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# Identification of Presenilin 1-Selective γ-Secretase Inhibitors with Reconstituted γ-Secretase Complexes

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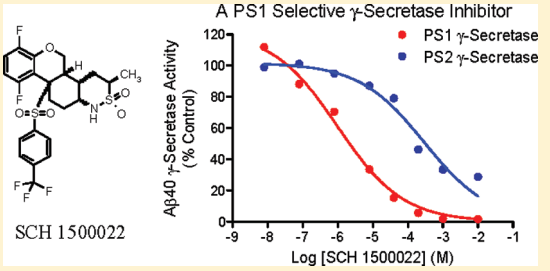
**ABSTRACT:** Accumulation of the β-amyloid (Aβ) peptides is one of the major pathologic hallmarks in the brains of Alzheimer's disease (AD) patients. Aβ is generated by sequential proteolytic cleavage of the amyloid precursor protein (APP) catalyzed by β- and γ-secretases. Inhibition of Aβ production by γ-secretase inhibitors (GSIs) is thus being pursued as a target for treatment of AD. In addition to processing APP, γ-secretase also catalyzes proteolytic cleavage of other transmembrane substrates, with the best characterized one being the cell surface receptor Notch. GSIs reduce Aβ production in animals and humans but also cause significant side effects because of the inhibition of Notch processing. The development of GSIs that reduce Aβ production and have less Notch-mediated side effect liability is therefore an important goal. γ-Secretase is a large membrane protein complex with four components, two of which have multiple isoforms: presenilin (PS1 or PS2), aph-1 (aph-1a or aph-1b), nicastrin, and pen-2. Here we describe the reconstitution of four γ-secretase complexes in Sf9 cells containing PS1−aph-1a, PS1−aph-1b, PS2−aph-1a, and PS2−aph-1b complexes. While PS1−aph-1a, PS1−aph-1b, and PS2−aph-1a complexes displayed robust γ-secretase activity, the reconstituted PS2−aph-1b complex was devoid of detectable γ-secretase activity. γ-Secretase complexes containing PS1 produced a higher proportion of the toxic species Aβ42 than γ-secretase complexes containing PS2. Using the reconstitution system, we identified MRK-560 and SCH 1500022 as highly selective inhibitors of PS1 γ-secretase activity. These findings may provide important insights into developing a new generation of γ-secretase inhibitors with improved side effect profiles.




γ-Secretase is best known for its involvement in the proteolytic processing of amyloid precursor protein (APP) that generates β-amyloid peptide (Aβ), the major component in amyloid plaques that is a hallmark of Alzheimer's disease (AD) pathology (reviewed in ref 1). Processing of APP releases Aβ peptides of 37−42 residues (reviewed in refs 2−4). The longer forms, especially Aβ42, are more hydrophobic, prone to aggregation, and critical in the pathogenesis of AD. Blocking Aβ production with γ-secretase inhibitors (GSIs) is expected to slow or prevent the progression of AD and has thus become a major drug development effort in the past decade.

In addition to APP, the substrates of γ-secretase include many single-transmembrane domain proteins (reviewed in ref 5), with the best characterized one being the cell surface receptor Notch. γ-Secretase processing of Notch generates the Notch intracellular domain (NICD), which is subsequently transported to the nucleus and acts as a transcription factor. Notch-mediated signaling activities are crucial for embryonic development as well as cell differentiation in adult animals.[6] Achieving a significant therapeutic window between Aβ reduction and side effects due to inhibition of Notch processing has been a major challenge for developing GSIs as a treatment for AD.

γ-Secretase is a large membrane protein complex containing four essential components: presenilin (PS), aph-1, nicastrin (NCT), and pen-2.[2] PS is the catalytic subunit that contains the active site of the enzyme, but all components are essential as the absence of any of them leads to a complete loss of γ-secretase activity. Of the four components in the γ-secretase complex, both PS and aph-1 have two isoforms in humans. PS1 and PS2 are ∼60% homologous in sequence, and a similar degree of homology is found between aph-1a and aph-1b. The presence of different PS and aph-1 isoforms suggests the potential for heterogeneity of native γ-secretase such that there may be up to four different subtypes of γ-secretase complexes. The existence of two splicing variants of aph-1a (long and short forms) may further contribute to the heterogeneity of native γ-secretase.

Little is known about the physiologic function of each individual γ-secretase complex. The biochemical characterization of the γ-secretase complex has been conducted mostly with the native enzyme from various cells and tissues, each of which likely contains a variable mixture of γ-secretase isoforms. The first generation of GSIs was developed using native enzyme preparations for screening and evaluation before the molecular identification of γ-secretase components. Understanding whether any of these compounds are selective for specific γ-secretase complexes may provide important insights into their in vivo efficacy and side effect

**Received:** January 6, 2011
**Revised:** April 27, 2011
**Published:** April 29, 2011





dx.doi.org/10.1021/bi200026m | *Biochemistry* 2011, 50, 4973–4980

profiles. In addition, developing GSIs selective for a particular γ-secretase complex may offer a means of achieving efficacy while minimizing the mechanism-based side effects caused by inhibition of the cleavage of γ-secretase substrates other than APP. Compounds with selectivity against a specific γ-secretase complex can also be used as a tool to dissect the biological roles of individual complexes.

We have previously reported the reconstitution of the γ-secretase complex in Sf9 cells by co-infection of cells with baculoviruses expressing PS1, aph-1a, NCT, and pen-2.[7] In this study, we describe the further reconstitution and validation of γ-secretase complexes with different combinations of PS and aph-1 isoforms. GSIs with selectivity for PS1 have been identified by screening with reconstituted PS1−aph-1a and PS2−aph-1a γ-secretase complexes.

## EXPERIMENTAL PROCEDURES

**Expression Constructs.** Human PS1, PS2, and NCT cDNAs were obtained from P. St George-Hyslop (University of Toronto, Toronto, ON). PS1 N-terminal fragment (NTF) and C-terminal fragment (CTF) and PS2 NTF and CTF expression constructs were generated by polymerase chain reaction (PCR) amplification of PS1 or PS2 cDNAs and encode amino acid residues 1−291 (PS1 NTF), 292−467 (PS1 CTF), 1−297 (PS2 NTF), and 298−448 (PS2 CTF), respectively. Human pen-2 and the short splice variant of aph-1a [aph-1a (S)] were cloned from a human brain cDNA library (Clontech). A myc tag and six-His tag were introduced at the C-terminus of NCT and aph-1a (S). Myc and six-His tags were introduced at the C-terminus of TMP21. All clones were confirmed by DNA sequencing. Baculoviruses carrying these cDNAs were generated using the Bac-to-Bac system (Invitrogen) according to the manufacturer's instructions. The N160 and C99 expression system has been described previously.[8]

**Reagents.** L-685458 was purchased from Calbiochem. LY-411575, LY-450139, BMS-299498, MRK-560, SCH 697466, and SCH 1500022 were synthesized as reported previously.[9−13]

**Expression of γ-Secretase Complexes in Sf9 Cells, Membrane Preparation, and Measurement of γ-Secretase Activity.** Sf9 cells grown to a density of $1 \times 10^6$ cells/mL were co-infected with four baculoviruses expressing each of the four different components of the γ-secretase complex. The cells were harvested 72 h later, and membranes were prepared as reported previously.[7] To measure γ-secretase activity, 10 μg of solubilized membrane proteins was incubated with purified C99 or N160 substrate in a 50 μL reaction mixture containing 50 mM acetate (pH 6), 150 mM NaCl, 2 mM EDTA, and 0.25% CHAPSO. The reaction mixture was incubated at 37 °C for 2 h. For detection of Aβ40 and Aβ42 products, the C99 reactions were analyzed with a sandwich immunoassay using Meso Scale Discovery (MSD) technology with ruthenylated antibodies G2-10 and G2-11,[14] respectively, in combination with biotinylated antibody 4G8.

**Western Blot Analysis of Protein Expression.** PS1 NTF expression and PS2 NTF expression were detected using antibodies from Covance. PS1 CTF and PS2 CTF were detected using antibodies from Calbiochem. The anti-myc antibody used for detecting the expression of NCT and aph-1 was purchased from Roche Biochemicals. pen2 expression was detected using the anti-pen2 N-terminal antibody (Oncogene). The antibody used to detect NICD was purchased from Cell Signaling.

**Mass Spectrometry Analysis of Aβ Peptides Generated from Reconstituted γ-Secretase Complexes.** The spectrum of Aβ peptides generated by reconstituted γ-secretase was analyzed using surface-enhanced laser desorption ionization (SELDI) mass spectrometry. A γ-secretase reaction mixture (50 μL) was applied to a PS20 protein chip array (Bio-Rad) that had been precoated with 1 μg of antibody W02. The array was incubated overnight at 4 °C. Nonspecific protein binding to the array was alleviated after it had been washed three times in phosphate-buffered saline (PBS) with 0.2% Tween 20 and then three times in PBS. The array was rinsed briefly with water and air-dried before being subjected to analysis with a SELDI ProteinChip Reader (Bio-Rad).

## RESULTS

**Reconstitution of γ-Secretase Complexes Containing Different PS and aph-1 Isoforms.** To express individual γ-secretase complexes, we co-infected Sf9 cells with baculoviruses expressing PS1 or PS2, aph-1a (S) (aph-1a hereafter), or aph-1b, NCT, and pen-2. This led to four complexes with common components of NCT and pen-2 but different PS and aph-1 combinations, namely, PS1−aph-1a, PS1−aph-1b, PS2−aph-1a, and PS2−aph-1b. The expression levels of NCT, pen-2, and aph-1a or aph-1b were comparable in these four complexes (Figure 1C). In PS1−aph-1a, PS1−aph-1b, and PS2−aph-1a complexes, a significant amount of PS was processed to NTF and CTF (Figure 1A). Processing of PS to NTF and CTF has been reported in cells overexpressing PS and in native tissues and is also consistent with our previous report of reconstitution of the γ-secretase complex in Sf9 cells and with a report describing the reconstitution of the γ-secretase complex in yeast.[15] In Sf9 cells expressing the PS2−aph-1b complex, however, the majority of PS2 remained in full-length form (Figure 1B). This result suggests that the PS2−aph-1b complex is not processed efficiently in this reconstitution system.

γ-Secretase activity of the reconstituted complexes was also measured by adding purified C99 substrate to CHAPSO-solubilized membrane extracts from cells expressing the various complexes. PS1−aph-1a, PS1−aph-1b, and PS2−aph-1a γ-secretase complexes exhibited robust γ-secretase activity as measured by Aβ40 production, while no activity was detected with the PS2−aph-1b γ-secretase complex (Figure 2A). Similar profiles of γ-secretase activities were also observed when using a modified Notch substrate N160. PS1−aph-1a, PS1−aph-1b, and PS2−aph-1a complexes processed N160 to generate NICD product, while the PS2−aph-1b complex was inactive in this assay, as well (Figure 2B). Generation of both Aβ40 and NICD by the reconstituted γ-secretase complexes was blocked by inclusion of a γ-secretase inhibitor (Figures 2B and 4B).

The observation that significant full-length PS and processed NTF and CTF were detected in the reconstituted PS1−aph-1a, PS1−aph-1b, and PS2−aph-1a complexes, but not in the PS2−aph-1b complex, raises the question of whether PS processing is the factor limiting the γ-secretase activity of the PS2−aph-1b complex. Therefore, we tested co-expression of PS2 NTF and CTF instead of full-length PS2 to reconstitute PS2 γ-secretase complexes. Co-expression of PS2 NTF and CTF with aph-1a, NCT, and pen-2 produced an active γ-secretase complex, but the activity of the complex with aph-1b was close to the background level (Figure 3).

**Characterization of PS1−aph-1a and PS2−aph-1a γ-Secretase Complexes.** We focused our efforts on the PS1−aph-1a

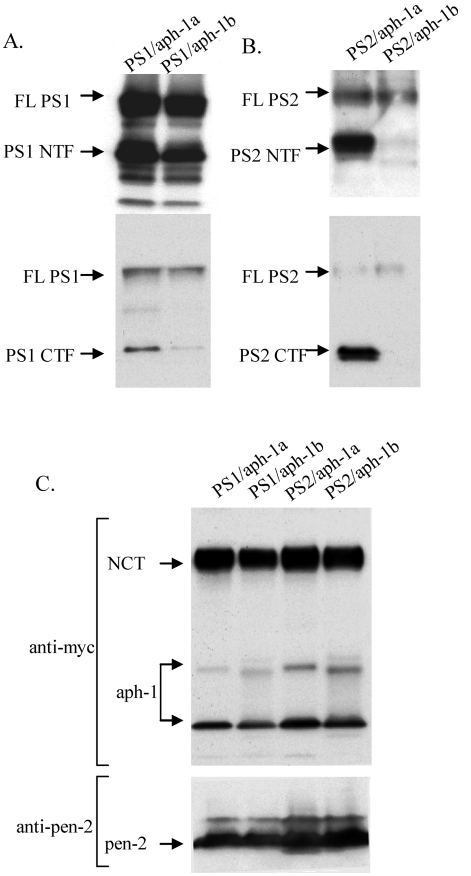



**Figure 1.** Expression of four individual $\gamma$-secretase complexes in Sf9 cells. Membrane fractions were prepared from Sf9 cells expressing the specified $\gamma$-secretase complexes. The membrane proteins were separated on NuPage gels and subjected to Western blot analysis after being transferred to nitrocellulose membranes. (A) Western blot analysis of PS1 expression in Sf9 cells using anti-PS1 NTF and anti-PS1 CTF antibodies. (B) Western blot analysis of PS2 expression in Sf9 cells using anti-PS2 NTF and anti-PS2 CTF antibodies. (C) Western blot analysis of NCT, aph-1, and pen-2 expression using the anti-myc antibody (NCT and aph-1) and the anti-pen-2 antibody (pen-2). Two aph-1a bands were detected, which is consistent with our previous observation.[7] The top band is likely dimer aggregation.

and PS2−aph-1a $\gamma$-secretase complexes to study the role of PS1 and PS2 in $\gamma$-secretase activity in more detail. Both PS1−aph-1a and PS2−aph-1a $\gamma$-secretase complexes displayed substrate-dependent A$\beta$40 and A$\beta$42 production (Figure 4). The $K_m$ values of A$\beta$40 and A$\beta$42 activity are 1 and 2 $\mu$M, respectively, for the PS1−aph-1a complex and 0.3 and 0.4 $\mu$M, respectively, for the PS2−aph-1a complex. Interestingly, the $V_{max}$ values for A$\beta$40 and A$\beta$42 production were comparable for the PS1−aph-1a $\gamma$-secretase complex, but A$\beta$42 activity was much lower for the PS2 $\gamma$-secretase complex (Figure 4). The average A$\beta$42:A$\beta$40 ratio at substrate concentrations between 0.5 and 4 $\mu$M was 0.6 for the PS1−aph-1a complex and 0.2 for the PS2−aph-1b complex. A$\beta$ products generated from the different $\gamma$-secretase complexes were further analyzed using SELDI mass spectrometry. Mass spectrometry revealed that a variety of A$\beta$ species were produced by the reconstituted $\gamma$-secretase complexes, including A$\beta$43, A$\beta$42, A$\beta$40, A$\beta$38, and A$\beta$37 (Figure 5). Production of all A$\beta$ products was blocked when a $\gamma$-secretase inhibitor was included in the reaction mixture (data not shown). Consistent with data from the

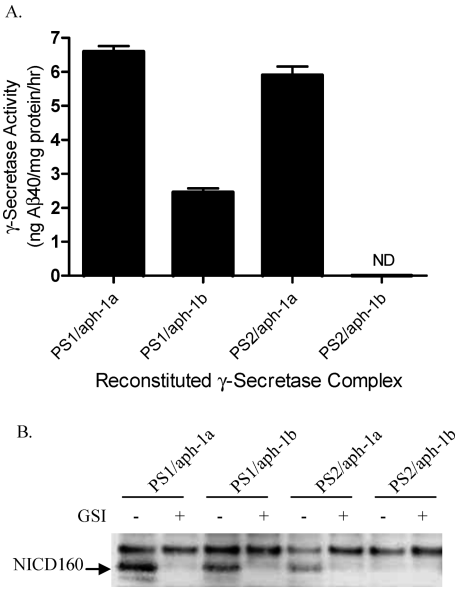



**Figure 2.** $\gamma$-Secretase activity of reconstituted $\gamma$-secretase complexes. (A) A$\beta$40 production in reconstituted complexes. (B) NICD production by reconstituted $\gamma$-secretase complexes. As expected, NICD production was reduced by inclusion of the GSI LY-450139 (2 $\mu$M).

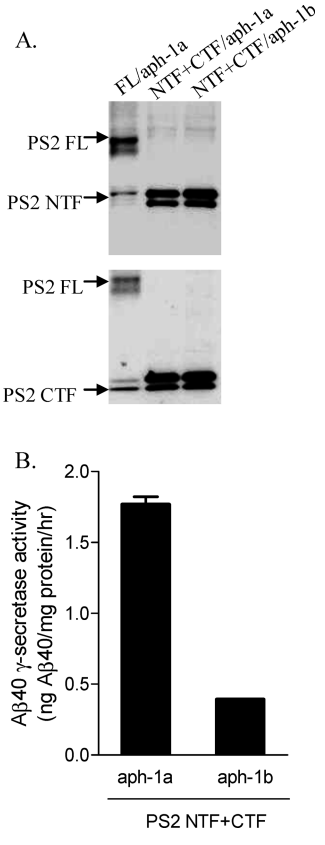



**Figure 3.** Reconstitution of $\gamma$-secretase complexes with preprocessed PS2 NTF and CTF. Baculoviruses of PS2 NTF and CTF expression constructs were used to infect Sf9 cells together with the baculoviruses carrying aph-1a or aph-1b, NCT, and pen-2. (A) Western blot analysis of full-length PS2, NTF, and CTF expression. (B) A$\beta$40 $\gamma$-secretase activities.


dx.doi.org/10.1021/bi200026m |*Biochemistry* 2011, 50, 4973–4980

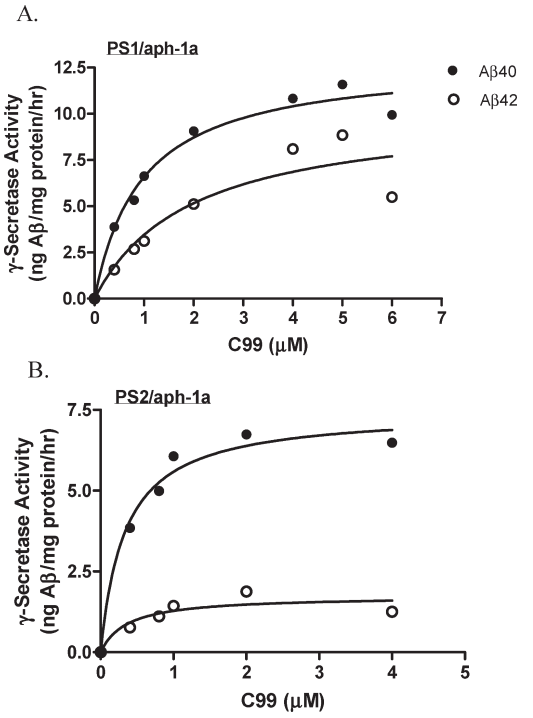



**Figure 4.** Comparison of reconstituted PS1—aph-1a and PS2—aph-1a $\gamma$-secretase complex activity. A$\beta$40 production and A$\beta$42 production were assessed by incubating reconstituted $\gamma$-secretase complexes with the C99 substrate at the specified concentrations. The specific activity was calculated from duplicate measurements of the MSD signal. The data are representative of more than five independent experiments. (A) Production of A$\beta$40 and A$\beta$42 by the PS1—aph-1a $\gamma$-secretase complex. (B) Production of A$\beta$40 and A$\beta$42 by the PS2—aph-1a $\gamma$-secretase complex.

activity assay shown in Figure 4, the PS2—aph-1a $\gamma$-secretase complex generated primarily A$\beta$40 (Figure 5B), while the PS1—aph-1a $\gamma$-secretase complex generated relatively more A$\beta$37, A$\beta$38, A$\beta$42, and A$\beta$43 in addition to A$\beta$40 (Figure 5A).

Several structurally distinct $\gamma$-secretase inhibitors were then tested with PS1—aph-1a and PS2—aph-1a $\gamma$-secretase complexes (Figure 6). Most of these compounds are nonselective between PS1 and PS2 complexes, as represented by LY-411575 (Figure 7A). The ratios of the $IC_{50}$ values for these inhibitors at the PS2—aph-1a and PS1—aph-1b $\gamma$-secretase complexes are summarized in Table 1, with a ratio of 1 signifying equal potency for PS1 and PS2 $\gamma$-secretases and a higher number indicative of a PS1-selective compound. BMS-299897 was a compound with modest selectivity for the PS1 over the PS2 $\gamma$-secretase complex, with a PS2:PS1 ratio of 3.2 (Table 1). MRK-560 was a significantly more selective for the PS1 complex (Figure 7B), while SCH 1500022 was highly selective for PS1 $\gamma$-secretase (Figure 7C). The PS2:PS1 ratios for MRK560 and SCH 1500022 were 37 and 250, respectively (Table 1).

## DISCUSSION

**Reconstitution of Four $\gamma$-Secretase Complexes.** The presence of presenilin and aph-1 isoforms suggests that native $\gamma$-secretase activity is a heterogeneous mixture of various complexes and that various tissues may express different $\gamma$-secretase complexes. It is clear from genetic knockout studies that the different isoforms of presenilin and aph-1 play overlapping as

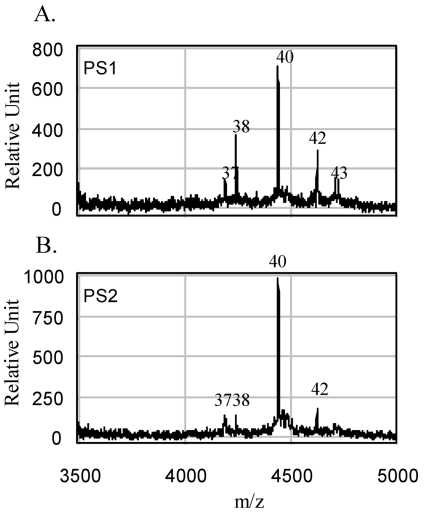



**Figure 5.** Mass spectrometric analysis of A$\beta$ products generated from reconstituted PS1—aph-1a and PS2—aph-1a $\gamma$-secretase complexes. $\gamma$-Secretase reactions were conducted as described in Experimental Procedures, and A$\beta$ products were captured on a PS20 ProteinChip (Bio-Rad) precoated with the W02 antibody. Mass spectrometry was conducted on a SELDI ProteinChip Reader (Bio-Rad). The A$\beta$ profile generated by the PS1—aph-1a $\gamma$-secretase complex (A) and the PS2—aph-1a $\gamma$-secretase complex (B).

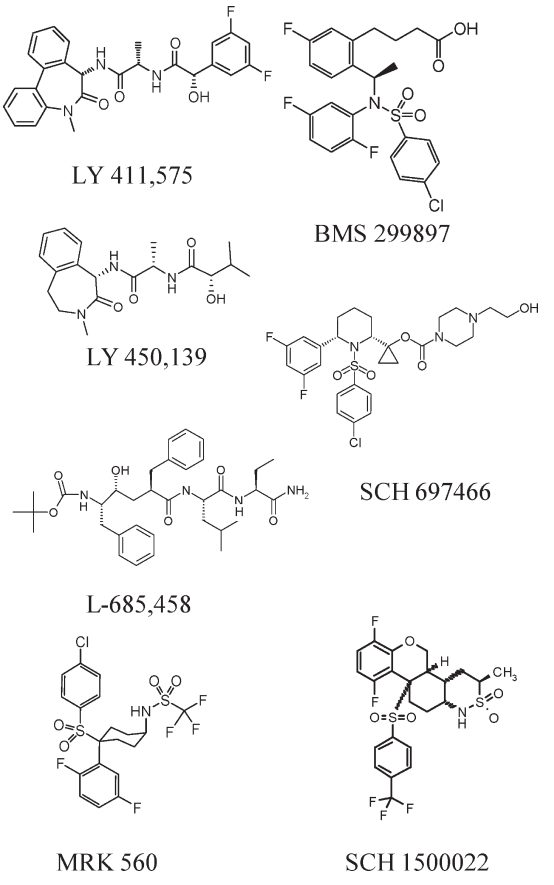



**Figure 6.** Structures of $\gamma$-secretase inhibitors used in this study.

well as distinct roles in various physiological activities;[16−18] inhibiting specific $\gamma$-secretase complexes may therefore permit

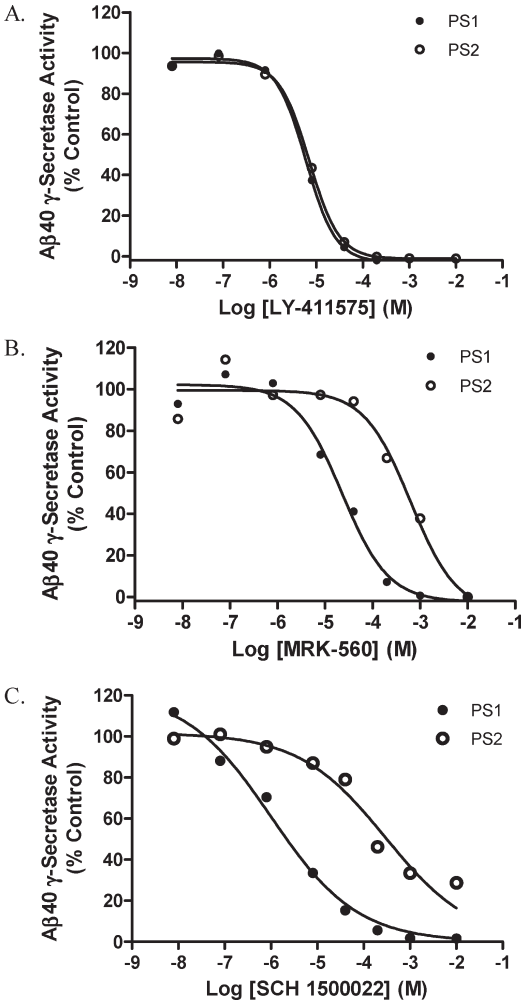



**Figure 7.** Potency of different classes of GSIs at the PS1−aph-1a and PS2−aph-1a γ-secretase complexes. PS1−aph-1a and PS2−aph-1a γ-secretase complex activity was measured in the presence of GSIs at specified concentrations: (A) LY-411575, (B) MK-560, and (C) SCH 1500022.

Aβ reduction while sparing side effects caused by inhibition of Notch processing mediated by other complexes. We have expanded our previous study from reconstituting the γ-secretase complex of PS1, aph-1a, NCT, and pen-2 in Sf9 cells[7] to reconstituting four complexes containing different PS and aph-1 isoforms. This system allows us to assess the biochemical properties of each individual γ-secretase complex.

Of the four complexes reconstituted, all display robust γ-secretase activity except for the PS2−aph-1b complex, which is consistently inactive in γ-secretase assays (Figure 2). While the PS2−aph-1b complex was not processed to NTF and CTF like the other reconstituted γ-secretase complexes (Figure 1B), the lack of processing does not appear to fully explain the lack of activity because reconstitution of preformed PS2 NTF and CTF, aph-1b, NCT, and pen-2 also resulted in little if any activity (Figure 3B). PS2−aph-1b γ-secretase complex activity was previously reported for a complex isolated from HEK293 cells.[19] Because reconstitution of the γ-secretase complex in Sf9 cells does not incorporate host cell proteins,[7] one possible explanation for this discrepancy is that an additional component is required for the assembly of a functionally active PS2−aph-1b γ-secretase complex. Another possibility is that the PS2−aph-1b complex may be involved in

**Table 1. Summary of GSI Selectivity between PS1−aph-1a and PS2−aph-1a γ-Secretase Complexes**

| | $IC_{50}$ (nM) | | |
|---|---|---|---|
| | PS1−aph-1a | PS2−aph1a | PS2:PS1 $IC_{50}$ ratio |
| L-685458 | 133 | 206 | 1.5 |
| LY-411575 | 5 | 5 | 1.0 |
| LY-450139 | 311 | 137 | 0.4 |
| BMS-299897 | 206 | 659 | 3.2 |
| MRK-560 | 9 | 320 | 37 |
| SCH 697466 | 148 | 174 | 1.2 |
| SCH 1500022 | 1 | 275 | 250 |

γ-secretase-independent physiologic processes, which has been suggested for PS1 (reviewed in ref 5); therefore, the processing of APP is not its major biological function.

Although FAD mutations in PS1 and PS2 genes all lead to an increased Aβ42:Aβ40 ratio in vitro, there are more than 150 mutations associated with the PS1 gene but only six associated with the PS2 gene (http://www.molgen.ua.ac.be/admutation), suggesting PS2 plays a lesser role in AD pathogenesis. Biochemical characterization of reconstituted PS1−aph-1a and PS2−aph-1a complexes revealed distinct product profiles. Both PS1−aph-1a and PS2−aph-1a γ-secretase complexes generated Aβ40 and Aβ42, but the Aβ42:Aβ40 ratio was significantly higher for the PS1−aph-1a complex (Figures 4 and 5). Given that Aβ42 is the more hydrophobic and amyloidogenic form of Aβ, our data provide further evidence that PS1 γ-secretase activity plays a major part in amyloidogenesis in AD brains and should be the main target for developing a subtype-selective GSI.

**PS1-Selective γ-Secretase Inhibitors.** The development of GSIs for the treatment of AD has been hampered by the Notch-related side effects. Interestingly, animals treated with GSIs share a similar side effect profile with those carrying genetic knockout or knockdown of γ-secretase components. PS1 knockout and deficient mice display a variety of phenotypes, suggesting its prominent role in Aβ production and Notch processing.[16] PS2 knockout mice, on the other hand, are largely normal,[17] suggesting that most of its functions may be redundant and/or can be compensated by PS1. However, when PS1 activity is reduced genetically, loss of PS2 gene exacerbates the phenotypes of PS1 deficiency, leading to myeloproliferative disease,[20] skin and autoimmune disease,[21] tyrosinase-mediated coat color change,[22] and learning and memory disorders.[23] In addition, the phenotypes associated with aph-1a- and nicastrin-deficient mice are similar to those seen in mice with both PS1 and PS2 deficiency and are much more pronounced than those seen in mice with PS1 deficiency alone.[24,25] These findings suggest that PS2 γ-secretase complexes have biological functions distinct from those of PS1 γ-secretase. One implication from these findings is that PS1-selective GSIs that spare the biological pathways mediated by PS2 may have the advantage of an improved side effect profile.

The reconstituted system provides an important tool for screening and identifying subtype-specific GSIs. Our analysis of several well-studied GSIs revealed different selectivity with PS1−aph-1a and PS2−aph-1a γ-secretase complexes. The non-selective feature of L-685458 (Table 1) is consistent with its proposed mechanism as transition state inhibitor binding to the active site of γ-secretase as well as presenilin-like proteases such as SPP.[26,27] Of note is the fact that there is a trend of correlation

between PS1 selectivity and the side effect profile in vivo. LY-411575 is a potent nonselective GSI (Figure 7A and Table 1) and has been reported to cause Notch-mediated toxicity, including intestinal goblet cell hyperplasia, thymus atrophy, B-cell development, and coat color change.[28−30] Similar side effects are also found with nonselective inhibitors LY-450139 and SCH 697466 (L. Hyde, manuscript in preparation). BMS-299897 is a compound with modest selectivity (Table 1). It does not cause Notch toxicity in vivo, although interpretation of this data may be complicated as it causes autoinduction with chronic dosing that reduces the drug exposure.[9,31] MRK-560 is the first compound we identified with significant PS1 selectivity. It is a potent GSI in vivo,[10] which is consistent with the finding from genetic studies that PS1 $\gamma$-secretase complexes play a major role in A$\beta$ production. Chronic dosing with MRK-560 in mice does not cause Notch-mediated side effects as reported with previous GSIs, even though the compound does not show selectivity between inhibition of APP and Notch processing in vitro.[32] These findings provide strong evidence for our hypothesis that PS1-selective GSIs should have improved therapeutic windows. It should be noted that this mechanism differs from the Notch-sparing approach that aims to selectively inhibit APP processing. Both MRK-560 and SCH 1500022 are potent inhibitors in in vitro Notch cleavage assays (refs 33−35 and and unpublished data of J. Lee). This is likely because most of the cell lines used in assays have PS1 $\gamma$-secretase as the dominant enzyme form. However, the advantage of a PS1-selective GSI would emerge in in vivo testing where PS2-mediated biological activities are spared.

Our results are consistent with the findings in PS1 and PS2 knockout embryonic fibroblasts[36] that L-685458 and the DAPT-like compound LY-411575 are nonselective GSIs. The selectivity of BMS-299897 in knockout embryonic fibroblasts (40-fold) is significantly greater than in our reconstituted system (4-fold). The cause of this discrepancy is not clear. This compound is categorized as moderately selective in both assays, suggesting the reconstituted system can identify PS1-selective compounds with a rank order similar to that in the PS1 and PS2 knockout fibroblast assay.

A recent study demonstrates that knockout of the aph-1b gene in mice significantly reduces the amyloid plaque load in aged APP mice but has no Notch-mediated phenotypes as aph-1a knockout mice do.[37] Although this suggests that developing GSIs targeting aph-1b $\gamma$-secretase complexes may have the advantage of robust A$\beta$ reduction with a reduced side effect profile, this approach faces the challenge that aph-1 is not the catalytic subunit. The function of aph-1 in the $\gamma$-secretase complex has yet to be elucidated, and the impact and/or feasibility of a small molecule binding to aph-1b and allosterically regulating enzyme activity needs to be further evaluated. Indeed, all GSIs for which binding to $\gamma$-secretase has been studied have been found to bind to the catalytic PS1 and PS2 subunits.[33−35] The compounds we have tested do not display selectivity between PS1−aph-1a and PS1−aph-1b $\gamma$-secretase complexes (data not shown).

Despite the hurdles in developing GSIs as treatments for AD, it is still being pursued vigorously.[38,39] Chronic treatment with BMS-708613 in humans leads to a robust reduction of the level of A$\beta$ in cerebrospinal fluid without causing significant Notch-related side effects, suggesting a therapeutic window can be achieved with GSIs. On the other hand, abnormal Notch activities are associated with certain cancers such as T-cell acute lymphoblastic leukemia.[40] GSIs have been pursued as potential anticancer and/or chemosensitizing agents.[41,42] Taken together, $\gamma$-secretase is still a promising drug target. Understanding the role of individual $\gamma$-secretase complexes may provide important insights into developing more effective drugs with reduced side effects.

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## ABBREVIATIONS

AD, Alzheimer's disease; APP, amyloid precursor protein; CTF, presenilin C-terminal fragment; GS, $\gamma$-secretase; GSI, $\gamma$-secretase inhibitor; NCT, nicastrin; NICD, Notch intracellular domain; NTF, presenilin N-terminal fragment; PS, presenilin.

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