INTRODUCTION

Over the years, various investigations have come up with novel therapeutic interventions to prevent and/or treat neoplastic disorder and associated concerns such as metastasis. Metastasis, spreading of cancer lesions from primary site of carcinogenesis to secondary sites, requires cellular phenotype transition (epithelial-mesenchymal transition) which involves reprogramming cytoskeletal elements [1]. Research evidences also report that early stages of malignant transformation of neoplastic cells essentially involve alteration of cytoskeletal structure of cells [2]. A good proportion of chemotherapeutic agents act through interfering microtubule function in transformed cells. Among these, tubulin-binding drugs are most successful and constitute a greater affinity of the newly designed ligands for binding to tubulin was predicted. The predictive binding free energy ($E_{binding}$) of these derivatives (ranging from $-10.5178$ to $-16.8473$ kcal/mol) based on linear interaction energy method with a surface generalized born continuum salvation model showed improved binding affinity with tubulin as compared to the lead compound, natural α-Noscapine ($-5.505$ kcal/mol). The binding energy of ligand determined using LigX, i.e., MM/GBVI was found to be $-23.208$ kcal/mol.

Conclusion: We found that designed derivative compounds have better efficacy as compared noscapine and triazole.

Keywords: Noscapine, Triazole, Tubulin heterodimer, Molecular docking, Surface generalized born.

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with tubulin, leading to altered conformation causing arrest of cell division. Noscapine causes alteration in the steady-state dynamics of microtubule assembly before and during metaphase [6].

Triazole (C₃H₅N₃) is a heterocyclic compound having a five-membered ring of two carbon atoms and three nitrogen atoms [7]. There are two sets of isomer, which differs in their relative positions of the three nitrogen atoms. The in vitro studies performed by National Cancer Institute (NCI), USA, using a panel of 60 human cancer cell lines (NCI60), have shown that 1,2,3 triazole and its derivative posse anticancer activity [7]. On an average, it takes about 10–15 years and US$ 500–800 million to introduce a drug in the market. The use of computer-aided drug designing to improve the efficacy of the drug discovery and will accelerate the development pipeline in the pharmaceutical industry. Utilizing tools and applications of bioinformatics for in silico development and synthesis of novel drugs for the treatment of various diseases will improve the effectiveness with reduced cost and time.

Combinatorial chemistry of many derivatives of noscapine has been used along with reasonable speculative prediction model of most potent derivative (Figs 1 and 2). Despite being primary tool for speculative calculation, the use of molecular docking alone may result in decrease in accuracy. To overcome this issue and find more precise predictive information about derivatives, the present study employs methodologies to rescore docking results. The present investigation exercises molecular mechanics in combination with implicit continuum salvation-based scoring function including linear interaction energy method with a surface generalized born continuum salvation model, salvation-based scoring function including linear interaction energy exercises molecular mechanics in combination with implicit continuum methodologies to rescore docking results. The present investigation predicts information about derivatives, the present study employs speculative calculation, the use of molecular docking alone may result potent derivative (Figs 1 and 2). Despite being primary tool for speculative calculation, the use of molecular docking alone may result in decrease in accuracy. To overcome this issue and find more precise predictive information about derivatives, the present study employs methodologies to rescore docking results. The present investigation exercises molecular mechanics in combination with implicit continuum salvation-based scoring function including linear interaction energy method with a surface generalized born continuum salvation model, salvation-based scoring function including linear interaction energy exercises molecular mechanics in combination with implicit continuum methodologies to rescore docking results.

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In silico screening and ligand preparation

Selection of protein file

The criteria for the selection of protein file (PDB) were that it should be from the human source and should have a resolution between 0.5 and 2.00 Å. Thus, PDB file of ABTUBMDS was selected. The PDB was downloaded from RCSB protein data bank. The allowable and disallowable regions were checked from Ramachandran plot and ERRAT report of PDB. The water molecules were removed from the monomer. The hydrogens were added to the protein molecule and energy was minimized using Merck molecular force field (MMFF).

Ligand preparation

Ligands from the series of 3-(1H-1,2,3-triazole-4-yl)propan-1-ol derivative of noscapine (Table 1) to (3R,4S)-5-(1H-1,2,3-triazol-4-yl) tetrahydrofuran-2,3,4-triazole derivative of noscapine were prepared by the combinatorial approach in MOE Builder (MOE 2008.10). The 3D structure was energetically minimized using molecular mechanics followed by MMFF. All the conformers were then energetically minimized up to the RMS gradient of 0.001 and saved in a separate folder. Dielectric properties were kept constant. Gradient type used was analytical with a maximum number of cycles were 1000. The potential energy of the conformers was calculated using MOE potential energy calculator.

LogP value

Log P stands for the partition coefficient of any compound; it measures how well a substance partitions between a lipid (oil) and water. It helps in understanding the behavior of drug molecules, as different ionic species of molecule differ in its physiochemical and biological property so it is important to be able to predict which ionic form of the molecule is present at the site of action.

Log P = 1 means 10:1 organic: aqueous
Log P = 0 means 1:1 organic: aqueous
Log P > 1 means 1:10 organic: aqueous

Studies have found: (these may not apply to every class of chemicals)

• Optimum CNS penetration around Log P = ±0.7 (Hansch)
• Optimum Oral absorption around Log P = 1.8
• Optimum Intestinal absorption Log P = 1.35
• Optimum Colonic absorption Log P = 1.32
• Optimum Sublingual absorption Log P = 5.5
• Optimum Percutaneous Log P = 2.6 (and low mw).

Formulation and dosing forms:

• Low Log P (below 0) Injectable
• Medium (0-3) Oral
• High (3-4) Transdermal
• Very High (4-7) Toxic buildup in fatty tissue.

Lipinski’s “rule of 5” for drugs

Chris Lipinski of Pfizer derived an easy to use “rule of thumb” for drug-likeness in molecules after surveying the world’s marketed drugs.

The rule states that for reasonable absorption:

• Keep H-Bond donors <5 (sum of OH and NHs)
• Keep mW <500
• Log P should be <5
• No more than 10 H bond acceptors.

Energy minimization

The energy minimization application in MOE calculates atomic coordinates that are local minima of a molecular energy function. Energy minimization comprises finding a set of atomic coordinates that match up to a local minimum of the molecular energy function (such as the
<table>
<thead>
<tr>
<th>S. No</th>
<th>R-Group</th>
<th>LogP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>propan-1-ol</td>
<td>0.34±0.8</td>
</tr>
<tr>
<td>2.</td>
<td>ethanol</td>
<td>1.34±0.18</td>
</tr>
<tr>
<td>3.</td>
<td>cyclohexanol</td>
<td>1.69±0.15</td>
</tr>
<tr>
<td>4.</td>
<td>cyclopropane</td>
<td>1.90±0.22</td>
</tr>
<tr>
<td>5.</td>
<td>thiophene</td>
<td>1.32±0.22</td>
</tr>
<tr>
<td>6.</td>
<td>1-methylcyclopentanol</td>
<td>0.73±0.18</td>
</tr>
<tr>
<td>7.</td>
<td>pyridine</td>
<td>0.19±0.18</td>
</tr>
<tr>
<td>8.</td>
<td>ethanol</td>
<td>2.82±0.93</td>
</tr>
<tr>
<td>9.</td>
<td>butan-2-one (trans,trans,trans)-cyclohexene</td>
<td>3.74±0.17</td>
</tr>
</tbody>
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(Contd...)
potential energy model). This is done by applying large-scale non-linear optimization techniques to calculate a conformation (near to the starting geometry) for which the forces on the atoms are zero.

**Force field optimization**
The truncated Newton (TN) method is the most efficient large-scale non-linear optimization method known. It exhibits superlinear convergence even in highly non-linear conditions. The TN method attempts to use curvature information to improve convergence. The Newton direction $p$ satisfies:

$$G_k(x_k) p = - \text{grad} \ U(x_k)$$

Where, $G_k$ is the second derivative, or Hessian, of $U$. These equations are called the Newton equations. TN solves the Newton equations approximately using an iterative linear equation solver. The iterative linear equation solver (based on the Linear Conjugate Gradient method) is terminated after a small number of iterations, hence, the name TN. The energy of both receptor and ligand is minimized.

**Binding site**
The different site of ligand binding is detected using "site finder" in MOE (MOE 2008.10). The purpose of Site Finder is to calculate possible active sites in a receptor from the 3D atomic coordinates of the receptor. Site Finder falls into the category of geometric methods since no energy models are used. Instead, the relative positions and accessibility of the receptor atoms are considered along with a rough classification of chemical type. The Site Finder methodology is based on Alpha Shapes (AlphaShapes) which are a generalization of convex hulls developed in (Edelsbrunner, 1995). About 46 binding sites are detected to which binding of ligand is performed.

**Surface and mapping**
The surfaces and maps application is used to generate visual representations of molecular surfaces and can be used to predict the preferred locations of ligand atoms (in the absence of the ligand itself). The surfaces and maps application is capable of calculating a number of molecular surfaces - surfaces near to the Van der Waals or solvent accessible surfaces of a molecule. The reentrant surface, or Connolly surface, consists of the boundary of the volume of space defined by removing all points inside the Van der Waals surface of the spherical probe (that represents the solvent) located at all positions for which the probe does not contact the Van der Waals volume of a molecule.

**Molecular docking**
The purpose of the dock application is to search for favorable binding configurations between small- and medium-sized ligands and a not too flexible macromolecular target, which is usually a protein. For each ligand, a number of configurations called poses are generated and scored in an effort to determine favorable binding modes. The top scoring poses are written out to a database for further analysis. Docking simulation was carried out using MOE-Dock. The default parameters of MOE-Dock program were used to find the correct conformations of the ligands and to obtain minimum energy structures. Ligands were allowed to be flexible (rotation of bonds was allowed) in the docking algorithm. At the end of docking, the best conformations of the ligands were analyzed for their binding interactions. The parameters used for docking were as follows: Triangle Matcher placement with timeout (300 s) and the number of Return poses=1000, Rescoring1: London dG, Retain=10, and force field refinement with a final gradient of 0.01 with a maximum of 500 iterations. The docking of the 28 CCBs into the
Ligand interaction

The ligand interactions application provides a means to visualize an active site of a complex in diagrammatic form. The diagram itself consists of the selected ligand as the centerpiece, which is drawn using the traditional schematic style for molecules. A selection of interacting entities, which includes hydrogen-bonded residues, close but non-bonded residues, solvent molecules, and ions are drawn about the ligand, their positions in 2D being chosen to be representative of the observed 3D distances, as well as taking into account esthetic considerations. Additional properties such as solvent-accessible surface area and the ligand proximity outline are also shown.

RESULTS

Selection of protein file

ABTUB protein file is downloaded from the public database library in the PDB format and is then exported to the MOE window where partial charges and hydrogen is added, and the potential energy of the receptor is fixed using MOE/EDIT function tool (Fig 3).

Ligand preparation

Using combinatorial approach, 20 derivatives of ligand are made. Using Molecular Builder tool (MOE 2008.10), ligand is made by adding different functional group containing triazole pharmacophore to the biaryl pharmacophore skeleton of noscapine (Fig 4). Mainly addition of triazole is made at the 13\textsuperscript{th} carbon position in the skeleton of noscapine. On the basis of it, about 20 stable structures were drawn.

The energy of all the 20 ligands is minimized and saved in MDB file format. However, the ligand 1 [2-(3\textsuperscript{H}-pyrazol-5-yl)ethanol ammoniate (1:1) derivative of noscapine] showed maximum activity in overall process; hence, the overall property of the ligand (Fig 5) is deduced using software ChemDraw, the property generated is as follows:

Site generation

Using Site Finder tool in MOE/compute/site finder, sites are generated at alpha center and dummy atoms are created. All the total of 46 binding sites was generated (Fig 6) to which there is a possibility of ligand binding. A list of site is generated to which each and every site is saved in MOE format for further use.

Surface and map generation

A Gaussian surface is generated (Figs7-9) at alpha dummies atom surrounding the Van der Waals surface of a molecule which is solvent in accessible gaps with constant activity, colored by constant properties and the transparency of back side is set to more than 75% approximately. Using graphics, object manager back and front is made glossy.

Molecular docking

Docking is performed for every ligand to each site and is searched for maximum binding energy score value (Figs10 and 11). However, the binding energy value of ligand 1 with that of site 2 showed a maximum result. Docking is also performed with native triazole and noscapine molecule to the same site.

The overall binding score was $-16.8035$, with retain of 10, then the same was exceeded to retain a value of 100, leading to more optimized result, i.e., $-16.0380$. While the overall binding of native noscapine to the same site is $-12.0377$, and that of native triazole molecule is $-6.7858$. Hence, the binding efficacy of the new fused molecule of both the native compounds has increased.
Ligand interaction
With the help of LigX plot, interaction plot is generated which provides a detailed knowledge of the type of bonds and amino acids playing a role in the interaction. The interaction report and diagram of triazole and noscapine and then the ligand is attached one by one. The interacting amino acid in case of native triazole is aspartic acid and in case of noscapine is glycine, asparagine, and tyrosine; whereas, in case of my ligand 1 [2-(3H-pyrazol-5-yl)ethanol ammoniate (1:1) derivative of noscapine] has lysine, glutamine, threonine, asparagine, proline, and tyrosine; hence, we can say that the overall binding efficiency of the new fused compound has increased from its native molecule (Fig 12).

MD simulation
MD simulation is carried out for 200 picosec in two phases, i.e., neutral and heating phase. The value calculated out after equilibrium simulation is 0.001 and the correlation plot is shown below. The basic principle behind MD simulation is that every particle has its own vibrational motion and frequency which changes from one state to other state and then to a zeroth stage. Thus, MD simulation helps in identifying the stability of substance at two-point stage and then time back to its zeroth stage and the kinetic energy of motion is deduced.

DISCUSSION
The side chain addition is done keeping in mind their partition coefficient. The ligand derivative generated was force field energy optimized, and the activity was deduced by calculating the binding energy and free energy binding (MM/GBVI) of ligand and native compound. The result generated has shown an elevated result of drug efficacy from its lead compound, i.e., noscapine and triazole. Native binding energy generated by docking is in the range of 4 for triazole and 11 in the case of noscapine while to an astonishing my lead compound showed binding energy in the range of 16, which is much more efficient. Similar to that, the MM/GBVI energy of triazole cannot be estimated as it is a small molecule with high vibrational energy, while that of noscapine showed an energy of −16.272 kcal/mol and that of my ligand has −23.208 kcal/mol not only this ligand interaction showed an increase in binding amino groups but also the percent of binding was 13% with aspartic acid for triazole, 12% with tyrosine, and 74% with glycine for noscapine. While the ligand has 75% with tyrosine, 24% and 21% proline at two sites, 44% with aspartic acid, 48% and 57% with threonine from two sites, 73% and 63% with glutamic acid at two sites, and 18% with lysin, this shows that the overall binding affinity has increased and thus the drug can be screened for in vivo and ex vivo experiment to support the in silico result, and finally, it can be used as one of the oral drugs of cancer in future as the logP result shows it to be non-toxic and oral route administration is predicted.

CONCLUSION
In the present study a greater affinity of the newly designed ligands for binding to tubulin was determined for 2-(3H-pyrazol-5-yl) ethanol ammoniate (1:1) derivative of noscapine. The predictive binding free energy (Gbind, pred) of all twenty derivatives were ranging from −10.5178 to −16.8473 kcal/mol based on linear interaction energy.
method with a surface generalized Born continuum salvation model which shows improved binding affinity with tubulin as compared to the lead compound. Natural α-Noscapine (−5.505 kcal/mol). Therefore the derivatives can be further evaluated for its anticancer activity by using invitro and invivo techniques.

REFERENCES