SUPPLEMENTARY MATERIAL

Unprecedented antioxidative cyclic ether from the red seaweed *Kappaphycus alvarezii* with anti-cyclooxygenase and lipoxidase activities

Fasina Makkar and Kajal Chakraborty*

Affiliation

*Central Marine Fisheries Research Institute, Ernakulam North P.O., P.B. No. 1603, Cochin 682018, India*

* Corresponding author. Address: Marine Bioprospecting Section of Marine Biotechnology Division, Central Marine Fisheries Research Institute, Ernakulam North, P.B. No. 1603, Cochin, India. Tel.: +91 484 2394867; fax: +91 484 2394909. E-mail address: kajal_cmos@yahoo.com (K. Chakraborty).

Acknowledgements

Financial support from the Science and Engineering Research Board (SERB) of Department of Science and Technology, Ministry of Science and Technology, New Delhi, India in the form of a research grant (SR/S1/OC-96/2012 SERB) is gratefully acknowledged. The authors thank the Director, Central Marine Fisheries Research Institute, for his guidance and support. Thanks are due to the Head, Marine Biotechnology Division, Central Marine Fisheries Research Institute for facilitating the research activity. One of us, F.M. acknowledges Department of Science and Technology, for the award of fellowship.
Abstract:
An unprecedented non-isoprenoid oxocine carboxylate cyclic ether characterized as (3S, 4R, 5S, 6Z)-3-((R)-hexan-2'-yl)-3, 4, 5, 8-tetrahydro-4-methyl-2H-oxocin-5-yl acetate was isolated from the ethyl acetate-methanol extract of the red seaweed *Kappaphycus alvarezii*. The structure, as well as its relative stereochemistry, was proposed on the basis of extensive spectral data. The antioxidative activity of the isolated metabolite was found to have significantly greater as determined by 2,2'-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activities (IC$_{50}$ ~ 0.3 mg/mL) compared to α-tocopherol (IC$_{50}$ > 0.6 mg/mL), and was comparable to the synthetic antioxidants butylated hydroxytoluene and butylated hydroxyanisole (IC$_{50}$ ~ 0.35-0.34 mg/mL). The compound exhibited greater activity against COX-2 (cyclooxygenase-2) than COX-1 (cyclooxygenase-1) isoform, and therefore, the selectivity index remained significantly lesser (anti-COX-1IC$_{50}$: anti-COX-2IC$_{50}$ 0.87) than synthetic anti-inflammatory drugs (0.02-0.44). No significant difference of *in vivo* 5-lipoxidase activity (IC$_{50}$ 0.95 mg/mL) than ibuprofen (IC$_{50}$ 0.93 mg/mL) indicated the potential anti-inflammatory properties of the title compound.

Keywords: *Kappaphycus alvarezii*; oxocine carboxylate cyclic ether; anti-inflammatory; antioxidant; structure-activity relationship

Supplemental file Legend

**Table S1.** NMR spectroscopic data of (3S, 4R, 5S, 6Z)-3-((R)-hexan-2'-yl)-3, 4, 5, 8-tetrahydro-4-methyl-2H-oxocin-5-yl acetate in CDCl$_3$

**Figure S1.** (A) $^1$H-$^1$H COSY (B) HSQC spectra of (3S, 4R, 5S, 6Z)-3-((R)-hexan-2'-yl)-3, 4, 5, 8-tetrahydro-4-methyl-2H-oxocin-5-yl acetate. Key $^1$H-$^1$H COSY couplings were shown as the bold face bonds.

**Figure S2.** Mass fragmentation pattern of (3S, 4R, 5S, 6Z)-3-((R)-hexan-2'-yl)-3, 4, 5, 8-tetrahydro-4-methyl-2H-oxocin-5-yl acetate
Figure S3. 2D NMR correlations of (3S, 4R, 5S, 6Z)-3-((R)-hexan-2'-yl)-3, 4, 5, 8-tetrahydro-4-methyl-2H-oxocin-5-yl acetate. (A) Key $^1$H-$^1$H COSY couplings (bold face bonds). (B) HMBC couplings (single-barbed arrow). (C) Key NOESY correlations (long range H–H couplings are indicated as double barbed arrow) of (3S, 4R, 5S, 6Z)-3-((R)-hexan-2'-yl)-3, 4, 5, 8-tetrahydro-4-methyl-2H-oxocin-5-yl acetate.
Table S1. NMR spectroscopic data of (3S, 4R, 5S, 6Z)-3-((R)-hexan-2'-yl)-3, 4, 5, 8-tetrahydro-4-methyl-2H-oxocin-5-yl acetate in CDCl$_3$\textsuperscript{a}

| C. No. | $^{13}$C NMR (δ, ppm) | $^{1}$H NMR (int., mult., J in Hz) | $^{1}$H-$^{1}$H COSY | HMBC ($^{1}$H-$^{13}$C) |
|--------|----------------------|---------------------------------|----------------------|----------------------|
| 2      | 68.08                | 4.21 (2H; m)                   | 8-H                  | -                    |
|        |                      |                                 | 7- H, 9-H,          |                      |
| 3      | 38.78                | 1.69 (1H, m)                   | 11-H                 | C-10, C-16          |
|        |                      |                                 | 3-H, 8-H, 10-H      |                      |
| 4      | 22.68                | 1.3 (1H, m)                    | H                    | C-12                |
| 5      | 28.93                | 2.01 (1H; m)                   | 4-H, 9-H            | C-10                |
| 6      | 124.81               | 5.38 (1H; m)                   | 3-H                  | -                    |
| 7      | 107.6                | 5.27 (1H; m)                   | 6-H                  | -                    |
| 8      | 68.08                | 4.15 (2H; m)                   | 5-H                  | C-2, C-12           |
| 1'     | 14.12                | 0.92 (3H; t)                   | 11-H                 | -                    |
| 2'     | 23.48                | 1.4 (1H; m)                    | 8-H, 12-H           | C-16                |
| 3'     | 34.08                | 2.32 (2H; m)                   | 14-H                 | -                    |
| 4'     | 29.18                | 1.63 (1H; m)                   | 13-H, 15-H          | -                    |
| 5'     | 31.92                | 1.28 (2H; m)                   | 14-H                 | C-12                |
| 6'     | 10.38                | 0.88 (3H; m)                   | -                    | -                    |
| 1''    | 162.79               | -                               | -                    | -                    |
| 2''    | 51.48                | 3.68 (3H; s)                   | -                    | -                    |
| 3''    | 13.88                | 0.9 (3H, m)                    | 9-H                  | C-11                |

\textsuperscript{a} NMR spectra recorded using Bruker AVANCE III 500 MHz (AV 500) spectrometer in CDCl$_3$ as aprotic solvent at ambient temperature with TMS as the internal standard (δ 0 ppm).
Values in ppm, multiplicity and coupling constants ($J$ Hz) are indicated in parentheses. The assignments were made with the aid of the $^1$H-$^1$H COSY, HSQC, HMBC and NOESY experiments.
Figure S1.
Figure S3