

Supporting Information for

**Gut Bacterial Metabolites from Tryptophan and
Phenylalanine Induce Melatonin Synthesis and
Extend Sleep Duration in Mice**

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Table S1. Primer list used in this study.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Accession no.
Aanat1	TTGGACACAGCCCTTGGA AA	CAGTGCGCTCACTACTGAC A	NM_200704.1
Aanat2 ¹	CAGGGCAAAGGCTCCATC T	CAGGGCAAAGGCTCCATC T	NM_131411.3
Tphla ¹	ACTCTATCCCTCACACGC CT	TGTTGTCTTCACGGGAGTC G	NM_178306.3
Hiomt ¹	GACCTGTTTGAAGCCCTC TACA	ACAGATGGTCTTGTACGGT GTC	NM_001114900 9.1
EF1- α ²	CTTCTCAGGCTGACTGTG C	CCGCTAGCATTACCCTCC	AY422992

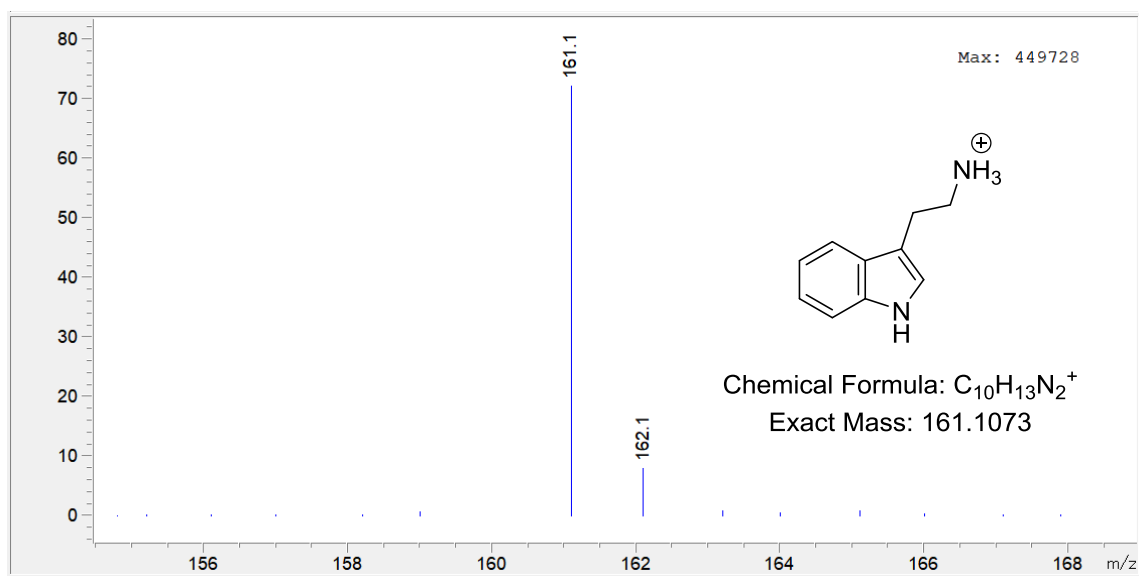
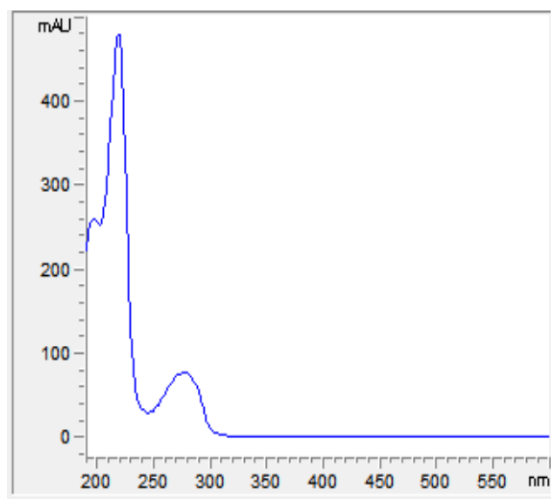


Figure S1. UV-Vis and MS spectrum of **1**.

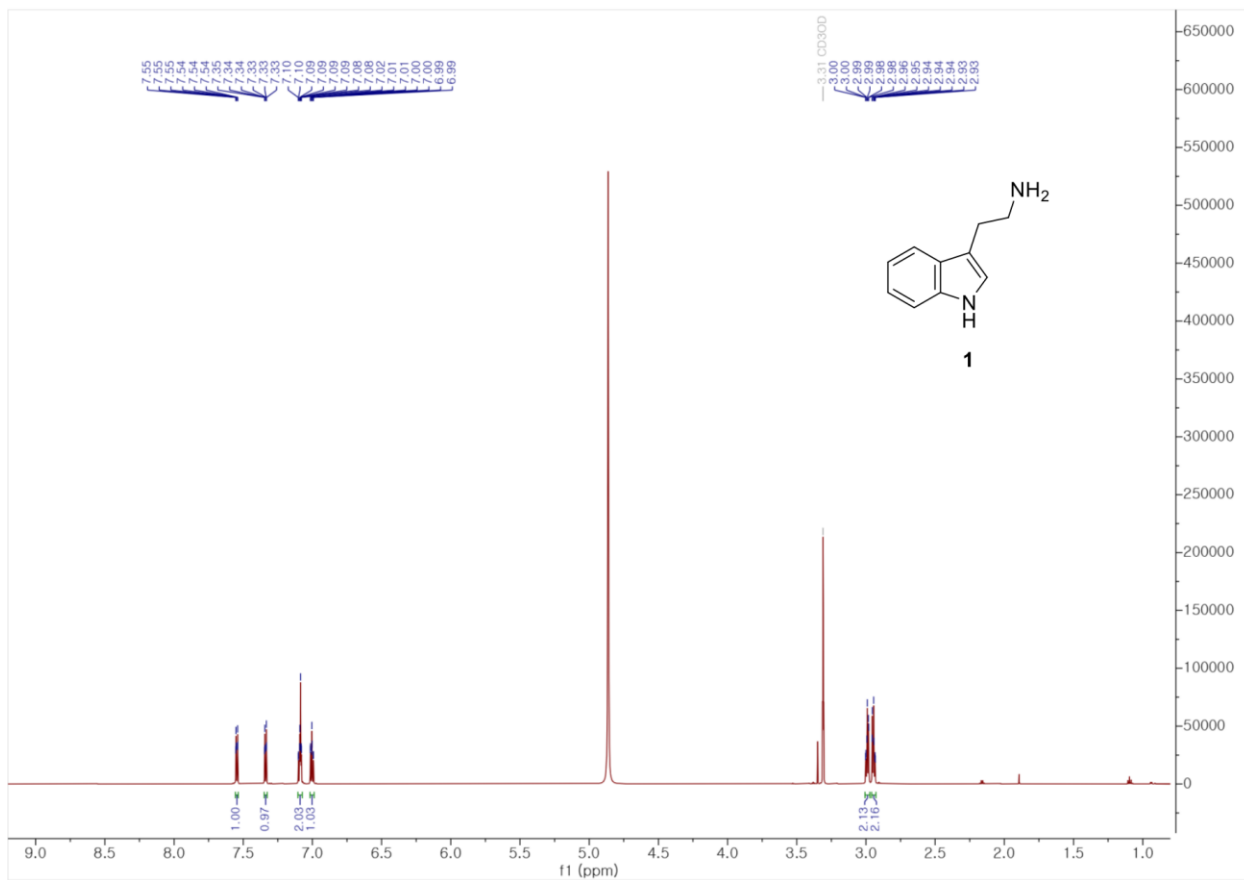


Figure S2. The ^1H NMR spectrum of natural **1** in methanol- d_4 .

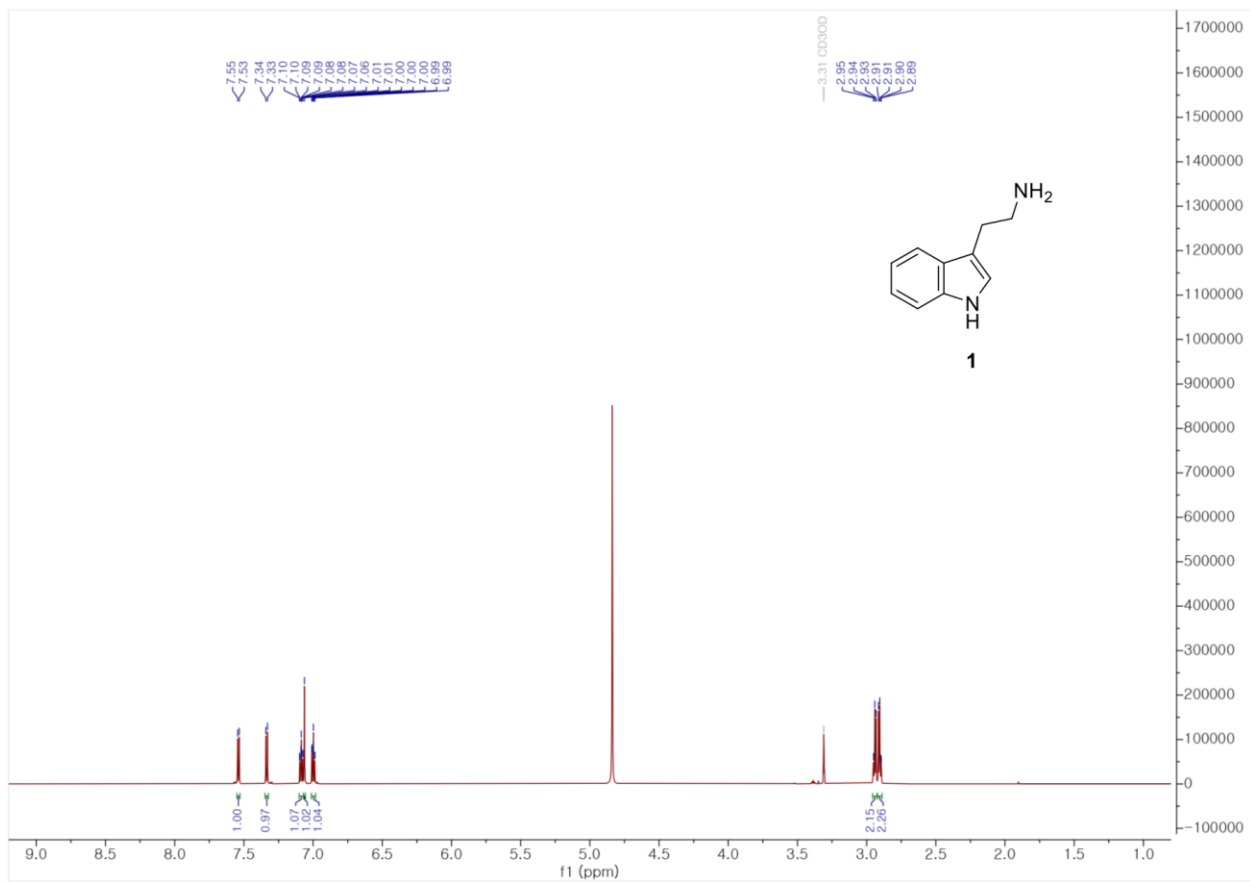


Figure S3. The ^1H NMR spectrum of standard **1** in methanol- d_4 .

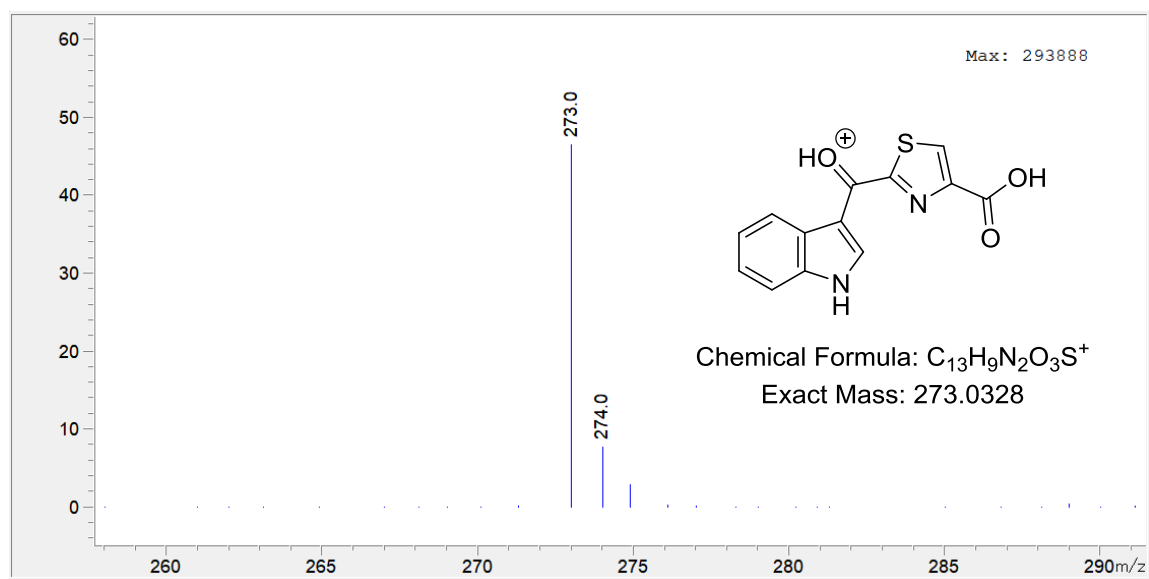
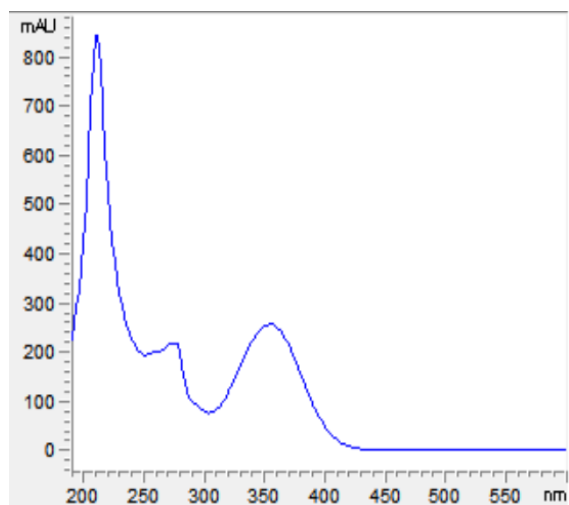


Figure S4. UV-Vis and MS spectrum of **2**.

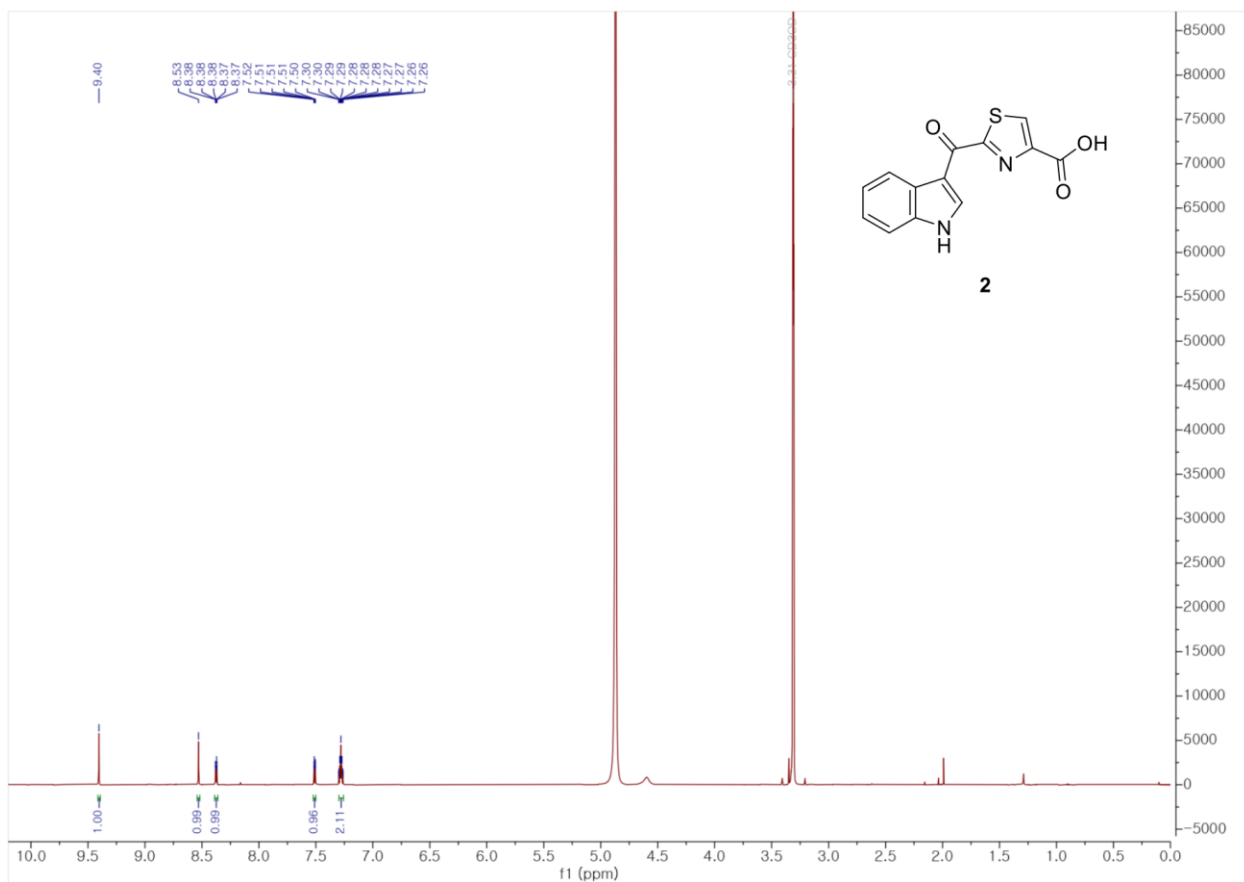


Figure S5. The ^1H NMR spectrum of **2** in methanol- d_4 .

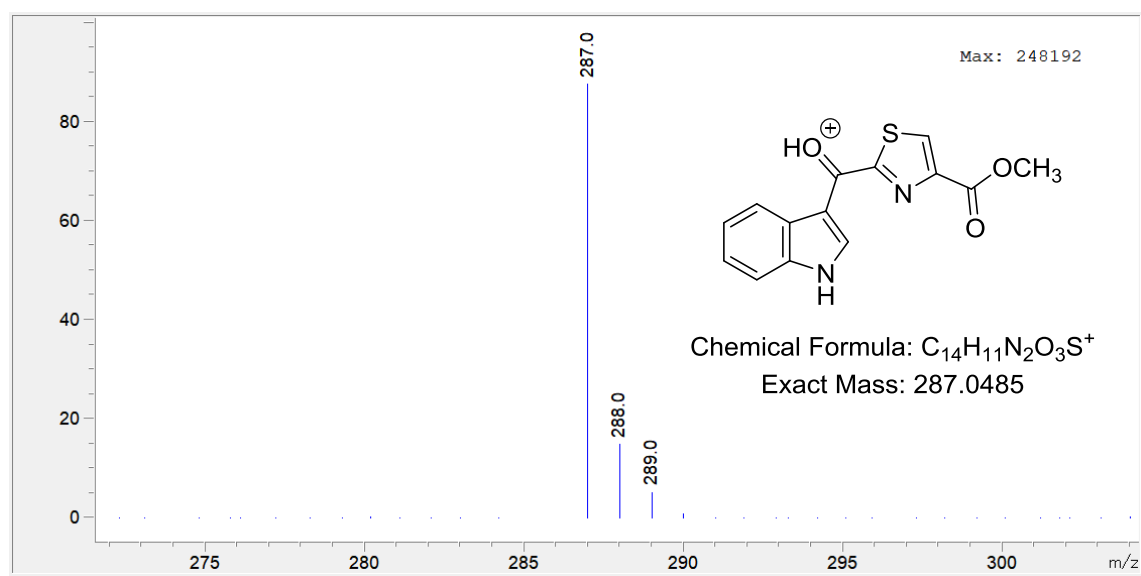
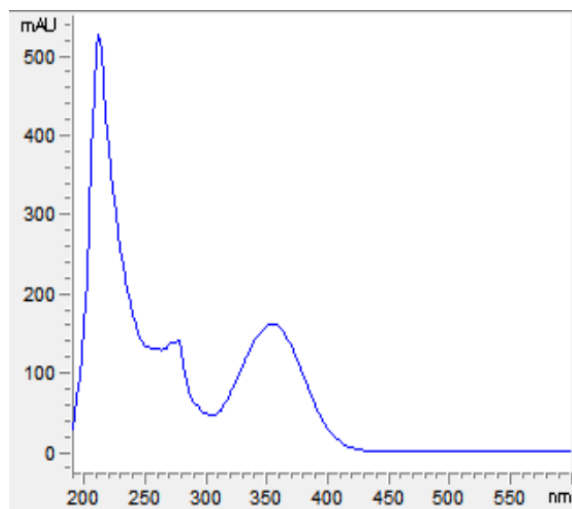


Figure S6. UV-Vis and MS spectrum of **3**.

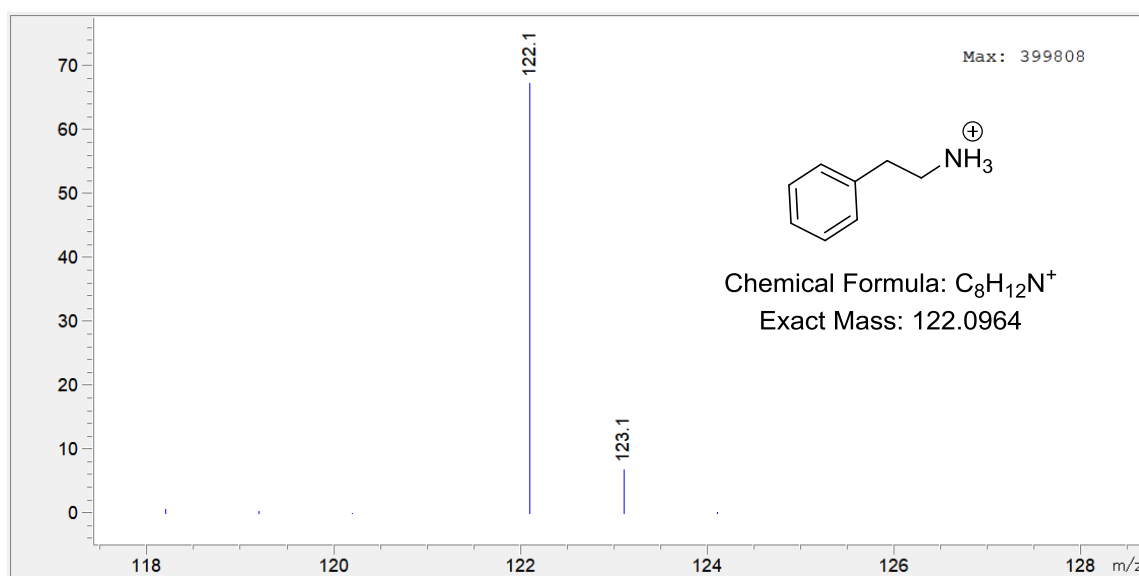
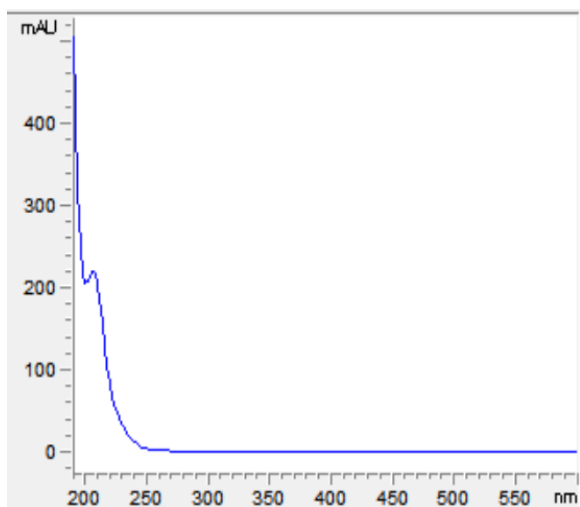


Figure S8. UV-Vis and MS spectrum of **4**.

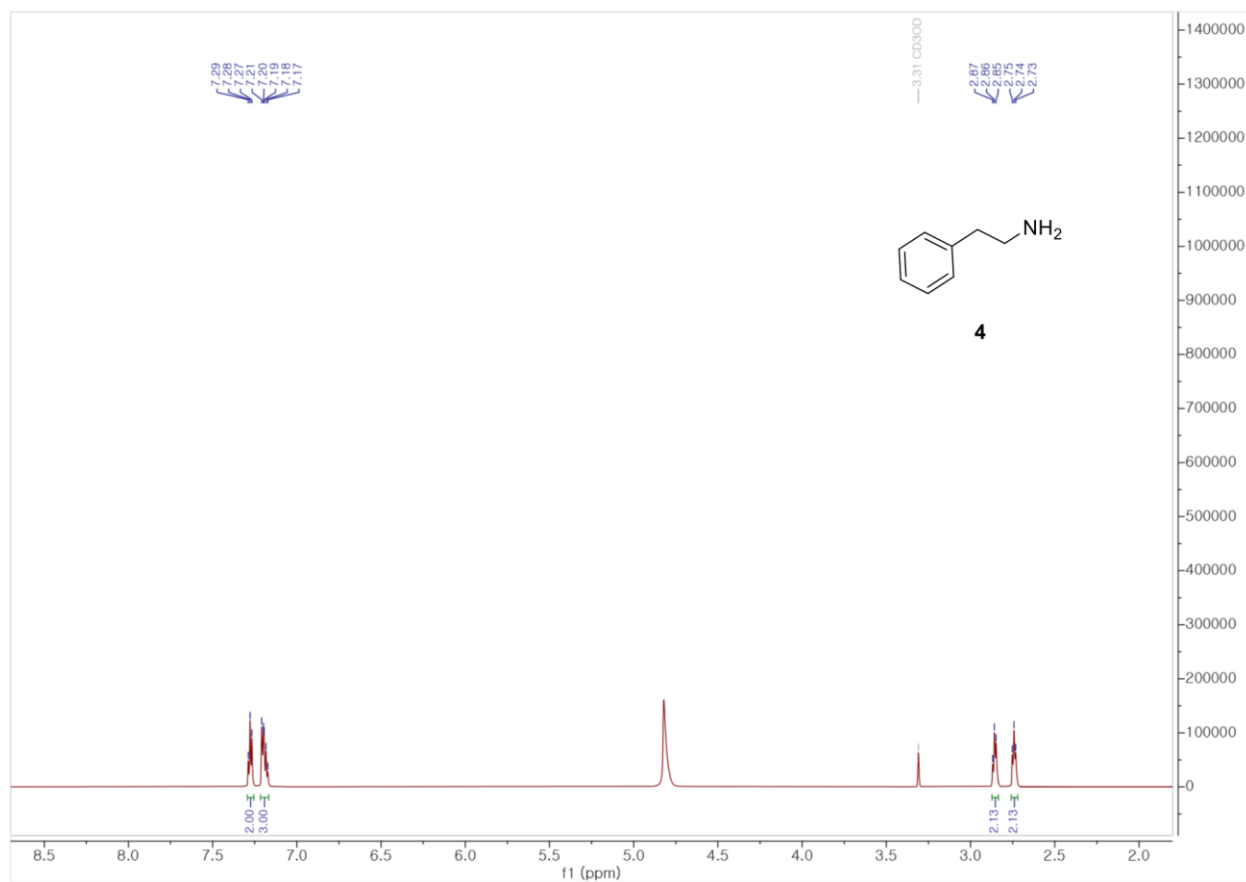


Figure S9. The ¹H NMR spectrum of natural **4** in methanol-*d*₄.

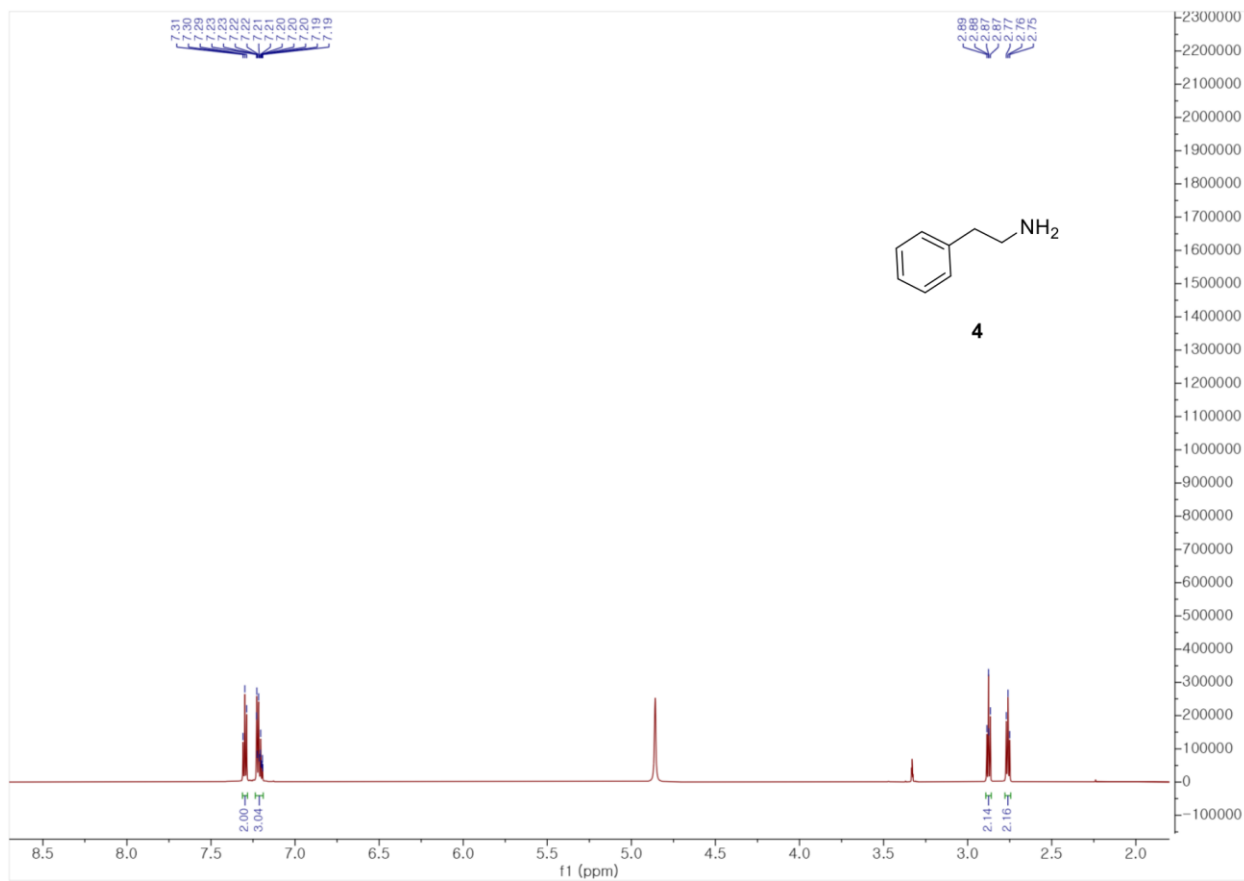


Figure S10. The ^1H NMR spectrum of standard **4** in methanol- d_4 .

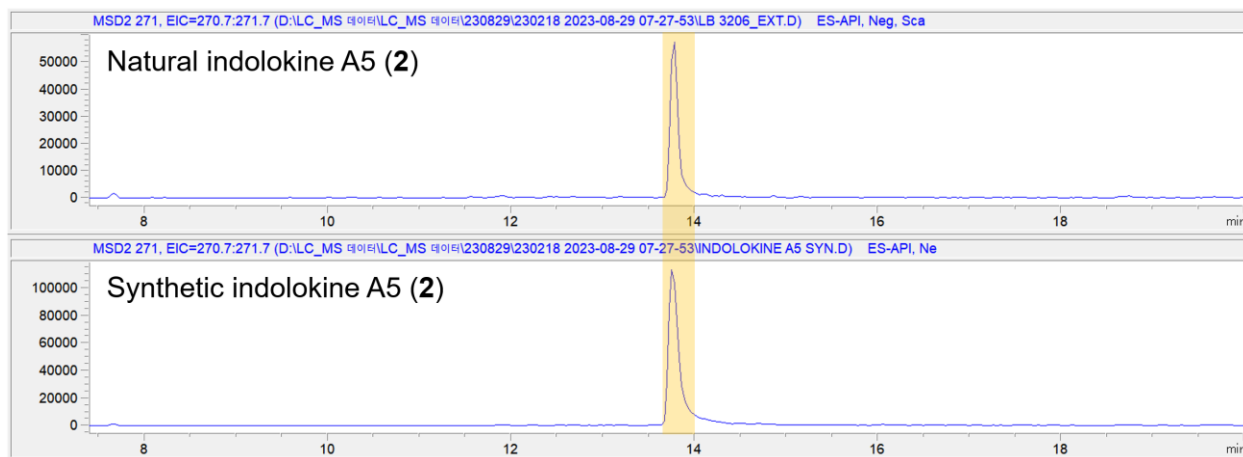


Figure S11. EIC chromatogram of natural and synthetic **2**.

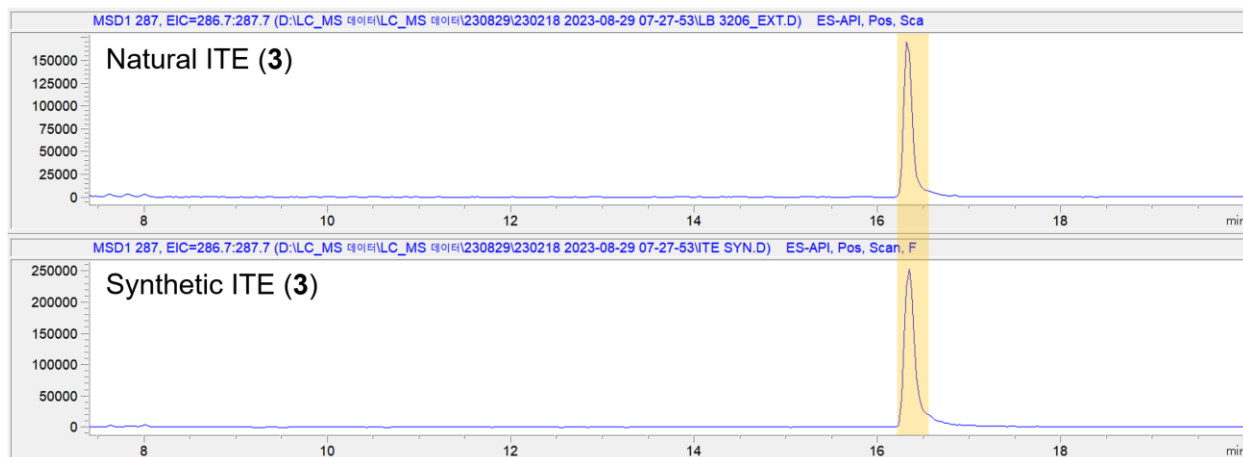
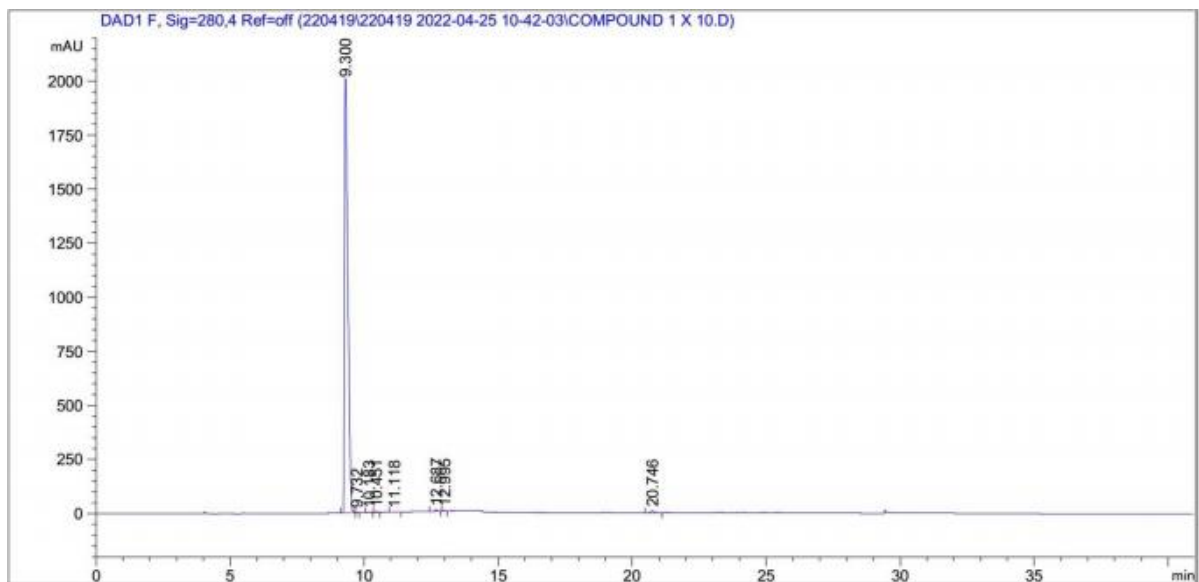
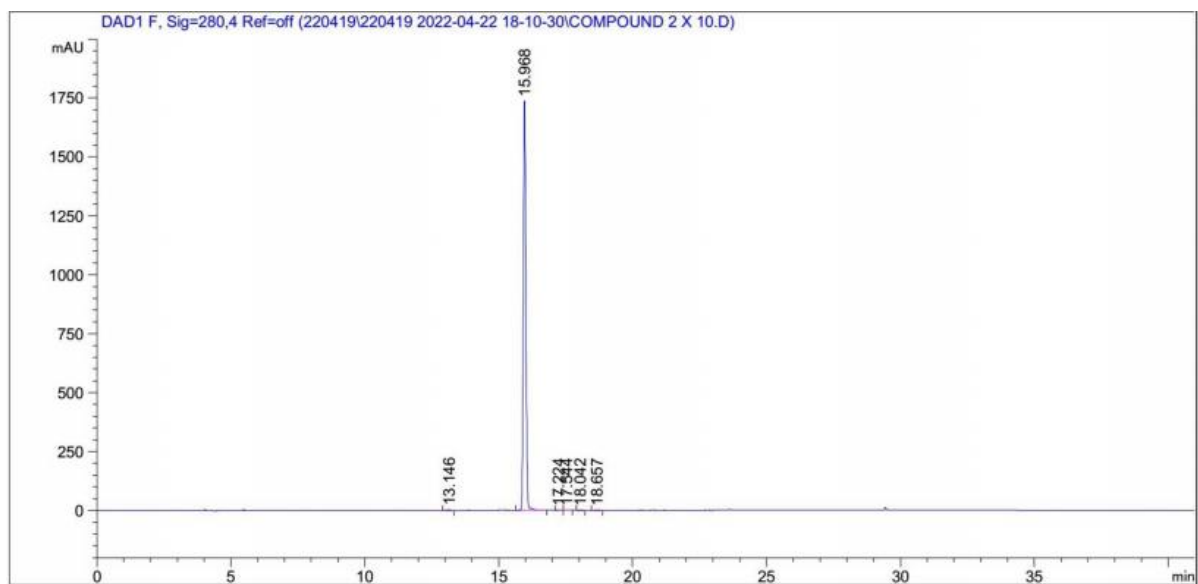


Figure S12. EIC chromatogram of natural and synthetic **3**.



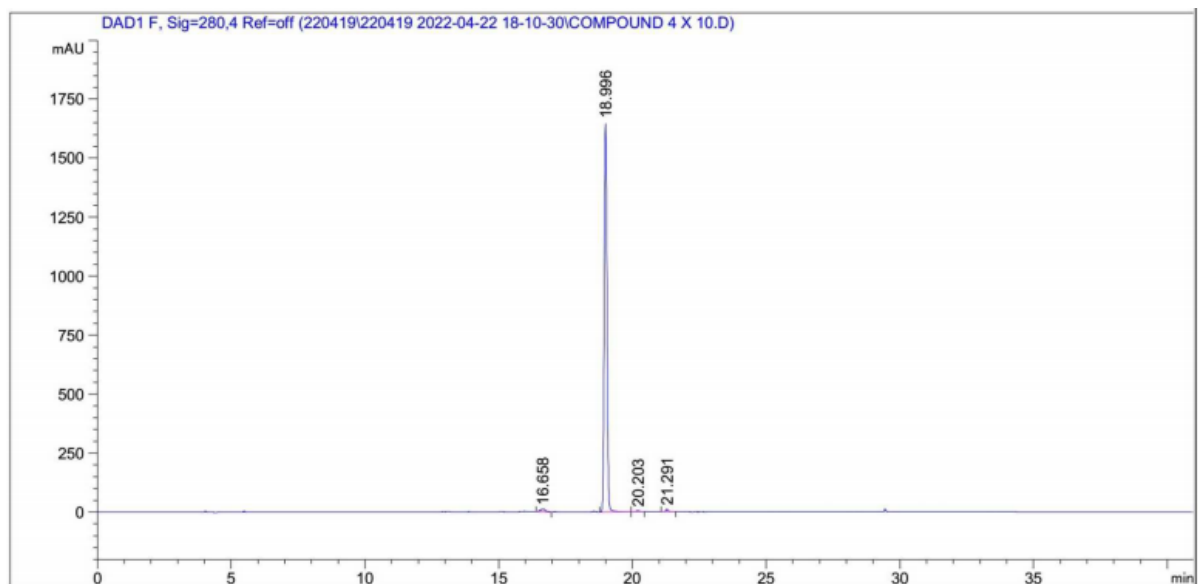
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.300	MF R	0.1459	1.76387e4	2014.62793	99.0068
2	9.732	FM R	0.0831	9.14649	1.83467	0.0513
3	10.183	MM R	0.1085	5.96750	9.16734e-1	0.0335
4	10.451	BB	0.0733	18.89737	3.98949	0.1061

Figure S13. Assessment of purity through HPLC chromatogram analysis for **1**.



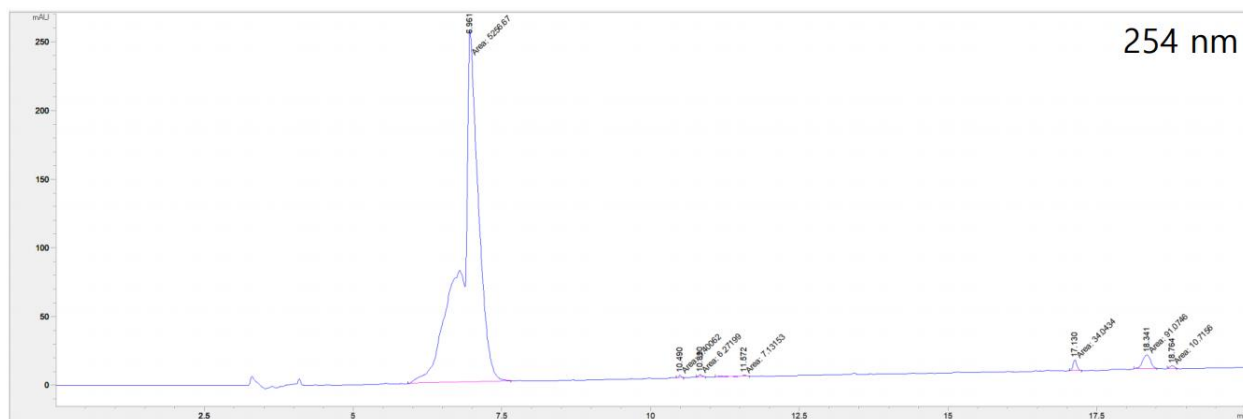
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.146	MM R	0.1505	26.07818	2.88743	0.2272
2	15.968	BB	0.1027	1.14220e4	1737.56995	99.5070
3	17.224	MF R	0.1544	9.07871	9.80179e-1	0.0791
4	17.544	FM R	0.1548	8.43920	9.08660e-1	0.0735

Figure S14. Assessment of purity through HPLC chromatogram analysis for **2**.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.658	BB	0.2096	159.23422	11.49630	1.3339
2	18.996	BB	0.1105	1.16644e4	1648.73877	97.7159
3	20.203	BB	0.1195	38.31491	4.88788	0.3210
4	21.291	BB	0.1109	75.10945	10.32176	0.6292

Figure S15. Assessment of purity through HPLC chromatogram analysis for **3**.



#	Time	Area	Height	Width	Area%	Symmetry
1	6.961	5256.7	256	0.3423	97.142	1.095
2	10.49	5.4	1.7	0.0517	0.100	0.985
3	10.83	6.3	1.7	0.0628	0.116	0.809
4	11.572	7.1	5.9E-1	0.2002	0.132	2.899
5	17.13	34	7.8	0.0731	0.629	0.924
6	18.341	91.1	9.8	0.1547	1.683	1.138
7	18.764	10.7	2.1	0.0861	0.198	0.948

Figure S16. Assessment of purity through HPLC chromatogram analysis for **4**.

Reference

(1) Dharmajyoti Devi, S.; Mondal, G.; Khan, Z. A.; Sarma, H. K.; Chattoraj, A. Differential gene expression and immunohistochemical localization of the key melatonin biosynthesizing enzymes in the testis of zebrafish (*Danio rerio*). *Biol. Rhythm Res.* **2022**, *53* (9), 1347-1363.

(2) Nayak, S.; Khozin-Goldberg, I.; Cohen, G.; Zilberg, D. Dietary supplementation with ω 6 LC-PUFA-rich algae modulates zebrafish immune function and improves resistance to streptococcal infection. *Front. Immunol.* **2018**, *9*, 1960.