CHAPTER-1

Insilico Screening, Validation and Elucidation of Mechanism of Action of Novel Aglycones against Angiogenesis Targets using AutoDock Tools 4.0
1.1 Introduction

Angiogenesis is a process of generation of new blood vessels from pre-existing ones. In a healthy person this process is under stringent control and only occurs during embryonic development, endometrial regulation, reproductive cycle and wound repair. However, in many pathological conditions such as solid tumor progression, metastases, diabetic retinopathy, hemangiomas, arthritis, psoriasis and atherosclerosis, it appeared to be driven by persistent up-regulated angiogenesis (Rodriguez-Nieto et al., 2002; Folkman, 1995). Tumor cells adopt angiogenesis to serve their metabolic needs thereby contributing in the subsequent development of cancer. Tumor microenvironment factors trigger the simultaneous production cum release of angiogenic stimuli by tumor cells which in turn increase the permeability of existing vessels and activate endothelial cells thereby triggering them to migrate, proliferate and finally differentiate to give rise to capillary tubules.

Vascular endothelial growth factor (VEGF) is one of the key angiogenic factors adopted by tumor cells to promote angiogenesis (Ferrara and Alitalo, 1999). It is an important signaling protein involved in both vasculogenesis and angiogenesis. Similarly, basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) are also essential pre-requisites for the execution of the process of angiogenesis (Rodriguez-Nieto et al., 2002; Kanda et al., 1999; Mustonen and Alitalo, 1995). There have been well studied drugs/compounds which efficiently inhibited the angiogenesis process using VEGFR, bFGF and/or EGF receptors (Hartmann et al., 2009; Kim et al., 2004). Several anti-angiogenic drugs (e.g., axitinib, gefitinib, motesanib, and lapatinib) had been developed for the treatment of various solid tumors, however, the inherent side-effects led to their withdrawal from the clinical trials (Pfizer, 2009a; Pfizer, 2009b; Havrilla, 2008; Choueiri, 2008; Biospectrum Asia, 2008; Golsteyn, 2005). This has further prompted researchers to explore the novel drug leads from various natural sources which have comparatively less or no toxic side-effects.

Several natural compounds such as aeroplysinin-1, curcumin and halofuginone have been studied in the wet lab for their anti-angiogenic potential (Rodriguez-Nieto et al., 2002;
Elkin et al., 2000; Thaloor et al., 1998; Kreuter et al., 1990). Aeroplysinin-1 displayed a very strong cytotoxic effect on EGF – dependent tumor cell lines, due to inhibition of the ligand-dependent tyrosine kinase activity (Kreuter et al., 1990). The capillary tube formation in HUVE cells was also shown to be inhibited remarkably by halofuginone while aeroplysinin-1 completely inhibited its formation. Moreover, curcumin was shown to be less effective compared to halofuginone and aeroplysinin-1 (Rodriguez-Nieto et al., 2002; Elkin et al., 2000; Thaloor et al., 1998; Kreuter et al., 1990). Further, in the chemoinvasion assay, halofuginone and aeroplysinin-1 completely inhibited the ability of BAE cells to invade through the Matrigel-coated filters (Rodriguez-Nieto et al., 2002; Elkin et al., 2000). Curcumin once again was shown to be ineffective (Thaloor et al., 1998). Aeroplysinin-1 and halofuginone inhibited the vascular endothelial cell differentiation while curcumin again showed less significant effects (Rodriguez-Nieto et al., 2002; Elkin et al., 2000; Thaloor et al., 1998; Kreuter et al., 1990).

The development of an unknown compound to an effective anticancer drug takes a long scientific journey, efforts and money. In the past several decades, there have been numerous wet lab studies defining anticancer molecular targets which subsequently been validated by innumerable compounds in the wet lab. However, with the development of novel bioinformatics tools and softwares, it is wiser and advisable to use these relatively inexpensive and faster tools in the elucidation of mechanism(s) for novel compounds to be developed as anticancer drugs using specific molecular anticancer targets. Our approach was to validate the wet lab results of the model compounds (aeroplysinin-1, curcumin, halofuginone) along with some of the well-known anticancer anti-angiogenic drugs (gefitinib, lapatinib, axitinib, motesanib) acting through anti-EGFR and anti-VEGFR pathways. The methodology adopted in the present study emphasizes the application of widely used AutoDock Tools 4.0 to selectively prove and validate in vivo/in vitro data which can provide preliminary information on the anti-angiogenic potential of the compounds prior to test in the expensive wet lab experiments.

Following the validation of the above approach with model compounds and reference drugs, we extended the work to explore and verify other natural compounds as multi-
target anti-angiogenic leads. In this connection, we selected those compounds which are present in the most consumed vegetables by the world population such as potatoes, tomatoes and egg plants (brinjal). Briefly, glycoalkaloids (GAs) are the naturally-occurring, nitrogen-containing plant steroids found mainly in the plants of the Solanaceae family, particularly the potato, having a carbohydrate side chain attached to the 3-hydroxy position. Aglycones which are the steroidal parts of the GAs, lack the carbohydrate side chain, e.g., solanidine from α-chaconine and α-solanine, α-solasodine from α-solasonine and α-tomatidine from α-tomatine (Korpan et al., 2004; Bushway et al., 1983). Earlier report showed the effectiveness of these GAs against the liver cancer cells (Lee et al., 2004), however, the tendency of these GAs to inhibit even normal human liver cells and failure to pass through the Lipinski’s drug-likeness criteria directed our work towards the screening of aglycones of these GAs as potential anti-angiogenic leads (Lee et al, 2004). Moreover, the aglycones have been shown to be associated with reduced toxicity level at lower concentrations (Van Gelder, 1990).

In the present chapter, we have conducted insilico interaction and inhibition studies on the selected GAs and their respective aglycones against the EGFR, VEGFR-1, VEGFR-2 using the AutoDock Tools 4.0 and other online bioinformatics software. The compounds have been further checked for drug-likeness and filtered through insilico toxicity parameters as to propose these novel compounds as potent anti-angiogenic leads with enhanced efficacy and reduced toxicity.

1.1.1 Strategy
The whole work has been divided into two parts: one part deals with the validation and assessment studies of insilico platform potential to wet lab setup in preliminary and faster screening of compounds for anti-angiogenic potential. The second part deals with the implementation of the validated insilico tools for the screening of GAs and their aglycones for anti-angiogenic potential.
1.2 Materials

1.2.1 Compounds under Study

1.2.1.1 Standard anti-angiogenic drugs: gefitinib, lapatinib, axitinib and motesanib have been discussed in detail in the Review of Literature (Figure 1.1).

Figure 1.1: Chemical structures of reference drugs with anti-angiogenic potential.

1.2.1.2 Aeroplysinin-1: an antibacterial brominated compound produced by the sponges (Figure 1.2).

1.2.1.3 Curcumin: Curcumin (diferuloylmethane), a principal curcuminoid of the popular Indian spice turmeric, is a prominent member of the ginger family (Zingiberacea) (Figure 1.2).

1.2.1.4 Halofuginone: a low molecular weight quinazolinone alkaloid isolated from the plant Dichroa febrifuga (Figure 1.2).
A. Aeroplysinin-1  

B. Curcumin

C. Halofuginone

Figure 1.2: Chemical structures of aeroplysinin-1, curcumin and halofuginone

1.2.1.5 Aglycones: The α-solanidine [solanid-5-en-3β-ol], a class of solanidine aglycones, was obtained as the steroidal part of α-chaconine and α-solanine. They possess a prominent indolizidine substructure, where N connects the spirostan E and F rings (Figure 1.3). α-solasodine [solasod-5-en-3β-ol] and α-tomatidine [5α-tomatidan-3β-ol], form a part of spirosolane aglycones, and were obtained from GA’s α-solasonine and α-tomatine. They have a conventional steroidal frame with a spiro-azaketal functionality present in the side chain (Figure 1.3). Both of these aglycones share a C-22 spiro substructure which can be subdivided into 2 diastereoisomeric groups, 25R- and 25S-spirosolanes. The methyl group is in equatorial position in both series, where (25R)-spirosolanes are 22αN-configurated and (25S)-spirosolanes are 22βN-configurated. The isomer α-solasodine (22R, 25R) is the dominating congener of the 25R-series (22αN-configurated) whereas α-tomatidine, the saturated derivative of tomatidenol (22S, 25S), obtained from α-tomatine is usually 22βN-configurated. Further, in the chapter these steroidal alkaloid aglycones will be referred for simplicity as solanidine, solasodine, and tomatidine.
Figure 1.3: Detailed chemical structures of aglycones: solanidine, solasodine and tomatidine

1.2.2 Softwares Used

All the docking studies have been carried out with the help of AutoDock Tools 4.0. Visualization of the molecules has been done through Accelrys Discovery Studio Visualizer 2.5.5. The drug-likeliness and insilico toxicity studies have been checked through online bioinformatics softwares.
1.3 Methodology

1.3.1 Drug-likeliness of Compounds under Study

The drug-likeliness of the aeropylinin-1, curcumin, halofuginone, standard drugs, GAs (α-chaconine, α-solanine, α-solasonine and α-tomatine) and their aglycones [solanidine, solasodine and tomatidine], were checked against ‘The Lipinski Rule of Five’, using the Lipinski Filter facility available online at Supercomputing Facility for Bioinformatics & Computational Biology, Indian Institute of Technology, New Delhi, India. The Lipinski’s rule, formulated by Christopher A. Lipinski in 1997, is a rule to evaluate whether a given chemical compound with a certain pharmacological, biological and ADME (absorption, distribution, metabolism and excretion) activity, has the potential that would likely make it orally active drug in humans (Lipinski et al., 1997).

1.3.2 Molecular Docking

Molecular docking experiments were performed using the AutoDock Tools 4.0 (Morris et al., 1998; Morris et al., 1996; Goodsell and Olson, 1990), a suite of automated docking tool developed at the Scripps Research Institute, Molecular Graphics Laboratory, USA, which uses an empirical scoring function based on the free energy of binding (Huey et al., 2007; Morris et al., 1998). Among the stochastic search algorithms available on the AutoDock suite, we chose the Lamarckian Genetic Algorithm (LGA) which combines global search (Genetic Algorithm alone) to local search Solis and Wets algorithm (Solis and Wets, 1981) to find the binding conformations of the ligand to the receptor. Genetic algorithms are the class of evolutionary computational models, in which the solution to an adaptative problem is spread among a genetic pool. In molecular docking, the solution corresponds to the best binding position and conformation for the ligand, and it is represented on a chromosome like data structure containing translation, orientation, and torsion genes. Basically, a genetic algorithm tries to mimic natural process of evolution and during this course gives rise to new generations until an optimum solution is achieved. The solutions are evaluated in terms of Binding energy (kcal/mol) and Inhibition constant (Ki). To achieve faster energy evaluation, AutoDock represents the macromolecule as a 3D grid, in which each point stores precalculated affinity potentials.
for all atom types of the ligand allowing flexibility to the ligand, while keeping the macromolecule rigid and fixed during docking.

The docking protocol involved four main steps as mentioned below:

a) Receptor preparation: Crystal structures of EGFR (PDB ID: 2GS2), bFGF (PDB ID: 1BFF), VEGFR-1 in complex with N-(4-2Chloropheny)-2-[(Pyridin-4-ylmethyl)amino]benzamide (PDB ID: 3HNG) and VEGFR-2 in complex with motesanib (PDB ID: 3EFL) were retrieved from BrookHaven Protein Data Bank (www.pdb.org). The PDB files were energy minimized using GROMACS and the missing residues were corrected using repair missing atoms option available in AutoDock. The non-essential water molecules were removed and polar hydrogens were merged with the PDB file. The complexes bound to receptor molecule were further removed.

b) Ligand preparation: As for the preparation of ligands, the molecular formula and SMILES notations for the known drugs, aeroplysinin-1, curcumin, halofuginone, steroidal GAs and their aglycones were obtained from Pubchem database (Wang et al., 2009) (http://pubchem.ncbi.nlm.nih.gov) and their 3D structures were built using the online demonstration of CORINA for generating 3D coordinates available at http://www.molecular-networks.com. The ligand files were further minimized and were converted into .pdbqt format.

c) Docking using a search algorithm: Subsequently, after preparing the receptor.pdbqt file and ligand.pdbqt file, the grid parameters file (.gpf) and docking parameters file (.dpf) were prepared. Based on previously reported structural information, grid across the active-site regions for the comparative AutoDock simulations of drugs, aeroplysinin-1, curcumin, halofuginone, GAs and aglycones with EGFR, bFGF, VEGFR-1 and VEGFR-2 were constructed (Fan et al., 2005; Wood et al., 2004; Muller et al., 1997). Gefitinib and lapatinib drugs were considered standard against EGFR, while axitinib and motesanib were taken standard against VEGFR’s, for comparative study. The grid was sketched as 60* 60*62 with its grid centre 57.301 -0.505 -19.959 around EGFR, 36*42*54 with its grid centre 22.534 25.493 13.24 around bFGF, 70*70*68 around
VEGFR-1 with its grid centre 5.665, 17.465, 33.112 and 60*56*50 around VEGFR-2 with its grid centre 37.762, 33.217 17.537, as such that the ligand was allowed to rotate freely inside the grid. The genetic algorithm parameters in AutoDock were set to default values, by which the program itself determines the optimal run parameters depending on the nature of the ligand and the receptor active site. “Number of genetic algorithm runs”, “Crossover frequency”, and “Mutation rates” parameters were thus automatically adjusted by the AutoDock.

d) Analysis of the binding conformation using a scoring function: The docking results generated the .glg and .dlg files of which the .dlg file was loaded along with the receptor file and was analyzed for the receptor-ligand interactions. The results were clustered using a root mean square deviation (RMSD) cutoff value and the best scoring conformation in each cluster was selected. The generated docked structures were furthermore minimized in the end. The interactions were studied in terms of binding energy (kcal/mol) and inhibition constant (µM) along with the number of hydrogen bonds formed with the surrounding amino acid residues. The figures of the best docked solutions of all ligands with EGFR, bFGF, VEGFR-1 and VEGFR-2 were generated using the Accelrys Discovery Studio Visualizer 2.5.5.

The experiments were repeated three times with 20 generations in each run to improve the precision level of result.

1.3.3 Insilico Toxicity Studies

The novel selected aglycones, proposed to be anti-angiogenic in our study were further analyzed insilico for any toxicity availing the online services of Organic chemistry portal available at www.organicchemistry.org. The study gave us an idea about the existence of possible mutagenic, tumorigenic, irritant and reproductive properties in the aglycones.
1.4 Results and Discussion

1.4.1 Validation Studies

This involved the comparative observation of the wet lab data and our insilico data of model compounds (aeroplysinin-1, curcumin, halofuginone) in relation to the standard anti-angiogenic drugs.

1.4.1.1 Comparative anti-angiogenic potential of model compounds in wet lab

Angiogenesis is an integral part of the cancer metastases and invasion and it is this unique ability of cancer which provides an efficient cancer treatment strategy. Various wet lab results as extracted and compiled in Table 1.1 showed that halofuginone had the lowest inhibition constant of 0.96 µM against the vascular endothelial cell differentiation compared to the aeroplysinin-1 and curcumin which showed inhibition constants of 2.1 and 25 µM, respectively (Rodriguez-Nieto et al., 2002; Elkin et al., 2000; Thaloor et al., 1998; Kreuter et al., 1990). Furthermore, in case of capillary tube formation and migration-invasion of cells, halofuginone was found to be the most effective compound followed by aeroplysinin-1 and curcumin (Table 1.1).

Table 1.1: Comparative inhibition pattern of capillary tube formation, vascular endothelial cells and migration-invasion of cells by aeroplysinin-1, curcumin and halofuginone

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Capillary tube formation (µM)</th>
<th>Migration and invasion of cells (µM)</th>
<th>Vascular endothelial cells (µM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeroplysinin-1</td>
<td>3</td>
<td>3</td>
<td>2.10</td>
<td>Rodriguez-Nieto et al, 2002</td>
</tr>
<tr>
<td>Curcumin</td>
<td>10-25</td>
<td>NS</td>
<td>25</td>
<td>Thaloor et al, 1998</td>
</tr>
<tr>
<td>Halofuginone</td>
<td>0.12</td>
<td>0.12</td>
<td>0.96</td>
<td>Elkin et al, 2000</td>
</tr>
</tbody>
</table>

NS=Not significant
1.4.1.2 Drug-likeliness study of model compounds

Our model compounds (aeroplysinin-1, curcumin and halofuginone) showed the drug-likeliness properties within the recommended range, as suggested by Lipinski (Table 1.2). The molecular weights of all the three compounds were found to be below 500 Dalton and the H-bond donors within normal limit of 5 and H-bond acceptors existed within the desired range of 10 indicating that these compounds have drug-likeliness (Table 1.2). The log P values were also below 5 for all the three compounds.

Table 1.2: Prediction of drug-likeliness of aeroplysinin-1, curcumin and halofuginone

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular weight (Dalton)</th>
<th>H-bond acceptor</th>
<th>H-bond donor</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeroplysinin-1</td>
<td>337.0</td>
<td>3</td>
<td>2</td>
<td>1.02</td>
</tr>
<tr>
<td>Curcumin</td>
<td>368.0</td>
<td>6</td>
<td>2</td>
<td>3.15</td>
</tr>
<tr>
<td>Halofuginone</td>
<td>413.5</td>
<td>5</td>
<td>2</td>
<td>1.07</td>
</tr>
</tbody>
</table>

*Criteria for the drug-likeliness:
A Molecular weight of most of the drugs available in market is <500 Dalton;
B H-bond Acceptor <10; C H-bond donor <5;
D Log P value <5; (calculated from http://www.scfbio-iitd.res.in)

1.4.1.3 Validation of the wet lab data of the model compounds and/or reference drugs on the insilico platform

Our docking simulation studies using the current anti-angiogenic molecular targets of EGFR, bFGF and VEGFR-1 with the model test compounds and reference drugs (gefitinib, lapatinib and axitinib) produced promising results in terms of binding energies (kcal/mol) and inhibition constant (µM).
Table 1.3: Docking studies of EGFR, bFGF, VEGFR-1 with aeroplysinin-1, curcumin and halofuginone.

<table>
<thead>
<tr>
<th>Receptors</th>
<th>EGFR</th>
<th>bFGF</th>
<th>VEGFR-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compounds</td>
<td>Binding energy (kcal/mol)</td>
<td>Inhibition constant (µM)</td>
<td>Hydrogen bonds</td>
</tr>
<tr>
<td>Aeroplysinin-1</td>
<td>-4.23</td>
<td>789.41</td>
<td>3 H (GLU738 LYS721 THR830)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>-4.73</td>
<td>341.15</td>
<td>2 H (LYS692 LYS721)</td>
</tr>
<tr>
<td>Halofuginone</td>
<td>-7.37</td>
<td>3.96</td>
<td>2 H (GLU780 CYS773)</td>
</tr>
</tbody>
</table>

#Calculated free energy of binding (∆G) in kcal/mol.
*Calculated inhibition constant Kᵢ from AutoDock.

**EGFR:** Aeroplysinin-1 produced a moderate binding energy of -4.23 kcal/mol against EGFR compared to curcumin and halofuginone with respective values of -4.73 and -7.37 kcal/mol (Table 1.3) (Figure 1.4). The inhibition constant of halofuginone was found to be 3.96 µM which was almost 86-fold less than curcumin and 200-fold less than aeroplysinin-1 suggesting halofuginone to be the strongest anti-angiogenic compound in agreement with the results shown in Table 1.1. Further, gefitinib, lapatinib and axitinib produced binding energies against EGFR with respective values of -7.00, -7.36 and -8.5 kcal/mol along with 7.36, 4.06 and 0.58 µM of inhibition constants (Table 1.4). These results are quite comparable to the halofuginone suggesting it as an effective anti-angiogenic compound.
Aeroplysinin-1  Curcumin  Halofuginone

Figure 1.4: Docked structure of model compounds with EGFR

Table 1.4: Docking studies of EGFR, bFGF and VEGFR-1 with reference drugs

<table>
<thead>
<tr>
<th>Receptors</th>
<th>EGFR</th>
<th>bFGF</th>
<th>VEGFR-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>#Binding energy (kcal/mol)</td>
<td>*Inhibition constant (µM)</td>
<td>Hydrogen bonds</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>-7.00</td>
<td>7.36</td>
<td>1 H (MET769)</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>-7.36</td>
<td>4.06</td>
<td>2 H (MET769 GLU780)</td>
</tr>
<tr>
<td>Axitinib</td>
<td>-8.5</td>
<td>0.58</td>
<td>1 H (LYS721)</td>
</tr>
</tbody>
</table>

#Calculated free energy of binding (ΔG) in kcal/mol.
*Calculated inhibition constant Ki from AutoDock.

**bFGF:** However, in relation to bFGF, aeroplysinin-1, curcumin and halofuginone expressed moderate binding energies of -3.79, -3.06 and -2.47 kcal/mol, respectively, along with higher inhibition constants of 1680, 5700 and 15,580 µM, respectively (Table
1.3) (Figure 1.5). Moreover, gefitinib, lapatinib and axitinib also produced moderate binding energies of -2.88, -3.81 and -3.97 kcal/mol (Table 1.4). These results suggest that these compounds and reference drugs may not effectively act through inhibition of bFGF.

![Docked structure of model compounds with bFGF](image)

**Figure 1.5: Docked structure of model compounds with bFGF**

**VEGFR-1:** Further, significant interaction of aeroplysinin-1 was seen against VEGFR-1 with a good binding energy of -6.23 kcal/mol and inhibition constant of 27.23 µM (Table 1.3). Similar binding energy and inhibition constant were also observed for curcumin with respective values of -6.08 kcal/mol and 34.97 µM (Table 1.3) (Figure 1.6). However, halofuginone resulted as the most effective compound with -8.96 kcal/mol binding energy and 0.27 µM inhibition constant (Table 1.3). Gefitinib, lapatinib and axitinib also showed similar type of interactions with VEGFR-1 resulting in respective values of -6.56, -6.08 and -9.81 kcal/mol (Table 1.4).
The wet lab results against the capillary tube formation, migration-invasion of cells and vascular endothelial cell differentiation with all the model compounds suggested that halofuginone was the most effective compound with the lowest inhibition constant. Our insilico results with the angiogenic targets (EGFR and VEGFR-1) clearly validates that halofuginone is better anti-angiogenic compound compared to the curcumin and aeroplysinin-1 (Arif et al., 2011). Recent report also suggests that synthetic analogues of aeroplysinin-1 were better drug candidates than aeroplysinin-1 in the inhibition of prostate cancer cell proliferation (Sallam et al., 2010).

In conclusion, our comparative observations with the model compounds and reference drugs between the wet lab and insilico approaches are in significant agreement which has led to open up the hidden potential of simple insilico approach for preliminary screening of the new compounds using the anti-angiogenic molecular targets. The results can also elucidate the underlying mechanism(s) of angiogenesis up to certain extent for test compounds.

**Figure 1.6: Docked structure of model compounds with VEGFR-1**
1.4.2 Screening, Validation and Elucidation of mechanism of action of aglycones against angiogenesis

Most of the anti-angiogenic drugs currently administered for treatment of cancer are already being withdrawn from the market due to the associated side-effects like hypertension and proteinuria which are the class effects of anti-VEGF drugs, while others have failed to prove their prolonged survival rate in the clinical trials (Pfizer, 2009; Havrilla, 2008; Biospectrum Asia, 2008; Golsteyn, 2005). Furthermore, these drugs lack expected efficiency and have to be administered in combination with other drugs for proper therapeutic effect. Lapatinib is administered to the patients along with capecitabine which itself induces nausea, diarrhea and hand and foot syndrome (Havrilla, 2008; Moy et al., 2007). Some of these drugs like sorafenib were even clastogenic and mutagenic (Wishart et al., 2008).

In such a situation, on the footsteps of the above conducted validation strategy, our insilico studies clearly showed the naturally-occurring aglycones (solanidine, solasodine and tomatidine) as effective anti-angiogenic lead compounds. The aglycones were in general exceptionally comparable to the standard drugs while the GAs did not show the drug-like potential. Our aglycones showed substantially lowered inhibition constant than the standard anti-angiogenic drugs targeting EGFR, VEGFR-1 or VEGFR-2 with almost no toxicity compared to these drugs. The detailed work is as under mentioned.

1.4.2.1 Drug-likeliness test of GAs and their aglycones, compared with standard drugs.

The compounds under study were preliminary checked for their expected drug-likeliness before proceeding for further studies. The GAs failed to exist within the recommended parameters of drug-likeliness, as given by Lipinski (Table 1.5). The molecular weight of α-chaconine, α-solane, α-solasonine and α-tomatine crossed the 800 Dalton mark, which was supposed to be below 500 Dalton for a compound to be like a drug. The H-
bond donors in GAs were also greater than normal limit of 5 and even molar refractivity
did not exist within the desired range of 40-130.

On the other hand, aglycones (solanidine, solasodine and tomatidine) were found to be
well within the desired limits of drug-likeliness parameters, in agreement with the
standard drugs, gefitinib and axitinib. All the values for H-bond donors, H-bond
acceptors and molar refractivity for aglycones were found to be exceptionally good for
being potent lead. Even the values for molecular weight, logP, molar refractivity of
aglycones were in close vicinity to the values for the respective parameters of the
standard drugs (Table 1.5). The study clearly ruled out the possible use of GAs as active
drug leads, however, the aglycones were further studied for their anti-angiogenic
potential.

**Table 1.5: Prediction of drug-likeliness of the selected drugs, GA’s and their
steroidal aglycones**

<table>
<thead>
<tr>
<th></th>
<th>Molecular Weight (Dalton)\textsuperscript{A}</th>
<th>H Bond Acceptor\textsuperscript{B}</th>
<th>H Bond Donor\textsuperscript{C}</th>
<th>Log P\textsuperscript{D}</th>
<th>Molar Refractivity\textsuperscript{E}</th>
<th>PSA\textsuperscript{F}</th>
<th>Protein Binding\textsuperscript{G}</th>
<th>BBB\textsuperscript{H}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib</td>
<td>423</td>
<td>6</td>
<td>1</td>
<td>3.854</td>
<td>119.387</td>
<td>68.74</td>
<td>82.655</td>
<td>-0.97</td>
</tr>
<tr>
<td>Axitinib</td>
<td>386</td>
<td>3</td>
<td>2</td>
<td>4.541</td>
<td>113.364</td>
<td>91.68</td>
<td>96.456</td>
<td>0.10</td>
</tr>
<tr>
<td>α-Chaconine</td>
<td>851</td>
<td>7</td>
<td>8</td>
<td>5.111</td>
<td>215.608</td>
<td>220.46</td>
<td>82.061</td>
<td>-1.11</td>
</tr>
<tr>
<td>α-Solanine</td>
<td>867</td>
<td>7</td>
<td>9</td>
<td>4.711</td>
<td>215.804</td>
<td>240.69</td>
<td>67.282</td>
<td>-1.49</td>
</tr>
<tr>
<td>α-Solasonine</td>
<td>883</td>
<td>7</td>
<td>10</td>
<td>4.543</td>
<td>214.444</td>
<td>258.71</td>
<td>43.219</td>
<td>-1.65</td>
</tr>
<tr>
<td>α-Tomatine</td>
<td>1033</td>
<td>9</td>
<td>13</td>
<td>3.403</td>
<td>245.912</td>
<td>337.86</td>
<td>46.209</td>
<td>-1.67</td>
</tr>
<tr>
<td>Solanidine</td>
<td>397</td>
<td>1</td>
<td>1</td>
<td>4.637</td>
<td>118.355</td>
<td>23.47</td>
<td>72.016</td>
<td>0.75</td>
</tr>
<tr>
<td>Solasodine</td>
<td>413</td>
<td>1</td>
<td>2</td>
<td>4.269</td>
<td>119.680</td>
<td>41.49</td>
<td>72.581</td>
<td>0.38</td>
</tr>
<tr>
<td>Tomatidine</td>
<td>415</td>
<td>1</td>
<td>2</td>
<td>4.349</td>
<td>119.704</td>
<td>41.49</td>
<td>72.581</td>
<td>0.38</td>
</tr>
</tbody>
</table>

\*Criteria for the drug-likeliness:
\textsuperscript{A}Molecular weight of most of the drugs available in market is <500 Dalton; \textsuperscript{B}H-bond Acceptor <10;
\textsuperscript{C}H-bond donor <5; \textsuperscript{D}logP value <5; \textsuperscript{E}Molar refractivity values between 40-130 (calculated from http://www.scfbio-iitd.res.in)
\textsuperscript{F}Polar Surface Area between 0.00-150 (calculated from http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py)
\textsuperscript{G}PB, \textsuperscript{H}BBB= Blood brain barrier (calculated from https://secure.chemsilico.com/index.php)
1.4.2.2 Structure/biological activity relationships of the selected aglycones

Furthermore, the aglycones under study, solanidine, solasodine and tomatidine are structurally similar in that they share common combination of six heterocyclic hexagonal and pentagonal rings. The presence of additional groups, positioning of double bonds and differential arrangement of these rings make them slightly structurally as well as functionally different (Friedman et al., 1996).

Solanidine and solasodine share common A-D rings, with minor differences in E and F rings. Solanidine has fused spirostan nitrogenous E and F rings forming indolizidine group, while solasodine F ring is nitrogen heterocyclic and E ring is oxygen heterocyclic, forming spiro-azaketal substructures (Figure 1.3). Tomatidine, on the other hand, has a slightly different arrangement than solanidine and solasodine (Figure 1.3). Tomatidine E ring is an oxygen heterocyclic and lacks the double bond at 5, 6 position in B ring. Ring F is nitrogen heterocyclic of which the 25-methyl group is epimeric.

Additionally, previous feeding studies in mice concluded that solasodine induced significant hepatomegaly in mice while tomatidine was ineffective (Friedman et al., 1996). Thereby, owing to significant structural similarity of solasodine and solanidine, as mentioned above and knowing that the structural differences in the F-ring appear to have little effect, the absence of 5,6 double bond in tomatidine may likely be responsible for the observed differences in potency of aglycones in inducing liver enlargement (Friedman et al., 1996).

1.4.2.3 Docking simulations of the selected aglycones with RTKs

The docking simulation results of aglycones and standard drugs have been discussed below under separate sections covering different RTK’s under target.

**EGFR:** Docking simulations of aglycones, solanidine [solanid-5-en-3β-ol], solasodine [solasod-5-en-3β-ol] and tomatidine [5α-tomatidan-3β-ol], with EGFR showed promising
results in terms of binding energies (kcal/mol) and inhibition constant (µM), which was quite comparable to the already available anti-EGFR drugs in the market. The compounds solanidine and solasodine specially showed good binding energies of -8.50 and -8.23 kcal/mol, respectively, compared to the lapatinib (-7.36 kcal/mol) (Table 1.6). Tomatidine also expressed a moderate binding energy of -7.53 kcal/mol. Our results clearly demonstrate that these aglycones exhibited similar types of interactions with EGFR as other quinazoline inhibitors like lapatinib and gefitinib.

### Table 1.6: Docking studies of EGFR with reference drugs and steroidal aglycones

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Energy* (Kcal/mol)</th>
<th>Ki** (µM)</th>
<th>Hydrogen bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Drugs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gefitinib</td>
<td>-7.00</td>
<td>7.360</td>
<td>1 H bond with Met769</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>-7.36</td>
<td>4.060</td>
<td>2 H bond with Met769 and Glu780</td>
</tr>
<tr>
<td><strong>Aglycones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solanidine</td>
<td>-8.50</td>
<td>0.590</td>
<td>No H bonds formed</td>
</tr>
<tr>
<td>Solasodine</td>
<td>-8.23</td>
<td>0.923</td>
<td>2 H bonds with Thr830 and Asp831</td>
</tr>
<tr>
<td>Tomatidine</td>
<td>-7.53</td>
<td>3.020</td>
<td>1 H bond with Met769</td>
</tr>
</tbody>
</table>

*Calculated free energy of binding (∆G) in kcal/mol.

**Calculated inhibition constant Ki from AutoDock.

The docked complexes of aglycones with EGFR, revealed solanidine bound in the same ATP binding cleft of EGFR as other kinase inhibitors. The fused nitrogenous E and F rings comprising indolizidine group of solanidine, was surrounded with Met769 and Leu694 amino acid residues (Figure 1.7, panel B). The N° of the fused spirostan E and F rings lies in close vicinity to Met769 amino acid residue. The remaining A, B, C and D rings of the molecule are oriented deep in the back of the ATP binding cleft and seem to
be involved in predominantly hydrophobic interactions, within the hydrophobic pocket composed of Val702, Ala719 and Leu820 amino acid residues. No polar interactions of solanidine with EGFR were visible (Figure 1.7, panel B).

In similar fashion to solanidine, the fused nitrogenous spiro-azaketal E and F rings of solasodine were also found to be present in the same binding pocket, surrounded with Met769 and Leu694 amino acid residues of the ATP binding cleft, while B, C and D rings were involved in mainly hydrophobic interactions with Ala719 at the top and Val702 amino acid residues at the bottom. An important aspect with solasodine interaction, was the formation of hydrogen bonds between hydroxyl (OH) group of A ring with Thr830 and Asp831 amino acid residues. These hydrogen bonds enhanced the stability of solasodine molecule with the closely associated polar Thr and Asp amino acids. The docked complex of solasodine with EGFR represented a good balance between the hydrophobic and hydrophilic environmental forces of surrounding amino acid residues present in the ATP binding cleft (Figure 1.7, panel C).

Tomatidine again was also found to be present in a similar conformation with EGFR, within the ATP binding cleft. The spiro-azaketal F ring was hydrogen bonded to the hinge region between NH₂ and COOH terminal lobes of the kinase, with the formation of hydrogen bond between N atom of the F ring with main chain Met769. The D, E and F rings were surrounded by Leu820, Ala719, Val702 amino acid residues form the top, side and bottom. Furthermore, A, B and C rings were found to be present, at the back side of ATP binding cleft, surrounded by Asp831, Thr830 and Arg817 amino acid residues (Figure 1.7, panel D). No pi-pi, t-shaped or cation-pi interactions of any of the above aglycones with EGFR were visible.
Figure 1.7: Docked structures of gefitinib and aglycones with EGFR. Ligand [drug/aglycones (solanidine, solasodine, tomatidine)] represented in light green. Receptor (EGFR) as stick model, colored by element. Hydrogen bonds represented as dotted lines in green. Amino acid residues labeled dark green.
**VEGFR-1:** Similarly, the docking studies of aglycones with VEGFR-1 also showed promising results. Tomatidine emerged as the most potent compound with a binding energy of -9.5 kcal/mol, followed by solanidine and solasodine with values of -8.74 and -8.46 kcal/mol, respectively. The results are quite comparable to the standard drugs, axitinib and motesanib, with respective values of -9.81 and -8.10 kcal/mol (Table 1.7). In addition, the docked complexes obtained in the process helped us to elucidate the mechanism of action of these aglycones against VEGFR-1.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Energy* (Kcal/mol)</th>
<th>Ki** (µM)</th>
<th>Hydrogen bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Drugs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axitinib</td>
<td>-9.81</td>
<td>0.064</td>
<td>1 H bond with Asp1040</td>
</tr>
<tr>
<td>Motesanib</td>
<td>-8.10</td>
<td>1.160</td>
<td>3 H bonds with Asp1040, His1020, Asp1022</td>
</tr>
<tr>
<td><strong>Aglycones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solanidine</td>
<td>-8.74</td>
<td>0.394</td>
<td>No H bonds formed</td>
</tr>
<tr>
<td>Solasodine</td>
<td>-8.46</td>
<td>0.631</td>
<td>No H bonds formed</td>
</tr>
<tr>
<td>Tomatidine</td>
<td>-9.50</td>
<td>0.109</td>
<td>2 H bonds with Arg1021, Asp1040</td>
</tr>
</tbody>
</table>

*Calculated free energy of binding (ΔG) in kcal/mol.
**Calculated inhibition constant Ki from AutoDock.

Actually, recent trend in this area has shifted towards the anti-angiogenic drugs targeting the VEGFR-2, like axitinib and motesanib, even though VEGFR-1 binds to VEGF with a 50-fold higher affinity (Muller *et al.*, 1997). In continuation with this, the docked complexes of aglycones with VEGFR-1 in our study, showed the similar type of interactions, as of axitinib and motesanib. The indolizidine group of solanidine was found
to be buried inside the VEGFR-1 protein, while surrounded with Leu882 and Asp1040 amino acid residues from the top and the bottom. The N⁹ atom of the spirostan F ring was present in the close vicinity to O atom of polar Asp1040 amino acid residue. The B, C, D rings were present on the exterior of the surface, surrounded by Glu878 and His1020 residues. Furthermore, the O atom of A ring was found in close vicinity to Arg1021 amino acid residue, however no polar interactions were visible. Further, no pi-pi, t_shaped or cation-pi interactions of solanidine with VEGFR-1 were seen either (Figure 1.8, panel B).

The second aglycone, solasodine was bound to VEGFR-1 in a slightly different mode than solanidine. The spiro-azaketal nitrogenous fused E and F rings of solasodine were found to be surrounded by Ile1038 amino acid residue at bottom and Ile881 from the top. In addition, the A,B,C and D rings were found to be buried inside the protein in a curved manner, surrounded by Val909, Glu878 and Leu1029 amino acid residues. Especially the A ring of the solasodine was found deeply buried in the hydrophobic pocket composed of Val841, Ala859 and Leu883 amino acid residues (Figure 1.8, panel C).

The third aglycone, tomatidine was also found to be bound in a similar fashion as solanidine, with its A and B rings lying between Leu882 and Asp1040 amino acid residues. The OH group of the A ring was hydrogen bonded with polar Asp1040 residue. The B,C,D and E rings were present on the exterior of the surface surrounded by Cys1018, Asp1022 and Arg1021 amino acid residues. Moreover, the O atom of the spiro-azaketal ring of tomatidine molecule was found to be hydrogen bonded with Arg1021. The proper balance between the hydrophobic forces and hydrogen bond mediated hydrophilic forces, in the docked complex, supports tomatidine as the most potent inhibitor of VEGFR-1 amongst the aglycones (Figure 1.8, panel D). Furthermore, no pi-pi, t_shaped or cation-pi interactions of solasodine and tomatidine with VEGFR-1 were visible.
A. Axitinib  
B. Solanidine  
C. Solasodine  
D. Tomatidine

Figure 1.8: Docked structures of axitinib and aglycones with VEGFR-1. Ligand [drug/aglycones (solanidine, solasodine, tomatidine)] represented in light green. Receptor (VEGFR-1) as stick model, colored by element. Hydrogen bonds represented as dotted lines in green. Amino acid residues labeled dark green.
VEGFR-2: Docking simulations of VEGFR-2 with axitinib and motesanib, in the class of known anti-angiogenic drugs, produced good binding energies of -7.17 and -6.29 kcal/mol, respectively. However, the aglycones were able to produce better binding energies with solanidine resulting in -8.81 kcal/mol, followed by tomatidine (-8.24 kcal/mol) (Table 1.8). Solasodine also expressed a moderate binding energy of -6.84 kcal/mol.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Energy* (Kcal/mol)</th>
<th>Ki** (µM)</th>
<th>Hydrogen bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Drugs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axitinib</td>
<td>-7.17</td>
<td>5.590</td>
<td>1 H bond with Gly846</td>
</tr>
<tr>
<td>Motesanib</td>
<td>-6.29</td>
<td>24.54</td>
<td>2 H bonds with Asp1046, Glu885</td>
</tr>
<tr>
<td><strong>Aglycones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solanidine</td>
<td>-8.81</td>
<td>0.348</td>
<td>No H bonds formed</td>
</tr>
<tr>
<td>Solasodine</td>
<td>-6.84</td>
<td>9.670</td>
<td>No H bonds formed</td>
</tr>
<tr>
<td>Tomatidine</td>
<td>-8.24</td>
<td>0.906</td>
<td>1 H bonds with Ile1044</td>
</tr>
</tbody>
</table>

*Calculated free energy of binding (ΔG) in kcal/mol.
**Calculated inhibition constant Ki from AutoDock.

The docked complexes of aglycones again revealed a similar type of interaction with VEGFR-2 as of standard drugs motesanib and axitinib. Solanidine was found docked in the ATP binding position of VEGFR-2 with indolizidine group covered from top by Asp1046 residue and Ile888 amino acid residue at the bottom. The A,B,C and D rings were present at the back side of the pocket, surrounded by Val916, Lys868 and Cys1045 amino acid residues. The O atom of the A ring of solanidine molecule was found in close association with aromatic ring of Phe1047 amino acid residue. No polar interactions were
visible; however, the straight and buried conformation of solanidine molecule into VEGFR-2 favored the stability of the complex and henceforth enhanced its potency (Figure 1.9, panel B).

Furthermore, for solasodine, the fused N containing spirostan E and F ring was covered from top by Asp1046 residue and Ile888 amino acid residue at the bottom, as like with solanidine. The A, B, C and D rings again were present at the back side of the pocket, surrounded by Val916, Lys868 and Cys1045 amino acid residues. No polar interactions were visible. But the extra torsion at the spiro-azaketal group of solasodine molecule and protruded conformation of A ring in the VEGFR-2 complex, might have reduced the potency of solasodine against solanidine. (Figure 1.9, panel C).

In a similar manner, the A ring of the third aglycone tomatidine, was found docked in the ATP binding pocket of VEGFR-2, surrounded with Cys1045 and Leu889 amino acid residues, with the formation of hydrogen bond between the OH group of the A ring and Ile1044 amino acid residue. The B, C and D ring were present in a flat conformation surrounded by Asp1046 and Ile888 amino acid residues. The spiro-azaketal ring of tomatidine molecule made a torsional bend and was found embedded inside the active site, in close vicinity to Asp1028 and Arg1027 amino acid residues. The presence of hydrogen bonds along with the deep hydrophobic interactions of tomatidine molecule inside the active site makes it an efficient lead as an anti-VEGFR-2 agent (Figure 1.9, panel D). In addition, no pi-pi, t-shaped or cation-pi interactions of any of the above aglycones with VEGFR-2 were visible.
Figure 1.9: Docked structures of axitinib and aglycones with VEGFR-2. Ligand [drug/aglycones (solanidine, solasodine, tomatidine)] represented in light green. Receptor (VEGFR-2) as stick model, colored by element. Hydrogen bonds represented as dotted lines in green. Amino acid residues labeled dark green.
1.4.2.4 Insilico toxicity studies

The tested aglycones showed no toxic effects when screened through insilico toxicity channel available at Organic Chemistry portal. Solanidine, solasodine and tomatidine were reported negative for mutagenic, tumorigenic and irritant property. In addition, solasodine and tomatidine were also found to be negative for reproductive effect with an exception to solanidine (Table 1.9)

Table 1.9: Insilico toxicity studies of the selected steroidal aglycones

<table>
<thead>
<tr>
<th>Compounds</th>
<th>^AM</th>
<th>^BT</th>
<th>^CIE</th>
<th>^DRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanidine</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Yes</td>
</tr>
<tr>
<td>Solasodine</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Tomatidine</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

^-AM Mutagenicity
^-BT Tumorigenicity
^-CIE Irritant Effect
^-DRE Reproductive Effect
(Analyzed by using Organic Chemistry portal at [www.organic-chemistry.org](http://www.organic-chemistry.org))

The most important benefit of these aglycones is that they are naturally-occurring compounds in the members of Solanaceae family, particularly potatoes (Norgia et al., 2008; Bushway et al., 1983). Bioavailability and natural occurrence are positive factors for these anti-angiogenic leads. Potato is one of the most commonly consumed foods all over the world, and these aglycones possessing anti-angiogenic and anticancer properties are present in sufficient amount in it (Norgia et al., 2008; Bushway et al., 1983).

Though studies suggested that these aglycones could be toxic when consumed in large amounts but with relatively high LD$_{50}$ values (about 1.75 mg/kg body weight) in animal
models their toxicity seems to be insignificant compared to the inherent side-effects of the standard anti-angiogenic drugs (Friedman et al., 2006; Friedman et al., 1996). Although solanidinederived compounds have higher toxicity, the solasodine alkaloids may also affect male fertility and disrupt cell membrane, thereby resulting in alterations in the liver and central nervous system (Friedman et al., 2006; Roddick et al., 2001; Friedman et al., 1996). Some of the GAs intoxication symptoms include colic pain in the abdomen and stomach, gastroenteritis, diarrhea, vomiting, burning sensation and headache. In more severe cases, neurological symptoms, including drowsiness and apathy, confusion, weakness, and vision disturbances, followed by unconsciousness and, in some cases, death have also been reported. Some adverse effects may also be due to alkaloid-induced teratogenicity (Gaffield and Keeler, 1996; Gaffield and Keeler, 1994; Harvey et al., 1986; Renwick et al., 1984). Solanidine and solasodine were reported to be slightly teratogenic in animal models, but tomatidine, the non-nitrogen containing analog of solasodine, was not teratogenic. The teratogenicity and embryotoxicity of the spirosolane derivatives, solasodine and solanidine have been attributed to the presence of a nitrogen atom bonded in a position analogous to hormone-binding sites and to the basicity of the nitrogen atom of the spiroketimine (Gaffield and Keeler, 1994). Additionally, the use of solanidine and solasodine may be responsible for the neural tube defects in fetus and hence may require certain precautions by the pregnant women (Gaffield and Keeler, 1996; Gaffield and Keeler, 1994; Harvey et al., 1986; Renwick et al., 1984).

Moreover, a comparative study between different chacotriose- and/or solatriose-based GAs in their effects on target membranes highlights the importance of the carbohydrate moiety in the synergistic effect (Sanchez-Mata et al., 2010; Friedman et al., 2006; Friedman and McDonald, 1997). Further, it should also be kept in mind, that the real situation in plants may be different. In many solanaceous species, “paired” presence of GAs with different carbohydrate moieties is due to the adaptive response to the acquired immunity against the pathogens. For example, eggplant contains, besides α-solasonine also α-solamargine, noted for more potent biological activity of membrane disruption than α-solasonine (Sanchez-Mata et al., 2010; Friedman and McDonald, 1997). Similarly,
dehydrotomatine and α-tomatine have been shown to act synergistically in protecting the tomato plant against phytopathogens (Friedman et al., 2002). Thus, a separate study involving the paired effect of different aglycones present in natural products is also a matter of great interest.

Furthermore, little information regarding genotoxicity and carcinogenicity of GAs derived from *S. indicum* has been reported. Pure α-solanine and extracts from potatoes were negative in the Ames test both with strains TA98 and TA100, and in the presence or absence of activation system (Ness et al., 1984). The α-solanine (25 and 250 µM) tested negative in a DNA-cell-binding assay using Ehrlich ascites cells and *E. coli* cells mixed with ³²P-labelled nucleic acids (Kubinski et al., 1981). Further, in one of our study, these GAs and aglycones also did not cause any DNA damage *per se* in an *in vitro* system suggesting lack of their genotoxic potential (Arif J.M. et al., manuscript under preparation). Moreover, no long-term carcinogenicity studies are reported in the literature.

In addition, the negative in silico toxicity results of these aglycones obtained through online organic chemistry portal also supported them as new generation lead compounds against the angiogenesis. However, at a time, when most of the anti-angiogenic drugs present in the market are associated with severe side-effects to humans, the oral administration of these naturally-occurring aglycones can be treated as an effective preventive strategy to combat angiogenesis and subsequently cancers.
1.6 Conclusions

Our comparative observations with the model compounds and reference drugs between the wet lab and insilico methods opens up the hidden potential of the simple insilico approach for preliminary and faster screening of the new compounds using the angiogenic molecular targets. Furthermore, our insilico docking results clearly suggest for the first time that the steroidal alkaloid aglycones (solanidine, solasodine and tomatidine) remarkably possess specific RTKs inhibitory capability in comparison to standard drugs which makes them as potential new generation natural anti-angiogenic drugs with little or no toxicity (Akhtar et al., 2011).
1.7 References


Kreuter, M.H.; Leake, R.E.; Rinaldi, F.; Muller-Klieser, W.; Maidhof, A.; Muller, W.E.G. and Schroder, H.C. (1990), ‘Inhibition of intrinsic protein tyrosine kinase activity of EGF-receptor kinase complex from human breast cancer cells by the


