CONCLUSION AND FUTURE DIRECTION

Microtubules have long been considered an ideal target for anticancer drugs because of the essential role they play in mitosis, forming the dynamic spindle apparatus. As such, there is a wide variety of compounds currently in clinical use and in development that act as antimitotic agents by altering microtubule dynamics. Despite the initial impressive response by these MT binding drugs such as taxanes and vinca alkaloids, their potential is somewhat restricted by the development of multi drug resistance, toxicity, hypersensitivity and limited bioavailability. Therefore, there is a continual need to develop novel drugs that are efficacious, well-tolerated, non-toxic, orally available, can overcome resistance to other chemotherapeutic and display better pharmacologic profiles. Noscapine (an opium alkaloid) has recently shown great potential as a microtubule binding anti-cancer agent. Noscapine alters tubulin dynamics and leads to programmed cell death and its relatively non toxic profile, oral availability and efficiency against various drug resistant cell lines indicates its potential as a chemotherapeutic agent for the treatment of human cancers to treat various malignancies in the clinic. Furthermore, it was found to be effective against a wide variety of cancer cells (according to the NC 60 cell line screen) but the effective concentration was found to be in high micromolar ranges. Therefore, attempts were required to be made to design a new generation of noscapine derivatives for better therapeutic outcome. The initial efforts in this have already been encouraging as indicated by the development of few more potent derivatives, but complete elimination of the disease could not be achieved. Thus, the paramount goal is to develop a more potent derivative of noscapine by structural modifications.

Availability of structure activity data of many derivatives of noscapine led to develop a reasonable QSAR prediction model and thus guided in rational design of more potent derivatives of noscapine. We have designed novel derivatives of noscapine based on quantitative structure activity relationship of known noscapine derivatives. To achieve this we have first determined the bioactive conformation of noscapine derivatives using quantum mechanics optimization in reference to experimentally determined noscapine structure. The IC_{50} value of already existing noscapine derivatives with CEM cancer cell line was determined and used for developing the QSAR model. To obtain quantitatively the effects of various structural parameters of the noscapine derivatives on their biological activity, QSAR analysis with different types of molecular descriptors was operated. We used genetic function approximation algorithm of variable selection and generated robust QSAR models with high predictability for the external data set. The QSAR model guided us towards designing two new derivatives, 9-azido-noscapine and reduced 9-azido-noscapine. The activity of these new compounds was predicted using the developed QSAR model, which motivated

us further towards synthesis of these compounds. Validation of the model was achieved by experimentally determining value of pIC_{50} for both the compounds (5.585 M) which turned out to be very close to predicted pIC_{50} (5.731 and 5.710 M). Thus, this model was established to be a good rapid screening tool which could be used in future to uncover new and more potent anti-tumor drugs based on noscapine derivatizations.

Noscapine binds to tubulin composed of α- and β-tubulin, which exist as various isotypes. Although, noscapinoids bind at the interphase between α- and β-tubulin; their interaction is more biased towards β-tubulin. The distribution of the isotypes of β-tubulin is different among cells of different tissue of origin. Furthermore, their drug-binding properties are significantly different. Also the existence of different isotypes of tubulin and their differential expression in cancer cells has been related to resistance towards the currently used chemotherapeutic agents. Effectiveness of noscapine in a wide range of cancers and various drug resistant cancer cell lines generated an interest to computationally investigate the details of interaction of noscapinoids towards different isotypes of β-tubulin. We found that the binding score of a specific noscapinoid with each type of tubulin isotype is different. Specifically, amino-noscapine has the highest binding score with $\alpha\beta_{I}$, $\alpha\beta_{II}$, $\alpha\beta_{III}$ and $\alpha\beta_{IV}$ isotypes. More importantly, both amino-noscapine and bromo-noscapine have the highest binding affinity with $\alpha\beta_{III}$ (overexpression of $\alpha\beta_{III}$ has been associated with resistance to a wide range of chemotherapeutic drugs for several human malignancies) in the pattern of amino-noscapine (-34.70 and -46.23 kcal/mol), bromo-noscapine (-32.05 and -38.09 kcal/mol) and noscapine (-28.02 and -38.86 kcal/mol) based on both MM-PBSA and MM-GBSA calculations. The interaction analysis revealed that amino acids, Ser 239, Leu253, Ile 368, consistently contribute to the binding energy for all the three ligands, indicating their importance in interaction with the ligands targeted towards $\alpha\beta_{III}$-tubulin isotype. The information gained in this study could pave a way for more efficient design of novel noscapinoid, which could be specifically targeted towards cancer cells. Knowledge of the isotype specificity of noscapinoid may allow for development of novel therapeutic agents based on this drug.

In pursuit of developing novel noscaponoids, we have rationally designed six new biaryl substituted noscapine analogues which possess increased tubulin-binding and anti-proliferative activity using structure based design approach. Initially a library of noscapine derivatives was screened out using molecular docking, molecular dynamics simulation, and binding free energy calculation. The predictive binding affinity indicated that the newly designed noscapinoids bind to tubulin with a greater affinity. Six new derivatives with improved predicted binding affinity than the lead molecule were selected for further analysis. MM-PBSA and MM-GBSA methods have

been used to further investigate the mechanistic details of the interaction of these new derivatives with tubulin. The analysis suggests that newly designed noscapinoids bind to tubulin with a greater affinity than the parent compound in the following order of magnitude: 5e > 5f > 5d > 5c > 5b > 5a. Analysis of the binding energy contribution of amino acids in the binding site also reveals that Leu253 is consistently important, contributing to the binding energy among all the noscapinoids.

Guided by the computational findings, these new biaryl type α-noscapine congeners were synthesized from 9-bromonoscapine using optimized Suzuki reaction conditions for further experimental evaluation. Most importantly, our results show that biaryl substituted noscapine analogues have increased affinity to tubulin and the substitution impacted their therapeutic potential for a variety of cancer types. It was also demonstrated that this series of noscapinoids are able to arrest mitosis and inhibit cell proliferation with significantly higher efficiency than noscapine in various human cancer cell lines. These compounds perturbed DNA synthesis, delayed the cell cycle progression at S phase and G2M phase as well as induced apoptosis in cancer cells. *In vivo* toxicological evaluation of one of the compounds **5e** did not reveal any toxicity in the vital organs such as liver, kidney, spleen, lung, heart, brain and duodenum. In addition, there was no significant difference in hematological and blood biochemical parameters between the treated and untreated groups. Hence the newly designed noscapinoid, 5e is a safe and effective anticancer drug with a potential for the oral treatment of cancer and holds great promise for further clinical studies.

Our data thus generate compelling evidence that these analogs indicate a great potential for further preclinical and clinical evaluation. Also the analysis of interaction of noscapinoids with different isotypes can be further exploited for better and more efficient design of noscapinoids. Knowledge of the isotype specificity of noscapinoids may lead to the improvement of both efficacy and specificity of cancer treatments in different tissues. Thus future holds promises both in the form of further clinical evaluation of already designed noscapinoids in this study and more efficient and specific targeting of cancers of different tissue origin by exploiting the interaction mechanism of noscapinoids towards different tubulin isotypes.