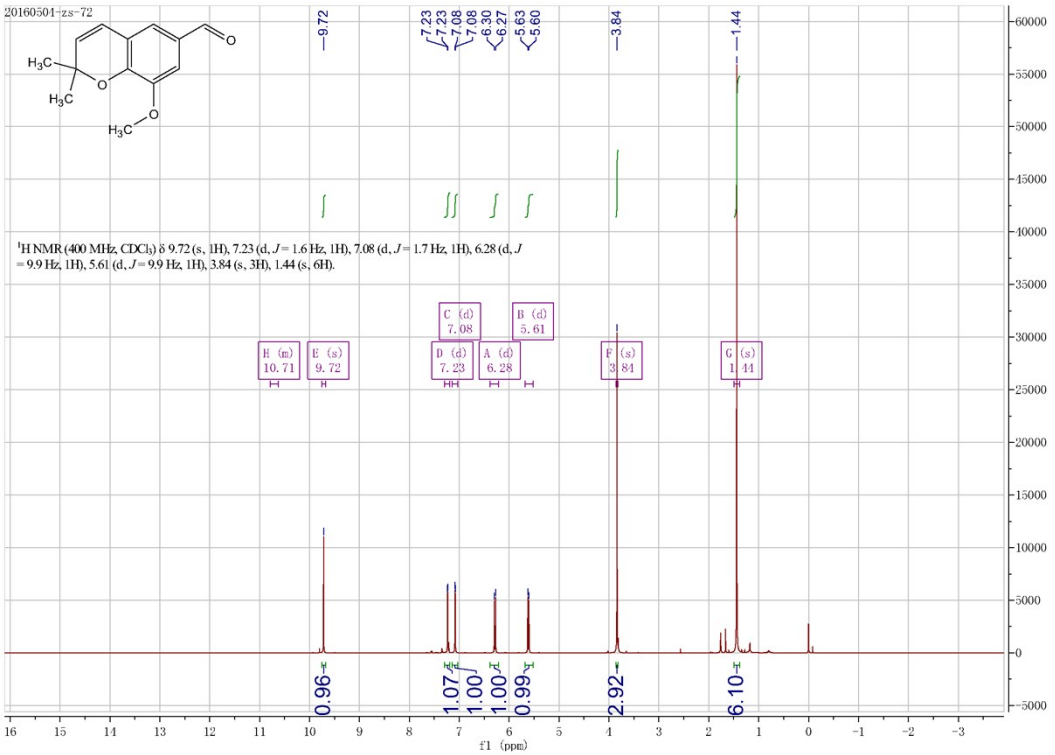

20160315-zs-155-c
^{13}C NMR (101 MHz, $CDCl_3$) δ 194.50 (s), 152.57 (s), 148.91 (s), 148.13 (s), 146.00 (s), 131.20 (s), 130.80 (s), 130.20 (s), 124.80 (s), 122.17 (s), 121.88 (s), 120.76 (s), 113.50 (s), 112.21 (s), 109.73 (s), 77.80 (s), 56.35 (s), 56.12 (s), 28.30 (s).
f1 (ppm)

SI3. NMR spectrums of intermediate compounds

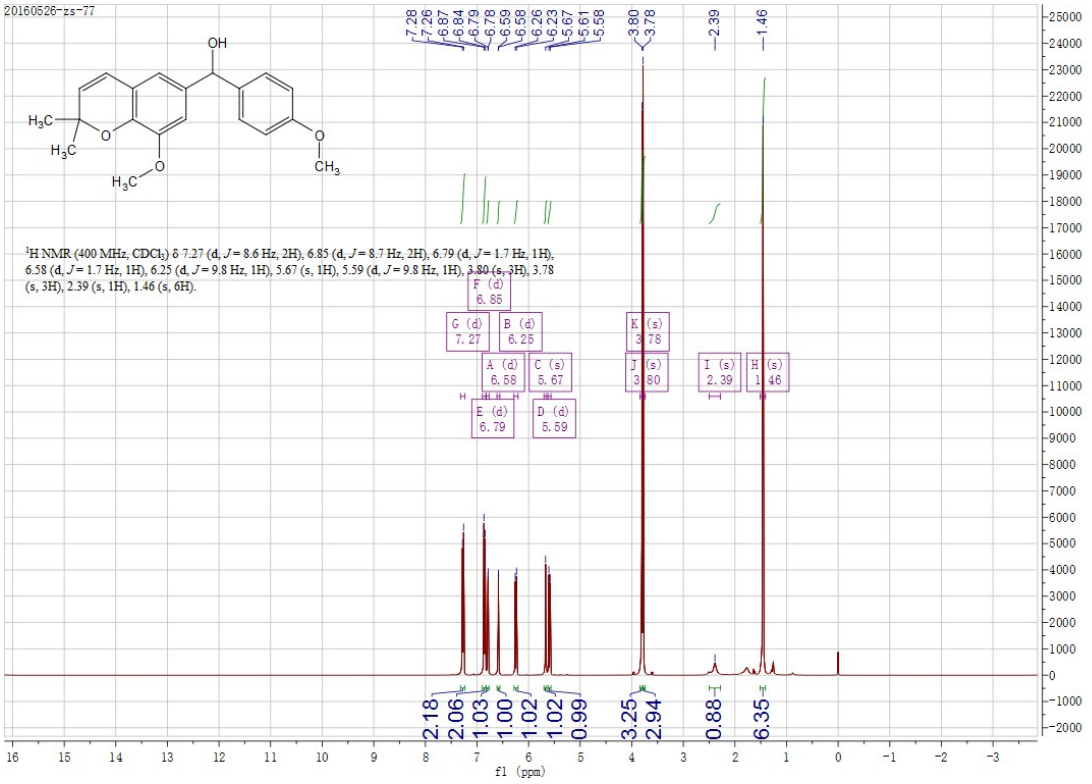
2

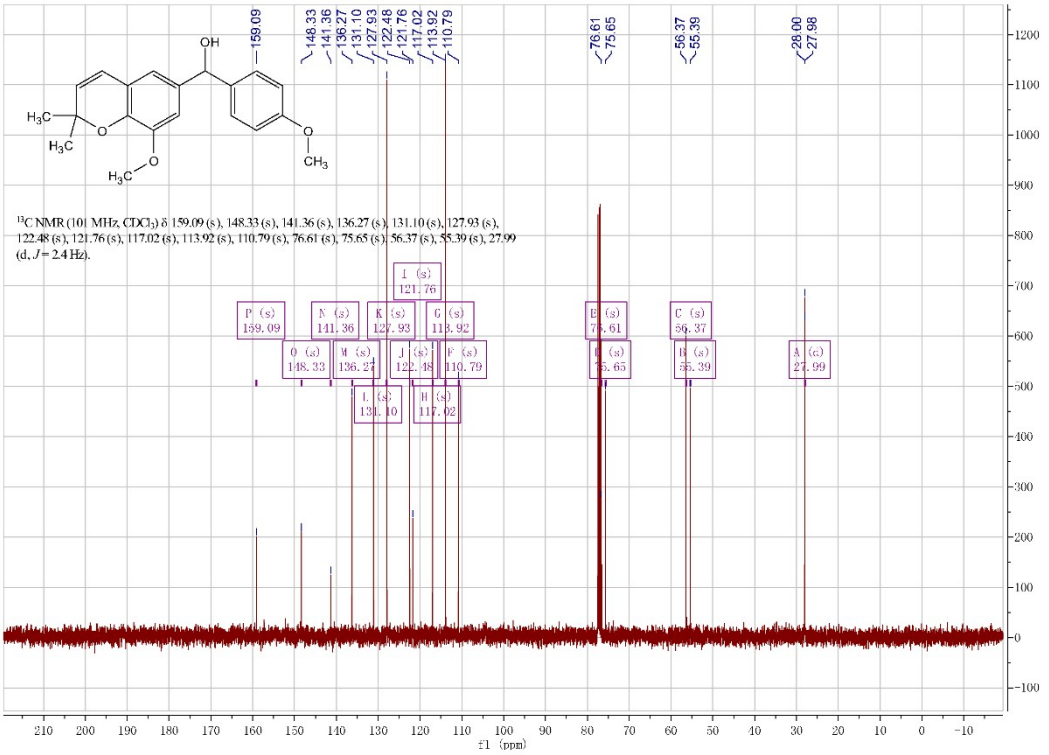


3

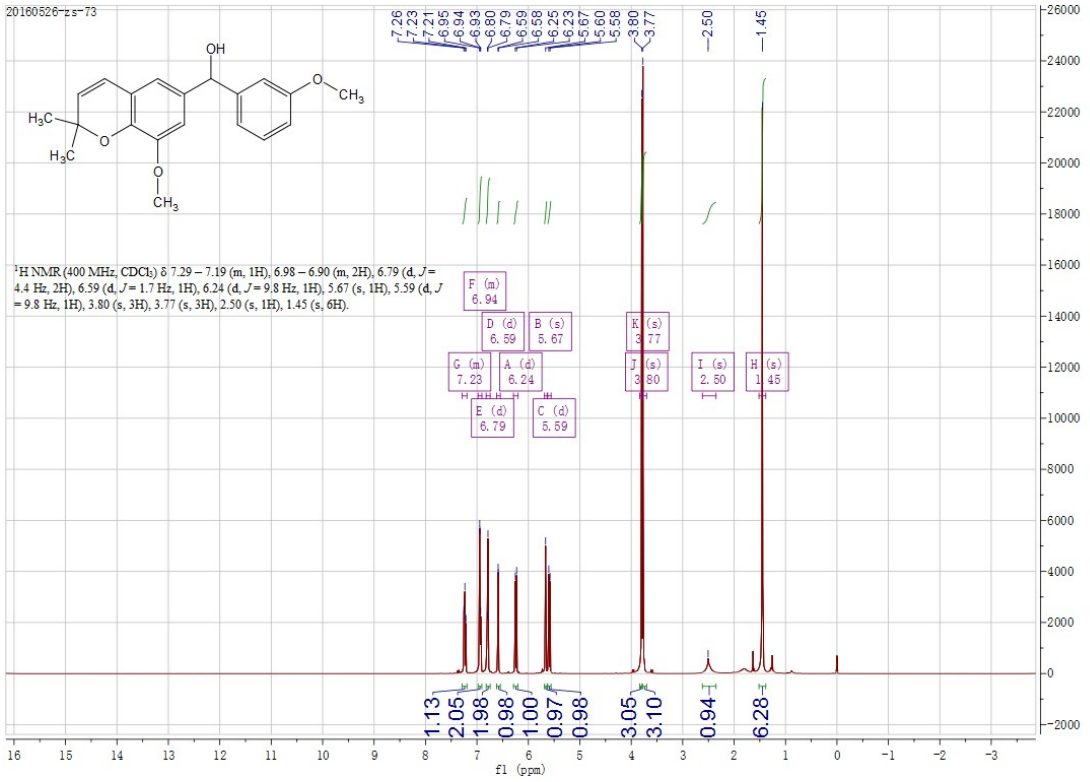


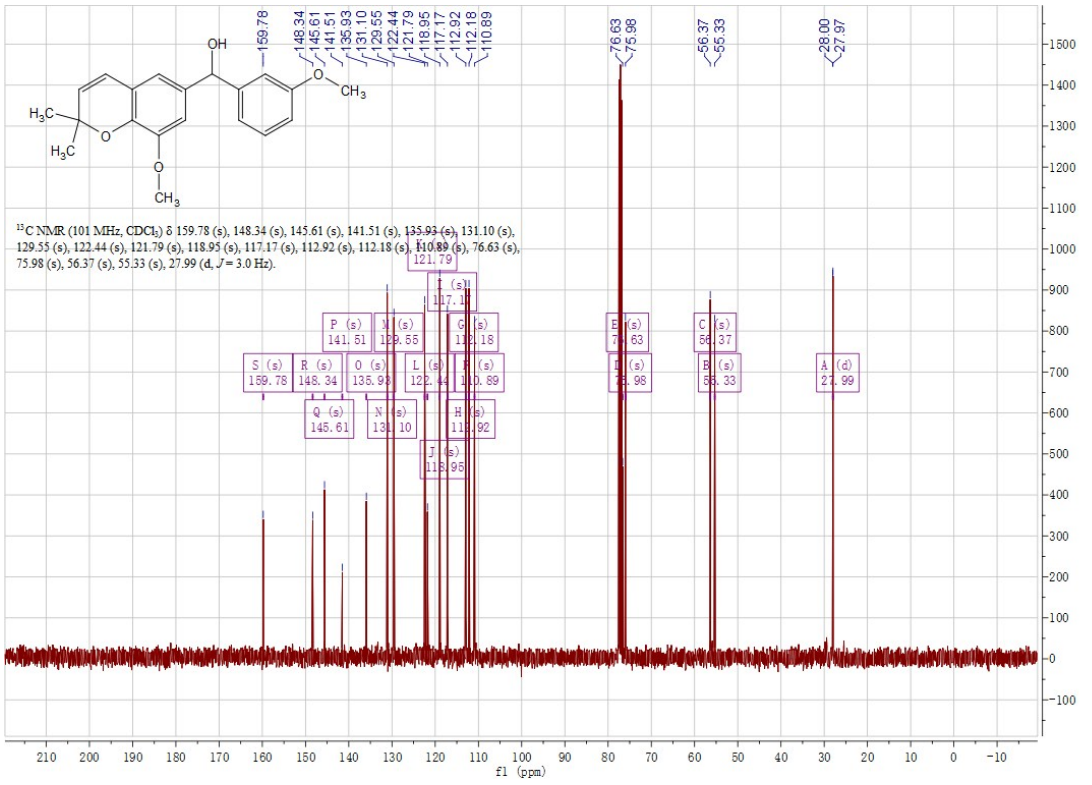
4a


20160526-zs-77
OH
H3C
H3C
O
O
H3C
O
CH3
7.28
7.26
6.87
6.84
6.79
6.78
6.59
6.58
6.26
6.23
5.67
5.61
5.58
3.80
3.78
2.39
1.46
1H NMR (400 MHz, CDCl3) δ 7.27 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 6.79 (d, J = 1.7 Hz, 1H), 6.58 (d, J = 1.7 Hz, 1H), 6.25 (d, J = 9.8 Hz, 1H), 5.67 (s, 1H), 5.59 (d, J = 9.8 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 2.39 (s, 1H), 1.46 (s, 6H).
F (d) 6.85
G (d) 7.27
B (d) 6.25
K (s) 3.78
A (d) 6.58
C (s) 5.67
J (s) 3.80
I (s) 2.39
H (s) 1.46
E (d) 6.79
D (d) 5.59
2.18
2.06
1.03
1.00
1.02
1.02
0.99
3.25
2.94
0.88
6.35
f1 (ppm)

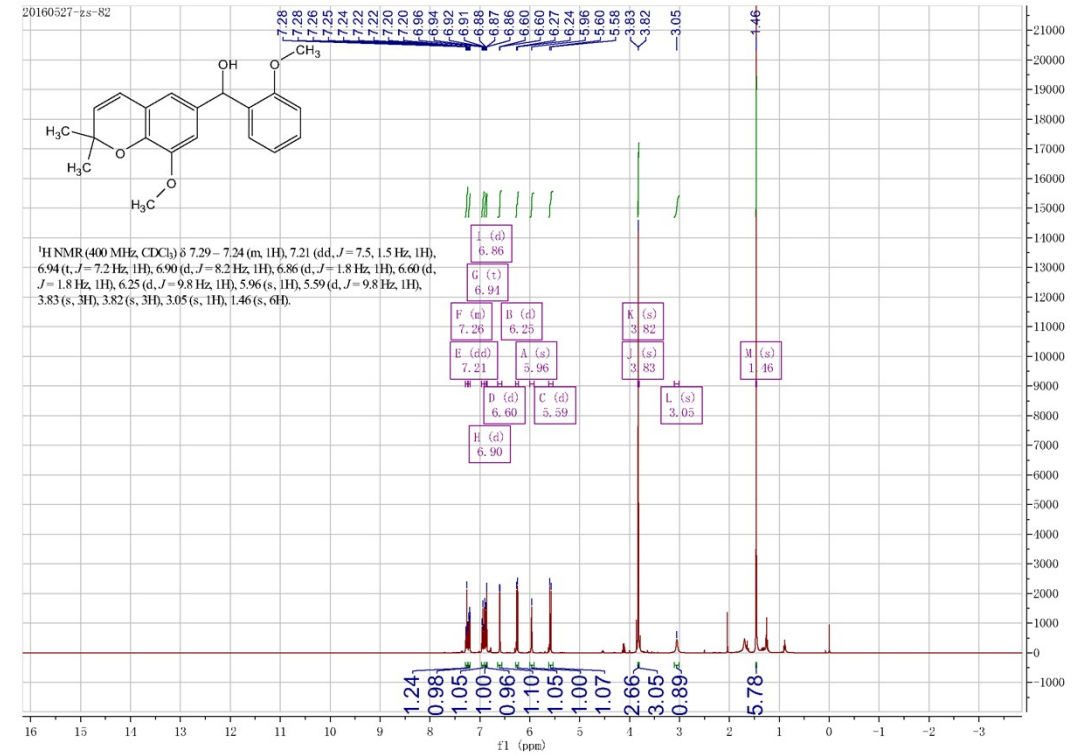

OH
H3C
H3C
O
O
H3C
O
CH3
159.09
148.33
141.36
136.27
131.10
127.93
122.48
121.76
117.02
113.92
110.79
76.61
75.65
56.37
55.39
28.00
27.98
13C NMR (101 MHz, CDCl3) δ 159.09 (s), 148.33 (s), 141.36 (s), 136.27 (s), 131.10 (s), 127.93 (s), 122.48 (s), 121.76 (s), 117.02 (s), 113.92 (s), 110.79 (s), 76.61 (s), 75.65 (s), 56.37 (s), 55.39 (s), 27.99 (d, J = 2.4 Hz).
L (s) 121.76
P (s) 159.09
N (s) 141.36
K (s) 127.93
G (s) 113.92
E (s) 76.61
C (s) 56.37
O (s) 148.33
M (s) 136.27
J (s) 122.48
F (s) 110.79
D (s) 75.65
B (s) 55.39
A (d) 27.99
I (s) 131.10
H (s) 117.02
f1 (ppm)

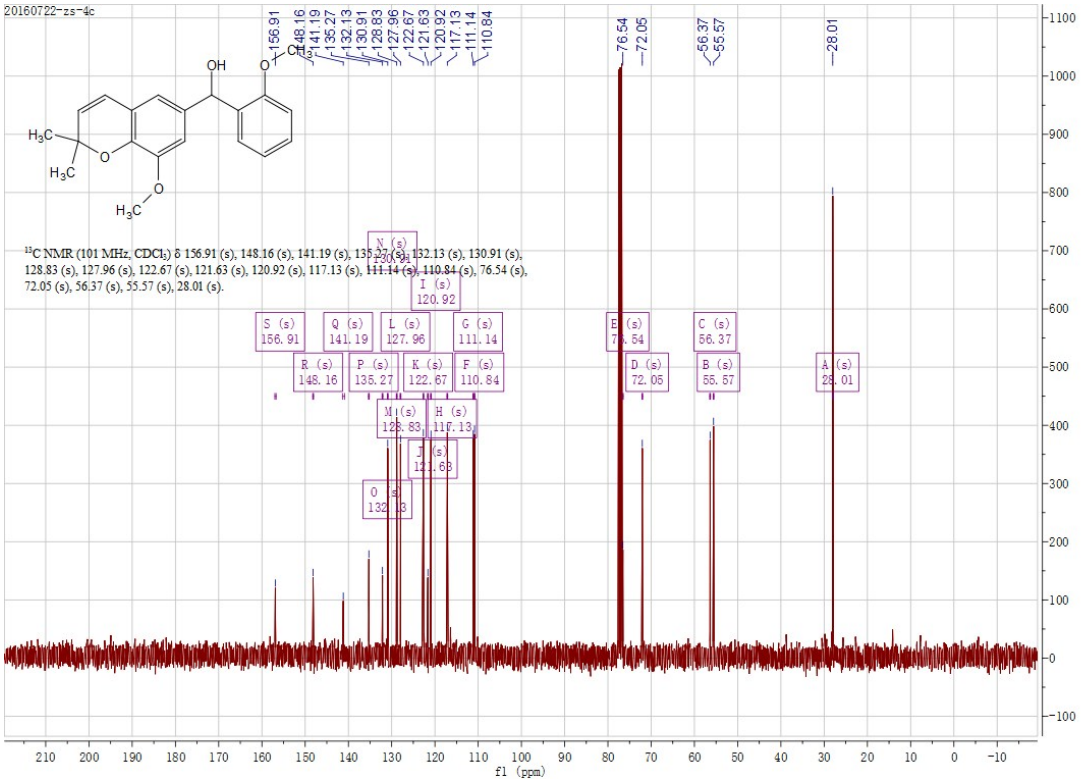
4b


20160526-zs-73
OH
O
CH3
H3C
O
H3C
O
CH3
1H NMR (400 MHz, CDCl3) δ 7.29 – 7.19 (m, 1H), 6.98 – 6.90 (m, 2H), 6.79 (d, J = 4.4 Hz, 2H), 6.59 (d, J = 1.7 Hz, 1H), 6.24 (d, J = 9.8 Hz, 1H), 5.67 (s, 1H), 5.59 (d, J = 9.8 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 2.50 (s, 1H), 1.45 (s, 6H).
f1 (ppm)

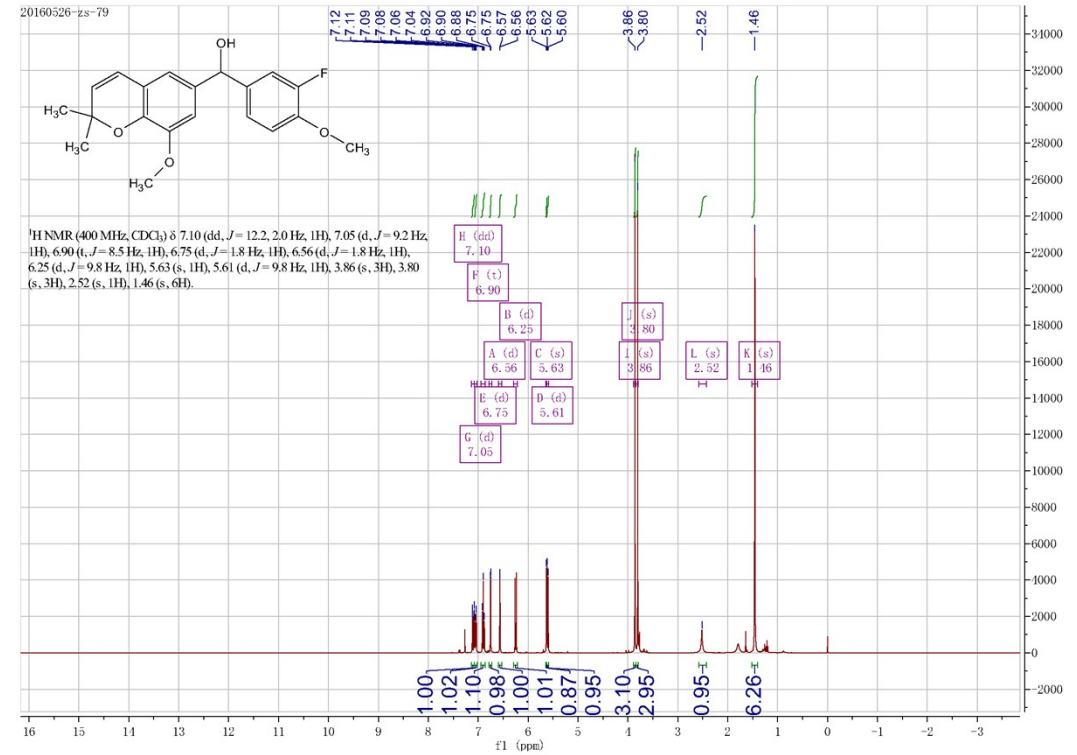

OH
O
CH3
H3C
O
H3C
O
CH3
13C NMR (101 MHz, CDCl3) δ 159.78 (s), 148.34 (s), 145.61 (s), 141.51 (s), 135.93 (s), 131.10 (s), 129.55 (s), 122.44 (s), 121.79 (s), 118.95 (s), 117.17 (s), 112.92 (s), 112.18 (s), 110.89 (s), 76.63 (s), 75.98 (s), 56.37 (s), 55.33 (s), 27.99 (d, J = 3.0 Hz).
f1 (ppm)

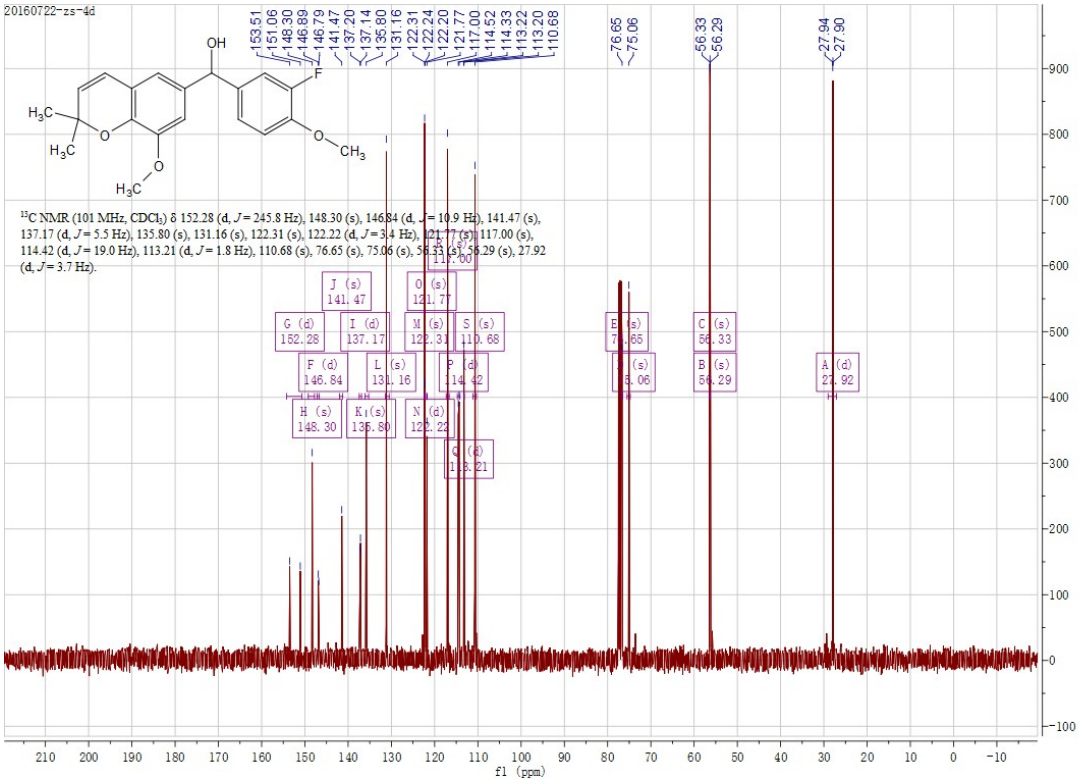
4c


20160527-zs-82
¹H NMR (400 MHz, CDCl₃) δ 7.29 – 7.24 (m, 1H), 7.21 (dd, J = 7.5, 1.5 Hz, 1H), 6.94 (t, J = 7.2 Hz, 1H), 6.90 (d, J = 8.2 Hz, 1H), 6.86 (d, J = 1.8 Hz, 1H), 6.60 (d, J = 1.8 Hz, 1H), 6.25 (d, J = 9.8 Hz, 1H), 5.96 (s, 1H), 5.59 (d, J = 9.8 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.05 (s, 1H), 1.46 (s, 6H).
f1 (ppm)

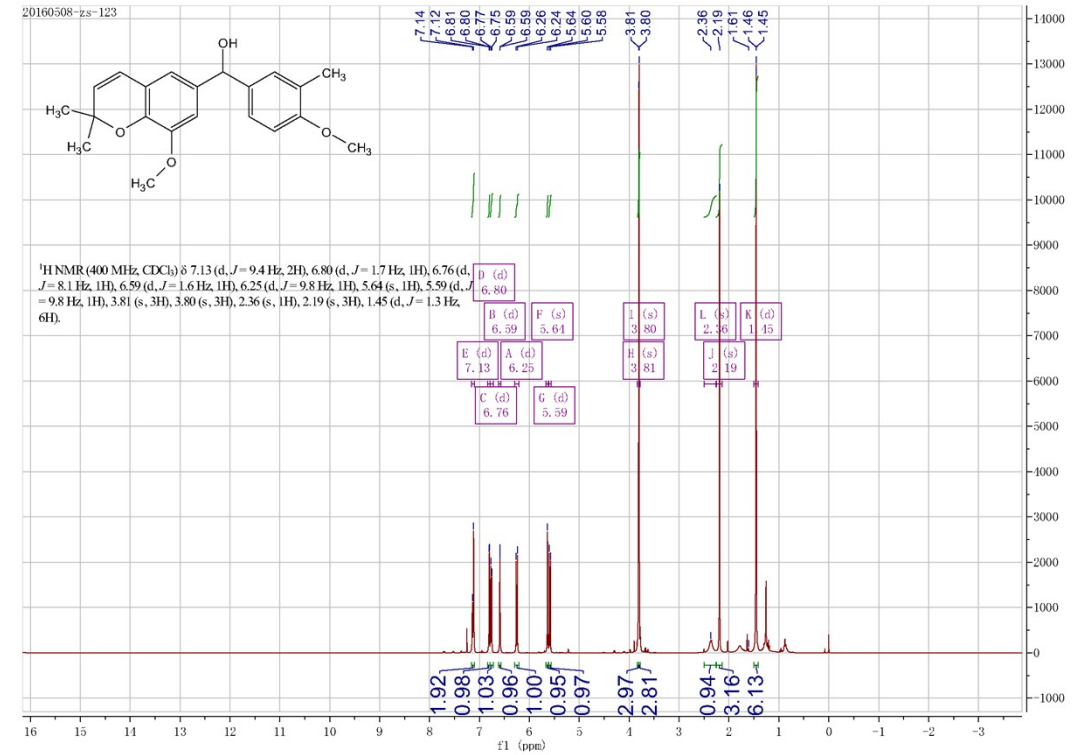

20160722-zs-4c
¹³C NMR (101 MHz, CDCl₃) δ 156.91 (s), 148.16 (s), 141.19 (s), 135.27 (s), 132.13 (s), 130.91 (s), 128.83 (s), 127.96 (s), 122.67 (s), 121.63 (s), 120.92 (s), 117.13 (s), 111.14 (s), 110.84 (s), 76.54 (s), 72.05 (s), 56.37 (s), 55.57 (s), 28.01 (s).
f1 (ppm)

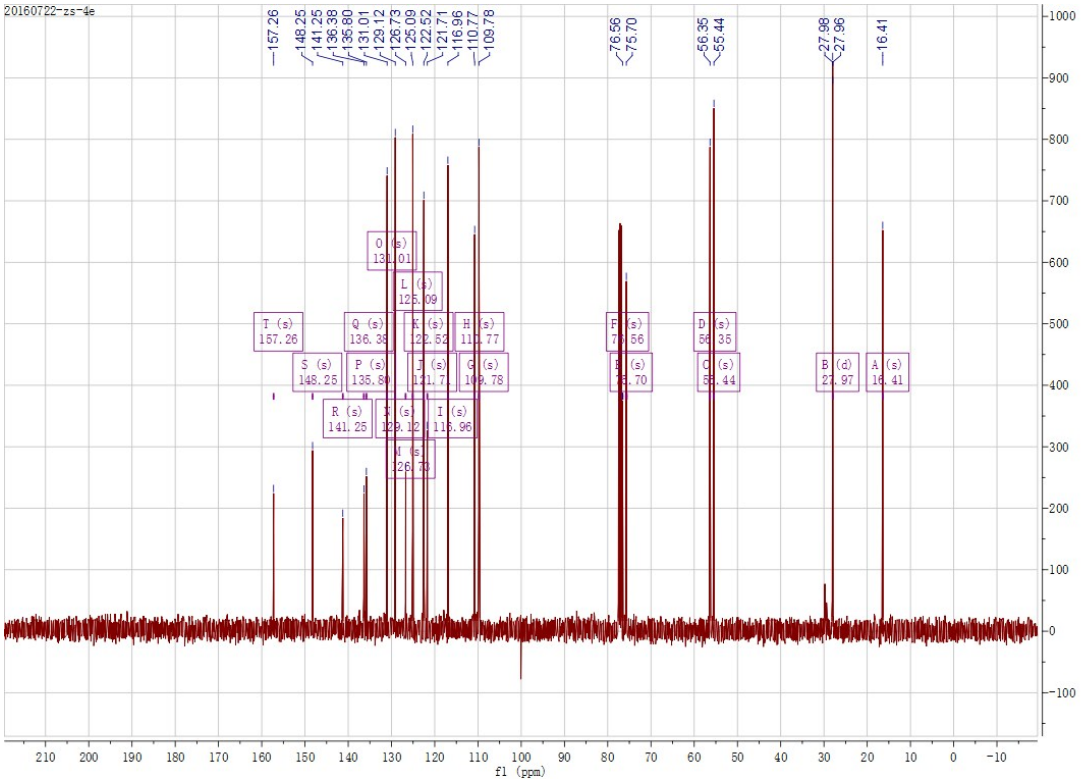
4d


20160526-zs-79
OH
F
O
O
O
H_3C
H_3C
H_3C
CH_3
7.12
7.11
7.09
7.08
7.06
7.04
6.92
6.90
6.88
6.75
6.57
6.56
5.63
5.62
5.60
3.86
3.80
2.52
1.46
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (dd, J = 12.2, 2.0 Hz, 1H), 7.05 (d, J = 9.2 Hz, 1H), 6.90 (t, J = 8.5 Hz, 1H), 6.75 (d, J = 1.8 Hz, 1H), 6.56 (d, J = 1.8 Hz, 1H), 6.25 (d, J = 9.8 Hz, 1H), 5.63 (s, 1H), 5.61 (d, J = 9.8 Hz, 1H), 3.86 (s, 3H), 3.80 (s, 3H), 2.52 (s, 1H), 1.46 (s, 6H).
H (dd) 7.10
F (t) 6.90
B (d) 6.25
A (d) 6.56
C (s) 5.63
E (d) 6.75
D (d) 5.61
G (d) 7.05
J (s) 3.80
I (s) 3.86
L (s) 2.52
K (s) 1.46
1.00
1.02
1.10
0.98
1.00
1.01
0.87
0.95
3.10
2.95
0.95
6.26
f1 (ppm)

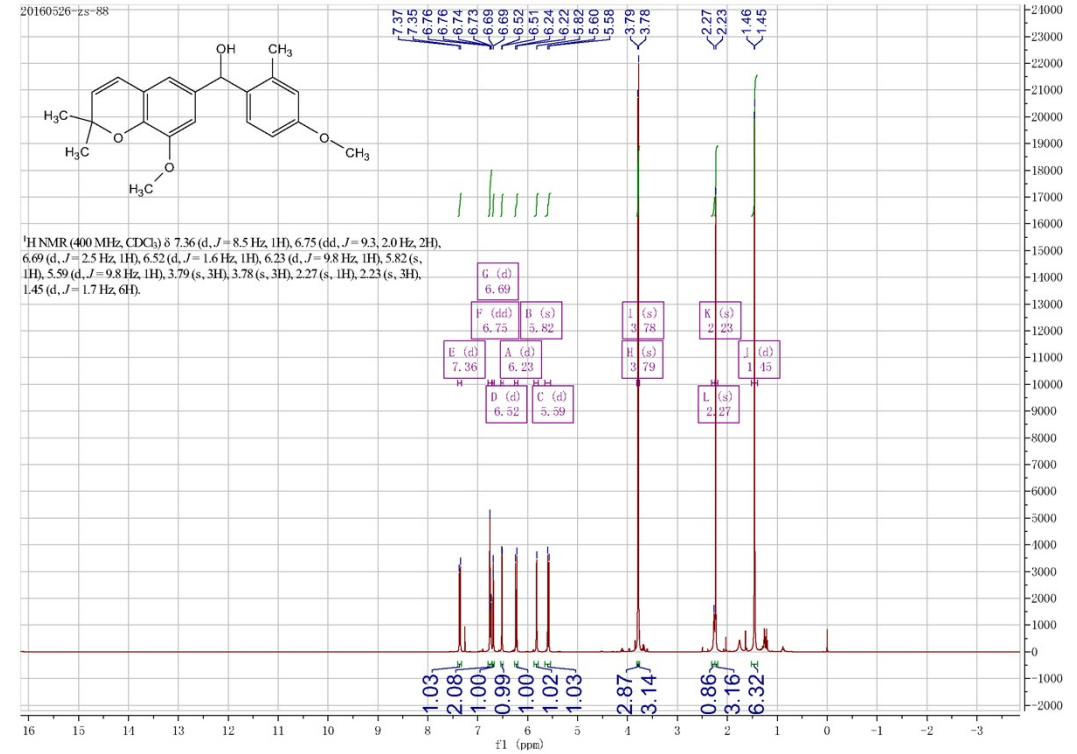

20160722-zs-4d
OH
F
O
O
O
H_3C
H_3C
H_3C
CH_3
153.51
151.06
148.30
146.89
146.79
141.47
137.20
137.14
135.80
131.16
122.31
122.24
122.20
121.77
117.00
114.52
114.33
113.22
113.20
110.68
76.65
75.06
56.33
56.29
27.94
27.90
^{13}C NMR (101 MHz, $CDCl_3$) δ 152.28 (d, J = 245.8 Hz), 148.30 (s), 146.84 (d, J = 10.9 Hz), 141.47 (s), 137.17 (d, J = 5.5 Hz), 135.80 (s), 131.16 (s), 122.31 (s), 122.22 (d, J = 3.4 Hz), 121.77 (s), 117.00 (s), 114.42 (d, J = 19.0 Hz), 113.21 (d, J = 1.8 Hz), 110.68 (s), 76.65 (s), 75.06 (s), 56.33 (s), 56.29 (s), 27.92 (d, J = 3.7 Hz).
J (s) 141.47
O (s) 121.77
G (d) 152.28
I (d) 137.17
M (s) 122.31
S (s) 110.68
E (s) 76.65
C (s) 56.33
F (d) 146.84
L (s) 131.16
P (d) 114.42
B (s) 56.29
A (d) 27.92
H (s) 148.30
K (s) 135.80
N (d) 122.22
Q (d) 113.21
f1 (ppm)

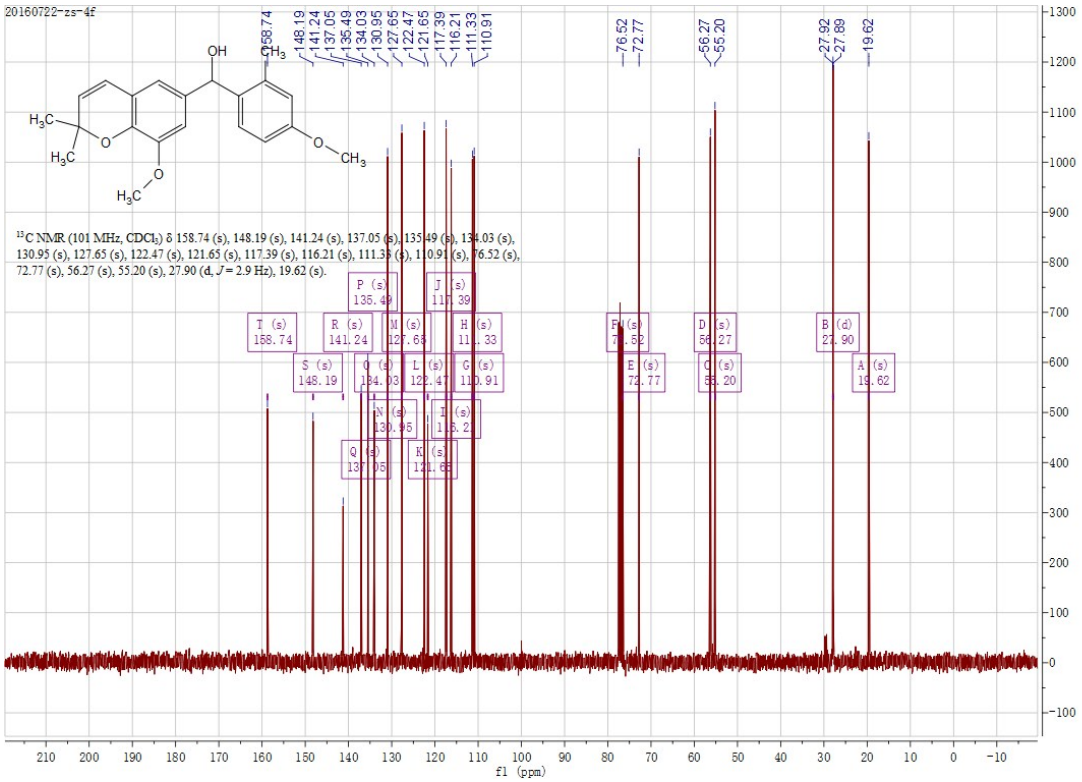
4e


20160508-zs-123
OH
CH3
H3C
O
H3C
O
CH3
O
H3C
7.14
7.12
6.81
6.80
6.77
6.75
6.59
6.59
6.26
6.24
5.64
5.60
5.58
3.81
3.80
2.36
2.19
1.61
1.46
1.45
1H NMR (400 MHz, CDCl3) δ 7.13 (d, J = 9.4 Hz, 2H), 6.80 (d, J = 1.7 Hz, 1H), 6.76 (d, J = 8.1 Hz, 1H), 6.59 (d, J = 1.6 Hz, 1H), 6.25 (d, J = 9.8 Hz, 1H), 5.64 (s, 1H), 5.59 (d, J = 9.8 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 2.36 (s, 1H), 2.19 (s, 3H), 1.45 (d, J = 1.3 Hz, 6H).
D (d) 6.80
B (d) 6.59
F (s) 5.64
I (s) 3.80
L (s) 2.36
K (d) 1.45
E (d) 7.13
A (d) 6.25
H (s) 3.81
J (s) 2.19
C (d) 6.76
G (d) 5.59
1.92
0.98
1.03
0.96
1.00
0.95
0.97
2.97
2.81
0.94
3.16
6.13
f1 (ppm)

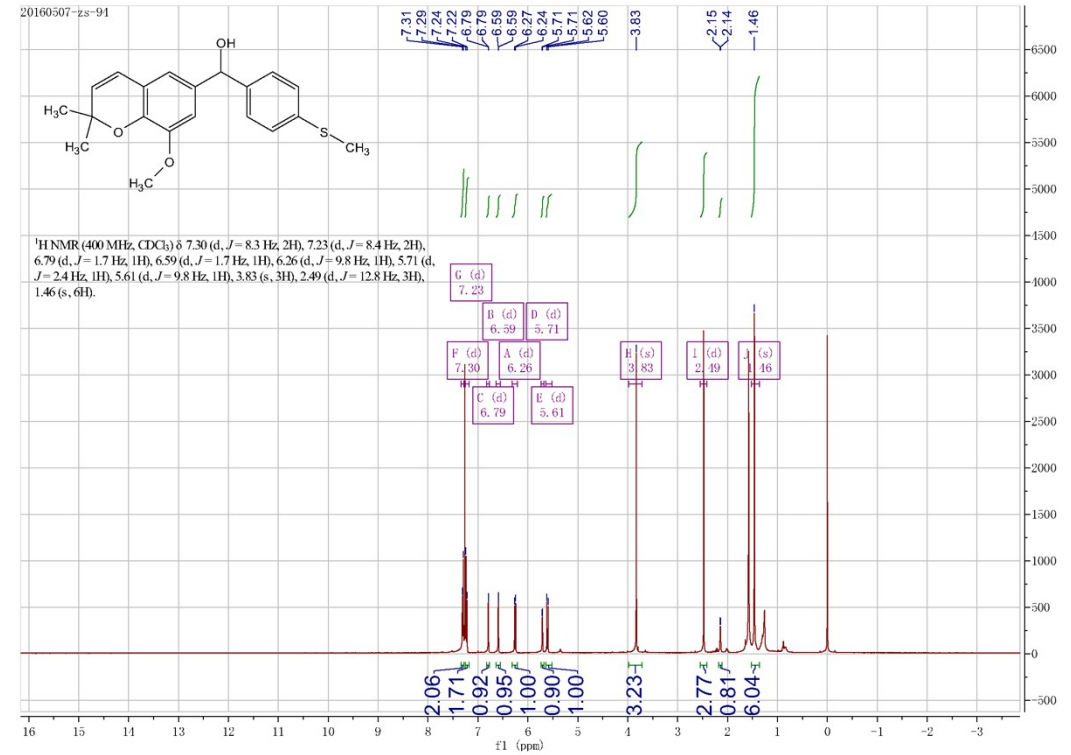

20160722-zs-4e
157.26
148.25
141.25
136.38
135.80
131.01
129.12
126.73
125.09
122.52
121.71
116.96
110.77
109.78
76.56
75.70
56.35
55.44
27.98
27.96
16.41
Q (s) 131.01
L (s) 125.09
T (s) 157.26
Q (s) 136.38
K (s) 122.52
H (s) 110.77
F (s) 76.56
D (s) 56.35
S (s) 148.25
P (s) 135.80
J (s) 121.71
G (s) 109.78
E (s) 75.70
C (s) 55.44
B (d) 27.97
A (s) 16.41
R (s) 141.25
N (s) 129.12
I (s) 116.96
M (s) 126.73
f1 (ppm)

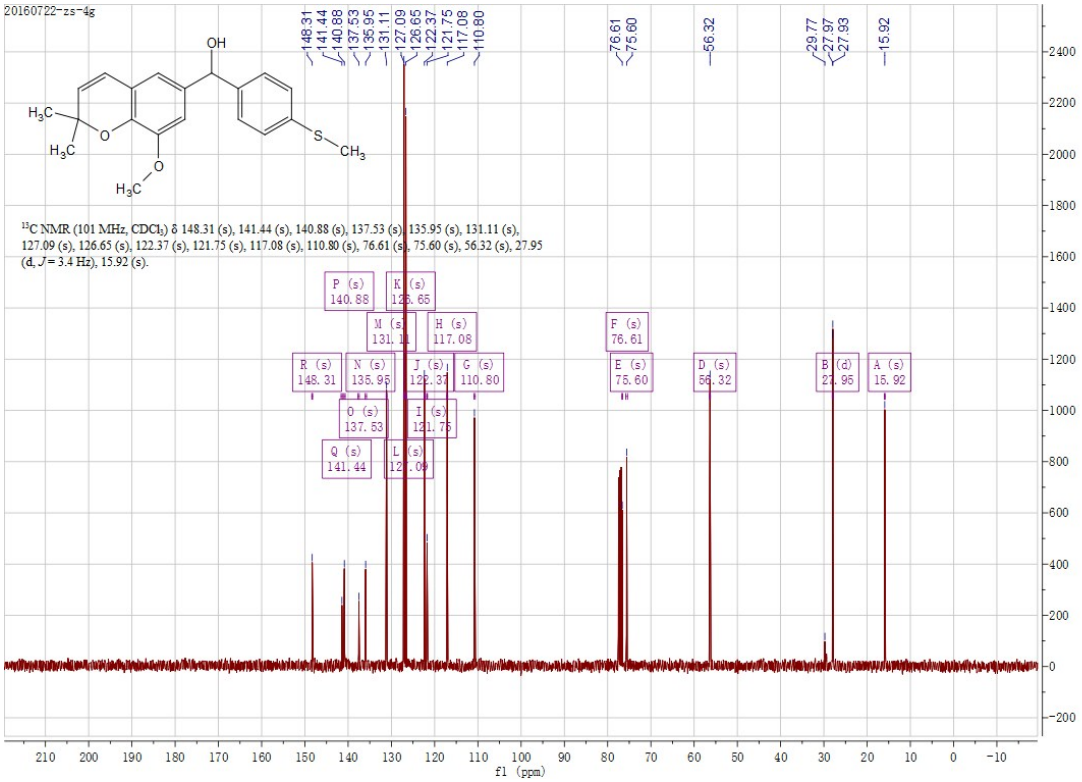
4f


20160526-zs-88
OH
CH3
H3C
O
H3C
O
O
CH3
H3C
1H NMR (400 MHz, CDCl3) δ 7.36 (d, J = 8.5 Hz, 1H), 6.75 (dd, J = 9.3, 2.0 Hz, 2H), 6.69 (d, J = 2.5 Hz, 1H), 6.52 (d, J = 1.6 Hz, 1H), 6.23 (d, J = 9.8 Hz, 1H), 5.82 (s, 1H), 5.59 (d, J = 9.8 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 2.27 (s, 1H), 2.23 (s, 3H), 1.45 (d, J = 1.7 Hz, 6H).
f1 (ppm)

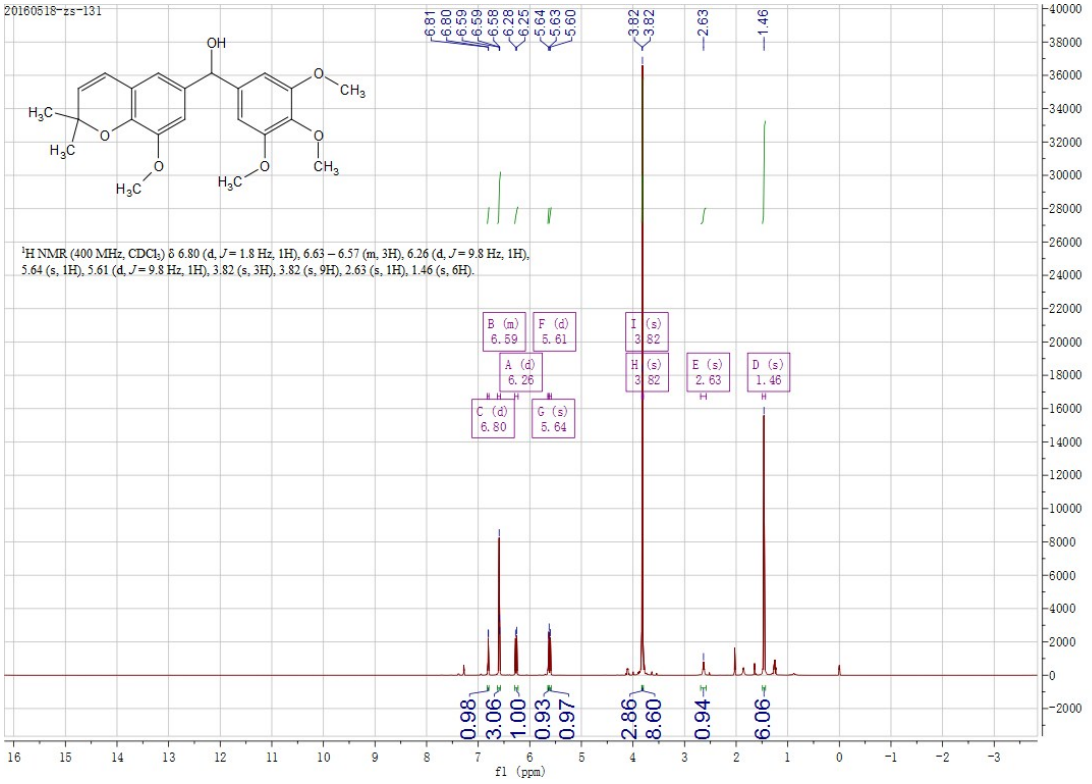

20160722-zs-4f
OH
CH3
H3C
O
H3C
O
O
CH3
H3C
13C NMR (101 MHz, CDCl3) δ 158.74 (s), 148.19 (s), 141.24 (s), 137.05 (s), 135.49 (s), 134.03 (s), 130.95 (s), 127.65 (s), 122.47 (s), 121.65 (s), 117.39 (s), 116.21 (s), 111.33 (s), 110.91 (s), 76.52 (s), 72.77 (s), 56.27 (s), 55.20 (s), 27.90 (d, J = 2.9 Hz), 19.62 (s).
f1 (ppm)

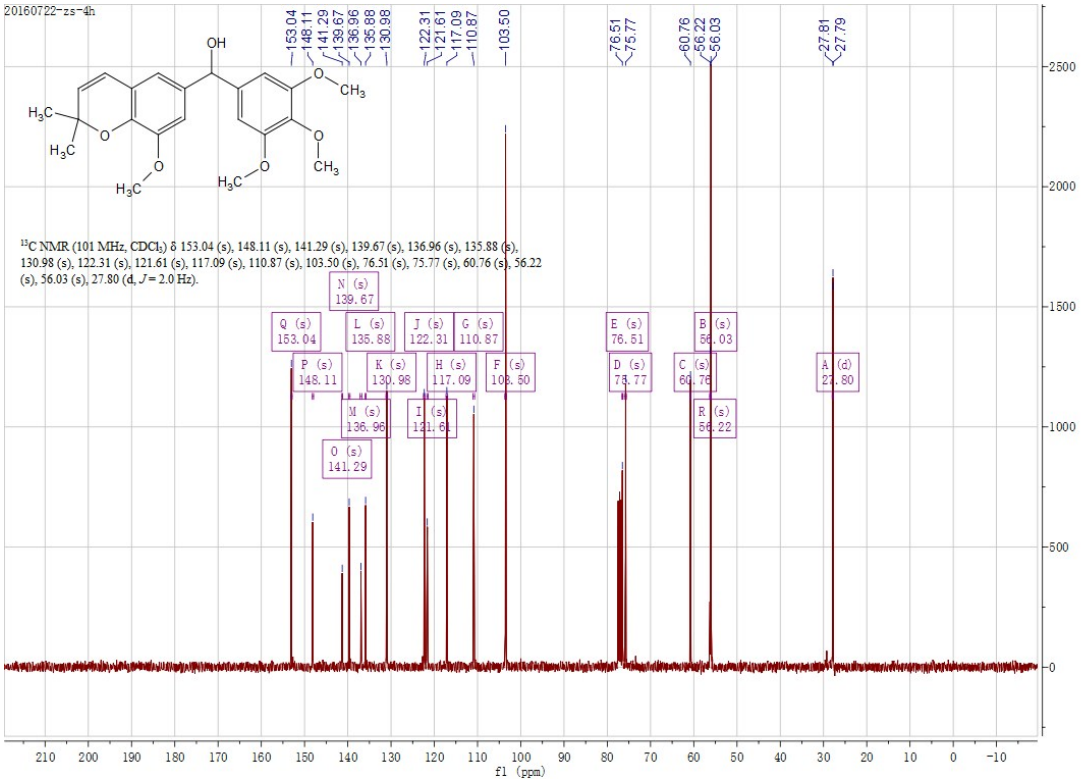
4g


20160507-zs-91
OH
H3C
O
H3C
O
H3C
S
CH3
7.31
7.29
7.24
7.22
6.79
6.59
6.59
6.27
6.24
5.71
5.71
5.62
5.60
3.83
2.15
2.14
1.46
1H NMR (400 MHz, CDCl3) δ 7.30 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 1.7 Hz, 1H), 6.59 (d, J = 1.7 Hz, 1H), 6.26 (d, J = 9.8 Hz, 1H), 5.71 (d, J = 2.4 Hz, 1H), 5.61 (d, J = 9.8 Hz, 1H), 3.83 (s, 3H), 2.49 (d, J = 12.8 Hz, 3H), 1.46 (s, 6H).
G (d) 7.23
B (d) 6.59
D (d) 5.71
F (d) 7.30
A (d) 6.26
C (d) 6.79
E (d) 5.61
H (s) 3.83
I (d) 2.49
J (s) 1.46
2.06
1.71
0.92
0.95
1.00
0.90
1.00
3.23
2.77
0.81
6.04
f1 (ppm)

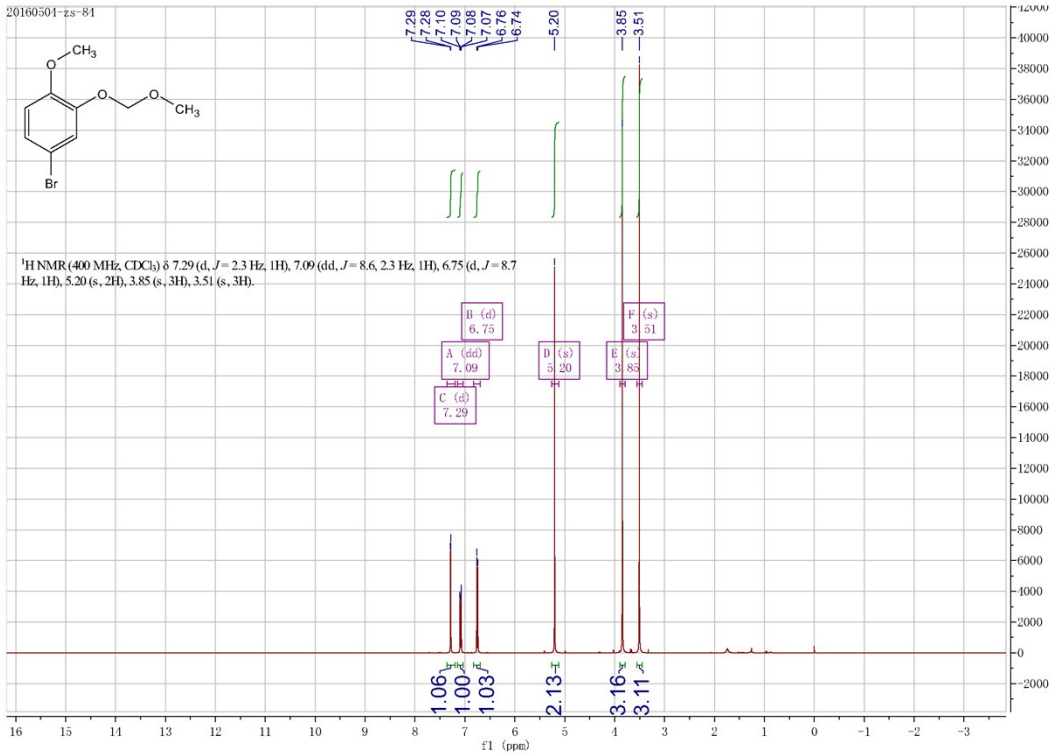

20160722-zs-4g
OH
H3C
O
H3C
O
H3C
S
CH3
148.31
141.44
140.88
137.53
135.95
131.11
127.09
126.65
122.37
121.75
117.08
110.80
76.61
75.60
56.32
29.77
27.97
27.93
15.92
13C NMR (101 MHz, CDCl3) δ 148.31 (s), 141.44 (s), 140.88 (s), 137.53 (s), 135.95 (s), 131.11 (s), 127.09 (s), 126.65 (s), 122.37 (s), 121.75 (s), 117.08 (s), 110.80 (s), 76.61 (s), 75.60 (s), 56.32 (s), 27.95 (d, J = 3.4 Hz), 15.92 (s).
P (s) 140.88
K (s) 126.65
M (s) 131.11
H (s) 117.08
R (s) 148.31
N (s) 135.95
J (s) 122.37
G (s) 110.80
O (s) 137.53
I (s) 121.75
Q (s) 141.44
L (s) 127.09
F (s) 76.61
E (s) 75.60
D (s) 56.32
B (d) 27.95
A (s) 15.92
f1 (ppm)

4h

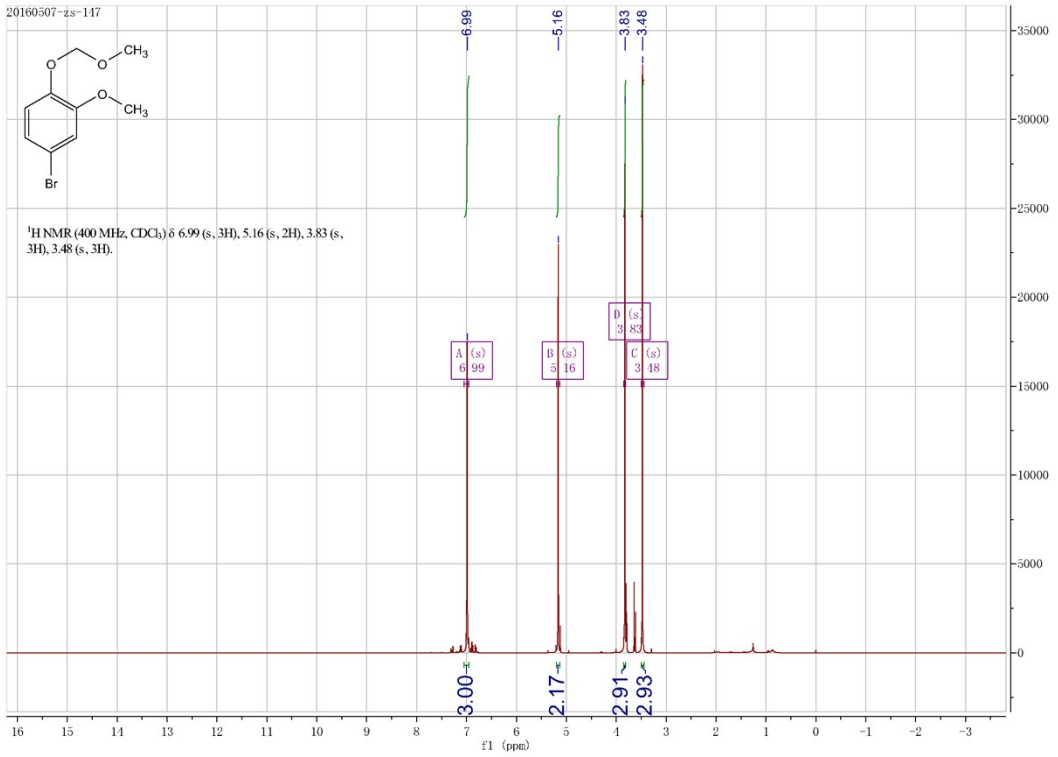

20160518-zs-131
6.81
6.80
6.59
6.59
6.58
6.28
6.25
5.64
5.63
5.60
3.82
3.82
2.63
1.46
OH
O
CH3
H3C
O
H3C
O
H3C
O
O
CH3
¹H NMR (400 MHz, CDCl3) δ 6.80 (d, J = 1.8 Hz, 1H), 6.63 – 6.57 (m, 3H), 6.26 (d, J = 9.8 Hz, 1H), 5.64 (s, 1H), 5.61 (d, J = 9.8 Hz, 1H), 3.82 (s, 3H), 3.82 (s, 9H), 2.63 (s, 1H), 1.46 (s, 6H).
B (m) 6.59
F (d) 5.61
I (s) 3.82
A (d) 6.26
H (s) 3.82
E (s) 2.63
D (s) 1.46
C (d) 6.80
G (s) 5.64
0.98
3.06
1.00
0.93
0.97
2.86
8.60
0.94
6.06
f1 (ppm)


20160722-zs-4h
153.04
148.11
141.29
139.67
136.96
135.88
130.98
122.31
121.61
117.09
110.87
103.50
76.51
75.77
60.76
56.22
56.03
27.81
27.79
OH
O
CH3
H3C
O
H3C
O
H3C
O
O
CH3
¹³C NMR (101 MHz, CDCl3) δ 153.04 (s), 148.11 (s), 141.29 (s), 139.67 (s), 136.96 (s), 135.88 (s), 130.98 (s), 122.31 (s), 121.61 (s), 117.09 (s), 110.87 (s), 103.50 (s), 76.51 (s), 75.77 (s), 60.76 (s), 56.22 (s), 56.03 (s), 27.80 (d, J = 2.0 Hz).
N (s) 139.67
Q (s) 153.04
L (s) 135.88
J (s) 122.31
G (s) 110.87
E (s) 76.51
B (s) 56.03
P (s) 148.11
K (s) 130.98
H (s) 117.09
F (s) 103.50
D (s) 75.77
C (s) 60.76
A (d) 27.80
M (s) 136.96
I (s) 121.61
R (s) 56.22
O (s) 141.29
f1 (ppm)

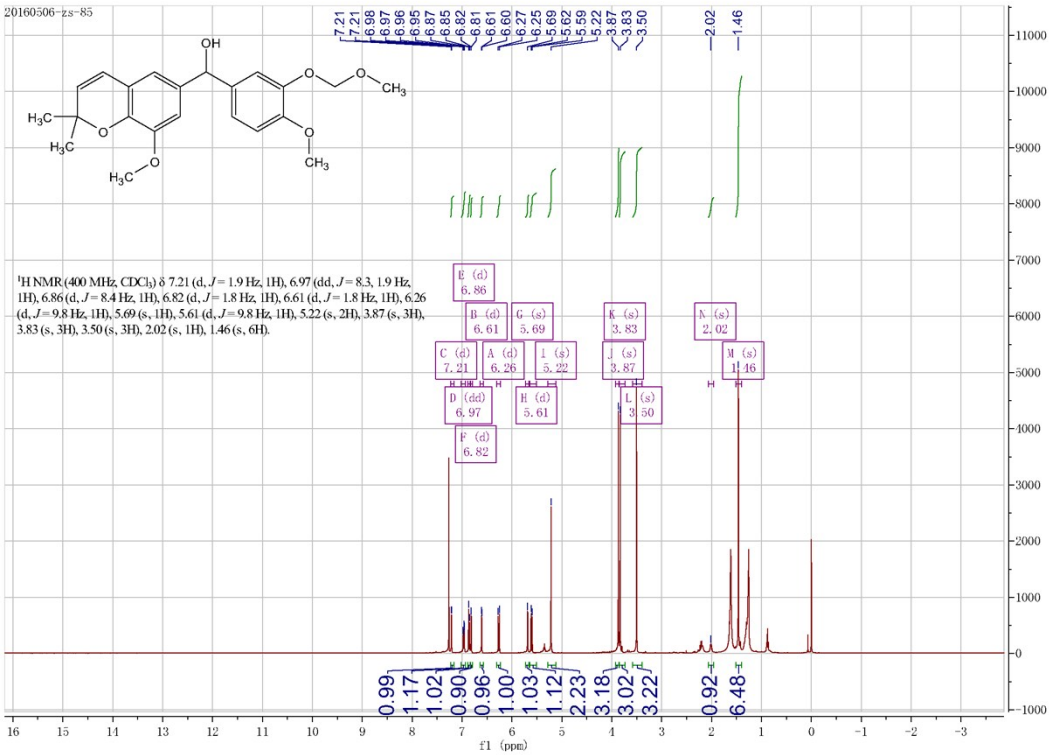
7a


20160501-zs-81
7.29
7.28
7.10
7.09
7.08
7.07
6.76
6.74
5.20
3.85
3.51
CH3
O
O
O
CH3
Br
1H NMR (400 MHz, CDCl3) δ 7.29 (d, J = 2.3 Hz, 1H), 7.09 (dd, J = 8.6, 2.3 Hz, 1H), 6.75 (d, J = 8.7 Hz, 1H), 5.20 (s, 2H), 3.85 (s, 3H), 3.51 (s, 3H).
B (d) 6.75
A (dd) 7.09
C (d) 7.29
D (s) 5.20
E (s) 3.85
F (s) 3.51
1.06
1.00
1.03
2.13
3.16
3.11
f1 (ppm)

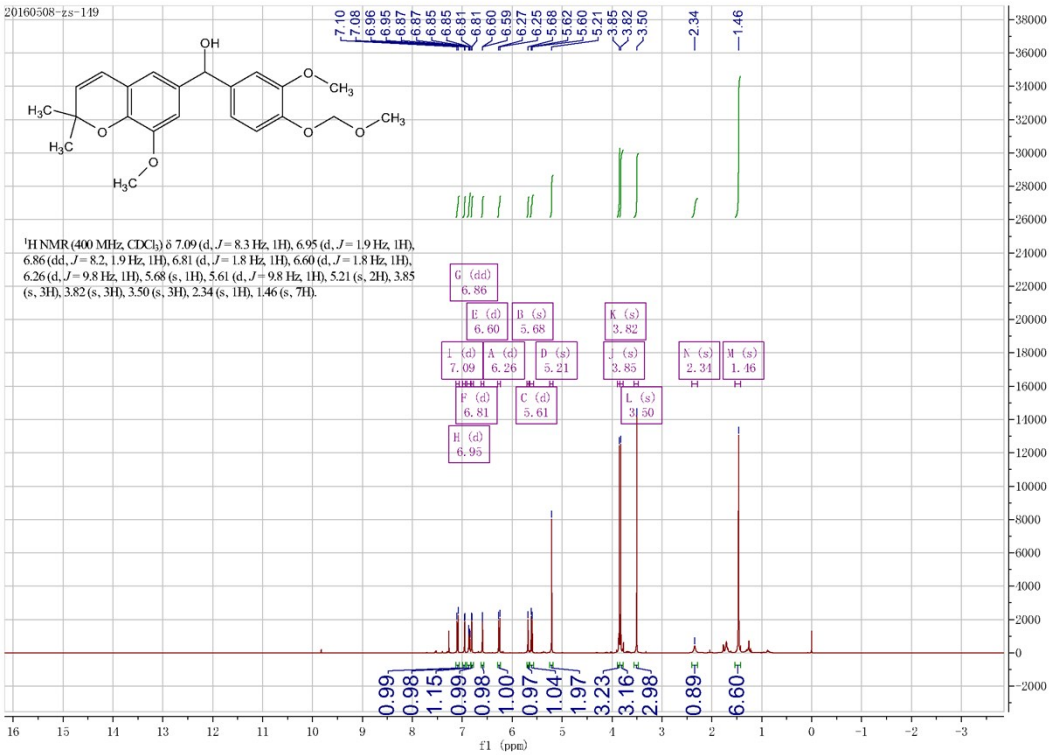
7b


20160507-zs-117
6.99
5.16
3.83
3.48
O
O
CH3
O
CH3
Br
1H NMR (400 MHz, CDCl3) δ 6.99 (s, 3H), 5.16 (s, 2H), 3.83 (s, 3H), 3.48 (s, 3H).
A (s) 6.99
B (s) 5.16
D (s) 3.83
C (s) 3.48
3.00
2.17
2.91
2.93
f1 (ppm)

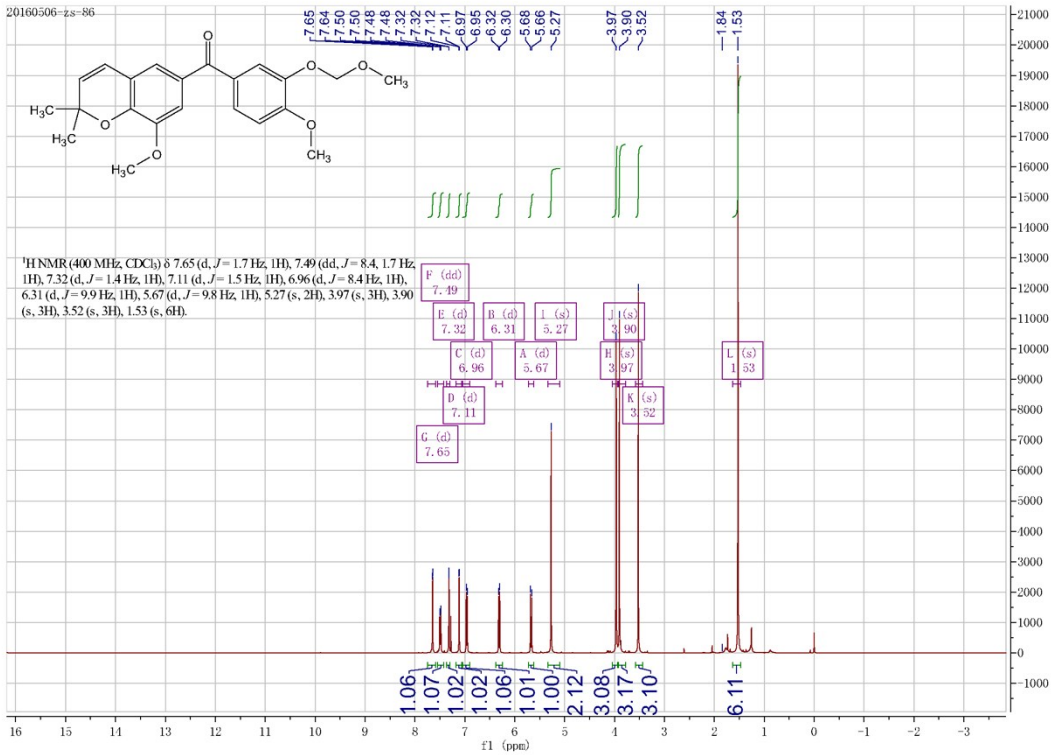
8a


20160506-zs-85
¹H NMR (400 MHz, CDCl₃) δ 7.21 (d, J = 1.9 Hz, 1H), 6.97 (dd, J = 8.3, 1.9 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 1.8 Hz, 1H), 6.61 (d, J = 1.8 Hz, 1H), 6.26 (d, J = 9.8 Hz, 1H), 5.69 (s, 1H), 5.61 (d, J = 9.8 Hz, 1H), 5.22 (s, 2H), 3.87 (s, 3H), 3.83 (s, 3H), 3.50 (s, 3H), 2.02 (s, 1H), 1.46 (s, 6H).
f1 (ppm)

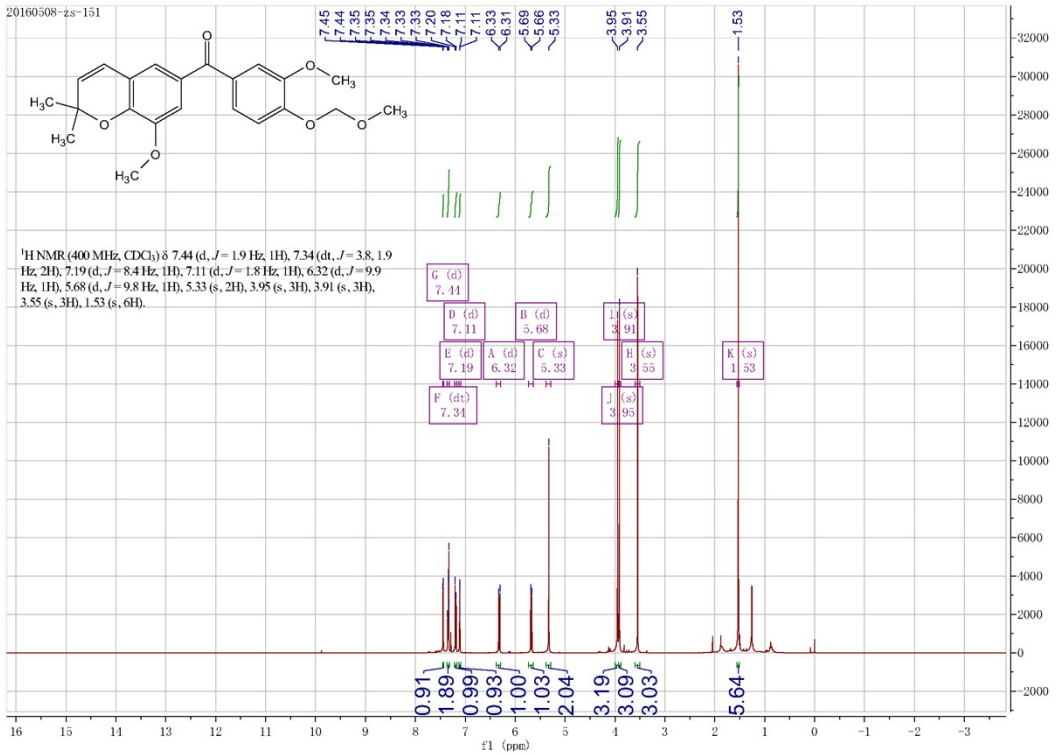
8b


20160508-zs-119
¹H NMR (400 MHz, CDCl₃) δ 7.09 (d, J = 8.3 Hz, 1H), 6.95 (d, J = 1.9 Hz, 1H), 6.86 (dd, J = 8.2, 1.9 Hz, 1H), 6.81 (d, J = 1.8 Hz, 1H), 6.60 (d, J = 1.8 Hz, 1H), 6.26 (d, J = 9.8 Hz, 1H), 5.68 (s, 1H), 5.61 (d, J = 9.8 Hz, 1H), 5.21 (s, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 3.50 (s, 3H), 2.34 (s, 1H), 1.46 (s, 7H).
f1 (ppm)

9a

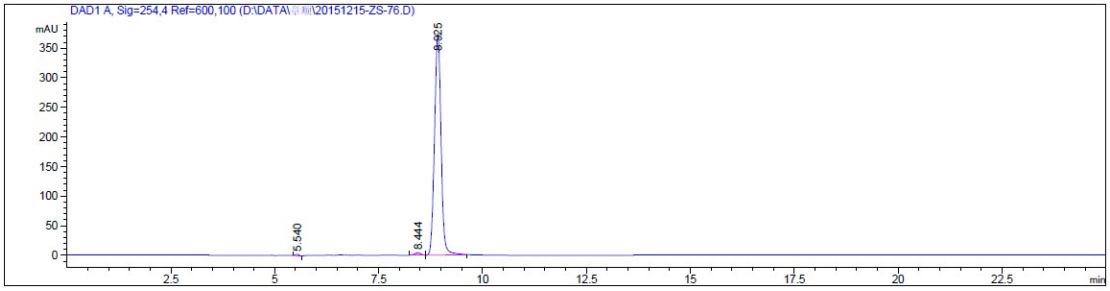

20160506-zs-86
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 1.7 Hz, 1H), 7.49 (dd, J = 8.4, 1.7 Hz, 1H), 7.32 (d, J = 1.4 Hz, 1H), 7.11 (d, J = 1.5 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 6.31 (d, J = 9.9 Hz, 1H), 5.67 (d, J = 9.8 Hz, 1H), 5.27 (s, 2H), 3.97 (s, 3H), 3.90 (s, 3H), 3.52 (s, 3H), 1.53 (s, 6H).
f1 (ppm)

9b


20160508-zs-151
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 1.9 Hz, 1H), 7.34 (dt, J = 3.8, 1.9 Hz, 2H), 7.19 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 1.8 Hz, 1H), 6.32 (d, J = 9.9 Hz, 1H), 5.68 (d, J = 9.8 Hz, 1H), 5.33 (s, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 3.55 (s, 3H), 1.53 (s, 6H).
f1 (ppm)

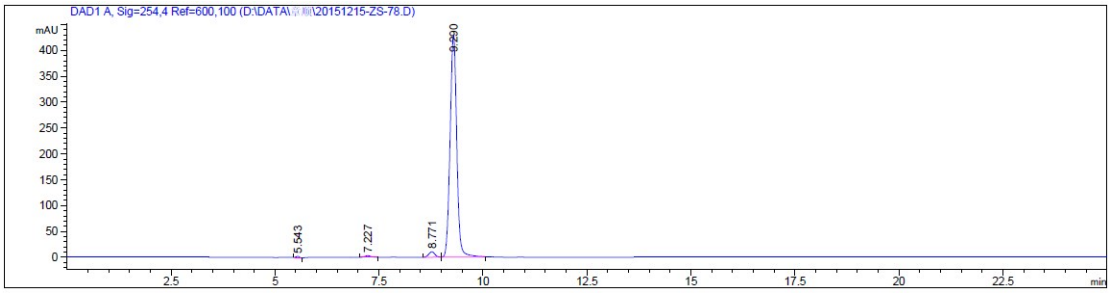
SI4. HPLC chromatograms of target compounds

5a



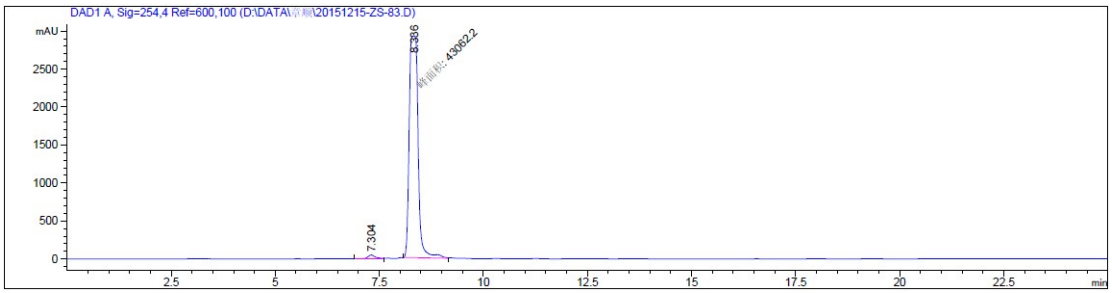
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.540	BV	0.0975	15.74513	2.57083	0.3796
2	8.444	BV	0.1583	41.80822	4.12309	1.0079
3	8.925	VB	0.1697	4090.62256	373.79340	98.6126

5b



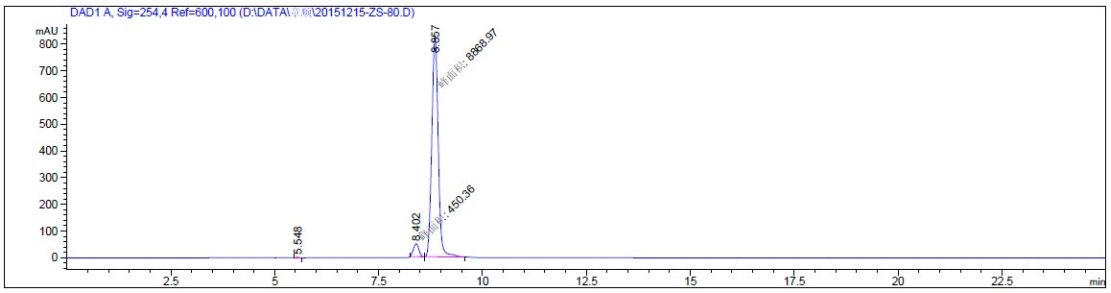
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.543	BV	0.0939	15.96751	2.66602	0.3223
2	7.227	BB	0.1518	32.21017	3.24606	0.6501
3	8.771	BV	0.1601	110.26850	10.70960	2.2254
4	9.290	VB	0.1723	4796.52930	429.47940	96.8023

5c



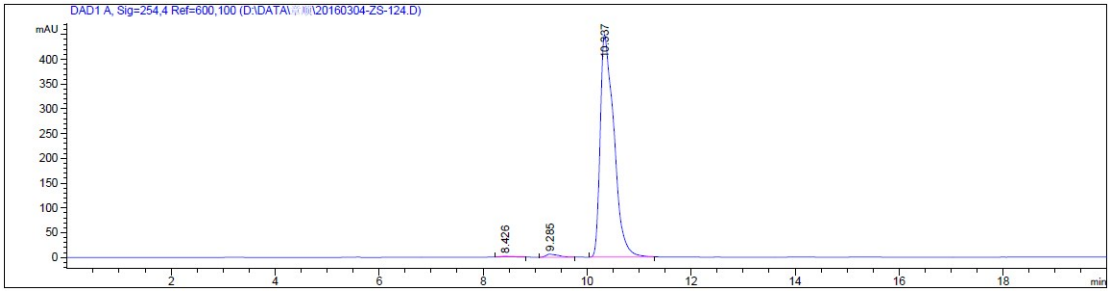
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	7.304	VV	0.1972	681.65546	50.59747	1.5583
2	8.336	MM	0.2459	4.30622e4	2918.20850	98.4417

5d



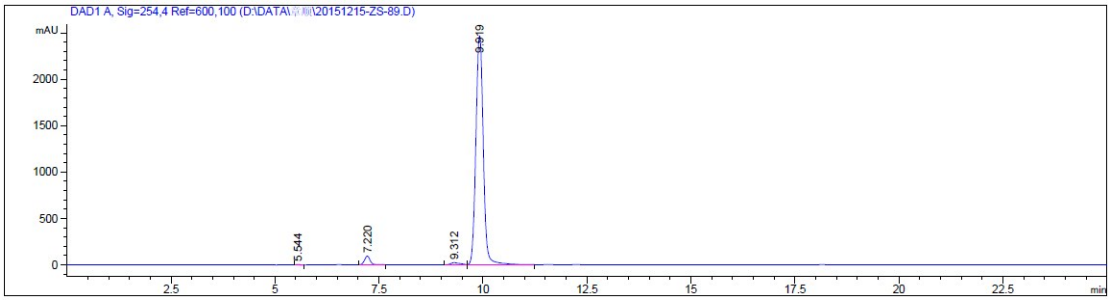
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.548	BV	0.0903	15.71390	2.68394	0.1683
2	8.402	MM	0.1537	450.36014	48.83542	4.8244
3	8.857	MM	0.1785	8868.97266	828.01196	95.0073

5e



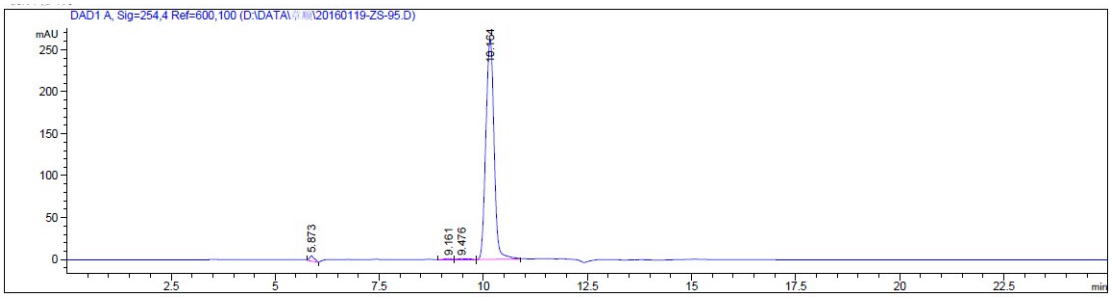
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	8.426	BB	0.2175	30.49657	1.91793	0.3650
2	9.285	BB	0.2288	101.01812	6.11150	1.2092
3	10.337	BB	0.2514	8222.62598	449.47678	98.4258

5f



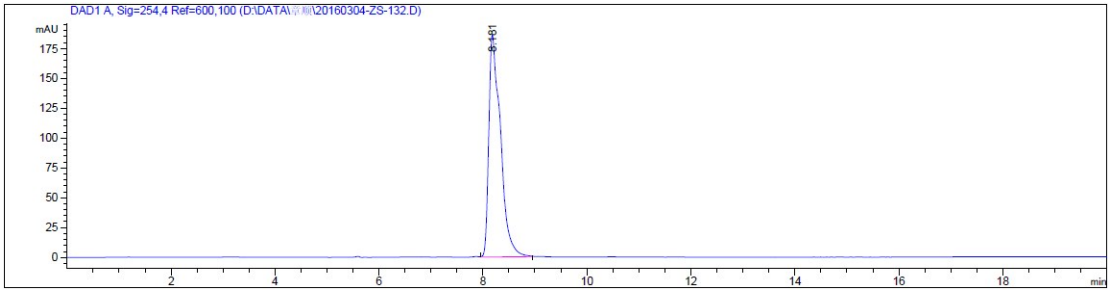
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.544	BB	0.0821	30.13395	5.48157	0.0947
2	7.220	BB	0.1431	874.80896	93.51264	2.7493
3	9.312	BV	0.2183	362.07480	23.68715	1.1379
4	9.919	VB	0.1924	3.05517e4	2468.21704	96.0180

5g



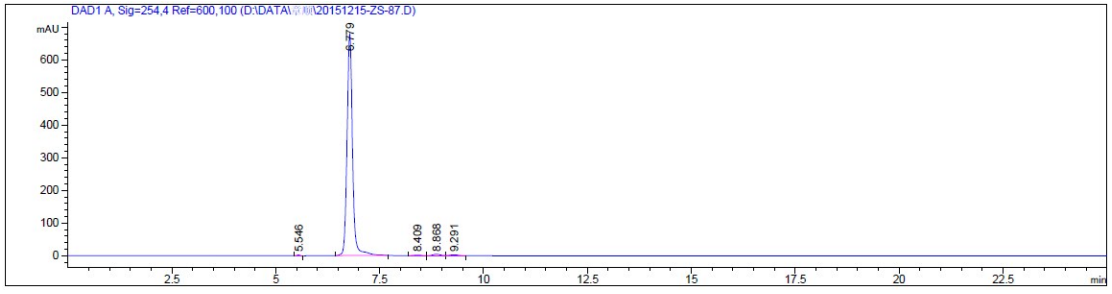
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.873	BV	0.1123	51.53109	6.37678	1.3986
2	9.161	BV	0.1911	14.41676	1.20895	0.3913
3	9.476	VV	0.3033	23.73493	1.05976	0.6442
4	10.164	VB	0.2201	3594.84668	262.02167	97.5660

5h



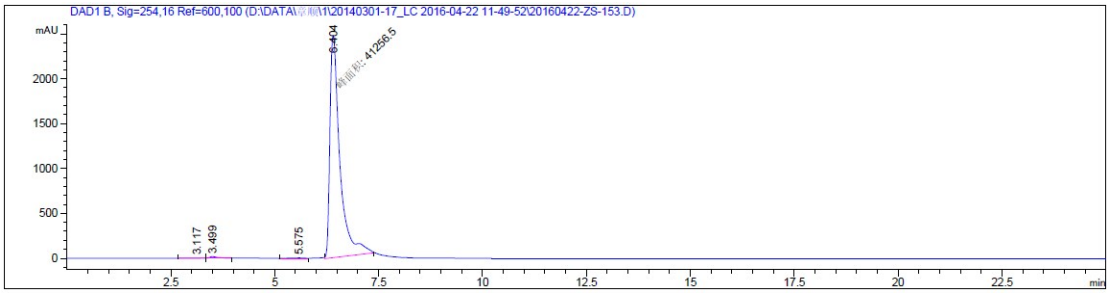
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	8.181	BB	0.2138	2906.07471	186.52052	100.0000

10a



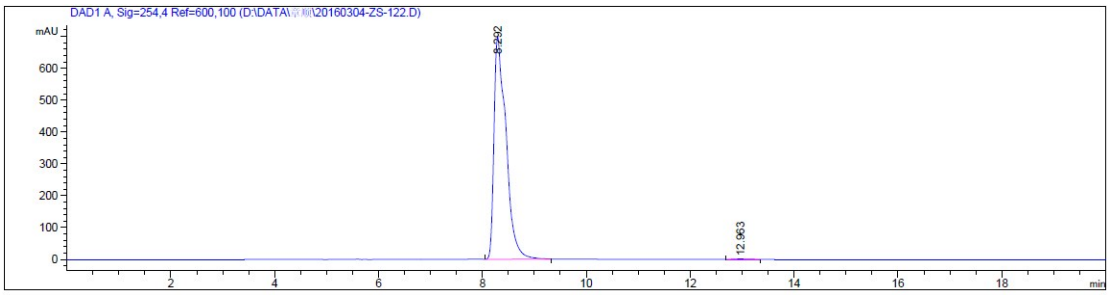
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.546	BV	0.0906	16.03418	2.72853	0.2531
2	6.779	BB	0.1389	6219.03418	679.02905	98.1823
3	8.409	BV	0.1917	16.36669	1.34712	0.2584
4	8.868	VV	0.1700	50.14239	4.49978	0.7916
5	9.291	VB	0.1918	32.59187	2.50542	0.5145

10b



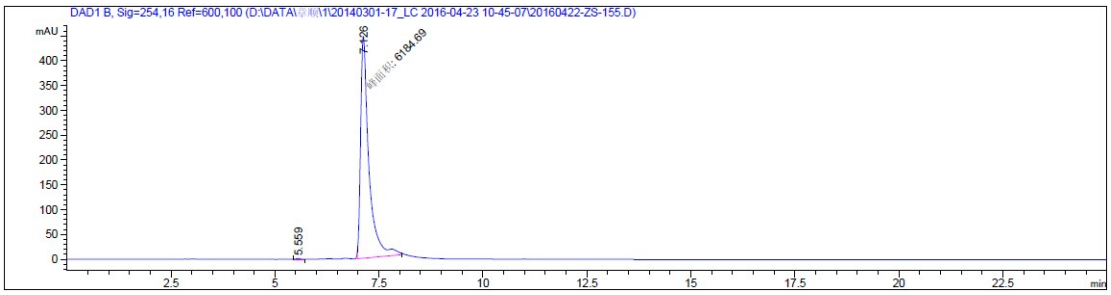
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	3.117	BV	0.1272	30.20098	3.16855	0.0725
2	3.499	VB	0.1867	231.96599	17.72483	0.5568
3	5.575	BV	0.2751	140.16789	6.46084	0.3365
4	6.404	MM	0.2777	4.12565e4	2475.90112	99.0342

11



峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	8.292	BB	0.2212	1.12037e4	698.16962	99.7690
2	12.963	BB	0.2616	25.93790	1.47176	0.2310

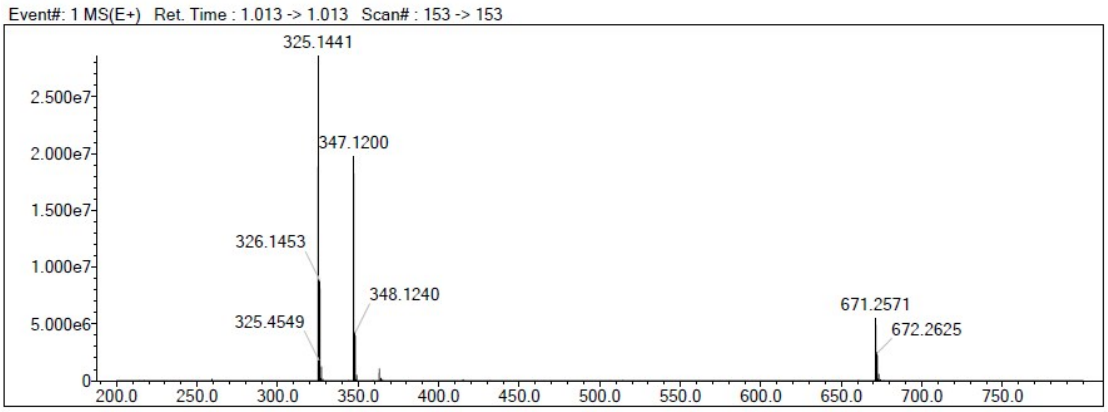
12

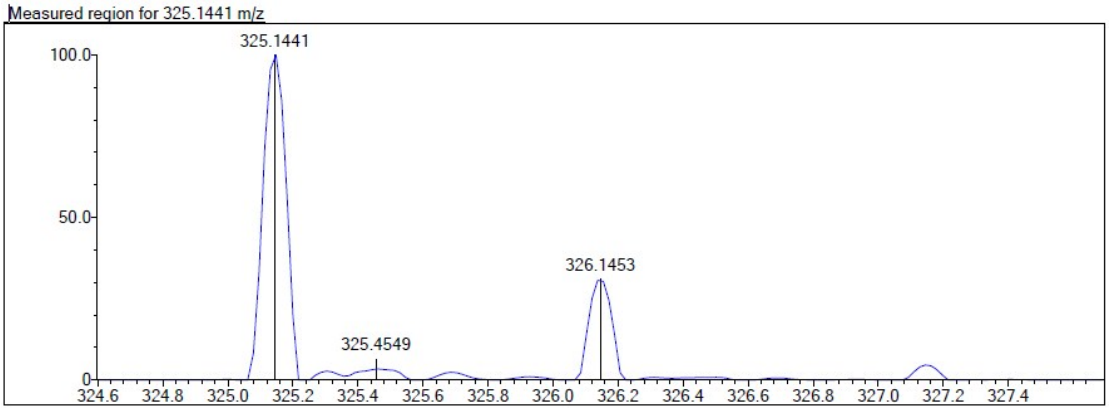


峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.559	BB	0.0922	8.34995	1.42908	0.1348
2	7.126	MM	0.2305	6184.69385	447.12988	99.8652

SI5. HR-MS of target compounds and some of intermediate compounds

5a

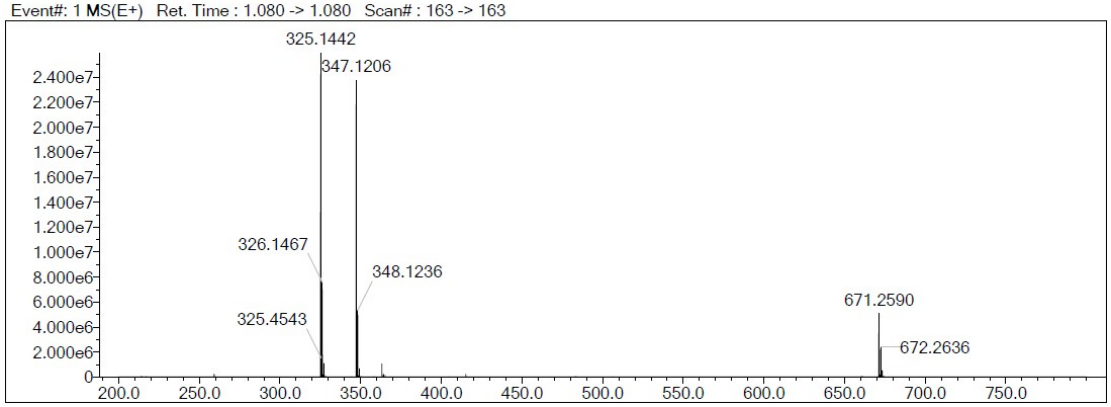




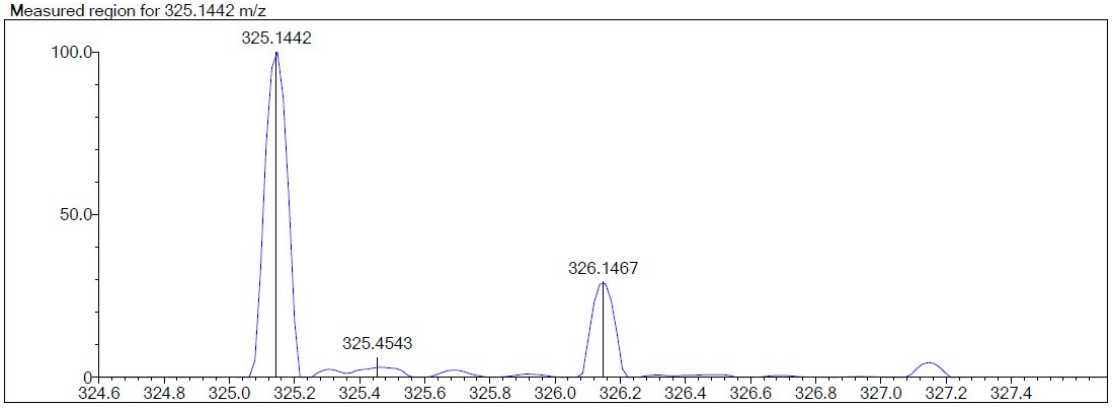
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	58.24	C20 H20 O4	[M+H]+	325.1441	325.1434	0.7	2.15	59.97	11.0

5b

Event#: 1 MS(E+) Ret. Time : 1.080 -> 1.080 Scan# : 163 -> 163



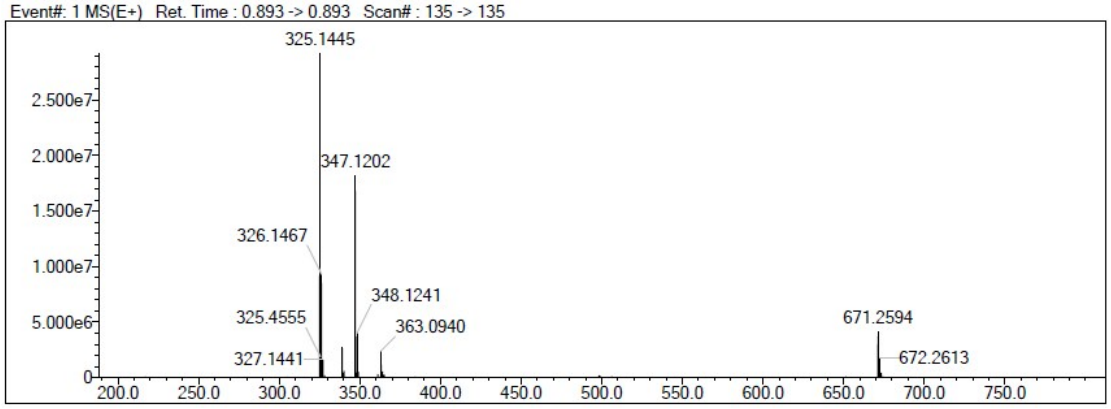
Measured region for 325.1442 m/z



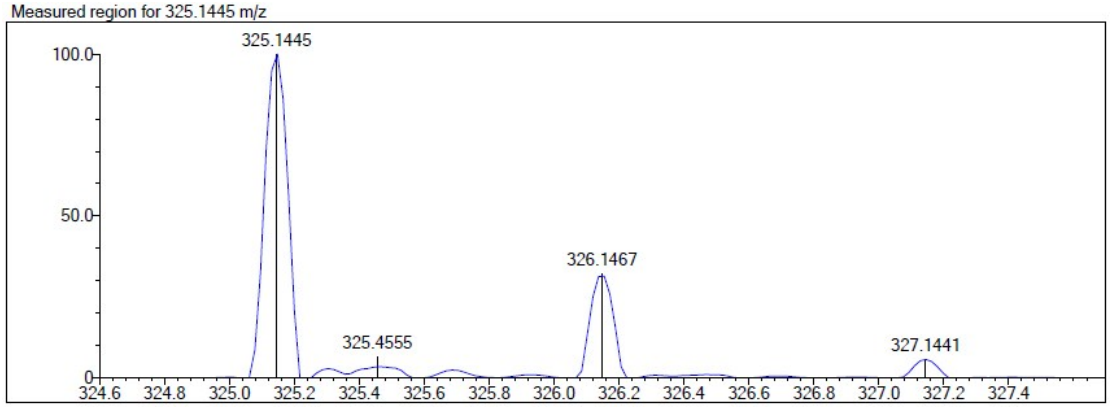
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	61.80	C20 H20 O4	[M+H]+	325.1442	325.1434	0.8	2.46	64.14	11.0

5c

Event#: 1 MS(E+) Ret. Time : 0.893 -> 0.893 Scan# : 135 -> 135

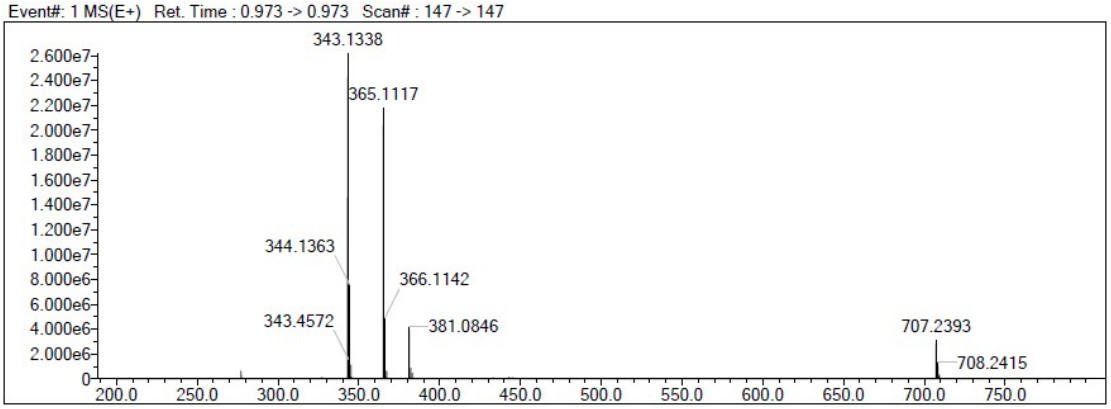


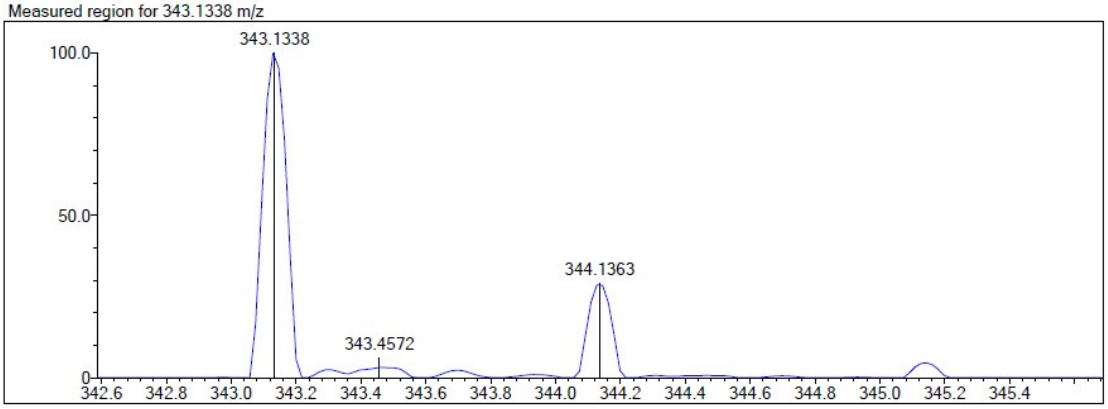
Measured region for 325.1445 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	49.62	C20 H20 O4	[M+H]+	325.1445	325.1434	1.1	3.38	52.76	11.0

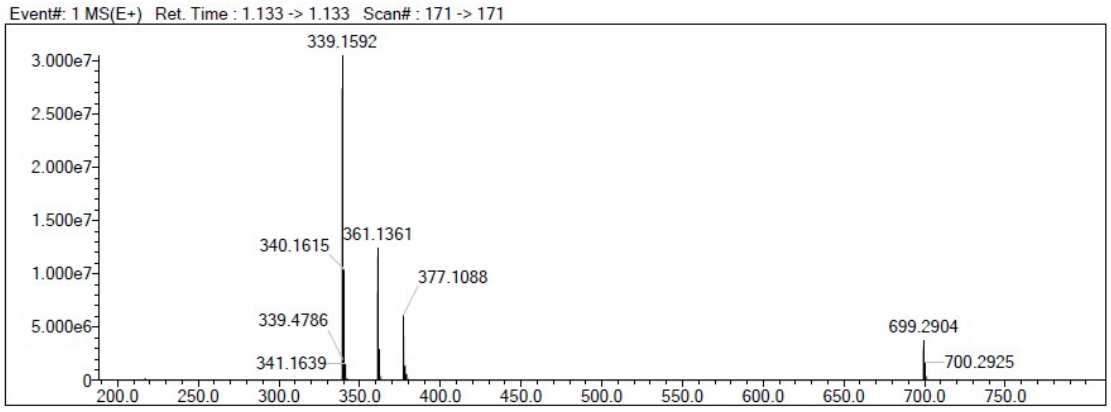
5d

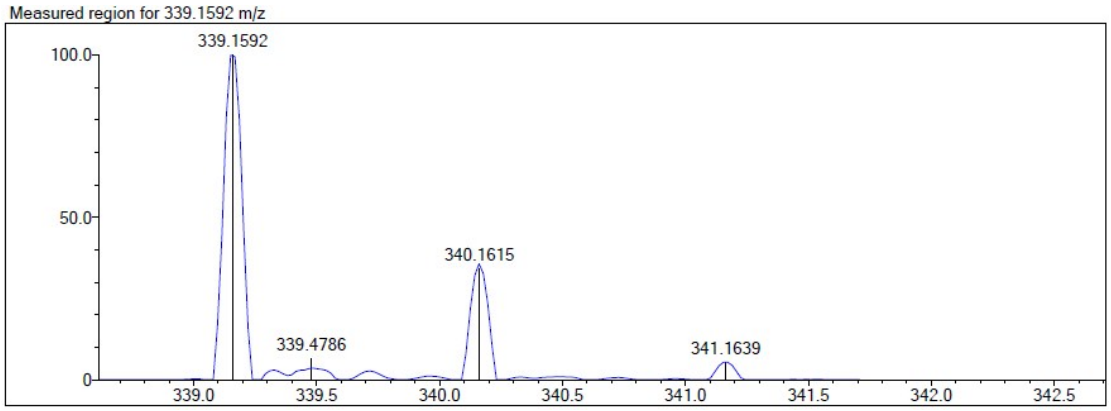




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	74.47	C20 H19 O4 F	[M+H]+	343.1338	343.1340	-0.2	-0.58	74.47	11.0

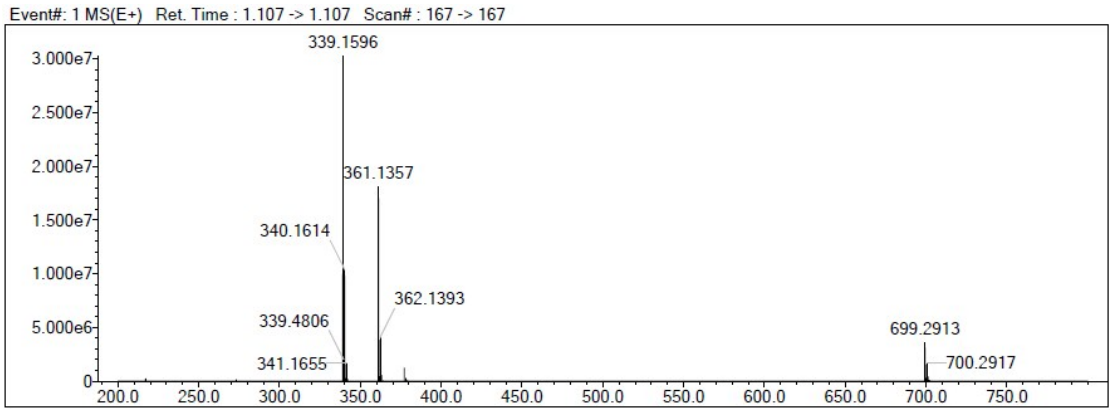
5e

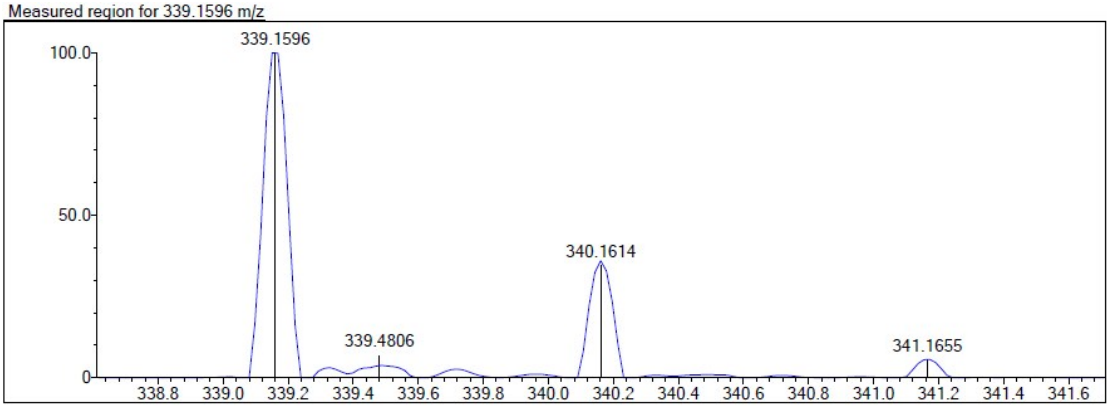




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	60.69	C21 H22 O4	[M+H]+	339.1592	339.1591	0.1	0.29	60.69	11.0

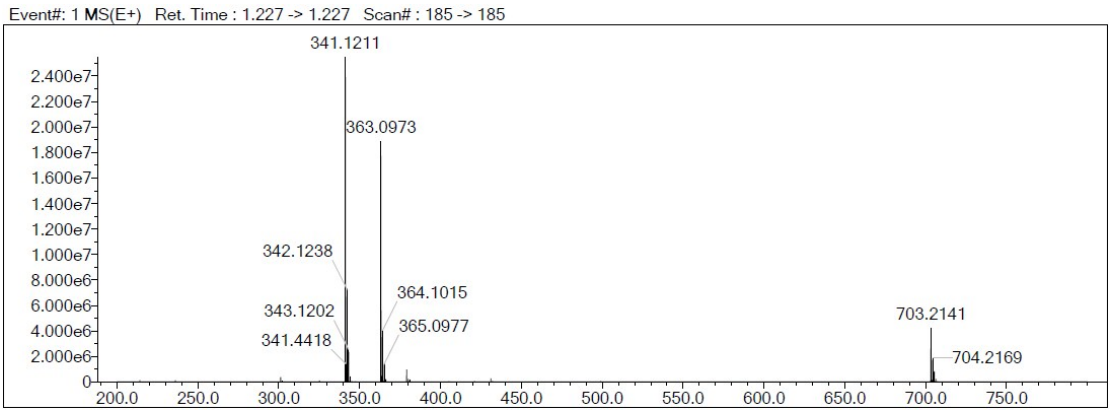
5f

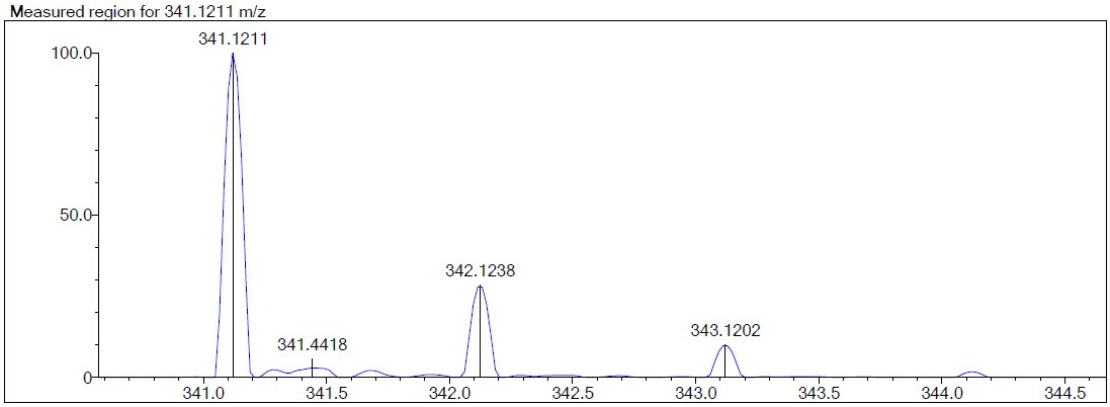




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	59.79	C21 H22 O4	[M+H]+	339.1596	339.1591	0.5	1.47	60.50	11.0

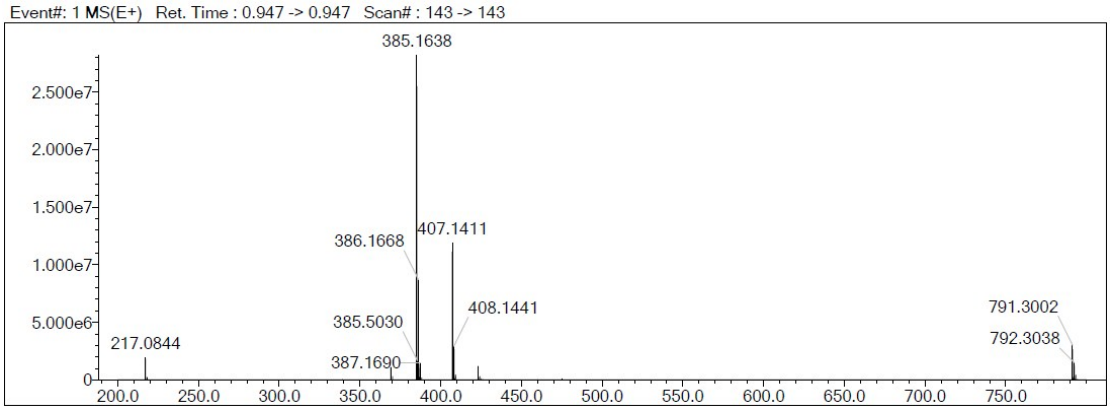
5g

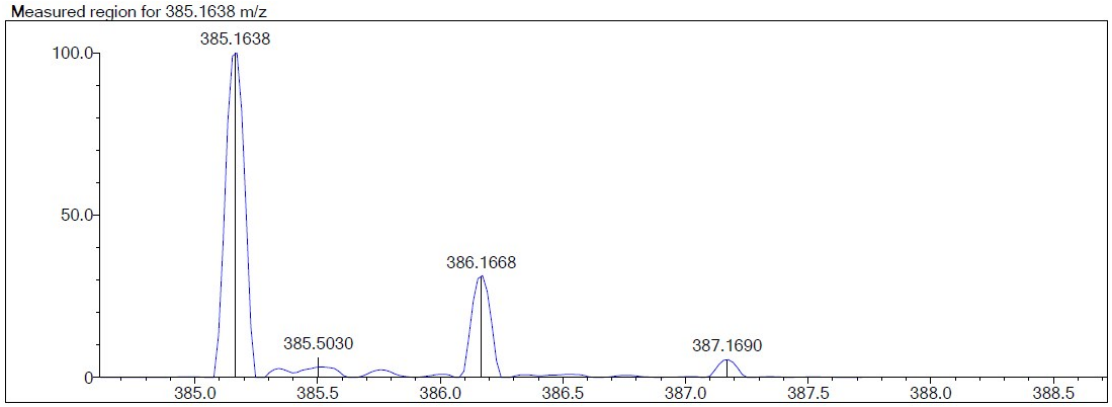




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	82.95	C20 H20 O3 S	[M+H]+	341.1211	341.1206	0.5	1.47	83.94	11.0

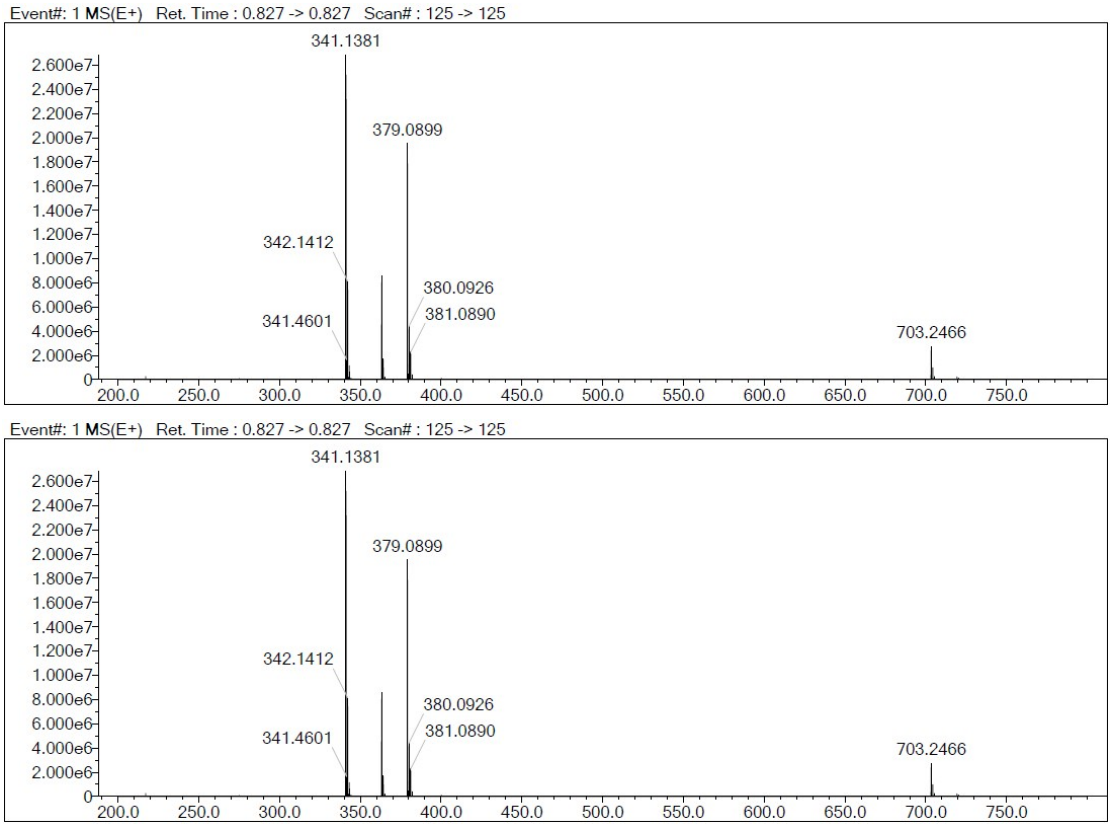
5h





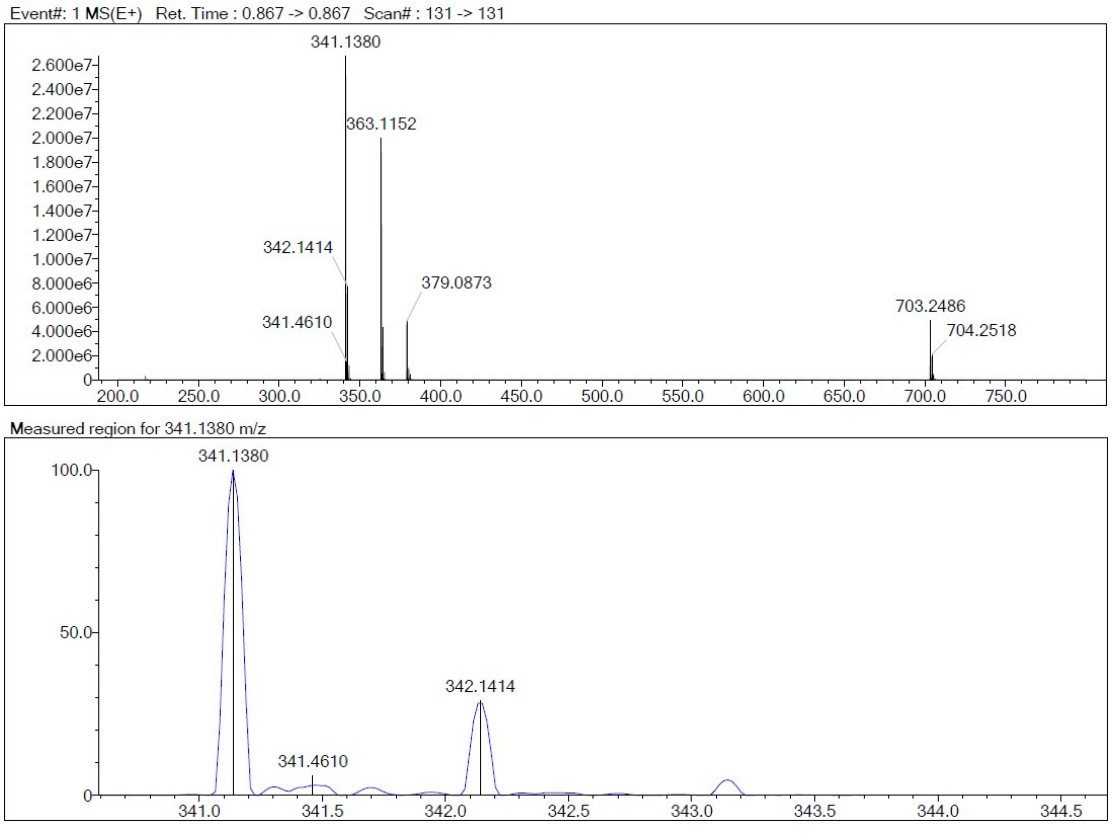
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
4	71.49	C22 H24 O6	[M+H]+	385.1638	385.1646	-0.8	-2.08	73.47	11.0

10a



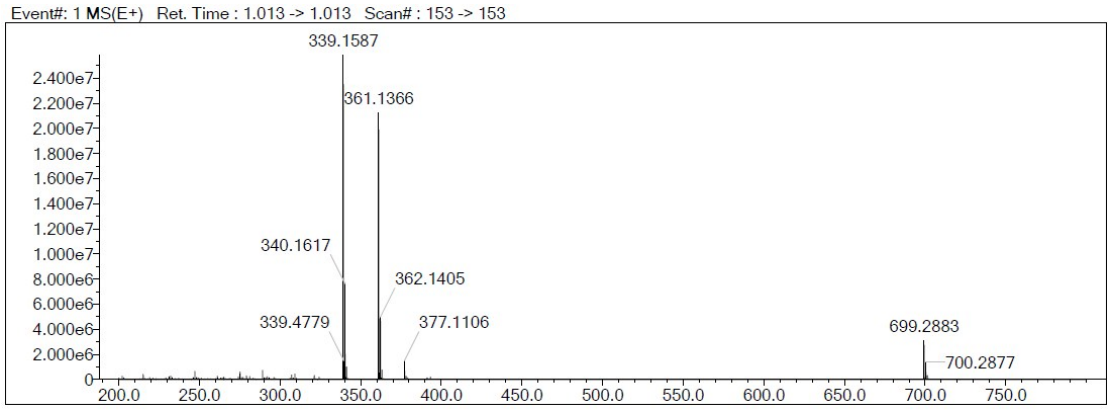
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	69.04	C20 H20 O5	[M+H]+	341.1381	341.1384	-0.3	-0.88	69.04	11.0

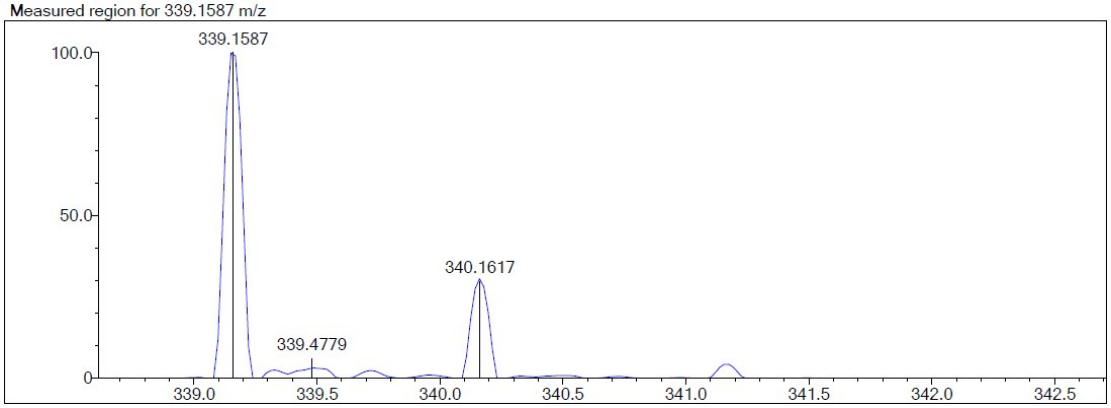
10b



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	72.09	C20 H20 O5	[M+H]+	341.1380	341.1384	-0.4	-1.17	72.40	11.0

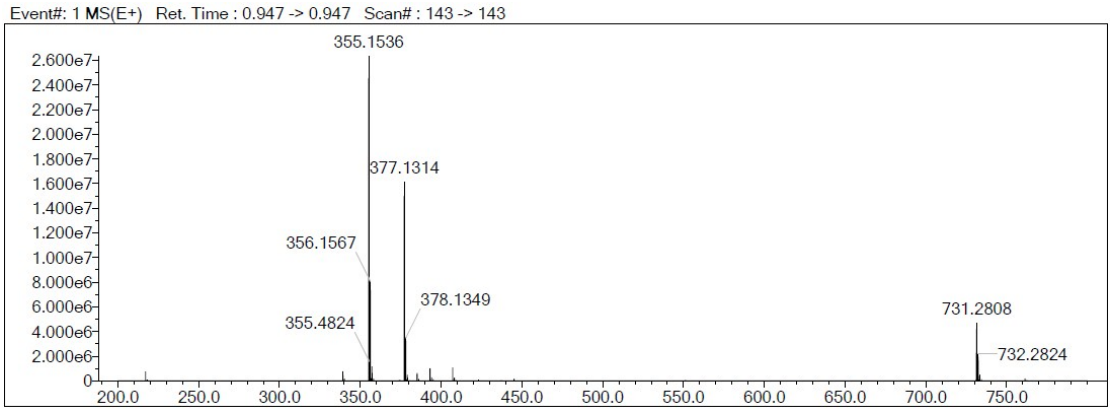
11

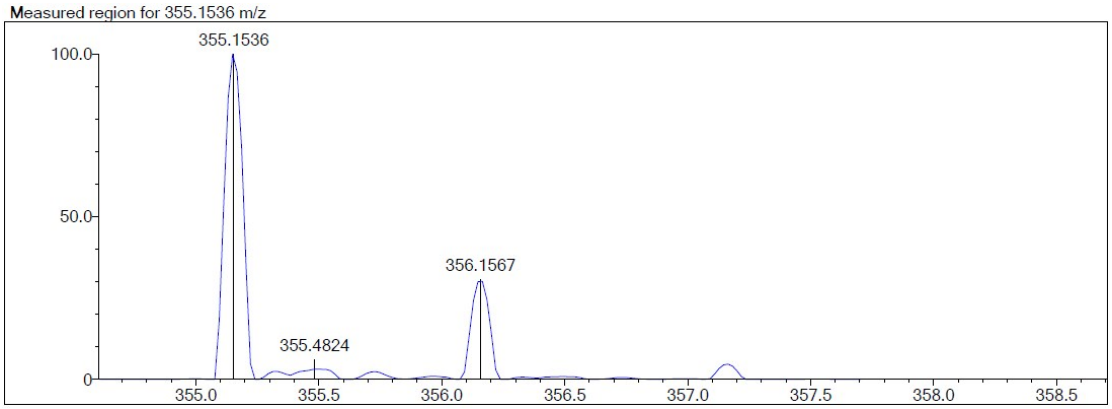




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	76.86	C21 H22 O4	[M+H]+	339.1587	339.1591	-0.4	-1.18	77.21	11.0

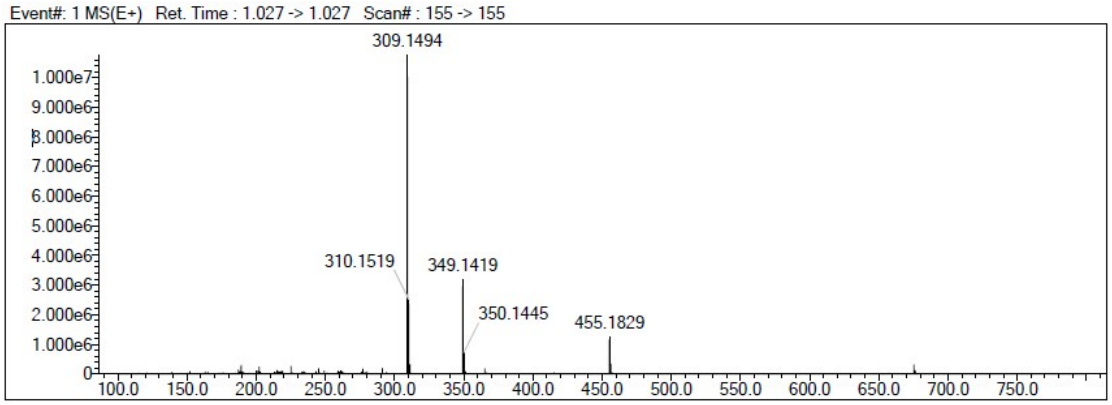
12

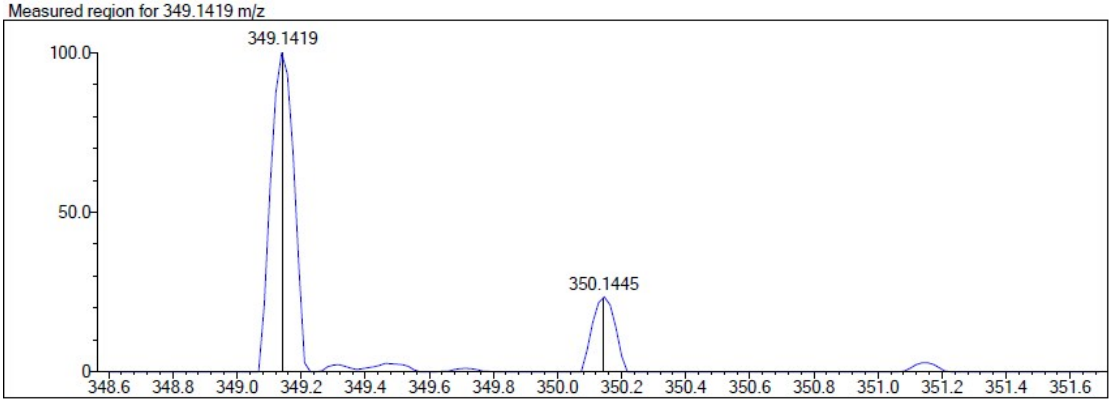




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	71.62	C21 H22 O5	[M+H]+	355.1536	355.1540	-0.4	-1.13	71.86	11.0

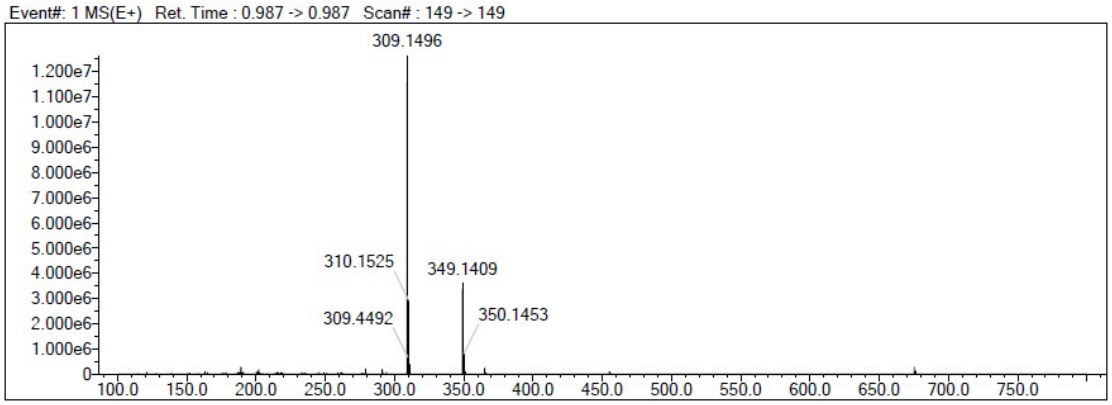
4a

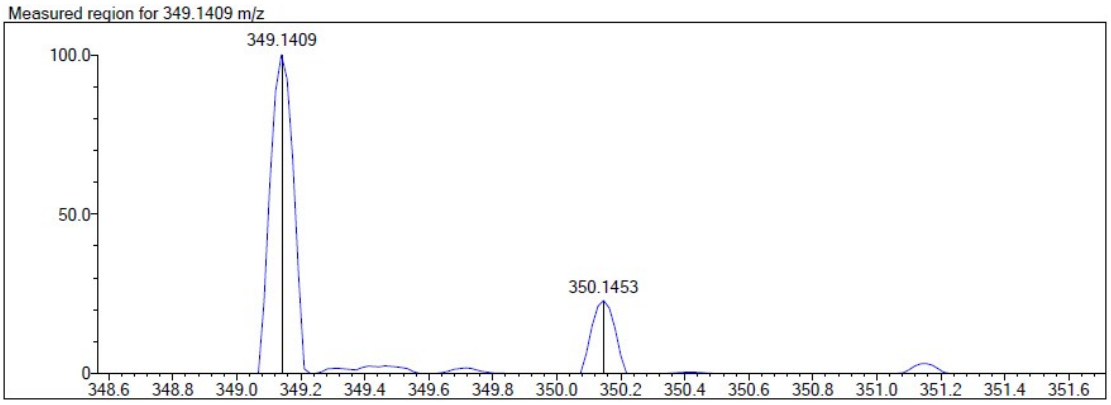




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	85.32	C20 H22 O4	[M+Na]+	349.1419	349.1410	0.9	2.58	88.83	10.0

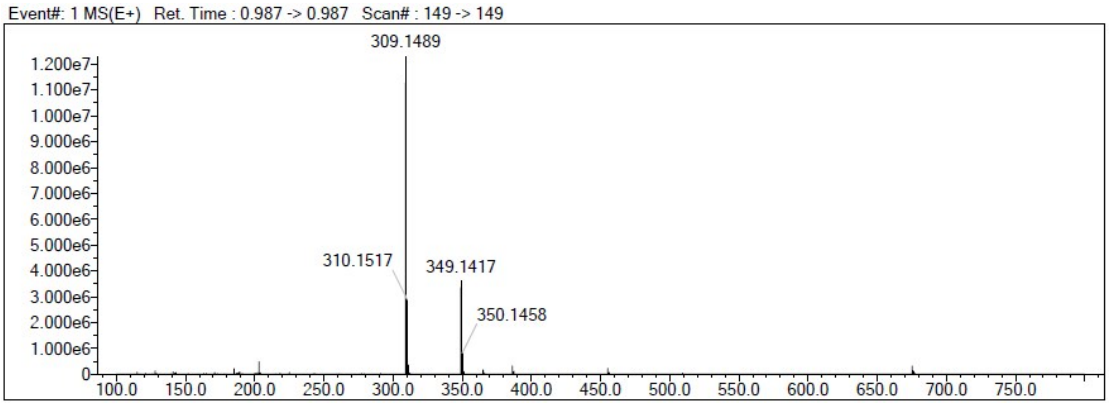
4b

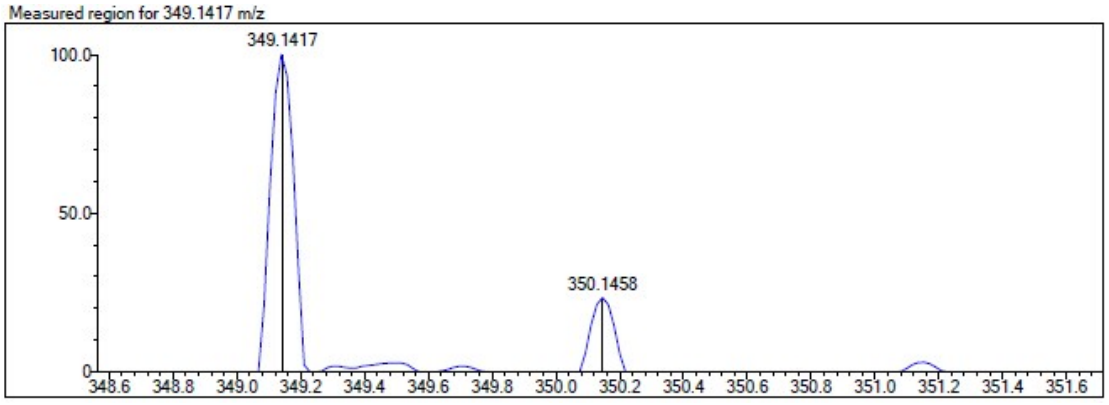




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	88.52	C20 H22 O4	[M+Na]+	349.1409	349.1410	-0.1	-0.29	88.52	10.0

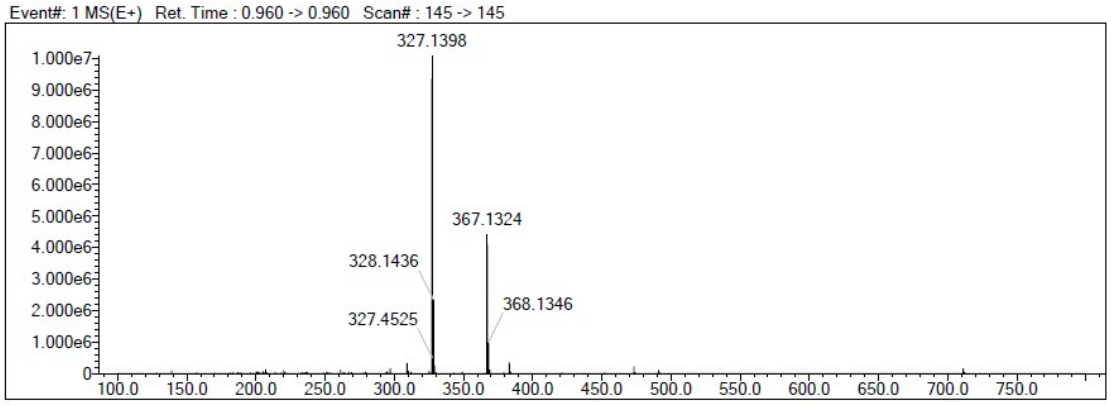
4c

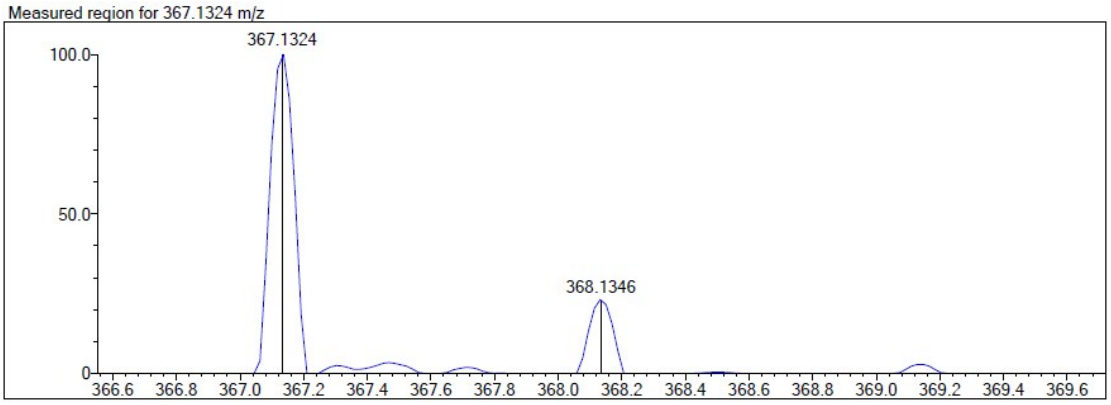




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	85.88	C20 H22 O4	[M+Na]+	349.1417	349.1410	0.7	2.00	88.08	10.0

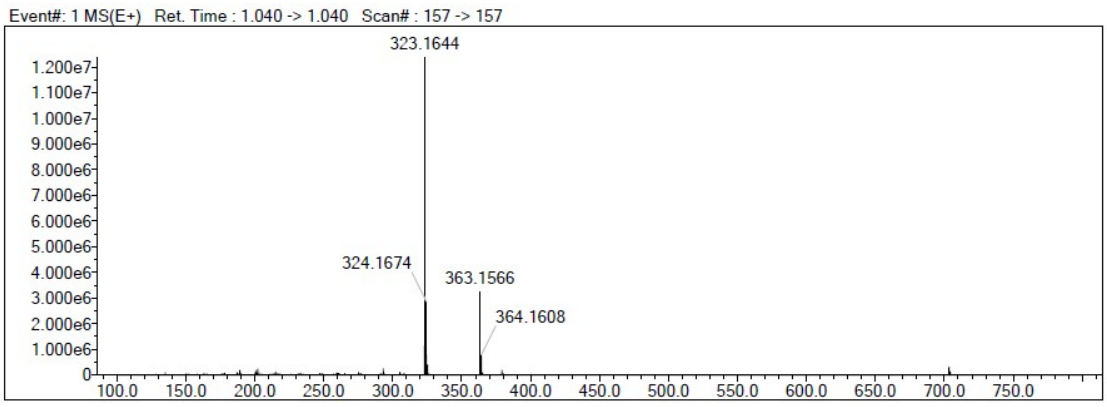
4d

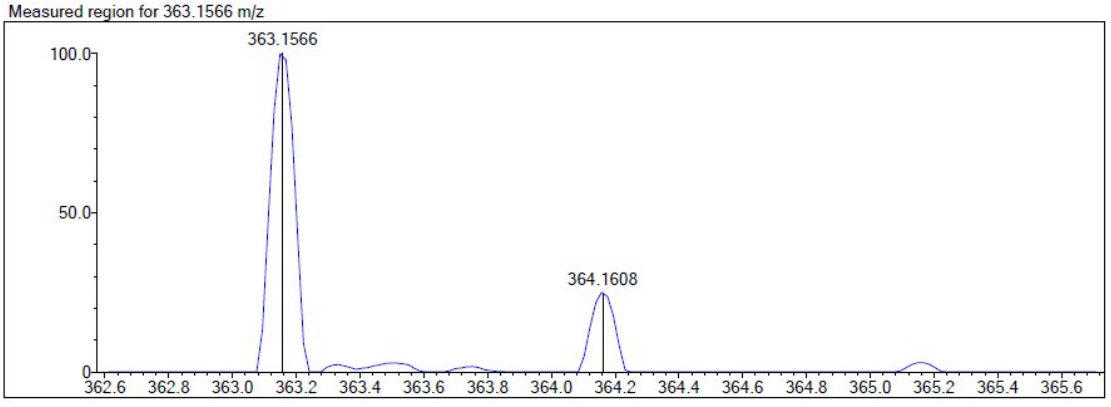




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	77.37	C20 H21 O4 F	[M+Na]+	367.1324	367.1316	0.8	2.18	79.72	10.0

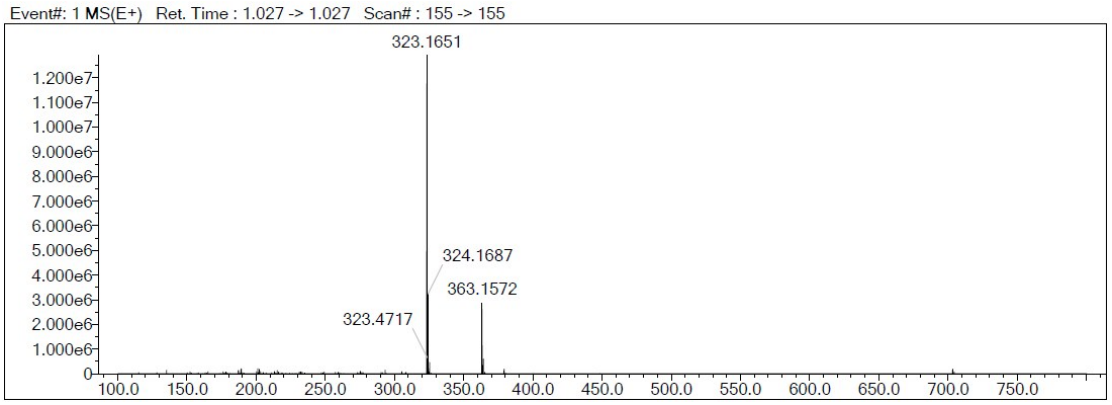
4e

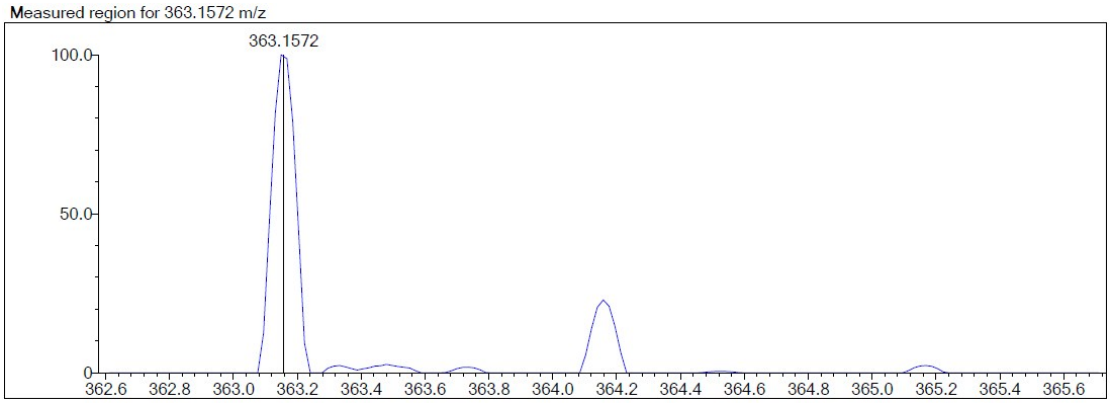




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	85.60	C21 H24 O4	[M+Na]+	363.1566	363.1567	-0.1	-0.28	85.60	10.0

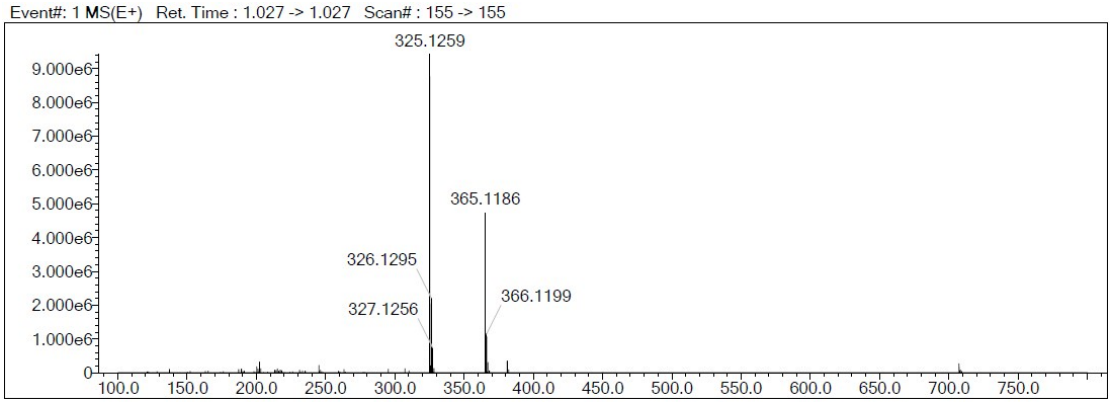
4f

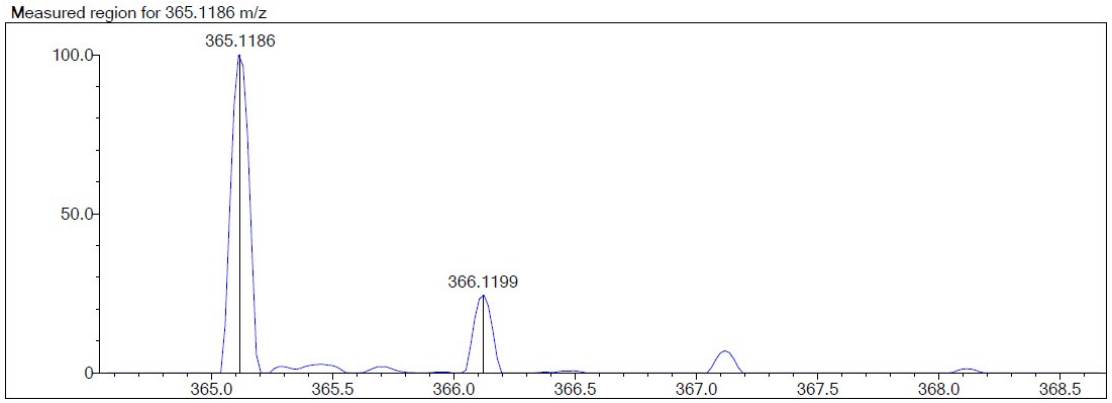




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	88.22	C21 H24 O4	[M+Na]+	363.1572	363.1567	0.5	1.38	89.06	10.0

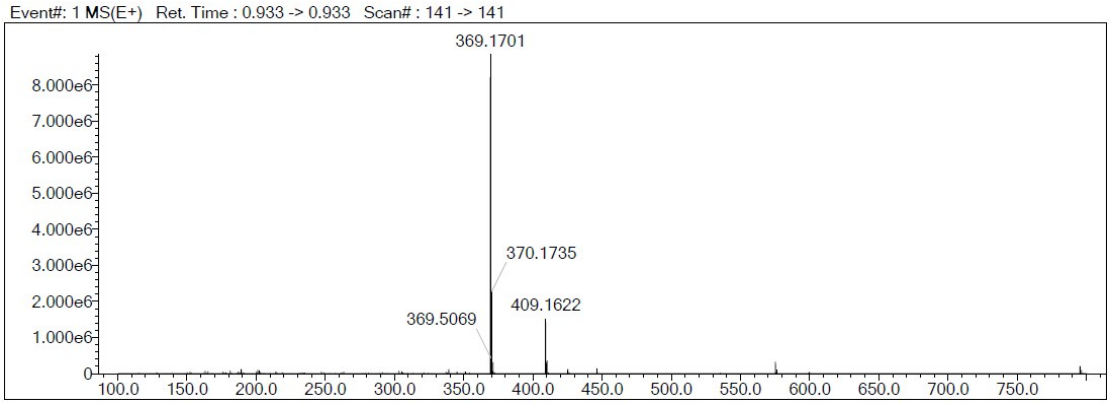
4g

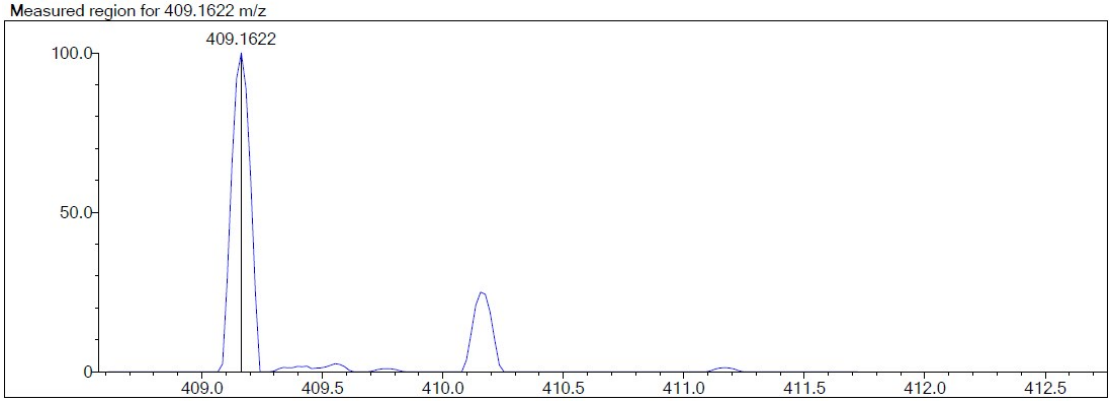




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	82.52	C20 H22 O3 S	[M+Na]+	365.1186	365.1182	0.4	1.10	82.72	10.0

4h

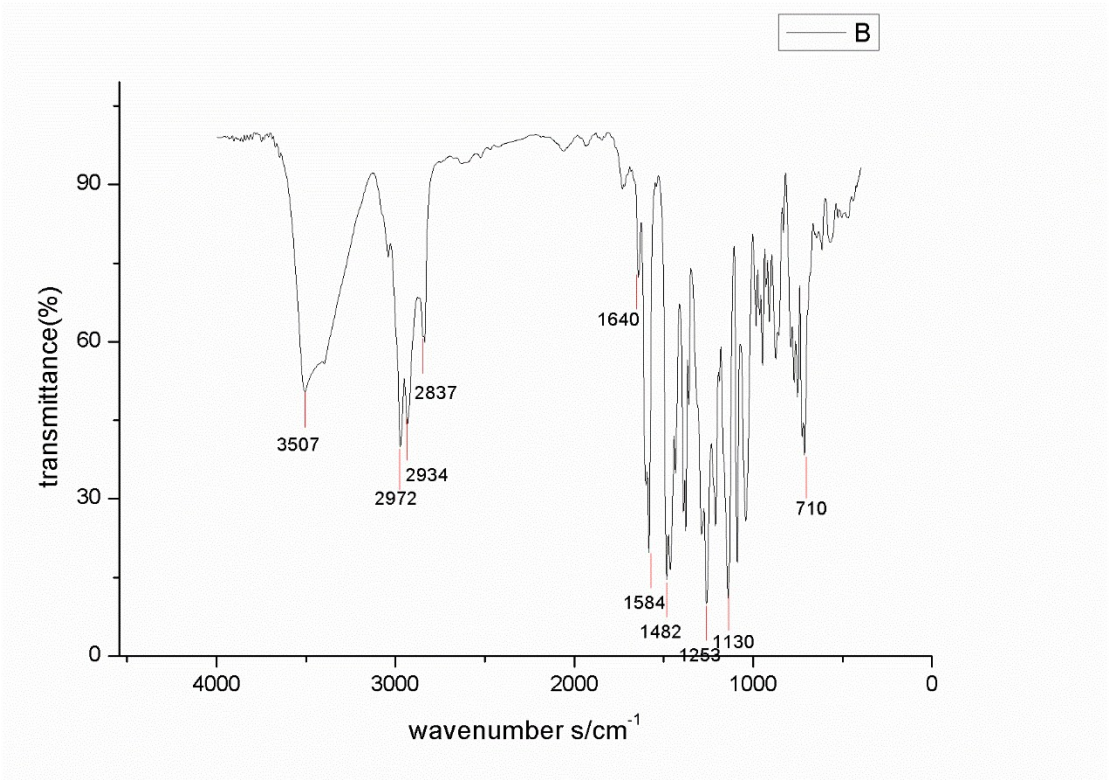




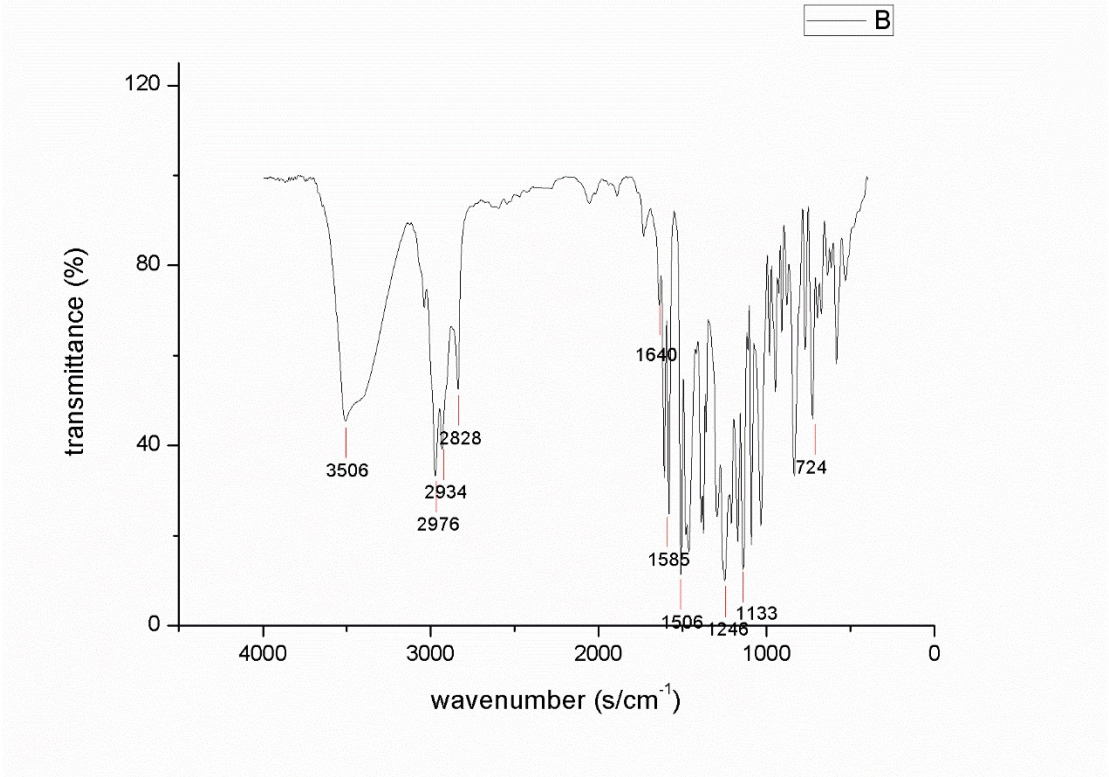
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	77.46	C22 H26 O6	[M+Na]+	409.1622	409.1622	0.0	0.00	77.46	10.0

SI6. IR of some of intermediate compounds

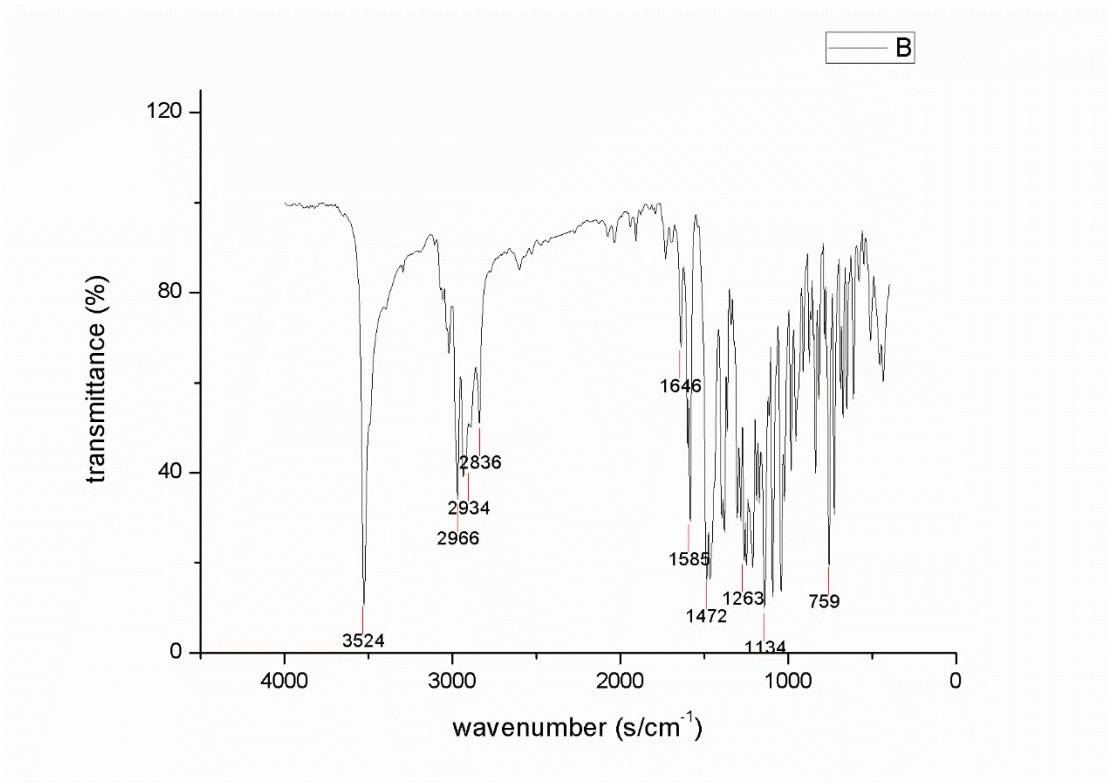
4a



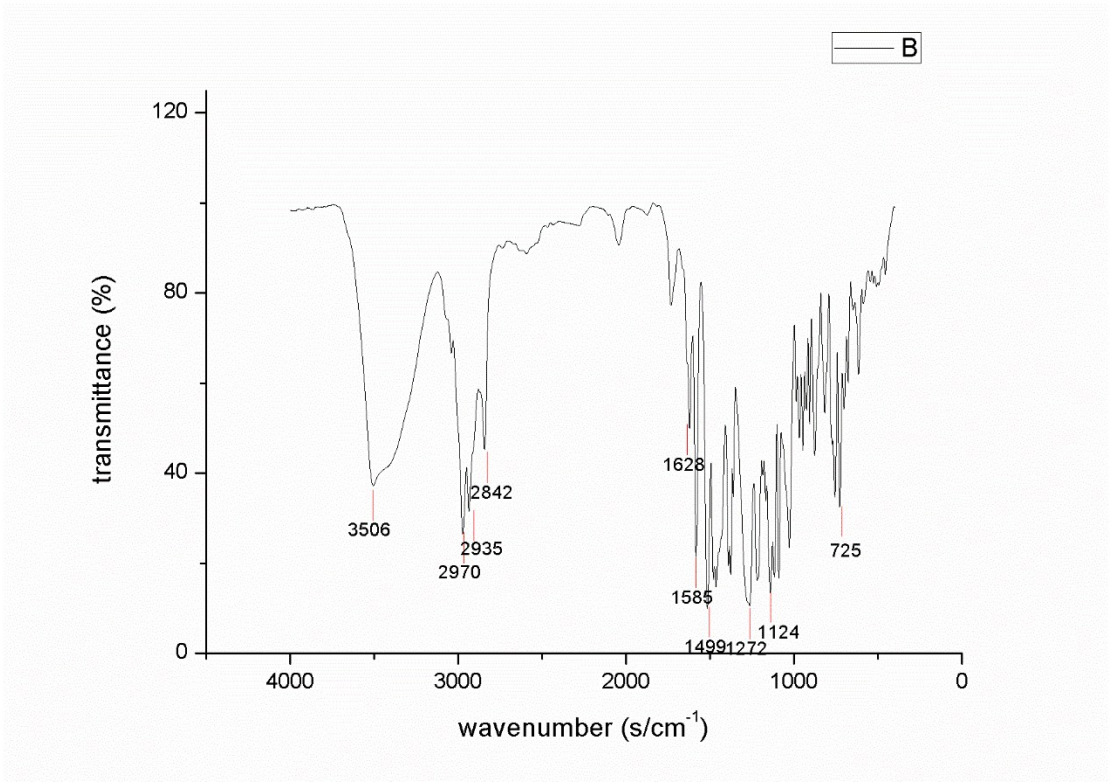
4b



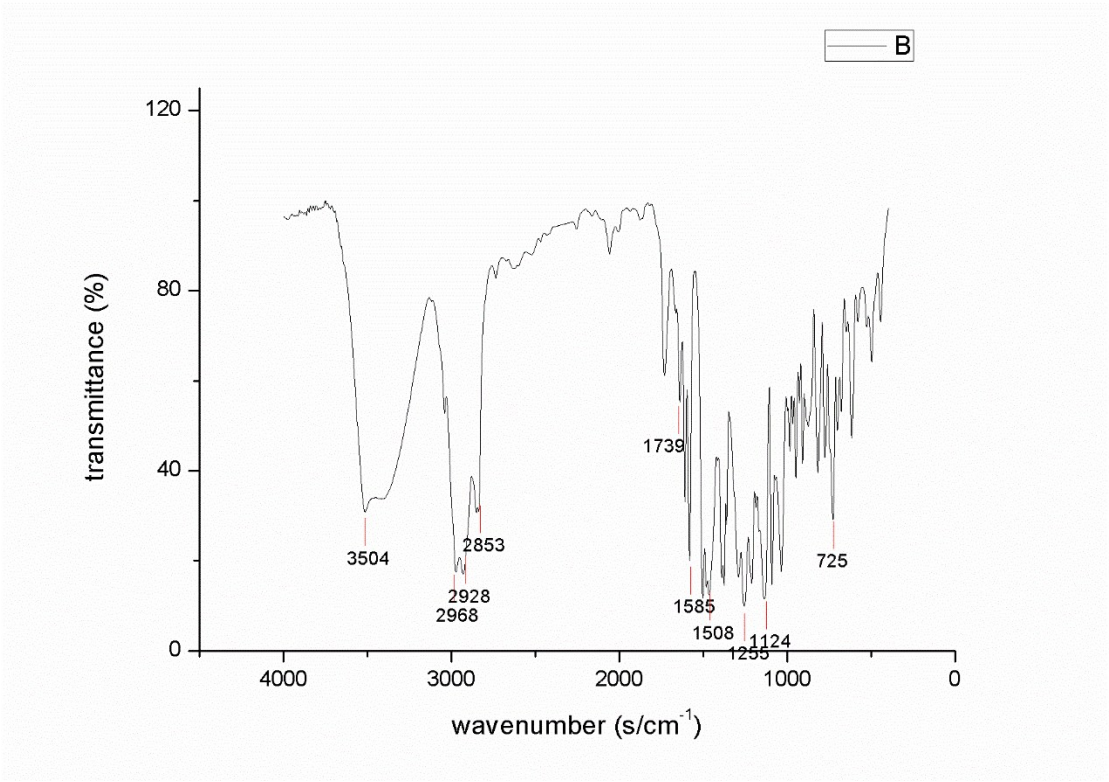
4c


B
120
80
40
0
transmittance (%)
3524
2966
2934
2836
1646
1585
1472
1263
1134
759
4000
3000
2000
1000
0
wavenumber (s/cm-1)

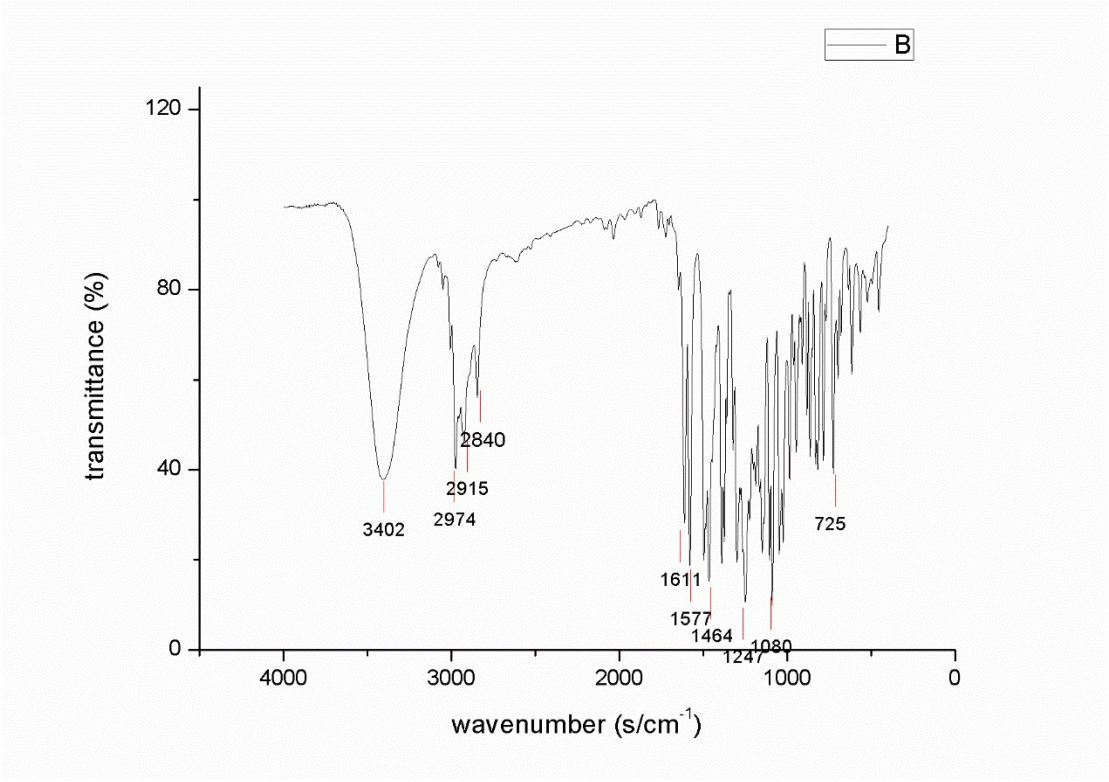
4d


B
120
80
40
0
transmittance (%)
3506
2970
2935
2842
1628
1585
1499
1272
1124
725
4000
3000
2000
1000
0
wavenumber (s/cm-1)

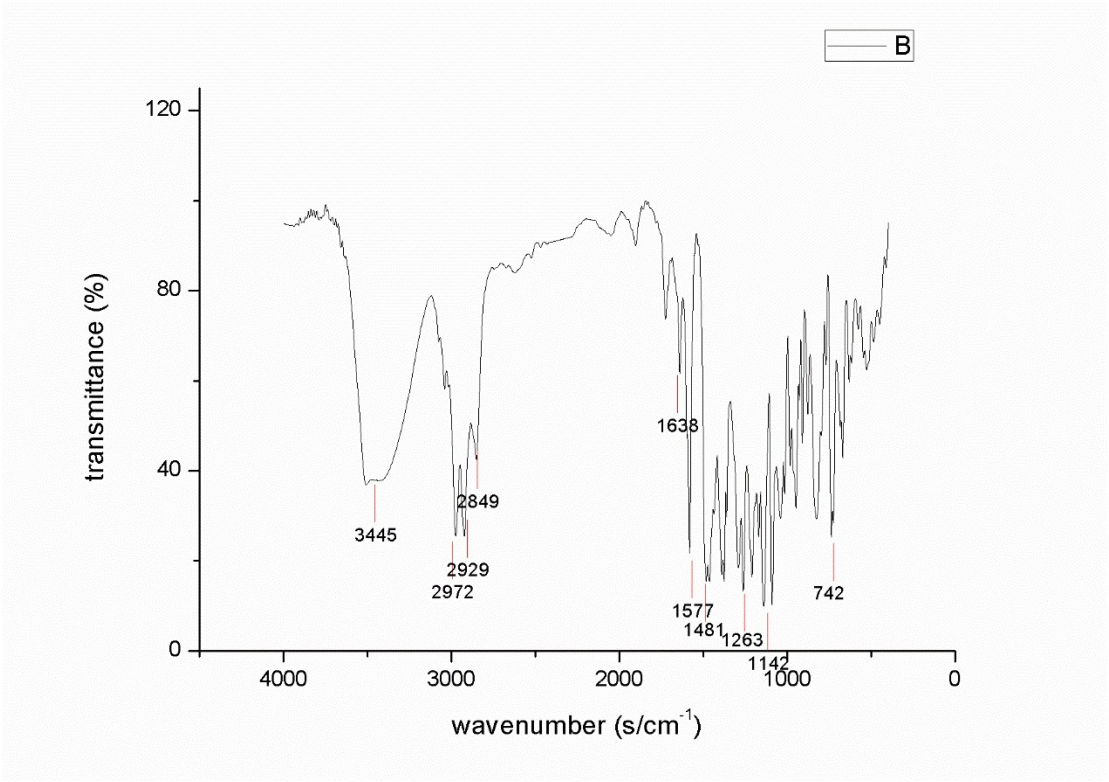
4e


B
120
80
40
0
transmittance (%)
3504
2928
2968
2853
1739
1585
1508
1255
1124
725
4000
3000
2000
1000
0
wavenumber (s/cm-1)

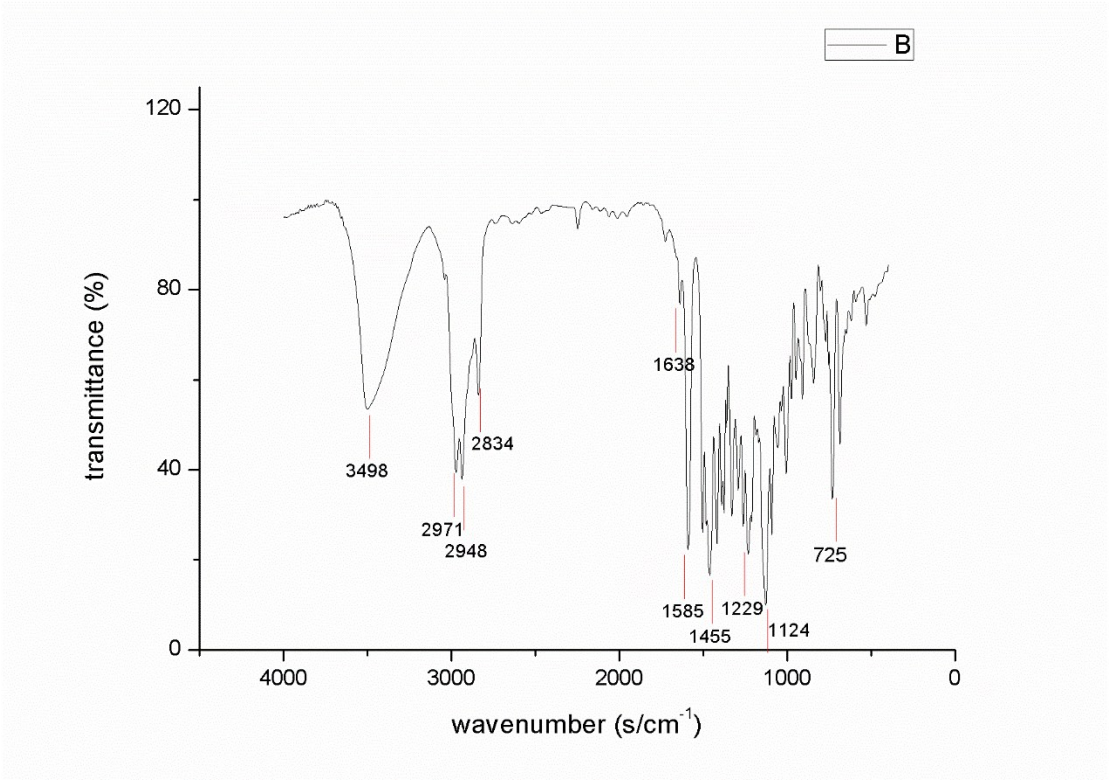
4f


B
120
80
40
0
transmittance (%)
3402
2974
2915
2840
1611
1577
1464
1247
1080
725
4000
3000
2000
1000
0
wavenumber (s/cm-1)

4g


B
120
80
40
0
transmittance (%)
3445
2849
2929
2972
1638
1577
1481
1263
1142
742
4000
3000
2000
1000
0
wavenumber (s/cm-1)

4h


B
120
80
40
0
transmittance (%)
3498
2834
2971
2948
1638
1585
1455
1229
1124
725
4000
3000
2000
1000
0
wavenumber (s/cm-1)