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Sulfur-containing therapeutics in the treatment of Alzheimer's disease

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Abstract

Sulfur is widely existent in natural products and synthetic organic compounds as organosulfur, which are often associated with a multitude of biological activities. OBenzothiazole, in which benzene ring is fused to the 4,5-positions of the thiazolerganosulfur compounds continue to garner increasing amounts of attention in the field of medicinal chemistry, especially in the development of therapeutic agents for Alzheimer's disease (AD). AD is a fatal neurodegenerative disease and the primary cause of age-related dementia posing severe societal and economic burdens. Unfortunately, there is no cure for AD. A lot of research has been conducted on sulfur-containing compounds in the context of AD due to their innate antioxidant potential and some are currently being evaluated in clinical trials. In this review, we have described emerging trends in the field, particularly the concept of multi-targeting and formulation of disease-modifying strategies. SAR, pharmacological targets, in vitro/vivo ADMET, efficacy in AD animal models, and applications in clinical trials of such sulfur compounds have also been discussed. This article provides a comprehensive review of organosulfur-based AD therapeutic agents and provides insights into their future development.

Graphical Abstract

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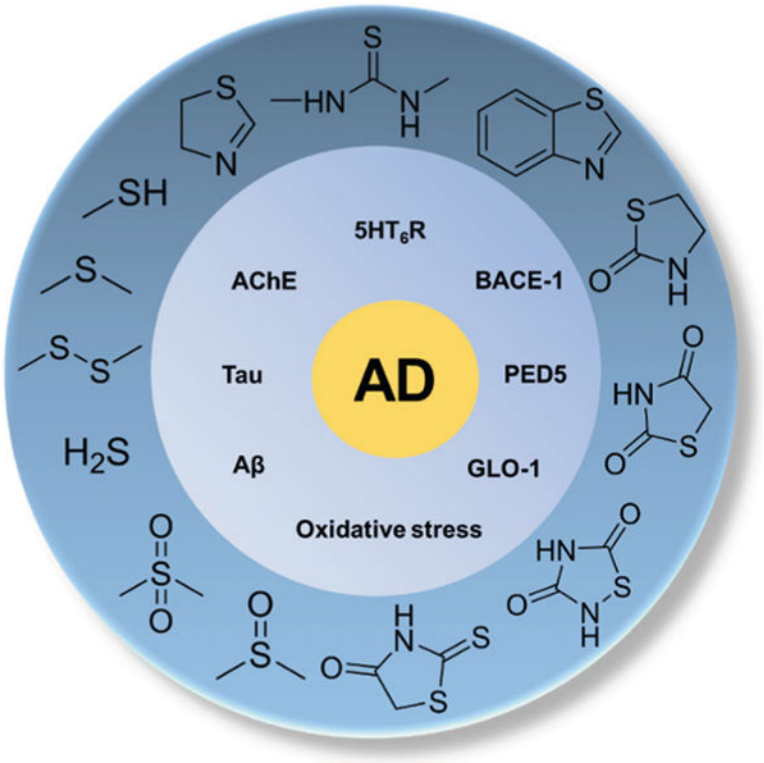
Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.



Keywords

AD; SAR; ADMET; Clinical trials

Introduction

Sulfur-containing compounds [1] are often associated with foul odors but are widely used in various biological applications. The oxidation state of sulfur can range from −2 to +6, so sulfur-containing compounds exist in different forms [2]. Common classes of such compounds include chemical element sulfur, sulfides, thiols, disulfides, thioesters, thioketones, thioureas, sulfur-containing heterocyclic compounds with −2 oxidation state; sulfoxides and thiocarboxylic acids with +4 oxidation state; while sulfones and sulfonamides with +6 oxidation state. Sulfur is one of the core chemical elements and an elemental nutrient needed for biochemical functions in all living organisms [3]. For example, several amino acids (cysteine, cystine, and methionine) and vitamins (biotin and thiamine) are organosulfur compounds. Many bioactive sulfur-containing compounds, such as glutathione, hydrogen sulfide, and taurine also play an important role in the living organisms by maintaining cellular redox homeostasis. In addition, organosulfur compounds are also used in pharmaceuticals, dyes, and agrochemicals. For example, antibacterial sulfonamides [4] is one such well-known sulfur-containing class of drugs. Most β-lactam antibiotics [5], including penicillin, cephalosporin, and monobactams also contain sulfur.

Alzheimer's disease (AD) is a chronic, fatal neurodegenerative disease that usually manifests slowly and gradually worsens over time [6]. It is prominently characterized by progressive cognitive dysfunction, which includes difficulty remembering, problems with language, disorientation, mood swings, loss of motivation, and other behavioral issues [7]. As dementia worsens, patients often withdraw from family and society. This cognitive dysfunction further leads to biological functions loss and, ultimately to death. More than 5 million Americans were living with AD in 2010 and the number is increasing annually, expected to reach about 15 million by 2050 [8]. It is estimated that the cost of medical care for AD will increase to $1.1 trillion by 2050 [9]. Unfortunately, despite numerous efforts and investments, there is currently no cure for this fatal disease. This is because the pathophysiology and precise molecular mechanisms of AD are still poorly understood, while advanced age is the main risk factor [10]. It is reported that 1 in 10 adults over the age of 65 has Alzheimer's dementia. The only clinically approved drugs for AD focus on increasing neurotransmission to improve cognition, but unfortunately, these drugs only offer temporary relief from worsening dementia symptoms [11].

Over the past two decades, efforts in understanding the biology and clinical pathophysiology and therapeutic drug discovery have yielded some important insights to AD therapy [12]. AD has a complex pathophysiology that includes aggregation of pathological proteins, impaired neurotransmission, increased oxidative stress, or microglia-mediated neuroinflammation. Hundreds of candidate drugs targeting these processes are advanced to clinical trials, which include γ-secretase inhibitors, β-secretase (BACE) inhibitors, immunotherapy for amyloid beta (Aβ) clearance, Tau aggregation inhibitors, serotonin 5-hydroxytryptamine receptor 6 ($5HT_6R$) antagonists, acetylcholinesterase (AChE) inhibitors, but with limited success [11]. Many sulfur compounds are part of these promising drug candidates targeting key enzymes involved in AD pathophysiology and have demonstrated neuroprotection in preclinical models of AD. The advantages of sulfur are multipronged. First, the importance of thiol redox homeostasis is well documented in AD onset and progression [13]. Sulfur-containing endogenous compounds such as glutathione (GSH) are considered a biomarker for AD [14]. Many thiol-containing compounds display antioxidant activity to balance the increased oxidative stress observed in AD pathology [15]. Furthermore, sulfur is incorporated as a crucial pharmacophore in the design of bioactive compounds targeted toward enzymes involved in AD pathogenesis. For example, sulfonamide is the key pharmacophore utilized in the development of BACE-1 inhibitors and $5HT_6R$ antagonists [16]. Additionally, sulfur is a bioisostere of oxygen and widely used to improve lipophilicity and metabolism stability [17].

In this review, we focus mainly on sulfur-containing compounds as therapy options for AD. The design and structure-activity relationships, pharmacological targets, in vitro/vivo ADMET, efficacy in AD animal models, and applications in clinical trials of such sulfur compounds are described based on the chemical class. Table 1 comprehensively summarizes all the sulfur therapeutics subjected to clinical testing.

Organic thiols, disulfides, and prodrug compounds

Design rationale and pharmacological target

Cysteine based thiols: Cellular availability of Cysteine (Cys, **1**) is the rate-limiting factor for the synthesis of antioxidant GSH and maintenance of cellular redox potential. Supplementation with Cys and its prodrugs have been used as GSH precursors. Among Cys based prodrugs, N-acetyl cysteine (NAC, **2**) has been extensively studied. NAC reduces reactive oxygen species (ROS) and apoptosis by replenishing and maintaining GSH stores in the brain [18]. NAC also has been shown to suppress the inflammatory nuclear factor-kappa B, which is involved in AD pathogenesis [19]. NAC activated the extracellular signal-regulated kinase (Ras-ERK) pathway in PC12 cells preventing neuronal death by a non-antioxidant mechanisms [20]. Additionally, NAC enabled neurogenesis and differentiation of neuronal stem cells [21]. However, poor bioavailability and low membrane penetration has limited its clinical application [22, 23]. N-acetylcysteine amide (NACA) and N-Acetylcysteine ethyl ester (NACET) derivatives (**3**) of NAC were synthesized to increase GSH content and have been reported to possess antioxidant and anti-inflammatory activities [24] (Fig. 1).

Similar to GSH, Cys is easily oxidized. Hence strategies to mask thiol in the form of thiazolidine-4-carboxylic acids were applied to prodrug design. Among these, L-thiazolidine-4-carboxylic acid (TCA, **4**) [25, 26] and L-2-oxothiazolidine-4-carboxylate (OTC, **5**) [27, 28] are the most studied prodrugs. These prodrugs are hydrolyzed by enzyme prolinase to release Cys and show improved half-life ($t_{1/2}$) in plasma. Naturally occurring thiol protected cysteine analogs from Allium plants such as S-allyl cysteine (SAC), S-methyl cysteine (SMC), S-ethyl cysteine (SEC), and S-propyl cysteine (SPC) [29] also enhanced plasma and tissue GSH levels (Fig. 1).

GSH based thiols: GSH is the most abundant and important endogenous antioxidant that counteracts ROS and nucleophilic compounds such as 4-hydroxynonenal (HNE) and acrolein in the brain. Mandal et al. reported reduced GSH levels in the hippocampi and frontal cortices of mild cognitive impairment (MCI) and AD patients, which is directly associated with cognitive function [30]. Hence, maintaining or restoring cellular GSH levels and inhibiting its degradation is considered a beneficial pharmacological strategy for preventing or halting AD progression.

GSH (**6**) can react with ROS or reduce lipid peroxides through glutathione peroxidase (GPx) to protect cells against oxidative stress injury. GSH can be regenerated from glutathione disulfide (GSSG) by glutathione reductase (GR) using equivalents of NADPH. Under normal physiological conditions, cellular biosynthesis of GSH is homeostatically controlled through ATP-dependent steps. Enzymes such as γ-glutamylcysteine synthetase (GCS) and GSH synthetase are important for the synthesis of GSH, deficiency of which has been implicated in AD progression. In extracellular spaces of cells, GSH is degraded exclusively by expressed γ-glutamyl transpeptidase (GGT) to form cysteinylglycine and glutamate. Levels of cysteine/cystine and GSH/glutathione disulfide (GSSG) redox pairs are maintained by NADPH dependent enzyme systems such as thioredoxin and glutaredoxin, GR, and GPx (Fig. 2).

The challenge in the application of GSH (**6**) is its poor stability and bioavailability in biological systems. GSH has a short $t_{1/2}$ in plasma (<3 min) and poor cell membrane permeability, administration of high doses is necessary to reach therapeutic concentrations. Researchers have synthesized several carboxylic ester and thiol modified GSH prodrugs (**7–8**) (monomethyl, monoethyl (GEE), diethyl, isopropyl esters, S-acetyl GSH), which effectively increased intracellular GSH levels in human lymphoid cells, fibroblasts, endothelial cells, and rat hepatocytes in vitro compared to native GSH [31–34]. GEE significantly elevated intracellular GSH levels in the neuronal cells and provided neuroprotection against H_2O_2-induced oxidative stress [35]. L-cysteine-glutathione (L-CySSG, **8**), linked L-Cys with thiol of GSH through a disulfide link [36]. The disulfide bond was reduced by GR to release a molecule of GSH and Cys. L-CySSG improved the stability of GSH toward oxidation and the released Cys enabled the synthesis of GSH (Fig. 2).

We and others have an active interest in the role of the glyoxalase (GLO) enzyme system in the progression of AD and developing substrates of GLO-1 to restore its function. GLO is involved in detoxification of toxic dicarbonyls such as methylglyoxal using GSH as a cofactor and has implications in glycation induced toxicity of $A\beta_{1–42}$. Supplementation of GSH, however, is unable to restore GLO-1 activity due to its hydrolysis by ubiquitous GGT resulting in loss of activity. We have synthesized a GLO-1 substrate, Ψ-GSH (**9**) [37], by replacing the γ- glutamyl-cysteinyl amide bond in GSH with a ureide linkage. Ψ-GSH was found to be stable against GGT-mediated breakdown and was able to substitute for GSH in enzymatic reactions, a property crucial for preventing and halting damage caused by AD pathology (Fig. 2).

Alpha-lipoic acid: The anti-AD effect of lipoic acid (ALA, **10**) is attributed to multitude of properties. ALA activated AChE and increased glucose uptake, thus providing more acetyl-CoA to generate acetylcholine (ACh). ACh is a neurotransmitter used by all cholinergic neurons and is transported into synaptic vesicles by vesicular ACh transporter [38, 39], where it plays an important role in the peripheral and central nervous systems. A variety of studies have shown that ACh modulates the memory process, such as acquisition, encoding, consolidation, reconsolidation, extinction, and retrieval [40] (Fig. 3).

ALA increased intracellular GSH levels by chelating redox-active transition metals, thus inhibiting the formation of hydroxyl radicals and Aβ aggregation. Levels of several antioxidant enzymes including catalase, GR, glutathione-S-transferase (GST), NADPH, and quinone oxidoreductase-1 (NQO1) were enhanced by ALA [41]. Also, ALA prevented the induction of iNOS, inhibited TNFα-induced activation of NF-κB [42], levels of which are increased in AD. Furthermore, ALA reduced the levels of lipid peroxidation products responsible for mitochondrial and cell signaling disruption by GPx-mediated inactivation of hydrogen peroxide and by scavenging generated HNE and acrolein [43] (Fig. 3).

The chemical activity of ALA is mainly due to the dithionate ring, whose sulfur atoms confer high electron density to ALA making it an efficient antioxidant. The long carbon chain and ring system make it more lipophilic than other natural antioxidants. ALA could easily cross the blood–brain barrier (BBB) and keep a uniform uptake profile throughout the central nervous system (CNS), which is beneficial against AD. Modifications of ALA

mainly focused on linking the free carboxylic acid to other AD therapeutic agents. A hybrid of ALA with a derivative of tacrine, an AChE inhibitor (AChEI) approved for AD treatment, was synthesized [44]. The ALA-tacrine hybrid (**11**) could bind to both, catalytic and peripheral sites of AChE (IC_{50} = 0.25 nM), and reduced Aβ aggregation (IC_{50} = 45 μM). Further studies found that (R)-LA-taurine enantiomer was twice as potent as (S)-enantiomer (IC_{50} = 0.23 nM vs. 0.47 nM, respectively) as an AChEI, which is consistent with better inhibitory potential of R-LA isomer than L-LA [45]. In other research, a melatonin-ALA-tacrine hybrid was synthesized and tested (**12**) [46]. The length of the linker between the ALA and tacrine was vital for its potency. The analog with a 6-carbon linker was the most active with IC_{50} of 1.25 nM for butyrylcholinesterase (BChE), IC_{50} of 3.62 nM for AChE and exhibited antioxidant potential. A similar design rationale was adapted for synthesis of ALA hybrid with benzoquinone moiety of memoquin by using a polyamine linker (**13**), which showed both AChE and BChE inhibitory activities [47]. Compared to memoquin, all the hybrid molecules with different linker lengths showed moderate inhibition of AChE, however, were more potent inhibitors of Aβ aggregation. The hybrid with the shortest linker (n = 1, m = 1) was the most active inhibitor of these enzymes (Fig. 3).

In vitro/in vivo ADMET

Cys based thiols: NAC (**2**) can enter cells without active transport and is rapidly hydrolyzed to yield Cys. However, because NAC is negatively charged at physiological pH, it displays poor membrane permeability, and bioavailability [22]. Low bioavailability of NAC is one of the major limitations for its application in oxidative stress-related diseases such as AD. The C_{max} and $t_{1/2}$ were 554 mg/L and 5.7 h, respectively, after intravenous administration in patients at 150 mg/kg [48]. NAC has a relatively small volume of distribution (536 ml/kg). Oral NAC is rapidly absorbed, but its bioavailability is low (9.1%) because of significant first-pass metabolism [49]. The T_{max} of NAC was 1.4 ± 0.7 h and the mean elimination $t_{1/2}$ was 2.5 ± 0.6 h after a single daily dose [50]. Oral NAC causes nausea, vomiting, diarrhea, flatus, and gastroesophageal reflux, while intravenously NAC may cause anaphylactic reactions [51].

The NAC esters were hydrolyzed within 1 h in plasma to NAC, whereas thiol protected prodrugs offered extended stability ($t_{1/2}$ of 18 h for rats and over 100 h for humans) [52]. Oral NAC and NACET administered at equivalent dosages reached comparable C_{max} (69 ± 10 μM vs. 96 ± 15 μM) but different T_{max} (10 min vs. 120 min) in rats [53]. Intravenous administration of both compounds yielded relatively low concentrations of NACET in plasma (C_{max} = 75 ± 12 μM, $t_{1/2}$ = 0.36 ± 0.06 h), while NAC reached high plasma concentrations (C_{max} = 1250 ± 220 μM) with longer half-life ($t_{1/2}$ = 4.35 ± 0.57 h). These studies demonstrated that oral bioavailability of NACET is 58.5 ± 8.8%, while that of NAC is below 4.8 ± 1.2% [52–54].

GSH based thiols: GSH (**6**) has a very short half-life ($t_{1/2}$ = 10 min) and a high dose is needed to maintain its therapeutic concentrations[55]. Moreover, GSH synthesis is hampered in AD brain. Therefore, GSH analogs and prodrugs could potentially substitute for GSH while possessing a favorable pharmacokinetics (PK) profile. Intraduodenal administration of GEE at 0.5 and 5 mmol/kg dose in rats resulted in a C_{max} of 3.6 ± 0.9 and 46.3 ± 3.5

nmol/mL, with a corresponding T_{max} of about 45 min. However, no significant increase in GSH and Cys plasma levels were observed after administration of 0.15 and 0.5 mmol/kg of GSH [56].

Ψ-GSH (**9**), a ureide analog of GSH, is stable toward GGT mediated breakdown [37]. The elimination half-life of ψ-GSH ($t_{1/2}$ = 1.227 h) was 3 times longer than GSH ($t_{1/2}$ = 0.495 h) in mice after i.p. administration in mice (500 mg/kg). The maximum serum concentration of ψ-GSH (C_{max} = 109.7 μg/mL) was much higher than that of GSH (C_{max} = 43.97 μg/mL) and it was able to cross the BBB. This compound was also orally bioavailable [37]. No appreciable toxicity of ψ-GSH was observed at doses up to 2000 mg/kg.

ALA and derivatives: Cell permeability assessment using the CACO-2 transwell model showed that ALA is rapidly traversed through cell monolayer in a pH-dependent manner [57]. PK analysis of oral ALA at 8.25 mg/kg dose displayed serum C_{max} of 16.03 μg/mL (range: 10.6–33.8 mcg/mL) with rapid renal clearance and plasma $t_{1/2}$ of 30 min in mice [58]. There are no literature reports on the maximum allowable exposure limit for ALA in humans. In mice, a LD50 of 400–500 mg/kg has been reported [59]. A dose of 2400 mg/day ALA was used to assess adverse health effects in human clinical trials, however, this study failed to find any adverse effects of the treatment [60].

Efficacy in animal models—Although there are reservations for the use of NAC (**2**) in human AD trials, the administration of NAC showed beneficial effects against oxidative damage in AD murine models [61]. The ability of in vivo NAC to reduce protein carbonyls levels, lipid peroxidation, and protein nitration was demonstrated in the APP (Amyloid Precursor Protein)/Presenilin-1 (PS1) mice. It also enhanced the activities of GPx and GR [62]. NAC-treatment improved cognition reduced neuronal loss, and tau expression in specific regions of the brain in mice [63, 64]. There are reports in the literature on the significant anti-Aβ efficacy of NAC in TgCRND8 transgenic mouse model [65] and alleviation of oxidative damage in ApoE$^{-/-}$ mice [61].

Among various GSH analogs, Ψ-GSH (**9**) was shown to mitigate the buildup of AD indicators in the APP/PS1 mouse model [66]. Aβ deposition and oxidative stress indicators (lipid peroxidation, protein carbonyl, and ROS) were also dramatically reduced in the ψ-GSH-treated group. Transgenic AD mice treated with ψ-GSH showed significant learned behavior and long term cognitive/reference memory improvement. In vivo engagement of GLO-1 enzyme by ψ-GSH was demonstrated by reduction levels of methylglyoxal in brain. Further advancement of this class of compounds is currently underway in our laboratories.

Biological evaluation of ALA (**10**) has been extensively reviewed elsewhere [60, 67, 68]. In relation to AD, the effects of ALA supplements on hippocampus-dependent memory were investigated in aged Tg2576 mice [69]. The study demonstrated significant cognitive benefits of ALA treatment by restoration of spatial learning and memory in the Morris water maze. However, ALA treatment did not have an apparent effect on Aβ plaque burden [69]. In another study, young and aged Tg2576 mice were fed with the R-LA diet for 10 months [70]. The study showed significant improvement in oxidative stress markers, however, no cognitive improvement was noticed in the Y-maze assay [70]. Another study evaluated the

neuroprotective effect of ALA in the P301s tau transgenic mice [71]. ALA treatment significantly improved the spatial memory and cognition capacity of the mice in the Morris water maze and novel object recognition test. The protective effect against tau-induced neurotoxicity and attenuation of cognitive dysfunction were attributed to antioxidant and anti-inflammatory activities of ALA [71].

Clinical trials

Cysteine based thiols: In a placebo-controlled trial (n = 34), 6-month treatment of NAC (**2**) (600 mg) showed improvement in cognitive function. However, NAC was given as a nutraceutical formulation containing other antioxidants including alpha-tocopherol, vitamin B_{12}, and S-adenosylmethionine [72]. Interestingly a similar study in a larger group of AD subjects (n = 106) also showed a significant improvement over the placebo group [73]. A nutraceutical Memory XL supplement containing 600 mg of NAC (2 pills/day for 6 months) was administered to patients (n = 8) with MCI in phase II clinical trial that was terminated due to lack of beneficial outcomes (NCT00903695). In another randomized double-blind phase I clinical trial, 60 participants (NCT03493178) were treated with a combination of NAC and glycine for 12 weeks, and the improvement in cognitive function was measured for another 12 weeks post treatment. The primary outcome of cognitive function was measured using free and cued selective reminding tests as well as delayed recall scores. In addition, blood concentrations of GSH, Cys, and glycine were measured. NAC, given in combination has shown improvements in cognition assessment tests and this benefit declined upon cessation of the supplementation.

GSH based thiols: Dietary nutritional assessment in patients with MCI and AD displayed reduced plasma levels of antioxidant micronutrients like vitamin C, vitamin A, and vitamin E. Besides, GSH levels were found to be depleted in patients compared to healthy subjects [74]. However, studies using oral supplementation of GSH have shown poor results. In a randomized double-blind study involving healthy adult subjects, oral administration of GSH (500 mg, twice daily) did not significantly improve plasma GSH concentration (total, oxidized, or ratio levels) and failed to show a reduction in oxidative stress markers. Another study involving 7 healthy volunteers, showed that even a high dose (3 g, oral administration), failed to improve plasma levels of GSH, Cys, and glutamate after 4 h. This has been mainly attributed to intestinal degradation by trans-peptidases [75, 76]. In contrast, a 6-month randomized double-blind study of GSH supplementation showed increased plasma levels of GSH after oral administration at doses 250 and 1000 mg, and this increase was reversed 1 month after stopping the GSH supplementation [77]. In a randomized crossover study with healthy volunteers, the sublingual form of GSH (450 mg) showed improved bioavailability when compared with oral GSH administration [78].

Lipoic acid: Preclinical studies in animal models have shown that ALA (**10**) can ameliorate cognitive impairment in mild to moderate AD. In a pilot study with nine probable AD patients, treatment for 1 year with 600 mg of ALA stabilized cognitive function as measured by mini-mental state examination (MMSE) and cognitive scale using ADAS-Cog scores [79]. In a slightly larger phase II clinical trial involving 39 patients with MCI, 600 mg of ALA was used alone or in combination with fish-oil containing omega-3 fatty acids. In this

study, oxidative stress as measured by peripheral F2-isoprostane did not show any significant reduction after 12-month treatment. However, MMSE-based cognitive decline showed slower progression in ALA-treated groups compared to placebo [80]. In another double-blind randomized study with 78 subjects with MCI, treatment with 900 mg/day of ALA for 16 weeks in combination with antioxidants like vitamin C, vitamin E did not change the levels of Aβ or tau-phosphorylation. No changes in peripheral F2-isoprostane were observed either. Cognitive decline measurement by MMSE and ADAS-Cog did not show any significant change in the ALA-treated group over placebo [81].

Hydrogen sulfide donors and elemental sulfur

Design Rationale and pharmacological target—For a long time, hydrogen sulfide (H_2S, **14**), like other gas transmitters NO or CO, was considered a poisonous gas with a pungent odor. However, Abe and Kimura found that H_2S selectively improved the N-methyl-D-aspartate receptor (NMDAR) mediated function, and its role in the CNS was discovered [82]. Since then, H_2S is recognized as generated endogenously by living organisms and has an important role in the regulation of physiological and pathological processes [83]. H_2S has been reported to be produced mainly through cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST). Recently, researchers discovered relatively high concentrations of H_2S (47–166 μmol/L) in the brains of mammals [84] and that changes in its levels are relevant to AD pathogenesis [85, 86].

Under physiological conditions, H_2S exists in three forms: the neutral molecular form (H_2S) and two different ionic forms (HS^- and S^{2-}), but the bioactive form among these, is still unknown. Increasing evidence from both in vivo and in vitro studies suggest that H_2S has potential therapeutic value for the treatment of AD [87]. H_2S could protect against AD-related oxidative stress [88], Aβ release [89], apoptosis [90], and inflammation [91]. It has been reported to reduce Aβ plaques by decreasing β-and γ-secretase enzyme expressions, which has a protective effect on neurons of AD mice [92]. H_2S has also shown neuroprotective effects by increasing the levels of Bcl2, an antiapoptotic protein, and by decreasing the expression of apoptotic proteins like Bax [93, 94]. As inflammation plays a key role in neurodegeneration, the anti-inflammatory action of H_2S-releasing agents could play a beneficial role in treating AD

Although H_2S has good solubility in water, it is still very unstable and is easily oxidized in air. Also, as a gas, it is difficult to measure its precise concentration, needs a special equipment for use, and has an unpleasant odor [95]. Hence several different forms of sulfur-containing precursors or prodrugs of H_2S were developed [96, 97].

Elemental sulfur: Biological evaluation of elemental Sulfur (**15**) as a source of H_2S has been a topic of recent investigations. Oral administration of sublimed sulfur formulation was studied as a potential AD therapeutic due to its effect on β-galactosidase mediated autophagy and homocysteine (Hcy)/H_2S signaling pathway [98]. Besides, sulfurous water has been reported to protect peripheral mononuclear blood cells in AD patients by preventing H_2O_2 induced oxidative DNA damage while also preventing homocysteine-induced cytotoxicity [99].

Inorganic sulfide salts: NaSH (16) and Na_2S (**17**) are two widely used inorganic H_2S donors that are reported to show antioxidant effects primarily through modulation of GSH levels in the brain. GSH levels in rat brains measured by HPLC revealed an increased transport of GSH into mitochondria by H_2S-releasing NaSH [88, 100, 101]. Protein expression studies using western blot showed NaSH-induced H_2S release, downregulation of APP, and BACE-1 [102]. Reduced lipopolysaccharide (LPS)-induced inflammation as measured by TNF-α levels, was observed in murine microglial cells upon addition of NaSH [103]. NaSH has also been reported to reduce Aβ-induced pro-inflammatory cytokines like IL-1 and IL-6 [104].

Upon hydrolysis, both compounds generate H_2S quickly in the buffer. Under physiological conditions, HS^-/H_2S ratio is about 3:1 [105]. Compared to H_2S gas, these salts are readily available and do not need special equipment to produce or use. Similar to H_2S, its salts are also unstable, for example, the $t_{1/2}$ of $Na_2S{\bullet}9H_2O$ solutions (10–100 μM concentrations) range from 0.5 min to 5 min [106]. Additionally, the release of H_2S from these salts cannot be controlled, and a sudden spike in H_2S concentration may cause problems such as blood pressure change or imbalance in redox potential [107]. Lastly, all sulfide salts have unpleasant odor, which prevent their use in the clinic.

Allyl sulfide: S-Allylcysteine (SAC, **18**), diallyl disulfide (DADS, **19**), and diallyl trisulfide (DATS, **20**) are the main bioactive components in garlic [108]. SAC showed cytoprotective effects by preventing Aβ-induced cell death in PC12 cells [109]. Thioflavin fluorescence studies and size exclusion HPLC studies showed prevention of Aβ aggregation in vitro by allyl sulfides [110]. S-Propargyl-cysteine (SPRC, **21**) which is a SAC structural analog, can be used to modulate endogenous H_2S levels [111]. One of the benefits of these allyl sulfides compared to the salts is that the release of H_2S can be controlled. It is reported that the release of H_2S from DATS occurs at a steady rate over a prolonged time in relatively low concentrations, while the same concentration of Na_2S causes immediate release of H_2S at 10 times higher concentration than DATS [112] (Fig. 4).

Recently Cheng et al. linked H_2S-releasing allyl sulfide moiety (ACS81, **22**) with tacrine, later being a potent acetylcholinesterase (AChE) inhibitor, to form a tacrine-H_2S donor hybrid (THS, **23**) [93] (Fig. 4).

Lawesson's reagent and analogs: Lawesson's reagent (**24**), which is used as a sulfurization agent in organic synthesis [113], also releases H_2S upon hydrolysis and could be used as an H_2S donor [114, 115]. Compared to inorganic sulfides, the H_2S release rate from Lawesson's regent is slower. However, it is spontaneous enough to cause an immediate H_2S spike and the reagent also suffers from poor water solubility limiting its further applications [116]. Attempts have been made to improve solubility by modifing Lawesson's reagent. GYY4137 (**25**), a water-soluble derivative of Lawesson's reagent, is one such successfully modified H_2S donor [117]. SAR studies have shown that replacement of all sulfur groups of GYY4137 with oxygen eliminated its cytotoxicity against different types of cells. GYY4137 inhibited LPS-induced pro-inflammatory ROS production in human THP-1 and SH-SY5Y cells after 24 h [118]. GYY4137 generated H_2S at a slower rate when compared to parent Lawesson's reagent and the sulfide salts. Further modifications of GYY4137 to tune H_2S

release rate included substitution of the C–P bond with C–O bond, and substitution of morphine with phenylamine (**26**). These analogs stabilized GYY4137 and further decreased the releasing rate of H_2S [119] (Fig. 5).

1,2-dithiole-3-thiones (DTT): 1,2-Dithiole-3-thiones (DTT) has been widely considered as a H_2S donor [120, 121]. Although its H_2S-release mechanism is still not fully understood, some research has indicated hydrolysis of DTT forming H_2S and 1,2-dithiol-3-one [114].

To reduce the side effects of nonsteroidal anti-inflammatory drugs (NSAID), DTT was introduced as a DTT-NSAID hybrid. These compounds reduced the side effects of NSAIDs such as gastrointestinal ulceration and bleeding and enhanced their anti-inflammatory effect [122–124]. Several of these hybrids have been evaluated as AD therapeutics[125]. For such hybrids, phenol was introduced at the 5-position of the heterocycle forming 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione (ADT-OH, **27**), and the –OH group of the phenol was esterified with a carboxylic acid of the NSAIDs. One such example is direct linking of aspirin with ADT-OH (ACS14, **28**). Other modifications included the inclusion of mitochondria-targeted triphenylphosphine (TPP), which modified cellular bioenergetics and demonstrated protection against neuronal damage observed in AD (AP39, **29**) [126]. Although DTT-NSAID hybrids showed promising bioactivities related to H_2S release both in vitro and in vivo, some issues remained that hindered their further development. First, the H_2S release mechanism of DTT was still unclear and high reactivity of DTT was a major concern for undesired side effects. Also, the unpleasant odor of these compounds was also an obstacle for clinical use (Fig. 6).

Organic isothiocyanates: Organic isothiocyanates (ITCs) isolated from some botanical species such as cabbage, broccoli, rocket salad, and cauliflower, have been proposed as a new H_2S-donor family in a recent discovery. ITCs are formed by enzymatic hydrolysis of natural glucosinolates (GLS) by myrosinase [127]. Interestingly, it was found that the ITCs not only share the pharmacological profile of H_2S such as antioxidant [128] and anti-inflammatory [129] properties, but demonstrate impressively overlapping molecular mechanisms. In 2014, it was first discovered that certain natural ITCs in Brassicaceae exhibited H_2S-releasing properties [130]. Follow-up studies indicated the chemotherapeutic potential of various ITCs [131], such as allyl isothiocyanate (AITC, **30**), benzyl isothiocyanate (BITC, **31**), phenethyl isothiocyanate (PEITC, **32**), sulforaphane (SFN, **33**), and erucin (ERN, **34**). It is reported that almost all selected ITCs have Cys-dependent H_2S release mechanism and demonstrate absence or decreased H_2S release [132] without Cys. This is proposed to be due to the nucleophilic reaction of cysteine thiol with isothiocyanates resulting in the formation of dithiocarbamic adducts, followed by subsequent intramolecular cyclization to obtain dihydrothiazole derivatives and H_2S release [133]. The cyclization is the rate-limiting step and the electron-withdrawing nature of the isothiocyanate dictates the rate of H_2S release. The advantages of these ITCs are good water solubility, low molecular weights [134], and easily amenable to further modifications [135]. The challenge however is that ITCs like H_2S are highly volatile and possess high reactivity, posing problems in their pharmaceutical applications [136] (Fig. 7).

A strategy was recently developed to overcome the limitations of ITCs by their incorporation in drug-like compounds forming multifunctional drugs [127, 137]. Similar to fragment-based design, these hybrid drugs take advantage of the H_2S-releasing property of the ITC moiety, and improved ADMET properties and pharmacological effects of the drug-like entity. One such recent example is the development of Memit (**35**), an isothiocyanate of memantine resulting from the replacement of its primary amine functional group [138]. Memantine is a well-known neuroprotective drug that was approved in 2003 by the FDA as a treatment option for moderate to severe AD [139]. It is an uncompetitive antagonist of the voltage-dependent NMDA receptors and targets NMDAR pathological hyperfunction. Memit (**35**) decreased the ROS levels and also showed cytoprotective effects by reducing Aβ aggregation in rat microglial cells. In vitro aggregation studies using thioflavin fluorescence showed reduced $A\beta_{1–42}$ self-aggregation in the presence of Memit [140]. The compound displays slow and prolonged H_2S releasing property (Fig. 7).

To further expand this class of H_2S-releasing ITCs, Rapposelli [135] developed hybrids of rivastigmine (**36**, **37**), an FDA approved AChE inhibitor for AD, with SFN and ERN [141]. SFN activated Nrf2 pathway, demonstrating cytoprotective effects by reduction of oxidative damage in astrocytes [128]. Furthermore, the increased expression of Nrf2 by SFN has been reported to protect the BBB after cerebral insult in rat brain [128, 142]. The hybrids were comparable in the H_2S release efficacy and produced H_2S levels similar to those achieved by DADS in a murine microglial cell line (BV-2) (Fig. 7).

In vitro/in vivo ADMET—The rate constant (k) for H_2S volatilization is 0.13 min^{-1} and the half-life is 5 min ($t½ = 0.693/k$) in buffer solutions. H_2S is highly diffusible and most endogenous H_2S is released along with CO_2 through exhalation. The release of H_2S from donors occurs under acidic conditions (pH of 5.4–7.4) and the normal distribution of H_2S in plasma and other tissues is at 30–100 μM [143]. It therefore is usually stored in mitochondria as bound sulfur. H_2S is primarily oxidized in mitochondria to thiosulfate and further oxidized to sulfate in the kidneys. Exogenously administered NaSH (**16**) and Na_2S (**17**) directly release H_2S and most of the released H_2S is eliminated from the body as thiosulfate, sulfite metabolites through urine. In plasma, H_2S forms sulfhemoglobin and is metabolized by cytochrome C oxidase in the liver [93, 144, 145]. The toxicity of H_2S is mostly concentration-dependent as it provides therapeutic benefits at low concentrations (100–160 μM), however, induces acute toxicity at higher doses mediated through its CNS and pulmonary actions.

Efficacy in animal models—Many H_2S donors have demonstrated therapeutic potential in AD models. NaSH has been most studied amongst all. Cognitive and behavioral studies demonstrated beneficial effects of NaSH pretreatment against Aβ-induced memory impairment in a rat model on Morris water maze. Treatment with NaSH lowered the levels of APP, PS1, and Aβ proteins and improved the cognitive functions in 3 × Tg-AD mice [140]. Anti-inflammatory effects of NaSH treatment were also observed in the LPS injected rat model by reduced expression of pro-inflammatory cytokines (IL-1β) and TNFα [146]. Additionally, NaSH treatment attenuated neurodegeneration and neuroinflammation in homocysteine treated mice [147]. H_2S donors in garlic extract showed increased expression

of presynaptic proteins like synaptophysin and SNAP25 in the transgenic mouse model with APP mutation [148]. Another H_2S releasing compound, a conjugate of tacrine with a H_2S releasing moiety (ACS81), was tested in an aluminum chloride-induced AD model. The compound improved cognitive and locomotor functions in these mice. Besides, such conjugation with H_2S donor improved the safety profile of tacrine while retaining the AChE inhibition in the hippocampus of rat brains [93].

Clinical trials—Although there is promising in vitro and in vivo animal data on the therapeutic potential of H_2S, so far there have been no conclusive clinical trials studying the cognitive benefits of H_2S or its donors. A phase I clinical trial involving H_2S donor SG1002 showed good tolerance by healthy subjects at oral doses of 200–800 mg/kg. At 800 mg/kg, SG1002 showed elevated levels of H_2S for up to 12 h [149]. SG1002 is an inorganic mixture, containing S_8, Na_2SO_4, $Na_2S_2O_3$, $Na_2S_3O_6$, $Na_2S_4O_6$, and $Na_2S_5O_6$. In a clinical trial involving AD patients, sulfurous water demonstrated protection against cellular H_2O_2 and homocysteine-induced oxidative damage. In this study, peripheral mononuclear blood cells of AD patients were analyzed for oxidative damage to the DNA [99]. An exploratory study in healthy volunteers ($n = 16$) showed decreased homocysteine levels after the administration of elemental sulfur formulation at 200 mg/day for a 30-day treatment period. Increased homocysteine accumulation is usually associated with lowered GSH production, a hallmark of AD.

Alkyl sulfide derivatives

Design rationale and pharmacological target—Organic sulfides are compounds with the connectivity C–S–C formula. This substitution is considered a bioisostere for ether linkage by replacing the oxygen atom of the ether with sulfur and is widely used in drug development. Compared to ethers, sulfides are less volatile, higher melting, and less hydrophilic [150]. These properties arise from the polarizability of the sulfur center, which is greater than that for oxygen in ethers. Several sulfide-containing compounds are widely used in AD therapy.

MMP inhibitors: Nerve growth factor-dependent cholinergic neurons undergo degeneration during the prodromal and advanced stages of AD. One of the contributing factors is the degradation of mature NGF by matrix metalloproteinases (MMP). Postmortem brain analysis of patients diagnosed with MCI and mild AD showed higher MMP-9 activity compared to healthy volunteers [151]. Batimastat (**38**) was the first sulfide-containing MMP inhibitor that was tested in patients [152]. MMPs are a superfamily of endopeptidases which are capable of degrading components of the extracellular matrix, remodeling tissues, shedding cell surface receptors, and processing various signaling molecules. Recently, the role of MMPs (matrixes) in AD has gained interest. The capacity of several MMPs (MMP-2, −3, −9, and MT1-MMP) in degrading APP leading to generation and aggregation of Aβ was discovered [153]. Increased mRNA levels of certain gelatinases (MMP-2 and MMP-9) have been found in the hippocampus of the 5xFAD model, which leads to degradation of tight junction proteins at the BBB, leading to BBB leakage and thus exacerbating chronic inflammation in AD [154]. Moreover, MMP-9 has been reported to present neurotoxicity in

the hippocampus due to its interactions with nitric oxide, leukocyte infiltration in the damaged CNS, and other indirect mechanisms [155, 156] (Fig. 8).

In addition to the effect of MMPs on APP metabolism and neuroinflammation in AD, increasing evidence has reported functional links between MMPs and tau protein. Tau is reported to be a substrate for both MMP-3 and MMP-9, but only MMP-9 caused the increase in toxic forms of tau [157, 158]. Another study found that MMP-3 and MMP-10 levels in CSF is related to tau or p-tau levels in AD patients [159, 160]. Batimastat (BB-94, **38**) is a relatively nonspecific MMP inhibitor; inhibits MMP-1, −2, −3, −7, and −9 with IC_{50} in the range of 3–20 nM, respectively [161, 162]. Compared to batimastat, next-generation compounds such as prinomastat (**39**) and tanomastat (**40**) demonstrated improvement in vivo PK profiles [152]. Tanomastat (Bay12–9566, **40**) is an orally bioavailable potent inhibitor of MMP −2, −3, and −9 with IC_{50} of 11, 134, 301 nM, respectively [163]. Prinomastat (AG-3340, **39**) inhibits MMP-2, 9, 13, and 14 (IC_{50}: MMP-3, 6.3 nM; MMP-9, 5.0 nM) [164]. These inhibitors were designed to fit the binding cavity of MMPs and chelate the catalytic zinc atom by a zinc binding group. The presence of sulfide groups helped to improve BBB penetration of these inhibitors (Fig. 8).

Sulindac sulfide: Similar to the MMP inhibitors, the sulfide group was also utilized in the development of γ-secretase modulators [165]. Under physiological conditions, around 90% of APP is cleaved by α-secretase. If APP is cleaved by β-secretase under disease conditions, the amino acid fragment is further cleaved by γ-secretase generating different species, of which $Aβ_{40}$ and $Aβ_{42}$ are the most abundant species in the brain and CSF. Sulindac sulfide (**41**), an NSAIDs, can reduce the production of $Aβ_{42}$ through modulation of γ-secretase [166]. Such γ-secretase modulators exhibit better pharmacological profiles than γ-secretase inhibitors by selectively decreasing the $Aβ_{42}$ levels without any effect on other functions of γ-secretase. In addition to γ-secretase modulatory activity, sulindac sulfide attenuated Aβ neurotoxicity by acceleration of Aβ fibrils formation, thus reducing exposure to toxic soluble aggregates of Aβ [167]. However, higher doses of the compound were required to achieve the therapeutic drug concentration in the CNS due to poor brain penetration and aqueous solubility (Fig. 8).

In vitro/in vivo ADMET—Because of poor aqueous solubility, the bioavailability of batimastat (**38**) was low when administered by oral or parenteral routes. The peak plasma concentrations were reached 1 h after dosing. The $t_{½}$ of batimastat was 19.1 days after intraperitoneal injection in patients with malignant ascites [164, 168]. Fever, hepatotoxicity, and acute bowel toxicity were reported in clinical trials [169, 170].

The bioavailability of tanomastat (**40**) was 70–98% after oral administration in mice, rats, guinea pigs [171]. The peak plasma concentration was reached within 0.5–2 h. In rats and dogs, tanomastat was mainly metabolized in the liver and then excreted by the biliary mechanism. In healthy human volunteers, PK profile of tanomastat was linear at doses up to 100 mg/day. Hepatotoxicity was a major concern with in rodents and dogs. Additionally, depressed erythropoiesis and tubular nephropathy were reported in female rats [171].

Prinomastat (**39**) is a lipophilic MMP inhibitor that crosses the BBB. The $t_{½}$ of prinomastat was 2–5 h after oral administration in patients [162, 172] and the T_{max} was around 1 h. It has been reported that prinomastat causes moderate but reversible arthralgia, myalgia, muscoloskeletel syndrome at high doses [173].

No data on the PK of sulindac sulfide is available.

Efficacy in animal models—Batimastat (**38**), tanomastat (**40**), and prinomastat (**39**) have shown anticancer activities in numerous animal models, but there is no data available of their efficacy in an AD animal models. One study reported a reversible, short-term memory impairment after intracortical administration of batimastat at a dose of 0.0001M and led to longer-lasting memory impairments after administration into the brains of neonatal rats [174].

The efficacy of sulindac sulfide (**41**) has been investigated in the LPS-induced mouse model. Oral administration of sulindac sulfide (3.75 or 7.5 mg/kg) was initiated 3 weeks before the injection of LPS. The pretreatment mitigated memory impairment in this model as assessed by the Morris water maze test and passive avoidance test. Sulindac sulfide at the tested doses also significantly reduced $A\beta_{42}$ levels in the brain and suppressed the activation of astrocytes by LPS. Moreover, the compound significantly decreased the number of apoptotic cells (3.75 mg/kg, 11.4 ± 2.8%; 7.5 mg/kg. 6.1 ± 1.8%) [104].

Clinical trials

MMP inhibitors: Analysis of plasma and CSF samples in AD patients ($n = 14$) by gelatin zymography showed significantly increased MMP-3 activity, whereas MMP-2 activity was significantly decreased in CSF. Interestingly this study showed a reduction in MMP-9 levels in plasma. A similar study in a group of 56 patients with AD-related dementia and vascular dementia showed a correlation between total tau protein and MMP-3, MMP-10 levels in CSF of both AD and vascular dementia group, whereas MMP-9 was significantly increased in the vascular dementia group. Both groups showed an increase in phosphorylated tau levels [160]. Significant issues with bioavailability and dose-limiting toxicities have been noted in clinical trials for MMP inhibitors. For example, phase III clinical trial of batimastat (**38**) was terminated due to peritonitis from intraperitoneal injections as the drug was not suitable for oral administration [175, 176]. Prinomasat (**39**) and tanomastat (**40**) exhibited relatively poor selectivity and their clinical evaluation was halted due to their nonspecific side effects. In phase I clinical trials, patients on prinomastat (1–100 mg twice daily oral dose) exhibited joint and muscle pains which were dose-related but the mechanism is unknown [172]. Plasma analysis of volunteers in phase I studies of tanomastat showed asymptomatic reversible effects on platelets and mild anemia [174, 177].

Sulindac sulfide: Analysis of six NSAID clinical trials with more than 13,000 dementia-free patients showed that the use of NSAIDS lowered AD risk by 23%. However, only some NSAIDS like sulindac sulfide have reported Aβ lowering potential in preclinical studies. A pooled study analysis of clinical trials comparing Aβ lowering NSAIDS like sulindac sulfide (**41**) found that any NSAID use regardless of its reported Aβ lowering effect, reduced the AD risk in dementia-free patients. The risk of AD in these patients was analyzed by reported

outcomes such as neurocognitive tests, neuroimaging, and clinical evaluations [178]. Chronic NSAID use has been associated with increased adverse effects including gastrointestinal, renal, and cardiovascular effects. Chronic NSAID use increases the risk of peptic ulcers in older patients (>65 years of age). In addition, monitoring of renal function is recommended in patients with long half-life NSAIDS due to the risk of acute renal failure. Nonselective NSAIDS also increased the risk of heart failure in older adults [179].

Sulfoxide, sulfone, and sulfonic acid derivatives

Design rationale and pharmacological target

Sulindac: Sulindac (**42**) is an FDA approved NSAID for the treatment of acute or chronic inflammatory conditions. It is a prodrug and the active metabolite is sulindac sulfide. In the liver, the oxidized form sulindac is reduced to sulindac sulfide, which helps to maintain constant blood level and reduce gastrointestinal side effects. We have already discussed sulindac sulfide in the alkyl sulfide. Sulindac is also a nonselective cyclooxygenase (COX) inhibitor and has been applied for the treatment of AD. Sulindac and its sulfide could be oxidized to the inactive sulfone form through metabolic processes. Generally, the presence of the indene ring resulted in decreased CNS and gastrointestinal side effects but also reduced aqueous solubility. Substitution of 5-fluorine with methoxy group resulted in increased analgesic activity and the Z-isomer was more potent than E-isomer [180–183]. Sulindac (**42**) has COX inhibition activity at lower concentrations but shows Aβ lowering effect at higher concentrations (>50 μM). Several studies have reported that NSAIDs like sulindac target γ-secretase activity and generated variants of amyloid peptides with lower neurotoxicity compared to $A\beta_{1–42}$. Protein expression studies in CHO cells overexpressing APP and PS1 showed $A\beta_{42}$ lowering effect of sulindac [184]. ELISA assays in this cell model showed that this decrease in Aβ levels occurs at 40–60 μM concentrations. Fluorescence studies further showed interactions of sulindac with the lipid bilayer of the APP protein, thereby altering the membrane structure. However direct action on Aβ peptides is much debated. Molecular modeling studies using NMR reported direct interaction of sulindac with amyloid fibrils [185], but atomic force microscopy studies revealed this interaction to be weak [186]. Aggregation studies in neuronal cell cultures showed increased formation of large oligomeric aggregates with sulindac that have reduced toxicity [187] (Fig. 9).

Sulfones: A sulfone is another important sulfur-containing group which is widely used in pharmacology. Compared to sulfonamide, sulfone needs more harsh conditions for synthesis, which limits its application in drug design [188]. In the design of $5HT_6R$ antagonists, the sulfonamide group was substituted with the sulfone group to enhance stability [189]. Details of the sulfonamide SAR studies are discussed in the sulfonamide section. Here we focus on sulfone containing $5HT_6R$ antagonists with promising applications in AD. SB-742457 (**43**) [190] is a sulfone containing quinolone derivative, which demonstrated $5HT_6R$ antagonistic potency along with high selectivity (>100-fold vs. 84 other similar receptors) and excellent oral bioavailability in phase III clinical trials for AD. Intepirdine (SB-742457, **43**) has been used in combination with AChEIs like donepezil and has shown beneficial effects including improved cognition in early phase I and phase II clinical trials [191]. SAM315 (**44**) and SAM 531 (**45**), both contain an indazole ring linked

to a naphthalene ring through a sulfonyl group and an electron-rich scaffold attached at the 3 positions of indazole [192]. AVN-322 (**46**) and AVN-211 (**47**) [193–196] are pyrazolopyrimidine derivatives that exhibited excellent selectivity (>2500-fold) within a panel of more than 65 relevant therapeutic targets and are currently being evaluated in phase II clinical trials for AD (Fig. 10).

Cyclic sulfone hydroxyethylamine (Fig. 11) is an important scaffold utilized in the development of BACE-1 inhibitors. This class of compounds displayed improved BBB permeability due to the presence of a secondary amine in the scaffold [197]. The sulfone hydroxyethylamines retained essential H-bond interactions with the BACE-1 catalytic diad and the flap region but reduced flexibility compared to sulfonamide-containing compounds described in the sulfonamide section. The best compound (**48**) reported in this series displayed high potency against BACE-1 (enzymatic IC_{50} = 2 nM) and >200-fold selectivity over cathepsin D [197]. Pharmacodynamic studies in transgenic AD animal models also demonstrated significant effects on AD pathology by reduction of brain Aβ levels.

Sulfonic acids: Taurine (**49**) is a sulfur-containing amino acid with neuroprotective and neuromodulatory effects and plays an important role in addressing pathophysiological changes in AD [198, 199]. Taurine displays an antioxidant mechanism by inhibiting ROS production in mitochondria. Taurine and its analog homotaurine have been reported to reduce Aβ-induced neurotoxicity in neuronal cell cultures by activating GABA receptors. It does not seem to inhibit glutamate-induced neurotoxicity [200, 201]. IHC studies in mice brains showed no effect of taurine treatment on Aβ deposition [202]. Taurine has been reported to modulate synaptic activity by influencing the levels of proteins like synapsin [198]. The sulfonic acid group in taurine presents unique physical properties, which include very low solubility in water (10.48 g/100 mL at 25 °C), low pKa value, and difficulty crossing the BBB. Its cyclical conformation with intramolecular hydrogen bonding also presents passive diffusion. The concentrations of taurine in the CNS are thus dependent on a complex taurine-specific transporter at the BBB, expressions of which are reported to decline under disease conditions or oxidative stress. These properties limit the utility of taurine as an AD therapeutic. Several other taurine-based analogs were developed to address these problems. Critical reviews on taurine and its analogs [199, 203] have been published, herein we focus on the application of these compounds in AD therapy. Compared to taurine, its analog homotaurine (**50**) with 4 carbon aliphatic chain shows preferential binding to soluble monomeric Aβ peptide and maintains Aβ in a non-fibrillar form, thereby reducing the oligomer-induced aggregation and plaque formation [203]. Also, the neuroprotective activity of homotaurine is reported partly due to its inhibition of Aβ-induced caspase activity in neuronal cell cultures [201]. N-protected taurine analogs, γ-L-glutamyltaurine (**51**) [204], or N-pivaloyltaurine (**52**) improved stability of the parent compound along with enhanced lipophilicity [205]. Another modification involved introduction of sulfonic acid group and cyclic amine in the form of piperidine in piperidine 3-sulfinic acid (PSA, **53**) and aniline 2-sulfinic acid (ANSA, **54**), both of which displayed neuroprotective effects [206]. Additionally, linking taurine to another pharmacophore resulted in the formation of hybrid compounds, such as taurepar (**55**) and tauropyrone (**56**), which displayed increased lipophilicity and improved neuroprotective activities [207, 208] (Fig. 12).

In vitro/in vivo ADMET

Sulindac: Sulindac (**42**) is orally bioavailable (90%) and reaches peak plasma concentration in 1 h after a single oral dose of 400 mg. Sulindac metabolites, sulfide, and sulfone reach peak plasma concentrations after 2 h. The plasma concentration of sulindac and its metabolites after a single dose or a 2-week treatment appears to be two times higher in older subjects compared to younger subjects. At 100 mg dose, sulfone metabolite is more prominent in the plasma whereas, at 400 mg, sulfide metabolite is the highest. Sulindac and its metabolites are extensively bound to albumin in the plasma (>90%). Its conversion to sulindac sulfide is reversible, whereas the formation of sulfone metabolite by oxidation is irreversible. The liver and kidneys are the major sites of metabolism for sulindac and it is excreted primarily in the urine. However, the sulfide metabolite is reoxidized to sulindac before elimination in the urine. The main adverse effect related to sulindac is hepatotoxicity; however, this is very rarely observed (0.1% patients). The mechanism of hepatotoxicity seems to be through hypersensitivity-induced hepatitis [180].

Sulfone: $5HT_6R$ are almost exclusively expressed in the brain tissue with no reported expression in peripheral tissues. Antagonists that are highly selective to $5HT_6R$ therefore show minimal adverse effects related to peripheral distribution [209]. Some sulfone-based $5HT_6R$ antagonists like AVN-322 (**46**) showed good oral bioavailability and BBB penetration in animal models including BALB/c mice and Wistar rats [191]. Intepirdine (SB-742457, **43**) showed 76% oral bioavailability in rats at 0.3 mg/kg dose.

Sulfonic acids: In humans, endogenous taurine is predominantly intracellular and has very little presence in the plasma. However, oral supplementation of taurine markedly increased plasma taurine levels for up to 8 h. In one study, oral administration of 4 g of taurine increased plasma concentrations in healthy volunteers from 40 to 690 μM in 1.5 h after administration. Taurine (**49**) is widely distributed across the body including skeletal muscle. Physical activity seems to increase the concentration of taurine in muscle tissue where it is converted to acetyl taurine. Taurine is mainly transported by tauT and PAT1 transporters. However, the transporters have low capacity and therefore saturation occurs even after 10 mg of supplementation, resulting in excretion of excess taurine. Taurine is mainly excreted by urine in healthy humans and excretion is reduced during starvation or kidney failure. It is interesting to note that de novo synthesis of taurine in the brain is very low and is highest in hepatocytes. It has been reported that taurine can be transported across the BBB by a sodium-dependent carrier mechanism in neuronal culture and this uptake can be blocked by β-alanine [210].

Efficacy in animal models

Sulindac: Several animal studies have reported the therapeutic benefit of sulindac (**42**) in AD models. In the LPS-induced AD model, pretreatment with oral sulindac for 3 weeks at 3–7 mg/kg dose reduced Aβ production and inhibited memory impairment in male ICR mice [104]. In aged Fischer 344 rats, behavioral studies using radial arm water maze and contextual fear conditioning showed improved learning and memory in animals upon pretreatment with sulindac. Interestingly, HPLC data showed aged mice with higher levels of sulfone metabolite in the blood [181]. In Sprague-dawley rats, intraperitoneal infusion of

sulindac increased GSH levels and showed neuroprotective effects after ischemic reperfusion injury [211].

Sulfonic acids: Oral administration of 1 g/kg/day taurine (**49**) in double transgenic mice (APPswe/PSdE9) ameliorated cognitive deficits. In an oligomeric Aβ-infusion model, mice treated with 250 mg/kg/day taurine showed improved performance on the Y-maze test [212]. Treatment with taurine in 5 × FAD transgenic mice showed increased glutamate uptake by the brain. Several preclinical studies also showed that chronic administration of taurine was well tolerated by the animals [202, 213]. In TgCRND8 mice, 30–100 mg/kg/day treatment with homotaurine caused a significant reduction in Aβ plaque burden and dose-dependent reduction in plasma Aβ levels [214]. Oral pretreatment with taurine (50 mg/kg/day) for 15 days also inhibited streptozotocin-induced cognitive impairment and neurotoxicity due to GSH depletion in aged Wistar rats [215]. Passive avoidance tasks in aged FVB/NJ mice upon chronic taurine treatment (0.05% in water) showed marked improvement, indicating the beneficial effect of taurine on age-related cognitive decline [216]. Taurine also protected mice from chemical-induced memory impairment, without affecting motor coordination and locomotor activity. In aged dogs, taurine supplementation (500 mg/kg) improved T-maze performance at 8 and 12 months [217].

Clinical trials

Sulindac: Sulindac sulfone, also known as Exisulind is approved for use as an antineoplastic agent. Early clinical studies showed that Exisulind was well tolerated by patients without any serious adverse effects. This drug has not been tested for AD, although preclinical data shows therapeutic potential of sulindac and its metabolites in AD [218].

Sulfones: Inhibition of the 5-HT_6 receptor improves cholinergic and glutamatergic neurotransmission and has been hypothesized to improve AD-related cognitive impairment, learning, and memory deficits in preclinical models. However, small-molecule inhibitors of $5HT_6$ have failed to provide safety profile and therapeutic benefit in phase II and phase III clinical trials in healthy and AD patients. In two randomized clinical trials for SB-742457 (**43**) (Intepirdine) in patients with mild to moderate AD, treatment (15 and 35 mg daily) failed to show any cognitive benefits over placebo. The first study involved 574 patients on intepirdine alone and in the second one, patients (n = 684) were also given donepezil. Cognitive assessment was done using the ADAS-Cog score [219]. A phase I clinical study exploring tolerability and PK properties of SAM-315 (**44**) in 56 healthy volunteers was discontinued (NCT00479440). AVN-322 has shown good tolerability in healthy and MCI subjects; however, a phase II trial of this drug has been discontinued by the sponsoring companies. Similar compounds like intepirdine and latrepirdine (dimebon) also failed to provide therapeutic benefit in combination or as monotherapy in AD patients when compared to donepezil alone in phase III randomized clinical trials [220].

Sulfonic acids: A randomized, placebo-controlled, double-blind phase II clinical study in 58 patients with MCI showed that homotaurine (**50**) supplementation for 3 months at different doses was well tolerated. However, MMSE and ADAS-Cog tests showed no significant benefits over the placebo group [203]. In a larger phase III clinical trial in 1052 patients with

mild to moderate AD, homotaurine supplementation with AChEI for 18 months failed to show significant therapeutic benefit [201]. However, in a phase I study involving 127 healthy volunteers ALZ-801, a valine-conjugated prodrug of homotaurine showed good tolerability after single-dose and 14-day treatment. A phase III clinical study with 300 participants in being planned for 2021 [221]. In 20 patients with MCI and carrying the ApoE4 gene, supplementation of taurine (**49**) for 1 year slightly improved episodic short-term memory. Serum analysis in these patients showed no significant change in the levels of pro-inflammatory cytokines over time [222]. In another study involving 245 patients with MCI, homotaurine (100 mg/day) showed significant improvement in the MMSE score after 8–12 months of treatment [217].

Sulfonamide derivatives

Design rationale and pharmacological target—The sulfonamide group is an important sulfur-containing motif used in medicinal chemistry. Since the discovery of sulfonamide-based antibacterial drugs, more and more compounds in this category are found to have a wide range of biological activities such as antibacterial, antifungal, anti-inflammatory, antioxidant, anticancer, antiviral, and anti-malarial [223]. Sulfonamide group which is considered a bioisostere of acrylamide has unique features such as stability against hydrolysis, resistance to reduction at sulfur [224]. Because of these unique properties, sulfonamide compounds were explored for their varied effects on key enzymes like BACE-1 and AChE in AD pathology [225]. Some of the well-studied targets of sulfonamide compounds are discussed here.

BACE-1 inhibitors: Sulfonamide is a part of originally designed BACE-1 inhibitors. BACE-1 plays an important role in the formation of neurotoxic Aβ [226]. The inhibition of BACE-1 was considered a promising strategy to target AD. It has been shown that in transgenic mice, BACE-1 knockout reduced $A\beta_{1–42}$ production and subsequent plaque formation in the brain [227]. The catalytic site of BACE-1 consists of several sub-pockets with flexibility toward binding to a ligand. Through X-ray crystallography of sulfonamide-based inhibitors binding to BACE-1, tight binding interactions of sulfonamide to S2 sub-pocket of active sites were discovered [228].

First-generation peptidomimetic BACE-1 inhibitors contained phenyl methanesulfonamide motif to mimic the peptide bond (Fig. 13 left). The sulfone oxygen of sulfonamide interacted with Asn233 and Ser325 by hydrogen bond, but also made ionic interactions with Arg235 [229]. The second sulfonamide oxygen formed hydrogen bonds with Thr232 and Asn233. Van der Waal interactions with Thr231, 232, Asn233, and Arg235 were also found in the S2 unit. Amide binding to S3 unit could be replaced by different hydrophobic groups (**57**, **64**, **65**, **66**, **67**) while still maintaining high levels of enzyme inhibition [230]. The methylbenzylamide in this position displayed the highest binding affinity. Interestingly, in one of the studies, the (R)-methylbenzylamide substitution in the S3 unit showed better potency than (S)-methylbenzylamide [231]. Substitution at the S1 site was generally considered to be a Leu-Ala isostere to mimic the transition state. R_2 could be hydrophobic groups such as isobutene (**57**, **58**) or benzyl (**59**, **60**, **61**), which indicated that the S1 pocket is tolerant of bulky substitutions [232]. The amide in the S1 site was critical for BACE-1

inhibition. The introduction of 1,3,4-oxadiazole (**62**, **63**) as an ester mimic to the amide group resulted in a non-transition-state inhibitor [233]. Compared to traditional transition state ligands for BACE-1, both the inhibition efficacy and the stability of these compounds were improved. R3 group could be a variety of motifs such as another amino acid (**57**), amine (**59**), benzylamine (**60**) to occupy the S1' pocket, which helped in the improvement of the potency, selectivity, and metabolic stability. Another modification of this category was linking the 3 and 5 positions of the phenyl ring in phenylmethanesulfonamide motif to form macrocyclic inhibitors (**68**) [234]. These macrocyclic inhibitors displayed low nanomolar inhibition of BACE-1 (IC_{50} as low as 2 nM) and some of them showed better brain penetration and stability compared to noncyclic inhibitors [235] (Fig. 15).

Another important modification was involving the sulfonamide in a ring system (**69**) (Fig. 13 right). Different ring sizes of cyclic sulfonamides were synthesized and tested, with the most potent inhibition presented by a six-membered ring analog [236]. These compounds also showed good selectivity for BACE-1 over BACE-2 and cathepsin D. Interactions of cyclic sulfonamides with the binding pocket are different from the noncyclic compounds and the sulfone oxygen formed H-bond with Asn-294. Substituted aryl group was preferred as the R group, while substitution of fluorine at R1 site improved both inhibition and selectivity against BACE-1. Amine derivatives in X position were more potent than ether or alkyl derivatives and the ethyl substituent in R2 position was optimal for potency and selectivity. Different benzylic rings and their meta-substituted analogs were preferred at the R3 position for BACE-1 inhibitory activity (Fig. 15).

To improve the PK properties of the above mentioned compounds, tricyclic structures (Fig. 14 left) were introduced by linking nitrogen in 1 and 3 positions of the benzylic core, which are the major metabolic sites of these compounds (**70**) [237]. The SAR studies found that a seven-membered ring A was well-tolerated and displayed the highest potency. A five-membered ring B and nitrogen substitution at X, Y sites rather than carbon were more favorable for inhibitory activity [238]. These compounds displayed drastically improved PK properties compared to the compounds discussed before, with better oral bioavailability and brain penetration [239].

By combining sulfonamide and guanidine scaffolds (Fig. 14 right), MK-8931 (**71**) (verubecestat/Merck) was synthesized and tested in AD models. It is the first BACE-1 inhibitor to reach phase III clinical trials [240]. MK-8931 (**71**) has been reported to inhibit BACE-1 and BACE-2 enzymes, but no other aspartyl proteases. Compared to other sulfonamide inhibitors, it has better oral bioavailability, cellular penetration, and brain permeability. It is a nanomolar inhibitor of BACE-1 with IC_{50} as low as 2.2 nM and 45,000-fold selectivity over cathepsin D. The strong hydrogen bond interactions between the amidine moiety of MK-8931 and the BACE-1 catalytic dyad of aspartic acids were obvious in their co-crystal structure, which contributed to its high potency. Further studies showed that substitution of fluorine at X was vital for BACE-1 inhibitory activity and R groups occupying the S3 site were quite tolerant to electron-donating or electron-withdrawing groups (Fig. 15).

5HT$_6$R antagonists: 5-HT$_6$R, or serotonin receptors, which are a member of the G protein-coupled receptor (GPCR) family and ligand-gated ion channels, almost exclusively expressed in the central and peripheral nervous systems and neurotransmitter serotonin acts as a natural ligand. Interestingly, more than 80% of 5-HT$_6$R antagonists contain a sulfonyl group and a heterocyclic ring.

Further SAR studies showed four common key structural elements of 5-HT$_6$R antagonists (Fig. 16): a positive ionizable atom (PI), an aromatic ring-hydrophobic site (AR), a hydrogen bond acceptor group (HBA), and a hydrophobic site (HYD). The PI was generally represented by a positively charged amine such as piperazine and (dimethylamino)-ethyl fragment, which could form electrostatic interactions with the carboxylic acids of Asp3, 32, and the thiol groups of Cys3, 36. The AR region was mainly occupied by indole or indole-like π–electron donor systems, which formed π–π stacking interactions with Phe6, 52. The HBA was either a sulfonamide or sulfone group, which formed hydrogen bonds with Ser5, 43, and Asn6, 55. The HBA also linked the HYD and AR parts together. The HYD was open to diverse aromatic or heteroaromatic rings, which allowed insertion into a hydrophobic pocket formed by Ala5, 42, Val3, 33, and Phe5, 36. A report recently described significant developments in the field of medicinal chemistry and pharmacology of 5-HT$_6$R antagonists as potential therapies for AD [189]. Here we focus on the clinical findings and some recent developments in sulfonamide-based compounds.

SB-271046 (**72**) was the first 5-HT$_6$R antagonist reported to enter into phase I clinical trials [241]. SAR study of SB-271046 showed that lipophilic aryl sulfonyl moieties, such as halogen-substituted aromatic rings (**73**), were beneficial for 5-HT$_6$R affinity. Although SB-271046 provided excellent in vitro antagonistic potency against 5-HT$_6$R (IC_{50} = 2.0 nM), moderate half-life ($t_{1/2}$ = 4.8 h), and excellent oral bioavailability (F > 80%) in rat, but further development efforts were halted due to its insufficient BBB permeability. Additional modifications within this family of compounds focused on improving the PK profile. One such modification was protection of the free amine in piperazine (**74**) to improve logP. In addition, further studies demonstrated that reversed sulfonamides retained the 5-HT$_6$R antagonistic potency of the parent molecule while improved the brain permeability (Fig. 17).

Another lead compound in this class is SUVN-502 (**75**) [242]. SAR studies found that inclusion of 5-substituted indole rings was important for the antagonistic potency and electron-donating group such as the methoxy group was favored at this position. Substitution of small alkyl groups like methyl or ethyl were tolerated on the piperazine nitrogen, and bulky substitutions here caused significant loss of potency. Halogen, especially bromine substitution at the 2 position of sulfonamide aromatic ring, improved the 5-HT$_6$R antagonistic potency, while substitution at any other position or multi-substitution was not favored. SUVN-502 displayed high selectivity (>1000-fold) for 5-HT$_6$R over 5-HT$_2$A receptors and showed no affinity for 5-HT$_1$A, 5- HT$_2$C, 5-HT$_4$B, and 5-HT$_7$ receptors. Furthermore, by changing the indole ring in SUVN-502 to pyrrolo[3,2−c]quinolone (**76**), a novel class of 5-HT$_6$R antagonists was obtained and these analogs reversed phencyclidine-induced memory deficits and displayed distinct procognitive properties in AD mouse models [243] (Fig. 17).

Other sulfonamide derivatives such as R1485 (**77**) with benzoxazine ring or SAM760 (**78**) with tertiary sulfonamide using the amine of the indole ring improved brain penetration, bioavailability, and reduced cardiotoxicity compared to earlier analogs (Fig. 17).

Recently multitarget-directed ligands combining 5-HT_6R antagonists with other key AD-related enzyme inhibitors were developed. For example, free amine in the 4-piperazinyl sulfonamide structure of 5-HT6R antagonists was successfully conjugated with MAO inhibitor (**79**) [244] and AChE inhibitor (**80**) [245] (Fig. 17).

γ-Secretase inhibitors: γ-Secretase enzymes are involved in Aβ production during sequential proteolysis of APP. Inhibition of γ-secretase by small-molecule γ-secretase inhibitors is considered a promising treatment strategy for AD. Most of the compounds targeting the γ-secretase enzyme in AD either lowered the production of Aβ through enzyme inhibition or modulated enzyme activity resulting in shorter amyloid fragments with lower aggregation potential [246]. Aryl sulfonamide-containing inhibitors were developed to avoid undesired cleavage of Notch protein and the resultant downstream signaling that cause adverse gastrointestinal and dermatological events and deterioration of cognition [247]. Some reviews on the identification of aryl sulfonamide inhibitors and further advances have been published [246, 247]. Here we focus on the clinical applications and some recent development of these compounds in the context of AD.

SAR studies of sulfonamide-based inhibitors found that aryl sulfonamide was essential for inhibitory activity. The preferred aryl group was mainly phenylsulfonamide, but other aryl groups such as thiophene displayed a significant increase in potency [246]. It was also found that the aryl sulfonamide group was sensitive to modifications and the 4-chlorophenyl group was the optimal substituent. Both mono and di substitution of the sulfonamide nitrogen were attempted. For mono substitution of the sulphonamide nitrogen, the reported groups were, methylpentan-1-ol (**81**), cyclohexane (**82**), or cycloheptane (**83**) [247]. For di substitution, different substituted aryl groups and side chains containing branched allyl group or bulky ring systems such as azepan-2-one (**84**) were tolerated. These modifications improved the potency, brain/plasma ratio, and Notch-sparing selectivity. Besides, sulfonamide nitrogen was also a part of a ring system in the form of morpholine (**85**) or piperidine (**86**), which offered better potency and stability [248] (Fig. 18).

The lead resulting from above mentioned SAR is avagacestat (**87**) containing the key 4-chlorophenyl-sulfonamide pharmacophore, which was the first γ-secretase inhibitor to be tested in a clinical trial for AD but discontinued after phase I [249]. Further SAR studies focused on improving stability and brain penetration [250]. Begacestat (**88**) containing 5-chlorothiophene moiety displayed improved potency [251], while the trifluoromethyl group offered improved stability [252] (Fig. 18).

Other compounds like ELND006 (**89**), ELND007(**90**) [253], or SCH 697466 (**86**) [254], which introduced piperidine rings in the arylsulfonamide scaffold improved Notch-sparing selectivity. However, this scaffold displayed poor PK properties. Hence further modifications still focused on improving bioavailability and stability. Compared to avagacestat and

begacestat, these advanced compounds not only showed better Notch-sparing selectivity but also enhanced bioavailability and metabolic stability (Fig. 18).

AChE inhibitors: Loss of cholinergic neurons and the subsequent reduction of ACh levels have been associated with loss of synapse, cognitive impairment and dementia observed in AD patients [40, 255]. AChE and BuChE negatively affect the levels of neurotransmitters and contribute to the initiation of Aβ self-assembly [256]. AChE or BuChE inhibitors could increase neurotransmitters concentration, thus supporting cognitive improvement [257]. However, modulation of ACh levels through enzyme inhibitors has only shown to be effective for a short duration as AD therapy. In addition, although cholinesterase inhibitors like donepezil, memantine, rivastigmine improved cognition and improved management of AD, they were unable to reverse the disease progression [258]. Despite this, recent studies have shown that modulation of the cholinergic system could still provide therapeutic benefit in AD by influencing the cholinergic loss, cognitive impairment, and amyloid-related pathologies. Sulfonamide was developed as a new scaffold to improve the potency of AChE or BuChE inhibitors. It was reported that the oxygen in the sulfonamide group formed H-bond interactions with residues in the peripheral anionic site (PAS) and the amide moiety of the sulfonamide interacted with the catalytic or acetylation site (CAS) of AChE [259]. The first developed sulfonamide-based inhibitor was dimethyl piperidine aryl sulfonamides (**91**) [260]. Further SAR studies found that the 2,6-dichloro substituted aryl sulfonamides presented the best potency and improvement in memory function in animal studies. Replacement of piperidine with N-substituted piperazine or different substituted aryl groups (**92**) [261], bi (**93**) [262], or tri (**94**) [263] sulfonamide compounds were designed. However, these compounds failed to show any improvement over the parent mono-sulfonamide. Introduction of the sulfonamide to existent AChEIs was another useful strategy to improve the potency [264]. The hybrid tacrine-sulfonamide derivatives (**95**) showed ~sixfold improvement in potency compared to tacrine alone. An AChEI was also conjugated with an MAO-B inhibitor by sulfonamide linker (**96**) [265] (Fig. 19).

NLR family pyrin domain containing 3 (NLRP3) inhibitors: NLRP3 inflammasome is recognized as a novel target for the treatment of AD. NLRP3 or crypyrin, is a sensor molecule, predominantly expressed in macrophages. Its complex with adapter proteins such as ASC and procaspase-1 is known as NLRP3 inflammasome, which plays a crucial role in the immune response. Activation of NLRP3 inflammasome negatively influences Aβ aggregation and tau phosphorylation, leading to the progression of AD pathology and cognitive deficits.

Sulfonamide is one of the most used motifs in the development of NLRP3 inhibitors [266]. SAR studies indicated that the presence of sulfonyl and benzamide moieties in the inhibitor structure is necessary for NLRP3 inhibitory activity [267]. Small-molecule inhibitors like MCC950 (**97**), CRID1 (**99**), JC121 (**102**), and JC124 (**103**) that prevented activation and formation of caspases and interleukins via NLRP3 have been studied. CP424174 (**98**) selectively inhibited posttranslational processing of IL-1β and presented a potent anti-inflammatory response [268]. Also, immunofluorescence staining showed that TAK242 (**100**), a specific inhibitor of toll-like receptor (TLR4) decreased Aβ-induced NLRP3

activation in microglial cells of APP/PS1 transgenic mice [269]. These compounds have been reviewed by Fulp et al. (**97–103**) [267]. However, none of them have been studied clinically as potential AD therapeutics (Fig. 20).

Phosphodiesterase 5 (PDE5) inhibitors: Phosphodiesterases (PDEs), which interfere with signaling pathways of secondary messengers such as NO, cGMP, PKG, CREB, and histone deacetylases (HDACs), have gained much attention as drug targets for neurodegenerative diseases [270]. Some marketed PDE5 inhibitors for the treatment of erectile dysfunction and arterial pulmonary hypertension, including sildenafil (**104**), and vardenafil (**105**), have been tested as treatment options for AD [271, 272]. These PDE5 inhibitors showed restoration of cognitive function in AD mouse models [273]. In addition to enzyme inhibition, sildenafil (**104**) has also been reported to improve cerebral blood flow and suppress neuronal apoptosis and neuroinflammation [274] (Fig. 21).

Generally, guanine or guanine-like groups were critical for binding to the active site of PDE5 and their inhibitory activities [275]. The sulfonamide group was chosen due to lower lipophilicity and increased solubility [276]. Vardenafil is a better selective PDE5 inhibitor than sildenafil due to the arrangement of the nitrogen atom in the heterocyclic core, which offers a higher affinity toward PDE5 [277]. The alkyl substituent on the piperazine ring in vardenafil does not interfere with binding but enhances its permeability. New generation of PDE5 inhibitors lodenafil (**106**), udenafil (**107**), and mirodenafil (**108**) offer more selectivity, but none of them have been approved by the FDA (Fig. 21).

In vitro/in vivo ADMET

BACE-1 inhibitors: Although many studies report the high efficacy of BACE-1 inhibitors in vitro, very few of them retain their potency in vivo. This is due to difficulty of peptidomimetics like piperazine-sulfonamides in accessing BACE-1 enzymes across the BBB. In addition, these drugs are substrates for efflux pumps like P-gp which limit their availability for effective brain penetration to show efficacy in the clinical setting [278]. However, with recent advances using high-throughput screening and X-ray crystallography, several small-molecule inhibitors were developed that can effectively cross the BBB [279]. Mass spectrometry analysis of APP mice treated with isonicotinamide sulfonamide (**66**) showed brain concentrations closer to its IC_{50}. However, PK studies showed high clearance due to susceptibility to efflux pumps like P-gp. PK studies involving rats and monkeys showed good oral availability of verbucestat at 3 mg/kg and chronic toxicology studies showed a good safety profile while retaining its pharmacological action [280, 281].

$5HT_6$R antagonist: $5HT_6$R antagonist SB-271046 (**72**) showed good oral bioavailability with a minimum oral effective dose of 0.1 mg/kg and T_{max} of 4 h. It showed a plasma half-life of 4.8h in rats with very low brain penetration (10%). SB-399885 (**73**), however, had higher brain penetration. SB-271046 presented a very low clearance rate (7.7 ml/min/kg), but it was found to have no effect on P450 enzymes indicating low hepatotoxicity. SAM760 is orally bioavailable and predominantly metabolized in the liver by CYP3A (85%). The primary metabolite of SAM760 (**78**) was found to be benzene sulfinic acid formed by thiol reductive cleavage. It has an average half-life of 27–34 h [282].

γ-Secretase inhibitors and modulators: γ -Secretase inhibitors pose a similar PK challenge due to poor substrate specificity and bioavailability. Sulfonamide pyrazole (**89,90**) compounds showed well in vitro inhibition of γ-secretase (IC_{50} = 4 nM), but poor oral bioavailability in Sprague-Dawley rats at 100 mg/kg dose. Additionally, the compound showed a very high clearance rate [283, 284]. Notch-sparing inhibitor BMS-708163 (**87**), a diazolylbenzyl sulfonamide, showed good selectivity toward APP-cleavage and was well tolerated in healthy subjects upon single oral administration of 400 mg and a daily dose of 150 mg for 4 weeks. It also showed a T_{max} of 1–2 h in blood with a plasma half-life of 40 h [249]. Another sulfonamide ELND-006 (**89**) showed good brain penetration in PDAPP mice at 0.3 mg/kg as measured by LC–MS [285]. In vivo toxicity studies in these animal models also showed a good safety profile for MK-8931 [281, 283].

AChE inhibitors: Well-known cholinesterase inhibitors like tacrine are generally well absorbed orally and achieve peak concentration in <2 h. Tacrine is highly protein-bound and metabolized extensively by CYP450. Hepatotoxicity is one of the main adverse effects of these compounds. Computational methods showed that piperazine-sulfonamides (**92**), inhibitors of BuAChE showed good solubility and absorption parameters, while also showing moderate BBB penetration. However, while AMES toxicity prediction showed no DNA damage, the compounds showed mild hepatotoxic effects indicated by their inhibition of CYP450 [278, 279].

NLRP3 inhibitors: As NLRP3 mediates ATP sensitive potassium channels, one of the main adverse effects of NLRP3 inhibition is cardiotoxicity. Microsomal stability studies showed that MCC950 (**97**) was very stable in the liver with over 70% remaining after 1 h. The compound showed very less binding to CYP enzymes indicating a lack of significant hepatotoxicity. At the therapeutic IC_{50} of 10 nM, the compound did not show any cardiotoxicity as measured by hERG safety assay. PK studies in C57BL/6 mice using 3 mg/kg iv dose and an oral dose of 20 mg/kg showed that MCC950 has good oral bioavailability (68%) and a plasma half-life of 3 h [286]. Another well-studied NLRP3 inhibitor glyburide, an antidiabetic medication. It is almost completely absorbed from the gastrointestinal tract and single-dose administration in normal subjects showed peak absorption in 4 h with a terminal half-life of about 10h. It is extensively bound to serum proteins (99%). Liver enzyme CYP2C9 is thought to be the main metabolizing enzyme for glyburide. Glyburide and its metabolites (trans and cis hydroxyl derivatives) are excreted by both bile and urine [287].

PDE5 inhibitors: PDE5 inhibitors like sildenafil (**104**) and vardenafil (**105**) are orally bioavailable and their PK and metabolism have been well studied [288]. Oral sildenafil dose has a bioavailability of 41% with a plasma half-life of 4.5 h and a T_{max} of 0.5–2.5 h. It is rapidly cleared from the body with concentration barely detectable after 24 h due to first-pass metabolism.

Efficacy in animal models

BACE-1 inhibitors: BACE-1 inhibitors like MK-8931 (**71**) showed a reduction in Aβ accumulation in brains of Sprague-Dawley rats and cynomolgus monkeys. MK-8931 also

reduced the plasma and brain cortex levels of $A\beta_{40}$ in the animals. In addition, MK-8931 reduced Aβ production in the spinal cord and cortex of monkeys [280, 281].

CRND8 mice treated with piperazinyl sulfonamides (**67**) showed lower plasma levels of $A\beta_{40}$, but not in the cortex of the treated animals [278]. Piperazine derivatives also prevented the loss of synapse by reducing calcium stores in APP knock-in mice [279].

5HT$_6$R antagonist: In aged mice inhibition of $5HT_6R$ by SB-271046 (**72**) and SB-399885 (**73**) reversed the deficits in non-spatial recognition memory and working memory [289]. Animals treated with 3–30 mg/kg of SB-271046 and SB-399885 showed a dose-dependent improvement in working memory assessed by spontaneous alternation in T-maze. In Wistar rats, repeated oral administration of SB-271046 and SB-399885 at 30 and 10 mg/kg dose, respectively, reversed scopolamine-induced amnesia [290]. However, these compounds had no significant effect on spatial learning. Preclinical studies in AD rats showed significant cognitive improvements on radial arm maze and water maze tests after SUVN 502 treatment (**75**) by modulating glutamate levels [274].

γ-Secretase inhibitors and modulators: Preclinical studies in rodent models showed that γ-secretase inhibition reduced Aβ accumulation in the brain and also improved cognition [291]. Begacestat (**88**) caused a robust decrease in Aβ levels in the brain, plasma, and CSF and also improved the performance on contextual fear conditioning tests [292, 293].

NLRP3 inflammasome inhibitor: NLRP3 deficient mice have been reported to show lowered levels of inflammatory cytokines as well as reduced brain caspase levels [294]. Benzene sulfonamides such as JC124 (**103**) lowered oxidative stress levels in CRND8 mice. Treatment with JC124 also provided neuroprotective effects by preserving synaptic functions and lowering brain levels of Aβ in mice [295]. In the LPS-induced AD mouse model, sulfonamide MCC950 (**97**) inhibited caspase activation and also improved cognitive function as measured by spontaneous T maze [286]. Sulfonamides CP-424174 (**98**), showed selective inhibition of IL-1 but not of IL-6 or TNFα [296]. TLR4 inhibitor TAK242 (**100**) provided neuroprotection by inhibiting inflammatory cytokine production and decreasing Aβ-induced NLRP3 activation in microglial cells in APP/PS1 mice [269].

PDE5 inhibitors: Several PDE5 inhibitors have shown promise in preclinical AD models. Treatment of female Tg2576 mice with sildenafil (**104**) (15 mg/kg, i.p) for 5 days caused an increase in transcription of memory-related genes and improved cognitive performance on Morris water maze. Besides, chronic treatment with sildenafil for 5 weeks caused reduction of tau phosphorylation in mice brains [297]. In APP/PS1 mice, sildenafil treatment (3 mg/kg, i.p) showed improvement in behavioral tasks like fear conditioning test and radial arm water maze test. Sildenafil treatment improved contextual learning and working memory in the transgenic animals [298].

Clinical trials—Several sulfonamide derivatives have shown efficacy in preclinical models of AD. However, very few have been developed for clinical trials owing to their poor bioavailability and efficacy issues. BACE-1 inhibitor MK-8931 (**71**) was developed by Merck and entered phase III clinical trial, however, it failed to show efficacy over the

placebo. Plasma levels of Aβ showed no significant change over placebo. Similarly, no improvement in cognitive assessment was observed. A phase III randomized, placebo-controlled, parallel-group, double-blind clinical trial was conducted to study the efficacy of MK-8931 (oral, 12 mg/dose) in prodromal AD patients. MK-8931 showed a reduction in $A\beta_{40}$ levels in healthy and AD patients over 36 h [281]. In a phase III study with 40 participants, treatment with MK-8931 (40 mg/day) showed decreased cognitive performance on ADAS−Cog compared with the placebo group. In addition, psychiatric problems like anxiety, depression, and nightmares worsened with MK-8931 [299]. Other BACE-1 antagonists like LY3314814, CNP520 similarly failed to show efficacy over the placebo in clinical trials. Difficulty in developing secretase inhibitors is due to off target effects of these compounds. For example, phase II clinical trial of BACE-1 inhibitor LY2886721 developed by Lily, was terminated due to hepatotoxicity observed in patients.

Phase I clinical trial for SUVN-502 (**75**) showed that it is well tolerated for up to 200 mg of single oral dose in healthy young and old adult volunteers. A phase IIa clinical trial (NCT02580305) for SUVN-502 in combination with donepezil and memantine was done in 563 patients with mild to moderate AD. The placebo-controlled trial compared SUVN-502 at doses 50 and 100 mg for 26 weeks with cognition assessment using ADAS-Cog as the primary outcome. The treatment however failed to produce significant benefit over placebo treatment. SAM760 (**78**) developed by Pfizer underwent multiple phase I clinical trials. These trials included single and multiple ascending doses and evaluated the safety and tolerability of the drug. In phase II placebo-controlled study, 30 mg oral dose of SAM-760 given as capsule showed no added benefit over donepezil [282].

Several clinical trials studied the safety and tolerability of γ-secretase inhibitor avagacestat (**87**). In a phase II study (n = 200), 25–125 mg/day oral dose of avagacestat showed gastrointestinal and dermatological side effects. In addition, higher doses of worsened cognitive performance on ADAS-Cog score in a 24-week study [300]. A phase I study involving coadministration of begacestat (**88**) and donepezil in 47 randomized healthy subjects was recently completed (NCT00959881).

AChE inhibitors like tacrine, donepezil, and galantamine have traditionally been used for symptomatic relief of AD-related cognitive impairment. These inhibitors have also been reported for side effects involving the peripheral cholinergic system such as dyspepsia and appetite loss. Most of these side effects seem to be dose dependent. Another serious side effect observed involves syncope and fall caused by cholinergic bradycardia [301].

NLRP3 containing inflammasome activation offered promising results in preclinical models and has been studied as a therapeutic target for AD. Inzomelid, an inhibitor structurally related to compound, has completed phase I clinical trial in healthy adults for the cryopyrin-associated periodic syndrome. The trial included 80 participants and safety and tolerability was measured as primary endpoints. NLRP3 inhibition in plasma was measured as a secondary outcome but no data was provided (NCT04015076). NLRP3 inhibitors like glyburide (glibenclamide) and gli-pizide have been studied for their effects on memory and cognition in patients with diabetes [302].

PDE inhibitor sildenafil (**104**) is given as a single dose, decreased cerebral vascular reactivity while improving cerebral metabolic oxygen and blood flow in a small number of AD patients ($N = 14$) [303]. However, in cannabis-induced memory impairment, pretreatment with vardenafil at 20 mg/dose failed to preserve cognitive function. This was a randomized double-blind study of 15 patients diagnosed with cannabis-induced cognitive deficits [304]. In patients with erectile dysfunction, a pilot study ($n = 27$) found that treatment with 100 mg of udenafil at 3-day intervals for 2 months improved cognitive function on MMSE assessment battery and verbal learning tests [305].

Thiazole, benzothiazole, and thiadiazolidinone derivatives

Design rationale and pharmacological target/mechanism—The thiazole and thiazole derivatives are present as a part of many natural and synthetic compounds with a wide range of biological activities, such as antioxidant, antibacterial, antifungal, antitubercular, diuretic, anti-inflammatory, anti-virus, and anticancer activities [306]. The thiazole ring is highly reactive due to the presence of an acidic proton, an electron-donating group (–S–) and an electron-accepting group (C–N) in the ring system. Thiazole fused with other ring systems offers some useful scaffolds such as benzothiazole, phenolic thiazole. Several reviews already describe the synthesis, bioactivity, and therapeutic application of such compounds [307, 308]. Herein, we focus on the recent developments and applications of such scaffolds in the development of AD therapeutics.

Thiazole: Thiazole is a widely used motif in the development of AChEIs. For example, Acotiamide (**109**) is a thiazole ring containing drugs presenting potent AChE inhibitory activity for the treatment of postprandial fullness, upper abdominal bloating, and early satiation due to functional dyspepsia [309]. It is reported that the planar structure of thiazole in these compounds led to the important electrostatic interactions with the catalytic residues Phe330 and Trp84 present at the anionic site (CAS) of AChE [310]. Modification of C-2 of thiazole is widely explored in the design of AChEIs due to its high reactivity [311]. Substitution at this position was either an aromatic ring or a linker attached to an aromatic system, which enhanced the interaction of the scaffold with the catalytic pocket of AChE [312]. The linker was mostly an amine (**110**) [313], imine (**111**, **112**) [314, 315], amide (**113**) [316], or other groups with H-bond donors. For example, by linking benzyl rings to the C-2 position of thiazole through an amide bond (**114**), new thiazole-based AChEI was synthesized [310]. Further SAR studies found that benzyl substitution in the C-4 position was also necessary for inhibitory activity. The substitution of an electron-withdrawing group in the benzoyl ring at the C-2 position further improved the potency. These compounds in addition to be potent AChEIs, also exhibited inhibition of Aβ aggregation and BACE-1 activity, thus making them prospective lead molecules for AD. Other modifications of the thiazole was coupling molecules with distinct biological activity to form multi-targeted AChEIs. For example, by fusing the pharmacophoric features of AchEIs, donepezil and diarylthiazole, benzylpiperidine-linked diarylthiazoles were designed and evaluated as potential multitarget directed ligands (**115**) for treatment of AD [317]. Docking studies proved binding of the diaryltriazine and benzylpiperidine portions to the peripheral anionic site (PAS) and the catalytic active site (CAS) of AChE, respectively. Thiazole containing inhibitors displayed significant in vivo anti-AChE, antioxidant and antiapoptotic properties,

while some of them also exhibited moderate to high inhibition of AChE-induced $A\beta_{1-42}$ aggregation [318] (Fig. 22).

Thiazoles were also used in the development of cyclin-dependent kinase 5 (Cdk5) inhibitors. Cdk5 belongs to the serine/threonine-protein kinase family and plays an important role in tau phosphorylation contributing to AD pathogenesis [319]. Like other Cdks, the activity of Cdk5 depends on its interaction with activators, such as p35, p25, and p39. By using high-throughput screening with Cdk5/p25, N-(5-isopropyl-thiazol-2-yl)isobutyramide was found as a leading compound (**116**), which showed potent inhibition of cdk5 and cdk2/cyclin E (IC_{50} = ca. 320 nM) [320]. In the presence of increasing concentrations of ATP, the thiazole-based compounds were less effective at inhibiting Cdk5/p25, suggesting their competitive binding at the ATP site [321]. Further SAR studies found that the cyclobutane (**117**) was the best substitution for 5-position of the thiazole ring, and too bulky or small substituents resulted in reduction of inhibitory potency [322]. The C-2 position could be attached with a linker to an aromatic ring system. Further modifications included linking thiazole rings to another pharmacophore to enhance the binding affinity (**118**) [321] (Fig. 22).

Benzothiazoles: Benzothiazole, in which benzene ring is fused to the 4,5-positions of the thiazole ring, is one of the most important thiazole derivatives. Benzothiazole is also a widely used scaffold in AD therapeutic development [323] as a part of tau or Aβ aggregation inhibitors due to its high affinities for β-sheet structures [324]. It is reported that the tau aggregation process correlates with cognitive decline and neurodegeneration in AD, thus inhibitors of such aggregation are beneficial against neuronal dysfunction in AD [325]. Benzothiazole inhibitory scaffolds capable of tau aggregation antagonistic activity in vitro have been identified for this purpose. Most of them are polymethine members of the broad π-delocalized lipophilic cation (DLC) family that passively cross cell membranes and accumulate intracellularly depending in part on membrane polarization [326] (Fig. 23).

Cyanine dyes, which are identified by a polymethine chain linking two benzothiazoles, have shown Aβ and tau aggregation inhibitory properties. For example, N744 (**119**) [327] showed tau aggregation inhibition with IC_{50} of 300nM. SAR studies found that modifications of the benzothiazole heterocycle or the polymethine bridge drastically influenced the tau aggregation inhibitory potency [326]. Although at high concentrations self-assembly of N744 was observed which caused the loss of inhibitory activity [328]. Another example of cyanine dye is riluzole (**120**), a medication used to treat amyotrophic lateral sclerosis [329]. A phase II clinical trial was initiated to evaluate riluzole as a treatment option for mild AD [330]. A prodrug of riluzole, troriluzole (**121**), was also evaluated in phase II clinical trial for AD [331]. Riluzole is a multitargeted agent that showed neuronal protection by reducing both tau and Aβ aggregation [332]. Another target for riluzole included NMDA receptors and glutamate receptors, which play an important role in AD [333] (Fig. 23).

There are many synthetic dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) inhibitors with benzothiazole as their core structure [334]. DYRK1A, a member of eukaryotic serine-threonine protein kinase, belongs to the CMGC group of kinases [335]. Inhibition of DYRK1A has beneficial effects on cognitive dysfunctions observed in Down syndrome and AD [336]. Like most tyrosine kinase inhibitors, DYRK1A inhibitors compete

with the ATP-binding pocket at the catalytic site of the kinase [337]. Researchers have found that DYRK1A phosphorylates tau protein and APP in human neuroblastoma cells [338]. Consequently, overexpression of DYRK1A was reported to enhance the levels of phospho-APP and Aβ in the brain [339]. TG003 (**122**) is the lead DYRK1A inhibitor that showed moderate inhibition of DYRK1A (IC_{50} = 930 nM) [340]. Further SAR studies found that the hydroxyl group at the five position of the benzothiazole ring is vital for potency as it forms crucial hydrogen bond interactions with Leu241 in the active site of DYRK1A. The carbonyl group is also indispensable for the interaction, but could be replaced by an amide group (**123**). Further modifications were focused on the fusion of the benzothiazole ring with other ring systems such as pyrimidine (**124**, **125**) [341, 342] or dihydroisobenzofuran (**126**) [343] to improve inhibitory activity (Fig. 23).

Benzothiazole scaffold has also been incorporated as a part of Aβ binding alcohol dehydrogenase (ABAD) inhibitors. ABAD is a mitochondrial protein and overexpression of ABAD in neuroblastoma cells enhances the cytotoxic effects of Aβ. The binding of Aβ to ABAD has been shown to disrupt the enzymatic activity of ABAD toward specific substrates [344], such as those known to possess neuroprotective effects. For example, the Aβ–ABAD interaction disrupts the neuronal balance of estradiol/estrone, which leads to a reduction in the levels of estradiol [345, 346]. Reduced estradiol levels enhance the formation of Aβ leading to tau hyperphosphorylation and affecting glycogen synthase kinase-3β (GSK-3β) activity [347–349]. Targeting the Aβ–ABAD interaction has emerged as a novel therapeutic strategy for AD. Frentizole (127), an FDA approved immunosuppressant drug, was identified as an Aβ–ABAD interaction inhibitor (IC_{50} = 200 μM) and considered a potential treatment option for AD [350]. SAR studies identified that the urea pharmacophore is essential for the inhibitory activity. The presence of a benzothiazole ring with a halogen group also enhances activity. Inclusion of a terminal phenyl ring with polar substituents enhanced ABAD inhibition activity (**128**). Frentizole-based compounds (**127**, **128**) were tested for cellular cytotoxicity and potency in HEK293 mts17β-HSD10 cells. These compounds showed 10–30% cytotoxicity at 25 μM by lactate dehydrogenase assay, however, the measured IC_{50} values for ABAD inhibition ranged from 2 to 10 μM [351]. Further studies on the frentizole chemotype were aimed at changes to the linker moiety, resulting in the identification of a benzothiazole amine phosphonate analog (**129**) [352] and a guanidine analog (**130**) [353] with improved PK profiles (Fig. 23).

Phenothiazine: Phenothiazine is a well-studied structure, which shows tau and Aβ aggregation inhibitory properties. The first reported inhibitor in this category is methylene blue [354] (MB, **131**), which has both reduced (Leuco methylene blue, **132**) and oxidized forms while the oxidized form is the active form with the inhibitory activities. SAR studies found that redox cycling was important for its potency and any substitution on the nitrogen of thiazine resulted in the loss of activities [355]. MB (**131**) is also an MAO and NO-synthase inhibitor and promotes cell survi-val. TRx0237 (methylene blue, **131**) was evaluated in clinical trials for AD. Other phenothiazine derivatives, for example, toluidine blue O (TBO, **133**) and thionine (TH, **134**) were investigated and showed tau and Aβ aggregation inhibitory activities [356]. TBO (**133**) and TH (**134**) also showed AChE and BChE inhibition [357, 358] and effects on secreted $Aβ_{40}$, $Aβ_{1–42}$, sAPPα, and intracellular

APP levels in PS70 cells [356]. Future studies focused on introduction of phenothiazine into other pharmacophores forming multitargeted drugs [359–361] (Fig. 24).

Thiadiazolidinone derivatives: Thiadiazolidinone is another widely used motif with a favorable ADMET profile, which is introduced in the design of AD therapeutics. These derivatives have been shown to engage multiple biological targets and present neuroprotective effects [362–364].

Rhodanine core is the crucial part of the most effective tau aggregation inhibitors [365] and it belongs to the thiazolidine family (Fig. 25). The inhibition mechanism of rhodanine-based compounds is still poorly understood. It is reported that the rhodamine core, which serves as a carboxylic acid bioisostere with low electronegativity, can engage in hydrogen bond and Van der Waals interactions with the tau protein. Further SAR [366] studies of these compounds found that carboxylic acid (**135**) substituent at the N5 position of rhodanine core was preferred and the optimal linker length between the rhodanine to the carboxylic acid was two carbons. At 3-position, an electron-rich heteroaromatic substitution (**136**) was deemed necessary. The presence of an aromatic substitution on such heteroaromatic side chain was also important and bulky substituents like naphthalene (**137**) were tolerated suggesting importance of hydrophobic and/or π–stacking interactions (Fig. 28).

Thiadiazolidine (TDZD) itself was first reported as a non-ATP competitive GSK-3β Inhibitor [367]. It is found that over-activity or/and expression of GSK3 in AD patients accounts for memory impairment, tau hyper-phosphorylation, Aβ production, and other inflammatory responses [368]. Consequently, many structural analogs of TDZD such as triazoles, hydantoins, dithiazolidindiones, rhodanines, maleimides, and its derivatives were synthesized and evaluated in GSK-3β inhibition assay [369, 370].

These SAR studies concluded that the nature of the heteroaromatic ring and particularly, the presence of the sulfur atom in the TDZD scaffold is essential for the inhibitory activity (Fig. 26 left). One of the reasons is that the sulfur atom of TDZDs interacted with the thiol group of Cys199 of GSK-3β, which was close to the heteroaromatic ring of TDZD in the ATP-binding site. The aromaticity of the N4 substituent was found to be important, indicating the presence of hydrophobic interactions with a non-polar residue of the binding site, for example, Tyr216. The size of the N2 substituent of the TDZD ring enhanced the inhibitory potency because of additional cation–π interactions between the N2-phenyl ring and the positively charged arginine residues [371, 372]. In this category, compounds such as tideglusib (**138**) (R = naphthyl, Ar = Ph) and TDZD-8 (**139**) (R = Me, Ar = Ph) were considered potential AD therapeutics (Fig. 28).

Another class of compounds incorporating this moiety is imino-thiadiazoles (ITDZ, **140**) [373]. By changing the carbonyl groups in positions 3 and 5 of thiadiazole with different imino and aryl/alkyl moieties, the stability and cell permeability of the ITDZ scaffold was improved (Fig. 26 right). While TDZD-based compounds are non-competitive inhibitors of GSK-3β, ITDZs are competitive inhibitors in the low micromolar range. SAR studies showed hydrogen bond interactions of the imino hydrogen with Phe67, while the thiadiazole formed S–π interaction with Phe67. Aromatic substitution at the 3-position formed π–π

stacking interactions with Phe93 and was vital for the inhibitory activity. Hydrogen bond donor groups such as amine were preferred on the imino nitrogen due to its interaction with charged Asp 90. Aromatic substitution at the N-2 position was tolerant of various sizes and electronic character.

Structural similarity between tau aggregation inhibitors and GSK-3 inhibitors resulted in the generation of thiadiazole with dual GSK-3β and tau-aggregation inhibitory activities [374]. Among the thiadiazoles heterocycle, 2,4-thiazolidinedione (TZD, **141**) scaffold was chosen for further modifications. The SAR studies found that an electron-rich heteroaromatic ring such as indole in 5 position, resulted in low IC_{50} against GSK-3β. Such compounds were also selective to GSK-3β and showed minimal interactions with homologous kinases, such as CK1δ, CK1ε, and Cdc7. The tested compounds also showed anti-aggregation effects on tau fibrils due to the maintenance of the thiazolidine structure and planarity of the molecule.

The chemical structure of TZD derivatives is also shared by agonists of the peroxisome proliferator-activated receptor (PPARγ) [375] such as rosiglitazone (**142**), pioglitazone (**143**) [376], and troglitazone (**144**) [377, 378]. Functionally, PPARγ is involved in the regulation of glucose metabolism, lipid uptake/oxidation, inflammation, and the expression of immunoregulatory genes. In AD, PPARγ plays multiple roles in influencing the pathology of this disease.

The full agonists of PPARγ present some common pharmacophores: polar acidic head groups and a hydrophobic tail separated by an aromatic or aliphatic linker [379]. Acidic hydrogen on the thiazolidinedione ring of these compounds formed a hydrogen bond with Tyr473 and this interaction leads to significant stabilization of the helix of PPARγ near the active site, thus stabilizing active conformation of the receptor leading to gene transcription [380, 381]. Other important residues, His449, His323, and Ser289 formed a polar pocket to further stabilize the active site of PPARγ (Fig. 27). TZD based PPARγ agonists inhibited neuroinflammation through inactivation of NFκB–dependent promoter [382, 383]. Pioglitazone upregulated neuronal expression of PPARγ as evaluated by RT-PCR and western blot analysis [384]. Elevated PPARγ levels blocked BACE expression, thus inhibiting the generation of intracellular Aβ peptide as determined by luciferase assay [385]. One of the disadvantages of these compounds is that they not only influence the trans-expression function of PPARγ but also its transactivation which is responsible for their clinical adverse effects such as heart failure [379, 386, 387]. Further modification focused on the hydrophobic tail to eliminate these treatment-related side effects [388] (Fig. 28).

In vitro/in vivo ADMET

Thiazoles: PK of Acotiamide (**109**), the lead thiazole-based AChE inhibitor, was evaluated in preclinical animal models and human subjects. In one of the studies, acotiamide (10 mg/kg) was administered to Sprague-Dawley rats ($n = 6$) after overnight fasting (12 h), and mean values for C_{max}, T_{max}, and $t_{½}$ were 327.80 ± 44.63 μg/mL, 0.25 h, and 3.67 ± 0.95 respectively [389]. A single oral dose of acotiamide (100 mg) was rapidly absorbed in healthy volunteers with mean values for C_{max}, T_{max}, $t_{½}$ were 30.82 ng/mL, 2.42 h, and 13.31 h, respectively [390]. Acotiamide is predominantly metabolized by uridyl diphosphate-glucosyltransferase 1 family polypeptide A9 (UGP1A9) and UGT1A8 [391]. The drug is not

associated with significant drug–drug interactions with cytochrome P450 (CYP) substrates at clinically effective doses [391]. No specific clinically significant safety concerns are documented for chronic administration of acotiamide (>50 weeks) [392]. In other studies, the hybrid AChEI containing benzylpiperidine-linked diarylthiazoles (**115**) after single oral administration of 2000 mg/kg dose displayed mean values for C_{max}, T_{max}, and $t_{½}$ as 21.20 ± 1.99 ng/mL, 3.66 ± 0.57 h, and 19.42 ± 4.69 h, respectively. No apparent heart, liver, and kidney toxicity was observed after 14 days of treatment in rats [317].

Benzothiazoles: Benzothiazole containing tau aggregation inhibitor, riluzole (**120**) presented a satisfactory 60% bioavailability after oral ingestion with T_{max} of 2 h peak, and the plasma $t_{½}$ of 12 h, indicating well in vivo stability. The BBB permeability was confirmed by analysis of mouse brain homogenates; however, the drug was also found to be a substrate for P-gp efflux transporters [393, 394]. Riluzole was found to be metabolized mostly in the liver by CYP450-dependent hydroxylation and glucuronidation [395].

PK analysis of benzothiazole phosphonate (**129**) was conducted by intravenous injection to mice at 10 mg/kg dose. Mean values for C_{max}, T_{max}, and $t_{½}$ were 4.8 ng/mL, 0.03 h, and 8.3 h in the brain and 2129.4 ng/mL, 0.03 h, and 5.5 h in plasma. Detectable levels in the brain suggested the ability of benzothiazole-based ABAD inhibitors to cross the BBB [396].

Benzothiazole containing DYRK1A and CDK5 inhibitors are relatively new, and no ADMET studies are reported in the literature.

Phenothiazine: Oral administration of 10mg of MB (**131**) to adult male volunteers showed high oral bioavailability (72%) and was recovered mainly in urine as leuco methylene blue (**132**) [397]. When administered as prophylaxis for ifosfamide-associated encephalopathy, MB exhibited a 5 h half-life, a high-volume distribution, and good brain permeability [398]. It is reported that bioavailability correlates with significant liver accumulation after oral administration, which is also found in the cyanine dyes-based tau aggregation inhibitors described before [399].

Among TDZD derivatives, the stability of tideglusib (**144**) was tested in mouse liver microsomes and found to be highly susceptible to metabolism ($t_{½}$ = 16 min). The plasma $t_{½}$ was 5.12 h and T_{max} was 0.25 h [400]. In preclinical studies, no significant toxicity was observed after tideglusib treatment (200 mg/kg, p.o.) [401].

PK profiles of PPAR agonists have been extensively studied. Pioglitazone (**143**), rosiglitazone (**142**) and troglitazone (**144**) are absorbed within 2–3 h after oral administration [402, 403]. These drugs are mainly metabolized by CYP 450 in the liver. CYP2C8 and CYP3A4 are the principal enzymes for biotransformation of pioglitazone and troglitazone, while CYP2C9 and CYP2C8 are responsible for the metabolism of rosiglitazone [402–404]. The plasma $t_{½}$ of pioglitazone and rosiglitazone (pioglitazone: 3–7 h; rosiglitazone: 3–4 h) is shorter than that of troglitazone (7.6–24 h). The adverse effects of pioglitazone included ventricular hypertrophy with congestion of liver and kidneys [405]. Rosiglitazone has been related to cardiovascular risk, especially heart failure [406], while troglitazone is associated with severe hepatotoxicity [407].

Efficacy in animal models—Thiazole derivatives have been studied for therapeutic potential in AD due to their reported anti-inflammatory and neuroprotective activities in addition to their enzyme inhibitory activity described above. Phenyl-butadienyl-benzothiazole derivatives (PBB) have been used as ligands for visualizing tau-related pathologies by PET imaging in transgenic mouse models [408].

In TauP301L mice expressing hereditary tauopathy, riluzole (**120**) treatment (12 mg/kg/day in drinking water) improved the performance of the mice on radial arm maze and Morris water maze. Immunoblotting of tissue samples showed attenuation of tau hyperphosphorylation by riluzole in the hippocampus region of P301 mice [409]. In transgenic 5 × FAD mice, treatment with riluzole (12 mg/kg, oral) for 5 months improved hippocampus-dependent memory. ELISA assays of brain tissue samples showed a reduction in the toxic Aβ oligomers. However, no change in the mRNA level of APP was observed [410]. Riluzole also attenuated memory defects in rat models at 10 mg/kg oral dose and improved spatial memory in Morris water maze and passive avoidance tests [332].

TDZD-based compound, TDZD-8 (**139**), has been one of the most useful pharmacological tools used to study GSK-3β function. TDZD-8 showed protective effects against $A\beta_{1–42}$ neurotoxicity in vivo [411]. The okadaic acid (OKA)-induced AD-like zebrafish model has been used to study the effects of TDZD-8 on AD phenotype [412]. TDZD-8 (1 μM) treatment reduced the ratio of active/inactive GSK-3β in OKA-induced zebrafish model, along with the reduction in the levels of phosphorylated tau. TDZD-8 treatment also restored memory dysfunction in this model as assessed by the spatial alternation paradigm [412].

Another GSK 3β inhibitor, tideglusib (**138**) was tested in a double transgenic APP/tau mouse model. The treatment reduced Aβ burden by up to 59% and also successfully prevented spatial memory impairment in the Morris water maze test [413]. Interestingly, the treatment also caused upregulation of brain insulin growth factor 1 (IGF-1) in both APP/PS1 transgenic and wild type mice, promoting hippocampal neurogenesis in vitro and in vivo [414, 415]. Kainic acid-induced excitotoxic injury to hippocampal neurons in adult rats was prevented by tideglusib treatment through activation of the PPARγ nuclear receptor [416].

PPARγ agonists offered promising results in mouse models of AD. When tested in APPV717I-transgenic mice, pioglitazone at a dose of 40 mg/kg/day reduced $A\beta_{1–42}$ deposition by ~25 and 33% in the hippocampus and frontal cortex of the brain compared to non-treated transgenic mice [417]. In this study, pioglitazone (**143**) also reduced glial activation and BACE-1 expression. Rosiglitazone (**142**) reversed insulin abnormalities and normalized stress responses in Tg2576 mice [418]. In another study, rosiglitazone treatment attenuated learning and memory deficits in Tg2576 mice [419]. This study demonstrated that rosiglitazone reduced brain $A\beta_{1–42}$, but did not change $A\beta_{1–42}$ deposition [419]. Troglitazone (**144**) treatment reduced $A\beta_{1–42}$ burden in the transgenic J20 mice [420]. The study also demonstrated enhancement of phagocytic pathology after troglitazone treatment, while reducing the expression of pro-inflammatory cytokines.

Clinical trials

Thiazole and benzo thiazole: Acotiamide (Z-338, **109**), has been approved for use in functional dyspepsia. In multiple clinical trials, it was shown to be well-tolerated and safe at doses of 100 mg for 52 weeks (NCT01973790). Riluzole (**120**) has been initially approved for use in lateral sclerosis. In preclinical studies, it inhibited glutamate receptor activity and showed neuroprotective effects. A randomized double-blind phase II study (NCT01703117) in 42 patients taking donepezil started in 2013. In this study, the effect of riluzole on neuronal marker N-acetyl aspartate was measured using PET scan after 6 months of treatment. In addition, changes in cognition were measured between 36 months. This study is expected to conclude in November 2020. Troriluzole (**121**), a prodrug of riluzole, is currently in phase II/III clinical trials (NCT03605667). In this randomized open-label study, 350 elderly volunteers with mild AD will be given 280 mg drug or placebo for 48 weeks. Changes in ADAS-Cog score and clinical dementia rating (CDR-sum of boxes) will be evaluated.

TRx0237 (**131**), a second-generation phenothiazine derivative is a tau aggregation inhibitor. A phase II clinical trial evaluating the safety of oral TRx0237 at 250 mg/day dose was discontinued. A phase III study with more than 800 participants failed to show cognitive improvement in MCI subjects (NCT01689246). Another phase III study with 180 participants, comparing the effect of 200 mg drug in frontotemporal dementia failed to show improvement in cognitive score measured by ADAS-Cog. Another phase II/III trial in 180 patients with all-cause dementia is currently underway.

TDZD derivatives: Thiazolidinediones like rosiglitazone (**142**), in a 6-month clinical trial in patients with AD and MCI, showed preservation of memory function, but blood Aβ levels remained unchanged [421]. At 8 mg dose, rosiglitazone improved cognitive function in mild to moderate AD patients. However, a phase III clinical trial of rosiglitazone as monotherapy in patients with mild to moderate AD failed to show the therapeutic benefit over that of placebo after 24 weeks [422]. Rosiglitazone treatment showed an adverse effect of peripheral edema (15% compared with 7% of placebo). A double-blind, placebo-controlled randomized controlled trial was done in 25 nondiabetic patients with AD to explore the tolerability of the pioglitazone. At 45 mg dose pioglitazone was well tolerated however, peripheral edema was also observed in these patients. Secondary outcomes involving cognitive impairment showed no significant improvement in drug-treated group over placebo [423]. Another adverse event associated with thiazolidinediones like pioglitazone (**143**) has been the risk of heart failure. A meta-analysis of as many as 19 clinical trials in more than 16,000 patients showed that treatment of pioglitazone did not increase death due to myocardial infarction, but it did increase the risk of serious heart failure when compared to placebo treatment [424].

Thiourea derivatives

Design rationale and pharmacological target

Dihydrothiazine: Dihydrothiazine is another widely used motif in the development of BACE-1 inhibitors. Dihydrothiazine was independently discovered using fragment-based drug discovery or high-throughput screening methods [425–429]. The SAR [430, 431]

studies found that the amidine group formed a strong salt bridge with catalytic aspartates Asp32 and Asp228 in the S2 subunit, and sulfur in the thiazine ring improved the potency and stability compared to the corresponding N or O analog [432]. The substituted aromatic group within the S1 pocket led to important π interactions, which could be either phenyl or thiazole. Substitution of fluorine on this aromatic ring improved the inhibitory potency. This scaffold was used in the development of the next generation of BACE-1 inhibitors and some of the compounds were tested in clinical trials [433]. Here we focus on the recent progress in the inhibitor design and their applications in AD clinical trials (Fig. 29).

The aminothiazine LY2811376 (**145**) developed by Eli Lilly, is one of the lead compounds in this category [427]. LY2811376 showed modest potency (IC_{50} = 0.24 μM) with tenfold selectivity toward BACE-1 over BACE-2. In vitro cell culture experiments in human kidney cells overexpressing APP and in primary neuronal cell culture of PDAPP transgenic model showed reduction in amyloid production after LY2811376 treatment [427]. Besides, it showed little activity toward other aspartyl proteases like cathepsin D. In addition to dihydrothiazine, dihydrothiazine derivatives with fused tetrahydrofuran (THF), tetrahydropyran (THP), cyclopropane, cyclohexyl- and cyclopentyl-rings, were also used in the development of the BACE-1 inhibitors to improve potency and selectivity [433]. Amongst these compounds, E-2609(elenbecestat, **146**), LY-3202626 (**147**), LY-2886721 (**148**), PF06751919 (**149**), Amgen (**150**), and Janssen (**151**) [434] were evaluated or planned to be evaluated in clinic trials. LY2886721, another BACE-1 inhibitor showed a similar reduction in Aβ levels in primary neuronal cell culture, albeit at much lower EC_{50} levels compared to LY2811376 [435]. LY3202626, the last of these classes of compounds developed by Eli Lilly, cleaves APP protein to release C99 fragments. This upstream inhibition lowers Aβ levels in AD patients for a much longer term [436] (Fig. 30).

One of the strategies to enhance the selectivity was to target the flap region, a flexible antiparallel β-hairpin which controls substrate access in the S2′ pocket [437]. This flap is one of the regions where residue differences exist between BACE-1 and BACE-2. Introduction of the bulky group into the dihydrothiazine backbone or fused ring with dihydrothiazine, such as methyl, fluoromethyl [438], isoxazole (**152**) [439], pyrazole [438], stabilized the flap region of BACE-1, and improved the selectivity [440]. Further SAR demonstrated that incorporation of different spirocycles to the dihydrothiazine backbone (**153**, **155**) improved the BACE-1 selectivity by 550-fold over BACE-2 [441] (Fig. 30).

The introduction of amide linkage on phenyl substitution of the dihydrothiazine scaffold improved the BACE-1 inhibitory activity. Such an amide linker was generally installed between two aromatic rings allowing the compounds to enter the deep S3 pocket, thus achieving selectivity over cathepsin D [439]. For example, compared to LY2811376 (**147**), JNJ-54861911 (**154**) [442] (atabecestat) containing the amide linker between two aromatic rings displayed better potency (IC_{50} = 9.8 nM). BACE-1 inhibitors like elenbecstat, atabecestat (JNJ-54861911 (**154**)), and PF-06751979 (**149**) were reported to lower Aβ levels in CSF and plasma samples in mice through BACE inhibition. Further modifications were focused on improving selectivity over cathepsin D and BACE-2, enhancing the BACE-1 inhibitory activity, improving BBB permeability and metabolic stability. Further modifications involved substitution of the aromatic ring with CN, $OCHF_2$, OCH_3, CHF_2,

allowing deeper insertion into the S3 pocket [443]. One of the problems of amide linker, however, was its metabolic instability. Other linkers such as substituted ethane groups [156] were used to avoid this metabolic liability [444] (Fig. 30).

Thione backbone is used in the development of ABAD inhibitors, which is a relatively new target for AD [445]. A potent ABAD inhibitor, AG18051 (**157**), containing a pyrazolo[3,4-d]pyrimidine-4(1H)-thione backbone (IC_{50} = 92 nM) has been reported [446]. SAR studies have shown that the pyrazole ring is important for the ABAD inhibitory activity and substitution of phenyl ring attached to pyrazole, especially with a hydroxyl group (**158**), improved the potency. The bulky amide group (**159**) is tolerated and did not significantly influence the activity (Fig. 31).

Thiourea: Pyrimidinylthiourea derivatives were designed as potential multifunctional anti-AD agents [447]. The SAR studies found the thiourea moiety to be an indispensable scaffold for AChE inhibitory activity. The 4,6-disubstituted pyrimidine was also critical for AChE inhibition and the imidazole ring was the most preferred substituent at the C-6 position of the pyrimidine ring (**160**). The imidazole ring could be substituted at the 4 positions with aminomethyl moieties to modulate BBB permeability. These compounds were potent AChEIs (AChE, IC_{50} = 0.067 μM, SI > 597), and displayed specific metal-chelating ability, and significant antioxidant effects. Another modification involved incorporation of MAO-B inhibitory scaffold to the four positions of imidazole ring to target both AChE and MAO-B (**161**) [448]. Thiourea scaffold has also been used as a metal-chelating motif to link AChE inhibitor pharmacophore (**162**) with $A\beta_{40}$ aggregation inhibitor motif (**163**) [449], M1 inhibitor with AChE inhibitor (**164**) [450], and MAO-A inhibitor with ABAD inhibitor (**165**) [451] (Fig. 32).

Thiourea is also a key pharmacophore of somatostatin receptor-4(sst_4) agonists. It is reported that sst_4 not only plays an important role in learning and memory processes, but also modulates formation of soluble $A\beta_{1–42}$ [452]. NNC 26–9100 (**166**) was the first non-peptide agonist that displayed high affinity and selectivity for sst4 [453, 454]. SAR [454] studies found that the thiourea group was critical for sst4 activity and an imidazole substitution on thiourea was preferred. The bulky group was also needed as a secondary substituent on thiourea, for example, an indole group increased the selectivity for sst4. Other modifications, for example, incorporation of thiourea in a ring system forming 2-thiohydantoin (**167**), could not improve the potency [455]. Fluorescent probe assays showed marked antioxidant potential of these compounds in vitro by inhibition of ROS production. MTT assays in neuronal cells SH-SY5Y, showed low cytotoxicity for thiourea derivatives even at concentrations of 100 μM (Fig. 33).

Thiourea has also been used in the development of glutaminyl cyclase (QC) inhibitors. QC, a metalloenzyme upregulated in the brains of AD patients, is responsible for the generation of pyroglutamate Aβ. This modified form of Aβ is considered more toxic, aggregation-prone and forms a major part of Aβ plaques in humans. The SAR studies showed the importance of thiourea functionality for potency [456]. Replacing thiourea moiety with other groups decreased the activity dramatically. Imidazole ring and an aromatic substituent on each side of the thiourea were essential for QC inhibition (**168**). The imidazole could engage the active

site Zn^{2+} [457]. The aromatic substitution of amine in thiourea is needed. It is reported that the addition of an electron-rich ring system to the four positions of aromatic substituent through a flexible ring enabled insertion into the active site (**169**), thus enhancing the inhibitory activity [458] (Fig. 34).

In vitro/in vivo ADMET

Dihydrothiazine: PK studies of LY2811376 (**145**) in humans using an oral dose of 90 mg achieved peak plasma concentration at 2 h, while the maximum concentration in CSF was reached at 5 h and was threefold higher than the plasma level. The mean plasma half-life was 40 h. In vivo studies in APP, transgenic mice showed high brain penetration of LY2811376 after an oral dose of 100 mg/kg, achieving brain exposure more than the IC_{50} levels needed for the Aβ lowering activity. This study also showed its rapid clearance. A 3-month toxicology study in rats showed that LY2811376 at oral doses of more than 30 mg/kg has increased the risk of photogeneration in eyes and neurodegeneration in brain glial cells. However, a follow-up study in BACE-1 knockout mice found it to be unrelated to BACE inhibition [427]. For LY3202626 (**148**) and LY2886721 (**147**), oral administration of 10–70 mg dose in healthy volunteers resulted in a plasma T_{max} of 4–5 h and a mean half-life of 18–24 h. Similar to LY2811376, this drug also showed increased accumulation in CSF compared to plasma. Metabolite profiling studies of plasma samples showed active metabolism of LY3202626 by demethylation and amide hydrolysis. In vitro studies in hepatocytes also showed oxidation and formation of sulfenic acid intermediates by LY3202626 [435, 459]. PK analysis of PF-06751979 after oral administration at 50–130 mg in human subjects showed plasma T_{max} of 4 h and half-life of 29–33 h. The T_{max} however increased to 6 h in fed state compared to fasting subjects and the half-life was more than 40 h in older subjects. About 10% of the drug was excreted unchanged in the urine. Long term studies in dogs for up to 9 months showed that PF-06751979 (**149**) is well tolerated without BACE-2 inhibition related side effects [434]. In TgrasH2 mice, treatment with elenbecestat for 26 weeks at an oral dose of up to 300 mg/kg was well tolerated and did not show any adverse effects [460]. Elenbecestat (E2609, **146**) showed a plasma $t_{½}$ of 12–16 h after single oral dosing of 30 mg/kg in nonhuman primates [461]. Oral administration of JNJ-54861911 (**154**) in healthy volunteers offered plasma T_{max} of up to 2 h after a single dose and 4 h after multiple dosing, indicating rapid absorption. In addition, single dosing showed a half-life of 9–16 h, which increased to 18 h after multiple dosing for 14 days. JNJ-54861911 showed more plasma binding and less CSF accumulation [462].

Thiourea: In vitro membrane permeation assays displayed good BBB permeability of pyrimidine thiourea derivatives (**160**). In vivo concentrations in plasma were 3 times higher than that in the brain, indicating plasma protein binding of the drug [447]. QC inhibitor PDB150 (**168**) was reported to have poor brain penetration. PET imaging in animals (mice and rats) treated with radiolabeled PDB150 confirmed poor BBB permeability. Additional experiments with liver microsome from Sprague-Dawley rats showed that PDB150 has a half-life of 1 h [463].

Efficacy in animal models

Dihydrothiazine: Preclinical studies of LY series of BACE-1 inhibitors showed Aβ lowering action in AD mouse models. In female PDAPP transgenic mice, oral administration of LY2811376 (**145**) and LY2886721 (**147**) at doses 10–100 mg/kg showed a dose-dependent decrease in Aβ. ELISA studies of the brain cortex showed a similar dose-dependent decrease in C99 levels, a product of APP proteolysis. Similar studies in beagle dogs showed lowered plasma Aβ levels for up to 14 h by 5 mg/kg LY2811376. CSF levels of Aβ also remained low for up to 9 h [427]. ELISA assay of brain samples from male 129/sve treated with subcutaneous PF-06751979 (**149**) for 5 days at 50 mg/kg showed reduced $A\beta_{42}$ levels. Dogs treated with JNJ-54861911 (**154**) for 14 days at a dose of 0.3 mg/kg/day orally, showed a 50% reduction in CSF and brain Aβ levels. In a similar study in monkeys at doses of 0.3–3 mg/kg, JNJ-54861911 significantly and dose-dependently reduced Aβ in CSF and plasma over 6 h. IHC analysis showed that repeated oral administration of JNJ-54861911 reduced the number and area of the Aβ plaques in the APP/PS1 mice [462].

Thiourea: In the Scopolamine-induced amyloid AD model, oral administration of 200 mg/kg pyrimidinethiourea derivatives (**160**) improved cognitive performance on Morris water maze [447]. IHC analysis of brains from humans and animal models of AD showed early deposition of pyroglutamate Aβ in humans and nonhuman primates whereas it occurs later in transgenic murine models [464]. Several QC inhibitors have shown promise in different transgenic animal models of AD [465, 466].

Clinical trials

Dihydrothiazine: Although BACE-1 inhibitors showed amyloid lowering action in vitro and preclinical models, most of them failed in clinical trials due to risks of toxicity. For example, a safety study of a single oral dose of LY2811376 (**145**) by Eli Lilly in 30 healthy volunteers was discontinued after phase I due to the risk of retinal toxicity and neurodegeneration. In this study, LY2811376 (500 mg/day) was given as an oral capsule (NCT00838084). A similar compound, LY2886721 (**147**) reached phase 2 after showing good tolerability and safety in phase I study with 170 healthy volunteers and AD patients treated with an oral dose of 15–70 mg/day. Single ascending dose and multiple ascending dose studies for up to 26 weeks were conducted to determine the tolerability safety and efficacy of the drug. Treatment showed lower levels of Aβ in CSF samples of patients, however, the study was terminated at phase II due to abnormal liver biochemical results in some patients (NCT01561430). It is not clear if this is related to BACE-1 inhibition. For LY3202626 (**148**), two phase I trials were conducted to evaluate the PK parameters and a third trial to understand the effect of food and dosage form in 30 healthy volunteers. Phase I study in 136 healthy and AD patients showed that the drug was well tolerated at doses 0.1–45 mg and the treatment showed dose-dependent lowering of $A\beta_{40}$ in CSF with a 90% reduction at 26 mg/day. A phase II trial with 316 volunteers with mild AD was terminated due to the lack of efficacy attributed to test parameters (NCT02791191). The patients received LY3202626 at doses 3–12 mg/day orally for 52 weeks. Tau PET scan to measure neurofibrillary pathology burden was used as the primary outcome and plasma Aβ levels were measured as a secondary outcome in this study. A second phase II trial involved the combination of

LY3202626 with donanemab, an antibody that targets amyloid aggregates. However, in 2018, the BACE-1 investigation arm in this clinical trial was discontinued.

Phase I trials for Atabecestat (JNJ-54861911, **154**), in healthy volunteers, showed well tolerability, safety, and lowered CSF amyloid levels. Three similar phase I randomized placebo-controlled studies were done in 15, 30, and 45 volunteers at an oral dose of 10–50 mg for 4 weeks [467]. Phase II study in 90 patients with early AD treated with JNJ-54861911 at 5–25 mg/day for 6–12 months day was terminated following elevated liver enzymes in 12 patients [468]. Some patients also reported worsening cognitive defects upon long term treatment. Another phase IIb/3 study was terminated due to liver enzyme related adverse events (NCT02569398). In this double-blind, placebo-controlled study ($n = 557$), patients were amyloid positive and asymptomatic for AD (at risk of developing dementia) and were receiving 5–25 mg/day for up to 54 months.

Phase I study of elenbecestat (**146**) in 500 healthy volunteers showed well tolerability and safety. Single and multiple dosing at 5–800 mg showed dose-dependent reduction in CSF Aβ levels after 14 days. A phase II study in 70 volunteers with MCI showed a 50% reduction in CSF amyloid levels in patients treated with elenbcestat at 5–50 mg/day. At 50 mg/day, elenbecestat showed positive cognitive outcomes as measured by ADAS-Cog score. However, some subjects reported nightmares as a possible side effect (NCT02322021). A phase III clinical trial is currently active ($n = 1181$) in patients with early AD treated with 50 mg oral elenbecestat, for 24 months with a 12-month extension. The study is expected to be completed in 2023 (NCT02956486).

A phase I trial for PF-06751979 (**149**) developed by Pfizer, was done in 46 healthy volunteers to study the safety and PK PD parameters. Doses of 100–700 mg/day were given in single and multiple ascending studies (NCT02793232). Results showed a reduction in CSF Aβ levels. Two other phase I studies in smaller groups found similar safety and dose-dependent reduction in plasma Aβ levels [460]. However, in 2018, Pfizer discontinued research involving neuroscience research including PF-06751979.

Conclusion

In summary, major classes of sulfur-containing compounds with multitude of pharmacological targets and their preclinical and clinical applications in the development of AD therapeutics are presented. A number of these sulfur-containing compounds that were advanced to human clinical trials have offered limited success. In this review, pharmacological targets, ADMET, and clinical applications of various sulfur-containing compounds with respect to AD are discussed. Due to the complex underlying pathophysiology of AD, therapeutics targeting single pathological events in AD have not been successful, and thus emerging need for multipronged treatment strategies to tackle this devastating disease [469]. Many sulfur-containing compounds belong to disease-modifying drugs [470], which act on multiple targets involved in the pathogenesis of the disease [11]. These compounds are used alone as multifunctional and multitarget therapies [471] or as a part of combination treatments [471, 472] and are expected to provide more efficient therapeutic approaches. Incorporation of sulfur pharmacophore offers improved pharmacokinetic properties along with engagement of multiple targets involved in the

complex pathophysiology of AD when fused or merged with other pharmacophores resulting in multipronged approach toward AD therapy. For example, lipoic acid linked with AChE inhibitors formed complexes which targeted both neuroinflammation and oxidative stress in AD pathogenesis (section Organic Thiols, disulfides, and prodrug compounds.) Some the thiol compounds, on other hand, were themselves used as a linker to link or fuse different pharmacological ligands to produce multitargeted therapies. For example, thiourea linker has been used as a part of AChE inhibitor-MAO or Aβ aggregation inhibitor complex, MAO inhibitor-ABAD inhibitor complex, which are discussed in section Thiourea derivatives. Additionally, repurposing of some of the clinically approved sulfur-containing drugs such as riluzole for AD, presents an extremely efficient strategy due to superior PK and known safety profiles of these medications [473].

Organosulfur is essential for vital functions in the human body and generally presents low toxicity. Pharmacological limitations of sulfur based compounds and their applications as AD therapeutics are either due to instability of thiol pharmacophore or metabolism of these compounds in physiological systems. For example, biothiols, thiol based drugs and H_2S releasing compounds (sections organic thiols and hydrogen sulfide donors), are known for their efficient antioxidant property. However, in presence of oxygen, these drugs can simultaneously lead to formation of thiyl radicals and superoxides. Inactivation of these harmful thiyl radicals could be impaired under disease conditions. A sustained and controlled release of H_2S is necessary for pharmacological action of H_2S donors and prevention of any potential toxicity. Also, sulfur usually in the form of thiol, imparts an unpleasant odor to these compounds and this requires formulation as prodrugs [107].

Sulfonamides that form a major class of these sulfur-containing drugs, are well known for the “sulfa-allergy” presumably caused by hypersensitivity of patients to sulfonamides. Sulfonamides also inhibit the folate biosynthesis and some metabolites cause renal insufficiency. However, newer generation of more soluble sulfonamides have been developed to address these issues. Apart from hypersensitivity, drugs like alkyl sulfides have been investigated for hepatotoxicity due to their interaction with cytochrome P450 enzyme system. Sulfur containing drugs like sulindac are given as sulfoxide prodrugs due to their increased solubility and absorption characteristics (section alkyl sulfides). Sulfonic acid derivatives such as taurine and its analogs suffer from limited BBB permeability and require much higher doses to elicit pharmacological response in AD.

Protocols addressing the shortcomings of organic sulfur compounds are constantly evolving and the advantages of such therapeutics as mono- and combination AD therapy are increasingly evident. With recent focus on conducting AD clinical trials in prodromal or preclinical populations with positive diagnostic markers, treatment options such as multitarget oriented sulfur-containing therapeutics hold promise and warrant further investigations.

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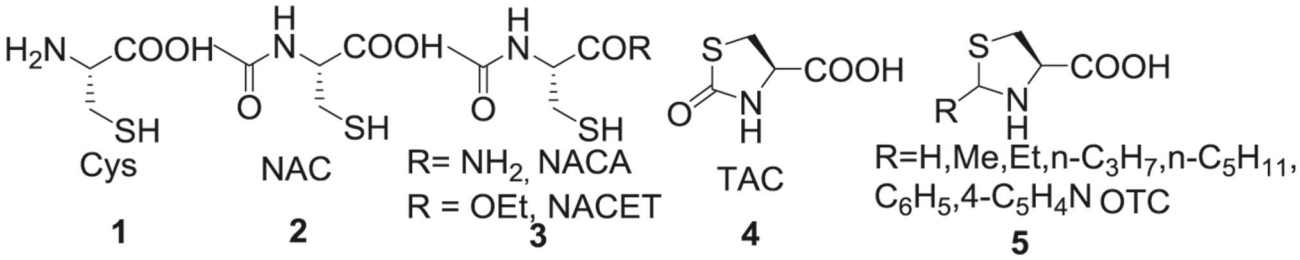

Fig. 1.
Cysteine based thiol derivatives

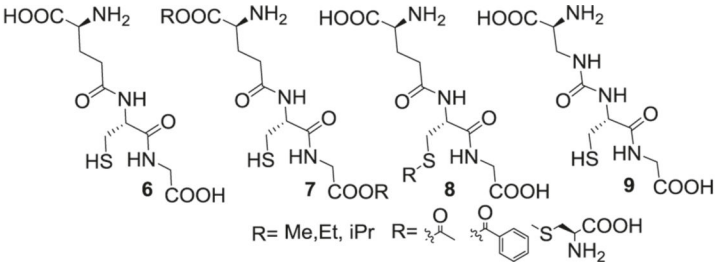

Fig. 2.
GSH based thiol derivatives

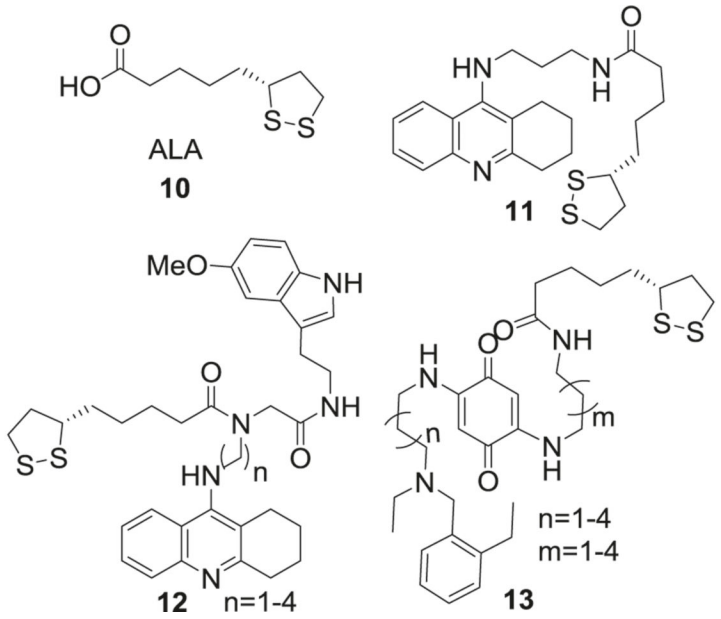

Fig. 3.
Alpha-lipoic acid and its analogs

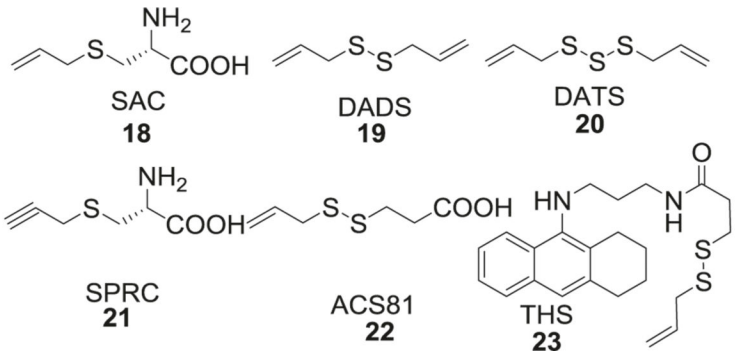

Fig. 4.
Allyl sulfide derivatives

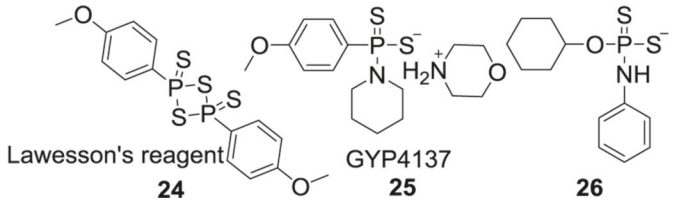

Fig. 5.
Lawesson's reagent and its analogs

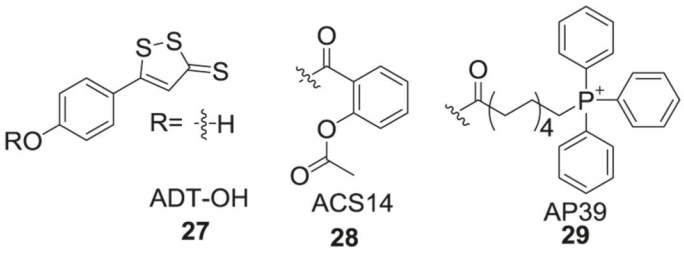

Fig. 6.
1,2-Dithiole-3-thiones (DTT) and its analogs

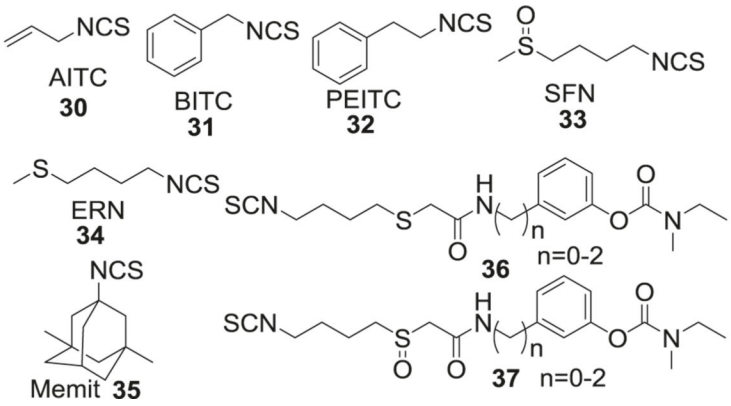

Fig. 7.
Organic isothiocyanates (ITCs)

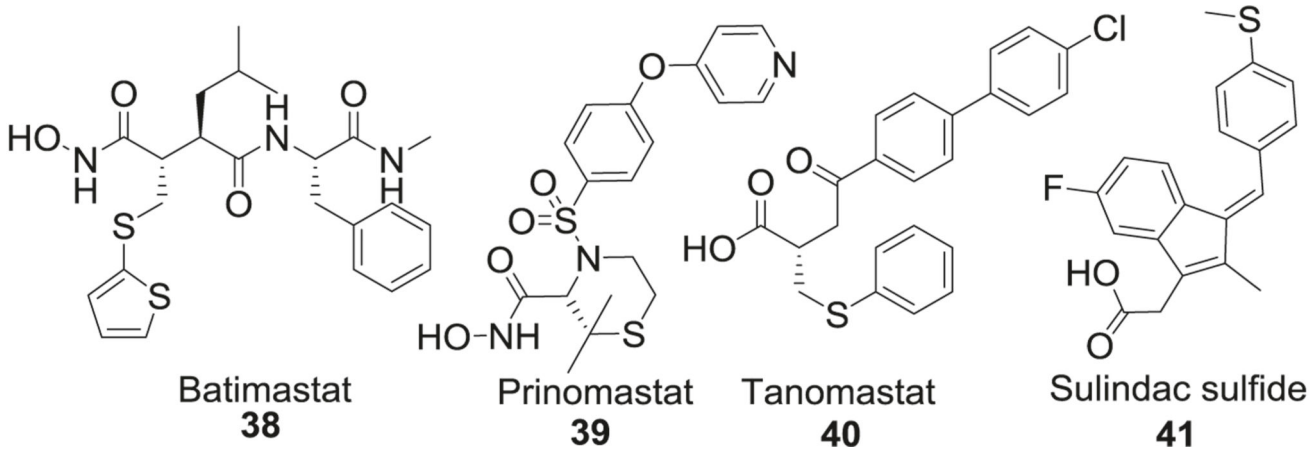

Fig. 8.
Alkyl sulfide-containing AD therapeutics

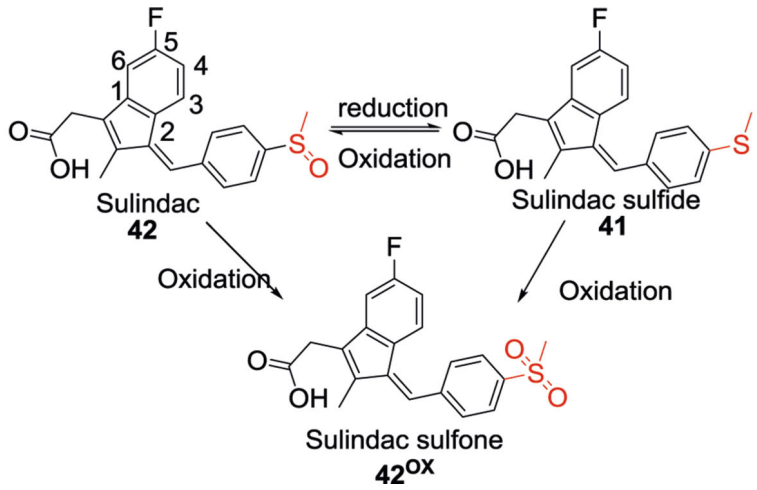

Fig. 9.
Sulindac and its metabolites

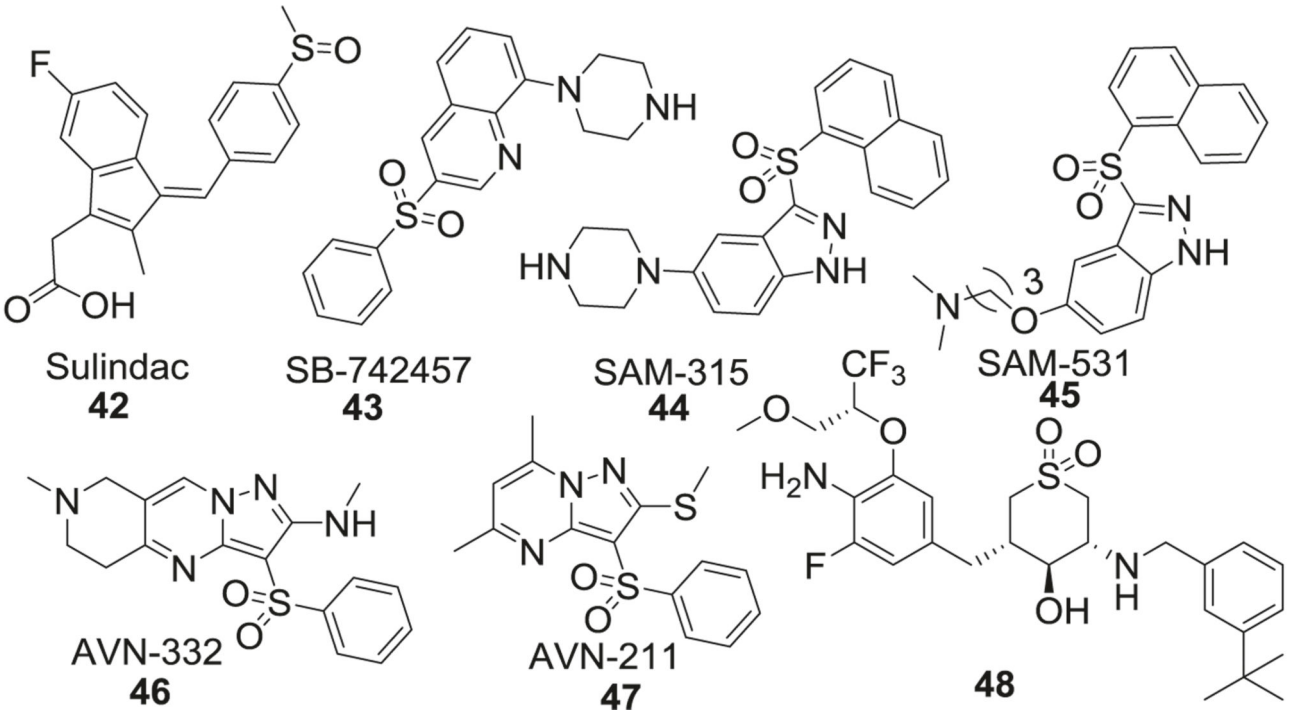

Fig. 10.
Analogs containing sulfoxide and sulfone functional groups

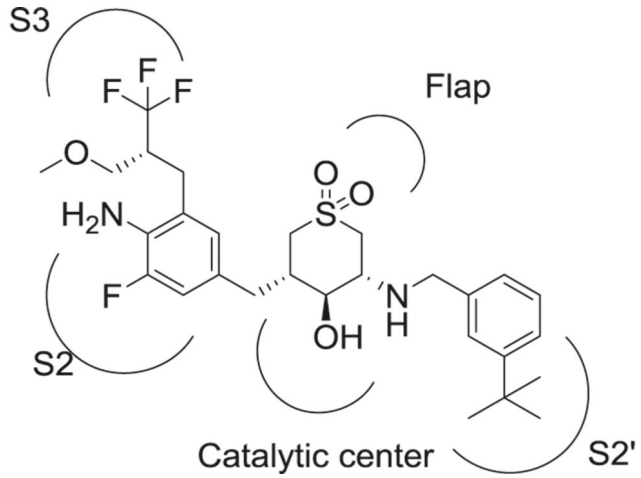

Fig. 11.

Interactions of cyclic sulfone hydroxyethylamine **48** with BACE-1

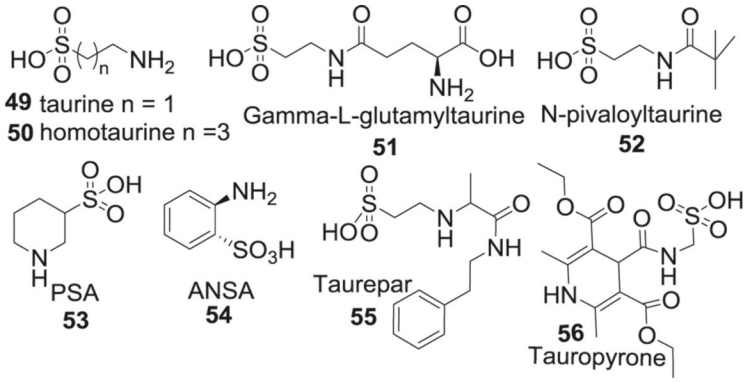

Fig. 12.
Analogs containing sulfonic acid functional group

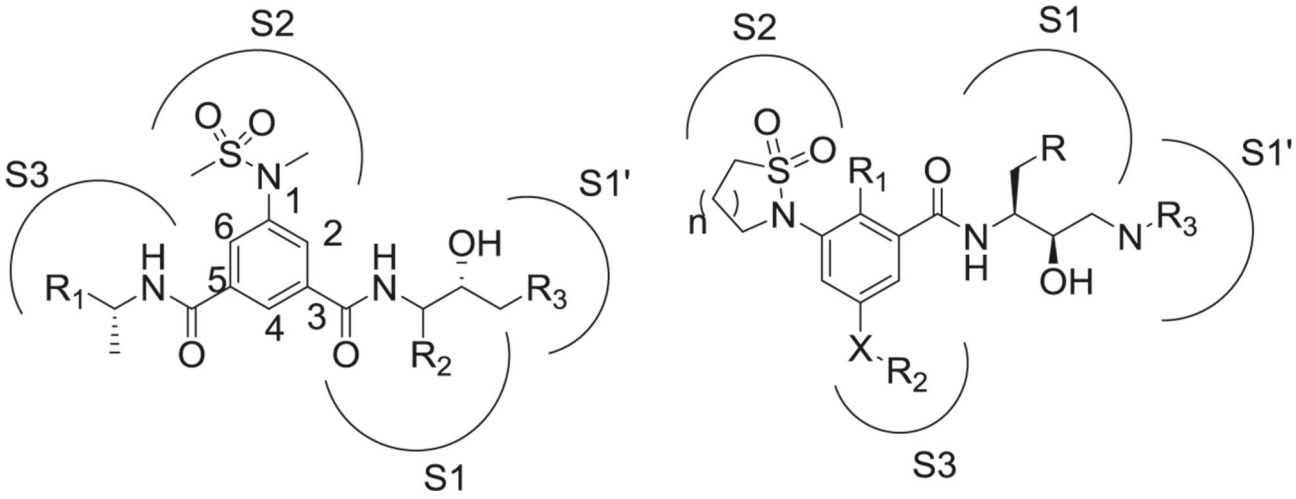

Fig. 13.

Interactions of methanesulfonamide (left) and cyclic sulfonamide (right) analogs with BACE-1 active site

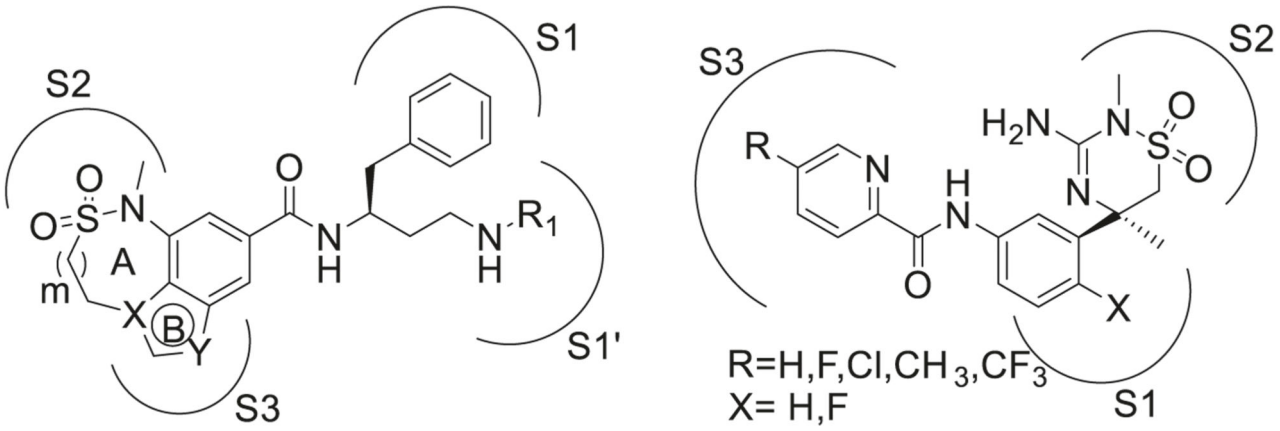

Fig. 14.

Active site interactions of tricyclic (left) and guanidine (right) sulfonamides with BACE-1 active site

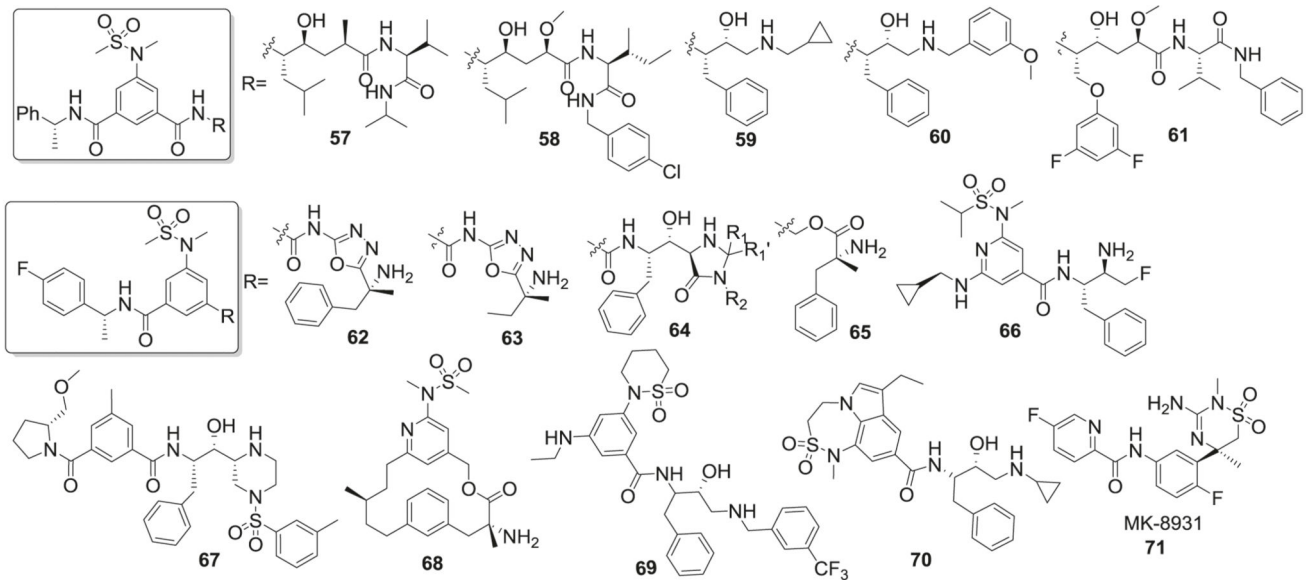

Fig. 15.
Structures of sulfonamide-containing BACE-1 inhibitors

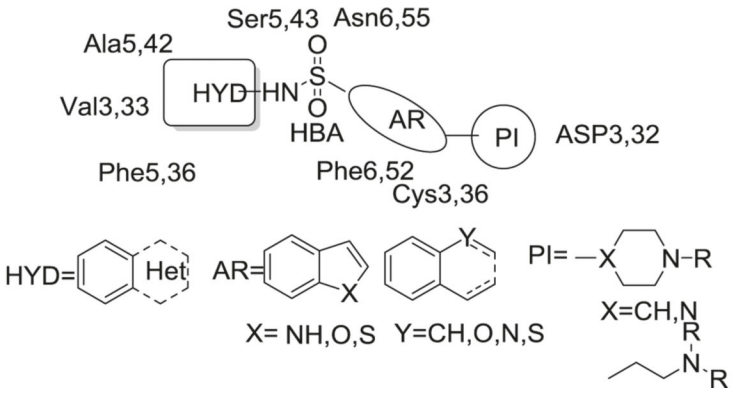

Fig. 16.

Active site interactions of sulfonamide-containing $5HT_6R$ antagonists

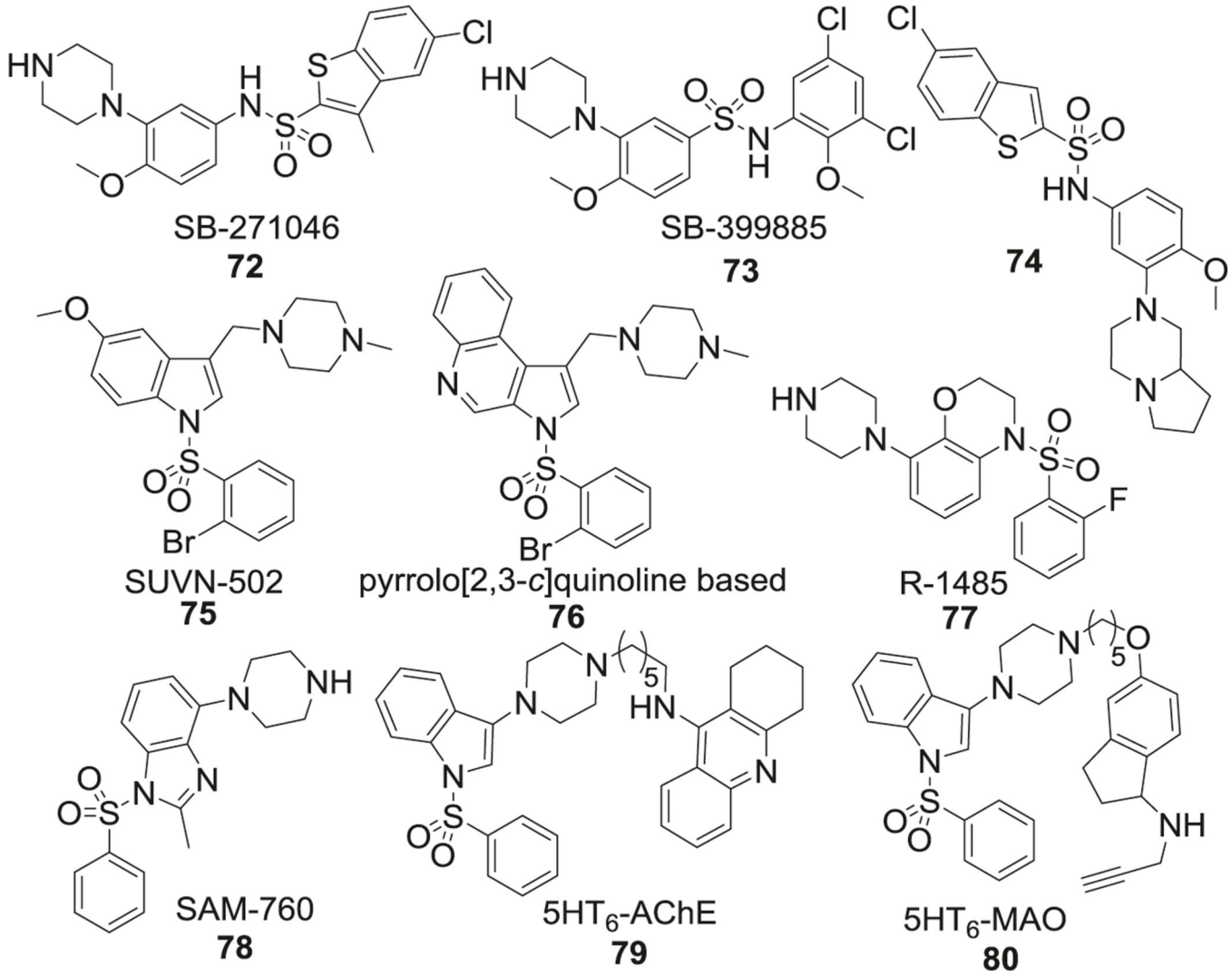

Fig. 17.
Sulfonamide-based $5HT_6$ receptor antagonists

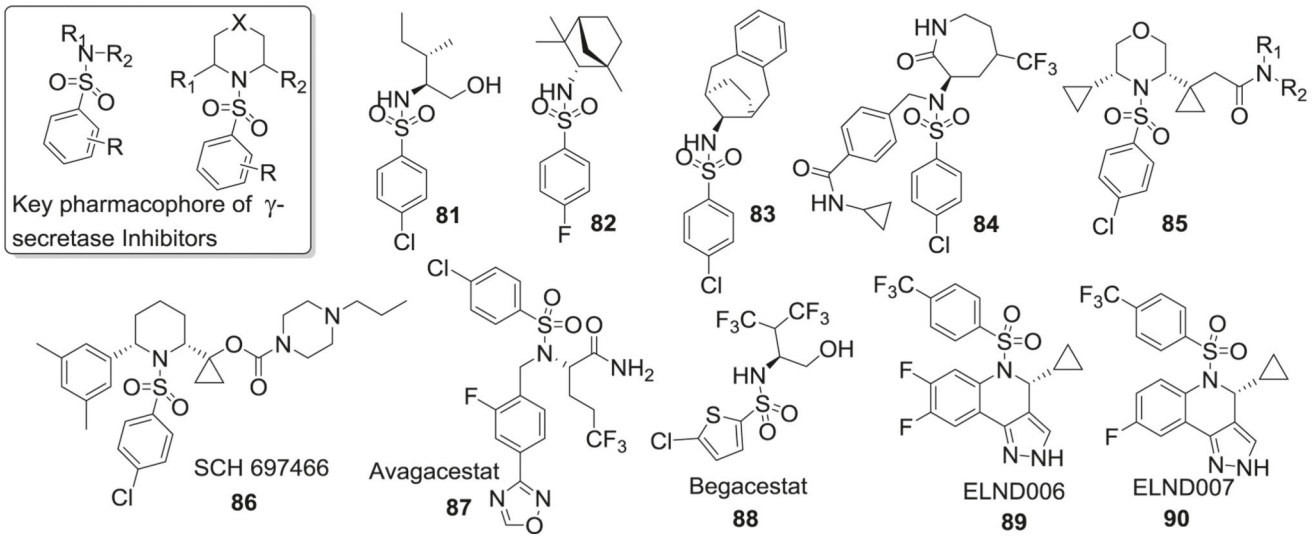

Fig. 18.
Sulfonamide-containing γ-secretase inhibitors

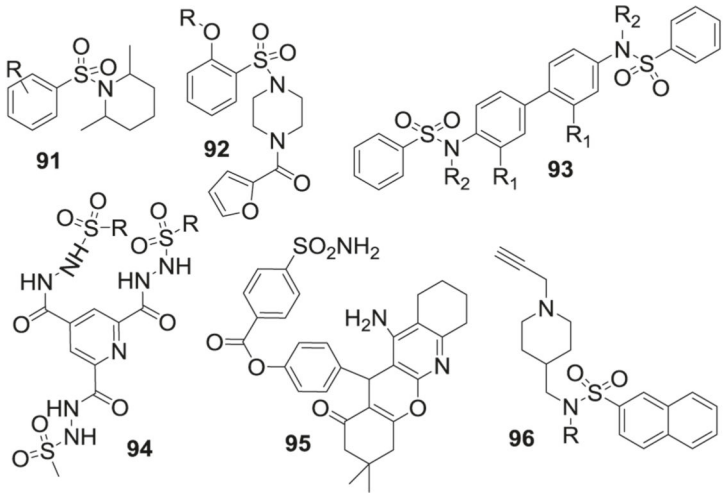

Fig. 19.
Sulfonamide-containing inhibitors of AChE

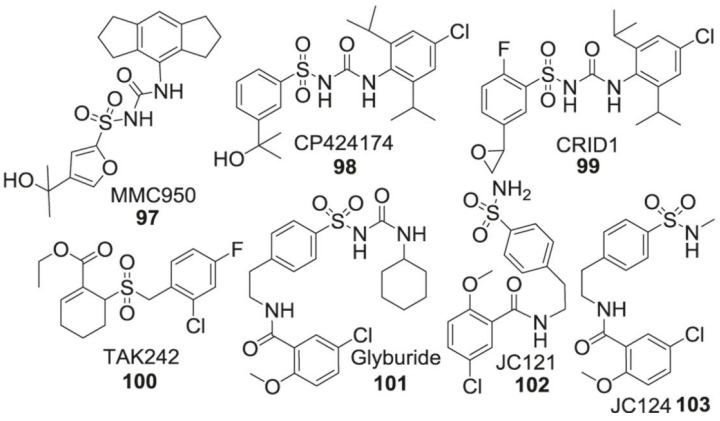

Fig. 20.
Sulfonamide-containing NLRP3 inhibitors

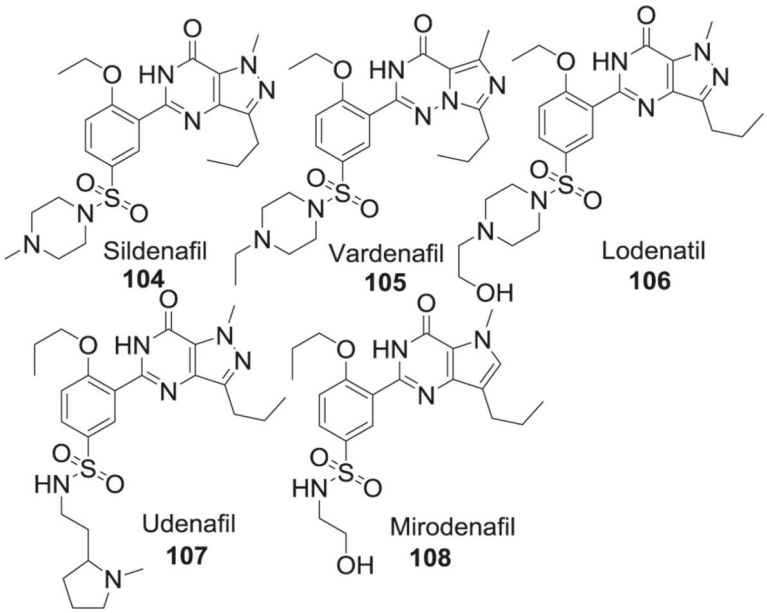

Fig. 21.
Sulfonamide-containing PDE5 inhibitors

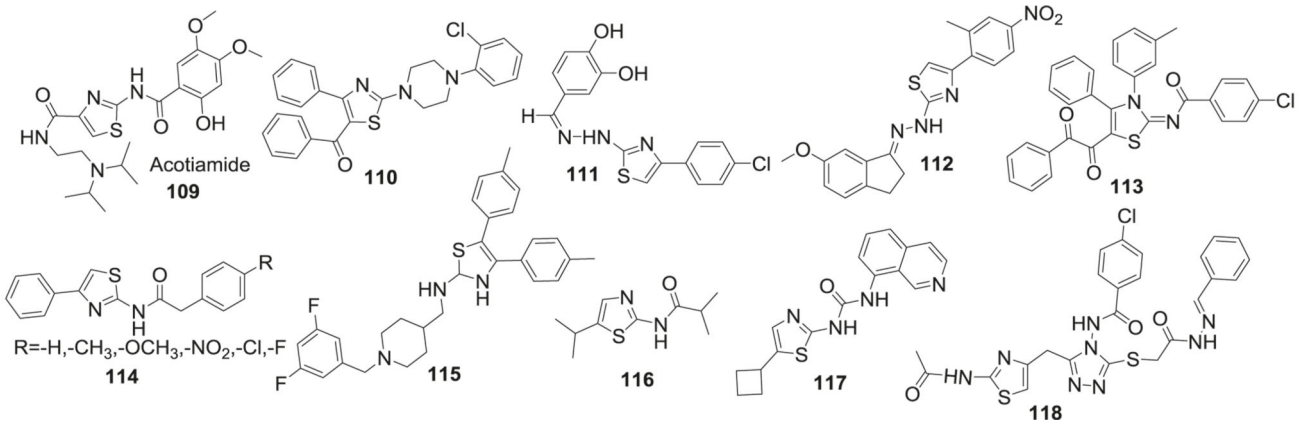

Fig. 22.
Structures of thiazole containing AChE inhibitors

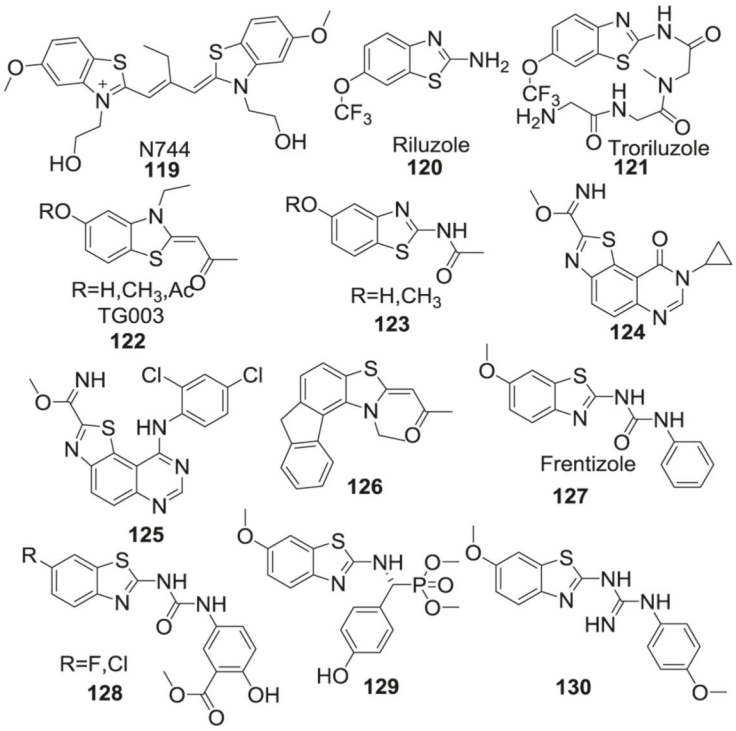

Fig. 23.
Benzothiazole containing AD therapeutics

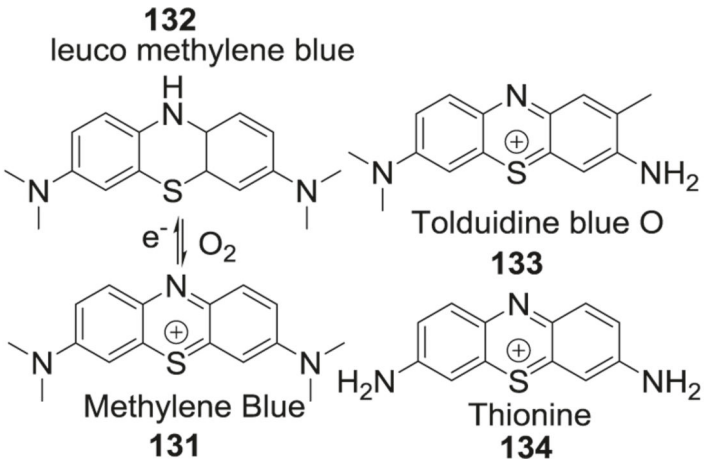

Fig. 24.
Phenothiazine containing AD theapeutics

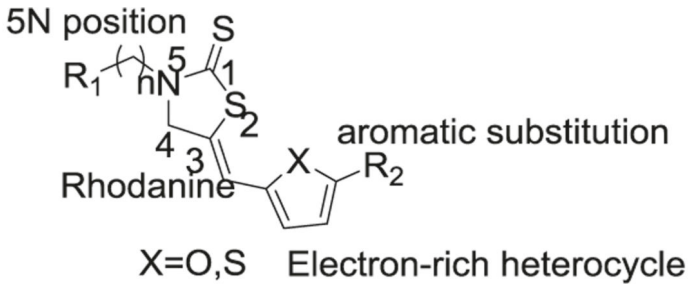

Fig. 25.
SAR for rhodanine derivatives as tau aggregation inhibitors

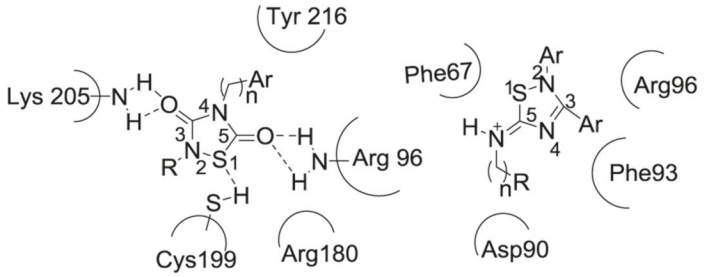

Fig. 26.
Interactions of TZD derivatives with GSK-3β active site, left: TDZD, right: ITDZ

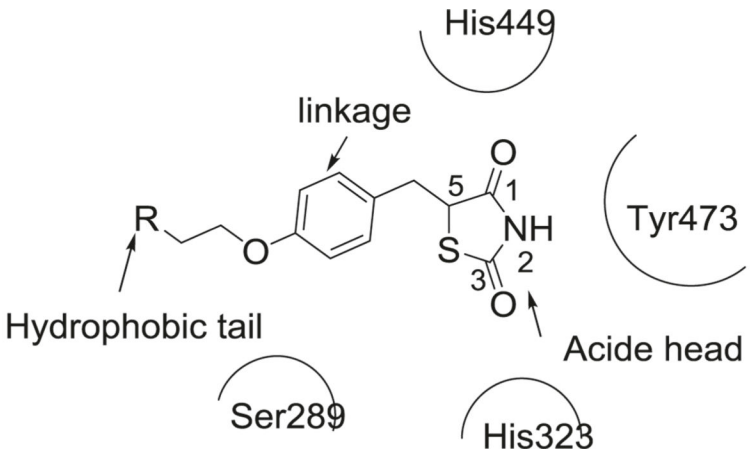

Fig. 27.
Interactions of TZD derivatives with PPAR-γ active site

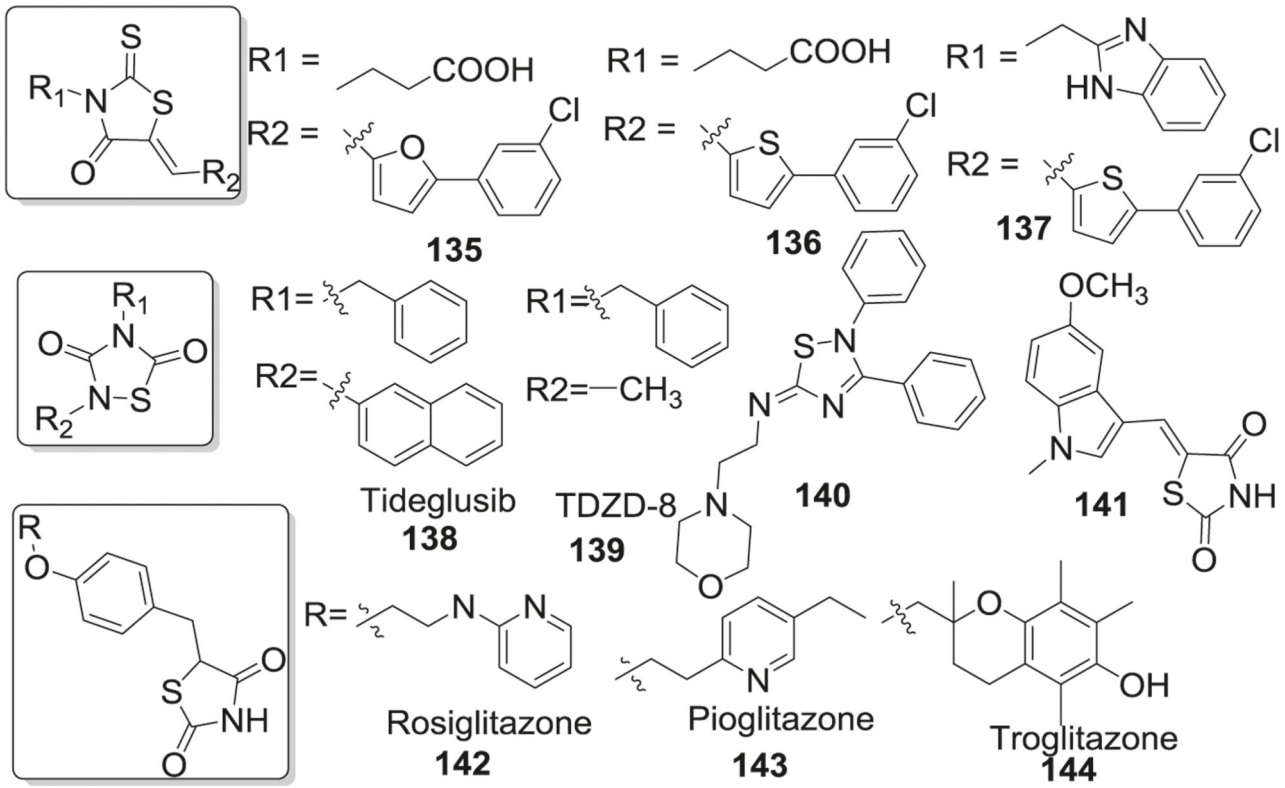

Fig. 28.
Structures of PPARγ agonists containing thiadiazolidinone ring

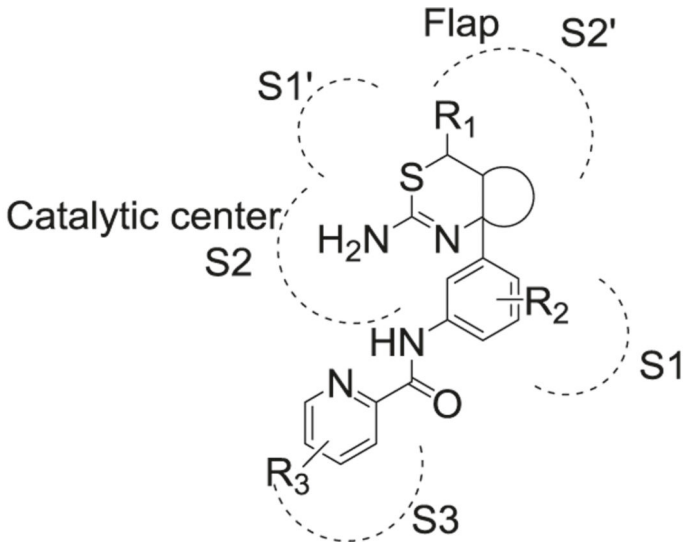

Fig. 29.
Interactions of dihydrothiazine derivatives with BACE-1 active site

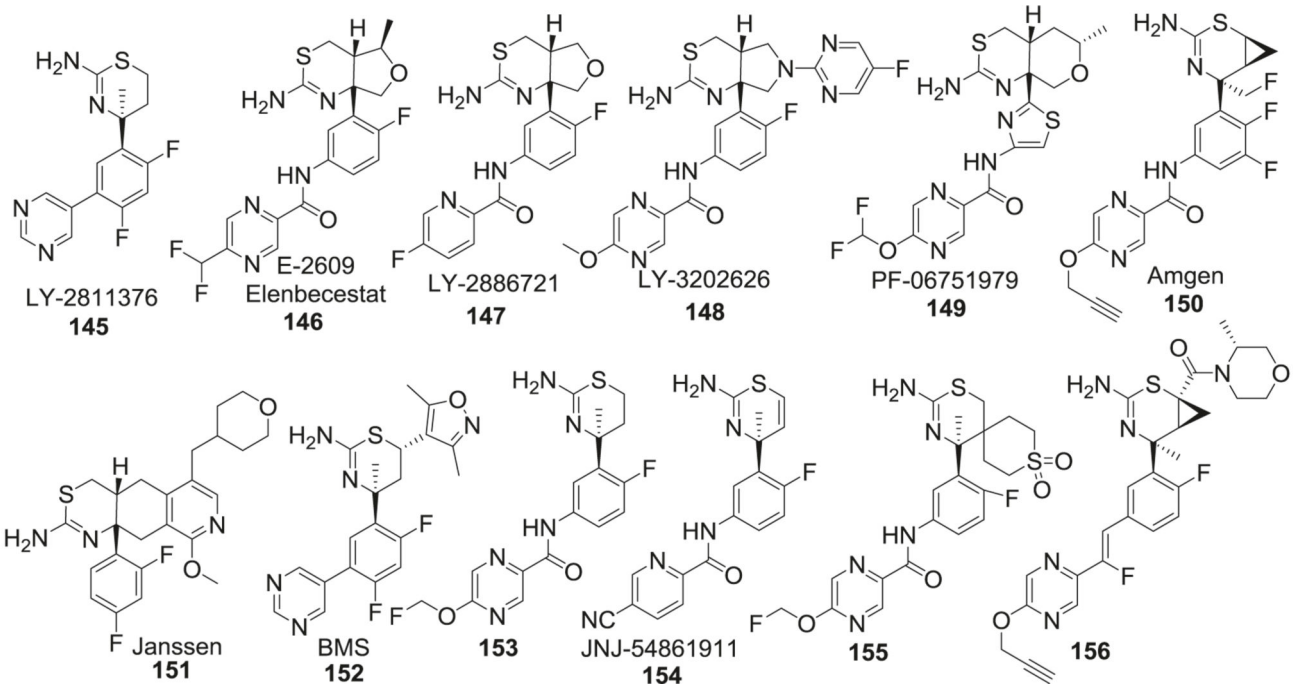

Fig. 30.
Dihydrothiazine containing BACE-1 inhibitors

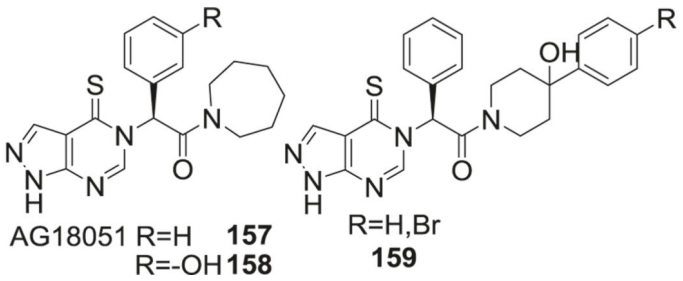

Fig. 31.
Thiamide containing ABAD inhibitors

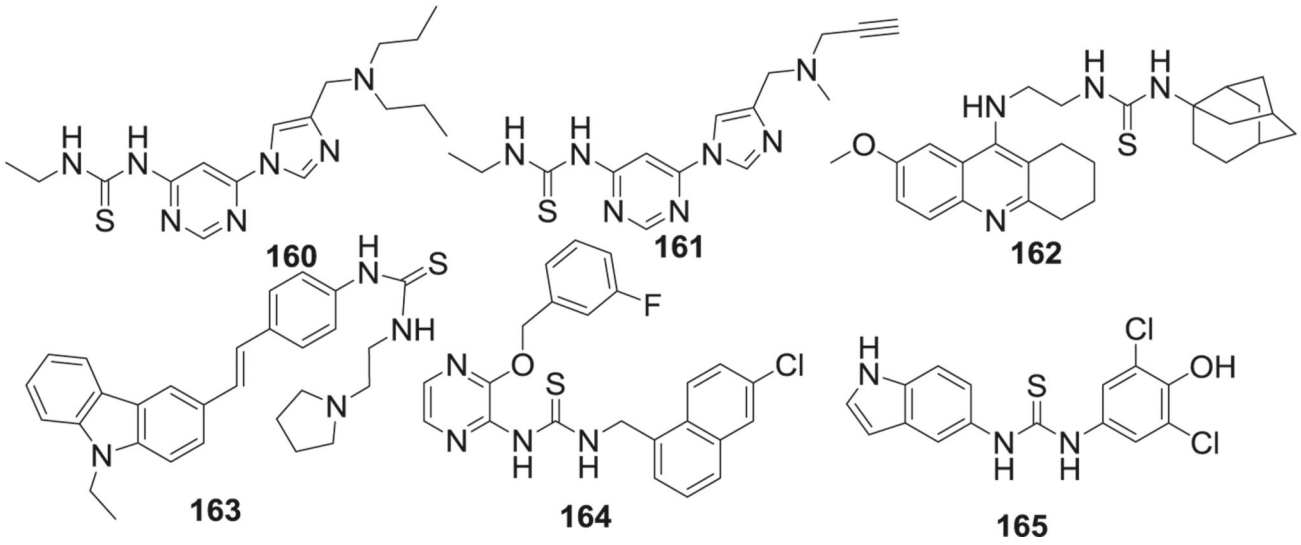

Fig. 32.
Thiourea containing AChE inhibitors

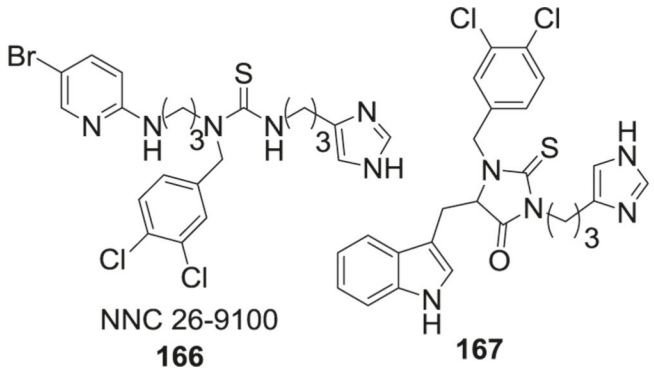

Fig. 33.
sst4 agonists containing thiourea scafflold

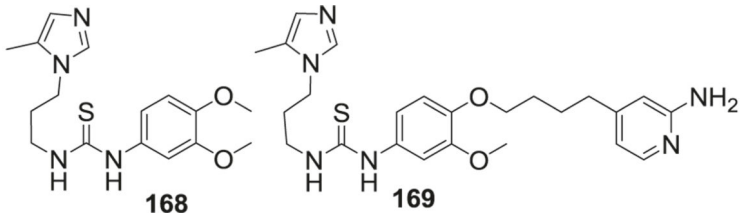

Fig. 34.
QC inhibitors containing thiourea scaffold

Table 1

Sulfur-containing therapeutics in clinical trials

Target (reference)	Drug	Trial status	Outcome	Clinical trial identifier
Cysteine based thiols	NAC	Phase II terminated	No benefit over placebo	NCT00903695
		Phase I	NA	NCT03493178
		Recruiting		
	Cerefolin NAC	Observational	Unknown	NCT01370954
	NAC/vitamin supplement	Phase II completed	Improved cognition and baseline performance	NCT01320527
Glutathione	GSH	Nutritional intervention	Recruiting	NCT03448055
			Completed; observational study	NCT01713816
Alpha lipoic acid [84, 85]	Fish oil/APA	Phase I/Phase II	Completed; no significant benefit	NCT00090402
				NCT01058941
	Lipoic acid/vitamin E/vitamin C	Phase I	No change in Tau pathology	NCT00117403
$5HT_6$ receptor antagonist [224]	Interpirdine (SB-742457)	Phase III	Terminated due to lack of efficacy	NCT02586909
				NCT02585934
	SAM-315	Phase II	Failed to show benefit over donepezil	NCT00710684
				NCT00708552
				NCT00348192
				NCT00224497
		Phase I	Completed	NCT00551772
		Phase I	Discontinued	NCT00480467
				NCT00479440
				NCT00474552
Sulfonic acid	Homotaurine (Tampiprostate, ALZ-801, 3APS, Alzhemed)	Phase III	Discontinued, failed to improve cognition	NCT00088673
				NCT00217763
				NCT00314912
		Phase II completed	No significant benefit over placebo	NCT04422743
		Phase I	Completed; well tolerated in healthy subjects	NCT04157712
				NCT04585347
Sulfonamide, BACE-1 inhibitors [286, 305]	MK-8931 (Verubecestat)	Phase III terminated	Failed to show efficacy	NCT01953601
	MK-8931 (Verubecestat) [286]			

Target (reference)	Drug	Trial status	Outcome	Clinical trial identifier
		Phase II/ Phase III Phase II discontinued	Reduced Aβ levels in MCI patients, discontinued due to adverse events	NCT01739348
		Phase I	Completed; well tolerated in subjects	NCT02910739 NCT01537757 NCT01496170
	Lanabecestat (LY3314814)	Phase III	Terminated, no efficacy over placebo	NCT02783573 NCT02972658
		Phase II/ Phase III	Terminated, no efficacy over placebo	NCT02245737
		Phase I	Completed; well tolerated, no interactions with other drugs	NCT02406261 NCT02540668
	LY2886721	Phase I	Completed; well tolerated, lowered plasma Aβ levels in AD patients	NCT01534273 NCT01807026 NCT01227252 NCT01133405
		PhaseI/Phase II	Phase II terminated due to hepatotoxicity	NCT01561430
	SUVN-502	Phase IIa	Completed; no significant benefit terminated, no significant benefit	NCT02580305
	SAM-760 [287]	Phase II		NCT01712074
		Phase I	Completed; well tolerated in healthy subjects and AD patients	NCT01213355 NCT02005991 NCT01159496 NCT00948662
γ-Secretase inhibitor	Avagacestat [306]	Phase II	Terminated due to gastrointestinal effects	NCT00890890 NCT00810147
		Phase I	Completed; well tolerated in healthy subjects	NCT01454115 NCT01039194 NCT01042314 NCT00901498 NCT01057030 NCT00828646

Target (reference)	Drug	Trial status	Outcome	Clinical trial identifier
	Begacestat	Phase I	Completed; results unknown	NCT00959881
Thiazole, glutamate receptor inhibition	Riluzole	Phase II	Completed	NCT01703117
	Troriluzole (BHV-4157)	Phase II/Phase III	Active, ongoing	NCT03605667
Phenothiazine, tau aggregation inhibition	TRX 0237 (LMTM)	Phase III	Completed; failed to show efficacy	NCT01689233
				NCT01689246
				NCT01626378
				NCT02245568
		Phase III		
		Phase III	Ongoing	NCT03446001
		Phase II	Terminated for administrative reasons	NCT01626391
TDZD	Rosiglitazone, Rosiglitazone XR	Phase III	terminated; no effects on cognition or memory	NCT00550420
			function	NCT00348140
				NCT00490568
				NCT00428090
		Phase II	Completed	NCT00381238
				NCT00334568
				NCT00242593
		Phase I	Completed; well tolerated in healthy and AD subjects	NCT00733785
				NCT00688207
				NCT00468897
Thiourea	LY2811376	Phase I	Completed; renal toxicity and neurodegeneration found	NCT00838084
	LY3202626	Phase II	Terminated; low likelihood of statistical significance	NCT02791191
		Phase I	Completed; well tolerated in AD patients and reduced Aβ in CSF samples	NCT02555449
				NCT02323334
				NCT03023826
	Atabecestat (JNJ-54861911)	Phase II/ PhaseIII	Terminated due to changes in liver enzyme levels	NCT02569398
		Phase II	Completed; liver related adverse events	NCT02260674
		Phase I	Completed; well-tolerated in healthy and AD patients	NCT01827982
				NCT01887535

Target (reference)	Drug	Trial status	Outcome	Clinical trial identifier
				NCT01978548
	Elenbecestat	Phase III	Active	NCT03036280
Thiourea, BACE-1 inhibitor	PF-06751979	Phase I	Completed; well tolerated and reduced Aβ levels in blood	NCT03126721
				NCT02509117
				NCT02793232