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Polypharmacology in Alzheimer's Disease: Integrating AI, Network Pharmacology, and Experimental Validation

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Abstract

Alzheimer's disease (AD), the leading cause of dementia, is a progressive neurodegenerative disorder characterized by amyloid-β plaques, tau tangles, oxidative stress, mitochondrial dysfunction, and neuroinflammation. Current monotherapies, designed using a "one drug-one target" approach, provide only symptomatic relief without altering disease progression. Polypharmacology, through the development of multi-target-directed ligands (MTDLs), offers a promising therapeutic strategy that simultaneously modulates multiple interconnected pathways in AD pathology. This review highlights the integration of computational and experimental approaches in the discovery of MTDLs. In silico tools, such as molecular docking, pharmacophore modeling, QSAR, network pharmacology, and artificial intelligence, facilitate the prediction and design of polypharmacological agents. In vitro, in vivo, and omics-based studies have validated their therapeutic relevance. Hybrid molecules, including ladostigil, M-30, ASS234, donecopride, and chalcone–rivastigmine hybrids, demonstrate multi-faceted neuroprotection by targeting cholinergic dysfunction, amyloid aggregation, oxidative stress, and neuroinflammation. Despite challenges such as off-target effects and translational limitations, advances in AI-driven platforms, systems biology, and human-relevant models, such as brain organoids, are expected to accelerate the development of disease-modifying therapies. Polypharmacology represents a paradigm shift in AD treatment, moving beyond symptomatic relief towards mechanism-informed interventions with the potential to slow or halt disease progression.

Keywords: *Polypharmacology, Neurodegenerative diseases, Multi-target-directed ligands, Computational drug discovery, Artificial intelligence, Molecular docking, Network pharmacology*

1. INTRODUCTION

Alzheimer's disease (AD), the leading cause of dementia, is an increasingly significant global health concern, particularly in countries with growing elderly populations. The Alzheimer's Association reports that, in the United States, there are currently over five million individuals aged 65 and older living with Alzheimer's disease. This figure is projected to rise to almost 14 million by 2060 (1). Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder characterized by a gradual decline in cognitive and functional abilities. The disease advances through preclinical, mild, moderate, and severe stages, ultimately resulting in severe cognitive deficits and physical incapacity. The hallmark pathological features include the accumulation of extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein, predominantly in the cortical and limbic regions of the brain (2).

Currently approved pharmacotherapies for AD are primarily palliative and offer limited disease-modifying effects. Acetylcholinesterase inhibitors, such as donepezil, galantamine, and rivastigmine, are used in the mild-to-moderate stages of AD to alleviate symptoms by enhancing cholinergic neurotransmission (3). For moderate-to-severe AD, memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist, is used to reduce excitotoxicity and preserve cognitive function (3). These drugs, while beneficial in providing symptomatic

relief, are primarily designed based on a "one drug–one target" paradigm, targeting individual molecular pathways implicated in AD pathogenesis. However, this reductionist approach has shown limited clinical success, especially considering the multifactorial nature of AD, which involves interrelated processes such as protein misfolding, oxidative stress, mitochondrial dysfunction, neuroinflammation, synaptic degradation, and vascular impairment (4).

Given the complexity and overlapping pathophysiological mechanisms involved in AD, there is a growing consensus that single-target interventions may be insufficient to meaningfully alter disease progression. This has led to the exploration of multi-target therapeutic strategies as more robust alternatives to single-target ones. Traditionally, this has involved combination therapies or the use of multiple drugs that act independently on various targets. For instance, the co-administration of donepezil and memantine is a clinically approved approach for managing the symptoms of moderate-to-severe AD. However, combination therapies often suffer from drawbacks, such as drug–drug interactions, adverse effects such as bradycardia or psychosis, and challenges in optimizing pharmacokinetic compatibility among the component drugs (5)(6).

To address these limitations, a polypharmacological approach has emerged as a promising therapeutic strategy. This strategy involves the rational design or identification of single molecules capable of modulating multiple targets simultaneously, known as multi-target-directed ligand (MTDL) design. Unlike combination therapy, polypharmacological agents offer the advantages of a unified pharmacokinetic profile, simplified dosing regimens, and reduced risk of adverse interactions, ultimately improving patient adherence and therapeutic efficacy (7).

Polypharmacology is particularly well-suited for tackling complex neurodegenerative disorders, such as AD, in which pathological cascades are deeply interconnected. The goal is not merely symptomatic relief but also to target multiple synergistic pathways involved in the onset and progression of the disease. With the advancement of computational drug discovery tools, including molecular docking, pharmacophore modeling, machine learning, and network pharmacology, it is now possible to predict, design, and optimize multi-target compounds more efficiently and precisely than ever before (7).

This review focuses on the evolving landscape of polypharmacology in neurodegenerative diseases, particularly AD. It explores the scientific rationale behind multi-target strategies, compares them with conventional combination therapies, and highlights recent advances in computational techniques that support MTDL discoveries. In addition, this review presents experimental validations and case studies of several hybrid molecules with demonstrated efficacies in preclinical models. Through an integrated approach that bridges computational intelligence with biological experimentation, polypharmacology holds promise for reshaping the therapeutic paradigm for Alzheimer's disease and other neurodegenerative disorders.

2. COMMON PATHOLOGICAL MECHANISMS

According to the dominant theory of AD pathogenesis, amyloid beta (Aβ) buildup and aggregation appear to be the primary causes of widespread neuronal death over the past 25 years. Hyperphosphorylation of tau proteins and increased Aβ peptide levels, which oligomerize and aggregate to form Aβ plaques, are typical markers of Alzheimer's disease pathogenesis. Patients with AD frequently show hypoperfusion, Aβ accumulation, and ultrastructural alterations in vessel shape (8).

Chronic oxidative stress in the brain augments protein folding, mitochondrial failure, and proinflammatory pathways, leading to increased neuronal death in patients with neurodegenerative diseases (9). The metals (iron and copper) present in the brain react with hydrogen peroxide to generate hydroxyl radicals, and the high energy demand of the brain renders it susceptible to oxidative stress (OS). This highly reactive and destructive OH radical destroys vital cellular constituents in the brain, including proteins, lipids, and DNA. Chronic oxidative stress disrupts the equilibrium between the production and neutralization of reactive oxygen species (ROS), leading to their accumulation in tissues and cells. Increased ROS levels result in DNA damage, lipid peroxidation, and protein peroxidation, causing neuronal injury and death (10).

Glutamate is the core excitatory neurotransmitter of the brain. It is thought to be involved in approximately 66% of all brain synapses and is almost universal in the central nervous system. Additionally, glutamatergic neurons are significant because they influence cognition by radiating to other parts of the brain, such as the cholinergic neurons (11). Glutamate itself does not cause the disease linked to glutamatergic neurons in AD;

rather, pre-and postsynaptic glutamate receptor levels do. Only one of the three types of postsynaptic glutamate receptors, the N-methyl-D-aspartate (NMDA) receptor, has been shown to cause AD. It appears that the brains of patients with AD experience persistently low neurotransmission as a result of NMDA receptor activation (12). This dysregulation of the glutamate NMDA receptor drives a vicious cycle of neuronal injury, whereby persistent receptor activation causes persistent calcium influx into the neuron, disrupting regular signal transmission. Furthermore, it causes more Amyloid Precursor Protein (APP) to be produced, which is linked to increased rates of plaque growth and tau protein hyperphosphorylation (hence the formation of NFT), followed by neuronal toxicity (13,14).

Acetylcholine is a neurotransmitter that plays a significant role in memory-related brain regions, and specific features of cognitive impairment are associated with a decline in cholinergic activity. Cholinergic neuron counts drastically decrease in late-stage AD, and in certain regions of the brain, the loss exceeds 75%. Cholinergic anomalies are the most noticeable neurotransmitter alterations in patients with AD. The two types of postsynaptic receptors to which acetylcholine binds are nicotinic and muscarinic receptors. Acetylcholine, glutamate, serotonin, and norepinephrine are neurotransmitters that are essential for mood and memory and are released in response to presynaptic nicotinic receptors. All these neurotransmitters have been linked to AD pathologies (15).

Inflammation may be triggered by injured neurons, NFTs, and β-amyloid deposits as a typical response to cellular damage. Proinflammatory cytokines, reactive oxygen species, proteinases, and complement proteins are potentially lethal substances released by activated microglia during AD pathogenesis (16). This is a typical reaction to cellular damage that often goes unchecked in AD, causing more harm than good to the cells. Cytokines trigger inflammatory responses that may cause myelin injury and encourage oligodendrocyte and neuronal apoptosis or programmed cell death (17). Prostaglandins, which are generated by cyclooxygenases COX-1 and COX-2, are elevated in the AD brain, indicating inflammation as a component of AD pathophysiology. It is interesting to note that, like other forms of cellular damage associated with AD, inflammation in AD is persistent and restricted to certain regions of the brain (18).

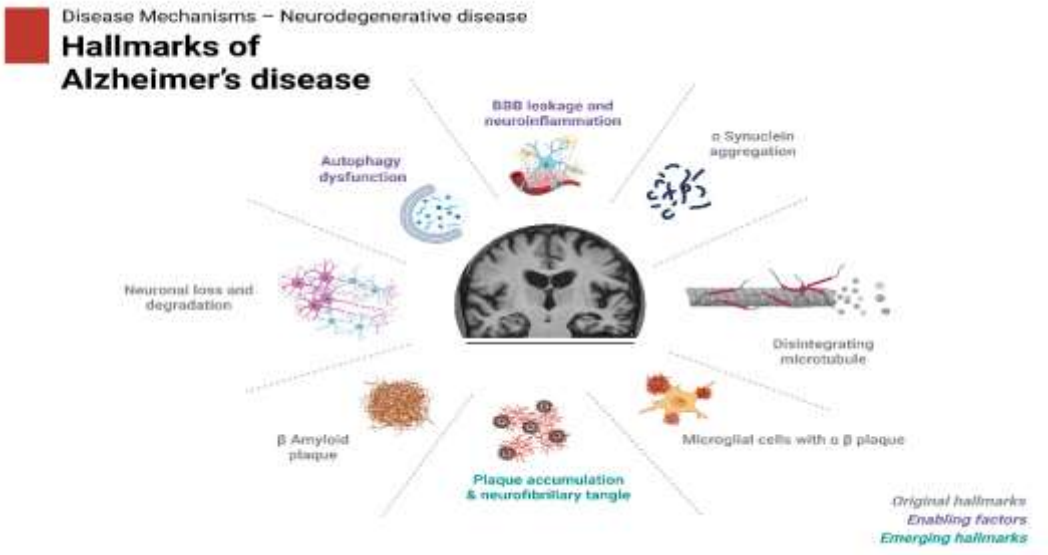


Figure 1: Hallmarks of Alzheimer's disease

3. LIMITATIONS OF CURRENT MONOTHERAPIES

Despite decades of research, pharmacological management of neurodegenerative diseases remains largely symptomatic, with limited success in altering disease progression. Most approved drugs for conditions such as Alzheimer's disease (AD), Parkinson's disease (PD), and Amyotrophic Lateral Sclerosis (ALS) are monotherapies designed to target a single molecular pathway. However, these diseases are inherently multifactorial, involving complex and interacting pathological processes such as protein misfolding, oxidative stress, mitochondrial dysfunction, neuroinflammation, and synaptic loss (19–21). Consequently, targeting only one pathway may not be sufficient to halt or significantly slow disease progression (22).

Another major limitation of monotherapy is the biological adaptability of the brain. When a single target is pharmacologically inhibited, compensatory mechanisms may activate alternative pathological routes, thereby diminishing the drug efficacy over time. This phenomenon can lead to treatment resistance and therapeutic failure, especially in complex and progressive diseases such as AD, in which multiple signaling pathways are dysregulated simultaneously (23,24).

Moreover, many monotherapies exhibit limited efficacy when administered during the later stages of neurodegenerative diseases. By the time most patients are diagnosed, significant and often irreversible neuronal loss has occurred in the brain. This late intervention window reduces the potential impact of single-target drugs, which are often more effective when used preventively or in the early stages of the disease (25).

Finally, the clinical translation of monotherapy-based treatments has been met with considerable setbacks. A large proportion of drug candidates targeting single molecules fail in phase II and III clinical trials, particularly in Alzheimer's disease (23). These failures highlight a critical disconnect between disease complexity and the oversimplified therapeutic strategies employed for treatment. Consequently, there is a growing consensus that more holistic approaches, such as polypharmacology, combination therapies, or multi-target-directed ligands (MTDLs), may offer a more promising path for managing neurodegenerative disorders (24,25).

4. POLYPHARMACOLOGY

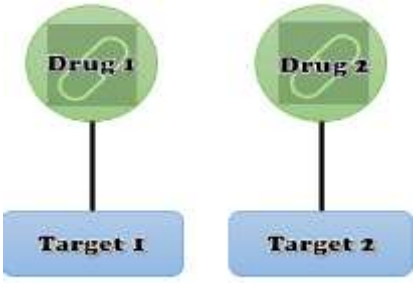
Polypharmacology refers to the strategic design or identification of single drugs that can simultaneously modulate multiple molecular targets and biological pathways. This approach diverges from the traditional "one drug, one target" model, offering a more holistic therapeutic paradigm, particularly for complex multifactorial diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and other neurodegenerative disorders. Multi-target-directed ligands (MTDLs) have a more predictable pharmacokinetic profile owing to the "novel" polypharmacology concept, which simplifies dosage schedules and offers an opportunity to prevent drug-drug interactions and increase patient compliance (26).

Polypharmacology can emerge through intentional rational drug design, where molecules are engineered to interact with multiple targets, or through drug repurposing, where existing drugs exert effects beyond their originally intended mechanisms (27,28). Advances in computational chemistry, systems biology, and multi-omics profiling have accelerated the prediction, evaluation, and optimization of multi-target properties of lead compounds. Importantly, polypharmacological agents can offer synergistic benefits, reduce the need for combination therapy, and potentially minimize drug resistance and side effects when designed appropriately (29). In the context of neurodegeneration, polypharmacology is emerging as a promising therapeutic strategy to simultaneously address multiple dysfunctional pathways, potentially slowing disease progression rather than merely alleviating symptoms. Consequently, multi-target-directed ligands (MTDLs) and hybrid molecules are gaining traction in preclinical and clinical research pipelines, reflecting a paradigm shift in the treatment philosophy for neurodegenerative diseases (30).

This review explores the emerging concept of polypharmacology as a promising therapeutic strategy for neurodegenerative diseases, moving beyond the traditional "one drug, one target" paradigm. By examining the complex and multifactorial nature of conditions such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis, this review highlights the urgent need for therapeutic approaches capable of simultaneously modulating multiple interconnected pathological pathways. This article discusses the rationale, design, and development of multi-target-directed ligands (MTDLs), emphasizing their potential to offer improved efficacy and disease-modifying benefits. This review also critically evaluates the limitations of current monotherapies, such as inadequate efficacy and lack of neuroprotection, and outlines how polypharmacology can overcome these limitations. Finally, we provide a comprehensive understanding of how polypharmacology may shift the treatment paradigm in the management of neurodegenerative diseases.

Table No.1: Combination therapy vs. multi-target drugs

Aspect	Multi-Target Drugs (MTDLs)	Combination Drugs (Drug Combinations)

Definition	Single compound designed to modulate multiple pathogenic targets in neurodegenerative diseases.  Single drug binding to multiple targets	Co-administration of two or more drugs, each targeting a specific pathological mechanism.  Two or more drugs for distinct targets
Mechanism of Action	Integrated action on multiple targets: e.g., cholinesterase inhibition + antioxidant + MAO-B inhibition.	Separate drugs act independently or synergistically on different pathways.
Complexity of Dosing	Simpler — one molecule, one dose.	More complex — multiple agents, different dose optimizations.
Drug–Drug Interactions (DDIs)	Lower risk of classical DDIs (since it's a single compound).	Higher potential for DDIs due to multiple drugs.
Pharmacokinetics	Unified (one drug, one ADME profile).	Each drug has a distinct pharmacokinetic profile.
Clinical Flexibility	Less flexible; fixed activity profile.	More flexible; individual drugs can be titrated or switched.
Development Complexity	High — requires rational design to achieve balanced activity at different targets.	Moderate — existing drugs can be tested in combination.

5. RATIONALE FOR POLYPHARMACOLOGY IN NEURODEGENERATION

Currently, AD treatments can be broadly divided into two groups according to the disease stage. For mild-to-severe cases, galantamine, rivastigmine, and donepezil, which are acetylcholinesterase inhibitors, are recommended to provide temporary symptomatic relief. For moderate-to-severe AD, memantine, an N-methyl D-aspartate (NMDA) antagonist, is used as a monotherapy to control symptoms. The primary goal of these medications is to restore physiological acetylcholine levels. They are primarily selective molecules that target specific proteins (the "one compound-one target" method). Traditional monotherapies targeting a single molecular pathway have repeatedly failed to produce meaningful disease-modifying outcomes in clinical trials, typically offering only transient relief (6).

This therapeutic inefficacy has prompted growing interest in polypharmacology, which involves the use or design of drugs that simultaneously modulate multiple targets in the body. Rather than combining multiple agents, polypharmacology often utilizes multi-target-directed ligands (MTDLs), which are single molecules with affinities for more than one disease-relevant target. This strategy offers the potential for synergistic efficacy, reduced drug resistance, and more comprehensive modulation of the disease mechanisms (26). The rationale for polypharmacology is supported by systems biology and multi-omics data, which reveal intricate interdependencies between various pathological cascades. For instance, amyloid-β accumulation in AD exacerbates tau pathology, oxidative stress, and inflammatory responses, indicating that single-target therapies may be insufficient to halt disease progression (6).

Moreover, computational tools and AI-driven drug discovery platforms have enhanced our ability to predict polypharmacological profiles, optimize pharmacokinetics, and design ligands with favorable therapeutic indices (6).

6. POLYPHARMACOLOGICAL AGENTS IN NEURODEGENERATIVE DISEASE

In recent years, numerous polypharmacological agents have been developed to address the multifaceted pathologies of neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). These agents are typically formulated to target essential interconnected pathways, such as amyloid-β aggregation, tau hyperphosphorylation, oxidative stress, mitochondrial dysfunction, neuroinflammation, and neurotransmitter imbalance.

6.1 Ladostigil (TV 3326)

Ladostigil integrates features from both rasagiline and rivastigmine, incorporating the propargylamine group characteristic of rasagiline and the carbamate element typical of rivastigmine. Referred to as TV 3326, this molecule effectively inhibits both butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE), and maintains its efficacy longer than rivastigmine. Furthermore, it achieves over 80% inhibition of MAO-A and MAO-B in the brain.

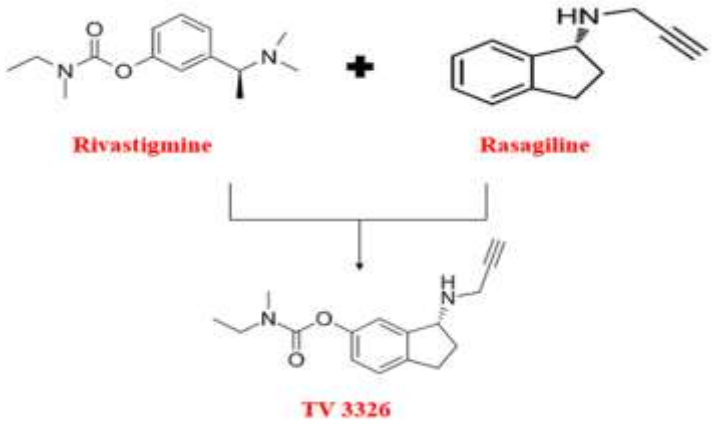


Figure 2: Hybrid molecule Ladostigil (TV3326)

It is well known that neurodegenerative diseases like AD also exhibit gliosis and OS. These characteristics were diminished during in vivo research using a rat model. Therefore, TV 3326 may help reduce neurodegeneration and halt the progression of the disease. Overall, it is reasonable to conclude that TV 3326 has several therapeutic benefits, making it useful for the treatment of Alzheimer's disease (31).

Figure 3: Mechanistic pathways targeted by TV3326 in neuroprotection

6.2 M-30

M-30 was synthesized by integrating the propargyl moiety of rasagiline into the framework of our innovative brain-permeable neuroprotective iron chelator, VK-28.

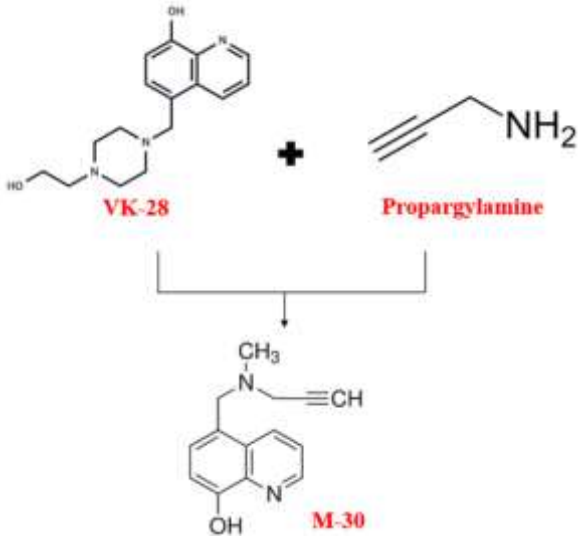


Figure 4: Hybridisation of VK-28 and propargylamine to form hybrid molecule M-30

The multifunctional, nontoxic, and brain-permeable iron chelator drug, M-30, was designed to prevent iron-induced OS as a consequence of reactive hydroxyl radical generation via its interaction with hydrogen peroxide (Fenton Reaction). It also inhibits the formation of reactive hydroxyl radicals from hydrogen peroxide generated by MAO and potentiates the pharmacological action of accumulated dopamine formed from l-dihydroxyphenylalanine (l-DOPA). Among a series of multifunctional iron chelators, M-30 was found to be a highly potent inhibitor of both MAO-A and MAO-B activities and the most effective inhibitor of lipid peroxidation in the brain. Multiple neuroprotective benefits of M-30 have been demonstrated, such as stimulation of neuronal differentiation, control of APP and Aβ levels, and pro-survival/neuro-rescue actions (32).

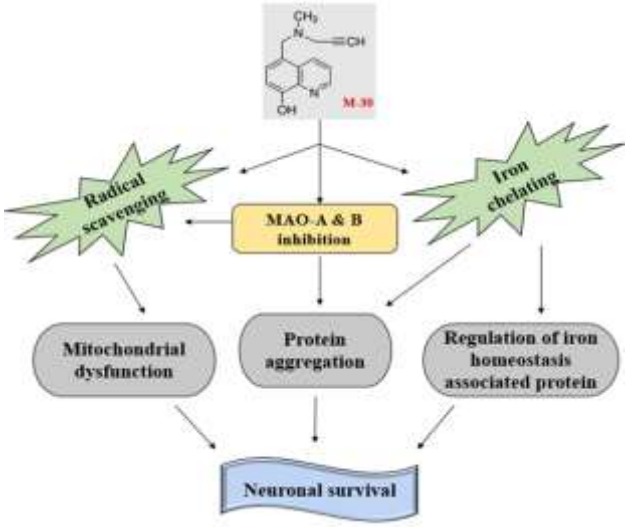


Figure 5: Mechanistic pathways targeted by M-30

6.3 ASS234

The most promising hit compound among a new series of hybrid derivatives, ASS234, was later found to be the most potent MAOI, exhibiting the strongest activity against AChE inhibition. These compounds consist of an N-benzyl piperidine moiety and PF9601N's indolyl propargylamine moiety.

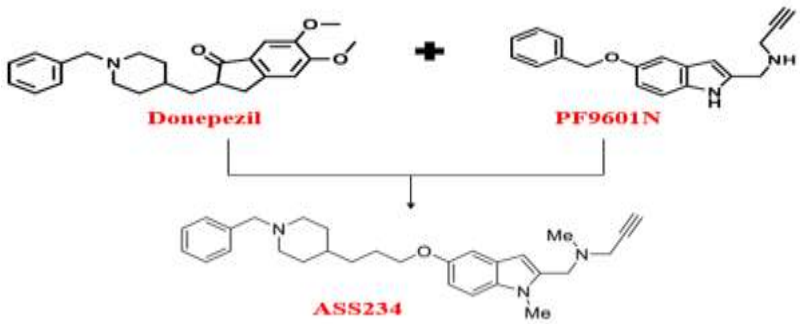


Figure 6: Hybridisation of donepezil and PF9601N to form hybrid molecule ASS234

ASS234 can control apoptosis, prevent Aβ aggregation, and pass through the blood-brain barrier (BBB). Additionally, in the submicromolar range, it can inhibit the BuChE activity. ASS234 prevents the self-aggregation of Aβ peptide fragments (Aβ1–40 and Aβ1–42) and their AChE-dependent aggregation in SH-SY5Y cells. Furthermore, ASS234 dramatically decreased Aβ1–42 mediated toxicity in a dose-dependent manner within the micromolar range. Antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD-1), are depleted by Aβ1-42. ASS234 may directly or indirectly inhibit the mitochondrial apoptotic pathway in cancer cells. These results suggest that ASS234 may regulate the intrinsic apoptotic mechanism, preventing the death of neurons associated with AD. Additionally, ASS234 may be a strong HSP inducer that could stop AD cell death and protein misfolded aggregation. In addition to showing a favorable preclinical safety profile, possible disease-modifying profile, meaningful neuroprotective and anti-apoptotic actions, and promising MAO/AChE inhibitory potency profile, ASS234 can also control multiple signalling pathways involved in brain activity. Additionally, managing a single chemical entity eliminates the difficulty of providing many single-drug entities, improves patient compliance, and simplifies the dosing regimens. Taken together, these studies and findings emphasize the potential effectiveness of MTDL ASS234 in the fight against AD (33).

6.4 ITH12674

Melatonin and sulforaphane were combined to create ITH12674, which has a dual drug–prodrug mode of action. ITH12674 exhibited a better neuroprotective profile than that of sulforaphane or melatonin. By boosting the expression of haem oxygenase-1 and lowering the production of free radicals, ITH12674 protected organotypic cultures of hippocampal slices exposed to oxygen and glucose deprivation and reoxygenation from stress. It also increased GSH concentrations in cortical neurons, decreased reactive oxygen species production, and improved the Nrf2-antioxidant response element transcriptional response in the transfected HEK293T cells. ITH12674 enhances its neuroprotective qualities by combining the signalling pathways of the parent chemicals (34).

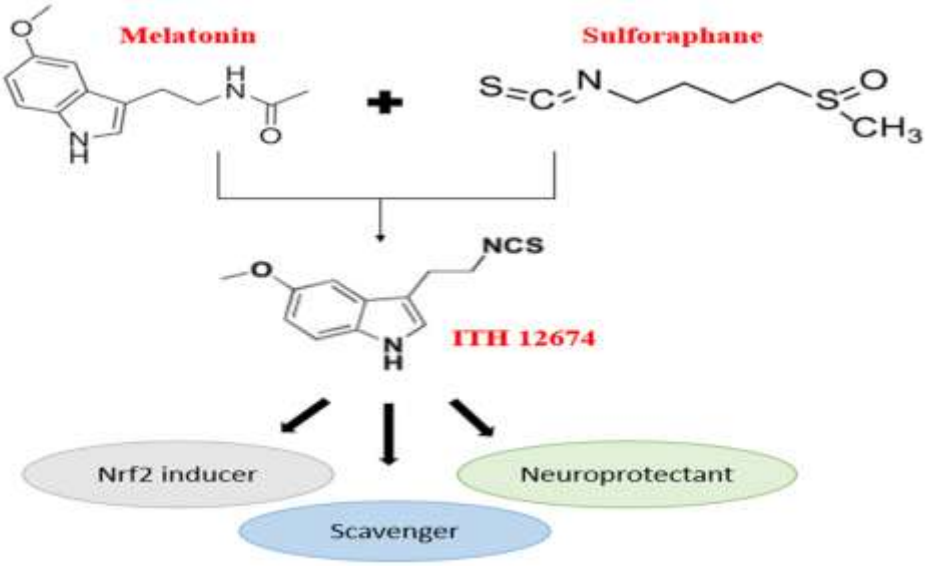


Figure 7: Synthesis and neuroprotective actions of ITH12674

6.5 Donecopride

Donecopride is a hybrid molecule that combines two pharmacological actions into a single chemical structure. Donecopride is a hybrid of the piperidine ring system of RS67333 (or similar 5-HT_4 receptor agonists) and benzyl and/or aryl groups of donepezil (an AChE inhibitor), allowing simultaneous action on both targets.

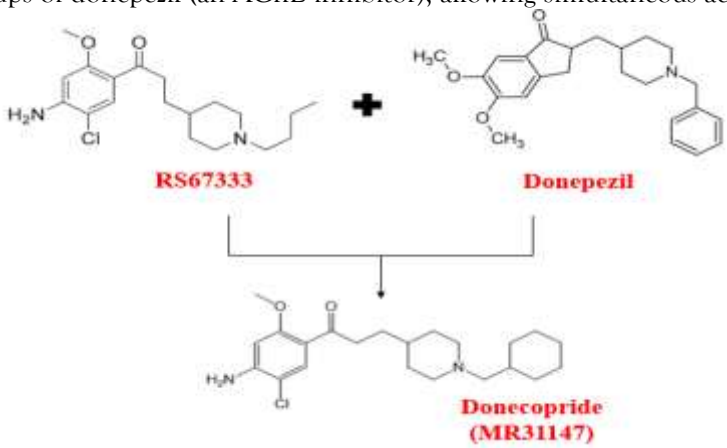


Figure 8: Synthesis of donecopride

Activation of 5-HT_4 receptors enhances the release of acetylcholine (ACh), stimulates synaptic plasticity, and promotes non-amyloidogenic processing of amyloid precursor protein (APP), leading to the production of neuroprotective soluble APP-α fragments instead of toxic amyloid-β peptides. Inhibiting AChE prevents the breakdown of ACh in the synaptic cleft, boosting cholinergic neurotransmission, which is severely impaired in patients with AD. Thus, by modulating both the cholinergic and serotonergic systems and promoting non-amyloidogenic APP processing, donecopride represents a step towards disease modification (35).

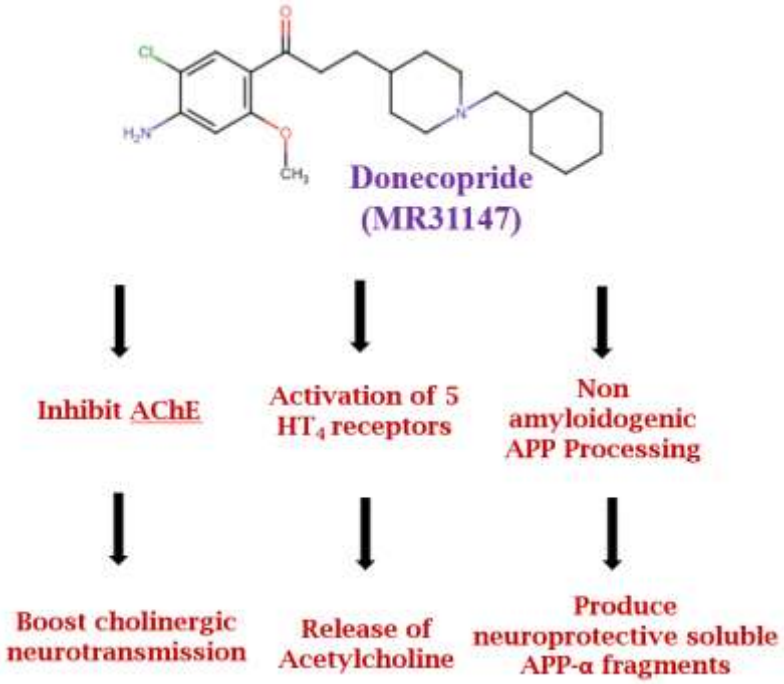


Figure 9: Mechanistic actions of donecoopride

6.6 β-carboline - cinnamic acid hybrid

To develop effective multi-target therapeutic agents for Alzheimer's disease (AD), Chen et al. (2021) designed and synthesized a novel series of β-carboline–cinnamic acid hybrid compounds. These hybrids combine a neuroprotective β-carboline scaffold with an antioxidant-rich cinnamic acid moiety to achieve synergistic biological effects. Among the synthesized derivatives, compound 6d emerged as the most promising candidate, exhibiting potent dual inhibition of acetylcholinesterase (AChE; IC_{50} = 0.32 μM) and butyrylcholinesterase (BuChE; IC_{50} = 0.52 μM). Furthermore, this compound demonstrated strong antioxidant activity, effectively scavenging DPPH radicals, and offered significant neuroprotection against oxidative stress-induced cellular

damage in SH-SY5Y neuroblastoma cells. Importantly, these hybrids exhibited minimal cytotoxicity, addressing a major limitation associated with traditional cholinesterase inhibitors, such as tacrine. The incorporation of β-carboline and cinnamic acid pharmacophores into a single molecular framework underscores the potential of such hybrid molecules as promising multi-target-directed ligands (MTDLs) for AD treatment. This study highlights the strategic value of hybridization approaches in overcoming the multifactorial challenges associated with neurodegenerative disorders (35).

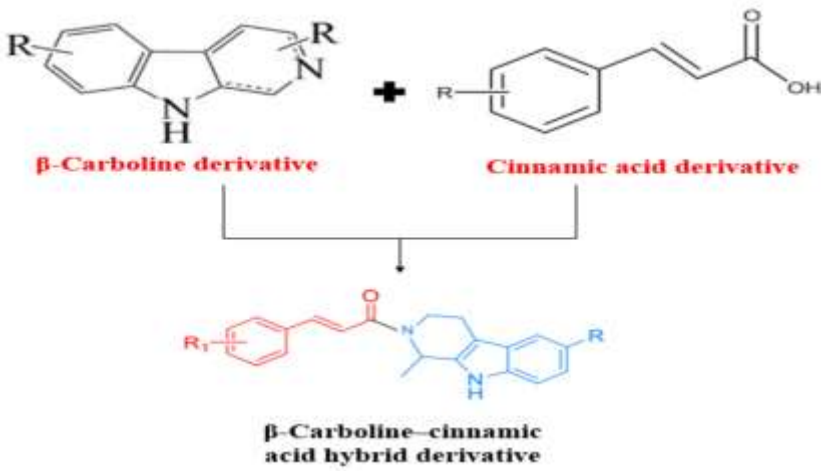


Figure 10: Synthesis of β-carboline–cinnamic acid hybrids

6.7 4′-amino chalcone-rivastigmine hybrids

In the pursuit of effective multi-target strategies for Alzheimer's disease (AD), Xiao et al. (2021) reported the design, synthesis, and biological evaluation of a novel series of 4′-amino chalcone–rivastigmine hybrid compounds. This study capitalizes on the complementary pharmacological properties of chalcones, which are known for their antioxidant, anti-inflammatory, and anti-amyloid activities, and rivastigmine, a clinically approved acetylcholinesterase (AChE) inhibitor. By hybridizing these two scaffolds through rational linker design, the authors aimed to develop multifunctional agents capable of simultaneously addressing multiple pathological hallmarks of AD. Among the synthesized compounds, several hybrids exhibited potent dual inhibitory activities against both AChE and butyrylcholinesterase (BuChE), significant antioxidant capacities, and effective inhibition of β-amyloid (Aβ) aggregation. Furthermore, the hybrids exhibited acceptable blood–brain barrier permeability, a crucial feature of central nervous system drug candidates. These findings support the concept of polypharmacology in neurodegeneration, emphasizing that the combination of multiple bioactive pharmacophores into a single molecule can lead to superior therapeutic profiles compared to those of traditional single-target drugs. Thus, the study by Xiao et al. provides valuable insights into hybrid-based multi-target drug design as a promising avenue for Alzheimer's disease therapy (36).

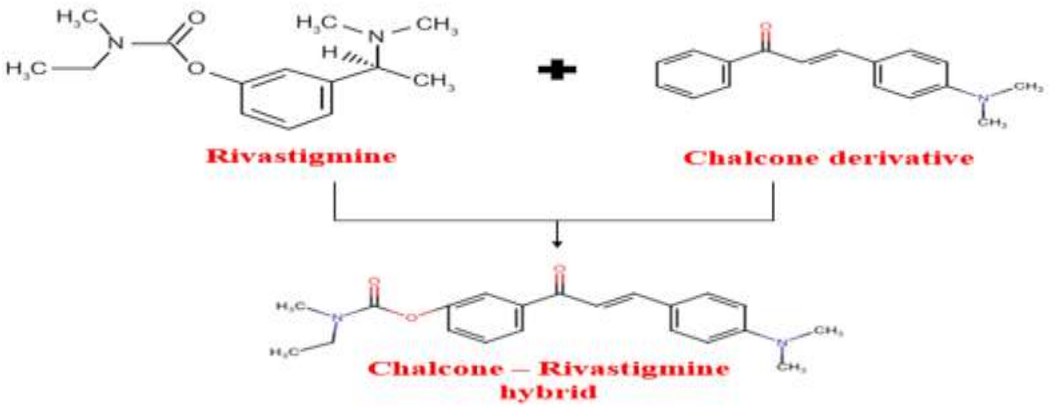


Figure 11: Synthesis of Chalcone – Rivastigmine hybrid (37)

6.8 Quinolinetrione-tacrine hybrids

Uliassi et al. engineered a series of quinoline trione–tacrine hybrids by tethering the classic AChE inhibitor tacrine to a 2,5,8-quinoline trione core via 2–4-methyl amide linkers, thereby creating single molecules capable

of dual cholinesterase blockade, anti-amyloid aggregation, antioxidant protection, and improved hepatotoxicity. All hybrids inhibited human AChE (IC_{50} = 5.58–667 nM) and BChE (IC_{50} = 109–2 830 nM), with selectivity indices of up to 507. In fibrillogenesis assays, lead hybrid 3 reduced $A\beta_{1-42}$ aggregation by over 50% at 10 μM, matching classic antiaggregants, whereas in menadione-challenged SH-SY5Y cells (10 μM), it restored ROS to near baseline —mirroring Trolox—whereas tacrine was inactive, and compounds 2 and 5 acted as pro-oxidants. Importantly, hybrid 3 exhibited only ~14% HepG2 viability loss at 10 μM, significantly lower than the ~25% loss observed with tacrine or tacrine-quinone leads, indicating an improved safety margin compared to the leads. Molecular docking into Torpedo californica AChE (PDB 4TVK) showed dual-site engagement, with the tetrahydroacridine core occupying the catalytic gorge and the quinolinetrione moiety binding to the peripheral anionic site via hydrogen bonds and π–π stacking, thus rationalizing the observed nanomolar potency and selectivity (38).

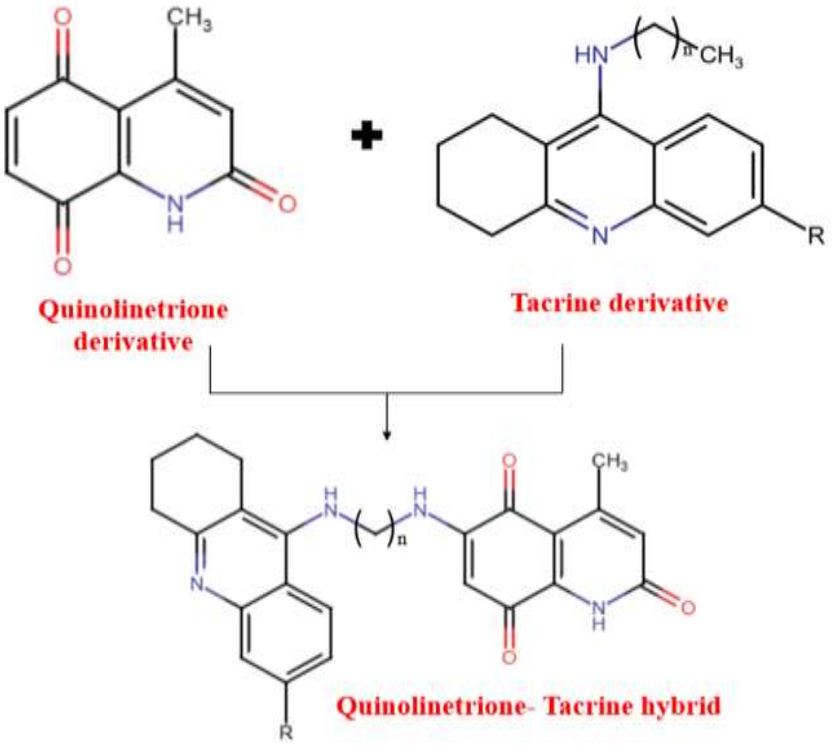


Figure 12: Synthesis of Quinolinetrione- Tacrine hybrid

6.9 Ibudilast

Ibudilast is a multi-target medication that is a non-selective phosphodiesterase inhibitor and TLR4 antagonist. It reverses the molecular phenotypes of patients with Alzheimer's disease, inhibits IRAK1 as an off-target, and modifies several pathways associated with the disease. Ibudilast primarily increases intracellular cAMP levels, which alters various signalling cascades and physiological processes (39). According to growing preclinical evidence, ibudilast may have significant anti-neuroinflammatory effects on various neurological conditions, including chronic cerebral hypoperfusion, peripheral and central neuropathic pain, opioid withdrawal, HIV-1-associated neurocognitive disorders (HAND), cerebral aneurysms, transient cerebral ischemia, ischemic brain injury, post-stroke vertigo, tactile allodynia, and cognitive impairment caused by oxaliplatin, tacrolimus-induced neurotoxicity, and cocaine use disorder. Ibudilast can reduce neuroinflammation by blocking microglial activation, downregulating the proinflammatory cytokines TNF-α, IL-1β, and IL-6, upregulating the anti-inflammatory cytokine IL-10, and regulating IRAK1, IRAK3, and TRAF6. By restoring the potential of the mitochondrial membrane, ibudilast can prevent mitochondrial damage and increase the levels of neurotrophic factors such as GDNF, NT-3, NT-4, and NGF. By controlling the mTORC1-TFEB pathway, it can also affect the autophagy-lysosomal system. Ibudilast also suppresses aberrant protein aggregation by promoting the clearance of TDP-43 and SOD1 aggregates. It protects against glutamate-induced neurotoxicity by reducing Ca2+ influx and neuronal apoptosis by downregulating caspase-3 and upregulating bcl-2. Ibudilast may also prevent oxidative stress by reducing reactive oxygen species (ROS) production (40).

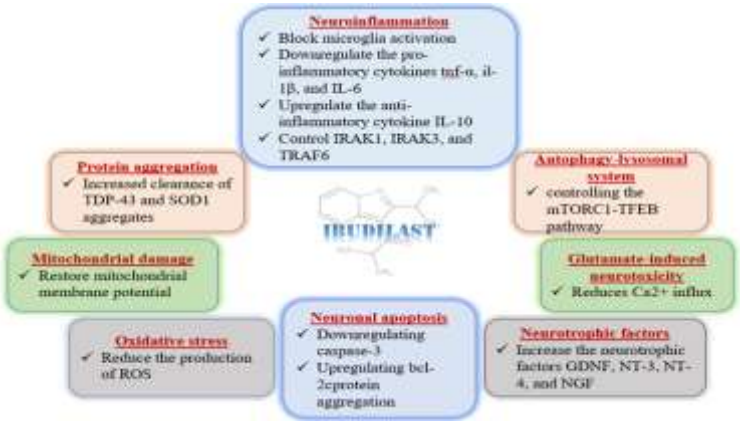


Figure 13: Mechanistic pathways of Ibudilast

7. PATENTS:

Table No. 2: Selected Patents Relevant to Polypharmacology and Alzheimer's Disease

S. No	Patent No.	Publication Date	Title	References
1.	US 9, 738, 605 B2	Aug. 22, 2017	Hybrid compounds of curcumin and melatonin as neuroprotectants for neurodegenerative disorders	(41)
2.	US 2018 / 0157786 A1	Jun. 7, 2018	Predicting drug - target interactions and uses for drug repositioning and repurposing	(42)
3.	US 11,842,793 B2	Dec. 12, 2023	Methods and tools for detecting, diagnosing, predicting, prognosticating, or treating a neurobehavioral phenotype in a subject	(43)
4.	US 2017/0147743 A1	May 25, 2017	Rapid identification of pharmacological targets and anti-targets for drug discovery and repurposing	(44)

8. INTEGRATION OF COMPUTATIONAL AND EXPERIMENTAL APPROACHES

Polypharmacology is defined as the ability of a drug to interact with multiple targets and is gaining prominence in the treatment of complex, multifactorial diseases involving multiple dysregulated pathways that rarely respond adequately to single-target therapies. Consequently, drug discovery is shifting from the traditional "one drug–one target" paradigm to a "multi-target-directed ligands" (MTDLs) approach, wherein a single compound is designed to modulate several targets simultaneously (45).

Given the complexity of neurodegenerative diseases, a comprehensive understanding of disease pathology and drug action at the systemic level is essential. This requires the integration of computational (in silico) approaches, which can rapidly screen and predict interactions across large biological networks, and experimental (in vitro/in vivo) methods, which provide empirical validation and mechanistic insights (46).

Computational methods, such as molecular docking, network pharmacology, and machine learning, can identify potential polypharmacological compounds by simulating interactions with multiple biological targets. These methods enable researchers to prioritize candidate molecules before experimental testing, significantly reducing the cost and time required (47). For example, network pharmacology maps the interactions between drugs, targets, and disease pathways, revealing critical nodes (targets) for therapeutic intervention (48).

However, computational predictions must be validated experimentally to ensure their biological relevance. In vitro assays help confirm target binding and functional effects, whereas in vivo models assess the efficacy, toxicity, and pharmacokinetics. Without empirical validation, computational models may overlook off-target effects and misinterpret dynamic biological responses. Thus, a cyclical workflow in which computational

predictions inform experiments and experimental data refine computational models is increasingly regarded as the gold standard in modern drug discovery (49,50).

Thus, the integration of computational and experimental strategies represents a powerful complementary approach for the discovery and development of multi-target therapeutics for neurodegenerative diseases. This aligns with the principles of precision medicine and systems pharmacology, which aim to address disease complexity through a holistic, iterative framework.

9. COMPUTATIONAL APPROACHES IN POLYPHARMACOLOGICAL DRUG DISCOVERY

The rapid advancement of computational tools has transformed early phase drug discovery, particularly in polypharmacology for complex disorders such as neurodegenerative diseases. These approaches allow researchers to explore vast chemical and biological datasets, predict drug-target interactions, and prioritize candidates for experimental validation, thus significantly reducing both the cost and time.

9.1 Molecular Docking

Molecular docking is a structure-based computational technique used to predict the preferred orientation of a small molecule (ligand) when bound to a specific target protein and to estimate the strength of the interaction using scoring function. Docking plays a central role in virtual screening workflows and early phase drug discovery by enabling the rapid assessment of large chemical libraries against known or putative biological targets of interest.

In the context of polypharmacology, molecular docking facilitates the identification of compounds capable of interacting with multiple targets, an approach particularly valuable for the treatment of complex diseases such as Alzheimer's and Parkinson's diseases. Docking simulations allow for the comparative evaluation of binding affinities and modes across structurally diverse proteins, supporting the design of multi-target ligands.

Docking involves two principal components: pose prediction and scoring. Pose prediction generates multiple potential binding conformations of a ligand within the active site of the target, whereas scoring functions rank these poses based on the estimated binding energies derived from factors such as hydrogen bonding, hydrophobic interactions, electrostatics, and desolvation effects. Despite their utility, scoring functions often have limitations in accurately ranking ligands, particularly in multi-target scenarios, and may benefit from further refinement using molecular dynamics simulations.

Modern docking programs increasingly incorporate receptor and ligand flexibility, which is critical when working with dynamic or conformationally adaptable targets, such as kinases, G-protein-coupled receptors (GPCRs), and enzymes implicated in neurodegenerative pathways. These flexible docking protocols enhance the reliability of predictions and provide more realistic models of drug-target interactions.

Overall, molecular docking serves as a foundational in silico tool in multi-target drug discovery, offering insights into binding mechanisms and supporting the prioritization of candidates for subsequent experimental validation (51,52).

9.2 Molecular Dynamics (MD) Simulations

Molecular Dynamics (MD) simulations are advanced computational techniques that enable the investigation of the time-resolved behavior of biological macromolecules and their interactions with potential drug candidates at atomic resolution. Unlike static molecular docking approaches, MD simulations offer a dynamic and physiologically relevant perspective by considering critical factors, such as protein conformational flexibility, solvent effects, and thermal fluctuations. This time-dependent insight is particularly valuable in the context of polypharmacology, as it allows for a detailed evaluation of how a single ligand interacts with multiple targets over extended simulation periods. By capturing these dynamic interactions, MD simulations contribute significantly to the understanding of the stability, binding mechanisms, and potential efficacy of multi-target drug candidates. MD simulations have become increasingly accessible owing to advances in software, hardware (notably, GPUs), and force field accuracy. These improvements now allow researchers, even those without a background in computational chemistry, to conduct meaningful simulations to evaluate drug-target interactions. After a ligand is docked to multiple targets, MD simulations can evaluate the stability of each drug-target complex over time and reveal the behavior of protein targets in physiological environments. Many disease-related proteins, such as amyloid-β or tau in Alzheimer's disease, are intrinsically disordered or adopt multiple conformations. MD helps capture these conformational ensembles, which can influence binding affinities and selectivity. Although docking provides an initial approximation of binding, it often

ignores solvent effects and protein flexibility. MD simulations refine these poses by allowing the system to relax and evolve under more realistic conditions, potentially identifying new binding modes and off-target interactions. MD simulations are particularly valuable for multi-target drug design, where small differences in binding modes across targets can have profound implications for drug efficacy and toxicity. In diseases such as Parkinson's or Alzheimer's, where therapeutic targets include enzymes (e.g., MAO-B), receptors (e.g., muscarinic receptors), and misfolded proteins (e.g., tau), MD simulations offer a nuanced view of how a single compound may act on all of them (53).

9.3 Pharmacophore modeling

Pharmacophore modeling is a ligand-based computational approach that identifies the essential molecular features required for optimal ligand–biological target interactions. These features typically include hydrogen bond donors and acceptors, hydrophobic centers, aromatic rings, and charged groups arranged in a specific three-dimensional (3D) spatial configuration.

As discussed by Schuster et al. (2006), pharmacophore models serve as abstract representations of the steric and electronic characteristics necessary for molecular recognition, allowing researchers to screen databases for new compounds with similar activity profiles, even when their chemical structures differ substantially. This approach is particularly useful when structural information on the target protein is limited or unavailable.

In polypharmacology, pharmacophore modeling is instrumental in identifying compounds that can bind to multiple targets. By generating shared or merged pharmacophore models, researchers can identify ligands that satisfy the pharmacophoric requirements of several proteins. This strategy has been employed to identify multi-target ligands for complex disorders, such as neurodegenerative diseases, where modulating more than one pathological pathway is often necessary for therapeutic efficacy (54).

Pharmacophore models can be derived from known active ligands (ligand-based pharmacophore modeling), protein-ligand complexes (structure-based pharmacophore modeling), or a combination of both for improved accuracy of the model. Virtual screening using pharmacophore models allows the rapid identification of hit compounds with the desired features. These hits can be further refined through molecular docking and validated using molecular dynamics simulation. Overall, pharmacophore modeling provides a conceptually intuitive yet computationally powerful tool for multi-target drug design, enabling the discovery of structurally diverse compounds with similar functional activities across multiple disease-relevant targets (55).

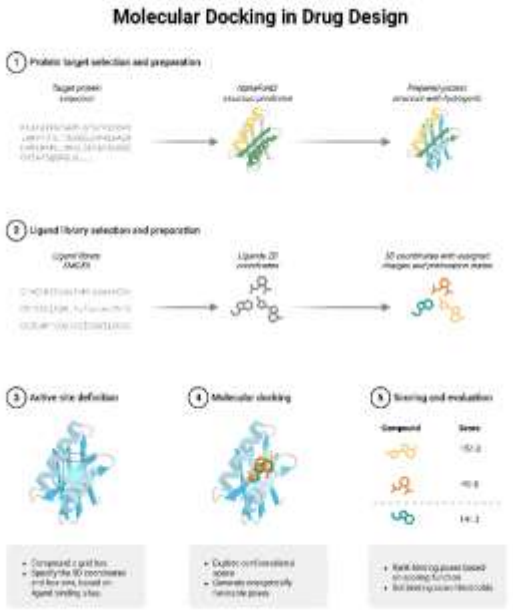


Figure 14: Applications of pharmacophore modelling

9.4 Quantitative Structure–Activity Relationship (QSAR) Modeling

Quantitative Structure–Activity Relationship (QSAR) modeling is a ligand-based computational approach that establishes mathematical correlations between the structural or physicochemical properties of chemical compounds and their biological activities. It relies on the assumption that molecules with similar structures

exhibit similar biological activities. These models are built using molecular descriptors such as hydrophobicity (e.g., logP), electronic characteristics, topological indices, and steric factors (56). These descriptors are statistically analyzed using methods such as multiple linear regression (MLR), partial least squares (PLS), support vector machines (SVM), or random forest algorithms to develop predictive models that can estimate biological activity from chemical structure (57).

QSAR modeling is critical in polypharmacology, particularly in the identification and optimization of compounds that can modulate multiple targets. In complex diseases such as Alzheimer's and Parkinson's diseases, multi-target drugs are often more effective than single-target agents. For instance, dual inhibitors targeting acetylcholinesterase (AChE) and monoamine oxidase B (MAO-B) have shown therapeutic potential. QSAR models can aid in the design of such compounds by predicting their activity profiles against both targets, thereby accelerating the discovery of multi-target-directed ligands (MTDLs) (58,59).

One of the key advantages of QSAR models is their cost-effectiveness and efficiency in early phase drug discovery. They enable the virtual screening of large chemical libraries, significantly reducing the number of compounds that must be synthesized and tested (56). Furthermore, QSAR models can provide mechanistic insights by identifying the molecular features that most strongly influence biological activity, thereby guiding rational drug design.

However, QSAR modeling has its limitations. The accuracy of the predictions depends heavily on the quality, size, and diversity of the training data set. Furthermore, traditional QSAR models often lack the ability to capture the three-dimensional and dynamic features of molecular interactions. To overcome this, advanced 3D-QSAR methods, such as Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA), have been developed to incorporate spatial and steric properties (60,61).

9.5 Network Pharmacology

Network pharmacology is an emerging discipline that integrates systems biology, bioinformatics, and pharmacology to understand the complex interactions between drugs, their targets, and the pathways involved in the development of diseases. Unlike traditional "one drug–one target" approaches, network pharmacology embraces the multi-target nature of most drugs, particularly in complex diseases such as Alzheimer's and Parkinson's diseases, in which multiple dysregulated pathways contribute to pathology (46).

At its core, network pharmacology models the biological system as a complex network of nodes (proteins, genes, and drugs) and edges (interactions), enabling the study of drug–target–pathway–disease relationships. Using databases such as DrugBank, STRING, STITCH, and DisGeNET, researchers can identify known and predicted interactions and construct drug-target-pathway networks to pinpoint critical nodes (or hubs) that may serve as polypharmacological intervention points (62).

Network pharmacology has been instrumental in revealing how existing drugs can modulate multiple targets in neuroinflammation, oxidative stress, and protein aggregation in the context of neurodegenerative diseases. It identifies the synergistic effects of multi-target therapies (e.g., donepezil's dual action on acetylcholine and amyloid pathways) and facilitates drug repurposing by mapping approved drugs onto neurodegenerative disease networks to discover novel indications for their use. Furthermore, network pharmacology integrates omics data (transcriptomics, proteomics, and metabolomics) to contextualize drug effects at the system level, offering insights into the mechanisms of action and potential side effects of drugs (63). This systems-based approach supports a more holistic and personalized drug discovery model, which is critical for diseases with multifactorial etiologies, such as Alzheimer's disease (64).

Modern computational tools such as Cytoscape, Gephi, and R packages allow for the visualization and analysis of biological networks. These platforms help identify key regulatory hubs, feedback loops, and bottlenecks that are often overlooked in reductionist approaches. The integration of machine learning further enhances the predictive power of these complex datasets (65).

9.6 Artificial Intelligence (AI) and Machine Learning (ML)

Artificial Intelligence (AI) and Machine Learning (ML) are revolutionizing drug discovery by enabling data-driven predictions of drug–target interactions, biological activities, and disease mechanisms. These approaches are particularly valuable in polypharmacology, where understanding the complex interactions among drugs, multiple targets, and biological networks is crucial. In neurodegenerative diseases such as

Alzheimer's and Parkinson's diseases, AI/ML tools facilitate the identification and optimization of multi-target-directed ligands (MTDLs) and the repurposing of existing drugs (66).

ML algorithms, including support vector machines (SVM), random forests (RF), k-nearest neighbors (k-NN), gradient boosting machines, and deep learning models such as convolutional neural networks (CNNs) and recurrent neural networks (RNNs), are trained on large datasets of chemical structures, gene expression profiles, and known drug–target interactions. These models learn patterns and generate predictive insights into new compounds, such as their binding affinity to multiple targets, potential side effects, and efficacy across pathways (67).

For example, deep learning-based models have shown exceptional performance in predicting drug–target interactions and off-target effects with higher accuracy than traditional QSAR or docking approaches. This is especially useful in neurodegenerative drug discovery, where ML models can identify compounds capable of modulating multiple protein targets involved in amyloid formation, oxidative stress, neuroinflammation, and tau pathology (68).

AI has also been integrated with network pharmacology and multi-omics data to enable system-level modeling of diseases. For instance, AI-driven tools can prioritize key regulatory genes or hub proteins in Alzheimer's disease using transcriptomic and proteomic data, thereby guiding the discovery of multi-target drugs. Similarly, ML models can assist in the virtual screening of compound libraries, thereby reducing the time and cost of early phase drug discovery processes. AI's ability to improve drug development methodologies has been demonstrated through its use in virtual screening and careful creation of medications. Researchers can precisely evaluate medication options by identifying and classifying target cells using AI's computing power of AI. This computational efficiency demonstrates AI's revolutionary potential of AI to improve healthcare outcomes globally and extends to polypharmacology, chemical synthesis, and drug repurposing. The variety of techniques available for drug development is highlighted by cutting-edge AI systems and software, such as IBM Watson, DeepChem, DeepTox, ORGANIC, and Hit Dexter. Despite its promise, the application of AI/ML faces challenges, including data quality, interpretability of black-box models, and the need for robust validation. Efforts are ongoing to develop explainable AI (XAI) frameworks that can provide mechanistic understanding along with predictions (69).

10. EXPERIMENTAL APPROACHES FOR VALIDATING COMPUTATIONAL PREDICTIONS

Translating computational predictions into biologically relevant outcomes requires robust experimental validation using a combination of in vitro, in vivo, and omics-based approaches. These methods not only confirm the bioactivity of the predicted compounds but also elucidate their mechanisms of action and safety profiles in complex biological systems.

10.1 In Vitro Approaches in Polypharmacology Research

In vitro assays are essential for initial screening and providing mechanistic insights. Cell-based models, such as SH-SY5Y human neuroblastoma cells and iPSC-derived neurons, simulate key neurodegenerative features, including oxidative stress, protein aggregation, and mitochondrial dysfunction (70,71). Enzyme inhibition assays assess the interaction of candidate compounds with targets such as acetylcholinesterase (AChE), monoamine oxidase B (MAO-B), and beta-secretase (BACE1), providing functional validation of binding predictions (72).

10.2 In Vivo Preclinical Models

In vivo studies offer systemic insights into the efficacy, pharmacokinetics, and long-term safety of these treatments. Transgenic rodent models, such as 5xFAD and APP/PS1 for Alzheimer's disease, MPTP-treated mice for Parkinson's disease, and SOD1-G93A for ALS, mimic disease-specific pathologies and enable behavioral, biochemical, and histological evaluations (73,74). Behavioral assays, such as the Morris water maze, rotarod, and Y-maze, assess cognitive and motor functions, while immunohistochemistry and brain tissue analyses validate neuroprotective effects at the cellular level (75,76).

10.3 Omics-Based Validation

Omics technologies, including transcriptomics, proteomics, and metabolomics, provide a system-level perspective of drug action. RNA sequencing (RNA-seq) and mass spectrometry-based proteomics revealed gene and protein expression changes in response to treatment, validating the engagement of the

computationally predicted pathways. Multi-omics integration enables deeper insights into the polypharmacological and potential off-target effects of candidate drugs (77,78).

10.4 Translational Relevance and Integration

Together, these experimental strategies establish a critical link between computational drug discovery and translational neurotherapeutic research. By corroborating computational predictions with experimental evidence, researchers can confidently advance multi-target drug candidates for clinical development (79). This integrative approach enhances the success rate and reliability of therapeutics designed for complex neurodegenerative conditions (80).

11. CHALLENGES AND LIMITATIONS IN POLYPHARMACOLOGICAL DRUG DEVELOPMENT

Despite its promise, the integration of computational and experimental strategies in polypharmacology faces several scientific, technical, and translational challenges that must be addressed. These limitations underscore the need for cautious interpretation of the results and continuous refinement of methodologies to enhance the efficiency and reliability of drug discovery.

Polypharmacology aims to target multiple disease pathways simultaneously; however, this strategy introduces significant complexity. Multi-target drugs can enhance efficacy but also increase the risk of unintended interactions that may lead to adverse effects in patients. Designing molecules with balanced potency across different targets while maintaining optimal absorption, distribution, metabolism, and excretion (ADME) profiles remains a significant challenge.

Computational tools, such as molecular docking, pharmacophore modeling, and machine learning, have revolutionized early stage drug discovery processes. However, these models often lack the biological complexities of living systems. For example, molecular docking assumes a mostly rigid target structure and may not fully capture protein conformational flexibility, solvent effects, or allosteric modulation, which are crucial factors in real-world biological interactions (52,81).

The success of machine learning and AI approaches is significantly influenced by the quality and diversity of training datasets. Incomplete or biased datasets can lead to overfitting, false positives, and poor model performance on unseen data. Moreover, target prediction algorithms may not account for pathway-level redundancies, cross-talk, or compensatory mechanisms that occur in complex diseases such as Alzheimer's and Parkinson's (82).

Although invaluable for high-throughput screening and mechanistic insights, in vitro systems often fail to capture the systemic, metabolic, and immune responses observed in whole organisms. For instance, a compound exhibiting neuroprotective activity in SH-SY5Y cells or primary neurons may not be effective in animal models because of poor bioavailability, metabolic instability, or off-target toxicity (83).

Additionally, blood–brain barrier (BBB) permeability, pharmacokinetics, and drug-drug interactions are rarely assessed in vitro, limiting the translational power of these results. Even successful in vivo results in rodent models may not translate to human patients because of species differences in drug metabolism, immune response, and disease pathology (84).

A major challenge in polypharmacology research is the lack of standardization in computational and experimental workflows. Variability in docking algorithms, scoring functions, in vitro assay conditions, and animal model usage makes it difficult to compare results across studies and hinders reproducibility.

Furthermore, effectively merging multi-omics data with computational forecasts necessitates the establishment of strong bioinformatics systems, compatible databases, and uniform reporting standards; without these, the full potential of systems-level drug discovery remains untapped (85).

12. FUTURE DIRECTIONS IN POLYPHARMACOLOGICAL DRUG DISCOVERY

The future of polypharmacological drug discovery for neurodegenerative diseases lies in the integration of advanced computational frameworks, system-level modeling, and translationally relevant validation strategies. Hybrid approaches that combine artificial intelligence (AI) with systems biology are expected to significantly enhance target prediction and drug optimization by enabling a holistic understanding of disease networks. Graph neural networks and deep learning models trained on curated protein–protein interaction datasets are increasingly capable of identifying compounds that modulate entire pathological pathways rather than isolated targets, while minimizing off-target effects (86). Furthermore, the emergence of end-to-end drug discovery

pipelines, encompassing virtual screening, ADMET profiling, and automated high-throughput screening, facilitates iterative hit-to-lead optimization in a time- and cost-efficient manner (67). As multi-omics data from CRISPR screens, single-cell analyses, and patient-derived models become more accessible, the development of closed-loop systems that continuously refine computational predictions based on experimental feedback will be essential (87). Additionally, human-relevant preclinical models, such as brain organoids and organ-on-chip platforms, are poised to improve the predictive accuracy of therapeutic responses, bridging the gap between in silico design and clinical application (80). Collectively, these innovations represent a transformative shift towards more precise, effective, and mechanism-informed therapies for treating neurodegenerative disorders.

13. CONCLUSION

The multifactorial nature of neurodegenerative diseases necessitates a shift from traditional single-target drug design to polypharmacological strategies that can modulate the interconnected pathological pathways. The integration of computational tools such as molecular docking, pharmacophore modeling, QSAR, and artificial intelligence with experimental validation platforms has significantly accelerated the identification and optimization of multi-target therapeutics. In vitro and in vivo models combined with high-throughput omics technologies provide critical validation of the predicted targets and mechanisms. Despite these advances, challenges remain, including off-target effects, species-specific differences in experimental models, and limitations in current computational accuracy. Moving forward, the development of hybrid platforms that combine AI with systems biology, along with iterative feedback from experimental data, will be essential for enhancing the predictive reliability and translational success. By incorporating interdisciplinary feedback, this framework has the potential to transform the drug discovery landscape for neurodegenerative diseases, paving the way for treatments that are not only more effective but also capable of altering the course of these diseases.

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