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Review Article

Review on Multitarget Drug Design Based on Computational Strategies for the Treatment of Alzheimer's Disease

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ABSTRACT

The multi-target drugs have recently attracted much attention as promising tools due to their advantages in the treatment of complex diseases and health conditions linked to drug resistance issues. The pharmaceutical industry is increasingly linking cheminformatics in the search for the development of novel drugs to be used in the treatment of diseases. These computational studies have the benefit of being less expensive and optimize the study time. Amongst the techniques used is the development of multitarget directed ligands (MTDLs), which has become an ascending technique, mainly due to the improvement in the quality of treatment including several drugs. Multitarget therapy is more effective than traditional drug therapy that emphasizes maximum selectivity for a single target. In this review a multitarget drug study was presented as a promising strategy for the treatment of Alzheimer's disease. It is aimed at collecting the latest and most interesting target combinations which have been reported in recent years for AD therapy. Computer-Aided Drug Design (CADD) techniques is discussed as a tool in the projection of multitarget compounds against this disease.

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INTRODUCTION

As stated by Richard Morphy, the multitarget drugs are defined as compounds that are designed to modulate multiple targets of relevance to a disease, with the overall objective of increasing efficacy and/ or improving safety (1).

The multitarget drug is an important key that can open many locks. Study into a multifunctional compound can follow two main paths: comprehensive experimental analyses, or by first using computer aided rational drug design; after that screening and identifying potential targets, optimizing the research, and avoiding higher expenses(2), (3). Moreover, multitarget therapy is more effective than traditional drug therapy, which emphasizes maximum selectivity for a single target. Through interacting with different targets, complex diseases of multifactorial origin are cured with superior effectiveness and in less time. Requiring smaller doses for simultaneous targets. Furthermore, multitarget therapies have lower toxicity and reduced side effects(1).

On the other hand, despite the therapeutic potential of multitarget compounds, there are challenges in terms of their discovery and development. Such as the difficulty in selecting the accurate combination of targets involved in the diseases of interest, requiring the understanding of target-disease action mechanism relationships and adverse effects profile, along with creating similar levels of action intensity for each target and design the

molecules to avoid interactions with undesirable targets. Moreover, it should be noted that only one part of the molecule can interact with each of the suggested targets, and the other part may become an obstacle to the binding event, reducing binding action as a result of enteric and entropy based approaches. Thus, to include some degree of flexibility in the molecule that can assist the pharmacological characteristics, however, an additional flexibility might interfere negatively with the binding affinity (because of the unfavorable entropic loss associated with the binding event) or the bioavailability of the drug (4). These critical issues extremely reflected in the design of these compounds (5),(6).

In recent years, the term "one-target-one-disease" has been growing during the discovery of new drugs mainly due to the complexity of some diseases(7). Another term is "cocktail therapy" which is based on the combination of numerous drugs in clinical practice. These drugs can act on several targets and may have synergistic effects in treatment. On the other hand, these effects may be hindered by several disadvantages, which involve the pharmacokinetics of these compounds, the number of side effects that each drug can cause, and the possible interactions between the drugs administered(8), (9).

As a result a new approach has emerged termed multitarget-directed ligands (MTDLs) that is being used as a technique to overcome unwanted clinical effects. This technique is based on the principle "one-compound multiple-targets" that is, the use of one compound that can interact with more than one target, assuring superior effectiveness and rarer side effects(10). Computer-aided drug design (CADD), *in silico* methods were developed to facilitate the screening of new compounds with multitarget characteristics. Many studies have been reported in the literature addressing these methods targeting multiple targets (11). Recently, many such techniques are key tools in multi-target drug discovery (MTDD) to find drug leads that at the same time interact with multiple target agents. These methods are classified into fragment-based and combinatorial approaches. Conventionally, for each individual target and to find virtual hits, combinatorial approaches perform parallel searches to determine which hit interacts with more than one target. Because of such inquires, new drugs for targets to use for one disease and/or to decrease the resistance, have been found (12). Design and development of a single chemical entity that acts simultaneously at multiple molecular targets is gaining major consideration in drug discovery. Alternatively, several multi-target molecular entities are currently emerging from the drug discovery programs, and some of these agents are now in clinical use for the management of various hematologic malignancies and solid tumors(13). FDA approved multikinase inhibitors for use in cancer therapy as Vandetanib, Regorafenib, Ponatinib, Crizotinib, Cabozantinibin cited in review of Gentile et al. Some other examples as Lapatinib (Tykerb) is a reversible, ATP-competitive inhibitor of the HER2 and EGFR tyrosine kinases (14), and Duvelisib, a novel oral dual inhibitor of PI3K-δ, γ, is clinically active in advanced hematologic malignancies (15). This review discusses studies that used *in silico* tools to discover new compounds that have potential multitarget activity for Alzheimer disease.

Alzheimer disease

AD is a complex multifactorial disease with diverse clinical symptoms. It causes the gradual onset of multiple cognitive deficits, affecting language, episodic memory and attention(16). At present, there are 17 million AD patients worldwide, and it is expected that this number will reach 70 million by 2050 if an efficient therapy is not developed(17). The etiopathology of Alzheimer's disease is extremely complex and heterogeneous(18). AD is related to increased levels of the amyloid β peptide (Aβ) and the hyperphosphorylated tau protein, along with loss of neurons and synapses. Moreover, there is some evidence pointing to the role of oxidative stress, metal ion deregulation, inflammation and cell cycle regulatory failure in its pathogenesis(19). Treatments targeting only one of these AD-related subpathologies have not yet been successful in the search for a disease-modifying treatment. Thus, multi-target drugs (MTDs) aiming simultaneously at several subpathologies are expected to be

a better approach(20). Currently the term multitarget has been intensifying and solidifying studies in this area as a way to control the multiple targets responsible for the pathogenesis of the disease, known as Multi-Target Directed Ligands (MTDL)(21) . The MTDL concept has been exploited to design different ligands hitting different biological targets(22). Moreover, MTDLs that are designed to target different pathological cascades of AD are more likely to be efficient against multifactorial AD in comparison to MTDLs designed against a single pathway of AD progression(23).For example,

Triazinone derivatives that acts simultaneously on the enzymes BACE-1 and GSK-3B

Since a significant increases in the enzyme of beta-site amyloid precursor protein cleaving enzyme1 (BACE1) protein and activity have been observed in the AD brain(24) also, Glycogen synthase kinase 3 beta (GSK-3B) is responsible for the hyperphosphorylation of tau protein, an important component of NFTs, which confers it a key role in the pathogenesis of AD simultaneously(25). Therefore, modulating of these enzymes can be a promising strategy for the treatment of Alzheimer's disease. Triazinones (Figure-1) are considered as multitarget molecules that may represent a promising starting point in developing lead compounds for Alzheimer disease. In the study it was reported that the triazinones can modulate the enzymes BACE-1 and GSK-3B, with IC50 values ranging from 18–0.01 μM on BACE-1 and 14–0.78 μM on GSK-3B. In studying the interactions through the molecular docking study of the compounds with both enzymes, they found that the compounds, mainly compound 3, acquired dual-target profiles because they had strong interactions with both enzymes tested(26).

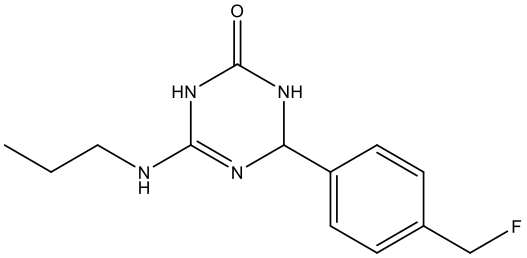

FIGURE 1 - Triazinone derivatives(27)

2-aminotiazole derivative that can act on PARP-1 enzymes and BACE-1

The increase of Poly [ADP-ribose] polymerase1 (PARP-1) activity and the accumulation of PAR were confirmed in the brain of patients with Alzheimer's disease (AD), mainly in neurons of the frontal and temporal lobes and in skin fibroblasts and lymphoblasts. Moreover, it has been reported that PARP-1 gene polymorphisms are highly associated with the development of AD(28). Considering the deregulation of these specific proteins in Alzheimer disease, Zhang et al. conducted studies *in silico* and found that derivatives 2-aminotiazoles (Figure-2) can act against the enzymes PARP-1 and BACE-1, with predictive activities that provided a basis for further progression and structural optimization of new effective inhibitors for the treatment of Alzheimer's disease(29).

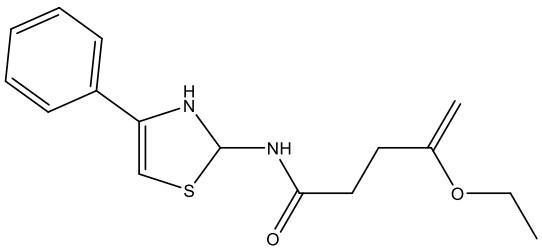

FIGURE 2 - Derivative 2-aminotiazole(27)

Hybrid with an indole moiety reported to act on the enzymes PLA2, LOX-5, COX-2, and cholinesterase A and B

Phospholipase A_2 (PLA_2), lipoxygenase-5 (LOX-5) and Cyclooxygenase-2 (COX-2) are some of the major enzymes involved in neuroinflammatory processes. Since NI is closely associated with AD, therefore, compounds that can inhibit cholinesterase enzymes in addition to enzymes related to inflammation will be better therapy for neurodegenerative disorders (30)

In a study, 67 hybrid molecules were designed with indole (Figure-3), and that by means of screening only 13 hybrids were selected that were submitted to molecular docking analysis of the enzymes phospholipases A2 (PLA2), 5-lipoxygenase (LOX-5), cyclooxygenase-2 (COX-2), and cholinesterase A and B . As a result, when calculating their interaction energies, they observed that 3 hybrid compounds had strong interactions with the study targets, with better energy values compared to standard reference drugs(30).

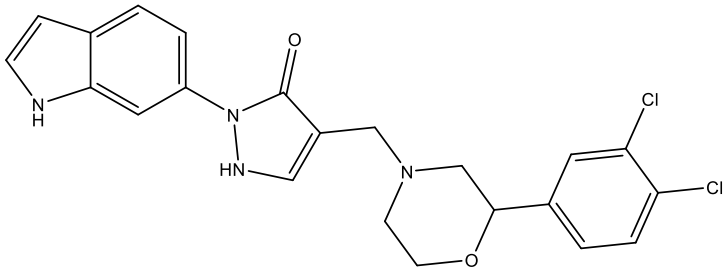

FIGURE 3 Hybrid with an indole moiety(27)

Tacrine-phenothiazine derivative with multitarget action against the enzymes AChE, tau, amyloid-A4 (ABPP), GSK-3α, GSK-3β, MAO-B, and PS-1

Tacrine is a potent inhibitor of cholinesterases (acetylcholinesterase and butyrylcholinesterase) that shows limiting clinical application because of its liver toxicity. In spite of this, analogues of tacrine are considered as a model inhibitor of cholinesterases in the therapy of Alzheimer's disease(31).

In studies of Hui et al., they performed tests *in silico* and in vitro on various tacrine-phenothiazine derivatives (Figure-4) in the discovery of multitarget action. Several tacrine-phenothiazine hybrids (T1–T26) were designed for inhibiting acetylcholinesterase and tau protein involved in Alzheimer's disease. In their study they reported inhibitory compounds, with relevance to the compound T5, of the enzyme AChE, besides action of hyperphosphorylation of the enzyme tau and aggregation of beta amyloid(32).

In another *in silico* study, Speck-Planche et al. analyzed 483 compounds from a database and performed multi-target QSAR analysis using an LDA model. In their study, the descriptors were computed, and a model was constructed for the prediction of inhibitors that could act on proteins amyloid-A4 (ABPP), GSK-3α, GSK-3β, monoamine oxidase –B (MAO-B), and presenilin 1 **(**PS-1), all associated with Alzheimer disease(27).

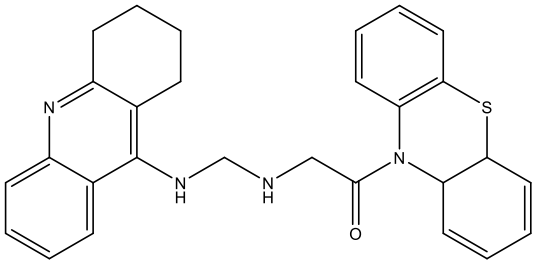

FIGURE 4 - Tacrine-phenothiazine derivative(27)

Phenylpyrazole derivative that can inhibit the enzymes AChE and MAO A and B

Activated monoamine oxidase-B MAO-B in the brains of patients with AD is a biomarker for AD. Studies have shown that activated MAO contributes to cognitive dysfunction, destroys cholinergic neurons, causes disorder of the cholinergic system and leads to the formation of amyloid plaques and NFTs. Thus, MAO inhibitors may be considered as promising therapeutic agents for AD(33).

In a study, a series of phenyl pyrazole derivatives (Figure-5) have been stated as inhibitors of acetylcholinesterase (AChE) and of (MAO-A and B), with selective activities in the micro and nanomolar range of 0.06 μM to AChE and 2.69 μM to MAO A and B (34)). In the same study, structure-activity relationships were analyzed, where they presented that the derivatives with the chlorine moiety were more effective as compared to fluoro derivatives while reverse trend was observed in MAO-B inhibitory activity, however the molecular modeling data did not present in agreement with the experimental study, from which the strongest interactions occurred with the acetylcholinesterase(34).

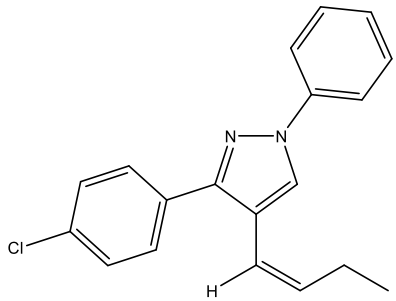

FIGURE 5 - Phenylpyrazole derivative(27)

Baicalin, which has been reported in the literature to inhibit NMDA, 5-lipoxygenase, COMT, and MAO-B enzymes

AD is the most frequent dementia accompanied with cognitive damages. Catechol-O-Methyltransferase (COMT) plays a significant role in cognition across different diagnostic entities, and therefore COMT can be considered as a target for many neuropsychiatric disorders including AD and other dementias, that are characterized with cognitive impairments(35).

Among the natural compounds described in the literature are baicalin (Figure-6), a flavonoid obtained from the plant Scutellaria baicalensis Georgi, which causes reduced activity of ONOO- and, in turn, neurotoxicity at a concentration of 10–50 mg/kg(36). For the same compound, studies of its activity in the inhibition of the calcium influx N-Methyl-D-aspartic acid or N-Methyl-D-aspartate (NMDA) and 5-lipoxigenase, which leads to a decrease in neuronal death, was found at a concentration of 1–5 μM (37). In the studies conducted by Gao et al., *in silico* analysis of molecular docking and structure-based pharmacophore was performed, searching for activity against COMT and MAO-B proteins. In this study it was realized that baicalin has the potential to modulate the activity of the two proteins mentioned and guaranteed a protective role in neurotoxicity, however the activity of baicalin against the NMDA enzyme did not show an inhibitory effect in a [3H] MK-801 binding study(38).

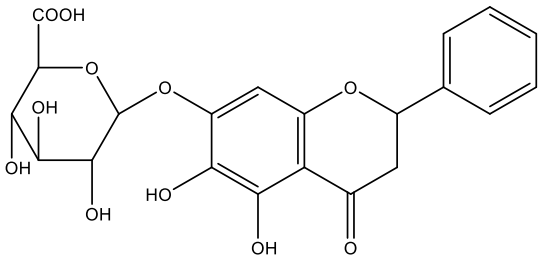

FIGURE 6 - Baicalin(39)

Huperzine A acts as a multitarget by inhibition of AChE, ROS, NF-ķB, and NMDA enzymes

Excessive generation of Reactive Oxygen Species (ROS) leads to several detrimental effects including DNA, lipid, and protein damage. ROS are, however, scavenged by defence mechanisms, known as enzymatic and nonenzymatic antioxidants. An imbalance in this oxidant-antioxidant status could determine the extent of cell injury. Oxidative damage due to ROS has been involved in the pathogenesis of neurodegenerative diseases, cancer, diabetes, and aging(40)

Huperzine A (HupA) (Figure-7), a novel alkaloid isolated from the Chinese herb Huperzia serrata, is a potent, highly specific and reversible inhibitor of acetylcholinesterase (AChE). Compared with tacrine, HupA has better penetration through the blood-brain barrier, higher oral bioavailability, and longer duration of AChE inhibitory action without promoting toxicity (41),(42). Molecular docking studies confirmed a higher specificity of interaction between AChE and huperzine A, of which the aromatic ring portion of the compound was responsible for the activity by binding to the aminophenol moiety of the enzyme, thus promoting its inhibition(43). Inhibitory activities have also been stated for ROS enzymes at 0.1 mg/kg (44), NF-ķB at 0.1 mg/kg (45), and NMDA 10 nM in calcium influx throughout neurotoxicity mechanisms (46).

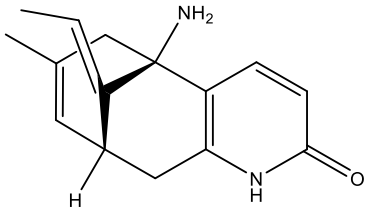

FIGURE 7 - Huperzine A(41)

Honokiol acts with multi-targeting activity by inhibiting the enzymes NF-ķB, NMDA, γ-secretase, STAT3, mTORC1 and mTORC2, and MEK1 and MEK2

Honokiol (Figure-8) is derived from Magnolia grandiflora and has been presented to be a compound with multi-target features acting in the blockade of the translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ķB) and by inhibiting NO production in glial cells at a concentration of 0.7–70 mg/kg(47). In addition to these targets, Cui et al. revealed the inhibition of NMDA mediated neurotoxicity(48). Honokiol has also been described to stimulate the expression of Apolipoprotein E (ApoE), ATP-binding cassette transporter (ABCA1), and ATP-binding cassette sub-family G member 1 (ABCG1) in cells(49),(49). In addition to these targets, it has also been stated to have activity against Aβ through the regulation of autophagy, reducing the phosphorylation of Protein kinase B Akt and mechanistic target of rapamycin (mTOR), and inhibiting γ-secretase through the negative regulation of the expression of the complex γ-secretase (49),(50). In studies by Saeed et al., *in silico* molecular docking studies were examined with the AKT kinase domain enzymes, Signal Transducer And Activator of Transcription 3 (STAT3) DNA binding domain, both mTOR complexes (mTORC1 and mTORC2), and

both homologous Mitogen-Activated Protein Kinase Kinase MAPKKs (MEK1 and MEK2). In the study, it was seen that the compound bound to the same binding sites as the standard drugs erlotinib and gefitinib(51). The two are anti-epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors approved for treatment of advanced non- small cell lung cancer NSCLC patients. They can restore memory deficits associated with Alzheimer's disease(52).

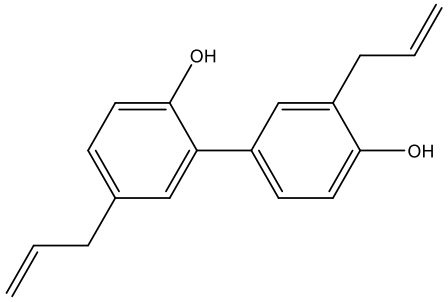

FIGURE 8 - Honokiol(53)

CONCLUSION

In this review, the importance of multitarget compounds in the treatment of the most complicated and progressive neurodegenerative diseases: Alzheimer disease was discussed. Several factors including amyloid-β (Aβ) deposits, oxidative stress, dyshomeostasis of biometals and low levels of acetylcholine (ACh) have been demonstrated to be associated with AD pathogenesis. Hence, targeting them requires an in-depth knowledge of their structural and their activities. Accordingly, new therapeutic developments that target these features of AD pathology and guarantee disease modification are currently under progress. In this review also, It is verified that the use of *in silico* methods in drug design emerges as an advantageous technique, since this study proposes better outcomes with lower expenses, as well as performs screening for more than one receptor that can interact with the drug, allowing the validation of a larger number of activities that can be tested latter. The multitarget treatments presented in this review to qualify the chemical structure in the discovery of new targets, therefore supporting the increase of activity against diseases. Based on this information, compounds with multi-targeting properties can be designed which may provide new perspectives for the pharmacological treatment of AD. The search for multi-targeting drugs will continue and is probably our last hope in coming up with an effective treatment to eradicate complex diseases.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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