Biochimica et Biophysica Acta 1828 (2013) 2898–2907

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

γ-Secretase inhibitors and modulators ☆

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ARTICLE INFO

Article history:
Received 24 April 2013
Accepted 4 June 2013
Available online 17 June 2013

Keywords:
γ-Secretase inhibitor
γ-Secretase modulator
Cancer
Alzheimer's disease

ABSTRACT

γ-Secretase is a fascinating, multi-subunit, intramembrane cleaving protease that is now being considered as a therapeutic target for a number of diseases. Potent, orally bioavailable γ-secretase inhibitors (GSIs) have been developed and tested in humans with Alzheimer's disease (AD) and cancer. Preclinical studies also suggest the therapeutic potential for GSIs in other disease conditions. However, due to inherent mechanism based-toxicity of non-selective inhibition of γ-secretase, clinical development of GSIs will require empirical testing with careful evaluation of benefit versus risk. In addition to GSIs, compounds referred to as γ-secretase modulators (GSMs) remain in development as AD therapeutics. GSMs do not inhibit γ-secretase, but modulate γ-secretase processivity and thereby shift the profile of the secreted amyloid β peptides (Aβ) peptides produced. Although GSMs are thought to have an inherently safe mechanism of action, their effects on substrates other than the amyloid β protein precursor (APP) have not been extensively investigated. Herein, we will review the current state of development of GSIs and GSMs and explore pertinent biological and pharmacological questions pertaining to the use of these agents for select indications. This article is part of a Special Issue entitled: Intramembrane Proteases.

Contents

1. Introduction

In concert with three other proteins, APH1, PEN2, and Nicastrin, presenilin 1 (PSEN1) or 2 (PSEN2) function as the catalytic core of the intramembrane cleaving protease called γ-secretase [1–3]. This multi-subunit protease cleaves within the transmembrane domains (TMDs) of over 100 type 1 membrane proteins [4]. γ-Secretase was originally identified as the protease responsible for the generation of Aβ, and thus considered a prime therapeutic target in Alzheimer's disease (AD) [5,6]. However, it was soon recognized that γ-secretase catalyzed cleavages regulate a variety of signaling events by untethering the cytoplasmic domain of various transmembrane proteins from the membrane, allowing these domains to transduce signals to the nucleus [7,8]. It is now clear that regulated intramembrane proteolysis carried out by γ-secretase is another means for cells to transmit and regulate signals across a lipid bilayer, though in other cases γ-secretase may also play a role in transmembrane protein turnover [9,10].

γ-Secretase is an unusual protease. It is highly promiscuous in terms of the transmembrane domain (TMD) sequences it cleaves. Although not

☆ This article is part of a Special Issue entitled: Intramembrane Proteases.

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http://dx.doi.org/10.1016/j.bbamem.2013.06.005

absolute, major determinants of cleavage appear to be prior ectodomain shedding and co-localization of γ-secretase with the resulting membrane stub in subcellular compartments [11,12]. Studies of amyloid β protein precursor (APP) and Notch 1 indicate that γ-secretase initially cleaves the TMD of a protein at a site near the cytoplasmic face of the membrane [8,13–15]. In APP this initial cleavage is then followed by 3–5 sequential di, tri, or tetrapeptide cleavages [14]. Thus, γ-secretase cleavage of transmembrane protein results in two potentially biologically active fragments: the cytoplasmic intracellular domain and a small secreted peptide. Fig. 1 illustrates the typical processing of a generic type 1 membrane protein by γ-secretase. For most substrates, the initial γ-secretase cleavage site and the extent of processivity have not been defined.

Primarily because γ-secretase was a therapeutic target in AD, a plethora of γ-secretase inhibitors (GSIs) have been developed that effectively inhibit γ-secretase cleavage in humans [16]. Indeed, "druggability" of γ-secretase has *not* been an issue even when the identity of the target was unknown in these blind screens. γ-Secretase is a highly tractable therapeutic target and numerous orally-bioavailable, brain penetrant GSIs have been developed [16,17] (see Fig. 2 for examples). Many of these GSIs are highly potent and show excellent bioavailability and pharmacokinetic properties. In AD the efficacy of GSIs has been tied to inhibition of amyloid β protein (Aβ); thus, in AD, GSIs have been conceptualized as "Aβ production inhibitors" [16]. GSIs can decrease Aβ production in human and mouse brain and chronic administration decreases Aβ deposition in amyloid β protein precursor (APP) mouse models [18–21]. These GSIs have been important tools in the AD field, but also have served as essential elements of preclinical proof of concept studies for many different disease indications. In addition to GSIs, compounds referred to as γ-secretase modulators (GSMs) that modulate processivity of γ-secretase have been identified and remain in development as potentially inherently safe ways to selectively target Aβ42 in AD.

Herein, we will review the development status of both GSIs and GSMs. For GSIs we will largely focus on the efforts to i) repurpose these compounds for indications other than AD ii) design substrate selective GSIs. For GSMs we will discuss the current development status and open questions regarding potential utility in AD.

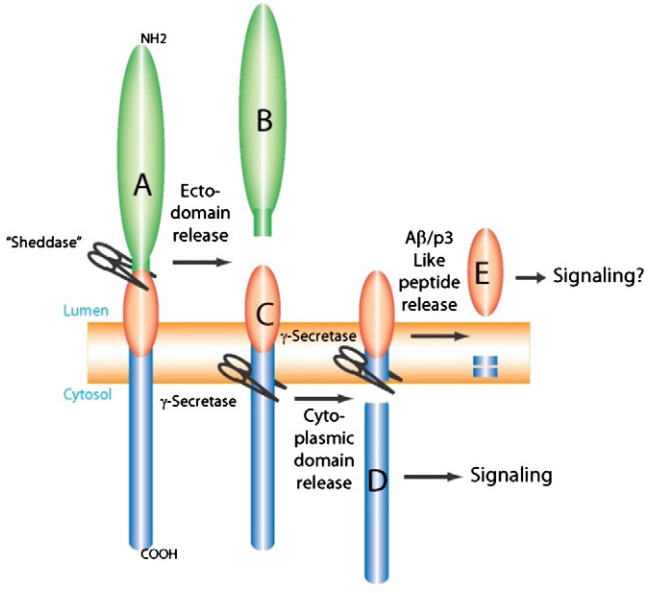

Fig. 1. A model of stepwise γ-secretase cleavage. Typical processing of a type 1 transmembrane protein (A), by a "sheddase" followed by sequential γ-secretase cleavages results in release of multiple potentially bioactive protein fragments: the ectodomain (B), a transmembrane carboxyl terminal fragment (CTF) or stub (C), the cytoplasmic domain (D) and an Aβ/p3 like domain (E). Thus, γ-secretase generates potentially bioactive fragments (D) by an initial cleavage and (E) by processive step-wise cleavages. GSIs inhibit the initial cleavage and GSMs alter processivity.

2. GSIs

In the mid to late 1990s, cell-based drug screens conducted by multiple groups searching for inhibitors of Aβ production identified a number of compounds that dramatically inhibited Aβ secretion and increased levels of APP carboxyl terminal fragments (CTFs) produced by prior α- or β-secretase catalyzed ectodomain shedding [22–29]. At the time the first compounds with these effects on APP processing were identified, the protease targeted was unknown, but the cleavage activity was referred to as γ-secretase. Thus, compounds with this profile were named GSIs. Because γ-secretase cleaved APP within its transmembrane domain and generated multiple Aβ peptides, there were many hypotheses regarding the nature of the activity and the proteases responsible [30,31]. Furthermore, at that time, there was general resistance to the concept that a protease could cleave peptide bonds normally present within the transmembrane domain (TMD) of a protein, fueling further speculation regarding the nature of the protease responsible. Several inhibitor studies also demonstrated that γ-secretase possessed multiple pharmacologically dissociable cleavage activities indicating that it may be more than one protease [32,33]. However, genetic, GSI binding, biochemical and mutational analyses soon demonstrated that γ-secretase was a multi-protein complex with the PSEN1 or PSEN2 acting as the catalytic core, and three accessory proteins, APH1, PEN2, and Nicastrin, needed for complex assembly and stability in cells [1–3,34]. Although it remains formally possible that small-molecules that inhibit γ-secretase cleavage could bind one of the other subunits, GSI binding studies suggest that the target of most GSIs is PSEN1 and 2.

PSEN1 and 2 are now known to be part of a larger family of intramembrane cleaving aspartyl proteases which include five human homologs referred to as signal peptide peptidases (SPP (HM123), SPPL3, SPPL2a,b,c) [35–37]. SPPs differ from PSENs in that they cleave the transmembrane domain of type 2 as opposed to type 1 membrane proteins, and at least for SPP (HM13), the apparent lack of requirement for co-factors for activity [35,38,39]. The differential cleavage specificity appears to be determined by the opposite orientation of the two catalytic aspartate residues between SPPs and PSEN family members. Of note, the recent crystal structure of an SPP from archaeon *Methanoculleus marisnigri* JR1 was established [40]. This structure showed that the catalytic aspartates residues that reside within opposing transmembrane domains are in close proximity to each other and the lipid membrane surface.

Some, but not all, GSIs inhibit signal peptide peptidases as well, though this has not been systemically studied [36,41]. When considering biological activities of various GSIs this is an important and understudied caveat that could influence both biological response as well as potential toxicities. Indeed, it has been reported that SPP, and not PSEN1 or PSEN2, is the major binding target of a GSI in some cells, which likely reflects the fact that in most cells SPP is much more abundant then PSEN/γ-secretase [42]. Although largely outside the scope of this current review, SPP family members have been proposed to be potential therapeutic targets in malaria, various viral infections, and more recently in B-cell related disease [43–50]. Thus, GSIs which target SPPs can be useful probes to examine the biological consequences of SPP inhibition.

As noted above GSIs were initially developed as "Aβ inhibitors". As accumulation of Aβ aggregates in the brain is proposed to trigger AD, and Aβ aggregate formation is a concentration dependent phenomenon, the rationale for GSI development was strong [51]. However, there are concerns that Aβ inhibitors have to be given for a prolonged period of time, may work only as prophylactic therapies or in the protracted, asymptomatic, prodromal phase where Aβ accumulates, and will be increasingly ineffective as Aβ loads increase in the brain [52]. Indeed, this assertion is supported by several *in vivo* preclinical

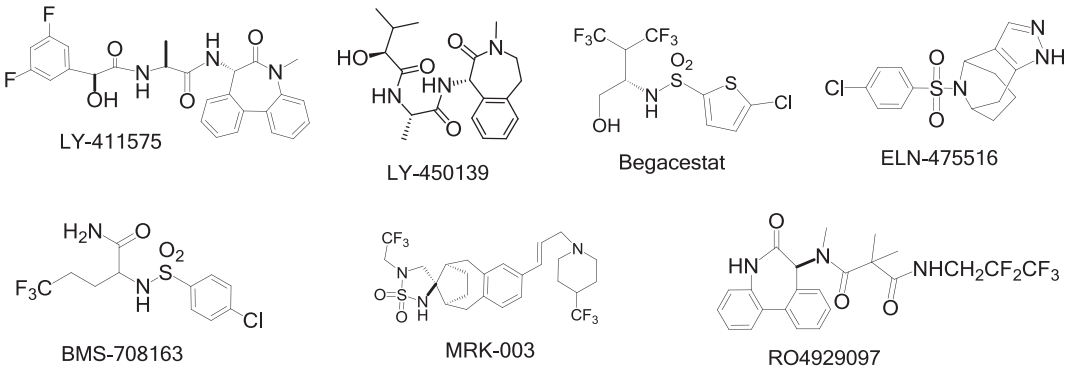

Fig. 2. Examples of GSIs. Begacestat, BMS-708163 and ELN-475516 have been reported to be Notch-sparing GSIs.

studies that show that targeting Aβ with a GSI or other modality is much more effective in a prevention paradigm [21,53,54]. To date, the clinical experience in humans with AD is that long-term GSI treatment designed to produce moderate levels of inhibition of γ-secretase is associated with unacceptable side-effects and lack of clinical efficacy [55–58]. Thus, unless there is an unanticipated breakthrough, γ-secretase inhibition is not likely to be a viable chronic treatment strategy for AD. Furthermore because of these safety issues, it is almost certain that GSIs will not be suitable for testing in asymptomatic individuals at risk for AD.

Outside of the CNS, therapeutic inhibition of γ-secretase has been most often associated with reduced Notch 1 signaling; GSIs are often thought of in these settings as "Notch 1 inhibitors" [59–62]. γ-Secretase has now been proposed to be a therapeutic target in various cancers [59,61,63–81], immunologic disorders including graft *versus* host disease [82,83], vasculitis [84], macular degeneration [85], diabetic nephropathy [86,87], ischemic reperfusion injury in the kidney [88], ischemic stroke [89], traumatic brain injury, [90], hearing loss [91] and fibrosis [92]. It is also likely that additional disease indications may emerge. Currently, a main focus of the repurposing of γ-secretase inhibitors (GSIs) has been in cancer with multiple human trials underway (Table 1). Both GSI monotherapy and combination therapies with other agents are being explored.

The development of GSIs for most cancers as well as other indications has been primarily based on the premise that GSIs act by inhibiting the cleavage of Notch 1, as inhibition of γ-secretase cleavage blocks Notch 1 signaling [59,78,93,94]. Although studies in T-cell lymphoblastic leukemia (T-ALL) unequivocally demonstrate that Notch 1 plays a central role in T-ALL tumor development [79]; the role of Notch 1 signaling has not been as critically examined in most solid tumors. Notch 1 signaling has a normal function in maintenance, development and cell fate. It also has been shown to promote cell survival, angiogenesis and treatment resistance in numerous cancers, both through direct Notch 1 signaling and crosstalk with other key oncogenic pathways [94–99]. However, a comprehensive examination of the mechanism of action of GSIs in cancer and other indications, as well as their effects on angiogenesis and immunity in immunocompetent models has not been conducted [59,100]. Thus, if GSIs show efficacy for cancer other indications, it may be because they synergistically alter multiple signaling pathways. It is also important to consider the findings that GSI based inhibition of Notch 1 and perhaps other substrates of γ-secretase, can actually promote oncogenic transformation in certain tissues such as the skin [61]. Thus, even if acute toxicities can be managed, there are concerns that GSI like many anti-cancer therapies could promote other cancers.

Table 1
GSIs in trial for Cancer.

Indication	GSI	Single agent	Combination
Breast cancer	RO4929097[193,194] MK0752[123]	Yes	Yes
Glioma/GBM	RO4929097	Yes	Yes
Pancreatic cancer	RO4929097	Yes	Yes
Lung cancer	RO4929097	Yes	Yes
Melanoma	RO4929097	Yes	Yes
Leukemia/lymphoma	RO4929097 MK0752 PF-03084014 [64,81]	Yes	Yes
Ovarian cancer	RO4929097	Yes	Yes
Kidney cancer	RO4929097	No	Yes
Colorectal cancer	RO4929097	No	Yes
Sarcomas	RO4929097	No	Yes
Endometrial cancer	RO4929097	No	Yes
Prostate cancer	RO4929097	No	Yes

With rare exceptions, the biological role of γ-secretase cleavage of substrates other than Notch 1 has been ignored during proof of concept preclinical repurposing studies. For example, in the GSI AD trial, many individuals noted changes in hair color, apparently due to inhibition of tyrosinase, another γ-secretase substrate [101]. In a few studies other Notch paralogs and VEGFR1 have been considered to be targets [70,102,103]. Given that tools are not readily available to perform facile, detailed studies on the impact of γ-secretase cleavage on γ-secretase substrates other than APP and Notch 1; it will be important to develop these tools to better understand the biological consequences of GSI based therapies [4]. Indeed, the biology of γ-secretase is complex and our understanding of it suffers from the lamplight effect. As a field we have largely focused only on what we can easily see — cleavage of Notch 1 and APP [104].

Sub-unit composition and subcellular localization of γ-secretase within the target cell may influence both activity on a given substrate and response to a given GSI. Zhao and colleagues have shown that sulfonamide based GSIs selectively inhibit PSEN1 over PSEN2, whereas the GSIs DAPT and L685458 showed minimal selectivity [105]. Similarly, recent data suggest that the GSI MRK-560 preferentially targets PSEN1 over PSEN2 and that this selectivity, at least in mice, increases the tolerability of this GSI [106]. De Strooper and colleagues also show that heterogeneity with respect to the Aph1 subunit is important with respect to viability and overall phenotype of mice, indicating that selective targeting of Aph1b γ-secretase complexes may be less toxic [107]; however, it is not clear whether selective targeting of Aph1b γ-secretase complexes is feasible. Other studies also provide some evidence that various γ-secretase complexes may have differential sensitivity to GSI and different substrate preferences [108–110]. In many cases it is not clear if substrate preference, differential spatiotemporal expression patterns of the various γ-secretase subunits, or a combination of these factors, contributes to the phenotype. Outside of AD, the fact that γ-secretase is a heterogeneous activity has largely been ignored.

3. Avoiding GSI toxicity, substrate selective GSIs and other strategies

Given the toxicities associated with inhibition of Notch 1, especially those associated with altered proliferation and maturation of gut epithelium, there has been considerable effort to develop APP selective

inhibitors or at least inhibitors that would reduce *in vivo* toxicity sufficiently to enable significant reduction of Aβ production in the brain [111]. Unfortunately, so called "Notch-sparing" APP selective GSIs that can be shown to preferentially inhibit γ-secretase APP relative to Notch 1 in preclinical studies have not shown reduced toxicity nor increased Aβ lowering in humans [112–119]. Furthermore, recent studies suggest that one potential issue is that substrate selectivity has not always been assessed in matched assays, and when GSIs inhibitory profiles are examined in matched assays substrate selectivity is absent [117,120]. Thus, it is possible that the apparent substrate selectivity is really attributable to different *in vitro* assays. Discrepant IC50 values may arise when using reporter assays for γ-secretase activity that rely on translocation of endogenous or transcription factor tagged intracellular domains of a given substrate. In these assays, γ-secretase cleavage liberates the intracellular domain and enables it to translocate to the nucleus where it activates a reporter gene [121,122]. It is not clear that these reporter assays accurately reflect effects on cleavage, as there are often large differnces between inhibitor IC50s in the reporter assays versus direct cleavage assays. One thought is that the overexpression of and cleavage of substrate may over-saturate the transcriptional reporter; thus; significant inhibition of cleavage can be observed before the reporter activity is decreased.

Based on the collective experience to date, it is likely that a much more comprehensive assessment of GSI selectivity for inhibition of multiple substrates, as well as biomarkers to track inhibition of non-APP substrates *in vivo* will be needed to support future efforts to develop substrate selective GSIs. Similarly, development of GSIs designed to target a specific γ-secretase complex will be facilitated by development of such biomarkers that can facilely track inhibition of multiple substrates. The most likely biomarker candidates are the Aβ-like peptides produced by γ-secretase cleavage, as these are likely to be present in body fluids and detectable using sandwich ELISAs or mass-spectrometry. Alternatively one might collect peripheral blood and examine the accumulation of substrate derived carboxyl terminal fragments in peripheral leukocytes, but this will likely limit the number of substrates that could be assayed. Until such assays are available we will not know whether we can predict with any accuracy the differential *in vivo* activity of a given GSI using current preclinical models.

With the repurposing of GSIs for cancer, one of the key findings from the early human trials is that subacute dosing with GSIs is reasonably well-tolerated especially when dosing regimens are altered so that dosing is not continuous but intermittent [123]. Alternatively, administration of glucocortocoids with GSI dramatically attenuated gastrointestinal toxicity [124]. In this regard, development of additional tools that better enable assessment of GSI activity on multiple substrates may help to optimize individual dosing so that maximal clinical benefit is achieved while minimizing side-effects. Of course in cancer as opposed to AD, there is generally a willingness to accept some level of toxicity if there are any signs of efficacy.

One important question that remains is whether all current GSIs are biologically equivalent. Though many GSIs currently being used for cancer trials are considered "pan-GSI inhibitors" this labeling may be a misnomer. GSI inhibitory activity is often only established for Aβ and Notch 1 [16]. The net action of GSIs may be influenced by multiple factors within a target cell [9,107,109,125,126]. These factors not only include the variable subunit composition of the γ-secretase complexes but also a) the expression of the substrate in the target cell, b) the location of the substrate, c) sheddase expression, and d) activation of the sheddase. Thus, GSI action could be unexpectedly influenced by any of these factors. Clearly, given the investment in repurposing GSIs, additional studies directly comparing biological actions of various GSI used in clinical trials in various model systems are warranted.

γ-Secretase cleavage is remarkably promiscuous, but somehow regulated [9]. To our knowledge there is no type 1 membrane protein which has been shown to be processed by a sheddase in which the membrane stub is not subsequently processed by γ-secretase. Furthermore, mutational studies and comparison of the TMD sequences cut by γ-secretase reveal that there is little sequence specificity to γ-cleavage [32,127–]. Clearly, ligand binding induced ectodomain shedding is one regulatory step in γ-cleavage, but for many constitutively shed and processed proteins there must be other ways of regulating signaling [131]. Previously, we and others have shown that γ-secretase cleavage of APP was located in cholesterol rich buoyant membranes (lipid rafts) and that cholesterol depletion blocked cleavage [12,132–134]. Subsequently, a number of labs have confirmed these findings for APP, and recently this was extended to show that γ-secretase interacts with tetraspanins and this interaction facilitates γ-secretase localization in raft domains [135,136]. Another study suggested that Notch 1 γ-secretase cleavage occurs on the cell surface whereas APP occurs in intracellular compartments [131]. These data indicated that co-localization of ectodomain-shed substrate CTF and γ-secretase, helped to regulate cleavage. In contrast during development, γ-secretase appears to be active in both raft and non-raft membranes. How the potential altered localization of γ-secretase influences activity and response to a GSI is not known, but again an area worthy of further study.

4. GSMs

γ-Secretase cleavage of APP generates a number of Aβ peptides [32,137]. In most cells Aβ1–37, 38, 39, and 42 are produced at low levels (typically each represents 5–20% of total Aβ detected) and the major species generated is Aβ1–40 (typically over 50% of total Aβ). Other Aβ peptides can also be variably detected at low levels including Aβ1–34, 1–36, 1–41 and 1–43. Shifts in the relative production of these various Aβ peptides towards Aβ1–42, is tightly associated with risk for AD [138,139]. Mutations in *APP* and *PSEN1/2* that elevate the relative level of Aβ42 by even as little as 30% deterministically cause early onset AD [140], and it now appears that the deposition process likely begins 20 years before the onset of dementia in these individuals [141]. Seminal biochemical studies show that Aβ1–42 aggregates into amyloid fibrils and other assemblies much more readily than Aβ1–40 [142,143], and transgenic modeling studies show that AD-associated *APP* and *PSEN* mutations increases Aβ42 levels and accelerate Aβ deposition [144,145]. In addition other studies using various fusion protein strategies to express Aβ1–42 and Aβ1–40 in the absence of APP overexpression show that Aβ42 is required to drive Aβ deposition, and that Aβ1–40 may actually inhibit Aβ deposition [146–149].

Some early studies of peptidic GSIs showed that although they reduced total Aβ levels and increased APP CTF; they also shifted the profile of Aβ species produced, in some cases increasing the absolute level of longer Aβ1–41,42, and 43 [30,32,33]. As discussed earlier these data fueled some speculation that multiple proteases contributed to the generation of the various Aβ peptides. Subsequently, we identified a subset of non-steroidal anti-inflammatory agents (NSAIDs) such as ibuprofen, sulindac and indomethacin, as prototypic agents capable of lowering Aβ42 selectively *in vitro* and *in vivo* by targeting the γ-secretase complex [150–152]. The classic signature of these first generation GSMs differed from previous GSIs in that they did not alter total Aβ production, or increase APP CTFs and no alterations in the generation of several other γ-secretase substrates, but instead decreased Aβ1–42 levels and increased Aβ1–38 [152]. These data suggested that it may be possible to use GSMs as therapeutic agents for AD as they would selectively target the longer more pathogenic forms of Aβ. In addition to these classic GSMs, other compounds referred to as inverse GSMs (iGSMs) were also identified [153,154]. These compounds were often structurally related to the GSMs but typically lacked an acidic group, and increased, rather than lowering, Aβ1–42. In some, but not all cases, these compounds also decreased levels of shorter Aβ peptides including (Aβ1–37, 38, 39). Since the initial identification of NSAID-based GSMs there has been a major effort to improve potency, pharmacokinetic properties and find new non-acidic classes of GSMs. This has led to the identification of a number of acidic GMSs with dramatic increases in potency

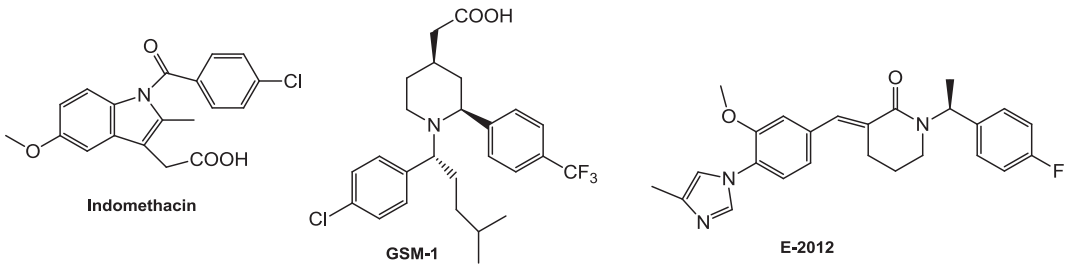

Fig. 3. Examples of GSMs. Indomethacin is an NSAID GSM, GSM-1 is an example of a potent 2nd generation acidic GSM and E2012 is an example of a non-acidic GSM.

and brain penetrance, and non-acidic GSMs based around a common scaffold that are also much more potent than the first generation NSAID-based GSMs (Fig. 3) [155–172]. More recently triterpenoid natural product derived GSMs have been identified [173,174]. Overall there are subtle differences between these GSMs that may be therapeutically important. While the more potent acidic GSMs show the classic GSM signature of lowering Aβ1–42 and increasing Aβ1–38 [175], non-acidic GSMs increase Aβ1–37 and 1–38 and lower both Aβ1–40 and 1–42 [171]. In contrast, the triterpenoid GSM appears to be even more distinct lowering both Aβ1–38 and 1–42 [173,174]. These differential properties and comparison to GSIs are described in Table 2.

Until recently the mechanism of how GSMs shifted γ-secretase cleavage was poorly understood. Today, building on the sequential cleavage model developed by Ihara and colleagues [14], there is evidence that GSMs act as processivity enhancers, and iGSMs as inhibitors of processivity [120]. At least for APP and Notch, neither acidic, non-acidic GSMs nor iGSMS significantly alter the initial γ-secretase cleavage site [150,152,176,177], but they do appear to primarily alter the subsequent number of processivity cleavage events. For classic GSMs this means that they increase cleavage of Aβ1–42 to Aβ1–38; for many iGSMs, they appear to do the opposite. Although not directly established non-acidic GSMs appear would be proposed to increase processivity of Aβ1–40 and Aβ1–42 to Aβ1–37 and Aβ1–38. The novel triterpenoid GSMs recently described by Satori Pharmaceuticals, are somewhat unusual as they decrease both Aβ1–38 and Aβ1–42 [173,174]. How this unusual type of modulation occurs is unclear, but could be accounted for either by effect on the substrate product line that is determined by the initial γ-secretase cleavage (*i.e.* a shift to cleavage that initially generates Aβ1–49) or decreased processivity along the Aβ1–42 product line. If the latter is the case, then an even longer Aβ peptide should accumulate.

Notably, for the more well studied GSMs there is clear evidence that these compounds do not alter Notch 1 processing to any great extent [129,150,152]. They preserve Notch 1 signaling and thus are thought to be inherently safe. Although it is almost certain that GSMs will to varying extents modulate cleavage of some other γ-secretase substrates, there is no evidence to date that this will have significant biological impact. GSMs are not likely to alter signaling events mediated by the initial γ-cleavage and subtle shifts in the length of the short Aβ like fragments produced by subsequent γ-cleavages are unlikely to have significant liabilities.

There remains some controversy over the binding site of GSMs. Low potency NSAID-based GSMs have been shown to bind substrate [171,178,179], whereas more potent acidic GSMs and non-acidic GSMs have been shown to bind PSEN1 or PEN-2 [171,180–182]. The target of the triterpenoid natural product has not been established. Notably, there is evidence that the effect of GSMs is substrate selective and that sequences within substrate dramatically influence processivity of γ-secretase [129,130]. Collectively these binding studies and substrate selective effects of GSMs suggest that there may tripartite interactions between GSM, γ-secretase and substrate that subtly alter processivity perhaps by altering residence time of the substrate within the active site of γ-secretase. Another area of some controversy is whether APP and PS1 mutations influence the potency of GSMs. Though potency of 1st generation GSM were reported to be altered by AD-linked PS1 and APP mutations, other studies with more potent GSMs showed little effect on potency [175,183].

5. Clinical development of GSMs

A Phase III study of the NSIAD based GSM Tarenflurbil (R-flurbiprofen) in patients with mild AD showed no benefit on cognitive or functional outcomes [184]. Tarenflurbil is a weak GSM with low CNS penetrance limiting general inferences based on this trial with respect to clinical efficacy of more potent, CNS penetrant GSMs [11,151,160,185]. Many companies and academic groups have ongoing GSM development programs and several more potent GSM have entered early phase clinical trials. TheGSM, CHF5074, based on R-flurbiprofen developed by Chiesi Pharmaceuticals has advanced the furthest in human testing completing a phase II trial [157]. Recent data suggest that while this compound may possess GSM activity it may have a rich pharmacology and may have additional mechanism(s) of action besides altering Aβ42 [186–188]. One clear challenge in the development of potent GSMs that have been recently reviewed [160], is the balance between liophillicity and potency. Although potency and brain penetration have been dramatically improved in 2nd and 3rd generation GSMs, this has been associated with increases in lipophillicty of the compounds. It has been proposed that this lipophillicity as well as potential glucuronidation of acidic GSMs contributes to what are thought to be off-target, primarily liver toxicities [160].

As with the GSIs, there are again major concerns that GSM therapy in patients with symptomatic AD is almost certain to fail, unless the compound has additional mechanism of action not linked to Aβ that prove to beneficial [52]. Although Aβ1–42 or other longer Aβ peptides are

Table 2
Comparison of effects of GSIs, GSMs and iGSMs.

	Total Aβ	Aβ1–37	Aβ1–38	Aβ1–40	Aβ1–42	APP CTF	Notch	EC50↓ Aβ42[a]	Binding target
GSI	↓	↓	↓	↓	↓	↑	↓	~500 pM	PSEN1/2
NSAID-GSM	=	=	↑	=	↓	=	=	~10 μM	APP/Substrate
2nd generation acidic GSMs	=	=	↑	=	↓	=	=	~50 nM	PSEN
Non-acidic	=	↑	↑	=	↓	=	=	~50 nM	PSEN/PEN2
Triterpenoid	=	=	↓	=	↓	=	=	~50 nM	?
iGSM	=	↓ or =	↓ or =	=	↑	=	=	~10 μM	APP/substrate

[a] These EC50s are approximations of the most potent compounds reported.

critical for initiating Aβ deposition, shorter Aβ accumulate in the AD brain [189]. Thus, shifting Aβ cleavage after nucleation events have occurred is not likely to dramatically alter Aβ deposition kinetics. Thus, a major challenge is whether a sufficiently safe GSM can be developed that can potentially be used as a prophylactic agent in asymptomatic individuals. A final issue of clinical relevance relates to the biology of the shorter Aβ peptides. There has been little systematic investigation of these peptides. Although one report suggested that Aβ1–38 may behave *in vitro* like Aβ1–42, this important finding has not been reproduced [190]. Further study of these short peptides may reveal unique properties that might help to guide development of GSMs. For example Aβ1–40 appears to act an inhibitor of Aβ1–42 nucleation and aggregation *in vivo* [191,192], but whether Aβ1–37 or 1–38 inhibit aggregation *in vivo* is unknown. Given that non-acidic GSMs decrease Aβ1–40 whereas acidic GSMs do not, the distinct action of these two classes of GSMs could have major impact on efficacy.

6. Conclusions

From a biological perspective γ-secretase is both a fascinating and complex enzyme that is increasingly being scrutinized as a therapeutic target for conditions other than AD. There are many gaps in our knowledge that need to be answered to optimally move forward with development of GSIs for cancer and other indications and the continued development of GSMs for AD. Future studies addressing the contribution of other substrates to effects of GSIs and even GSMs, and efforts to determine if all GSI are biologic equivalents certainly will be key steps in these development efforts.

Acknowledgements

This work was supported by P01 AG20206 (EHK, TEG) and P01 AG025531 (BAO, TEG, LM).

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