

Supplementary data

Supplementary experimental procedure

Cell death rate

Caco-2 cells were plated at a density of 1×10^4 cells in a 96-well culture plate at 37°C in 5% CO₂. Heat-treated OLL2712 were added at 100 µg/mL and cultured for 24 h. The cells were washed twice with Hanks' balanced salt solution, and stained with propidium iodide (PI) and Hoechst33342 for 30 min at 37°C. The cells were examined under a fluorescence microscope (Keyence, BZ-X810). PI and Hoechst 33342 were detected using TRITC (excitation: 545/25 nm, emission: 605/70 nm, Keyence) and DAPI filters (excitation: 360/40 nm, emission: 460/50 nm, Keyence), respectively. The cell death rate was calculated as the percentage of PI-positive cells among all cells.

Lactate dehydrogenase (LDH) assay

Cell viability was assessed by measuring the release of LDH using Cytotoxicity LDH Assay Kit-WST (Dojindo Laboratories) according to the manufacturer's instructions.

Table S1. Primer sequences in real-time polymerase chain reaction (PCR) used in this study

<i>Gene</i>	Sequences (5'→3')
<i>GAPDH</i>	Takara, HA067812
<i>CLDN1</i> [56]	Forward GTGCGATATTTCTTCTTGCAGG
	Reverse TTCGTACCTGGCATTGACTGG
<i>F11R</i>	Forward ACCTTCTTGCCAACTGGTATCA
	Reverse AGCACGATGAGCTTGACCTTG
<i>MYD88</i>	Forward GGCTGCTCTCAACATGCGA
	Reverse CTGTGTCCGCACGTTCAAGA
<i>OCN1</i> [57]	Forward TCAGGGAATATCCACCTATCACTTCAG
	Reverse CATCAGCAGCAGCCATGTACTCTTCAC
<i>TJPI</i>	Forward ACCAGTAAGTCGTCCTGATCC
	Reverse TCGGCCAAATCTTCTCACTCC
<i>CLDN4</i> [56]	Forward GGCTGCTTTGCTGCAACTGTC
	Reverse GAGCCGTGGCACCTTACACG

Not referenced PCR primer sequences were obtained from Primerbank

(<https://pga.mgh.harvard.edu/primerbank/>) [58].

Supplementary References

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57. Braniste, V.; Leveque, M.; Buisson-Brenac, C.; Bueno, L.; Fioramonti, J.; Houdeau, E. Oestradiol decreases colonic permeability through oestrogen receptor beta-mediated up-regulation of occludin and junctional adhesion molecule-A in epithelial cells. *J. Physiol.* 2009, 587, 3317–3328. <https://doi.org/10.1113/jphysiol.2009.169300>.
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