Chapter - 1

INTRODUCTION

Computer aided drug design is a rapid growing field and it has emerged as a major player to design new drugs in a rational manner. In this work, the application of computational drug design techniques including structure-based drug design, ligand-based three-dimensional quantitative structure-activity relationship (3D-QSAR) methods and combined virtual screening approaches were studied using a well known anti-cancer target 3-phosphoinositide dependent kinase-1 (PDK1). These computational drug design approaches have been successfully applied for screening and optimization of new lead molecules against PDK-1 kinase, a well known anti cancerous agent.

Drug design also known as rational drug design, is an approach identified new molecules based on the available information of the biological target molecule (Madsen et al., 2002). The drugs are generally a small organic molecule which either activates or inhibits the function of a biological target molecule. However these drugs need to be designed in such a way so as not to affect functioning of any other important related molecules in biological system (Jurgen., 2000; Rother et al., 2006)

An earlier effort on designing a new drug has always been a hit and trial method. Later in 1960's some concepts were build up between quantitative properties of a drug and the biological activity. These developments means the use of computer aided drug designing to a new

era in drug discovery system. The conventional way of drug designing although gives a novel drug, but is an expensive, time consuming activity that usually fails. However, with the introduction of information technology, it can be done more efficiently in silico, thereby saving huge money, time and man power. The approach of whole drug discovery process consists of seven basic steps: disease selection, target selection, lead compound identification, lead optimization, preclinical trials, clinical trial testing and pharmacogenomic optimization (Roderick ., 2006)

Figure: 1.1 Various stages of computer aided drug designing adopted form (Hammer., 2002)

During the last decades the area of drug designing has been assisted by modern science using computational and experimental approaches. Computer-assisted drug design (CADD) process uses computational chemistry to design, improve, or study drugs and drug like biologically active molecules (Richards., 1994). The main goal is to predict whether a given small molecule will bind to a target binding sites and if so how strongly. Now a days CADD approaches are being implemented in all important stages of drug discovery process like target identification, lead identification, lead optimization, prediction of absorption, distribution, metabolism, elimination, and toxicity (ADMET) properties and design of compound libraries(Young., 2009) CADD approach is always quicker and cheaper process than previous used conventional drug designing approaches (Young., 2009) This approach plays an important role in lead identification as well as lead optimization. The major achievement of

this approach has been successful development of marketed drugs like HIV protease inhibitor Viracept and the anti-influenza drug Relenza (Shoichet., 2004). In both these cases, computational methods were used to envisage the activity of the designed inhibitor prior to synthesis and testing (Reddy et al., 2005).Thus this approach plays a key role to assist the experimental work as well as prioritize the synthesis of the next compounds. Recent report also revealed that the computer aided drug designing process play a important role in open source drug designing projects (Orti et al., 2009; Delano., 2005)

In recent applications of CADD, to find out a ligand (the putative drug molecule) that will bind favorably to the receptor binding site. This ligand receptor association may incorporate several interactions like hydrophobic, electrostatic, and hydrogen-bonding. Apart of these interactions solvation energies of ligand as well as receptor site also play an important role in ligand and receptor binding (Richards., 1994; Wang et al., 2005).

The approach of CADD is basically dependent up on the amount of data available about the ligand and receptor. Before proceeding to CADD method one should have 3-dimensional structural data for the receptor molecule as well as X-ray or NMR data of receptor ligand complex, used as reference data for designing new lead molecule.

Taking this into account the data that is accessible defines two fundamental methodologies for computer based drug design (Waterbeemd et al., 1997)

1. Analogue or ligand based drug designing (LBDD).
2. Structure or target based drug designing (SBDD)

Ligand base approach or indirect approach make use of ligand molecule or series of ligand molecule as starting point and explore the properties of these ligand molecule for designing of better lead molecule (Ooms., 2007; Veselovsky et al., 2003). Based on the study of these known ligands a hypothetical information of receptor active site can be proposed (Costanzi et al., 2008).The receptor based drug designing approach is applicable when proper information about receptor site is available either in the form of X-ray/NMR data or homology modeling data. These receptor structures are used as starting point for designing new drug molecule (Congreve et al., 2005) With the accessibility of the receptor site, the issue is to design ligand that will interact more favorably at the binding site of the receptor, which is a docking issue (Hillisch et al., 2004), while exhibiting (proposing) pharmacological activity.

Analogue-based drug design (or indirect drug design) relies on knowledge small ligands molecules that bind to the active site of receptor molecule. These series of small molecules may be used to derive a pharmacophore model which defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. In other words, a model of the biological target may be built based on the knowledge of what binds to it and this model in turn may be used to design new molecular entities that interact with the target. Alternatively, a quantitative structure-activity relationship (QSAR) in which a correlation between calculated properties of molecules and their experimentally

determined biological activity may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs.

An interesting example of small-molecules designed using a ligand-based approach is the case of tubulin inhibitors (Chiang et al. 2009). Tubulin polymerization, an essential component of cell cycle progression and cell division, represents an important target in anticancer therapy. Several antimitotic agents – like vinblastine and colchicine – have already been discovered and are clinically used, despite often low bioavailability, significant toxicity, rapid acquired resistance, and the resulting over expression of drug-resistant pumps that eject these antimitotic inhibitors from the cell. However, due to these unfavourable properties, researchers have devoted substantial effort to discover new agents with more tolerable and effective properties, especially since it is believed that antimitotic agents could work to diminish blood supply to cancerous tumors. To solve this problem ligand based approach is used to design new class of small molecules against tubulin by using a set of 21 indole-derivatives (Liou et al. 2006) by designing of successful 3 dimensional pharmacophore.

Structure-based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as X-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and

the intuition of a medical chemist (Anderson 2003; Gane and Dean 2000; Klebe 2000). In recent years, several cases of successful applications of structure-based drug design have been reported (Combs 2007; Coumar et al. 2009; Khan et al. 2010; van Montfort and Workman 2009). Given the three dimensional structure of a target molecule, chemical compounds having potentially high affinity for this target can be designed rationally with the aid of computational methods. Based on a binding site-derived pharmacophore model, a pattern of putative interaction sites, the results consist of a collection of virtual ligands complementary to a three dimensional structure of the binding pocket.

A successful example of structure-based pharmacophore modelling in cancer treatment can be found in the identification of PUMA inhibitors (Mustata et al. 2011). PUMA, the p53 upregulated modulator of apoptosis, is induced by a wide range of apoptotic stimuli through both p53- dependent and -independent mechanisms. This cancer treatment target is central in mitochondria-mediated cell death by interacting with all known anti apoptotic Bcl-2 family members (Yu and Zhang 2009). Over the years, it has become increasingly apparent that apoptosis acts as a barrier against oncogenesis. Deregulated apoptosis contributes to tumor formation, tumor progression, and impaired responsiveness to anticancer therapies, and recent studies suggest that the function of PUMA is compromised in cancer cells (Yu and Zhang 2009).

The present research work entitled **"Computation Identification and Evaluation of PDK-1 inhibitors as potential anticancer drug"** is divided in to three major parts:

1. The **first part** defines the designing of 3 dimensional ligand based pharmacophore (3D QSAR) and pharmacophore based virtual screening of small molecule inhibitors from NCI and Maybridge database.

2. The **second part** includes the molecular docking based screening of natural compounds against PDK-1 kinase. Further validation of active hit (Myricetin) was done by molecular dynamic simulation study.

3. The **third part** of work attempts towards the screening of new 95% similar analogues from PubChem database. Further pharmacokinetics validation was done by *Insilico* ADMET study of screened molecules.