CHAPTER FIVE

Secretase Inhibitors and Modulators as a Disease-Modifying Approach Against Alzheimer's Disease

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1. INTRODUCTION

With an aging population across the world, the prevalence of Alzheimer's disease (AD) and the consequent burden to society are rapidly rising. The currently approved medications for AD only offer symptomatic treatment of limited duration without affecting the progression of the

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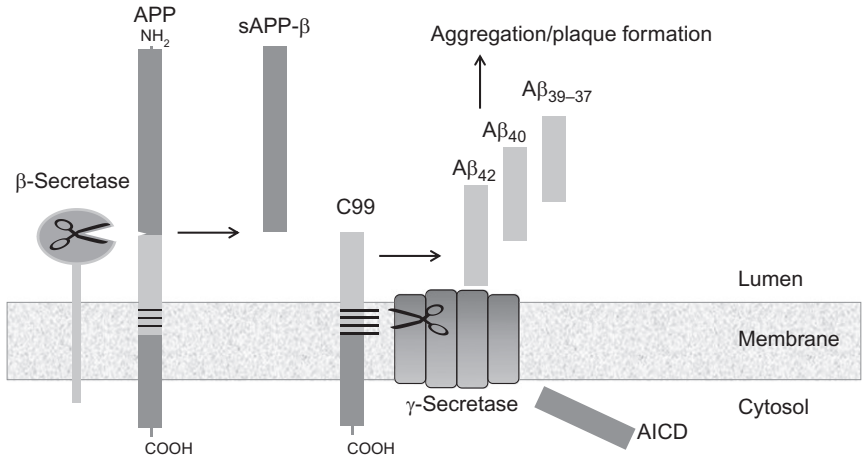


Figure 5.1 Production of amyloid peptides by the sequential cleavage of BACE1 and GS.

disease. Therefore, disease-modifying approaches are urgently needed. A hallmark pathology of AD is the presence of amyloid plaques in the brain, which are mainly aggregates of amyloid beta (Aβ) peptides, among which Aβ42 is the most neurotoxic. These peptides are formed via proteolytic processing of the amyloid precursor protein (APP) by two aspartyl proteases: beta-site APP-cleaving enzyme 1 (BACE1 or β-secretase) and γ-secretase (GS) (Fig. 5.1). Consequently, BACE1 and GS are attractive targets to prevent both the build-up of the amyloid plaques and the formation of toxic amyloid dimers and oligomers.

This report highlights key progress and findings toward γ-secretase inhibitors (GSIs)/modulators and β-secretase inhibitors, since the last time these topics were reviewed in this journal.[1]

2. INHIBITORS OF GS

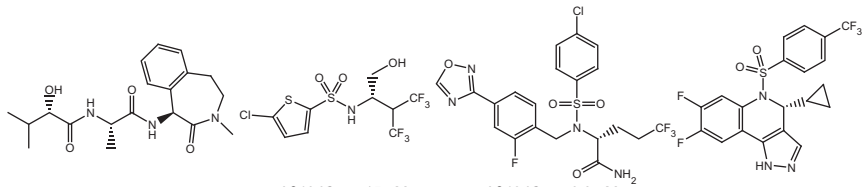
2.1. Function of GS

GS is a member of the intramembrane-cleaving aspartyl protease family consisting of four integral membrane proteins: presenilin (PS) 1 or 2, nicastrin (Nct), anterior pharynx-defective 1 (Aph-1), and presenilin enhancer protein 2 (Pen-2).[2] PSs constitute the aspartic protease catalytic subunit of the GS complex which cleaves C99, the 99-residue membrane stub generated after cleavage of APP by BACE1, in a progressive manner at the ε, ζ, and γ sites, resulting in Aβ species of varying lengths.[3] Initial proteolysis releases the APP intracellular domain (AICD) in the cytoplasm, leaving

membrane-embedded Aβ49 or Aβ48. GS then successively cleaves Aβ49 and Aβ48 by releasing tripeptides to give rise to Aβ40 and Aβ42, respectively.[4] These are either released extracellularly or further cleaved to generate shorter isoforms Aβ39–Aβ37, of which Aβ38 results from the product line containing Aβ42. The GS complex also functions in many other cellular processes, and more than 50 different substrates have been identified to date, including Notch,[5] creating the potential for adverse effects upon inhibition of GS.

2.2. Current status of GSIs

Several GSIs have moved to the clinic, and their progress has been reviewed recently.[6,7] The most advanced compound, semagacestat **1** (LY450,139) entered two large Phase III trials in mild-to-moderate AD patients which were prematurely interrupted in August 2010.[7] Instead of slowing disease progression, **1** was associated with a statistically significant decline in cognition. In addition, an increased risk of skin cancer was reported. Multiple reasons have been proposed to explain the lack of efficacy and observed side effects,[7] including the rebound effect on Aβ42 at low levels of **1**, poor selectivity versus Notch cleavage (threefold *in vitro*), and accumulation of neurotoxic C99.[8]

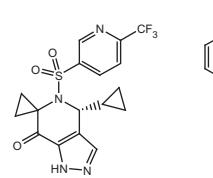


APP IC_{50} = 15 nM
1, Semagacestat

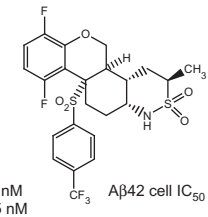
Aβ40 IC_{50} = 15 nM
2, Begacestat

Aβ40 IC_{50} = 0.3 nM
3, BMS-708163

APP IC_{50} = 0.34 nM
4, ELND006



γ-APP cell-free IC_{50} = 1.4 nM
γ-Notch cell-free IC_{50} = 15 nM
5



Aβ42 cell IC_{50} = 0.5 nM
6

Two other GSIs studied in the clinic, begacestat **2** (GSI-953) and ELND006 **4**, have 17- and 16-fold Notch sparing selectivity, respectively, in cellular assays.[9,10] Despite achieving Aβ lowering in human, development of both compounds has been halted, with **4** showing liver toxicity.[7,11] Avagacestat **3** (BMS-708163) is another potent GSI[12] undergoing clinical

testing. Dosing **3** at 200 and 400 mg reduced Aβ42 levels in CSF by 32% and 34%, respectively. In a Phase II study with **3**, a trend for cognitive decline and potentially Notch-related side effects were noted at the high dose, despite a reported 193-fold selectivity against Notch.[12,13] Further development of **3** is targeting prodromal AD.[7]

Medicinal chemistry efforts around GSIs have been reviewed recently.[6] Since then, new arylsulfonamide-containing GSIs have been reported. For instance, pyrazole sulfonamide-based dihydroquinoline derivative **5**, with optimized PK properties, reduced mouse brain Aβ40 levels by 27% (1 mg/kg, p.o.).[14] Tetracyclic sulfones have also been reported as potent GSIs.[15] For instance, **6** (SCH 1500022) demonstrated subnanomolar cellular activity and reduced mouse brain Aβ40 levels by 57% (10 mg/kg, p.o.). Moreover, **6** was identified as a selective PS1 inhibitor, which may provide insights into developing new GSIs with improved side-effect profiles.[16]

3. MODULATORS OF GS

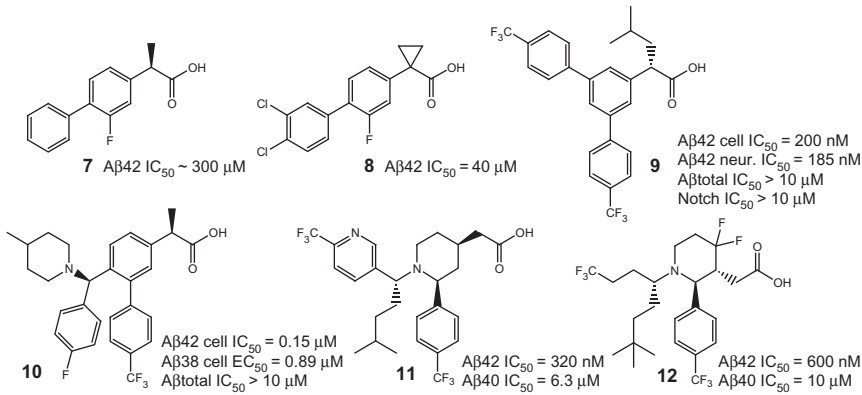
In comparison with GSIs, modulators (GSMs) cause a product shift from the longer Aβ peptide isoforms to shorter, more soluble, and less amyloidogenic isoforms, without inhibiting APP or NOTCH proteolytic processing. As such, modulating GS may avoid some of the adverse effects observed with GSIs. Since the late stage clinical failure of the NSAID-derived GSM tarenflurbil in 2008, considerable progress has been made toward discovering more potent and better brain penetrable compounds derived from both NSAID- and non-NSAID scaffolds.[17]

3.1. Mechanism of modulation

The molecular mechanism of GSMs is still a subject of debate.[18] Recent photoaffinity labeling studies with carboxylic acid-[19] and imidazole-derived GSMs[20] suggest that both bind to the N-terminal fragment of PS, although their binding sites only partially overlap.[20] All GSMs reduce Aβ42 levels without a major affect on total Aβ levels, but reduction of Aβ40 tends to be more pronounced for non-NSAID-derived GSMs.[21] The enhanced processing of the longer Aβ peptides toward the shorter isoforms Aβ39–Aβ37 supports the hypothesis that GSMs actually activate GS and thus counter the loss of GS function linked to many familial AD causing mutations.[22] In a study to further discriminate between modulation and inhibition, sustained cognitive improvement was achieved with a GSM, but not with GSIs, the latter leading to accumulation of C99.[8]

3.2. NSAID-derived modulators

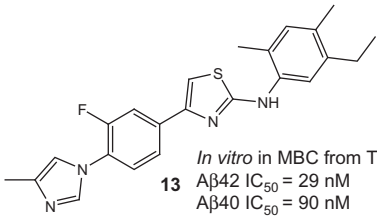
The failure of tarenflurbil **7** to improve conditions in mild AD patients has been attributed to insufficient potency (Aβ42 $IC_{50} \sim 300$ μM) and a plasma-to-CSF ratio of only 0.5–1%.[23] A slight improvement in these parameters has been achieved with CHF5074 (**8**) (Aβ42 IC_{50} = 40 μM, brain penetration 3–5%), which is currently undergoing clinical testing.[24] Medicinal chemistry programs around new NSAID-derived GSMs have led to increasingly potent and brain-penetrant compounds. The orally active NSAID-derived GSM **9** (JNJ-40418677) was reported to inhibit Aβ42 with an IC_{50} of 200 nM and have excellent brain penetration with a B/P ratio around 1 in mice.[25] In a preventive study, chronic treatment of Tg2576 mice with **11** dose dependently reduced both plaque number and area.[25] Brain penetration of NSAID-derived GSMs is improved by introduction of a basic nitrogen atom as in **10** (BIIB042). A brain concentration of 4.6 μM of **10** was achieved in mice (10 mg/kg, p.o.), resulting in a 40% reduction in brain Aβ42.[26] Replacement of the central phenyl ring with piperidine, as in **11** and **12**, has been reported by several groups. Heterocyclic aromatic groups were introduced in order to lower the lipophilicity of the compounds, with **11** demonstrating high brain levels (4 μM) in mice (5 mg/kg, p.o.).[27] 4,4-Difluoropiperidine **12** resulted in improved pharmacokinetic properties and dose-dependently lowered Aβ42 in rats (ED_{50} 5 mg/kg, with brain/plasma levels at 1/3.7 μM).[28]



3.3. Imidazole-derived modulators

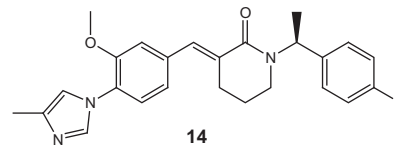
A class of noncarboxylic acid, imidazole-containing GSMs has been developed, which contains an anilinothiazole core represented by **13**. In line with NSAID-derived **9**, chronic treatment with **13** resulted in a significant

inhibition of plaque deposition.[21] Related analog **14** (E-2012) had progressed toward a Phase I clinical trial and reduced plasma Aβ42 levels dose dependently, with a maximum reduction of ~50% after a 400-mg dose.[29] Further development of **14** was suspended as lenticular opacity was observed in a preclinical study in rats.[17]



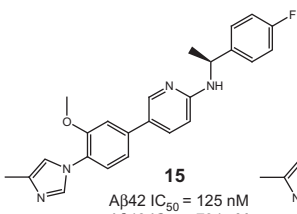
13

In vitro in MBC from Tg2576
Aβ42 IC_{50} = 29 nM
Aβ40 IC_{50} = 90 nM
Aβ38 EC_{50} = 170 nM
Aβtotal unchanged



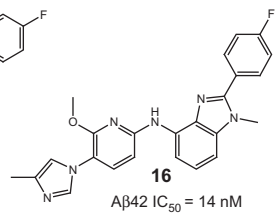
14

These two series have served as a starting point for a substantial amount of work that has been recently reviewed.[17] For example, a novel series of potent pyridazine- and pyridine-derived GSMs have been described, exemplified by **15**, which reduced rat brain Aβ42 by 28% with a corresponding brain concentration of 8.9 μM.[30] Several chemical subclasses with additional conformational restrictions led to a further increase in *in vitro* and *in vivo* potency. Among these, benzimidazole **16** induced a 63% lowering of Aβ42 levels and a 91% increase of Aβ38 levels in mouse brain (30 mg/kg, p.o.).[31] Potent pyrazolopyridine **17** produced a 45% reduction of rat CSF Aβ42 levels at a corresponding brain concentration of 1.22 μM.[32] Application of amide isosteres to **14** resulted in cyclic hydroxylamidines, as exemplified by **18**.[33] In rat, treatment with **18** (3 mg/kg, p.o.) reduced cortical Aβ42 by 37%. Replacement of the characteristic imidazole moiety in these series with other heterocycles, as exemplified with **19**, has also been reported.[34]



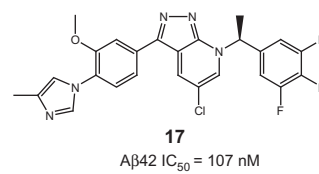
15

Aβ42 IC_{50} = 125 nM
Aβ40 IC_{50} = 794 nM



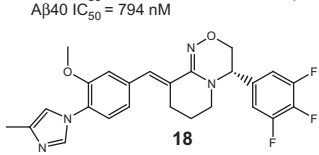
16

Aβ42 IC_{50} = 14 nM



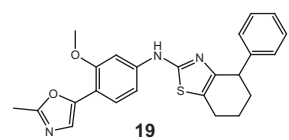
17

Aβ42 IC_{50} = 107 nM



18

Aβ42 IC_{50} = 33 nM
Aβ40 IC_{50} = 123 nM
Aβtotal IC_{50} = 20 μM

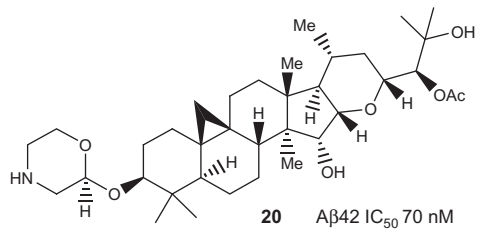


19

Aβ42 IC_{50} = 44 nM

3.4. Triterpene-derived modulators

Structurally distinct compounds such as **20**, semi-synthetically derived from triterpene glycosides extracted from ginkgo or black cohosh, were reported as GSMs with a unique profile as they selectively lowered both Aβ42 as well as Aβ38 while sparing Aβ40.[35] In mice, **20** had a B/P ratio of 1.6 and an oral bioavailability of 37% (30 mg/kg, p.o).[36]



20 Aβ42 IC_{50} 70 nM

4. INHIBITORS OF β-SECRETASE

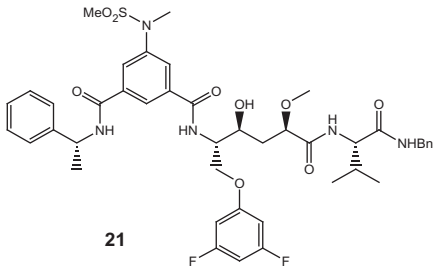
4.1. BACE as a druggable target

Membrane-bound aspartyl protease BACE1 catalyzes the initial cleavage step in the formation of Aβ peptides and thus has been a prime target for interference in Aβ production since its discovery in 1999. Although potent inhibitors have been developed over the past decade, it has been extremely difficult to combine *in vitro* potency with sufficiently high drug concentrations in brain to achieve optimal *in vivo* efficacy. Since the last report in this series in 2007, considerable progress has been made in this regard, which will be highlighted in the following sections. Several reviews on the biology[37] and medicinal chemistry challenges[38,39] of BACE1 have been published in recent years, including a book entirely devoted to this target.[40] Although key questions related to the desired level of inhibition and on- and off-target selectivity remain unanswered, over the past 2 years, a growing number of companies moved into human clinical trials with BACE inhibitors.

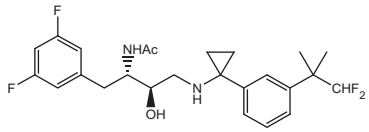
4.2. Main classes of BACE inhibitors

BACE1 inhibitors have been designed starting from various approaches, ranging from substrate-derived inhibitors to fragment-based screening.[41] Structure-based design has been routinely applied, with over 170 crystal structures of the soluble, catalytic domain of both apo and complexed BACE1 deposited in the PDB. Most BACE1 inhibitors contain a "warhead" which interacts via hydrogen bonds with the catalytic aspartyl dyad. Historically, a large group of BACE1 inhibitors are substrate transition-state

analogs (TSAs) mimicking the tetrahedral intermediate formed during catalysis. They include (nor)statine, aminostatine, hydroxyethyleneamine, hydroxyethylene, and aminoethylene TSAs. An overview of these structures can be found in several recent reviews.[39,41,42] TSA-derived inhibitors are often highly potent, as exemplified by hydroxyethylene TSA **21** (BACE1 IC_{50} = 0.3 nM),[43] but often lack sufficient brain exposure for *in vivo* efficacy. However, compound **22** with a modest BACE1 IC_{50} of 230 nM, but good permeability and reduced PgP liability showed about 30% reduction in guinea pig brain and CSF Aβ levels (30 mg/kg).[44]

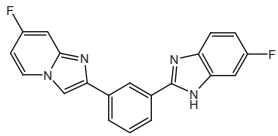


21



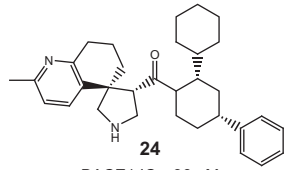
22

The first BACE compound to enter the clinic, CTS-21166, has been described as a TSA, although the structure remains undisclosed.[45] Animal studies indicated good PK/PD properties, including a brain/plasma ratio of 0.44, and oral bioavailability. Dosing CTS-21166 for 6 weeks in rats (4 mg/kg, i.p.) reduced brain Aβ levels by 35–38% and plaque load by 40%. In a human proof-of-concept study, CTS-21166 reduced plasma Aβ levels by up to 80% at the highest dose (225 mg, i.v.), but no CSF data were available. Despite the apparent success of CTS-21166, in general, TSAs display poor drug-like properties due to their bulkiness and often still peptidic nature. In recent years, this has translated in a significant decrease in newly reported TSAs, especially in the patent literature. Compounds **23**–**26** represent alternative heterocyclic classes of inhibitors.



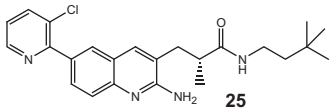
23

BACE1 IC_{50} 18 nM



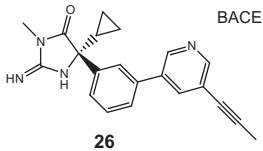
24

BACE1 IC_{50} 36 nM



25

BACE1 IC_{50} 11 nM



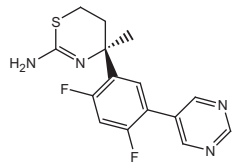
26

BACE1 IC_{50} 5.4 nM

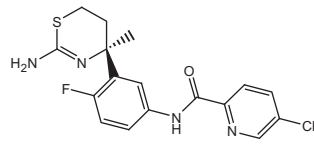
Extensive rigidification of hydroxyethylene TSAs, to remove all peptidic nature, led to **23**.[46] In **24**, the pyrrolidine nitrogen forms a hydrogen bond network with the catalytic aspartyl residues.[47] Introduction of the weakly basic pyridyl nitrogen in **24** resulted in an acceptable PK profile and oral bioavailability. Fragment-based screening led to heterocyclic scaffolds with amidine or guanidine motifs such as in **25** and **26**.[48,49] These motifs form an optimal hydrogen-bonding network with the catalytic aspartates, as apparent from cocrystal structures with BACE1. The latter represent a majority of the recently reported structures and will be discussed further in the next section.

4.3. Amidine- and guanidine-derived inhibitors

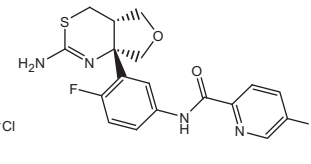
The surge in patents around amidine- and guanidine-containing scaffolds is undoubtedly related to the combination of improved potency and blood–brain barrier penetration resulting in good to excellent *in vivo* reduction of Aβ levels in brain and CSF. A representative of this class is aminothiazine **27** (LY2811376), which was shown to be highly brain penetrant in PDAPP mice with a B/P ratio of ~2 and reduced CSF Aβ levels in dog with ~70% (9 h after 5 mg/kg, p.o.). In a Phase I clinical trial, **27** reduced CSF Aβ dose dependently, with an average reduction of CSF Aβ40 over 24 h of ~56% after a 90-mg oral dose.[50] Further development of **27** was stopped due to adverse eye effects in rats, which importantly was shown to be unrelated to BACE1 inhibition. Elongation of the biaryl moiety in **27** to amides as in **28** has led to an increase in potency while maintaining excellent *in vivo* efficacy (85% reduction in mouse brain Aβ40 levels, at 100 mg/kg, s.c.).[51] In addition, **28** also inhibits BACE2 with an IC_{50} of 6 nM, thus may find application in the treatment of diabetes.[52]



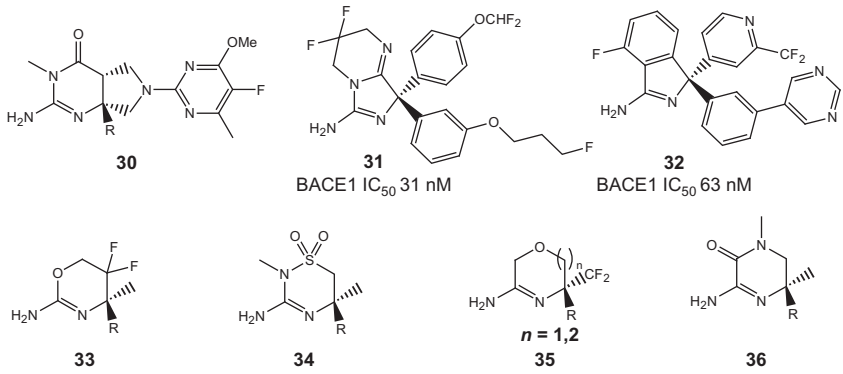
27
BACE1 IC_{50} 239 nM
BACE2 IC_{50} 2880 nM



28
BACE1 IC_{50} 18 nM
BACE2 IC_{50} 6 nM



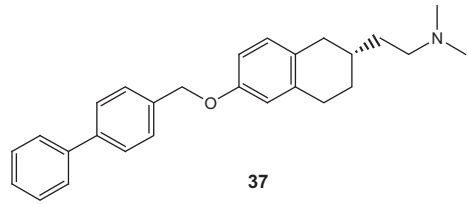
29
BACE1 IC_{50} 20 nM



Ring-fused analogs have also been reported, with **29** reducing CSF Aβ levels by 62% (10 mg/kg, p.o.).[53] As further exemplified by **30** and **31**, many variations of substitution pattern, ring size, and ring fusion have appeared.[54,55] Alternative amidine-containing scaffolds, as exemplified by **32–36**, have also been described as potent inhibitors.[56–60] As for **28**, some of these compounds have limited to no selectivity over BACE2.[57] Lowering brain Aβ levels by all these variations is greatly dependent upon the level of brain exposure. Despite a reduced efflux ratio *in vitro*, **31** only achieved high brain concentrations and Aβ reduction *in vivo* by concomitant dosing with a PgP inhibitor.[55] In contrast, a 60-mg/kg dose of a brain-penetrant derivative of **36** resulted in a 73% reduction in mouse brain Aβ.[60]

4.4. Alternative methods for inhibiting BACE activity

All of the inhibitor classes mentioned above target the catalytic site by occupying the APP substrate pockets. Recently, highly selective BACE1 antibodies have been reported which target an exosite and reduce Aβ levels *in vitro* and *in vivo*.[61,62] Brain uptake can be elegantly increased by engineering a dual specific antibody with high affinity for BACE1 and low affinity for the transferrin receptor.[63] The antibodies were shown to bind noncompetitively and were highly selective over BACE2 and Cathepsin D. TAK-070 (**37**) is a small molecule which was also shown to bind noncompetitively to BACE1.[64] It was only binding to full-length BACE1, but not to truncated BACE1 lacking the transmembrane domain. Chronic treatment of Tg2576 mice reduced cerebral Aβ deposition and normalized behavioral impairments in cognitive tests.



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5. CONCLUDING REMARKS

Consensus is growing that for a successful disease-modifying therapy of AD with amyloid-targeting drugs, treatment has to take place before the disease has progressed too far and most likely before disease symptoms become apparent (prodromal AD).[65] The need for early and chronic treatment will require high safety margins. Inhibition of either GS or BACE has a potential for on-target-related side effects, as they both process multiple proteins. While this has become manifest in clinical trials with GSIs, BACE1 knockout animals display a fairly normal phenotype. GSMs may circumvent the Notch-related toxicity related to GSIs, but the relatively high micromolar concentrations required for reduction of Aβ levels increases the likelihood for off-target side effects.

Although the validity of the amyloid hypothesis is supported by genetics, so far clinical trial results with GSM tarenflurbil and GSI semagacestat have been disappointing. Currently ongoing Phase III trials with the monoclonal antibodies bapineuzumab and solanezumab, for which the first data are expected in 2012, may provide renewed support for the amyloid hypothesis.[66] Multitargeted anti-Alzheimer agents, for example, displaying dual BACE/acetyl cholinesterase inhibition or GSM/PPARγ activity, may provide additional value.[67] With the more potent GSMs, PS1-selective GSIs, and brain-penetrant BACE inhibitors described in this report, new tools have become available to clinically test the therapeutic potential of intervening in the amyloid peptide production.

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