Journal of Medicinal Chemistry Perspective

J. Med. Chem. **2011,** *54,* 669–698 **669**
DOI: 10.1021/jm101168r

γ-Secretase Modulators as Potential Disease Modifying Anti-Alzheimer's Drugs

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Received September 8, 2010

Introduction

Alzheimer's disease (AD[a]) is an incurable degenerative disease that was first described by the German psychiatrist and neuropathologist Alois Alzheimer in 1906, from whom it takes its name. He reported the existence of two abnormal structures, senile plaques and neurofibrillary tangles, in the brain of his patient Auguste D., who was suffering from dementia.[1] AD is an age-related neurodegenerative disorder, which progresses through symptoms including confusion, aggression, irritability, loss of vocal and motor control, long- and short-term memory loss, and gradual loss of bodily functions, which ultimately results in death.[2]

More than 26 million people worldwide suffer from AD, and a recent estimate predicts that this number will quadruple by 2050 to more than 106 million.[3] To date, the disease therapeutics consists of just five FDA approved drugs, which only treat cognitive decline and negative symptoms of the disease. The drugs fall into two classes: the first four, donepezil, rivastigmine, tacrine, and galantamine, are acetylcholine esterase inhibitors, and the fifth most recent compound, memantine, is an NMDA antagonist.[4] Thus, the development of a treatment or cure for AD that may slow or reverse the progression of the disease in the form of a so-called "disease-modifying anti-Alzheimer's drug" (DMAAD) represents a huge unmet medical, social, and economic need. The development of DMAADs relies on the understanding of the disease progression and pathway and on identifying the molecular targets of therapeutic interest.

AD is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. The neuronal loss is caused by the existence of extracellular senile plaques and intercellular neurofibrillary tangles, both entities that characterize the disease.[5] The plaques are dense, mostly insoluble deposits of amyloid-β (Aβ) that are produced from amyloid precursor protein (APP) by two sequential proteolytic reactions (Figure 1).

APP is primarily cleaved by the action of β-secretase (BACE) at the N-terminus of Aβ, producing soluble β-APPs fragments and a membrane bound C-99 fragment. The latter then serves as the substrate for subsequent cleavage by γ-secretase. This results in the formation of APP intracellular domain (AICD) and various Aβ species of differing lengths ranging from 37 to 49 amino acids. Of these, Aβ_{42} constitutes between 5% and 10% of the overall Aβ population and is more hydrophobic and prone to aggregation than the shorter isoforms. It is believed to be neurotoxic when aggregated, resulting in the formation of amyloid plaques. Investigations have revealed a link between these plaques and the pathogenesis of AD.[6−9] γ-Secretase cleaves the APP transmembrane domain in a progressive, stepwise manner at the ε, ζ, and γ sites, resulting in Aβ species of varying length.[9,10] Published results suggest that this stepwise cleavage may occur via two different routes.[11] These initiate with the formation of Aβ_{49} and Aβ_{48} and then proceed via the cleavage at approximately every three residues, i.e., every helical turn of the substrate. The two product lines are ε49-ζ46-γ43-γ40 and ε48-ζ45-γ42. Further cleavage will subsequently generate the other isoforms Aβ_{39}, Aβ_{38}, and Aβ_{37}, of which Aβ_{38} results from the product line containing Aβ_{42}.[10,11] This process is represented schematically in Figure 1.

The pathway from the monomeric Aβ to the plaques progresses through many stages of aggregation where non-fibrillary and fibrillary aggregates of different dimensions are formed. Aβ_{42} is key to these intermediates because it has a high propensity to form these aggregates with itself and other proteins. Importantly, all mutations of APP observed in early onset Alzheimer's disease (EOAD) or familial Alzheimer's disease (FAD) induce a relative increase in the production of Aβ_{42}, thus pointing to a relationship between Aβ_{42} and the development of AD. The neurofibrillary tangles that are observed intracellularly are a result of the aggregation of hyperphosphorylated tau protein.[5] Although many older individuals develop some plaques and tangles as a consequence of aging, the brains of AD patients have a greater number of them in specific brain regions such as the temporal lobe.[12]

In 1992, soon after the discovery of FAD causing mutations in the genes that encode for the key proteins that are involved

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[a] Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β peptide; AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; Aph-1, anterior pharynx-defective 1; AUC, area under the curve; BACE, β-amyloid cleavage enzyme; CNS, central nervous system; COX, cyclooxygenase; CTF, C-terminal fragment; DMPK, drug metabolism and pharmacokinetics; DMAAD, disease-modifying anti-Alzheimer's drug; EOAD, early onset Alzheimer's disease; FDA, Food and Drug Administration; FAD, familial Alzheimer's disease; GS, γ-secretase; GSI, γ-secretase inhibitor; GSM, γ-secretase modulator; HTS, high-throughput screening; ICDs, intracellular domains; LOAD, late onset Alzheimer's disease; MCI, mild cognitive impairment; Nct, nicastrin; NICD, Notch intracellular domain; NMDA, *N*-methyl-D-aspartic acid; NMR, nuclear magentic resonance; NSAID, nonsteroidal anti-inflammatory drug; PS-1, presenilin; PS-2, presenilin-2; Pen-2, presenilin enhancer protein 2; PSEN, presenilin; RIP, regulated-intramembrane proteolysis; SAD, sporadic Alzheimer's disease; sAPP, soluble APP fragment.

Published on Web 12/09/2010

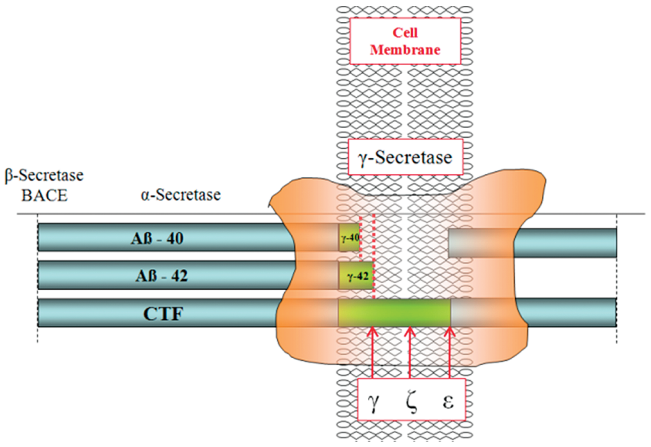

Figure 1. Processing of APP through the amyloidogenic pathway. After initial cleavage by β-secretase and release of the soluble APP, the remaining APP segment is further cleaved by γ-secretase. First, ε-cleavage by γ-secretase releases the AICD. Second, γ-secretase cleavage releases the Aβ peptide, of which there can be longer and shorter forms (e.g., Aβ_{40} and Aβ_{42}), depending on the exact cleavage site by γ-secretase.[9]

in the production of APP or the γ-secretase components presenilin (PS-1) and presenilin-2 (PS-2), John Hardy proposed the amyloid cascade hypothesis. This states that amyloid is at the center of the pathophysiology of AD: amyloid deposits in the CNS are the primary cause of and instigate the process that drives a pathological cascade that eventually culminates in the manifestation of the disease.[13] Since then, the scientific community has generally, but not universally, accepted the amyloid hypothesis, suggesting that amyloid formation and/or deposition has a key role in the development of AD.

The amyloid cascade hypothesis is based on several facts and lines of evidence, the most compelling being (i) the disease pathology and post-mortem data, (ii) the biological properties of the Aβ peptides, (iii) the evidence gathered from transgenic animal models that enforce some previously observed aspects of the disease,[14,15] and (iv) the genetic evidence related to the disease. AD cases are divided into two groups, either "early onset AD" (EOAD) or "late onset AD" (LOAD), with the division set at 65 years old.[16] EOAD has a large genetic component. The incidence rate in affected families is 50% per generation, which is statistically typical for autosomal-dominant inheritance. A single copy of the mutant gene is enough to predispose the carrier to 100% likelihood of being affected with AD. Although cases of EOAD represent only a minor percentage (< 10%) of all AD patients, they have helped to contribute to the understanding of AD.[17,18]

Familial history of AD represents the second largest risk factor behind that of age. It is hypothesized that a genetic predisposition can influence the development of the disease.[19] Of the cases of AD, only 5−10% are EOAD, of which only 50% are FAD. These are usually diagnosed when a patient has a first degree relative with history of AD. FAD results from a mutation in the APP, PS-1, or PS-2. More than 160 FAD mutations in PS-1 have been identified. Sporadic Alzheimer's disease (SAD) is the name given to non-FAD cases. FAD and SAD follow similar disease progression, which has led to the acceptance of a "linear" amyloid hypothesis. Both FAD and SAD result in an increase in Aβ plaque load. All FAD mutations lead to an increased ratio of Aβ_{42}/Aβ_{40}. This results in an acceleration of the formation of amyloid plaques, as demonstrated in transgenic mice.[20] In SAD the increase is due to more heterogeneous and more complex mechanisms.[21] A recent development has been the proposal of a "dual pathway" model for SAD. This assumes that for SAD, the cascades for tau and Aβ are linked, although this signal network still remains to be discovered.[22] Further elucidation of the signal pathways responsible for both forms of AD will aid the identification of future biomarker and therapeutic targets for AD.

It is also acknowledged that amyloid deposits precede symptoms by many years. These amyloid deposits predominantly contain the more aggregation prone, cytotoxic Aβ_{42}.[23] The shorter Aβ peptides are speculated to be less toxic or even neuroprotective.[24,25] Over recent years the hypothesis has been met with some opposition, mainly concentrated around two observations. First, the degree of dementia does not correlate with the amount of Aβ plaque loading, since some dementia patients show no Aβ-plaques post-mortem.[26] Second, it has been shown, using neuroimaging techniques in vivo, that plaques exist in people who are cognitively intact. These results have triggered the discussion that the plaques are not responsible for AD but could be a protective measure such as a site for depositing toxins.[27,28] Despite the data gathered from genetic and pathological analyses of AD, the exact mechanism of how the Aβ plaques exert their toxicity is not understood. Some scientists believe that the brain levels of soluble Aβ appear to correlate better with severity of cognitive impairment than with the number and density of plaques found in the brain. Recently, it has been postulated that the soluble Aβ species are to blame for AD, since it has been shown that these species correlate better with the degradation of the synapses and cognitive impairment.[29−31] It has been reported that soluble Aβ species inhibit critical neuronal activities, including "long term potentiation".[32] Regardless of whether plaques or soluble Aβ species are causative in the AD disease pathway, an intervention in the production of the Aβ_{42} peptides or an increase in the Aβ_{40}/Aβ_{42} ratio could potentially have a beneficial effect regardless of the mechanism.

Pharmacological Intervention in Aβ Peptide Production

Since the biosynthesis of Aβ_{42} and other isoforms ranging from 37 to 49 amino acids are dependent on β- and γ-secretase via cleavage of APP, the inhibition or modulation of β- or γ-secretase is an obvious therapeutic target, with the goal of decreasing the concentration of Aβ and in particular Aβ_{42}. Other routes to reduce Aβ plaques could be the stimulation of the clearance pathways for the Aβ oligomers or the prevention of the aggregation of Aβ_{42}.[6] Another therapy, although less tangible from a druggability perspective, could be the activation/stimulation of α-secretase. The processing of APP by α-secretase is considered to be protective and reduces the risk of AD. α-Secretase cleaves APP between amino acids 16 and 17 in the Aβ sequence, preventing the formation of Aβ_{42} and resulting in soluble APP α ectodomain and a membrane-bound carboxy-terminal fragment.[33]

β-Secretase would be an obvious target, and many research groups have targeted this secretase in the hope of decreasing the concentration of all isoforms of Aβ.[34] The progress toward drug candidates in this field has been very slow because of the difficulty of designing and synthesizing orally bioavailable small molecules with the physiochemical characteristics necessary to gain entry to the intracellular compartments where the endogenous BACE enzyme is localized and that can

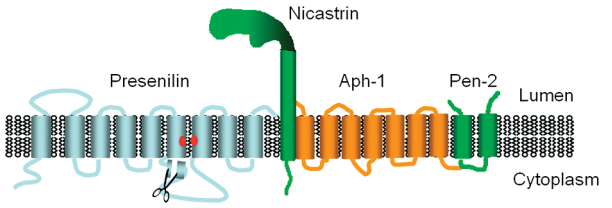

Figure 2. γ-Secretase complex. The catalytic aspartyl residues are indicated in red and are in the presenilin protein.[33] The figure is from Prof B. De Strooper and is produced with his permission.

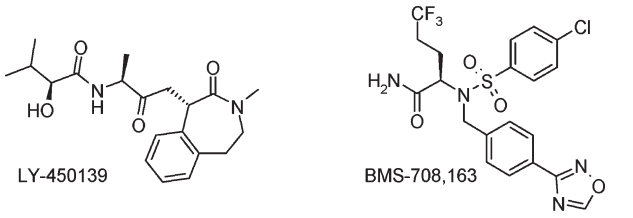

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Figure 3. Eli Lilly and BMS GSI compounds.

interact with the large substrate binding site of the BACE enzyme.[35] The other critical enzyme required for the production of Aβ species and obvious therapeutic target is γ-secretase, which, as the key subject of this Perspective, will be discussed in more detail in the following section.

What Is γ-Secretase?

γ-Secretase is a member of the intramembrane cleaving aspartyl protease family consisting of multiple subunits. γ-Secretase is unusual, as it cleaves single-pass transmembrane proteins at residues within the transmembrane domain in a process called regulated intramembrane proteolysis (RIP), in which it is thought to play a key role.[36−38] γ-Secretase is known to have multiple substrates, and to date, more than 50 have been identified in addition to APP. These include Notch, Jagged, and Nectin-1α.[39] Signal pathways are activated by RIP, allowing intracellular domains (ICDs) to translocate to the nucleus, as in the case of Notch. RIP plays an essential role in the activity of developed cells and is partly responsible for the maturation of cells.

The γ-secretase protease complex consists of four main proteins: anterior pharynx-defective 1 (Aph-1), presenilin enhancer protein 2 (Pen-2), presenilin (PSEN), and nicastrin (Nct) (Figure 2).[40] Presenilins constitute the aspartic protease catalytic subunit of γ-secretase; they are a family of related multipass transmembrane proteins that function as a part of the γ-secretase protease complex. Vertebrates have two presenilin genes, namely, *PSEN1* (located on chromosome 14 in humans) that encodes for presenilin 1 (PS-1) and *PSEN2* (located on chromosome 1 in humans) that encodes for presenilin 2 (PS-2). Human PS-1 contains 467 amino acids and has been identified as having nine-transmembranal topography, exhibiting a cytosolic amino terminus and a luminal carboxy terminus vide infra (Figure 2). PS-2 differs from PS-1 in that it lacks four amino acid residues between amino acids 26 and 29, close to the amino terminus.[41] As already stated, in addition to PS-1, γ-secretase contains Pen-2, Aph-1, and Nct. Aph-1 is a seven-transmembranal protein with a cytosolic carboxy terminus.[42] Pen-2 is a two-transmembranal protein with both the C terminus and N terminus in the lumen.[43] The final component of γ-secretase is Nct, a type 1 membrane glycoprotein with an enlarged luminal domain, and its ectodomain is responsible for the maturation of the complex.[44,45] The overall stoichiometry of γ-secretase complex has been discovered to be monomeric, containing one copy of each of the four components.[46] This was verified by transmission electron microscopy, although unfortunately the resolution has not been sufficient to orientate individual components.[47] An important characteristic identified in γ-secretase is the mode in which water molecules enter the γ-secretase environment and gain access to the aspartate catalytic site in the lipid bilayer. This water is crucially involved in the hydrolysis of the substrates. Using cysteine scanning mutagenesis of Pen-1, two groups confirmed independently that transmembranes 6 and 7 form a water containing cavity with two catalytic aspartates in proximity to each other.[48−50] Further structural elucidation to aid drug discovery using NMR and X-ray crystallography will undoubtedly take many years because of the complexity of analyzing such a multicomponent complex exhibiting 19 transmembranal domains.

γ-Secretase Inhibition

γ-Secretase inhibition has been and remains an active field. Many research programs have existed during the past decade, and the progress in this field has been recently reviewed in a Perspective.[6] γ-Secretase inhibitors (GSIs) can be classified into three subgroups, depending on where they bind to the γ-secretase complex: (i) active site binding GSIs, (ii) substrate docking-site-binding GSIs, and (iii) alternative binding site GSIs, which can be further divided into carboxamide and arylsulfonamide containing GSIs.[51]

The first generation of GSIs showed undesirable side effects attributed to the inhibition of Notch processing by γ-secretase. These consisted of interference of the natural maturation of B- and T-lymphocytes, resulting in gastrointestinal tract toxicity and increased susceptibility to infection.[52−54] Many orally bioavailable, brain penetrating GSIs have been identified and have been shown to decrease Aβ production in both human and mouse brains.[55−58] Several GSIs were undergoing clinical trials, of which LY-450139 (semagacestat, **1**, Figure 3) was in phase III clinical trials. GSI **1** does not show an appreciable selectivity over Notch inhibition, resulting in a narrow safety window between the Aβ lowering effect and the undesired side effects. Recently, Eli Lilly has announced that they halted the development of semagacestat (**1**) because of results from two long-term phase III studies.[59] The results actually showed a decline in cognition and the ability to perform daily tasks when compared to placebo. In addition, **1** was shown to be associated with an increased risk in skin cancer, which possibly can be attributed to Notch processing.[60] Although still unclear, the negative trial results may be attributed to several factors including the stimulation of GS due to a rebound effect, thus resulting in overall stimulation of Aβ production, or the inhibition of GS resulting in the inhibition of processing of not only APP but also other substrates relevant for cognition.[61−64] The Notch signaling pathway, for example, has been linked to the formation of long-term memories.[65] Later generations of GSIs have attempted to address these unwanted side effects, developing compounds that show selectivity for APP over other substrates, especially Notch. The first compound to reach clinical trials from this generation of Notch sparing activity was BMS-708,163 (**2**). Its mode of action/binding remains undisclosed, and the extent to which it will result in a safer profile is still unknown. The area of GSIs is still active, but the synthesis of therapeutic agents still

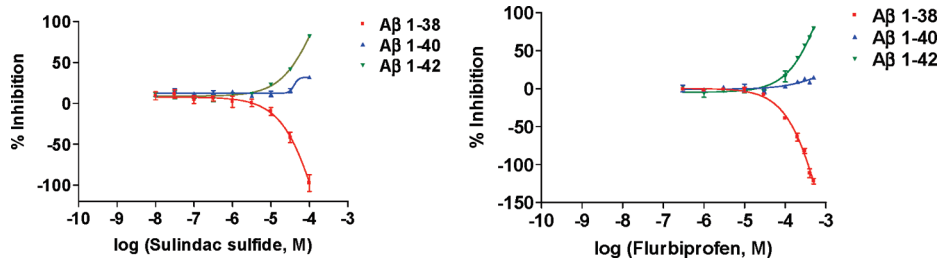


Figure 4. Percentage inhibition of the Aβ-isoforms by **4** and **5**.

must overcome many unknown obstacles, including a lack of knowledge in the factors affecting selectivity of Aβ_{42} versus Aβ_{40} and Notch processing, the possibility of processing other substrates, and the potential side effects caused by long-term dosing, which can be illustrated by the recently reported increase in the occurrence of skin cancer upon treatment with **1**.[60]

An unsolved problem associated with the GSI class is the occurrence of the late rebound effect in plasma levels of Aβ. To date, the reason for this rebound effect, which consists of an increase in the levels of Aβ when concentrations of the drug decrease, is unknown. This phenomenon has been observed in both animals and humans.[61−64] Interestingly, studies in monkeys have revealed that the rebound effect is dependent on the potency and dosing of the GSI. It has been hypothesized that it could be caused by activation of two different active sites on the γ-secretase complex.[64] However, this does not answer all the observations, and it is clear that a full understanding of the pharmacodynamics of γ-secretase remains elusive.[66]

γ-Secretase Modulation

More recently, the so-called γ-secretase modulation, an alternative approach to intervene pharmacologically with the activity of γ-secretase, has become the subject of intense investigation. A γ-secretase modulator (GSM) is defined as a molecule that changes the relative proportion of the Aβ isoforms while maintaining the rate at which APP is processed. The first generation of GSMs originated from an epidemiological study revealing a reduced occurrence of AD in patients using nonsteroidal anti-inflammatory drugs (NSAIDs). This observation resulted in the discovery that a subset of NSAIDS, including indomethacin (**3**), sulindac sulfide (**4**), flurbiprofen (**5**), and ibuprofen (**7**), were able to modulate the production of Aβ peptides in vitro and in vivo.[67,68] These NSAIDs were shown to selectively reduce the production of Aβ_{42} while simultaneously increasing the levels of Aβ_{38} without significantly changing the levels of Aβ_{40}. Typical dose-response curves illustrating this phenomenon are shown in Figure 4 for sulindac sulfide **4** and flurbiprofen **5**.

In addition, compounds causing reverse modulation, increasing the levels of Aβ_{42} and decreasing the levels of Aβ_{38}, were identified.[69] These include celecoxib (**8**) and fenofibrate (**9**) (Figure 5). Enzyme kinetic studies imply that these NSAIDS are noncompetitive with respect to APP substrate, suggesting an interaction with a different active site.[69] The site of cleavage within the Notch transmembranal domain was similarly affected by these modulators and inverse modulators, but this subtle change did not inhibit the release of the intracellular domain and thus has no effect on Notch signaling.[70] As already mentioned, the inhibition of Notch processing is considered a major hurdle in the development of safe GSIs. The lack of Notch inhibition by GSMs may

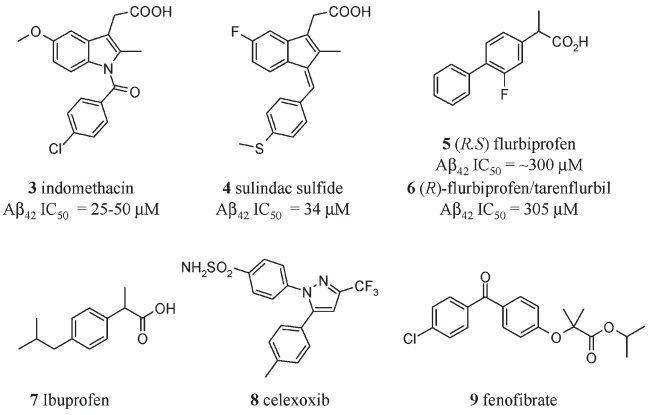


Figure 5. NSAID GSMs.

therefore present a considerable advantage over the GSI approach. However, the approach of γ-secretase modulation using NSAID derivatives represented some challenges in the form of continued dosing of cyclooxygenase 1 (COX1) inhibitors, as most of the NSAIDs exhibited activity against COX1. This resulted in significant gastrointestinal and renal toxicity and would preclude the prolonged clinical use of compounds with a combined GSM−COX1 activity. However, the COX1 activity was shown to be independent of the γ-secretase modulatory activity or vice versa. Flurbiprofen (**5**) is used for pain relief and inflammation and is administered as a racemate. However, the *R*-enantiomer, called tarenflurbil (**6**), is devoid of COX1 activity while retaining γ-secretase modulatory activity.[68]

Since the discovery of the NSAID series, other small molecules, both NSAID and non-NSAID derived, have been explored with improved in vitro potency. GSM research has resulted in the publication of around ~80 patent applications and many journal articles related to both the NSAID and non-NSAID series of GSMs, with a high concentration of these being published in the past 3 years. The validation of γ-secretase modulation as a target for the treatment of AD is now under investigation. Reports on GSMs derived from multiple chemical classes have now appeared, demonstrating acute reduction in the concentration of Aβ_{42} in brain or CSF in nontransgenic mice. The first reports of the effects on plaque formation are also appearing, in addition to a limited amount of clinical data. Before going into more detail on the various GSMs and their pharmacological profiles, we will first address the information available on the molecular mechanism of GSMs.

How Do γ-Secretase Modulators Work?

Although the precise molecular mechanism of γ-secretase modulation on Aβ production is still unclear, recent studies using NSAID derived GSMs start to give some indications of

the molecular interaction of these molecules with the γ-secretase complex.

The initial studies describing the $A\beta_{42}$ lowering effects of NSAIDS like **3**, **4**, and **5** have clearly ruled out the involvement of COX1 and COX2 and other known NSAID targets as a mechanism involved in selective modulation of APP cleavage.[71] NSAIDs and their more potent carboxylic acid derivatives have been shown to retain their ability to reduce $A\beta_{42}$ in cell free and partially purified γ-secretase preparations.[68,72,73] Since then, two main hypotheses have evolved to explain the action of GSMs, one via enzyme-targeting and the other via substrate-targeting of the GSMs. The first one, enzyme-targeting, suggests an interaction of the compounds with γ-secretase, and data have been generated by several groups indicating that GSMs interact with presenilin allosterically, resulting in a modification of the enzyme's conformation responsible for the altered cleavage specificity.[74−76] The enzyme-targeting of GSMs has been further substantiated by the finding that certain NSAID GSMs can also modulate, but not inhibit, the γ-secretase cleavage site of Notch in a similar ranking as observed in the cleavage of APP.[70] The second, more recent hypothesis originated from photo-cross-linking experiments using biotinylated and photo-affinity labeled derivatives of the GSM **6** and the inverse GSM **9** which were shown to label APP and its C-terminal derivatives.[77] They were therefore concluded to indirectly modulate γ-secretase via substrate targeting. This theory is still controversial. For example, subsequent NMR studies on the interaction between APP and GSMs, including **6** and **9**, revealed no evidence of specific binding, and the interaction was described as nonspecific binding due to aggregation of C-99 fragment under the experimental conditions used.[78]

APP has been shown to form dimers via two sites in the ectodomain as well as a site in the transmembrane sequence (TMS) consisting of three consecutive GxxxG motifs.[79,80] Mutations in the Aβ sequence aimed at disrupting the dimer interface have been shown to attenuate the TMS-dimerization strength and result in a reduced formation of $A\beta_{42}$ in favor of $A\beta_{38}$.[79] Likewise, the APP−TMS dimers have been shown to be destabilized by the NSAIDs sulindac sulfide and indomethacin in a concentration-dependent manner, which correlated to their ability to reduce the formation of $A\beta_{42}$.[81] In this study, sulindac sulfide **4** and analogues were shown by plasma resonance analysis and NMR studies to directly bind to the Aβ sequence. Additional docking studies suggested that the compounds bind to the GxxxG dimerziation motifs in the APP−TMS, thereby modulating the APP−TMS interactions similarly to the mutational studies. In another recent mutation analysis study on APP, the APP substrate targeting of GSMs was questioned again.[82] Mutations of residues in the proposed GxxxG GSM-binding site (Gly-29, Gly-33) as well as in the basic amino acid residue Lys-28, which has been proposed to interact with the carboxylic acid group present in the NSAID derived GSMs, were all responsive to treatment with **73**, a potent carboxylic acid GSM.[83] $A\beta_{42}$ increasing FAD mutations within the γ-secretase cleavage site domain of APP also responded to GSMs such as **4** and **73**. In contrast, the same compounds were shown to display different or no effects on $A\beta_{42}$ and $A\beta_{38}$ levels when preselinin mutants were used, indicating an interaction of the GSMs with the enzyme.[83] Interestingly, this study also demonstrated that mutations that showed no effect on $A\beta_{42}$ upon treatment with GSMs did display a robust increase in $A\beta_{38}$, showing that the production of $A\beta_{38}$ and $A\beta_{42}$ is not interdependent.

Another hypothesis on the molecular mechanism of GSMs has been proposed based on the alteration of the membrane architecture by sulindac sulfide, potentially resulting in a conformational shift of the membrane embedded enzyme−substrate complex favoring the processing toward shorter Aβ peptides.[84] Studies using GSMs as well as mutations targeting either γ-secretase components (PS-1, Pen2, and Aph1) or γ-secretase substrate (APP) and that are known to change $A\beta_{42}$ production have all been shown to cause conformational changes in PS-1 via Forster resonance energy transfer (FRET) based approaches.[85] It can therefore be expected that there is no uniform molecular mechanism or common allosteric binding site for the various classes of GSMs known today. Most of the mechanistic studies with GSMs mentioned above have been carried out using weak NSAID derived first generation compounds. The high concentration of these often lipophilic compounds required to achieve efficacy could lead to nonspecific interactions with membranes, substrates, or the enzyme complex. Similar studies using more recently reported GSMs from structurally distinct chemical classes and considerably more potent (up to 1000-fold) may help to obtain more conclusive information on the molecular mechanism of γ-secretase modulation.

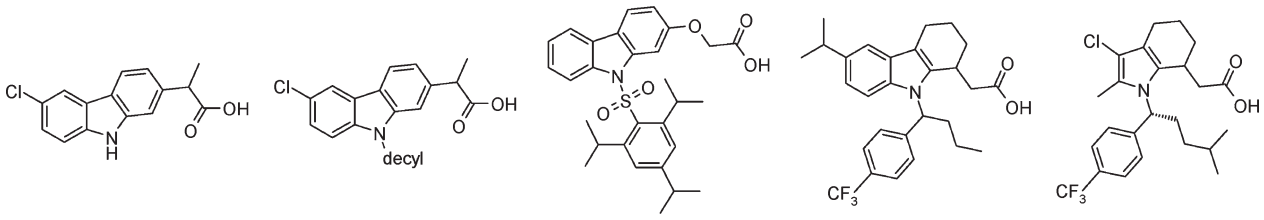
Chemical Classes of γ-Secretase Modulators

The terminology used in published material on γ-secretase ligands is not always clear. The terms and concepts of modulation/modulator and inhibition/inhibitor are not always used with the same meaning. Often, careful analysis of the available material is needed to clarify whether the subject matter deals with true inhibitors or modulators, as defined in this Perspective.

For this review, we have divided the GSM field into two main classes: (i) NSAID derived carboxylic acids, the discovery of which has already been explained in the previous sections; (ii) non-NSAID derived compounds, which originate from work by Neurogenetics and which do not contain a carboxylic acid group.[86] Subsequent work by Eisai led to the first compound from this series reaching the clinic, which has prompted many companies to further investigate this class of GSMs. A large part of this Perspective will deal with a comprehensive overview of chemotypes derived from this series. A series of triterpenoids derived from ginseng have also been described as GSMs and will be briefly discussed in a later section.

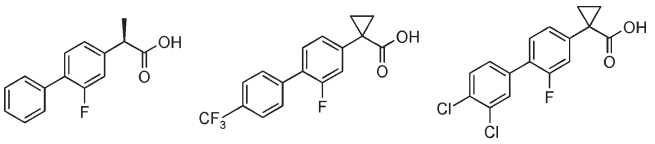
NSAID Derived GSMs

The discovery in 2001 that NSAIDS such as indomethacin (**3**), sulindac sulfide (**4**), and ibuprofen (**7**) modulate γ-secretase has spurred the exploration of carboxylic acid analogues.[67] The early contributions to this class of GSMs have been regularly reviewed up to 2008.[87−91] This Perspective will focus on those carboxylic acid series, for which the pharmacological data were recently published, especially in vivo. In addition, we will discuss the most recent medicinal chemistry that has been patented and published around this area. Compounds **11**−**14** represent key early examples derived from the NSAIDS carprofen (**10**)[92,93] and **3**[94,95] (Figure 6). Recently, analogues of **11** and **12**, containing even longer (up to 18 carbon alkyl chains) lipophilic N-substituents, were postulated to be substrate-targeting GSMs in line with some of the mechanistic work described earlier.[74,77] The carboxylic acid functionality was proposed to interact with a lysine residue of APP located close to the membrane interface



10, Carprofen; $A\beta_{42}$ IC_{50} = 76 μM | **11** $A\beta_{42}$, IC_{50} = 2.9 μM | **12** $A\beta_{42}$, IC_{50} = 7.5 μM | **13** | **14**

Figure 6. NSAID derived GSMs.



6 (R)-flurbiprofen | **15** CHF5022 | **16** CHF5074

Figure 7. Second generation NSAID derived GSMs.

(for example, Lys-624), with the lipophilic substituents serving as membrane anchors.[96]

Tarenflurbil has served as a starting point for Chiesi with the aim of increasing $A\beta_{42}$ inhibitory potency while removing COX inhibitory activity.[97] The replacement of the α-methyl substituent in **6** (Figure 7) by a cyclopropyl group led to a complete removal of COX inhibition. The $A\beta_{42}$ inhibition could be improved via the addition of substituents on the terminal phenyl ring. This has culminated in **15** (CHF5022) and **16** (CHF5074), which were found to be 3- and 7-fold more potent than **6** in inhibiting $A\beta_{42}$ secretion in vitro (IC_{50} of 92, 40, and 268 μM, respectively).[98] No COX1 (at 100 μM) or COX2 (at 300 μM) inhibition was observed for **15** and **16**. Brain penetration of both compounds can still be considered poor, with a brain/plasma ratio of about 10% for **15** and 5% for **16** in mice after 100 or 300 (mg/kg)/day for 4−5 days. However, after prolonged 4-week dosing of **16** in mice, brain levels were considerably higher (mean plasma/brain levels of (580 μM)/(20 μM)) than for **6** (mean plasma/brain levels of (74 μM)/(1.3 μM)). Although plasma $A\beta_{42}$ levels were dose-dependently decreased by **15** and **16**, no significant changes in brain $A\beta_{40}$ and $A\beta_{42}$ levels were observed after 4−5 days of dosing for either compound or after a 4-week treatment with **15**.[98] To evaluate the long-term effects on Aβ brain pathology, two studies have been published investigating the chronic treatment of transgenic mice with **16**. In the first study, aged Tg2576 transgenic mice expressing the Swedish mutated form of human APP were treated for 17 weeks with **16**-medicated diet (375 ppm, ∼60 (mg/kg)/day, corresponding to a brain concentration of **16** of 6.4 μM after 17 weeks).[99] This resulted in a significant reduction in brain plaque load. In addition, $A\beta_{40}$ and $A\beta_{42}$ levels were reduced. In the second study, a similar chronic treatment of 6-month-old hAPP mice expressing the Swedish and London mutations for a 6-month period confirmed the reduced plaque load. A reduction in area occupied by plaques was observed in both cortex (32%) and hippocampus (42%), as well as a reduction in the number of plaques (28% in cortex and 34% in hippocampus). Moreover, behavioral testing after the 6-month treatment in the Morris water maze model showed an improvement in spacial memory deficit compared to controls or transgenic animals treated with ibuprofen **7**.[100] In contradiction to the first chronic treatment study, despite the reduced brain plaque burden, no significant effect on total brain and CSF Aβ levels was observed, which is in line with the results obtained after short-term treatment described above.[98] Although no convincing explanation of this discrepancy has been given, it could be due to a proposed dual, synergistic mechanism of action of some GSMs, lowering the production of $A\beta_{42}$ and inhibiting Aβ aggregation. The differences in outcome of the two chronic treatment studies with **16** could also be related to the use of different strains of transgenic mice. The additional London mutation used in the second study is a mutation in the γ-secretase cleavage site of APP and could therefore have an influence on the modulation by a GSM. Compound **16** was also evaluated in animal models of nonspatial memory using subchronic (4 weeks) and chronic (10 months) treatment of 6-month-old, plaque-free hAPPsw transgenic mice. After subchronic treatment with 375 ppm **16** (∼60 (mg/kg)/day), nonspatial memory was evaluated with the novel object recognition test and was found to be completely restored in transgenic mice treated with **16**.[101] This was accompanied by a complete reversal of the impairment in hippocampal synaptic plasticity, as indicated by long-term potentiation (LTP) measurements in parasagittal hippocampal slices. After a 10-month treatment with vehicle, 125 ppm (∼20 (mg/kg)/day) and 375 ppm (∼60 (mg/kg)/day) of **16**, the recognition index as determined in the novel object recognition test was dose-dependently increased from 48.9% in the vehicle treated mice to 62.2% and 64.1% in the 125 and 375 ppm dosed animals, respectively.[102] In a similar 9-month chronic study, **16** was shown to restore hippocampal neurogenesis potential, which was accompanied by a complete reversal of contextual memory deficit, as demonstrated in a contextual fear conditioning test and compared to treatment with vehicle.[103]

The micromolar $A\beta_{42}$ inhibitory potency of compounds like **15** and **16** is only moderate at best, and other groups have tried to improve further on this. Like Chiesi, researchers at Cellzome initiated efforts through the introduction of additional substituents on the biphenylacetic acid core of flurbiprofen. The first patents resulting from these efforts claim compounds such as **17** and **18** (Figure 8), which focused on the exploration of the α-substitution and introduction of lipophilic terminal phenyl substituents.[104,105] Reported activities are in the range of 24−84 μM, similar to those of Chiesi, and are likewise claimed to display no significant COX inhibition. A difference of in vivo activity of the two enantiomers of **6** was reported, with a 36% reduction in brain $A\beta_{42}$ levels for the *R*-enantiomer compared to 17% for the *S*-enantiomer when dosed orally at 50 mg/kg in APPLd2 transgenic mice.[105] In contrast to this, a separate application has been filed claiming

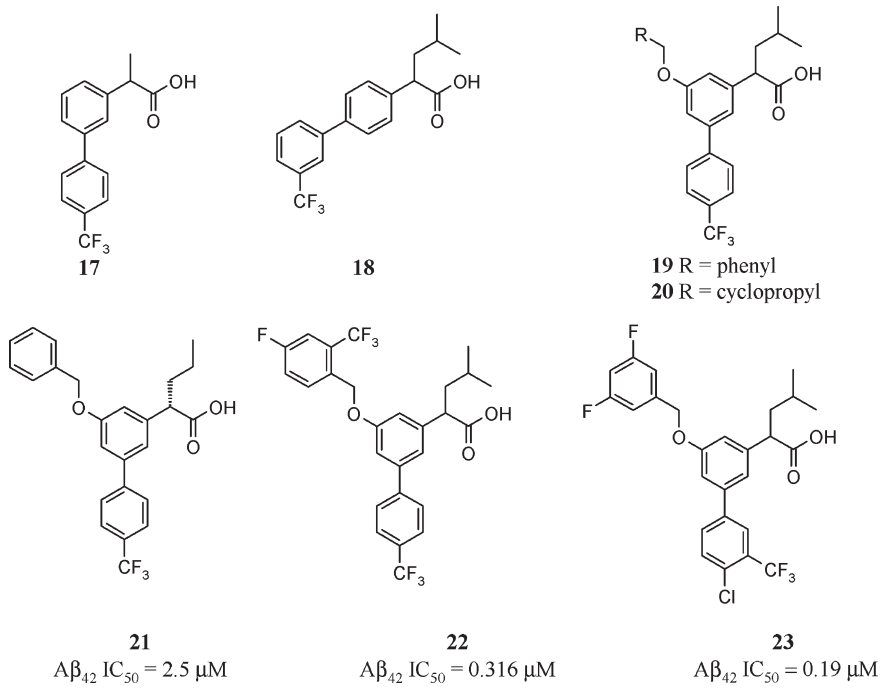


Figure 8. NSAID derived GSMs from Cellzome.

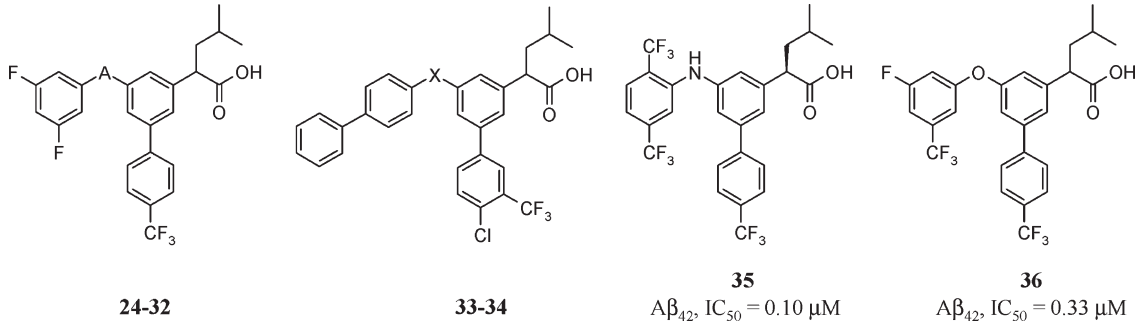


Figure 9. OrthoMcNeil/Janssen NSAID derived GSM series.

specifically the *S*-enantiomers in this series.[106] The introduction of additional lipophilic benzyloxy and cyclopropylmethyloxy substituents, as exemplified by **19** and **20**, led to compounds with Aβ_{42} lowering activities of $< 10\ \mu$M, as well as an increased brain/plasma ratio of about 0.3.[107] A subset of compounds in this series were claimed in a separate application.[108] Comparisons of the reported data on **21** with **22** and **23** illustrate the relevance of the additional lipophilic substituents on the benzyl group, as well as the isobutyl group, leading to Aβ_{42} lowering activities of $< 1\ \mu$M. Oral dosing of **22** and **23** at 30 mg/kg in nontransgenic mice led to a reduction in Aβ_{42} brain levels after 4 h of 25% and 42%, respectively, with brain/plasma concentrations at 4 h of (5.1 μM)/(18.4 μM) for **22** and (6.5 μM)/(24.5 μM) for **23**.

Further exploration of the 3,5-disubstituted arylacetic acid motif, in collaboration with researchers from OrthoMcNeil/Janssen, has led to several additional patent applications. In Figure 9 and Table 1, representative compounds are given with a replacement of the methyleneoxy linker in **19**−**23** by heteroatoms (**24**−**26**),[109,110] an amide linker (**32**),[111] or various carbon linkers (**27**−**31**).[112] The reported in vitro potencies of these compounds indicate that the Aβ_{42} lowering activity is increased by adding extra lipophilic bulk, as exemplified by **33** and **34**.

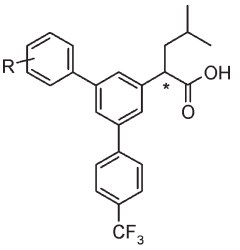
Table 1. OrthoMcNeil/Janssen NSAID Derived GSM Series

compd	A	IC_{50} (μM)
24	O	0.77
25	NH	73% at 3 μM
26	NMe	0.45
27	CH_2	0.74
28	C=O	50% at 1 μM
29	CH_2CH_2	0.41
30	CH=CH	0.45
31	CC	53% at 1 μM
32	$CONH_2$	34% at 1 μM
compd	X	IC_{50} (μM)
33	CH=CH	0.15
34	CH_2CH_2	0.21

Oral dosing of 30 mg/kg **35** and **36** in mice led to a reduction in Aβ_{42} brain levels of 18% and 43% after 4 h, respectively. A similar experiment with **35** in rats led to a reduction in Aβ_{42} brain levels of 30% after 4 h.

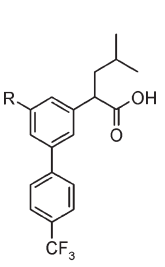
A series of terphenyl, 3,5-diaryl-substituted arylacetic acids have also been claimed, with an indication of their in vivo activity in both mice and rats (Table 2).[109] Lowering of brain Aβ_{42} levels have been reported up to 58%, 4 h after oral dosing at 30 mg/kg in mice. Since it is not apparent from the

Table 2. OrthoMcNeil/Janssen NSAID Terphenyl, 3,5-Diaryl-Substituted Arylacetic GSM Series

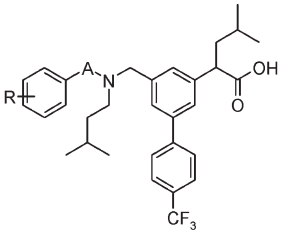


37-43

compd	R	stereochemistry at *	in vitro IC_{50} (μM)	in vivo, rat 30 mpk po, 4 h, % lowering Aβ_{42}
37	3,4,5-trifluoro	*RS*	0.19	20
38	3-F, 4-Cl	*RS*	0.25	28
39	4-OCF_3	*RS*	0.25	20
40	4-CF_3	*RS*	0.19	29
41	4-CF_3	*R*	0.14	27
42	4-CF_3	*S*	0.09	40
43	3,5-diCF_3	*RS*	0.08	na



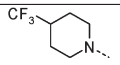
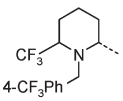
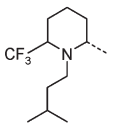
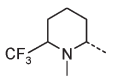
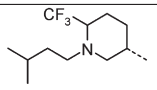
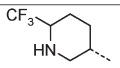
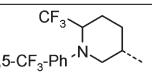
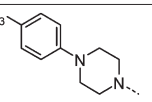
44-54



55, **R** = 4-CF_3 **A** = bond, Aβ_{42} IC_{50} = 0.25 μM
56, **R** = 4CF_3 **A** = CH_2, Aβ_{42} IC_{50} = 0.22 μM
57, **R** = 3,4-diF **A** = CH_2 Aβ_{42}, IC_{50} = 0.065 μM

Figure 10. OrthoMcNeil/Janssen NSAID basic nitrogen containing GSM series.

Table 3. OrthoMcNeil/Janssen NSAID Basic Nitrogen Containing GSM Series

compd	R	In Vitro IC_{50} (μM)
44		0.43
45		0.44
46		0% at 1μM
47		0.31
48		0.11
49		0.42
50		0.37
51		8% at 1μM
52		0.31
53		5% at 1μM
54		63% at 1μM

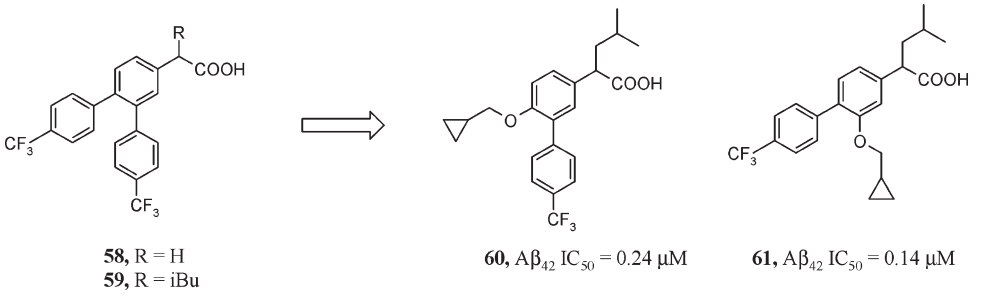
experiments whether transgenic or nontransgenic animals were used, the data shown in Table 2 are the reported in vivo activities in rats.

Again, the compound with the highest reported in vitro potency, **43**, is among one of the most lipophilic representatives. In a likely attempt to reduce the lipophilicity, a series of analogues have been disclosed that incorporate piperidine, piperazine,[113] and other substituents displaying a basic nitrogen as exemplified by **55**−**57** (Figure 10 and Table 3).[110] Although potency in the range 100−400 nM has been achieved, the most potent compounds still contain the largest lipophilic substituents, and the presence of piperidine- or piperazine-NH appears to be detrimental to the modulatory activity.

Recently, data have been presented on the chronic oral treatment of **42** and the effects on plaque formation.[114] Tg2576 mice of 6 months old, thus starting before the appearance of plaques, were treated with 20, 60, and 120 (mg/kg)/day over a 7-month period. This resulted in a dose dependent reduction in the brain area occupied by plaques, as well as the number of plaques. In addition, analysis of the soluble Aβ fraction in brain showed a dose-dependent reduction of all measured Aβ species except Aβ_{38}.

While the Cellzome/OrthoMcNeil optimization on the NSAID derived GSM series has been focused around 3,5-disubstituted phenylacetic acids, Merck has been exploring 3,4-diaryl substituted analogues as exemplified by **58** and **59** (Figure 11).[115] As with the 3,5-diaryl series, trifluoromethylaryl substituents are preferred. Although most exemplified compounds do not carry an additional α-substituent, a limited set of compounds with various α-substituents has been exemplified, including the isobutyl substituted **59**.

Very similar 3,5-diaryl and 3,4-diaryl substituted arylacetic acids have been claimed by EnVivo.[116,117] However, the efforts of EnVivo on the NSAID derived GSMs have been mainly focused around a reduction in the extreme lipophilicity present in most of the highly active compounds described so far while attempting to maintain potency and to increase brain penetration. In order to achieve this, the exploration of EnVivo has focused on optimization of the cyclopropyloxy substituted **60**.[116−118] As with the compounds described by Cellzome, in this series the isobutyl group as α-substituent was shown to be optimal. The relative position of the alkyloxy and aryl groups appears to have minor influence on the in vitro potency. However, while **60** did not demonstrate Aβ_{42} lowering activity in vivo in a rat model, **61** did show a reduction of about 20−30%, most likely due to increased brain levels over

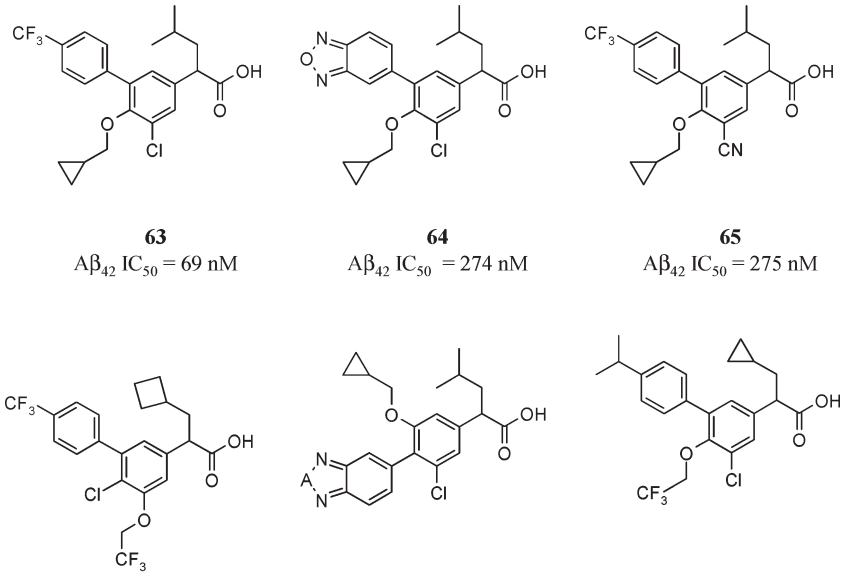


58, R = H
59, R = iBu

60, $A\beta_{42}$ IC_{50} = 0.24 μM

61, $A\beta_{42}$ IC_{50} = 0.14 μM

Figure 11. EnVivo NSAID derived GSM series.



63
$A\beta_{42}$ IC_{50} = 69 nM

64
$A\beta_{42}$ IC_{50} = 274 nM

65
$A\beta_{42}$ IC_{50} = 275 nM

66

67

68
in vivo activity
23% red $A\beta_{42}$ @30mpk

Figure 12. Examples of EnVivo NSAID derived GSM series.

60 (10 μM, brain/plasma ratio of 0.11 for **61** vs 2.7 μM, brain/plasma ratio 0.08 for **60**). A species difference has been observed in this series between rat and human cell lines, with the compounds being less potent in rat neuronal cell lines. This may be of relevance in translating animal in vivo data to humans.

As an extension to this series, tetrasubstituted phenyl compounds have been claimed in patent applications, with some typical examples shown in Figure 12.[119] Although the structure remains undisclosed, data have been presented on EnVivo's preclinical candidate EVP-0015962 (**62**, structure not disclosed).[120,121] The in vitro $A\beta_{42}$ lowering activity of this compound was reported as an IC_{50} of 0.12 μM in a human cellular assay and 0.489 μM in a neuronal cellular assay, without any effect on Notch-processing. Oral dosing of 10 and 30 mg/kg in rats led to a reduction in $A\beta_{42}$ brain levels of 22% and 38%, respectively, with brain levels of 2.8 and 8.3 μM. Chronic treatment of transgenic Tg2576 mice with a 20 or 60 (mg/kg)/day dosing over a 6-month period led to a lowering of brain plaque load of >81% at 20 mg/kg and >95% at 60 mg/kg. **62** was also tested in the contextual fear conditioning model after 30−33 weeks of treatment of Tg2576 mice at 20 or 60 (mg/kg)/day dosing or in the Morris water maze model after 47 weeks of treatment at 20 (mg/kg)/day.[121] In these experiments, cognitive deficits in contextual and spatial working memory, respectively, could be reversed. Surprisingly, for the Morris water maze model, no significant effect was observed in the 60 (mg/kg)/day group, despite significant reduction in soluble and formic acid extractable $A\beta_{42}$ and decrease in $A\beta$ aggregates at both doses of **62**. The modulatory nature of **62** was demonstrated in these in vivo studies by the increase in $A\beta_{38}$ with no overall changes in the amount of total $A\beta$ peptides.

Researchers at Merck have tried to lower lipophilicity by replacing the central phenyl ring present in **58** by piperidine, as exemplified by **69**.[122] Subsequent work by GSK has led to *N*-benzyl substituted piperidines. Additional alkyl substituents on the benzylic carbon atom resulted in potent compounds (**70**) with submicromolar activity but still with extremely high lipophilicity.[123] It is worth noting that similar alkyl-substituted *N*-benzyl groups were already present in early, indomethacin derived GSMs developed by Merck (Figures 6, 13, and 14).[94,95] Attempts to reduce the lipophilicity by replacing the benzylic aryl group with heterocyclic aromatic groups resulted in compound **71**, which demonstrated good pharmacokinetic properties in mouse, rat, and dog.[123] When the compound was dosed orally to mice at 5 mg/kg, high brain levels of 4.2 μM were observed 2 h after dosing, with a brain/plasma ratio of 0.74.

Further in vitro and in vivo efficacy data have subsequently been reported for **72**, which demonstrated the modulatory nature of **72**.[124] A time-course study in TASTPM transgenic mice, dosed at 100 mpk with **72**, showed a significant decrease

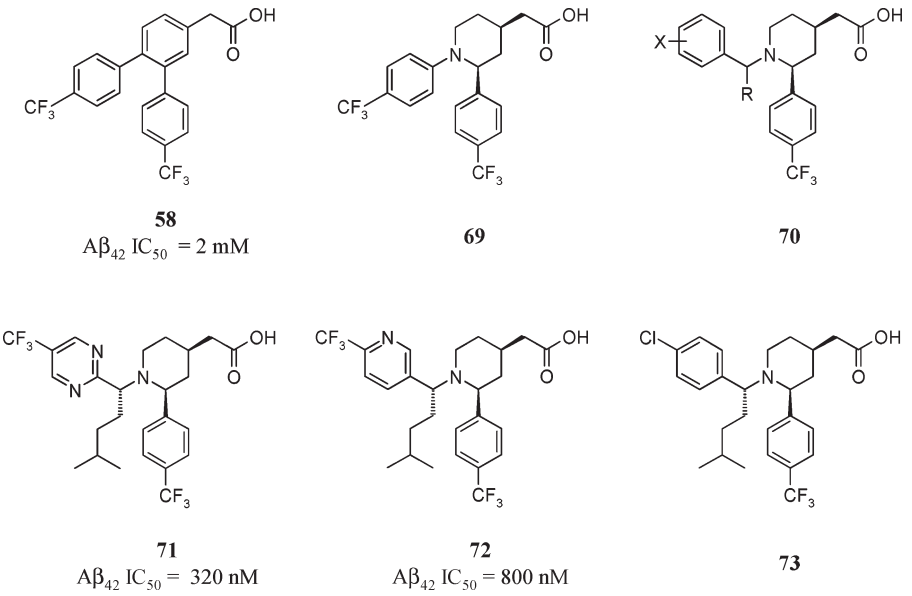


Figure 13. Examples of Merck/GSK NSAID derived GSM series.

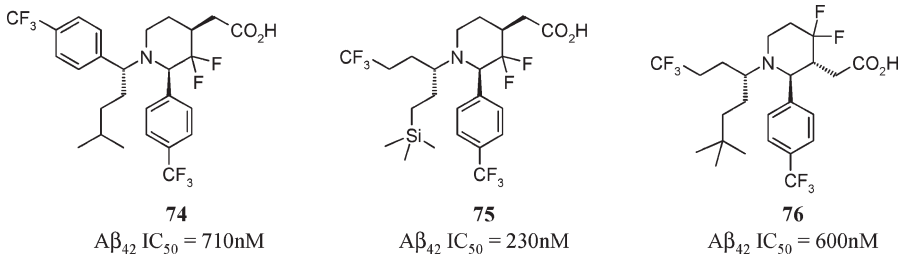


Figure 14. Examples of Merck NSAID derived GSM series with improved PK properties.

in Aβ_{42} brain levels (about 20%) at 3, 6, and 15 h, while at the same time points an increase in Aβ_{38} was observed (30−37%), with no effect on Aβ_{40} levels. The compound showed good brain penetration with levels in brain and plasma at the 6 h time point of 54.7 and 32.9 μM, respectively. Data on the analogous **73** in transgenic mice have been presented by the group of Haass.[82] In APP-Swe mice, a dose-dependent increase in Aβ_{38} and a decrease in Aβ_{42} were observed upon treatment with **73** at 3, 10, and 30 mpk, with levels in Aβ_{40} and total Aβ remaining unchanged. Interestingly, when performing the same experiment in the double mutant strain APP-Swe × PS2 N141I mice, no significant decrease in Aβ_{42} was observed, despite robust dose-dependent increases in Aβ_{38}. In combination with similar results obtained from in vitro experiments,[125] this indicates that Aβ_{38} generation is not directly linked to Aβ_{42} production. Reported nonsynchronous changes in Aβ_{42} and Aβ_{38} levels for **62** point in a similar direction.[120]

In a continuing effort to improve the pharmacokinetic properties of the piperidine acetic acids series of GSMs, analogues have been prepared where fluorine has been incorporated into the piperidine ring.[126] Three examples, **74**[127] **75**,[128] and **76**,[127] are shown in Figure 14. Both 3,3-difluoro- and 4,4-difluoropiperidine analogues showed similar activity in lowering Aβ_{42}, demonstrating the allowance of a flexible orientation of the lipophilic piperidine substituents relative to the carboxylic acid group. In addition, the phenyl ring of the benzylic substituent, as in **74**, could be replaced by an ethyl linker, as in **75** and **76**. When **75** and **76** are dosed in APP-YAC transgenic mice (10 mg/kg po, 7 h), both compounds showed a potent reduction in Aβ_{42} of 64% and 84%, respectively, without a significant change in Aβ_{40} levels. Compound **76** also demonstrated a dose dependent lowering of Aβ_{42} (ED_{50} = 5 mg/kg, brain EC_{50} = 1 μM, and plasma EC_{50} = 3.7 μM) in rats, and no adverse Notch effects were observed after dosing for 7 days in rats at 250 mg/kg per day.

Some of the initial NSAIDs showing GSM activity, such as indomethacin and ibuprofen, also show agonism toward the peroxisome proliferator-activated receptor γ (PPARγ). PPARγ agonists may have beneficial effects in AD at multiple levels, including core pathological processes in the brain as well as on peripheral risk factors such as serum glucose levels and insulin sensitivity.[129] An attempt to design dual GSM and PPARγ agonists has been described as a novel strategy for the prevention of AD.[130] This series originated from the high throughput screening hit **77** (Figure 15). The carboxylic acid group is present as a key structural element for both PPARγ agonism and NSAID-type GSM activity. Therefore, the exploration focused around variations of the R1 and R2 substituents. For R1, an alkyl group was found to be required for GSM activity, with an *n*-butyl group being optimal. For R2, a variety of aryl and (cyclo)alkyl substituents were tolerated, leading to compounds **79** and **80** exhibiting an optimal, combined PPARγ and GSM activity. The selectivity for COX1 and COX2 was only marginally improved compared to **77** and may not be sufficient to prevent COX-inhibition-related side effects. No activities have been presented for the single enantiomers of **79** and **80**. As for flurbiprofen, a clear difference in activity for COX inhibition may exist between the enantiomers. It is noted that only

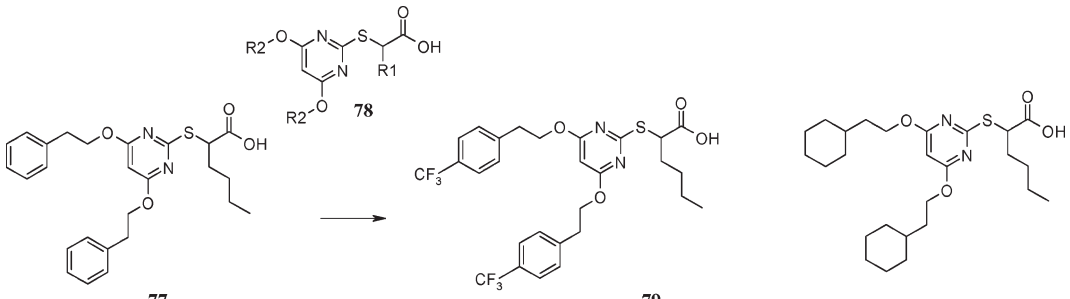


Figure 15. GSM series with PPARγ activity.

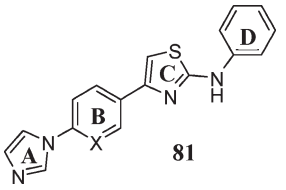


Figure 16. Neurogenetics 2004.

symmetrically R2 disubstituted compounds have been described and tested.

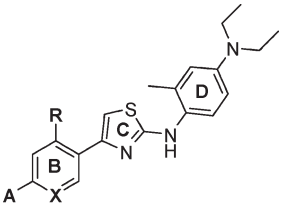
Non-NSAIDS Derived GSMs

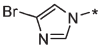
One of the first GSM series not bearing a carboxylic acid (non-NSAID derived) was identified by Neurogenetics in 2004 (which later became TorreyPines Therapeutics; the compounds are now under investigation by NeuroGenetic Pharmaceuticals).[86] The exemplified compounds consisted mostly of four consecutive linked (hetero)aromatic rings designated as A, B, C, and D (Figure 16) which were focused around aryl- or heteroarylimidazoles with an anilinothiazole. This nomenclature of describing the rings A−D will be used throughout the Perspective to remove ambiguity.

At first, although only ranges of activity were reported, it is apparent that the imidazolephenyl A−B ring system was optimal for in vitro activity (Table 4). Addition of an extra methyl or halogen at the 4-position of the imidazole on **82** led to equipotent compounds (**83**, **84**). Replacement by 4-ethylimidazole (**85**), 2,4-dimethylimidazole (**86**), or 2-methylimidazole (**87**) resulted in a loss in potency. Maintaining the 2-methyl-4*N*-diethylanilinothiazolephenyl B−C−D ring system constant while varying the imidazole was attempted without apparent success. More discrete modifications of the 4-methyl-1*H*-imidazole (**83**) with its replacement by 1,2,3-triazole (**88**) or 2-methyl-1*H*-imidazole (**89**) led to a decrease in activity. Replacing the unsubstituted phenyl B ring by a pyridine (**90**) or pyrimidine ring led to an increase in potency, while introducing a CF_3 substituent ortho to the thiazole as in **91** was detrimental for activity.

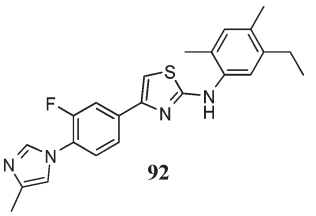
A key feature required for potency is the ortho methyl substituent on the aniline. Compounds in which the aniline D-ring is 2,4-, 2,4,5-, and 2,5-substituted were all reported to have activity below 200 nM. Overall, the more active analogues in this series have a very lipophilic character. The most potent compounds in this series lowered secreted Aβ_{42} levels with IC_{50} values of 5−50 nM in human neuroblastoma cells overexpressing APP and 20−200 nM in primary mixed brain

Table 4. Imidazole Replacements or Modifications



compd	A	X	R	Aβ$_{42}$ or Aβ$_{40}$ (μM)
82		C	H	0.2-1
83		C	H	0.2-1
84		C	H	0.2-1
85		C	H	1.1-5
86		C	H	1.1-5
87		C	H	1.1-5
88		C	H	1.1- 5
89		C	H	5.1-10
90		N	H	<0.2
91		C	CF_3	5.1-10

cultures from Tg2576 mice.[131] Moreover, some compounds lowered Aβ_{42} in both plasma and brain of Tg2576 mice after oral administration.[132] Compound **92** in particular showed favorable PK properties (T_{max}(po) = 3 h, Cl(iv) = 0.2 (L/h)/kg, $t_{1/2}$(po) = 2 h, F = 49%) (Figure 17). In vitro (mixed brain cultures from Tg2576 mice), **92** dose dependently lowered Aβ_{42} (IC_{50} = 29 nM) and Aβ_{40} (IC_{50} = 90 nM) and concomitantly increased Aβ_{38} (EC_{50} = 170 nM) without changing Aβ_{total} levels, which is characteristic for a modulator.



92

Figure 17. NeuroGenetic GSM.

Upon oral dosing (50 mg/kg) once daily for 3 days in C57BI/6 mice, **92** showed a plasma exposure of ~70 and ~65 μg·h/mL in brain, giving a brain plasma ratio of 0.93. It was also found to be efficacious with significant lowering of Aβ_{42} and Aβ_{40} peptide upon chronic dosing (50 (mg/kg)/day po, resulting in ~4 μM plasma concentration) using 8- to 15-month-old Tg2576 mice. Histopathological studies revealed a statistically significant reduction in plaque load in treated versus untreated animals with, respectively, 7.6% versus 2.8% in the cortex and 1.9% versus 0.5%.in the hippocampal area. This compound was well tolerated without any weight loss or GI related toxicity.[132]

As it will become apparent below, extensive exploration has been carried out around this series by many pharmaceutical companies.

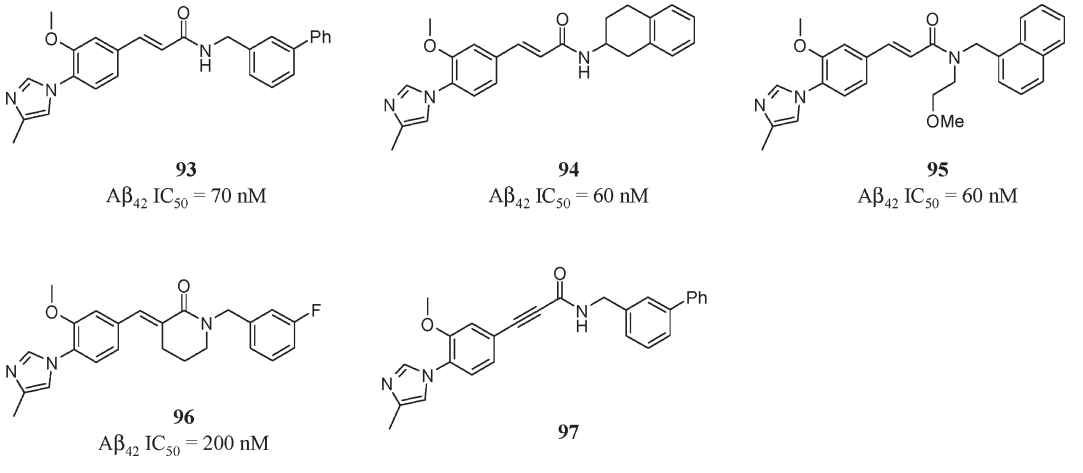
Cinnamides

Work around this series by Eisai has resulted in diarylcinnamide derivatives.[133] The arylimidazole feature exhibited in the compounds described by Neurogenetics is still present, but now the central aminothiazole group is replaced by an α,β-unsaturated amide fragment. Amide variations described by Eisai and their activity are reported in Figure 18. In particular, conversion of the lipophilic diphenyl compound **93** to incorporate a chiral center resulted in **94** with maintained nanomolar activity. Alkylation of the amide NH as in **95** did not seem to have a major effect on potency. Cyclization to afford piperidone **96** resulted in a loss of potency. Changing the double bond by a triple bond as in **97** was also attempted; however, no data were reported.

This initial Eisai application also included a number of variations of **96**, including modifications of ring size and replacement of the piperidone ring by other heterocycles such as **98**, **99**, and **100** (Figure 19).

Further elaboration of the piperidone and morpholinone core was reported in two additional applications.[134,135] These cyclizations were achieved by locking the α-methylbenzyl group to form a bicycle, which resulted in morpholinone **101** and/or piperidone example **102** (Figure 20). Replacing the methoxy group by fluorine in the B-ring (**103** vs **104**) resulted in compounds with slightly reduced activity. In a separate patent application Eisai described 3-methyl-1,2,4-triazole **105** as a replacement of the imidazole.[136] In general this substitution resulted in compounds that were shown to be less active than the corresponding imidazole. The introduction of an extra hydrogen bond donor in the form of a primary or secondary alcohol increased the potency more than 5-fold (**106** vs **107**), leading to the most potent compounds in the series, such as **103** and **107**. This increase in potency suggests the importance of a hydrogen bond donor in this region. The influence of this hydroxyl group on brain penetration and overall pharmacokinetic profile remains unclear.

The work around this cinnamide series culminated in the selection of compound E-2012 (**108**, structure not disclosed) which entered the clinic in 2006. According to press releases by Eisai, lenticular opacity was observed in a high-dose group of a 13-week preclinical safety study in rats, running in parallel to the phase I study, prompting a suspension of the phase I clinical study. Eisai conducted an additional 13-week multiple dosing study in rats to re-evaluate reproducibility and recovery potential and examined the no adverse effective level, the mechanism causing lenticular opacity, and an exploratory marker. Examination of follow-up data from the phase I study was also conducted. After submitting these data to the FDA early 2008, Eisai resumed clinical studies with **108**. Although the structure of **108** has not been officially disclosed, on the



93
Aβ_{42} IC$_{50}$ = 70 nM

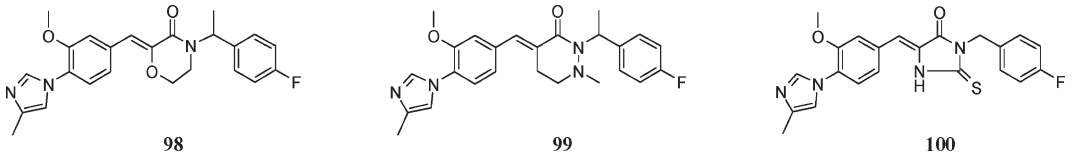
94
Aβ_{42} IC$_{50}$ = 60 nM

95
Aβ_{42} IC$_{50}$ = 60 nM

96
Aβ_{42} IC$_{50}$ = 200 nM

97

Figure 18. Eisai cinnamides.



98 99 100

Figure 19. Eisai non-NSAID GSM series.

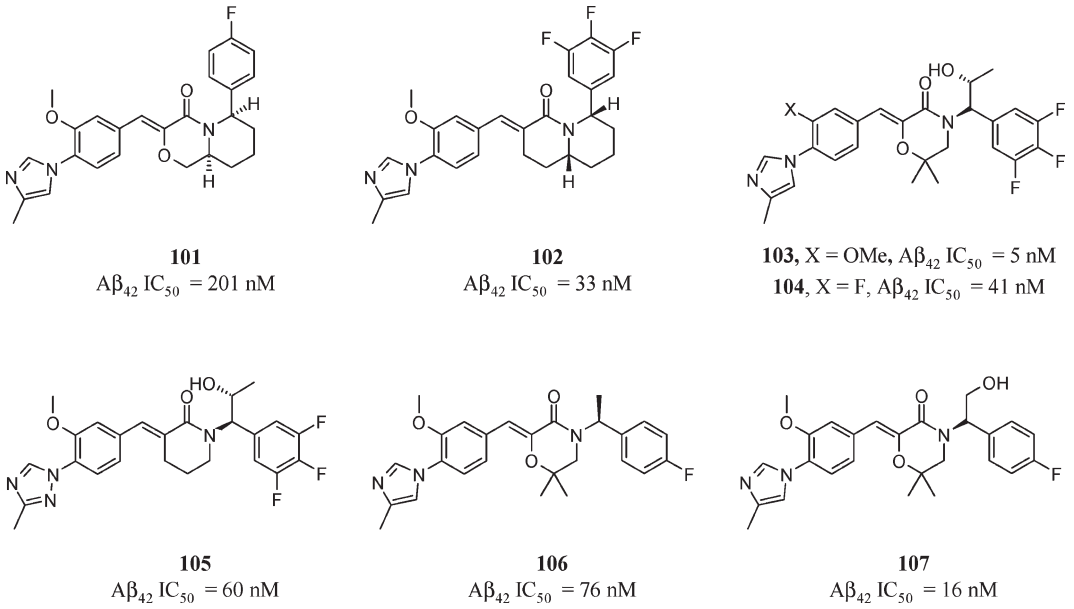


Figure 20. Morpholinone and piperidone series.

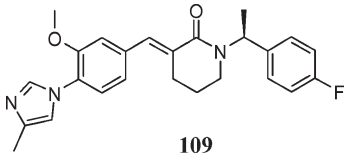


Figure 21. Tentative structure of E-2012 (**108**).

basis of a number of patent applications such as process patents,[137,138] salt patent,[139] and prodrug[140] and photoaffinity labeling patent[141] applications, it may be tentatively assigned as **109** (Figure 21).

108 reduced the production of Aβ_{40} and Aβ_{42} in the rat cortical neuron culture dose-dependently without significant cytotoxicity.[142] The reported IC_{50} values of **108** for Aβ_{40} and Aβ_{42} were 330 and 92 nM, respectively. When dosed orally once a day for 3 days, **108** decreased the levels of Aβ_{40} and Aβ_{42} in rat CSF, brain, and plasma 6 h after the final dose in a dose dependent manner. Especially, in rat CSF, **108** significantly decreased Aβ_{42} levels by 16.6% and 47.2% at doses of 10 and 30 mg/kg, respectively. The reduction in Aβ_{42} levels in brain at doses of 10 and 30 mg/kg were 17.1% and 42.9%, respectively. Aβ_{42} levels in plasma were decreased by 59.2%, 90.8%, and 96.1% at doses of 3, 10, and 30 mg/kg, respectively. The compound had no effect on Notch processing in vitro up to 3 μM. MALDI-TOF analysis revealed that **108** reduced Aβ_{40} and Aβ_{42} and increased shorter Aβ peptides, such as Aβ_{37} and Aβ_{38}, without changing total Aβ levels.[143] **108** did not induce the accumulation of APP-CTFs, suggesting that E2012 modulates, but does not inhibit, the cleavage of APP-CTF by γ-secretase. Likewise, production of Notch intracellular domain (NICD) was not inhibited by **108**. All these data combined confirm the γ-secretase modulatory nature of **108**, compared to the classical GSIs.

In recent applications, Eisai pursued the isosteric replacement of the amide moiety by a 1,2,4-triazole or an imidazole.[144] Some representative compounds are shown below (Figure 22). Only a few examples of bis-imidazole compounds have been described. Worth noting is an increase in potency when the imidazole is methylated (**110** vs **111**), which is in contrast to the reduction in potency reported for cyclized compound **112**. The monocyclic 1,2,4-triazole **113** was also converted to a bicycle constraining the benzylic position **114**, and unlike in the work described vide supra with the morpholinone **101** and piperidone **102**, this time it enhanced the primary activity, although the importance of the newly created chiral center is not known (activity for enantiomeric pairs not reported).

The importance of this particular variation is apparent by the filing of two additional patents on this bicyclic triazole subseries.[145,146] In particular, the terminal aryl substitution was examined further, as shown in Figure 23. The presented data indicate that the position and nature of the substituent do not greatly influence the primary activity which varies between 11 and 50 nM, with **122** being the most potent.

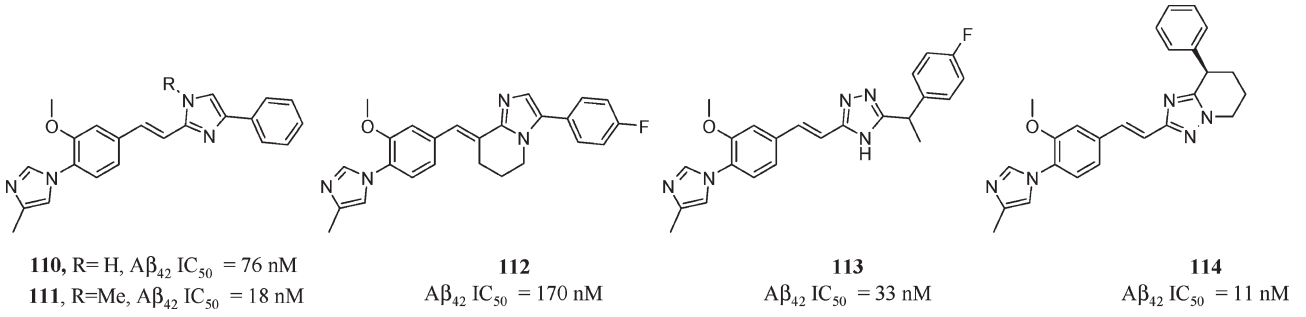


Figure 22. Eisai cinnamide bioisosteric replacements.

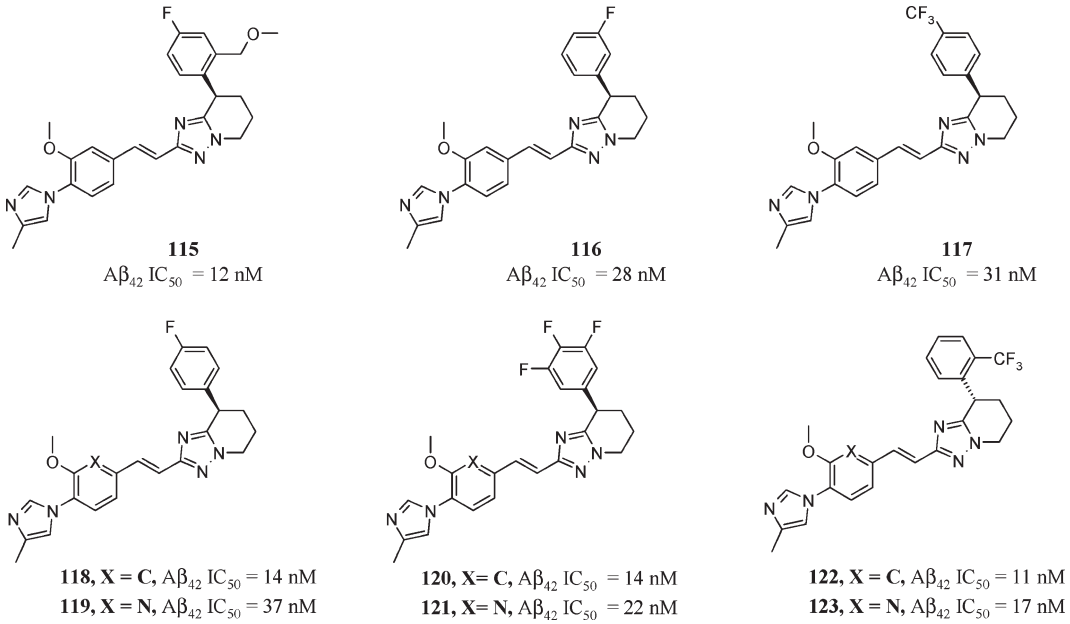


Figure 23. Bicyclic triazole series from Eisai.

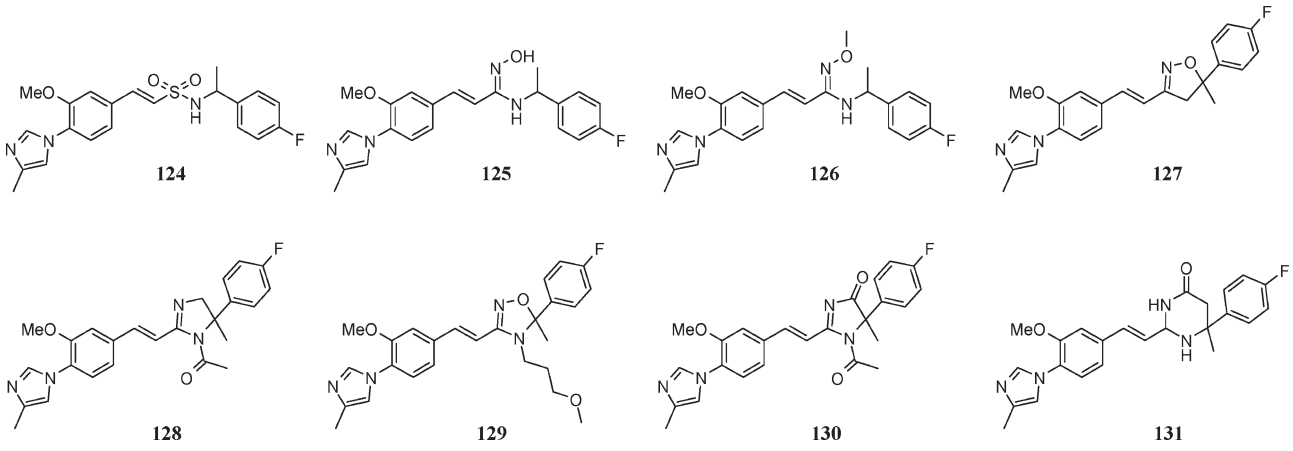


Figure 24. Schering cinnamide mimetics.

Introduction of nitrogen next to the methoxy, as in **119**, **121**, and **123**, kept the activity in the same order of magnitude. Worth noting is a recent process patent containing both enantiomers of **123**, indicating an interest in these compounds which might be undergoing more advanced toxicological studies.[146]

In an apparent continuation on the cinnamide series, Schering has been investigating closely related structures by maintaining the cinnamide double bond while modifying the amide group (Figure 24).[147] Compounds **124**, **125**, and **126** are examples of amide replacement by sulfonamide, hydroxyl, or methoxy amidines, respectively. Monocyclic nonaromatic heterocycles such as in compounds **127**−**131** containing, respectively, 4,5-dihydro[1,2,4]oxadiazole, 4,5-dihydroimidazole, 4,5-dihydroimidazolone, or tetrahydropyrimidine-4-one serve as an amide mimetic but also lock the benzylic group in analogy with the imidazole and triazole bioisosteric amide replacements reported by Eisai as in **110** and **114**, respectively.[148−151]

Conformational restricted derivatives of these cinnamide mimetics have also been patented with examples **132**−**137** shown in Figure 25.[149,150,152] They are analogous to the piperidones of Eisai shown in Figure 19.

In the same patent application a range of 6,5- (**139**−**143**), 6,6-fused bicycles (**144**−**146**), and more recently 6,7-bicycles such as **147** were exemplified (Figure 26). Worth noting is the positioning of the *p*-fluorophenyl D ring, which is in a similar orientation in all of these bicyclic systems. All the heterocyclic cores described by Schering are nonaromatic, in contrast to the imidazole and triazoles previously described by Eisai (Figure 22 and 23).

In an additional variation, the same orientation of the *p*-fluorophenyl group could be achieved via cyclization to form 4,5-dihydro[1,2,4]oxadiazole and oxazole bicycles **148** and **149**, respectively (Figure 27).

This lead hopping strategy based on Neurogenetics and Eisai structures was also carried out in the case of the iminohydantoins such as **150** and **151**, which were converted to their 5,5-, 5,6-, or 5,7-bicyclic analogues such as **152** (Figure 28).[153,154]

The best compounds of these series such as **150**, **151**, and **152** were found to exhibit low nanomolar in vitro activity. However, after dosing at 30 mg/kg in a CRND8 transgenic mouse study, they did not significantly lower brain or CSF Aβ levels, despite reaching considerable micromolar compound concentrations in the brain (Table 5).[155]

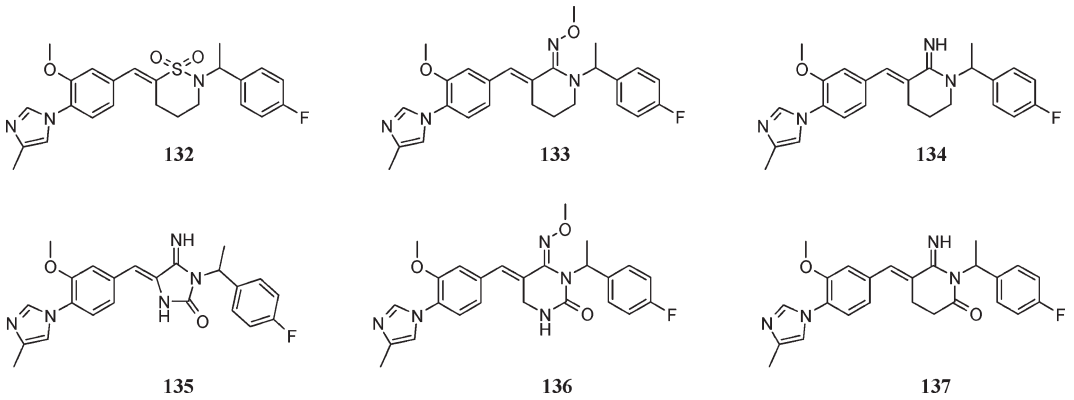


Figure 25. Schering pyridone analogues.

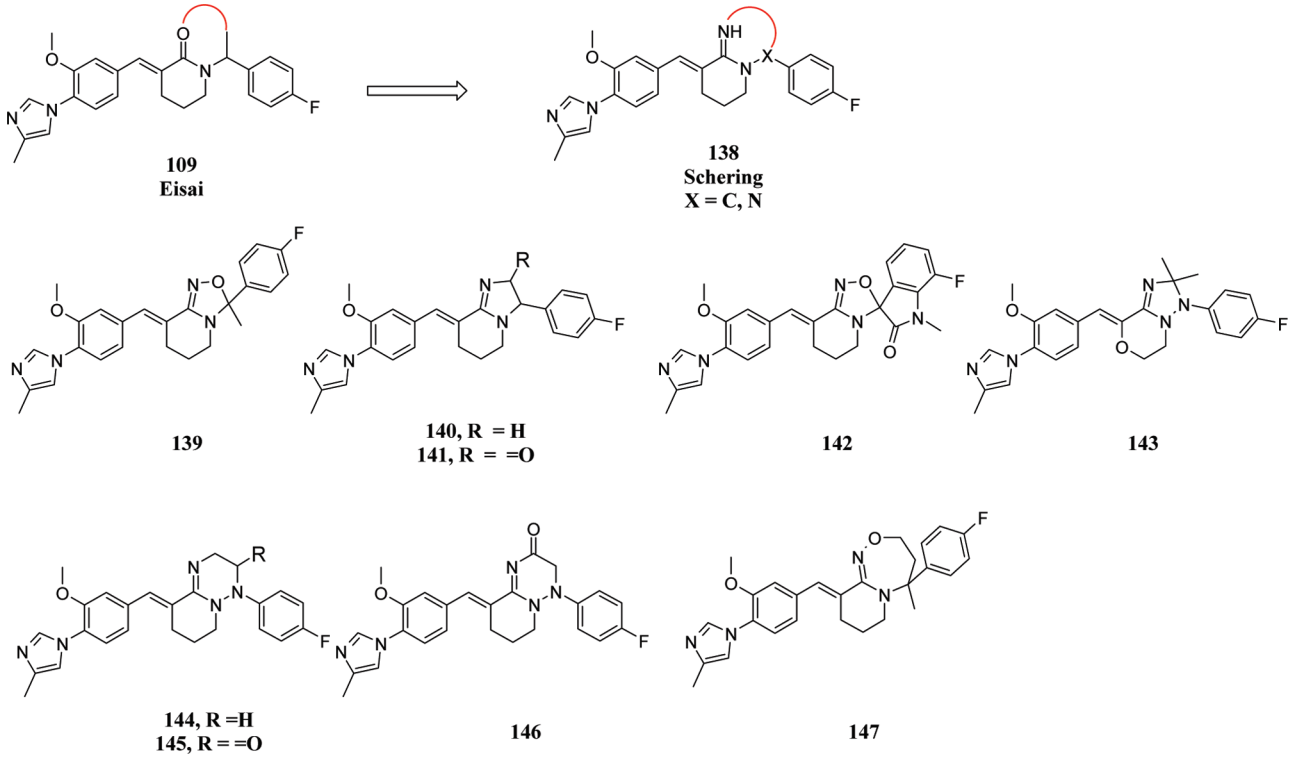


Figure 26. Schering fused-bicyclic series.

Recently, work covering variations to the A ring imidazole similar to those already reported by Neurogenetics has been published.[156] This was accomplished using the bicyclic 4, 5-dihydro[1,2,4]oxadiazole system **153** bearing a terminal primary alcohol, hinting again at the relevance of an hydrogen bond donor in this region of the molecule (Figure 29). Five-membered heterocycles such as 1,2,3-triazole, thiazole, oxazole, 1,2,4-triazole, pyrazole, and halo or alkyl substituted imidazole were reported (**154**). Replacement of the imidazole by six-membered heterocycles, in particular substituted pyridine, pyrazine, pyridazine, and pyrimidine, was also exemplified.

Yet another modification of the cinnamide derived analogues was achieved by a conformational restriction via cyclization onto the β-carbon of the α,β-unsaturated amide, resulting in **155** and **156** (Figure 30).

Schering also undertook modifications of the cinnamide double bond, presumably using the Eisai piperidone compound **109** as a starting point (Figure 31).[157] Isooxazolidinyl

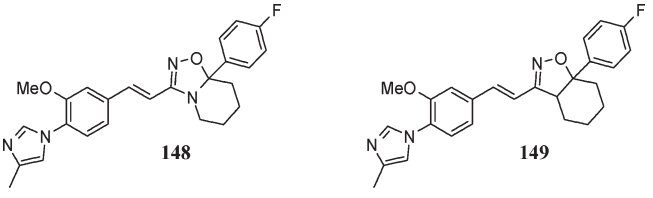


Figure 27. Conformationally restrained bicyclic oxadiazole and oxazole GSMs.

157, cyclopropyl **158**, and substituted piperidine **159** are among the spirocyclic variations that were exemplified. In vitro IC_{50} values for these compounds were reported to be above 200 nM, again hinting at planarity as a pharmacophoric requirement for this class of GSMs.

The efforts of Schering around the cinnamide subseries of GSMs have been focused on the introduction of conformational restriction in the cinnamide compounds, resulting in the production of an impressive list of bicycles acting as

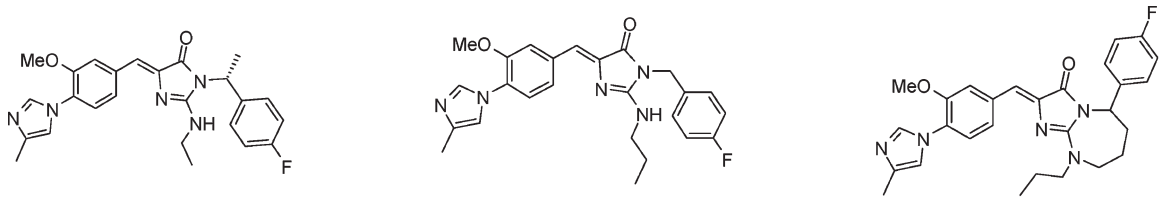


Figure 28. Iminohydantoins series of GSMs.

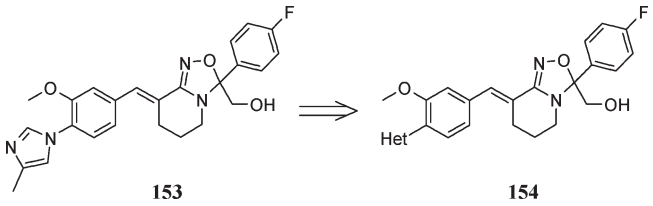


Figure 29. Core for the A ring exploration.

Table 5. In Vivo Data for Merck Iminohydantoins Series[155]

compd	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{total}/A\beta_{42}$	$A\beta_{42}$ (%) CSF
150	88	159	−12
151	85	70	−10
152	73	215	−8

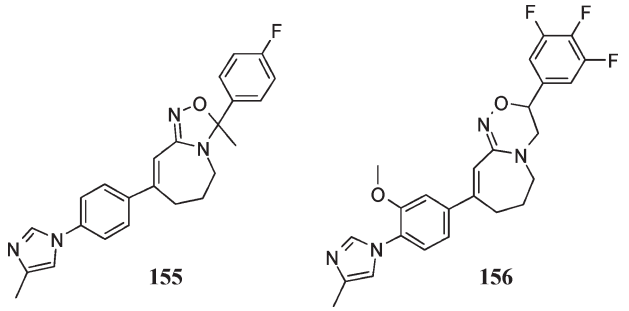


Figure 30. Cyclization incorporating the double bond.

cinnamide isosteres. It is not clear at this time if the Schering investigation resulted in any optimized compounds that are currently progressing further toward clinical trials.

The work described up to now has stayed close to the double bond feature initiated by Eisai. Considerable work by several groups has also dealt with the replacement of this double bond linker and/or other heterocyclic variations of the initial Neurogenetics thiazole central core.

Non-Cinnamide Linked B−C Compounds

A number of additional patents by Schering have appeared describing further variations of the cinnamide double bond present in Eisai's **109** and most of the patents by Schering described before. In a recent paper, Schering postulated that the use of a hydrogen bond donor instead of the methylene could result in an intramolecular hydrogen bond as shown in Figure 32.[158] This intramolecular hydrogen bond was hypothesized to exist between the hydroxyl and the carbonyl of the pyridazone core in **160**, thus constraining the conformation of B with respect to C, mimicking the orientation of the cinnamide **109**.

This initial compound **160** displayed a weak potency compared to **109**.[158] A set of compounds was synthesized using alternative linkers between rings B and C. This included keto-linked **161** and amine-linked **162** and **163**. The greatly improved potency of **163** compared to **162** resulted in the discovery that the methoxy substituent at C-5 was disrupting the intramolecular H-bond, creating a steric clash and twisting the two ring systems, resulting in loss of planarity and potency.

Further C-ring optimization resulted in the identification of pyridone **164** (Figure 33, IC_{50} = 148 nM). Maintaining this core allowed the exploration of the available chemical space at the C-5 position. A number of functional groups were introduced at C-5, of which **165** (IC_{50} = 159 nM) is a representative. However, these compounds did not result in an increase in potency or selectivity. Interestingly, substituting CHF_2 in **166** for CF_3 in **169** resulted in a marginal decrease in both potency and $A\beta_{42/40}$ selectivity, from 44 to 101 nM and a selectivity difference from 450 to 155, respectively. The introduction of a benzylic methyl substituent did not have a dramatic effect on in vitro potency but resulted in favorable rat PK and improved brain concentrations from 391.3 ng/g for **165** to 917.3 ng/g for **168** at 10 mpk 6 h after dosing. This modification generated a chiral center, which was key for activity, as the *S* enantiomer **168** was 3 times more potent than the corresponding *R* enantiomer.

Optimization was performed around **168** which led to the identification of **166**, possessing a CHF_2 group on the C-5 position, with an optimal in vitro profile. All compounds **164**−**169** showed a $A\beta_{42/40}$ selectivity ratio greater than 130-fold. The most potent analogue **166** also had the highest $A\beta_{42/40}$ ratio of 450.

Compound **166** was further profiled in vivo, showing good efficacy in the CRND8 transgenic mouse model reducing plasma $A\beta_{42}$ by 85% at 30 mpk. In a nontransgenic rat model this resulted in a 40% reduction in $A\beta_{42}$ in the CSF and 26% in the brain at 100 mpk. Good AUC, DMPK profile, and no adverse side effects were mentioned.[158]

Subsequent patent applications disclosed the introduction of conformational constraint of the previously described pyridazone and pyridone cores present in **163** and **164**, respectively. Two types of cyclizations were performed. Pyridone **164** was cyclized, incorporating the benzyl attached to the nitrogen, to afford **170** (Figure 34).[159] This fixes the orientation of the D-ring into a vertical conformer, coinciding with previous work by Eisai and also Schering (vide supra with the synthesis of their bicyclic triazoles, such as **118** (Figure 23)).

Tricyclic pyridazones **171** and **172** resulted from additional cyclization between rings B and C.[160] This introduced planarity and restricted the rotation of ring B with respect to C, thus fixing the methoxy on one side of the molecule. Interestingly, to date, this is the only example of this modification.[160] Unfortunately, no data were provided for **171** and **172** or their analogues. This could have yielded an indication for the preferred orientation of the methoxy substituent.

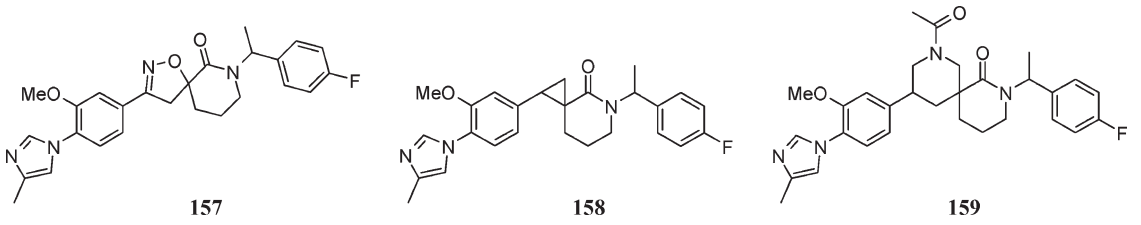


Figure 31. Double bond replacements.

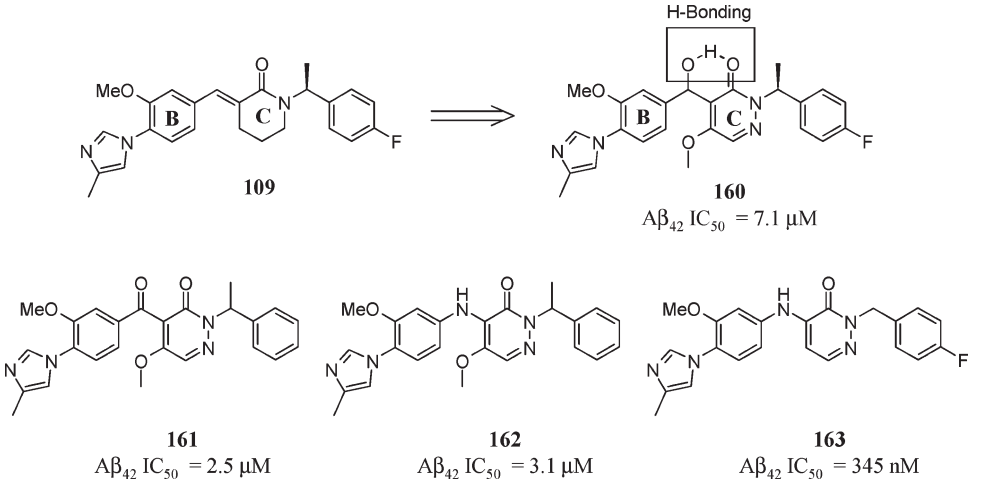


Figure 32. Introduction of intramolecular H-bond.[158]

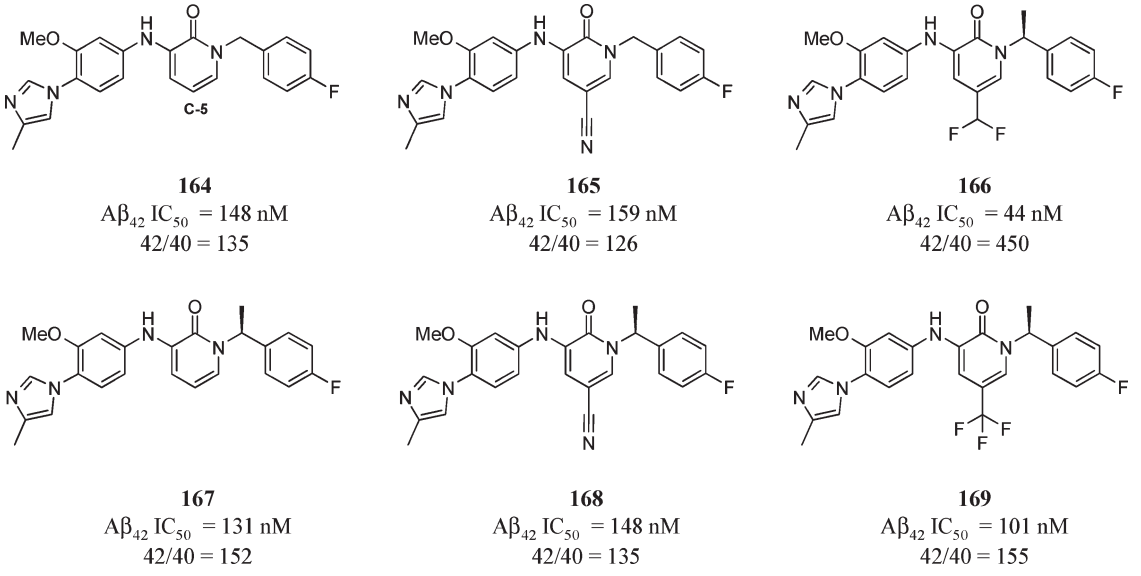


Figure 33. Optimized aminopyridones.

Further conformational constraint introduced by Schering resulted from the cyclization of the two carbonyls in **174** to afford five- or six-membered heterocycles exemplified by **173** and **175**, respectively (Figure 35).[161,162]

Differently constrained pyridazones have also been claimed by converting the linker into a heterocycle, resulting in the formation of 5,6-bicyclic systems, including 5*H*-furo-[2,3-*d*]pyridazin-4-one, dihydropyrrolo[2,3-*d*]pyridazin-4-one systems as exemplified by **177** and **178**, respectively (Figure 36). In addition 5*H*-thiazolo[4,5-*d*]pyridazin-4-one and 1,5-dihydro-imidazo[4,5-*d*]pyridazin-4-one cores were disclosed.[163]

Analogues with an amino linker between the B and C rings have also been claimed by other companies. Ortho-McNeil-Janssen disclosed their efforts in the field in three patents (Figure 37).[164−166] Imidazolopyridines **179** and **180** can be seen as constrained analogues of the Schering pyridones such as **164**. This positions the D ring in an orientation that has been adopted before by other groups, including Eisai and Schering, and seems to enhance activity.

The introduction of a methyl in the 3-position of the imidazopyridine resulted in a greater than 6-fold increase in in vitro potency [**179** (IC_{50} = 101 nM) to **180** (IC_{50} = 14 nM)]. This, however, is not directly translated into in vivo potency.

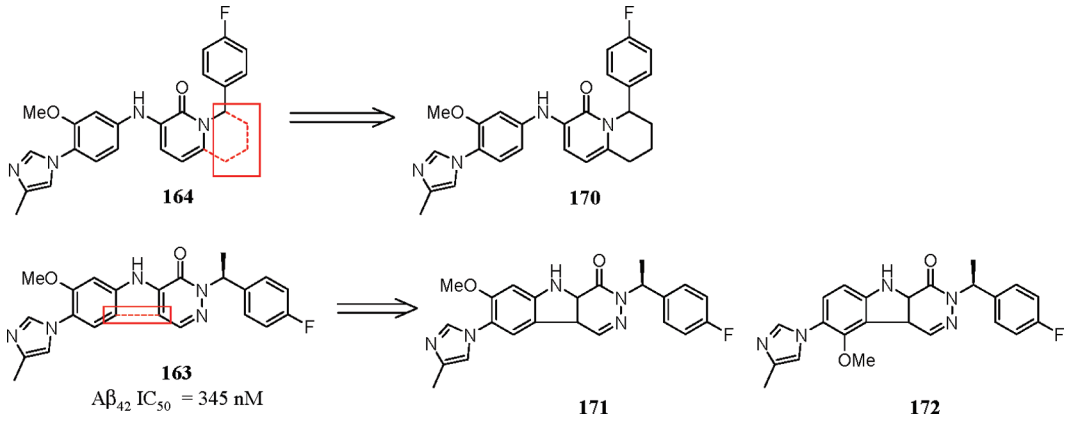


Figure 34. Constrained analogues of the aminopyridazone and aminopyridones.[159,160]

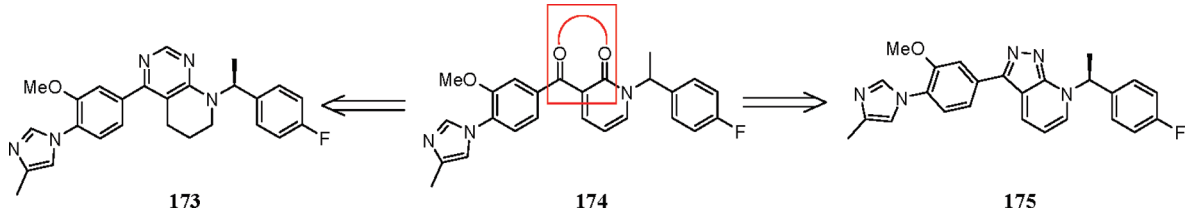


Figure 35. Constrained analogues of keto-pyridones.[161,162]

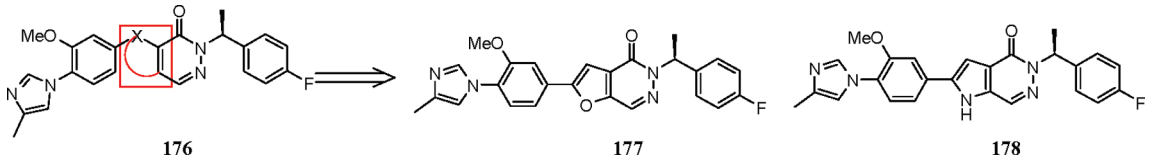


Figure 36. Constrained analogs of pyridazones.

In a subsequent application, it was shown that the high potency of the 3-methylimidazolopyridines could be matched by the use of the *N*-methyl substituted (aza)benzimidazoles, as exemplified by **181** and **182**. These two cores were used to investigate the effect on potency when the imidazole or triazole A ring was replaced with carbon-linked heterocycles such as oxazoles and pyridines. Some of these A-ring variations have also been applied by other groups. The data presented in this patent application demonstrated that these variations can result in potent GSMs, both in vitro and in the case of **183** in vivo.

Several patent applications by Roche have also disclosed series containing an amine linker between the B and C rings. The C ring variations disclosed consisted of five- or six-membered heterocycles, including bicyclic combinations, which resemble the conformational restriction of the D ring, as present in Eisai bicyclic triazole series presented in Figure 23.[167−170]

Representatives of the different subseries of Roche have been grouped in Figure 38. There are two thiazole analogues **185** and **186**, with their constrained **187** being the most potent compound (IC_{50} = 40 nM). Further examples of five-membered heterocycles are evident in the 1,2,4-triazoles **188** and **189**, showing alkylation on the nitrogen to introduce the D ring. There are also examples of six-membered C rings in the form of pyrimidines **190** and **191** and triazine **192**. Further constrained analogues **193**−**195** were disclosed, being achieved by cyclization from the pyrimidine incorporating the benzylic carbon and orientating the D-ring vertically again as seen in compounds from Eisai (vide supra, Figure 23). In analogy with the structures in Figure 37, replacement of the imidazole by carbon-linked heterocycles maintained GSM activity.

Independently, AstraZeneca disclosed a chemical series with an aminopyrimidine core similar to that of Roche (Figure 39).[171] The pyrimidine heterocycle is also part of a bicyclic system, but in contrast to the Roche work, the D ring is attached to an alternative position as exemplified in **196** and **197**. SAR was generated around the A, C, and D rings in investigations that were similar to those previously described by other companies.

Bristol-Myers Squibb has also concentrated on amino-linked bicyclic systems, disclosing bicyclic thiazole compounds similar to those disclosed by Roche (**187**) but also claiming bicyclic triazole compounds as depicted in Figure 40.[172] The patent exemplifies mostly seven-membered bicyclic triazoles, such as **199**, **200**, and **201**; however, some six-membered analogues are also exemplified, **198**. The *S* enantiomer is shown as "more active"; however, the activity of one of the enantiomers is divulged while the other enantiomer is assigned a range of activity (Figure 40).[172]

In contrast to the 4-methylimidazole A ring, which was preferred in most series described before, in this patent most exemplified compounds contain 4-chloroimidazole. The seven-membered ring was also substituted with hydroxyls and other functional groups, resulting in potent compounds such as **201** (IC_{50} = 2 nM).[172]

TorreyPines Therapeutics disclosed a series of compounds that, instead of the aniline thiazole used in one of their previous series, contained a urea as its bioisosteric replacement (**202**−**204**,

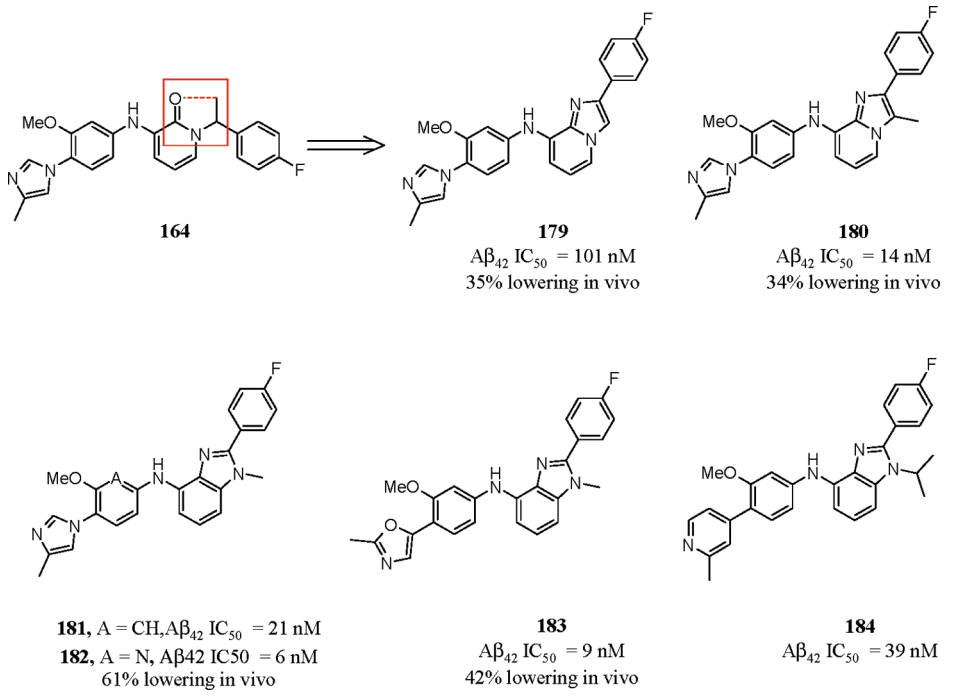


Figure 37. Examples of the chemical series from Ortho-McNeil-Janssen.[164−166]

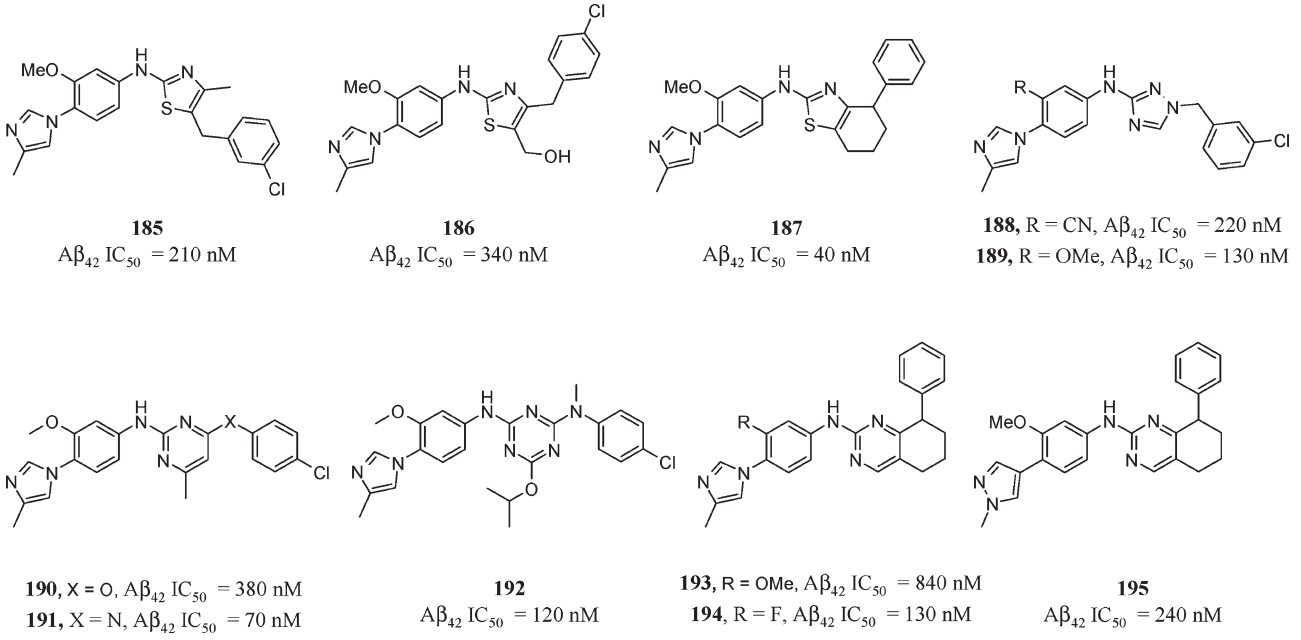


Figure 38. Roche GSM compounds.

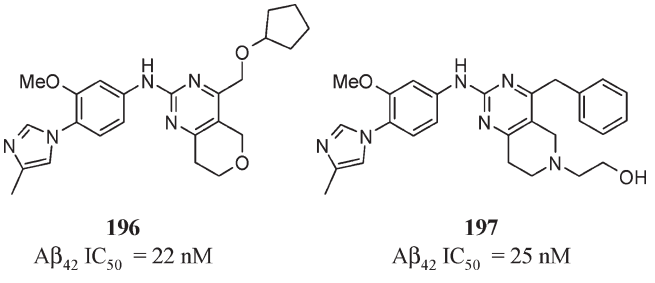


Figure 39. Examples of the AstraZeneca series.

Figure 41).[173] The exploration concentrated on the modification of the substitution pattern of the D ring.[175]

Amgen also disclosed a series of ureas, which exhibited a significant difference, namely, a 2-methylpyridine **205** as the A-ring instead of the 4-methylimidazole (Figure 42).[174] In this series, activity seems to be dependent on the distance between the C and the D rings. Compounds with short linkers between rings C and D like **206** were found to be less active (IC_{50} = 3.3 μM) than those with a longer more flexible linker, **205** (IC_{50} = 131 nM).

Amgen also described a series of amide containing GSMs.[175] The amides, as in the urea series, contained 2-methylpyridine as the A ring (**208**−**210**, Figure 43). The tolerance of this group was illustrated by reporting in vitro potency, with IC_{50} up to 100 nM, demonstrating that 2-methylpyridine could be used as an isosteric replacement of the 4-methylimidazole.

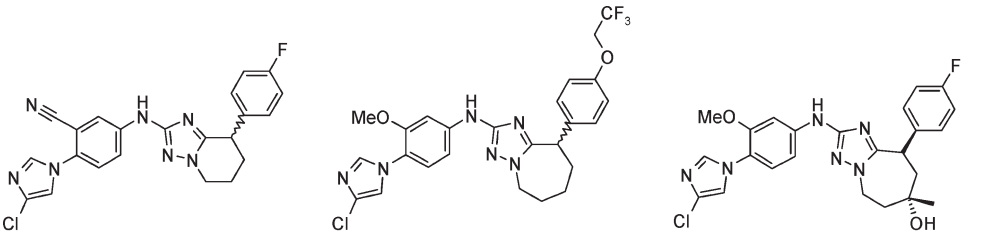


Figure 40. Examples of bicyclic triazoles from Bristol-Myers Squibb.

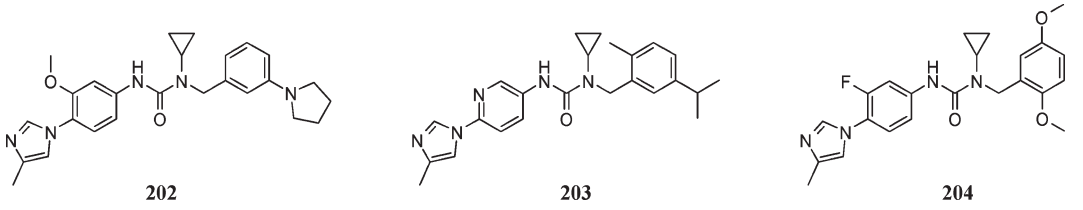


Figure 41. Ureas from Torreypines Therapeutics.

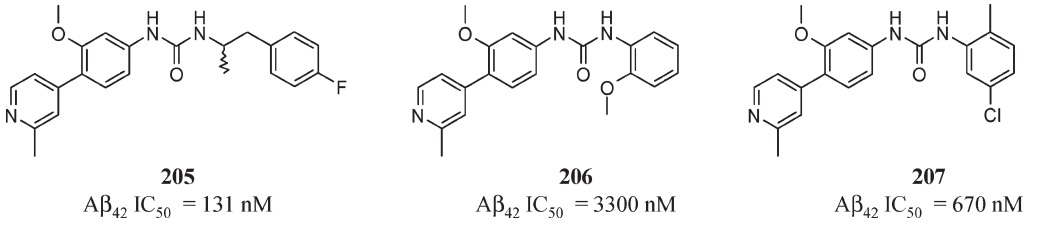


Figure 42. Amgen's ureas.

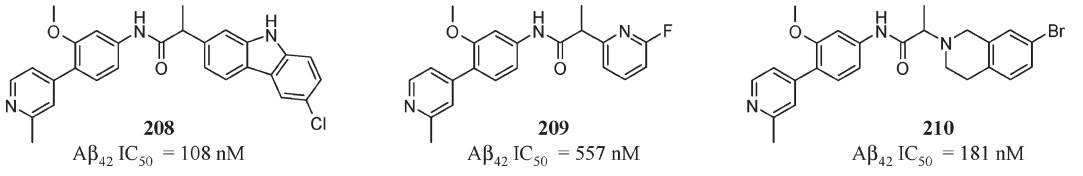


Figure 43. Example amides from Amgen.

Directly Attached B−C Rings

Merck has published three patent applications describing compounds having a 1,2,3-triazole as the central core (ring C).[176−178] Compounds having a methylene linker between the C and D rings, such as **211**, displayed low potency. Other examples exhibiting an amide between rings C and D, such as **212**, resulted in a 25-fold increase in potency. The next generation of triazoles combines the amide and the D ring, which resulted in the introduction of an azepinone. In this series, the linker between ring B and heterocycle A was varied, including ether **215**, anilino **216**, and alkyne **217** (Figure 44).[177]

Eisai recently disclosed in two patent applications a series of compounds without a linker between the B and C rings, and some representatives are shown below (Figure 45).[179,180] The applications describe monocyclic and bicyclic triazole series of which **218**, **219** and **220**, **221**, respectively, are examples. Eisai previously disclosed similar compounds that contained a double bond between rings B and C, as shown in Figures 22 and 23. The removal of this linker resulted in compounds with IC_{50} values in a similar low nanomolar range.

GSK disclosed their activity in the GSM field with a series of pyridazine analogues (**222** and **223**, Figure 46).[181] The investigation mainly concentrated on modifications around the D ring. This series seems to share some analogy to the anilinothiazole series from Neurogenetics, **81**, where the pyridazine can be seen to replace the thiazole core. Noteworthy is the similarity that it shows to Schering compound **173** (Figure 35).

GSMs that show a dissimilarity to the non-NSAID derived compounds discussed up to here have been published by Merck.[182,183] This series is based around piperazinylpyrimidine, **224**, which was identified via a screening campaign and selectively inhibited the production of $A\beta_{42}$ (IC_{50} = 1912 nM) over that of $A\beta_{40}$ (IC_{50} = 3736 nM) (Figure 47). The initial investigations demonstrated that the 4-methoxyphenylpiperazine was critical for activity. A key structural modification that could be performed was the introduction of the gem dimethyl on the piperazine **225**, which resulted in a 10-fold increase in potency.

Modifications of the D ring to replace the 1,4-dianilino moiety resulted in the identification of **226** (Figure 48). The introduction of polar groups at the 6-position of the pyrimidine core, as in **227** and **228**, resulted in compounds with better

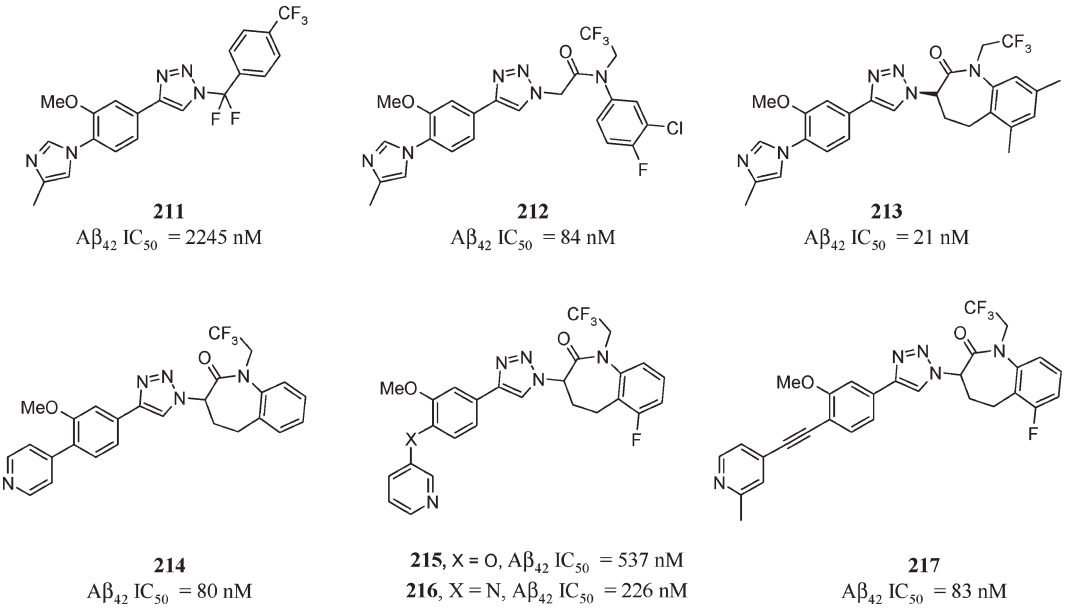


Figure 44. Examples of the 1,2,3-triazoles from Merck.[176,177]

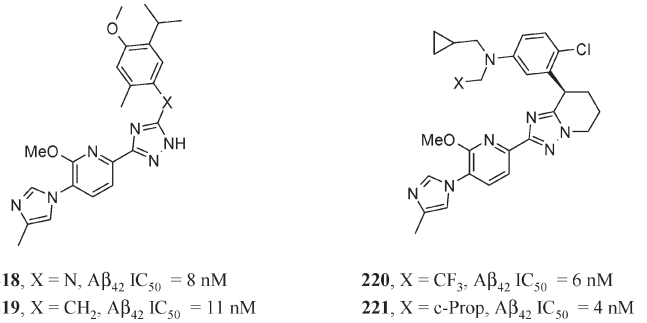


Figure 45. Monocyclic and bicyclic triazoles from Eisai.[179,180]

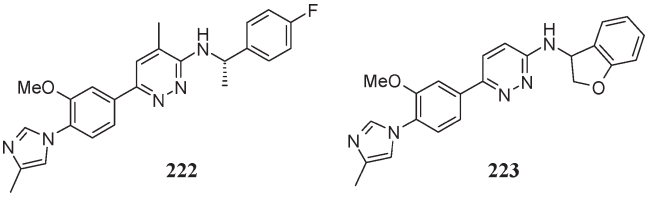


Figure 46. Examples from the GSK patent.[181]

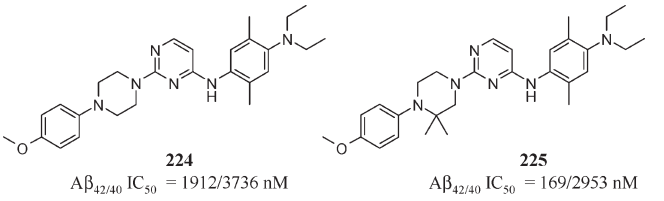


Figure 47. Hit and lead from the Merck series.[182]

physicochemical parameters. The more potent compounds showed a 180-fold selectivity over Notch.[182]

A subsequent publication showed the optimization of **228**, resulting in the identification of a novel purine series **230**−**235** (Table 6).[183] Introduction of additional heteroatom functionality in the form of bicyclic systems and hence polarity in analogy to the compounds **227** and **228** resulted in the discovery of purine **229**. Thus, by use of compound **229** as a starting point, the optimization of the AB ring system was attempted. In contrast to the pyrimidine piperazine series, these purines could tolerate a wide variety of substituents, as displayed in Table 6.[183] It was speculated that the increased tolerance to the A−B ring combinations resulted from a change in binding mode for the purine series. This would orient the A−B ring system into a less confined situation in the active site, thus tolerating more diversity.

Both Notch and in vivo assays were performed on some of the more potent compounds. Notch selectivity was shown to be at least 300-fold. The in vivo potency for compounds **229** and **233** was evaluated using the APP-YAC transgenic mouse model. The compounds were dosed at 100 mg/kg po, and the results are displayed in Table 7.

A summary of the NSAID and non-NSAID chemical series as well as the chemical motifs and structural requirements necessary to obtain GSM activity will be discussed later in the conclusion and perspective section.

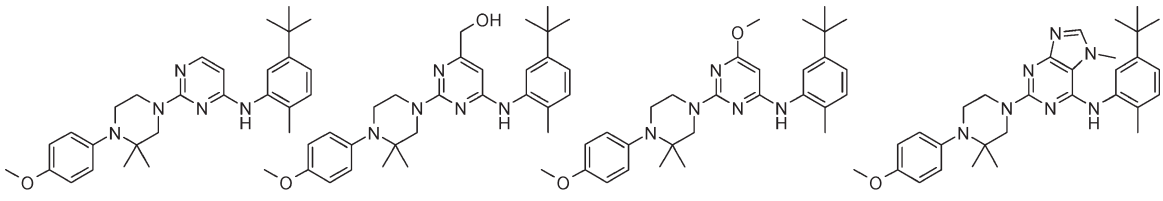
Ginseng Derived GSMs

Ginseng has long been known as a remedy for age-related disorders including memory loss, which prompted the testing of its extracts as potential therapies of AD. A patent application describes a series of ginseng extracts and their Aβ lowering capabilities.[184] The in vitro and in vivo activity of the ginsenosides has also been reported, showing **236** and **237** to be among the most potent analogues (Figure 49).[185] **237** was reported to have an in vitro IC_{50} for the reduction of Aβ_{42} of about 25 μM in a CHO cell line and an IC_{50} of 200 μM for the reduction of Aβ_{40}. In vivo, a 20−30% reduction in Aβ_{40} and Aβ_{42} was observed in a Tg2576 mouse model after an oral dose of 25 mg/kg, with the maximum reduction observed 18 h after dosing.[185]

Satori Pharmaceuticals described a series of GSMs with a similar triterpene core in two patent applications, as exemplified by **238**−**240** (Figure 50).[186,187] No biological data were given.

Clinical Trials of GSMs

The initial indications obtained from epidemiological studies revealing a reduced occurrence of AD in patients using



226 $A\beta_{42/40}$ IC_{50} = 255/5565 nM

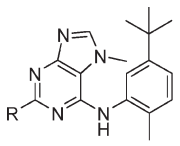
227 $A\beta_{42/40}$ IC_{50} = 34/823 nM

228 $A\beta_{42/40}$ IC_{50} = 260/5700 nM

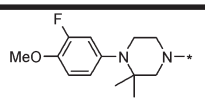
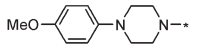
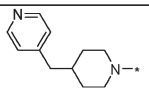
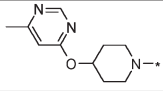
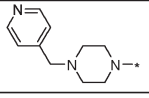
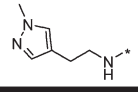
229 $A\beta_{42/40}$ IC_{50} = 21/350 nM

Figure 48. Merck piperazinylpyrimidine series.[182,183]

Table 6. Purine Series and in Vitro Potency[183]



230 - 235

compd	R	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{40}$ IC_{50} (nM)
230		21	270
231		39	730
232		30	310
233		16	120
234		12	130
235		67	400

NSAIDs have since then been followed by clinical trials investigating the effects of NSAIDs on preventing or slowing the progression of AD.[188,189] Despite initial evidence of **3** having beneficial effects in slowing cognitive decline in patients with mild to moderate AD,[190] large-scale clinical trials assessing cognitive outcomes following NSAID administration have been disappointing, suggesting that NSAID treatment is ineffectual once memory decline and associated pathology have already developed. A large prevention trial with the NSAIDs naproxen and **8** has been the Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT). Although initial results did not indicate any improvement in cognitive decline or the prevention of AD, a follow-up analysis of the clinical trial did show a benefit of naproxen in the preservation of cognitive function in patients with healthy brain.[191] This cannot be attributed to GSM activity, however, since naproxen is inactive as a GSM. The expectations were certainly higher for the first selective (non-COX-inhibiting) GSM, **6** (tarenflurbil), to enter the clinic. The results of a 12-month phase II study indicated some positive effects on cognition in mildly affected AD patients as measured by the Alzheimer's Disease Cooperative Study Activities on Daily Living scale (ADCS-ADL) and Clinical Dementia Rating Sum of Boxes (CDR-SB) scale. However, no effects were observed using two other relevant cognitive measurements, namely, the Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-Cog) and the Mini-Mental State Examination (MMSE). Moderately affected AD patients actually showed a clinical deterioration compared to placebo on the CDR-SB scale. A phase III study subsequently showed a similar detrimental effect of **6** (800 mg b.i.d.) on the CDR-SB scale of mild patients during an 18-month multicenter clinical trial. The negative result of the phase III clinical study with tarenflurbil has questioned the viability of γ-secretase modulation to treat AD or even the Aβ hypothesis of AD in general. However, strong arguments exist, suggesting that the pharmacological profile of tarenflurbil does not meet the requirements for a successful DMAAD drug.[192] The low potency in combination with poor brain penetration early on resulted in no effects on $A\beta_{40}$ or $A\beta_{42}$ levels in CSF or even a significant effect on plasma Aβ levels during 3-week dosing in healthy volunteers with tarenflurbil at 200, 400, or 800 mg b.i.d. Currently, two additional GSMs, the NSAID-derived **16** and the non-NSAID derived **108**, have entered the clinic. For both compounds, phase I data have been reported recently.[193,194] Six ascending oral doses of **16** (25, 50, 100, 200, 400, and 600 mg) were evaluated for safety parameters and pharmacokinetic properties. This included the measurement of the main metabolite (**16**-glucoronide), whose levels peaked at 4−5 h and accounted for about 30% of the parent compound. Although the data were still blinded, results indicated that the drug was well tolerated after single oral administration to healthy subjects of doses up to and including 600 mg. The pharmacokinetics of **16** appears linear in the studied dose range, with plasma exposure increasing in a predictable dose-proportional manner.

Table 7. In Vivo Data for Merck Purine Series[183]

compd	$A\beta_{42}$ (%)	$A\beta_{40}$ (%)	brain (μM)	plasma (μM)
229	−73	not significant	7.8	23
233	−69	−26	7.8	27

For **108**, ascending doses of 1−400 mg were evaluated for pharmacokinetic parameters. In addition, pharmacodynamic parameters were measured in the form of $A\beta_{40}$ and $A\beta_{42}$ levels in plasma. Single oral doses of **108** reduced plasma levels of $A\beta_{40}$ and $A\beta_{42}$ in healthy subjects in a dose-related manner. As expected for a GSM, $A\beta_{42}$ levels were decreased to a greater extent than $A\beta_{40}$ (maximum reductions at 400 mg of ~50% and ~30%, respectively), occurring 4−6 h postdose without a rebound effect commonly observed with GSIs. Data obtained from rat studies with **108** were found to be predictive of the results obtained in humans for plasma Aβ effects. The effects on rat CSF and brain Aβ followed similar trends, supporting the use of CSF as a surrogate matrix to further evaluate **108** effects on brain Aβ in humans. **108** initially

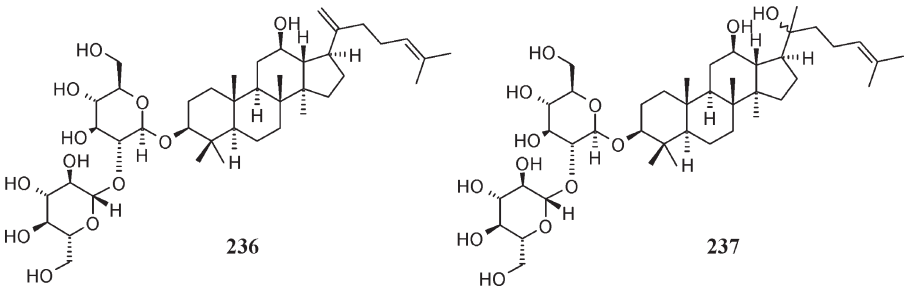


Figure 49. Ginsenoside GSMs.[184,185]

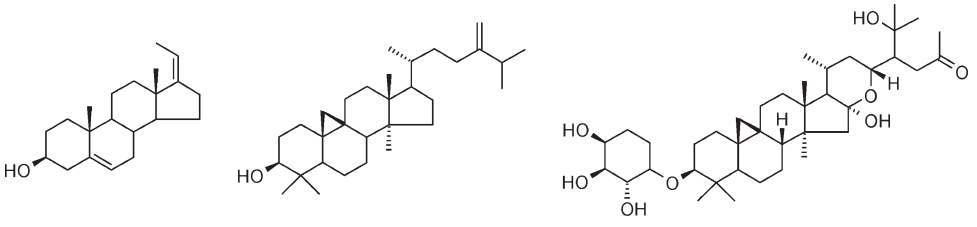


Figure 50. GSMs from Satori Pharmaceuticals.[186,187]

entered the clinic in 2006. Despite the initial drawbacks encountered in a 13-week safety preclinical study in rats, running in parallel with the phase I study (vide supra), clinical studies with **108** were resumed. On the basis of communications to investors, a more potent, second generation GSM (E-2212, structure not disclosed) has since then replaced **108** in the clinic. It was reported to be a more potent GSM both in vitro and in vivo and is expected to possess a more predictable animal and human PK.[195]

Perspective/Conclusion and Future Directions

Since the identification that some of the NSAIDs, such as **3**−**7**, display γ-secretase modulatory activity, this class of compounds has been extensively investigated, leading to the identification of compounds that show improved γ-secretase modulation potency and selectivity. The investigations have involved the profiling of many series of compounds, initially exhibiting in vitro potency in the range of 5−10 μM, which has been subsequently improved. The latest series can be grouped into a third generation of compounds, described by Cellzome-OrthoMcNeil/Janssen, Merck, and EnVivo between 2006 and 2009, and in general they constitute the more potent examples of the NSAID derived GSM, with typical potency being submicromolar.

The various investigations have culminated with the discovery of *R*-flurbiprofen/tarenflurbil (**6**) from Myriad and **16** from Chiesi, which entered into clinical trials. However, for both compounds it can be argued that they do not have the pharmacological profile requirements for a successful DMAAD, especially their low micromolar potency in combination with poor brain penetration, which are limiting factors for expected efficacy. For **6** the outcome of phase III clinical trials has indeed been negative. Next generation of GSMs have considerably improved on potency and brain penetration but generally suffer from high lipophilicity. The NSAID derived GSMs exhibit an amphiphilic nature, combining a carboxylic acid functionality with high lipophilicity. Several groups have tried to address this problem by the introduction of polarity, such as phenyl replacements by piperidines, or the introduction of additional basic nitrogen atoms.[109,113,123] However, in search of potency, lipophilicity tends to increase again.

The phase I clinical trial of **16** included a measurement of the main metabolite (**16**-glucoronide), which was observed in levels up to 30% of the parent compound.[193] A possible liability of the acid series could be the formation of reactive metabolites such as acylglucoronides, as demonstrated by **16**, and could be inherent to the carboxylic moiety. Overall, the NSAID derived GSMs are likely to have suboptimal properties and their high lipophilicity could result in low free fraction, poor solubility, and liver toxicity.

Most of the non-NSAID derived GSMs seem to have been derived from the original Neurogenetics reported structures (Figure 16, Table 4)[86] and subsequent Eisai cinnamide derivatives. Further elaboration of these initial compounds via hit-hopping exercises, isosteric replacement of critical functionalities, and cyclization or otherwise conformational restriction has led to multiple chemical subseries.

The pattern of four consecutive linked rings designated A, B, C, and D as introduced in Figure 16 can be found in most of the variations currently published. In Figure 51, we have attempted to capture the observations for the various described subseries in this class into a generalized structure. The work of many of the research groups has clearly focused on finding C ring variations, including noncyclic alternatives such as urea or amide spacers, presumably aimed at the creation of novel chemical space within this class of compounds. This has led to subseries that share the presence of a hydrogen bond acceptor in the center of the molecules but differ considerably in the distance between the A and D rings. A high degree of planarity for the orientation of the B and C rings seems to be particularly necessary to achieve good potency. A multitude of cyclizations have been described as well, from which the conformational restriction of the D-ring in a position more or less aligned with the direction of the aforementioned H-bond acceptor appears to be of relevance for increasing potency. The imidazole A ring is present as the key heterocycle in many series, but other heterocyclic replacements containing H-bond acceptors have also been identified. The D ring clearly has an important role in providing hydrophobic interactions,

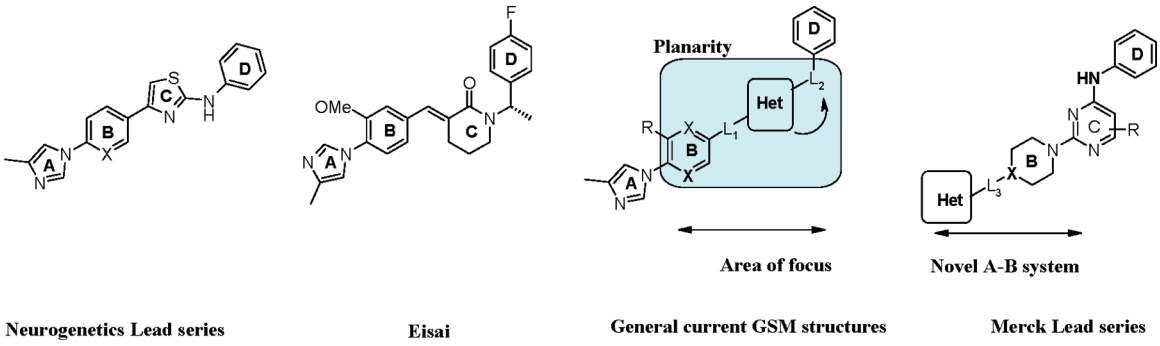


Figure 51. General structural characteristics of the non-NSAID derived GSMs.

with the more potent molecules containing aryl D-rings with often multiple additional lipophilic substituents. The overall high lipophilicity of the series, a property shared with the NSAID derived series, might be regarded as a suboptimal feature that could result in unwanted toxicity. The HTS derived Merck series (Figures 47 and 48) structurally deviates from the other non-NSAID Neurogenetics/Eisai derived series. However, the characteristic similar A−B−C−D ring system can be recognized with a good overlap of the SAR especially on the pyrimidine/purine C-ring and lipophilic substitution patterns present on the aromatic D-ring, also resulting in highly lipophilic compounds. It would be of interest to find out if the various series, NSAID or non-NSIAD derived, show an overlap in their binding site or mode, for example, via competitive binding experiments.

Caution is required around the comparison of the potencies and biological activities reported in this paper. Different assay conditions have been used by the various research groups, making a direct comparison of potencies challenging. Regarding reported in vivo data, a distinction needs to be made between data from transgenic animals versus wild type animals, as well as the source of Aβ sampling (CSF, brain, or plasma). Nevertheless, the field of GSM has clearly progressed from compounds exhibiting micromolar cellular potencies and poor brain penetration to single digit nanomolar potencies with improved CNS properties.

Despite the impressive progress in the GSM area, much work remains to be done. To date, the required structural features that lead to potent GSM and modulation of Aβ production are fairly well understood but the knowledge around the mechanism of action and the way the drug interacts to initiate the response are still vague.

γ-Secretase modulation could result in the identification of a disease-modifying anti-Alzheimer's drug. Since the identification of the first γ-secretase modulator 10 years ago, the scientific progress into the understanding of the molecular basis of AD has been substantial. γ-Secretase is involved in the last step of the Aβ production and thus conceptually constitutes an ideal target for therapeutic intervention resulting in a DMAAD. The concept of manipulating the cleavage specificity of the γ-secretase complex by interactions with a small molecule in order to reduce the concentration of the potentially toxic Aβ_{42} has now clearly been demonstrated with compounds derived from distinct chemical classes. In contrast to GSIs, for several of these compounds the complete lack of Notch inhibition has been reported, as well as the absence of a rebound effect. Therefore, potentially, a therapeutically beneficial intervention in the amyloid cascade can be achieved without the undesired side effects associated with inhibition of γ-secretase. Additionally, the modulatory mechanism of GSMs results in a shift in cleavage from Aβ_{42} to the shorter isoforms. This relative decrease in Aβ_{42} caused by GSMs may actually be of greater benefit than reduction of absolute levels of all Aβ species resulting from GS or BACE inhibition. It counters the reversed shift observed in most, if not all, FAD mutations resulting into EOAD.[196] Additionally, the potential neuroprotective action of the shorter Aβ species[24,25] and recent reports on the role of Aβ species on memory function[197] are also in favor of the minimally invasive way the GSMs modulate Aβ production. Nevertheless, the reduction of the concentration of the toxic Aβ_{42} species remains an unproven therapeutic hypothesis for the treatment of AD. It relies on our understanding of the amyloid hypothesis, which to date is still limited. Apart from pertinent questions around the toxicity of Aβ_{42} and its relation to the development of AD, we have very little idea as to the functions performed by APP and AICD, the possible function of Aβ, or the relationship between Aβ and tau.

Key information required for the rational design of compounds is an understanding of how these compounds interact with the substrate. To date, this information has been gathered for GSIs, as they are the more advanced research area. For GSMs the information is much more limited and the enzyme- versus substrate-targeting of the GSMs is still hotly debated. So far, the mechanistic work on GSMs has been limited to mostly weak NSAID derived GSMs and the reverse modulator fenofibrate. With the recent identification of more potent and structurally distinct series of GSMs this investigation can be continued. A patent describing photoaffinity labeled analogues of Eisai's **109** is a first indication of work in progress in this area for non-NSAID derived GSMs.[141]

Compared to the structural elucidation of β-secretase, the structure of γ-secretase has proven very difficult to resolve, since it is a multicomponent, multiple pass transmembrane protein complex of high molecular weight. High resolution structural information, preferably in the presence of GSMs, will be needed to elucidate further the molecular interaction of GSMs with the GS complex or substrate. As a therapeutic target, γ-secretase modulation will benefit from the identification of more druggable starting points. This will allow the research groups the freedom and ease to generate compounds that are optimized for CNS investigations. So far, the intense medicinal chemistry research efforts have centered around a handful of original starting points and have resulted in a series with overall limited structural diversity. This could be indicative of the difficulty in finding true novel GSM hits. Further structural information on the substrate−GS−GSM interaction may be of help in broadening the understanding into the available chemical space.

The disappointing results obtained to date from clinical trials for both GSM (**6**) and GSIs have brought into question the mode of action, including the amyloid hypothesis. However, the data gathered so far have been generated from the use of suboptimal compounds. A further problem of the clinical outcome is the point at which the patients are involved. Is it at a stage in the disease progression where the treatment could be effective or even appropriate? Data presented in a recent review suggests that AD treatment is only effective in certain phases of the disease and that they only show benefit in mild and moderate AD.[198] The generation of DMAADs will also benefit from the identification of reliable biomarkers that may allow the diagnosis of presymptomatic AD and MCI, allowing the physician and clinician to start the treatment/dosing at the appropriate point in the disease progression. For several GSMs, including **16**, **42**, and **92**, a preventive effect on plaque load has been demonstrated upon chronic treatment in transgenic mice. A preventive approach for AD by using a GSM and consequential early and chronic dosing would increase the safety demands for this type of drug considerably with few, if any, side effects being tolerated. Most reported in vivo studies with the current, highly lipophilic GSM compounds describe the need for fairly high levels of compounds required to achieve efficacy. This will make the preventive approach unlikely to be attainable via the use of GSMs.

For GSM as a research area, the now available, potent compounds may enable the investigation of the molecular interaction with γ-secretase, by either inhibition or modulation. This will advance our understanding of γ-secretase, which will be further evolved by the interpretation of the results gathered from the various ongoing and planned clinical trials.

Acknowledgment. The authors thank our many colleagues at Ortho-McNeil-Janssen Pharmaceutica and Cellzome, who have contributed to the GSM project. In particular the authors thank Drs. G. Macdonald, M. Mercken, F. Bischoff, A. Velter, L. Quaghebeur, Prof. B. De Strooper, and M. Vanderhallen for their help with reviewing this document.

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Harrie J. M. Gijsen studied Molecular Sciences at the Wageningen University in The Netherlands, where he received his Ph.D. degree from the Department of Organic Chemistry in 1993. From 1994 to 1996 he has done postdoctoral studies at The Scripps Research Institute with Dr. Chi-Huey Wong and The Ohio State University with Dr. Leo Paquette. After working in process chemistry at DSM in The Netherlands for 1.5 years, he joined the Department of Medicinal Chemistry at Janssen Research and Development in Belgium in 1998. He has worked in the areas of gastrointestinal diseases (5-HT_4) and pain (CB_2 and TRPA1). Currently, he is Principal Scientist in the Neuroscience Medicinal Chemistry group and a chemistry team leader on Alzheimer's programs, most notably γ-secretase modulation.

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