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Design, synthesis and biological evaluation of di-substituted noscapine analogs as potent and microtubule-targeted anticancer agents

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ABSTRACT

Noscapine is an opium-derived kinder-gentler microtubule-modulating drug, currently in Phase I/II clinical trials for cancer chemotherapy. Here, we report the synthesis of four more potent di-substituted brominated derivatives of noscapine, 9-Br-7-OH-NOS (**2**), 9-Br-7-OCONHEt-NOS (**3**), 9-Br-7-OCONHBn-NOS (**4**), and 9-Br-7-OAc-NOS (**5**) and their chemotherapeutic efficacy on PC-3 and MDA-MB-231 cells. The four derivatives were observed to have higher tubulin binding activity than noscapine and significantly affect tubulin polymerization. The equilibrium dissociation constant (K_D) for the interaction between tubulin and **2**, **3**, **4**, **5** was found to be, 55 ± 6 μM, 44 ± 6 μM, 26 ± 3 μM, and 21 ± 1 μM respectively, which is comparable to parent analog. The effects of these di-substituted noscapine analogs on cell cycle parameters indicate that the cells enter a quiescent phase without undergoing further cell division. The varying biological activity of these analogs and bulk of substituent at position-7 of the benzofuranone ring system of the parent molecule was rationalized utilizing predictive in silico molecular modeling. Furthermore, the immunoblot analysis of protein lysates from cells treated with **4** and **5**, revealed the induction of apoptosis and down-regulation of survivin levels. This result was further supported by the enhanced activity of caspase-3/7 enzymes in treated samples compared to the controls. Hence, these compounds showed a great potential for studying microtubule-mediated processes and as chemotherapeutic agents for the management of human cancers.

Microtubules, formed by α and β-tubulin heterodimers, are essential constituents of the cytoskeleton in eukaryotic cells and are involved in a number of important structural and regulatory functions, including the maintenance of cell shape and intracellular transport machinery as well as cell growth and mitosis.[1–3] Over the past few decades, microtubule-active drugs have met with abundant success in the oncology clinic for a wide-spectrum of malignancies.[4,5] Nonetheless, several impediments associated with their clinical use, such as non-specific toxicity, drug resistance, and water insolubility, have resulted in a sub-optimal realization of their clinical potential.[6,7] Microtubules, which are involved in a complex process of cell division, are now a well-established target for the chemotherapy of many types of cancers.[8,9] Two major classes of tubulin-interacting drugs either over-polymerize or de-polymerize the involved subunits leading to devastation of cytoskeleton in later stages of cell division. For example, drugs such as Taxol perturb the normal microtubule assembly dynamics

Abbreviations: FBS, fetal bovine serum; ATCC, American type cell culture; MTT, tetrazolium bromide solution; DNA, deoxyribonucleic acid; DMSO, dimethylsulfoxide; IC_{50}, inhibitory concentration 50%; Nos, noscapine; EM011, bromonoscapine; PBS, phosphate buffered saline; K_D, dissociation constant; FACS, fluorescence-activated cell sorting; PARP, poly ADP ribose polymerase.

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by 'hyperpolymerizing' the microtubules[4] whereas compounds like maytansinoids inhibit microtubule assembly and interfere with the polymerization dynamics.[10] In addition to above, a variety of synthetic small molecules have also been used as inhibitors of polymerization, which compete with colchicine binding site of tubulin. Few Colchicine like compounds are in clinical trials, for example, Combretastatin A-4P (CA4P),[11] ZD6216,[12] *N*-(3-hydroxy-4-methoxyphenyl)-3,4,5-trimethoxy benzenesulfonamide,[13] and 1-methyl-1H-indole-5-sulfonic acid (3,4,5-trimethoxyphenyl)amide.[14] A list of various microtubule polymerizing and de-polymerizing agents that are currently in use and in clinical development are presented in Table 1. Another important microtubule polymerization inhibitor, noscapine, was studied extensively for its tubulin binding anticancer properties in the last decade.[15,16] It has been known for some time that noscapine can act as a potent anticancer agent in certain in vivo models.[17] Although it appears to be a weak inhibitor of microtubule polymerization, its low cost, ready availability and a favorable toxicity profile allow further exploration of this natural product.

Currently, noscapine is in phase I/II of clinical trials for the treatment of non-Hodgkin's lymphoma and the clinical trials employing it for the treatment of multiple myeloma have recently been completed (www.clinicaltrials.gov). In our previous report we discovered noscapine as a structural variant of colchicine like toxin.[18] Our group has been involved in the synthesis and anticancer property evaluation of many of the noscapinoids. These include the derivatization of the isoquinoline ring system of the molecule to prepare chloro, bromo, fluoro and iodo analogs. Also, more noscapinoids were prepared using nitration reaction on aromatic ring of the isoquinoline ring system and also by complete reduction of lactone moiety in the benzofuranone ring system followed by halogenation reactions. Ever since, several groups including ours, have been actively engaged in the synthesis of in silico guided, more potent noscapine analogs with potentially better pharmacological profiles.[8,19,20] Recently, we reported the synthesis of second-generation 7-position benzofuranone noscapine analogs that offered better anti-proliferative activity than the founding molecule.[21] Anderson et al. synthesized novel noscapine analogs, which have shown potential as S-phase arresting anti-mitotic agents.[22] Their approach involved exploitation of the methyl ether cleavage reaction on the benzofuranone ring system using Grignard reagent.

Here, we describe the chemical synthesis of a third generation di-substituted bromo analogs of noscapine, which were evaluated for their tubulin polymerization and antiproliferative properties. In silico molecular modeling data were employed to rationalize and comprehend their observed biological activity patterns. They were examined for their anti-proliferative activity against prostate and breast cancer cells. This has contributed to an enhanced understanding of structure-based drug design to facilitate drug discovery and development of a novel class of tubulin-active, non-toxic agents.

In silico docking and molecular simulation studies: Molecular simulations and in silico docking studies were performed to gain insights into how the compounds **2**, **3**, **4** and **5** docked into the active site independently at the interface of the alpha and beta domains of the tubulin dimer (Fig. 1A–D). The binding orientations were held similar for all of the ligand–tubulin complexes. The docking results suggest that noscapine structure can be divided into two distinct halves (a) the tetrahydroisoquinoline moiety which can insert deep and interact closely with the tubulin dimer

Table 1
Microtubule targeting cancer therapeutics

S/N	Tubulin antagonistic activity	Drug/drug candidate	Clinical development status
1	Polymerization	Vinblastine	In clinical use
2	antagonists	Vincristine	In clinical use
3		Vinorelbine	In clinical use
4		Vinflunine	Phase III
5		Cryptophycin 52	Phase III done
6		Halichondrins	Phase I
7		Dolastatins	Phase I and II done
8		Hemiasterlins	Phase I
9		Colchicine	Failed trials (toxicity)
10		Combretastatins	Phase I
11		2-Methoxy-estradiol	Phase I
12		E7010	Phase I and II
13	De-polymerization	Paclitaxel (taxol)	In clinical use
14	antagonists	Docetaxel (taxotere)	Phase I, II and III
15		Epothilon	Phase I, II and III
16		Discodermolide	Phase I
17		Griseofulvin	Phase I

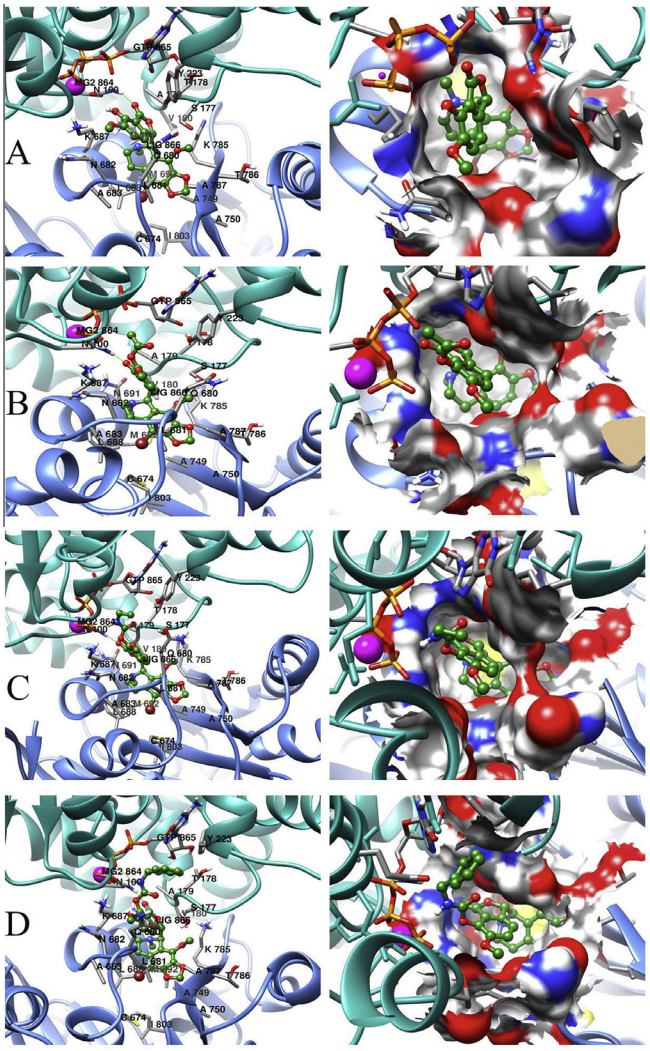

Figure 1. Molecular docking analysis of di-substituted noscapine analogs. Conformations of compounds in tubulin active site, hydrogen bond interactions (left) and compound orientation in binding pocket (right) for compounds **2**, **3**, **4** and **5** (**A**, **B**, **C**, and **D**), respectively. Compounds are shown in ball and stick model with green color.