Molecular Pharmacology

Supplementary Information

5-Cholesten-3β,25-diol 3-sulfate Decreases Lipid Accumulation in Diet-induced Nonalcoholic Fatty Liver Disease Mouse Model

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Supplementary Figure 1. Mass spectral analysis of 25HC3S.

Characterization of the chemically synthesized and purified 5-cholesten-3β,25-diol 3-sulfonate was analyzed by negative ion-triple quadrapole mass spectrometer (Peking University, China), which shows the same molecular mass ion, *m/z* 481 as the "authentic" nuclear oxysterol and the purified product was not contaminated by the starting material, 25-hydroxycholesterol, *m/z* 401.

Supplementary Figure 2. Proton nuclear magnetic resonance spectroscopy of 25HC3S.

1H NMR analysis shows that the proton resonance at C3 with multiple small (1.5 Hz) splits near 3.65 ppm in the spectrum of 25-hydroxycholesterol (starting material) is shifted to 4.20 ppm in the product spectrum, which confirms that a HSO3− group is added at the C3 position of 25-hydroxycholesterol. The results indicate that the synthesized molecule is 5-cholesten-3β,25-diol 3-sulfonate (25HC3S).

Supplementary Table. 1. Primer sets for real time RT-PCR analysis of gene expression

Name	GenBank No.	Forward Sequence	Reverse Sequence
SREBP-1c	NM_011480	AGCAGCCCCTAGAACAAACAC	CAGCAGTGAGTCTGCCTTGAT
ACC1	NM_133360	ATGGGCGGAATGGTCTCTTTC	TGGGGACCTTGTCTTCATCAT
FAS	NM_007988	AGAGATCCCGAGACGCTTCT	GCCTGGTAGGCATTCTGTAGT
LXRα	NM_013839	GAGCCGACAGAGCTTCGTC	GCGTGCTCCCTTGATGACA
CPT1	NM_013495	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
PPARα	NM_011144	AGAGCCCCATCTGTCCTCTC	ACTGGTAGTCTGCAAAACCAAA
ACOX1	NM_015729	TCCAGACTTCCAACATGAGGA	CTGGGCGTAGGTGCCAATTA
MCAD	NM_007382	AGGGTTTAGTTTTGAGTTGACGG	CCCCGCTTTTGTCATATTCCG
SCAD	NM_007383	ACAGTGGATCACCCCTTTCAC	ACCCATGAGTCACCCTCTTCC
PPARγ	NM_008904	TATGGAAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
FABP1	NM_017399	CTGACACCCCCTTGATGTCC	ATGAACTTCTCCGGCAAGTAC
FATP1	NM_011977	CGCTTTCTGCGTATCGTCTG	GATGCACGGGATCGTGTCT
GPAM	NM_008149	ACAGTTGGCACAATAGACGTTT	CCTTCCATTTCAGTGTTGCAGA
MTTP	NM_008642	CTCTTGGCAGTGCTTTTTCTCT	GAGCTTGTATAGCCGCTCATT
PLTP	NM_011125	CGCAAAGGGCCACTTTTACTA	GCCCCCATCATATAAGAACCAG
SREBP-2	NM_033218	TGAAGGACTTAGTCATGGGGAC	CGCAGCTTGTGATTGACCT
HMGR	NM_008255	AGCTTGCCCGAATTGTATGTG	TCTGTTGTGAACCATGTGACTTC
ABCA1	NM_013454	AAAACCGCAGACATCCTTCAG	CATACCGAAACTCGTTCACCC
ABCG1	NM_009593	GCTCCATCGTCTGTACCATCC	TGTTCTGATCCCCGTACTCCC
ABCG5	NM_031884	ATTATGTGCATCTTAGGCAGCTC	CGTAGGAGAAGCAGTCTTGGAA
CYP7α	NM_007824	AACGGGTTGATTCCATACCTGG	GTGGACATATTTCCCCATCAGTT
CYP27α	NM_024264	GACAACCTCCTTTGGGACTTAC	GTGGTCTCTTATTGGGTACTTGC
LDLR	NM_010700	AGTGGCCCCGAATCATTGAC	CTAACTAAACACCAGACAGAGGC
SRB1	NM_016741	TTTGGAGTGGTAGTAAAAAGGGC	TGACATCAGGGACTCAGAGTAG
CD36	NM_007643	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
G6Pase	NM_008061	TCGGAGACTGGTTCAACCTC	AGGTGACAGGGAACTGCTTTAT
PCK1	NM_011044	CTGCATAACGGTCTGGACTTC	CAGCAACTGCCCGTACTCC
GCK	NM_010292	AGGAGGCCAGTGTAAAGATGT	CTCCCAGGTCTAAGGAGAGAAG
Pklr	NM_013631	TCAAGGCAGGGATGAACATTG	CACGGGTCTGTAGCTGAGTG
IL1α	NM_010554	CTGATGAAGCTCGTCAGGCAG	TGGTGCTGAGATAGTGTTTGTC
IL1β	NM_008361	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
NFκB (Rela)	NM_009045	GCGCGGGGACTATGACTTG	GCCCGGTTATCAAAAATCGGAT
TNFα	NM_013693	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
IκBα	NM_010907	TGAAGGACGAGGAGTACGAGC	TTCGTGGATGATTGCCAAGTG
GAPDH	NM_008084	CATGTTCCAGTATGACTCCACTC	GGCCTCACCCCATTTGATGT
β-actin	NM_007393	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT