COMPUTER AIDED DESIGN OF POTENT NOSCAPINE ANALOGS AND EVALUATION AS ANTI-CANCER DRUGS

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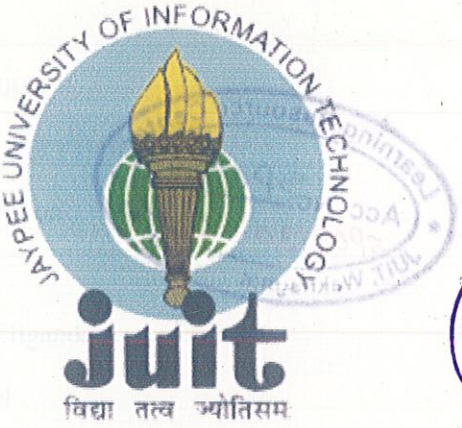

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CERTIFICATE

This is to certify that the work titled **"COMPUTER AIDED DESIGN OF POTENT NOSCAPINE ANALOGS AND EVALUATION AS ANTI-CANCER DRUGS"** submitted by "**Pranika Singh and Garima Thakur**" in partial fulfillment for the award of degree of B.Tech. ofJaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of Supervisor: .....

Name of Supervisor: Dr. Pradeep K. Naik

Designation:Associate Professor of Biotechnology and Bioinformatics,

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Date: 28 MAY, 2013

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Last but not the least, our family and the one above all of us, the omnipresent God, for answering our prayers for giving us the strength to plod on ,thank you so much Dear Lord.

PranikaSingh(091506)

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ABSTRACT

As our knowledge of microtubule-targeting drugs increases, we realize that the mechanism underlying the anti-cancer activity of these agents may mainly lie in their inhibitory effects on spindle microtubule dynamics.There is increasing evidence showing that even minor alteration of microtubule dynamics can engage the spindle checkpoint, arresting cell cycle progression at mitosis and eventually leading to apoptotic cell death. The effectiveness of microtubule-targeting drugs for cancer therapy has been impaired by various side effects, notably neurological and hematological toxicities. Drug resistance is another notorious factor that thwarts the effectiveness of these agents, as with many other cancer chemotherapeutics. It was shown previously that an antitussive plant alkaloid, noscapine, binds tubulin, displays anticancer activity, and has a safe pharmacological profile in humans. With this discovery many efforts has been made to design more potent analogs of noscapine and some promising results were also found , but still the IC50 values remain in the high micromolar ranges (~21.1 to 100 μM). So here we tried to develop more analogs of noscapine with an effective glide score, whose IC50 value can be evaluated in future to know whether or not they are more potent than other analogs present.

Signature of the student Singh, Garima

Name of Student Pranika Singh, Garima Thakur

Date 28/04/2013

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CHAPTER 1

INTRODUCTION

Cancer is a class of diseases characterized by out-of-control cell growth.Normal cells in the body follow an orderly path of growth, division, and death. Unlike regular cells, cancer cells do not experience programmatic death, called apoptosis, and instead continue to grow and divide. This leads to a mass of abnormal cells that grows out of control.

The treatment of cancer has undergone evolutionary changes as understanding of the underlying biological processes has increased. Non-hematological cancers can be cured if entirely removed by surgery, but this is not always possible. When the cancer has metastasized to other sites in the body prior to surgery, complete surgical excision is usually impossible. Radiation therapy (also called radiotherapy, X-ray therapy, or irradiation) is the use of ionizing radiation to kill cancer cells and shrink tumors. Radiation therapy are localized and confined to the region being treated by damaging their genetic material. Although radiation damages both cancer cells and normal cells, most normal cells can recover from the effects of radiation and function properly.Chemotherapy is the treatment of cancer with drugs ("anticancer drugs") that can destroy cancer cells. Many agents currently used for cancer chemotherapy are cell-cycle-specific growth inhibitors.The majority of chemotherapeutic drugs can be divided into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumour agents. All of these drugs affect cell division or DNA synthesis. Some newer agents do not directly interfere with DNA. These include monoclonal antibodies and the new tyrosine kinase inhibitors, which directly target a molecular abnormality in certain types of cancer (chronic myelogenousleukemia, gastrointestinal stromal tumors).These are examples of targeted therapies.

The MT targeting drugs earlier used to fall in two classes: one that depolymerize microtubules (such as vinca alkaloids) and the others that over polymerize MTs and bundle them (such as taxanes and epothilone).Both the polymerization and depolymerization of microtubulesare important for the proper execution of celldivision machinery, and by interfering with these processesmicrotubule-binding drugs have become usefultools for inhibiting mitotic progression [1, 2].The mechanism by which these drugs disruptmicrotubule

dynamics has led to the discovery anddevelopment of important agents for the clinical management of cancer. These anti- microtubule agents are frequently toxic to normal tissues and are effective only for certain types of cancer [3-6]. Hence, new andbetter chemotherapeutic drugs are needed with less toxicity and with better therapeutic value.

Noscapine, an alkaloid from opium, was discovered in Dr.joshi's lab that turned out to be a promising tubulin bindingagent and is in Phase II clinical trials as an anticancer agent.[7]. Since the 1960s, noscapine has been widely usedas an cough suppressant in humans and in experimental animals, with very few side effects and no addiction liability. In addition, its water solubility and feasibility for oral administration are valuable advantage over many other drugs for cancer therapy (for a review see ref. #8). Recent studies show that noscapine binds stoichiometrically to tubulin (one noscapine molecule per α-βtubulin dimer), alters tubulin conformation, and arrests mammalian cells at mitosis[9].

Noscapine inhibits microtubule dynamics by prolonging an attenuated 'pause' state in which growth or shortening is not detectable, it does not bundle microtubules or cause microtubule depolymerization, but rather slows microtubule dynamics.Noscapine and its derivatives do not cause detectable toxicity to normally dividing cells and do not cause serious detectable neuropathies in animal models.As noscapine was present in the market for many years, the compound itself cannot be patented.Although noscapine is cytotoxic in a variety of different cancer cell lines in the public library of the U.S. National Cancer Institute (60-cell screen), the IC50 values remain in the high micromolar ranges (~21.1 to 100 μM) [10-13]. Opportunities must now be explored to acquire better and more effective derivatives. Some of the initial efforts have already been encouraging. Derivates of Noscapine have been developed and tested and the results have been promising in terms of activity which is better than the lead compound, without compromising the toxicity profile of noscapine .

The details were published in 2003 for "Brominated Derivatives of Noscapine "[14]as a result of efforts made by Emory researchers, in collaboration with scientists at University of Delhi, India.Several halo-derivativesof noscapine have been synthesized and evaluated for their cytotoxic activities [7, 15].While, noscapine is a powerful anti-microtubule agent,it had to be said that some analogs of this compounds such as9- nitro- noscapine has the potential to be used in thetreatment of resistant cancer when other microtubule agentsare ineffective.The effectiveness of noscapine was improved by many of the efforts to decrease the dissociation constant (Kd) from 144 to 86 lM by nitronoscapine (2), 80 lM by F-noscapine(3), 54 lM by

Br-noscapine (4), 40 lM by Cl-noscapine (5), and 22 lM by I-noscapine (6) [8, 9].Amino-noscapine[(S)-3-((R)-9-amino-4-methoxy-6-methyl-5,6,7,8-tetrahydro[16-18]dioxolo[4,5-g]isoquinolin-5-yl)-6,7-dimethoxy isobenzofuran-1(3H)-one] that has higher tubulin binding activity(predicted DGbind = -6.438 kcal/mol and experimental DGbind = -6.628 kcal/mol) than noscapine.In our study we are trying to develop more potent analogues of noscapine. Theoretical calculations, in particular the molecular docking method seems to be a proper tool for the same.

1.1 Computational Methods

When the structure of the target protein is known, receptor-based docking can be employedComputational docking can be described as the process of modeling the binding orientation of a specific ligand to a specific protein of interest, i.e. a "receptor". Docking provides a understanding of the mechanism involved in protein-ligand binding in general, as well as helps to understand the details of the interactions in a specific protein or protein-ligand complex of interest. This approach aims to predict correctly the structure of the intermolecular complex formed between the target receptor and the ligand. To correctly dock a molecule, two technical challenges imply, (1) the pose generation (docking) of the ligand in the active site and (2) the evaluation of the different poses (scoring). Scoring requires estimation of the binding energy between protein and ligand and produces a relative rank-ordering between different ligand, docked to the same target.

Computer-aided drug design techniques are nowadays established and have emerged as key strategy to help assessing compounds. Predicting chemical and biological properties with computational models identifies compounds that are likely to fail in primary, secondary and further downstream screen at significantly lower costs. Integration of computational approaches into the drug discovery process is nicely reviewed by Chin [19].

1.1.1Glide Docking

Glide is designed to assist in high-throughput screening of potential ligands based on binding mode and affinity for a given receptor module. One can compare ligand scores with those of other rest ligands or compare ligand geometrics with those of reference ligands. We used the Glide program [20] as our docking engine. The Glide docking algorithm performs a series of

hierarchical searches for locations of possible ligand affinity within the binding site of a receptor. A rough positioning and scoring algorithm is applied during the initial search step, followed by torsional energy optimization on an OPLA-AA non-bonded potential energy grid for enduring candidate poses. The pose conformations of the very best candidates are further refined by using Monte Carlo sampling. Selection of the final docked pose is accomplished using a Glide score, which is a model energy function that combines empirical and force field based terms. The Glide score is a modified and extended version of the ChemScore function [21].

1.1.2Prime MM-GBSA

This application is used to predict the free binding energy between a receptor and a ligand. MM-GBSA is a method that combines OPLS molecular mechanics energies (*E*MM), surface generalized Born solvation model for polar solvation (G_{SGB}), and a nonpolar solvation term (G_{NP}). The G_{NP} term comprises the non-polar solvent accessible surface area and vander Waals interactions. The total free energy of binding is calculated as:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

$$G = E_{MM} + G_{SGB} + G_{NP}$$

Thus in this study we have applied this approach to design morepotent noscapinoid and to evaluate them as anti cancer drug.

CHAPTER 2

MATERIALS AND METHODS

2.1Protein Modelling and preparation:

PDB ID 1SA0 was taken for our calculations .It has 5 chains two alpha ,two beta and one stathmin chain. We removed one alpha one beta and stathmin chain, now we have a pdb file having an α-β dimer complexed with ligand colchicine ,GTP and a GDP molecule.One more problem with the tubulin structure is that there are missing residues in the structure.(residues for which coordinates could not be found with the experimental techniques),(fig 1) since we are working with the interaction of tubulin with different molecules, absence of some residues would affect the interactions as the molecules may interact differently in presence and absence of the missing residues. Also we need to do a lot of molecular modeling calculations and gaps in structure which could affect the interactions so we needed to fix our structure first and fill the gaps.

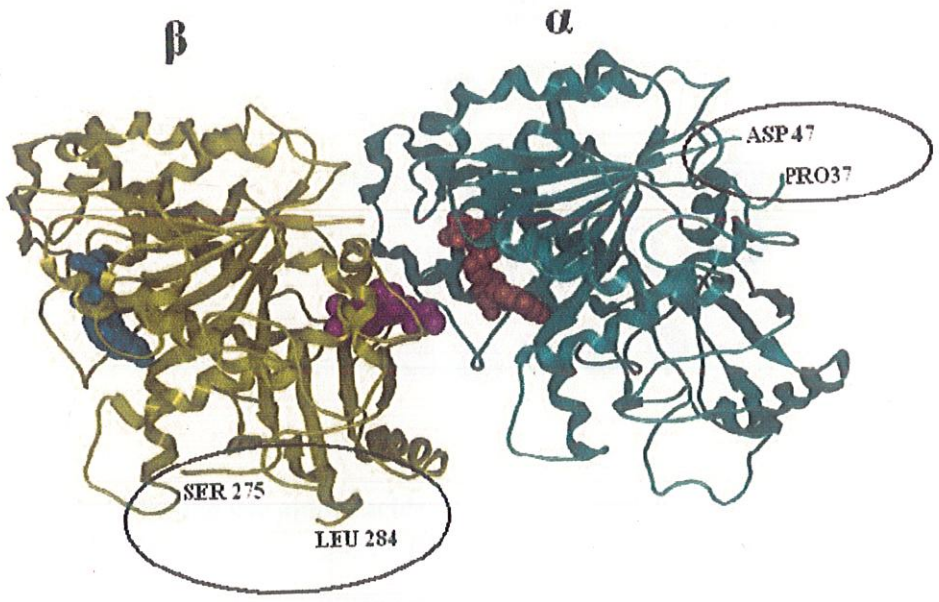
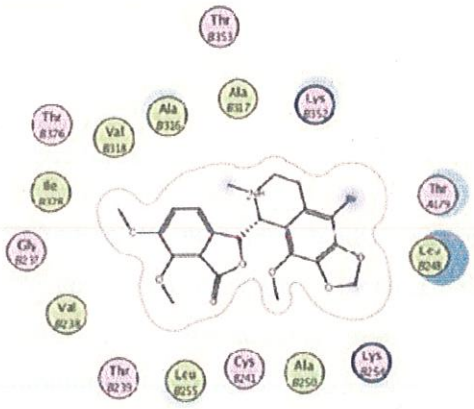


Fig 2.1: Structure of tubulin dimer along with ligand colchicine(pink),GTP(Brown)and GDP(blue) showing the gaps(encircled) and the end residues of gaps ASP 47 and PRO 37 in A chain and SER275 andLEU384 in B chain .

The protein was modelled using multiple templates (using MOE) and the gaps were filled. The structure was subjected to energy minimization using OPLS 2005 forcefield and minimization algorithm PRCG. The structure was validated using Procheck, Errat and verify 3D.

2.2 Noscapine Binding site:

The computationally determined noscapinoid binding pocket of tubulin is considerably hydrophobic. The ligand plot generated from the tubulin–colchicine and tubulin–Br-noscapine docked complexes revealed identical sets of amino acids, interacting with both ligands (Fig). The binding modes and key protein–ligand interactions are shown in Fig. 4C. The molecular superposition of bound conformation of colchicine, noscapine, and Br-noscapine indicates that these compounds have more or less identical binding mode with tubulin. Macromodel surface representation of binding site according to residue charge (electropositivecharge) is shown in Fig.2.2.



Binding site amino acids			
THR A 179	CYS B239	LEU B253	LYS B350
GLY B235	LEU B246	ALA B314	THR B351
VAL B236	ALA B248	ALA B315	THRB366
THR B237	LYS B252	VAL B316	ILE B368

Table 2.1:The binding site we got from literature which we further used for docking.

2.3 Designing and Preparation of ligands :

The ligands were designed manually by replacing various functional groups on noscapine's side chains. An initial dataset of 20 analogues were designed. Molecular structures of noscapine and its analogs were built using the builder feature in Maestro (Schrodinger package). Each structure was assigned an appropriate bond order using Ligprep (version 2.3, Schrodinger Inc.). Ligprep utility produces a number of structures from each input structure with various ionization states, tautomers, stereochemistries, and ring conformations. The program automatically generated all possible stereoisomers (default value of 32 was used) for each ligand. Furthermore, a unique low-energy ring conformation for each stereoisomer with correct chirality was generated with the help of Ligprep. All structures were subsequently subjected to molecular mechanics energy minimization using Impact (version 5.6, Schrodinger Inc.) with default settings: maximum cycles 100, conjugate gradient minimizer, initial step size 0.05, maximum step size 1.0, gradient criteria 0.01. Partial atomic charges were assignedto the molecular structures using the 2005 implementation of the OPLS-AA force field. These optimized structures were used for Glide (grid-based ligand docking with energetics) docking.

2.3.1 The Glide Docking Protocol

All the ligands were docked to the noscapine binding site using Glide version 4.0. All docking calculations were performed using the "Standard precision" (SP) mode of Glide docking. The docking results were refined using "Extra Precision" (XP) mode of Glide docking (version 4.5, Schrodinger Inc.) with the 2005 implementation of the OPLS-AA force field. Briefly, Glide approximates a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using a series of hierarchical filters. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand pose. The binding site is defined in terms of two concentric cubes: the bounding box, which must contain the mass center of any acceptable ligand pose, and the enclosing box, which must contain all the atoms of a ligand pose for successful docking into the binding site. Glide also performed conformational searches for each input structure during docking process. A set of initial ligan conformations is generated through exhaustive search of the torsional minima and the

conformers are clustered in a combinatorial fashion. Each cluster, characterised by a common conformation of the core and an exhaustive set of side-chain conformations, is docked as a single object in the first stage. The search begins with a rough positioning and scoring phase that significantly narrows the search space and reduces the number of poses to be further considered to a few hundred. These selected poses are energy minimized on precomputed OPLS-AA van der Waals and electrostatic grids for the receptor. In the final stage, the 5–10 lowest-energy pose obtained in this fashion are subjected to aMonte Carlo sampling in which nearby torsional minima are examined, and the orientation of peripheral groups of the ligand is refined. The minimized poses are then rescored using the GlideScore function. In this work the bounding box of size 12 °A ×12 °A ×12 °A was defined in tubulin and centered at the centroid of the noscapine binding site by selecting the residues of the noscapine binding site as mentioned in naik et al. The larger enclosing box with an edge length of 12 °A was also defined (which occupied all the atoms of the docked poses).The scale factor of 0.4 for van der Waals radii was applied to atoms of protein with absolute partial charges less than or equal to 0.25. Five thousand poses per ligand were generated during the initial phase of the docking calculation out of which best 1000 poses per ligand were chosen for energy minimization. Energy minimization protocol included dielectric constant of 4.0 and 1000 steps of conjugate gradient minimizations. Upon completion of each docking calculation, 100 poses per ligand were generated and the best docked structure was chosen using a GlideScore (Gscore) function. GlideScore is a more sophisticated version of ChemScore with force field-based components and additional terms accounting for solvation and repulsive interactions. The choice of the best pose is made using a model energy score (Emodel) that combines the energy grid score, Gscore, and the internal strain of the ligand. Glide docking is widely used by pharmaceutical industries and academic institutes to study drug–target interactions and to design new drug candidates with improved activities because of its better accuracy .

2.3.2 Ligand & Structure-Based Descriptors (LSBD) Protocol

Further refinement of docking results and calculation of binding affinity was performed.using Prime MM-GBSA. The Prime MM-GBSA calculations were performed using the Ligand & Structure-Based Descriptors (LSBD) application of the Schrödinger software package. These calculations were applied on the ligand-receptor complex structures obtained from Glide docking. Prime MM-GBSA calculates the free energy of binding between a ligand and a receptor. This method combines OPLS molecular mechanics energies (EMM), surface generalized Born solvation model for polar solvation (GSGB), and a nonpolar solvation term

(GNP) in order to calculate the total free energy of binding between the receptor and the ligand as follows. GNP term comprises the nonpolar solvent accessible surface area and van der Waals interactions. The total free energy of binding is calculated as:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

$$G = E_{MM} + G_{SGB} + G_{NP}$$

The docked poses were minimized using the local optimization feature in Prime (version 2.2, Schro¨dinger) and the energies were calculated using the OPLS 2005 force field and the GBSA continuum solvent model. During energy minimization, all the residues of the protein beyond 12 Å from the bound ligand were kept frozen.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Protein Modelling and preparation:

The protein was modelled using multiple templates and the gaps were filled. The structure was subjected to energy minimization using OPLS 2005 forcefield and minimization algorithm PRCG(Polak–Ribiere Conjugate Gradient algorithm).

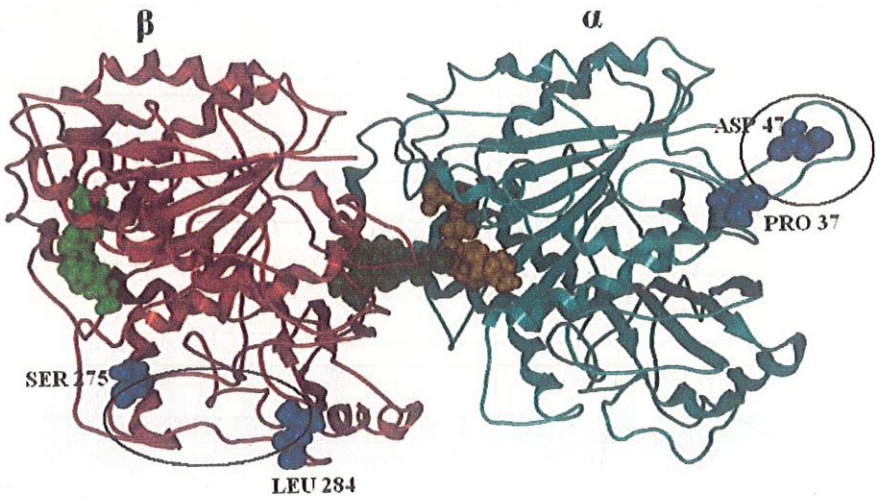


Fig 3.1: Modelled tubulin dimer along with ligand colchicine(dark green),GTP(Brown)and GDP(light green) having no gaps as is evident from the figure gap between the end residues of gaps ASP 47 and PRO 37 in A chain and SER275 and LEU384 in B chain are filled.

The structure was further validated using Procheck, Errat and verify 3D. Conclusions have been drawn from the following that structure has maximum number if residues (78.2%) in the most favourable regions as seen in Ramachandran Plot , 96.76% of atoms have 3D-1D score > 0.2 . Errat score is well-suited for evaluating the progress of crystallographic model building and refinement. The program works by analyzing the statistics of non-bonded interactions between different atom types. In this it gives a good score of 90.789 .

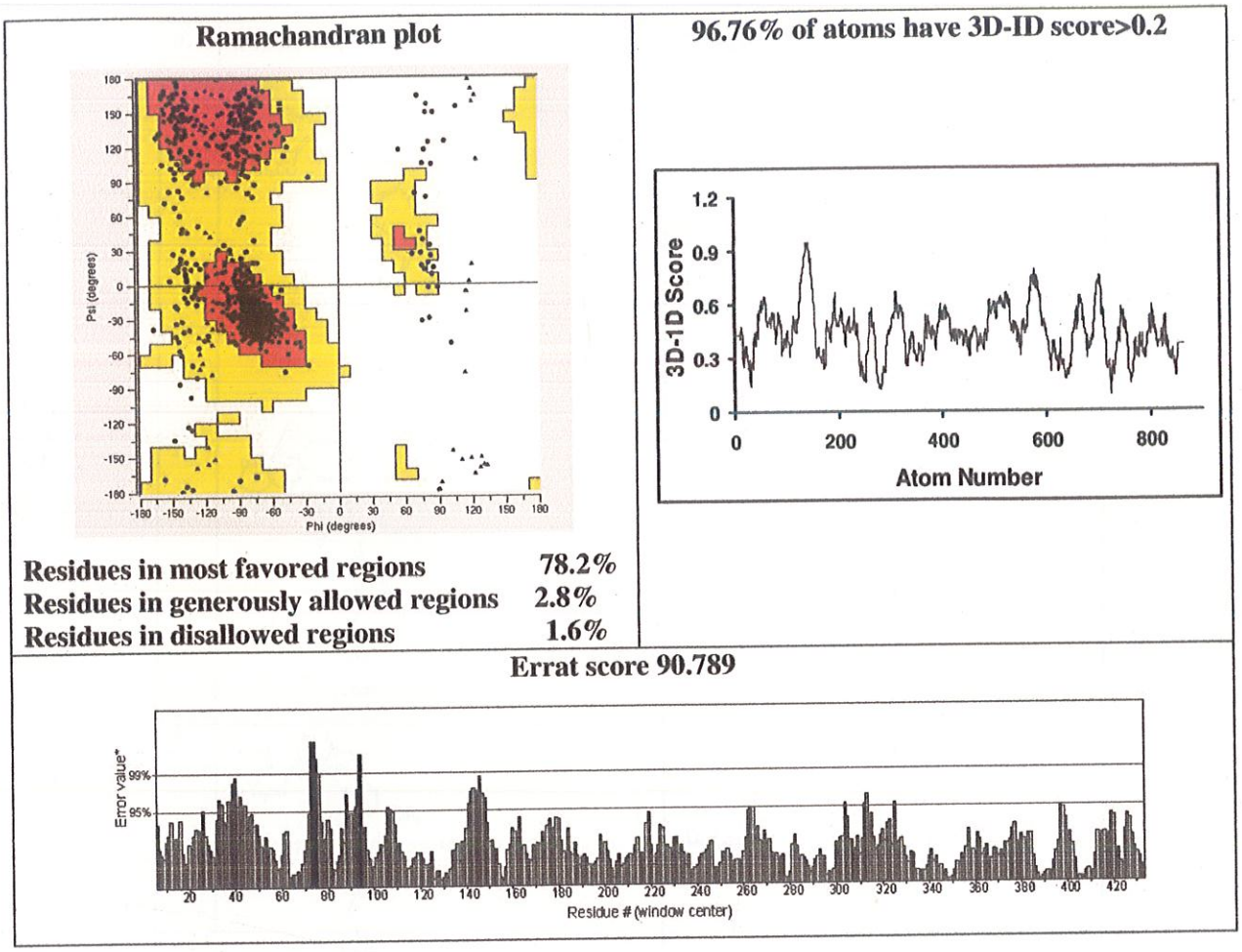


Fig 3.2: Validation results of tubulin dimer wherein 78.2%of residues are in most favoured regions .also the model bears an errat score of 90.789 and 96.76% of the residues have a 3D - 1D score >0.2.

3.2 Glide Docking :

Molecular docking methods are widely used by academic institutes and pharmaceutical industries to study drug–target interactions in order to understand the basic electronic/stericfeatures required for therapeutic action and to design new drug candidates with improved activities. These docking calculations provide insight into interactions of ligands withamino acids in the binding pocket of a target and to predict the corresponding binding affinities of ligands. Their Glide score values of noscapine and its analogues ranged from –4.0305kcal/mol to–10.4517 kcal/mol which is good glide score.The docked complexes were rescored with Prime MM-GB/SA.The following table gives the structures with glide score and MM-GB/SA score.

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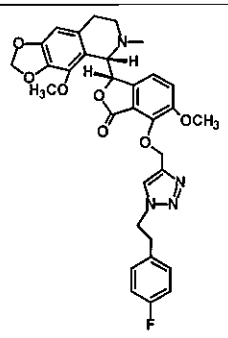
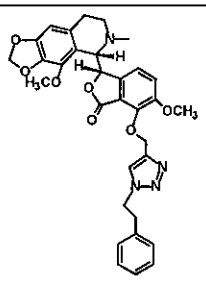
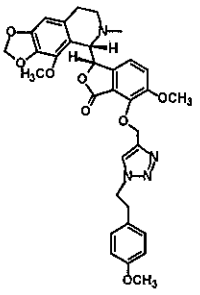
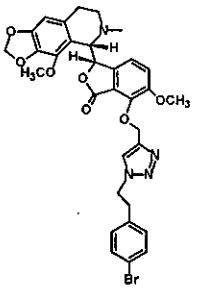
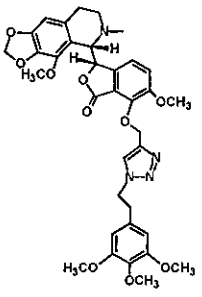
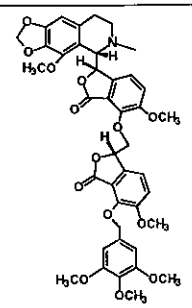
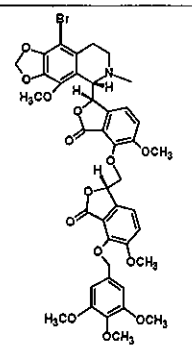
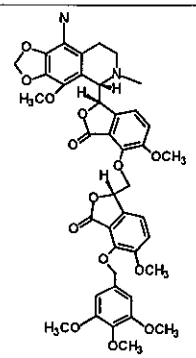
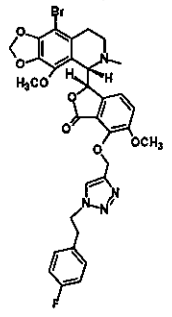
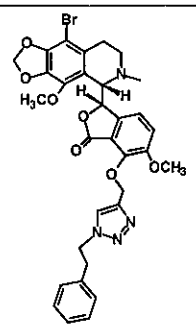
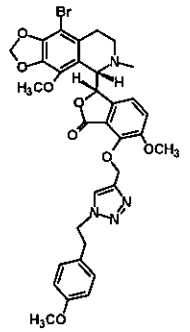
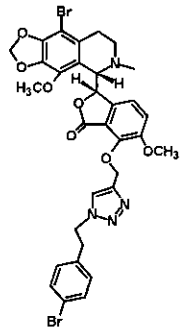
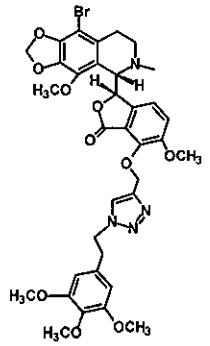
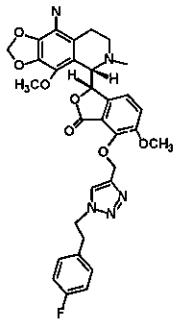
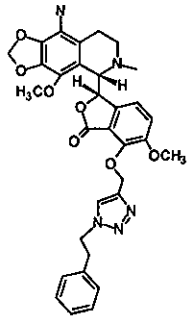
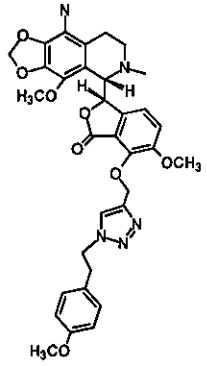
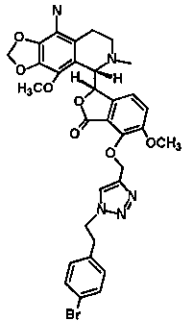
1		-9.09953	-68.25
2		-7.37255	-81.91
3		-6.73996	-76.70
4		-8.19607	-66.36
5		-7.74396	-100.40

6		-10.5776	-40.77
7		-6.64811	-48.63
8		-8.90331	-70.47
9		-9.49454	-76.73

10		-7.79809	-85.05
11		-6.66332	-79.72
12		-6.11554	-84.40
13		-5.13951	-104.55

14		-8.81243	-67.21
15		-4.46388	-75.52
16		-7.24486	-104.56
17		-5.94684	-76.39

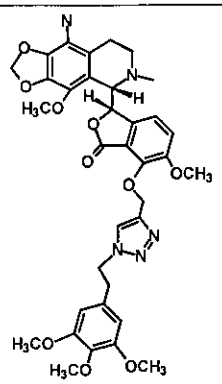
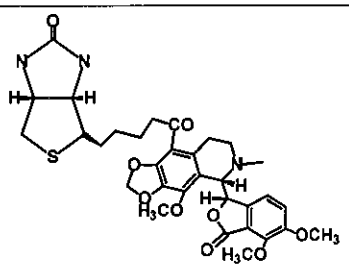
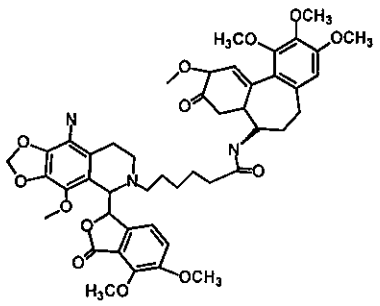
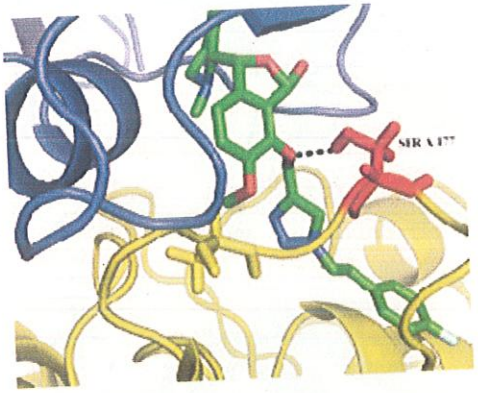
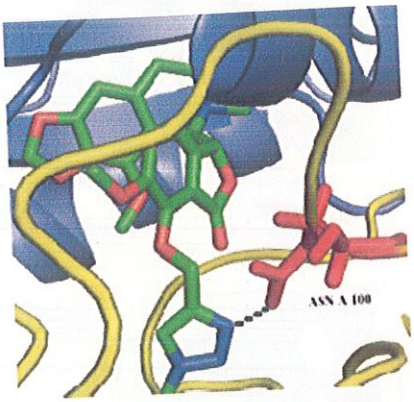
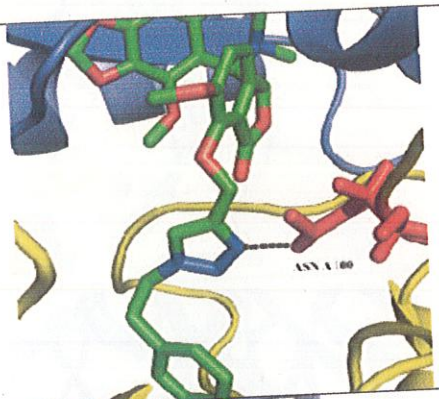
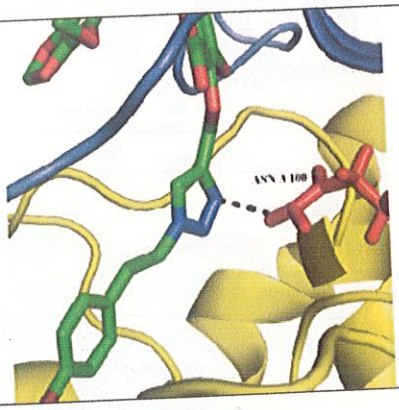
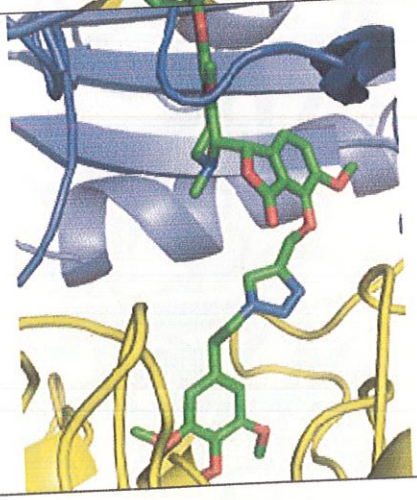
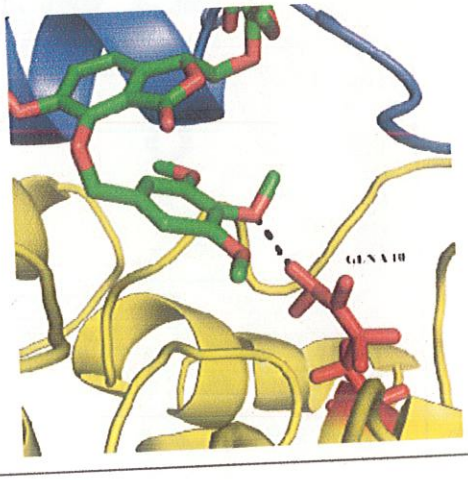
18		-8.01918	-81.92
19		-10.4517	-100.52
20		-4.0305	-69.82

Table3.1: The structure of the noscapine analogs with their respective glide scores and binding energies.

3.3 Insight into the interaction in the docked complexes using Pymol AND Ligplot:

Since all the noscapinoids are found to have promising glide scores binding affinity , so we were motivated to further investigate the mechanistic details of the interaction of these newly designed analogues with tubulin. For more clear view of the interaction between ligand and target we have analyzed the different poses of noscapinoids and their hydrogen bonding pattern using Pymol viewer andLigplot respectively.

The docked conformations and the of these noscapinoids is shown below :

1. 	2. 
3. 	4. 
5. 	6. 

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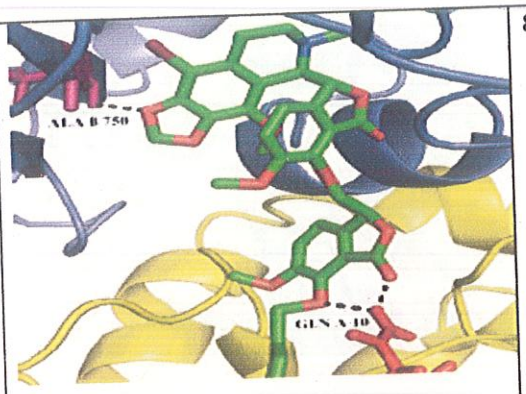

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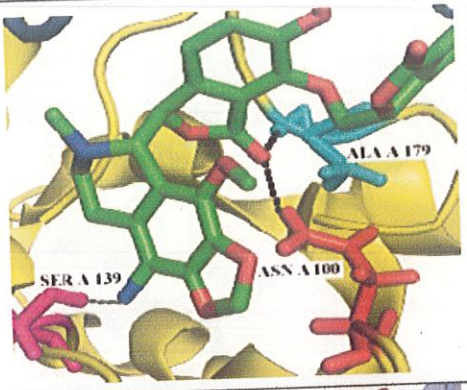

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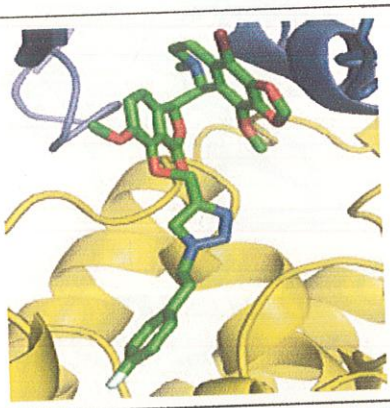

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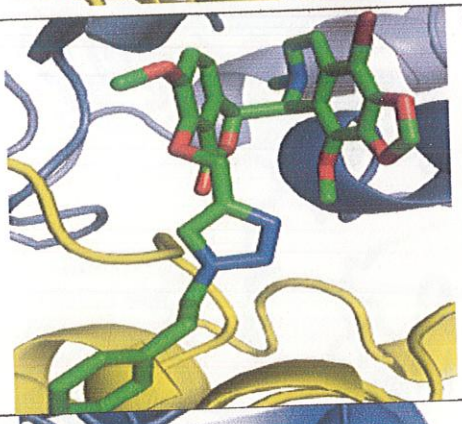

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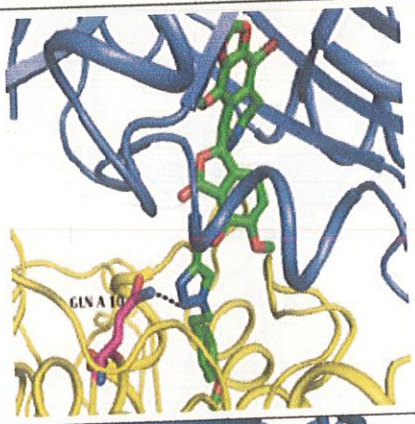

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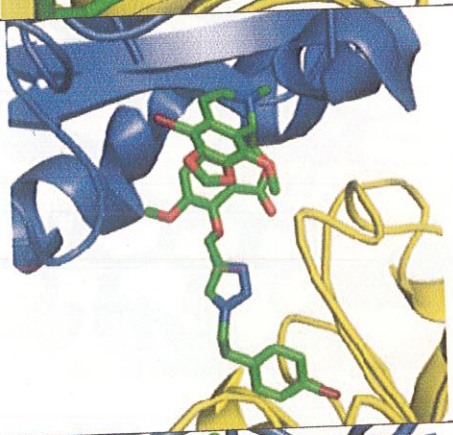

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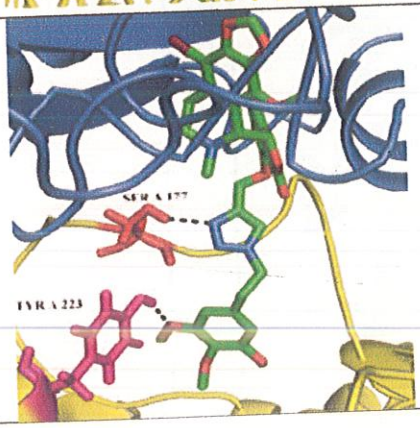

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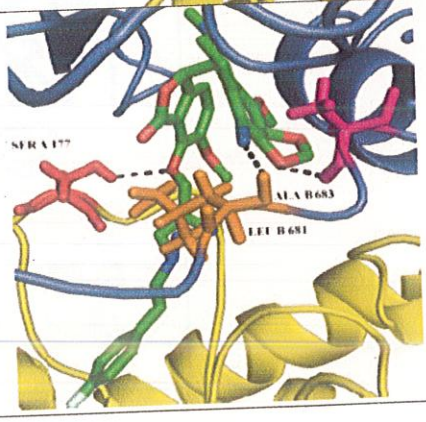


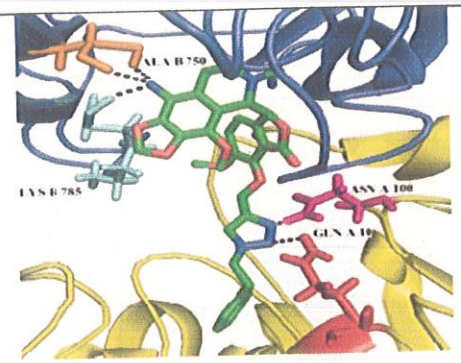
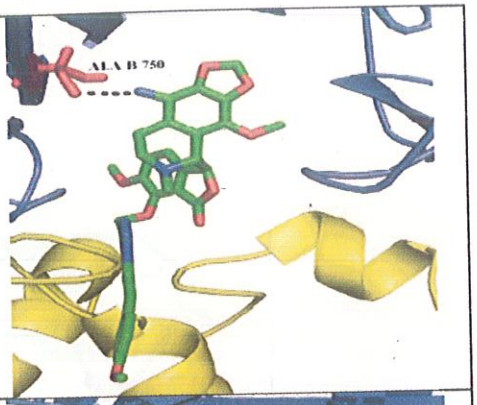
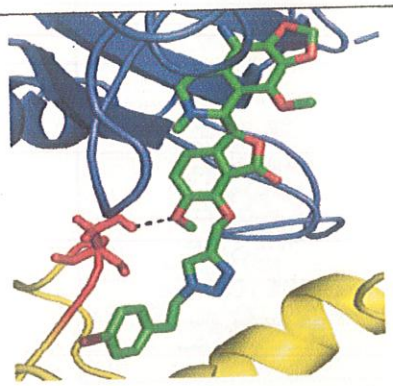
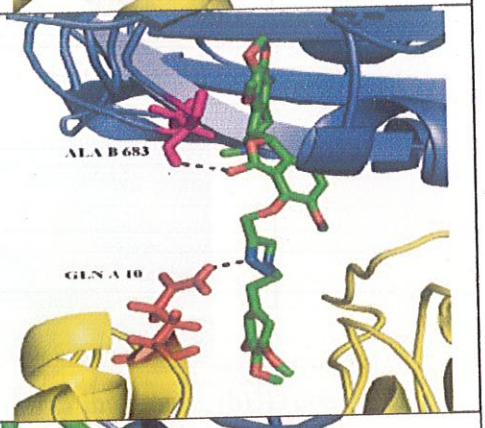
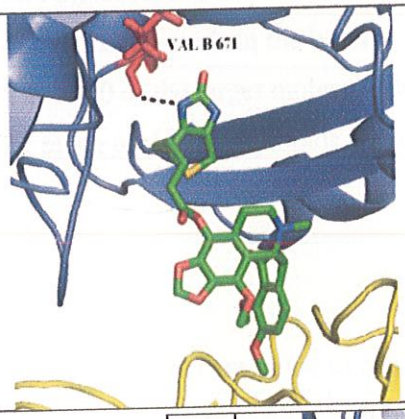
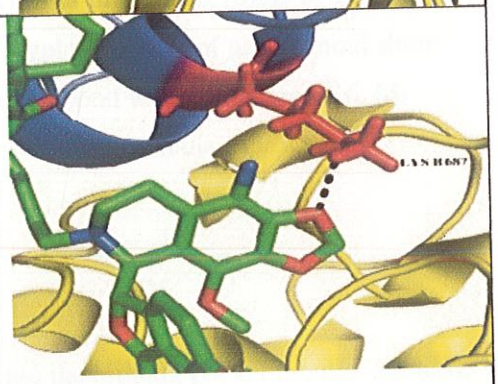
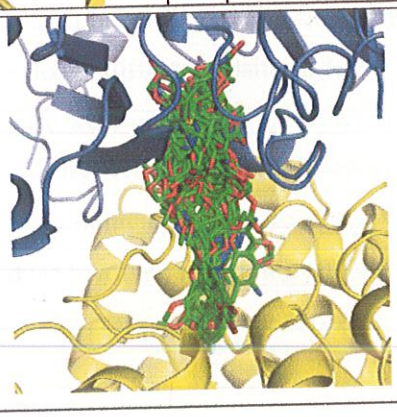
15.	
16.	
17.	
18.	
19.	
20	
All	

Table 3.2: The docked complexes of various designed noscapinoids with the target.

Noscapinoid 6 was found to give the most promising result . It has the maximum glide score among all i.e. -10.5776.

MOLECULE 6

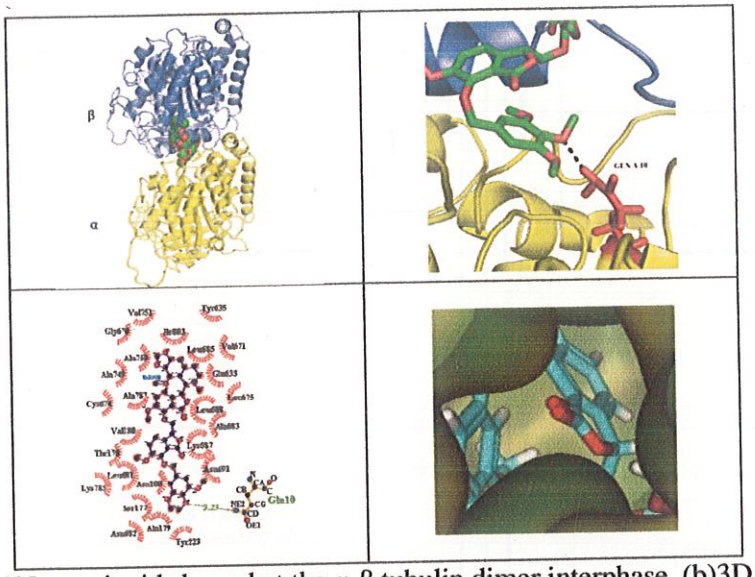
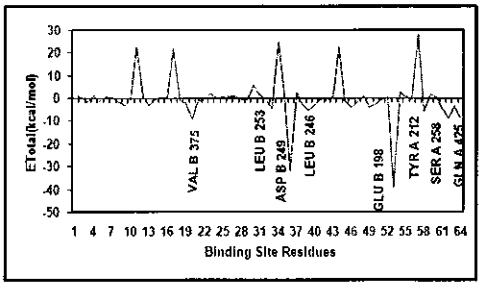
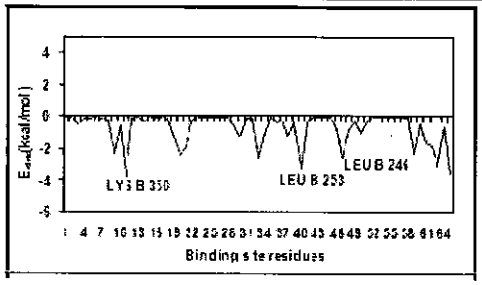
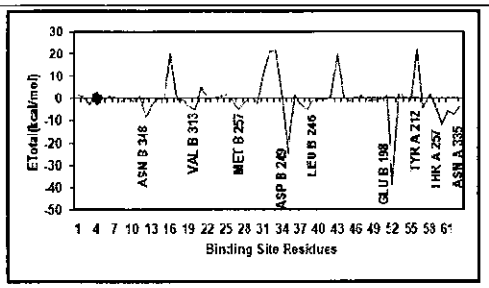
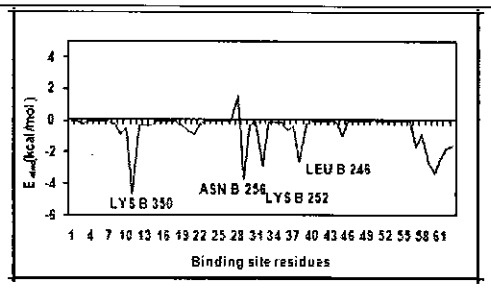
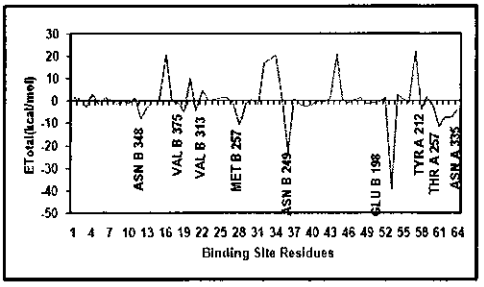
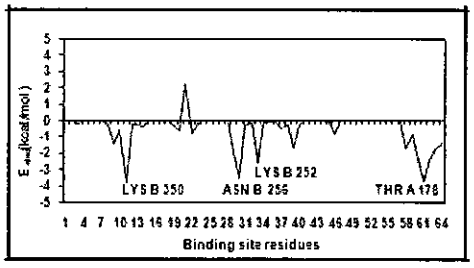
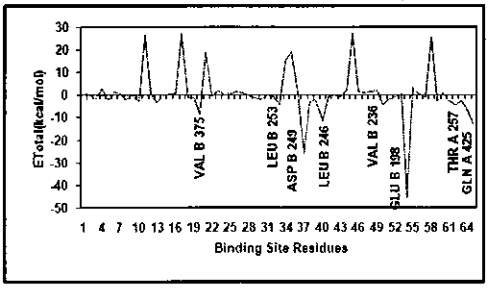
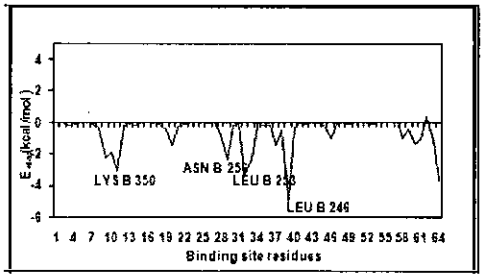
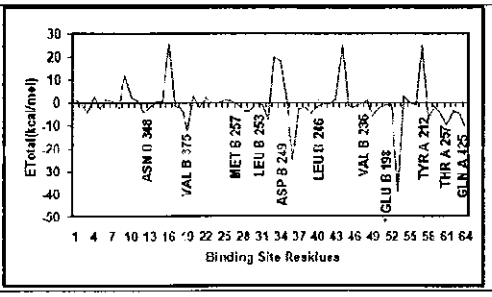
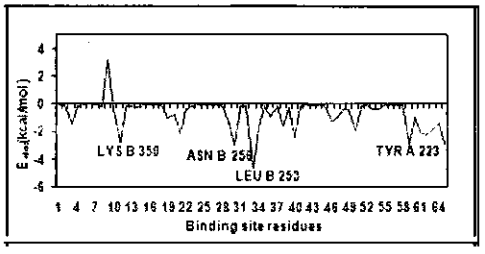
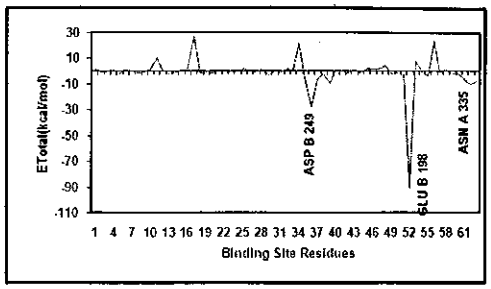
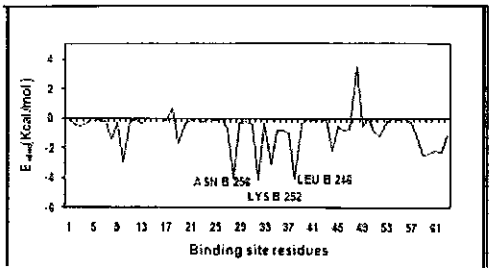
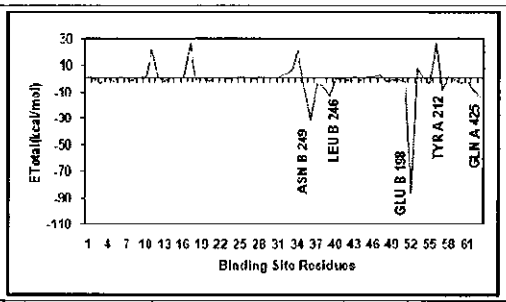
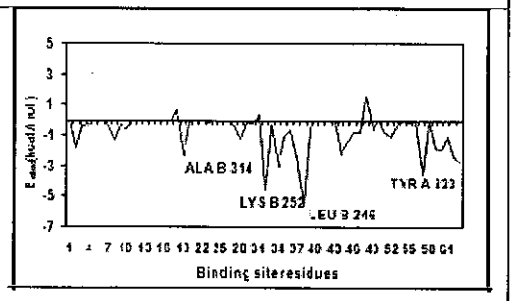
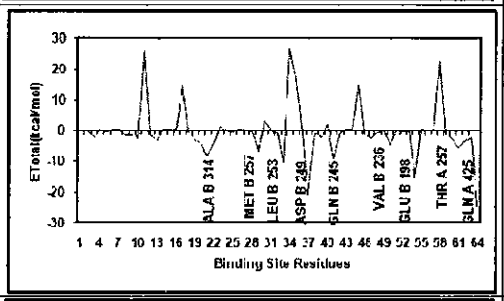
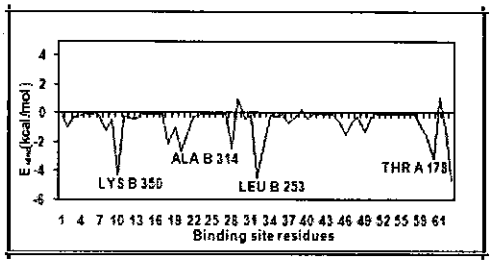
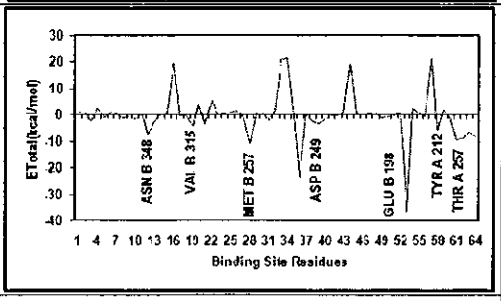
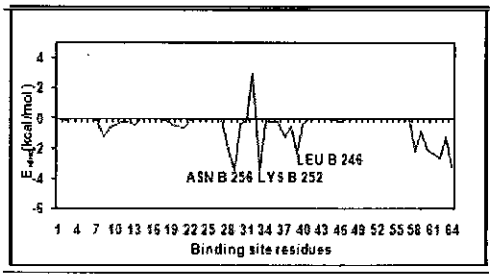
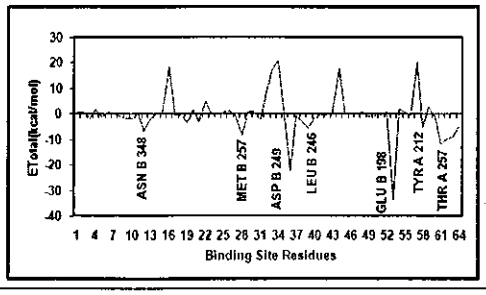
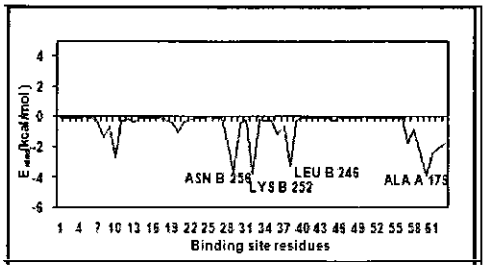


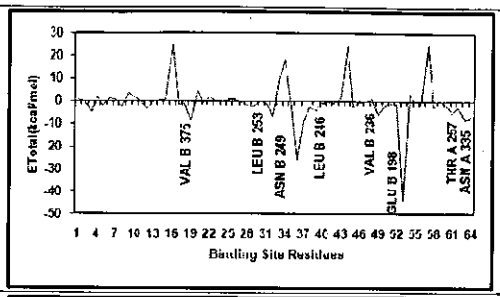
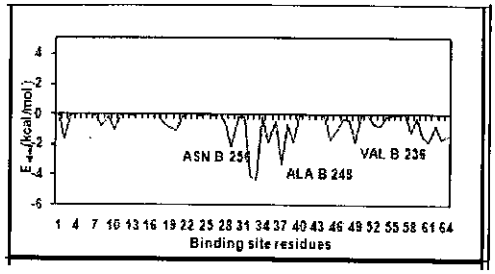
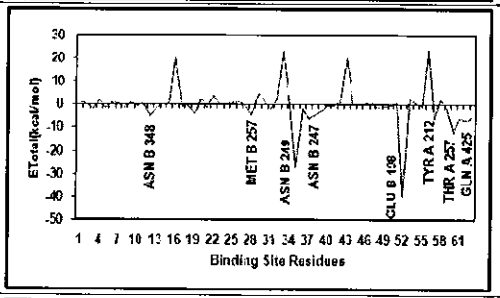
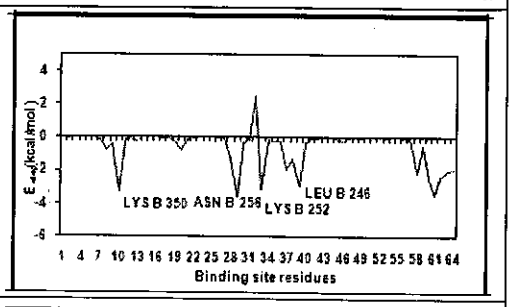
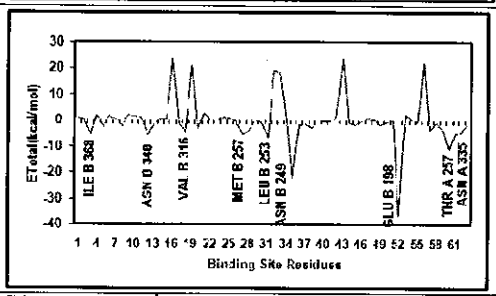
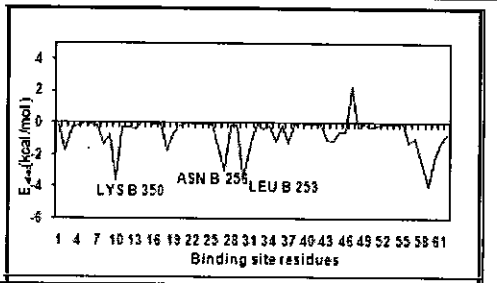
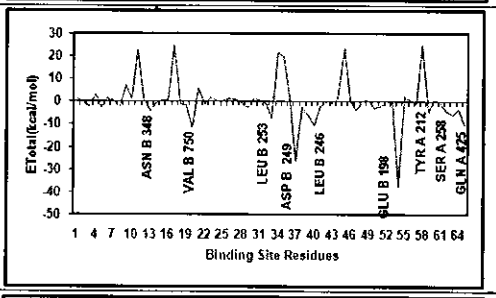
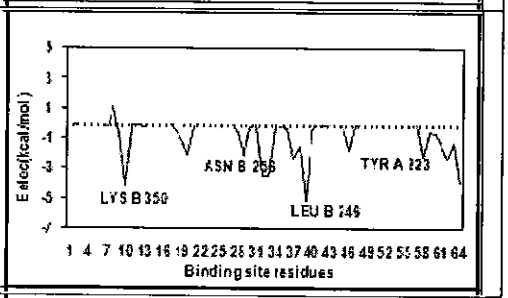
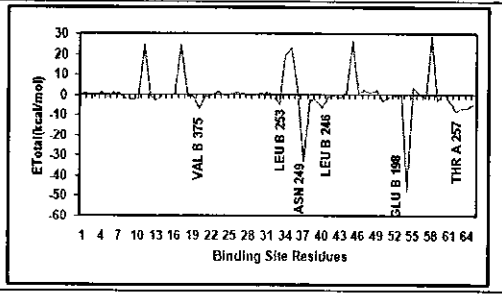
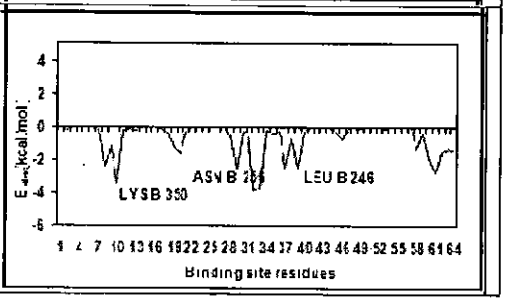
FIG 3.3 : a)Noscapinoids bound at the α-β tubulin dimer interphase, (b)3D conformation view of the noscapinoids in the binding pocket.(c)Ligplot analysis of noscapinoid shows that GLN A 10 of the target molecule makes hydrogen bond with the molecule 6. (d) Binding mode of the noscapinoids in the binding pocket (surface view).

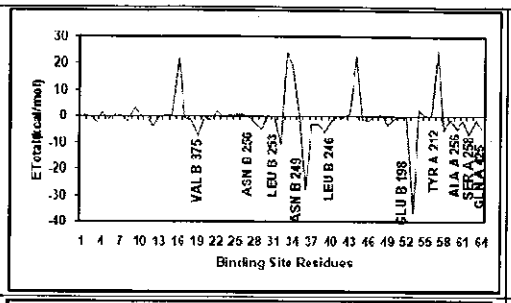
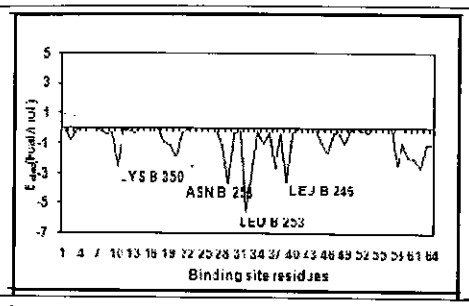
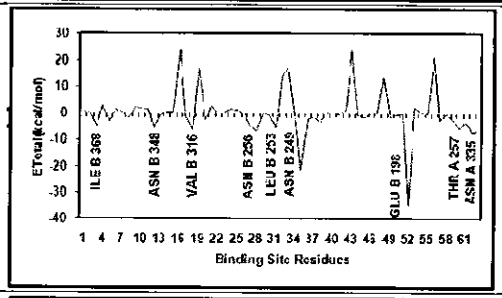
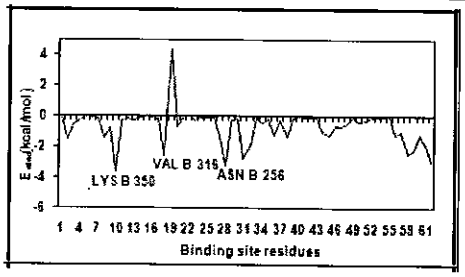
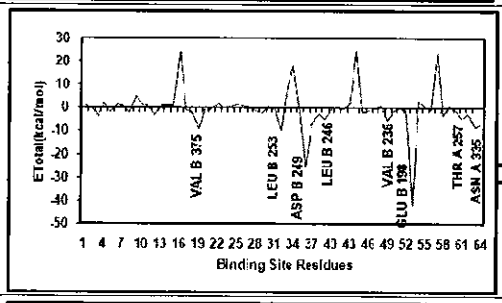
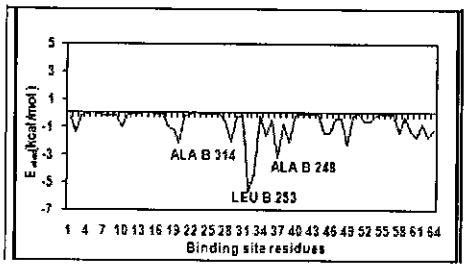
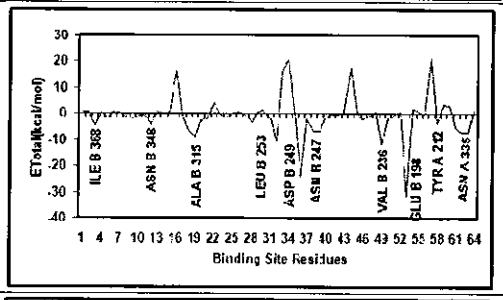
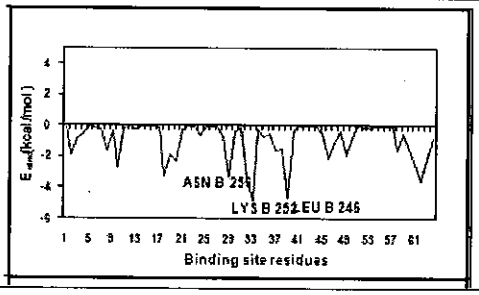
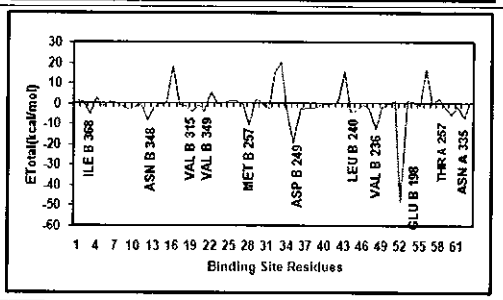
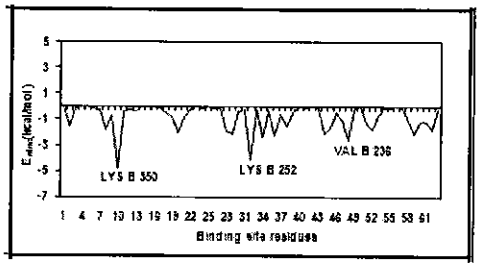
Since all the noscapinoids are found to be well fitted into the binding pocket tubulin and have shown to be bound differently with the tubulin dimer as revealed by the ligplotanalysis,owing to the different functional groups added at different positions to the lead compound noscapine. With the above analysis we were motivated to further investigate the contribution of the amino acids to the binding affinity. We carried out the energy decomposition analysis of the amino acids, within 12Å of the binding pocket, interacting with the Ligand molecules to find out the most important residues involved in binding and their contribution in the binding affinity. The results of energy decomposition are shown in the tables.

3.4:The graphs were made for Strain energy, Vander walls energy , Coloumbs energy and total energy.Some residues were deleted for better view of the graph.

MOL	E TOTAL	E COL
1		
2		
3		
4		
5		

6	 ETotal(kcal/mol) ASP B 249 GLU B 198 ASN A 335 Binding Site Residues	 ASN B 256 LEU B 246 LYS B 252 Binding site residues
7	 ETotal(kcal/mol) ASN B 249 LEU B 246 GLU B 198 TYR A 212 GLN A 425 Binding Site Residues	 ALA B 314 LYS B 252 Binding site residues
8	 ETotal(kcal/mol) ALA B 314 MET B 257 LEU B 253 ASP B 249 GLN B 245 VAL B 236 GLU B 198 THR A 257 GLN A 425 Binding Site Residues	 LYS B 350 ALA B 314 LEU B 253 THR A 178 Binding site residues
9	 ETotal(kcal/mol) ASN B 348 VAL B 315 MET B 257 ASP B 249 GLU B 198 TYR A 212 THR A 257 Binding Site Residues	 LEU B 246 ASN B 256 LYS B 252 Binding site residues
10	 ETotal(kcal/mol) ASN B 348 MET B 257 ASP B 249 LEU B 246 GLU B 198 TYR A 212 THR A 257 Binding Site Residues	 ASN B 256 LYS B 252 LEU B 246 ALA A 175 Binding site residues

11	 ETotal(kcal/mol) VAL B 375 LEU B 253 ASN B 249 LEU B 246 VAL B 236 GLU B 198 THR A 257 ASN A 335 Binding Site Residues	 ASN B 256 ALA B 248 VAL B 236 Binding site residues
12	 ETotal(kcal/mol) ASN B 348 MET B 257 ASN B 249 ASN B 247 GLU B 198 TYR A 212 THR A 257 GLN A 425 Binding Site Residues	 LYS B 350 ASN B 256 LYS B 252 LEU B 246 Binding site residues
13	 ETotal(kcal/mol) ILE B 368 ASN B 348 VAL B 316 MET B 257 LEU B 253 ASN B 249 GLU B 198 THR A 257 ASN A 335 Binding Site Residues	 LYS B 350 ASN B 256 LEU B 253 Binding site residues
14	 ETotal(kcal/mol) ASN B 348 VAL B 750 LEU B 253 ASP B 249 LEU B 246 GLU B 198 TYR A 212 SER A 258 GLN A 425 Binding Site Residues	 E elec(kcal/mol) LYS B 350 ASN B 256 LEU B 246 TYR A 223 Binding site residues
15	 ETotal(kcal/mol) VAL B 375 LEU B 253 ASN 249 LEU B 246 GLU B 198 THR A 257 Binding Site Residues	 LYS B 350 LEU B 246 Binding site residues

16		
17		
18		
19.3		
19.4		

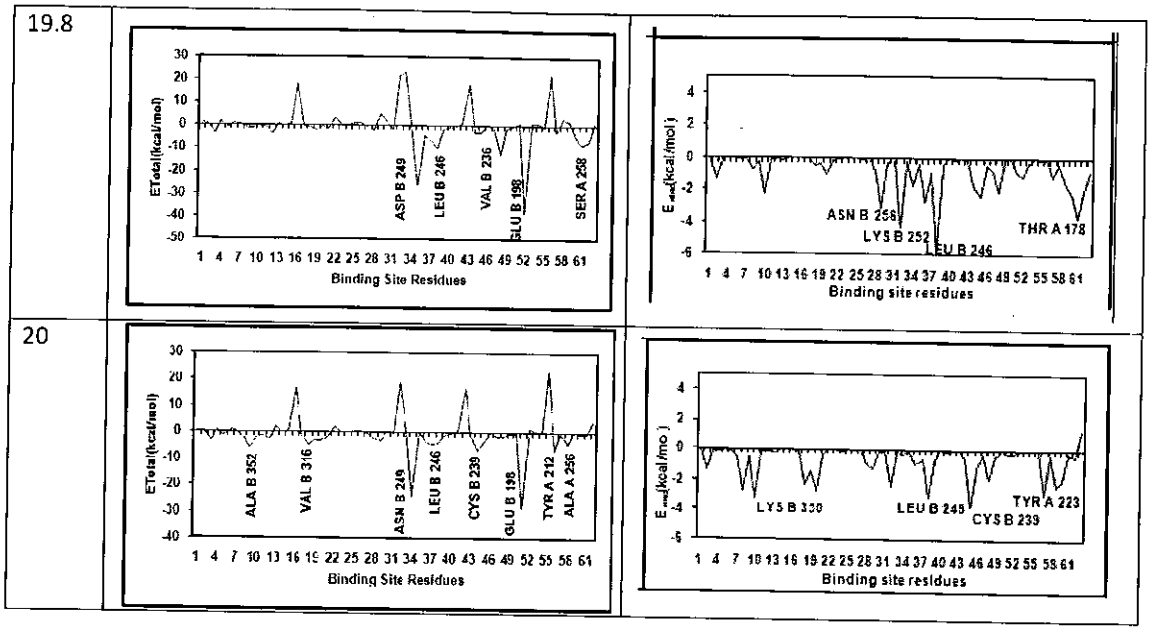


Table 3.3E Total energy graph shows that residues like ASP B 249, GLU B 198, LEU B 246, GLU B249, ASN A 335, ASN B 348, LEU B 253, VAL B 375 and TYR A 212 contributed majorly.E Columb's energy graph shows that residues like LEU B 246 , LYS B 350 and ASN B 256 contributed majorly.

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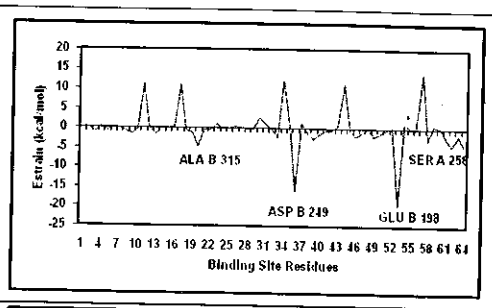
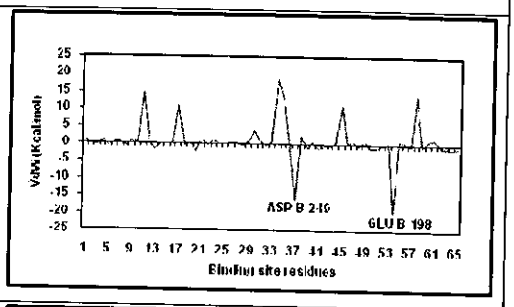
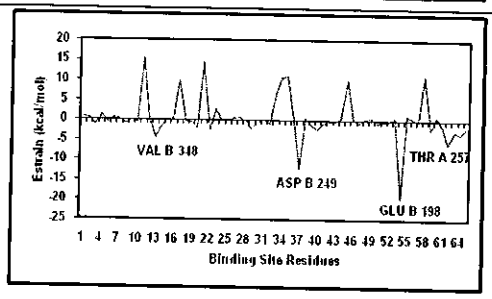
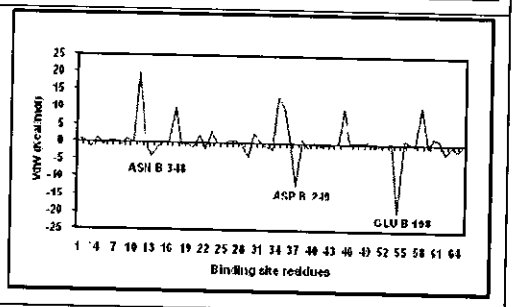
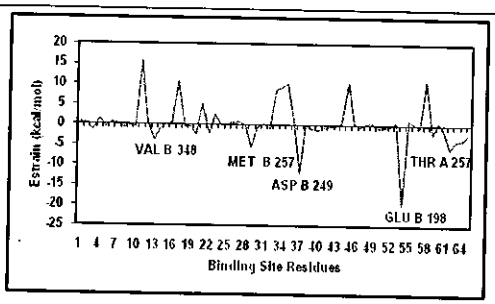
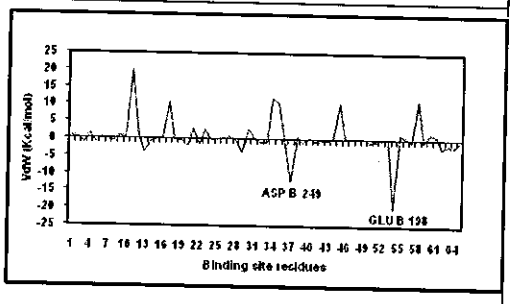
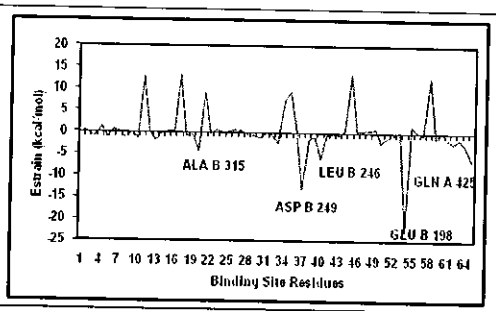
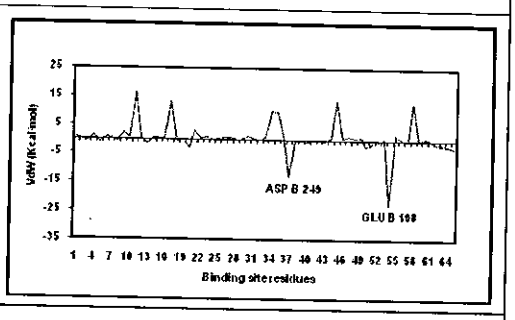
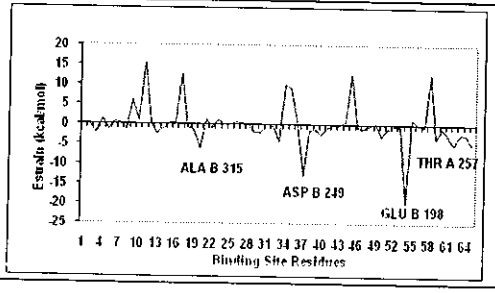
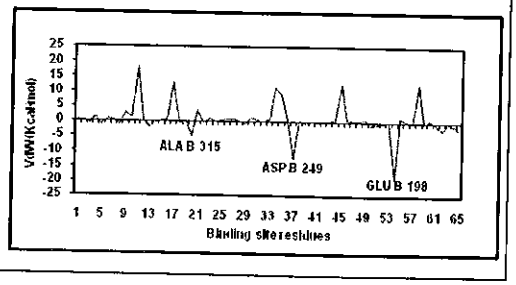
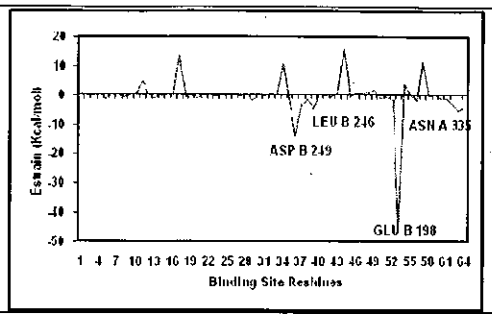
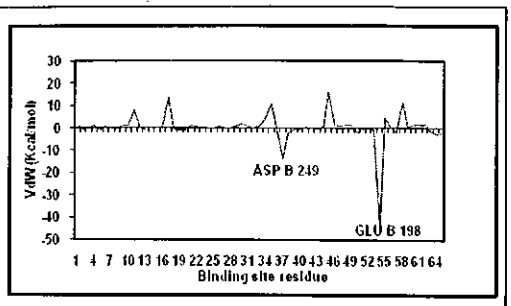
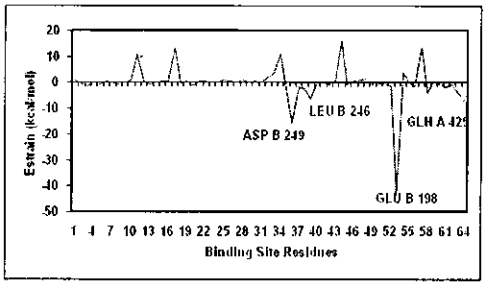
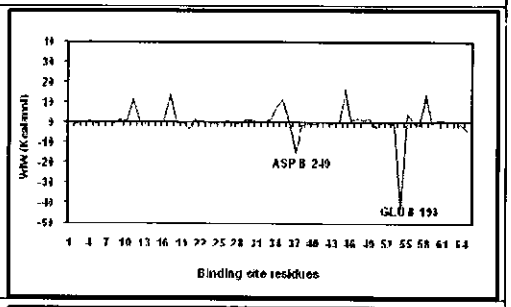
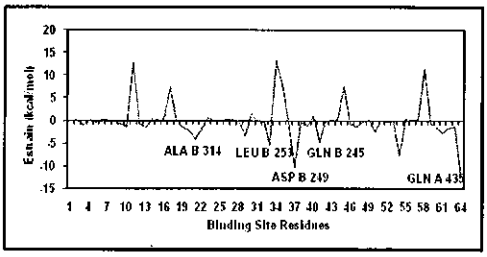
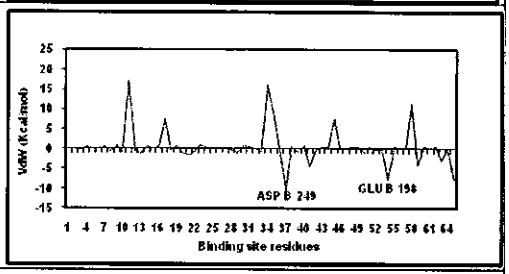
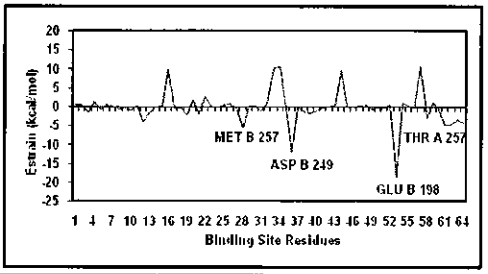
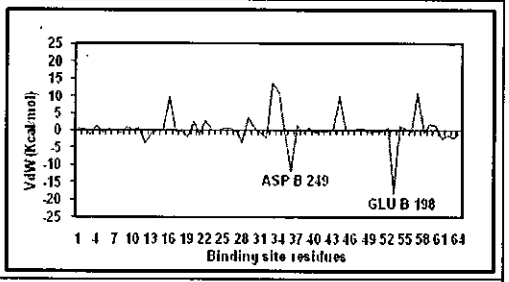
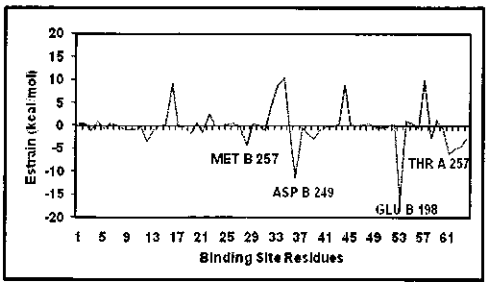
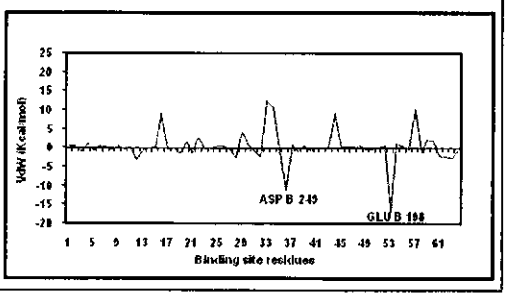
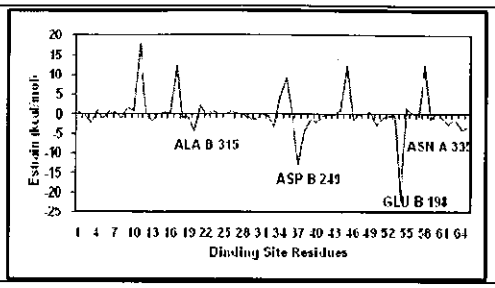
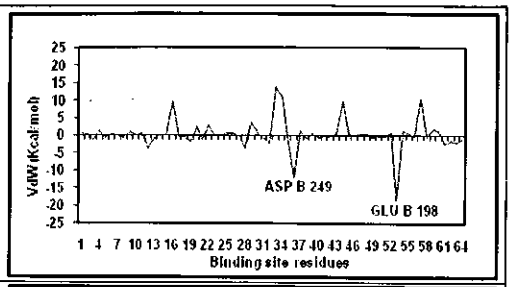
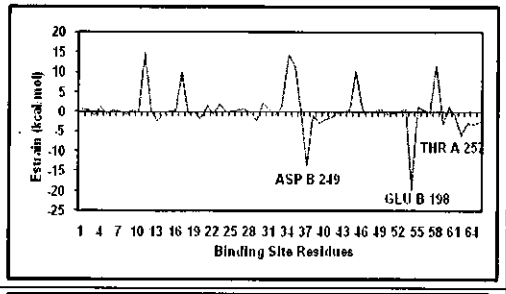
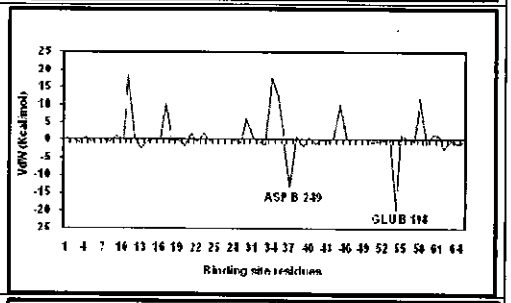
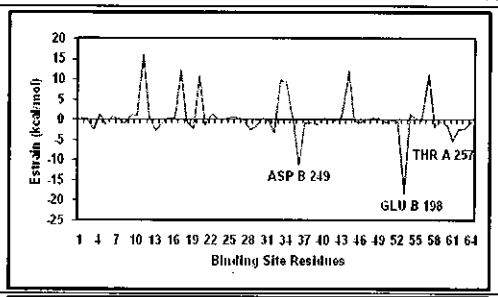
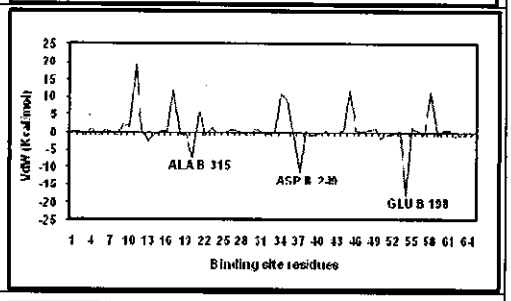
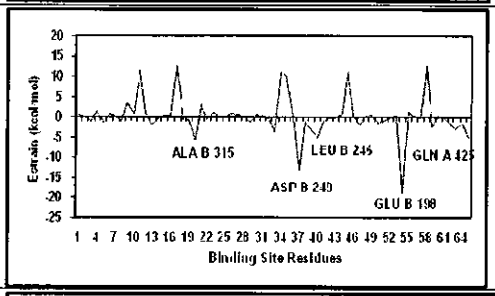
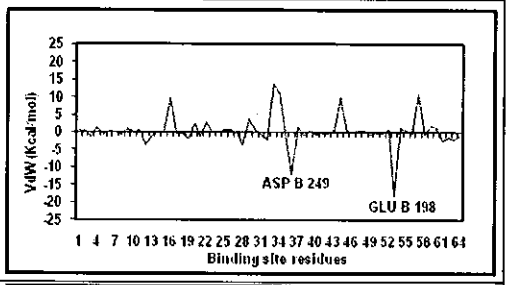
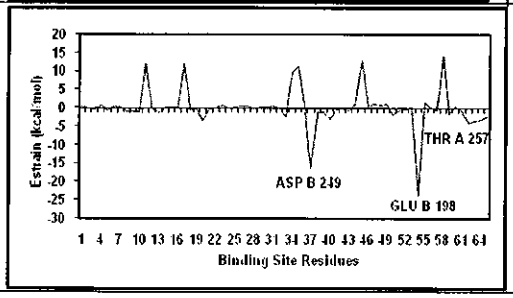
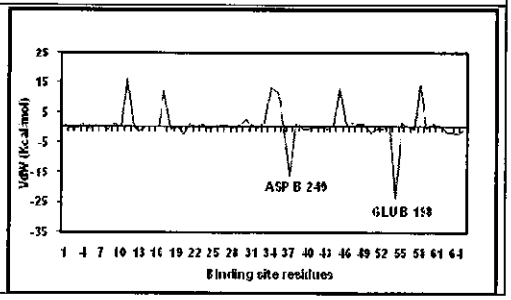
1		
2		
3		
4		
5		

6	 Estrain (Kcal/mol) LEU B 246 ASN A 335 ASP B 249 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residue
7	 Estrain (Kcal/mol) LEU B 246 GLN A 425 ASP B 249 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residues
8	 Estrain (Kcal/mol) ALA B 314 LEU B 253 GLN B 245 ASP B 249 GLN A 435 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residues
9	 Estrain (Kcal/mol) MET B 257 THR A 257 ASP B 249 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residues
10	 Estrain (Kcal/mol) MET B 257 THR A 257 ASP B 249 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residues

11	 Estrain (kcal/mol) ALA B 315 ASP B 249 ASN A 33 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residues
12	 Estrain (kcal/mol) ASP B 249 THR A 25 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residues
13	 Estrain (kcal/mol) ASP B 249 THR A 257 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ALA B 315 ASP B 249 GLU B 198 Binding site residues
14	 Estrain (kcal/mol) ALA B 315 LEU B 245 GLN A 425 ASP B 249 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residues
15	 Estrain (kcal/mol) ASP B 249 THR A 257 GLU B 198 Binding Site Residues	 VdW (Kcal/mol) ASP B 249 GLU B 198 Binding site residues

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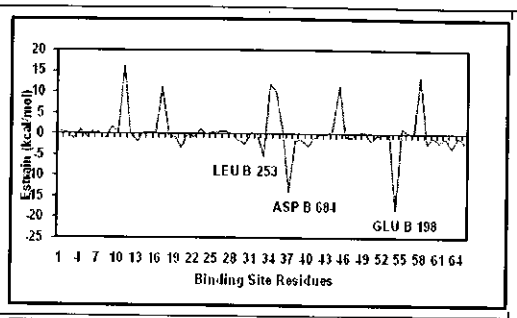

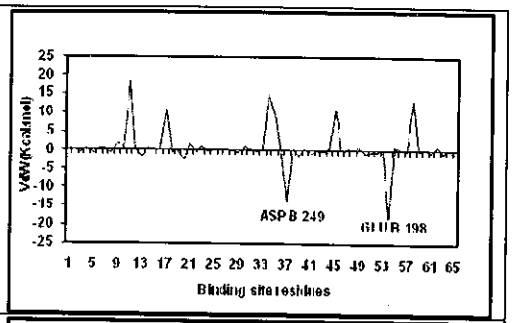

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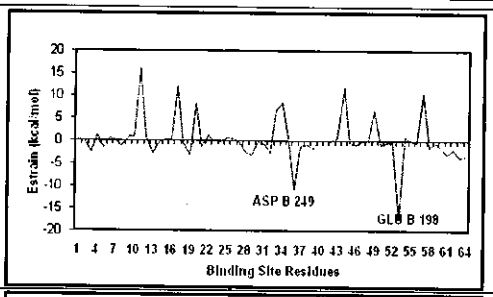

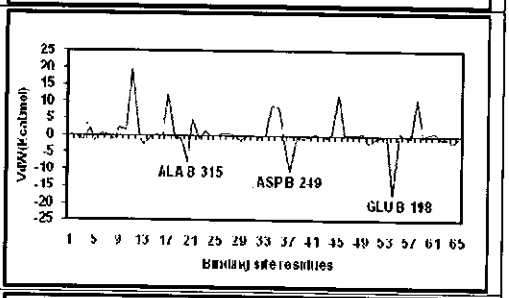

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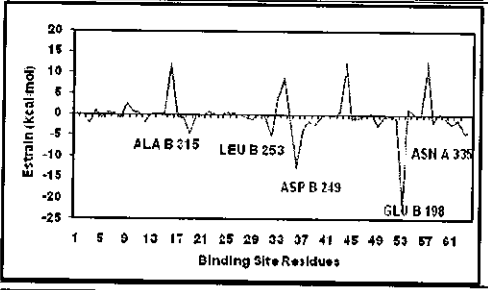

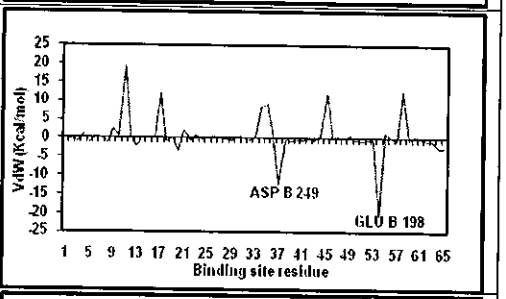

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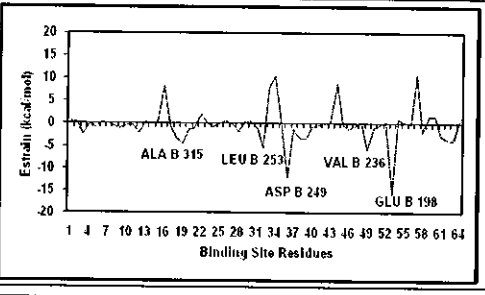

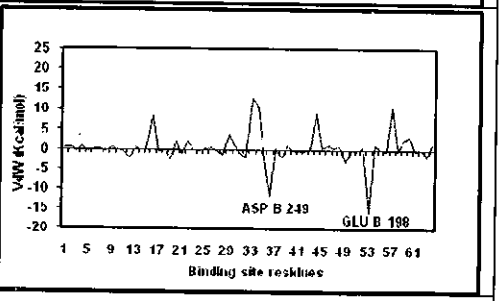

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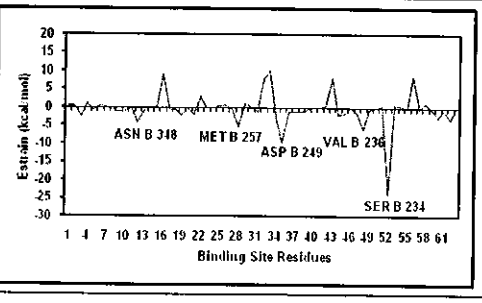
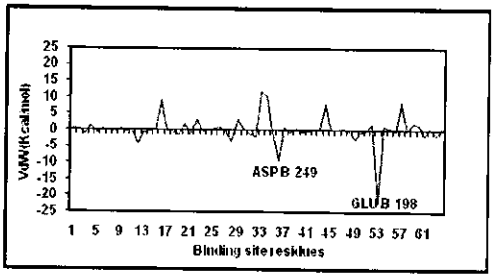




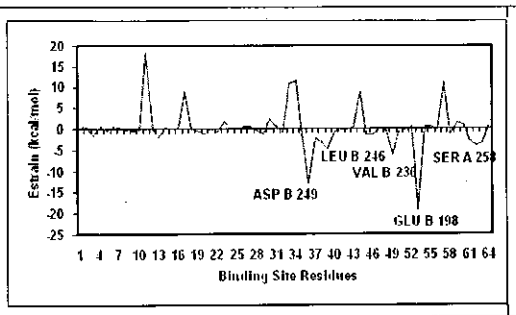
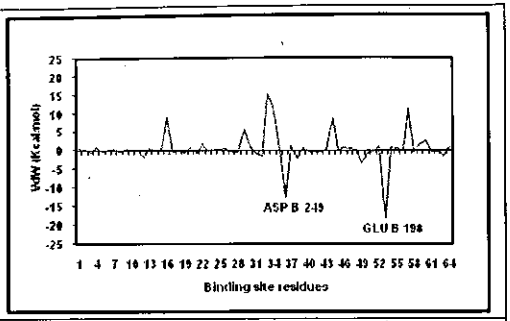
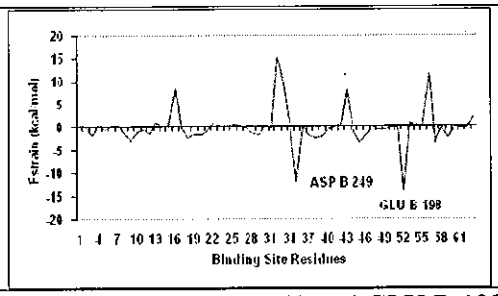
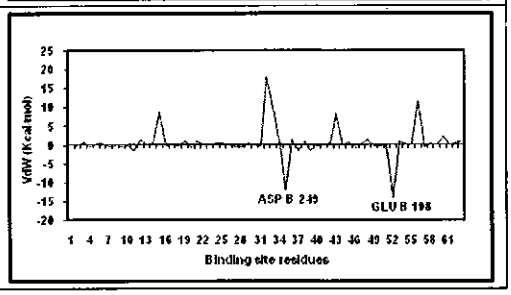
19.8		
20		

Table 3.4 The residues ASP B 249 and GLU B 198 contributed majorly in both vander walls energy and strain energy .

3.4 CONCLUSION:

The binding site of noscapine was analysed and important residues were noted down. The analogs of noscapine were designed and docking was done using glide docking . Energy decomposition was done to find the most important residues involved in interaction. Noscapine analog 6 was found to have maximum glide score of -10.5776 which is an excellent score. We can further sent them for experimental validation .

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