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Bridging the gap in Alzheimer's therapy: strategic design and SAR evolution of coumarin-based hybrids as potent MTDLs

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Due to their multitargeting potential, coumarin-based hybrid molecules represent a new and valuable drug development strategy for the treatment of Alzheimer's disease (AD). This review summarizes recent advances in the design, synthesis, and evaluation of coumarin hybrids as multi-target-directed ligands (MTDLs) for AD. This review covers various coumarin hybrid classes, including those incorporating triazole, thiazole, quinoline, chalcone, and other pharmacophores, highlighting their diverse mechanisms of action, such as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition, amyloid-beta (Aβ) aggregation prevention, BACE-1 inhibition, MAO-B modulation, antioxidant activity, and metal ion chelation. Structure–activity relationship (SAR) studies have identified key structural features for optimal activity, including linker length, substitution patterns, and physicochemical properties influencing CNS penetration. Molecular docking and dynamics simulations reveal information about drug–target interactions and demonstrate the ability to engage multiple targets. Coumarin hybrids are already showing ecological *in vivo* efficacy in animal models, and also the desired safety window and metabolic stability make them potential clinical candidates. Future research directions include incorporating emerging therapeutic targets, advanced computational design, and a focus on the microbiome–gut–brain axis to develop more effective and disease-modifying AD therapeutics.

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1. Introduction and pathophysiology of Alzheimer's disease

Alzheimer's disease (AD) is one of the most debilitating neurodegenerative disorders, characterized by a gradual decline of cognition, memory deterioration, and behavioral abnormalities, finally resulting in a dementia state and then death. AD pathogenesis encompasses several interconnected processes such as cholinergic dysfunction, aggregation and accumulation of amyloid-beta (Aβ), hyperphosphorylation of tau protein, oxidative stress, neuroinflammation, dysregulation of metal ions, and mitochondrial malfunction. While several therapeutic agents do exist, such as the acetylcholinesterase (AChE) inhibitors donepezil, rivastigmine, and galantamine, and the NMDA receptor antagonist memantine, currently approved by the FDA, these treatments offer only symptomatic relief without disease-modifying outcomes. The multifactorial aspects of AD have led to efforts towards designing multitarget-directed ligands (MTDLs), which can simultaneously modulate several pathologic routes.[1–5]

The underlying cause is cholinergic dysfunction characterized by low levels of the neurotransmitter acetylcholine, due to its excessive degradation by both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). This cholinergic deficiency is directly associated with cognitive decline and amnesic impairment. Herein, these data support the measure of BACE-1 (β-secretase) inhibition and amyloid-beta aggregation reduction as taking a central stage in Alzheimer's therapy. Recent studies have indicated monoamine oxidase B (MAO-B), another potential target, where inhibition has been observed to induce mitigation of oxidative stress and neurodegeneration.[6,7]

Coumarin is a privileged scaffold used in medicinal chemistry with a bicyclic 2*H*-chromen-2-one core having diverse pharmacophoric characteristics. The coumarin scaffold has its own AChE inhibition activity, MAO-B inhibition, and anti-Aβ aggregation property.[3,8–11]

This review summarizes the literature, with the latest data, from over 50 peer-reviewed papers regarding coumarin-based hybrid molecules as multi-targeted ligands for the disease modification of AD, focusing on cholinesterase inhibition activity and structure–activity relationships, molecular docking analysis, *in vivo* efficacy validation, blood–brain barrier penetration, Pharmacokinetic–Pharmacodynamic (PK–PD) mismatch, and metabolic consideration of coumarin-based hybrids. This review summarizes the state of coumarin in drug discovery and perhaps offers a path to further progress and tailoring to improve the biological activity of coumarin.

2. Synthesized coumarin hybrids in modulation of AD: a paradigm for multi-target anti-AD agents

2.1 Coumarin–triazole hybrids

The 1,2,3-triazole moiety can be synthesized using "click chemistry" and offers structural diversity, hydrogen bonding capacity, and metabolic stability. The strategic dual pharmacophore merging through triazole linkers had yielded hybrid

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compounds that efficiently bind in both the catalytic active site (CAS) and peripheral anionic sites (PAS) of AChE enzyme.[12,13]

A landmark series of coumarin–triazole hybrids demonstrated remarkable dual inhibition of both AChE and BuChE enzymes. The active analogue, compound [**1**] (Fig. 1), belonging to the 8-acetylcoumarin core, exhibited exceptional potency with IC_{50} values of 2.57 μM for AChE, 3.26 μM for BuChE, and 10.65 μM for BACE-1, showcasing multitarget engagement.[12] Notably, this compound crossed the blood–brain barrier *via* passive diffusion and effectively inhibited the self-aggregation of amyloid-beta monomers. The acetyl coumarin core resided at the PAS region, while the benzyl triazole moiety at the catalytic site of AChE, Trp286, joined both coumarin core rings through π–π stacking. Arg296 and Phe296 hydrogens were linked with the C8-acetyl group. The benzyl ring interacted with Trp86 at the anionic subsite; finally, the triazole and coumarin aryl rings stacked with Tyr341 (π–π). Molecular dynamics showed ligand–enzyme complex stability and durability over 50 ns. The protein and ligand RMSD values for the AChE complex remained below 3 Å, demonstrating exceptional stability. Over 95% of the simulation was devoted to Phe295–ligand hydrogen bonding. Hydrophobic interactions with Trp86 and Tyr341 lasted 79% and 56%.[12]

3-Acetyl coumarin scaffold revealed similar results to 8-acetyl coumarin. Dar *et al.*, 2025 attached it to the *N*-bromophenyl-1,2,3-triazole ring through an oxy methyl linker in position 7, compound [**2**]. It had a good inhibition activity against AChE with $IC_{50} = 2.18$ μM relative to the standard inhibitor, eserine. It was proven as a mixed inhibitor with $K_i = 8.13 \pm 0.18$ μM (Fig. 1).[14]

Tacrine was linked to the coumarin scaffold through a 1,2,3-triazole ring. When the linker was elongated, it led to a multi-targeting effect and good activity against AChE and BuChE, compound [**3**] (Fig. 1). Compound [**3**] exhibited a well-balanced inhibitory profile against AChE and BuChE ($IC_{50} = 0.080 \pm 0.007$ μM and 0.044 ± 0.004 μM, respectively) in comparison to the tacrine, which had IC_{50} values of 0.17 ± 0.04 and 0.029 ± 0.005 μM for AChE and BuChE, respectively. It also inhibits the self-induced Aβ aggregation (58.4% ± 2.1% at 20 μM) in relation to curcumin (46.6% ± 2.5% at 20 μM), and inhibited MAO-B ($IC_{50} = 0.18 \pm 0.01$ μM) compared to iproniazid ($IC_{50} = 3.96 \pm 0.35$ μM).[15]

SAR analysis of 1,2,3-triazole-linked tacrine–coumarin hybrids showed key design concepts. Compound [**4**] (Fig. 1) was the strongest anti-AChE derivative ($IC_{50} = 27$ nM). The medication showed strong anti-BuChE action ($IC_{50} = 6$ nM), surpassing tacrine and donepezil as reference medicines. Evaluations showed limited BACE-1 inhibitory activity and neuroprotectivity against $A\beta_{25-35}$-exposed PC12 cells. Finally, compound [**4**] effectively restored scopolamine-induced

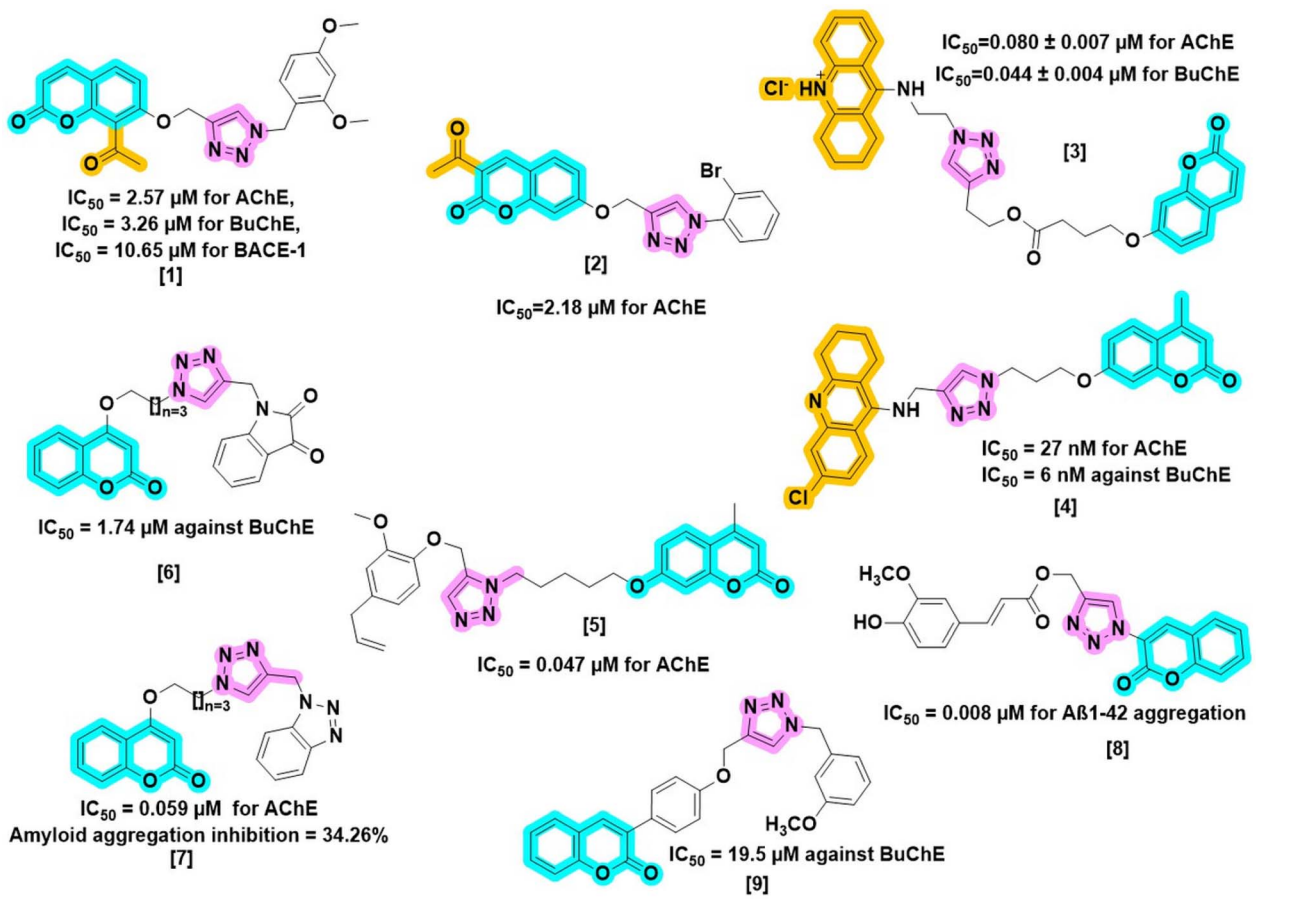

Fig. 1 Reported coumarin–triazole hybrids in modulation of AD.

memory loss in rats in Morris's water maze task. These findings stressed the need for optimum linker length and substitution patterns for multi-target balance.[16]

Triazole-tethered coumarin–eugenol hybrids selectively inhibited AChE ($IC_{50} = 0.047$ μM) and showed desirable $A\beta_{1-42}$ aggregation inhibition (72.21% at 50 μM). Molecular docking and simulation studies validated accommodating binding positions with AChE and the $A\beta_{1-42}$ monomer. Notably, compound [**5**] not only guarded DNA against hydroxyl radicals but was also the most active compound in enhancing memory and learning ability in mice models of scopolamine-induced cognitive deficits, when compared to analogs with shorter (n = 2–5) alkyl linkers. Designation of the triazole-linked eugenol moiety was essential for antioxidant and anti-amyloidogenic activity (Fig. 1).[17]

Singh *et al.* demonstrated the dual-site binding of compound [**5**] through kinetics and molecular docking. The coumarin scaffold was located in the peripheral anionic site (PAS), the triazole nucleus was deeply embedded into the catalytic active site (CAS) and stacked with Trp86, and the eugenol moiety was associated with His447. Given that the six-carbon alkyl chain sufficiently spanned the gorge, the oxygen of the ether linkage could H-bond to Tyr124. The stability of the AChE-compound [**5**] combination was confirmed by molecular dynamics simulations lasting 100 ns. The ligand maintained an average RMSD of 1.24 Å within the active site.

New coumarin–triazole–isatin hybrids (21 compounds) inhibited butyrylcholinesterase selectively over AChE. Lead compound [**6**] (Fig. 1) showed substantial BuChE inhibition (IC_{50} = 1.74 μM), surpassing donepezil's efficacy. Molecular docking proved dual binding to catalytic and peripheral locations, while enzyme kinetics showed mixed-type inhibitory mechanisms. SAR research showed that linker flexibility and substituent steric/electronic effects affect isatin activity.[18]

Beyond cholinesterase inhibition, coumarin–triazole hybrids demonstrate potent anti-amyloid-beta aggregation activity through multiple mechanisms. Some compounds inhibit Aβ self-assembly, while others prevent AChE-induced aggregation, a critical secondary function of this enzyme. For instance, coumarin–benzotriazole hybrid, compound [**7**], (Fig. 1) inhibited copper-induced $A\beta_{1-42}$ aggregation (34.26% at 50 μM) and demonstrated metal chelating properties for Cu^{2+}, Fe^{2+}, and Zn^{2+} ions, along with DNA protective potential against hydroxyl radical damage.[19]

Coumarin triazolyl hybrid, compound [**8**], (Fig. 1) had a good neuroinflammatory response in amyloid beta aggregation-mediated oxidative stress and neuroinflammation. It decreased the expression of the proinflammatory cytokines, such as TNF-α, by 1.09 ± 0.09% relative to the $A\beta_{1-42}$-treated group (1.73 ± 0.22%). It also inhibited the expression of IL-1β by 0.71 ± 0.14% compared to the $A\beta_{1-42}$-treated group (1.53 ± 0.13%).[20]

When attached to 3-methoxy *N*-benzyl triazole through a phenyl oxy methylene linker at position 3, the coumarin core gave a moderate activity against BuChE with $IC_{50} = 19.5 \pm 0.9$ μM and no activity against AChE, compound [**9**] (Fig. 1). On the other hand, it had an exceptional neuroprotective impact, surpassing the standard medicine (quercetin) on the PC12 cell model damaged by H_2O_2, and greatly diminished aggregation of amyloid $A\beta_{1-42}$, exhibiting a potency 1.44 times greater than donepezil and inhibited 15-LOX (IC_{50} = 39.1 μM). Compound [**9**] exhibited more efficacy than butylated hydroxytoluene (BHT), the reference antioxidant agent, in diminishing H_2O_2 levels induced by amyloid β in BV2 microglial cells. Docking results on BuChE showed that the benzyl moiety interacted with Trp82, whereas the triazole ring formed T-shaped connections with His438 and Trp82. Finally, the coumarin core was stabilized with Tyr332.[21]

In conclusion, molecular docking studies have given extensive mechanistic details of the interactions of coumarin–triazole hybrids with cholinesterases. Collectively, these studies show that potent inhibitors bind to the extended AChE binding site, with critical interactions localized in both the CAS (catalytic active site) and PAS (peripheral anionic site). The triazole linker provides the proper distance between the coumarin and the second pharmacophore, which may be engaged simultaneously at spatially separated sites.[16]

Molecular dynamics simulations revealed that ligand–enzyme complexes are relatively stable, while most active compounds displayed the binding free energies from −8 to −12 kcal mol^{-1}. Both coumarin's aromatic rings and the attached pharmacophores undergo π–π stacking interactions with key aromatic residues (notably Trp86, Tyr121, and Phe330 in the AChE gorge), while the triazole and adjacent polar groups make crucial hydrogen bonds with Glu202 and other active site residues.[12,16]

2.2 Coumarin–thiazole and coumarin–oxadiazole hybrids

Coumarin–thiazole derivatives represent another important class of multifunctional agents. Ibrar *et al.* synthesized a series of 15 coumarin–thiazole hybrids, compounds [**10a–o**], which were evaluated for AChE and BuChE inhibition. In the thiazole series, the amino-containing derivative (Fig. 2) emerged as the most active AChE inhibitor ($IC_{50} = 0.87 \pm 0.09$ μM). Molecular docking studies revealed that potent members interacted with the active site *via* π–π stacking with aromatic residues and water-bridged hydrogen bonding networks (MOE scores ranging from −10.19 to −11.03 kcal mol^{-1}).[22]

A novel series of *N*-substituted thiazole–coumarin derivatives (120 compounds) was designed and synthesized through systematic pharmacophore hybridization. Molecular property filter analysis and molecular docking identified 11 promising hybrids, with compound [**11**] (Fig. 2) exhibiting strong binding interactions. *In vitro* anticholinesterase activity results demonstrated that the compound showed potent activity with IC_{50} values of 2.00 ± 0.09 μM against AChE and 34.93 ± 0.62 μM against BuChE.[23]

Eight coumarin–oxadiazole derivatives were synthesized by Ibrar *et al.* The alkylated agent, compound [**12a**] (Fig. 3), was identified as the lead candidate for AChE inhibition ($IC_{50} = 6.07 \pm 0.23$ μM), with compounds [**12a–c**] showing exceptional BuChE inhibition (IC_{50} = 0.15 μM). The oxadiazole series derivatives showed high anti-butyrylcholinesterase activity with

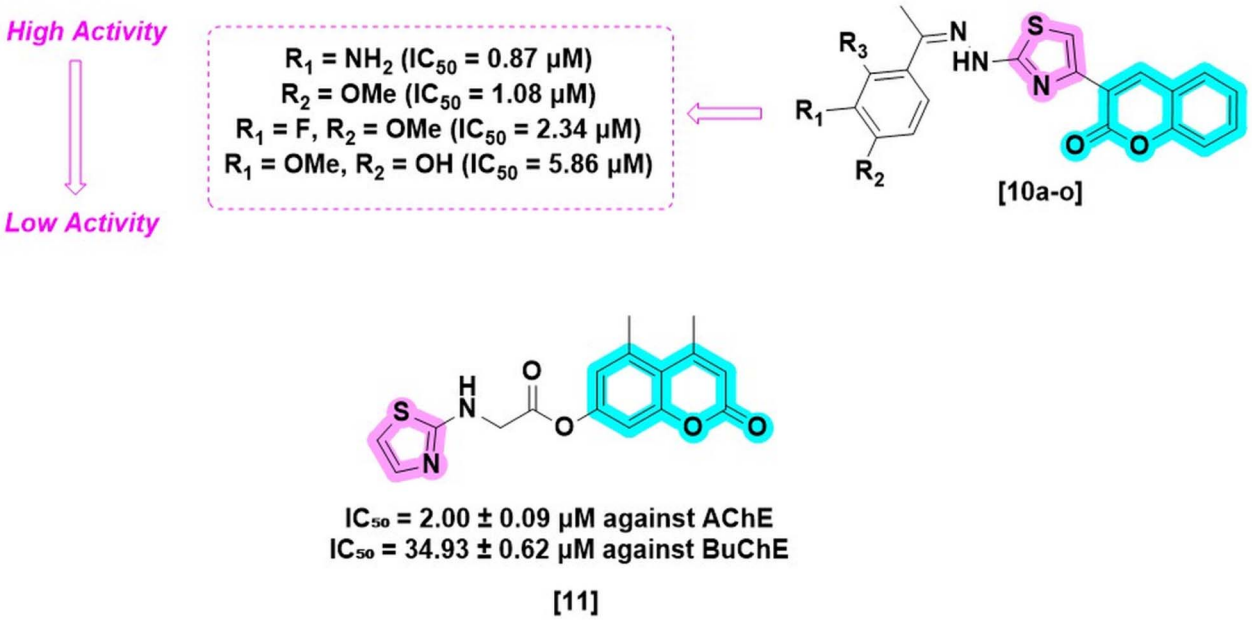

Fig. 2 Reported coumarin–thiazole derivatives against AChE enzyme.

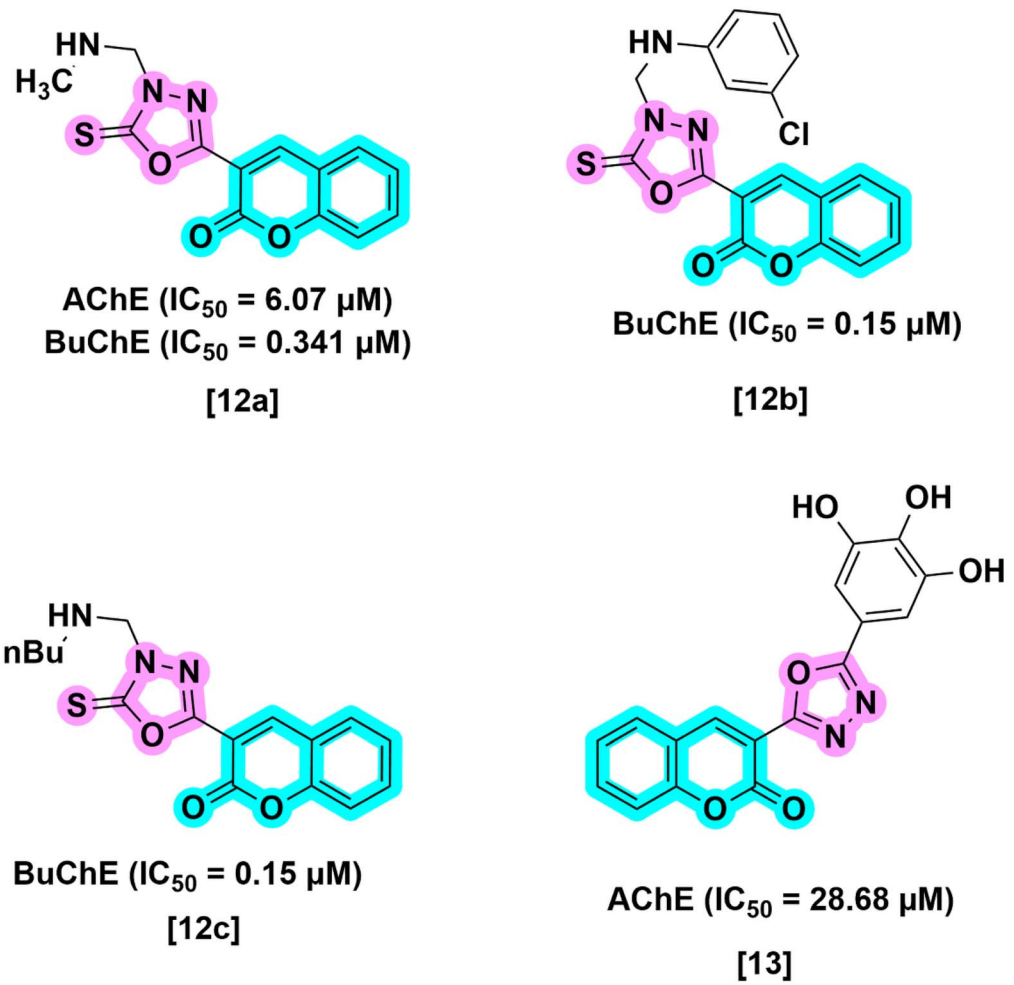

Fig. 3 Reported coumarin–oxadiazole derivatives against AChE and BuChE enzymes.

compounds binding energies of −9.9 to −8.2 kcal mol^{-1}, featuring important Trp82-bridged interactions.[22]

4-methylcoumarin is linked by an oxymethylene bridge to 1,3,4-oxadiazole, which has a trihydroxy phenyl group at position 2. Compound [**13**] acted as a multi-target ligand with inhibition of AChE with IC_{50} = 28.68 μM, with a high selective index = 1.65, and it imparted mild inhibition of BuChE with IC_{50} = 28.68 μM. Compound [**13**] (Fig. 3) also had good antioxidant activity with IC_{50} = 65.57 μM and anti-inflammatory effect against COX with 71.74% inhibition. Docking studies against AChE showed that the oxygen atom of the oxadiazole moiety engaged with Tyr121, supported by pi–pi stacking contacts with Trp84, Phe330, and Phe331, as well as pi–alkyl interactions with Tyr334. Finally, the m-OH group established a vital hydrogen bond with Phe288.[24]

2.3 Coumarin–quinoline hybrids: multi-target directed ligands

Coumarin and quinoline scaffolds have been strategically combined to create potent multi-target-directed ligands. A series of 24 novel coumarin–quinoline hybrid compounds were rationally designed and synthesized, targeting Aβ aggregation, cholinesterase inhibition, and BACE1 inhibition simultaneously. The combination of the two synthetic scaffolds is known for its distinct neuroprotective and enzymatic inhibitory properties. This strategy sought to develop multi-target-directed ligands (MTDLs) that can concurrently regulate cholinergic signaling and oxidative stress. In compound [**14**], the coumarin scaffold was attached to the quinoline through a five-carbon alkyl chain as a linker. Compound [**14**] emerged as a promising lead, exhibiting effective Aβ binding and significantly attenuating Aβ-induced SH-SY5Y cell death by lowering oxidative stress and decreasing cellular apoptosis.[25]

Importantly, compound [**14**] (Fig. 4) demonstrated excellent blood–brain barrier permeability, and intragastric administration to 7 month-old APP/PS1 transgenic mice resulted in improved cognitive function. This improvement was supported by protection of hippocampal and cortical neurons from necrosis, attenuation of oxidative stress and inflammation, and reduction in Aβ deposition. It demonstrated considerable efficacy and strong selectivity for BuChE compared to acetylcholinesterase (AChE). It inhibited human BuChE with a value of 0.15 M, while its activity against AChE was quite weak (>5 M). This selectivity profile was clinically significant for moderate-to-advanced stages of Alzheimer's disease, characterized by an increase in BuChE levels concomitant with a decrease in AChE levels. These findings highlight the potential of coumarin–quinoline hybrids as a novel class of AChE inhibitors.[25]

Earlier coumarin–quinoline hybrids showed small structural variations contributing to dual acetyl/butyrylcholinesterases activity or selectivity toward one enzyme. Compound [**15**] (Fig. 4) proved to be the most promising compound in its series, displaying selective AChE inhibition and serving as an excellent iron chelator with 72.87% iron chelation at 100 μM. Molecular docking studies established the nature of interaction between binding pockets and target enzymes, revealing well-defined π-stacking with Phe330 and hydrogen bond interactions with Tyr121 residues.[26]

2.4 Coumarin hydrazones hybrids

Three compounds derived from an 8-methoxy coumarin scaffold, linked to a substituted aryl hydrazone at position 3, were synthesized and evaluated as anti-AD compounds [**16–18**] (Fig. 5).[27] The aryl group in compound [**16**] consisted of hydroxy naphthol, in compound [**17**] it comprised a trifluoromethyl phenyl moiety, and finally, compound [**18**] featured a pyridine moiety. The synthesized hybrids had significant inhibitory efficacy against three principal Alzheimer's disease targets, namely AChE, BuChE, and MAO-A.

For the first target, (AChE), lead compounds [**16–18**] demonstrated notable potency with values of 7.40 μM, 8.01 μM, and 8.54 μM, respectively. Compound [**16**], including a hydroxy naphthol moiety, was recognized as the most effective AChE inhibitor in the series. The three compounds showed a good overall inhibitory activity against BuChE, ranging from 65.41 to 79.30 μM. The hybrids demonstrated remarkably low micromolar potency against the last target, MAO-A, with [**16**] (1.44 μM), [**17**] (1.51 μM), and [**18**] (1.87 μM).[27]

As revealed by the docking scores and analysis of biological activity, compound [**16**] was found to bind more efficiently to

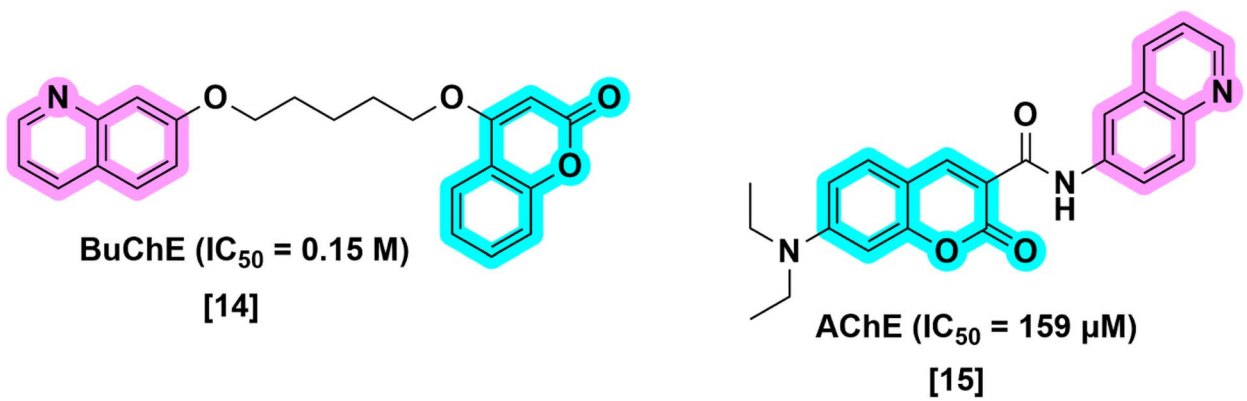

Fig. 4 Reported coumarin–quinoline hybrids against AChE and BuChE enzymes.

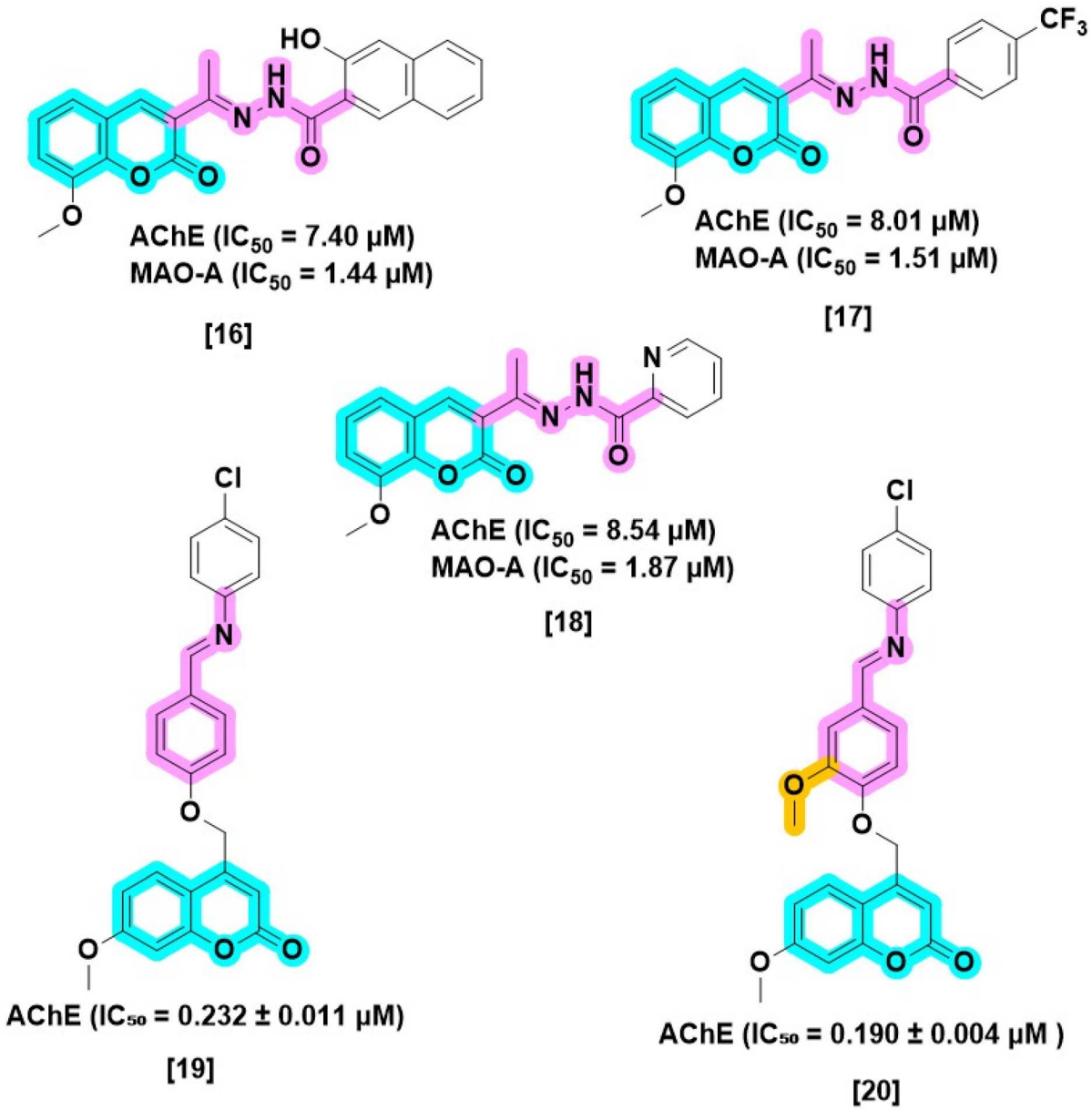

Fig. 5 Coumarin–hydrazones as multi-targets in AD.

hAChE and MAO-A than any other compound. Therefore, it has the highest binding affinity to hAChE and MAO-A from this binding site, which is largely due to extensive hydrophobic interactions. As discussed in [**16**], a number of polar functional groups, including hydroxyl and amine moieties, served to enhance these interactions and overall binding. Compound [**17**] demonstrated enhanced affinity for the hBuChE target in computational models, due to the interaction of its phenyl group with hydrophobic residues, notably phenylalanine (PHE) and tyrosine (TYR), in the binding pocket. The stability of the [**17**]-hBuChE complex was enhanced by essential hydrogen bonding and π-stacking interactions. The docking simulations indicated that these structures with a balanced combination of hydrophobic moieties, aromatic rings for π-stacking, and polar groups for hydrogen bonding exhibited increased enzyme affinity and enhanced inhibition efficacy.[27]

A related series of coumarin–Schiff base hybrids showed excellent inhibitory activity against AChE with IC_{50} values ranging from 87.84 to 515.59 ng ml^{-1}, with hybrids [**19**] and [**20**] (Fig. 5) showing the most potent activity (IC_{50} values of 0.232 ± 0.011 and 0.190 ± 0.004 μM, respectively), surpassing the reference drug galantamine.[28]

2.5 Coumarin–indole and coumarin–carbazole hybrids: neuroprotective multi-functional agents

Coumarin–indole hybrids represent an emerging class of multifunctional agents combining the AChE inhibitory and metal-chelating properties of coumarin with the antioxidant

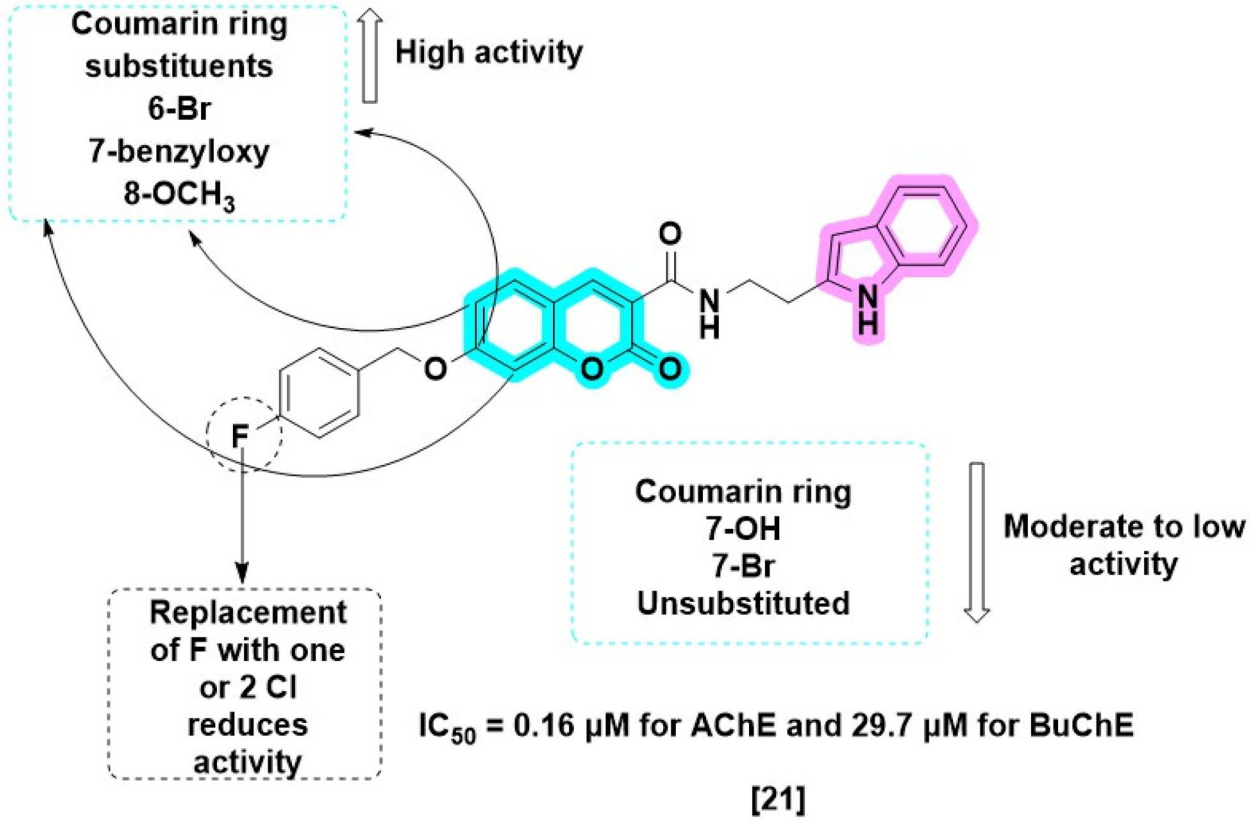

Fig. 6 Reported coumarin–indole hybrid for AD, along with SAR study.

and neuroprotective capabilities of indole scaffolds. These hybrids have demonstrated superior activity in multiple *in vitro* and *in silico* models of AD pathology.

Ghanie-Nasab *et al.* created and tested *N*-(2-(1*H*-indol-3-yl) ethyl)-2-oxo-2*H*-chromene-3-carboxamides for inhibiting AChE and Aβ aggregation. Due to its capacity to connect with PAS on ChE, coumarin was combined with tryptamine's indole scaffold linked *via* ethyl carboxamide. Cyclization of substituted salicylaldehyde with diethyl malonate in ethanol with piperidine and alkaline hydrolysis produced substituted coumarin-3-carboxylic acids. Carboxamide-linked coumarin–indole hybrids were obtained by condensing coumarin acid chlorides with tryptamine and potassium carbonate in dry toluene. These compounds can be produced in 5 min by microwave irradiation with acetonitrile solvent. Coumarin–indole hybrids showed potent inhibitory activity (IC_{50} = 0.16–43.8 μM) and selectivity for AChE over BuChE. The most effective AChE inhibitor was *N*-(2-(1*H*-indol-3-yl)ethyl)-7-((4-fluorobenzyl)oxy)-2-oxo-2*H*-chromene-3-carboxamide, compound [**21**], (Fig. 6), (IC_{50} = 0.16 μM for AChE and 29.7 μM for BuChE; SI = 185.6). Its ability to bind at both PAS and CAS on the enzyme made it a dual site inhibitor in molecular docking tests.[29] During its interaction with Trp84, the fluorophenyl fragment was positioned towards the bottom of the active site. Other π–π interactions were discovered between the coumarin ring and Tyr334 and indole with Trp279 residue at PAS. Kinetic investigations reinforced the mixed inhibition by compound [**21**] (0.49 μM). According to SAR research, substituting the coumarin ring can boost or reduce anti-ChE action. A benzyloxy group at the 7th position, -methoxy group at the 8th position, or -bromo group at the 6th position increased AChE inhibitory activity, while -bromo or -hydroxy groups at the 7th position created inert molecules. The addition of an *O*-alkyl/benzyl group at the 7th position showed mild to moderate inhibitory efficacy and outperformed unsubstituted coumarin hybrid compounds. Replacement of a fluorine atom on the benzyl moiety with one or two chlorine atoms reduced anti-AChE action.[29]

The carbazole scaffold is a natural phytochemical formed in plants with different biological activities. The carbazole scaffold was connected to the 4-methyl coumarin using oxy pentyl

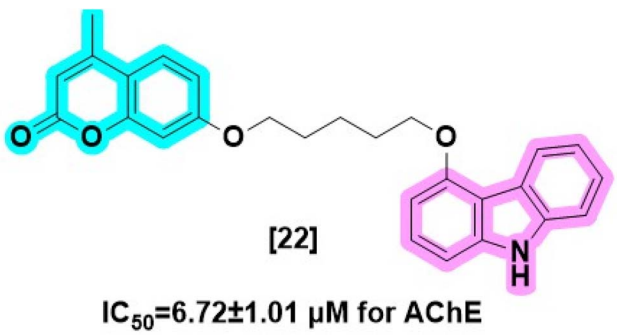

Fig. 7 Reported coumarin–carbazole hybrid for AD.

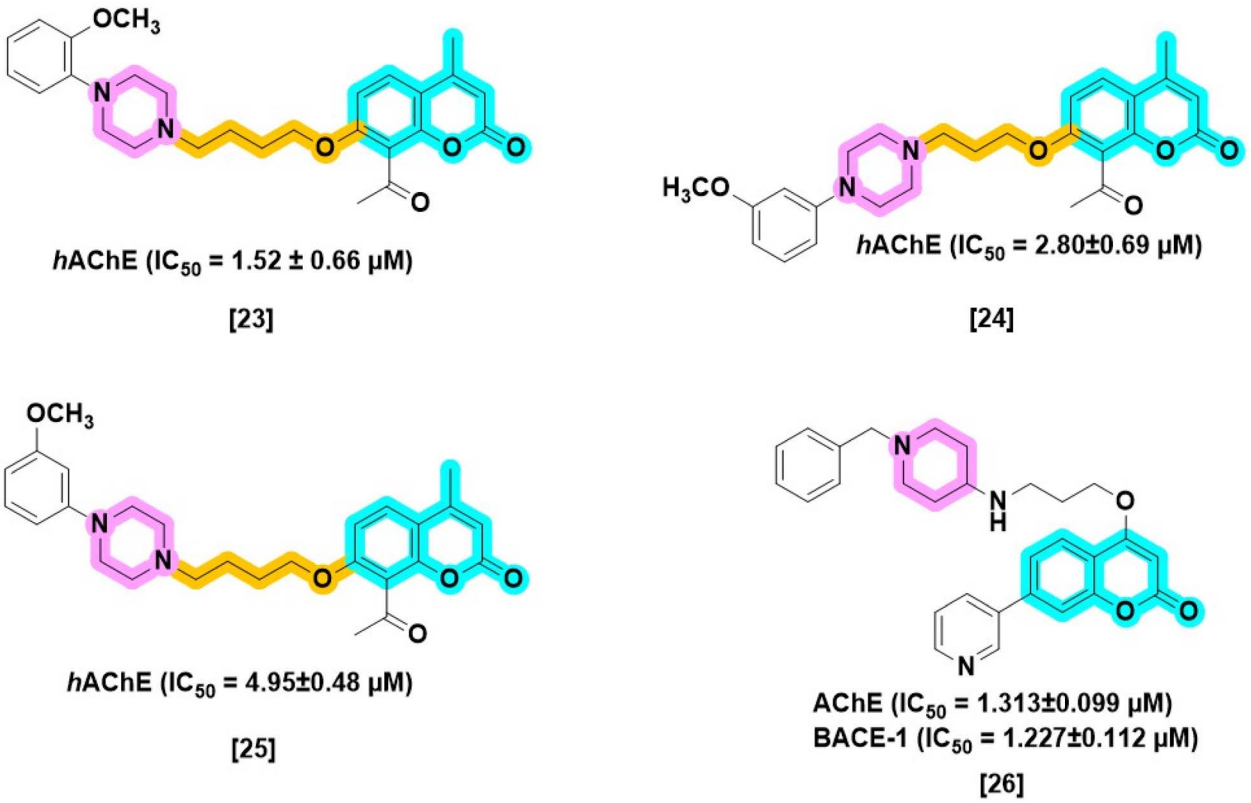

Fig. 8 Coumarin–piperazine and coumarin–piperidene hybrids in modulation of AD.

linker, compound [**22**] (Fig. 7). It had a moderate inhibitory activity for AChE with $IC_{50} = 6.72 \pm 1.01$ μM compared to tacrine with $IC_{50} = 0.18 \pm 0.02$ μM.[30]

2.6 Coumarin–piperazine and coumarin–piperidene hybrids

Arylpiperazinyl–coumarin hybrids, compounds [**23–25**], (Fig. 8), were designed to concurrently regulate cholinergic signaling and monoamine oxidase (MAO) activity, targeting both cognitive impairments and oxidative stress in Alzheimer's disease (AD). Coumarin was tri substituted in position 8 with acetyl group and in position 4 with methyl group while the 7-OH group was attached to substituted phenyl piperazinyl through a four carbon or three carbon alkyl chain as a linker giving a good activity as a MTDLs.[31] Compound [**23**], formed of *O*-methoxyphenyl piperazinyl attached to the coumarin scaffold through an four carbon alkyl chain linker, showed a good inhibition against hAChE than compounds [**24**] and [**25**] with $IC_{50} = 1.52 \pm 0.66$ μM. Compounds [**24**] and [**25**] are *m*-methoxyphenyl piperazinyl attached to the coumarin scaffold through different linkers. In the case of compound [**24**], the linker is a three-carbon alkyl chain, while in compound [**25**], it is a four-carbon alkyl chain linker. Compounds [**24**] and [**25**] had IC_{50} against hAChE equal to 2.80 ± 0.69 and 4.95 ± 0.48 μM, respectively. Compared to AChE, most derivatives were largely inactive against BuChE or displayed significantly higher values.[31]

Computational simulations revealed a dual-site binding mechanism in which the hybrid molecules span the whole approximately 20 Å deep enzymatic gorge of hAChE. The CAS-binding component was in "folded" conformations (such as compound [**24**]), the coumarin moiety bonded to the catalytically active site (CAS), forming hydrophobic contacts with Trp86 and hydrogen bonds with Gly121 and His447. In "extended" conformations (such as compound [**23**]), the 2-methoxyphenyl substituent approached the CAS region, establishing π-interactions with Trp86 and His447. The protonated nitrogen of the piperazine ring served as a cationic core, emulating the quaternary ammonium of acetylcholine to stabilize.[31]

These flexible alkyl chains (3-carbon propylene or 4-carbon butylene) constituted crucial structural spacers. MD simulations suggested that the butylene linker in compound [**23**] promoted an extended conformation to sufficiently cover the distance between sites, while the propylene linker in compound [**24**] induced a folded conformation that reoriented the coumarin core toward the CAS. Regarding the PAS-binding domain, the most potent hAChE inhibitors [**23**] and [**25**] employed a planar coumarin scaffold that filled the peripheral anionic site (PAS). It made strong double stacking contacts with Trp286 and π–alkyl interaction with Leu289. The 8-acetyl substituent formed important hydrogen bonds with PAS residues Ser293 and Arg296, thus improving the stability of the complex at the entrance of the enzyme.

In conclusion, the arylpiperazinyl–coumarin hybrids provided a "bridge-like" binding mechanism, wherein the piperazine and its aryl moiety secured the catalytic depths, while the acetylated coumarin core protected the peripheral

entrance, potentially obstructing both acetylcholine hydrolysis and aggregation.

Compounds [**24**] and [**25**] (Fig. 8), were identified as highly balanced multi-target drug-like molecules (MTDLs), exhibiting robust and concurrent inhibition of both human acetylcholinesterase (hAChE) and human monoamine oxidase A (hMAO-A).[31]

Liu *et al.* designed and synthesized *N*-benzylpiperidine–coumarin hybrid comprised of a coumarin core connected to an *N*-benzylpiperidine fragment through an oxypropyl amine linker at C-4 of coumarin and a pyridine ring attached to C-7 of the coumarin scaffold, compound [**26**]. IT showed multi-targeting effect against AChE, BACE-1, and GSK-3β. IT inhibited AChE with $IC_{50} = 1.313 \pm 0.099$ μM and was more selective to AChE over the BuChE with SI = 24.623. Kinetic enzyme studies showed an increase in the slope and intercept with an increase in the inhibition concentration, which indicated a competitive inhibitor. IT showed inhibitory effect against BACE-1 with $IC_{50} = 1.227 \pm 0.112$ μM and GSK-3β with 19.30% inhibition at 20 μM.[32]

Docking studies showed for AChE that the *N*-benzyl group interacts with Trp86 (CAS), the piperidine ring formed a hydrophobic interaction with Tyr341, but the coumarin core was stabilized at the PAS by Trp286. While the docking studies in GSK-3 showed that the coumarin core engaged in a hydrogen bond interaction with the Lys85 side chain, the NH of the side chain established a hydrogen bond with the critical amino acid Val135. Cys199 and Phe67 established hydrophobic interactions with the coumarin core. Nonetheless, the attachment of the side chain directed the compound into a potentially unfavorable orientation for binding to GSK-3β, resulting in a reduction of binding energy between the compound and GSK-3β. On the other hand, upon docking of that compound to BACE-1, it was revealed that it had hydrogen bond interactions with the essential active-site amino acid Gly230. In the absence of interactions with Asp228, compound [**26**] exhibited additional Pi–Pi interactions involving Ile110 and Tyr198 with the coumarin core and tail of the structure.[32]

2.7 Coumarin–pyridine hybrid

When the 3-fluoro benzyloxy coumarin scaffold was attached to the hydroxypyridinone in position 4 through a suitable linker, as shown in compound [**27**] (Fig. 9), it had a good activity against MAO-B enzyme, with an IC_{50} of 99.3 nM compared to the pargyline reference drug that had an IC_{50} of 86.9 nM. Docking studies showed the interaction of coumarin with lipophilic amino acid residues at the substrate cavity, hydroxy pyridinone interacted with FAD, and the 3-fluorobenzyl group was located at the entrance of the cavity of the enzyme. That compound also showed a good activity as an iron chelating agent and a good antioxidant effect.[33]

A series of hydroxypyridinone–coumarin hybrids was rationally developed, synthesized, and biologically assessed for iron ion chelation and MAO-B inhibition. Most compounds chelated iron ions well and had moderate to good anti-MAO-B activity. Compound [**28**] (Fig. 9) showed the most potential against MAO-B, with an IC_{50} of 14.7 nM, this compound had a shorter linker than [**27**]. Furthermore, [**28**] effectively protected U251 cells and greatly improved cognitive function in scopolamine-induced AD mice. The probable ligand–receptor interaction was determined

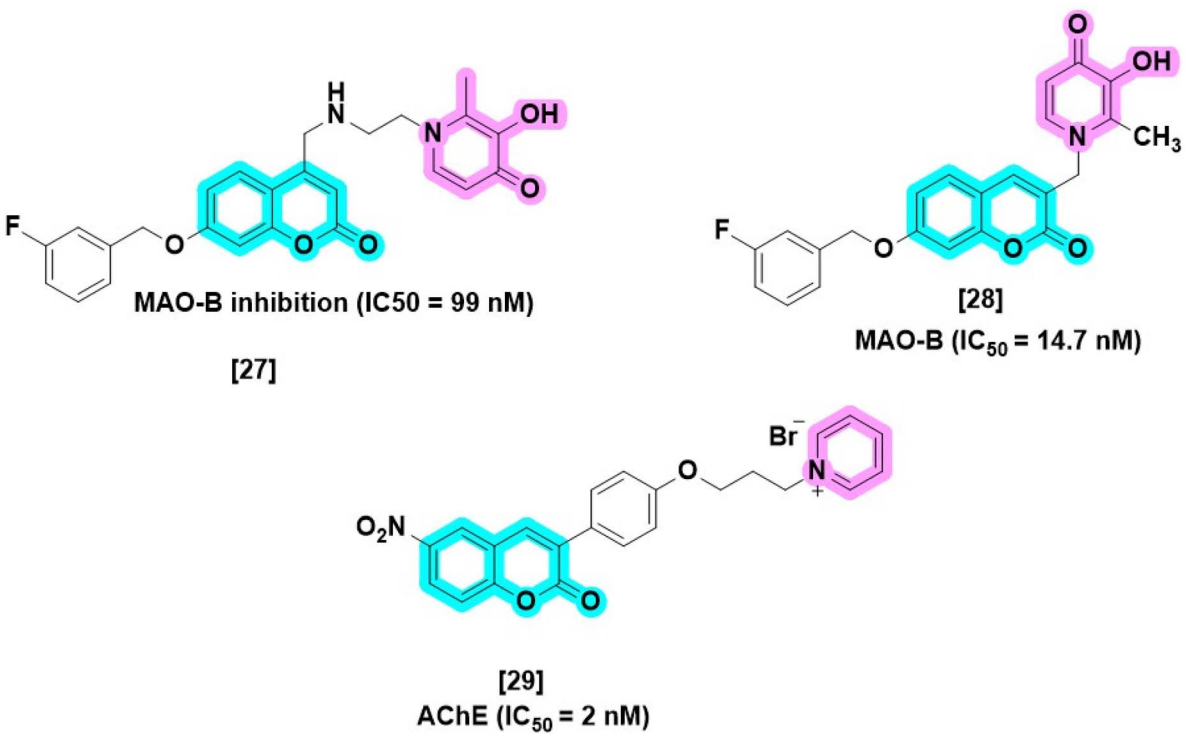

Fig. 9 Reported coumarin–pyridinium salt and hydroxypyridinone hybrids against AD.

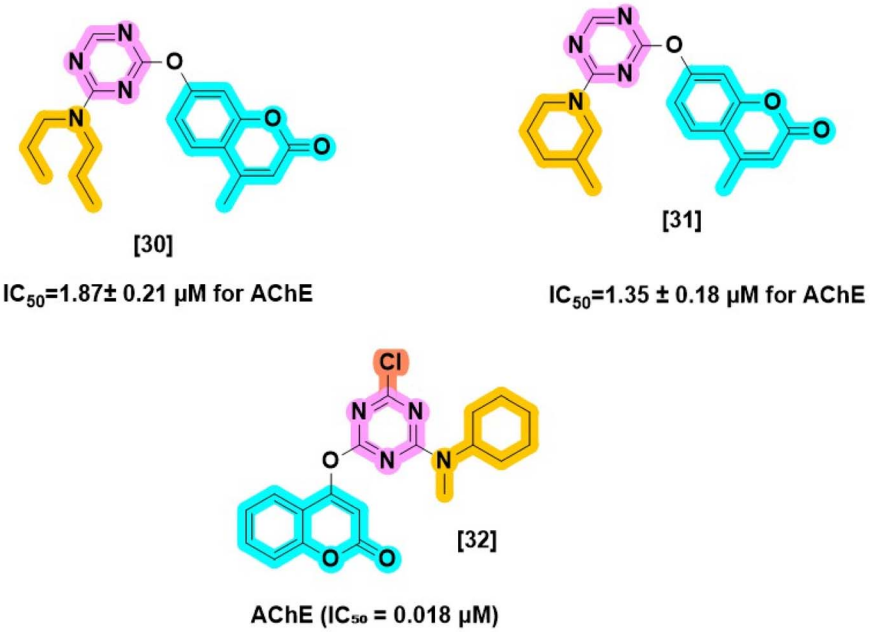

Fig. 10 Reported coumarin–1,3,5-triazine hybrids against AChE enzyme.

by molecular docking, and structure–activity connections were presented[34]

Novel 3-phenyl-coumarin derivatives containing pyridinium salts represent another important hybrid class. A series of coumarin-based scaffolds linked to pyridine derivatives *via* flexible aliphatic linkages demonstrated exceptional potency. Compound [**29**] (Fig. 9), emerged as the best AChE inhibitor (IC_{50} = 2 nM) with acceptable BuChE inhibition activity (IC_{50} = 24 nM), approximately 100 times more active than the standard drug donepezil. This compound could significantly protect PC12 and SH-SY5Y cells against H_2O_2-induced cell death and amyloid toxicity, superior to standard drugs.[2]

Docking studies results for AChE showed that it interacted with Trp84 (anionic site) and established π–π stacking with Phe330. The nitro group at position 6 established hydrogen bonds with Phe288 and Arg289. Finally, the 3-carbon chain length of the aliphatic linker was identified as the optimum length to maintain high potency.

2.8 Coumarin–1,3,5-triazine derivatives

The 4-methyl coumarin scaffold was attached to a substituted 1,3,5-triazine ring at position 4 of triazine, as in compounds [**30**, **31**] (Fig. 10). Compound [**31**], which is substituted at position 4 of the triazine ring with methyl piperidine, had a good activity against AChE with $IC_{50} = 1.35 \pm 0.18$ μM compared to donepezil with $IC_{50} = 0.052 \pm 0.004$ μM. When substituted with a dipropyl amino group in position 4 of the triazine ring, it decreased the IC_{50} = 1.87 ± 0.21 μM compared to compound [**30**]. During enzyme kinetics studies, both of these compounds acted as mixed inhibitors with K_i = 9.77 μM and 12.56 μM for compounds **30** and **31**, respectively. Both compounds can inhibit the Aβ aggregation according to the propidium iodide displacement assay, which indicates both agents can be effectively conjugated to the PAS site of acetylcholinesterase.[35]

Comparative molecular docking revealed that compound [**32**] (Fig. 10), a coumarin–1,3,5-triazine demonstrated the strongest inhibitory effect on acetylcholinesterase with an IC_{50} value of 0.018 μM, closest to the donepezil control (IC_{50} = 0.016 μM). Enzyme kinetic studies indicated mixed-mode inhibition, while molecular docking confirmed stable interaction with key amino acids in both the catalytically active site and peripheral anionic site. The chloro-substituted triazine, along with the cyclohexane group had a positive impact on the inhibitory effect of the compound.[36]

2.9 Aminocoumarin enaminones and 6-aminocoumarin-based derivatives

The synthesis of 6-aminocoumarin enaminones as Multi-Target Directed Ligands (MTDLs) showed a notable progress in targeting the different pathways of (AD). This class of drugs was particularly designed to concurrently regulate cholinergic insufficiency, glycogen synthase kinase 3 (GSK-3) activity, and the aggregation of both amyloid-beta and tau proteins. Specifically, compounds [**33**] and [**34**] (Fig. 11), formed of the 6-amino coumarin scaffold attached to a hydrophobic tail, substituted aryl group, or heterocycle through enaminone as a linker. The substituted aryl group in [**33**] was a 4-methoxy phenyl moiety, while in the compound [**34**], it was a thiophene ring.[1]

Both compounds [**33**] and [**34**] showed good activity against hAChE with IC_{50} = 28.88 nM and 26.03 nM, respectively, and hBuChE with IC_{50} = 131.90 nM and 90.02 nM, respectively. Enzyme kinetics study against AChE showed a mixed inhibitory effect, which means binding to the PAS and CAS binding sites of the enzyme.[1]

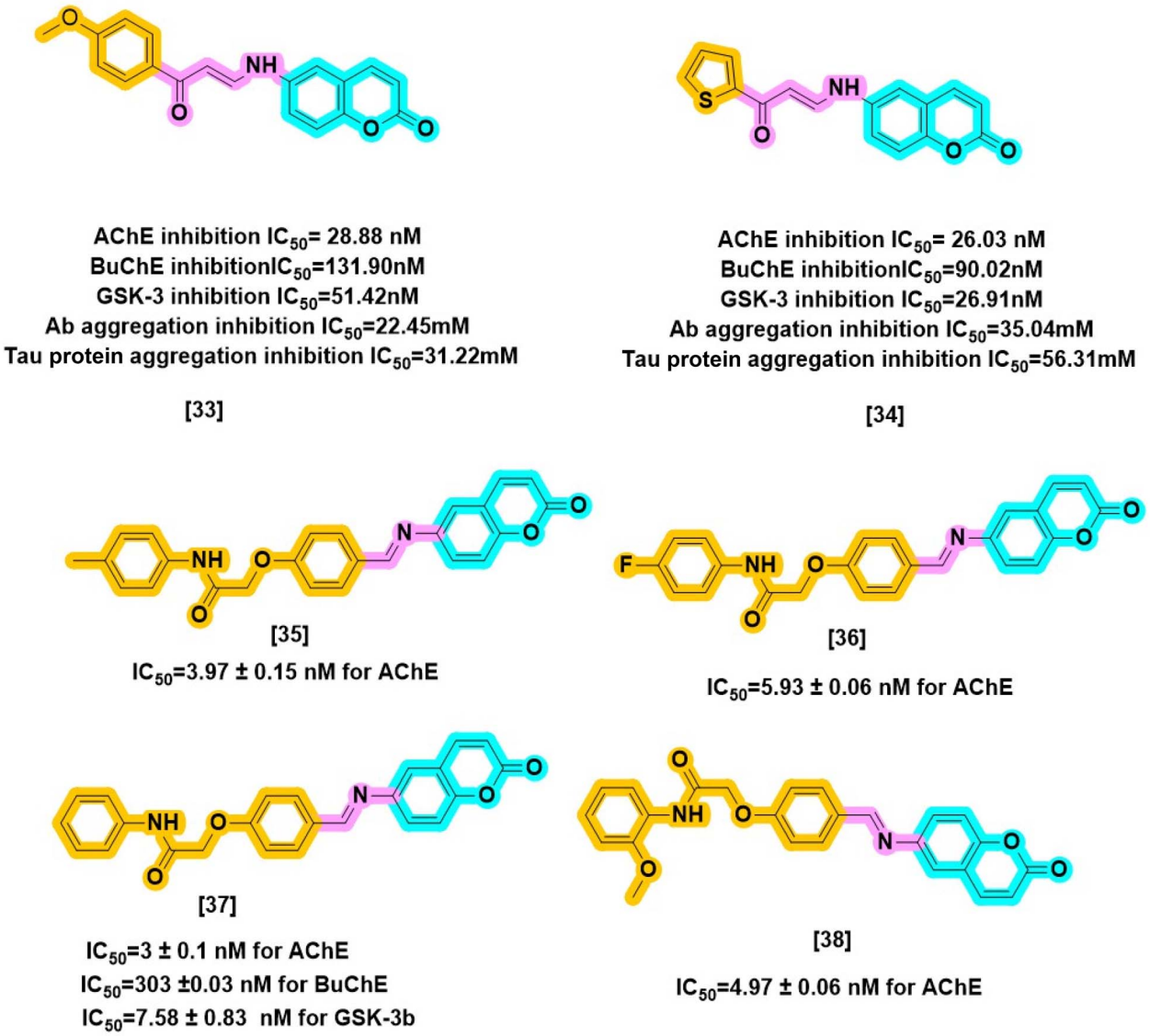

Fig. 11 Reported aminocoumarin enaminones and 6-aminocoumarin-based hybrids against AD.

As for GSK-3 enzyme inhibition, both compounds acted in the nanomolar range (Fig. 11), exhibiting 4 to 8 times greater efficiency compared to donepezil (IC_{50} = 219.10 ± 5.82 nM). Protein aggregation studies indicated similar results where compounds [**33**] and [**34**] demonstrated superior efficacy compared to donepezil (IC_{50} = 75.31 ± 3.53 μM) in inhibiting beta amyloid aggregation, with [**33**] IC_{50} = 22.45 ± 1.05 μM and [**34**] IC_{50} = 35.04 ± 1.64 μM. Finally, they inhibited the tau protein aggregation more effectively than donepezil (Fig. 11).[1]

6-Amino coumarin scaffold was reportedly connected to the 2-phenoxy-*N*-substituted phenylacetamide derivative with azomethine linkage, compounds [**35–38**], (Fig. 11). They had a promising activity against AChE with IC_{50} = 3.97, 5.93, 3, and 4.97 nM, respectively, compared to donepezil with IC_{50} = 7.03 nM. Compound [**37**] showed a multi-targeting effect as in BuChE with IC_{50} = 303 ± 0.03 nM compared to donepezil with IC_{50} = 606 ± 0.17 nM, GSK-3β inhibition with IC_{50} = 7.58 ± 0.83 nM relative to the reference broad-spectrum kinase inhibitor staurosporine (8.63 ± 0.94 nM). Finally, compound [**37**] had a significant activity in the iron chelating capacity compared to the iron chelator EDTA, thus decreasing oxidative stress effects.[37]

2.10 Coumarin–chalcone hybrids

Coumarin–chalcone derivatives represent a versatile class of hybrids combining the rigid bicyclic coumarin core with the flexible α,β-unsaturated ketone of chalcones. These compounds have demonstrated multifaceted effects on AD pathology.

Chiu *et al.*, synthesized a group of 4-hydroxy coumarin hybridized to different chalcone derivatives, compound [**39**], (Fig. 12), having the dimethylamino phenyl moiety, which provided a neuroprotective effect. It worked as a CREB enhancer, reduced Aβ and tau aggregation (EC50 s of 14 μM and 10 μM, respectively), and provided antioxidative protection.[38]

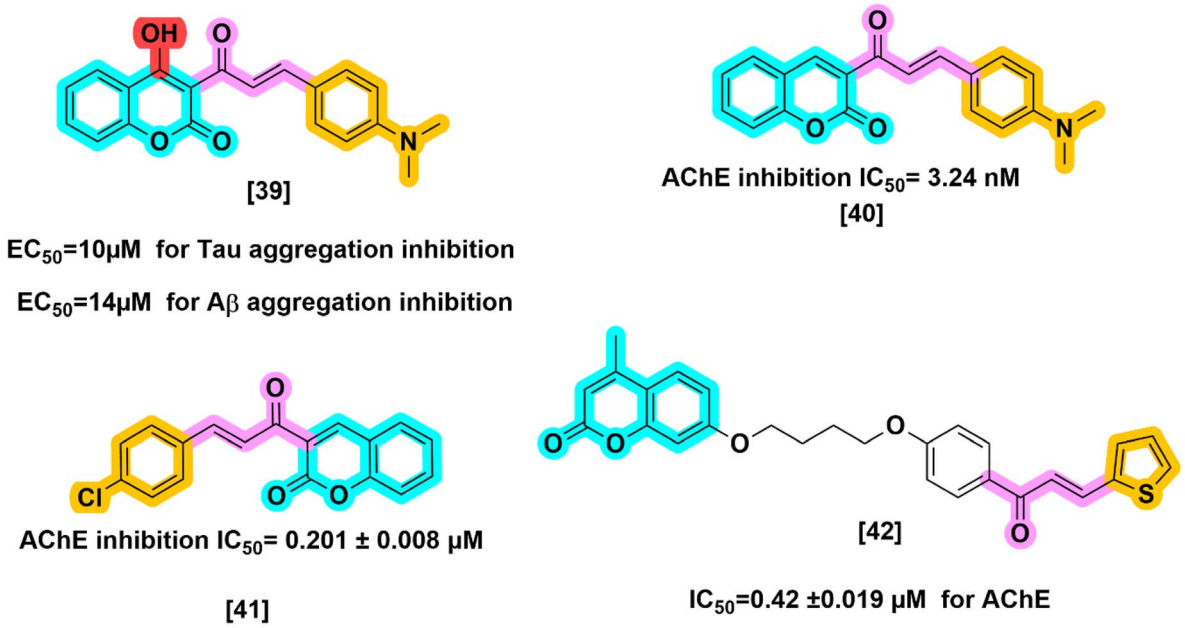

Fig. 12 Coumarin–chalcone hybrids as multi-targets in AD.

In another study, substitution with *N*,*N*-dimethyl amino phenyl moiety, compound [**40**] chalcone–coumarin derivatives were synthesized, and several properties were evaluated. All of the synthesized coumarin–chalcone compounds showed strong acetylcholinesterase activity. Notably, compound [**40**] showed a high inhibitory effect (IC_{50} = 3.23 nM).[39]

Hasan *et al.*, synthesized a series of 14 novel chalcone–coumarin derivatives, which exhibited AChE inhibition with IC_{50} values ranging from 0.201 ± 0.008 to 1.047 ± 0.043 μM.

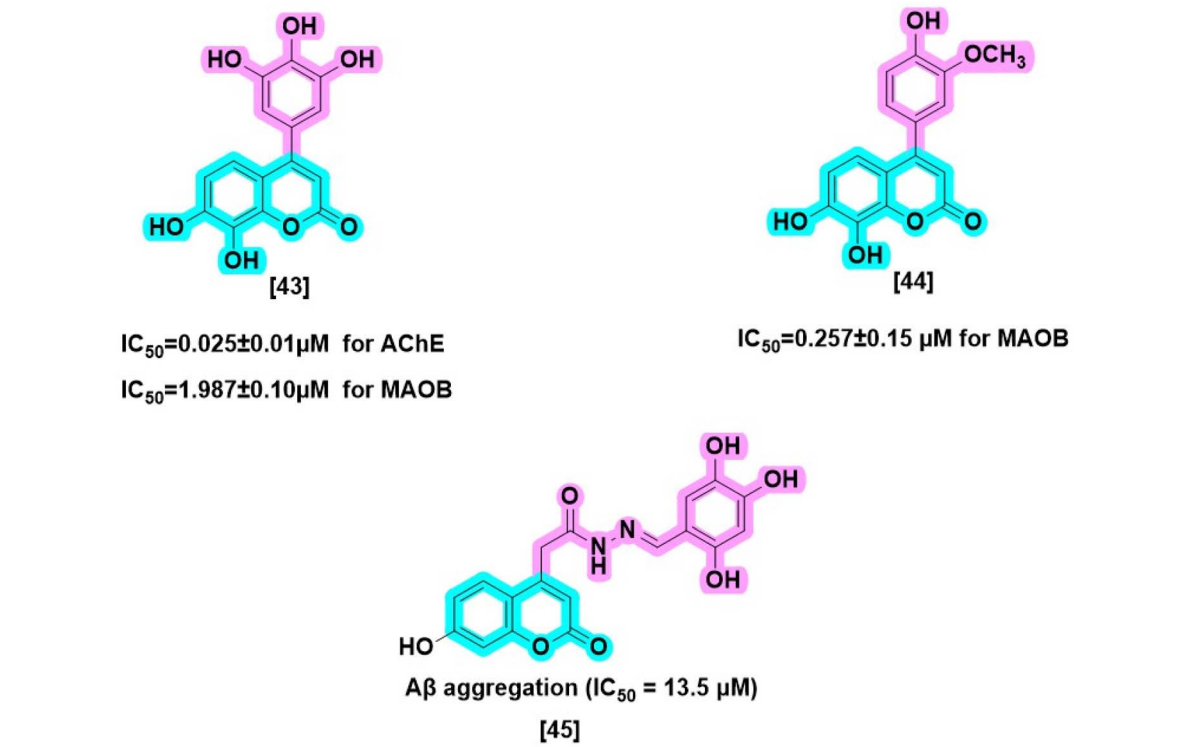

Fig. 13 4-Arylcoumarin derivatives as multi-targets in AD.

Hybrid, compound [**41**], (Fig. 12), with chloro substitution on the chalcone ring-B showed superior potency in the micromolar range ($IC_{50} = 0.201 \pm 0.008$ μM) compared to galantamine ($IC_{50} = 1.142 \pm 0.027$ μM).[40]

Upon replacement of the phenyl ring of chalcone with the substituted thiophene and increasing the length of the linker from ethylene to a pentyl chain, Aso Hameed *et al.*, designed and synthesized eight promising agents against AChE. Compound [**42**] stood out with $IC_{50} = 0.42 \pm 0.019$ μM compared to the galantamine ($IC_{50} = 1.142 \pm 0.027$ μM). That compound revealed a high safety profile according to the MTT assay, which showed no substantial toxicity in normal human liver cells at the maximum tested concentration of 1000 μg ml^{-1}.[41]

2.11 4-Arylcoumarin derivatives in AD

Different substituted phenol rings linked directly to the dihydroxy coumarin scaffold at position 4, as in compounds [**43**, **44**] (Fig. 13), acted as multi-targeting agents. Compound [**43**] exhibited inhibitory activity against AChE with $IC_{50} = 0.025 \pm 0.01$ μM acting as a mixed inhibitor compared to donepezil ($IC_{50} = 0.029 \pm 0.01$ μM) and a moderate inhibition against MAO-B with $IC_{50} = 1.987 \pm 0.10$ μM in comparison to rasagiline ($IC_{50} = 0.230 \pm 0.05$ μM). It also had a good antioxidant activity ($IC_{50} = 10.101 \pm 0.04$ mmol g^{-1}) compared to ascorbic acid ($IC_{50} = 9.868 \pm 0.01$ mmol g^{-1}). On the other hand, compound [**44**] had a weak inhibition against AChE ($IC_{50} = 10.938 \pm 0.47$ μM) but a promising inhibition of MAO-B ($IC_{50} = 0.257 \pm 0.15$ μM) and a good antioxidant activity ($IC_{50} = 10.089 \pm 0.01$ mmol g^{-1}).[42]

Insertion of a hydrazone linker between 7-hydroxy coumarin and the trihydroxy phenyl group through a hydrazone linker in position 4 yielded compound [**45**], (Fig. 13), which had high inhibitory activity against Aβ aggregation with an IC_{50} of 13.5 μM and a good antioxidant activity exceeding the ascorbic acid standard at values of 5 and 10 μg ml^{-1} at DPPH antioxidant assay and adequate permeability across the blood–brain barrier.[43]

2.12 Miscellaneous coumarin derivatives in modulation of AD

8-methoxy coumarin scaffold attached to benzofuran at C-3 by an amidic linkage of an aliphatic chain and a suitable length of a butyl alkyl linker as in compound [**46**]. It showed a good inhibition activity against AChE with $IC_{50} = 0.18 \pm 0.05$ μM related to donepezil 0.031 ± 0.05 μM. It had moderate antioxidant activity. The design of that compound mimicked donepezil structure and occupies the PAS and CAS binding sites of AChE.[9]

Benazzouz-Touami *et al.*, attached a methyl pyrazole ring directly to the coumarin scaffold at position number 3, while substituting C-6 with a nitro group, compound [**47**]. It had good inhibitory activity against AChE with $IC_{50} = 4.41 \pm 0.53$ μg ml^{-1} relative to galantamine as the standard drug with $IC_{50} = 6.27 \pm 1.15$ μg ml^{-1}. It also had a good antioxidant activity according to the DPPH assay, and according to the cupric reducing antioxidant capacity (CUPRAC) assay with a value equal to 66.9 ± 2.27 μg ml^{-1} compared to α-tocopherol showing a reading of 19.92 ± 1.46 μg ml^{-1}.[44]

When 3-acetyl-4-hydroxycoumarin reacted with a primary aliphatic amine as N^1,N^1-dimethylpropane-1,3-diamine in the presence of *p*-toluenesulfonic acid and absolute ethanol with stirring under reflux, it yielded compound [**48**] (Fig. 14). It acted as a multi-targeting agent as an AChE inhibitor, an antioxidant, and as an inhibitor of self-induced and Cu^{2+}-induced aggregation of β-amyloid. Compound [**48**] exhibited a good selectivity towards AChE over BuChE.[45]

The docking studies of the AChE enzyme revealed that compound [**48**] bonded to the active site, where the dimethylamino substituent established carbon–hydrogen interactions with Glu199 and His440 (CAS), and a pi–sigma interaction with Trp84. The phenolic hydroxyl group formed hydrogen bonding with Gln69 (3.1 Å), Gly123 (2.7 Å), and Trp84 (2.0 Å), whilst the coumarin pyran ring formed a pi–pi stacking interaction with Trp84. In the $A\beta_{1-42}$, the molecule aligned with the hydrophobic surface of the Aβ helix, establishing hydrogen bonds with Gln15 (2.1 Å and 2.9 Å) and Val12 (2.2 Å).[45]

Kamel *et al.*, attached 4-methylcoumarin to the indene ring through the oxyacetohydrazide linker to synthesize compound [**49**], showing a good activity against AChE with $IC_{50} = 0.802$ μM and antioxidant activity with DPPH scavenging activity of 57.14 ± 2.77% (Fig. 14). The docking studies results supported the *in vitro* assay against AChE inhibition, where hydrogen bonds were established between the carbonyl group of coumarin and Tyr130 (2.5 Å), and the chromene moiety formed arene–arene interactions with Trp84, while the acetohydrazide (C=O) formed a hydrogen bond with Tyr121 (2.9 Å). The indene moiety interacted with Trp279. Overall, the indene moiety imparted considerable lipophilicity to the coumarin at the 7-position, enhancing its inhibitory efficacy. The oxyacetohydrazide linker stabilized the molecule within the enzyme gorge, facilitating essential arene–arene interactions between the indene ring and Trp279 in the AChE pocket.[46]

In vivo studies of [**49**] showed improvement of cognition in mice and no effect on the blood profile, hepatic enzyme levels (AST, ALT, and ALP), or total urea, which indicated the safety of the drug. Also, compound [**49**] significantly improved MDA and GSH levels, achieving 90.64% and 27.17%, respectively, compared to the standard medication, which recorded 90.64% and 35.03% for MDA and GSH, respectively.[46]

7,8-Dihydroxycoumarin derivatives, compound [**50**], (Fig. 14) proved multi-target activity, through inhibition of AChE and antioxidant effect. It demonstrated significant AChE inhibition (92.116% at 500 μg ml^{-1}), comparable to the conventional medication galantamine. It demonstrated the most potent DPPH radical scavenging activity in the study with an IC_{50} value = 10.06 μM. Also, it reduced the metal dyshemostasis through FRAP and CUPRAC assays, which were employed to assess the capability of the compound to decrease ferric ions (Fe^{3+}) and cupric ions (Cu^{2+}). The assays showed that Compound [**50**] exhibited significant Fe^{3+} reducing capability and moderate reducing of Cu^{2+} ability. Docking studies for AChE showed a notable ligand efficiency by engaging in pi–pi stacking

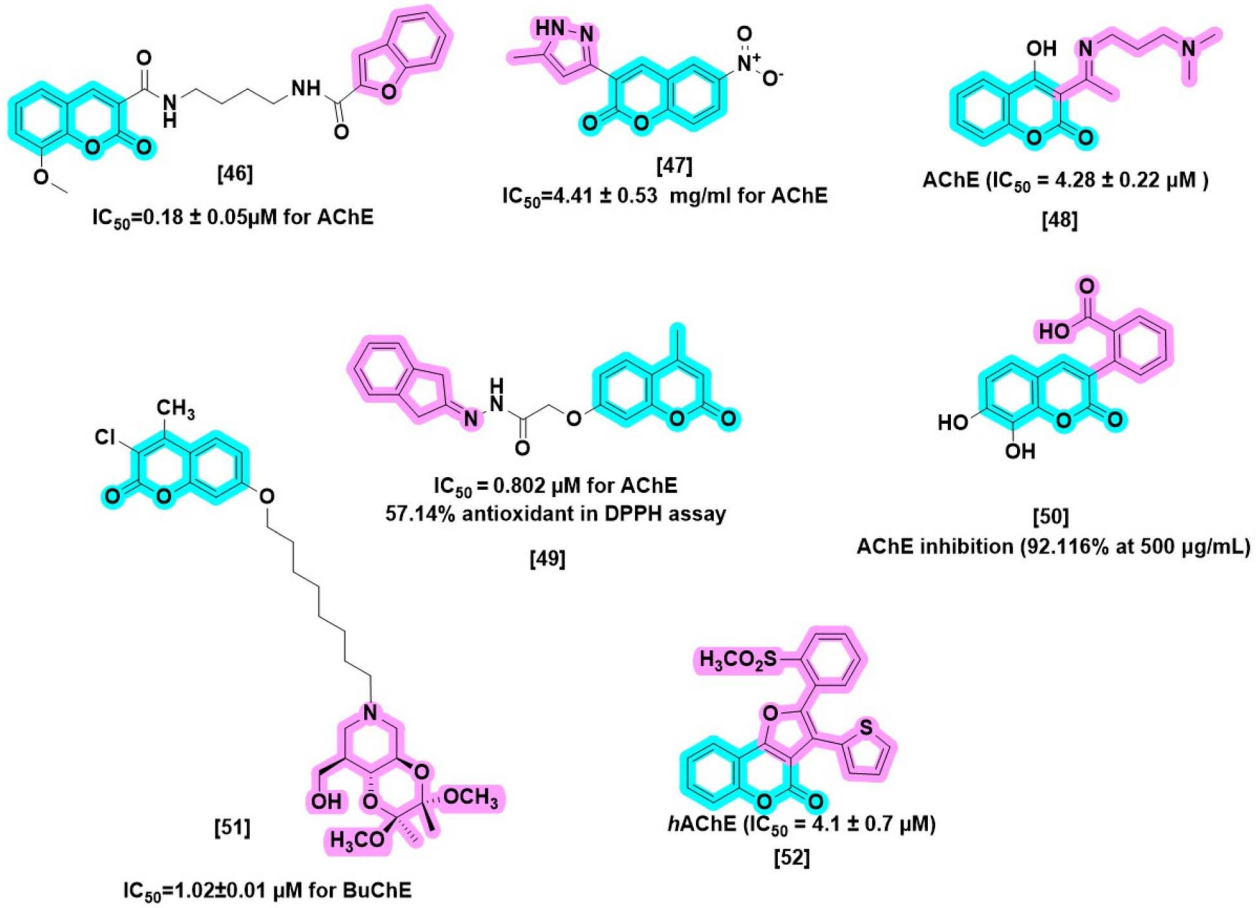

Fig. 14 Miscellaneous coumarin derivatives in modulation of AD.

interactions with Trp286, a critical PAS residue, and establishing a hydrogen bond with Tyr341.[47]

Isofagomine (azasugar) linked to the substituted coumarin scaffold through an octyl tether linker yielded compound [**51**] by Santos Evangelista *et al.* That hybrid was designed to treat cancer and Alzheimer's disease. It had a good inhibition activity against hBuChE with $IC_{50} = 1.02 \pm 0.01$ μM in comparison to the galantamine with $IC_{50} = 20.1 \pm 3.1$ μM. It showed more selective activity against BuChE than AChE with up to 39-fold.[48]

The achiral molecule [**52**], characterized by a 2-substituted thienyl ring and a methylsulfonyl-substituted benzene ring, proved to be a promising prototype. It was distinguished by its dual-inhibitory profile and lack of cytotoxic effects at therapeutic dosages.[49]

Compound [**52**] acted as a multi-target-directed ligand (MTDL) by concurrently regulating cholinergic signaling and monoamine oxidase activity. It demonstrated low-micromolar potency against hAChE, with an IC_{50} of 4.1 ± 0.7 μM. It had a pronounced selectivity for AChE compared to butyrylcholinesterase (hBChE), demonstrating merely 21% inhibition of hBChE at a concentration of 10 μM. Also, it had a highly selective inhibitory effect upon human Monoamine Oxidase B (hMAO-B), with a submicromolar value of 0.561 ± 0.085 μM.[49]

Lineweaver–Burk plots classified compound [**52**] as a mixed-type or non-competitive inhibitor of hAChE, with an inhibition constant of 9.7 ± 0.6 μM. This kinetic behavior indicates the ligand's capacity to occupy the peripheral anionic subsite (PAS) of the enzyme, a characteristic associated with the inhibition of amyloid beta deposition into fibrils. Computational simulations offered a structural explanation for the dual-target profile of [**52**]. Consistent with kinetic data, compound [**52**] was localized within the PAS and the mid-gorge. The sulfonyl-substituted phenyl ring connected to the PAS *via* an arene–arene interaction resembling a "sandwich" with the indole side chain of Trp286 and the side chain of Tyr124.[49]

The type of binding of [**52**] was located in the entrance cavity of hMAO-B. Stabilization is invoked through arene–arene stacking of the coumarin motif with Phe103, and the lactone carbonyl forms an essential hydrogen bond with the His115 side chain. It could not comfortably fit into the substrate cavity due to its bulky structure, where Ile199 and Tyr326 controlled access.[49]

2.13 Benchmarking coumarin hybrid scaffolds against multifunctional Alzheimer's targets

To effectively guide the future rational design of novel coumarin hybrids for Alzheimer's disease (AD) therapy, a comparative summary was established to evaluate the primary structural

scaffolds against their respective biological profiles and target multi-potency.

The raw dataset reveals clear trends across different structural families:

• Exceptional multi-target potency: aminocoumarin enaminones and 6-aminocoumarin derivatives (specifically compounds **33**, **34**, and **37**) stand out for highly potent multi-target activity, showing nanomolar IC_{50} values against acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and glycogen synthase kinase-3 beta (GSK-3beta) simultaneously.

• Most potent AChE selectivity: the coumarin–pyridine hybrid (**29**) demonstrates exceptional potency against AChE with an IC_{50} of just 2 nM, paired with a strong 24 nM inhibition against BuChE.

• Target diversity: while traditional scaffolds heavily prioritize AChE and BuChE inhibition, specific hybrid strategies successfully expand into disease-modifying mechanisms. These include MAO-B inhibition (notably the coumarin–pyridine hybrid **28** at 14.7 nM), $Abeta_{1-42}$ aggregation prevention (coumarin–triazole **8** at 8 nM), and metal chelation.

Compound number	Biological target	Biological activity	Reference
Coumarin–triazole hybrids			
(**1**)	AChE	$IC_{50} = 2.57$ μM	12
	BuChE	$IC_{50} = 3.26$ μM	
	BACE-1	$IC_{50} = 10.65$ μM	
(**2**)	AChE	$IC_{50} = 2.18$ μM	14
(**3**)	AChE	$IC_{50} = 0.080$ μM	15
	BuChE	$IC_{50} = 0.044$ μM	
	MAO-B	$IC_{50} = 0.18$ μM	
	Aβ aggregation	58.4% at 20 μM	
(**4**)	AChE	$IC_{50} = 27$ nM	16
	BuChE	$IC_{50} = 6$ nM	
(**5**)	AChE	$IC_{50} = 0.047$ μM	17
(**6**)	BuChE	$IC_{50} = 1.74$ μM	18
(**7**)	AChE metal chelating properties	$IC_{50} = 0.059$ μM	19
	Copper-induced $Aβ_{1-42}$ aggregation	34.26% at 50 μM	
(**8**)	$Aβ_{1-42}$ aggregation	$IC_{50} = 0.008$ μM	20
	proinflammatory cytokines (TNF-α, IL-1β)	1.09 ± 0.09%, 0.71 ± 0.14% respectively	
(**9**)	BuChE	$IC_{50} = 19.5$ μM	21
Coumarin–thiazole and coumarin–oxadiazole hybrids			
(**10a**)	AChE	$IC_{50} = 0.87$ μM	22
(**10b**)		$IC_{50} = 1.08$ μM	
(**10c**)		$IC_{50} = 2.34$ μM	
(**10d**)		$IC_{50} = 5.86$ μM	
(**11**)	AChE	$IC_{50} = 2.00$ μM	23
	BuChE	$IC_{50} = 34.93$ μM	
(**12a**)	AChE, BuChE	$IC_{50} = 6.07$, 0.341 μM	22
(**12b**)	BuChE	$IC_{50} = 0.15$ μM	
(**12c**)	BuChE	$IC_{50} = 0.15$ μM	
(**13**)	AChE	$IC_{50} = 28.68$ μM	24
	Antioxidant activity	$IC_{50} = 65.57$ μM	
Coumarin–quinoline hybrids			
(**14**)	BuChE	$IC_{50} = 0.15$ μM	25
(**15**)	Iron chelating	72.87% iron chelation at 100 μM	26
Coumarin hydrazones hybrids			
(**16**)	AChE	$IC_{50} = 7.40$ μM	27
	MAO-A	$IC_{50} = 1.44$ μM	
(**17**)	AChE	$IC_{50} = 8.01$ μM	27
	MAO-A	$IC_{50} = 1.51$ μM	
(**18**)	AChE	$IC_{50} = 8.54$ μM	27
	MAO-A	$IC_{50} = 1.87$ μM	
(**19**)	AChE	$IC_{50} = 0.232$ μM	28
(**20**)	AChE	$IC_{50} = 0.190$ μM	28
Coumarin–indole and coumarin–carbazole hybrids			
(**21**)	AChE	$IC_{50} = 0.16$ μM	29
	BuChE	$IC_{50} = 29.7$ μM	
(**22**)	AChE	$IC_{50} = 6.72$ μM	30
Coumarin–piperazine and coumarin–piperidene hybrids			
(**23**)	AChE	$IC_{50} = 1.52$ μM	31
(**24**)	AChE	$IC_{50} = 2.80$ μM	31
(**25**)	AChE	$IC_{50} = 4.95$ μM	31
(**26**)	AChE	$IC_{50} = 1.313$ μM	32
	BACE-1	$IC_{50} = 1.227$ μM	
	GSK-3β	19.30% inhibition at 20 μM	
Coumarin–pyridine hybrid			
(**27**)	MAO-B	$IC_{50} = 99$ nM	33
(**28**)	MAO-B	$IC_{50} = 14.7$ nM	34
(**29**)	AChE	$IC_{50} = 2$ nM	2
	BuChE	$IC_{50} = 24$ nM	
Coumarin–1,3,5-triazine hybrid			
(**30**)	AChE	$IC_{50} = 1.87$ μM	35
(**31**)	AChE	$IC_{50} = 1.35$ μM	35
(**32**)	AChE	$IC_{50} = 0.018$ μM	36
Aminocoumarin enaminones and 6-aminocoumarin-based derivatives			
(**33**)	AChE	$IC_{50} = 28.88$ nM	1
	BuChE	$IC_{50} = 131.90$ nM	
	GSK-3β	$IC_{50} = 51.42$ nM	
	$Aβ_{1-42}$ aggregation	$IC_{50} = 22.45$ μM	
	Tau protein aggregation	$IC_{50} = 31.22$ μM	
(**34**)	AChE	$IC_{50} = 26.03$ nM	1
	BuChE	$IC_{50} = 90.02$ nM	
	GSK-3β	$IC_{50} = 26.91$ nM	
	$Aβ_{1-42}$ aggregation	$IC_{50} = 35.04$ μM	
	Tau protein aggregation	$IC_{50} = 56.31$ μM	
(**35**)	AChE	$IC_{50} = 3.97$ nM	37
(**36**)	AChE	$IC_{50} = 5.93$ nM	37
(**37**)	AChE	$IC_{50} = 3.00$ nM	37
	BuChE	$IC_{50} = 303$ nM	
	GSK-3β	$IC_{50} = 7.58$ nM	
(**38**)	AChE	$IC_{50} = 4.97$ nM	37
Coumarin–chalcone hybrids			
(**39**)	$Aβ_{1-42}$ aggregation	$IC_{50} = 14$ μM	38
	Tau protein aggregation	$IC_{50} = 10$ μM	
(**40**)	AChE	$IC_{50} = 3.24$ nM	39
(**41**)	AChE	$IC_{50} = 0.201$ μM	40

(*Contd.*)

Compound number	Biological target	Biological activity	Reference
(**42**)	AChE	$IC_{50} = 0.42$ μM	41
4-Arylcoumarin derivatives			
(**43**)	AChE	$IC_{50} = 0.025$ μM	42
	MAO-B	$IC_{50} = 1.987$ μM	
(**44**)	MAO-B	$IC_{50} = 0.257$ μM	42
(**45**)	$A\beta_{1-42}$ aggregation	$IC_{50} = 13.5$ μM	43
Miscellaneous coumarin derivatives			
(**46**)	AChE	$IC_{50} = 0.18$ μM	9
(**47**)	AChE	$IC_{50} = 4.41$ μg ml^{-1}	44
(**48**)	AChE	$IC_{50} = 4.28$ μM	45
(**49**)	AChE	$IC_{50} = 0.802$ μM	46
	Antioxidant property	57.14%	
(**50**)	AChE	92.116% at 500 μg ml^{-1}	47
(**51**)	BuChE	$IC_{50} = 1.02$ μM	48
(**52**)	AChE	$IC_{50} = 4.1$ μM	49
	MAO-B	$IC_{50} = 0.561$ μM	

3. Structure–activity relationship (SAR) insights and pharmacophore features

A detailed series of structure–activity relationship (SAR) investigations across various coumarin hybrids classes have uncovered crucial features of bioactivity (FAB) and have provided infallible directionality, toward the rationalization of structural contributions to biological activity. Owing to steric clashes, appropriate linker lengths (typically 3–6 carbon atoms or comparable aromatic linkers) are crucial for AChE inhibition since dual-site binding is believed to be optimal. Electron-donating and electron-withdrawing groups on aromatic rings significantly influence activity, with the electronic properties of substituents affecting both binding affinity and BBB permeability. Here, the electronic properties of substituents are known to affect both binding affinity and BBB permeability.[50]

Enzyme selectivity is determined by the nature of the heterocyclic moiety attached to coumarin. Hybrids containing triazole moiety show a preference for AChE inhibition, while thiazole and oxadiazole substituents generally improve the selectivity towards BuChE. Type of substitution on coumarin nucleus (3-, 4-, 7- or 8-) plays an important role in the degree of inhibition, where generally 7-hydroxycoumarins have shown a much higher degree of activity due to their potential hydrogen binding capability (Fig. 15).[12,20]

4. Molecular docking and computational implications

4.1 Acetylcholinesterase enzyme–coumarin interaction

4.1.1 Dual-binding mode and linker concept. Inhibition of AChE by dual site binding (CAS + PAS) is an excellent known tactic, and hybrids with coumarin can act as dual binding site inhibitors of AChE. For example, coumarin–piperazine hybrids and biscoumarins occupy both CAS and PAS in docking, with one coumarin ring stacking in PAS (Trp286/Tyr72/Tyr341) and the other or a different aromatic fragment binding near CAS (Trp86, Phe338, catalytic gorge region) (Fig. 16a).[31,51–53]

The AChE active site gorge is ~20 Å in depth, and larger ligands that bridge both PAS and CAS typically exhibit greater inhibition than ligands binding to only one of these two regions.[54,55]

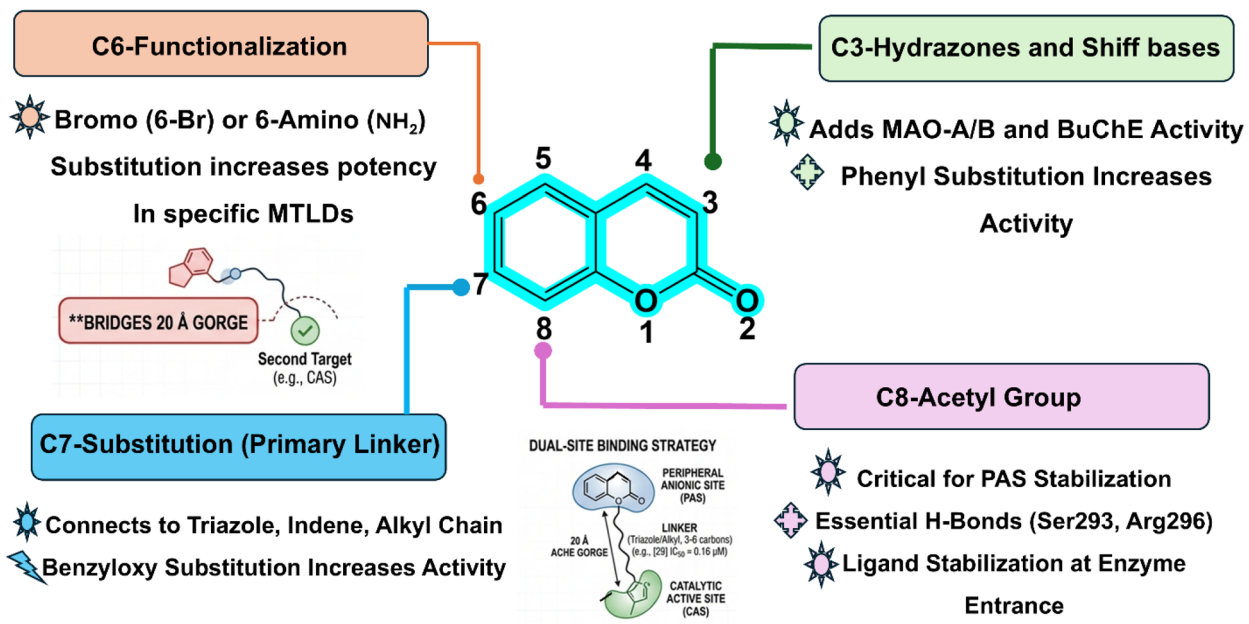

Fig. 15 SAR study of reported benzopyrones as anti-AD.

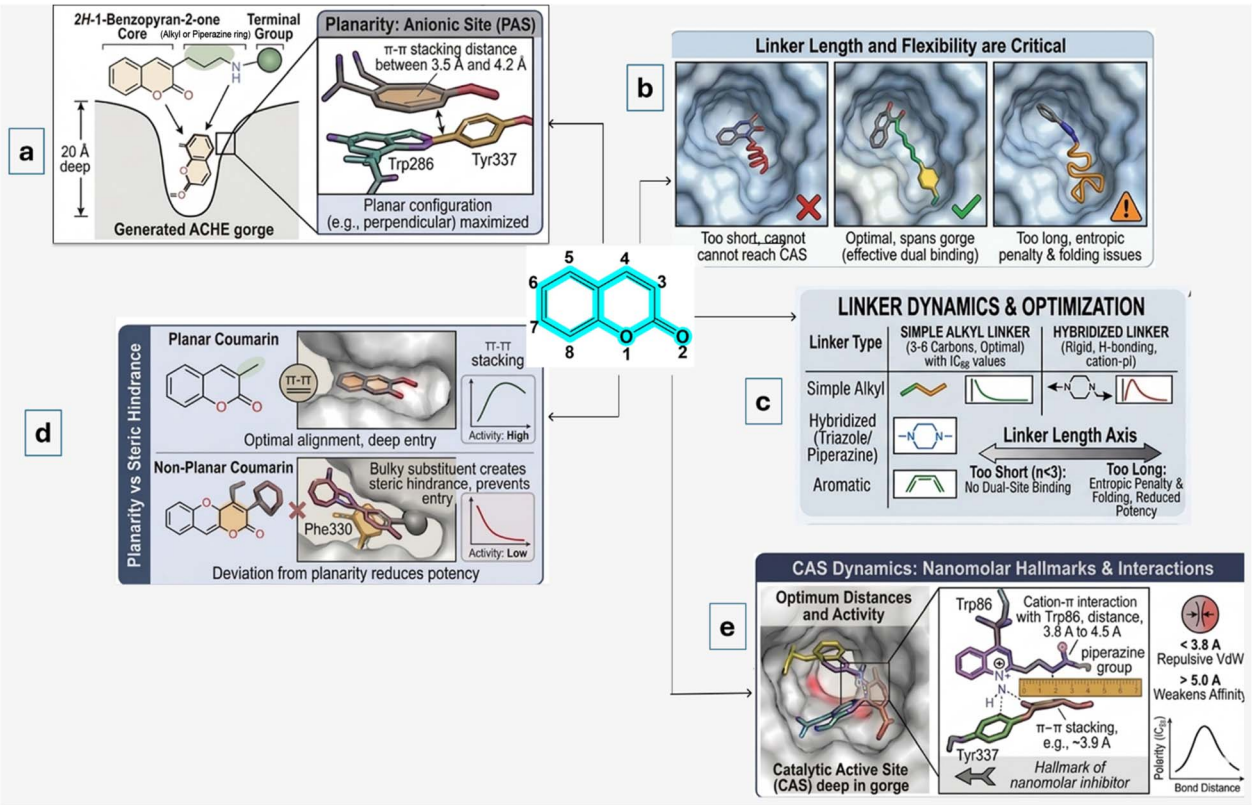

Fig. 16 Action and interaction of coumarin derivatives with CAS and PAS of AChE enzyme; (a) planarity of coumarin scaffold; (b) linker length and flexibility; (c) linker optimization; (d) planarity and potency of coumarin; (e) interaction with the CAS of AChE enzyme.

Coumarin scaffolds include ensaculin, biscoumarin, and 7-*O*-arylpiperazinyl coumarins that may also bind PAS by π–π stacking with PAS Trp286 and other PAS aromatics (*e.g.* Tyr72, Tyr341), while a positively charged amine (piperidine/piperazine) or further aromatic groups may engage with CAS region residues (*e.g.*, Trp86, Tyr337, Phe338).[31,52,53]

Modeling and SAR consistently show that too short a spacer prevents simultaneous PAS–CAS engagement, whereas long, highly flexible spacers can incur entropic penalties and less optimal binding, reducing potency.[31,52,53,55]

4.1.2 Linker dynamics and hybridization. Several coumarin-based or coumarin-inspired dual-site ligands incorporate alkyl linkers (*i.e.*, containing 3–6 methylene groups) between the PAS and CAS, showing that linkers that are too short do not access both subsites, while longer linkers are likely to be more flexible, thus increasing any entropic penalty (Fig. 16b).[31,52,53]

Spacer elongation leads to enhanced AChE inhibition (up to a certain extent) followed by plateau/decline for several series (*e.g.*, biscoumarins, coumarin–piperazine hybrids).[51,53]

Dual site ligands often utilize piperazine or piperidine containing linkers; protonated nitrogens frequently participate in cation–π interactions with Trp86 or Tyr341 in CAS, while proximal aromatic rings form π-stacking interactions with residues in PAS such as Trp286.[31,52,53,56]

Docking of biscoumarin and ensaculin reveals the proximal coumarin stacked on Trp86 in the CAS and the distal coumarin stacked on Trp286 in the PAS, with an alkoxy chain and piperazine spacer formed hydrophobic and cation–π contacts extending along the gorge.[53]

SAR data, along with other reviews, have asserted linear alkyl linkers of up to several carbons in length, in addition to heterocyclic segments (piperazine, piperidine, triazole) as effective though not uniquely optimal spacers for dual-site AChE ligands (Fig. 16c).[31,52,53,56]

4.1.3 Ring interaction and planarity. There are also a number of aromatic side chains lining up the gorge, notably Trp86 and Phe338 in the CAS, as well as Tyr337, Tyr341, Tyr124, Trp286, Phe295, and Phe330 in the mid-gorge and the surface active site (PAS).[52–56]

A coplanar orientation of the coumarin nucleus is preferred for optimal inhibitory activity. Planarity favors ideal alignment with the aromatic amino acid side chains (tyrosine 337, phenylalanine 338, or phenylalanine 330) that line the gorge of the enzyme (Fig. 16d). The heterocyclic ring of the coumarin needs to be preferably kept at a specific orientation, which is usually perpendicular or parallel to the indole rings of tryptophan residues to maximize the quadrupole interactions in docking simulation. The presence of planarity deviances or heavy, nonplanar substituents replacing hydrogen atoms at the coumarin backbone may create steric hindrance, preventing the derivative from reaching deep within the tight pocket of the enzyme to be targeted.[57]

Planar benzopyranone (benzopyran-2-one) docks well into the aromatic gorge for coumarin derivatives; both ensaculin (KA-672) and biscoumarin dock with one coumarin ring parallel to Trp86 and the other stacked against Trp286.[53]

Docking of ensaculin (KA-672) and other coumarin hybrids shows that planarity promotes favorable stacking and H-bonding interactions with Tyr124, Tyr133, Phe295, and the oxyanion hole region.[53]

Docking studies have also established that the coumarin ring system consistently docks in the PAS, most often being fastened by π–π stacking with surrounding residues such as Trp286.[2,58] The planar geometry is critical for the coumarin scaffold to glide into the shallow and narrow enzyme gorge and optimally positions to aromatic residues.[59]

The 7-position (*e.g.*, 7-*O*-alkoxy/arylpiperazinyl) is a common target of substitutions for incorporating linkers that allow the coumarin to remain at PAS and the "tail" to penetrate into CAS (for example, 7-*O*-arylpiperazine coumarins and biscoumarins).[31,53,57]

4.1.4 "Optimum" bond distances *vs.* activity. In reported docking studies, π–π stacking distances in the 3.5–4.0 Å range between aromatic ligands (such as coumarin, benzyl, indanone) and Trp86/Trp286, and cation–π distances 3.5–5 Å between protonated amines and aromatic residues (Tyr341, Trp86, Trp286) have been reported (Fig. 16e).[53,56,57] Second, if a protonated nitrogen is included with this linker (as is common within donepezil-like motifs), a cation–pi interaction at 3.8 Å to 4.5 Å within the CAS to Trp86 is often a hallmark of promising inhibition.[57] In biscoumarin and KA 672, π–π distances of ∼3.6–3.7 Å to Trp86 and Trp286 and cation–π contact around 3.5–5.0 Å have been reported, values that are typical of strong aromatic interactions.[53]

4.2 MAO-B interaction and linker dynamics

Coumarin derivatives can act as potent, selective, and reversible MAO-B inhibitors. Coumarin core normally fills the hydrophobic substrate cavity of the enzyme, but is typically retained near the FAD co-factor.[60–63] In many designed ligands, the coumarin (or related heteroaromatic) core binds deep in the cavity of the substrate, while a secondary aromatic or bulky group extends towards the entrance cavity; these two areas are connected by a linker which must cross the region of the "bottleneck", defined by residues like Ile199/Tyr326 (Fig. 17).[60,61,64,65]

Computational and SAR studies have shown that MAO-B inhibition and selectivity are generally favored by moderate, metabolically stable, aliphatic linkers, associated with a short to moderate length, unlike excessive bulk or excessive length, which reduce potency or change selectivity, probably by forcing nonoptimal, strained binding poses or by allowing alternative binding modes better compatible with MAO-A.[60,61,64] The precise "optimal" carbon count can vary across scaffolds, but multi-target coumarin hybrids and other MAO-B inhibitor series repeatedly identify relatively short C2–C4 segments or compact polar linkers (*e.g.*, amides, heterocycles) as effective spacers between a cavity-anchoring aromatic core and an entrance-pocket substituent.[31,60–62,64,66]

4.2.1 Ring orientation and planarity. One important structural feature that confers high affinity is the planar configuration of the coumarin scaffold, which is ideally suited for binding to narrow, aromatic pockets within the enzyme. Coumarin rings are inherently planar benzopyrones with extensive π delocalization, and crystallographic/quantum chemical studies reveal that common substituents maintain this planarity.[67] Potent inhibitors of MAO-B typically place an

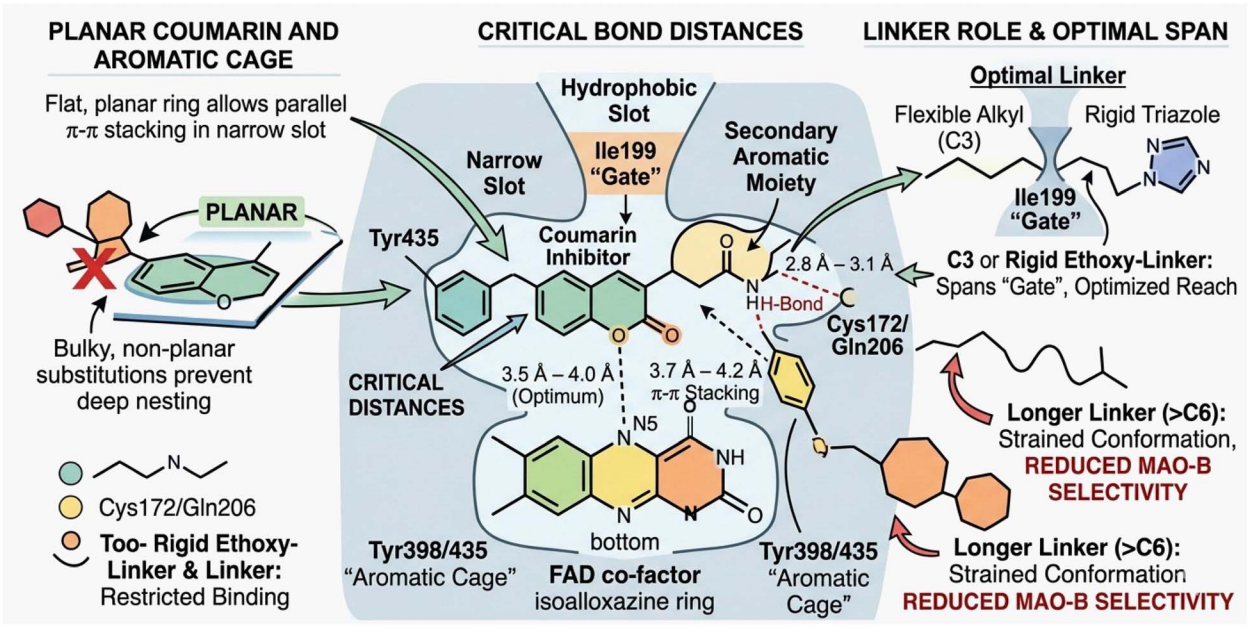

Fig. 17 Mao-B/coumarin interaction and linker dynamics.

aromatic or heteroaromatic ring system within the aromatic cage formed by Tyr398 and Tyr435, where they engage in π–π or π–alkyl interactions.[60–62,64] This is ideal for accommodation in the hydrophobic "slot" of MAO B, to which it is ideally suited due to its planar structure.[60–62]

The structure–activity data for coumarin and coumarin hybrid MAO inhibitors suggest substitution patterns that enable the coumarin plane to orient along the longitudinal axis of the binding cavity while allowing linkers as well as secondary aromatic groups in the other half to project toward the entrance region.[31,61,63] MAO-targeted coumarin chemistry has commonly utilized substitutions at positions corresponding to either a 3 or 7-substitution on coumarin, often maintaining the π-conjugated core while facilitating vectorial expansion into neighboring sub-pockets.[49,62,68] In a similar vein, very bulky or strongly out-of-plane substituents in close proximity to the ring can disrupt deep penetration and reduce π-stacking due to steric clashes or torsional strain.[61,62,64,65,67]

4.2.2 Key noncovalent contacts and activity correlation. Comprehensive *in silico* study of various coumarin MAO-B inhibitors shows frequent interaction motifs, first, aromatic stacking and hydrophobic contacts with Tyr435 and Tyr326 (and often Tyr398) are highly correlated with high MAO-B potency,[62,63,66] second, hydrogen bonds involving residues such as Cys172 and Gln206 regularly appear in the top-scoring poses and mediate selectivity.[31,62,66]

Docking and 3D QSAR modeling show that for a good inhibitor, an H bond acceptor or polar atom should be positioned so it is within hydrogen bonding distance (usually 2.5–3.0 Å in individual complexes) of specific residues (for example, Gln206, Cys172, or tyrosine hydroxyls), while also maintaining favorable π stacking distances between the aromatic core and Tyr435/Tyr398 on the order of what is commonly observed in aromatic complexes (3.5–4.0 Å in individual docked structures).[31,60,61,63,64,69] However, these values should be regarded as representative distances from specific complexes rather than strict universal cut-offs, and quantitative distance–activity relationships across series remain limited.

Overall, current computational and medicinal-chemistry work supports a design strategy for coumarin-based MAO-B inhibitors in which; (1) a planar coumarin or coumarin like scaffold docks into the Tyr398/Tyr435 aromatic cage; (2) a bulge or hinge induces favorable accommodation while a short to medium, non-bulky linker bridges into the entrance cavity; (3) H bonding groups are positioned to interact with residues such as Cys172 and Gln206 to complement π stacking and hydrophobic interactions to effect potency and selectivity (Fig. 17).[7,31,49,62,63,67]

5. Neuroprotection, antioxidant and metal chelation of coumarin derivatives in AD

Coumarin hybrids have strong antioxidant and neuroprotective properties, also by other mechanisms apart from cholinesterase inhibition. These compounds quenched free radicals and decreased oxidative stress markers in neuronal cell models. The DPPH and ABTS assays have demonstrated antioxidant activities comparable or superior to reference compounds like vitamin C and Trolox.[28]

The incorporation of metal-chelating functionalities into coumarin hybrids addresses the metal-ion dyshomeostasis central to AD pathogenesis. Coumarin–8-hydroxyquinoline conjugated Cu^{2+}, Zn^{2+}, and Fe^{2+} effectively through their nitrogen-rich and oxygen-rich chelation sites. Metal chelation ratios ranged from 1 : 1 to 2 : 1 (ligand : metal), with kinetic studies confirming equilibrium binding constants in the micromolar to nanomolar range (Duarte *et al.*, 2019). As in Compound [7] showed Cu^{2+}, Fe^{2+}, and Zn^{2+} ions metal chelation,[19] compound [**37**] had iron chelation activity with $IC_{50} = 0.162 \pm 0.01$ mg ml^{-1} relative to EDTA (0.073 ± 0.01 mg ml^{-1}),[37] compound [**50**] reduced Fe^{+3} and Cu^{2+} levels according to FRAP and CUPRAC assays[47] and compound [**15**] chelating iron with 72.87% at 100 μM.[26]

6. *In vivo* efficacy and behavioral studies

Several lead coumarin hybrids have demonstrated efficacy in various animal models of AD[5,11,70] (Fig. 18). Tacrine–coumarin hybrid, compound [**4**], (Fig. 1), significantly reversed scopolamine-induced memory deficits in rats as assessed by Morris water maze task performance.[16] More recent studies with coumarin–quinoline hybrids confirmed cognitive improvements in 7 month-old APP/PS1 transgenic mice, with protection of hippocampal and cortical neurons from necrosis and reduction in Aβ deposition.[25]

The most extensively validated compound [**5**], (Fig. 1), (coumarin–eugenol hybrid), exhibited an LD_{50} of 300 mg kg^{-1} and showed significant improvements in memory and learning behavior in scopolamine-induced cognition impairment mouse models. Both Y-maze tests and Morris water maze tests documented cognitive improvement comparable to standard anti-AD medications.[17]

Indene–coumarin hybrids demonstrated no signs of toxicity or adverse events with normal hepatic enzyme levels and total urea, and insignificant differences in blood profiles through *in vivo* assertion as in compound [**49**].[46]

7. Blood–brain barrier penetration, pharmacokinetic–pharmacodynamic (PK–PD) mismatch and metabolic consideration of coumarin based hybrids

One of the recurring tragedies in AD drug discovery is that of the "potent *in vitro*, silent *in vivo*" phenomenon. Despite low nanomolar IC_{50} values against AChE and Aβ-aggregation in cell-free assays, cognitive improvements in transgenic mouse models elicited by many coumarin hybrids are not achieved. This discrepancy is primarily due to a marked PK–PD mismatch

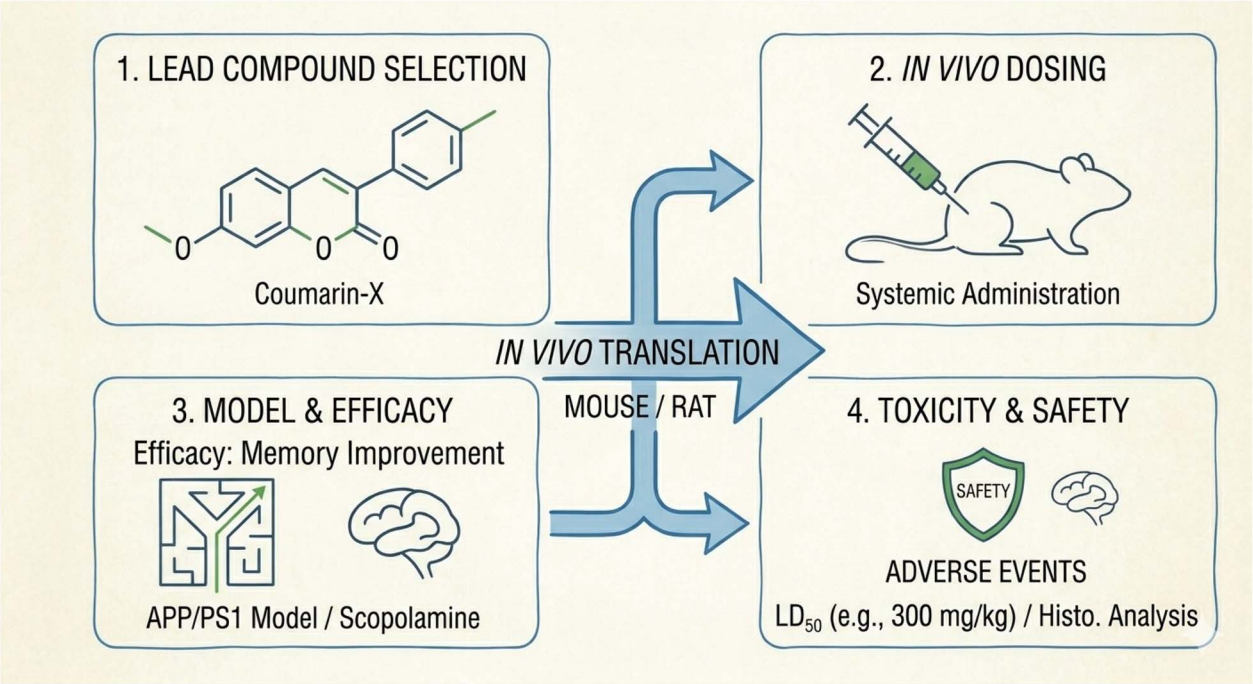

Fig. 18 *In vivo* translation of coumarin-hybrids in AD animal models.

with respect to BBB permeation and P-glycoprotein (P-gp) mediated efflux.[71] The coumarin nucleus itself is small and lipophilic, sufficient to serve as a fragment for CNS penetration. But, in many cases, the practice of building large, high-molecular-weight "Multi-Target Directed Ligands" (MTDLs) by simply attaching a bulky triazole, piperazine, or tacrine unit tips the molecule outside of the realm of Lipinski's rule of five and Veber's criteria.[16,72]

This is due in part to the fact that the blood–brain barrier is not just a physical wall, but a functional gatekeeper, over which many of the members of the ABC transporters (*e.g.*, P-gp (MDR1)) hold dominion.[73] Several coumarin scaffolds have been studied for both blood–brain barrier (BBB) behavior and P-glycoprotein (P-gp) interaction. Twelve coumarins from Angelicae Pubescentis Radix (including umbelliferone, osthol, scopoletin, peucedanol, ulopterol, angepubebisin, psoralen, xanthotoxin, bergapten, isoimperatorin, columbianadin, and columbianetin acetate) were tested in an MDCK-pHaMDR *in vitro* BBB model that overexpresses human MDR1/P-gp; most showed high apparent permeability consistent with passive diffusion, whereas peucedanol had transport significantly affected by P-gp and was modulated by the P-gp inhibitor verapamil.[74]

Most coumarins showed $P_{app\ (AP\ \rightarrow\ BL)} \sim 10^{-5}$ cm s^{-1}, comparable to caffeine, and were classified as well-absorbed across a BBB surrogate; peucedanol and isoimperatorin were moderately absorbed. Efflux ratios (BL → AP/AP → BL) were generally <2, indicating predominantly passive diffusion for most compounds.[74]

Drugs-like 7-*O*-arylpiperazinyl-4-methylcoumarins (eight derivatives) were evaluated for CNS "drug-likeness"; physicochemical profiling and modeling indicated they can cross the BBB and they were predicted to act as P-gp inhibitors, suggesting they may reduce efflux rather than be efficiently pumped out.[75]

Computational ADMET predictions (using tools such as SwissADME and pkCSM) consistently indicate that coumarin hybrids maintain favorable drug-like properties, with most derivatives adhering to Lipinski's rule of five and Veber's criteria for good oral bioavailability. Metabolic stability studies in human microsomal preparations have shown that several lead compounds resist first-pass hepatic metabolism, suggesting favorable pharmacokinetic profiles for oral administration.[25]

The development of effective anti-AD agents requires optimal blood–brain barrier (BBB) penetration, a critical challenge addressed through careful hybrid design. PAMPA-BBB (parallel artificial membrane permeability assay) studies have confirmed that many coumarin hybrids, particularly those with balanced lipophilicity ($\log P$ between 2–3), achieve good BBB permeability comparable or superior to donepezil.[19,25,32]

The coumarin carbonyl (lactone) oxygen often participates in key H-bonds or electrostatic interactions within enzyme active sites, analogous to how carbonyl/heteroatom acceptors interact with the AChE oxyanion region or MAO-B cofactor cavity.[31,76]

For example, in MAO-B docking for coumarin hybrids, the lactone carbonyl oxygen forms a hydrogen bond with Arg42, helping to anchor the coumarin ring and stabilize binding. Loss or chemical modification of this carbonyl would be expected to disrupt such contacts.[31]

Coumarin-based AD ligands are routinely evaluated with ADME or broader pharmacokinetic profiling, confirming that metabolism is a critical design constraint, but most AD-focused

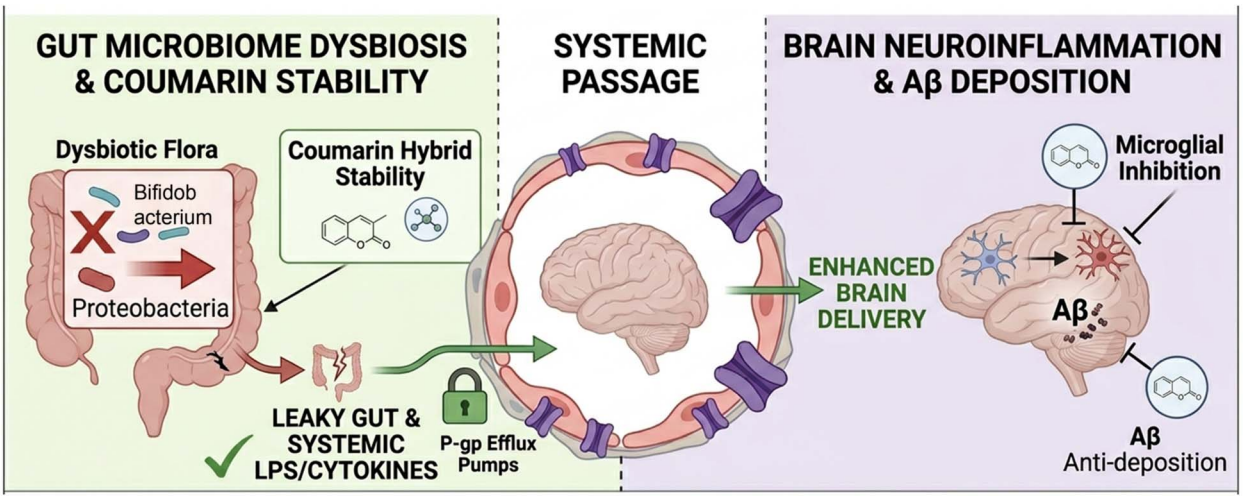

Fig. 19 Coumarin-hybrids and microbiome gut–brain axis in AD.

coumarin/AChE studies emphasize potency and binding rather than detailed lactone ring-opening pathways.[77]

If the coumarin hybrid's design relies on the intact lactone for target binding, as is the case with many AChE inhibitors, where the carbonyl oxygen interacts with the oxyanion hole, metabolic ring-opening renders the drug inactive.

8. The microbiome–gut–brain axis: a new frontier for coumarin hybrids

The traditional "neurocentric" view of Alzheimer's Disease (AD) is rapidly being superseded by an integrated systemic perspective that places the gut microbiome at the heart of neuroinflammation.[78] While the coumarin scaffold is celebrated for

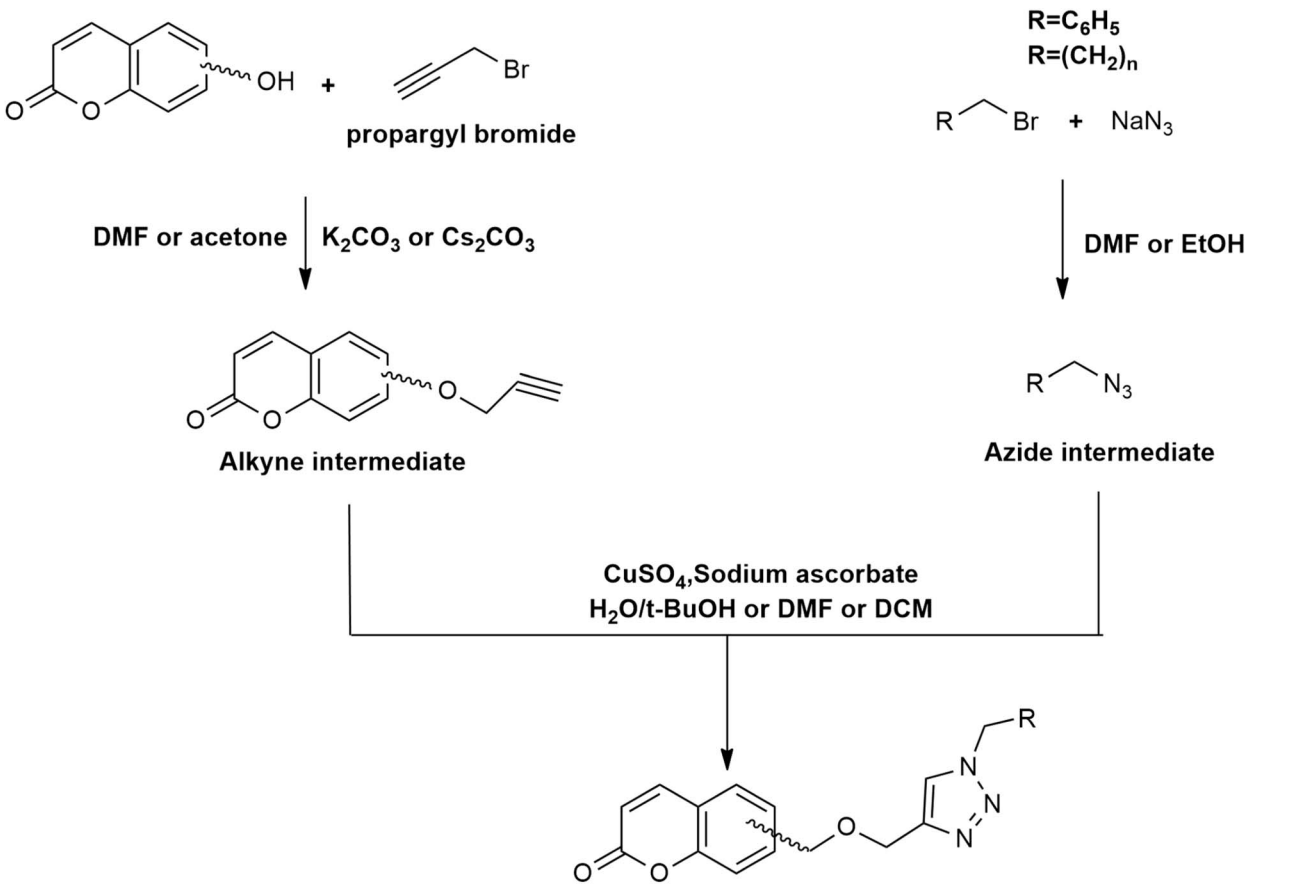

Fig. 20 General synthetic pathway for synthesis of triazoles–coumarin hybrids.

its enzyme-inhibitory properties, its potential as a modulator of the MGB axis remains an under-explored "gold mine" in medicinal chemistry. Emerging evidence suggests that the gut microbiota of AD patients exhibits significant dysbiosis, characterized by a decrease in anti-inflammatory taxa like *Bifidobacterium* and an increase in pro-inflammatory Proteobacteria. This dysbiosis leads to increased intestinal permeability, often termed "leaky gut" which allows bacterial lipopolysaccharides (LPS) and pro-inflammatory cytokines to enter systemic circulation, eventually breaching the blood–brain barrier (BBB) to trigger microglial activation and Aβ deposition (Fig. 19).[79,80]

Coumarin-based hybrids, particularly those derived from natural sources like esculetin or umbelliferone, possess inherent prebiotic-like qualities.[81] Therefore, a coumarin hybrid designed for AD should not only be evaluated for its ability to inhibit AChE in the brain but also for its stability in the gastrointestinal tract and its impact on microbial diversity.

9. General synthetic approaches of promising coumarin-hybrids MTDLs in AD

9.1 Coumarin–triazole hybrids

Copper-catalyzed Azide–Alkyne Cycloaddition (CuAAC), a regioselective click reaction, is the major method for synthesizing coumarin–triazole hybrids from different articles.[12,14] The 1,2,3-triazole linker is produced *via* synthesizing a terminal alkyne intermediate, forming an azide intermediate, and cycloaddition. Propargylation of the hydroxylated or amine-containing core with propargyl bromide in the presence of base, such as K_2CO_3 or Cs_2CO_3, in DMF or acetone substitutes the coumarin core or secondary pharmacophore (such as tacrine, isatin, or benzotriazole) with a terminal alkyne. The reaction of bromoalkyl derivatives or benzyl halides with sodium azide (NaN_3) in DMF or ethanol produces most azide intermediates. To ensure high yield and purity of the final hybrid, the alkyne and azide intermediates are joined using copper(II) sulphate ($CuSO_4$) and sodium ascorbate as reducing agents in a solvent mixture of water and an organic solvent like *tert*-butanol, DMF, or DCM (Fig. 20).

9.2 Synthetic strategy of coumarin–quinoline hybrid

In the case of the reaction of 7-oxyalkyl halide, coumarin reacts with 8-hydroxy quinoline using acetonitrile (MeCN) under reflux in the presence of K_2CO_3 to yield that hybrid.[25] On the other hand, the reaction of coumarin-3-carboxylic acid with 6-amino quinoline to yield the amide linkage using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC HCl) and *N*,*N*-dimethylaminopyridine (DMAP) as a catalyst in anhydrous dichloromethane (DCM) (Fig. 21).[26]

9.3 Synthetic pathway of coumarin hydrazone hybrid

Coumarin–hydrazone hybrids are synthesized by directly reacting an acetyl coumarin derivative with different hydrazide derivatives using a direct condensation approach. The acetyl coumarin derivative is dissolved in absolute ethanol, and then the hydrazide derivative is added with 3–4 drops of glacial acetic acid, which acts as a catalyst. The reaction is done under reflux at 80 °C for 5–8 hours. Then, filtration of the precipitate with washing with hot ethanol to get pure coumarin hydrazone hybrid (Fig. 22).[27]

9.4 Synthetic pathway of coumarin thiazole hybrid

These hybrids are obtained using an acid-catalyzed one-pot process that concurrently integrates three components: the bromoacetyl coumarin intermediate, various substituted acetophenones, and thiosemicarbazide, and reflux in acetic acid (AcOH) for 4–5 hours (Fig. 23).[22]

9.5 Synthetic pathway of coumarin indole hybrid

The coumarin-3-carboxylic acid is first converted to acid chloride by refluxing with thionyl chloride ($SOCl_2$) for 5–6 h. That acid chloride reacts with tryptamine and K_2CO_3 in dry toluene under reflux for 14–16 h (Fig. 24).[29]

10. Future directions and therapeutic implications

Recent research (2025–2026) emphasizes coumarin-based hybrids as Multi-Target Directed Ligands (MTDLs) that simultaneously inhibit acetylcholinesterase (AChE) and address other Alzheimer's-related factors like amyloid-beta (Aβ) aggregation.

Extensive research on coumarin-based hybrids has established this scaffold class as highly promising for next-generation anti-AD therapeutics. Future opportunities include: (1) rational design of compounds with enhanced selectivity toward specific enzymatic targets; (2) incorporation of additional pharmacophores targeting recently identified AD pathways (*e.g.*, neuroinflammatory mediators, tau kinases); (3) development of prodrugs and formulations improving BBB penetration and CNS bioavailability; (4) structure-based design of compounds with reduced off-target interactions and improved safety profiles; and (5) investigation of combination therapeutic approaches synergizing multiple coumarin hybrids or combining them with existing AD medications.

Some newer findings point to these coumarin MTDLs doing more than just easing symptoms. They might actually change the disease progression. A few of them break up amyloid deposits that are already there. What's interesting is how they tackle so many issues at once, from low acetylcholine to plaques, tau problems, stress from oxidation, and brain inflammation. All the previous positions them as leading candidates for clinical development.

Strategies now mix coumarin with fresh pharmacophores aimed at targets like GSK-3, tau kinases, or even PDE inhibitors. This could mean better protection for the brain overall. Coumarins are easy to tweak structurally, so we might end up with stuff that's more targeted, handles the body better, and provides more safety compared to current clinical standards.

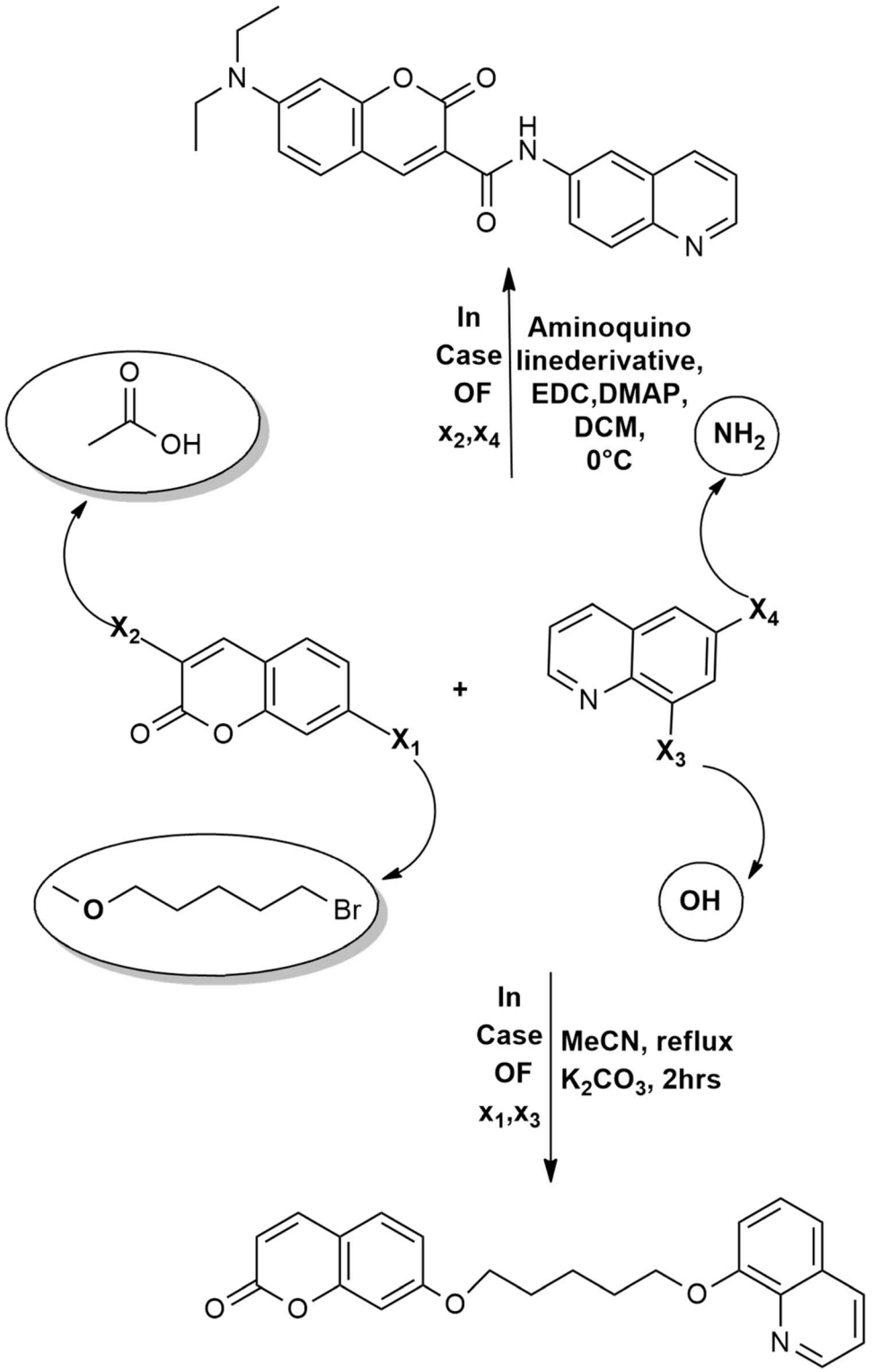

Fig. 21 General synthetic pathways of coumarin–quinoline hybrids.

Computational tools are speeding things up too. Virtual screening on coumarin libraries, QSAR models, and even AI designs help find new active ones fast. These approaches, validated by experimental testing in enzyme assays, cellular models, and animal models, provide a systematic pathway toward clinical candidates.[27]

For the next batch of hybrids, adding shielding groups or bioisosteric replacements (such as quinolinones) that preserve the structural geometry while enhancing metabolic longevity.

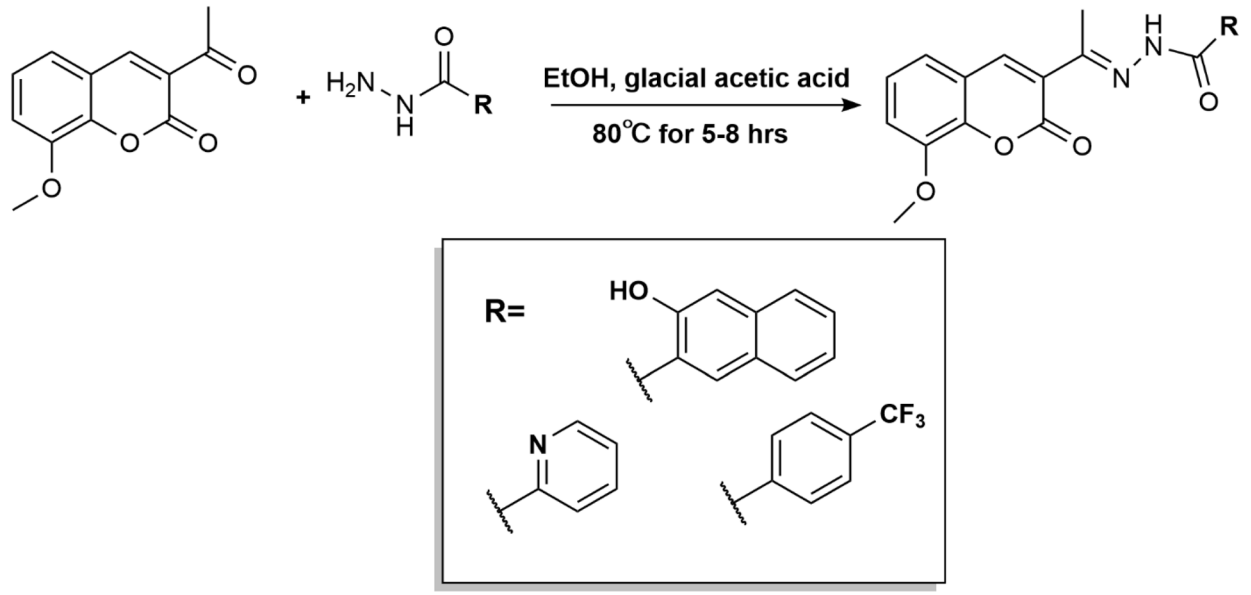

Fig. 22 Synthetic pathway of coumarin hydrazone hybrid.

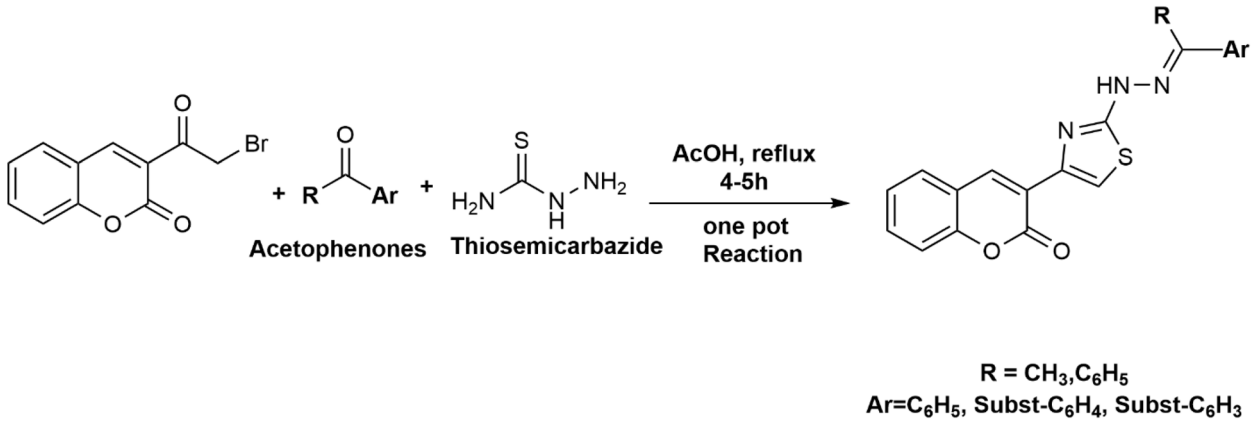

Fig. 23 Synthetic pathway of coumarin thiazole hybrid.

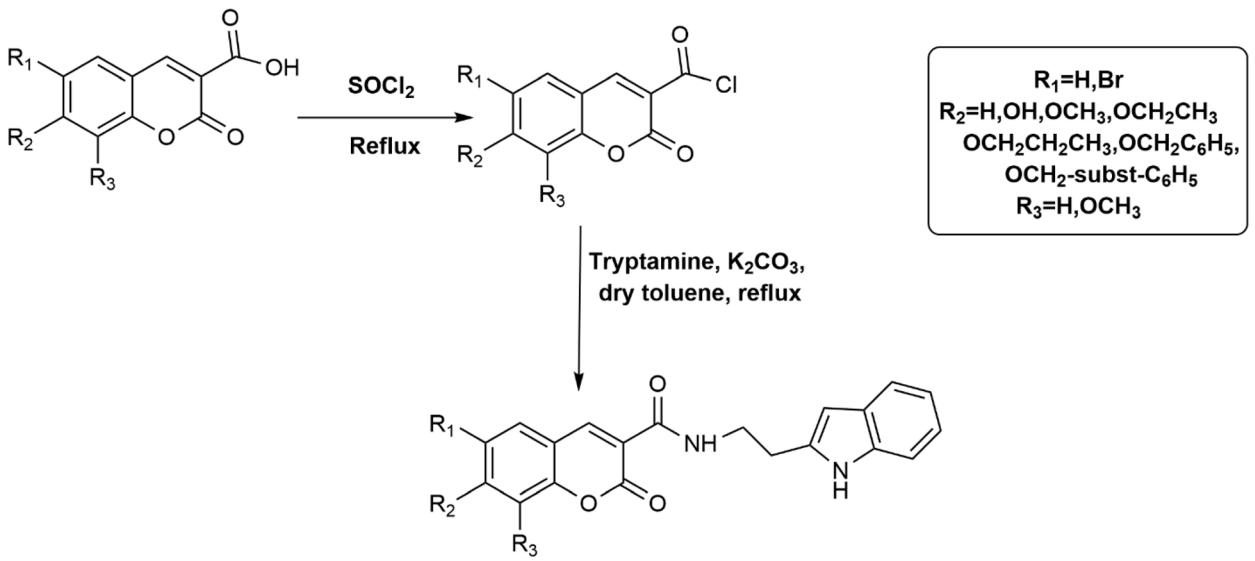

Fig. 24 Synthetic pathway of coumarin indole hybrid.

Only by matching the potent *in vitro* activity with robust pharmacokinetic profiles can these hybrids move from the laboratory bench to clinical relevance.

Future design strategies must prioritize "P-gp avoidance" as much as "target affinity." This can be achieved through the strategic use of "prodrug" hybrids that utilize nutrient transporters (like the LAT1 large neutral amino acid transporter) to bypass efflux pumps.

The dose that gets to the brain matters, but so does how the compound or its breakdown products or its microbial metabolites mitigate the systemic inflammatory "fire" before it ever reaches the CNS. By incorporating a section on the MGB axis, researchers can shift the narrative from simple symptomatic relief to a holistic systemic intervention.

11. Conclusion

Coumarin hybrids offer this wide platform for fighting Alzheimer's. The coumarin–triazole ones have been tested by different groups and show great potency across multiple angles of the disease. Mixing coumarin with triazoles, indoles, thiazoles, quinolines, chalcones, and more lets these molecules modulate multiple AD targets, including AChE, BuChE, stop amyloid buildup, block BACE-1, tweak MAO-B, fight oxidation, and act as metal ion chelators.

Structure–activity relationship studies have delineated critical design principles, including optimal linker lengths, appropriate substitution patterns on both the coumarin and attached heterocyclic moieties, and careful consideration of physicochemical properties for CNS penetration. Docking and simulations explain how they interact with targets, backing up the multi-hit approach.

The demonstrated *in vivo* efficacy in animal models, combined with favorable safety profiles, metabolic stability, and BBB penetration, positions coumarin hybrids as serious contenders for clinical development. Ongoing research incorporating emerging therapeutic targets (tau phosphorylation pathways, neuroinflammatory mediators) and advanced computational design methodologies promises further refinement of this promising drug class. The versatility of coumarin chemistry and the accessibility of efficient synthetic routes (particularly click chemistry) facilitate continued structural optimization and the discovery of even more potent multifunctional agents.

Conflicts of interest

All authors of this article declares no conflict of interest.

Abbreviations

AD	Alzheimer's disease
MTDLs	Multi-target directed ligands
AChE	Acetylcholinesterase
BuChE	Butyrylcholinesterase
Aβ	Amyloid β
BACE-1	β-Secretase
MAO-B	Monoamine oxidase B
SAR	Structure–activity relationship
CNS	Central nervous system
PK–PD	Pharmacokinetic–pharmacodynamic
CAS	Catalytic active site
PAS	Peripheral anionic site
RMSD	Root-mean-square deviation
TNF-α	Tumor necrosis factor α
IL-1β	Interleukin 1β
BHT	Butylated hydroxytoluene
COX	Cycloxygenase
FAD	Flavin adenine dinucleotide
GSK-3	Glycogen synthase kinase 3
MDA	Malondialdehyde
GSH	Glutathione
FAB	Features of bioactivity
BBB	Blood brain barrier
P-gp	P-glycoprotein
ADMET	Absorption, distribution, metabolism, excretion, toxicity
PAMPA	Parallel artificial membrane permeability assay
MGB axis	Microbiota–gut–brain axis
LPS	Lipopolysaccharide
QSAR	Quantitative structure activity relationship
PDE	Phosphodiesterase
LAT1	Large neutral amino acid transporter

Data availability

No primary research results, software or code have been included, and no new data were generated or analyzed as part of this review.

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