IDENTIFICATION OF ANTICANCER DRUG THROUGH STUDYING THE ACTIVATION MECHANISM OF CASPASE PROTEIN: AN *IN SILICO* APPROACH

MD. ARIF KHAN
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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

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Md. Arif Khan

30 August, 2018

SUMMARY

Apoptosis, a process of programmed cell death controlled by a defined apoptotic pathway, plays an important role in the development of all multicellular organisms. Any alterations in apoptotic pathways have been implicated in Cancer, one of the most deadly diseases in the world and Bangladesh as well. The pathway of Apoptosis is executed with a cascade of sequential activation of initiator and effector caspases, a family of proteases found in Apoptotic pathways to induce Apoptosis during abnormal cell growth in the cell cycle. Due to limited number of successful inhibitors in the market as well as pharmacological constraints of designed and tested peptide and peptidomimetic inhibitors against Caspase protein, In this study, *In Silico* approach like Virtual Screening with Molecular Docking applied to identify potential lead compounds against Pro-Caspase 7, one of the effector proteins of Apoptotic signaling proteins and 1571 drug-like molecules downloaded from the Binding database and ZINC database and identified four potential lead compounds. Noncovalent interactions like hydrogen bond, halogen bond, hydrophobic interaction, electrostatic interaction are examined among all the identified potential lead compounds and Pro-Caspase 7 after Molecular Docking study. In addition, Molecular dynamics study conducted to test the feasibility of the identified compounds in biological systems and again checked Molecular Docking energy and binding interactions for the Pro-Caspase 7 protein. Molecular dynamics study significantly increased binding energies among Pro-Caspase 7 and the first and third potential lead compounds and that are -15.8 and -14.3 $kcalmol^{-1}$ respectively. Pharmacoinformatics analysis predicts that all potential lead compounds are non-carcinogenic and nonmutagenic. And, hence considering Molecular Docking study, Molecular dynamics study and Pharmacoinformatics study, the identified four potential lead compounds can induce Pro-Caspase 7 to Caspase which leads to Apoptosis and ultimately works for Cancer treatment.

Although this *in silico* study helps the researchers and pave the way for Anti-Cancer drug development, further wet lab assessment of these potential lead compounds has to be performed.

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CHAPTER-1
INTRODUCTION

Apoptosis plays an essential role in the development of all multicellular organisms and the homeostasis of adult tissues [Steller, 1995; Jacobson *et al.*, 1997; Horvitz, 1999]. Though the phenomenon had been described for almost a century, in 1972, Kerr, Wyllie, and Currie first coined the term "apoptosis" in order to differentiate naturally occurring developmental cell death from necrotic cell death that results from acute tissue injury [Kerr *et al.*, 1972]. Alterations in apoptotic pathways have been implicated in many diseases, such as cancer and neurodegenerative disorders [Thompson, 1995; Yuan and Yankner, 2000].

Cancer cells, in particular, are known to have a decreased sensitivity to proapoptotic signals when compared to normal cells. It is well established, however, that anticancer drugs are effective at inducing the cell death program by a variety of mechanisms [Fulda and Debatin, 2004; Kabore *et al.*, 2004; Meng *et al.*, 2006]. Cancer is the reason for one in eight deaths worldwide. Cancer cases are expected to flow 57% worldwide in the next 20 years, an impending "Human disaster" that will need a renewed focus on hindrance to combat, according to the WHO (World Health Organization). Like many other countries in the world, cancer is one of the major killer diseases in Bangladesh [Garcia *et al.*, 2007; Hume and Christensen, 2014].

The mechanism of apoptosis is remarkably conserved across species, executed with a cascade of sequential activation of initiator and effector caspases [Thornberry and Lazebnik, 1998; Budihardjo *et al.*, 1999]. Caspases are a family of proteases that are involved in the execution of apoptosis and the inflammatory response. Apoptosis is primarily executed by active caspases, which are derived from the inactive pro-caspase zymogens through proteolytic cleavage [Chai *et al.*, 2012]. If the caspase protein remains inactive during the cell cycle process, the apoptosis process remains silent that ultimately induces cancer development. So, activation of caspase is necessary for the human body to start apoptosis. To date, Hundreds of peptide and peptidomimetic inhibitors have been designed and tested, but only a few have advanced to clinical trials because of poor drug-like properties and pharmacological constraints. There is the limited number of successful inhibitors found in the market. At this moment, identification of new drug would be helpful for overcoming clinical and financial

challenge to the nation [Festjens *et al.*, 2006; Fleischer *et al.*, 2006; Lamkanfi *et al.*, 2007; MacKenzie *et al.*, 2010; Hensley *et al.*, 2013; Koff *et al.*, 2015].

Recently, it has been proposed that initiator and effector caspases of apoptosis are required for non-apoptotic functions [Newton, 2003; Woo *et al.*, 2003; Huh *et al.*, 2003; Fernando *et al.*, 2005; Kuranaga *et al.*, 2006; Siegel, 2006]. Concerning caspase-3, an active caspase-3 fragment is immunostained in the proliferative region in rat brain [Yan *et al.*, 2001], caspase-3 is upregulated just prier to mitosis [Hsu *et al.*, 2006], and the treatment with a caspase-3 inhibitor induces cell death at late mitosis [Swe & Sit, 2000]. Caspase-8 is pointed out to play an essential role in the development and activation of immune cells [Siegel, 2006; Yan *et al.*, 2001]. In addition, the activity of caspase-3, -7, -8, and -9 is elevated in some tumor cells [Yang *et al.*, 2003; Vakkala *et al.*, 1999; Nakopoulou *et al.*, 2001]. We have recently reported a possible involvement of caspase-7 to the cell cycle progression at mitosis [Hashimoto *et al.*, 2008]. The clear evidence, however, has not been available for the role of caspases in the regulation of cell proliferation.

The aim of the study has focused on the design of inhibitors against caspases through virtual screening of drug databases and molecular docking of that selected drugs. Ligand and receptor-based virtual screening approaches will be adopted in the present research.

Virtual screening has been defined as the "automatically evaluating very large libraries of compounds" using computer programs [Walters *et al.*, 1998]. As this definition suggests, VS has largely been a numbers game focusing on how the enormous chemical space of over 10^{60} conceivable compounds can be filtered to a manageable number that can be synthesized, purchased, and tested [Bohacek *et al.*, 1996]. Although searching the entire chemical universe may be a theoretically interesting problem, more practical VS scenarios focus on designing and optimizing targeted combinatorial libraries and enriching libraries of available compounds from in-house compound repositories or vendor offerings. As the accuracy of the method has increased, virtual screening has become an integral part of the drug discovery process [McGregor *et al.*, 2007]. Virtual screening is a computational technique that represents popular and costs efficient tool for developing potential lead drugs [Kitchen *et al.*, 2004; Sliwoski *et al.*, 2013; Song *et al.*, 2009]. The main objective of this method is the reduction of the vast virtual chemical space related to the drug-like molecules by screening

them against a particular protein receptor. From the receptor-drug interaction, an appropriate number of drug-like molecules that strongly and specifically bind with the receptor protein can be isolated leading to a potential drug candidate. There are two kinds of virtual screening techniques: ligand-based and structure-based [Drwal and Griffith, 2013; Aparoy *et al.*, 2012; Wilson and Lill, 2011]. There is a rise of success stories reported by use of structure-based virtual screening among which docking based screening is the most popular one. The aim of virtual screening is to identify molecules of novel chemical structure that bind to the macromolecular target of interest. Thus, success of a virtual screen is defined in terms of finding interesting new scaffolds rather than the total number of hits. Interpretations of virtual screening accuracy should therefore be considered with caution. Low hit rates of interesting scaffolds are clearly preferable over high hit rates of already known scaffolds. Structure-based drug design is clearly becoming a valuable and integral part of the drug discovery, and is perhaps the most elegant approach for discovering compounds exhibiting high specificity and efficacy. At the present time, a number of recent successful drugs have in part or in whole emerged from a structure-based research approach. Understanding the molecular basis of a drug action and exploring the chemical interactions involved in the complex processes of drug delivery and reaction with a variety of biological molecules are among the most important goals of contemporary drug design [Wilson and Lill, 2011; Lionta *et al.*, 2014; Toledo *et al.*, 2014].

Molecular docking is an essential aspect of computer-aided drug design and widely used protocol to evaluate the binding affinity of a ligand to its target receptor without considering receptor conformers. However, biological phenomenon of ligand-receptor binding is a dynamic process, where both ligands and receptors can morph into different conformations [Seeliger *et al.*, 2010; Murga *et al.*, 2008]. A major limitation in molecular docking method is that of failure to account for receptor flexibility [Kokh *et al.*, 2011; Totrov and Abagyan, 2008]. It is important to consider receptor flexibility as binding affinity predicted by docking against rigid receptor may not provide the accurate image of binding affinity and binding interactions between a ligand and a receptor [Totrov and Abagyan, 2008]. Each docking mode is termed as a ‘pose’ [Rahman *et al.*, 2016; Rahman *et al.*, 2016A]. In order to identify the energetically most welcoming/favorable pose, each pose is evaluated (‘scored’) based on its compatibility to the target [Saleh *et al.*, 2016; Shawon *et al.*, 2016].

Molecular dynamics (MD) is a computer simulation method for studying the physical movements of atoms and molecules. The atoms and molecules are allowed to interact for a fixed period of time, giving a view of the dynamic evolution of the system. In the most common version, the trajectories of atoms and molecules are determined by numerically solving Newton's equations of motion for a system of interacting particles, where forces between the particles and their potential energies are often calculated using interatomic potentials or molecular mechanics force fields. The method was originally developed within the field of theoretical physics in the late 1950s but is applied today mostly in chemical physics, materials science and the modelling of biomolecules [Fermi *et al.*, 1955; Alder & Wainwright, 1959; Rahman, 1964]. MD simulation of protein structure can be performed in an aqueous environment to generate conformations predictive of those taken by a protein under physiological conditions. Alternatively, different crystallographic structures of the same protein bound to different ligands or with different mutations can be used [Totrov and Abagyan, 2008].

Objectives of Specific Aims and Possible Outcome

The objectives of the research can be summarized as follows:

The key focus will be on the development of selective inhibitor by computational approach against Pro-Caspase 7. The overall process summarized below briefly:

- Ligand Identification to activate Caspase 7 through Virtual screening (VS) of drug molecules from BindingDB and ZINC database.
- In subsequent steps, selected suitable drugs will be used for further computer-aided drug design approach.

The aim of the present study is depicted in Fig 1.

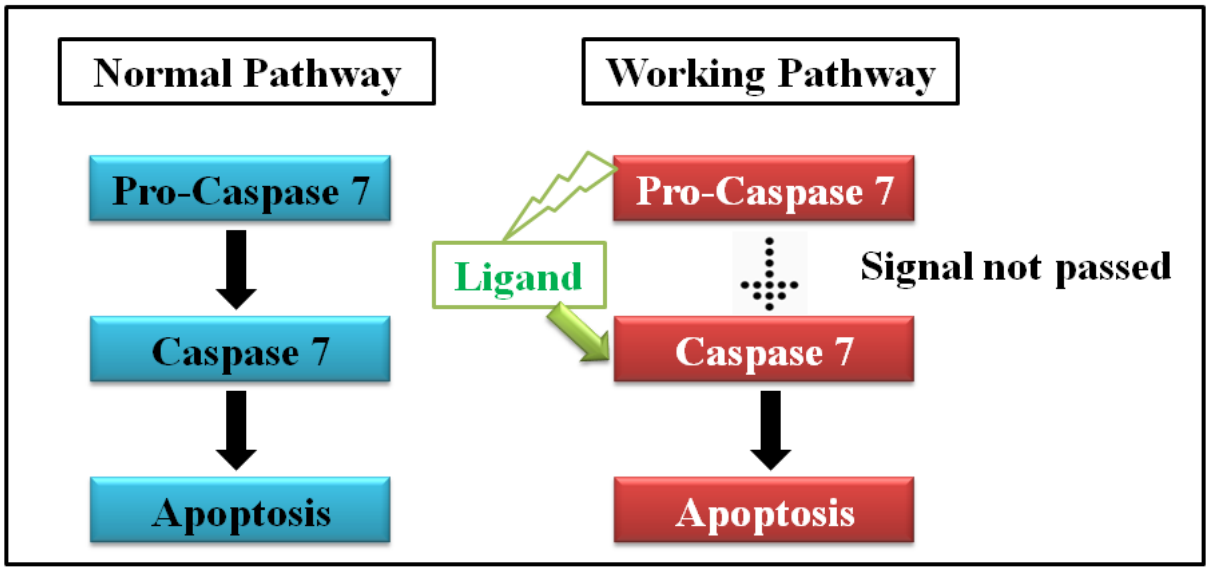

Figure 1 Specific aims and possible outcome of the research.

CHAPTER-2

REVIEW OF LITERATURE

Scientific communities have been analyzing the mechanism of Apoptosis in various part of the world for several decades. Researchers published their findings in different national and international scientific journals. Nowadays, a new research cannot be launched without back ground information. Review of literature is the easiest method to obtain sufficient information and knowledge. Moreover, to evaluate one's findings, review of literature plays a vital role. In this chapter we have discussed related previous studies which have been published in different peer review scientific journals.

2.01 Details about Apoptosis

Though the phenomenon had been described for almost a century, in 1972, Kerr, Wyllie, and Currie first coined the term "apoptosis" in order to differentiate naturally occurring developmental cell death from necrotic cell death that results from acute tissue injury [Kerr *et al.*, 1972].

Apoptosis is a type of cell death in which a cell uses specialized machinery to dismantle itself. Under normal growth and developmental conditions, apoptosis is a cell suicide mechanism that enables eumetazoans to control cell number, that is, to maintain homeostasis and to eliminate damaged cells. A healthy adult human produces approximately ten billion cells each day by mitosis and a similar number are removed by apoptosis [Jacobson *et al.*, 1997]. Disregulation of the cell death mechanism results in a loss of homeostasis. Indeed, alterations in the cell death program have been implicated in several diseases, including neurodegenerative disorders, inflammatory diseases and cancer [Fadeel & Orrenius, 2005]. Cancer cells, in particular, are known to have a decreased sensitivity to proapoptotic signals when compared to normal cells. It is well established, however, that anticancer drugs are effective at inducing the cell death program by a variety of mechanisms [Kabore *et al.*, 2004; Fulda & Debatin, 2004, Meng *et al.*, 2006].

Apoptosis is a highly regulated process that may be triggered by a variety of stimuli including, but not limited to, virus infection, toxic stress, environmental insults and hormones [Earnshaw *et al.*, 1999]. The morphology of the cell changes during apoptosis due to cytoplasmic shrinkage, active membrane blebbing, chromatin condensation and

fragmentation of membrane-enclosed vesicles [Wyllie *et al.*, 1980]. In addition, the nuclear DNA is degraded, the cytoskeleton in dismantled and cell cycle progression is halted [Kaufmann, 1989; Canman *et al.*, 1992]. In short, every aspect of the cell is disrupted so that the contents are dismantled and packaged into vesicles, called apoptotic bodies, which are phagocytozed by macrophages or surrounding tissue [Jin & El-Deiry, 2005].

2.02 Types of Apoptosis Signaling Pathways

There are two distinct molecular signaling pathways that lead to apoptotic cell death: (a) the *intrinsic*, or mitochondria-mediated pathway, and (b) the *extrinsic*, or extracellular activated pathway [Danial & Korsmeyer, 2004; Cory & Adams, 2002, Nagata, 1997]. The intrinsic pathway is usually activated in response to intracellular stress signals, which include DNA damage and high levels of reactive oxygen species (ROS), as well as by viral infection and activation of oncogenes. The extrinsic pathway is triggered by the binding of an extracellular ligand to a receptor on the plasma membrane. Both pathways activate proteolytic enzymes called caspases that mediate the rapid dismantling of cellular organelles and architecture.

2.03 Details about Caspase Proteins and Its classification

A family of cysteine-dependent aspartate-directed proteases, known as caspases, is intimately involved in apoptosis. The cleavage of key proteins in the cell by caspases leads to the morphological and biochemical changes observed in apoptosis. For example, the cleavage of ICAD (inhibitor of CAD) by caspases releases the DNase CAD (caspase activated DNase) from an inactive complex and ultimately results in the cleavage of nuclear DNA by CAD [Enari *et al.*, 1998; Thornberry *et al.*, 1992; Martinon & Tschopp, 2004; Ahn *et al.*, 2002]

Caspases (**c**ysteinal**asp**artate-specific prote**ases)** [Alnemri *et al.*, 1996] are enzymes which utilize a catalytic cysteine to cleave their peptide substrates after specific aspartate residues. The first caspase was discovered in 1992 and because of its function was named interleukin-1 converting enzyme (ICE) [Cerretti *et al.*, 1992; Thornberry *et al.*, 1992] but was later renamed to caspase- 1. In 1993, Ced-3 from *C. elegans* was found to be homologous to ICE [Miura *et al.*, 1993] and the corresponding human protein CPP32 (later named caspase-3) was found in 1994 [Fernandes-Alnemri *et al.*, 1994]. The official caspase nomenclature was

decided on in 1996 to alleviate the confusion that went along with discovery of ten different caspases, some with multiple names [Alnemri *et al.*, 1996].

Caspases are divided into two main categories based on their function: apoptotic caspases and inflammatory caspases. The apoptotic caspases are further divided into two categories based on time of entry into the apoptotic cascade: initiator caspases and effector caspases [Fig 2].

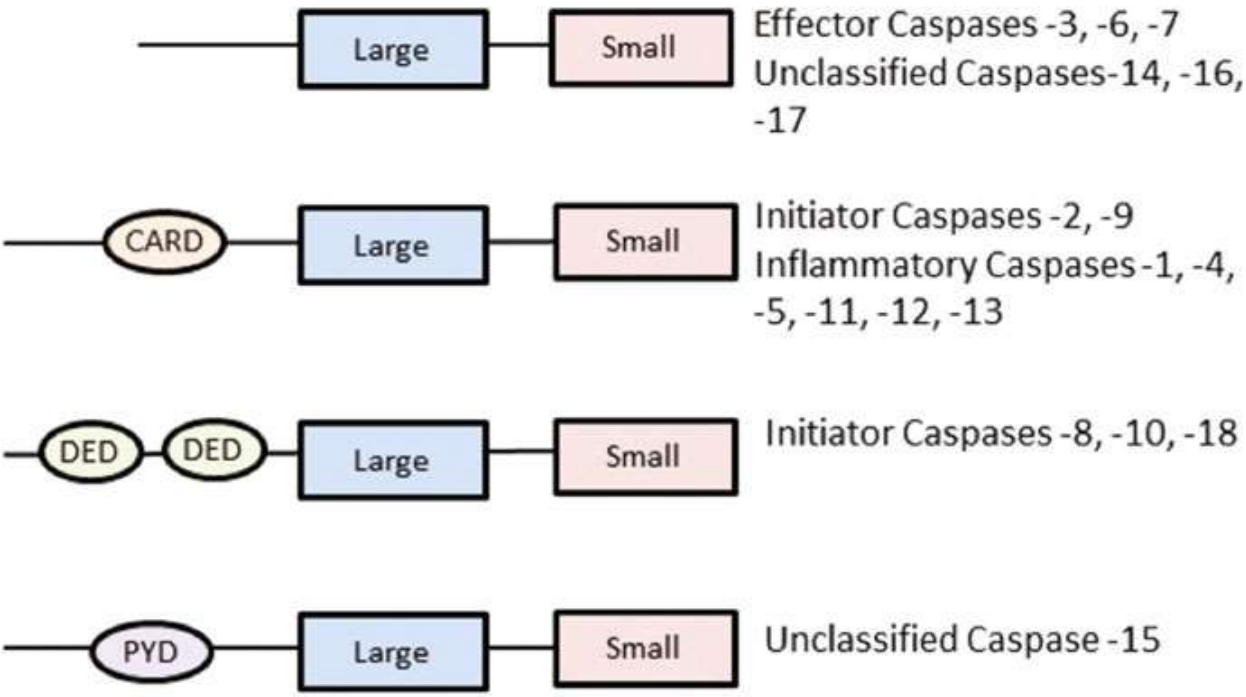

Figure 2 Domain arrangements of mammalian caspases

2.03.1A Apoptotic Caspases (Initiator Caspases)

Initiator caspases are stable monomers in the cell until they are activated by dimerization. Once dimerized, initiator caspases have sufficient activity to autoprocess, cleaving their prodomain and intersubunit linker. An induced proximity model for dimerization was first invoked for caspases-8 and -10 but now seems to be generalizable to initiator caspases as a whole. This model says that activation complexes increase the local concentration of the initiator caspases, enabling them to dimerize [Muzio *et al.*, 1998]. The prodomains of initiator caspases contain either a CARD (caspase activation and recruitment domain) or DED (death effector domain), which allow initiator caspases to bind to activation complexes [Fig. 2]. The initiator caspases-2 and -9 are involved in the intrinsic pathway, which is

activated by mitochondrial damage, cytotoxic stress, chemotherapeutic drugs or certain developmental cues [Gerl, 2004]. Activation of caspase-2 leads to release of cytochrome *c* from the mitochondria, which then binds to Apaf-1 and forms the heptameric apoptosome. The apoptosome binds procaspase-9 to dimerize and therefore activate it. Once active, caspase-9 activates downstream effector caspases.

2.03.1B Apoptotic Caspases (Effector Caspases)

The effector caspases-3, -6, and -7, are found as inactive dimers in the cell. They are activated once an initiator caspase cleaves their intersubunit linkers. Because they do not require death scaffolds for dimer formation [Milam & Clark, 2009; Pop *et al.*, 2001], their prodomains are short and lack the CARD and DED domains typical of initiator caspases. Their prodomains are, however, likely to be involved in targeting within the cell [Mao *et al.*, 1998; Baliga *et al.*, 2002; Colussi *et al.*, 1998; Yaoita , 2002].

2.03.2 Inflammatory Caspases

Similarly to the initiator caspases, the inflammatory caspases-1, -4, -5, -11, -12, and -13 are activated by dimerization. Their prodomains contain a CARD which allows them to bind to activation complexes. Similarly to apoptosome formation, a multiprotein complex called the inflammasome consists of a NOD-like receptor such as NLRP1, an adaptor protein such as ASC (apoptosis-associated speck like protein containing a CARD), and the inflammatory procaspase, particularly procaspase-1 [Martinon *et al.*, 2002]. In some cases, the procaspase can also be recruited to CARD domains in the receptor directly, without the aid of an adaptor molecule [Faustin *et al.*, 2007].

Once the inflammatory caspases become active, they are activators of cytokines through cleavage of their preforms. In monocytes and macrophages, caspase-1 activates interleukin-1β (IL-1β) [Thornberry *et al.*, 1992] and interleukin-18 (IL-18). These cytokines mediate innate immunity and inflammation [Newton & Dixit, 2012].

The mouse caspase-11 is a homolog of human caspase-4 [Wang *et al.*, 1998]. In humans, caspase-12 is generally truncated due to a premature stop codon, but in some people of African descent, a read-through mutation causes expression of the full length protein, causing

increased risk of sepsis due to decreased inflammatory and immune response to endotoxins [Saleh *et al.*, 2004]. Caspase-13 is a bovine ortholog of human caspase-4 [Koenig *et al.*, 2001].

2.03.3 Other or Unclassified Caspases

Caspase-14 expression is restricted to epidermal keratinocytes and is involved in differentiation [Rendl *et al.*, 2002]. Like the effector caspases, it has a short prodomain with no adaptor regions. Several caspases are not yet classified: 15, 16, and 17 [Eckhart *et al.*, 2008]. Caspase- 15 is expressed in several mammalian species including pigs, dogs, and cattle [Eckhart *et al.*, 2005]. It contains a pyrin-like region in its prodomain similar to that found in zebrafish caspases caspy and caspy2 [Masumoto *et al.*, 2003]. Caspase-16 is found in marsupials and placental mammals and contains a short prodomain with no adaptor regions [Eckhart *et al.*, 2008]. Caspase-17 is found in vertebrates except for marsupials and placental mammals and also does not contain adaptor regions in its prodomain. Caspase-18 is found in opossums and chickens and, like caspases-8 and -10, contains two DED regions in its prodomain, so it is likely also an initiator apoptotic caspase [Eckhart *et al.*, 2008].

2.04 Structure of Caspase Proteins

Caspases are expressed as proenzymes (*zymogens*) called procaspases, which then become activated to the mature caspase form. Procaspase structure can be divided into three domains: an N-terminal prodomain, a large subunit, and a small subunit. The first step in maturation is dimerization. Then, proteolytic processing removes the prodomain and cleaves a loop called the intersubunit linker between the large and small subunits.

The secondary structure of mature caspases consists of six core β-strands in a slightly twisted sheet in each monomer, with two main helices on one face (the “front”) of the protein and three helices on the other face (the “back”) of the protein [Fig. 3]. The first four core β-strands and helices 1–3 form the large subunit, whereas the last two core “-strands and helices 4–5 form the small subunit. The dimer interface consists of the final β-strand from each monomer, side by-side in an antiparallel manner. The two monomers are related through a C2 axis of symmetry such that one monomer is “upside-down” compared to the other monomer.

Five loops are important for the formation of the active site. Once the intersubunit linker is cleaved, the two halves of the cleaved linker are called L2 and L20. Active site loops L1, L2, L3, and L4 come from one monomer, and loop L20 comes from the other. The catalytic cysteine is part of loop L2, and the catalytic histidine is part of a loop extending from the C terminal end of β3 [In Bose, 2015].

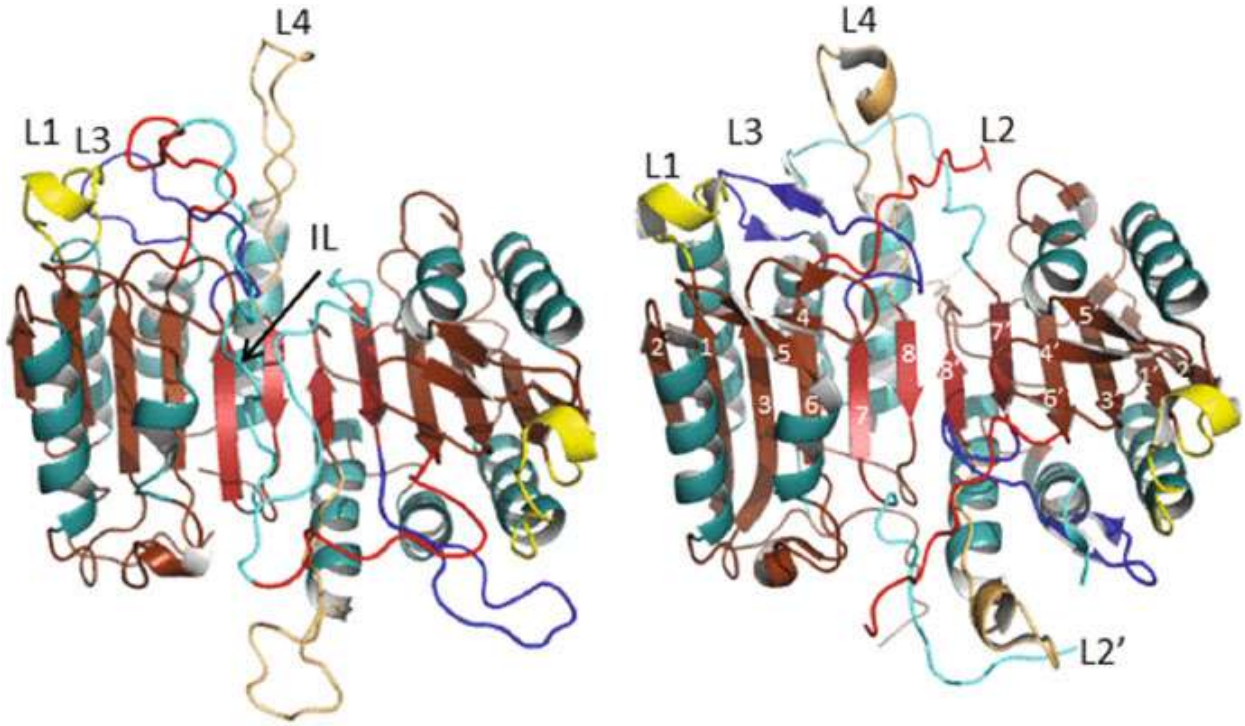

Figure 3 Procaspase-3 model and crystal structure of caspase-3. Active site loop coloring: *yellow*DL1, *red*DL2, *cyan*DL20, *blue*DL3, *tan*DL4

2.05 Structure of Pro-Caspase 7 and Its Activation

Procaspase-7, in particular, is expressed as a 303-aa residue polypeptide chain. Upon activation *in vivo*, a short N-terminal peptide is removed, and, more importantly from the perspective of generating catalytic activity, an Ile-Gln-Ala-Asp-2-Ser-Gly site is cleaved, giving rise to a 175-residue large chain and a 105-residue small chain, comprising the active caspase-7. The only known exception to this mode of activation is caspase-9, whose zymogen does not require proteolytic processing for activity [Stennicke *et al.*, 1999; Rodriguez & Lazebnik, 1999].

The first crystal structures of active caspase-1 [Walker *et al.*, 1994; Wilson *et al.*, 1994], and later the homologous structures of caspases -3, -7, and -8 [Rotonda *et al.*, 1996; Blanchard *et*

al., 1999; Watt *et al.*, 1999; Wei *et al.*, 2000; Mittl *et al.*, 1997], revealed that the active caspases comprise a heterotetramer consisting of two closely associated large and two small subunits, aligned together by means of the central small subunits about a 2-fold axis. The large subunits harbor the catalytic residues *His-237* and *Cys-285* [Walker *et al.*, 1994; Wilson *et al.*, 1994], whereas the small subunits contribute mainly to formation of the substrate binding region. The preference for substrate cleavage after Asp residues is explained by the side chains of *Arg-179* and *Arg-341* lining the S1 specificity pocket. In contrast, no structure of an uncleaved caspase zymogen has been reported so far, thus limiting the understanding of the procaspase inactivity and the mechanism of procaspase activation.

The structure of cleaved caspase-9 [Renatus *et al.*, 2001] has allowed postulating a zymogen conformation, in that the asymmetric dimers have captured two conformations, a catalytically competent and an incompetent one. We have postulated that the zymogen form of caspase-9 is restrained by dislocation of the catalytic and the substrate binding sites. To disclose the general mechanism(s) responsible for caspase activation, the C285A variant of human procaspase-7 has crystallized and has solved its crystal structure.

The zymogen of the executioner caspase 7 is maintained in a similar inactive conformation to the caspase 9 zymogen [Chai *et al.*, 2001; Riedl *et al.*, 2001]. The primary difference is that pro-caspase 7, unlike pro-caspase 9, is already a dimer and the driving force for zymogen activation is proteolysis. This releases the N-termini of the small subunits and C-termini of the large subunits, thereby allowing them to form the four-stranded loop bundles that are required to order the catalytic site.

The structures of the caspase 7 zymogen seem to rule out previously proposed models in which the two subunits of the active caspase molecule arise from different subunits of each zymogen by a domain swapping mechanism. Rather, the two catalytic domains in the zymogen are associated by simple dimerization, no domain swapping takes place, and the driving force for activation is the large movement of the inter-chain connector that helps to align the substrate-binding region and catalytic machinery [Riedl *et al.*, 2001].

2.06 Function of Caspase Proteins

2.06.1 Apoptosis

The activation of caspases commits the cell to apoptosis. The main hallmarks of apoptosis include rounding of cells and retraction from neighbors, membrane blebbing to form vesicles called apoptotic bodies, nuclear fragmentation, chromatin condensation, hydrolysis of genomic DNA to approximately 200 bp fragments, and translocation of phosphatidylserine (PS) to the external surface of cells as an "eat me" signal to phagocytes. The apoptotic caspases are necessary for conferring all of these phenotypes.

In addition to the systematic dismantling of the cell, caspases are also involved in producing "find-me" signals to cause chemotaxis of phagocytes to apoptotic cells [Lauber *et al.*, 2003; Knies *et al.*, 1998; Chekeni *et al.*, 2010]. The recruitment of phagocytes keeps cells from releasing their contents into extracellular space and activating an immune response which could be harmful to the tissue.

When the number of apoptotic cells is too great for consumption by phagocytes, secondary necrosis can occur. When this happens, the cell releases its contents into extracellular space. However, immune cells are somehow able to recognize the cells undergoing apoptosis (and secondary necrosis) differently from necrotic cells. This is likely due to the actions of caspases. Caspases keep danger-associated molecular patterns (DAMPs) and alarmins from being activated [Martin *et al.*, 2012]. This can be thought of as a "tolerate me" signal.

Caspases are also involved in turning off transcription and translation [Taylor *et al.*, 2008]. This keeps any infecting viral particles from replicating using the host's machinery. They also fragment the Golgi, ER, and mitochondria [Frank *et al.*, 2001, Lane *et al.*, 2002].

2.06.2 Inflammatory Response

In contrast to the actions of apoptotic caspases, which systematically dismantle the cell to avoid an immune response, the actions of inflammatory caspases lead to cell lysis and activation of the immune response in a process called pyroptosis [Lamkanfi, 2011]. In order to activate an immune response, caspases cleave cytokine IL-1β and IL-18 to produce the mature form [Miao *et al.*, 2011].

In addition to activation of cytokines, procaspase-1 is also able to activate the pro-inflammatory transcription factor NF-κB [Lamkanfi *et al.*, 2004]. Rather than using its

catalytic activity, the CARD domain of procaspase-1 binds to a CARD domain in the kinase RIP2, which is involved in NF-κB activation.

2.06.3 Other Functions

Caspase expression is kept below a certain threshold required for apoptosis by IAPs (inhibitor of apoptosis proteins). At these sub-threshold levels they are able to play roles that are neither apoptotic nor inflammatory. Caspase-3 activity was found to be important for differentiation of erythroblasts, [Zermati *et al.*, 2001] skeletal muscle, [Fernando *et al.*, 2002] bone marrow stromal stem cells, [Miura *et al.*, 2004] and neural stem cells [Fernando *et al.*, 2005].

Caspase-3 has several other non-apoptotic functions in nerve cells. In addition to neural cell differentiation, caspase-3 has also been implicated in neuronal migration and plasticity, [Gulyaeva, 2003] axon pruning, and synapse elimination [Hyman & Yuan, 2012].

Caspases have been shown to play a role in cell migration and invasion under certain circumstances [Rudrapatna *et al.*, 2013]. They can also induce neighboring cells to proliferate to replace dying cells in a process called apoptosis-induced proliferation [Ryoo & Bergmann, 2012]. These roles for caspases have implications for cancer: moderate activation of caspases could, in fact, cause cancer to progress rather than regress [Rudrapatna *et al.*, 2013; Ryoo & Bergmann, 2012]. In addition to its apoptotic function, caspase-8 has an anti-apoptotic function when it forms a heterodimer with FLIPL (a protein similar to caspase-8 but lacking a catalytic site) [Oberst *et al.*, 2011]. This protein complex is able to activate the NF-›B signaling pathway leading to proliferation [Kataoka & Tschopp, 2004]. In another pro-survival capacity, the caspase- 8/FLIPL complex is also able to inhibit RIPK3-dependent necrosis [Oberst *et al.*, 2011].

2.07 Types of Apoptosis Signaling Pathways

Caspases are themselves activated either by so called extrinsic or intrinsic mechanisms. The extrinsic pathway for initiator caspase activation ultimately is responsible for the elimination of unwanted cells that are produced during development or that have tumorogenic qualities [Boatright & Salvesen, 2003]. This pathway is initiated by ligation of a transmembrane death receptor in response to an extracellular signal, followed by recruitment and activation of

initiator caspases as a part of a multiprotein complex [Fig. 4]. Caspases-8 and -10 are the initiator caspases that are activated by way of the extrinsic pathway. In contrast, the intrinsic pathway primarily is responsible for the removal of cells in response to cytotoxic stress, chemotherapeutic drugs, mitochondrial damage and certain developmental cues [Boatright *et al.*, 2003]. The mitochondria release cytochrome c into the cytoplasm in response to one or more of these cues [Fig. 4]. The increase in the cytoplasmic concentration of cytochrome c is sensed by the protein Apaf-1 (apoptosis activating factor 1), which leads to recruitment of caspase-9 to a multiprotein complex, called the apoptosome, followed by activation of the caspase in a cofactor-dependent manner. The end result of initiator caspase activation is the downstream activation of the effector caspases-3, -6 and -7, which ultimately are responsible for cleavage of intracellular proteins that lead to the dismantling of the cell.

2.07.1 The Intrinsic Apoptosis Signaling Pathway

Bcl-2 family members act by regulating the efflux of apoptogenic proteins from mitochondria. Bcl-2 proteins contain from one to four Bcl-2 homology (BH) domains. The number and combination of the BH domains dictate whether the proteins are proapoptotic or antiapoptotic. Antiapoptotic Bcl-2 members contain all four BH domains and include Bcl-2, Bcl-xL, Mcl-1, Bcl-w, and Bf1-1/A1. Proapoptotic members lack the BH4 domain and are divided into two groups, the "BH3-only" members and the multidomain BH1-3 proapoptotic members Bax and Bak. In mouse cells, deletion of Bax and Bak is sufficient to prevent mitochondrial outer membrane permeabilization (MOMP) induced by upstream apoptotic events [Lindsten *et al.*, 2000; Wei *et al.*, 2001]. Bax and Bak normally exist as inactive monomers. Bax resides in the cytosol or loosely attached to intracellular membranes [Suzuki *et al.*, 2000], and Bak is bound by Mcl-1, Bcl-xL, or voltage-dependent anion channel protein 2 (VDAC-2) in the mitochondrial outer membrane [Scorrano *et al.*, 2003; Willis *et al.*, 2005].

The generalized scheme of intrinsic pathway activation is the oligomerization of Bax and Bak in the mitochondrial outer membrane to activate MOMP, thus permitting release of apoptogenic factors such as cytochrome c, second mitochondria–derived activator of caspase/direct inhibitor of apoptosis (IAP) binding protein with low pI (Smac/DIA-BLO), and Omi stress-regulated endoprotease/high temperature requirement protein A2 (Omi/ HtrA2). Once released, cytochrome c binds apoptotic protease-activating factor 1 (Apaf-1), which recruits pro-caspase-9, promoting its self-activation. Activated caspase-9 cleaves the downstream effectors caspase-3 and caspase-7, which rapidly cleave intracellular substrates.

Proteins of the IAP family, including X-linked IAP (XIAP), c-IAP1, and c-IAP2, can bind and inhibit the active sites of caspase-3, caspase-7, and caspase-9. When released from mitochondria, Smac/DIA-BLO and Omi/HtrA2 can bind these IAPs and prevent their inhibition of the activated caspases [Du *et al.*, 2000; Suzuki *et al.*, 2001; Verhagen *et al.*, 2000]. Antiapoptotic Bcl-2 proteins block oligomerization of Bax and Bak, or their associations with BH3-only proteins, thus preventing MOMP [Boatright *et al.*, 2003]. The BH3-only proteins are universally proapoptotic, and each can act either to antagonize antiapoptotic members or activate proapoptotic members. Members of the BH3-only family include Bid, Bad, Bim, Puma, Noxa, Bmf, and several others. BH3-only proteins need to be activated in order to elicit their death signal. The proapoptotic activity of BH3-only proteins appears to be kept in check by either transcriptional control (mainly by p53) or post-translational events. For example, cellular stresses, such as ionizing radiation (IR) or chemotherapy, activate a DNA damage response that stabilizes the p53 tumor suppressor protein. p53 acts to either arrest the cell division cycle by transcriptionally activating the cyclin-dependent kinase inhibitor p21, giving the cell time to repair the damage, or else it helps to mediate apoptotic cell death. p53 also activates proapoptotic genes, including those encoding Bax and the BH3- only proteins Puma, Noxa, and Bid [El-Deiry, 2003]. Another role for p53 has been identified showing that p53 acts directly to increase MOMP by binding Bcl-2 family members and helping mediate Bax and Bak dimerization [Leu *et al.*, 2004; Mihara *et al.*, 2003].

2.07.2 The Extrinsic Apoptosis Signaling Pathway

The extrinsic pathway is activated by members of two protein families, the tumor necrosis factor (TNF) family and the receptors for these ligands (TNFR) [Locksley *et al.*, 2001]. Most TNF family members bind receptors that activate signals involved in proinflammatory responses and do not signal cell death. The TNF ligands that can induce apoptosis are TNF-α, FasL (also known as CD95L), and TNF receptor apoptosis-inducing ligand (TRAIL; also known as Apo2L) [LeBlanc & Ashkenazi, 2003; Peter & Krammer, 2003]. After extracellular ligand binding, the cytoplasmic end of the TNFR recruits initiating caspases. TRAIL binding to its death inducing receptors acts in a manner similar to FasL, while TNF-mediated signaling is more complex [Wajant *et al.*, 2003]. The ligand-bound Fas or TRAIL death receptors (DR4 and DR5) recruit the adapter protein Fas-associating death domain-containing protein (FADD) [Chinnaiyan et al., 1995]. Bound FADD recruits initiator caspase-8 and

caspase-10, and this assembly of proteins (receptor, FADD, and caspases) is termed the death-inducing signaling complex(DISC) [Kischkel *et al.*, 1995]. Recruitment of caspase-8/10 to the DISC leads to their auto proteolytic cleavage [Donepudi *et al.*, 2003; Boatright *et al.*, 2003]. Caspase-8/10 activity can be blocked by a protein with which they share high homology, FLIP (FADD-like interleukin-1β-converting enzyme inhibitory protein). FLIP can oligomerize with caspase-8/10 but lacks critical residues in its caspase domain, including the catalytic cysteine, suggesting it to be a dominant-negative inhibitor. In some cells, named type I cells, activation of effector caspases by activated caspase-8/10 alone is sufficient to induce apoptosis [Scaffidi, 1998]. In type II cells, activated caspase-8/10 stimulates the release of factors from mitochondria. The BH3-only protein Bid connects the extrinsic pathway to mitochondria. Bid is cleaved by caspase-8, resulting in its myristyolization of a newly exposed glycine residue to form tBid. tBid is then targeted to membranes where it promotes Bax and Bak oligomerization [Li *et al.*, 1998, Luo *et al.*, 1998].

Apoptotic cell death is as a key element in maintaining immune homeostasis and preventing the emergence of lymphomas or the development of autoimmunity [Bidere *et al.*, 2006]. Cells derived from the hematopoietic progenitor cells (CD8+ T cells, natural killer cells [NK], dendritic cells) have the capacity to mediate cell death through the use of the extrinsic pathway. For example, antigen stimulation of T cells causes the induction of FasL, TNF, and TRAIL that mediates contact-dependent destruction of their targets [Brunner *et al.*, 1995; Dhein *et al.*, 1995; Ju *et al.*, 1995; Zheng *et al.*, 1995; Janssen *et al.*, 2005]. Cytotoxic immune cells can also induce apoptosis through exocytosis of specialized granules that contain perforin and caspase-like proteases, called granzymes [Barry & Bleackley, 2002]. Entry of these proteins into target cells activates apoptosis, through both caspase-dependent and independent mechanisms.

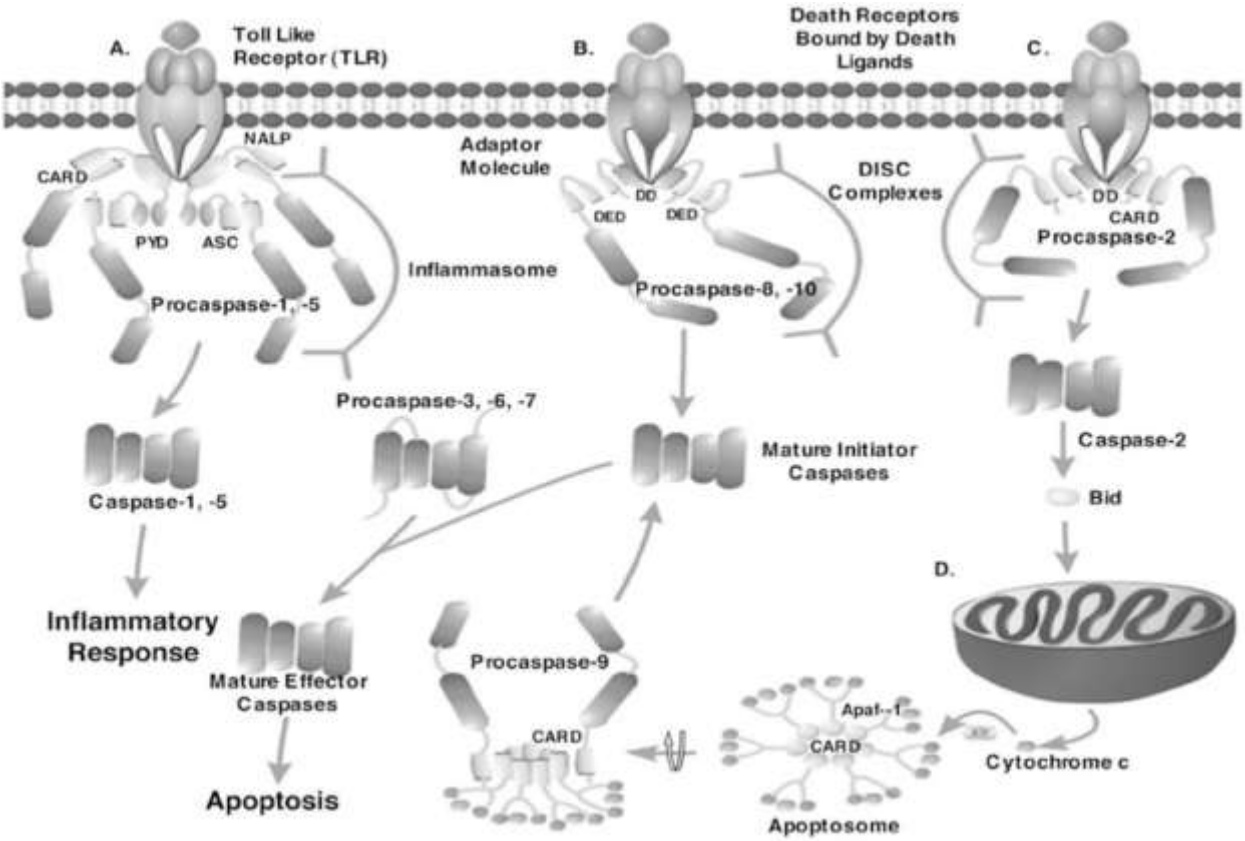

Figure 4 The Caspase Cascade. A) Inflammatory caspase activation: a ligand binds to a toll-like receptor (TLR), which signals a NALP protein to bind to the TLR. ASC interacts with the pyrin domain (PYD) of NALP via PYD:PYD interactions. The CARD domain of ASC interacts with the CARD domain of procaspase-1 and the CARD domain of NALP interacts with the CARD domain of procaspase-5, forming the inflammasome. The inflammasome complex promotes dimerization of caspases-1 and -5, leading to their activation and the inflammatory response. B) The extrinsic apoptotic pathway: a death ligand binds to a death receptor, which signals an adaptor molecule to bind to the receptor via death domain (DD) interactions. The DED motif of the adaptor molecule interacts with the DED of procaspases-8 and -10, forming a DISC complex. Dimerization (mechanism unknown) results in maturation and full activity. Caspases-8 and -10 then process executioner caspases. C) Procaspase-2, a unique caspase, is activated when a ligand binds to a death receptor, which signals an adaptor molecule to bind via interactions with the death domain. The CARD of the adaptor molecule interacts with the CARD of procaspase-2 to promote dimerization in a DISC-like complex. Upon removal of the prodomain, caspase-2 cleaves Bid, a protein responsible for the increased permeability of the mitochondria. D) The intrinsic apoptotic pathway: an increase in the cytosolic concentration of cytochrome c leads to the formation of

the apoptosome. The apoptosome is composed of Apaf-1 monomers that form a heptameric structure when cytochrome c binds to the WD40 motifs of Apaf-1, in an ATP-dependent manner, leading to interactions of the CARDs. The CARD of procaspase-9 then interacts with the CARD of Apaf-1, increasing the local concentration of procaspase-9 monomers and thereby promoting dimerization and activation. Caspase-9 then processes effector caspases, which leads to apoptosis. Effector caspases are activated by cleavage of their prodomain and intersubunit linker [Boatright & Salvesen, 2003].

2.08 Details about Cancer

Alterations in apoptotic pathways have been implicated in many diseases, such as cancer and neurodegenerative disorders [Thompson, 1995; Yuan and Yankner, 2000]. Human bodies are made up of billions of cells that grow up, divide, and then die in a predictable manner. Cancer occurs when something goes wrong with this system, causing uncontrolled cell division and growth. Cancer is not a single disease; it is a group of more than 100 different and distinctive diseases. Cancer known medically as a malignant neoplasm, is a broad group of diseases involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumors are cancerous; benign tumors do not invade neighboring tissues and do not spread throughout the body. Over 200 different known cancers affect humans [Cancer Research UK, 2012].

The causes of cancer are diverse, complex, and only partially understood. Many things are known to increase the risk of cancer, including tobacco use, dietary factors, certain infections, exposure to radiation, lack of physical activity, obesity, and environmental pollutants [Anand *et al.*, 2008]. These factors can directly damage genes or combine with existing genetic faults within cells to cause cancerous mutations [Vogelstein and Kinzler, 2002]. Approximately 5–10% of cancers can be traced directly to inherited genetic defects [American Cancer Society, 2013]. Many cancers could be prevented by not smoking, eating more vegetables, fruits and whole grains, eating less meat and refined carbohydrates, maintaining a healthy weight, exercising, minimizing sunlight exposure, and being vaccinated against some infectious diseases [Anand *et al.*, 2008; Kushi *et al.*, 2012].

Cancer can be detected in a number of ways, including the presence of certain signs and symptoms, screening tests, or medical imaging. Once a possible cancer is detected it is diagnosed by microscopic examination of a tissue sample. Cancer is usually treated with chemotherapy, radiation therapy and surgery. The chances of surviving the disease vary greatly by the type and location of the cancer and the extent of disease at the start of treatment. While cancer can affect people of all ages, and a few types of cancer are more common in children, the risk of developing cancer generally increases with age. In 2007, cancer caused about 13% of all human deaths worldwide (7.9 million). Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world [Jemal *et al.*, 2011].

2.09 Frequency of Cancer in Bangladesh

Bangladesh, at 142 million people, is the 9th most populous country in the world. There are 13 to 15 lac cancer patients in Bangladesh, with about 2 lac patients newly diagnosed with cancer each year [Hussain, 2013; Noronha *et al.*, 2012]. Cancer is the most important killer diseases in Bangladesh as like as many other countries. Among many types of cancer, lung cancer was the commonest cancer among the patients. A total of 1,231 patients were found in 2007, of them 86% were males. The number of lung cancer patients was increasing year by year. About 52% had a history of smoking and almost all of them were male. One third of them were smoking for three decades. Most (95%) of the female lung cancer patients were non smokers. Following Lung cancer, Breast cancer was the commonest cancer among the female patients in Bangladesh, which formed the 2nd most common cancer as a whole. The mean age of patients was 41 years, with largest proportion of patients in 35-54 year age group. Among all breast cancer patients 89% were married. At the time of first reporting two-third had histopathological diagnosis and one-fourth had cytological diagnosis. A vast majority (96%) had duct cell carcinoma. A total of 1,116 breast cancer patients received treatment prior to NICRH. Cervical cancer is the second most common cancers among females (21.5%) and ranked third among the whole group. Out of 1,718 cervical cancer patients around 91% were married while 8% were widowed. Majority (97%) of the cervix cancer patients was housewives. A scenario of cancer patients per district is given in Fig. 5 [Cancer Registry Report, 2008].

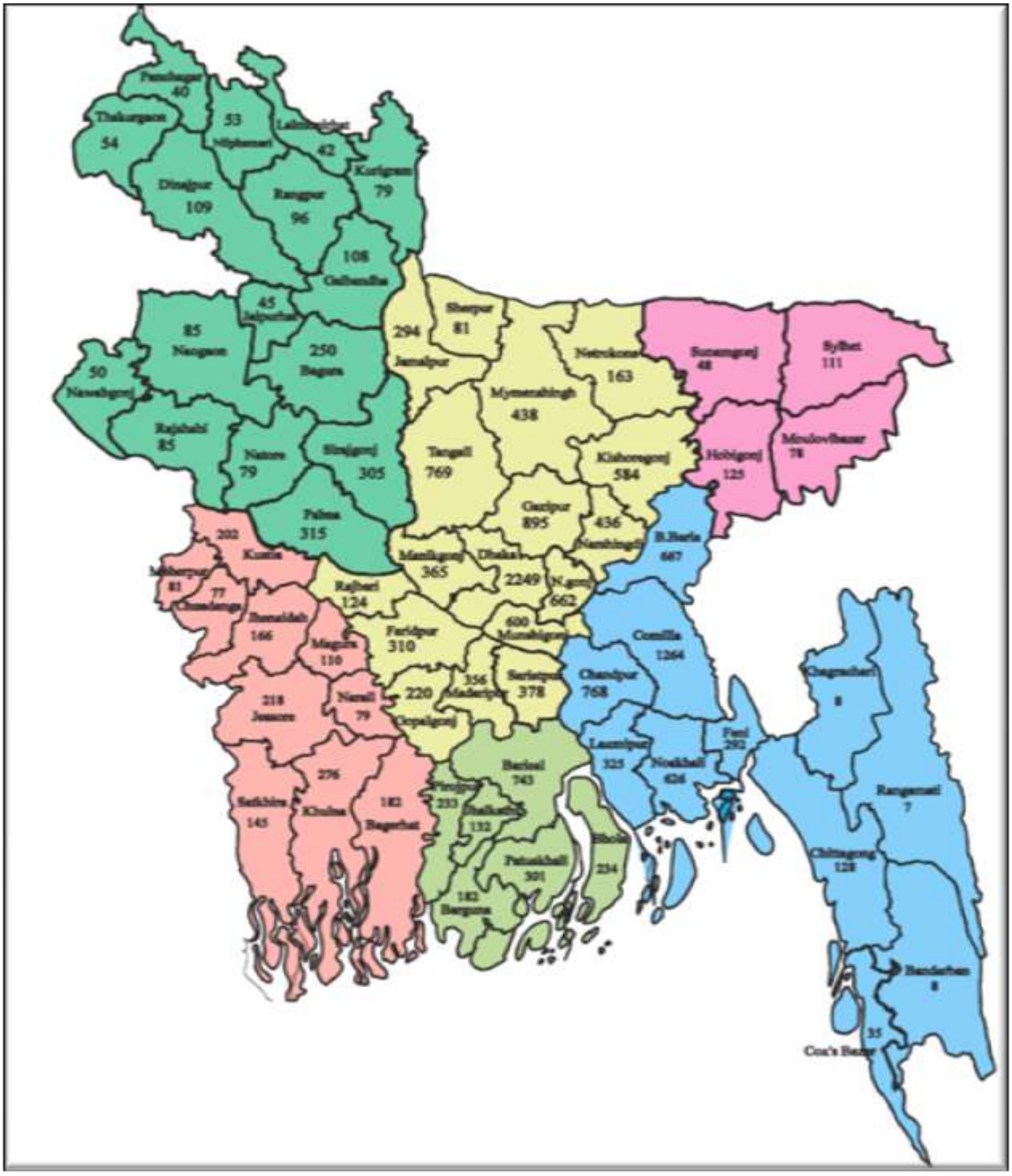

Figure 5 Map of Bangladesh showing current resident per districts of the patients [Cancer Registry Report, 2008**].**

2.10 Data mining on Different Cancer around the World

Lung cancer is characterized by outgoing growth of cell in tissues of the lung. If lung cancer left untreated, this type of growth can broaden beyond the lung called metastasis into near tissue or different parts of the body. Primary lung cancers are carcinomas that originate from epithelial cells [In Broaddus *et al.*, 2016]. The most common cause of lung cancer is continuing exposure to tobacco smoke, [O'Reilly et al., 2007] which causes 80–90% of lung cancers. It is anticipated that 8 to 14% of lung cancer is occurred by inherited factors [El-Telbany & Ma, 2012].The risk of lung cancer is increased 2.4 times in relatives of people. This is possible due to a combination of genes [Ferlay *et al.*, 2010] Worldwide, It is the most frequent cancer in terms of both incidence and mortality.

Breast cancer is originating from breast tissue, generally from the inner lining of milk ducts or the lobules that provide the ducts with milk [Cancer, 2012]. It occurs in humans as well as in other mammals. It is 100 times more common in women than in men, although men tend to have poorer outcomes due to late in diagnosis [Cavalieri *et al.*, 2006]. Breast cancer, like other cancers, occurs because of an interaction between an environmental factor and a genetically susceptible host. Worldwide, breast cancer is the most familiar invasive cancer in women. It comprises 22.9% of invasive cancers in women [Ferlay *et al.*, 2010].

Colorectal cancer commonly known as colon cancer or bowel cancer is occurred by uncontrolled cell growth in the colon or rectum, in the appendix, or parts of the large intestine. Colorectal cancer is the third most commonly diagnosed cancer in the world, but it is comparatively more common in developed countries. Around 60% of this cancer was diagnosed in the developed world.

Ovarian cancer is a cancerous growth arising from the ovary. There is good evidence that in some women genetic factors are important. Carriers of certain BRCA mutations are notably at risk. The BRCA1 and BRCA2 genes account for 5%–13% of ovarian cancers [Soegaard *et al.*, 2009] and certain populations are at a higher risk of both breast cancer and ovarian cancer, often at an earlier age than the general population [Wooster & Weber, 2003]. Patients with a personal history of breast cancer or a family history of breast and/or ovarian cancer, especially if diagnosed at a young age, may have an elevated risk and should be tested for the "cancer gene".

Pancreatic cancer is a malignant neoplasm starting from transformed cells increasing in tissues forming the pancreas. Adenocarcinoma is accounting for 95% of these tumors arising within the exocrine part of the pancreas. Minorities arise from islet cells, and are categorized as neuroendocrine tumors. It is the eighth most common cause of cancer-related deaths in the worldwide [Cancer, 2011].

2.11 Links between Apoptosis, Proliferation and the Cell Cycle

The cell cycle is divided into four phases, and the cellular decision to initiate mitosis or to become quiescent (G0 state) occurs during the G1 phase. Oncogenes have a dual role: they can induce both proliferation and apoptosis. As somatic cells proliferate, the cell-cycle progression is regulated by positive and negative signals. Apoptosis and mitosis share common morphological features such as cell shrinkage, chromatin condensation and membrane blebbing. Additionally, cell-cycle genes such as *p53*, *RB* and *E2F* have been shown to participate in both the cell cycle and in apoptosis. Thus, the balance between apoptosis and proliferation must be strictly maintained to sustain tissue homeostasis. The link between apoptosis and proliferation is suggested by studies that have demonstrated the presence of large numbers of dying cells in proliferating cell populations *in vivo*. Cell proliferation, differentiation and death are fundamental processes in multicellular organisms, and several lines of evidence link apoptosis to proliferation. Firstly, uncontrolled proliferation can be associated with a high level of apoptosis.

A number of dominant oncogenes (e.g., *c-Myc)* appear to induce apoptosis, which suggests that the cell proliferation and apoptosis pathways are closely linked [Evan *et al.*, 1992]. Reid *et al*. demonstrated that myeloid progenitors derived from the bone marrow of *CCR*-/- mice (ligand for MCP-1 chemokine) show an increased cycling rate and enhanced apoptosis [Reid *et al.*, 1999]. Traver *et al*. showed that expression of the myeloid activation marker Mac-1 correlates with Fas expression levels. They also showed that exposure to granulocyte macrophage-colony stimulating factor (GMCSF) and interleukin (IL)-3 increased Fas expression on myeloid progenitor cells [Traver *et al.*, 1998]. These examples infer a positive relationship between apoptosis and proliferation. However, an inverse relationship was proposed by Koury, who suggested that when over-production of progenitor cells occurs, the excess undergo apoptosis. This means that an increase in the level of demand could be met by a reduction in apoptosis [Koury, 1992]. In this case, proliferation may remain constant while the rate of apoptosis changes; thus, there is no strict relationship between proliferation and

apoptosis. This hypothesis is supported by radiolabelled iron ferrokinetic studies in hypertransfused mice that showed continued production of erythroid progenitor cells but no increase in their number, indicating the direct involvement of apoptosis in this situation [Testa, 1979]. As both hypotheses involve changes in the level of proliferation and apoptosis, the difference between them may be only a matter of degree. A final possibility that must be considered is that apoptosis in haemopoietic progenitor cell populations is linked to pathology or abnormality such as growth factor deprivation or oncogene expression [Alenzi, 2004].

2.12 Contribution of Caspase(s) to the Cell Cycle Regulation at Mitotic Phase

Cell cycle is controlled by the ubiquitin-mediated proteolysis of the key regulators [Vodermaier, 2004; Pines, 2006; Nakayama & Nakayama, 2006; Van Leuken *et al.*, 2008]. Two major classes of ubiquitin ligases, the SKP1-CUL1-F-box-protein (SCF) complex and the anaphase-promoting complex/cyclosome (APC/C), play central roles in the cell cycle regulation through the ubiquitination of various cell cycle regulators. The SCF complex functions from late G1 phase to early M phase, whereas APC/C is active from anaphase to the end of G1 phase. There are two activators for APC/C, cell division cycle 20 (Cdc20) and Cdh1, which bind with APC/C and recognize respective substrate proteins. APC/ CCdc20 targets securin and cyclin B1 for their destruction, and promotes sister chromatid separation. APC/CCdh1 facilitates exit from M phase and maintains G1 phase by mediating the degradation of a variety of substrates including PLK1, cyclin B1, and geminin.

2.13 *In Silico* Approaches and databases used for Drug Design

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Molecular docking can predict an *optimized orientation* of ligand on its target. It can predict different binding modes of ligand in the groove of target molecule. This can be used to

develop more potent, selective and efficient drug candidates [Shoichet *et al.*, 2002; Gschwend *et al.*, 1996].

Virtual Screening can be used to select in house database compounds for screening, choose compounds that can be purchased externally, and to choose which compound should be synthesized next. Given a set of structurally diverse ligands that binds to a receptor, a model of the receptor can be built by exploiting the collective information contained in such set of ligands. These are known as pharmacophore models. A candidate ligand can then be compared to the pharmacophore model to determine whether it is compatible with it and therefore likely to bind [Sun, 2008]. Another approach to ligand-based virtual screening is to use 2D chemical similarity analysis methods to scan a database of molecules against one or more active ligand structure [Willett *et al.*, 1998]. A popular approach to ligand-based virtual screening is based on searching molecules with shape similar to that of known actives, as such molecules will fit the target's binding site and hence will be likely to bind the target.

There are a number of prospective applications of this class of techniques in the literature [Rush *et al.*, 2005; Ballester *et al.*, 2009]. Pharmacophoric extensions of these 3D methods are also freely-available as webservers [Li *et al.*, 2016; Sperandio *et al.*, 2009]. Structure-based virtual screening involves docking of candidate ligands into a protein target followed by applying a scoring function to estimate the likelihood that the ligand will bind to the protein with high affinity [Romano, 2007; Cavasotto & Orry, 2007; Kooistra *et al.*, 2016]. Webservers oriented to prospective virtual screening are available to all [Irwin *et al.*, 2009; Li *et al.*, 2014].

A major breakthrough in lead identification in the recent years occurred with the availability of fast and cheap computers on one hand and commercially available databases of compounds with more than a million small molecules, on the other. With the exponential rise in the number of viable novel drug targets, computational methods are being increasingly applied to accelerate the drug discovery process. This resulted in Virtual Screening (VS) technologies using *in silico* (computer-aided molecular drug design and chemo-bioinformatics) techniques, such as, high throughput docking, homology searching and pharmacophore searches of 3D databases. VS, has become an integral part of the drug discovery process. The generic definition of VS is significantly wide and may encompass

many different methods. It is perhaps the cheapest way to identify a lead and several cases have already proven successful using this technology. The dominant technique for the identification of new lead compounds in drug discovery is the physical screening of large libraries of chemicals against a biological target. This conventional experimental method like High Throughput Screening (HTS), continues to be the best method for rapid identification of drug leads. HTS identifies lead molecules by performing individual biochemical assays with over millions of compounds. However, the huge cost and time consumed with this technology has lead to the integration of cheaper and effective computational methodology, namely Virtual High Throughput Screening (vHTS), which is an established computational screening method to identify drug candidates from large collection of compound libraries. vHTS is widely applied to screen *in silico* collection of compound libraries to check the binding affinity of the target receptor with the library compounds. This is achieved by using a scoring function which computes the complementarity of the target receptor with the compounds. This computational screening of databases has become increasingly popular in the pharmaceutical research.

Beginning in theoretical physics, the method of MD gained popularity in materials science and since the 1970s also in biochemistry and biophysics. MD is frequently used to refine 3-dimensional structures of proteins and other macromolecules based on experimental constraints from X-ray crystallography or NMR spectroscopy. In physics, MD is used to examine the dynamics of atomic-level phenomena that cannot be observed directly, such as thin film growth and ion-subplantation, and also to examine the physical properties of nanotechnological devices that have not or cannot yet be created. In biophysics and structural biology, the method is frequently applied to study the motions of macromolecules such as proteins and nucleic acids, which can be useful for interpreting the results of certain biophysical experiments and for modeling interactions with other molecules, as in ligand docking. In principle MD can be used for ab initio prediction of protein structure by simulating folding of the polypeptide chain from random coil.

Most tests of virtual screening studies in the literature are retrospective. In these studies, the performance of a VS technique is measured by its ability to retrieve a small set of previously known molecules with affinity to the target of interest (active molecules or just actives) from a library containing a much higher proportion of assumed inactives or decoys. By contrast, in prospective applications of virtual screening, the resulting hits are subjected to experimental

confirmation (e.g., IC_{50} measurements). There is consensus that retrospective benchmarks are not good predictors of prospective performance and consequently only prospective studies constitute conclusive proof of the suitability of a technique for a particular target [Irwin, 2008; Good & Oprea; 2008; Schneider, 2010; Ballester, 2011].

Because molecular systems typically consist of a vast number of particles, it is impossible to determine the properties of such complex systems analytically; MD simulation circumvents this problem by using numerical methods. However, long MD simulations are mathematically ill-conditioned, generating cumulative errors in numerical integration that can be minimized with proper selection of algorithms and parameters, but not eliminated entirely.

For systems which obey the ergodic hypothesis, the evolution of one molecular dynamics simulation may be used to determine macroscopic thermodynamic properties of the system: the time averages of an ergodic system correspond to microcanonical ensemble averages. MD has also been termed "statistical mechanics by numbers" and "Laplace's vision of Newtonian mechanics" of predicting the future by animating nature's forces and allowing insight into molecular motion on an atomic scale [Schlick, 1996; Stigler, 2005].

The results of MD simulations can be tested through comparison to experiments that measure molecular dynamics, of which a popular method is NMR spectroscopy. MD-derived structure predictions can be tested through community-wide experiments in Critical Assessment of protein Structure Prediction (CASP), although the method has historically had limited success in this area. Michael Levitt, who shared the Nobel Prize partly for the application of MD to proteins, wrote in 1999 that CASP participants usually did not use the method due to "*a central embarrassment of molecular mechanics, namely that energy minimization or molecular dynamics generally leads to a model that is less like the experimental structure.*" [Koehl & Levitt, 1999] Improvements in computational resources permitting more and longer MD trajectories, combined with modern improvements in the quality of force field parameters, have yielded some improvements in both structure prediction and homology model refinement, without reaching the point of practical utility in these areas; many identify force field parameters as a key area for further development [Raval *et al.*, 2012; Beauchamp *et al.*, 2012; Piana *et al.*, 2014].

Limits of the method are related to the parameter sets used, and to the underlying molecular mechanics force fields. One run of an MD simulation optimizes the potential energy, rather

than the free energy of the protein, meaning that all entropic contributions to thermodynamic stability of protein structure are neglected, including the conformational entropy of the polypeptide chain (the main factor that destabilizes protein structure) and hydrophobic effects (the main driving forces of protein folding) [Jaremko *et al.*, 2013]. Another important factor are intramolecular hydrogen bonds, which are not explicitly included in modern force fields, but described as Coulomb interactions of atomic point charges [Myers & Pace, 1996]. This is a crude approximation because hydrogen bonds have a partially quantum mechanical and chemical nature. Furthermore, electrostatic interactions are usually calculated using the dielectric constant of vacuum, although the surrounding aqueous solution has a much higher dielectric constant. Using the macroscopic dielectric constant at short interatomic distances is questionable. Finally, van der Waals interactionsin MD are usually described by Lennard-Jones potentials based on the Fritz London theory that is only applicable in vacuum. However, all types of van der Waals forces are ultimately of electrostatic origin and therefore depend on dielectric properties of the environment. The direct measurement of attraction forces between different materials (as Hamaker constant) shows that "the interaction between hydrocarbons across water is about 10% of that across vacuum" [Israelachvili, 2011]. The environment-dependence of van der Waals forces is neglected in standard simulations, but can be included by developing polarizable force fields.

Cavities on a proteins surface as well as specific amino acid positioning within it create the physicochemical properties needed for a protein to perform its function. CASTp (http://cast.engr.uic.edu) is an online tool that locates and measures pockets and voids on 3D protein structures. This new version of CASTp includes annotated functional information of specific residues on the protein structure. The annotations are derived from the Protein Data Bank (PDB), Swiss-Prot, as well as Online Mendelian Inheritance in Man (OMIM), the latter contains information on the variant single nucleotide polymorphisms (SNPs) that are known to cause disease. These annotated residues are mapped to surface pockets, interior voids or other regions of the PDB structures. The updated CASTp web server can be used to study surface features, functional regions and specific roles of key residues of proteins.

Characterizing protein functions is an increasingly important challenging problem that has been approached from both the sequence and structure levels. The fact that only 4922 of the 35 000 Protein Data Bank (PDB) structures contain any type of functional annotation

illustrates the widening gap between their ability to resolve the proteins structure and our ability to locate functionally important residues and to obtain a comprehensive understanding of the structural basis of protein function [Berman, 2000]. The 3D structure of a protein and its surface topography can provide important information for understanding protein function, if a broad knowledge base of the functionally important residues and where they are located on the protein structures is provided. This update of the CASTp web server incorporates functional information about a large set of annotated residues on PDB structures obtained from annotations in PDB, Swiss-Prot and Online Mendelian Inheritance in Man (OMIM) [Dundas *et al.*, 2006].

The Protein Data Bank (PDB) was founded in 1971 to provide a repository for three-dimensional (3D) structure data of experimentally determined biological macromolecules [Berman, 2000; Berman *et al.*, 2003; Bernstein *et al.*, 1977]. The PDB archive contains 3D coordinate data, information about the chemical content such as polymer sequence and ligand chemistry, information about the experiment used to derive the structure and some qualitative descriptions of the structure. When the PDB was in its infancy, the archive contained seven structures composed of loosely structured free text. Today, the PDB archive contains close to 40 000 structures and relies upon strict ontologies that define the content of these entries. The data contained in the PDB are generated and submitted by scientists from around the globe to sites in the United States, Europe and Asia. The worldwide PDB (wwPDB) was established in 2003 to formally recognize the international nature of the PDB archive [Berman *et al.*, 2003; Henrick *et al.*, 2005] and to ensure that the data files remain uniform in content and format. The founding members are the RCSB PDB (USA) [Berman, 2000], the Macromolecular Structure Database at the European Bioinformatics Institute (MSD-EBI) and the Protein Data Bank Japan (PDBj) at Osaka University [Golovin, 2004]. These wwPDB sites share responsibilities in data deposition, processing and distribution of the PDB archive, and agree to support a single, standardized archive of structural data. The BioMagRes- Bank (BMRB) at the University of Wisconsin-Madison (USA) became a member in 2006 and will be a deposition site for primary experimental data and PDB data [Ulrich *et al.*, 1989].

A wwPDB Advisory Committee (wwPDBAC) consists of representatives appointed by each member site as well as representatives of the international X-ray, NMR and electron microscopy (EM) communities. wwPDBAC meets yearly and provides advice about policies

governing the content, format and distribution of the PDB data files. The website (http://www.wwpdb.org/) contains the formal agreement for the operation of the wwPDB organization, links to the deposition and access sites, and news and announcements about policies and projects related to the wwPDB [Berman *et al.*, 2007].

2.14. Expected outcome from the present research

This present research has several expected outcomes as follows:

1. Computational study will facilitate in-depth analysis on the designed drugs as well as the target receptors. Theoretical calculation will help us to select the best drug from thousand potential candidates. Computer-aided drug design (CADD) is a ration choice for designing and developing new therapeutic candidates. Development of a lead drug requires billions of dollars investment for several years. Computer-aided drug design has been effective in reducing the extensive trial-and-error investigation cycle and enormous costs associated with drug discovery and development. Computer simulation not only reduces the cost of chemical and time; it also saves the environmental pollution associated to pharmaceutical waste. Theoretical calculations can also help us to visualize the chemical system or process occurred in the test-tube and thus advances our understanding in chemistry and biology.

2. At least one research publication will be emerged from this present research.

CHAPTER-3
MATERIALS AND METHODS

A scientific research cannot be thought without appropriate methodology, guidelines, and planning. This study was performed in Department of Chemistry, Military Institute of Science and Technology (MIST), Mirpur Cantonment, Dhaka-1216, Bangladesh as well as The Red-Green Computing Centre, 218 Elephant Road [Level 6, Suite 14], Dhaka-1205, Bangladesh.

The flow-chart representing the overall procedures of the Identification of Anticancer Drug against Caspase Protein is illustrated in Fig 6.

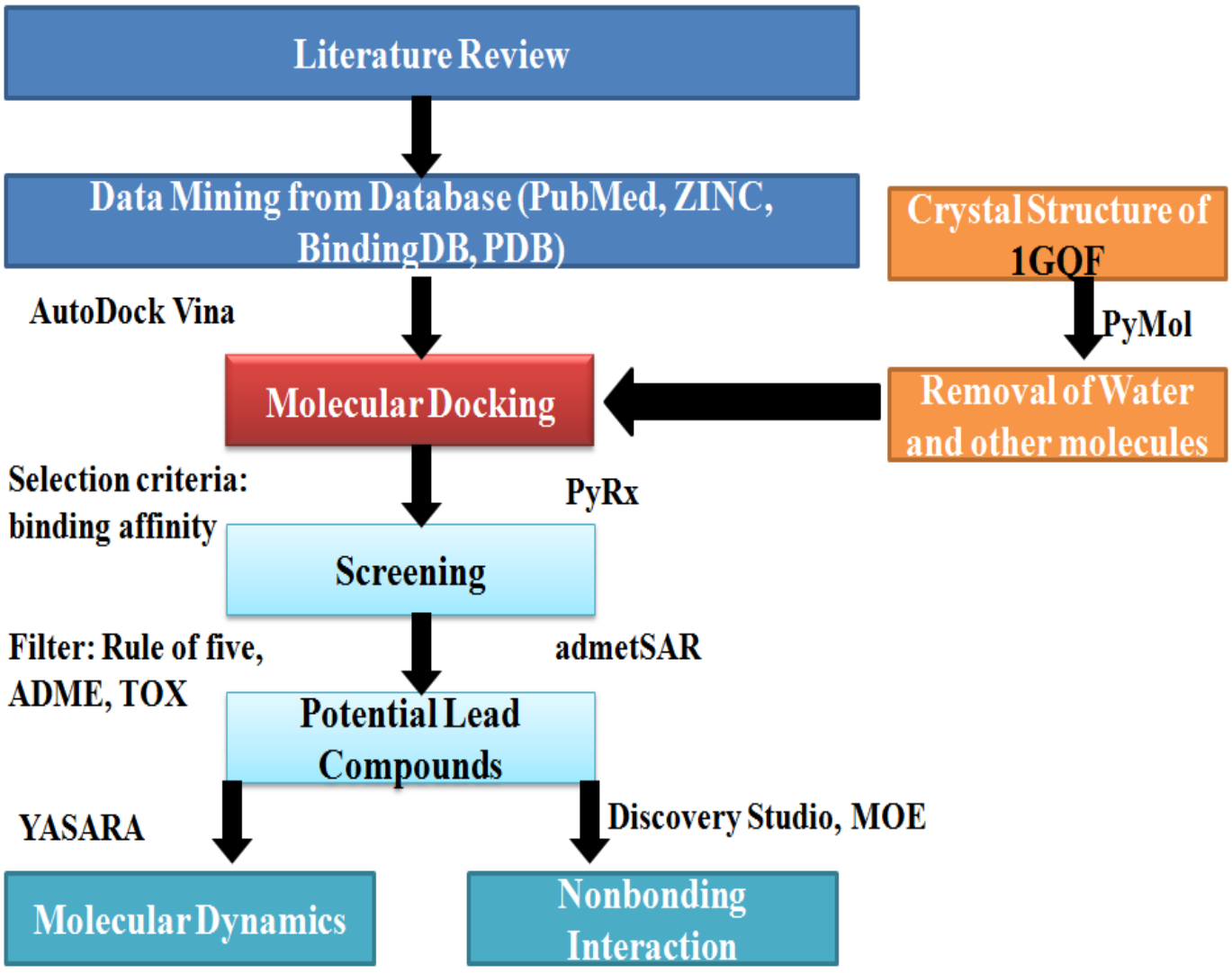

Figure 6 Steps for Identification of Anticancer Drug against Caspase Protein in the present study

The materials and methods we used to complete this research have been described below.

3.01 Structural preparation of Caspase protein

Crystal structure of Human Procaspase 7 was collected from the Protein Data Bank (PDB) database [Rose *et al.*, 2017] (PDB ID: 1GQF) containing Sulfate Ion with it. Prior to docking, the Sulfate Ion was removed from the protein structure and the remaining protein structure was saved in PDB format, which is the supported format for docking in AutoDockVina software (version1.1.2, May 11, 2011) [Trott and Oslon, 2010]. The X-ray diffraction resolution of the protein is 2.9Å. The graphical representation of the prepared structure of Pro-Case 7 is given below in Fig 7.

Figure 7 Molecular modeling of Pro-Caspase 7 Protein (PDB ID: 1GQF)

3.02 Collection of Drug like Chemical Molecules

Drug-like chemical structure library of 917 compounds were downloaded from the ZINC database and 654 compounds were downloaded from the BindingDB database for molecular docking simulation studies. A critical barrier to entry into structure-based virtual screening is the lack of a suitable, easy to access database of purchasable compounds. ZINC has a library of 727 842 molecules, each with 3D structure, using catalogs of compounds from vendors (the size of this library continues to grow). The molecules have been assigned biologically relevant protonation states and are annotated with properties such as molecular weight, calculated LogP, and number of rotatable bonds. Each molecule in the library contains vendor and purchasing information and is ready for docking using a number of popular docking programs. Within certain limits, the molecules are prepared in multiple protonation states and multiple tautomeric forms. In one format, multiple conformations are available for the molecules. This database is available for free download (http://zinc.docking.org) in several common file formats including SMILES, mol2, 3D SDF, and DOCK flexibase format. A Web-based query tool incorporating a molecular drawing interface enables the database to be searched and browsed and subsets to be created. Users can process their own molecules by uploading them to a server. This database brings virtual screening libraries to a wide community of structural biologists and medicinal chemists.

BindingDB, www.bindingdb.org, is a publicly accessible database of experimental protein-small molecule interaction data. Its collection of over a million data entries derives primarily from scientific articles and, increasingly, US patents. BindingDB provides many ways to browse and search for data of interest, including an advanced search tool, which can cross searches of multiple query types, including text, chemical structure, protein sequence and numerical affinities. The PDB and PubMed provide links to data in BindingDB, and vice versa; and BindingDB provides links to pathway information, the ZINC catalog of available compounds, and other resources.

The BindingDB website offers specialized tools that take advantage of its large data collection, including ones to generate hypotheses for the protein targets bound by a bioactive compound, and for the compounds bound by a new protein of known sequence; and virtual compound screening by maximal chemical similarity, binary kernel discrimination, and support vector machine methods. Specialized data sets are also available, such as binding

data for hundreds of congeneric series of ligands, drawn from BindingDB and organized for use in validating drug design methods. BindingDB offers several forms of programmatic access, and comes with extensive background material and documentation [Irwin and Shoichet, 2005; Mysinger & Shoichet, 2010; Gilson *et al.*, 2015].

3.03 Active Site Analysis

Computed Atlas of Surface Topography of proteins (CASTp) server was used to identify the active site and pocket of the Pro-Caspase 7. CASTp (http://sts.bioe.uic.edu/castp/calculation.php) provides an online resource for locating, delineating, and measuring concave surface regions on 3D structures of proteins including pockets located on protein surfaces and voids buried in the interior of proteins (Tian *et al.*, 2018).

3.04 Molecular Docking and Virtual Screening

Virtual screening is a very useful application when it comes to identifying hit molecules as a beginning for medicinal chemistry. As the virtual screening approach begins to become a more vital and substantial technique within the medicinal chemistry industry the approach has had an expeditious increase [Lavecchia & Giovanni, 2013]. After protein preparation and collection of drug-like molecules, the next step was docking simulations and virtual screening. Initially, 1571 lead compounds from ZINC and BindingDB database were screened using PyRx program to perform docking simulations [Dallakyan and Olson, 2015]. During each docking, receptor is kept rigid while ligands remained flexible.

To dock the compounds against Pro-Caspase 7, the center grid box was positioned at the center of the protein structure and was expanded in x, y and z directions until the grid box fully covered the protein structure. AutoDock Vina protocol was employed for docking which can produce several binding modes and predict binding affinities between the drug-receptor complex [Trott and Oslon, 2010]. The protein was kept rigid but the torsional rotation was allowed for all rotatable bonds of optimized drug structures to perform flexible docking. The overall fold of the homodimeric procaspase-7 resembles that of the active tetrameric caspase-7. Each monomer is organized in two structured sub domains connected by partially flexible linkers, which asymmetrically occupy and block the central cavity, a typical feature of active caspases [Riedl et al., 2001]. To dock the compounds against 1GQF,

the center grid box were set at 0.3189, 39.8716 and 21.87A° and box size was set at 74.7865, 79.1043 and 65.5113A° in x, y, and z direction, respectively. Next, the docked pose of lowest binding free energy conformer with the respective protein was analyzed using PyMOL Molecular Graphics System (version 1.7.4) [DeLano, 2015] and Accelrys Discovery Studio 4.1 [Dassault Systemes, 2016]. This also helped to ensure that ligands are binding at the correct binding pocket of the macromolecule structure after docking.

3.05 Protein-Ligand Interaction Analysis

A non-covalent interaction differs from a covalent bond in that it does not involve the sharing of electrons, but rather involves more dispersed variations of electromagnetic interactions between molecules or within a molecule [Anslyn, 2004]. The chemical energy released in the formation of non-covalent interactions is typically on the order of 1-5 kcal/mol (1000–5000 calories per $6.02*10^{23}$ molecules) [Lodish *et al*., 2000]. Non-covalent interactions can be classified into different categories, such as electrostatic, π-effects, and hydrophobic effects [Anslyn, 2004; Lodish *et al.*, 2000].

The hydrogen bond, hydrophobic interactions, ion pair interactions and water bridges between ligand and active site residues play important role in accommodation of small molecule into the catalytic domain of a protein [Salmas *et al.*, 2015; Leonis *et al.*, 2014; Salmas *et al.*, 2015a; Salmas *et al.*, 2015b; Salmas *et al.*, 2015c; Durdagi *et al.*, 2015]. A detailed analysis of residues involved in the interaction between ligands and target protein was conducted to visualize and interpret the hydrogen bond interactions, hydrophobic interactions of the best poses of drugs with the respective protein using the Accelrys Discovery Studio 4.1 [Dassault Systemes, 2016], and Web MOE [MOE, 2017]. To identify the hydrogen bond, maximum distance and minimum donor angle were considered with default parameters. In the case of face to face, π-π stacking interaction, angle and distance between the rings were set with default parameters also.

3.06 Molecular dynamics (MD) simulations

MD simulations help in better understanding of biological systems. It provides time-dependent investigations of protein-ligand interactions and conformational dynamics of studied complex systems. A molecular dynamics simulation requires the definition of a potential function, or a description of the terms by which the particles in the simulation will

interact. In chemistry and biology this is usually referred to as a force field and in materials physics as an interatomic potential. Potentials may be defined at many levels of physical accuracy; those most commonly used in chemistry are based on molecular mechanics and embody a classical mechanics treatment of particle-particle interactions that can reproduce structural and conformational changes but usually cannot reproduce chemical reactions.

The reduction from a fully quantum description to a classical potential entails two main approximations. The first one is the Born–Oppenheimer approximation, which states that the dynamics of electrons are so fast that they can be considered to react instantaneously to the motion of their nuclei. As a consequence, they may be treated separately. The second one treats the nuclei, which are much heavier than electrons, as point particles that follow classical Newtonian dynamics. In classical molecular dynamics, the effect of the electrons is approximated as one potential energy surface, usually representing the ground state.

When finer levels of detail are needed, potentials based on quantum mechanics are used; some methods attempt to create hybrid classical/quantum potentials where the bulk of the system is treated classically but a small region is treated as a quantum system, usually undergoing a chemical transformation.

In the current study we carried out MD simulation on the docked four complex using AMBER14 force field [Maier *et al.*, 2015] implemented in YASARA dynamics program [Krieger *et al.*, 2004; Krieger *et al.*, 2003]. 4109 water molecules were added and system was neutralized by adding NaCl salt at 0.9% concentration. A cut-off radius of 8.0 Å was used for short-range van der Waals and Coulomb interactions. The Particle-Mesh Ewald (PME) method [Tom *et al.*, 1993] was applied to calculate the long-range electrostatic interactions. MD simulation was equilibrated for 100 picoseconds (ps) followed by 10 nano second (ns) production at 298 K. A time step of 2-5 femto second (fs) will be used for the overall simulations. To study the change in binding affinity and binding interactions of the four complexes after 10 ns MD simulation, drug structures were retrieved from the MD simulated drug protein complex and rigid docking was performed against the simulated protein structure using Autodock Vina protocol. To perform rigid docking, torsional rotations of all rotatable bonds were disallowed. The docked poses were analyzed using same protocol as stated before.

3.07 Pharmacoinformatics study

Pharmacoinformatics is also referred to as pharmacy informatics. According to the article "Pharmacy Informatics: What You Need to Know Now" by the University of Illinois at Chicago Pharmacoinformatics may be defined as: “the scientific field that focuses on medication-related data and knowledge within the continuum of healthcare systems. It is the application of computers to the storage, retrieval and analysis of drug and prescription information. Pharmacy informaticists work with pharmacy information management systems that help the pharmacist safe decisions about patient drug therapies with respect to, medical insurance records, drug interactions, as well as prescription and patient information. Different computational tools and web databases were exploited for the pharmacoinformatics elucidation of active compounds that might have the potentiality to exhibit anti-cancer activity by interacting with the pro-caspase 7 protein. Pharmacophore is an ensemble of steric and electronic features that are needed to have an optimal supramolecular interaction or interactions with a biological target structure in order to precipitate its biological response. Choose a representative as a set of actives; most methods will look for similar bindings. It is preferred to have multiple rigid molecules and the ligands should be diversified, in other words ensure to have different features that don't occur during the binding phase. This technique is used when merging the results of searches by using unlike reference compounds, same descriptors and coefficient, but different active compounds. This technique is beneficial because it is more efficient than just using a single reference structure along with the most accurate performance when it comes to diverse actives [Leach *et al.*, 2010]. The pharmacophoric library screening, ADMET (absorption, distribution, metabolism, excretion, and toxicity) and QSAR (quantitative structural-activity relationship) properties were carried out by Osiris property explorer [Sander, 2001], Molinspiration [Mishra and Raghava, 2011], AcTor [Judson *et al.*, 2008], admetSAR [Cheng *et al.*, 2012], and ACD/I-Lab [Masunov, 2001].

Briefly, Osiris property explorer [Sander, 2001] computes various drug related properties like tumorigenicity, mutagenicity, irritation, reproductive effect, drug likeness, and drug-score prediction based on chemical structure. Molinspiration offers broad range of Chemoinformatics software tools that support molecule manipulation and fragmentation, calculation of various molecular properties such as QSAR molecular modeling, and drug

design [Ertl *et al.*, 2000]. The ACToR (Aggregated Computational Toxicology Resource) database, that locates many types and sources of data, has information about *in vitro* bioassays and *in vivo* toxicology assays on chemical structure derived from more than 150 sources [Judson *et al.*, 2008]. The admetSAR (absorption, distribution, metabolism, excretion, and toxicity structure-activity relationship database) online database has been utilized to predict the data related to drug absorption, metabolism and carcinogenicity for the selected drug molecules [Cheng *et al.*, 2012]. Structure Data File (SDF) and simplified molecular-input line-entry system (SMILES) strings were utilized throughout the generation process [Cheng *et al.*, 2012] and ACD/I-Lab [Masunov, 2001] handles existing ADMET-associated information from the available literature. Default parameters of these online servers are used for the pharmacoinformatics analysis.

CHAPTER-4
RESULTS

After conducting virtual screening through molecular docking, we have selected four potential lead compounds based on docking energies. The chemical formula of the four potential lead compounds are $C_{30}H_{31}N_3O_3$, $C_{28}H_{22}BrN_5O_4S$, $C_{26}H_{21}F_3N_2O_5S$, and $C_{29}H_{24}FN_5O_5S$ and IUPAC name are 4-(1,3-dioxooctahydro-4,6 ethenocyclopropa[f]isoindol-2(1H)-yl)-N-[4-(pyridin-4-ylmethyl)phenyl]cyclohexanecarboxamide,2-[1-[(4 bromo phenyl) methyl] -2-oxo-5-[(2S)-2-(pyridin-3-yloxy methyl) pyrrolidin-1-yl] sulfonylindol-3-ylidene] propane dinitrile, 5- [(2S)-2- [(3,5- difluoro phenoxy) methyl] pyrrolidin-1-yl] sulfonyl-1-[(4-fluorophenyl)methyl]indole-2,3-dione, and 2-[1-[[4-(2-fluoroethoxy)phenyl]methyl]-2-oxo-5-[(2S)-2-(pyridin-3-yloxy methyl) azetidin-1-yl] sulfonyl-indol-3-ylidene] propane dinitrile respectively. Considering long IUPAC name, we denoted all the four compounds as D1, D2, D3, and D4 respectively.

4.01 Active Site Analysis of Pro-Caspase 7

The active site area of the insulin receptor protein and the number of amino acid residues involved in it were determined with the CASTp server. This provides a significant insight of the docking simulation study to locate the active site cleft and the amino acid residues that interact with different ligands. The preeminent active site is found with 2209.665 areas and a volume of 1591 amino acids. The active site of the Pro-Caspase 7 graphically presented in Fig 8 and the active site amino acids revealed from CASTp server tabulated in Table 1.

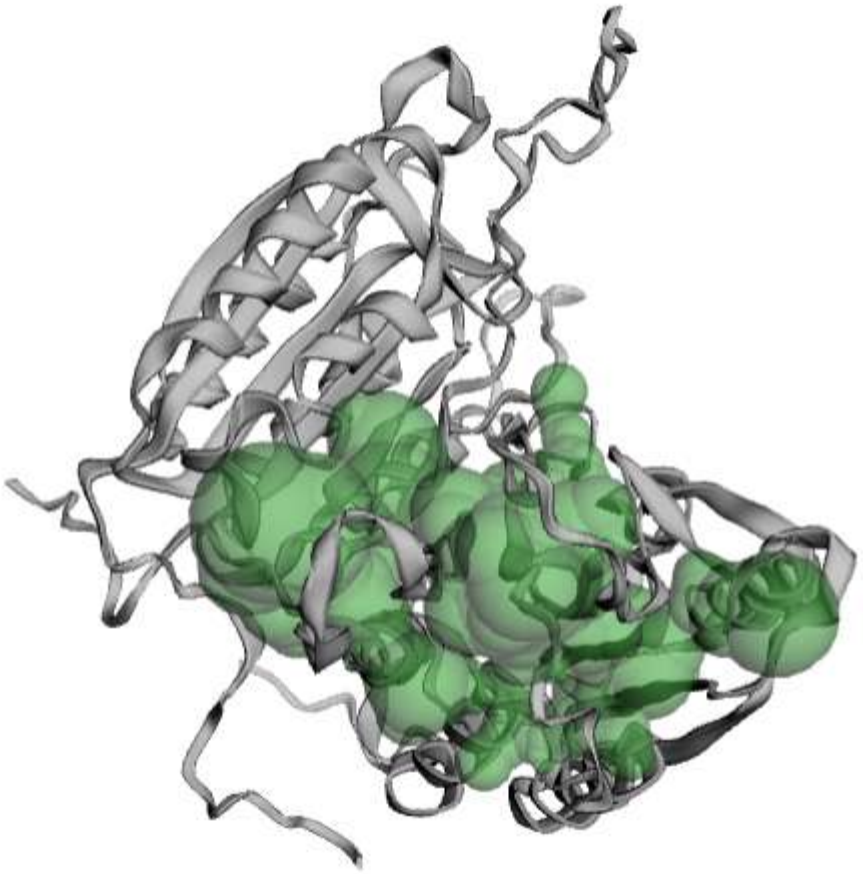

Figure 8 Active site amino acids of Pro-Caspase 7 protein (PDB ID: 1GQF) (Green color round shapes indicate the active site residues)

Table 1 Active site amino acid residues analysis of Pro-Caspase 7 protein

Properties	Binding Pocket amino acid residues
Volume	1591.375
Area	2209.665
Amino Acid residues	TYR153, ARG266, GLY267, ASP268, ARG269, CYS270, LYS271, LEU274, GLU275, LYS278, VAL323, GLU324, ALA325, ASP 326, THR393, LYS396, ASN170, LYS 171, PHE173, LYS175, MET176, GLY177, VAL178, ARG179, ASN180, GLY181, THR182, ASP185, LEU235, SER236, HIS237, GLY238, GLU239, GLU240, ASN 241, VAL242, ILE243, ILE258, ILE282, GLN283, ALA284, ALA285, ARG286, GLY287, THR288, GLU289, LEU290, ASP291, ASP292, GLY293, ILE294, GLN295, ALA296, SER298, GLY299, PRO300, ILE301, ASN311, ASP312, THR313, ASP314, ALA315, ASN316, PRO317, TYR319, LYS320, TYR331, SER332, THR333, VAL334, PRO335, GLY336, TYR337, TYR338, SER339, TRP340, SER342, PRO343, GLY344, ARG345, GLY346, SER347, TRP348, ASP375, ALA378, ARG379, HIS379, PHE380, HIS381, GLU382, LYS383, LYS384, GLN385, ILE386, PRO387, CYS388.

4.02 Binding Affinity and Interaction of selected drug molecules with 1GQF

The binding affinity of the selected drug molecules (D1, D2, D3 and D4) against 1GQF is −10.8 kcal mol^{-1}, −10.8 kcal mol^{-1}, −10.7 kcal mol^{-1}, and −10.6 kcal mol^{-1} respectively. The chemical structure of the selected drug with Molecular Docking energy presented in Fig 9.

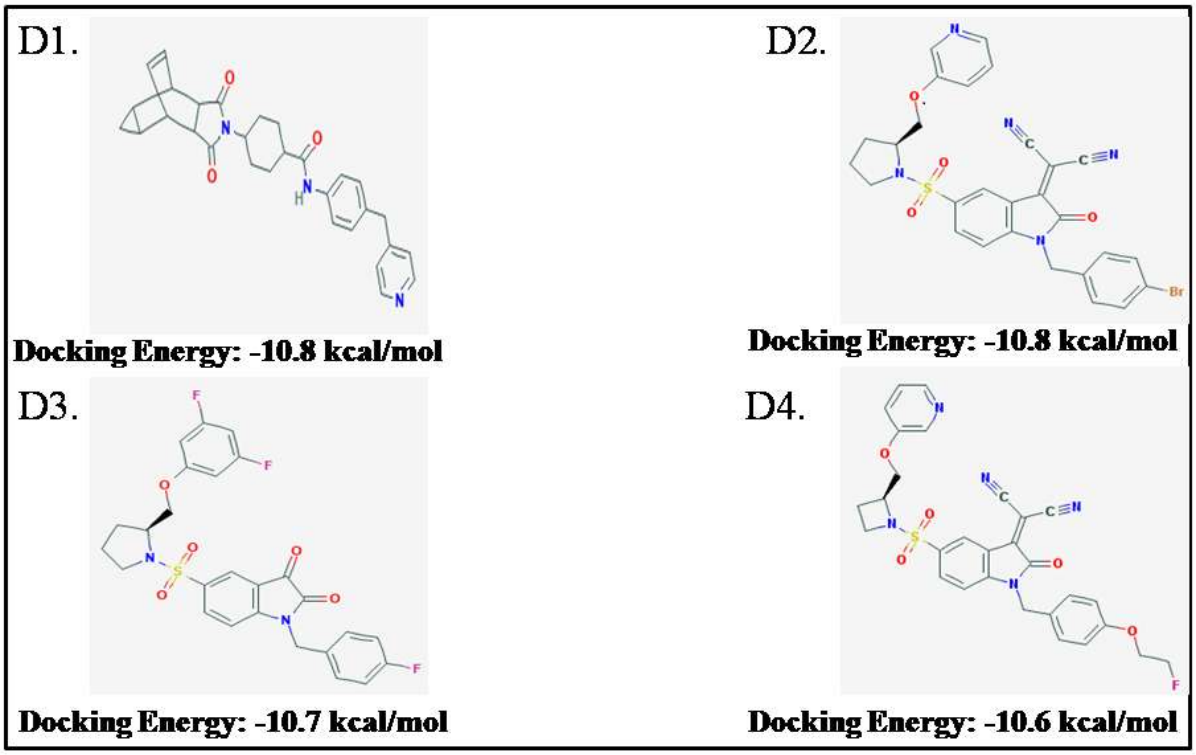

Figure 9 Chemical structures of the selected compounds with molecular docking energy

The surrounding binding pocket residues (generated by MOE program) showing interaction with the drug molecules residues of 1GQF which interact with D1, D2, D3 and D4 are demonstrated in Fig 10,11,12,13. All the drug molecules have significant interaction with different amino acid residues. Nonbonding interactions like hydrogen bond, halogen bond, hydrophobic interaction, electrostatic interaction are examined by Discovery Studios Software version 4.1 and summarized in Table 2.

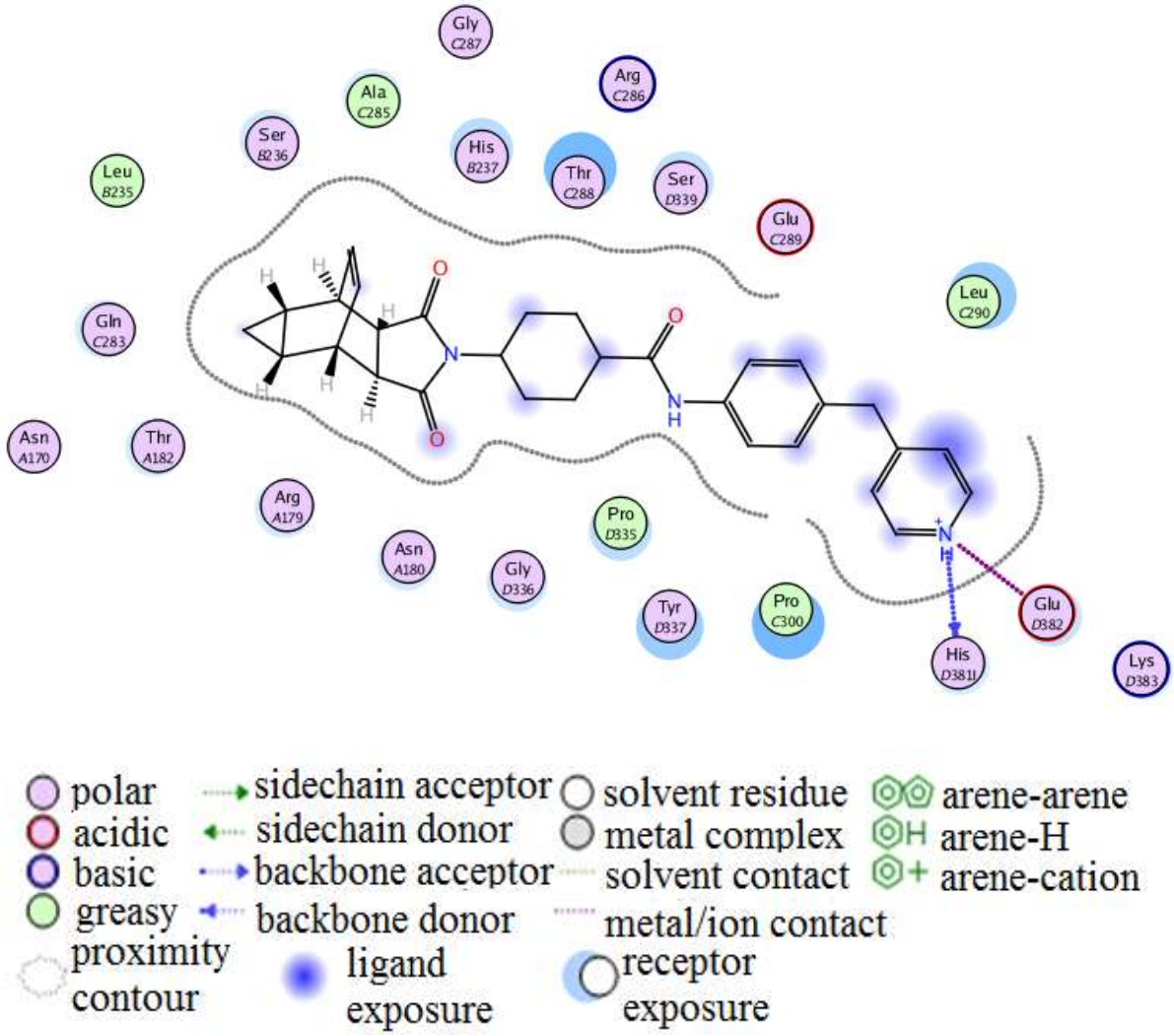

Figure 10 Docking simulation analysis of the drug molecule D1 with Pro-Caspase 7 (PDB ID: 1GQF) binding pocket residues showing interaction with the drug molecule

In case of D1, we found three hydrogen bonds, two hydrophobic interactions and an electrostatic interaction between drug molecules with 1GQF. There have no halogen bond found in this stage [Fig 10]. On the other hand, hydrogen bond and hydrophobic interactions are significantly increased in the interaction between D2 and 1GQF except halogen bond and electrostatic interactions. There has no halogen bond interaction in D2 also [Fig 11].

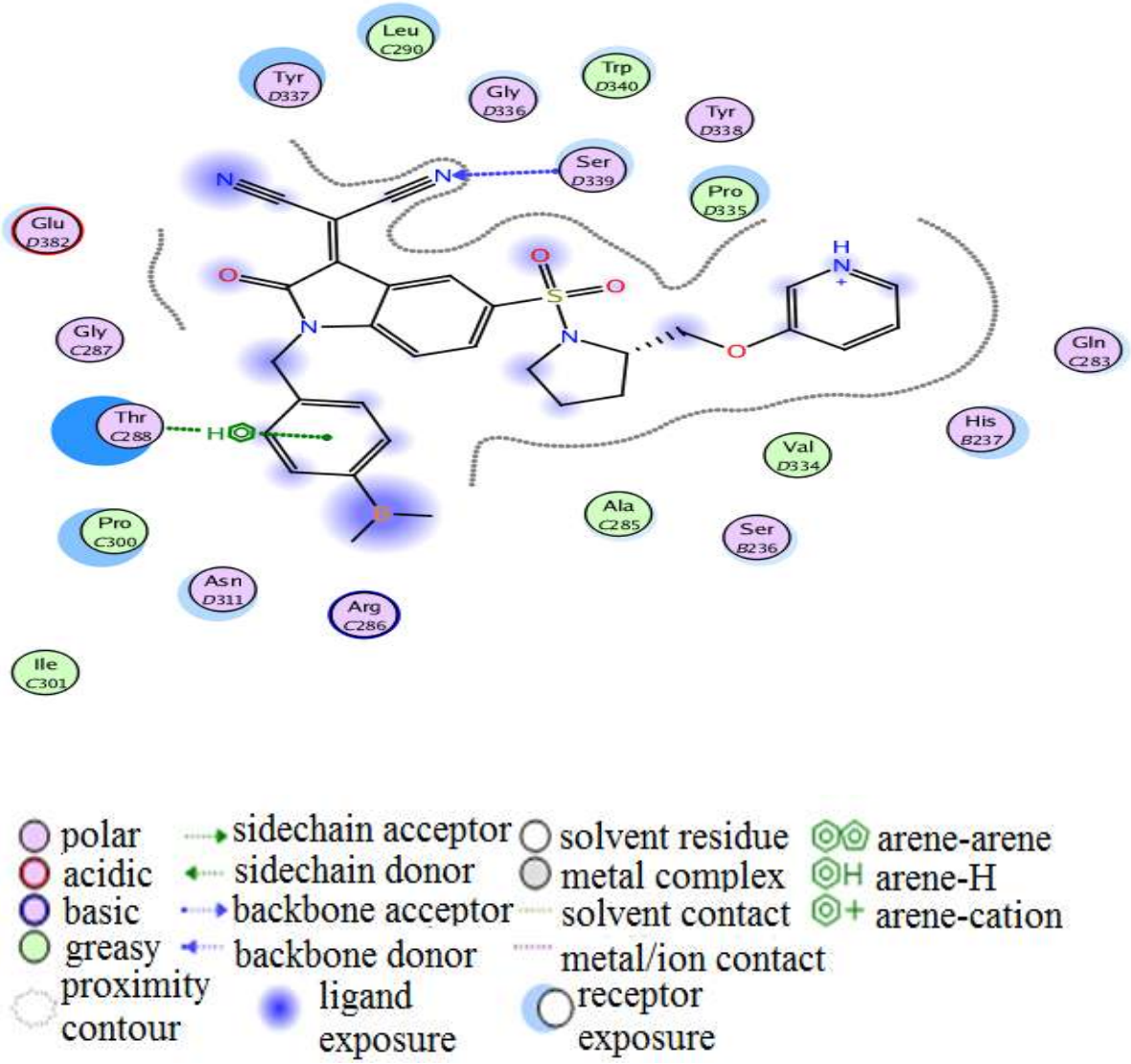

Figure 11 Docking simulation analysis of the drug molecule D2 with Pro-Caspase 7 (PDB ID: 1GQF) binding pocket residues showing interaction with the drug molecule

The highest number of halogen bond and hydrophobic interactions found in the third interaction between drug molecules D3 and 1GQF and its total six and eight in number respectively. But, there have only five hydrogen bond interaction [Fig 12].

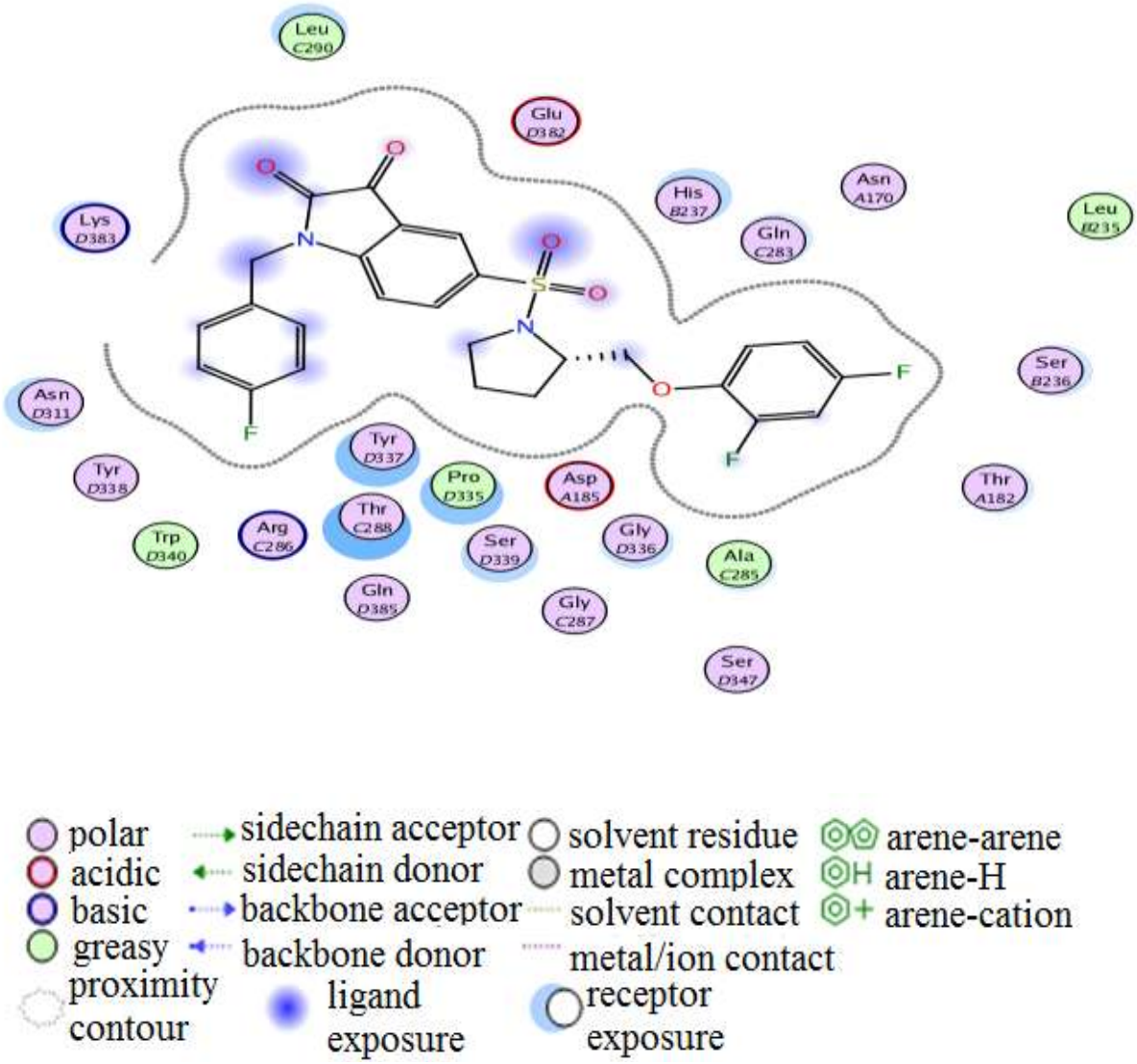

Figure 12 Docking simulation analysis of the drug molecule D3 with Pro-Caspase 7 (PDB ID: 1GQF) binding pocket residues showing interaction with the drug molecule

Non-covalent interactions such as hydrogen bond, halogen bond and hydrophobic interaction are involved in the binding of drug molecules D4 with 1GQF (Fig 13). The D4-1GQF complex is stabilized by eleven hydrogen bonds, seven hydrophobic interactions and one halogen bond [Table 2].

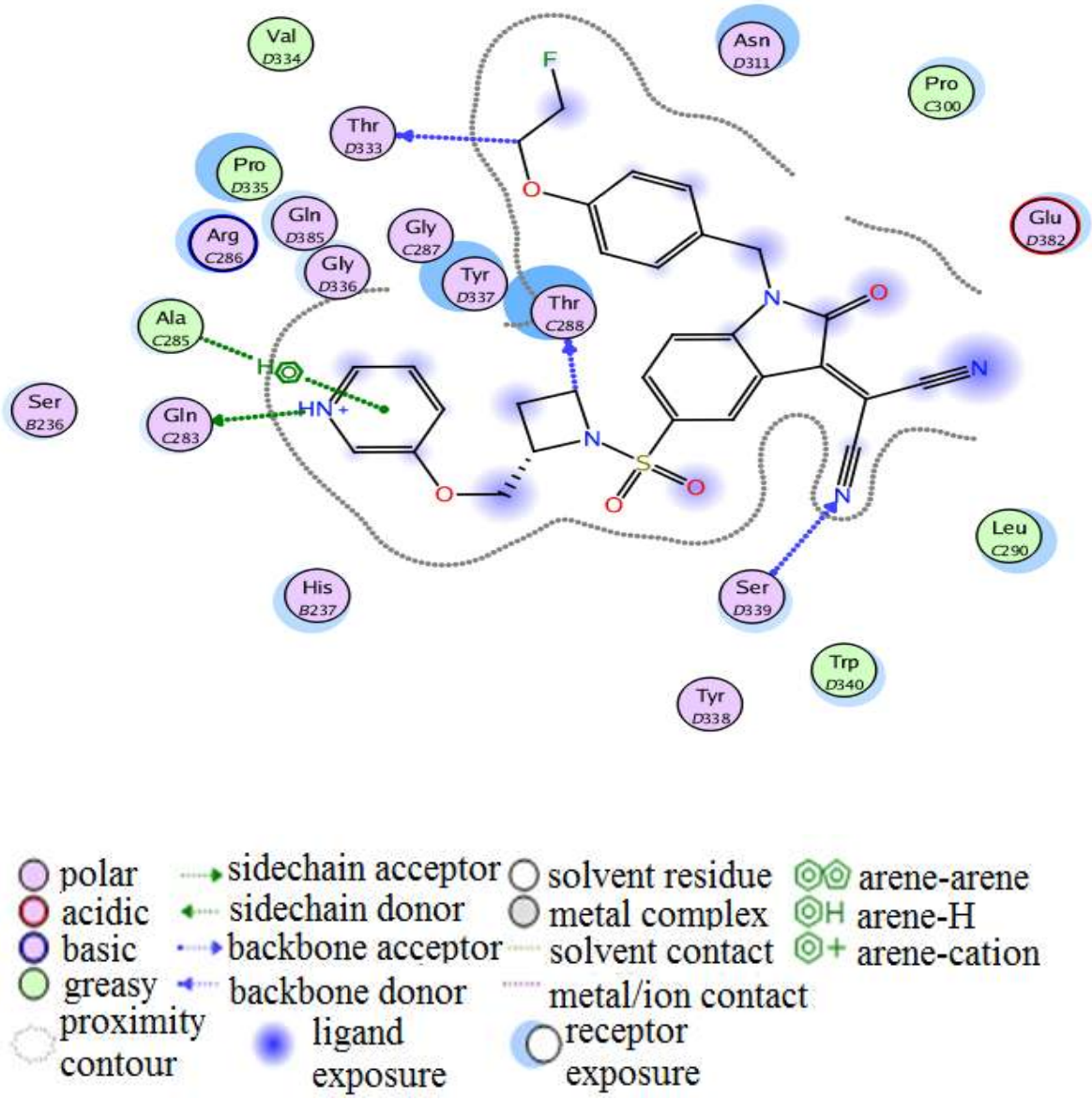

Figure 13 Docking simulation analysis of the drug molecule D4 with Pro-Caspase 7 (PDB ID: 1GQF) binding pocket residues showing interaction with the drug molecule

Table 2 Binding energy and nonbonding interaction of the drug compound after Molecular docking

Compound	Docking against Pro-Caspase 7 (1GQF)							
	Binding energy (kcal/ mol)	Hydrogen bond		Halogen bond		Hydrophobic Interaction		Electrostatic Interaction	
		(A acid-L atom)	Distance (A°)	(A acid-L atom)	Distance (A°)	(A acid-L atom)	Distance (A°)	(A acid-L atom)	Distance (A°)
D1	-10.8	HIS237 [N-H…O]	2.92			LEU290 [Alkyl…Pi]	5.19	GLU382 [O…H-N]	3.48
		HIS381 [N-H…O]	2.33			PRO300 [Alkyl…Pi]	4.2		
		HIS381 [C-H…O]	3.09						
D2	-10.8	GLY336 [N-H…O]	3.01			TYR337 [Pi-Pi T-shaped]	5.22		
		TYR337 [N-H…O]	2.8			TYR337 [Pi-Pi T-shaped]	4.8		
		SER339 [N-H…O]	3.06			ALA285 [Alkyl]	4.71		
		SER236 [C-H…O]	3.09			ALA285 [Alkyl]	5.18		
		THR288 [C-H…O]	2.92			HIS237 [Alkyl…Pi]	4.83		
		THR288 [C-H…O]	2.56			PRO335 [Pi…Alkyl]	4.81		
						PRO300 [Pi…Alkyl]	4.68		
D3	-10.7	TYR337 [N-H…O]	2.96	GLN385 [H…F]	2.56	TYR337 [Pi-Pi T-shaped]	5.12		
		GLN385 [N-H…O]	2.56	SER236 [H…F]	2.69	TYR337 [Pi-Pi T-shaped]	5.59		
		SER236 [N-H…O]	2.69	ASN170 [O…F]	3.13	TYR337 [Pi-Pi T-shaped]	5.03		
		GLY336 [N-H…O]	2.8	LEU235 [C…F]	3.19	ALA285 [Alkyl]	4.34		
		TYR337 [N-H…O]	3.25	GLN283 [O…F]	3.22	HIS237 [Alkyl…Pi]	5		
				LYS383 [O…F]	3.57	PRO335 [Pi…Alkyl]	4.67		
						LYS383 [Pi…Alkyl]	5.32		
						PRO335 [Pi…Alkyl]	4.95		
D4	-10.6	TYR337 [N-H…O]	2.71	ASN311 [O…F]	3.33	TYR337[Pi-Pi T-shaped]	5.31		

SER339 [N-H…O]	3.01	TYR337[Pi-Pi T-shaped]	5.01
SER339 [N-H…O]	3.21	ALA285 [Alkyl]	4.62
SER339 [N-H…O]	3.13	ARG286 [Alkyl]	3.94
GLN385 [N-H…O]	3.12	HIS237 [Alkyl…Pi]	5
GLN283 [O…H-N]	2.16	PRO335 [Alkyl…Pi]	5.05
ARG286 [C-H…F]	3.74	PRO335 [Alkyl…Pi]	4.36
PRO335 [N-H…O]	3.4		
SER236 [C-H…O]	2.65		
THR288 [C-H…O]	2.27		
THR333 [C-H…O]	2.4		

4.03 Molecular dynamics (MD) simulations analysis

MD simulations (10 ns for the each individual system) were performed for the four selected complexes. The conformational convergences of the systems were checked using the RMSD values of the CA (Alpha) atoms to their initial forms. Judging from the results in Fig 14, reasonable converges for all the systems was observed. Then again, molecular docking simulation study was performed for the four drug molecules by using PyRx program [Dallakyan and Olson, 2015]. Before starting the docking, the 10 ns MD simulated protein and drug molecule complexes were separated through PyMOL Molecular Graphics System (version 1.7.4) [DeLano, 2015]. PyRx program [Dallakyan and Olson, 2015] used to dock the extracted drug molecules with procapase 7 protein and significant changes happen in case of docking energy and the binding energies of the selected drug molecules (D1, D2, D3 and D4) against 1GQF are −15.8 kcal mol^{-1}, −10.1 kcal mol^{-1}, −14.3 kcal mol^{-1}, and −10.1 kcal mol^{-1} respectively. To monitor the changes of Protein-Ligand interactions after MD simulation, the Accelrys Discovery Studio 4.1 [Dassault Systemes, 2016] and Web MOE [MOE, 2017] generated results analyzed. The Protein-ligand interactions of the docking simulation are shown in Fig 15, 16, 17, 18 sequentially from compounds D1 to D4. All the drug molecules have significant interaction with different amino acid residues. Nonbonding interactions like hydrogen bond, halogen bond, hydrophobic interaction, electrostatic interaction are examined by Discovery Studios Software version 4.1 and summarized in Table 3.

Flexibility of the target binding site is an essential but frequently overlooked aspect to be considered in molecular docking. Enzymes and receptors can undergo conformational changes during the molecular recognition process [Lin, 2011]. In some cases these structural rearrangements are small and the ligand fits in a binding site with little mobility. Otherwise, some proteins undertake significant conformational changes, which can involve elements of secondary and tertiary structure. Such flexibility issues can be handled by the use of techniques such as MD [Salsbury, 2010].

Molecular dynamics applies Newton's equations of motion, as described in classical mechanics, to specify the position and speed of each atom in the system under study. As a result, the trajectory and temporal evolution of a ligand-receptor complex can be examined [Nichols *et al*., 2011]. Initially, a specific configuration is attributed to the atoms with the purpose to reproduce the temperature and pressure of the real system. From the computation of the forces acting on each particle, it is possible to determine the position and velocity of each of these atoms at a posterior time. These calculations are repeatedly performed until the molecular trajectories are integrated for a given time interval [Salsbury, 2010].

In all the cases of drug-protein complex, RMSD values of the CA (Alpha) atoms remains similar in terms of backbone. In case of D1-Pro-Caspase 7 complex, backbone and CA (Alpha) atoms remains near about 4 RMSD (A^0) except during 6ns backbone exceeds 4.5 RMSD (A^0). In case of D2-Pro-Caspase 7 complex, backbone and CA (Alpha) atoms remains near about 4 RMSD (A^0) up to 8ns later both exceeds 4.5 RMSD (A^0). In case of D3-Pro-Caspase 7 complex, backbone and CA (Alpha) atoms exceed 4 RMSD (A^0) in 2ns and remains same up to 10ns. In case of D4-Pro-Caspase 7 complex, backbone and CA (Alpha) atoms rise gradually from the starting point and touches 5 RMSD (A^0) in 5ns and remains same up to 7ns. During 8ns, both move forward to 5.5 RMSD (A^0) and later go down to 5 RMSD (A^0) again and remain same up to last.

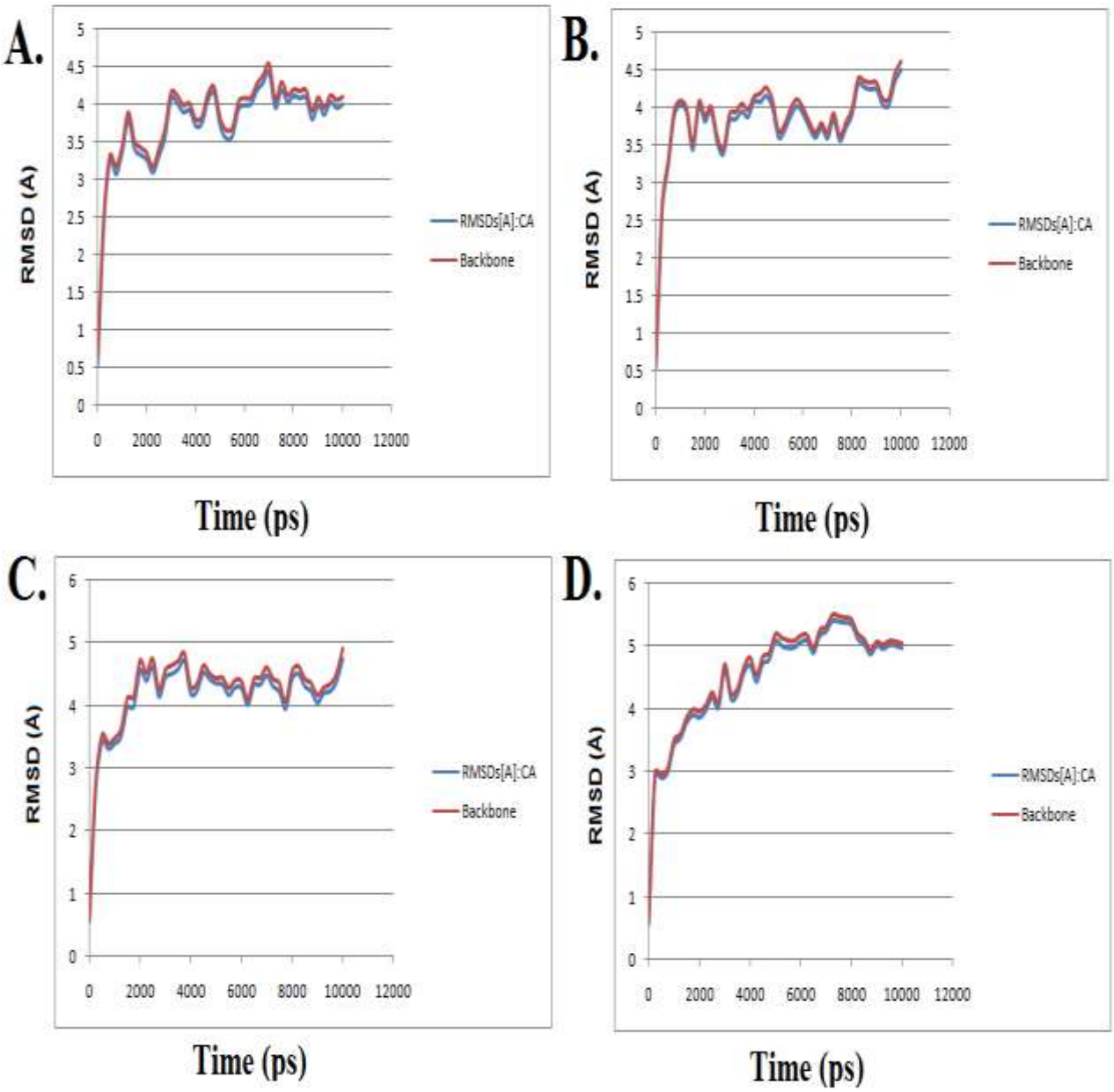

Figure 14 RMSD values of CA (alpha) atoms of the proteins in the complexes with drug molecules along the 10 ns simulations. In this figure, A= the drug molecule D1 with Pro-Caspase 7 (PDB ID: 1GQF), B= the drug molecule D2 with Pro-Caspase 7 (PDB ID: 1GQF), C= the drug molecule D3 with Pro-Caspase 7 (PDB ID: 1GQF), D= the drug molecule D4 with Pro-Caspase 7 (PDB ID: 1GQF).

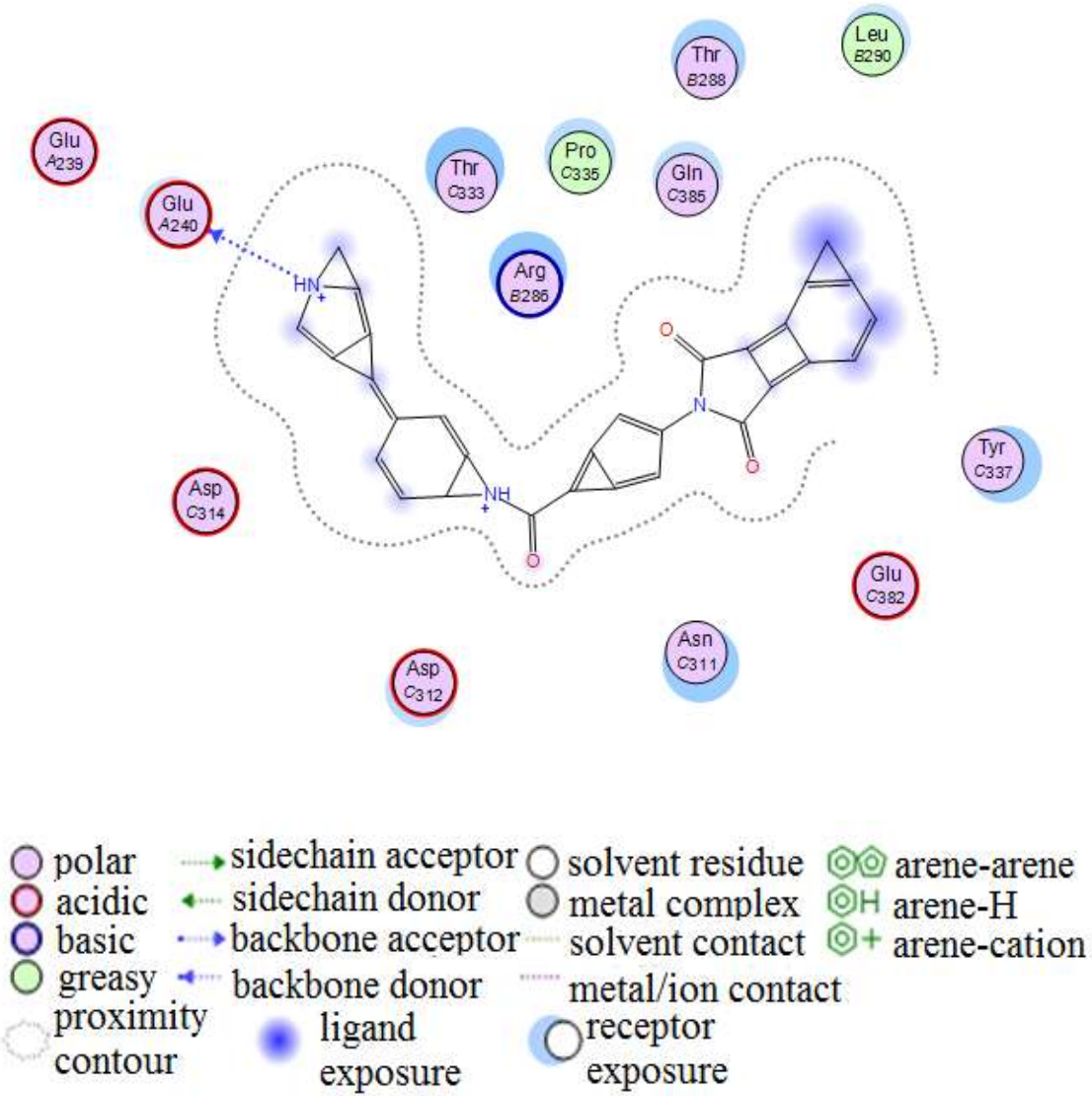

Figure 15 Molecular Docking simulation analysis of the drug molecule D1 with Pro-Caspase 7 (PDB ID: 1GQF) binding pocket residues showing interaction with the drug molecule after Molecular dynamic study

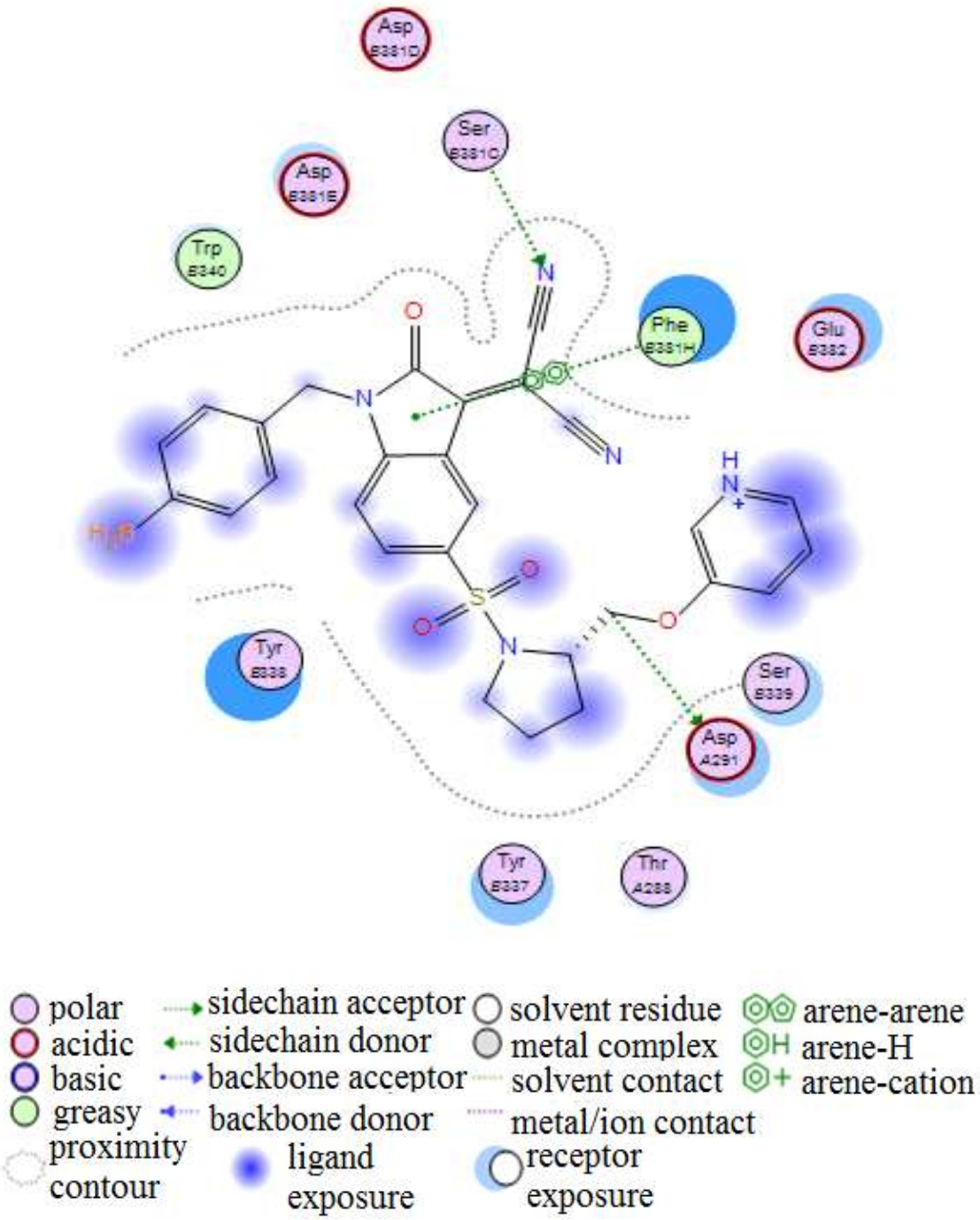

Figure 16 Molecular Docking simulation analysis of the drug molecule D2 with Pro-Caspase 7 (PDB ID: 1GQF) binding pocket residues showing interaction with the drug molecule after Molecular dynamic study.

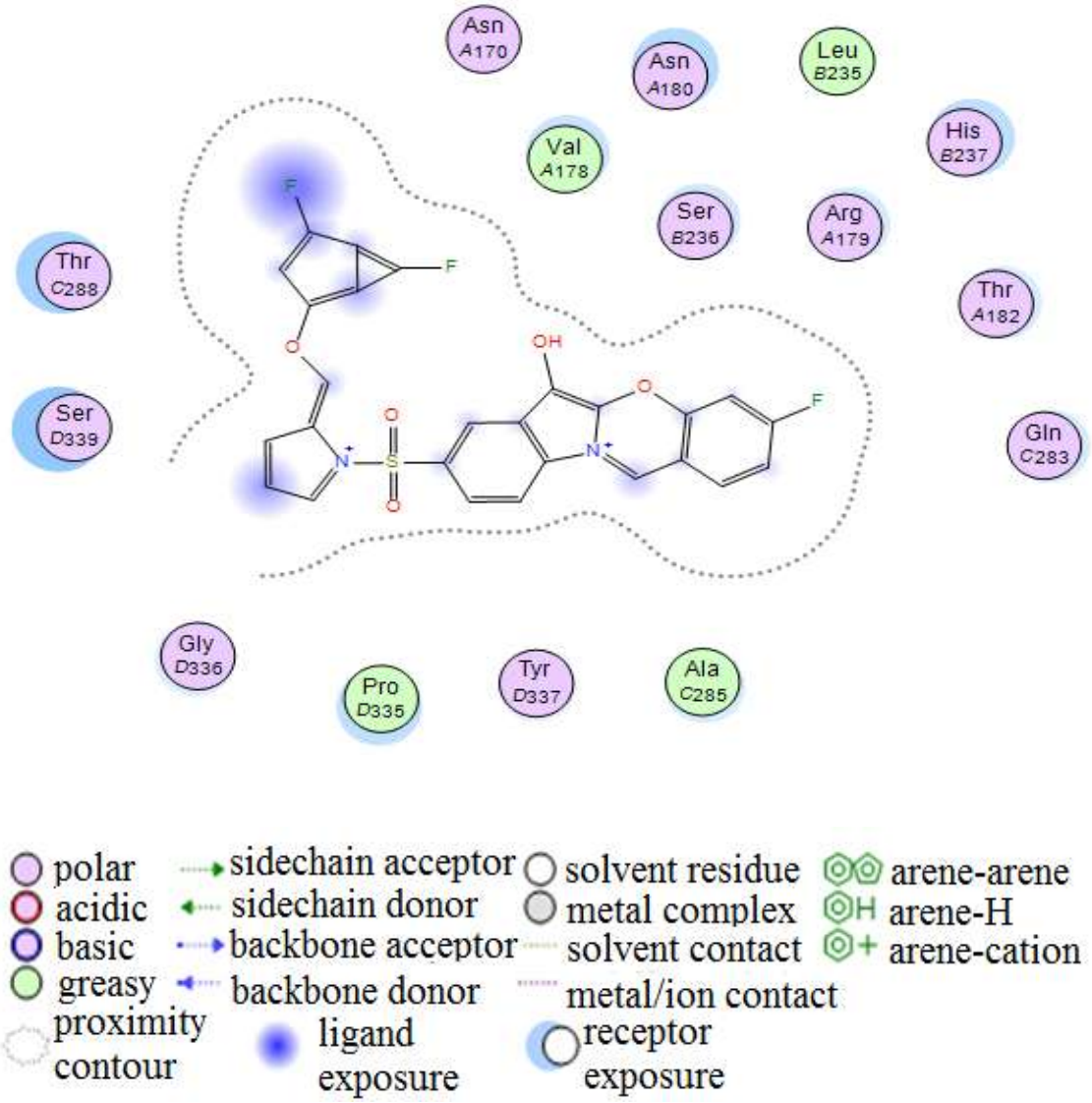

Figure 17 Molecular Docking simulation analysis of the drug molecule D3 with Pro-Caspase 7 (PDB ID: 1GQF) binding pocket residues showing interaction with the drug molecule after Molecular dynamic study

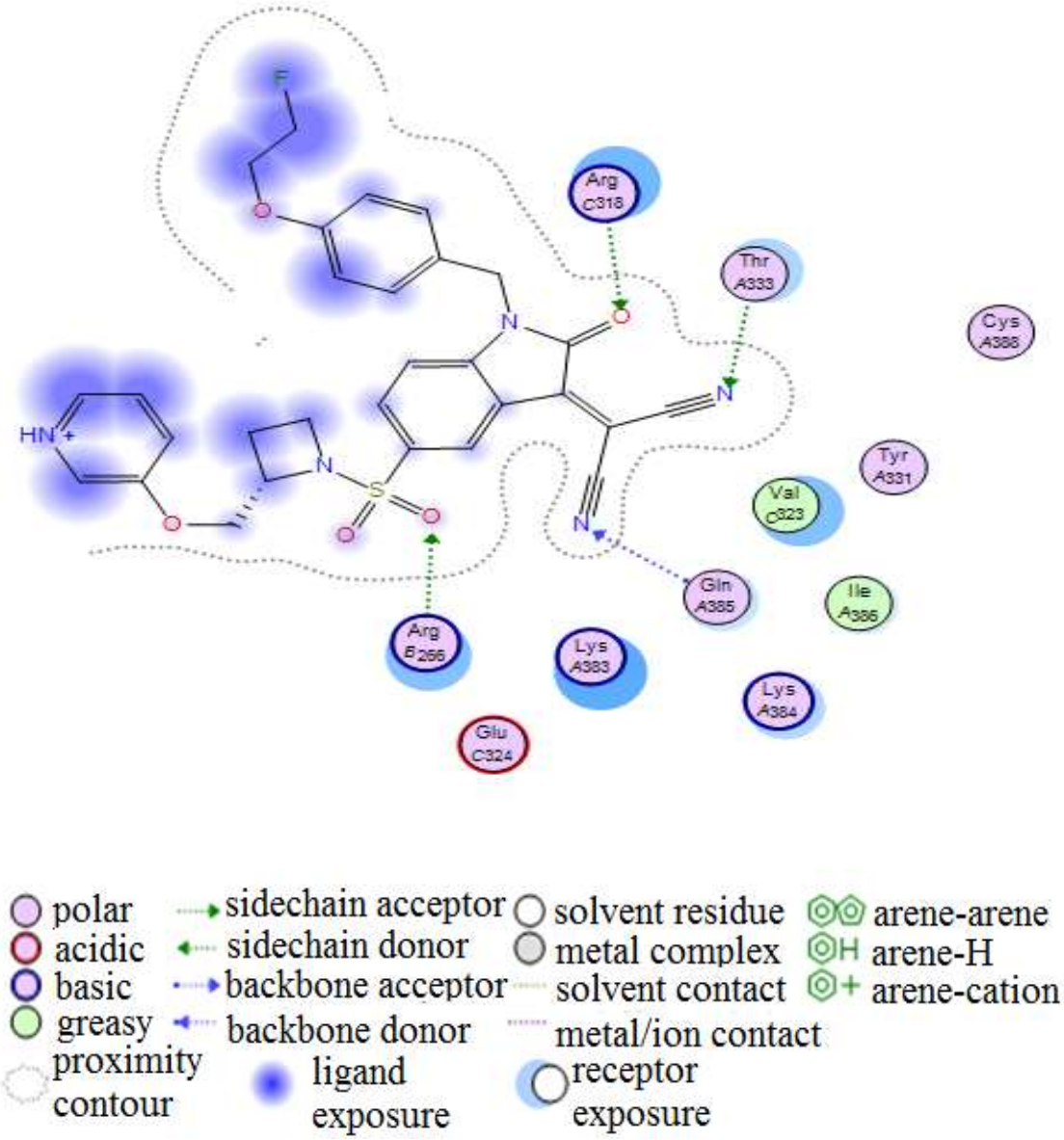

Figure 18 Molecular Docking simulation analysis of the drug molecule D4 with Pro-Caspase 7 (PDB ID: 1GQF) binding pocket residues showing interaction with the drug molecule after Molecular dynamic study

Table 3 Binding energy and nonbonding interaction of the drug Compound Molecular dynamics analysis

Compound	Docking against Pro-Caspase 7 (1GQF)							
	Binding energy (kcal/ mol)	Hydrogen bond		Halogen bond		Hydrophobic Interaction		Electrostatic Interaction	
		(A acid-L atom)	Distance (A°)	(A acid-L atom)	Distance (A°)	(A acid-L atom)	Distance (A°)	(A acid-L atom)	Distance (A°)
D1	-15.8	ASN311 [N-H…O]	2.74			PRO335 [Alky]	5.39		
		GLU240 [O…H-N]	2.18			TYR337 [Alkyl…Pi]	5.36		
						TYR337 [Alkyl…Pi]	4.85		
						PHE381 [Pi-Pi T-shaped]	4.36		
D2	-10.1	ASP381 [C-H…O]	2.58						
		ASP291 [C-H…O]	2.70			PHE381 [Pi-Pi T-shaped]	3.86		
		PHE381 [N-H…O]	2.89						
D3	-14.3	ASN180 [N-H…F]	3.53	ASN180 [N…F]	3.53				
		ASN180 [N-H…F]	3.60	ASN180 [N…F]	3.60				
		THR182 [O-H…F]	2.99	THR182 [O…F]	2.99				
		SER339 [O-H…O]	3.15	LEU235 [C…F]	3.17				
		ARG179 [C-H…O]	3.55	VAL176 [O…F]	3.23				
D4	-10.1	THR333 [O-H…N]	2.94			LYS383 [Alkyl]	4.73	ARG266 [O…H-N]	4.41
		ILE386 [N-H…O]	3.10			VAL323 [Alkyl…Pi]	5.08		
		ARG266 [N-H…O]	2.86			VAL323 [Alkyl…Pi]	4.14		
		ARG318 [C-H…O]	3.01						
		ARG318 [O…H-C]	2.49						

4.04 Pharmacophore Study of Drug Compounds

The Pharmacophore and QSAR properties of the compounds were analyzed by using different software mentioned in methods section and the results are listed in Tables 4 and 5. Among the ADMET properties, human intestinal absorption is highest for Drug 1 and 2 (D1&D2) (1 out of 1.0) while Caco-2 permeability is highest for Drug 4 (D4) (0.6033 out of

1). The other two compounds showed very high human intestinal absorption but in case of Caco-2 permeability, Drugs showed moderate permeability. No drug seems to cross the blood brain barrier as revealed by their score ranging from 0.7188 to 0.988. All the four drugs are distributed in Mitochondria. In terms of metabolism, The Cyp450 2c9 Substrate score remained more or less same while the highest and lowest Cyp450 2c9 inhibitor score found in Drug 3 and Drug 4 respectively. The toxicity study like human ether-a-go-go-related (hERG) gene inhibitory score of the fur drug molecules were ranging from 0.75 to 0.95 and all of the drug molecules are categorized as weak inhibitor. AMES toxicity test is employed to know whether a compound is mutagenic or not. All the drug compounds displayed negative AMES toxicity test which means that the ligands are non-mutagenic. Carcinogenic profile also revealed that the drugs were non-carcinogenic. Important information obtained from admetSAR server was the computed LD50 dose in rat model. Comparing the LD50 doses, a compound with lower dose is more lethal than the compound having higher LD50. From our observation, we found that D2 had almost the same LD50, compared to the D4 (2.5956 versus 2.6042, resp.). Drug 1 (D1) had the lowest LD50 of 2.46 and was most toxic among the selected drugs.

Table 4 ADMET properties of selected drug compounds. (Most data of ADMET properties were measured according to a measuring scale of 1.0.)

Properties	CID-4678256 D1	CID-16749271 D2	CID-25178949 D3	CID-25217031 D4
		Absorption		
Blood-Brain Barrier	0.988	0.7688	0.904	0.7188
Human Intestinal Absorption	0.996	1	0.9973	1
Caco-2 Permeability	0.5344	0.5905	0.5905	0.6033
		Distribution		
	Mitochondria	Mitochondria	Mitochondria	Mitochondria
		Metabolism		
Cyp450 2c9 Substrate	0.7479	0.7698	0.7265	0.7666
Cyp450 2c9 Inhibitor	0.795	0.7781	0.892	0.6611
		Toxicity		

Carcinogens	NC (0.9309)	NC (0.767)	NC (0.6989)	NC (0.7483)
AMES toxicity	0.7377	0.5988	0.6003	0.5868
Human ether-a-go-go-related (hERG) gene Inhibition	WI (0.9463)	WI (0.7485)	WI (0.9174)	WI (0.8418)
LD50 in Rat (mol/kg)	Nontoxic (2.4584)	Nontoxic (2.5956)	Nontoxic (2.491)	Nontoxic (2.6042)
Mutagenicity	No	No	No	No
Irritating effects	No	No	No	No

Table 5 illustrates the various ADMET parameters obtained from admetSAR tool. Ligand properties were found in acceptable range for all the tested drugs [Table 5]. Among the four drugs, the highest molecular weight found in Drug 3 (D3) and its molecular formula and IUPAC name are $C_{28}H_{22}BrN_5O_4S$ and 2-[1-[(4-bromophenyl)methyl]-2-oxo-5-[(2S)-2-(pyridin-3-yloxymethyl)pyrrolidin-1-yl]sulfonylindol-3-ylidene]propanedinitrile respectively. In case of hydrogen, the highest number of hydrogen bond acceptor and rotatable bond fund in same drug named drug 4 (D4). All the drugs are poorly soluble except drug 2 (D2) which is molecularly soluble. TPSA or Topological Polar Surface Area indicates the surface belonging to polar atoms in the compound. The highest TPSA found in drug 4 (D4) on the other hand the lowest TSPA found in drug 1 (D1). In case of Bioavilibility, all the four drugs showed same score. Finally, the toxicity profile of the selected drug molecules checked and found all were non toxic.

Table 5 ADMET and QSAR properties of selected drug molecules.

Ligand	CID-4678256	CID-16749271	CID-25178949	CID-25217031
Properties	D1	D2	D3	D4
IUPAC Name	4-(1,3-dioxooctahydro-4,6-ethenocyclopropa[f]isoindol-2(1H)-yl)-N-[4-(pyridin-4-ylmethyl)phenyl]cyclohexanecarboxamide	2-[1-[(4-bromophenyl)methyl]-2-oxo-5-[(2S)-2-(pyridin-3-yloxymethyl)pyrrolidin-1-yl]sulfonylindol-3-ylidene]propanedinitrile	5-[(2S)-2-[(3,5-difluorophenoxy)methyl]pyrrolidin-1-yl]sulfonyl-1-[(4-fluorophenyl)methyl]indole-2,3-dione	2-[1-[[4-(2-fluoroethoxy)phenyl]methyl]-2-oxo-5-[(2S)-2-(pyridin-3-yloxymethyl)azetidin-1-yl]sulfonylindol-3-ylidene]propanedinitrile
Molecular Formula	$C_{30}H_{31}N_3O_3$	$C_{28}H_{22}BrN_5O_4S$	$C_{26}H_{21}F_3N_2O_5S$	$C_{29}H_{24}FN_5O_5S$
Molecular Weight (g/mol)	481.596	604.479	530.518	573.599
Number of H donor	1	0	0	0
Number of H acceptor	4	8	9	10
Number of rotatable bond	5	7	7	10
Class	Poorly Soluble	Molecularly Soluble	Poorly soluble	Poorly Soluble
TPSA	79.37	129.09	85.69	138.33
Bioavilibility	0.55	0.55	0.55	0.55
Toxicity	No	No	No	No

CHAPTER-5
DISCUSSION

Compounds screening to drug molecules have been an active area of research for many years. In recent years, computational compound screening has been pursued extensively due to the expensive nature of investigational screening procedures [Bajorath, 2002]. Virtual screening (VS), a computational technique used in drug discovery to search libraries of small molecules in order to identify those structures which are most likely to bind to a drug target, typically a protein receptor or enzyme, adopted in this study to screen potential compounds downloaded from databases[Rollinger *et al.*, 2008; Raster, 2008]. The main objective of this present study was to identify the drug-receptor interactions and it's in silico analysis of potential drug molecules against Apoptosis executioner protein Pro-Caspase 7. The experimentally determined three-dimensional (3D) structure of Pro-Caspase 7 was utilized to identify the potential ligands.

5.01. Binding Affinity and Non-Bonding Interaction

Molecular docking is an important tool in computational drug design which can predict the predominant binding mode(s) of a ligand with the target protein [Morris and Lim-Wilby, 2008]. Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Molecular docking can predict an *optimized orientation* of ligand on its target. It can predict different binding modes of ligand in the groove of target molecule. This can be used to develop more potent, selective and efficient drug candidates [Shoichet *et al.*, 2002; Gschwend *et al.*, 1996].

Non-covalent interactions [Schally, 2007] are critical in maintaining the three-dimensional structure of large molecules, such as proteins and nucleic acids. In addition, they are also involved in many biological processes in which large molecules bind specifically but transiently to one another. These interactions also heavily influence drug design, crystallinity

and design of materials, particularly for self-assembly, and, in general, the synthesis of many organic molecules [Cockroft *et al.*, 2007; Brown *et al.*, 2009; Eisler, 2010]. A number of conventional hydrogen bonds, carbon hydrogen bonds and hydrophobic interactions are observed between the drug molecules and Procaspase protein. In case of hydrophobic interactions, a couple of types of hydrophobic interactions such as alkyl, pi-pi T-shaped, pi-alkyl, and Alkyl-pi are found and presented in Table 2.

In biology, hydrogen bonding is essential for DNA structure. Depending on the nucleotide sequence, a regular helical structure of the DNA helix is dictated by specific hydrogen bonding patterns [Zhurkin *et al.*, 2005]. The structures and reactivity of many molecular systems can be determined by intermolecular hydrogen bonding [Zhao *et al.*, 2007; Zhao and Han, 2008]. Among various factors, hydrogen bonding is the one which can affect selectivity of nucleotide incorporation by a DNA polymerase [Lee *et al.*, 2008]. For drug binding, hydrogen bonds have important function in determining the accuracy of ligand binding [Wade and Goodford, 1989]. Both conventional and nonconventional hydrogen bonds are observed in the drug and protein complex. The C-H...O interaction, known as nonconventional hydrogen bond, slightly weaker than its classical O-H…O hydrogen bonding, is believed to be critical in a large number of biomacromolecules' crystal structures [Kuduva and Craig, 1999; Meadows *et al.*, 2000]. In case of drug molecules 4 (D4), the highest eleven hydrogen bonds are observed in which three hydrogen bonds are nonconventional (C-H… O) and these bonds are formed with Ser235 (2.65 A˚), Thr288 (2.27A˚), and Thr333 (2.4 A˚) [Table 2 and Fig 13]. This nonconventional hydrogen bond plays crucial role in biological systems. The strongest hydrogen bond found in Gln283 (2.16 A˚) of D4-Procaspase complex. In literature, it is suggested hydrogen bond of <2.3 A° can increase binding affinity by several magnitudes [Wade and Goodford, 1989].

Short oxygen–halogen interactions have been known in organic chemistry since the 1950s and recently have been exploited in the design of supramolecular assemblies. A survey of protein and nucleic acid structures revealed similar halogen bonds as potentially stabilizing inter- and intramolecular interactions that can affect ligand binding and molecular folding [Auffinger, 2004]. In this present study, in total, seven halogen bonds found within the interaction of drug molecule 3 and 4 (D3 & D4) where four are oxygen-halogen bond. The amino acids involved in oxygen-halogen interactions are Asn170 (3.13 A°), Gln 283 (3.22 A°), Lys383 (3.57 A°), and Asn311 (3.33 A°) [Fig 12 and Fig 13].

The concept of the halogen bond (or X-bond) has become recognized as contributing significantly to the specificity in recognition of a large class of halogenated compounds [Scholfield *et al.*, 2013]. Halogenated compounds are important inhibitors against proteins, including those that are involved in carcinogenesis. There have been extensive reviews on the role of X-bonds in the recognition of various inhibitors against several classes of protein kinases [Voth and Ho, 2007; Liao, 2007]. The structures of halogenated inhibitors in complex with epidermal growth factor receptor and maltripase showed that X-bonding can be generalized to other antitumor targets [Yun *et al.*, 2007]. Finally, the five-order of magnitude lower Ki of a brominated compared with nonbrominated inhibitor against the tumor suppressor protein aminopeptidases-N (APN) was suggested to be associated with X-bonds rather than general hydrophobic effects, indicating that this concept is becoming invoked even in the absence of specific structural evidence [Maiereanu *et al.*, 2011].Two recent studies showed that the X-bonding concept can be incorporated at the design stage to increase the affinity of ligands as potential anticancer drugs [Wilcken *et al.*, 2012; Carpenter *et al.*, 2010].

Importantly among the four selected drug molecules, two of them are halogenated compounds and form halogen bonds between drug molecules and Pro-caspase protein complex. In this case, drug molecules contained Fluorine and formed oxygen-fluorine, and hydrogen-fluorine bond, which may have positive effect on the protein–ligand stability, as well as in the binding affinity and selectivity. Interestingly, these two drug molecules formed highest non-bonding interactions and Pro-caspase 7 (1GQF) is a cancer causing protein. So, these halogen atom containing drug molecules might play significant role in the activation of Pro-Caspase 7 to Caspase 7 and activate it to start apoptosis process.

5.02 Molecular dynamics (MD) simulations analysis

Usually the ligand stabilizes a subset of several possible conformations of the receptor, shifting the equilibrium toward the minimum energy structures [Durrant & McCammon, 2011]. In such cases, MD simulations can produce alternative conformational states corresponding to these ligand-induced structures. Also, when no suitable crystallographic structures for a particular molecular target are available (*i.e.*, structures with inaccessible or

poorly defined binding sites), MD can be applied to generate a set of docking convenient structures [Harvey & Fabritiis, 2012]. Accordingly, potential conformational states are sampled by MD simulations based on the available crystallographic data, and accessible conformations (*i.e.*, those with accessible and well-defined binding cavities) can be selected for molecular docking [Salsbury, 2010]. MD can additionally be used to estimate the stability of a ligand-receptor complex proposed by molecular docking [Alonso *et al.*, 2006]. When a MD-generated ligand conformation deviates by more than a given RMSD value from the corresponding docking solution, the predicted ligand-receptor complex can be considered unstable [Chen, 2015].

After Molecular dynamics, it was found that the binding affinity of the first (D1) and third (D3) drug molecules significantly increased and the molecular docking energy are -15.8 kcal mol−1 and -14.3 kcal mol−1 respectively. On the other hand, the binding affinity of the second (D2) and fourth (D4) drug molecules slightly decreased and the molecular docking energy is -10.1 kcal mol−1 for both of the molecules. Active site analysis performed to identify the active site cavity amino acid residues of Pro-Caspase 7 and later checked with the Molecular docking interacting residues. It has found that all of the active site residues of Pro-Caspase 7 interacted with the potential drug compounds and formed different bonds like hydrogen bond, halogen bond, hydrophobic interaction, electrostatic interaction.

5.03 Pharmacoinformatics Elucidation

A good drug candidate is absorbed in required time and well distributed throughout the system for its effective metabolism and action. Toxicity is another very important factor which often overshadows the ADME behavior. Failure of drugs at clinical trial stage due to adverse effects generated because of their toxicity proves very expensive and detrimental in the drug development process. *In silico* ADMET tools presents an array of opportunities which help in accelerating the discovery of new targets and ultimately lead to compounds with predicted biological activity. Table 4 depicts the drug-likeness properties of test compounds with least binding energies predicted using OSIRIS Property Explorer.

The drugs show positive response for blood brain barrier (BBB) criteria, predicting that drugs will go through BBB. ADMET properties reveal that D2 and D4 had best Human Intestinal Absorption (HIA) scores (1.0 out of 1.0). Greater HIA denotes that the compound could be

better absorbed from the intestinal tract upon oral administration. However, the selected drug molecules show weak inhibitory property for human ethera-go-go-related gene (hERG). Inhibition of hERG can lead to long QT syndrome [Sanguinetti and Tristani-Firouzi, 2006], so more study on this aspect is necessary.

A lower molecular weight would again enhance the absorption rate and thus most of the drugs are tried to be kept at the lowest possible molecular weight [Sander, 2001]. An increased TPSA is associated with diminished membrane permeability and compounds with higher TPSA were better substrates for p-glycoprotein. Thus comparing the compounds, lower TPSA was favorable for drug-like property. It was also predicted that a molecule with better CNS penetration should have lower TPSA value [Blake, 2000; Chico *et al.*, 2009].

CHAPTER-5
CONCLUSION AND FUTURE RECOMMENDATION

Apoptosis, an energy-dependent process, is a process of programmed cell death in which caspase protein activation plays a key role. As a number of the key apoptotic proteins that are regulated in the activation or inactivation mechanism of apoptotic pathways have been identified, in this study, it is prioritized identifying potential anti-cancer drug molecules against Pro-Caspase 7, one of the effector caspases of Caspase superfamily. Molecular Docking study is investigated for Pro-Caspase 7 with 917 Drug-like compounds of BindingDB database and 654 Drug-like compounds of ZINC database and identified 4 potential lead compounds those showed strong predicted binding affinity for the important residues of Pro-Caspase 7. In addition to Molecular Docking study, Molecular dynamics conducted to test the feasibility of the identified compounds in biological systems and again checked binding interactions for the Pro-Caspase 7 protein. The details analysis of nonbonding interactions identified between the selected potential lead compounds and Pro-Caspase 7 may help to develop the new anticancer drug which can effectively target the Pro-Caspase 7 and ultimately activated the apoptosis pathway.

Our study discloses that after Molecular dynamics there have found the significant difference in binding affinity and binding interactions that play a major role in the drug-receptor interaction. For instance, the binding energies among Pro-Caspase 7 and two potential lead compounds after the MD significantly improved to -15.8 and -14.3 kcal mol^{-1} from −10.8 and −10.7 kcal mol^{-1}, respectively. Pharmacoinformatics analysis predicts that all potential lead compounds are non-carcinogenic and they have no mutagenic and irritating effects. This study results in the identification of potential hit for initiation of apoptosis which ultimately works for cancer treatment.

Remarkable progress, has been made during the past few years, in almost all the areas concerning with drug design and discovery, although it is a complex process, involving the application of many different fields of knowledge. Drug design, discovery and development are thought as an intense, lengthy and interdisciplinary endeavor. Drug design, sometimes referred to as rational drug design or more simply rational design, is the inventive process of

finding new medications based on the knowledge of a biological target. Compounds screening leading to drug design have been an active area of research for many years. Due to the wearisome and expensive nature of investigational screening procedures, Computational compound screening has been pursued extensively here in order to identify novel lead compounds. This *in silico* study helps the future researchers around the world including Bangladesh to select Hits to Lead compounds by conducting Bioassay screening, clinical trials, and other laboratory-based experiments.

CHAPTER-6
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