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Lanostane Triterpenoids from the Mushroom *Naematoloma fasciculare*

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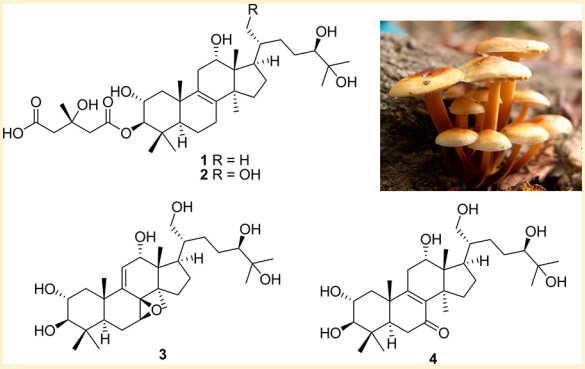
Supporting Information

ABSTRACT: In our continuing search for structurally interesting and bioactive metabolites from Korean wild mushrooms, bioassay-guided fractionation and a chemical investigation of the MeOH extracts of the fruiting bodies of the mushroom *Naematoloma fasciculare* resulted in the isolation of four new lanostane triterpenoids (**1**−**4**), together with 11 known compounds (**5**−**15**). The structures of **1**−**5** were determined by a combination of 1D and 2D NMR and HRMS. The absolute configuration of the 3-hydroxy-3-methylglutaryl group as a side chain in **1** and **2** was determined by the alkaline methanolysis method. The full NMR data assignment of the known compound fasciculol G (**5**) is reported for the first time. Compounds **1**−**15** were tested for their antiproliferative activities against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) and evaluated for their inhibitory effects on nitric oxide production in a lipopolysaccharide-activated murine microglial cell line.



In our continuing search for structurally interesting and bioactive metabolites from Korean wild mushrooms,[1−5] we have collected scores of endemic mushroom species in the mountainous areas during the hot humid summer and prepared MeOH extracts of the mushrooms for antitumor-activity screening tests. Among the collected wild mushrooms, the extract of the mushroom *Naematoloma fasciculare* (Strophariaceae) showed significant cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines. The bitter poisonous mushroom *N. fasciculare* (Japanese name: Nigakuritake), which is widely distributed worldwide, grows on the stumps of old trees in tufts.[6] This mushroom is well known for its diverse phytochemicals (ergosterols, triterpenoids, and sesquiterpenoids),[6−13] and particularly for the presence of lanostane triterpenoids as the toxic components.[8−12] Lanostane triterpenoids from this mushroom inhibit the growth of Chinese cabbage seedlings and have antimicrobial and calmodulin inhibitory activities.[8−12]

In the course of the search for new bioactive metabolites of the poisonous mushroom *N. fasciculare*, we isolated four new lanostane triterpenoids (**1**−**4**), together with 11 known compounds (**5**−**15**), from the MeOH extracts of the fruiting bodies using a bioassay-guided fractionation technique. The isolated compounds (**1**−**15**) were evaluated for their antiproliferative activities against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) and for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial cell line.

RESULTS AND DISCUSSION

Fresh fruiting bodies of *N. fasciculare* were collected at Donggureung, Guri of GyeongGi-do, Korea, and extracted with 80% aqueous MeOH to give an extract that showed cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cells. A chemical investigation of the MeOH extract led to the isolation of 15 lanostane triterpenoids (**1**−**15**). The known compounds were identified as fasciculol E (**6**),[12,14] fasciculic acid B (**7**),[12] fasciculic acid F (**8**),[15] fasciculol D (**9**),[16] fasciculol F (**10**),[16] fasciculol B (**11**),[16] fasciculol C (**12**),[16] fasciculol H (**13**),[17] fasciculol I (**14**),[17] and sublateriol C (**15**),[18] by comparison of their spectroscopic and physical data with reported values.

Fasciculol J (**1**) was obtained as a colorless gum. The molecular formula was determined to be $C_{36}H_{60}O_9$ from the molecular ion peak $[M + Na]^+$ at m/z 659.4132 (calcd for $C_{36}H_{60}O_9Na$, 659.4135) in the positive-ion HRESIMS. The IR spectrum exhibited absorptions of hydroxy (3357 cm^{-1}) and carbonyl (1683 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed the presence of signals due to eight tertiary methyl

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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Fasciculols J (1) and K (2)

	1		2	
position	δ_C (CD_3OD)[a]	δ_H (J in Hz)	δ_C (CD_3OD)[a]	δ_H (J in Hz)
1	40.6 t	1.26 m; 2.04 m	40.3 t	1.25 m; 2.06 m
2	66.4 d	3.83 td (10.5, 4.0)	66.5 d	3.82 td (10.0, 4.5)
3	84.4 d	4.55 d (10.5)	84.3 d	4.56 d (10.0)
4	38.6 s		38.5 s	
5	50.3 d	1.27 m	50.3 d	1.26 m
6	17.9 t	1.53 m; 1.71 m	17.8 t	1.55 m; 1.74 m
7	27.6 t	2.07 m	27.4 t	2.06 m
8	135.3 s		135.1 s	
9	132.2 s		132.4 s	
10	37.8 s		37.8 s	
11	33.2 t	2.09 br s	32.5 t	2.08 br s
		2.65 br d (8.0)		2.68 br d (8.0)
12	72.1 d	4.01 br d (8.0)	72.8 d	4.02 br d (8.0)
13	49.2 s		49.7 s	
14	49.1 s		49.6 s	
15	31.8 t	1.28 m; 1.71 m	31.3 t	1.22 m; 1.74 m
16	27.6 t	1.53 m; 2.08 m	26.0 t	1.56 m; 2.09 m
17	42.6 d	2.23 q (9.5)	37.7 d	2.45 q (9.5)
18	15.5 q	0.66 s	15.9 q	0.64 s
19	19.0 q	1.11 s	18.7 q	1.11 s
20	36.1 d	1.44 m	42.8 d	1.40 m
21	16.5 q	1.04 d (6.5)	60.5 t	3.72 dd (11.0, 2.5)
				3.78 dd (11.0, 4.5)
22	33.1 t	1.36 m; 1.55 m	27.4 t	1.45 m; 1.64 m
23	27.6 t	1.33 m; 1.53 m	27.9 t	1.41 m; 1.58 m
24	78.3 d	3.23 br d (9.5)	77.9 d	3.24 br d (10.0)
25	72.5 s		72.4 s	
26	24.2 q	1.16 s	24.2 q	1.16 s
27	23.5 q	1.13 s	23.4 q	1.13 s
28	27.6 q	0.91 s	27.5 q	0.91 s
29	15.8 q	0.91 s	16.5 q	0.91 s
30	23.8 q	1.08 s	22.7 q	1.08 s
1′	171.3 s		171.6 s	
2′	46.3 t	2.64 d (15.5)	46.0 t	2.68 d (15.5)
		2.72 d (15.5)		2.74 d (15.5)
3′	69.9 s		70.1 s	
4′	26.7 q	1.39 s	26.6 q	1.40 s
5′	46.1 t	2.42 d (15.5)	45.8 t	2.60 d (15.5)
		2.57 d (15.5)		2.54 d (15.5)
6′	173.8 s		173.9 s	

[a]The assignments were based on DEPT, HMQC, and HMBC experiments.

[δ_H 0.66, 0.91, 0.91, 1.08, 1.11, 1.13, 1.16, and 1.39], a secondary methyl [δ_H 1.04 (d, J = 6.5 Hz)], and four oxygenated methine [δ_H 3.23 (br d, J = 9.5 Hz), 3.83 (td, J = 10.5, 4.0 Hz), 4.01 (br d, J = 8.0 Hz), and 4.55 (d, J = 10.5 Hz)] groups. The ^{13}C NMR spectrum (Table 1) showed 36 carbon signals, which were attributed to nine methyl, 10 methylene, and seven methine groups, as well as 10 quaternary carbons, including two olefinic carbons [δ_C 132.2 and 135.3], two oxygenated quaternary carbons [δ_C 69.9 and 72.5], and two carbonyl groups [δ_C 171.3 and 174.7] determined by analysis of the DEPT and HMQC spectra. These data suggested that compound **1** is a lanostane triterpenoid, which was also confirmed by comparison of its data with those of fasciculol B (**11**) and fasciculic acid B (**7**).[12,16] Analysis of the ^{1}H and ^{13}C NMR data revealed that the ^{1}H and ^{13}C NMR spectra of **1** were very similar to those of **7**, with an apparent difference in the signals for H-2 [δ_H 3.83 (1H, td, J = 10.5, 4.0 Hz)] and H-3 [δ_H 4.55 (1H, d, J = 10.5 Hz)] in **1** compared to the corresponding signals [δ_H 5.55 (1H, td, J = 11.0, 4.5 Hz, H-2) and 3.50 (1H, d, J = 11.0 Hz, H-3)] in **7**.[12] This downfield shift [δ_H 4.55; δ_C 84.4] observed at H-3 (C-3) suggested that the 3-hydroxy-3-methylglutaryl group in **1** is linked at C-3, which was confirmed by the HMBC correlation between H-3 (δ_H 4.55) and C-1′ (δ_C 171.3) (Figure 1). The structure of **1** was supported by the cross-peaks in the ^{1}H−^{1}H COSY and HMBC spectra (Figure 1). The relative configuration of **1** was established to be identical to **11** by analysis of the NOESY data (Figure 2) and by comparing the coupling constants and chemical shifts with those of fasciculol B (**11**) and fasciculic acid B (**7**).[12,16] The absolute configuration of **1** was determined by an alkaline methanolysis of **1**.[19,20] Treatment of **1** with 3% sodium methoxide (NaOMe) in MeOH yielded two compounds, fasciculol B (**1a**)[10,16] and methyl (*S*)-3-hydroxy-3-methylglutarate (**1b**), which was identified by comparison of its ^{1}H NMR, specific rotation of $[\alpha]_D^{25}$ +6.5 (*c* 0.08, $CHCl_3$), and MS data.[19,20] Thus, the absolute configuration of C-3′ of **1** was determined to be *S*. Finally, comparing the optical rotation of **1a** with that of fasciculol B (**11**) allowed us to establish the absolute configuration of the main triterpene skeleton, which is identical to that of **11**.[10,16]

Fasciculol K (**2**) was obtained as a colorless gum with a molecular formula of $C_{36}H_{60}O_{10}$ deduced by the molecular ion peak [M + Na]$^+$ at m/z 675.4082 (calcd for $C_{36}H_{60}O_{10}Na$, 675.4084) in the HRESIMS. The ^{1}H and ^{13}C NMR spectra (Table 1) were quite similar to those of **1**, the only difference being that a secondary methyl group [δ_H 1.04 (d, J = 6.5 Hz); δ_C 16.5] in **1** was replaced with an oxygenated methylene group [δ_H 3.72 (dd, J = 11.0, 2.5 Hz) and 3.78 (dd, J = 11.0, 4.5 Hz); δ_C 60.5] in **2**. The location of this oxygenated methylene group was further confirmed to be C-21 by the HMBC correlations of H-21/C-17 (δ_C 37.7), C-20 (δ_C 42.8), and C-22 (δ_C 27.4), suggesting that compound **2** was a C-21-hydroxylated product of **1**. The complete assignments of the ^{1}H and ^{13}C NMR data of **2** were determined by DEPT, HMQC, HMBC, and NOESY spectra. The relative configuration of **2** was established to be identical to **1** based on the coupling constants and a NOESY experiment and by comparison of the ^{1}H and ^{13}C NMR data with those of **1**. The absolute configuration at C-3′ in **2** was determined using alkaline methanolysis of **2**, which demonstrated the *S*-configuration for C-3′, the same as that of **1**.

Fasciculol L (**3**) was obtained as a colorless gum. The HRESIMS displayed a molecular ion peak [M + Na]$^+$ at m/z 545.3457 (calcd for $C_{30}H_{50}O_7Na$, 545.3454), consistent with a molecular formula of $C_{30}H_{50}O_7$. The ^{1}H and ^{13}C NMR data (Table 2) were similar to those of sublateriol C (**15**),[18] with the added presence of signals for an epoxy group [δ_H 3.10 (1H, d, J = 6.5 Hz, H-7); δ_C 55.8 (C-7) and δ_C 61.4 (C-8)][21,22] in **3** instead of signals for a double bond at δ_H 5.66 (br d, J = 5.9 Hz, H-7); δ_C 124.6 (C-7) and δ_C 143.6 (C-8) in **15**.[18] The location of the epoxy group was confirmed to be C-7/C-8 by ^{1}H−^{1}H COSY correlations starting at H-5 via H-6 and ending at H-7 in combination with HMQC and HMBC correlations of H-7/C-5, H-11/C-8, and H-30/C-8 (Figure 1). This result suggested that the $\Delta^{7,8}$ double bond in **15** was oxidized as an epoxy group in **3**. The 7α,8α-configuration of the epoxide ring was deduced from

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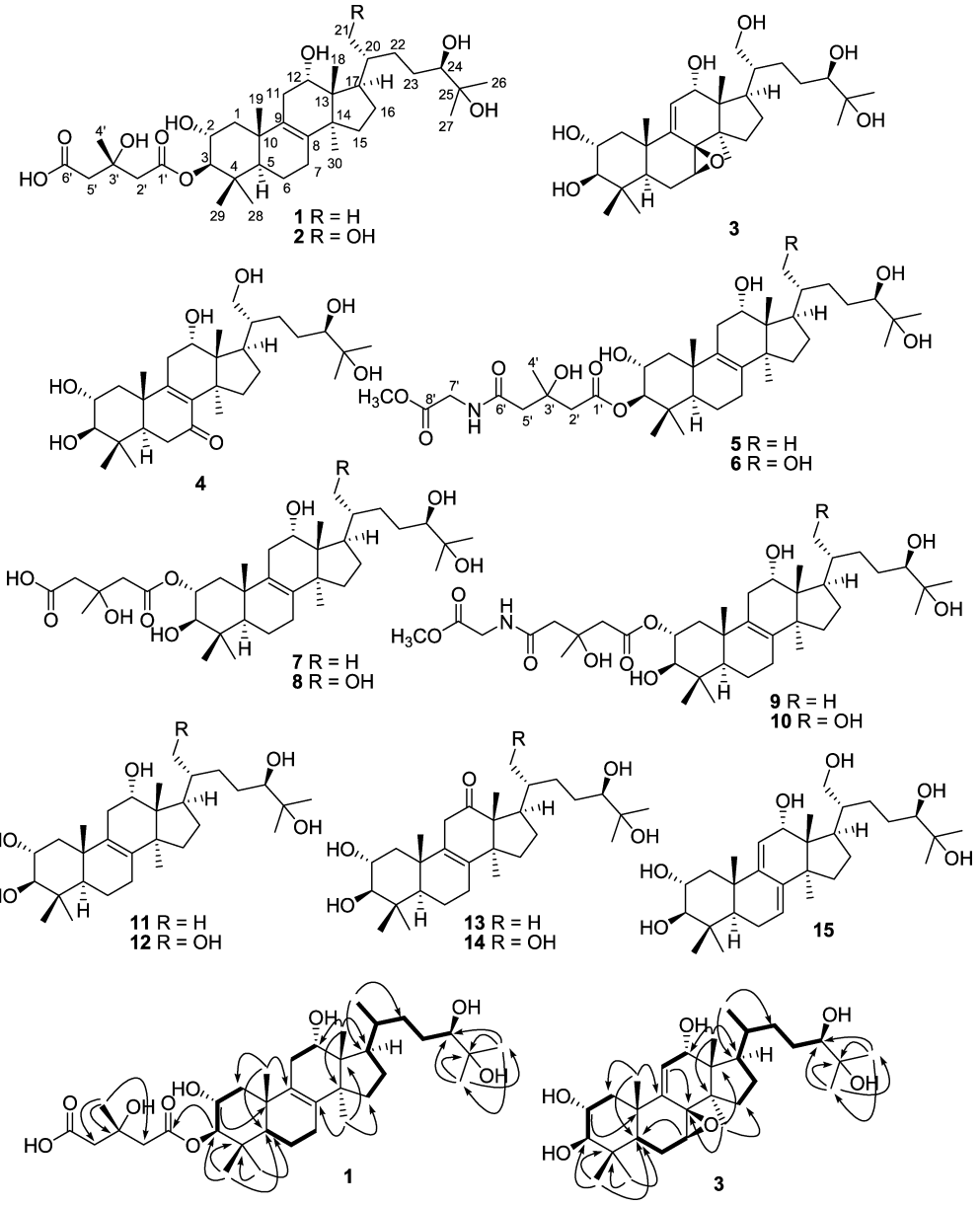

Figure 1. $^{1}H-^{1}H$ COSY (bold lines) and key HMBC (arrows) correlations for **1** and **3**.

the correlations of H-7/H-5 and H-7/H-30 in the NOSEY spectrum (Figure 2). The remaining relative configuration of **3** was established to be identical to **15** based on the coupling constants and a NOESY experiment and by comparison of the ^{1}H and ^{13}C NMR data with those of **15**.

Fasciculol M (**4**) was obtained as a colorless gum with a molecular formula of $C_{30}H_{50}O_7$ based on the molecular ion peak $[M + Na]^+$ at m/z 545.3457 (calcd for $C_{30}H_{50}O_7Na$, 545.3454) by HRESIMS. The ^{1}H and ^{13}C NMR data (Table 2) were similar to those of fasciculol C (**12**),[16] with a noticeable difference being the presence of a signal for a ketone group (δ_C 199.7), which was determined to be at C-7 by the HMBC correlations from H-5 (δ_H 1.71) to C-7, from H-6 (δ_H 2.35 and 2.53) to C-7, and from H-30 (δ_H 1.11) to C-7. Full assignments of the ^{1}H and ^{13}C NMR data of **4** were performed by DEPT, HMQC, HMBC, and NOESY spectra. The relative configuration of **4** was elucidated to be identical to **12** on the basis of the coupling constants and a NOESY experiment (Figure 2) and by comparison of the ^{1}H and ^{13}C NMR data with those of **12**.

Fasciculol G (**5**) is a known compound reported by Ikeda et al. from this mushroom.[23] The NMR data of fasciculol G (**5**) have not been reported, although its melting point, MS, IR, and specific rotation were published by Ikeda et al.[23] We performed full NMR data assignments (Table 2) of **5** by analysis of 2D NMR data (including DEPT, HMQC, HMBC, and NOESY). To the best of our knowledge, the NMR data of fasciculol G are reported for the first time in this study.

Compounds **1**–**15** were evaluated for their antiproliferative activities against the A549, SK-OV-3, SK-MEL-2, and HCT-15 human cancer cell lines using the SRB bioassay.[24] Doxorubicin was used as a positive control. All isolated triterpenoids except for compounds **1** and **2** showed cytotoxic activity against the four human cancer cell lines with IC_{50} values of 2.29–28.48 μM (Table 3). The newly isolated compounds **3** and **4** exhibited significant cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} (**3**): 6.59, 7.08, 8.26, and 8.53

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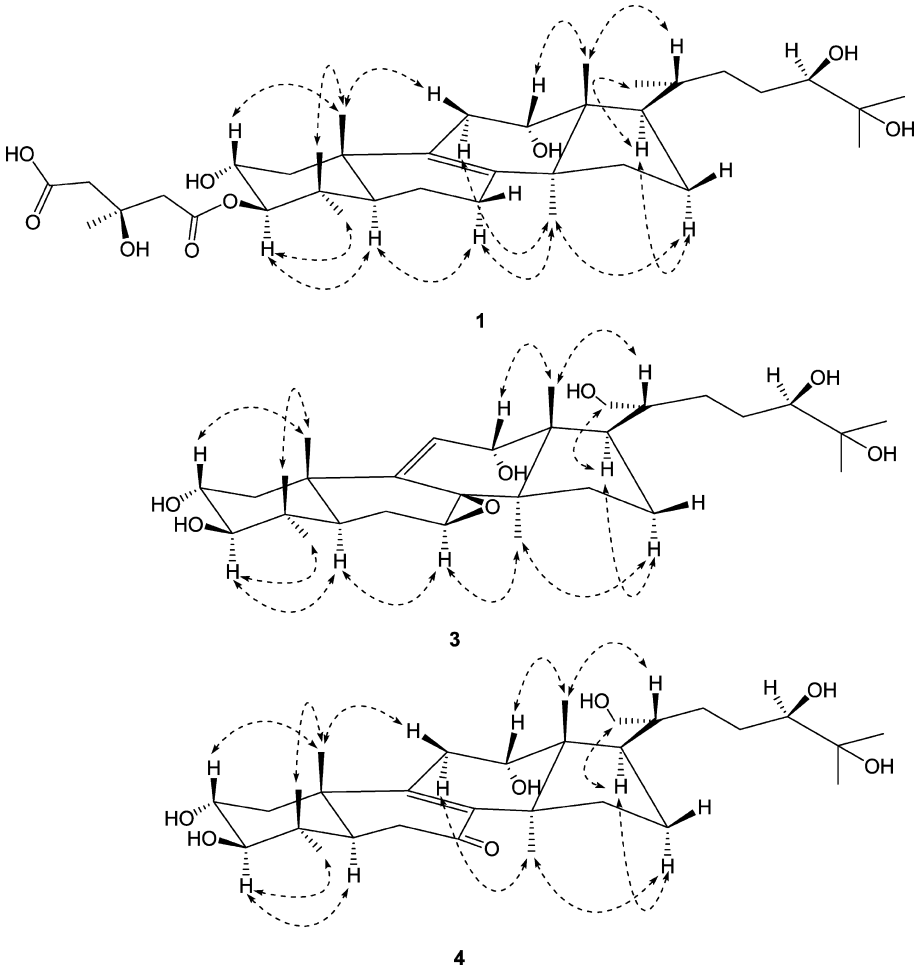

Figure 2. Key NOESY (dashed arrow) correlations for **1**, **3**, and **4**.

μM, and IC_{50} (**4**): 3.99, 7.36, 4.77, and 8.50 μM, respectively). Moreover, compounds **11**, **12**, and **15** displayed considerable cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} (**11**): 7.85, 8.53, 5.17, and 8.22 μM, IC_{50} (**12**): 2.37, 2.82, 2.29, and 3.06 μM, and IC_{50} (**15**): 4.47, 3.29, 4.54, and 7.71 μM, respectively). Interestingly, compounds **5**, **6**, **9**, and **10**, with an *N*-glycyl-3-hydroxy-3-methylglutaryl group as a side chain, showed significant selective cytotoxicity against the SK-MEL-2 cell line (IC_{50} (**5**): 8.60 μM, IC_{50} (**6**): 9.06 μM, IC_{50} (**9**): 9.16 μM, and IC_{50} (**10**): 5.73 μM). The presence of a 3-hydroxy-3-methylglutaryl group linked at C-3 in compounds **1** and **2** markedly reduced the activity when compared to compounds **7** and **8**, with the side chain connected to C-2. Compounds **13** and **14**, with a ketone functionality at C-12, had good selective cytotoxicity against the SK-OV-3 cell line (IC_{50} (**13**): 9.86 and IC_{50} (**14**): 7.98 μM). A number of lanostane-type triterpenes isolated from fungi have recently been reported to have potential as anticancer agents, and these compounds were found to arrest the cell cycle in either the G_1 or the G_2/M, affect the transporting proteins MDR and MRP, increase levels of p53 and Bax, decrease MMP expression, induce apoptosis, or inhibit not only the phosphorylation of Erk1/2 but also the activation of NF-κB and AP-1.[25] Thus, it appears that the active compounds **3**–**15** isolated from the mushroom *N. fasciculare* may be valuable in further studies addressing their cytotoxic mechanism and for in vivo experiments.

Based on the expanded understanding that cancer progression is associated with inflammatory responses, the anti-inflammatory activities of compounds **1**–**15** were also evaluated by examining their effects on LPS-induced NO production in murine microglia BV-2 cells. Among the tested compounds, compounds **4** and **7** moderately inhibited NO production, with IC_{50} values of 49.9 and 93.1 μM without cell toxicity, respectively (Supporting Information, Table 1S). The remaining compounds did not show any significant effects. Some cell toxicity was observed in cells treated with compounds **5**, **9**–**12**, **14**, and **15**, whereas other compounds had no influence on cell viability.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded using an Agilent 8453 UV–visible spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. ESIMS and HRESIMS spectra were recorded on a Micromass QTOF2-MS. Semipreparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector. Chromatographic separation was performed on an Econosil RP-18 10 μ column (250 × 10 mm i.d.). Silica gel 60 (Merck, 230–400 mesh) and RP-C_{18} silica gel (Merck, 230–400 mesh) were used for column chromatography. Silica Waters Sep-Pak Vac 6 cc and C_{18} Waters Sep-Pak Vac 6 cc cartridges were also used for column chromatography. TLC was performed using Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates. Spots

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Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Fasciculols L (3), M (4), and G (5)

	3		4		5	
position	δ_C (CD_3OD)[a]	δ_H (J in Hz)	δ_C (CD_3OD)[a]	δ_H (J in Hz)	δ_C (CD_3OD)[a]	δ_H (J in Hz)
1	45.2 t	1.46 m	42.6 t	1.42 m	40.3 t	1.26 m; 2.00 m
		2.18 dd (17.5, 4.5)		2.18 dd (17.5, 4.5)		
2	67.8 d	3.66 td (10.0, 4.5)	67.9 d	3.70 td (10.0, 4.5)	65.8 d	3.82 td (10.0, 4.0)
3	82.3 d	2.88 d (10.0)	81.9 d	2.97 d (10.0)	84.1 d	4.57 d (10.0)
4	39.1 s		38.9 s		38.6 s	
5	48.5 d	0.89 m	50.2 d	1.71 dd (14.5, 3.0)	50.4 d	1.28 m
6	21.2 t	1.86 m; 2.09 m	36.1 t	2.35 dd (14.5, 3.0)	17.9 t	1.53 m; 1.72 m
				2.53 t (14.5)		
7	55.8 d	3.10 d (6.5)	199.7 s		27.5 t	2.08 m
8	61.4 s		138.2 s		135.2 s	
9	145.8 s		164.0 s		132.2 s	
10	37.8 s		40.5 s		37.6 s	
11	125.2 d	5.91 br d (5.5)	33.7 t	2.33 br d (21.0)	32.9 t	2.08 br s
				3.05 dd (21.0, 8.0)		2.68 br d (8.0)
12	73.6 d	4.22 br d (5.5)	72.1 d	4.10 br d (8.0)	72.1 d	4.01 br d (8.0)
13	48.2 s		49.9 s		49.3 s	
14	47.8 s		47.6 s		49.2 s	
15	26.5 t	1.30 m; 1.72 m	33.1 t	1.38 m; 1.98 m	31.7 t	1.28 m; 1.69 m
16	26.2 t	1.57 m; 2.03 m	27.7 t	1.53 m; 2.12 m	27.5 t	1.55 m; 2.10 m
17	39.2 d	2.43 q (10.0)	36.5 d	2.38 q (9.5)	42.6 d	2.23 q (9.5)
18	16.0 q	0.81 s	15.9 q	0.61 s	15.5 q	0.66 s
19	22.7 q	1.14 s	17.5 q	1.24 s	18.8 q	1.11 s
20	42.3 d	1.46 m	42.5 d	1.36 m	36.1 d	1.41 m
21	60.5 t	3.72 dd (11.0, 0.5)	60.6 t	3.71 dd (11.0, 1.5)	16.5 q	1.05 d (6.5)
		3.76 dd (11.0, 2.0)		3.79 dd (11.0, 3.5)		
22	26.7 t	1.44 m; 1.67 m	26.7 t	1.44 m; 1.64 m	34.2 t	1.37 m; 1.58 m
23	27.8 t	1.42 m; 1.57 m	27.9 t	1.42 m; 1.58 m	27.5 t	1.33 m; 1.55 m
24	77.9 d	3.26 br d (10.5)	77.9 d	3.25 br d (10.5)	78.3 d	3.23 d (11.0)
25	72.4 s		72.4 s		72.4 s	
26	24.2 q	1.16 s	24.3 q	1.16 s	24.2 q	1.16 s
27	23.4 q	1.13 s	23.4 q	1.13 s	23.5 q	1.13 s
28	27.5 q	1.02 s	27.0 q	1.01 s	27.5 q	0.91 s
29	15.4 q	0.84 s	15.5 q	0.90 s	15.7 q	0.91 s
30	23.0 q	1.09 s	23.8 q	1.11 s	23.5 q	1.08 s
1′					171.7 s	
2′					46.8 t	2.73 s
3′					70.1 s	
4′					27.5 q	1.41 s
5′					46.7 t	2.59 s
6′					172.7 s	
7′					40.3 t	3.95 br s
8′					172.6 s	
OMe					53.9 q	3.72 s

[a]The assignments were based on DEPT, HMQC, and HMBC experiments.

were detected on TLC under UV light or by heating after spraying with anisaldehyde–sulfuric acid.

Mushroom Material. Fresh fruiting bodies of *N. fasciculare* were collected at Donggureung, Guri of GyeongGi-do, Korea, in August 2009. A voucher specimen (SKKU-2009-08) of the mushroom was authenticated by one of the authors (K.R.L.) and was deposited at the herbarium of the School of Pharmacy, Sungkyunkwan University, Korea.

Extraction and Isolation. Air-dried and powdered *N. fasciculare* fruiting bodies (88 g) were extracted twice with 80% aqueous MeOH (each 2.0 L × 3 days) at room temperature and filtered. The filtrate was evaporated under vacuum to obtain a crude MeOH extract (9.4 g), which was suspended in distilled water and then successively partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, yielding 900 mg, 1.8 g, and 2.8 g of residues, respectively. Each fraction was evaluated for cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines using the SRB bioassay. We selected the $CHCl_3$-soluble fraction for the current phytochemical study, as the $CHCl_3$-soluble fraction was the most cytotoxic against the tested tumor cell lines. The $CHCl_3$-soluble fraction (1.8 g) was separated by silica gel (230–400 mesh) column chromatography [65 g, 1.5 × 32 cm, eluted with $CHCl_3$–MeOH, 20:1 (1.8 L) and 4:1 (1.0 L)] to yield 10 fractions (Fr. A, 20:1, 0.3 L; Fr. B, 20:1, 0.3 L; Fr. C, 20:1, 0.3 L; Fr. D, 20:1, 0.3 L; Fr. E, 20:1, 0.3 L; Fr. F, 20:1, 0.3 L; Fr. G, 4:1, 0.2 L; Fr. H, 4:1, 0.2 L; Fr. I, 4:1, 0.2 L; and Fr. J, 4:1, 0.4 L). Fraction F (115 mg) was subjected to fractionation with passage over a C_{18} Waters Sep-Pak Vac 6 cc (80% aqueous MeOH) to give 13 subfractions (F1–F13). Subfraction F4 (8 mg) was purified by semipreparative HPLC (80% MeOH(aq)) with a Shodex refractive index detector, using an Econosil RP-18 10 μ column (250 × 10 mm i.d.), to yield compound **14** (3 mg).

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Table 3. Cytotoxicity of Compounds 1−15 against Four Human Cancer Cell Lines Using the SRB Bioassay

	IC_{50} (μM)[a]			
compound	A549	SK-OV-3	SK-MEL-2	HCT-15
1	>30	>30	>30	>30
2	>30	>30	>30	>30
3	6.59	7.08	8.26	8.53
4	3.99	7.36	4.77	8.50
5	>30	>30	8.60	>30
6	>30	>30	9.06	>30
7	27.20	22.17	16.60	28.48
8	25.52	16.17	25.23	27.96
9	>30	>30	9.16	>30
10	>30	>30	5.73	>30
11	7.85	8.53	5.17	8.22
12	2.37	2.82	2.29	3.06
13	>30	9.86	>30	>30
14	>30	7.98	>30	>30
15	4.47	3.29	4.54	7.71
doxorubicin[b]	0.02	0.01	0.01	0.13

[a] IC_{50} value of compounds against each cancer cell line, which was defined as the concentration (μM) that caused 50% inhibition of cell growth in vitro. [b] Doxorubicin as a positive control.

Subfraction F7 (7 mg) was purified by separation with semipreparative HPLC (83% MeOH(aq)) to give compound **13** (3 mg). Compound **10** (3 mg) was obtained from subfraction F8 (6 mg) by separation with semipreparative HPLC (85% MeOH(aq)). Subfraction F9 (25 mg) was purified by semipreparative HPLC (90% MeOH(aq)) to afford compounds **5** (8 mg) and **9** (6 mg). Fraction G (58 mg) was subjected to purification by passage over a Silica Waters Sep-Pak Vac 6 cc ($CHCl_3$−MeOH, 4:1) to give compound **6** (6 mg). Fraction H (150 mg) was subjected to fractionation by passage over a C_{18} Waters Sep-Pak Vac 6 cc (80% MeOH(aq)) to afford 10 subfractions (H1−H10). Subfraction H3 (7 mg) was purified by separation with semipreparative HPLC (73% MeOH(aq)) to furnish compound **4** (4 mg). Subfraction H6 (8 mg) was purified by semipreparative HPLC (75% MeOH(aq)) to give compound **3** (3 mg). Compound **15** (5 mg) was isolated from subfraction H7 (43 mg) by separation with semipreparative HPLC (75% MeOH(aq)). Fraction I (108 mg) was subjected to fractionation by passage over a C_{18} Waters Sep-Pak Vac 6 cc (80% MeOH(aq)) to afford eight subfractions (I1−I8). Subfraction I5 (18 mg) was subjected to purification with passage over a Silica Waters Sep-Pak Vac 6 cc ($CHCl_3$−MeOH, 8:1) to obtain compound **12** (10 mg). Compound **11** (5 mg) was isolated from subfraction I6 (11 mg) by passage over a silica Waters Sep-Pak Vac 6 cc ($CHCl_3$−MeOH, 8:1). Subfraction I7 (35 mg) was purified by separation with semipreparative HPLC (85% MeOH(aq)) to give compounds **1** (7 mg), **7** (8 mg), and **8** (4 mg). Fraction J (52 mg) was subjected to fractionation with passage over a C_{18} Waters Sep-Pak Vac 6 cc (80% MeOH(aq)) to give two subfractions (J1 and J2). Finally, subfraction J2 (10 mg) was purified by separation with semipreparative HPLC (70% MeOH(aq)) to furnish compound **2** (6 mg).

*Fasciculol J (**1**):* colorless gum; $[\alpha]_D^{25}$ +15.8 (*c* 0.35, MeOH); IR (KBr) ν_{max} 3357, 2946, 2833, 2496, 2235, 1683, 1451, 1121, 1030, 674 cm^{-1}; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; positive HRESIMS *m/z* 659.4132 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_9Na$, 659.4135).

*Fasciculol K (**2**):* colorless gum; $[\alpha]_D^{25}$ +17.1 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3357, 2946, 2833, 2495, 2236, 1685, 1451, 1121, 1032, 674 cm^{-1}; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; positive HRESIMS *m/z* 675.4082 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_{10}Na$, 675.4084).

*Fasciculol L (**3**):* colorless gum; $[\alpha]_D^{25}$ +13.0 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3358, 2945, 2832, 2494, 2235, 1451, 1121, 1030, 674 cm^{-1}; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 2; positive HRESIMS *m/z* 545.3457 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_7Na$, 545.3454).

*Fasciculol M (**4**):* colorless gum; $[\alpha]_D^{25}$ +21.9 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3357, 2945, 2832, 2495, 2235, 1667, 1451, 1121, 1031, 674 cm^{-1}; UV (MeOH) λ_{max} (log ε) 250 (2.72) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 2; positive HRESIMS *m/z* 545.3457 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_7Na$, 545.3454).

*Fasciculol G (**5**):* colorless gum; $[\alpha]_D^{25}$ +6.4 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3355, 2944, 2833, 2495, 2235, 1687, 1451, 1121, 1032, 674 cm^{-1}; UV (MeOH) λ_{max} (log ε) 213 (3.84) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 2; positive HRESIMS *m/z* 730.4508 $[M + Na]^+$ (calcd for $C_{39}H_{65}NO_{10}Na$, 730.4506).

Alkaline Methanolysis of 1 and 2. Compounds **1** and **2** (each 3.0 mg) were treated with 3% NaOMe in MeOH at room temperature for 2 h. Each reaction mixture was then passed through an Amberlite IRA-67 column (Rohm and Haas) for neutralization and was evaporated under vacuum to obtain a residue, which was purified on a Sephadex LH-20 column (Pharmacia) using MeOH to give the main triterpene body [fasciculol B (**1a**; 0.7 mg) from **1** and fasciculol C (**2a**; 0.6 mg) from **2**] and methyl (*S*)-3-hydroxy-3-methylglutarate [**1b** (0.8 mg) from **1** and **2b** (0.5 mg) from **2**] as a viscous syrup.[16,19,20]

1a: colorless gum; $[\alpha]_D^{25}$ +75.4 (*c* 0.07, MeOH); ^1H NMR and ESIMS data were identical to those of fasciculol B.[16] **1b:** viscous syrup; $[\alpha]_D^{25}$ +6.5 (*c* 0.08, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 3.73 (3H, s, OMe), 2.72 (1H, d, *J* = 16.5 Hz, H-2), 2.67 (2H, s, H-4), 2.58 (1H, d, *J* = 16.5 Hz, H-2), 1.40 (3H, s, H-6); ESIMS *m/z* 177 $[M + H]^+$. **2a:** colorless gum; $[\alpha]_D^{25}$ +63.5 (*c* 0.06, MeOH); ^1H NMR and ESIMS data were identical to those of fasciculol C.[16] **2b:** viscous syrup; $[\alpha]_D^{25}$ +6.0 (*c* 0.05, $CHCl_3$); ^1H NMR and ESIMS data were identical to those of **1b**.

Cytotoxicity Testing. The cell lines used were A549 (non-small-cell lung adenocarcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). These cancer cell lines were provided by the National Cancer Institute (NCI). An SRB bioassay was used to determine the cytotoxicity of each compound against the cell lines mentioned above.[24] The assays were performed at the Korea Research Institute of Chemical Technology. Doxorubicin was used as a positive control. Doxorubicin cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines was IC_{50} 0.02, 0.01, 0.01, and 0.13 μM, respectively.

Measurement of NO Production and Cell Viability. Murine microglia BV-2 cells were plated into a 96-well plate (3×10^4 cells/well). After 24 h, cells were pretreated with samples for 30 min and then stimulated with 100 ng/mL of LPS for another 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture media using the Griess reaction. The supernatant (50 μL) was harvested and mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% *N*-1-napthylethylenediamine dihydrochloride in 5% phosphoric acid). Absorbance at 540 nm was measured after 10 min using a microplate reader. Sodium nitrite was used as the standard to calculate the NO_2^- concentration. Cell viability was assessed by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. N^G-Monomethyl-L-arginine (L-NMMA, Sigma, USA) was tested as a positive control. L-NMMA is a nonspecific NO synthase inhibitor.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR data of **1**−**5** and inhibitory effects on NO production of compounds **1**−**15** in LPS-activated BV-2 cells (Table 1S). These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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