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PERSPECTIVE

Peloruside, Laulimalide, and Noscapine Interactions with Beta-Tubulin

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ABSTRACT This article reviews the recent findings regarding the binding sites, binding modes and binding affinities of three novel antimitotic drugs peloruside, laulimalide and noscapine with respect to tubulin as the target of their action. These natural compounds are shown to bind to β-tubulin and stabilize microtubules for the cases of peloruside A and laulimalide, and prolong the time spent in pause for noscapine. Particular attention is focused on β-tubulin isotypes as targets for new cancer chemotherapy agents and the amino acid differences in the binding site for these compounds between isotypes. We propose a new strategy for antimitotic drug design that exploits differential distributions of tubulin isotypes between normal and cancer cells and corresponding differential affinities between various drug molecules and tubulin isotypes.

KEY WORDS chemotherapy · docking · laulimalide · noscapine · peloruside · tubulin

INTRODUCTION

There is a critical need for a fundamental understanding of the interactions of cancer chemotherapy drugs, such as peloruside A (1), laulimalide (2), and noscapine (3), with cancer cells, and in particular with their molecular targets, in order to improve treatment outcomes. Normal cells grow and divide according to a regulated cycle, known as the cell cycle. At the onset of cancer, it is believed that some normal cells exhibit what is called genomic instability and mutate due to various, generally unknown, factors. These cells begin to grow and divide at an unregulated (usually faster) rate and show an increasing number of phenotypic abnormalities. According to the seminal paper by Hanahan and Weinberg (4), virtually all cancers can be characterized by the following six hallmarks: (a) self-sufficiency in growth signals, (b) insensitivity to anti-growth signals, (c) tissue invasion and metastases, (d) limitless replicative potential, (e) sustained angiogenesis, and (f) evasion of apoptosis.

Chemotherapy uses drugs that either damage deoxyribonucleic acid (DNA), inhibit signaling pathways for growth and division, or interfere with mitosis through binding with microtubules (MTs). A key force-generating component in cellular division involves MTs with motor proteins and kinetochore complexes. Finding a ligand that binds with high affinity to the MTs of cancerous cells, while simultaneously binding to the MTs of healthy cells with significantly lower affinity, is the focus of much cancer research. Currently used antimitotic drugs interfere with the cell cycle, specifically during mitosis when MT formation and dynamics are essential for correct cell division. Tubulin has been the target for numerous small molecule ligands for several decades. The action of these drugs results in the alteration of MT dynamics, ultimately leading to cell cycle arrest and apoptosis. Many of these ligands are currently used clinically for the treatment of several types of cancer (especially breast and ovarian) and include the drugs paclitaxel and vinblastine as well as their derivatives. These drugs bind to one of several distinct binding sites within β-tubulin, all of which

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have been identified through crystallographic determination. Paclitaxel is one of the most successful cancer therapeutic agents and it binds to and results in the stabilization of MTs within all cells. Derivatives of paclitaxel, such as docetaxel, have been synthesized to address the limited solubility of paclitaxel. While structural information exists for several drug-binding conformations within β-tubulin, the information is unfortunately unable to reveal the precise mechanism of MT stabilization within the taxane binding site.

Taxanes (e.g. docetaxel and paclitaxel) and vinca alkaloids (e.g. vinblastine, vinorelbine and vincristine) serve as stabilizing and destabilizing agents against MTs, respectively (5). However, these two families of compounds bind in distinct locations on β-tubulin, and often cause serious toxicity due to their respective over-polymerization or depolymerization action (6), as well as off-target interactions. Colchicine is another well-known antimitotic agent that binds to the β-monomer of the tubulin hetero-dimer whose distinct binding site has been identified to be located in close proximity to the intra-domain region of the two tubulin monomers (7). Colchicine is representative of a special type of antimitotic behavior when a drug binds to the α/β-tubulin dimer and prevents MT polymerization. If the MTs cannot form, the cell cannot undergo division and eventually apoptosis (cell death) occurs. Colchicine has been known as a highly toxic chemotherapeutic agent (8) and due to its toxicity has not been approved for treatment of cancer. Another class of antimitotic drugs are the vinca alkaloids; these drugs bind to MTs and cause them to depolymerize into free α/β-tubulin dimers. Antimitotic drugs such as paclitaxel, laulimalide, and peloruside are MT stabilizing agents. These drugs bind to MTs and prevent them from depolymerizing into free α/β-tubulin dimers. Interestingly, paclitaxel binds to the inside of MTs and prevents them from depolymerizing (9). The depolymerization/polymerization dynamics of MTs is essential in cell division, specifically when the chromosomes are being pulled to either side of the cell in anaphase. Without MTs, the cell is unable to complete the cell cycle and therefore goes into apoptosis. X-ray and NMR structures of several drugs can be found in the Protein Data Bank (PDB: http://www.rcsb.org/pdb/) and the interested reader is directed there for further information on the structures of the tubulin/drug complexes.

As stated above, several drugs currently used in chemotherapy work by interfering with the process of cellular division by binding in a well-defined way to the α/β-tubulin heterodimer (10) which is the building block of MTs. It is worth noting that there are ten (eight expressed by proper genes) common isotypes of β-tubulin that are expressed in varying degrees in the tissues and organs of the human body. An extensive body of work exists in the literature concerning the distribution, the structural differences and functional implications of the tubulin isotypes such as MT dynamics, in various organisms including human organs and tissues (11-13). This information serves as an important motivating factor in seeking isotype-specific drug targeting. Obviously, targeting cancer cells with their differential expression of tubulin isotypes and mutants would be expected to broaden the therapeutic window of novel drugs that have increased specificity and selectivity due to their isotype binding profiles. Moreover, all of the common isotypes of β-tubulin can have both germline and somatic mutations with potentially significant consequences for drug binding and therapeutic outcomes. Recent research by Leandro-García *et al.* (14) has provided quantitative measures of the mRNA expression levels of different isotypes of β-tubulin for different types of normal and cancer cells. This is only the first step in this direction, and caution must be exercised due to the fact that mRNA expression levels do not necessarily reflect protein levels of the different isotypes, particularly since it is known that the regulation of tubulin synthesis is complex. Moreover, protein expression levels in tumor tissues may vary as a result of exposure to cytotoxic agents.

As mentioned above, tubulin isotypes (15) are expressed in varying degrees in various healthy cells in the human body. The β-tubulin isotypes consist of 421 amino acid residues (excluding their C-termini) and approximately 1–20% of the residues differ between the isotypes (16). Only one or two residues differ between the various isotypes within the binding sites of drug molecules, and therefore a single amino acid mutation within the binding site can affect the binding affinity of a drug and result in the drug being ineffective against that tubulin mutation/isotype. It is known (14) that although there may be a dominant form of β-tubulin within a certain organ, other isotypes are also found in lower amounts within the same organ. The percentage of each isotype may also change when tissue turns cancerous; it is possible that this property can be exploited in sophisticated drug design strategies aimed to more effectively eliminate cancer cells. The change in tubulin isotype distribution could be exploited such that a specific drug could be chosen to maximize cancer cell death while simultaneously minimizing damage to healthy tissues. However, due to a number of molecular mechanisms (e.g. isotype expression changes, somatic mutations, p-glycoproteins, MDR proteins, etc.) cancer cells can still become resistant to this antimitotic agent over the course of treatment, which would require designing of a second line of treatment strategy.

According to Leandro-García *et al.* (14), colon and prostate cancer cells have βI as the most abundant β-tubulin isotype, and βIVb as the second most abundant isotype. Additionally, ovarian, kidney, breast, lung and larynx cancer all have the opposite distribution (βIVb as the most abundant and βI as the second most abundant isotype). Also, increased expression of the βIII isotype in some tissues

has been associated with cancerous cells, and thus determining which drug will bind to this isotype is extremely important. An increase in βIII isotype has been observed for lung cancer, which is the second most prevalent form of cancer (13.9% of cancer cases) and also accounts for over one-quarter (27%) of all cancer deaths (17). Finding a drug that binds strongly and specifically to this isotype is of great importance.

PELORUSIDE AND LAULIMALIDE

It is interesting to note that most of the cancer drugs used or investigated today have been extracted from natural sources or are modifications of natural products. A new class of polyketide macrolides isolated from deep-sea sponges has been discovered to induce a phenotype similar to, and synergistic with, paclitaxel. These macrolides display a cellular phenotype highly similar to what is observed with paclitaxel. MT dynamics are altered at low concentration and the mitotic spindle is disrupted in a dose-dependent manner, ultimately leading to multipolar spindle formation and MT bundling at high concentration. As with paclitaxel, the resulting mitotic block leads to variable cell fates resulting from mitotic slippage, but with a significant population of cells undergoing apoptosis. In spite of these numerous similarities, competitive binding studies with paclitaxel have highlighted that laulimalide, and a related macrolide peloruside A, do not occupy the taxane-binding site, as we discuss below.

Peloruside has been isolated from the marine sponge, *Mycale hentscheli*, which grows in the Pacific Ocean around New Zealand (18), and laulimalide has been isolated from the marine sponge, *Cacospongia mycofijiensis*, which grows around Vanuatu (2). Laulimalide is currently being investigated for use in combination therapy with other antimitotic drugs, and has not yet reached a clinical trial stage (19). Preclinical and clinical trials of peloruside A are currently being held up due to the short supply of the marine sponge that contains peloruside A (20). Nonetheless, both of these drugs have been demonstrated to be potent MT stabilizers with substantial promise for future clinical development.

Peloruside A and B (Fig. 1) are new potential antimitotic agents which have been extensively studied since 2000 (18,20-24). Additionally, laulimalide and isolaulimalide, shown in Fig. 1, (isolaulimalide forms from laulimalide under acidic conditions) were investigated for their antimitotic behavior by Mooberry *et al.* (2) who found that laulimalide had an IC_{50} only one order of magnitude less than that of paclitaxel, but isolaulimalide was a thousand times less potent. The IC_{50} values of peloruside A (25) and laulimalide (26-28) are given in Table I with respect to several cancer cell lines determined by growth inhibition assays. Laulimalide potently inhibits cellular proliferation against numerous cancer cell lines with IC_{50} values in the low nM range. Importantly, it is also very active against multidrug-resistant (MDR) cancer cell lines which overexpress PgP, and is effective against both paclitaxel-resistant and epothilone-resistant cells that have β-tubulin mutations modifying the paclitaxel binding site (29).

The ADMET Predictor program (30) was run to predict the pharmacokinetic and pharmacodynamic properties of peloruside A and laulimalide. The predicted octanol/water partition coefficient (Log P) values were calculated in ADMET Predictor using a method described by Moriguchi *et al.* (31). The Log P values were found to be –0.69 for peloruside A and 2.08 for laulimalide. The human jejunal effective permeability (P_{eff}) values were calculated to be 0.66 $cm/s \times 10^4$ for peloruside A and 0.03 $cm/s \times 10^4$ for laulimalide. The solubility in simulated fasted state intestinal fluid (FaSSIF) was in the range of 1.02 to 0.07 mg/mL. A toxicity risk index for laulimalide and peloruside A was also computed by ADMET Predictor. The toxicity risk is calculated on the basis of several properties such as acute toxicity in rats, carcinogenicity in rodents, and Ames test mutagenicity. A toxicity risk value of less than or equal to three corresponds with 90% of a subset of the World Drug Index; both laulimalide and peloruside A fall within this threshold. ADMET Predictor also calculates compliance with the Lipinski Rule of Five (32). Using the toxicity risk that was calculated, it can be conjectured which drugs would be potential candidates for further pre-clinical studies. Laulimalide has been shown to be extremely toxic, and has a relatively high toxicity risk value of 3. Peloruside A, a less potent antimitotic agent, has a toxicity risk value of 1. These values are consistent with the experimental findings that laulimalide is slightly too toxic.

Molecular docking, nuclear magnetic resonance (NMR), and mass shift perturbation mapping (MSPM) (33,34) studies were performed on the complexes of both peloruside A and laulimalide with tubulin hetero-dimer (35). It has been hypothesized that both peloruside A and laulimalide share the same binding pocket. An NMR study on peloruside A and laulimalide binding to MTs was performed by Pineda *et al.* (9). A possible binding site was shown on the surface of α-tubulin. Additionally, docking studies were performed and an alternative site was found on the surface of β-tubulin. The docking studies were corroborated by MSPM and both studies show similar results. The most probable binding sites/modes for peloruside A and laulimalide are depicted in Fig. 2; the key residues shown in the images were determined through docking and molecular mechanics simulations. Docking calculations were performed by Bennett *et al.* (35) and Huzil *et al.* (1), for peloruside A and laulimalide, respectively. The docking predictions of Bennett *et al.* (35) were experimentally validated by mass shift perturbation mapping. Some key residues in the binding site of all ten

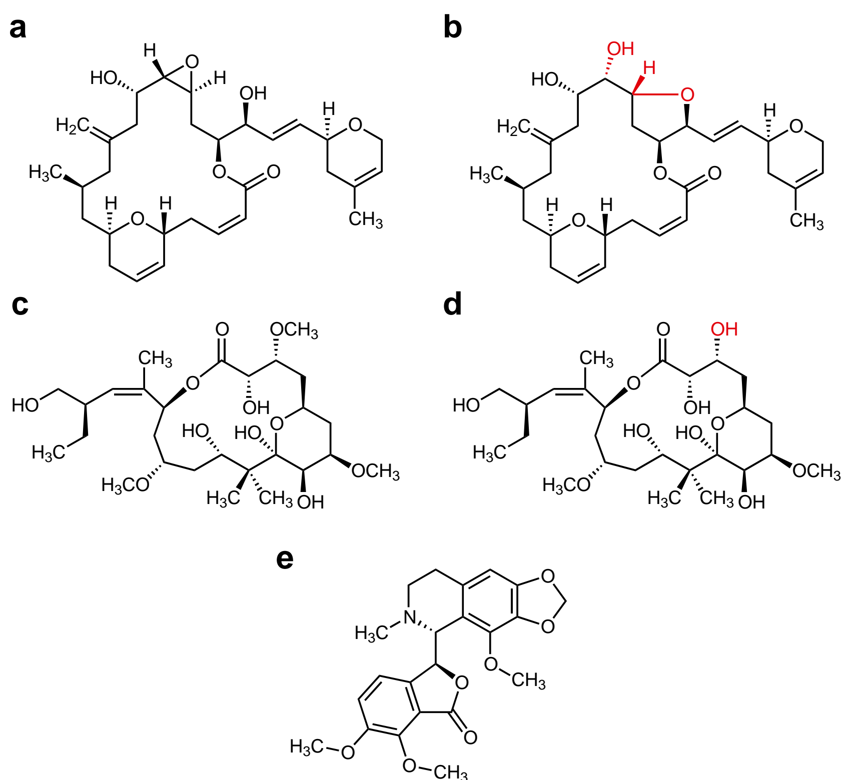

Fig. 1 Chemical structures of laulimalide (**a**), isolaulimalide (**b**), peloruside A (**c**), peloruside B (**d**), and noscapine (**e**). The differences in isolaulimalide compared with laulimalide are highlighted in red. Similarly, the differences in peloruside B compared with peloruside A are highlighted in red.

isotypes of β-tubulin (βI, βIIa, βIIb, βIII, βIVa, βIVb, βV, βVI, βVII, and βVIII) from the work done by Huzil *et al.* (16), are given in Fig. 3. It is apparent from Fig. 3 that at least one of the key residues mutates within the peloruside A/laulimalide binding site between the isotypes, and hence this can be further exploited in the redesign of these compounds for greater specificity and selectivity. Specifically, introducing an additional hydrogen bond between a derivative of laulimalide or peloruside with a functional group placed at a relevant position to make contact with Ser296 in βII may stabilize the interactions. This stabilization would not exist in βI (and all the remaining tubulin isotypes), and would only affect the interaction of the derivative with MTs containing βII tubulin. The absence of Ser296, which is replaced by Ala296 in βI and the remaining (less abundant) tubulin isotypes, would result in the destabilization of this interaction, and therefore an effective reduction of laulimalide/peloruside activity in MTs containing non-βII tubulin isotypes. This should affect the sensitivity of, for example, ovarian cancer cells, in which βII tubulin is expressed more abundantly than in the corresponding normal tissue, and offer a broader therapeutic window for drugs designed specifically with such features in mind.

Another docking/MSPM study was performed by Nguyen *et al.* (36). These studies also predicted a similar binding site to that of Bennett *et al.* (35) and Huzil *et al.* (1). Nguyen *et al.* (36) also performed docking simulations starting with the binding site identified by Huzil *et al.* (1) for both laulimalide and peloruside A, and a slightly different structure of the protein-ligand interaction was found. The protein-ligand binding mode was determined to have more favorable hydrophobic and electrostatic interactions for peloruside A. Experimental studies with paclitaxel, epothilone B, and discodermolide were performed in order to confirm that laulimalide and peloruside bind to a different site on tubulin.

Begaye *et al.* (37) and Kanakkanthara *et al.* (38) investigated mutations in the β-tubulin binding site for peloruside A and

Table I Experimental IC_{50} (μM) Values for Peloruside A (25) and Laulimalide (26-28) with Several Cancer Cell Lines Determined By Growth Inhibition Assays. 1A9, PTX10, PTX22, A8, B10, SK-OV-3, and SKVLB are Ovarian Cancer Cell Lines. MDA-MB-435 is an M14 Melanoma Cell Line. PTX10, PTX22, and A8 are Paclitaxel-Resistant. SK-OV-3 is Resistant to Diphtheria Toxin, Cisplatin, and Adriamycin. SKVLB is Resistant to Vinblastine. ND Indicates Cases with No Data

Ligand	1A9	PTX10	PTX22	A8	B10	MDA-MB-435	SK-OV-3	SKVLB
Peloruside A	0.0215	0.0510	0.0210	0.0170	0.0290	ND	ND	ND
Laulimalide	0.0039	0.0060	0.0063	0.0092	0.0150	0.00574	0.01153	1.21

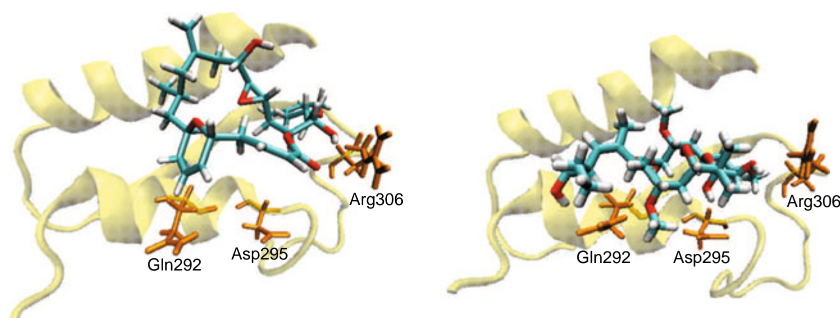

Fig. 2 Laulimalide (*left*) and peloruside (*right*) bound to β-tubulin, showing the key residues Gln292, Asp295, and Arg306. The tubulin structure is based on the 1JFF tubulin PDB structure, and laulimalide and peloruside are docked into their binding sites based on previous work (1,35).

used peloruside A resistant cell lines. Again, MSPM and molecular docking identified the binding site on β-tubulin. The region identified was close to the outer surface of the MT, and confined in a cavity surrounded by a continuous loop of the folded protein so as to center on Tyr340. The peloruside A resistant lines of the human ovarian carcinoma cell line 1A9 were also used in this study to better characterize this binding site and investigate the effect of mutations of residues within the binding pocket. The peloruside resistant lines were shown to have 10–15 fold resistance to peloruside A. Additionally, the peloruside A resistant cell lines showed resistance to laulimalide, whereas other MT stabilizers did not show additional resistance due to the mutations within the peloruside/laulimalide binding pocket.

All studies of peloruside A and laulimalide have shown a distinct binding site different from the binding site of the other MT stabilizing agents. Molecular docking and hydrogen deuterium exchange studies all provide the same binding site, and similar binding modes.

NOSCAPINE

Noscapine, 5-(4,5-Dimethoxy-3-oxo-1,3-dihydro-isobenzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-6-ium, (Fig. 1), is a benzylisoquinoline alkaloid derived from plants of the Papaveraceae (poppy) family. Noscapine has been used as an antitussive (cough suppressant) agent for over half a century; however, its recent tests as an antimitotic agent made it a focus of attention in the cancer chemotherapy community. Noscapine binds to MTs (39) and exhibits antimitotic properties that could lead to a new class of less toxic anti-cancer drugs. Noscapine (originally extracted from opium) showed potential to be effective in treating many forms of cancer such as breast (40), small cell lung (41), colon (42), prostate (43), and drug resistant lymphomas (44).

Experimental studies of halogenated derivatives of noscapine have also been performed revealing that the halogenated complexes have an increased antimitotic activity in several cell lines (39). The chlorinated and brominated noscapine derivatives show particularly interesting properties. The chlorinated derivative shows promising results against resistant ovarian cancer cells (45), and the brominated noscapine has been shown to be particularly effective as an antimitotic (46) and an anti-inflammatory agent (47). Another clinical application of this compound was as an anti-stroke agent (48). The compound in combination with two other important opiate drugs (morphine and heroin) has exhibited electrochemical oxidation properties, where the response of electrodes using these compounds shows excellent electrocatalytic activity (49).

However, in regard to the chemotherapeutic use of noscapine, it is still an investigational anti-cancer agent (50). Unlike taxanes and vinca alkaloids, it is water-soluble and readily crosses the blood–brain barrier (51). It exhibits some toxic effects such as fixed drug eruption (which refers to the development of one or more annular or oval erythematous patches as a result of systemic exposure to a drug) (52), but no toxicity was identified to the duodenum, spleen, liver or hematopoietic cells as determined by pathological microscopic examination of their tissues and flow cytometry (51). It has also been demonstrated to slow down the growth rate of dorsal root ganglion cultures by affecting axonal degeneration (51). This is a common reaction to a drug (e.g. rash, lesion) at the exposed site, or after occasional oral administration of a drug. Most fixed drug eruptions are caused by a limited number of chemical substances such as

Fig. 3 Amino acid sequence alignments for ten β-tubulin isotypes in the region near the laulimalide/peloruside binding site, positions 290–350. The amino acid numbering corresponds to the βI isotype, with position 1 at the initial methionine (16).

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       290                                                         350
βI     TQQVFDAKNMMAACDPRHGRYLTVAAVFRGRMSMKEVDEQMLNVQNKNSSYFVEWIPNNVK
βIIa   TQQMFDSKNMMAACDPRHGRYLTVAAIFRGRMSMKEVDEQMLNVQNKNSSYFVEWIPNNVK
βIIb   TQQMFDSKNMMAACDPRHGRYLTVAAIFRGRMSMKEVDEQMLNVQNKNSSYFVEWIPNNVK
βIII   TQQMFDAKNMMAACDPRHGRYLTVATVFRGRMSMKEVDEQMLAIQSKNSSYFVEWIPNNVK
βIVa   TQQMFDAKNMMAACDPRHGRYLTVAAVFRGRMSMKEVDEQMLSVQSKNSSYFVEWIPNNVK
βIVb   TQQMFDAKNMMAACDPRHGRYLTVAAVFRGRMSMKEVDEQMLNVQNKNSSYFVEWIPNNVK
βV     TQQMFDARNMMAACDPRHGRYLTVATVFRGPMSMKEVDEQMLAIQSKNSSYFVEWIPNNVK
βVI    TQQMFDARNTMAACDLRRGRYLTVACIFRGKMSTKEVDQQLLSVQTRNSSCFVEWIPNNVK
βVII   TQQMFDAKNMMAARDPRHGRYLTAAAIFQGRMPMREVDEQMFNIQDKNSSYFADWFPNNVK
βVIII  TQQMFDAKNMMAACDPRHGRYLTAAAIFRGRMPMREVDEQMFNIQDKNSSYFADWLPNNVK
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barbiturates, sulphonamides, tetracyclines, carbamazepine and many of the antitussive agents (52).

Noscapine is also in Phase I and II clinical trials for various human cancers in spite of the fact that its mechanism of action as a stabilizer or destabilizer of MTs has not been clearly determined (53). Noscapine alone and in combination with doxorubicin acts against triple negative breast cancer (TNBC) and potentiates the anti-cancer activity of doxorubicin in a synergistic manner against TNBC tumors. This occurs via inactivation of the NF-KB and anti-angiogenic pathways while stimulating apoptosis. These findings suggest potential benefit for the use of orally administered noscapine and doxorubicin in combination therapy for treatment of more aggressive TNBC (54). Briefly, the oral administration of chemotherapeutic agents is an advantage since the utility of some of the administered anti-cancer drugs is limited due to the development of drug-resistance and need of intravenous infusion over a long period of time, which is associated with toxicities. This has led to a search for MT-targeting agents that can be administered orally with less toxicity. Oral administration of noscapine has shown a significant reduction in tumor volume while this anti-tumor activity causes no or minimum toxicity. More comprehensive elucidation of the relevant experiments and corresponding references are found in the referenced paper by Chougule *et al.* (54). Its combination with gemcitabine also increases anti-cancer activity of the latter against non-small cell lung cancer in an additive to synergistic manner via anti-angiogenic and apoptotic pathways (54). It significantly decreases TMZ-resistant glioma cell growth-invasion and increases survival of animals with TMZ-resistant gliomas (55).

Noscapine is capable of inducing a dose-dependent apoptosis of gastric cancer cells. Treatment with noscapine up-regulates Bax and Cytochrome c (Cyt-c) protein, and down-regulates Bcl-2 protein. Activation of caspase-3 and caspase-9 suggests that apoptosis is mediated by mitochondrial pathways. In a xenograft tumor mouse model, noscapine injection successfully inhibited tumor growth via apoptosis induction (56).

The discovery of this plant-derived compound as a highly potent anti-cancer agent makes it a potentially valuable target for extensive clinical investigations, where it could be exploited as a lead compound for designing new agents with improved bioavailability, physico-chemical and pharmaco-clinical properties, and lesser toxicity. For this purpose MTs and their major building block, tubulin heterodimers, have been the main targets. Several noscapine derivatives have been synthesized and studied *in silico* and *in vitro*. Joshi, Aneja, and colleagues, have synthesized and biologically evaluated a set of C-9 halogenated, nitrated and aminated derivatives of noscapine, among which the latter (amino noscapine) was found to be the most potent tubulin-binding agent (57,58).

Anderson *et al.* (59) synthesized N-methylated analogues of noscapine and evaluated their anti-tumor potentials. They discovered an O-benzyl analogue of noscapine that caused S-phase arrest. Its activity was further improved by removing the benzyl group and by substitution of an amine, which has provided the compound with its capability to arrest HEK293EBNA cells in the G2/M phase. Their investigations found that the phenol and aniline analogues of N-methylated noscapine show significant improvements in MT inhibition and cytotoxicity as compared to the parent compound, noscapine (59).

A clear understanding at the atomic level of modeling is required to explain noscapine's mode of action when binding to tubulin, while taking into consideration the fact that the function of the polymeric MTs as dynamic systems depends on association and dissociation of the α/β-tubulin hetero-dimer at the two MT ends (60). This mechanism depends on the activity of GTPs bounded in both β- and α-tubulin subunits (61). The GTP molecule in the exchangeable site of the β-subunit is hydrolyzable to GDP following association of β-tubulin with the MT. This event takes place after depolymerization of the MT and therefore the GTP-binding site becomes exchangeable. The GTP molecule in the α-tubulin subunit, in contrast, is unavailable for hydrolysis and therefore remains unchanged (62). GTP binding status and the hydrolysis process are the key factors causing the so-called dynamic instability of MTs. The characteristic tread-milling manner in MT length alteration (63) may be impacted or controlled by a small ligand such as noscapine.

Since the crystal structure of the complex of the noscapine-tubulin hetero-dimer has not yet been solved, several groups have conducted studies of noscapine aimed at addressing the issue with the use of *in silico* computational simulations. A possible binding site of noscapine, Br-noscapine, and amino-noscapine, has been investigated *in silico* (57,64,65), indicating that the binding site may lie at the α/β-tubulin interface near the colchicine binding site. Experimental competition and fluorescence experiments were performed (66), and the results of these experiments indicate that noscapine does not compete with colchicine. Alisaraie and Tuszynski (67) have also studied the noscapine binding site *in silico*, employing molecular docking and molecular dynamics (MD) simulations. As a result, the predicted binding site of noscapine was found at the intra-domain region of the α- and β-tubulin (Fig. 4). The halogenated and nitrated derivatives of the lead compound were also individually studied by docking into the noscapine-binding site. Based on the calculated binding scores, a substantial advantage of the nitrated and brominated compounds compared to noscapine was found, which is consistent with the corresponding experimentally measured dissociation constants (58,68). The information obtained from docking was improved upon by studying the noscapine-tubulin complex in a dynamic mode and by including water molecules.

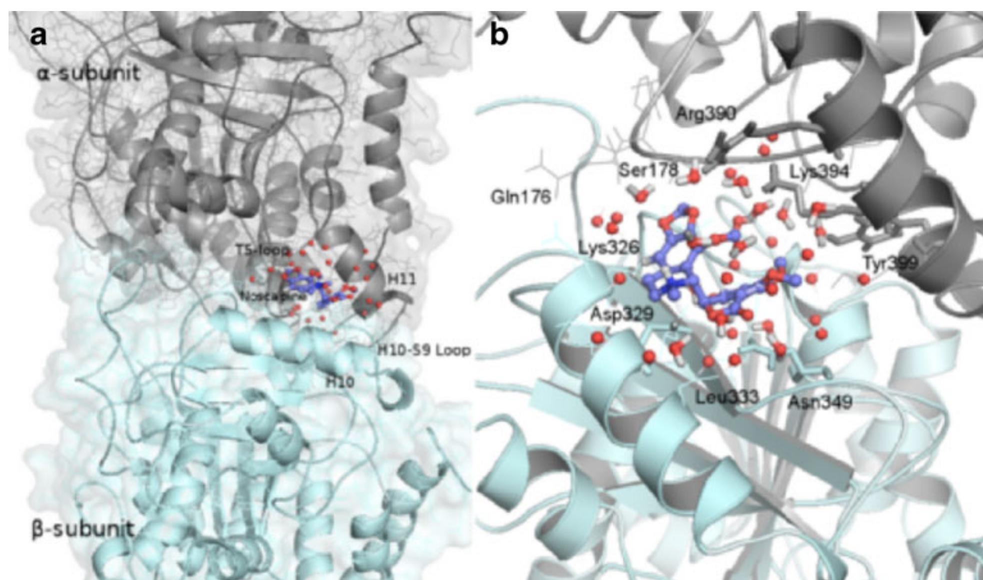

Fig. 4 A predicted binding site from Alisaraie and Tuszynski (67) of noscapine to the β-tubulin monomer. The left panel (**a**) depicts an α/β-tubulin dimer with noscapine bound to it. The right panel (**b**) shows the key resides on the α/β-tubulin dimer that contribute to binding.

The α- and β-tubulin subunits have two GTP binding sites, known as the exchangeable site (E-site), and the non-exchangeable site (N-site). Within individual MD experiments GTP molecules were added to this complex system in the N- and E-sites. It was observed that in the α-tubulin subunit the water molecules surrounding the binding site accompany noscapine and shift it toward the central region of the intra-domain interface via pulling forces of the hydrogen bond network, which are mediated by surrounding water molecules between noscapine and its neighboring amino acids. This event and the dynamic nature of the surrounding environment of noscapine were found to force it toward GTP in the N-site, since the distance between the centers of mass of GTP and noscapine decreased during 20 ns of the MD simulation.

Distance variations between the centers of mass of GTP and the important elements of the N-site, in both liganded and unliganded tubulin revealed that structural elements of tubulin in these regions function differently. Namely, they cause a partially closed conformation of the N-site and therefore provide a tighter packing with the monomer of the neighboring protofilament upon noscapine binding. On the other hand, a shift in some other elements of the N-site away from GTP causes a partially open conformation for the site, forcing GTP to adopt a new binding pose in order to maintain its stable status in the N-site. Upon binding of noscapine, an increased stability of the tubulin elements at the E-site components, and a reduction of dynamical motions of parts of tubulin located alongside the protofilament, were observed. Since these elements interfere with the longitudinal interactions in MTs, a positive effect on MT polymerization upon noscapine binding could be expected.

The identification of the key interacting moieties of noscapine and implementing its major interacting parts as the seed scaffold led to the design of novel analogues of noscapine with predicted binding energies stronger than that of noscapine and its known halogenated/nitrated derivatives with improved physico-chemical properties (67). The designed molecules were introduced as a new generation of noscapine-based compounds and proposed for future investigations involving preclinical development aimed at finding potent and well-tolerated anti-cancer drugs as the ultimate goal of research in this area.

CONCLUSIONS

In this article we have reviewed the recent findings regarding the binding sites, binding modes and binding affinities of three novel antimitotic drugs peloruside, laulimalide and noscapine with respect to tubulin as the target of their action. These natural compounds from both marine sponges and plants, respectively, are shown to bind to β-tubulin and stabilize MTs for the cases of peloruside A (22) and laulimalide (2), and prolong the time spent in pause phase without actually stabilizing MTs for noscapine (64,69). We have discussed their status in pre-clinical development and clinical trials together with the advantages they offer and challenges they pose. Particular attention has been focused on tubulin isotypes as targets for new cancer chemotherapy agents and the amino acid differences in the binding site for these compounds between different tubulin isotypes. We have also discussed the efforts made in creating derivatives and analogues of these parent compounds aimed at improving the pharmacological profiles of the resultant agents. In this connection we have proposed a new strategy for antimitotic drug design that exploits differential distributions of tubulin isotypes between normal and cancer cells and the corresponding

differential affinities between various drug molecules and tubulin isotypes.

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