SUPPLEMENTARY MATERIAL

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

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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 ($IC_{50} \sim 0.3$ mg/mL) compared to α -tocopherol ($IC_{50} > 0.6$ mg/mL), and was comparable to the synthetic antioxidants butylated hydroxytoluene and butylated hydroxyanisole ($IC_{50} \sim 0.35$ - 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-1 $_{IC50}$: anti-COX-2 $_{IC50}$ 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 $CDCl_3^a$

Figure S1. (A) 1H - 1H 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 1H - 1H 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 ^1H - ^1H 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 CDCl₃^a

C. No.	¹³ C NMR (δ, ppm)	¹ H NMR (int., mult., J in Hz) ^b	¹ H- ¹ H COSY	HMBC (¹ H- ¹³ C)
2	68.08	4.21 (2H; m)	8-H	-
3	38.78	1.69 (1H, m)	7-H, 9-H, 11-H	C-10, C-16
4	22.68	1.3 (1H, m)	3-H, 8-H, 10-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

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

^bValues in ppm, multiplicity and coupling constants (J = Hz) are indicated in parentheses. The assignments were made with the aid of the ¹H-¹H COSY, HSQC, HMBC and NOESY experiments.

Figure S2

Figure S3