H), 2.87 (m, 4 H), 2.04 (m, 2 H), 0.92 (m, 12 H); MS (FAB, m/e + 1) 546, 450 (-CF_3CO), 349 (-CF_3CO Val), 333 (-CF_3CO Val, Me). Anal. ($C_{25}H_{33}F_3N_3O_7$) C, H, F, N.

Acknowledgment. This work was supported in part by the Rutgers University Research Council, the Rutgers Busch Fund, the BRSG from NIH, Hoffmann-La Roche Inc., Nutley, NJ, and Ciba-Geigy, Ardsley, NY. We are grateful to Dr. F. Scheidl of Hoffmann-La Roche, Nutley, NJ, for performing the elemental analysis, and to Mr. Phil Hoops for some of the NMR spectra.

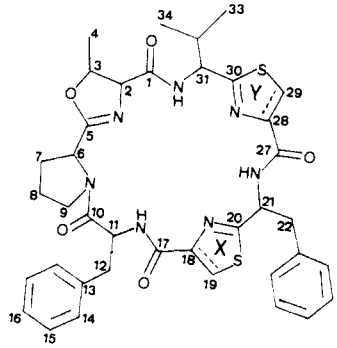
Structure-Activity Relationships of the Lissoclinamides: Cytotoxic Cyclic Peptides from the Ascidian *Lissoclinum patella*

Clifford J. Hawkins,† Martin F. Lavin,‡ Karen A. Marshall,‡ Anna L. van den Brenk,† and Diane J. Watters*,†

Department of Chemistry, University of Queensland, St. Lucia, Queensland 4067, Australia, and Queensland Institute of Medical Research, Bramston Terrace, Herston, Queensland 4006, Australia. *Received July 28, 1989*

Two new lissoclinamides (lissoclinamides 7 and 8) have been isolated from the aplousobranch ascidian *Lissoclinum patella*. These lissoclinamides are cyclic heptapeptides with the same structural features as lissoclinamides 4 and 5 reported earlier, containing an oxazoline ring, one proline, one valine, two phenylalanine residues, and thiazole and/or thiazoline rings. All four peptides have the same sequence of amino acids around the ring and differ from one another only in their stereochemistry or the number of thiazole and thiazoline rings. The cytotoxicities of the compounds were tested with human fibroblast and bladder carcinoma cell lines and normal lymphocytes. Slight changes in structure resulted in marked differences in the cytotoxicities of these compounds. The most potent is lissoclinamide 7, containing two thiazoline rings, which rivals didemnin B in cytotoxicity in vitro.

Cytotoxic cyclic peptides from marine organisms are showing great promise as potential antineoplastic agents. Didemnin B from the ascidian *Tridemnum solidum* is now in phase II clinical trials.[1,2] In a recent paper we described the isolation and characterization of three new cyclic peptides from *L. patella*: patellamide D, lissoclinamide 4, and lissoclinamide 5.[3] We now report the structures of two new lissoclinamides from the same species, lissoclinamides 7 and 8. The four lissoclinamides are made up of the same amino acids, in the same sequence, yet display dramatic differences in their cytotoxicity in vitro. In the present study structural-functional correlations are investigated.



	X	Y
lissoclinamide 4	thiazole	thiazoline
lissoclinamide 5	thiazole	thiazole
lissoclinamide 7	thiazoline	thiazoline
lissoclinamide 8	thiazole	thiazoline

Results and Discussion

Isolation and Structure Determination. *L. patella* was extracted with methanol/toluene and the extract was chromatographed on a preparative reverse-phase column as previously described.[3] The elution profile is shown in Figure 1. The peaks corresponding to lissoclinamides 7 and 8 have been marked L7 and L8. The fractions corresponding to these compounds were pooled and rechromatographed on the reverse-phase column or further purified by Sephadex LH-20 chromatography with methanol/water (80:20). The structures of the pure compounds were determined by a combination of high-resolution electron-impact mass spectrometry (HREIMS), acid hydrolysis followed by chiral gas chromatography, and two-dimensional NMR techniques.

HREIMS of lissoclinamide 7 ($C_{38}H_{45}N_7O_5S_2$) gave a molecular ion peak of m/z 743.2935 (calcd m/z 743.2927). Chiral gas chromatography of the *N*-pentafluoropropionyl isopropyl esters yielded L-threonine, L-proline, DL-phenylalanine, DL-valine, and L-cysteine (double the peak observed in lissoclinamides 4 and 8). The NMR data for this compound are presented in Table I. The assignments were determined by ^1H-^1H COSY 45 and ^1H-^{13}C COSY experiments. The sequence was established with COLOC and COSY 45 correlations namely C-1 to NH-1 and H-31 to NH-1. This places the valine residue at position 31; thus the sequence is the same as in lissoclinamide 4.[3]

As described in our previous paper,[3] we are unable to determine the stereochemistry of amino acids adjacent to thiazole rings, and hydrolysis of amino acids adjacent to thiazoline rings leads to racemization.

Due to racemization of the amino acids adjacent to the thiazoline rings, we can make only a tentative statement about the stereochemistry at these positions. The large proportion of D-phenylalanine obtained (D/L = 0.64) would suggest this configuration at position 21. (The phenylalanine at C-11 is always of the L configuration, so if the phenylalanine at C-21 is completely racemized, one would expect a D/L ratio of 0.25.)

D- and L-valine were obtained in equal proportions, so it is not possible to comment on the stereochemistry at position 31 for this compound.

HREIMS of lissoclinamide 8 ($C_{38}H_{43}N_7O_5S_2$) gave a molecular ion peak of m/z 741.2785 (calcd m/z 741.2767). Chiral gas chromatography of the *N*-pentafluoropropionyl

† University of Queensland.
‡ Queensland Institute of Medical Research.

(1) Rinehart, K. L.; Gloer, J. B.; Cook, J. C.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857.
(2) Stewart, J. A.; Tong, W. R.; Hartshorn, J. N.; McCormack, J. J. *Proc. Am. Soc. Clin. Oncol.* **1986**, *5*, 33.
(3) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; van den Brenk, A. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1349.

0022-2623/90/1833-1634$02.50/0

Table I. NMR Assignments for Lissoclinamide 7

carbon no.	δ ^{13}C	δ ^{1}H (J, Hz)	^{1}H–^{1}H couplings (COSY 45) (directly bonded protons)	^{1}H–^{13}C long range couplings (COLOC)
1	172.13			H2, H3, NH-1
2	75.78	4.27 d (4.4)	H3	H4
3	81.19	4.89 m	H4, H2	H4
4	21.77	1.42 d (3 H) (6.4)	H3	H2
5	169.74			H2, H8
6	56.49	4.55 m	H7	
7	28.79	2.25, 1.90 m	H6	H9
8	25.39	1.98, 1.90 m	H9	
9	47.13	3.63, 3.02 m	H8	H7
10	170.88			
11	51.95	4.89 m	H12, NH-2	H12
12	39.46	2.69, 2.90 dd, dd (13.3, 8.9; 13.3, 6.4)	H11	H11
13	135.76			H12
14	129.74	7.08–7.31 m (5 H)		H12
15	128.67	7.08–7.31 m (5 H)		
16	127.44	7.08–7.31 m (5 H)		
17	169.51			H19, H18
18	78.19	4.64 m	H19	
19	36.24	3.54, 3.20 m	H18	
20	173.05			H22, H18, H21
21	51.73	5.26 m	NH-3, H18, H22	H22
22	38.91	3.08 d (2 H) (5.1)	H21	H21
23	134.81			H22, H21
24	129.58	7.08–7.32 m (5 H)		H22
25	128.43	7.08–7.32 m (5 H)		
26	127.07	7.08–7.32 m (5 H)		
27	170.88			H28, H29
28	79.77	5.21 m	H29	
29	34.80	3.54 m (2 H)	H28	
30	179.83			H28, H31
31	56.46	4.96 m	NH(1) H32, H28	H33, H34
32	30.77	2.48 m	H33, H34, H31	H33, H34, H31
33	19.70	0.96 d (3 H) (7.0)	H34, H32	H34
34	15.97	0.64 d (3 H) (6.4)	H33, H32	H33
NH(1)		7.73 d (10.2)	H31	
NH(2)		7.22	H11	
NH(3)		6.90 d (8.9)	H21	

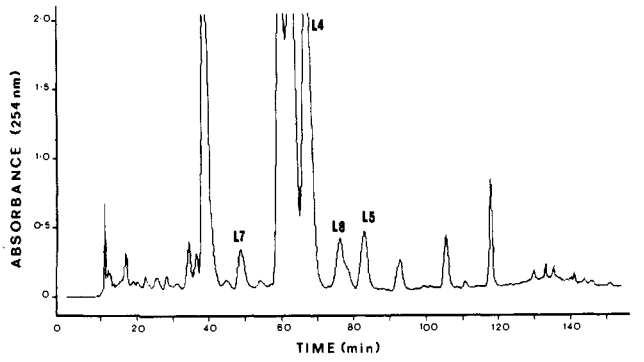


Figure 1. HPLC of *L. patella* extract. The extract was applied to a Whatman Partisil 10 ODS-3 column (C18) in methanol/water (77:23) and eluted isocratically. Lissoclinamides 4, 5, 7, and 8 are labelled L4, L5, L7, and L8, respectively.

isopropyl esters yielded L-threonine, L-cysteine, L-phenylalanine, L-proline, and DL-valine. The NMR data for lissoclinamide 8 are given in Table II. The assignments were determined by ^{1}H–^{1}H COSY 45 and ^{1}H–^{13}C COSY. The sequence of amino acids around the ring was again determined by long-range ^{1}H–^{13}C couplings in a COLOC experiment in combination with ^{1}H–^{1}H couplings detected by the 2D COSY 45 experiment.

The sequence is unambiguously established from the following correlations: H-31 to NH-1 and C-1 to NH-1, thus placing the valine residue at position 31. This is the same sequence as seen in the other lissoclinamides. The only difference between lissoclinamides 4 and 8 must therefore reside in the stereochemistry of one or two of the amino acids. For lissoclinamide 8 we consistently obtained an excess of D-valine over L-valine, albeit a small one (D/L = 1.22), and therefore tentatively assigned the D configuration to this residue. However, lissoclinamide 4 did not show the corresponding excess of L-valine. Dreiding models show the isopropyl group of the D-valine has unfavorable steric interactions that are relieved by conformational changes to the peptide ring. This steric hindrance may allow the observation of the steric preference in the hydrolyzed amino acid. Lissoclinamides 7 and 8 comprised 0.4% and 0.5% of the dried crude extract, respectively.

Recently another group has independently characterized lissoclinamides 4 and 5.[4] They also described lissoclinamide 6 as a trace component which is a diastereomer of lissoclinamides 4 and 8.

These authors tentatively assigned the configurations at C-21 and C-31 as L and D, respectively, in lissoclinamides 4 and 5, and D and D in lissoclinamide 6. The most likely configuration of lissoclinamide 8 is thus likely to be D and L, respectively. We have not yet found lissoclinamide 6 in our preparations nor the presumed diastereomer with the L,L configuration.

Conformational Information. The ^{3}J(NH–CH) vicinal coupling constants were used to obtain information about the dihedral angle (θ) between NH and α-CH.[5] For

(4) Schmitz, F. J.; Ksebati, M. B.; Chang, J. S.; Wang, J. L.; Hossain, M. B.; van der Helm, D. *J. Org. Chem.* **1989**, *54*, 3463.

Table II. NMR Assignments for Lissoclinamide 8

carbon no.	δ ^{13}C	δ ^{1}H (J, Hz)	^{1}H–^{1}H couplings (COSY 45) (directly bonded protons)	^{1}H–^{13}C long range couplings (COLOC)
1	171.27			H2, H3, NH-1
2	75.06	4.24 d (4.1)	H3	
3	82.09	4.80 dd (4.1, 6.2)	H2, H4	H4
4	21.80	1.45 d (3 H) (6.2)	H3	H3
5	169.41			H2, H3
6	56.51	4.58 t (7.9)	H7	
7	28.77	1.82, 2.16 m	H6, H8	H9
8	25.20	1.78 m	H7	
9	47.10	2.30, 3.36 m		
10	170.36			H11, H12
11	52.79	4.97 m	H12, NH-2	H12
12	40.78	3.18, 2.90 dd, dd (13.2, 5.3; 13.2, 5.3)	H11	
13	135.79			H12
14	129.84	7.07–7.32 m (5 H)		
15	128.56	7.07–7.32 m (5 H)		
16	127.27	7.07–7.32 m (5 H)		
17	159.49			
18	148.21			H19
19	123.22	7.96 s		
20	168.22			H19, H21, H22
21	52.98	5.51 m	H22	
22	43.59	3.35, 2.94 dd (13.5, 4.7; 13.5, 5.9)	H21	
23	135.58			H22
24	129.78	7.07–7.32 m (5 H)		
25	128.53	7.07–7.32 m (5 H)		
26	127.27	7.07–7.32 m (5 H)		
27	170.99			H28, H29, NH-3
28	79.14	5.24 dd (10.6, 7.6)	H29	
29	35.72	3.61, 3.65 dd, dd (11.4, 7.6; 11.4, 10.6)	H28	
30	175.13			H28, H31
31	55.23	4.70 t (9.8)	NH-1	H33, H34
32	33.64	2.49 m	H33, H34	
33	19.60	1.00 d (3 H) (6.7)	H32	
34	19.46	1.00 d (3 H) (6.7)	H32	
NH(1)		7.73 d (10.0)	H31	
NH(2)		8.49 d (7.9)	H11	
NH(3)		8.26 d (7.3)	H21	

lissoclinamide 8 the coupling constants are J[NH(1)–CH(31)], 10.0 Hz; J[NH(2)–CH(11)], 7.9 Hz; and J[NH(3)–CH(21)], 7.3 Hz. These correspond to dihedral angles of $150° \leq \theta \leq 170°$, $140° \leq \theta \leq 155°$ or $10° \leq \theta \leq 25°$, and $140° \leq \theta$ 150° or $10° \leq \theta \leq 30°$, respectively. These compare with values of $155° \leq \theta \leq 180°$, $140° \leq \theta \leq 150°$ or $25° \leq \theta \leq 35°$, 130° or 40°, respectively obtained for lissoclinamide 4.[3] If these compounds differ in the configuration at C-31, with lissoclinamide 8 having the D configuration as suggested by the isomer ratio following hydrolysis, the similar values for θ for the NH(1)–CH(31) group reflect the different orientations of the group relative to the peptide ring. The D-valyl group's nonbonded interactions with the surrounding atoms force the peptide group to adopt an orientation perpendicular to the plane of the ring with NH(1) trans to CH(31), consistent with the above value of θ from the coupling constant. For the L isomer the preferred orientation for the valyl group would be axial to the peptide ring. The derived value of θ between 155° and 180° would have NH(1) directed toward the center of the peptide ring.

The α-CH–β-CH_2 vicinal coupling constants for the phenylalanine residues can be analyzed by the method of Feeney to yield populations of the rotamers about C-α–C-β,[6] where rotamer I has the phenyl group between CO and α-CH, rotamer II has it between α-CH and NH, and rotamer III has it between CO and NH. The coupling constants for lissoclinamide 8 are both 5.3 Hz at positions 11 and 12 and 4.7 and 5.9 Hz at positions 21 and 22. The following solutions were obtained. Position 11: p_I, 0.17; p_{II}, 0.25; p_{III}, 0.58; where p_I, p_{II}, p_{III} represent the mole fractions of rotamers I, II, and III, respectively. Thus rotamer III is preferred, placing the phenyl group between CO and NH. Position 21: p_I, 0.22; p_{II} 0.17; p_{III}, 0.61; or p_I, 0.11; p_{II} 0.32; p_{III}, 0.57. Again rotamer III is the preferred orientation of the phenyl ring.

For lissoclinamide 7 the ^{3}J(NH–CH) vicinal coupling constants are J[NH(1)–CH(31)], 10.2 Hz; and J[NH(3)–CH(21)], 8.9 Hz. These correspond to dihedral angles of $155° \leq \theta \leq 180°$ and $145° \leq \theta \leq 160°$. The coupling constant for J[NH(2)–CH(11)] could not be determined because of the coincidence of the proton resonances of NH-2 with the aromatic proton resonances.

The α-CH–β-CH_2 vicinal coupling constants for the phenylalanine residues of lissoclinamide 7 are 8.9 and 6.4 Hz at positions 11 and 12 and 5.1 Hz at positions 21 and 22. Substituting these values into Feeney's equation gives the following solutions. For position 11: p_I, 0.68; p_{II}, 0.30; p_{III}, 0.02. For position 21: p_I, 0.14; p_{II}, 0.23; p_{III}, 0.63. Thus at position 11 the preferred orientation of the phenylalanine ring is between CO and α-CH, and at position 21 the dominant orientation is between CO and NH.

The analysis of the NMR data by Feeney's method has highlighted differences in the conformations of the lisso-

(5) Bystrov, V. F. *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 41.
(6) Feeney, J. *J. Magn. Reson.* **1976**, *21*, 473.

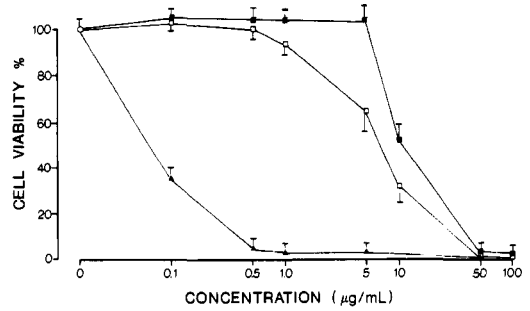


Figure 2. Cytotoxicity of the lissoclinamides toward T24 (transitional bladder carcinoma) cells. Cells were plated at a density of 10^5/mL and exposed to the compounds at the indicated concentrations for 1 h. After a further 48 h, cell survival was assayed by a colorimetric assay using MTT. Each experiment was performed three times with eight assays at each point. The error bars show the standard deviation of the mean: Lissoclinamide 5, (■); lissoclinamide 7, (▲); lissoclinamide 8, (□).

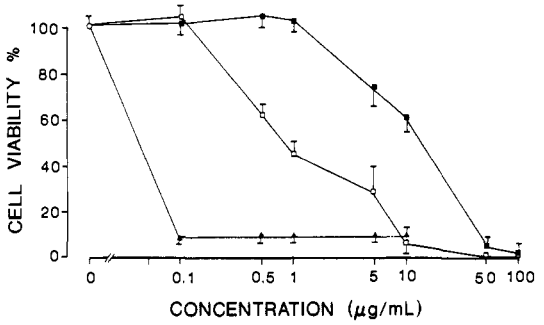


Figure 3. Cytotoxicity of the lissoclinamides toward MRC5CV1 cells. For details see the legend of Figure 2.

clinamides. In our previous paper the preferred orientations of the phenylalanines at position 11 and 21 in lissoclinamides 4 and 5 were shown to be similar. Indeed these compounds were tentatively assigned the same stereochemistry by Schmitz et al.[4] For both compounds at positions 11 and 21, either of the rotamers I or II was preferred. In lissoclinamide 8 rotamer III is preferred at both positions and in lissoclinamide 7, rotamer I is dominant at position 11 and rotamer III is dominant at position 21.

Biological Activity. The cytotoxicities of the lissoclinamides were examined in vitro using transitional bladder carcinoma cells (T24), SV40-transformed fibroblasts (MRC5CV1), and normal peripheral blood lymphocytes. Cell viability assays were performed by using a colorimetric assay[7-9] which relies on the conversion of MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a blue formazan dye by live mitochondria. The cell survival curves for the three different cell types are shown in Figures 2–4 and the IC_{50} values (concentration required for 50% growth inhibition) for the compounds with the three different cell types are summarized in Table III. The IC_{50} values for lissoclinamides 4 and 5 reported in this study are in good agreement with those reported by Schmitz and co-workers using a lymphocytic leukemia cell line.[4] It is obvious that small structural variations can lead to marked differences in activity, spanning nearly 3 orders of magnitude. Lissoclinamide 7 is by far the most cytotoxic with an IC_{50} value of 0.04 μg/mL for a 1-h exposure. For comparison, didemnin B with a 1-h exposure

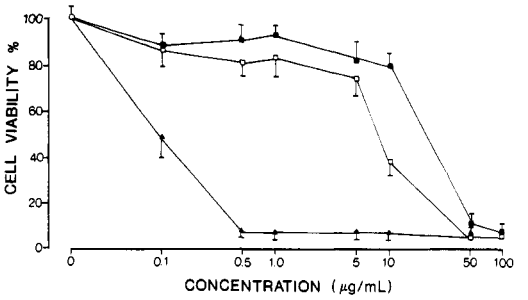


Figure 4. Cytotoxicity of the lissoclinamides toward normal peripheral blood lymphocytes. Lymphocytes were prepared and cultured as described in the Experimental Section. The cell viability was determined after 24 h as described in the legend for Figure 2.

Table III. Comparison of the Cytotoxicities of the Lissoclinamides in 3 Different Cell Types

	IC_{50}, μg/mL		
	T24	MRC5CV1	lymphocytes
lissoclinamide 5	10	15	20
lissoclinamide 7	0.06	0.04	0.08
lissoclinamide 8	6	1	8

has a mean IC_{50} value of 0.046 μg/mL for cultured tumor cells.[10] The presence of two thiazole rings leads to a considerable decrease in activity (lissoclinamide 5). This is interesting in light of the many biologically active compounds containing thiazole rings, e.g., the dolastatins,[11] thiostrepton,[12] botromycin,[13] dysidenin,[14] and distamycin analogues.[15]

Previous studies[3] showed lissoclinamide 4 to be more cytotoxic (IC_{50} 0.8 μg/mL for T24 cells) and lissoclinamide 5 to be less cytotoxic (IC_{50} 100 μg/mL) than the present study. We are now using the MTT colorimetric assay instead of [*methyl*-^{3}H]thymidine incorporation. This discrepancy could be explained by the use of different assays. It has been demonstrated that the thymidine incorporation assays parallel the clonogenic assay for measurement of cytotoxicity.[9] On the other hand, the MTT assay gives lower IC_{50} values than the clonogenic assay in some cases.[16] A number of isolates have now given consistent results with the MTT assay. It is also possible that an undetectable level of cytotoxic impurity was present in the original samples of lissoclinamide 4, which may be due to seasonal or other variation.

Shioiri and co-workers[17] have compared the cytotoxicities of natural and synthetic cyclic peptides. They examined dolastatin 3 (the proposed structure and its 15 isomers), ascidiacyclamide, the patellamides (correct and originally assigned structures), and ulithiacyclamide. The conclusions from that study were that the oxazoline function is essential for cytotoxicity and that the cyclic skeleton may not be essential as even small linear peptides

(7) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
(8) Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271.
(9) Holt, P. S.; Buckley, S.; Norman, J. O.; DeLoach, J. R. *Toxicon* **1988**, *26*, 549.
(10) Jiang, T. L.; Liu, R. H.; Salmon, S. E. *Cancer Chemother. Pharmacol.* **1983**, *11*, 1.
(11) Pettit, G. R.; Kamano, Y.; Brown, P.; Gust, D.; Inoue, M.; Herald, C. L. *J. Am. Chem. Soc.* **1982**, *104*, 905.
(12) Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature* **1970**, *225*, 233.
(13) Waisvicz, J. M.; van der Hoeven, M. G.; te Nijenhuis, B. *J. Am. Chem. Soc.* **1957**, *79*, 4524.
(14) Kazlauskas, R.; Lidgard, R. O.; Walls, R. J.; Vetter, W. *Tetrahedron Lett.* **1977**, 3183.
(15) Bailly, C.; Houssin, R.; Bernier, J.-L.; Henichart, J.-P. *Tetrahedron* **1988**, *44*, 5833.
(16) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
(17) Shioiri, T.; Hamada, Y.; Kato, S.; Shibata, M.; Kondo, Y.; Nakagawa, H.; Kohda, K. *Biochem. Pharmacol.* **1987**, *36*, 4181.

having an oxazoline ring showed moderate cytotoxicity. The importance of the oxazoline ring for cytotoxicity is doubtful since dolastatin 10, which does not contain a thiazoline or oxazoline ring, has been shown to be the most active antineoplastic agent presently known.[18] However, a role for the oxazoline ring in cytotoxicity cannot be ruled out. From the above discussion it is apparent that one possible determinant of cytotoxic activity is the overall conformation of the molecule. In the absence of further knowledge about the mode of action of these peptides, such discussions are purely speculative. The mechanism of action of the lissoclinamides is currently under investigation in our laboratory.

Experimental Section

Collection of Animals and Extraction of Cytotoxic Compounds. Specimens of *L. patella* were collected from Heron Island Reef on the Great Barrier Reef, Queensland, Australia. The animals were processed and extracted as previously described.[3] Briefly, the frozen ascidians were homogenized in methanol/toluene (3:1) and extracted with 1 M sodium nitrate, and the resulting aqueous layer was extracted with chloroform. The dried chloroform extract was chromatographed on a preparative Whatman ODS-3 reverse-phase HPLC column. Fractions corresponding to lissoclinamides 7 and 8 were pooled, evaporated, and further purified on Sephadex LH-20.

NMR Spectroscopy. Spectra were obtained as described previously.[3] The spectral widths for the ^1H–^1H COSY experiments were 3101.7 Hz for lissoclinamide 7 and 3657.6 Hz for lissoclinamide 8. For the COLOC experiments spectral widths were as follows: lissoclinamide 7, 3176 and 18939.4 Hz; and lissoclinamide 8, 3602.3 and 16835.0 Hz.

Mass Spectroscopy. A Kratos MS25 RFA instrument was used. High-resolution electron impact mass spectra were recorded at 70 keV with a resolving power of 3000.

Acid Hydrolysis and Chiral Gas Chromatography. These experiments were performed exactly as described in the previous publication.

Cell Culture and Cytotoxicity Testing. SV40 transformed fibroblasts (MRC5CV1) and transitional bladder carcinoma cells (T24) were maintained in RPMI 1640 medium (Commonwealth Serum Laboratories) containing 10% fetal calf serum, at 37 °C in a humidified atmosphere of 5% CO_2 in air. Human lymphocytes were isolated from whole blood with Ficoll Paque (Pharmacia-LKB) as recommended by the manufacturer. Phytohemagglutinin [PHA, (GIBCO)] was added at 1 μL/mL. The lymphocytes were plated into 96-well microtiter plates at a density of 1×10^5/mL. After PHA stimulation for 72 h, the lissoclinamide compounds were added in a range from 100 to 0.1 μg/mL. After 24 h surviving cells were assayed as described below.

For cytotoxicity data, MRC5CV1 and T24 cells were plated at a density of 1×10^4/100 μL in 96-well microtiter plates. After overnight incubation the cells were exposed to varying concentrations of the lissoclinamides for 1 h. After a 48-h incubation, 10 μL of MTT (5 mg/mL) was added per well and the cultures were incubated for 4 h at 37 °C. Thereafter 100 μL/well of 0.08 M HCl in 2-propanol was added. Samples were mixed until all the blue product was dissolved, and the absorbance at 540 nm was determined in a Titertek Multiscan MC instrument using a reference wavelength of 690 nm.

Acknowledgment. We are grateful to Graham MacFarlane and Dr. John MacLeod (Research School of Chemistry, Australian National University, Canberra) for the mass spectroscopic data. The assistance of Lynette Lambert with the NMR experiments and Vince Alberts with the gas chromatography is much appreciated. This investigation was supported by grants from the Australian Research Council and the Queensland Cancer Fund. We thank Barbara Muller for typing the manuscript.

Registry No. Lissoclinamide 4, 120853-16-9; lissoclinamide 5, 120853-17-0; lissoclinamide 7, 126297-39-0; lissoclinamide 8, 126452-98-0.

(18) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinnan, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883.