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# To Design Novel Derivatives of Noscapine (Fourth Generation) Using Structure Based Computer Aided Molecular Designing Techniques

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## ABSTRACT

*Microtubules are responsible to maintain the genetic stability when cell divides. Involvement of these microtubules leads to apoptosis and the drug that binds with microtubule like docetaxel, paclitaxel and the vinca alkaloids are presently using for the treatment of various malignancies.*

*Chemotherapy has negative effects like toxicity, non-selective actions and depolymerizing effects, overpolymerizing effects on microtubule. Systematic screening of new compounds that interfere with microtubules, noscapine, which is an opium alkaloid, was discovered that binds to tubulin stoichiometrically, arrests mammalian cells in mitosis and changes its conformation upon binding. noscapinoids alters the steady-state dynamics of microtubule assembly. it do not show immunological and neurological toxicities and inhibit tumorigenesis in vivo albeit at high concentrations (~ 300 mg/kg body weight). Although noscapine acts as cytotoxic agent in various different cancer cell. A new series of noscapine derivatives consisting of 18 compounds showed improved docking score ranging from-5.8 kcal/mol to -8.17 kcal/mol compared to the lead molecule, noscapine. The predictive binding affinity calculated based on MM-GBSA also revealed improved ΔGbind ranging from -45.12 kcal/mol to -100.75 kcal/mol. collectively, the study reported here identified potential derivatives of noscapine pertaining to chemical synthesis and experimental evaluation as anti-cancer drugs.*

***KEYWORDS**: **Microtubules, Chemotherapy, noscapine, MM-GBSA***

## Introduction-

Cancer causes of death. Cells display uncontrolled growth, invasive intrusion, destruction of adjacent tissues, and metastasis to other locations in the body.

## Microtubule Structure and Dynamics

Microtubules (MTs) are main vibrant structural components in cells. They are important in the growth and maintenance of cell shape, in cell reproduction and division, in cell signaling, intracellular transport of vesicles and organelles and in cellular movement (Lodish et al., 1999).

The a- and b-tubulin monomer each has a molecular mass of about 50 kDa The head-to-tail association of tubulin dimer gives microtubules an intrinsic polarity in their structure.

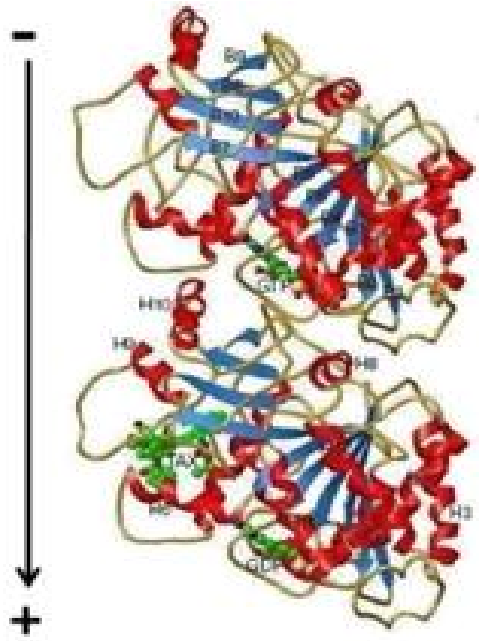


**Figure 6.**Ribbon diagram showingthe α and β-monomers of tubulin from X-ray crystallographic data at a resolution of 3.7 Å viewed from inside of the microtubules. The tubulin consisted of alpha helix, beta strands and loops. The GTP (non-hydrolyzable) is shown to bind to the α-monomer whereas GDP (formed from the hydrolysis of GTP, hydrolyzabe site of GTP) and Taxotere are bound to β-monomer. Colchicine bound at the interface between α- and β- tubulin. The arrow indicates the growth of the protofilaments during microtubule formation (Nogales et al., 1998).

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## Noscapine: a Plant Derived Alkaloid

Noscapine was originally discovered by French pharmacist and Professor Pierre-Jean Robiquet in 1817. He isolated two natural compounds from opium (Papaversomniferum): codeine and noscapine (Warolin et al., 1999). Noscapine (21%) is one of the more abundant opium alkaloids, the other prominent alkaloids being morphine (42%), codeine (12%), papaverine (18%), thebaine (6.5%) sanguinarine, berberine and tubocurarine. The pioneering step has been achieved by Perkin and Robinson (1910), who could obtain noscapine from meconine and cotarnine in the presence of potassium carbonate combined with fractional crystallization.

## Discovery of Noscapine as a New Anti-microtubule Drug

Noscapine is non-narcotic, phthalideisoquinoline alkaloid derived from the opium poppy

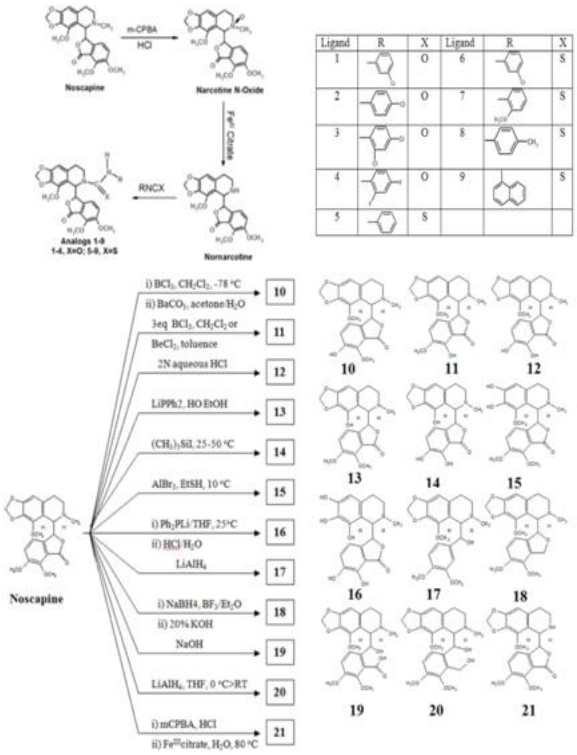


**Figure** Aryl-derivatives of noscapine. These derivatives were synthesized by fictionalization by deleting or substitution of various functional groups as mentioned in the noscapine scaffold. All these derivatives showed very little or no improvement in cytotoxicity activity of noscapine.

## Computer-aided Design of Potent and Novel Noscapine Analogues

This is why computer-assisted drug design (CADD) approaches have been widely used in the pharmaceutical industry to improve the productivity of the drug discovery and development pipeline (Gomeni et al., 2001; For the unrevealed protein structures several methods of ligandbased drug designing are using like pharmacophore analysis and QSAR. Molecular docking is used for structure-based approaches of known target structures to design 3D structures with improved potency

Active data availability of many noscapine derivatives leads to grow a reasonable theoretical prediction model and thus guided in rational design of more potent derivatives of noscapine.

Exacting the method of docking appears to be a primary tool. Docking algorithms provide good-quality binding pose for a compound that fits into the binding cavity of target receptor and evaluates its binding affinity using scoring function. However, the scoring functions used by docking algorithms are very simple. They generally do not include shape complementarity parameters between binding cavity and the ligand as well as the solvation effect in calculating the electrostatic interactions energy between protein and ligand. Thus using of molecular docking only in designing or screening of ligands may be problematic

## Materials and Methods

### How to prepare ligands

All the structure of derivatives of noscapine 4 a-m builded by the help of molecular builder. Structure minimising the energy by macromodel ,OPLS 2005 force field with the help of PRCG algorithm, minimising step should be 1000 and energy gradient 0.001 Assign the bond to each protein using ligprep and optimise the ligands using hybrid density functional theory.

### How to Preparation Protein

For molecular docking and rescoring used co crystallised podophyllotoxin tubulin complex. Schrödinger's protein preparation (multistep) used for the protein preparation .

Identify the missing hydrogen atoms by the use of Maestro Interface. Remove the entire water

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molecule from complex and optimise the hydrogen bond with the help of PPrep Wizard.

With the help of homology modelling fill all the missing amino acid i.e from 37 to 47 in A chain and in B chain 275 to 284 this we done on different templet like PDB ID: 3DU7 ,C-chainand PDB ID: 3RYC, D-chain respectively, using Prime.

**Rescoring using Prime/MM-GBSA approach**

In favor of every ligand, the pose with the lowest Glide score was rescored using Prime/MM-GBSA approach (Lyne et al., 2006). Approach used for calculation of free energy for set of ligands to receptor. Docked pose were minimized using local optimization feature in Prime The binding free energy ($\Delta G_{bind}$) is then estimated using equation:

$$\Delta G_{bind} = E_{R:L} - (E_R + E_L) + \Delta G_{solv} + \Delta G_{SA}$$

where $E_{R:L}$ is energy of the complex, $E_R + E_L$ is sum of the energies of the ligand and unliganded receptor, using the OPLS-AA force field, $\Delta G_{solv}$ ($\Delta G_{SA}$) is the difference between GBSA solvation energy (surface area energy) of complex and sum of the corresponding energies for the ligand and unliganded protein.

**Results and discussion**

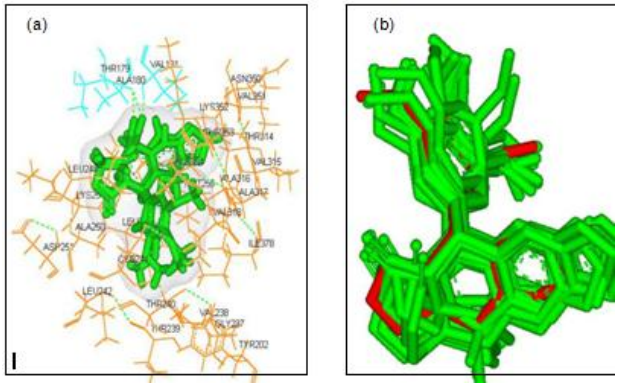
**Docking method validation**

Crystal structure of tubuline-podophyllotoxin complex (PDB_ID: 1SA1) used to validate the glide-XP protocol of docking. Co crystallised podophyllotoxine ligand moves outside the active site and dock it back in the active site. To validate the result top 10 configurations were taken after docking .For every configuration we calculate the RMSD and the value will be from 0.02 to 0.85 Å.

The docking score and RMSD from the docking simulation of 10 lowest configuration of cocrystal podophyllotoxin in tubulin protein (ISA1)

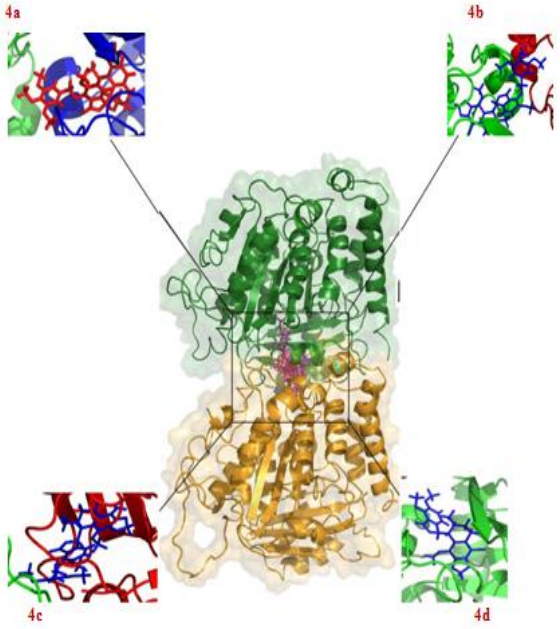
.[a]$\Delta G_{score}$ = $E_i$-$E_{lowest;}$[b]RMSD = RMSD between docked and crystallographic podophyllotoxin structure; [c]RMSD = RMSD between docked poses corresponding to each configuration. Whereas the RMSD value calculated out of 10 accepted poses for each configuration was found in between 0.59 – 1.33 Å. This discloses that the docked configurations have similar site or place of binding and same orientations.

These docking results illustrate that the best-docked podophyllotoxin complex agrees well with its crystal structure and that Glide (XP)-docking protocol successfully reproduces the crystal tubulin-podophyllotoxin complex.




Glide 4.0 in XP mode have been used to dock the newly designed noscapinoid derivatives onto the noscapinoid binding site of tubulin. All the 18 noscapinoids were found to be good binder with tubulin.

**Figure 11.**Superposition of the docked configurations of co-crystallized podophyllotoxin: (a)with binding site and (b) only the superposed structure (red one represents the X-ray podophyllotoxin structure). RMSD (heavy atoms) = 0.02 to 0.85 Å.



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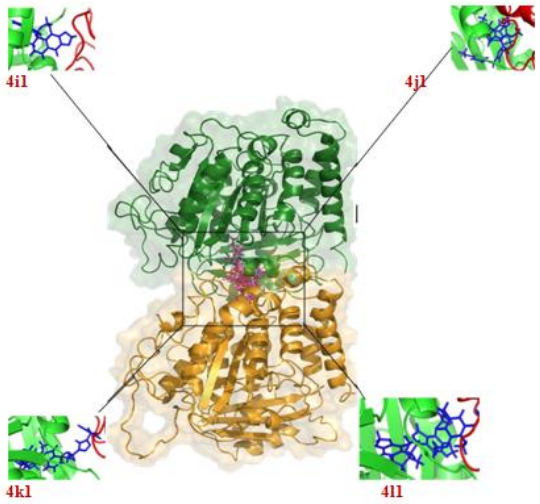



| Configuration | Glide Score | [a]$\Delta G_{score}$ | [b]RMSD (Å) | [c]RMSD (Å) |
|---|---|---|---|---|
| 1 | -10.26 | 0 | 0.85 | 0.60 |
| 2 | -10.20 | -0.06 | 0.02 | 0.86 |
| 3 | -9.80 | -0.46 | 0.68 | 1.33 |
| 4 | -9.72 | -0.54 | 0.57 | 1.26 |
| 5 | -9.50 | -0.76 | 0.04 | 0.67 |
| 6 | -9.25 | -1.01 | 0.04 | 0.67 |
| 7 | -8.78 | -1.48 | 0.80 | 0.59 |
| 8 | -8.47 | -1.79 | 0.13 | 1.02 |
| 9 | -7.87 | -2.39 | 0.03 | 0.79 |
| 10 | -7.72 | -2.54 | 0.07 | 0.90 |

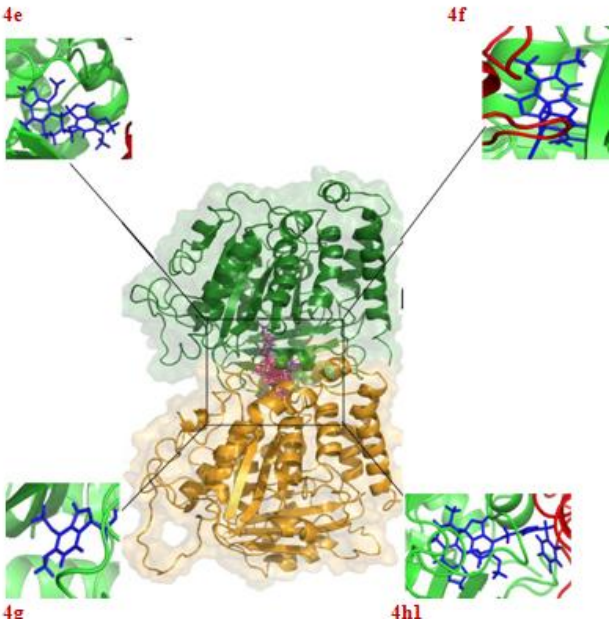
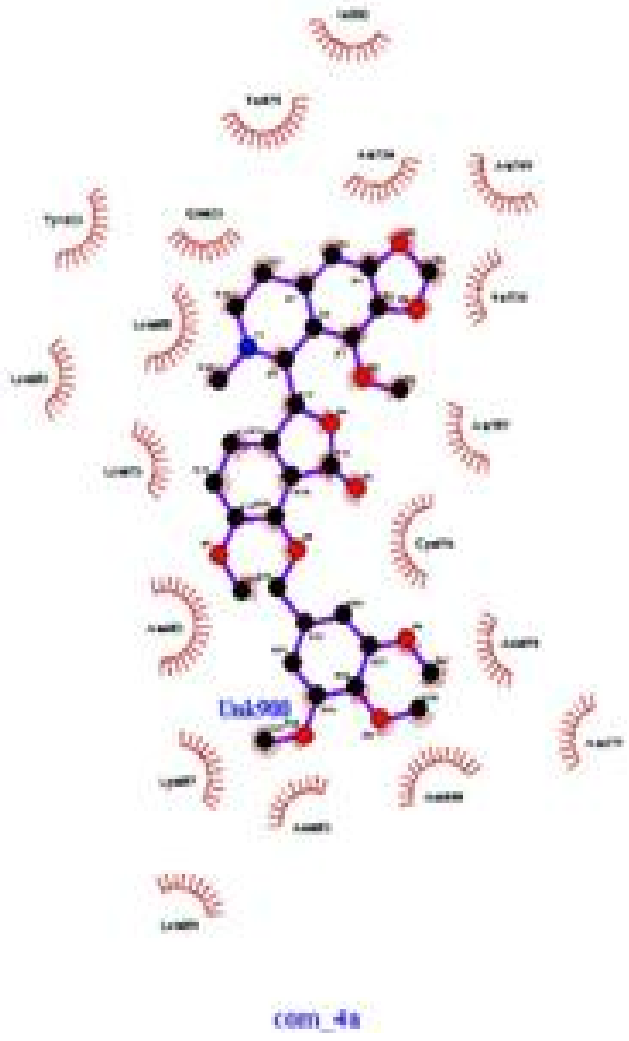



**Fig12.** The newly designed noscapinoids**4a-4f**are well accommodated in noscapinoidbinding site of noscapinoid at the interface in between the- and β-tubulin. It is the snapshot of the ligands **4a-f**, obtained by the MM-GBSA calculation. Binding site is represented as macromodel surface according to residue charge (Electronegative charge, blue; neutral, yellow, red, electropositive charge) as implemented in Pymol.

**Ligplots for all the derivatives**

**Component_4a**



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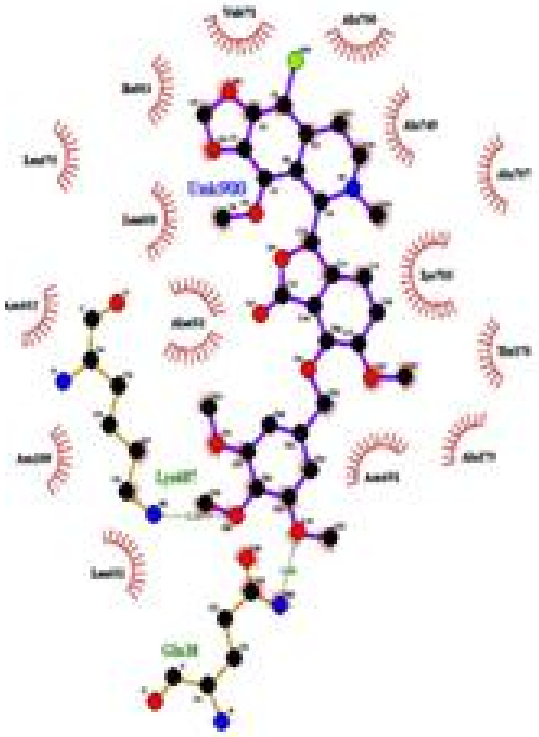
**component_4b**

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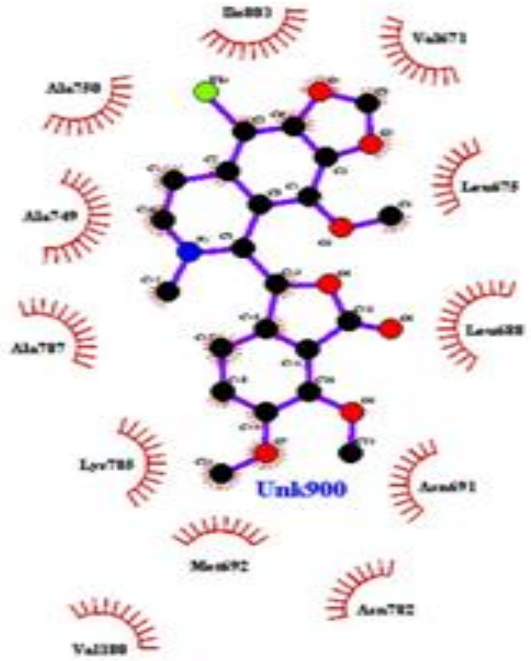


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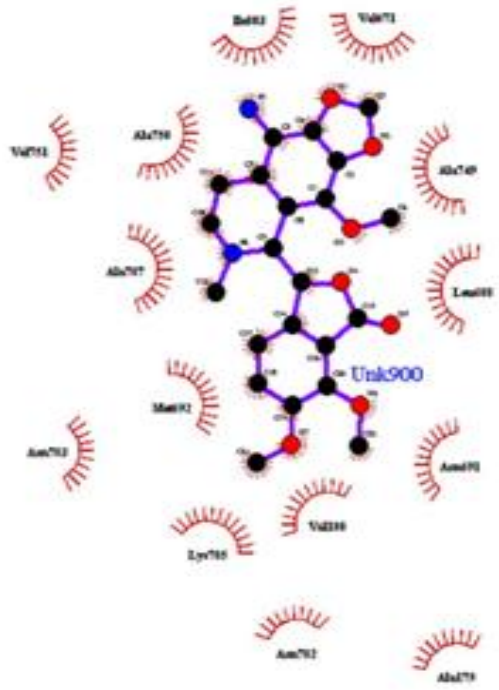


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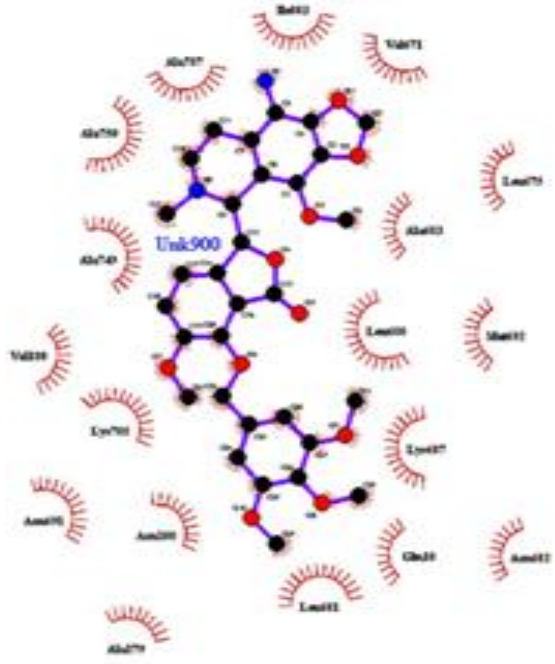


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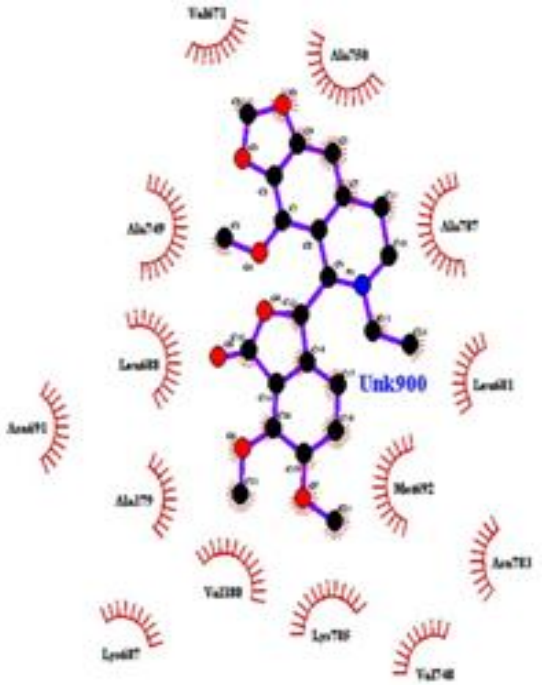
**Component_4f**

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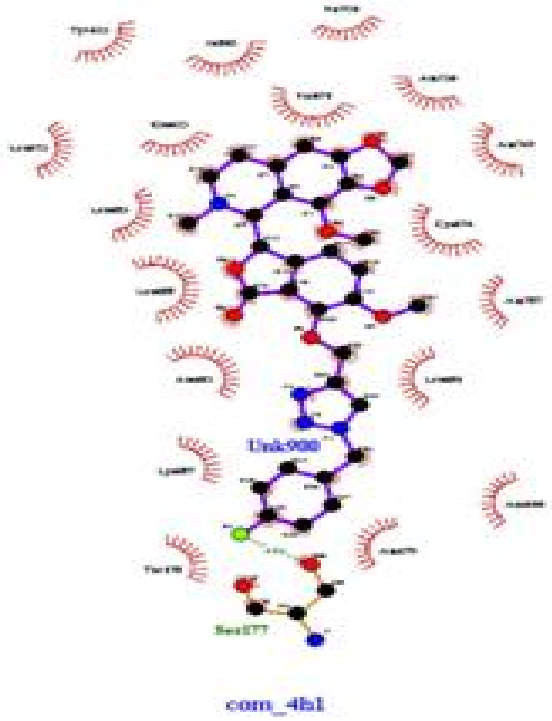


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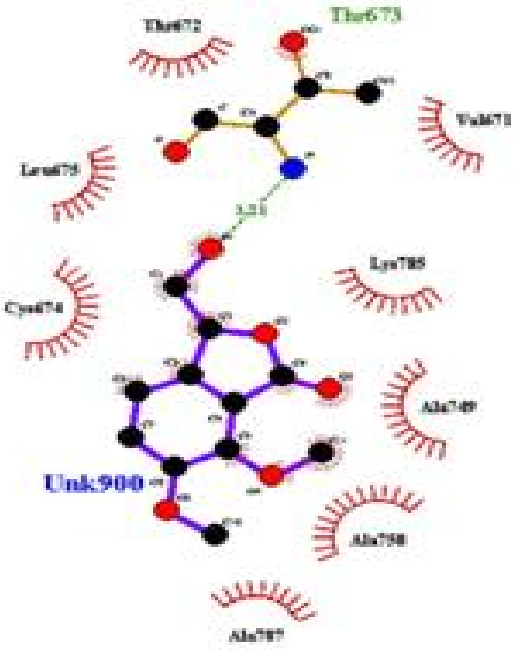


com_4g

**Component_4i1**


com_4i1

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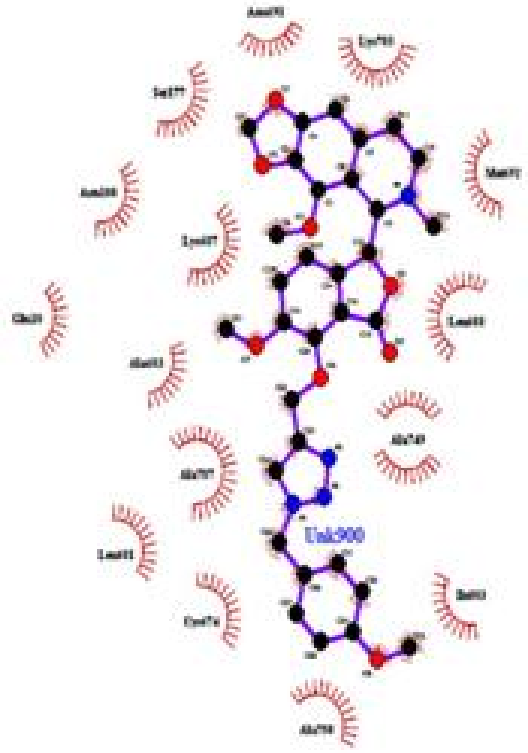
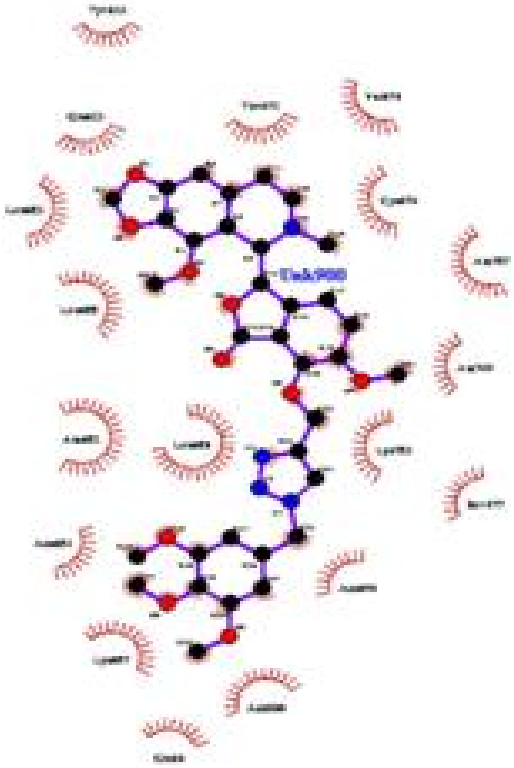
**Component_4j1**




com_4j1

**Component_4l1**

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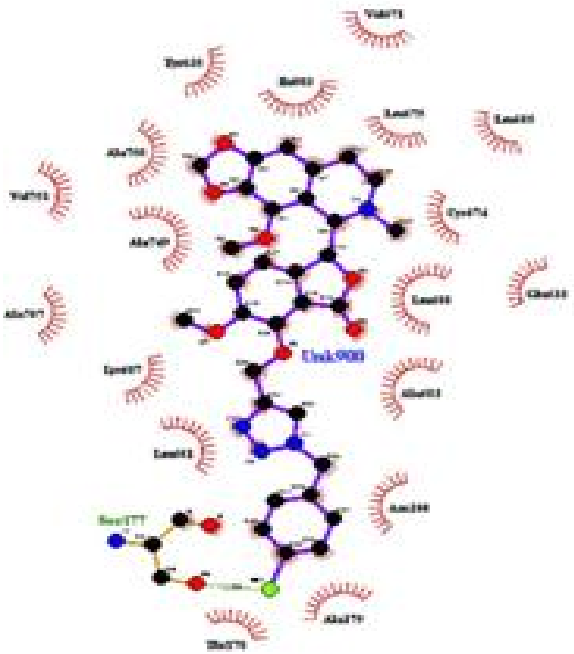


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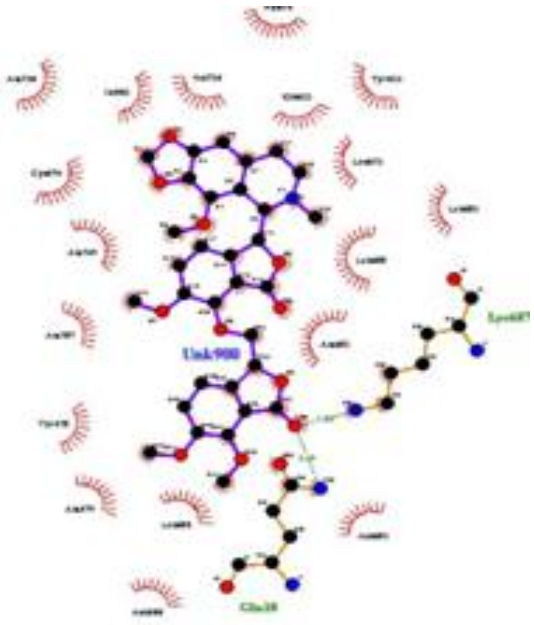
**Component_4m**

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For every ligands, the lowest glide scores rescored using primeMM-GBSA approach. For binding free energy we use this approach ($\Delta G_{bind}$) for set of ligands to receptor. Table 4 reveals the $\Delta G_{bind}$ energy and its components of all the newly designed noscapinoids. All these derivative showed better binding affinity, rang from -45.12 kcal/mol to -100.75 kcal/mol. So we can conclude that the structural derivatives of noscapine used in the study is far more focused to tubulin binding site based on both glide score and primeMM-GBSA approache.

## Conclusion

First we complied 18 nascapinoid library after the modification in the scaffold structure of noscapine We have done docking and rescoring by the help of prime MM-GBSA for the interaction of Ligand-Tubulin and will predict the binding-affinity of noscapine derivatives.After the simulation of docking, flexible dock shows the binding structure of crystal structure . All these testsconfirm the docking protocol acquire in the job.

With the help of docking we can see that it can dock inhibitors into receptors similar to the complex to the crystal structure with podophyllotoxin. Many types of noscapine analogues studied in docking simulation.Analysis showed that all analogous are binding in a same manner. The magnitude of the binding affinity can be a key factor that decides the activeness of an individual inhibitor. The key factor i.e. the magnitude of binding affinity will decide the energy of individual inhibitors. After the assessment of the binding affinity will find a way to estimate the inhibitors activity. In any type of calculation of binding energy the correct binding structure of every ligands resolute first prior to binding energy estimation. Noscapine crystal structure is not available but podophyllotoxin's crystal structure is available with tubuline. To determine the binding structure of noscapine analogues with tubulin we use flexible docking . The optioned structure is very similar for the set of analogues.

The newly designed noscapinoids determine the better binding affinity compared to noscapine. Taken together these analogs specify a significantprospective for further clinical evaluation and preclinical.

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