Chapter 2

Literature Review

2.0 Literature Review

Abstract: Based on the complicated pathogenesis of AD and the limited single-target drugs, MTDLs strategies may be more fruitful for the treatment of AD. A potential multi-target lead could be either a known molecule that has shown desired interactions with the specific target or developed by merging multiple known single-target molecules or their common substructures. These merged molecules are usually smaller and directly link two distinct structures via a flexible chain. However, despite the therapeutic potential of MTDLs, there are many challenges regarding their discovery and development. This includes the selection of precise combination of targets involved in the diseases of interest, the understanding of target-disease mechanisms and safety profile.

2.1 Multi-target directed ligands (MTDLs):

The construction of molecular functional assemblies with well-defined pharmacophore properties is a tremendously challenging task for contemporary medicinal chemists to address unresolved human-related diseases. Multidisciplinary oriented synthetic strategies are highly demanding in the field of current drug discovery. The term “one-target-one-disease-one drug” has been growing during the discovery of new drugs in recent years, mainly due to the complexity of diseases. Among these drugs, another term is “cocktail therapy,” which is based on the combination of several drugs in clinical practice, which can act on several targets and may have synergistic effects and wider therapeutic window [Bansal and Silakari 2014, Talevi 2015, Viana et al. 2018]. Design and development of a single chemical molecule that acts concurrently at multiple molecular targets is attaining major consideration in drug discovery.

Conversely, several multi-target molecular entities are presently being developed from the drug discovery programs, and some of these compounds are now in clinical use for the management of various hematologic malignancies and solid tumors[Gentile et al. 2017]. It is also necessary to understand extent of each target and interactions of molecule with unwanted targets [Morphy and Rankovic 2005, Morphy and Rankovic 2007].

2.2 Rational combination of multiple targets for MTDLs

The design and validation of target selection is crucial for MTDLs drug discovery. The ideal target combination may afford greater therapeutic efficacy through synergies. It is generally easier to design MTDLs for highly related targets in the same super family. If targets belong to different super families. Thus their endogenous ligand should be similar or even identical. If so, the binding sites of multiple targets are more likely to accommodate a shared ligand frame. Interestingly, there are also few examples of targets from different super families, and endogenous ligands are also unrelated [Läubli et al. 2018, Morphy et al. 2004, Seshacharyulu et al. 2012].

2.2.1 Target combination based on clinical observations

Combination therapies such as drug cocktails are common topics of clinical studies, but the issues of complex PK profiles, drug−drug interaction often limit their clinical applications. The clinical data of drug on target / off target interactions are important to considered before designing and selection of target combinations. The understanding of enhanced efficacy may also offer the possible target combination.

2.2.2 Target combination based on *in silico* technique

In silico technique is one of the feasible approaches for screening suitable target combinations. Classical computer aided drug design (CADD) methods, such as

pharmacophore studies, 3D-similarity searches, and molecular docking, have been implemented in serial or parallel to fulfil this goal (Figure2.1) [Ma et al. 2010b]. Many modern tools have been extended for discovery of ligands with the required therapeutic profile. A pharmacophore model with numerous key features can be developed for each target, based on the chemical structures of known ligands or the 3D structure of the binding site. Multiple conformations of virtual ligands are mapped onto pharmacophore model and fitness evaluations are performed. In docking-based methods, the ligands are put into the binding site and then evaluated using different scoring functions. One simple approach is to use these methods sequentially or in parallel to screen molecules that can bind multiple targets [Zhang et al. 2017].

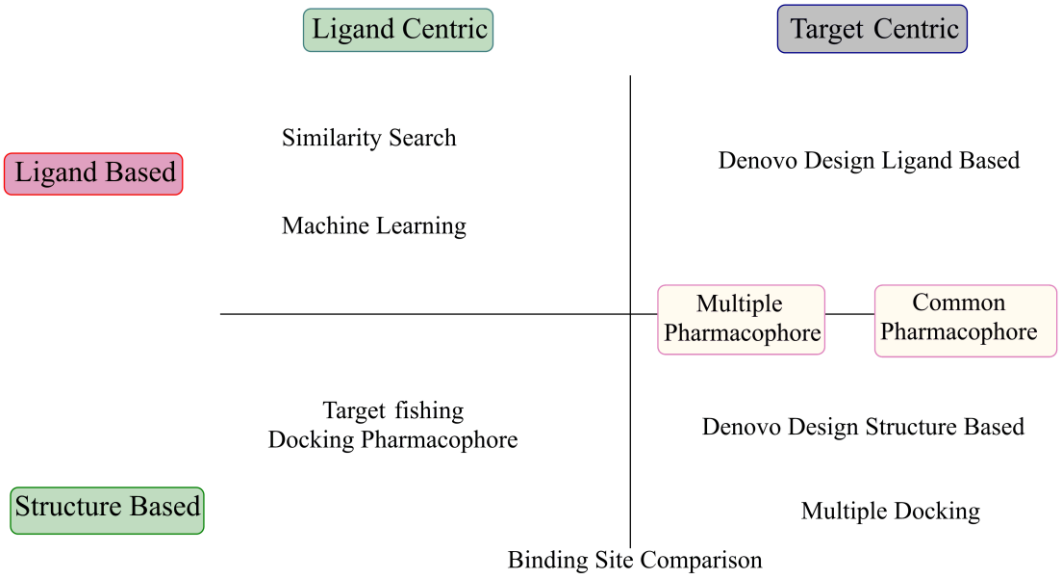


Figure 2.1. An overview of computational methods for multi-target drug design. Methods used to discover MTDLs are classified as either "ligand-centric" or "target-centric".

Generation of a common feature model is a key step for this type of design strategy. Instead of directly building a common pharmacophore from known pharmacophores, researches also use the information from a large-scale data set of docked compounds to build a site-moiety map that was used to search for MTDLs [Hsu et al. 2012].

2.2.3 Target combination based on phenotypic screening

Phenotypic screening is another approach for selection of target combinations [Moffat et al. 2014]. *In vitro* cellular and tissue models can be used for screening large number of compound combinations for synergies. Biological assays that generate multidimensional readouts are usually known as high-content screening, which are valuable approaches for MTDLs development [Koutsoukas et al. 2011]. If the screening systems are more complex, entire animals may be employed, enabling sophisticated readouts including even behavioural changes. The accurate biological profiles of compounds on specific targets may provide great understanding in MDTLs design [Cho and Kwon 2012, Lee et al. 2012].

2.3 MTDLs lead generation

After selection of the target combination and suitable chemical scaffolds, lead generation plays vital role in MTDLs development. For hit to lead generation in MTDLS, there are generally two approaches: knowledge-based approach and screening approach.

2.3.1 Knowledge based approach

Knowledge based lead generation is also known as pharmacophore-based approach. This includes the combination of selective ligands for multiple targets into a single compound, which incorporates the activities of these ligands. In the order of pharmacophore overlapping, the MTDLs are classified into three types; linked, fused, and merged types [Zhang et al. 2017].

A linked MTDL typically contains underlying pharmacophores bridged by a linker that does not exist in any one of the original ligands. Linked MTDLs are usually too large for favourable bioavailability or accessing intracellular compartments. Moreover, the

linker may hinder the interaction between targets and ligands. The linker should not join a ligand at a position with steric effect. Sometimes, the linker itself works as a pharmacophore by interacting with the target. Therefore, linkers should be tailored so as to enhance interactions between the targets and linkers. According to the property of linkers, the linked MTDLs are further divided into two categories: cleavable or non-cleavable (Figure 2.2) [Morphy and Rankovic 2005, Savelieff et al. 2018].

2.3.2 Screening approach

Focused screening is the main-stream screening method instead of "irrational" high throughput screening (HTS). In focused screening, compound classes which are already identified to be effective toward one of the targets of interest are consequently screened for another one. It is a favourable approach for targets that are kinases: MTDLs are often identified via cross-screening of compounds in kinase panel profiling. Although, the screening of compound collections has provided useful insights, this appears to be a less common approach than pharmacophore combination. This could be due to the lower probability of the screening of compounds delivering appropriate combinations of activities, or perhaps due to logistical difficulties of conducting multiple screens [Jenwitheesuk et al. 2008, Ma et al. 2010a].

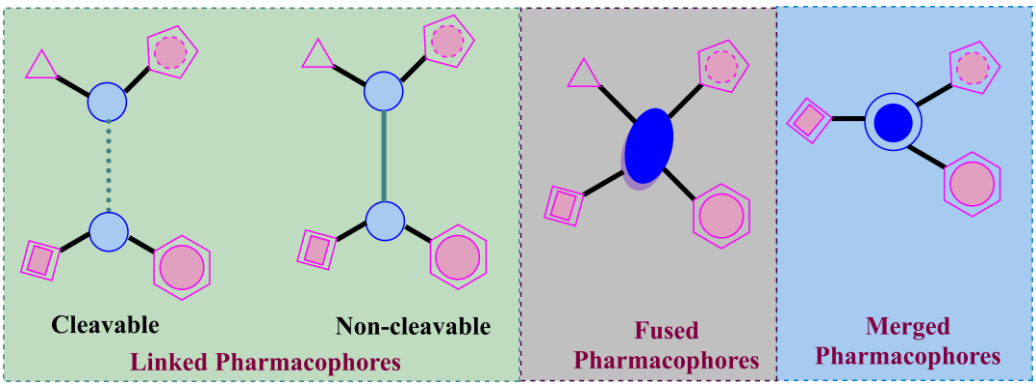


Figure 2.2. Rational design of multitarget-directed ligands (MTDLs).

2.4 MTDLs lead optimization

After the lead generation, the next challenging task is to develop the optimized lead ligand with balanced activities and better physicochemical properties. This phase is classified in two approaches; design-in and design-out approaches.

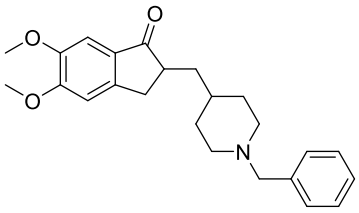
2.4.1 Design-in approach

The pharmacophore of ligand is designed based on the functional groups which are necessary for biological target. The goal of the approach is to enhance the affinity to one target while retaining other target. Although, establishing the systematic structure activity relationship (SAR) towards both the target should be necessary. The SAR data of selective templet ligands evidently provide many clue for optimization. In this, merged pharmacophore strategy is workable to get optimized hybrid molecule [Bottegoni et al. 2012, Woo et al. 2011].

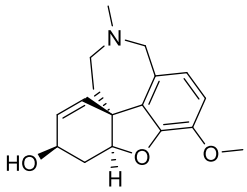
2.4.2 Design-out approach

Generally, design-out approach begins with a molecule that concurrently acts on all targets of interest, involving undesired ones [Proschak et al. 2018]. By minimising the affinity to undesired targets, selectivity to the targets of interest may be improved. The binding pattern of the lead molecule can be characterized by X-ray crystal structure analysis. The prediction of differences in binding patterns between desired and undesired targets provides the direction to lessen the off-target (s) potencies [Morphy and Rankovic 2003]. The design-out approach has been applied successfully to develop MTDLs in various disease treatments [Reichard et al. 2002].

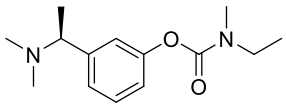
Creating the optimal ratio of affinities to several targets via *in vitro* assays is critical; it often causes side effects [Ramsay et al. 2018]. In most cases, the aim is to balance *in vitro* activities within an order of magnitude for several targets, under the assumption



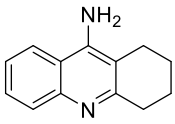
Donepezil (DNZ)
Approved
AChE inhibitor



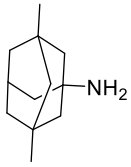
Galantamine
Approved
AChE inhibitor



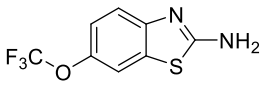
Rivastigmine
Approved
AChE inhibitor



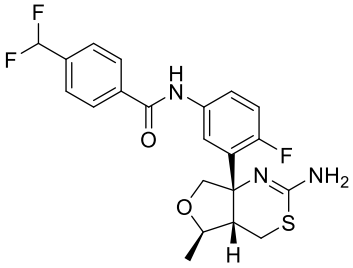
Tacrine
Approved and Withdrawn
AChE inhibitor



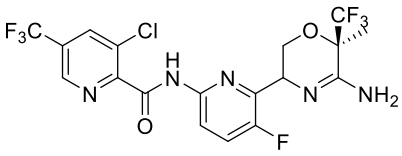
Memantine
Approved
NMDA receptor antagonist



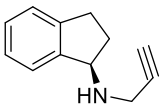
Riluzole (L1)
Phase II
Sodium channel Blocker



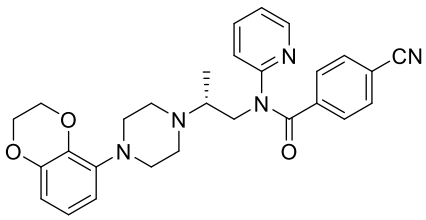
Elenbecestat (L2)
Phase III
BACE-1 inhibitor



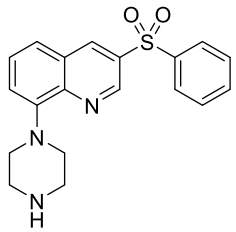
Umibecestat (L3)
Phase III
BACE-1 inhibitor



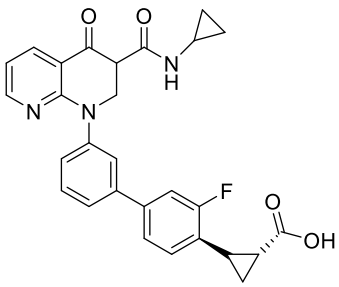
Rasagiline (L4)
Phase II
MAO-B inhibitor



Lecozotan (L5)
Phase III
5-HT$_{1A}$ receptor antagonist



Intepirdine (L6)
Phase III
5-HT6 receptor agonist



MK-0952 (L7)
Phase II
PDE4 inhibitor

Figure 2.3. Approved and clinical drugs for treatment of AD.

that it may achieve similar degrees of *in vivo* receptor occupancy. Ideally, the template ligands to be combined/merged should contain similar physicochemical profiles and *in vivo* activities of the same order of magnitude, because, it is hard to obtain a workable MTDLs if the template ligands are completely dissimilar from each other in terms of Pharmacokinetic and Pharmacodynamic profiles and activities [Morphy and Rankovic 2005, 2009, Morphy and Rankovic 2007].

2.5 Recent advancements of MTDLs in AD drug discovery

MTDLs strategy for AD therapy is a breakthrough direction in current scenario compared with single target drugs, and multi target drugs will become more crucial and effective in controlling the disease progression. AD disease pathology involves different cell signalling pathways can interact with each other and forms a disease network which this results in a poor curative effect of single target drugs.

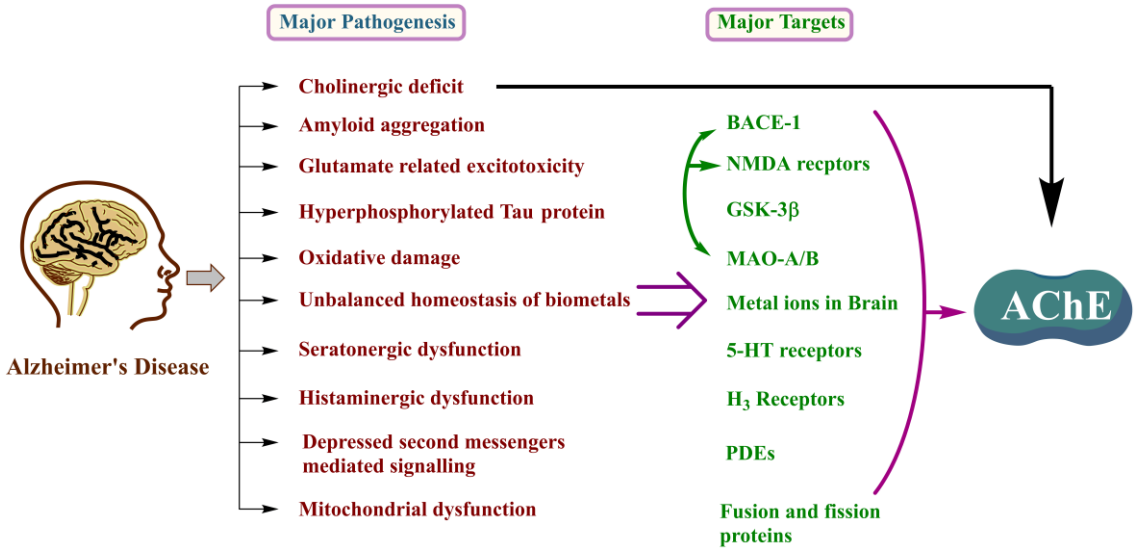


Figure 2.4. Multitarget-design strategies involving in AD.

Currently, investigations on AD have led to discovery of several drug targets which are involved in the disease progression, such as acetylcholinesterase (AChE), beta-secretase 1 (BACE-1), glycogen synthase kinase 3 beta (GSK-3β), monoamine oxidase (MAOs),

metal ions in the brain, NMDA receptors, 5-HT receptors, and phosphodiesterase (PDEs). All these drug targets are extensively being studied by the global research communities for the development of potent MTDLs. Some multi target combinations are depicted in Figure 2.4. Among them, AChE is the key target to be considered MTDLs are designed to involve this target to increase the ability and reduce the failure percentage of new drugs. In short, AChE is the most popular in AD drug discovery process. Drugs which act through AChE and other single target strategies are included in Figure 2.3.

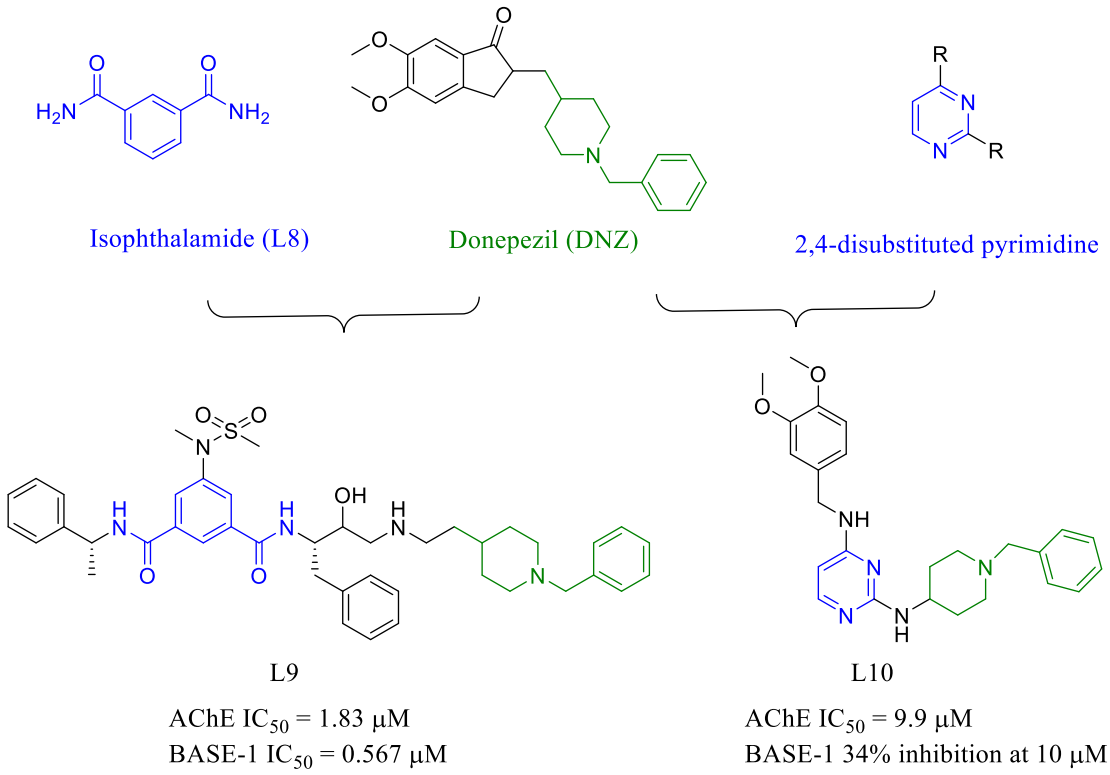


Figure 2.5. MTDLs strategy involved in AChE and amyloid-beta.

Design of MTDLs based on AChE inhibitors, DNZ and tacrine, is preferred because of US-FDA approval with evident AChE inhibition capacity. Zhu and co-workers reported a series of hybrid molecules with AChE and BACE-1 dual inhibition and the scaffold was developed based on DNZ and isophthalamide (L8) fused with three types of

linkers. Among the series, compound (L9) showed multi target potential for AD with better enzyme inhibitions (AChE IC_{50} = 1.83 µM; BACE-1 IC_{50} = 0.567 µM). Additionally, it also produced good inhibitory effects on Aβ production in APP-transfected HEK293 cells (IC_{50} = 98.7 nM) and mild anti-oxidative effect against H_2O_2 induced PC12 cell injury at 10 µM. Further, *in vivo* animal experiments on APP transgenic mice showed 29% reduction in $A\beta_{1-40}$ production [Stachel et al. 2004, Zhu et al. 2009].

Another hybrid compound (L10) by Praveen group was developed by fusing with DNZ and 2,4-disubstituted pyrimidine It showed dual inhibition on AChE (IC_{50} = 9.9 µM) and BACE-1 (34% inhibition at 10 µM). The compound also exhibited 17.4 % self-induced $A\beta_{1-40}$ aggregation inhibition at 100 µM and 81.0% neuroblastoma cell viability at 40 µM [Mohamed et al. 2012, Mohamed et al. 2011].

Fernandez-Bachiller and co-workers reported a novel AChE inhibitor from tacrine and 4-oxo-4H-chromene (L11) hybrids. The most potent hybrid (L12) showed dual inhibition against human AChE (IC_{50} = 8.0 nM) and BACE-1(IC_{50} = 2.8 µM) and 1.3 fold potent anti-oxidant activity than trolox (anti-oxidant) along with good CNS permeability in parallel artificial membrane permeation assay (PAMPA-BBB) [Fernández-Bachiller et al. 2012]. Another potent hybrid (L15) reported by Munoz-Torrero group was obtained through a fused long alkylamine linker with huprine Y (L13) and rhein (L14), showed excellent inhibitory activities against human AChE and BACE-1 and 47.9% Aβ aggregation activity at 10 µM [Camps et al. 2000, Viayna et al. 2014]. Bartolini co-workers also reported a potent tacrine-benzofuran fused hybrid (L17), which showed an interesting inhibitory profile against human AChE and BACE-1 (IC_{50} = 0.86 nM for AChE; IC_{50} = 1.35 µM for BACE-1) and 61.3% inhibition

towards self-induced $A\beta_{1-40}$ aggregation at 10 µM. Further *in-vivo* studies demonstrated that compound (L17) produced cognitive improvement in scopolamine treated ICR mice and was free from significant hepatotoxicity [FJ et al. 2017, Zha et al. 2016].

Hui and co-workers reported tacrine-phenothiazine based novel hybrid (L19) with AChE inhibition of IC_{50} = 89 nM. The compound exhibited 39.5 % down regulation of tau protein levels at 100 µM in tau hyperphosphorylation induced okadaic acid in N2α cells.

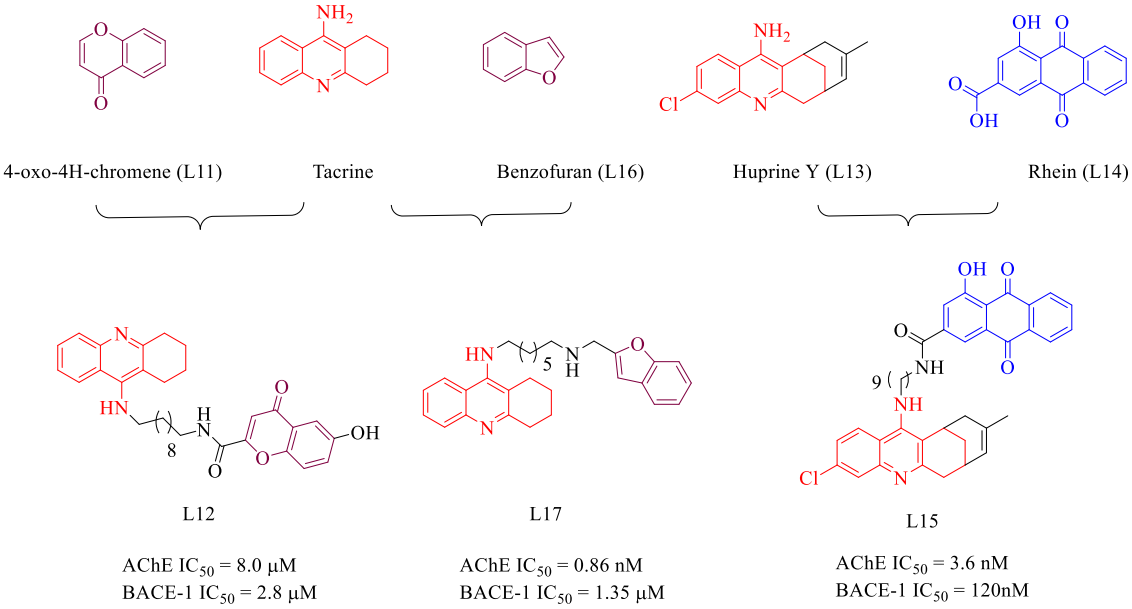


Figure 2.6. MTDLs strategy involving AChE and amyloid-beta.

The molecular docking studies of compound (L19) on AChE (Mol Dock score = -183.585 kJ/mol) and GSK-3β (Mol Dock score = -148.821 kJ/mol) demonstrated significant binding affinities[Hui et al. 2014]. Jiang and co-workers reported a novel hybrid fused with tacrine, compound (L20) (GSK-3β inhibitor with IC_{50} = 1.1 nM). Among the series, compound (L21) exhibited dual target inhibition against human AChE (IC_{50} = 6.5 nM) and GSK-3β (IC_{50} = 66 nM). Further compound (L21) also showed 46% inhibition on Aβ self-aggregation at 20 µM concentration, tau protein

hyperphosphorylation in mouse neuroblastoma N2α-Tau cells, hepatoprotectivity and significant *in-vivo* cognitive improvement in scopolamine treated ICR mice[Jiang et al. 2018].

Ladostigil (L22), from Avraham Pharma, is a potent inhibitor of AChE, BuChE, MAO-A and MAO-B in brain. It is currently in phase II clinical trial for the treatment of cognitive impairment and Alzheimer's type dementia. It is developed by combining carbamate moiety of ChE inhibitor rivastigmine and indole amine scaffold of MAO-B inhibitor rasagiline (L4) [Rosini et al. 2016, Weinreb et al. 2016].

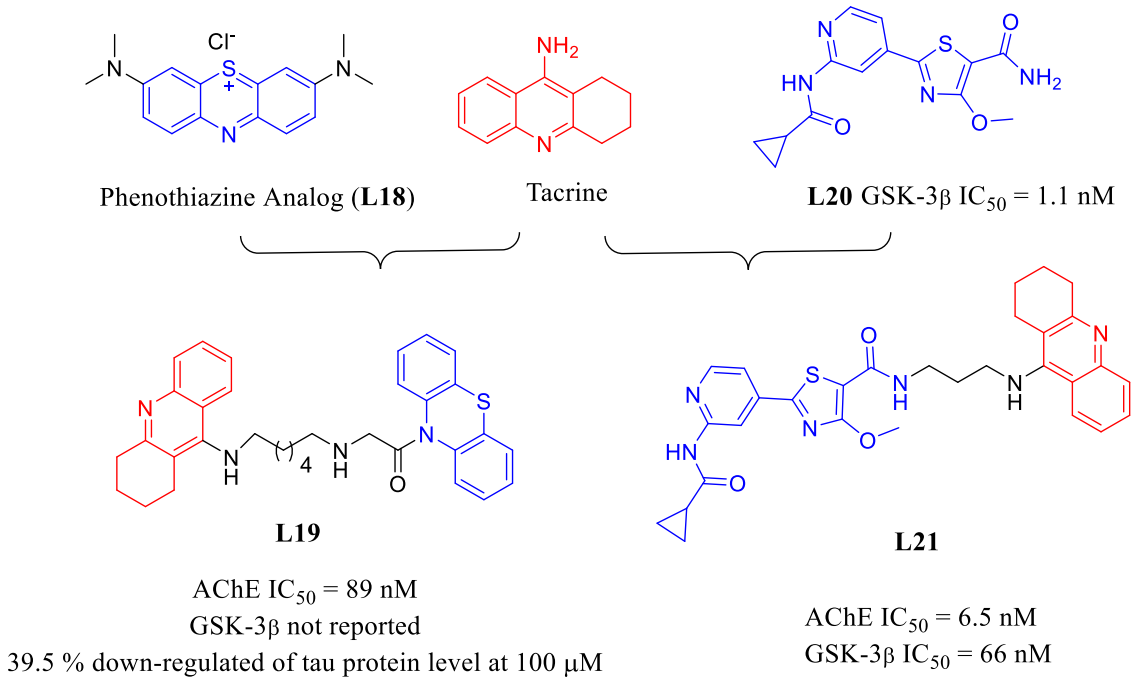


Figure 2.7. MTDLs strategy involving AChE and GSK-3β.

MAO inhibitors evidently upsurge monoaminergic neurotransmission, decrease ROS formation, oxidative stress and exert pharmacological effects including antioxidant property, neuroprotective and cognitive improvement, which serve valuable strength in the treatment of AD [Xu et al. 2018]. The Unzeta group reported a promising hybrid compound (L24) by fusing of benzylpiperidine moiety of DNZ with M30 (L23). It was

a potent brain selective MAOs inhibitor. The compound (L24) exhibited excellent ChEs inhibition along with selective MAO-A inhibition (AChE IC_{50} = 29 nM; BuChE IC_{50} = 39 nM; MAO-A IC_{50} = 10.1 µM) and strong metal chelating capacity to Cu^{2+} and Zn^{2+} ions [Wu et al. 2016]. Xie and co-workers developed a novel hybrid (L26) by fusing tacrine and coumarin (L25), a natural compound containing AChE inhibitory activity. The compound (L26) showed ability to inhibit AChE (IC_{50} = 92 nM), 67% self-induced Aβ aggregation at 20 µM, Cu^{2+} and Fe^{2+} ion chelating properties.

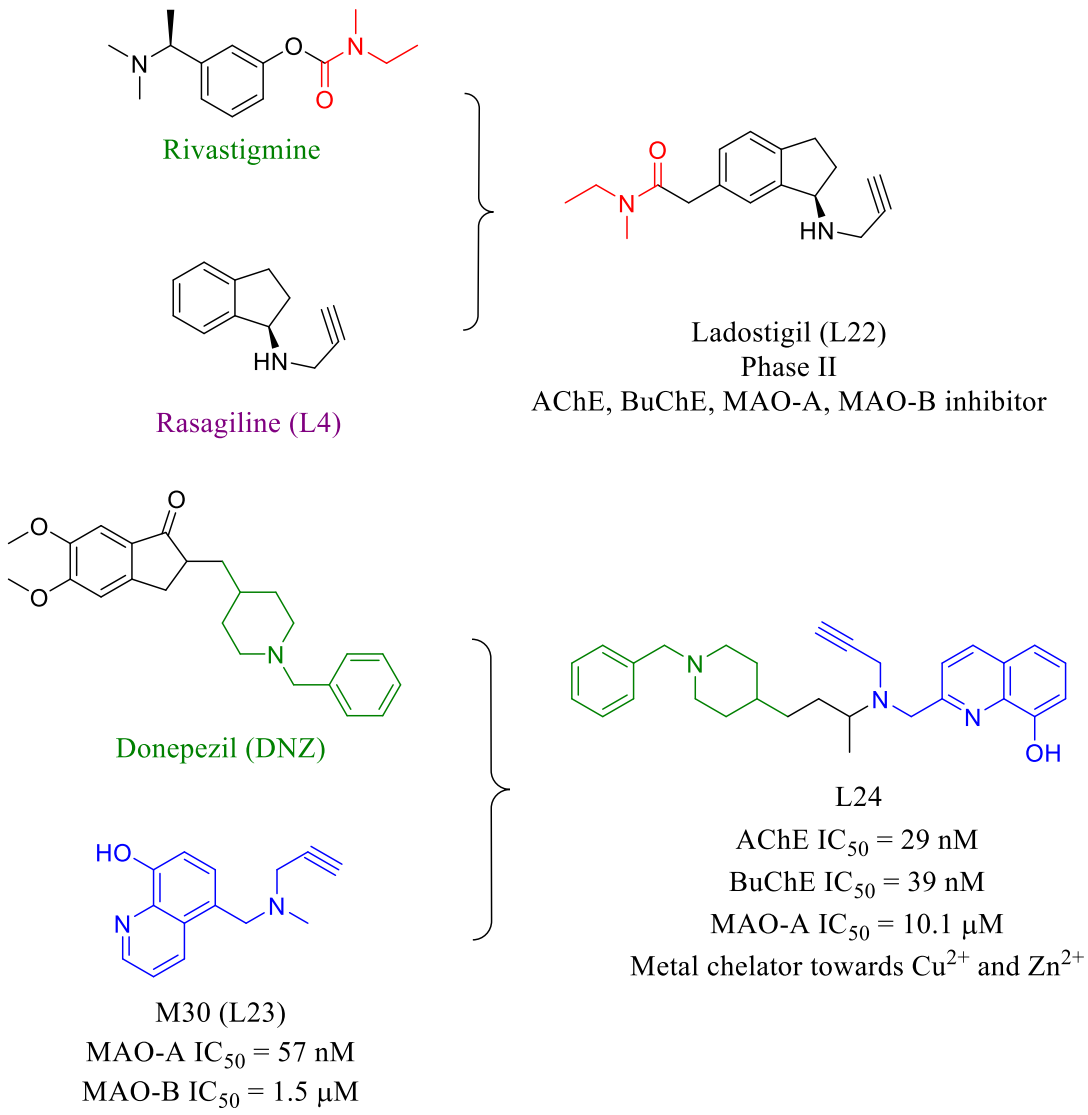


Figure 2.8. MTDLs strategy involving AChE and MAOs.

Hybrid (L28) reported by Fernandez-Bachiller's group, is obtained by combining tacrine and clioquinol (L27). It also exhibited potent AChE inhibitory activity (IC_{50} = 5.5 nM) and Cu^{2+} ion chelating ability based on UV-visible spectrometry [Fernández-Bachiller et al. 2010].

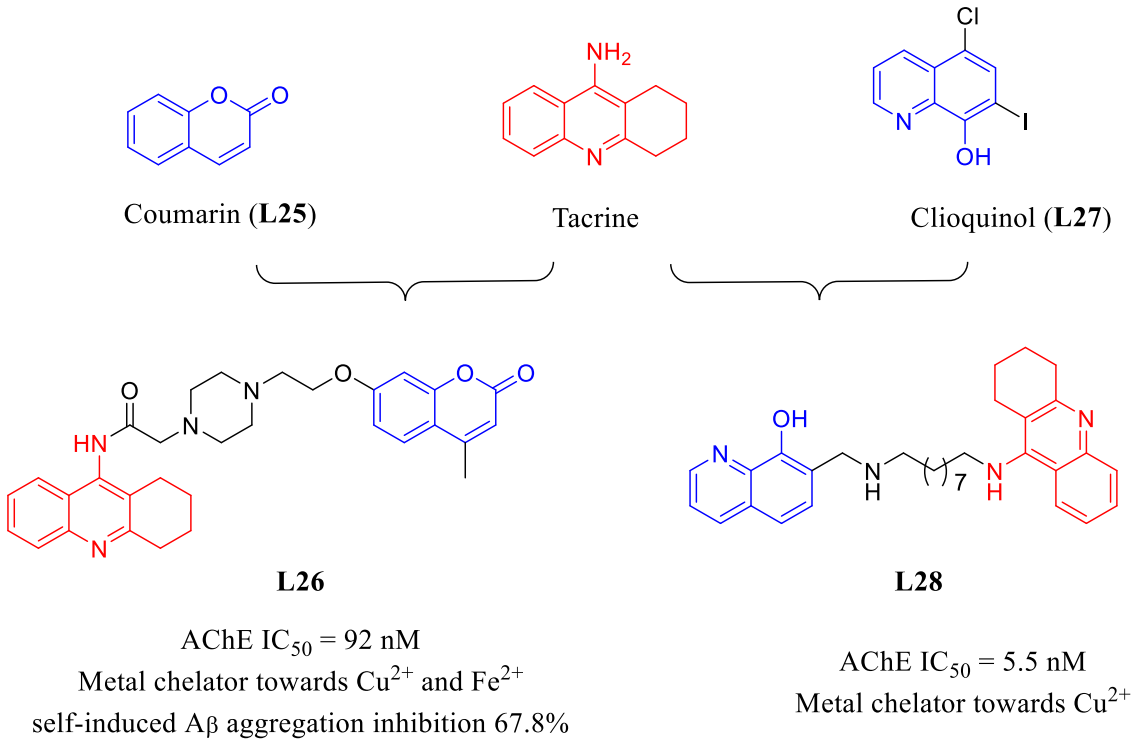


Figure 2.9. MTDLs strategy involving AChE, amyloid-beta and metal chelation.

The AChE and NMDARs multi-target strategy is a valuable approach for cholinergic and glutamatergic systems and is becoming an important prospect because of excessive activation of NMDARsis implicated in the degenerative process of cholinergic neurons in AD. NMDARs antagonist can reduce neurodegeneration and AChE inhibitors can recover memory and cognition [Parsons et al. 2013]. Namzaric is a prescription medicine from Allergan approved in 2015 for moderate to severe AD, which comprised of memantine and DNZ.

Memagal (L29) is a novel hybrid compound developed by linking galantamine and memantine. This hybrid compound showed remarkable inhibitory potency against AChE (IC_{50} = 1.16 nM) and NMDARs ([^{3}H] MK-801 binding assay K_i = 4.6 µM; NR2B containing NMDARs ([^{3}H] ifenprodil binding assay K_i = 4.6 µM). Further investigations revealed that L29 inhibit NMDA mediated neurotoxicity (IC_{50} = 0.28 nM) in SH-SY5Y cell viability assay with potent neuroprotective property [Simoni et al. 2012].

Gazova Z et. al. evaluated the multi-target efficiency for a series of 7-methoxytacrine – memantine heterodimers. The compound consisted of 7-methoxytacrine (AChE inhibitor) and adamantylamine moiety (NMDAR antagonist) connected through methylene-thiourea linker (L30) and exhibited AChE (IC_{50} = 0.47 µM), BACE-1 (IC_{50} = 224.7 µM), NMDAR (Ki = 1.8 µM GluN1/GluN2B) inhibition and effectively suppressed Aβ peptide amyloid fibrillization. It also acted as antagonist for both M1 muscarinic (IC_{50} = 4.02 µM) and muscle type nicotinic receptors (IC_{50} = 0.90 µM). Multiple potencies of L30 demonstrated potent clinical impact of slowing or blocking the neurodegenerative process related to AD [Gazova et al. 2017].

Dimebon (L31) is a withdrawn antihistamine drug having an ability to antagonise NMDARs (IC_{50} = 10-70 µM) and inhibit AChE (IC_{50} = 42 µM) at higher concentrations. This was evaluated by Pfizer in Phase III clinical trials for moderate to severe AD, but it was discontinued due to insufficient understanding of its mechanism of action[Bezprozvanny 2010]. Interestingly, the optimized dimebon analog (L32) showed potent multi-target profile against both AChE (IC_{50} = 0.195 µM) and NMDARs (IC_{50} = 11 µM) and 67.0% self-induced Aβ aggregation inhibition. Makhaeva and co-workers reported another dimebon analog (L34) from fused phenothiazine (L33) ring.

Compound L33 showed dual BuChE (IC_{50} = 0.52 µM) and NMDARs ([^{3}H] MK-801 binding assay IC_{50} = 14.6 µM; [^{3}H] ifenprodil binding assay IC_{50} = 13.4 µM) inhibition potency.

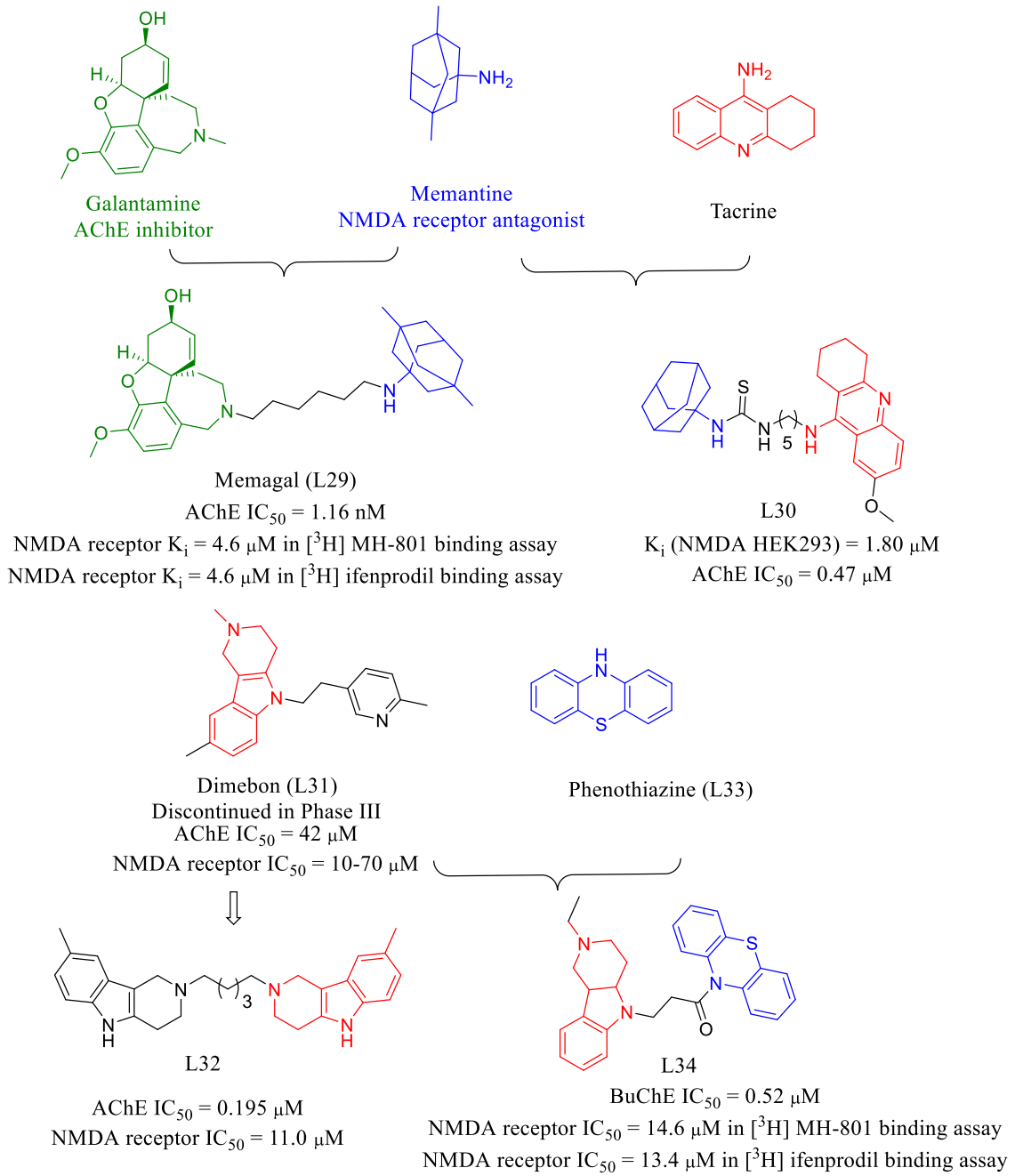


Figure 2.10. MTDLs strategy involving AChE and NMDA.