Section B

3D Molecular docking and Antitopoisomerase activities of the derived compounds

1. Introduction

Topoisomerases are ubiquitous enzymes that direct and modify the topological state of DNA.[41-46] They play crucial role in several aspects of DNA metabolism like replication, transcription, recombination and chromosomal segregation at mitosis.[45,46] These enzymes act by sequential breakage and reunion of either one DNA strand (Topoisomerase I) or both DNA strands (Topoisomerase II).[45,46] All vertebrates have two highly similar, though functionally discrete, Topo II isoforms, α and β.[31,41-46] Multiple studies have shown the Topo IIα levels increase during cell proliferation and this enzyme appears to be the isoform involved in mitosis.[41-46] The pharmacological inhibition of this enzyme has therefore been identified as target for anticancer drug development.[41-46] To date several topoisomerase inhibitors have been identified. Among them plant derived camptothecin and podophyllotoxins/etoposides have notable therapeutic efficacy as antitumor drugs. Despite their numerous applications, one cannot ignore the toxicity associated to these compounds.[47,48] Therefore, the development of newer drugs with lesser side effects, more chemical stability and better efficacy is indispensable.

In the present study 3D molecular docking of structurally modified friedelan derivatives, highly oxidized on ring A were studied on crystal structure of topoisomerase IIα (1bgw for topoisomerases IIα, PDB)[49] was performed to evaluate the binding energies as well as their mode of interaction. Finally, the molecules were tested for their ability to inhibit the catalytic activity of topoisomerases IIα.

2. 3D Molecular Docking studies of Friedelan analogs

To determine whether the friedelan analogs have potential as topoisomerase inhibitor, the parent compounds (**1** and **29**) and their hemisynthetic derivatives ((**59**, **61** and **45**) were docked into the central catalytic domain of the enzyme (1bgw PDB for topoisomerase IIα)[49] by using AutoDock 4.

A previously modeled lupane bound structure was used as a starting point for calculating the lowest energy conformation of the bound ligands. The amino acids ARG 1016, HIS

1012, TYR 805, LYS 812 and LYS 713, have been shown to be present in the triterpenoids (lupane skeleton) binding domain of Topo IIα.[49] An initial docking was therefore performed considering these amino acid residues as flexible for binding of the present triterpenoid derivatives. Although, the calculated binding energies are negative for all friedelan derivatives (ligands) against the reported residues, the lowest value is obtained for LYS 713 (feasible binding, -9.46 Kcal/mol for **1**, -8.54 Kcal/mol for **29**, -8.13 Kcal/mol for **59** and -8.81 Kcal/mol for **61**). However, in docked complexes LYS 713 is not within 3.5 Å from the ligand. Hence, despite good binding energy values, there remain scientific limitations to consider any type of noncovalent interactions (H-bonding or electrostatic interaction) between the ligands and the flexible residues. These observations preclude a similar/common binding for lupane and friedelan derivatives in Topoisomerase IIα (1bgw, PDB).

Table 2 Estimated binding energy of different compounds against LYS 713

Entry	Compound	Estimated energies (Kcal/mol)				
		BIE	IME	INE	TE	UE
1	**1**	-9.46	-9.46	+0.00	+0.00	+0.00
2	**29**	-8.54	-8.84	+0.01	+0.30	-0.01
3	**59**	-8.13	-9.32	-0.26	+1.19	+0.26
4	**61**	-8.81	-8.81	+0.00	+0.00	+0.00

BIE, Binding energy; IME Intermolecular energy; INE, Internal energy; TE, Torsional energy; UE, Unbound system's energy

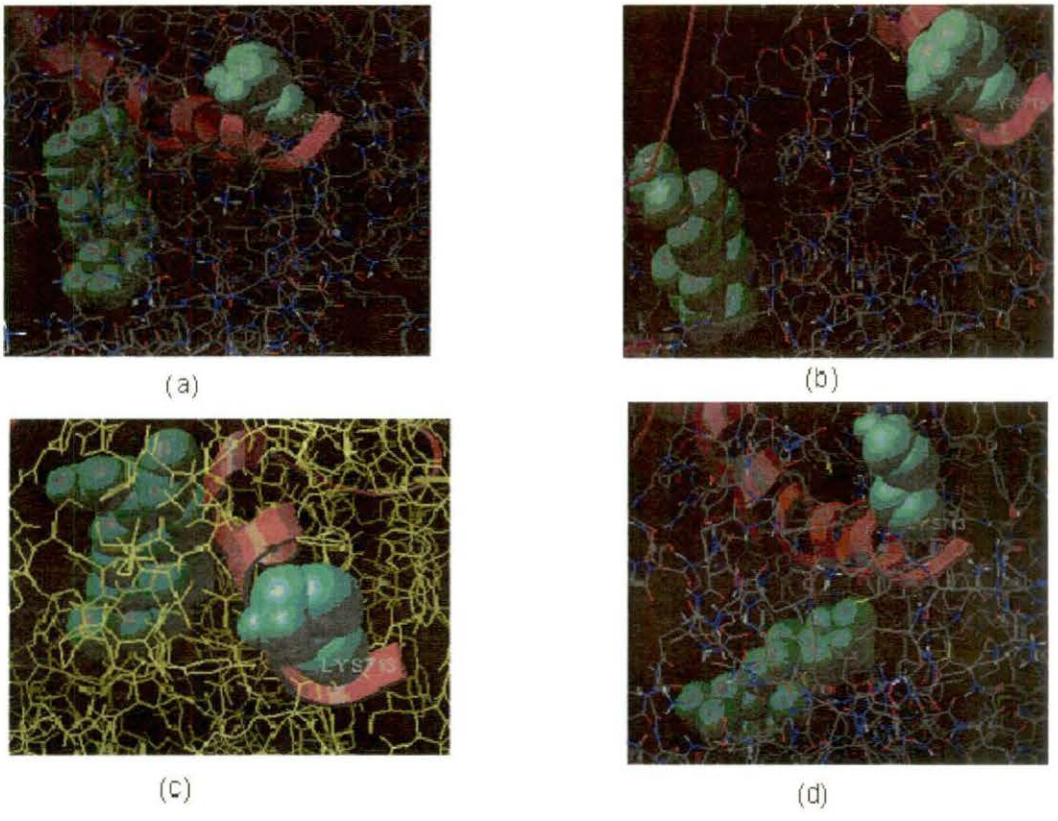

Figure 30 Detailed view of docked structures of compounds **1** (a), **29** (b), **59** (c) and **61** (d) against LYS713 of Topo IIα.

To obtain a better understanding of the binding site, amino acids neighbors within 3.5 Å from the ligand were considered for final docking studies. Interestingly, the calculated binding energies of the ligands to the residues that are well within 3.5 Å are acceptable values in every case.

The results of molecular docking of hemisynthetc friedelan analogs on TopoIIα are overlaid in figure 3. The compound **1** binds to the hydrophilic region of the enzyme involving ASN 765, ASN 752, GLY 766, TYR 760, ARG 1172, indicating the bulk of intermolecular interactions as non-ionic. In addition, the carbonyl group in ring A of the compound binds via a hydrogen bond to amino group of LEU 791. It also forms a water mediated hydrogen bond with ASN 769 (Figure 3a). Compound **29** differs from compound **1,** in having an additional beta- hydroxyl group at position C-2. The presence of the beta hydroxyl group modifies the binding properties of the molecule to the DNA-binding domain of the Topo II enzyme. Most of the interactions between compound **29** and TopoII are essentially hydrophobic and the molecule interacts with aliphatic side

chains of TopoII, including ALA 778, ALA 781, ALA 723, GLY 633 and ALA743 (Figure 3b). Presence of the carbonyl group and non bonding electron pair in oxygen atom of the lactone ring impart some polar properties to the compound **61**. From the results in Figure 3d, it is evident that lactone ring in the molecule interacts with a polar region of the enzyme containing ASP 513, GLU 512, TYR 511, ARG 508. Moreover, carbonyl group in lactone ring also forms hydrogen bond with hydroxyl group of TYR 511 and side chain amino group of ARG 508. On the other hand, rest of the nonpolar part of compound **61** binds to an alanine rich hydrophobic pocket of TopoII consisting of aliphatic side chains of ALA 778, ALA 779, ALA 780 and ALA 781, and also to ILE 553.

Compounds **59** and **45** both are seco-friedelan derivatives and they have certain structural similarities. Their skeletons contain carboxylic acids, which on deprotonation will generate the carboxylate ion. Hence, it can be predicted that both **59** and **45** will bind at similar sites in the structural space of the enzyme and the binding site should have a condensed positive charge for the best fitting of the ligands. As shown in figure 3c and 3e, compounds **59** and **45** bind to a condensed positively charged polar site of the enzyme that permits attractive electrostatic interactions with the neighboring residues. In addition, GLY 703, GLN 704 and LYS 701 residues are in common in their binding domain, thus confirming the prediction. The compound **59** also showed three H-bond interactions with the neighboring THR 745, SER 741 and TYR 735 residues.

From the present docking studies it can be inferred that some subtle change in molecular structure of the drug molecule alters the ligand binding domain in the drug target. These phenomena is very interesting as well as desirable for drug designing because repeated application of same drug/compound leads to the development of resistance to the action of the drug , due to unavoidable conformational modifications in the drug target. A suitable change in structure or more precisely specific modification in the structural arrangement/connectivity of the drug molecule may enable it to bind with some other nearby residues within the radius of binding domain. As a result, the newer molecule may again act as a good inhibitor to the host molecule and subsequently can show its drug efficacy.

Table 3 Names of the neighbouring residues

Entry	Ligand	Neighbouring residues		BIE (Kcal/mol)
		Within 3.5 A	Within 2.5 A	
1	**1**	ARG 691 GLN 744 GLN 751 GLY 703 TYR 735	ARG 691 GLY 703[a] TYR 735	-9.48 -8.82
2	**29**	ARG 691 GLN 704 GLN 744 SER 741 THR 745 TYR 735	ARG 691 SER 741 TYR 735	-8.51 -10.00 -9.95
3	**59**	ALA 831 ARG 907 ASN 757 ASN 829 GLN 704 GLN 744 GLY 830 LYS 701 SER 756	ASN 757 ASN 829 GLN 704	-8.82 -8.88 -8.39
4	**61**	ALA 780 ARG 508 ARG 782 ASP 513 GLN 632 GLN 704 GLU 512 ILE 553 LYS 515 TRY 511	GLN 632 LYS 515	-8.82 -8.82
5	**45**	ALA 780 ARG 508 ARG 782 GLN 632 ASP 513 GLY 633 LYS 515 LEU 631 GLU 512 ILE 553 TYR 511 PHE 628	GLN 632 ASP 513 GLY 633[a] LYS 515	-8.01 -9.38 -5.61

BIE, Estimated binding energy of the most stable conformation
a, For this residue no active torsion is present

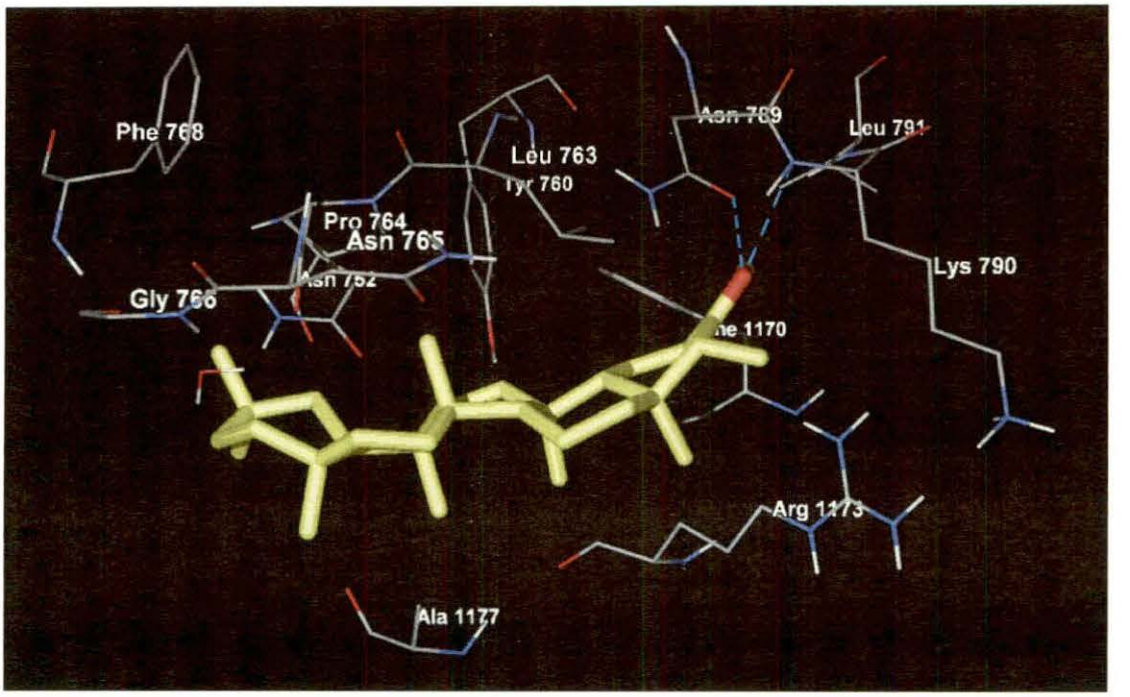

Figure 31 Detailed docked view of friedelin, **1** on the 1bgw, pdb

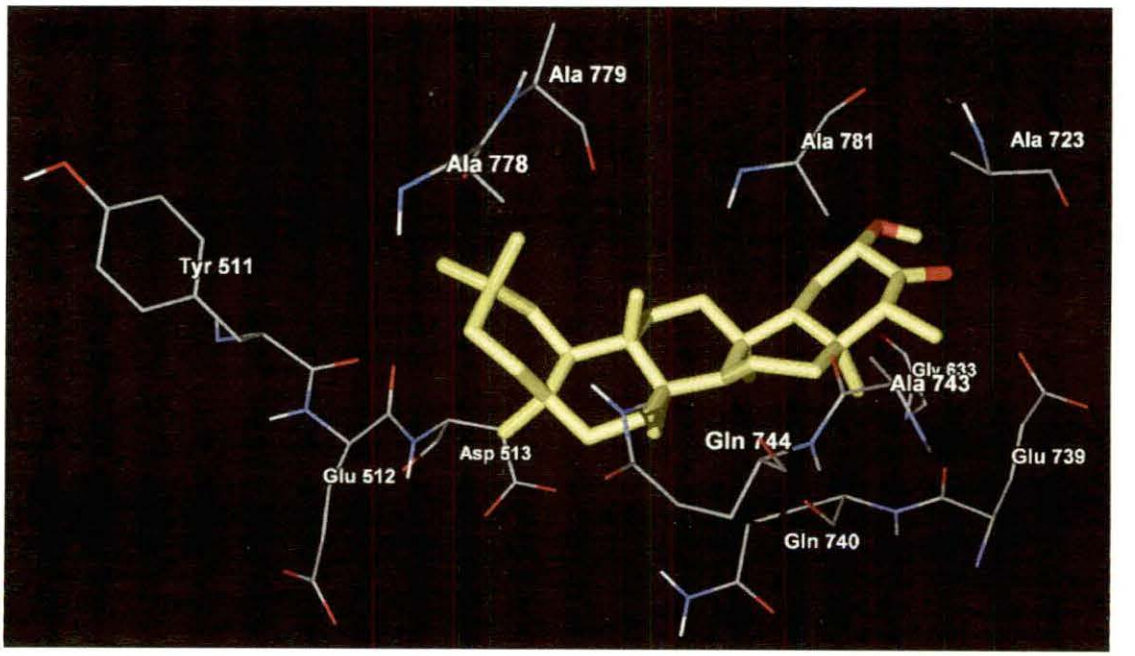

Figure 32 Detailed docked view of cerin, **29** on the 1bgw, pdb

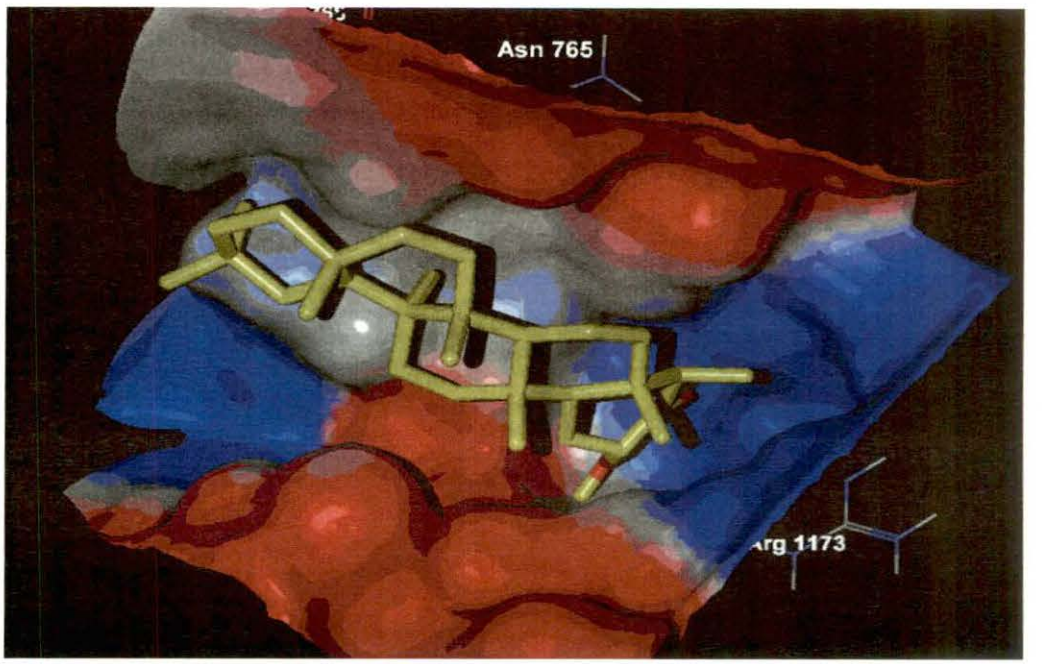

Figure 33 Electrostatic interaction view of cerin, **29** inside the hydrophobic pocket of the binding site in the protein

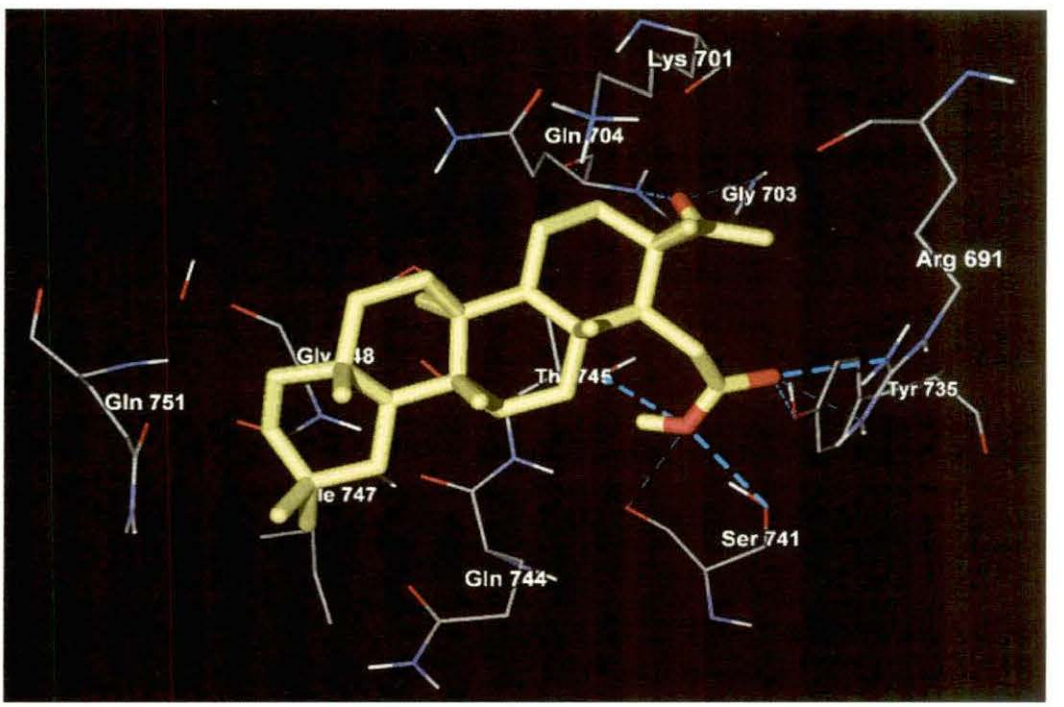

Figure 34 Detailed docked view of compound **59** on the 1bgw, pdb

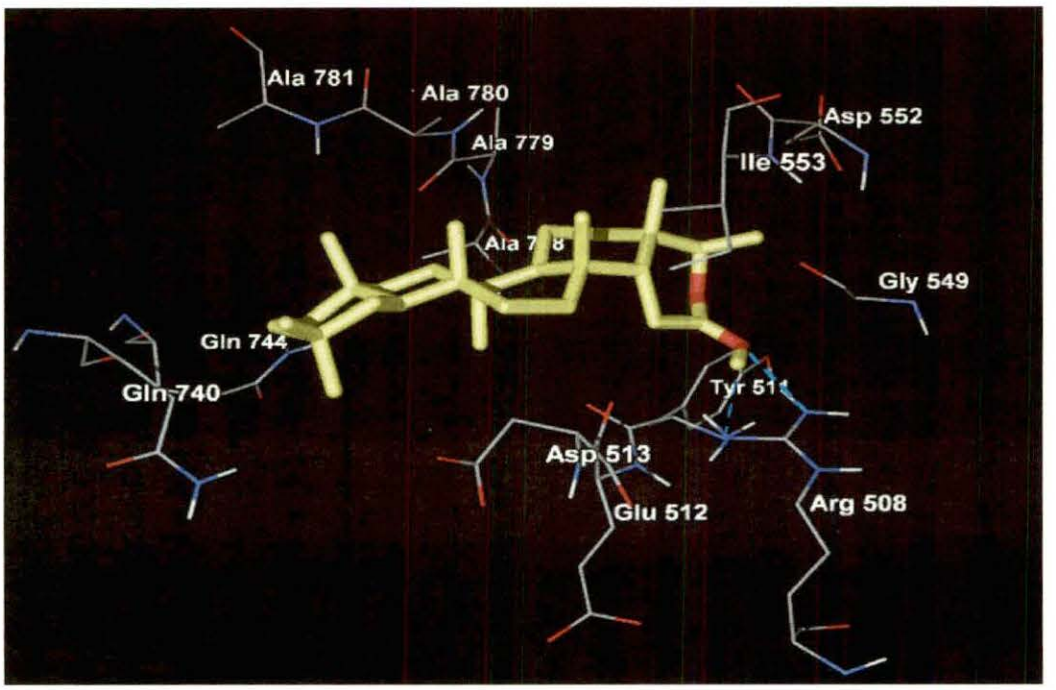

Figure **35** Detailed docked view of compound **61** on the 1bgw, pdb

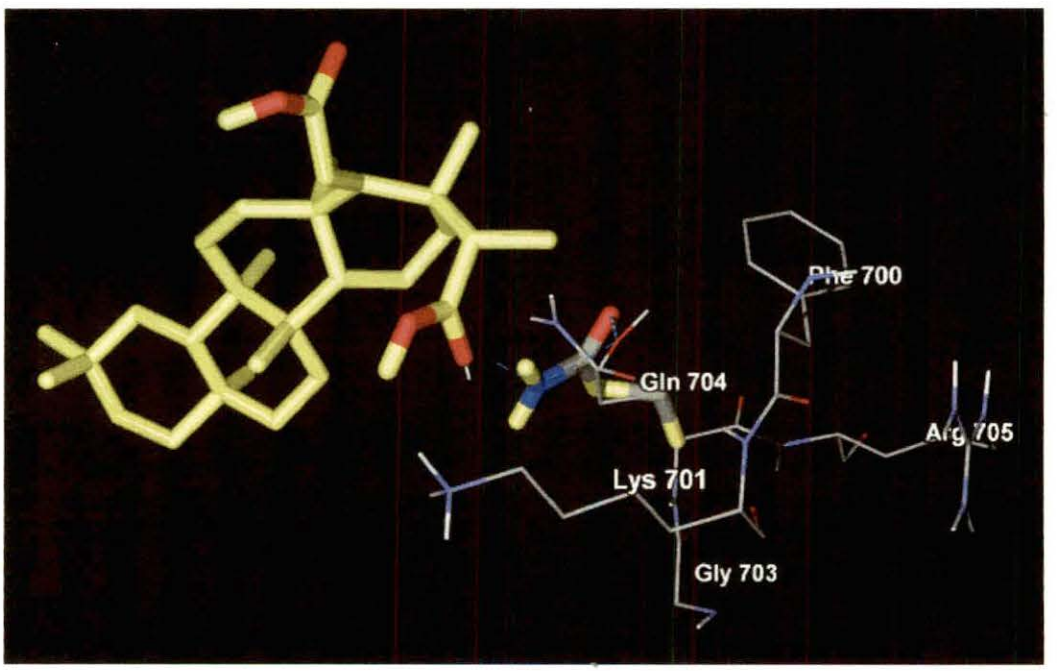

Figure 36 Detailed docked view of compound **45** on the 1bgw, pdb

Additionally, the estimated binding energies of ten different conformations of compounds **59** and **45** against the surrounding residues (only that within 2.5A) are presented in figure 38.

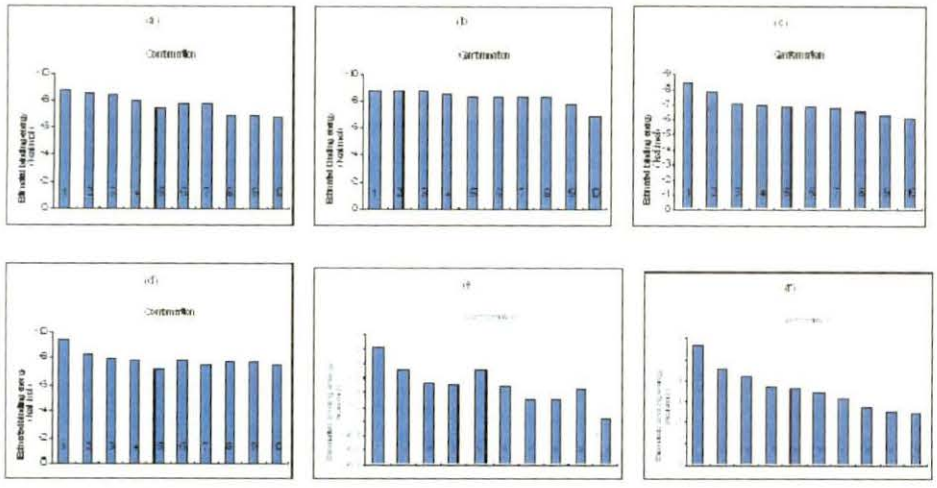

Figure 38 Plots of estimated binding energy (Kcal/mol) vs the ten possible conformations of the ligand; plots (a), (b) and (c) are for compounds **59** for ASN 757, ASN 829 and GLN 704 residues respectively, plots (d), (e) and (f) are for compound **45** for ASP 513, GLN 632 and LYS 515 residues respectively

3. Topoisomerase inhibitory activity of Friedelan derivatives

Inhibition of catalytic activity of topoisomerase constitutes a useful strategy for the identification of potential antitumor agents. Topo IIα creates transient breaks in supercoiled DNA resulting in DNA relaxation. The relaxed DNA can be distinguished from supercoiled DNA by gel electrophoresis analysis. The results of molecular docking studies on interaction of hemisynthetic friedelan derivatives with DNA binding domain of human Topo IIα, was further confirmed by examining DNA relaxation activity of Topo IIα. The ATP dependent relaxation of supercoiled DNA by the enzyme was monitored in absence or presence of hemisynthetic friedelan derivatives **59** and **45**. From the results in figure-39, it is evident that the inhibitory effects of compounds **59** and **45** were dose dependent. They showed complete inhibition of the catalytic activity of Topo IIα at 100 and 50 μM concentrations; whereas partial inhibition of the activity was observed at a concentration of 25μM. Compounds **59** and **45** couldn't be studied due to their partial solubility in DMSO.

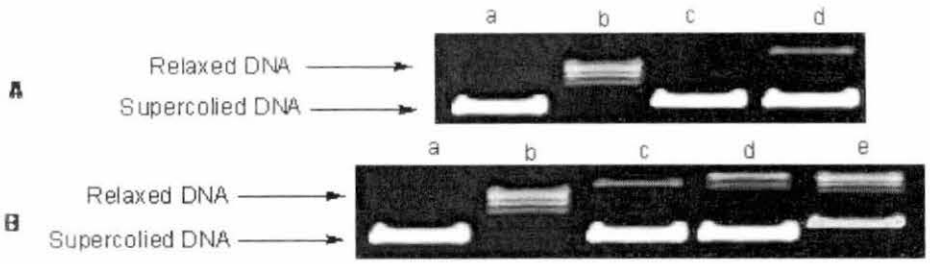

Figure 39 Effect of compound **3a** (**A**) and **5a** (**B**) on DNA relaxation activity by DNA topoisomerase IIα (**A**), Lane a: supercoiled DNA alone; Lane b: control no drug (2 unit of topo II); Lanes c and d: 100 and 50 μM of **3a** respectively. (**B**) Lane a;supercoiled DNA alone; Lane b: control no drug (2 unit of topo II); Lanes c, d and e: 100, 50 and 25μM of **5a** respectively.

4. Conclusion

The present author has successfully synthesized some oxygenated friedelan derivatives. All the molecules were characterized by spectral data and by comparison to that reported in literature. 3D molecular docking of these derivatives in the central catalytic domain of topoisomerase IIα (1bgw PDB for topoisomerase IIα) revealed the nature of the binding and the type of interactions between the synthesized compounds and the enzyme. The topoisomerase IIα inhibitory activity was further confirmed by *in vitro* experiments. This is the first report of the antitopoisomerase activities of friedelan derivatives. The present author believes that the findings will definitely enrich the modern drug designing towards the invention of newer plant based chemotherapeutics to fight against human ailments. In addition the findings may provide a better understanding of the structure activity relationship towards the topoisomerase inhibitory activity of the pentacyclic triterpenoids, whether the carboxylation at ring A is truly the key factor of the defined activity or not.