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Lissoclibadins 8–14, polysulfur dopamine-derived alkaloids from the colonial ascidian *Lissoclinum* cf. *badium*

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ABSTRACT

Seven new polysulfur alkaloids, lissoclibadins 8 (**1**)–14 (**7**), were isolated from an ascidian, *Lissoclinum* cf. *badium*, collected in Indonesia. The structures were assigned on the basis of their spectroscopic data and computational modeling study. Lissoclibadin 8 (**1**) had four identical dopamine-derived units, and this is the first instance of a tetramer. Lissoclibadin 9 (**2**) was the second example of trimers next to lissoclibadin 1 (**8**). Lissoclibadins 10 (**3**)–12 (**5**) were symmetric dimers, and 13 (**6**) had a dopamine and ethyl units connected by sulfide and disulfide bonds. Lissoclibadin 14 (**7**) was a varacin-type monomer. These compounds inhibited the colony formation of Chinese hamster V79 cells and cell proliferation of murine leukemia L1210 cells.

1. Introduction

Ascidians are a rich source of biologically active nitrogenous substances with high chemical diversity.[1,2] More than 80% of new compounds from ascidians contained nitrogen, and about 70% of nitrogenous compounds are alkaloids. During our ongoing studies on bioactive alkaloidal components from ascidians, we have isolated seven new polysulfur aromatic alkaloids, lissoclibadins 8 (**1**)–14 (**7**) (Fig. 1), from the colonial ascidian, *Lissoclinum* cf. *badium*, collected in Indonesia.[3] This ascidian gave lissoclibadins 1 (**8**)–7 (**14**) and four known compound (**15**–**18**) (Fig. 1).[4–6] The related polysulfur alkaloids derived from dopamine have been reported from ascidians of the genera *Eudistoma*,[7] *Lissoclinum*,[7–12] and *Polycitor*.[13] These alkaloids were constructed with one,[7–10,12,13] two,[4–6,9,11,14] and three[4,5] dopamine units. In the present investigation, we isolated a tetramer for the first time, a trimer, four dimers, and a monomer. These new compounds showed cytotoxicity against murine and human tumor cell lines. We report herein the isolation, structures, and bioactivity of seven new lissoclibadins (**1**–**7**).

2. Results and discussion

Seven new compounds, lissoclibadins 8 (**1**)–14 (**7**), were isolated from *L.* cf. *badium* collected at Manado, Indonesia, in September, 2006, together with **8**, **10**, **11**, **14**, **15**, **17**, and **18**.[4–6]

2.1. Structure of lissoclibadin 8 (1)

The molecular formula ($C_{52}H_{76}N_4O_8S_{12}$) and weight (1268) were determined from HRFABMS and NMR data (Table 1). Two sets of ^1H and ^{13}C NMR signals were assigned by the analysis of ^1H–^1H COSY, HMQC, HMBC, and NOE difference spectra of **1** to two identical aromatic amine moieties (unit **a** in Fig. 2). Each unit had a hexa-substituted benzene ring and two methylenes, two OMe, an SMe, and an NMe_2 group. The ^1H–^1H COSY spectrum of **1** showed the connectivity of the two methylenes at C-7 and -8. NOEs were observed between H_3-9/H_3-10, H_3-10/H_3-11, and NMe_2/H_2-7 and -8 in

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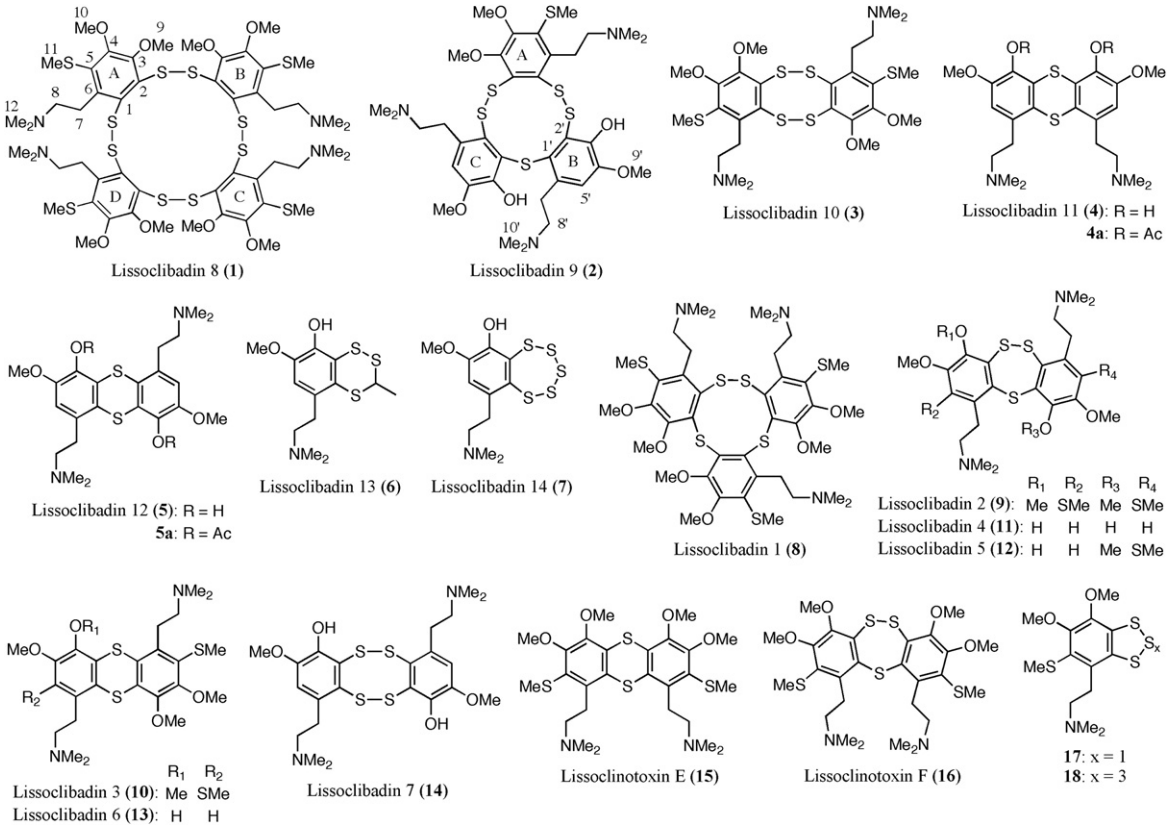

Figure 1. Structures of new lissoclibadins 8 (**1**)–14 (**7**) and compounds **8**–**18**.

each unit and between H_3-9 in one unit and H_3-11′ in another unit. These data and HMBC correlations revealed that the two identical aromatic units depicted as unit **a** (Fig. 2). Therefore, **1** had four identical aromatic amine units. On subtraction of the sum of four units from the molecular formula of **1**, there remained eight sulfur atoms. The fragment ion peaks observed in the FABMS of **1** at *m*/*z* 318 [(unit **a**+2S+H)$^+$], 635 [(2×unit **a**+4S+H)$^+$], and 952 [(3×unit **a**+6S+H)$^+$] showed that four units were, respectively, connected through a disulfide bond.

Four geometric isomers were possible for lissoclibadin 8 with the orientation of four units, cyclo[–A(1,2)–S–S–B(2,1)–S–S–C(1,2)–S–S–D(2,1)–S–S–] (clockwise, structure **1** shown in Fig. 1), cyclo[–A(1,2)–S–S–B(1,2)–S–S–C(1,2)–S–S–D(1,2)–S–S–] (**1a**), cyclo[–A(2,1)–S–S–B(2,1)–S–S–C(1,2)–S–S–D(1,2)–S–S–] (**1b**), and cyclo[–A(1,2)–S–S–B(2,1)–S–S–C(1,2)–S–S–D(1,2)–S–S–] (**1c**). Since **1** gave two sets of NMR signals, **1** has a center of symmetry, a C_2 axis, or a plane of symmetry in the molecule. An NOE was detected between an OMe signal at δ_H 3.96 (H_3-9) and an SMe signal at 2.45 (H_3-11′) in the different aromatic units in the NOE difference spectra of **1**. Considering these NMR data, we have employed Monte Carlo conformational analysis in H_2O on tetra-cation isomers (**1**, **1a**, **1b**, and **1c**) with MMFF94 force field utilizing Spartan'04.[15] Conformers of four isomers predicted to be the global energy minima revealed that isomers **1b** and **1c** had asymmetric conformations (C_1), that is, **1b** and **1c** should show four sets of NMR signals. Conformers of **1** and **1a** had C_i symmetry. Therefore, isomers **1** and **1a** give two sets of NMR signals. The closest distance between H_3-9 and H_3-11′ in conformers of **1** and **1a** were about 3.3 and 5.2 Å, respectively. Three-dimensional structures of **1** are shown in Figure 3. A C_i symmetric structure is seen in Figure 3a, and an S-shaped conformation and close positions of H_3-9 and H_3-11′ are observed in Figure 3b (view of Fig. 3a from the right side of B). Consequently, **1** will be the most probable structure of lissoclibadin 8. This is the first example of tetrameric polysulfur aromatic alkaloid.

Table 1
^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for lissoclibadin 8 (**1**) in CD_3OD

Unit A (C)				Unit B (D)			
C#	δ_C	δ_H, mult.	HMBC	C#	δ_C	δ_H, mult.	HMBC
1	134.8			1′	136.9[a]		
2	140.0[b]			2′	143.5[b]		
3	155.8			3′	158.1		
4	158.5			4′	159.1		
5	137.0[a]			5′	136.5		
6	139.9[c]			6′	139.5[c]		
7	29.5	2.62, m, 3.05, m	1, 6	7′	30.6	3.82, m, 4.01, m	1′, 5′
8	57.5	2.64, m, 2.84, m		8′	58.2	3.38, m, 3.49, m	
9	61.9	3.96, s	3	9′	60.7	3.17, s	3′
10	61.4	4.04, s	4	10′	62.2	4.09, s	4′
11	19.5	2.60, s	5	11′	19.3	2.45, s	5′
12	42.9	2.73, br s	8, 12	12′	43.6	3.13, br s	8′, 12′

[a, b, c] Signals may be interchangeable for the same letters.

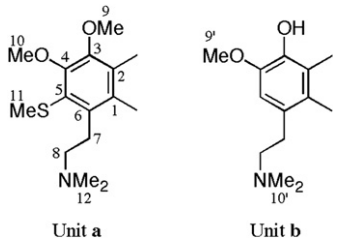

Figure 2. Partial structures of units **a** and **b**.

2.2. Structure of lissoclibadin 9 (2)

Lissoclibadin 9 (**2**) showed three sets of NMR signals (Table 2). The molecular weight (799) and formula ($C_{35}H_{49}N_3O_6S_6$) were deduced from HRFABMS and NMR data. Three aromatic amine moieties were assigned by the analysis of ^{1}H–^{1}H COSY, HMQC, HMBC, and NOE difference spectra of **2**. NMR data for one of three units (unit A) was very similar to those for **1** and identified the structure of unit A as unit **a** (Fig. 2). The other two units were identical and each had a pentasubstituted benzene ring and two methylenes, an OH, an OMe, and an NMe_2 group. The connectivity of H_2-7 and -8 was assigned by the ^{1}H–^{1}H COSY spectrum. Key HMBC correlations were detected from H-5′ to C-1′, -3′, and -7′, H_3-9′ to C-4′, and NMe_2 to C-8′ (Table 2). NOEs were observed between H-5′/H_3-9′ and H-5′/H_2-7′. Therefore, these two units were assigned as unit **b** (Fig. 2). The sum of three units was 160 Da (S_5) less than the molecular weight (formula) of **2**. FABMS data for **2** showed two prominent fragment ions at *m*/*z* 318 [(unit **a**+2S+H)$^+$] and 483 [(2×unit **b**+3S+H)$^+$]. Therefore, unit A (unit **a**) was connected with units B and C through two disulfide bonds and units B and C (both unit **b**) through a sulfide bond. Thus, the gross structure of lissoclibadin 9 was determined as a tetracyclic tribenzopentathiepin, which was the second example of trimeric polysulfur aromatic alkaloids next to lissoclibadin 1 (**8**) obtained from the same ascidian.

Four geometric isomers were possible for lissoclibadin 9 with the orientation of three aromatic amine units, cyclo[–A(2,1)–B(2,1)–C(2,1)–] (clockwise, structure **2** shown in Fig. 1), cyclo[–A(2,1)–B(1,2)–C(1,2)–] (**2a**), cyclo[–A(2,1)–B(2,1)–C(1,2)–] (**2b**), and cyclo[–A(2,1)–B(1,2)–C(2,1)–] (**2c**). Since no NOE was detected to connect each unit, we have performed Monte Carlo conformational analysis in H_2O on four tri-cation isomers as similar to lissoclibadin 1 (**8**)[4,5] with MMFF94 force field using Spartan'04. The isomer **2** had the lowest energy minimum value and the other isomers showed relatively higher energy values (Table 3).

From these results, we have selected the isomer **2** (Fig. 4) as the most probable structure of lissoclibadin **9**. However, the other isomers cannot be excluded since biogenetic enzymes may construct a thermodynamically more unfavorable structures.[4,5,14]

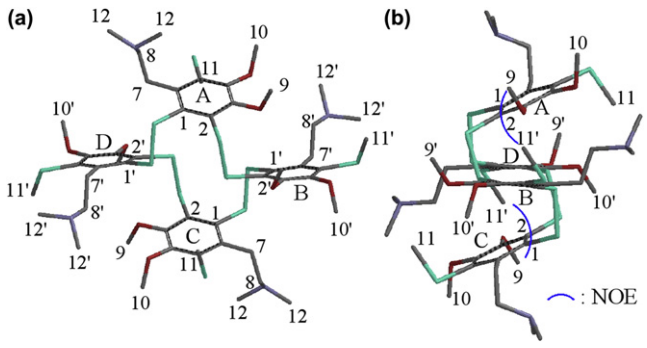

Figure 3. 3D structures (tri-cation in H_2O) of lissoclibadin 8 (**1**) (H atoms are omitted for clarity).

2.3. Structure of lissoclibadin 10 (3)

The molecular formula ($C_{26}H_{38}N_2O_4S_6$) and NMR data (Table 4) showed that **3** was a dimer of symmetric structure. The aromatic unit assigned by the analysis of ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra of **3** was unit **a** in Figure 2. The sum of the two units was 128 Da (S_4) less than the molecular weight (formula) of **3**. Two fragment ion peaks at *m*/*z* 318 [(unit **a**+2S+H)$^+$] and 273 [(318−NMe_2)$^+$] suggested that two aromatic units were connected through two disulfide bonds. Two compounds, lissoclibadin 7 (**14**)[6] and lissoclinotoxin D (Fig. 5),[9] have been reported as examples of this type of dimers. The antiparallel (*trans*-type) orientation of lissoclinotoxin D was selected on the basis of a computational modeling study,[9,14] and antiparallel structures of **3** and **14** were drawn following the study on lissoclinotoxin D. However, parallel (*cis*-type) structures cannot be excluded. Interestingly, each one isomer of these dimers (two disulfide bonds) has been isolated, although two sets of isomers were obtained for the other two types of dimers, **4** versus **5** and **10** versus **15** (two sulfide bonds) and **9** versus **16** (disulfide/sulfide bonds).

2.4. Structures of lissoclibadins 11 (4) and 12 (5)

Lissoclibadins 11 (**4**) and 12 (**5**) had the same molecular weight (450) and formula ($C_{22}H_{30}N_2O_4S_2$) and showed each one set of NMR signals (Table 4). The analysis of ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra of **4** and **5** revealed that both compounds had unit **b** in Figure 2 as the aromatic amine moiety. Therefore, two units were connected through two sulfide bonds and **4** and **5** were isomers of parallel and antiparallel structures. Since methylation of OH groups in **4** and **5** was unsuccessful, acetyl derivatives (**4a** and **5a**) were prepared and measured the NOE difference spectra. An NOE was detected between OAc (2.43) and NMe_2 (3.00) in the NOE difference spectra of **5a**, but not **4a**. Therefore, parallel (**4**) and antiparallel (**5**) structures were assigned to lissoclibadins 11 and 12, respectively.

2.5. Structure of lissoclibadin 13 (6)

HRFABMS and NMR data (Table 5) revealed the molecular formula ($C_{13}H_{19}NO_2S_3$) and weight (317) of **6**. The ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra of **6** showed the presence of unit **b** (Fig. 2) as an aromatic amine unit and a partial structure of –S–$CH(CH_3)$–S– (C_2 group), which was also suggested by a fragment ion peak at *m*/*z* 258 [(M−$SCHCH_3$+H)$^+$] in the FABMS of **6**. Therefore, the C_2 group was connected with the aromatic amine unit through sulfide and disulfide bonds. Although H-1′ did not show HMBC correlation to aromatic carbons, an NOE was observed between NMe_2 and H_3-2′ in the NOESY spectrum of **6**. Consequently, the structure of lissoclibadin 13 (**6**) was assigned as shown in Figure 1. Compound **6** has a chiral carbon at C-1′, but its stereochemistry has not been determined.

2.6. Structure of lissoclibadin 14 (7)[16]

The analysis of ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra of **7** revealed the presence of unit **b** (Fig. 2) as an aromatic amine unit. On subtraction of unit **b** from the molecular formula ($C_{11}H_{16}NO_2S_5$) of **7**, there remained five sulfur atoms. Therefore, **7** was assigned as a varacin-type monomer as shown in Figure 1.[16]

2.7. Biological activity

Compounds **1**, **2**, and **4**–**7** were tested their effects on the colony formation of Chinese hamster V79 cells. This bioassay reflects the direct action of compounds on the cells.[17] Results from three

Table 2
^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for Lissoclibadin 9 (**2**) in CD_3OD

Unit A				Units B and C						
C#	δ_C	δ_H, mult.	HMBC	C#	δ_C	δ_H, mult.	HMBC	δ_C	δ_H, mult.	HMBC
1	142.8			1′	136.1			127.6		
2	139.8			2′	123.7			130.0		
3	156.1			3′	150.3			145.6		
4	157.8			4′	149.1			151.6		
5	133.8[a]			5′	116.2	7.01, s	1′, 3′, 7′	112.8	6.94, s	1′, 3′, 7′
6	135.0			6′	132.4			133.9[a]		
7	28.8	2.65, m, 2.78, m		7′	31.3	3.42, m		31.6	3.42, m	
8	57.8	3.12, m		8′	59.8	3.40, m		58.5	3.68, m	
9	61.3	4.04, s	3	9′	56.9	3.91, s	4′	56.6	3.84, s	4′
10	61.3	3.96, s	4	10′	43.7[b]	2.96, s	8′, 10′	43.6[b]	3.04, s	8′, 10′
11	19.6	2.34, s	5							
12	43.5[b]	2.86, s	8, 12							

[a, b] Signals may be interchangeable for the same letters.

different experiments, including **8**–**14**, and **16**–**18**, are summarized in Table 6. Cytotoxicity of lissoclibadins 1 (**8**)–14 (**7**), except for 10 (**3**), was also examined against murine leukemia L1210 cells, and Table 6 shows the results from the same experiment. Lissoclibadin 12 (**5**) was not active at 20.0 μM (9.0 μg/mL) and 11 (**4**) also showed a reduced activity. Although lissoclibadin 10 (**3**) could not be examined against V79 and L1210 cells because of the sample availability, this compound showed similar magnitudes of antiproliferative activity (data not shown)[18] against human breast MDA-MB-231 and large-cell lung NCI-H460 cell lines to those of lissoclibadins 1 (**8**) and 2 (**9**).[17]

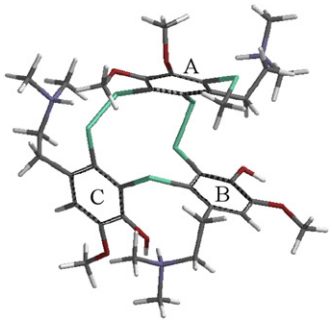

Figure 4. 3D structure (tri-cation in H_2O) of lissoclibadin 9 (**2**).

3. Experimental section

3.1. General experimental procedures

NMR spectra were measured on a JEOL JNM-AL-400 or JNM-LA-600 spectrometer. Mass spectra were obtained by a JEOL JMS-MS 700 mass spectrometer (FAB mode, *m*-nitrobenzyl alcohol or glycerol as the matrix). UV and IR spectra were recorded on a Hitachi U-3310 spectrophotometer and a Perkin–Elmer Spectrum One FT-IR spectrometer, respectively. Fetal bovine serum (FBS) was obtained from GIBCO after checking the lot, and all other reagents and chemicals for bioassay were of the highest grade available commercially.

3.2. Ascidian

L. cf. *badium* was collected by scuba diving at −5 to −21 m off the coral reef in Manado, Indonesia in September 2006. The voucher specimen is deposited at the Nagoya University Museum as NUM-Az0391.

3.3. Extraction and isolation

The ascidian (about 500 g) was cut into small pieces and soaked in EtOH on a boat immediately after collection. The organism was further extracted three times with EtOH. The extract (13.2 g) was dissolved in 1% NH_4OH–MeOH (1:1, 150 mL) and adsorbed on an ODS column (400 mL). The ODS column was washed with the same solvent mixture (500 mL) and eluted successively with MeOH (1.3 L), 0.1% TFA–MeOH [4:1 (3 L) and 1:1 (2.5 L)], and MeOH (containing 0.1% TFA, 3 L). The MeOH eluate (1.95 g) was separated by an LH-20 column (300 mL) with 0.1% TFA–MeOH (gradient elution) into four fractions (Fr. 2-1–2-4). Fr. 2-2 (70 mg) was subjected to ODS HPLC (10–50% CH_3CN–0.1% TFA, linear gradient) to give **4** (3 mg), **14** (2 mg), **17** (2.7 mg), and Fr. 2-2-3, which was purified by ODS HPLC (30–45% MeOH–0.1% TFA, linear gradient) to afford **2** (2 mg). Fr 2-3 (27 mg) gave **11** (5 mg) and **14** (6 mg) by ODS HPLC (10–50% CH_3CN–0.1% TFA, linear gradient). Compound **7** (4.5 mg) was purified from Fr. 2-4 (150 mg) by ODS HPLC (30–80% CH_3CN–0.1% TFA, linear gradient). The MeOH (0.1% TFA) eluate (0.63 g) from the above ODS column was chromatographed on ODS (250 mL) with 0.1% TFA–MeOH (gradient) into 13 fractions (Fr. 5-1–5-13). Fr. 5-4 (55 mg) was separated by ODS HPLC (18–35% CH_3CN–0.1% TFA, linear gradient) to give **5** (3 mg) and **6** (2 mg). Compounds **1** (6 mg) and **10** (8 mg) were purified from Fr. 5-8 (45 mg) by ODS HPLC (45% CH_3CN–0.1% TFA), and **3** (2 mg), **8** (16 mg), **15** (28 mg), and **18** (2.5 mg) were obtained by ODS HPLC (25–50% CH_3CN–0.1% TFA, linear gradient) from Fr. 5-13 (100 mg).

Compounds **1** and **2** were also isolated from the remaining fractions collected in March 2006,[6] and **3** was first obtained from the ascidian collected in September 2005.

*3.3.1. Lissoclibadin 8 (**1**).* Isolated as a tetrakis-TFA salt; yellow film; UV λ_{max} (MeOH) nm (log ϵ): 263 (4.30), 310 (4.00); IR ν_{max} (KBr) cm^{-1}: 1681, 1380, 1204, 1178, 1126, 1062, 1017; FABMS *m*/*z* 1269 [$(M+H)^+$], 952, 635, 318, 273; HRFABMS [$(M+H)^+$, *m*/*z* 1269.2356,

Table 3
Relative energy values of energy minima of four possible isomers of lissoclibadin 9

Isomer (in H_2O, tri-cation)	**2**	**2a**	**2b**	**2c**
kJ/mol (kcal/mol)	0.0 (0.0)	+25.1 (+6.0)	+48.1 (+11.5)	+20.3 (+4.9)

Table 4
^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for lissoclibadins 10 (**3**), 11 (**4**), and 12 (**5**) in CD_3OD

C#	**3**			**4**			**5**		
	δ_C	δ_H, mult.	HMBC	δ_C	δ_H, mult.	HMBC	δ_C	δ_H, mult.	HMBC
1	137.5			127.6			127.3		
2	139.7			125.1			125.5		
3	154.9			145.5			145.7		
4	157.1			148.8			148.7		
5	135.1			113.2	6.94, s	1, 3, 4, 7	113.6	6.95, s	1, 3, 4, 7
6	138.6			126.8			125.4		
7	30.0	3.71, m, 3.81, m		30.5	3.33, m	1, 5, 6, 8	30.3	3.35, m	1, 5, 6, 8
8	57.5	3.20, br s	12	59.4	3.31, m	7, 10	58.7	3.23, m	7, 10
9	62.0	3.93, s	3	56.9	3.88, s	4	56.9	3.88, s	4
10	60.8	3.97, s	4	43.7	2.99, s	8, 10	43.6	3.01, s	8, 10
11	19.0	2.49, s	5						
12	43.4	3.00, br s	8, 12						

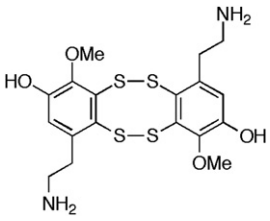

Figure 5. Structure of lissoclinotoxin D.

Calcd for $C_{52}H_{77}N_4O_8S_{12}$, 1269.2390]; ^{1}H and ^{13}C NMR data, see Table 1.

*3.3.2. Lissoclibadin 9 (**2**).* Isolated as a tris-TFA salt; yellow film; UV λ_{max} (MeOH) nm (log ϵ): 258 (4.10), 335 (3.56); IR ν_{max} (KBr) cm^{-1}: 1681, 1464, 1387, 1202, 1180, 1131, 1067; FABMS *m/z* 800 [$(M+H)^+$], 483, 318, 273; HRFABMS [$(M+H)^+$, *m/z* 800.1998, Calcd for $C_{35}H_{50}N_3O_6S_6$, 800.2024]; ^{1}H and ^{13}C NMR data, see Table 2.

*3.3.3. Lissoclibadin 10 (**3**).* Isolated as a bis-TFA salt; yellow film; FABMS *m/z* 635 [$(M+H)^+$], 318, 273; HRFABMS [$(M+H)^+$, *m/z* 635.1219, Calcd for $C_{26}H_{39}N_2O_4S_6$, 635.1234]; ^{1}H and ^{13}C NMR data, see Table 4.

*3.3.4. Lissoclibadin 11 (**4**).* Isolated as a bis-TFA salt; yellow film; UV λ_{max} (MeOH) nm (log ϵ): 239 (4.37), 260 (4.31), 300 (4.05), 309 (4.07); IR ν_{max} (KBr) cm^{-1}: 3341, 1679, 1484, 1462, 1391, 1278, 1199, 1180, 1133, 1070; FABMS *m/z* 451 [$(M+H)^+$]; HRFABMS [$(M+H)^+$, *m/z* 451.1748, Calcd for $C_{22}H_{31}N_2O_4S_2$, 451.1725]; ^{1}H and ^{13}C NMR data, see Table 4.

Table 6
Cytotoxicity (IC_{50}) against Chinese hamster V79 and murine leukemia L1210 cells

	V79[a]	V79[b]	V79[c]	L1210
1	0.14 (0.18)[d]			2.00 (2.54)
2	0.63 (0.50)			0.38 (0.30)
4	>20.0 (>9.0)			>20.0 (>9.0)
5	7.90 (3.56)			>20.0 (>9.0)
6	0.44 (0.14)			2.20 (0.70)
7	0.70 (0.25)			1.80 (0.64)
8	0.21 (0.19)	0.20 (0.18)	0.40 (0.35)	1.50 (1.33)
9			0.08 (0.05)	2.04 (1.23)
10			0.34 (0.19)	2.79 (1.59)
11		0.71 (0.34)		1.94 (0.94)
12		0.058 (0.031)		0.97 (0.53)
13		0.057 (0.029)		0.63 (0.32)
14		0.17 (0.09)		2.17 (1.12)
16			0.28 (0.17)	
17			0.19 (0.07)	
18			0.15 (006)	

[a] Present study.
[b] Reported in Ref. 6.
[c] Reported in Ref. 17.
[d] IC_{50} value as μM (μg/mL).

*3.3.5. Lissoclibadin 12 (**5**).* Isolated as a bis-TFA salt; yellow film; UV λ_{max} (MeOH) nm (log ϵ): 242 (4.04), 260 (3.98), 299 (3.62), 309 (3.68); IR ν_{max} (KBr) cm^{-1}: 3363, 1678, 1485, 1463, 1398, 1274, 1202, 1134, 1073; FABMS *m/z* 451 [$(M+H)^+$], 406 [$(M-NHMe_2+H)^+$], 361

Table 5
^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for lissoclibadins 13 (**6**) and 14 (**7**) in CD_3OD

C#	**6**				**7**		
	δ_C	δ_H, mult.	HMBC	NOESY	δ_C	δ_H, mult.	HMBC
1	128.8				136.6		
2	130.6				131.8		
3	144.2				150.3		
4	148.5				151.6		
5	111.4	6.90, s	1, 3, 6, 7	9	115.3	6.98, s	1, 3, 4, 6,7
6	127.2				133.8		
7	29.8	3.28, m	1, 5, 8		32.0	3.28, m	1, 5, 6, 8
8	59.1	3.34, m	6, 7, 10	10	59.6	3.30, m	7, 10
9	56.8	3.90, s	4	5	57.0	3.94, s	4
10	43.6	2.96, s	8, 10	8, 2′	43.6	2.96, s	8, 10
1′	53.2	4.94, q (6.4)					
2′	23.6	1.70, d (6.4)	1′	10			

[(M−2NHMe$_2$+H)$^+$]; HRFABMS [(M+H)$^+$, *m*/*z* 451.1721, Calcd for $C_{22}H_{31}N_2O_4S_2$, 451.1725]; ^{1}H and ^{13}C NMR data, see Table 4.

*3.3.6. Lissoclibadin 13 (**6**).* Isolated as a TFA salt; yellow film; $[\alpha]_D$ −6.1 (*c* 0.2, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 262 (3.53), 314 (3.20); IR ν_{max} (KBr) cm^{-1}: 1680, 1467, 1398, 1206, 1133, 1067; FABMS *m*/*z* 318 [(M+H)$^+$], 258 [(M−SCHCH$_3$+H)$^+$]; HRFABMS [(M+H)$^+$, *m*/*z* 318.0640, Calcd for $C_{13}H_{20}NO_2S_3$, 318.0657]; ^{1}H and ^{13}C NMR data, see Table 5.

*3.3.7. Lissoclibadin 14 (**7**).* Isolated as a TFA salt; yellow film; UV λ_{max} (MeOH) nm (log ϵ): 245 (4.05), 355 (3.42); IR ν_{max} (KBr) cm^{-1}: 1678, 1466, 1391, 1204, 1180, 1137, 1064; FABMS *m*/*z* 354 [(M+H)$^+$], 290 [(M−S$_2$+H)$^+$], 258 [(M−S$_3$+H)$^+$]; HRFABMS [(M+H)$^+$, *m*/*z* 353.9799, Calcd for $C_{11}H_{16}NO_2S_5$, 353.9785]; ^{1}H and ^{13}C NMR data, see Table 5.

3.4. Acetyl derivatives (4a and 5a) of 4 and 5

Acetic anhydride (20 μL) was added to a solution of **4** (2.5 mg) and 4-dimethylaminopyridine (4.0 mg) in CH_2Cl_2 (0.5 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at rt for 1 h and evaporated. The residue was purified by ODS HPLC (10–80% MeOH–0.1% TFA, linear gradient) to give 2.0 mg of **4a**: ^{1}H NMR (600 MHz, CD_3OD) δ 7.16 (1H, s, H-5), 3.86 (3H, s, H$_3$-9), 3.41 (4H, m, H$_2$-7 and -8), 3.02 (6H, s, two H$_3$-10), 2.36 (3H, s, OAc).

Compound **5** (1.5 mg) was treated by the similar procedure as above to afford 1.1 mg of **5a**: ^{1}H NMR (600 MHz, CD_3OD) δ 7.16 (1H, s, H-5), 3.87 (3H, s, H$_3$-9), 3.32 (4H, m, H$_2$-7 and -8), 3.00 (6H, s, two H$_3$-10), 2.43 (3H, s, OAc).

3.5. Relative plating efficiency

Chinese hamster V79 cells were grown as a monolayer in Eagle's MEM (Nissui Seiyaku Co. Ltd, Tokyo, Japan) with 10% heat-inactivated FBS. Two hundred cells were seeded onto a 60/15 mm Petri dish in 4 mL of MEM with 10% FBS and incubated overnight at 37 °C. Samples were dissolved in DMSO, and 4 μL of each sample was added to the dish, which were incubated for another four days. The numbers of colonies in the sample dishes were counted and compared with those in the control dishes. The relative plating efficiency of the sample against V79 cells at a given concentration (0.01–10 μM) was described as the ratio of the number of colonies in the sample dish to that in the control culture as described in previous papers.[19,20]

3.6. Cytotoxicity

L1210 cells were incubated in RPMI 1640 using 96-well assay plates. Samples were dissolved in MeOH, and 10 μL of each sample solution was poured in a well and the solvent evaporated in a clean bench. The suspension (4×10^4 cells/mL, 100 μL) of L1210 was added into each well and incubated at 37 °C for 72 h in a CO_2 incubator. The number of vital cells in the sample wells was compared with those in the control wells using XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide] (cell proliferation kit II®).

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