Final Report for R&D Projects [Year 2018-2021]

Section-A : Project Details

A1. Project Title: Development of synthetic derivatives of natural compound, Noscapine as tubulin binding chemotherapeutic agent and evaluation of its synergistic effect with Taxotere for management of human breast cancer

A2. DBT Sanction Order No. & Date: BT/PR24726/NER/95/833/2017, Dated: 23.03.2018

A3. Name of Principal Investigator:

Parent Institute:	Dr. Anand Ramteke, Professor
Collaboration Institute:	Dr. Pradeep K. Naik, Professor & Head

Name of Co-PI/Co-Investigator:

Parent Institute:	Dr. Suvendra Ray, Professor & Head
Collaboration Institute:	Dr. Srinivas Kantevari, Principal Scientist Dr. Sabita Mohapatra, Professor

A4. Parent Institute: Tezpur University, Assam
Collaboration Institute: Sambalpur University, Odisha

A5. Address with Contact Nos. (Landline & Mobile) & Email:

PI, Parent Institute:	Dept. of Molecular Biology & Biotechnology, Tezpur University, Napaam – 784 028, Assam
PI, Collaboration Institute:	Dept. of Biotechnology & Bioinformatics, Sambalpur University, Jyoti Vihar – 768 019, Sambalpur, Odisha, Mobile: 94792678802, Email: pknaik1973@gmail.com
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A6. Total Cost: Rs. 70.50 Lakhs

A7. Duration: 3.0 Years

A8. Approved Objectives of the Project:

Tezpur University:

1. To evaluate and establish the *in vitro* anticancer efficacy of novel microtubule-interfering agent (design above) using breast cancer cells. Towards this end we will determine the effect of novel Noscapinoid to (i) inhibit cellular proliferation, (ii) perturb spindle architecture, (iii) binding affinity with tubulin, (iv) affect cell cycle kinetics, and (v) induce apoptosis in breast cancer cells.

2. To determine the combination dose regimen of promising Noscapinoid and Docetaxel (a clinically used taxane for metastatic breast cancer therapy) and evaluate its therapeutic outcome using *in vitro* models.

Sambalpur University:

1. To rationally design novel and potent derivative of Noscapine (called Noscapinoid) with high binding affinity onto α- and β-tubulin complex followed by chemical synthesis and structural characterization.
2. To evaluate the *in vivo* therapeutic efficacy of promising Noscapinoid as an inhibitor of localized and metastatic breast cancer using breast cancer model established in animal.
3. To evaluate the toxicity if any of promising Noscapinoid based on histopathology and hematology studies using animal model.
4. To determine the combination dose regimen of promising noscapinoid and Docetaxel (a clinically used taxane for metastatic breast cancer therapy) and evaluate its therapeutic outcome using *in vivo* model as well as toxicity analysis

A9. Specific Recommendations made by the Task Force (if any): Nil

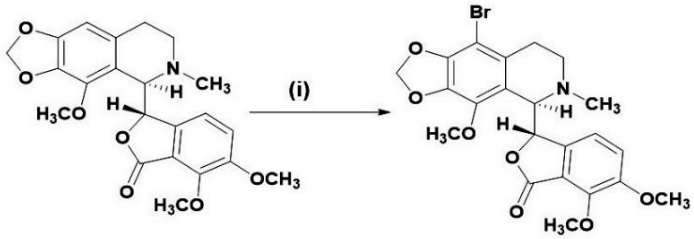
Section-B : Scientific and Technical Progress

B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period (2500-3500 words for final reports)

Design of noscapine derivatives

Several derivatives of noscapine have been developed previously, which are at a different level of preclinical and clinical trials. The availability of several derivatives along with their experimental activity was utilized to develop several new derivatives through structure-activity relationship study. All these derivatives of noscapine were found to bind tubulin with high affinity (docking score ranged from -4.41 to -8.99 kcal/mol and docking energy ranged from -37.13 to -57.61 kcal/mol). We approach to chemically synthesized a panel of novel derivatives of noscapine by derivatization at different site points of the noscapine scaffold (synthetic scheme 1-3) followed by experimental evaluation in single as well as in combination regimen with docetaxel.

Chemical synthesis of 9-Bromo-Noscapine (Br-Nos):

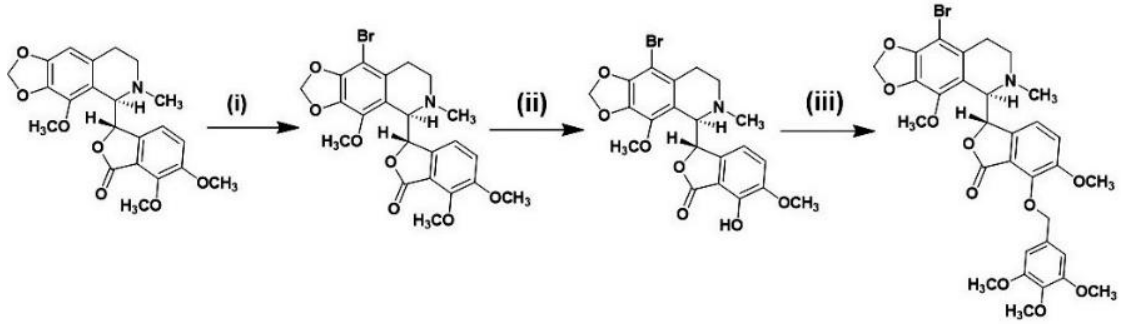


Reaction Scheme 1. Synthesis of 9-Bromo-Noscapine: Reaction Conditions. (i) 48% HBr, bromine water, room temperature, 2h, 90% yield.

mp 170 ^{o}C; $[\alpha]_D^{25}$= -106.8 (c=1, Dichloromethane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.96 (d, J= 8.30 Hz, 1H), 6.26 (d, J= 8.30 Hz, 1H), 6.02 (s, 2H), 5.39 (d, J= 4.72 Hz, 1H), 4.27 (d, J= 4.72 Hz, 1H), 4.07 (s, 3H), 3.99 (s, 3H), 3.87 (s, 3H), 2.83-2.74 (m, 1H), 2.67-2.57 (m, 1H), 2.51 (s, 3H), 2.49-2.42 (m, 1H), 2.02-1.91 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.9, 152.2, 147.6,

146.4, 141.1, 139.9, 134.1, 130.2, 119.5, 118.9, 118.2, 117.4, 101.0, 95.5, 81.2, 62.2, 60.8, 59.3, 56.7, 48.3, 45.1, 25.8. MS (ESI) m/z 492 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{22}H_{22}NO_7Br$ $[M+H]^+$:492.0657, found: 492.0636.

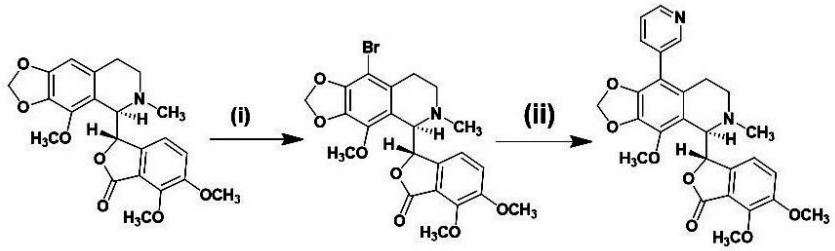
Chemical synthesis of Bromo-Trimethoxy Benzyl-Noscapine (Br-TMB-Nos):



Reaction Scheme 5. Reaction Conditions: (i) Br_2-H_2O in 48% Aqueous HBr, 2 h, RT, 90% (ii) NaN_3/NaI, DMF, 4 h, 135–140 ^oC, 75% (iii) 3,4,5 Trimethoxybenzyl bromide/NaH/ TBAI, Toluene: NMP (1:1), 70 ^oC, 2 h, 82% yield.

Mp 76 °C; [α] D25 = -63.7 (c=1, $CHCl_3$). IR, (KBr): 2941, 2839, 1760, 1593, 1499, 1445, 1373, 1124, 1035, 1008, 936, 820, 727, 633, 580, 525, cm 1. ^1H NMR (500MHz, $CDCl_3$): δ 7.01 (d, J=8.24 Hz, 1H), 6.89 (d, J= 6.86 Hz, 1H), 6.87 (s, 2H), 6.24 (d, J= 8.08 Hz, 1H), 6.03 (d, J= 1.3 Hz, 1H), 6.01 (d, J= 1.3 Hz, 1H), 5.52 (d, J= 4.27 Hz, 1H), 5.32 (q, J= 11.29 Hz, 2H), 4.34 (d, J= 4.27 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.87 (s, 6H), 3.82 (s, 3H), 2.61-2.51 (m, 2H), 2.49 (s 3H), 2.42-2.34 (m, 1H), 1.90-1.81 (m, 1H). ^{13}C NMR (125MHz, $CDCl_3$): δ 168.1, 152.9, 152.5, 146.4, 145.8, 141.0, 139.7, 137.4, 134.0, 132.6, 130.2, 120.1, 118.6, 118.2, 117.6, 105.2, 101.0, 95.5, 81.0, 75.6, 60.7, 60.6, 59.2, 56.7, 56.0, 48.0, 44.9, 25.6. MS (ESI) m/z: 659 $[M+H]^+$. HRMS (ESI) Calcd for $C_{31}H_{33}BrNO_{10}$ $[M+H]^+$: 659.21364, found: 659.21084.

Chemical synthesis of 9-3-Pyridyl-Noscapine (Py-Nos):



Reaction Scheme 6: Synthesis of 9-3-Pyridyl noscapine: Reaction Conditions. (i) 48% HBr, Bromine water, room temperature, 2h, 90% (ii) 3-Pyridyl boronic acid, $Pd(TPP)_4$, $NaHCO_3$, EtOH/Toluene,120^oC, 48 h, 62%.

Yield: 62%; mp: 193 ^0C; [α]D 25 124.25 (c = 1, dichloro methane); ^1H NMR (500 MHz, $CDCl_3$): δ 8.52 (s, 1H), 8.43 (s, 1H) 7.56 (d, J= 7.64 Hz, 1H), 7.30 (t, J= 6.65 Hz, 1H), 6.98 (d, J= 8.54 Hz, 1H), 6.12 (d, J= 7.61 Hz, 1H), 5.99 (s, 1H), 5.92 (s, 1H), 5.43 (d, J= 4.74 Hz, 1H), 4.43 (d, J= 4.74 Hz, 1H), 4.11 (s, 3H), 4.08 (s, 3H), 3.88 (s, 3H), 2.67–2.60 (m, 1H), 2.55 (s, 3H), 2.22–2.14 (m, 2H), 1.79–1.69 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.9, 152.3, 150.7, 148.36, 147.6, 146.4, 140.7, 140.2, 137.3, 133.7, 130.7, 130.3, 130.2, 123.1, 120.4, 118.2, 117.5, 100.9, 81.8, 62.2, 61.0, 59.4, 56.8, 50.6, 46.6, 26.8; IR (KBr): 3412, 2938, 1756, 1637, 1497, 1445, 1273, 1082, 1032, 943,

815, 714 cm^{-1} ; MS (ESI): m/z 513 $[M+Na]^+$; HRMS (ESI): Calcd for $C_{27}H_{26}N_2O_7$ $[M+Na]^+$; 513.1637; found: 513.1615.

In vitro assessments of cytotoxicity of designed compounds

Inhibition of cellular proliferation of MCF-7 was assessed by MTT assay. Briefly, MCF-7 cells (3 X 10^3) were seeded into 96 well plates. After post-attachment, the cells were treated with different concentrations of noscapine derivatives (Br-Nos, Br-TMB-Nos, Py-Nos) and docetaxel in single as well as in combination regimen. The IC_{50} values for the concentration of drugs needed to destroy a 50 % cell have been calculated using the online tool Quest GraphTM IC_{50} Calculator. The anti-proliferative activity for Br-Nos, Br-TMB-Nos, Py-Nos increases with the increasing concentration in single as well as in combination regimen with Docetaxel (DOX) (Figure 1a-f). The IC_{50} value amounted to 35.17 µM and 20.92 µM, respectively for 48 h and 72 h with Bromo-Nos. In contrast, the IC_{50} value amounted to 0.521 µM and 0.093 µM, respectively for 48 h and 72 h with DOX. Approximately 50% inhibition of cellular proliferation was found with the combination treatment of Bromo-Nos (30 µM) and DOX (0.01 µM). The IC_{50} value for Br-TMB-Nos was found to be 10.461 µM and 8.266 µM for 48 h and 72 h of treatments. In contrast, the IC_{50} value for DOX was found to be 0.033 µM and 0.014 µM for 48 h and 72 h of treatments. Our results showed that the combination dose of Br-TMB-Nos (25 µM) and DOX (0.01 µM) revealed a reduction in cell survival to ~50% at 48 h and 72 h of treatment. The IC_{50} values were 11.0 µM and 8.4 µM, respectively, for the Py-Nos after 48 h and 72 h of treatment. Likewise, the IC_{50} value amounted to 0.028 µM and 0.015 µM with DOX for 48 h and 72 h, respectively. Approximately 50% inhibition of cellular proliferation was achieved in the combination regimen of Py-Nos (10 µM) and DOX (0.1 µM) after 48 h and 72 h post-treatment. The dose dependent cytotoxicity of DOX has been reduced considerably with the combination dose regimen with derivatives of noscapine.

Drug combination effect study using isobologram analysis

The cumulative effect of drugs in terms of additive, synergistic, or antagonistic effect is studied by the most classical approach called Isobologram analysis. This method has been proven and mathematically demonstrated. As the combinations of noscapinoids and docetaxel have been used in a non-constant dose ratio, a normalized isobologram for the designed drugs at their ED_{50} was constructed.

The fractional inhibitory concentration (FIC) was interpreted by the following formula:

$$FIC = \frac{Conc.of\ drug\ in\ combination\ to\ produce\ IC_{50}}{Conc.\ of\ drug\ alone\ require\ to\ produce\ IC_{50}}$$

The sum FIC value for each of the preparations determined by the following formula was used to classify the drug–drug interaction.

$$Sum\ FIC = \frac{IC_{50}\ of\ drug\ A\ in\ combination}{IC_{50}\ of\ drug\ A\ alone} + \frac{IC_{50}\ drug\ B\ in\ combination}{IC_{50}\ of\ drug\ B\ alone}$$

Sum FIC < 0.5 represents substantial synergism, sum FIC < 1 represents synergism, sum FIC = 1 represents additive interaction, sum FIC ≥ 1 represents antagonism. An Isobologram was plotted to show the drug interaction as per the method proposed earlier (Pandey et al., 2016).

The interaction between the noscapine derivatives (Br-TMB-Nos and Py-Nos) with Docetaxel has been investigated using their sum FICs and isobologram plots. At 48 h and 72 h, the cumulative

FICs value was 0.8 and 0.76 respectively for Br-TMB-Nos. The isobolographic plot of Br-TMB-Nos and DOX revealed that the two drugs have synergistic antiproliferative efficacy after 48 h and 72 h of administration (Figure 2a). Similarly, for the Py-Nos the sum FICs value was found to be 0.49 and 0.62 respectively at 48 h and 72 h. The isobologram of Py-Nos with DOX suggested that the combination regimen has synergistic antiproliferative activity at 48 h and 72 h exposure (Figure 2b) (sum FIC < 1).

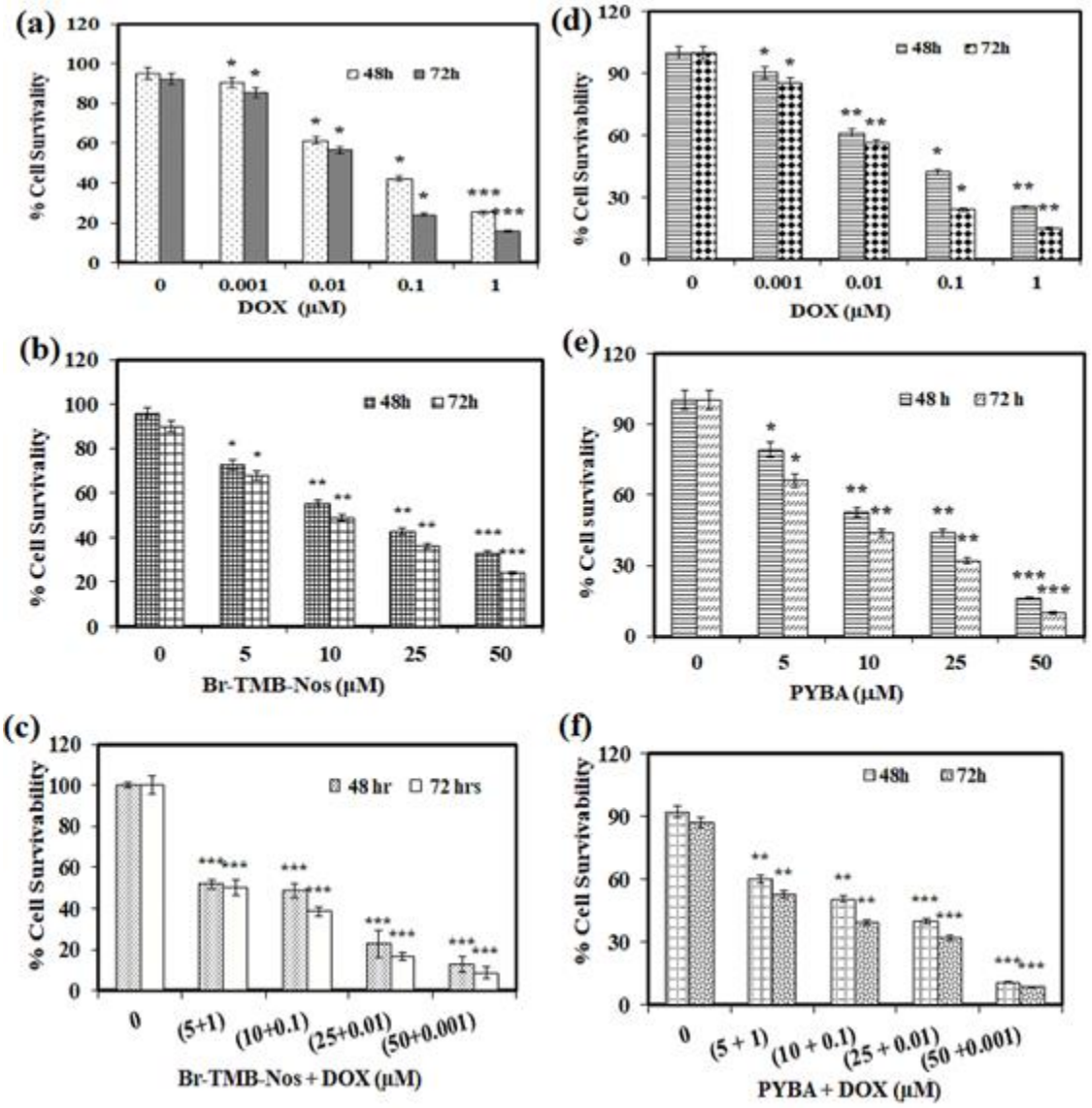


Figure 1. Reduction in percentage of cell survivability with the treatment of (a) DOX (b) Br-TMB-nos in single as well as in (c) combination regimen at different concentration after 48 h and 72 h post-treatment. Similarly, (d) DOX (e) PYBA-nos in single as well as in (f) combination regimen at different concentration inhibit cellular proliferation of human breast cancer cell, MCF-7 after 48 h and 72 h treatment. The graph is presented as mean ± SD, (n = 3), and considered significant if *p < 0.05, **p < 0.01, ***p < 0.001 compared to the control.

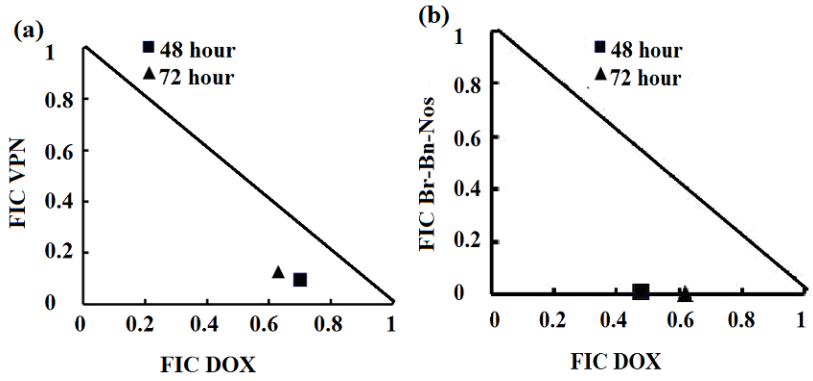


Figure 2. Representative figures of Isobolograms analysis showing *in vitro* interactions between (a) Br-TMB-Nos and DOX (b) PYBA-Nos and DOX. Sum FIC < 0.5 represents substantial synergism, sum FIC < 1 represents synergism, sum FIC 1 represents additive interaction, sum FIC >1 represents antagonism.

Effects of noscapinoids and dox using single and in combination regimen on cell cycle progression analysis

For cell cycle analysis, MCF-7 ($1X10^5$) cells were seeded in a 6-well culture plate overnight and then the cells were treated with IC_{50} concentrations of noscapinoids (PYBA-Nos, Br-Nos, Br-TMB-Nos) in single and in combination regimen with DOX. The cells were analyzed in a flow cytometer (FACS Calibur) for the effect of drug treatment on different phases of cell cycle. The FACS data suggests high deposition of cells in the G2/M phase at 24 hours of treatment with 25 µm of noscapine derivatives and DOX (0.01 µm) in single and in combination regimens (25 µm Noscapine derivatives+0.01 µm DOX), relative to untreated cells. A characteristic hypodiploid DNA content peak (sub-G1) was shown to increase at 24 h of treatment, indicating dying cells (Figure 3).

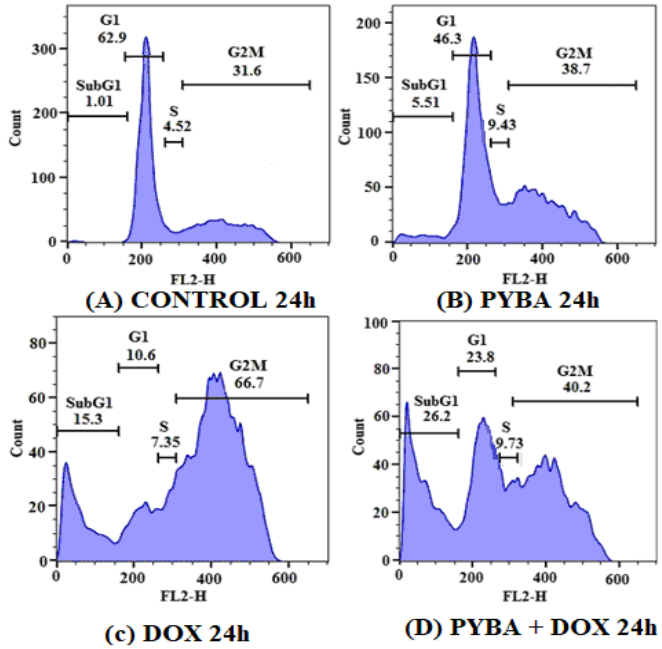


Figure 3. A representative figure (A) to (D) depicts cell cycle distribution of MCF-7 cells in a two-dimensional disposition as determined by flow cytometry at 24h of treatment with 25 µM of PYBA-Nos, 0.01 µM of DOX as single regimen and 25 µM of PYBA-Nos+0.01 µM of DOX in combination regimen. Results represent cell cycle progression at mitosis followed by the appearance of a characteristic hypodiploid (sub-G1) DNA peak is indicative of apoptosis.

Induction of apoptosis to MCF-7 cancer cells with the treatment of noscapinoids and DOX in single and in combination regimen

MCF-7 cells (5 X 10^4) were seeded in 35 mm plates. After 24 hours of attachment, cells were treated with IC_{50} concentrations of noscapinoids (PYBA-Nos, Br-Nos, Br-TMB-Nos) and docetaxel in single as well as in combination regimen (Noscpine derivatives 25 µM+DOX 0.01 µM) for a duration of 24 h at 37 °C in 5% CO_2. Percentage of apoptotic cells was assessed using BD FACS Calibur.

The amount of early and late apoptotic cells was 26.1 % and 19.9 % with the combination treatment of PYBA-Nos (25 µm) + DOX (0.01 µm) which were remarkably high compared to single regimen treatment with 25 µm of PYBA-Nos (13.0 % and 8.56 %) or 0.01 µm of DOX (11.0 % and 3.99 %) (Figure 4). The percentage of early apoptotic cells measured were 5.33%, 13.5%, 27.8%, and estimated late apoptotic cells were 6.37%, 4.77%, 13.7%, respectively with the treatment of Br-TMB-Nos and DOX in single as well as in combination regimen. Parenthetically, significantly high percentage of early apoptotic cells of 12,5%, 14.1% and 21.3% as well as late apoptotic cells of 7.90%, 10.5% and 32.1%, respectively were found with the treatment of Br-Nos and DOX in single as well as in combination regimen.

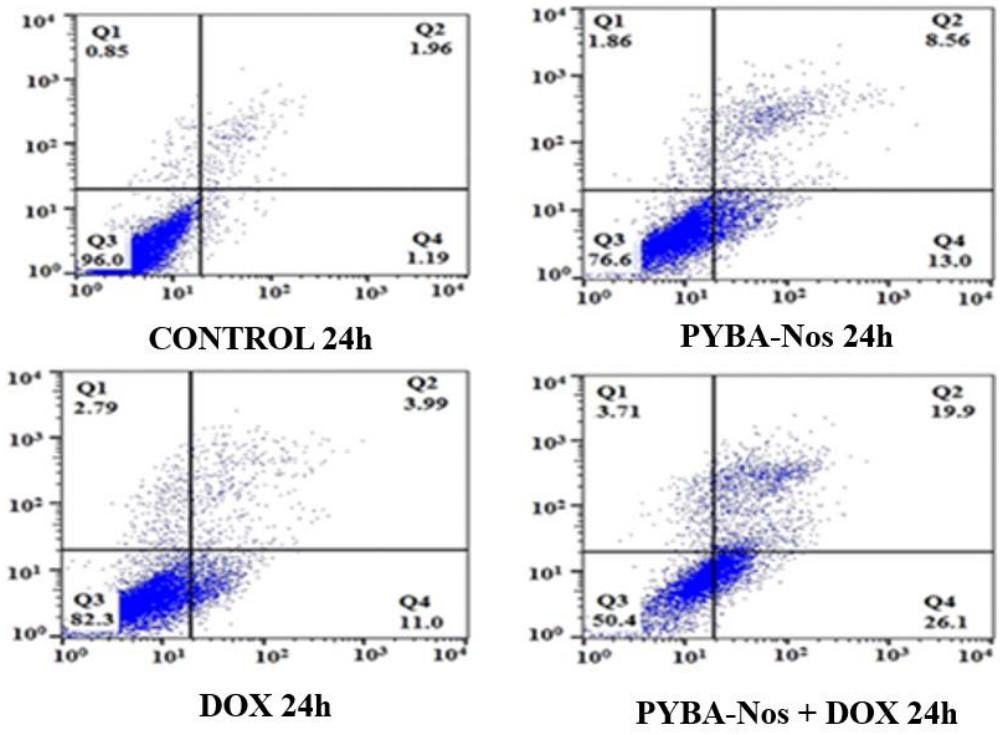


Figure 4. Flow cytometry analysis of phosphatidylserine (PS) exposure in MCF-7 cells treated with PYBA-Nos alone and in combination with DOX based on flow cytometric analysis for 24 hours and compared with non-treated control cells.

Tubulin binding activity of noscapinoids and DOX in single as well as in combination treatment (Tryptophan Quenching Assay)

The tubulin-binding affinity of noscapinoids and DOX in single as well as in their combination was determined by quenching of fluorescence intensity of tubulin with the treatment of noscapine

derivatives in single as well as in combination regimen with docetaxel. The decrease in fluorescence intensity with the treatment of Br-TMB-Nos (25 μm) and DOX (0.1 μm) suggested the binding of both the compounds to tubulin. The percentage reduction in fluorescence intensity was 30.03 % and 46.89 %, respectively in the presence of 25 μm Br-TMB-Nos and 0.1 μm DOX. Further reduction in fluorescence intensity of 65.7% respectively (Figure 5a) were observed in the combination treatment (DOX 0.01 μm + Br-TMB-Nos 25 μm). The proportional percentage of reduction in fluorescence intensity was 32.59 % and 50.03 % respectively in the presence of 25 μm PYBA-Nos and 0.1 μm DOX and 61 % in the combination of DOX (0.01 μm) and PYBA (25 μm) (Figure 5b). The dynamic reduction in fluorescence intensity of tubulin in presence of noscapinoids and docetaxel in single as well as in combination regimen indicated the binding of both the compounds with tubulin.

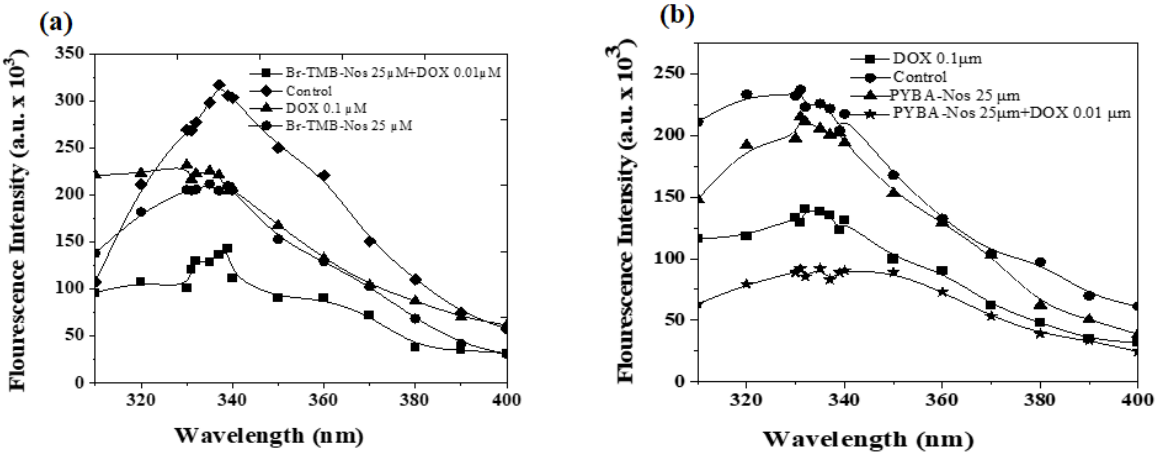


Figure 5. Representative figures of decrease of fluorescence intensity of tubulin by (a) PYBA-Noscapine and (b) Br-TMB-Noscapine in single as well as in combination regimen with docetaxel (DOX). Tubulin (2 μM) was incubated with indicated concentration of noscapinoids and DOX alone as well as in combination and the emission spectra were collected (310 nm - 400 nm). The graph is a representative of three independent experiments.

Effects of noscapinoids and DOX on ANS-tubulin fluorescence in single and combination treatment

In order to further investigate the conformational changes in tubulin due to binding of noscapinoids and DOX in single as well as in combination regimen, we probed the purified tubulin with ANS (8-anillino-1-naphthalenesulfonic acid). ANS is a fluorescent probe that bound to hydrophobic patches on proteins and improves the fluorescence when attached to protein. An increase in ANS fluorescence of tubulin suggests a loss of protein structural integrity. Purified tubulin with the treatment of PYBA-Nos and DOX showed an increase in tubulin-ANS fluorescence intensity (Figure 6a). It displayed a 25.07% increase in fluorescence intensity at 25 μM PYBA-Nos, and 42.39% in presence of 0.1 μM of DOX compared to unbound tubulin. Similarly, in combination treatment with PYBA-Nos (25 μM) and DOX (0.01 μM) the tubulin-ANS fluorescence intensity was further increased to 52.25%. We also analysed the effect of Br-TMB-Nos (25 μm) and DOX (0.1 μm) on transitions of tubulin conformation. Treatment of tubulin with Br-TMB-Nos and DOX in single and in combination demonstrated a significant improvement in tubulin-ANS fluorescence intensity (Figure 6b). The fluorescence intensity was increased to 34.28 % with the treatment of 25 μM of Br-TMB-Nos, 42.29 % with 0.1 μM of DOX compared to unbound tubulin. In contrast, combination effect of both the compounds, DOX 0.01 μM + Br-

TMB-Nos 25 µM, increased the fluorescence intensity of the tubulin-ANS to 52 % respectively (Figure 4.29b).

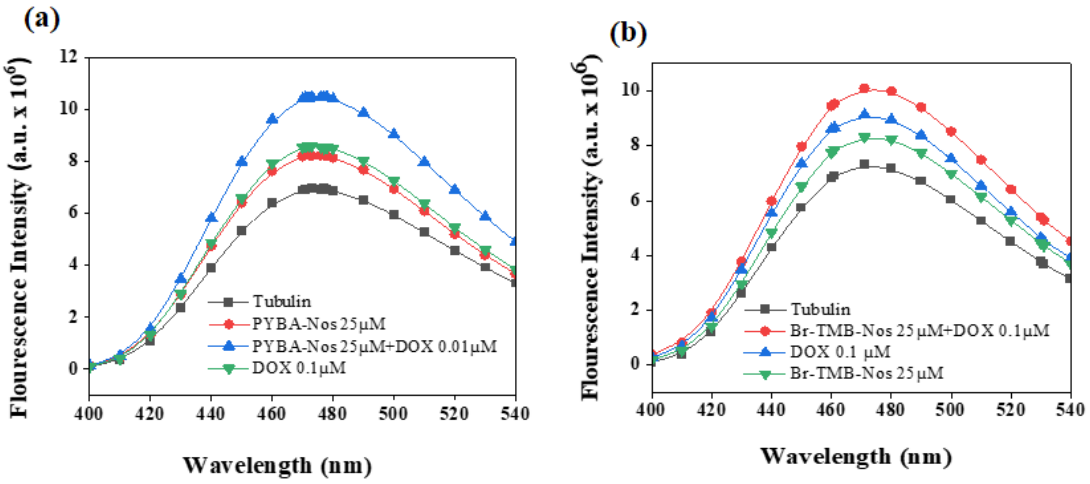


Figure 6. Representative figures of enhancement of tubulin-ANS fluorescence by (a) PYBA-Noscapine and (b) Br-TMB-Noscapine in single as well as in combination regimen with docetaxel (DOX). Tubulin (2 µM) was incubated with Noscapinoids at desired concentration with DOX and in their combination, regimen followed by incubation with ANS (50 µM). The samples were excited at 380 nm and the emission spectra were collected (400 nm – 500 nm).

Reduction in tumor volume with treatment of Br-TMB-Nos and DOX in single and in combination regimen against MCF-7 xenograft animal model

All experimental protocols involved in this study were approved by Institutional Animal Ethics Committee of National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad (1548/PO/Re/2011/CPCSEA). About 8 to 10 weeks old female BALB/c athymic nude mice were housed in the Animal Care Facility. Suspensions of $1x10^6$ human breast adenocarcinoma estrogen receptor positive cell line MCF-7 cells in 0.2 ml of PBS were inoculated subcutaneously into the anterior flank. After 7-10 days when the tumors were palpable, treatment of the test compound, Br-TMB-Nos in single as well as in combination with docetaxel were administrated by oral gavage.

Treatment with Br-TMB-Nos (150 mg/kg/day), DOX (1.5 mg/kg/week, i.v), or in combination (Br-TMB-Nos 300 mg/kg/day+DOX 1.0 mg/kg/week, i.v,) considerably decreased tumour volume in comparison to control ($P < 0.001$) (Figure. **7**A). Tumor volume was reduced to 630 mm^3 with combination treatment, 960 mm^3 with DOX and 1145 mm^3 from the tumor size of 1630 mm^3 from the untreated control group on day 40 post tumour implantation. On 40^{th} day tumor cell inoculation, mice were sacrified and tumors were removed and weighted. All untreated mice developed solid tumors in sizes ranging from 4.5g to 10.5 g (mean 7.8 ± 2.0 g). Whereas, among the treated groups the tumor size was significantly regressed and showed only small palpable tumors. Figure 7B shows the mean tumor weight±standard error of control and experimental mice. Compared to untreated control mice, inhibition of tumor growth by the treatment of Br- TMB-Nos and DOX in single and in combination regimen was statistically significant ($p < 0.001$). It is clear from these data that combination treatment of both Br-TMB-Nos and DOX reduces the tumor size quite dramatically compared to single regimen treatment of Br-TMB-Nos and DOX. In addition,

we did not observe any apparent weight loss after drug treatment compared to control group of mice (Figure 7C).

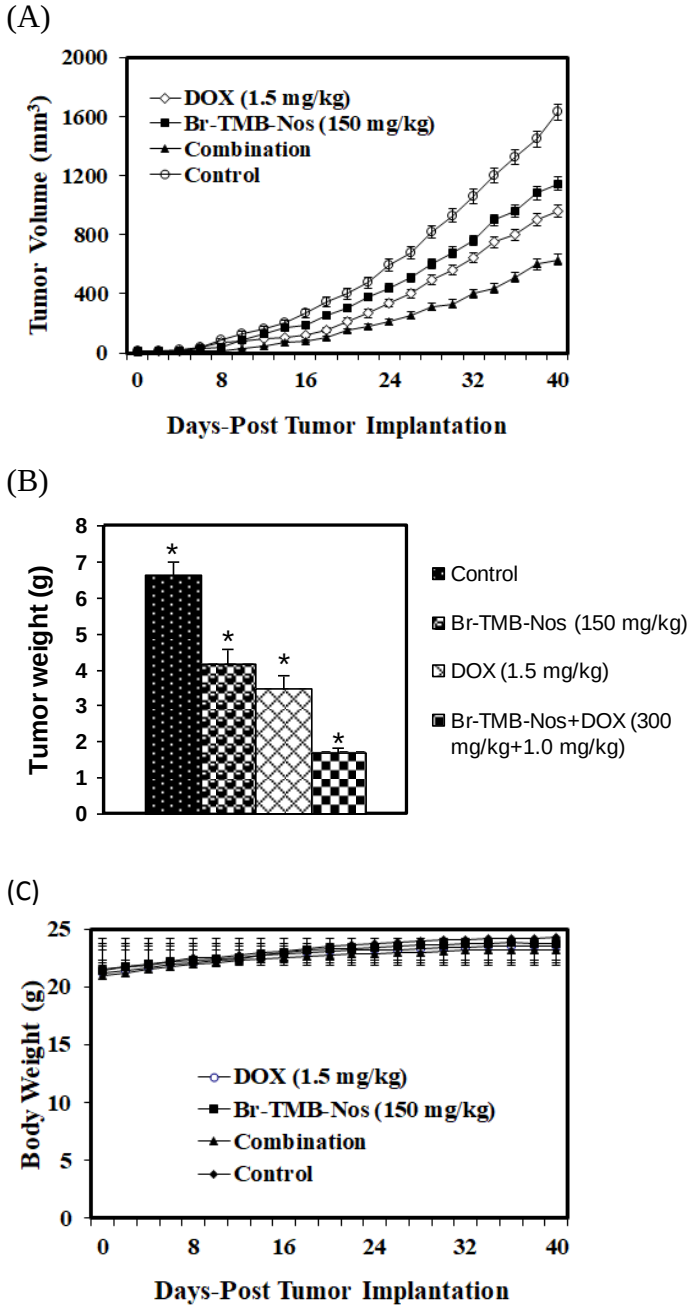


Figure 7. (A) Progression profile of tumor growth kinetics of in-vivo antitumor effect of therapeutics doses of Br-TMB-Nos and DOX alone and in combination regimen on human MCF-7 tumor xenograft model (tumor volumes, $mm^3 \pm$ SEM), (B) and measurement of body weight following Br TMB-Nos alone, DOX alone and in combination regimen. Female nude mice with xenograft MCF-7 tumors received various treatments for 30 days starting on day 7 post tumor implantation. The mice were treated with Br-TMB-Nos (150 mg/kg/day), DOX (1.5 mg/kg i.v.) and Br-TMB-Nos 300 mg/kg/day+DOX 1.0 mg/kg/week. Control group received vehicle only. Statistical significance of the difference in tumor volume of treatment groups compared with control. $P<0.01$ (*, significantly different from untreated controls; **, significantly different from Br-TMB-Nos and DOX single treatments). This experiment was repeated twice.

Treatment of Br-TMB-Nos and DOX in single and in combination does not cause any detectable toxicity

Treatment with the compound Br-TMB-Nos at daily doses of 150 mg/kg, DOX at a tolerated dose of 1.5 mg/kg body weight once in a week and in their combination treatment (Br-TMB-Nos 300 mg/kg/day+DOX 1.0 mg/kg/week, iv) failed to reveal any detectable pathological abnormalities in normal tissues involved in normal cell proliferation. Figure 4.31 collated at 200x magnification

of H&E staining of paraffin-embedded 5.0 micron-thick sections of the liver, kidney, spleen, lung, heart, colon, and brain. Microsections of brain did not reveal any infracted areas. The liver showed normal hepatic lobular architecture. The kidneys revealed normal glomeruli, proximal and distal tubules, interstitium and blood vessels. The heart muscle showed normal morphology among the groups. The lung tissue showed normal alveoli and bronchial airways.

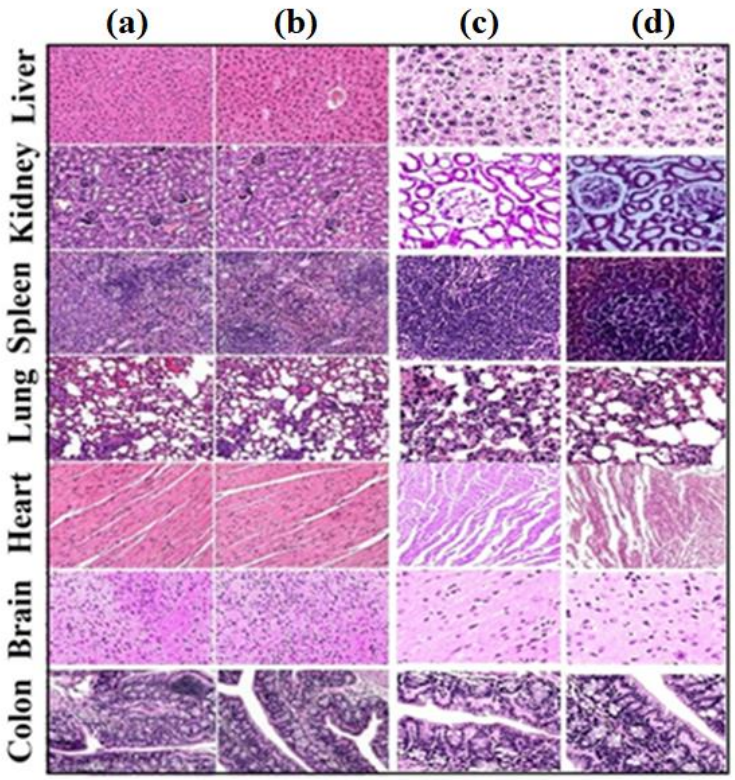


Figure 8. Represent H&E staining of paraffin-embedded 5.0 micron-thick sections of (a)Vehicle, (b) Br-TMB-Nos (150 mg/kg/day), (c) DOX (1.5 mg/kg/week), (d) (Br-TMB-Nos 300 mg/kg/day + DOX 1.0 mg/kg/week, the colon, brain, heart, lung, spleen, kidney and liver at a magnification of 200x.

B2. Summary and Conclusions of the Progress made so far:

In a quest of experimentally validate the chances of combination effects of noscapionoids, (PYBA-Nos, Br-Nos, Br-TMB-Nos) with docetaxel (DOX), these noscapinoids were chemically synthesised as per the synthetic scheme published earlier. We have performed extensive cellular and biochemical assays to validate the improvement in anticancer activity when both the class of molecules were applied in combination compared to their single regimen treatment. Antiproliferative activity of noscapinoids and docetaxel in single as well as in combination regimen using MCF-7 breast cancer cells revealed significant improvement in their activity when applied in combination compared to single regimen treatment. Further, isobolographic method of evaluation between both the class of molecules revealed a synergistic antiproliferative activity. Similarly, the combination regimen of noscapinoids and docetaxel effectively interfered with the cell cycle progression of cancer cells compared to their single regimen treatment. It was also observed that cytotoxicity of noscapinoids is mediated by perturbed DNA synthesis, delayed cell cycle progression at S phase, enhanced fragmentation of DNA in the sub G1 population, arrest of cell cycle at G2/M phase and subsequently induced apoptosis. The induction of apoptosis to cancer cell is clearly evidenced by altered plasma membrane asymmetry and fragmentation of nuclear DNA. Further, the theoretical prediction of co-binding of both the molecules with microtubule was

experimentally validated based on tubulin binding assay with purified tubulin. We found significantly high binding affinity between both the molecules when applied in combination compared to single regimen treatment. The antitumor activity of noscapinoids and docetaxel in their single regiment treatment and in their combination, effect was evaluated by taking a xenograft nude mice model and administrating both the molecules in single as well as in combination regimen. Our *in vivo* results demonstrated that a combination treatment of both the molecules exhibited a synergistic effect. The tumor volume was found to be significantly regressed in combination treatment compared to single regimen treatment. Surprisingly, no toxicity has been noticed with the vital organs in either single or in combination treatment, indicating that high doses of noscapinoids and low doses of docetaxel have a favourable toxicity profile.

B3. Details of New Leads Obtained, if any: Rationally designed 3 novel derivatives of tubulin binding anticancer agent, noscapine (the lead molecule) with high binding affinity with tubulin. These molecules were chemically synthesized in high yield and structurally characterized.

B4. Details of Publications, technology developed & Patents, if any emanated from the project: 02 (communicated)

- Shruti Gamya Dash, Anand Ramteke and Pradeep Kumar Naik (2022) Anticancer potential of brominated derivative of Noscapine in combination with Docetaxel as potent tubulin binding agent. *Communicated in 3 Biotech*.

- Shruti Gamya Dash, Anand Ramteke, Srinivas Kantevari, Santosh Kumar Guru, and Pradeep Kumar Naik (2022). Evaluation of combination effect of docetaxel and 9-3-pyridyl-noscapine against breast cancer cell line. *Communicated in Chemical Biology and Drug Design*

B5. Benefits gained:

- **Scientific & Technical expertise gained:**

  Rational design of derivatives of noscapine and screening of promising derivatives using computational techniques, chemical synthesis of design compounds, purification of tubulin from goat brain, experimental evaluation of binding affinity of noscapine derivatives with purified tubulin, apoptosis assay and cell cycle progression assay.

- **No. of NER manpower (including PI & staffs) trained in the Non-NER Institute:** 01

- **No. of visits by Non-NER Researchers to NER Institutes and vise-versa:** 01

- **Training in any new techniques, if any:** Computer aided drug design techniques, tubulin isolation and binding assay with ligands, cellular study using cancer cell lines

Section-C : Details of Grant Utilization#

Tezpur University, Tezpur, Assam

C1. Equipment Acquired or Placed Order with Actual Cost:

1. Inverted Florescent Microscope (Make Zeiss) with accessories and 2. Deep Freezer Actual cost inclusive of all taxes is Rs. 1340000.00

C2. Manpower Staffing and Expenditure Details:

Research Fellow (monthly emolument @ Rs 12000.00) Rs 56880.00

C3. Details of Recurring Expenditure:

Total recurring expenditure: Rs. 986673.00

C4. Financial Requirements for the Next Year with Justifications:

The project has been sanctioned for 03 years. However, only the grant for the 1st year has been received and other two years has not been released.

Sambalpur University, Odisha

C1. Equipment Acquired or Placed Order with Actual Cost:

Cryostat Microtome (Make: Medimeas, Model: MCM-ST) with accessories
Actual cost inclusive of all taxes is Rs. 6,98,997.00

C2. Manpower Staffing and Expenditure Details:

Research Fellow (monthly emolument @ Rs 15,400) Rs. 3,07,067.00

C3. Details of Recurring Expenditure:

Total recurring expenditure: Rs. 2,99,600.00

C4. Financial Requirements for the Next Year with Justifications:

The project has been sanctioned for 03 years. However, only the grant for the 1st year has been received and other two years has not yet released.

#Grant utilization details (UC&SE, Assets Certificate & manpower details) also required to be submitted separately as per the prescribed format

(Signature of PI) **(Signature of Co-PI)**

Instructions:

(i) All the information needs to be provided; otherwise the Progress Report will be treated as incomplete. In case of 'Nil' / 'Not Applicable' information, the same may be indicated.

(ii) In case of multicentre project, a combined Progress Report should be submitted incorporating the progress of all components. The Project Co-coordinator/ PI will be responsible for this.

*(iii) *Please indicate the reporting period [i.e. Year 1/2/3/4/5].*

(iv) Submission of Progress Report by the end of the 11th month of grant sanction is linked with further continuation of the project and timely release of funds for the next year.

Final Project Report

Project Title: "Development of synthetic derivatives of natural compound, Noscapine as tubulin binding chemotherapeutic agent and evaluation of its synergistic effect with Taxotere for management of human breast cancer"

Sanction Order No. & Date: BT/PR24726/NER/95/833/2017, Dated: 23.03.2018

Submitted By

Principal Investigators:

Parent Institute:
Dr. Anand Ramteke, Professor
Dept. of Molecular Biology & Biotechnology, Tezpur University, Napaam – 784 028, Assam

Collaboration Institute:
Dr. Pradeep K. Naik, Professor & Head
Dept. of Biotechnology & Bioinformatics, Sambalpur University, Jyoti Vihar – 768 019, Sambalpur, Odisha,

FINALCONSOLIDATED STATEMENT OF EXPENDITURE
(FOR FINAL SETTLEMENT OF ACCOUNTS)

1. **Title of the Project** : "Development of synthetic derivatives of natural compound, Noscapine as tubulin binding chemotherapeutic agent and evaluation of its synergistic effect with Taxotere for management of human breast cancer"
2. **Sanctioned Project Cost** : Rs 7050000.00
3. **Revised cost if any** :NIL
4. **Duration of the project** :Three Years (3 Yrs)
5. **Sanction Order No. & Date** : BT/PR24726/NER/95/833/2017 Dated 23/03/2018
6. **Date of commencement of Project** : March 23, 2018
7. **Extension if any** :NIL
8. **Date of completion of project** :March 22, 2021

Details of grant, expenditure and balance

S.No.	Heads	Sanctioned Cost (Rupees)	Year-wise Release made (Rupees)				Year-wise Expenditure incurred (Rupees)				
			1st Yr	2nd Yr	3rd Yr	Total	1st Yr	2nd Yr	3rd Yr	Total	Balance (Rupees)
A. Non-Recurring											
	Equipment	1200000.00	1200000.00	0.00	0.00	1200000.00	0.00	1200000.00	0.00	1200000.00	0.00
B. Recurring											
1	Manpower	1030000.00	330000.00	0.00	0.00	330000.00	12000.00	44880.00	0.00	56880.00	273120.00
2	Consumables	1300000.00	500000.00	0.00	0.00	500000.00	500862.00	0.00	0.00	500862.00	(-)862.00
3	Travel	150000.00	50000.00	0.00	0.00	50000.00	83078.00	59073.00	0.00	142151.00	(-)92151.00
4	Contingency	150000.00	50000.00	0.00	0.00	50000.00	21780.00	0.00	0.00	21780.00	28220.00
5	Overhead	400000.00	200000.00	0.00	0.00	200000.00	125000.00	140000.00	0.00	265000.00	(-)65000.00
	Total	3030000.00	1130000.00	0.00	0.00	1130000.00	742720.00	1443953.00	0.00	2186673.00	143327.00
C.	Interest Earned	0.00	39682.00	3583.00	3583.00	0.00	0.00	0.00	0.00	0.00	46848.00
	Grand Total (A+B+C)	4230000.00	2330000.00	0.00	0.00	2330000.00	742720.00	1443953.00	0.00	2186673.00	190175.00

(PROJECT INVETIGATOR)

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

(FINANCE OFFICER)

Finance Officer
Tezpur University

UTILISATION CERTIFICATE
(for the financial year April 2020 to March 2021)

1. Title of the project/scheme : **"Development of synthetic derivatives of natural compound, Noscapine as tubulin binding chemotherapeutic agent and evaluation of its synergistic effect with Taxotere for management of human breast cancer"**
2. Name of the Organisation : **Tezpur University**
3. Principal Investigator : **Dr. Anand Ramteke**
   Deptt. of Biotechnology sanction order No. & date of sanctioning the project :
   BT/PR24726/NER/95/833/2017
   Dated 23/03/2018
4. Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given : **Rs 143327.00**
5. Amount received from DBT during the financial year *(Please give No. and dates of sanction orders showing the amounts paid)* : **NIL**
6. Other receipts/interest earned, if any, on the DBT grants : **Rs 46848.00**
7. Total amount that was available for expenditure during the financial year (Sl. nos. 5, 6 and 7) : **Rs 143327.00**
8. Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed) : **NIL**
9. Unspent balance refunded, if any (Please give details of cheque No. etc.) : **Rs 190175.00**
10. Balance amount available at the end of the financial year : **Rs 190175.00**
11. Amount allowed to be carried forward to the next financial year vide letter No. & date : NIL

Ramteke

1. Certified that the amount of **Rs NIL** mentioned against col. 9 has been utilised on the project / scheme for the purpose for which it was sanctioned and that the balance of **Rs Rs 190175.00** remaining unutilized at the end of the year is deposited to Bharatkosh (Receipt No.)

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled / are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Sanction letter
2. Cash book
3. Ledge book

(PROJECT INVESTIGATOR)
(Signed and stamped)

(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

Details of Assets acquired wholly or substantially out of Govt. grants Register to be maintained by Grantee Institution

Name of the Sanctioning Authority:		Department of Biotechnology, Govt. of India
1.	Sl. No.	419-420 in the Register of Grants
2.	Name of the Grantee Institution	Tezpur University
3.	No. & Date of sanction order	BT/PR24726/NER/95/833/2017 Dated 23/03/2018
4.	Amount of the sanctioned grant	Rs 2330000.00
5.	Brief purpose of the grant	For Research on breast cancer
6.	Whether any condition regarding the right of ownership of Govt. in the Property or other assets acquired out of the grant was incorporated in the grant-in-aid sanction order.	NO
*7.	Particulars of assets actually credited or acquired.	Acquired
8.	Value of the assets as on	Rs 1340000.00
9.	Purpose for which utilised at present	Research
10.	Encumbered or not	NO
11.	Reasons, if encumbered	
12.	Disposed of or not	NO
13.	Reasons and authority, if any, for Disposal	NA
14.	Amount realised on disposal	NA
15.	Remarks	NIL

(PROJECT INVESTIGATOR)

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

*** List of equipment purchased indicating the item wise costs may please be provided.**

Sl. No.	Name of Sanctioned Equipment	Cost of Purchase (in INR)
1.	Inverted Florescent Microscope	1340000.00
2.	Deep Freezer	
	Custom duty, clearing, Freight and insurance	100000.00
	Taxes/Levise etc	63810.00
	Total	1340000.00
	Amount sanctioned for equipment	1200000.00
	Excess expenditure towards Custom duty, clearing, Freight and insurance/taxes etc **is adjusted from overhead**	140000.00

(PROJECT INVESTIGATOR)

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

(FINANCE OFFICER)

Finance Officer
Tezpur University

Annexure A

Manpower Staffing Details (In the financial year wise manner)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING PROJECT PERIOD
Mr. Joydeep Singha	JRF	01/10/2018	31/10/2018	Rs 12000.00	Rs 12000.00	Rs 12000.00
Ms. Kankana Goswami	JRF	19/06/2019	30/09/2019	Rs 12000.00	Rs 44880.00	Rs 44880.00

(Signature of Principal Investigator)

(Signature of Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University