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Article

Combination Regimen of Amino-Noscapine and Docetaxel for Evaluation of Anticancer Activity

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Abstract: Docetaxel (DOX) is one of the clinically used chemotherapeutic agents for the treatment of breast cancer, instead of its severe side effects. Noscapine, an antitussive opium alkaloid, on the other hand, has shown antitumor activity against a number of cancers without any significant side effects. Since both the compounds targeted tubulin, it is interesting to see the combined effect of both the compounds for the management of breast cancer. The binding energy of -3.49 and -4.18 kcal/mol respectively was noted by the molecular docking of amino-noscapine and DOX on the microtubule. In contrast, the binding energy was improved significantly (-6.27 kcal/mol) when the DOX was docked to the co-complex of aminonoscapine and tubulin, indicating the combined effect of both the ligands. The cell-killing potential represented in terms of IC_{50} value was 38.07 μM and 28.4 μM for a treatment duration of 48 h and 72 h for amino-noscapine. Parenthetically the IC_{50} value was 0.61 μM and 0.08 μM for DOX respectively for the treatment duration of 48 h and 72 h. The cytotoxic effect of DOX was reduced significantly (to 0.05 μM) in combined treatment with amino-noscapine (20 μM). Apropos to the cytotoxic effect, both the compounds induced apoptosis to cancer cell by interfering with the cell cycle progression. Again, the induction of apoptosis was more effective in the combined treatment of both the compounds. This investigation offers a promising concept for the combination of DOX and amino-noscapine treatment to improve effectiveness in the treatment of breast cancer.

Keywords: Anti-tumor activity, Amino-Noscapine, MCF-7, Molecular Docking, Tubulin binding affinity, Alkaloids, Cancer therapy.

Introduction

Microtubule-interfering drugs like taxol derivatives and vinca alkaloids are effective chemotherapy agents for a variety of human cancers. However, these drugs are plagued by severe toxicity to patients [1-6]. More importantly, the patients have shown resistance to taxol derivatives. The success of taxol in the management of aggressive breast and ovarian cancers is a chance to identify other compounds that target microtubules but are less toxic, soluble in aqueous solutions and substantially effective both in single as well as in combination with currently available drugs such as docetaxel (low doses). Noscapine, a safe anti-tussive agent,

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is an excellent choice of treatment to use, which is not detrimental to healthy cells [7-8]. Joshi *et al.* have recently conducted multiple experiments to determine the mode of action of noscapine. [7-10].

It is a novel selective tubulin-binding anticancer drug that does not alter tubulin's organization (monomer/polymer ratio) across a wide range of concentrations [7,12]. This is a unique advantage over currently available antimicrotubule drugs that either inhibit the disassembly of the microtubule (taxanes, epothilone) or inhibit the assembly of tubulin (vincas, eribulin, estramustine) and therefore do not trigger any hemo and neuronal toxicity. In addition, the derivatives of noscapine inhibit cell proliferation and cause G2/M arrest in different human cancer cells followed by apoptotic cell death [13-19]. We have demonstrated that these compounds bind with a better binding affinity to tubulin. While several synthesized derivatives of noscapine showed promising *in vitro* activity against tumor cell lines, it would be unable to achieve complete elimination of the disease despite increased dosages. Over the course of this decade, it has become well understood that more toxic drugs are not necessarily better at their maximum tolerated dose (MTD) and that there is an opportunity to reduce their dose levels through the use of combination drug regimens that demonstrate synergistic interactions [20].

Different groups of compounds that include a ring structure and a nitrogen atom are alkaloids. The chemical structure of these alkaloids and their biosynthetic precursors is of significant concern. Due to their various significant biological activity and medicinal applications, alkaloids have been thoroughly researched. As an example, ephedrine was tested for antiasthmatic activity, morphine was tested for analgesic action and vinblastine was tested for its anticancer activity [21-23]. The widely used insole alkaloids include serotonin and other associated compounds. It is anticipated that about 2000 compounds are being classified as indole alkaloids. However, alkaloids are the most bioactive compounds in natural herbs. Some alkaloids were already used as chemotherapeutic agents viz. camptothecin (CPT), a common inhibitor of topoisomerase I (Topo I) [24] and vinblastine, a tubulin-binding agent. Vinblastine, strychnine, ajmaline, vincamine, vincristine and ajmalicine are among the most researched participants because of their pharmacological activities [25].

The existence of several drug binding sites on tubulin suggests that the rational combination of two or more drugs may increase the effectiveness of anticancer activity and reduce toxic side effects. The combination of docetaxel and other agents has earlier shown to increase the anticancer efficacy with lung cancer [26-30] and breast cancer [31]. In the current study, we approach to evaluate the anticancer activity with combination regimens of amino-noscapine and Docetaxel for the management of breast cancer.

Material and methods

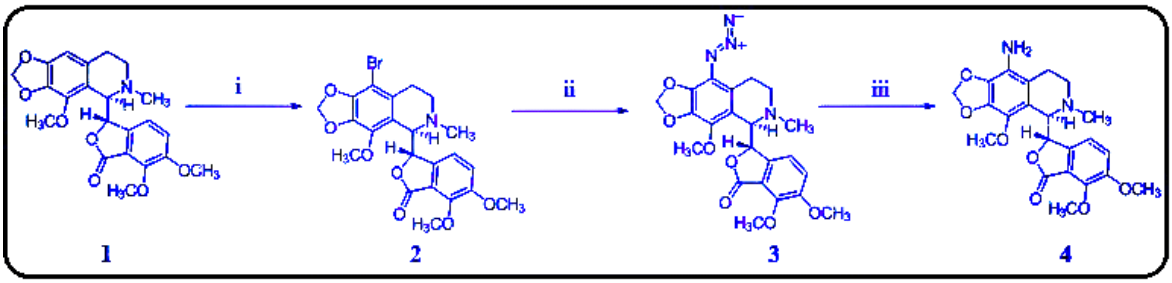
Chemistry

The amino-noscapine was synthesized from the lead molecule, noscapine as per the method described earlier [32,33]. First of all, bromo-noscapine was synthesized from the noscapine by adding hydrobromic acid (~40 ml) and bromine water (~250 ml). The bromo-noscapine was converted to azido-noscapine in presence of sodium azide (40.63 mmol) and sodium iodide (4.063 mmol). Finally, amino-noscapine was synthesized from azido-noscapine by adding a solution of $SnCl_2$ in THF (10 ml), thiophenol and triphenylamine. The detailed of the synthetic scheme was mentioned below. The amino-noscapine was structurally elucidated based on ^1H NMR (300 MHz), ^{13}C NMR (75 MHz), HRMS, etc. as reported previously [34] and the final product as supporting information.

Molecular modelling

Protein preparation

The crystal structure of tubulin-amino noscapine (PDB ID: 6Y6D, resolution 2.20 Å) [35] was used for molecular docking of amino-noscapine and DOX. To prepare the protein structure, the protein preparation wizard (Schrödinger) was used and Macromodel (Schrödinger) was used for energy minimization [36]. For energy minimization, the OPLS 2005 force field with the Polak-Ribiere Conjugate Gradient (PRCG) algorithm with 1000 steps and an energy gradient of 0.001 kcal/mol was used.



Scheme 1. Synthesis of 9-amino-noscapine. (i) HBr/Br_2-H_2O at 25°C/h, (ii) NaN_3/NaI in DMF at 80°C/15h, (iii) $SnCl_2$/PhSH/Et_3N in THF at 25°C/2h

Ligand preparation

The amino-noscapine and DOX molecular framework were developed using the maestro molecular builder (version 17.4, Schrodinger). Using Macromodel (version 17.4, Schrodinger) and OPLS 2005 force field, the constructed structures were energy minimized. A PRCG algorithm with 1000 steps and an energy gradient of 0.001 was used for energy minimization. Using Ligprep, a suitable bond order was allocated to each ligand. In addition, the ligands were DFT optimized using Jaguar (version 17.4, Schrödinger, LLC) applying Becke's three-parameter exchange potential and the Lee-Yang-Parr correlation functional (B3LYP) [37-38] with base set 3-21G* [39-41].

Molecular docking

Using Glide-XP (Extra Precision) (Schrödinger) [42], both amino-noscapine and DOX were docked to ±²-tubulin heterodimer at their respective binding cavities. The recorded noscapinoid [35] and DOX binding pockets [13] were established using a concentrated grid box at the center of the binding site using the Glide grid-receptor generation software. In order to contain the center mass of the docked ligand, a bounding box of size 14Å x 14Å x 14Å was specified. Also, specified larger enclosing box of size 20Å x 20Å x 20Å that occupied all the docked pose atoms. Using Glide-XP on the respective binding sites, both Amino-noscapine and DOX were docked. The DOX was docked onto the amino-noscapine and tubulin complex and the Amino-noscapine was docked onto the DOX and tubulin complex. The binding of ligands was evaluated using a parameter of the Glide XP score. For the ligand docking method, the scale factor of 0.4 for van der Waals radii was implemented to protein atoms with exact partial charges less than or equal to 0.25. Out of the 10000 poses sampled, 1000 were extracted by minimization (conjugate gradients) and favorable Glide docking performance was further evaluated by 30 structures with the lowest energy conformation.

Biology

Cell culture and reagents

Noscapine and docetaxel were obtained from Sigma. All the chemical reagents and media used for cell culture were obtained from Mediatech, Cellgro. The human breast cancer cell line, MCF7 was obtained from the cell repository of the National Center for Cell Science Pune, Maharashtra, India. The cells were grown in a 5% CO_2 and 95% humidity in Dulbecco's modified Eagle medium (DMEM, Pan Biotech) at a favorable temperature of 37°C, supplemented with 10 % fetal bovine serum (FBS) and antibiotics. For bioassays using trypsin-EDTA (0.25%), having a confluence with 70-80 % cells were subcultured.

In vitro cell proliferation MTT assay

The human breast cancer cell lines MCF-7 (3×10^3 cells per well) were seeded into 96-well plates. After incubation for 24 h (when cells reached 70-80% confluency), the medium was aspirated and the cells were treated with several concentrations of amino-noscapine alone (10, 25, 50, 100 µM), DOX alone (0.001, 0.01, 0.1,1,10 µM) and in combination of amino-noscapine and DOX (10 µM amino-noscapine+0.001 µM DOX, 15 µM amino-noscapine +0.01 µM DOX, 20 µM amino-noscapine +0.05 µM DOX, 25 µM amino-noscapine +0.1 µM DOX, 30 µM amino-noscapine +0.5 µM DOX). After 24h incubation,

10 µL of MTT (1 mg/mL) solution was added and the plates were incubated for an additional 4 h at 37°C and the absorbance was measured in a plate reader (Varioskan, Thermo Scientific) at 570 nm. The value of IC_{50} (the concentration of the drugs required to prevent cell proliferation by 50 %) of amino-noscapine alone, DOX alone and in the combination regimen of amino-noscapine + DOX was determined. The experiments were repeated in triplicates.

Cell cycle analysis

In Dulbecco's Modification of Eagle's Medium (DMEM), MCF7 cells were cultivated with 4.5 g/l glucose and L-glutamine, supplemented by 10 % bovine fetal serum and 1 % penicillin/ streptomycin. The MCF7 cells (1 X 10^5) were seeded with 35 mm plates. Cells were treated with amino-noscapine alone (25 µm), DOX alone (0.5 µm) or in combination with amino-noscapine and DOX (25 µm amino-noscapine+0.05 µm DOX) at 37°C temperatures and 5 % CO_2 after 24 hours. Cells were sampled after 24 h of treatment, followed by analysis using flow cytometry. The cells were briefly centrifuged, two times washed with ice-cold phosphate-buffered saline (PBS) and fixed with 70 % ethanol. The tubes containing the cell pellets have been deposited for 24 h at -20°C. Thereafter, the cells were centrifuged at 1000 x g for 10 minutes, and the supernatant was removed. The pellet was resuspended in 30 µl of phosphate/ citrate buffer (0.2 M Na_2HPO_4/0.1 M citric acid, pH 7.5) at room temperature for 30 min. Cells were then washed with 5 ml of PBS and incubated with 0.5 ml of propidium iodide (5 µg/ml in 0.1 % Triton-X in PBS) and 5 µg/ml of RNase A for 45 minutes in dark.

Apoptosis assay

Choline phospholipids such as phosphatidylcholine and sphingomyelin (PS) are exposed to the external leaflet during apoptosis, whereas amino-phospholipids (phosphatidylserine, phosphatidylethanolamine) are positioned exclusively on the lipid bilayer's cytoplasmic surface. The identification of PS by the fluorochrome-tagged 36 KDa anticoagulant protein Annexin V permits apoptotic incidence to be reliably calculated. Only in the presence of mM concentrations of divalent calcium ions, this probe reversibly binds to phosphatidylserine residues. Apoptosis in cancer cells has been identified by Annexin-V-FITC detection kit (Sigma-Aldrich, USA) based on the instruction provided by the manufacture.

In short, $5x10^4$ cells per well were seeded on a 35 mm plate and incubated for 24 h with a complete medium. After 24 hours, cells were treated with amino-noscapine alone (25 µm), DOX alone (0.5 µm) or in the combination regimen of amino-noscapine and DOX (25 µm amino-noscapine+0.5 µm DOX) at a temperature of 37°C and 5% CO_2. Cells were trypsinized and stained with surface marker antibodies (biotin-conjugated Annexin V, FITCconjugated streptavidin) and propiumdium iodide (PI). Cells were allowed to suspend in 1X binding buffer and incubated with Annexin-V-FITC conjugate for 20 minutes in dark conditions at room temperature. Flow cytometer data with 488 nm excitation for PI and emission at 530 nm were collected. Viable cells (Annexin V- / PI-), early apoptotic cells (Annexin V+ / PI-), late apoptotic/necrotic cells (Annexin V+ / PI+) and late necrotic cells (Annexin V- / PI+) were identified and determined their percentage, assessed using BD FACS Calibur (San Jose, CA, USA).

Tubulin purification

Microtubules were isolated and purified from the goat brain through alternative cycles of GTP-dependent polymerization and depolymerization in PEM buffer (50 mM pipes, 3 mM MgSO4, 1 mM EGTA, pH 6.8) [43,44]. The purified microtubules were preserved at -80°C. The purified tubulin was estimated using the Bradford method as well as by SDS PAGE [45].

Tryptophan quenching assay

Tubulin (2 µM) was incubated in a water bath with amino-noscapine at a concentration of (20 µM), DOX (0.5 µM) and in combination regimen (DOX 0.05 µM+ amino-noscapine 25 µM) in PEM buffer (50 mM pipes, 3 mM MgSO4, 1 mM EGTA, PH 6.8) for 45 minutes at 35°C. The samples were excited at 295 nm and emission was measured at 310-400 nm. For the spectrofluoro-

metric titrations, a FlouroMax® 4 spectrofluorometer (Horiba Scientifc, Edison, NJ) assisted by Fluor Essence 3.5 software was used. The experiments were repeated twice.

ANS (8-Anilino-1-naphthalene sulfonic acid)-binding assay

ANS binding assay was performed to verify the structural integrity of the tubulin in presence of amino-noscapine and DOX in a single as well as in combination regimen. Tubulin (2 µM) was incubated with two concentrations of amino-noscapine (25 µM and 50 µM), DOX (0.5 µM) and in combination regimen (DOX 0.05 µM+ amino-noscapine 25 µM, DOX 0.05 µM+ amino-noscapine 50 µM) at 35ºC for 30 min in PEM buffer. ANS (50 µM) was added and the samples were incubated in dark at 25ºC for 15 minutes. The samples were excited at 350 nm and the emission was measured at 410-470 nm using a Flourolog 3 spectrofluorometer (Horiba Scientific, Edition, NJ) assisted by fluorescence 3.5 software. The assays were repeated two times.

Results and discussion

Molecular modelling

It was reported that both noscapinoid and docetaxel bind to tubulin at a different binding site. Noscapinoids bind at the α- and β-tubulin interfaces [46, 47], while docetaxel binding was biased towards β-tubulin [48] (**Figure 1**). The difference in the mode of interactions of amino-noscapine and DOX with the residues of tubulin was also investigated in protein-ligand interaction profiler (PLIP), which is an automated detection and visualization tool of non-covalent protein-ligand interaction patterns from 3D structures. Results for each binding site were provided as 3D interaction diagrams for manual inspection (with PyMOL). The differences in amino acids for binding of amino-noscapine with tubulin independently and in combination with DOX (**Figure 2**) were mainly because of the change in conformation of tubulin due to binding of DOX. This clearly explains the changes in the binding mode of amino-noscapine and DOX when both the ligands were docked together with the tubulin

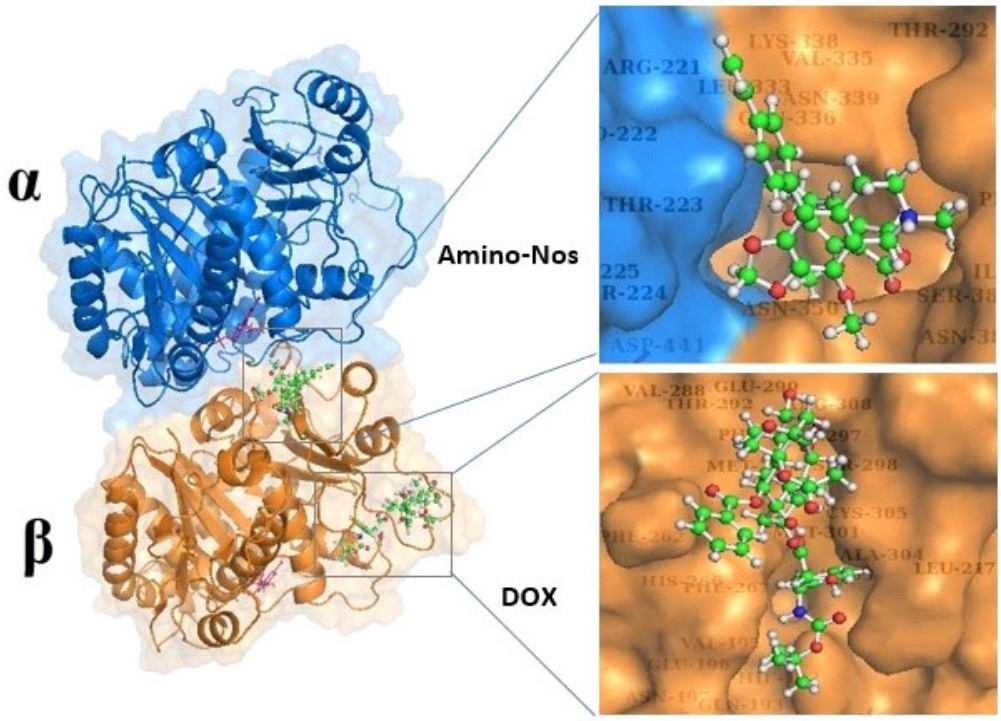


Figure 1. Both amino-noscapine and Docetaxel are well accomodated inside their respective tubulin binding site. (b) Snapshot of both the ligands obtained. The binding site is represented as macromodel surface according to α- and β-tubulin (α-tubulin is represented in green colour and β-tubulin is represented in brown colour)

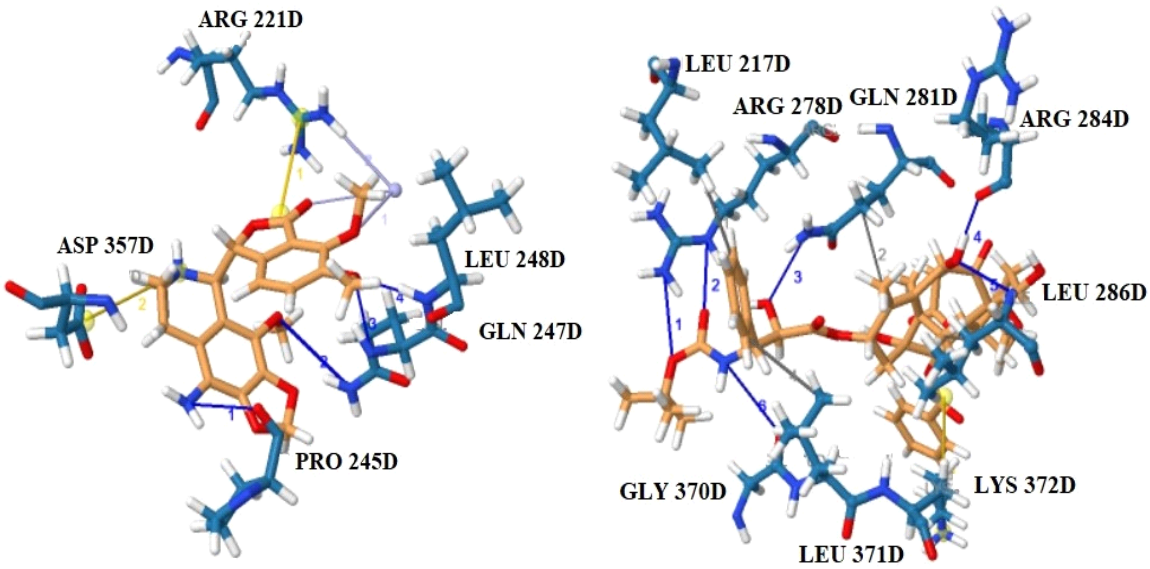


Figure 2. 3D ligplot representing the mechanism in which (a) Docetaxel and (b) Amino-noscapine interact with the binding sites of the amino acid. The protein-ligand interaction profiler (PLIP) was used to analyze the docked complex of DOX and Amino-noscapine with tubulin. In the figure, the hydrogen bonds involved in the binding of ligands are represented

Table 1. Molecular docking results (Glide XP_{score}) and the relevant energy parameters of amino-noscapine and docetaxel in single as well as in combination with tubulin

Ligands	Glide XPscore	Glide lipo	Glide Hbond	Glide Ecoul	Glide Evdw	Glide Emodel	Glide Energy
Amino-noscapine	-3.49	-1.56	-0.62	-31.16	-5.97	-42.37	-37.13
Docetaxel	-4.18	-0.99	-0.47	-28.17	-8.93	-44.44	-37.65
Amino-noscapine+Docetaxel	-6.27	-1.72	-0.01	-43.27	-6.46	-54.22	-49.97

so there is a chance of synergistic effect [48].

Towards determination of binding affinity of both the ligands in single as well as in combination, we have docked them at their respective binding pocket on tubulin in two cycles of molecular docking. It was found that both amino-noscapine and DOX docked well into their binding site with a binding score of -3.494 kcal/mol and -4.18 kcal/mol respectively (**Table 1**). The DOX-tubulin complex was taken in the second cycle and the amino-noscapine was docked onto the noscapinoid binding site. The presence of DOX on its binding site interferes with the binding of amino-noscapine with a reduced docking score of -6.27 kcal/mol. It is maybe because of the alteration of the secondary conformation of tubulin due to the binding of DOX [49].

Biology

In vitro cell proliferation MTT assay

The MTT assay was used to determine the effect of amino-noscapine and docetaxel individually and in their combination on cell viability. Amino-noscapine showed a dose-dependant cytotoxicity effect after 24 h in MCF-7 cell line. The antiproliferative activity increases with the increasing concentration of amino-noscapine and DOX in single as well as in combination. The percentage of cell survivability was reduced to ~50 % at 38.7 µM and 28.4 µM respectively at 48 h and 72 h of treatment with amino-noscapine (**Figure 3a**). In contrast, the DOX significantly revealed the dose-dependant cytotoxicity value of 0.61 µM and 0.08 µM respectively at 48 h and 72 h (**Figure 3b**). Further, the combination dose of amino-noscapine

(20 µM) and DOX (0.05 µM) revealed a reduction in ~50 % cell survival at 48 h and 72 h of treatment. The decrease in the concentration of DOX in combination with amino-noscapine in comparison to single regimen treatment revealed a chance of combined effect of both the drugs (**Figure 3c**).

Cell cycle analysis

The effect of amino-noscapine and DOX on the cell at the required concentration on the cell cycle profile of MCF-7 was evaluated by the induction of cell death using fluorescently labeled DNA deposition as a promising predictor of cell cycle

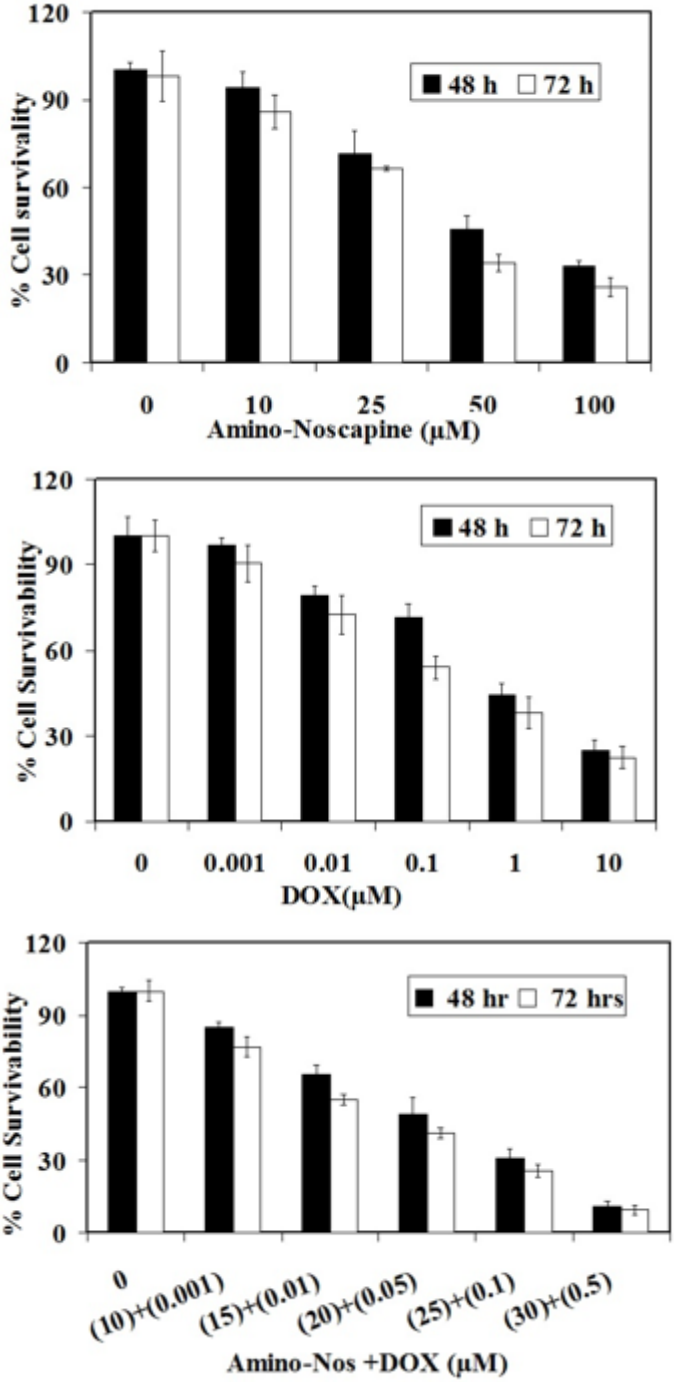


Figure 3. Reduction in percentage of cell survivability with the treatment of Amino-noscapine and Docetaxel in single as well as in combination regimen for MCF-7 cancer cell

progression and cell death. The un-replicated pattern of 2N DNA following characteristics of the G1 phase, while the duplicated 4N DNA cells describe the G2 and M phases. (**Figure 4**). During DNA duplication among peaks of 2N and 4N, the cells exhibit the S stage when DNA is synthesized. Less than 2N DNA appears in population groups of dying cells which deteriorate their DNA to varying degrees.

Treatment of MCF-7 cells with 25 µM treated test compounds for 24 h resulted in massive disruptions of the cell cycle profile. High cell aggregation during the G2/M transition phase at 24 h of treatment compared to untreated cells was observed in the FACS analysis (**Table 2**). The sub-G1 population with the treatment of amino-noscapine was increased to 9.62 %, whereas with DOX it was increased to 15.4 % and in combination, it further increased to 32.6 % in comparison to control.

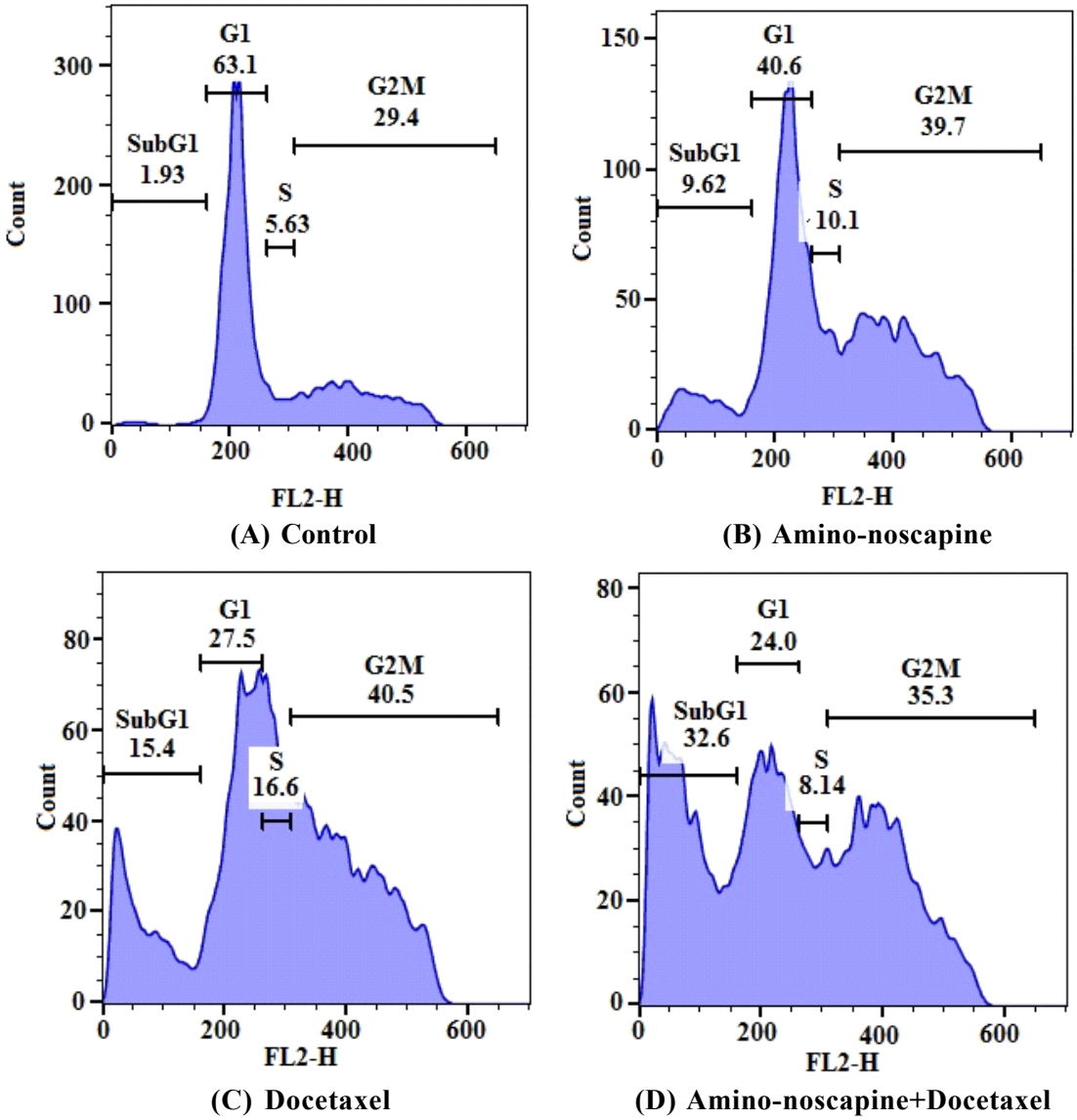


Figure 4. Amino-noscapine and docetaxel suppress the progression of the cell cycle at mitosis, indicated by characteristic hypodiploid (sub-G1) DNA peak, indicative of apoptosis. Figure A-D demonstrates the two-dimensional cell cycle distribution analysis as assessed by flow cytometry in MCF-7 cells treated with 25 µM of amino-noscapine, 0.5 µM of docetaxel single regimen, and 25 µM of amino-noscapine + 0.05 µM of docetaxel single regimen combination regimen.

Table 2. Effect of amino-noscapine (25 μM), docetaxel (0.5 μm) and their combination regimen (25 μm amino-noscapine + 0.05 μm docetaxel) on cell cycle progression of MCF-7 cells for 24 hours

	24 hr			
	Sub-G1	G_0/G_1	S	G_2/M
Control	1.93	63.1	5.63	29.4
Amino-noscapine (25 μm)	9.62	40.6	10.1	39.7
Docetaxel (0.5μm)	15.4	27.5	16.6	40.5
Amino-noscapine (25 μm) + Docetaxel (0.05 μm)	32.6	24.0	8.14	35.3

Apoptosis assay

The apoptotic phase is characterized by alterations in the lipid composition of the cell membrane, *i.e.*, phosphatidylserine, usually translocated to the outer leaflet on the inner leaflet of the cell membrane, which can be determined by using fluorescent binding Annexin V. In contrast, the cell impairment DNA-binding fluorescent dye *i.e.,* propidium iodide can only enter the cells at the stage of late apoptosis when membrane permeability is compromised. Apoptotic cells can be quantified by FACS analysis. There were very few apoptotic cells (~2 %) in the untreated cell cultures, which were assigned as the background cell death. MCF7 cells treated with amino-noscapine for 24 hours showed both early and late apoptosis (4.12 % and 2.42 %) respectively. In comparison, DOX demonstrates early and late apoptosis activation, 10.9 % and 3.80 % after 24 hours respectively. The combination treatment of DOX (0.05) and amino-noscapine (20) showed an increased rate of early and late apoptosis (15.1 % and 11.9 %) post 24 hours treatment (**Figure 5**).

Tryptophan quenching assay

The innate ability of proteins is to display the intrinsic fluorescence, which has provided a way to understand the environmental changes after interaction with the quencher. Tubulin is auto-fluorescence due to the presence of tryptophan amino acid. Tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) are the three natural amino acids that are fluorescent, but Trp does have the highest fluorescence quantum yield [50]. Thus, any alteration in its conformation with ligand binding decreases emission fluorescence - a tool used to recognize a ligand binding. The dynamic reduced fluorescence intensity in presence of increasing concentration of amino-noscapine and DOX in single as well as in combination, indicate the binding of both the compounds with tubulin. The relative percentage of decrease in fluorescence intensity was 28.87 % in presence of 20 μM amino-noscapine, 25.31 % in presence of 0.5 μM of DOX and 64 % in combination of DOX (0.05 μM) and amino-noscapine (25 μM) (**Figure 6**).

ANS-binding assay (8-anilino-1-naphthalene-sulfonic acid)

The impact of amino-noscapine (25 and 50 μM) and DOX on tubulin conformation changes using ANS binding assay, a fluorescent probe that binds to the protein's hydrophobic patches. Treatment of tubulin with amino-noscapine (25 and 50 μM) showed an increase in tubulin-ANS fluorescence intensity in a way dependent on the concentration (**Figure 7**). It showed 45 % and 62.4 % increase in fluorescence intensity at 25 and 50 μM of amino-noscapine, whereas 55.39 % in presence of DOX (0.5 μM) in comparison to unbound tubulin. Similarly, the tubulin-ANS fluorescence intensity was increased to 79 % in combination treatment of DOX (0.5 μM) and amino-noscapine (25 μM) as well as 88 % in combination of amino-noscapine (50 μM) and DOX (0.5 μM). The relative increase in tubulin-ANS fluorescence intensity in combi-nation treatment of amino-noscapine and DOX compared to a single regimen indicate the synergistic effect in the binding of both the compounds onto their respective binding sites.

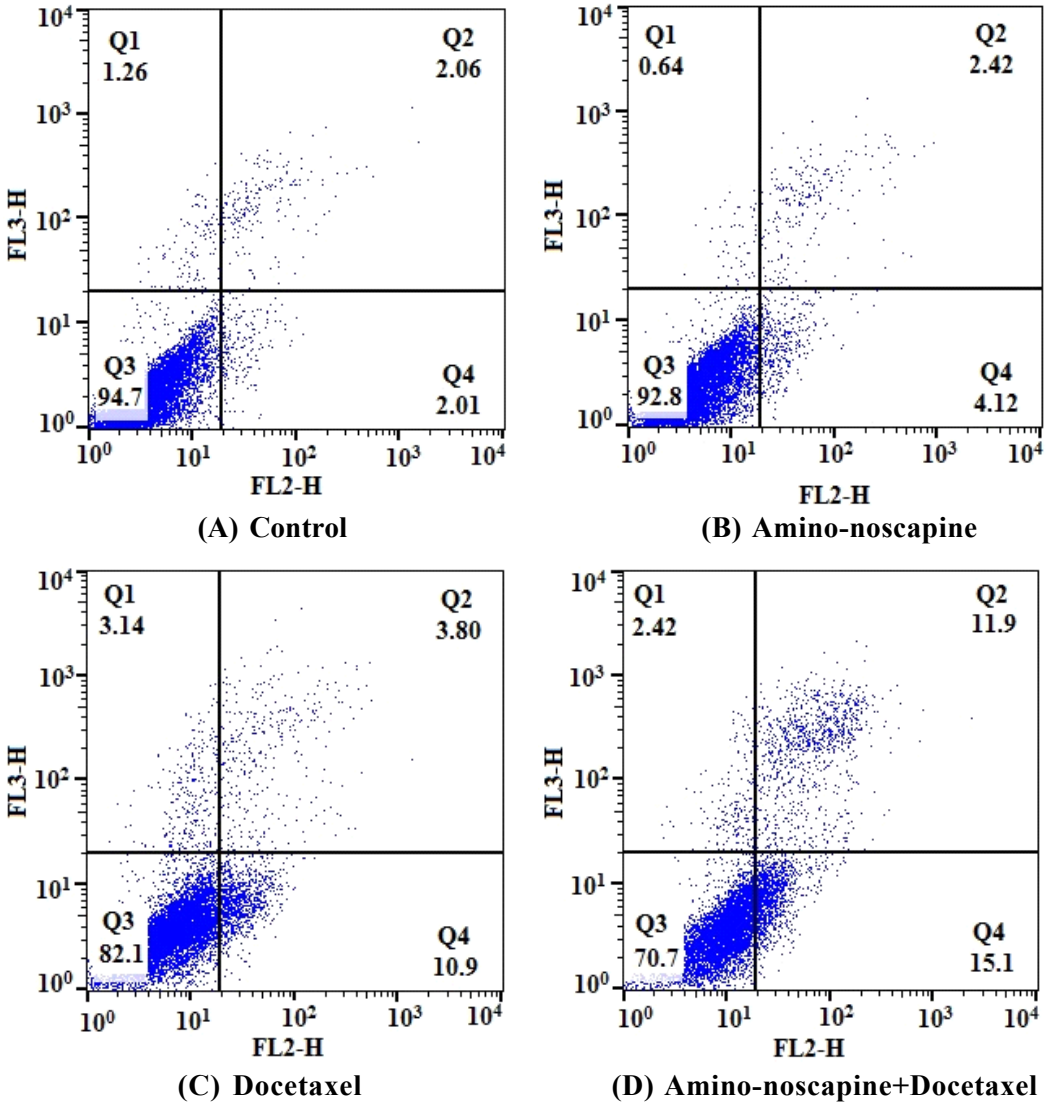


Figure 5. Analysis of apoptosis cell death induced by Amino-noscapine alone and in combination with Docetaxel based on flow cytometric analysis. In combination with 7-Amino-Actinomycin (7-AAD), Annexin V PE conjugate was used to distinguish the difference between 3 subpopulations: PE-and 7-AAD-the population indicates viable cells (lower left quadrant); PE-and 7-AAD+the population indicates early apoptotic cells (lower right quadrant); PE+ and 7-AAD+the population indicates late apoptotic cells (lower right quadrant) (top right quadrant).

Conclusion

We conclude that both amino-noscapine and docetaxel bind tubulin, alter the conformation of tubulin and inhibit the cell cycle at G2/M stage leading to induction of apoptosis. Regardless of its impressive anticancer bustle, docetaxel has a comparatively squat therapeutic index and its clinical significance is limited due to acute and chronic toxicities such as dose cumulative cardio-toxicity, myelosuppression, and immuno-suppression. Consequently, the dose-dosage regimen can be decreased in combination with other chemotherapeutic agents. However, the binding affinity of amino-noscapine increased to many folds in presence of docetaxel on its binding site, indicating the combined effect of both the molecules. Further, the antiproliferative and apoptosis activity increased significantly when both amino-noscapine and docetaxel were treated in combination compared to their single regimen

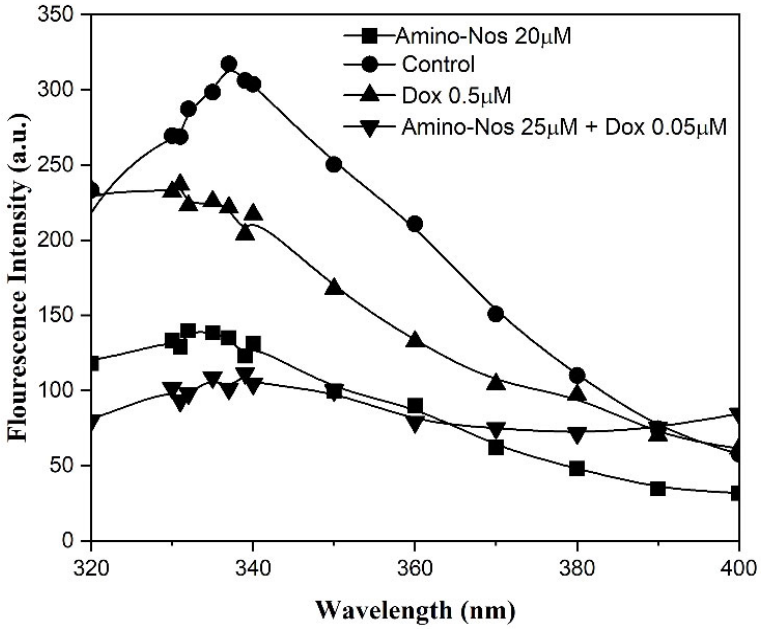


Figure 6. Interactions of amino-noscapine with tubulin alone and in combination with DOX showing concentration-dependent quenching of the intrinsic tubulin fluorescence emission intensity

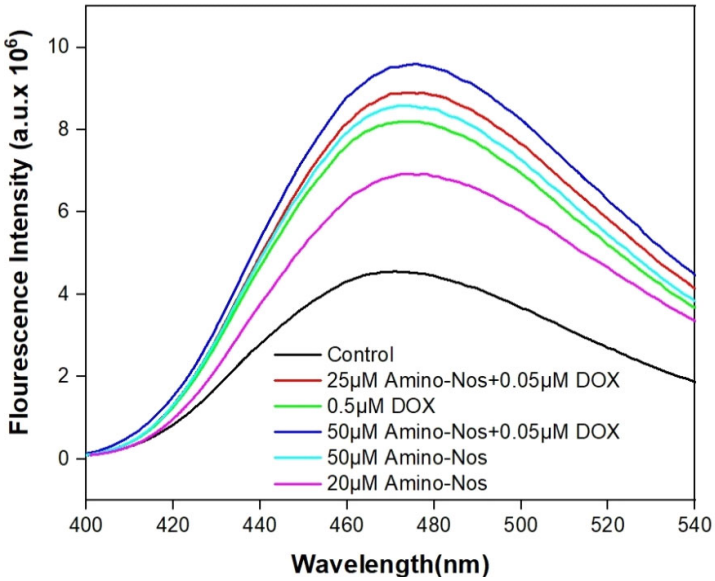


Figure 7. Interactions of the amino-noscapine with tubulin alone and in combination with DOX shown by concentration-dependent elevation of tubulin-ANS fluorescence

treatment. All these results taken together established a proof-of-concept that a rational combination of amino-noscapine and docetaxel could generate synergistic effects on cancer treatment, which is a highly promising and novel approach for the treatment of breast cancer.

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