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Design of Novel Tripeptides with Macrophage Migration-Enhancing Activity

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Abstract: A new approach to the generation of biologically active peptides from non-peptide lead was demonstrated. *C,N*-termini protected Phe-Phe-His derivatives possessing macrophage migration-enhancing activity were designed on the basis of the structure of imidazoline derivative **1**, and these structure-activity relationships were also described.

Keywords: Antiinflammatories; Mimetics; Peptides and polypeptides; Solid phase synthesis

Introduction

During the past decade, a wide variety of non-peptides have been designed on the basis of the structure of biologically active peptides,[1] and some of them have been developed as useful therapeutic agents. On the other hand, *de novo* design of peptides based on the structure of the small organic molecules has not been attempted so far. This new designing approach will be one of the useful methods to create new drugs, bio-materials, and so on. Furthermore, the peptides thus designed are expected to provide a clue for understanding functions of unknown physiologically important peptides and proteins.

We focused on *cis*-2-(4-chlorophenyl)-4,5-diphenyl-2-imidazoline (**1**) as a non-peptide lead which was found to exhibit potent macrophage migration-enhancing activity, and high efficacy for picryl chloride-induced delayed type hypersensitivity, and type II collagen-induced arthritis in mice at our laboratory.[2] It is speculated that the compound induces the dispersion of infiltrated macrophages from the inflammatory sites, and exhibits anti-inflammatory activity that is performed physiologically by the macrophage migration-enhancing factor.

In this paper, we wish to report the preliminary results of design and synthesis of the peptides with macrophage migration-enhancing activity.

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Design

According to the structure-activity relationships of **1** and its derivatives, it was elucidated that the essential requirements for the activity consisted of three phenyl rings and basic imidazoline moiety.[3] It was supposed that aromatic amino acids, *e.g.* phenylalanine, tryptophan, and tyrosine, and basic amino acids, *e.g.* histidine, arginine, and lysine can substitute for aromatic ring moieties and the basic moiety of **1**, respectively. These ideas prompted us to design tripeptide analogs Type **A-C** as a prototype as shown in Figure 1. Three dimensional analysis of **1** and these peptides suggested that three phenyl rings of **A-C** can be superimposed over the phenyl rings of **1**.

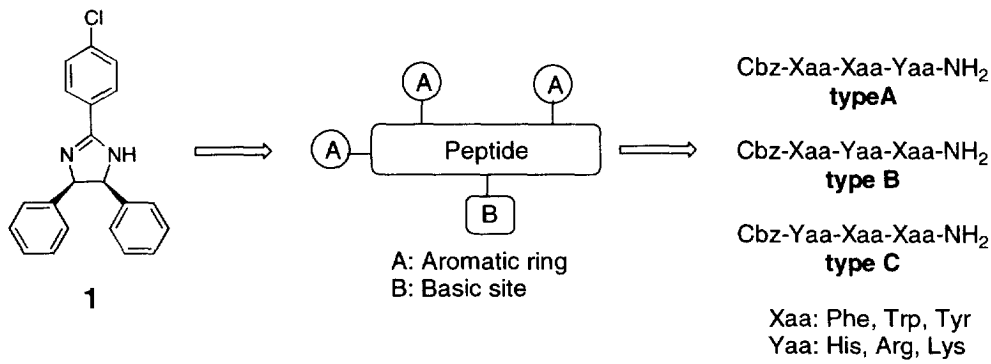

Figure 1. Designing Strategy of the Peptides

Synthesis and Screening

N-Cbz tripeptide amide derivatives (**2-19**) were synthesized by the Rink amide resin-Fmoc strategy using an automatic solid phase synthesizer (Advanced ChemTech ACT-350, Shimadzu PSSM-8). Deprotection and cleavage from the resin were carried out with trifluoroacetic acid in the presence of cation scavengers.[4] Their analogs (**20-29**) were synthesized by the solution phase method. All peptides were purified by RP-HPLC.

Macrophage migration-enhancing activity of the compounds was determined using rabbit alveolar macrophages according to the procedure reported.[5] Their activities at the concentration of 10^{-7}M were expressed by the migration indexes (MI), which were calculated by the following formula: MI=(area of enhanced migration by a peptide - area of normal migration / area of enhanced migration by 5mM L-fucose – area of normal migration)x100. The results are summarized in Table 1.

Results and Discussion

A Type **A** peptide Cbz-Phe-Phe-His-NH_2 (**2**) exhibited the migration activity as expected, whereas the corresponding Type **B** and **C** peptides (**3**, **4**) were inactive. Substitution of the

Table 1. Macrophage Migration-Enhancing Activity of **1-29**.

Entry	Compound	MI
1	Imidazoline Derivative	140
2	Cbz-Phe-Phe-His-NH_2	94
3	Cbz-Phe-His-Phe-NH_2	-1
4	Cbz-His-Phe-Phe-NH_2	1
5	Cbz-Trp-Phe-His-NH_2	14
6	Cbz-Phe-Trp-His-NH_2	7
7	Cbz-Trp-Trp-His-NH_2	4
8	Cbz-Tyr-Phe-His-NH_2	-28
9	Cbz-Phe-Tyr-His-NH_2	-23
10	Cbz-Tyr-Tyr-His-NH_2	-25
11	Cbz-Phe-Phe-Arg-NH_2	8
12	Cbz-Phe-Phe-Lys-NH_2	3
13	Cbz-Ala-Phe-His-NH_2	2
14	Cbz-Val-Phe-His-NH_2	3
15	Cbz-Leu-Phe-His-NH_2	4
16	Cbz-Phe-Leu-His-NH_2	4
17	Cbz-D-Phe-Phe-His-NH_2	2
18	Cbz-Phe-D-Phe-His-NH_2	0
19	Cbz-Phe-Phe-D-His-NH_2	-1
20	Cbz-Phe-His-NH_2	18
21	Cbz-Phe-Phe-Phe-His-NH_2	15
22	H-Phe-Phe-His-NH_2	7
23	Cbz-Phe-Phe-His-OH	-6
24	Cbz-Phe-Phe-His-OMe	97
25	Cbz-Phe-Phe-His-OEt	95
26	Ac-Phe-Phe-His-NH_2	61
27	Boc-Phe-Phe-His-NH_2	96
28	Pht-Phe-Phe-His-NH_2	-1
29	Fmoc-Phe-Phe-His-NH_2	122

phenylalanine moieties of **2** by tryptophan lost activities (**5-7**), and that by tyrosine notably induced macrophage migration inhibitory activities (**8-10**). The phenylalanine moiety of **2** is essential for the activity, because replacement of that amino acid by the aliphatic amino acids such as alanine, valine, and leucine diminished the activity (**13-16**). The histidine residue can not be replaced by other basic amino acids such as arginine and lysine (**11**, **12**). All the absolute configuration of amino acids of **2** should be *S*, since its diastereomers (**17-19**) did not show any activity. Elongation or reduction of the length of the peptide main chain lost activities (**20**, **21**). The effects of the substituents at *N* and *C* termini of **2** on the activity were examined. The

results elucidated that both termini should be protected (**22**, **23**). Substituents of the *N*-terminal had a drastic influence (**26-29**), while the *C*-terminal can be substituted for by an ester group (**24**, **25**). Fmoc-Phe-Phe-His-NH_2 (**29**) showed the most potent migration activity in these series.

Conclusion

We demonstrated a new approach for the generation of the peptides with macrophage migration-enhancing activity on the basis of the structure of non-peptide lead. Lead optimization is under investigation in this laboratory.

The methodology designing peptides from non-peptides will be utilized for the drug discovery and understanding functions of the physiologically important factors.

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References and Notes

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