INTRODUCTION

There are so many uneasy and cruel health conditions affecting human beings that are collectively termed as diseases or disorders. To achieve wellness and comfort over such conditions one of the methods is to use specific drug molecules which act on the human body relieving such uneasy conditions with or without very minimum of other responses. The designing of such new chemical molecules and making them available to the patients is a very complex, time consuming and costly process. It may take from 12 to 15 years of time and cost around 350 million dollars on an average for a new drug to be introduced into the market. To minimize the cost and time factors, rational drug design approach is required.[1]

Drug design, occasionally referred to as rational drug design or purely rational design, is a process of identifying new medicines on the basis of prior knowledge of the biological target. A drug is in general an organic small molecule that either activates or inhibits the function of a biological system such as a protein, that consequences in a remedial advantage to the patient. Drug design involves the design of small molecule that acts complementary in shape and charge to the biological target with which it interacts and thus binds to it. Drug design techniques normally rely on computer modeling techniques. Such type of modeling is known as computer-aided drug design (CADD), or rational drug design/computer aided molecular modeling/*in silico* drug design.[2,3]

The work presented in this thesis involves the use of different computational techniques and is divided into three different sections. **Section I** deals with the design and systematic validation of computational models developed for some anti-cancer agents. The focus of research work in cancer is on the development of computational models on aurora kinase inhibitiors. **Section II** deals with the design, synthesis and biological evaluation of some novel heterocyclic derivatives against Alzheimer's disease. **Section III** concerns with the development of i*n silico* model of ACAT receptor inhibitors and generation of a pharmacophore model along with a 3D-QSAR model for inhibitors of the target using molecules previously reported from this laboratory.

These three sections are discussed in brief in this section. Details of the three sections are given individually at respective places.

Biochemically kinases are the enzymes that catalyze the phosphorylation of substrates with the transport of phosphate group from high energy phosphate donating molecules to the substrates. The kinases are broadly divided into three major classes viz. lipid, carbohydrate and protein kinases on the basis, whether the substrate to be phosphorylated is a protein, lipid, or carbohydrate. This can influence their action, reactivity, and ability to bind other molecules. Therefore, the kinases perform very critical and important role in metabolism, protein regulation, cell signaling, cellular transport and many other cellular pathways.[4]

Protein kinases are enzymes that modify other proteins by phosphorylation. This typically results in a functional change of the target protein (i.e. substrate) by altering enzyme activity, association with other proteins or cellular location. There are more than 500 protein kinases which are associated with different physiological functions in the body. Kinase activity modifies almost up to 30% of all human proteins and is known to control the majority of the cellular pathways, particularly those related to signal transduction. Protein kinases are mainly of four types, which include Tyrosine specific protein kinases, Serine/threonine specific protein kinases, Histidine specific protein kinases and mixed kinases, according to the name of the amino acid they phosphorylate in the cascade and are majorly associated with the signal transduction.[4,5]

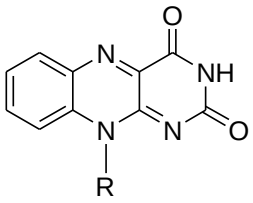
Serine/threonine protein kinases (EC 2.7.11.1), phosphorylate the OH group of serine or threonine. Out of >500 human protein kinases, at least 125 are serine/threonine kinases. In **Section I** of this thesis, designing and systematic validation of the computational models developed on some anti-cancer agents have been described in detail. Here the focus is on the development of computational models on aurora kinase inhibitiors. Aurora kinases are mitotic serine/threonine kinases that participate in the cell cycle and formation of the mitotic spindle assembly, and have been widely considered as prospective targets for a new class of anticancer drugs.[6,7] The aurora kinase family is divided into three subclasses: aurora A kinase, aurora B kinase and aurora C kinase. Aurora A localizes mostly in the centrosomes, late in the S phase and near the beginning of G1 phase. It is maximally expressed in the G2/M phase of the cell cycle, and is a means of accurate centrosome maturation and separation.[8] Aurora B localizes mainly in the spindle midzones and functions in the attachment of the mitotic spindle to the kinetochore of the centrosome. It controls chromosome segregation and cytokinesis.[9] Aurora C shows similar functions to those of Aurora B and is required for cell division.[10] Inhibition of any of the isoforms of aurora kinases has shown cancer cell death by apoptosis and mitotic catastrophe. [11-14]

In section I of this thesis, two different Quantitative structure activity relationship (QSAR) models have been developed on two different data sets obtained from the literature.

QSAR or quantitative structure property relationship (QSPR), is a mathematical process in which chemical structures are quantitatively correlated with some well defined process, such as biological activity or chemical reactivity. When physicochemical properties or structures are expressed in numbers, one can form a mathematical relation between the structure and the activity. The mathematical expression can then be used to predict the biological response of other chemical structures. The QSAR is based on the assumption that there is an underlying relationship between the molecular structure and biological activity.[11]

The first model generated here is a **3D-QSAR** model to study the structural requirements for a series of imidazo[1,2-*a*]pyrazine derivatives as aurora A kinase inhibitors. For the development of this model a data set of 51 imidazo[1,2-*a*]pyrazine aurora A kinase inhibitors were retrieved from publications previously reported by Merck Research Laboratories. For the development of this model, different chemoinformatics tools like AutoDock and Tripos Sybyl were used. The second model generated here is a **4D-QSAR** model to identify the requisite features for the inhibition of aurora A kinase enzyme. The 4D-QSAR approach is a grid-based technique, first proposed by Hopfinger *et al*. As compared to the conventional 3D-QSAR, this method uses a fourth dimension, i.e. conformational flexibility. To date, very few studies based on 4D-QSAR technique have been reported and, to the best of our knowledge, this is first report of 4D-QSAR on aurora kinase inhibitors. This work pertains to the development of a 4D-QSAR model for a series of 31 benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6(11*H*)-one derivatives as aurora A kinase Inhibitors and its methodical validation. The extent of validation utilized for this 4D-QSAR model remains unreported to date. The softwares used for this model development include AutoDock, OpenBabel, GROMACS, MATLAB, Schrodinger, Gaussian, PRODRG server and LQTA-QSAR tool. The outcome from both of the models is described in detail in **section I**. Along with routine validation methods, both the models were validated in detail and systematically by using various validation parameters to standardize the results. Various validation techniques used and their significance in the model are described in detail in subsequent sections.

In **Section II** the design, synthesis and biological evaluation of some novel heterocyclic derivatives against Alzheimer's disease have been described. Alzheimer's disease (AD) is an overwhelming neurodegenerative disorder characterized by a progressive and irreversible decline in cognitive functions. It typically develops leisurely, and gradually worsens as brain cells shrink and die. Eventually, Alzheimer's proves fatal, and at present, there is no complete cure for it. AD affects the cholinergic regions of the central nervous system (CNS) associated with cognitive functions and awareness.[15] Here, some novel cholinesterase inhibitors (which include both acetylcholinesterase as well as butyrylcholinesterase inhibition) have been designed and synthesized for the treatment of AD. The basic scaffolds for the five different series of compounds synthesized and described in this thesis are as follows:



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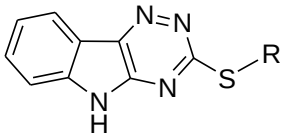

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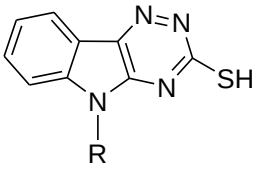

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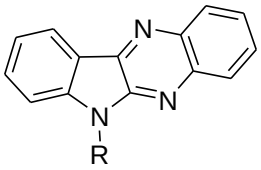
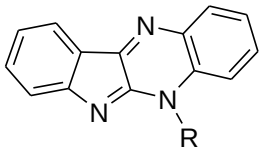


IV



V

In all, a total of 65 compounds were synthesized, characterized by IR, NMR and Mass spectroscopic techniques and evaluated for their cholinesterase inhibition activity by Ellman's method. Further, the potent compounds from Series I were evaluated for their ability to prevent β-amyloid (Aβ) aggregation in presence and absence of *h*AChE by Thioflavin-T (ThT) and Congo red (CR) binding assays respectively. Further, to evaluate the cytotoxic profile of the potent compounds from series I, cell viability assay on SH-SY5Y human neuroblastoma cells was performed. The synthesis and biological evaluation of these compounds in detail have been described in this section of this thesis.

The acyl-coenzyme A: cholesterol O-acyltransferases (ACAT) is a small family of enzymes comprising of three homologous members, namely acyl-coenzyme A: cholesterol O-

acyltransferase 1 and 2 (ACAT-1 and ACAT-2), and acylcoenzyme A: diacylglycerol acyltransferase 1 (DGAT-1). ACAT has generated much interest as a potential target for exploring atherosclerotic disease process by both lipid and non-lipid mechanisms. ACAT family enzymes perform important biological functions. ACAT-1 and ACAT-2 are critical for the *in vivo* cholesterol homeostasis. At the single cell level, they prevent building up of free excess cholesterol in the cell membranes. Under pathophysiological condition, these enzymes convert excess cholesterol into cholesteryl esters in cholesterol-loaded macrophages. These macrophages are gradually converted into foam cells, which is a hallmark of early lesions of atherosclerosis.[16]

Till date there is no enzyme structure identified for ACAT. This brings the limitations in the development of better and effective ACAT inhibitors for the treatment of atherosclerosis. In our lab researchers are engaged in syntheses of ACAT inhibitors. On the basis of biological activity data obtained from our laboratory, a predictive and robust pharmacophore model along with a 3D-QSAR model for ACAT inhibitors have been developed. Further, possible enzyme structures for ACAT 1 and 2 were developed to depict possible active sites of the enzymes for better drug design. The software's used here for this study included the use of Glide and PHASE modules of Schrodinger, MODELLER, online server Phyre and i-Tasser. The procedure and results obtained from this study are explained in detail in **section III** of this thesis.

All the three sections are described sequentially in the coming sections.