

LIST OF FIGURES

- Figure 1.1** Continental distribution of cancer cases. Visual depiction of the estimates projected by the American Cancer society in 2007.
- Figure 1.2** Year wise total cancer prevalence in India [ICMR, 2006; ICMR, 2009].
- Figure 1.3** The most common cancers found in India in comparison to USA.
- Figure 1.4** Proportions of various mechanisms/drugs currently in-use in anti-cancer therapy.
- Figure 1.5** Molecular structure of tubulin hetero-dimer consisting of α - and β -tubulin.
- Figure 1.6** Microtubules having 13 linear protofilaments forming the wall of the hollow tube and these protofilaments in turn result from a head-to-tail assembly of tubulin dimmers.
- Figure 1.7** Microtubule dynamics and the measured dynamic parameters.
- Figure 1.8** The structure of various tubulin interacting agents sharing structural similarity with the promising tubulin binding agent Noscapine (from opium poppy).
- Figure 1.9** Noscapine as tubulin binding agent. Titration of fluorescence spectra of tubulin and Scatchard plot.
- Figure 1.10** Figure showing that noscapine increases the average time cellular microtubules remain inactive (pause duration).
- Figure 1.11** Halogenated derivatives of noscapine.
- Figure 1.12** Aryl-derivatives of noscapine.
- Figure 1.13** Design strategy for new α -noscapine analogues.
- Figure 1.14** The overview of structure based drug design cycle.
- Figure 1.15** The schematic representation of various steps of QSAR model building and prediction.
- Figure 2.1** View of noscapine with the atom-labelling scheme.
- Figure 2.2** Two-dimensional NOESY spectrum of noscapine.
- Figure 2.3** Two most probable noscapine conformations in solution derived from the NOESY spectroscopy followed by NAMFIS analysis.
- Figure 2.4** Elucidation of dynamic structure of noscapine.
- Figure 2.5** Schematic representations of different steriomolecular parameters used in the study.
- Figure 2.6** Relationship between predicted and experimental biological activities of training set compounds as per the final QSAR equation.
- Figure 2.7** Relationship between predicted and experimental biological activities of test set compounds as per the final QSAR equation.
- Figure 2.8** Synthesis of 9-azido-noscapine and reduced 9-azido-noscapine.
- Figure 3.1** Multiple sequence alignment of the stretches of amino acids located in or near the 12 Å of the binding site of noscapinoids in α -tubulin in the tubulin heterodimer.

- Figure 3.2** Snapshots of pdb file 1SA0 showing errors in numbering in β chain.
- Figure 3.3** Representation of gaps (missing residues) in the crystal structure (1SA0) of tubulin dimer showing the gaps in the structure.
- Figure 3.4** Screen shot of missing residues in α (A) and β (B) chain shown in the pdb file (1SA0).
- Figure 3.5** Chemical structure of noscapine and its derivatives used in the study.
- Figure 3.6** Multiple sequence alignment of β_I - β_{VIII} isotypes showing mismatches at certain places.
- Figure 3.7** Modeled tubulin dimer along with ligand colchicine, GTP and GDP with gaps filled.
- Figure 3.8** Figure showing the superimposition of the 1SA0 template and the modeled tubulin dimer.
- Figure 3.9** Typical snapshots of pdb file showing $C\alpha$ coordinates of the filled gap in α (A) chain and β (B).
- Figure 3.10** Pairwise alignment between sequences of modeled structure of chain A and chain B and template 1SA0 chains A & B along with secondary structure alignment.
- Figure 3.11** Structure validation diagrams based on Programs Errat, Verify 3D and Procheck (Ramachandran plot).
- Figure 3.12** Time series of the root-mean-square deviations (RMSD) for the $C\alpha$ carbon atoms of template ($\alpha\beta$) and all the 8 tubulin isotypes ($\alpha\beta_I$ to $\alpha\beta_{VIII}$) during 10 ns of MD simulation starting from the initial structure.
- Figure 3.13** Superimposition of $\alpha\beta$ -tubulin isotypes ($\alpha\beta_I$ to $\alpha\beta_{VIII}$) with the template structure (1SA0).
- Figure 3.14(a-h)** All-vs-all structure superimposition and comparison of the $\alpha\beta$ -tubulin isotypes.
- Figure 3.15** Superimposition of the noscapinoid binding site of the template structure (1SA0) and all the 8 isotypes of tubulin ($\alpha\beta_I$ - β_{VIII}).
- Figure 3.16(a-h)** All-vs-all superimposition and comparison of the noscapinoid binding site of the amino acids within 12 Å diameter of noscapinoid binding site of the different $\alpha\beta$ -tubulin isotypes.
- Figure 3.17** The hydrogen bonding pattern of amino-noscapine with respect to all the $\alpha\beta$ -tubulin isotypes.
- Figure 3.18** Differences in the electrostatic and van der Waals energy contribution of residues within the 12 Å diameter of noscapinoid binding site of docked amino-noscapine with respect to β_I - β_{VIII} -tubulin isotypes.
- Figure 3.19** Typical snapshot of binding mode of amino-noscapine with tubulin obtained after MD simulation.
- Figure 3.20** The root-mean square deviations (RMSD) of $C\alpha$ carbon atoms of tubulin during 10 ns of MD simulation of the docking complexes of $\alpha\beta_{III}$ with amino-noscapine, bromo-noscapine and noscapine.

- Figure 3.21** Root mean square fluctuation (RMSF) of residues around the ligand binding site of $\alpha\beta_{III}$ (13-393) complexed with amino-noscapine, bromo-noscapine and noscapine.
- Figure 3.22** Per residue binding free energy (δG_{bind}) contribution of $\alpha\beta_{III}$ for the binding of noscapine, bromo noscapine and amino-noscapine.
- Figure 3.23** Energy decomposition of the key residues (contributing $\delta G_{\text{bind}} < - 1.0$ kcal/mol) in $\alpha\beta_{III}$ that are involve in the binding of noscapine, bromo noscapine and amino-noscapine.
- Figure 4.1** Natural and synthetic analogues that are acting as microtubule targeting agents.
- Figure 4.2** Molecular structure of newly designed noscapinoids, **5a-5f** in this study.
- Figure 4.3** Molecular structure of previously reported noscapinoids used in the training set.
- Figure 4.4** Optimized Suzuki coupling reaction conditions for synthesis of noscapine derivatives **5a-f**.
- Figure 4.5** Design strategy for new biaryl type α -noscapine congeners
- Figure 4.6** The root-mean square deviations (RMSD) of C α carbon atoms of tubulin during 10 ns of MD simulation of tubulin–ligand complexes with respect to initial structures as a function of time.
- Figure 4.7** Root mean square fluctuation (RMSF) of the residues of tubulin within 20 Å diameter (includes 13-393 residues) of the docked ligands in the bound form and in the unbound form of tubulin hetrodimer.
- Figure 4.8** The newly designed noscapinoids **5a-5f** shown to be well accommodated in the noscapinoid binding site at the interface between α - and β - tubulin.
- Figure 4.9** Typical snapshot of the binding mode of noscapinoids **5a-5f** with tubulin.
- Figure 4.10** Total binding energy (δG_{bind}) contribution on per residue basis in tubulin-drug complexes.
- Figure 4.11** Decomposition of the binding energy on per residue basis of the key residues in tubulin-drug complexes.
- Figure 4.12** A view of **5b**, (crystal structure) showing the atom-labelling scheme.
- Figure 4.13** The tubulin fluorescence emission intensity is quenched by noscapinoids 5a, 5c, 5d and 5e in a concentration dependent manner
- Figure 4.14** Panels showing morphological evaluation of nuclei stained with DAPI in the absence and presence of the analogues (25 μ M each).
- Figure 4.15** Noscapine analogs inhibit cell cycle progression at mitosis followed by the appearance of a characteristic hypodiploid (sub-G1) DNA peak, indicative of apoptosis.
- Figure 4.16** Panels represent H&E staining of paraffin-embedded 5 micron-thick sections of the liver, kidney, spleen, lung, heart, duodenum and brain at magnifications 200x and 400x.
- Figure 4.17** Panel showing blood biochemical parameters between control and treated groups

LIST OF TABLES

Table 1.1	Drugs currently used for cancer treatment.
Table 1.2	Inhibitors of tubulin polymerization in clinical development.
Table 1.3	Promoters of tubulin polymerization in clinical development.
Table 2.1	Chemical structures of noscapine and its congeners used in the study.
Table 2.2	Crystal data and structure refinement for noscapine.
Table 2.3	Experimental and theoretical values of the isoquinoline and isobenzofuranone ring parameters (bond lengths in Å, bond angles and torsional angles in degrees).
Table 2.4	Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{Å}^2 \times 10^3$) for noscapine.
Table 2.5	Anisotropic displacement parameters ($\text{Å}^2 \times 10^3$) for noscapine.
Table 2.6	The results of multiple linear regression (MLR) analysis with different type of descriptors.
Table 2.7	Predicted anti-tumor activity of the training set compounds.
Table 2.8	Predicted anti-tumor activity of the test set compounds.
Table 2.9	Predicted biological activity (pIC_{50}) obtained from the QSAR models and experimental biological activity for the designed set of noscapinoids.
Table 3.1	Tissue distribution of β -tubulin isotypes in normal cells.
Table 3.2	Mismatches in amino acids comprising the noscapinoid binding site among different β isotypes in comparison to the template (1SA0).
Table 3.3	All-vs-all structure comparison of the $\alpha\beta$ -tubulin isotypes.
Table 3.4	Physico-chemical properties of the noscapinoid bind site of different $\alpha\beta$ -tubulin isotypes as calculated from SiteMap.
Table 3.5	All-vs-all comparison of the amino acids within 12 Å diameter of noscapinoid binding site of the different $\alpha\beta$ -tubulin isotypes.
Table 3.6	Docking results (Glide XP) of noscapinoids with different $\alpha\beta$ -tubulin isotypes.
Table 3.7	Binding free energy and its components (kcal/mol) for the receptor, $\alpha\beta_{\text{III}}$ heterodimer and noscapine derivatives.
Table 3.8	Hydrogen bonding (H-bond) patterns between the residues of $\alpha\beta_{\text{III}}$ with amino-noscapine, bromo-noscapine and noscapine.
Table 4.1	Grouping of the experimental animals as acute, sub-acute and vehicle (control) treatment and the dosage and duration of dosage.
Table 4.2	Molecular docking results (Glide XP) as well as calculated energies based on LIE-SGB model of training set noscapine derivatives.

Table 4.3	Molecular docking results (Glide XP) as well as calculated energies based on LIE-SGB model of newly designed noscapinoids (5a-5f).
Table 4.4	Change in binding free energy and its components (kcal/mol) for the noscapine derivatives 5a-5f binding with tubulin.
Table 4.5	IC ₅₀ values of noscapine derivatives 5a-5f for various cancer cell types.
Table 4.6	Effect of noscapine derivatives on cell cycle progression of MCF-7 cells treated with 25 μM solution for the indicated time.