INTRODUCTION

In the modern world, the drugs affecting central nervous system plays a significant role and these drugs are of special interest to mankind.

Neurodegenerative diseases (NDDs) are a major global health burden in the world particularly with our ageing population. The two most prevalent NDDs are Alzheimer's disease (AD) and Parkinson's disease (PD). As of 2010, > 35 million people worldwide suffered from dementia, with the vast majority due to AD [Wimo and Prince 2010]. Similarly, as of 2005, > 4 million people worldwide suffered from PD [Dorsey *et al.* 2007]. Similarly, as of 2005, > 4 million people worldwide suffered from PD [Dorsey *et al.* 2007]. Moreover, risk for both of these NDDs increases with age, with both of these diseases projected to double in numbers over the next two decades [Dorsey *et al.* 2007; Wimo and Prince 2010]. Other diseases such as epilepsy, Huntington's disease (HD), depression, anxiety, post-stroke neurodegeneration etc., also exert a great human toll on the affected individuals, their families and society. As a consequence, the economic burden associated with these incurable, NDDs is enormous and continues to grow [Dorsey *et al.* 2013; Kowal *et al.* 2013]. Thus, research into these diseases is therefore of great importance. **Figure 1.1.** illustrates the World Health Organization (WHO) report 2006 on NDDs.

1.1. Neurodegenerative diseases: biggest challenge of present century

NDDs represent, nowadays, one of the main causes of death in the industrialized countries. NDDs are a heterogeneous group of disorders characterized by progressive degeneration of the structure and function of central nervous system (CNS) or peripheral nervous system (PNS). They are characterized by loss of neurons in particular regions of the nervous system. It is believed that this nerve cell loss underlies the subsequent decline in cognitive and motor functions that patients experience in these diseases. The NDDs sharing such multifactorial pathogenic mechanism includes, among others, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), depression, anxiety, post-stroke neurodegeneration, etc.

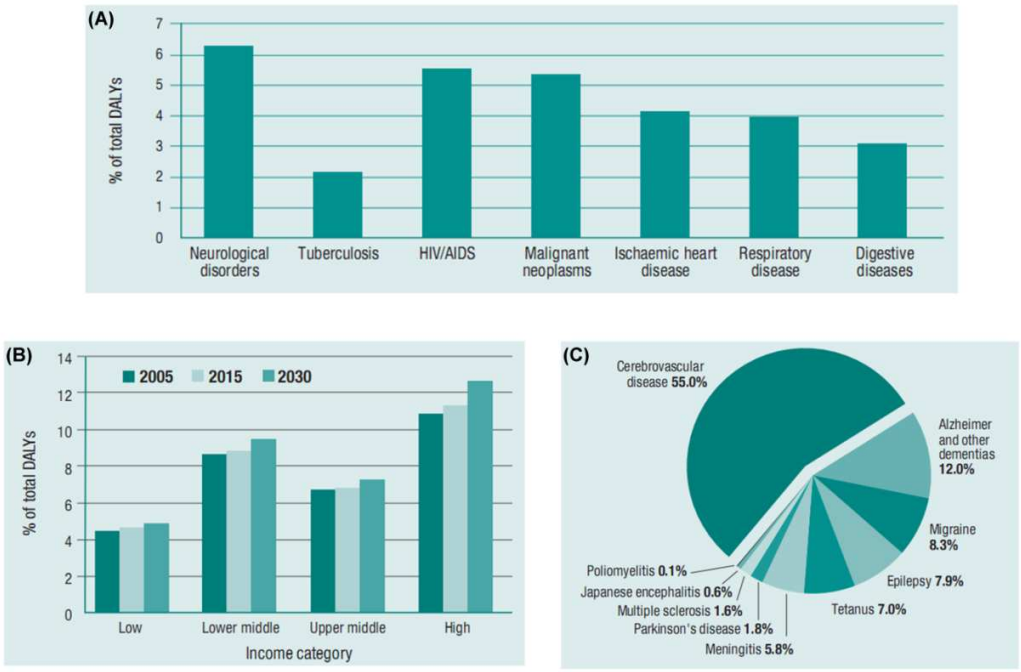


Figure 1.1. NDDs: A report from WHO. (A) Comparison of percentage of total DALYs for NDDs and other selected diseases (DALYs – Disability adjusted life years). (B) NDDs as a percentage of total DALYs for 2005, 2015 and 20130 across World Bank Income Category. (C) DALYs for individual NDDs as percentage of total NDDs. [From WHO library cataloguing-in-Publication data 2006]

Even though each of these diseases has its own molecular mechanisms and clinical manifestations, a number of common pathways might be recognized in diverse pathogenic cascades. A variety of mutant genes and environmental toxins have been implicated in the cause of NDDs but the mechanism remains largely unidentified and needed to be explored [Mandemakers 2007; Jellinger 2009].

Literatures have evidenced the role of numerous factors leading to NDDs, viz. abnormality in the dynamics of protein resulting in malfunctioning in protein aggregation and misfolding, formation of reactive oxygen species (ROS) causing free radical formation and oxidative stress, damaged bioenergetics and mitochondrial dysfunction, increased concentration of metals and pesticides resulting in metal dyshomeostasis and phosphorylation impairement, etc. all occurring simultaneously or independently [Baldi 2003] (**Figure 1.2.**). In addition to the above mentioned factors, it is worth noting that

amyloid oligomers such as amyloid-β (Aβ) and α-synuclein have been extensively reported to permeabilize both the cell and mitochondrial membranes causing neuronal damage [Caughey *et al.* 2003; Kagan *et al.* 2002].

Even though a lot of research has been carried out in this field to understand the pathophysiology of these diseases, scientists are still in search of new therapeutic approaches to the problem, molecular mechanisms involved and associated viable drug targets.

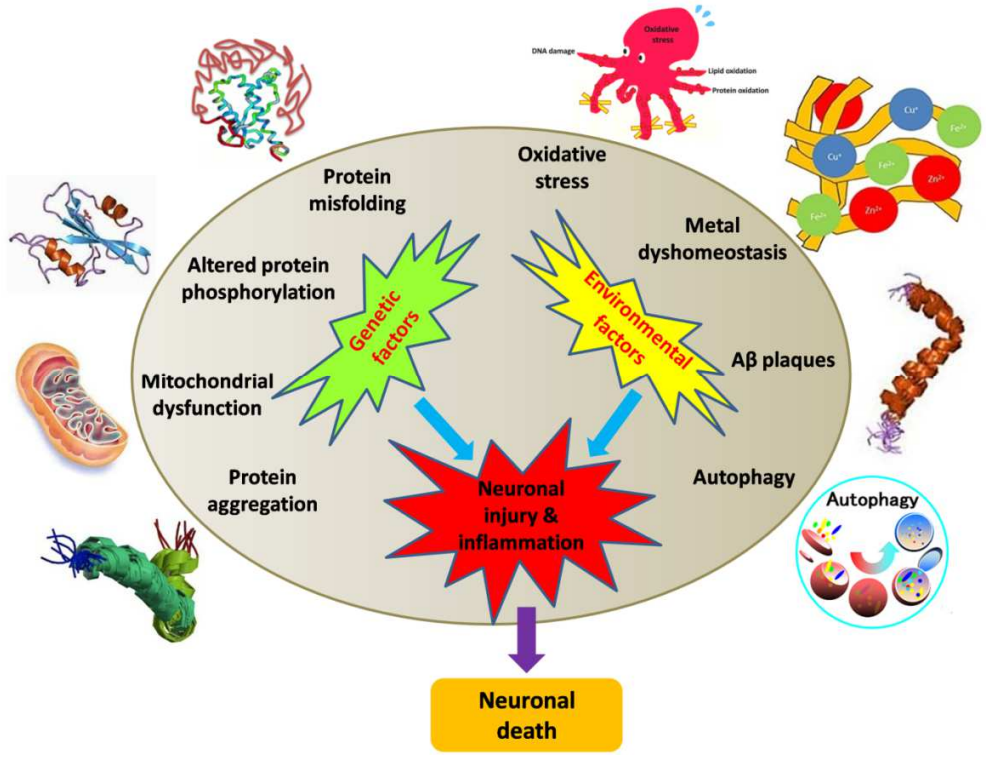


Figure 1.2. Multifactorial events leading to NDDs

An early contribution of neuroprotective strategies for these diseases seems particularly desirable because isolated treatments cannot be as effective. This has inspired active research in the development of new drugs that could mark an important advance in the treatment of these complex diseases.

1.2. Strategies employed for anti-neurodegenerative drug discovery

Indeed, an array of agents have been discovered following the strategy of 'one-drug-one-target' approach (mono-target model; **Figure 1.3.**) and are currently used in various multi-factorial pathologies although their therapeutic efficacy is limited to an essentially palliative and/or symptomatic effect. As a result, the discovery of genuine disease-

modifying agents addressing a multi-factorial disease through a single-target mechanism was a limiting factor which resulted in its failure.

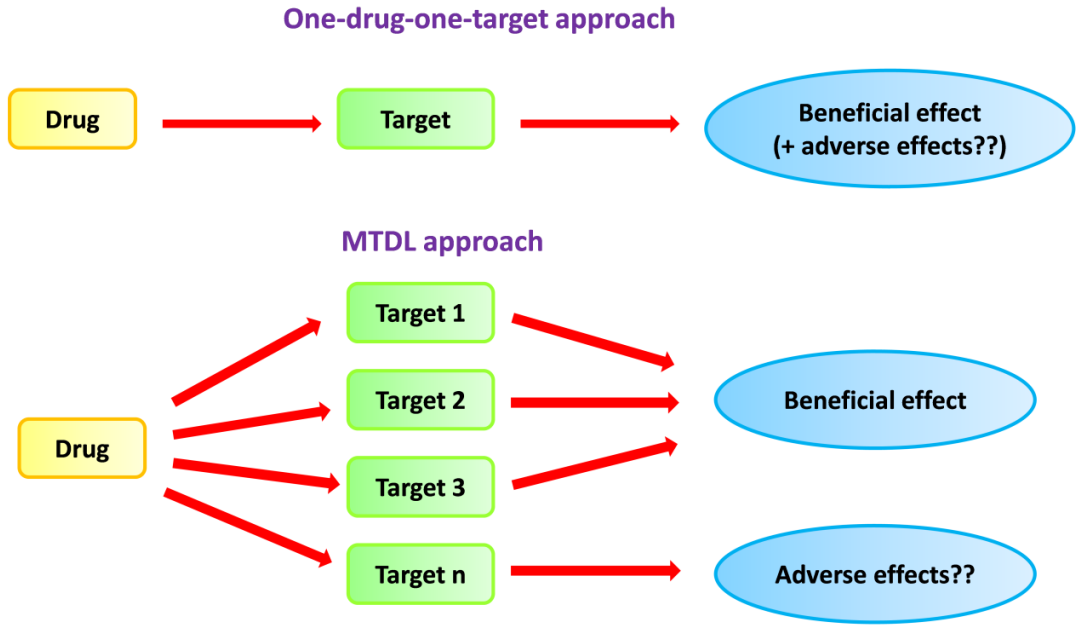


Figure 1.3. Neurodegenerative drug discovery approaches. (a) Target-focused approach based on one-drug-one-target paradigm (b) MTDL approach based on one-drug-multiple-target paradigm

The failure of this paradigm insisted the researchers to explore out of the ordinary ways for the treatment of such multi-factorial diseases leading to the invention of the 'polypharmacology approach' focussed on addressing concurrently various biological targets. Accordingly, a combination of two or more drugs was used for targeting a single biochemical process in such a way so as to exert beneficial therapeutic effects by modulating different targets [Korcsmaros *et al*. 2007]. However, low patient compliance, multiple dosing, increased risks of drug-drug interactions and multiple competing metabolic transformations resulted in the restricted use of this therapy.

1.2.1. MTDL approach

Nowadays, an emerging strategy widely followed for the development of multi-factorial disease-modifying drugs is based on the multi-target-directed-ligand (MTDL) design, an innovative shift from the traditional one-drug-one-target approach to one-drug-multiple-target approach based on the assumption that a single, properly designed molecular entity might be able to interfere with a number of cellular events simultaneously causing

maximum therapeutic benefit against a multi-factorial disease more efficiently in comparison to drug cocktails.

Multi-target drug discovery is an emerging area of increasing interest to the drug design chemists. Drugs that modulate several targets have the potential for an improved balance of efficacy and safety compared to single target agents [Morphy *et al.* 2004]. More than 70% of FDA (Food and Drug Administration, USA) approved drugs are known to derive their therapeutic benefit by virtue of interacting with "multiple targets" [Cavalli *et al.* 2008]. Based on these evidences, presently it is widely accepted that a more effective therapy for the multi-factorial nature of NDDs would result from the development of the "one drug, multiple target" strategy, also called the multi-target-directed-ligand (MTDL) approach [Morphy *et al.* 2005].

MTDL approach suggests the use of compounds with multiple activities at different biological targets (**Figure 1.3.**). MTDL approach is an alternative to combination therapy with two or more drugs.

1.2.2. Rational design strategies for MTDL

To obtain MTDLs, the design strategy involves the assimilation of different pharmacophores of diverse drugs in the same structure to obtain hybrid fragments. Primarily, each pharmacophore of the hybrid drug should retain the ability to interact with its specific site(s) on the targets producing consequential pharmacological response.

Three major approaches are employed in the design of molecular skeleton of a multi-target molecular entity (**Figure 1.4.**).

a) **Conjugation (Spacer-coupled linkage) approach**: It consists of molecular heterodimerization of two structurally different scaffolds, each accountable for a different pharmacological activity. These molecules are structurally connected through an appropriate linker (spacer) resulting in a metabolically stable single entity that can be broken down to release the two active moieties after enzymatic activity.

b) **Fusion (Direct-coupling) approach**: Single molecular entity is formed by the fusion of two separate molecules possessing different pharmacological activity, coupled through a single covalent bond. There is an absence of linker in this approach.

c) **Hybridization approach**: It is based on the recognition of the key structural fragments accounting to a defined pharmacological activity in different molecules. These structural fragments are then combined in a unique and appropriate manner resulting in the formation of molecular hybrid. These molecules do not need enzymatic activation to exert the pharmacological effect.

However, further optimization of the potential multi-target lead compounds is needed in order to appropriately stabilize their *in-vitro* and *in-vivo* pharmacological activities.

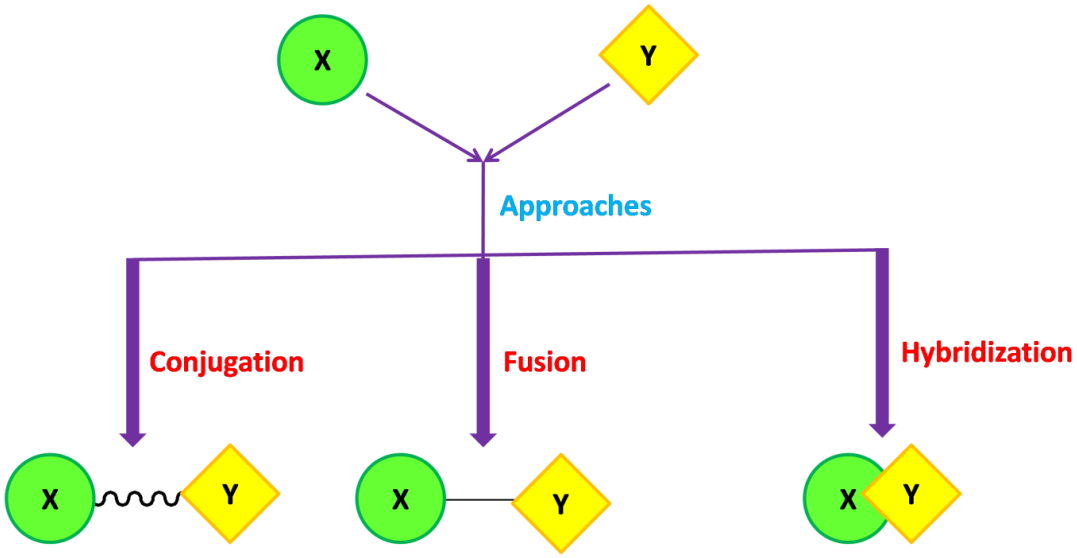


Figure 1.4. Schematic representation of MTDL strategy

1.2.3. MTDLs and NDDs

Because of the multifactorial nature of NDDs and diverse cerebral mechanisms implicated in their treatment, MTDLs have come out as novel drug candidates with beneficial therapy of NDDs. In this context, monoamine oxidases (MAOs) i.e. monoamine oxidase A (MAO-A) and/or monoamine oxidase B (MAO-B) and their inhibitors and acetylcholinesterase (AChE) and its inhibitors have been a steady source of surprises and have been regarded as attractive and potential targets with the highest prospect for therapeutic use in the treatment of widespread NDDs.

1.3. Monoamine oxidases: an overview

The discovery of monoamine oxidases dates back to early 1928 by a young doctoral student, Mary L. C. Bernheim (née) [Hare 1928]. She hypothesized that the enzyme may be protective and may be involved in the metabolism of potentially toxic exogenous amines. Subsequent studies by Blaschko [Blaschko *et al.* 1937] and Zeller [Zeller *et al.*

1938] demonstrated that MAOs play an important role in controlling the concentration of neurotransmitters into the synaptic cleft.

1.3.1. Molecular characteristics of MAO

Monoamine oxidases (MAOs; amine-oxygen oxidoreductase; EC 1.4.3.4) are the enzymes containing the flavin adenine dinucleotide (FAD) cofactor covalently bonded to a cysteine residue [Reyes-Parada *et al.* 2005], and are involved in catalyzing the oxidative deamination of biogenic amines *viz.* neuroamines, vasoactive and exogenous amines, and xenobiotic amines including monoamine neurotransmitters and hormones in the brain and the peripheral tissues by catalyzing their oxidative deamination [Strolin-Benedetti and Tipton 1998; Singer and Ramsay 1995; Richards *et al.* 1998] to prevent their possible function as false neurotransmitters, thereby resulting in the modulation of their concentrations in brain and peripheral tissues [Berry *et al.* 1994; Dostert *et al.* 1982]. The broad array of substrates includes several notable biogenic amines: indoleamines such as serotonin (5-hydroxytryptamine, 5-HT), and tryptamine; catecholamines such as dopamine (DA), norepinephrine (NE) and epinephrine [Johnston 1968]; trace amines, such as β-phenylethylamine (PEA) [Knoll and Magyar 1972], tyramine and octopamine [Youdim and Riederer 1993]; and MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxin (which immediately converts to MPP^+) [Javitch *et al.* 1985] (**Figure 1.5.**).

The enzyme exists in the two isoforms [Binda *et al.* 2003; Ma *et al.* 2004; De Colibus *et al.* 2005; Son *et al.* 2008], namely MAO-A and MAO-B, which are located predominantly in the outer membrane of mitochondria of neuronal, glial and other cells [Johnston 1968]. These two forms have the molecular weight of approximately 60 kDa. MAO-A and MAO-B are composed of 527 and 520 amino acid residues respectively and shares about 70% amino acid sequence identity and represent separate X-chromosome related gene products [Bach *et al.* 1988]. Thus, they can be differentiated by separate encoding genes [Bach *et al.* 1988; Grimsby *et al.* 1991], tissue and cell distribution [Westlund *et al.* 1985], their substrate selectivity and inhibitor sensitivity [Youdim and Finberg, 1991; Gottowik *et al.* 1993; Geha *et al.* 2001]. The active forms of the enzymes are homodimers with subunit molecular weights, determined from their cDNA structure; of 59,700 and 58,800; respectively.

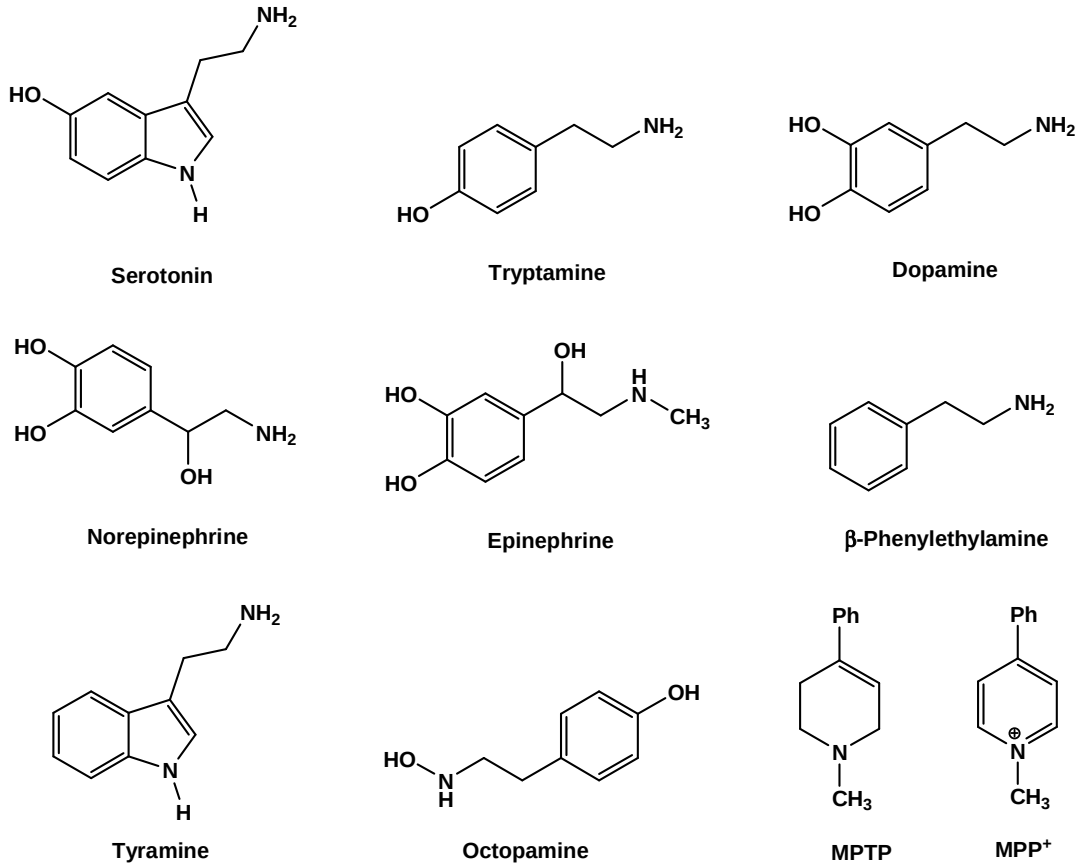


Figure 1.5. Substrates of MAO

The substrate specificity and tissue distribution of MAO isozymes are presented in **Table 1.1.**

The presence of MAO-A in glial cells should be responsible for degradation of serotonin in brain regions rich in serotonergic neurons while the role of MAO-B in serotonergic neurons is to eliminate any foreign amines and prevent them from reaching the synaptic cleft. Noradrenergic neuron contains both MAO-A and MAO-B isoforms and are responsible for catalyzing the oxidative deamination of norepinephrine efficiently. However, because the uptake of norepinephrine into the synaptic vesicle is strongly favourable over binding to MAO, the substrate can escape metabolic degradation to some extent [O' Carroll *et al.* 1987]. Although this finding is in apparent contrast with the pharmacological evidence that serotonin levels are enhanced only following MAO-A, but not MAO-B inhibition. However, the reasons of this mismatch are still needed to be established.

Table 1.1. Substrate specificity and tissue distribution of MAO isozymes

	MAO-A	**MAO-B**	**MAO-A & MAO-B**
Substrates	5-HT, Epinephrine, Norepinephrine, Octopamine	Benzylamine, Phenylethylamine, N-phenylamine, Methylhistamine, Octylamine, N-acetylputrescine, Milacemide, MPTP	Dopamine, Tyramine, Tryptamine, Kynuramine, 3-Methoxytyramine
Tissue distribution	Brain (catecholaminergic neurons of the locus ceruleus, the reticular formation and presynaptic terminals of dopaminergic neurons), Gut, Liver, Placenta, Skin, Fibroblasts	Brain (posterior hypothalamus and dorsal raphe nucleus of serotonergic and histaminergic neurons, as well as in astrocytes), Blood Platelets, Lymphocytes	Human basal ganglia

The difference in the molecular nature of these two isoform leads to the discovery of two kinds of genes associated with MAO: MAO-A and MAO-B genes, whose discovery was a big breakthrough in the field of neuropharmacology. This discovery allowed better understanding of the pharmacological functions of these molecules. The immunologically distinct isozymes are coded for by the separate but closely related genes located on the X chromosome (Xp 11.4 – Xp 11.23) [Kochersperger *et al.* 1986; Lan *et al.* 1989]; in the opposite direction with tail-to-tail orientation. Moreover, both genes display identical number of exons and intron-exon organisation [Ou *et al.* 2006], suggesting that the two genes are likely derived from the duplication of a common ancestral gene.

1.3.2. Role of MAO in neurodegeneration

MAO-A preferentially catalyzes the deamination of serotonin and norepinephrine. Mild retardation and prominent behavioural abnormalities in males [Brunner *et al.* 1993] and aggressive behaviour in mice [Cases *et al.* 1995] has been observed in case of loss of MAO-A function. Thus MAO-A inhibitors are used for the treatment of mental disorders, particularly depression [Lipper *et al.* 1979; Robinson *et al.* 1973]. They are indicated in

panic attack disturbances, in dementia-associated depression (non-severe forms), in atypical depression [West and Dally 1959], in social phobia [Fyer and Gorman 1986] as well as in anxiety [Rudorfer and Potter 1989; Palhagen *et al.* 2006]. They have also been shown to have mild antimuscarinic effects. MAO-A is more sensitive to inhibitors like clorgyline and moclobemide. Both genes encoding MAO-A and MAO-B are deleted in atypical Norrie disease [Sims *et al.* 1989].

On the other hand, MAO-B has higher affinity for β-phenylethylamine and is selectively inhibited by low concentrations of selegiline (L-deprenyl) and rasagiline [Weyler *et al.* 1990; Lang and Lees 2002]. Selective MAO-B inhibitors are used as adjuvants for the treatment of Alzheimer's disease (AD) [Cesura and Pletscher 1992; Youdim *et al.* 2004] and Parkinson's disease (PD) [Riederer *et al.* 2004; Guay 2006].

The rapid degradation of brain monamines, such as 5-HT, NE and DA is essential for the proper functioning of synaptic neurotransmission and is critically important for the regulation of emotional and other brain functions. MAO catalyses the oxidative deamination of monoamines according to the following equations (**Figure 1.6.**):

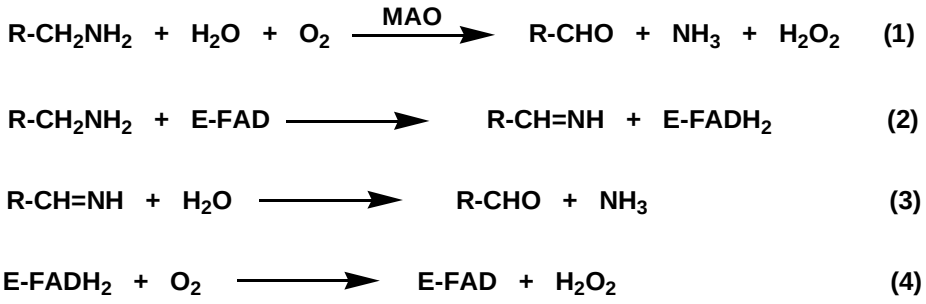


Figure 1.6. Catalytic reaction pathways of MAO

Thus, MAO causes the oxidative metabolic degradation of the various primary, secondary and tertiary amines resulting in their conversion to corresponding aldehydes [Waldmeier 1987] with simultaneous production of ammonia and hydrogen peroxide (Eqn. (1)) [Edmondson *et al.* 2004]. This reaction is hypothesized to proceed by means of a 'Pingpong mechanism', wherein the amine is first oxidized to an imine by the MAO-bound FAD moiety viz. E-FAD (FAD reduction) (Eqn.(2)), followed by hydrolysis to the corresponding aldehyde with the release of ammonia (deamination) (Eqn. (3)). Consequentially, MAOs are regenerated by the reaction with oxygen (O_2) producing hydrogen peroxide (H_2O_2) (FAD reoxidation) (Eqn. (4)). The aldehydes in the above scheme are typically unstable and are then further rapidly metabolized to carboxylic acids

or alcohols by aldehyde dehydrogenases or alcohol dehydrogenases [Yan *et al*. 2004]. The acidic metabolites e.g. 5-Hydroxyindole acetic acid (5-HIAA) obtained from the metabolism of 5-hydroxytryptamine (5-HT, serotonin) or dihydroxyphenylacetic acid (DOPAC) obtained from degradation of dopamine are commonly used as the measure of *in-vitro* or *in-vivo* MAO activity [Green and Kelly 1976].

As from the above scheme, various potentially neurotoxic byproducts such as ammonia and hydrogen peroxide are also generated [Strolin-Benedetti and Dostert 1989; Zhang and Piantadosi 1991]. The hydrogen peroxide generated can stimulate the production of reactive oxygen species (ROS) thereby inducing protein disruption, lipid peroxidation, mitochondrial and DNA damage and neuronal apoptosis causing neuronal injury and inflammation which gradually results into a wide variety of NDDs (**Figure 1.7.**).

For all these reasons, MAO may be considered one of the most interesting potential target in the design of multifunctional ligands that might exhibit a disease-modifying activity towards multi-factorial NDDs.

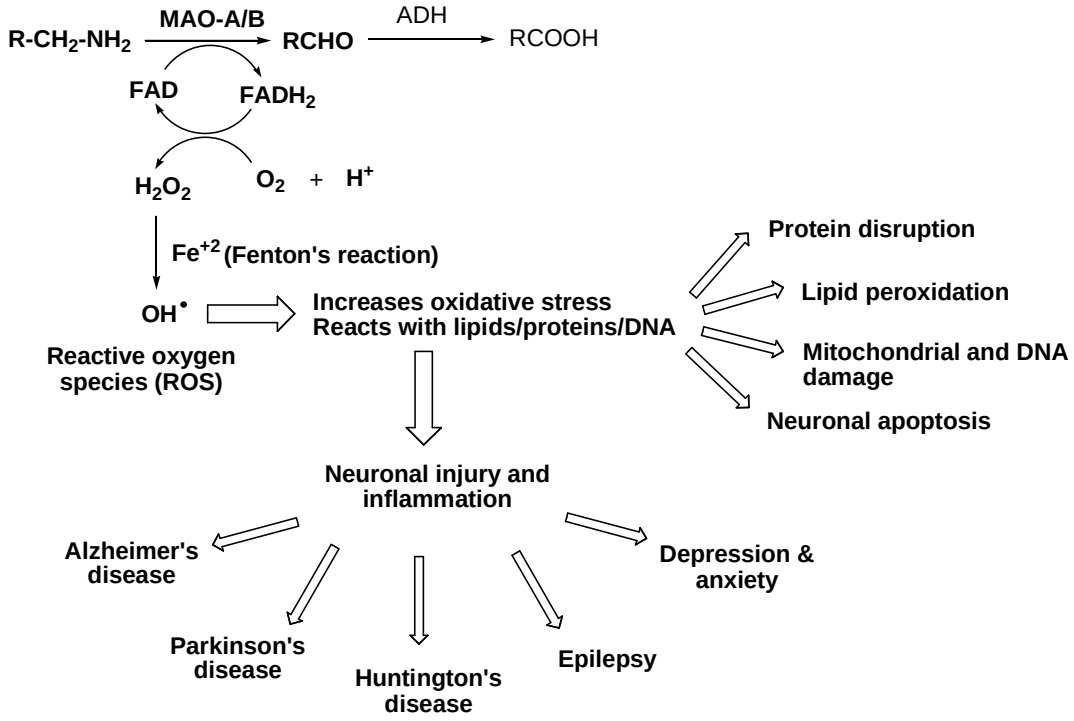


Figure 1.7. MAO catalyzed formation of cytotoxic chemical species and their consequential neuronal effects

1.3.3. Three dimensional structure of MAO

The determination of the crystal structure of MAO-A and MAO-B has resolved the catalytic mechanism involved in the selective interactions between the enzymes and their ligands or substrates, and has provided a better understanding of the pharmacophoric requirements for the rational design of potent and selective inhibitors with therapeutic potential. The RSCB Protein Data Bank (PDB) availability of experimentally determined co-crystals of MAO-A and MAO-B has allowed researchers to also perform computational studies with the aim of proposing preferred binding modes, thereby aiding in the rational design of new MAO inhibitors.

1.3.3.1. Crystal structure of human MAO-A

The crystal structure of human MAO-A (hMAO-A) was determined for the first time by Son and co-workers, in complex with harmine, a reversible MAO-A inhibitor, in 2008, at a resolution of 2.2 Å, by means of X-ray crystallographic studies. Accordingly, human MAO-A crystallized as a monomer and exhibited the solution hydrodynamic behaviour of a monomeric form [Son *et al.* 2008].

Further, the structure of human MAO-A may be divided into two domains, namely a membrane binding domain (indicated in green colour) and an extra-membrane domain. The extra-membrane domain may be further divided into the substrate/inhibitor binding region (indicated in red colour) and a FAD binding region (indicated in blue colour) (**Figure 1.8. (A)**).

The active site cavity of hMAO-A is hydrophobic in nature and has an approximate volume of 550 $Å^3$ and is formed by a cavity-shaping loop consisting of residues 210-216 [Youdim *et al.* 2006]. Further, loop comprising of the residues 108-118 is thought to regulate access to the active site. The width of the entrance is too narrow for compounds such as harmine to pass through during the steady-state phase. As a result, the flexibility of loop 108-118 together with anchoring of the enzyme into the membrane seems to be essential for substrate/inhibitor access to the active site [Son *et al*. 2008].

The study of co-crystallized structure of hMAO-A with harmine revealed that harmine interacted with the amino acid residues Tyr69, Asn181, Phe208, Val210, Gln215, Cys323, Ile325, Ile335, Leu337, Phe352, Tyr407, Tyr444 and FAD within the active centre cavity of hMAO-A. Additionally, the space between the inhibitor (harmine) and the aforementioned residues was occupied by seven water molecules. Gln215 of hMAO-

A was found to exhibit π- π interactions with coplanar aromatic rings of harmine [Son *et al*. 2008].

Literatures have revealed the similarity between the structures of hMAO-A and human MAO-B (hMAO-B). However, substrate/inhibitor selectivity between hMAO-A and hMAO-B may be attributed to residues Ile335 and Phe208 in hMAO-A, which corresponded to Tyr326 and Ile199 of hMAO-B. One of the most unique characteristics in hMAO-A being the residue Phe208 which is situated at the analogous position of residue Ile-199 in hMAO-B and consequently resulted in the elimination of an entrance cavity in hMAO-A. Thus, hMAO-A consisted of only a single cavity [De Colibus *et al*. 2005] that occupied a bigger volume compared to hMAO-B consisting of two cavities.

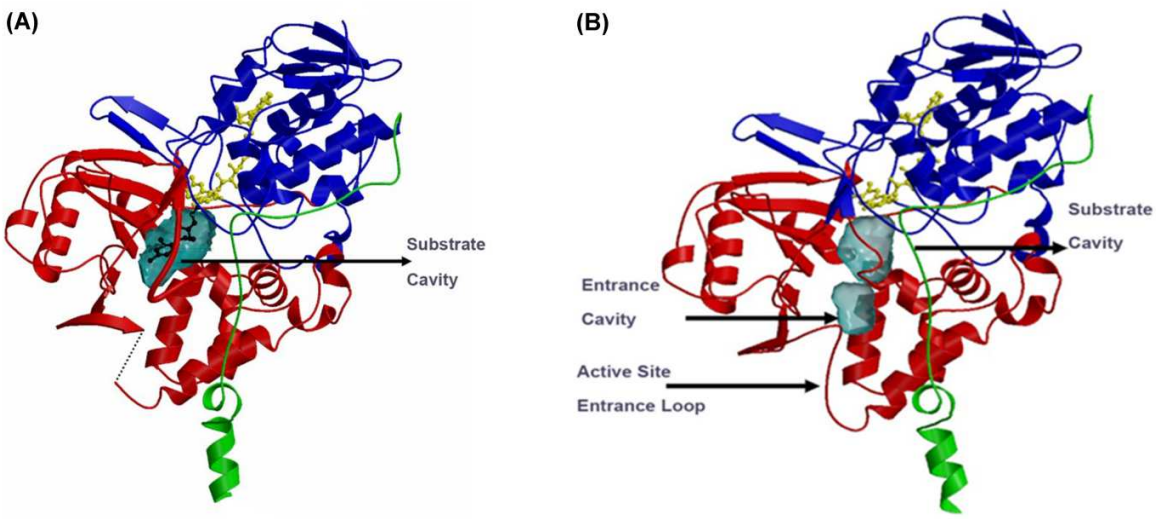


Figure 1.8. Ribbon diagram of monomeric unit of (A) human MAO-A enzyme and (B) human MAO-B enzyme. The membrane binding domain, the substrate binding domain and the flavin binding domain is represented in green, red and blue colour respectively. The covalent flavin moiety is shown in ball and stick model in yellow.

1.3.3.2. Crystal structure of human MAO-B

The first elucidation of the crystal structure of human MAO-B (hMAO-B) was determined by Binda et al., in 2002, at a resolution of 3.0 Å [Binda *et al.* 2002] and was later improved to the resolution to 1.7 Å, including co-crystals with various inhibitors [Binda *et al.* 2004]. Accordingly, in contrast to hMAO-A, hMAO-B crystallized as a dimer [Binda *et al*. 2002] with a large surface contact area between the monomers [De

Colibus *et al.* 2005]. Each of the monomers of hMAO-B consisted of a globular domain which is anchored to the outer mitochondrial membrane via a C-terminal helix [Binda *et al.* 2004; Binda *et al.* 2007].

Similar to hMAO-A, the structure of hMAO-B also consisted of two domains, namely a membrane binding domain (indicated in green colour) and an extra-membrane domain, further consisting of a substrate/inhibitor binding region (indicated in red colour) and a FAD binding region (indicated in blue colour) (**Figure 1.8. (B)**).

The active site of the hMAO-B consisted of two cavities (**Figure 1.8. (B)**), a flat hydrophobic substrate cavity with a volume of ~420 $Å^3$ and the entrance cavity with a volume of ~290 $Å^3$ [Binda *et al.* 2002, Youdim *et al.* 2006; Binda *et al.* 2007]. The hydrophobic environment may be attributed to several aromatic and aliphatic amino acid residues that line the substrate cavity. Furthermore, the substrate cavity is partitioned from the entrance cavity by the residue Tyr326, which is positioned in close proximity to the junction of the two cavities [Hubalek *et al.* 2005]. Residues Tyr326, Ile199, Leu171 and Phe168 formed the boundary between the two cavities [Binda *et al.* 2002; Son *et al.* 2008] with loop 99–112 covering the entrance cavity of 290 $Å^3$ [Binda *et al.* 2002; Youdim *et al.* 2006]. The entrance cavity is lined by the residues Phe103, Pro104, Trp119, Leu164, Leu167, Phe168, Leu171, Ile199, Ile316 and Tyr326 [Binda *et al.* 2002]. It was found that the substrate negotiated access into the entrance cavity [Youdim *et al.* 2006] and this required movement of loop 99–112 [Binda *et al.* 2002]. Residue Ile199 acted as the "gateway" between the two cavities. The conformation of Ile199 side chain depended upon the nature of the substrate or inhibitor. The conformation of this residue concluded (decided/determined) if the substrate and entrance cavity are either fused or separated [Hubalek *et al.* 2005]. Thus, fusion of the substrate and entrance cavity via residue Ile199 resulted in the accommodation of larger ligands [Hubalek *et al.* 2005; Legoabe *et al.* 2012a].

As mentioned before, substrate/inhibitor selectivity between hMAO-A and hMAO-B may be attributed, in part, to residue Phe208 in hMAO-A which corresponded to Ile199 in hMAO-B [Son *et al*. 2008]. Binding of large MAO-B selective inhibitors may be blocked in MAO-A due to the increased bulk of the phenyl side chain of the MAO-A residue Phe-208 [Edmondson *et al.* 2009; Legoabe *et al.* 2012a]. Residue Tyr326, found at the junction of the substrate and entrance cavity [Hubalek *et al.* 2005], was also considered to

play a role in the substrate/inhibitor selectivity of the two MAO isoforms as Tyr326 prevents the binding of MAO selective inhibitors to the MAO-B active site [Son *et al*. 2008; Legoabe *et al.* 2012], thereby aiding in selectivity towards MAO-A or MAO-B.

However, the biological significance of the C-terminal of membrane binding domain of MAO-B is still unclear [Son *et al.* 2008]. The C-terminal was a transmembrane α-helix that is thought to be responsible for anchoring the enzyme to the outer mitochondrion membrane, while the rest of the protein was exposed to the cytoplasm [Youdim *et al*. 2006]. It was thought that the transmembrane helix is 27 amino acids long and initiated at Val489 [Binda *et al.* 2002]. At the end of loop 99–112, two residues (Pro109 and Ile110) also interacted with the membrane [Hubalek *et al.* 2005]. Interestingly, truncation of the C-terminal may lead to a decrease in MAO-B catalytic activity but does not significantly change inhibitor specificity [Son *et al.* 2008].

1.3.3.3. Flavin binding domain

Flavin adenine dinucleotide (FAD) is considered an essential entity which imparted the catalytic activity to the MAO enzyme. It is covalently bound to MAO apoenzyme through a thioether linkage which is formed between cysteine397 (Cys397) residue and the 8α-methylene of isoalloxazine ring [Kearney *et al.* 1971; Bach *et al*. 1988]. This binding was found to be located near the C-terminus of MAO enzyme [Edmondson *et al.* 2004].

Table 1.2. shows the comparison of amino acid residues which are involved in the formation of active site of human MAO-A and MAO-B isoforms. The amino acid residues Ile335 in hMAO-A and Tyr326 in hMAO-B plays an important role in determining the substrate and inhibitor specificities in human MAO-A and MAO-B. All the amino acid residues are same in case of both MAO-A and MAO-B except the amino acid residues Leu171, Cys172, Ile199 and Tyr326 which is present in MAO-B only, whereas in case of MAO-A these residues are replaced by Ile180, Asn181, Phe208 and Ile335 respectively (**Figure 1.9.**).

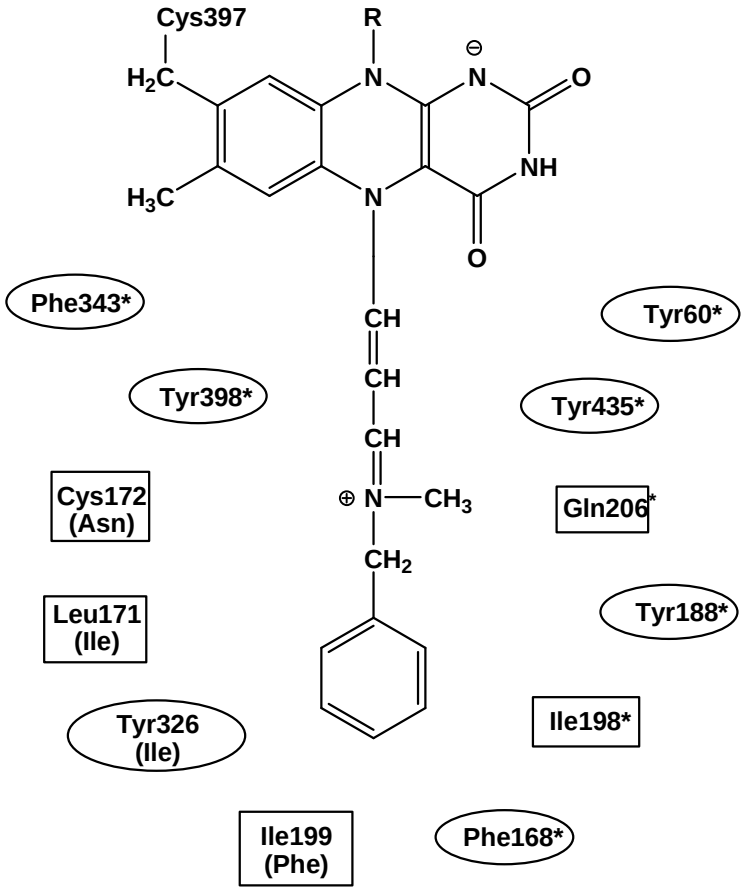


Figure 1.9. Schematic representation of active site of MAO-B. Asterisk represents the amino acid residues that are conserved in hMAO-A and hMAO-B both. The residues in parentheses represents the replacement side chains of MAO-A for non-conserved amino acids. Ellipsoidal frames represent the aromatic side chains while rectangular boxes represent the other residues [Binda *et al*. 2002].

Table 1.2. Comparison of amino acid residues involved in the formation of active site of human MAO-A and MAO-B [Krueger *et al.* 1995; Veselovsky *et al.* 2004]

Human MAO-A	**Human MAO-B**	**Human MAO-A**	**Human MAO-B**
Tyr69	Tyr60	Phe208	Ile199
Phe177	Phe168	Gln215	Gln206
Ile180	Leu171	Ile335	Tyr326
Asn181	Cys172	Phe352	Phe343
Tyr197	Tyr188	Tyr407	Tyr398
Ile207	Ile198	Tyr444	Tyr435

1.3.4. Monoamine oxidase inhibitors

Monoamine oxidase inhibitors (MAOIs) are the agents which inhibits the activity of monoamine oxidase (MAO), thereby preventing the breakdown of monoamine neurotransmitters, hence increasing their availability. MAOIs may be divided into two categories namely, reversible and irreversible inhibitors based on the interactions of the MAOIs with the enzymes. **Table 1.3.** lists the various non-selective (**Table 1.3 (A).**) and selective MAO inhibitors (**Table 1.3 (B). and Table 1.3 (C).**) with their therapeutic actions along with molecules in clinical trials (**Table 1.3 (D).**).

Table 1.3 (A). Non-selective MAO-A/MAO-B inhibitors

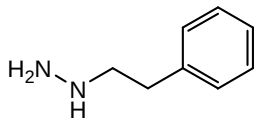
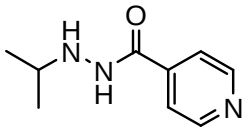
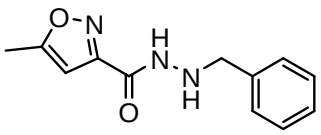
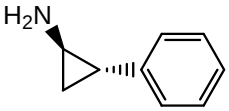
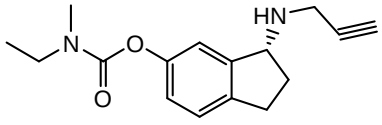
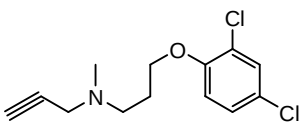
Name	Structure	Type of MAO inhibition	Clinical uses
Phenelzine (FDA)		Irreversible	Depression/ Anxiety
Iproniazid (FDA)		Irreversible	Depression
Isocarboxazid		Irreversible	Depression
Tranylcypromine (FDA)		Irreversible	Depression/ Anxiety
Ladostigil		Irreversible	Depression, AD, PD

Table 1.3 (B). Selective MAO-A inhibitors

Name	Structure	Type of MAO inhibition	Clinical uses
Clorgyline		Irreversible	Depression

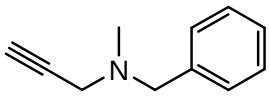
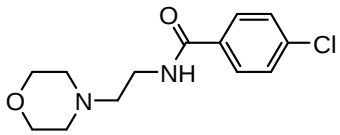
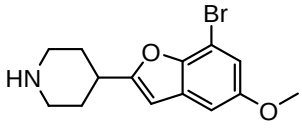
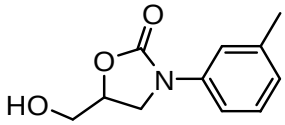
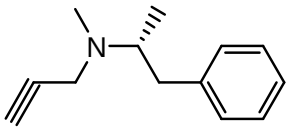
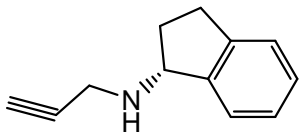
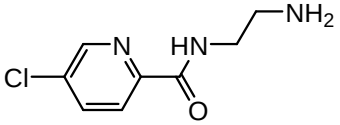
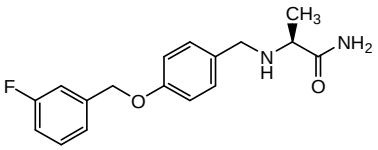
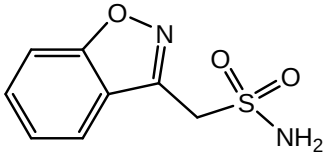
Pargyline (FDA)		Irreversible	Depression
Moclobemide (FDA)		Reversible	Not approved in US
Brofaromine (FDA)		Reversible	Depression
Toloxatone		Reversible	Depression

Table 1.3 (C). Selective MAO-B inhibitors

Name	**Structure**	**Type of MAO inhibition**	**Clinical uses**
Selegiline (L-deprenyl) (FDA)		Irreversible	PD
Rasagiline (FDA)		Irreversible	PD
Lazabemide (FDA) (Ro 19-6327)		Reversible	Anti-PD in clinical trials
Safinamide (FDA)		Reversible	Anti-PD in clinical trials, antiepileptic
Zonisamide (FDA)		Reversible	Antiepileptic and anti-PD in Japan

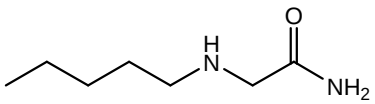
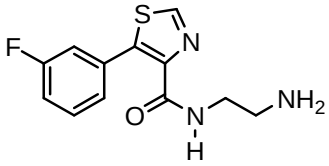
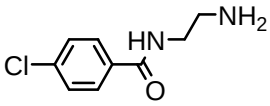
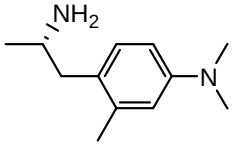
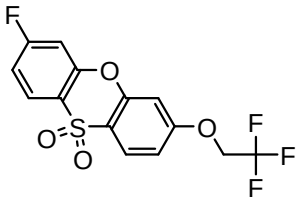
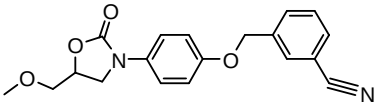
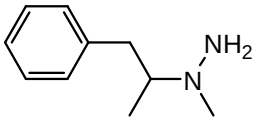
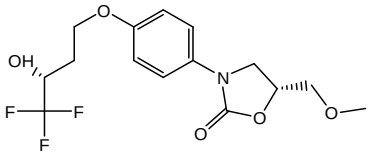
Milacemide		Reversible	Anti-PD

Table 1.3 (D). Molecules in clinical trials

Name	**Structure**	**Type of MAO inhibition**	**Clinical uses**
Ro 41-1049		Selective and reversible MAO-A inhibition	
Ro 16-6491		Reversible MAO-B inhibition	
Amiflamine		Reversible MAO-A inhibition	
Tyrima (CX157)		Reversible MAO-A inhibition	In 2013, discontinued for depression and anxiety
Cimoxatone		Reversible MAO-A inhibition	
Metfendrazine		Non-selective and irreversible MAO-A inhibitor	Antidepressant
Befloxatone (M370503)		Reversible MAO-A inhibition	

1.3.4.1. Mode of action of MAOIs

5-HT and NE are the important components of the neurotransmitter system, the metabolism of which occurs by MAO-A and results in the depletion of their levels which is directly linked to depression. Inhibition of MAO-A results in decreased degradation of these monoamines thereby demonstrating their increased brain levels. Thus MAO-A inhibitors rather than the B inhibitors potentiate pharmacological and behavioural actions mediated by 5-HT and NA and hence act as antidepressants [Finberg and Youdim 1983]. However, MAO-B inhibitors are ineffective as antidepressants since there is no direct effect of MAO-B on the metabolism of either 5-HT or NE.

MAO-B is an enzyme involved in the metabolism of dopamine. It converts dopamine to its corresponding carboxylic acid via an aldehyde intermediate. Thus, MAO-B regulates both the free intraneuronal concentration of dopamine and the releasable stores. MAO-B inhibitors bind to and inhibit MAO-B, preventing dopamine degradation. This results in greater stores of dopamine available for release. This makes the selective MAO-B inhibitor a useful drug in the treatment of Parkinson's disease because the dopamine level of the parkinsonian human brain basal ganglia is dramatically decreased. Although not all features of their anti-Parkinson action is known, studies on brains obtained at autopsy from patients on (-) deprenyl showed that the selective inhibition of MAO-B with a concomitant increase of dopamine, but not of serotonin, in the basal ganglia may be responsible for its mode of action. [Youdim 1986]. In addition, there have been reports that MAO-B converts some environmentally derived amine substrates, namely protoxins, into toxins which may cause damage to the neurons and perhaps contribute to the cause or decline of function in PD [Herraiz *et al.* 2013]. Inhibition of MAO-B may thus halt the process, and may decrease the rate of degeneration of various neurodegenerative diseases.

1.3.4.2. Adverse effects of MAOIs

MAO is responsible for the metabolism of tyramine, a trace amine found in some foods that-act as a sympathomimetic. MAO inhibition allows accumulation of excess tyramine resulting in hypertensive crisis due to the release of norepinephrine called as "cheese effect"; therefore, patients availing MAO inhibitor (MAOI) are recommended to follow dietary restrictions to avoid tyramine-rich foods [Anderson *et al.* 1993]. Hypertensive crisis may also be precipitated by using MAOIs in conjunction with other drugs that have

vasoconstrictive properties, that act as sympathomimetics, or that inhibit the reuptake of norepinephrine. Moreover, serotonin syndrome is another serious adverse effect that can potentially occur when using an MAOI with another drug that inhibits the reuptake of serotonin [Boyer and Shannon 2005]. Hepatotoxicity also represents one of the severe side effects of MAOIs. Besides, the common side effects include dry mouth, nausea, diarrhoea or constipation, headache, drowsiness, dizziness or light-headedness, skin reaction at the patch site, sudden drop in blood pressure upon standing up called as orthostatic hypotension, etc.

1.4. Acetylcholinesterase: an overview

Acetylcholinesterase (AChE, EC 3.1.1.7) belongs to ubiquitous class of serine hydrolase family of enzymes which catalyzes the hydrolysis of the ester bond in acetylcholine (ACh) leading to the release of choline and acetic acid, thereby causing termination of ACh activity and hence cholinergic neurotransmission [Massoulie *et al.* 1993; Taylor 1994].

Its catalytic mechanism is among the most efficient known, rate approaches that of a diffusion-controlled reaction, the substrate turnover is 25000 molecules sec^{-1}, and each turnover lasts about 40 µs. The hydrolysis reaction proceeds by nucleophilic attack of the carbonyl carbon, involving acylation of the enzyme and release of choline. This is followed by a rapid hydrolysis of the acylated enzyme yielding acetic acid, and restoring the esteratic site (**Figure 1.10.**).

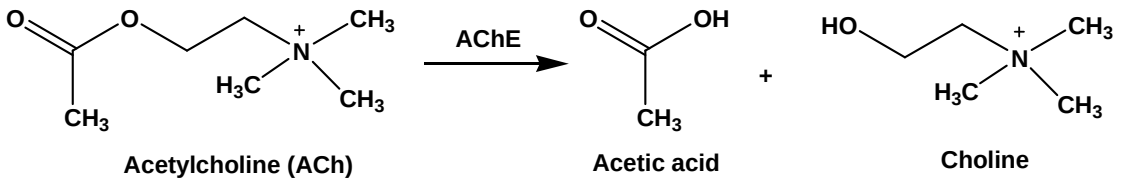


Figure 1.10. Hydrolysis of ACh by AChE

1.4.1. Molecular characteristics of AChE

AChE is present both in the CNS and PNS. In the CNS, ACh is believed to be involved in learning, memory, and mood while in the PNS, ACh plays an important role in skeletal muscle contraction and movement. AChE is abundantly found in nervous tissue, muscles [Brimijoin, 1983], blood cells and plasma [Heller and Hanahan 1972; Szelenyi *et al.* 1982]. The molecular weight of AChE is 32 KDa.

It exists in complex molecular polymorphs of quaternary structure displaying similar catalytic properties but differing in hydrodynamic parameters and ionic or hydrophobic interactions [Massoulie *et al.* 1993]. Because of the selective loss of molecular forms of AChE in AD [Atack *et al.* 1983], understanding of its molecular polymorphism is of great interest. The various molecular forms of AChE includes (a) the asymmetric form which are mainly restricted to the neuromuscular junction in the extracellular matrix, (b) the globular form, secreted as soluble forms, which exists as monomers, dimers or catalytic tetramers and are preferentially expressed in mammalian central nervous system; these are anchored to the membrane by a hydrophobic domain, while (c) other forms are confined to nerves, muscles, erythrocytes and lymphocytes and are linked to the membrane by a glycolipid anchor.

The principal biological role of AChE is the termination of impulse transmission at cholinergic synapses as a result of rapid hydrolysis of the neurotransmitter acetylcholine, within both the CNS and PNS, and hence its localization is mainly related to cholinergic system. The localization and function of AChE is presented in **Table 1.4.**

Table 1.4. Localization and function of AChE

Localization	Brain	Periphery
	Cholinergic Neuron	Neuromuscular junction, Heart, Liver
Function	Brain cholinergic, Neurotransmission, Memory and cognition	Peripheral cholinergic neurotransmission, Cardiovascular regulation, Neuromuscular activity, Papillary control

1.4.2. Role of AChE in neurodegeneration

According to the ‘‘cholinergic hypothesis’’, impairment in the cholinergic function is of crucial significance in NDDs such as AD especially in the brain areas associated with learning, memory, behaviour and emotional responses that include the neocortex and the hippocampus. Brain atrophy is the most evident clinical finding in NDDs in which the levels of ACh are decreased due to its rapid hydrolysis by the enzyme AChE [Ladner and Lee 1998].

Another hypothesis is ‘‘amyloid hypothesis’’ according to which AChE produces secondary non-cholinergic functions that includes beta-amyloid (Aβ) deposition in the form senile plaques/neurofibrillary tangles in the brain of affected individuals [Castro and

Martinez 2001; 2006; Selkoe 2002; Bartolini *et al.* 2003]. The deposition of Aβ has been considered to play an important role in the initiation as well as in the progression of AD [Rees *et al.* 2003]. These deposits further trigger an extensive array of neurotoxic events including oxidative stress, tau hyperphosphorylation, inflammation, apoptosis and calcium dysregulation. Therefore, a rational approach to enhance the cholinergic neurotransmission and thus, the cognitive functions is to inhibit the activity of AChE leading to increase in the availability of ACh in the synaptic cleft. Thus, AChE inhibition has emerged as a critical target regarding the effective management of NDDs like AD, since its inhibition not only provides the symptomatic relief by increasing the synaptic availability of ACh, but also may be critical in inhibiting the progression of disease by preventing the deposition of Aβ. The reason behind the increased potential efficacy of AChE inhibitors stems from the occasional discovery of a non-catalytic action of the enzyme, whose peripheral anionic site (PAS) proved to be the important structural motif involved in the promotion of the aggregation of Aβ to form amyloid plaques [Inestrosa *et al.* 2008].

Thus, blockade of the enzyme AChE causes inhibition of the hydrolysis of ACh which results in the increase in the levels of ACh in cholinergic synapses and can subsequently affect a number of pathogenic processes and thereby provides a possible treatment for AD and other NDDs. Apart from its role in cholinergic neurotransmission, it is also involved in non-cholinergic responses like cell differentiation and proliferation, stress and amyloid formation (**Figure 1.11.**). Thus AChE inhibition acts as one of the viable and winning strategy to solve cognitive and behavioural symptoms associated with various NDDs.

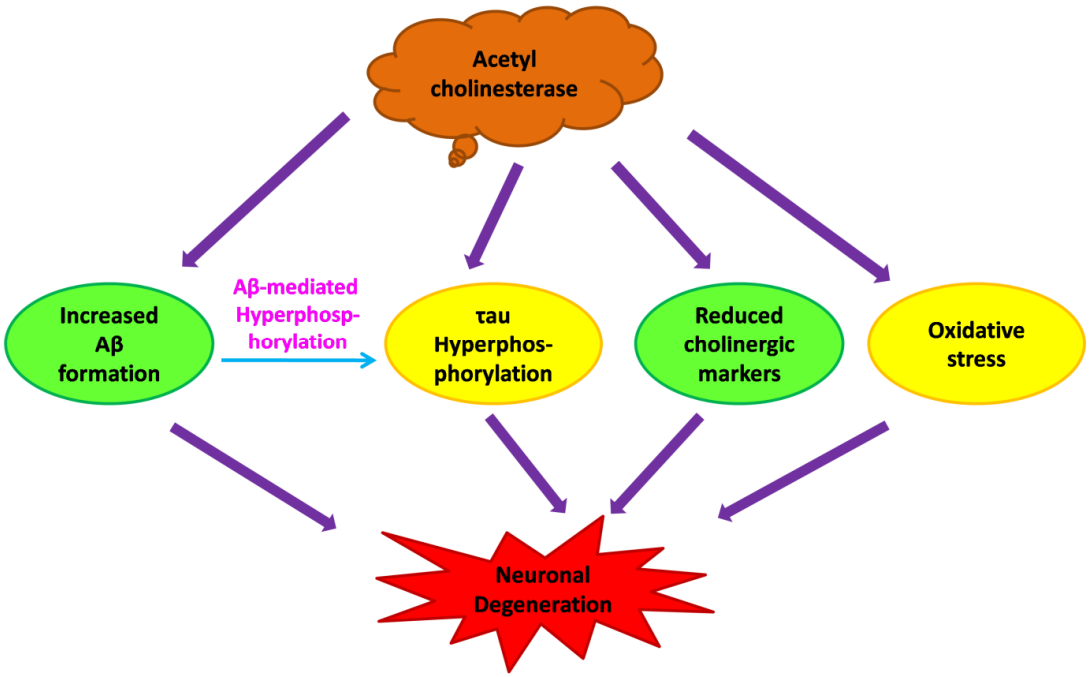


Figure 1.11. Role of AChE in neurodegeneration

1.4.3. Three dimensional structure of AChE

The crystal structure of AChE was elucidated for the first time from the electric organ tissue of *Electrophorus electricus* and the crystals were isolated in tetrameric form. [Leuzinger and Baker 1967]. However, though their preliminary characterization was reported [Chothia and Leuzinger 1975; Schrag *et al.* 1988], no information regarding the structural data were obtained. Later in 1991, Sussman and coworkers determined the first crystal structure of *Tc*AChE at a resolution of 2.8 Å [Sussman *et al.* 1991]. This was followed by the discovery of a series of 3D structures of *Tc*AChE complexed with a broad repertoire of inhibitors, including anti-Alzheimer drugs, [Greenblatt *et al.* 2003] as well as the structures of mouse [Bourne *et al.* 2003] and *Drosophila* [Harel *et al.* 2000] AChE, and those of human [Kryger *et al.* 2000], *Torpedo* [Harel *et al.* 1995] and mouse [Bourne *et al.* 1995] AChE complexed with the snake venom toxin, fasciculin.

Recently, in 2012, Cheung and co-workers presented a high resolution (resolution = 2.35 Å) crystal structure of human AChE, in complex with the drug ligand; donepezil, an Alzheimer's disease drug; revealing the various binding sites and the interactions involved between the ligand and the human enzyme and the amino acid residues critical for the inhibition of the human enzyme. This discovery has brought an important

breakthrough in the field of development of anti-acetylcholinesterase agents and to understand the subtle factors that govern AChE inhibition activity [Cheung *et al.* 2012].

1.4.3.1. Crystal structure of human AChE

The ribbon diagram of human AChE (hAChE; PDB Code: 4EY7) is represented in **Figure 1.12. (A)**.

The enzyme is an α/β hydrolase having the molecular weight of 65612, containing 537 amino acids and consisting of 12-stranded mixed β-sheet surrounded by 14 α-helices (**Figure 1.12. (B)**). The enzyme has a β-sheet platform that bears the catalytic machinery and is rather similar in all members of the family.

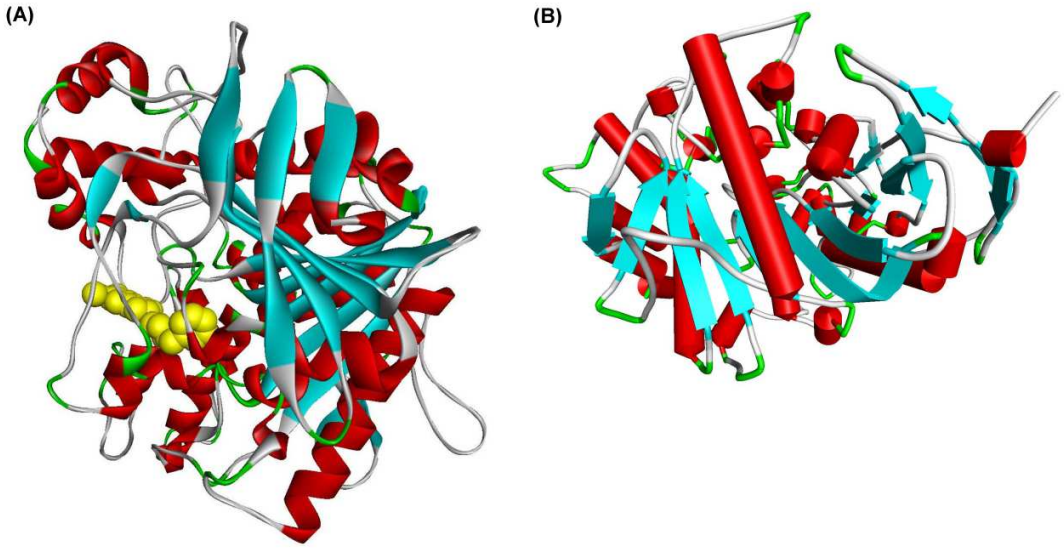


Figure 1.12. Structure of hAChE (A) Ribbon diagram. Donepezil (yellow) lies in the gorge of hAChE. (B) Schematic diagram showing β-strands (cyan) and α-helices (red).

Indeed, the three members of the catalytic triad (Glu-His-Ser) appeared in the same order along the polypeptide chain in all α/β hydrolase enzymes (Ollis *et al.* 1992). The α-helices and loops are then ascribed by the task of handling the specificity element, substrates of different members of the family being very varied. It is ellipsoidal in shape, with the approximate dimensions of 45 x 60 x 65 Å. [Sussman *et al.* 1991; Silver 1974]. The X-ray crystallographic structure of hAChE consists of a deep and narrow hydrophobic gorge, about 20 Å long, which penetrates more than halfway into the enzyme, and widens out close to its base (**Figure 1.13.**). It is composed of two distinct binding sites: the active site and the peripheral anionic site (PAS).

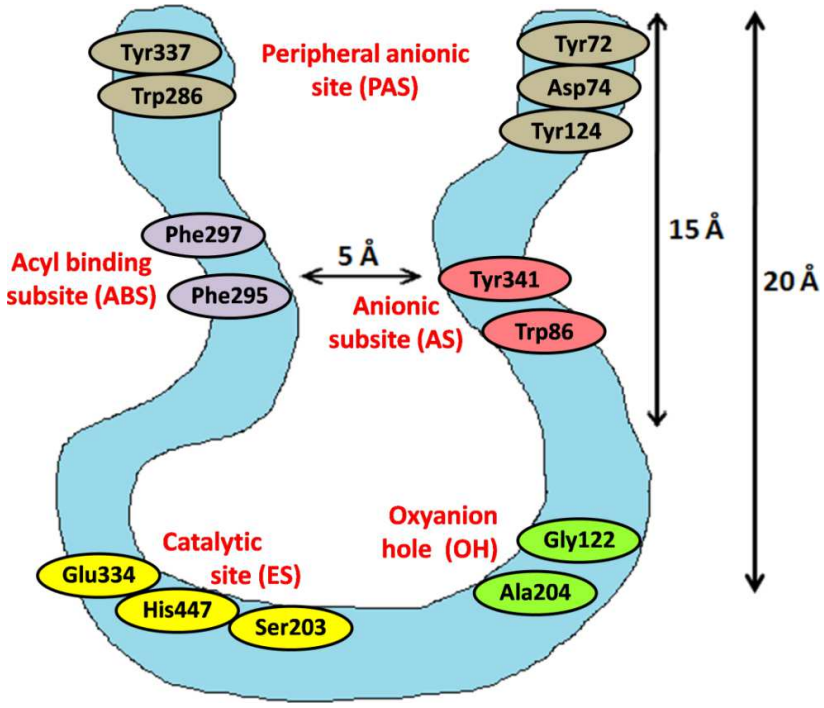


Figure 1.13. Schematic representation of binding sites of hAChE

1.4.3.2. Active site of AChE

The active site is situated at the bottom of the narrow gorge and is further composed of several sub-domains: (a) Catalytic/esteratic/ecstatic site (ES) (b) Anionic subsite (AS)/anionic substrate binding site/choline-binding subsite or hydrophobic subsite (c) Acyl binding site (ABS) and (d) the "oxyanion hole" (OH).

(a) Catalytic/esteratic/ecstatic site (ES)

This site comprises the catalytic triad Serine (Ser)-203; Histidine (His)-447; Glutamic acid (Glu)-334 and acts the catalytic machinery of the enzyme. AChE uses the Ser-His-Glu catalytic triad to enhance the nucleophilicity of the catalytic serine. The strongest hydrogen bond between His-447 and Ser-203 improves the ability of Ser for a nucleophilic attack on the substrate and Glu-334 stabilizes the histidinium cation of the transition state during the hydrolysis of ACh (**Figure 1.14.** and **Figure 1.15.**).

(b) Anionic subsite (AS)/anionic substrate binding site/choline-binding subsite or hydrophobic subsite

It consists of small number of negatively charged molecules and the aromatic residues viz. Trp86, Tyr341 and Glu202 that interact with quaternary ammonium

part of ACh through π – π cation interactions (**Figure 1.14.**). The quaternary nitrogen of ammonium part of ACh interacts with π-electrons of the aromatic groups of tryptophan, glutamate and tyrosine residues (Trp86, Tyr341 and Glu202) [Silver 1974; Lockridge *et al.* 1987]. Trp86 is an important residue for binding of ACh and its substitution by an aliphatic residue (alanine) results in a large decrease in reactivity toward ACh [Vellom *et al.* 1993].

(c) Acyl binding site (ABS)

This site contains Phe295 and Phe297 (**Figure 1.14.**) that bind to acetyl group of ACh and is responsible for the selectivity of the substrate by preventing the access of the larger members of choline ester series.

(d) The "oxyanion hole" (OH)

This domain consists of three amino acid residues (peptide residues) i.e. Gly121, Gly122 and Ala204 as hydrogen bond donors that stabilize the tetrahedral intermediate state of ACh in catalytic process (**Figures 1.14.** and **1.15.**).

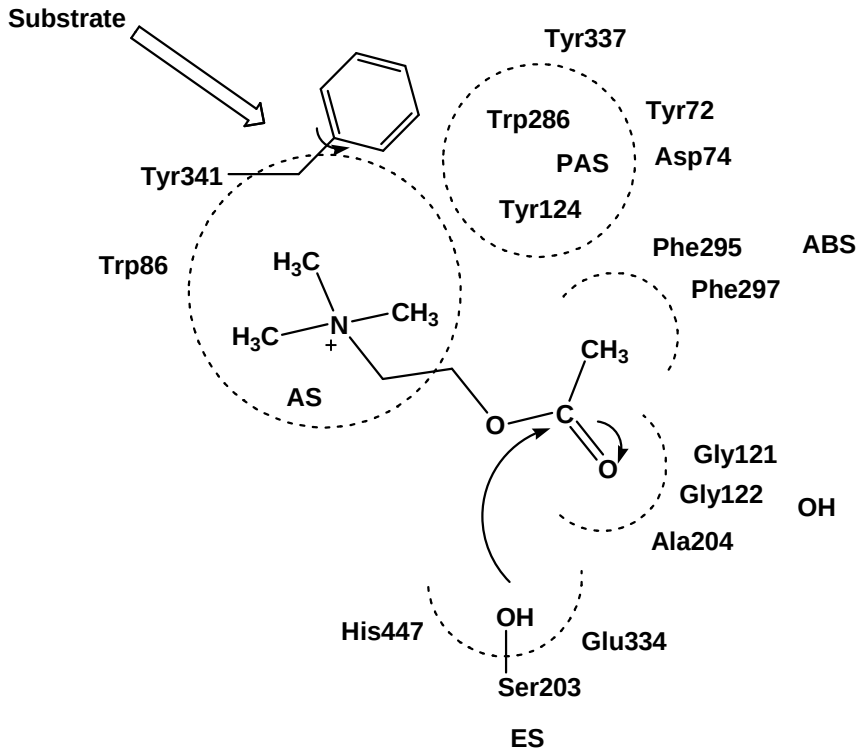


Figure 1.14. Binding orientation of ACh within the binding pockets of hAChE (ES, AS, ABS, OH and PAS)

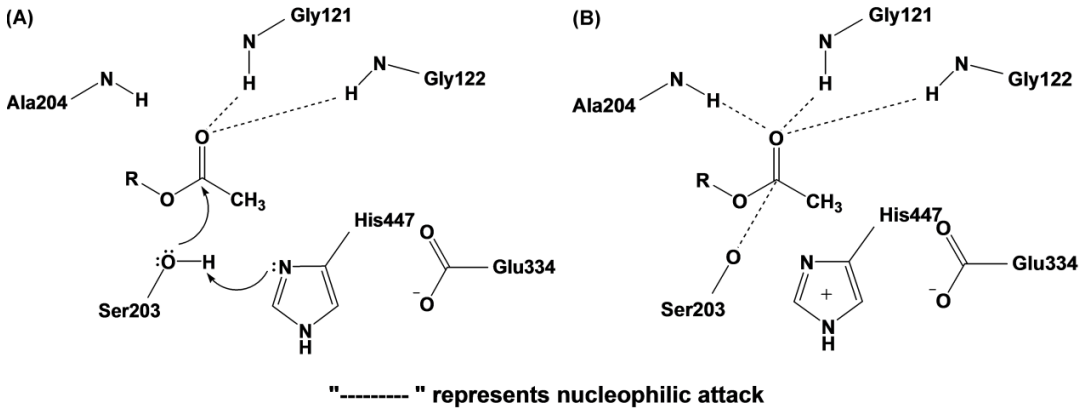


Figure 1.15. (A) Interaction of ACh with the catalytic triad and 'oxyanion hole' of hAChE. (B) The transfer state of substrate, tetrahedral intermediate, 'oxyanion hole' and the catalytic triad.

1.4.1.1. Peripheral anionic site

Peripheral anionic site (PAS) is located at the surface of AChE at a distance of 20 Å from an active site (**Figure 1.13.**). It is comprised of 5 amino acid residues Tyr72, Asp74, Tyr124, Trp286 and Tyr337 forming the cage around the entrance of the active site gorge. It binds to ACh and allosterically modulates catalysis which represents the first step in the catalytic pathways. Besides, PAS has also been identified as a site promoting non-cholinergic functions viz. cell proliferation and differentiation, cell adhesion and neurite outgrowth in developing and transformed neural cells and amyloidosis via interaction with the amyloid β-peptide in AD. In addition, PAS is associated with the surface loops that incorporate several of its residues. The large omega loop Cys69–Cys96 incorporates Tyr72 and Asp74. The latter section of this loop forms part of the outer wall of the gorge, and includes Trp86, the principal component of the anionic site. The surface loops 275- 305 lies on the opposite side of the gorge and includes Trp286. There are ten acidic residues in the area surrounding the PAS, forming the "annular electrostatic motif" and results in increased concentration of negative charge, which is shared by AChE. The aromatic PAS residues, with Trp286 at their core, appear to act synergistically. The aromatic ring of Tyr72 and Tyr124 flank the indole of Trp286, displaying a variety of interaction modes, including stacking, aromatic-aromatic and π-cation, depending upon the nature of the ligand and interact with charged groups of ligands. It is possible that the

π electron system of the indole may be polarised by the adjacent Glu285, which may result in enhancement of the stability of the interaction modes.

1.4.1.2. Mechanism of action of catalytic triad

The catalytic triad has been termed the "charge relay system". In hAChE, as indicated above, the triad includes Ser203, the imidazole ring of His447 and the carboxylic group of Glu334. During the binding of ACh to AChE, the charge relay system causes electron shifts yielding to the acylation of the enzyme. The tetrahedral intermediate collapses to the acylated enzyme by acid catalyzed exulsion of choline by His447. The acylated enzyme complex is finally rapidly hydrolyzed, regenerating active enzyme by releasing acetic acid.

1.4.2. Clinically approved AChE inhibitors

AChE inhibitors (AChEIs) can be classified into different groups, based on their mode of interaction with the AChE enzyme. Inhibition can be either reversible, by competitively preventing the substrate from reaching the active site; pseudo-irreversible, by covalent reaction with the active site serine, inactivating the catalytic ability of the enzyme or irreversible. Competitive inhibitors act by blocking substrate at the active site, non-competitive inhibitors by binding to the PAS.

To date only four AChEIs have been clinically approved by regulatory agencies such as U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). Tacrine [1,2,3,4-tetrahydroacridin-9-amine; Cognex®; THA; IC_{50} (nM): hAChE; 30, hChE; 95] [Qizilbash *et al.* 2000] was the first clinically approved AChE inhibitor followed by donepezil [(RS)-2-[(1-benzyl-4-piperidyl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one; Aricept®; DNP; IC_{50} (nM): hAChE; 5.7, hChE; 14] [Birks and Harvey 2006], galantamine [(4aS,6R,8aS)-5,6,9,10,11,12-hexahydro-3-methoxy-11-methyl-4aH-[1]benzofuro[3a,3,2-ef][2]benzazepin-6-ol; Razadyne®; GAL; IC_{50} (nM): hAChE; 3800, hChE; 575] [Olin and Schneider 2002] and rivastigmine [(S)-3-[1-(dimethylamino)ethyl]phenyl-N-ethyl-N-methylcarbamate; Exelon®; RIV; IC_{50} (nM): hAChE; 4150, hChE; 9120] [The Cochrane database of systematic reviews 2009] have been developed and are successfully available in the market (**Figure 1.16.**).

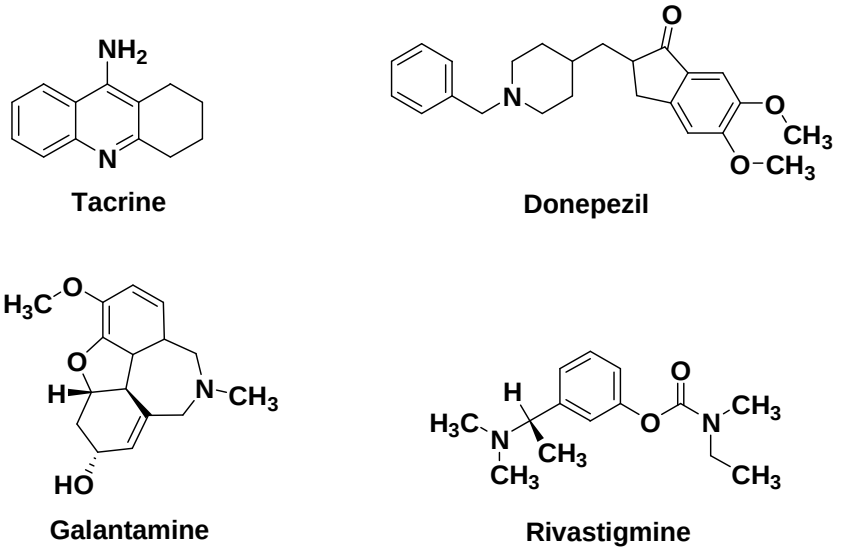


Figure 1.16. FDA approved AChE inhibitors

All these drugs have achieved various levels of clinical use for easing the effects of dementia in the first six months following diagnosis. However, none of these medications have shown good long-term tolerability or efficacy over a wide range of patients. The development of resistance, side effects, cost and persistent progression of disease are major limitation of these drugs. In view of the limited number of AChE inhibitors currently available clinically, the search for new AChE inhibitors is of great interest worldwide. Therefore, there has been a continuous research related to the synthesis of more potent and highly efficacious AChE inhibitors for the management of NDDs (AD). Accordingly, scientists have modified the main template moieties of clinically available AChE inhibitors in the search of more potent compounds. Others have also used other scaffolds to obtain drugs that may inhibit the AChE and overcome the symptoms of the disease.

1.4.2.1. Mode of action of AChE inhibitors in NDDs

Genetic, biochemical, and behavioural research suggested that physiologic generation of the neurotoxic Aβ peptide from sequential amyloid precursor protein (APP) proteolysis is the crucial step in the development of NDDs like AD. AChE inhibitors act by decreasing the APP thereby reducing the deposition of amyloid-β (Aβ) through amyloid pathway. Further, it also attempts to improve the cholinergic neurotransmission by increasing the synaptic availability of ACh through cholinergic neurotransmission by increasing the synaptic availability of ACh through cholinergic pathway (**Figure 1.17.**).

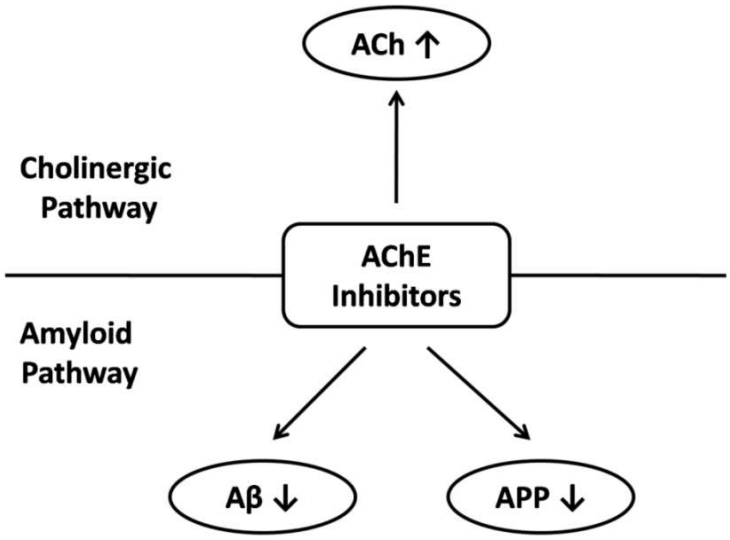


Figure 1.17. Dual role of AChE inhibitors in the management of NDDs

1.4.2.2. Adverse effects of AChE inhibitors

It has been reported that AChE inhibitors induce adverse events resulting from activation of the peripheral cholinergic systems which includes nausea, vomiting and diarrhoea. Tolerance to these side effects often develops and can usually be managed. However, among the potentially serious side effects includes abdominal pain, lack of appetite, dizziness, yellowing of the skin, bradycardia, sudden or substantial weight loss, hypotension, hypersecretion, bronchoconstriction, GI tract hypermotility, decreased intraocular pressure [Consumer Reports: Drug Effectiveness Review Project 2012; Inglis 2002] and SLUDGE syndrome [Wagner and Promes 2007]. Actions of AChE inhibitors on the neuromuscular junction resulted in prolonged muscle contraction [Consumer Reports: Drug Effectiveness Review Project 2012; Inglis 2002].

1.5. Recent advances in the discovery of anti-neurodegenerative agents

The safety and efficacy of more than 400 pharmaceutical treatments are being investigated in clinical trials worldwide, and approximately one-fourth of these compounds are in Phase III clinical trials (as of 2008), which is the last step prior to review by the regulatory agencies. One area of clinical research is focused on treating the underlying disease pathology.

In the perspective of NDDs, the most widely adopted recent approach is to discover the multi-target-directed ligands effective in encompassing dual inhibition of both the

enzymes MAO (MAO-A and/or MAO-B) and AChE and this have been regarded as putative therapy to treat people suffering from NDDs. It has been extensively reported that MAO inhibitors, targeting both MAO-A and MAO-B and AChE inhibitors may possess enhanced benefits against NDDs such as prevention of oxidative stress conditions resulting due to generation of reactive oxygen species (ROS) through MAO inhibition, reduction of Aβ aggregation and increased cholinergic neurotransmission due to the blockade of the dual binding site of AChE i.e. both the catalytic anionic site (CAS) and the PAS of the enzyme.

The experimental evidences supporting this research line includes the following facts: (a) the MAO-B activity in the temporal, parietal and frontal cortex part of the brain is found to increase upto 3-fold in patients suffering from AD in comparison to control patients [Saura *et al.* 1994]; (b) the interconnection between cognitive and behavioural disorders associated with decreased concentration of AChE in brain and the observed modification in other neurotransmitter systems mediated by biogenic amines whose level is influenced by the catabolic action of MAOs [Saura *et al*. 1994].

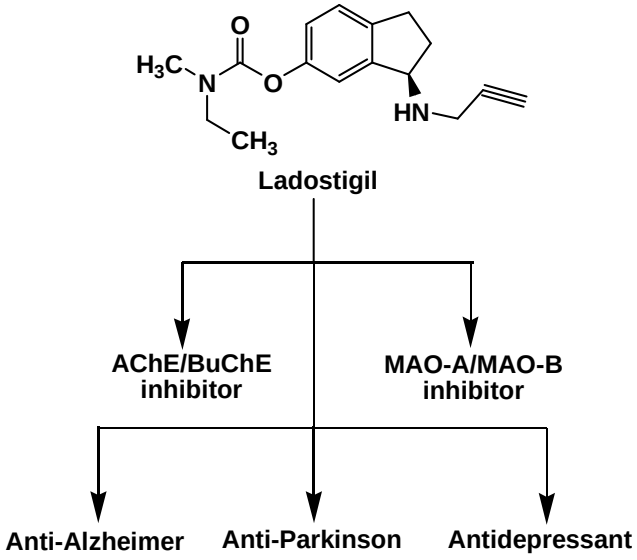


Figure 1.18. Various actions of ladostigil

Several heterocyclic compounds have been reported as possible leads for further development based on a well-balanced profile of AChE and MAO-B inhibitory activities. Ladostigil, a dual inhibitor of MAO and AChE has finished phase II clinical studies for the treatment of dementia with PD-like symptoms and depression (**Figure 1.18.**)

[Youdim *et al.* 2006]. **M30** and **HLA20** are other examples of neuroprotective agents in clinical trials exhibiting multifunctional character (**Figure 1.19.**) [Zheng *et al.* 2005].

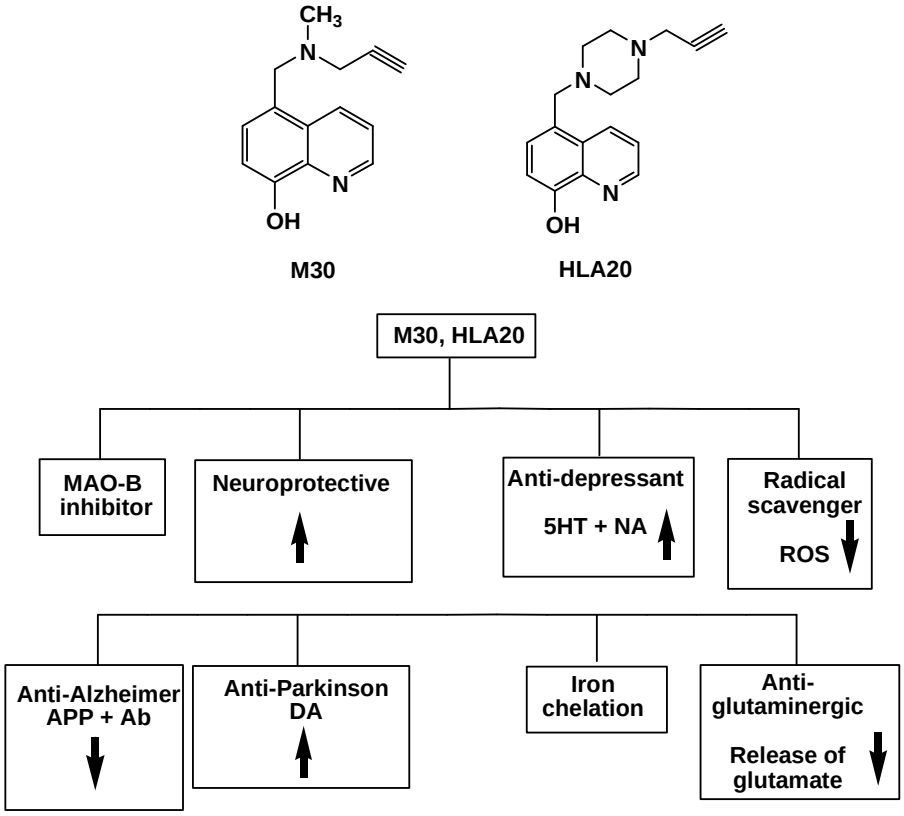


Figure 1.19. Various actions of **M30** and **HLA20**

1.6. Conclusion

The huge complexity of NDDs calls for an enormous effort to search the moieties capable of modulating multiple CNS targets and then to promote a real improvement in the treatment of NDDs. The multifactorial nature of these diseases rules out the use of the consolidated monotherapy and put scientists on the way of discovering multipotent agents hitting cooperatively different targets underlying the onset and/or progression of the disease. The use of MTDL strategy is grounded based on the idea that a MAO inhibitor and an AChE inhibitor could exhibit a more efficient therapeutic effect in the cure of NDDs when both are used synergistically, thereby limiting the sources of ROS-associated neurotoxicity.