**Supplementary Information**

# Discovery of flavonoids from *Scutellaria baicalensis* with inhibitory activity against PCSK 9 expression: Isolation, synthesis and their biological evaluation

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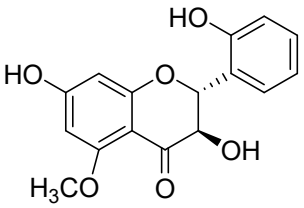
# Figure legends

# List of Table

# Compound **1**




- Appearance: yellow powder
- $[\alpha]_D^{20}$ 95.11° (*c* 0.31, $CD_3OD$)
- UV $\lambda_{max}^{MeOH}$(log ε): 284.5 (4.55)
- HRESIMS *m/z* $[M+H]^+$ 303.0856 (calcd for $C_{16}H_{15}O_6$ 303.0990)

CD [MeOH, nm (Δε)] : 276 (-0.39), 299 (-0.13), 328 (+0.21).

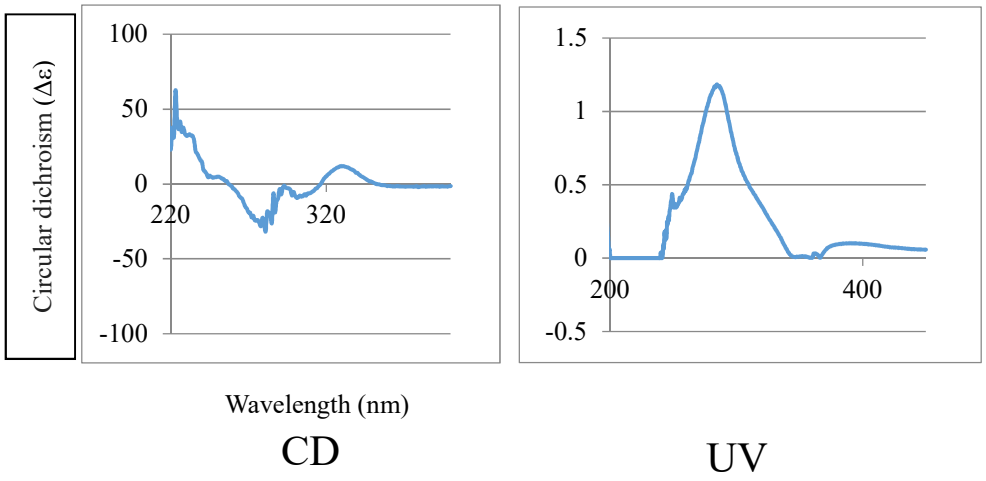
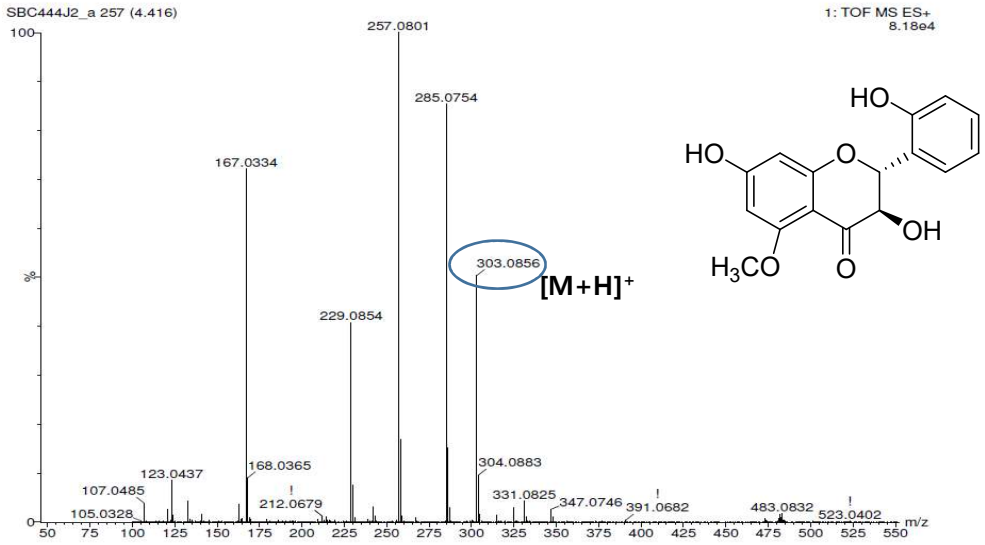


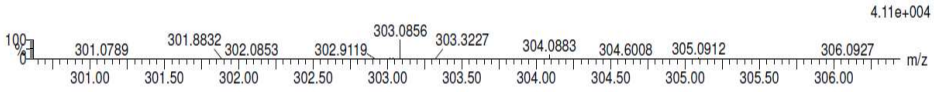

**Fig. 1** CD and UV spectrum of compound **1**




**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 100.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
57 formula(e) evaluated with 8 results within limits (up to 100 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 O: 0-50
SBC444J2_a 257 (4.416)
1: TOF MS ES+




| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | | | |
| Maximum: | | 5.0 | 100.0 | 50.0 | | | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula |
| 303.0856 | 303.0869 | -1.3 | -4.3 | 9.5 | 187.4 | 0.147 | 86.32 | C16 H15 O6 |
| | 303.0810 | 4.6 | 15.2 | 18.5 | 193.7 | 6.387 | 0.17 | C23 H11 O |
| | 303.0927 | -7.1 | -23.4 | 0.5 | 197.2 | 9.875 | 0.01 | C9 H19 O11 |
| | 303.0716 | 14.0 | 46.2 | 5.5 | 194.5 | 7.242 | 0.07 | C12 H15 O9 |
| | 303.1021 | -16.5 | -54.4 | 13.5 | 190.7 | 3.469 | 3.12 | C20 H15 O3 |
| | 303.0657 | 19.9 | 65.7 | 14.5 | 189.6 | 2.282 | 10.20 | C19 H11 O4 |
| | 303.1080 | -22.4 | -73.9 | 4.5 | 194.0 | 6.765 | 0.12 | C13 H19 O8 |
| | 303.0564 | 29.2 | 96.3 | 1.5 | 198.5 | 11.253 | 0.00 | C8 H15 O12 |

**Fig. 2** Mass spectrum of compound **1**

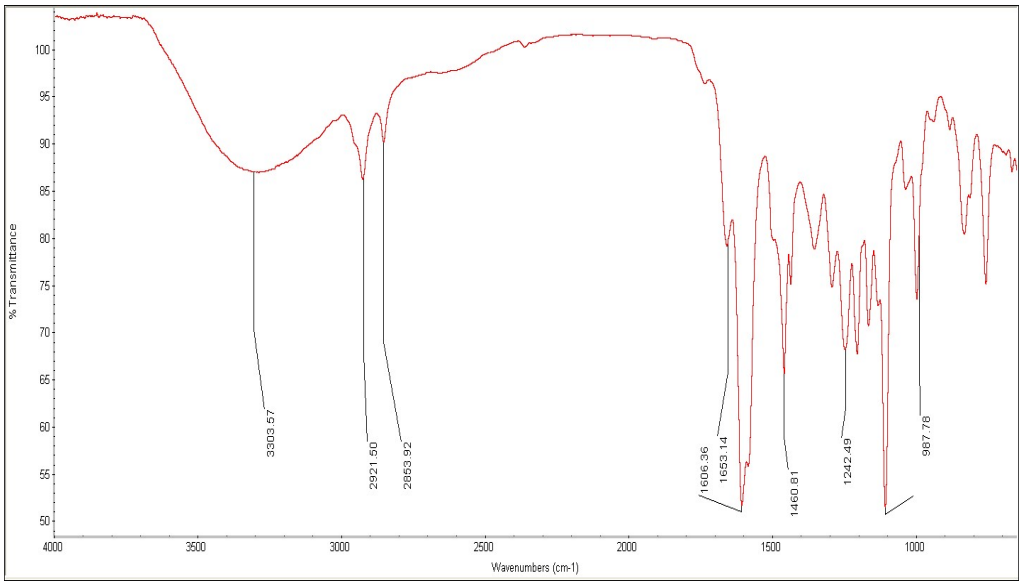



**Fig. 3** IR spectrum of compound **1**

The IR spectrum of compound **1** was recorded by Attenuated Total Reflection (ATR) method in $CD_3OD$.

Band assignment: IR spectrum of **1** exhibited typical absorption bands at 3303 $cm^{-1}$ due to the stretching vibration of hydroxyl group. The band at 1653 $cm^{-1}$ can be attributed to the stretching vibration of carbonyl group.

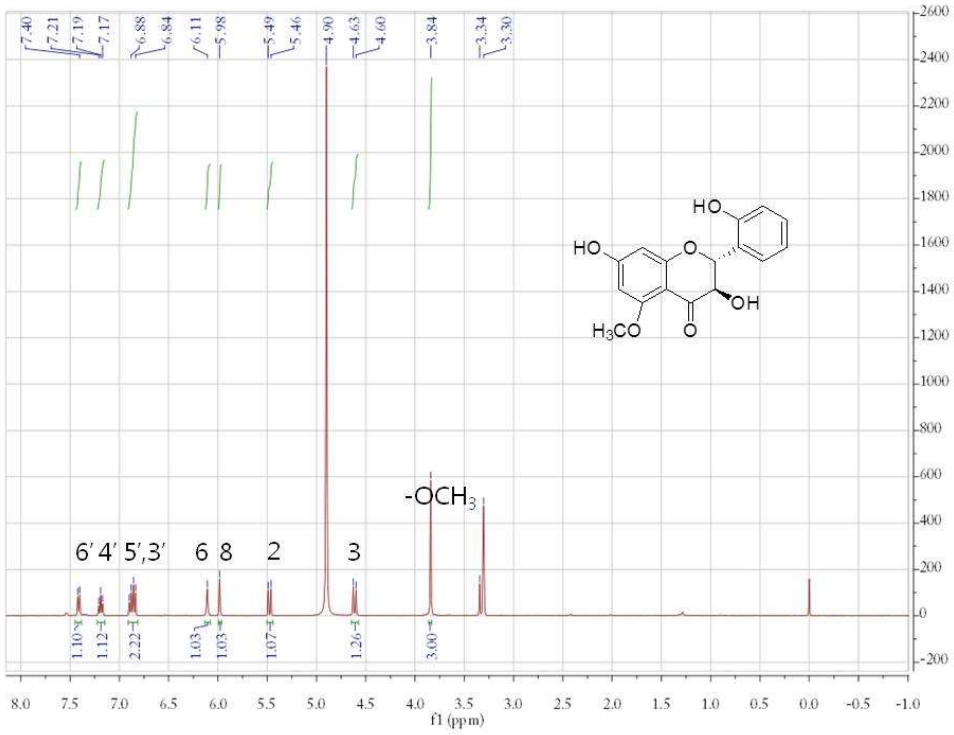



**Fig. 4** $^{1}$H-NMR (400 MHz, $CD_3OD$) spectrum of compound **1**

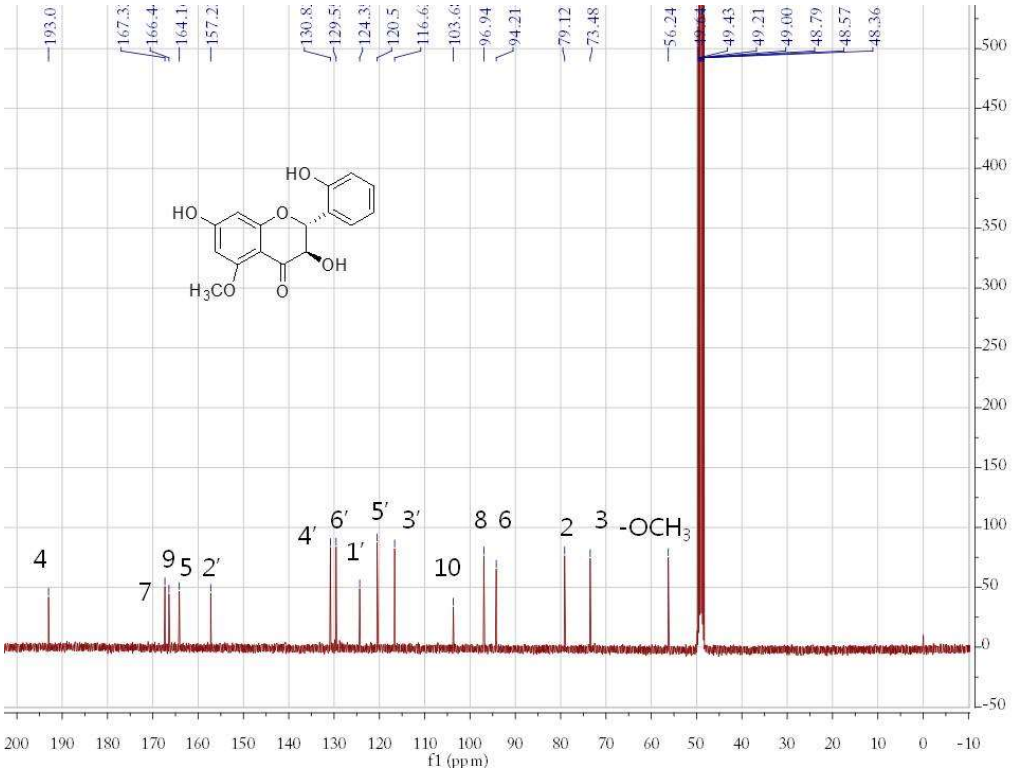



**Fig.5** $^{13}$C-NMR (100 MHz, $CD_3OD$) spectrum of compound **1**

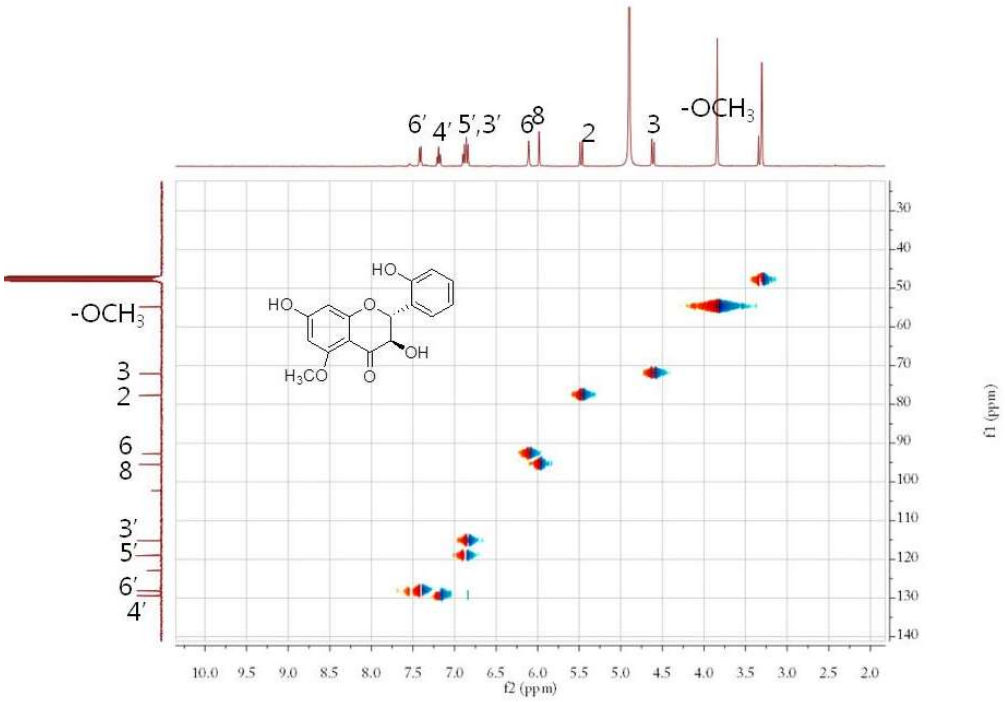



**Fig. 6** $^{1}H$-$^{13}C$-HSQC spectrum of compound **1**

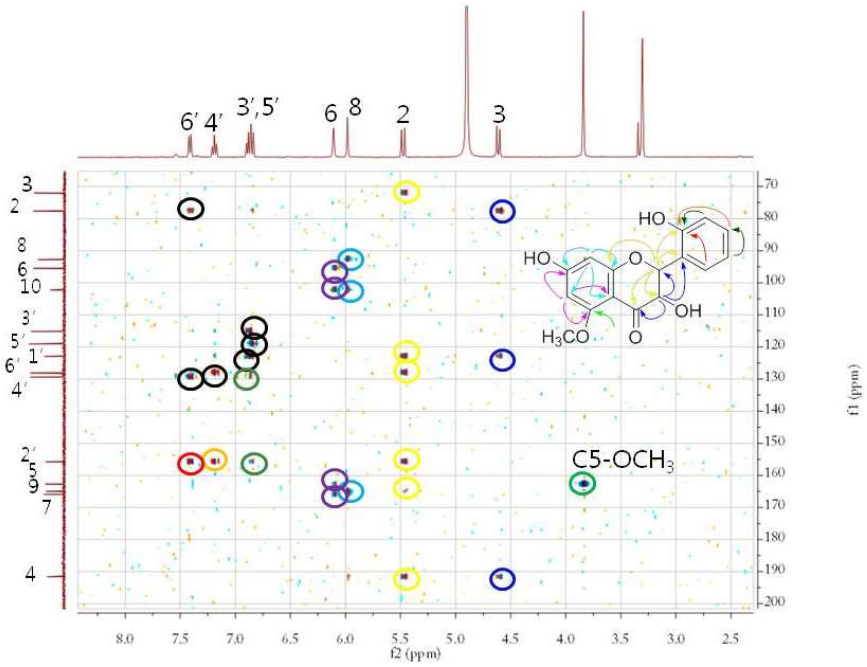



**Fig. 7** $^{1}H$-$^{13}C$-HMBC spectrum of compound **1**

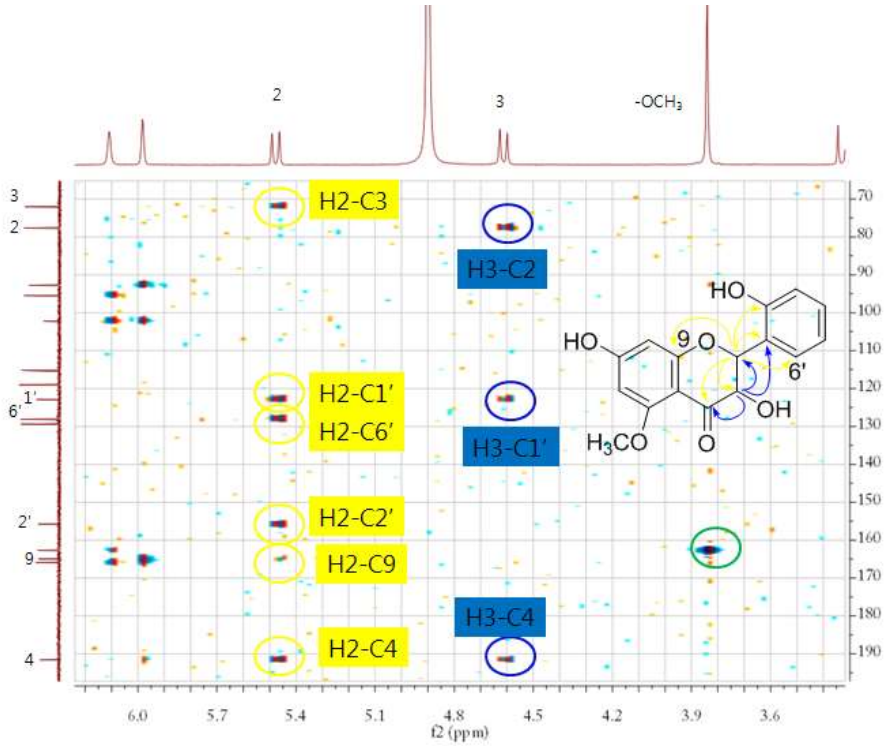



**Fig. 8** $^{1}$H-$^{13}$C-HMBC spectrum of compound **1** (confirmation of correlations of H2 and H3)

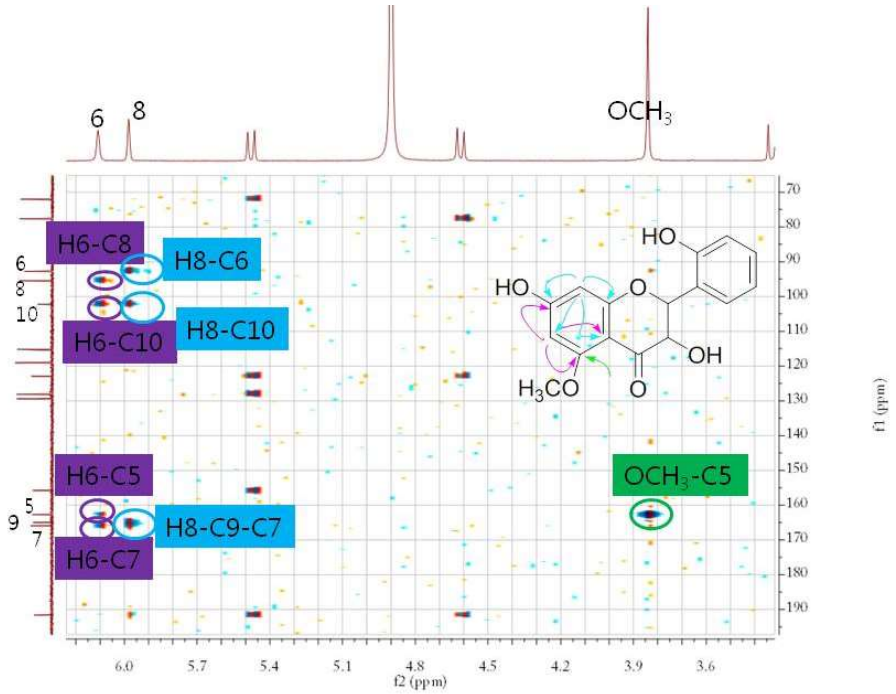



**Fig. 9** $^{1}$H-$^{13}$C-HMBC spectrum of compound **1** (confirmation of correlations of H6 and H8 and methoxyl group)

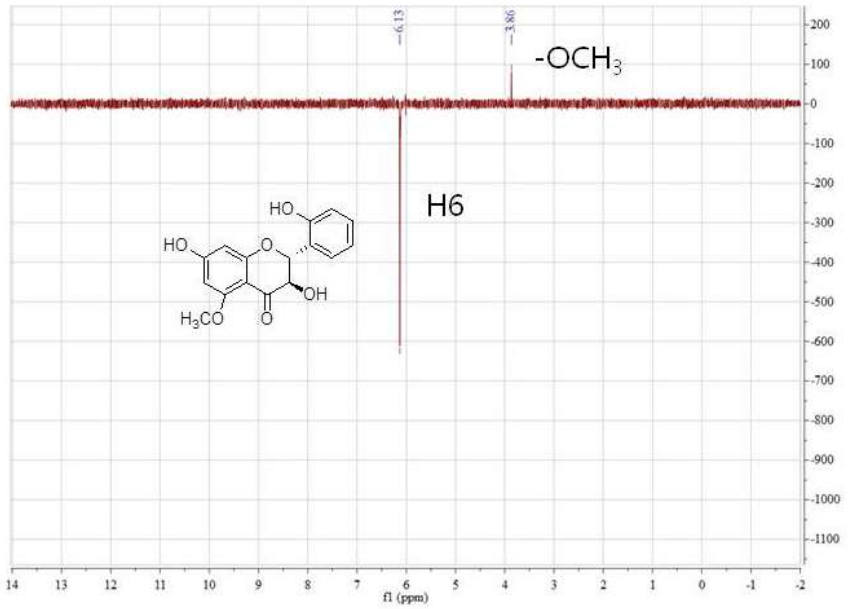



**Fig. 10** NOESY difference spectrum for H6 irradiation of compound **1**.

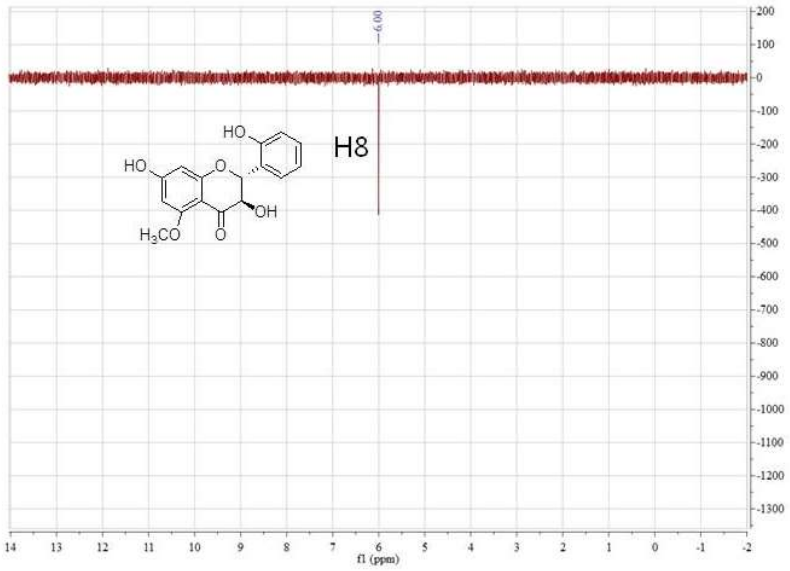



**Fig. 11** NOE difference spectrum for H8 irradiation of compound **1**.

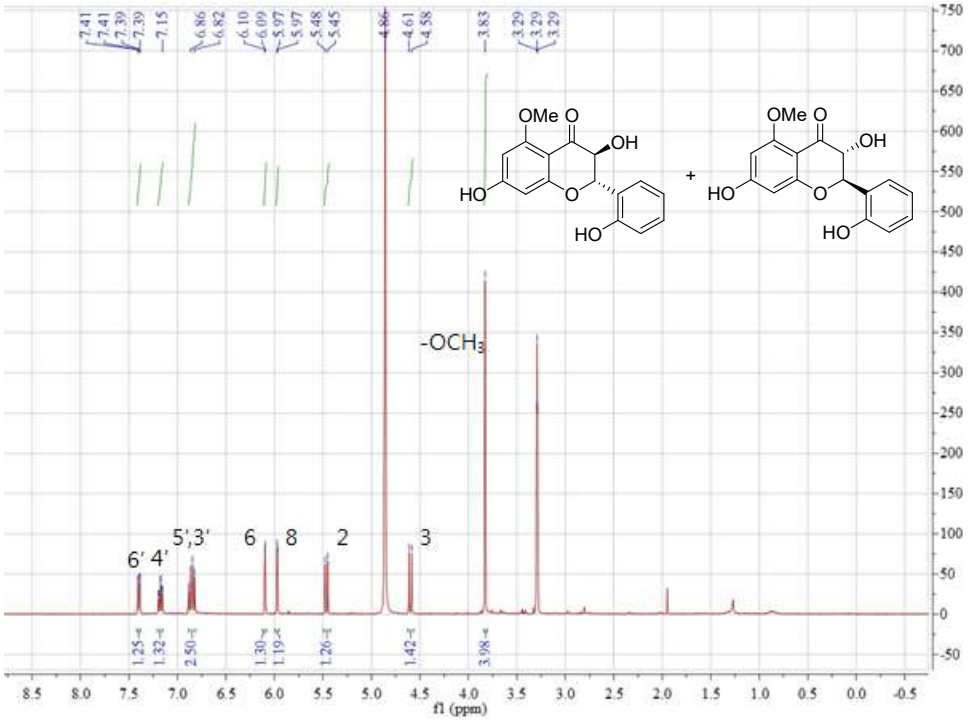



**Fig. 12** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **1a**

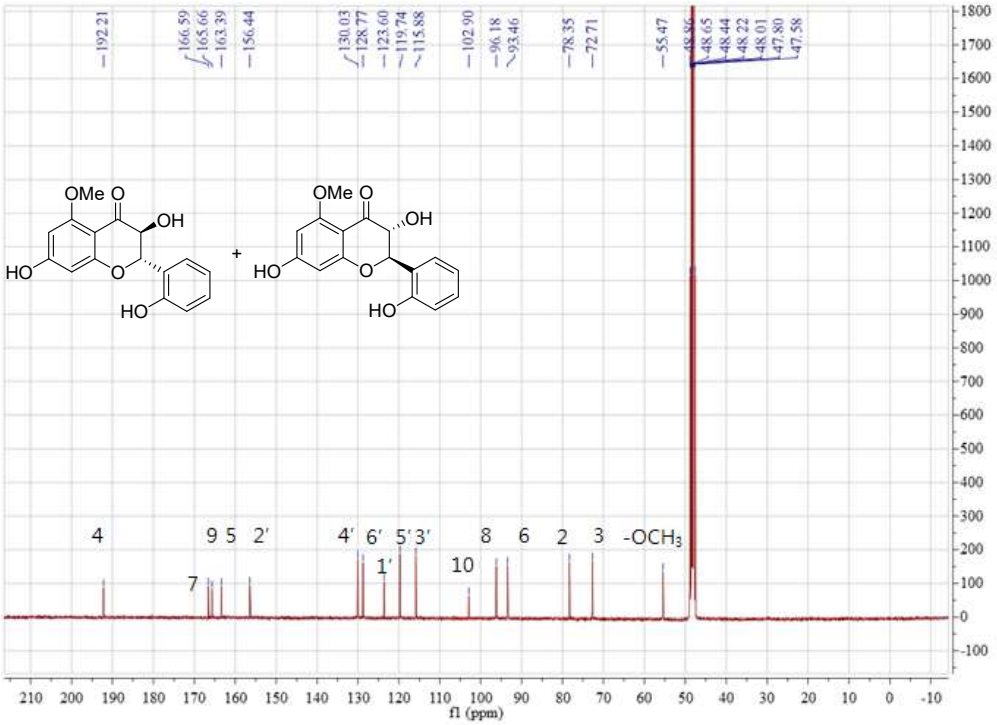
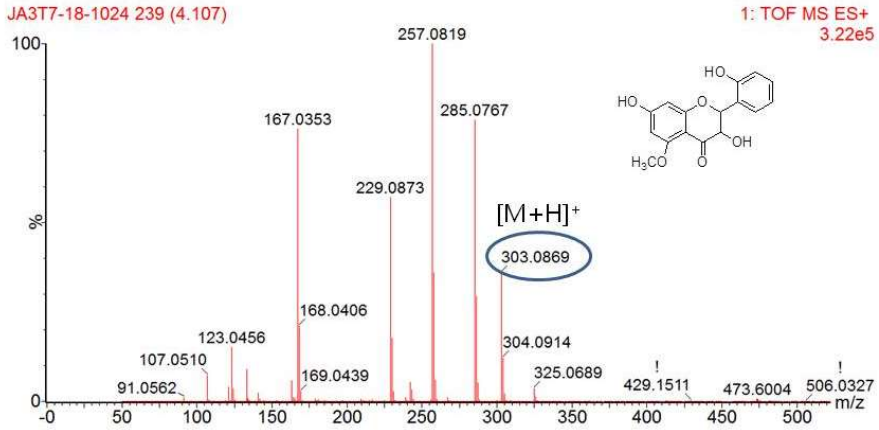



**Fig. 13** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **1a**

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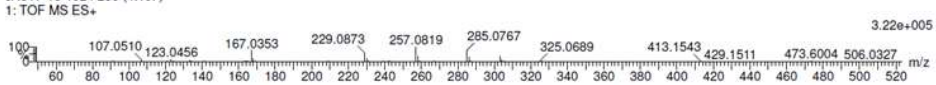
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
57 formula(e) evaluated with 7 results within limits (up to 100 closest results for each mass)
Elements Used:
C: 0-100 H: 0-100 O: 0-50
JA3T7-18-1024 239 (4.107)
1: TOF MS ES+



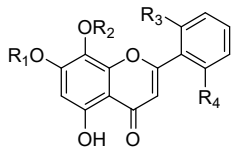

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | | | |
| Maximum: | | 5.0 | 100.0 | 50.0 | | | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula |
| 303.0869 | 303.0869 | 0.0 | 0.0 | 9.5 | 458.3 | 3.849 | 2.13 | C16 H15 O6 |
| | 303.0927 | -5.8 | -19.1 | 0.5 | 461.6 | 7.124 | 0.08 | C9 H19 O11 |
| | 303.0810 | 5.9 | 19.5 | 18.5 | 454.7 | 0.238 | 78.80 | C23 H11 O |
| | 303.1021 | -15.2 | -50.2 | 13.5 | 456.6 | 2.203 | 11.05 | C20 H15 O3 |
| | 303.0716 | 15.3 | 50.5 | 5.5 | 460.3 | 5.842 | 0.29 | C12 H15 O9 |
| | 303.1080 | -21.1 | -69.6 | 4.5 | 460.3 | 5.864 | 0.28 | C13 H19 O8 |
| | 303.0657 | 21.2 | 69.9 | 14.5 | 457.0 | 2.608 | 7.37 | C19 H11 O4 |

**Fig. 14** Mass spectrum of compound **1a**

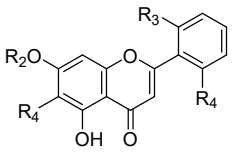
Table of $^{1}H$-and$^{13}C$-NMR spectroscopic (in $CD_3OD$) of Compound **1** and **1a**

| | 1 | | 1a | |
|---|---|---|---|---|
| No. | $\delta_H$ *a)* | $\delta_C$ *b)* | $\delta_H$ *a)* | $\delta_C$ *b)* |
| 1 | - | - | - | |
| 2 | 5.49(1H, d, *J*=11.2 Hz), | 79.1 | 5.48 (d, *J* = 11.2 Hz) | 78.3 |
| 3 | 4.62(1H, d, *J*=11.2 Hz) | 73.5 | 4.61 (d, *J* = 11.2 Hz) | 72.7 |
| 4 | - | 193.0 | | 192.2 |
| 5 | - | 164.1 | | 164.0 |
| 6 | 6.11 (1H, s) | 94.2 | 6.09 (d, *J* = 2.0 Hz) | 93.5 |
| 7 | - | 167.3 | | 166.6 |
| 8 | 5.98 (1H, s) | 96.9 | 5.97 (d, *J* = 2.0 Hz) | 96.2 |
| 9 | - | 166.4 | | 165.7 |
| 10 | - | 103.7 | | 102.9 |
| 1' | - | 124.3 | | 123.6 |
| 2' | - | 157.2 | | 156.4 |
| 3' | 6.84 (1H, d, *J*=8.4Hz) | 116.6 | 6.82 (1H, d, *J*=8.5Hz) | 115.9 |
| 4' | 7.17 (1H, t, *J*=7.6 Hz) | 130.8 | 7.17 (1H, td, *J* =7.6, 1.5 Hz) | 130.3 |
| 5' | 6.88 (1H, d, *J*=7.6 Hz) | 120.5 | 6.88 (1H, d, *J*=7.6Hz) | 119.8 |
| 6' | 7.41 (1H, d, *J*=7.6Hz) | 129.5 | 7.41 (1H, dd, *J* =7.6, 1.5 Hz) | 128.8 |
| $OCH_3$ | 3.84 (3H, s) | 56.2 | 3.83 (3H s,) | 55.5 |

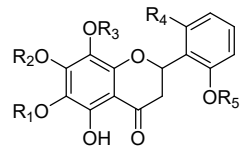
a) Recorded at 400 MHz. *b*) 100 MHz.



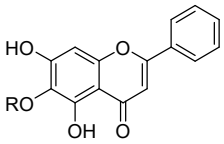
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **1** | H | H | H | H |
| **2** | $CH_3$ | $CH_3$ | H | H |
| **3** | $CH_3$ | $CH_3$ | OH | OH |
| **4** | H | $CH_3$ | OH | H |



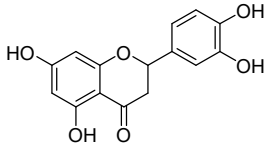
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **5** | H | H | H | H |
| **6** | $CH_3$ | H | H | H |
| **7** | H | H | OH | OH |
| **8** | $CH_3$ | $CH_3$ | OH | OH |
| **9** | H | glcA | H | H |



| | R1 | R2 | R3 | R4 | R5 |
|---|---|---|---|---|---|
| **10** | $CH_3$ | H | H | H | H |
| **11** | $CH_3$ | $CH_3$ | $CH_3$ | H | H |



| | R |
|---|---|
| **12** | H |
| **13** | $CH_3$ |



**14**

| No. | Compounds |
|---|---|
| 1 | norwogonin |
| 2 | moslosooflavone |
| 3 | viscidulin II |
| 4 | scutevulin |
| 5 | baicalein |
| 6 | oroxylin A |
| 7 | 2-(2,6-dihydroxyl phenyl)-5,6,7-trihydroxy-flavone |
| 8 | mosloflavone |
| 9 | baicalin |
| 10 | 5,7,8-trihydroxy-6-methoxylflavone |
| 11 | alnetin |
| 12 | dihydrobaicalein |
| 13 | dihydrooroxylin A |
| 14 | 5,7,30,40-tetrahydroxy flavanone |

**Fig. 15** Structures of isolated compounds from *S. baicalensis* in the same laboratory

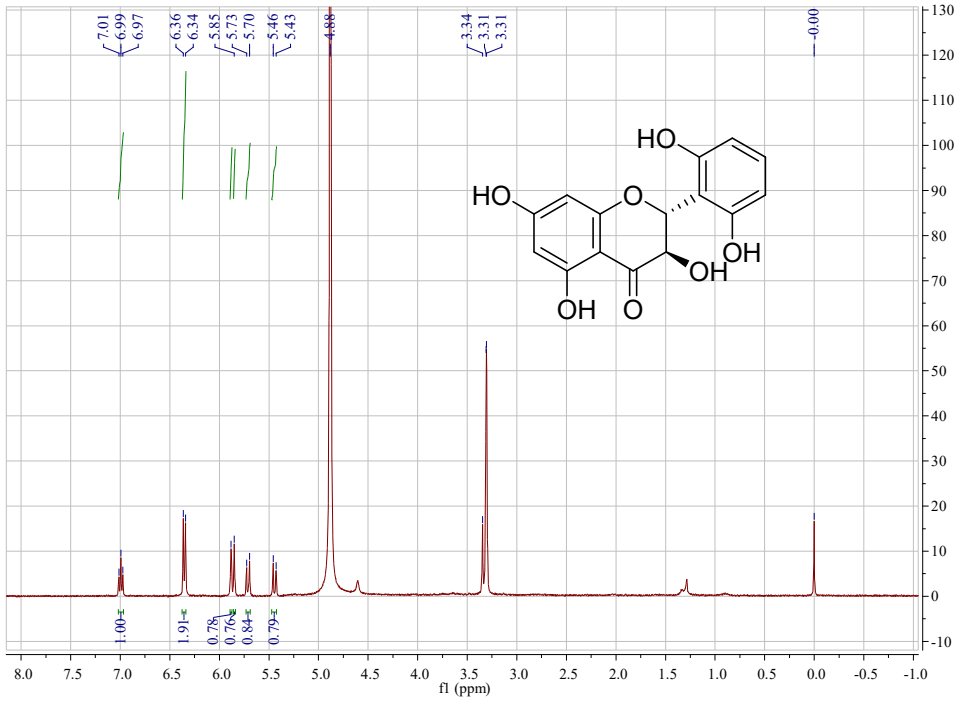



**Fig. 16** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **2**

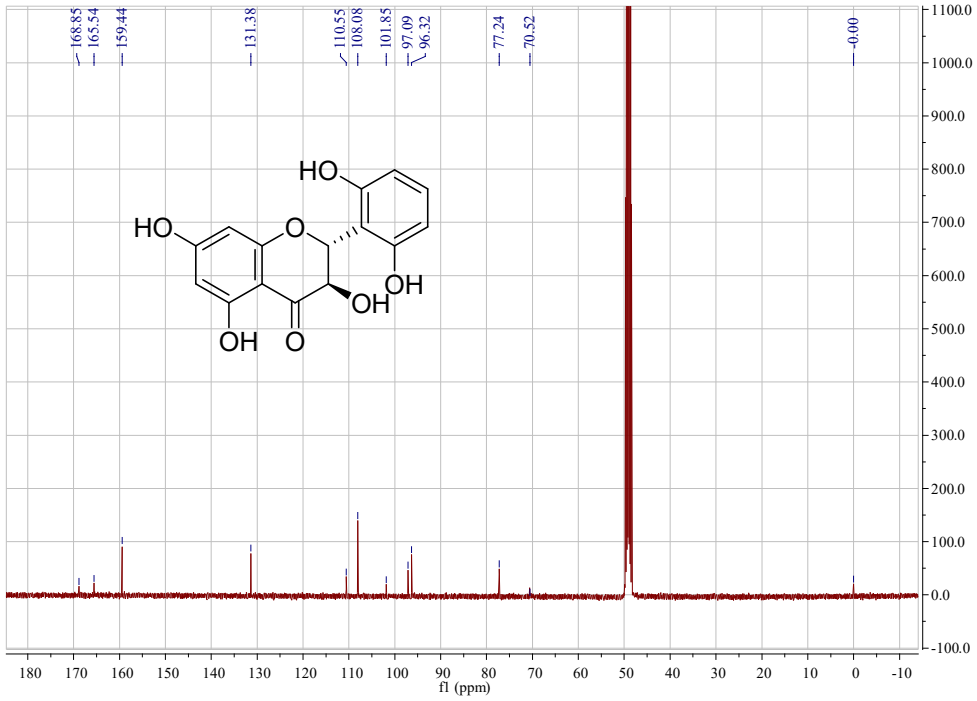



**Fig. 17** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **2**

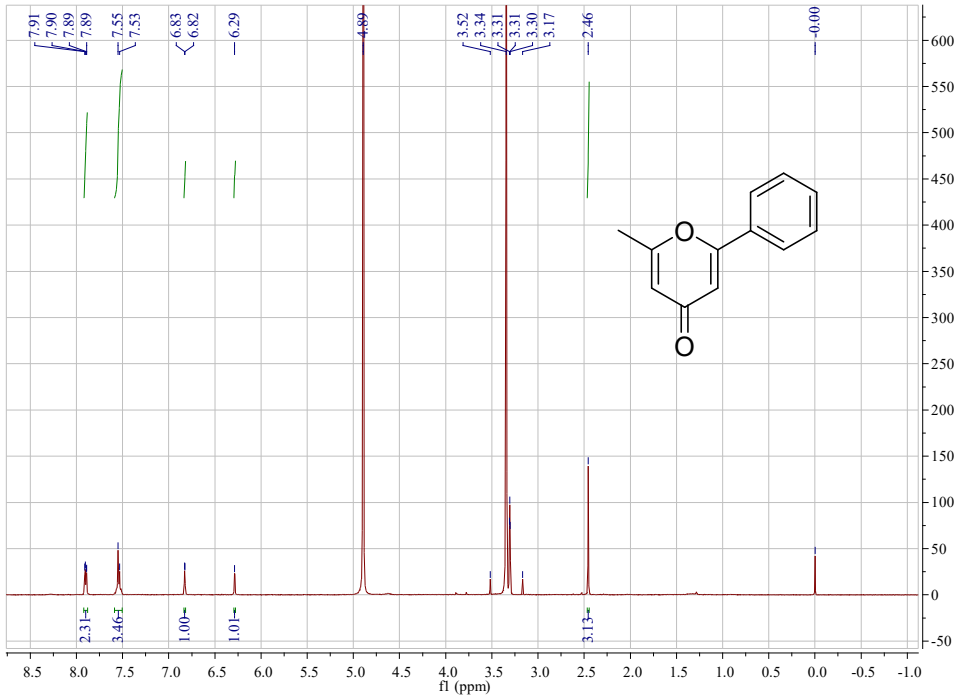



**Fig. 18** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **3**

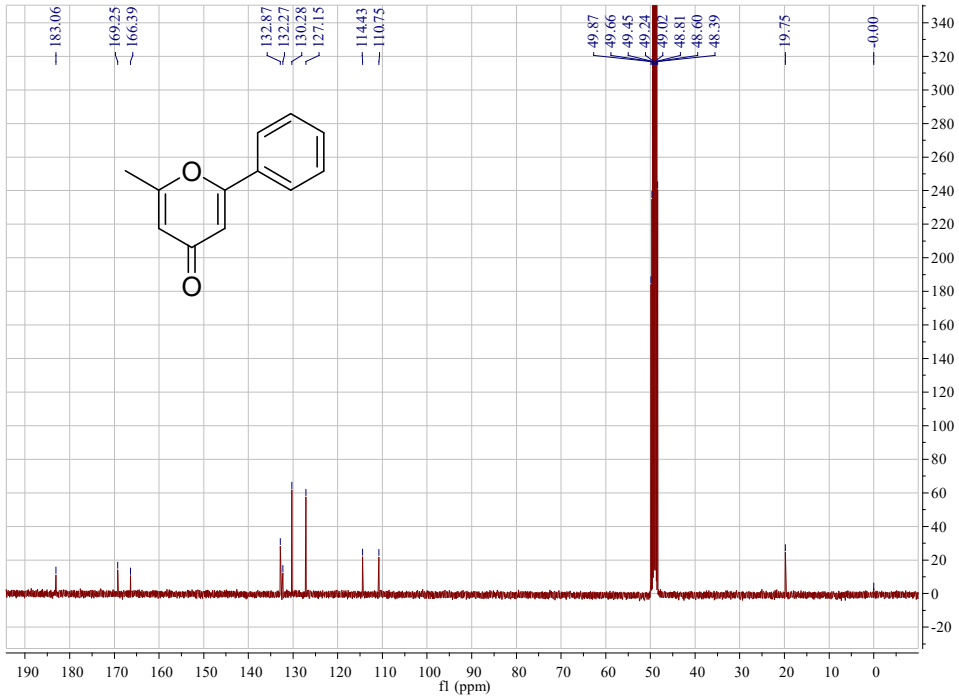



**Fig. 19** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **3**

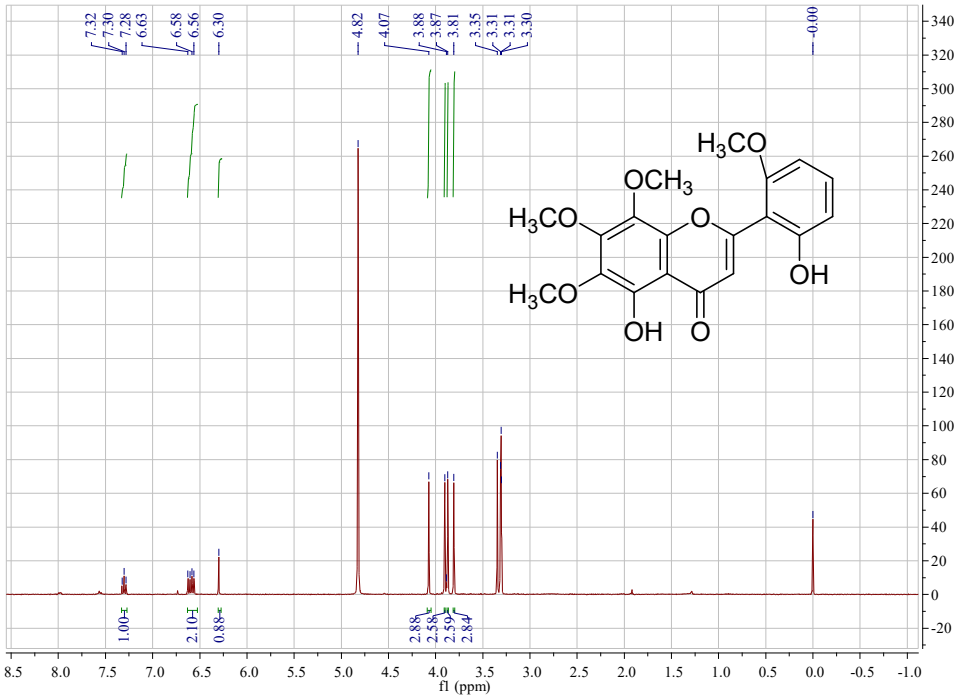



**Fig. 20** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **4**

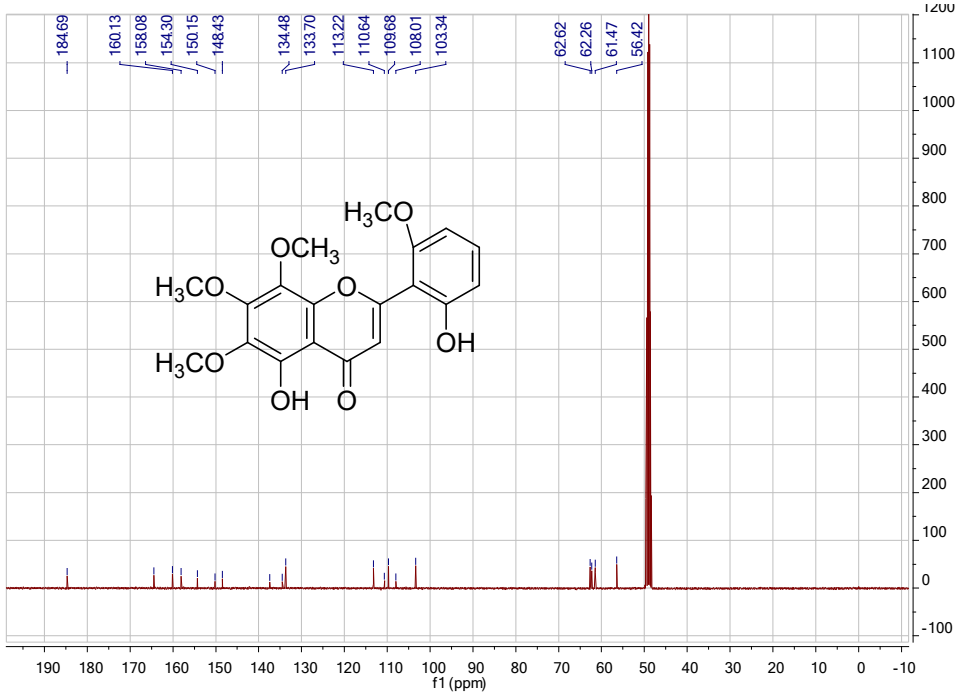



**Fig. 21** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **4**

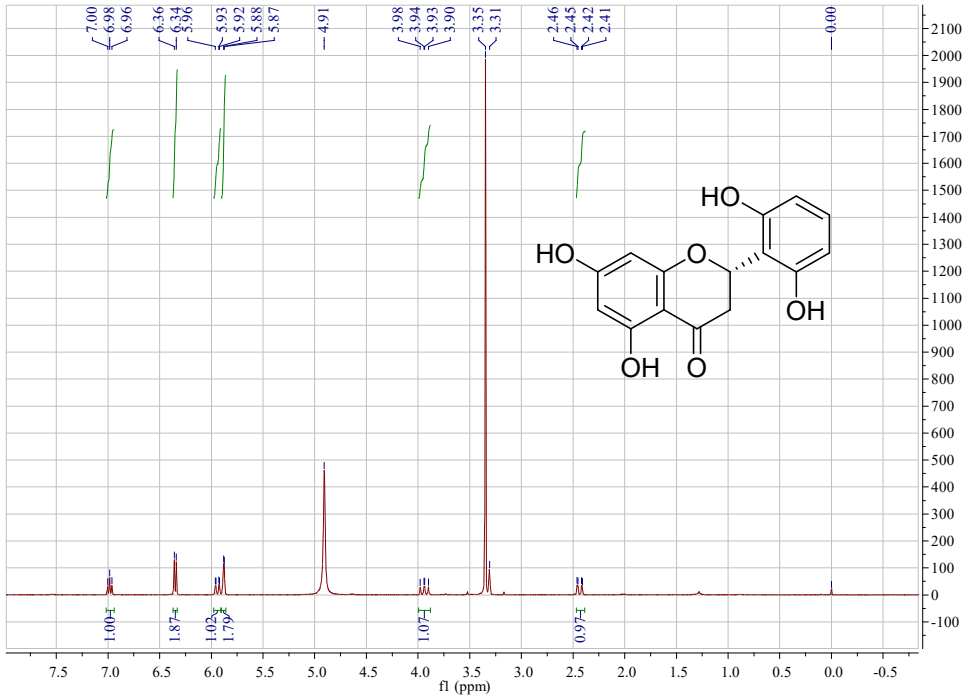



**Fig. 22** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **5**

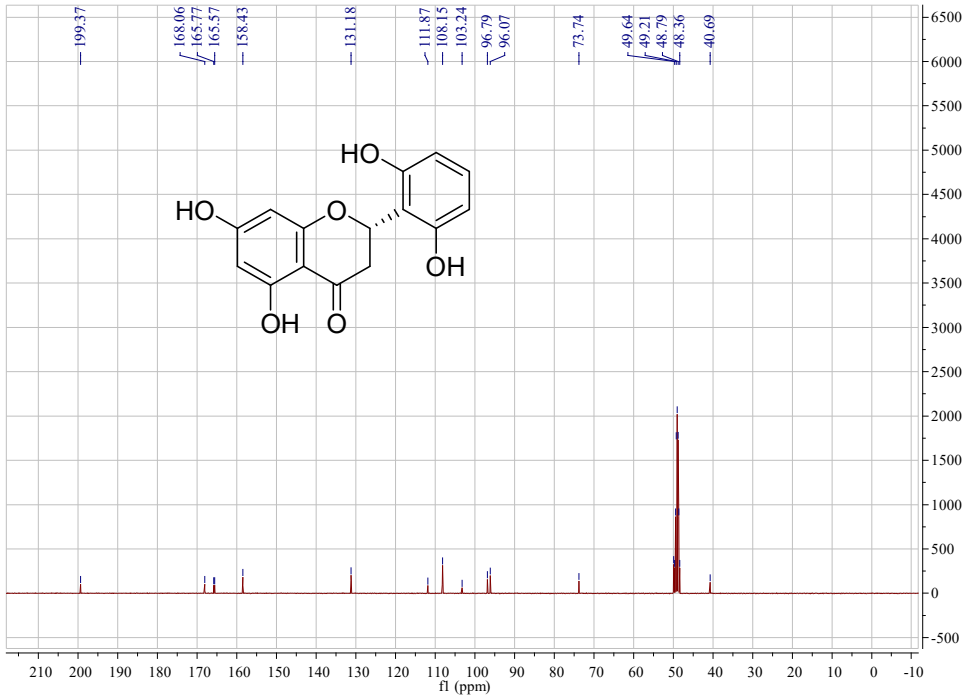



**Fig. 23** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **5**

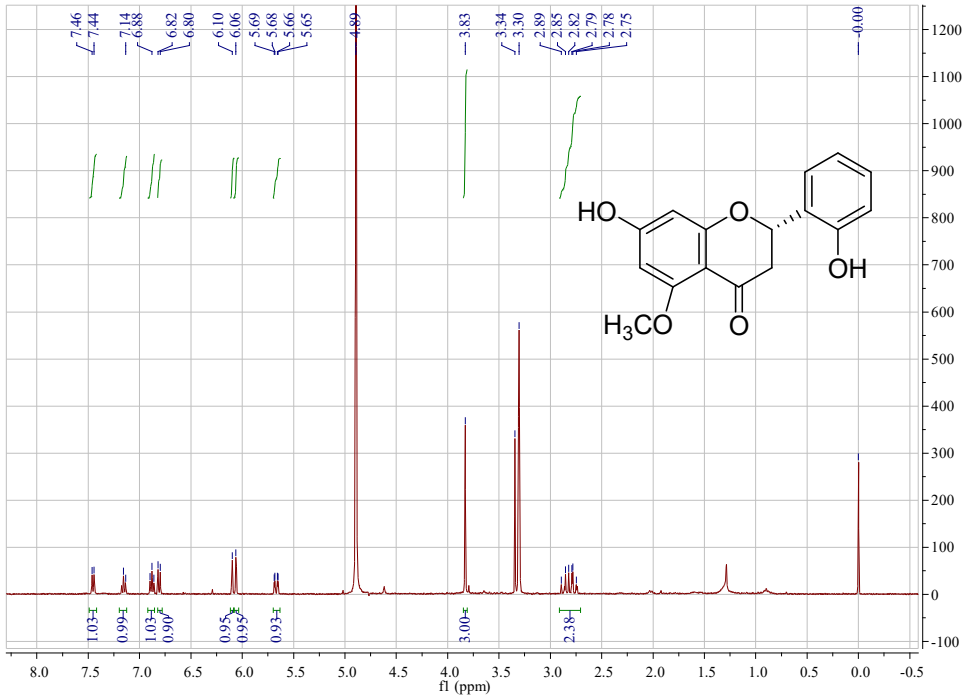



**Fig. 24** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **6**

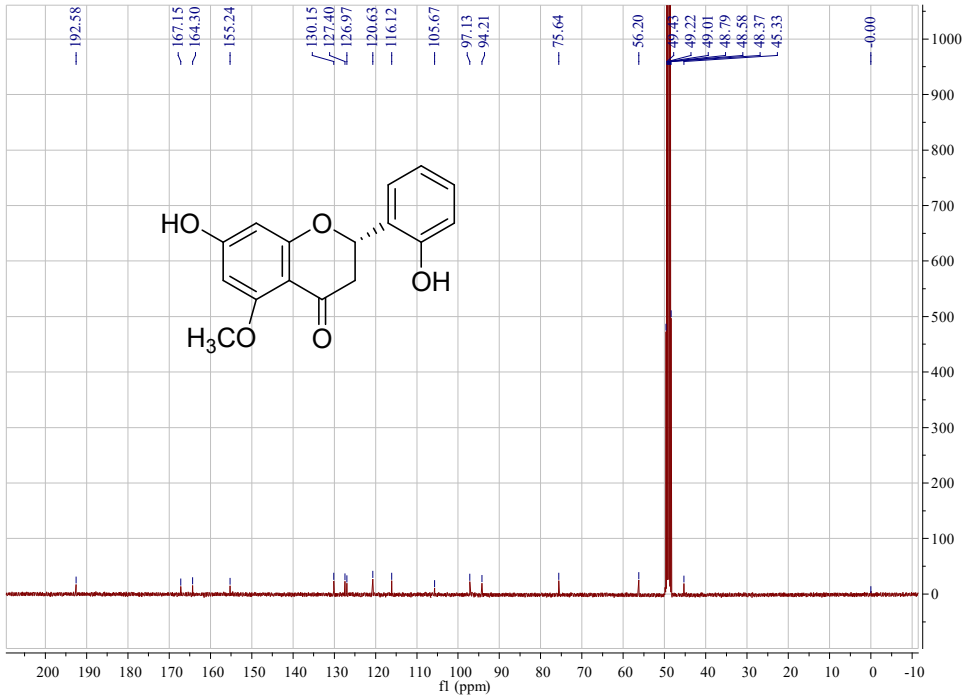



**Fig. 25** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **6**

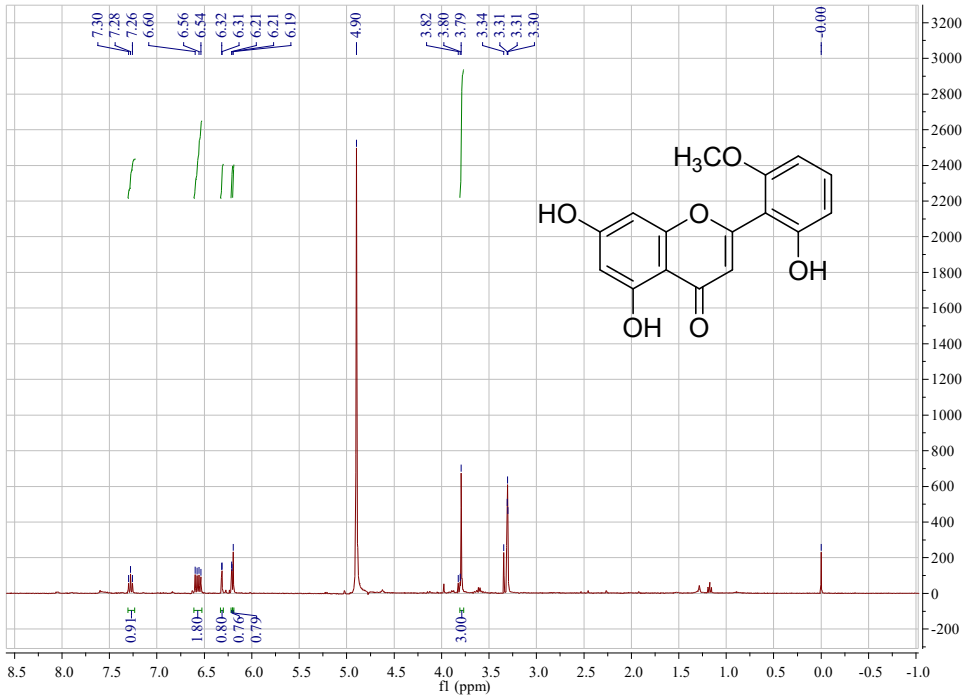



**Fig. 26** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **7**

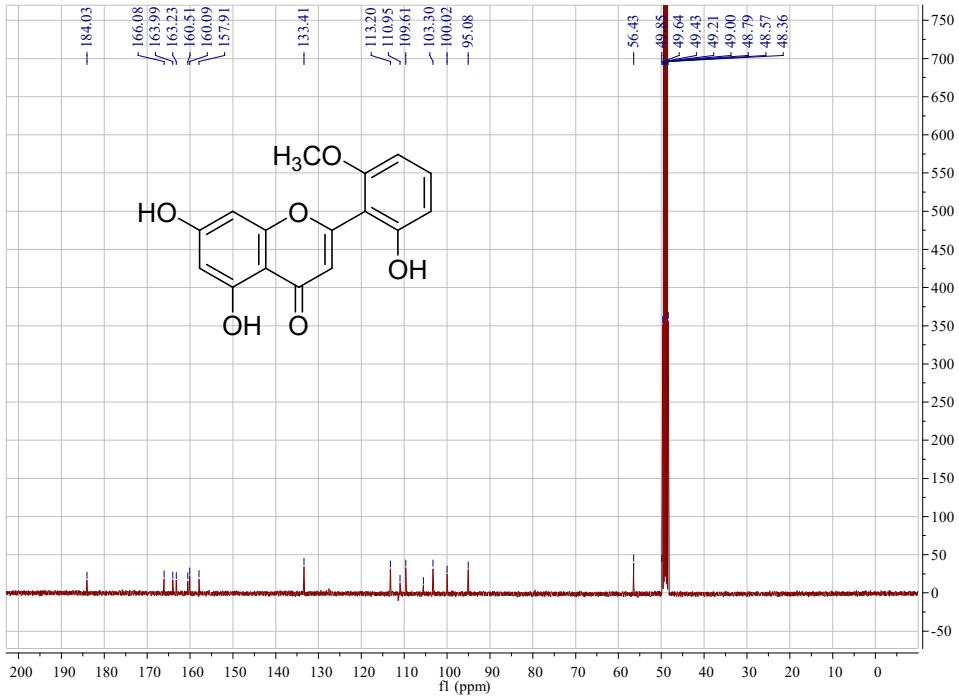



**Fig. 27** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **7**

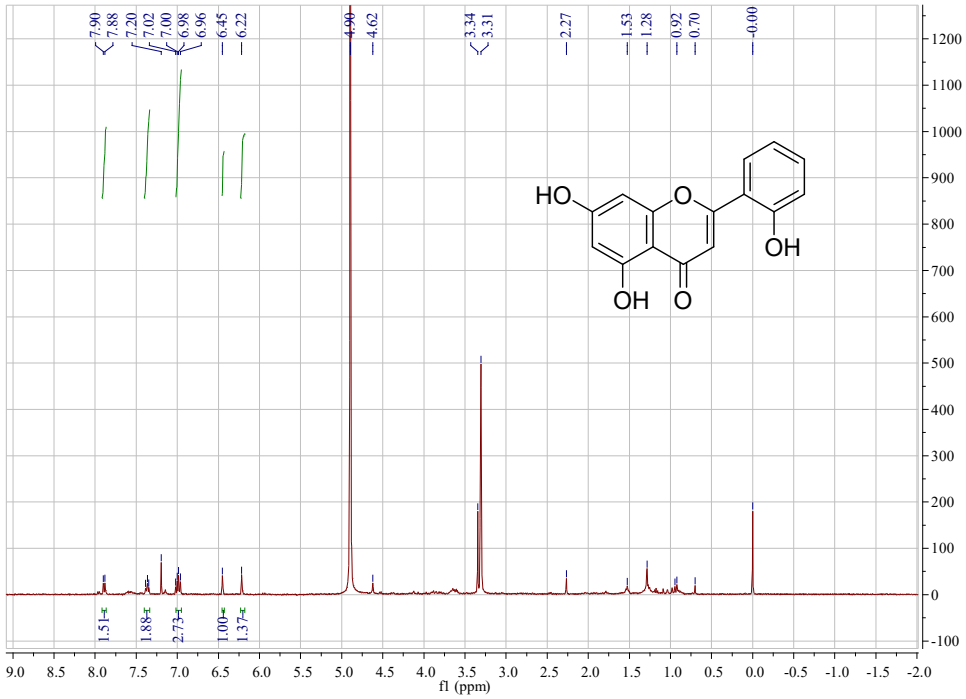



**Fig. 28** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **8**

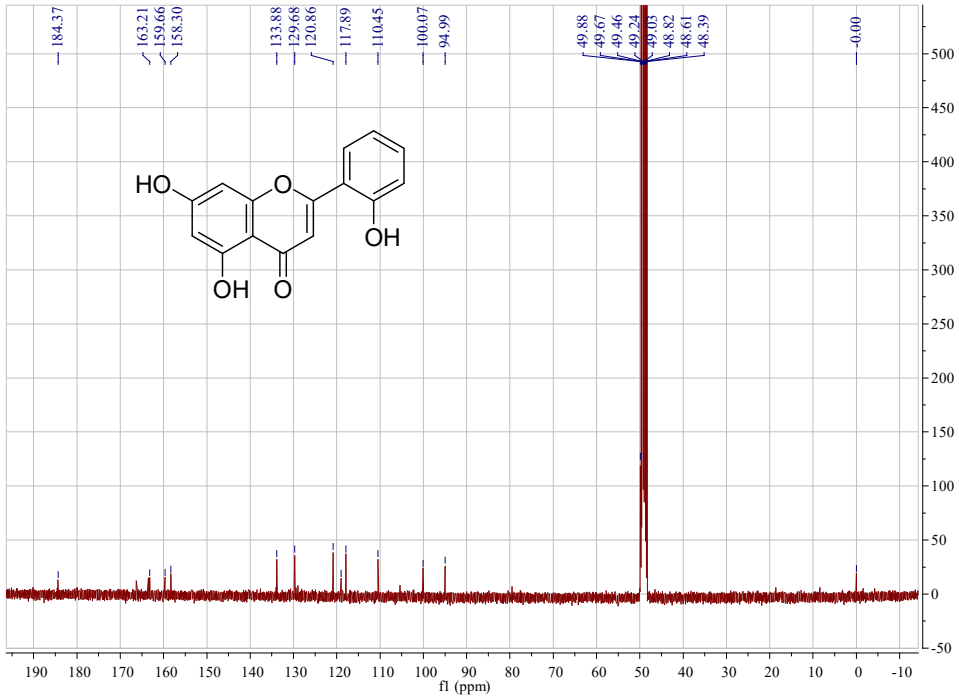



**Fig. 29** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **8**

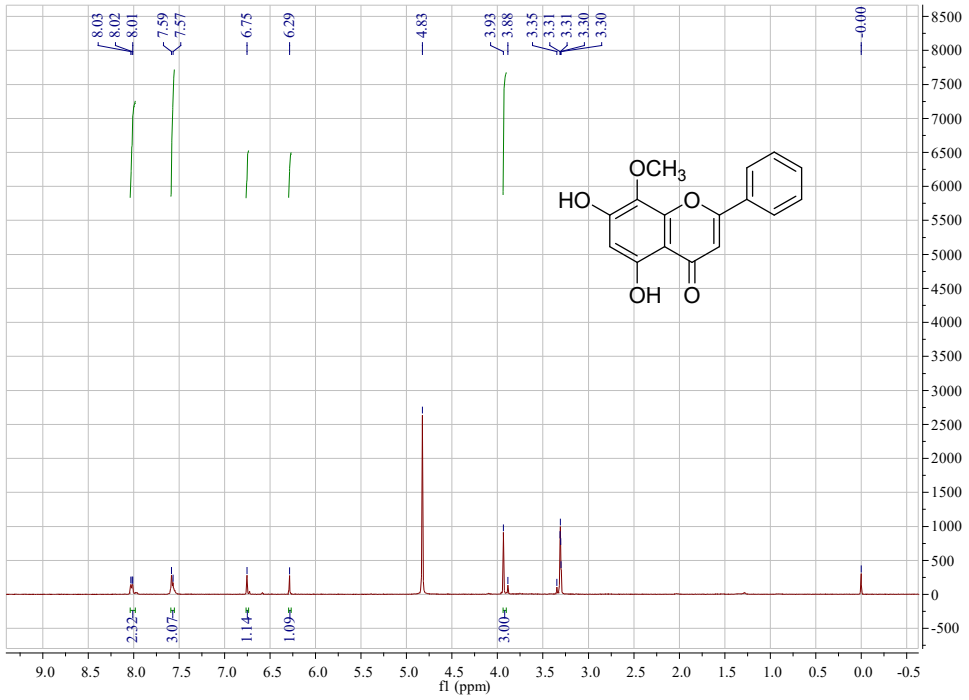



**Fig. 30** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **9**

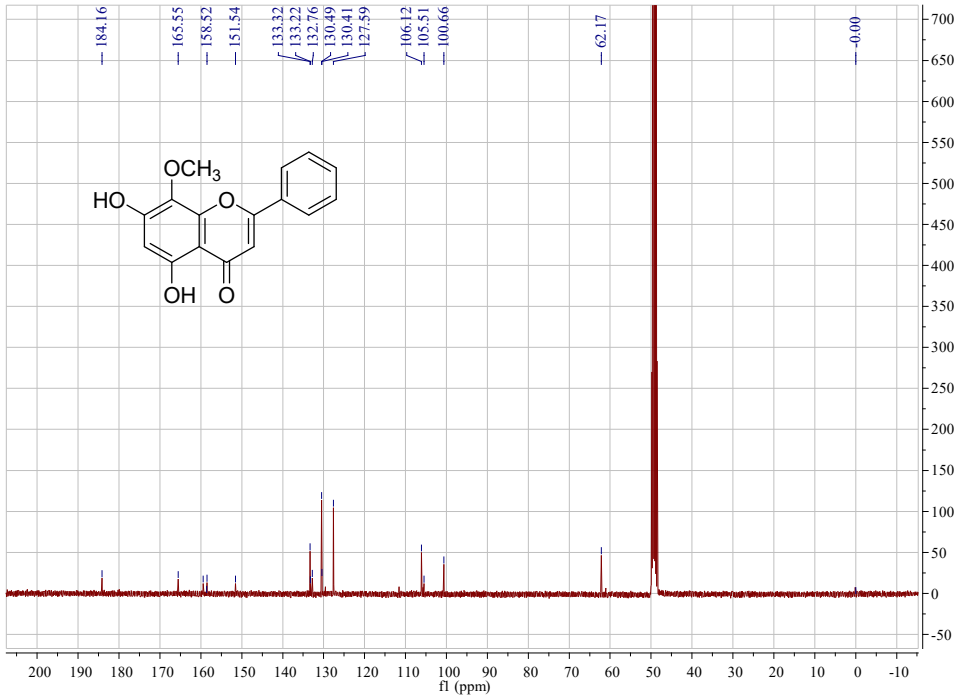



**Fig. 31** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **9**

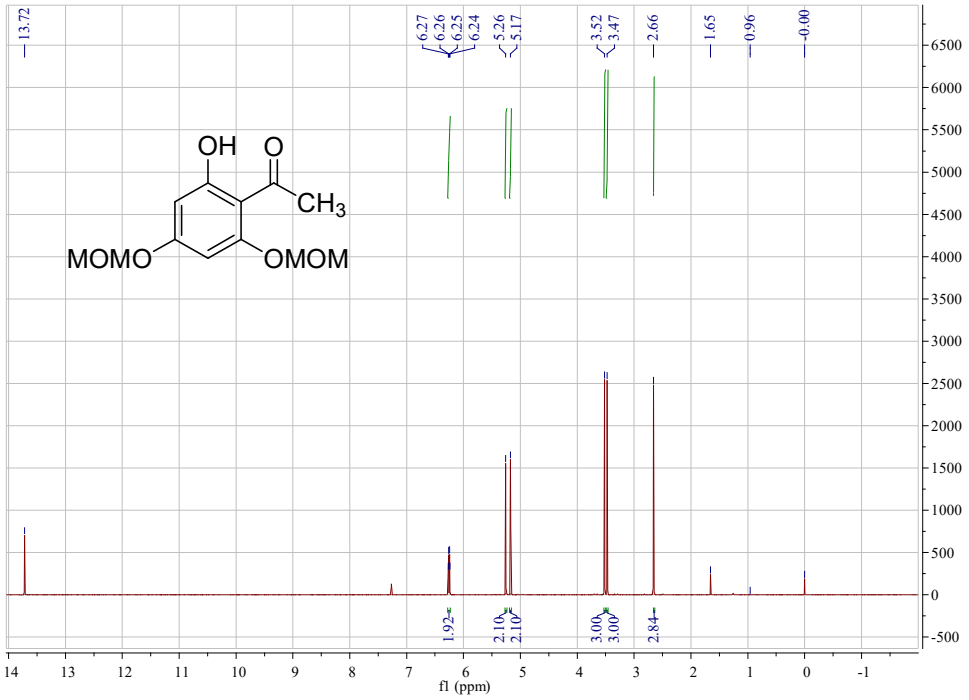



**Fig. 32** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **11**

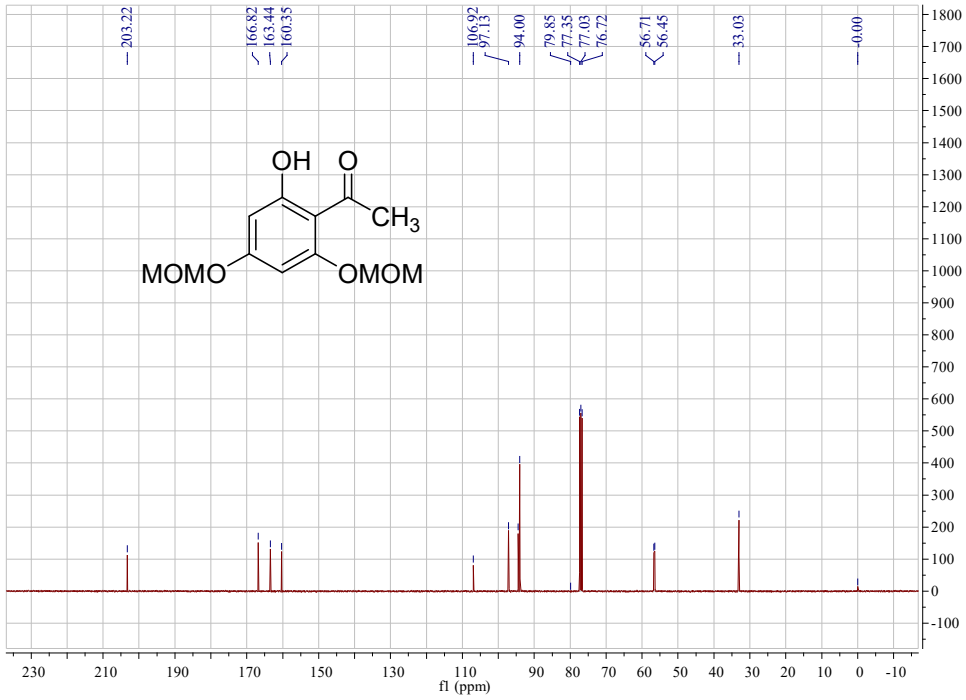



**Fig. 33** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **11**

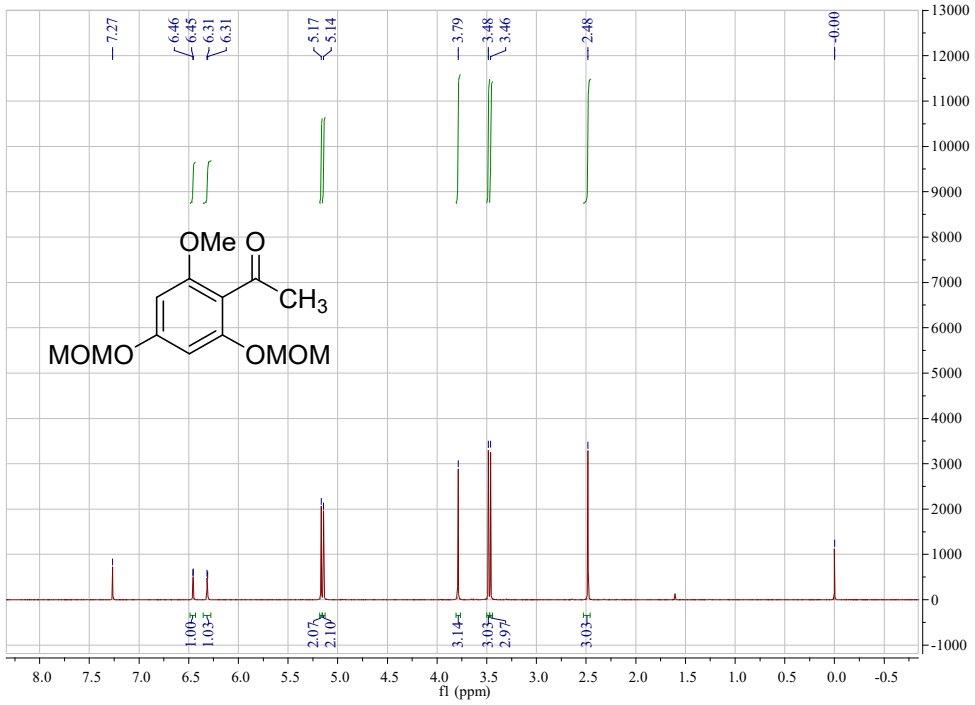



**Fig. 34** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **12**

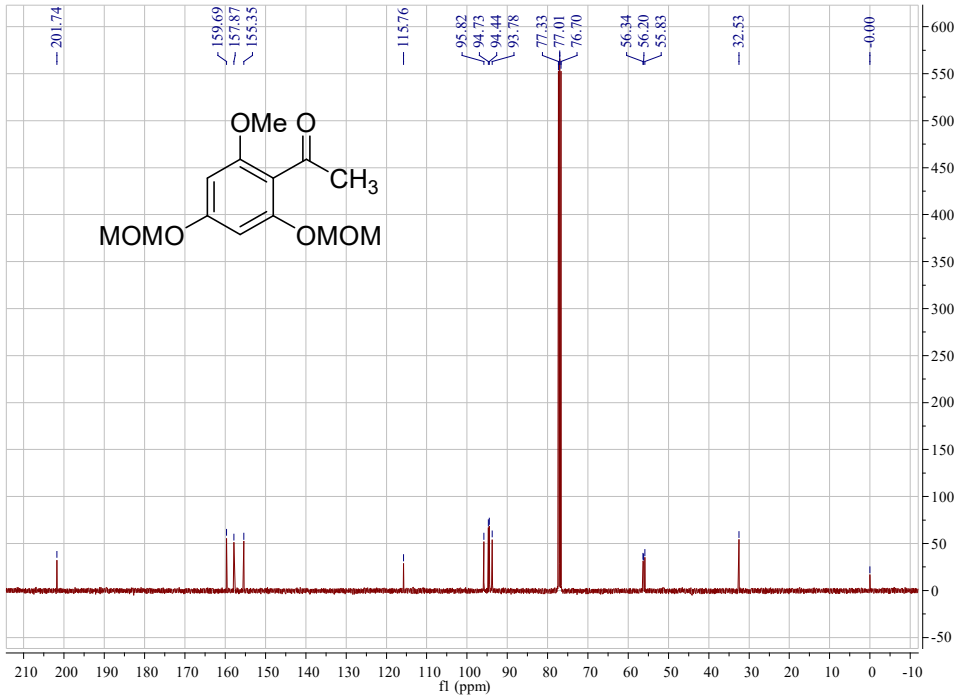



**Fig. 35** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound 1**2**

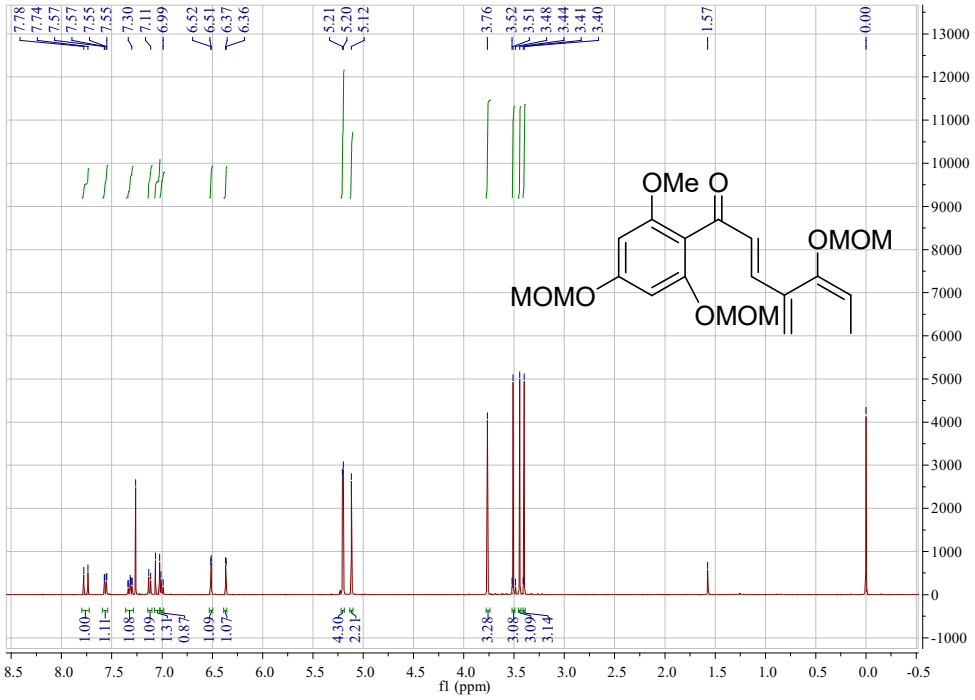



**Fig. 36** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **14**

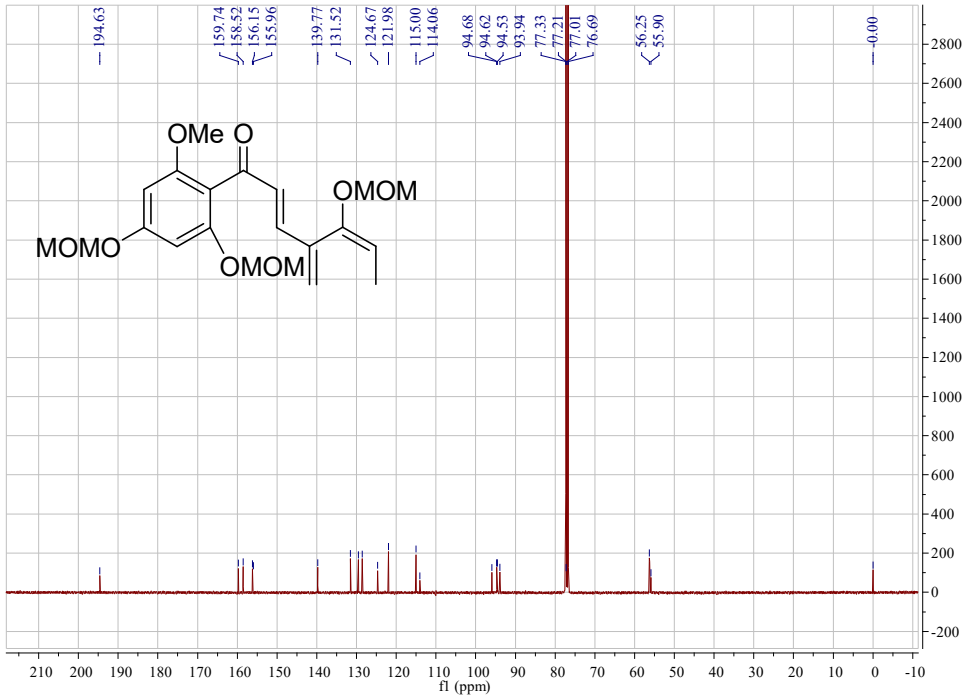



**Fig. 37** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **14**

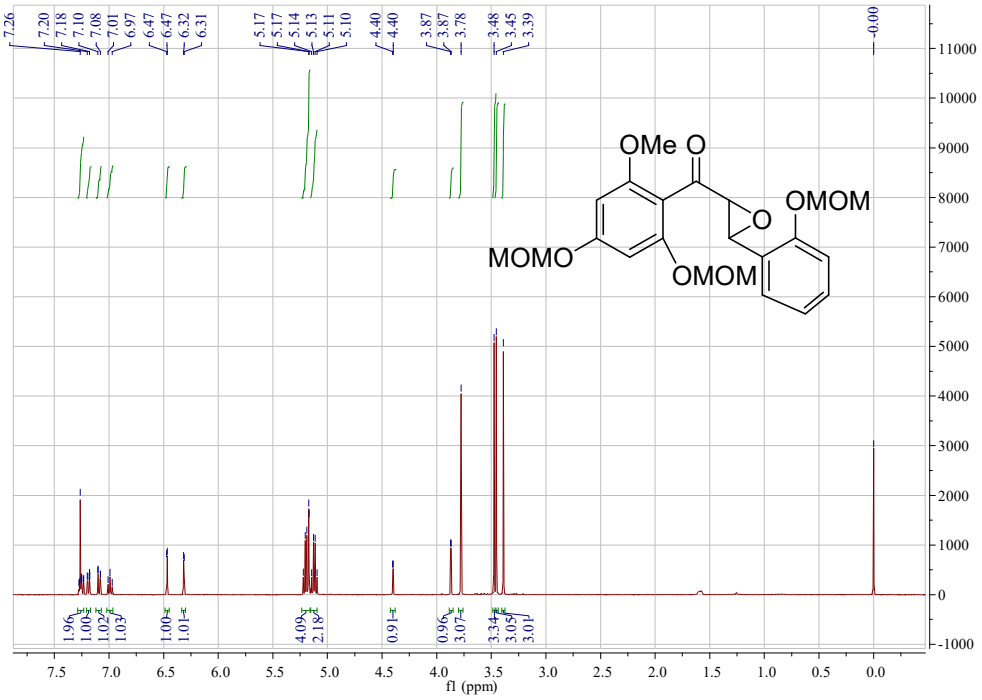



**Fig. 38** $^{1}$H-NMR (400Hz, $CD_3OD$) spectrum of compound **15**

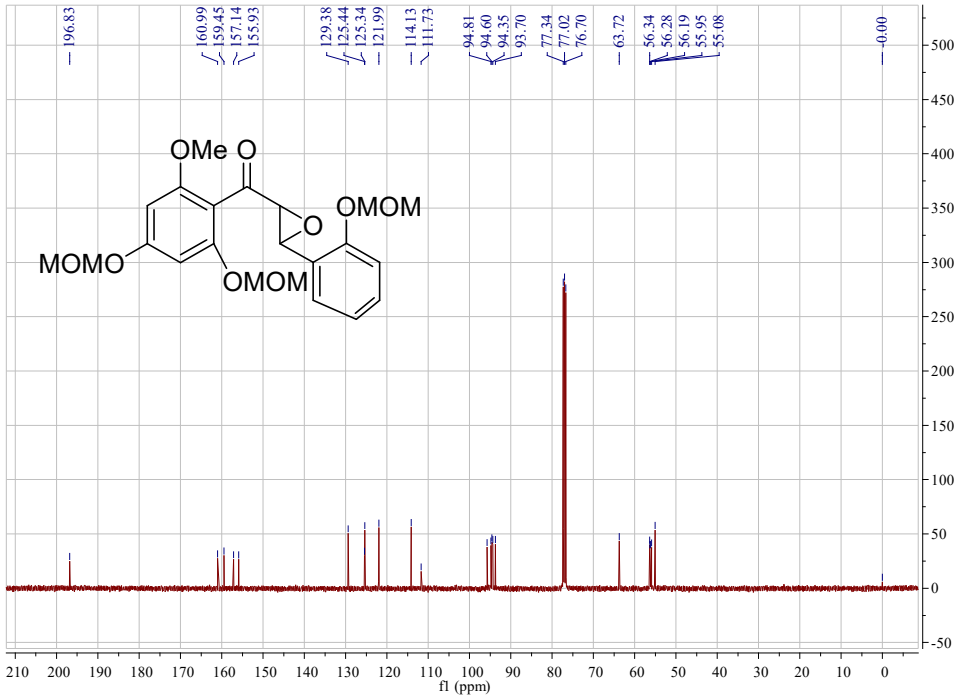



**Fig. 39** $^{13}$C-NMR (100Hz, $CD_3OD$) spectrum of compound **15**

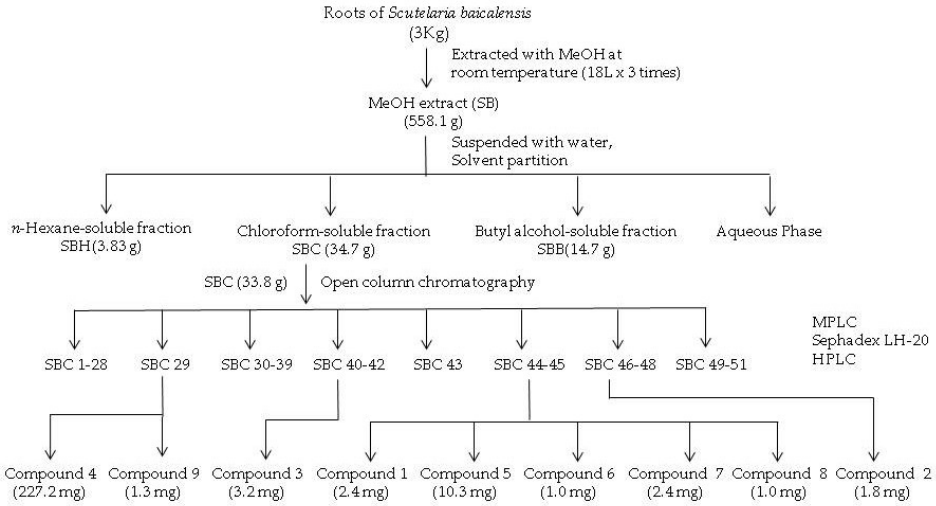



**Fig. 40** Isolation scheme of *S. baicalensis*.