Supporting Information

Synthesis, in vitro and in vivo evaluation of new hybrids of millepachine and phenstatin as potent tubulin polymerization inhibitors

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SI1. Biological assay

MTT assay. The cells grown in the logarithmic phase were seeded into a 96-well plates (5×103 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).

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 MgCl2, 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 60 sec 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, 3×10^4 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 0.1% 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 (3×10^5cells/well) and incubated in the presence / absence of compound 5i 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 12,000 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 CaCl2 at pH 7.4) at room temperature for 15 min. PI solution was then added to the medium for another 10 min-incubation. Almost 12,000 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,

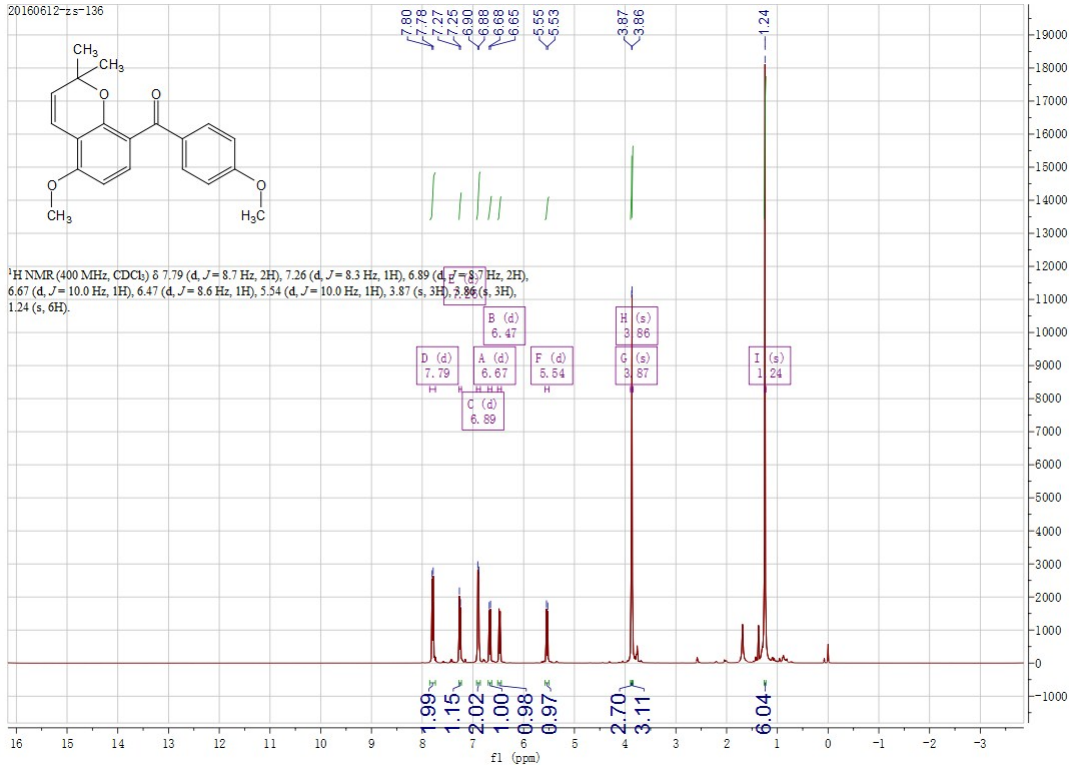
which indicated by green fluorescence. We applied two methods which including flow cytometry and fluorescence microscopy to detected the MMP. For flow cytometry analysis, A549 cells were plated in 6-well plates (3×10^5 cells/well) and grown for 24 h, and treated with compound 10a at the indicated concentrations 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 (3×10^5 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).

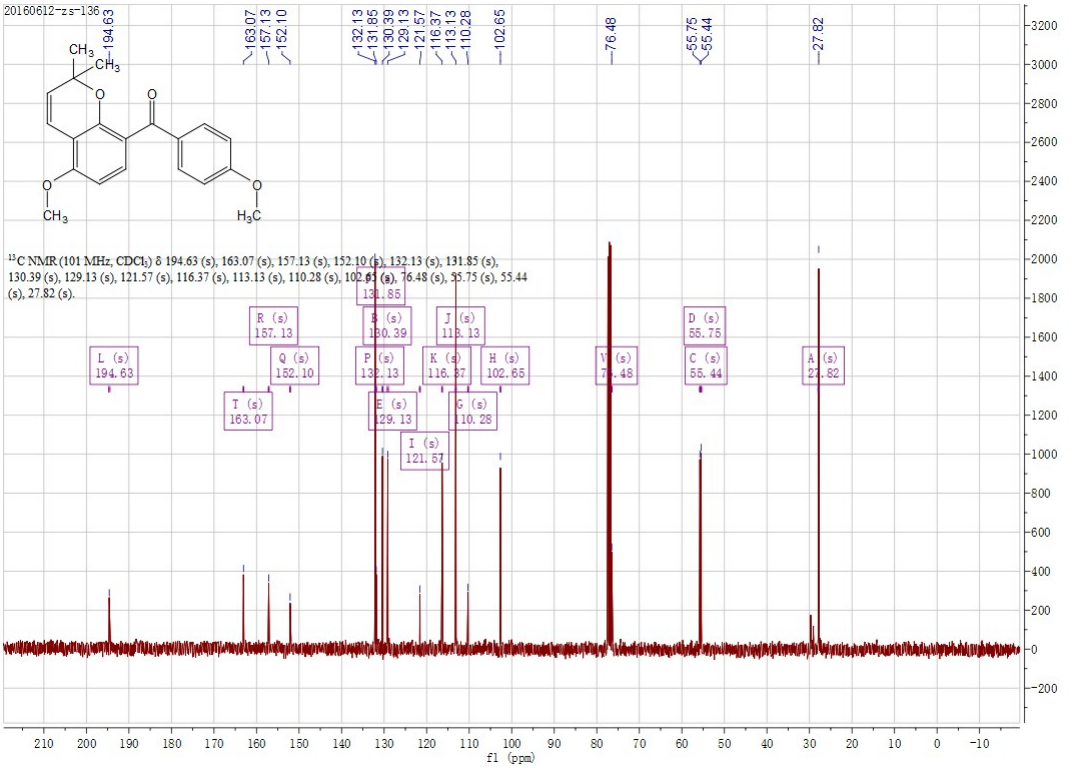
Western bolt analysis. A549 cells (5.0×10^5 cells/dish) were incubated with or without 5i at various concentrations for 24 h or 48 h. After incubation, the cells were collected by centrifugation and washed twice with phosphate-buffered saline chilled to 0 °C. The cells were then homogenised in RIPA lysis buffer containing 150 mM NaCl, 50 mM Tris (pH 7.4), 1% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100, 0.1% (w/v) SDS and 1 mM EDTA (Beyotime, China). The lysates were incubated on ice for 30 min, intermittently vortexed every 5 min, and centrifuged at 15,000 × g for 15 min to harvest the super-natants. Next, the protein concentrations were determined by a BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, Illinois, USA). The protein extracts were reconstituted in loading buffer containing 62 mM Tris–HCl, 2% SDS, 10% glycerol, and 5% b-mercaptoethanol (Beyotime, China), and the mixture was boiled at 100 °C for 3 min. An equal amount of the proteins (50 mg) were separated by 8–12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred to nitrocellulose membranes (Amersham Biosciences, Little Chalfont, Buckingham-shire, UK). Then, the membranes were blocked with 5% non-fat dried milk in TBS containing 1% Tween-20 for 90 min at room temperature and were then incubated overnight with specific primary antibodies (CST, USA) at 4 °C. After three washes in TBST, the membranes were incubated with the appropriate HRP- conjugated secondary antibodies at room temperature for 2 h. The blots were developed with enhanced chemiluminescence (Pierce, Rockford, Illinois, USA) and were detected by an LAS4000 imager (GE Healthcare, Waukesha, Wisconsin, USA). The intensities of the blots were quantified by ImageQuantTL (GE Healthcare) software.

Anti-tumour effect of 5IP in a xenograft model in vivo. The reference compound CA-4P and the test compound 5iP were completely dissolved in isotonic saline. In the A549 xenograft studies, when the size of a growing tumour reached about 100 mm^3, the xenograft tumour-bearing nude mice were randomly placed into three groups: the vehicle-treated group, the CA-4P- treated group, and the 5iP-treated group at 9 mice per group. The mice were injected intraperitoneally (ip) with CA-4P and compound 5iP at a dose of 30 mg/kg body weight every other day for the entire period of observation, whereas mice in the control group were treated with an equivalent volume of saline. The tumour size and the body weight of the mice were measured every other day after drug treatment. At the end of the observation period, the animals were euthanized by cervical dislocation. This study conformed to the Guide for the Care and Use of Laboratory Animals as published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Institutional Ethics Review Board of Sun Yat-Sen University.

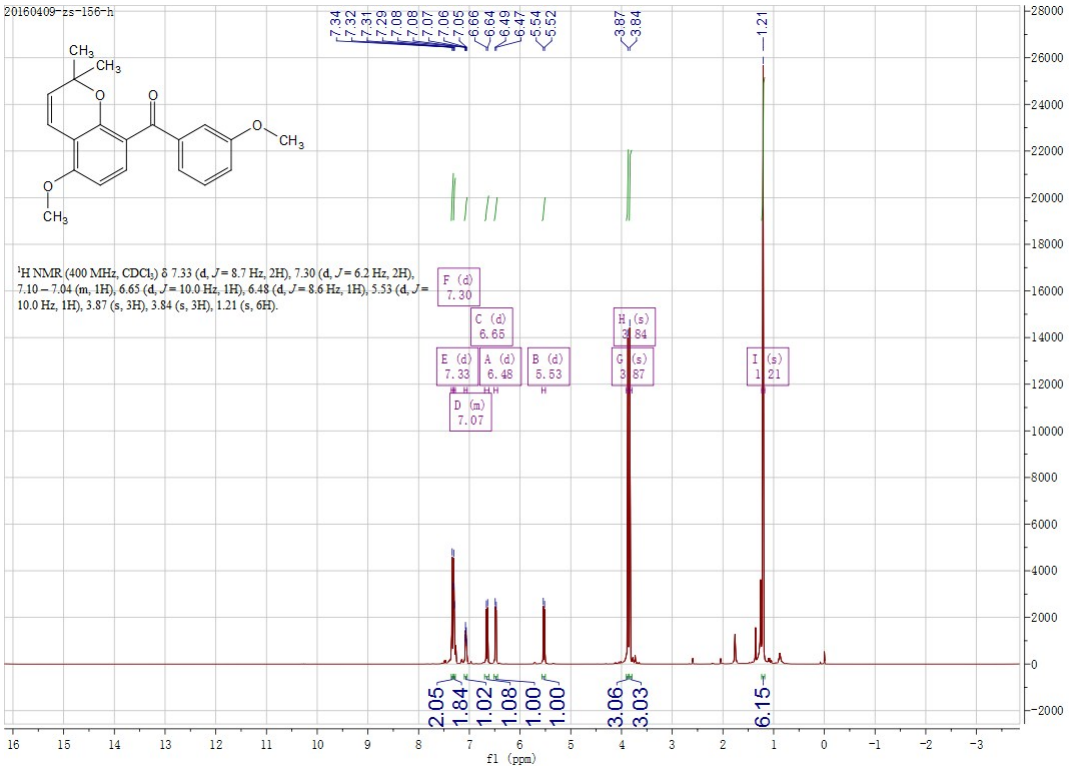
SI2. NMR spectrums of target compounds

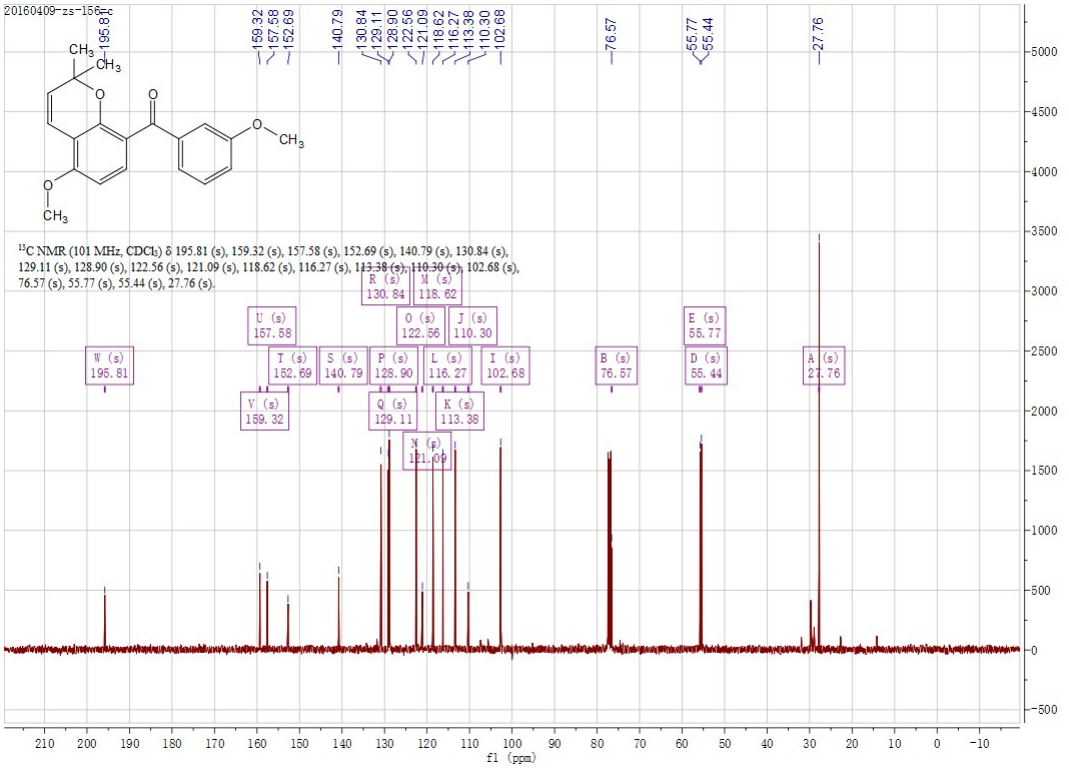
4a



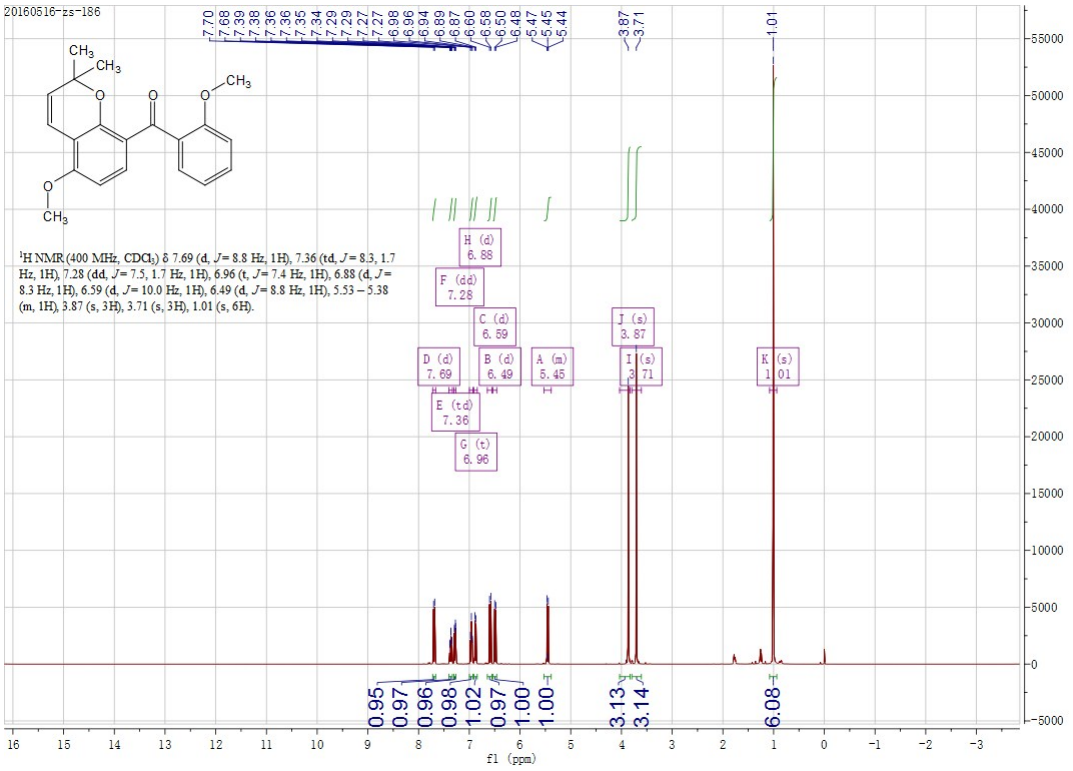


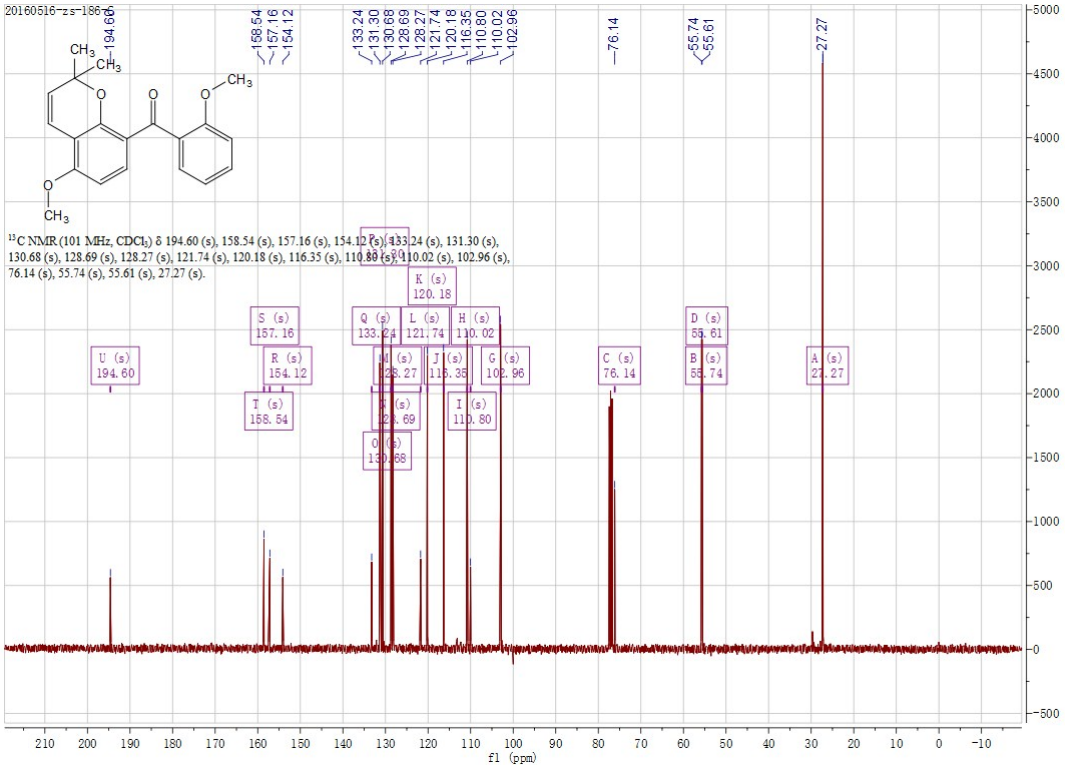
4b


20160409-zs-156-h
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 6.2 Hz, 2H), 7.10 – 7.04 (m, 1H), 6.65 (d, J = 10.0 Hz, 1H), 6.48 (d, J = 8.6 Hz, 1H), 5.53 (d, J = 10.0 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 1.21 (s, 6H).
f1 (ppm)

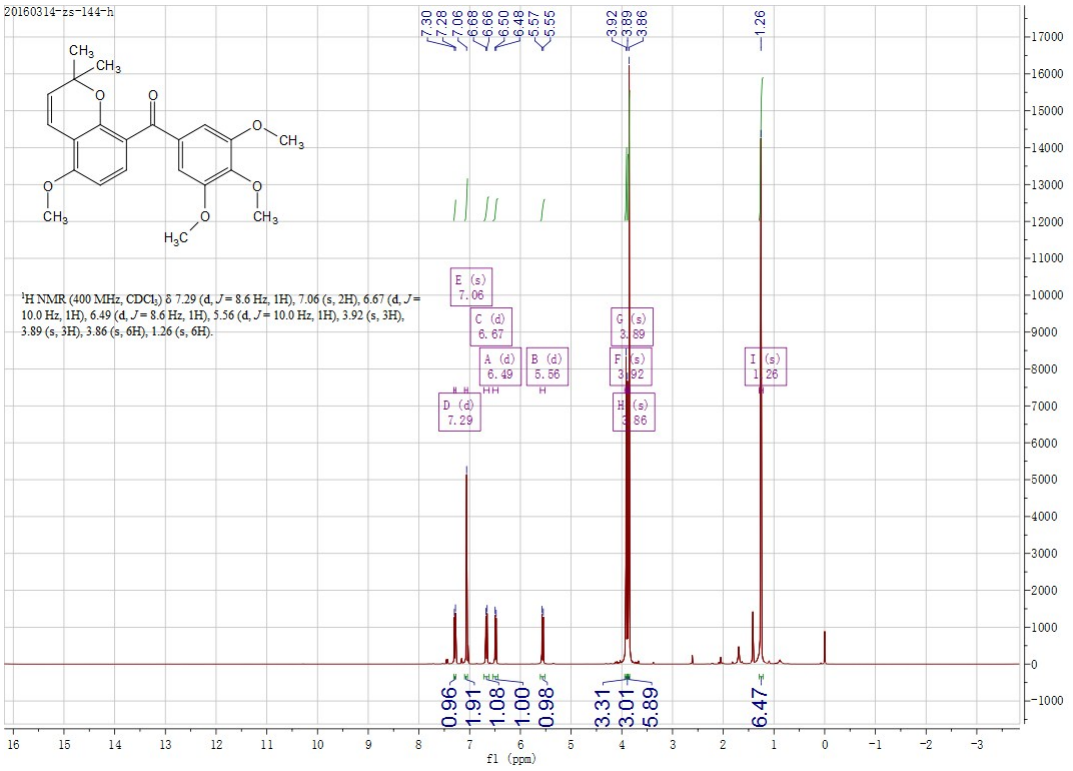

20160409-zs-156-c
^{13}C NMR (101 MHz, $CDCl_3$) δ 195.81 (s), 159.32 (s), 157.58 (s), 152.69 (s), 140.79 (s), 130.84 (s), 129.11 (s), 128.90 (s), 122.56 (s), 121.09 (s), 118.62 (s), 116.27 (s), 113.38 (s), 110.30 (s), 102.68 (s), 76.57 (s), 55.77 (s), 55.44 (s), 27.76 (s).
f1 (ppm)

4c


20160516-zs-186
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.69 (d, J = 8.8 Hz, 1H), 7.36 (td, J = 8.3, 1.7 Hz, 1H), 7.28 (dd, J = 7.5, 1.7 Hz, 1H), 6.96 (t, J = 7.4 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 6.59 (d, J = 10.0 Hz, 1H), 6.49 (d, J = 8.8 Hz, 1H), 5.53 – 5.38 (m, 1H), 3.87 (s, 3H), 3.71 (s, 3H), 1.01 (s, 6H).
H (d) 6.88
F (dd) 7.28
C (d) 6.59
J (s) 3.87
D (d) 7.69
B (d) 6.49
A (m) 5.45
I (s) 3.71
K (s) 1.01
E (td) 7.36
G (t) 6.96
f1 (ppm)


20160516-zs-186
^{13}C NMR (101 MHz, CDCl$_3$) δ 194.60 (s), 158.54 (s), 157.16 (s), 154.12 (s), 133.24 (s), 131.30 (s), 130.68 (s), 128.69 (s), 128.27 (s), 121.74 (s), 120.18 (s), 116.35 (s), 110.80 (s), 110.02 (s), 102.96 (s), 76.14 (s), 55.74 (s), 55.61 (s), 27.27 (s).
K (s) 120.18
S (s) 157.16
Q (s) 133.24
L (s) 121.74
H (s) 110.02
D (s) 55.61
U (s) 194.60
R (s) 154.12
M (s) 128.27
J (s) 116.35
G (s) 102.96
C (s) 76.14
B (s) 55.74
A (s) 27.27
T (s) 158.54
P (s) 128.69
I (s) 110.80
O (s) 130.68
f1 (ppm)

4d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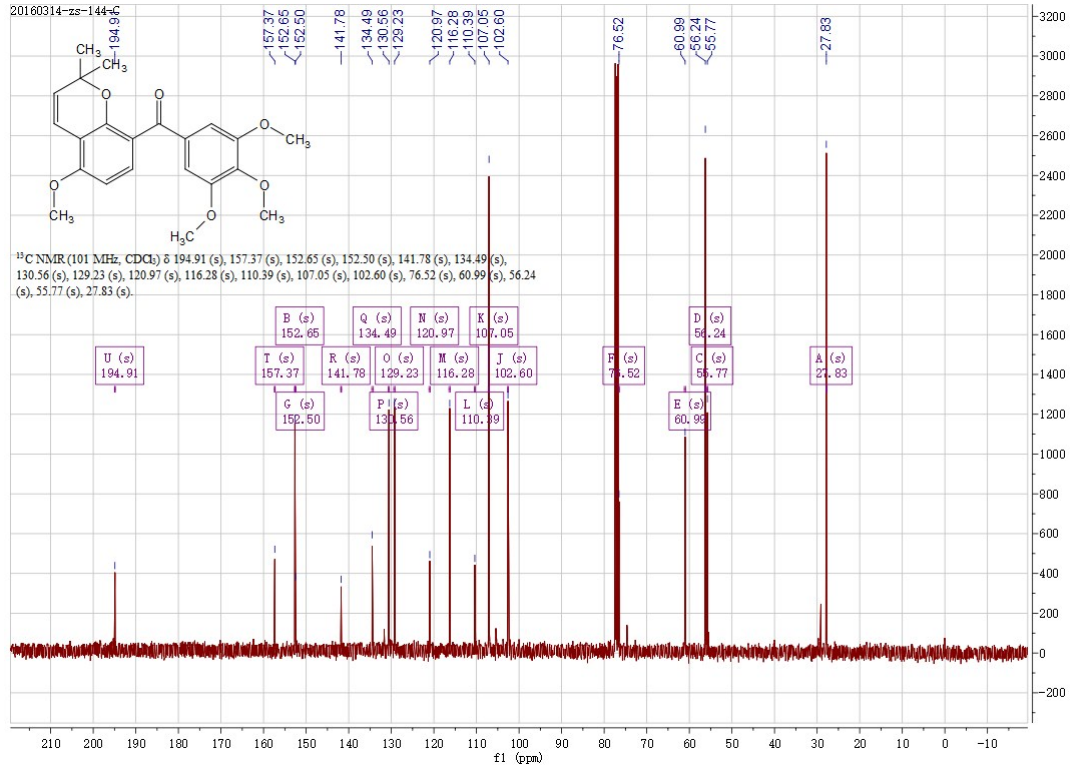
f1 (ppm)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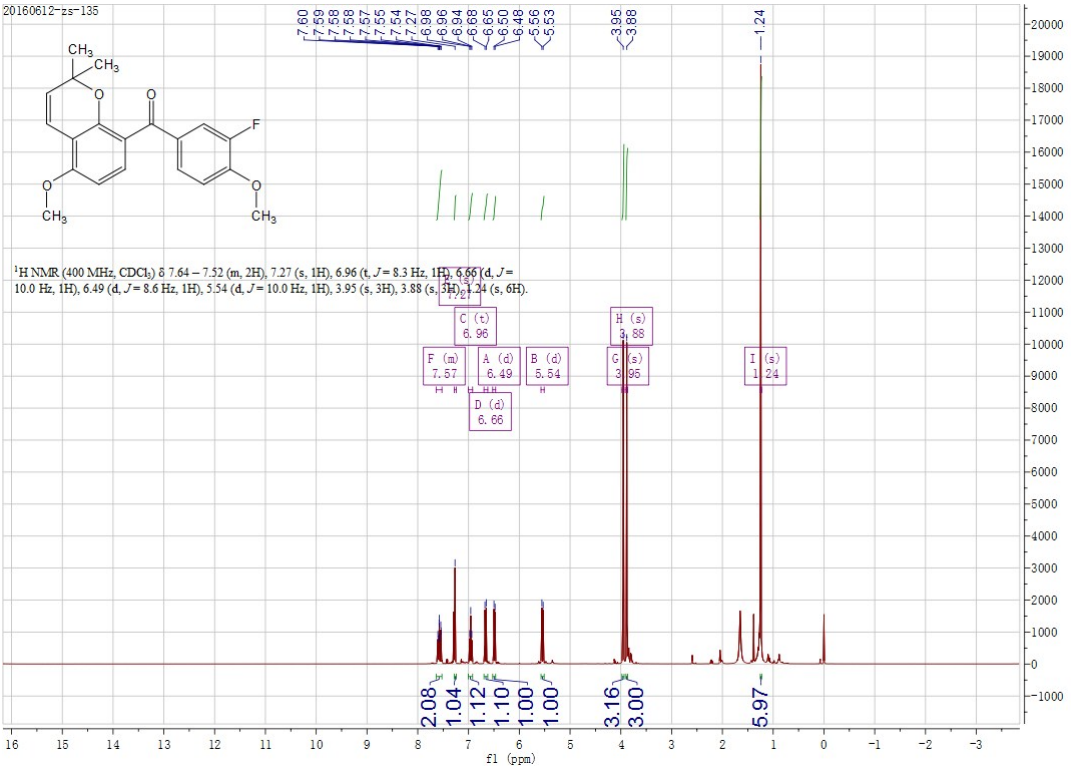
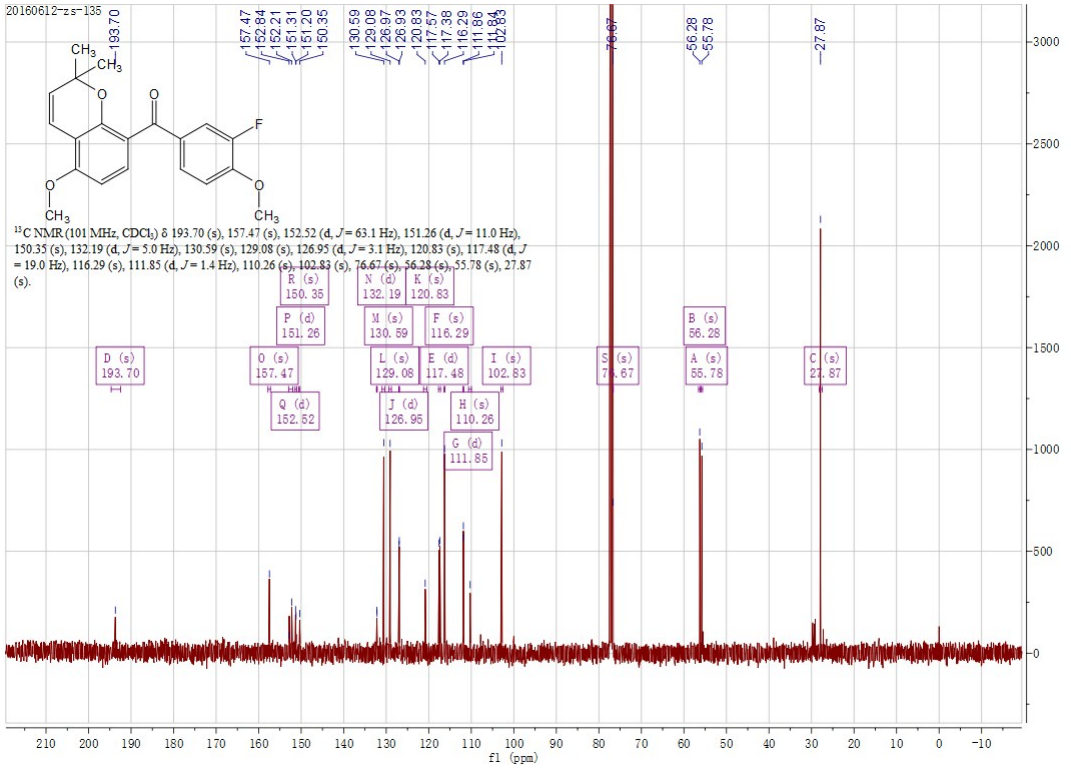
^{13}C NMR (101 MHz, CDCl$_3$) δ 194.91 (s), 157.37 (s), 152.65 (s), 152.50 (s), 141.78 (s), 134.49 (s), 130.56 (s), 129.23 (s), 120.97 (s), 116.28 (s), 110.39 (s), 107.05 (s), 102.60 (s), 76.52 (s), 60.99 (s), 56.24 (s), 55.77 (s), 27.83 (s).
f1 (ppm)

4e


20160612-zs-135
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.64 – 7.52 (m, 2H), 7.27 (s, 1H), 6.96 (t, J = 8.3 Hz, 1H), 6.66 (d, J = 10.0 Hz, 1H), 6.49 (d, J = 8.6 Hz, 1H), 5.54 (d, J = 10.0 Hz, 1H), 3.95 (s, 3H), 3.88 (s, 3H), 1.24 (s, 6H).
f1 (ppm)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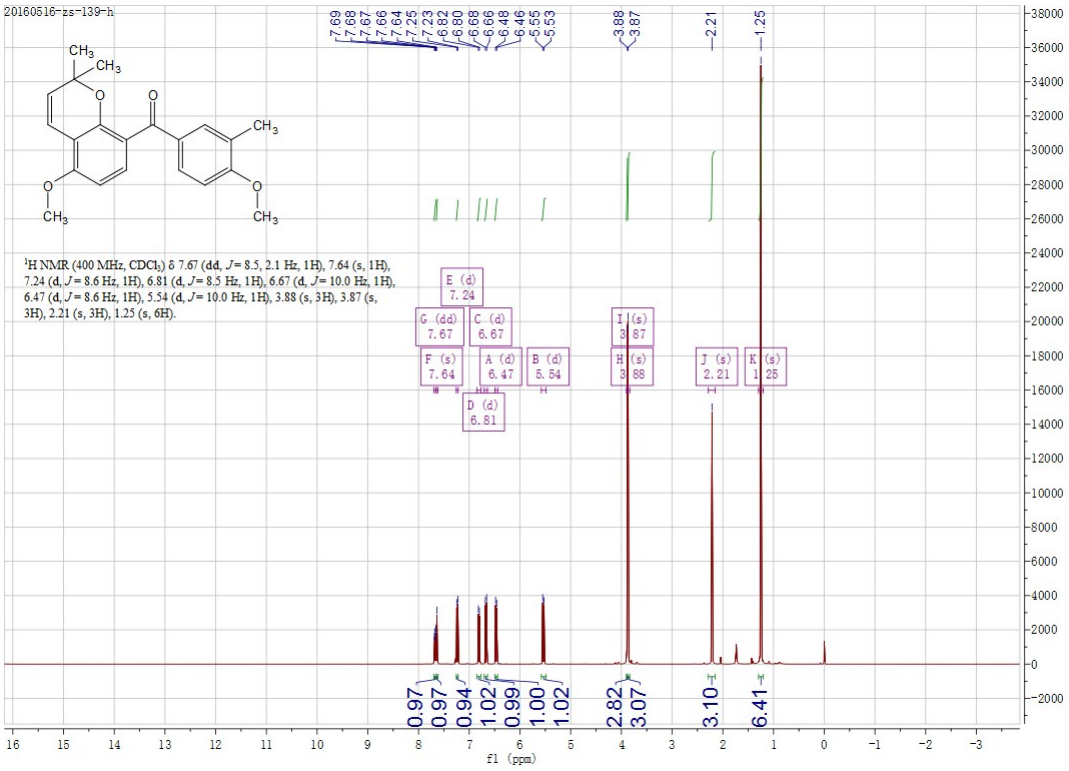
^{13}C NMR (101 MHz, CDCl$_3$) δ 193.70 (s), 157.47 (s), 152.52 (d, J = 63.1 Hz), 151.26 (d, J = 11.0 Hz), 150.35 (s), 132.19 (d, J = 5.0 Hz), 130.59 (s), 129.08 (s), 126.95 (d, J = 3.1 Hz), 120.83 (s), 117.48 (d, J = 19.0 Hz), 116.29 (s), 111.85 (d, J = 1.4 Hz), 110.26 (s), 102.83 (s), 76.67 (s), 56.28 (s), 55.78 (s), 27.87 (s).
f1 (ppm)

4f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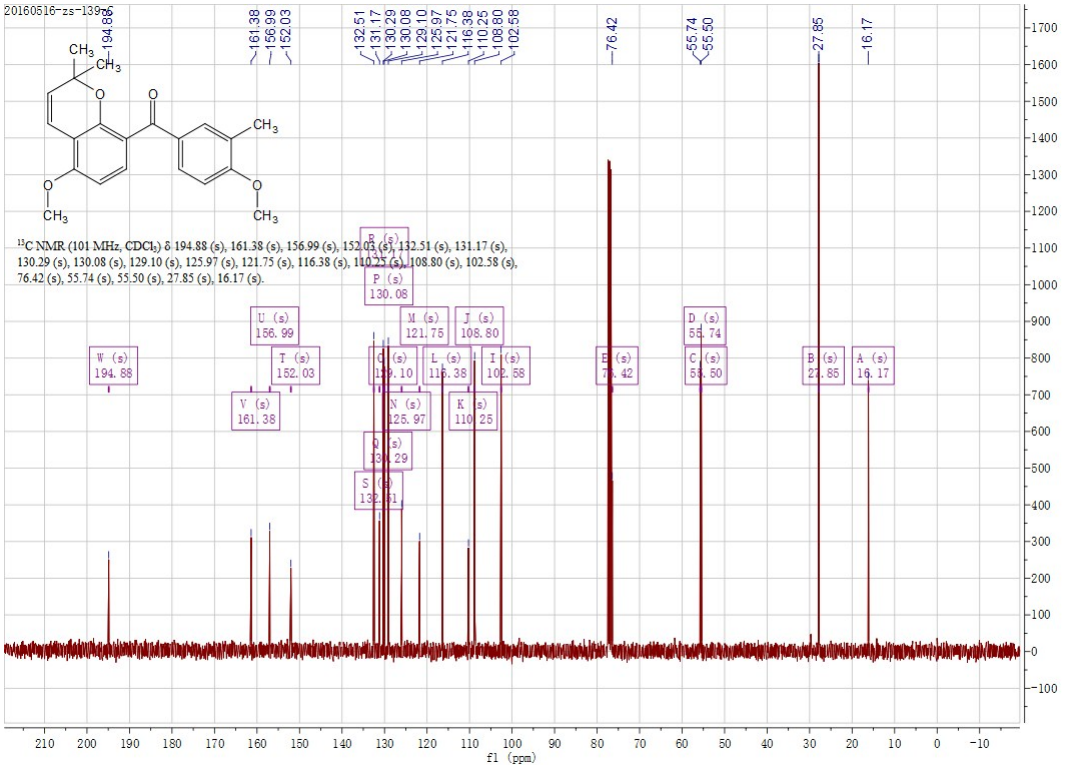
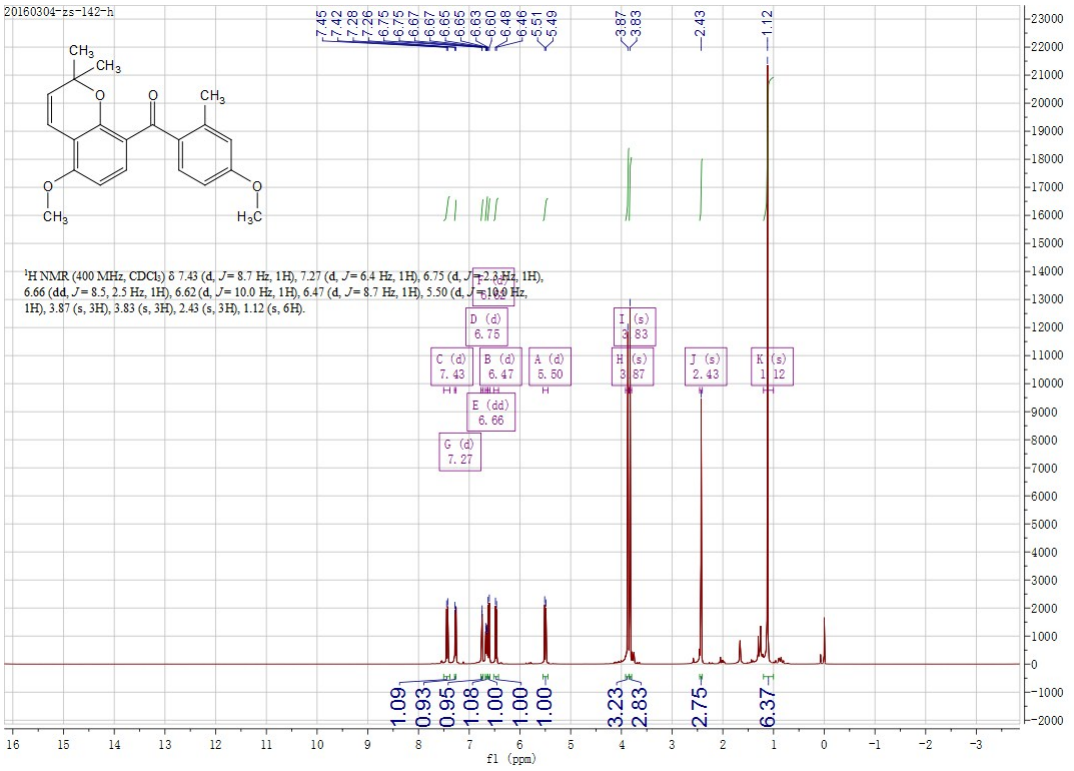
f1 (ppm)


20160516-zs-139-c
¹³C NMR (101 MHz, CDCl₃) δ 194.88 (s), 161.38 (s), 156.99 (s), 152.03 (s), 132.51 (s), 131.17 (s), 130.29 (s), 130.08 (s), 129.10 (s), 125.97 (s), 121.75 (s), 116.38 (s), 110.25 (s), 108.80 (s), 102.58 (s), 76.42 (s), 55.74 (s), 55.50 (s), 27.85 (s), 16.17 (s).
f1 (ppm)

4g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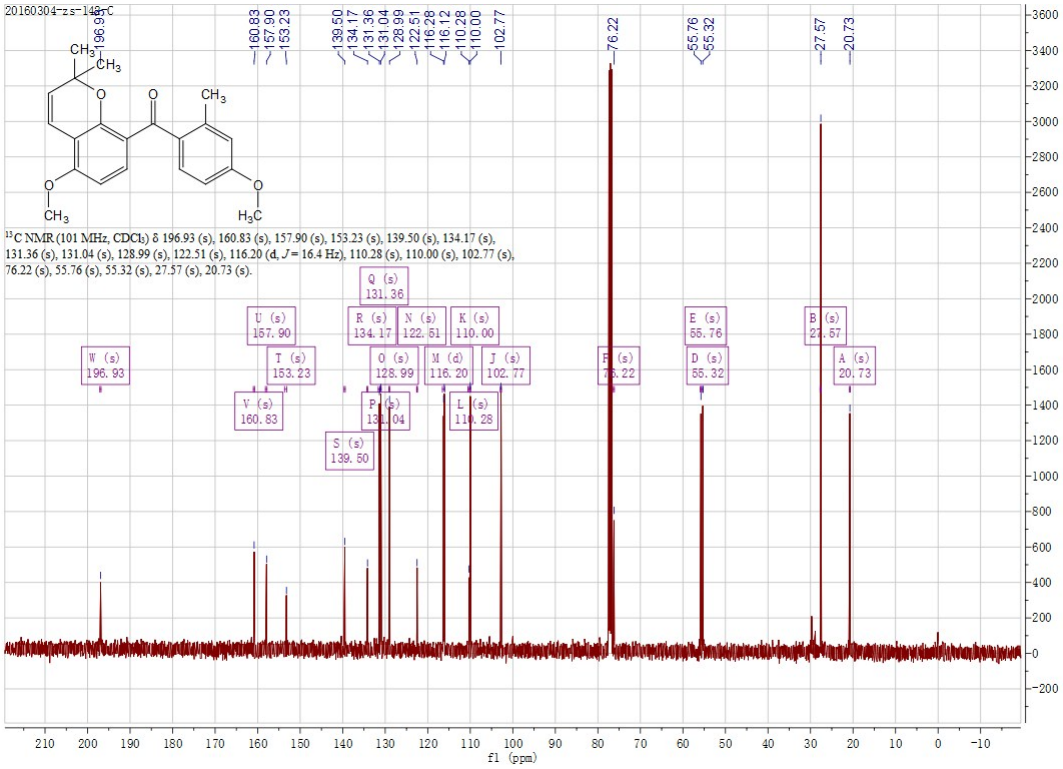
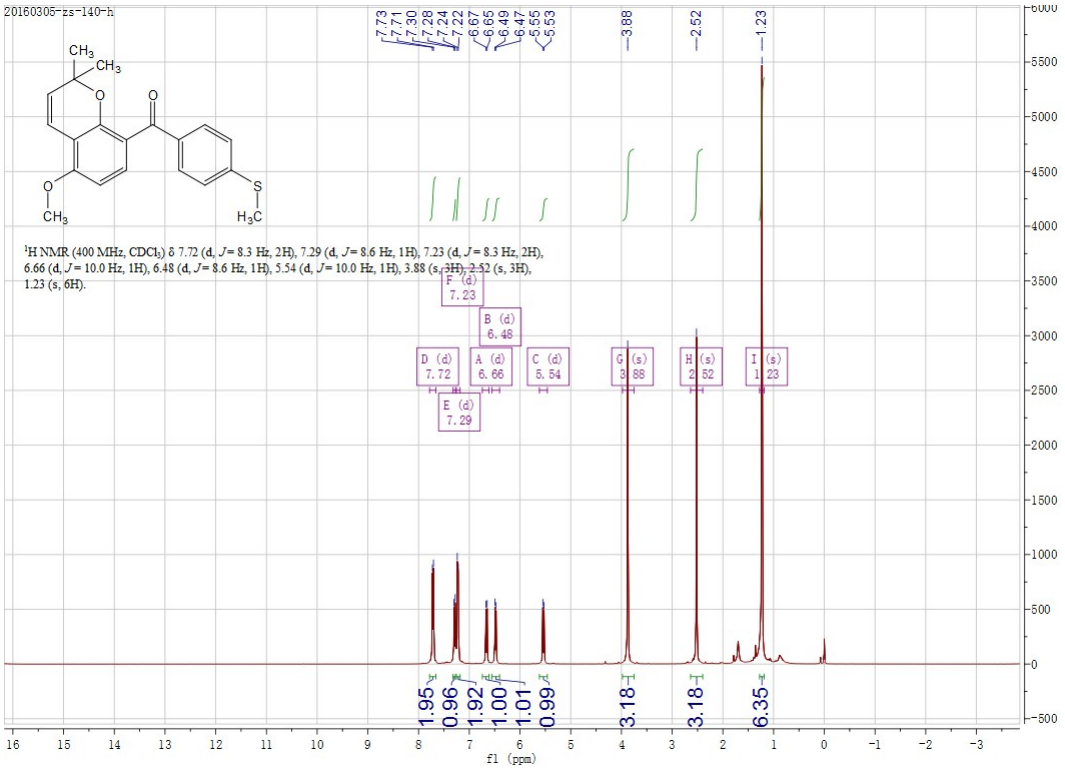
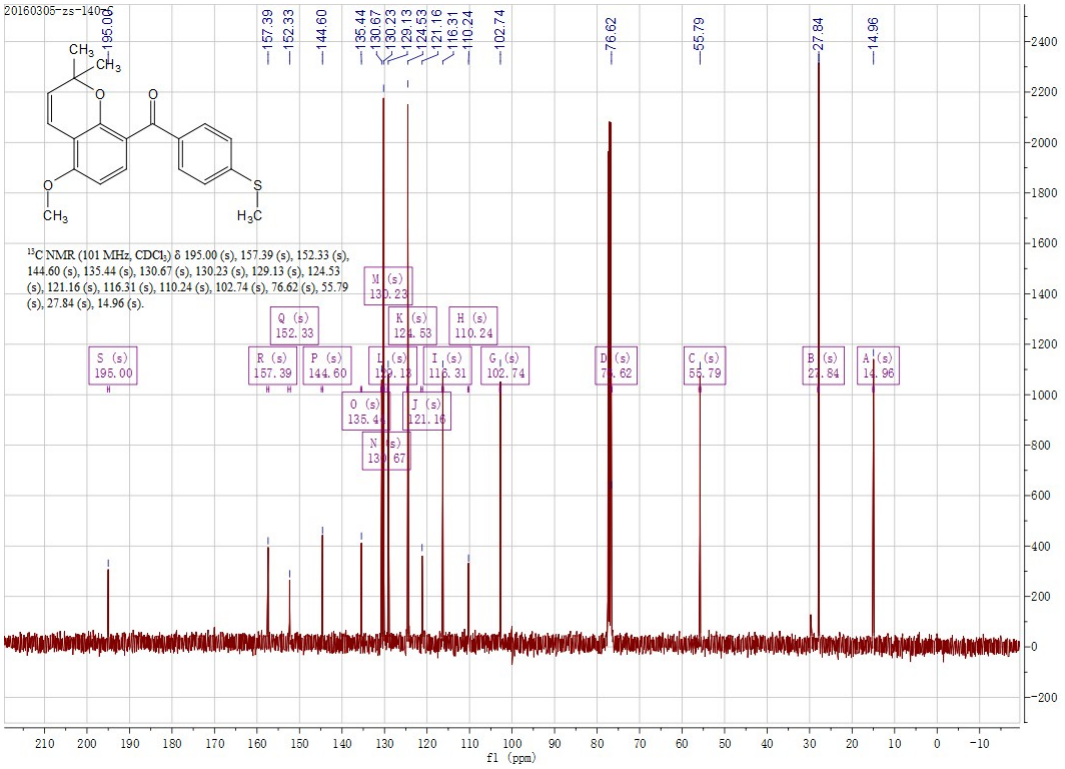
f1 (ppm)

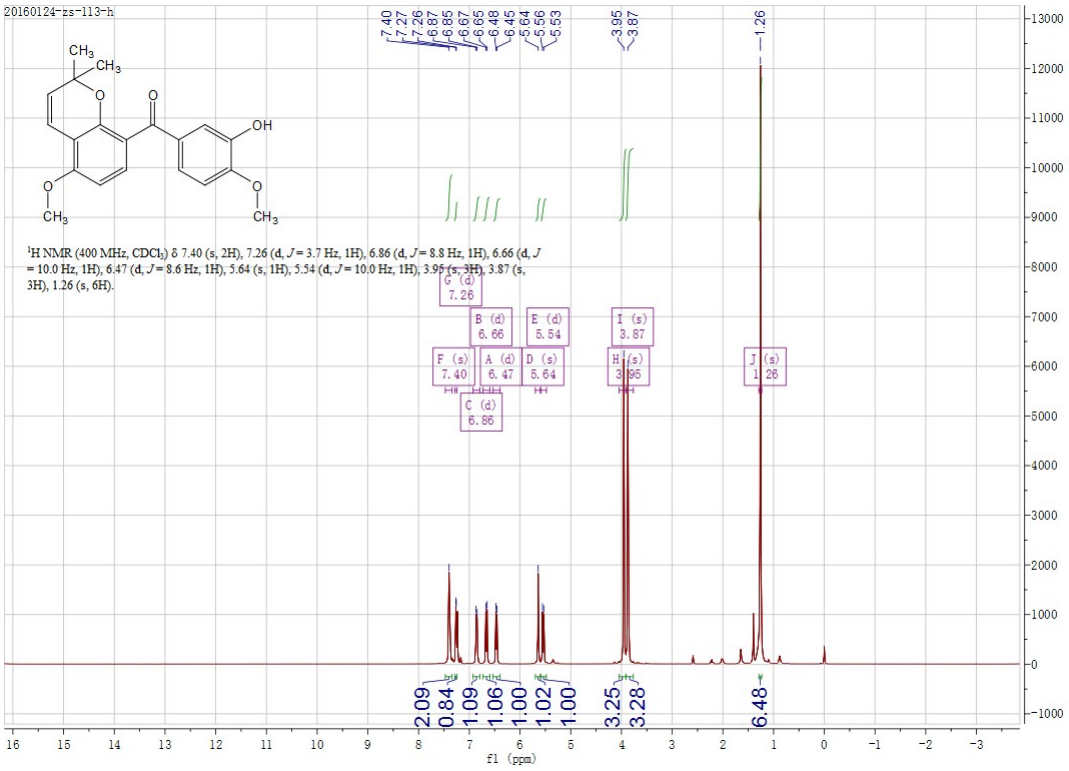

20160304-zs-142-C
^{13}C NMR (101 MHz, CDCl$_3$) δ 196.93 (s), 160.83 (s), 157.90 (s), 153.23 (s), 139.50 (s), 134.17 (s), 131.36 (s), 131.04 (s), 128.99 (s), 122.51 (s), 116.20 (d, J = 16.4 Hz), 110.28 (s), 110.00 (s), 102.77 (s), 76.22 (s), 55.76 (s), 55.32 (s), 27.57 (s), 20.73 (s).
f1 (ppm)

4h


20160306-zs-140-h
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 8.6 Hz, 1H), 7.23 (d, J = 8.3 Hz, 2H), 6.66 (d, J = 10.0 Hz, 1H), 6.48 (d, J = 8.6 Hz, 1H), 5.54 (d, J = 10.0 Hz, 1H), 3.88 (s, 3H), 2.52 (s, 3H), 1.23 (s, 6H).
f1 (ppm)


20160306-zs-140-c
^{13}C NMR (101 MHz, $CDCl_3$) δ 195.00 (s), 157.39 (s), 152.33 (s), 144.60 (s), 135.44 (s), 130.67 (s), 130.23 (s), 129.13 (s), 124.53 (s), 121.16 (s), 116.31 (s), 110.24 (s), 102.74 (s), 76.62 (s), 55.79 (s), 27.84 (s), 14.96 (s).
f1 (ppm)

5i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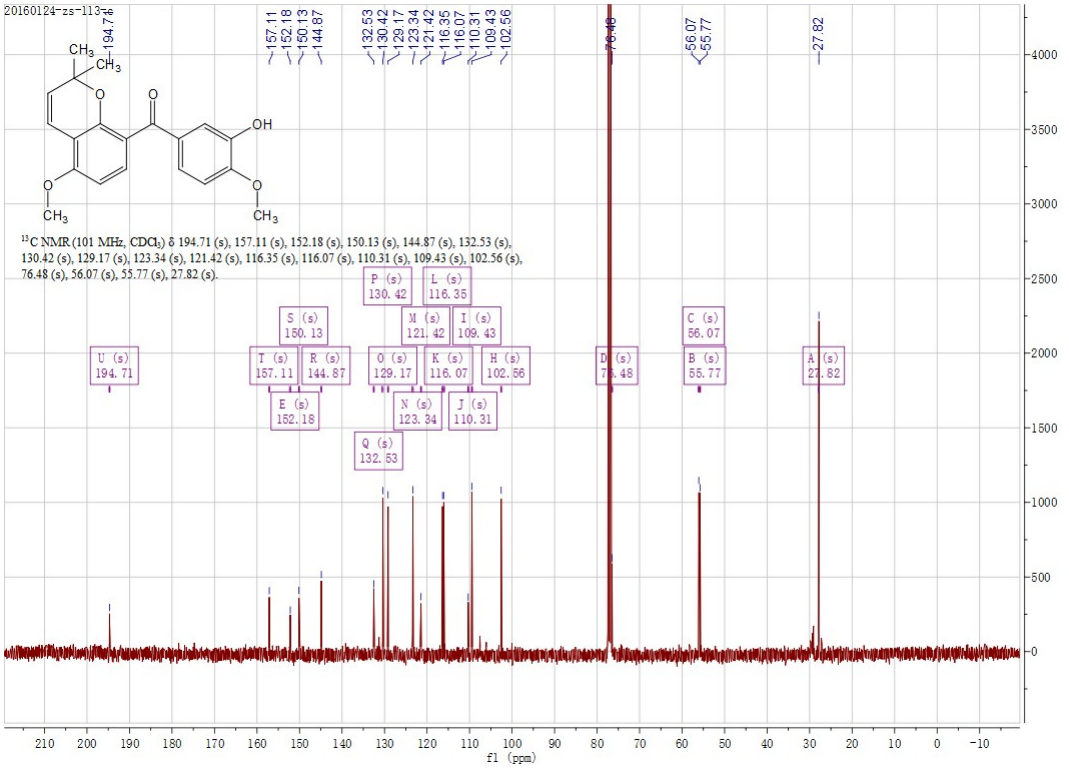
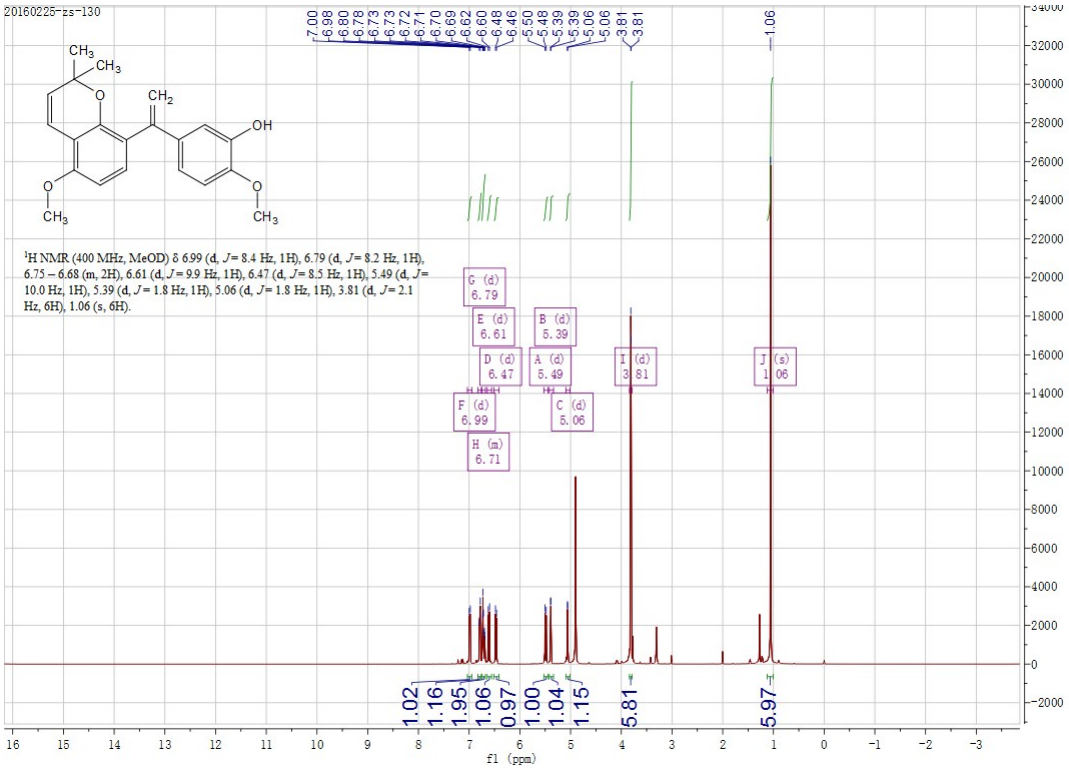
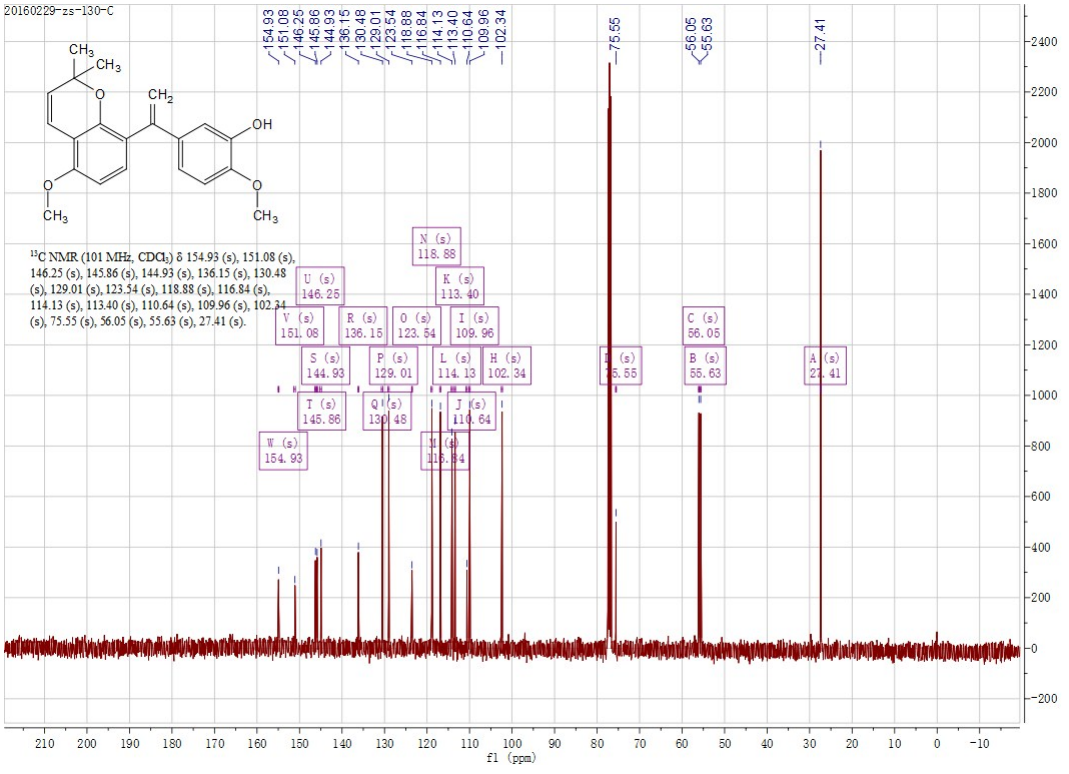
f1 (ppm)

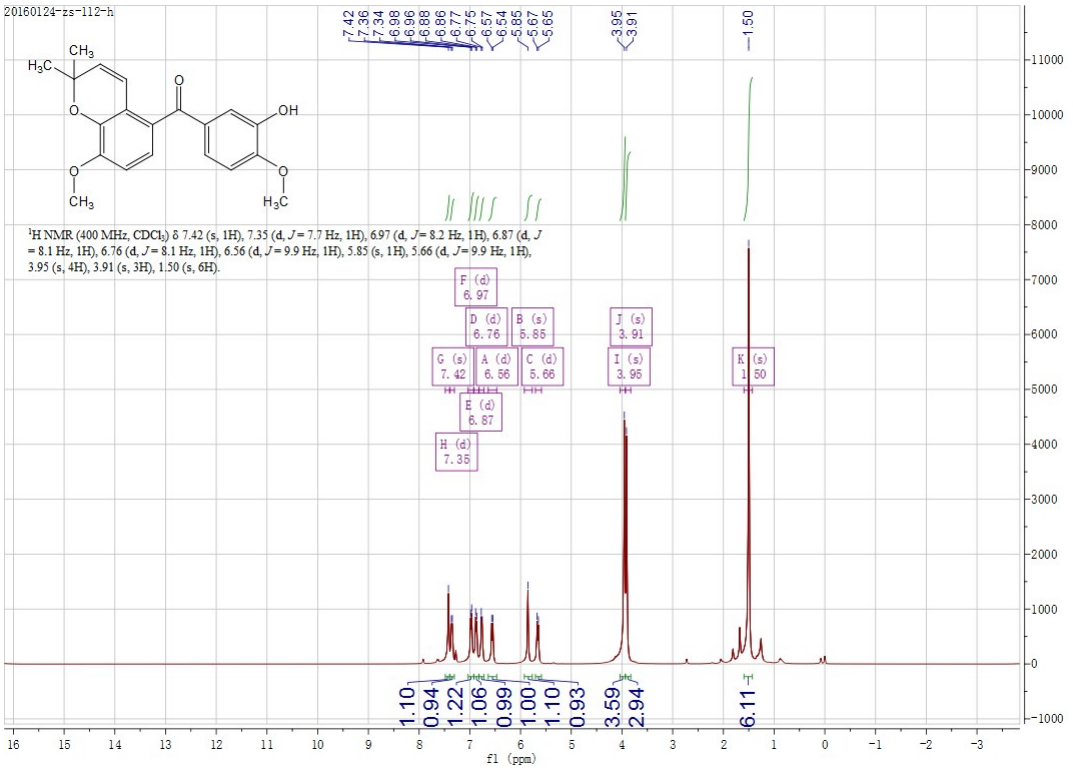

20160124-zs-113-c
^{13}C NMR (101 MHz, CDCl$_3$) δ 194.71 (s), 157.11 (s), 152.18 (s), 150.13 (s), 144.87 (s), 132.53 (s), 130.42 (s), 129.17 (s), 123.34 (s), 121.42 (s), 116.35 (s), 116.07 (s), 110.31 (s), 109.43 (s), 102.56 (s), 76.48 (s), 56.07 (s), 55.77 (s), 27.82 (s).
f1 (ppm)

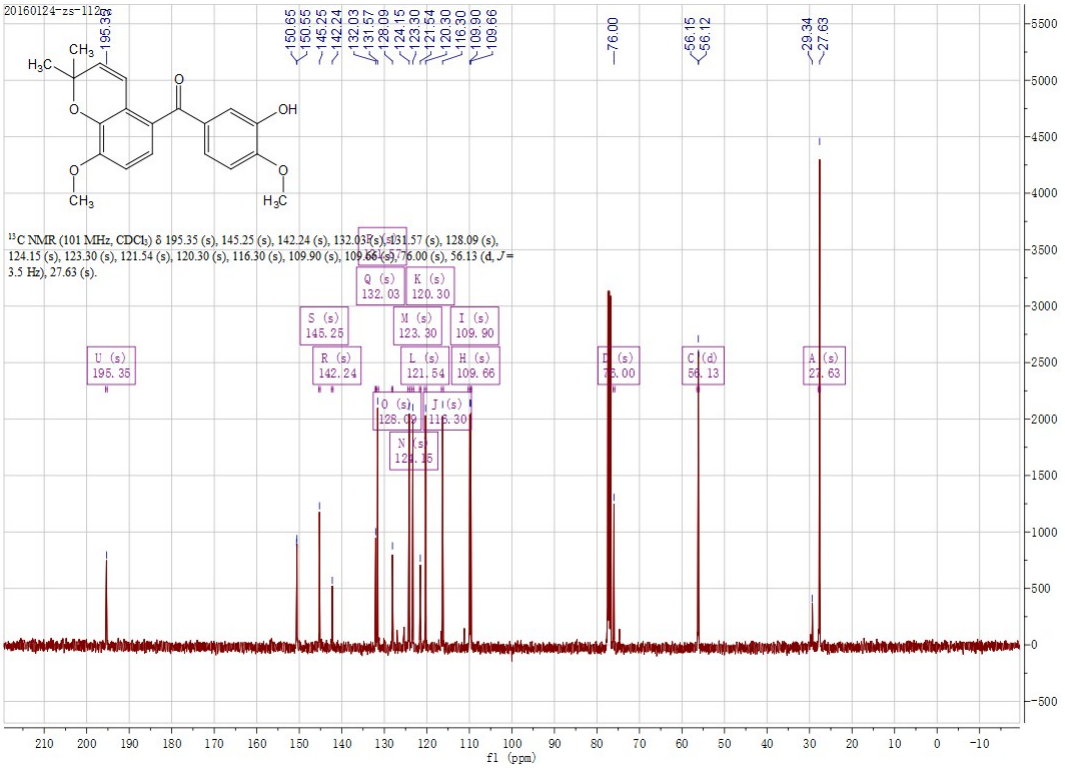
6i


20160225-zs-130
^{1}H NMR (400 MHz, MeOD) δ 6.99 (d, J = 8.4 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1H), 6.75 – 6.68 (m, 2H), 6.61 (d, J = 9.9 Hz, 1H), 6.47 (d, J = 8.5 Hz, 1H), 5.49 (d, J = 10.0 Hz, 1H), 5.39 (d, J = 1.8 Hz, 1H), 5.06 (d, J = 1.8 Hz, 1H), 3.81 (d, J = 2.1 Hz, 6H), 1.06 (s, 6H).
f1 (ppm)

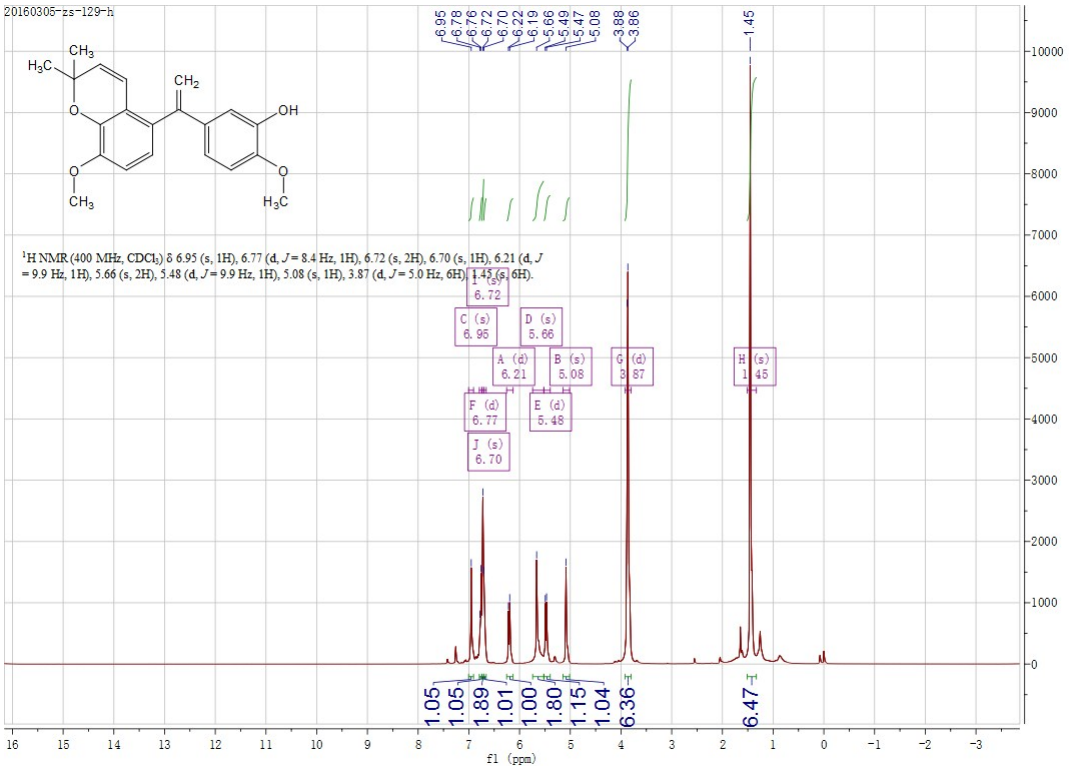

20160229-zs-130-C
^{13}C NMR (101 MHz, CDCl$_3$) δ 154.93 (s), 151.08 (s), 146.25 (s), 145.86 (s), 144.93 (s), 136.15 (s), 130.48 (s), 129.01 (s), 123.54 (s), 118.88 (s), 116.84 (s), 114.13 (s), 113.40 (s), 110.64 (s), 109.96 (s), 102.34 (s), 75.55 (s), 56.05 (s), 55.63 (s), 27.41 (s).
f1 (ppm)

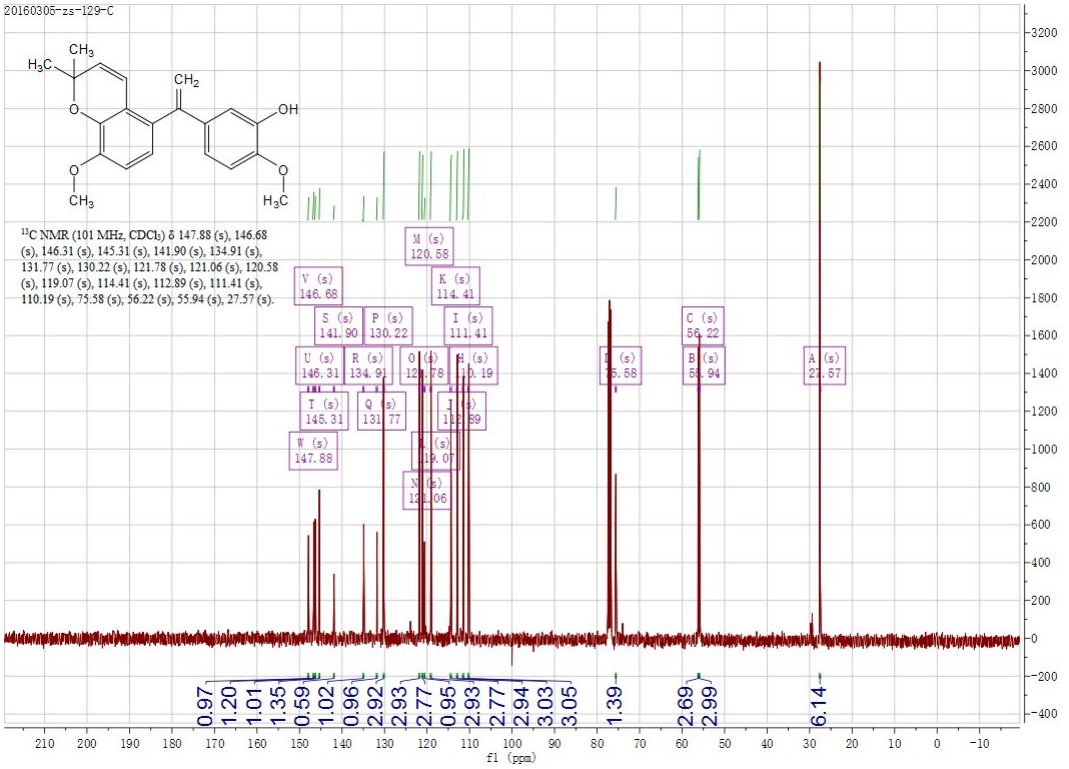
11


20160124-zs-112-h
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42 (s, 1H), 7.35 (d, J = 7.7 Hz, 1H), 6.97 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 6.76 (d, J = 8.1 Hz, 1H), 6.56 (d, J = 9.9 Hz, 1H), 5.85 (s, 1H), 5.66 (d, J = 9.9 Hz, 1H), 3.95 (s, 4H), 3.91 (s, 3H), 1.50 (s, 6H).
f1 (ppm)


20160124-zs-112-c
^{13}C NMR (101 MHz, CDCl$_3$) δ 195.35 (s), 145.25 (s), 142.24 (s), 132.03 (s), 131.57 (s), 128.09 (s), 124.15 (s), 123.30 (s), 121.54 (s), 120.30 (s), 116.30 (s), 109.90 (s), 109.66 (s), 76.00 (s), 56.13 (d, J = 3.5 Hz), 27.63 (s).
f1 (ppm)

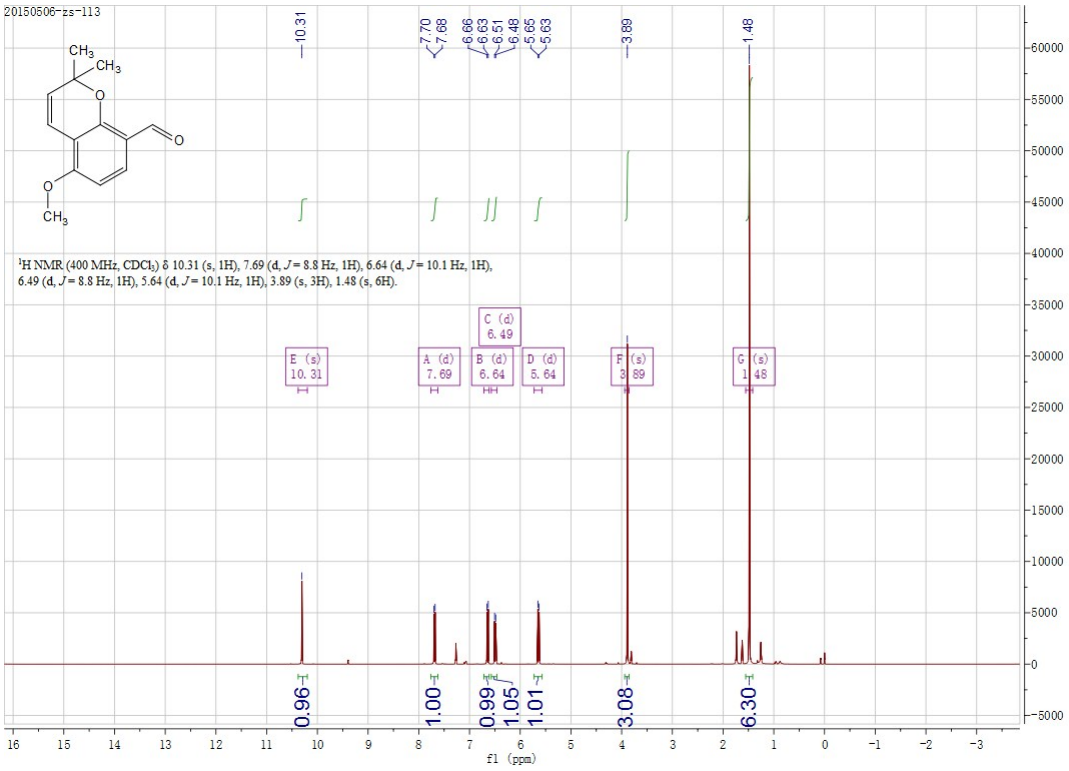
12


20160305-zs-129-h
^{1}H NMR (400 MHz, CDCl$_3$) δ 6.95 (s, 1H), 6.77 (d, J = 8.4 Hz, 1H), 6.72 (s, 2H), 6.70 (s, 1H), 6.21 (d, J = 9.9 Hz, 1H), 5.66 (s, 2H), 5.48 (d, J = 9.9 Hz, 1H), 5.08 (s, 1H), 3.87 (d, J = 5.0 Hz, 6H), 1.45 (s, 6H).
f1 (ppm)

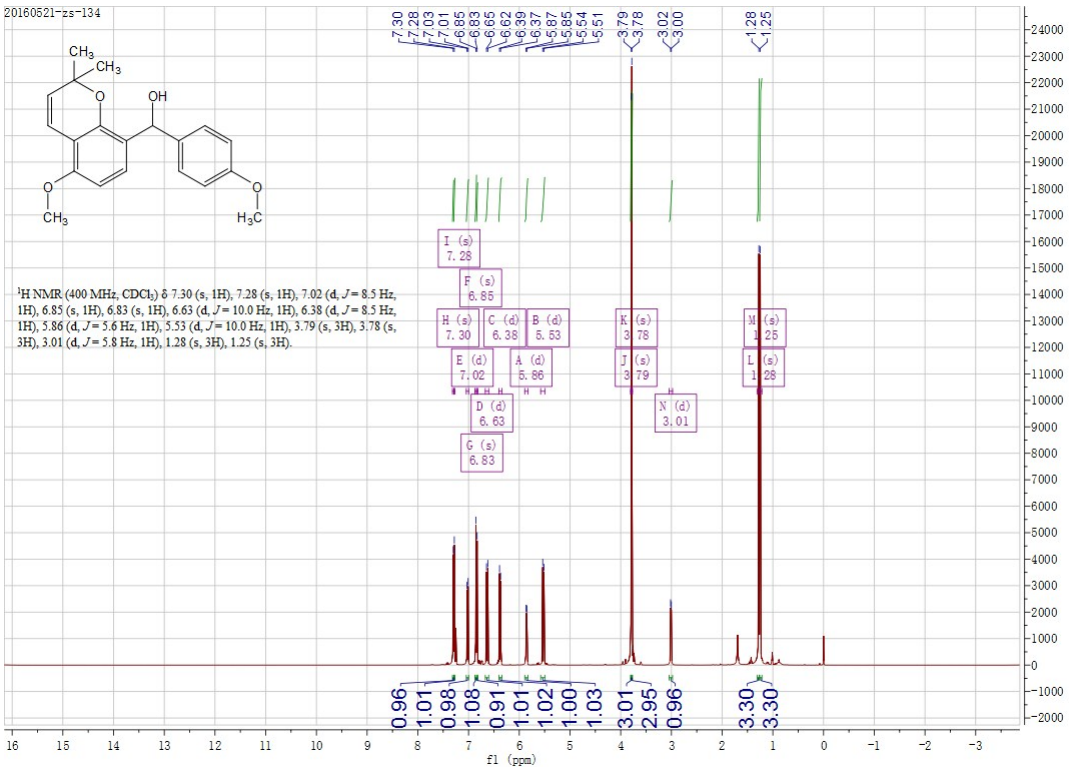

20160305-zs-129-C
^{13}C NMR (101 MHz, CDCl$_3$) δ 147.88 (s), 146.68 (s), 146.31 (s), 145.31 (s), 141.90 (s), 134.91 (s), 131.77 (s), 130.22 (s), 121.78 (s), 121.06 (s), 120.58 (s), 119.07 (s), 114.41 (s), 112.89 (s), 111.41 (s), 110.19 (s), 75.58 (s), 56.22 (s), 55.94 (s), 27.57 (s).
f1 (ppm)

SI3. ^{1}H NMR spectrums of intermediate compounds

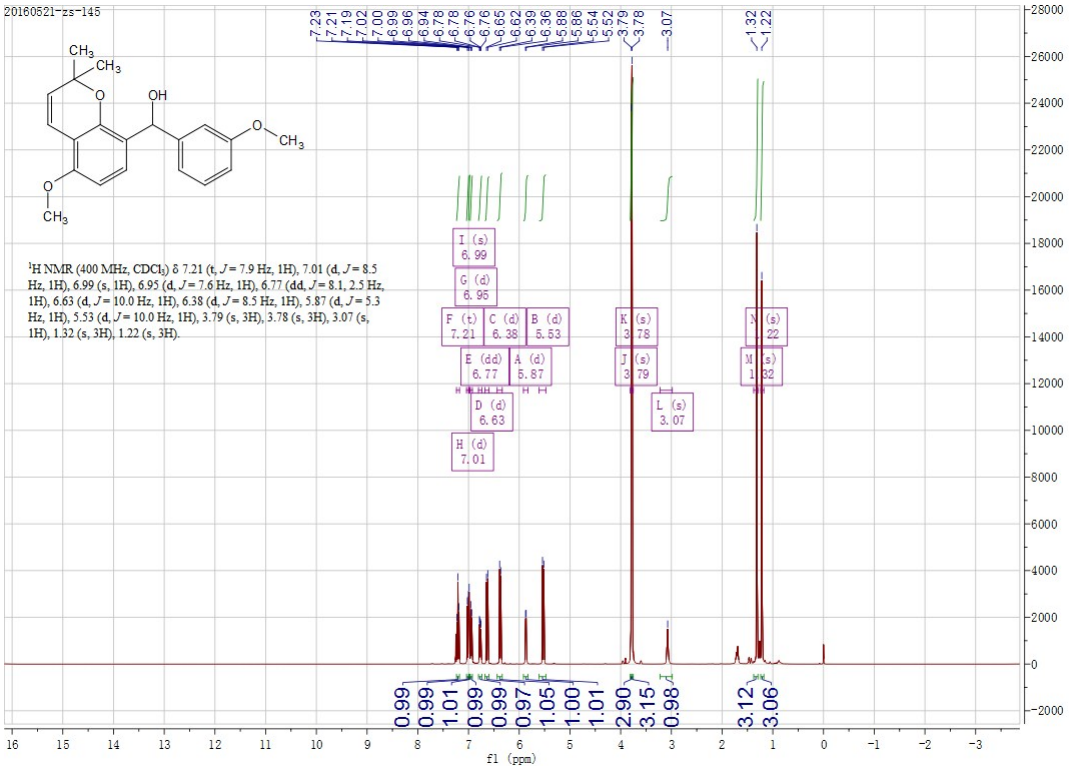
2



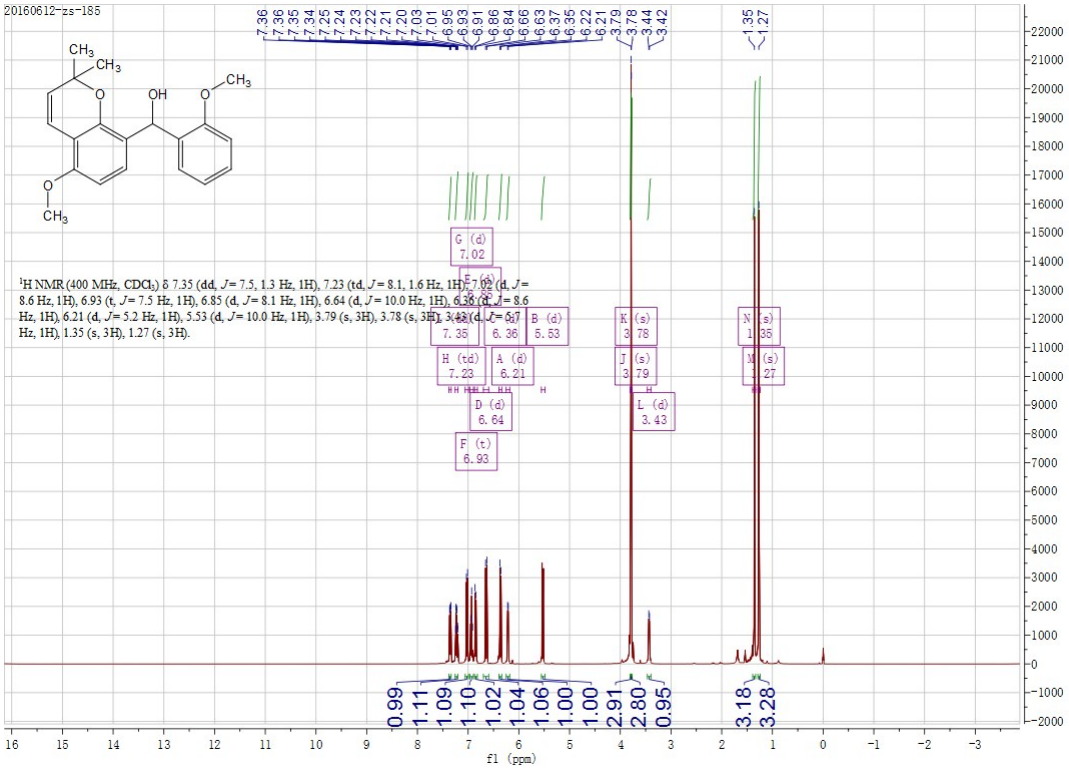
3a



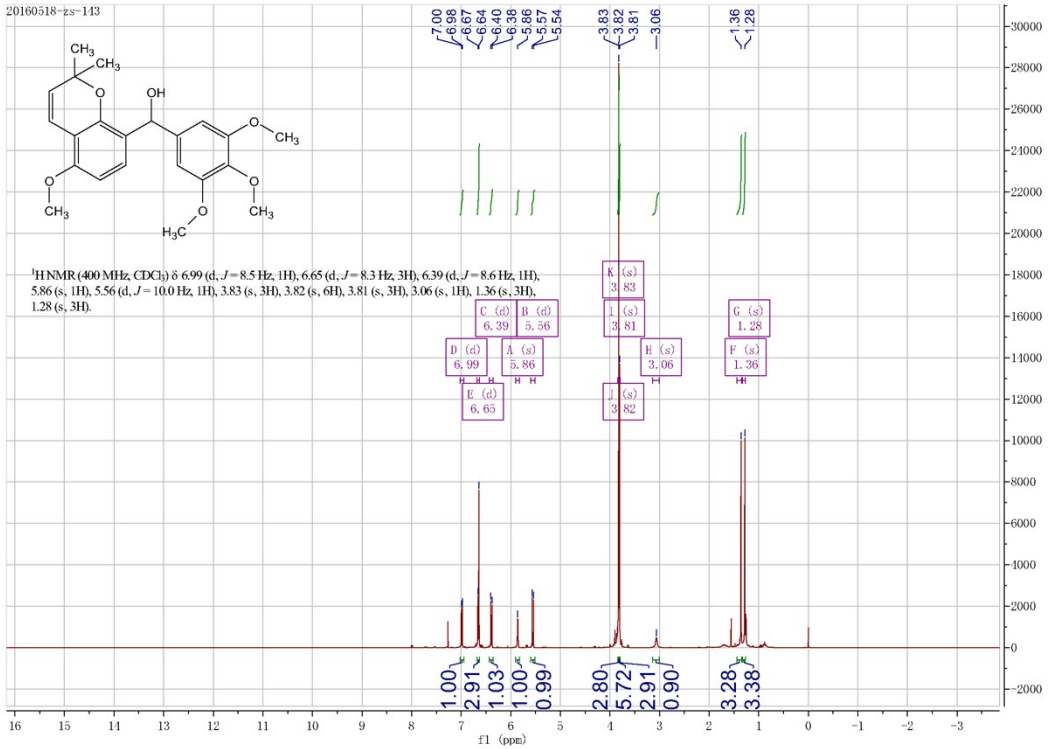
3b


20160521-zs-145
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.21 (t, J = 7.9 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.99 (s, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.77 (dd, J = 8.1, 2.5 Hz, 1H), 6.63 (d, J = 10.0 Hz, 1H), 6.38 (d, J = 8.5 Hz, 1H), 5.87 (d, J = 5.3 Hz, 1H), 5.53 (d, J = 10.0 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.07 (s, 1H), 1.32 (s, 3H), 1.22 (s, 3H).
f1 (ppm)

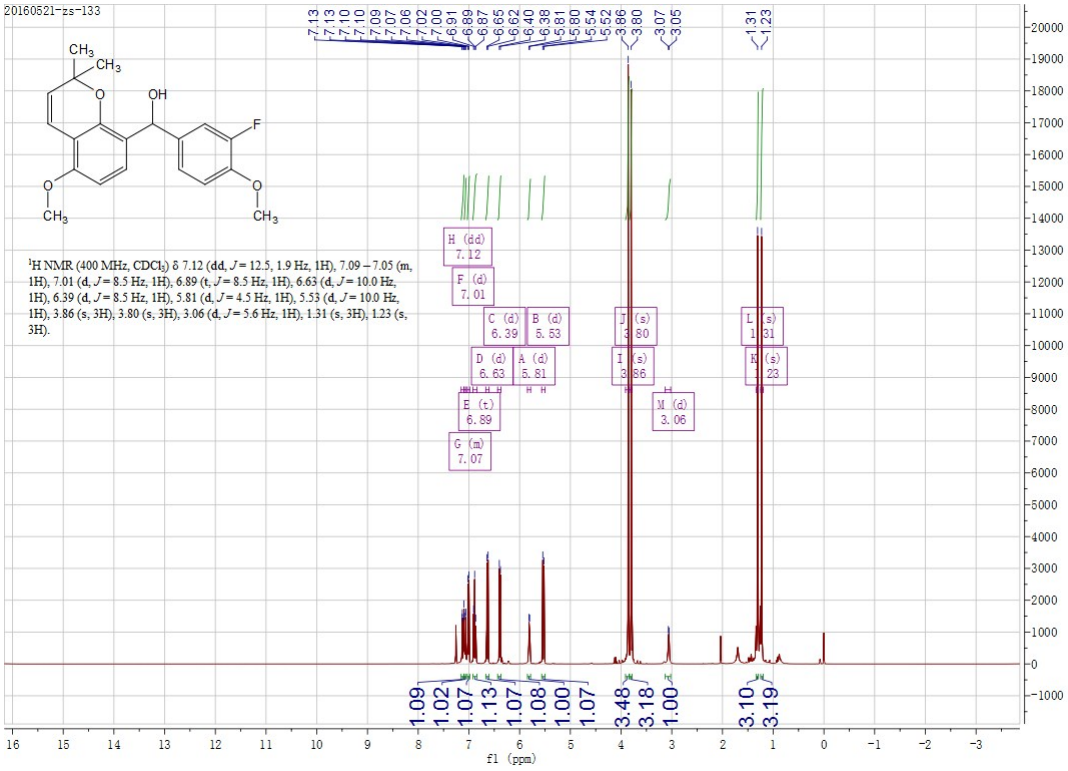
3c


20160612-zs-186
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.35 (dd, J = 7.5, 1.3 Hz, 1H), 7.23 (td, J = 8.1, 1.6 Hz, 1H), 7.02 (d, J = 8.6 Hz, 1H), 6.93 (t, J = 7.5 Hz, 1H), 6.85 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 10.0 Hz, 1H), 6.36 (d, J = 8.6 Hz, 1H), 6.21 (d, J = 5.2 Hz, 1H), 5.53 (d, J = 10.0 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.43 (d, J = 5.7 Hz, 1H), 1.35 (s, 3H), 1.27 (s, 3H).
f1 (ppm)

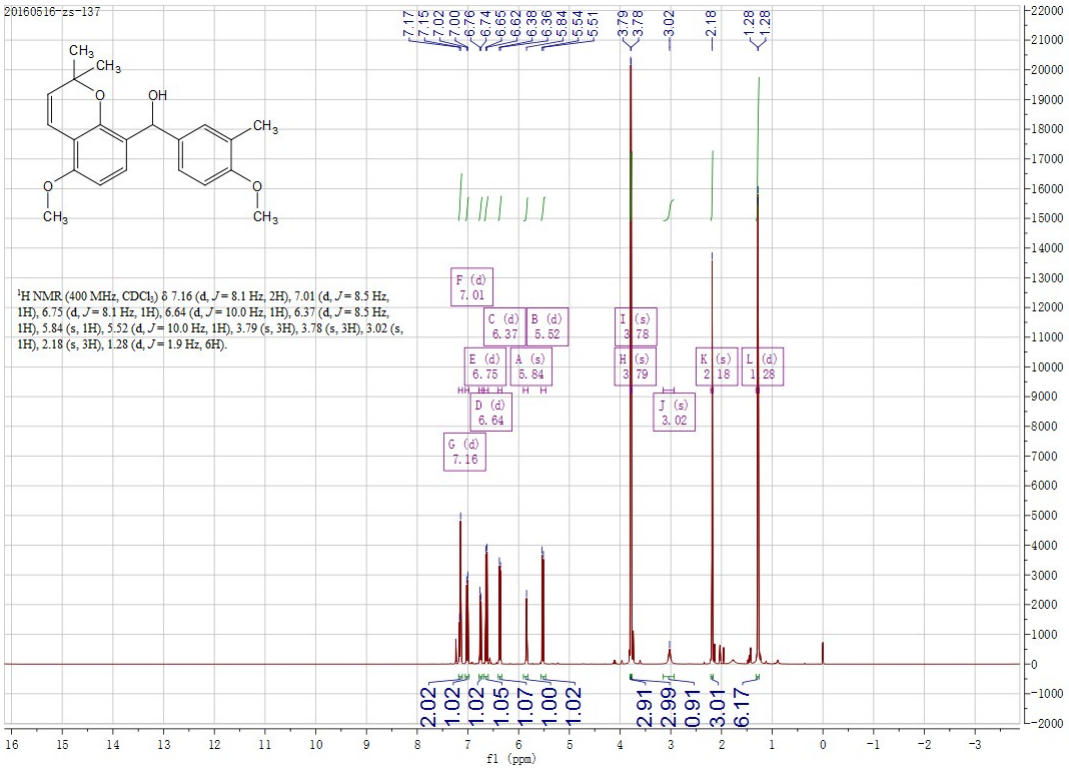
3d


20160518-zs-143
¹H NMR (400 MHz, CDCl₃) δ 6.99 (d, J = 8.5 Hz, 1H), 6.65 (d, J = 8.3 Hz, 3H), 6.39 (d, J = 8.6 Hz, 1H), 5.86 (s, 1H), 5.56 (d, J = 10.0 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 6H), 3.81 (s, 3H), 3.06 (s , 1H), 1.36 (s , 3H), 1.28 (s, 3H).
f1 (ppm)

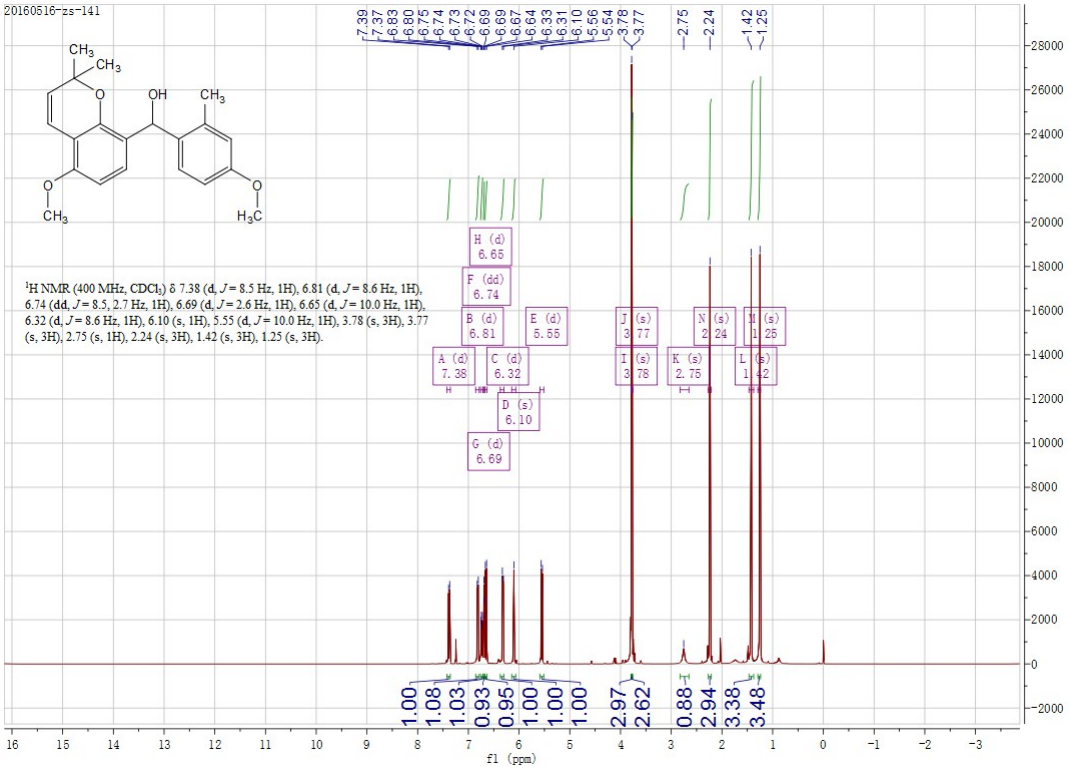
3e


20160521-zs-133
¹H NMR (400 MHz, CDCl₃) δ 7.12 (dd, J = 12.5, 1.9 Hz, 1H), 7.09 – 7.05 (m, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.89 (t, J = 8.5 Hz, 1H), 6.63 (d, J = 10.0 Hz, 1H), 6.39 (d, J = 8.5 Hz, 1H), 5.81 (d, J = 4.5 Hz, 1H), 5.53 (d, J = 10.0 Hz, 1H), 3.86 (s, 3H), 3.80 (s, 3H), 3.06 (d, J = 5.6 Hz, 1H), 1.31 (s, 3H), 1.23 (s, 3H).
f1 (ppm)

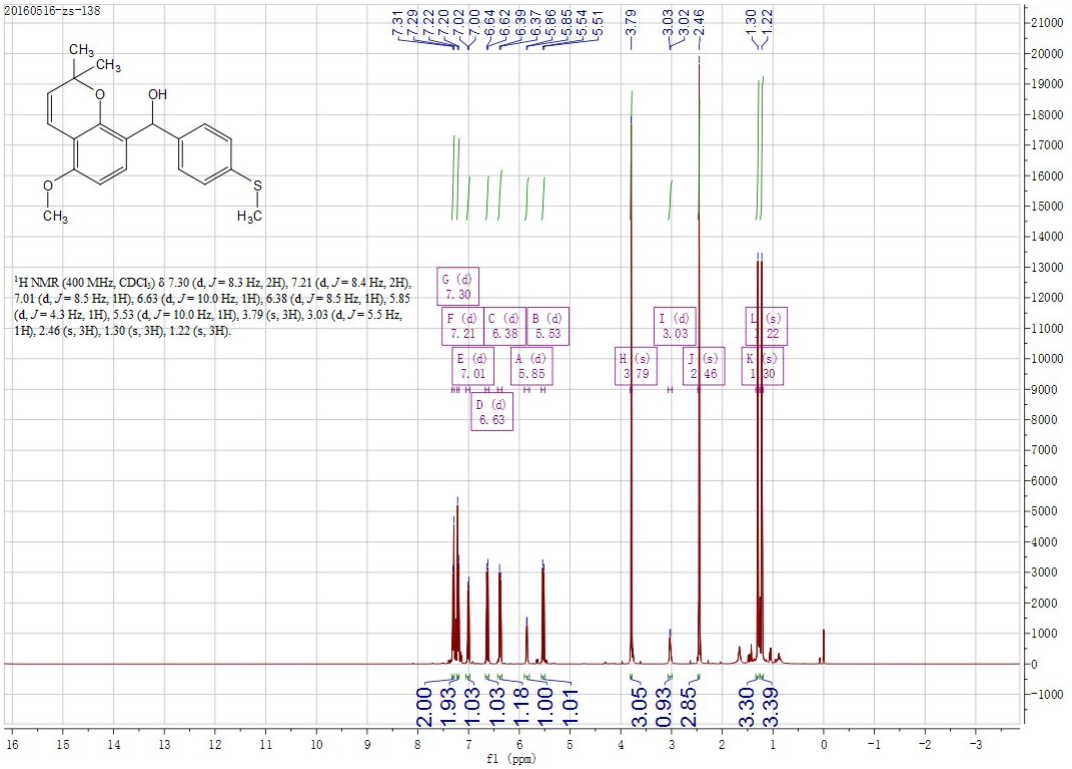
3f


20160516-zs-137
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, J = 8.1 Hz, 2H), 7.01 (d, J = 8.5 Hz, 1H), 6.75 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 10.0 Hz, 1H), 6.37 (d, J = 8.5 Hz, 1H), 5.84 (s, 1H), 5.52 (d, J = 10.0 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.02 (s, 1H), 2.18 (s, 3H), 1.28 (d, J = 1.9 Hz, 6H).
f1 (ppm)

3g

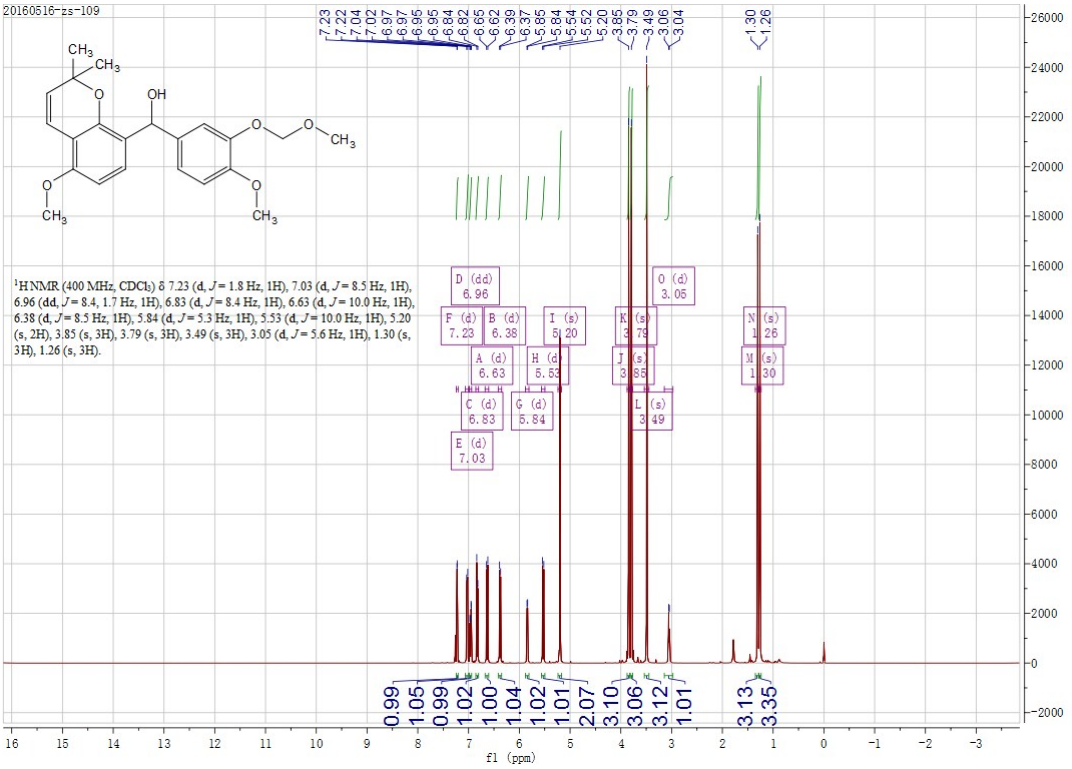

20160516-zs-141
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 8.5 Hz, 1H), 6.81 (d, J = 8.6 Hz, 1H), 6.74 (dd, J = 8.5, 2.7 Hz, 1H), 6.69 (d, J = 2.6 Hz, 1H), 6.65 (d, J = 10.0 Hz, 1H), 6.32 (d, J = 8.6 Hz, 1H), 6.10 (s, 1H), 5.55 (d, J = 10.0 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 2.75 (s, 1H), 2.24 (s, 3H), 1.42 (s, 3H), 1.25 (s, 3H).
f1 (ppm)

3h



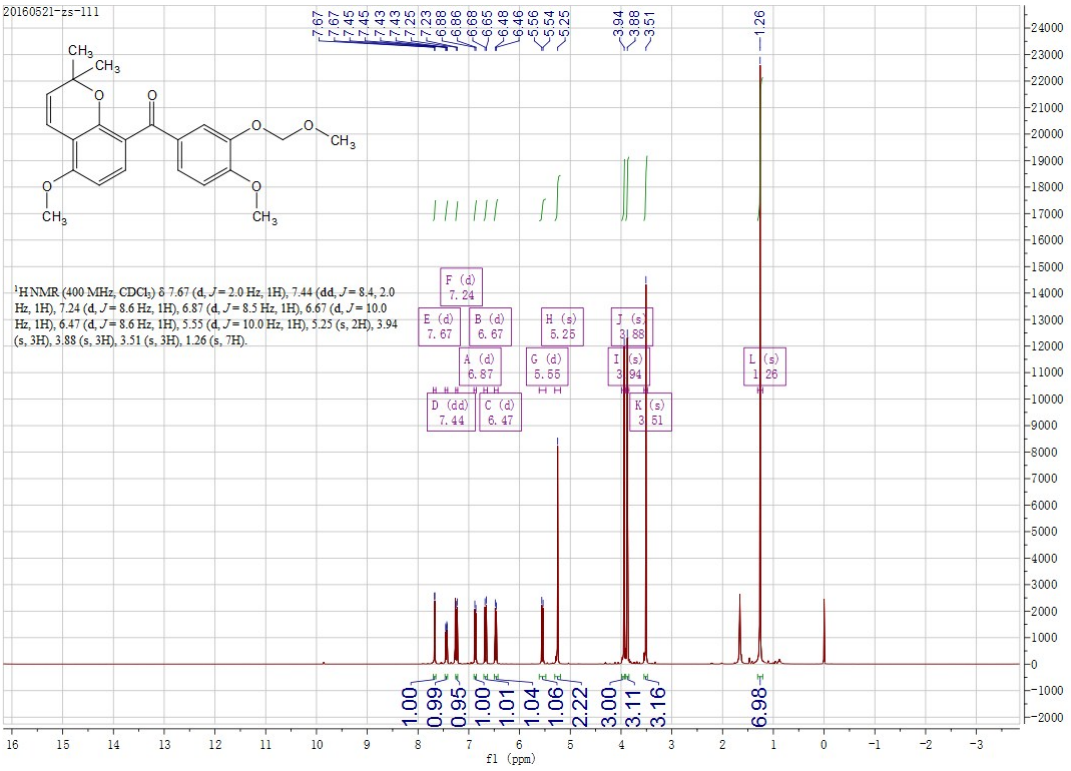
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, J = 8.3 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.5 Hz, 1H), 6.63 (d, J = 10.0 Hz, 1H), 6.38 (d, J = 8.5 Hz, 1H), 5.85 (d, J = 4.3 Hz, 1H), 5.53 (d, J = 10.0 Hz, 1H), 3.79 (s, 3H), 3.03 (d, J = 5.5 Hz, 1H), 2.46 (s, 3H), 1.30 (s, 3H), 1.22 (s, 3H).

3i

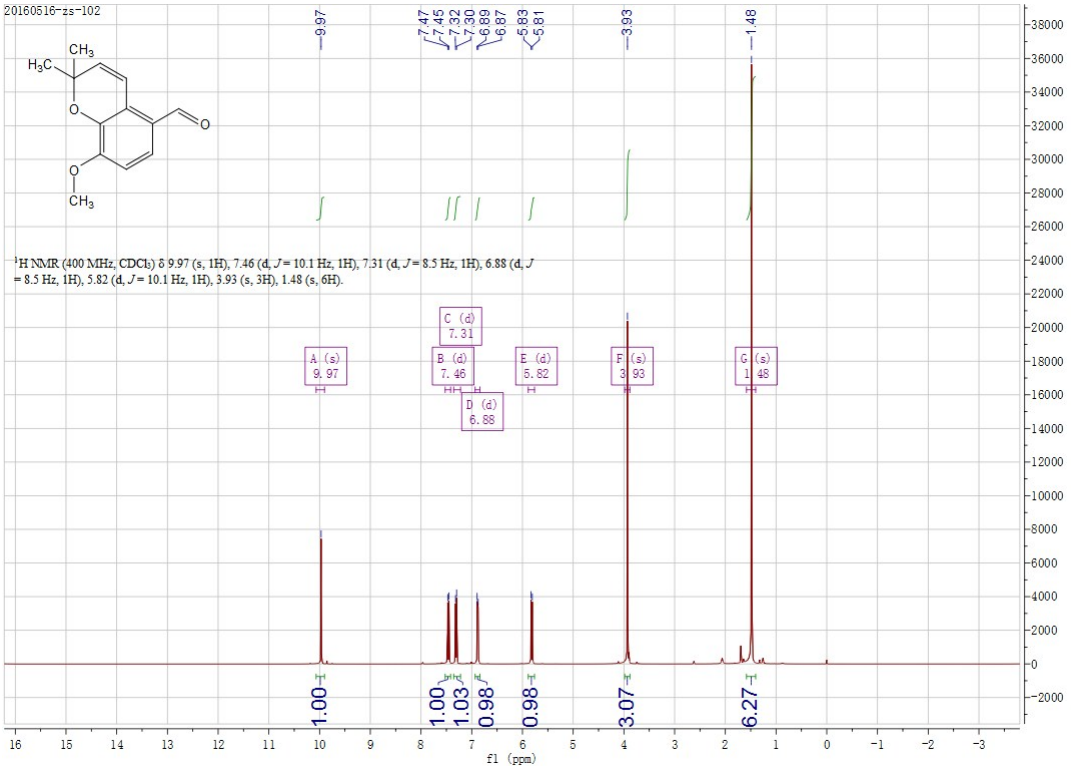


^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J = 1.8 Hz, 1H), 7.03 (d, J = 8.5 Hz, 1H), 6.96 (dd, J = 8.4, 1.7 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.63 (d, J = 10.0 Hz, 1H), 6.38 (d, J = 8.5 Hz, 1H), 5.84 (d, J = 5.3 Hz, 1H), 5.53 (d, J = 10.0 Hz, 1H), 5.20 (s, 2H), 3.85 (s, 3H), 3.79 (s, 3H), 3.49 (s, 3H), 3.05 (d, J = 5.6 Hz, 1H), 1.30 (s, 3H), 1.26 (s, 3H).

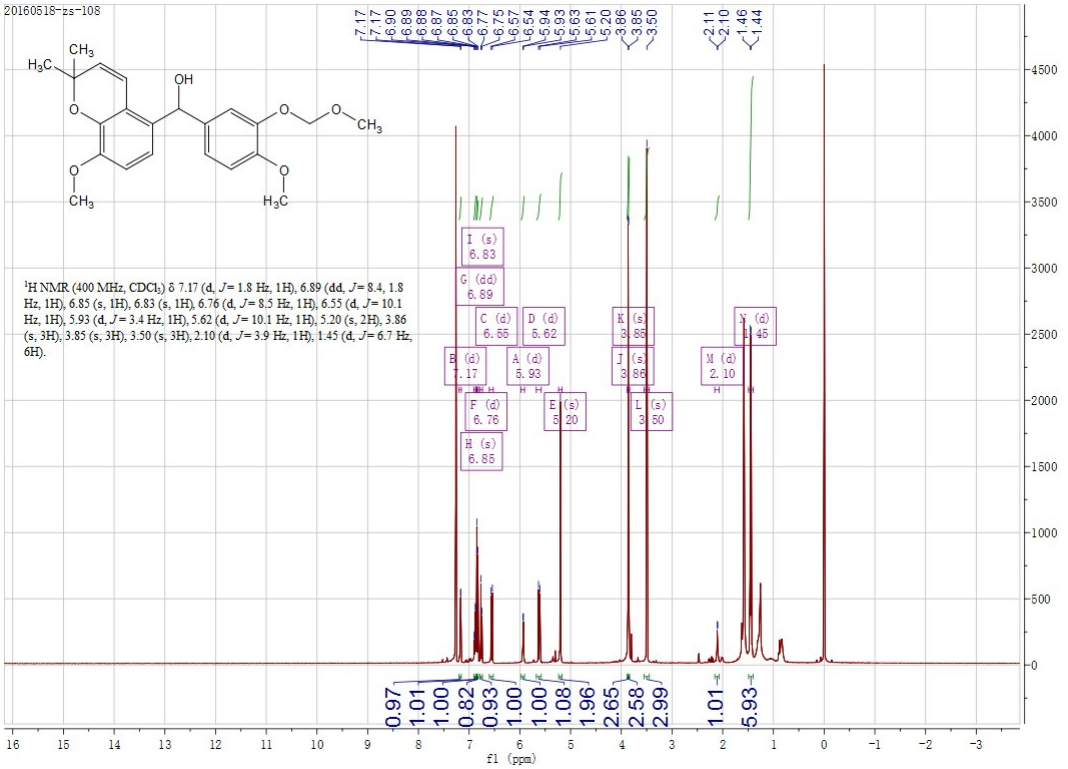
4i


20160521-zs-111
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 2.0 Hz, 1H), 7.44 (dd, J = 8.4, 2.0 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.67 (d, J = 10.0 Hz, 1H), 6.47 (d, J = 8.6 Hz, 1H), 5.55 (d, J = 10.0 Hz, 1H), 5.25 (s, 2H), 3.94 (s, 3H), 3.88 (s, 3H), 3.51 (s, 3H), 1.26 (s, 7H).
f1 (ppm)

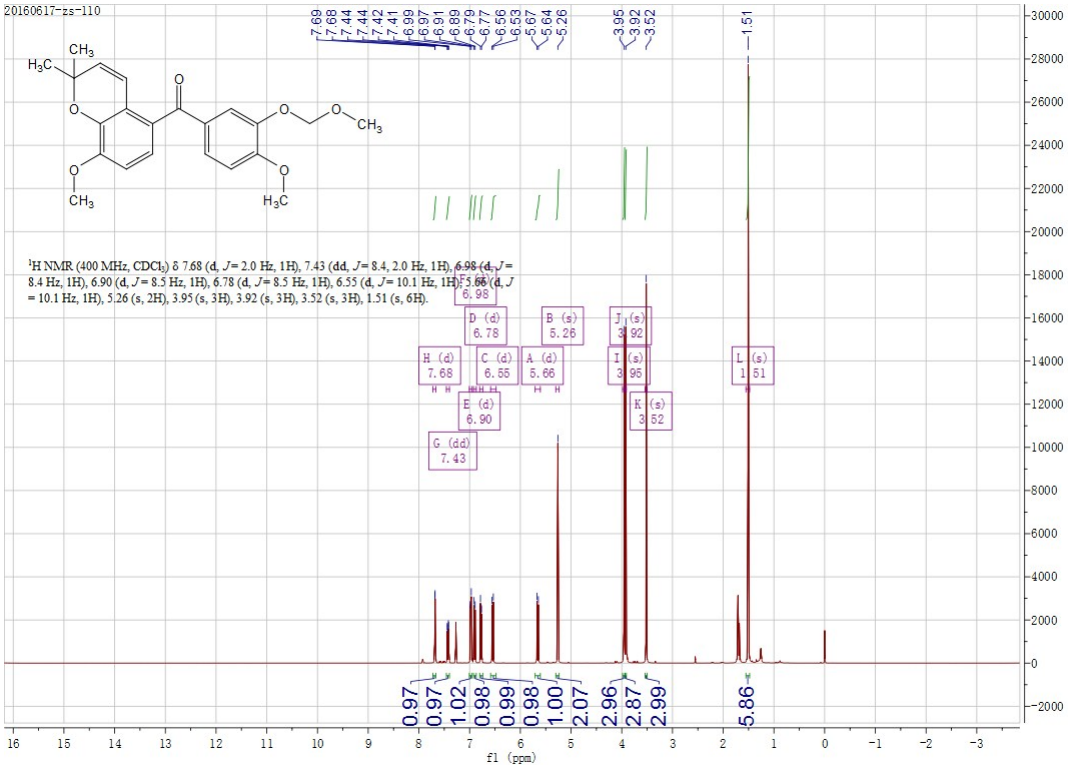
8


20160516-zs-102
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (s, 1H), 7.46 (d, J = 10.1 Hz, 1H), 7.31 (d, J = 8.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 5.82 (d, J = 10.1 Hz, 1H), 3.93 (s, 3H), 1.48 (s, 6H).
f1 (ppm)

9

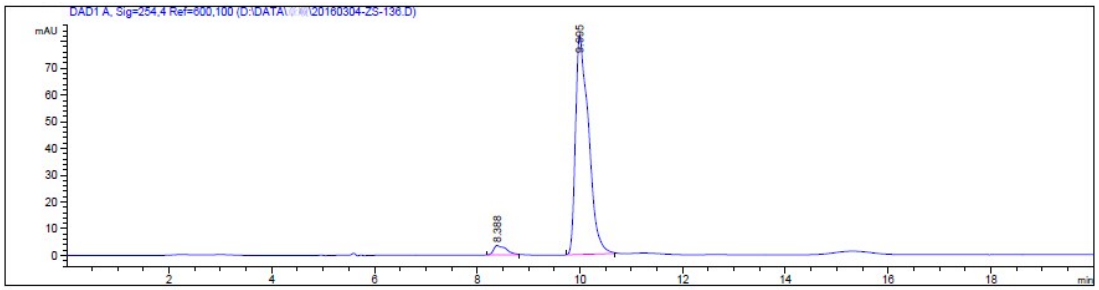

20160518-zs-108
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.17 (d, J = 1.8 Hz, 1H), 6.89 (dd, J = 8.4, 1.8 Hz, 1H), 6.85 (s, 1H), 6.83 (s, 1H), 6.76 (d, J = 8.5 Hz, 1H), 6.55 (d, J = 10.1 Hz, 1H), 5.93 (d, J = 3.4 Hz, 1H), 5.62 (d, J = 10.1 Hz, 1H), 5.20 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.50 (s, 3H), 2.10 (d, J = 3.9 Hz, 1H), 1.45 (d, J = 6.7 Hz, 6H).
f1 (ppm)

10


20160617-zs-110
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.68 (d, J = 2.0 Hz, 1H), 7.43 (dd, J = 8.4, 2.0 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 6.78 (d, J = 8.5 Hz, 1H), 6.55 (d, J = 10.1 Hz, 1H), 5.66 (d, J = 10.1 Hz, 1H), 5.26 (s, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.52 (s, 3H), 1.51 (s, 6H).
f1 (ppm)

SI4. HPLC chromatograms of target compounds

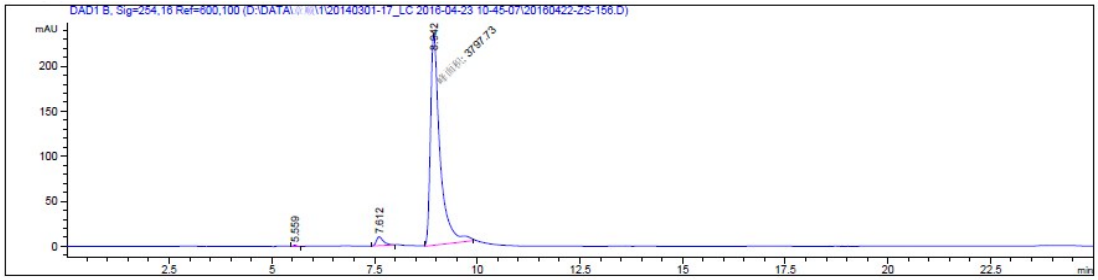
4a



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	8.388	BB	0.2307	60.62122	3.52326	4.0156
2	9.995	BB	0.2444	1449.02405	81.92206	95.9844

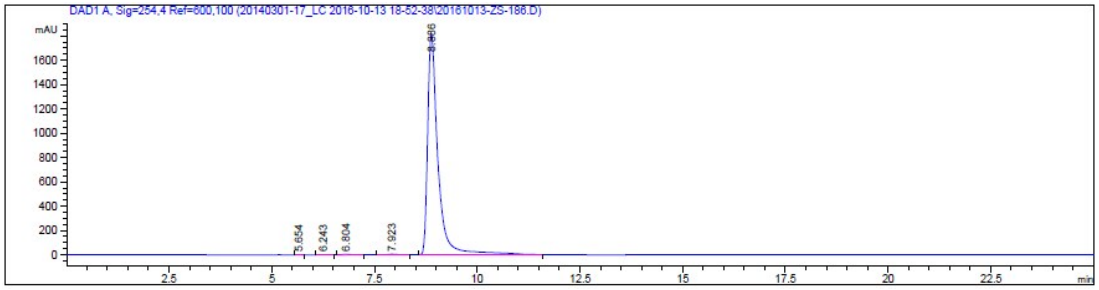
4b



信号 1: DAD1 B, Sig=254,16 Ref=600,100

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	0.0856	6.54361	1.19967	0.1669
2	7.612	BB	0.1798	117.08617	9.77253	2.9859
3	8.942	MM	0.2688	3797.72778	235.43219	96.8473

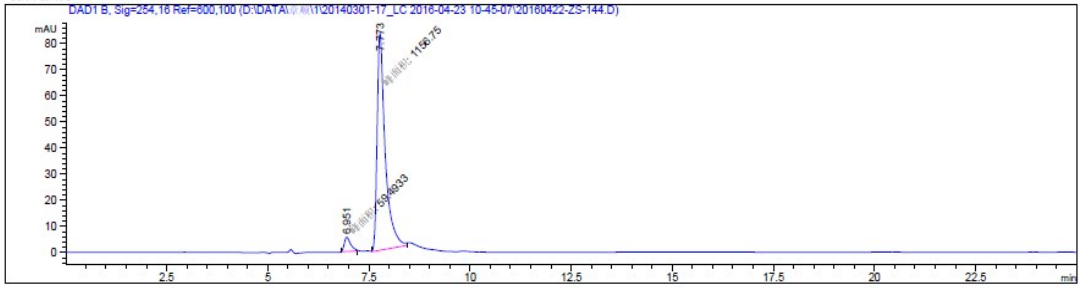
4c



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.654	BV	0.1253	12.59872	1.64593	0.0394
2	6.243	BB	0.2611	30.14365	1.59033	0.0943
3	6.804	BB	0.2298	77.17100	4.74335	0.2413
4	7.923	BB	0.2411	91.71185	5.42738	0.2868
5	8.886	BB	0.2564	3.17651e4	1813.41248	99.3382

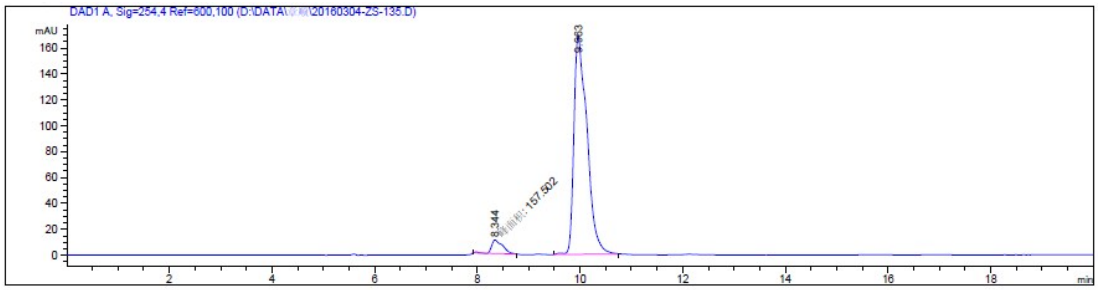
4d



信号 1: DAD1 B, Sig=254,16 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	6.951	MM	0.1812	59.49333	5.47114	4.8916
2	7.773	MM	0.2322	1156.74609	83.03009	95.1084

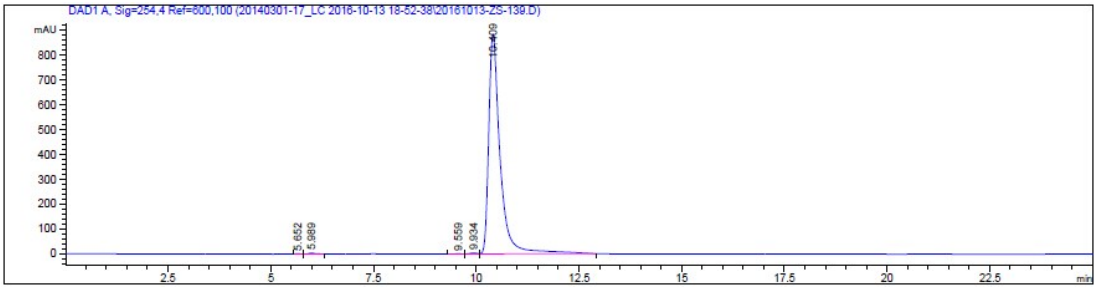
4e



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	8.344	MM	0.2499	157.50227	10.50275	4.9854
2	9.963	BB	0.2450	3001.77686	169.14580	95.0146

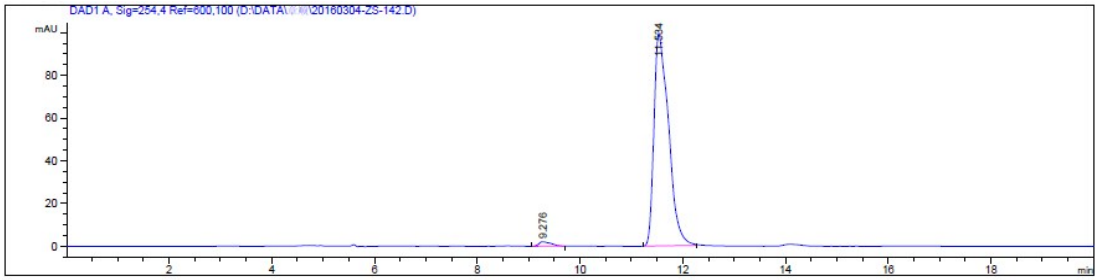
4f



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.652	BV	0.1253	12.57450	1.64275	0.0726
2	5.989	VB	0.1852	46.26952	3.71876	0.2672
3	9.559	BV	0.2081	14.92657	1.11542	0.0862
4	9.934	VV	0.2105	49.58898	3.65041	0.2864
5	10.409	VB	0.2823	1.71941e4	886.35571	99.2877

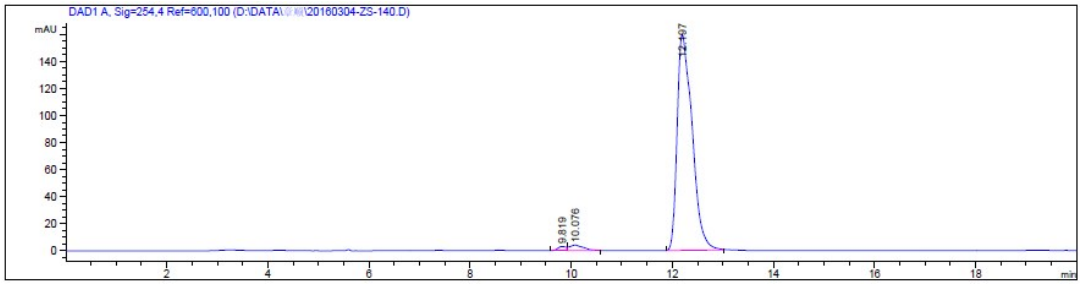
4g



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	9.276	BB	0.2249	34.25296	2.11545	1.7185
2	11.534	BB	0.2759	1958.95276	99.41146	98.2815

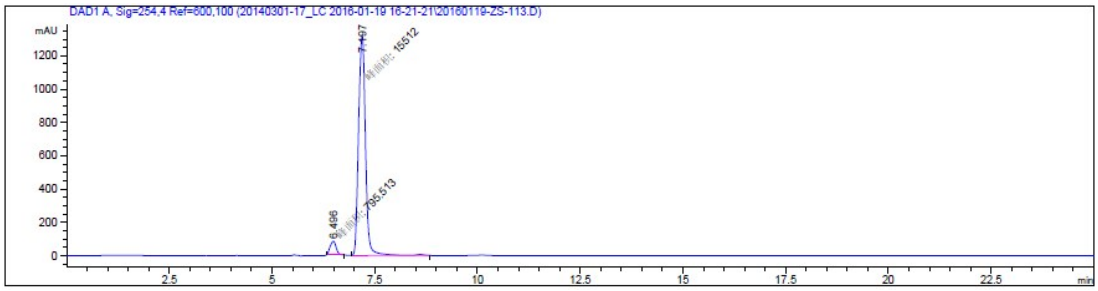
4h



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	9.819	BV	0.1603	32.90554	3.08911	0.9689
2	10.076	VB	0.2684	79.05352	4.03792	2.3276
3	12.197	BB	0.2878	3284.34595	159.76161	96.7035

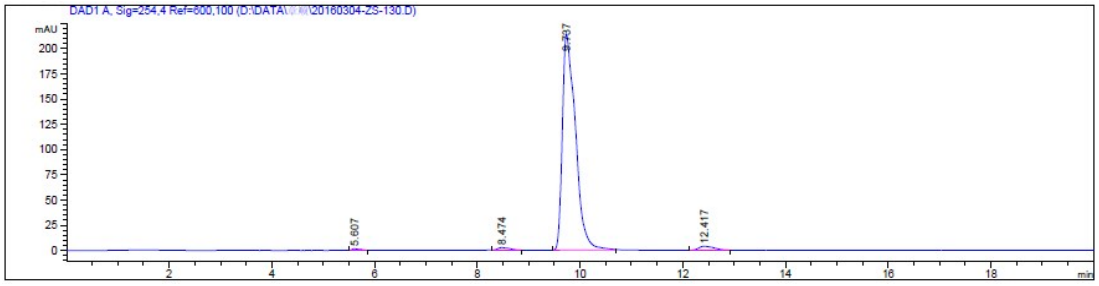
5i



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	6.496	MM	0.1642	795.51337	80.75768	4.8782
2	7.197	MM	0.1951	1.55120e4	1324.97241	95.1218

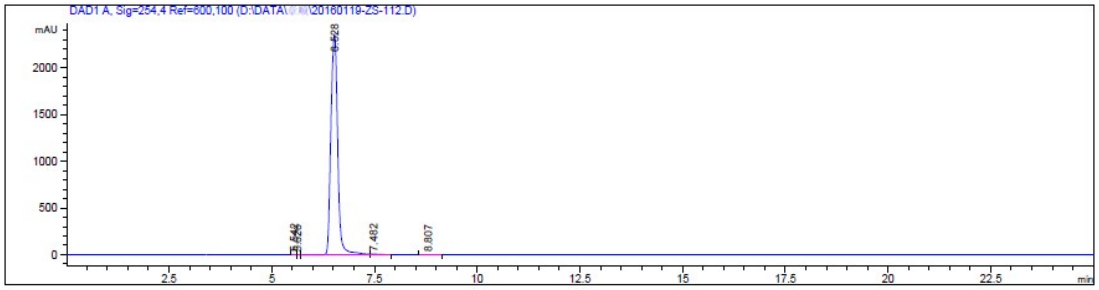
6i



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.607	BB	0.1335	15.75866	1.61961	0.4010
2	8.474	BB	0.2163	40.44462	2.56084	1.0293
3	9.737	BB	0.2446	3792.26196	214.17491	96.5090
4	12.417	BB	0.2872	80.97546	4.01626	2.0607

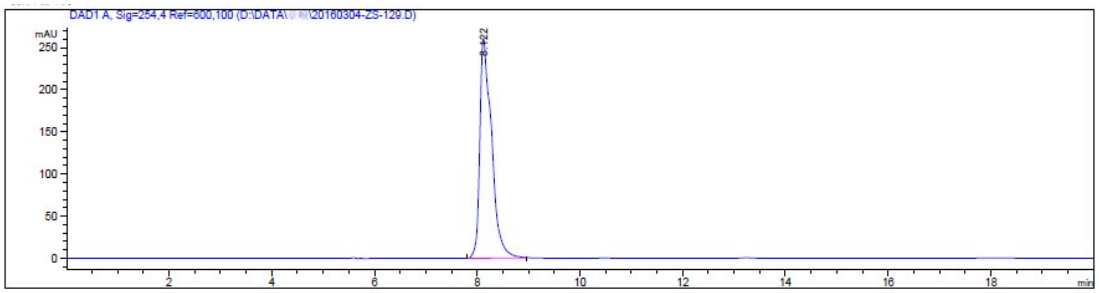
11



信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	5.542	BV	0.0716	25.59680	5.37053	0.0940
2	5.626	VV	0.0589	10.20242	2.77225	0.0375
3	6.528	VV	0.1838	2.70743e4	2360.26611	99.4243
4	7.482	VB	0.2482	105.68037	6.35030	0.3881
5	8.807	BB	0.2152	15.28425	1.09275	0.0561

12

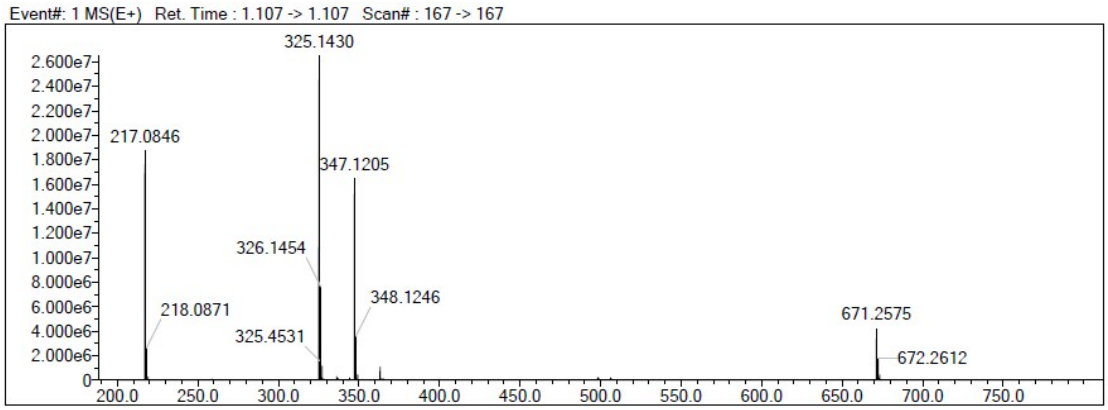


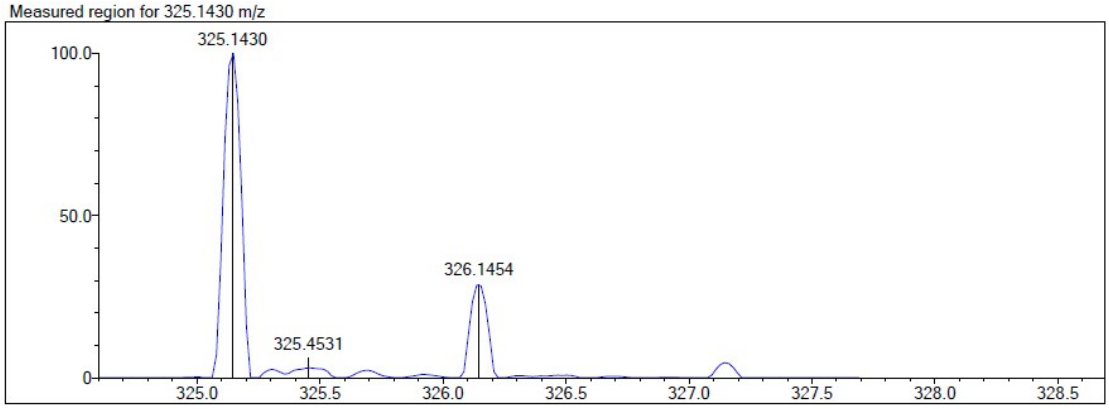
信号 1: DAD1 A, Sig=254,4 Ref=600,100

峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	8.122	BB	0.2176	4146.81494	260.64896	100.0000

SI5. HR-MS of target compounds

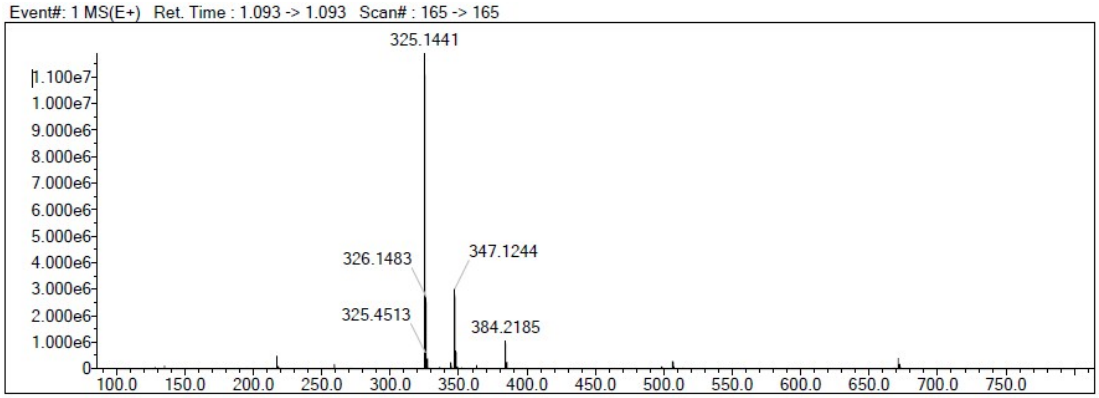
4a

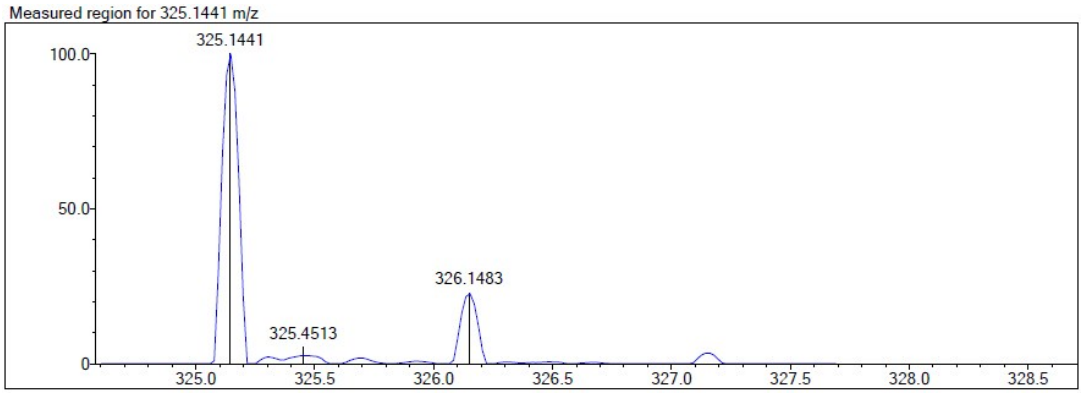




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	64.77	C20 H20 O4	[M+H]+	325.1430	325.1434	-0.4	-1.23	65.15	11.0

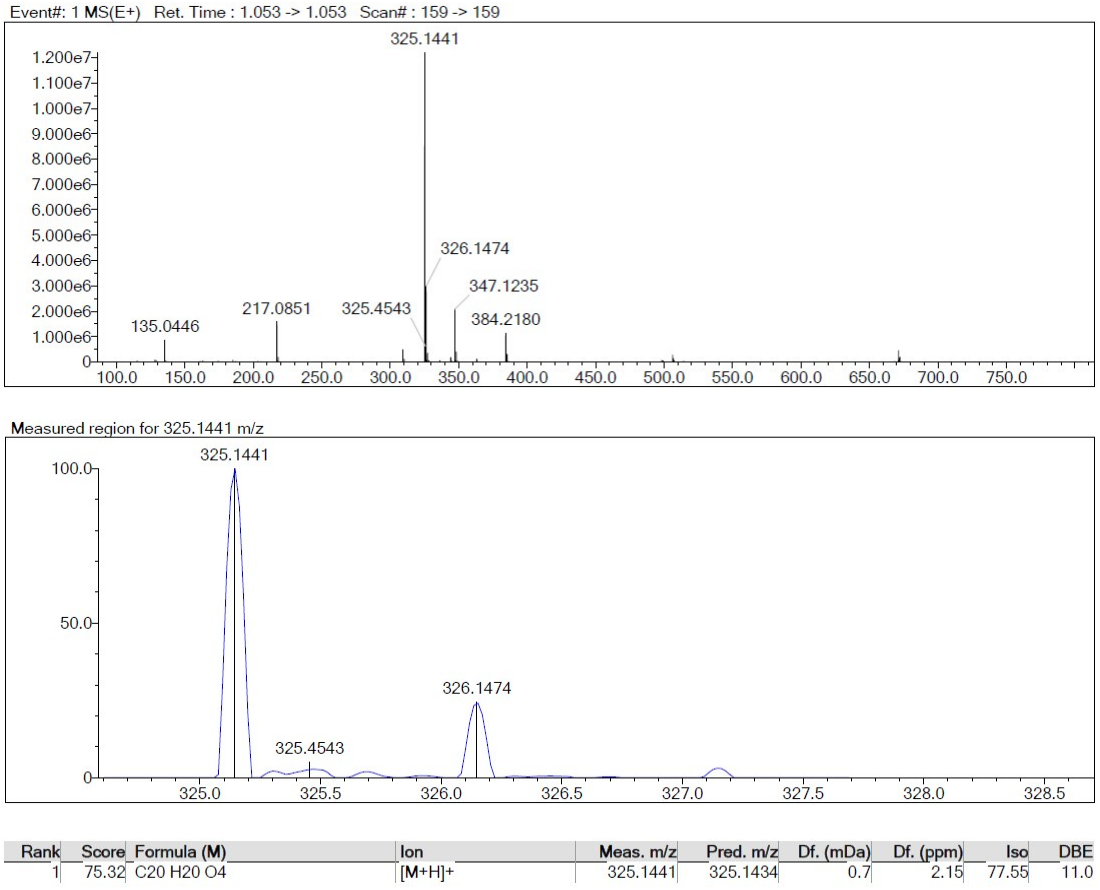
4b





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	78.10	C20 H20 O4	[M+H]+	325.1441	325.1434	0.7	2.15	80.41	11.0

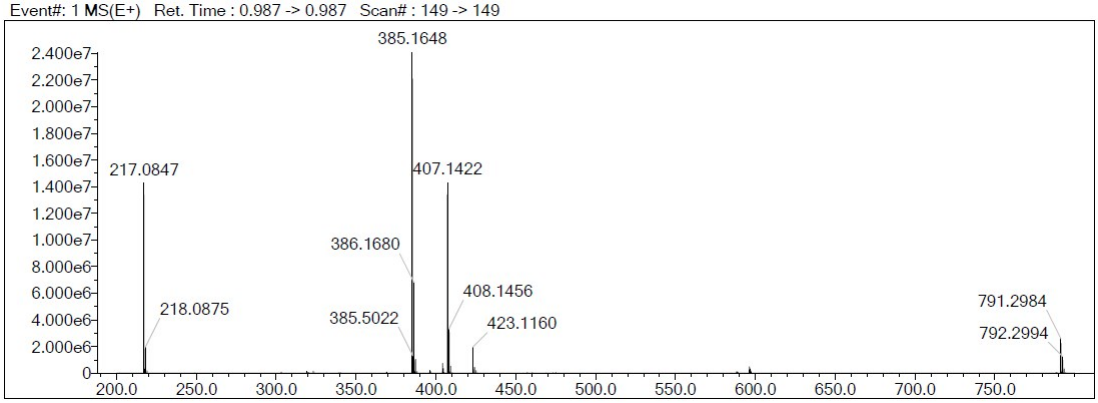
4c



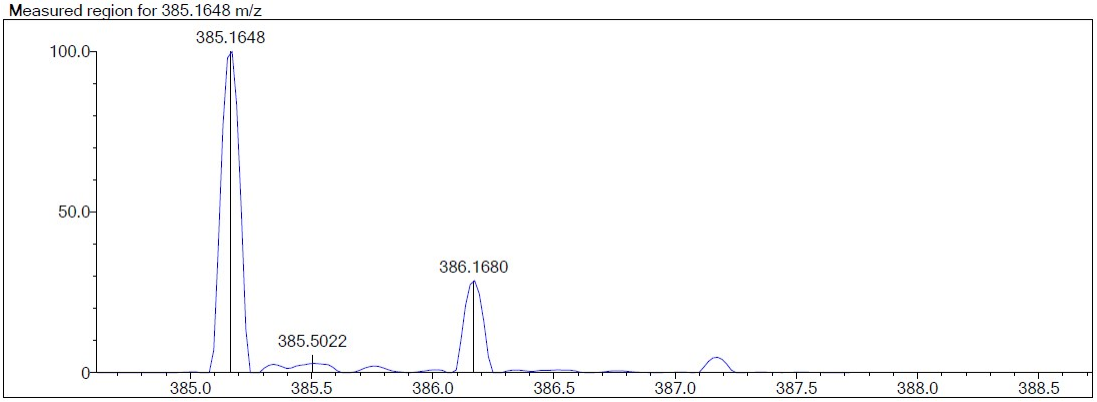
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	75.32	C20 H20 O4	[M+H]+	325.1441	325.1434	0.7	2.15	77.55	11.0

4d

Event#: 1 MS(E+) Ret. Time : 0.987 -> 0.987 Scan# : 149 -> 149



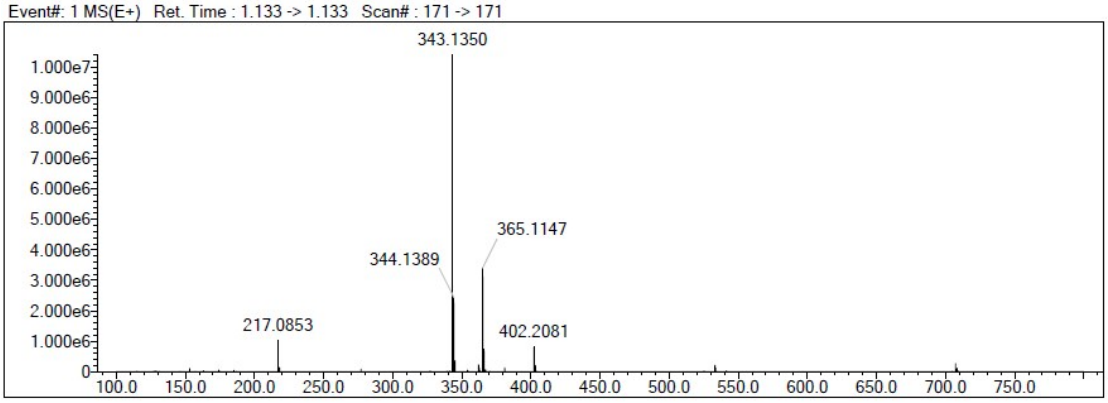
Measured region for 385.1648 m/z



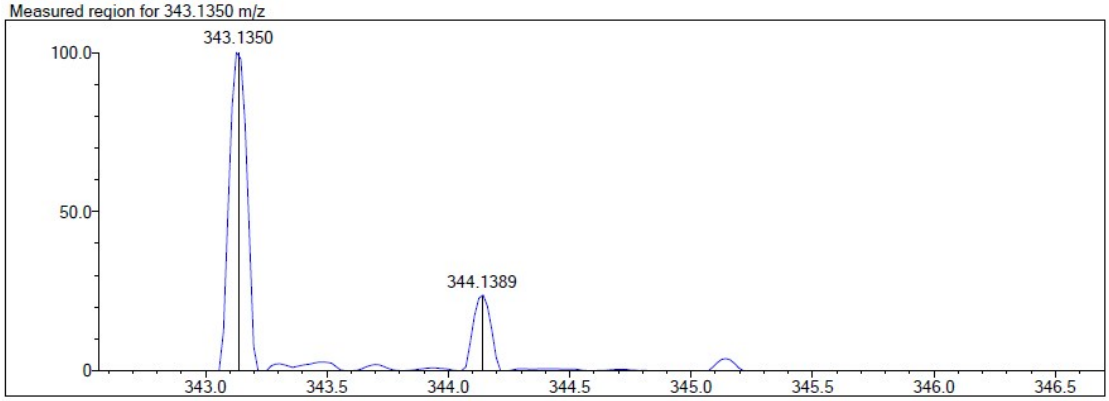
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	84.95	C22 H24 O6	[M+H]+	385.1648	385.1646	0.2	0.52	84.95	11.0

4e

Event#: 1 MS(E+) Ret. Time : 1.133 -> 1.133 Scan# : 171 -> 171

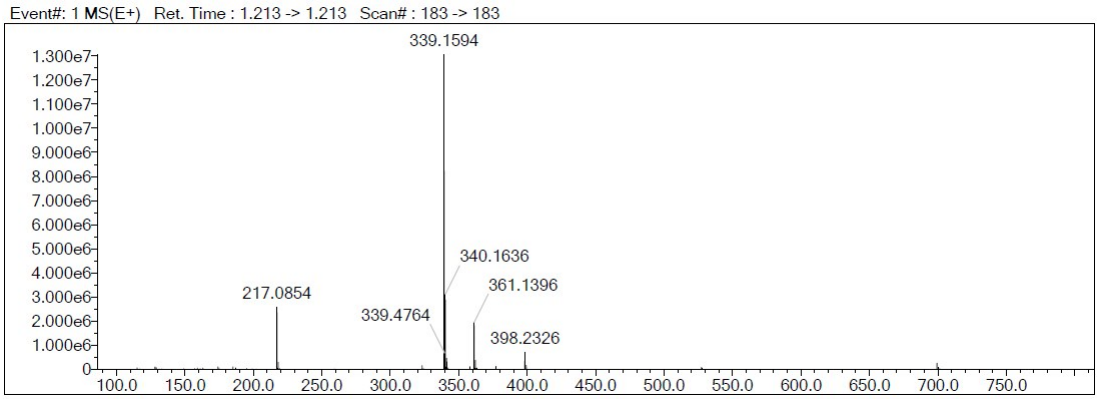


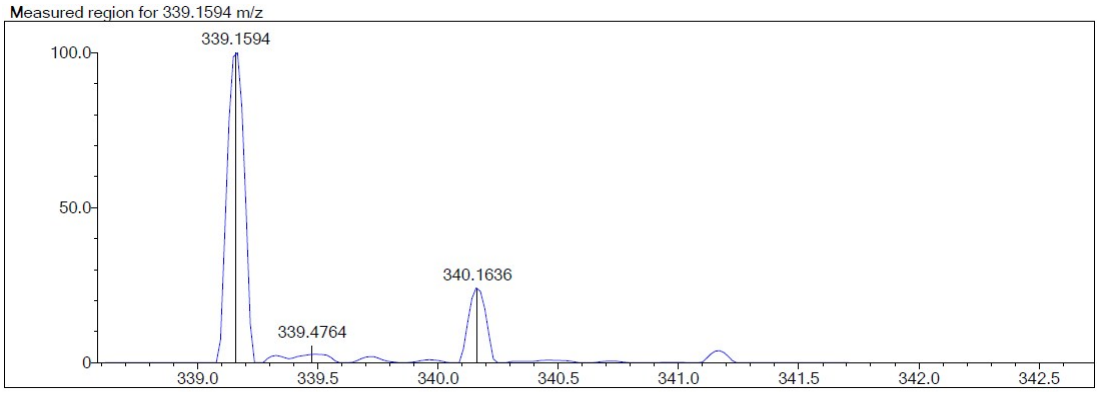
Measured region for 343.1350 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	82.62	C20 H19 O4 F	[M+H]+	343.1350	343.1340	1.0	2.91	86.76	11.0

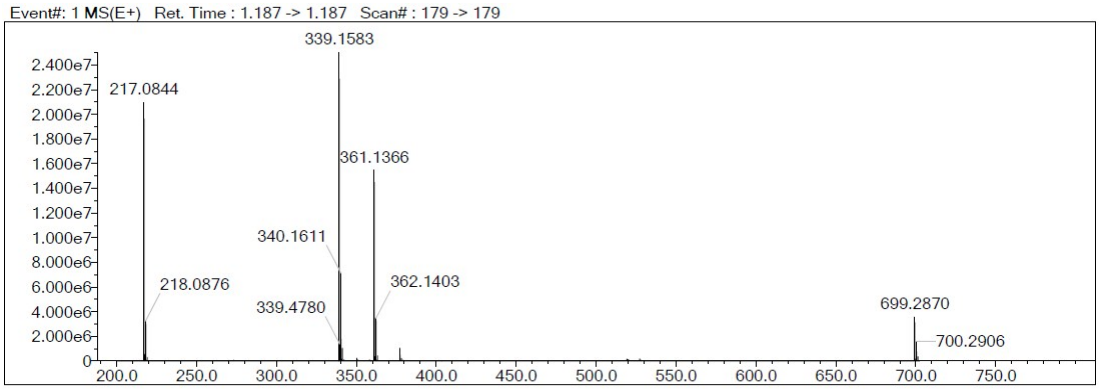
4f



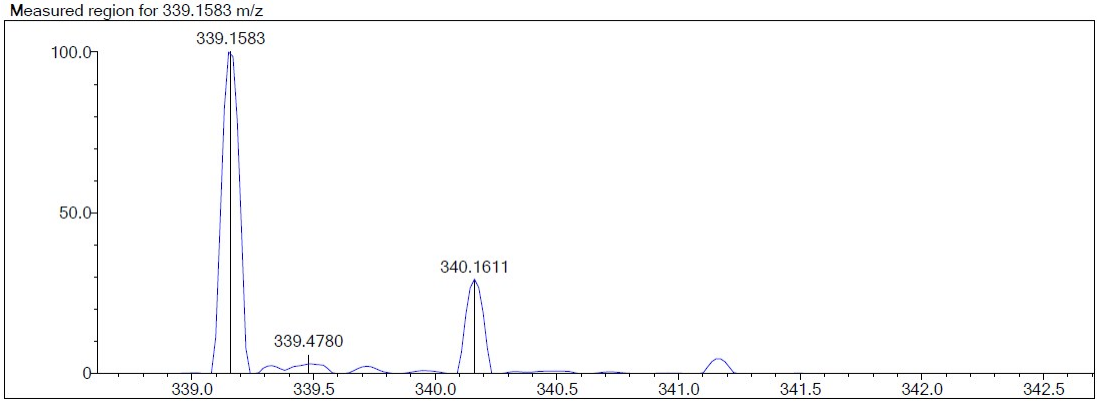


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	88.71	C21 H22 O4	[M+H]+	339.1594	339.1591	0.3	0.88	88.71	11.0

4g



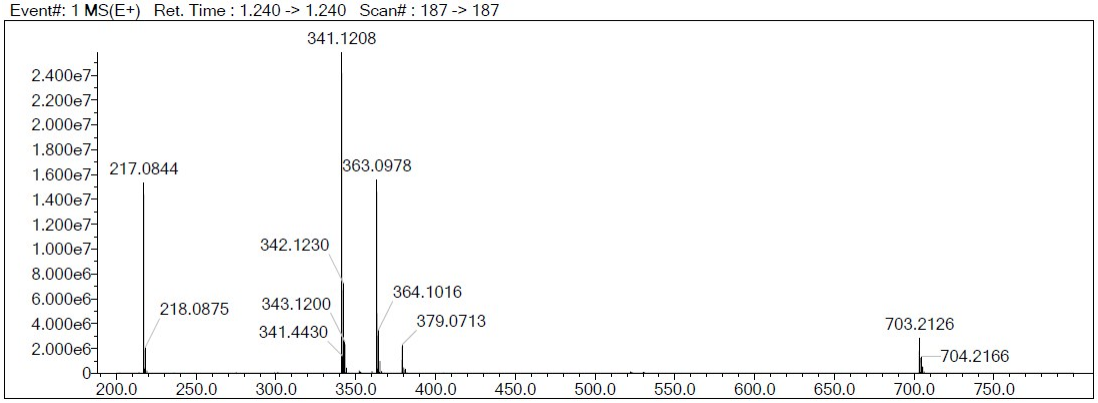
Measured region for 339.1583 m/z



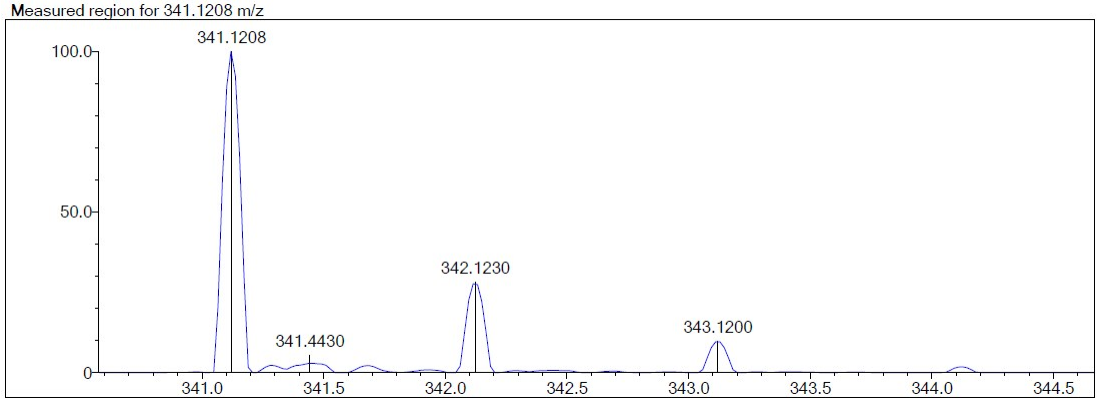
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	76.94	C21 H22 O4	[M+H]+	339.1583	339.1591	-0.8	-2.36	79.65	11.0

4h

Event#: 1 MS(E+) Ret. Time : 1.240 -> 1.240 Scan# : 187 -> 187

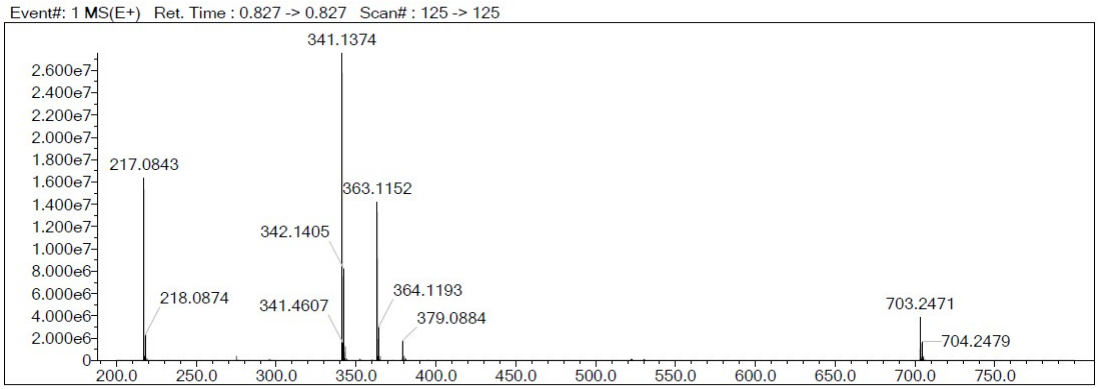


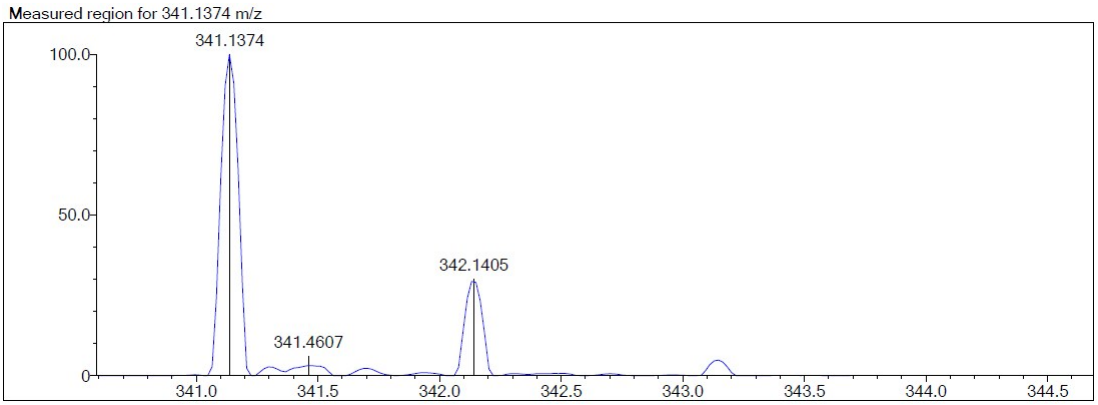
Measured region for 341.1208 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	84.62	C20 H20 O3 S	[M+H]+	341.1208	341.1206	0.2	0.59	84.62	11.0

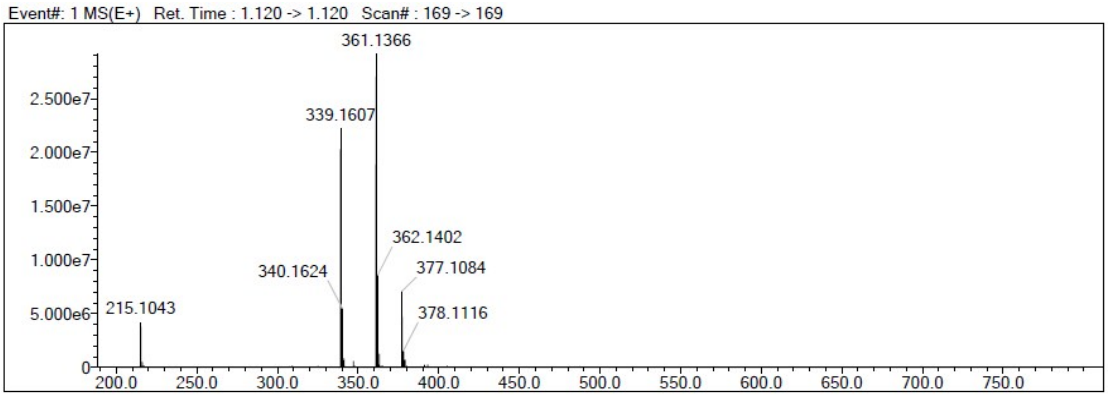
5i

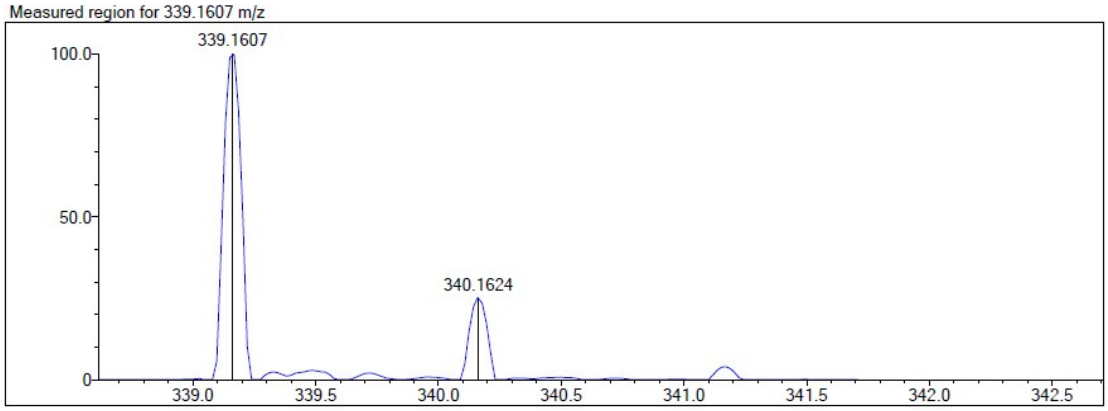




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	65.77	C20 H20 O5	[M+H]+	341.1374	341.1384	-1.0	-2.93	69.11	11.0

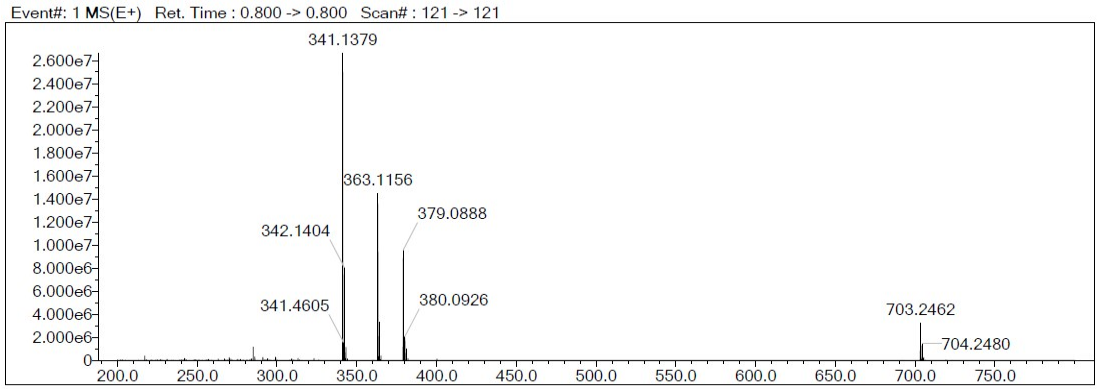
6i

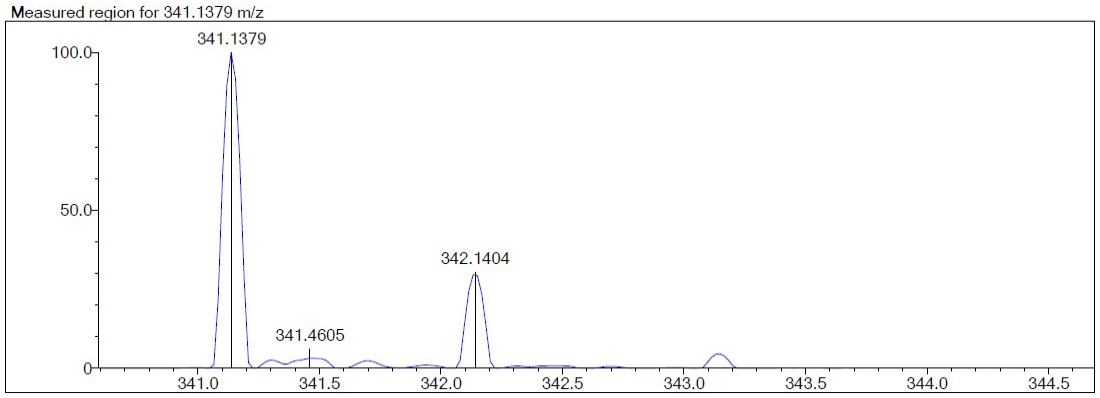




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	81.63	C21 H22 O4	[M+H]+	339.1607	339.1591	1.6	4.72	90.00	11.0

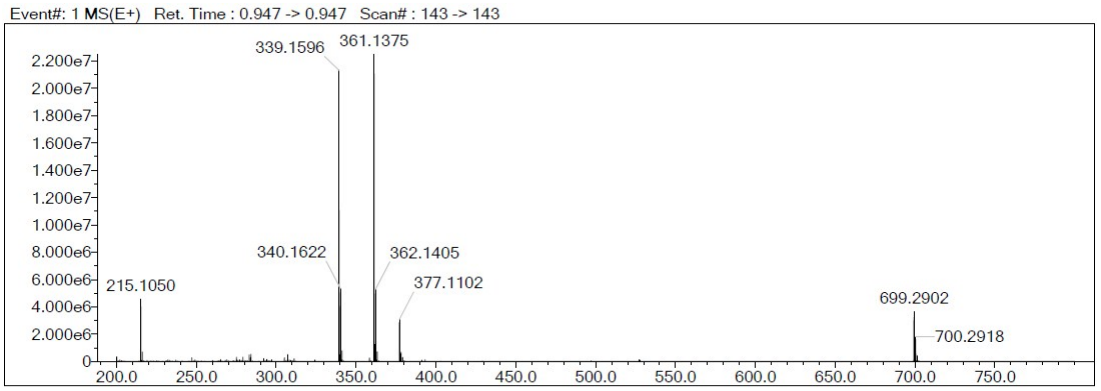
11

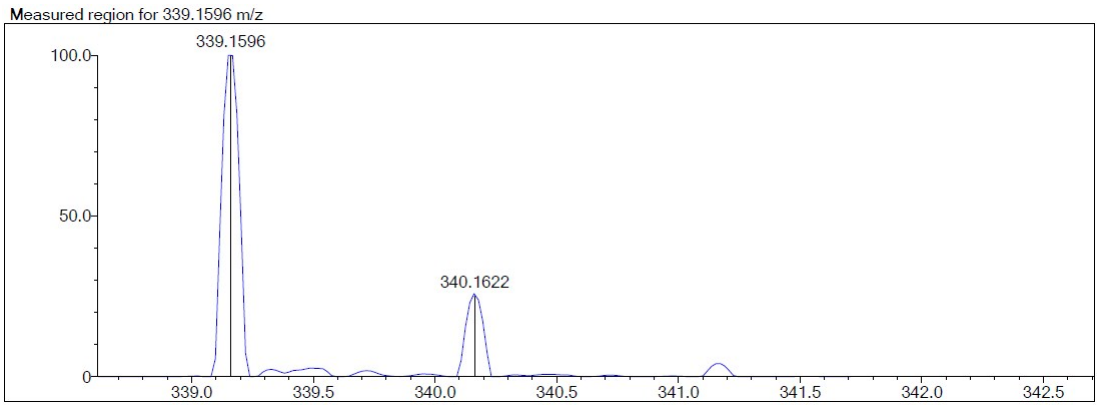




Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	67.80	C20 H20 O5	[M+H]+	341.1379	341.1384	-0.5	-1.47	68.61	11.0

12





Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	90.54	C21 H22 O4	[M+H]+	339.1596	339.1591	0.5	1.47	91.62	11.0