Supplementary information

Dehydroeburicoic Acid, a Dual Inhibitor against Oxidative Stress in Alcoholic Liver Disease

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Table of Contents

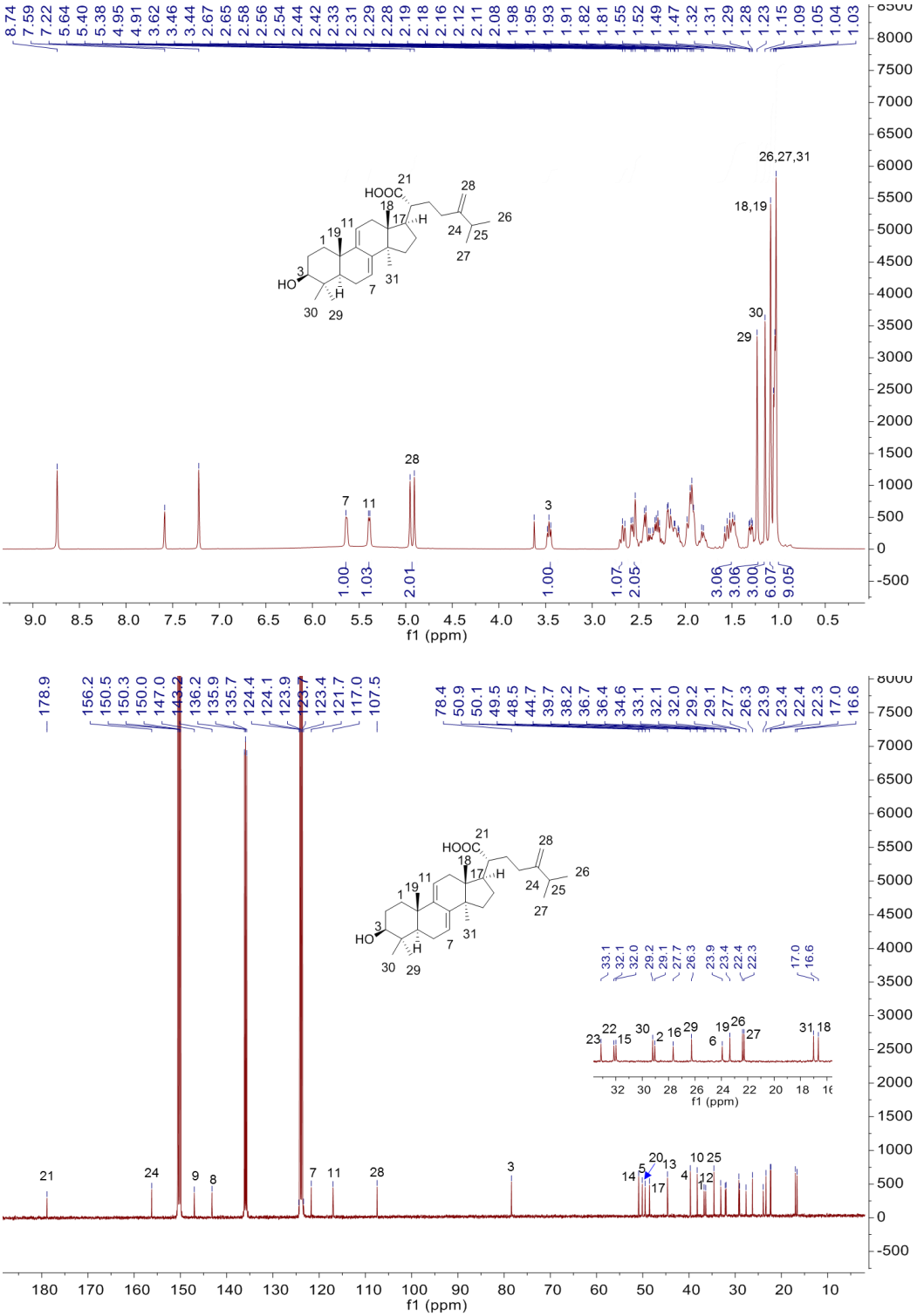
Supplementary file S1

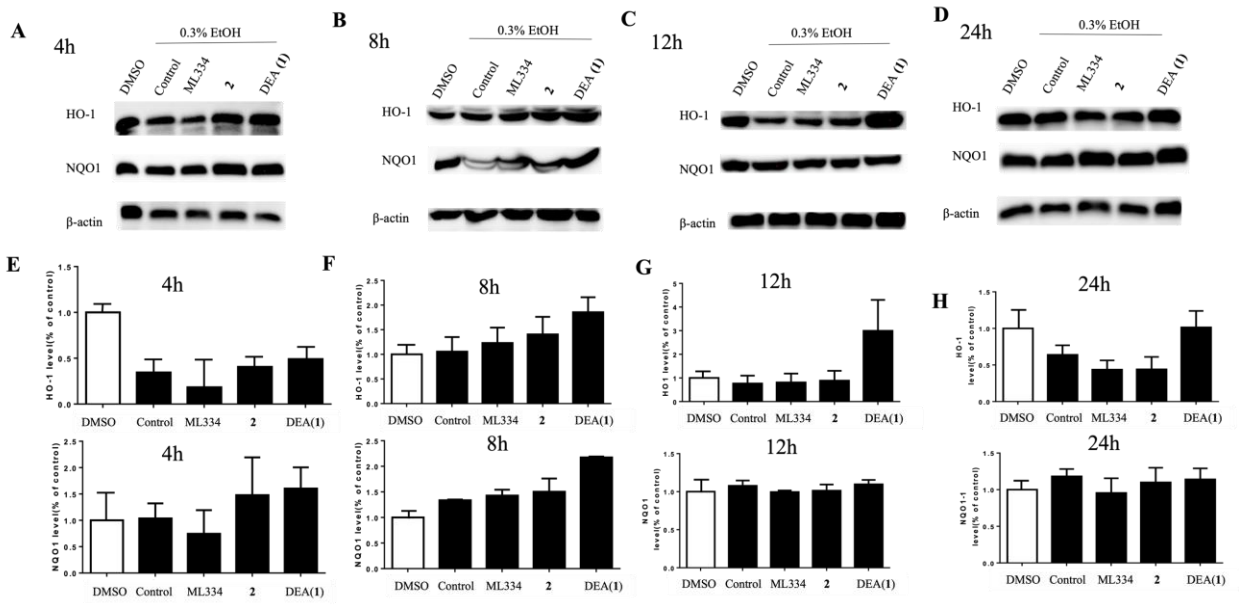
NMR data of dehydroeburicoic acid

Dehydroeburicoic acid: ^{1}H NMR (400 MHz, pyridine-d5) δ: 3.46 (1H, t, J = 7.9 Hz, H-3), 5.63 (1H, s, H-7), 5.39 (1H, d, J = 5.1 Hz, H-11), 1.09 (3H, m, H-18), 1.09 (3H, m, H-19), 1.03 (6H, m, H-26, 27), 4.91 (1H, s, H-28a), 4.95 (1H, s, H-28b), 1.23 (3H, s, H-29), 1.15 (3H, m, H-30), 1.03 (3H, m, H-31). ^{13}C NMR (100 MHz, pyridine-d5) δ: 36.7 (C-1), 29.1 (C-2), 78.4 (C-3), 39.7 (C-4), 50.1 (C-5), 23.9 (C-6), 121.7 (C-7), 143.2 (C-8), 147.0 (C-9), 38.2 (C-10), 117.0 (C-11), 36.4 (C-12), 44.7 (C-13), 50.9 (C-14), 32.0 (C-15), 27.7 (C-16), 48.5(C-17), 16.6 (C-18), 23.4 (C-19), 49.5 (C-20), 178.9 (C-21), 32.1 (C-22), 33.1 (C-23), 156.2 (C-24), 34.6 (C-25), 22.4 (C-26), 22.3 (C-27), 107.5 (C-28), 26.3 (C-29), 29.2 (C-30), 17.0 (C-31)

Supplementary file S2

Supplementary material Figure S1. ^{1}H and ^{13}C NMR spectra of dehydroeburicoic acid.





Supplementary Figure S2. The time dependent of ML334, compound **2**, and compound DEA (**1**) induces antioxidant factor expression in the ALD cell model. (A–D) Effects of 30 μM ML334, 30 μM of **2**, and DEA (**1**) on NQO1, HO–1 protein levels in LO2 cells after 4, 8, 12, 24 h treatment. (E–H) Quantification analysis of 4 h of HO–1 (upper) and NQO1 (lower) (E), 8 h of HO–1 (upper) and NQO1 (lower) (F), 12 h of HO-1 (upper) and NQO1 (lower) (G), 24 h of HO–1 (upper) and NQO1 (lower) (H) in WB.

Supplementary file S3

Supplementary material Table S1. Primer sequences used for PCR analysis.

RT-PCR primers	Forward primer	Reverse primer	Accession number	Amplicon size
β-actin	5'CATGTACGTTGCTATCCAGGC	5'CTCCTTAATGTCACGCACGAT	AK225414	250
NQO1	5'GAAGAGCACTGATCGTACTGGC	5'GGATACTGAAAGTTCGCAGGG	NG01150 4	312
HO1	5'GCCCTTCAGCATCCTCAGTTC	5'GTTTGAGACAGCTGCCACAT	NM002133	68
SOD2	5'CGTGACTTTGGTTCCTTTGAC	5'ATTTGTAAGTGTCCCCGTTCC	NG008729	116
Nrf1	5'CCAAGTGAATTATTCTGCCG	5'TGACTGCGCTGTCTGATATCC	KJ901609	494
PGC-1α	5′-GTCACCACCCAAATCCTTAT	5'ATCTACTGCCTGGAGACCTT	EU280319	131