SYNOPSIS OF THE THESIS SUBMITED TO SAMBALPUR UNIVERSITY

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ABSTRACT OF THE DISSERTATION

Drugs that target mitotic spindle, such as taxol, vinca-drugs, and estramustine have been in the clinic for the treatment of different types of breast cancers. However, these drugs are known to cause severe dose-limiting toxicities in patients such as peripheral neuropathy, systemic toxicity, and allergic reactions. More importantly patients are developing resistance against taxol. Thus, wonderful promise of taxol in managing breast cancers justifies further effort to discover novel mitotic inhibitors. Better yet, it would be additionally useful if other novel anti-mitotic agents have less side effects and easily administered. Our initial efforts towards this end have been quite encouraging in that we have rationally designed and chemically synthesized a battery of derivatives of natural lead molecule, Noscapine (opium alkaloid, used as anticough medicine and identified as tubulin binding anticancer agent). Some of these compounds were demonstrated as more potent compared to Noscapine as anticancer agent. In a quest of making novel derivatives of noscapine, the present study focusing on rational design of five new classes of noscapinoids such as (1) 1,3-diynyl-noscapinoids, (2) 9-arylimino noscapinoids, (3) N-arylalkylamino-noscapinoids, (4) N-imidazopyridine-noscapinoids and (5) 9-Urea noscapinoids, followed by chemical synthesis and exhaustive experimental evaluation as potent anticancer agents with little or no toxicity that holds great promise for clinical use.

A novel class of noscapine derivatives known as 9-arylimino noscapinoids was designed by substituting arylimino groups (Schiff bases) at the C-9 position. These molecules were docked with αβ-tubulin complex and a panel of three top-scoring molecules, 9-((E)-((5-bromothiophen-2-yl)methylene)amino)-4-methoxy-6-methyl-5,6,7,8 -tetrahydro-[1,3] dioxolo [4,5-g] isoquinolin-5-yl)-6,7dimethoxyisobenzofuran-1(3H)-one **(4),** 9-((E)-(2,5-difluorobenzylidene)amino)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3] dioxolo[4,5-g]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3H)-one **(5)** and 9-((E)-(4-bromobenzylidene)amino) -4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]iso quinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3H)-one **(6)** based on docking score were screened out. These molecules bind tubulin with robust predicted binding energy of -37.24 kcal/mol and -45.41 kcal/mol for **4**, -39.73 kcal/mol and -47.74 kcal/mol for **5**, and -43.62 kcal/mol and -49.72 kcal/mol for **6** respectively compared to noscapine (-34.47 kcal/mol and -40.27 kcal/mol) using molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) and molecular mechanics/generalized Born surface area (MM/GBSA). These three molecules were chemically synthesized and demonstrated experimentally to bind tubulin with high affinity compared to noscapine. The anti-proliferative activity of **4-6** revealed inhibitory concentration (IC_{50} value) in between 3.6 to 32.6 μM using MCF-7 and

MDA-MB-231 human breast cancer cell lines and a group of primary breast tumor cells. All three molecules were shown to inhibit the mitotic progression at the G2/M phase and induce apoptosis to cancer cells at a different level. Thus, we conclude that 9-arylimino noscapinoids **4-6** have tremendous potential as chemotherapeutic agents for the treatment of breast cancer.

In our next attempt, we envisaged developing 9-N-arylmethylamino derivatives of noscapine to enhance the anticancer activity of noscapine. The scaffold structure of noscapine was tailored by inducing a N-aryl methyl pharmacophore at the C-9 position to generate a library of derivatives, followed by a screening of top-ranked three derivatives (S)-3-((R)-9-((Anthracen-9-ylmethyl)amino)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3] dioxolo[4,5-g]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3H)-one **(15)**, (S)-3-((R)-9-(([1,1'-Biphenyl]-4-ylmethyl)amino)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dio xolo[4,5-g]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3H)-one **(16)** and (S)-3-((R)-9-((2,5-difluorobenzyl)amino)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo [4,5-g]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3H)-one **(17)** using molecular docking. These derivatives were synthesized and analysed for their *in vitro* cytotoxicity against breast cancer cell lines (MCF-7 and MDA-MB-231). Further, inhibition of cell cycle progression and induction of apoptosis to cancer cells was determined using FACS. Antiproliferative activity with the treatment of 15-17 revealed IC50 values ranging between 19.4 to 47.3 μM in two human breast cancer cell lines (MCF 7 and MDA-MB-231) without affecting the normal healthy cells (cytotoxicity is < 5% at 100 μM) using human embryonic kidney cell (293T). These derivatives induce apoptosis to cancer cells by arresting the mitotic cell cycle in the G2/M-phase by interfering with microtubules. The 9-(N-arylmethylamino) derivatives of noscapine have a good probability of becoming a novel therapeutic agent for the treatment of breast cancer.

In our furher attempt, a panel of 1,3-diynyl-noscapinoids such as (*S*)-3-((*R*)-9-(cyclopropylbuta-1,3-diyn-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3] dioxolo[4,5-*g*]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3*H*)-one **(20)**, (*S*)-3-((*R*)-9-((4-fluorophenyl)buta-1,3-diyn-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro [1,3] dioxolo [4,5-*g*]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3*H*)-one **(21)** and (*S*)-6,7-dimethoxy-3-((*R*)-4-methoxy-6-methyl-9-((2-(trifluoromethyl)phenyl)buta-1,3-diyn- 1-yl)-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl)isobenzofuran-1(3*H*)-one **(22)** were strategically designed to increase the anticancer activity of the lead molecule, noscapine. Structure-activity analyses revealed strong predicted free energy of binding ($\Delta G_{bind,pred}$) of -6.694, -7.294 and -7.468 kcal/mol, for **20-22** respectively compared to

noscapine (experimental free energy of binding ("$\Delta G_{bind,expt}$") is -5.246 kcal/mol). These novel derivatives were demonstrated to bind tubulin by fluorescence quenching assay and Far-UV circular dichroism. Further, they were tested to exhibit potent cytotoxic activity compared to noscapine using two human breast cancer cell lines. The IC_{50} value for noscapine, **20, 21** and **22** has been derived to be 35.2, 27.3, 18.7 and 12.7 µM using MCF7 and 39.6, 31.4, 22.5 and 16.1 µM using MDAMB-231. These derivatives were found to arrest cell cycle in the G2/M-phase followed by apoptosis and appearance of TUNEL-positive cells. Thus, we conclude that 1,3-diynyl derivatives of noscapine have great potential to be a novel therapeutic agent for breast cancers.

In a quest of making a new class of noscapine derivatives, we have developed N-imidazopyridine-noscapinoids (**7-11**) by coupling imidazo[1,2-a]pyridine pharmacophore to the N-atom of the isoquinoline ring based on our *in silico* efforts. These compounds were found to bind with high affinity to tubulin based on molecular docking, MD simulation and MM-PBSA. The predicted $\Delta G_{binding}$ varies from -183.79 to -150.66 KJ/mol for the N-imidazopyridine-noscapinoids which is higher then the lead molecule, noscapine ($\Delta G_{binding}$ is -132.63 KJ/mol) and satisfies all the properties essential for ADME. These novel derivatives were chemically synthesized and validated their anticancer activity based on cellular studies using two human breast cancer cell lines, MCF-7 and MDAMB-231, as well as with a panel of primary breast cancer cells isolated from patients. Interestingly, all these derivatives inhibited cellular proliferation in all cancer cells ranging between 5.3 to 34.0 µM, without affecting the normal healthy cells (IC_{50} value > 1500 µM), indicating that these compounds were not toxic to normal healthy cells. Out of the N-imidazopyridine-noscapinoids **(7-11),** N-5-Bromoimidazopyridine-noscapine **(9)** showed promising antiproliferative activity using both the cell lines (IC_{50} value is 5.3 and 7.8 µM against MCF-7 and MDAMB-231) and was selected for the detailed investigation. Unlike previously reported derivatives of noscapine that arrest cells in the S-phase, this novel derivative effectively inhibit proliferation of cancer cells, perturb cell cycle in the G2/M-phase followed by induction of apoptosis. FACS analysis revealed the percentage of early apoptotic cells to 15% and late apoptotic cells to 35% with treatment of N-5-Bromoimidazopyridine-noscapine **(9)** which were significantly high compared to controlled untreated cells. The induction of apoptosis to MDAMB-231 cells by the treatment of N-5-Bromoimidazopyridine-noscapine at its IC_{50} concentration is revealed from the morphological changes such as membrane blebbing, cellular shrinkage, chromatin condensation and formation of apoptotic bodies. Moreover, treatment with N-5-Bromoimidazopyridine-noscapine **(9)** significantly elevated the reactive oxygen species

and the loss of mitochondrial membrane potential which might have a function in the induction of apoptosis. The compound **9** was also found to significantly regressed the implanted tumour in nude mice as xenografts of MCF-7 cells without any apparent side effects after drug administration. Thus, we conclude that N-imidazopyridine-noscapinoids have great potential to be a novel therapeutic agent for breast cancers.

Additionally, we have presents a series of noscapine urea congeners **7a-7h** as potential tubulin binding agents. This series of compounds were designed thorugh in silico combinatorial chemistry by coupling of urea pharmacophore at the C-9 position of the noscapine scaffold. The binding affinity of these urea noscapine congeners **7a-7h** with tubulin was theoretically predicted based on combination of molecular docking, MD simulation and MM-PBSA. Both the docking acore and $\Delta G_{bind,pred}$ reveled strong binding affinity of urea congeners wih tubulin compared to noscapine. The $\Delta G_{bind,pred}$ varies in between – 186.3 to -140.9 KJ/mol, whereas for noscapine it is -132.6 KJ/mol. Further, all these compounds have qualified the ADME and drug like characteristics based on in silico prediction. Inspired by *in silico* predictive activity, we have strategically synthesised these urea noscapine congeners **7a-7h** followed by experimental evaluation using a panel of establiehd breast cancer cell lines (MCF-7 and MDAMB-231, primary breast tumor cells obtained from the patients and normal healthy cell line (293T). Interestingly all these derivatives reduced cellular proliferation in all the cancer cells with a IC50 concentration between 4.77 to 48.45 μM, without affecting the normal healthy cells (IC_{50} is > 285.4 μM. In particular, the compound **7g** was found to be most promising among the library based on IC_{50} value (IC_{50} value is 4.77 and 6.56 μM for the MCF-7 and MDAMB-231 cell lines) and was selected for the detailed investigation.The treated breast cancer cell line, MDAMB-231 with IC_{50} value of **7g** underwent several morphological changes such as membrane blebbing, numerous fragmented nuclei, and appearance of apoptotic bodies, indicating induction of apoptosis. It has been further confirmed by flow cytometry analysis which revealed early apoptosis (22%) and late apoptosis (68%) to MDAMB-231 cells. The urea noscapine congener **7g** was also found to arrest cell cycle in the G2/M-phase followed by apoptosis and appearance of TUNEL-positive cells at IC_{50} concentration. Further, treatment with **7g** significantly elevated the reactive oxygen species and the loss of mitochondrial membrane potential which might have a function in the induction of apoptosis. The compound **7g** was also found to significantly regressed the implanted tumour in nude mice as xenografts of MCF-7 cells without any apparent side effects after drug administration. Although the lead compound, noscapine, is already in clinical trials,

urea noscapine congener represents an additional edge over noscapine because of its higher potency, without compromising the nontoxic profile of noscapine.

In conclusion, five different classes of noscapinoids were developed by coupling active pharmacophore such as arylimino groups (Schiff bases), N-aryl methyl, 1,3-diynyl, imidazo[1,2-*a*] pyridine and urea group at the C-9 position with noscapine scaffold based on in silico combinatorial approach. We have primarily focused on these functional groups because they are recognized as key pharmacophores in several anticancer drugs utilized in the clinic. All the noscapinoids developed were found to enhance the anticancer activity to several folds. Thus, these noscapinoids have great potential to be a novel therapeutic agent for breast cancers.