Chapter

Multi-Target-Directed Ligands in Alzheimer's Disease Therapy

Eugenie Nepovimova and Kamil Kuca

Abstract

So far, the only clinically approved drugs that are effective in Alzheimer's disease (AD) are those neurotransmitters oriented in their mode of action and focus, in particular, on the functional significance of acetylcholine or glutamate in the brain. Current AD drugs can, therefore, reduce the severity of cognitive symptoms, improve the quality of life, and stabilize the symptoms for some years, but they are not able to significantly modify the course of the disease. Complex disorders such as neurodegenerative diseases tend to result from multiple molecular abnormalities, not from a single defect. Moreover, a single target is unlikely to help in such cases because the cells can often find ways to compensate for a protein whose activity is affected by a drug. Thus, these limitations of the conventional "one-target, one-molecule" paradigm have triggered a recent shift in efforts to create drugs that hit more than one target simultaneously. The term multi-target-directed ligands (MTDLs) have been proposed to describe these hybrid molecules that are effective in treating complex diseases. Within our contribution, we would like to present general overview of MTDL design strategy in AD therapy, its positives and negatives, and finally summary of such multipotent compounds evaluated in clinical trials.

Keywords: Alzheimer's disease, therapy, multi-target-directed ligands, drug design, ladostigil, ANAVEX 2-73

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with unknown etiology. Currently, no causal treatment is available, probably due to multiple factors involved in pathophysiology of the disease. Recently, it has become clear that "one-target, one-molecule" therapy is not effective to complex diseases with multifactorial pathogenesis. Thus, novel approach, called multi-target-directed ligand (MTDL) strategy, has been developed. Hybrid compounds resulting from this drug design strategy have to be capable to act at diverse biological targets simultaneously. Discovery and subsequent launch of such multipotent drug candidates on the pharmaceutical market would greatly facilitate and improve therapeutic strategies of Alzheimer's disease.

2. Drug design history leading to multi-target-directed ligand strategy

The "one-target, one-molecule" philosophy has resulted in many approved drugs and will likely continue to be the benchmark in the time to come [1]. This paradigm

has been driven by the notion that a single target's selective modulation can help create the needed extend of efficiency while simultaneously bringing down the risk of off-target side effects. On the other hand, current research has shown that the failure of such compounds is largely owed to poor safety and poor efficiency, observed in the last 10 years. It has therefore been put forward that the biology networks' intrinsic robustness and redundancy are the main culprits when it comes to highly selective drugs failing to ensure that the needed impact or result is present [2]. Furthermore, substances that focus on one target likely prove to be ineffective or insufficient when the treatment is being focused on complex illnesses, including diabetes mellitus, neurodegenerative disorders, cardiovascular diseases, and cancer that come laced with several pathogenic aspects [1].

After three decades, it seems that this approach is not effective in terms of possible success. Some of these substances only prove to be helpful to a specific set of the population [3]. When a "one-target, one-molecule" drug is not effective enough to address an illness, the next route involves several drugs administered together. Such approach is sometimes denoted as "cocktail of drugs," where multiple substances are mixed together to tackle the illness [1]. These mixtures typically contain two or more substances that come together to produce a more holistic impact [4]. This approach helps not only to increase the efficiency of the therapy but also to address and bring down the side effects – such a situation is not possible when only a single drug is being used to provide therapy. This positive situation has been observed within the treatment of several maladies, including hypertension, HIV, and cancer. However, the benefits of taking several drugs can become shaky if the patient does not comply with the regime properly. This is especially typical for situations when the illness is asymptomatic [2].

In recent times, multicomponent drugs have become more popular, where two agents or more are mixed into a single tablet, so that patient's compliance can be improved alongside the dosing schedules [2, 4]. Such combinations are called "fixed drug combinations" (FDCs). On the other hand, the problems that stem from highly complicated pharmacodynamics/pharmacokinetics necessitate formulations that have the right kind of sophistication due to the occurrence of possible drug-drug interactions, which could have a considerable impact on the costs and risks of designed FDCs [2].

Two independent scientific groups of Inestrosa and Brimijoin found out that the active site of enzyme acetylcholinesterase (AChE; E.C. 3.1.1.7) is close enough to its allosteric peripheral site and that these two sites can be spanned by one molecule at the same time. This discovery has launched rational design of novel class of therapeutic agents – dual-binding site acetylcholinesterase inhibitors (AChEIs) [5]. Inestrosa and Brimijoin in their studies demonstrated that AChE interacts through its allosteric site with amyloid peptide (Aβ) and acts thus like a pathological chaperone inducing a conformational change favoring Aβ aggregation [6, 7]. In this respect, ligands that can simultaneously interact with both sites could produce many merits comparing to active site inhibitors. Namely, such dual-binding site inhibitors considerably increase the inhibitory potential toward AChE, thus providing symptomatic relief, facilitating memory process, and, at the same time, exerting neuroprotective preventive effect [8]. Positive effects of dual-binding site inhibitors, AD's multifactorial aspect, and the routine of use of combination therapy in clinical practice prompted drug designers to pay more attention to development of more complex medicaments that in turn use dual-binding site inhibitors as an appropriate starting point [9].

Cancer, depression, neurodegenerative maladies, cardiovascular diseases, and other complex disorders typically result from several abnormalities at the molecular level, not because of a single issue. Moreover, modulation of one single target would

probably not show any significance in such cases since the cells will likely find routes through which the protein can be compensated after its activity is affected by the medicine. Thus, these limitations of the conventional "one-target, one-molecule" paradigm have induced a shift in pharmaceutical companies' research to develop therapeutics that can address more than one problem. Many research groups and pharma companies now look for compounds that can address multiple issues and are even attempting to develop the so-called promiscuous drugs [3, 10]. With this new drug design strategy, two or more compounds, binding with a very high selectivity to their respective targets, are used as the starting blocks, and their structural elements are combined into a single molecule to incorporate activity at both targets. Hence, this approach normally involves the use of two or more different pharmacophoric moieties (in most cases, at least one is directly related to AChEI being a pillar of standard AD therapy) to include into a single framework [4, 10]. The term multi-target-directed ligand has been proposed to describe these hybrid molecules that could be effective in treating complex diseases [1].

3. Advantages and disadvantages of MTDLs

The use of such promiscuous drugs may provide some advantages: (i) in terms of the disease, various pathways can be effectively targeted via a single multipotent molecule, thus increasing its efficiency; (ii) drugs of a promiscuous nature do not always overactivate or suppress a network or pathway; (iii) single molecular species, although consisting of several pharmacophores, show a complex ADMET profile; (iv) drug-drug interactions' risk should be reduced; and (v) the drug regimen of the patients taking MTDLs should be greatly simplified [1, 11]. However, beside the advantages, there are also several drawbacks. A key problem linked to the use of promiscuous drugs has to do with how hard it is to optimize potencies for two different targets while using one medicine. Taking into account all the advantages and disadvantages, therapy that uses one medicine with several biological activities will prove to be inherently better than FDCs or cocktails of drugs.

4. Classification of MTDLs

Depending on the extent to which the frameworks of selective pharmacophores have been integrated, three different classes of MTDLs can be distinguished (**Figure 1**). The first class is represented by linked MTDLs, whose molecular frameworks have not been integrated but have been connected via a specific linker not found in either of the starting selective pharmacophores. Sometimes, pharmacophores in linked MTDLs contain a metabolically cleavable linker, purposely designed to exude *in vivo* two ligands that can independently interact with related targets. Such scenario could be considered as a half-way between real MTDLs and FDCs. However, in most cases, the linker is designed to be metabolically stable, yielding a single compound capable of interacting with two targets simultaneously. In particular, dual-binding site inhibitors are the best representatives of linked subclass. Fused MTDLs constitute the second class. In fused MTDLs, the frameworks are linked directly. However, medicinal chemists generally aspire to maximize the degree of framework overlap in order to design as simplest and smallest molecules as possible with favorable physicochemical properties. Therefore, it would probably not be surprising that the most common and sought after are merged MTDLs, where the frameworks are integrated by the use of commonalities in the structures of the starting compounds [2].

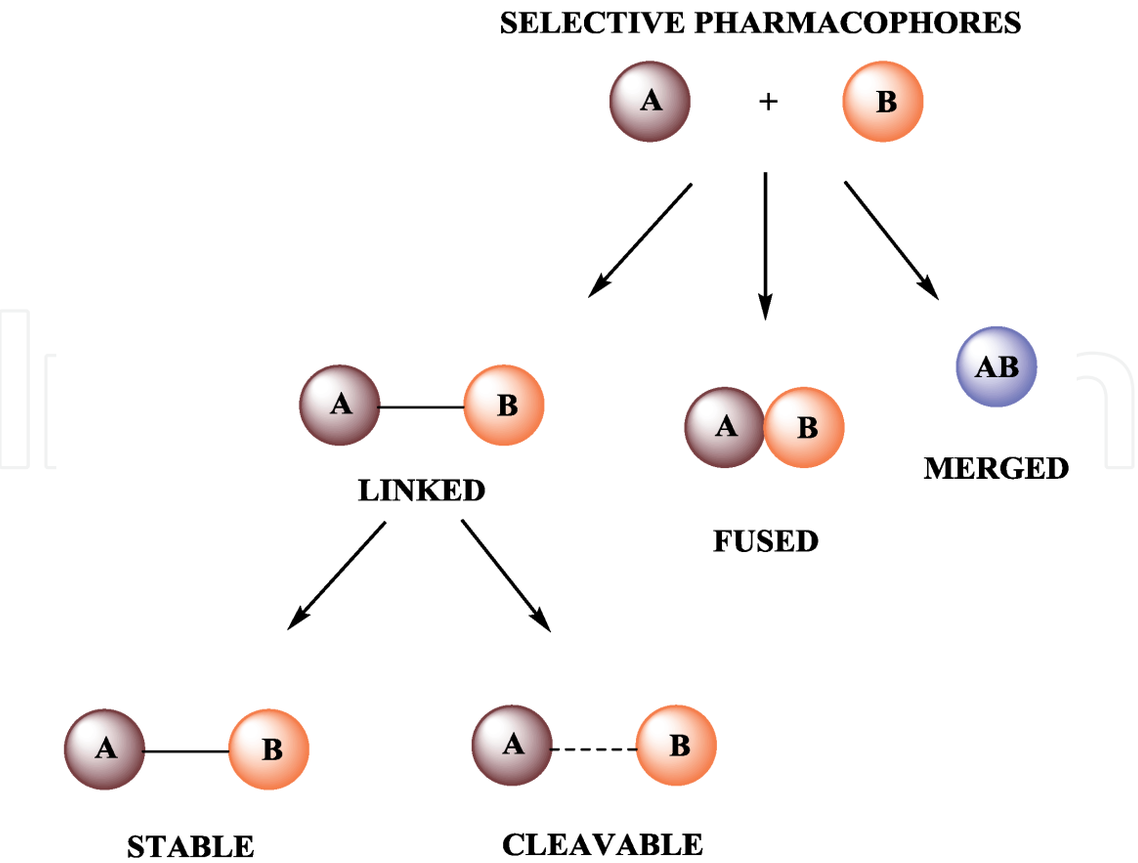

Figure 1.
Classification of MTDLs.

5. MTDLs in clinical trials

In Alzheimer's disease drug development pipeline for year 2019 issued annually by Alzheimer's and Dementia, there was no MTDL currently assessed in AD clinical trials [12]. The only drug candidate with multimodal action found within the mentioned list was ANAVEX 2-73. However, for completeness of the subchapter, we have decided to include also ladostigil as the only real representative of MTDLs ever evaluated in clinics.

Ladostigil (TV3326; **Figure 2**) is a dual cholinesterase (ChE) and brain-selective monoaminooxidase-A (MAO-A) and monoaminooxidase-B (MAO-B) inhibitor indicated for the treatment of dementia comorbid with extrapyramidal disorders and depression [13]. The design of this MTDL is based on the combination of carbamate rivastigmine and *N*-propargyl scaffold of anti-Parkinsonian drug and irreversible selective MAO-B inhibitor, rasagiline [14, 15].

Rasagiline is an irreversible inhibitor of MAO-B used as a monotherapy to treat symptoms of early Parkinson's disease (PD) or as an adjunct therapy in more advanced cases of PD [16]. Rivastigmine is a nonselective AChE and butyrylcholinesterase (BChE; E.C. 3.1.1.8) inhibitor [17]. It could be also classified as pseudo-irreversible

Figure 2.
Chemical structure of ladostigil.

ChE inhibitor since the duration of inhibition is longer than its elimination half-life [18]. It is indicated for the treatment of mild-to-moderate dementia associated with Alzheimer's disease type and PD [18]. Rivastigmine has also proven efficacy in decreasing psychiatric symptoms and cognitive deficits [19]. This fact together with the continued beneficial effect observed in rivastigmine-treated patients after drug withdrawal indicated disease-modifying effect [20].

In rodents, oral administration of ladostigil was shown to antagonize scopolamine-induced spatial memory impairments, pointing out that it is able to sufficiently penetrate the blood-brain barrier [21]. Apart from MAO and ChE inhibition, ladostigil has shown to possess a broad scale of neuroprotective activities against a variety of neurotoxins and neuronal cell culture models of neurodegeneration [20].

All these perspective preclinical results forwarded ladostigil to clinical evaluation. In 2011, Avraham Pharmaceuticals evaluated a 6-month trial of ladostigil in Phase II in 201 people with mild-to-moderate Alzheimer's disease. However, this trial missed its primary endpoint on the ADAS-cog11, and thus, development for Alzheimer's disease was terminated [22, 23]. In January 2012, the same company started the second Phase II study, in this case evaluating a lower dose of ladostigil for its ability to delay progression from mild cognitive impairment (MCI) to AD. This study enrolled 210 people with a clinical diagnosis of MCI. In September 2016, the company disclosed that ladostigil missed its primary endpoint in this trial as well [22, 24].

ANAVEX 2-73 (blarcamesine; **Figure 3**) is an experimental drug in Phase II clinical trial for Alzheimer's disease, Phase I for epilepsy, and preclinical trials for amyotrophic lateral sclerosis, Parkinson's disease, Rett syndrome, and stroke [25, 26]. From the pharmacological point of view, this small molecule acts as a muscarinic receptor agonist and activator of sigma-1 receptors.

Within preclinical trials, ANAVEX 2-73 alleviated scopolamine- and dizocilpine-induced learning impairments, pointing out to its antimuscarinic and neuroprotective effect mediated by NMDA receptors [27, 28]. The sigma-1 receptors are small transmembrane stress-reducing survival proteins, mainly located on the endoplasmic reticulum membrane of cells. Moreover, these receptors are known to modulate cellular processes relevant to neurodegeneration. In particular, ANAVEX 2-73 is thought to help to restore cellular balance by targeting protein misfolding, oxidative stress, mitochondrial dysfunction, inflammation, and cellular stress [29]. More recently, the effect of ANAVEX 2-73 on the main hallmarks, that is, $A\beta_{1–42}$ seeding and tau hyperphosphorylation, of Alzheimer's disease has been studied. The results of such experiment revealed that ANAVEX 2-73 significantly blocked an increase in $A\beta_{1–42}$ levels in hippocampus, suggesting that it may alleviate amyloid load in AD model. In addition, the data presented within the same study suggested that modulation of both receptors, that is, muscarinic and sigma-1, targets GSK-3β activity and that inhibiting of this kinase efficiently decreases tau hyperphosphorylation and Aβ accumulation in AD model [25].

Phase I clinical trial, assessing safety and pharmacokinetics, with ANAVEX 2-73 was successfully completed in healthy volunteers in Germany. The maximum

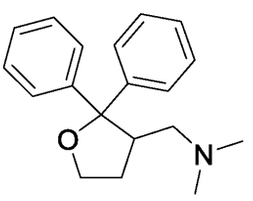

Figure 3.
Chemical structure of ANAVEX 2-73.

tolerated dose in men was determined to be 55 mg. The results of Phase II clinical trial on patients with mild-to-moderate Alzheimer's disease showed a significant association between the dosage of ANAVEX2-73 and the cognitive and function improvements [29].

6. Conclusion

While AChEI itself is an ever evolving branch of AD research, the rationale for MTDL design strategy clearly stems from the AD's multifactorial etiological basis. In a meanwhile, novel therapeutic targets continually emerge. Optimization of the therapeutic potential of dual-binding site AChEIs by adding biological activities, such as one from the arsenal against neurodegeneration, is an ongoing process for medicinal chemists. Several approaches are being deployed to design MTDLs; however, all of them use the combination of different smaller fragments of a given specific activity in a single molecule. Future work on such design strategy will involve fine tuning of pharmacokinetic and activity profiles of novel drug candidates for the purpose of modulating the selected molecular targets at the similar levels. Additionally, more clinical trials are required to prove the MTDL concept. The way ahead is not a short one; however, it is extremely possible that MTDLs could become the future treatment against AD and other similar complex multifactorial diseases, including infectious disorders, cancer, cardiovascular maladies, and so on.

Acknowledgements

This work was supported by the University of Hradec Kralove (Faculty of Science, VT2019-2021).

Conflict of interest

The authors declare no conflict of interest.

Author details

Eugenie Nepovimova and Kamil Kuca*
Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove, Czech Republic

*Address all correspondence to: kamil.kuca@uhk.cz

References

[1] Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, et al. Multi-target-directed ligands to combat neurodegenerative diseases. Journal of Medicinal Chemistry. 2008;**51**(3):347-372

[2] Morphy R, Rankovic Z. Designing multiple ligands—Medicinal chemistry strategies and challenges. Current Pharmaceutical Design. 2009;**15**(6):587-600

[3] Frantz S. Drug discovery: Playing dirty. Nature. 2005;**437**(7061):942-943

[4] Rampa A, Belluti F, Gobbi S, Bisi A. Hybrid-based multi-target ligands for the treatment of Alzheimer's disease. Current Topics in Medicinal Chemistry. 2011;**11**(22):2716-2730

[5] Muñoz-Torrero D. Acetyl-cholinesterase inhibitors as disease-modifying therapies for Alzheimer's disease. Current Medicinal Chemistry. 2008;**15**(24):2433-2455

[6] Inestrosa NC, Alvarez A, Pérez CA, Moreno RD, Vicente M, Linker C, et al. Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: Possible role of the peripheral site of the enzyme. Neuron. 1996;**16**(4):881-891

[7] Rees T, Hammond PI, Soreq H, YounkinS, BrimijoinS. Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex. Neurobiology of Aging. 2003;**24**(6):777-787

[8] Castro A, Martinez A. Peripheral and dual binding site acetylcholinesterase inhibitors: Implications in treatment of Alzheimer's disease. Mini Reviews in Medicinal Chemistry. 2001;**1**(3):267-272

[9] Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Melchiorre C. From dual binding site acetylcholinesterase inhibitors to multi-target-directed ligands (MTDLs): A step forward in the treatment of Alzheimer's disease. Mini Reviews in Medicinal Chemistry. 2008;**8**(10):960-967

[10] Bolognesi ML, Rosini M, Andrisano V, Bartolini M, Minarini A, Tumiatti V, et al. MTDL design strategy in the context of Alzheimer's disease: From lipocrine to memoquin and beyond. Current Pharmaceutical Design. 2009;**15**(6):601-613

[11] Espinoza-Fonseca LM. The benefits of the multi-target approach in drug design and discovery. Bioorganic & Medicinal Chemistry. 2006;**14**(4):896-897

[12] Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline. Alzheimers & Dementia (NY). 2019;**5**: 272-293

[13] Weinreb O, Amit T, Bar-Am O, Youdim MBH. Ladostigil: A novel multimodal neuroprotective drug with cholinesterase and brain-selective monoamine oxidase inhibitory activities for Alzheimer's disease treatment. Current Drug Targets. 2012;**13**(4):483-494

[14] Weinstock M, Bejar C, Wang RH, Poltyrev T, Gross A, Finberg JP, et al. TV3326, a novel neuroprotective drug with cholinesterase and monoamine oxidase inhibitory activities for the treatment of Alzheimer's disease. Journal of Neural Transmission. Supplementum. 2000;**60**:157-169

[15] Korábečný J, Nepovimová E, Cikánková T, Špilovská K, Vašková L, Mezeiová E, et al. Newly developed drugs for Alzheimer's disease in relation to energy metabolism, cholinergic and monoaminergic neurotransmission. Neuroscience. 2018;**370**:191-206

[16] Gallagher DA, Schrag A. Impact of newer pharmacological treatments on quality of life in patients with Parkinson's disease. CNS Drugs. 2008;**22**(7):563-586

[17] Zemek F, Drtinova L, Nepovimova E, Sepsova V, Korabecny J, Klimes J, et al. Outcomes of Alzheimer's disease therapy with acetylcholinesterase inhibitors and memantine. Expert Opinion on Drug Safety. 2014;**13**(6): 759-774

[18] Dhillon S. Rivastigmine transdermal patch: A review of its use in the management of dementia of the Alzheimer's type. Drugs. 2011;**71**(9):1209-1231

[19] Farlow MR. Update on rivastigmine. The Neurologist. 2003;**9**(5):230-234

[20] Weinreb O, Amit T, Bar-Am O, Youdim MBH. A novel anti-Alzheimer's disease drug, ladostigil neuroprotective, multimodal brain-selective monoamine oxidase and cholinesterase inhibitor. International Review of Neurobiology. 2011;**100**:191-215

[21] Weinstock M, Gorodetsky E, Poltyrev T, Gross A, Sagi Y, Youdim M. A novel cholinesterase and brain-selective monoamine oxidase inhibitor for the treatment of dementia comorbid with depression and Parkinson's disease. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2003;**27**(4):555-561

[22] Ladostigil [Internet]. 2017. Dostupné z: http://www.alzforum.org/therapeutics/ladostigil

[23] Avraham closes its AD program and concentrates on the development of ladostigil to MCI, due to failure of its clinical trial [Internet]. 2017. Dostupné z: http://www.avphar.com/closing-ad-program/

[24] Clinical Data [Internet]. 2017. Dostupné z: http://www.avphar.com/ladostigil/clinical-data/

[25] Villard V, Espallergues J, Keller E, Vamvakides A, Maurice T. Anti-amnesic and neuroprotective potentials of the mixed muscarinic receptor/sigma 1 (σ1) ligand ANAVEX2-73, a novel aminotetrahydrofuran derivative. Journal of Psychopharmacology Oxford England. 2011;**25**(8):1101-1117

[26] Blarcamesine. Anavex Life Sciences. AdisInsight [Internet]. Dostupné z: https://adisinsight.springer.com/drugs/800033840

[27] Maurice T, Hiramatsu M, Itoh J, Kameyama T, Hasegawa T, Nabeshima T. Behavioral evidence for a modulating role of sigma ligands in memory processes. I. Attenuation of dizocilpine (MK-801)-induced amnesia. Brain Research. 1994;**647**(1):44-56

[28] Senda T, Matsuno K, Kobayashi T, Mita S. Reduction of the scopolamine-induced impairment of passive-avoidance performance by sigma receptor agonist in mice. Physiology & Behavior. 1997;**61**(2):257-264

[29] PhD EM. Anavex 2-73 [Internet]. Alzheimer's News Today. 2017. Dostupné z: https://alzheimersnewstoday.com/anavex-2-73/