PART II
MOLECULAR DOCKING AND MOLECULAR DYNAMICS STUDIES
CHAPTER VI
6.1 Introduction

Molecular docking predicts the binding modes and affinities of small molecules within the binding site of particular receptor targets and is currently used as a standard computational tool in drug design. Targets are most often proteins and enzymes that are typically key molecules involved in specific metabolic or cell signalling pathways related to a particular disease state. The small molecule, known as ligand usually fits within a protein’s cavity which is predicted by the search algorithm. These protein cavities become active when they come in contact with any external compounds and are thus called as active sites. Molecular docking estimates the interaction between the ligand and target at the atomic level and characterizes the behaviour of the small molecules in the active site of target proteins.

By identifying the correct conformation, position and orientation of ligands in the binding pocket of a protein and assessing its affinity to the receptor protein, docking procedure predicts the activity of the small molecule. The three dimensional pose of the bound ligand can be visualized using different visualizing tools like Pymol and Rasmol, which help in inferring the best fit of ligand. A binding interaction between a ligand and an enzyme may result in
activation or inhibition of the enzyme. Interactions between protein and ligand are a consequence of forces between the molecules which may be electrostatic in nature as in charge-charge or dipole-dipole interactions or they may be electrodynamic forces like van der Waals interactions. Steric forces are generated when atoms in different molecules come into very close contact with one another and start affecting each other’s reactivity & free energy. Solvent-related forces such as hydrogen bonds, hydrophobic and hydrophilic interactions, generated due to chemical reactions between the solvent and the protein or ligand also play a crucial role.

The three dimensional structures of the protein and ligand are the fundamental prerequisites for a docking program. Biophysical techniques such as X-ray crystallography and NMR spectroscopy can resolve the structures of proteins to a resolution of a few angstroms at which it becomes possible to examine precisely the interactions between the atoms of the protein and atoms of the bound ligand. The structure of the target protein is usually retrieved from databases such as RCSB Protein Data bank. The docking process involves the sampling of conformations of the ligand in the active site of the protein and ranking these conformations using a scoring function.

**Search algorithms** are the methods used to predict the possible conformations of a protein-ligand complex. The principles of docking methods employ search algorithms based on Molecular dynamics, Monte Carlo methods, Genetic algorithms, Fragment-based methods, Point complementary methods, Distance geometry methods and Systematic searches. The search algorithm
generates an optimum number of configurations and examines all possible binding modes in a given complex. The energy of the resulting complexes and of individual interactions is estimated.

**Scoring functions** are mathematical methods used to predict the strength of the non-covalent interaction between the protein and ligand. They differentiate the correct poses from incorrect poses or compounds that bind strongly from inactive compounds in a reasonable computation time. The scoring function indicates the probability that a given pose represents a favourable binding interaction. A pose is a "snapshot" of the protein-ligand complex. Most scoring functions are physics based molecular mechanics force fields that estimate the binding affinity, between two molecules after they have been docked. The three broad categories of scoring functions are knowledge-based, empirical and those based on molecular mechanics force fields.

An empirical scoring function takes into account the physics of the binding process including a lipophilic-lipophilic term, hydrogen bond terms, a rotatable bond penalty and contributions from protein-ligand, coloumb-vdW energies.

**Docking Methodologies** may be categorised into the following three types.

**Rigid Docking** - Docking method wherein the ligand and the receptor are treated as rigid bodies and the internal geometries of both the receptor and ligand are kept fixed during docking.
**Flexible Ligand Docking** - Docking method in which the ligand is treated as flexible while the receptor is kept rigid. Docking determines a suitable position for the ligand in receptor environment while maintaining the receptor’s rigidity.

**Induced fit or Flexible Docking** - Usually, macromolecules are known to experience conformational changes on ligand binding so as to maximize energetically favourable interactions with the ligand or solvent. The receptor may alter its binding site so that it conforms more appropriately to the shape and binding mode of the ligand. The driving force behind most induced fit mechanisms is hydrophobic interactions or hydrophobic collapse of the receptor around the bound ligand. Conformational flexibility does not make domain, tertiary or secondary structure motions imperative but may consist of only side-chain adjustments. In Induced Fit docking both the ligand and side chain of the protein is kept flexible and the energy for different conformations of the ligand fitting into the protein is calculated.

The molecular docking carried out in this thesis studies the *structure - antimicrobial* activity of the 14 small molecules that have been characterized using X-ray Crystallography. Target selection was based on the reported biological functions of each of the five classes of derivatives and on structural similarities of the molecule under study with co-crystallized ligand of the chosen target proteins.
The small molecules were subjected to induced fit protocol and the docking scores, glide energy, hydrogen bond and hydrophobic interactions with suitable targets were observed and reported.

6.2 Materials and Methods

Molecular docking was performed on Centoes 6 Linux workstation using Maestro (2014 -3). The three-dimensional crystal structures of target proteins were downloaded from the Protein Data Bank (PDB) (http://www.rcsb.org). GLIDE-6.0 searches were performed for understanding docking interactions between ligand and target protein. Glide helps in properly assigning the required bond orders and ionization states using an OPLS-AA (Optimized Potential Liquid Simulation for All Atom) force field. No hydrogen atoms were minimized until the average root mean square deviation reached default value of 0.3 Å. Energy minimization of the ligands to be docked had to be carried out in order to free the three dimensional structure of the molecules from steric clashes. Two types of algorithms were used to obtain the perfect three dimensional structures. The Steepest descent method was followed for initiating a minimization on a starting geometry that contains large steric clashes. However as the convergence in the steepest descent method is very poor towards the end of minimization the Conjugate gradient method, an effective optimization method was further carried out to improve the convergence of the algorithm. Ligprep 2.3 module (Schrodinger) was employed for ligand preparation. Induced fit docking (IFD) was performed to predict ligand binding modes and structural movements in the Ligand Binding Domain (LBD) of protein using Glide and Prime modules.
The prepared proteins were loaded in the workstation and the grid values were calculated to about 20Å in order to cover all the active site amino acids. About 20 conformational images were created and analyzed for the best conformational pose based on the docking score and glide energy. The PyMol Molecular Graphics software was used to analyze the hydrogen bond interactions and preparation of high resolution images. Ligplots were generated to examine the hydrogen bond and hydrophobic interactions.

6.3 Molecular Docking Studies of Isoxazole derivatives with Bromodomain and Extra-C Terminal Domain Protein (BET-BRD4)

6.3.1 Introduction

The Bromodomain and Extra-C Terminal Domain (BET) family of proteins play a vital role in controlling expression of genes crucial for proper cell growth. Inhibition of their activity has been reported to have profound effects on cell proliferation and hence they are crucial therapeutic targets for cancer. The BET family of proteins are the first characteristic chromatin readers against which small-molecule inhibitors have been developed (Bolden et al., 2014). Members of the BET family, including Bromodomain2 (BRD2), Bromodomain3 (BRD3), Bromodomain4 (BRD4) and Bromodomain testis associated (BRDT), regulate gene expression by engaging transcriptional regulators to specific genomic locations. BRD4 remains bound to transcriptional start sites of genes expressed during the M/G1 transition, influencing mitotic progression (Dey et al., 2009). It is also a critical mediator of transcriptional elongation. BRD4 has
been reported to regulate diverse biological processes, including the cell cycle (Dey et al., 2009), inflammation (Huang et al., 2009, Nicodeme et al., 2010), maintenance of higher-order chromatin structure (Wang et al., 2012), DNA damage signalling (Floyd et al., 2013) and male contraceptive properties (Matzuk et al., 2012).

BRD4 possess two bromodomains implicated in the interaction with chromatin. It recognizes and binds acetyl-lysine residues of histone and non-histone proteins (Wu & Chiang, 2007). Studies have suggested that an ideal bromodomain inhibitor would bind in the acetyl-lysine recognition site, engaging ASN140 in direct hydrogen-bonding interactions and ILE146 in hydrophobic interactions (Fillippakopoulos et al., 2012).

The known potent inhibitors of BRD4 interact predominantly with ASN140 through triazole or isoxazole moieties. JQ1 is a novel thieno-triazolo-1,4-diazepine BET inhibitor which binds competitively to acetyl-lysine recognition motifs of bromodomains (Fillippakopoulos et al., 2010). It possesses significant anti-proliferative effect in leukemia by inhibiting growth and inducing apoptosis of human Acute Myeloid Leukemia (AML) cells and T cells (Fu et al., 2015). On comparing the binding modes of certain amino isoxazole derivatives with those of JQ1, it has been reported that both formed similar hydrogen bonding contacts with the asparagine residue, ASN140. This interaction is critical for recognition of the acetyl lysine substrate suggesting that an isoxazole could serve as an alternative for the triazole ring present in JQ1 (Gehling et al., 2013). The greater potency of the isoxazole derived benzoazepine-type
compounds when compared to the corresponding triazole analogs, suggested that the isoxazoles were especially well-suited to engage in the key interaction with ASN140 (Albrecht et al., 2016). Hence the target human BRD4 in complex with benzoisoxazoloazepine 3 (PBB ID:5HM0) was chosen for docking studies with the isoxazole derivatives.

6.3.2 Results and Discussions

The docking scores, glide energy and hydrogen bond interactions of the isoxazole derivatives and the co-crystallized ligand (CCL) are given in Table 6.1. The Pymol diagrams showing hydrogen bond interactions of ISZ1, ISZ2 and CCL (62V) at the active site of BRD4 (5HM0) protein are depicted in Figs. 6.3.1, 6.3.3 and 6.3.5, respectively. Ligplot diagrams showing hydrogen bond and hydrophobic interactions of ISZ1, ISZ2 and CCL (62V) at the active site of BRD4 (5HM0) protein are depicted in Figs. 6.3.2, 6.3.4 and 6.3.6, respectively.

The structurally conserved features of Brd4 include three domains – two N-terminal bromodomains (BD1 and BD2) and a central extra terminal (ET) domain. The BD1 domain is within residue 44-168 and is the site that interacts with the histone complex of DNA. The general structure of the BRD4 - BD1 domain include a left-handed four-helix bundle (αA, αB, αC, αZ) (Sanchez & Zhou, 2009), as well as two varying loop regions (ZA and BC) that determine substrate specificity (Fillippakopoulos et al., 2010). The four helices form a left-handed twist that makes up the hydrophobic core of the domain, and the two loops form a cleft that acts as a recognition site for the acetylated lysines in histone. The conserved features of the acetylated lysine (KAc) binding site are
necessary for binding of acetylated lysine and they are required for ensuring shape complementarity and optimal positioning of inhibitors. KAc recognition is facilitated through a direct hydrogen bonding interaction between the oxygen of the acetylated lysine of LYS14 of histone H3 and the amino group of the conserved ASN140 in BRD4-BD1, located in the BC loop (Ember et al., 2014). Additional contacts with the acetylated lysine are mediated by TYR139 and ILE146 (Vollmuth et al., 2009). Binding of BRD4 to acetylated lysine facilitates gene expression.

The 5HM0 protein structure contains a single chain of 125 amino acid residues. The two isoxazole ligands were seen to dock well in the KAc site of the target. The oxygen atom of the isoxazole moiety in ISZ1 interacts with the amino group of ASN140 mimicking the crucial interaction between LYS14 of histone H3 and the amino group of the conserved ASN140. The ligplots show that both ISZ1 and ISZ2 are involved in hydrophobic interactions with ILE146. ISZ1 engages in the two interactions necessary for an ideal bromodomain inhibitor. The hydrogen bonding distance between the isoxazole and ASN140 in ISZ1 was close to that observed in the literature structures (range 2.9–3.2 Å) (Gehling et al., 2013). In ISZ2 the oxygen of the chromene system interacts with the TYR97 residue.

Re-docking of cocrystallized ligand (62V) to the target protein was carried out. A compound is considered to be more stable than that of the co-crystal when it forms good hydrogen bond interactions at the active site and has the lower glide score and glide energy than that of the co-crystal. In the current
study, on comparing these parameters for the complexes after docking, it can be concluded that the two isoxazole ligands can be considered as potential inhibitors. Some of the key residues involved in hydrophobic interactions with the three ligands are TYR139, MET132, VAL87, ILE146, PHE83, PRO82 and LEU94 as seen from the ligplot.

Table 6.1: Hydrogen bond interactions of ISZ1, ISZ2 and CCL at the active site of 5HM0

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Glide Energy (kcal/mol)</th>
<th>Docking Score</th>
<th>Interactions at the active site</th>
<th>Donor Acceptor Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISZ1</td>
<td>−44.423</td>
<td>−8.737</td>
<td>ASN140 (N―H···O)</td>
<td>2.81</td>
</tr>
<tr>
<td>ISZ2</td>
<td>−42.742</td>
<td>−9.678</td>
<td>TYR 97 (O―H···O)</td>
<td>3.20</td>
</tr>
<tr>
<td>CCL (62V)</td>
<td>−42.196</td>
<td>−8.299</td>
<td>ASN140 (N―H···O)  TYR97 (O―H···N)</td>
<td>2.91  3.23</td>
</tr>
</tbody>
</table>

6.4 Molecular Docking Studies of Acrylate derivatives with Human FactorXa (FXa)

6.4.1 Introduction

Thromboembolic disease is caused by the improper functioning of the blood coagulation process. Studies have shown that selective inhibitors of the serine proteases in the coagulation cascade have been key targets for anti-thrombotic drug development (Lassen & Laux, 2008). As FactorXa occupies a key position in the coagulation cascade and has limited roles outside of
Fig 6.3.1. Interaction diagram of ISZ1 in the active site of 5HM0 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.3.2. LIGPLOT showing the hydrogen bonds and non-bonded contacts between ISZ1 and 5HM0.
Fig 6.3.3. Interaction diagram of ISZ2 in the active site of 5HM0 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.3.4. LIGPLOT showing the hydrogen bonds and non-bonded contacts between ISZ2 and 5HM0.
Fig 6.3.5. Interaction diagram of CCL in the active site of 5HM0 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.3.6. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CCL and 5HM0.
coagulation, this key physiological activator of prothrombin has become a central target for novel anticoagulants (Lefkovits et al., 1996; Ko et al., 1996). By combining with Factor Va and calcium ions on membrane surfaces, FXa forms the prothrombinase complex, which then converts prothrombin to thrombin. Thrombin in turn converts fibrinogen into fibrin leading to clot formation (Kumar et al., 1995; Senden et al., 1998). One molecule of FXa catalyzes the formation of approximately 1000 thrombin molecules. Hence it is only rational that instead of targeting thrombin which is lower down in the coagulation cascade, FXa could be a more effective strategy for anticoagulation (Herault et al., 1997). The inhibition of FXa compared to thrombin may allow the effective control of thrombogenesis with minimal effect upon bleeding. This may diminish thrombin-mediated activation of both coagulation and platelets without affecting the activity of existing thrombin level necessary for primary haemostasis, a characteristic that is desirable for antithrombotic agents (Leadley, 2001; Babine et al., 1997).

The docking of the two acrylate derivative ligands in the structure of human FXa in complex with N-{N'-(2-Methyl-1-Benzofuran-5-Yl)-N-(3s)-2-Oxo-1-(2-Oxo-2-Pyrrolidin-1-Ylethyl)azepan-3-Yl] carbamimidoyl} pyridine-3-Carboxamide (PDB ID: 3K9X) is reported herein.

6.4.2 Results and Discussions

The docking scores, glide energy and hydrogen bond interactions of the acrylate derivatives and the co-crystallized ligand (CCL) are given in Table 6.2. The Pymol diagrams showing hydrogen bond interactions of ACR1, ACR2 and
CCL (MBM1) at the active site of FXa (3K9X) protein are depicted in Figs. 6.4.1, 6.4.3 and 6.4.5 respectively. Ligplot diagrams showing hydrogen bond and hydrophobic interactions of ACR1, ACR2 and CCL (MBM1) at the active site of FXa (3K9X) protein are depicted in Figs. 6.4.2, 6.4.4 and 6.4.6, respectively.

Molecular docking studies demonstrated that the title compounds bind well in the active site of FXa and could be potential inhibitors. The active site of Factor Xa is divided into four sub pockets S1, S2, S3 and S4. The anionic S1 subpocket lined by residues ASP189, SER195 and TYR228 determines the major component of selectivity and binding. The S4 pocket is lined by the residues TYR99, PHE174 and TRP215 (Nazare et al., 2012). FactorXa inhibitors generally bind in an L-shaped conformation, where one group of the ligand occupies the anionic S1 pocket and another group of the ligand occupies the aromatic S4 pocket (Rai et al., 2001).

The binding of the ligands ACR1 and ACR2 to FXa is seen to involve interaction with residues SER195, GLN192, TYR99, GLY193. The docking studies indicate that ACR1 binds with residues in the S1 and S4 pockets signifying potential for good inhibitory activity. The benzene ring of ACR1 occupies the hydrophobic pocket S4 bounded by the residues TYR99, TRP215 and PHE174. Interaction with GLY193 is exhibited by ACR1 and the co-crystallized ligand.

One of the most widely used methods for validating a docking protocol is the re-docking of co-crystallized ligand to the target protein. The co-crystallized
ligand was extracted and redocked into the active site of the receptor. The docking scores, glide energy and interactions of the acrylate ligands are comparable with that of the co-crystallized ligand. The key residues involved in hydrophobic interactions are GLY218, CYS220, TRP215 as seen from the ligplot. The results indicate that ACR1 shows good FXa inhibitory activity and can be considered as potential anticoagulant agent.

Table 6.2: Hydrogen bond interactions of ACR1, ACR2 and CCL at the active site of 3K9X

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Glide Energy (kcal/mol)</th>
<th>Docking Score</th>
<th>Interactions at the active site</th>
<th>Donor-Acceptor Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR1</td>
<td>−56.003</td>
<td>−8.570</td>
<td>SER195 (O—H⋯O)</td>
<td>2.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GLN192 (N—H⋯O)</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GLN61 (N—H⋯O)</td>
<td>2.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TYR99 (N—H⋯O)</td>
<td>2.89</td>
</tr>
<tr>
<td>ACR2</td>
<td>−47.161</td>
<td>−5.550</td>
<td>GLY193 (N—H⋯O)</td>
<td>2.99</td>
</tr>
<tr>
<td>CCL (MBM 1)</td>
<td>−67.245</td>
<td>−9.098</td>
<td>GLY193 (N—H⋯O)</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SER195 (O—H⋯O)</td>
<td>2.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SER195 (N—H⋯O)</td>
<td>2.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TYR99 (O—H⋯O)</td>
<td>3.15</td>
</tr>
</tbody>
</table>
Fig 6.4.1. Interaction diagram of ACR1 in the active site of 3K9X showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.4.2. LIGPLOT showing the hydrogen bonds and non-bonded contacts between ACR1 and 3K9X.
Fig 6.4.3. Interaction diagram of ACR2 in the active site of 3K9X showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.4.4. LIGPLOT showing the hydrogen bonds and non-bonded contacts between ACR2 and 3K9X.
Fig 6.4.5. Interaction diagram of CCL in the active site of 3K9X showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.4.6. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CCL and 3K9X.
6.5 Molecular Docking Studies of Indole derivatives with Cyclin Dependant Kinase 2 (CDK2)

6.5.1. Introduction

A series of complexes of cyclin and cyclin-dependent kinase (CDK) are key regulators of cell division cycle in higher eukaryotes. The activity of the catalytic kinase subunit is dependent on the regulatory cyclin subunit. The binding of cyclin to CDK, induces a large conformational change of the T-loop of CDK. This exposes the catalytic ATP-binding sites of CDK to which potential substrates will interact (Jeffrey et al., 1995). The cyclin-dependent kinase 2 (CDK2) is a serine or a threonine protein kinase activated by cyclin E in late G1 phase of cell division and plays a critical role in the G1-S phase transitions in cell cycle (Aleem et al., 2004). Hence CDK2 has become a popular drug target for potential anticancer therapies (Fischer, 2004). CDK2 has also been a much studied drug target for proliferative diseases such as psoriasis, restenosis and for the prevention of chemotherapy-associated side effects (Meijer et al., 1999). Most of the CDK2 inhibitors in clinical development are found to target exclusively the catalytic ATP-binding pocket of CDK2.

Indoles are important nitrogen containing heterocyclic molecules, found extensively in biological systems which have been reported to possess anticancer activity. Modification in their structure has offered a high degree of diversity that has proven useful for the development of new therapeutic agents having improved potency and lesser toxicity. The structure of CDK2 in complex with 4-\{((2-oxo-1,2-dihyro-3h-indol-3-ylidene)methyl]amino\}-n(1,3-thiazol-2-yl)
benzenesulfonamide (PDB ID:1KE8) was used for docking with the indole derivatives.

6.5.2. Results and Discussions

The docking scores, glide energy and hydrogen bond interactions of the indole derivatives and the co-crystallized ligand (CCL) are given in Table 6.3. The Pymol diagrams showing hydrogen bond interactions of IND1, IND2, IND3 and CCL (LS4) at the active site of CDK2 (1KE8) are depicted in Figs. 6.5.1, 6.5.3, 6.5.5 and 6.5.7, respectively. Ligplot diagrams showing hydrogen bond and hydrophobic interactions of IND1, IND2, IND3 and CCL (LS4) at the active site of CDK2 (1KE8) are depicted in Figs. 6.5.2, 6.5.4, 6.5.6 and 6.5.8, respectively.

The protein CDK2 consists of a single chain of 282 amino acid residues. The deep ATP binding catalytic cleft of CDK2 where protein substrate binding and catalysis occurs is found to lie between an amino terminal and a carboxy terminus lobe (Pavletich, N., 1999). The two main regions in the CDK2 subunit that are critical to its function are the PSTAIRE region and T-loop region. The triad of residues LYS33, GLU51 and ASP145 are essential to all eukaryotic kinases and are critical to ATP phosphate orientation (Brown & Nobel, 1995). CDK2 is inactive in the absence of cyclin and activation requires extensive structural changes in its active site. In the inactive form of CDK2 several important amino-acid side chains in the active site are incorrectly positioned, so that the phosphates of ATP are not ideally oriented for the kinase reaction. Upon cyclin binding changes involving extensive hydrogen bonding, reorient the
PSTAIRE region with a rotation of about 90° about its helical axis. Due to this reorientation and movement, the side chain of GLU51 relocates into the ATP binding catalytic cleft. The residue ASP145 in the T-loop now forms ligands to the Mg atoms present in the active site. This opens the substrate binding site and the CDK2/cyclin complex can phosphorylate the serine and threonine residues of proteins bound to them thus activating the bound proteins.

The study reveals that the binding of the three ligands IND1, IND2 and IND3 to CDK 2 involve interactions with residue ASP145 which is a critical residue in ATP phosphate orientation, responsible for holding the phosphates in place. Hence, it can be concluded that these ligands could be potential inhibitors of CDK2 activity.

Among the 20 hydrogen bonding sites used by small molecules in X-ray structures of CDK2 one of the most frequently used is the main chain CO of GLU81 located deep in the ATP binding site. From the docking studies it is seen that the ligand IND1 and the co-crystallized ligand exhibit similar hydrogen bond interaction with GLU81. The key residues involved in hydrophobic interactions with the discussed ligands are ILE10, ALA31, VAL18, PHE80, GLN85 and LEU134 as seen from the ligplot.
Table 6.3: Hydrogen bond interactions of IND1, IND2, IND3 and CCL at the active site of 1KE8

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Glide Energy (kcal/mol)</th>
<th>Docking Score</th>
<th>Interactions at the active site</th>
<th>Donor-Acceptor Distance(Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND1</td>
<td>-48.889</td>
<td>-8.274</td>
<td>(O—H⋯O) GLU81 (N—H⋯O) ASP145</td>
<td>3.27 2.89</td>
</tr>
<tr>
<td>IND2</td>
<td>-52.233</td>
<td>-8.332</td>
<td>(N—H⋯O) ASP145</td>
<td>2.10</td>
</tr>
<tr>
<td>IND3</td>
<td>-56.113</td>
<td>-7.306</td>
<td>(N—H⋯O) ASP145 (N—H⋯O) ILE10</td>
<td>2.91 3.27</td>
</tr>
<tr>
<td>CCL (LS4)</td>
<td>-60.969</td>
<td>-10.734</td>
<td>ASP86 (N—H⋯O) LEU83 (N—H⋯O) LEU 83 (N—H⋯O) GLU81 (N—H⋯O) HIS84 (N—H⋯O)</td>
<td>3.09 2.84 3.28 3.05 2.91</td>
</tr>
</tbody>
</table>

6.6. Molecular Docking Studies of Hydrazine Carbothioamide derivatives with Human Carbonic Anhydrase Isozyme II (CAII)

6.6.1. Introduction

Carbonic anhydrases (CA) are zinc metalloenzymes which catalyze the reversible hydration of carbon dioxide:

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}^-_3 + \text{H}^+$$

These reactions are important to a variety of biological processes, including regulation of respiration and gas exchange (Henry et.al., 2000), regulation of acid-base equilibria (Shah et al., 2005), development and function of bone, (Gay et al., 2000), metabolism (Chegwidden et al., 2000), signalling and
Fig 6.5.1. Interaction diagram of IND1 in the active site of 1KE8 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.5.2. LIGPLOT showing the hydrogen bonds and non-bonded contacts between IND1 and 1KE8.
Fig 6.5.3. Interaction diagram of IND2 in the active site of 1KE8 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.5.4. LIGPLOT showing the hydrogen bonds and non-bonded contacts between IND2 and 1KE8.
Fig 6.5.5. Interaction diagram of IND3 in the active site of 1KE8 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.5.6. LIGPLOT showing the hydrogen bonds and non-bonded contacts between IND3 and 1KE8.
Fig 6.5.7. Interaction diagram of CCL in the active site of 1KE8 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.5.8. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CCL and 1KE8.
memory (Sun et al., 2002), intestinal transport of ions (Swenson, 1991), muscle function (Geers et al., 2000), the nervous system (Parkkila et al., 1997), etc.

The carbonic anhydrases are widespread in nature and found in animals, plants and certain bacteria. In humans carbonic anhydrase has at least fourteen isoenzymes with different physiological functions (Scozzafava et al., 2000). These isozymes are involved in respiration and transport of CO\textsubscript{2} or bicarbonate between metabolizing tissues and the lungs, pH homeostasis, electrolyte secretion in a variety of tissues, and biosynthetic reactions such as lipogenesis, gluconeogenesis, ureagenesis and photosynthesis.

Aromatic heterocyclic sulphonamides are a class of CA inhibitors that have been well studied for the development of antiglaucoma, antitumour, antiobesity and anticonvulsant drugs (Pastorekova et al., 2006). Although a lot of progress has been achieved in the development of isozyme-specific inhibitors, development of new potent CA inhibitors with improved activity, selectivity, solubility, reduced toxicity and side effect is much needed (Clare & Supuran, 2006). Induced fit docking was performed of the four hydrazine carbothioamide ligands into the active site of CA and the following results were obtained. For docking the crystal structure of human carbonic anhydrase isoyme II in complex with 4-[(6-chloro-5-nitropyrimidin-4-yl)amino]benzenesulphonamide (PDB ID:3M40) was downloaded from PDB.
6.6.2. Results and Discussions

The docking scores, glide energy and hydrogen bond interactions of the hydrazine carbothioamide (CTA) derivatives and the co-crystallized ligand (CCL) are given in Table 6.4. The Pymol diagrams showing hydrogen bond interactions of CTA1, CTA2, CTA3, CTA4 and CCL (J45) at the active site of CAII (3M40) protein are depicted in Figs. 6.6.1, 6.6.3, 6.6.5, 6.6.7 and 6.6.9, respectively. Ligplot diagrams showing hydrogen bond and hydrophobic interactions of CTA1, CTA2, CTA3, CTA4 and CCL (J45) at the active site of CA II (3M40) protein are depicted in Figs. 6.6.2, 6.6.4, 6.6.6 and 6.6.8 and 6.6.10, respectively.

The CAII enzyme is a functional 29-kDa monomer with 258 amino acid residues consisting of a 10-stranded, twisted β sheet. The active site is situated at the bottom of a 15 Å cone-shaped cavity that leads to the center of the protein (Liljas et al., 1972). A zinc ion coordinated tetrahedrally by 3 histidine residues (HIS94, HIS96 and HIS119) and a water molecule or hydroxide ion as a fourth ligand (WAT263) is the main feature of the active site (Sjoblom et al., 2009). Zinc plays the crucial role of facilitating the water to create a proton H+ and a nucleophilic hydroxide ion, in carbonic anhydrase. The nucleophilic water molecules attack the carbonyl group of carbon dioxide to convert it into bicarbonate. This is achieved through the +2 charge that the zinc ion has, which attracts the oxygen of water, deprotonates water, thus converting it into a better nucleophile so that the newly converted hydroxyl ion can attack the carbon dioxide.
The binding cavity of CA has hydrophobic as well as a hydrophilic faces. The residues of the hydrophobic face known as the hydrophobic pocket, consists of VAL121, VAL143, LEU198 and TRP209 (Christianson & Fierke, 1996) and is believed to bind CO$_2$ adjacent to the Zn(II)-bound hydroxide (Liang & Lipscomb 1990; Christianson et al.,1996). The hydrophilic face of the binding cavity consists of eight residues of which THR199 and THR200 are nearest to the entrance to the cavity, while HIS64 is located on the opposite side of this entrance. The other five residues namely TYR7, ASN62, ASN67, GLN92 and GLU106 are involved in an intricate network of hydrogen bonds with nine ordered water molecules in the active site (Eriksson et al., 1988; Liljas et al., 1972).

The docking of all the four CTA derivatives shows co-ordination of oxygen of the ligand with Zn(II) and this could be a compelling reason for these compounds to be considered as potential inhibitors. In addition the oxygen atoms of the ligands interact with the amide group in the imidazole ring of HIS119 and HIS94. The oxygen atoms in the chromene ring of CTA1, CTA2 and CTA3 interact with the imidazole ring amide group of HIS119. In CTA4 the two oxygen atoms of the substituted benzene ring are seen to interact with His 94. In all the four cases, the oxygen atoms involved in coordination with Zn (II) are also involved in a hydrogen bond interaction with the amide group of the crucial histidines. These results validate the prospect that the catalytic activity of CA involving the Zn(II) could be inhibited by these HCTA derivatives. The four ligands further show hydrophobic interactions with VAL121, VAL143, LEU198 and TRP209, the key residues of the hydrophobic face of the binding cavity.
For validating the docking protocol the re-docking of co-crystallized ligand to the target protein is carried out. In this study, the co-crystallized ligand was extracted from the receptor proteins and re-docked into the active site of the receptor. The glide energy and the docking scores of the CTA ligands indicate that they are bound stably in the active site. The CTA ligands are found to exhibit greater hydrophobic interactions with the protein than the co-crystallized ligand.

Table 6.4: Hydrogen bond interactions of CTA1, CTA2, CTA3, CTA4 and CCL at the active site of 3M40

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Glide Energy (kcal/mol)</th>
<th>Docking Score</th>
<th>Interaction at the active site</th>
<th>Donor-Acceptor Distance(Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTA1</td>
<td>-53.079</td>
<td>-5.533</td>
<td>HIS119 (N—H⋯O)</td>
<td>3.21</td>
</tr>
<tr>
<td>CTA2</td>
<td>-48.804</td>
<td>-6.615</td>
<td>HIS119 (N—H⋯O) THR200</td>
<td>3.26 3.26</td>
</tr>
<tr>
<td>CTA3</td>
<td>-53.137</td>
<td>-5.576</td>
<td>HIS119 (N—H⋯O)</td>
<td>3.18</td>
</tr>
<tr>
<td>CTA4</td>
<td>-49.737</td>
<td>-6.900</td>
<td>HIS94 (N—H⋯O) PRO201</td>
<td>3.04</td>
</tr>
<tr>
<td>CCL (J45)</td>
<td>-47.995</td>
<td>-5.915</td>
<td>HIS119 (N—H⋯O) HIS94 (N—H⋯O)</td>
<td>3.29 2.77</td>
</tr>
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</table>
Fig 6.6.1. Interaction diagram of CTA1 in the active site of 3M40 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.6.2. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CTA1 and 3M40.
Fig 6.6.3. Interaction diagram of CTA2 in the active site of 3M40 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.6.4. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CTA2 and 3M40.
Fig 6.6.5. Interaction diagram of CTA3 in the active site of 3M40 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.6.6. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CTA3 and 3M40.
Fig 6.6.7. Interaction diagram of CTA4 in the active site of 3M40 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.6.8. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CTA4 and 3M40.
Fig 6.6.9. Interaction diagram of CCL in the active site of 3M40 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.6.10. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CCL and 3M40
6.7. Molecular Docking Studies of Carbazole derivatives with Topoisomerase IIβ

6.7.1. Introduction

Topoisomerases are enzymes that regulate conformational changes in DNA topology such as DNA supercoiling and separating of interlocked chromosomes. Topoisomerase II (Topo II) is an ATP-dependent enzyme which plays an important role in DNA replication, transcription, chromosome condensation & de-condensation, DNA recombination and untangling of replicate chromosome. Topoisomerase II makes double-strand breaks and passes double-stranded DNA through the nick to permit relaxation of over-coiled DNA (Kellner et al., 2002). It uses ATP to pass an intact helix through a transient double-stranded break in DNA to modulate DNA topology (Watt & Hickson, 1994). After strand passage, the DNA backbone is religated and the DNA structure is restored. It is an important target for a number of widely used anticancer agents currently in use such as etoposide, doxorubicin, daunorubicin, etc. However, emerging tumor resistance and several side effects, such as hematological toxicity, nausea and vomiting and hair loss are associated with these anticancer agents (Karki et al., 2012; Trojan et al., 2005). Therefore, efforts have been made by many research groups to find new chemicals with improved bioactivity.

Studies have shown that antitumour activity of N-substituted carbazoles inhibited topoisomerase II activity at a concentration of 2.5 µm which is ten times lower concentration than the concentration required by standard drug etoposide (Nakamura et al., 2002). Carbazole derivatives have been reported to
bind with DNA through intercalation and thus interfere with topoisomerase II activity.

Humans express two, type II topoisomerase isoforms, termed topoisomerase IIα and topoisomerase IIβ (Pflugfelder et al., 1998). To analyse the molecular details of the mode of interaction with Topo IIβ, the three carbazole derivatives compounds were docked into the enzyme’s DNA binding sites. For docking the structure of Topo II β in complex with DNA & etoposide was downloaded from the PDB (PDB ID:3QX3).

6.7.2. Results and Discussions

The docking scores, glide energy and hydrogen bond interactions of the carbazole derivatives and the co-crystallized ligand (CCL) are given in Table 6.5. The Pymol diagrams showing hydrogen bond interactions of CAR1, CAR2, CAR3 and CCL (EVP) at the active site of TopoIIβ (3QX3) protein are depicted in Figs. 6.7.1, 6.7.3, 6.7.5 and 6.6.7, respectively. Ligplot diagrams showing hydrogen bond and hydrophobic interactions of CAR1, CAR2, CAR3 and CCL (EVP) at the active site of TopoIIβ (3QX3) protein are depicted in Figs. 6.7.2, 6.7.4, 6.7.6 and 6.7.8, respectively.

The PDB structure of TopoIIβ consists of two chains (A & B) of 671 amino acid residues complexed with 4 strands of nucleic acids (C, D, E & F) and 6 magnesium ions. Each chain of protein has a co-crystallised etoposide molecule. From the results obtained from docking of ligands, it is seen that CAR2 and CAR3 show interactions with guanine base (DG10) of the nucleic
acid fragment D. The oxygen attached to the carbazole moiety in compound CAR2 interacts via bifurcated hydrogen bonds with the guanine base (DG10D) of the DNA fragment D and the amino acid residue LYS456. The docking scores and glide energy of all the carbazole ligands are comparable with that of the co-crystallised ligand. Induced fit docking procedure was validated by removing drug compound from the active site and re-docking it with target. Analysis of the docked structure of the anticancer drug etoposide to fragment of human topoisomerase complexed to DNA revealed that the inhibitor forms hydrogen bonds with ASP479 residue and also with a guanine fragment (DG10D) of DNA. The key residues involved in hydrophobic interactions with the ligands GLN778, ARG503, DA12F, DC8C, DT 9D, DG 13F as seen from the ligplots.

Table 6.5: Hydrogen bond interactions of CAR1, CAR2, CAR3 and CCL at the active site of 3QX3

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Glide Energy (kcal/mol)</th>
<th>Docking Score</th>
<th>Interaction at the active site</th>
<th>Donor-Acceptor Distance(Å)</th>
</tr>
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<tbody>
<tr>
<td>CAR1</td>
<td>-58.796</td>
<td>-5.939</td>
<td>DG13F (N—H···O)</td>
<td>2.99</td>
</tr>
<tr>
<td>CAR2</td>
<td>-53.354</td>
<td>-6.343</td>
<td>LYS456 (N—H···O)</td>
<td>2.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DG 10D (O—H···O)</td>
<td>2.92</td>
</tr>
<tr>
<td>CAR3</td>
<td>-63.557</td>
<td>-9.288</td>
<td>DG10D (O—H···O)</td>
<td>3.03</td>
</tr>
<tr>
<td>CCL (EVP)</td>
<td>-81.779</td>
<td>-10.160</td>
<td>ASP479 (N—H···O)</td>
<td>3.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ASP479 (O—H···O)</td>
<td>2.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DG10D (O—H···O)</td>
<td>2.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DG10D (O—H···O)</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(O—H···S) MET782</td>
<td>3.26</td>
</tr>
</tbody>
</table>
Fig 6.7.1. Interaction diagram of CAR1 in the active site of 3QX3 showing key hydrogen bonds between inhibitor and enzyme

Fig 6.7.2. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CAR1 and 3QX3
Fig 6.7.3. Interaction diagram of CAR2 in the active site of 3QX3 showing key hydrogen bonds between inhibitor and enzyme

Fig 6.7.4. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CAR2 and 3QX3
Fig 6.7.5. Interaction diagram of CAR3 in the active site of 3QX3 showing key hydrogen bonds between inhibitor and enzyme.

Fig 6.7.6. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CAR3 and 3QX3.
Fig 6.7.7. Interaction diagram of CCL in the active site of 3QX3 showing key hydrogen bonds between inhibitor and enzyme

Fig 6.7.8. LIGPLOT showing the hydrogen bonds and non-bonded contacts between CCL and 3QX3