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A Series of C-Terminal Amino Alcohol Dipeptide Aβ Inhibitors

Albert W. Garofalo,[a,*] David W. G. Wone,[a] Angela Phuc,[a] James E. Audia,[b] Cheryl A. Bales,[a] Harry F. Dovey,[a] Darren B. Dressen,[a] Beverly Folmer,[a] Erich G. Goldbach,[a] Ashley C. Guinn,[a] Lee H. Latimer,[a] Thomas E. Mabry,[b] Jeffrey S. Nissen,[b] Michael A. Pleiss,[a] Stephen Sohn,[a] Eugene D. Thorsett,[a] Jay S. Tung[a] and Jing Wu[a]

[a]*Elan, 800 Gateway Blvd, South San Francisco, CA 94080, USA*
[b]*Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA*

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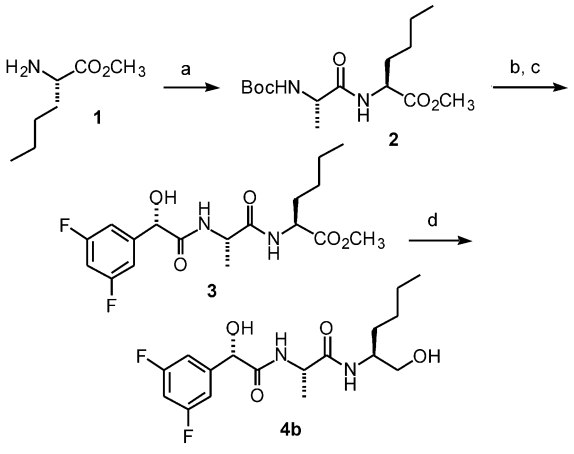
Abstract—Potent, small molecule Aβ inhibitors have been prepared that incorporate an alanine core bracketed by an N-terminal arylacetyl group and various C-terminal amino alcohols. The compounds exhibit stereospecific inhibition as demonstrated in an in vitro assay.

β-Amyloid peptide (Aβ) has been implicated in the pathogenesis of Alzheimer's Disease (AD). This protein is a 39–43 amino acid peptide derived from amyloid precursor protein (APP) by the action of β- and γ-secretases. β-Secretase has recently been identified[1] and acts on the extracellular portion of APP to form the 99 amino acid peptide, CTFβ, which contains the N-terminus of Aβ. γ-Secretase has not been definitively characterized but acts on a portion of APP within the lipid bilayer to create the C-terminal end of Aβ. Inhibition of either of these enzymes is an approach being pursued to reduce the production of Aβ in the hope of altering AD progression. Herein we describe a series of amino alcohol dipeptides designed to inhibit Aβ formation at the level of γ-secretase.

Previously, peptidic esters and amides have been disclosed that inhibit Aβ formation.[2] The peptidic esters and amides were shown to be effective with no observable in vitro cell toxicity.[3] Continued medicinal chemistry efforts with those compounds led to the development of the dipeptide amino alcohols. We looked at both primary and secondary C-terminal alcohols and observed a large difference in activity between the two. The synthesis of the amino alcohol analogues used in this study is illustrated for compound **4b** in Scheme 1. Carbodiimide coupling of L-norleucine methyl ester (**1**) with Boc-protected L-alanine afforded dipeptide **2**.[4] Deprotection in neat trifluoroacetic acid followed by a second carbodiimide coupling with (*S*)-3,5-difluoromandelic acid[5] gave **3** (3,5-difluorophenylacetic acid was employed for compounds **4a**, **c**, **d**, **f**, **g**, **i**, **k**, **m**, and **o**). Reduction of the ester with $LiBH_4$ in THF then gave amino alcohol **4b**.



Scheme 1. Synthesis of compound **4b**. (a) Boc-Ala-OH, EDC·HCl, HOBT, NMM, THF; (b) CF_3CO_2H; (c) (*S*)-3,5-difluoromandelic acid, EDC·HCl, HOBT, NMM, THF; (d) $LiBH_4$, THF.

*Corresponding author. Tel.: +1-650-877-7647; fax: +1-650-877-7486; e-mail: al.garofalo@elan.com

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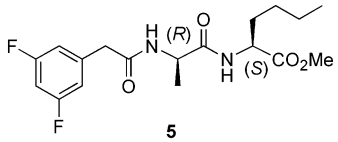
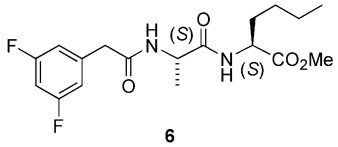
Results and Discussion

We examined the ability of these compounds to inhibit Aβ formation in a human kidney cell line (HEK 293) stably transfected with the gene for APP_{751} harboring the Swedish double mutation.[6] This cell line secretes both the 40 and 42 residue forms of Aβ. Inhibition relating to the amount of Aβ 40 and 42 was measured in the cell culture medium.[3] Previously observed SAR with dipeptide esters established the importance of a central L-amino acid in those inhibitors. To illustrate this point, diastereomeric dipeptide esters **5** and **6** with both natural and unnatural configuration at the alanine center are shown in which a clear advantage is observed for the (*S,S*)-diastereomer, **6**, over the (*R,S*)-diastereomer, **5**.



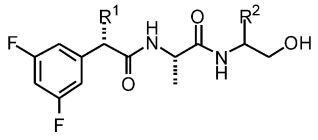
5
ED_{50} 9.4 μM



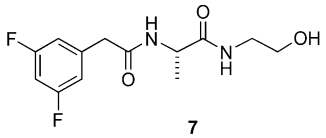
6
ED_{50} 0.015 μM

We continued our studies with dipeptide alcohols using L-alanine as the core amino acid. As shown by comparison of primary (*S*)-amino alcohols **4a**–**c** with (*R*)-amino alcohols **4d**–**f**, the stereochemistry of the amino alcohol has a large influence on activity with the *S*-configuration being preferred (Table 1). The importance of an aliphatic group of correct configuration at C2 of the amino alcohol was also shown by the synthesis of compound **7**. This glycinol derivative, lacking substitution at C2, has an ED_{50} of >10 μM.

Table 1. ED_{50} values for primary amino alcohol dipeptides



Compd	R^1	R^2	ED_{50} (μM)
4a	H	(*S*)-*n*-Bu	0.46
4b	OH	(*S*)-*n*-Bu	0.60
4c	H	(*S*)-C_6H_5	0.18
4d	H	(*R*)-*n*-Bu	>29
4e	OH	(*R*)-*n*-Bu	24.5
4f	H	(*R*)-C_6H_5	>10
4g	H	(*S*)-$CH_2C_6H_{11}$	0.80
4h	OH	(*S*)-$CH_2C_6H_{11}$	0.20
4i	H	(*S*)-$CH_2C{\equiv}CH$	10.0
4j	OH	(*S*)-$CH_2C{\equiv}CH$	2.30
4k	H	(*S*)-CH_2Ph	2.70
4l	OH	(*S*)-CH_2CH_2Ph	0.27
4m	H	(*S*)-$CH_2CH_2CH_2Ph$	0.87
4n	OH	(*S*)-$CH_2CH_2CH_2Ph$	0.13
4o	H	(*S*)-$CH_2CH_2SCH_3$	0.49
4p	OH	(*S*)-$CH_2CH_2SCH_3$	0.96

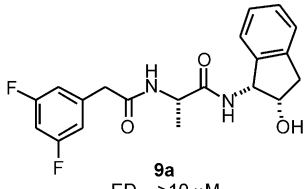


7
ED_{50} >10 μM

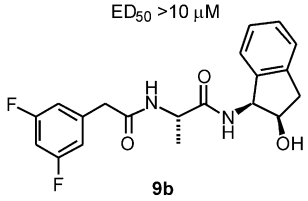
With the exception of compounds **4a** and **4o**, N-terminal (*S*)-3,5-difluoromandelic acid derivatives generally exhibited higher potency than the 3,5-difluorophenylacetic acid derivatives. The most active compound in the series was the aminophenylpentanol derivative, **4n**. It was observed that within the mandelic acid series of compounds (**4b**, **h**, **j**, **l**, **n**, and **p**), increasing cellular activity corresponded roughly with increasing cLog *P* (Table 2).[7]

The effect of substitution at C1 of the amino alcohol was examined with the compounds listed in Table 3. These secondary alcohols have greatly reduced potency compared to the primary alcohols. Three sets of diastereomeric pairs were prepared. The ED_{50} values obtained show a slight preference for the (2*S*,1*R*) analogues over the (2*R*,1*S*) analogues (cf. **8e** with **8f**). Alkylation to form the *N*-methyl amides, **8c** and **8d**, offered no improvement in activity.

We also prepared a diastereomeric pair of amino indanol based derivatives, **9a** and **9b**. As with other secondary alcohols, the (2*S*,1*R*) configuration was preferred. The ED_{50} of **9b** is comparable to that of the primary amino alcohols that were examined. The enhanced activity of this compound compared to other secondary alcohols may be due to the restricted conformation imposed by the indanol ring that places the hydroxyl group in a favorable position.

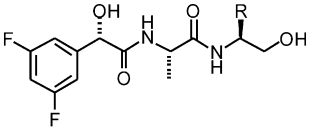


9a
ED_{50} >10 μM



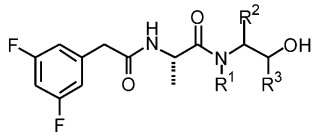
9b
ED_{50} 0.63 μM

Table 2. Comparison of ED_{50} and cLog *P* values



Compd	R	ED_{50} (μM)	cLog *P*
4b	(*S*)-*n*-Bu	0.60	1.25
4h	(*S*)-$CH_2C_6H_{11}$	0.20	0.91
4j	(*S*)-$CH_2C{\equiv}CH$	2.30	0.08
4l	(*S*)-CH_2CH_2Ph	0.27	2.02
4n	(*S*)-$CH_2CH_2CH_2Ph$	0.13	2.44
4p	(*S*)-$CH_2CH_2SCH_3$	0.96	0.26

Table 3. ED_{50} values for secondary amino alcohol dipeptides



Compd	R^1	R^2	R^3	ED_{50} (μM)
8a	H	(*R*)-Me	(*S*)-Ph	>10
8b	H	(*S*)-Me	(*R*)-Ph	>10
8c	Me	(*R*)-Me	(*R*)-Ph	>10
8d	Me	(*S*)-Me	(*S*)-Ph	>10
8e	H	(*R*)-Ph	(*S*)-Ph	>10
8f	H	(*S*)-Ph	(*R*)-Ph	3.5
8g	H	(*S*)-CH_2Ph	(*R*)-$CH_2CO_2CH_3$	>10

In conclusion, we have prepared a novel series of potent compounds for inhibiting Aβ. The majority of the primary amino alcohols showed sub-micromolar activity in vitro with compound **4n** being the most active. Secondary acyclic amino alcohols were prepared which exhibited micromolar activity. This activity could be improved with the formation of cyclic amino alcohol derivatives of specified configuration.

References and Notes

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4. Compounds **4e** and **4f** were similarly prepared from their commercially available amino alcohols.

5. Enantiomerically pure (*S*)-3,5-difluoromandelic acid was obtained by optical resolution of racemic 3,5-difluoromandelic acid (Oakwood Products, Inc.) with (1*R*,2*R*)-(−)-pseudoephedrine in isopropanol.

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