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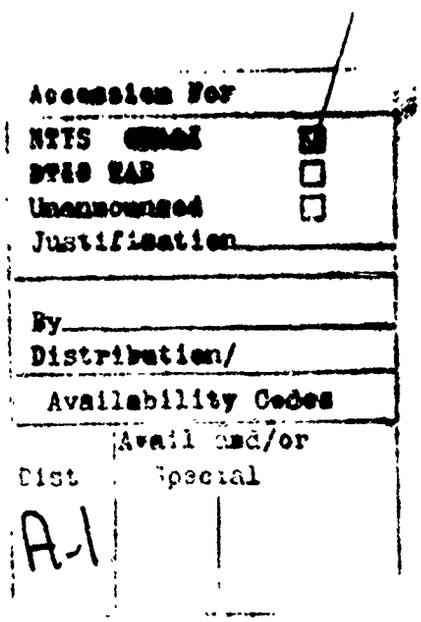

Novel trifluoromethyl-containing peptides as inhibitors for angiotensin-converting enzyme and enkephalin-aminopeptidase

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Introduction

There is an increasing interest in the incorporation of fluoroamino acids as well as fluoro-isosteres into physiologically active peptides and enzyme inhibitors [1]. We have been interested in the unique lipophilicity, size and polarity of trifluoromethyl (TFM) group. In the present study, we examined the effects of TFM on the activity and specificity of angiotensin-converting enzyme (ACE) inhibitors and enkephalin analogs.

Although many analogs of the potent ACE inhibitors, captopril and enalaprilat, have been synthesized, there is a paucity of information in the literature regarding the synthesis and activity of fluorinated congeners [2]. Accordingly, it would

Table 1 *ACE inhibitory activity of trifluoromethyl-containing peptides*

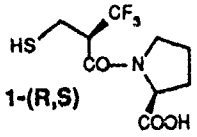
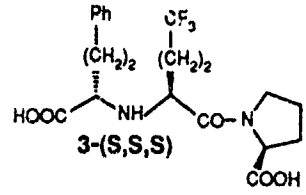
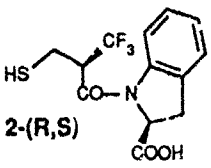
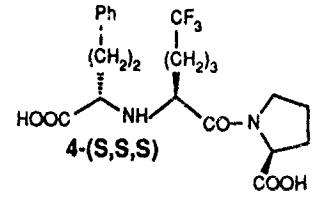
Inhibitor	IC_{50}(M)	Inhibitor	IC_{50} (M)
 **1-(R,S)**	3×10^{-10}	 **3-(S,S,S)**	6×10^{-8}
1-(S,S)	5×10^{-7}	**3-(R,S,S)**	1×10^{-3}
 **2-(R,S)**	8×10^{-8}	 **4-(S,S,S)**	3×10^{-8}
2-(S,S)	6×10^{-6}	**4-(R,S,S)**	2×10^{-4}

Table 2 *Analgesic activity of TFM-enkephalins (i.c.v., mouse)*

Enkephalins	ED_{50} (mol/mouse)	Enkephalins	ED_{50} (mol/mouse)
Tyr-D-TFNV-Gly-Phe-Met-NH_2	7.5×10^{-12}	Tyr-Gly-L-TFNV-Phe-Met-NH_2	2.2×10^{-8}
Tyr-D-Nval-Gly-Phe-Met-NH_2	3.5×10^{-11}	Tyr-L-TFNV-Gly-Phe-Met-NH_2	2.5×10^{-8}
Tyr-Gly-L-TFNV-Phe-Met-NH_2	1.4×10^{-7}	Methionine-Enkephalin	7.0×10^{-7}
Tyr-Gly-D-TFNV-Phe-Met-NH_2	1.2×10^{-8}	Tyr-D-Ala-Gly-Phe-Met-NH_2	4.5×10^{-11}
Tyr-D-TFNL-Gly-Phe-Met-NH_2	6.5×10^{-11}	Morphine · HCl	7.0×10^{-11}
Tyr-D-TFNV-Gly-(N-Me)Phe-Met-NH_2	2.0×10^{-12}	Sedapain™	5.0×10^{-11}

be of interest to examine the influence that TFM incorporation might have on such ACE inhibitors.

Enkephalins and their analogs have been extensively studied regarding their analgesic activity as well as functions as neurotransmitter [3]. However, no systematic study has been performed on the incorporation of TFM-amino acids into enkephalins. Thus, we looked at the effects of TFM-amino acids on analgesic activity (in vivo) as well as receptor binding ability (in vitro) so that we can distinguish the effects based on receptor binding from inhibition of degradation by endogenous enzymes.

Results and Discussion

A series of new TFM-containing peptides are synthesized as potential inhibitors for ACE, which are TFM analogs and homologs of captopril and enalaprilat (Table 1). As Table 1 shows, the direct substitution of TFM for methyl provides a very potent captopril analog, **1-(R,S)**. The SAR study by means of the π-SCF-molecular mechanics program (PIMM) developed by Lindner [4] as well as the MM calculations of active cite conformations by SYBIL 5.0 program indicates that **1-(R,S)** should be at least 5 times better than (S,S)-captopril ($IC_{50}=4\times10^{-9}$M), and the latter calculation suggests that **1-(R,S)** should be more than 1000 times better than **1-(S,S)**, which are consistent with the observed results. Stereoelectronic effects on conformation and lipophilicity of TFM would account for the excellent activity of **1-(R,S)**. Incorporation of both TFM and an indoline residue unexpectedly gives a less potent captopril analog, **2-(R,S)**. Enalaprilat analogs derived from replacement of the alanine residue with (S)-TFM-norvaline (L-TFNV) [5], **3-(S,S,S)**, and (S)-TFM-norleucine (L-TFNL) [5] residues, **4-(S,S,S)**, gave moderately potent peptides. The other diastereomers of **2-4** exhibited 2-5 order of magnitude weaker activities as predicted.

A series of TFM-containing enkephalin analogs were synthesized and their in vivo analgesic activity determined (Table 2). These modified enkephalins are derived from replacement of (a) Gly^2 and Gly^3 by D-TFNV, L-TFNV and D-TFNL and (b) Phe^4 by (N-Me)Phe^4. As Table 2 shows, [D-$TFNV^2$, Met-$NH_2{}^5$]- and [D-$TFNV^2$, (N-Me)Phe^4, Met-$NH_2{}^5$]-enkephalins prove to be extremely potent in vivo with respect to methionine-enkephalin (Met-Enk) (10^5-fold stronger). The in vitro binding assay to μ-, δ-, and κ-receptors revealed that [D-$TFNV^2$,

Table 3 *Receptor binding assay for [D-TFNV², Met-NH₂⁵]enkephalin (D-TFNV-Met-Enk)*

Enkephalins	Receptor	Tissue	Ligand[a]	IC_{50} (M)
D-TFNV-Met-Enk	μ	cerebrum (rat)	[³H]-PL-01[illegible]	5×10^{-10}
Met-Enk	μ	cerebrum (rat)	[³H]-PL-0[illegible]7	2×10^{-9}
D-TFNV-Met-Enk	δ	cerebrum (rat)	[³H]-DPD[illegible]	2×10^{-9}
Met-Enk	δ	cerebrum (rat)	[³H]-D[illegible]PE	1×10^{-9}
D-TFNV-Met-Enk	k	cerebellum (guinea pig)	[³H]-U-[illegible]593	4×10^{-7}
Met-Enk	k	cerebellum (guinea pig)	[³H]-U-69593	$> 1 \times 10^{-5}$

[a] [³H]-PL-017 = [³H]Tyr-Pro-MePhe-D-Pro-NH; [³H]-DPDPE = [³H][D-Pen², D-Pen⁵]enkephalin; [³H]-U-69593 = [³H] (5α,7α,8β)-(-)-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5[illegible]c-8-yl]benzene acetamide.

Met-NH_2^5]enkephalin is only several times better binder to μ-site than Met-Enk (Table 3). Therefore, it is concluded that the observed remarkable increase in potency is mainly due to the inhibition of degradation by enkephaline-aminopeptidase.

Acknowledgements

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