SUPPLEMENTARY MATERIAL

A new curvularin glycoside and its cytotoxic and antibacterial analogues from marine actinomycete *Pseudonocardia* sp. HS7

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Abstract

Five curvularin macrolides (**1–5**) were isolated from the cultured broth of marine actinomycete *Pseudonocardia* sp. HS7 that was obtained from the cloacal aperture of sea cucumber *Holothuria moebii*. The structures of these isolates were characterized as (11*S*,15*R*)-11-hydroxycurvularin (**1**), (11*R*,15*R*)-11-hydroxycurvularin (**2**), curvularin-7-*O*- α -D-glucopyranoside (**3**), *trans*-dehydrocurvularin (**4**), and curvularin (**5**) based on their NMR and HRESIMS data as well as chemical degradation. Compound **3** is a new macrolide with a rare α -D-glucopyranose substituent. Compounds **1–4**, **5a** and **5c** (the acyl products of **5**), suppressed the proliferation of all six tested cancer cell lines and **4** is the most active compound with IC₅₀ values ranging from 0.59 to 3.39 μ M. The 11-hydroxycurvularins **1** and **2** also showed antibacterial activity inhibiting the growth of *Escherichia coli*.

Keywords: *Pseudonocardia* sp. HS7; curvularin glycoside; cytotoxic; antibacterial activity

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Figure S1. HPLC chromatogram of compounds **1–5** and **5a–5c**

Figure S2. ^1H -NMR spectrum of compound **3** (in acetone- d_6)

Figure S3. ^1H -NMR spectrum of compound **3** (in acetone- d_6)

Figure S4. ^1H -NMR spectrum of compound **3** (in acetone- d_6)

Figure S₅. ¹H-NMR spectrum of compound **3** (in acetone-*d*₆)

Figure S₆. ¹³C-NMR spectrum of compound **3** (in acetone-*d*₆)

Figure S7. ^{13}C -NMR spectrum of compound **3** (in acetone- d_6)

Figure S8. ^{13}C -NMR spectrum of compound **3** (in acetone- d_6)

Figure S₉. ¹³C-NMR spectrum of compound **3** (in acetone-*d*₆)

Figure S₁₀. ¹H-NMR spectrum of compound **3** (in methanol-*d*₄)

Figure S11. ^1H -NMR spectrum of compound **3** (in methanol- d_4)

Figure S12. ^1H -NMR spectrum of compound **3** (in methanol- d_4)

Figure S13. ^{13}C -NMR spectrum of compound **3** (in methanol- d_4)

Figure S14. ^{13}C -NMR spectrum of compound **3** (in methanol- d_4)

Figure S15. ^{13}C -NMR spectrum of compound **3** (in methanol- d_4)

Figure S16. HSQC spectrum of compound **3** (in methanol- d_4)

Figure S17. HSQC spectrum of compound **3** (in methanol- d_4)

Figure S18. HSQC spectrum of compound **3** (in methanol- d_4)

Figure S19. HRESIMS spectrum of compound **3** (Top: measured result, Bottom: predictable result)

Figure S20. ¹H-NMR spectrum of compound **5** (in methanol-*d*₄)

[illegible][illegible]

Figure S23. ^1H -NMR spectrum of compound **5** (in methanol- d_4)

Figure S24. ^{13}C -NMR spectrum of compound **5** (in methanol- d_4)

Figure S25. ^{13}C -NMR spectrum of compound **5** (in methanol- d_4)

Figure S26. ^{13}C -NMR spectrum of compound **5** (in methanol- d_4)

Figure S27. ^1H -NMR spectrum of compound **5a** (in methanol- d_4)

Figure S28. ^1H -NMR spectrum of compound **5a** (in methanol- d_4)

Figure S29. ^1H -NMR spectrum of compound **5a** (in methanol- d_4)

Figure S30. ^{13}C -NMR spectrum of compound **5a** (in methanol- d_4)

Figure S31. ^{13}C -NMR spectrum of compound **5a** (in methanol- d_4)

Figure S32. ^{13}C -NMR spectrum of compound **5a** (in methanol- d_4)

Figure S33. HRESIMS spectrum of compound **5a**

16S rDNA sequence of strain HS7

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GGGCCGCCGGCTTCGGGTGTTACCAACTTTCGTGACGTGACGGGCGGTGTGTACAAG
GCCCCGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACTCCAAC
TCACGGGGTTCGAGTTGCAGACCCCGATCCGAAGTGAAGTCACTTTAAGGGATTCCG
TCCACCTCACGGTCTCGCAGCCCTCTGTATGAGCCATTGTAGCATGTGTGAAGCCCTG
GACATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAG
TTGACCCCGGCAGTCTCCCATGAGTCCCCACCACTACGTGCTGGCAACATGGAATAA
GGGTTGCGCTCGTTGCG-GGACTTAA-CCCAACATCTCACGACACGAGCTGACGACAG
CCATGCACCACCTGCACACAGGCCACAAGGGAAACGACATCTCTGCCGCGATCCTGT
GCATGTCAAACCCAGGTAAGGTTCTTCGCGTTGCATCGAATTAATCCACATGCTCCG
CCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTCAGCCTTGCGG
CCGTACTCCCCAGGCGGGGCGCTTAATGCGTTAGCTGCGGCGCAGAAACCGTGGAAT
GGTCCCCACACCTAGCGCCCAACGTTTACGGCGTGGAATACCAGGGTATCTAATCCT
GTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTATCGGCCAGAGACCCGCCTTCG
CCACCGGTGTTCTCCTGATATCTGCGCATTTACCGCTACACCAGGAATTCCAGTCT
CCCCTGCCGAAGTCAAGTGATGCCCGTATCGACCGCACGCTCAGAG
TTAAGCCCCAAGTTTTACGGCCGACGCGACACACCGCCTACGAGCTCTTTACGCCC
AATAATTCCGGACAACGCTCGCACCCCTACGTGTTACCGCGGCTGCTGGCACGTAGTT
GGCCGGTGCTTCTTCTCCAGGTACCGTCACAAAAGCTTCGTCCCTGGCGAAAGAGGT
TT-ACAACCCGAAGGCCGTCATCCCCACGCGGCGTCGCTGCGTCAGGCTTCCGCCCCA
TTGCGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTG
TCTCAGTCCCAGTGTGGCCGGTCGCCCTCTCAGGCCGGCTACCCGTCACCGCCTTGGT
AGGCCATCACCCACCAACAAGCTGATAGGCCGCGGGCCCATCCCCACCGAAAAA
ACTTTCCACCACCAACATGCGTTGAGAGGTCTATCCGGTATTAGACCCAGTTTCCC
GGGCTTATCCCAGAGTGGAGGGCAGGTCACCCACGTGTTACTACCCGTTTCGCCGCT
CGTGTACCCCGAAAGGGCCTACCGCTCGACTGC

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Table S1. Sequences producing significant alignments

Accession	Description	Max score	Total score	Query coverage
KJ413158.1	<i>Pseudonocardia</i> sp. UGM020518-05_2010	2510	2510	99%
EU928995.1	<i>Pseudonocardia</i> sp. NMG020605-08	2510	2510	99%
EU928990.1	<i>Pseudonocardia</i> sp. JS020524-10_B1F6	2510	2510	99%
EF588224.2	<i>Pseudonocardia</i> sp. UGM020518-05	2510	2510	99%
FJ948116.2	<i>Pseudonocardia</i> sp. PT1CWM2701	2510	2510	99%
EU283932.1	<i>Pseudonocardia</i> sp. CC011120-01	2510	2510	99%
EF588220.1	<i>Pseudonocardia</i> sp. SP020602-02	2510	2510	99%
EF588211.1	<i>Pseudonocardia</i> sp. CC011213-35	2510	2510	99%
EF588208.1	<i>Pseudonocardia</i> sp. AL040116-05	2510	2510	99%

Experimental

General experimental procedures

HRESIMS data were acquired on an Agilent 6230 TOF LC/MS spectrometer. All NMR spectra were recorded on a Bruker 500 spectrometer using standard pulse programs and acquisition parameters. Chemical shifts were reported in δ (ppm) referencing to NMR solvent used. GC analysis was conducted on an Agilent 6890N gas chromatograph system using a DB-624 capillary column (30 m $\times$ 0.53 mm, 3.0 μ m, Agilent Technologies). N₂ (4.0 mL/min) was used as the carrier gas and FID as detector. The detector and injection port temperatures were set at 260°C and 240°C, respectively. The column temperature was 200°C in 2 min, raised with 20°C/min to 250°C, and then kept at 250°C for 15 min. D-glucose was purchased from Sigma-Aldrich. HPLC separation was performed on an Agilent 1260 HPLC system with a DAD detector using a Zorbax SB-C18 column (250 $\times$ 9.4 mm, 5 μ m, Agilent Technologies). All solvents (HPLC and analysis grade) used for this study were purchased from Sinopharm Chemical Reagent Co Ltd (Shanghai, China). Nutrient Broth (NB), Mueller Hinton Broth (MHB), and Gause's-agar were obtained from Hangzhou Microbial Reagent Co. Ltd. (Hangzhou, China), Thermo Fisher Scientific Inc (USA), and Guangdong Huankai Microbial Science and Technology Co. Ltd. (Guangzhou, China), respectively. Human glioma U251, U87-MG, SHG-44 and rat

glioma C6 cells, and colorectal cancer HCT-15 and SW620 cells were purchased from the Cell Bank of the Chinese Academy of Sciences. Bacteria *Staphylococcus aureus* ATCC and *Escherichia coli* ATCC were gifts from Dr. Zhongjun Ma and Dr. Pinmei Wang.

Sea cucumber material

Fresh sea cucumbers *Holothuria moebii* Ludwig were collected from the Turtle Islet in the South China Sea close to Shanwei City, China, in April 2014. A voucher sample (SW-SC201404) was authenticated by one of the authors (Z. Zhang) and deposited in the Laboratory of Institute of Marine Biology, Ocean College, Zhejiang University, China.

Isolation and identification of strain HS7

The strain HS7 was isolated from the cloacal aperture of a sea cucumber *H. moebii* sample. The sea cucumber sample was surfacely disinfected with 75% ethanol for 60 s and then washed with sterile distilled water three times. The cloacal aperture of surface-disinfected sea cucumber was dissected and immersed in 50 mL sterile water and then shook on a rotary shaker at 180 rpm at 28°C for 10 min. A 200 µL sample of the suspension was dispersed across a Gause's-agar plate supplemented with 2% sea salt and incubated at 28°C for 6 days. The single colony (strain HS7) that grew well was picked with sterile needles and transferred on Gause's-agar medium. Working stocks were prepared on Gause's agar slants and stored at 4°C for further use.

The taxonomic identity of strain HS7 was determined by 16S rDNA analysis. The top sequence using BLAST (nucleotide sequence comparison) was compared to the GenBank database. A voucher strain (*Pseudonocardia* sp. HS7) of this actinomycete was preserved at the Laboratory of Institute of Marine Biology, Ocean College, Zhejiang University, China.

Fermentation of strain HS7

Homogenized colony of isolate HS7 was transferred into a 500 mL Erlenmeyer flask containing 200 mL of seed broth that was incubated for 4 days at 28°C on a rotary shaker (180 rpm). The seed broth (5 mL) was then transferred into 500 mL Erlenmeyer flasks containing 200 mL of Gause's broth medium supplemented with 2% sea salt. The flasks were incubated at 28°C on a rotary shaker at 180 rpm for 7 days. A total of 10.0 L fermentation broth was made for this study.

Isolation of compounds 1–5

The fermentation broth of isolated strain HS7 (10.0 L) was filtered to give pellets and fermentation solution. The pellets and fermentation solution were respectively extracted by

EtOAc three times. The combined EtOAc part was evaporated under reduced pressure to give a residue, which had activity against glioma cells. This active residue was redissolved in 3.0 mL DMSO and then separated by HPLC using a Zorbax SB-C18 column at the conditions of flow rate at 1.2 mL/min, column temperature at 25 °C, and UV detection at 256 nm. Water and MeOH were employed as mobile phases A and B, respectively. The gradient procedure was 0.0–20.0 min with 60% B to 100% B, 21.0–28.0 min with 100% B, 28.1–36.0 min with 60% B. After HPLC separation, each of fractions from peaks at 13.3, 14.7, 17.6, and 19.2 min was dried *in vacuo* to furnish a mixture of **1** and **2** (23.2 mg), **3** (6.6 mg), **4** (10.2 mg), and **5** (500 mg), respectively. The mixture of **1** and **2** was further purified by HPLC using an isocratic solvent of MeOH/H₂O (50:50) to give **1** (10.6 mg, *t_R* 25.1 min) and **2** (8.3 mg, *t_R* 26.4 min) (Figure S1).

Compound 3: colorless amorphous powder; molecular formula C₂₂H₃₀O₁₀, UV: $\lambda_{\max}$ 223, 272, 305 nm; ¹H NMR data (500 MHz, in acetone-*d*₆ or methanol-*d*₄) and ¹³C NMR data (125 MHz, in acetone-*d*₆ or methanol-*d*₄), see Tables S₂ and S₃; HRESIMS *m/z* [M+Na]⁺ 477.1730 (calcd for C₂₂H₃₀NaO₁₀, 477.1737) and [M–H][–] 453.1752 (calcd for C₂₂H₂₉O₁₀, 453.1761).

Acidic hydrolysis of compound 3

Compound **3** (2.0 mg) was hydrolyzed in 2 mL 1N HCl at 80°C for 2 h in a water bath. The reaction mixture was neutralized with 1 N NaOH and then extracted with EtOAc two times (each 5 mL) to give two parts of EtOAc partition and water solution. The EtOAc part was purified by HPLC to give the aglycone (2.1 mg) of **3**. The aglycone and curvularin (**5**) had very close optical rotation values { [α]_D²⁰ –22.8 (*c* 0.10, MeOH) for the aglycone; –23.6 (*c* 0.10, MeOH) for **5**} and a same HPLC retention time (*t_R*: 14.7 min). The water solution was dried under reduced pressure to give a residue. This residue was first treated with 10 mg hydroxylamine hydrochloride in 2 mL pyridine at 90°C for 30 min in a water bath. After cooling, it was mixed with 2 mL acetic anhydride at 90°C for 1 h in a water bath. Then the reaction products were concentrated *in vacuo* to dryness and finally dissolved in 2 mL chloroform. The sugar acetyl derivatives were identified as D-glucose (*t_R* 11.9 min) by GC analysis with standard D-glucose (*t_R* 12.0 min) as reference.

Preparation of compounds 5a–5c

Compound **5** (30.6 mg) was dissolved in pyridine/Ac₂O (2:1, 9 mL) and the mixture was allowed to stand at room temperature for 24 h. After addition of 30 mL of H₂O, the resulting mixture was evaporated *in vacuo* to dryness and then separated by HPLC (mobile phase: MeOH/H₂O, 65:35;

flow rate: 1.0 mL/min; detection wavelength: 256 nm) to afford **5a** (6.3 mg, t_R 18.3 min), **5b** (14.1 mg, t_R 19.6 min), and **5c** (11.3 mg, t_R 22.3 min).

Compound 5a: colorless amorphous powder; molecular formula $C_{18}H_{22}O_6$, UV: λ_{max} 225, 275 nm; 1H NMR data (500 MHz, in methanol- d_4) and ^{13}C NMR data (125 MHz, methanol- d_4), see Tables S₂ and S₄; HRESIMS m/z $[M+Na]^+$ 357.1308 (calcd for $C_{18}H_{22}NaO_6$, 357.1314) and $[M-Ac+Na]^+$ 315.1200 (calcd for $C_{16}H_{20}NaO_5$, 315.1208).

The ^{13}C and 1H NMR data of known compounds **1**, **2**, **4**, **5**, **5b**, and **5c** were summarized in Tables S₂, S₃, and S₄.

Biological activity assay

Tumor cell culture

Rat glioma C6 and human glioma U251 cells were cultured in DMEM (Dulbecco's Modified Eagle Medium, Gibco) with 10% FBS (Fetal Bovine Serum, PAA Laboratories Inc.), human glioma U87-MG cells in MEM (Minimum Essential Medium, Gibco), human glioma SHG-44 and colorectal cancer HCT-15 cells in RPMI-1640 (Roswell Park Memorial Institution 1640, Gibco), and human colorectal cancer SW620 cells were grown in Leibovitz's L15 medium. SW620 cells were incubated at 37°C in a humidified incubator without CO₂, while other cells were incubated at 37°C in a humidified incubator with 5% CO₂. Cells after third generation were used for experiment.

Sulforhodamine B (SRB) assay

SRB assay as described in the previous publications (Xin et al., 2012; Yu et al., 2014) was used to determine the activity of isolated compounds suppressing the proliferation of glioma U87-MG, U251, SHG-44, C6 cells, and colorectal cancer HCT-15 and SW620 cells. Doxorubicin (DOX) were used as positive controls.

Statistical Analysis

Data were analyzed by statistical software Statistical Product and Service Solutions (SPSS, PASW Statistics 18) and all data were expressed as means $\pm$ SD (standard deviation).

Antibacterial assay

Preparation of stock solution of tested compounds

The stock solution of different purified compounds and positive control drug norfloxacin was prepared in 50% DMSO and final concentration was 100 μ g/mL.

Determination of antibacterial activity by spot method

Escherichia coli and *Staphylococcus aureus* were grown in nutrient broth (NB) inside shaking incubator at 180 rpm for 12 h and temperature was 28°C and then the optical density (OD) of the cultured seed broth was 0.08 to 0.1 by measured at 550 nm. A mixture of 250 µL of culture seeds and 25 mL of Mueller Hinton Agar (MHA, 21 g MHB and 17 g agar in 1000 mL distilled water) was poured on the surface of Petri plates. After the surface of plates was dried, a 5 µL aliquot of tested compounds (100 µg/mL) was spotted onto the surface of the plates and let that dry for half an hour inside the cabinet. The plates were incubated at 37°C for 5 h for *E. coli* and 10 h for *S. aureus*. All positive samples were identified by clear zones of inhibition.

Determination of MIC and MBC by micro broth dilution method

MIC and MBC of the positive compounds against *E. coli* and *S. aureus* were determined by micro broth dilution method and norfloxacin was used as positive control. 96-well plates were used to make dilutions of the tested compounds. The stock solution of tested compounds was diluted with 50% DMSO to make a series of concentrations and the final volume was 200 µL. After that 2 µL from 10⁸cf/mL of culture was added and the plates were incubated at 37°C for 12 h overnight. Next day, plates were observed by eye and those dilutions, which were completely cleared along with the next dilutions, were plated over nutrient agar plates. The agar plates were incubated at 37°C for 12 h and the results were recorded next day. The dilution that inhibited the growth of bacteria was assigned as MIC and the dilution that completely killed the bacteria was considered as MBC of that compound.

Table S2. ¹³C NMR data for compounds **1–5**, **3a**, and **5a–5c** (125 MHz, in methanol-*d*₄)

No	1	2	3	3*	3a*[#]	4	5	5a	5b	5c
1	172.8	172.6	172.8	171.1	171.0	173.2	173.0	172.4	172.5	171.8
2	40.4	40.6	40.6	39.2	39.1	42.6	40.6	40.0	39.6	39.2
3	137.5	138.2	136.6	136.1	135.5	137.2	137.3	136.8	136.2	135.7
4	112.4	112.8	114.1	113.7	113.9	112.4	112.4	118.3	117.5	117.0
5	161.7	162.0	161.2	160.0	159.8	162.5	161.3	160.8	157.6	152.7
6	102.9	103.1	102.9	102.6	102.7	103.0	102.9	110.1	109.6	124.1
7	160.0	160.9	158.1	157.5	157.4	162.3	159.6	151.1	153.6	149.2
8	120.7	120.2	124.1	124.3	124.7	118.0	121.1	126.6	127.5	133.2
9	206.7	205.8	209.9	207.0	207.0	200.0	209.9	207.0	209.7	206.6
10	54.9	53.7	44.8	44.7	44.0	133.5	44.8	44.4	44.3	44.0
11	67.8	68.7	24.0	23.6	23.4	154.4	24.0	23.8	23.4	23.2
12	35.7	35.1	27.9	27.9	27.8	35.3	27.9	27.8	28.1	28.1
13	23.2	19.4	25.0	25.2	24.7	25.6	25.0	25.1	25.4	25.1
14	32.7	32.5	33.1	33.2	33.4	34.4	33.1	33.5	33.4	33.9
15	74.9	72.5	73.9	73.0	72.8	74.4	74.0	74.2	74.4	74.4
16	21.6	19.3	20.6	20.8	20.7	20.5	20.6	20.7	20.8	20.8
Glc/Ac										
1'			99.8	99.5	102.3			170.6	170.7	170.1, 170.3
2'			74.4	73.1	74.6			21.2	21.1	21.1, 21.2
3'			75.0	74.8	77.8					
4'			71.3	71.5	71.3					
5'			75.2	75.0	78.2					
6'			62.3	62.6	62.6					

The data* were recorded in acetone-*d*₆. [#] The NMR data of **3a** were from reference (Zhang and Gunatilaka, 2005).

Table S3. ¹H NMR data for compounds **1–3**, and **3a** (500 MHz, in methanol-*d*₄, *J* = Hz)

No	1	2	3	3*	3a*[#]
2	3.57, 1H, dd (15.7, 6.1); 3.93, 1H, dd (15.7, 9.7)	3.58, 1H, dd (15.6, 6.1); 3.93, 1H, dd (15.6, 9.8)	3.59, 1H, d (9.8); 3.61, 1H, d (9.8)	3.69, 1H, d (11.5) ; 3.70, 1H, d (11.5)	3.72, 2H, m
4	6.22, 1H, d (2.3)	6.22, 1H, d (2.2)	6.41, 1H, d (2.1)	6.49, 1H, d (2.2)	6.51, 1H, d (2.1)
6	6.27, 1H, d (2.3)	6.28, 1H, d (2.2)	6.77, 1H, d (2.1)	6.81, 1H, d (2.2)	6.68, 1H, d (2.1)
10	3.23, 2H, m	3.22, 2H, m	2.80, 1H, m; 3.18, 1H, m	2.80, 1H, m; 3.25, 1H, m	2.86, 1H, m; 3.15, 1H, m
11	4.05, 1H, m	4.06, 1H, m	1.41, 1H, m; 1.79, 1H, m	1.41, 1H, m; 1.77, 1H, m	1.35, 1H, m; 1.79, 1H, m
12	1.37, 1H, m; 1.45, 1H, m	1.42, 1H, m; 1.45, 1H, m	1.24, 1H, m; 1.42, 1H, m	1.25, 1H, m; 1.43, 1H, m	1.26, 1H, m; 1.43, 1H, m
13	1.39, 1H, m; 1.47, 1H, m	1.43, 1H, m; 1.46, 1H, m	1.28, 1H, m; 1.46, 1H, m	1.27, 1H, m; 1.45, 1H, m	1.27, 1H, m; 1.44, 1H, m
14	1.58, 1H, m; 1.65, 1H, m	1.62, 1H, m; 1.68, 1H, m	1.48, 1H, m; 1.60, 1H, m	1.46, 1H, m; 1.61, 1H, m	1.41, 1H, m; 1.65, 1H, m
15	4.96, 1H, m	4.97, 1H, m	4.92, 1H, m	4.90, 1H, m	4.97, 1H, m
16	1.12, 3H, d (6.3)	1.12, 3H, d (6.3)	1.13, 3H, d (6.3)	1.11, 3H, d (6.3)	1.13, 3H, d (6.3)
Glc-1'			5.51, 1H, d (2.3)	5.49, 1H, d (3.1)	4.94, 1H, d (7.6)
2'			3.54, 1H, m	3.60, 1H, m	3.42, 1H, m
3'			3.56, 1H, m	3.62, 1H, m	3.51, 1H, mm
4'			3.43, 1H, t (9.8)	3.47, 1H, t (9.6)	3.39, 1H, m
5'			3.77, 1H, m	3.79, 1H, m	3.49, 1H, m
6'			3.69, 1H, m; 3.76, 1H, m	3.71, 1H, m; 3.77, 1H, m	3.69, 1H, m; 3.89, 1H, m

The data* were recorded in acetone-*d*₆. [#] The NMR data of **3a** were from reference (Zhang and Gunatilaka, 2005).

Table S4. ^1H NMR data for compounds **4**, **5** and **5a–5c** (500 MHz, in methanol- d_4 , $J = \text{Hz}$)

No	4	5	5a	5b	5c
2	3.44, 1H, d (16.6); 3.72, 1H, d (16.6)	3.61, 1H, d (16.0); 3.85, 1H, d (16.0)	3.69, 1H, d (16.0); 3.82, 1H, d (16.0)	3.68, 1H, d (16.0); 3.76, 1H, d (16.0)	3.76, 2H, s
4	6.25, 1H, d (2.3)	6.22, 1H, d (2.3)	6.52, 1H, d (2.3)	6.56, 1H, d (2.1)	7.02, 1H, d (2.2)
6	6.29, 1H, d (2.3)	6.26, 1H, d (2.3)	6.65, 1H, d (2.3)	6.60, 1H, d (2.1)	7.04, 1H, d (2.2)
10	6.49, 1H, d (15.6)	2.72, 1H, dddd (9.7, 2.7); 3.18, 1H, dddd (9.7,2.7)	2.68, 1H, dddd (9.6, 2.9); 2.85, 1H, dddd (9.6,2.9)	2.81, 1H, dddd (9.8, 2.8); 3.13, 1H, dddd (9.8, 2.8)	2.77, 1H, dddd (9.6,2.8); 2.83, 1H, dddd (9.6, 2.8)
11	6.56, 1H, m	1.59, 1H, m; 1.74, 1H, m	1.55, 1H, m; 1.75, 1H, m	1.59, 1H, m; 1.79, 1H, m	1.46, 1H, m; 1.84, 1H, m
12	1.26, 1H, m; 1.40, 1H, m	1.26, 1H, m; 1.40, 1H, m	1.26, 1H, m; 1.42, 1H, m	1.26, 1H, m; 1.44, 1H, m	1.30, 1H, m; 1.41, 1H, m
13	1.29, 1H, m; 1.43, 1H, m	1.29, 1H, m; 1.43, 1H, m	1.30, 1H, m; 1.46, 1H, m	1.30, 1H, m; 1.46, 1H, m	1.28, 1H, m; 1.43, 1H, m
14	1.49, 1H, m; 1.56, 1H, m	1.49, 1H, m; 1.56, 1H, m	1.49, 1H, m; 1.60, 1H, m	1.47, 1H, m; 1.61, 1H, m	1.44, 1H, m; 1.61, 1H, m
15	4.80, 1H, m	4.95, 1H, m	4.95, 1H, m	4.96, 1H, m	4.99, 1H, m
16	1.19, 3H, d (6.3)	1.11, 3H, d (6.3)	1.14, 3H, d (6.3)	1.13, 3H, d (6.4)	1.14, 3H, d (6.3)
Ac-2'			2.26, 3H, s	2.25, 3H, s	2.22, 3H, s; 2.24, 3H, s