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Targeting amyloid-β in Alzheimer's disease: A critical analysis of clinical trials and their implications for drug development

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ABSTRACT

Alzheimer's disease (AD), a prevalent neurodegenerative dementia, is characterized by amyloid-β (Aβ) plaques and neurofibrillary tangles, with Aβ playing a central pathogenic role. Approved AD drugs, primarily acetylcholinesterase inhibitors, only alleviate symptoms without modifying disease progression. Aβ-targeting strategies aim to inhibit Aβ production or enhance its clearance, leveraging early deposition for proactive intervention. Preclinical studies show Aβ reduction mitigates neurodegeneration, but clinical trials reveal challenges: γ-secretase inhibitors face off-target toxicities and limited efficacy, while BACE1 inhibitors suffer from safety issues or failure to improve cognition. Despite setbacks, advancing understanding of AD pathogenesis and optimized drug design/ trial protocols sustain the potential of Aβ-targeted therapies. This review aims to advance Aβ-targeted therapies for AD by integrating lessons from prior clinical trials and outlining strategic directions for future research and development.

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Introduction

Alzheimer's disease (AD), a progressive neurodegenerative disorder predominantly afflicting the elderly, stands as the most prevalent form of dementia (1). Currently, AD ranks sixth among the leading causes of death in Americans aged 65 and older. AD-related mortality has been on a steady upward trajectory (2). Despite significant advances in unraveling the pathogenesis of AD, which is characterized by two hallmark lesions in the central nervous system (CNS) - amyloid plaques and neurofibrillary tangles (NFTs) - the intricate mechanisms governing their formation remain incompletely understood. Amyloid plaques are primarily composed of extracellular deposits of amyloid-β (Aβ) peptides. These peptides are generated by sequential proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. NFTs appear as intraneuronal filamentous aggregates formed by hyperphosphorylated tau protein, a microtubule-associated protein (3). The development of both amyloid plaques and NFTs is accompanied by the progressive loss of neuronal synapses and pyramidal neurons, thereby driving neurodegeneration in AD.

Clinically, the initial symptoms of AD typically make their appearance roughly 20 years after the onset of structural brain changes commence (4, 5). As the disease progresses, it leads to a reduction in cerebral glucose metabolism and causes brain atrophy (5, 6), progressively impairing cognitive functions, including verbal communication and environmental responsiveness, primarily in regions governing cognition, memory, and language. AD risk factors include non-modifiable elements (genetics, sex, age) and modifiable factors (education, physical activity, sleep patterns, diet, smoking/alcohol habits) (6). Over the course of more than three decades of clinical evaluation, only five AD therapeutics have demonstrated conclusive efficacy to gain regulatory approval. Currently approved agents primarily ameliorate cholinergic deficits in the AD brain by inhibiting acetylcholinesterase (i.e., cholinesterase inhibitors). Since memantine received United States Food and Drug Administration (FDA) approval in 2003 as an N-methyl-D-aspartate (NMDA) receptor antagonist, no new disease-modifying therapies have emerged, and subsequent trials have consistently failed to meet their primary endpoints (7). In the past five years, Aβ-targeting monoclonal antibodies, such as lecanemab, aducanumab, and donanemab, have received FDA approval for the treatment of AD. However, these drugs still face challenges

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regarding safety, efficacy, and clinical application. Against this backdrop, early-phase drug development remains critical. Currently, the ClinicalTrials.gov database documents more than 300 small-molecule agents in clinical trials for AD, targeting diverse pathological mechanisms, such as APP receptors, 5-hydroxytryptamine (5-HT) receptors, tau protein, acetylcholinesterase (AChE), and Aβ peptides. This target diversity underscores the multifaceted therapeutic strategies under investigation during early clinical development, reflecting efforts to address AD's complex pathogenesis through varied mechanistic angles.

This review primarily focuses on the clinical progress of Aβ-targeting drugs for AD. Phase I trials represent the first step in evaluating new therapeutic agents in humans. Within this scope, we will examine the various classes of Aβ-targeting drugs that have entered phase I trials, mainly including γ-secretase inhibitors and β-secretase (BACE1) inhibitors. Ultimately, this review aims to contribute to the advancement of Aβ-targeting therapies for AD by synthesizing the lessons learned from earlier clinical trials and providing direction for future research and development efforts.

Aβ-targeting therapy: Mechanistic basis and clinical significance

The mechanistic rationale for Aβ-targeting therapies stems from Aβ's central role as a pathogenic driver in AD. Aβ peptides—particularly the aggregation-prone $Aβ_{42}$ isoform—function as key initiators of the neurodegenerative cascade. Biosynthetically, Aβ is generated through sequential proteolytic cleavage of the APP. While α-secretase-mediated cleavage directs APP toward a non-amyloidogenic pathway producing soluble APPα fragments, sequential cleavage by BACE1 and γ-secretase generates Aβ peptides. These peptides, especially $Aβ_{42}$, exhibit a high propensity for self-assembly, progressing from soluble oligomers to insoluble fibrils that accumulate as extracellular amyloid plaques.

These Aβ species exert multifaceted neurotoxic effects (Figure 1). Aβ oligomers, recognized as the most pathologically active species, directly disrupt synaptic plasticity and neurotransmission—critical early events in cognitive decline. They activate microglia and astrocytes, triggering a proinflammatory response characterized by the release of cytokines and reactive oxygen species, which propagate oxidative stress and neuronal damage. Additionally, Aβ oligomers promote tau hyperphosphorylation via activation of kinases such as glycogen synthase kinase-3β (GSK-3β), facilitating the formation of NFTs and exacerbating neuronal dysfunction. Beyond tau-mediated effects, Aβ oligomers enhance acetylcholinesterase activity, reducing synaptic acetylcholine levels and worsening cholinergic neurotransmission deficits—a hallmark of AD-related cognitive impairment. They also selectively damage 5-HT neurons, inducing serotonin deficiency and further dysregulating serotonergic signaling pathways involved in cognition. Collectively, these interconnected mechanisms—Aβ aggregation, neuroinflammation, oxidative stress, synaptic dysfunction, tau pathology, and neurotransmitter imbalance—drive progressive neuronal loss and cognitive deterioration.

APP is cleaved by α-secretase to generate secreted sAPPα and membrane-bound C83 (via the nonamyloidogenic pathway), or by β-secretase to produce sAPPβ and membrane-bound C99 (via the amyloidogenic pathway). Subsequent processing of C99 by γ-secretase leads to extracellular secretion of Aβ, which is associated with multiple downstream pathological effects. Aβ oligomers (AβOs) induce Tau hyperphosphorylation via activating GSK-3β to form neurofibrillary tangles, increasing AChE activity to reduce acetylcholine levels, and damaging 5-HT neurons, leading to serotonin reduction, ultimately causing cognitive impairment.

Clinically, Aβ-targeting strategies hold transformative potential for AD management. Aβ deposition begins decades before the onset of clinical symptoms, establishing a critical window for early intervention to halt or delay disease progression—distinct from current symptomatic therapies, which merely alleviate manifestations without modifying underlying pathology. Preclinical studies support this approach: reducing Aβ production, inhibiting aggregation, or enhancing clearance in animal models mitigates neurodegeneration and preserves cognitive function. Furthermore, validated Aβ biomarkers—including cerebrospinal fluid (CSF) $Aβ_{42/40}$ ratios and positron emission tomography (PET) imaging of amyloid plaques—enable the identification of at-risk individuals during preclinical or prodromal stages, facilitating targeted therapeutic intervention. This capacity for early, biomarker-guided treatment underscores the clinical significance of Aβ-targeting therapies in shifting AD management from reactive symptom control to proactive disease modification.

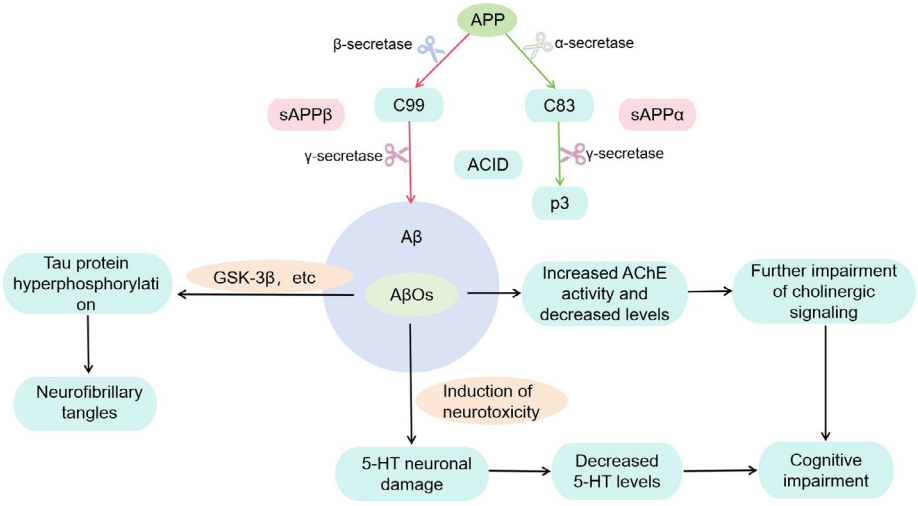

Figure 1. Mechanistic diagram of enhanced Aβ toxicity in AD
AD: Alzheimer's disease

Overview of approved small-molecule drugs for AD

Acetylcholine (ACh), the first identified neurotransmitter, serves as a pivotal signaling molecule in cholinergic neurons spanning the peripheral and CNS (8). Cholinergic neurotransmission is integral to cognitive processes such as attention, memory consolidation, and learning, functions profoundly impaired in AD. The cholinergic hypothesis of AD pathogenesis posits that progressive degeneration of basal forebrain cholinergic neurons, coupled with abnormally elevated AChE activity, leads to depletion of synaptic ACh levels, directly contributing to cognitive decline (9, 10). Beyond its role in ACh hydrolysis, AChE exhibits non-cholinergic pathological functions, including promoting Aβ aggregation and stabilizing NFT-Aβ complexes—processes that exacerbate the core histopathological features of AD (11). Disruption of cortical and hippocampal cholinergic inputs further impairs information processing, attention, and the use of contextual cues, which are critical for sustained cognitive function and behavioral decision-making (12). Pharmacological inhibition of AChE thus represents a rational strategy to enhance cholinergic neurotransmission, with demonstrated efficacy in ameliorating attention, memory, and learning deficits in AD. To date, five small-molecule drugs have received FDA approval for AD treatment, four of which are acetylcholinesterase inhibitors (AChEIs); the fifth, memantine, acts via a distinct mechanism as an NMDA receptor antagonist.

Donepezil, a second-generation, CNS-selective reversible AChEI with minimal peripheral cholinergic side effects, Donepezil (AChE, IC_{50} = 11.6 nM) is the most widely prescribed AD therapeutic. Its high selectivity for brain AChE minimizes gastrointestinal and cardiovascular adverse events, a key advantage over earlier agents. Clinical trials demonstrate superior efficacy in improving cognitive and functional outcomes in mild-to-moderate AD compared with other AChEIs, with sustained benefits observed with long-term use (13). Mechanistically, Donepezil has also been reported to modulate APP processing and reduce Aβ production in preclinical models. Rivastigmine is a pseudo-irreversible AChEI (AChE, IC_{50} = 4.15 μM) with dual inhibitory activity against both AChE and butyrylcholinesterase (BuChE). Rivastigmine exhibits a favorable safety profile in elderly populations. It is approved for mild-to-moderate AD and has demonstrated consistent efficacy in improving cognitive function and delaying functional decline (14). Its ability to inhibit BuChE, an enzyme that compensates for AChE activity in advanced AD, may confer advantages in later-stage disease. Galantamine is a reversible AChEI (AChE, IC_{50} = 500 nM) with a unique dual mechanism: in addition to inhibiting AChE, it acts as a positive allosteric modulator (PAM) of nicotinic acetylcholine receptors (nAChRs). This dual action enhances cholinergic neurotransmission both by increasing ACh availability and potentiating receptor responsiveness (15, 16). Galantamine is approved for mild-to-moderate AD and has shown benefits in maintaining cognitive function in long-term studies, with a safety profile comparable to other AChEIs. Zunveyl (Benzgalantamine) was approved by the FDA in 2024 and is an AChE inhibitor (IC_{50} = 18.6 μM) derived from Galantamine. Preclinical studies indicate enhanced CNS penetration and prolonged half-life compared to its parent compound. While detailed clinical trial data remain emerging, its approval was based on improved tolerability in elderly patients and sustained efficacy in cognitive endpoints, building on the mechanistic foundation of Galantamine (Figure 2) (17).

Memantine (Cell effect, EC_{50} = 2.48 μM), the only non-cholinergic agent approved for AD, acts as a low-affinity, uncompetitive antagonist of NMDA receptors, which are critical for synaptic plasticity and memory formation (Figure 2). In AD, excessive glutamate-mediated NMDA receptor activation leads to excitotoxicity, contributing to neuronal death. Memantine modulates this overactivation by blocking pathological NMDA receptor signaling while preserving physiological synaptic transmission—a mechanism that distinguishes it from non-selective NMDA antagonists. Approved for moderate-to-severe AD, memantine is often used in combination with AChEIs, with clinical trials demonstrating synergistic benefits in slowing cognitive decline and reducing behavioral disturbances (7).

Collectively, approved small-molecule drugs for AD primarily address symptomatic deficits, with limited impact on underlying neurodegeneration. Their efficacy is constrained by the advanced stage of disease at which most patients initiate treatment and by the complex, multifactorial nature of AD pathogenesis. Nonetheless, these agents remain foundational in clinical practice, underscoring the enduring relevance of cholinergic and glutamatergic systems as therapeutic targets and the need for disease-modifying strategies.

Small-molecule drugs targeting Aβ

Aβ accumulation in the brain, particularly in hippocampal and entorhinal regions, is widely regarded as the initiating event in AD. This framework posits that Aβ deposition triggers downstream pathology, including tau hyperphosphorylation, NFT formation, synaptic loss, and cognitive decline. Biomarker data support Aβ's early pathogenic role, with sequential changes including altered

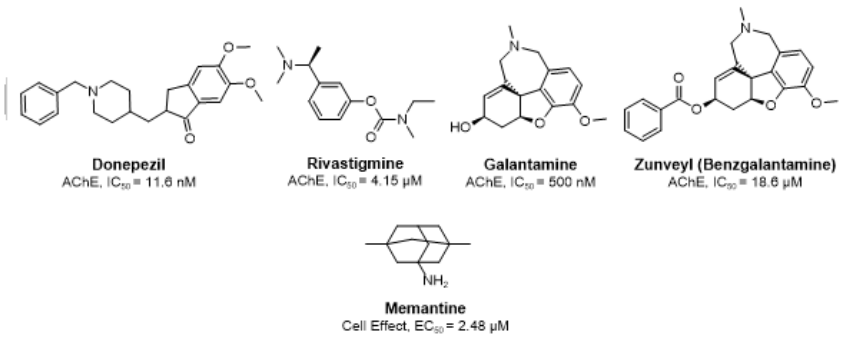

Figure 2. Approved AChEI inhibitors
AChEI: Acetylcholinesterase inhibitors

CSF Aβ levels, cerebral Aβ accumulation (detectable via PET), and subsequent tau elevation, brain atrophy, and cognitive impairment, spanning the preclinical to clinical stages. While ~ 44% of cognitively normal older adults show Aβ deposition, longitudinal studies confirm that such individuals face accelerated cognitive decline and neurodegeneration, with Aβ accumulation preceding clinical onset by 15–20 years.

Over the past two decades, Aβ-targeted therapeutic strategies have focused on two core approaches: (1) inhibiting Aβ biosynthesis (e.g., γ-secretase inhibitors, BACE1 inhibitors) (Table 1) and (2) enhancing Aβ clearance (e.g., anti-Aβ monoclonal antibodies, vaccines). Despite consistent reductions in cerebral Aβ burden across clinical trials, none have demonstrated clinically meaningful improvements in cognitive function or disease progression (18). This persistent biomarker-efficacy disconnect has sparked critical reevaluation of the amyloid cascade hypothesis, prompting exploration of refined models that integrate Aβ with tau pathology, neuroinflammation, and synaptic dysfunction. It also highlights the need to reconsider trial design (e.g., earlier intervention, combination therapies) and biomarkers (e.g., soluble Aβ oligomers vs plaques) to better align with AD's complex pathogenesis.

Drugs targeting γ-secretase

Avagacestat (BMS-708163) is an orally active γ-secretase inhibitor designed to selectively suppress A**β** synthesis while preserving Notch signaling—a critical feature to avoid the off-target toxicities. Early development of avagacestat was guided by systematic structure-activity relationship (SAR) investigations aimed to enhance potency and selectivity. Initial studies revealed that opening the caprolactam ring of the lead compound 1 significantly improved *in vitro* inhibitory potency against Aβ40 (IC_{50} = 4.1 ± 1.2 nM). These analogs were optimized for three key attributes: inhibitory potency, Notch selectivity, and physicochemical

Table 1. Small-molecule drugs targeting γ-secretase and β-secretase (BACE1)

Drug	NCT Number	Sponsor	Target	Study start	Status	Phase	Ref.
Avagacestat (BMS-708163)	NCT00860275	Bristol-Myers Squibb	γ-secretase	2009	Completed	1	19-22
	NCT01454115			2007	Completed	1	
	NCT00726726			2008	Completed	1	
	NCT00901498			2009	Completed	1	
	NCT00810147			2009	Completed	2	
	NCT01079819			2010	Completed	1	
	NCT00979316			2009	Completed	1	
	NCT01057030			2010	Completed	1	
	NCT01042314			2010	Completed	1	
	NCT00890890			2009	Terminated	2	
	NCT01002079			2010	Completed	1	
	NCT01039194			2010	Completed	1	
Semagacestat (LY-450139)	NCT00594568	Eli Lilly and Company	γ-secretase	2008	Completed	3	23-29
	NCT00762411			2008	Completed	3	
	NCT00765115			2006	Completed	1	
	NCT00244322			2005	Completed	2	
	NCT01035138			2009	Completed	3	
GSI-136	NCT00718731	Pfizer	γ-secretase	2008	Completed	1	30
	NCT00719394			2008	Completed	1	
CHF 5074	NCT01421056	Chiessi Farmaceutici S.p.A.	γ-secretase	2009	Completed	1	31-37
	NCT01602393			2012	Completed	2	
	NCT00954252	CERESPIR		2011	Completed	2	
	NCT01303744			2011	Completed	2	
	NCT01258452			2011	Completed	1	
	NCT01723670			2012	Withdrawn	2	
	NCT01203384			2010	Completed	1	
NGP 555	NCT02534480	Neuro Genetic Pharmaceuticals Inc	γ-secretase	2015	Completed	1	38-40
	NCT02537938			2016	Completed	1	
Begacestat (GSI-953)	NCT00479219	Pfizer	γ-secretase	2007	Completed	1	41, 42
	NCT00441987			2007	Completed	1	
	NCT00547560			2007	Completed	1	
	NCT00959881			2009	Completed	1	
	NCT04585347			2015	Completed	1	
ALZ-801	NCT04693520	Alzheon Inc.	BACE1	2020	Active, not recruiting	2	45, 46
	NCT06304883			2024	Active, not recruiting	3	
	NCT04157712			2015	Completed	1	
	NCT04770220			2013	Completed	3	
Elenbecestat (E2609)	NCT01600859	Eisai Inc.	BACE1	2012	Completed	1	63-65
	NCT01716897			2012	Completed	1	
	NCT02322021			2014	Terminated	2	
	NCT02859207			2016	Completed	1	
	NCT02956486			2016	Terminated	3	
	NCT01294540			2010	Completed	1	
Atabecestat (JNJ-54861911)	NCT02406027	Janssen Pharmaceutical K.K.	BACE1	2015	Terminated	2	51-53
	NCT02360657			2015	Completed	1	
	NCT01978548			2013	completed	1	
	NCT02260674			2014	Completed	2	
	NCT01827982			2013	Completed	1	
	NCT02180269			2014	Completed	1	
	NCT01887535			2013	Completed	1	
	NCT02260700			2013	Completed	1	
	NCT02197884			2014	Completed	1	
	NCT02211079			2014	Completed	1	
	NCT03587376			2018	Completed	1	
	NCT02569398			2015	Terminated	1, 3	
LY-2811376	NCT00838084	Eli Lilly and Company	BACE1	2008	Completed	1	44
LY2886721	NCT01561430	Eli Lilly and Company	BACE1	2012	Terminated	1, 2	44, 54
	NCT01807026			2013	Completed	1	
	NCT01133405			2010	Completed	1	
	NCT01227252			2010	Completed	1	

Continued Table 1.

LY3202626	NCT02791191 NCT03367403 NCT02323334	Eli Lilly and Company	BACE1	2016 2017 2014	Terminated Completed Completed	2 2 1	44
Verubecestat (MK-8931)	NCT02910739 NCT01496170 NCT01537757 NCT01953601 NCT01739348	Merck Sharp & Dohme LLC	BACE1	2016 2011 2012 2013 2012	Completed Completed Completed Terminated Terminated	1 1 1 3 2, 3	54-57
Lanabecestat (AZD-3293)	NCT02040987 NCT02126514 NCT01795339 NCT02245737 NCT02972658 NCT02972658	AstraZeneca	BACE1	2014 2014 2013 2014 2017 2016	Completed Completed Completed Terminated Terminated Terminated	1 1 1 2, 3 3 3	58-62
AZD 3839	NCT01348737	AstraZeneca	BACE1	2011	Completed	1	66-70

properties (e.g., solubility, blood-brain barrier penetration) (Figure 3). Notably, stereochemical configuration emerged as a critical factor of activity: (R)-configured amino acid sulfonamide derivatives consistently outperformed their (S)-counterparts in inhibitory efficacy. Further refinement of acyclic carboxamides led to the identification of N-methyl substituted benzamide (compound 2), which exhibited exceptional $A\beta_{40}$ inhibitory activity (IC_{50} = 0.14±0.05 nM) (19). Preclinically, avagacestat demonstrated robust Aβ-lowering activity: *in vitro* assays confirmed potent inhibition of $A\beta_{40}$ (IC_{50} = 0.3 ± 0.15 nM) with about 193-fold selectivity for Aβ production over Notch processing. In animal models, it significantly reduced cerebral Aβ burden without inducing Notch-related toxicities, validating the selectivity achieved through structural optimization (19). In addition, Phase I clinical trials further confirmed target engagement: therapeutic doses led to measurable reductions in plasma $A\beta_{40}$ and $A\beta_{42}$ levels (20, 21). However, despite promising preclinical and phase I data, higher doses were plagued by dose-limiting adverse events—including skin rashes, gastrointestinal disturbances, and occasional worsening of cognitive function—while lower doses failed to translate Aβ reduction into meaningful clinical benefits (22).

Semagacestat (LY-450139), a γ-secretase inhibitor developed by Eli Lilly, was the first to advance to phase III trials, with an IC_{50} of 12.1 nM against $A\beta_{40}$ (Figure 4) (23). Clinical studies have shown that single-dose administration reduced plasma $A\beta_{40}$ by up to 40% in healthy volunteers and AD patients in short-term observations (24, 25). Dose-escalation trials confirmed dose-dependent increases in plasma and CSF drug concentrations, accompanied by proportional plasma Aβ reductions (26). In a phase II trial, 100 mg and 140 mg daily doses achieved 58% and 65% reductions in plasma Aβ40, respectively; the 140 mg regimen was tolerable over 14 weeks but required intensive monitoring (27). Notably, despite significant plasma Aβ lowering (consistent with γ-secretase inhibition), CSF Aβ levels remained largely unchanged. Semagacestat ultimately failed in phase III trials, showing no cognitive improvement and causing severe adverse effects (28). Like avagacestat, it effectively reduced Aβ levels across species but failed to alter disease progression; some patients even experienced accelerated symptom worsening (29). These outcomes highlight the challenges of translating preclinical Aβ-lowering effects into clinical benefit, particularly regarding CNS penetration, off-target toxicity, and disease-stage intervention timing.

GSI-136 [(S)-N-(5-chlorothiophene-2-sulfonyl)-β,β-diethylalaninol] was developed from high-throughput screening of (S)-4-chlorophenylsulfonylisoleucinol analogs. This compound inhibits $A\beta_{40/42}$ production with an EC_{50} of 28 nM; the β,β-diethyl moiety contributes to superior

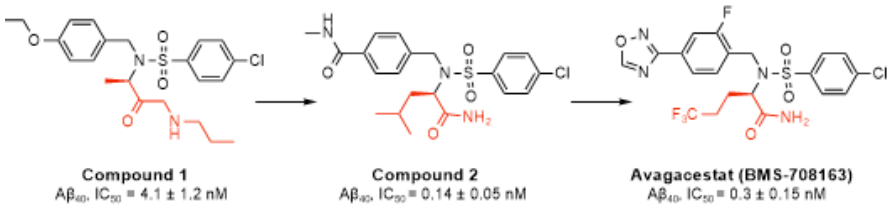

Figure 3. Structural optimization of avagacestat (BMS-708163) derivatives

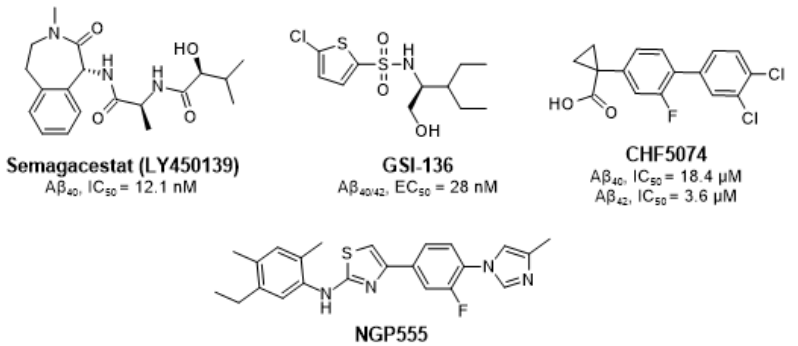

Figure 4. Chemical structures of semagacestat (LY-450139), GSI-136, CHF5074, and NGP555

potency relative to other tested derivatives. Preclinical evaluations confirmed its ability to effectively reduce *in vivo* Aβ production. Notably, selectivity profiling revealed an EC_{50} of 266 nM in Notch-1 cleavage assays, resulting in a 9.5-fold selectivity ratio for β-CTF over Notch-1 cleavage (Figure 4) (30). Currently, GSI-136 is in two phase I clinical trials, with limited publicly available data on human outcomes to date.

CHF5074 is a non-COX-inhibitory derivative of nonsteroidal anti-inflammatory drugs (Figure 4), which functions as a selective γ-secretase modulator (GSM) with preferential $Aβ_{42}$-lowering activity (IC_{50} = 3.6 μM vs 18.4 μM for $Aβ_{40}$). Unlike traditional γ-secretase modulators (GSMs), it avoids APP C-terminal fragment processing and Notch signaling, therefore minimizing off-target toxicities (31-33). Preclinical studies in transgenic AD models demonstrate multifaceted neuroprotection, including dose-dependent reduction of cerebral amyloid burden, reversal of contextual/spatial memory deficits, and restoration of hippocampal long-term potentiation (34-36). Mechanistic investigations reveal unexpected roles in promoting axonal regeneration and astrocytic plasticity via Rho-GTPase pathway modulation (37). Additionally, CHF5074 polarizes microglia toward a neuroprotective phenotype, further mitigating neuroinflammation. In clinical trials involving patients with mild cognitive impairment (MCI), CHF5074 improves performance on validated cognitive assessments, with greater efficacy in APOE4 carriers. These findings underscore its multiple mechanisms of action, including Aβ modulation and broader neuroprotective effects, positioning CHF5074 as a promising candidate for disease-modifying therapy in early AD.

NGP555 is a γ-secretase modulator that has been shown to effectively reduce Aβ42 levels while increasing those of less toxic $Aβ_{38}$ and $Aβ_{37}$ forms (Figure 4). Preclinical studies underscore its multifaceted potential in AD: it regulates cerebral amyloid biomarkers, alleviates core pathological features, and prevents cognitive decline in animal models (38-40). Notably, NGP555 offers additional neuroprotective benefits by enhancing synaptic function, an effect directly tied to its suppression of $Aβ_{42}$ production. Unlike earlier GSMs with broader off-target activity, it modulates γ-secretase without disrupting Notch cleavage or the cleavage of other critical substrates, thereby reducing the risk of toxicity. However, a key translational challenge persists for NGP555 and the broader GSM class: improvements in Aβ biomarkers (such as reduced CSF $Aβ_{42}$ levels and amyloid plaque burden) have not yet been definitively linked to clinically meaningful cognitive protection, nor has an adequate long-term therapeutic window been established. Addressing this gap remains essential to advancing GSMs like NGP555 into clinical practice for AD.

Begacestat (GSI-953) is a selective γ-secretase inhibitor exhibiting anti-$Aβ_{40}$ activity ($Aβ_{40}$, IC_{50} = 15 nM). Phase I trials confirmed its efficacy in reducing plasma amyloid peptide concentrations. Cellular Notch assays demonstrate 15-fold selectivity for inhibiting APP cleavage over Notch processing. High-dose GSI-953 significantly reduced $Aβ_{1-40}$ levels in the brain, CSF, and plasma compartments, whereas low-dose treatment decreased Aβ1-40 levels in the brain and plasma (41). Notably, begacestat ameliorated contextual memory deficits in Tg2576 transgenic AD mice, suggesting therapeutic potential requiring further clinical validation. During lead optimization, SAR studies of lead compounds 3 ($Aβ_{40}$, IC_{50} = 5449 nM) and 4 ($Aβ_{40}$, IC_{50} = 2214 nM) enabled the development of potent γ-secretase inhibitors with minimal Notch-1 interference. Metabolic stabilization was achieved by replacing the metabolically labile methyl moiety in compound 5 ($Aβ_{40}$, IC_{50} = 25 nM) with a trifluoromethyl group, yielding analog 6 ($Aβ_{40}$, IC_{50} = 16 nM) with enhanced metabolic stability and superior in vivo activity. Subsequent side-chain optimization culminated in the discovery of begacestat, a potent and selective γ-secretase inhibitor (Figure 5) (42). This structural evolution enhanced inhibitory potency and metabolic stability while minimizing Notch pathway disruption, offering critical insights for developing safer AD therapeutics.

Drugs targeting BACE1

Since BACE1's discovery in 1999, researchers have pursued highly effective BACE1 inhibitors with optimized CNS activity. Early efforts employed transition-state mimicry and substrate-based design, yielding high-affinity peptidic/peptidomimetic inhibitors. However, excessive topological polar surface area (TPSA) and molecular weight compromised blood-brain barrier penetration, limiting cerebral Aβ reduction after oral administration. This impasse was overcome by guanidine-based inhibitors that exhibited physicochemical properties better suited to CNS therapeutics. Through structure-based drug design and pKa fine-tuning, several clinical candidates emerged, including elenbecestat (E-2609 (1), LY-2811376 (2), LY-2886721 (3), LY3202626 (4), verubecestat (MK-8931 (5), lanabecestat (AZD-3293 (6), and AZD3839 (7)). All BACE1 inhibitor programs were terminated due to inadequate efficacy, retinal/hepatic toxicity, or cognitive worsening (43). Compounds 1 and 3 induced hepatotoxicity via thiazine-derived reactive metabolites, while compound 2 caused retinal toxicity through cathepsin D (CatD) inhibition (44). These failures underscore the critical

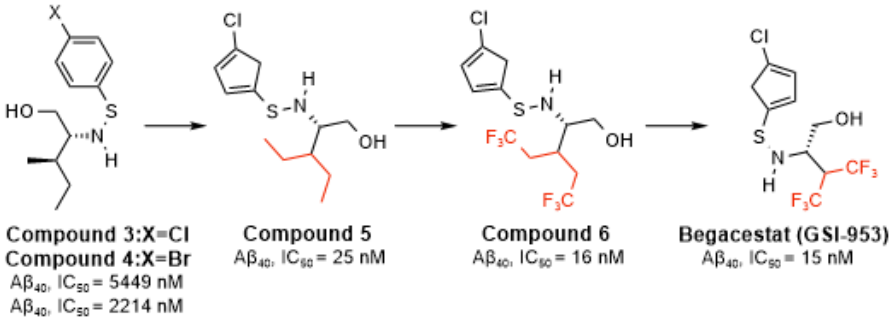

Figure 5. Structural optimization of begacestat (GSI-953)

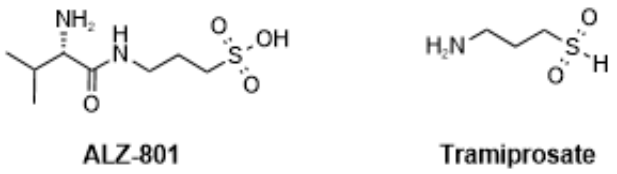

Figure 6. Chemical structures of ALZ-801 and tramiprosate

importance of structural vigilance to minimize reactive metabolite formation and CatD inhibition. Compounds 5-7 failed clinical endpoints despite testing in MCI or mild-to-moderate AD populations. Given that Aβ deposition begins 15 years pre-symptom, intervention may have been too late in MCI patients. Notably, compound 1 accelerated cognitive decline in phase II/III preclinical AD trials – a phenomenon also seen with inhibitors 4 and 5 (44).

These clinical setbacks necessitate deeper investigation of the biological functions of BACE1 and its homolog, BACE2. Crucially, BACE2 is expressed in the CNS and processes multiple neuronal substrates, while existing BACE1 inhibitors may exhibit insufficient selectivity between these paralogs. These insights provide critical guidance for developing safer and more effective BACE-targeted therapeutic strategies.

Agents blocking Aβ production

ALZ-801 is an orally administered tramiprosate prodrug structurally optimized for enhanced efficacy. Functioning as a small-molecule Aβ inhibitor, it exerts therapeutic effects by selectively preventing the misfolding and oligomerization of $Aβ_{42}$ monomers. At therapeutic doses, ALZ-801 blocks amyloid oligomer formation in the brain (45). Unlike tramiprosate, ALZ-801 is hydrolyzed by hepatic and plasma amidases to release the active compound (Figure 6) (46), avoiding gastrointestinal adverse effects (e.g., nausea/vomiting) associated with direct tramiprosate administration.

Clinical studies indicate that while tramiprosate lacked significant efficacy in the overall mild-to-moderate AD population, it demonstrated measurable cognitive improvement specifically in APOE ε4 homozygous patients (47). Subsequent analysis revealed enhanced clinical benefits in APOE ε4 homozygous patients with mild AD (48). Notably, tramiprosate did not induce amyloid-related imaging abnormalities with edema (ARIA-E) in APOE ε4 homozygous patients, a favorable safety attribute attributable to its lack of Aβ fibril interference (49). Building on these findings, a phase III trial of ALZ-801 is being conducted in APOE ε4 homozygous early-stage AD patients to evaluate therapeutic efficacy and safety (50).

Agents reducing Aβ production

Atabecestat (JNJ-54861911), an orally active BACE1 inhibitor, exhibits potent target engagement with IC_{50} values of 9.8 nM against BACE1 and 1.1 nM for Aβ production. Its development stemmed from SAR studies on lead compounds with a 1,3-thiazine scaffold. The initial lead (compound 7) showed moderate potency (BACE1, IC_{50} = 73.3 nM; Aβ, IC_{50} = 2.63 nM) and minimal cellular toxicity (Figure 7) (51). Further structural optimization focused on enhancing potency and selectivity: incorporation of an amide linker yielded compound 8, with improved BACE1 inhibitory activity (IC_{50} = 6.7 nM) and Aβ-lowering efficacy (IC_{50} = 0.84 nM), alongside superior selectivity over CatD, a critical off-target protease (52). Further refinements targeted pharmacokinetic properties: introducing double bonds or fluorine atoms to the thiazine head group reduced the compound's pKa. The double-bond-modified analog, atabecestat, notably diminished P-glycoprotein (P-gp)-mediated efflux (53), and enhanced intracellular accumulation and therapeutic efficacy in the CNS. Atabecestat demonstrates significant blood-brain barrier penetration and robustly reduces CSF Aβ levels, confirming target engagement in the CNS. However, its clinical development was halted following hepatotoxicity observed in clinical trials, potentially linked to reactive metabolites generated from the thiazine moiety, underscoring the challenge of mitigating metabolic toxicity in heterocyclic BACE1 inhibitor design.

LY2811376, developed by Eli Lilly, is an oral BACE1 inhibitor that reduces Aβ production through specific inhibition of β-secretase activity. During development, aminobenzothiazine (1) and aminothiazine (2) were optimized as lead compounds due to favorable ligand efficiency and physicochemical properties. Notably, LY2811376 exhibited potent BACE1 inhibition (BACE1, IC_{50} = 0.24 μM), representing ~3300-fold and 34,000-fold potency enhancements over compounds 9 (BACE1, IC_{50} = 790 μM) and 10 (BACE1, IC_{50} = 8200 μM), respectively (Figure 8). LY2811376's LEAN value of 0.30 (versus initial 0.26) demonstrates improved drug design parameters, signifying enhanced bioactivity with maintained/reduced lipophilicity. Development was discontinued during phase I trials after long-term rat studies revealed retinal epithelial toxicity. Subsequent investigations attributed this toxicity to off-target protease inhibition (44).

LY2886721 is a highly selective BACE1 inhibitor (BACE1, IC_{50} = 20.3 nM). Its cellular IC_{50} is 10.7 nM, whereas its IC50 against CatD exceeds 300 μM. Preclinical studies confirmed efficacy in reducing amyloid levels in plasma and CSF (44), but clinical development was discontinued

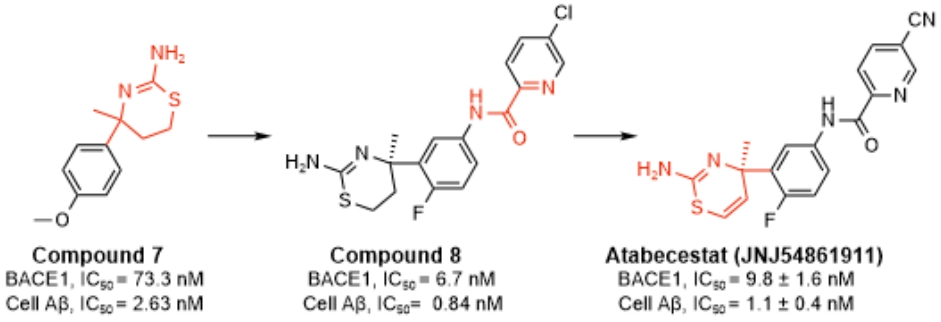

Figure 7. Structural optimization of atabecestat (JNJ-54861911)

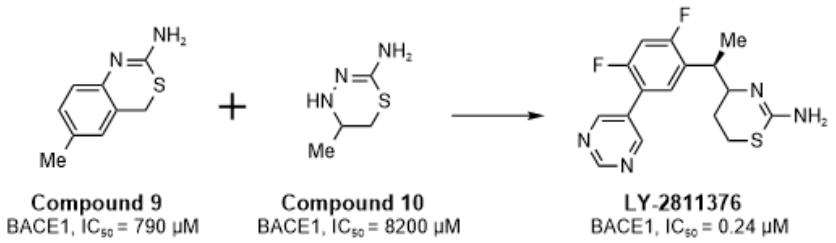

Figure 8. Structural optimization of LY2811376

during phase II trials due to abnormal hepatic enzyme elevation (54). In subsequent optimization, compounds 12, 13, and LY3202626 were synthesized. The IC_{50} values for BACE1 inhibition are 0.603 nM, 0.78 nM, and 0.615 nM, respectively (Figure 9). These derivatives exhibit ≥25-fold greater BACE1 inhibitory activity and improved ligand efficiency compared with LY2886721. While lacking selectivity against BACE2, these compounds exhibit exceptional selectivity for CatD, with ratios ranging from 105,000 to 220,000. Among them, the CatD IC_{50} values for compounds 12, 13, and LY3202626 are 80 μM, 169 μM, and 65 μM, respectively. Compound 12 (Cell, IC_{50} = 0.481 nM) showed comparable activity in BACE1 enzymatic assays and PDAPP neuronal cultures, whereas compounds 13 (Cell, IC_{50} = 0.309 nM) and LY3202626 (Cell, IC_{50} = 0.275 nM) exhibited superior cellular potency (44). In clinical trials, LY3202626 demonstrated good tolerability across various doses and showed effective blood-brain barrier penetration. However, in the phase II clinical trial involving patients with mild AD, although LY3202626 significantly reduced Aβ levels, it did not show clinically significant improvements in cognitive function or neurofibrillary tangle burden. Additionally, the drug reported an increase in treatment-emergent adverse events at certain doses, particularly in the psychiatric disorders system organ class.

Verubecestat (MK8931), a potent BACE1 inhibitor characterized by a diaroylamide-substituted 3-imino-1,2,4-thiadiazinane 1,1-dioxide scaffold, exhibits nanomolar inhibitory activity against $Aβ_{40}$ (IC_{50} = 2.1 nM) (Figure 10). Its high potency arises from the guanidine moiety, which forms robust hydrogen bonds with residues of the catalytic dyad in BACE1's active site. SAR studies systematically optimized fragments derived from lead compounds 14 (BACE1, K_d = 550 μM) and 15 (BACE1, K_d = 15 μM) (54). Preclinically, verubecestat demonstrates efficient blood-brain barrier penetration, with sustained concentrations in rat brain tissue for up to 12 hr (55). However, clinical development was halted due to safety concerns. Long-term trials revealed a significantly higher incidence of neuropsychiatric adverse events (including anxiety, depression, and sleep disturbances) in treated patients compared to controls (56). Merck subsequently terminated its AD development program for verubecestat (57). Verubecestat's failure underscores the importance of

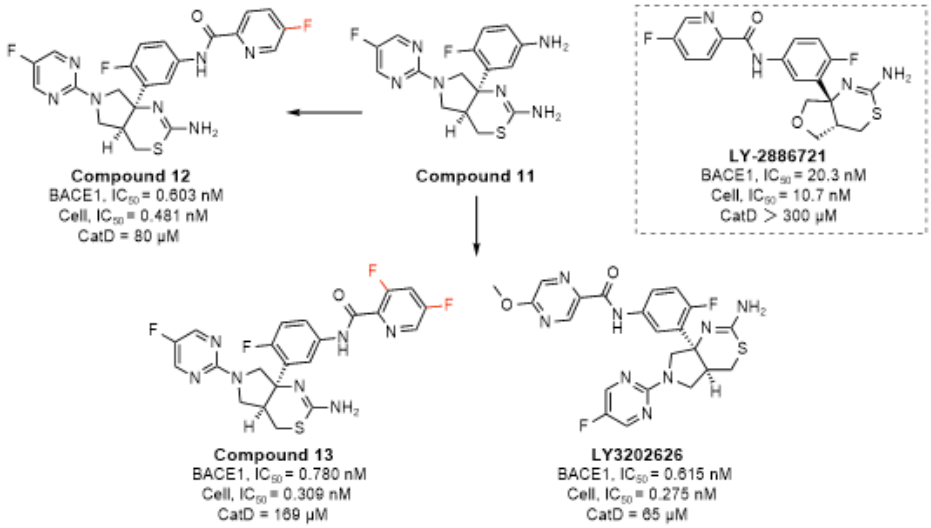

Figure 9. Structural optimization of LY3202626 and LY2886721

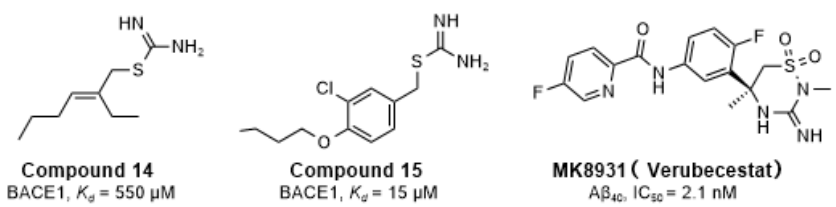

Figure10. Structural optimization of verubecestat (MK8931)

evaluating neuropsychiatric safety profiles early in clinical development, particularly for agents targeting pathways with broad neurobiological roles.

Lanabecestat (AZD3293), a BACE1 inhibitor with a K_i of 0.4 nM for BACE1, exhibits efficient blood-brain barrier penetration but minimal selectivity between BACE1 and BACE2. Preclinical investigations demonstrated robust reductions in $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels across brain tissue, CSF, and plasma in animal models (58, 59). Pharmacodynamic studies further quantified its Aβ-lowering efficacy: at doses of 15 mg and ≥ 50 mg, plasma Aβ levels were reduced by ≥ 64% and ≥ 78%, respectively, with corresponding CSF Aβ reductions of ≥ 51% and ≥ 76% (60). Two pivotal trials co-sponsored by Eli Lilly and AstraZeneca evaluated its clinical potential: AMARANTH (phase II/III; NCT02245737), a 104-week study involving 539 participants, and DAYBREAK-ALZ (phase III; NCT02783573), a 78-week trial with 76 participants. Both trials confirmed good tolerability but revealed no significant slowing of cognitive or functional decline. Additionally, treated patients exhibited higher incidences of neuropsychiatric adverse events, weight loss, and hair pigmentation changes (61). Metabolic profiling identified AZ13569724 as the primary circulating metabolite, with approximately one-tenth the BACE1 inhibitory activity of lanabecestat and steady-state plasma concentrations reaching about 33% of the parent compound (Figure 11) (62).

Elenbecestat (E-2609) is a potent inhibitor of BACE1 with an IC_{50} value of 7 nM (Figure 11). Phase I trial data confirmed dose-dependent reductions in CSF $A\beta_{42}$ levels following single ascending oral doses (5–800 mg), with the 800 mg dose achieving a 92% reduction (63). Notably, since CSF $A\beta_{42}$ concentrations typically correlate inversely with cerebral $A\beta_{42}$ deposition, this marked reduction may reflect enhanced clearance of soluble $A\beta_{42}$ rather than reduced plaque burden, which highlights the complexity of interpreting Aβ biomarker changes in AD. In terms of safety, elenbecestat (administered at 5, 15, or 50 mg/day) demonstrated favorable tolerability, with no serious adverse events, including hepatotoxicity, reported. However, the study was limited by a small sample size (n=43) and a 61% completion rate, with preliminary findings indicating only modest attenuation of cognitive decline in patients with MCI to moderate AD (64). Subsequent phase III trials (MISSION AD1: NCT02956486; MISSION AD2: NCT03036280) enrolled 2,100 patients with mild AD to evaluate the efficacy and safety of once-daily 50 mg elenbecestat. However, the phase III program was discontinued in 2019 based on interim analyses indicating an unfavorable risk-benefit profile. This outcome underscores the persistent challenges in translating preclinical BACE1 inhibitory activity into meaningful clinical benefit for AD, emphasizing the critical need to balance efficacy, safety, and biomarker interpretability in therapeutic development (65).

AZD3839, a highly selective and orally bioavailable small-molecule BACE1 inhibitor, was developed through fragment-based lead generation to leverage an aminoisoindole scaffold to optimize target engagement and pharmacokinetic properties (66). The discovery process began with compound 16 with an amidine-binding motif as the initial template for optimization (67). Subsequent scaffold-hopping strategies led to the identification of bicyclic aminoimidazole 17 (BACE1, K_i = 23 nM) (68). Early derivatives, such as compound 18, demonstrated good BACE1 inhibitory activity (BACE1, K_i = 20 nM) but suffered from poor membrane permeability and insufficient brain exposure. To address these limitations, researchers pursued fluoro-substituted isoindole derivatives to shield the amidine functionality. These modifications significantly enhanced the membrane permeability of compound 19 (BACE1, K_i = 93 nM) and reduced its P-gp efflux ratio to 10, compared to >35 for compound 18. Further optimization focused on fine-tuning lipophilicity and molecular weight by replacing the CF_3 group in compound 20 (BACE1, K_i = 93 nM) with CHF_2 restored BACE1 potency (BACE1, K_i = 26 nM) while optimizing pharmacokinetic properties, culminating in the development of AZD3839 (Figure 12) (66). Preclinical evaluations across cellular systems and animal models confirmed the compound's ability to reduce Aβ peptides (66, 69). In clinical trials, AZD3839 demonstrated dose- and concentration-dependent reductions in plasma $A\beta_{40}$ and $A\beta_{42}$ levels, accompanied by favorable safety and tolerability profiles across all tested doses (70). However, AZD3839 encountered challenges in clinical trials. The compound was found to induce a dose-dependent prolongation of the QT interval, a phenomenon that could potentially lead to cardiac repolarization issues. Despite its favorable efficacy in reducing Aβ levels, this overlap with QT interval prolongation resulted in the termination of its further clinical development.

Conclusion

AD remains a significant challenge in neurodegenerative research, characterized by its complex pathogenesis and the limited efficacy of current therapeutic strategies. This review underscores the central role of Aβ in AD initiation and progression, while critically evaluating the clinical progress of small-molecule therapies targeting Aβ, particularly γ-secretase and β-secretase inhibitors.

Currently approved drugs, such as NMDA receptor

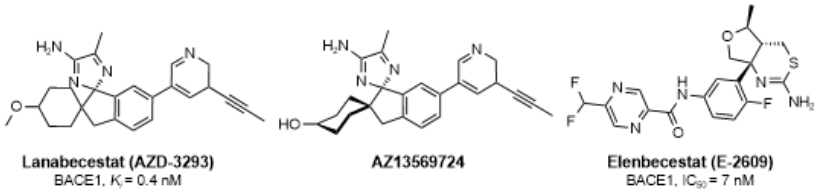

Figure 11. Chemical structures of lanabecestat (AZD3293), AZ13569724, and elenbecestat (E-2609)

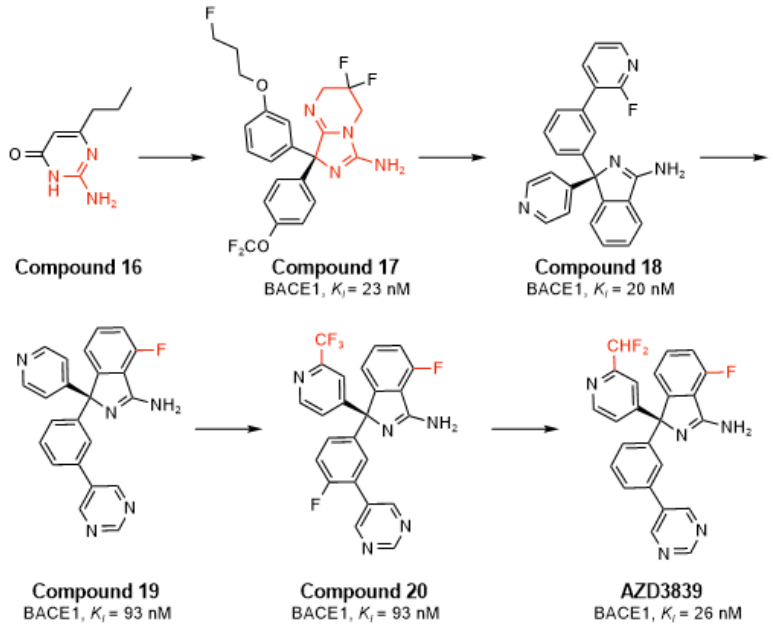

Figure 12. Structural optimization of AZD3839

antagonists and acetylcholinesterase inhibitors, only ease symptoms. They don't stop the underlying neurodegeneration (71). In recent years, the FDA has approved a few Aβ-targeting monoclonal antibodies, including lecanemab, aducanumab, and donanemab. These therapeutic agents exert neuroprotective effects by clearing Aβ plaques and have been demonstrated to modestly slow the clinical progression of early-stage AD. But they only help early-stage patients, like those with MCI or mild dementia. They don't help people with moderate or severe AD. Furthermore, they need intravenous administration and carry adverse effect risks. These are extra hurdles for clinical use (72,73). These downsides underscore the need for disease-modifying therapies, and Aβ-targeted strategies rooted in the amyloid cascade hypothesis hold promise here —Aβ deposits early, decades before clinical symptoms emerge, offering a window for proactive intervention. Preclinical studies consistently demonstrate that reducing Aβ production, inhibiting aggregation, or enhancing clearance mitigates neurodegeneration and preserves cognitive function in animal models, reinforcing the rationale for these approaches (74).

However, clinical translation has been hindered by significant challenges. γ-secretase inhibitors, including avagacestat and semagacestat, effectively reduce Aβ levels but suffer from off-target toxicities (e.g., Notch signaling disruption) and a failure to translate biomarker reductions into meaningful cognitive benefits. Similarly, BACE1 inhibitors such as elenbecestat, verubecestat, and lanabecestat show potent Aβ-lowering activity in preclinical and clinical trials but have been terminated due to safety concerns (hepatotoxicity, neuropsychiatric adverse events) or inability to slow cognitive decline. These setbacks underscore the complexity of AD pathogenesis, which involves hyperphosphorylation, neuroinflammation, synaptic dysfunction, and neurotransmitter imbalance, beyond Aβ alone (75).

Moving forward, advancing Aβ-targeted therapies requires a multifaceted approach. First, refining drug design to enhance selectivity and reduce off-target effects is critical. Second, optimizing clinical trial design, including earlier intervention in preclinical or prodromal stages, where Aβ deposition is detectable but cognitive decline is minimal, which may better align with the timeline of pathological progression. Third, integrating Aβ-targeted strategies with therapies addressing tau pathology, neuroinflammation, or synaptic dysfunction could synergistically modify disease course, given AD's multifactorial nature.

In summary, while Aβ remains a validated therapeutic target, its clinical translation demands a deeper understanding of AD's interconnected pathology and innovative strategies to bridge the gap between biomarker changes and clinical efficacy. With continued advances in drug design, biomarker validation, and trial methodology, Aβ-targeted therapies may yet fulfill their potential as disease-modifying treatments for AD.

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Authors' Contributions

M H performed data processing and collection, drafted the manuscript, and prepared visualizations; Z L provided final approval of the version to be published, provided supervision, and acquired funding; T S, B X, J Z, S L, and J Y performed data processing and collection and provided critical revision or editing of the article; J M provided supervision and funding acquisition; P W provided final approval of the version to be published, provided supervision, and acquired funding.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Declaration

During the preparation of this work, the authors used AI-assisted technologies (DeepSeek and Kimi) to rephrase, reduce plagiarism, and improve language and grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

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