*Supporting Information*

# A Sterol from Soft Coral Induces Apoptosis and Autophagy in MCF-7 Breast Cancer Cells

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|---|---|---|
| S3 | Expression of p62 and LC3B in compound **1**-treated MDA-MB-231 breast cancer cells for 48 h. | 5 |
| S4 | Left, Western blot analyses of LC3B in compound **2** or compound **3** or 50 μM troglitazone (TRO) for 48 h in MCF-7 cells. Three independent experiments were performed, and the fold changes of LC3B-II/β-actin is presented in right panel. Points, means; bars, S.D. (n = 3). | 6 |

**Supplementary materials and methods**

*Inhibition effects of compound 1 relative to DMSO control on the cell proliferation in MDA-MB-231 cells.*

Cells (5x103) were treated with compound 1 or DMSO in 5% FBS-supplemented DMEM/F12 medium in 96-well plates. After 24 h, the medium was removed, replaced by 200 μL DMEM/F12 containing 0.5 mg/mL of MTT, and cells were incubated in the CO2 incubator at 37°C for 2 h. Supernatants were aspirated from the wells, and the reduced MTT dye was solubilized in 200 μL/well DMSO. Absorbance at 570 nm was measured using a plate reader.

*Cell cycle analysis of compound 1 in MDA-MB-231 cells*

Cells (2x105/3 mL) were treated with DMSO or compound 1 for 48 h and washed twice with PBS, followed by staining with propidium iodide (PI) and analyzed by flow cytometry and the multicycler (ModFitLT 3.0) software program.

*Western blot analysis*

Western blot analysis was performed as previously report (Weng et. al, (2017) Phytother. Res., 31, 1722-1730).

**Figure S1**

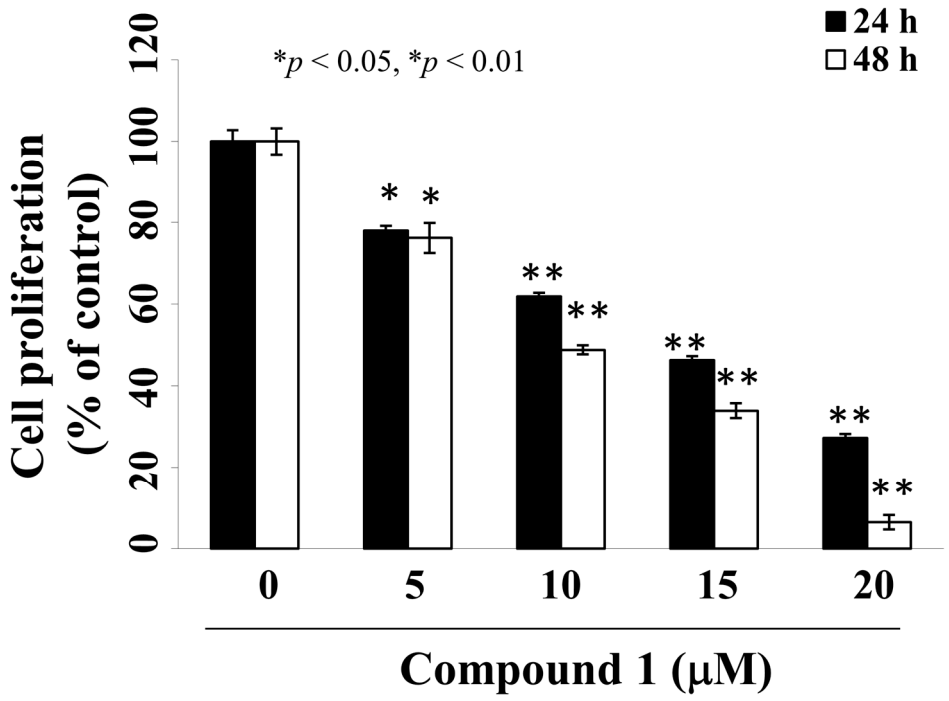

24 h
48 h
*p < 0.05, *p < 0.01
Cell proliferation (% of control)
0
20
40
60
80
100
120
* *
**
**
**
**
**
**
0
5
10
15
20
Compound 1 (μM)

**Figure S2**

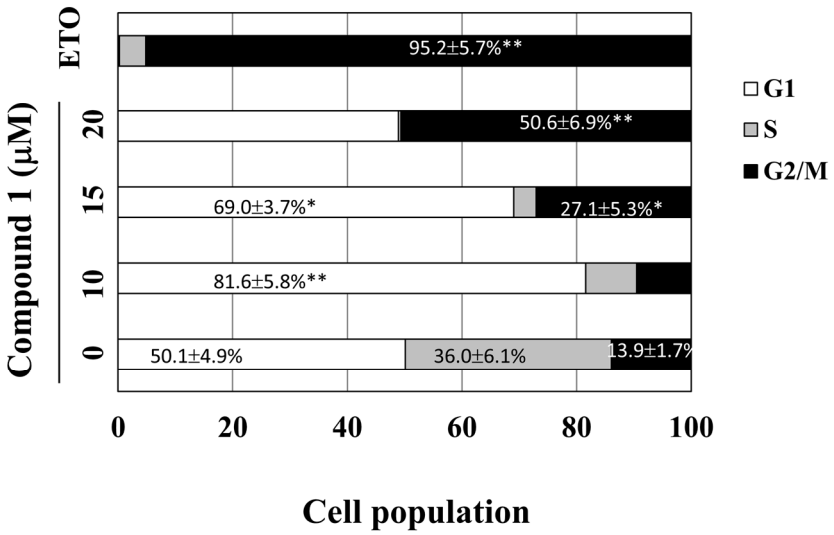

Compound 1 (μM)
ETO
20
15
10
0
95.2±5.7%**
50.6±6.9%**
69.0±3.7%*
27.1±5.3%*
81.6±5.8%**
50.1±4.9%
36.0±6.1%
13.9±1.7%
G1
S
G2/M
0
20
40
60
80
100
Cell population

**Figure S3**

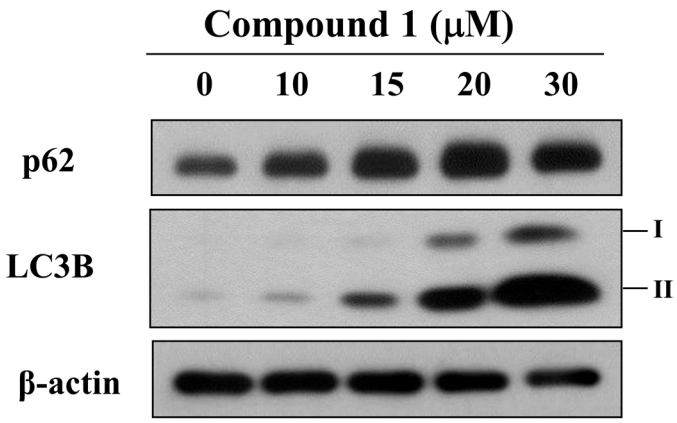
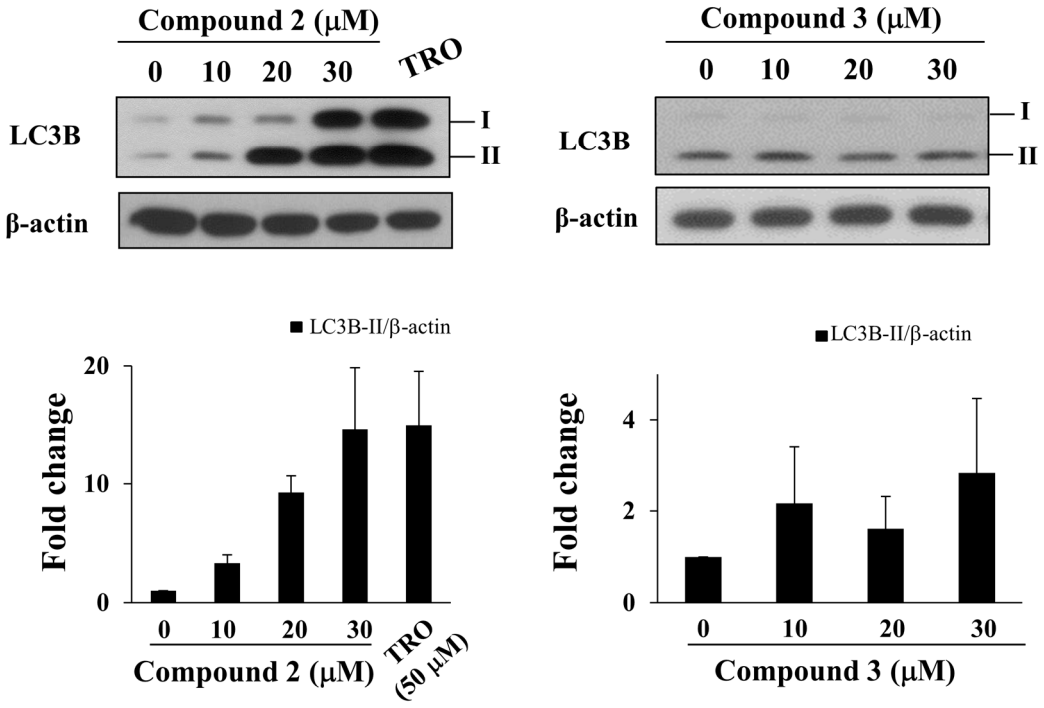


**Figure S4**


Compound 2 (μM)
0
10
20
30
TRO
LC3B
I
II
β-actin
Compound 3 (μM)
0
10
20
30
LC3B
I
II
β-actin
LC3B-II/β-actin
Fold change
20
10
0
0
10
20
30
TRO (50 μM)
Compound 2 (μM)
LC3B-II/β-actin
Fold change
4
2
0
0
10
20
30
Compound 3 (μM)