



# A REVIEW ON INDOLE–CHALCONE DERIVATIVES AS POTENTIAL MULTITARGET THERAPEUTIC AGENTS FOR ALZHEIMER’S DISEASE

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## ABSTRACT :

Alzheimer’s disease (AD) is a neurodegenerative disorder of gradual progression that leads to cognitive decline and multiple pathological processes. A key issue in the treatment of AD is the complexity of the disease mechanism, as a result of which single-target drugs fail to show efficacy. Multitarget-directed ligands (MTDLs) appear to be a promising way to treat such diseases as they modulate several disease-related targets. Indole–chalcone hybrids are of particular interest due to the presence of two pharmacophores with complementary properties. This review discusses the possibilities of using hybrid molecules of indole and chalcone for the treatment of AD. The literature data concerning the treatment of AD with indole derivatives, chalcone-based drugs, and directly related indole–chalcone hybrids were analysed. Particular attention was drawn to the study of the structure–activity relationship, synthesis and methods of study of pharmacological activity of derivatives of these two heterosystems, such as inhibition of cholinesterase enzyme, monoamine oxidase, formation of aggregates of amyloid- $\beta$  peptide and the reduction of oxidative stress and neuroinflammation. Based on the analysis, it was found that some indole–chalcone derivatives showed good monoamine oxidase-B and butyrylcholinesterase inhibitory activities but were weak in terms of acetylcholinesterase inhibition. Data on the activity of these hybrids against other disease mechanisms are lacking. However, the search for optimal ligands worthy of further detailed study is still relevant. Possible problems in this field include the lack of data on the ability to penetrate the blood–brain barrier, low overall efficacy and insufficient study of the interaction of such molecules with various disease-related targets. Targeted optimisation of promising leads, increased multitarget activity and specificity, and comprehensive preclinical study of their pharmacological characteristics will allow further exploration of these hybrid molecules.

**Keywords:** Alzheimer’s disease, multitarget-directed ligands, indole, chalcone, cholinesterase, monoamine oxidase, amyloid- $\beta$ , neuroinflammation

## 1. INTRODUCTION: DISEASE BURDEN AND THE LIMITATIONS OF EXISTING THERAPIES

Alzheimer’s disease (AD) is the most prevalent form of dementia globally, and its incidence will inevitably increase as the population ages. The Global Burden of Disease study estimated that 57.4 million people worldwide were living with dementia in 2019, with this number expected to reach 152.8 million by 2050 [1]. The burden of AD is likewise substantial in the United States, where an estimated 7.2 million individuals aged 65 years and older were living with Alzheimer’s dementia. The related costs are also considerable, with total care expenses estimated at US\$384 billion in 2025. Unless prevention and treatment improve substantially, the number of people with the disease in the US is expected to reach 13.8 million by 2060 [2].

Although understanding of the disease has advanced, effective treatments and disease-modifying strategies remain unavailable. The cholinesterase inhibitors donepezil, rivastigmine, and galantamine, together with memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist, are the only FDA-approved medications for treating AD symptoms. However, their effectiveness is limited, and they do not alter the progression of the disease [3,4]. The recently approved anti-amyloid monoclonal antibodies lecanemab and donanemab demonstrated some ability to slow cognitive and functional decline during the early stages of the disease. Even so, the clinical benefit was relatively modest, and treatment requires neuroimaging confirmation of amyloid positivity while also carrying a risk of amyloid-related imaging abnormalities (ARIA) [5]. Consequently, new therapeutic strategies are needed that address multiple disease mechanisms and possess favourable pharmacokinetic characteristics, including oral bioavailability and the capacity to cross the blood–brain barrier (BBB).

Multitarget-directed ligands (MTDLs) have emerged as promising candidates in the search for disease-modifying drugs for AD. Hybrid compounds containing chalcone and indole motifs are of particular interest because both parent structures have been shown to contribute to anti-amyloid, anti-inflammatory, and neuroprotective effects. This review discusses the rationale for developing multitarget therapeutics to treat AD, emphasising indole – chalcone hybrids. The main sections of the review cover an overview of AD pathophysiology, an examination of currently available treatment options and unmet needs, and a detailed account of the pharmacology of indole-based, chalcone-based, and hybrid compounds. The review also examines the medicinal chemistry of indole – chalcone hybrids, including their synthesis, structure-activity relationships, and pharmacological properties. The concluding section of the review will emphasise the challenges and opportunities associated with using indole – chalcone hybrids in drug discovery.

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## 2. PATHOPHYSIOLOGICAL NETWORK UNDERLYING ALZHEIMER'S DISEASE

### 2.1 Cholinergic dysfunction and role of cholinesterase

One of the major neurochemical changes associated with Alzheimer's disease (AD) is the progressive loss of cholinergic neurons in the basal forebrain. This loss leads to reduced cholinergic signalling in key brain regions such as the cortex and hippocampus, and is the basis of the cholinergic hypothesis of Alzheimer's disease (AD). Cholinergic dysfunction also interacts with disease-associated processes such as amyloid- $\beta$  (A $\beta$ ) accumulation and tau pathology, and therefore remains an attractive therapeutic target, including as part of combination treatments [6,7]. Acetylcholinesterase (AChE) is a key enzyme in the metabolism of acetylcholine, but its peripheral anionic site may be implicated in promoting A $\beta$  aggregation in AD [8], and so compounds that target both catalytic and peripheral sites may provide multifunctional therapeutic potential [8]. Butyrylcholinesterase (BuChE) is another enzyme that may contribute to acetylcholine hydrolysis and whose role becomes more prominent as the disease progresses. Consequently, compounds that possess dual inhibitory activity towards AChE and BuChE are of interest for the treatment of AD [8,9].

### 2.2 Amyloid- $\beta$ aggregation, tau pathology and the anti-amyloid antibody era

The accumulation of extracellular plaques formed from A $\beta$  deposits, together with the formation of intracellular neurofibrillary tangles of hyperphosphorylated tau, are pathological hallmarks of AD [10]. Despite the progress that has been made with monoclonal anti-amyloid antibodies, clinical benefits are often modest, and treatments are associated with amyloid-related imaging abnormalities (ARIA) and require appropriate monitoring of amyloid status [5]. Thus, small molecules that prevent A $\beta$  aggregation remain of interest as an adjunct research strategy, and may provide additional therapeutic rationale if they can also influence AChE-associated A $\beta$  aggregation [8,11].

### 2.3 Glial Activation and Neuroinflammatory Signalling

Neuroinflammation represents an integral part of the pathological network underlying AD, and may contribute to various disease mechanisms. Following activation, microglia and astrocytes undergo changes in their activity and phenotype, which can impact on the production of soluble inflammatory mediators and may contribute to dysregulated A $\beta$  clearance, altered tau-associated pathology and synaptic dysfunction [10,12,13].

Cholinesterase inhibition also provides therapeutic rationale for targeting neuroinflammation through the modulation of inflammatory cytokine expression and suppression of NF- $\kappa$ B-mediated signalling [15]. However, the role of glial activation and inflammatory processes is complex and cannot be fully explained by an amyloid-centric view of AD pathophysiology. Han and colleagues discuss the contribution of microglia- and astrocyte-driven inflammatory cascades to disease development, and highlight the need to consider the role of neuroinflammation alongside the other pathological processes [12]. Other studies also demonstrate the interactions between amyloid accumulation, tau pathology, cholinergic dysfunction, mitochondrial impairment and neuroinflammation [14], and so the modulation of inflammatory signalling represents another therapeutic strategy for the treatment of AD. However, the potential clinical value of this approach depends on the ability to demonstrate substantial anti-inflammatory properties without adversely impacting the physiological functions of activated glial cells [10,15].

### 2.4 Oxidative Stress and Monoamine Oxidase-B Activity

Oxidative stress is an important pathological process that contributes to neuronal dysfunction and loss in AD, and the balance between the production of reactive species and the endogenous antioxidant capacity of the brain may be disrupted [16]. Monoamine oxidase-B (MAO-B), which catalyses the oxidation of biogenic amines, is a target for the treatment of AD because of the role it plays in the generation of hydrogen peroxide, a reactive oxygen species [17]. Consequently, selective and reversible inhibition of MAO-B may provide therapeutic benefit by modulating monoamine metabolism and reducing the contribution of this enzyme to oxidative stress [18].

Pharmacological considerations also highlight the importance of developing compounds with selective and reversible inhibitory activity, because irreversible inhibitors permanently inactivate the enzyme, and activity is restored only through the synthesis of new enzyme [18]. The intrinsic pharmacological properties of an MAO-B inhibitor are therefore of prime importance, and while selectivity and reversibility are advantageous properties, potency and selectivity should be considered in relation to the clinical context [18]. Targeting two disease-associated processes (e.g. cholinergic dysfunction and oxidative stress) with a single molecule is one option explored within multitarget-directed ligand (MTDL) research to identify compounds with enhanced therapeutic potential [19].

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## 3. MULTITARGET-DIRECTED LIGANDS: THERAPEUTIC RATIONALE AND DESIGN CHALLENGES

The traditional approach of developing single-target drugs has failed to provide an adequate solution for the multifactorial nature of the disease because targeting a single molecular site is insufficient to modulate the numerous pathological processes underlying the condition [3, 4, 20]. As a result, recent reviews on the topic highlight multitarget-directed ligands (MTDL) as a strategy to address these complexities, defined as “drugs with multiple independent mechanisms of action targeting two or more discrete molecular entities” [4, 21].

An MTDL is a “single compound able to engage two or more pharmacological or biochemical targets to modify several pathological events in a given disease” [3, 21]. Depending on the design concept, such drugs can simultaneously inhibit two enzyme systems, for instance, cholinesterases and monoamine oxidase (MAO), and influence different aspects of the pathophysiology of AD, including the aggregation of amyloid- $\beta$  (A $\beta$ ) and tau proteins,

the production of oxidative stress or neuroinflammation [3, 21]. In comparison to combination therapies using multiple single-target drugs, MTDL offer the advantage of potentially optimised pharmacokinetics and reduced side-effects due to the absence of polypharmacy, although this depends on the individual compound [20]. At the same time, by engaging different molecular targets in a balanced manner, an MTDL can provide greater therapeutic efficacy than a single-target drug with a high potency, which can have detrimental off-target effects [20]. Among the reported design approaches to develop MTDL, hybrid molecules containing pharmacophore elements connected via a chemical linker, and bifunctional compounds formed by the merging of two pharmacologically active moieties seem the most promising [3, 20].

However, the design of MTDL poses a significant challenge for medicinal chemistry. Since a single compound needs to have a balanced profile of activity across different targets, optimising its structure to achieve sufficient potency for each of them can be difficult [3, 21]. Additionally, an MTDL's molecular weight, lipophilicity, and other pharmaceutically relevant properties should be optimised to ensure that the drug can effectively pass the blood-brain barrier and reach the target tissues [3, 21]. Therefore, the selection of an appropriate molecular framework that can be modified to contain the additional pharmacophore elements required to engage multiple targets is crucial for the development of an MTDL. Particularly, indole and chalcone scaffolds can be promising lead compounds because they can be chemically modified to possess multiple pharmacological activities. However, the therapeutic potential of these scaffolds should be validated by pharmaceutical studies assessing their drug-likeness.

## 4. PHARMACOPHORE RATIONALE FOR INDOLE–CHALCONE DERIVATIVES

### 4.1 Indole as Privileged Neuroactive Scaffold

Indole has emerged as a privileged pharmacophore in medicinal chemistry, and its potential utility in the design of compounds targeting neurodegenerative processes has been highlighted in several recent review articles [22], which consider pharmacological issues and data pertinent to indole-derived compounds reported in the literature from 2018 to 2023. Some of the features that may be relevant to the design of multifunctional neuroprotective agents for the treatment of Alzheimer's disease (AD) include:

1. Melatonin- and tryptophan-related features. Thus, the indole ring is a constituent of both melatonin and tryptophan, and this pharmacophore has been exploited in attempts to obtain compounds with antioxidant activity and neuroprotective properties. More recently, indole hybridisation approaches have also exploited the potential of indole-based pharmacophores for the inclusion of cholinesterase-inhibiting pharmacophores such as those present in rivastigmine-related structures [8].
2. Potential application to the design of cholinesterase inhibitors (donepezil type). Indole derivatives have been targeted as alternative scaffolds to develop AChE inhibitors. The potential of indole-based compounds to interact with aromatic residues present in the binding gorge of the enzyme has been demonstrated, and such derivatives can be subject to pharmacophore hybridisation strategies in order to obtain multifunctional AChE inhibitors [8, 9].
3. Monoamine oxidase (MAO) inhibitory potential. The pharmacophore relevant to MAO inhibition is represented by the indolylpropargylamine moiety, and hybridisation studies have also exploited the opportunity to combine this pharmacophore with that present in donepezil (the benzylpiperidine portion), resulting in hybrids with enhanced inhibitory properties towards MAO-A and MAO-B ( $IC_{50}$  = 5.2 and 43 nM respectively), as well as towards AChE and BuChE (0.35 and 0.46  $\mu$ M respectively) [19].
4. Reports of multifunctional indole derivatives. The multifunctional nature of contilisant and its recently reported belinostat-based hybrids also illustrate the type of pharmacological profile that could be targeted in hybridisation approaches based on the indole nucleus; in this case, the hybrid compounds were found to display inhibitory activity towards cholinesterases and MAO-B and HDACs [17]. These data illustrate the potential of the indole nucleus as a pharmacophore in the design of multifunctional neuroprotective agents. However, the pharmacological and toxicological relevance of each hybrid would have to be considered individually.

### 4.2 Chalcone as a Tunable Scaffold and Linker

Chalcones are open-chain compounds consisting of two aromatic rings linked through a conjugated  $\alpha,\beta$ -unsaturated carbonyl system, typically referred to as the enone. As a precursor in flavonoid biosynthesis, they provide a versatile chemical framework for medicinal chemistry and have relatively simple structures, a variety of substituents and synthetic accessibility, making them attractive for multitarget-directed ligands (MTDL) for Alzheimer's disease (AD) [23]. This potential pharmacological relevance is supported by a number of complementary activities:

- Cholinesterase and monoamine oxidase inhibition. Chalcone-based derivatives and their hybrids have been explored for inhibitory activity against acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B). Study of structure–activity relationships suggests that modifications to substituents in either aromatic rings can influence inhibitory potency and target selectivity, presenting opportunities to tailor pharmacological profiles [18, 23].
- Inhibition of amyloid-related processes. Certain chalcone-containing hybrids have demonstrated activity against amyloid- $\beta$  (A $\beta$ ) aggregation and have also been explored for effects on  $\beta$ -secretase and cholinesterases [11, 15]. This suggests that chalcone-based structures may contribute to multitarget strategies targeting amyloid-related pathology and cholinergic dysfunction, although the extent of these effects depends on the compound and would require experimental confirmation.
- Antioxidant and anti-inflammatory potential. The conjugated enone group can act as a Michael acceptor, allowing suitable chalcone derivatives to interact with biological nucleophiles and potentially modulate cellular signalling pathways. Investigation of chalcones have looked at the activation of the Kelch-like ECH-associated protein 1/nuclear factor erythroid 2-related factor 2 (Keap1/Nrf2) antioxidant pathway and suppression of nuclear factor kappa B (NF- $\kappa$ B)-associated inflammatory signalling [24]. Cardamonin, a naturally occurring chalcone, has also been reviewed for its potential neuroprotective effects involving oxidative stress, Nrf2-related responses and neuroinflammation [16]. These

properties make chalcone derivatives interesting candidates for further investigation, although their activity and safety depends on molecular structure and biological context.

#### 4.3 Structural basis for derivative design

The combination of indole and chalcone scaffolds rationalises the design of multitarget-directed hybrids for the treatment of Alzheimer's disease. The integration of these two structural elements by a conjugated  $\alpha$ ,  $\beta$ -unsaturated carbonyl group via a Claisen-Schmidt condensation strategy allows the presentation of complementary structural features within a hybrid compound. The indole ring may participate in aromatic and cation- $\pi$  interactions within cholinesterase binding sites, while its substitution pattern and overall structure may determine its affinity for monoamine oxidase B (MAO-B) and its radical scavenging ability [8, 9, 19, 22]. Meanwhile, the  $\alpha$ ,  $\beta$ -unsaturated carbonyl moiety, which serves as a conjugated linker, may contribute to molecular recognition, although experimental evidence is required to confirm its role as a Michael acceptor involved in Nrf2 activation or NF- $\kappa$ B suppression [18, 24]. Additionally, the substitution pattern of the second aromatic ring may contribute to the modulation of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity, MAO-B selectivity and inhibition, and lipophilicity of the hybrid molecule [18, 23, 25]. Hydroxy, methoxy, and halogen substituents may affect electronic distribution and hydrogen-bonding interactions, influencing antioxidant activity and blood-brain barrier (BBB) penetration, among other pharmacological properties [8, 18]. Altogether, these structural elements provide a rational basis for the optimisation of chalcone-indole hybrids. However, the impact of these structural features on the inhibition of cholinesterases, MAO-B activity, amyloid- $\beta$  (A $\beta$ ) aggregation, oxidative stress, inflammation, and BBB penetration should be validated experimentally.

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## 5. LITERATURE REVIEW OF REPORTED ANTI-ALZHEIMER'S CHEMOTYPES

### 5.1 Indole-Based Multitarget Ligands

Indole-based compounds are interesting candidates for the treatment of Alzheimer's disease (AD) as various substituents may be introduced at the heterocycle to fine-tune target affinity and biological activity. Gupta and co-workers targeted hydroxy- and methoxy-substituted benzylidene indole derivatives and identified IND-30 as a promising hit within the series. They found it to inhibit human acetylcholinesterase (AChE) with an IC<sub>50</sub> value of 4.16  $\mu$ M and preferentially target this enzyme over butyrylcholinesterase (BuChE) with a 22.92-fold selectivity. IND-30 was furthermore associated with radical-scavenging activity, did not display cytotoxic effects on SH-SY5Y cells under the described test conditions, and reduced AChE-induced amyloid- $\beta$  (A $\beta$ ) aggregation by about 50% at 20  $\mu$ M. Yet, its AChE-inhibitory properties were found to be lower than those of the clinical reference donepezil, which displayed an IC<sub>50</sub> of 0.03  $\mu$ M in the same study [8]. A similar approach by Azmy and co-workers involved the preparation of indole derivatives with oxathiolanyl, pyrazolyl, and pyrimidinyl moieties. Three of the investigated compounds were found to inhibit AChE with IC<sub>50</sub> values ranging from 0.042 to 2.54  $\mu$ M and BuChE with IC<sub>50</sub> values ranging from 0.207 to 3.003  $\mu$ M. Molecular docking and molecular dynamics simulations corroborated the likelihood that these scaffolds could bind to both cholinesterases [9]. In a hybridisation approach, Schäker-Hübner and co-workers coupled the indole-based pharmacophore of contilisant to the histone deacetylase inhibitor belinostat, obtaining compounds with good inhibitory activity across HDAC 1 and 6 and cholinesterases as well as monoamine oxidase B (MAO-B) [17]. Finally, Bolea and co-workers described donepezil-indolylpropargylamine hybrids as early proof-of-concept molecules for the design of bifunctional cholinesterase inhibitors and monoamine oxidase modulators [19]. Overall, this collection of articles demonstrates the versatility of an indole-based core structure to provide multitarget ligands with various degrees of success for the treatment of AD. The described chemotypes were shown to inhibit cholinesterases, modulate A $\beta$  aggregation, scavenge radicals, and target additional enzymes relevant to the disease. Yet, caution should be exercised when comparing the activity of the described compounds, as differences in the assay conditions, target affinity, selectivity, and the extent of cellular activity have been observed across reports. More work is clearly required to identify optimal candidates for the treatment of AD based on the described hits and to obtain a thorough understanding of their mechanism of action.

### 5.2 Chalcone-Based Multifunctional Agents

A report from 2025 on chalcones targeting AD has concluded that oxindole-chalcones are effective AChE inhibitors, chalcone rivastigmine conjugates excel as BuChE inhibitors, and 4-aminochalcone rivastigmine and morpholine-chalcone hybrids are potent MAO-B inhibitors [23]. Amongst the most recent examples, a benzoylpiperazine-thiophene chalcone conjugate (compound 2e) inhibited AChE, BuChE and A $\beta$ 42 aggregation with IC<sub>50</sub> of 7.01, 5.39 and 4.47  $\mu$ M, reduced reactive oxygen species and inflammatory cytokines in microglial cells and exerted its action via the NF- $\kappa$ B pathway [15]. A set of 32 donepezil-chalcone compounds have been tested in vitro and in vivo; the best ones inhibited A $\beta$ 1–42 aggregation (IC<sub>50</sub> 3.40  $\mu$ M), induced its disaggregation (IC<sub>50</sub> 4.53  $\mu$ M) and inhibited BACE-1 (IC<sub>50</sub> 1.82  $\mu$ M) [11].

### 5.3 Indole-Chalcone Conjugates: Current Evidence

Investigations on indole-chalcone conjugates and related hybrids have yielded promising evidence of the capacity of these chemotypes to modulate multiple targets relevant to Alzheimer's disease (AD). Sasidharan and co-workers synthesised eleven indole-based chalcone derivatives (IC1–IC11) bearing electron-donating or electron-withdrawing substituents on the phenyl ring. Most compounds exhibited potent and selective inhibition of human monoamine oxidase B (hMAO-B) with reversible and competitive inhibition reported. IC9 showed the most promising activity with a K<sub>i</sub> value of 0.01  $\pm$  0.005  $\mu$ M and high selectivity index (SI = 120) in the reported enzyme assay. It also exhibited antioxidant activity in the ABTS assay and acceptable cell viability at tested concentrations. Parallel artificial membrane permeability assay experiments (PAMPA) were performed to assess the potential for a series to cross the blood-brain barrier (BBB), although such assays provide preliminary estimates of permeability rather than definitive evidence of brain

exposure [18]. These findings offer biochemical and preliminary physicochemical justification for the further development of indole-based chalcones as potential MAO-B inhibitors.

Expanding on this approach, Tang and co-workers synthesised 31 chalcone derivatives incorporating indole and benzothiophene moieties and evaluated their cholinesterase- and monoamine oxidase-inhibitory activities. The series generally showed weak acetylcholinesterase (AChE) inhibition, whereas selected derivatives showed substantial butyrylcholinesterase (BuChE) inhibition. Compound 1a, identified as an indol-3-yl-containing chalcone, produced 85.55% BuChE inhibition at the reported screening concentration, whereas compound 1h produced 76.43% inhibition. Further evaluation of selected compounds identified derivatives with activity against MAO-A and MAO-B, and molecular docking analyses suggested potential interactions with the corresponding enzyme binding sites. The more active compounds also showed no detectable cytotoxicity toward L929 cells under the reported experimental conditions. Nevertheless, the percentage inhibition values obtained at a screening concentration do not establish  $IC_{50}$  values, and further concentration-dependent testing is required to characterise potency and selectivity [25].

Related indole-only chemotypes (IND-30 [8]; the oxathiolanyl-, pyrazolyl- and pyrimidinyl-substituted indoles [9]; the donepezil–indolylpropargylamine hybrids [19]; the contilisant–belinostat hybrids [17]) and chalcone-based hybrids (compound 2e [15]; the donepezil–chalcone hybrids [11]), described in Sections 5.1 and 5.2, show that cholinesterase, MAO, A $\beta$  aggregation, BACE-1 and HDAC endpoints can each be addressed through hybrid design. These findings are relevant to the design of indole–chalcone conjugates, although the biological activities of these related compounds should not be assumed to apply automatically to indole–chalcone conjugates.

Overall, the available literature provides encouraging evidence for MAO-B inhibition by indole-based chalcones and BuChE inhibition by selected indole–benzothiophene chalcones. Related indole and chalcone hybrids also demonstrate the feasibility of targeting cholinesterases, A $\beta$  aggregation, BACE-1, and other enzymes. However, these findings represent different chemotypes and levels of experimental evidence and cannot be assumed to imply that a single indole–chalcone hybrid might exhibit all of these activities. Future research should therefore prioritise systematic structure–activity relationship studies, quantitative evaluation of AChE, BuChE, and MAO-A/B inhibition, investigation of A $\beta$  aggregation and neuroinflammatory pathways, and assessment of BBB permeability, cellular safety, and in vivo efficacy. Such studies will help determine whether rational integration of indole and chalcone scaffolds can produce balanced multitarget activity with properties suitable for further AD drug development.

Table 1 summarises the activity data discussed in Sections 5.1–5.3. Assay conditions differ between studies, so values should not be compared directly across rows.

**Table 1. Reported activities of the indole-based, chalcone-based and indole–chalcone compounds discussed in Section 5.**

| Compound / series                                                                  | Reported activity                                                                                                                                              | Additional findings                                                                                                                      |
|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| IND-30 (benzylidene indole)                                                        | hAChE $IC_{50}$ $4.16 \pm 0.063$ $\mu$ M; 22.92-fold selectivity for AChE over BuChE; reduced AChE-induced A $\beta$ aggregation by about 50% at 20 $\mu$ M    | Radical scavenging; no cytotoxicity in SH-SY5Y cells; donepezil $IC_{50}$ 0.03 $\mu$ M in the same study                                 |
| Oxathiolanyl-, pyrazolyl- and pyrimidinyl-substituted indoles (compounds 5 and 11) | AChE $IC_{50}$ 0.042 and 0.052 $\mu$ M (compounds 5 and 11); across three compounds, AChE $IC_{50}$ 0.042–2.54 $\mu$ M and BuChE $IC_{50}$ 0.207–3.003 $\mu$ M | Docking and molecular dynamics support binding to both cholinesterases                                                                   |
| Donepezil–indolylpropargylamine hybrid                                             | MAO-A $IC_{50}$ 5.2 nM; MAO-B $IC_{50}$ 43 nM; AChE $IC_{50}$ 0.35 $\mu$ M; BuChE $IC_{50}$ 0.46 $\mu$ M                                                       | Early proof-of-concept cholinesterase/MAO hybrid                                                                                         |
| Contilisant–belinostat hybrids                                                     | Activity against HDAC1, HDAC6, cholinesterases and MAO-B                                                                                                       | Potency and selectivity to be assessed per compound                                                                                      |
| Compound 2e (benzoylpiperazine–thiophene chalcone)                                 | $IC_{50}$ 7.01, 5.39 and 4.47 $\mu$ M for AChE, BuChE and A $\beta$ 42 aggregation, respectively                                                               | Reduced ROS and inflammatory cytokines in microglial cells; acts via the NF- $\kappa$ B pathway                                          |
| Donepezil–chalcone hybrids (best compounds)                                        | A $\beta$ 1–42 aggregation $IC_{50}$ 3.40 $\mu$ M; disaggregation $IC_{50}$ 4.53 $\mu$ M; BACE-1 $IC_{50}$ 1.82 $\mu$ M                                        | 32 compounds tested in vitro and in vivo                                                                                                 |
| IC1–IC11 (IC9 most active)                                                         | hMAO-B $K_i$ $0.01 \pm 0.005$ $\mu$ M for IC9; selectivity index 120                                                                                           | Reversible, competitive inhibition; antioxidant activity (ABTS); acceptable cell viability; PAMPA used for preliminary BBB estimate      |
| Indole- and benzothiophene-containing chalcones (compounds 1a and 1h)              | BuChE inhibition 85.55% (1a) and 76.43% (1h) at the screening concentration; weak AChE inhibition overall                                                      | MAO-A/MAO-B activity in selected compounds; no detectable cytotoxicity in L929 cells; percentage inhibition does not establish $IC_{50}$ |

## 6. PROPOSED SYNTHETIC AND PHARMACOLOGICAL EVALUATION STRATEGY

### 6.1 Molecular Design and Synthetic Approach

Rational design of indole–chalcone hybrids requires combining the indole scaffold with a substituted aromatic ring via a conjugated  $\alpha,\beta$ -unsaturated carbonyl linker, to allow modification of the electronic, steric, and physicochemical properties of the resulting compound and its multitarget activity against multiple Alzheimer's disease (AD)-related targets. Indole-3-carbaldehyde derivatives, with and without N-substitution or substitution at the 5-position, could be starting materials for structural diversity. A reported approach to chalcones is to apply the base-catalysed Claisen–Schmidt condensation of indole-derived aldehydes with appropriately substituted acetophenones, affording chalcone analogues with various substituents on the second aromatic ring [25].

Hydroxy and methoxy substituents could be applied to explore their effects on antioxidant activity, molecular interactions, and physicochemical properties. Previously reported substituted indole derivatives displayed cholinesterase inhibition and radical-scavenging activity, providing a rationale for exploring related structural modifications in related hybrid compounds [8]. However, the effect of individual substituents on biological activity and blood–brain barrier (BBB) permeability should be experimentally established. After synthesis, the identity of the compounds should be confirmed by complementary analytical techniques, including Fourier-transform infrared (FT-IR) spectroscopy, proton and carbon-13 nuclear magnetic resonance ( $^1\text{H}$  and  $^{13}\text{C}$  NMR) spectroscopy, and mass spectrometry, as appropriate [8, 25].

A general synthetic route is shown in Figure 3.

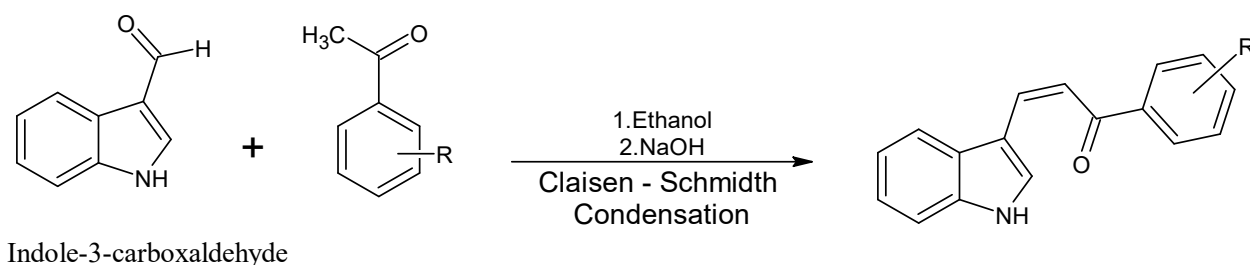


**Figure 3.** Proposed synthesis of indole–chalcone hybrids by base-catalysed Claisen–Schmidt condensation of indole-3-carbaldehydes with substituted acetophenones [25]. R<sup>2</sup>, optional substituent at C-5 of the indole; R<sup>3</sup>, substituent(s) on the acetophenone ring. N-substituted indole-3-carbaldehydes may be used in the same way.

### 6.2 Proposed Pharmacological Evaluation Framework

A stepwise strategy for pharmacological evaluation is proposed to identify indole–chalcone hybrids with balanced activity profile, acceptable preliminary safety, and physicochemical properties for further development as potential anti-AD agents. Molecular docking against acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), monoamine oxidase B (MAO-B), and  $\beta$ -site amyloid precursor protein-cleaving enzyme 1 (BACE-1) could be considered initially to identify plausible binding interactions and focus experiments. Molecular dynamics simulations could complement computational investigations to provide more insight into the stability of the predicted protein–ligand complexes, while computational absorption, distribution, metabolism, and excretion (ADME) evaluations could support preliminary evaluation of drug-likeness and potential BBB permeability. However, computational predictions should be validated with appropriate experimental studies [8, 9].

The next step should involve the biochemical evaluation of the prioritised compounds. AChE and BuChE inhibition could be investigated via the Ellman colourimetric assay, while MAO-A and MAO-B activity could be explored using validated enzyme assays with appropriate controls. Dose–response studies should be undertaken to determine half-maximal inhibitory concentration (IC<sub>50</sub>) values and compare activity between investigated targets. Where appropriate, kinetic and reversibility studies should be undertaken to elucidate the mode of inhibition. These investigations are important given that considerable differences in potency and selectivity among indole-based chalcones and related derivatives have been reported [8, 18].

Given the relevance of amyloid- $\beta$  (A $\beta$ ) aggregation in AD, selected compounds should be investigated using appropriate aggregation assays, including thioflavin-T-based measurement of A $\beta$ <sub>1–42</sub> aggregation and assays exploring acetylcholinesterase-induced A $\beta$  aggregation. Such experiments could provide more information on whether the compounds influence amyloid aggregation beyond their enzyme-inhibitory effects. Appropriate controls for the assays are critical to differentiate between genuine inhibition of aggregation and interference with fluorescent signals or other measurement artefacts. Previous studies with indole derivatives and chalcone-containing hybrids offer precedents for investigating these endpoints [8, 11, 15].

Antioxidant activity could be explored initially with chemical radical-scavenging assays, such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. While these assays could provide an initial indication of radical scavenging, they are not sufficient on their own to establish antioxidant or neuroprotective effects in a biological system. Compounds that display promising biochemical activity could be prioritised for cell-based investigations. These may include the assessment of cytotoxicity in SH-SY5Y neuroblastoma cells, protection against A $\beta$ -associated injury, and the modulation of inflammatory responses in appropriately validated microglial models. Measurement of inflammatory mediators and nuclear factor kappa B (NF- $\kappa$ B)-associated signalling could help establish the ability of the selected compounds to influence neuroinflammatory pathways. Previous studies of indole-based chalcones and related chalcone derivatives provide a foundation for these experimental approaches [8, 15, 18].

Finally, compounds that show consistent multitarget activity, acceptable cellular tolerability, and preliminary physicochemical properties could be considered for further pharmacokinetic and in vivo evaluation. Experimental assessment of brain exposure is particularly important as predicted BBB

permeability does not necessarily translate into adequate central nervous system penetration. Where appropriate, based on the preceding results, animal studies could investigate cognitive outcomes, relevant biochemical or pathological endpoints, and preliminary safety. Progression to these studies should depend on the preceding evidence and ethical requirements. Related studies with donepezil–chalcone hybrids provide a precedent for investigating multiple AD-related biochemical endpoints, but they do not establish the efficacy of newly proposed indole–chalcone compounds [11].

Overall, candidate compounds should be prioritised on the basis of the balance of activity profiles across relevant targets, rather than the potency against a single enzyme alone. This reflects the core principle behind multitarget-directed ligand (MTDL) design, to identify compounds with a useful combination of complementary activities and acceptable selectivity, cellular tolerability, and drug-like properties. A systematic progression from computational evaluation to biochemical, cellular, and, where appropriate, in vivo studies would be a better basis for establishing the therapeutic potential of indole–chalcone hybrids for AD.

The proposed stages are summarised in Table 2 and Figure 4.

**Table 2. Proposed stepwise pharmacological evaluation of indole–chalcone hybrids.**

| Stage                                   | Methods                                                                                                                         | Purpose / readout                                                                              |
|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| 1. In silico                            | Docking (AChE, BuChE, MAO-B, BACE-1); molecular dynamics; ADME and BBB prediction                                               | Plausible binding interactions, stability of complexes, drug-likeness; focuses experiments     |
| 2. Enzyme assays                        | Ellman assay (AChE, BuChE); MAO-A and MAO-B assays; dose–response, kinetic and reversibility studies                            | IC <sub>50</sub> values, selectivity, mode of inhibition                                       |
| 3. Aβ aggregation                       | Thioflavin-T assay (Aβ1–42); AChE-induced aggregation assay                                                                     | Effect on aggregation beyond enzyme inhibition; controls for fluorescence interference         |
| 4. Antioxidant and cell-based assays    | DPPH assay; SH-SY5Y cytotoxicity; protection against Aβ-associated injury; microglial inflammation; NF-κB-associated signalling | Preliminary radical scavenging; cellular tolerability; neuroinflammatory effects               |
| 5. Pharmacokinetics and in vivo studies | Brain exposure; cognitive outcomes; biochemical or pathological endpoints; preliminary safety                                   | Confirmation of CNS penetration and efficacy of compounds with consistent multitarget activity |

## 8. CONCLUSIONS AND FUTURE PERSPECTIVES

- Alzheimer's disease involves multiple interconnected pathological mechanisms, including cholinergic dysfunction, amyloid-beta and tau accumulation, oxidative stress, and neuroinflammation.
- Indole–chalcone hybrids offer a promising multitarget-directed ligand (MTDL) approach by combining potentially beneficial cholinesterase-inhibitory, MAO-B-inhibitory, antioxidant, and anti-inflammatory properties.
- Future studies should focus on balanced AChE, BuChE, and MAO-B inhibition, alongside investigations of amyloid-beta aggregation, tau pathology, and neuroinflammatory pathways.
- Experimental evaluation of blood–brain barrier permeability, pharmacokinetic properties, safety, and in vivo efficacy will be essential to establish their therapeutic potential in Alzheimer's disease.

### Abbreviations

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AChE, acetylcholinesterase; AD, Alzheimer's disease; ADME, absorption, distribution, metabolism and excretion; ARIA, amyloid-related imaging abnormalities; Aβ, amyloid-β; BACE-1, β-site amyloid precursor protein cleaving enzyme 1; BBB, blood–brain barrier; BuChE, butyrylcholinesterase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; HDAC, histone deacetylase; MAO, monoamine oxidase; MTDL, multitarget-directed ligand; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; PAMPA, parallel artificial membrane permeability assay; ROS, reactive oxygen species.

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### Declaration

Conflict of interest: The authors declare that they have no known financial, commercial, or personal conflicts of interest that could have influenced the work reported in this review article. All authors confirm that the research was conducted independently and that no external organisation or individual had any role that could bias the content or conclusions of this manuscript.

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