BIOCHEMISTRY
including biophysical chemistry & molecular biology

Current Topic

pubs.acs.org/biochemistry

Development and Mechanism of γ-Secretase Modulators for Alzheimer's Disease

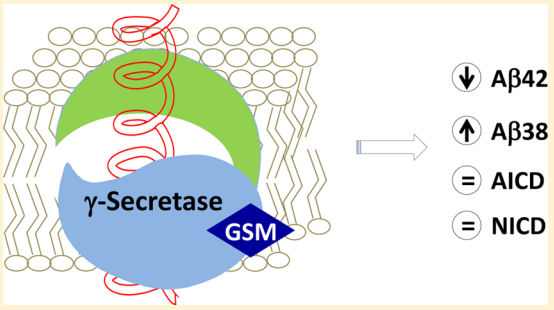
Christina J. Crump,[†,‡] Douglas S. Johnson,[§] and Yue-Ming Li*[,†,‡]

[†]Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10065, United States

[‡]Department of Pharmacology, Weill Graduate School of Medical Sciences of Cornell University, New York, New York 10021, United States

[§]Neuroscience Medicinal Chemistry and Chemical Biology, Pfizer Worldwide Research and Development, Cambridge, Massachusetts 02139, United States

ABSTRACT: γ-Secretase is an aspartyl intramembranal protease composed of presenilin, Nicastrin, Aph1, and Pen2 with 19 transmembrane domains. γ-Secretase cleaves the amyloid precursor proteins (APP) to release Aβ peptides that likely play a causative role in the pathogenesis of Alzheimer's disease (AD). In addition, γ-secretase cleaves Notch and other type I membrane proteins. γ-Secretase inhibitors (GSIs) have been developed and used for clinical studies. However, clinical trials have shown adverse effects of GSIs that are potentially linked with nondiscriminatory inhibition of Notch signaling, overall APP processing, and other substrate cleavages. Therefore, these findings call for the development of disease-modifying agents that target γ-secretase activity to lower levels of Aβ42 production without blocking the overall processing of γ-secretase substrates. γ-Secretase modulators (GSMs) originally derived from nonsteroidal anti-inflammatory drugs (NSAIDs) display such characteristics and are the focus of this review. However, first-generation GSMs have limited potential because of the low potency and undesired neuropharmacokinetic properties. This generation of GSMs has been suggested to interact with the APP substrate, γ-secretase, or both. To improve the potency and brain availability, second-generation GSMs, including NSAID-derived carboxylic acid and non-NSAID-derived heterocyclic chemotypes, as well as natural product-derived GSMs have been developed. Animal studies of this generation of GSMs have shown encouraging preclinical profiles. Moreover, using potent GSM photoaffinity probes, multiple studies unambiguously have showed that both carboxylic acid and heterocyclic GSMs specifically target presenilin, the catalytic subunit of γ-secretase. In addition, two types of GSMs have distinct binding sites within the γ-secretase complex and exhibit different Aβ profiles. GSMs induce a conformational change of γ-secretase to achieve modulation. Various models are proposed and discussed. Despite the progress of GSM research, many outstanding issues remain to be investigated to achieve the ultimate goal of developing GSMs as effective AD therapies.



γ-SECRETASE AND Aβ PEPTIDES

γ-Secretase modulators (GSMs) have moved to the forefront of Alzheimer's disease (AD) research because of their potential as disease-modifying agents and despite an unclear mechanism of action. GSMs make up a class of compounds that selectively reduce the level of formation of pathogenic Aβ42 species yet do not affect the total amount of Aβ produced.[1] Moreover, they have little effect on γ-secretase-dependent Notch processing because the generation of Notch intracellular domain (NICD) is not inhibited.[1] Several reviews[2−7] have highlighted the progress made in developing the next generation of GSMs. This review focuses on recent progress in molecular probe development and studies that aim to elucidate the mechanism of action of GSMs.

Although the precise pathological mechanism of AD remains elusive, it is widely believed that Aβ peptides, the major constituents of amyloid plaques,[8,9] play a central role in AD through a process named the "amyloid cascade hypothesis".[10] In this hypothesis, Aβ peptides form a neurotoxic species that triggers a pathological cascade and ultimately leads to neurodegeneration and dementia. Aβ peptides are excised from the amyloid precursor protein (APP) through two proteases: β- and γ-secretases (Figure 1A). This process also generates sAPPβ and APP intracellular C-terminal domain (AICD), which could have different biological roles.[11] Alternatively, APP can be processed by α- and γ-secretases to generate αCTF, sAPPα, P3, and AICD with varying biological activities.[11] Recent studies suggest that α-secretase cleavage can function as a negative feedback regulator to modulate γ-secretase for Aβ production,[12,13] in addition to competing with β-secretase for APP substrates.[14−17]

Received: March 22, 2013
Revised: April 23, 2013
Published: April 24, 2013

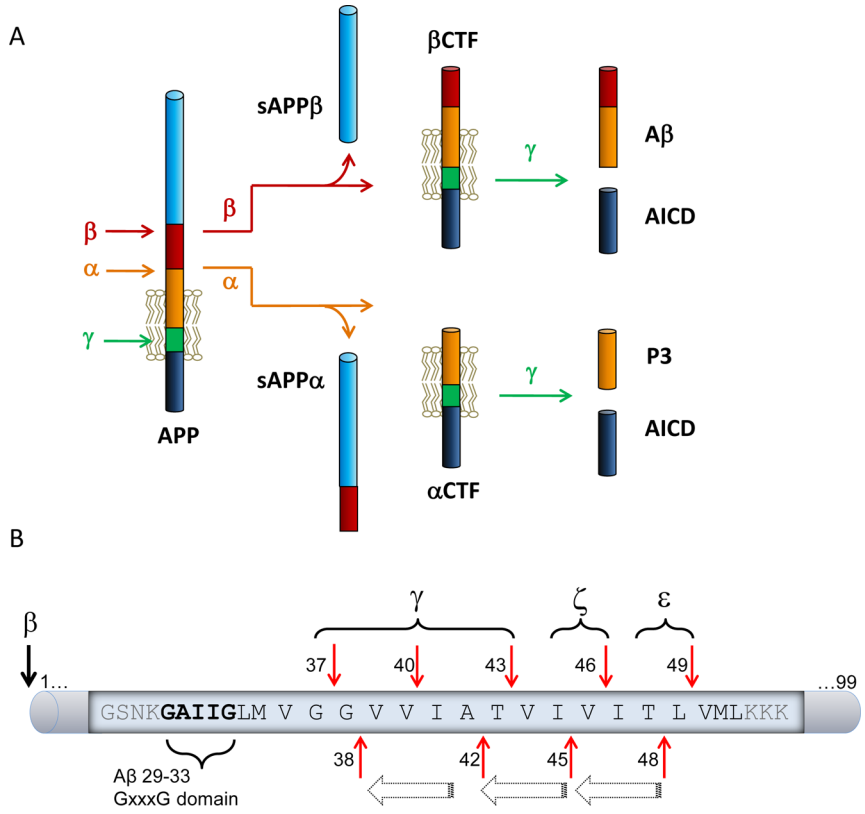


Figure 1. (A) Illustration of APP processing by *α*-, *β*-, and *γ*-secretases and the corresponding products. (B) Sequence of the membrane and nearby regions of the *β*-CTF substrate and relevant cleavages. Thick horizontal arrows represent the hypothesized processive cleavages by *γ*-secretase. Vertical red arrows show locations of *γ*-, *ζ*-, and *ε*-cleavages.

γ-Secretase cleaves APP at multiple sites, including *γ*-, *ζ*-, and *ε*-cleavages[18,19] (Figure 1B), to generate A*β* species with heterogeneous C-termini, which are 37−46 amino acids long.[20,21] Compelling evidence indicates that these A*β* peptides can be generated through a processive mechanism that travels from the *ε*-site to the *γ*-site and removes three to four amino acids at each step.[22] It has also been proposed that there are two *γ*-secretase product lines: one from A*β*49 to A*β*46, A*β*43, A*β*40, and A*β*37 and the other from A*β*48 to A*β*45, A*β*42, and A*β*38. However, recent studies showed that A*β*38 can be generated from A*β*42 and A*β*43,[23] suggesting that both product lines can be crossed with various combinations. Furthermore, multiple studies have shown that the *γ*- and *ε*-cleavages are not always correlated.[12,24−30] Mutations in APP and PS1 lead to different effects on *γ*- and *ε*-cleavages, and even within *γ*-sites (such as A*β*42 and A*β*38).[12,24,27−29] In addition, interaction of *γ*-secretase with other proteins and/or different assay conditions can dissociate these events.[25,26,30] The question of whether these findings reflect the fact that *γ*- and *ε*-cleavages are differentially regulated during sequential processing or just indicate that they represent independent events merits further investigation.

Among the different forms of A*β* species, the role of A*β*40 and A*β*42 in AD has been intensively investigated. While both A*β*40 and A*β*42 have been implicated in AD,[10] A*β*42 is more prone to aggregation and is believed to play a critical role in the initiation of AD pathogenesis.[31,32] However, recent studies suggest that the A*β*42/A*β*40 ratio, rather than the total amount of A*β*, exhibits a better correlation with the age of onset of FAD.[33] Moreover, *in vitro* and animal studies showed that A*β*40 can play a role in preventing A*β*42 aggregation, and therefore, reduction of A*β*40 that alters the A*β*42/A*β*40 ratio may lead to enhanced amyloidogenesis.[34−39] Direct evidence demonstrating that A*β*40 inhibits amyloid deposition came from the studies of bitransgenic (BRL-A*β*40/Tg2576) mice in which the overexpression of the A*β*40 peptide significantly reduced the extent of amyloid deposition.[35]

Nonselective inhibition of *γ*-secretase drastically affects the processing and metabolism of APP proteins, which have been shown to regulate various neuronal and synaptic functions conferred by distinct APP domains.[11,40] Furthermore, the accumulation of APP *β*CTF that results from *γ*-secretase inhibition has been implicated in neurotoxicity.[41] Also, it has been shown that *γ*-secretase inhibitors (GSIs) can cause an elevation in the level of A*β* when administered at low concentrations, and withdrawal of GSIs leads to a rebound increase in A*β* plasma levels.[42] In addition, it has been found that an increased concentration of *β*CTF can augment the A*β*42/A*β*40 ratio.[43] Together, these data suggest that total inhibition of APP processing could actually aggravate AD pathology.

Autosomal dominant inheritance of mutations in three genes [the amyloid precursor protein (APP), presenilin-1 (PS1), and presenilin-2 (PS2)] causes early onset and familial AD (FAD).[44−46] Although how these FAD mutations cause the disease is controversial,[47] it appears that the overwhelming majority of mutations lead to an increase in the A*β*42/A*β*40 ratio,[48] further supporting the A*β* hypothesis. It is worth mentioning a recent discovery showing that an APP mutation, which reduces the level of A*β* production, protects against AD and age-related cognitive decline,[49] providing another line of support for the amyloid cascade hypothesis.

dx.doi.org/10.1021/bi400377p | *Biochemistry* 2013, 52, 3197−3216

Notch1 was the second γ-secretase substrate identified after APP, and functional γ-secretase knockouts result in a notch phenotype.[50−52] The Notch signaling pathway plays an essential role in cell fate decisions during development.[53] Notch signaling also plays an important role in the adult brain, which includes the maintenance and differentiation of neuronal stem cells, structure, and synaptic plasticity as well as neuron survival.[54,55] In addition, Notch can act as a proto-oncogene or tumor suppressor in some cancers.[56] Notch1 is processed at least three times (S1−S3 cleavages) for its signaling. First, Notch is cleaved by a furin-like protease (S1 site) in the Golgi that converts a single chain into a heterodimer.[57] Next, binding of ligands to Notch receptors triggers two sequential proteolytic events (S2 and S3): Notch is cut by ADAM metalloproteases at site 2 (S2) and then by γ-secretase at site 3 (S3), which is within the transmembrane domain[58] and analogous to the ε-site of APP (Figure 2).[19] Following the S3 cleavage, the Notch

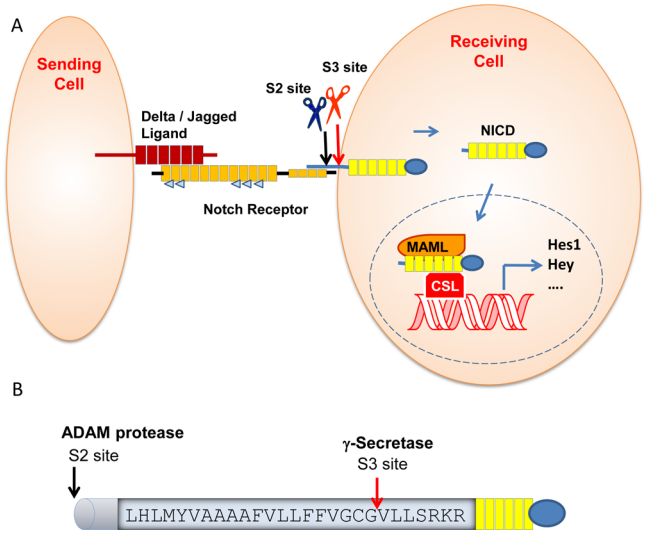


Figure 2. Illustration of the notch signaling cascade (A) depicting activation by a sending cell, which induces S2 cleavage by an ADAM protease, followed by S3 cleavage by γ-secretase within the membrane domain. Subsequently, notch intracellular domain (NICD) is released from the membrane and translocates to the nucleus where it can turn on target genes. (B) Sequence of the membrane domain and S3 site cleavage of the Notch1 receptor.

intracellular domain (NICD) is released from the membrane tether and translocates to the nucleus, where it activates transcription of target genes. NICD binds the CSL (*CBF-1/Su(H)/Lag-1*) transcription factor, thereby dissociating corepressors and recruiting coactivators such as mastermind (MamL), ultimately leading to the activation of effector genes.[53,57] There are five Notch ligands (Dll-1, -3, and -4 and Jagged-1 and -2) and four mammalian Notch receptors (N1−4). All four receptors have been shown to be cleaved by γ-secretase.[59]

The wide spectrum of γ-secretase substrates has made it even more challenging to develop target-based therapy. More than 90 putative γ-secretase substrates have been reported,[60] reflecting the diverse functions of this protease. However, it is worth considering that many of the experimental studies have demonstrated only that γ-secretase can cleave these protein substrates. Deeper investigation is required to determine how many of these proteins are bona fide physiological substrates of γ-secretase, and which ones are most likely to cause detrimental side effects when γ-secretase is inhibited. The phase III clinical trial of semagacestat, a nonselective γ-secretase inhibitor, was terminated because of slightly worse cognition scores and an increase in the risk of skin cancer compared to placebo.[61] Although the precise mechanism that caused these adverse effects is unknown, increased incidents in skin cancer are likely associated with γ-secretase-dependent Notch1 signaling that functions as a tumor suppressor.[62,63] In addition, semagacestat treatment also led to a lightening of hair color,[64] which could be associated with tyrosinase, a substrate of γ-secretase.[65] Therefore, it is critical to know how many substrates are affected by *in vivo* inhibition of γ-secretase, and what the consequences of these events are.

γ-Secretase is an intramembranal complex that relies on the assembly of an active enzyme complex that is composed of a quartet of proteins: Nicastrin (NCT), presenilin (PS), Pen-2, and Aph-1 with 19 putative transmembrane domains (Figure 3).[66] All four proteins are obligatory for cellular γ-secretase

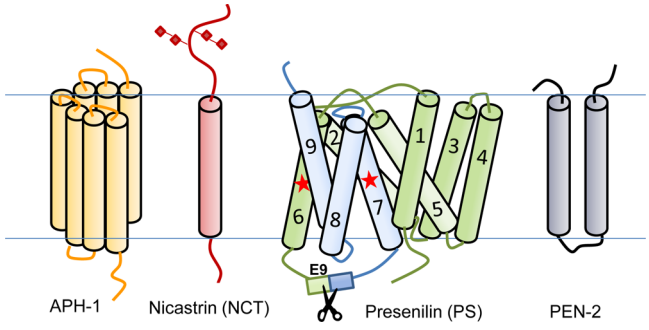


Figure 3. Four essential components of γ-secretase. Presenilin, the catalytic center, is depicted in zymogen form before endoproteolysis of exon 9 and according to the predicted structure by Li et al.[73] Stars represent the relative locations of the two aspartic acid residues required for catalysis.

activity.[67] PS is the catalytic subunit of γ-secretase[68−70] and belongs to a unique family of GxGD-type aspartyl proteases.[71,72] Recently, the crystal structure of a PS/signal peptide peptidase (SPP) homologue (PSH) from the archaeon *Methanoculleus marisnigri* has offered insights into how the transmembrane domains and catalytic dyad are organized in PS1.[73] Both PS1 and PS2 polypeptides undergo endoproteolysis, whereby the N- and C-terminal cleavage products (NTF and CTF, respectively) remain associated as heterodimeric integral membrane proteins.[74] There are two isoforms of presenilin (PS1 and PS2) and three isoforms of Aph-1 (Aph-1aS, Aph-1aL, and Aph-1b). At least six active γ-secretase complexes have been reported (2 presenilins × 3 Aph-1s).[76,77] Remarkably, PS1 and PS2 are not engaged in the same complex, but both of them coexist in the same cells,[75] indicating a tight and precise control of the assembly of the γ-secretase complex. Aph-1 and NCT play critical roles in the assembly, trafficking, and stability of γ-secretase as well as substrate recognition.[66,78,79] Lastly, Pen-2 facilitates the endoproteolysis of PS into its N-terminal (NTF) and C-terminal (CTF) fragments, thereby yielding a catalytically competent enzyme.[66,78,80−82] Although a γ-secretase complex of ∼200 kDa, which contains only one copy of each subunit, is catalytically active,[83] the endogenous γ-secretase complex appears to possess a higher molecular mass ranging from 500

dx.doi.org/10.1021/bi400377p | *Biochemistry* 2013, 52, 3197−3216

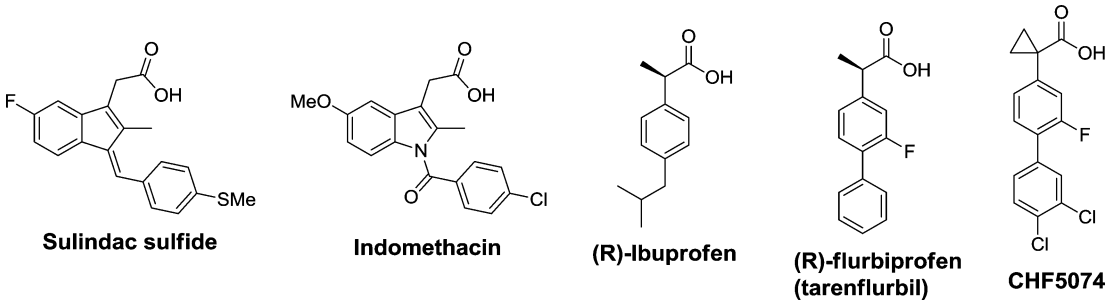


Figure 4. Structures of NSAID-based GSMs.

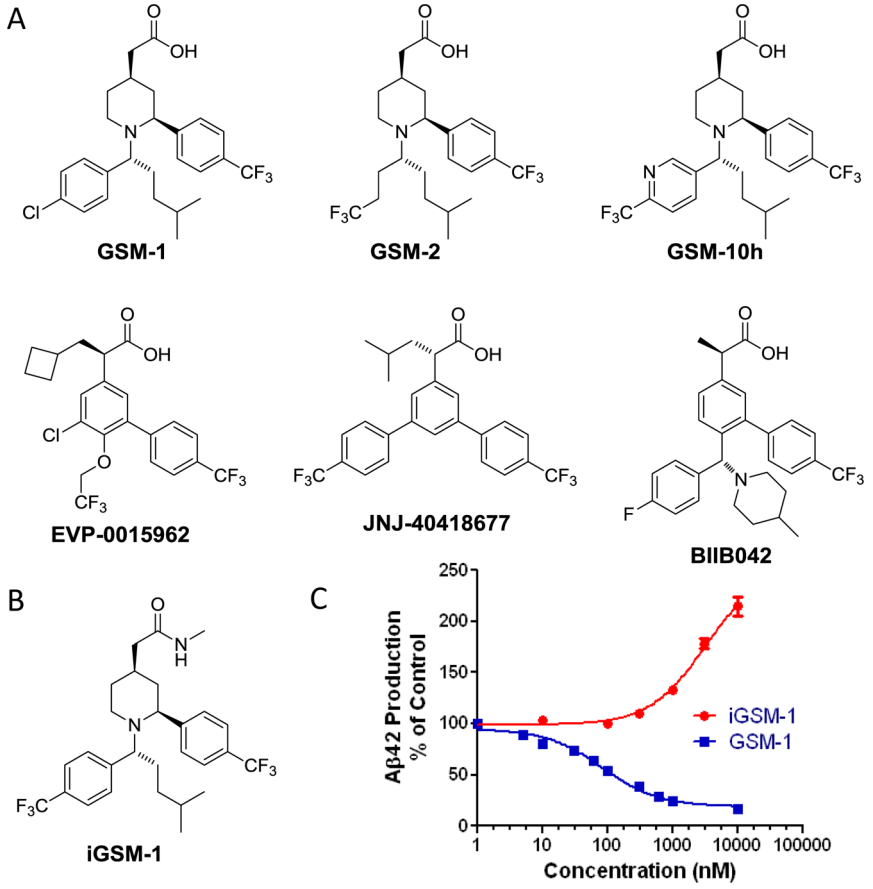


Figure 5. (A) Structures of second-generation NSAID-derived GSMs with the acetic acid chemotype. (B) Structure of an inverse GSM (iGSM). (C) Comparison of the Ab42 profile for GSM-1 and iGSM-1.

to 2000 kDa.[83−87] Taken together, these studies suggest that the quaternary protein complex[83] may be the basic functional γ-secretase unit in cells, and additional cofactors and/or varying stoichiometries of subunits exist in the high-molecular mass γ-secretase complexes for modulating γ-secretase activity and specificity. Nonessential factors, such as CD147, TMP21, γ-secretase activating protein (GSAP), β-arrestin-1, β-arrestin-2, Erin-2, syntexin-1, voltage-dependent anion channel 1 (VDAC1), contactin-associated protein 1 (CNTNAP1), TPPP, and NDUFS7, have been found to be associated with the γ-secretase complex and to modulate γ-secretase activity and specificity;[26,88−94] however, the functional significance of some of these interactions has been contended.[95−97] Moreover, γ-secretase has been shown to interact with tetraspanin-enriched microdomains, or lipid rafts.[98] It has been suggested that different γ-secretase complexes can contribute to substrate specificity,[99,100] which is exemplified by genetic knockout of Aph-1b in a mouse AD model that improved the disease-relevant phenotypic features without Notch-related side effects.[100]

Another unique feature of γ-secretase is that only a small fraction of the four protein complex is catalytically active[85,101] and the total amount of PS protein is not always correlated with γ-secretase activity.[75,101,102] Lai et al. found that <14% of PS1 is engaged in active γ-secretase complexes.[75] Activity-based probes designed from transition state GSIs have been used broadly to study the active γ-secretase complex because they do not bind to the inactive complex.[68,75,99,102−104]

DISCOVERY AND DEVELOPMENT OF GSMS

First-Generation Nonsteroidal Anti-Inflammatory Drug (NSAID) GSMs. The concept of γ-secretase modulation

dx.doi.org/10.1021/bi400377p | *Biochemistry* 2013, 52, 3197−3216

Table 1. Effect of Second-Generation Acid GSMs on the Production of Aβ Peptides

Acid GSM	Cell-based Aβ42 IC_{50}	In vivo studies	Aβ profile		
			38	40	42
GSM-1	348 nM [123]	guinea pig[124]	↑	-	↓
GSM-10h	300 nM[114]	rat[115], mouse[125]			↓
GSM-2	65 nM[41]	mouse [41] [126]			↓
EVP-0015962	67 nM[117]	mouse[117]	↑	-	↓
JNJ-40418677	200 nM[118]	mouse[118]	↑	-	↓
BIIB042	170 nM[119]	mouse, rat, monkey[119]			↓

was discovered when a subset of NSAIDs, such as ibuprofen, indomethacin, and sulindac sulfide, were found to selectively lower the level of formation of Aβ42 in favor of Aβ38 without inhibiting Notch1 cleavage.[1] Furthermore, the effect of these NSAIDs on Aβ modulation was dissociated from their COX activity.[1] GSMs have many unique characteristics: (1) reducing the level of Aβ42 production, (2) promoting shorter forms of Aβ species (Aβ38 or Aβ37), (3) having no significant effect on the total amount of Aβ produced or accumulation of βCTF, and (4) lacking an inhibitory effect on Notch cleavage and other substrates. Not surprisingly, these ideal properties have inspired the development of GSMs as potential disease-modifying agents for AD treatment. Of note, although the role of Aβ37 or Aβ38 in AD is unknown, it is believed that the short forms are less pathogenic than Aβ42.

The NSAID GSMs selectively lower Aβ42 levels with a concomitant increase in Aβ38 levels, without inhibiting the proteolysis of Notch1. The first-generation GSMs include the NSAIDs: ibuprofen, indomethacin, sulindac sulfide, flurbiprofen, and the close analogue CHF5074 (Figure 4). These compounds provided the first evidence that γ-secretase could be specifically modulated to reduce the more pathogenic Aβ42 species. However, their weak *in vitro* potencies (Aβ42 IC_{50} > 10 μM) and poor brain penetration have limited their development. Despite its weak potency (Aβ42 IC_{50} ∼ 200−300 μM),[105] R-flurbiprofen (tarenflurbil) was advanced into clinical studies, and a hint of efficacy was seen in a phase II trial in a subgroup of patients with mild AD.[106] However, the phase III clinical trial of R-flurbiprofen did not achieve statistically significant improvement compared to placebo.[107] R-Flurbiprofen is a weak GSM, and whether it crossed the blood−brain barrier and significantly lowered Aβ42 levels in the clinical studies is unknown. Chiesi has prepared flurbiprofen analogues with improved Aβ42 inhibitory potency leading to CHF5074 (Aβ42 IC_{50} = 41 μM).[108] This compound has been advanced into clinical trials and was found to lower the levels of the soluble CD40 ligand, a marker of microglia activation, but not Aβ42 in plasma or CSF, so it is now being termed a microglial modulator.[109]

Second-Generation GSMs. A key goal in the development of second-generation GSMs has been to improve the potency and brain availability, and advances toward this end have resulted in GSMs with encouraging preclinical profiles in recent years.[2,3] Structurally, second-generation GSMs can be generally divided into three categories: NSAID-derived carboxylic acid GSMs, non-NSAID-derived heterocyclic GSMs, and natural product-derived GSMs.

NSAID-Derived Carboxylic Acid GSMs. Next-generation NSAID-derived GSMs, including GSM-1, GSM-2, GSM-10h, EVP-0015962, JNJ-40418677, and BIIB042, with improved *in vitro* potency and brain penetration have been reported (Figure 5A). Merck and GSK have substituted the core aryl ring with a piperidine ring and optimized the substituent on the piperidine nitrogen to generate a potent series of piperidine acetic acid GSMs. This series is exemplified by GSM-1 and close analogues GSM-2 and GSM-10h, which have become the prototypical second-generation acid GSMs and have been extensively investigated from cellular to animal studies (see Table 1). Overall, this class of GSMs reduces Aβ42 production, promotes the generation of Aβ38, and has little effect on Aβ40 production, total Aβ levels, or AICD and NICD production.

The first *in vitro* characterization of GSM-1 appeared in 2008 when it was shown to significantly decrease the level of Aβ42 and increase the level of Aβ38 in cells expressing either WT PS1 or WT PS2.[28] In contrast, cells expressing PS1 L166P or PS2 N141I FAD mutants showed no change in Aβ42 with GSM-1 treatment, but a robust increase in the level of Aβ38 was still observed. [28] This observation translated to an *in vivo* setting because administration of GSM-1 to Tg2576 mice resulted in a dose-dependent reduction in the level of brain Aβ42 and an increase in the level of Aβ38, whereas administration to APP-Swe/PS2N141I double transgenic mice showed no significant change in the level of brain Aβ42 despite robust increases in the level of Aβ38. Furthermore, the levels of Aβ40 and total Aβ were unchanged, which is consistent with the profile of the NSAID GSMs discussed above. Therefore, one must be careful when transgenic PS FAD models are chosen for GSM studies because certain mutants could give rise to false-negative results for the effect of GSMs on Aβ42. However, a study by Kretner et al. that looked at >20 different PS1 FAD mutations found that the majority of the mutations responded well to GSM-1, with the exception of L166P.[110] This finding suggests that GSMs could be considered as a candidate therapy for prevention trials in asymptomatic AD patients with PS1 FAD mutations.[111,112] The Aβ profile in response to GSM-1 has also been characterized for a number of

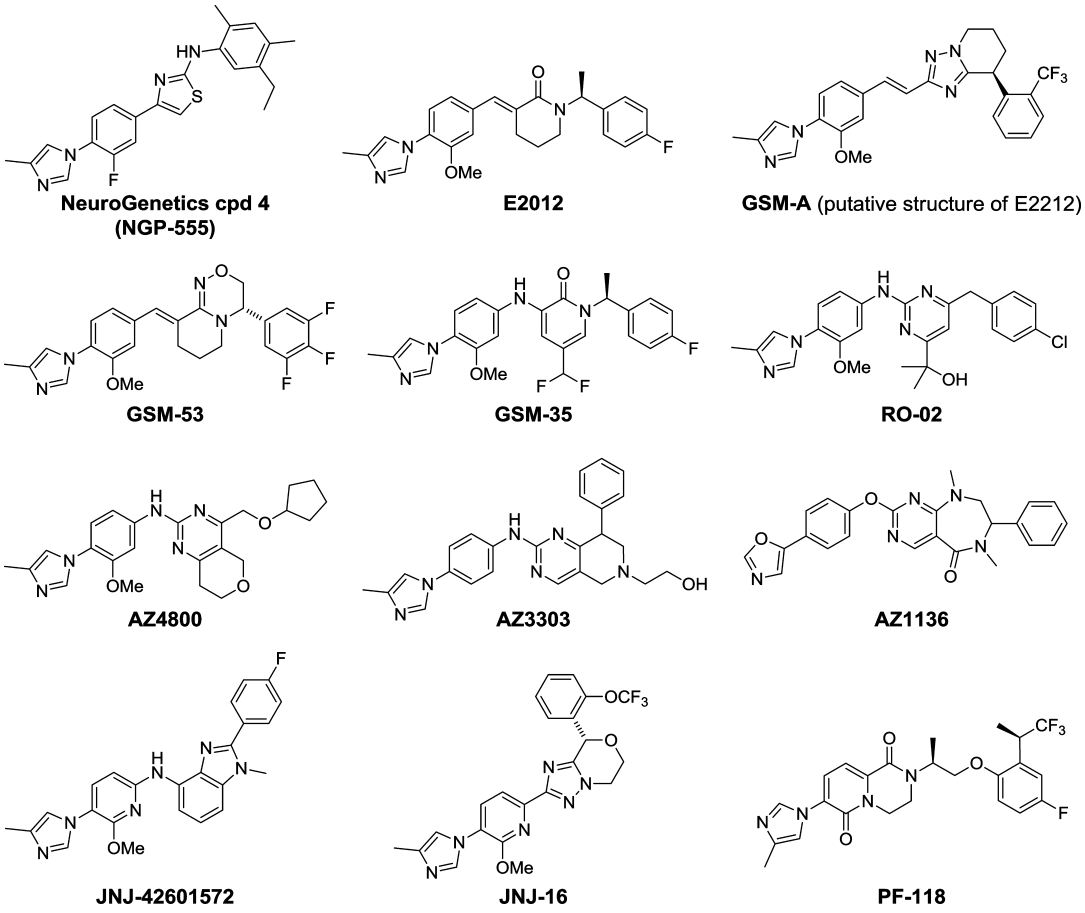


Figure 6. Structures of non-NSAID-derived heterocyclic GSMs containing the arylimidazole chemotype.

FAD-associated APP mutations in both cell-free and cell-based assays.[113] GSM-1 lowered the level of Aβ42 robustly for each APP mutant, but the reciprocal increase in the level of Aβ38 was attenuated in several cases (i.e., T43F, V44F, and I45F); for certain mutants, levels of Aβ39 (i.e., V46I and V46F) and Aβ41 (i.e., V44F) were lowered by GSM-1 treatment. Therefore, it appears that the relationship between Aβ42 and Aβ38 is not always interdependent, and the effect of GSMs on each FAD mutant should be considered independently.

GSM-10h, a pyridyl analogue of GSM-1 with lower lipophilicity, has also demonstrated excellent bioavailability and good central nervous system (CNS) penetration.[114] Additionally, acute and subchronic administration of GSM-10h to rats decreased the level of Aβ42 in plasma, CSF, and brain.[115] Furthermore, GSM-10h did not cause Aβ rebound in rat plasma or accumulation of β-CTF.[116]

Recently, a study was conducted comparing the efficacy of two GSIs (LY450139 and BMS-708,163) and GSM-2, a piperidine acetic acid (Figure 5A).[41] These compounds were administered to wild-type and 5.5-month-old Tg2576 mice for 8 days, and Y-maze tests were conducted to evaluate spatial working memory. Only GSM-2 ameliorated the cognitive deficit in Tg2576 mice. While all three drugs reduced hippocampal Aβ42 levels, β-CTF levels increased with the two GSIs but were unchanged with GSM-2. Subchronic treatment with LY450139 actually impaired normal cognitive function in WT mice, while treatment with GSM-2 had no effect. These data suggest that the cognitive impairment associated with GSI treatment could be due, at least in part, to elevation of the β-CTF level.[41]

EnVivo and Janssen have returned to the phenyl acetic acid core of flurbiprofen and added additional substituents on the core aryl ring to generate potent compounds such as EVP-0015962[117] and JNJ-40418677.[118] Chronic treatment with EVP-0015962 in Tg2576 mice reduced the levels of soluble (Tris-buffered saline-extractable), insoluble (formic acid-extractable), and aggregated Aβ42; the amyloid plaque load in the hippocampus; and cognitive deficits in the contextual fear-conditioning test.[117] The major concern with this compound is its high lipophilicity with a clogP of 6.8 (measured logD of 3.88), and it remains to be seen if the promising preclinical profile can be matched by an acceptable safety profile. Acute treatment with JNJ-40418677 reduced brain Aβ42 levels in WT mice with a concomitant increase in the level of Aβ38, while total Aβ levels in brain were not affected. In contrast, chronic administration in Tg2576 mice from 6 to 13 months of age resulted in dose-dependent reductions in the levels of of all Aβ species in soluble and deposited fractions. As with the other second-generation acids, the increase in the potency of JNJ-40418677 came at the expense of increased lipophilicity. No data were reported on the safety profile other than the fact that dosing for 7 months was tolerated with no weight loss.[118] Biogen has disclosed a phenyl acetic acid GSM (BIIB042) that appears to be a hybrid of flurbiprofen and GSM-1.[119] High drug concentrations in the brain appear to be necessary to achieve a robust decrease in the level of Aβ42, and

dx.doi.org/10.1021/bi400377p | *Biochemistry* 2013, 52, 3197−3216

Table 2. Effect of Second-Generation Non-Acid GSMs on the Production of Aβ Peptides

Non-Acid GSM	Cell-based Aβ42 IC_{50}	In vivo data	Aβ profile				
			37	38	39	40	42
E2012	92 nM[130], 143 nM[137]	rat[130], dog[128], guinea pig[137]	↑	↑	↓	↓	↓
GSM-A	8-33 nM[124]	guinea pig[124]		↑		↓	↓
NGP-555	10 - 29 nM[127],	mouse[127]	↑	↑		↓	↓
GSM-53	33 nM[133]	rat, dog, monkey[134]	↑	↑	↓	↓	↓
GSM-35	44 nM[135]	rat[135]					
RO-02	~14 nM[136]		↑	↑		↓	↓
AZ4800	26 nM[129]	mouse[129]	↑	↑	↓	↓	↓
AZ3303	74 nM[129]	mouse[129]	↑	↑	↓	↓	↓
AZ1136	990 nM[129]		↑	↓	↑	↓	↓
JNJ-42601572	16 nM[138]	rat & mouse[138], dog[139]	↑	↑		↓	↓
JNJ-16	56 nM[140]	dog[140]					↓
PF-118	<10 nM						
Merck-8	36-77 nM[141, 142]						
RO-18	158 nM						
BMS-3	< 10 nM						
Satori-1	100 nM[143]		↑	↓	↑	-	↓
SPI-1810	100 nM[144]	mouse[144]	↑	↓	↑	-	↓

this may be due in part to the high level of binding of protein to BIIB042 (>99.9% protein-bound in all species tested).

The carboxylic acid moiety is critical for both first- and second-generation NSAID and NSAID-derived GSMs. Multiple studies have shown that if a carboxylic acid GSM is converted to the corresponding ester or amide (Figure 5B), the compound behaves as an inverse GSM (iGSM) and actually increases the level of Aβ42 production (Figure 5C).[120−123]

Non-NSAID-Derived Heterocyclic GSMs. The first examples of non-NSAID-derived heterocyclic GSMs were reported in the patent literature by Neurogenetics in 2004 and by Eisai in 2005 and are characterized by the presence of an arylimidazole moiety.[3] Since then, several additional members from this class have been disclosed (Figure 6).

In contrast to the NSAID-like GSMs, the imidazole GSMs alter the cleavage site preference of γ-secretase such that levels of both Aβ42 and Aβ40 decrease, while those of Aβ37 and Aβ38 increase, albeit to different degrees depending on the compound (Table 2).[127−129] The prototypical imidazole GSM is exemplified by E2012 and has been used as a standard by many laboratories. E2012 lowers the levels of Aβ42, Aβ40, and Aβ39 and increases the levels of Aβ37 and, to a lesser extent, Aβ38 (Table 2).[128−130] E2012 entered phase I trials in 2006, and it represents the first non-NSAID GSM to enter clinical development. Following the observation of lenticular opacity in a 13-week rat safety study, clinical development of E2012 was temporarily halted. Subsequent safety studies in rats and monkeys, however, did not show ocular toxicity, and the clinical trial was allowed to proceed in April 2008. Dose-dependent reductions in the levels of Aβ42 and Aβ40 were observed in plasma in the phase I clinical trial.[131] Eisai recently reported that E2012 was not developed further in favor of an improved compound, E2212. E2212 was reported to be more potent both *in vitro* and *in vivo* than E2012 and to have a wider safety margin. The first human study began in January 2010 (doses ranging from 10 to 250 mg, ClinicalTrials.gov identifier NCT01221259); however, the present status of development is not known.

Neurogenetics has recently disclosed a detailed *in vitro* and *in vivo* characterization of imidazole GSM compound 4,[127] which was identified as NGP-555.[132] This compound reduced levels of Aβ42 and Aβ40 while concomitantly elevating levels of Aβ38 and Aβ37 without inhibiting NICD or AICD formation (Table 2). Administration of NGP-555 to 8-month-old Tg2576 mice for 7 months showed a significant reduction in plaque density and the level of amyloid deposition. The compound appeared to be tolerated well with no change in body weights and intestinal goblet cell densities. However, in contrast to results from acute dosing and cell-based assays where an increase in Aβ38 levels was observed, levels of all brain Aβ peptides (Aβ42, Aβ40, and Aβ38) were lowered in the soluble DEA-extractable, denaturing SDS-extractable, and formic acid-extractable brain fractions from Tg2576 mice dosed chronically with NGP-555 from 8 to 15 months of age. The reasons for the decrease in the level of Aβ38, most surprisingly in the soluble fraction from DEA brain extracts, are not known. Nonetheless, this is a

dx.doi.org/10.1021/bi400377p | *Biochemistry* 2013, 52, 3197−3216

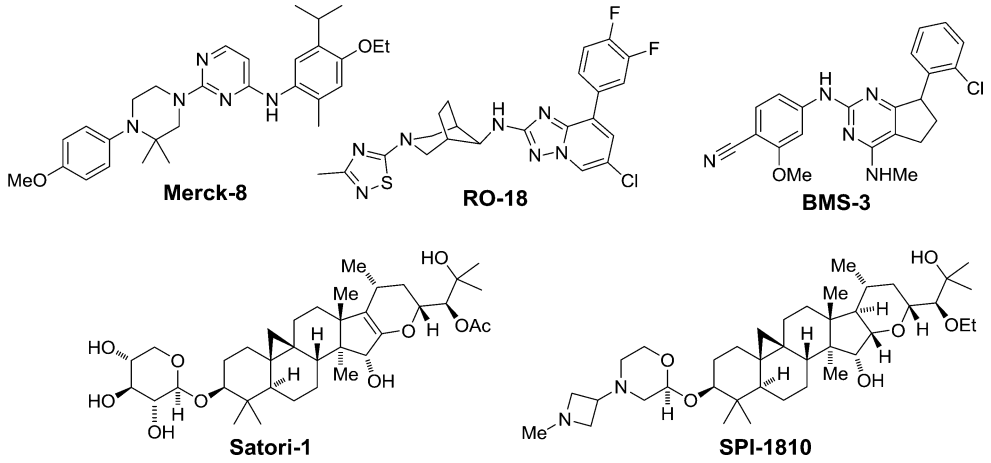


Figure 7. Additional GSMs with distinct chemotypes.

significant study because it was the first demonstration that a non-NSAID GSM could lower plaque density and amyloid load in a transgenic mouse model of AD.

The identification of the heterocyclic imidazole-containing GSM class by Neurogenetics and Eisai has spurred intense research activity throughout the industry as is evident by the large number of publications and patent applications related to this chemotype that have been published over the past several years (Figure 6). For example, Merck/Schering Plough has reported analogues of E2012, exemplified by GSM-53, that incorporate a conformational constrained fused oxadiazine as an amide replacement.[133,134] Merck, Hoffman LaRoche, AstraZeneca, and Janssen have each disclosed variations of the arylimidazole series that incorporate an aminoheterocycle. For example, Merck replaced the methylenepiperidinone of E2012 with an aminopyridone to give GSM-35.[135] Hoffman LaRoche used a similar strategy but replaced the pyridone with a pyrimidine to give the aminopyrimidine GSM RO-02.[136] AstraZeneca has also explored this chemical space and disclosed the Aβ profiles and binding characteristics of several amino-pyrimidine GSMs, exemplified by AZ4800.[129] AZ4800 reduced levels of Aβ42, Aβ40, and Aβ39 in HEK-APPswe cells and cell membranes but increased levels of Aβ38 and Aβ37 by 750 and 300%, respectively. Interestingly, the close analogue AZ3303 increased levels of Aβ37 more than levels of Aβ38, and another analogue, AZ1136, actually decreased levels of Aβ38 and increased levels of Aβ39 (Table 2). Taken together with the Aβ profiles of E2012 and NGP-555, it is apparent that small structural changes can greatly influence the relative amounts of Aβ37 and Aβ38 that are generated, although the mechanistic basis for this is not clear.

Despite the improvement in potency for the second-generation GSMs, many are still very lipophilic, which puts them at a higher risk of having off-target toxicity. As a result, it is clear from the recent patent literature that an important goal within industry is to lower the lipophilicity of candidate GSMs to improve the druglike properties while maintaining the improved potency (improved lipophilic efficiency). For example, Janssen has removed the linker altogether and attached a triazolo-oxazine heterocycle directly to the arylimidazole to give JNJ-16.[140] This compound has a good pharmacokinetic profile in dog and lowered the CSF Aβ42 level by 30−40% (20 mg/kg). The reduced lipophilicity of JNJ-16 (clogP = 3.1) relative to those of earlier GSMs translated into an improved safety profile compared to that of JNJ-42601572. Another example can be seen in a patent application from Pfizer in which the aryl core has been replaced with a bicyclic pyrido-pyrazinedione core as in PF-118. This compound is reported to lower the level of Aβ42 in CHO-APP cells with an IC_{50} of <10 nM, while possessing improved lipophilicity (clogP = 3.1).[145]

Additional GSMs with Distinct Chemotypes. All of the non-NSAID-derived heterocyclic GSMs discussed so far contain an arylimidazole (or similar heterocycle such triazole or pyridine), but alternative cores are starting to emerge (Figure 7). For example, Merck has disclosed a series of GSMs in which the arylimidazole has been replaced with a 4-methoxyphenylpiperazine as in Merck-8.[141,142] Furthermore, a recent patent from Hoffmann-LaRoche highlighted a series of bridged amino-piperidines represented by RO-18[146] in which a large portion of the exemplified compounds contain a thiadiazole left-hand ring. Additionally, BMS has disclosed GSMs in which the ubiquitous left-hand heterocycle (imidazole, triazole, and pyridine) has been replaced with a nitrile.[147] For example, BMS-3 is reported to have an Aβ42 IC_{50} of <10 nM. Despite this structural diversity, the basic pharmacophore is maintained in that two H-bond acceptors are separated by a conformationally constrained cyclic core with a lipophilic aryl group on the right-hand side.

A truly structurally distinct chemotype has been introduced by Satori Pharmaceuticals (Figure 7). They have disclosed a new series of GSMs that were isolated from the black cohosh plant with the triterpene glycoside Satori-1 as the initial hit.[143] Subsequent optimization to improve metabolic stability and CNS disposition led to SPI-1810.[144] These GSMs have a distinct Aβ profile in that they lower the levels of both Aβ42 and Aβ38 but maintain total Aβ levels by increasing the levels of Aβ39 and Aβ37.

■ MECHANISM OF ACTION OF GSMS

To determine the mechanism of action of GSMs, the following critical questions have to be addressed. (1) What are the targets of GSMs? (2) Do different classes occupy the same or overlapping binding sites of the target(s)? (3) What is the molecular basis for cleavage shifting and substrate specificity? (4) Do different classes of GSMs have similar mechanisms?

Notable Chemical Biology and Biochemistry Techniques. Because of the lack of high-resolution structural information and intrinsic complexity of the γ-secretase complex, investigators have had to use creative methods to study the mechanism of action of the diverse and myriad small molecules

dx.doi.org/10.1021/bi400377p | *Biochemistry* 2013, 52, 3197−3216

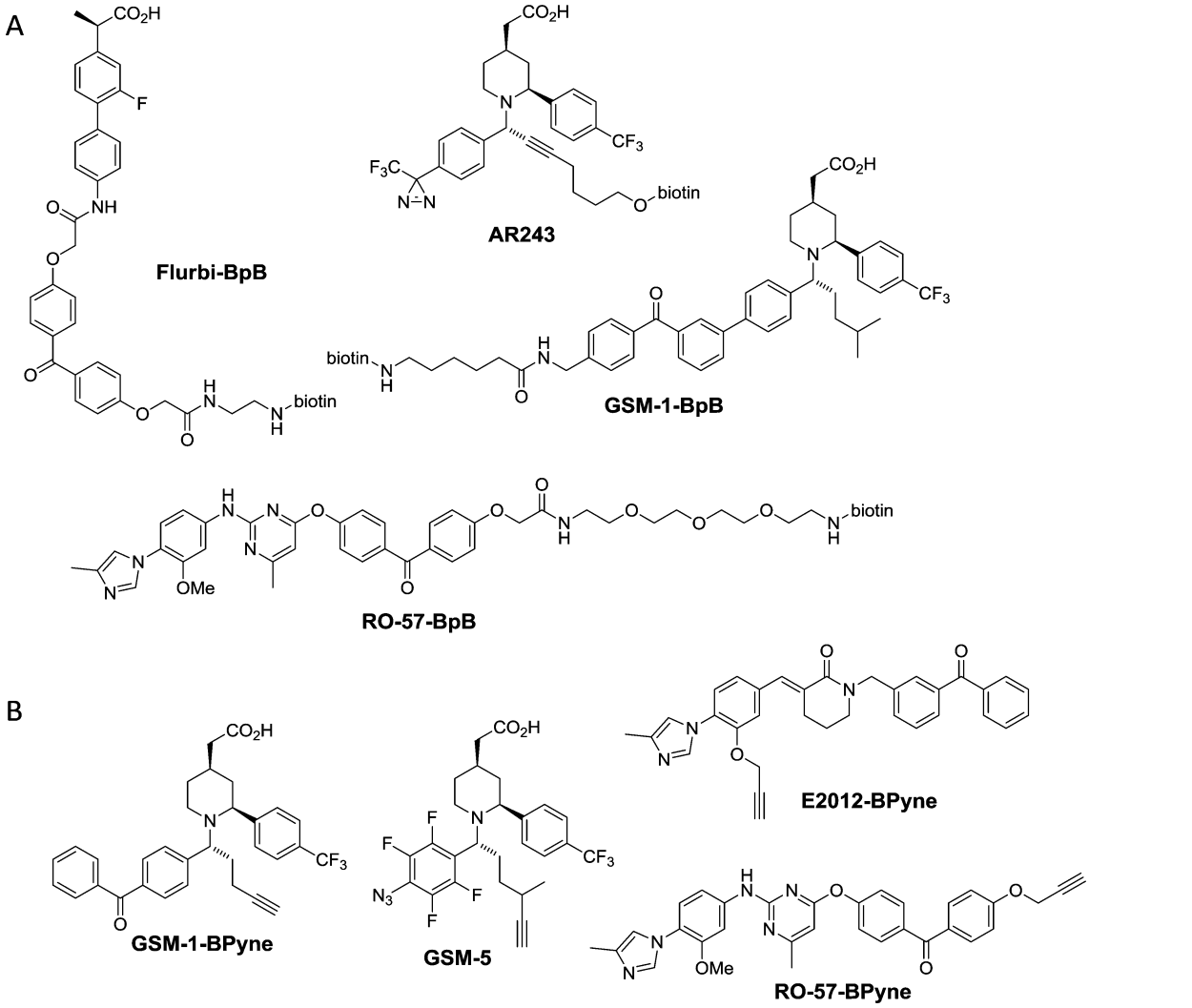


Figure 8. Structures of GSM-derived photoaffinity probes containing (A) biotin or (B) a clickable alkyne handle.

that target γ-secretase activity. Photoaffinity labeling (PAL) has widely been used for target identification of small molecules.[148] PAL has been instrumental in not only the identification of presenilin as the catalytic component of γ-secretase[68] but also the determination of the target of many γ-secretase inhibitors.[12,149−151] Common cross-linking moieties include the photoreactive benzophenone, diazirine, and phenylazide motifs.[152] Many GSM photoprobes contain a biotin tag for affinity purification of the labeled enzyme (Figure 8A). However, incorporation of a bulky biotin group could reduce the potency of parental compounds. Therefore, employing PAL with a smaller alkyne tag can be beneficial; moreover, the alkyne is more versatile because either a biotin or fluorescent tag can be "clicked" on using a copper-catalyzed azide−alkyne cycloaddition (CuAAC) reaction[153,154] (Figure 8B).

Another challenge is to detect small molecule-induced conformational changes in γ-secretase within the lipid bilayer. Currently, three select approaches that have been used for such studies are fluorescence lifetime imaging (FLIM), photophore walking, and the surface cysteine accessibility method (SCAM). By using FLIM, which measures the decay rate of a fluorophore rather than the intensity, one can measure the distance or, more importantly, changes in the distance between two fluorescence resonance energy transfer (FRET) pairs. Studies have looked at both the distance between the substrate and enzyme (APP C-terminus and PS1 loop)[155] and distances from the CTF to NTF domains within PS1 itself.[155,156] The conformational changes within presenilin have been studied by two methods: one used a pair of FITC- and CY3-labeled antibodies that bind two different epitopes on presenilin-1,[155] while the other used a G-PS1-R fusion protein that has a GFP tag on the N-terminus and an RFP tag within the C-terminal loop of presenilin.[123,156] Through these studies, it appears that γ-secretase adopts an "open" conformation when GSMs bind, resulting in an increased distance between the N- and C-termini of PS1 as measured by a longer fluorescence lifetime of the donor fluorophore.

The "photophore walking" approach[157] has been developed to detect conformational changes in the γ-secretase active site. Requirements of photophore walking probes include (1) an ability to interact with the active site and (2) the fact that photoactivatable groups are incorporated into different side chains along the probe and, therefore, cross-link to different subpockets within the active site. Because the efficiency of photolabeling depends on the contact region and proximity to residues within the active site, conformational changes induced by GSMs that alter the orientation or distance between a subpocket and the photophore can lead to different cross-

dx.doi.org/10.1021/bi400377p | *Biochemistry* 2013, 52, 3197−3216

Table 3. Summary of Evidence for the Target of NSAID GSMs

GSM used in this study	method	target	ref
fenofibrate, R-flurbiprofen	biotinylated probes label recombinant C100 and APP-CTF83 from cells	APP	120
ibuprofen, flurbiprofen, indomethacin, fenofibrate	conformational changes induced by GSMs and/or C99, NotchΔEC, or helical peptide substrates were monitored using a FRET-based FLIM assay	APP−PS1 border, PS1 with docking	155, 156
sulindac sulfide	circular dichroism and electron spin resonance with SDS-solubilized C100 and C100 mutant substrates	C100 and C100 dimer	167
sulindac sulfide, indomethacin	surface plasmon resonance (SPR) and solution state NMR	Aβ42	168
sulindac sulfide, sulindac sulfone, R-flurbiprofen	noncompetitive displacement of [^{3}H]GSIs	γ-secretase	164, 165
sulindac sulfide	*in vitro* assay shows noncompetitive inhibition of γ-secretase	γ-secretase, not APP	163
sulindac sulfide	noncompetitive displacement of [^{3}H]L-685,458	SPP	166
R-flurbiprofen, indomethacin, fenofibrate, sulindac sulfide	monitored binding with TROSY NMR of GSMs with [U-^{15}N]C99, or titration of C99 while measuring ^{19}F GSMs	not APP	169
sulindac sulfide, flurbiprofen, sulindac sulfone	measure binding with ^{15}N HSQC and SPR	not Aβ	170

linking efficiencies. By exploiting the complementarity of more than one probe, one can examine changes within the active site of γ-secretase by comparing differences in labeling efficiency of each probe in the presence or absence of a modifier. This approach has been used to characterize different inhibitors and GSMs,[12,154,158,159] as well as to investigate the conformational changes caused by PS1 FAD mutants.[157] Importantly, the structure−activity relationship (SAR) of the active site-directed photoprobes[68,160−162] indicates that the subpockets within γ-secretase have enough plasticity for interacting with different sized side chains because substitution of Phe with BPA (benzoylphenylalanine) at different positions did not alter the potency of these probes.[68,160−162] This method allows investigation of endogenous γ-secretase in any cell type or tissue. The surface cysteine accessibility method (SCAM) is another interesting and unique methodology that has been used to characterize γ-secretase modulators. This method allows the identification of which amino acid residues are embedded in the membrane and what environmental changes, such as GSM binding, can alter the water accessibility of certain amino acids on presenilin.[123]

Mechanism of Action. *First-Generation NSAID GSMs.* Initially, it was reported that NSAIDs bound to the γ-secretase complex at some undefined allosteric site because of their noncompetitive inhibition of γ-secretase[163] and noncompetitive displacement of radiolabeled GSIs.[164,165] Furthermore, it was shown that sulindac sulfide could also noncompetitively displace [^{3}H]L-685,458 from SPP, an aspartyl intramembrane protease, thus suggesting that NSAID GSMs also had a binding site for SPP.[166] Using FLIM-based FRET imaging, Lleó et al. showed that the presence of NSAID GSMs resulted in an increase in the distance between the APP C-terminus and the loop region of PS1 as determined by a measured increase in the lifetime of the donor fluorophore, the FITC-labeled C-terminus of APP.[155] Through similar methods, they were also able to show an increase in the distance between PS1-NTF and PS1-CTF fragments upon GSM binding, suggesting a conformational change to PS1 upon NSAID binding[155] (Table 3).

However, in 2008 Kukar et al. published a paradigm-shifting paper suggesting that GSMs bound to the substrate APP rather than to the γ-secretase complex.[120] Using benzophenone- and biotin-containing molecular probes derived from fenofibrate and flurbiprofen [Flurbi-BpB (Figure 8)], they found incorporation of the probe in APP-CTF83 (αCTF), but not APP-CTF99 (βCTF), from CHAPSO-solubilized H4-APP-alkaline phosphatase cells. Moreover, they were unable to find any labeling of γ-secretase complex subunits purified from CHO cells.[120] Both probes showed a dose-dependent (10−150 μM) increase in the level of binding to a recombinant APP-C100-Flag substrate, which is essentially the βCTF substrate of γ-secretase required for Aβ production. Furthermore, they show that labeling of C100-Flag by the fenofibrate probe can be partially challenged with multiple NSAID GSMs at 100 μM, and fenofibrate prefers binding to APP(C100)-Flag compared to Notch(C100)-Flag substrate. Using a series of truncated Aβ peptides, they mapped the binding site of the GSMs to Aβ28−36 (see Figure 1), which includes the beginning of the transmembrane domain of APP.[120] This finding not only offers a straightforward explanation of substrate selectivity but also provides an interesting mechanism for modulation of γ-secretase through targeting of the substrate, rather than the enzyme. Similarly, Espeseth et al. had previously reported on a series of APP binding compounds that also inhibited Aβ42 production.[171]

Munter et al. demonstrated not only that the GxxxG motif that corresponds to residues 29−33 within Aβ was important for dimerization of the APP transmembrane domain but also that an increase in dimerization strength within the TM region is correlated with an increase in the level of Aβ42 production relative to those of other Aβ species.[172] Conversely, if the GxxxG motif is mutated and/or disrupted so that dimerization is lost, then γ-secretase cleavage is altered so that there is an increase in the level of Aβ38 production but a decrease in the level of Aβ42.[172] This led to the hypothesis that GSMs may bind to the GxxxG motif in βCTF and alter the transmembrane dimerization of APP, resulting in modulation of cleavage from Aβ42 to Aβ38 production. Support for this hypothesis emerged from the work of Richter et al. that suggests that sulindac sulfide, and to a lesser degree indomethacin, could inhibit dimerization of the APP TM domain in a β-galactose-based dimerization assay using a ToxR fusion protein with residues 29−42 of the APP membrane.[168] They also showed that sulindac sulfide could directly bind to immobilized Aβ42 as measured by surface plasmon resonance (SPR) and that incubation of 100 μM Aβ42 with 300 μM sulindac sulfide yielded NMR chemical shifts at several residues, including a few within the purported binding domain of GSMs.[168] Further studies using SPR showed that sulindac sulfide prefers binding a C100 mutant that has an increased propensity for dimerization compared to that of wild-type C100.[167] Similarly, sulindac sulfide prefers binding the wild type with respect to a G33I mutant that disrupts the GxxxG motif and does not readily

Table 4. Summary of the Evidence for the Targeting of Second-Generation GSMs

GSM used in study	method	target	ref
GSM-1 (acid GSM)	biotinylated or clickable photoprobe labels PS1-NTF in cell membranes or with recombinant PS proteins	PS1-NTF, PS1-FL, PS1-ΔE9 without APP	123, 154
BB25 AR80 (acid GSM)	biotinylated photoprobes label PS1-NTF in cell membranes and live cells	PS1-NTF	178
NGP-555 (imidazole GSM)	pull-down assay with GSM immobilized Affigel matrix	Pen-2 ≫ PS1-NTF > PS1-CTF	127
RO-57 (imidazole GSM)	biotinylated photoprobes label PS-NTF in cell membranes	PS1-NTF, PS2-NTF	136
E2012 (imidazole GSM)	clickable photoprobes label PS1-NTF in cell membranes, recombinant PS proteins, and live cells and neurons	PS1-NTF, PS1-ΔE9 without APP	179

form dimers.[167] However, this work has not been repeated with more potent second-generation GSMs, so the functional significance awaits further study.

Beel et al. studied the biochemical nature of the interaction between βCTF and GSMs by using recombinant purified [U-^{15}N]C99 in LMPG micelles monitored by ^{1}H−^{15}N TROSY protein NMR, but they found no specific binding between C99 and R-flurbiprofen, fenofibrate, indomethacin, or sulindac sulfide.[169] Instead, they found only a few chemical shifts that were nonspecific in nature and did not correspond to the purported Aβ28−33 binding region of GSMs.[169] Interestingly, they found that GSMs did in fact bind to aggregated C99 and that the aggregated protein seems to promote the formation of GSM aggregates.[169] Similarly, in response to findings that GSMs bind directly to Aβ,[167,168] Barrett et al. performed additional SPR and protein NMR experiments with the Aβ42 peptide.[170] They again found that GSMs only nonspecifically bind to Aβ, and this binding can be eliminated with micelle formation.[170] Importantly, they also show using dynamic light scattering (DLS) that sulindac sulfide forms aggregates at concentrations above 50 μM.[170] Furthermore, Page et al. examined the effect of GSMs on multiple APP FAD mutations together with systemic phenylalanine scanning mutagenesis near the γ-secretase cleavage site (including the GxxxG domain) and found that the overwhelming majority of mutants responded well to the second-generation NSAID-derived GSM-1, and that the iGSM fenofibrate was also responsive to G33I and K28E mutations, thus further creating uncertainty about the binding of GSMs to the GxxxG domain.[113] Recently, NMR structural studies revealed that βCTF exists as a monomer and the GxxxG motif plays an important role in cholesterol binding.[173]

If the first hypothesis is that GSMs bind γ-secretase and the second is that GSMs bind directly to the APP substrate, then the third is that the compounds bind both. The latter theory is supported by several FRET-based FLIM assays with fluorescently tagged PS1 in APP/APLP2 knockout cells.[156] Uemura et al. showed that the conformational changes induced by NSAID GSMs in PS1 first require substrate docking by either C99, NotchΔEC, or a helical peptide.[156] It is interesting to note, however, that neither NotchΔEC nor the helical peptide contains the GxxxG motif, suggesting that substrate dimerization is not necessary for NSAID-induced PS1 conformational changes.

If NSAIDs do indeed bind solely to APP, and this is how selectivity is achieved, then one would expect NSAIDs to be selective for APP and not to bind other γ-secretase substrates. There is controversy about whether NSAID GSMs affect any substrates other than APP. Several groups have claimed that NSAIDs have an effect on Notch, by either reducing Nβ[174] or by binding to N100-Flag,[120] but in both of these studies, higher concentrations of NSAIDs were required for Notch than for APP. However, other groups have found that NSAIDs have no effect on Nβ,[175,176] CD44-β, or an APP−Notch TMD chimera.[176] Furthermore, NSAIDs have been shown to noncompetitively compete for binding of SPP[166] and to alter the cleavage site of the SPP substrate Prl.[177] These data suggest that NSAID GSM binding is unlikely to be entirely on the substrate but could be on the interface between the substrate and enzyme.

There is likely a complicated binding mechanism for GSMs, perhaps on the interface between the enzyme and the substrate or perhaps in the presence of multiple binding sites, and unfortunately, the high concentrations required because of the low efficacy of first-generation compounds complicates the interpretation of findings.

Second-Generation GSMs. Despite the large structural variation among second-generation GSMs, all work done to date on the more potent (IC_{50} < 300 nM) GSMs shows invariably that γ-secretase is indeed the target of these molecules. By immobilizing an imidazole-based second-generation GSM, Kounnas et al. first showed that this GSM could pull down components of the γ-secretase complex such as Pen-2, PS1-NTF, and PS1-CTF, but not APP.[127] Shortly thereafter, several independent laboratories simultaneously designed photo-cross-linking probes based on multiple second-generation GSMs and indisputably showed specific labeling of PS1-NTF but not APP[123,136,154,178,179] (Table 4).

The NSAID-derived piperidine acetic acid GSM-1 directly binds to PS1-NTF using photoaffinity probes GSM-1-BpB, GSM-1-BPyne, and GSM-5 (see Figure 8).[123,154] Furthermore, GSM-1-BpB was suggested to bind to residues 78−100 of TMD1 of PS1-NTF.[123] It appears that upon binding to this region of PS1, GSM-1 is able to induce an overall conformational change in γ-secretase as visualized by a FLIM study[123] as well as a conformational change within the active site of γ-secretase.[154] Interestingly, GSM-1-BpB was found to also bind full-length PS1,[123] the zymogen of γ-secretase.[68,70] Importantly, GSM-1-BPyne and GSM-5 bind to a reconstituted PS1 mutant, PS1ΔE9, in liposomes without any substrates present.[154] Together, these data paint a very different picture than what has previously been hypothesized for NSAID GSMs: GSM-1 can bind PS1 independent of any substrates and can presumably bind an inactive enzyme. Interestingly, the GSM-1 probes were also able to specifically label SPP,[123,154] a structurally related intramembrane aspartyl protease, which was also reported for the NSAID sulindac sulfide.[166]

The Roche imidazole-based GSMs were also found to directly label PS1-NTF and PS2-NTF.[136] Competition studies with labeling of the RO-57-BpB probe showed good

competition with E2012, but not NGP-555 like GSMs, all of which belong to the imidazole class of second-generation GSMs. Moreover, sulindac sulfide (100 μM) could compete for RO-57-BpB binding, but neither GSM-1 nor fenofibrate had any effect on RO-57-BpB labeling of PS1, although GSM-1 was found to partially block RO-57-BpB labeling of PS2, suggesting there could be partial overlap in the binding sites.[136] This also raises a critical issue for cross-talk studies regarding concentrations and the solubility of competing compounds. It can be addressed by conducting the competition in a dose responsive fashion in which compounds maintain solubility under assay conditions, further elucidating the nature of the competition.

Recently, using a series of reciprocal labeling experiments with GSM-1- and E2012-based photoaffinity probes, our groups have shown that the two compounds have distinct binding sites on PS1-NTF.[179] Moreover, unlike that of GSM-1-BPyne, labeling of PS1-NTF by E2012-BPyne is significantly potentiated in the presence of L458, showing direct cross-talk between the E2012 binding site and the active site of the enzyme.[179] Surprisingly, binding of L458 has no effect on RO-57-BPyne labeling,[179] suggesting that E2012 and RO-57 could have distinct effects even though both are from the same imidazole class of GSMs. In contrast to GSM-1-BpB, E2012-BPyne specifically labels PS1-NTF (active γ-secretase) but not full-length PS1 (inactive γ-secretase). Furthermore, the GSI BMS-708,163 binds to PS1-NTF, and the binding site does not overlap with the sites that interact with GSM-1 or E2012 (Figure 9).[159,179]

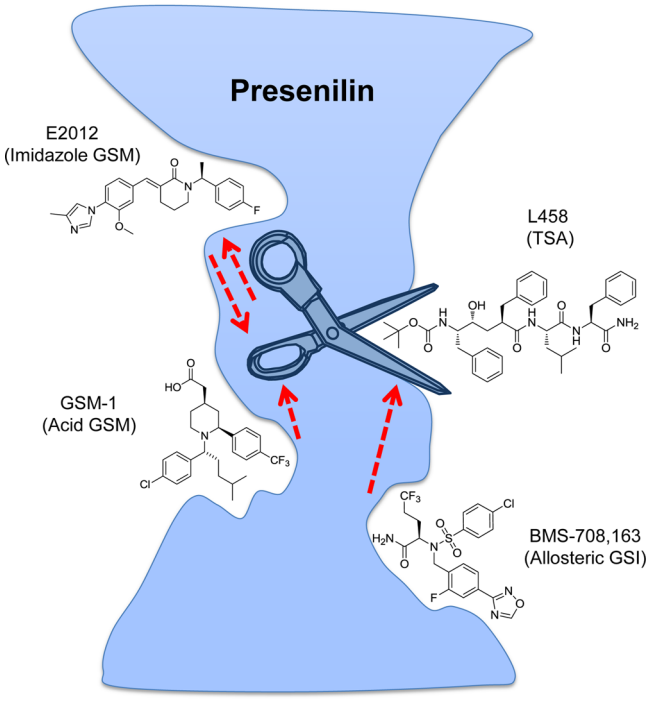


Figure 9. Model for different binding sites of GSMs and GSIs. The active site of γ-secretase is represented by a pair of scissors. GSMs alter the "handle" of the scissors, thereby manipulating the way the enzyme cuts and/or the location of the cleavage sites. In contrast, an allosteric GSI will shut the blades, whereas a transition state analogue (TSA) will block the blades of the scissors, preventing substrate binding and cleavage of the substrate.

Taken together, although both piperidine acetic acid GSMs and E2012-like GSMs target PS1, it appears they occupy different sites within the γ-secretase complex (Figure 9). Consequently, they lead to varying pharmacological effects on Aβ species (Tables 1 and 2), such that acid GSMs reduce the level of Aβ42 production and enhance that of Aβ38, whereas imidazole GSMs differentially decrease th levels of Aβ42 and Aβ40 and concurrently increase the levels of Aβ38 and/or Aβ37. It is noteworthy to point out there is great diversity within the imidazole GSMs, which are exemplified by NGP-555, E2012, and RO-57 (Figure 6).[136,179] Therefore, the interplay between different subtypes of imidazole GSMs and other classes of GSMs should be carefully examined.

The next key question is how the binding of second-generation GSMs to γ-secretase induces a conformational change that has been detected by FLIM,[156] SCAM,[123] or photophore walking[154] (Figure 10A). We propose two alternative models of how GSM-induced conformation, such as the S1 subpocket alteration,[154] leads to γ-secretase modulation (Figure 10). (1) Acid GSMs mainly affect the sequential processing cycle of Aβ42 to Aβ38,[180] which has been suggested to be due to slower dissociation of the Aβ42 substrate from the γ-secretase complex allowing further processing to Aβ38 (Figure 10B).[23] The overall result is reduction in the level of Aβ42 and elevation of the level of Aβ38. However, E2012 and many imidazole GSMs are known to preferentially increase the level of Aβ37 (Table 2), which presumably represents a fifth γ-secretase cleavage from the Aβ49 product line. Therefore, imidazole GSMs could bind to γ-secretase in a way that alters the sequential cycles of Aβ42 to Aβ38 and Aβ40 to Aβ37 to achieve γ-secretase modulation. (2) Alternatively, the GSM-induced conformational change could specifically block Aβ42 or Aβ40 production and potentiate Aβ38 or Aβ37 generation based on the independent cleavage model in which all sites of cleavage are parallel (Figure 10C). However, the newly discovered natural product GSMs (Figure 7) inhibit both Aβ38 and Aβ42 while increasing the levels of Aβ37 and Aβ39, suggesting a mechanism different from that of imidazole and acid GSMs. These Satori compounds could be operating by a mechanism similar to model 1 in which both the Aβ40 and Aβ42 peptide substrates have a slower dissociation rate, resulting in further processing to Aβ37 and Aβ39 peptides, or alternatively by model 2 in which Aβ38 and Aβ42 cleavage is specifically and independently blocked while Aβ37 and Aβ39 cleavage is enhanced. However, more information is clearly needed to determine how these natural product compounds compare with imidazole and acid GSMs.

SUMMARY AND FUTURE PERSPECTIVE

APP is processed into three major species, sAPP, Aβ, and AICD. GSMs cause a shift from Aβ42 to shorter less toxic Aβ species and have little effect on the generation of AICD and NICD, thus allowing their signaling roles to remain intact. As a result, GSI-mediated adverse effects should not be a concern, offering the hope that GSMs will become promising disease-modifying agents. Indeed, comparative studies of GSIs and GSMs in mice have supported such a notion.[41] Moreover, γ-secretase contains distinct sites that interact with different GSMs, which highlights the fact that γ-secretase can be modulated in multiple ways.[179] Although modulation of γ-secretase holds much promise, significant questions remain to be answered. First, although it is clear that GSMs are different from GSIs, it is not known if other safety issues will emerge

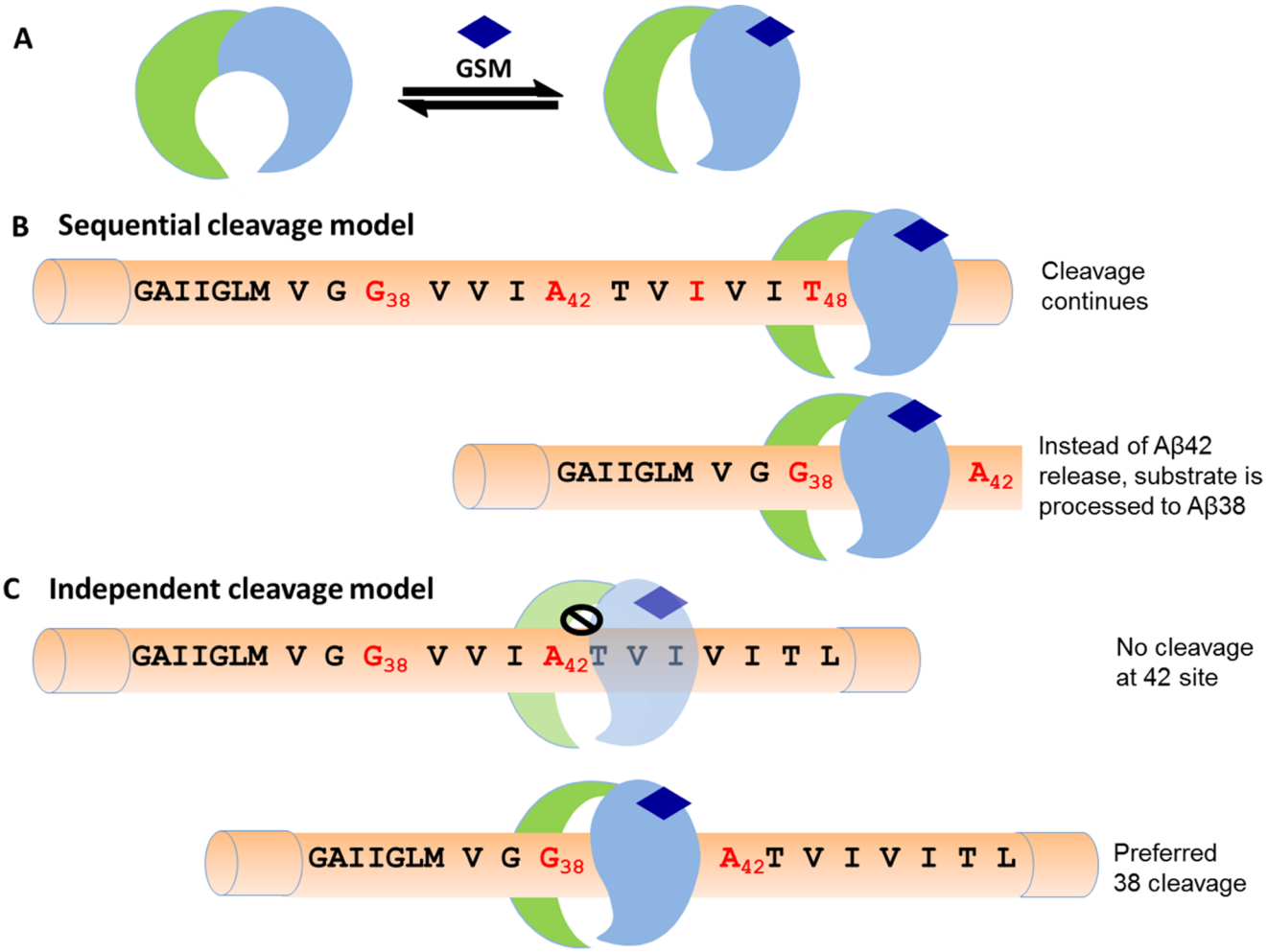


Figure 10. Proposed models for the mechanism of GSMs. (A) GSM binding leads to a conformational change in the active site, such as the S1 subpocket. (B) Sequential cleavage model. GSM binding has little effect on the processivity of γ-secretase at 48 and 45 sites; however, a tighter association of γ-secretase with Aβ42 results in a reduced rate of release of Aβ42 and an increase in the rate of generation of Aβ38. (C) Independent cleavage model. All cleavage sites are parallel; GSM binding inhibits Aβ42 cleavage but enhances Aβ38 cleavage and has little effect on other cleavages, including AICD production.

with the chronic treatment of GSMs. Second, while much progress has been made in understanding the target of GSMs, more work is needed to determine their precise binding sites and the molecular basis for their mechanism of action. The mechanism that regulates when γ-secretase cleavage of APP ends and Aβ is released is not well-understood, and it will be important to understand the influence GSMs have on that process. In addition, because different GSMs have distinct Aβ profiles, it may be difficult to determine the mechanism without considering the full Aβ profile, including shorter Aβ peptides such as Aβ37. It will be important to find efficient ways to quantitatively measure all Aβ species. Furthermore, it has been shown recently that mutation of lysine 624 of APP (K28A of Aβ) shifts the final γ-secretase cleavage site to favor shorter Aβ species such as Aβ1−33 and Aβ1−34, suggesting a pivotal role for this charged residue in preventing the continuation of APP cleavage by γ-secretase.[181] Further studies that help elucidate the precise mechanism of action of GSMs are highly anticipated. Third, it would be interesting to consider if different classes of GSMs could be used as a combination therapy or in combination with a BACE inhibitor. Finally, it is unknown whether successful GSM clinical trials could be conducted without the availability of effective biomarkers for early diagnosis of AD. It has been suggested that the pathological process of AD starts more than 10 years before clinical symptoms manifest.[182] Because some second-generation GSMs have been found to be responsive to several PS1 and APP mutations,[110,113] perhaps a prevention trial in asymptomatic patients with FAD mutations could be considered similar to the DIAN trial that is being planned.[111,112] Clearly, the development of GSMs for the treatment AD not only relies on the discovery of effective drug candidates but also is dependent on the progress of AD research in molecular pathogenesis, biomarkers, diagnosis, and other therapeutic developments. Undoubtedly, with the recognition that AD is the fastest growing threat to human health, an interdisciplinary approach and significant effort are required to drive these critical issues toward resolution for the development of effective AD therapies.

AUTHOR INFORMATION

Corresponding Author

*Address: 1275 York Ave., Box 459, ZRC 1904, New York, NY 10065. E-mail: liy2@mskcc.org. Phone: (646) 888-2193.

Funding

This work was supported by National Institutes of Health (NIH) Grants 1R01NS076117 and 2R01AG026660 (Y.-M.L.), a training grant in the pharmacological sciences (NIH Grant T32 GM073546 to C.J.C.), Alzheimer Association Grant IIRG-08-90824, the American Health Assistance Foundation (Y.-M.L.), Pfizer (Y.-M.L.), the Geoffrey Beene Cancer Research Center of MSKCC, Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research, the Experimental Therapeutics Center of MSKCC, and the William Randolph Hearst Fund in Experimental Therapeutics.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

αCTF, α-secretase-cleaved C-terminal fragment of APP; βCTF, β-secretase-cleaved C-terminal fragment of APP; AD, Alzheimer's disease; ADAM, disintegrin and metalloproteinase domain-containing protein; AICD, APP intracellular domain; APH-1, anterior pharynx defective-1; APLP2, amyloid β precursor-like protein 2; APP, amyloid precursor protein; Aβ, β-amyloid peptide; CHAPSO, 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonate; COX, cyclooxygenase; CSF, cerebral spinal fluid; CSL, CBF1/Su(H)/Lag-1, also known as the RBP-Jκ family; CuAAC, copper(I)-catalyzed azide−alkyne cycloaddition; FAD, familial Alzheimer's disease; FITC, fluorescein isothiocyanate; FLIM, fluorescence lifetime imaging microscopy; FRET, Förster (fluorescence) resonance energy transfer; GSAP, γ-secretase activating protein; GSI, γ-secretase inhibitor; GSK, GlaxoSmithKline; GSM, γ-secretase modulator; HES1, Hairy and enhancer of split-1; HEY, Hairy/enhancer-of-split related with YRPW motif protein; iGSM, inverse γ-secretase modulator; LMPG, lyso-myristoylphosphatidylglycerol; MamL, mastermind-like; NCT, Nicastrin; NICD, Notch intracellular domain; NMR, nuclear magnetic resonance; NotchΔEC, Notch with the extracellular domain removed; NSAIDs, nonsteroidal anti-inflammatory drugs; PAL, photoaffinity labeling; PEN2, presenilin enhancer 2; PS, presenilin; PS1-CTF, presenilin-1 C-terminal fragment; PS1-NTF, presenilin-1 N-terminal fragment; PS1ΔE9, presenilin-1 with exon 9 removed; sAPPα, soluble APP, α-secretase-cleaved; sAPPβ, soluble APP, β-secretase-cleaved; SPP, signal peptide peptidase; TM, transmembrane; TMD, transmembrane domain; TROSY, transverse relaxation optimized spectroscopy; TSA, transition state analogue.

■ REFERENCES

(1) Weggen, S., Eriksen, J. L., Das, P., Sagi, S. A., Wang, R., Pietrzik, C. U., Findlay, K. A., Smith, T. E., Murphy, M. P., Bulter, T., Kang, D. E., Marquez-Sterling, N., Golde, T. E., and Koo, E. H. (2001) A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. *Nature 414*, 212−216.

(2) Oehlrich, D., Berthelot, D. J., and Gijsen, H. J. (2011) γ-Secretase Modulators as Potential Disease Modifying Anti-Alzheimer's Drugs. *J. Med. Chem. 54*, 669−698.

(3) Pettersson, M., Kauffman, G. W., am Ende, C. W., Patel, N. C., Stiff, C., Tran, T. P., and Johnson, D. S. (2011) Novel γ-secretase modulators: A review of patents from 2008 to 2010. *Expert Opin. Ther. Pat. 21*, 205−226.

(4) Bulic, B., Ness, J., Hahn, S., Rennhack, A., Jumpertz, T., and Weggen, S. (2011) Chemical Biology, Molecular Mechanism and Clinical Perspective of γ-Secretase Modulators in Alzheimer's Disease. *Curr. Neuropharmacol. 9*, 598−622.

(5) Wolfe, M. S. (2012) γ-Secretase inhibitors and modulators for Alzheimer's disease. *J. Neurochem. 120* (Suppl. 1), 89−98.

(6) Wagner, S. L., Tanzi, R. E., Mobley, W. C., and Galasko, D. (2012) Potential Use of γ-Secretase Modulators in the Treatment of Alzheimer Disease. *Arch. Neurol.*, 1−4.

(7) Xia, W., Wong, S. T., Hanlon, E., and Morin, P. (2012) γ-Secretase modulator in Alzheimer's disease: Shifting the end. *J. Alzheimer's Dis. 31*, 685−696.

(8) Glenner, G. G., and Wong, C. W. (1984) Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun. 120*, 885−890.

(9) Glenner, G. G., and Wong, C. W. (1984) Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. Biophys. Res. Commun. 122*, 1131−1135.

(10) Hardy, J., and Allsop, D. (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol. Sci. 12*, 383−388.

(11) Zheng, H., and Koo, E. H. (2011) Biology and pathophysiology of the amyloid precursor protein. *Mol. Neurodegener. 6*, 27.

(12) Tian, Y., Bassit, B., Chau, D., and Li, Y. M. (2010) An APP inhibitory domain containing the Flemish mutation residue modulates γ-secretase activity for Aβ production. *Nat. Struct. Mol. Biol. 17*, 151−158.

(13) Tian, Y., Crump, C. J., and Li, Y. M. (2010) Dual role of α-secretase cleavage in the regulation of γ-secretase activity for amyloid production. *J. Biol. Chem. 285*, 32549−32556.

(14) Nitsch, R. M., Slack, B. E., Wurtman, R. J., and Growdon, J. H. (1992) Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science 258*, 304−307.

(15) Skovronsky, D. M., Moore, D. B., Milla, M. E., Doms, R. W., and Lee, V. M. (2000) Protein kinase C-dependent α-secretase competes with β-secretase for cleavage of amyloid-β precursor protein in the trans-Golgi network. *J. Biol. Chem. 275*, 2568−2575.

(16) Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R., Jasionowski, M., Haass, C., and Fahrenholz, F. (1999) Constitutive and regulated α-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl. Acad. Sci. U.S.A. 96*, 3922−3927.

(17) Postina, R., Schroeder, A., Dewachter, I., Bohl, J., Schmitt, U., Kojro, E., Prinzen, C., Endres, K., Hiemke, C., Blessing, M., Flamez, P., Dequenne, A., Godaux, E., van Leuven, F., and Fahrenholz, F. (2004) A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *J. Clin. Invest. 113*, 1456−1464.

(18) Zhao, G., Mao, G., Tan, J., Dong, Y., Cui, M. Z., Kim, S. H., and Xu, X. (2004) Identification of a new presenilin-dependent ζ-cleavage site within the transmembrane domain of amyloid precursor protein. *J. Biol. Chem. 279*, 50647−50650.

(19) Sastre, M., Steiner, H., Fuchs, K., Capell, A., Multhaup, G., Condron, M. M., Teplow, D. B., and Haass, C. (2001) Presenilin-dependent γ-secretase processing of β-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. *EMBO Rep. 2*, 835−841.

(20) Qi-Takahara, Y., Morishima-Kawashima, M., Tanimura, Y., Dolios, G., Hirotani, N., Horikoshi, Y., Kametani, F., Maeda, M., Saido, T. C., Wang, R., and Ihara, Y. (2005) Longer forms of amyloid β protein: Implications for the mechanism of intramembrane cleavage by γ-secretase. *J. Neurosci. 25*, 436−445.

(21) Kakuda, N., Funamoto, S., Yagishita, S., Takami, M., Osawa, S., Dohmae, N., and Ihara, Y. (2006) Equimolar production of amyloid β-protein and amyloid precursor protein intracellular domain from β-carboxyl-terminal fragment by γ-secretase. *J. Biol. Chem. 281*, 14776−14786.

(22) Takami, M., Nagashima, Y., Sano, Y., Ishihara, S., Morishima-Kawashima, M., Funamoto, S., and Ihara, Y. (2009) γ-Secretase:

Successive tripeptide and tetrapeptide release from the transmembrane domain of β-carboxyl terminal fragment. *J. Neurosci. 29*, 13042−13052.
(23) Okochi, M., Tagami, S., Yanagida, K., Takami, M., Kodama, T. S., Mori, K., Nakayama, T., Ihara, Y., and Takeda, M. (2013) γ-Secretase Modulators and Presenilin 1 Mutants Act Differently on Presenilin/γ-Secretase Function to Cleave Aβ42 and Aβ43. *Cell Rep. 3*, 42−51.
(24) Hecimovic, S., Wang, J., Dolios, G., Martinez, M., Wang, R., and Goate, A. M. (2004) Mutations in APP have independent effects on Aβ and CTFγ generation. *Neurobiol. Dis. 17*, 205−218.
(25) Chen, F., Hasegawa, H., Schmitt-Ulms, G., Kawarai, T., Bohm, C., Katayama, T., Gu, Y., Sanjo, N., Glista, M., Rogaeva, E., Wakutani, Y., Pardossi-Piquard, R., Ruan, X., Tandon, A., Checler, F., Marambaud, P., Hansen, K., Westaway, D., George-Hyslop, P. S., and Fraser, P. (2006) TMP21 is a presenilin complex component that modulates γ-secretase but not ε-secretase activity. *Nature 440*, 1208−1212.
(26) He, G., Luo, W., Li, P., Remmers, C., Netzer, W. J., Hendrick, J., Bettayeb, K., Flajolet, M., Gorelick, F., Wennogle, L. P., and Greengard, P. (2010) γ-Secretase activating protein is a therapeutic target for Alzheimer's disease. *Nature 467*, 95−98.
(27) Moehlmann, T., Winkler, E., Xia, X., Edbauer, D., Murrell, J., Capell, A., Kaether, C., Zheng, H., Ghetti, B., Haass, C., and Steiner, H. (2002) Presenilin-1 mutations of leucine 166 equally affect the generation of the Notch and APP intracellular domains independent of their effect on Aβ 42 production. *Proc. Natl. Acad. Sci. U.S.A. 99*, 8025−8030.
(28) Page, R. M., Baumann, K., Tomioka, M., Perez-Revuelta, B. I., Fukumori, A., Jacobsen, H., Flohr, A., Luebbers, T., Ozmen, L., Steiner, H., and Haass, C. (2008) Generation of Aβ38 and Aβ42 is independently and differentially affected by familial Alzheimer disease-associated presenilin mutations and γ-secretase modulation. *J. Biol. Chem. 283*, 677−683.
(29) Czirr, E., Cottrell, B. A., Leuchtenberger, S., Kukar, T., Ladd, T. B., Esselmann, H., Paul, S., Schubenel, R., Torpey, J. W., Pietrzik, C. U., Golde, T. E., Wiltfang, J., Baumann, K., Koo, E. H., and Weggen, S. (2008) Independent generation of Aβ42 and Aβ38 peptide species by γ-secretase. *J. Biol. Chem. 283*, 17049−17054.
(30) Quintero-Monzon, O., Martin, M. M., Fernandez, M. A., Cappello, C. A., Krzysiak, A. J., Osenkowski, P., and Wolfe, M. S. (2011) Dissociation between the processivity and total activity of γ-secretase: Implications for the mechanism of Alzheimer's disease-causing presenilin mutations. *Biochemistry 50*, 9023−9035.
(31) Iwatsubo, T., Odaka, A., Suzuki, N., Mizusawa, H., Nukina, N., and Ihara, Y. (1994) Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: Evidence that an initially deposited species is Aβ42(43). *Neuron 13*, 45−53.
(32) Jarrett, J. T., Berger, E. P., and Lansbury, P. T., Jr. (1993) The C-terminus of the β protein is critical in amyloidogenesis. *Ann. N.Y. Acad. Sci. 695*, 144−148.
(33) Kumar-Singh, S., Theuns, J., Van Broeck, B., Pirici, D., Vennekens, K., Corsmit, E., Cruts, M., Dermaut, B., Wang, R., and Van Broeckhoven, C. (2006) Mean age-of-onset of familial Alzheimer disease caused by presenilin mutations correlates with both increased Aβ42 and decreased Aβ40. *Hum. Mutat. 27*, 686−695.
(34) Deng, Y., Tarassishin, L., Kallhoff, V., Peethumnongsin, E., Wu, L., Li, Y. M., and Zheng, H. (2006) Deletion of presenilin 1 hydrophilic loop sequence leads to impaired γ-secretase activity and exacerbated amyloid pathology. *J. Neurosci. 26*, 3845−3854.
(35) Kim, J., Onstead, L., Randle, S., Price, R., Smithson, L., Zwizinski, C., Dickson, D. W., Golde, T., and McGowan, E. (2007) Aβ40 inhibits amyloid deposition in vivo. *J. Neurosci. 27*, 627−633.
(36) Wang, R., Wang, B., He, W., and Zheng, H. (2006) Wild-type presenilin 1 protects against Alzheimer disease mutation-induced amyloid pathology. *J. Biol. Chem. 281*, 15330−15336.
(37) Murray, M. M., Bernstein, S. L., Nyugen, V., Condron, M. M., Teplow, D. B., and Bowers, M. T. (2009) Amyloid β protein: Aβ40 inhibits Aβ42 oligomerization. *J. Am. Chem. Soc. 131*, 6316−6317.
(38) Yan, Y., and Wang, C. (2007) Aβ40 protects non-toxic Aβ42 monomer from aggregation. *J. Mol. Biol. 369*, 909−916.
(39) Jan, A., Gokce, O., Luthi-Carter, R., and Lashuel, H. A. (2008) The ratio of monomeric to aggregated forms of Aβ40 and Aβ42 is an important determinant of amyloid-β aggregation, fibrillogenesis, and toxicity. *J. Biol. Chem. 283*, 28176−28189.
(40) Zheng, H., and Koo, E. H. (2006) The amyloid precursor protein: Beyond amyloid. *Mol. Neurodegener. 1*, 5.
(41) Mitani, Y., Yarimizu, J., Saita, K., Uchino, H., Akashiba, H., Shitaka, Y., Ni, K., and Matsuoka, N. (2012) Differential effects between γ-secretase inhibitors and modulators on cognitive function in amyloid precursor protein-transgenic and nontransgenic mice. *J. Neurosci. 32*, 2037−2050.
(42) Lanz, T. A., Karmilowicz, M. J., Wood, K. M., Pozdnyakov, N., Du, P., Piotrowski, M. A., Brown, T. M., Nolan, C. E., Richter, K. E., Finley, J. E., Fei, Q., Ebbinghaus, C. F., Chen, Y. L., Spracklin, D. K., Tate, B., Geoghegan, K. F., Lau, L. F., Auperin, D. D., and Schachter, J. B. (2006) Concentration-dependent modulation of amyloid-β in vivo and in vitro using the γ-secretase inhibitor, LY-450139. *J. Pharmacol. Exp. Ther. 319*, 924−933.
(43) Yin, Y. I., Bassit, B., Zhu, L., Yang, X., Wang, C., and Li, Y. M. (2007) γ-Secretase Substrate Concentration Modulates the Aβ42/Aβ40 Ratio: Implications for Alzheimer Disease. *J. Biol. Chem. 282*, 23639−23644.
(44) Goate, A., Chartier-Harlin, M.-C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M., and Hardy, J. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature 349*, 704−706.
(45) Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., et al. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature 375*, 754−760.
(46) Levy Lahad, E., Wasco, W., Poorkaj, P., Romano, D. M., Oshima, J., Pettingell, W. H., Yu, C. E., Jondro, P. D., Schmidt, S. D., Wang, K., et al. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science 269*, 973−977.
(47) Shen, J., and Kelleher, R. J., III (2007) The presenilin hypothesis of Alzheimer's disease: Evidence for a loss-of-function pathogenic mechanism. *Proc. Natl. Acad. Sci. U.S.A. 104*, 403−409.
(48) Bertram, L., and Tanzi, R. E. (2012) The genetics of Alzheimer disease. *Prog. Mol. Biol. Transl. Sci. 107*, 79−100.
(49) Jonsson, T., Atwal, J. K., Steinberg, S., Snaedal, J., Jonsson, P. V., Bjornsson, S., Stefansson, H., Sulem, P., Gudbjartsson, D., Maloney, J., Hoyte, K., Gustafson, A., Liu, Y., Lu, Y., Bhangale, T., Graham, R. R., Huttenlocher, J., Bjornsdottir, G., Andreassen, O. A., Jonsson, E. G., Palotie, A., Behrens, T. W., Magnusson, O. T., Kong, A., Thorsteinsdottir, U., Watts, R. J., and Stefansson, K. (2012) A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature 488*, 96−99.
(50) Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J., Trumbauer, M. E., Chen, H. Y., Price, D. L., Van der Ploeg, L. H., and Sisodia, S. S. (1997) Presenilin 1 is required for Notch1 and DII1 expression in the paraxial mesoderm. *Nature 387*, 288−292.
(51) Shen, J., Bronson, R. T., Chen, D. F., Xia, W., Selkoe, D. J., and Tonegawa, S. (1997) Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell 89*, 629−639.
(52) De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A., and Kopan, R. (1999) A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. *Nature 398*, 518−522.
(53) Artavanis-Tsakonas, S., Rand, M. D., and Lake, R. J. (1999) Notch signaling: Cell fate control and signal integration in development. *Science 284*, 770−776.

(54) Louvi, A., and Artavanis-Tsakonas, S. (2006) Notch signalling in vertebrate neural development. *Nat. Rev. Neurosci. 7*, 93−102.
(55) Ables, J. L., Breunig, J. J., Eisch, A. J., and Rakic, P. (2011) Not(ch) just development: Notch signalling in the adult brain. *Nat. Rev. Neurosci. 12*, 269−283.
(56) Lobry, C., Oh, P., and Aifantis, I. (2011) Oncogenic and tumor suppressor functions of Notch in cancer: It's NOTCH what you think. *J. Exp. Med. 208*, 1931−1935.
(57) Kopan, R., and Ilagan, M. X. (2009) The canonical Notch signaling pathway: Unfolding the activation mechanism. *Cell 137*, 216−233.
(58) Kopan, R., and Goate, A. (2000) A common enzyme connects notch signaling and Alzheimer's disease. *Genes Dev. 14*, 2799−2806.
(59) Saxena, M. T., Schroeter, E. H., Mumm, J. S., and Kopan, R. (2001) Murine notch homologs (N1−4) undergo presenilin-dependent proteolysis. *J. Biol. Chem. 276*, 40268−40273.
(60) Haapasalo, A., and Kovacs, D. M. (2011) The many substrates of presenilin/γ-secretase. *J. Alzheimer's Dis. 25*, 3−28.
(61) Eli Lilly and Co. (2010) Lilly Halts Development of Semagacestat for Alzheimer's Disease Based on Preliminary Results of Phase III Clinical Trials. http://newsroom.lilly.com/releasedetail.cfm?releaseid=499794 (August 17, 2010).
(62) Xia, X., Qian, S., Soriano, S., Wu, Y., Fletcher, A. M., Wang, X. J., Koo, E. H., Wu, X., and Zheng, H. (2001) Loss of presenilin 1 is associated with enhanced β-catenin signaling and skin tumorigenesis. *Proc. Natl. Acad. Sci. U.S.A. 98*, 10863−10868.
(63) Nicolas, M., Wolfer, A., Raj, K., Kummer, J. A., Mill, P., Van Noort, M., Hui, C. C., Clevers, H., Dotto, G. P., and Radtke, F. (2003) Notch1 functions as a tumor suppressor in mouse skin. *Nat. Genet. 33*, 416−421.
(64) Fleisher, A. S., Raman, R., Siemers, E. R., Becerra, L., Clark, C. M., Dean, R. A., Farlow, M. R., Galvin, J. E., Peskind, E. R., Quinn, J. F., Sherzai, A., Sowell, B. B., Aisen, P. S., and Thal, L. J. (2008) Phase 2 safety trial targeting amyloid β production with a γ-secretase inhibitor in Alzheimer disease. *Arch. Neurol. 65*, 1031−1038.
(65) Wang, R., Tang, P., Wang, P., Boissy, R. E., and Zheng, H. (2006) Regulation of tyrosinase trafficking and processing by presenilins: Partial loss of function by familial Alzheimer's disease mutation. *Proc. Natl. Acad. Sci. U.S.A. 103*, 353−358.
(66) Takasugi, N., Tomita, T., Hayashi, I., Tsuruoka, M., Niimura, M., Takahashi, Y., Thinakaran, G., and Iwatsubo, T. (2003) The role of presenilin cofactors in the γ-secretase complex. *Nature 422*, 438−441.
(67) Edbauer, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H., and Haass, C. (2003) Reconstitution of γ-secretase activity. *Nat. Cell Biol. 5*, 486−488.
(68) Li, Y. M., Xu, M., Lai, M. T., Huang, Q., Castro, J. L., DiMuzio-Mower, J., Harrison, T., Lellis, C., Nadin, A., Neduvelil, J. G., Register, R. B., Sardana, M. K., Shearman, M. S., Smith, A. L., Shi, X. P., Yin, K. C., Shafer, J. A., and Gardell, S. J. (2000) Photoactivated γ-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature 405*, 689−694.
(69) Esler, W. P., Kimberly, W. T., Ostaszewski, B. L., Diehl, T. S., Moore, C. L., Tsai, J. Y., Rahmati, T., Xia, W., Selkoe, D. J., and Wolfe, M. S. (2000) Transition-state analogue inhibitors of γ-secretase bind directly to presenilin-1. *Nat. Cell Biol. 2*, 428−434.
(70) Ahn, K., Shelton, C. C., Tian, Y., Zhang, X., Gilchrist, M. L., Sisodia, S. S., and Li, Y.-M. (2010) Activation and intrinsic γ-secretase activity of presenilin 1. *Proc. Natl. Acad. Sci. U.S.A. 107*, 21435−21440.
(71) Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature 398*, 513−517.
(72) Steiner, H., Kostka, M., Romig, H., Basset, G., Pesold, B., Hardy, J., Capell, A., Meyn, L., Grim, M. L., Baumeister, R., Fechteler, K., and Haass, C. (2000) Glycine 384 is required for presenilin-1 function and is conserved in bacterial polytopic aspartyl proteases. *Nat. Cell Biol. 2*, 848−851.
(73) Li, X., Dang, S., Yan, C., Gong, X., Wang, J., and Shi, Y. (2013) Structure of a presenilin family intramembrane aspartate protease. *Nature 493*, 56−61.
(74) Thinakaran, G., Borchelt, D. R., Lee, M. K., Slunt, H. H., Spitzer, L., Kim, G., Ratovitsky, T., Davenport, F., Nordstedt, C., Seeger, M., Hardy, J., Levey, A. I., Gandy, S. E., Jenkins, N. A., Copeland, N. G., Price, D. L., and Sisodia, S. S. (1996) Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron 17*, 181−190.
(75) Lai, M. T., Chen, E., Crouthamel, M. C., DiMuzio-Mower, J., Xu, M., Huang, Q., Price, E., Register, R. B., Shi, X. P., Donoviel, D. B., Bernstein, A., Hazuda, D., Gardell, S. J., and Li, Y. M. (2003) Presenilin-1 and Presenilin-2 Exhibit Distinct yet Overlapping γ-Secretase Activities. *J. Biol. Chem. 278*, 22475−22481.
(76) Shirotani, K., Edbauer, D., Prokop, S., Haass, C., and Steiner, H. (2004) Identification of Distinct γ-Secretase Complexes with Different APH-1 Variants. *J. Biol. Chem. 279*, 41340−41345.
(77) Shirotani, K., Tomioka, M., Kremmer, E., Haass, C., and Steiner, H. (2007) Pathological activity of familial Alzheimer's disease-associated mutant presenilin can be executed by six different γ-secretase complexes. *Neurobiol. Dis. 27*, 102−107.
(78) Niimura, M., Isoo, N., Takasugi, N., Tsuruoka, M., Ui-Tei, K., Saigo, K., Morohashi, Y., Tomita, T., and Iwatsubo, T. (2005) Aph-1 contributes to the stabilization and trafficking of the γ-secretase complex through mechanisms involving intermolecular and intramolecular interactions. *J. Biol. Chem. 280*, 12967−12975.
(79) Shah, S., Lee, S. F., Tabuchi, K., Hao, Y. H., Yu, C., LaPlant, Q., Ball, H., Dann, C. E., III, Sudhof, T., and Yu, G. (2005) Nicastrin functions as a γ-secretase-substrate receptor. *Cell 122*, 435−447.
(80) Capell, A., Beher, D., Prokop, S., Steiner, H., Kaether, C., Shearman, M. S., and Haass, C. (2005) γ-Secretase complex assembly within the early secretory pathway. *J. Biol. Chem. 280*, 6471−6478.
(81) Prokop, S., Shirotani, K., Edbauer, D., Haass, C., and Steiner, H. (2004) Requirement of PEN-2 for stabilization of the presenilin N-/C-terminal fragment heterodimer within the γ-secretase complex. *J. Biol. Chem. 279*, 23255−23261.
(82) Luo, W. J., Wang, H., Li, H., Kim, B. S., Shah, S., Lee, H. J., Thinakaran, G., Kim, T. W., Yu, G., and Xu, H. (2003) PEN-2 and APH-1 coordinately regulate proteolytic processing of presenilin 1. *J. Biol. Chem. 278*, 7850−7854.
(83) Sato, T., Diehl, T. S., Narayanan, S., Funamoto, S., Ihara, Y., De Strooper, B., Steiner, H., Haass, C., and Wolfe, M. S. (2007) Active γ-secretase complexes contain only one of each component. *J. Biol. Chem. 282*, 33985−33993.
(84) Li, Y. M., Lai, M. T., Xu, M., Huang, Q., DiMuzio-Mower, J., Sardana, M. K., Shi, X. P., Yin, K. C., Shafer, J. A., and Gardell, S. J. (2000) Presenilin 1 is linked with γ-secretase activity in the detergent solubilized state. *Proc. Natl. Acad. Sci. U.S.A. 97*, 6138−6143.
(85) Gu, Y., Sanjo, N., Chen, F., Hasegawa, H., Petit, A., Ruan, X., Li, W., Shier, C., Kawarai, T., Schmitt-Ulms, G., Westaway, D., St George-Hyslop, P., and Fraser, P. E. (2004) The presenilin proteins are components of multiple membrane-bound complexes that have different biological activities. *J. Biol. Chem. 279*, 31329−31336.
(86) Edbauer, D., Winkler, E., Haass, C., and Steiner, H. (2002) Presenilin and nicastrin regulate each other and determine amyloid β-peptide production via complex formation. *Proc. Natl. Acad. Sci. U.S.A. 99*, 8666−8671.
(87) Evin, G., Canterford, L. D., Hoke, D. E., Sharples, R. A., Culvenor, J. G., and Masters, C. L. (2005) Transition-state analogue γ-secretase inhibitors stabilize a 900 kDa presenilin/nicastrin complex. *Biochemistry 44*, 4332−4341.
(88) Zhou, S., Zhou, H., Walian, P. J., and Jap, B. K. (2005) CD147 is a regulatory subunit of the γ-secretase complex in Alzheimer's disease amyloid β-peptide production. *Proc. Natl. Acad. Sci. U.S.A. 102*, 7499−7504.
(89) Chen, F., Hasegawa, H., Schmitt-Ulms, G., Kawarai, T., Bohm, C., Katayama, T., Gu, Y., Sanjo, N., Glista, M., Rogaeva, E., Wakutani, Y., Pardossi-Piquard, R., Ruan, X., Tandon, A., Checler, F., Marambaud, P., Hansen, K., Westaway, D., St George-Hyslop, P.,

and Fraser, P. (2006) TMP21 is a presenilin complex component that modulates γ-secretase but not ε-secretase activity. *Nature 440*, 1208−1212.
(90) Teranishi, Y., Hur, J. Y., Gu, G. J., Kihara, T., Ishikawa, T., Nishimura, T., Winblad, B., Behbahani, H., Kamali-Moghaddam, M., Frykman, S., and Tjernberg, L. O. (2012) Erlin-2 is associated with active γ-secretase in brain and affects amyloid β-peptide production. *Biochem. Biophys. Res. Commun. 424*, 476−481.
(91) Hur, J. Y., Teranishi, Y., Kihara, T., Yamamoto, N. G., Inoue, M., Hosia, W., Hashimoto, M., Winblad, B., Frykman, S., and Tjernberg, L. O. (2012) Identification of novel γ-secretase-associated proteins in detergent-resistant membranes from brain. *J. Biol. Chem. 287*, 11991−12005.
(92) Frykman, S., Teranishi, Y., Hur, J. Y., Sandebring, A., Yamamoto, N. G., Ancarcrona, M., Nishimura, T., Winblad, B., Bogdanovic, N., Schedin-Weiss, S., Kihara, T., and Tjernberg, L. O. (2012) Identification of two novel synaptic γ-secretase associated proteins that affect amyloid β-peptide levels without altering Notch processing. *Neurochem. Int. 61*, 108−118.
(93) Thathiah, A., Horre, K., Snellinx, A., Vandewyer, E., Huang, Y., Ciesielska, M., De Kloe, G., Munck, S., and De Strooper, B. (2013) β-Arrestin 2 regulates Aβ generation and γ-secretase activity in Alzheimer's disease. *Nat. Med. 19*, 43−49.
(94) Liu, X., Zhao, X., Zeng, X., Bossers, K., Swaab, D. F., Zhao, J., and Pei, G. (2013) β-Arrestin1 regulates γ-secretase complex assembly and modulates amyloid-β pathology. *Cell Res. 23*, 351−365.
(95) Vetrivel, K. S., Zhang, X., Meckler, X., Cheng, H., Lee, S., Gong, P., Lopes, K. O., Chen, Y., Iwata, N., Yin, K. J., Lee, J. M., Parent, A. T., Saido, T. C., Li, Y. M., Sisodia, S. S., and Thinakaran, G. (2008) Evidence that CD147 modulation of β-amyloid (Aβ) levels is mediated by extracellular degradation of secreted Aβ. *J. Biol. Chem. 283*, 19489−19498.
(96) Hussain, I., Fabregue, J., Anderes, L., Ousson, S., Borlat, F., Eligert, V., Berger, S., Dimitrov, M., Alattia, J. R., Fraering, P. C., and Beher, D. (2013) The role of γ-secretase activating protein (GSAP) and imatinib in the regulation of γ-secretase activity and amyloid-β generation. *J. Biol. Chem. 288*, 2521−2531.
(97) Vetrivel, K. S., Gong, P., Bowen, J. W., Cheng, H., Chen, Y., Carter, M., Nguyen, P. D., Placanica, L., Wieland, F. T., Li, Y. M., Kounnas, M. Z., and Thinakaran, G. (2007) Dual roles of the transmembrane protein p23/TMP21 in the modulation of amyloid precursor protein metabolism. *Mol. Neurodegener. 2*, 4.
(98) Wakabayashi, T., Craessaerts, K., Bammens, L., Bentahir, M., Borgions, F., Herdewijn, P., Staes, A., Timmerman, E., Vandekerckhove, J., Rubinstein, E., Boucheix, C., Gevaert, K., and De Strooper, B. (2009) Analysis of the γ-secretase interactome and validation of its association with tetraspanin-enriched microdomains. *Nat. Cell Biol. 11*, 1340−1346.
(99) Placanica, L., Chien, J. W., and Li, Y. M. (2010) Characterization of an atypical γ-secretase complex from hematopoietic origin. *Biochemistry 49*, 2796−2804.
(100) Serneels, L., Van Biervliet, J., Craessaerts, K., Dejaegere, T., Horre, K., Van Houtvin, T., Esselmann, H., Paul, S., Schafer, M. K., Berezovska, O., Hyman, B. T., Sprangers, B., Sciot, R., Moons, L., Jucker, M., Yang, Z., May, P. C., Karran, E., Wiltfang, J., D'Hooge, R., and De Strooper, B. (2009) γ-Secretase heterogeneity in the Aph1 subunit: Relevance for Alzheimer's disease. *Science 324*, 639−642.
(101) Beher, D., Fricker, M., Nadin, A., Clarke, E. E., Wrigley, J. D., Li, Y. M., Culvenor, J. G., Masters, C. L., Harrison, T., and Shearman, M. S. (2003) In vitro characterization of the presenilin-dependent γ-secretase complex using a novel affinity ligand. *Biochemistry 42*, 8133−8142.
(102) Placanica, L., Tarassishin, L., Yang, G., Peethumnongsin, E., Kim, S. H., Zheng, H., Sisodia, S. S., and Li, Y. M. (2009) Pen2 and Presenilin-1 Modulate the Dynamic Equilibrium of Presenilin-1 and Presenilin-2 γ-Secretase Complexes. *J. Biol. Chem. 284*, 2967−2977.
(103) Placanica, L., Zhu, L., and Li, Y. M. (2009) Gender- and age-dependent γ-secretase activity in mouse brain and its implication in sporadic Alzheimer disease. *PLoS One 4*, e5088.
(104) Teranishi, Y., Hur, J. Y., Welander, H., Franberg, J., Aoki, M., Winblad, B., Frykman, S., and Tjernberg, L. O. (2010) Affinity pulldown of γ-secretase and associated proteins from human and rat brain. *J. Cell. Mol. Med. 14*, 2675−2686.
(105) Eriksen, J. L., Sagi, S. A., Smith, T. E., Weggen, S., Das, P., McLendon, D. C., Ozols, V. V., Jessing, K. W., Zavitz, K. H., Koo, E. H., and Golde, T. E. (2003) NSAIDs and enantiomers of flurbiprofen target γ-secretase and lower Aβ42 in vivo. *J. Clin. Invest. 112*, 440−449.
(106) Wilcock, G. K., Black, S. E., Hendrix, S. B., Zavitz, K. H., Swabb, E. A., Laughlin, M. A., and Tarenflurbil Phase II Study investigators (2008) Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: A randomised phase II trial. *Lancet Neurol. 7*, 483−493.
(107) Green, R. C., Schneider, L. S., Amato, D. A., Beelen, A. P., Wilcock, G., Swabb, E. A., Zavitz, K. H., and Tarenflurbil Phase 3 Study Group (2009) Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: A randomized controlled trial. *J. Am. Med. Assoc. 302*, 2557−2564.
(108) Peretto, I., Radaelli, S., Parini, C., Zandi, M., Raveglia, L. F., Dondio, G., Fontanella, L., Misiano, P., Bigogno, C., Rizzi, A., Riccardi, B., Biscaioli, M., Marchetti, S., Puccini, P., Catinella, S., Rondelli, I., Cenacchi, V., Bolzoni, P. T., Caruso, P., Villetti, G., Facchinetti, F., Del Giudice, E., Moretto, N., and Imbimbo, B. P. (2005) Synthesis and biological activity of flurbiprofen analogues as selective inhibitors of β-amyloid$_{1-42}$ secretion. *J. Med. Chem. 48*, 5705−5720.
(109) Imbimbo, B. P., Frigerio, E., Breda, M., Fiorentini, F., Fernandez, M., Sivilia, S., Giardino, L., Calza, L., Norris, D., Casula, D., and Shenouda, M. (2012) Pharmacokinetics and Pharmacodynamics of CHF5074 After Short-term Administration in Healthy Subjects. *Alzheimer Disease & Associated Disorders*, DOI: 10.1097/WAD.0b013e3182622ace.
(110) Kretner, B., Fukumori, A., Gutsmiedl, A., Page, R. M., Luebbers, T., Galley, G., Baumann, K., Haass, C., and Steiner, H. (2011) Attenuated Aβ42 responses to low potency γ-secretase modulators can be overcome for many pathogenic presenilin mutants by second-generation compounds. *J. Biol. Chem. 286*, 15240−15251.
(111) Mullard, A. (2012) Sting of Alzheimer's failures offset by upcoming prevention trials. *Nat. Rev. Drug Discovery 11*, 657−660.
(112) Miller, G. (2012) Stopping Alzheimer's Before It Starts. *Science 337*, 790−792.
(113) Page, R. M., Gutsmiedl, A., Fukumori, A., Winkler, E., Haass, C., and Steiner, H. (2010) β-Amyloid precursor protein mutants respond to γ-secretase modulators. *J. Biol. Chem. 285*, 17798−17810.
(114) Hall, A., Elliott, R. L., Giblin, G. M., Hussain, I., Musgrave, J., Naylor, A., Sasse, R., and Smith, B. (2010) Piperidine-derived γ-secretase modulators. *Bioorg. Med. Chem. Lett. 20*, 1306−1311.
(115) Hawkins, J., Harrison, D. C., Ahmed, S., Davis, R. P., Chapman, T., Marshall, I., Smith, B., Mead, T. L., Medhurst, A., Giblin, G. M., Hall, A., Gonzalez, M. I., Richardson, J., and Hussain, I. (2011) Dynamics of Aβ42 reduction in plasma, CSF and brain of rats treated with the γ-secretase modulator, GSM-10h. *Neurodegener. Dis. 8*, 455−464.
(116) Li, T., Huang, Y., Jin, S., Ye, L., Rong, N., Yang, X., Ding, Y., Cheng, Z., Zhang, J., Wan, Z., Harrison, D. C., Hussain, I., Hall, A., Lee, D. H., Lau, L. F., and Matsuoka, Y. (2012) γ-Secretase modulators do not induce Aβ-rebound and accumulation of β-C-terminal fragment. *J. Neurochem. 121*, 277−286.
(117) Rogers, K., Felsenstein, K. M., Hrdlicka, L., Tu, Z., Albayya, F., Lee, W., Hopp, S., Miller, M. J., Spaulding, D., Yang, Z., Hodgdon, H., Nolan, S., Wen, M., Costa, D., Blain, J. F., Freeman, E., De Strooper, B., Vulsteke, V., Scrocchi, L., Zetterberg, H., Portelius, E., Hutter-Paier, B., Havas, D., Ahlijanian, M., Flood, D., Leventhal, L., Shapiro, G., Patzke, H., Chesworth, R., and Koenig, G. (2012) Modulation of γ-secretase by EVP-0015962 reduces amyloid deposition and behavioral deficits in Tg2576 mice. *Mol. Neurodegener. 7*, 61.
(118) Van Broeck, B., Chen, J. M., Treton, G., Desmidt, M., Hopf, C., Ramsden, N., Karran, E., Mercken, M., and Rowley, A. (2011) Chronic treatment with a novel γ-secretase modulator, JNJ-40418677, inhibits

amyloid plaque formation in a mouse model of Alzheimer's disease. *Br. J. Pharmacol. 163*, 375−389.
(119) Peng, H., Talreja, T., Xin, Z., Cuervo, J. H., Kumaravel, G., Humora, M. J., Xu, L., Rohde, E., Gan, L., Jung, M.-y., Shackett, M. N., Chollate, S., Dunah, A. W., Snodgrass-Belt, P. A., Arnold, H. M., Taveras, A. G., Rhodes, K. J., and Scannevin, R. H. (2011) Discovery of BIIB042, a Potent, Selective, and Orally Bioavailable γ-Secretase Modulator. *ACS Med. Chem. Lett. 2*, 786−791.
(120) Kukar, T. L., Ladd, T. B., Bann, M. A., Fraering, P. C., Narlawar, R., Maharvi, G. M., Healy, B., Chapman, R., Welzel, A. T., Price, R. W., Moore, B., Rangachari, V., Cusack, B., Eriksen, J., Jansen-West, K., Verbeeck, C., Yager, D., Eckman, C., Ye, W., Sagi, S., Cottrell, B. A., Torpey, J., Rosenberry, T. L., Fauq, A., Wolfe, M. S., Schmidt, B., Walsh, D. M., Koo, E. H., and Golde, T. E. (2008) Substrate-targeting γ-secretase modulators. *Nature 453*, 925−929.
(121) Narlawar, R., Baumann, K., Czech, C., and Schmidt, B. (2007) Conversion of the LXR-agonist TO-901317: From inverse to normal modulation of γ-secretase by addition of a carboxylic acid and a lipophilic anchor. *Bioorg. Med. Chem. Lett. 17*, 5428−5431.
(122) Zall, A., Kieser, D., Hottecke, N., Naumann, E. C., Thomaszewski, B., Schneider, K., Steinbacher, D. T., Schubenel, R., Masur, S., Baumann, K., and Schmidt, B. (2011) NSAID-derived γ-secretase modulation requires an acidic moiety on the carbazole scaffold. *Bioorg. Med. Chem. 19*, 4903−4909.
(123) Ohki, Y., Higo, T., Uemura, K., Shimada, N., Osawa, S., Berezovska, O., Yokoshima, S., Fukuyama, T., Tomita, T., and Iwatsubo, T. (2011) Phenylpiperidine-type γ-secretase modulators target the transmembrane domain 1 of presenilin 1. *EMBO J. 30*, 4815−4824.
(124) Oborski, C. E., Iyer, R., Maguire, B. A., Bora, G., Atchison, K., Pozdnyakov, N., Wood, K., Parker, C., Subashi, T. A., Pettersson, M., Johnson, D. S., and Bales, K. R. (2012) Pharmacological assessment of γ-secretase activity from rodent and human brain. *Neurosci. Med. 3*, 149−161.
(125) Hussain, I., Harrison, D. C., Hawkins, J., Chapman, T., Marshall, I., Facci, L., Ahmed, S., Brackenborough, K., Skaper, S. D., Mead, T. L., Smith, B. B., Giblin, G. M., Hall, A., Gonzalez, M. I., and Richardson, J. C. (2011) TASTPM mice expressing amyloid precursor protein and presenilin-1 mutant transgenes are sensitive to γ-secretase modulation and amyloid-β(4)(2) lowering by GSM-10h. *Neurodegener. Dis. 8*, 15−24.
(126) Mitani, Y., Yarimizu, J., Akashiba, H., Shitaka, Y., Ni, K., and Matsuoka, N. (2013) Amelioration of cognitive deficits in plaque-bearing Alzheimer's disease model mice through selective reduction of nascent soluble Aβ42 without affecting other Aβ pools. *J. Neurochem. 125*, 465−472.
(127) Kounnas, M. Z., Danks, A. M., Cheng, S., Tyree, C., Ackerman, E., Zhang, X., Ahn, K., Nguyen, P., Comer, D., Mao, L., Yu, C., Pleynet, D., Digregorio, P. J., Velicelebi, G., Stauderman, K. A., Comer, W. T., Mobley, W. C., Li, Y. M., Sisodia, S. S., Tanzi, R. E., and Wagner, S. L. (2010) Modulation of γ-secretase reduces β-amyloid deposition in a transgenic mouse model of Alzheimer's disease. *Neuron 67*, 769−780.
(128) Portelius, E., Van Broeck, B., Andreasson, U., Gustavsson, M. K., Mercken, M., Zetterberg, H., Borghys, H., and Blennow, K. (2010) Acute effect on the Aβ isoform pattern in CSF in response to γ-secretase modulator and inhibitor treatment in dogs. *J. Alzheimer's Dis. 21*, 1005−1012.
(129) Borgegard, T., Jureus, A., Olsson, F., Rosqvist, S., Sabirsh, A., Rotticci, D., Paulsen, K., Klintenberg, R., Yan, H., Waldman, M., Stromberg, K., Nord, J., Johansson, J., Regner, A., Parpal, S., Malinowsky, D., Radesater, A. C., Li, T., Singh, R., Eriksson, H., and Lundkvist, J. (2012) First and second generation γ-secretase modulators (GSMs) modulate amyloid-β (Aβ) peptide production through different mechanisms. *J. Biol. Chem. 287*, 11810−11819.
(130) Hashimoto, T., Ishibashi, A., Hagiwara, H., Murata, Y., Takenaka, O., and Miyagawa, T. (2010) E2012: A novel γ-secretase modulator—pharmacology. *Alzheimer's Dementia 6* (Suppl.), S242.
(131) Nagy, C., Schuck, E., Ishibashi, A., Nakatani, Y., Rege, B., and Logovinsky, V. (2010) E2012, a novel γ-secretase modulator, decreases plasma amyloid-β (Aβ) levels in humans. *Alzheimer's Dementia 6* (Suppl.), S574.
(132) http://www.neurogeneticpharmaceuticals.com/about-ngp.html.
(133) Huang, X., Zhou, W., Liu, X., Li, H., Sun, G., Mandal, M., Vicarel, M., Zhu, X., Bennett, C., McCraken, T., Pissarnitski, D., Zhao, Z., Cole, D., Gallo, G., Zhu, Z., Palani, A., Aslanian, R., Clader, J., Czarniecki, M., Greenlee, W., Burnett, D., Cohen-Williams, M., Hyde, L., Song, L., Zhang, L., Chu, I., and Buevich, A. (2012) Synthesis and SAR Studies of Fused Oxadiazines as γ-Secretase Modulators for Treatment of Alzheimer's Disease. *ACS Med. Chem. Lett. 3*, 931−935.
(134) Sun, Z. Y., Asberom, T., Bara, T., Bennett, C., Burnett, D., Chu, I., Clader, J., Cohen-Williams, M., Cole, D., Czarniecki, M., Durkin, J., Gallo, G., Greenlee, W., Josien, H., Huang, X., Hyde, L., Jones, N., Kazakevich, I., Li, H., Liu, X., Lee, J., Maccoss, M., Mandal, M. B., McCracken, T., Nomeir, A., Mazzola, R., Palani, A., Parker, E. M., Pissarnitski, D. A., Qin, J., Song, L., Terracina, G., Vicarel, M., Voigt, J., Xu, R., Zhang, L., Zhang, Q., Zhao, Z., Zhu, X., and Zhu, Z. (2012) Cyclic hydroxyamidines as amide isosteres: Discovery of oxadiazolines and oxadiazines as potent and highly efficacious γ-secretase modulators in vivo. *J. Med. Chem. 55*, 489−502.
(135) Huang, X., Aslanian, R., Zhou, W., Zhu, X., Qin, J., Greenlee, W., Zhu, Z., Zhang, L., Hyde, L., Chu, I., Cohen-Williams, M., and Palani, A. (2010) The Discovery of Pyridone and Pyridazone Heterocycles as γ-Secretase Modulators. *ACS Med. Chem. Lett. 1*, 184−187.
(136) Ebke, A., Luebbers, T., Fukumori, A., Shirotani, K., Haass, C., Baumann, K., and Steiner, H. (2011) Novel γ-secretase enzyme modulators directly target presenilin protein. *J. Biol. Chem. 286*, 37181−37186.
(137) Lu, Y., Riddell, D., Hajos-Korcsok, E., Bales, K., Wood, K. M., Nolan, C. E., Robshaw, A. E., Zhang, L., Leung, L., Becker, S. L., Tseng, E., Barricklow, J., Miller, E. H., Osgood, S., O'Neill, B. T., Brodney, M. A., Johnson, D. S., and Pettersson, M. (2012) Cerebrospinal fluid amyloid-β (Aβ) as an effect biomarker for brain Aβ lowering verified by quantitative preclinical analyses. *J. Pharmacol. Exp. Ther. 342*, 366−375.
(138) Bischoff, F., Berthelot, D., De Cleyn, M., Macdonald, G., Minne, G., Oehlrich, D., Pieters, S., Surkyn, M., Trabanco, A. A., Tresadern, G., Van Brandt, S., Velter, I., Zaja, M., Borghys, H., Masungi, C., Mercken, M., and Gijsen, H. J. (2012) Design and synthesis of a novel series of bicyclic heterocycles as potent γ-secretase modulators. *J. Med. Chem. 55*, 9089−9106.
(139) Borghys, H., Tuefferd, M., Van Broeck, B., Clessens, E., Dillen, L., Cools, W., Vinken, P., Straetemans, R., De Ridder, F., Gijsen, H., and Mercken, M. (2012) A canine model to evaluate efficacy and safety of γ-secretase inhibitors and modulators. *J. Alzheimer's Dis. 28*, 809−822.
(140) Gijsen, H. J., and Mercken, M. (2012) γ-Secretase Modulators: Can We Combine Potency with Safety? *Int. J. Alzheimer's Dis. 2012*, 295207.
(141) Hahn, S., Bruning, T., Ness, J., Czirr, E., Baches, S., Gijsen, H., Korth, C., Pietrzik, C. U., Bulic, B., and Weggen, S. (2011) Presenilin-1 but not amyloid precursor protein mutations present in mouse models of Alzheimer's disease attenuate the response of cultured cells to γ-secretase modulators regardless of their potency and structur. *J. Neurochem. 116*, 385−395.
(142) Rivkin, A., Ahearn, S. P., Chichetti, S. M., Kim, Y. R., Li, C., Rosenau, A., Kattar, S. D., Jung, J., Shah, S., Hughes, B. L., Crispino, J. L., Middleton, R. E., Szewczak, A. A., Munoz, B., and Shearman, M. S. (2010) Piperazinyl pyrimidine derivatives as potent γ-secretase modulators. *Bioorg. Med. Chem. Lett. 20*, 1269−1271.
(143) Findeis, M. A., Schroeder, F., McKee, T. D., Yager, D., Fraering, P. C., Creaser, S. P., Austin, W. F., Clardy, J., Wang, R., Selkoe, D., and Eckman, C. B. (2012) Discovery of a novel pharmacological and structural class of γ-secretase modulators derived from the extract of *Actaea racemosa*. *ACS Chem. Neurosci. 3*, 941−951.

(144) Hubbs, J. L., Fuller, N. O., Austin, W. F., Shen, R., Creaser, S. P., McKee, T. D., Loureiro, R. M., Tate, B., Xia, W., Ives, J., and Bronk, B. S. (2012) Optimization of a natural product-based class of γ-secretase modulators. *J. Med. Chem. 55*, 9270−9282.
(145) Pfizer (2012) WO 2012/131539.
(146) Hoffmann-La Roche AG (2012) WO 2012/116965.
(147) Bristol-Myers Squibb Co. (2012) WO 2012/103297.
(148) Geoghegan, K. F., and Johnson, D. S. (2010) Chemical proteomic technologies for drug target identification. *Annu. Rep. Med. Chem. 45*, 345−360.
(149) Seiffert, D., Bradley, J. D., Rominger, C. M., Rominger, D. H., Yang, F., Meredith, J. E., Jr., Wang, Q., Roach, A. H., Thompson, L. A., Spitz, S. M., Higaki, J. N., Prakash, S. R., Combs, A. P., Copeland, R. A., Arneric, S. P., Hartig, P. R., Robertson, D. W., Cordell, B., Stern, A. M., Olson, R. E., and Zaczek, R. (2000) Presenilin-1 and -2 are molecular targets for γ-secretase inhibitors. *J. Biol. Chem. 275*, 34086−34091.
(150) Kornilova, A. Y., Bihel, F., Das, C., and Wolfe, M. S. (2005) The initial substrate-binding site of γ-secretase is located on presenilin near the active site. *Proc. Natl. Acad. Sci. U.S.A. 102*, 3230−3235.
(151) Fuwa, H., Takahashi, Y., Konno, Y., Watanabe, N., Miyashita, H., Sasaki, M., Natsugari, H., Kan, T., Fukuyama, T., Tomita, T., and Iwatsubo, T. (2007) Divergent synthesis of multifunctional molecular probes to elucidate the enzyme specificity of dipeptidic γ-secretase inhibitors. *ACS Chem. Biol. 2*, 408−418.
(152) Brunner, J. (1993) New photolabeling and crosslinking methods. *Annu. Rev. Biochem. 62*, 483−514.
(153) Crump, C. J., am Ende, C. W., Ballard, T. E., Pozdnyakov, N., Pettersson, M., Chau, D. M., Bales, K. R., Li, Y. M., and Johnson, D. S. (2012) Development of clickable active site-directed photoaffinity probes for γ-secretase. *Bioorg. Med. Chem. Lett. 22*, 2997−3000.
(154) Crump, C. J., Fish, B. A., Castro, S. V., Chau, D. M., Gertsik, N., Ahn, K., Stiff, C., Pozdnyakov, N., Bales, K. R., Johnson, D. S., and Li, Y. M. (2011) Piperidine acetic acid based γ-secretase modulators directly bind to Presenilin-1. *ACS Chem. Neurosci. 2*, 705−710.
(155) Lleo, A., Berezovska, O., Herl, L., Raju, S., Deng, A., Bacskai, B. J., Frosch, M. P., Irizarry, M., and Hyman, B. T. (2004) Nonsteroidal anti-inflammatory drugs lower Aβ42 and change presenilin 1 conformation. *Nat. Med. 10*, 1065−1066.
(156) Uemura, K., Farner, K. C., Hashimoto, T., Nasser-Ghodsi, N., Wolfe, M. S., Koo, E. H., Hyman, B. T., and Berezovska, O. (2010) Substrate docking to γ-secretase allows access of γ-secretase modulators to an allosteric site. *Nat. Commun. 1*, 130.
(157) Chau, D. M., Crump, C. J., Villa, J. C., Scheinberg, D. A., and Li, Y. M. (2012) Familial Alzheimer Disease Presenilin-1 Mutations Alter the Active Site Conformation of γ-Secretase. *J. Biol. Chem. 287*, 17288−17296.
(158) Shelton, C. C., Zhu, L., Chau, D., Yang, L., Wang, R., Djaballah, H., Zheng, H., and Li, Y. M. (2009) Modulation of γ-secretase specificity using small molecule allosteric inhibitors. *Proc. Natl. Acad. Sci. U.S.A. 106*, 20228−20233.
(159) Crump, C. J., Castro, S. V., Wang, F., Pozdnyakov, N., Ballard, T. E., Sisodia, S. S., Bales, K. R., Johnson, D. S., and Li, Y. M. (2012) BMS-708,163 Targets Presenilin and Lacks Notch-Sparing Activity. *Biochemistry 51*, 7209−7211.
(160) Xu, M., Lai, M. T., Huang, Q., DiMuzio-Mower, J., Castro, J. L., Harrison, T., Nadin, A., Neduvelil, J. G., Shearman, M. S., Shafer, J. A., Gardell, S. J., and Li, Y. M. (2002) γ-Secretase: Characterization and implication for Alzheimer disease therapy. *Neurobiol. Aging 23*, 1023−1030.
(161) Chun, J., Yin, Y. I., Yang, G., Tarassishin, L., and Li, Y. M. (2004) Stereoselective Synthesis of Photoreactive Peptidomimetic γ-Secretase Inhibitors. *J. Org. Chem. 69*, 7344−7347.
(162) Yang, G., Yin, Y. I., Chun, J., Shelton, C. C., Ouerfelli, O., and Li, Y. M. (2009) Stereo-controlled synthesis of novel photoreactive γ-secretase inhibitors. *Bioorg. Med. Chem. Lett. 19*, 922−925.
(163) Takahashi, Y., Hayashi, I., Tominari, Y., Rikimaru, K., Morohashi, Y., Kan, T., Natsugari, H., Fukuyama, T., Tomita, T., and Iwatsubo, T. (2003) Sulindac sulfide is a noncompetitive γ-secretase inhibitor that preferentially reduces Aβ42 generation. *J. Biol. Chem. 278*, 18664−18670.
(164) Beher, D., Clarke, E. E., Wrigley, J. D., Martin, A. C., Nadin, A., Churcher, I., and Shearman, M. S. (2004) Selected non-steroidal anti-inflammatory drugs and their derivatives target γ-secretase at a novel site. Evidence for an allosteric mechanism. *J. Biol. Chem. 279*, 43419−43426.
(165) Clarke, E. E., Churcher, I., Ellis, S., Wrigley, J. D., Lewis, H. D., Harrison, T., Shearman, M. S., and Beher, D. (2006) Intra- or intercomplex binding to the γ-secretase enzyme. A model to differentiate inhibitor classes. *J. Biol. Chem. 281*, 31279−31289.
(166) Iben, L. G., Olson, R. E., Balanda, L. A., Jayachandra, S., Robertson, B. J., Hay, V., Corradi, J., Prasad, C. V., Zaczek, R., Albright, C. F., and Toyn, J. H. (2007) Signal peptide peptidase and γ-secretase share equivalent inhibitor binding pharmacology. *J. Biol. Chem. 282*, 36829−36836.
(167) Botev, A., Munter, L. M., Wenzel, R., Richter, L., Althoff, V., Ismer, J., Gerling, U., Weise, C., Koksch, B., Hildebrand, P. W., Bittl, R., and Multhaup, G. (2011) The amyloid precursor protein C-terminal fragment C100 occurs in monomeric and dimeric stable conformations and binds γ-secretase modulators. *Biochemistry 50*, 828−835.
(168) Richter, L., Munter, L. M., Ness, J., Hildebrand, P. W., Dasari, M., Unterreitmeier, S., Bulic, B., Beyermann, M., Gust, R., Reif, B., Weggen, S., Langosch, D., and Multhaup, G. (2010) Amyloid β 42 peptide (Aβ42)-lowering compounds directly bind to Aβ and interfere with amyloid precursor protein (APP) transmembrane dimerization. *Proc. Natl. Acad. Sci. U.S.A. 107*, 14597−14602.
(169) Beel, A. J., Barrett, P., Schnier, P. D., Hitchcock, S. A., Bagal, D., Sanders, C. R., and Jordan, J. B. (2009) Nonspecificity of binding of γ-secretase modulators to the amyloid precursor protein. *Biochemistry 48*, 11837−11839.
(170) Barrett, P. J., Sanders, C. R., Kaufman, S. A., Michelsen, K., and Jordan, J. B. (2011) NSAID-Based γ-Secretase Modulators Do Not Bind to the Amyloid-β Polypeptide. *Biochemistry 50*, 10328−10342.
(171) Espeseth, A. S., Xu, M., Huang, Q., Coburn, C. A., Jones, K. L., Ferrer, M., Zuck, P. D., Strulovici, B., Price, E. A., Wu, G., Wolfe, A. L., Lineberger, J. E., Sardana, M., Tugusheva, K., Pietrak, B. L., Crouthamel, M. C., Lai, M. T., Dodson, E. C., Bazzo, R., Shi, X. P., Simon, A. J., Li, Y., and Hazuda, D. J. (2005) Compounds that bind APP and inhibit Aβ processing in vitro suggest a novel approach to Alzheimer disease therapeutics. *J. Biol. Chem. 280*, 17792−17797.
(172) Munter, L. M., Voigt, P., Harmeier, A., Kaden, D., Gottschalk, K. E., Weise, C., Pipkorn, R., Schaefer, M., Langosch, D., and Multhaup, G. (2007) GxxxG motifs within the amyloid precursor protein transmembrane sequence are critical for the etiology of Aβ42. *EMBO J. 26*, 1702−1712.
(173) Barrett, P. J., Song, Y., Van Horn, W. D., Hustedt, E. J., Schafer, J. M., Hadziselimovic, A., Beel, A. J., and Sanders, C. R. (2012) The amyloid precursor protein has a flexible transmembrane domain and binds cholesterol. *Science 336*, 1168−1171.
(174) Okochi, M., Fukumori, A., Jiang, J., Itoh, N., Kimura, R., Steiner, H., Haass, C., Tagami, S., and Takeda, M. (2006) Secretion of the Notch-1 Aβ-like peptide during Notch signaling. *J. Biol. Chem. 281*, 7890−7898.
(175) Wanngren, J., Ottervald, J., Parpal, S., Portelius, E., Stromberg, K., Borgegard, T., Klintenberg, R., Jureus, A., Blomqvist, J., Blennow, K., Zetterberg, H., Lundkvist, J., Rosqvist, S., and Karlstrom, H. (2012) Second generation γ-secretase modulators exhibit different modulation of Notch β and Aβ production. *J. Biol. Chem. 287*, 32640−32650.
(176) Sagi, S. A., Lessard, C. B., Winden, K. D., Maruyama, H., Koo, J. C., Weggen, S., Kukar, T. L., Golde, T. E., and Koo, E. H. (2011) Substrate sequence influences γ-secretase modulator activity, role of the transmembrane domain of the amyloid precursor protein. *J. Biol. Chem. 286*, 39794−39803.
(177) Sato, T., Nyborg, A. C., Iwata, N., Diehl, T. S., Saido, T. C., Golde, T. E., and Wolfe, M. S. (2006) Signal peptide peptidase: Biochemical properties and modulation by nonsteroidal antiinflammatory drugs. *Biochemistry 45*, 8649−8656.

(178) Jumpertz, T., Rennhack, A., Ness, J., Baches, S., Pietrzik, C. U., Bulic, B., and Weggen, S. (2012) Presenilin Is the Molecular Target of Acidic γ-Secretase Modulators in Living Cells. *PLoS One 7*, e30484.
(179) Pozdnyakov, N., Murrey, H. E., Crump, C. J., Pettersson, M., Ballard, T. E., Am Ende, C. W., Ahn, K., Li, Y. M., Bales, K. R., and Johnson, D. S. (2013) γ-Secretase modulator (GSM) photoaffinity probes reveal distinct allosteric binding sites on presenilin. *J. Biol. Chem. 288*, 9710.
(180) Chavez-Gutierrez, L., Bammens, L., Benilova, I., Vandersteen, A., Benurwar, M., Borgers, M., Lismont, S., Zhou, L., Van Cleynenbreugel, S., Esselmann, H., Wiltfang, J., Serneels, L., Karran, E., Gijsen, H., Schymkowitz, J., Rousseau, F., Broersen, K., and De Strooper, B. (2012) The mechanism of γ-secretase dysfunction in familial Alzheimer disease. *EMBO J. 31*, 2261−2274.
(181) Kukar, T. L., Ladd, T. B., Robertson, P., Pintchovski, S. A., Moore, B., Bann, M. A., Ren, Z., Jansen-West, K., Malphrus, K., Eggert, S., Maruyama, H., Cottrell, B. A., Das, P., Basi, G. S., Koo, E. H., and Golde, T. E. (2011) Lysine 624 of the amyloid precursor protein (APP) is a critical determinant of amyloid β peptide length: Support for a sequential model of γ-secretase intramembrane proteolysis and regulation by the amyloid β precursor protein (APP) juxtamembrane region. *J. Biol. Chem. 286*, 39804−39812.
(182) Bateman, R. J., Xiong, C., Benzinger, T. L., Fagan, A. M., Goate, A., Fox, N. C., Marcus, D. S., Cairns, N. J., Xie, X., Blazey, T. M., Holtzman, D. M., Santacruz, A., Buckles, V., Oliver, A., Moulder, K., Aisen, P. S., Ghetti, B., Klunk, W. E., McDade, E., Martins, R. N., Masters, C. L., Mayeux, R., Ringman, J. M., Rossor, M. N., Schofield, P. R., Sperling, R. A., Salloway, S., Morris, J. C., and Dominantly Inherited Alzheimer, N. (2012) Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N. Engl. J. Med. 367*, 795−804.