

## SUPPLEMENTARY MATERIAL

### Isolation, purification and tyrosinase inhibitory activity of anthocyanins and their novel degradation compounds from *Solanum tuberosum* L.

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**Abstract:** To explore the composition of anthocyanins and expand their biological activities, anthocyanins were systematically isolated and purified from tubers of *Solanum tuberosum* L., and their tyrosinase inhibitory activity was investigated. In this study, two new anthocyanin degradation compounds, norpetanin (**9**) and 4-*O*-(*p*-coumaryl) rhamnose (**10**), along with 17 known anthocyanins and their derivatives, were isolated and purified from an acid-ethanolic extract of fresh purple potato tubers. Their structures were elucidated via 1D and 2D NMR and HR-ESI-MS and compared with those reported in the literature. The extracts were evaluated for anthocyanins and their derivatives using a tyrosinase inhibitor screening kit and molecular docking technology, and the results showed that petanin, norpetanin, 4-*O*-(*p*-coumaryl) rhamnose, and lyciruthenylpropanoid D/E possessed tyrosinase inhibitory activity, with 50% inhibiting concentration (IC<sub>50</sub>) values of 122.37 ± 8.03, 115.53 ± 7.51, 335.03 ± 12.99, and 156.27 ± 11.22 μM (Mean ± SEM, *n* = 3), respectively. Furthermore, petanin was validated against melanogenesis in zebrafish; it was found that it could significantly inhibit melanin pigmentation (*p* < 0.001), and the inhibition rate of melanin was 17% compared with the normal group. This finding may provide potential treatments for diseases with abnormal melanin production, and high-quality raw materials for whitening cosmetics.

**Keywords:** acylated anthocyanins; norpetanin; 4-*O*-(*p*-coumaryl) rhamnose; molecular docking; petanin; tyrosinase inhibitor.

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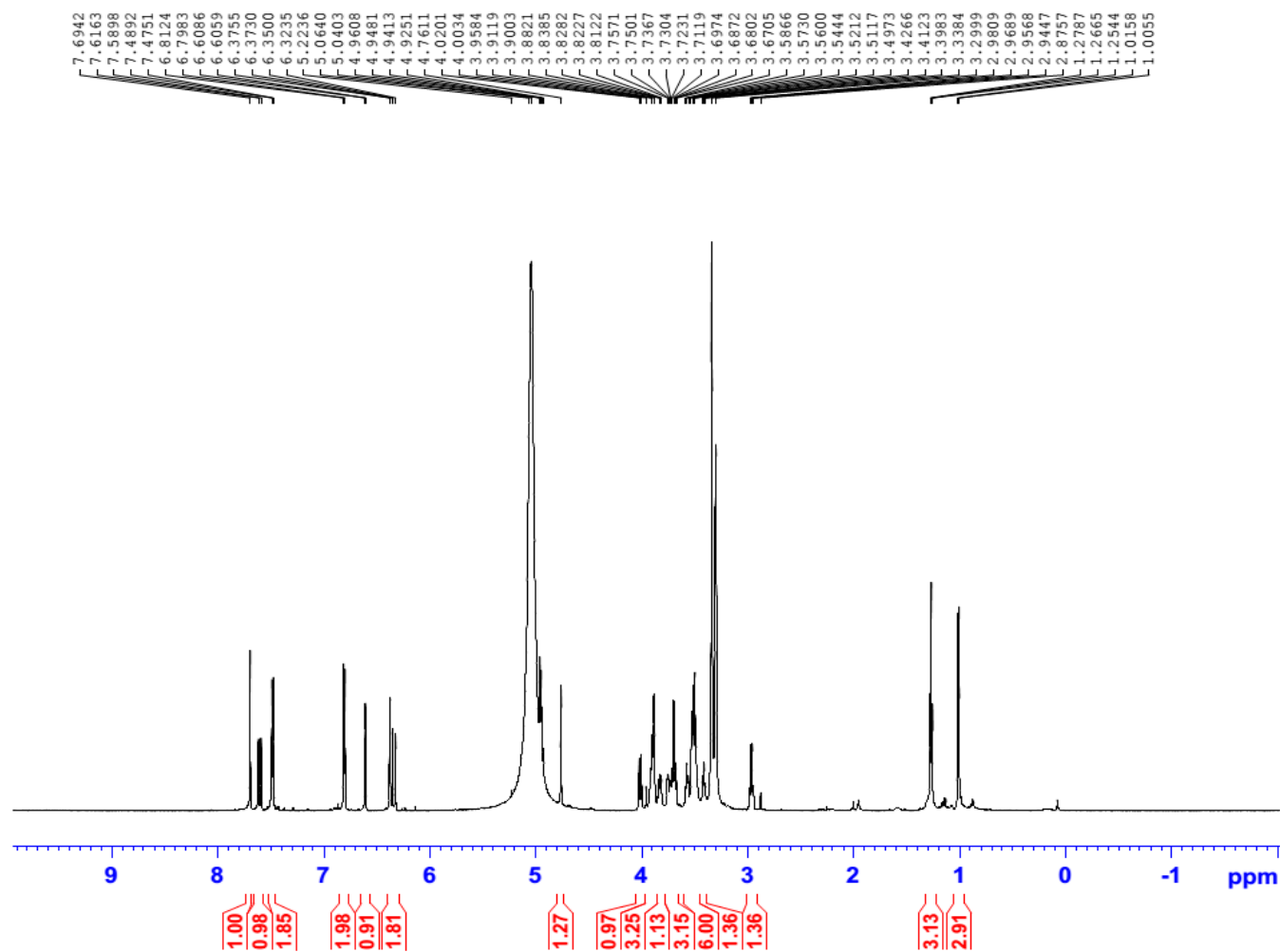
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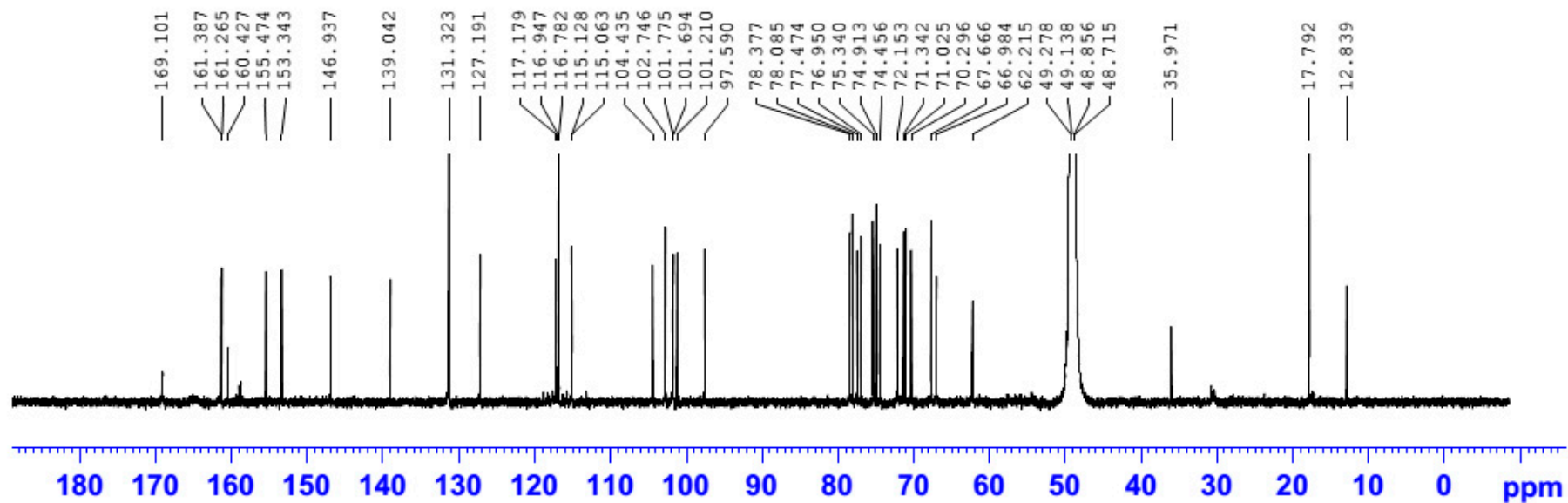
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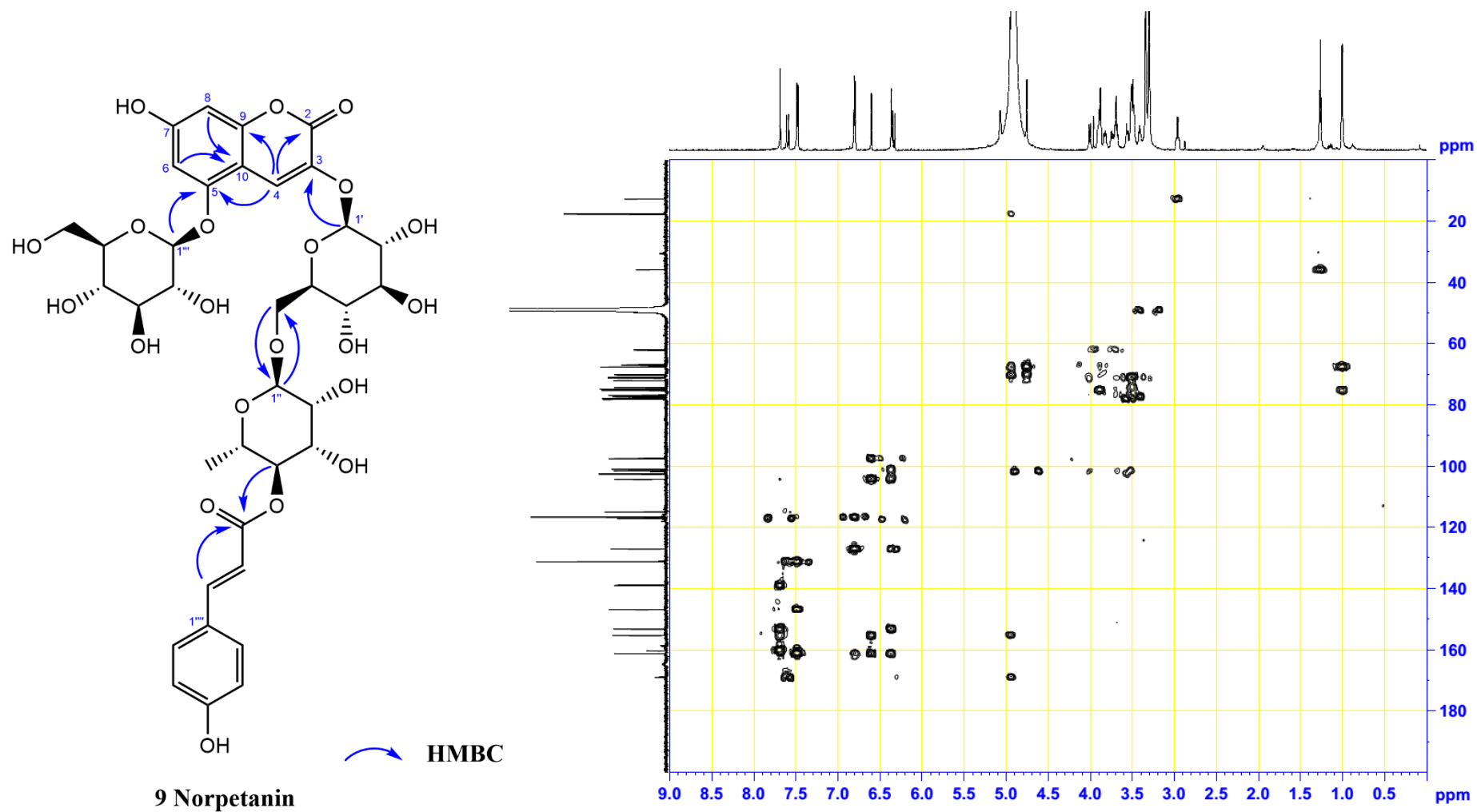


Figure S3. HMBC spectrum and key HMBC correlations of **9**

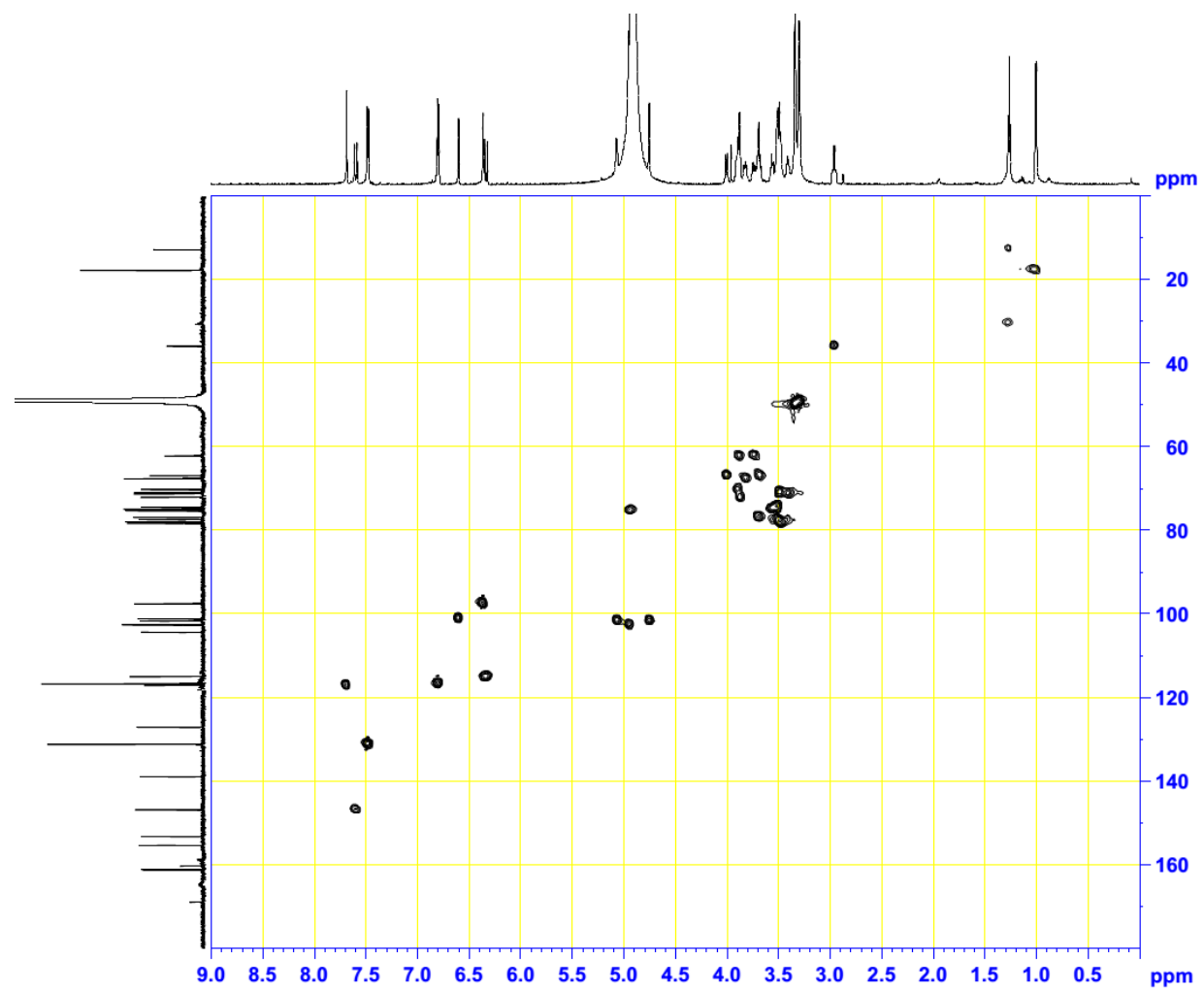


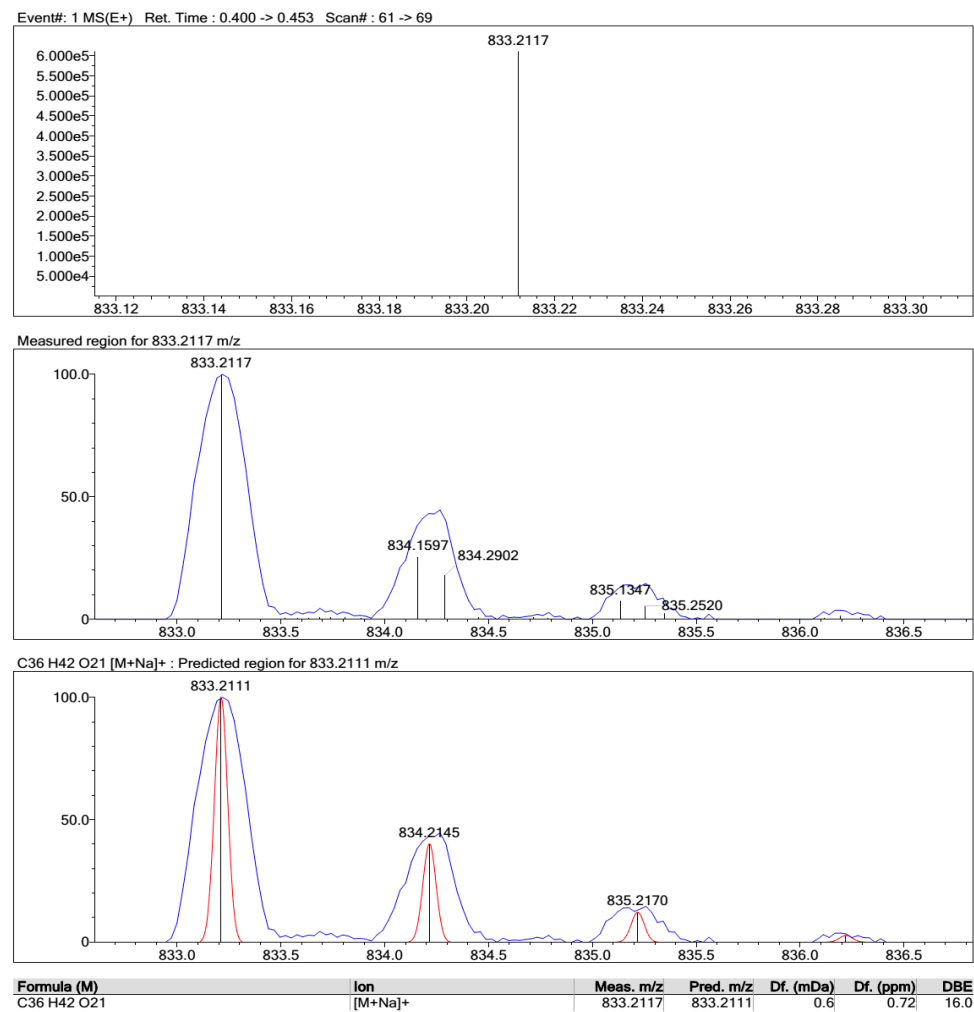
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**Figure S6.** HR-ESI-MS spectrum of **9**



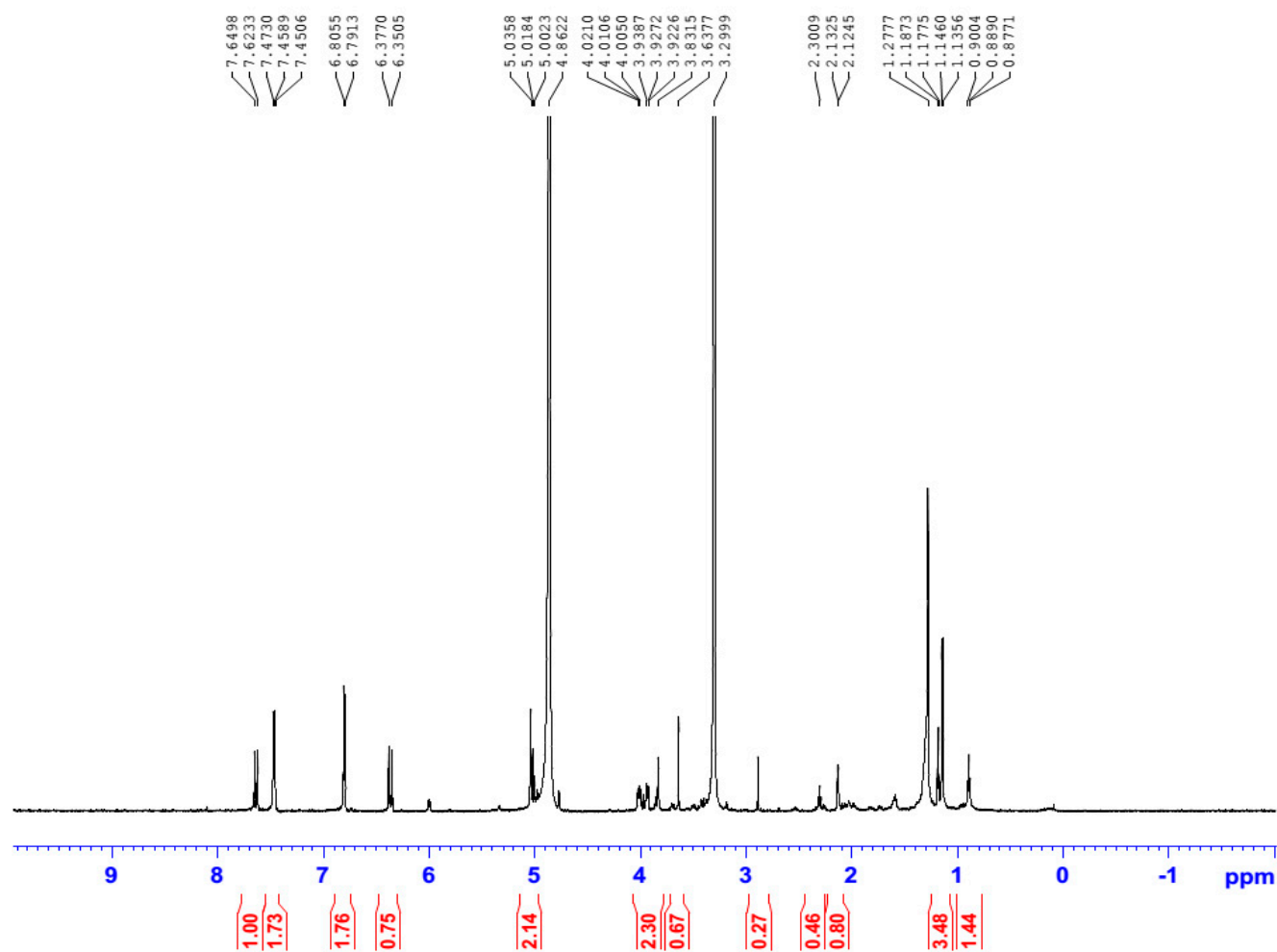


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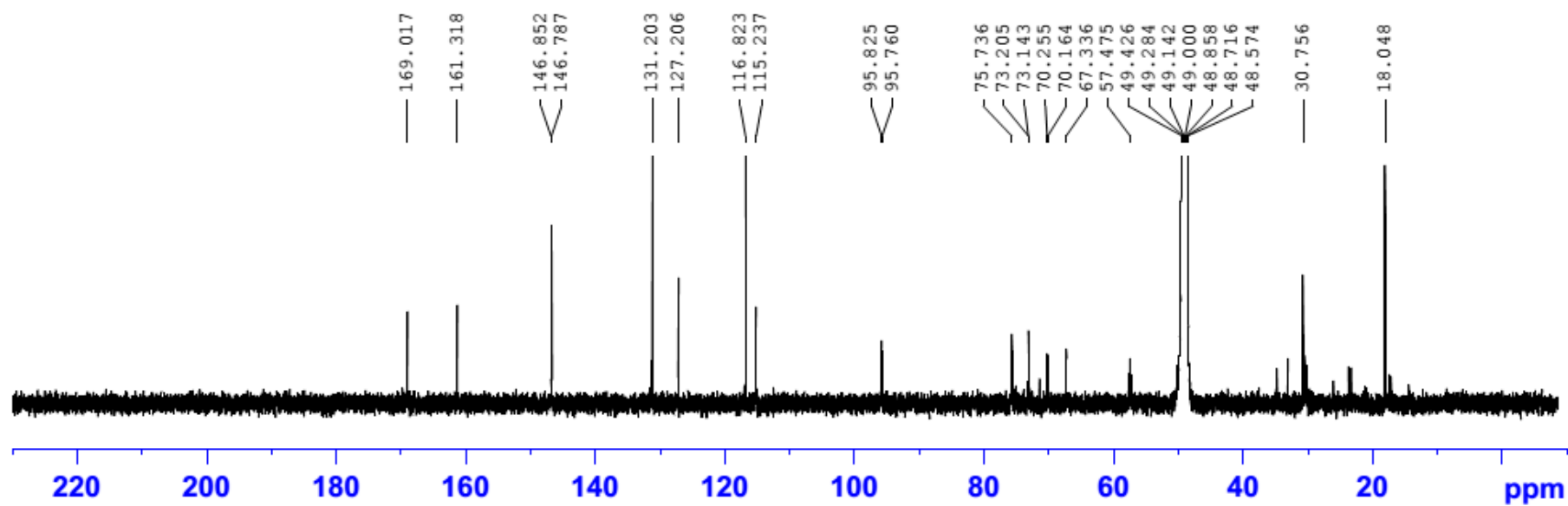
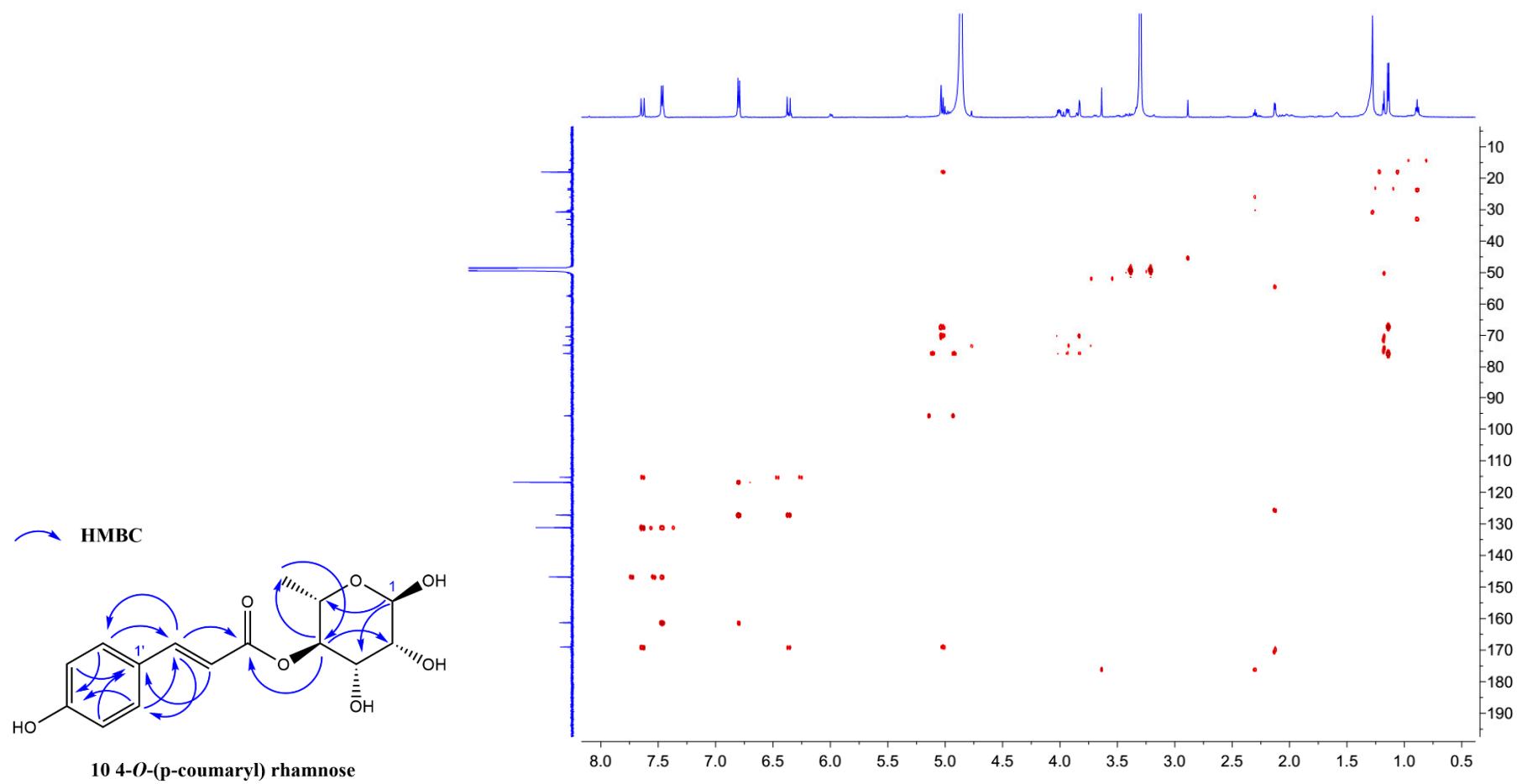
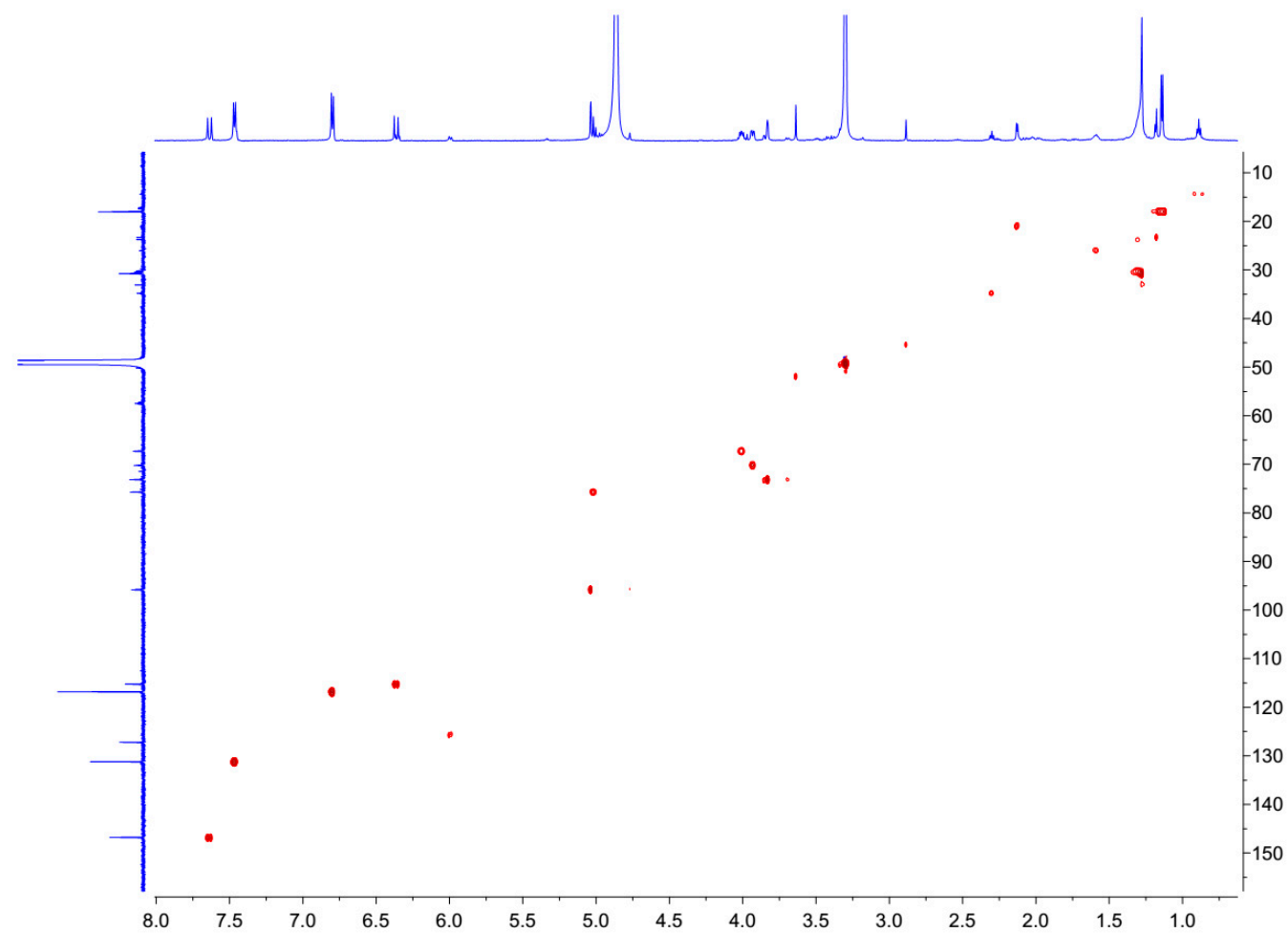


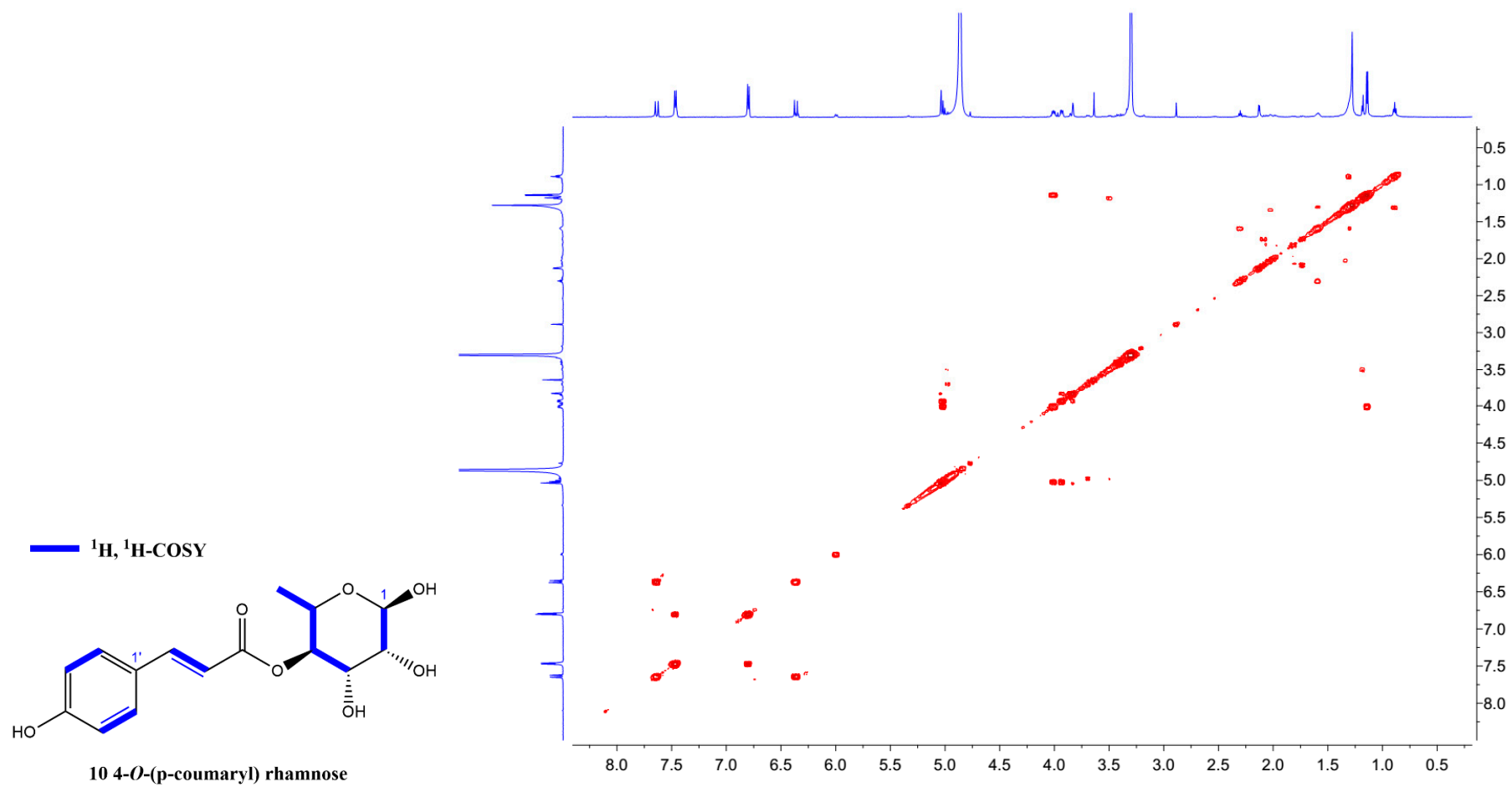
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**Figure S9.** HMBC spectrum and key HMBC correlations of **10**



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**Figure S11.**  $^1\text{H}$ ,  $^1\text{H}$ -COSY spectrum and key  $^1\text{H}$ ,  $^1\text{H}$ -COSY correlations of **10**

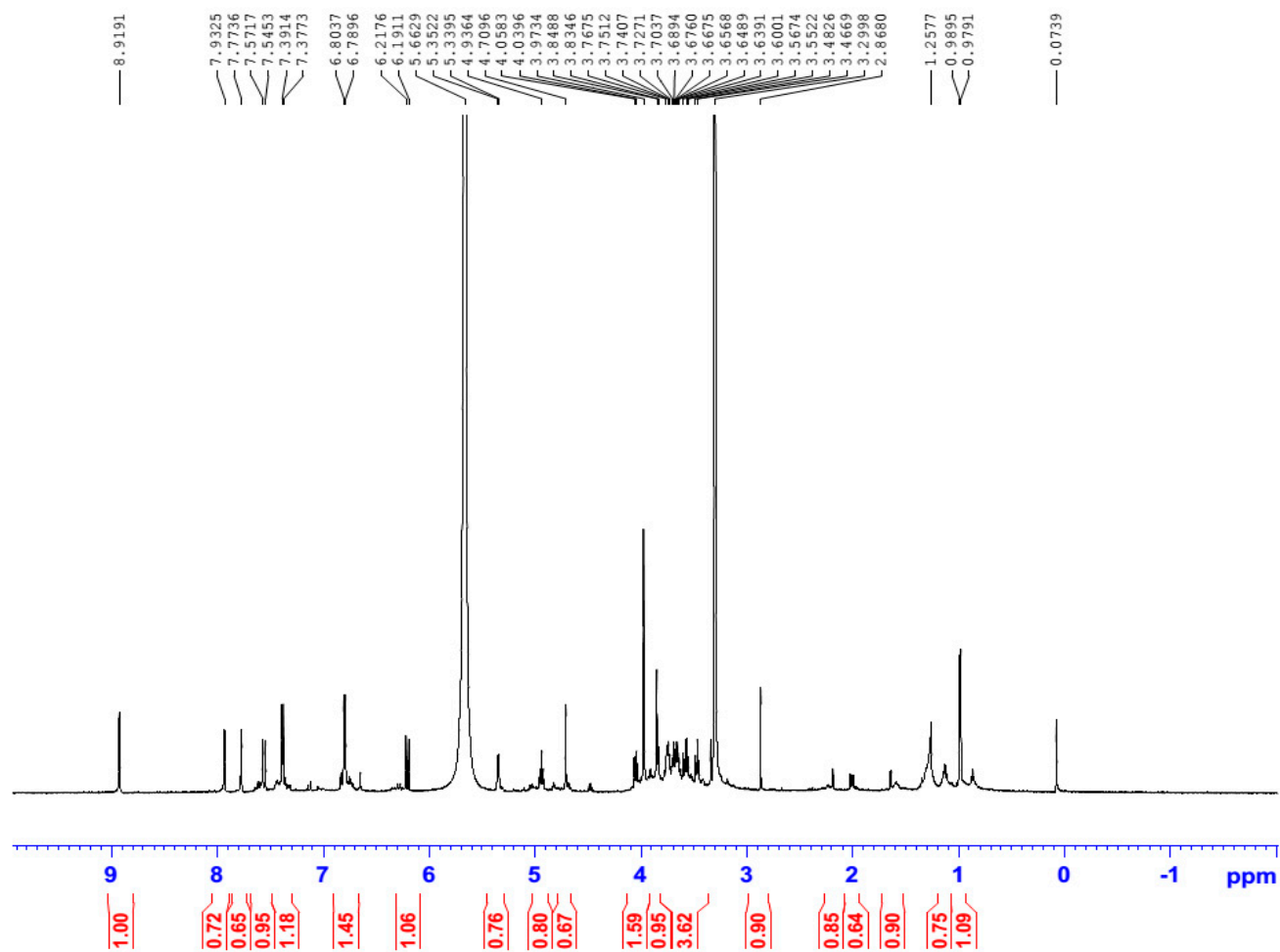
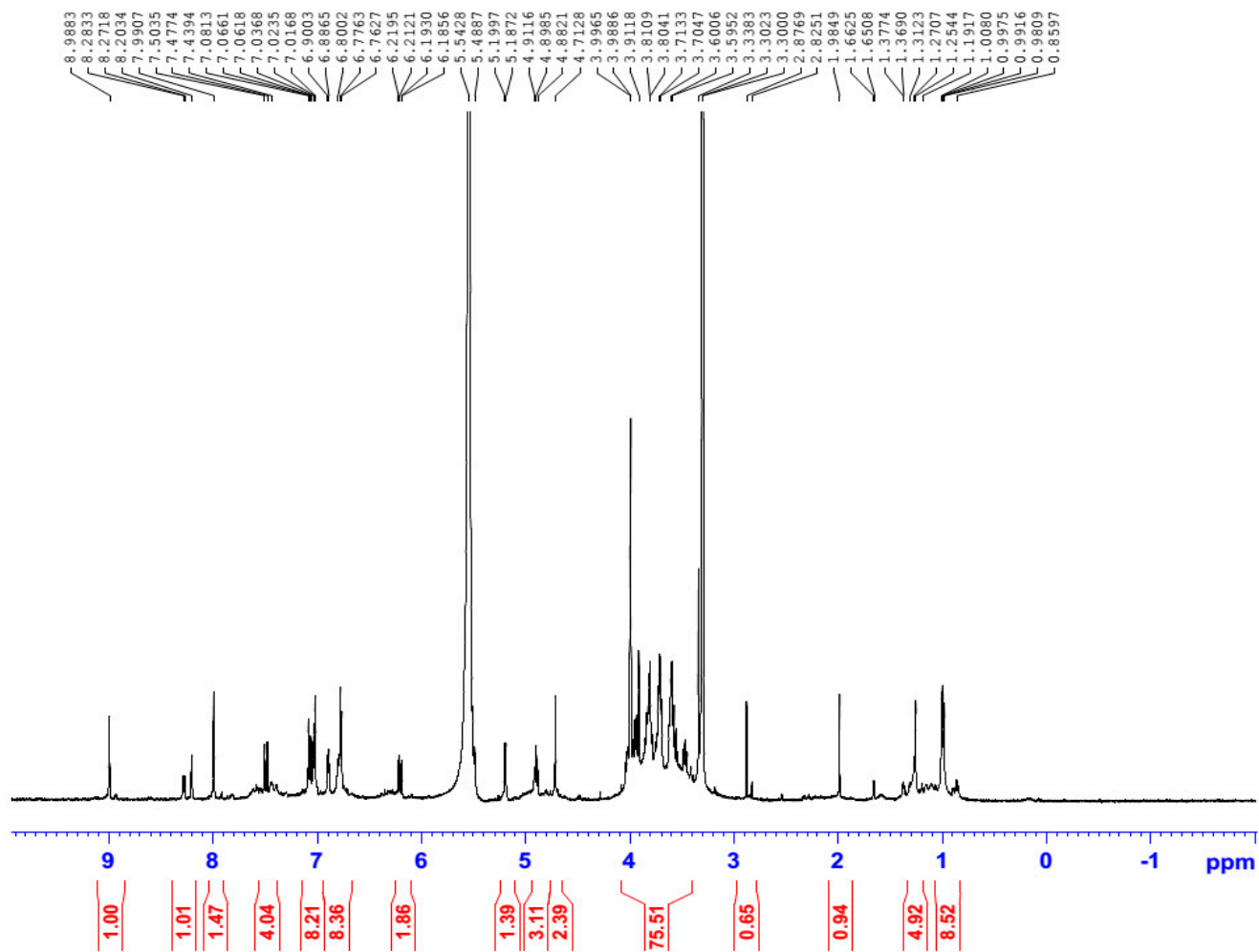
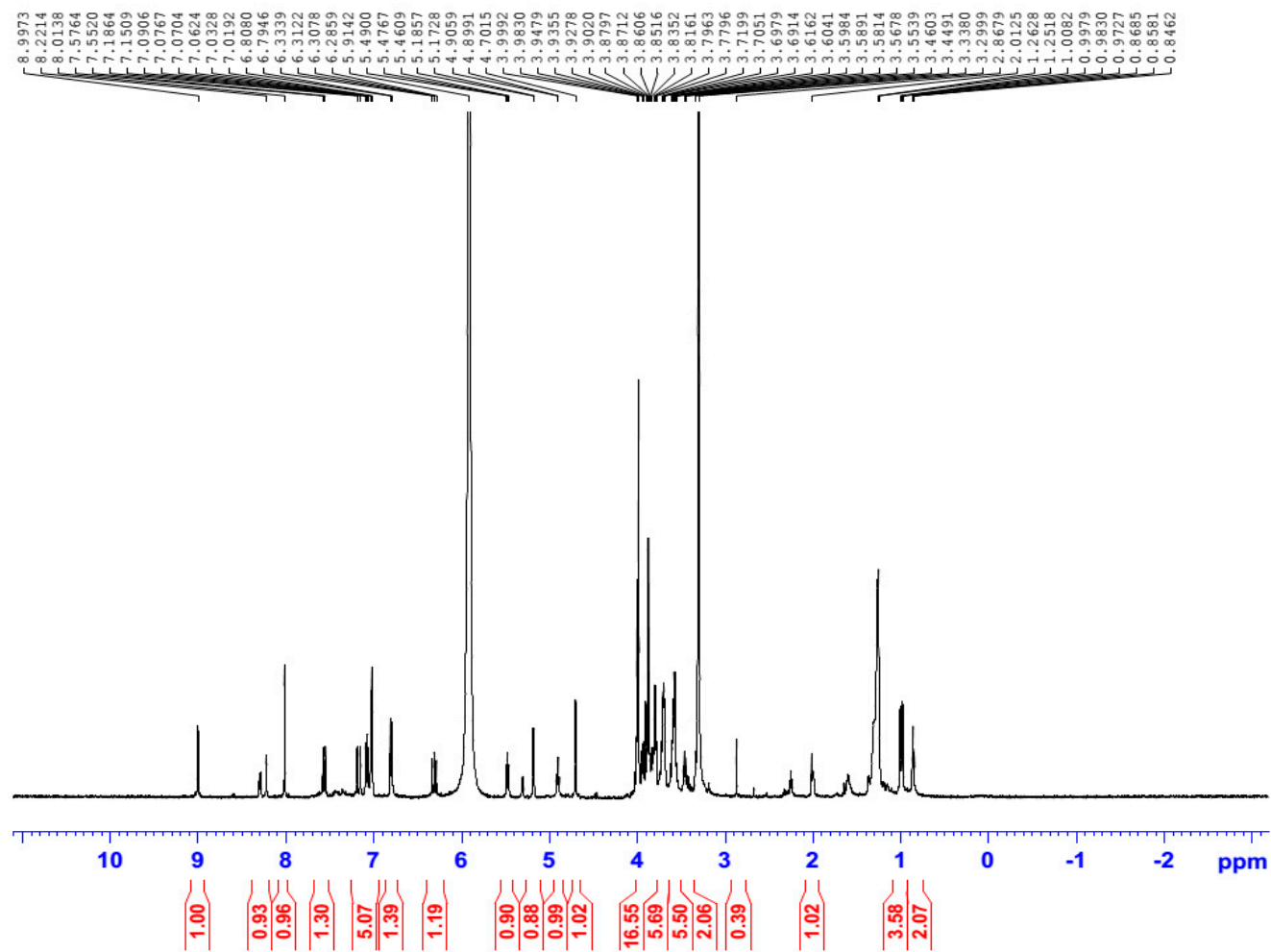


Figure S12. <sup>1</sup>H NMR spectrum (600 MHz, CD<sub>3</sub>OD/CF<sub>3</sub>COOD (9:1)) of **3**

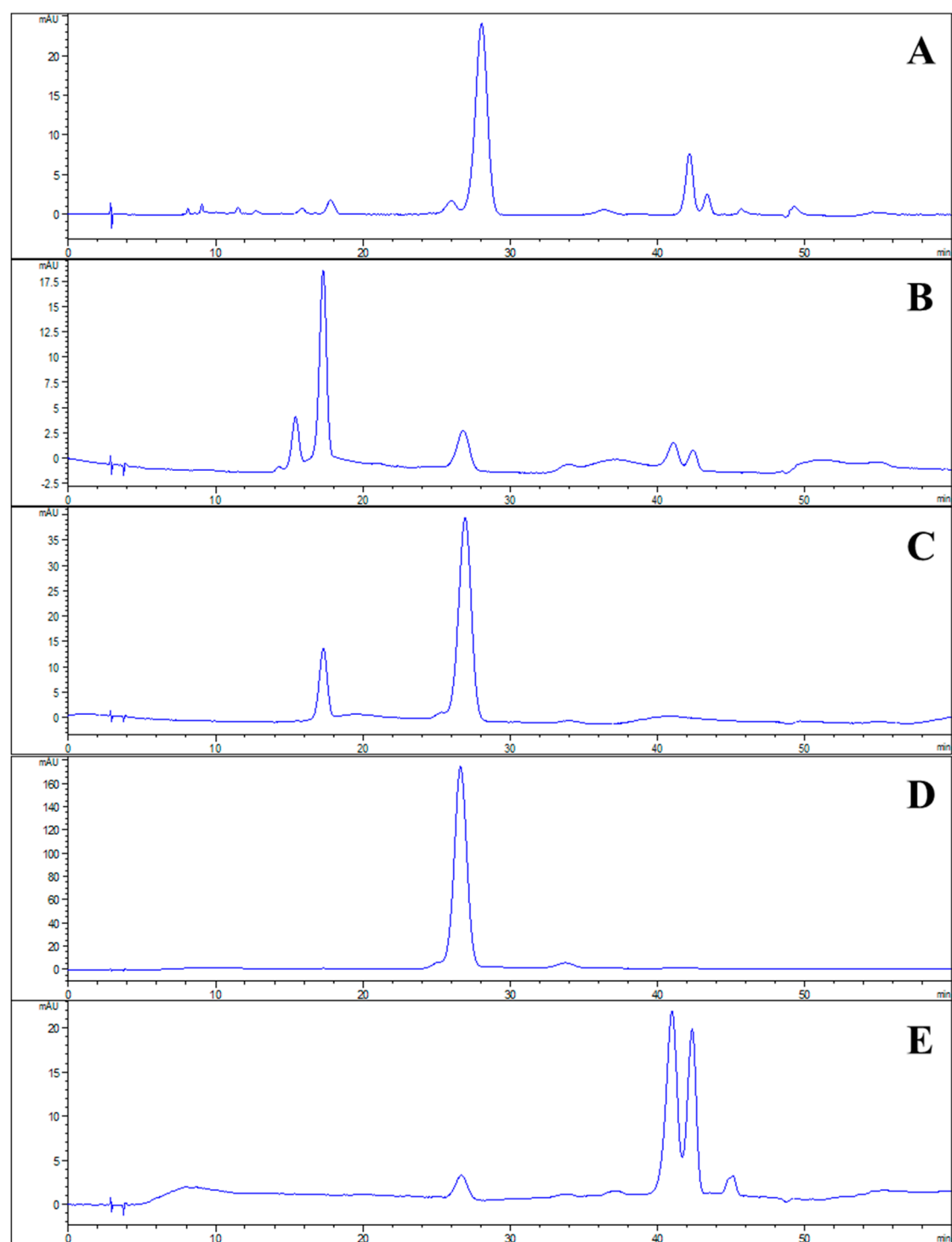


**Figure S13.**  $^1\text{H}$  NMR spectrum (600 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  (9:1)) of **12**

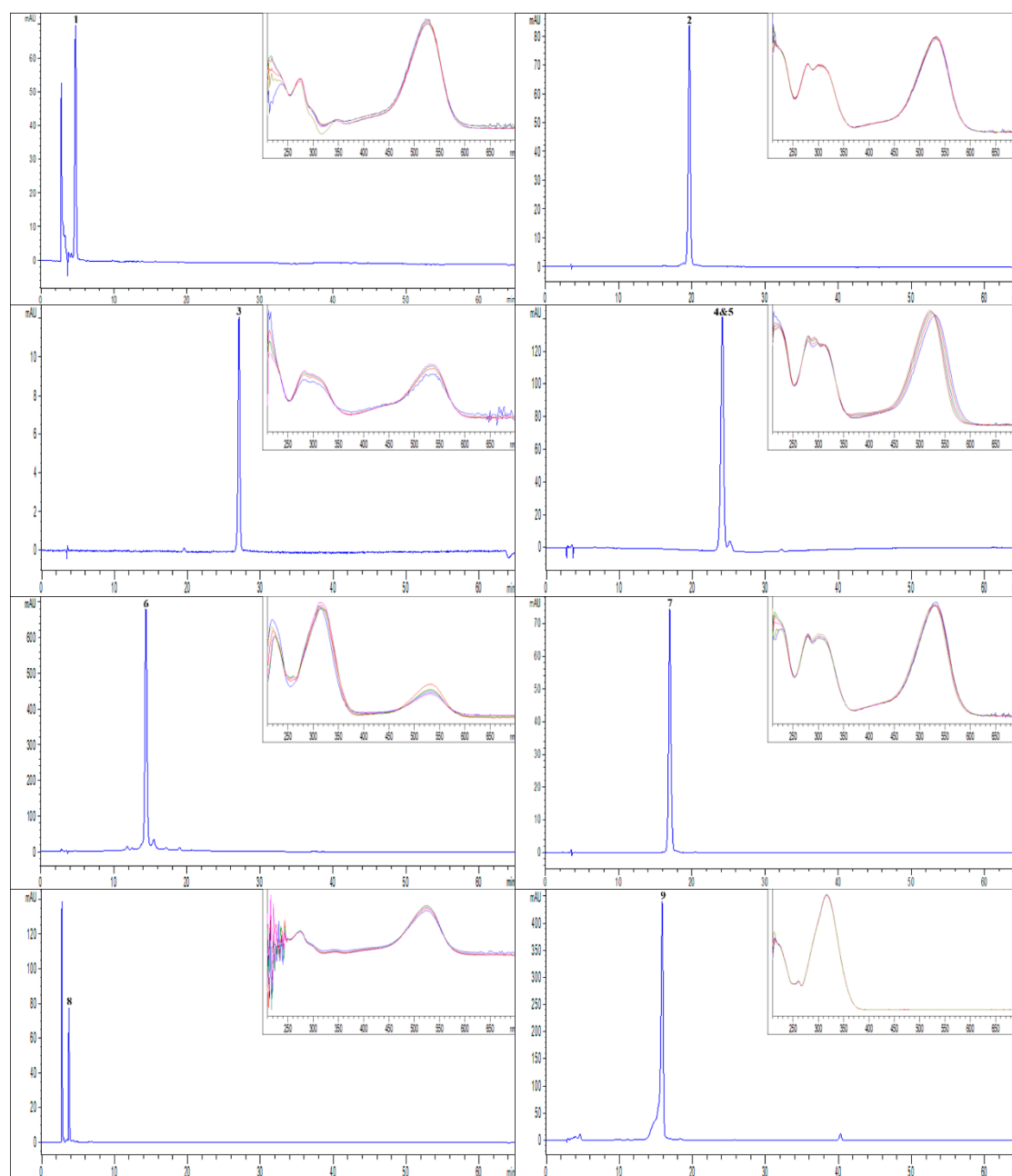


**Figure S14.**  $^1\text{H}$  NMR spectrum (600 MHz,  $\text{CD}_3\text{OD}/\text{CF}_3\text{COOD}$  (9:1)) of **15**

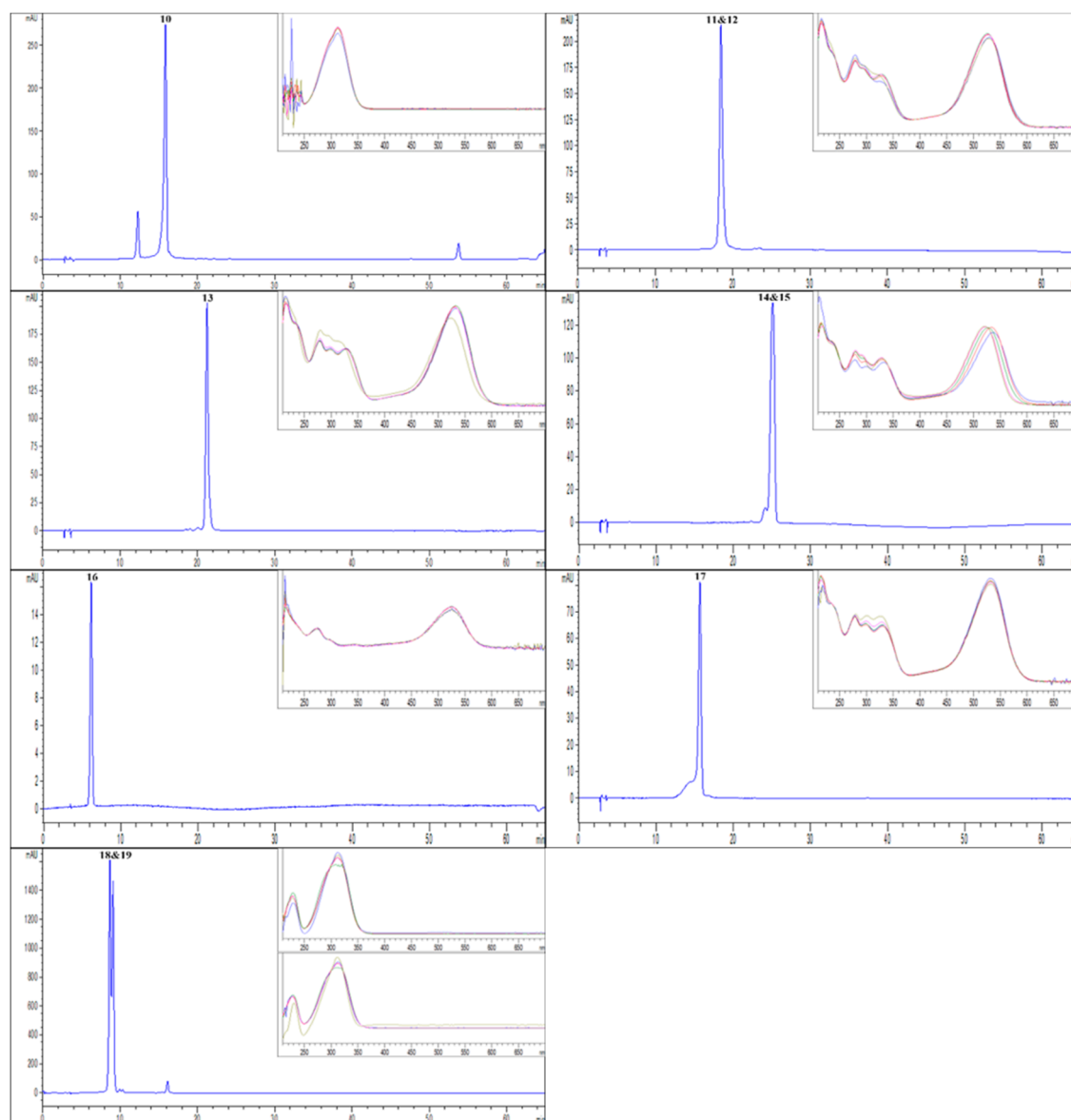




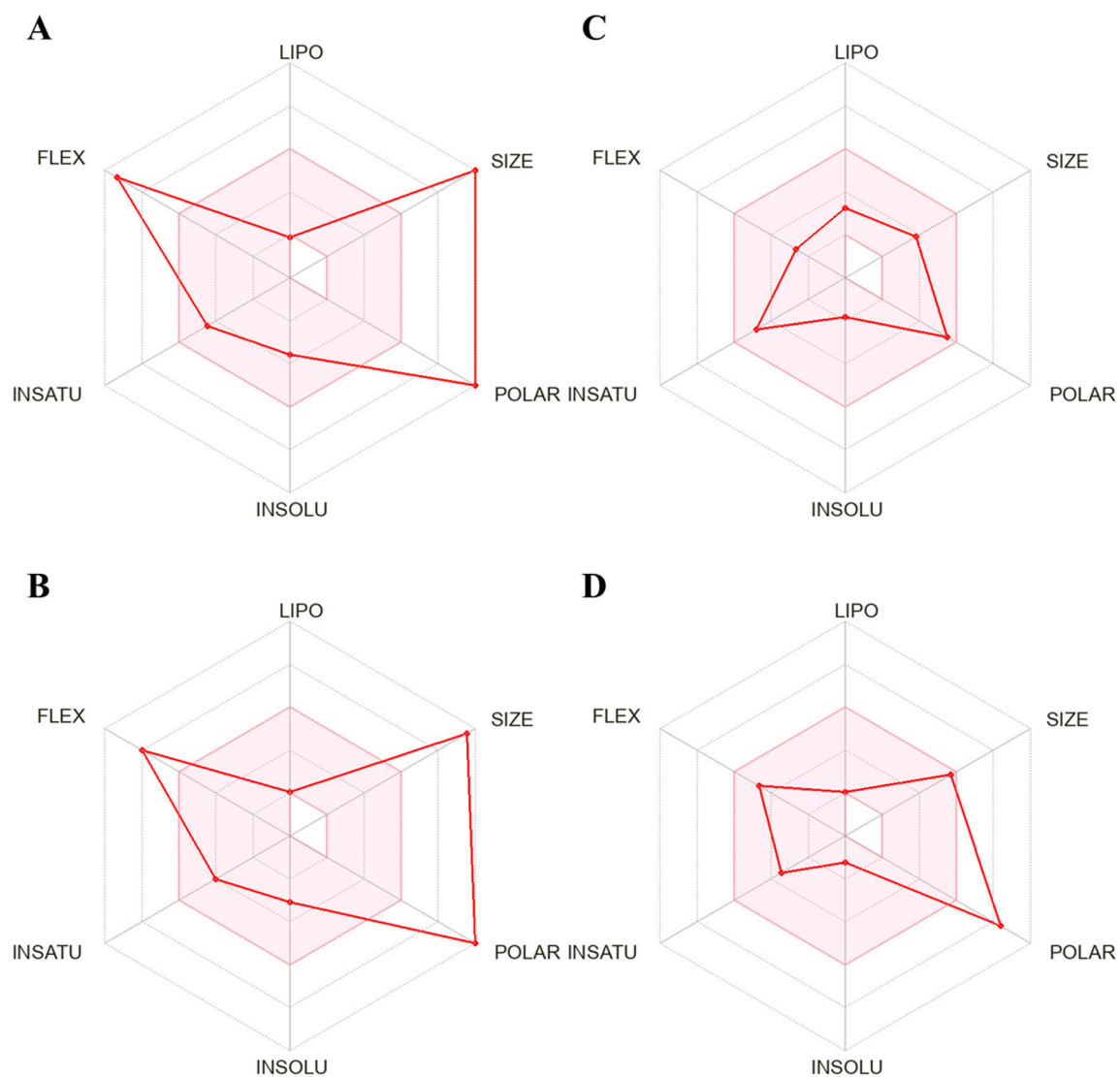
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**Figure S16.** HPLC chromatography and spectrogram of **1-9**



**Figure S17.** HPLC chromatography and spectrogram of 10~19



**Figure S18.** ADMET of (A) petanin, (B) norpetanin, (C) 4-O-(p-coumaryl) rhamnose, (D)

Lyciruthenylpropanoid D/E

**Table S1.** Retention time, purity and  $\lambda_{\text{max}}$  of anthocyanins and their degradation products by HPLC

<b>Compound</b>	<b>Retention time</b>	<b>Peak purity</b>	<b><math>\lambda_{\text{max}}</math></b>
<b>No.</b>	<b>(min)</b>	<b>(%)</b>	<b>(nm)</b>
<b>1</b>	4.731	92.429	272, 528
<b>2</b>	19.659	99.433	218, 280, 302, 531
<b>3</b>	27.072	100.000	218, 281, 300, 535
<b>4&amp;5</b>	24.097	92.654	220, 280, 292, 311, 520
<b>6</b>	14.379	91.233	223, 318, 531
<b>7</b>	15.564	99.421	228, 280, 300, 310, 530
<b>8</b>	3.719	97.602	278, 300, 342, 525
<b>9</b>	15.863	94.839	221, 260, 317
<b>10</b>	15.854	85.905	313
<b>11&amp;12</b>	18.471	97.557	218, 280, 298, 330, 529
<b>13</b>	21.241	95.082	218, 280, 300, 330, 530
<b>14&amp;15</b>	25.088	94.316	218, 280, 298, 330, 521
<b>16</b>	6.169	100.000	273, 300, 341, 528
<b>17</b>	15.679	95.536	218, 280, 300, 330, 531
<b>18&amp;19</b>	8.671/ 9.082	50.182/ 45.092	230, 311

**Table S2.** Tyrosinase inhibitory activity of anthocyanins and their degradation products (0.60 mM)

NO.	Compound name	Molecular weight	Inhibitory ratio at 0.60 mM (%)
1	Petunidin 3-O-rutinoside-5-O-glucoside	787.2291	83.05 ± 2.76
2	<b>Petanin</b>	<b>933.2659</b>	93.25 ± 0.95
3	Petunidin 3-O-trans-p-coumaroylrutinoside	771.2131	64.46 ± 3.09
4/ 5	Peonidin 3-O-trans-p-coumaroylrutinoside-5-O-glucoside	917.2710	66.74 ± 2.79
	Malvidin 3-O-trans-p-coumaroylrutinoside-5-O-glucoside	947.2816	
6	Petunidin 3-O-trans-caffeoylrutinoside-5-O-glucoside	949.2608	87.01 ± 2.59
7	Delphinidin 3-O-trans-p-coumaroylrutinoside-5-O-glucoside	919.2503	79.79 ± 2.35
8	Delphinidin 3-O-rutinoside-5-O-glucoside	773.2135	86.47 ± 1.75
9	<b>Norpetanin</b>	<b>810.2219</b>	94.31 ± 2.36
10	<b>4-O-(p-coumaryl) rhamnose</b>	<b>310.1053</b>	73.56 ± 0.90
11/ 12	Malvidin 3-O-trans-caffeoylrutinoside-5-O-glucoside	963.2765	47.01 ± 3.19
	Peonidin 3-O-trans-caffeoylrutinoside-5-O-glucoside	933.2659	
13	Petunidin 3-O-feruloylrutinoside-5-O-glucoside	963.2765	48.83 ± 2.59
14/ 15	Malvidin 3-O-feruloylrutinoside-5-O-glucoside	977.2921	67.29 ± 2.85
	Peonidin 3-O-feruloylrutinoside-5-O-glucoside	947.2816	
16	Petunidin 3,5-di-O-glucoside	641.1712	90.40 ± 1.58
17	Peonidin 3-O-rutinoside-5-O-glucoside	771.2342	57.79 ± 2.81
18&19	<b>Lyciruthenylpropanoid D</b>	<b>472.1581</b>	90.30 ± 4.79
	<b>Lyciruthenylpropanoid E</b>		

**Table S3.** Maximum tolerated concentration of Petanin in zebrafish (n=30)

<b>Group</b>	<b>Content (%)</b>	<b>Death count (Tail)</b>	<b>Death rate (%)</b>	<b>Phenotype</b>
<b>Normal</b>	/	0	0	No obvious abnormality
	0.002	0	0	ns
	0.02	0	0	ns
	0.05	0	0	ns
	0.1	0	0	ns
<b>Petanin</b>	<b>0.15</b>	<b>1</b>	<b>3</b>	<b>ns</b>
	0.20	6	20	/
	0.25	12	40	/
	0.5	30	100	/

Note: ns means that there is no significant difference compared with the normal group.