Docking Analysis of Ribulose 1, 5 bisphosphate carboxylase from Selected Medicinal Plants in Evolutionary Relationship

[1]GOBALAN K 1st, AHAMED JOHN S , 2nd RAJA A 3rd
[1]ASSISTANT PROFESSOR 1st , ASSOCIATE 2nd , [3]ASSISTANT PROFESSOR 3rd
[1] PG and Research Department of Biotechnology 1st
[1], Jamal Mohamed College, Trichy 20

Abstract: Multiple sequence alignments are an indispensable tool in bioinformatics. Many applications rely on accurate multiple alignments, including protein structure prediction, phylogeny and the modelling of binding sites. In this thesis we dissected and analyzed the crucial algorithms and data structures required to construct such a multiple alignment. Based upon that dissection, we present a novel graph-based multiple sequence alignment programs and a new method for multi-read alignments occurring in assembly projects. The advantage of the graph-based alignment is that a single vertex can represent a single character, a large segment or even an abstract entity such as a gene. This gives rise to the opportunity to apply the consistency based progressive alignment paradigm to alignments of genomic sequences. The proposed multi-read alignment method outperforms similar methods in terms of alignment quality and it is apparently one of the _rst methods that can readily be used for insert sequencing. An important aspect of this thesis was the design, the development and the integration of the essential multiple sequence alignment components in the SeqAn library. SeqAn is a software library for sequence analysis that provides the core algorithmic components required to analyze large-scale sequence data. SeqAn aims at bridging the current gap between algorithm theory and available practical implementations in bioinformatics. Hence, we always describe in conjunction to the theoretical development of the methods, the actual implementation of the data structures and algorithms in order to strengthen the use of SeqAn as an experimental platform for rapidly developing and testing applications. All presented methods are part of the open source SeqAn library that can be downloaded from our website, www.seqan.de.

IndexTerms **– BLAST, FASTA, Multiple Sequence alignment, Pair Wise**

I. INTRODUCTION

Traditional medicines include herbal medicines composed of herbs, herbal materials, herbal preparations, and herbal products that contain many active ingredients. They are used in every country in the world, and have been relied upon to support, promote, retain and regain human health for millennia Traditional medicines (products) are a part of the larger field which includes procedures and practitioners, as well as products (WHO, 2002). In much of the developing world, 70–95% of the population relies on these traditional medicines for primary health care. In Mali, like many African countries 75% of the population depends on traditional medicine for primary health care (Imperato, 1981; WHO, 2002). The traditional medicine is mainly based on plants, and most of these plants have never been investigated for their chemical composition or pharmacological properties. Many plants and animals are beneficial to human beings for medicine, or for biological control of pests, parasites, or pathogens Phylogeny can help guide a search for potentially beneficial species based on what is known about their close relatives if a valuable medicine can be produced from natural compounds in plant species, it would make sense to search for similar compounds in the closely related plant species. Or, if wasp species 3 helps control pest caterpillar C, then wasp species 4 may be a good candidate for natural control of pest caterpillar D. Plant secondary chemicals have proven to be a vast reservoir for useful pharmaceuticals — these include analgesics, diuretics, laxatives, tranquilizers, contraceptive agents, and cough drops. Clinically proven drugs derived from higher plants include morphine, codeine, atropine, quinine, digitalis, and many others. Scientists have only examined about 1 percent of existing plant species for such useful pharmaceuticals. Currently, spider, snake, and fish phylogenies are being used in a survey to search for medically valuable compounds in the venoms of these animals. Powerful anticancer and antistroke drugs have already been isolated from venoms of snakes. The powerful anti-cancer drug Paclitaxel (Taxol) caused a sensation when it was discovered in the bark of Pacific Yew Trees, Taxus brevifolia. However, almost 100,000 pounds of bark were needed for clinical trials alone, and bark collection kills the tree. It was estimated that if Paclitaxel were used regularly to treat melanomas and ovarian cancer in the U.S., 360,000 trees would have to be destroyed each year. (!!!!!) In the 90's, phylogenetic studies of yew trees guided scientists to other species of Taxus that could be used as sources for taxol (including Taxus baccata) Now Taxus genes in transformed bacterial genomes produce paclitaxel at large scales. Now a day human genome sequence field produce more number of biological data are produced and the biological data were deposited in public and private databases in a structure and searchable format to indentify the functional annotation of the particular protein sequence like PROSITE, PFAM, PDB. Currently many multiple sequence alignment free software's available, as their only input, the sequences to be aligned without employing any other source of external information in the alignment process. But when such softwares make use of the data available in public databases, the quality of the produced alignments will be improved. This happens because constraints will be extracted from the used data and employed during the alignment calculation. In this thesis, we have selected ten potential

medicinal plants from different family, then the protein sequence were retrieved from NCBI, then the sequence were further taken into multiple sequence alignment using .... finally we construct Phylogenetic tree to find out the evolutionary relationship between the medicinal plants. Phylogenetics is important because it enriches our understanding of how genes, genomes, species (and molecular sequences more generally) evolve. Through phylogenetics, we learn not only how the sequences came to be the way they are today, but also general principles that enable us to predict how they will change in the future. This is not only of fundamental importance but also extremely useful for numerous applications. Heterocyclic like benzothiazine compounds having many medicinal properties especially cancer field The ten Benzothiazine Derivatives were synthesized then the compounds were further taken into molecular docking studies, ADME properties prediction and Anti-cancer activity was carried out for best two compounds against leukemia cancer.

II.Materials and Methods

I.Multiple sequence alignment: CLUSTALW

In 1994 one of the first and most popular MSA methods was published, denoted ClustalW [46]. It had its roots in the progressive Feng and Doolittle method from 1987. The two major issues of the progressive approach are the local minimum problem and the choice of parameter settings. The focus in ClustalW was on the latter where the parameter weights are adjusted based on empirical knowledge and biological reasoning, during the progress of the alignment algorithm. The justification of this procedure is that as long as identities are dominating the alignment most fixed scoring schemes will find a sufficiently accurate solution. However, when only few identities are present the importance of non-identical positions becomes imminent and another scoring scheme would be more appropriate. Furthermore, gaps will not occur randomly and are more likely to occur in regions without regular secondary structure elements (i.e. in loops). The algorithm also gives lower gap opening costs for existing gaps and higher gap opening costs to the surrounding residues in order keep the existing gaps and not introduce new ones. The adjusted weights are derived from the branch length of the guidance tree. The guidance tree in ClustalW is built by the neighbor-joining method which is good at estimating individual branch lengths and coping with unequal evolutionary rates in different lineages. Taken together, this procedure tends to keep regions of biological importance aligned and introduce gaps only in regions that are less critical for fold or function.

II. Molecular docking Ligands preparation

Chemdraw freeware is one of the chemical compounds drawing package that allows us to draw chemical structures including organics, organometallics, polymers, and Markush structures. It also includes features such as calculation of molecular properties e.g., molecular weight, density, molar refractivity etc., 2D and 3D structure cleaning and viewing, functionality for naming structures, and prediction of logP. We have drawn ten synthesized compounds and saved in mol file format and eventually input file for molecular docking studies.

III.Retrieval of Epidermal growth factor receptor Protein Protein Data Bank

The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived (i.e., secondary) databases that categorize the data differently. For example, both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations; GO categorize structures based on genes.

IV.Importing the Prepared PIM-1 KINASE and ligand Structures

The complex for this exercise is actually in two files, one containing the receptor and one containing the Ligands.

1. Click the Import structures toolbar button.
2. From the Files of type menu, ensure that Maestro is chosen.
3. If the options are not displayed, click Options.
4. Ensure that Import all structures, Replace Workspace, and Fit to screen following import are all selected.
5. From the Include in Workspace option menu, ensure that First Imported Structure is chosen.
6. Navigate to the structures directory and select the file 1fjs_prep_recep.mae.gz.
7. Click Open.

The prepared protein is displayed in the Workspace. The protein structure is displayed in ribbon representation. The structure includes solvent molecules (glycerine) and ions (Ca^{2+} and Cl^{-}), but does not include the ligand.

Ligand Docking

Glide ligand docking jobs require a set of previously calculated receptor grids and one or more ligand structures. Preparation of the ligands before docking is strongly recommended. If a correct Lewis structure cannot be generated for a ligand, it is skipped by the docking job. Glide also automatically skips ligands containing unparametrized elements, such as arsenic, or atom types not supported by the OPLS force fields, such as explicit lone pair “atoms.”

QikProp

QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule’s properties with those of 95% of known drugs. QikProp also flags 30 types of reactive functional groups that may cause false positives in high-throughput screening (HTS) assays.

Invitro anti cancer activity

The epithelial human leukemic cancer cell line MDA-MB-231 (human Caucasian triple-negative, claudin-low, breast carcinoma, lacking the oestrogen, progesterone and human epidermal growth factor receptors (ER, PR and HER2)) was purchased from the European Collection of Cell Cultures (Salisbury, UK). The cells were cultured as monolayers at 37°C in a humidified atmosphere of 5% CO_2. Cultures were maintained in DMEM-HG medium supplemented with 10% (v/v) FBS, 1% (v/v) penicillin/streptomycin and sodium bicarbonate. Cells were subcultured at 80% confluence, using 0.05% trypsin-EDTA (1x) in PBS.

III.RESULTS AND DISCUSSION

Multiple Sequence Alignment

1. Plant scientific name: **Hybanthus enneaspermus (Graham, R.M., 1983)**
Tamil name: **Oridhazh thamarai**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase

>gi|195468853|dbj|BAG66930.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast) [Hybanthus enneaspermus]
SVGFKAGVKDYKLTYYTPDYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTSVWTDGLTSLDRYKGRCYDI
EPVAGEENQYIAYVAYPLDLFEEGSVTNMLTSIVGNVFGFKALRALRLEDLRIPTSYTKTFQGPPHGIQVERDKLNKYGR
PLLGCTIKPKLGLSAKNYG

2. Plant scientific name: **Solanum trilobatum** (griffithii C.B.Clarke 1753)
Tamil name: **thoodhuvalai**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format:
>gi|421958256|gb|AFX72966.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast) [Solanum trilobatum]
SVGFKAGVKEYKLTYYTPEYQTKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLTSLDRYKGRCYRI
ERVVGEKDQYIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIPPAYVKTFQGPPHGIQVERDKLNKYG
RPLLGCTIKPKLGLSAKNYGRAVYECLRGGLDFTKDDENVNSQPF

3. Plant scientific name: **Terminaliaarjuna**
Tamil name: **ArjunTree**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format
>gi|340747732|gb|AEK66716.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast) [Terminalia arjuna]
AAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLTSLDRYKGRCYHIEPVAGEENQYICYVAYPLDL
FEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIPTAYIKTFQGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRA
VYECLRGGLDFTKDDENVNSQPFMRWRDRFLFCAEGIYKAQAET

4. Plant scientific name: **Solanum Xanthocarpum (Carl Linnaeus 1753)**
Tamil name: **Kantankattiri**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format:
>gi|340747732|gb|AEK66716.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast) [Terminalia arjuna]
AAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLTSLDRYKGRCYHIEPVAGEENQYICYVAYPLDLFEEGSVTN
MFTSIVGNVFGFKALRALRLEDLRIPTAYIKTFQGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECLRG
GLDFTKDDENVNSQP

5. Plant scientific name: **Momordica charantia** (Schaefer and Renner)
Tamil name: **Pavakai**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format:
>gi|108951054|gb|ABG24929.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast)

[Momordica charantia]
PGVPPEEAGAAVAAESSTGTWTTVWTDGLTSLDRYKGRCYGIEPVPGEESQFIAYVAYPLDLFEEGSVTNMFTSIVGNVF
GFKALRALRLEDLRIPPAYIKTFQGPPHGIQVERDKLNK

6. Plant scientific name: **Justicia adhatoda** (Shrub, Singhee)
Tamil name: **Adathodai**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format:

>gi|383929174|gb|AFH56430.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast) [Justicia adhatoda]
TKASVGFKAGVKEYKLTYYTPEYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLTSLDRYKGRC
YNIEPVLGETDQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIPPAYIKTFQGPPHGIQVERDKLNKY
GRPLLGCTIKPKLGLSAKNYGRACYECLRGGLDFTKDDENVNSQPFMRWRDRFL

7. Plant scientific name: **Couroupita guianensis (**C. guianensis 1881)
Tamil name: **Nagalingam flower**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format: >
gi|4033944|emb|CAB02228.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast) [Couroupita guianensis]
KASVGFKAGVKDYKLTYYTPEYKTLDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLTTLDRYKGRCY
HIEPVPGEDNQYIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIPPAYIKTFQGPPHGIQVERDKLNKYG
RPLLGCTIKPKLGLSAKPGAVANRVALEACVQARNEGRDLAREGNEIIRAASKWSPELAAACEVWKEIKFEFPAMD

8. Plant scientific name: **Aloe vera (**Barbadensis Miller)
Tamil name: **Sotru Katrazhai**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format: >
gi|37361677|gb|AAQ91152.1| ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast) [Aloe vera]
YTPDYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLTSLDRYKGRCYHIEAVAGEENQFIAYVA
YPLDLFEEGSVTNMFTSIVGNVFGFKAXRALRLEDLRIPPAYSKTFQGPPHGIQVERDKLNKYGXPLLGCXIKPKLGLSA
KNYGRAVYECLRGGLDFTKDDENVNXQPFMRWRDRXLFCAEAIYKAQAETGEIKGHYLNATAGTCEEMIKRAVCA

9. Plant scientific name: **Azadirachta indica (**A. Juss)
Tamil name: **Veppai**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format: >
gi|32815200|gb|AAM98870.1| ribulose 1,5-bisphosphate carboxylase, partial (chloroplast) [Azadirachta indica]
FKAGVKDYKLTYYTPDYVTKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLTSLDRYKGRCYNIEPV
AGEENQYICYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIPPAYSKTFQGPPHGIQVERDKLNKYGRPLL
GCTIKPKLGLSAKNYGRAV

10. Plant scientific name: **Lagenaria siceraria** (Whitaker and Carter **1954)**
Tamil name: **Surakkai**

protein name: ribulose-1,5-bisphosphate carboxylase/oxygenase
protein sequence in FASTA format: >

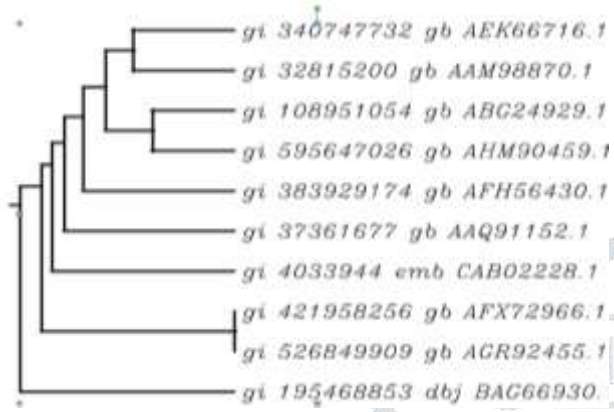
gi|595647026|gb|AHM90459.1| ribulose 1 5-bisphosphate carbosylase/oxygenase large subunit (chloroplast) [Lagenaria siceraria]
MSPQTETKASVGFKAGVKDYKLTYYTPEYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLTSLD
RYKGRCYGIEPVPGEENQYIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDLRIPTAYIKTFQGPPHGIQVER
DKLNKYGRPLLGCTIKPKL

CLUSTAL 2.1 Multiple Sequence Alignments

Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: gi|195468853|dbj|BAG66930.1| 441 aa

Sequence 2: gi|421958256|gb|AFX72966.1| 202 aa
Sequence 3: gi|526849909|gb|AGR92455.1| 192 aa
Sequence 4: gi|340747732|gb|AEK66716.1| 195 aa
Sequence 5: gi|108951054|gb|ABG24929.1| 421 aa
Sequence 6: gi|383929174|gb|AFH56430.1| 213 aa
Sequence 7: gi|4033944|emb|CAB02228.1| 468 aa
Sequence 8: gi|37361677|gb|AAQ91152.1| 422 aa
Sequence 9: gi|32815200|gb|AAM98870.1| 462 aa
Sequence 10: gi|595647026|gb|AHM90459.1| 475 aa

Phylogenetic tree analysis



ADME Properties Prediction

We analyzed 44 physically significant descriptors and pharmaceutically relevant properties of 10 compounds, amongwhich were molecular weight, H-bond donors, H-bond acceptors,log p, log p MDCK, log Kp, humoral absorption according to Lipinski's rule of 5**.** Lipinski's rule of 5 is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including its ADME. These compounds were further evaluated for their drug-like behaviour through analysis of pharmacokinetic parameters required for absorption, distribution, metabolism and excretion (ADME) by use of QikProp. For the ten compounds, the partition coefficient (QPlogPo/w) and water solubility (QPlogS), critical for estimation of absorption and distribution of drugs within the body ranged between 1.139 to 1.852 and -2.982to 1.783. Cell permeability (QPPCaco2), a key factor governing drug metabolism and its access to biological membranes, ranged from 58 to 1924, QPPMDCK ranges from 43 to 1505. Overall, the percentage human oral absorption for the compounds ranged from 66 to 95%.

Table 2: ADME properties of the ten molecules as verified by using QikProp (Schrodinger.9.5)

Ligand	QPlogS	Caco2	% human oral absorption	QPlogKhsa	LogBB	MW	HBD	HBA	QPlog (o/w)	MDCK
1	-2.878	635	86	-0.315	-0.607	237.27	1.00	5.00	1.444	428
2	-2.982	741	89	-0.381	-0.572	279.31	0.00	5.50	1.831	507
3	-2.326	61	66	-0.618	-0.942	223.24	2.00	5.00	1.139	43
4	-2.538	959	91	-0.351	-0.396	237.27	0.00	4.00	1.852	705
5	-2.499	58	68	-0.638	-0.850	251.25	0.00	4.50	1.570	44
6	-1.783	134	74	-0.805	-0.514	223.24	0.00	4.50	1.464	109
7	-2.149	68	70	-0.553	-0.781	209.21	0.00	3.00	1.672	52
8	-2.596	695	88	-0.425	-0.499	279.31	0.00	5.50	1.720	544
9	-2.126	1924	95	-0.603	-0.120	251.30	0.00	5.50	1.564	1505
10	-2.202	757	87	-0.471	-0.412	223.24	0.00	4.00	1.404	545

Predicted aqueous solubility; S in mol/L (acceptable range; -6.5 to 0.5);
Percentage of human oral absorption; (<25% is poor and >80% is high);
Prediction of binding to human serum albumin; (acceptable range; -1.0 to 1.5);
Prediction of brain/blood; (acceptable range; -3.0 to 1.2);
Molecular weight (< 500Da);
Hydrogen bond donor (< 5);
Hydrogen bond acceptor (< 10);
Predicted octanol /water partition co-efficient log p (acceptable range; -2.0 to 6.5).
Predicted apparent MDCK cell permeability in nm/s.

Molecular docking Studies

Binding mode of compound 1 with PIM kinase1

The binding conformation of compound 1within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound1 were -7.622Kcal/mol and -48.908kcal/mol, respectively. Upon the examination of docking features between compound1 and Pim1 it was found only one hydrogen bond interaction. The hydrogen atom of the compound 1 was interacted with negative charged residue of ASP 128with bond length (2.50Å). Furthermore that the following residues are mainly involved in hydrophobic interactions PHE 49, VAL 52, ILE 185, LEU 174, VAL 126, and LEU 44.

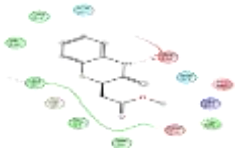


Figure 1: 2D & 3D structure of target protein PIM1 with drug molecule **1**.

Binding mode of compound 2 with PIM kinase1

The binding conformation of compound2 within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 2 were -7.478Kcal/mol and -45.769kcal/mol, respectively. Upon the examination of docking features between compound 2 and Pim1.it was found only one hydrogen bond interactions.

Figure 2: The 2D &3 D structure of target protein Pim1 with drug molecule 2.

Binding mode of compound 3 with PIM kinase1

The binding conformation of compound3within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 3 were -6.891Kcal/mol and -54.758kcal/mol, respectively. Upon the examination of docking features between compound **3** and Pim1.it was found only one hydrogen bond interaction. The hydrogen atom of the compound 3 were nicely interacted with side chain hydrogen atom of the negative charged residue of ASP 128 with bond length (1.98Å).

Figure 3: 2D & 3D structure of target protein **PIM1** with drug molecule **3**.

Binding mode of compound 4 with PIM kinase1

The binding conformation of compound 4within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 4 were -6.006Kcal/mol and -43.741kcal/mol, respectively. it was found no hydrogen bond interactions. Interestingly many hydrophobic interactions were found between drug compounds 4 with pim1.

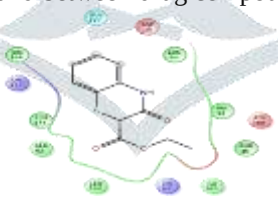


Figure 4: 2D & 3D structure of target protein PIM1 with drug molecule **4**.

Binding mode of compound 5 with PIM kinase1

The binding conformation of compound 5within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 5 were-6.001Kcal/mol and -48.591kcal/mol, respectively. It was found only two hydrogen bond interactions. The side chain hydrogen atom of the positive charged residue of ARG 122 were strongly interacted with oxygen atom of the compound 5 then the backbone hydrogen atom of the hydrophobic residue of VAL 126 were nicely interacted with oxygen atom of the compound 5 with bond distance (1.83Å, 2.2398Å). Furthermore that the following residues are mainly involved in hydrophobic interactionsLEU44, LEU 174, PRO 125, PRO123.

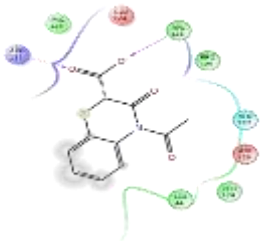


Figure 5: 2D & 3D structure of target protein PIM1 with drug molecule **5**.

Binding mode of compound 6 with PIM kinase1

The binding conformation of compound 6within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 6 were -5.941Kcal/mol and -21.314kcal/mol, respectively. It was found no hydrogen bond interactions. Interestingly many hydrophobic interactions were found between drug compound 6 with pim1. That the following residues are mainly involved in hydrophobic interactions Val 52, PHE 49, ILE 185, LEU 44, LEU 120, ALA 65, and LEU 174.

Figure 6: 2D & 3D structure of target protein PIM1 with drug molecule **6**.

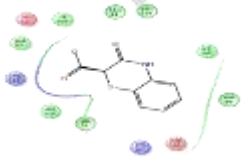
Binding mode of compound 7 with PIM kinase1

The binding conformation of compound 7within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 7 were -5.476Kcal/mol and -35.234kcal/mol, respectively. It was found no hydrogen bond interactions. Interestingly many hydrophobic interactions were found between drug compound 7 with pim1. That the following residues are mainly involved in hydrophobic interactions Val 52, PHE 49, ILE 185, LEU 44, LEU 120, ALA 65, LEU 174.

Figure 7: 2D & 3D structure of target protein PIM with drug molecule **7**.

Binding mode of compound 8 with PIM kinase1

The binding conformation of compound 8within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 8 were-5.301Kcal/mol and -38.521kcal/mol, respectively. It was found only two hydrogen bond interactions. The side chain hydrogen atom of the positive charged residue of ARG 122 were strongly interacted with oxygen atom of the compound 8 then the backbone hydrogen atom of the hydrophobic residue of VAL 126 were nicely interacted with oxygen atom of the compound 8 with bond distance (1.131Å,



1.118Å). Furthermore that the following residues are mainly involved in hydrophobic

Figure 8: 2D & 3D structure of target protein PIM1 with drug molecule **8**.

Binding mode of compound 9 with PIM kinase1

The binding conformation of compound 9within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 9 were -3.251Kcal/mol and -21.31kcal/mol, respectively. It was found no hydrogen bond interactions. Interestingly many hydrophobic interactions were found between drug compound 9 with pim1. That the following residues are mainly involved in hydrophobic interactions Val 52, PHE 49, ILE 185, LEU 44, LEU 120, ALA 65, LEU 174.

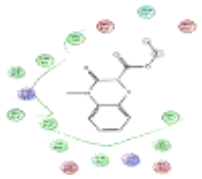


Figure 9: 2D & 3D structure of target protein PIM1 with drug molecule **9**.

Binding mode of compound 10 with PIM kinase1

The binding conformation of compound 10within the active site of the Pim1 has been analyzed. The glide score and glide energy values for compound 10 were -3.136Kcal/mol and -20.221kcal/mol, respectively. It was found no hydrogen bond interactions. Interestingly many hydrophobic interactions were found between drug compound 10 with pim1. That the following residues are mainly involved in hydrophobic interactions Val 52, PHE 49, ILE 185, LEU 44, LEU 120, ALA 65, and LEU 174.

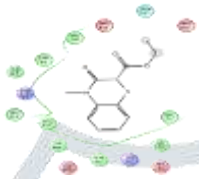
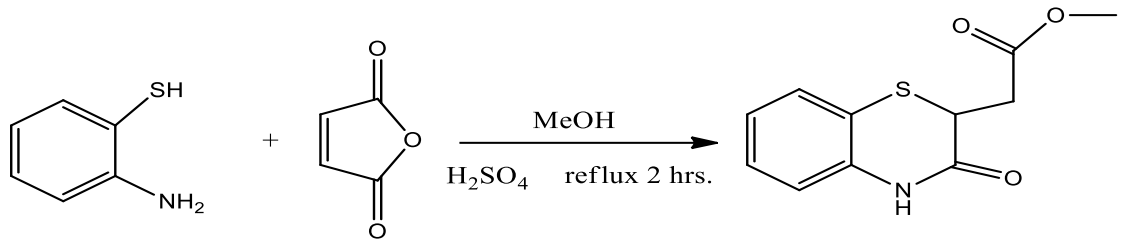


Figure 10: 2D & 3D structure of target protein PIM1 with drug molecule **9**.

Binding mode of compound 10 with PIM kinase1

Compound Name	Glide Score	Glide Energy	Interacting Residues	Distance (Å)
1	-7.622	-48.908	ASP 128	2.50
2	-7.478	-45.769	VAL 126	1.79
3	-6.891	-54.758	ASP 128	1.98
4	-6.006	-43.741	-	-
5	-6.001	-48.591	ARG 122 VAL 126	1.83 2.2398
6	-5.941	-21.314	-	-
7	-5.476	-35.234	-	-
8	-5.301	-38.521	ARG 122 VAL 126	1.131 1.118
9	-3.251	-21.31	-	-
10	-3.136	-20.221	-	-

Compound Name
Glide score (Kcal/mol);
Glide energy (Kcal/mol).
Interacting residues;
Distance between the protein and ligand (Å);



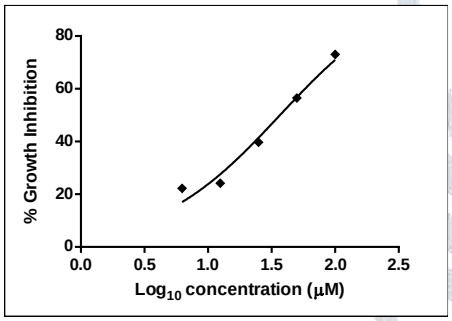
2-Aminobenzenethiol **maleic anhydride** **1**

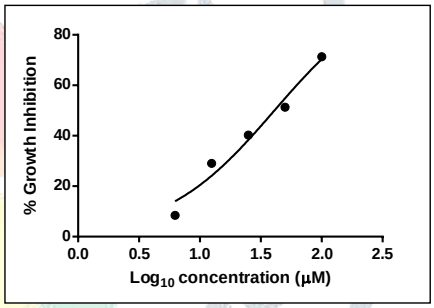
The formation of compound **1** was explained on the basis of an IR band at 1730 and 1690 cm^{-1} due to acid and cyclic carbonyl groups respectively. This was also confirmed from the ^{1}H-NMR signal at δ 4.85 for one proton is due to thiazine ring proton. The bunch of signals between 6.99-7.28 (m, 4H, ArH) are attributed to aromatic protons, signal at δ 11.1 is due to –COOH proton and the signal at 10.90 is due to ring N-H proton.

The formation of compound was explained on the basis of an IR band at 1740 and 1690 cm^{-1} due to ester and cyclic carbonyl groups respectively. This was also confirmed from the ^{1}H-NMR signal at δ 3.6 due to -OCH_3 group. The pair of doublet of doublets at δ 2.57 and 2.92 each of one proton is due to –CH_2 group, The doublet of doublets at δ 3.85 for one proton is due to thiazine ring proton. The bunch of signals between 6.99-7.32 (m, 4H, ArH) are attributed to aromatic protons and the signal at 10.60 is due to ring N-H proton.

MTT ASSAY

The effect of 1 and 2 on proliferation of K562 leukemic cells was verified using MTT assay. K562 cells were treated with 6.25, 12.5, 25, 50 and 100 µg/ml of compounds and were subjected to MTT assay after 48 h (Fig. 11). Results showed that cell viability was affected at higher concentration in case of the benzothiazine compounds 1 and 7. The IC_{50} values of the compounds **1** and **7** were 36.82 µg/ml and 40.94 µg/ml respectively. Complementing to the docking data compound I showed higher activity when compared to compound **7**.





Compound **1**
IC_{50} = 36.82 µg/ml

Compound**2**
IC_{50} = 40.94 µg/ml

Figure 11: MTT assay- %Growth Inhibition of **1**and **2**against K562 Cells

Table 4: MTT assay results

Concentration of sample (µg/ml)	**% viability**	
	1	**7**
6.25	77.74 ± 3.6	91.56 ± 1
12.5	75.82 ± 9.7	70.99 ± 3.7
25	60.30 ± 2.8	59.79 ± 8.1
50	43.48 ± 5.8	48.69 ± 9.2
100	26.95 ± 1.4	28.71 ± 3.7

Table 5: MTT assay results

Concentration of sample (µg/ml)	% Inhibation	
	1	7
6.25	22.25 ± 3.6	8.439 ± 1
12.5	24.17 ± 9.7	29.00 ± 3.7
25	39.69 ± 2.8	40.20 ± 8.1
50	56.51 ± 5.8	51.30 ± 9.2
100	73.04 ± 1.4	71.28 ± 3.7

Conclusion

PIM1 is commonly over expressed in human cancers and represents an interesting novel therapeutic target. Due to its anti-apoptotic activity PIM1 is referred to as a proto-oncogene involved in the progression of different cancer types. In the present work ten benzothiazine molecules were selected which have drug like properties tested by means of determining their ADME properties and docked with PIM1 protein to study their interactions. From the docking studies two compounds 1 and 7 were selected for synthesis and their anticancer was determined against K562 cell lines by using MTT assay. The best fit compound 1 which has the least score in the docking study showed a good anticancer activity against K562 cell lines. So compound 1 act as an effective PIM1 inhibitor and may serve as a template for further studies.

References

1. Aho TL, Sandholm J, Peltola KJ, et al. Pim-1 kinase promotes inactivation of the pro-apoptotic Bad protein by phosphorylating it on the Ser112 gatekeeper site. FEBS Lett 2004; 571:43-9.
2. Arung ET, Wicaksono BD, Handoko YA, Kusuma IW, Yulia D, Ferrry S (2009) J Immunol Methods 8:217-324.
3. Bachmann M, Hennemann H, Xing PX, et al. The oncogenic serine/threonine kinase pim-1 phosphorylates and inhibits the activity of Cdc25C-associated kinase 1 (C-TAK1): a novel role for Pim-1 at the G2/M cell cycle checkpoint. J Biol Chem 2004; 279:48319-28.
4. Bachmann M, Kosan C, Xing PX, et al. The oncogenic serine/threonine kinase pim-1 directly phosphorylates and activates the G2/M specific phosphatase Cdc25C. Int J Biochem Cell Biol 2006; 38:430-43.
5. Bakavoli M, Nikpour M, Rahimizadeh M, Saberi MR, Sadeghian H. Design and synthesis of pyrimido[4,5-b][1,4]benzothiazine derivatives, as potent 15-lipoxygenase inhibitors. Bioorg Med Chem 2007;15(5):2120-6.
6. Barange DK, Batchu VR, Gorja D, Pattabiraman VR, Tatini LK, Babu JM, *et al.* Rigioselective construction of six-membered fused heterocyclic rings via Pd/C-mediated C-C coupling followed by iodocyclization strategy a new entry to 2*H*-1,2- benzothiazine-1,1-dioxides. Tetrahedron 2007; 63:1775-89.
7. Barazarte A, Lobo G, Gamboa N, Rodrigues JR. Synthesis and antimalarial activity of pyrazolo and pyrimido benzothiazine dioxide derivatives. Eur J Med Chem 2008;30:1-8.
8. Bhattacharya N, Wang Z, Davitt C, et al. Pim-1 associates with protein complexes necessary for mitosis. Chromosoma 2002; 111:80-95.
9. Brault L, Gasser C, Bracher F, et al. PIM serine/threonine kinases in the pathogenesis and therapy of hematologic malignancies and solid cancers. Haematologica 2010;95:1004-15.
10. Bullock AN, Debreczeni J, Amos AL, et al. Structure and substrate specificity of the pim-1 kinase. J Biol Chem 2005; 280:41675-82.
11. Cen B, Mahajan S, Zemskova M, et al. Regulation of Skp2 levels by the pim-1 protein kinase. J Biol Chem 2010; 285:29128-37.
12. Chen J, Kobayashi M, Darmanin S, et al. Hypoxia-mediated up-regulation of pim-1 contributes to solid tumor formation. Am J Pathol 2009; 175:400-11.
13. Cuypers HT, Selten G, Quint W, et al. Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. Cell 1984; 37:141-50.
14. Dabholkar VV, Gavande RP. Synthesis of pyrazolyl 1,4-benzothiazine derivatives. Heteroletters 2011; 13:255-61.
15. Dar AA, Goff LW, Majid S, et al. Aurora kinase inhibitors--rising stars in cancer therapeutics? Mol Cancer Ther 2010; 9: 268-78.
16. Eiring AM, Harb JG, Neviani P, et al. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. Cell 2010;140:652-65.
17. Fabian MA, Biggs WH III, Treiber DK, et al. A small molecule-kinase interaction map for clinical kinase inhibitors. Nat Biotechnol 2005; 23: 329-36.
18. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127: 2893-917.
19. Fringuelli R, Schiaffella F, Utrilla Navarro MP, Milanese L, Santini C, Rapucci M, *et al* 1,4-benzothiazine analogues and apoptosis Structure-activity relationship. Bioorg Med Chem 2003;11(15):3245-54.

20. González-Gómez JC, Santana L, Uriarte E, Brea J, Villazón M, Loza MI, *et al.* New arylpiperazine derivatives with high affinity for alpha1A, D2 and 5-HT2A receptors. Bioorg Med Chem Lett 2003; 13(2):175-8.
21. Grandolini G, Luana P, Ambrogi V. Synthesis of some new 1,4-benzothiazine and 1,5-benzothiazepine tricyclic derivatives with structural analogy with TIBO and their screening for anti-HIV activity. Eur J Med Chem 1999; 34:701-9.
22. Greten TF, Korangy F, Manns MP, Malek NP. Molecular therapy for the treatment of hepatocellular carcinoma. Br J Cancer 2009; 100: 19-23.
23. Gu JJ, Wang Z, Reeves R, Magnuson NS. PIM1 phosphorylates and negatively regulates ASK1-mediated apoptosis. Oncogene 2009; 28: 4261-71.
24. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74

Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.
25. Hoover D, Friedmann M, Reeves R, Magnuson NS. Recombinant human pim-1 protein exhibits serine/threonine kinase activity. J Biol Chem 1991; 266: 14018-23.
26. Hori M, Kataoka T, Shmizu H, Imai Y. Ring expansion reactions of benzothizolines to benzothiazine. Chem Pharm Bull 1973; 5: 27.
27. Jakobsen JN, Sorensen JB. Review on clinical trials of targeted treatments in malignant mesothelioma. Cancer Chemother Pharmacol 2011; 68: 1-15.
28. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
29. Jung CH, Ro SH, Cao J, et al. mTOR regulation of autophagy. FEBS Lett 2010;584:1287-95.
30. Katayama H, Sen S. Aurora kinase inhibitors as anticancer molecules. Biochim Biophys Acta 2010;1799:829-39.
31. Kim O, Jiang T, Xie Y, et al. Synergism of cytoplasmic kinases in IL6-induced ligand-independent activation of androgen receptor in prostate cancer cells. Oncogene 2004;23:1838-44.
32. Lens SM, Voest EE, Medema RH. Shared and separate functions of polo-like kinases and aurora kinases in cancer. Nat Rev Cancer 2010;10:825-41.