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Hydroxytriamides as potent γ-secretase inhibitors

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Abstract—Using a cell-based assay, we have identified optimal residues and key recognition elements necessary for inhibition of γ-secretase. An (*S*)-hydroxy group or 3,5-difluorophenylacetyl group at the amino terminus and *N*-methyltertiary amide moiety at the carboxy terminus provided potent γ-secretase inhibitors with an $IC_{50} < 10$ nM.

Alzheimer's disease (AD) is a chronic neurodegenerative disorder affecting the elderly. The disease is associated with dementia, loss of neurons, reduced levels of several major neurotransmitters, senile plaques and neurofibrillary tangles.[1] To date all therapeutic approaches have relied on neurotransmitter replacement therapies. Although these treatments have provided modest palliative improvement, the underlying disease progression remains unchecked. Recent genetic evidence strongly implicates abnormal proteolytic processing of β-amyloid precursor protein (β-APP) to Aβ40 and elevated amounts of Aβ42 in amyloid plaque formation.[2] Amyloid deposition is one of the major pathological hallmarks of Alzheimer's disease and therapeutic approaches that modulate abnormal processing of APP may delay the onset and progression of the disease.[3] Aβ40 and Aβ42 are generated through the combined action of β- and γ-secretases. Recently, β-secretase has been cloned and recombinant protein isolated in pure form. γ-Secretase activity has been isolated in association with the presenilin complex.[4] Cellular assays to measure Aβ40 and Aβ42 levels have also been described.[5] These assays have permitted the discovery of several selective small-molecule inhibitors (**A**, **B**, **C**, **D** and **E**) of Aβ production that inhibit γ-secretase activity (Fig. 1).[6,7] A substrate-based difluoro ketone **F** was also developed as a selective inhibitor of γ-secretase.[8]

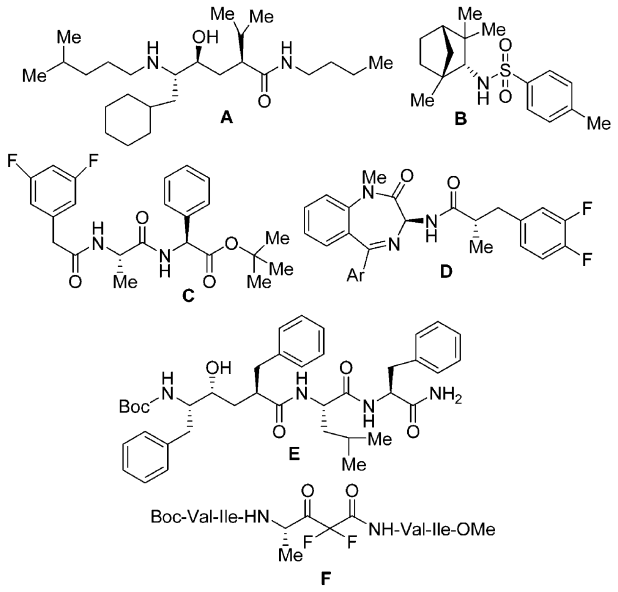

Figure 1. Known inhibitors of γ-secretase.

Keywords: Hydroxytriamides; γ-Secretase.
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Herein we report the SAR within a novel series of γ-secretase inhibitors evaluated in a cell-based assay measuring Aβ production.[9] Screening of our corporate compound collection provided the hydroxyethylene isostere **1** ($IC_{50}=4.7$ μM) as a lead compound that inhibited Aβ production in tissue culture cells. The lead compound and subsequent analogues exhibited a profile consistent with inhibition of γ-secretase. Cells treated with the compounds produced dose dependent decreases in Aβ levels and concomitant increases in 9 and 11 kDa membrane bound substrates of γ-secretase. A cell free assay that specifically detects γ-secretase cleavage of membrane bound precursors was used to profile select compounds in the series and show that γ-secretase is specifically inhibited.[10–13]

Truncation of both the amino- and carboxy-termini of **1** afforded ketone **2** ($IC_{50}=300$ nM) with improved potency. Treatment of Weinreb amide **3** with *iso*-pentylmagnesium bromide afforded ketone **2** in 50% yield. Surprisingly, amide **3** ($IC_{50}=130$ nM) was found to be more potent than either ketone **2** or alcohol **4** ($IC_{50}=1200$ nM) respectively. Incorporation of structural features from the inhibitors **2** and **3** led to the design and synthesis of *N*-methylbutyl amide **5** (Fig. 2). Synthesis of the triamide is readily achieved through peptide coupling of *N*-(*t*-butoxycarbonyl)-amino acids with *N*-methylbutyl amine followed by trifluoroacetic acid (TFA) mediated deprotection.[14] For example, treatment of *N*-(*t*-butoxycarbonyl)-cyclohexylalanine (Boc-Cha) **6** with ethyl chloroformate and diisopropylethylamine afforded the mixed anhydride which was subsequently reacted with *N*-methylbutylamine (Scheme 1). The resulting amide was treated with TFA to afford the amine salt **7**. Reaction of *N*-(*t*-butoxycarbonyl)leucine under mixed anhydride coupling conditions (diisopropylethylamine and ethyl chloroformate) with amine salt **7** afforded the *N*-Boc dipeptide which was subsequently deprotected via treatment with TFA affording amine salt **8**. Triamide **5** was obtained in 55% overall yield by PyBop-mediated coupling of aminodipeptide **8** with phenylacetic acid.

Analogue **5** proved to be a potent γ-secretase inhibitor ($IC_{50}=17$ nM). Encouraged by the potency of **5**, we elected to assess the effect of various secondary and tertiary C-terminal amides on γ-secretase inhibition (Table 1). Within the *N*-phenylacetylLeu-Cha-R series of compounds, tertiary amides were consistently more potent than the secondary amides (compound **5** and **9**). These tertiary amides likely possess enhanced cellular penetration as a result of fewer solvated amide bonds.[15] The conformational constraint imposed by the *N*-methyl group also may play a role in influencing the potency as the proton NMR spectrum of **5** reveals *cis–trans* isomerism about the Cha-R bond. Among the tertiary amides assayed, *n*-butyl and *iso*-butyl moieties were optimal (compound **5** and **10**). In contrast, the methyl and ethyl esters displayed only modest potencies (**16** and **17**).

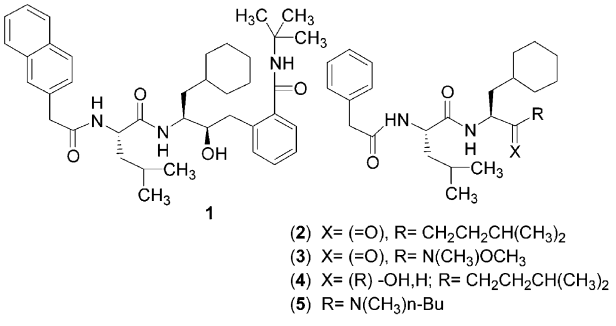
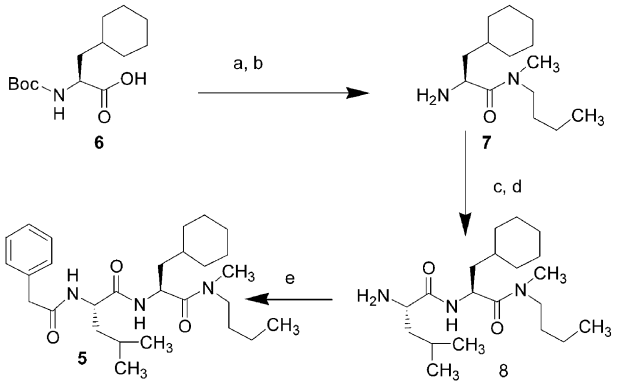
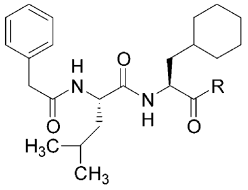


Figure 2. Lead compound and analogues modified.



Scheme 1. Reagents and conditions: (a) EtOC(O)Cl, EtN(*i*-Pr)$_2$, CH_2Cl_2, then NH(Me)*n*-Bu (90%); (b) 20% CF_3CO_2H in CH_2Cl_2 (95%); (c) Boc leucine, EtOC(O)Cl, EtN(*i*-Pr)$_2$ (85%); (d) 20% CF_3CO_2H in CH_2Cl_2 (95%); (e) $PhCH_2CO_2H$, PyBop, EtN(*i*-Pr)$_2$ (80%).

Table 1. Structure–activity relationships at the carboxy terminus



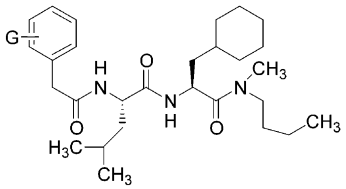
Compd	R	R_1	R_2	IC_{50} (nM)[a]
5	NR_1R_2	Me	$(CH_2)_3Me$	17
9	NR_1R_2	H	$(CH_2)_3Me$	240
10	NR_1R_2	Me	$CH_2CH(Me)_2$	13
11	NR_1R_2	H	$CH_2CH(Me)_2$	150
12	NR_1R_2	Me	CH_2Ph	39
13	NR_1R_2	Me	Me	102
14	NR_1R_2	Et	$(CH_2)_3Me$	55
15	piperidyl	na	na	50
16	OR_1	Me	na	800
17	OR_1	Me	na	900

na, not applicable.

[a] Values are means of two experiments with 16 data points in each experiment; intra-assay variance <10%.

Several strategies were employed to identify the optimal structural requirements at the amino terminus. A variety of substituents were introduced on the phenylacetyl ring to probe electrostatic effects (Table 2). In general, *meta*-halogens afforded greater potency than either *ortho*- or *para*-substitution. Introduction of an amino group or electron donating methoxy group provided less active compounds. The most potent compound in this series was obtained through the introduction of two

Table 2. Structure–activity relationships at the amino terminus



Compd	G	IC_{50} (nM)[a]
5	H	17
18	2-F	280
19	3-F	20
20	4-F	36
21	3-Cl	52
22	3-CH_3	454
23	3-OMe	480
24	3-NH_2	>2500
25	4-NH_2	1100
26	3,5-di-F	6
27	3,5-di-CF_3	408
28	3,5-di-OMe	800

[a] Values are means of two experiments with 16 data points in each experiment; intra-assay variance <10%.

meta-fluorine atoms on the aromatic ring. This SAR finding is consistent with other recently disclosed functional γ-secretase inhibitors.[7]

Removal of the methylene group tethering the phenyl ring to the amide carbonyl (Fig. 3, compound **29**) dramatically attenuated activity. Insertion of an additional methylene group (compound **30**) resulted in a 10-fold loss in potency. Furthermore, introduction of an unsaturated bond in **30** led to a dramatic loss of potency (compound **31**). Clearly the methylene group was playing a pivotal role in the inhibitory profile of these compounds. Hydroxylated compounds **32** and **33** were synthesized to investigate the impact of hydrogen bonding and stereospecificity (Table 3). Introduction of a (*S*)-hydroxyl moiety (compound **32**) resulted in a significant enhancement in potency (IC_{50} = 1 nM). The ketoamide **34**, which is expected to be in the hydrate form also was found to be very potent (IC_{50} = 1 nM). To investigate the role of the hydroxyl group further, the methoxy (**35** and **36**), substituted methoxy (**37** and **38**) and substituted hydroxy (**39**) analogues were prepared.

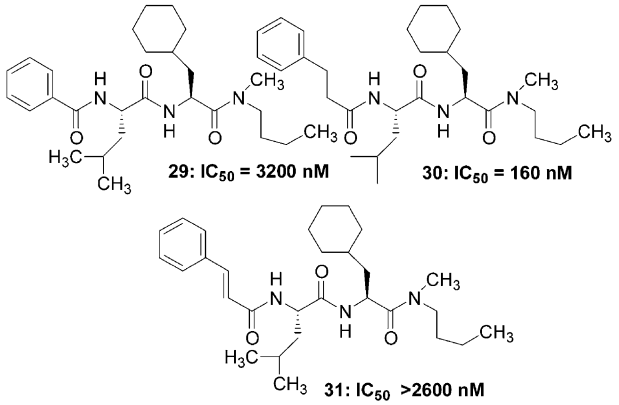
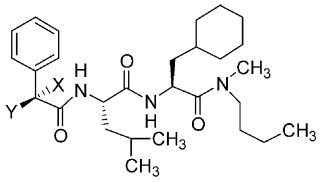


Figure 3. Modifications at the amino terminus.

Table 3. Substitution at the benzylic position



Compd	X	Y	IC_{50} (nM)[a]
32	H	OH	1
33	OH	H	240
34	O	O	1
35	H	OMe	2000
36	OMe	H	>2500
37	Me	OMe	>2500
38	OMe	Me	>2500
39	Me	OH	>2500

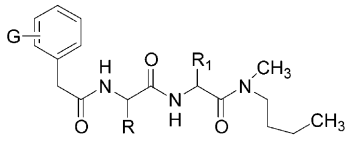
[a] Values are means of two experiments with 16 data points in each experiment; intra-assay variance <10%.

As revealed in Table 3, methylation of the hydroxy group or substitution on the carbon carrying the hydroxy group was not tolerated.

It is conceivable that compounds of the triamide class represented by **5** are undergoing oxidation to give hydroxylated compounds such as **32** under the conditions of the cell-based assay. To address the possibility of cellular oxidation of these compounds, cells treated with compound for 18 h were lyopholized, extracted with acetonitrile, and analyzed by HPLC as well as mass spectrometry. These analytical methods were unable to detect the presence of hydroxy analogues **31**, **32** or keto compound **33** in the cellular extracts.

To identify optimal substituents appended to the internal amino acid residues, a variety of amino acid substitutions were explored (Table 4). Cyclohexylalanine in compound **5** was replaced with basic, acidic, and neutral amino acids. The hydrophobic nature of the recognition subsite was evident from the reduced potency of

Table 4. Structure–activity relationships at the sub-sites



Compd	G	R	R_1	IC_{50} (nM)[a]
40	3,5-di-F	(*S*)-Leu	(*S*)-Asp	na
41	3,5-di-F	(*S*)-Leu	(*S*)-Lys	na
5	H	(*S*)-Leu	(*S*)-Cyclohexylalanine	17
42	H	(*S*)-Leu	(*R*)-Cyclohexylalanine	1000
26	3,5-di-F	(*S*)-Leu	(*S*)-Cyclohexylalanine	6
43	3,5-di-F	(*S*)-Ala	(*S*)-Cyclohexylalanine	32
44	3,5-di-F	(*S*)-Gly	(*S*)-Cyclohexylalanine	300
45	3,5-di-F	(*S*)-Phe	(*S*)-Cyclohexylalanine	2400
46	3,5-di-F	(*S*)-Pro	(*S*)-Cyclohexylalanine	na
47	3,5-di-F	(*R*)-Leu	(*S*)-Cyclohexylalanine	na

na = not active; IC_{50} >2500 nM.

[a] Values are means of two experiments, with 16 data points in each experiment; intra-assay variance <10%.

compounds **40** and **41** compared with the more potent compounds **26** and **43**. A similar SAR emerged when small (**44**), medium (**43**) and large (**45**) hydrophobic residues were introduced in place of leucine. It was clear from the inhibition data that leucine and alanine were the preferred residues among those assayed. The stereochemical requirements at both subsites were investigated by incorporating *R*-amino acids. The strong preference for *S*-stereochemistry was evident from poor activity displayed by compounds **42** and **47**.

Incorporation of constrained amino acids was conducted to define key interactions with the enzyme. The inhibition data for the inhibitors **48–52** against γ-secretase is presented in Figure 4. Pipecolic acid at the amino terminus resulted in a dramatic loss of affinity (**48**: IC_{50} >2500 nM). On the other hand, introduction of (*S*)-tetrahydroisoquinoline-3-carboxylic acid (Tic) at the carboxy terminus in compound **49** afforded moderate inhibition (IC_{50} = 66 nM). Replacement of either the C-terminal carbonyl or the central carbonyl with a methylene moiety lowered activity (compound **50** and **51**). However, the activity could be restored by the incorporation of a β-sheet mimetic such as *cis*-aminoindanol (compound **52**).[16]

Although there are potential limitations in the use of a cell-based assay to determine subtle enzyme–inhibitor interactions, the relative potency of compounds within this series provided direction for synthesis of compounds that consistently demonstrated γ-secretase cleavage inhibition. The situation is more complex for the demonstration of in vivo efficacy, where many other parameters can influence the outcome. Oral administration of a single 200 μmol/kg dose to Tg2576 βAPP-Swedish transgenic mice[17] of compound **32** resulted in little (15%) or no reduction of Aβ levels in the CNS. This single dose of 200 μmol/kg gave a concentration of 1833 ± 196 nM in the plasma, but only 65 ± 7 nM in the brain three h after dosing. The lack of significant activity in the brain is likely due to its inadequate CNS penetration.

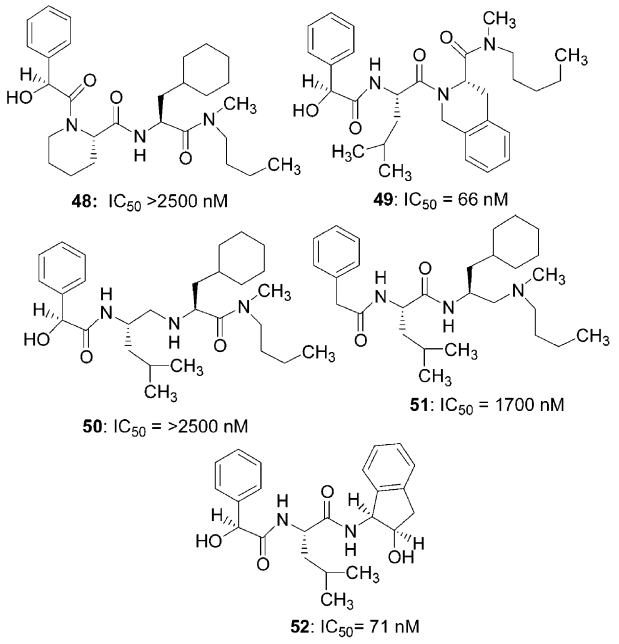


Figure 4. Constrained inhibitors of γ-secretase.

In summary we have explored the SAR of a series of γ-secretase inhibitors exemplified by compound **5** and its analogues. We have identified two moieties for optimal γ-secretase inhibition. An hydroxy group with (*S*)-stereochemistry or a keto group is optimal at the amino terminus. A *N*-alkyl tertiary amide functionality at the carboxy terminus is optimal for potency in this series.[18] Medium and large hydrophobic groups are preferred at the central hydrophobic residues. Compounds **6**, **32** and **34** are the most potent γ-secretase inhibitors identified so far in this series. Investigations are underway in our laboratories to improve the pharmacokinetic properties of these hydroxy triamides. Compounds such as hydroxy amides with good pharmacokinetic profiles will be required to determine the role of amyloid in Alzheimer's disease and to evaluate γ-secretase as a target for therapeutic intervention.

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18. All compounds gave satisfactory spectroscopic data consistent with the proposed structures. Data for **5**: IR (KBr): 3294, 3065, 2925, 2870, 1631, 1553, 1495, 1449, 1367, 1254, 696 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4–7.2 (m, 5H), 6.6–6.4 (m, 2H), 5.85–5.75 (m, 1H), 5.0–4.9 (m, 1H), 4.5–4.4 (m, 1H), 3.6 (s, 2H), 3.45–3.15 (m, 2H), 2.9 (m, 3H), 1.9–1.8 (m, 1H), 1.8–0.8 (m, 27H); MS (ES), 470 $(M\text{-}H)^-$; Data for **26**: IR (KBr): 3282, 3055, 2946, 2830, 1636, 1558, 1490, 1459, 1373, 1264, 694 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.84–6.78 (m, 3H), 6.73–6.66 (m, 1H), 6.44–6.37 (m, 1H), 4.97–4.92 (m, 1H), 4.53–4.46 (m, 1H), 3.51–3.39 (s, 2H), 3.33–3.20 (m, 2H), 3.02–2.90 (s, 3H), 1.89–0.86 (m, 29H); MS (ESI) 508 (M + H); Data for **32**: IR (film): 3285, 2924, 2852, 1679, 1630, 1519, 1449, 1277, 1061, 729, 696 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4–7.2 (m, 5H), 6.85–6.6 (m, 2H), 5.1 (s, 1H), 4.95–4.80 (m, 1H), 4.4–4.3 (m, 1H), 3.4–2.8 (m, 6H), 1.9–1.75 (m, 1H), 1.70–0.70 (m, 28H); MS (ES), 488 $(M + H)^+$.