Research Article

Pharmacological active Cyclic Peptides from the Roots of *Stellaria dichotoma*

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Cyclic peptides, dichotomins A-K has been isolated from the roots of plant *Stellaria dichotoma*. Their structures have been elucidated by various methods. These cyclic peptides show various pharmacological activities like cytotoxic activity, cyclooxygenase inhibitory activity and vasorelaxant effect.

Key-words: Cyclic peptides, Cytotoxic activity, Cyclooxygenase inhibitory activity, Vasorelaxant effect.

INTRODUCTION

Cyclic peptides are polypeptide chains, whose amino and carboxyl termini are themselves linked together with a peptide bond, forming a circular chain. Cyclic peptides are interesting natural products exhibiting a wide range of biological activities. As part of our continuing study of cyclic peptides from higher plants.[1,2] Since cyclolinopeptide A was isolated and determined from *Linum usitatissimum* (linseed oil, Linaceae) in 1959 by Kaufmann and Tobschirbel,[3,4,5,6] about 455 cyclopeptides have been discovered from higher plants during the past half century, belonging to 26 families, 65 genera, and 120 species. In particular, plants of the Caryophyllaceae and Rhamnaceae families commonly contain cyclopeptides.

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Stellaria is a genus of about 90-120 species flowering plants in the family Caryophyllaceae, with a cosmopolitan distribution. Common names include stitchwort and chickweed. Chickweeds are used as food plants by the larvae of some Lepidoptera species including Angle Shades, Heart and Dart, Riband Wave, Setaceous Hebrew Character and the *Coleophora* case-bearers *C. coenosipennella* (feeds exclusively on *Stellaria spp*), *C. lineolea* (recorded on *S. graminea*), *C. lithargyrinella* (recorded on *S. holostea*), *C. solitariella* (feeds exclusively on *S. holostea*) and *C. striatipennella*. Some species, including *Stellaria media*, are used as leaf vegetables, often raw in salads[7].

Dichotomin A-K has been isolated from the roots of *Stellaria dichotoma* and their biological assays has been performed.[8] In

this review article, mainly isolation methods and biological assays performed for the various cyclic peptides from the roots of *Stellaria dichotoma* are described.

Cyclic peptides from roots of *Stellaria dichotoma*

Table 1:Dichotomins with their structures

S. No.	Cyclic peptide	Structure
1	Dichotomin A	Cyclo-(Gly-L-Thr-L-Phe-L-Leu-L-Tyr-L-Val)
2	Dichotomin B	Cyclo-(Gly-L-Thr-L-Phe-L-Leu-L-Tyr-L-Thr)
3	Dichotomin C	Cyclo-(Gly-L-Thr-L-Phe-L-Leu-L-Tyr-L-Ala)
4	Dichotomin D	Cyclo-(Gly-L-Val-Gly-L-Phe-L-Tyr-L-Ile)
5	Dichotomin E	Cyclo-(Gly-L-Tyr-L-Ala-L-Phe-L-Ala)
6	Dichotomin F	Cyclo-(L-Pro-L-Tyr-L-Phe-L-Val-L-Leu-L-Pro-L-Ser-L-Val-L-Tyr)
7	Dichotomin G	Cyclo-(L-Pro-L-Leu-L-Pro-L-Ile-L-Pro-L-Pro-L-Phe-L-Tyr-L-Ser)
8	Dichotomin H	Cyclo-(L-Pro-L-Thr-L-Phe-L-Tyr-l-Pro-L-Leu-L-Ile-L-Ala)
9	Dichotomin I	Cyclo-(L-Pro-L-Thr-L-Phe-L-Tyr-l-Pro-L-Leu-L-Ile-L-Val)
10	Dichotomin J	Cyclo-(Gly-Ile-Phe-Leu-Tyr-Ala)
11	Dichotomin K	Cyclo-(Pro-Tyr-Tyr-Val-Ile-Pro-Ala-Val-Ile)

Dichotomins A-E

Cyclic peptides, dichotomins A-E were isolated from the roots of *Stellaria dichotoma L. var. lanceolata Bge*.[9] The methanollc extract of the roots of S. *dichotoma* L. var. *lanceolata* Bge. was partitioned between n-BuOH and H_2O. The n-BuOH soluble material was subjected to Diaion HP-20 column (H_2O - MeOH), and 80% MeOH eluted fractions were chromatographed on a silica gel column, followed by HPLC on ODS to yield five peptidic compounds, named dichotomins A - E (0.007 %, 0.0004 %, 0.003 %, 0.0012 %, 0.0002 %).

Their structures were elucidated on the basis of extensive 2D NMR, chemical degradation and X-ray crystallographic analysis.[9]

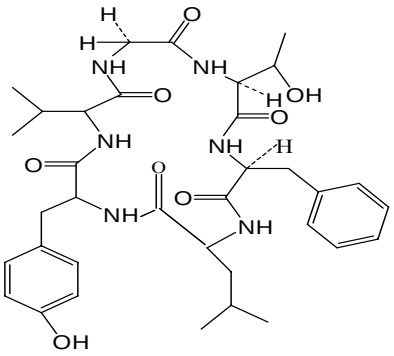
Dichotomins A, B, C and E showed cytotoxic activity.[9] Dichotomins A, B, C and E showed cell growth inhibitory activities against P-388 lymphocytic leukemia cells. IC_{50} values were found out to be 2.5 µg/ml, 3.5 µg/ml, 5.0 µg/ml and 2.0 µg/ml respectively.

While dichotomin D showed potent cyclooxygenase inhibitory activity (72.6 % inhibition at 100 µM).

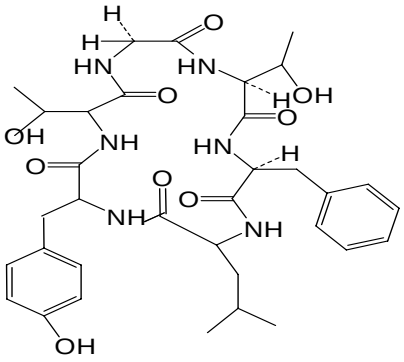
Cytotoxic Activity was performed on P388 cells. The MTT (3-[4,S-d~methylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) calorimetric assay was performed in a 96 well plate. The blue formazan produced by the mitochondrial dehydrogenase of arable cells was measured spectrophotometri-cally. 100 µl of RPMI-1640 medium supplemented with 5 % fetal calf serum and 100 µg/ml of kanamycin and containing mouse P388 leukemia cells (3 x 104 cells/ml) was added to each well. After overnight incubation (37 C, 5 % CO_2), 100, 30, 10, 3, 1, 0.3 and 0.1 µg/ml

of sample solutions were added to the wells and the plates were incubated for 48 h Then, 20 µl of MTT was added to each well and the plates were incubated for 4 h. The resulting formazan was dissolved in 100 µl of 10 % SDS (Sodium dodecyl sulfate) containing 0.01 N HCI. Each well was mixed gently with a pipet for 1 or 2 min and the plate was read on a microplate reader (Tosoh MPR-A4i) at 540 nm. The IC50 (kg/ml) value was defined as the concentration of sample which achieved 50 % reduction of viable cells with respect to the control.

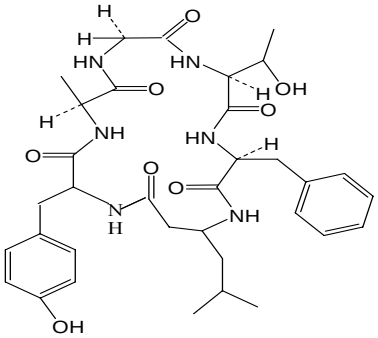
Assay for cyclooxygenase inhibitory activity was performed by the use of cyclooxygenase + PGH_2/PGE_2 isomerase kit (Eldan Tec. Co. Ltd., Israel); that is, 2 ml of samples in various concentrations and indomethacin (1×10^{-4} M) solutions, and 10 ml of cofactors solution (includes epinephrine, tryptophan, hydroquinone, and GSH) were added to 100 ml of sheep vesicular gland microsome solutions (0.2 mg/ml) that were dissolved in 50 mM Tris-HCl buffer solution. Their mixture solutions were preincubated with shaking for 3 mins at 25 ºC. After preincubation, 2 ml of arachidonic acid solution (1mg/ml) was added to the above enzymatic solutions and incubated continually for further 3 mins. At the end of the reaction was added 10 ml of $FeCl_3$ solution (25 mM) to the reaction mixtures were centrifuged at 4 ºC for 10 min. The contents of PGE_2 in the supernatant solutions were determined by using the prostaglandin E_2 enzyme immunoassay kit (Cayman Chemical Co.).[9]



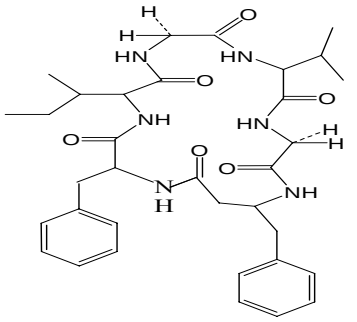
(Dichotomin A)



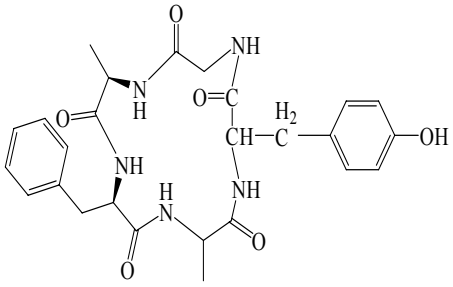
(Dichotomin B)



(Dichotomin C)



(Dichotomin D)



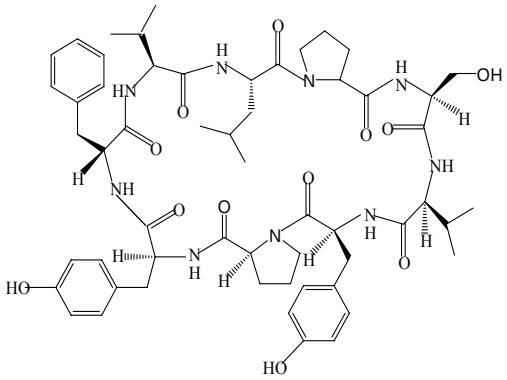
(Dichotomin E)

Dichotomins F and G

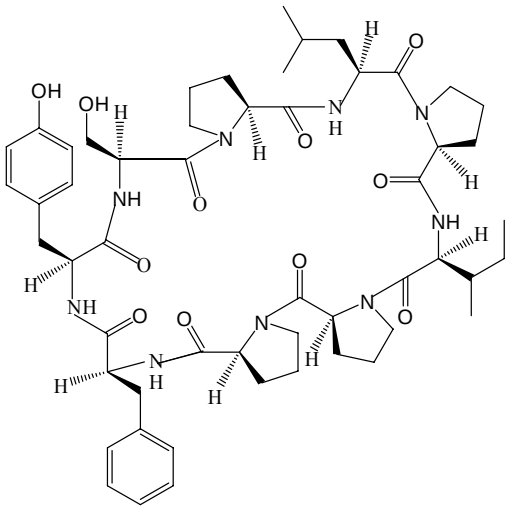
Two cyclic peptides, dichotomins F and G, were also isolated from the roots of *Stellaria dichotoma L. var. lanceolata Bge*.[10] The MeOH extract of the roots of S. dichotoma L. var. lanceolata Bge. was partitioned between n-BuOH and H_2O. The n-BuOH-soluble material was subjected to Diaion HP-20 column (H_2O-MeOH), 80 % and 100 % MeOH eluted fractions were chromatographed on a Si gel column, followed by HPLC on ODS to yield two peptides, which were named as dichotomin F (0.0003 %) and G (0.0006 %).

The structures were elucidated by chemical degradation, ESIMS-MS, and extensive 2D NMR methods.[10]

Dichotomins F and G showed moderate cyclooxygenase inhibitory activities.[10] Dichotomin F showed 72.6 % inhibition at 100 µM and dichotomin G showed 62.6 % inhibition at 100 µM. Assay for cyclooxygenase inhibitory activity was performed by the use of cyclooxygenase + PGH_2/PGE_2 isomerase kit (Eldan Tec. Co. Ltd., Israel).



(Dichotomin F)



(Dichotomin G)

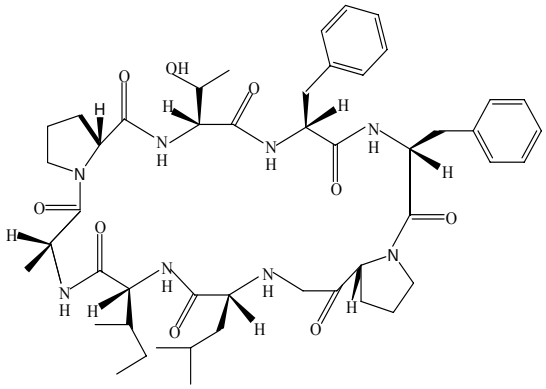
Dichotomin H and dichotomin I

Two cyclic octapeptides, dichotomin H and dichotomin I, were isolated from the roots of *Stellaria dichotoma var.*

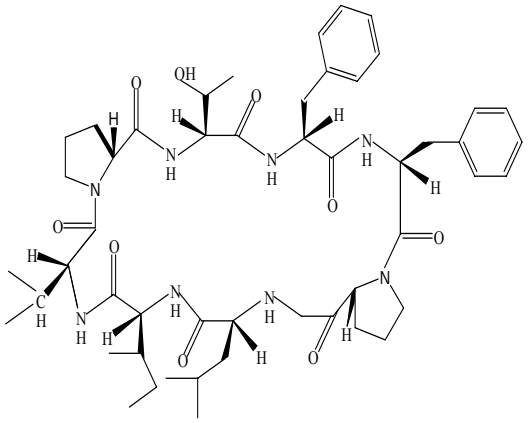
lanceolata.[11] The concentrated methanolic extracts of the roots of *Stellaria dichotoma* var. *lanceolata* was extracted with l-butanol. The 1-butanol phase was concentrated, successively treated with HP-20 with gradient system (water-methanol), silica gel column chromatography with gradient system (methylene chloride-methanol), and was finally subjected to HPLC on ODS column chromatography with 38 % CH_3CN and 67 % methanol to furnish two new cyclic octapeptides, dichotomins H (0.0001 % yield) and I (0.0005 % yield).

Their structures were elucidated by extensive two dimensional NMR methods and chemical degradation.[11]

Cytotoxic activity was shown by dichotomin H and dichotomin I. Dichotomins H and I showed a moderate cell growth inhibitory activity against P-388 cells. IC_{50} values were 3.0 µg ml^{-1} and 2.3 µg ml^{-1}. Cytotoxic activity was performed on P388 cells.[11]



(Dichotomin H)



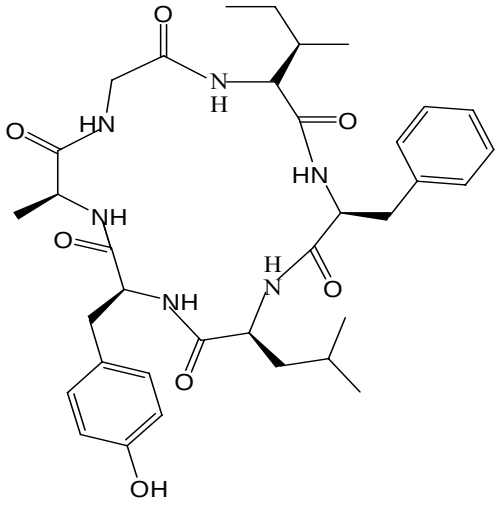
(Dichotomin I)

Dichotomins J and K

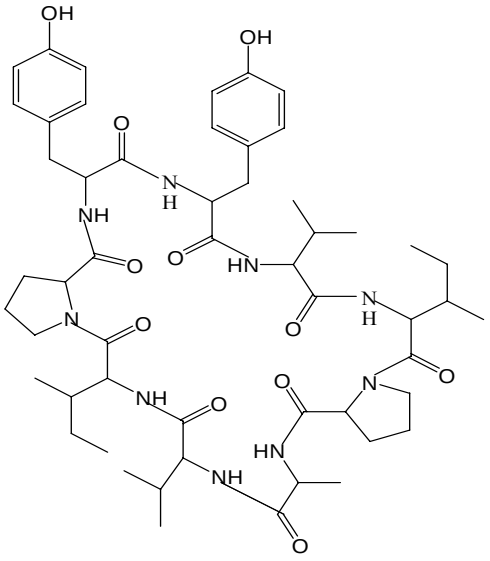
Two cyclic peptides, dichotomins J and K, were isolated from the roots of *Stellaria dichotoma*.[12] The n-BuOH-soluble material was subjected to Diaion HP-20 column (H_2O-MeOH), 80 % MeOH eluted fractions were chromatographed on a Si gel column, followed by purification by C_{18} HPLC to yield two peptides, which were named as dichotomin J (0.0001 %) and K (0.0002 %).

Their structures were elucidated by chemical degradation and extensive 2D NMR methods.[12]

Dichotomins J and K showed a moderate vasorelaxant effect on rat aorta.[12] Vasorelaxant effects of dichotomin J and K were examined in contractions of isolated rat thoracic aorta. The vasodilator effect of dichotomin J was shown to be more potent than that of dichotomin K. Vasodilator assay was performed as follows. A male wistar rat weighing 230 g was sacrificed by bleeding from carotid



(Dichotomin J)



(Dichotomin K)

arteries under anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS: 118 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$ and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparation 3 mm in length. The tissue was placed in a well oxygenated (95 % O_2, 5 % CO_2) bath of 10 mL of KHS solution at 37 ºC with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting bath was replaced every 20 min. After equilibration, each aortic ring was contracted by treatment with 3×10^{-7} M norepinephrine (NE). The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (Ach), and aortic rings in which 80 % relaxation occurred were regarded as tissues with endothelium. When the NE-induced contraction reached plateau, each sample was added.

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