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Noscapine and Its Analogues as Anti-Cancer Agents

Mohit Tripathi, Panyala Linga Reddy and Diwan S. Rawat*

^aDepartment of Chemistry, University of Delhi, Delhi-110007, India; E-mail: dsrawat@chemistry.du.ac.in

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Dedicated to Professor Richard A. Gibbs (1961 - 2014)

Abstract: *Noscapine, a phthalideisoquinoline alkaloid, originally supposed to be a medically insignificant constituent of the opium poppy until the discovery of its anti-tussive properties in the 1960s, is yet again in focus after the recent discovery of its anti-mitotic potential. Together with its synthetic analogues and further unearthing of their anti-cancer pathways, their development as an oral and 'gentler' anti-cancer medication is one of the most keenly observed advancement in the realm of cancer therapeutics. This is more significant, considering the toxicity and ineffectiveness of other anti-mitotic agents such as Taxol in the treatment of several types of cancers. Here, an overview of the development of noscapine and its analogues as effective anticancer agents is presented together with their synthetic aspects and their future prospects.*

Keywords: noscapine, analogues, anti-cancer, microtubule, anti-mitotic

1. INTRODUCTION

Nature has bestowed various gifts upon man and one of the most significant is the arsenal of natural products possessing curative or therapeutic properties. What started as a modest exploration of traditional medicine to isolate and design modern drugs, has given rise to numerous therapeutic ingredients to treat a multitude of human diseases. For example, 85 of the 175 drugs approved for the treatment of cancer from 1940 to 2010, are either natural products or directly derived therefrom [1]. Often these nature-derived drugs exhibit multiple therapeutic effects which are discovered

during or sometimes after their development as a drug for a particular disease. A notable example is Curcumin and its analogues which are currently under development stage for their wide range of activities such as anti-cancer, anti-inflammatory, anti-Alzheimer's, anti-malarial, anti-bacterial [2-4] etc. Noscapine, a phthalideisoquinoline alkaloid, discovered in 1817 from opium (*Papaver somniferum*) under the denomination of "Narcotine" [5] is one of the more abundant opium alkaloid (21% of the total opiate content). Initially it was thought to be medically inactive and remained grossly neglected for around one and a half century. However, with the discovery of its cough-

suppressant properties in 1960s [6], a surge in demand for this alkaloid was observed and it was successfully used as a safe anti-tussive agent for decades to follow [7]. Still, its therapeutic potential continued to be underrated until the discovery of its anti-tumour activity by the pioneering work of Joshi *et al.* [8]. This study showed that noscapine binds stoichiometrically to tubulin, alters its conformation, affects microtubule assembly, and arrests mammalian cells in mitosis. Furthermore, it was also shown to shrink murine thymoma and human breast and bladder tumors *in vivo*. This ground-breaking research has spurred a significant interest in the scientific world and noscapine with its synthetic analogues, offers a plethora of opportunities for the treatment of cancer. This short review is particularly aimed towards the development of noscapine and its analogues during the last few years as a safe anti-cancer agent, methods for their synthesis, their mechanism(s) of action and the opportunities and challenges this pharmacophore presents to be fully developed as an anti-cancer medication.

2. HISTORICAL ASPECTS AND DISCOVERY OF ANTI-CANCER PROPERTIES

2.1 Isolation of noscapine from opium and early uses

Pierre-Jean Robiquet, a famous French chemist, conducted a series of studies for the enhancement of methods for isolation of morphine (Figure 1) from opium, between 1815 and 1835. It was during this study when he isolated two natural compounds from opium: noscapine (1817) and codeine (1832). Noscapine, formerly known as narcotine, anarcotine or gnoscopine, was classified as an alkaloid. Its content in the opium seed is between four to twelve percent and is the second most abundant opium alkaloid (21% of total opiate content) after morphine (42%).

It was considered to be an insignificant

substance, with minimal or no therapeutic value by the scientific community until the late nineteenth century, when Sir Robert Williams, a medical expert of the Royal Commission on Opium concluded that noscapine was responsible for opium's ability to prevent and treat malaria. This commission was established by the British Empire to investigate the infamous Anglo-Asian opium trade and it eventually defended the opium production and trade by promoting the drug as an anti-malarial [9]. Its use as an anti-malarial continued for many years, until a comprehensive study published in 1930 disapproved its use for this purpose [10]. Noscapine was also investigated for its analgesic (pain-relieving) properties as found in other opioids. However, it was found that the alkaloid on its own has a very slight analgesic effect but when taken with morphine, it acts synergistically to escalate morphine's sedative effects [11].

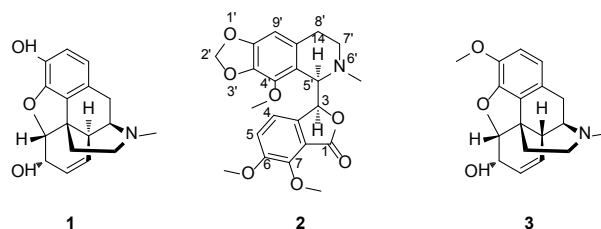


Figure 1: Opium alkaloids morphine (1), noscapine (2) and codeine (3).

2.2 Discovery of noscapine's anti-tussive properties

Noscapine's cough-suppressing effect was first put forward in 1930, and was described in more detail in 1954 [12]. Its general antitussive effect and positive symptomatic outcome in patients suffering from bronchial asthma was acknowledged through reports over the course of the next few years [6,13-16]. Since 1960s, it has been widely used as an antitussive medication throughout the world. It has been administered orally in tablets, lozenges or syrup, and rectally in suppository form. It is still available as

a prescription or OTC medication in most European countries, North and South American nations and Asian countries like China, Japan and Korea.

2.3 Discovery of anti-cancer properties of noscapine

Noscapine was found to possess significant cytotoxic properties *in vitro* in the cell culture studies performed for the United States National Cancer Institute (NCI) in 1958 [17]. Similar findings were reported in 1954 [18]. However, in a subsequent study, it failed to show any considerable anti-tumour effects *in vivo* [19] and no further studies were carried out to determine its effect on treating cancer. This molecule was left overlooked for a good 40 years until the re-discovery of its anti-cancer effects by Keqiang Ye, a graduate student working under Dr H.C. Joshi at Emory University School of Medicine in Atlanta [8,20] as a part of their work towards the exploration of microtubule-targeting compounds. It was found that noscapine shared chemical similarity with colchicine and podophyllotoxin and binds in a similar way to tubulin which leads to a conformational change subsequently affecting microtubule assembly, and consequently arrests mitosis. Treating animals which had been transplanted with human breast-cancer tumours, a reduction in tumour

volume by 80% was demonstrated within three weeks. Similar results were obtained against solid murine lymphoid tumors (even when the drug was administered orally) and against human bladder tumors implanted in nude mice. Most importantly, this was done without any visible toxicity to the animals' vital organs. The authors concluded that noscapine reduces tumor size quite dramatically, with no obvious weight loss nor any detected tissue toxicity after noscapine treatment. A subsequent report in 2000 confirmed noscapine's effects against murine lymphoma [21] and that the anti-mitotic effect was specific to noscapine. Moreover, its anti-cancer effect was backed by its oral bioavailability and safety, and it was found that it did not inhibit primary immune responses in the treated animals. Further explorations on the anti-cancer mechanism of noscapine were conducted and strategies to make synthetic derivatives of noscapine and their evaluation as successful anti-cancer agents were performed in the subsequent years.

3. NOSCAPINE: CHEMICAL ASPECTS

3.1 Structural analysis and chemical properties

Chemically, noscapine (molecular formula, $C_{22}H_{23}NO_7$) is a phthalideisoquinoline alkaloid,

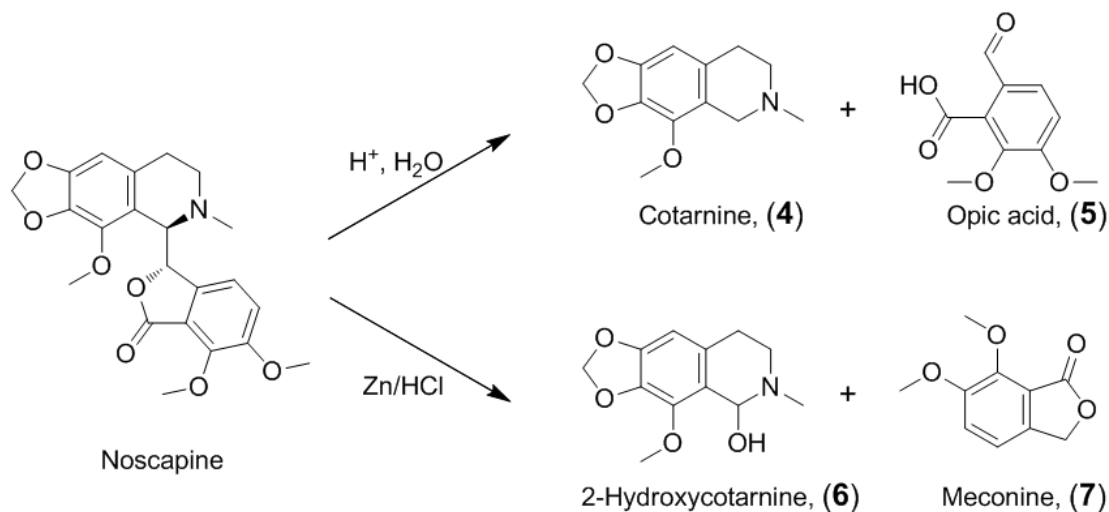


Figure 2: Chemical degradation of noscapine

i.e. its structure has a substituted phthalide ring and a substituted 1,2,3,4-tetrahydroisoquinoline ring, linked together *via* a single rotatable C-C bond, which in turn, involves two chiral centres. However, natural noscapine which is S,R (S stereochemistry at phthalide-carbon and R at isoquinoline-carbon) is evidently the only clinically active form of the four possible stereoisomers (Figure 1) [22]. It is an optically active substance and pure natural noscapine is levorotary ($[\alpha]_D^{20} -200.5^\circ$; c 1.0, CHCl_3). The phthalide lactone is unstable and opens in basic media. Also, the C-C bond connecting the chiral centres is reactive and in aqueous acidic solutions, it gets dissociated into cotarnine, (4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinoline) **4** and opic acid, (6-formyl-2,3-dimethoxybenzoic acid) **5**. Treatment of noscapine with Zn/HCl results in its dissociation into hydrocotarnine, (2-hydroxycotarnine) **6** and meconine, (6,7-dimethoxyisobenzofuran-1(3H)-one) **7** (Figure 2).

The metabolism of noscapine in animals has also been studied and O-demethylated metabolites were identified, in addition to the above-mentioned dissociation products [23,24]. However, it has been demonstrated that noscapine metabolites do not inhibit the proliferation of E.G7-OVA cells at doses comparable to the noscapine dose [21]. In other words, anti-tumour effects of noscapine are not due to its metabolites.

3.2 Chemical synthesis of noscapine

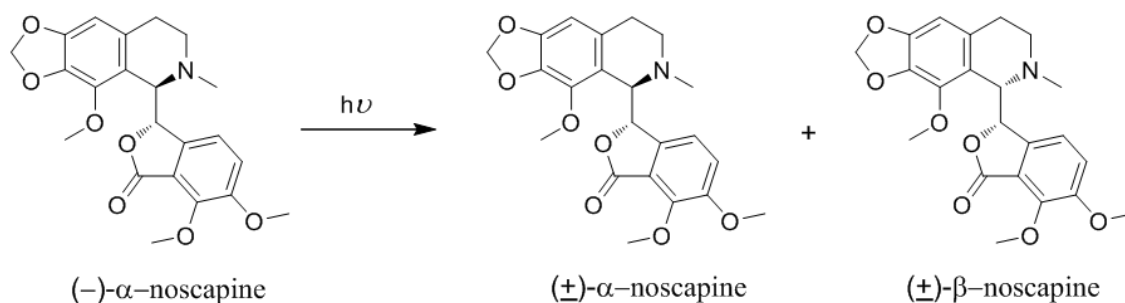


Figure 3: Photoepimerization and racemisation of $(-)\text{-}\alpha\text{-noscapine}$.

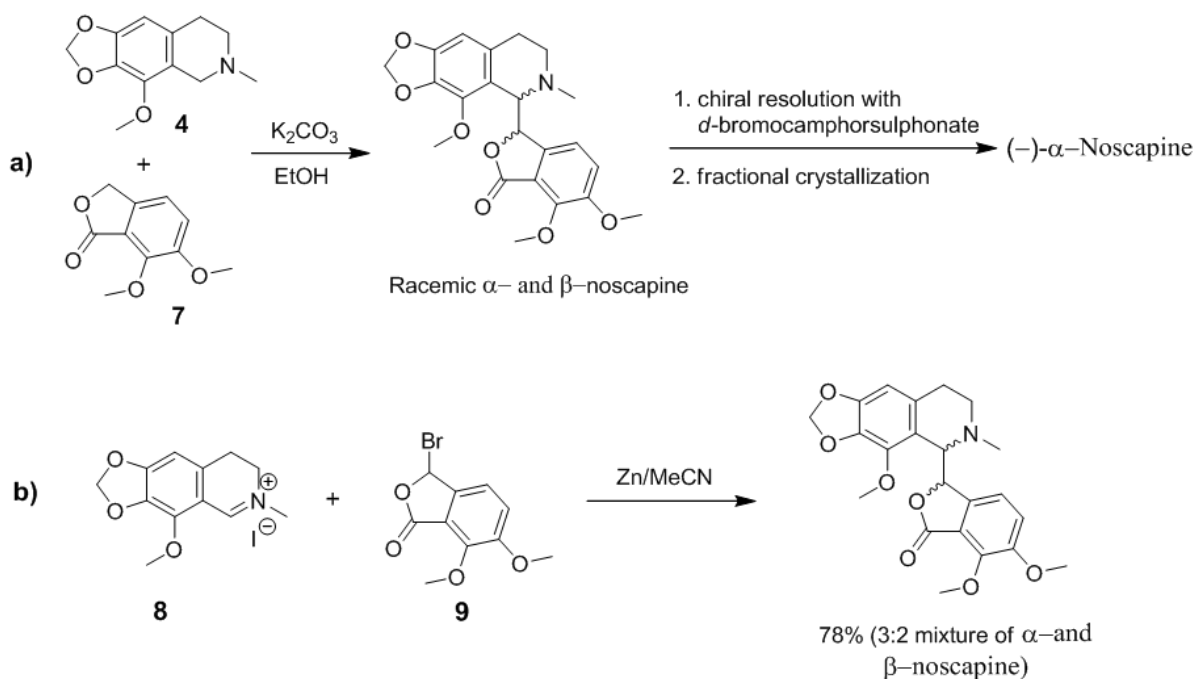
Traditionally, $(-)\text{-}\alpha\text{-noscapine}$ is isolated from the harvested opium poppy, which till date is the only economical source of optically pure noscapine. But, this requires the culturing and harvesting of opium, which is controversial and has too many regulatory issues as it is also the source of morphine (major opioid), which is converted into heroin, both of which are notoriously used as illicit drugs and for drug-trade. Methods to increase its content in opium, optimisation of its isolation techniques and biotechnological interventions to produce optically pure noscapine in high yields in plant cultures are underway [25-27]. But for these methods to be fully successful, prior knowledge of noscapine's bio-synthesis in plants is necessary, which is still far from being completely understood [28-30]. However, with the discovery of its medicinal properties, including the recent discovery as an anti-cancer agent, and some fascinating recent findings on its effect on stroke [31] and fibrosis [32], substantial interest has been generated on its chemical synthesis. However, since naturally isolated $(-)\text{-}\alpha\text{-noscapine}$ is the only clinically active isomer of four possible stereoisomers, synthesis of optically pure noscapine is a challenging task for the synthetic chemists. This task is made more daunting due to the possible photoepimerization and racemisation (Figure 3) [33].

Nevertheless, several attempts to successfully synthesize noscapine with the desired stereochemistry have been made. One of the first steps towards its synthesis was achieved in

1911 when racemic noscapine was synthesized from the condensation of meconine (**4**) and cotarnine (**7**), by the use of potassium carbonate (Scheme 1a). Its resolution with *d*-bromocamphorsulphonate and subsequent fractional crystallization [34] gave the desired *l*-noscapine, but the overall yield was quite low. A similar methodology towards its synthesis was reported by Shono *et al.* in 1983 [35], wherein they reported zinc-promoted reductive coupling of 3-bromo-meconine (**9**) to the iminium salt of cotarnine (**8**) to yield a 3:2 mixture of α - and β -noscapine in 78% yield (Scheme 1b).

(**16**) to iminium intermediate (**8**), which was prepared *via* Bischler-Napieralski reaction (Scheme 3). The use of the ionic liquid butylmethylimidazolium-tetrafluoroborate as an additive in the addition reaction improved the dr ratio of erythro and threo products to 4:1 [38a].

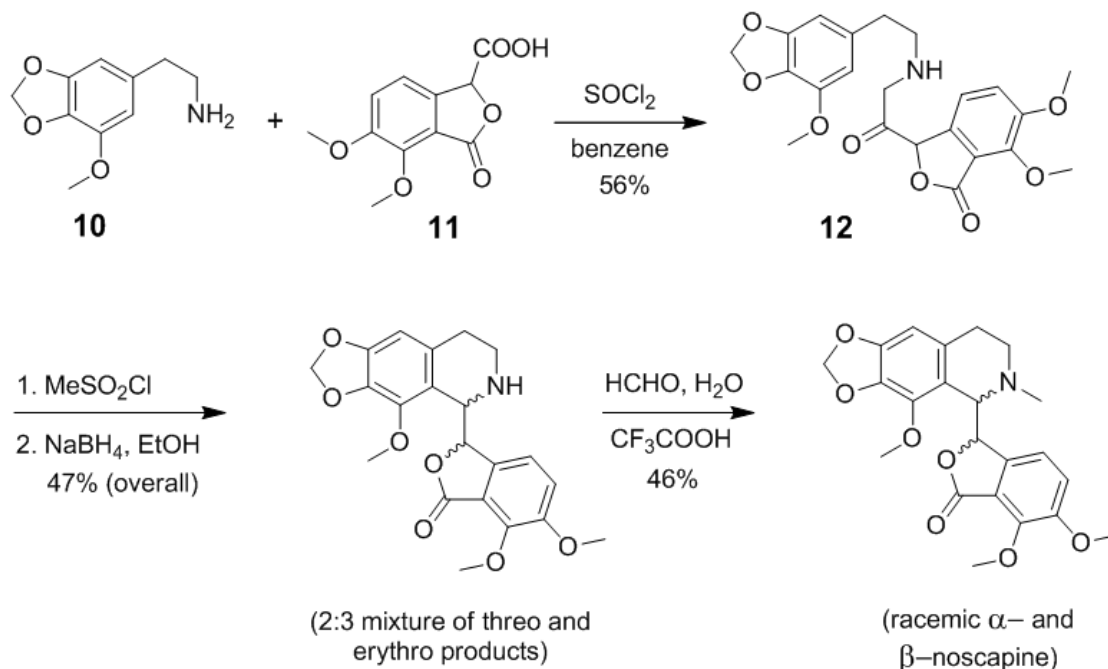
In 2011, Xu and co-workers improved the earlier methodology of Szantay *et al.* *via* installation of $-Br$ in the C-9' as a removable blocking group to avoid the formation of undesired regioisomers in Bischler-Napieralski cyclization [38b]. It was



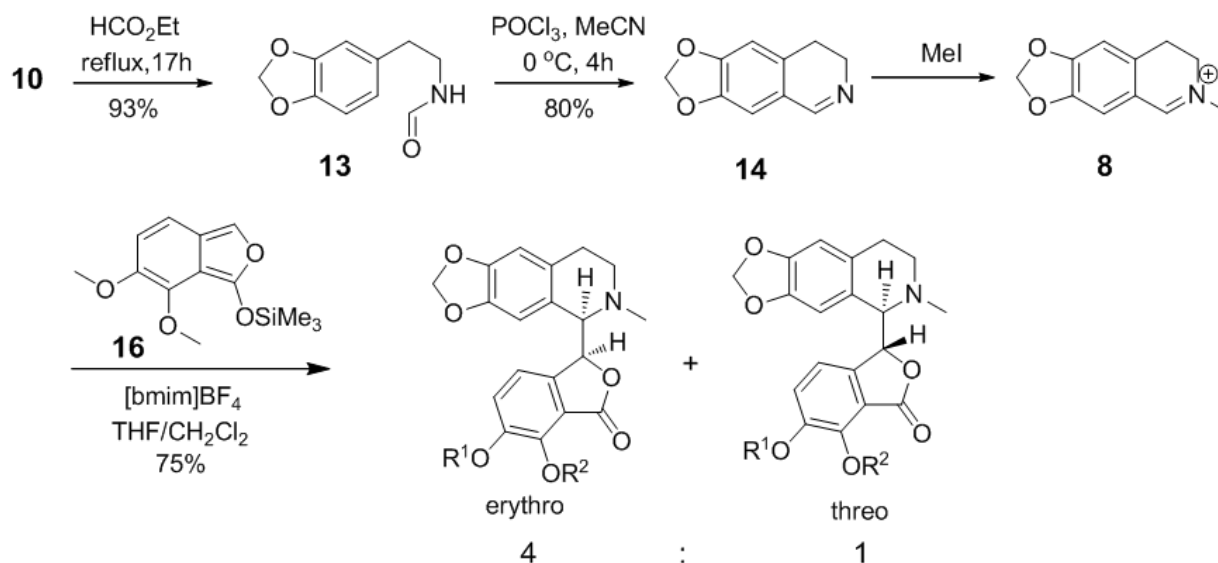
Scheme 1: Synthesis of noscapine through condensation of meconine and cotarnine derivatives.

Another alternative method of its synthesis was by the use of Bischler-Napieralski reaction for constructing the isoquinoline nucleus of the alkaloid, as demonstrated by Bogner *et al.* [36] and Szantay *et al.* [37]. They first constructed the C5'-C3 bond which was then followed by a Bischler-Napieralski reaction resulting in formation of the isoquinoline skeleton (Scheme 2). In 2010, Santos *et al.* demonstrated a short route to noscapine and other related alkaloids through the diastereoselective addition of 1-siloxy-isobenzofuran derivative

then followed by a highly diastereoselective reduction with $NaBH_4$ at low temperature ($-78\text{ }^{\circ}C$), affording the desired erythro-type phthalide tetrahydroisoquinoline structure (dr ratio 23:1). Subsequently, Eschevier-Clarke reaction to furnish *N*-methylation and finally, atmospheric hydrogenation in the presence of Raney Ni-W2 to remove the blocking group ($-Br$) and further recrystallization afforded pure ($\pm$)- α -noscapine (Scheme 4). Recently, Zhao *et al.* have synthesized racemic-noscapine [39] *via* a similar protocol as used by Santos *et al.* Instead



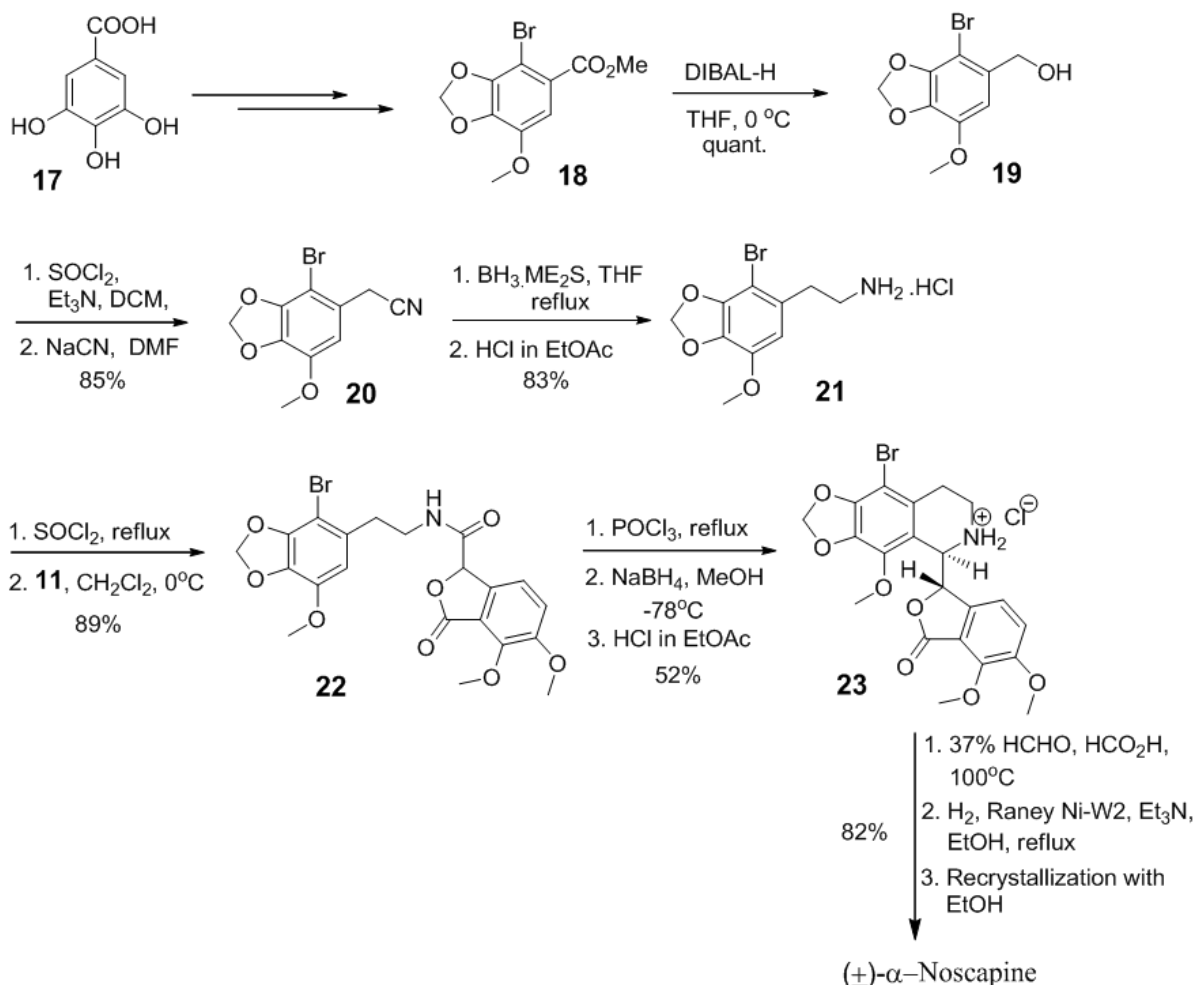
Scheme 2: Noscapine synthesis involving the use of Bischler-Napieralski reaction [37]

Scheme 3: Noscapine synthesis by Santos *et al.*

of the 1-siloxy-isobenzofuran derivative, they synthesized noscapine through the condensation of 3-trimethylsilyl meconin derivative and the iodized salt cotarnine derivative as the key step.

From the preceding discussion, it can be realized that excluding the work of Xu *et al.*,

all other methods provide a mixture of other diastereomers and none of the methods furnished optically pure (–)- α -noscapine. Hence, ample opportunities exist in the asymmetric synthesis of this alkaloid in a shortest route possible, including combinatorial methods, which can provide ample ways to synthesise its derivatives



Scheme 4: Blocking group directed synthesis of (±)-α-noscapine

as well.

4. NOSCAPINE: DETAILED MECHANISM OF ANTI-CANCER ACTION

Noscapine's anti-cancer effect, as mentioned before, has been primarily attributed to its microtubule-interfering effect by binding with tubulin in a 1:1 ratio and alteration of its conformation [8]. However, it was suggested that noscapine does not bind to tubulin as strongly as colchicine and this weak interaction makes it safer to use than other microtubule targeting agents such as Taxol, which show serious side effects and toxicity. The anti-mitotic effect of noscapine triggers cell apoptosis in cancer cells, the mechanism of which is poorly understood.

Several significant pathways for its anticancer activity have been discovered till date and are described in the proceeding sections.

4.1 Noscapine's anti-mitotic effect

Microtubules are structures involved in cell division. These are highly dynamic cytoskeletal fibres that are composed of tubulin subunits (α -tubulin and β -tubulin heterodimers arranged in the form of thin filamentous tubes with μ M dimensions, Figure 4). They show two types of non-equilibrium dynamics —treadmilling (net growth at one microtubule end and balanced net shortening at the opposite end) and dynamic instability (individual microtubule-ends switch between phases of growth and

shortening) — both of which are crucial to mitosis and cell division [40-42]. Microtubule exists in a continuous dynamic state of growing and shortening by reversible association and dissociation of α/β -tubulin heterodimers at both the ends. This dynamic behavior and resulting control over the length of the microtubule is vital to the proper functioning of the mitotic spindle in mitosis i.e., cell division. Microtubule-active drugs generally bind to one of three main classes of sites on tubulin, (i) the paclitaxel site, (ii) the Vinca domain and (iii) the colchicine domain and target microtubules and their dynamics, and have been used with great success in the treatment of cancer. Both extensive dynamic instability and treadmilling occur in mitotic spindles [43] and suppression of these by antimetabolic drugs seems to reduce spindle tension and slows or prevents progression from metaphase into anaphase. The cell then remains blocked in a prometaphase/metaphase-like state and eventually undergoes apoptosis [44].

number of microtubules attached to each pair of kinetochore. This fails to deactivate the spindle assembly checkpoint, and the active checkpoint is responsible for the sustained mitotic arrest, preventing the onset of anaphase and eventually cell apoptosis [23,48]. One question may arise that how noscapine differentiates between the normal cells and cancer cells. The answer lies in the characteristic feature of the cancer cell—bypassing cellular mechanisms for uncontrolled multiplication. Consequently, checkpoint-compromised cancer cells evade the mitotic-arrest and ‘slip’ into the next G1 phase. This leads to daughter cells having nucleus with abnormal ploidy. Such cells have a further propensity to undergo massive poly- or aneuploidy leading to the activation of apoptotic pathways and cell-death [45,49]. On the contrary, healthy somatic cells have very strong checkpoints, and it is conceived that they resume cell division after the noscapine concentration decreases below the threshold level in a few hours [26].

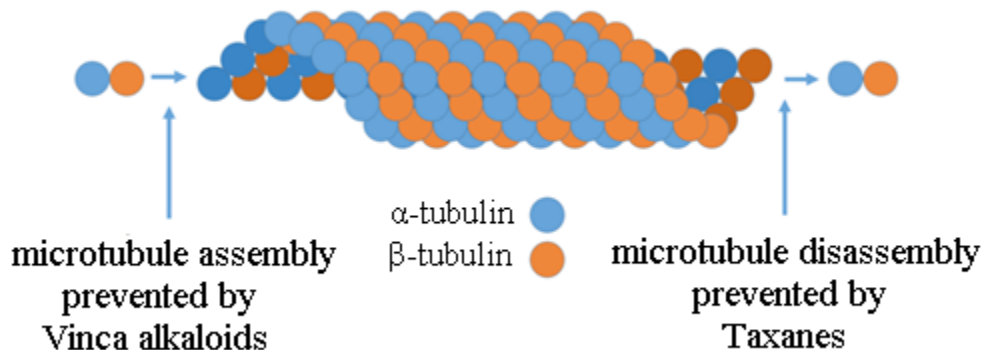


Figure 4: Structure of a Microtubule and action of taxanes and vinca alkaloids [45].

Noscapine, like other microtubule-active drugs, suppresses microtubule dynamics, arrests mammalian cells at mitosis and ultimately leads to their apoptosis [8,46-47]. Careful observation of individual polymerizing microtubules *in vitro* revealed that noscapine increased the amount of time microtubules spent in an attenuated pause state rather than engaging into active depolymerization and repolymerization. Thereby, noscapine reduces tension as well as the

4.2 Centrosome declustering

The centrosome is an organelle that serves as a microtubule organizing center (MTOC) during division. It is duplicated during S phase, and the two copies move to opposite sides of the cell and help in setting-up the spindle apparatus for mitosis. Upon division, each daughter cell receives one centrosome. However, cancer cells are found to have aberrant number of

centrosomes. This should have eventually led to multipolar mitosis resulting in severe aneuploidy and activation of apoptotic pathways causing cell death. However, cancer cells paradoxically manage to survive by means of clustering of centrosomes which allows the formation of a 'pseudo-bipolar' mitotic spindle, evading multipolarity [49,50]. Noscapinoids evidently induce centrosome declustering by the gentle reduction of microtubule dynamicity [51], ultimately causing cell-apoptosis.

4.3 Cell apoptosis pathways

Joshi *et al.* showed that sustained activation of the p34(cdc2) kinase during mitotic arrest is required for subsequent apoptosis [46]. Another report stated that cell-apoptotic pathway is preceded by activation of c-Jun NH2-terminal kinases (JNK) [52]. Mahmoudian *et al.* observed that noscapine can induce apoptosis in both apoptosis-proficient and apoptosis-resistant leukemic cells, with typical features such as increase in the activity of caspase-2, -3, -6, -8 and -9, poly(ADP ribose)-polymerase (PARP) cleavage, detection of phosphatidylserine on the outer layer of the cell membrane, nucleation of chromatin, and DNA fragmentation. A p53-dependent pathway that essentially involves the function of p21 for apoptosis in colon cancer cells was also observed [53]. Noscapine was also found to induce apoptosis in human glioma cells by an apoptosis-inducing factor (AIF)-dependent pathway [54].

4.4 Anti-angiogenic effects of noscapine

Newcomb *et al.* [55] showed that noscapine was an inhibitor of the hypoxia-inducible factor-1 α (HIF-1 α) pathway in hypoxic human glioma cells and human umbilical vein endothelial cells. HIF-1 α acts as a transcription factor for production of vascular endothelial growth factor (VEGF), a potent promoter of angiogenesis. Thus, noscapine, through its inhibition of HIF-1 α , has been shown to inhibit production of VEGF which in turn identified

its anti-angiogenic properties, another possible way for anti-cancer effect. The same group demonstrated that noscapine enhanced the sensitivity of GL261 glioma tumors to radiation, resulting in a significant tumor growth delay and an anti-angiogenic mechanism was found to be responsible [56].

4.5 Inhibition of NF-kappaB (NF- κ B) signalling pathway.

In 2010, Aggarwal *et al.* [57] reported that noscapine inhibits the proliferation of leukemia cells and sensitize them to tumor necrosis factor and chemotherapeutic agents by suppressing the NF-kappaB signalling pathway. This finding was important as the transcription factor NF-kappaB has been linked with inflammation, survival, proliferation, invasion, and angiogenesis in tumors. Therefore, noscapine may show its anti-cancer effects by modulating the NF-kappaB activation pathway. It was shown that noscapine (i) inhibits I κ B kinase, leading to inhibition of phosphorylation and degradation of I κ B- α ; (ii) suppressed phosphorylation and nuclear translocation of p65, leading to inhibition of NF-kappaB reporter activity; and (iii) it inhibited the activity of the NF-kappaB-containing cyclooxygenase-2 promoter.

5. PHARMACOKINETIC AND TOXICITY PROFILE OF NOSCAPINE

Before the discovery of its anti-cancer effects, noscapine has been used widely as an oral anti-tussive agent. The common form of noscapine which is used in cough suppression is noscapine hydrochloride. However, free noscapine base possesses significantly higher bioavailability than that of noscapine hydrochloride [58,59]. The terminal half-life of noscapine, which was independent of formulation or dose size, has been established to be 4.5 h [59]. Joshi *et al.* investigated the pharmacokinetics of noscapine in mice at oral tumour-suppressive doses [60]. It was found to distribute rapidly

and widely in all tissues and had a mean bioavailability of 31.5% over the studied dose range. Noscapine can pass the blood-brain barrier and thus can inhibit gliomas, and was indeed found to show such positive outcomes in mice [61]. In the early 1990s, it was suggested that noscapine could be contraindicated in women of childbearing potential because of potential genotoxicity [62]. But this decision was criticized, and subsequent animal studies have shown that such vulnerabilities were not found with recommended therapeutic doses of noscapine [63]. However, the effective dose for tumour-suppression (ED_{50}) for noscapine is around 300mg/kg which is bit on a higher side and hence, development of its derivatives, nano-encapsulation and targeted drug delivery have been used to combat this constraint.

6. DEVELOPMENT OF NOSCAPINE ANALOGUES

To fully utilize the medicinal potential of this alkaloid, attempts to synthesize its semi-synthetic derivatives have resulted in limited but important derivatives. Their number is limited due to the constraints posed by the synthesis of this alkaloid which, as seen, still needs to be pondered upon to provide optically

pure analogues in a concise way. Nevertheless, these analogues were found to possess much better therapeutic indices and improved pharmacological profiles. Approaches for preparation of various noscapine derivatives are illustrated in Figure 5. Usually, these derivatives have been prepared from naturally occurring noscapine, in which the basic skeleton is preserved and only some synthetically feasible derivatizations have been performed.

In 2003, 9-bromonoscapine (**24**) and its reduced partner (**25**) were synthesized by the team of Joshi *et al.* (Scheme 5) and were found to have higher tubulin binding activity than noscapine with improved effect on tubulin polymerization, differently from noscapine. Whereas noscapine-arrested cells have nearly normal bipolar spindles, cells arrested by **24** and **25** form multipolar spindles. In addition, these were able to arrest cell cycle progression at mitosis at concentrations much lower than noscapine [64]. These were found to be more active than noscapine in inhibiting the proliferation of various human cancer cells, including those resistant to paclitaxel and epothilone.

In 2006, Chandra *et al.* chemoselectively synthesized other halo-derivatives of noscapine

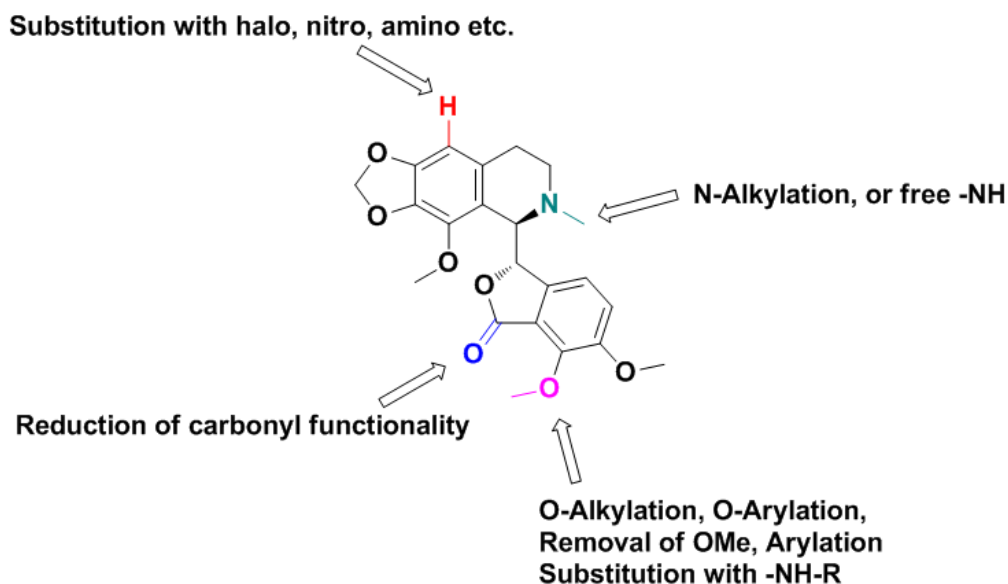
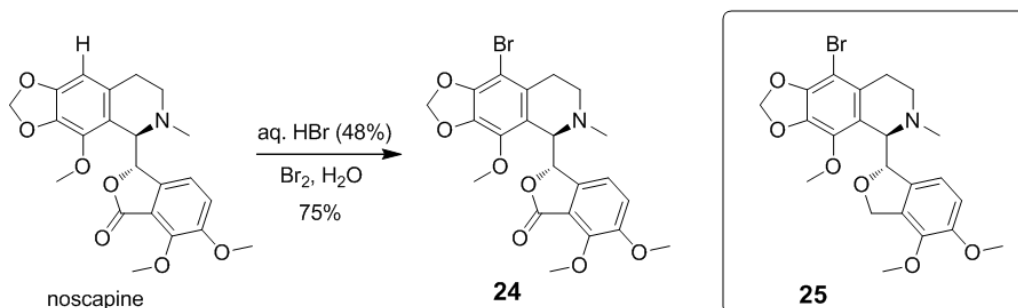


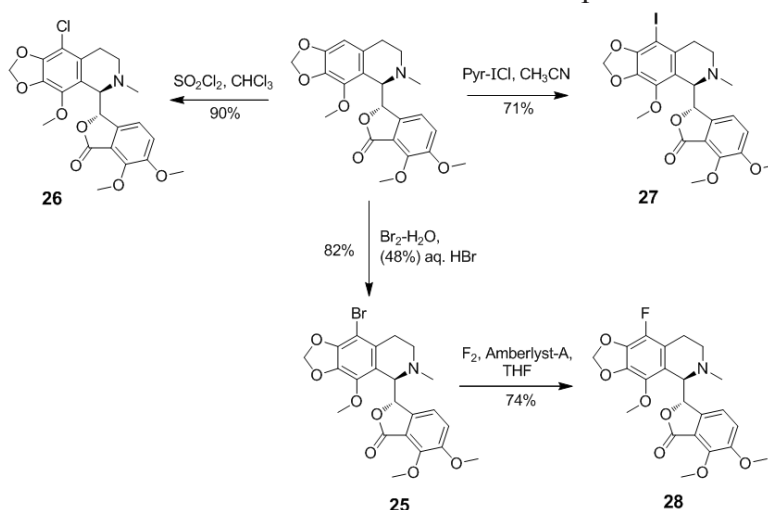
Figure 5: Strategies towards preparation of noscapine analogues.



Scheme-5: Bromonoscapine and reduced bromonoscapine

viz. 9-chloronoscapine (**26**) and 9-iodonoscapine (**27**), besides 9-bromonoscapine (**25**) and evaluated their *in vitro* cytotoxicity by MTT assay on U-87 human glioblastoma cell lines [65]. At concentrations of 50 μ M, derivatives **26,25** and **27** killed 87.8%, 51.2%, and 56.8% cells after 72 h, respectively, whereas noscapine killed only 40% of the cells; revealing 9-chloronoscapine **26**, as a potent cytotoxic agent than noscapine and 9-bromonoscapine. However at lower concentrations (1 μ M) both 9-chloro and 9-bromo analogues were found to show similar activities. A similar report by Joshi *et al.* [66] in the same year further reinforced these findings and found that all analogs except 9-iodonoscapine, caused selective mitotic arrest with a higher efficiency than noscapine, followed by apoptotic cell death as shown by immunofluorescence and quantitative FACS analyses. The IC₅₀ values of these halo-analogues are as shown in Table 1. In another

paper [67], 9-bromonoscapine was shown to be approximately 10- to 15-fold more potent than noscapine in inhibiting cell proliferation and induced apoptosis following G2-M arrest in hormone insensitive human breast cancers (MDA-MB-231). Furthermore, a loss of mitochondrial membrane potential, release of cytochrome-c, activation of the terminal caspase-3, and the cleavage of its substrates such as poly(ADP-ribose) polymerase, suggested an intrinsic apoptotic mechanism. Joshi *et al.* prepared 9-nitronoscapine as well, by the aromatic nitration of natural noscapine using silver nitrate in acetonitrile and trifluoroacetic anhydride (TFAA) at 25°C [68]. This derivative effectively inhibited cellular proliferation of lymphoma and ovarian cancer cells and even in drug-resistant ones. It was demonstrated to upset the progression of cell cycle by mitotic arrest, followed by apoptotic cell death associated with increased caspase-3 activation.



Scheme-6: Synthesis of 9-halo-noscapines

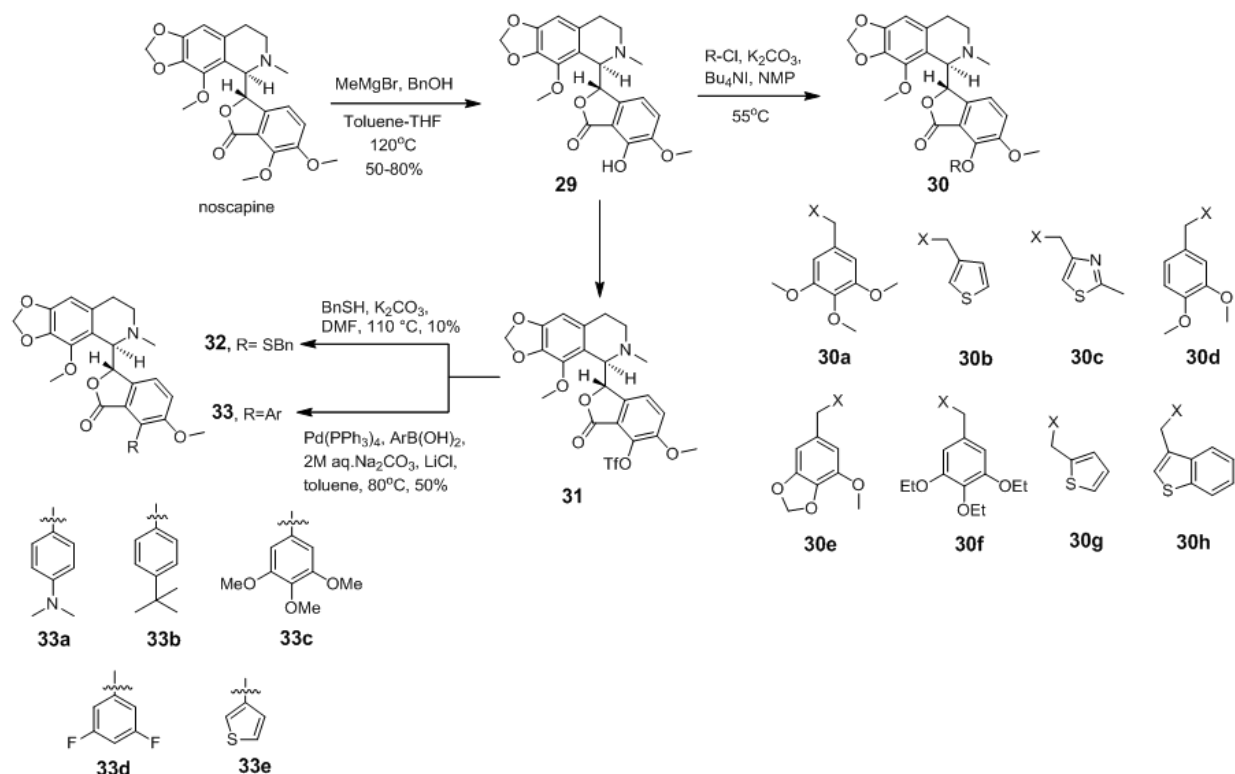
Table 1. IC₅₀ values of halogenated derivatives of noscapine [66]

Cancer Cell-line	IC ₅₀ (μM)				
	Noscapine	25	26	27	28
MCF-7	39.6±2.2	1.0±0.2	1.9±0.3	45.6±3.1	3.3±0.8
MDA-MB-231	36.3±1.8	3.3±0.4	3.5±0.4	25.6±2.4	8.2±0.6
CEM	16.6±2.4	1.9±0.2	1.2±0.3	38.9±3.5	2.3±0.7

Meanwhile in 2005, Anderson *et al.* synthesized several O-alkylated noscapine analogues through regio- and stereoselective *O*-demethylation at C-7 of the phthalide ring and subsequent reaction of the resulting product (**29**) with various alkyl-aryl or alkyl-heteroaryl halides to yield **30a-h** (Scheme 7). Some derivatives were synthesized by reacting the triflate (**31**) with arylboronic acids *via* Pd-catalysed C-C bond formation reactions to yield **33a-e** and also an S-benzyl derivative (**32**) was synthesized [69]. These derivatives were found to induce

an unexpected S-phase arrest of mammalian cells with the most potent compound being the 3,4,5-trimethoxybenzyl analogue (**30a**).

The same group published another report in the same year [70] in which they further derivatized the C-7 position of the phthalide ring by employing the triflate (**31**) and converting it into different analogues (**34a-d**) using a palladacycle catalyst (**I**) (Scheme 8). They also synthesized derivatives **35** and **36** from the triflate but through a different procedure, as shown (Scheme 8). The anilino-noscapine **34b**, along with the hydroxy-noscapine (**29**) was found to be the most potent analogue which arrested mammalian cells in the G2/M phase of the cell cycle at 0.1 μM and also affects tubulin polymerization. Moreover, **34b** was found to be orally bioavailable and was 250-fold more potent than noscapine in reducing cell proliferation in rapidly dividing cells (Tables 2 and 3).

Scheme 7: Synthesis of noscapine derivatives by Anderson *et al.* [69]

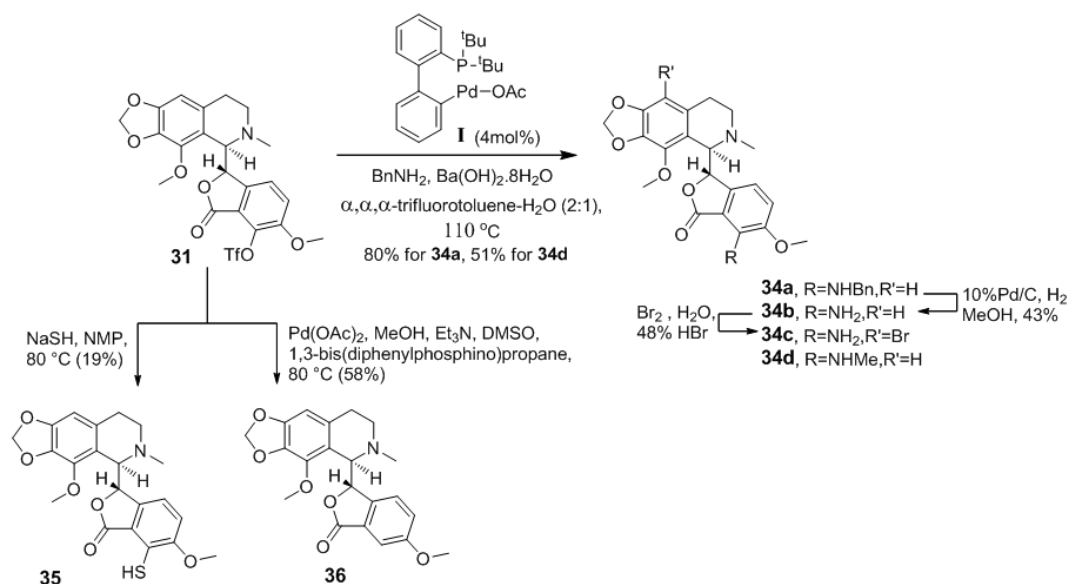
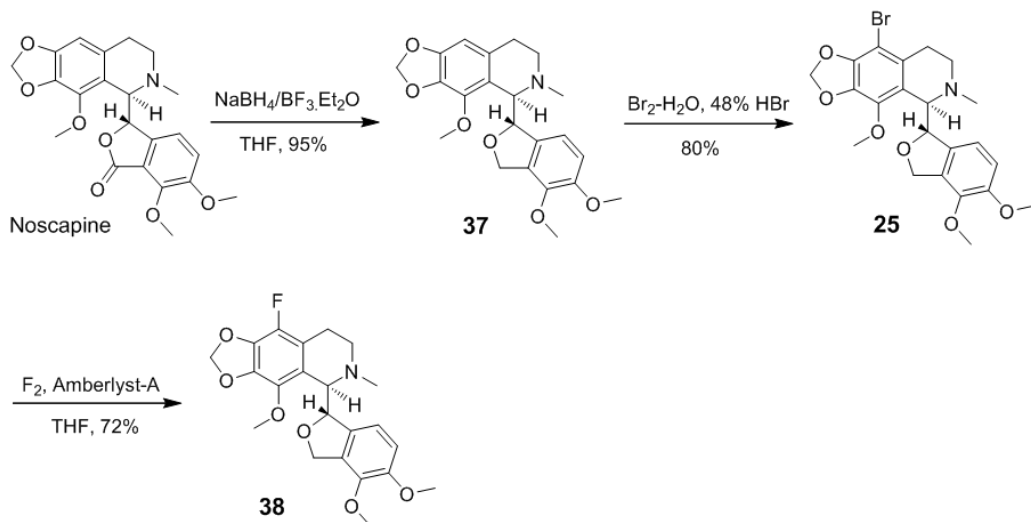
Scheme 8: Synthesis of C-7 anilino-noscapines and other analogues by Anderson *et al.* [70]

Table 2: Noscapine derivatives: Cell Cycle Results for HEK293 Cells [70]

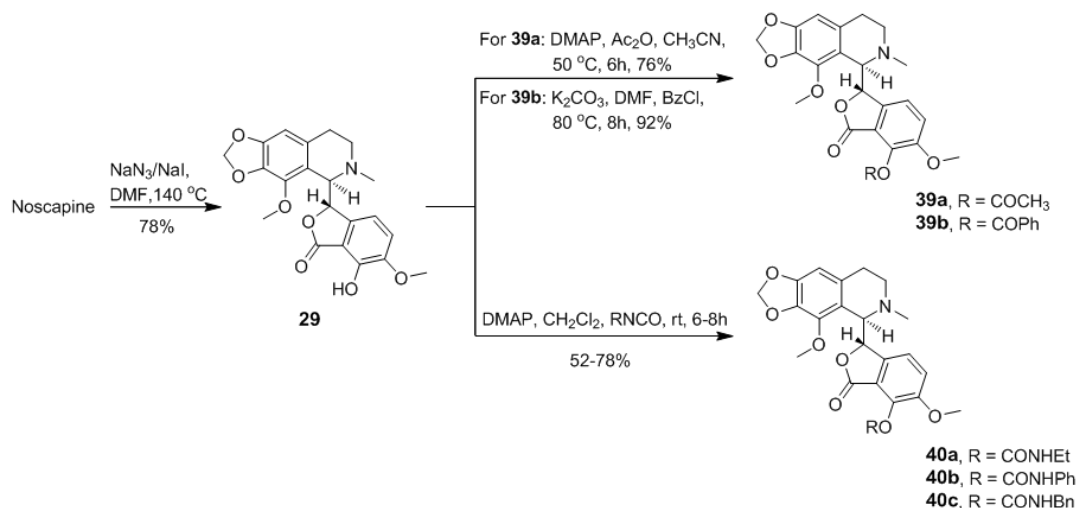
Compound	Concentration (μM)	%G1	%S	%G ₂ /M
DMSO	0.5%	48	39	13
29	1	3	9	88
31	50	56	35	9
34a	5	8	29	63
34b	0.1	1	7	92
34c	1	4	15	81
34d	0.5	8	25	67
35	5	3	7	90
36	1	4	6	90
Noscapine	50	19	31	50
Colchicine	10	3	14	83

Table 3: Tubulin Polymerization and A549 cell-growth assays for **29** and **34b**

Compound	Tubulin polymerization assay, EC ₅₀ (μM)	MTS assay, EC ₅₀ (μM)	Colony-forming assay, EC ₅₀ (μM)
29	0.7	0.6	0.5
34b	0.3	0.097	0.084
Noscapine	>50	25	Not determined



Scheme 9: Synthesis of reduced 9-fluoronoscapine.



Scheme 10: Second generation 7-position benzofuranone noscapine analogues [72].

Table 4: In vitro cytotoxicity (IC₅₀, μM) of noscapine analogs **39** and **40** [72]

Compound	In vitro cytotoxicity (IC ₅₀ , μM)				
	A549 CEM	CEM	MCF-7	MIA PaCa-2	PC-3
39a	3.2	15.5	166.0	1.0	9.3
39b	4.5	49.0	34.0	1.7	4.8
40a	5.6	1.7	49.0	49.0	6.6
40b	50.0	250.0	182.0	1.0	83.2
40c	25.0	7.1	74.1	24.5	49.0
Noscapine	73.0	20.0	45.0	70.0	51.0

In 2006, Aneja *et al.* synthesized reduced 9-fluoro-noscapine (**38**) [71] that showed potent anti-proliferative and anti-cancer activity in both hormone-responsive (MCF-7) and hormone non-responsive (MDA-MB-231) breast cancer cells. It was also effective against MCF-7/Adr, an adriamycin-resistant variant of MCF-7 cells. Immunofluorescence experiments displayed numerous micronuclei, revealing apoptotic cell death triggered by this noscapine analogue. It was synthesized from reduced noscapine (**37**) which was converted to 9-bromo intermediate using same method as used for 9-bromonoscapine, and subsequently to the desired product (**38**) with the use of Br/F exchange reaction by employing the fluoride form of Amberlyst-A26 (Scheme 9).

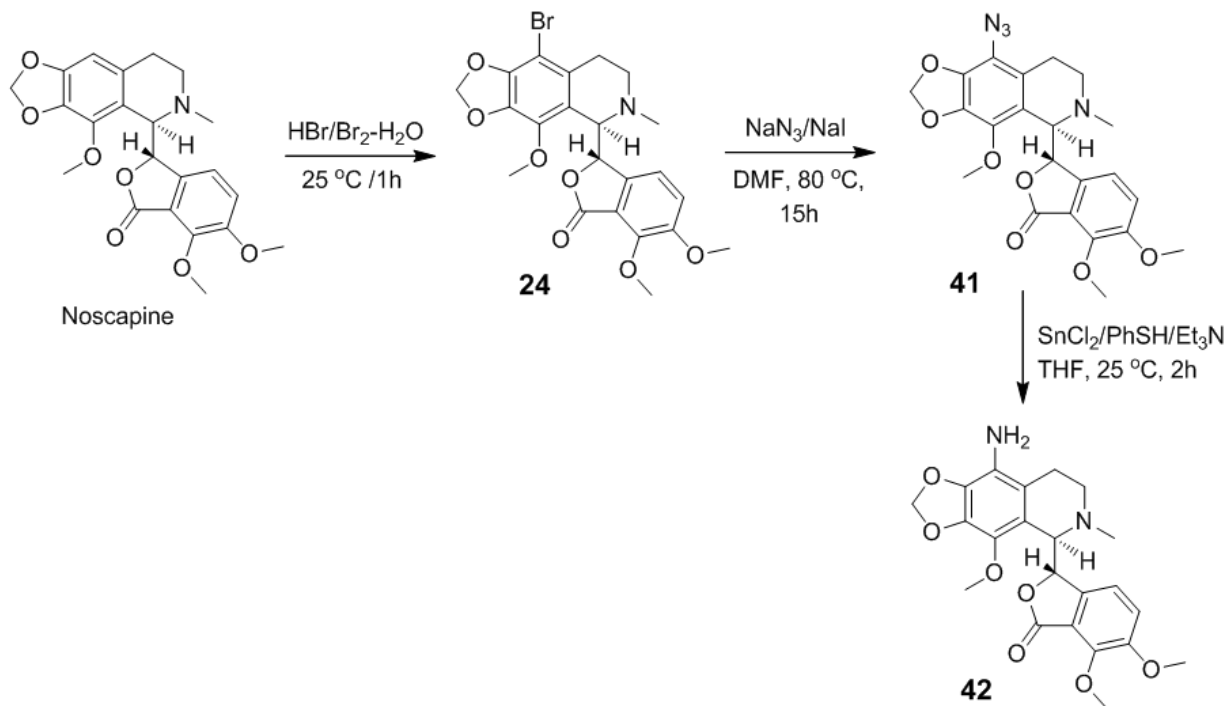
In 2011, Aneja *et al.*, based upon the previous reports on modification of C-7 position of the phthalide ring [69,70], synthesized second-generation 7-position benzofuranone noscapine analogues (**39** and **40**) [72] that differed in the steric bulk of the substituent (Scheme 10). The synthesized analogues were evaluated for their tubulin polymerization activity and their antiproliferative activity against representative cancer cell lines. However, these derivatives were not found to be as potent as the previous analogues, but still more potent than noscapine in most of the cell lines (Table 4).

In 2011, Naik *et al.* developed a LIE-SGB continuum solvation model and computed binding free energies (ΔG_{bind}) of noscapinoids. This LIE-SGB model guided them in designing a novel derivative of noscapine namely, 9-amino-noscapine (**42**) that was found to have higher tubulin binding activity (predicted $\Delta G_{bind} = -6.438$ kcal/mol and experimental $\Delta G_{bind} = -6.628$ kcal/mol) than noscapine, but did not considerably change the total extent of the tubulin subunit/polymer ratio [73]. Its preparation involved conversion of noscapine to 9-bromonoscapine (**24**) which was then subsequently converted to 9-azidonoscapine

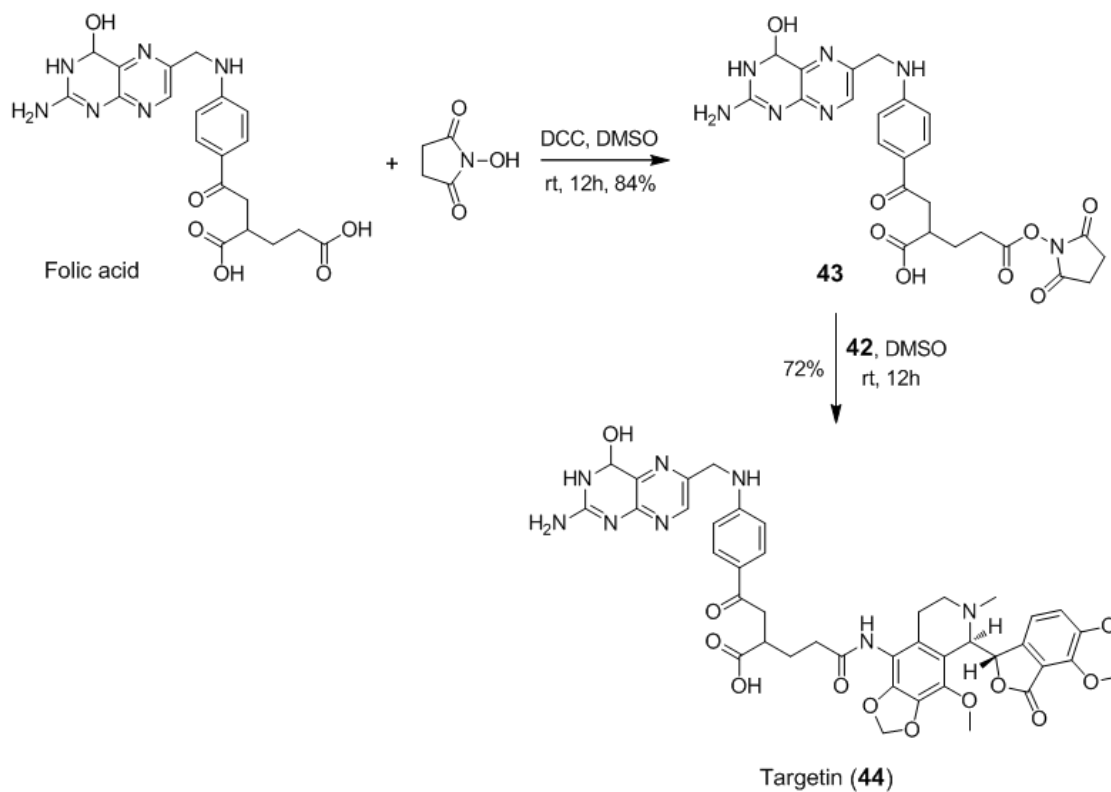
(**41**) and which on reduction gave the desired product i.e. 9-amino-noscapine (**42**) (Scheme 11). It was found to be the most potent among all the previously synthesised noscapinoids. In the subsequent year, Kantevari *et al.* reported a facile route for the synthesis of **42** [74]. They employed copper(I) iodide mediated *in situ* aromatic azidation and reduction of 9-bromo- α -noscapine with NaN_3 in DMSO at 130 °C in the presence of L-proline as an amino acid promoter. This protocol avoided isolation of 9-azido-noscapine intermediate.

In another study, a genetic function approximation algorithm of variable selection was used to generate a quantitative structure activity relationship (QSAR) model for noscapinoids [75]. This QSAR model showed that 9-azido-noscapine (**42**) and its reduced partner are more potent noscapine analogues. The experimentally determined value of pIC_{50} for both the compounds (5.585 M) turned out to be very near to the predicted pIC_{50} (5.731 and 5.710 M). In the following year, Joshi *et al.* used *in silico* methodologies to design a novel folate-noscapine analogue, Targetin (**44**) [76]. It was synthesized by coupling folic-acid activated ester, NHS-folate (**43**) with 9-amino-noscapine (**42**) (Scheme 12). Molecular docking experiments had predicted that **44** fits well inside the binding cavity at the interface between α - and β -tubulin and experimental results (tubulin binding studies and *in vitro* anti-cancer activity) corroborated well with these claims. It was found to be more potent than noscapine (IC_{50} in the range of 15–40 μM). Interestingly, ovarian cancer cells (SKOV3 and A2780), known to overexpress folate-receptor alpha (FOLR1), were much more sensitive to **44** (IC_{50} in the range of 0.3–1.5 μM).

In 2012, the Scammells group reported the synthesis and pharmacological evaluation of a series of 6'-substituted noscapine derivatives [77]. They first prepared nor-noscapine (N-desmethyl-noscapine) **46**, which was then



Scheme 11: Synthesis of 9-azido and 9-amino-noscapine



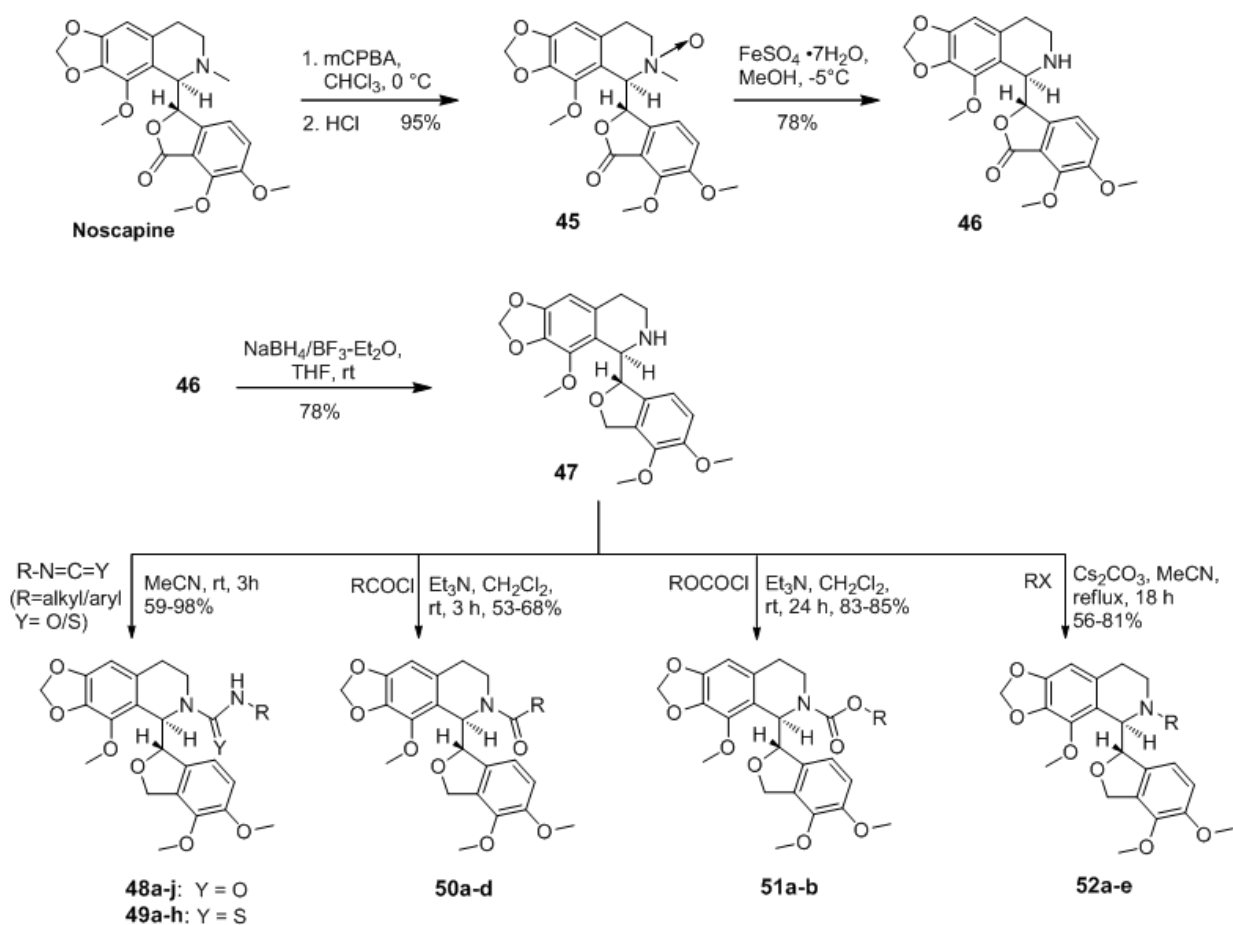
Scheme 12: Synthesis of Targetin

reduced to get intermediate **47**. Reaction of the latter with a range of alkyl-isocyanates, thioisocyanates, acid chlorides, chloroformates and halides resulted in the formation of the corresponding N-carbamoyl, N-thiocarbamoyl, N-acyl, N-carbamate and N-alkyl derivatives (**48**, **49**, **50**, **51**, **52** respectively). (Scheme 13). Evaluation of these derivatives against prostate cancer (PC3), breast cancer (MCF-7), and colon cancer (Caco-2) cell lines furnished that the N-urea (**48**) and N-thiourea (**49**) compounds were the most potent analogues within the series and the N-urea compounds showed more activity than their N-thiourea counterparts, with **48a** (R=NH₂) being the most potent derivative of the series (EC₅₀ = 3.58 - 6.7 μM).

and **34b** was published which gave important insights on the tubulin-binding and anti-mitotic mechanisms of the C-7-substituted derivatives [78]. It was shown that noscapine and its 7-aniline derivative (**34b**) does not compete for the same binding site nor does its small molecule crystal structure match its tubulin-bound conformation. It was more potent than noscapine but pharmacokinetic data suggests this compound to be highly cleared *in vivo*, thus showing lesser potential as an anti-mitotic.

In 2013, Waldmann *et al.* synthesized a noscapine-inspired collection of tetrahydroisoquinolines (**53**) [79]. The derivative **53d**, was found to be the most promising of all, being more potent than noscapine in HeLa cells (Figure 6) and was even effective in drug-resistant cell lines as well.

In 2012, a further study on derivatives **29**



Scheme 13: Synthesis of N-substituted noscapine derivatives.

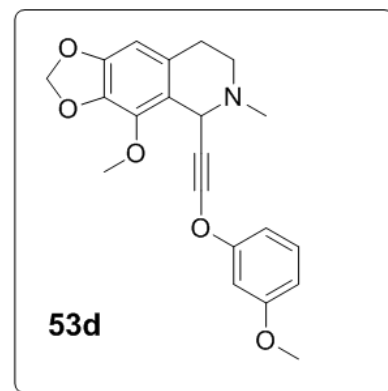
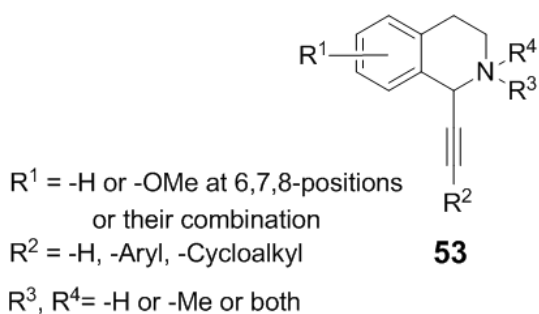
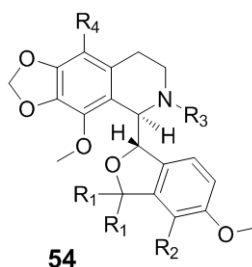


Figure 6: Noscapine-inspired collection of tetrahydroisoquinolines [79]

Table 5: Percentage increase in arrested cells trapped in G2/Mphase for **54** [80].

Comp-- ound	Structural Variations				Increase in G2/M [%]		
	R ₁	R ₂	R ₃	R ₄	PC3	MCF-7	PANC-1
48a [77]	H, H	OMe	C(O)NHEt	H	133	172	not determined
54a	H, H	OMe	H	Cl	142	215	138
54b	H, H	OMe	H	Br	154	209	132
54c	H, H	OMe	H	I	117	180	135
54d	H, H	OMe	C(O)NHEt	Cl	162	229	114
54e	H, H	OMe	C(O)NHEt	Br	159	233	119
54f	H, H	OMe	C(O)NHEt	I	146	227	120
54g	H, H	OMe	C(O)NHEt	NH ₂	85	31	92
54h	H, H	OMe	C(O)NHEt	Ph	3	-5	7
54i	H, H	OMe	C(O)NHEt	2-OMe-Ph	11	-21	-12
54j	H, H	OMe	C(O)NHEt	3-OMe-Ph	18	-6	5
54k	H, H	OMe	C(O)NHEt	4-OMe-Ph	17	-15	-8
54l	H, H	OMe	C(O)NHEt	2-F-Ph	14	-14	11
54m	H, H	OMe	C(O)NHEt	3-F-Ph	9	-17	12
54n	H, H	OMe	C(O)NHEt	4-F-Ph	14	-15	13
54o	=O	OMe	C(O)NHEt	Cl	21	9.9	37
54p	=O	OH	C(O)NHEt	Cl	155	238	98
54q	H, H	OH	C(O)NHEt	Cl	99	80	93

It had similar anti-mitotic action as noscapine and it was proposed that this derivative can overcome drug resistance due to P-glycoprotein expression.

Recently, Scammells and co-workers [80], have synthesised a number of noscapine derivatives in continuation of their earlier work [77] which had revealed that an N-ethylaminocarbonyl group at 6'-position improves anti-cancer activity. This was combined with other structural changes that were known to improve anticancer activity of noscapine *viz.* halo substitution in the 9'-position; regioselective *O*-demethylation to reveal a free -OH in the 7-position; and reduction of the lactone to the corresponding cyclic ether in the 1-position. The incorporation of new aryl substituents in the 9'-position was also investigated. This study has identified some potent derivatives that are able to induce G2/M cell-cycle arrest (Table 5) and that possess cytotoxic activity against the human prostate carcinoma cell line PC3, the human breast adenocarcinoma cell line MCF-7, and the human pancreatic epithelioid carcinoma cell line PANC-1. The compounds **54d-f** which are N-ethylaminocarbonyl derivatives of halo-noscapine, were identified as the most promising of all derivatives ($IC_{50} = 0.9-2.9 \mu M$ against the 3 cell lines). These were also active against drug-sensitive and -resistant breast cancer cell lines with IC_{50} values in the range of 1.24 to 4.07 μM [80].

7. CURRENT STATUS, CHALLENGES AND FUTURE PROSPECTS

With the discovery of noscapine's anti-cancer effects in 1998, much advancement has been made towards finding a 'gentler' class of cancer chemotherapeutics. Although it has been neglected for many decades, the time is ripe for noscapine and its derivatives to develop into successful anti-cancer agents. Already it has been used as a safe and oral anti-tussive preparation for decades, and as shown by researchers, that

although its tumour suppressive dose is high ($ED_{50} = 300 mg/kg$), it is still safer than other anti-mitotic agents currently available in the market. However, this high dose may impact its availability as a successful anti-cancer medication, due to the constraints of nature as its only source. To circumvent these problems, targeted drug delivery and nano-encapsulation of noscapine and its analogues is under investigation. For example, nanoencapsulation of noscapine using human serum albumin (HSA) with a particle size in the range of 150-300 nm and drug loading efficiency of 85-96% has shown enhanced bioavailability by delivering maximum amount of noscapine to target sites at a rate and concentration that permits optimal therapeutic efficacy, while reducing any undesirable side effects to a minimum [81]. Similarly, complexation of noscapine with β -cyclodextrin (β -CD) displayed 1.87-fold increase in bioavailability as compared to free noscapine [82]. 9-Bromo-noscapine **25** upon complexation with β -CD and methyl- β -CD has shown enhanced solubility upto 11-fold and 21-fold which in turn increases their anti-cancer activity [$IC_{50} = 28.5 \mu M$ (9-Br-Nos- β -CD) and 12.5 μM (9-Br-Nosmethyl- β -CD)] in PC-3 cells compared to free **25** (~200 μM) [83].

Noscapine and its analogues were shown to positively enhance the effects of other anti-cancer treatments, including radiation-therapy. For example, noscapine augments the effect of cisplatin in the treatment of non-small cell lung cancer [84] and sensitizes resistant ovarian cells to cisplatin [85]. Similar effects were observed for combination treatment of noscapine with other chemotherapeutic agents in glioblastoma cells [86]. It was also found to enhance the effect of radiation therapy and taxol-treatment in C6 glioma cells [87]. These findings are significant because these can be applied for the treatment of multiple cancers, including drug-resistant ones.

Till date, noscapine and its analogues have been

found to possess efficient anti-cancer activities against various types of cancer. Already it is under Phase I/II clinical trials. In 2007, results of phase I trial for non-Hodgkin's lymphoma and chronic lymphocytic leukaemia were disclosed to the public. The preliminary results were very encouraging showing that noscapine has been well tolerated, with no grade 3 or 4 haematological toxicities (however one grade III neurotoxicity consisting of depressed level of consciousness was experienced at the 3 g dose level) [26]. Another Phase I trial for its effects on patients with relapsed or refractory multiple myeloma has been completed, though its results are yet to be disclosed [88].

An immensely important task is the development of concise and efficient synthetic routes for synthesis of optically pure noscapine. These synthetic methodologies should provide enough room for further derivatization of this scaffold. It can be observed that till date, only minor changes have been done to the structure of this molecule and it is extremely important to fully explore any further possibilities to develop more potent and better derivatives.

To conclude, noscapine and its analogues have been established to be a much better and safe class of anti-cancer agents. Their use promises minimal or no side-effects compared to the currently used treatments. Although there are many challenges on the part of the scientific world to discover its full potential in not only cancer, but to other human ailments as well, the future prospects look bright for this novel, 're-discovered' class of anti-cancer agents.

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