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Ulicyclamide is cytotoxic against L1210 cells *in vitro* and inhibits both DNA and RNA syntheses

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Many bioactive compounds have been isolated from marine organisms and their structures which have been elucidated are quite varied. Cyclic peptides represent one group found, wherever ulithiacyclamide (UTC), ascidiacyclamide (AC), patellamide (PA), dolastatin 3, and didemnins, etc. are present. These compounds have recently been synthesized [1]. In a previous paper, we reported the structure–activity relationship among a series of cyclic peptides using UTC, AC, PA, dolastatin 3, and their synthetic intermediates [2]. These natural products, except for dolastatin 3, possess both the thiazole and oxazoline rings, the unusual amino acid moieties, as constituents. A study of this structure–activity relationship revealed that the oxazoline moiety has an important role in cytotoxicity. From investigations of the biological aspects of UTC, the most cytotoxic of the compounds tested, it was also found that cytotoxic activity might involve an inhibitory effect on protein synthesis [3].

Ulicyclamide (UC) (Fig. 1) is a cytotoxic cyclic peptide isolated from a tunicate *Lissoclinum patella* [4] and contains both oxazoline and thiazole moieties in its structure. It has been effectively synthesized by our group [5]. When the cytotoxicity of a synthetic UC intermediate (**2**) (Fig. 1) that has no oxazoline function in its structure, was tested against L1210 cells in culture, it was found that **2** was also cytotoxic, although the activity was less than that of UC. This contradicted our previous proposal that the oxazoline moiety is the essential constituent for cytotoxicity in the series of cyclic peptides tested [2]. Therefore, the biological aspects of UC and **2** were studied further. In this study, we report that both UC and **2** are cytotoxic and that both inhibit DNA and RNA syntheses.

Materials and methods

Chemicals. Ulicyclamide and its synthetic intermediates were synthesized according to our previous report [5].

Cell culture and growth inhibition assay. Mouse leukemia L1210 cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum at 37° in a humidified incubator with 5% CO_2 in air, as reported previously [2]. For the growth inhibition (cytotoxicity) assay, several concentrations of a test compound were added to cells *in vitro* and the suspension incubated at 37° for 48 hr. Then the number of surviving cells was counted as reported previously [2].

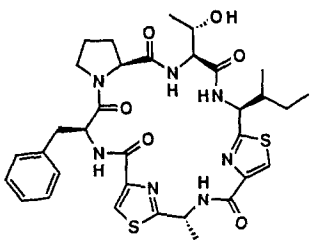
Inhibition of macromolecular synthesis. DNA, RNA, and protein syntheses were detected by measuring the incorporation of [^{3}H]-labelled precursors, uridine, thymidine and leucine into cells which were treated for 2 hr with the test compound in the presence of a precursor as reported previously [3].

Results and discussion

Cytotoxicity of UC and its two intermediates (linear peptide, **1** and cyclic peptide, **2** that has no oxazoline function) were tested against L1210 leukemia cells *in vitro*. Dose–response curves are shown in Fig. 2. The IC_{50} of UC was 13 μg/ml, which was comparable to that previously reported [4]. Linear intermediate, **1** was not cytotoxic even at a dose of 250 μg/ml. It is worth noting that compound **2**, which has no oxazoline function, showed fairly potent cytotoxicity, IC_{50} = 35 μg/ml. This contradicted our proposal reported previously that the oxazoline moiety has an important role in cytotoxicity, which was based on our

H-*L*-aThr-*L*-(ile)Thz-*D*-(ala)Thz-*L*-Phe-*L*-Pro-OH

1



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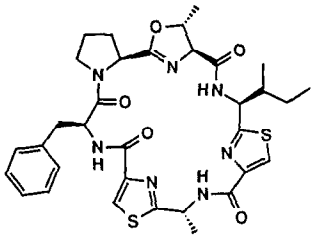
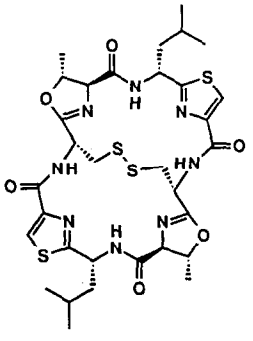


Ulicyclamide



Ulithiacyclamide

Fig. 1. Structures of ulicyclamide (UC) and its synthetic intermediates **1** and **2**, and ulithiacyclamide (UTC).

study of the structure–activity relationship using a series of cytotoxic cyclic peptides (UTC, AC, patellamides A, B, and C, and dolastatin 3) and their synthetic intermediates [2]. Since UTC was found to mainly inhibit protein synthesis, in order to determine whether UC and **2** display similar biological activity, we studied the effects of UC and **2** on macromolecular synthesis. Results are shown in Figs. 3 and 4. As we expected, UC did not inhibit protein synthesis, but produced a remarkable effect on both DNA and RNA syntheses and to the same extent (Fig. 3). This biological property is quite different from that of UTC. Compound **2**, which has no oxazoline function but is capable of cytotoxic activity, also showed a similar inhibitory effect on both DNA and RNA syntheses as UC (Fig. 4). The doses of UC and **2** required for 50% inhibition of both DNA and RNA syntheses were about 2.2 and 30 μg/ml, respectively. These results suggest that the oxazoline moiety of UC is not always responsible for its cytotoxicity, and that the cytotoxic mechanism involved is different from that of UTC. To date, we have tested the cytotoxic activity of about 40 derivatives of a series of cyclic peptides, however, only compound **2** showed cytotoxic activity while lacking an oxazoline moiety. When we reconsider the structural differences between UC and UTC (Fig. 1), we find that UC contains one oxazoline and one pyrrolidine moiety and two thiazole moieties situated side by side, while UTC contains two oxazoline and two thiazole moieties with each of them situated alternately. Further studies of the cytotoxic mechanism of UC and its related compounds are required on the basis of structural differences involving steric and adjacent effects.

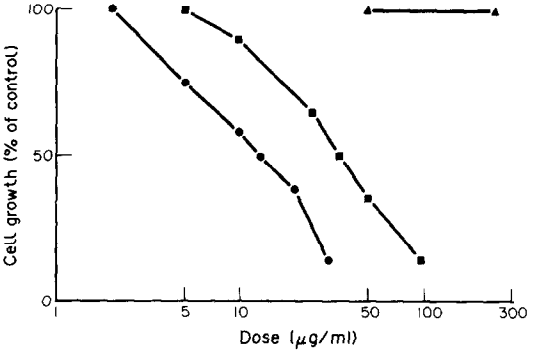


Fig. 2. Cytotoxicity of UC (●), **1** (▲), and **2** (■) against L1210 cells *in vitro*. After the cells were incubated with a test compound at 37° for 48 hr, the number of viable cells was counted. Cytotoxicity is expressed as % value of cell number relative to control cells.

In summary, ulicyclamide (UC), a cyclic peptide isolated from a tunicate *Lissoclinum patella*, was cytotoxic against cultured L1210 mouse leukemia cells even though it lacked an oxazoline function. The cytotoxicity of UC may be related to its inhibitory effect on both DNA and RNA

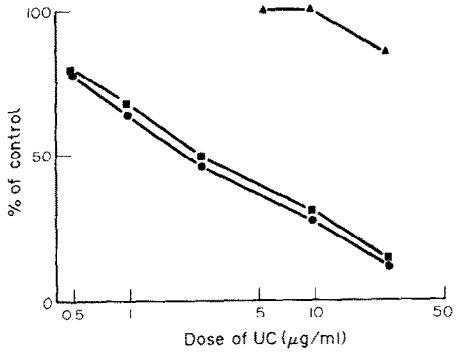


Fig. 3. Effect of UC on macromolecular synthesis. Exponentially growing cells were treated with UC for 2 hr in a medium containing [^{3}H]-labelled precursor (dThd, Urd, or Leu). Incorporation of labelled precursor into cells was measured. Incorporation ratio (%) relative to control cells is shown. DNA (●), RNA (■), and protein (▲) syntheses.

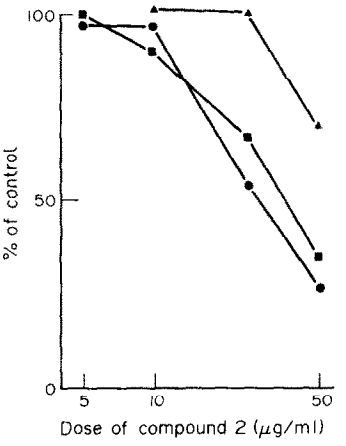


Fig. 4. Effect of compound 2 on macromolecular synthesis. See the legend of Fig. 3.

syntheses. This differs from ulithiacyclamide, a UC analogue, which is a potent cytotoxic agent that has an inhibitory effect on protein synthesis.

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Identification of multiple G_i subtypes and a novel G protein in bovine kidney cortex

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Heterotrimeric guanine nucleotide-binding proteins (G proteins*) comprise a specific family that serves as intermediaries in a variety of transmembrane signaling processes in eukaryotic cells [1, 2]. Pertussis toxin catalyzes ADP-ribosylation of certain G_α subunits, thereby uncoupling the G protein substrate from its corresponding receptor [1, 2]. Molecular cloning of cDNAs and genes encoding α subunits indicates the existence of at least four genes for the α subunits ($G_{i1\alpha}$, $G_{i2\alpha}$, $G_{i3\alpha}$ and $G_{o\alpha}$) of the G proteins serving as the substrate for pertussis toxin, besides two transducin

* Abbreviations: G protein, guanine nucleotide-binding protein; G_i, a G protein originally identified in terms of inhibition of adenylate cyclase; G_o, a G protein of unclear function, abundant in brain; G_x, α subunit of G protein; $G_{x(z)}$, a putative G protein encoded by cDNAs cloned from rat (G_x) and human (G_z) neural libraries; G_s, G protein involved in hormonal stimulation of adenylate cyclase; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; and DTT, dithiothreitol.