

Supplementary Information

Anti-Inflammatory Effects of 5 α ,8 α -Epidioxycholest-6-en-3 β -ol, a Steroidal Endoperoxide Isolated from *Aplysia depilans*, Based on Bioguided Fractionation and NMR Analysis

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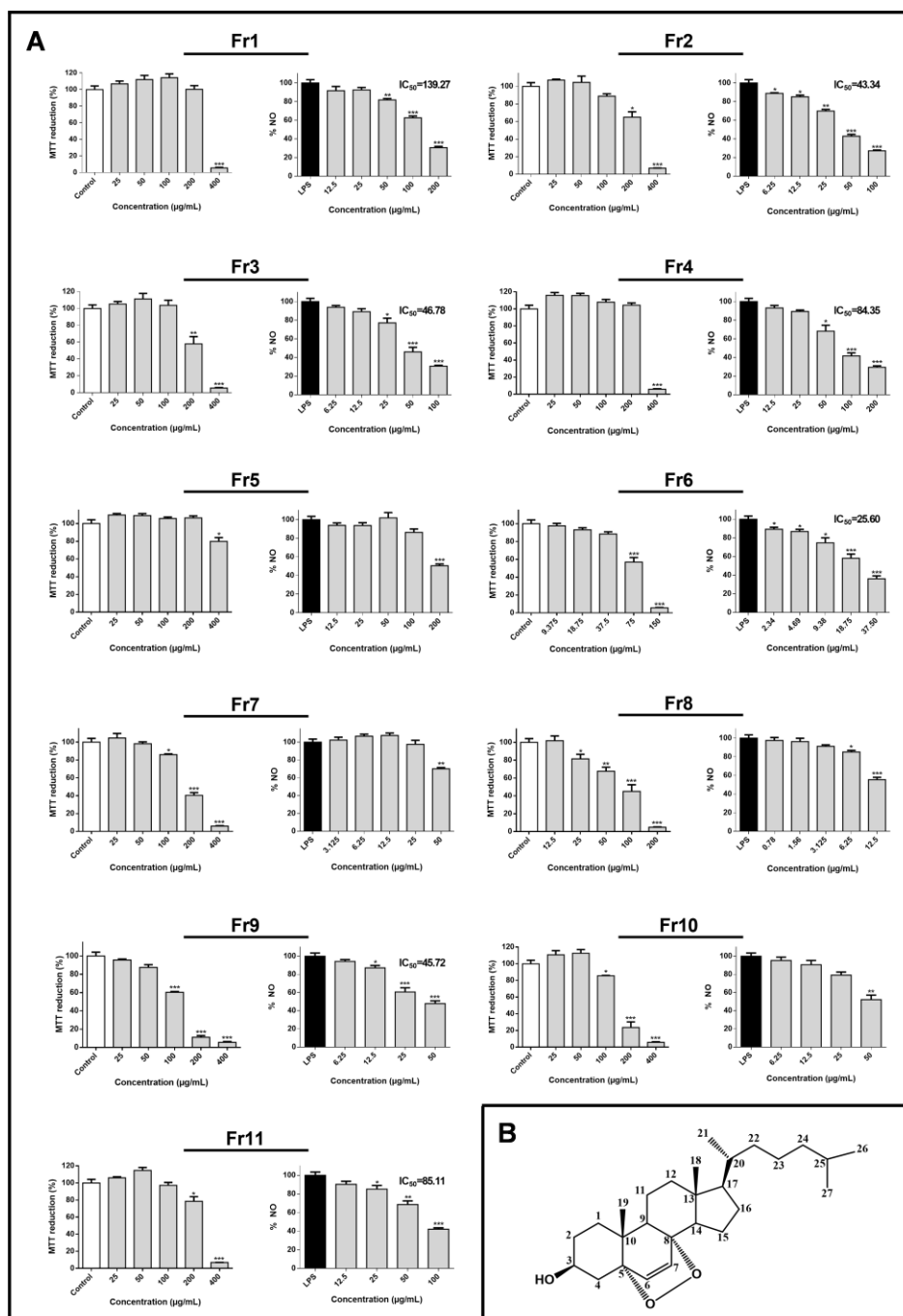


Figure S1. (A) Effect of the *A. depilans* non-polar extract fractions on MTT reduction and in NO levels of RAW 264.7 macrophages challenged with 1 µg/mL of LPS. Results are expressed as mean ± SEM of at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (B) Chemical structure of EnP(5,8) isolated from the non-polar fraction of *A. depilans* extract.

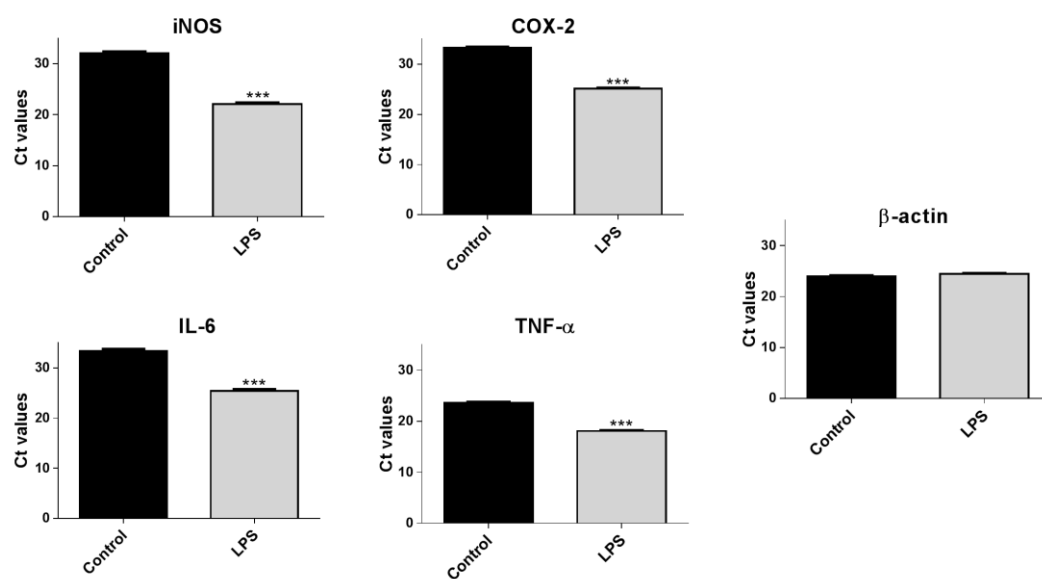


Figure S2. Number of cycles (Ct values) required to amplify 2 ng of cDNA obtained from mRNA extracted from non-treated (control) and treated (LPS) RAW 264.7 macrophages. β -actin was used as reference gene. Results represent the mean $\pm$ SEM of at least five independent experiments performed in duplicate. *** p < 0.001 (vs. control).

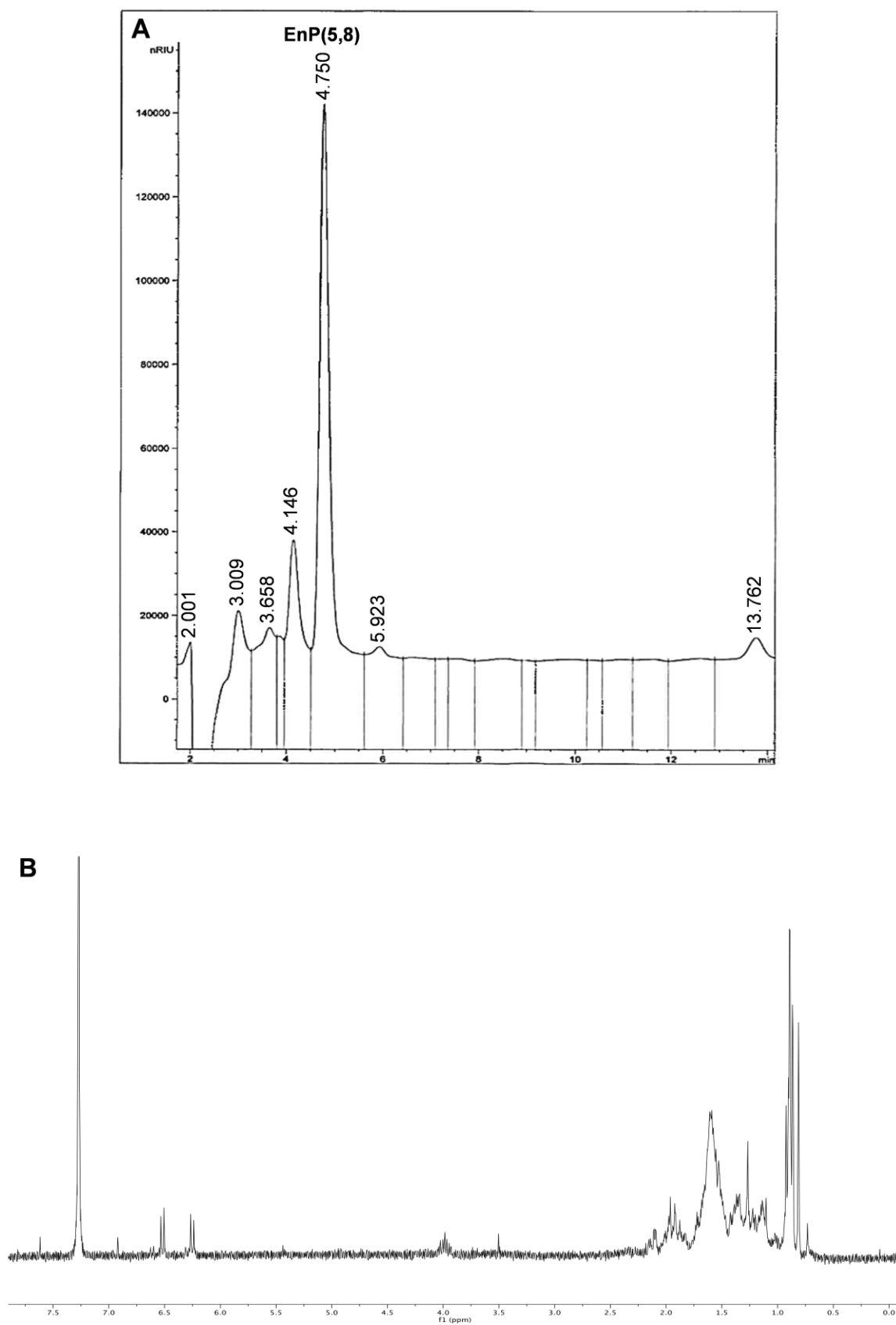


Figure S3: (A) HPLC chromatogram of SubFr5. (B) ^1H -NMR (500 MHz) spectra in CDCl_3 of the peak eluted at 4.75 min.

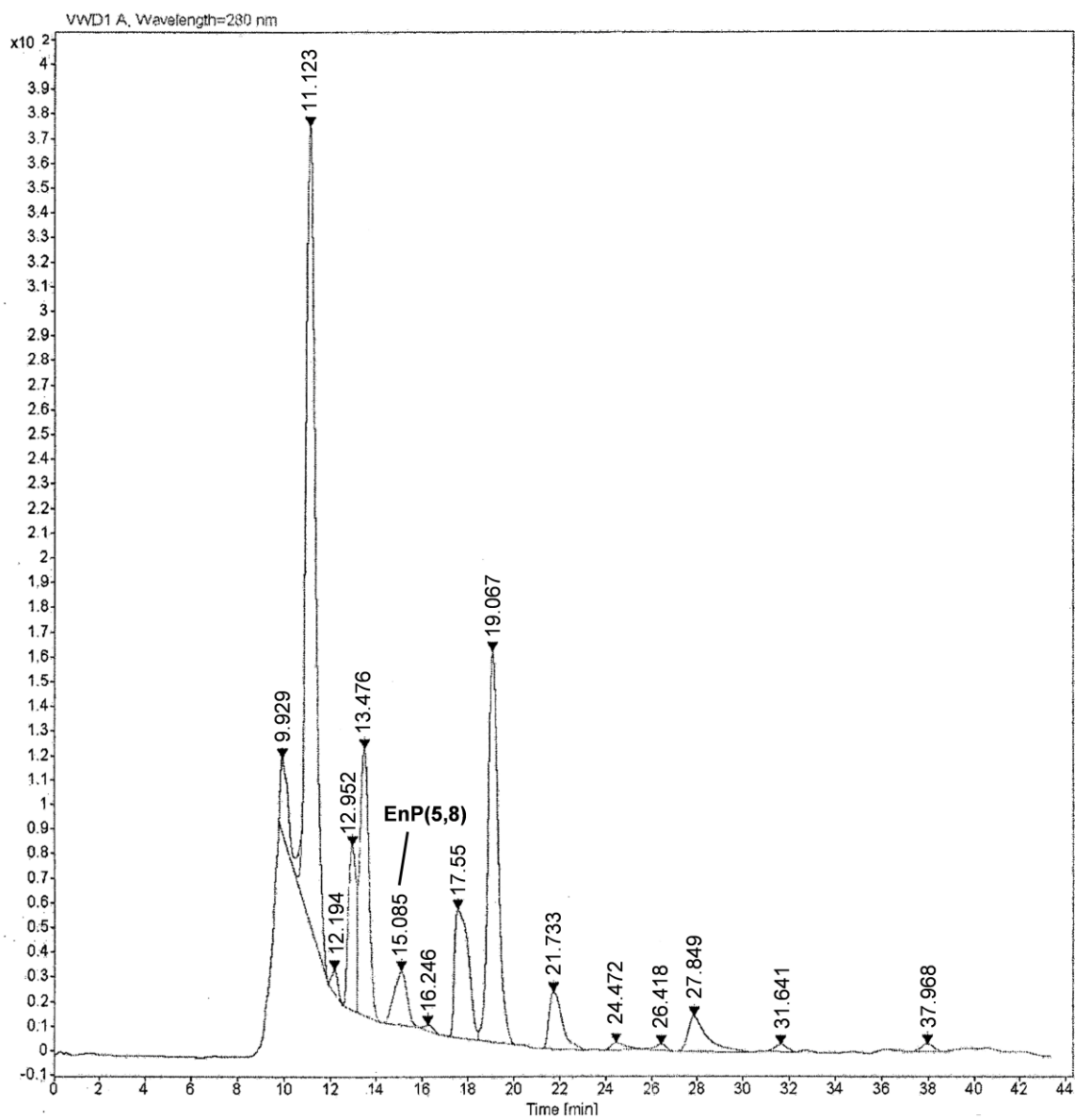


Figure S4. HPLC chromatogram of SubFr7.

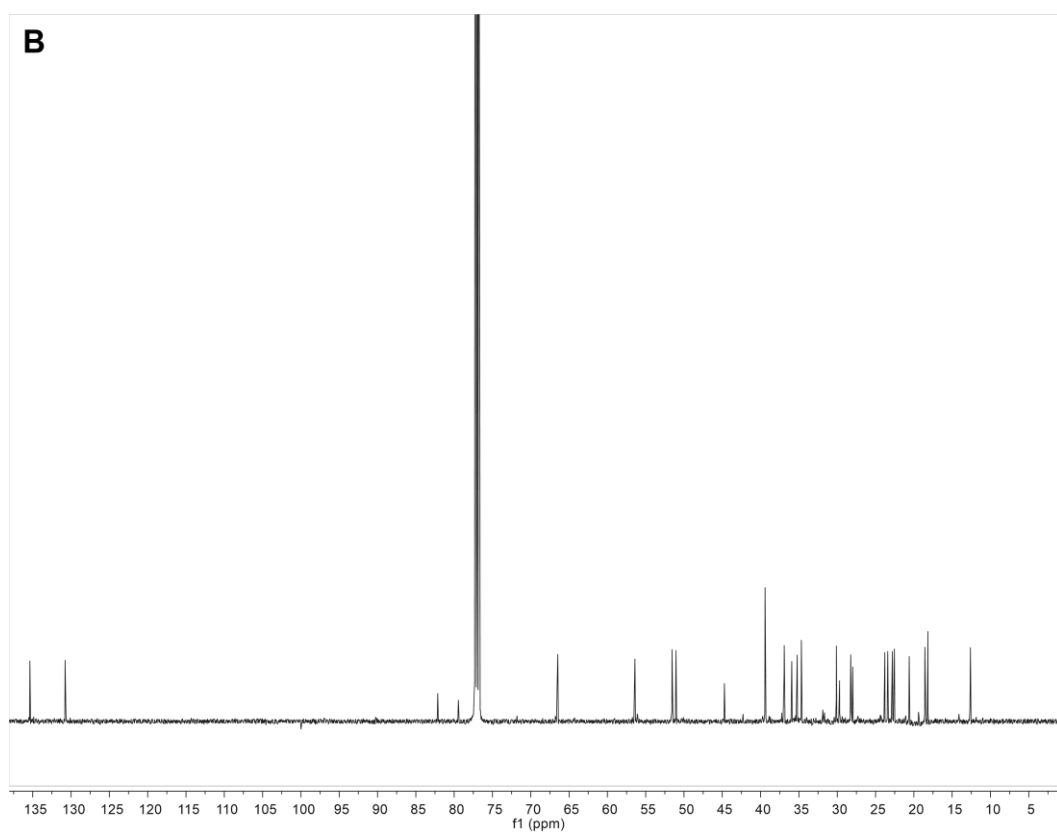
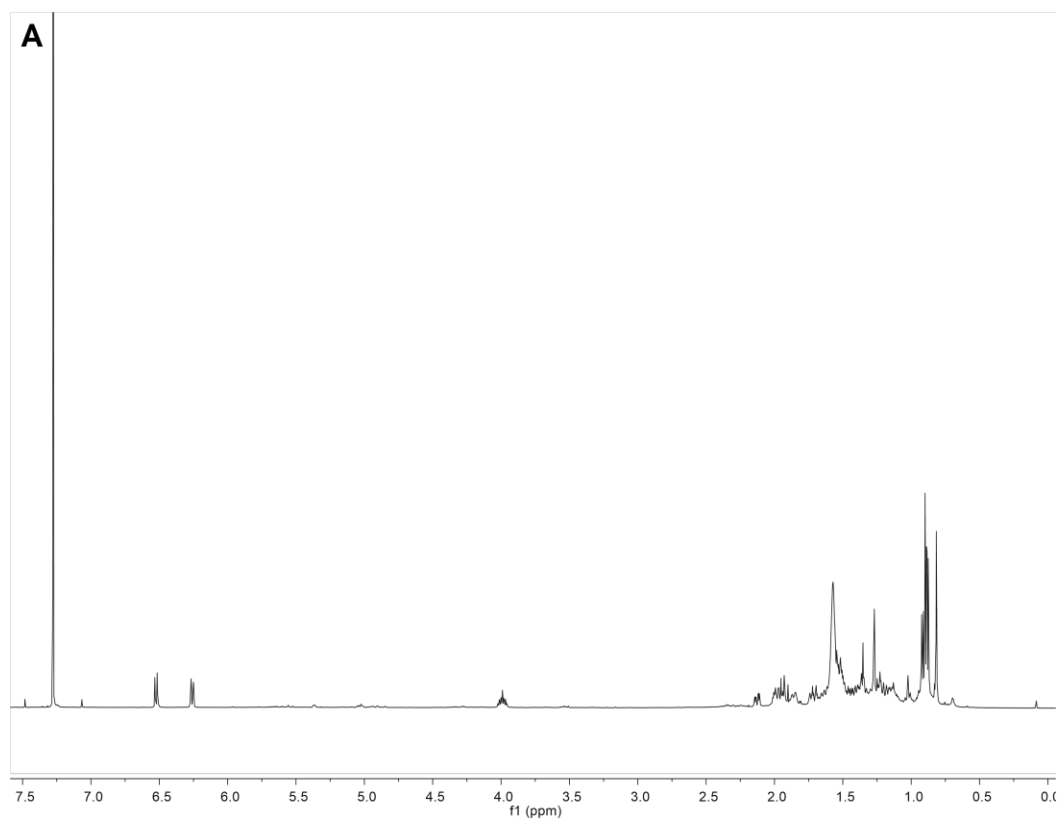


Figure S5. (A) ^1H -NMR (500 MHz), and (B) ^{13}C -NMR (125 MHz) spectra in CDCl_3 of the peak eluted at 15.08 min of Figure S4.