

## Supplementary Information

### Inhibitory Effect of *Trans*-tiliroside on Very Low Density Lipoprotein Secretion from HepG2 Cells and Mouse Liver

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**Table S1.** Effects of mevalonate on CHO secretion and cell viability in HepG2 cells

| Treatment  | Concentration<br>(mM) | CHO / protein<br>(% of control) | Cell viability<br>(%) |
|------------|-----------------------|---------------------------------|-----------------------|
| Control    | –                     | 100.0 ± 5.0                     | 100.0 ± 0.6           |
| Mevalonate | 1                     | 99.2 ± 3.7                      | 108.7 ± 3.7           |
|            | 5                     | 100.5 ± 4.5                     | 108.5 ± 5.0           |
|            | 10                    | 118.2 ± 8.3                     | 99.9 ± 4.5            |
|            | 20                    | 147.8 ± 5.7**                   | 88.9 ± 5.4            |
|            | 50                    | 119.0 ± 2.9                     | 28.7 ± 0.5**          |

Each value represents the mean ± S.E.M. ( $n = 4$ ). Significantly different from the control,

\*\*  $p < 0.01$  (Dunnett's test).

**Table S2.** Effects of *trans*-tiliroside (**1**) and flavonoids (**2–4**) on CHO secretion in HepG2 cells

| Treatment                             | Concentration<br>( $\mu$ M) | Mevalonate<br>(20 mM) | CHO / protein<br>(% of control) | Protein concentration<br>(% of control) |
|---------------------------------------|-----------------------------|-----------------------|---------------------------------|-----------------------------------------|
| Normal                                | –                           | –                     | 77.2 ± 1.4**                    | 103.0 ± 2.5                             |
| Control                               | –                           | +                     | 100.0 ± 2.3                     | 100.0 ± 1.0                             |
| <i>Trans</i> -tiliroside ( <b>1</b> ) | 10                          | +                     | 80.8 ± 1.7**                    | 105.1 ± 2.1                             |
|                                       | 30                          | +                     | 64.9 ± 0.5**                    | 107.3 ± 0.9*                            |
|                                       | 100                         | +                     | 27.7 ± 1.0**                    | 110.6 ± 1.7**                           |
|                                       | 100                         | –                     | 32.4 ± 1.4**                    | 109.9 ± 1.5**                           |
| Normal                                | –                           | –                     | 74.6 ± 0.3**                    | 106.0 ± 0.3                             |
| Control                               | –                           | +                     | 100.0 ± 1.8                     | 100.0 ± 1.4                             |
| Nobiletin ( <b>2</b> )                | 3                           | +                     | 102.1 ± 1.5                     | 101.9 ± 1.0                             |
|                                       | 10                          | +                     | 95.9 ± 2.6                      | 103.9 ± 1.6                             |
|                                       | 30                          | +                     | 73.4 ± 2.3**                    | 104.7 ± 3.6                             |
|                                       | 30                          | –                     | 60.0 ± 0.6**                    | 110.7 ± 0.5**                           |
| Normal                                | –                           | –                     | 76.7 ± 1.8**                    | 109.2 ± 3.5*                            |
| Control                               | –                           | +                     | 100.0 ± 1.3                     | 100.0 ± 1.3                             |
| Hesperidin ( <b>3</b> )               | 30                          | +                     | 94.1 ± 1.4                      | 103.2 ± 0.9                             |
|                                       | 100                         | +                     | 92.9 ± 3.5                      | 102.6 ± 2.2                             |
| Hesperetin ( <b>4</b> )               | 30                          | +                     | 90.4 ± 2.2*                     | 112.4 ± 2.1**                           |
|                                       | 100                         | +                     | 89.6 ± 1.5**                    | 109.7 ± 2.1**                           |

Each value represents the mean ± S.E.M. ( $n = 4$ ). Significantly different from the control, \*  $p < 0.05$ , \*\*  $p < 0.01$  (Dunnett's test).

**Table S3.** Effects of compounds **1** and **2** on HepG2 cell viability

| Treatment                             | Cell viability (%) |             |             |             |             |              |              |
|---------------------------------------|--------------------|-------------|-------------|-------------|-------------|--------------|--------------|
|                                       | Vehicle            | 1 $\mu$ M   | 3 $\mu$ M   | 10 $\mu$ M  | 30 $\mu$ M  | 100 $\mu$ M  | 300 $\mu$ M  |
| <i>Trans</i> -tiliroside ( <b>1</b> ) | 100.0 ± 2.7        | –           | 106.0 ± 2.2 | 109.6 ± 0.9 | 104.2 ± 2.7 | 101.2 ± 2.7  | 74.9 ± 5.8** |
| Nobiletin ( <b>2</b> )                | 100.0 ± 1.7        | 108.1 ± 3.6 | 106.6 ± 2.3 | 98.9 ± 3.1  | 90.0 ± 5.9  | 72.9 ± 3.7** | –            |

Each value represents the mean ± S.E.M. ( $n = 4$ ). Significantly different from the vehicle, \*\*  $p < 0.01$  (Dunnett's test).

**Table S4.** Effects of compounds **1** and **2** on apoB-100 secretion in HepG2 cells

| Treatment                             | Concentration<br>( $\mu$ M) | Mevalonate<br>(20 mM) | ApoB-100 / protein<br>(% of control) |
|---------------------------------------|-----------------------------|-----------------------|--------------------------------------|
| Normal                                | –                           | –                     | 97.3 $\pm$ 3.4                       |
| Control                               | –                           | +                     | 100.0 $\pm$ 3.1                      |
| <i>Trans</i> -tiliroside ( <b>1</b> ) | 30                          | +                     | 96.1 $\pm$ 4.9                       |
|                                       | 100                         | +                     | 82.9 $\pm$ 3.4*                      |
| Nobiletin ( <b>2</b> )                | 10                          | +                     | 74.2 $\pm$ 3.5**                     |
|                                       | 30                          | +                     | 18.9 $\pm$ 1.2**                     |

Each value represents the mean  $\pm$  S.E.M. ( $n = 4$ ). Significantly different from the control,

\*  $p < 0.05$ , \*\*  $p < 0.01$  (Dunnett's test).

**Table S5.** Effects of compounds **1** and **2** on plasma TG levels in Triton WR-1339-treated mice

| Treatment                             | Dose<br>(mg/kg) | Plasma TG (mg/dL) |                 |                  |                    | AUC <sub>0-4</sub><br>(mg·h/dL) |
|---------------------------------------|-----------------|-------------------|-----------------|------------------|--------------------|---------------------------------|
|                                       |                 | 0 h               | 1 h             | 2 h              | 4 h                |                                 |
| Control                               | –               | 123.0 $\pm$ 12.6  | 112.4 $\pm$ 8.4 | 257.2 $\pm$ 32.0 | 1187.7 $\pm$ 95.1  | 1747.5 $\pm$ 141.2              |
| <i>Trans</i> -tiliroside ( <b>1</b> ) | 50              | 138.6 $\pm$ 7.7   | 119.5 $\pm$ 7.0 | 229.2 $\pm$ 15.9 | 794.6 $\pm$ 114.2* | 1327.1 $\pm$ 133.4 <sup>#</sup> |
| Nobiletin ( <b>2</b> )                | 50              | 121.4 $\pm$ 8.9   | 115.4 $\pm$ 8.4 | 272.4 $\pm$ 25.8 | 1132.6 $\pm$ 111.7 | 1717.2 $\pm$ 155.6              |

Each value represents the mean  $\pm$  S.E.M. ( $n = 10$ – $11$ ). Significantly different from the control, \*  $p < 0.05$ , <sup>#</sup>  $p = 0.084$  (Dunnett's test).

**Table S6.** Plasma apoB-100 concentration at 4 h after Triton WR-1339 injection

| Treatment                             | Dose<br>(mg/kg) | Plasma apoB-100<br>(mg/mL) |
|---------------------------------------|-----------------|----------------------------|
| Control                               | –               | 0.56 $\pm$ 0.01            |
| <i>Trans</i> -tiliroside ( <b>1</b> ) | 50              | 0.50 $\pm$ 0.02**          |
| Nobiletin ( <b>2</b> )                | 50              | 0.54 $\pm$ 0.01            |

Each value represents the mean  $\pm$  S.E.M. ( $n = 10$ – $11$ ). Significantly different from the control,

\*\*  $p < 0.01$  (Dunnett's test).

**Table S7.** Effects of administration of compound **1** and **2** on body weight in mice

| Treatment                             | Dose<br>(mg/kg) | Body weight (g) |                |                |                |                |                |                |
|---------------------------------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                                       |                 | Day 1           | Day 2          | Day 3          | Day 4          | Day 5          | Day 6          | Day 7          |
| Control                               | –               | 43.9 $\pm$ 0.2  | 44.2 $\pm$ 0.3 | 43.5 $\pm$ 0.3 | 43.7 $\pm$ 0.4 | 43.8 $\pm$ 0.3 | 43.9 $\pm$ 0.4 | 39.2 $\pm$ 0.3 |
| <i>Trans</i> -tiliroside ( <b>1</b> ) | 50              | 43.6 $\pm$ 0.3  | 44.2 $\pm$ 0.2 | 43.5 $\pm$ 0.3 | 44.1 $\pm$ 0.4 | 44.0 $\pm$ 0.4 | 43.9 $\pm$ 0.4 | 39.5 $\pm$ 0.5 |
| Nobiletin ( <b>2</b> )                | 50              | 43.7 $\pm$ 0.3  | 44.3 $\pm$ 0.4 | 43.4 $\pm$ 0.4 | 44.0 $\pm$ 0.5 | 44.3 $\pm$ 0.5 | 44.5 $\pm$ 0.5 | 39.5 $\pm$ 0.5 |

Each value represents the mean  $\pm$  S.E.M. ( $n = 10$ – $11$ ). No significantly difference was observed. Mice were fasted for 18 h after sample administration at Day 6.

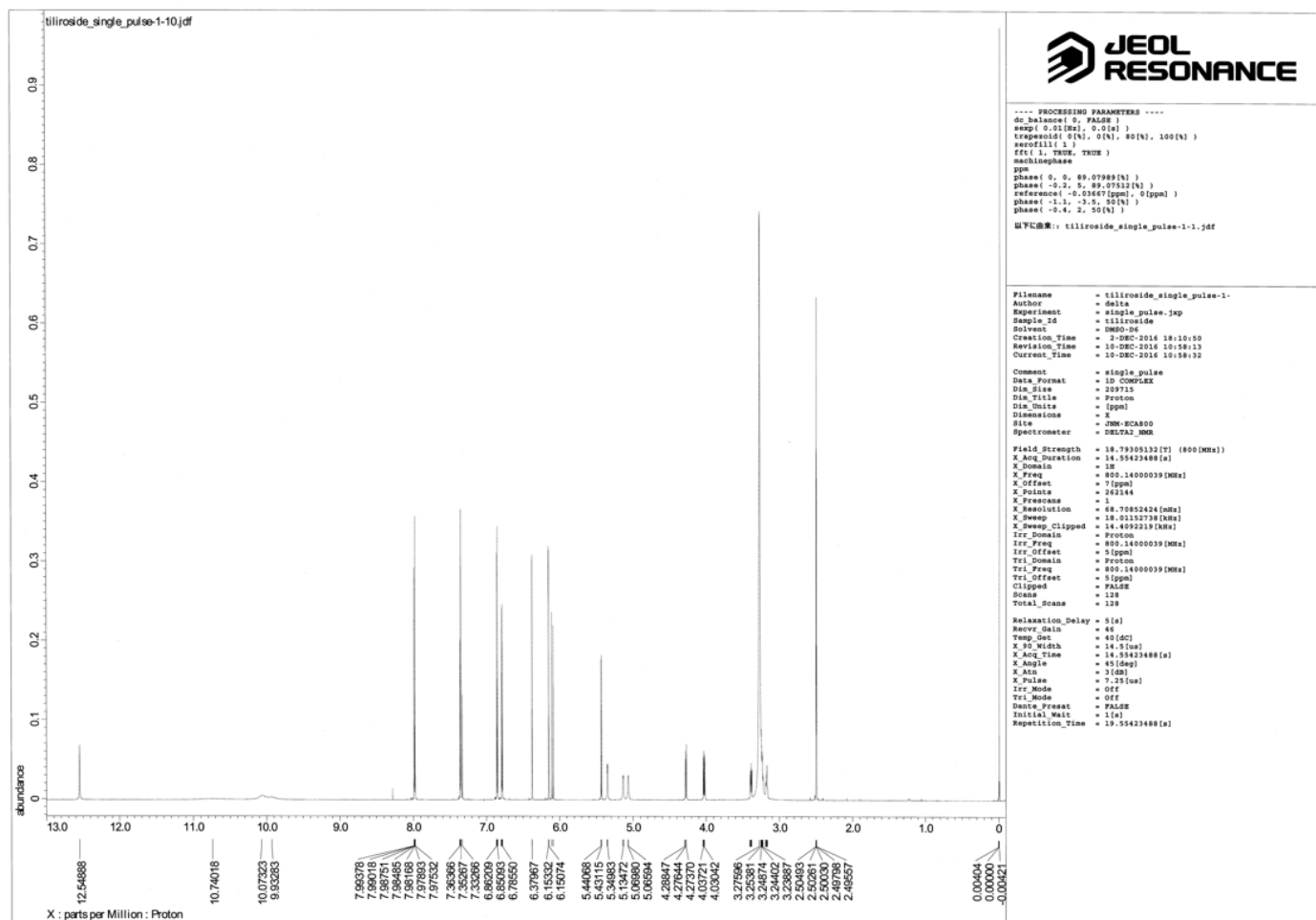
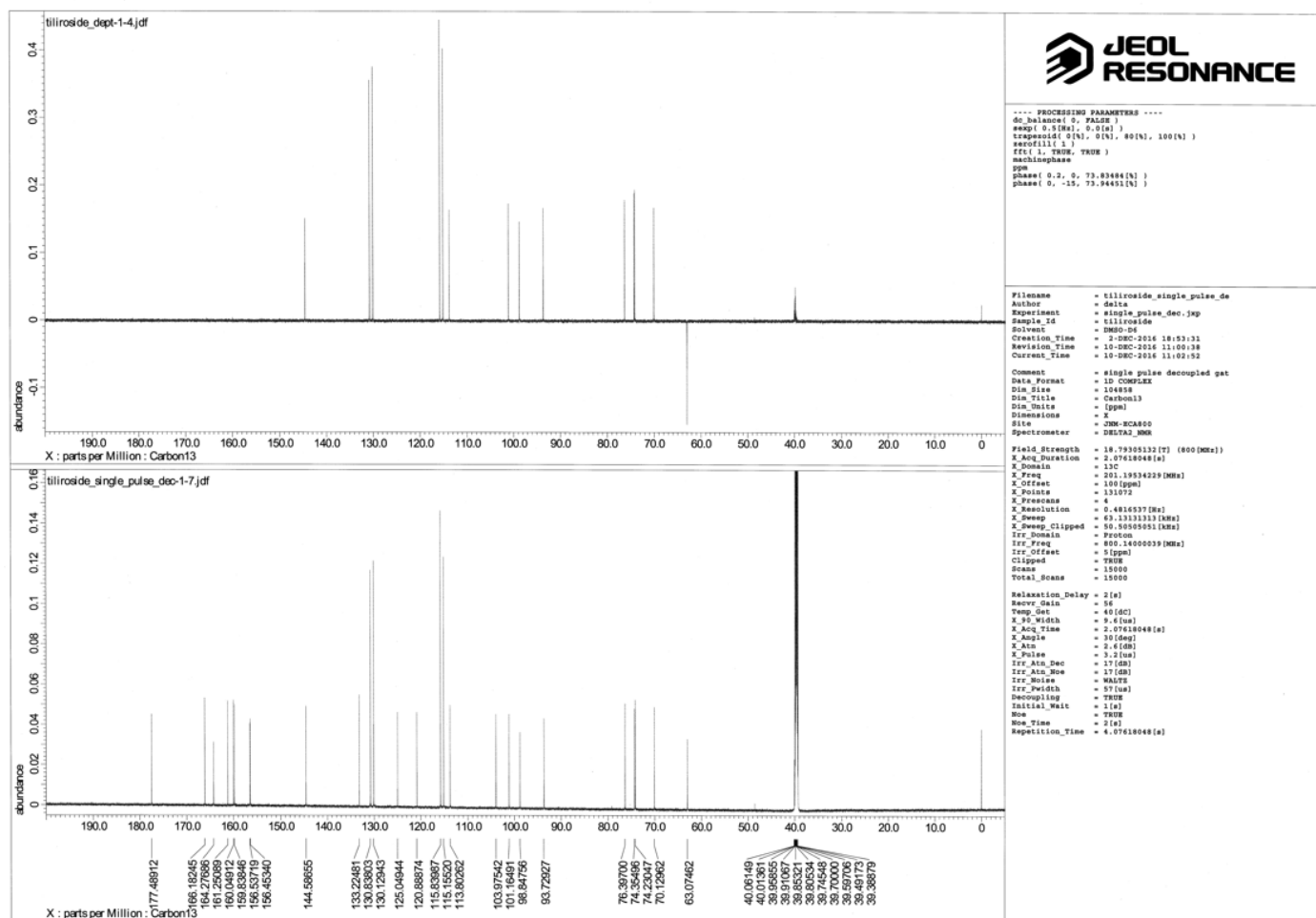


Figure S1.  $^1\text{H}$ -NMR (800 MHz,  $\text{DMSO}-d_6$ ) spectrum of compound 1



**Figure S2.**  $^{13}\text{C}$ -NMR (200 MHz,  $\text{DMSO}-d_6$ ) spectrum of compound **1**