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Cyclolinopeptides, cyclic peptides from flaxseed with osteoclast differentiation inhibitory activity

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ABSTRACT

Flaxseed (*Linum usitatissimum* seed) is widely used in food and natural health products. In our search for osteoclast differentiation inhibitors, some cyclic peptides isolated from flaxseed, known as the cyclolinopeptides, were discovered to have osteoclast differentiation inhibition activity. The osteoclast differentiation inhibition activity of cyclolinopeptides A–I (**1**–**9**) and their related derivatives (**10**–**14**) are described herein. Cyclolinopeptides F, H and I (**6**, **8** and **9**), in particular, showed potent osteoclast differentiation inhibition activity.

Osteoclasts are giant multinucleated cells formed by the fusion of mononucleated cells derived from hematopoetic tissue and are the only cell type that can resorb bone tissues. Together with osteoblasts, which are responsible for bone formation, osteoclasts play a critical role in bone remodeling.[1] Osteoclasts express osteoclast-specific tartrate-resistant acid phosphatase (TRAP) isoform 5b, which activity correlates to the osteoclast number and bone resorption.[2,3]

Excessive osteoclastic bone resorption, caused by elevated osteoclast number and activity, relative to osteoblastic bone formation is often associated with bone diseases, for example, osteoporosis.[4,5] Thus, a substance that inhibit osteoclast differentiation may play a role in the treatment of bone diseases due to high bone resorption rate.

Flax (*Linum usitatissimum* L.), one of the oldest cultivated crops, is a plant of the Linaceae family, whose seeds are used in industrial and natural health products.[6] In addition to its exceptionally high content of α-linolenic acid, an essential omega-3 fatty acid, flaxseed also contains lignans and cyclic peptides.[6–8]

Cyclic peptides contained in flaxseed are generally known as cyclolinopeptides (CLs), and so far, there are 25 known CLs.[9–17] Several CLs possess the same amino acid sequence but differs in the oxidation state of the methionine residue, for example, CL-B, CL-C and CL-K. The methionine (Met) can be oxidized to the methionine *S*-oxide (MetO), which can be further oxidized to the methionine *S*,*S*-dioxide ($MetO_2$). However, in fresh flaxseed the levels of MetO and $MetO_2$ are generally low, and will accumulate in flaxseed oil with storage.[7,18,19]

Some CLs have been reported to be biologically active. For example, CL-A exhibited immunosuppressive activity similar to that of cyclosporine,[20–22] inhibitory activity towards calcium dependent activation of T-lymphocyte cell division,[23] and antimalarial activity.[24] CL-B and E were also reported to show immunosuppressive activity.[10,25] However, the osteoclast differentiation inhibitory activity of CLs have never been reported before. The osteoclast differentiation inhibitory activity of CL-A–CL-I (**1**–**9**) isolated in our previous studies[10,11,25] are reported herein.

In our search for new osteoclast differentiation inhibitor,[26] we screened the compounds isolated in our previous phytochemical studies for osteoclast differentiation inhibitory activity using TRAP activity as indicator of osteoclast differentiation.[26,27] As a result, some cyclolinopeptides were found to show osteoclast differentiation inhibitory activity (Table 1). Most of the tested CLs dose-dependently inhibited osteoclast differentiation (Table 1) without affecting the cell viability. Compounds **6**, **8** and **9**, in particular, are several times more potent than the positive controls (curcumin: IC_{50} 7.50 μM, ipriflavone: IC_{50} 17.2 μM).

In addition, we have also synthesized compounds **10**–**14** from CL-E (**5**), CL-F (**6**) and CL-G (**7**) and tested their osteoclast differentiation inhibitory activity (Table 1). In brief, CL-E (**5**) was dissolved in TFA and cooled to 0 °C. Et_2S and HCl were then added for a final concentration of 1 M and 0.1 M, respectively, and the mixture was stirred for 1 h to afford **10**. CL-F (**6**) was treated similarly to afford

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Table 1
Amino acid sequences and osteoclast differentiation inhibitory activity of cyclolinopeptides from flaxseed oil

CL	Amino acid sequence	IC_{50} (μM)	CL	Amino acid sequence	IC_{50} (μM)
A (**1**)	*cyclo*-(Pro-Pro-Phe-Phe-Leu-Ile-Ile-Leu-Val)	12.2	H (**8**)	*cyclo*-(Pro-Phe-Phe-Trp-Ile-MetO-Leu-Met)	0.83
B (**2**)	*cyclo*-(Pro-Pro-Phe-Phe-Val-Ile-Met-Leu-Ile)	5.53	I (**9**)	*cyclo*-(Pro-Phe-Phe-Trp-Val-Met-Leu-MetO)	0.47
C (**3**)	*cyclo*-(Pro-Pro-Phe-Phe-Val-Ile-MetO-Leu-Ile)	14.9	(**10**)	*cyclo*-(Pro-Leu-Phe-Ile-Met-Leu-Val-Phe)	15.3
D (**4**)	*cyclo*-(Pro-Phe-Phe-Trp-Ile-MetO-Leu-Leu)	3.86	(**11**)	*cyclo*-(Pro-Phe-Phe-Trp-Val-Met-Leu-Met)	0.60
E (**5**)	*cyclo*-(Pro-Leu-Phe-Ile-MetO-Leu-Val-Phe)	>20	(**12**)	*cyclo*-(Pro-Phe-Phe-Trp-Val-MetO-Leu-Met)	0.59
F (**6**)	*cyclo*-(Pro-Phe-Phe-Trp-Val-MetO-Leu-MetO)	0.58	(**13**)	*cyclo*-(Pro-Phe-Phe-Trp-Ile-Met-Leu-Met)	0.49
G (**7**)	*cyclo*-(Pro-Phe-Phe-Trp-Ile-MetO-Leu-MetO)	4.04	(**14**)	*cyclo*-(Pro-Phe-Phe-Trp-Ile-Met-Leu-MetO)	4.67

CL-I (**9**), **11** and **12**, while CL-G (**7**) afforded CL-I (**8**), **13** and **14**. The CL-F series (**6**, **9**, **11** and **12**) showed similar osteoclast differentiation inhibitory activity indicating the oxidation states of the methionines have negligible effect on the activity. On the other hand, the oxidation states of the methionines seems to affect the activity of the CL-G series (**7**, **8**, **13** and **14**) and the CL-E series (**5** and **10**). In general, oxidation of methionine to methionine sulfoxide decreased the osteoclast differentiation inhibitory activity. For the CL-G series, the oxidation of Met[8] affect the activity more than the oxidation of Met.[6]

In conclusion, we showed that several cyclolinopeptides, obtained from flaxseed which is used as food and in natural health products, have potent osteoclast differentiation inhibition activity. This discovery further supports the use of flaxseed in natural health products.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.040.

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