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FASCIOSPYRINADINONE AND FASCIOSPYRINADINOL, NOVEL 3-ALKYLPYRIDINE SESQUITERPENOIDS FROM AN INDONESIAN MARINE SPONGE, AS SELECTIVE GROWTH INHIBITORS OF THE CANCER CELLS UNDER NUTRIENT STARVATION

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Dedicated to Professor Yasuyuki Kita on the occasion of his 77th birthday

Abstract – On the guidance of the bioassay using PANC-1 cells adapted to the glucose starvation, two novel pyridine sesquiterpenoids named fasciospyrinadinone (**1**) and fasciospyrinadinol (**2**) were isolated from the Indonesian marine sponge of *Petrosaspongia* sp. The chemical structures of the compounds were established on the basis of the 1D and 2D NMR spectra data. Then, we accomplished the first total syntheses of **1** and **2**. Compound **1** showed selective growth inhibitory activity against PANC-1 cells adapted to glucose starvation, with IC_{50} value of 13 μM.

INTRODUCTION

Pancreatic cancer tissue is known to contain hypoxic and nutrient-starved regions caused by its hypovascular nature.[1] Some cancer cells can adapt to these severe environments in tumors, resulting in acquiring drug resistance. In addition, they aggravate the cancerous pathology by promoting tumor growth, angiogenesis, and metastasis.[2] So, compounds that exhibit selective growth inhibitory activities under these conditions could be novel drug leads for cancer chemotherapy. Following these backgrounds, there have been several reports of the selective growth inhibitors against the cancer cells adapted to

nutrient starvation.[3-8] However, little is known about the adaptation mechanism of the cancer cells against nutrient starvation.

In the course of our study of bioactive substances from marine organisms, we also established a screening system searching for selective growth inhibitors against the cancer cells adapted to the glucose-deficient condition and isolated some active constituents.[9-14] Further bioassay-guided screening of the marine medicinal resources led us to isolate two novel pyridine sesquiterpenoids named fasciospyrinadinone (**1**) and fasciospyrinadinol (**2**), from an Indonesian marine sponge of *Petrosaspongia* sp. In this paper, we report about isolation, structure elucidation, total synthesis, and biological evaluation of **1** and **2**.

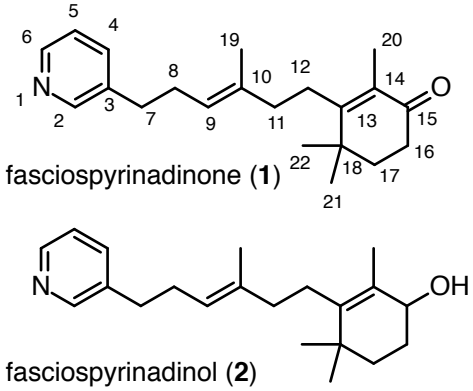


Figure 1. Chemical structures of fasciospyrinadinone (**1**) and fasciospyrinadinol (**2**)

RESULTS AND DISCUSSION

Recently, we have isolated four novel polyketides named biakamides A–D as selective growth inhibitors of the human pancreatic cancer PANC-1 cells adapted to nutrient starvation, from the marine sponge *Petrosaspongia* sp.[11] Further examination of the MeOH extract of the same sponge through bioassay-guided fractionation by SiO_2 column chromatography, octa-decyl silica (ODS) HPLC afforded two active substances, designated fasciospyrinadinone (**1**, 18 mg) and fasciospyrinadinol (**2**, 16 mg).

Fasciospyrinadinone (**1**) was obtained as yellow amorphous powders. The FAB-MS of **1** showed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 312, and the molecular formula was determined as $C_{21}H_{29}NO$ by high-resolution FAB-MS analysis. ^1H and ^{13}C NMR spectra for **1**, summarized in Table 1, also supported the molecular formula. Considering the ^{13}C NMR and HSQC spectra, compound **1** consisted of four methyls, six methylenes, one quaternary sp^3 cabon, five olefinic methines, four quaternary sp^2 carbons, and one carbonyl carbon. The detailed analysis of H–H COSY and HMBC spectra of **1** allowed us to deduce three partial structures A, B, and C as shown in Figure 2. ^1H and ^{13}C NMR signals in the aromatic region [δ_H 8.42 (s), 8.39 (d, J = 5.0 Hz), 7.63 (d, J = 8.0 Hz) and 7.29 (dd, J = 8.0 and 5.0 Hz); δ_C 149.8, 147.0, 137.0, 135.9 and 123.2] together with their COSY and/or HMBC correlations suggested

that the partial structure A was a 3-substituted pyridine ring. And, the partial structure C was easily deduced by the characteristic ^{13}C NMR signals of an α,β-unsaturated ketone [δ_C 197.5, 164.2 and 129.7] and typically shielded α-methyl group at δ_C 11.2. Furthermore, the connectivity between three partial structures was clarified by the HMBC correlations from H-7 to C-2/C-3/C-4 and from H-12 to C-13/C-14/C-18. Finally, the geometry of the double bond at $\Delta^{9(10)}$ was determined as *E*-configuration based on the NOESY correlation observed between H_2-8 and H_3-19. Thus, the gross structure of fasciospynadinone (**1**) was determined as shown in Figure 1.

Fasciospyrinadinol (**2**) was also obtained as yellow amorphous powders. The FAB-MS of **2** showed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 314, and the molecular formula was determined as $C_{21}H_{31}ON$ by high-resolution FAB-MS analysis. The ^1H and ^{13}C NMR spectra for **2** were quite similar to those of **1**, except that a secondary alcohol moiety (δ_C 68.1, δ_H 3.68) might be contained in **2** instead of a ketone group (δ_C 197.5) in **1** (Table 1). The IR absorption around 3345 cm^{-1} also supported the structure. Further, HMBC correlations from OH signal to C-14/C-15/C-16, and from H_1-15 to C-13/C-14/C-16/C-17/C-20 affirmed the above deduction. Therefore, the structure of **2** was determined as a reduced form of **1**. Then we attempted to determine the absolute configuration of **2** through modified Mosher's method. However, esterification of each (*R*) or (*S*)-MTPA and **2** gave two MTPA esters, and the spectral data of the respective MTPA esters were almost identical. It clearly shows that **2** was obtained as a racemate.

Table 1. ^1H and ^{13}C NMR data for fasciospyrinadinone (**1**) and fasciospyrinadinol (**2**) in DMSO-d_6

Position	**1**		Position	**2**	
	δ_C[a]	δ_H[b]		δ_C[a]	δ_H[b]
1			1		
2	149.8	8.42 (s)	2	149.7	8.41 (s)
3	137.0		3	137.0	
4	135.9	7.63 (d, 8.0)	4	135.9	7.62 (d, 8.0)
5	123.2	7.29 (dd, 8.0, 5.0)	5	123.2	7.28 (dd, 8.0, 5.0)
6	147.0	8.39 (d, 5.0)	6	147.0	8.38 (d, 5.0)
7	32.2	2.63 (t, 7.5)	7	32.3	2.62 (t, 7.5)
8	29.0	2.28 (dt, 7.0, 7.5)	8	29.0	2.26 (dt, 7.0, 7.5)
9	123.3	5.21 (t, 7.0)	9	122.5	5.15 (t, 7.0)
10	135.7		10	136.5	
11	38.0	2.01 (m)	11	39.4	1.93 (br)
12	29.6	2.20 (m)	12	27.7	1.93 (br)
13	164.2		13	138.9	
14	129.7		14	130.0	
15	197.5		15	68.1	3.68 (m)
16	33.8	2.36 (t, 7.0)	16	28.7	1.68 (m), 1.49 (m)
17	36.8	1.73 (t, 7.0)	17	34.7	1.56 (m), 1.24 (m)
18	36.0		18	35.0	

19	15.6	1.52 (s)	19	15.7	1.49 (s)
20	11.2	1.66 (s)	20	16.4	1.62 (s)
21	26.5	1.12 (s)	21	27.3	0.91 (s)
22	26.5	1.12 (s)	22	28.2	0.97 (s)
			OH		4.47 (d, 6.0)

[a] ^{13}C NMR: δ_C (ppm); [b] ^{1}H NMR: δ_H (ppm, *J* in Hz).

There have been some related sponge-derived metabolites.[15-19] Among them, fasciospyrinadine (**3**), isolated from the marine sponge of *Fasciospongia* sp., possessed a quite similar structure to those of **1** and **2**. Those compounds might be generated through non-enzymatic allylic oxidation of **3** at C-15, although we could not find compound **3** in the extract of the sponge *Petrosaspongia* sp.

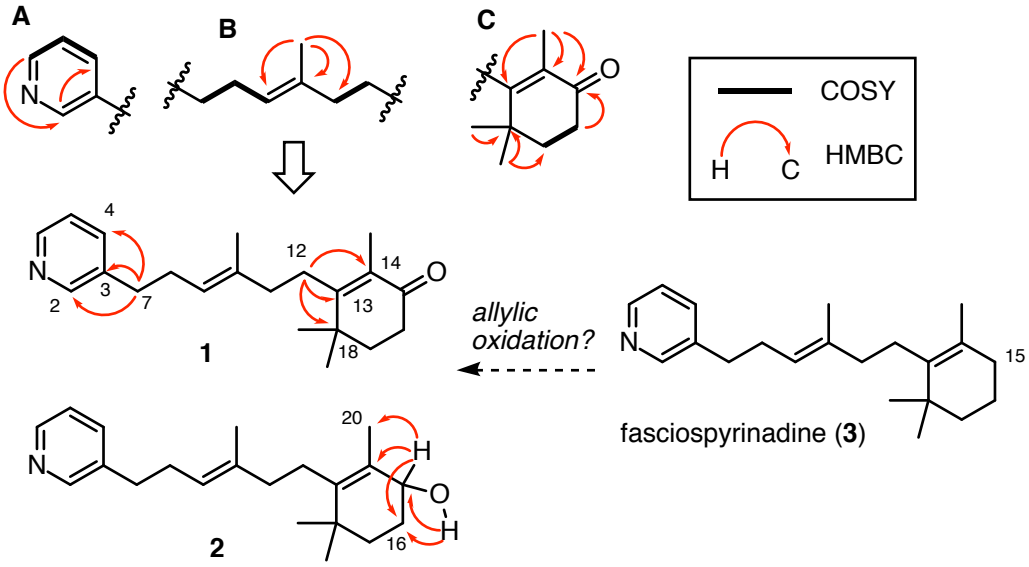
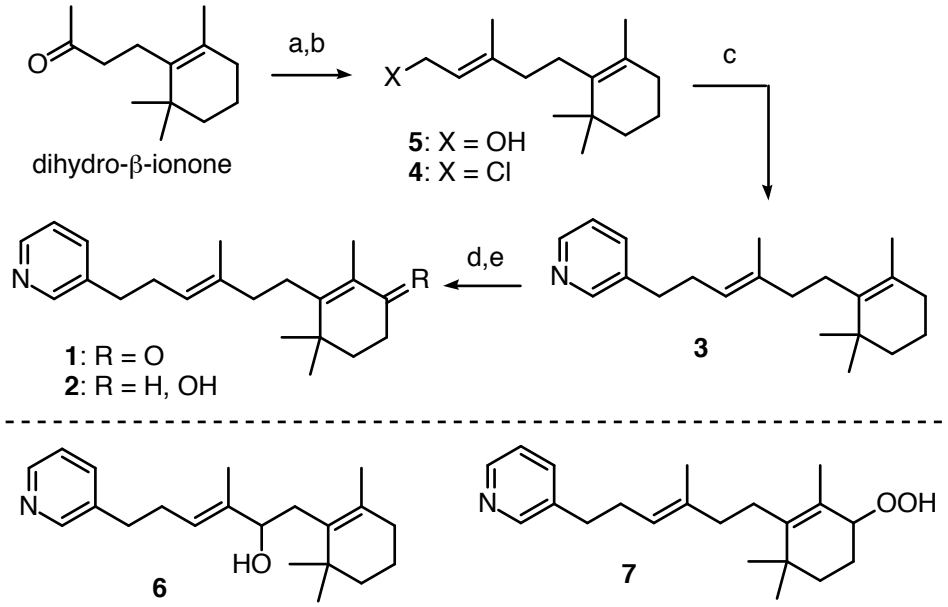


Figure 2. Partial structures and 2D NMR correlations of compounds **1** and **2**

Then, we attempted the total synthesis of fasciospyrinadinone (**1**) and fasciospyrinadinol (**2**), to supply sufficient amounts of the active compounds for further biological study, particularly *in vivo*. Based on the biosynthetic speculation as above, we anticipated that the selective allylic oxidation of C-15 position of fasciospyrinadine (**3**) might be possible leading to **1** and **2**. And, **3** could be easily elaborated through the coupling between 3-methylpyridine and the allylic halide compound **4**, derived from commercially available dihydro-β-ionone.

The actual synthesis of **1** and **2** was depicted in Scheme 1. Dihydro-β-ionone was converted to a known allylic alcohol **5**[20] in 89% overall yield. The concomitant geometrical isomer was separated by SiO_2 column chromatography. Subsequent Appel reaction provided the corresponding chloride compound **4** in 85% yield, which was reacted with the carbanion generated by mixing 3-picoline and lithium diisopropylamide (LDA) to provide **3** in good yield. Then, we attempted the allylic oxidation of **3**, using a

variety of oxidation conditions as depicted in Table 2. The use of selenium dioxide resulted in generating a regioisomer **6** (entry 1). In contrast, oxidations with $RuCl_3$/TBHP[21] or CuI/TBHP[22] provided the desired compound **1**, albeit in low yield (entry 2, 3). Among tested, the oxidation of **3** with the combination of $Cr(CO)_6$/TBHP[23] was found to be the best condition, to afford **1** in 35% yield (entry 4). Oxidation without metal catalyst provided the peroxide **7** (entry 5). Finally, **2** was obtained by Luche reduction of **1** in 79% yield. All of the spectral data of the synthetic **1** and **2** corresponds to those of the natural products.



Scheme 1. Total syntheses of compounds **1** and **2**. Reagents and conditions: a) $(EtO)_2P(O)CH_2CO_2Et$, BuLi, THF, –78 °C; DIBAL, THF, –78 °C; b) PPh_3, CCl_4, CH_2Cl_2, rt, 85%; c) 3-picoline, LDA, THF/DMPU, –78 °C to rt, 83%; d) $Cr(CO)_6$, TBHP, MeCN, 0 °C to rt, 35%; e) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0 °C, 79%.

Table 2. Screening of Reaction Conditions from **3** to **1**

Entry	Oxidant	Solvent	Time (h)	Yield [a]
1	SeO_2	1,4-dioxane	3	28% (**6**)
2	$RuCl_3 \cdot nH_2O$/TBHP	cyclohexane	24	19% (**1**)
3	CuI/TBHP	MeCN	24	22% (**1**)
4	$Cr(CO)_6$/TBHP	MeCN	24	35% (**1**)
5	TBHP	cyclohexane	24	26% (**7**)

[a] Yields of main product (shown in the parentheses) in each condition.

Fasciospyrinadinone (**1**) and fasciospyrinadinol (**2**) exhibited the growth inhibitory activity against PANC-1 cells under glucose-starved condition, with IC_{50} values of 13 μM and 18 μM, respectively (Table 3). In contrast, **1** and **2** did not show the cytotoxicity up to 100 μM treatment in the general glucose condition. The selective index (S.I.) value was estimated to be more than 5.6. Compound **6**, oxidation

regioisomer of **1**, exhibited the similar growth inhibitory activity, whereas fasciospyrinadine (**3**) lost the activity by half. These results suggested that the oxygen atom might be important for the growth inhibitory activity under glucose starvation.

Table 3. Growth inhibition of compounds **1**, **2**, **3**, and **6** against PANC-1 cells

	1		**2**		**3**		**6**	
	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.
Glucose (–)	13	-	18	-	40	-	14	-
Glucose (+)	>100	>7.7	>100	>5.6	>100	>2.5	>50	>3.5

$IC_{50} = \mu M$; S.I = selective index: IC_{50} in Glucose (+) medium / IC_{50} in Glucose (–) medium.

In summary, we isolated two novel sesquiterpenoids, designated fasciospyrinadinone (**1**) and fasciospyrinadinol (**2**), from an Indonesian marine sponge of *Petrosaspongia* sp. These compounds showed the selective growth inhibitory activity against PANC-1 cells under the glucose-starved condition. These structures were determined by 1D and 2D NMR spectra, which was confirmed by the total syntheses. Further structure-activity relationship (SAR) study, mechanistic analysis, and *in vivo* evaluations are now in progress.

EXPERIMENTAL

General Experimental

NMR spectra, referenced to tetramethylsilane (TMS), were measured on JEOL ECS-400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) and an Agilent NMR system (^{1}H: 600 MHz, ^{13}C: 150 MHz). ESI-TOF-MS was recorded on a Q-Tof Ultima API (Waters Co., MA, U.S.A.). FAB MS was recorded on a JEOL JMS SX-102 mass spectrometer. IR spectra were obtained with a JASCO FT/IR-5300 (KBr pellets). UV spectra were obtained with UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). Column chromatography was performed on Silica gel BW-200 (Fuji Silysia, Aichi, Japan), Cosmosil ODS ($75C_{18}$-OPN, Nacalai Tesque, Kyoto, Japan), and Cosmosil $5C_{18}$-MS-II (10 mm i.d. × 250 mm, Nacalai Tesque). TLC analysis was carried out by silica gel $60F^{254}$ (Merck Chemical, Darmstadt, Germany). HPLC was performed by Hitachi High Sensitivity Series system (UV-detector: L-4000H), respectively. Unless otherwise noted, all the reactions were performed under a N_2 atmosphere.

Cell Culture and Bioassay

Human pancreatic carcinoma PANC-1 cells was maintained in the Dulbecco's modified Eagle's medium (DMEM) supplemented with heat-inactivated 10% fetal bovine serum (FBS) and kanamycin (50 μg/mL) in a humidified atmosphere of 5% CO_2 at 37 °C. In the case of the condition of nutrient starvation,

PANC-1 cells was cultured in the Glucose Deficient Medium [Basal Medium (25 mM *N*-(2-hydroxyethyl) piperazine-*N*'-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) supplemented with 6.4 g/L NaCl, 700 mg/L $NaHCO_3$, 400 mg/L KCl, 265 mg/L $CaCl_2 \cdot 2H_2O$, 200 mg/L $MgSO_4 \cdot 7H_2O$, 125 mg/L NaH_2PO_4, 0.1 mg/L $Fe(NO_3) \cdot 9H_2O$, 15 mg/L Phenol red, 10 mL/L MEM vitamin solution (x100) (GIBCO, Carlsbad, CA, U.S.A.), 200 mmol/L L-glutamine solution (GIBCO), 50 mg/L kanamycin) containing 10% dialyzed FBS]. The General Glucose Medium [Basal Medium supplemented with 10% FBS and 2.0 g/L glucose (final 25 mM)] was also used for bioassay as the general culture conditions to compare the activity of the sample under the conditions of glucose starvation.

PANC-1 cells (1×10^4 cells/100 μL in 96 well plastic plate) were pre-incubated in the DMEM supplement with 10% FBS for 24 h. The medium was then replaced with either General Glucose Medium or Glucose Deficient Medium adapting to the nutrient starvation. After 12 h incubation, serial diluted samples were added, and the plates were incubated for an additional 12 h in a humidified atmosphere of 5% CO_2 at 37 °C. The cell proliferation was detected by WST-8 colorimetric reagent. The IC_{50} value was determined by linear interpolation from the growth inhibition curve. We assessed the selectivity of anti-proliferative activity (S.I.) on the basis of the difference of IC_{50} values in the General Glucose Medium and the Glucose Deficient Medium.

Extraction and Isolation of Active Compounds

The dried marine sponge of *Petrosaspongia* sp. (2.4 kg), which was collected in 2005 at Biak, Indonesia, was extracted with MeOH. On the guidance of bioassay, the MeOH extract (240 g, IC_{50} (Glucose Deficient Medium) = 50 μg/mL, IC_{50} (General Glucose Medium) = >100 μg/mL) was partitioned into a water-EtOAc mixture (1:1). The active EtOAc soluble portion was further partitioned into a hexane-90% MeOH mixture (1:1). The active 90% MeOH soluble portion [34 g, IC_{50} (Glucose Deficient Medium) = 10 μg/mL, IC_{50} (General Glucose Medium) = >30 μg/mL] was fractionated by SiO_2 gel column chromatography [$CHCl_3$ -MeOH-H_2O (lower phase)] to give six fractions (Fr. 1- Fr. 6). The active Fr. 2 [6.3 g, IC_{50} (Glucose Deficient Medium) = 5 μg/mL, IC_{50} (General Glucose Medium) = 30 μg/mL] was fractionated by SiO_2 gel column chromatography [$CHCl_3$-MeOH-H_2O (lower phase)] to give six fractions (Fr. 2-1 - Fr. 2-6). The active Fr. 2-2 (300 mg) was then further purified by ODS HPLC (Cosmosil MS-II, MeOH/H_2O = 75:25) to afford fasciospyrinadinone (**1**, 18 mg) and fasciospyrinadinol (**2**, 16 mg).

Fasciospyrinadinone (1)

Yellow amorphous powder. FAB-MS *m/z*: 312 $(M+H)^+$. HR-FAB-MS: Calcd for $C_{21}H_{30}ON$: 312.2327. Found 312.2332. UV λ_{max} (MeOH) nm (ε): 253 (43200). IR ν_{max} (KBr) cm^{-1}: 2955, 2928, 2859, 1665, 1424, 1030.

^{1}H NMR (600 MHz, δ_H), ^{13}C NMR (150 MHz, δ_C): summarized in Table 1.

Fasciospyrinadinol (2): Yellow amorphous powder.

FAB-MS *m/z*: 314 $(M+H)^+$. HR-FAB-MS: Calcd for $C_{21}H_{32}NO$ 314.2484. Found 314.2490. UV λ_{max} (MeOH) nm (ε): 215 (24700), 262 (14800). IR ν_{max} (KBr) cm^{-1}: 3345, 2936, 2868, 1453, 1030.

^{1}H NMR (600 MHz, δ_H), ^{13}C NMR (150 MHz, δ_C): summarized in Table 1.

Preparation of (*R*)- or (*S*)-MTPA ester of 2

(*R*)- or (*S*)-MTPA (4 equiv.), DMAP (5 equiv.), DCC (5 equiv.) were added to a solution of **2** in THF, and the whole mixture was stirred for 3 h. Sat. $NaHCO_3$ aq. was added to the mixture and the whole mixture was extracted with EtOAc. Removal of the solvent from EtOAc extract under reduced pressure gave a crude product, which was purified by SiO_2 column [*n*-hexane/EtOAc = 5:1 to 1:1] to give the corresponding MTPA esters (**2*R***, **2*R*'** or **2*S***, **2*S*'**) as inseparable mixture of diastereomers, respectively.

^{1}H NMR (600 MHz, $CDCl_3$: only key resonances are listed)

Data for **2*R***: δ 5.35 (1H, m, H-15), 1.9, 1.7 (total 2H, m, H-16), 1.5, 1.3 (total 2H, m, H-17), 1.65 (3H, s, H-20), 1.54 (3H, s, H-19), 0.99 (3H, s, H-21), 0.97 (3H, s, H-22).

Data for **2*R*'**: δ 5.36 (1H, m, H-15'), 2.0, 1.8 (total 2H, m, H-16'), 1.6, 1.4 (total 2H, m, H-17'), 1.47 (3H, s, H-20'), 1.51 (3H, s, H-19'), 1.02 (3H, s, H-21'), 0.98 (3H, s, H-22').

HR-ESI-TOF-MS: Calcd for $C_{31}H_{38}NO_3F_3Na$ 552.2701. Found 552.2687.

Data for **2*S***: δ 5.36 (1H, m, H-15), 2.0, 1.8 (total 2H, m, H-16), 1.6, 1.4 (total 2H, m, H-17), 1.47 (3H, s, H-20), 1.52 (3H, s, H-19), 1.02 (3H, s, H-21), 0.98 (3H, s, H-22).

Data for **2*S*'**: δ 5.35 (1H, m, H-15'), 1.9, 1.7 (total 2H, m, H-16'), 1.5, 1.3 (total 2H, m, H-17'), 1.65 (3H, s, H-20'), 1.54 (3H, s, H-19'), 0.99 (3H, s, H-21'), 0.97 (3H, s, H-22').

HR-ESI-TOF-MS: Calcd for $C_{31}H_{38}NO_3F_3Na$ 552.2701. Found 552.2722.

Synthesis

(*E*)-2-(5-Chloro-3-methylpent-3-en-1-yl)-1,3,3-trimethylcyclohex-1-ene (4)

Triphenylphosphine (223 mg, 0.85 mmol) and tetrachloromethane (132 mg, 0.85 mmol) were added to a solution of **5** [20] (63.1 mg, 0.28 mmol) in CH_2Cl_2 (1.9 mL) at rt, and the whole mixture was stirred for 22 h. Removal of the solvent under reduced pressure gave a crude product, which was purified by SiO_2 column chromatography (hexane/EtoAc = 6:1) to give **4** (57.9 mg, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.47 (t, *J* = 8.0 Hz, 1H), 4.11 (d, *J* = 8.0 Hz, 2H), 2.08 (s, 4H), 1.91 (t, *J* = 6.3 Hz, 2H), 1.77 (d, *J* = 1.2 Hz, 3H), 1.60 (s, 3H), 1.57 (m, 2H), 1.42 (m, 2H), 0.99 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 143.5, 136.5, 127.3, 119.7, 41.1, 39.9, 39.7, 34.9, 32.7, 28.5, 27.2, 19.8 19.5, 16.1.

IR (KBr) cm^{-1}: 2928, 2865, 1472, 1455. FAB-MS *m/z*: 240 $(M+H)^+$. HR-FAB-MS *m/z*: 240.1645 (Calcd for $C_{15}H_{25}Cl$: 240.1645).

Fasciospyrinadine (3)

n-BuLi (1.63 M in hexane, 0.59 mL, 0.95 mmol) was added to a solution of diisopropylamine (0.13 mL, 0.92 mmol) in tetrahydrofuran (THF) (2 mL) at –10 °C, and the whole mixture was stirred for 30 min at –10 °C. A solution of 3-picoline (0.092 mL, 0.95 mmol) in DMPU (0.11 mL, 0.91 mmol) was added to the mixture at −10 °C, and the whole mixture was stirred for 15 min. Then, a solution of compound **4** (75.9 mg, 0.32 mmol) in THF (1.2 mL) was added to the mixture at −78 °C, and the whole mixture was gradually warmed to rt with stirring overnight. Sat. NH_4Cl aq. was added to the reaction mixture, and the whole mixture was extracted with EtoAc. The combined organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure to give a crude product, which was purified by SiO_2 column chromatography (hexane/ EtoAc = 5:1) gave **3** (77.6 mg, 83%).

^1H NMR (600 MHz, $CDCl_3$) δ: 8.45 (d, *J* = 1.8 Hz, 1H), 8.43 (dd, *J* = 4.8, 1.8 Hz, 1H), 7.49 (dt, *J* = 7.7, 1.8 Hz, 1H), 7.19 (dd, *J* = 7.7, 4.8 Hz, 1H), 5.16 (t, *J* = 7.3 Hz, 1H), 2.65 (t, *J* = 7.3 Hz, 2H), 2.31 (q, *J* = 7.3 Hz, 2H), 2.01 (m, 4H), 1.90 (t, *J* = 6.3 Hz, 2H), 1.59 (s, 3H), 1.56 (m, 2H), 1.54 (s, 3H) 1.41 (m, 2H), 0.98 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 150.1, 147.2, 137.6, 137.4, 137.0, 135.9, 127.0, 123.1, 122.0, 40.2, 39.8, 35.0, 33.2, 32.7, 29.5, 28.6, 27.8, 19.8, 19.5, 16.0. IR (KBr) cm^{-1}: 2927, 2863, 1476, 1422, 1025. ESI-TOF-MS *m/z*: 320 $(M+Na)^+$. HR-ESI-TOF-MS *m/z*: 320.2352 (Calcd for $C_{21}H_{31}NNa$: 320.2354).

Fasciospyrinadinone (1)

Hexacarbonylchromium (48.1 mg, 0.22 mmol) and 70% *tert*-butyl hydroperoxide (0.18 mL, 1.31 mmol) were added to a solution of **3** (130 mg, 0.44 mmol) in MeCN (4.4 mL) at 0 °C, and the whole mixture was stirred for 24 h at rt. H_2O was added to the reaction mixture, and the whole mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a crude product, which was purified by SiO_2 column chromatography (hexane/ EtoAc = 2:1) to give **1** (47.3 mg, 35%).

^1H NMR (600 MHz, DMSO-d_6) δ: 8.42 (d, *J* = 1.7 Hz, 1H), 8.38 (dd, *J* = 4.8, 1.7 Hz, 1H), 7.62 (dt, *J* = 7.7, 1.7 Hz, 1H), 7.28 (dd, *J* = 7.7, 4.8 Hz, 1H), 5.21 (t, *J* = 7.4 Hz, 1H), 2.63 (t, *J* = 7.4 Hz, 2H), 2.36 (t, *J* = 6.7 Hz, 2H), 2.28 (q, *J* = 7.4 Hz, 2H), 2.20 (m, 2H), 2.01 (m, 2H), 1.73 (t, *J* = 6.7 Hz, 2H), 1.65 (s, 3H), 1.52 (s, 3H), 1.12 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 197.5, 164.2, 149.8, 147.1, 137.0, 135.9, 135.7, 129.7, 123.3, 123.2, 38.0, 36.8, 36.0, 33.8, 32.2, 29.6, 29.0, 26.5, 15.6, 11.2. IR (KBr) cm^{-1}:

2926, 2858, 1662, 1468, 1424, 1025. ESI-TOF-MS *m/z*: 334 $(M+Na)^+$. HR-ESI-TOF-MS *m/z*: 334.2132 (Calcd for $C_{21}H_{29}NONa$: 334.2147).

Fasciospyrinadinol (2)

Sodium borohydride (6.0 mg, 0.16 mmol) and cerium(III) chloride heptahydrate (14.0 mg, 0.038 mmol) were added to a solution of **1** (60.0 mg, 0.19 mmol) in MeOH (1.8 mL) at 0 °C, and the whole mixture was stirred for 30 min at 0 °C. Sat. NH_4Cl aq. was added to the reaction mixture, and the whole mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a crude product, which was purified by SiO_2 column chromatography (hexane/ EtoAc = 3:2) to give **2** (47.9 mg, 79%).

^1H NMR (600 MHz, DMSO-d_6) δ: 8.41 (d, J = 1.8 Hz, 1H), 8.38 (dd, J = 4.7, 1.8 Hz, 1H), 7.61 (dt, J = 7.7, 1.8 Hz, 1H), 7.28 (dd, J = 7.7, 4.7 Hz, 1H), 5.15 (t, J = 7.4 Hz, 1H), 4.47 (d, J = 6.1 Hz, 1H), 3.68 (m, 1H), 2.62 (t, J = 7.4 Hz, 2H), 2.26 (q, J = 7.4 Hz, 2H), 1.93 (m, 4H), 1.68 (m, 1H), 1.62 (s, 3H), 1.56 (m, 1H), 1.49 (m, 1H), 1.49 (s, 3H), 1.24 (m, 1H), 0.97 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 149.8, 147.1, 139.0, 137.1, 136.5, 136.0, 130.0, 123.2, 122.5, 68.1, 35.0, 34.7, 32.3, 29.0, 28.7, 28.2, 27.7, 27.4, 16.4, 15.7. IR (KBr) cm^{-1}: 3304, 2932, 2859, 1477, 1424, 1029. ESI-TOF-MS *m/z*: 336 $(M+Na)^+$. HR-ESI-TOF-MS *m/z*: 336.2301 (Calcd for $C_{21}H_{31}NONa$: 336.2303).

(*E*)-3-Methyl-6-(pyridin-3-yl)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)hex-3-en-2-ol (6)

Selenium dioxide (8.9 mg, 0.08 mmol) was added to a solution of **3** (23.9 mg, 0.08 mmol) in 1,4-dioxane (0.16 mL) at rt, and the whole mixture was stirred for 3 h. Sat. $NaHCO_3$ aq. and Sat. $NaHSO_3$ aq. was added to the reaction mixture, and the whole mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a crude product, which was purified by SiO_2 column chromatography (hexane/AcOEt = 2:1) to give **6** (7.1 mg, 28%).

^1H NMR (600 MHz, $CDCl_3$) δ: 8.45 (m, 2H), 7.50 (d, J = 7.7 Hz, 1H), 7.20 (dd, J = 7.7, 4.7 Hz, 1H), 5.46 (t, J = 7.1 Hz, 1H), 4.13 (dd, J = 10.1, 3.9 Hz, 1H), 2.68 (m, 2H), 2.36 (m, 3H), 2.17 (m, 1H), 1.99 (m, 2H), 1.77 (s, 1H), 1.67 (s, 3H), 1.62 (m, 2H), 1.60 (s, 3H), 1.45 (m, 2H), 1.03 (s, 3H), 1.02 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 150.0, 147.3, 138.9, 135.9, 133.5, 131.8, 123.5, 123.2, 76.4, 39.9, 34.9, 34.8, 33.1, 32.8, 29.3, 29.1, 28.7, 21.1, 19.3, 11.7. IR (KBr) cm^{-1}: 3303, 2925, 2862, 1423, 1054, 1027. ESI-TOF-MS *m/z*: 336 $(M+Na)^+$. HR-ESI-TOF-MS *m/z*: 336.2287 (Calcd for $C_{21}H_{31}NONa$: 336.2303).

(*E*)-3-(6-(3-Hydroperoxy-2,6,6-trimethylcyclohex-1-en-1-yl)-4-methylhex-3-en-1-yl)pyridine (7)

70% *tert*-Butyl hydroperoxide (0.80 mL, 5.84 mmol) was added to a solution of **3** (77.6 mg, 0.26 mmol) in cyclohexane (2.6 mL) at rt, and the whole mixture was stirred for 24 h. Sat. $NaHCO_3$ aq. was added to the reaction mixture, and the whole mixture was extracted with EtOAc. The combined organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a crude product, which was purified by SiO_2 column chromatography (hexane/EtOAc = 2:1) to give **7** (22.7 mg, 26%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.69 (s, 1H), 8.43 (m, 2H), 7.50 (dt, *J* = 7.8, 1.7 Hz, 1H), 7.20 (dd, *J* = 7.8, 4.8 Hz, 1H), 5.16 (m, 1H), 4.20 (s, 1H), 2.65 (t, *J* = 7.5 Hz, 1H), 2.30 (q, *J* = 7.5 Hz, 1H), 2.12 (m, 1H), 2.06 (m, 1H), 2.00 (m, 3H), 1.74 (s, 3H), 1.67 (m, 2H), 1.53 (s, 3H), 1.30 (m, 1H), 1.02 (s, 3H), 0.96 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.9, 147.1, 146.9, 137.4, 137.1, 136.1, 123.5, 123.2, 122.4, 83.9, 39.3, 35.5, 33.9, 33.1, 29.4, 28.7, 28.2, 26.4, 22.8, 17.8, 16.0. IR (KBr) cm^{-1}: 3136, 2933, 2857, 1476, 1425, 994. ESI-TOF-MS *m/z*: 352 $(M+Na)^+$. HR-ESI-TOF-MS *m/z*: 352.2239 (Calcd for $C_{21}H_{31}NO_2Na$: 352.2252).

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