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# Inhibitors of Aβ Production: Solid-Phase Synthesis and SAR of α-Hydroxycarbonyl Derivatives

Owen B. Wallace,* David W. Smith, Milind S. Deshpande, Craig Polson and Kevin M. Felsenstein

*Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

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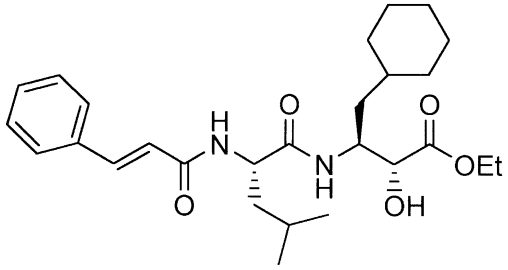
**Abstract**—Inhibitors of amyloid-β (Aβ) protein production have been widely pursued as a potential treatment for Alzheimer's disease. Following the identification of a 5 μM screening hit, SAR was initiated using solid-phase synthetic techniques. Two series of α-hydroxy esters and ketones which are sub-micromolar inhibitors of Aβ production were identified. The most potent α-hydroxyketone identified is approximately 30-fold more potent than the initial lead.


Alzheimer's disease (AD) is a progressive, neurodegenerative disorder characterised by the accumulation of neurofibrillary tangles and senile plaques in the brain. The tangles are composed of helical filaments of the hyperphosphorylated microtubule-associated protein tau, while the plaques consist of a 40–42 amino acid peptide known as amyloid-β protein (Aβ). There is a growing body of evidence which implicates Aβ as a causative agent of AD.[1,2] Aβ is formed by the action of two proteases on amyloid precursor protein (APP)—β-secretase at the N-terminus and γ-secretase at the C-terminus.[3,4] Proteolysis by another enzyme, α-secretase, may also occur in the middle of the Aβ peptide region of APP and precludes the formation of Aβ.[5] It has been proposed that inhibition of either β- or γ-secretase may lead to lower levels of Aβ in the brain, resulting in decreased plaque formation. Recently disclosed inhibitors of γ-secretase have included fenchylamine sulfonamides,[6] difluoro ketones[7] and orally active phenylglycine derivatives.[8] More potent hydroxyethyl[9] and hydroxyethyl urea[10] peptidomimetics have also been reported. We herein report the discovery of an α-hydroxyester derivative as an inhibitor of Aβ production in vitro and the optimisation of this lead using solid-phase synthesis.

Following a high throughput screening effort, cyclohexyl norstatine derivative **1** (Fig. 1) was identified as a weak inhibitor (5 μM) of Aβ production in a cell based assay.[11] Interestingly, ester **1** is structurally related to a series of dipeptide aldehydes identified as inhibitors of Aβ production using combinatorial chemistry.[12] In an effort to improve the potency of **1** and to probe the binding pockets of γ-secretase, a solid-phase synthesis of analogues was undertaken. Since the cyclohexylmethyl moiety is a common feature of several inhibitors of Aβ production,[13] we chose to fix this group at $P_1$ in order to expedite the synthesis. Our initial focus was to explore limited modifications at $P_2$ using four hydrophobic amino acids, and more extensive modifications at $P_3$ with the goal of replacing the potential Michael acceptor cinnamoyl capping group. Finally, we wished to replace the hydrolytically unstable ester while exploring SAR at $P_{1'}$.

Fmoc cyclohexylnorstatine ethyl ester **I** (Scheme 1) was prepared as described previously.[14] This was then coupled




**1**

**Figure 1.** Structure of Aβ inhibitor screening hit.

*Corresponding author at current address: Eli Lilly & Company, Indianapolis, IN 46285, USA. Fax: +1-317-433-0715; e-mail: owen.wallace@lilly.com

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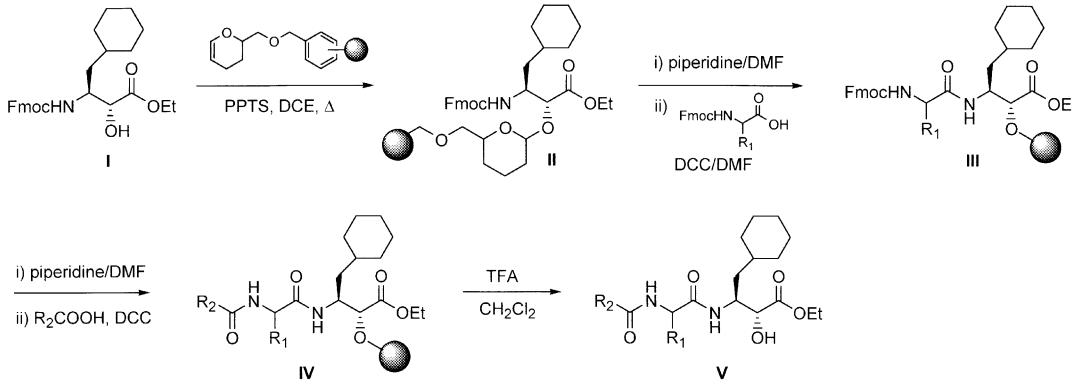


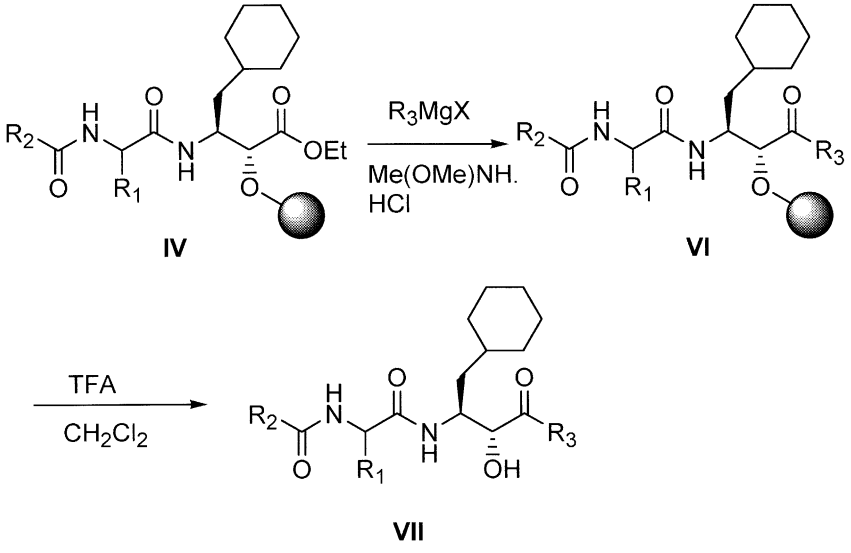

**Scheme 1.**

to polystyrene resin via Ellman's DHP linker[15] to afford resin-bound ester **II**. Removal of the Fmoc protecting group (20% piperidine/DMF) followed by DCC-promoted coupling with an Fmoc-protected amino acid (Val, D-Val, Leu and Phe) afforded ester **III**. Deprotection of the nitrogen followed by coupling with a diverse set of 24 aliphatic and aromatic carboxylic acids[16] afforded esters **IV** which were subsequently liberated from the resin (30% TFA/$CH_2Cl_2$) to yield α-hydroxyesters **V**.[17,18]

α-Hydroxyketones **VII** were synthesised from resin bound ester **IV** (Scheme 2) using a one-pot procedure previously developed in our laboratory.[19] Treatment of ester **IV** with *N,O*-dimethylhydroxylamine hydrochloride and excess Grignard reagent generated ketones **VI** which were removed from the polymer support under standard conditions to afford ketones **VII**.

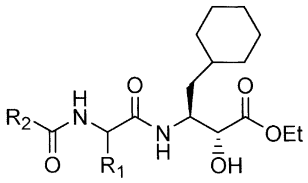
Compounds were evaluated using a cell-based ELISA assay.[11] Of the 24 aliphatic and aromatic $P_3$ substituents examined at the N-terminus (i.e., $R_2$), most resulted in decreased potency over our initial lead (**1**). Phenylacetyl derivative **2** (Table 1) did show some promise, however, and offered a modest gain in potency ($IC_{50}$ 3.4 μM). Introduction of an α-carbonyl was tolerated (example **3**) but α-alkyl substitution (example **4**) significantly decreased activity, as did replacement of the benzyl moiety with a phenyl ring (example **5**). Inverting the stereochemistry at $P_2$ (i.e., Val → D-Val) completely abrogated activity (example **6**). Replacement of the $P_2$ amino acid with phenylalanine also decreased cellular activity (example **7**), however replacement of the $P_2$ *iso*-propyl with *iso*-butyl was more rewarding. Example **8**, with a phenylacetyl unit at $P_3$ and *iso*-butyl at $P_2$, was a 1.6 μM inhibitor of Aβ production—3-fold more potent than the original lead. In general, aliphatic capping groups at $P_3$ resulted in weakly active compounds, with the exception being 4-methylpentanoic acid derivative **9** ($IC_{50}$ 1.5 μM).

Having identified a suitable surrogate for the potentially reactive $P_3$ cinnamoyl group of our screening lead, we now turned our attention to substitution of the phenyl ring of ester **8** using the methodology described in Scheme 1. A variety of substituents (F, Cl, Br, Me and OMe) were examined at the *ortho*, *meta* and *para* positions. A considerable decrease in potency was evident with ortho substitution (e.g., example **10** vs example **2**)[20] and, while substitution at the *para* position did not significantly decrease potency (e.g., example **11** versus example **8**), it failed to provide analogues with significantly increased activity. The meta position was




**Scheme 2.**

**Table 1.** Structures and in vitro activity of α-hydroxyester-based inhibitors of Aβ production



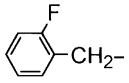
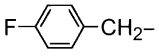
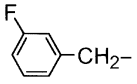
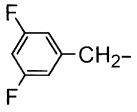
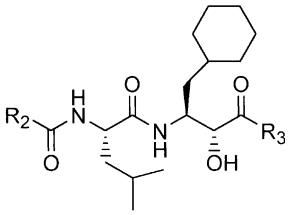
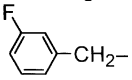
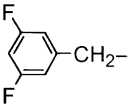
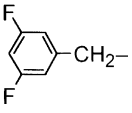
| Example # | $R_1$ | $R_2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **2** | (*S*)-*i*-Pr | $PhCH_2$– | 3.4 |
| **3** | (*S*)-*i*-Pr | PhCO– | 3.3 |
| **4** | (*S*)-*i*-Pr |  | >25 |
| **5** | (*S*)-*i*-Pr | Ph– | >25 |
| **6** | (*R*)-*i*-Pr | $PhCH_2$– | >25 |
| **7** | (*S*)-$CH_2Ph$ | $PhCH_2$– | 15.0 |
| **8** | (*S*)-*i*-Bu | $PhCH_2$– | 1.6 |
| **9** | (*S*)-*i*-Bu | $(CH_3)_2CHCH_2CH_2$– | 1.5 |
| **10** | (*S*)-*i*-Pr |  | 17.0 |
| **11** | (*S*)-*i*-Bu |  | 1.8 |
| **12** | (*S*)-*i*-Bu |  | 0.92 |
| **13** | (*S*)-*i*-Bu |  | 0.45 |

**Table 2.** Structures and in vitro activity of α-hydroxyketone and α-hydroxyacid-based inhibitors of Aβ production



| Example # | $R_2$ | $R_3$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **14** | $PhCH_2$– | –OH | 15.0 |
| **15** | $(CH_3)_2CHCH_2CH_2$– | $-CH_3$ | 0.62 |
| **16** | $(CH_3)_2CHCH_2CH_2$– | –*n*-Pr | 0.58 |
| **17** | $(CH_3)_2CHCH_2CH_2$– | –*I*-Pr | 0.55 |
| **18** | $(CH_3)_2CHCH_2CH_2$– | –Ph | 4.50 |
| **19** | $(CH_3)_2CHCH_2CH_2$– | $-CH_2Ph$ | 0.30 |
| **20** | $PhCH_2$– | $-CH_3$ | 0.90 |
| **21** | $PhCH_2$– | –*n*-Pr | 0.60 |
| **22** | $PhCH_2$– | –Ph | 0.90 |
| **23** | $PhCH_2$– | $-CH_2Ph$ | 0.40 |
| **24** |  | $-CH_2Ph$ | 0.40 |
| **25** |  | $-CH_3$ | 0.18 |
| **26** |  | $-CH_2Ph$ | 0.16 |

more tolerant to substitution, particularly with halogens. The *meta*-fluoro derivative **12** showed increased potency compared to its parent **8**, thus affording our first sub-micromolar inhibitor of Aβ production in this series. Gratifyingly, the effects of *meta*-fluoro substitution were additive as exemplified by 3,5-difluorophenyl derivative **13** which is a 0.45 μM inhibitor. Interestingly, the 3,5-difluorophenylacetyl substituent is a prominent feature of a recently disclosed phenylglycine-based γ-secretase inhibitor,[8] and amino alcohol dipeptide Aβ inhibitors.[21]

Our focus now turned to exploration of the $S_{1'}$ binding pocket. Hydrolysis of ester **8** (LiOH) yielded acid **14**, which displayed very weak inhibition of Aβ production in the cell-based assay (Table 2). However, the predicted poor cellular permeability because of the polar carboxylic acid functionality may be a contributing factor to the weak activity.

In order to decrease the peptidic nature of the compounds synthesised and potentially increase their hydrolytic stability, we chose to prepare ketones at $P_{1'}$ rather than concentrate on esters or amides.[22] Using the optimal substituents at $P_3$ (i.e., 4-methylpentanoyl and phenylacetyl) and at $P_2$ (*iso*-butyl) identified from our initial library, we synthesised a series of aliphatic and aromatic ketones at $P_{1'}$ (Table 2). In general, ketones are more potent than the corresponding ethyl esters. In the 4-methylpentanoyl series, methyl ketone **15** is greater than 2-fold more potent that ethyl ester **9**. Homologation of the ketone (example **16**) or introduction of branching (example **17**) failed to significantly increase activity further. Phenyl ketone **18** was considerably less potent than its aliphatic congeners. Rewardingly, benzyl ketone **19** yielded the most potent compound prepared in this series ($IC_{50}$ 0.30 μM).

In the phenylacetamide series, the benzyl ketone (**23**) is likewise more potent than its aliphatic ketone (**20**, **21**) or phenyl ketone (**22**) analogues. Somewhat surprisingly, the introduction of a single *meta*-fluoro substituent on the $P_3$ phenyl ring failed to increase potency as expected (example **24**). Introduction of two *meta*-fluoros, however, did result in a 3-fold increase in potency and afforded a 180 nM inhibitor of Aβ production in the cellular assay (example **25**). The benzyl ketone analogue **26** also displayed sub-200 nM potency ($IC_{50}$ 160 nM).

In summary, starting with ester **1** as a lead structure, a series of α-hydroxy esters has been prepared with improved potency with respect to lowering Aβ production in vitro. The most potent ester in this series is approximately 10-fold more potent than the initial 5 μM screening hit. The synthesis of ketones at $P_{1'}$ resulted in a general increase in activity and concomitantly eliminated the hydrolytically unstable ester bond. Benzyl ketone **26** is approximately 30-fold more potent than the original

screening hit. Since α-hydroxy carbonyl compounds have been previously shown to be inhibitors of aspartyl proteases,[23] the discovery of the inhibitors disclosed herein may add further evidence that an aspartyl protease mechanism is involved in γ-secretase activity.[24]

## Acknowledgements

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16. The 24 carboxylic acids used were: 1-phenyl-1-cyclopropanecarboxylic acid; 2,2-dichloro-1-methyl-cyclopropanecarboxylic acid; 2,4,5-trimethoxy-alpha-methylcinnamic acid; 2-oxo-6-pentyl-2*H*-pyran-3-carboxylic acid; 3-(3-methoxyphenyl)propionic acid; 3-(phenylsulfonyl)propionic acid; 3,4-(methylenedioxy)-phenylacetic acid; 3,5-bis(trifluoromethyl)phenylacetic acid; 3,5-difluorophenylacetic acid; 3-phenoxyphenyl-acetic acid; 4′-ethyl-4-biphenylcarboxylic acid; 4-isopropoxybenzoic acid; 4-methyl-pentanoic acid; 4-*n*-heptyloxybenzoic acid; 4-nitrobenzoic acid; 7-methoxy-coumarin-4-acetic acid; α-acetamidocinnamic acid; α-cyclopentylphenylacetic acid; benzoic acid; benzoylformic acid; mono-methyl *cis*-5-norbornene-endo-2,3-dicarboxylate; *N,N*-diethyl-3,6-difluorophthalamic acid; phenylacetic acid and *trans*-2,6-difluorocinnamic acid.
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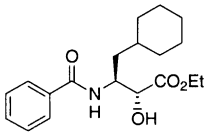


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