SUPPLEMENTARY MATERIAL

New sterols with anti-inflammatory potentials against cyclooxygenase-2 and 5-lipoxygenase from *Paphia malabarica*

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Acknowledgements

This research was supported by the project “Development of nutraceutical supplements from marine molluscs, macroalgae and shrimps” (grant number ICAR/CRP-HF/2016) under the ICAR Consortium Research Platform funded by the Indian Council of Agricultural Research (ICAR), New Delhi, India. The authors thank the Director, Central Marine Fisheries Research Institute for valuable guidance and support. Thanks are due to the Head, Marine Biotechnology Division, Central Marine Fisheries Research Institute for facilitating the research activity. M.J. and V.R. acknowledge Indian Council of Agricultural Research for fellowships.

ABSTRACT

Marine bivalves occupy a leading share in the total edible mollusks at the coastline regions of Southeastern Asia, and are found to possess significant nutritional and biologically potential. Various *in vitro* evaluation (antioxidant and anti-inflammatory) guided purification of ethyl acetate-methanol (EtOAc-MeOH) extract of bivalve clam, *Paphia malabarica* characterized two new sterol derivatives as 23-*gem*-dimethylcholesta-5-en-3 β -ol (**1**) and (22*E*)-24¹,24²-methyldihomocholest-5,22-dien-3 β -ol (**2**) collected from the southwest coast of Arabian Sea. Their structures were unambiguously assigned on the basis of 1D, 2D-NMR spectroscopy and mass spectrometry. The antioxidant and anti-inflammatory activities of **2** as determined

by DPPH/ABTS⁺ radical scavenging and anti-cyclooxygenase-2/5-lipoxygenase assays were significantly greater (IC₅₀ < 1 mg/mL) than **1** (IC₅₀ > 1 mg/mL). Structure activity relationship analysis revealed that the bioactivities of these compounds were directly proportional to the electronic and lipophilic parameters. This is the first report of the occurrence and characterization of 23-*gem*-dimethyl-3 β -hydroxy- Δ^5 -cholestane nucleus and C-30 dihomosterol from marine organisms.

Keywords: Bivalve clam; *Paphia malabarica*; 23-*gem*-dimethylcholesta-5-en-3 β -ol; (22*E*)-24¹,24²-methyl dihomocholest-5,22-dien-3 β -ol; anti-inflammatory activity

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Supplemental file Legends

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- Figure S17.** 2D NMR correlations observed in **1** and **2**. (A) Key ¹H-¹H COSY and HMBC correlations of **1** and (B) ¹H-¹H COSY and HMBC correlations of **2**. The key ¹H-¹H COSY couplings have been represented by the bold face bonds; the HMBC couplings are indicated as double barbed arrow.
- Figure S18.** 2D NMR correlations observed in **1** and **2**. (A) Key NOESY correlations of **1** and (B) NOESY correlations of **2**. The long range NOESY H-H couplings were represented by single barbed arrow.
- Figure S19.** HPLC chromatogram of **1** {R_t (HPLC, MeOH:ACN, 6:4 v/v): 5.997 min, wavelength: 230 nm}.
- Figure S20.** HPLC chromatogram of **2** {R_t (HPLC, MeOH:ACN, 6:4 v/v): 5.332 min, wavelength: 230 nm}.

Table S1. NMR Spectroscopic Data of **1** and **2** in CDCl₃^{a, b}

Position	δ_C , type	δ_H (<i>J</i> in Hz)	^1H - ^1H COSY	^1H - ^{13}C HMBC	Position	δ_C , type	δ_H (<i>J</i> in Hz)	^1H - ^1H COSY	^1H - ^{13}C HMBC
1a	37.22, CH ₂	1.84, t	H-2	C-3	1a	37.22, CH ₂	1.83, t	H-2	C-10, 3
1b	-	1.08, t	-	-	1b	-	1.08, t	-	C-3
2a	31.66, CH ₂	1.85, m	H-3	-	2a	31.83, CH ₂	1.82, m	H-3	C-3
2b	-	1.50, m	-	C-5	2b	-	1.50, m	-	C-5
3	71.82, CH	3.50, p	H-4	-	3	71.81, CH	3.50, p	H-4	-
4a	42.31, CH ₂	2.29, d	-	C-5, 6, 3	4a	42.33, CH ₂	2.28, d	-	C-3, 5, 6
4b	-	2.25, d	-	C-2, 10	4b	-	2.24, d	-	C-10, 2
5	140.76, C	-	-	-	5	140.76, C	-	-	-
6	121.72, CH	5.35, dd (5.13, 3.36)	H-7	C-4, 10, 7	6	121.71, CH	5.35, dd (5.28, 3.58)	H-7	C-10, 7
7a	31.91, CH ₂	1.98, t	H-8	-	7a	31.91, CH ₂	1.96, t	-	C-5, 6
7b	-	1.52, t	-	-	7b	-	1.56, t	-	-
8	46.06, CH	0.91, m	-	C-4, 7	8	46.05, CH	0.93, m	-	C-7
9	50.14, CH	0.95, t	H-11	-	9	50.14, CH	0.96, m	H-11	-
10	36.52, C	-	-	-	10	36.50, C	-	-	-
11	21.09, CH ₂	1.46, q	H-12	C-13	11	21.14, CH ₂	1.49, m	H-12	C-12, 14
12a	39.79, CH ₂	2.01, t	-	C-13	12a	39.72, CH ₂	2.01, t	-	C-13
12b	-	1.15, t	-	-	12b	-	1.15, t	-	C-20, 9
13	42.52, C	-	-	-	13	42.32, C	-	-	-
14	56.11, CH	1.10, m	H-15	C-16	14	56.70, CH	1.00, m	H-15	C-8, 9, 20
15a	24.21, CH ₂	1.56, t	H-16	C-16	15a	24.26, CH	1.57, m	H-16	C-16, 13

15b	-	1.06, m	-	-	15b	-	1.06, m	-	-
16	28.33, CH ₂	1.83, t	H-17	C-13	16	28.22, CH ₂	1.85, m	H-17	-
17	56.72, CH	1.02, m	H-20	C-21	17	56.16, CH	1.10, m	-	C-22, 14, 20, 18, 16
18	11.87, CH ₃	0.68, s	-	C-13, 12, 17	18	11.68, CH ₃	0.68, s	-	C-12, 20
19	19.41, CH ₃	1.01, s	-	C-5, 1, 9	19	19.40, CH ₃	1.01, s	-	C-5, 2, 1
20	36.27, CH	1.35, m	H-22	-	20	42.54, CH	1.86, t	H-22	-
21	18.72, CH ₃	0.92, d	-	C-13, 20	21	18.39, CH ₃	0.91, d	-	C-23
22	36.22, CH ₂	1.14, d	-	C-24, 25	22	135.83, CH	5.18, dd (12.4, 6.1)	-	-
23	39.61, C	-	-	-	23	131.71, CH	5.17, dt (16.1, 7.3)	H-24	-
23 ¹	28.01, CH ₃	1.59, s	-	C-25	24	41.90, CH ₂	2.03, m	H-24 ¹	C-25
23 ²	29.72, CH ₃	1.25, s	-	C-23	24 ¹	28.42, CH ₂	1.25, m	H-24 ²	-
24	23.83, CH ₂	1.30, d	H-25	C-23b	24 ²	35.72, CH	1.35, m	H-24 ³ , H-25	C24 ³ , 27, 25
25	35.71, CH	1.49, m	H-26, H-27	-	24 ³	20.53, CH ₃	0.92, d	-	-
26	19.31, CH ₃	0.86, d	-	C-26	25	28.02, CH	1.52, m	H-26, H-27	C-26, 27
27	22.82, CH ₃	0.87, d	-	C-20	26	19.89, CH ₃	0.86, d	-	-
-	-	-	-	-	27	22.69, CH ₃	0.87, d	-	-

^a NMR spectra recorded using Bruker AVANCE III 500 MHz (AV 500) spectrometer (Bruker, Karlsruhe, Germany) in CDCl₃ as aprotic solvent at ambient temperature with TMS as the internal standard (δ 0 ppm).

^b The ¹³C NMR spectra were recorded at 125 MHz.

The assignments were made with the aid of the ¹H-¹H COSY, HSQC, HMBC and NOESY experiments.

Figure. S1

Figure. S2

Figure. S3

Figure. S4

Figure. S5

Figure. S6

Figure. S7

Figure. S8

Figure. S9

Figure. S10

Figure. S11

Figure. S12

Figure. S13

Figure. S14

Figure. S16

Figure. S17

Figure. S18

Figure. S19

Figure. S20