Electronic supplementary materials *Mendeleev Commun.*, 2016, **26**, 191−192

Nambiscalarane, a novel sesterterpenoid comprising a furan ring, and other secondary metabolites from bioluminescent fungus *Neonothopanus nambi*

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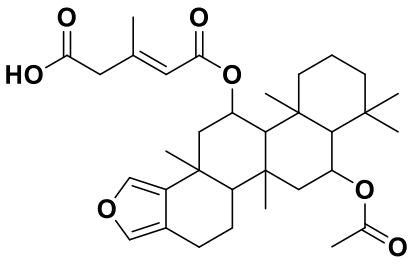
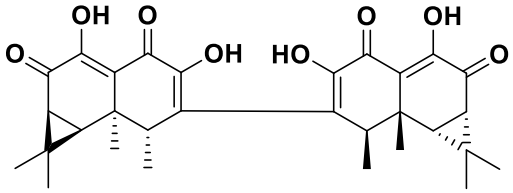
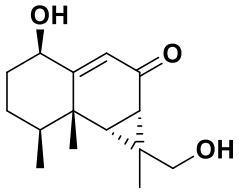


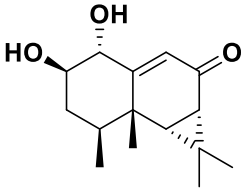
1. Nambiscalarane



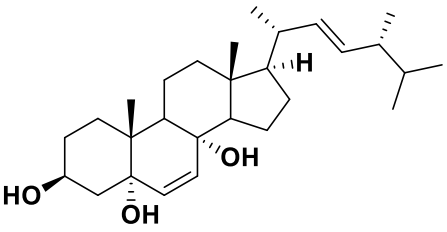
2. Aurisin Z



3. Nambinone A



4. Nambinone C



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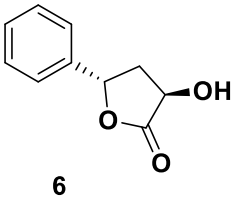

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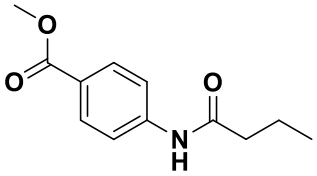

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NMR spectroscopy

NMR spectra were measured on Bruker Avance 700 or 800 MHz NMR spectrometer equipped with cryoprobe. The following spectra were used to identify chemical structure: ^{1}H, ^{13}C, ^{13}C-DEPT-135, 2D DQF-COSY, 2D NOESY (200ms, 400ms), 2D ^{1}H-^{13}C HSQC, 2D ^{1}H-^{13}C HMBC (J_{long}=6Hz, 9Hz), 2D ^{1}H-^{13}C ADEQUATE-11.

Mass spectrometry

Samples analysis was performed on an Agilent 6224 TOF LC/MS System (Agilent Technologies, Santa Clara, CA, USA) equipped with a dual-nebulizer ESI source. Data acquisition and analysis was performed by the MassHunter Workstation software (Agilent Technologies, Santa Clara, CA, USA).

Fungal Material

The mycelium *N. nambi* was collected in Southeast Vietnam and originally isolated and identified by Dao Thi Van (BIO LUMI Company Ltd., Ho Chi Minh, Vietnam private collection).

Mycelium was cultivated at 27 °C on Petri dishes (90 mm diameter) using a non-buffered 2.0% (w/v) agar medium, containing 0.3% (w/v) malt extract (Difco), 0.3% (w/v) yeast extract (Helicon), 0.5% bacto peptone (Difco) and 1.0% of dextrose. From the Petri dishes the mycelium was inoculated and grown in submerged liquid cultures under orbital shaking (150 rpm) in 750 mL Erlenmeyer flasks in 250 mL of the same medium, without agar. Media were prepared using an autoclave set at 120 °C for 30 min and a laminar flow hood. Mycelium was harvested after 14 days at room temperature, emitting observable light in contact with air. Harvested culture was filtered, washed with deionized water, dried under vacuum and flushed with argon before use. Mycelium samples were grinded frozen with liquid nitrogen using a mortar and pestle or disrupted in the blender with extraction solvent.

Extraction and isolation

Light-emitting mycelium of *N. nambi* (400 g), grown in shake flasks using yeast medium, was harvested, dried under reduced pressure, powdered and extracted with 20% methanol solution in chloroform. After solvent evaporation 6 g of extract were obtained. Extract was subjected to column chromatography using silicagel 60 Å (d=7 cm, l=18 cm), and $CHCl_3$-MeOH mixtures with increasing polarity (0-30%), yielding 7 fractions (A-G): fraction A (100 ml $CHCl_3$; 0.8 g), fraction B (200 ml $CHCl_3$-MeOH; 5%, 1.2 g), fraction C (200 ml $CHCl_3$-MeOH; 15%, 1.2 g), fraction D (100 ml $CHCl_3$-MeOH; 15%, 0.4 g), fraction E (100 ml $CHCl_3$-MeOH; 20%, 0.5 g), fraction F (100 ml $CHCl_3$-MeOH; 25%, 0.3 g), fraction G (100 ml $CHCl_3$-MeOH; 30%, 0.6 g).

The methanol-soluble part of the fraction C (0.72 g) was separated using HPLC (Diasorb-130-C16T as stationary phase) eluted with gradient H_2O-MeCN (50-100%) mixture to afford 8 mg of amide **7** (at 50% MeCN), R_f 0.55 (silicagel, $CHCl_3$-MeOH, 2%) and 9 mg of steroid **5** (at 60% MeCN), R_f 0.29 (silicagel, $CHCl_3$-MeOH, 3%).

Fraction D was subjected to LPLC, using silicagel 60 Å (d=2.5 cm, l=10 cm), eluted with 10% EtOH-EtOAc to yield nambiscalarane (**1**), R_f 0.73 (silicagel, EtOH-EtOAc 10%)

Methanol-soluble part of the fraction E (0.2 g) was also separated using HPLC (Diasorb-130-C16T as stationary phase) eluted with gradient H_2O-MeCN (40-100%) mixture, yielding 10 mg of nambinone (**2**) (at 60% MeCN), R_f 0.60 (silicagel, $CHCl_3$-MeOH, 10%), 11 mg of nambienones (**3**) (at 40% MeCN), R_f 0.49 (silicagel, $CHCl_3$-MeOH, 10%) and 9 mg of nambienones (**4**) (at 45% MeCN), R_f 0.49 (silicagel, $CHCl_3$-MeOH, 10%)

Mycelium of N. nambi was filtered from YM culture medium. Filtrate (50 ml), was acidified with hydrochloric acid to pH 2.5 then extracted with 5% methanol solution in chloroform. The resulting extract

was dried, 50 mg of residue was dissolved in 1 ml of chloroform and subjected to preparative TLC (Partisil PK6F,1000 µm), eluted with 10% methanol solution in chloroform to yield 8 mg of lactone **6** R_f 0.59 (silicagel, $CHCl_3$-MeOH, 10%).

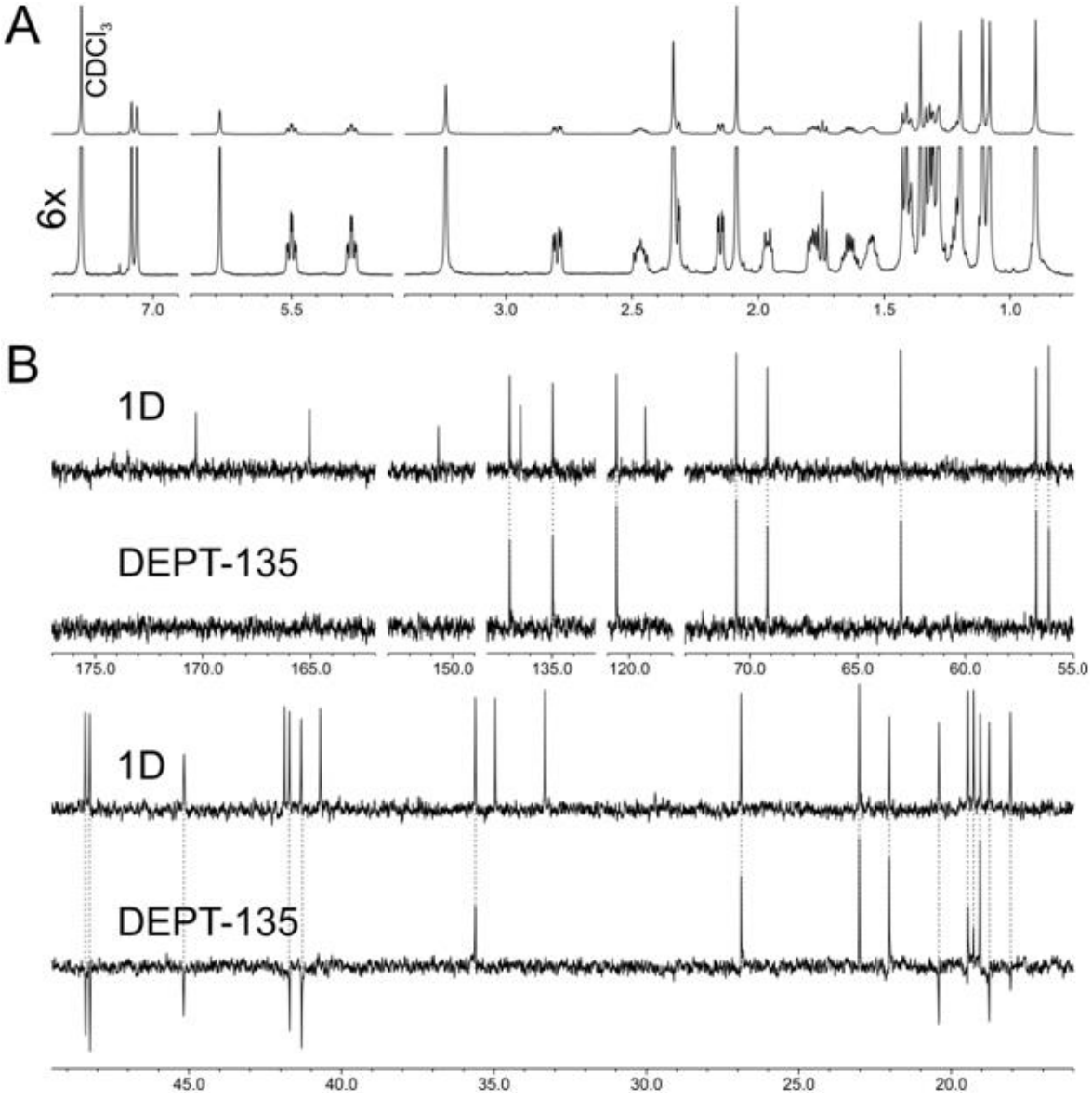
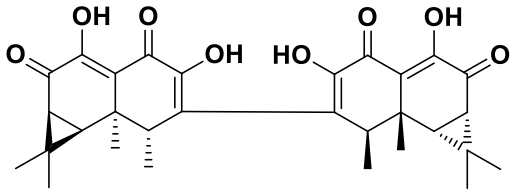


Figure S1 NMR spectra of **1 A** ^{1}H NMR and **B** ^{13}C NMR. Comparison of 1D ^{13}C (up) and DEPT-135 carbon spectrum (down) illustrates difference between CH_3, CH_2, CH and carbon atoms without attached protons.

Table S1 Chemical shifts, multiplicities and observed HMBC connectivities in NMR of nambiscalarane **1**.

Atom	δ ^{1}H, mult	δ ^{13}C	HMBC (^{1}H→^{13}C)
1	1.962 dt, d(13.5 Hz) ×t(5.2 Hz) 1.321 td, t(13.6 Hz) ×d(5.2 Hz)	41.37	C2, C3, C5, C9, C10, C22
2	1.551 m 1.416 m	18.78	C1, C3, C4, C10
3	1.402 m 1.215 m	41.75	C1, C2, C4, C5, C19, C20
4	—	33.35	
5	1.323 d(11.7 Hz)	56.77	C1, C3, C4, C6, C7, C9, C10, C19, C20, C22
6	5.262 td , t(11.3 Hz)×d(3.7 Hz)	70.68	C4, C5, C7, C10, C1''
7	2.150 dd(11.8 Hz, 3.7 Hz) 1.104 t(11.8 Hz)	48.45	C4, C5, C6, C8, C9, C11, C14, C21
8	—	40.71	
9	1.417 d(11.2 Hz)	63.12	C1, C5, C10, C8, C14, C11, C12, C21, C22
10	—	41.98	
11	5.498 td, t(11.1 Hz)×d(3.4 Hz)	69.18	C8, C9, C12, C13, C1'
12	2.322 dd(11.5 Hz, 3.4 Hz) 1.743 t(11.6 Hz)	48.33	C9, C10, C11, C13, C14, C15, C18, C23
13	—	35.00	
14	1.294 dd(13.7 Hz, 4.8 Hz)	56.18	C7, C8, C9, C12, C13, C18, C15, C16, C21, C23
15	1.783 dd(13.3 Hz, 7.8 Hz) 1.634 qd, q(12.5 Hz)×d(6.3 Hz)	18.13	C8, C13, C14, C16, C17
16	2.795 dd(16.3 Hz, 6.0 Hz) 2.465 ddd(16.0 Hz, 14.0 Hz, 6.0 Hz)	20.46	C14, C15, C17, C18, C24
17	—	119.22	
18	—	136.46	
19	1.081 s	35.66	C3, C4, C5, C20
20	0.897 s	23.09	C3, C4, C5, C19
21	1.196 s	19.51	C7, C8, C9, C14
22	1.108 s	19.31	C1, C5, C9, C10
23	1.353 s	26.94	C12, C13, C14, C18
24	7.058 d(1.1 Hz)	136.96	C17, C18, C25
25	7.081 d(1.1 Hz)	134.91	C17, C18, C24
1'	—	165.10	
2'	5.799 d(0.8 Hz)	120.51	C1', C3', C4', C6'
3'	—	150.88	
4'	3.231 s	45.55	C2', C3', C5', C6'
5'	—	174.50	
6'	2.331 d(0.8 Hz)	19.10	C1', C2', C3', C4'
1''	—	170.36	
2''	2.085 s	22.08	C1''



2. Aurisin Z

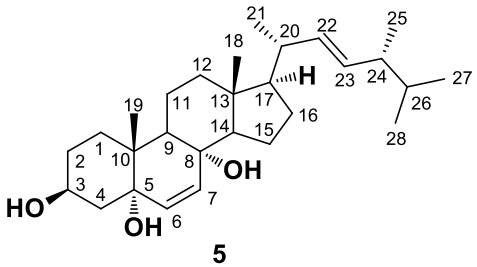
(1aS,1'aS,7R,7aS,7bR,7'R,7'aS,7'bR)-3,3',5,5'-tetrahydroxy-1,1,1',1',7,7a,7',7'a-octamethyl-7,7a,7',7'a-tetrahydro-1H,1'H-[6,6'-bi(cyclopropa[a]naphthalene)]-2,2',4,4'(1aH,1'aH,7bH,7'bH)-tetraone (**2**) yellowish solid, mp over 250 ^{o}C with decomposition.

^{1}H NMR (700 MHz, $CDCl_3$) δ 12.74 (bs, 1H), 6.44 (bs, 1H), 3.66 (q, J = 7.3, 1H), 2.12 (d, J = 7.8 Hz, 1H), 1.66 (d, J = 7.8 Hz, 1H), 1.390 (s, 3H), 1.385 (s, 1H), 1.33 (s, 3H), 1.28 (d, J = 7.3 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 13.1, 16.7, 26.5, 28.4, 29.9, 37.4, 37.7, 38.3, 38.9, 119.9, 130.2, 142.2, 157.4, 186.2, 190.6; HRMS m/z 523.2330 (calcd for $C_{30}H_{35}O_8$ $[M+H]^{+\bullet}$, 523.2326).

Table S2 Chemical shifts and multiplicities in NMR spectra of aurisin Z **2** and nambinones **3** and **4.**

Compound	Aurisin **Z** (**2**)		Nambinone **A** (**3**)		Nambinone **C** (**4**)	
Atom	δ ^{1}H, mult	δ ^{13}C	δ ^{1}H, mult	δ ^{13}C	δ ^{1}H, mult	δ ^{13}C
1	—	186.2	4.41^{eq}	73.3	4.21 bd, (9.8 Hz)	74.6
2	—	142.2	2.03^{eq} q(2.8 Hz)×d(14.3 Hz) 1.63^{ax} t(3.6 Hz)×t(13.9 Hz)	32.7	3.48 bt, (11 Hz)	74.0
3	—	130.2	1.87^{ax} d(2.9 Hz)×q(13.3 Hz) 1.43^{eq} q(3.2 Hz)×d(13.2 Hz)	24.8	1.56 q (12 Hz) 1.91-1.88 m	36.0
4	3.66 q (7.3 Hz)	38.3	1.82-1.77^{ax} m	38.7	1.96-1.92 m	35.3
5	—	38.9	—	38.5	—	39.4
6	1.66 d (7.8 Hz)	37.7	1.56 d(8.2 Hz)	36.2	1.41 d (8.1 Hz)	39.3
7	2.12 d (7.8 Hz)	37.4	1.91 dd(8.2 Hz, 1 Hz)	32.4	1.78 d (8.1 Hz)	34.9
8	—	190.6	—	196.4	—	196.2
9	—	157.4	5.92 d(1 Hz)	127.4	6.25 bs	122.0
10	—	119.9	—	165.4	—	164.7
11	—	28.4	—	30.2	—	24.5
12	1.33 s	29.9	1.26 s	11.8	1.22 s	29.7
13	1.39 s	16.7	3.52, 3.39 AB(11 Hz)	71.6	1.21 s	16.5

14	1.39 s	26.5	1.42 s	24.7	1.23 s	23.5
15	1.28 d (7.3 Hz)	13.1	1.12 d(6.8 Hz)	16.1	1.13 d (6.6 Hz)	15.8
OH-2	6.44 bs	—	—	—	2.64 d (2.8 Hz)	—
OH-9	12.74 bs	—	—	—	—	—
OH-1	—	—	1.69 bs	—	2.9 d (4.5 Hz)	—
OH-13	—	—	1.58 bs	—	—	—



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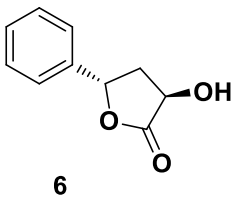
(3*S*,5*S*,8*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*R*,*E*)-5,6-dimethylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,5,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,5,8-triol (**5**) amorphous white powder.[1]

^{1}H NMR (700 MHz, $CDCl_3$) δ 6.52 (d, J = 8.6 Hz, 1H), 6.26 (d, J = 8.6 Hz, 1H), 5.25 (dd, J = 15.1 Hz, 7.7 Hz, 1H), 5.17 (dd, J = 15.1 Hz, 8.6 Hz, 1H), 4.02-3.98 (m, 1H), 2.15-2.10 (m, 1H), 2.05-1.23 (m, 22H, see Table S1), 1.02 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 6.8 Hz, 3H), 0.91 (s, 3H), 0.86-0.83 (m, 9H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 12.9, 17.6, 18.2, 19.7, 20.0, 20.7, 20.9, 23.5, 28.7, 30.2, 33.1, 34.8, 37.0, 39.4, 39.7, 42.8, 44.6, 51.2, 51.7, 56.3, 66.5, 79.4, 82.1, 130.7, 132.3, 135.2, 135.4. HRMS m/z 431.3513 (calcd for $C_{28}H_{47}O_3$ $[M+H]^{+\bullet}$, 431.3520).

Table S3 Chemical shifts in ^{1}H and ^{13}C NMR spectra of Ergosta-type steroid **5**.

Atom	δ ^{1}H	δ ^{13}C
1	1.98, 1.72	34.8
2	1.87, 1.56	30.2
3	4.00	66.5
4	2.13, 1.93	37.0
5	-	82.1
6	6.26	135.4
7	6.52	130.7
8	-	79.4
9	1.52	51.2
10	-	37.0
11	1.53, 1.25	23.5
12	1.98, 1.26	39.4
13	-	44.6
14	1.58	51.7
15	1.63, 1.44	20.7
16	1.78, 1.38	28.7
17	1.25	56.3
18	0.84	12.9
19	0.91	18.2
20	2.05	39.7
21	1.02	20.9
22	5.17	135.2
23	5.25	132.3
24	1.88	42.8
25	0.93	17.6
26	1.49	33.1
27	0.86	20.0
28	0.84	19.7



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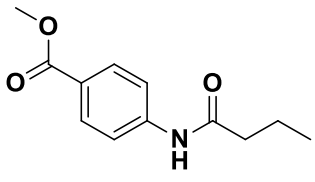
(3*R*,5*S*)-3-hydroxy-5-phenyltetrahydrofuran-2-one (**6**)[2]

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.41 (t, J = 7.5 Hz, 2H), 7.36 (t, J = 7.5 Hz, 1H), 7.31 (d, J = 7.5 Hz, 2H), 5.72 (dd, J = 7.7 Hz, 4.3 Hz, 1H), 4.6 (dd, J = 7.7 Hz, 7.6 Hz, 1H), 2.72-2.60 (m, 2H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 38.2, 67.2, 78.5, 125.0, 128.5, 128.9, 138.9, 176.8.; HRMS m/z 179.0708 (calcd for $C_{10}H_{11}O_3$ $[M+H]^{+\bullet}$, 179.0703).



7

Methyl 4-butyramidobenzoate (**7**)

Synthesis[3]:

To a solution of methyl 4-aminobenzoate hydrochloride (1.3 g, 6.9 mmol) and DIPEA (2.68 g, 20.1 mmol) in anhydrous $CHCl_3$ (25 mL) dropwise was added butyryl chloride (0.89 g., 8.32 mmol) at -10 °C. After stirring for 2h at ambient temperature, the reaction was washed successively with H_2O (2x20 mL), 0.5N HCl (20 mL), and again with H_2O (20 mL), dried over Na_2SO_4, and evaporated *in vacuo* to give methyl 4-(*n*-butyroylamino)-benzoate (1.25 g, 80%) as a colorless solid.

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.61 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.53 (br. s, 1H, N*H*), 3.89 (s, 3H, C*H*$_3$O), 2.36 (t, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_2$CH$_3$), 1.76 (h, *J* = 7.4 Hz, 2H, CH$_2$C*H*$_2$CH$_3$), 1.00 (t, *J* = 7.4 Hz, 3H, CH$_2$C*H*$_3$); ^{13}C NMR (176 MHz, $CDCl_3$) δ 13.7, 18.9, 39.7, 52.0, 118.8, 125.5, 130.8, 142.2, 166.6, 171.5; HRMS m/z 222.1129 (calcd for $C_{12}H_{16}NO_3$ $[M+H]^{+\bullet}$, 222.1125).

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[1] A. Rivera, O. L. Benavides and J. Rios-Motta, *Nat. Prod. Res.*, 2009, **23**, 293.

[2] B. Chen, H.-F. Yin, Z.-S. Wang, J.-H. Xu, L.-Q. Fan and J. Zhao, *Adv. Synth. Catal.*, 2009, **351**, 2959.

[3] F. Karaki, K. Ohgane, H. Fukuda, M. Nakamura, K. Dodo and Y. Hashimoto, *Bioorg. Med. Chem.*, 2014, **22**, 3587.