

Supplementary information

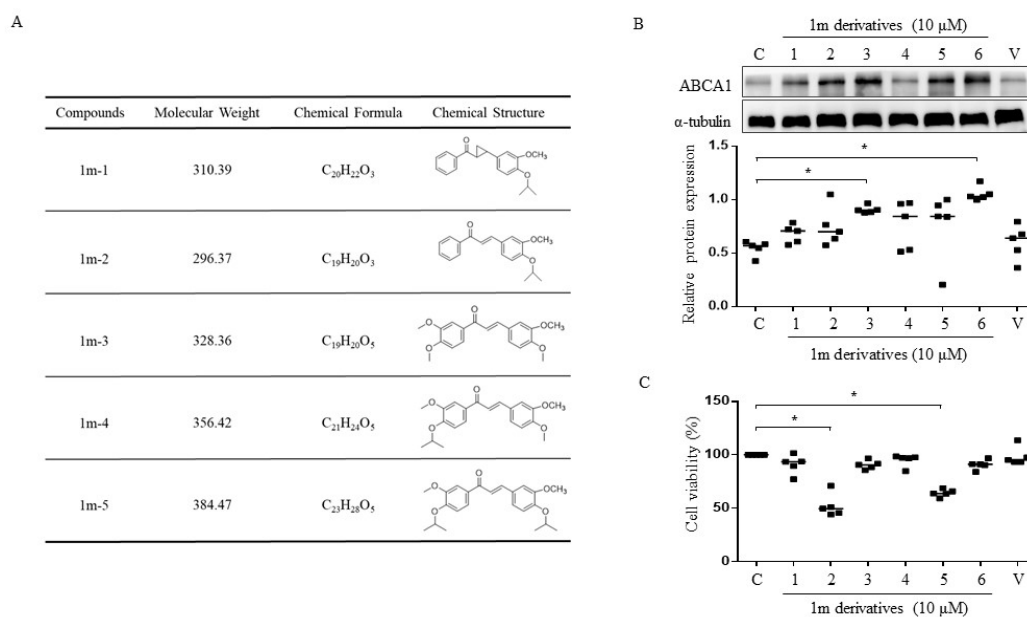
A chalcone derivative, 1m-6, exhibits atheroprotective effects by increasing cholesterol efflux and reducing inflammation-induced endothelial dysfunction

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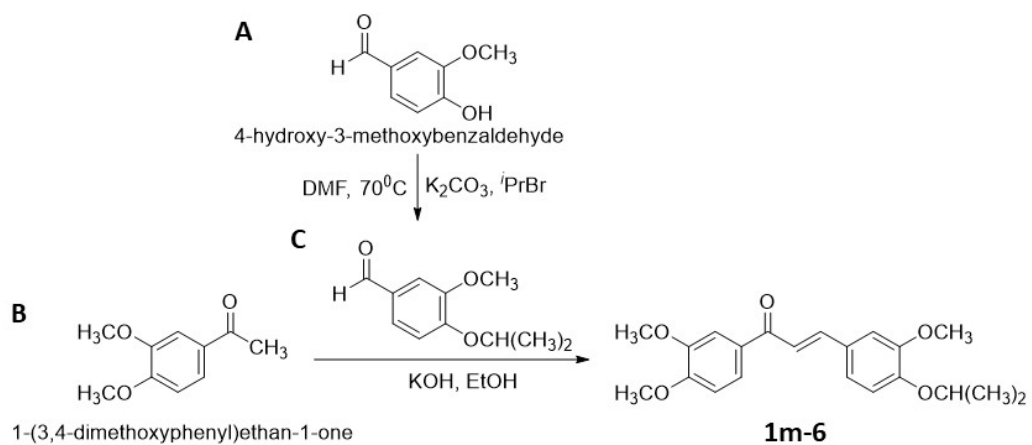
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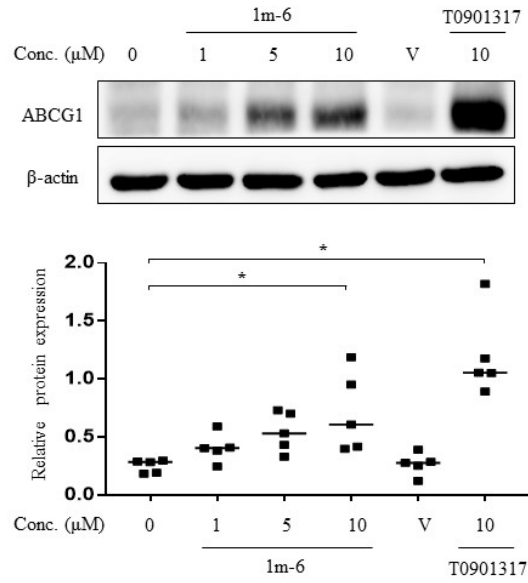
Supplementary Figures and Tables:



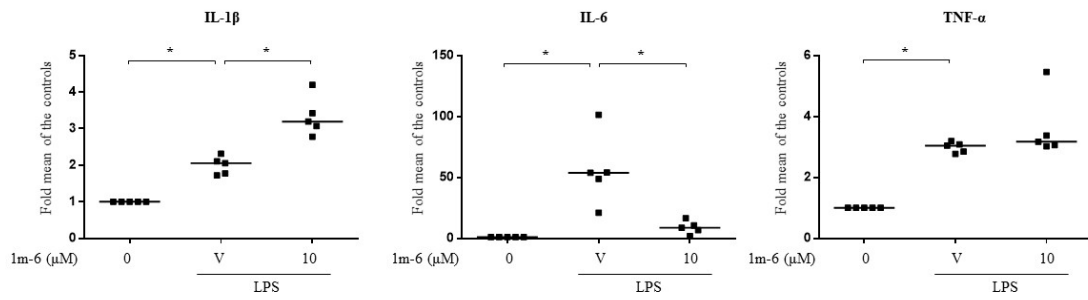
Supplementary Fig 1. Effects of 1m derivatives on ABCA1 expression in human THP-1 macrophages. (A) List of 1m derivatives. (B) Human THP-1 macrophages were treated with 1m derivatives or DMSO for 24 h. Cell lysates were collected and analyzed by western blot assay. Representative data and quantitative results expressed as the median with individual data of five independent experiments are shown. (C) Human THP-1 macrophages were treated with 1m derivatives or DMSO for 24 h and then incubated with MTT for another 6 h. Cell viability was measured using an ELISA reader. The results from five independent experiments are presented as the median with individual data. C indicates cells with no treatment. V indicates the vehicle (DMSO) control. The numbers, including 1 to 6, indicate 1m-1 to 1m-6. Significance is presented as * $p < 0.05$ versus the indicated group.



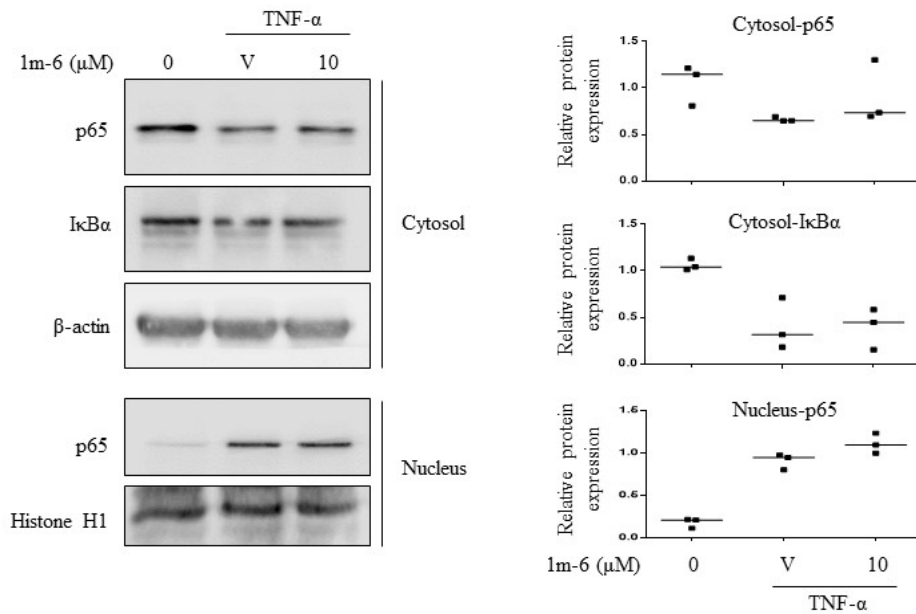
Supplementary Fig 2. The synthesis of 1m-6.



Supplementary Fig 3. Effects of 1m-6 on ABCG1 expression in human THP-1 macrophages. Human THP-1 macrophages were treated with 1m-6 (1, 5, and 10 μ M), T0901317 (10 μ M) or DMSO for 24 h. Cell lysates were collected and analyzed by western blot. Representative data and quantitative results of five independent experiments expressed as the median with individual data are shown. Conc. indicates concentration. V indicates the vehicle (DMSO) control. Significance is presented as * $p < 0.05$ versus the indicated group.



Supplementary Fig 4. Effects of 1m-6 on lipopolysaccharides (LPS)-induced cytokine production in human THP-1 macrophages. Human THP-1 macrophages were pre-treated with 10 μ M 1m-6 or DMSO for 2 h, and then stimulated with LPS (100 ng/ml) for 24 h. Cellular RNA was collected for qRT-PCR analysis. Data are shown as the median with individual data of five independent experiments. V indicates the vehicle (DMSO) control. Significance is presented as * $p < 0.05$ versus the indicated group.



Supplementary Fig 5. 1m-6 does not inhibit TNF- α -activated NF- κ B signaling in HUVECs. HUVECs were pretreated with 10 μ M 1m-6 or DMSO for 2 h and further stimulated with 10 ng/ml TNF- α for 30 minutes. The cytosol and nucleus cell lysates were collected using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific; Rockford, IL, USA) and analyzed by western blot. Representative data and quantitative results expressed as the median with individual data of three independent experiments are shown. V indicates the vehicle (DMSO) control. Significance is presented as * $p < 0.05$ versus the indicated group.

Supplementary Table 1. The primer sequences used for the determination of mRNA expression in the real-time PCR experiments

Gene	Accession number	Forward primer (5'-3')	Reverse primer (5'-3')
ABCA1	NM_005502.3	TGG CAG TGT CCA GCA TCT AA	GTA TTG TAG CAT GTT GGC GTG T
ABCG1	NM_207629.1	CGG GGA AAA GTC TGC AAT	GGT GCC AAA GAA AAG GGT
LXR α	NM_005693.3	AAG CCC TGC ATG CCT ACG T	TGC AGA CGC AGT GCA AAC A
SREBP1	NM_001005291.3	CCA TGG ATT GCA CTT TCG AA	GGC CAG GGA AGT CAC TGT CT
MCP-1	NM_002982.3	TGC AGA GGC TCG CGA GCT A	CAG GTG GTC CAT GGA ATC CTG A
RANTES	NM_002985.3	CTC CCC ATA TTC CTC GGA CA	GTT GAT GTA CTC CCG AAC CC
HO-1	NM_002133.3	GGG TGA TAG AAG AGG CCA AGA	AGC TCC TGC AAC TCC TCA AA
VCAM-1	NM_080682.2	CCG GAT TGC TGC TCA GAT TGG A	AGC GTG GAA TTG GTC CCC TCA
ICAM-1	NM_001544.5	GGC CTC AGT CAG TGT GA	AAC CCC ATT CAG CGT CA
IL-1 β	NM_000576.3	CAC GAT GCA CCT GTA CGA TCA	GTT GCT CCA TAT CCT GTC CCT
IL-6	NM_000600.5	CCA GGA GCC CAG CTA TGA AC	CCC AGG GAG AAG GCA ACT G
IL-8	NM_000584.4	TTG GCA GCC TTC CTG ATT TC	TCT TTA GCA CTC CTT GGC AAA AC
TNF- α	NM_000594.4	CCC ATG TTG TAG CAA ACC CTC	TAT CTC TCA GCT CCA CGC CA
STAT3	NM_139276.2	CTG CCC CAT ACC TGA AGA CC	AGG TGA GGG ACT CAA ACT GC
GAPDH	NM_001289745.2	ATG GGG AAG GTG AAG GTC G	TAA AAG CAG CCC TGG TGA CC

Supplementary Table 2. The primer sequences used for determination of miRNA expression in the real-time PCR experiments

Gene	Accession number	Primer (5'-3')
RNU6-2	NR_125730.1	ACG CAA ATT CGT GAA GCG TT
miR-10b	NR_029609.1	TAC CCT GTA GAA CCG AAT TTG T
miR-27a	NR_029501.1	TTC ACA GTG GCT AAG TTC CGC
miR-33	NR_029507.1	CAA TGT TTC CAC AGT GCA TCA C
miR-106b	NR_029831.1	CCG CAC TGT GGG TAC TTG CTG C
miR-128	NR_029824.1	GGG GGC CGA TAC ACT GTA CGA GA
miR-148a	NR_029597.1	TCA GTG CAC TAC AGA ACT TTG T
miR-145	NR_029686.1	GGA TTC CTG GAA ATA CTG TTC T
miR-155	NR_030784.1	CTC CTA CAT ATT AGC ATT AAC A
miR-206	NR_029713.1	TGG AAT GTA AGG AAG TGT GTG G
miR-758	NR_030406.1	TTT GTG ACC TGG TCC ACT AAC C