Current and emerging therapeutic targets of alzheimer's disease for the design of multi-target directed ligands.

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Current and Emerging Therapeutic Targets of Alzheimer's Disease for the Design of Synthetic Multi-Target Directed Ligands

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ABSTRACT

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease, and a major cause of death worldwide. The number of people suffering from this debilitating disorder is rising at an unprecedented rate, with a subsequent surge in healthcare costs. Only four drugs are clinically available for the treatment of AD symptoms, but they are not disease-modifying. Consequently, there is an urgent need for a cure. Although the cause of this debilitating condition remains poorly understood, it is believed that several factors may be involved in combination – including, health and lifestyle, environmental, and genetic factors. In recent years, a number of hallmarks of the disease have also been discovered, and it is believed that these factors may play an important role in the development of AD. Amyloid aggregation is one such factor which has been highly investigated, in addition to cholinesterase enzymes and tau aggregation. In the last decade, multi-target drugs have been increasingly investigated for their application to AD treatment. By combining two or more pharmacophores in a single compound, it is possible to synthesise a drug which can target several factors that are involved in AD development. This is a particularly attractive approach as it would avoid the use of combination therapies. As a result, it could reduce the burden on carers and families, and decrease healthcare and social care costs. Many active pharmacophores have been employed for the development of hybrid drugs, due to their abilities to inhibit the factors currently widely recognised to be involved in AD. These compounds have demonstrated promising results; however, research is still required to optimise the pharmacological profiles of the drugs, in addition to their potencies. Meanwhile, extensive research is continuously being performed into other potential targets for the treatment of AD. Based on the results obtained thus far, it is likely that multi-target compounds will continue to be increasingly studied in the future as potential treatments for AD.

Keywords: Alzheimer's disease; multi-target drugs; hybrid compounds; inhibitory activity

1 Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease and a prevalent cause of death worldwide. This slowly progressive disease is the main cause of dementia, and is characterised by memory and orientation loss, impaired judgement, language disturbances, and behavioural changes (e.g. irritability, depression).[1] These symptoms are due to a loss of neuronal cells in the brain. In most cases, it is believed that AD is caused by a combination of genetic, lifestyle and environmental factors that affect the brain over time. As with the majority of neurodegenerative diseases, AD is age-related and mainly affects people over 65 years old. In 2015, an estimated 46.8 million people worldwide suffered with AD, and this figure is expected to rise to 131.5 million in 2050 with a subsequent increase in the social and financial burden (shown in Figure 1).[2] The estimated total economic cost of dementia in the UK is £26 billion a year,[3] and this is projected to rise to £66 billion in 2050.[4]

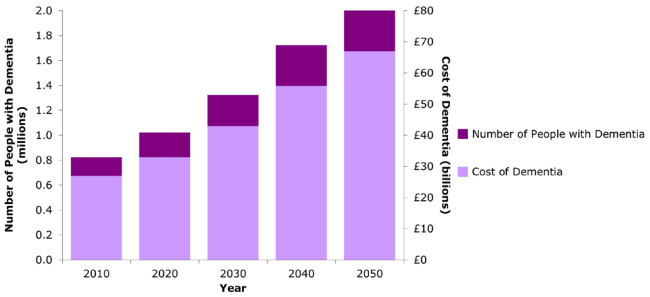


Figure 1: Predicted rise in the number of people with dementia, and the total cost of dementia, in the UK between 2010 and 2050 (data obtained from [3]).

No cure exists for this disease. Currently, only four drugs are approved to treat AD, and these are used to treat the symptoms only. These medications aim to improve the quality of life of the patient, but they are unable to halt the progression of the disease.[5] Drug development for AD faces one of the highest failure rates in any therapeutic area, which is due to the fact that the exact cause of AD is still poorly understood. As a result, drug candidates may be designed for the wrong target, or at least not the sole target involved in the disease.

2 Causes of AD

AD accounts for around 60-80% of the dementia cases in the UK.[6] This debilitating disorder is associated with a loss of neurons, deterioration of neurotransmitter systems, and the accumulation of abnormal proteins such as senile plaques and neurofibrillary tangles.[7] Diagnosis of AD is based on clinical findings; including memory loss, language impairment, and a decline in the recognition of familiar objects, people and places. Currently, technology does not allow the diagnosis of this disease on the sole basis of laboratory tests or neuroimaging.[7]

Although AD is the most common chronic neurodegenerative disease, the etiology of the disorder remains poorly understood. A number of hallmarks of the disease pathology have been identified. Therefore, it is currently theorised that a combination of factors may be responsible for the progression of the disease, but the exact initial cause is unknown and may be of varied origin. Factors influencing the risk of developing the disease have also been identified in recent years, including genetic and lifestyle factors (see Figure 2). However, the major risk factor is age, with an increase in the likelihood of developing the disease as age rises.[8] With a global rise in life expectancy, the prevalence of this disease is increasing exponentially. However, it is reported that the risk can be reduced by considering the factors that influence the development of the disease (e.g. by maintaining a healthy diet and lifestyle).

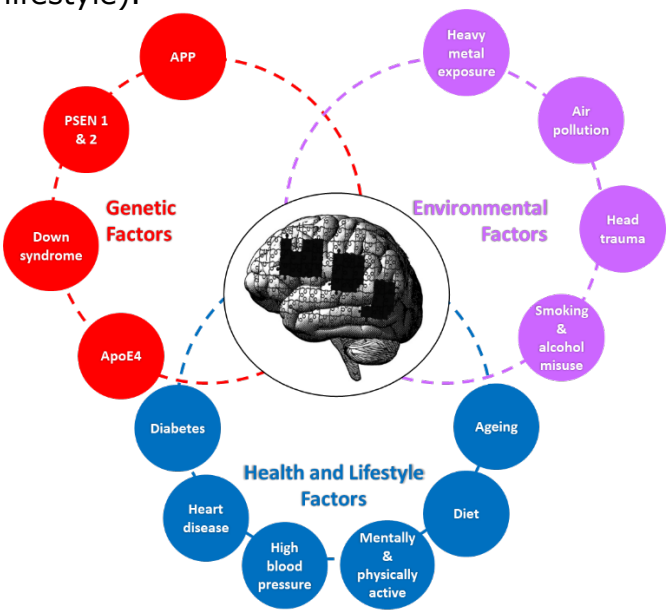


Figure 2: Factors influencing the risk of AD development.

2.1 Health and Lifestyle Factors

Factors associated with cardiovascular disease can also increase the risk of developing AD; including, obesity, high blood pressure, high cholesterol, and diabetes.[9] While the exact relationship between cardiovascular disease and Alzheimer's disease is unclear, it has been hypothesised that vascular disease could induce the onset of dementia due to their linked genetics and shared risk factors.[9] In order to avoid these risk factors, it is advised that people should lead a healthy lifestyle (e.g. stop smoking, consume less alcohol, maintain a healthy diet, and keep active mentally and physically), and have regular health checks.

2.2 Environmental Factors

Chronic low-level exposure to heavy metals and pesticides, such as lead and aluminium, have also been reported to cause a

progressive decline in mental functions and increase the incidence of neurodegenerative diseases. There is increasing evidence that exposure to air pollution and fungal pathogens is associated with the development of AD.[9] Airborne, iron-rich, strongly magnetic, combustion-derived nanoparticles have been studied and found to be highly oxidative and associated with mitochondrial dysfunction, accumulation of unfolded proteins, calcium homeostasis, and apoptotic signalling, which are all known hallmarks of AD and Parkinson's disease.[10]

2.3 Genetic Factors

Patients suffering from certain conditions have been found to be at a greater risk of developing AD. For example, the genetic defect exhibited in people with Down's syndrome can lead to a build-up of amyloid plaques in the brain over time which can lead to AD.[9]

Familial AD (FAD) is associated with an earlier onset of symptoms (patients in their 30s or 40s), and accounts for approximately 2-3% of cases of AD.[8] Several rare autosomal dominant point mutations are reported to be responsible for FAD. Mutations in the gene coding for amyloid precursor protein (APP) on chromosome 21 can increase the production of β-amyloid, or increase the incidence of the longer form of β-amyloid (42 amino acids) which has enhanced aggregation levels compared to the shorter form (40 amino acids).[11] Mutations have also been found to occur frequently in presenilin 1 (PSEN1) and presenilin 2 (PSEN2) genes, which encode the two subunits of the enzyme, γ-secretase. This enzyme is responsible for cleaving APP and forming Aβ peptides. Apolipoprotein E4 (ApoE4) with homozygosity has been established to be a major risk allele, with an approximately 8-fold increase in the risk of AD development.[8] ApoE4 is a lipid-binding protein involved in synaptic repair and maintenance of neuronal function.[11] However, Fish et al.[8] reported that it is still unknown whether an increase or decline of ApoE4 levels would be favourable.

3 Multifactorial Nature of AD

While the exact cause of AD is not yet known, it has been reported that several hallmarks are associated with the pathological development of the disease (see Figure 3); including acetylcholinesterase, tau protein, reactive oxygen species, metal ions, and β-amyloid.[12]

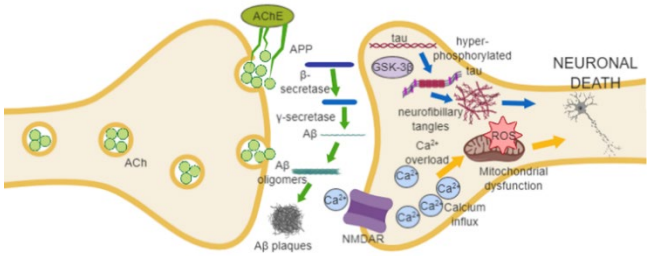


Figure 3: Factors involved in AD pathology and the major potential therapeutic targets: AChE, NMDAR, β-secretase, γ-secretase, Aβ oligomers, Aβ plaques, GSK-3β, neurofibrillary tangles, Ca^{2+} ions, and ROS.

3.1 Amyloid Aggregation

Amyloid beta (Aβ) peptides are proteins of typically 39-42 residues long, and are the main component of amyloid plaques: a well-recognised hallmark of AD. This protein is formed by the proteolytic cleavage of the amyloid precursor protein (APP), by β- and γ-secretase.[13] $Aβ_{1-42}$ is one of the most abundant isoforms of Aβ, and is highly prone to produce oligomers, which are highly toxic and considered to be the key neurotoxin in this disease. The accumulation of excess Aβ monomers and mutations within the APP gene, followed by the self-assembly of these peptides, can result in the production of insoluble Aβ aggregates which deposit extracellularly to form amyloid plaques around the neurons (see Figure 4). This process has been termed as the amyloid cascade hypothesis, which suggests that the aggregation of amyloid proteins is a stimulus for AD pathogenesis.[14] The amyloid hypothesis has dominated AD research and drug development for the last 25 years because it has been regarded as the most promising potential cause of AD. Although the association between amyloid plaques and AD development is still poorly understood, Aβ aggregates (e.g. oligomers) have been found to induce a higher

neurotoxicity than the insoluble fibrils, which is contrary to early research.[15] Therefore, it is now considered that several Aβ species may be responsible for initiating AD.

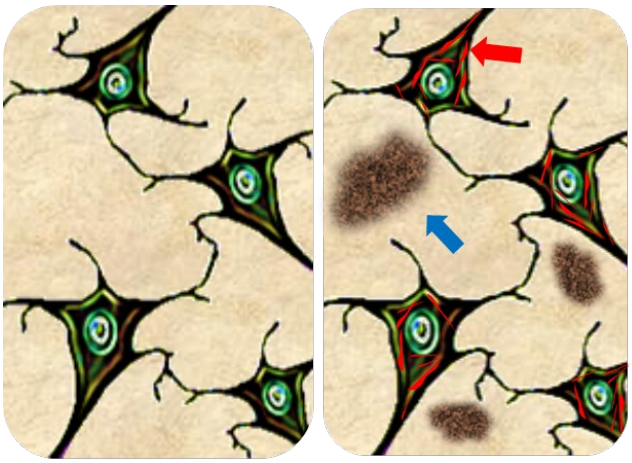


Figure 4: Diagram of normal neurons on the left, and neurons of an AD patient with neurofibrillary tangles within the neuronal cells (indicated with the red arrow) and amyloid plaques present extracellularly (blue arrow).

3.2 Oxidative Stress

Oxidative stress is associated with many pathological conditions, such as cancer, neurological diseases, diabetes, and asthma.[16] It is caused by an imbalance between pro-oxidants and antioxidants, whereby there is a lack of antioxidants or an over-production of oxidants. This results in a build-up of reactive oxygen species (ROS) and other free radicals that can potentially damage cell components; for example, lipids, proteins, and DNA. Oxidative stress can be caused by environmental factors, including chemicals, UV light, and infectious organisms. Alternatively, it can be intrinsically induced; for example, by the electron transport chain in mitochondria, some enzyme activities (e.g. NADH oxidase), and respiratory bursts from inflammatory cells.[16]

Examples of ROS include hydrogen peroxide, nitric oxide, superoxide anions, and hydroxyl and monoxide radicals. High ROS levels in the body can result in changes to DNA structure, modification of proteins and lipids, activation of several stress-induced transcription factors, and production of pro-inflammatory and anti-inflammatory cytokines.[17] This is due to the fact that ROS are highly reactive, and initiate a series of oxidation reactions with fundamental cellular molecules, generating toxic by-products and resulting in cell death.

The brain is particularly susceptible to oxidative stress as it contains high levels of unsaturated fatty acids, which are labile to free radical attack and lipid peroxidation. Moreover, the brain possesses reduced antioxidant activity compared to other tissues.[17]

The brain contains extremely high levels of oxygen, and the energy metabolism is governed by aerobic oxidation in the mitochondria. Crucially, this involves the electron transport system which has been shown to produce the majority of ROS.[17] The mechanisms of neuronal death are depicted in Figure 5.

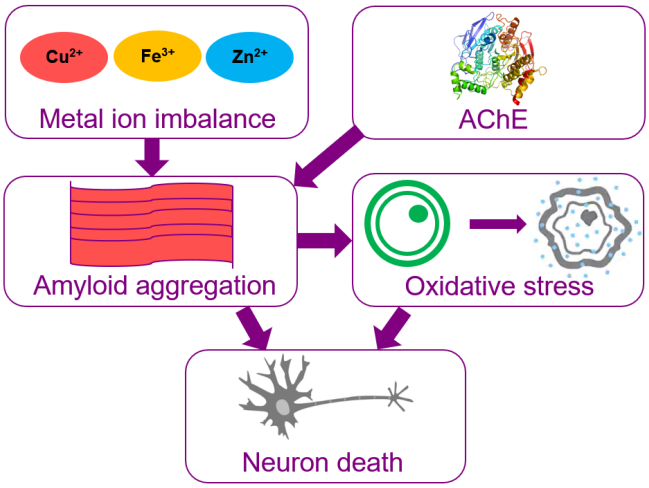


Figure 5: Neuronal demise in AD may be caused by oxidative stress which affects energy metabolism, and the interaction of transition metals and the rise in AChE levels which induce Aβ aggregation.

Lipid peroxidation can be generated by ROS, and is associated with the majority of neurodegenerative diseases. It involves free radical attacks on lipids found in the bilayer of cellular membranes, resulting in the inactivation of membrane-bound receptors and enzymes and the production of highly reactive compounds which can damage biological proteins and DNA.[16]

3.3 Metal Ion Imbalance

AD is also associated with an imbalance in metal ions. In particular, the excessive accumulation of metals such as copper, iron, zinc, and aluminium can induce toxic effects on neuronal cells.[18] The biometal disequilibrium is directly linked to a rise in amyloid aggregation. It is recognised that copper can strongly bind to Aβ, forming a toxic complex, and facilitating the formation of amyloid oligomers.[19] This interaction between metals and Aβ can also activate an inflammatory response, which can give rise to an increased production of ROS.[18]

3.4 Tau Aggregation

In addition to the extracellular accumulation of amyloid plaques, AD is also associated with the presence of neurofibrillary tangles (NFTs) within the neurons (see Figure 4). These are caused by the aggregation of hyperphosphorylated tau protein.[20] Currently, little is understood about the mechanisms of fibril formation. However, it has been reported that the level of tau in AD patients is four to five times higher than in healthy brains, and these proteins are in the form of abnormally phosphorylated tau.[21]

3.5 Cholinesterase Enzymes

Cholinesterases are extracellular enzymes found primarily in the central nervous system and at peripheral neuromuscular junctions. Their key role is in the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid.[22] This mechanism is critical for normal cell signalling, and for the prevention of overstimulation of the neurons. The two enzymes in the cholinergic nervous system are acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AD is associated with a profound deterioration of many different types of neurons, including cholinergic neurons.[23]

It is well recognised that there is a significant increase in the levels of AChE around amyloid plaques and NFT throughout each stage of the disease, and it has been suggested that AChE may directly interact with Aβ to promote the formation of plaques.[22] All the clinical drugs currently available are AChE inhibitors (donepezil, rivastigmine, and galantamine), with the exception of memantine which is a N-methyl-D-aspartate (NMDA) antagonist.[5] AChE possesses two distinct binding sites within the enzyme gorge (see Figure 6): the peripheral anionic site (PAS) at the entrance of the gorge, and the catalytic site (CAS) at the bottom.[22]

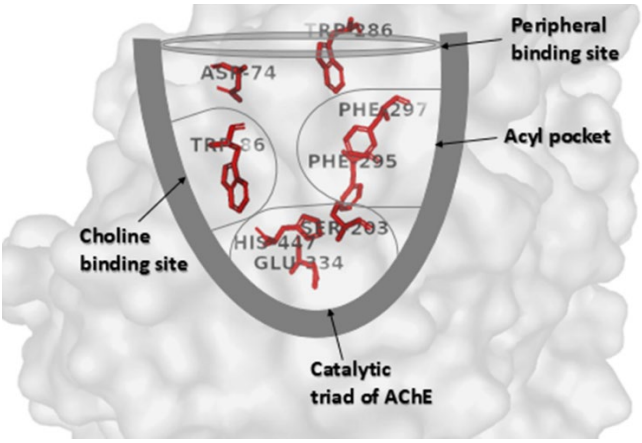


Figure 6: AChE binding sites.

It is recognised that AChE inhibitors bind to one or more of these sites on the enzyme in order to inhibit its activity and reduce the breakdown rate of acetylcholine; therefore, increasing the cholinergic neurotransmission.[22]

While the clinical AChE inhibitor drugs currently available have been shown to improve cognitive function, none of these treatments are capable of delaying or halting the progression of AD,[22] which further emphasises the multifaceted pathogenesis of AD.

3.6 Secretases

Abnormal processing of APP causes an accumulation of the neurotoxic forms of Aβ into plaques; thus, contributing to the characteristic neuronal dysfunction and death in the brains of AD patients.[24] The three major enzymes with a key role in the processing of APP are secretase enzymes: α-secretase, β-secretase, and γ-secretase (see Figure 7).[25]

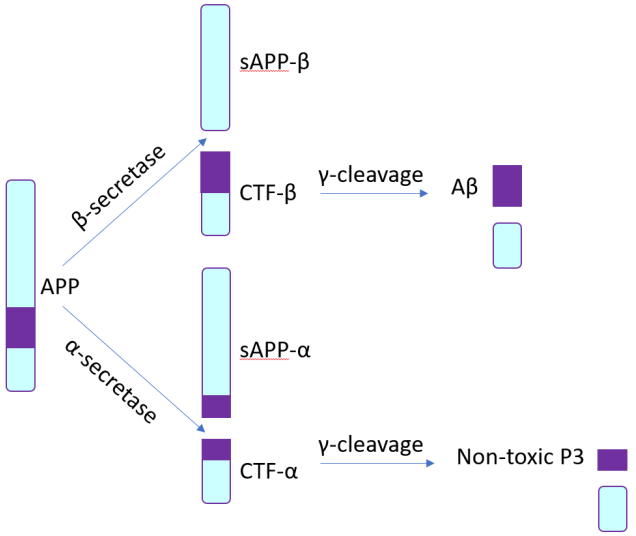


Figure 7: Role of secretase enzymes in APP processing.

The α-secretase enzyme is responsible for processing APP *via* a non-amyloidogenic pathway, whereby APP is cleaved to produce non-toxic soluble peptide fragments.[25] Therefore, activation of α-secretase is a potential target for reducing the production of amyloid plaques. Receptor agonists for muscarinic ACh receptors (mAChR) are capable of activating α-secretase, and are currently under development for the treatment of AD.[26] β-secretase, or β-site APP-cleaving enzyme 1 (BACE-1), is the aspartic protease that cleaves APP in the first step of the formation of the Aβ peptide.[27] BACE-1 has been the subject of recent interest as a potential target for AD, with most study focused on this particular secretase enzyme. The activity of BACE-1 is increased in the brains of AD patients; therefore, inhibitors of this enzyme have been investigated. However, inhibitors which have displayed high potencies *in vitro* tend to have limited bioavailability and mobility across the blood brain barrier as they have high molecular weights.[28] Therefore, current research is focused on developing highly lipophilic, smaller drugs. Finally, the γ-secretase enzyme is responsible for post-translational modifications of APP and production of Aβ.[25] The catalytic subunit of γ-secretase consists of presenilin 1. Although presenilin/γ-secretase inhibitors could lower Aβ production, they have also been shown to cause potentially fatal adverse effects as presenilin is also critical for processing the Notch signalling protein.[28] Notch plays an important role in cellular growth and function, and so its inhibition leads to intolerability and toxicity.[28] As a result, potential therapeutic agents must balance effectiveness and tolerability.

3.7 Sirtuin

Sirtuins are a class of enzymes with deacetylase and ADP-ribosyltransferase activity.[25] SIRT1 is the most widely studied sirtuin, and its activity has been linked to several processes associated with AD.[29] These include oxidative stress, inflammation, and glucose homeostasis. Consequently, patients with diabetes are at a greater risk of developing AD due to vascular and oxidative damage caused by the condition.[30] Interestingly, it has been suggested that SIRT1 has a positive effect in both *in vitro* and *in vivo* AD models, by reducing amyloid aggregation.[25] However, genetic studies have demonstrated that SIRT1 levels remain unchanged in AD models.[30] A contradictory hypothesis suggested that SIRT1 is down-regulated in patients with AD, and overexpression can provide neuroprotection as well as a reduction in amyloid deposition through the promotion of non-amyloidogenic processing of APP.[31] Resveratrol is one of several natural polyphenolic phytochemicals that can activate sirtuin activity. Resveratrol has been shown to be beneficial in AD models, exhibiting antioxidant properties and a protective effect against Aβ toxicity.[32] However, several other polyphenolic compounds (e.g. curcumin, benzoic acid, and quercetin) have been shown to have the same effects.[33] Furthermore, SIRT1 overexpression and resveratrol treatment in cellular models have exhibited the same beneficial inhibitory effects on Aβ fibril formation. Therefore, it has been hypothesised that the activation of SIRT1 by resveratrol was the fundamental process responsible for the neuroprotective effects observed.[34] However, there is evidence that the neuroprotective activity of resveratrol is linked to its chemical properties, and is independent from SIRT1 activation.[34] In studies where beneficial effects are reported for the role of SIRT1, resveratrol is also included for its positive effects. It could be

suggested that the role of resveratrol is the major contributing factor to the positive results.

3.8 Caspase

Caspases are a family of cysteine protease enzymes that play a vital role in apoptosis.[25] Apoptosis is a form of programmed cell death, which can be accelerated by the accumulation of amyloid plaques in the brains of AD patients. Inappropriate activation of apoptosis may contribute to several neurodegenerative diseases, and other conditions (such as cancer). The activation of caspases initiates a cascade of signalling events which results in the implementation of the characteristic apoptotic cellular changes, and accelerate cell death.[35] Recent evidence suggests that, as well as contributing to disease progression in later stages, caspases may be involved in promoting the initial processes associated with the disease; specifically, caspase-cleaved tau co-localises with the formation of NFTs.[25]

3.9 Glycogen Synthase Kinase-3

Glycogen synthase kinase-3 (GSK-3), a serine/threonine kinase, regulates several key cellular processes including glycogen synthesis, and glucose metabolism.[25] Dysregulation of GSK-3 is associated with the pathogenesis of cancer, diabetes, and AD. The overexpression of GSK-3 can lead to neurodegenerative alterations. It has been reported that GSK-3 is involved in the hyper-phosphorylation of tau and the generation of toxic Aβ, in addition to the loss of cholinergic neurons, inflammation, apoptosis, and synaptic loss.[36] Evidence suggests that GSK-3 is up-regulated in peripheral lymphocytes, and the hippocampus in the brains of AD patients.[25]

3.10 Monoamine Oxidase B

MAO-B is one of two isozymes of the enzyme, monoamine oxidase (MAO), which have a crucial role in the oxidative deamination of biogenic amines with the subsequent production of hydrogen peroxide.[37] MAO-B inhibitors are currently used to treat Parkinson's disease as they block the breakdown of dopamine in the brain. MAO-B activity has been found to be increased in the brains of AD patients. However, its role in AD pathogenesis is currently unclear. It has been suggested that MAO-B is associated with γ-secretase, and over-expression of MAO-B can enhance Aβ production in neurons.[38] The second MAO isoform, MAO-A, was also recently shown to have a role in AD with an increased activity in the cortex of AD brains. Levels of MAO-A immunoreactive neurons were reduced in patients with severe AD.[39]

3.11 Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter. Commonly referred to as the 'happy chemical', it plays a key role in well-being and mood regulation.[40] The exact function of the serotonin system remains poorly understood due to its complexity. However, it is known to be involved in modulating cognition, learning, and memory.[40] A significant reduction in the serotonin levels in the brains of AD patients has been reported.[40] Selective serotonin reuptake inhibitors (SSRIs) have been prescribed to patients suffering from depression for a number of years due to their ability to increase the level of serotonin available to bind to post-synaptic receptors. SSRIs achieve this by limiting the reuptake of serotonin into the presynaptic receptors. These drugs reportedly also enhance cognition in rodents and primates.[41] Recent research has focused on developing antagonists of the serotonin-6 receptor due to its high levels of expression in the central nervous system. Early trials showed promise, with improvements in short-term memory and cognition. At phase III, however, many drugs from this class are failing due to a lack of statistically significant clinical efficacy.[42] While this failure could be due to under-dosing, trials with combination therapies were also unsuccessful (where drugs were prescribed together with acetylcholinesterase inhibitors or memantine), and failed to produce an improvement in cognitive function.[42] Nevertheless, the promising results in earlier trials should not be ignored and continued development of these drugs could prove beneficial in terms of understanding the serotonin system and its role in AD.

3.13 N-Methyl-D-Aspartate Receptors

N-methyl-D-aspartate receptors (NMDAR) play a role in memory and learning, and function by allowing positive ions (Ca^{2+}) to cross the cell membrane while glutamate (agonist) is bound to it.[43] It is widely recognised that over-activation of these receptor sites are involved in the development of AD. Excessive activation of NMDAR results in an influx of Ca^{2+} ions and free radical generation which ultimately induces synaptic dysfunction and neuronal cell death.[43] It has been suggested that the glutamate uptake mechanisms are impaired in AD patients, which consequently enhances the glutamate availability to bind to NMDAR. Many NMDAR antagonists have proven unsuccessful due to debilitating adverse effects. It was found that, while inhibition of NMDAR over-activation was desired, the normal function of the receptor had to be maintained.[44] Memantine, the only NMDA receptor antagonist to be clinically approved for the treatment of AD, is able to alleviate some behavioural symptoms. Although it has been reported to delay the progression of the disease, only weak evidence exists for this. Memantine works by blocking the channel of NMDARs; however, it continues to allow the necessary physiological activation for essential brain function.[44] Despite numerous hypotheses, it is still not known how memantine can achieve this effect. Therefore, further investigation into this drug and its target may provide crucial information which could benefit not only the understanding of AD, but many other diseases that are also associated with NMDAR over-activation; including, multiple sclerosis and glaucoma.

3.14 Muscarinic and Nicotinic Acetylcholine Receptors

Acetylcholine receptors (AChR) are signalling integral membrane proteins that are stimulated by the binding of acetylcholine. Two subtypes of AChRs exist: muscarinic (mAChR) and nicotinic cholinergic receptors (nAChR).[25] These receptors are characterised by their respective agonists, muscarine and nicotine. mAChRs are G-protein coupled receptors, while nAChRs are ligand-gated ion channels. mAChRs are involved in memory and cognition. Within this subtype of AChR, there are several further subtypes; namely, M1-M5. The M1 type is reported to induce dephosphorylation of tau, while M2 inhibits the release of acetylcholine.[45] However, it has also been suggested that activity of M1-M4 mAChRs was significantly reduced in AD patients. The M2 receptor is reportedly associated with a decrease in β-secretase activity, while stimulation of M3 may increase γ-secretase activity; therefore, reducing the formation of amyloid plaques in the brain.[26] mAChR agonists have been developed for the treatment of AD, and were reported to reduce amyloid plaque deposition, oxidative stress and hyperphosphorylation of tau. However, the agonists were not specific and were found to activate other receptor subtypes.[26]

nAChRs are also related to cognitive function, and have been shown to inhibit the formation of amyloid plaques by promoting α-secretase activity.[26] When nAChR activity is inhibited, it is reported to significantly impair learning and memory and accelerate the aggregation of amyloid plaques.[45] It has been suggested that Aβ peptides can impair cognition by blocking nAChRs. As a result, activation of both mAChRs and nAChRs has been a promising route for the treatment of AD.

3.15 Nuclear Factor Erythroid 2-Related Factor 2

Nrf2 is a transcriptional pathway which is responsible for activating cellular defence genes.[46] These include genes for antioxidant and anti-inflammatory enzymes, as well as those involved in eliminating damaged proteins. Over-expression of Nrf2 has been reported to protect against Aβ toxicity.[46] Furthermore, GSK-3β down-regulates Nrf2 and, given that GSK-3β levels are increased in AD patients, it is likely that this significant reduction in Nrf2 is crucial to increasing neuronal susceptibility to oxidative stress.[47] Therefore, developing activators of the Nrf2 transcriptional pathway is an attractive approach to inhibit the progression of AD.

4 Current Clinical Drugs for the Treatment of AD

The drugs clinically available aim to inhibit the action of AChE, or the N-methyl-D-aspartate (NMDA) receptor.[2] AChE inhibitors are prescribed for mild to moderate AD, and include tacrine, donepezil, rivastigmine, and galantamine. Memantine is a NMDA receptor antagonist, and is used to treat severe cases of AD.[2] The structures of these drugs are shown in Figure 8.

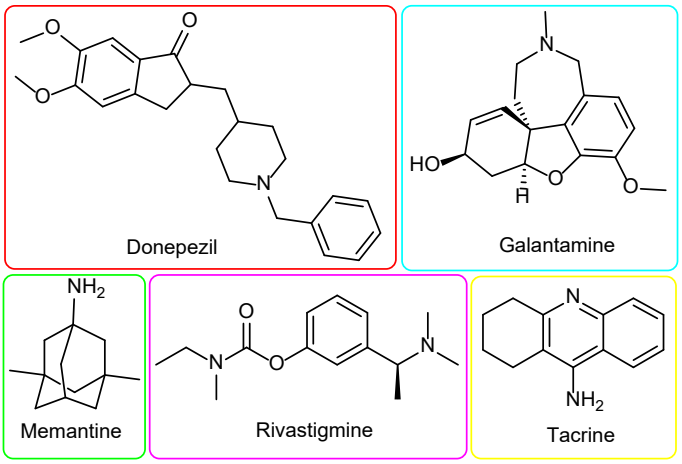


Figure 8: Chemical structures of the approved drugs for the treatment of AD.

4.1 Mechanisms of Action

Due to the cholinergic hypothesis of AD – where loss of acetylcholine neurons in the basal forebrain, and loss of enzymatic activity for acetylcholine synthesis is attributed to cognitive decline – it was proposed that AChE inhibitors could be used to impede the degradation of ACh by inactivating cholinesterases.[1] Donepezil is highly selective for AChE. Rivastigmine inhibits AChE and blocks the part of the active site responsible for plaque deposition.

Galantamine stimulates pre- and post-synaptic nicotinic receptors which produce increased levels of ACh.[1] NMDAR antagonists instead function by opposing the effects of glutamate, which is postulated to inhibit neurotransmission by means of excitotoxicity and is a factor in neurodegeneration.[44] The mechanisms of action of the AD drugs are shown in Figure 9.

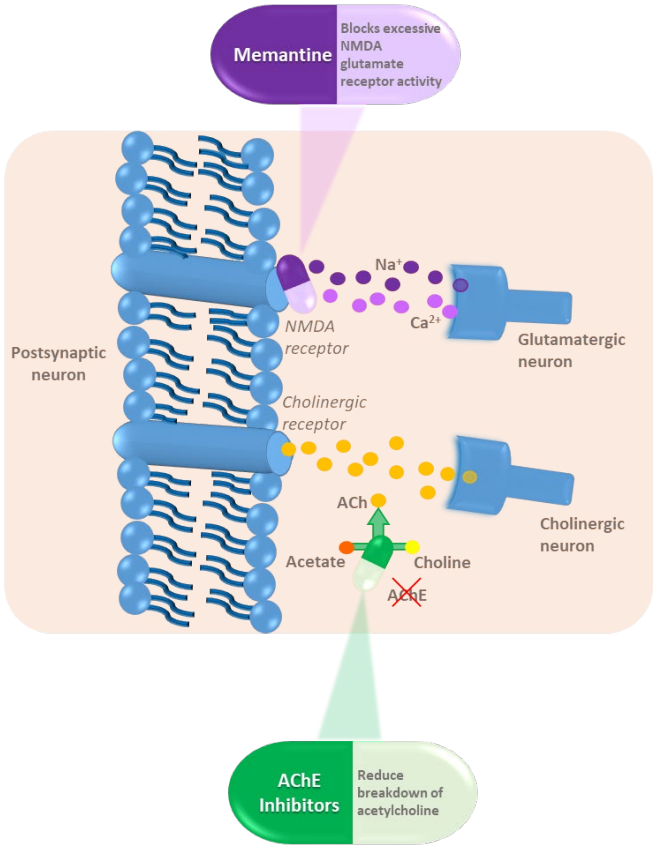


Figure 9: The mechanisms of action of cholinesterase inhibitors (tacrine, donepezil, rivastigmine, and galantamine) and NMDA antagonists (memantine).

4.2 Effectiveness of Drugs

Despite the differences between their mechanisms of action, the clinical effects of these AD drugs are similar. Generally, AChE inhibitors are modestly effective in terms of their ability to reduce the rate of cognitive decline. An AChE inhibitor is typically prescribed during the initial stages of the disease, and memantine is recommended when the patient has progressed to a moderate to severe stage of AD. However, combination therapy comprising donepezil and memantine is increasingly being administered since the synergistic effect between these drugs was reported.[1]

4.3 Side Effects

Tacrine was the first AChE inhibitor implemented in clinical practice in 1993. However, it was found to induce significant hepatotoxicity, so this drug was discontinued.[1] Adverse gastrointestinal effects (such as nausea, vomiting, and diarrhoea) are frequently reported with the use of cholinesterase inhibitors, which could

be ascribed to the increased acetylcholine levels in the peripheral nervous system. Rivastigmine was reportedly liable for the highest incidence of adverse effects compared to other cholinesterase inhibitors, leading to the introduction of a reduced dose administered in a dermal form with the aim of inducing fewer adverse side effects while maintaining a comparable efficacy.[48]

4.4 Cost

Despite the promising findings associated with cholinesterase inhibitors, their development has been impeded in the UK due to the cost of production. In 2005, the NHS in England proposed to halt the distribution of cholinesterase inhibitors and memantine for the majority of AD patients, citing the fact that the benefits of the drugs did not compensate for the expense. This view was met with widespread controversy, and so it was announced in 2006 that the NHS would continue the provision of cholinesterase inhibitors to patients with moderate to severe AD. However, access to memantine remains limited. In 2016/17, the total NHS expenditure was £144 billion with dementia medication costing £40 million.[49] Donepezil is the most commonly prescribed treatment with around 2.4 million prescriptions in 2016. It is also the cheapest drug clinically available at only £21 a year per patient.[50]

5 Development of Novel Drugs for the Treatment of AD

Despite the urgent requirement for novel AD treatments, the drug development process is extensive and complex (as shown in Figure 10) with an estimated 10-15 years between discovery and marketing approval, and an overall failure rate of greater than 95%.[51]

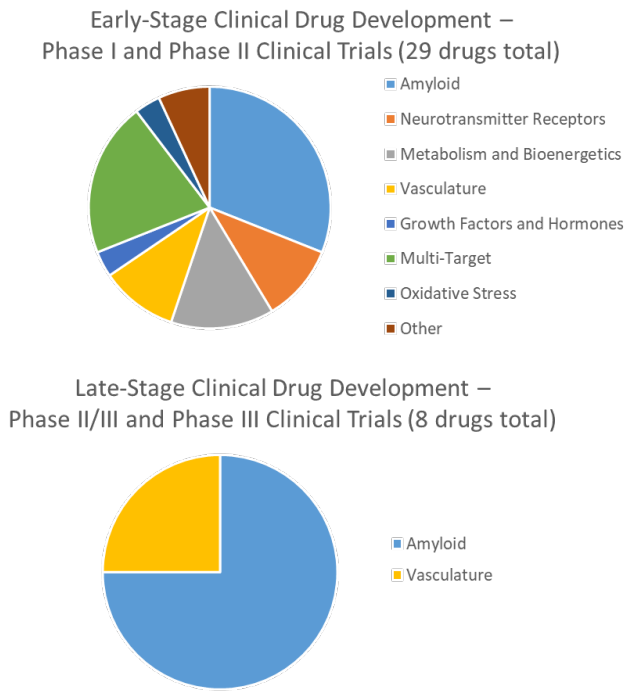


Figure 10: Compounds in clinical trials in 2019 for the treatment of AD, characterised by their desired targets. The purpose of each phase is to test: Phase I – safety and dosage; Phase II – efficacy and side effects; Phase III – efficacy and monitoring of adverse reactions; Phase IV – safety and efficacy. At each phase, the number of study participants and the length of the trial are increased (data obtained from [52]).

This high failure rate is most likely a result of the fact that the exact cause of AD is poorly understood and there could be various factors implicated in each stage of the disease. As a result, the therapeutic target cannot be accurately identified. A major reason for clinical failure is toxicity, which may be associated with the effects of the drug on the target and any physiological functions that include that target. Despite the fact that the amyloid hypothesis is regarded as one of the major pathological hallmarks of AD,[12] the failure of drugs developed against this target to demonstrate significant efficacy is considered to be due to ineffective target validation.[53] The proposed treatments may also be unsuccessful in practice as a result of the unpredictability of AD, and the rates of cognitive decline vary greatly between individuals. Therefore, it could be argued that trials involving the comparison of drugs

to a placebo are unreliable as each patient is affected by the disease in a different way. The recent discovery of several factors which are believed to be involved in AD development has greatly influenced the targets of novel drugs. Consequently, various therapy types are undergoing research and development, which can target the different distinct factors responsible for the progression of AD.

6 Multi-Target Compounds for the Treatment of AD

Historically, drug development has been based on the view that one molecule is used for one target to treat one disease. However, there is growing recognition that drugs against a single target may be inadequate as the pathology of most diseases – including neurodegenerative disorders, cancer, and diabetes – is multifactorial.[54]

In cases where a single drug is shown to be insufficient, it is common practice to prescribe multiple drugs with different targets in order to treat the various pathological pathways.[55] However, this can lead to complications in terms of patient compliance and quality of life, in addition to increased healthcare and social care costs. Combination therapies also have an increased risk of toxicity and adverse side effects due to potential drug-drug interactions, and a greater burden on carers and families of the patients.[56]

Consequently, a new strategy for drug design has been proposed whereby hybrid molecules are developed which consist of several distinct pharmacophores that act upon different targets involved in the disease, in order to create one multi-target-directed-ligand (MTDL) that has the capacity to interact with various targets and therefore treat complex multifactorial diseases.[57] Within the last decade, research has focused on developing multi-target agents particularly for the treatment of AD. Numerous noteworthy reviews have recently been published which cover the novel advances in the multi-target strategy for AD therapeutics, including the various chemical scaffolds that have been employed for MTDL development.[58-60]

However, the multi-target approach introduces new challenges for medicinal chemists; including the fact that hybrid molecules tend to be larger as a result of combining two or more pharmacophores.[61] This subsequently has an effect on the drug's bioavailability and ability to cross the blood-brain barrier, which reduces its efficacy *in vivo*. Achieving sufficient potencies against the various targets is another issue facing MTDL development.[62] Given that the exact etiology of AD remains unclear, identification of the appropriate biological targets is also challenging. As a result, hybrid drug development is based only on the available information, despite the fact that the probability of selecting the major targets of the disease is low. Table 1 below illustrates the benefits and challenges associated with the multi-target drug development strategy.

Table 1: MTDL development – benefits and challenges.

Benefits	Challenges
• Improve patient compliance and quality of life	• More likely to be larger and have poor bioavailability and solubility
• Reduce risk of toxicity and adverse side effects	• Higher lipophilicity can result in high clearance
• Reduce burden on carers and families	• Difficult to achieve sufficient potencies against every target
• Lower expense to NHS, including healthcare and social care costs	• Issues with identifying appropriate targets

Though no rationally designed MTDL has been clinically approved, there has been some success with positive *in vitro* results reported for various hybrid drugs.[62] Ladostigil, a hybrid drug combining pharmacophores from rivastigmine (an AChE inhibitor) and rasagiline (a MAO-B inhibitor) (see Figure 11), has also progressed to Phase III trials after exhibiting a capacity to slow neurodegenerative decline in patients with mild cognitive impairment. Therefore, it has potential to delay or prevent the onset of AD.[63] Ladostigil was reported to retain similar

levels of inhibitory activity against each target as its parent compounds, with a reduction in the activity of AChE by 25-40%, and MAO-B by 70-90% in rodents.[64] It was also shown to be capable of crossing the blood-brain barrier, and the adverse side effects commonly reported with the use of cholinesterase inhibitors (e.g. diarrhoea) were not observed even at increased dosages. This demonstrates the potential of combining multiple active moieties with distinct modes of action into a single hybrid drug which can target several factors that play a role in AD, with additive effects and reduced risk of adverse reactions.

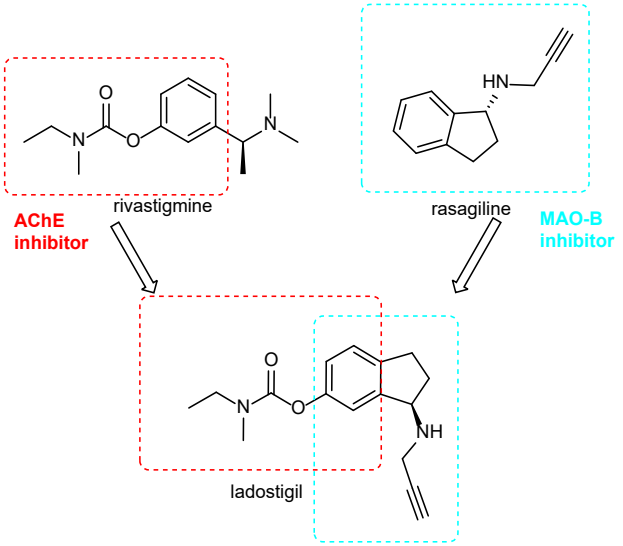


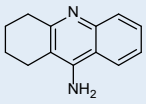
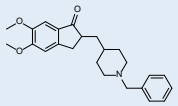
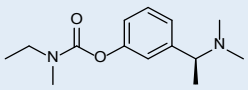
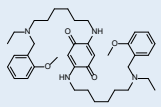
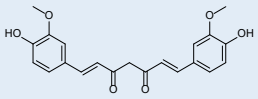
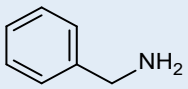
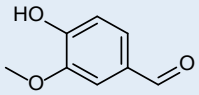
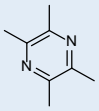
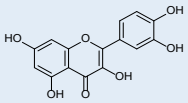
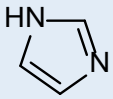
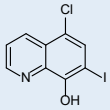
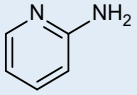
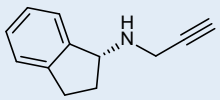
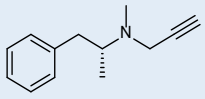
Figure 11: Mechanism for the development of ladostigil with the active moieties from rivastigmine and rasagiline (adapted from [64]).

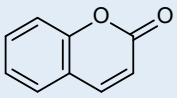
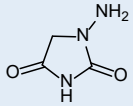
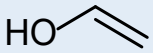
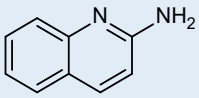
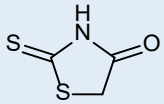
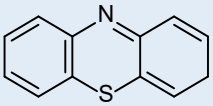
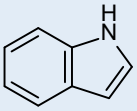
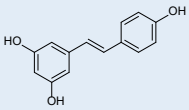
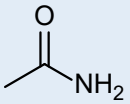
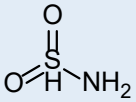
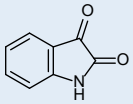
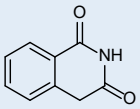
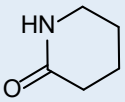
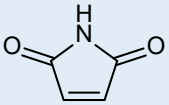
The use of natural products as AD therapeutics has also been thoroughly researched in the last decade. Natural products are particularly attractive for this purpose as they tend to induce fewer adverse side effects, and have lower toxicity levels.[65] Many classes of naturally-derived compounds have been shown to provide neuroprotection; for example, through inhibiting Aβ aggregation, BACE-1, and oxidative stress. Such structural classes of natural compounds include alkaloids, polyphenols, and flavonoids (e.g. hespiridine).[66] Research has also reported the potential of compounds obtained from marine sources as AD therapeutics, such as red algae, and poly-unsaturated fatty acids, including omega-3.[65] Recent advances in this area include a fungal metabolite, tenuazonic acid, with potential as an AChE inhibitor, antioxidant, and Aβ aggregation inhibitor.[67] Furthermore, an extract of amphibian skin from *Pseudis platensis* exhibited balanced potency against AChE, BuChE, MAO-B, and oxidative stress.[68] However, for the purpose of this report, the focus will remain on synthetic hybrid compounds with multi-target activity against AD targets.

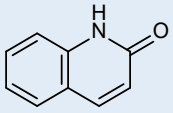
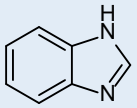
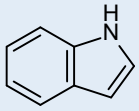
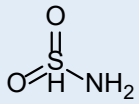
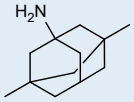
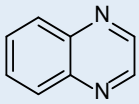
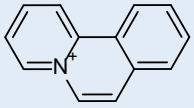
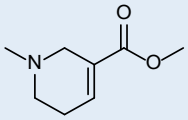
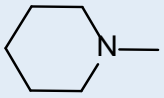
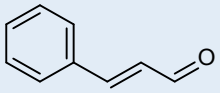
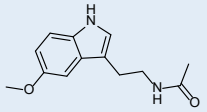
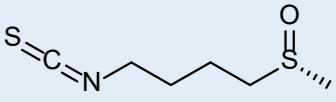
7 Multi-Target Compounds in Development for the Treatment of AD

Within the last decade, multi-target drugs have been developed by combining different pharmacophores with distinct targets which play a role in AD. These pharmacophores are commonly chosen based on previous research which report their ability to interact with the factors involved in the disease. Table 2 presents exemplar ligands that have been employed recently for MTDL development.

Table 2: Common ligands and their targets for the treatment of AD.

TARGET	LIGAND		REFERENCE
Cholinesterase enzymes	Tacrine		69
	Donepezil		70
	Rivastigmine		64
Amyloid aggregation	Memoquin		71
	Curcumin		72
	Benzylamine		73
Oxidative stress	Vanillin		74
	Ligustrazine		75
	Quercetin		76
Metal chelation	Imidazole		69
	Clioquinol		77
	Aminopyridine		78
MAO-B	Rasagiline		63
	Selegiline		79

	Coumarin		80
BACE-1	Aminohydatoin		81
	Hydroxyethylene		82
	Aminoquinoline		83
Tau	Rhodanine		84
	Nitrocatechol		85
	Phenothiazine		84
Sirtuin	Indole		86
	Resveratrol		32
	Carboxamide		86
Caspase	Sulfonamine		87
	Isatin		88
	Homophthalimide		88
GSK-3β	Cyclic amide		89
	Maleimide		90

	Quinolone		90
Serotonin	Benzimidazole		91
	Indole		92
	Sulfonamide		92
NMDAR	Memantine		93
	Quinoxaline		94
	Benzoquinolizium		95
mAChR and nAChR	Arecoline		26
	Thiadiazole		26
	Methylpiperidine		26
Nrf2	Cinnamaldehyde		96
	Melatonin		97
	Sulforaphane		47

7.1 Aβ Aggregation Inhibition and AChE Inhibition

Cen et al.[98] developed a series of tacrine-bifendate hybrid compounds (see Figure 12).

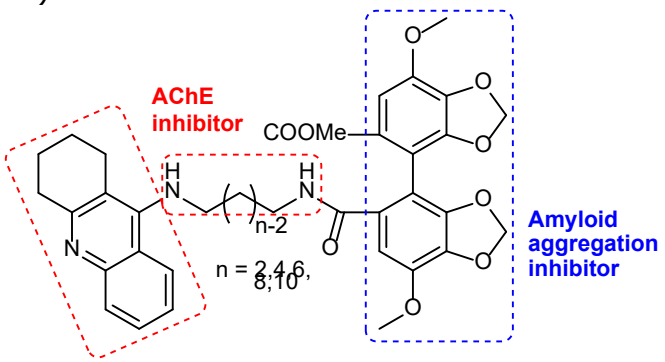


Figure 12: Structural design of tacrine-bifendate conjugates (adapted from [98]).

Bifendate was employed in order to reduce the hepatotoxicity of the drugs, as it was reported to protect mitochondria in the liver cells from tacrine-induced toxicity. A diamine linker chain was also used to increase AChE inhibitory activity. Cen et al.[98] supported the findings of Hiremathad et al.[69] where the linker chain length had the greatest effect on AChE inhibitory activity. Conversely, Cen et al.[98] reported that a chain length of 8 carbons demonstrated the highest potency. This could be due to the fact that the bifendate moiety is of a different size to hydroxyphenyl-benzimidazole, or that the conjugate moieties are interacting with different sites within the active site of the AChE enzyme. All the synthesised compounds had inhibitory activity in the nanomolar concentration range (27-944 nM), and were found to inhibit Aβ aggregation *in vitro* at greater potencies than tacrine and curcumin (65-90% inhibition, compared to 5% for tacrine and 53% for curcumin). Although it was reported that the most potent inhibitor demonstrated less hepatotoxicity in human hepatocyte cells compared to tacrine, the difference in cell viability at equal concentrations was not significant.

Atanasova et al.[99] proposed the combination of galantamine with the indole moiety in order to achieve dual site binding to AChE and consequently a rise in activity. The most active derivative (see Figure 13) was found to be 95 times more active than galantamine itself against AChE (IC_{50} of 0.011 μM determined in the Ellman's assay, compared to 1.07 μM of galantamine). Furthermore, it was suggested that derivatives with longer linkage chains were able to block Aβ deposition on AChE by binding to the same region proximal to PAS where the Ω-loop of Aβ was also shown to interact with the enzyme.

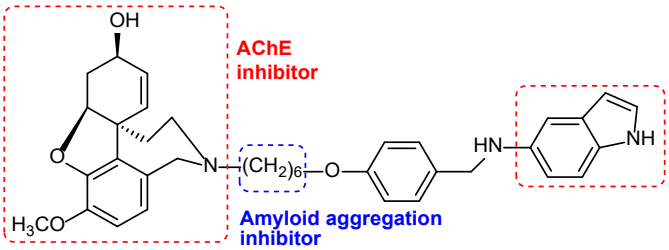


Figure 13: Structure of most potent galantamine derivative with indole moiety (adapted from [99]).

7.2 Aβ Aggregation Inhibition and Antioxidant

Orteca et al.[100] developed a series of curcumin derivatives in order to study their inhibitory activity against Aβ aggregation. It was observed that the derivatives which possessed an aromatic structure like vanillin demonstrated significant disruption of amyloid fibrils in the ThT assay (DC_{50} of 0.78 μM and 0.31 μM respectively in Figure 14).

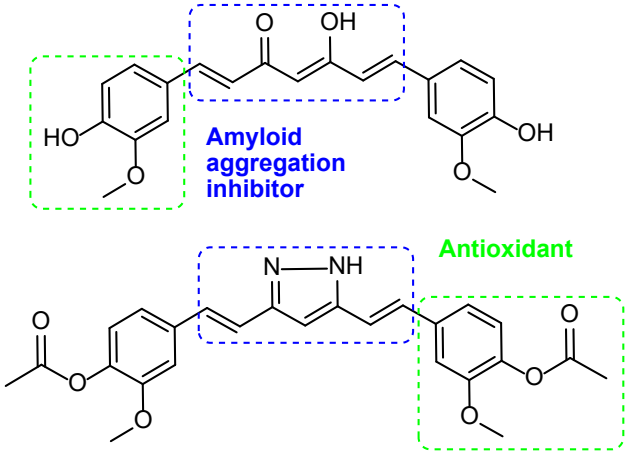


Figure 14: Structure of the most active curcumin derivatives containing the vanillin aromatic moiety (adapted from [100]).

The authors noted the importance of a highly conjugated structure, in addition to a rigid linker and polar substituents on the inhibitory activity of the compounds. Therefore, derivatives with the vanillin moiety were found to be the most active due

to the fact that they satisfied these criteria (e.g. DC_{50} of 0.73 μM for a vanillin derivative, compared to no observed activity for a compound with the same general structure but with no aromatic substituents). The derivatives exhibited protective effects on neuronal cells from glutamate toxicity (cell proliferation increased to 70-90% when curcumin derivatives were administered, compared to 35% when treated with glutamate alone). Therefore, it was reported that the vanillin aromatic structure within the curcumin derivatives was crucial to activity against Aβ fibrils and oxidative stress.

7.3 AChE Inhibition and Antioxidant

Li et al.[75] synthesised a series of tacrine derivatives conjugated with phenolic acid and ligustrazine. The latter was chosen based on its ability to protect cells from oxidative stress and inflammation, while phenolic acid was employed for its neuroprotective and hepato-protective capabilities. The compound which demonstrated the most potential as a neuroprotective agent is shown in Figure 15.

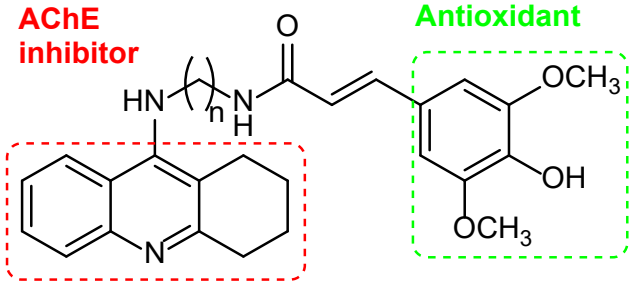


Figure 15: General structure of tacrine-phenolic acid-ligustrazine hybrid compounds (n=6 for the compound which displayed the highest potency as a neuroprotective agent) (adapted from [75]).

It was reported that the type of phenolic acid used had a major effect on the AChE inhibitory activity of the agent; for example, compounds with the sinapic acid moiety had greater potencies than those containing coumaric acid. However, data from other research papers was used in order to determine which structural features had an effect on the activity of the compounds. This approach may be less reliable as the results were reported as tacrine equivalents in the research paper used, rather than as a mean of several tests. Furthermore, the methods used by the authors may not be the same as those used in the referenced papers. It was reported that the most potent AChE inhibitor could prevent amyloid aggregation i*n vitro* with an IC_{50} of 65.2 nM. Yet, the concentrations tested were within the range 20 – 100 μM. A different concentration range was again used to determine the cytotoxicity of the compounds within cells, which was considerably lower (1.25 – 10 μM). Therefore, it is unclear if the compound was active at non-toxic concentrations.

Lamie et al.[101] also developed a series of phthalimide derivatives and studied their antioxidant activity, and other properties. The most active compound (see Figure 16) in terms of antioxidant activity had a value 18 times greater than Trolox. It should be noted that only the ORAC assay was employed in order to determine the antioxidant activity. Therefore, future work may include other assays (such as DPPH, and FRAP) to further investigate the antioxidant activity of the compound. On the other hand, other phthalimide derivatives displayed negligible antioxidant activity; therefore, the phthalimide moiety may not be responsible for any antioxidant activity. The compounds were also tested on various cell lines and no cytotoxicity was reported.

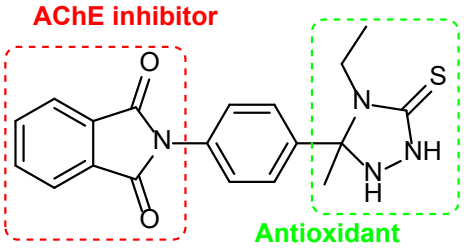


Figure 16: Structure of phthalimide derivative with greatest antioxidant activity (adapted from [101]).

7.4 AChE Inhibition, Aβ Aggregation Inhibition, and Antioxidant

Scipioni et al.[102] developed a multi-target directed ligand based on vanillin in combination with tacrine (Figure 17). This compound demonstrated promising activity *in vitro* against oxidative stress, AChE, and Aβ aggregation.

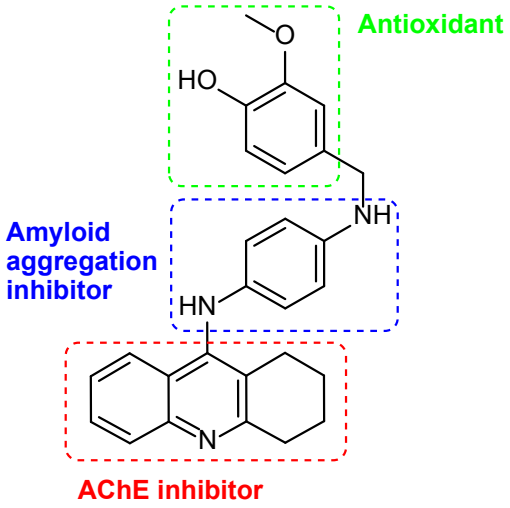


Figure 17: Structural design of vanillin-tacrine multi-target hybrid (adapted from [102]).

The antioxidant activity of the compound (DPPH IC_{50} = 20.5 μM) was reported to be due to structural features of the vanillin moiety; including electron delocalisation, a tertiary amine, and phenolic groups as the authors reported previously.[74] It was reported that the AChE inhibitory activity could be improved with a longer, more flexible linker chain (e.g. long, saturated alkyl chains with 8 methylene groups). This suggestion is confirmed by Li et al.[103], where it was reported that a short chain (e.g. 2 methylene groups) does not allow the terminal moiety to interact with the choline binding site. However, the authors disagreed with Scipioni et al.[102] in terms of the importance of the flexibility of the chain. Instead, they stated that an excessively long chain may increase flexibility and disturbance of the molecular structure such that the active moiety cannot interact with the binding site. Furthermore, Li et al.[103] suggested that a large terminal group can increase the rigidity and therefore increase the binding capacity to the enzyme. The Aβ aggregation inhibitory activity of the compound developed by Scipioni et al.[102] was suggested to be due to the low flexibility of the linker chain, and the longer length of the linker. On the other hand, Siposova et al.[104] reported that the conformational flexibility of ligands is important to allow the optimum interaction with the amyloidogenic protein binding sites as the molecule can easily adjust its conformation to fit. Tu et al.[105] also supported that a linker chain of sufficient length and flexibility is necessary for an effective Aβ inhibitor (a propyl linker was found to exhibit the greatest inhibitory activity against Aβ aggregation), as well as the presence of terminal groups that can interact with the residues that are involved in peptide aggregation.

7.5 AChE Inhibition, Aβ Aggregation Inhibition, and Metal Chelation

Santos et al.[12] developed a series of multi-functional metal chelators with the aim to target several factors that are involved in the development of AD. Amino-pyridine derivatives were reported to be capable of metal chelation, in addition to AChE inhibition *in vitro*. One of the synthesised compounds (see Figure 18) was shown to allow binding of Cu(II) and Zn(II), and interact with Aβ peptides due to the presence of the benzazole scaffold in the molecular structure. Significant AChE inhibitory activity was reported, with an IC_{50} of 52.4 nM. This could be due to the dual binding with the enzyme active site, which involved interactions with both the catalytic anionic site (CAS) and the peripheral anionic site (PAS) of the AChE. The PAS of the AChE enzyme is associated with the aggregation of Aβ peptides into fibrils.[64]

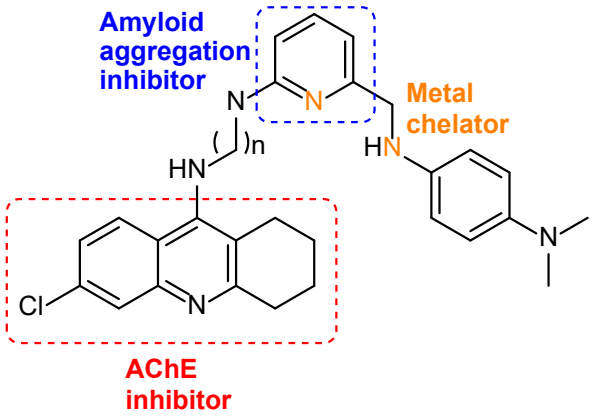


Figure 18: Chemical structure of a beta-aminopyridine chelating derivative (adapted from [12]).

Tacrine-hydroxyphenylbenzimidazole hybrids were synthesised (Figure 19), and were shown to inhibit AChE in the nanomolar concentration range and display radical scavenging and metal chelation abilities *in vitro*.[69]

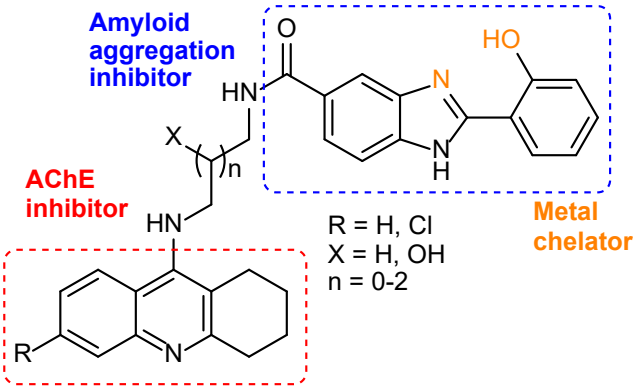


Figure 19: Structural design for tacrine-hydroxyphenylbenzimidazole hybrids (adapted from [69]).

Hiremathad et al.[69] reported that compounds with shorter chain linkers exhibited the greatest AChE inhibitory activity, and chloro-substitution also had a positive effect on activity. A hydroxyl substituent on the linker chain enhanced the AChE inhibition value as well as the radical scavenging ability. However, the radical scavenging abilities of the compounds were significantly lower than hydroxyphenyl-benzimidazole acid (EC_{50} around 500 μM instead of 160 μM). This moderate radical scavenging activity could be due to the combination with tacrine, which shows no significant activity against free radicals (EC_{50} greater than 1000 μM). The compounds exhibited moderate protection of neuroblastoma cells from induced oxidative stress and Aβ oligomerisation. However, the compound which demonstrated the greatest inhibitory activity against AChE, displayed the lowest activity against Aβ-induced cell toxicity. This test also did not include a positive control, and so the values obtained for the hybrid compounds could not be compared with a known inhibitor of oxidative stress or Aβ oligomerisation in this cell line. The concentrations at which each compound induces cell toxicity were also not reported.

7.7 AChE Inhibition and GSK-3β Inhibition

Jiang et al.[106] developed a series of hybrid compounds consisting of the AChE inhibitor, tacrine, and a GSK-3β inhibiting moiety, pyridothiazole (see Figure 20).

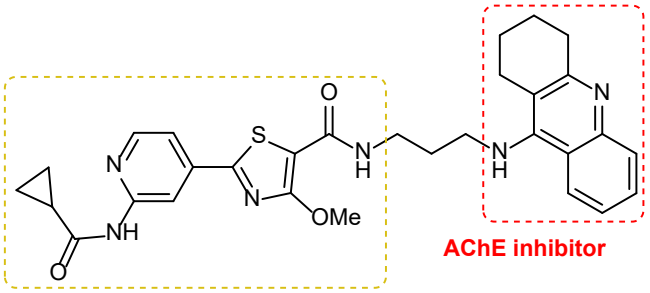


Figure 20: Structure of the most active AChE/GSK-3β inhibitor (adapted from [106]).

The most active compound was reported to inhibit AChE with an IC_{50} of 6.5 nM, and GSK-3β with an IC_{50} of 66 nM *in vitro*. The hybrid was also found to inhibit Aβ aggregation by 46% at 20 μM. On the other hand, all the compounds were expected to have an issue with toxicity due to the presence of the tacrine moiety. The toxicity of the most active compound was tested in human hepatocytes, and gave an IC_{50} of 35 μM. However, no controls were tested. As a result, it is unclear if an improvement in toxicity has been made compared to tacrine. Although the authors reported that the compounds did not demonstrate any hepatotoxicity in mice, no significant hepatotoxicity was also reported for tacrine. This may have been due to the fact that the drug was only administered once and measurements were recorded for a total of 36 hours.

7.8 AChE and MAO-B Inhibition

Phenolic derivatives have also attracted increased interest in recent years for their potential as multi-target drugs. Sang et al.[107] reported that derivatives of 2-acetyl-5-*O*-(amino-alkyl)phenol showed inhibitory activity *in vitro* against cholinesterases and MAOs, antioxidant and neuroprotective abilities, and metal chelation. The compound with the greatest activity (see Figure 21) could inhibit AChE with an IC_{50} of 0.96 μM, and MAO-B with an IC_{50} of 6.8 μM.

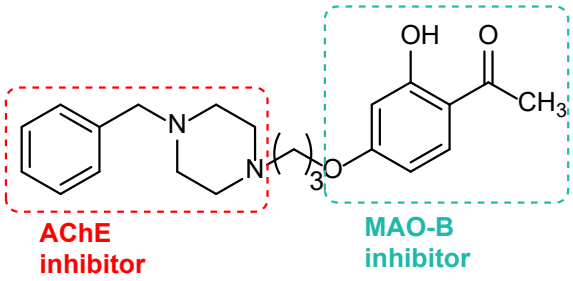


Figure 21: Chemical structure of the most active 2-acetyl-5-*O*-(amino-alkyl)phenol derivative (adapted from [107]).

It was also suggested that these compounds could inhibit MAO-A; however, no activity was reported when these drugs were tested on recombinant human MAO-A. The oxygen radical absorbance capacity (ORAC) for the compound in Figure 21 was shown to be 1.5 µM as a Trolox equivalent. Furthermore, it was reported to cross the blood-brain barrier efficiently, and interact with both the CAS and PAS simultaneously which could account for its high AChE inhibitory activity. The compound was found to protect cells against peroxide-induced injury with a viability of 73.3% at 10 µM. In the presence of $CuCl_2$, the UV peak at 314 nm for the phenolic derivative shifted to 356 nm. There was no change for the other biometals studied. Therefore, this demonstrates that the compound is a selective chelator for Cu(II). This selectivity is significant since Cu(II) is predominantly associated with the formation of amyloid plaques.[12]

7.9 AChE Inhibition and NMDAR Antagonism

A galantamine derivative in combination with memantine was developed by Reggiani et al.[93]. Memantine is the only clinically available NMDA receptor antagonist, and is prescribed to treat moderate to severe dementia. By fusing these moieties with different mechanisms of action, it was expected that the resulting hybrid would have multi-target capabilities. The compound (Figure 22) was found to achieve balanced potencies against AChE and NMDAR *in vitro*, with an IC_{50} of 0.695 µM and a K_i of 2.32 µM, respectively.

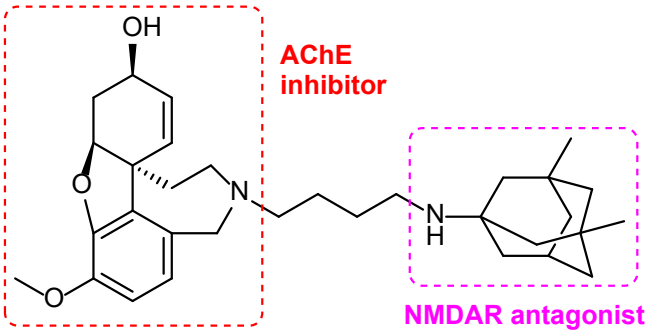


Figure 22: Structure of galantamine and memantine hybrid (adapted from [93]).

It was reported that the galantamine derivative could revert Aβ-induced neurotoxicity and inhibit behavioural impairment in rodents. However, it is crucial to note that the drug was administered with Aβ simultaneously. Therefore, the authors suggested that this compound would be suitable for treatment in the very early stages of the disease, but it is possible the Aβ was not allowed enough time to sufficiently aggregate and induce the characteristic toxic effects that are observed even during the early stages of AD. Nonetheless, the administration of the compound was not ideal (chronic infusion for 7 days directly into the brain). Thus, continued research into improving the pharmacokinetic profile and alternative delivery strategies are vital prior to the proposal of this compound as a potential treatment for AD.

7.10 AChE Inhibition and Nrf2 Induction

Benchekroun et al.[97] developed multifunctional compounds with the capacity to inhibit AChE and activate the Nrf2 transcriptional pathway by combining the tacrine moiety with melatonin. The authors also added ferulic acid to the chemical structure in order to reduce hepatotoxicity induced by the tacrine moiety, as well as increase antioxidant activity. Figure 23 shows the structure of the most active multi-target hybrid, with an IC_{50} of 1290 nM against AChE and the capacity to significantly induce Nrf2 at 3 µM *in vitro*. However, the value reported for AChE inhibition by tacrine was 420 nM, while this is usually around 230 nM.[103,106] The compound was also reported to have

antioxidant activity, with 9.11 TE in the ORAC assay, and neuroprotective effects against Aβ toxicity in a neuronal cell line (70.6% at 1 μM).

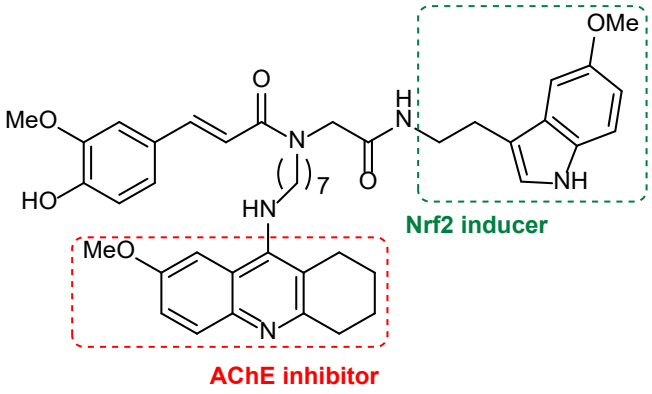


Figure 23: Chemical structure of tacrine-melatonin-ferulic acid hybrid (adapted from [97]).

7.11 BACE-1 Inhibition and GSK-3β Inhibition

Compounds combining the guanidine moiety and a cyclic amide group as BACE-1 and GSK-3β inhibitors respectively were developed by Prati et al.[89] After testing the effects of various substituents on the aromatic ring, the fluorinated compound shown in Figure 24 was found to be the most active. This hybrid compound exhibited an IC_{50} of 18.03 μM for BACE-1 inhibition, and 14.67 μM for GSK-3β inhibition *in vitro*.

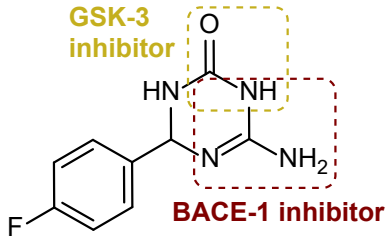


Figure 24: Structural design of the most active BACE-1/GSK-3β inhibitor (adapted from [89]).

The moderate BACE-1 inhibition levels were ascribed by the authors to be due to the fluorine substituent interacting with residues in the active site of the enzyme through polar and hydrophobic bonds. Despite the fact that this compound was not the most active, the authors proposed that this would be the most promising multi-target compound due to its moderate balanced potency against the two targets, and lower molecular weight. This suggestion was supported by Zheng et al.[108], who reported that partial inhibition of several targets would be more efficient and induce less adverse effects than complete inhibition of a single target.

7.13 Metal Chelation and Tau Aggregation

Silva et al.[109] employed nitrocatechol derivatives with the carboxamide moiety as multifunctional inhibitors of tau aggregation and copper chelators. The authors reported that nitrocatechol was essential for anti-aggregating activity, and the presence of a carboxamide group significantly enhanced this activity. Incorporating a cyano group in the chemical structure also appeared to improve inhibitory activity against tau aggregation, with over 70% aggregation inhibition at 50 μM for all compounds containing this moiety. The cyano group may also be associated with copper chelating activity, as only the compounds containing this moiety were reported to exhibit significant activity. The most active hybrid chelated copper, and inhibited 89.2% of tau aggregation at 50 μM *in vitro* (shown in Figure 25). None of the compounds were able to chelate iron, but Bagheri et al.[110] reported that copper is the major biometal involved in plaque formation and AD development.

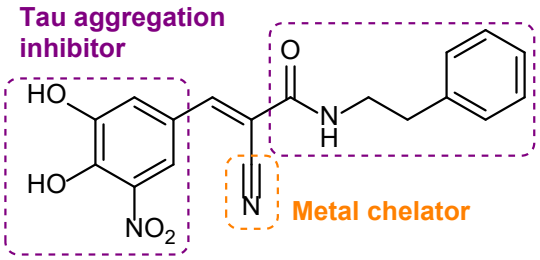


Figure 25: Structure of the most active nitrocatechol derivative with carboxamide (adapted from [109]).

8 Emerging Targets of AD for the Development of Multi-Target Drugs

Researchers are continuously identifying potential novel targets for the treatment of AD. Often, factors known to contribute to the development of similar disorders – such as other neurodegenerative diseases, or cancer – are found to have a similar

malfunction in AD. As a result, there may be drugs aimed at these particular targets already in development for the treatment of conditions other than AD. As it is discovered that these treatments could also be beneficial for AD, it is becoming increasingly common to repurpose drugs. Repurposing therapeutics for alternative diseases is often carried out with compounds that have been unsuccessful in advanced clinical trials for the particular disease they were aimed at, but showed promise in early trials. Therefore, when another condition is found to develop in a similar manner, the drug is trialled as a potential treatment for this condition instead. Repurposing is becoming more popular as an innovative strategy for drug discovery as it uses already approved and established drugs. This can therefore avoid the cost and high failure rates associated with traditional drug development approaches.[111]

Another technique which has been employed in recent years with the aim of generating more successful AD drugs is *in silico* drug design. Computer-based methods for screening potential ligands against drug targets are able to predict the efficacy of compounds, while reducing costs and accelerating the overall design process.[112] *In vitro* data is used to form predictive models; including quantitative structure-activity relationships (QSAR), datamining, molecular modelling, and machine learning. These models can also be used to optimise novel ligands by enhancing the interactions with the drug target, and improving the pharmacokinetic profile.[63]

8.1 Ubiquitin

The accumulation of misfolded proteins is associated with toxic effects, and ultimately the development of neurodegenerative diseases including AD. Typically, misfolded proteins are degraded by the cell via the ubiquitin proteasomal system (UPS). This system, along with autophagy and molecular chaperones, function to prevent the build-up of toxic levels of misfolded proteins. However, any disturbances in these systems can lead to the promotion of neurodegenerative diseases.[113] Synaptic proteins are predominantly degraded by means of the ubiquitin proteasomal pathway. Ubiquitination consists of the attachment of the ubiquitin protein to the target protein. Deubiquitinating enzymes (DUBs) recognise the specific linkage between the target protein and ubiquitin and remove and recycle ubiquitin, while the substrate is degraded by the proteasome. The UPP is responsible for the modulation of Aβ and tau levels, and it has been reported that a reduction in proteasomal activity is observed in AD brains. Therefore, UPS provides a promising target for AD treatment, particularly through the activation of enzymes involved in the system. For example, enhancing the activity of ubiquitin hydrolase is proposed to increase the levels of ubiquitin and has been shown to improve cognition in transgenic AD mouse models.[114]

8.2 AMP-Activated Protein Kinase

AMP (adenosine monophosphate)-activated protein kinase (or AMPK) plays a key role in regulating cellular metabolism, and is also involved in glucose uptake and lipid oxidation. Despite recent research that suggests the involvement in AMPK in AD development, it is still unclear what role it plays. It has been proposed that activation of AMPK inhibits tau phosphorylation and amyloid aggregation in neurons. However, this has been refuted by opposing suggestions that AMPK is responsible for phosphorylating tau and interfering with the normal binding between tau and microtubules. AMPK is also reported to reduce mTOR signalling, which is required for maintaining synaptic plasticity, and it can promote Aβ degradation.[115] Therefore, while the exact involvement of AMPK in AD is poorly understood, it is clear that it is likely to be involved in the pathology of the disease and consequently has the potential to be an important target for drug development.

8.3 C-Jun N-Terminal Kinases

C-Jun N-Terminal Kinases (JNK) are stress-activated signalling proteins. Research has suggested that JNK could play a role in the development of AD, as it has been found to induce neuronal death, oxidative stress, and the formation of phosphorylated tau.[45]

Furthermore, it has been reported that inhibition of JNK activity was able to prevent cell loss in an AD model.[115] Consequently, JNK poses a potentially important target for the development of AD therapeutics.

8.4 Mitochondria

There is growing evidence that mitochondria play an important role in AD.[116] Mitochondrial function reportedly declines in AD, and alterations in morphology and fusion/fission are observed. The expression of mitochondrial-encoded genes is also modified, and these are known to encode key proteins for the electron transport chain.[116] Despite ongoing research, no therapeutics against this target have been entered into clinical trials. However, a potential inhibitor of mitochondrial dysfunction has been reported with the ability to prevent excessive opening of the mitochondrial permeability pore.[117] Therefore, mitochondrial function remains an important therapeutic target for numerous chronic diseases, including AD.

9 Conclusions

Drug development for the treatment of AD in the future will include further research into the effectiveness and safety of these drugs in humans. In order to increase the success rates in clinical trials, it is essential to effectively validate these drugs and their targets.[53] Further research into the etiology of AD is required in order to be able to produce drugs that effectively target the major factors involved in the development of this disease.

Dosage optimisation and the reduction of adverse side effects are also crucial factors to address prior to the implementation of these drugs in clinical practice. Financial issues are potentially impeding the success of AD drug development. More recently, charities and government initiatives have been generating a rise in funding for AD research.[118]

Due to the complex pathology of AD, future research is likely to focus on multi-target drugs with the aim to target several factors that contribute to this disease simultaneously, and therefore limit their role in the development of AD. The use of only one multi-functional drug instead of a combination of single-target drugs will improve the patients' compliance and quality of life, in addition to lowered healthcare and social care costs. There is an urgent need for drugs that are capable of halting the progression of AD, and can be used as an early intervention. Current drugs in development have demonstrated promising results, with the proposal of further research including *in vivo* tests.[107] Multi-functional agents have the potential to transform the way neurodegenerative diseases are treated and managed.

10 Conflicts of Interest

There are no conflicts to declare.

11 Acknowledgements

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