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Sesquiterpene and Norsesquiterpene Derivatives from *Sanicula lamelligera* and Their Biological Evaluation

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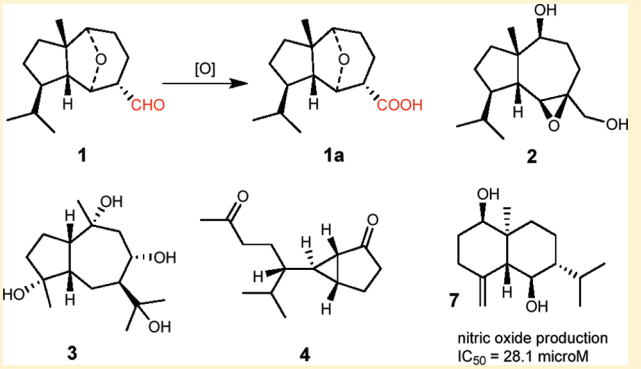
Supporting Information

ABSTRACT: Fourteen sesquiterpene and norsesquiterpene derivatives, comprising six different carbon skeletons, were isolated from *Sanicula lamelligera*. Saniculamoid A1 (**1a**) is an oxidation product of saniculamoid A (**1**), created by the transition of a formyl group to a carboxylic acid group after a period of storage in air. The known compounds **5**−**14** were identified in *Sanicula* plants for the first time. The compounds were evaluated for their anti-HIV-1, cytostatic, and nitric-oxide-production-inhibiting activities using in vitro cellular assays. The results showed that 1,5-naphthalenediol inhibited nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 cells with an IC_{50} value of 28.1 μM and was active toward five cancer cell lines with IC_{50} values in the 31.1−41.6 μM range.



Sanicula is a genus of the family Apiaceae. This genus has about 40 species worldwide, with 22 occurring in North America and 15 in the People's Republic of China. *Sanicula lamelligera* Hance, also known as *Fei-Jing-Cao* in Chinese, has been used as a folk medicine for the treatment of colds, cough, asthma, bleeding, gall, and amenorrhea.[1] Previous reports indicated that the extract of *S. lamelligera* exhibited significant antiviral activity against influenza A and also has expectorant effects.[2,3] However, the specific active compounds in this plant are unknown, apart from three previously identified compounds: rosmarinic acid and its glucopyranoside, and a triterpene glucoside.[4] In contrast, *S. europaea*, a traditional medicinal plant in Europe since the 12th century, has received much attention, and its constituent saponins have been shown to be the active compounds, with antimicrobial, hemolytic, and antioxidant activities.[5−8] As a continuation of our search for anti-infective and anti-inflammatory compounds from Traditional Chinese Medicines, we investigated the title plant and isolated 14 sesquiterpene and norsesquiterpene derivatives. In this paper, we describe their isolation, structural identification, and anti-HIV-1, cytostatic, and nitric-oxide-production-inhibiting activities.

Compound **1** was isolated as a colorless solid. Its molecular formula was determined to be $C_{15}H_{24}O_3$ by analysis of its EIMS, ^{13}C NMR, and DEPT spectra. The ^1H NMR spectrum showed a resonance at δ 9.83, corresponding to the presence of a formyl group. The ^{13}C NMR and DEPT spectra exhibited resonances (Table 1) for three methyl, four methylene, seven methine (including a formyl carbon), and one quaternary carbon, implying that **1** is a sesquiterpene similar to homalomenol D,[9] but differing in that a formyl group is attached to C-7 in **1** instead of a methyl and a hydroxy group, as in homalomenol D. The HMBC correlations of C*H*O/C-6, C-7, and C-8 confirmed this conclusion. The relative configuration of **1** was assigned from ROESY evidence (Figure 1), which showed correlations of H-10/H-15/H-5/H-7 and H-5/H-12/H-6, indicating that H-5, H-7, H-10, and CH_3-15 are β-oriented and that H-4 is α-oriented. When H-5 was irradiated, NOE signal enhancements of H-6, H-11, CH_3-13, and CH_3-15 were observed, and irradiation of CH_3-15 led to enhancement of the H-5 and H-10 resonances, which further supported the configuration assignments. The absence of a cross-peak between H-6 and H-7, together with the occurrence of H-6 as a doublet (J = 7.7 Hz), indicates that the dihedral angle of H−C(6)−C(7)−H approaches 90°. Notably, compound **1** is present in nature but is unstable in the pure form because of the presence of an *exo* formyl group. Compound **1** was oxidized to **1a**

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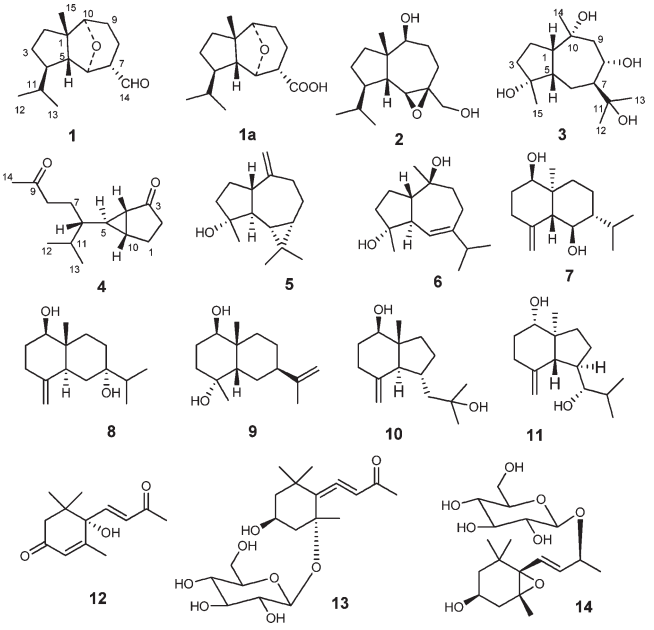
Table 1. NMR Spectroscopic Data for 1, 1a, and 2

	1		1a		2	
position	δ_H[a] (J in Hz)	δ_C[a] (J in Hz)	δ_H[a] (J in Hz)	δ_C[a] (J in Hz)	δ_H[a] (J in Hz)	δ_C[a] (J in Hz)
1		54.9 qC		54.6 qC		49.2 qC
2a	1.84 overlap	32.5 CH_2	1.84 m	32.6 CH_2	1.63 m	40.1 CH_2
2b	1.31 m		1.32 m		1.44 m	
3a	2.23 m	36.8 CH_2	2.24 m	36.6 CH_2	1.83 m	28.8 CH_2
3b	1.76 m		1.75 m		1.37 overlap	
4	1.59 overlap	48.4 CH	1.61 m	48.1 CH	1.70 overlap	57.1 CH
5	2.18 m	63.0 CH	2.13 t (8.3)	62.8 CH	1.36 m	52.7 CH
6	4.67 d (7.7)	77.0 CH	4.62 d (7.8)	78.4 CH	2.80 d (5.9)	63.0 CH
7	2.25 m	48.7 CH	2.53 d (5.5)	41.4 CH		62.4 qC
8a	2.08 m	14.9 CH_2	2.00 m	17.2 CH_2	2.03 m	25.6 CH_2
8b	1.95 m				1.37 overlap	
9a	1.86 overlap	24.4 CH_2	2.08 brs	24.4 CH_2	1.92 m	28.6 CH_2
9b	1.57 overlap		1.61 m		1.70 overlap	
10	3.66 d (3.9)	82.8 CH	3.77 brs	83.1 CH	3.74 dd (10.8, 5.6)	74.9 CH
11	1.37 m	35.0 CH	1.38 m	35.0 CH	1.52 m	33.8 CH
12	0.90 d (7.0)	22.1 CH_3	0.90 d (6.8)	22.0 CH_3	0.98 d (6.6)	21.7 CH_3
13	0.86 d (7.0)	21.7 CH_3	0.87 d (6.8)	21.6 CH_3	0.93 d (7.1)	21.0 CH_3
14a	9.83 s	203.6 CH		178.1 qC	3.78 d (12.7)	62.7 CH_2
14b					3.59 d (12.7)	
15	1.21 s	31.5 CH_3	1.23 s	31.6 CH_3	0.92 s	20.1 CH_3

[a] In $CDCl_3$.

after a period of storage in air, which made it difficult to collect sufficient physicochemical data. The structure of **1a** was identified by spectroscopic methods. As a result, compounds **1** and **1a** were assigned as shown and given the trivial names saniculamoids A and A1, respectively.



Compound **2** was obtained as a white solid. Its molecular formula was deduced as $C_{15}H_{26}O_3$ from the HRESIMS, ^{13}C NMR, and DEPT spectra, suggesting three degrees of unsaturation.

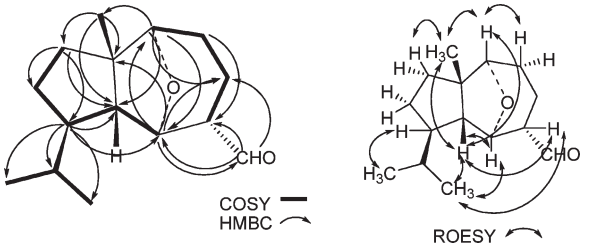


Figure 1. COSY, HMBC, and ROESY correlations of compound **1**.

The ^1H NMR spectrum showed resonances for two secondary methyl, one tertiary methyl, and two geminal protons at δ 3.78 (d, J = 12.7 Hz) and 3.59 (d, J = 12.7 Hz). The ^{13}C NMR and DEPT spectra indicated three methyl, five methylene (one oxygenated), five methine (two oxygenated), and two quaternary carbons (one oxygenated). The NMR spectroscopic data of **2** resembled those of 7-hydroxymethyl-1-isopropyl-3a-methyl-1,2,3,3a,4,5,6,8a-octahydroazulen-4-ol,[10] except that a $\Delta^{6,7}$ double bond is replaced by an epoxy group. The presence of an epoxy group is supported by the following evidence: (i) an upfield shift of H-6 (δ 2.80, J = 5.9) attributed to ring strain; (ii) HMBC correlations of H-14/C-6, C-7, C-8 and H-6/C-7; and (iii) the requirement for a degree of unsaturation. The ROESY correlations of CH_3-13/H-5/CH_3-15/H-2a, H-2b/H-10/H-8b, H-8a/H-14a, and H-4/H-6 suggest that H-5 and H-15 are β-oriented, whereas H-4, H-6, H-10, and H-14 are α-oriented. Consequently, the structure of **2** was identified as shown and given the trivial name saniculamoid B.

Compound **3** has the molecular formula $C_{15}H_{28}O_4$, deduced from its HRESIMS, ^{13}C NMR, and DEPT spectra. The ^1H NMR spectrum indicated four methyl groups, with one proton attached to an oxygenated carbon. The ^{13}C NMR and DEPT spectra

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Table 2. NMR Spectroscopic Data for 3 and 4

	3		4	
position	δ_H[a] (J in Hz)	δ_C[a] (J in Hz)	δ_H[b] (J in Hz)	δ_C[b] (J in Hz)
1a	2.68 m	53.0 CH	2.16 dd (9.3, 5.1)	22.7 CH_2
1b			2.04 m	
2a	1.80 m	26.2 CH_2	2.08 m	32.6 CH_2
2b	1.54 m			
3	1.67 overlap	37.7 CH_2		214.1 qC
4		83.9 qC	1.64 dd (4.0, 2.9)	34.2 CH
5	2.26 m	48.9 CH	1.06 ddd (10.1, 4.0, 2.8)	29.8 CH
6a	1.67 overlap	24.6 CH_2	0.70 m	46.1 CH
6b	1.51 m			
7a	1.64 m	53.7 CH	1.73 m	24.5 CH_2
7b			1.66 m	
8	4.28 m	70.1 CH	2.54 m	41.5 CH_2
9a	2.12 dd (13.9, 11.2)	44.9 CH_2		208.6 qC
9b	1.72 m			
10		73.6 qC	1.87 m	27.7 CH
11		75.7 qC	1.78 m	30.8 CH
12	1.29 s	26.9 CH_3	0.91 d (7.0)	19.4 CH_3
13	1.31 s	25.1 CH_3	0.91 d (7.0)	19.0 CH_3
14	1.16 s	32.2 CH_3	2.18 s	29.8 CH_3
15	1.31 s	31.0 CH_3		

[a] In CD_3OD. [b] In $CDCl_3$.

showed four methyl, four methylene, four methine (one oxygenated), and three oxygenated quaternary carbons. The NMR data (Table 2) suggested a guaiane-type sesquiterpene, similar to alismorientol A.[11] In the HMBC spectrum, the correlations of CH_3-14/C-1, C-9, C-10, CH_3-15/C-3, C-4, C-5, CH_3-12/C-10, C-11, and H-8/C-11, in conjunction with the chemical shifts of C-4, C-8, C-10, and C-11, indicated the locations of the four hydroxy groups. The ROESY correlations of CH_3-14/H-1, H-2b/H-9a, CH_3-12/H-8, and CH_3-15/H-5 suggested that these pairs of protons are coplanar. H-9a resonated as a doublet of doublets at δ 2.12 (dd, J = 13.9, 11.2 Hz), the large coupling constant indicating the *trans* relationship of H-8/H-9a. NOE enhancements of H-5 and CH_3-14 were observed when H-1 was irradiated. Similarly, the resonances for H-1 and CH_3-15 increased with irradiation of H-5. The above observations allowed the configurational assignments of **3**, as shown (Supporting Information: S2). The structure of compound **3** was thus determined as shown and named saniculamoid C.

The molecular formula of compound **4** was assigned as $C_{14}H_{23}O_2$ by its HRESIMS, ^{13}C NMR, and DEPT spectra. $^1H-^1H$ COSY, HSQC, and HMBC spectra established the planar structure of **4**, which is the same as that of chromolaevane dione (**4a**).[12] ROESY correlations of H-4/H-7/H-10 and H-5/H-4/H-10 were observed (Supporting Information: S2), suggesting that H-4 and H-10 are cofacial. The coupling constant for H-4 and H-5 is 4.0 Hz, indicating that these protons are *cis* oriented.[12] The coupling constant of H-5 and H-6 was 10.1 Hz, indicative of a *trans* relationship. The ^1H and ^{13}C NMR spectroscopic data (Table 2) agreed well with those of **4a**, except for a significant downfield shift of C-6 in **4** (**4**: δ 46.1; **4a**: δ 31.1), suggesting that **4** is a stereoisomer of **4a**. This conclusion was confirmed by the large difference in their specific rotations (**4**: $[\alpha]^{24}_D$ −52.2 {c 0.8, $CHCl_3$}; lit. **4a**: $[\alpha]^{20}_D$ +13.6 {c 0.1, $CHCl_3$}).[13] Therefore, **4** was identified as a new compound and named saniculamoid D.

Table 3. Anti-HIV-1 Activity of 1a and 2−14

compound	CC_{50} (μM)	EC_{50} (μM)	TI (CC_{50}/ EC_{50})
1a	194.9	162.3	1.2
2	357.9	125.6	2.9
3	>367.6	>367.6	
4	>450.5	195.5	>2.3
5	170.5	103.6	1.6
6	>420.2	>420.2	
7	68.1	31.9	2.1
8	>420.2	>420.2	
9	>420.2	150.4	>2.8
10	>420.2	>420.2	
11	397.9	166.8	2.4
12	>450.5	>450.5	
13	>259.1	>259.1	
14	>257.7	>257.7	
AZT	4071.9	4.5×10^{-3}	214331

The known sesquiterpenes were identified as 1*H*-cycloprop[*e*]azulen-7-ol (**5**),[14] (−)-alismoxide (**6**),[15] 1,5-naphthalenediol (**7**),[16] 4(15)-eudesmene-1α,7β-diol (**8**),[17] cyperusol C (**9**),[18] oppsit-4(15)-ene-1β,11-diol (**10**),[19] octahydro-4-hydroxy-3*R*-methyl-7-methylene-*R*-(1-methylethyl)-1*H*-indene-1-methanol (**11**),[20] (6*S*)-dehydrovomifoliol (**12**),[21] citroside B (**13**),[22] and phlomuroside (**14**)[23] by comparison with literature data. Compounds **5**−**14** were isolated from *Sanicula* plants for the first time.

Saponins have been reported from *Sanicula* plants.[24] However, sesquiterpene derivatives have not been reported in this genus. In the present study, we isolated a series of sesquiterpene and norsesquiterpene derivatives, including six different carbon skeletons, which add a new facet to the metabolites of this genus.

In accordance with the traditional medical applications of the herb, these compounds were evaluated for their in vitro antiviral,[25,26] cytostatic,[27,28] and anti-inflammatory effects using the cellular assays.[29] The results showed that compounds **1a**, **5**, and **7** are toxic to C8166 cells, with CC_{50} values of 194.9, 170.5, and 68.1 μM, respectively (Table 3). 1,5-naphthalenediol exhibited an EC_{50} value of 31.9 μM, with a therapeutic index (TI) of 2.1. An anti-inflammatory assay showed that 1,5-naphthalenediol inhibited nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 cells with an IC_{50} value of 28.1 μM, whereas the other compounds were inactive in this assay (IC_{50} value >64.4 μM), compared with MG-132 as a positive control, with an IC_{50} value of 0.439 μM. Five human tumor cell lines were used to evaluate the cytostatic activities of the compounds, revealing that only 1,5-naphthalenediol was active, with IC_{50} values of 31.1 (HL-60 cells), 32.4 (SMMC-7721 cells), 41.6 (A-549 cells), 35.3 (MCF-7 cells), and 37.0 μM (SW480 cells), whereas the other compounds were inactive (IC_{50} value >64.4 μM). DDP was used as a positive control, with IC_{50} values of 1.3 (HL-60 cells), 12.2 (SMMC-7721 cells), 13.0, (A-549 cells), 21.9 (MCF-7 cells), and 19.6 μM (SW480 cells), respectively.

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EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrometer. IR spectra were measured using a Tensor 27 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AV-400 or DRX-500, or an Avance III 600 spectrometer, with TMS as the internal standard. EIMS spectra were determined on a VG Autospec-3000 spectrometer; ESIMS and HRESIMS spectra were determined with an API QSTAR Pulsar 1 spectrometer. Silica gel (200−300 mesh; Qingdao Marine Chemical, Inc., People's Republic of China), MCI gel CHP 20P (75−150 μm; Mitsubishi Chemical, Co., Japan), RP-18 gel (40−63 μm; Daiso, Co., Japan), and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography.

Plant Material. Whole plants of *S. lamelligera* were collected from Mountain Emei, Sichuan Province, People's Republic of China, in October 2008, and were identified by one of the authors (X.Z.) at Hunan University of Traditional Medicine. A voucher specimen (FJC-200801) was deposited at Chengdu University of Traditional Chinese Medicine, Sichuan Province, People's Republic of China.

Extraction and Separation. The dried whole-plant powder of *S. lamelligera* (10 kg) was soaked with 80% aqueous ethanol (2 × 60 L) to produce an extract (810 g), which was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH (each 4 × 6 L). For detailed separation procedures, refer to the Supporting Information.

Saniculamoid A (***1***): colorless solid; ^{1}H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 236 (90), 219 (51), 218 (89), 208 (91), 189 (96), 96 (100).

Saniculamoid A1 (***1a***): colorless solid; $[\alpha]^{15}_D$ −35 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 234 (2.85), 203 (2.99) nm; IR (KBr) ν_{max} 3425, 2957, 2930, 2869, 1702, 1639, 1459, 1411, 1386, 1367, 1339, 1295, 1251, 1202, 1182, 1012, 988, 948, 878, 857, 792, 762, 616 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS (negative) *m*/*z* 251 $[M - H]^-$; HRESIMS (negative) *m*/*z* 251.1652 $[M - H]^-$ (calcd for $C_{15}H_{23}O_2$, 251.1647).

Saniculamoid B (***2***): white solid; $[\alpha]^{15}_D$ −8 (*c* 0.1, MeOH/$CHCl_3$, 1:1); UV (MeOH + $CHCl_3$, 1:1) λ_{max} (log ε) 204 (3.28), 194 (2.71) nm; IR (KBr) ν_{max} 3441, 2956, 2923, 2852, 1738, 1640, 1452, 1368, 1262, 1043, 1031, 930, 847, 805 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m*/*z* 277 $[M + Na]^+$; HRESIMS (positive) *m*/*z* 277.1779 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1779).

Saniculamoid C (***3***): colorless oil; $[\alpha]^{15}_D$ +5 (*c* 0.2, MeOH); UV (MeOH) λ_{max}(log ε) 389 (1.62), 203 (3.18) nm; IR (KBr) ν_{max} 3430, 2959, 2924, 2853, 1630, 1464, 1377, 1173, 1124, 1076, 1043, 915, 583 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS (positive) *m*/*z* 295 $[M + Na]^+$; HRESIMS (positive) *m*/*z* 295.1880 $[M + Na]^+$ (calcd for $C_{15}H_{28}O_4Na$, 295.1885).

Saniculamoid D (***4***): white oil; $[\alpha]^{24}_D$ −52 (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (3.47) nm; IR (KBr) ν_{max} 2959, 2930, 2873, 1721, 1464, 1415, 1367, 1286, 1186, 1042, 1013, 926, 896, 832, 800 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS (positive) *m*/*z* 245 $[M + Na]^+$, 233 $[M + H]^+$; HRESIMS (positive) *m*/*z* 233.1704 $[M + H]^+$ (calcd for $C_{14}H_{23}O_2$, 233.1698).

Anti-HIV-1 Assay. The cellular toxicity of the compounds was evaluated with the MTT method, as reported previously.[25] Briefly, cells were seeded in triplicate in a microtiter plate in the absence or presence of various concentrations of the compounds and incubated at 37 °C for 3 days. Wells without the compounds were used as the negative control. The percentage of viable cells was quantified on a Bio-Tek ELx 800 ELISA reader at 595/630 nm ($A_{595/630}$). The cytotoxic concentration that caused a reduction in viable cells of 50% (CC_{50}) was calculated from the dose−response curve. A syncytial assay was performed as follows.[26] Various concentrations of the compounds were added to a microtiter plate. C8166 cells (8×10^5/mL) were infected with HIV-1_{IIIB} at 1300 $TCID_{50}$ HIV-1 and then incubated at 37 °C for 3 days. AZT was used as the positive control. The cytopathic effect was determined by counting the numbers of syncytia. The percentage of syncytial cells in the treated culture relative to that in the infected control culture and the 50% effective concentration (EC_{50}) were calculated.

Cytostatic Assay. The cytostatic assay was performed with the MTT method, as reported previously, with slight modification.[27,28] Briefly, human tumor cells were seeded into 96-well plates and allowed to adhere for 12 h before the addition of the compounds. However, suspended cells were seeded immediately before the addition of the compounds, at an initial density of (1−2) × 10^5 cells/mL. Each cell line was incubated with different concentrations of the compounds for 48 h. Wells with DMSO were used as negative controls, and DDP was used as a positive control. Cell viability was measured, and the IC_{50} values were calculated.

Inhibition of Nitric Oxide Production Assay. The assay was performed as described previously.[29] Wells with DMSO were used as negative controls, and MG-132 was included as a positive control.

ASSOCIATED CONTENT

Supporting Information. Detailed separation procedures, COSY, HMBC, and ROESY correlations for compounds **1a** and **2**−**4**, 1D and 2D NMR spectra, and mass spectra of the new compounds are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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