SYNOPSIS OF THE THESIS SUBMITED TO SAMBALPUR UNIVERSITY

1. Title of the research topic	"Rational design of novel tubulin binding anticancer agents based on chemoinformatics evaluation of noscapinoids, their chemical synthesis and experimental validation"
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ABSTRACT OF THE DISSERTATION

Microtubules are important cytoskeletal structures that preserve genetic stability during cell division. The dynamics of these polymers, which may be defined as their growth rate at the plus ends, catastrophic shortening, frequency of transition between the two phases, pause between the two phases, release from the microtubule organising centre, and so on, are all critical for this function. Interfering with microtubule dynamics results in programmed cell death. Therefore microtubule-binding agents such as paclitaxel, docetaxel, and the vinca alkaloids are utilised in clinics to treat a variety of cancers. However, because of their non-selective action, these drugs are associated with severe toxicity (especially peripheral neuropathies, gastrointestinal damage, myelosuppression, and immunosuppression) mainly by overpolymerizing (by taxanes) or depolymerizing (by vincas) the microtubules. In a quest to find new tubulin-binding compounds for the treatment of cancer, noscapine, an opium alkaloid was discovered. It was found to binds stoichiometrically to tubulin, alter its conformation upon binding, and arrest mammalian cells in mitosis. Even at high doses, noscapine, unlike many other microtubule inhibitors, does not appreciably enhance or inhibit microtubule polymer mass. Instead, it alters the steady-state dynamics of microtubule assembly, principally by increasing the amount of time that microtubules spend in an attenuated (halt) state, in which neither microtubule growth nor shortening can be detected. Owing to the compromised cell cycle check points, cancer cells are preferentially destroyed by noscapine and its derivatives (together known as noscapinoids) without affecting normal cells. In addition, as a lead molecule, noscapine has the following advantages: (1) it retains activity against paclitaxel-resistant cell lines (1A9/PTX10, 1A9/PTX22) and epothilone-resistant cell lines (1A9/A8); (2) it has a favourable pharmacokinetics (clearance in 6-10 hours); (3) it is a poor substrate for drug-pumps (polyglycoproteins and MDR-related proteins. Despite the fact that noscapine is cytotoxic in a wide range of cancer cell lines (NCI 60 cell lines panel), the IC_{50} values (between 21.1 and 100 μM) are still in the high micromolar range. Research is focusing on rational design and chemical synthesis of noscapine derivatives to improve therapeutic outcomes. In persistence of our endeavours to create novel derivatives of noscapine with improved binding affinity with tubulin, we have designed five new classes of noscapinoids: (1) 1,3-diynyl-noscapinoids, (2) 9-arylimino noscapinoids, (3) N-arylalkylamino-noscapinoids, (4) N-imidazopyridine-noscapinoids and (5) 9-Urea noscapinoids.

A class of 9-arylimino noscapinoids was developed that binds to tubulin and displayed anticancer activity against a panel of breast cancer cells. These compounds were rationally designed by coupling of Schiff base containing imine groups at position C-9 of the isoquinoline ring of noscapine. Based on a combination of Glide docking and free energy of binding (FEB) calculation a panel of three 9-arylimino noscapinoids were screened out with improved binding affinity with tubulin compared to noscapine. These novel derivatives were strategically synthesized and validated their anticancer activity

based on cellular studies using two human breast adenocarcinoma, MCF-7 and MDAMB-231, as well as with a panel of primary breast tumor cells isolated from patients. Interestingly, all these derivatives inhibited cellular proliferation in all the cancer cells that ranged between 3.6 to 26.4 µM, which is 11.02 to 2.03 fold lower than that of noscapine. Unlike previously reported derivatives of noscapine that arrest cells in the S-phase, these novel derivatives effectively inhibit the proliferation of cancer cells, arrest the cell cycle in the G2/M-phase and induce apoptosis.

In our next attempt, the scaffold structure of noscapine was modified by inducting N-aryl methyl pharmacophore at C-9 position of the isoquinoline ring to rationally design and screened out three novel 9-(N-arylmethylamino) noscapinoids, with robust binding affinity with tubulin. The selected 9-(N-arylmethylamino) noscapinoids revealed improved predicted binding energy in comparison to the lead molecule. These derivatives were chemically synthesized and validated their anticancer activity based on cellular studies using two human breast adenocarcinoma, MCF-7 and MDA-MB-231, as well as with a panel of primary breast tumor cells. These derivatives inhibited cellular proliferation in all the cancer cells that ranged between 3.2 to 32.2 µM, which is 11.9 to 1.8 fold lower than that of noscapine. These novel derivatives effectively arrest the cell cycle in the G2/M-phase followed by apoptosis and the appearance of apoptotic cells.

In our furher attempt, 1,3-diynyl derivatives of noscapine were developed through *in silico* combinatorial approach and screened out a panel of promising derivatives that bind tubulin and display anticancer activity. The selected derivatives revealed improved predicted binding energy in comparison to noscapine. These 1,3-diynyl derivatives were strategically synthesized in high yields by regioselective modification of noscapine scaffold and HPLC purified (purity is > 96%). The decrease in intrinsic fluorescence of purified compared to control suggests their binding capability to tubulin. Their cytotoxicity activity was validated based on cellular studies using two human breast adenocarcinoma (MCF-7 and MDA-MB-231), a panel of primary breast tumor cells and one normal human embryonic kidney cell (293T). The 1,3-diynyl noscapinoids, inhibited cellular proliferation in all the cancer cells that ranged between 6.2 and 38.9 µM, without affecting the normal healthy cells (cytotoxicity is < 5% at 100 µM). Further, these novel derivatives arrest cell cycle in the G2/M-phase, followed by induction of apoptosis to cancer cells.

Additionally, a panel of urea-noscapine conjugates was developed to increase the anticancer potential of noscapine. These compounds were chemically synthesized and their antiproliferation activity was evaluated using human breast cancer cell lines (MCF-7

and MDA-MB-239), primary breast tumour cells and normal healthy cells using an MTT assay. All the urea-noscapine conjugates developed were inhibited the proliferation of breast cancer cell lines (MCF-7 and MDA-MB-231) without affecting the normal healthy cell. The most potent compound inhibited cell proliferation of MCF-7 (IC_{50} of 4.8 μM), and MDA-MB-231 (IC_{50} of 8.1 μM), primary breast tumour cells from different patients (IC_{50} ranges from 6.2 to 10.9 μM) and colony formation (IC_{50} 1.6 ± 0.35 μM) by arresting the cells at G2/M phase of the cell cycle. Further, it was found to effectively induce apoptosis which is facilitated by the elevated level of ROS. The compound was also found to significantly reduce the implanted tumour in the xenograft mice model without any toxicity to vital organs.

In our last attempt, another class of noscapinoids, N-imidazopyridine-noscapinoids was developed by coupling the imidazo[1,2-*a*] pyridine pharmacophore with noscapine scaffold. These novel derivatives were chemically synthesized and validated their anticancer activity based on cellular studies using two human breast adenocarcinoma, 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 the cancer cells that ranged between 3.7 to 15.8 μM, which is 11.8 to 2.7 fold lower than that of noscapine. These novel derivatives effectively inhibit the proliferation of cancer cells, arrest the cell cycle in the G2/M-phase followed by apoptosis.

In conclusion, five different classes of noscapinoids were developed by coupling active pharmacophore such as urea and imidazo[1,2-*a*] pyridine with noscapine scaffold based on in silico combinatorial approach. We have primarily focused on these functional groups because they are recognized as a key pharmacophore 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.