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A Potent Dipeptide Inhibitor of Dipeptidyl Peptidase IV

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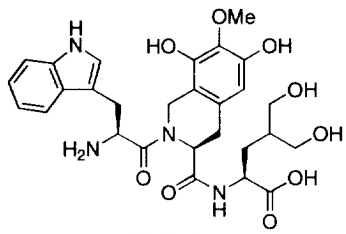
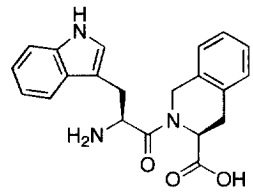
Abstract: A series of novel potent inhibitors of dipeptidyl peptidase IV (DPP-IV) has been developed. A brief structure-activity relationship of the inhibitors was investigated. The dipeptide TSL-225, tryptophyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, was identified with the critical structure for the inhibitory activity.

Keywords: Antiinflammatories; Enzyme inhibitor; Immunological activity; Peptides and polypeptides

Dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5, CD26)[1,2] is an extracellular membrane-bound enzyme present in a variety of mammalian cells and tissues, in particular on the surface of certain T-lymphocyte subsets. DPP-IV, a serine protease, is a post-proline cleaving enzyme which removes the dipeptides from the N-terminus of substrate proteins.[3] A number of functions for DPP-IV have been postulated so far.[4] It is supposed for example that this molecule plays a role in the metabolism or catabolism of collagen, which has a high frequency of Gly-Pro sequences.[5] Recently, it has been shown that inhibitors of DPP-IV block the proliferative response of T-cells to antigenic stimulation, and suppress IL-2 production.[6] Thus, inhibitors of DPP-IV may have therapeutic potential in the modulation of the autoimmune diseases such as rheumatoid arthritis.



1 (TMC-2)



42 (TSL-225)

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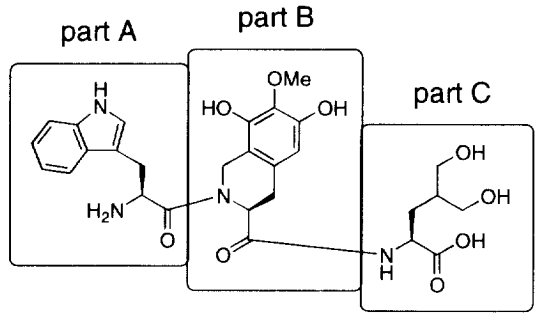
Though a variety of serine proteases inhibitors have been reported, few of these compounds show potent DPP-IV inhibitory activity.[7] Recently, TMC-2 (**1**) isolated from *Aspergillus oryzae* A374 was identified as a new specific and potent DPP-IV inhibitor (IC_{50} = 7.7 μM) by Ohnuki and co-workers.[8] TMC-2 has received considerable attention because of its unique structural features. It consists of three structural components which are (L)-tryptophan (Trp) and two unusual amino acids, (3*S*)-6,8-dihydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid and (L)-dihydroxy-leucine. We were interested in on finding the critical structure of TMC-2 required for the DPP-IV inhibitory activity on the basis of structure-activity relationships analysis. Combinatorial libraries of peptides and small organic compounds are of intense interest to the pharmaceutical industry.[9] Solid phase organic synthesis of discrete chemical mini-libraries is an adaptation of combinatorial chemistry that provides a powerful tool for rapid seed/lead optimization. In this paper, we wish to report the critical structure of TMC-2, which was determined by utilizing solid-phase combinatorial synthesis.

Chemistry

All compounds were synthesized by the Fmoc strategy using an automatic solid phase synthesizer. Deprotection and cleavage from the resin were carried out with trifluoroacetic acid in the presence of cation scavengers.[10] All compounds were of high enough purity for the purpose of inhibition assays after simple work-up such as isopropylether–water extraction or ether precipitation.

Results and Discussion

The strategy for finding the critical structure is as follows; The seed compound **1** was divided into three amino acid components and the effects of each part on the inhibition of DPP-IV were examined.



First, the effects of part A were examined (Table 1). The mixture type compounds **2–21** which consisted of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) [11] instead of the highly substituted Tic of TMC-2 were synthesized using a combinatorial synthesis technique.[12] The tryptophan derivatives **18** showed strong inhibition of DPP-IV, whereas other derivatives were only weakly inhibitory. The effects of part C were examined next (Table 2). Compounds **22–42** which consisted of Trp and Tic as part A and B, respectively were synthesized in parallel. The glutamine and serine derivatives (**26,36**) showed strong inhibitory activities whereas other derivatives had only moderate activity in the DPP-IV inhibition assay.

Table 1. H–Aaa–Tic–Xaa[a]–OH

compd	Aaa	% inhibition[b]	compd	Aaa	% inhibition[b]
2	Ala	1	**12**	Lys	7
3	Arg	10	**13**	Met	6
4	Asp	8	**14**	Phe	4
5	Asn	9	**15**	Pro	2
6	Glu	4	**16**	Ser	10
7	Gln	4	**17**	Thr	17
8	Gly	0	**18**	Trp	**61**
9	His	7	**19**	Tyr	12
10	Ile	6	**20**	Val	3
11	Leu	7	**21**	Cys	9

a) Xaa are the equimolar mixture of 19 natural amino acids except Cys
b) inhibitory activity of DPP-IV at 1 μM of each compd in the mixture sample

Table 2. H–Trp–Tic–Caa–OH

compd	Caa	% inhibition[a]	compd	Caa	% inhibition[a]
22	Ala	43	**33**	Met	43
23	Arg	38	**34**	Phe	29
24	Asp	38	**35**	Pro	20
25	Asn	46	**36**	Ser	**53**[c]
26	Glu	**62**[b]	**37**	Thr	37
27	Gln	49	**38**	Trp	37
28	Gly	34	**39**	Tyr	36
29	His	35	**40**	Val	19
30	Ile	32	**41**	Cys	49
31	Leu	25	**42**	none	**59**[d]
32	Lys	27			

a) inhibitory activity of DPP-IV at 10μM b) IC_{50} = 7.0 μM
c) IC_{50} = 7.9 μM d) IC_{50} = 5.7 μM

Table 3. H–Trp–Baa–OH

compd	Baa	% inhibition[a]	compd	Baa	% inhibition[a]
43	Glu	3	**46**	Trp	2
44	Phe	0	**47**	Tyr	10
45	Pro	15	**48**	Val	4

a) inhibitory activity of DPP-IV at 10μM

Interestingly, derivative **42** (TSL-225) which lacks part C also showed strong inhibitory activity (IC_{50} = 5.7 μM). These results suggest that tryptophan is the best amino acid for part A and that part C is not essential for the inhibition of DPP-IV. Lastly, to investigate the effects of part B, dipeptides **43–48** which contain Trp, as a standard *N*-terminal residue were synthesized. As shown in Table 3, none of them showed significant inhibitory activity.

In conclusion, the critical structure of TMC-2 for the DPP-IV inhibition is identified as H-Trp-Tic-OH (TSL-225) which showed potency equal to TMC-2. We are currently exploring the in vivo pharmacological activities of these compounds and further details will be reported in the near future.

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