Pak B with 5% Et_2O in hexane at 2 mL/min as the eluant and with the UV detector at 350 nm. The retention data are shown in Table VI. The solvents were from Burdick and Jackson, and they were degassed prior to use.

Nuclear Overhauser Effect (NOE) Determinations. All ^1H NOEs were measured on a Bruker WM-250 spectrophotometer operating in the pulse-FT mode. Each sample (ca. 5 mg) was dissolved in 0.5 mL of dry deuterioacetone (Merck acetone-d_6 "100%"), degassed by several freeze–pump–thaw cycles and sealed. The NOE values were determined from the ratio between the relative peak intensities with the irradiating field on resonance and off resonance for the saturated ^1H signal. At least five NOE measurements were made for each ^1H signal of each sample studied. The standard deviation was always 2% or less. The field strength of the irradiating field was determined for each sample by setting the decoupling power level so that a maximum increase in intensity of interacting protons was obtained without affecting other proton signals. In all experiments the pulse delay time used was sufficiently long to allow complete recovery of all signals. This time ranged from 40 to 60 s, depending on the sample.

The ambient probe temperature was 24 °C; the high-temperature experiments were run at 50 °C and the low temperature experiments at −50 °C. No significant changes were observed in the spectra or the NOE values at either the high or low temperatures.

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Registry No. *trans*-1, 3917-41-7; *cis*-1, 56013-13-9; 2, 67116-19-2; 3, 81121-53-1; 4, 81177-15-3; *cis*-5, 73192-75-3; *trans*-5, 73178-43-5; 6, 81176-73-0; 6e, 80009-89-8; 7, 81176-74-1; 7e, 80040-38-6; 8, 6703-19-1; 8e, 79985-66-3; 9e, 80009-88-7; *cis*-dimethyl β-methylglutaconate, 1712-35-2; *trans*-dimethyl β-methylglutaconate, 41527-39-3; *trans*-retinoic acid, 302-79-4; 13-*cis*-retinoic acid, 4759-48-2.

Antineoplastic Cyclic Peptides from the Marine Tunicate *Lissoclinum patella*

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The tunicate *Lissoclinum patella* produces a family of lipophilic cyclic peptides all of which contain an unusual fused oxazoline–thiazole unit. The structures of three of these peptides, patellamides A–C have been determined by chemical and spectral methods. The patellamides are cytotoxic, exhibiting IC_{50} values of 2–4 μg/mL against L1210 murine leukemia cells. Additionally, patellamide A was active against the human ALL cell line CEM with an ID_{50} of 0.028 μg/mL. Ulithiacyclamide, a peptide previously reported from *L. patella* was also tested for cytotoxicity and exhibited 50% inhibition at doses of 0.35 and 0.01 μg/mL for the L1210 and CEM tests, respectively.

As part of a program to isolate antineoplastic natural products from marine invertebrates, we have undertaken a systematic study of didemnid tunicates from Palau of the Western Caroline Islands. The didemnids seemed likely candidates for several reasons. It is well-known that tropical didemnid species harbor unicellular prokaryotic algae,[1,2] and even though nitrogen fixation is yet to be demonstrated in these symbionts, we felt the possibility warranted an investigation for novel nitrogenous metabolites. This was particularly intriguing in view of the activities encountered with terrestrial alkaloids.[3] Furthermore, cytotoxicity has been documented in extracts of tunicates,[4] and cytotoxic constituents have been isolated by Rinehart,[5] Fenical,[6] and Howard.[7]

As a result of preliminary studies, we recently reported the isolation of *N,N'*-diphenethylurea (1; see Chart I) from *Didemnum ternatanum*[8] and the cyclic peptides ulithiacylamide (2) and ulicyclamide (3) from *Lissoclinum patella*.[9] Both tunicates were collected on reef flats near Korror Island, Palau Islands. We now report the isolation of three additional cyclic peptides, patellamides A (4), B (5) and C (6) from *L. patella* collected at Eil Malk Island, Palau Islands. All six of these metabolites were tested for antitumor activity against L1210 murine leukemia cells cultured in vitro. As depicted in Table I, ulithiacyclamide (2) was the most potent having an IC_{50} of 0.35 μg/mL, whereas ulicyclamide (3) had an IC_{50} of 7.2 μg/mL. Patellamides A (4), B (5), and C (6) exhibited approximately equal activities with IC_{50} values of 3.9, 2.0, and 3.2 μg/mL,

Table I. Cytotoxicity Testing Results for Metabolites 1-6 from Marine Tunicates

compd	IC_{50}, μg/mL L1210	CEM
N,N'-diphenethylurea (1)	>10	
ulithiacyclamide (2)	0.35	0.01
ulicyclamide (3)	7.2	
patellamide A (4)	3.9	0.028
patellamide B (5)	2.0	
patellamide C (6)	3.2	

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(1) Lewin, R. A.; Cheng, L. *Phycologia* **1975**, *14*, 149–152.
(2) Newcomb, E. H.; Pugh, T. D. *Nature (London)* **1975**, *253*, 533–534.
(3) Pettit, G. R.; Ode, R. H. "Biosynthetic Products for Cancer Chemotherapy"; Plenum Press: New York, 1979; Vol. III and previous volumes.
(4) Weinheimer, A. J.; Matson, J. A.; Karns, T. K. B.; Hossain, M. B.; van der Helm, D. "Food and Drugs from the Sea"; Kahl, P. N., Sinderman, C. J., Eds.; University of Oklahoma: Norman, OK, 1978; pp 117–121.
(5) (a) Carter, G. T.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 7441–7442. (b) Cheng, M. T.; Rinehart, K. L., Jr. *Ibid.* **1978**, *100*, 7409–7411. (c) Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C., Jr. *Ibid.* **1981**, *103*, 1857–1859.
(6) Fenical, W. H. "Food and Drugs from the Sea"; Webber, H. H., Ruggieri, G. D., Eds.; Marine Technology Society: Washington, DC, 1974, p 388.
(7) Howard, B. M.; Clarkson, K.; Bernstein, R. L. *Tetrahedron Lett.* **1979**, 4449.
(8) Ireland, C. M.; Durso, A. R., Jr.; Scheuer, P. J. *J. Nat. Prod.* **1981**, *44*, 360–361.

0022-3263/82/1947-1807$01.25/0

Chart I

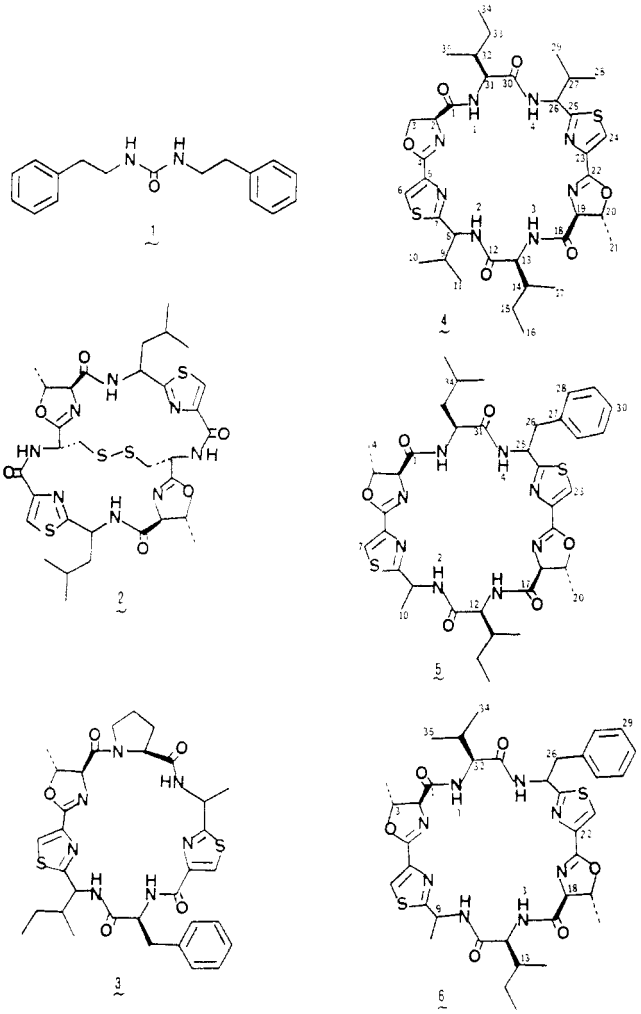
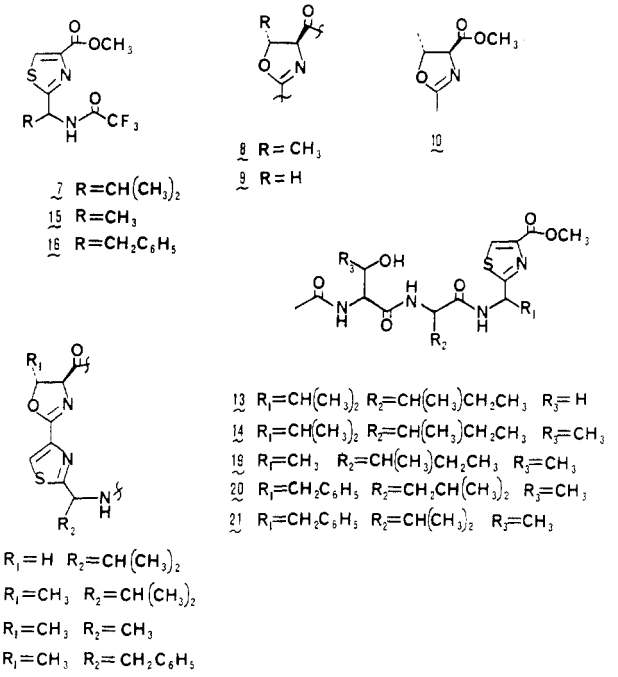


Chart II



respectively. *N,N'*-Diphenethylurea (**1**) had no activity. Patellamide A (**4**) and ulithiacyclamide (**2**) also inhibited the human ALL cell line (T cell acute leukemia) CEM with ID_{50} values of 0.028 and 0.010 μg/mL, respectively.

Methanol extraction of homogenized *L. patella* collected at Eil Malk yielded a dark green oil (0.60 g). Silica column chromatography of this oil by eluting with CH_2Cl_2/EtOAc (6/4) yielded four components that were visible under 254-nm light or after spraying with an *o*-tolidine reagent.[10] The first metabolite eluted from the column (80 mg) was identical in every respect with ulithiacyclamide (**2**). The remaining three components in order of elution were patellamides A (**4**, 100 mg), B (**5**, 140 mg), and C (**6**, 100 mg).

Patellamide A (**4**) had the molecular formula $C_{35}H_{50}N_8O_6S_2$ (high-resolution mass spectrum, obsd *m/z* 742.3280, calcd *m/z* 742.3297). Preliminary evaluation of spectral data [IR (CH_2Cl_2) 3395, 1675, 1655 cm^{-1}; ^1H NMR δ 7.83 (s, 2 H); ^{13}C NMR δ 160.5 s (2 C), 149.4 s (2 C), 123.0 (d, 2 C)] indicated a cyclic peptide containing two thiazole amino acids.[11] In support of these assignments, hydrolysis of **4** with 6 N HCl yielded L-serine, L-threonine, L-isoleucine, and thiazole **7** in an approximately 1:1:2:2 ratio. Amino acid assignments were made by GC/MS analysis of trifluoroacetyl methyl ester (TFA-ME) derivatives.

With the exception of **7**, all assignments were confirmed by comparison with authentic samples. The thiazole was assigned structure **7** on the basis of mass spectral evidence [*m/z* 310 (m^+), 268 ($m^+ - C_3H_6$), 267 ($m^+ - C_3H_7$)] plus signals in the ^1H and ^{13}C spectra of patellamide A for two valine units and the two thiazole rings (see Tables II and III). Complete electron-impact mass spectral fragmentation data for each amino acid TFA-ME are listed in the Experimental Section.

The absolute configuration for all amino acids except thiazole **7** were established by GC retention correlation of the TFA-ME derivatives on a column coated with an optically active liquid phase (SP-300, Supelco).[12] Retention times for D and L isomers are listed in the Experimental Section.

In addition to signals for two valine thiazoles and two isoleucines, the ^1H and ^{13}C spectra of patellamide A contained complimentary signals at δ 168.5 (s), 81.6 (d), 73.6 (d), 4.30 (d, 1 H, *J* = 4 Hz), 4.89 (m, 1 H), and 1.47 (d, 3 H, *J* = 6 Hz) and at δ 169.1 (s), 72.2 (t), 67.4 (d), 4.30 (dd, 1 H, *J* = 8, 4 Hz), and 4.80 (m, 2 H) for oxazolines **8** and **9**, respectively (Chart II). These signals were in good agreement with the data recorded for the oxazoline in ulicyclamide (**3**)[13] and are consistent with the isolation of serine and threonine from the acid hydrolysis. Furthermore, we have found that the multiplicity of the α-amino proton of a $\Delta^{2,3}$-oxazoline (C-4) is a diagnostic indicator of the type of substituent fused at the C-2 position. For example, this proton in ulithiacyclamide (H-2) occurs as a doublet of doublets (*J* = 8, 2 Hz), where the 2-Hz splitting represents homoallylic coupling to the α-cystinyl proton. This homoallylic coupling has been observed previously in similarly substituted $\Delta^{2,3}$-oxazolines.[14] Conversely, the corresponding proton in ulicyclamide appears as a doublet (*J* = 4 Hz), reflecting fusion to a thiazole. The difference in magnitude of vicinal coupling between hydrogens at positions 4 and 5 is also noteworthy.

(9) Ireland, C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 5688–5691.
(10) Krebs, K. G.; Heusser, D.; Wimmer, H. In "Thin Layer Chromatography"; Stahl, E., Ed. Springer-Verlag: West Berlin, 1969; pp 854–908, reagent no. 42.
(11) These ^{13}C and ^1H data are in agreement with the data recorded for the thiazole amino acids in ulicyclamide: δ 161.1 (s), 160.5 (s), 151.4 (s), 148.9 (s), 124.3 (d), 123.8 (d), 8.08 (s, 1 H), 8.03 (s, 1 H).
(12) Charles, R.; Beitler, B.; Gil-Av, E. *J. Chromatogr.* **1978**, *112*, 121–133.
(13) NMR δ 83.3 (d), 76.3 (d), 4.82 (dq, 1 H, *J* = 7, 4 Hz), 4.26 (d, 1 H, *J* = 4 Hz).
(14) Weinberger, M. A.; Greenhalgh, R. *Can. J. Chem.* **1963**, *41*, 1038–1041.

Table II. 270-MHz ^{1}H NMR Data for Patellamides A (4), B (5), and C (6)[a]

H at C no.	chemical shift, δ		
	4	5	6
1			
2	4.30 (dd, J = 8, 4 Hz)	4.38 (d, J = 4 Hz)[a]	4.35 (d, J = 4 Hz)[a]
3	4.80 (m, 2 H)	5.01 (m)	4.98 (m)
4		1.47 (d, J = 7 Hz)[b]	1.44 (d, J = 7 Hz)[b]
5			
6			
7	7.83 (s)	7.49 (s)[c]	7.50 (s)[c]
8	5.22 (m)		
9	2.32 (m)	5.39 (dq, J = 9, 7 Hz)	5.36 (dq, J = 10, 7 Hz)
10	1.13 (d, J = 7 Hz)[a]	1.74 (d, J = 7 Hz)	1.74 (d, J = 7 Hz)
11	1.08 (d, J = 7 Hz)[a]		
12		4.77 (dd, J = 11, 7 Hz)	4.77 (dd, J = 11, 8 Hz)
13	4.65 (dd, J = 8, 6 Hz)[b]	2.24 (m)	2.24 (m)
14	1.96 (m)	1.61 (m)	1.62 (m)
15		0.93 (t, J = 6 Hz)	0.89 (t, J = 7 Hz)
16	0.75 (t, J = 7 Hz)[c]	1.09 (d, J = 7 Hz)	1.09 (d, J = 7 Hz)
17	0.81 (d, J = 7 Hz)		
18		4.29 (d, J = 4 Hz)[a]	4.26 (d, J = 4 Hz)[a]
19	4.30 (d, J = 4 Hz)	5.01 (m)	4.98 (m)
20	4.89 (dq, J = 6, 4 Hz)	1.45 (d, J = 7 Hz)[b]	1.41 (d, J = 7 Hz)[b]
21	1.47 (d, J = 6 Hz)		
22			
23		7.39 (s)[c]	7.44 (s)[c]
24	7.83 (s)		
25		5.50 (ddd, J = 10, 10, 7 Hz)	5.50 (ddd, J = 9, 9, 7 Hz)
26	5.22 (m)	3.45 (dd, J = 14, 10 Hz)	3.43 (dd, J = 14, 9 Hz)
		3.31 (dd, J = 14, 7 Hz)	3.28 (dd, J = 14, 7 Hz)
27	2.32 (m)		
28	1.13 (d, J = 7 Hz)[a]	7.30 (m, 5 H)	7.39 (m, 5 H)
29	1.08 (d, J = 7 Hz)[a]		
30			
31	4.56 (dd, J = 10, 8 Hz)[b]		
32	1.96 (m)	5.01 (m)	4.53 (dd, J = 11, 8 Hz)
33		1.61 (m)	2.24 (m)
34	0.73 (t, J = 7 Hz)[c]	2.08 (m)	1.07 (d, J = 7 Hz)[d]
35	0.81 (d, J = 7 Hz)	1.07 (d, J = 6 Hz)[d]	1.04 (d, J = 7 Hz)[d]
36		1.05 (d, J = 7 Hz)	
N-1	7.95 (m)	7.62 (m)	7.62 (m)
N-2	7.41 (d, J = 10 Hz)	7.62 (d, J = 9 Hz)	7.62 (m)
N-3	7.95 (m)	7.62 (d, J = 11 Hz)	7.62 (m)
N-4	7.41 (d, J = 10 Hz)	7.62 (d, J = 10 Hz)	7.62 (m)

[a] Superscript letters indicate interchangeable signals.

In the case of ulithiacyclamide and model compound **10**, $^2J_{4,5}$ = 8 Hz.[15] In ulicyclamide where the C-2 substituent is thiazole, $^2J_{4,5}$ = 4 Hz. The large coupling in the former case can be explained if the ring is slightly puckered such that the two protons assume a pseudotransdiaxial arrangement. This conformation has the added advantage of minimizing eclipsing interactions between substituents at C-4 and C-5. The reduced coupling observed in ulicyclamide must arise from flattening of the ring which allows orbital overlap with the thiazole. Accordingly, the absence of homoallylic coupling in the ^{1}H spectrum of patellamide A allowed assignment of the fused oxazoline–thiazoles **11** and **12**. Significantly, assignment of constellations **11** and **12** plus two isoleucines accounted for all of the atoms in patellamide A, thus confirming its cyclic nature.

These data were consistent with structure **4** as well as a structure in which the oxazoline–thiazoles are in series. We previously established a methodology for hydrolyzing cyclic peptides containing fused thiazole–oxazolines such as **11** or **12** to acyclic derivatives in which the thiazole is the C terminal and threonine or serine the N terminal.[16] Manipulation of patellamide A under these conditions yielded a pair of tripeptide derivatives, **13** and **14**, only consistent with structure **4**. High-resolution mass spectra confirmed the molecular formulas of **13** and **14** as well as the serine and threonine N terminal (see Table IV). The oxazoline–thiazoles **11** and **12** in patellamide A (**4**) dictate that the C terminal be a valine–thiazole in both cases. Representative high-resolution mass spectral fragmentations for **13** and **14** are shown in Table IV.

Patellamide B (**5**) had the molecular formula $C_{38}H_{48}N_8O_6O_2$ (high-resolution mass spectrum, HRMS obsd m/z 776.3128, calcd m/z 776.3138). The 6 N HCl hydrolysis of patellamide B yielded L-threonine, L-isoleucine, L-leucine, and thiazoles **15** and **16** in an approximately 2:1:1:1:1 ratio, analyzed by the method described previously. Thiazole **15** was identical with an authentic sample obtained from ulicyclamide (**3**). Thiazole **16** was identified by mass spectral data [m/z 358 (m^+), 267 ($m^+ - C_7H_7$), 91 $(C_7H_7)^+$] and signals in the ^{1}H and ^{13}C spectra of **5** for phenylalanine: ^{1}H NMR δ 7.62 (d, 1 H, J = 10 Hz), 7.40 (m, 5 H), 5.50 (ddd, 1 H, J = 10, 10, 7 Hz), 3.45 (dd, 1 H, J = 14, 10 Hz), 3.31 (dd, 1 H, J = 14, 7 Hz); ^{13}C NMR 136.3 (s), 129.2 (d, 2 C), 128.7 (d, 2 C), 127.1 (d), 53.3 (d), 40.7 (t).

Consistent with the isolation of 2 equiv of threonine from the total acid hydrolysis, the ^{1}H and ^{13}C NMR spectra of patellamide B contained resonances for two threonine derived oxazolines:[9] ^{13}C NMR δ 168.2 (s), 168.0 (s), 82.5

(15) An 8-Hz coupling is also consistent with coupling observed in $\Delta^{2,3}$-oxazolines reported in ref 14.

(16) (i) 5% H_2SO_4/MeOH, (ii) Ac_2O/Pyr, (iii) 1% KOH/MeOH (iv) CH_2N_2.

Table III. ^{13}C NMR Assignments for Patellamides A (4), B (5), and C (6)[a]

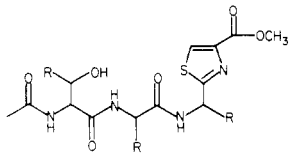
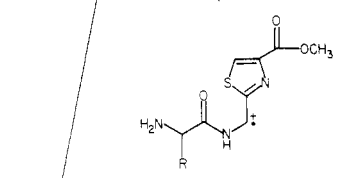
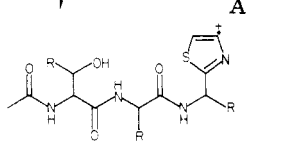
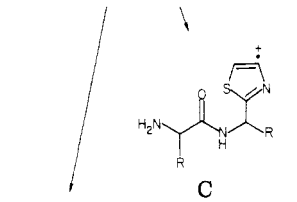
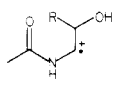
C	chemical shift, δ		
	4	5	6
1	169.5[a]	173.3[a]	173.2[a]
2	67.4	73.8	73.6
3	72.2	82.5[b]	82.3
4	169.1	23.2[c]	21.0[b]
5	149.4	168.2[d]	168.0[c]
6	123.0	147.6[e]	147.5[d]
7	160.5	123.6	123.7[e]
8	54.9	161.8[f]	161.8
9	37.1[b]	46.7	46.5
10	19.2[c]	21.0	20.6
11	19.2[c]	173.0[a]	173.0[a]
12	171.5[a]	52.5	52.3
13	52.4	32.9	32.6
14	33.3	25.1	24.8
15	24.9[e]	8.8	8.5
16	11.1[f]	15.0	15.0
17	15.0[g]	172.8[a]	172.6
18	171.8[a]	73.8	73.6
19	73.6	82.1[b]	82.3
20	81.6	21.8[c]	20.8[b]
21	21.7	168.0[d]	167.9[c]
22	168.5	147.2[e]	147.2[d]
23	149.4	123.6	123.5[e]
24	123.0	161.6	161.8
25	160.5	53.3	53.1
26	54.9	40.7	40.7
27	36.8[b]	136.3	136.1
28	17.9[c]	128.7	128.6
29	17.9[c]	129.2	129.1
30	171.5[a]	127.1	127.0
31	52.1[d]	170.8[a]	170.8[a]
32	33.3	47.8	55.8
33	24.7[e]	39.0	27.7
34	10.6[f]	25.1	19.6[f]
35	14.9[g]	21.0	19.1
36		21.0	

[a] Superscript letters indicate interchangeable signals.

(d), 82.1 (d), 73.8 (d, 2 C), 23.2 (q), 21.8 (q); ^1H NMR 5.01 (m, 2 H), 4.38 (d, 1 H, J = 7 Hz), 4.29 (d, J = 4 Hz), 1.47 (d, 3 H, J = 7 Hz), 1.45 (d, 3 H, J = 7 Hz). The 4-Hz doublets at δ 4.38 and 4.29 attributed to the α-amino (C-4) protons of the oxazolines again allowed us to assign the fused heteroaromatic units **17** and **18**. Carrying the analysis through, selective hydrolysis of patellamide B yielded a pair of tripeptides, **19** and **20**, thus confirming structure **5**. Partial structures **17** and **18** and high-resolution mass spectral fragmentation data dictatated that each tripeptide contained a thiazole at the C terminal and threonine at the N terminal. However, it could not distinguish between leucine and isoleucine. On the other hand, the ^1H NMR spectra readily made this distinction. Signals at δ 0.97 (d, 3 H, J = 7 Hz) and 0.95 (t, 3 H, J = 7 Hz) in the spectrum of **19** defined isoleucine. Whereas, the spectrum of **20** contained a pair of methyl doublets (J = 7 Hz) at δ 0.98 and 0.96 for the isopropyl group of leucine.

The most polar metabolite, patellamide C, had the molecular formula $C_{37}H_{46}N_8O_6S_2$ (high-resolution mass spectrum, obsd m/z 762.2973, calcd m/z 762.2981), apparently differing from patellamide B by a methylene unit. This relationship was confirmed by total acid hydrolysis which yielded two L-threonines, L-isoleucine, thiazoles **15** and **16**, and L-valine (in place of leucine). Additionally, the ^1H and ^{13}C spectra contained resonances for valine, isoleucine, and the oxazolines **17** and **18** (see Table II and III). More significantly, resonances for the latter three were virtually superimposable on the corresponding signals

Table IV. High-Resolution Mass Spectral Data for Patellamide Selective Hydrolysis Products[a]

		I	II	III
	14	470.2196	5.3	−0.2
	A	284.1071	5.3	0.2
	B	411.2099	26	3.4
	C	268.1485	24	0.2
	D	116.0710	21	−0.1
	13	456.2043	6	0.1
	A	284.1071	55	0.2
	B	397.1905	35	−0.4
	C	268.1485	28	0.2
	D	102.0555	70	
A				
	19	442.1880	4	−0.5
	A	284.1070	69	0.1
	B	383.1755	35	0.3
	C	240.1174	69	0.4
	D	116.0711	15	
B				
	20	518.2174	3	−2.4
	A	284.1071	99	0.2
	B	459.2063	7	−0.2
	C	299.1218	56	0.1
	D	116.0711	7	
C				
	21	504.2034	1	−0.7
	A	270.0915	76	0.2
	B	445.1907	4	−0.1
	C	285.1064	25	0.4
	D	116.0711	16	

[a] Column I, exact mass measurements (m/e); column II, intensity relative to base peak (percent); column III, deviance from calculated values in MMU.

in the spectra of patellamide B. These data led to the proposal of structure **6** for patellamide C. Confirmation of structure **6** was obtained by degrading patellamide C[16] to the tripeptides **19** and **21** (see Table IV for supporting evidence).

Antineoplastic peptides containing thiazole amino acids (e.g., bleomycin) have been reported from *Streptomyces* and related soil microbes. However, the lissoclinum peptides are the only peptides which contain a fused oxazoline–thiazole group. It is unclear whether the tunicate, symbiotic algae, or both produce these metabolites. Interestingly, Pettit recently reported the isolation of a family of thiazole-containing cyclic peptides from the herbivorous mollusc *Dolabella*.[17]

Experimental Section

Infrared spectra were recorded on a Beckman 620 MX spectrophotometer. Electron-impact mass spectra were recorded on an AEI MS-902 mass spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker WP-90 or HX-270 spectrometer. Chemical shifts are reported relative to Me_4Si (δ 0). Low-resolution GC/electron-impact mass spectra were recorded on an HP-5985 spectrometer. Gas chromatograms were recorded on a Varian Model 3700 gas chromatograph.

Collection of *Lissoclinum patella*. Specimens of *L. patella* were collected in Sept 1979 by snorkeling (−2 m) at Eil Malk Island (7° 10′ 01″ N, 134° 21′ 50″ E) in the Western Carolines. The

(17) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L; Inoue, M.; Brown, P.; Devens, G.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michael, C. *J. Nat. Prod.* **1981**, *44*, 482–485.

specimens were homogenized, stored in methanol for 1 month, and then shipped to the University of Connecticut. The homogenate was filtered and the methanol solution evaporated under vacuum to a watery residue. The residue was partitioned between CH_2Cl_2 and H_2O and the aqueous layer washed twice (100 mL) with CH_2Cl_2. The combined CH_2Cl_2 layers were dried over sodium sulfate and evaporated to yield a yellow oil (0.60 g). The oil was chromatographed on silica gel (Merck) with a 60:40 mixture of CH_2Cl_2/EtOAc to yield four components, **2** (80 mg), **4** (100 mg), **5** (140 mg), and **6** (100 mg), in order of elution.

Patellamide A (4): $[\alpha]_D$ +113.9° (*c* 0.27, CH_2Cl_2); IR (CH_2Cl_2) 3395, 3130, 3055, 2969, 2940, 2885, 1675, 1655, 1535, 1510, 1489 cm^{-1}; high-resolution mass measurement, obsd *m/z* 742.3280, $C_{35}H_{50}N_8O_6S_2$ requires *m/z* 742.3297.

Patellamide B (5): $[\alpha]_D$ +29.4° (c 0.34, CH_2Cl_2); IR (CH_2Cl_2) 3374, 3330, 1662, 1480 cm^{-1}; high-resolution mass measurement, obsd *m/z* 776.3128, $C_{38}H_{48}N_8O_6S_2$ requires *m/z* 776.3138).

Patellamide C (6): $[\alpha]$ +19° (*c* 0.21, CH_2Cl_2); IR 3380, 1675, 1655, 1535, 1510 cm^{-1}; high-resolution mass measurement, obsd *m/z* 762.2973, $C_{37}H_{46}N_8O_6S_2$ requires *m/z* 762.2981).

Methylation and N-Trifluoroacetylation of Amino Acid Standards. The amino acids (5 mg) were dissolved in MeOH (5 mL) in a 10-mL round-bottomed flask. Anhydrous HCl gas was bubbled through the solution until saturation (~30 s). The reaction was refluxed for 1 h, upon cooling the solvent was evaporated under vacuum and the residue suspended in CH_2Cl_2 (5 mL) and trifluoroacetic anhydride (5 mL) in a Pyrex threaded bomb sealed with a Teflon screw cap. The mixture was heated at 150 °C for 30 min. The cooled reaction was carefully evaporated under a stream of nitrogen being careful not to volatalize products. The residues were resuspended in CH_2Cl_2 (10 mL).

The data below are presented as follows: (A) GC/EIMS analysis (fragmentation data; 3% OV-17, 6 ft × 1/8 in.; program 60–250 °C at 10 °C/min, 5-min delay at 60 °C); (B) GC retention times for D and L isomers (12% SP-300, 12 ft × 1/8 in.; program 110–140 °C at 2 °C/min, 30-mm delay at 110°.

***N*-(Trifluoroacetyl)valine methyl ester:** (A) *m/z* 168 ($M^+ - COOCH_3$), 153, 69; (B) D 8.30 min, L 9.51 min.

***N*-(Trifluoroacetyl)isoleucine methyl ester:** (A) *m/z* 185 ($M^+ - C_4H_8$), 182 ($M^+ - COOCH_3$), 153, 69; (B) D 14.40 min, L 20.15 min.

***N*-(Trifluoroacetyl)leucine methyl ester:** (A) *m/z* 182 ($M^+ - COOCH_3$), 166, 140, 69; (B) D 26.39 min, L 32.44 min.

***N,O*-Bis(trifluoroacetyl)serine methyl ester:** (A) *m/z* 280 ($M^+ - OCH_3$), 252 ($M^+ - COOCH_3$), 214 ($M^+ - COCF_3$), 197 ($M^+ - CF_3COOH$), 138, 69; (B) D 26.54 min, L 29.54 min.

***N,O*-Bis(trifluoroacetyl)threonine methyl ester:** (A) *m/z* 294 ($M^+ - OCH_3$), 266, ($M^+ - COOCH_3$), 228 ($M^+ - COCF_3$), 211 ($M^+ - CF_3COOH$), 152, 69; (B) D 10.98 min, L 12.08 min.

Total Acid Hydrolysis of the Patellamides. The peptides (5 mg) and 6 N HCl (5 mL) were heated at 118 °C for 18 h in a Pyrex threaded bomb sealed with a Teflon screw cap. The cooled reaction mixture was transferred to a 10-mL round-bottomed flask and evaporated to dryness under vacuum. The hydrolysates were derivatized by following the procedure described in the previous section.

Patellamide A (4): L-serine, L-threonine, two L-isoleucines, and two valine–thiazoles **7** [*m/z* 310 (M^+), 279 ($M^+ - OCH_3$), 268, 267, 235, 208, 166, 69].

Patellamide B (5): two L-threonines, L-isoleucine, L-leucine, an alanine–thiazole [*m/z* 282 (M^+), 251 ($M^+ - OCH_3$), 235, 222, 207, 185, 170, 153, 140, 138, 69], and a phenylalanine–thiazole [*m/z* 358 (M^+), 327 ($M^+ - OCH_3$), 267, 235, 91, 69].

Patellamide C (6): two L-threonines, L-isoleucine, L-valine, an alanine–thiazole and a phenylalanine–thiazole.

Hydrolysis of the Patellamides to Acyclic Tripeptide *N*-Acetyl Methyl Esters. The peptides (10 mg) were refluxed in 5% H_2SO_4/MeOH for 1 h. The reaction was basefied to pH 12 with 10% aqueous NaOH and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to give an oil. The oil was N-acetylated by stirring in pyridine (100 μL) and acetic anhydride (500 μL) at room temperature for 24 h. Excess reactants were removed under vacuum. The crude product was saponified by stirring with 1% KOH/MeOH at room temperature for 1 h. After acidification to pH 1 with 10% aqueous HCl the product was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried over Na_2SO_4 and evaporated. The crude product was esterified with CH_2N_2. Chromatography of the products on silica gel TLC plates (EtOAc/MeOH, 90:10) gave two products from each peptide. The mass spectral fragmentations for each are listed in Table IV. ^1H NMR data for patellamide B hydrolysis products **19** and **20** are listed below.

19: δ 8.04 (s, 1 H), 7.84 (d, 1 H, *J* = 9 Hz), 7.61 (d, 1 H, *J* = 8 Hz), 6.66 (d, 1 H, *J* = 8 Hz), 5.38 (dq, 1 H, *J* = 7, 7 Hz), 4.75 (dd, 1 H, *J* = 10, 7 Hz), 4.47 (m, 1 H), 4.37 (dd, 1 H, *J* = 7, 2 Hz), 3.76 (s, 3 H), 2.06 (s, 3 H), 1.64 (d, 3 H, *J* = 8 Hz), 1.18 (d, 3 H, *J* = 7 Hz), 0.97 (d, 3 H, *J* = 7 Hz), 0.95 (t, 3 H, *J* = 7 Hz).

20: δ 8.02 (s, 1 H), 7.77 (d, 1 H, *J* = 9 Hz), 7.66 (d, 1 H, *J* = 8 Hz), 7.28–7.15 (m, 5 H), 6.56 (d, 1 H, *J* = 8 Hz), 5.56 (m, 1 H), 4.83 (m, 1 H), 4.31 (m, 2 H), 3.76 (s, 3 H), 3.29 (m, 2 H), 2.02 (s, 3 H), 1.73 (m, 2 H), 1.06 (d, 3 H, *J* = 6 Hz), 0.98 (d, 3 H, *J* = 7 Hz), 0.96 (d, 3 H, *J* = 7 Hz).

L1210 murine leukemia cells were grown as a stationary suspension culture in vitro in McCoy's 5A medium supplemented with 10% newborn calf serum, penicillin (100 μg/mL), streptomycin (100 μg/mL), and fungizone (0.25 μg/mL). Cultures were maintained at 37 °C in a humidified atmosphere of 90% air/10% CO_2. L1210 cells grown under these conditions have a doubling time of approximately 18 h.

Compounds were dissolved in sterile Me_2SO at concentrations such that a 40-μL addition of the solution to 4 mL of cell suspension delivered the desired final concentration of test compound. Routinely, 4 mL of L1210 cells (10^5 cells/mL) were added to 15 mL of disposable tissue culture tubes. The test compound was then added to the cells, and the cultures were incubated for 96 h. Cell concentration was measured electronically by using a Coulter particle counter (Model ZB_F).

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