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Abstract

Noscapine ($C_{22}H_{23}NO_7$), benzylisoquinoline alkaloid was reported to bind tubulin, limiting the process of division of cells at mitosis without changing the relatively stable state of tubulin's monomer/polymer ratio and selectively inducing apoptosis within cancer cells. This is a distinct advantage over presently exiting

antimicrotubular drugs, which at equimolar concentrations either overpolymerize (taxanes) or depolymerize (vincas) microtubules, resulting in a variety of debilitating toxicities such as leucocytopenias, diarrhea, alopecia, and peripheral neuropathies. Carbon consisting compound, noscapine hinders cellular growth over a broad range of cancer cells including those that are resistant to conventional chemotherapeutics. Another notable benefit of noscapine over currently existing antimitotic is its oral bioavailability. Although noscapine causes significant regression of localized tumor xenografts in mice models, a complete remission of the disease has not been established even at increased dosage. To improve therapeutic outcomes, several noscapine derivatives have been developed by modification of its scaffold structure at various positions. Several of these congeners have indeed been reported to block the progression of the cell cycle, massively reduce cellular proliferation, and cause apoptosis in a wide range of cancer cells both in vitro and in xenograft models of human cancers implanted in nude mice. In particular, the chemical modification at the C-9 position had a substantial effect on the therapeutic potential of noscapine. An overview of the development of C-9 derivatives of noscapine and their anticancer efficacy is presented together with their synthetic aspects and their future prospects.

Keywords (separated by '-')	Noscapine - Antitumor activity - Microtubule-binding drugs - Tubulin polymerization inhibitors - Antineoplastic drugs

Chapter 35
Noscapinoids: A Family of Microtubule-Targeted Anticancer Agent

Shruti Gamya Dash, Harish Chandra Joshi, and Pradeep Kumar Naik

1 Introduction

Cancer is generally an unusual and uncontrolled population cell, which at a later stage can invade tissues and metastasize to distant sites within the body. The normal cells convert into cancerous cells due to some mutations and the resultant changes in protein structure/function or the altered gene expression patterns that perturb cell proliferation or cell death. Although more prevalent at advanced ages, cancer can affect people of any age including the fetus and is currently one of the major causes of death. Moreover, the incidence of different types of cancer increases with increasing age worldwide according to the latest GLOBOCAN database. Although substantial advances in the treatment have successfully managed the severity and the advancement of this devastating disease, the complete cure is still largely elusive.

AQ1 Cancer is the world's second most prevalent disease with the highest mortality rate of around 0.3 million deaths per year. According to a 2020 Indian report [1], tobacco-related cancers are estimated to account for 3.7 lakhs (27.1%) of the total cancer burden [2]. Breast cancers are anticipated to reach 2.0 lakhs (14.8%) among women, and cervix cancer is reported to make a significant contribution of 0.75 lakhs (5.4%), whereas gastrointestinal tract cancers are estimated to contribute 2.7 lakhs (19.7%) of the total cancer incidence both for men and women (The national cancer registry program, India, 2020). Based on the cancer data compiled by ICMR from 2004 to 2010, the number of males, females, and total cancer patients were 390,809,

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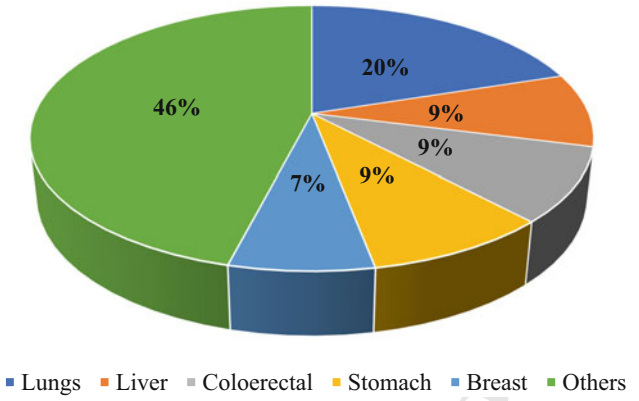

Fig. 1 Worldwide percentage distribution of cancer types. The incidence of lung cancer was found to be highest in number (20%)

428,545, and 819,354, respectively. It was depicted that the number of most cases of cancers has gradually improved over time. Among these, the cancers of the lung, the esophagus, belly, and mouth are the most common in men, whereas in women, these were mostly of the cervix and breast in India (Fig. 1).

2 Modalities of Treatment for Cancer

The modalities of treatment of cancer depend on their advancement, location and progression stage. Surgery, radiation-based therapy, chemotherapy, and combinations thereof are some of the most traditional and widely used therapeutic interventions. The modalities of treatment include hormone-based therapy, immunotherapy, anti-angiogenic modalities, DNA integrity/metabolism, tubulin-binding drugs, combination therapy, and even stem cell therapy in some blood cancers (Fig. 2) [3–6].

Traditionally chemotherapy, the use of chemicals to kill cancer cells is considered to be the most common in clinics. The chemical agents execute this through different mechanisms such as interference with the metabolism of DNA, the division of the cell, signal transduction, and cytotoxicity [7]. However, most of the chemotherapeutics also target normally growing blood cells in the bone marrow, lining of the gastrointestinal tract, hair cells within the hair follicles, thereby resulting in adverse effects such as leukocytopenia, immunocompromise, nausea, vomiting, diarrhea, hair loss, etc. [8]. These immunocompromised patients may thus acquire secondary complications due to sometimes lethal infections. A total of 132 cytotoxic chemotherapeutic drugs have been approved by the FDA. These drugs through a variety of their cytotoxic mechanisms often induce cell death (apoptosis or necrosis) in tumor cells [8, 9].

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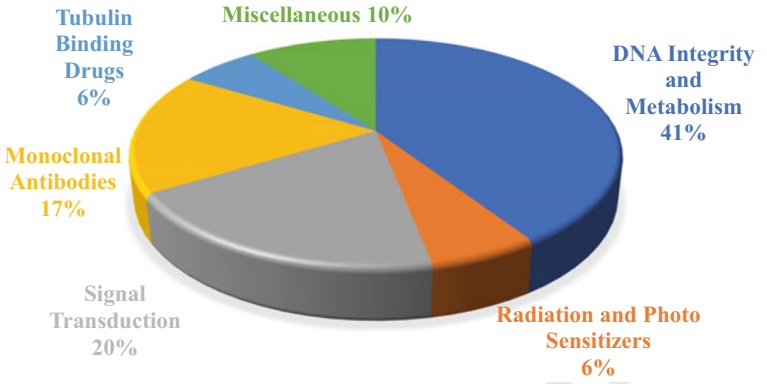

Fig. 2 Distributions of various mechanisms/drugs available currently in the management of cancer therapy

Among the microtubule-targeting chemotherapeutics in clinics, the most common are taxanes and several vinca alkaloids for the treatment of a wide range of human cancers. Taxanes are the most important class of anticancer agents. Paclitaxel (Taxol), derived from bark extracts of the pacific yew tree, *Taxus brevifolia*, or its derivative, is administered to patients with breast, ovarian, lung, head and neck, oesophageal, prostate, and bladder cancers [9]. Similarly, vinca alkaloids are probably the most frequently utilized microtubule agents in the clinic. These alkaloids have been isolated from *Catharanthus roseus*. Two major vinca alkaloids, vincristine and vinblastine, and a few structural variants such as vinorelbine, vindesine, and vinflunine, are commonly used in the clinic to diagnose various forms of cancer [10].

3 Microtubules: A Robust Target for Chemotherapy

3.1 Biology of Microtubule

Microtubules (MTs) are intracellular tubular structures that, together with actin and microfilaments, comprise the dynamic cytoskeleton of nearly all cell types. They continually arrange to form certain specialized super assemblies such as the mitotic apparatus for partitioning duplicated chromosomes during the cell division and then rearrange into normal interphase arrangements [9, 11, 12]. Thus, they are not only critical for cell proliferation, but also are required for subcellular trafficking, signaling, and migration. Microtubules assemble from tubulin, which itself is a dimer of α- and β-tubulin subunits each of a molecular weight ~50,000 Da. Both tubulin subunits comprise a chain of approximately 450 amino acids compacted into complex structures: a center of two β-sheets enveloped by α-helices and a bound

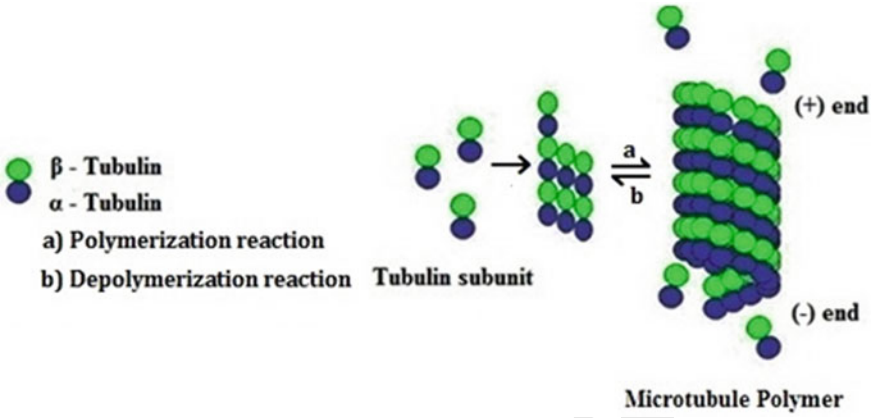

Fig. 3 Organization of microtubules

guanine nucleotide that is non-exchangeable when attached to the α-subunit and is freely exchangeable with the externally added guanine nucleotide when bound to the β-subunit (E-site) [13].

The unique functions of microtubules, as well as the mode of action of antimicrotubule agents, depend upon the dynamic equilibrium between α- and β-tubulin subunits and the microtubule polymer. Every monomer is asymmetric with approximate dimensions of 46 × 40 × 65 Å (width, height, and depth, respectively). Each tubulin subunit is split into three domains: the amino-terminal domain containing the nucleotide-binding region, the intermediate domain, and the carboxyl-terminal domain that coordinates drug interactions such as vinblastine and colchicine. The tightly bound α- and β-subunits form a single tubulin heterodimer. Tubulin dimers assemble head to tail to initiate assembly of a tubulin protofilament, 13 of which then associate sideways to form a microtubule cylinder that can elongate or shorten at the ends (Fig. 3).

The rates of polymerization/depolymerization and the threshold concentration of tubulin required for polymerization at either end of a microtubule varies. One end, called the "plus end," has faster kinetics and a lower critical concentration than the other "minus end." Thus, under some conditions, the plus end can elongate by adding new tubulin dimers, and at the same time, the minus end can shorten by losing tubulin dimers. If the rate of the growth and shortening is the same, the tubulin subunits can simply flux (or "treadmill") from the plus end toward the minus end of a fixed length microtubule lattice). This type of dynamic behavior of a microtubule is referred to as treadmilling. Also, within a population of slowly growing microtubules with a particular "growth rate," certain individual microtubules can transition catastrophically to depolymerization at a certain "catastrophe frequency" to a "shortening rate" until they disappear, or be "rescued" at a certain "rescue frequency" and "pause" before resuming growth. This type of dynamic behavior is termed "dynamic instability." All

these parameters of the dynamic instability, i.e., growth rate, catastrophe-frequency, rescue frequency, pause, and shortening rate can be measured.

Both types of microtubule dynamics described above require the hydrolysis of GTP at the exchangeable E-site of the beta-tubulin subunit [14, 15]. Magnesium ions (Mg^{2+}) are also required for assembly because GTP binds as an Mg-GTP complex [16].

The heterodimers of α- and β- tubulin arrange one above the other to form a polymer of protofilament and 13 of these protofilaments arranged sidewise to form a microtubule. The heterodimers polymerize at one end called (+) end and at the same time depolymerizes from another end, called as (-) end. The process of polymerization and depolymerization takes place simultaneously that gives dynamic structure to microtubule (a behavior called "treadmilling").

Within the cellular interior, these intrinsic dynamic behaviors of microtubules also depend upon the expression of many other microtubule stabilizing and destabilizing proteins. There are many microtubule-linked proteins (MAPs: tau, MAP1, MAP2, MAP4, XMAP215), regulatory proteins responsible for microtubule destabilization (stathmin, XKCM1, XKIF2, katanin) [17, 18]. The composition of cellular tubulin itself varies among different cell types due to the variable expression patterns of different tubulin isotypes and their post-translational modifications. Thus, the distinct tubulin pool of different cell types also differs in microtubule dynamics both directly due to the intrinsic assembly property of unique tubulin composition as well as indirectly via its differential interactions with various microtubule interacting proteins. This relevance of tubulin assembly and disassembly can also be hindered by different chemical agents that bind to a particular site in the β-tubulin subunit of the alpha-tubulin subunit or at their binding interfaces. Because cell division requires the most exquisite control of microtubule dynamics, these tubulin-binding agents often arrest cells in mitosis, ultimately resulting in cell death, through apoptosis and necroptosis.

3.2 Tubulin-Interacting Antimitotic Agents

The antimitotic agents act by binding specific sites on various structural domains of the tubulin heterodimer either on its unassembled or assembled forms. Three drug-binding sites have been outlined, the colchicine-binding site, the binding site of vinca alkaloids (α-tubulin) and that of the taxanes (β-tubulin) [19]. Colchicine primarily binds to β-tubulin near its α/β tubulin interface and modulates the peripheral protofilament interaction by inhibiting microtubule polymerization [20]. Vinca alkaloids prevent microtubule assembly by cross-linking the interdimer interactions, thereby sterically deforming the protofilament and inducing the formation of alternative polymers of tubulin [14, 21].

Although the microtubule-targeted drugs are successfully used in the treatment of a large spectrum of different cancers, some of these drugs sometimes also cause peripheral neuropathy, myeloid toxicity and neutropenia. Moreover, patients often develop drug resistance to a limited number of available and effective microtubule

drugs. Therefore, there is still an urgent need for expanding this series of effective drugs with the discovery of new bioavailable microtubule agents with minimal side effects that can overcome drug resistance. In a quest of finding such compounds, we explored naturally available alkaloids, in particular the opium alkaloids family. Opium alkaloids are widely used in clinics as analgesics, antimalarials, antispasmodics, etc. There are at least 25 active chemicals that can be derived from opium, called opiates. One of the alkaloids, noscapine, is being used as a safe antitussive drug [22] in the clinic for several decades and has recently been screened to have anticancer activity through tubulin binding [23].

3.3 Noscapine and Its Analogs: A Microtubule Modulating Agent

Noscapine ($C_{22}H_{23}NO_7$), (413.43 Da), a benzylisoquinoline alkaloid consisting of carbon, was initially described by Professor Pierre-Jean Robiquet in the year 1817 [24] from the opium plant (*Papaver somniferum*) (Fig. 4). From opium (*P. somniferum*), he identified two major compounds: codeine and noscapine [24, 25]. One of the most predominant opium alkaloids is noscapine (21%); other notable alkaloids include morphine (42%), codeine (12%), papaverine (18%), and thebaine (6.5%) tubocurarine, berberine, and sanguinarine. Since then, substantial progress has been accomplished in the development of complete synthetic methods. Nevertheless, the existence of noscapine's availability from natural sources is may be more cost-effective than synthetic alternatives. It was found that noscapine binds stoichiometrically to tubulin (one noscapine molecule for each αβ-tubulin dimer), modifies tubulin compliance, and arrests mammalian cells at the mitosis phase [23, 26]. Unlike vinca alkaloids and taxols, however, it does not induce overpolymerization, depolymerization, or any change in the general interphase MT organization. Because of its relatively low impact on the kinetic properties of dynamic instability of MTs, noscapine inhibits mitosis at prometaphase and arrests dividing cancer cells and normal cells in mitosis. Cancer cells, perhaps due to their mutations that compromise cell cycle checkpoints, often do not sustain arrested mitoses for a long time and undergo apoptosis while the arrested normal cells can resume mitosis after drug removal due to metabolic clearance [27]. It is reported previously that different diverse mechanisms were discovered to emerge the pathways of apoptosis in cancerous cells administered with noscapine and its congeners. These pathways involve the induction of stress-activated jun N-terminal kinase, mitochondrial depolarization, downward regulation of cell survival cascades, and upward regulation of pro-apoptotic signals, and eventually, all converging into caspase 3/7 activation. In comparison to the other MT interacting agents such as taxanes and vinca alkaloids, in treatment of cancer, noscapine has a number of advantages: (a) Noscapine induces apoptosis in a range of mammalian cancer cells, including drug-resistant varieties, by arresting them in mitosis [22, 28, 29]; (b) it is an insufficient

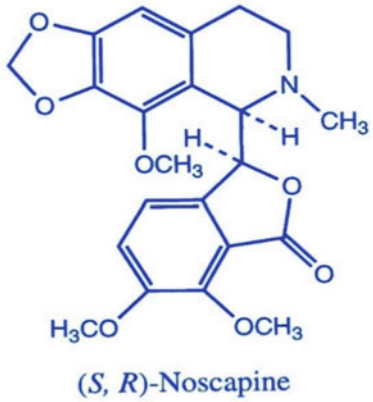

Fig. 4 Structure of *opium poppy* and the lead molecule, noscapine

target for drug efflux (poly glycoproteins and MDR-related proteins), which are a primary source of drug resistance [22]; (c) it suppresses the development of murine melanoma, lymphoma, glioblastoma, and human breast tumors transplanted in nude mice without causing harm to the rapidly proliferating cells of post-mitotic cells such neurons; (d) noscapine does not hinder primary humoral and cellular responses in mice [30]; (e) noscapine does not cause measurable immunological and neurological toxicity in mice, (f) noscapine is orally administered as opposed to other anti-MT drugs that require peritoneal injections or intravenous infusions with a risk of anaphylactic reactions and infection at the site of injection causing pain, blood vessel thrombosis or embolism; (g) noscapine has a mean bioavailability of ~30–32% over a dose range of 10–300 mg/kg in mice [29].

To further improve its efficacy, efforts were based on rational drug design and synthesis of new generations of noscapine derivatives for better therapeutic outcomes. Nevertheless, noscapine faces some difficulty as its two ring systems, i.e., the isoquinoline and the isobenzo-furanone are connected by a single rotating c–c bond between two chiral centers. Thus, ordinary chemical reactions necessarily lead to a racemic mixture of 4-stereoisomers of noscapine. Out of these, only one stereoisomer, the RS form, is biologically active [23, 27]. Perkin and Robinson (1910) were the first to obtain noscapine from meconine and cotarnine, in the presence of potassium carbonate combined with fractional crystallization, could. In 1958, tests in cell culture revealed that noscapine had cytotoxic properties. The antimitotic anticancer effect of noscapine was discovered back in 1998 [23, 27]. This was achieved through a structurally based justification for screening a modest library of naturally obtained molecules that shared structural similarities with highly cytotoxic MT depolymerizing drugs such as podophyllotoxin, MTC [2-methoxy-5-(2,3,4-trimethoxyphenyl)-2,4,6-cycloheptatrien-1-one] TKB [2,3,4-trimethoxy-4′-acetyl-1,1′-biphenyl], and colchicine. However, noscapine (**6**) has two chiral centers and four possible stereoisomers.

3.4 Noscapine is a Safe Cough Suppressant

The antitussive property of noscapine was suggested initially in the year 1930, and it was further comprehensively studied in 1954 [31]. Many researchers have already proven its antitussive activity and the relief it gives to bronchial asthma patients [32–35]. Noscapine has been commonly used as an antitussive drug in Europe, Japan, North and South America, and South Africa since the early 1960s. It is documented in several countries' pharmacopeias, including Europe, Japan, and the USA. It is administered orally in the form of tablets, lozenges, or syrup, or a rectal suppository form.

3.5 Noscapine's Potential Against Cancer

Antimicrotubule drugs interfere with the formation and proper function of microtubule assemblies such as the mitotic spindle, thereby preventing cell division. Exquisite regulation of coordinated microtubule growth, shortening, and treadmilling are all required for the mitotic function. Therefore, antimicrotubule drugs that either alter assembly or disassembly both interfere with cell division.

Noscapine has shown tremendous potential effectivity in vitro and in vivo against breast cancerous cells. By inducing apoptosis, it was demonstrated to suppress the development of murine and human breast tumors injected in mice. Noscapine's ability to prevent the growth of human MCF-7 breast cancerous cells, which are estrogen-positive receptors, was analyzed using in vitro proliferation assays. Noscapine brings about an 80% regression of human breast tumors grafted in athymic mice in vivo. Noscapine is also effective against hormone-insensitive, triple-negative breast cancer cells and in MDAMB-231 xenografts in nude mice. Noscapine-loaded estrone-conjugated gelatin nanoparticles (Nos-ES-GN) were designed to target estrogen receptor-positive breast cancer MCF-7 cells to overcome noscapine's short biological half-life, poor absorption, low aqueous solubility, and significant first-pass metabolism. The IC_{50} value of Nos-ES-GN seemed to be approximately 50% lower than that of the free drug. The same study found that estrogen receptor-positive (MCF-7) cells accumulated more estrone-conjugated noscapine-loaded gelatin nanoparticles than estrogen receptor-negative MDAMB-231 cells, indicating that estrone-conjugated nanoparticles have the potential to target estrogen receptor-positive breast cancer cells.

Consequently, experiments were carried out to assess the efficacy of noscapine against other cancer types. It includes ovarian cancer [36], malignant melanoma [37], bladder cancer [23], and glioblastoma [38]. Besides interfering with mitosis, it turns out that noscapine has several other metabolic effects that may explain its full repertoire of anticancer mechanisms. To determine the exact mechanisms by which noscapine prevents cancer growth, comprehensive experiments were performed by Dr. Joshi and his team and published in numerous medical journals [36, 39].

3.6 Anti-Angiogenic Effects of Noscapine

According to Newcomb et al. [40] noscapine inhibited the hypoxia-inducible factor-1α (HIF-1α) pathway in hypoxic human glioma cells and human umbilical vein endothelial cells. HIF-1α is a transcription factor that promotes the formulation of vascular endothelial growth factor (VEGF), a potent angiogenesis promoter. As a result of its inhibition of HIF-1α, noscapine has been shown to inhibit VEGF production, thereby identifying its anti-angiogenic properties, another possible mechanism for the anticancer effect. Also, noscapine increased the radiation sensitivity of GL261 glioma tumors delaying tumor growth via an anti-angiogenic mechanism.

3.7 Advancement of Noscapine Analogs as a Promising Drug Candidate

To improve noscapine's cytotoxicity activity, various analogs have been formulated and chemically synthesized (known as noscapinoids). A series of noscapinoid were synthesized by functionalizing the natural α-noscapine units of both isoquinoline and isobenzofuranone ring systems. Some of these derivatives have far better lists of treatments and better pharmacological profiles than the parent compound. Currently, more than three generations of noscapinoid have been developed, chemically synthesized, and their activities have been studied against cancer cells and normal cells [38, 41–44]. The first-generation noscapinoids include nitro, azido, amino, and halogenated (fluoro, chloro, bromo, and iodine) as analogs of α-noscapine by chemical functionalization of the 9th position of noscapine structural system, which is most widely explored by multiple groups [45]. The other two positions of modifications include the 6th and the 1st position of noscapine (Fig. 5). These three generations of noscapinoids [43, 44] represent chemical modifications of the functional groups of noscapine that have been demonstrated to drastically reduce its biological activity [46, 47] (Fig. 5).

3.8 9′-Halogenated Noscapine Analogs

These first-generation noscapine analogs developed by substitution of halogen groups at 9th position demonstrated better therapeutic effect compared to noscapine. For example, 9′-bromonoscapine (9′-Br-Nosc) and reduced 9′-bromonoscapine (Rd 9′-Br-Nosc) were able to bind more effectively to tubulin and were able to prevent mitosis at a much lower effective dose (ED50) than the parent compound noscapine. In certain cell lines, they showed as high as 20 to 40 times more potency than noscapine [42, 43].

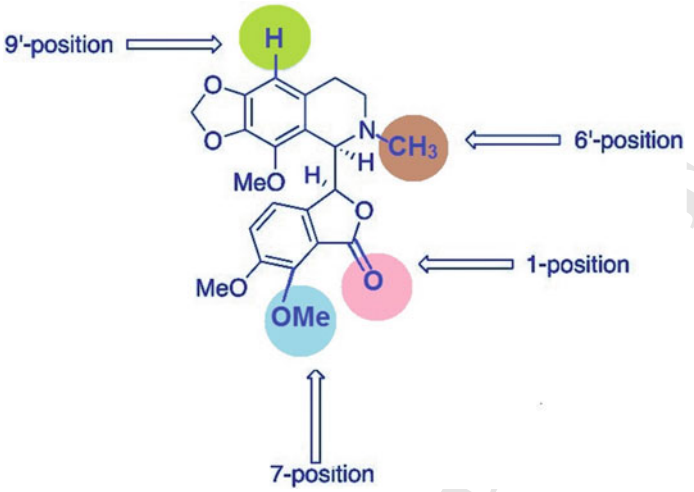

Fig. 5 Noscapine scaffold and sites of modification

A large spectrum of biological activity was also demonstrated by these compounds. Among the groups of noscapinoids, halogenated noscapinoids are implemented for their impact on the proliferation of cancer cells, antitumor potency, and associated risks [41]. The halogenated noscapine compounds, which are synthesized by chemical modifications, are outlined in Fig. 6. These compounds arrested mitosis at G2 and M phase much more efficiently than noscapine, leading to selective cancer cell apoptosis [48]. The computational blind docking approaches revealed a binding site at the interdimer region of the alpha (α) and beta (β) tubulin, overlapping with the colchicine-binding site for the noscapine and its derivatives with tubulin [43]. A cyclic ether derivative of 9′-fluoronoscapine (Fig. 7) was found to be an even more promising antibreast cancer agent [49]. This cyclic ether derivative of noscapine was chemically synthesized by the reduction of noscapine in the presence of boron trifluoride diethetate, and subsequent dropwise addition of a solution of sodium borohydride in dry THF at 0 °C.

3.9 Nitro-noscapine

The nitro-derivative (Fig. 8) of noscapine was developed by adding a nitro-group at the diversity point of the 9th position to the noscapine scaffold. It inhibits the growth of ovarian cancer cells of paclitaxel-resistant mutant cells, human lymphoblastoid cells, and their vinblastine- and teniposide-resistant variants [41]. Further, it also inhibits the cell cycle kinetics and induces apoptosis in cancerous cells. Surprisingly, there was no substantial inhibition of the growth of normal human fibroblast cells, demonstrating a specific effect for cancer cells [41, 50].

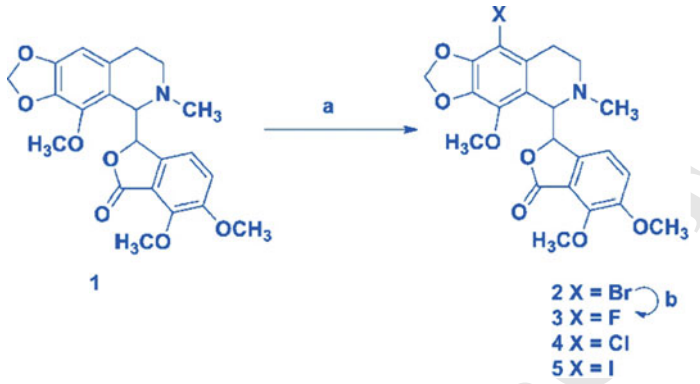

Fig. 6 Halogenated derivatives noscapine

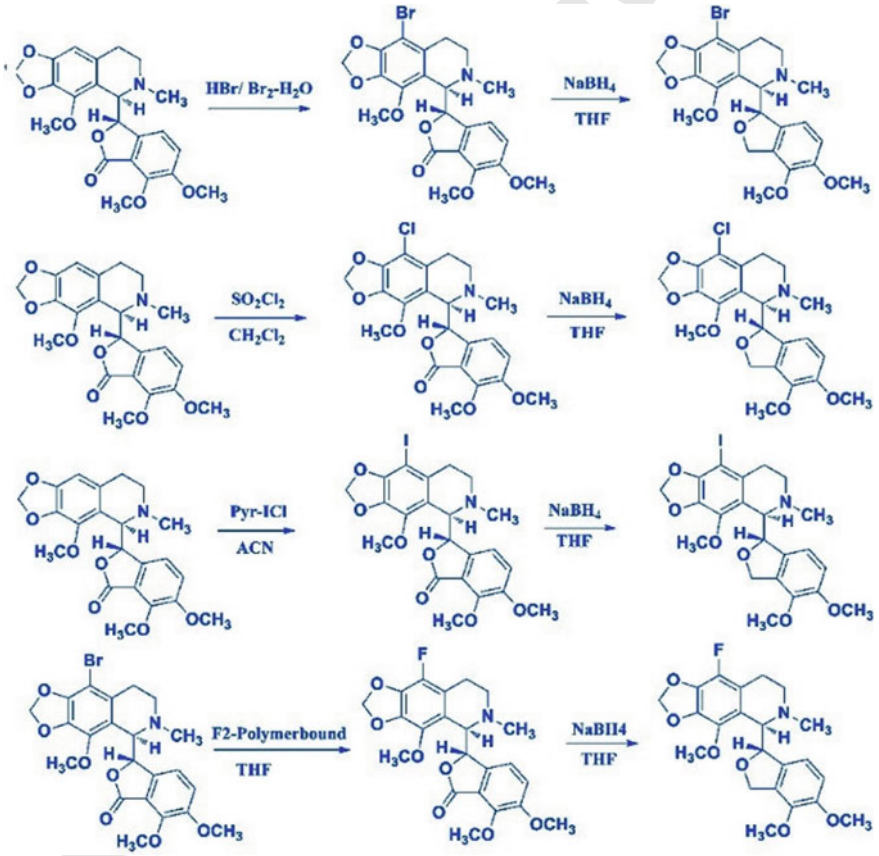

Fig. 7 Cyclic ether halogenated derivatives of noscapine (viz. Rd-9-F-nos; Rd-9-Cl-nos; Rd-9-Br-nos; Rd-9-I-nos

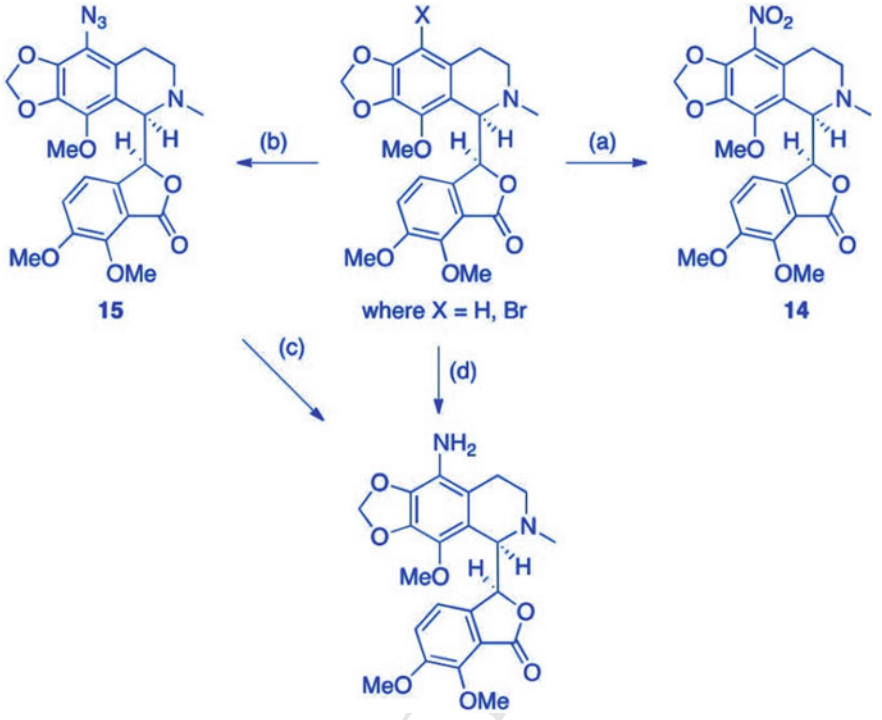

Fig. 8 Nitro-derivatives of noscapine

3.10 Azido Noscapine

An even more efficacious analog of noscapine is azido noscapine (Fig. 9). This noscapine derivative was created by converting noscapine to bromo-noscapine and afterward allowed to treat with sodium azide and sodium iodide. It was also more potent than other drugs at killing human acute lymphoblastic leukemia cells [42].

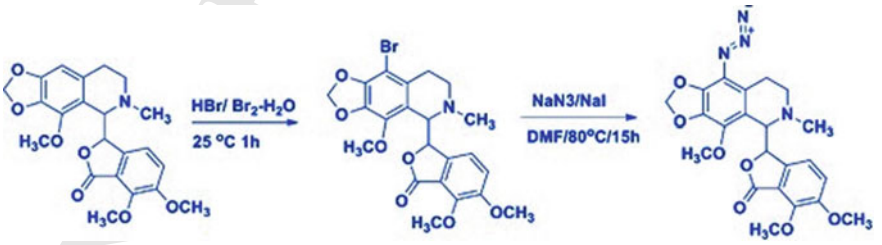

Fig. 9 Azido noscapine

3.11 *Amino Derivative of Noscapine*

The amino derivatives of noscapine were developed depending on the binding free energies of several noscapinoid, estimated in combination with a surface generalized Born (SGB) continuum solvation model using the linear interaction energy (LIE) method [43]. The assessment of the binding free energy revealed that the amino derivative of noscapine binds tubulin more strongly than the lead molecule. It inhibited the proliferation of cancer cells of different types more effectively compared to noscapine [43]. However, it did not directly influence the extent of polymerization/depolymerization of tubulin subunits [51, 52]. The amino derivatives of noscapine show promising anticancer activity in combination with docetaxel.

4 N-Substituted Derivatives of Noscapine

We proposed to add modifications as part of our efforts to design new noscapine derivatives at diversity point of 6' position (Fig. 5) by functionalization of "N" in isoquinoline unit of natural noscapine (named them as third-generation-noscapine analogs) which are anticipated to enhance biological activity. According to the earlier reports on functionalization at "N" mostly through urea-type linkages, and very few of these noscapinoids have been analyzed for their biological efficacy. We believe that urea-type linkage is not the right approach because it will cause delocalization of the electron density at isoquinoline N (Fig. 10).

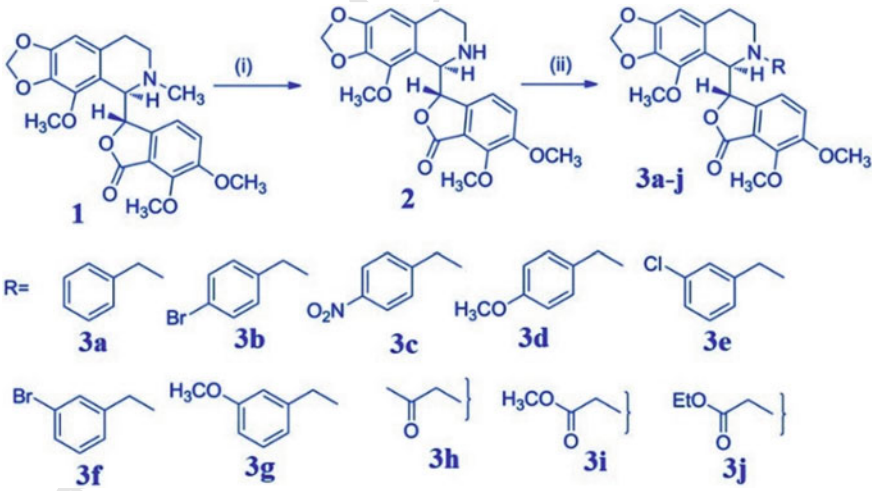

Fig. 10 N-substituated derivatives of noscapine

The third-generation noscapine congeners 3a-j, which vary in the side chain attached to isoquinoline "N" of natural α-noscapine, are described here as well. Depending upon the reasonable predictive mode, in silico molecular modeling, studies of these derivatives with tubulin complex have been used to evaluate their binding affinity as well as show prominent results in the cellular study.

4.1 Biaryl-Type Derivatives of Noscapine

As per the earlier literature, natural α-noscapine has biaryl-binding sites, which shows close similarity to colchicine. Colchicine's use as an anticancer agent is strictly limited because of its toxic side effects. Only a few natural products with biaryl architectural design are potent antimitotic agents that affect the tubulin-microtubule steady state [53] (Fig. 11).

Inspired by this, we propose to formulate novel biaryl type α-noscapine congeners by implementing a biaryl ring structure into the natural α-noscapine skeleton and testing them as chemotherapeutics agents. It is revealed that all of the newly developed biaryl noscapine derivatives (Fig. 12) bind tubulin with a higher affinity than that of the parent molecule and that the modification tends to affect their therapeutic efficacy for a variety of cancer types [46].

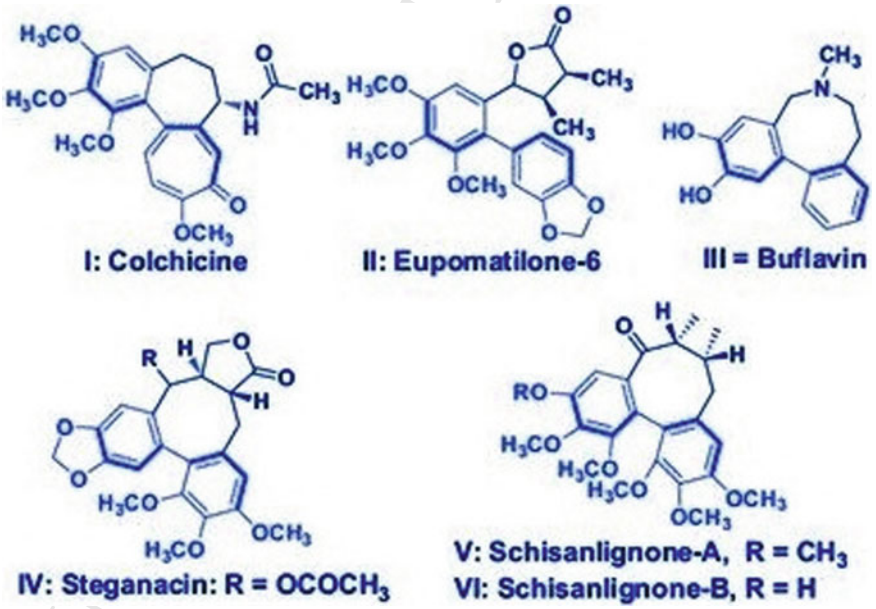

Fig. 11 Biaryl pharmacophore is a major defining component of natural and synthesized microtubule-targeting agents

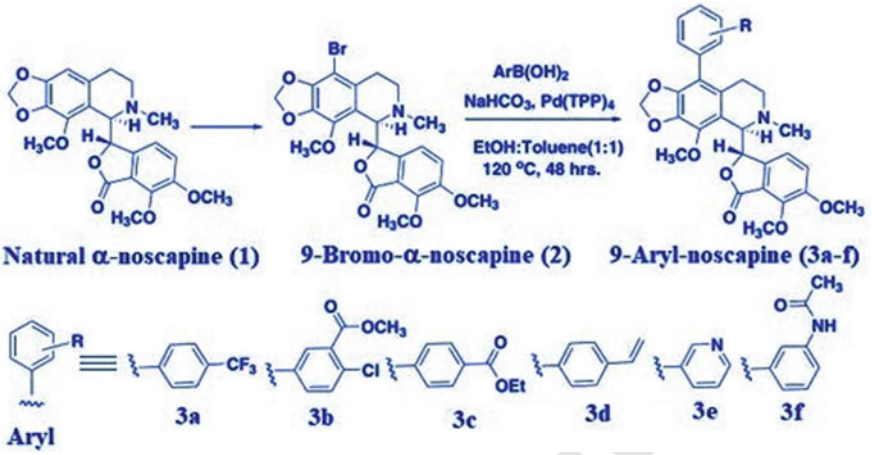

Fig. 12 Biaryl derivatives of noscapine

4.2 9-(4-vinylphenyl) Noscapine

Efforts have been focused on rational designing and synthesis of the new generation of noscapine derivatives. Noscapine docks onto b-tubulin near the interface between its dimerization partner, a-tubulin [54]. This is supported by the earlier finding of 1:1 stoichiometry of tubulin binding [23]. A closer look at the binding site revealed side chains surrounding the predicted binding pocket and the presence of empty space around position 9 of noscapine. In response to the *in-silico* findings, we have rationally coupled a bulky 4-vinyl phenyl functional group at the C-9 position of the noscapine scaffold in the context of improving a more potent derivative of noscapine (Fig. 13) [55, 56]. The inhibition of proliferative activity was significantly enhanced when the VPN, used for treatment in comparison to the parent noscapine. Also, this derivative of noscapine shows much efficacy in combinations with other cytotoxins and targeted agents to design preclinical studies [56].

4.3 Bromo-Trimethoxy Benzyl Noscapine

The C–C bond between isoquinoline and isobenzofuranone ring components of noscapine is labile to treatments with strong acids and bases. Therefore, it is often difficult and time-consuming to synthesize the novel TMB-Nos possessing 3,4,5-trimethoxybenzyl group appended at the 7th position on the lower isobenzofuran unit of noscapine. However, for the amalgamation of Br-OH-Nos and N-methyl pyrrolidone as starting material, we have optimized the reaction conditions without affecting

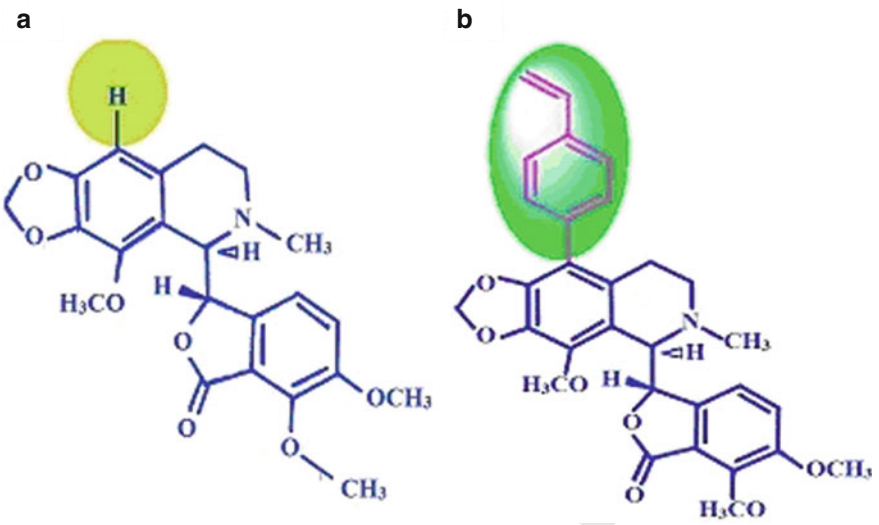

Fig. 13 Molecular structures of **a** noscapine **b** rationally designed derivative, 9-(4-vinylphenyl)-noscapine (VPN)

the sensitive C–C bond. These derivatives display superior anticancer efficiency than noscapine (Fig. 14) [57]. Furthermore, the newly synthesized TMB noscapine also found to have much potent and promising anticancer activity in combination with docetaxel [58].

5 Current Status, Challenges, and Future Prospects

Since the discovery of noscapine's antimitotic and anticancer effects in 1998, much progress has been made in the development of its derivatives as effective anticancer drugs. It has always been widely recognized that, in addition to treating cough, noscapine has a wide range of other potential applications that can effectively help a wide spectrum of patients, particularly those with cancer. Despite its high tumor suppressive dose (300 mg/kg), noscapine seems still safer than other antimitotic therapeutic agents. Noscapine also has another powerful property of synergizing with other anticancer treatments Qi et al. (2000). The ability to synthesize novel derivatives with improved efficacy against numerous cancer cell lines demonstrates noscapine's adaptability to further enhance the potential armory of anticancer drugs. Noscapine and its derivatives subtly modulate microtubule dynamicity, making them gentler than other microtubule-targeting anticancer drugs currently on the market [59–61]. Thus, a combination of sophisticated new methods in computational biology, bioinformatics, pharmacogenomics, engineering, and/or nanotechnology will continue to inspire the synthesis of new more effective analogs.

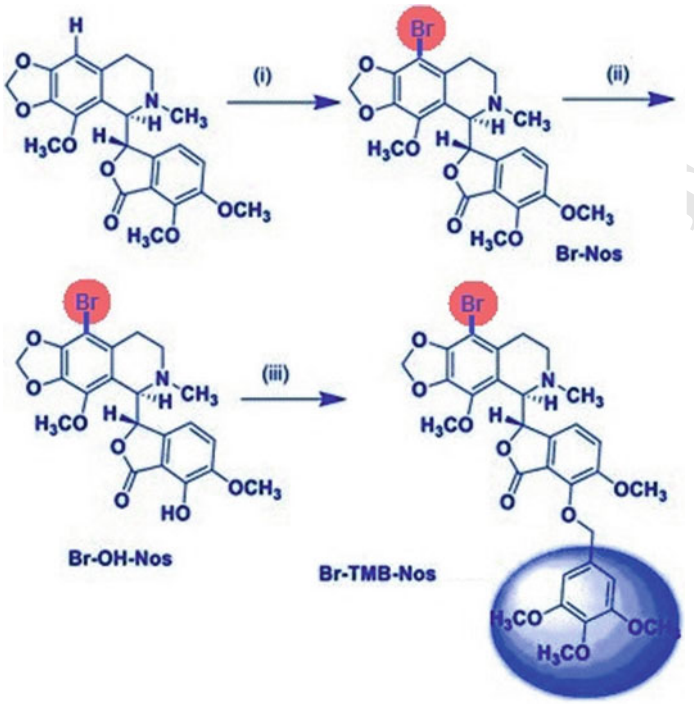

Fig. 14 Synthetic scheme of Br-TMB-noscapine

Conflicts of Interest The authors declare that they have no conflicts of interest that are relevant to the content of this manuscript.

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Chapter 35

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