

# Halochromic behavior and anticancer effect of new synthetic anthocyanidins complexed with $\beta$ -cyclodextrin derivatives

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## Supplementary material

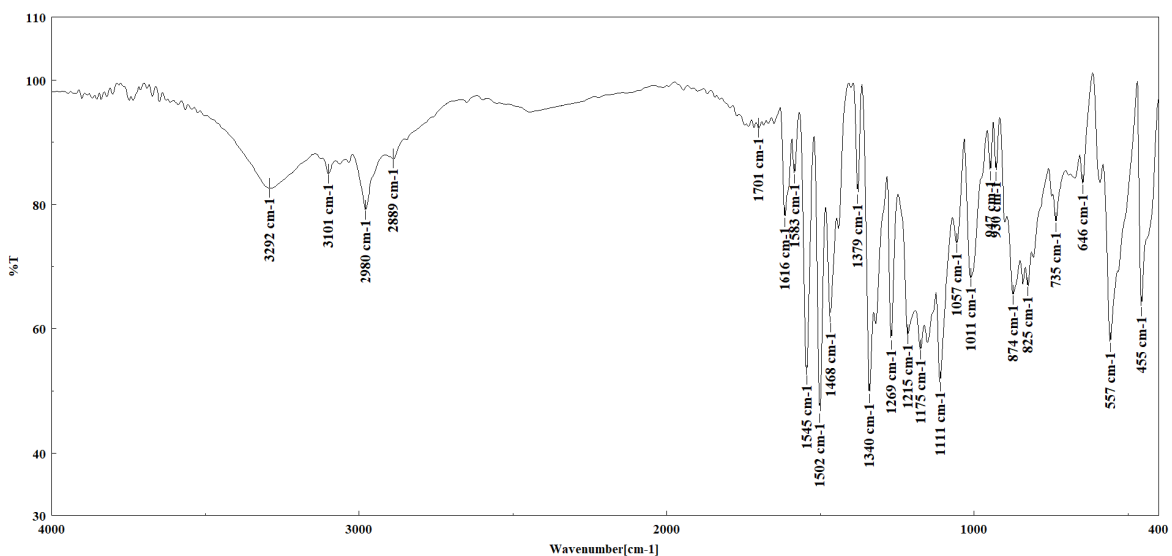


Figure S1. FT-IR spectrum of compound 5

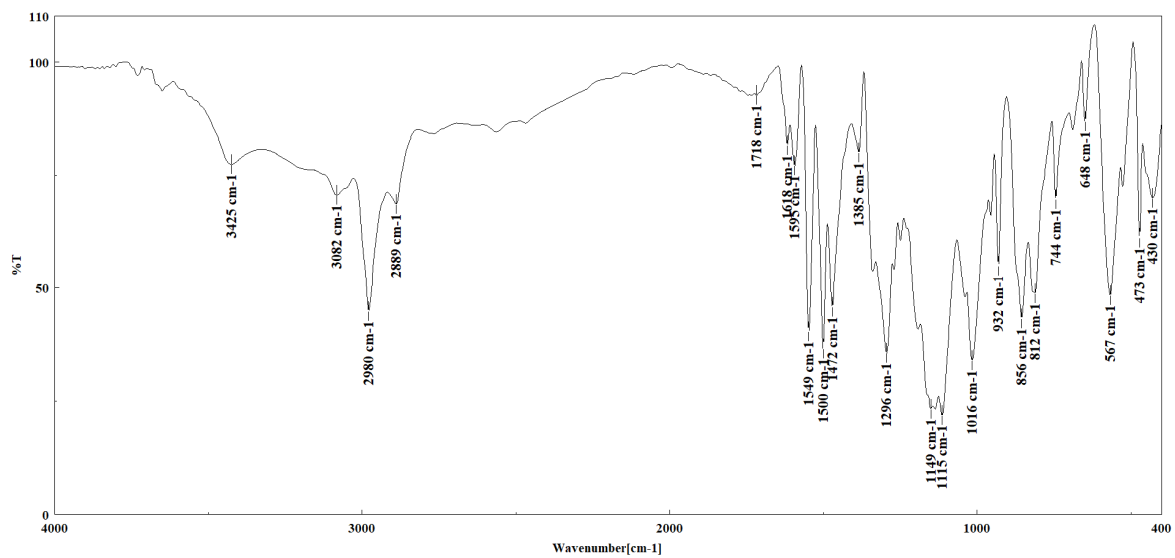


Figure S2. FT-IR spectrum of compound 1

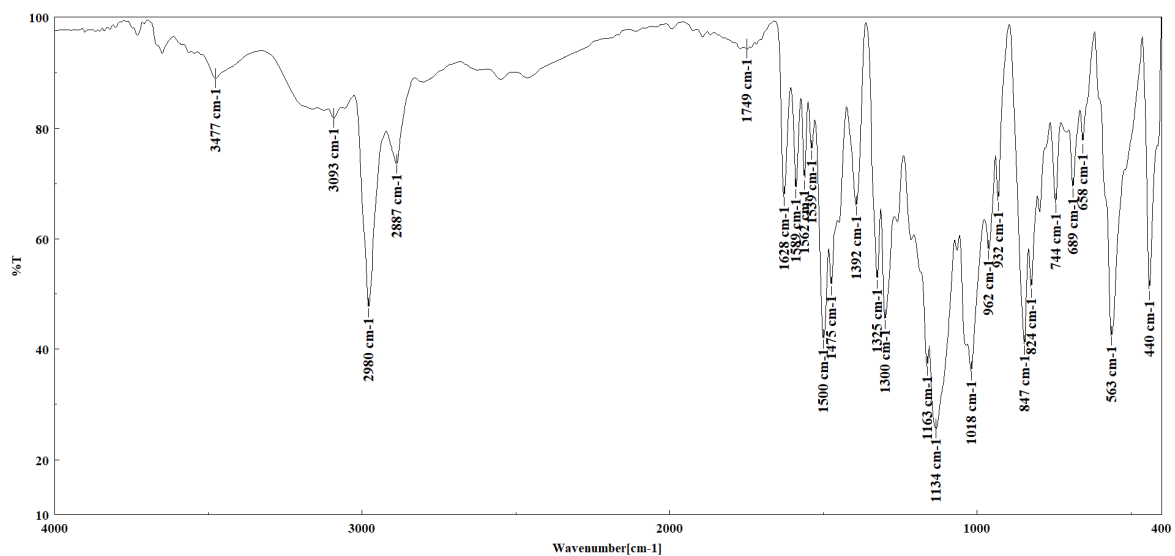


Figure S3. FT-IR spectrum of compound 2

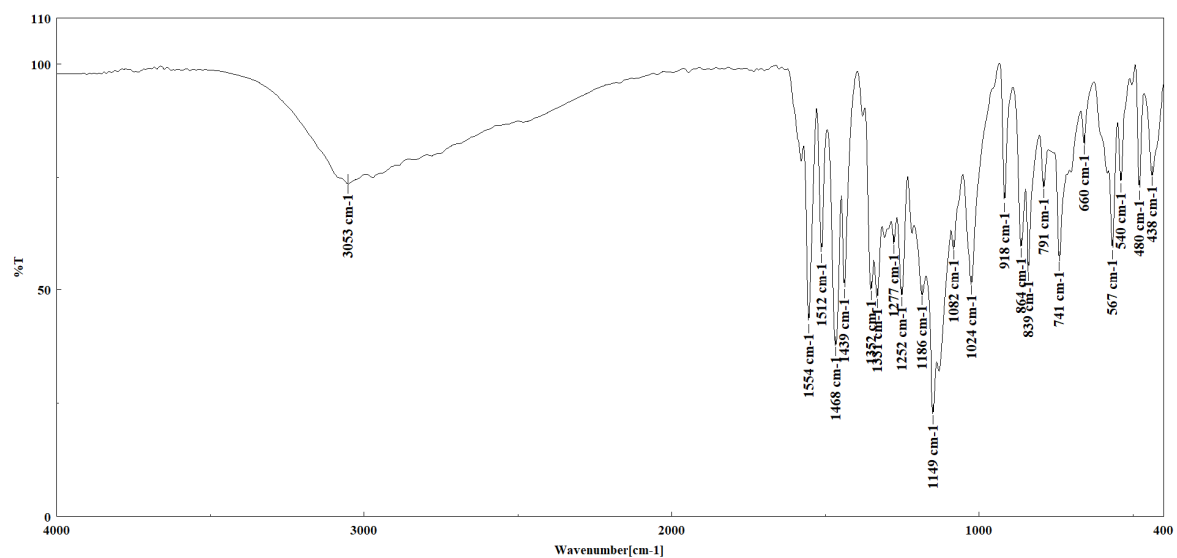


Figure S4. FT-IR spectrum of compound 3

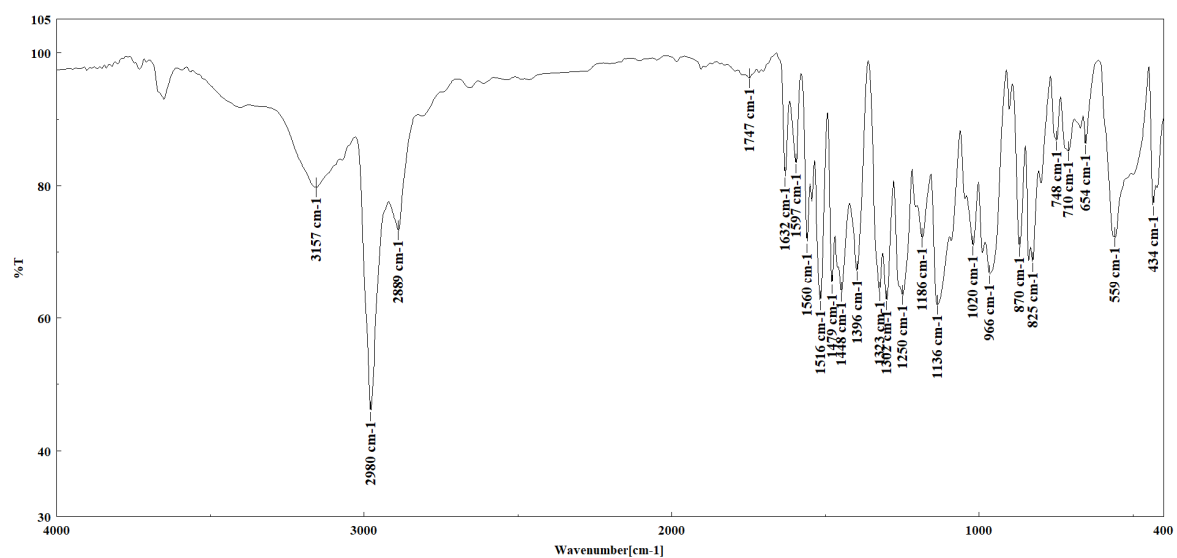


Figure S5. FT-IR spectrum of compound 4

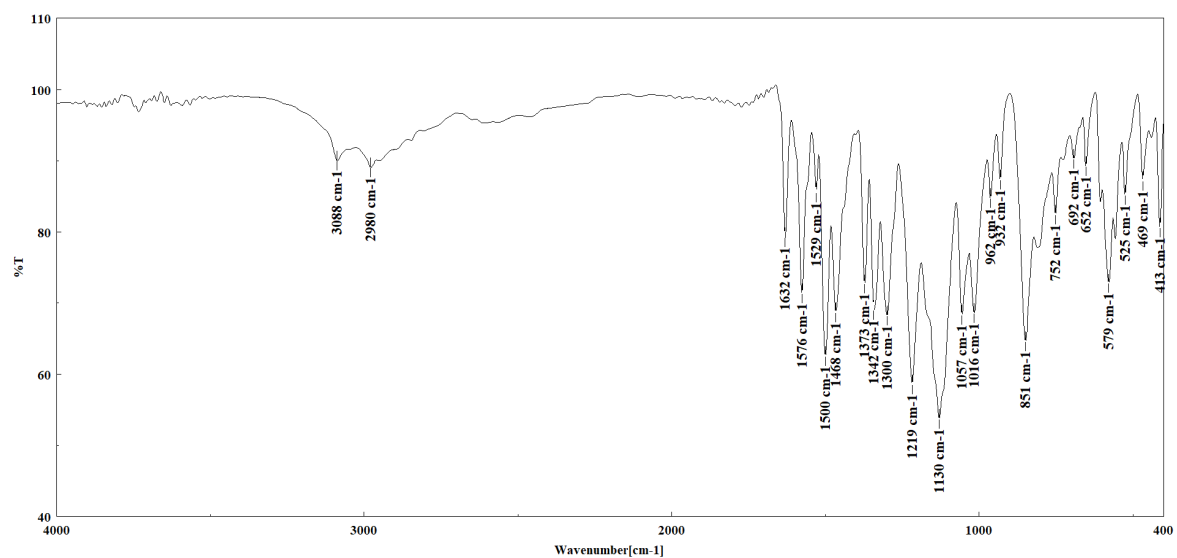


Figure S6. FT-IR spectrum of compound 6

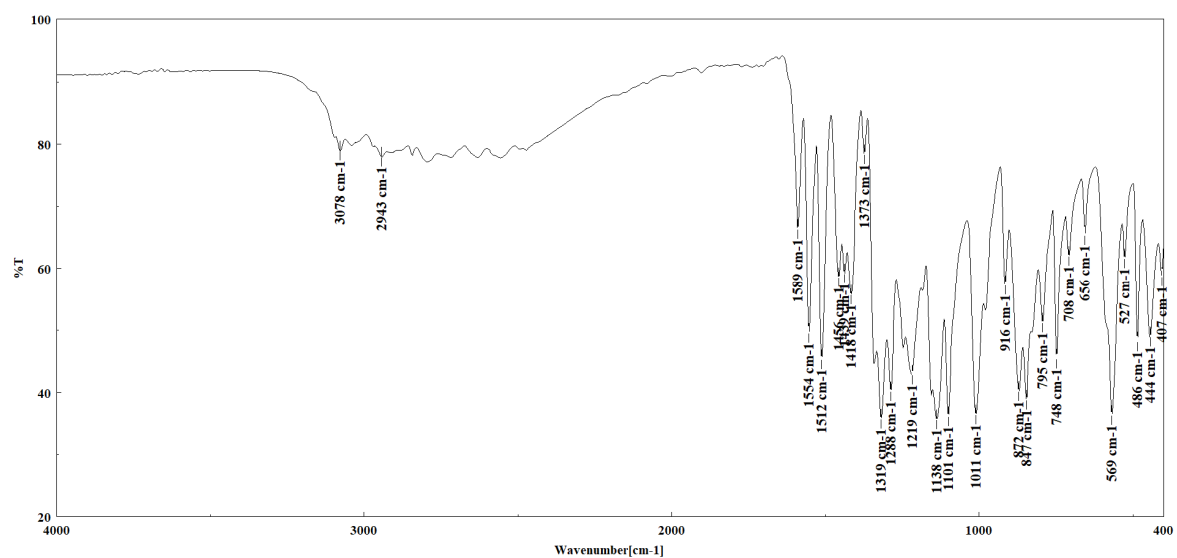


Figure S7. FT-IR spectrum of compound 7

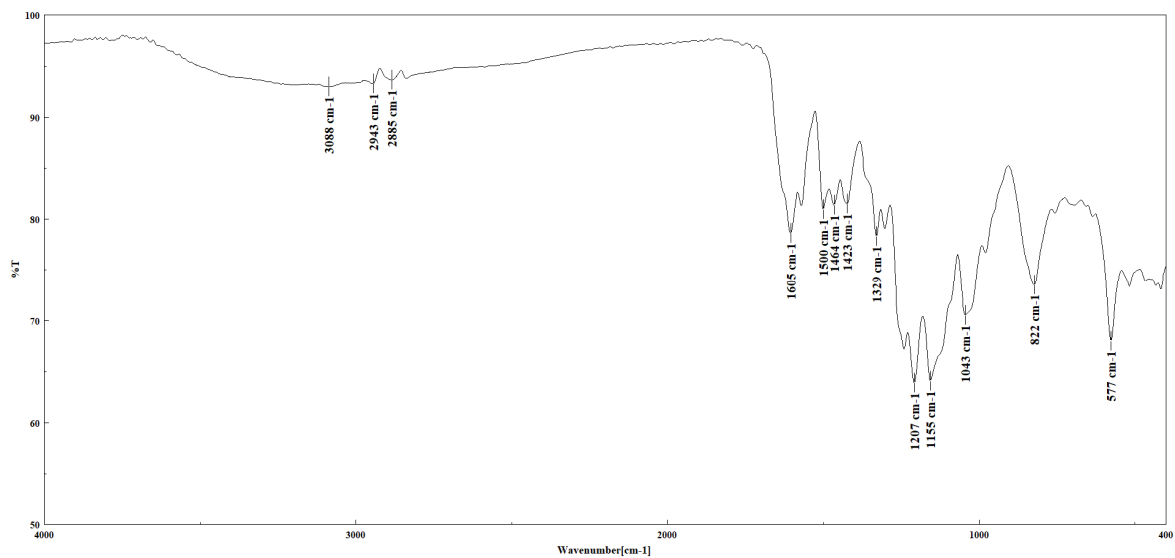


Figure S8. FT-IR spectrum of compound 8

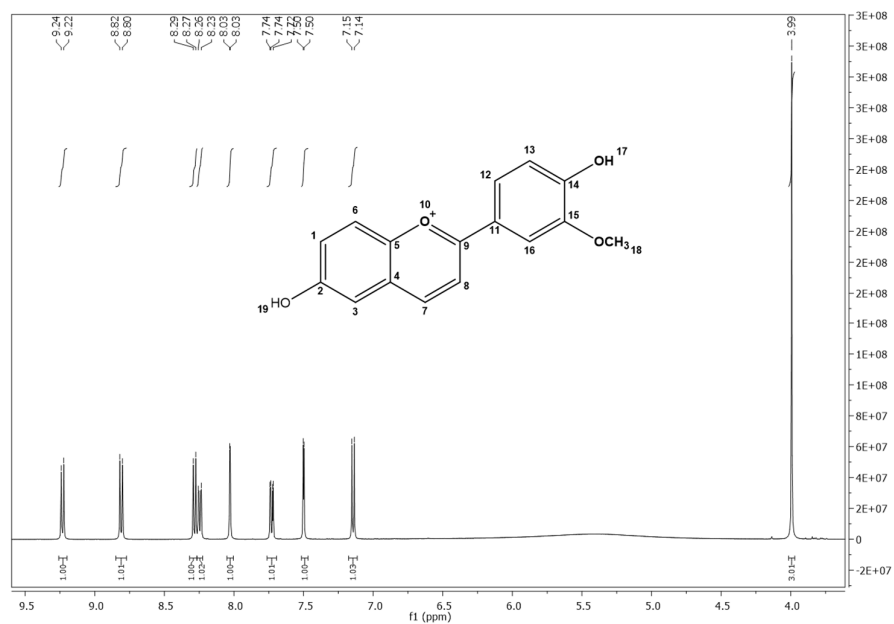


Figure S9.  $^1\text{H}$ -NMR spectrum of compound 1

$^1\text{H}$ -NMR (500 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 9.23 (d,  $J = 9.2$  Hz, 1H, **H7**), 8.81 (d,  $J = 9.2$  Hz, 1H, **H8**), 8.28 (d,  $J = 9.3$  Hz, 1H, **H6**), 8.24 (d,  $J = 10.8$  Hz, 1H, **H12**), 8.03 (d,  $J = 2.1$  Hz, 1H, **H13**), 7.73 (dd,  $J = 9.2, 2.9$  Hz, 1H, **H1**), 7.50 (d,  $J = 2.9$  Hz, 1H, **H3**), 7.14 (d,  $J = 8.6$  Hz, 1H, **H16**), 3.99 (s, 3H, **H18**).

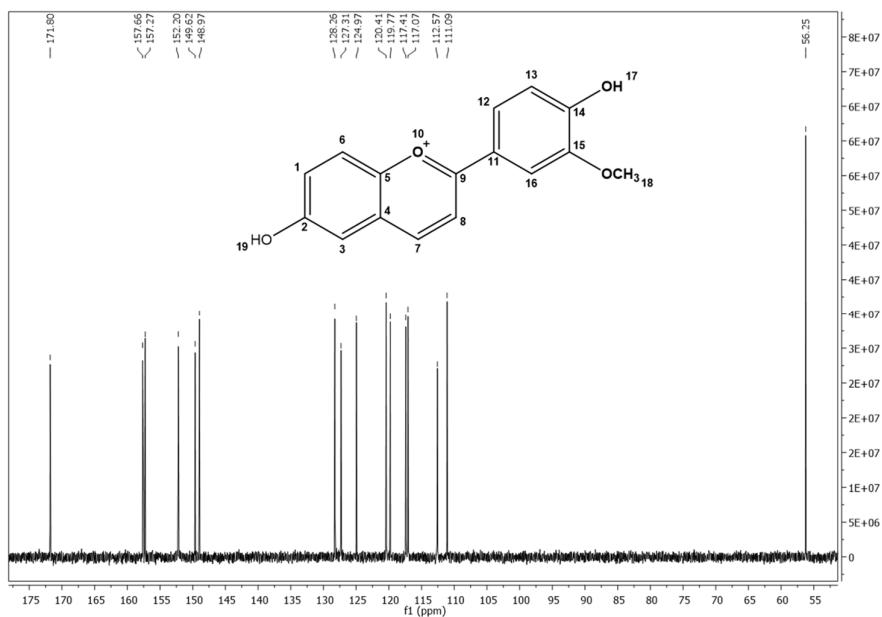


Figure S10. <sup>13</sup>C-NMR spectrum of compound 1

<sup>13</sup>C-NMR (125 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 171.8 (C9); 157.6 (C15); 157.2 (C2); 152.2 (C7); 149.6 (C14); 148.9 (C5); 128.2 (C1); 127.3 (C12); 124.9 (C4); 120.4 (C6); 119.7 (C11); 117.4 (C8); 117.0 (C16); 112.5 (C13); 111.0 (C3); 56.2 (C18).

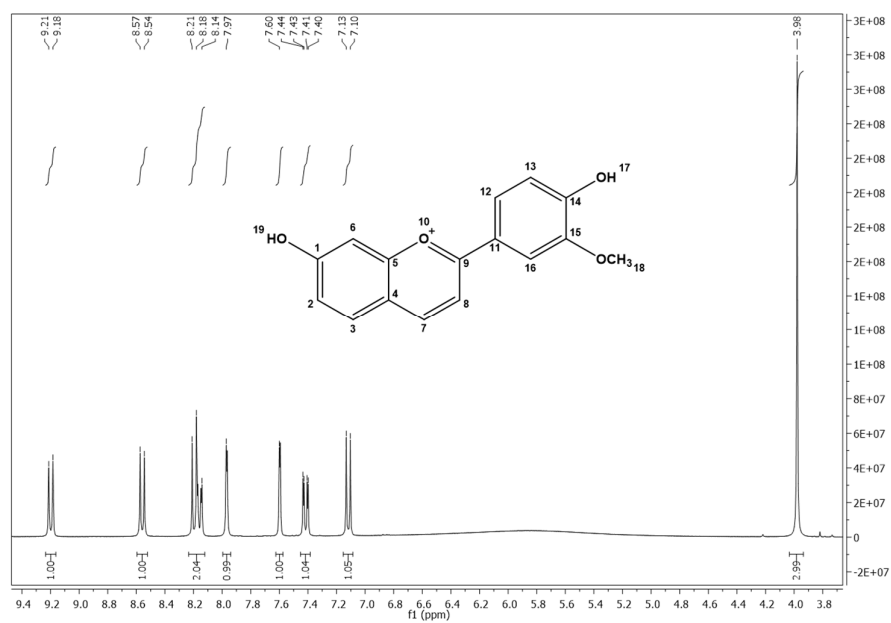


Figure S11. <sup>1</sup>H-NMR spectrum of compound 2

<sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 9.20 (d, *J* = 8.8 Hz, 1H, H7), 8.56 (d, *J* = 8.8 Hz, 1H, H8), 8.18 (t, *J* = 10.3 Hz, 2H, H3, H12), 7.97 (s, 1H, H16), 7.60 (s, 1H, H6), 7.42 (dd, *J* = 8.9, 2.2 Hz, 1H, H13), 7.12 (d, *J* = 8.6 Hz, 1H, H2), 3.98 (s, 3H, H18).

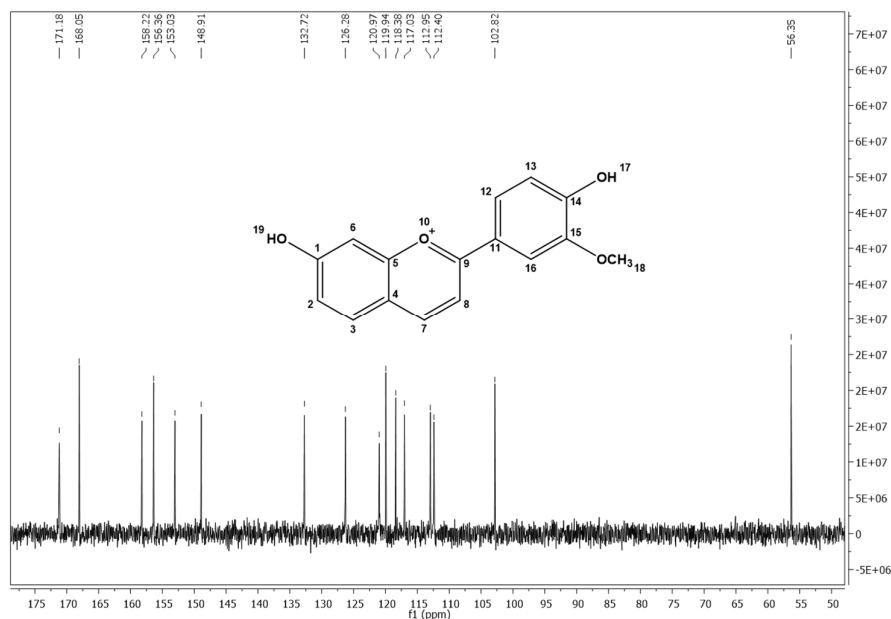


Figure S12.  $^{13}\text{C}$ -NMR spectrum of compound 2

$^{13}\text{C}$ -NMR (75 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 171.1 (C9); 168.0 (C1); 158.2 (C5); 156.3 (C14); 153.0 (C7); 148.9 (C15); 132.7 (C3); 126.2 (C12); 120.9 (C13); 119.3 (C4); 118.3 (C11); 117.0 (C2); 112.9 (C8); 112.4 (C16); 102.8 (C6); 56.3 (C18).

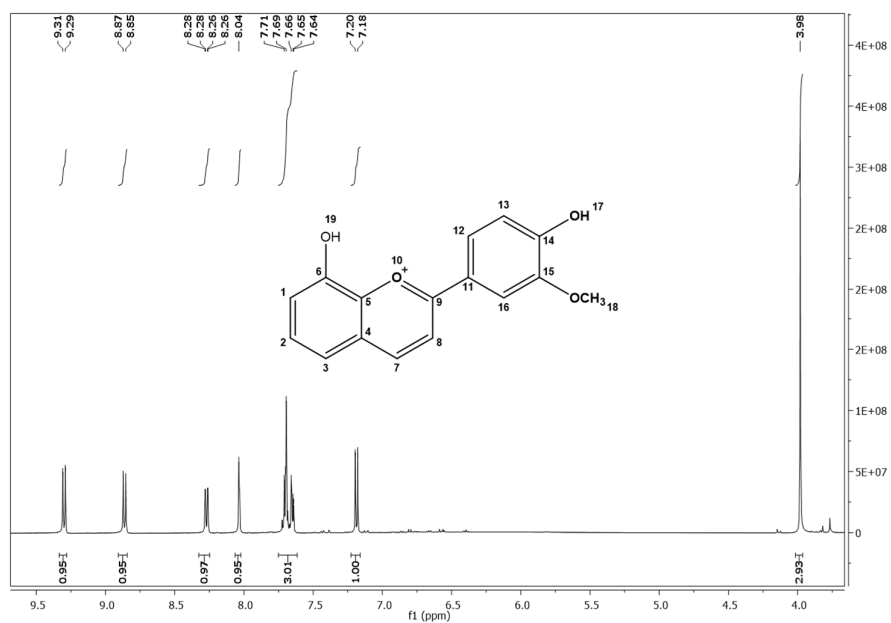


Figure S13.  $^1\text{H}$ -NMR spectrum of compound 3

$^1\text{H}$ -NMR (500 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 9.30 (d,  $J = 9.2$  Hz, 1H, H7), 8.86 (d,  $J = 9.2$  Hz, 1H, H8), 8.27 (dd,  $J = 8.7, 2.2$  Hz, 1H, H12), 8.04 (s, 1H, H16), 7.75 – 7.61 (m, 3H, H1, H2, H3), 7.19 (d,  $J = 8.7$  Hz, 1H, H13), 3.98 (s, 3H, H18).

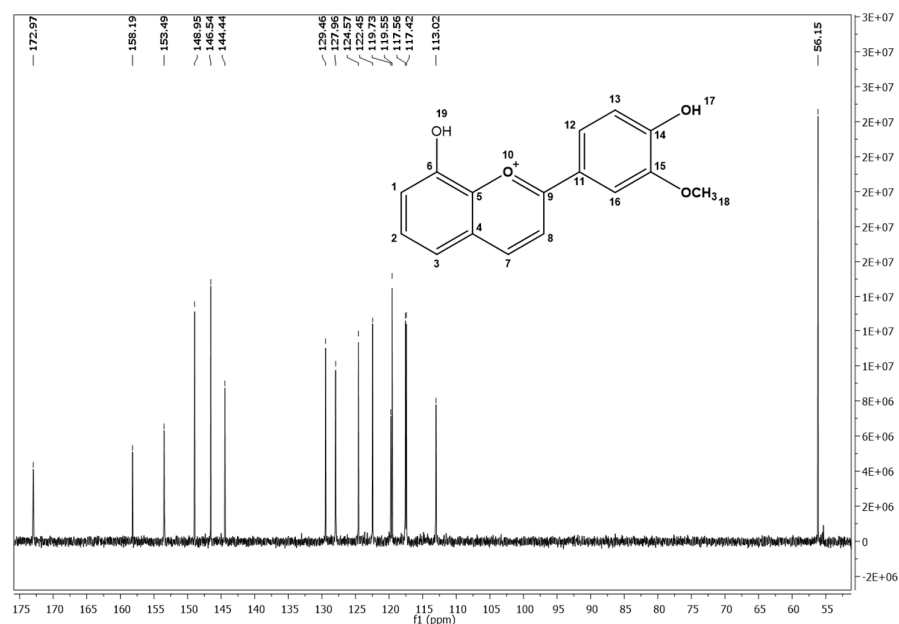


Figure S14.  $^{13}\text{C}$ -NMR spectrum of compound 3

$^{13}\text{C}$ -NMR (125 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 172.9 (C9); 158.1 (C14); 153.4 (C7); 148.9 (C15); 146.5 (C5); 144.4 (C6); 129.4 (C2); 127.9 (C12); 124.5 (C4); 122.4 (C3); 119.7 (C11); 119.5 (C1); 117.5 (C8); 117.4 (C13); 113.0 (C16); 56.1 (C18).

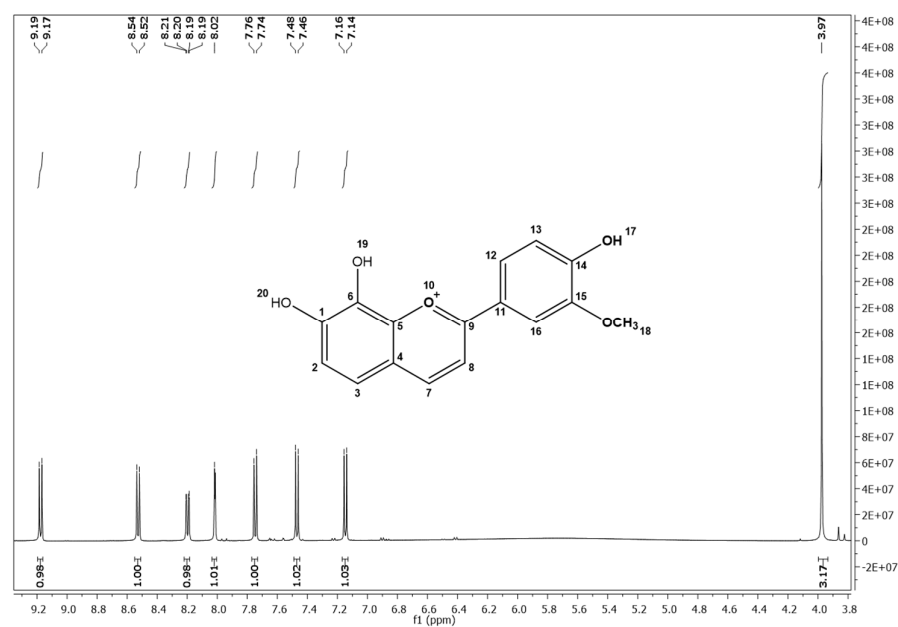


Figure S15.  $^1\text{H}$ -NMR spectrum of compound 4

$^1\text{H}$ -NMR (500 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 9.18 (d,  $J$  = 8.9 Hz, 1H, H7), 8.53 (d,  $J$  = 8.8 Hz, 1H, H8), 8.20 (dd,  $J$  = 8.6, 2.2 Hz, 1H, H12), 8.02 (s, 1H, H16), 7.75 (d,  $J$  = 8.8 Hz, 1H, H3), 7.47 (d,  $J$  = 8.8 Hz, 1H, H2), 7.15 (d,  $J$  = 8.6 Hz, 1H, H13), 3.97 (s, 3H, H18).

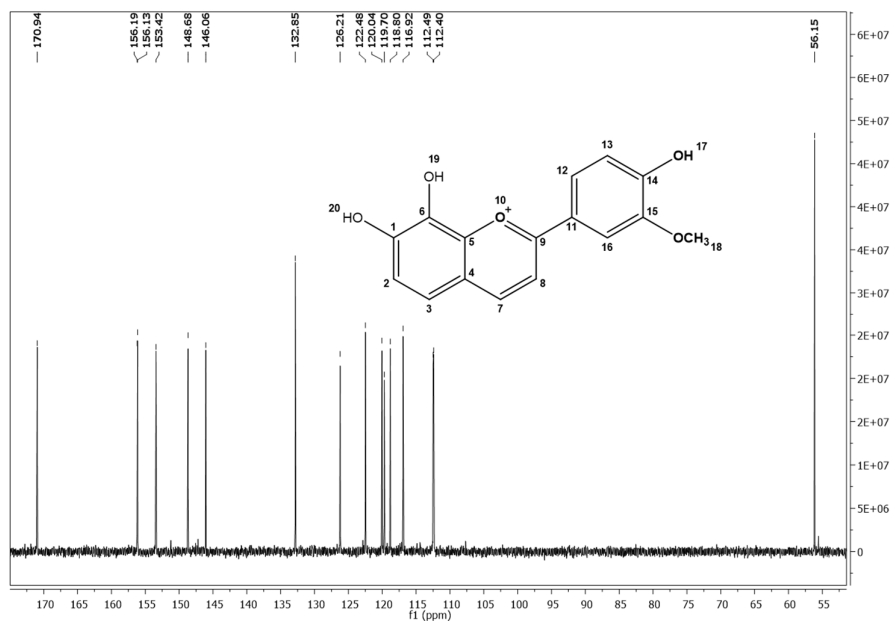


Figure S16.  $^{13}\text{C}$ -NMR spectrum of compound 4

$^{13}\text{C}$ -NMR (125 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 170.9 (C9); 156.2 (C15); 156.1 (C1); 156.1 (C14); 153.4 (C7); 148.6 (C14); 146.0 (C5); 132.8 (C6); 126.2 (C12); 122.4 (C3); 120.0 (C4); 119.7 (C2); 118.8 (C11); 116.9 (C13); 112.5 (C16); 112.4 (C8); 56.1 (C18).

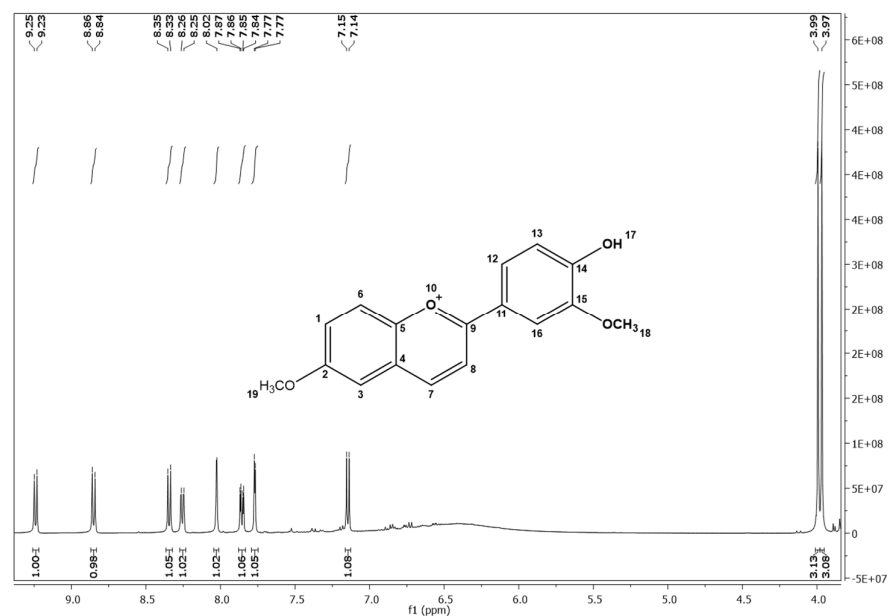


Figure S17.  $^1\text{H}$ -NMR spectrum of compound 5

$^1\text{H}$ -NMR (500 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 9.24 (d,  $J = 9.2$  Hz, 1H, H7), 8.85 (d,  $J = 9.2$  Hz, 1H, H8), 8.34 (d,  $J = 9.3$  Hz, 1H, H6), 8.25 (d,  $J = 8.6$  Hz, 1H, H12), 8.02 (s, 1H, H16), 7.86 (dd,  $J = 9.3, 3.0$  Hz, 1H, H1), 7.77 (d,  $J = 2.9$  Hz, 1H, H3), 7.15 (d,  $J = 8.6$  Hz, 1H, H13), 3.99 (s, 3H, H18), 3.97 (s, 3H, H19).

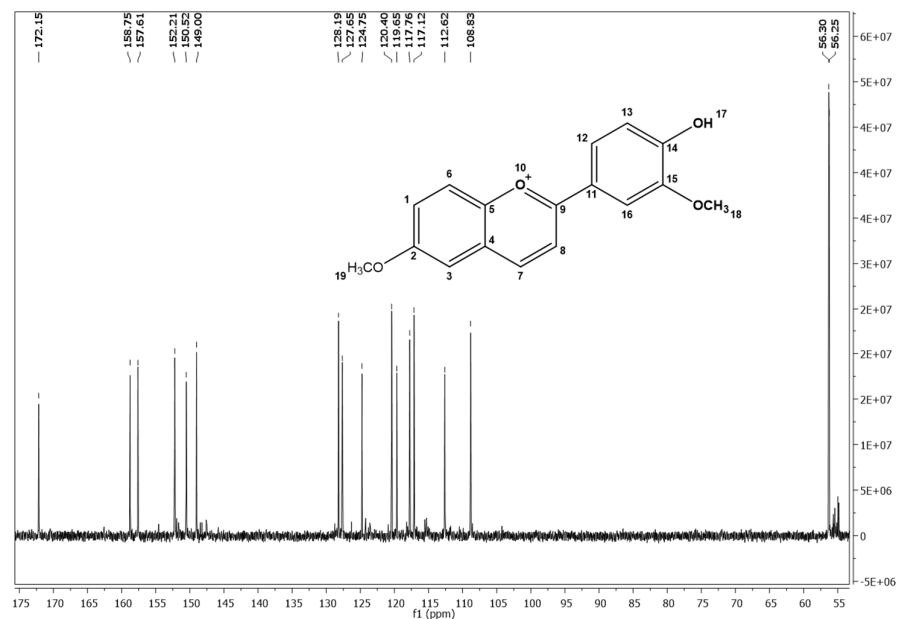


Figure S18.  $^{13}\text{C}$ -NMR spectrum of compound 5

$^{13}\text{C}$ -NMR (125 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 172.1 (C9); 158.7 (C2); 157.6 (C14); 152.2 (C7); 150.5 (C5); 149.0 (C15); 128.1 (C12); 127.6 (C1); 124.7 (C4); 120.4 (C6); 119.6 (C11); 117.7 (C8); 117.1 (C13); 112.6 (C16); 108.8 (C3); 56.3 (C18); 56.2 (C19).

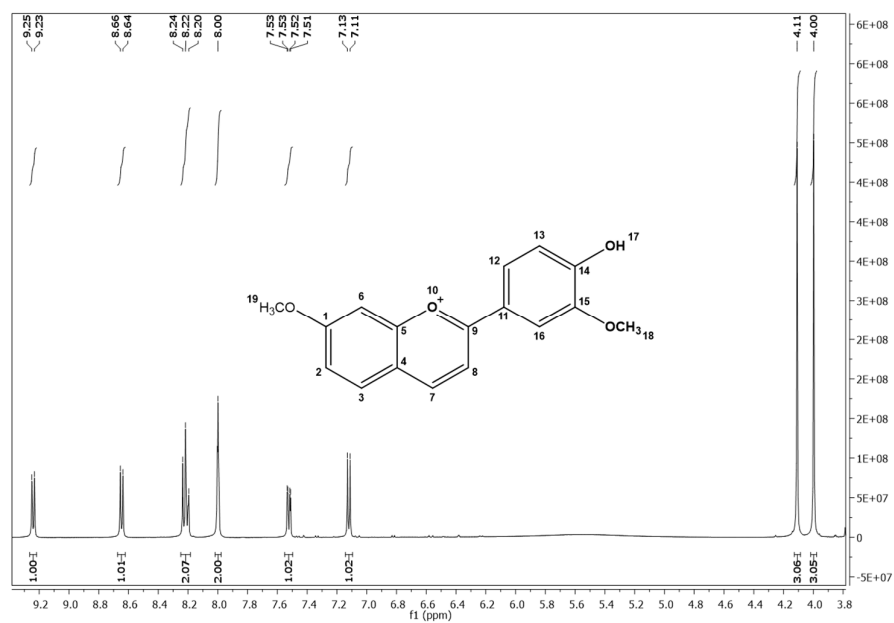


Figure S19.  $^1\text{H}$ -NMR spectrum of compound 6

$^1\text{H}$ -NMR (500 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 9.24 (d,  $J = 8.9$  Hz, 1H, **H7**), 8.65 (d,  $J = 8.9$  Hz, 1H, **H8**), 8.22 (t,  $J = 10.1$  Hz, 2H, **H3**, **H13**), 8.00 (s, 2H, **H6**, **H16**), 7.52 (dd,  $J = 9.0$ , 2.4 Hz, 1H, **H12**), 7.12 (d,  $J = 8.6$  Hz, 1H, **H2**), 4.11 (s, 3H, **H18**), 4.00 (s, 3H, **H19**).

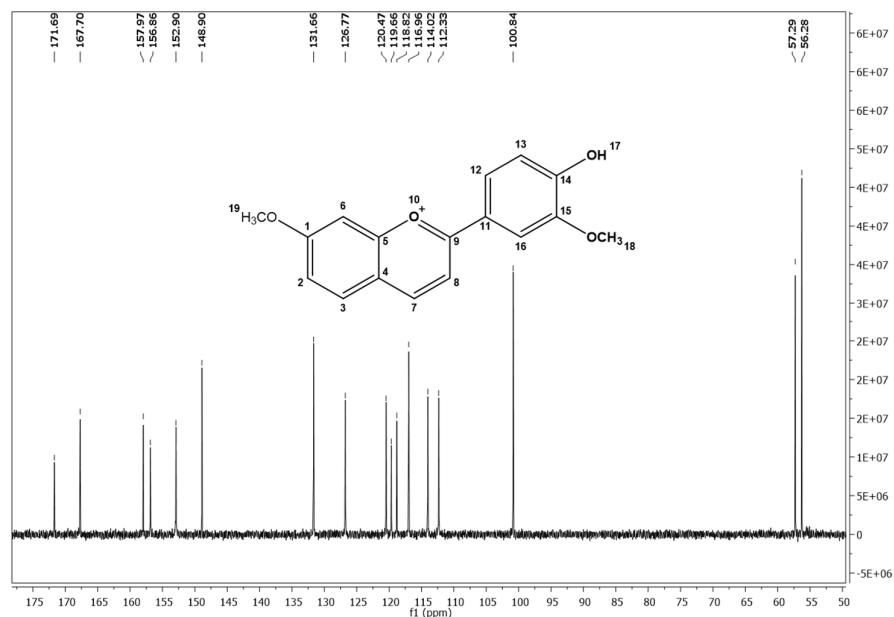


Figure S20.  $^{13}\text{C}$ -NMR spectrum of compound 6

$^{13}\text{C}$ -NMR (125 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 171.6 (C9); 167.7 (C15); 157.9 (C14); 156.8 (C5); 152.9 (C7); 148.9 (C1); 131.6 (C3); 126.7 (C13); 120.4 (C12); 119.6 (C11); 118.8 (C4); 116.9 (C2); 114.0 (C8); 112.3 (C16); 100.8 (C6); 57.2 (C18); 56.2 (C19).

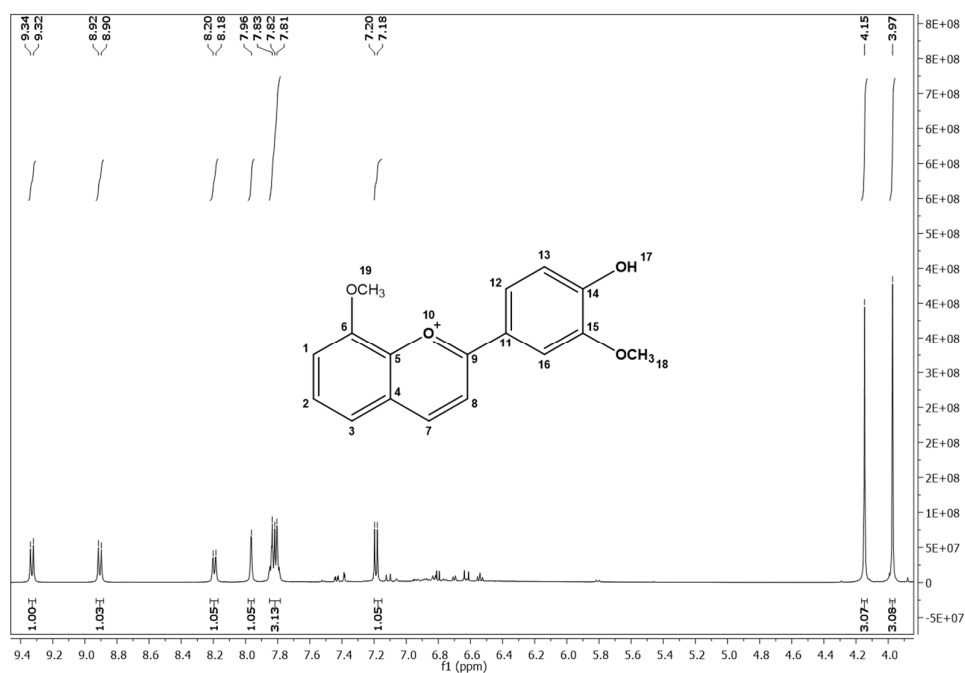


Figure S21.  $^1\text{H}$ -NMR spectrum of compound 7

$^1\text{H}$ -NMR (500 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 9.33 (d,  $J = 9.2$  Hz, 1H, H7), 8.91 (d,  $J = 9.2$  Hz, 1H, H8), 8.19 (d,  $J = 8.6$  Hz, 1H, H12), 7.96 (s, 1H, H16), 7.85–7.78 (m, 3H, H1, H2, H3), 7.19 (d,  $J = 8.6$  Hz, 1H, H13), 4.15 (s, 3H, H18), 3.97 (s, 3H, H19).

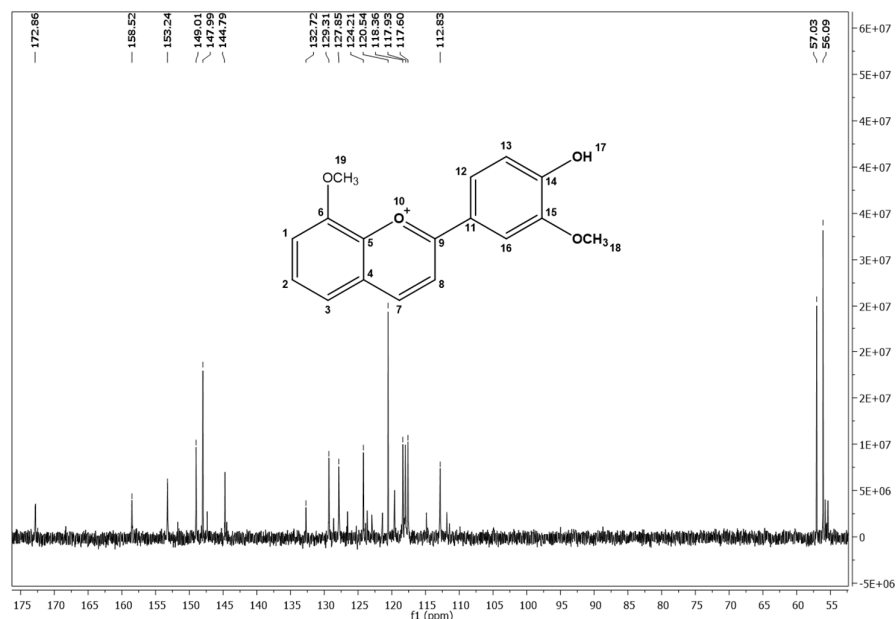


Figure S22.  $^{13}\text{C}$ -NMR spectrum of compound 7

$^{13}\text{C}$ -NMR (125 MHz,  $\text{DMSO-}d_6$ ,  $\delta$  ppm): 172.8 (C9); 158.5 (C14); 153.2 (C7); 149.0 (C15); 147.7 (C5); 144.7 (C6); 132.7 (C4); 129.3 (C2); 127.8 (C12); 124.2 (C11); 120.5 (C3); 118.3 (C1); 117.9 (C8); 117.6 (C13); 112.8 (C16); 57.0 (C18); 56.0 (C19).

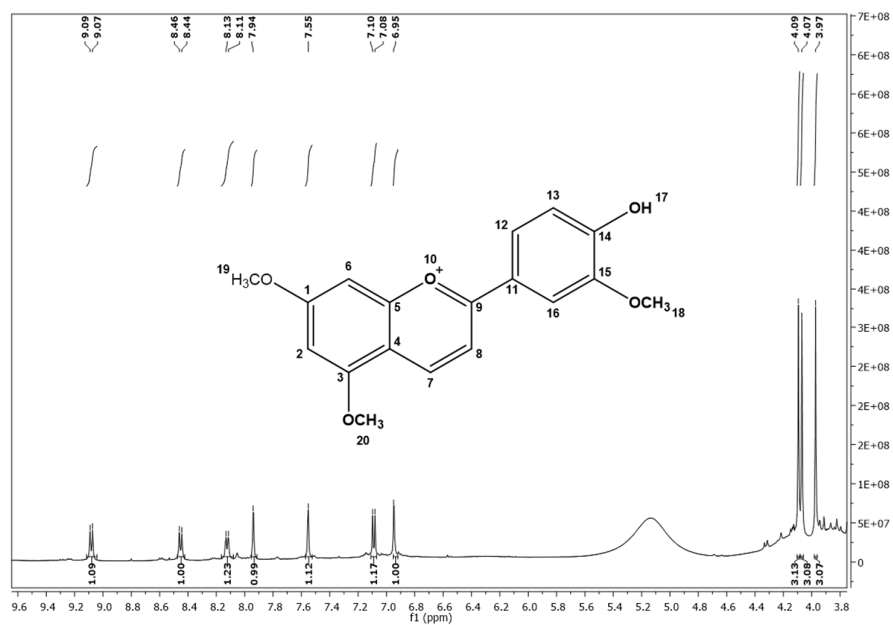


Figure S23.  $^1\text{H}$ -NMR spectrum of compound 8

$^1\text{H}$ -NMR (500 MHz,  $\text{DMSO-}d_6$ ,  $\delta$  ppm): 9.08 (d,  $J = 8.9$  Hz, 1H, **H7**), 8.45 (d,  $J = 8.9$  Hz, 1H, **H8**), 8.12 (d,  $J = 8.5$  Hz, 1H, **H12**), 7.94 (s, 1H, **H16**), 7.55 (s, 1H, **H6**), 7.09 (d,  $J = 8.6$  Hz, 1H, **H13**), 6.95 (s, 1H, **H2**), 4.09 (s, 3H, **H19**), 4.07 (s, 3H, **H20**), 3.97 (s, 3H, **H18**).

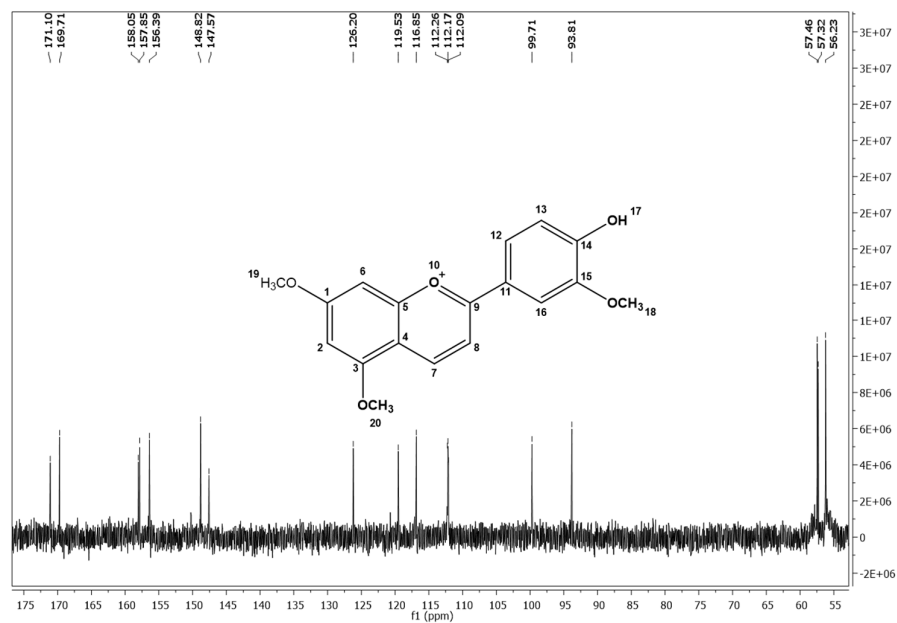


Figure S24.  $^{13}\text{C}$ -NMR spectrum of compound 8

$^{13}\text{C}$ -NMR (125 MHz,  $\text{DMSO}-d_6$ ,  $\delta$  ppm): 171.1 (C9); 169.7 (C19); 158.0 (C3); 157.8 (C14); 156.3 (C5); 148.8 (C15); 147.5 (C7); 126.2 (C12); 119.5 (C11); 116.8 (C13); 112.2 (C8); 112.1 (C16); 112.0 (C4); 99.7 (C2); 93.8 (C6); 57.4 (C19); 57.3 (C20); 56.2 (C18).

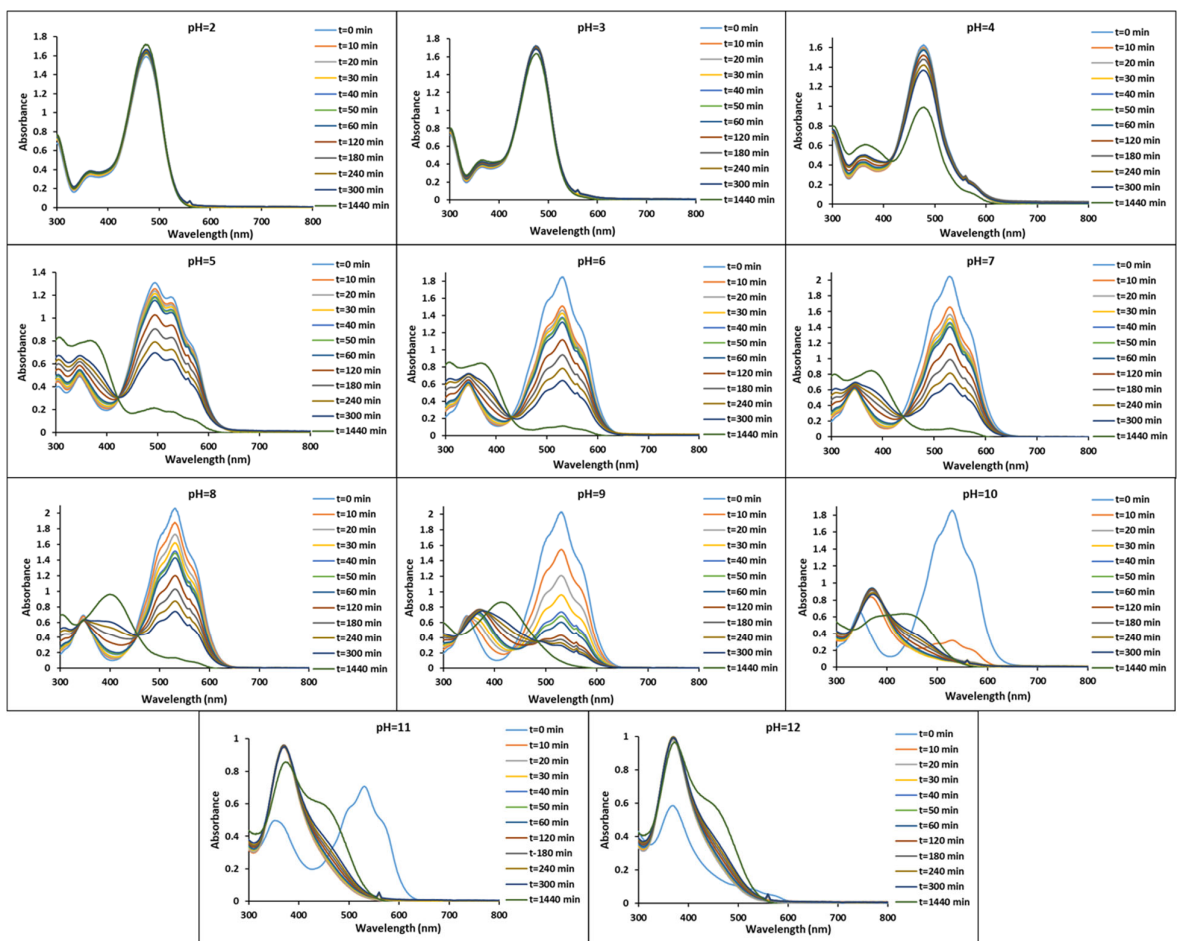


Figure S25. UV-Vis spectra of compound 5 solutions (7·10<sup>-5</sup> M in methanol:water 1:14) at pH values ranging from 2 to 12

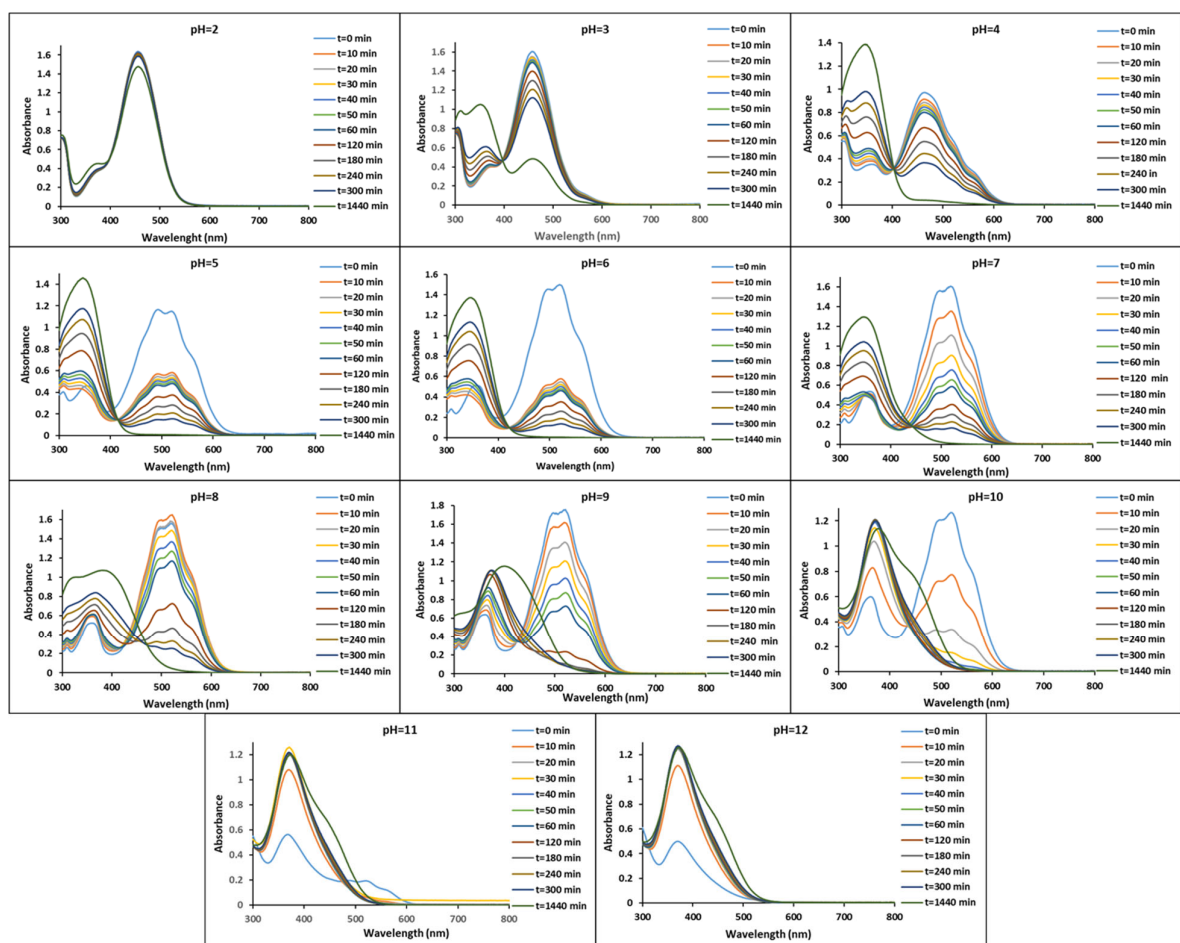


Figure S26. UV-Vis spectra of compound 7 solutions ( $7 \cdot 10^{-5}$  M in methanol:water 1:14) at pH values ranging from 2 to 12

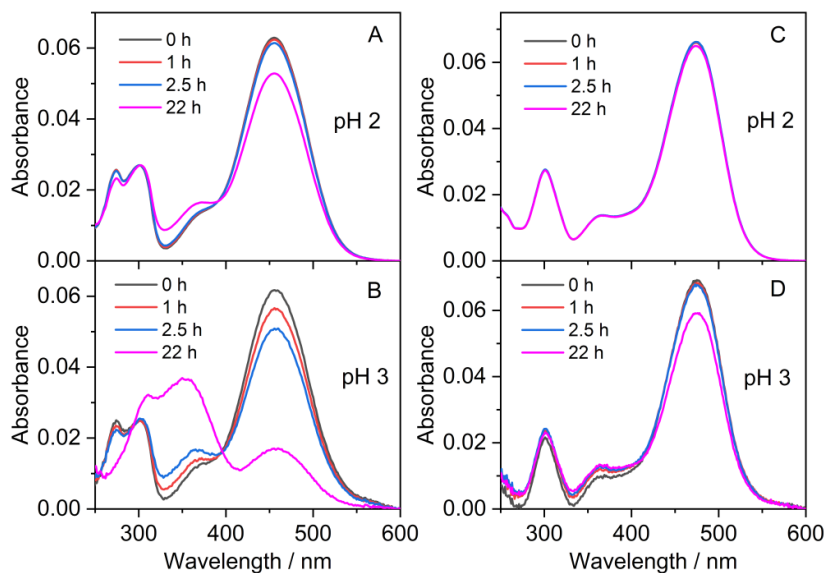


Figure S27. Absorption spectra for 7 at pH 2 (A) and pH 3 (B) as well as for 5 at pH 2 (C) and pH 3 (D) recorded at 0, 1, 2.5 and 22 h after preparation of the aqueous solutions.

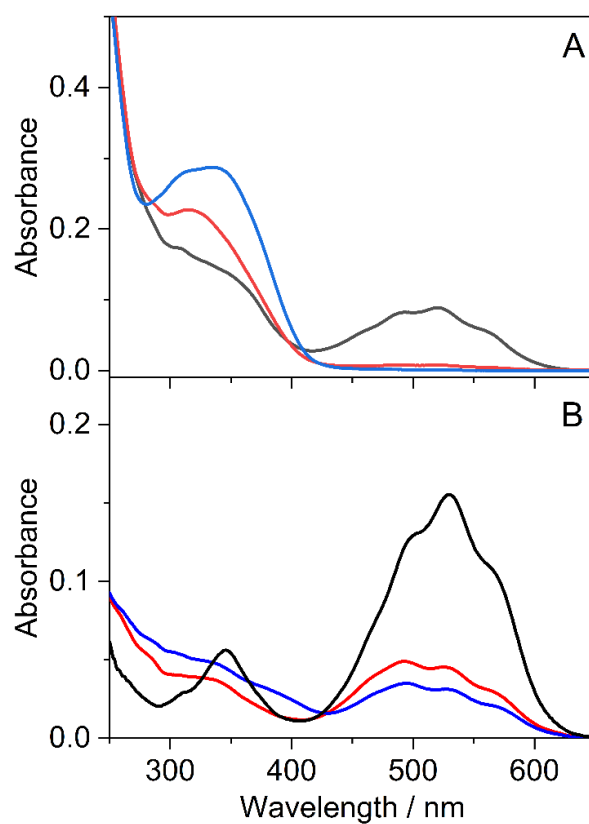


Figure S28. Absorption spectra of 7 (A) and 5 (B) immediately after the preparation of the samples in water (black lines) and in 11.43 mg/mL randomly methylated  $\beta$ -cyclodextrin aqueous solution (red lines). Blue lines display the spectra of the latter solutions in 3 h.

**Table S1.** t-test / Welch's t-test / Mann-Whitney U-test performed for each concentration groups (C1 – 132  $\mu$ M, C2 – 66  $\mu$ M) in both cell lines.

| Cell line | Sample | Normality test<br>(Shapiro Wilk)] | F-test                                                 | t-test / Welch's t-test /<br>Mann-Whitney U test |
|-----------|--------|-----------------------------------|--------------------------------------------------------|--------------------------------------------------|
| HCT116    | 1      | C1: p = 0.326<br>C2: p = 0.685    | F = 0.44, num df = 7,<br>denom df = 7,<br>p = 0.295    | t = -3.21, df = 14,<br>p < 0.01**                |
|           | 2      | C1: p = 0.331<br>C2: p = 0.989    | F = 0.88, num df = 7,<br>denom df = 6,<br>p = 0.864    | t = -5.64, df = 13,<br>p < 0.001***              |
|           | 3      | C1: p = 0.223<br>C2: p = 0.484    | F = 0.20, num df = 7,<br>denom df = 7,<br>p = 0.056    | t = -24.1, df = 13,<br>p < 0.001***              |
|           | 4      | C1: p = 0.771<br>C2: p = 0.969    | F = 0.39, num df = 7,<br>denom df = 6,<br>p = 0.242    | t = -4.36, df = 13,<br>p < 0.001***              |
| HepG2     | 1      | C1: p = 0.356<br>C2: p = 0.999    | F = 88.3, num df = 7,<br>denom df = 7,<br>p < 0.001*** | t = -7.81, df = 7.16,<br>p < 0.001***            |
|           | 2      | C1: p = 0.446<br>C2: p = 0.067    | F = 4.32, num df = 7,<br>denom df = 7,<br>p = 0.072    | t = 9.55, df = 14,<br>p < 0.001***               |
|           | 3      | C1: p = 0.696<br>C2: p = 0.386    | F = 0.25, num df = 7,<br>denom df = 7,<br>p = 0.091    | t = -24.1, df = 14,<br>p < 0.001***              |
|           | 4      | C1: p = 0.627<br>C2: p = 0.442    | F = 2.81, num df = 7,<br>denom df = 7,<br>p = 0.196    | t = 3.14, df = 14,<br>p < 0.01**                 |

**Table S2.** One-way-ANOVA / Kruskal-Wallis test performed for each concentration groups (C1 – 132  $\mu$ M, C2 – 66  $\mu$ M, C3 – 26  $\mu$ M) in both cell lines.

| Cell line | Sample | Normality test<br>(Shapiro Wilk)] | Homoscedasticity<br>(Bartlett test) | one-way-ANOVA /<br>Kruskal-Wallis      | post-hoc (Tukey HSD /<br>Nemenyi or Dunn)                         |
|-----------|--------|-----------------------------------|-------------------------------------|----------------------------------------|-------------------------------------------------------------------|
| HCT116    | 5      | C1: p = 0.266                     | p < 0.05*                           | Chi-sq = 5.12, df = 2<br>p > 0.05      | no differences                                                    |
|           |        | C2: p = 0.275                     |                                     |                                        |                                                                   |
|           |        | C3: p = 0.663                     |                                     |                                        |                                                                   |
|           | 6      | C1: p = 0.171                     | p = 0.241                           | F = 43.40, df = 2<br>p < 0.001***      | C1-C2: p < 0.001***<br>C1-C3: p < 0.001***                        |
|           |        | C2: p = 0.409                     |                                     |                                        |                                                                   |
|           |        | C3: p = 0.373                     |                                     |                                        |                                                                   |
|           | 7      | C1: p = 0.566                     | p < 0.001***                        | Chi-sq = 14.68, df = 2<br>p < 0.01**   | C1-C2: p < 0.01**<br>C1-C3: p < 0.01**                            |
|           |        | C2: p = 0.196                     |                                     |                                        |                                                                   |
|           |        | C3: p = 0.119                     |                                     |                                        |                                                                   |
| HepG2     | 8      | C1: p = 0.074                     | p < 0.05*                           | Chi-sq = 15.52, df = 2<br>p < 0.001*** | C1-C3: p < 0.001***<br>C2-C3: p < 0.01**                          |
|           |        | C2: p = 0.084                     |                                     |                                        |                                                                   |
|           |        | C3: p = 0.689                     |                                     |                                        |                                                                   |
|           | 5      | C1: p = 0.753                     | p < 0.05*                           | Chi-sq = 14.65, df = 2<br>p < 0.001*** | C1-C2: p < 0.01**<br>C1-C3: p < 0.01**                            |
|           |        | C2: p = 0.024                     |                                     |                                        |                                                                   |
|           |        | C3: p = 0.866                     |                                     |                                        |                                                                   |
|           | 6      | C1: p = 0.342                     | p < 0.001***                        | Chi-sq = 19.60, df = 2<br>p < 0.001*** | C1-C3: p < 0.001***<br>C2-C3: p < 0.05*                           |
|           |        | C2: p = 0.624                     |                                     |                                        |                                                                   |
|           |        | C3: p = 0.110                     |                                     |                                        |                                                                   |
|           | 7      | C1: p = 0.272                     | p < 0.001***                        | Chi-sq = 18.61, df = 2<br>p < 0.001*** | C1-C3: p < 0.001***<br>C2-C3: p < 0.05*                           |
|           |        | C2: p = 0.947                     |                                     |                                        |                                                                   |
|           |        | C3: p = 0.959                     |                                     |                                        |                                                                   |
|           | 8      | C1: p = 0.560                     | p = 0.993                           | F = 346.1, df = 2<br>p < 0.001***      | C1-C2: p < 0.001***<br>C1-C3: p < 0.001***<br>C2-C3: p < 0.001*** |
|           |        | C2: p = 0.858                     |                                     |                                        |                                                                   |
|           |        | C3: p = 0.887                     |                                     |                                        |                                                                   |

**Table S3.** One-way-ANOVA / Kruskal-Wallis test performed for each concentration groups (C1 – 132  $\mu$ M, C2 – 66  $\mu$ M, C3 – 26  $\mu$ M) in the complexed samples on HepG2 cells.

| Sample  | Normality test (Shapiro Wilk)]                  | Homoscedasticity (Bartlett test) | one-way-ANOVA / Kruskal-Wallis          | post-hoc (Tukey HSD / Nemenyi or Dunn)  |
|---------|-------------------------------------------------|----------------------------------|-----------------------------------------|-----------------------------------------|
| 5+SBECD | C1: p = 0.956<br>C2: p = 0.709<br>C3: p = 0.967 | p < 0.001***                     | Chi-sq = 19.23, df = 2,<br>p < 0.001*** | C1-C3: p < 0.001***                     |
| 7+RAMEB | C1: p = 0.342<br>C2: p = 0.386<br>C3: p = 0.246 | p = 0.193                        | F = 14.44, df = 2,<br>p < 0.001***      | C1-C3: p < 0.001***<br>C2-C3: p < 0.05* |

**Table S4.** t-test / Welch's t-test / Mann-Whitney U-test performed for each concentration groups (C1 – 132  $\mu$ M, C2 – 66  $\mu$ M, C3 – 26  $\mu$ M) to compare anthocyanidins (5, 7) with complexed ones (5+SBECD, 7+RAMEB) on HepG2 cell line.

| Concentration group | Samples     | Normality test (Shapiro Wilk)]      | F-test                                               | t-test / Welch's t-test / Mann-Whitney U test |
|---------------------|-------------|-------------------------------------|------------------------------------------------------|-----------------------------------------------|
| C1                  | 5 – 5+SBECD | 5: p = 0.753<br>5+SBECD: p = 0.956  | F = 1.17, num df = 7,<br>denom df = 6,<br>p = 0.861  | t = 3.84, df = 13,<br>p < 0.01**              |
| C2                  | 5 – 5+SBECD | 5: p = 0.240<br>5+SBECD: p = 0.709  | F = 0.98, num df = 7,<br>denom df = 7,<br>p = 0.982  | t = 3.81, df = 14,<br>p < 0.01**              |
| C3                  | 5 – 5+SBECD | 5: p = 0.866<br>5+SBECD: p = 0.967  | F = 0.05, num df = 6,<br>denom df = 7,<br>p < 0.01** | t = -3.97, df = 7.80,<br>p < 0.01**           |
| C1                  | 7 – 7+RAMEB | 7: p < 0.01**<br>7+RAMEB: p = 0.674 | F = 0.07, num df = 7,<br>denom df = 6,<br>p < 0.01** | W = 0, p < 0.01**                             |
| C2                  | 7 – 7+RAMEB | 7: p = 0.947<br>7+RAMEB: p = 0.992  | F = 0.60, num df = 7,<br>denom df = 6,<br>p = 0.523  | t = -11.3, df = 13,<br>p < 0.001***           |
| C3                  | 7 – 7+RAMEB | 7: p = 0.960<br>7+RAMEB: p = 0.246  | F = 0.47, num df = 7,<br>denom df = 7,<br>p = 0.340  | t = -11.2, df = 14,<br>p < 0.001***           |