CONVENTIONAL ORGANIC SOLVENTS AND IONIC LIQUID MEDIATED SYNTHESIS OF NEW AZOMETHINE COMPOUNDS AS POTENT CHOLINESTERASE INHIBITORS

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UNIVERSITI SAINS MALAYSIA

2016

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by

BASMA M. ABD RAZIK

Thesis submitted in fulfillment of the requirements for the degree of

Doctor of Philosophy

JANUARY 2016

DEDICATION

To Allah, who created the world.
My parents I owe you a debt of gratitude all that you have done for me. I dedicate this thesis to my parents. I hope that this achievement will complete their dream. My dedicate also to my son Ali and I thank the almighty for given you in my life.

ACKNOWLEDGEMENTS

I am grateful to many people who have assisted me to make this thesis a reality. Foremost, I am highly thankful to my main supervisor, Professor Dr. Hasnah Osman, for her guidance, comments, motivation, forgiveness and her unwavering support throughout the period of my candidature. I would like to express my appreciation to my co-supervisor, Dr. Vikneswaran Murugaiyah for his assistance and dedication in accomplishing this project. Special thanks also go to Associate Professor Dr. Abdussalam Salhin Mohamed Ali. I wish to take this opportunity to thank Universiti Sains Malaysia (USM) for providing me the giving me the chance to enroll as an Phd student in USM. I would like to thank School of Chemical Sciences, USM for providing me with the research facilities. My gratitude also goes to the staff of School of Chemical Sciences especially Mr. Megat, Mr. Zahari, Mr. Clement and Mr. Muhd Nizam Muhamad Isa for their great assistance during my research.

It is a pleasure to acknowledge the cordial working relationship that I enjoyed with all the lab mates in Lab 254 and their support and friendship. I am also appreciating the friendship that I have with all my fellow friends around School of Chemical Sciences and the happiness they had brought to me. I consider myself very fortunate to have known them all. I would also like to thank my friend Mohammad Oday Ezzat, Alireza Basiri, Yalda Kia, Nadirah Zawani, Nor Hashima and Wong Kok Tong for their help in my studies.

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LIST OF ABBREVIATIONS AND SYMBOLS

ACh	Acetylcholine
AChE	Acetylcholinesterase
AChI	Acetylthiocholine iodide
AD	Alzheimer's disease
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Å	Angstrom unit
BChE	Butyrylcholinesterase
BChI	S-butyrylthiocholine iodide
[bmim]Br	1-Butyl-3-methylimidazolum bromide
°C	Degree Celsius
ChEI's	Cholinesterase inhibitors
CHN	Carbon, hydrogen and nitrogen (Elemental analysis)
^{13}C NMR	Carbon Nuclear Magnetic Resonance
CT	Catalytic triad
d	Doublet
dd	Doublet of doublets
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
1D NMR	One Dimensional Nuclear Magnetic Resonance
2D NMR	Two Dimensional Nuclear Magnetic Resonance
DTNB	Dithiobisnitrobenzoic acid

FDA	Food and Drug Administration
FEB	Free binding energy
FT-IR	Fourier Transformer Infrared
Glu	Glutamic acid
Gly	Glycine
*h*BChE	Human butyrylcholinesterase
^{1}H-^{13}CHMBC	Heteronuclear Multiple Bond Correlation
^{1}H-^{13}CHMQC	Heteronuclear Multiple Quantum Correlation
^{1}H- ^{1}HCOSY	Correlation Spectroscopy
1H NMR	Proton Nuclear Magnetic Resonance
His	Histidine
Hz	Hertz
IC_{50}	Half-maximal inhibitory concentration
ILs	Ionic liquids
IR	Infra-red
J	Coupling constant
Leu	Leucine
m	Multiplet
mg	Milligram
MHz	Mega Hertz
mL	Milliliter
MOE	Molecular operating environment
mol	Mole
m.p.	Melting point
NMR	Nuclear Magnetic Resonance spectroscopy

ORTEP	Oak ridge thermal ellipsoid plot
PAS	Peripheral anionic site
Phe	Phenylalanine
ppm	Parts per million
Pro	Proline
s	Singlet
Ser	Serine
t	Triplet
*Tc*AChE	Torpedo california acetylcholinesterase
Thr	Threonine
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
Trp	Tryptophan
Tyr	Tyrosine
UV	Ultraviolet
Val	Valine
α	Alpha
β	Beta
γ	Gamma
%	Percentage
δ	Chemical shift
µg	Microgram
µL	Microlitre
µM	Micromolar

PELARUT ORGANIK KONVENSIONAL DAN CECAIR IONIK PERANTARA SINTESIS SEBATIAN AZOMETAN BARU BERPOTENSI SEBAGAI PERENCAT KOLINESTERASE YANG KUAT

ABSTRAK

Lima siri terbitan baru azometan telah disintesis melalui kondensasi tindak balas antara benzaldehid tentukarganti dan terbitan amino masing-masig dalam etanol suatu pelarut organik konvensional dan pelarut ionik hijau, 1-butil-3-metilimidazolium bromida ([bmim]Br). Pelarut ionik hijau, disebabkan kesan pemangkinan yang ketara, telah menunjukkan kelebihan yang luar biasa berbanding dengan etanol dari segi meningkatkan penghasilan produk dan mengurangkan masa tindak balas. Tindak balas yang dijalankan dalam pelarut ionik telah menunjukkan peningkatan yang ketara dar segi penghasilan produk daripada 18 kepada 29 % dan 6-15 kali pengurangan masa tindak balas berbanding dengan tindak balas yang dijalankan dalam etanol. Azometan ini telah dicirikan dengan menggunakan analisis unsur, teknik spektroskopi, IR, 1-D dan 2-D NMR juga X-ray kristalografi. Sebatian yang disintesiskan turut diuji aktivitinya terhadap penyakit Alzheimer's menggunakan asas kolorimetrik Ellman's. Dalam asas ini, aktiviti perencat kolinesterase bagi sebatian tesebut telah disaring secara '*in vitro'* terhadap enzim asetilkolinesterase (AChE) yang diesktrak daripada belut elektrik dan enzim butirilkolinesterase (BChE) yang diperoleh daripada serum kuda, yang mana kedua-dua enzim ini memainkan peranan utama dalam manifestasi dan perkembangan penyakit Alzheimer's. Keputusan siasatan telah menunjukkan bahawa azometan dalam siri **9** memaparkan aktiviti perencatan yang baik secara relatifnya berbanding dengan aktiviti perencatan bagi sebatian dalam siri **3**, **5**, **7** dan **11**. Kesan ini mungkin

disebabkan oleh kehadiran tiga pusat aromatik dalam struktur sebatian dalam siri **9**, yang memudahkan kemasukan dan penempatan perencat ini di dalam gaung tapak aktif AChE. Walaupun, sebatian dalam siri **5** juga terdiri daripada tiga pusat aromatik, tetapi kehadiran kumpalan karbonil dalam struktur molekul ini menghalang kemasukan dan penempatan sebatian tesebut dalam tapak aktif enzim AChE. Selain itu, sebatian **3g**, **3j**, **5j**, **7f**, **7g**, **7j**, **9f**, **9h**, **11h** telah menunjukkan aktiviti perencatan yang setara dengan aktiviti perecatan ubat piawai, galantamin. Kelakuan yang sama turut diperhatikan bagi sebatian **3j, 5j, 7j, 9h** dan **11j** dalam perencatan BChE. Suatu analisis permodelan molekul, '*in silico*' dengan menggunakan struktur kristal Torpedo californica AChE (*Tc*AChE) dan BChE (*h*BChE) manusia telah digunakan untuk mendedahkan orentasi dan mekanisma interaksi pengikatan bagi sebatian aktif masing-masing dalam gaung tapak aktif reseptor AChE dan BChE. Simulasi dinamik molekul terhadap siliko telah digunakan untuk mendapatkan maklumat bagi mencirikan interaksi antara sebatian aktif AChE dan BChE masing masing. Kestabilan kompleks ligan-protein telah dinilai berdasarkan dasar sisihan min persegi RMSD. Dalam kajian ini, nilai RMSD bagi sebatian aktif yang telah dikompleks masing masing dengan AChE dan BChE, adalah kurang daripada nilai RMSD bagi enzim kolinesterase tak kompleks. Keputusan ini menunjukkan bahawa konformasi kompleks ligan-protein telah mencapai keseimbangan dan mengurangkan perubahan disebabkan oleh ikatan yang kuat untuk mengikat protein dan sekaligus menyebabkan perencatan enzim kolinesterase. Semua keputusan ini adalah konsisten dengan permerhatian assai biologi.

CONVENTIONAL ORGANIC SOLVENTS AND IONIC LIQUID MEDIATED SYNTHESIS OF NEW AZOMETHINE COMPOUNDS AS POTENT CHOLINESTERASE INHIBITORS

ABSTRACT

Five new series of azomethine derivatives were synthesized by condensation of substituted benzaldehydes and amino derivatives in ethanol a conventional organic solvents and a green ionic solvent, 1-butyl-3-methylimidazolium bromide ([bmim]Br), respectively. The green ionic solvent [bmim]Br, due to its remarkable catalytic effect has several remarkable advantages over ethanol in terms of high product yields and short reaction time. Condensation reactions performed in an ionic solvent had resulted in a significant increase in product yields ranging from 18 to 29 % and 6-15 times decrease in reaction time as compared to similar reactions performed in ethanol. The azomethines were characterized using elemental analysis, FT-IR, 1-D and 2-D NMR spectroscopy as well as X-Ray crystallography. The synthesized compounds were also evaluated for their potency against Alzheimer's disease using the Ellman's colorimetric assay. In this assay, the cholinesterase inhibitory activities of the aforementioned compounds were screened *in vitro* against acetylcholinesterase (AChE) of *electric eel* and butyrylcholinesterase (BChE) extracted from equine serum, both of which play a major role in the manifestation and progression of Alzheimer's disease. The results revealed that azomethine derivatives in series **9** displayed relatively better AChE inhibitory activities than those in series **3**, **5**, **7** and **11**. This observation is presumably due to the presence of three aromatic cores in compounds series **9**, which may facilitate the insertion and

accommodation of these compounds in the AChE active site gorge. However, compounds of series **5** are also composed of three aromatic cores, but the presence of a carbonyl moiety in the structure of these molecules plausibly hinders their insertion and positioning in the active site gorge of the AChE enzyme. Besides, in term of AChE inhibition compounds **3g**, **3j**, **5j**, **7f**, **7g**, **7j**, **9f**, **9h**, and **11h** showed high inhibitory activities which are comparable to the inhibitory activity of the standard drug, galanthamine. A similar observation was seen for compounds **3j, 5j, 7j, 9h** and **11j** in BChE inhibition. An *in-silico* molecular modeling analysis was also employed by using the crystal structure of *Torpedo californica* AChE (*Tc*AChE) and human BChE (*h*BChE) to disclose the orientation and binding interaction mechanism of the active compounds inside the active site gorge of AChE and BChE receptors, respectively. Molecular dynamics (MD) simulation on silicon were used to obtain information in order to characterize the interactions between the active compounds and the related protein AChE and BChE, respectively. The stability of the ligand-protein complexes was evaluated based on their root mean square deviation (RMSD). In this study, the RMSD values of the active compounds, which complexed with AChE and BChE, respectively are less than those of the uncomplexed cholinesterase enzymes. This result indicates that the conformations of the ligand-protein complexes had achieved equilibrium and exhibit low fluctuation due to strongly tied up and binding with related proteins, thus leading to the inhibition of the cholinesterase enzymes. All these results are consistent with the observation in the biological assays.

CHAPTER ONE

INTRODUCTION

1.1. Ionic liquids

Currently, solvent-free reactions are the subject of considerable interest because of its advantages such as ease of the experimental procedure as well as workup, low cost, possibility of using acid or base sensitive substrates and environmentally benign processes [1, 2].

Ionic liquids are a class of novel solvents with very interesting properties including non-volatility, non-flammability, high thermal stability, high polarity because of their ionic nature, recyclability, non-contaminating nature and ability to dissolve a wide range of polar and non-polar materials [3]. The interesting behaviour of ionic liquids lies in the fact that they can be re--used after simple washing with a suitable solvent, thus rendering the process more economical.

The principle application of ionic liquids has been used as alternative solvents for synthesis and catalysis [4] and these solvents are found to be promising solvents in many of the organic reaction such as Diels-Alder, Bails-Hillman, Heck Reaction, esterification, isomerization reactions and many coupling reaction [5].

Researchers have demonstrated that there have been few other data on quantitative studies of nucleophilic substitutions in ionic liquids [4]. These data are compared to related reactions in molecular solvents, and used to show, where ionic liquids do

offer advantages over molecular solvents for nucleophilic substitutions. Although many experimental results were obtained with better yields in nucleophilic substitution reactions from other groups, there has been a controversy regarding the enhancement of nucleophilicity in ionic liquid [6].

1.2 Azomethine

Azomethine compounds as a bimolecular condensation products of primary amines with aldehydes-represent valuable intermediates in organic synthesis and at the same time, compounds with various applications [7]. They are important class of compounds due to their flexibility, structural similarities with natural biological substances and due to presence of imine (-N=CH-) which imports in elucidating the mechanism of transformation and rasemination reaction in biological system. These compounds could also act as valuable ligands whose biological activity has been shown to increase on complexation. Thousands of compounds have been synthesized and tested as cholinesterase inhibitors. They belong to different types of organic and organometallic classes such as alkaloids, physostigmine, and organophosphorus [8].

1.3. Definition of Alzheimer's disease (AD)

According to the World Alzheimer's Report, more than 35 million people worldwide are affected by Alzheimer's disease (AD) an irreversible neurodegenerative disorder [9]. AD is clinically characterized by vast cognitive impairments, memory loss as well as neuropsychiatric symptoms and pathologically characterized by the presence of extracellular β-amyloid plaques and intracellular neurofibrillary tangles [10,11].

A cholinergic hypothesis is proposed in order to clarify how the loss of cholinergic cells in the forebrain, cortex and hippocampus of AD patients' brain causes a severe memory loss and cognitive impairments due to dysfunctions in the cholinergic neurotransmission system [12]. Acetylcholinesterase (AChE) hydrolase acetylcholine in central and peripheral nervous systems terminates the impulse transmissions from the nerve cells to postsynaptic membrane or skeletal muscles and butyrylcholinesterase (BChE) is believed to protect AChE by hydrolyzing harmful toxins, which may damage or deactivate the AChE [13].

Active sites of the two previously mentioned enzymes are positioned at the bottom of a 20 Å long, narrow gorge from the enzyme surface with five major regions, which can accommodate and hydrolyze a substrate or inhibitor. The structure of BChE is very similar to that of AChE, except that the channel-lining aromatic residues in are BChE mostly replaced by aliphatic ones, such as leucine (**Leu**) and valine (**Val**), thus making the gorge in BChE's more spacious which can accommodate bulkier substrates [14].

1.4. Medicinal chemistry

Medicinal chemistry is the science of discovery and investigation of novel therapeutic agents and to develop them into useful drugs [15]. This science includes natural product chemistry or isolation of compounds from natural origins, organic synthesis chemistry, which involves the design and synthesis of new drug molecules and computational chemistry that relates the structures of molecules to their biological activities [16].

It is worth to know that modern history of medicinal chemistry began in 1785 with the application of foxglove plant in the treatment of dropsy (congestive heart failure) in 1785 by Withering [17,18].

1.4.1. Organic synthesis and drug discovery

There was a rapid advancement in organic synthesis, separation methods and biochemical techniques since the late 1940s and a rational approach toward the design, synthesis and chemical modifications of organic in order was adopted in order to improve their medicinal properties. The lead compounds that are discovered is a prototype compound, which has attractive pharmacological specifications along with some undesirable properties such as high toxicity, absorption difficulties, and insolubility or metabolism difficulties.

A lead compound is methodologically identified via screening techniques or from clinical investigations. However, rational approaches to drug design become a major route towards the discovery of lead compounds. In this method, firstly, the cause of the disease and its relevant biochemical pathways are identified. The natural receptor ligands or enzyme substrates are selected as initial lead compounds and their main functional groups, which are responsible for the activity of the lead compounds, such as pharmacophores and auxophores, which can be identified using pharmacodynamics. Eventually, with the aid of organic synthesis, the functional groups of the ligands or enzyme substrates are modified in order to synthesize the most appropriate drugs [19], the ones with optimal biological activities [15].

1.5. Problem statement

The elimination of volatile organic solvents in organic synthesis is the most important goal in green chemistry. One of the most efficient protocols to reach this aim is the substitution of volatile solvents with ionic liquids [20]. In recent years, ionic liquids have received increasing attention as benign reaction media in organic synthesis due to their unique properties. Work has tended to focus on using ionic liquids to synthesize various compounds and investigate their potential therapeutics properties for different diseases such as Alzheimer's disease.

Treatments of AD suffers from a shortage of clinically approved drugs, which are limited to a few cholinesterase inhibitors such as donepezil, galanthamine, rivastigmine and huperzine A, all of which with low to moderate clinical efficacy, and one *N*-methyl-D-aspartate receptor antagonists (e.g. memantine), which its efficacy is not approved [21]. These severe limitations of potent cholinesterase inhibitors, has prompted scientists worldwide to design / search for new inhibitors. It's clearly known that organic synthesis is a great tool to prepare a library of drug and their further modifications to amplify desired activities and minimize their inappropriate characteristics using for medicinal properties [15,22]. In the present study, new azomethines derivative were synthesized and evaluated for their cholinesterase enzymes inhibitory activities, Their molecular interactions and orientation with cholinesterase were studied using an *in silico* molecular docking and molecular dynamics simulation.

1.6. Objectives:

Objectives of this work are as follows:

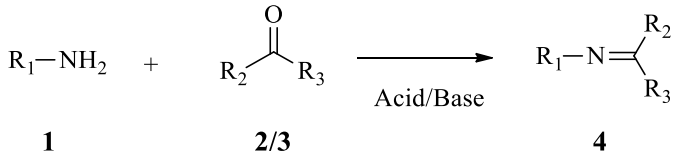
1. To compare the solvent efficiency using ethanol and an ionic solvent in the synthesis of five series of new azomethine derivatives.
2. To elucidate and characterize the structures of the newly synthetic compounds using elemental analysis as well as FT-IR, 1D, 2D NMR spectroscopy and determine the exact configuration of some derivatives by X-ray crystallography.
3. To evaluate the *in-vitro* inhibitory activities of the synthesized azomethine derivatives against acetylcholinesterase and butyrylcholinesterase enzymes.
4. To investigate the conformations of selective synthetic compounds and binding mechanism of these compounds with cholinesterase enzymes using molecular docking studies.
5. To study the microscopic interaction between selective synthetic compounds and cholinesterase enzymes using molecular dynamics simulation (MD).

CHAPTER TWO

Literature review

2.1. Formation of azomethines

Structurally, an azomethine is a nitrogen analog of an aldehyde or a ketone where in the carbonyl group (C=O) is replaced by azomethine or imine moiety [23]. This class of compounds are usually prepared by condensation of an aldehyde or ketone with a primary amine (R-NH_2) as depicted in Scheme 2.1. Azomethine is an important subset of aldimines, in which the substituent on the nitrogen atom (R') is an alkyl or aryl group.



1 **2/3** **4**

Scheme 2.1. Condensation of a primary amine (**1**) with an aldehyde (**2**) or ketone (**3**) to form an azomethine (**4**)

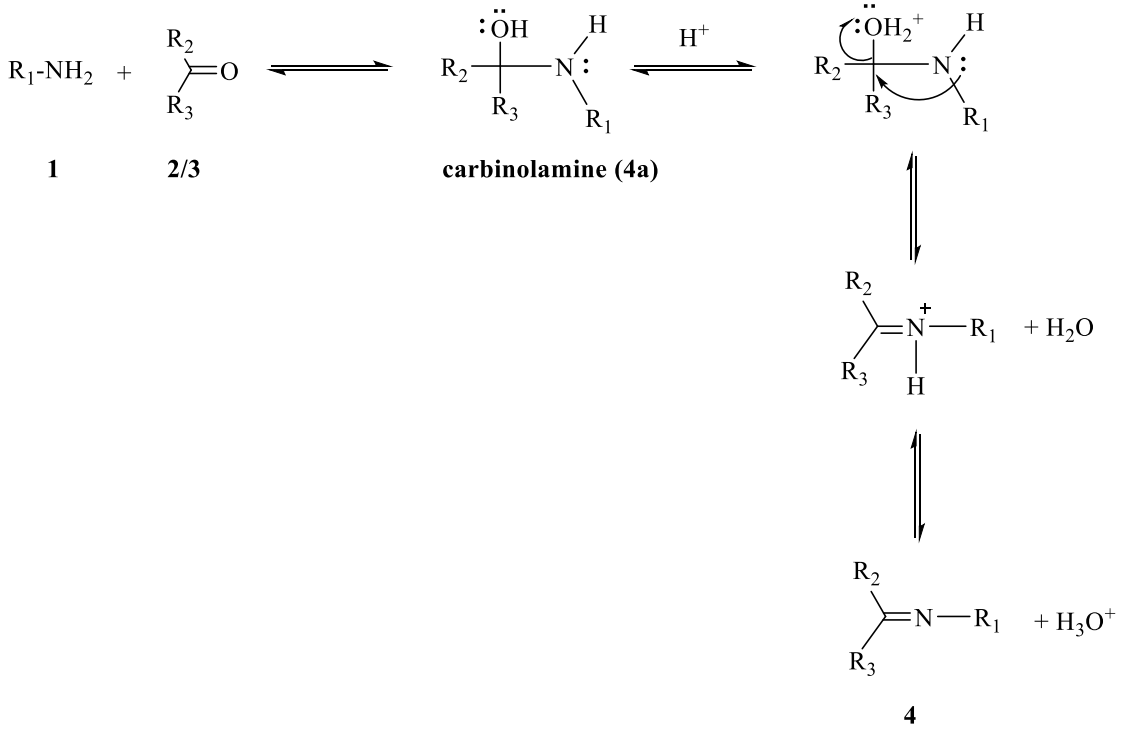
In the case of reacting a primary amine **(1)** with an aldehyde **(2),** wherein R_1 and R_2 can be alkyl or aryl moieties and R_3 is H however, by using a ketone **(3)** as a reactant, all R_1, R_2 and R_3 can be alkyl or aryl moieties.

It is worth mentioning that azomethine containing aryl substituents are basically more stable as well as more convenient to synthesize than those having alkyl substituents, which are also relatively unstable [24].

2.2. Synthesis of azomethines

The first synthetic methodology reported by Hugo Schiff involved the condensation of a carbonyl compound with a primary amine under distillation. In general, the formation of azomethines is a reversible reaction and it is acid or base catalyzed, or with heating. The reaction can be accelerated by constant separation of the product or by removal of water. Molecular sieves and *in situ* methods using dehydrating solvents such as tetramethyl *ortho*-silicate or trimethyl *ortho*-formate, are then used to completely remove water formed in the system [25].

The mechanism of azomethine formation is a nucleophilic addition of a primary amine **(1)** to the carbonyl group of an aldehyde **(2)** or a ketone **(3).** An unstable alcohol, carbinolamine **(4a),** is formed as an intermediate subsequently **4a,** undergoes dehydration to give the final product **(4).**



1 2/3 4

Scheme 2.2. Acid catalyzed formation of an azomethine

The dehydration of the carbinolamine at neutral pH is the slowest and therefore, the rate-determining step of azomethine formation. However, under mild acidic conditions, amine attack is the rate-determination step as shown in Scheme 2.2. [26].

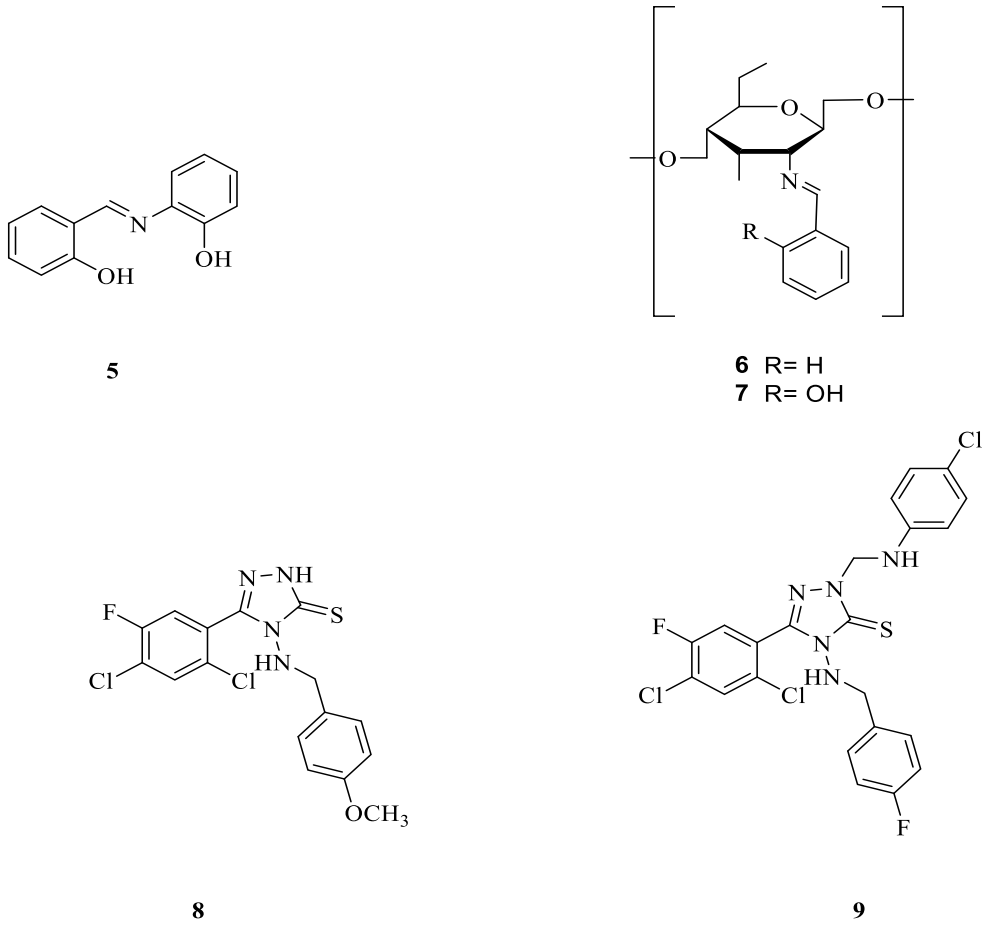
2.3. Biological importance of azomethine

Azomethines exhibit a wide range of biological activities, such as antifungal, antibacterial, antimalarial, anti-proliferative, anti-inflammatory, antiviral as well as cholinesterase (enzyme) inhibition.

2.3.1. Antifungal activity

Some promising antifungal azomethines are shown in (Figure 2.1(. *N*-(Salicylidene)-2-hydroxyaniline **5** at 500 ppm was found to inhibit the growth of *Alternaria brassicae* and *Alternaria brassicicola* by 67-68%. These fungi severely affect cruciferous crops (*e.g.* broccoli, cauliflower, mustard, cabbage and radish) [27]. Chitosan-derived azomethine such as **6** and **7**, could inhibit the growth of *Botrytis cinerea* and *Colletotrichum lagenarium* by 26–33% and 35–38%, respectively when used at 1000 ppm [28].

Azomethines with a 2,4-dichloro-5-fluorophenyl moiety, such as **8** and **9** could inhibit the growth of fungi of clinical interest, such as *Aspergillus fumigatus*, *Aspergillus flavus*, *Trichophyton entagrophytes* and *Penicillium marneffei*. The MIC values for these compounds were in the range of 6.3-12.5 μg/mL, and they are as potent as the referenced drug fluconazole [29].



5

6 R= H
7 R= OH

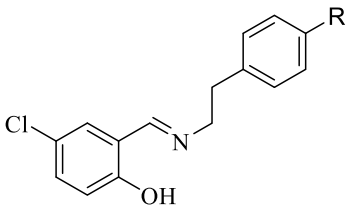
8

9

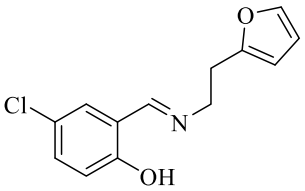
Figure 2.1. Antifungal azomethines

2.3.2. Anti-bacterial activity

Azomethines are also reported to show potent antibacterial activities (Figure 2.2). *N*-(Salicylidene)-2-hydroxyaniline **5** showed good inhibition against *Mycobacterium tuberculosis* H37RV, with a MIC value of 8 μg/mL [30]. The 5-chloro-salicylaldidene azomethines **10–13** obtained from condensation of 5-chloro-salicylaldehyde and primary amines, showed significant inhibitory activities against *Pseudomonas fluorescence*, with MIC values ranging from 2.5 to 5.2 μg/mL. The MIC value for the standard drug, kanamycin, is 3.9 μg/mL [31].



10 R=H
11 R=F
12 R=OH



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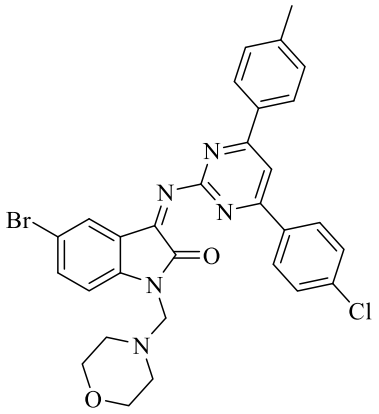

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Figure 2.2. Antibacterial azomethines

An antibacterial study performed by Pandeya and colleagues 1999 on 28 bacteria of clinical importance had disclosed that azomethine **14** had remarkable inhibition against *E. coli*, *Vibrio cholerae*, *Enterococcus faecalis* and *Proteus shigelloides* with a MIC value of 2.4, 0.3, 1.2, and 4.9 µg/mL, respectively. The MIC values for sulphamethoxazole as standard drug against the same bacterial strains were only 312-5000 µg/mL. Thus, compound **14** was significantly more potent than sulphamethoxazole [32].

2.3.3. Antiviral activities

2[1-[(3'-Allyl-2'-hydroxybenzylidene)amino]-3-hydroxyguanidine] is a substituted salicylaldehyde azomethine derived from 1-amino-3-hydroxyguanidine tosylate are good substrates to design new antiviral agents. From this family, compound **15** (Figure 2.3) showed very good inhibitory activity against mouse hepatitis virus (MHV) with an IC_{50} value of 3.2 μM [33].

Sriram and colleagues in 2006 have recently reported the synthesis and antiviral activities of abacavir-derived azomethines **16-19**. Abacavir is an analogue of nucleoside, which is capable of inhibiting the activity of reverse transcriptase, and used to treat human immunodeficiency syndrome (AIDS). These compounds were significantly effective against the human immunodeficiency virus-type 1 (HIV-1). The effective concentration (EC_{50}) necessary to achieve 50% protection of human leukemic cells (CEM) against the cytopathic effect of HIV-1 of these abacavir-derived azomethine was lower than 6 μM. Notably, compound **18** was the most potent azomethine, with an EC_{50} value of 50 nM. Compound **18** is toxic to CEM cells at concentrations higher than 100 μM, indicating its potential as a lead compound in the design of new anti-HIV drugs [34].

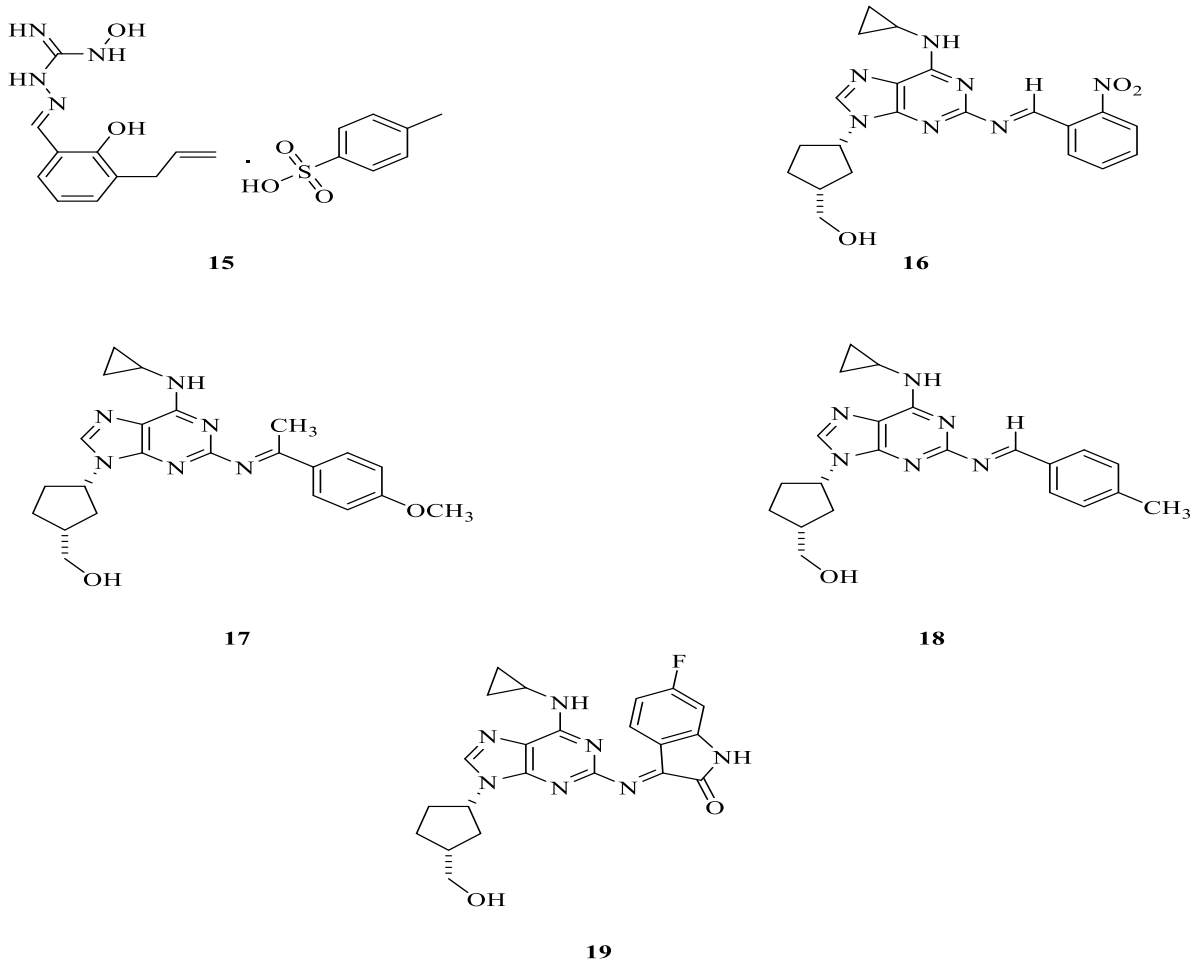

Figure 2.3. Azomethines with potent antiviral activities

2.3.4. Cholinesterase inhibitory activities

Chan *et al.*, 2012 reported the cholinesterase inhibitory activities of Cu(II) complexes derived from 2-(diphenylmethylene) hydrazinecarbothioamide azomethines. Were These complexes showed potent inhibition with IC_{50} values lower than 10 μM, where in complex **20**, exhibited the highest AChE and BChE inhibition with an IC_{50} value of 2.15 μmol/L and 2.16 μmol/L, respectively [35].

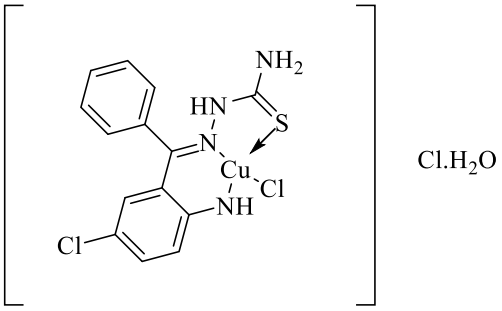

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Figure 2.4. Metal coordinated azomethine as a cholinesterase inhibitor

2.4. Ionic liquid mediated organic synthesis

Ionic liquids (ILs) are solvents of interest in synthetic organic chemistry due to their non-volatile nature, potential for recycling, ability to dissolve a variety of organic, inorganic, and metal complex materials, and especially due to their ability to activate various reactions [36]. Several commercially available ILs are shown in (Figure 2.5).

One of the main advantages of ionic liquids is their low, almost negligible, vapor pressure as compared to volatile and hence hazardous organic solvents. The strong ionic (Coulombic-) interactions in these substances result in the formation a negligible vapor pressure (unless decomposition occurs), a non-flammable, and a thermally, mechanically as well as electrochemically stable product. In addition to this very interesting combination of properties, and immiscibility with water or organic solvents that result in biphasic systems. This has prompted the claim that ionic liquids are environmentally benign, "green" solvents. The possibility to conduct chemical, biochemical, and analytical processes in an ionic, low coordinating, and highly solvating environment over a wide temperature range has

contributed to the enormous growth and expansion of the field of ionic liquids for use primarily as alternative solvents in organic reactions. Unlike conventional molecular solvents, the structures of ionic liquids can be modulated with ease. Thus, application of "task-specific" ionic liquids can provide additional benefits to a variety of processes [37].

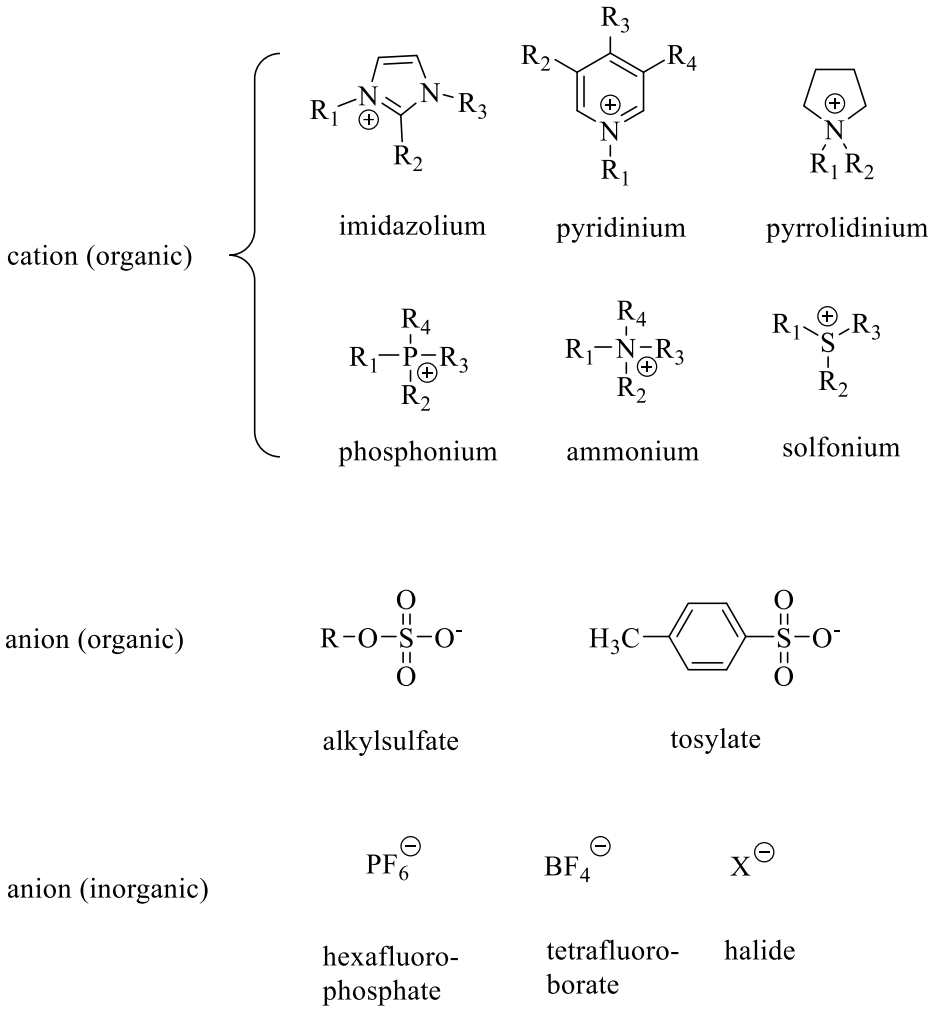

Figure 2.5. Commercially available ionic liquids

The choice of the cations has a strong impact on the properties of ionic liquids, which often determine their stability. The chemistry and functionality of an ionic liquid is generally controlled by the choice of the anions. The combination of a broad variety of cations and anions has led to a theoretically possible number of 1018 ionic

liquids. However, a realistic number will be smaller. Today, about 1000 ionic liquids have been reported in the literature, and approximately 300 are commercially available. It is worth mentioning that cation and inorganic anions such as $[AlCl4]^-$, $[BF4]^-$ or $[PF6]^-$ exist in a liquid state at room temperature. Ionic liquids based on imidazolium cation are especially favorable in various industrial applications [38].

2.4.1. The role of medicinal chemistry in Alzheimer's disease

The drug discovery for AD is complicated due to unclear origin and cause of the disease. However, based on the cholinergic hypothesis of AD, it was the first theory proposed to explain this disease. Thus, many commercially available drugs, which improve the symptomatic effects of this disease are cholinesterase inhibitors [15]. Donepezil the most common example, incorporates indene's core structure that effectively inhibits the enzyme and has been used to treat patient with mild to moderate AD [39]. Recent works have showed that monoamine oxidase (MAO) inhibitors may also be useful in the treatment of AD. An example of which is indene ladostigil. This compound includes a carbamate group associated with AChE inhibitory activity and a propargyl moiety, which is the active functional group in MAO inhibitors [40].

2.5. Alzheimer's disease

2.5.1. Fact and figures

There are an estimate of 35.6 million people worldwide living with dementia, based on the World Alzheimer report in 2012 [9]. This number is estimated to reach 65.7 million by 2030 and 115.4 million by 2050 [41]. Alzheimer's disease (AD) is the most prevalent form of dementia. It accounts for 60 to 80 % of dementia cases in the old population [42]. Researches showed that men are less affected by AD and other dementias than women are. According to findings, 16% of women at the age of 71 and older suffer from AD or other dementias as compared to 11% of men [43]. This phenomenon is probably due to the fact that women live longer than men [44]. Researchers also revealed that people with higher years of education seem to be at a lower risk for AD and other dementias [45].

2.5.2. Clinical symptoms of Alzheimer's disease

Severe impairments of cognitive abilities ensue AD, such as short-term and long-term memory losses, difficulty in planning for routine life and solving problems, confusion with time or disorientation in spaces as well as withdrawal from work and common social activities. However, at advanced stages of AD, patients also show abnormal behavioural activities including agitation, anxiety, delusion and depression that finally result in morbidity and mortality [46].

2.5.3. Pathology of Alzheimer's disease

The hippocampus is the area of the human brain, where it is mostly affected in patients with AD (Figure 2.6). The main and dominant pathological changes of the

brain, is the accumulation of β-amyloid (Aβ) plaques outside the neurons and neurofibrillary tangles, inside the neurons can be mostly found in this region.

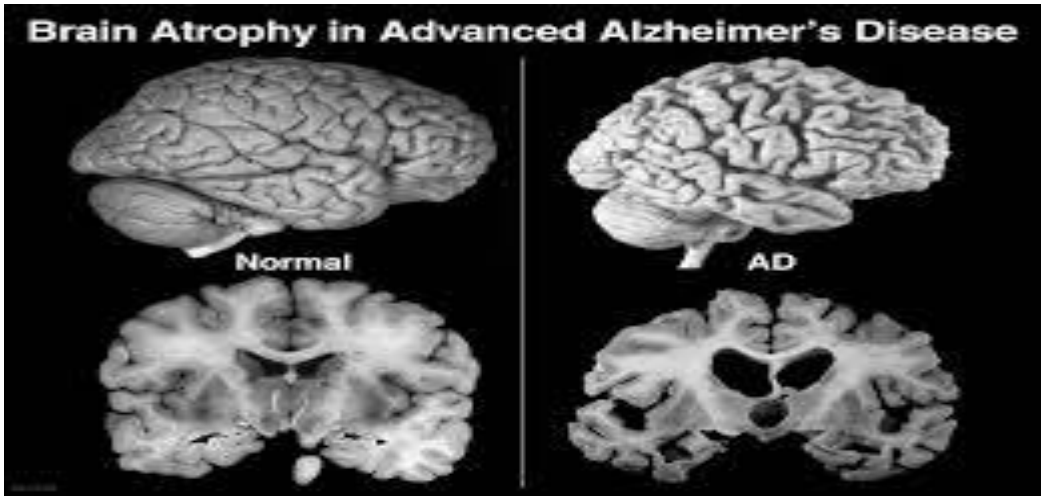

Figure 2.6. Schematic representation of healthy brain and severely affected AD brain

The β-amyloid plaques first appear in the frontal cortex, and then spread over the entire cortical region, while insoluble tangles initially appear in the limbic system and then progresses to the cortical region [10]. Atrophy and shrinkage of the parietal and temporal lobes of the brain are also observed in-patients with AD. Pathogenesis of AD can be explained by the amyloid, tau and cholinergic hypotheses.

2.5.3.1. Amyloid hypothesis

Accumulation of hydrophobic amyloid-β peptides outside the neurons in the basal forebrain due to an over expressed cleavage of amyloid precursor protein (APP) has resulted in the aggregation and deposition of insoluble plaques (senile plaques), which trigger a cascade of changes inside the brain, thus causing neuronal death and domination of AD [47].

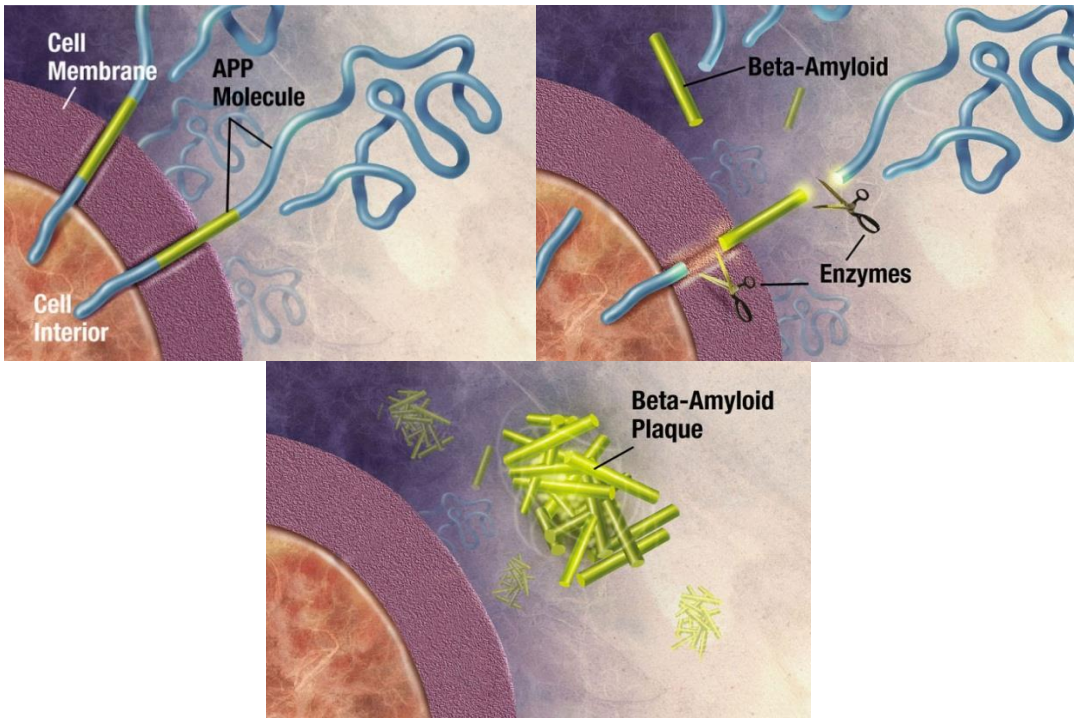

Figure 2.7. Formation of β-amyloid plaques from an over-expressed cleavage of APP [48]

Cholinergic neurotransmission in the basal forebrain is plausibly impaired by these neurotoxic plaques [49] a causal factor for cognitive symptoms of AD [50]. However, this plaque load may not correlate with the degree of dementia in humans. Studies revealed that many AD patients with severely impaired memory had no plaques at post-mortem analysis [51]. On the other hand using MRI techniques, huge plaque loads had been traced in cognitively normal people [52].

2.5.3.2. Tau hypothesis

High level of mutated tau proteins inside the neurons, will cause the build-up of insoluble neurofibrillary tangles, which impair nutrients transportation throughout the cell [53]. Tau proteins are structural components that support microtubules in addition to transporting nutrients, vesicles and other substantial compositions within

this cell. They also stabilize the growth of which is axons, a critical for neurons development and growth [54]. In AD patients, these proteins are abnormally hyper-phosphorylated and generate insoluble deposits within the cell thus contributing to cell death. Patients in advanced staged AD suffer from dramatic brain shrinkage due to extensive cell losses. This phenomenon begins to show up much longer before cognitive symptoms starts to develop and a dementia stage is reached [55].

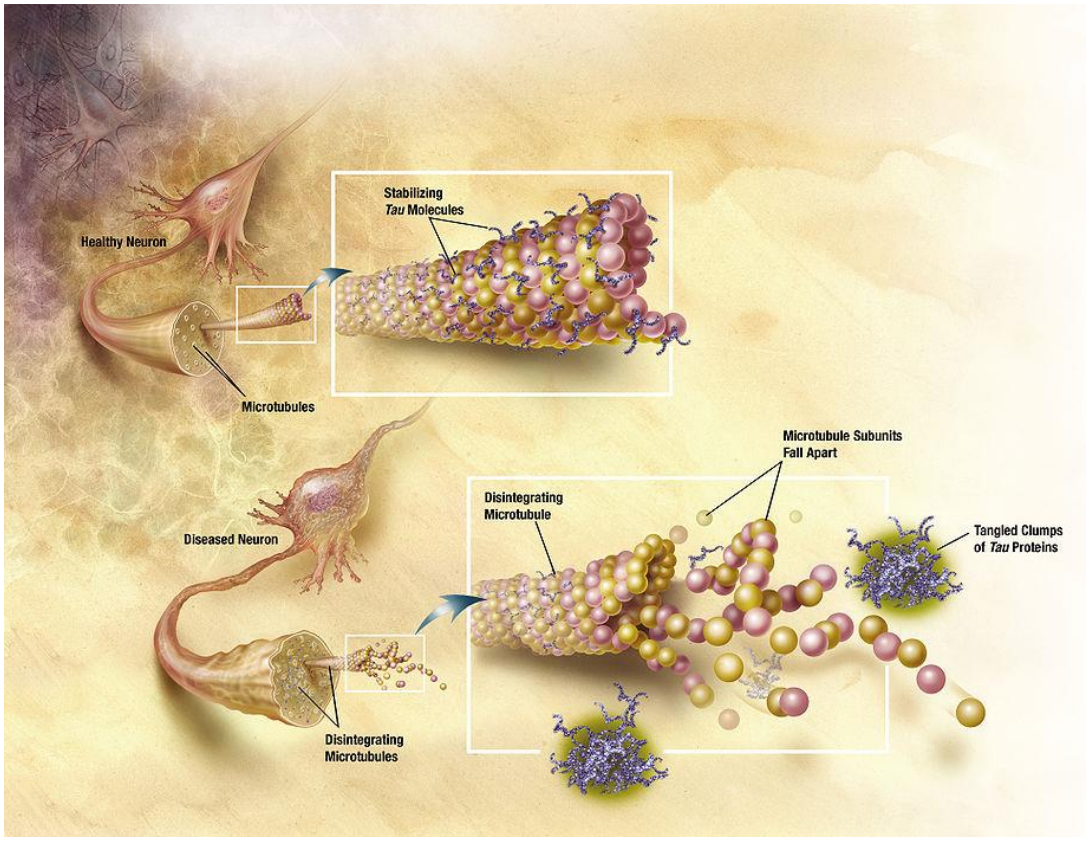

Figure 2.8. The role of Tau proteins in the formation of fibrillary tangles [48]

2.5.3.3. Cholinergic hypothesis

Based on the cholinergic hypothesis, in AD patients' brain, the activity of the enzyme responsible for the synthesis of acetylcholine neurotransmitter (ACh) significantly decreases [56, 57]. This phenomenon results in a decrease in ACh levels in hippocampus and basal forebrain of these patients, which leads to substantial memory loss and severe cognitive symptoms of AD [12].

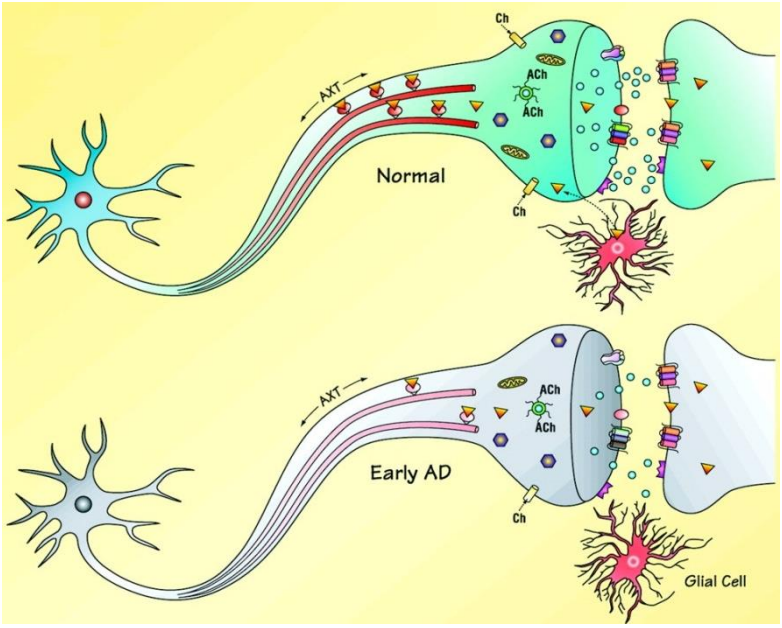

Figure 2.9. A Comparison of ACh concentration in a normal and AD afflicted human brain [58]

2.5.4. Cholinesterase enzymes

Cholinesterases (ChE's) catalyse the hydrolysis of ACh into choline and acetic acid, which is an essential process to regulate cholinergic transmission inside the human brain. Acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) (Figure 1.10) are the two ChE enzymes which exist in mammalian bodies [59].

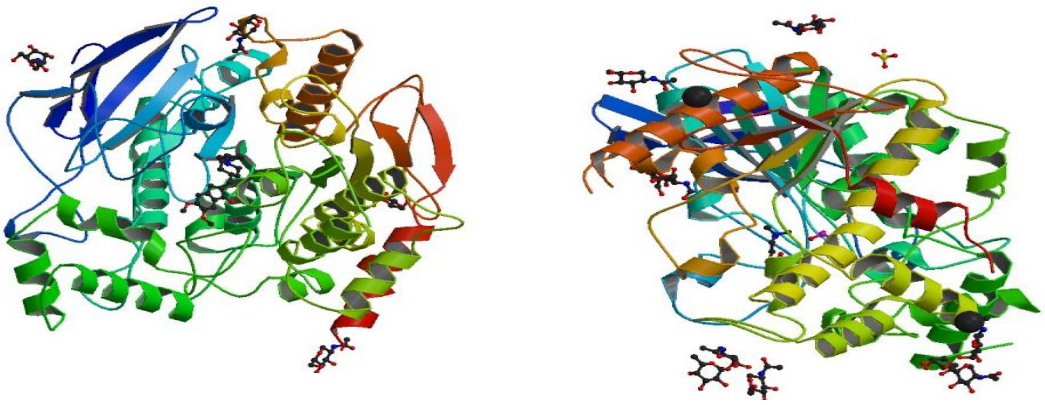

Figure 2.10. Representation of *Torpedo californía* AChE (left) and human BChE (right) [60]

2.5.4.1. Physiological functions of cholinesterase enzymes.

Acetylcholinesterase (AChE) plays an important role in the central and peripheral nervous systems. AChE terminates cell to cell nerve impulses transmissions through synaptic clefts and even nerve to skeletal muscles messages through fast hydrolysis of ACh [61].

Butyrylcholinesterase (BChE) is a non-specific enzyme, of which its physiological functions is still unclear. It has been supposed to hydrolyze herbal toxicants [62].

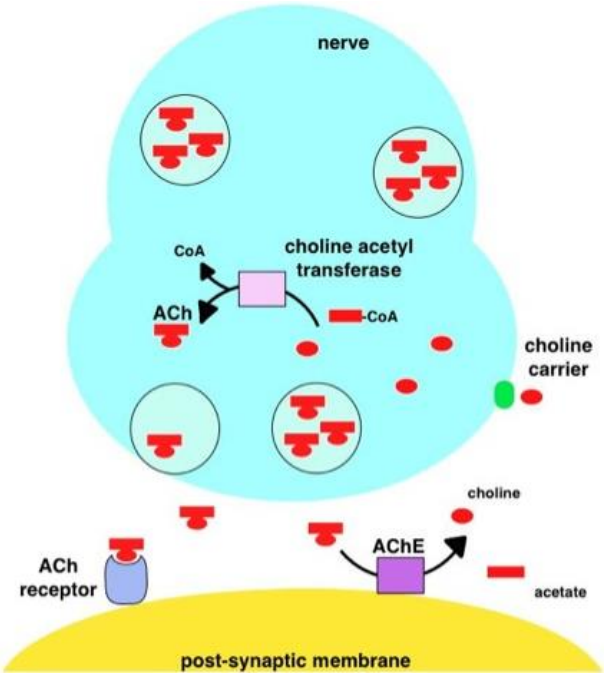

Figure 2.11. Mechanism of action of acetylcholinesterase

2.5.4.2. Structural specifications of AChE and BChE

The active sites of AChE and BChE enzymes are located at the bottom of a 20 Å cavity named as "aromatic gorge". Substrate or inhibitor transportation inside the aromatic gorge of AChE is facilitated by hydrophobic interactions with residues having aromatic side chains such as phenylalanine (**Phe**), tryptophan (**Trp**) and tyrosine (**Tyr**) [63]. However, in the active site of BChE, these aromatic residues are replaced with residues bearing hydrophobic side chains such as leucine (**Leu**) and valine (**Val**). Thus, active site of BChE is more spacious and non-specific to accommodate bulkier substrates [14].

2.5.4.3. Active sites of AChE and BChE enzymes

Active sites of AChE and BChE are classified into five regions, namely (i) catalytic triad, (ii) oxyanion hole, (iii) acyl pocket, (iv) choline binding site, and (v) peripheral anionic site.

Catalytic triad (CT) is the major site of both enzymes and it catalyses the acetylcholine hydrolysis. CT is composed of **Ser200, His440** and **Glu327** amino acids in *Tc*AChE and **His438**, **Ser198** and **Glu325** in *h*BChE [64]. Its mechanism of action is probably *via* nucleophilic addition of the hydroxyl group in serine to the carbonyl moiety of acetylcholine, thus resulting in an acyl-enzyme intermediate that is further hydrolysed to choline and acetic acid (Figure 2.12).

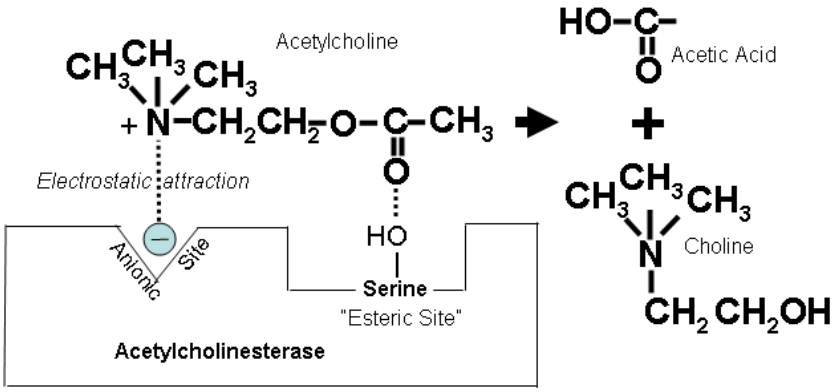

Figure 2.12. Acetylcholine hydrolysis in AChE active site [65]

2.5.5. Cholinesterase inhibitors for symptomatic treatment of Alzheimer's disease

Currently, cholinesterase inhibitors (ChEI's) are mainly prescribed for symptomatic treatment of AD patients. This method is the most promising and widely used to ameliorate cognitive impairments in these patients.