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Molecular-modeling based design, synthesis, and activity of substituted piperidines as γ-secretase inhibitors

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Abstract—Alzheimer's disease (AD) is a debilitating disease widely thought to be associated with the accumulation of beta amyloid (Aβ) in the brain. Inhibition of γ-secretase, one of the enzymes responsible for Aβ production, may be a useful strategy for the treatment of AD. Described below is a series of γ-secretase inhibitors designed from a scaffold identified by a ROCS [*J. Comput. Chem.* **1996**, *17*, 1653] search of the corporate database.

Alzheimer's disease (AD) is a debilitating disease of the elderly, which ultimately leads to death. The disease is typically recognized by the gradual deterioration of mental ability; AD is the most common form of dementia—loss of memory—in the elderly.

AD is pathologically characterized by the presence of two major lesions in the brain: extracellular amyloid deposits of beta amyloid protein (Aβ) in the form of plaques and amyloid angiopathy; and intracellular neurofibrillary tangles of aggregated hyperphosphorylated tau protein.[2] Recent evidence suggests that elevated Aβ levels in the brain not only precede tau pathology but also correlate with cognitive decline.[3] Additional studies have also shown that aggregated Aβ is toxic to neurons in cell culture[4] and has a detrimental effect on memory.[5] Together, this evidence implicates Aβ in a causative role in AD and suggests that reducing Aβ levels is a viable therapeutic strategy for the treatment of AD.

Aβ is a 39–42 amino acid peptide that is produced from a larger precursor protein called amyloid precursor protein (APP) by the sequential action of β- and γ-secretase. Although rare, cases of early onset of AD have been attributed to genetic mutations in APP or in presenilins 1 and 2, components of the γ-secretase complex, that lead to an overproduction of either total Aβ or its more aggregation-prone 42 amino acid isoform.[6–8] Furthermore, people with Down's Syndrome possess an extra copy of the chromosome that contains the gene that encodes APP; these people have elevated Aβ levels and invariably develop AD later in life.[9]

A plausible strategy for the treatment of AD suggested from the above information is to inhibit Aβ production via the inhibition of γ-secretase. Several γ-secretase inhibitors have been designed based upon the amino acid sequence of the APP cleavage site.[10] Importantly, the γ-secretase inhibitor *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *tert*-butyl ester (DAPT) has recently been shown to reduce Aβ protein levels in mice brains in vivo after oral administration.[11] Herein is described a series of γ-secretase inhibitors designed by molecular modeling and exhibiting some promising activity in our Aβ40/Aβ42 assay.

In our efforts to uncover new leads for the γ-secretase program, we chose to use the ROCS[1] program to find new scaffolds for lead development. The ROCS program identifies molecules that have a similar

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three-dimensional shape. The similarity search requires the input of a compound query to make comparisons against a compound database. In our search, the degree of shape similarity is scored by a Tanimoto coefficient.

As a starting point for the ROCS search, we used sulfonamide **1** (see Fig. 1), a recently reported potent inhibitor of Aβ production.[12] A conformational search based on the Monte Carlo method in Macromodel[13] was performed on **1**. The MMFF94 force field[14–18] was used in the energy minimization steps. The low-energy conformer of **1** was used as the query. Using a Tanimoto coefficient cut-off of 0.7, the search of an in-house database provided us with about 500 hits; one of these hits was compound **2**.

The hits were then analyzed to see which of them contained the chemical features believed to be important for γ-secretase activity or which could be readily modified to contain those features. We have identified, in particular, three features thought to be important for γ-secretase inhibition: two hydrophobes about 4 Å apart and a hydrogen bond acceptor about 6–8 Å from the hydrophobes (see Fig. 2).

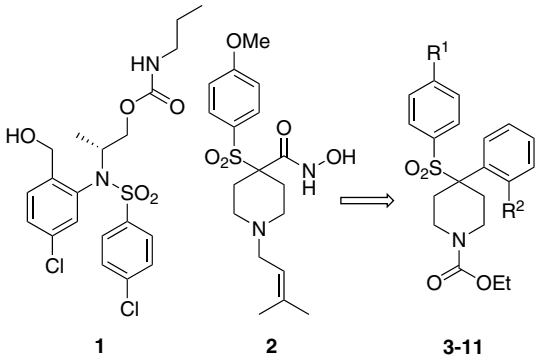


Figure 1. A ROCS search using the low-energy conformer of **1** identified hit **2** from the corporate database. Modification of hit **2** generated analogues **3**–**11**.

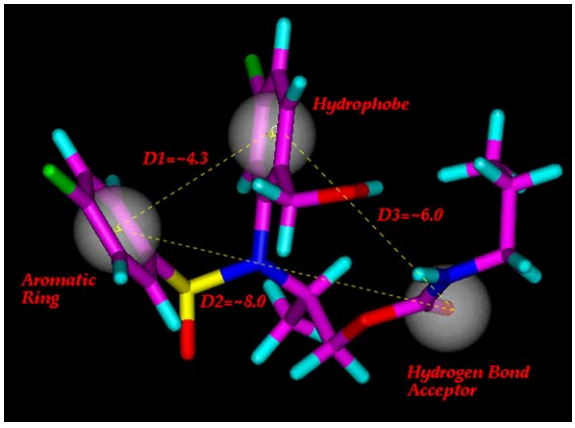


Figure 2. This figure depicts a schematic of the pharmacophore overlayed on compound **1**, shown as the low-energy conformer.

Overlap of the hydroxamic acid of **2** with one of the aromatic rings in **1** (see Fig. 3) suggested the replacement of the hydroxamic acid with a phenyl ring, satisfying the first requirement of our pharmacophore. Secondly, the replacement of the alkene moiety in **2** with a carbamate added the requisite hydrogen bond acceptor. These two modifications led us to compound **3**, which we also believed would be relatively straightforward to synthesize and would prove amenable to parallel synthesis if needed. Minimization of **3** with the MMFF94 force field and subsequent comparison with **1** (see Fig. 4) resulted in good overlap while maintaining the pharmacophore orientation and providing us with a potential new lead for our γ-secretase program.

While hit **2** was inactive in our Aβ40/Aβ42 assay, a sandwich ELISA employing 6E10 antibody for Aβ capture and Aβ40 or 42 C-terminal specific antibodies for

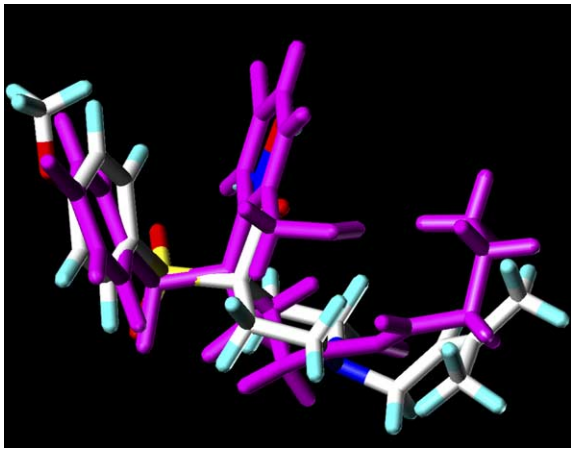


Figure 3. Overlay of compound **2** with compound **1** (magenta) generated from the ROCS software. Note the strong overlap of the aromatic rings and the tail portions. Also, note the close proximity of the hydroxamic acid to the aromatic hydrophobe of **1**.

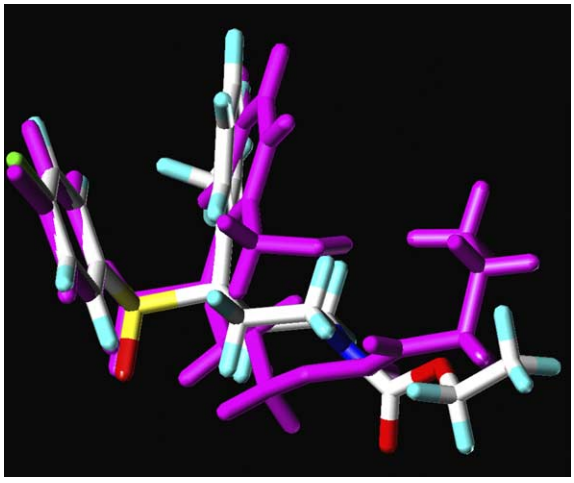


Figure 4. Overlay of compound **3** with compound **1** (magenta); these structures were minimized using the MMFF94 force field and then compared by mapping the aromatic regions, the sulfones, and the carbonyl oxygen of each molecule.

detection,[19] compound **3** gave good activity as an initial lead (see Table 1). This result prompted the synthesis of a small matrix of compounds to develop some SAR as well as to validate the chemistry for a future library. Eight additional compounds were prepared and their activities in the Aβ40/Aβ42 assay are presented in Table 1. The data suggests that there is both a steric and electronic component to the structure–activity relationship. Specifically, the data show that for R^1, Cl > OMe > H (see Table 1: compounds **3**, **6**, and **9**). Alternatively, the data show for R^2, that Me > OMe > H, (see Table 1: compounds **3**, **4**, and **5**).

Compound **3** was further tested in radiolabeled cellular assays employing either CHO cells stably expressing a wild-type recombinant human APP 695, or a human APP reporter construct containing the Swedish KM to NL mutation to determine the mechanism of action for the series.[21] The use of the wild-type construct gave an EC_{50} of 1.03 μM for Aβ reduction, while maintaining constant levels of APP and αAPPs, suggesting that the observed Aβ reduction was not caused by alterations in protein synthesis or secretion. Use of the Swedish mutant reporter construct gave an EC_{50} of 0.53 μM for Aβ reduction, an EC_{50} of 0.64 μM for elevation of the β C-terminal fragment (Ct-99) and a 3.8-fold increase in the level of Ct-99 over the control. Together the data confirms that the compound is acting via inhibition of γ-secretase.

Compounds **3**–**11** were prepared by a three-step sequence outlined in Scheme 1.[22] Addition of the appropriate substituted phenyl Grignard reagent to 1-carbethoxy-4-piperidone (**12**) afforded tertiary alcohols (**13**). Addition of the respective substituted thiol to a solution of the alcohol in ~88% formic acid gave the thioethers (**14**). Oxidation of the thioethers with oxone afforded the final products (**3**–**11**).

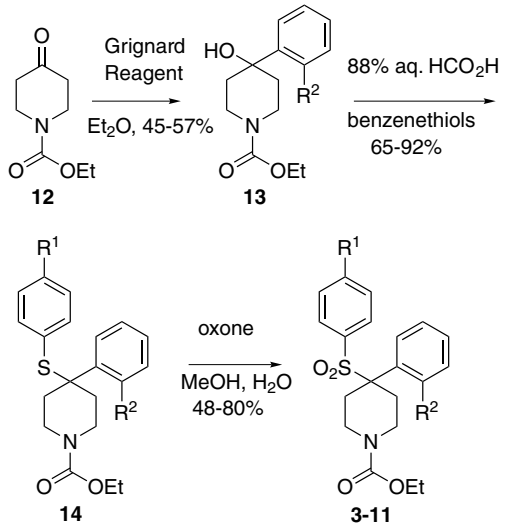
In summary a γ-secretase inhibitor was designed using a ROCS search; a small number of compounds were synthesized to expand the SAR of this series. Data suggest

Table 1. Summary of the in vitro inhibitory effects of compounds **3**–**11** on Aβ40 and Aβ42 in the ELISA assay[19]

Compound	R^1	R^2	EC_{50} (μM) Aβ40	EC_{50} (μM) Aβ42
1	—	—	0.1	<0.1
3	Cl	OMe	3.5	5.0
4	Cl	H	12.2	20.9
5[a]	Cl	Me	2.7	3.7
6	H	OMe	62.7	Increased[b]
7	H	H	84% at 100 μM	Increased[b]
8	H	Me	48.6	Increased[b]
9[a]	OMe	OMe	50.6	Increased[b]
10	OMe	H	97.0	Increased[b]
11	OMe	Me	24.5	Increased[b]

[a] Compound showed cell toxicity at 100 μM as measured using the MTS assay.[19]

[b] It is not uncommon to see increases in Aβ42 with weak inhibitors. See Ref. 20 for additional information.



Scheme 1. Synthetic scheme for the substituted piperidines.

that the compounds are acting via γ-secretase inhibition. However, activity exhibited by these compounds is only moderate. WO 02081435[23] describes a class of compounds similar to this series for the treatment of AD via inhibition of γ-secretase. Ultimately, our findings with this small library validated the ROCS search design strategy. Current efforts are directed to using this technique to identify more novel scaffolds.

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22. The synthesis of compound **10** described here serves as a representative example of the synthetic methodology used in this paper. (i) To a solution of 1-carbethoxy-4-piperidone (1.71 g, 10 mmol) in ether (20 mL) at 0 °C was added phenylmagnesium bromide (12 mL of a 1 M solution in ether). The mixture was stirred for 15 min then warmed to rt. After 2 h, the reaction mixture was quenched with saturated NH_4Cl (30 mL) then concentrated. The aqueous residue was extracted with EtOAc and the extracts washed with saturated NH_4Cl, and brine, dried over Na_2SO_4, filtered, concentrated, and dried to give a white solid (2.08 g), which was recrystallized from methyl-*tert*-butyl ether to give the desired product (1.21 g, 4.84 mmol) as a white solid, mp 154–156 °C. (ii) To a stirred solution of the alcohol (0.20 g, 0.80 mmol) from the previous step in 90% formic acid was added 4-methoxybenzenelthiol (0.12 g, 0.88 mmol). After 18 h, the reaction mixture was poured into saturated $NaHCO_3$ (50 mL) and extracted with EtOAc (60 mL). The EtOAc was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated to give a gum (0.296 g). The gum was purified by silica gel preparatory plate chromatography, developing with 20% EtOAc/hexane to give the desired product (0.25 g, 0.67 mmol) as a gum. (iii) To a solution of the gum from step 2 in methanol (4 mL) at 0 °C was added a solution of oxone (0.410 g, 0.67 mmol) in water (4 mL). After addition, the bath was removed and the reaction mixture stirred for 18 h. The reaction mixture was diluted with water (30 mL) and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give a viscous residue (0.180 g). The residue was purified by silica gel preparatory plate chromatography using 40% EtOAc/hexane, to give the desired product (0.14 g, 0.35 mmol) as a white solid, dec. 138–142 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (t, J = 7.1 Hz, 3H), 1.94–2.28 (m, 2H), 2.49–2.55 (m, 2H), 3.23–3.30 (m, 2H), 3.77 (s, 3H), 3.80–3.99 (m, 4H), 6.94 (d, J = 9.0 Hz, 2H), 7.13 (d, J = 9.0 Hz, 2H), 7.23 (dd, J = 1.2, 7.9 Hz, 2H), 7.27–7.35 (m, 3H); IR (solid) 2980, 1690, and 1500 cm^{-1}; [ES(+)], *m/z* 404 $(M+H)^+$; Anal. Calcd for $C_{21}H_{25}NO_5S$: C, 62.51; H, 6.24; N, 3.47 Found: C, 62.51; H, 5.96; N, 3.36.
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