CHAPTER 1

INTRODUCTION
1.1 General

Alzheimer's disease (AD) is the most common form of dementia and has been reported as the fourth cause of death by WHO records in 2009. In the early stages, the most commonly recognized symptom is memory loss, such as difficulty in remembering recently learned facts. The diagnosis is usually confirmed with behavioral assessments and cognitive tests, followed by a brain scan. The mean life expectancy following diagnosis is approximately seven years. Fewer than three percent of individuals live more than fourteen years after diagnosis.

The discovery of new pharmaceutical agents has gone through an evolution over the years and has been adding new technologies to this increasingly complex process. Following the successful trial of computational drug designing technique in the identification of inhibitor for HIV-protease [1], scientists started providing more attention to this new drug designing technique.

In the present work, ligands for Alzheimer’s disease have been collected from phytochemicals generated and are found to be highly promising. These compounds are biocompatible and the chance for the failure of these potential drugs during clinical trials would be minimum.

1.2 Alzheimer’s disease

Alzheimer's disease (AD) is a major cause of dementia and has been identified as a protein misfolding disease (proteopathy), caused by accumulation of abnormally folded A-beta (amyloid beta peptide) and tau proteins in the brain [2]. This peptide build up in the brain to form clumps called amyloid plaques (Fig. 1.1 and 1.2), which are characteristic of AD [3, 4].

Alzheimer's disease (AD) is reported as the fourth leading cause of death in developed and most of the developing nations (after heart disease, cancer, and stroke). More than 10 million new cases of AD are reported every year. By 2020, it is estimated that, more than one fifth of the population [5] will be affected by Alzheimer’s disease, making this disease as a major subject of concern.
1.3 Present treatment strategy of AD

There has been gratifying progress in the development of drugs for Alzheimer’s disease (AD). But the current generation of medications, the ‘cholinesterase inhibitors’ (CEIs), has produced only modest benefits. All the CEIs such as fortacrine (TAC) [6], donepezil (DON) [7], rivastigmine (RIV) [8] and metrifonate (MET) [9] have a narrow therapeutic window. Moreover efficacy of these drugs is found to be strongly dose dependent and the peripheral tolerance to the gastrointestinal side effects is developed slowly.

Muscarinic agonists have been the logical successors to CEIs. CEIs theoretically lead to activation of both presynaptic and postsynaptic muscarinic receptors. Only the latter is enabled in this medication, because presynaptic receptor activation leads into a decrease in synaptic function. The results from the muscarinic agonists have been disappointing. Although some changes have been
observed on cognitive assessments with two of the drugs, xanomeline [10] and SB202026 [11], the effects were no larger than those seen with the CEIs.

There is evidence supporting a biological role for estrogen in the pathogenesis of AD. Estrogen receptors are present on hippocampal and cholinergic neurons [12, 13]. Estrogen might function as an antioxidant or act to stimulate nerve growth factor release [14]. Prospective observational studies suggest a protective effect of estrogen on the subsequent development of AD [15, 16]. But at present, the data is insufficient to recommend estrogen replacement for the primary therapy of AD or as prevention for AD. The development of estrogen receptor analogs with activity restricted to the brain is being vigorously pursued.

Analogous to estrogen, clinical studies provide evidence that individuals using anti-inflammatory (AI) agents have a lower probability of developing AD [17–21]. The rationale for AI therapy is supported by immune-histochemical findings in the AD brain [22]. These include demonstrations of excess quantities of proteins known to be associated with an inflammatory response (such as α-1-antichymotrypsin, cytokines and complement) as well as activated microglia. These observations provide strong support for anti-inflammatory interventions aimed at slowing the AD pathological process. As knowledge of the mechanism of β−amyloid peptide (Aβ) action has been mounted up, Aβ itself appears to be an inducer of the inflammatory response [23]. At present, AI agents cannot be recommended for treatment of AD because evidence for benefit is only indirect and the toxicity of current drugs is considerable.

The evidence for oxidative stress in the brains of patients dying with AD is strong. Excess lipid peroxidation [24] and evidence for oxidative injury to neuronal DNA [25] and proteins [26] have been observed. There are several ways in which oxidant molecules could accumulate in the AD brain. Aβ itself might induce free radical production [27]. Elevated brain iron that has been observed in AD may also contribute to free radical generation [28]. The clinical trial using the antioxidants selegiline and α-tocopherol (vitamin E) (1000 IU twice daily) was the first to report convincing benefits of antioxidants [29]. Tocopherol is a fat-soluble substance that blocks lipid peroxidation. There is an extensive literature on
tocopherol supporting its role as an antioxidant, including in vitro evidence of improved cell survival in the presence of toxins, including Aβ [30]. On the other hand, there is no evidence of tocopherol deficiency in AD brain. Tocopherol is inexpensive and nontoxic. Selegiline will not be further discussed, as its effects were no greater than that of tocopherol. The modest success of α-tocopherol provides support for the hypothesis that oxidative stress plays a role in AD. Several future approaches are suggested by this study. One would be to develop more potent antioxidant compounds. The report that ‘γ-tocopherol [31] is more effective in blocking lipid peroxidation in vitro than α-tocopherol’ should be pursued.

Because the survival of cholinergic neurons in the basal forebrain is dependent on nerve growth factor (NGF), therapy with this and other growth factors has been conceptualized for some time. However, these proteins do not cross the blood brain barrier (BBB), and thus, intrathecal delivery is required. One case report has appeared on a patient who received intrathecal NGF [32]. Few conclusions can be drawn from that patient, other than to note that the results were not dramatic. There also have been reports of unanticipated side effects of NGF in experimental models.

An alternative to NGF itself is to use an orally administered agent that stimulates NGF activity. Several drugs currently under study may have this property. Estrogen is one. Propentophylline is another agent that appears to have NGF-enhancing properties [33] among its several actions. The other major effects of propentophylline are influencing microglia proliferation and possibly modulating inflammatory mechanisms in AD [34].

The prospects for more potent anti-AD therapies are slowly but steadily rising. Therapies of several different mechanisms are under investigation. One can only hope that medications that are more potent than the CEIs can be developed. Among the approaches likely to be studied intensively over the next decade are combination therapy regimens and attempts at either presymptomatic or very early symptomatic treatment.
1.4 Diagnosis of AD

Medical diagnosis of Alzheimer’s disease is hard, and symptoms are often dismissed as normal consequences of aging. Diagnosis is usually performed through a combination of extensive testing and elimination of other possible causes [35]. For instance, if the patient suffered a serious head injury any time in his past or some heart problems, it could account for the problems with memory or concentration that he is experiencing.

The evaluation should include an assessment for anxiety or depression, which can create Alzheimer's-like symptoms in older people as well as occur concurrently with Alzheimer's or another dementia. Depression, in particular, can result in a set of symptoms collectively known as pseudodementia. If a mood disorder is detected, it can be treated along with other disorders, such as Alzheimer's [36].

To assess memory, concentration and other cognitive skills of the patient, the mental status examination such as Mini Mental State Examinations (MMSE) [37] will be carried out, which is a research-based set of questions that results in a score that indicates a general level of impairment. Normally, if the score status is very high, there is less chance for AD. However, highly educated individuals have scored high on mental status exams even though they do have Alzheimer's disease.

Some neurological examinations will be carried out as major diagnostic tests. This should include an examination of the motor system (movement), reflexes, gait (walking), sensory functioning and coordination in order to detect problems with the nervous system that may be causing problems with thinking and behavior.

Recent trend is to analyze images of the brain using sophisticated technologies such as a CT scan (computed tomography), MRI (magnetic resonance imaging), PET scan (positron emission tomography) and electroencephalograms (EEGs) to identify changes in brain structure or size indicative of Alzheimer's [38] or to look for brain tumors, blood clots, strokes, normal pressure hydrocephalus
(NPH), or other abnormalities that might account for Alzheimer's-like symptoms [39].

There is no singular test that can conclusively diagnose Alzheimer's disease, although imaging technology designed to detect Alzheimer's plaques and tangles is rapidly becoming more powerful and precise. Still, a comprehensive, competent diagnostic workup by a skilled physician can pinpoint the cause of Alzheimer's-like symptoms with maximum accuracy.

1.5 Drug designing

Drug designing is the inventive research process of finding new medications based on the knowledge of the biological target [40] such as protein, DNA, RNA, metabolites etc (Fig. 1.3). Mainly, there are seven classes of therapeutic targets namely, normal receptors, nuclear receptor, ion channels, enzymes, hormones and cofactors and nucleic acids. The drug is most commonly an organic small molecule satisfying all the pharmacophoric requirements, which activates or inhibits the function of a biomolecule such as a protein responsible for the disease which in turn results in a therapeutic benefit to the patient. Modeling techniques for prediction of binding affinity are reasonably successful. However there are many other properties such as bioavailability, metabolic half life, lack of side effects, ADMET etc. that have to be optimized before a ligand can become a safe and efficacious drug.

Drugs may be designed to provide interaction with the active region and inhibit this key molecule. The vast majority of drugs work by inhibiting the action of the target by binding to the active site and thus preventing the native substrate from entering the site. Another approach is to enhance the normal pathway by promoting specific molecules that may have been affected in the diseased state. In addition, these drugs should also be designed in such a way that it is not affected by any other important ‘off-target’ molecules or antitargets that may be similar in appearance to the target molecule. The drug interactions with off-target molecules may lead to undesirable side effects.
The drug design process is a complex and interactive one, involving scientists from many disciplines working together to provide many types of information. Modern computational and experimental techniques have been very much developed to provide structural information about the biologically active molecules that are involved in disease processes [41].

The discovery of new pharmaceutical agents has gone through an evolution over the years and has been adding new technologies to this increasingly complex process [42]. The traditional way to discover new drugs has been to screen a large number of synthetic chemical compounds or natural products for desirable effects. This approach for the development of new pharmaceutical agents is not an ideal one for a number of reasons. The biggest drawback to the screening process is the requirement for an appropriate screening procedure. Although drugs are ultimately developed in the clinic, it is usually inappropriate to put chemicals of unknown efficacy directly into humans [43]. Consequently, other systems like an animal model system have to be developed. Secondary screening in animal model systems has additional problems such as:

- The animal model may not accurately reflect the human disease.
- The chemical may be extensively metabolized to a different compound in the animal before it reaches the target.
- The chemical may not be absorbed or distributed as it is in humans. In each of these cases, the active structure potentiality will not be identified.
Moreover chemical compounds discovered by this approach commonly do not have optimal structures for modulating the biological process. This in turn may require administration of larger quantities of the drug and increase the risk of unwanted side effects.

Computer-assisted drug design uses the principles of computational chemistry to discover, enhance, or study drugs and related biologically active molecules [44]. The most fundamental goal of this technique is to predict whether a given molecule will bind to a target and if so how strongly. Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when the small molecule binds to it. Semi-empirical, ab-initio quantum chemistry methods, or density functional theory are often used to provide optimized parameters for the molecular mechanics calculations and also to provide an estimate of the electronic properties of the drug candidate which will influence binding affinity.

Ideally, the computational method should be able to predict affinity before a compound is synthesized and hence in theory only one compound needs to be synthesized [45]. The reality however is that present computational methods provide at best only qualitative accurate estimates of affinity [46, 49]. Therefore in practice it still takes several iterations of design, synthesis, and testing before an optimal molecule is discovered. On the other hand, computational methods have accelerated discovery by reducing the number of iterations required and by providing more novel small molecular structures [47, 48] as lead molecules.

1.6 Computational drug designing

Fitting a drug molecule to the target and computing of the corresponding binding energy are two major computational steps in drug designing. The primary tool for this analysis is the “docking” technique, in which an automated algorithm positions the molecule in many different orientations in the active site to identify the lowest energy orientation [50]. This direct correlation between computational results and drug activity makes docking as the foundation of structure based drug design.
The following are some other typical criteria that may be necessary to identify a compound into a potential drug.

- Concentration-dependent activity
- Activity in both biochemical and cell-based assay
- Half maximal inhibitory concentration (IC50) below the threshold (perhaps low micromolar, or down to the nanomolar range)
- Some understanding of the structure activity relationship of the drug molecule.
- Knowing the binding kinetics
- Assessing the selectivity and stability
- Collection of well established and pure structure.
- Possibility for synthetic tractability
- Patentability of the compound.
- Presence of some apparent path for optimization (creating derivatives).
- Possibility for measurement and analysis of solubility and log D.
- Predictability of metabolic liabilities.
- Inclusion of pharmacokinetics (ADMETox) properties including the possibility for side effects. (e.g., whether the drug will block hERG channels, resulting in drug-induced cardiac arrhythmia), carcinogenicity etc [51].

1.7 Literature survey and identification of the research problem

After pointing out AD as a major degenerative neural disorder about 100 years back, researches entered in this field to find a suitable drug for controlling the development of AD. Still, it has attained full momentum very recently. The growing rate of AD has been pointed out by Ron Brookmeyer, Elizabeth Johnson, Kathryn Ziegler-Graham and H. Michael Arrighi[52]. In 2006 the worldwide prevalence of Alzheimer’s disease was 26.6 million. By 2050, prevalence will quadruple by that time 1 in 85 persons worldwide will be living with the disease. They concluded that about 43% of prevalent cases need a high level of care. If interventions could delay both disease onset and progression by a modest 1 year, there would be nearly 9.2 million fewer cases of disease in 2050 with nearly all the
decline attributable to decreases in persons needing high level of care. Pharmacological treatment protocol for AD has been included in the study of Robin Hsiung, Christopher Patterson, John W. Feightner, Angeles Garcia [53]. In this paper, the authors define the role of the primary care physician (PCP) in the management of Alzheimer’s disease (AD) and to propose a model for a work plan. In the research of Becker RE and Greig NH [54] to study the medical failure of AD treatment, they came to the conclusion that it is high time for introducing new methods and practices to control the disease. The association between anticholinergic drugs given to patients of cardio vascular patients and AD has been intensively studied by Uusvaara J, Pitkala KH, Tienari PJ, Kautiainen H, Tilvis RS and Strandberg TE [55]. Similarly risk of incident AD in diabetic patients has been studied by Daniel Kopf and Lutz Frölich [56]. Various sociological attitudes to aging and aging problems have been analyzed by the sociologists Dowling GA, Graf CL, Hubbard EM, Luxenberg JS [57]. The genetic factors involved in AD have been studied by Olgiati P, Serretti A etal. [58]. The present status of drug designing for AD has been reviewed by Marwan N. Sabbagh [59] supporting computational methods to drug designing.

Though there are no effective drugs for most of the neurodegenerative disorders in modern medicine, in the alternative medical practices like Ayurveda, Sidha and our traditional naturopathy, there are reports of success in treating some of the neurodegenerative diseases like Parkinson’s disease [60, 61, 62].

1.8 Objectives of this research work

The major objectives of the research work are:

- Identification and characterization of genes and proteins responsible for Alzheimer's disease.
- Study of the influence of the non coding RNA in controlling AD.
- Identification of phytochemicals generated from medicinal plants as potential drug molecules for AD.
- Possibility of applying gene silencing technique in the pre-transcriptional stage by preventing the mutation and thereby controlling the disease.
Conducting interactional studies by using a flexi-docking algorithm (CDOCKER).
Identification of multi-targeted potential drug molecules.
Analysis and characterization of ‘Protein-DNA’ based complexes found in AD patients.
Pharmacokinetic studies of the suggested molecules.
ADMETox study of the drug molecules.
Refining the potential drugs by slight structural modifications to suit pharmacophore properties like solubility, log D etc.
Drug delivery modeling.

1.9 Research methodology

Computational methods have been used to redefine drug likeness and characterize the targets- DNA, RNA and protein. The effect of non coding RNA in controlling AD has been studied. Mutations responsible for AD have been identified and characterized. ‘DNA-protein’ based complexes have been analyzed to study their stability. The microsatellite regions of the AD genes have been studied. AD Protein molecules have been characterized. Phytochemicals present in medicinal plants have been used as the ligand molecules [63, 64]. The major anti-Alzheimer’s drug molecules identified include limonene, A R Turmerone, curcumin, tryptophan, alpha-tocopherol, ar-turmerone arecoline, berberastine, berberine, bis-desmethoxycurcumin, choline, curcumin, galanthamine, huperzine-a, huperzine-b, lecithin naringenin, niacin, phosphatidyl-choline, physostigmine, quercetin, rosmarinic-acid, smilagenin, thiamin, tocopherol.

Docking studies have been conducted to identify DNA and protein targeted ligands from the phytochemicals. The major disadvantage of conventional docking technique is the poor flexibility of the target molecule, which is not permitting the molecule to adjust its conformation up on binding. Molecular Dynamic (MD) simulation can be used to treat both ligand and target in a flexible way, allowing for an induced fit of the receptor-binding site around the newly introduced ligand. In this work, CDOCKER tool of Accelrys’s Discovery Studio [65], has been used for the interactional studies, which works on a protocol consisting of combination of MD simulations and docking.
The solubility of all potential drug molecules in water has been computed. Structural changes were carried out by introducing $-NH_2$ and $-OH$ groups in different positions of the ligands. Moreover, the effect of dissolution of the ligand molecules in different solvent molecules such as glycerol, ethanol, glycol and glyceryl esters of Oleic, Myristic, Palmitic, Stearic, Lauric, Butyric, Caproic, Capric, Caprylic, Linoleic and Linolenic has been studied.

The drug delivery has been modeled by studying the Blood Brain Barrier (BBB). Pharmacokinetic studies of the potential drug molecules have been made.

1.10 Highlight of the research work

Receptor based drug designing technique has been used to identify potential drug for Alzheimer’s disease. DNA, RNA and protein have been made as the targets for the disease. The method involves a pharmacoinformatic high throughput screening approach. Ligand molecules are identified from the phytochemicals. As all the important pharmacoinformatic tools such as ADMETox, solubility, bioavailability have been tried before coming to the result, the possibility of success of the identified drug in the clinical trial is very high. Moreover, the method gives way in to a new culture for drug designing, with an initial screening of probable molecules by pharmacoinformatic techniques followed by pharmacological method to identify the real drug molecule from the short listed samples.

1.11 Organization of the Thesis.

The rest of the thesis is divided into nine chapters. An overview of Computational drug designing used in this research work is outlined in Chapter 2. This includes the introduction of common drug designing methods, computational tools and possibilities, machine learning approach for redefining drug likeness and common ADMETox and pharmacokinetic tools.

Chapter 3 gives an outline of biomolecular aspect of Alzheimer’s disease and common biochemical pathways to be analyzed during drug designing.

Protein based drug designing has been included in Chapter 4. The computational characterization of AD protein molecules through structural and
sequence analysis, thermodynamic studies and study of pockets and cavities has been included in this chapter.

DNA based drug designing through gene silencing technique is introduced in Chapter 5. Identification of microsatellite regions of the AD genes and study of mutations responsible for AD has been included in this chapter.

The influence of non-coding RNA on post-transcriptional gene silencing of Alzheimer disease has been included in Chapter 6. Analysis and characterization of ‘Protein-DNA’ based complexes of AD has been included in Chapter 7.

Identification and screening of ligand molecules for targeting Protein and DNA has been included in Chapter 8. The interactional studies, physiochemical characterization and ADMETox studies of the ligand molecules have been included in Chapter 9. The suggested drug modification and identification of potential drug for AD and modeling of BBB have been included in this chapter. Chapter 10 summaries the findings of research and explains the scope for further work.

1.12 Summary

Essentially, computational drug design involves identification of small molecules that are complementary in shape and charge to the biomolecular target. Modeling techniques for prediction of binding affinity are reasonably successful. However there are many other properties such as bioavailability, metabolic half life, lack of side effects, etc. that first must be optimized before a ligand can become a safe and efficacious drug.

The other characteristics can also be optimized using rational drug design techniques known as computational drug design and delivery system (CD3S). The target based drug designing is carried out in three phases-DNA based, RNA based and protein based. Ligand molecules have to be identified depending up on the target from a large number of preferably phytochemicals. The computational technique consists of the following steps.

1. Identification of target.
2. Characterization of target (identification of promoter/ suppressor, possible ORFs, surface property, thermodynamic behavior, active site identification).
3. Identification of ligand molecules from phytochemicals. (Other alternative medical practices can also be studied to trace the ligand molecules)
4. Modeling and simulation studies of ligand molecules to analyze the stability, potential factor, solvation effect etc.
5. QSAR studies and prediction of molecular properties.
6. Receptor-ligand interaction studies. (ligad poses, minimization, flexible docking, calculating binding energy)
7. Pharmacophore analysis (3D QSAR pharmacophore generation, building 3D database/figure prints, feature mapping and interaction generation)
8. ADMETox studies (ADMETox descriptors and toxicity prediction)
9. Identification of potential drugs for the disease.
10. Drug delivery modeling