Fenchylamine Sulfonamide Inhibitors of Amyloid β Peptide Production by the γ-Secretase Proteolytic Pathway: Potential Small-Molecule Therapeutic Agents for the Treatment of Alzheimer's Disease

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Introduction. The brain plaques associated with Alzheimer's disease (AD) are composed primarily of the amyloid β peptides (Aβ40,42) which are produced from the proteolytic processing of the β-amyloid precursor protein (APP).[1] The production of Aβ from APP proceeds via two cleavages which are catalyzed by distinct protease activities known as the secretases.[2] The cleavage of APP at the Aβ N-terminal residue is catalyzed by the recently cloned and characterized aspartic acid protease named β-secretase.[3] The Aβ C-terminal cleavage which occurs at the transmembrane region of APP is attributed to the action of the yet unknown protease(s) designated γ-secretase(s). In the brain of the AD patient, aggregates of Aβ peptides are deposited resulting in formation of the insoluble plaques and vascular deposits characteristic of AD pathology.[4] The overproduction of the relatively hydrophobic Aβ42 component has been particularly associated with plaque formation.[5] Genetic evidence suggests elevated brain levels of Aβ42 to be the cause of early-onset familial AD.[6]

Inhibition of the β- and γ-secretase proteolytic pathways would be expected to decrease the production of Aβ and potentially to slow the progression of AD. Cellular assays to measure inhibition of the overproduction of Aβ42 have recently been developed.[7] These assays have allowed the initiation of investigations toward the discovery of small-molecule inhibitors of Aβ production in cell culture. Among the reports of Aβ production inhibitors in the patent literature, the cyclohexylalanine-based statine **1**[8] and the lipophilic dimethylaminoethyl tetralin **2**[9] serve as examples of chemically stable small-molecule inhibitors of Aβ production in cell culture.

Inhibitors of Aβ Production in Cell Culture

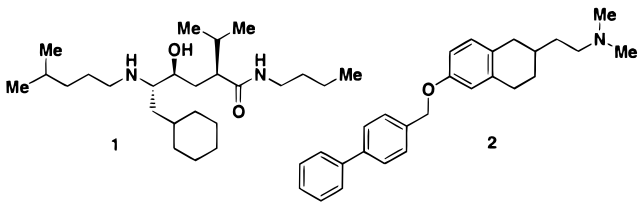

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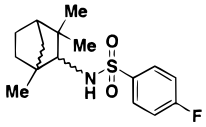
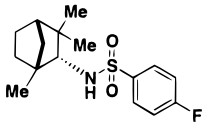
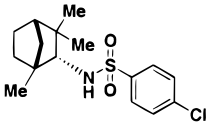
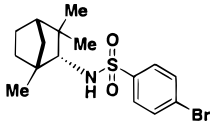
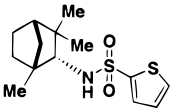
Table 1. Fenchylamine Sulfonamide γ-Secretase Inhibitors[a]

cmpd	structure	%inhibition[d]	Aβ42 IC_{50}(μM)	$\%APP_s$[e, 7]
3		31	5.0	20
4[b]		58	1.8 ± 0.4	23
5		26	11 ± 3	0
6		24	10 ± 4	0
7[c]		47	2.7 ± 0.2	32

[a] Elemental analyses obtained were within 0.4% of calculated values. Compound **4**: Anal. ($C_{16}H_{22}FNO_2S$) C, H, N, F, S. Compound **5**: Anal. ($C_{16}H_{22}ClNO_2S$) C, H, N, Cl, S. Compound **6**: Anal. ($C_{16}H_{22}BrNO_2S$) C, H, N, Br, S. Compound **7**: Anal. ($C_{14}H_{21}NO_2S_2$) C, H, N, S. [b] Compound **4**: a white solid; mp 128−129 °C; $[\alpha]_D^{20} = +33.7$ ($c = 0.05$, MeOH). [c] Compound **7**: a white solid; mp 138−139 °C; $[\alpha]_D^{20} = -43.6$ ($c = 0.05$, MeOH). [d] Selective inhibition of the production of Aβ42 by inhibition of the γ-secretase proteolytic pathway in HEK293 cells stably transfected with a double mutant form of human APP(K595N/M596L) at an inhibitor concentration of 2.5 μM (ref 7). Aβ40 was inhibited to a similar extent. Percent (%) inhibition values are the average of four experiments and IC_{50} values are the average of 4 experiments ± standard deviation. [e] The observed stimulation of the production of soluble β-amyloid precursor protein (APP_s) is consistent with the inhibition of the production of Aβ via the secretase pathways.

We report here the discovery of low-molecular-weight, chemically stable fenchylamine sulfonamide inhibitors of the production of Aβ in cell culture which operate via inhibition of the γ-secretase proteolytic pathway.

Results and Discussion. The stereochemically undefined fenchylamine sulfonamide sample (**3**) (Table 1) exhibited inhibition of Aβ42 production to the extent of 31% (@2.5 μM) with $IC_{50} = 5$ μM, and Aβ40 production was inhibited to a similar extent. Inhibition of Aβ production was accompanied by reduction of p3, a concomitant increase in β-secretase-cleaved APP C-terminal fragments, and no change in secreted $APP_s\beta$ (data not shown), but with an increase in secreted $APP_s\alpha$. These data indicate that the observed inhibition of Aβ production was not due to nonspecific toxicity effects, a general block in secretion, or inhibition of β-secretase cleavage but rather due to specific inhibition of the γ-secretase cleavage pathway.

Our subsequent analytical studies determined the inhibitor sample **3** to be a 3:1 mixture of stereoisomeric 4-fluorophenyl fenchylamine sulfonamides. We set out to identify the potent stereoisomer component of the

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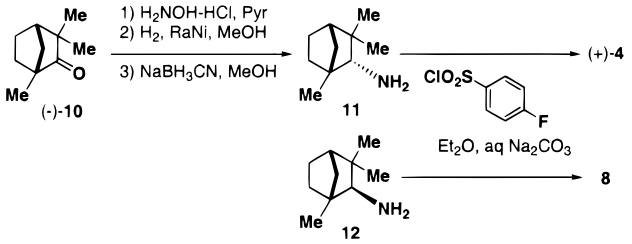
Scheme 1. Synthesis of Fenchylamine Sulfonamides from (1*R*)-(−)-Fenchone[a,b,10]

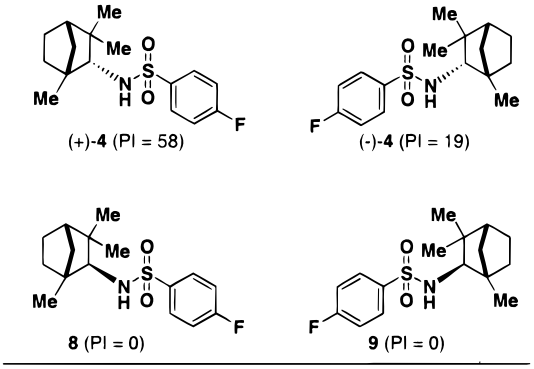


[a] The intermediate amines **11** and **12** were prepared according to a modification of the procedure of Girault (ref 10). (1*R*)-(−)- and (1*S*)-(+)-fenchone are commercially available from TCI. [b] The mixture of isomeric amines **11** and **12** was resolved by silica gel flash chromatography employing a mobile phase of hexane−EtOAc−NH_4OH (79:20:1). The lease polar *endo*-amine **11** eluted first.

mixture and then to utilize this information to develop a stereochemically defined series of γ-secretase inhibitors. Beginning from the commercially available enantiomers of fenchone (Scheme 1), we prepared all four stereoisomers and then determined the most potent inhibitor of the set to be the *endo*-isomer (+)-**4** which inhibited Aβ42 production by 58% (@2.5 μM) with $IC_{50} = 1.8\ \mu$M (Table 2). The enantiomeric *endo*-isomer (−)-**4** exhibited significantly less potency at 19% inhibition (Table 2). The *exo*-isomers **8** and **9** were each determined to be noninhibitory. The relative stereochemistry of (+)-**4** was unambiguously determined by X-ray crystallography. A stereochemical requirement had thus been established for this series of inhibitors of Aβ42 production.

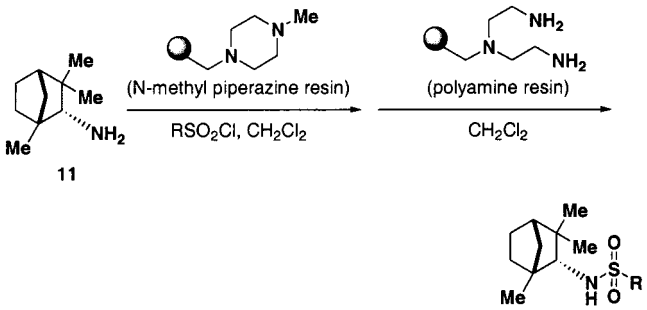
The structural requirements for inhibitory activity defined by the preceding studies guided our preparation of a stereochemically defined series of fenchylamine sulfonamide inhibitors based on the relative and absolute stereochemistry of the inhibitor (+)-**4**. The optically pure *endo*-fenchylamine **11** was readily prepared from the levorotatory enantiomer of fenchone (**10**) as depicted in (Scheme 1).[10] A mixture of the *endo*- and *exo*-fenchylamines **11** and **12**, respectively, was obtained by conversion of fenchone to its oxime followed by Raney nickel hydrogenation and subsequent sodium cyanoborohydride reduction of the intermediate imine. The mixture of amines **11** and **12** was resolved by flash chromatography to afford the desired *endo*-isomer **11** as the least polar component. Fenchylamine sulfonamides

Table 2. γ-Secretase Activity of the Stereoisomers of Inhibitor **4**[a]



[a] PI = % inhibition @ 2.5 μM; average of four experiments.

Scheme 2. Solution-Phase Parallel Synthesis of Fenchylamine Sulfonamide Library[a,11]



[a] The amine resins are commercially available from Novabiochem and were employed in a solution-phase parallel synthesis as previously described (ref 11).

were generally prepared from the amines **11** and **12** by reaction with various sulfonyl chlorides under the conditions provided for the synthesis of fenchylamine sulfonamides **4** and **8** (Scheme 1). The 4-chlorophenyl sulfonamide **5** and the 4-bromophenyl sulfonamide **6** established an initial structure−activity relationship for this class of inhibitors.

In an effort to prepare hundreds of fenchylamine sulfonamides derived from the optically pure *endo*-fenchylamine **11**, we initiated rapid analogue synthesis featuring a resin-bound amine catalyst and a resin-bound polyamine scavenging agent (Scheme 2).[11] A relatively potent and structurally distinct fenchylamine sulfonamide inhibitor of Aβ42 production was discovered as a result of this solution-phase parallel synthesis. The thiophene sulfonamide **7** exhibited an inhibition of Aβ42 production to the extent of 47% (@2.5 μM) with $IC_{50} = 2.7\ \mu$M. Inhibitor **7** also operated via the γ-secretase proteolytic pathway exhibiting an increase in $APP_s\alpha$ production consistent with mechanism-specific inhibition.

While the medicinal chemistry effort performed around this lead series was significantly more comprehensive than can be described in this Communication, it will suffice to say that the series of fenchylamine sulfonamides which are featured represent the best inhibitors among the hundreds of structural analogues screened. Numerous alterations of the bicyclic hydrocarbon moiety consistently met with a loss of inhibitory potency. Similarly, an exhaustive study which substituted alkyl and aryl sulfonamides for the 4-fluorophenyl sulfonamide moiety of inhibitor (+)-**4** also failed to identify more potent inhibitors. The thiophene sulfonamide **7** emerged unexpectedly from our parallel synthesis studies and currently stands as the other superior fenchylamine sulfonamide inhibitor of Aβ42 production via the γ-secretase pathway.

The Aβ production inhibitors described here are of immediate value as tools for in vitro pharmacological investigations directed toward the proteolytic processing of APP to produce Aβ. The inhibitors (+)-**4** and **7** reduce the production of Aβ42 and Aβ40 via the γ-secretase proteolytic pathway to a similar extent. They exhibit a graded stimulation of the production of $APP_s\alpha$, they show no effect on $APP_s\beta$, and they block the production of p3, strong indications of mechanism-specific inhibition. We did not observe stimulation of Aβ42 secretion with any of the compounds at any concentration tested.

A limited structure−activity relationship of fenchylamine sulfonamide inhibitors of Aβ formation by the γ-secretase proteolytic pathway has been described here. The relative and absolute stereochemical requirements for inhibitory activity in this series have been established. This information may help to guide future efforts toward the inhibition of the γ-secretase proteolytic pathway of APP processing and toward the development of small-molecule therapeutic agents for the treatment and prevention of AD.

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Supporting Information Available: Experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

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