

**SYNTHESIS AND CHARACTERIZATION OF PI3-K $\delta$ /HDAC6  
DUAL INHIBITOR AND CHEMO DRUG  
COENCAPSULATED BIODEGRADABLE POLYMERIC  
NANOPARTICLES FOR CANCER THERAPY**

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**CENTRE FOR BIOMEDICAL ENGINEERING  
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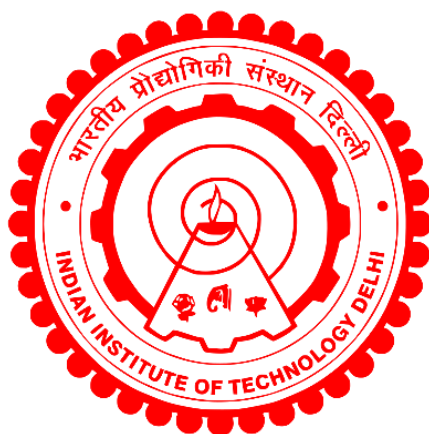
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**Centre for Biomedical Engineering**

**Submitted**

**in the fulfillment of the requirements of the degree of Doctor of Philosophy**

**to the**



**INDIAN INSTITUTE OF TECHNOLOGY DELHI**

**MARCH 2023**

**DEDICATED TO**  
**MY BELOVED FAMILY**

## **Certificate**

This is to certify that the thesis entitled “**Synthesis and characterization of PI3-K $\delta$ /HDAC6 dual inhibitor and chemo drug coencapsulated biodegradable polymeric nanoparticles for cancer therapy**” submitted by **Mr. Sachchidanand Tiwari** to the **Indian Institute of Technology Delhi** for the award of the degree of **Doctor of Philosophy** in Biomedical Engineering is a record of bonafide research work carried out by him. Mr. Sachchidanand Tiwari has worked under my guidance and supervision and has fulfilled the requirement for the submission of this thesis.

The results contained in this thesis are original and have not been submitted in partial or full, to any other university or institute for the award of any degree or diploma.

**Prof. Harpal Singh**

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## Abstract

Cancer is a leading cause of death worldwide. The available conventional treatment options of surgery, chemotherapy, hormonal therapy, and immunotherapy have increased overall cancer patient survival. Among them, chemotherapy is the major treatment option followed to treat all cancer types. Cancer initiation and progression depend on multiple receptors or signaling cascades. Phosphoinositide-3-kinases (PI3K) are a family of lipid kinase enzymes that transduce signals from cell surface receptors to downstream effectors of various cellular processes, including survival, proliferation, differentiation, metabolism, and angiogenesis. Various PI3K inhibitors have been evaluated in multiple cancer therapies. However, single PI3K inhibitor based treatment has limited therapeutic efficacy. Histone deacetylase (HDAC) inhibitors belong to the family of epigenetic enzymes that regulate the expression of various cancer related proteins, including p53 and p21. Recent studies have shown that dual targeted PI3K/HDAC inhibitors have synergistic mechanisms to inhibit cancer cell growth by simultaneously inhibiting phospho-AKT and c-Myc levels. Additionally, inhibition of phospho-AKT and c-Myc levels are synergized with DNA damaging anti-cancer agents (anthracyclines) and inhibitors of BCL2 family proteins. Combinatorial cancer treatment with multiple drugs suffers from low bioavailability, unsynchronized pharmacokinetics, off targeted toxicities, and drug resistance, which limit their therapeutic efficacy in cancer therapy. Nanoparticle (NP) based drug delivery systems can overcome almost all limitations associated with free anti-cancer drug treatment. NPs can also passively and actively target cancer cells, thus increasing drug accumulation in the tumor and minimizing anti-cancer agents' toxic effects on healthy cells. Moreover, enhanced bioavailability and sustained release of incorporated anti-cancer agents can be achieved through NP mediated drug delivery.

The present work is focused on the development of Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles for delivery of novel dual targeted PI3-K $\delta$ /HDAC6 inhibitor in combination with BCL-2/BCLxL inhibitor and anthracyclines for cancer therapy. In addition, tumor targeted delivery of anti-cancer agents encapsulated NPs were also evaluated to minimize the off targeted toxicity and maximize the therapeutic efficacy of encapsulated anti-cancer agents for cancer therapy. Pluronic<sup>®</sup> modified PLA block copolymers with different hydrophilic and hydrophobic block ratios were synthesized, characterized, and biologically evaluated for their potential drug carrier for cancer therapy. The synthesized hybrid block copolymers of mPEG-PLA and PLA-PEG-PPG-PEG-PLA were characterized using Gel permeation chromatography (GPC), Nuclear magnetic resonance (NMR), and Fourier transform infrared spectroscopy (FT-IR). These block copolymers were used to prepare NPs through the nanoprecipitation method, and the best block copolymeric nanoparticles (PLA/L61<sub>25</sub>/mPEG<sub>75</sub> NPs) based on size and stability was further selected for encapsulation of anti-cancer agents. The confocal microscopy studies confirmed the cellular internalization of rhodamine-b encapsulated PLA/L61<sub>25</sub>/mPEG<sub>75</sub> NPs block copolymeric NPs. Cytocompatibility and hemocompatibility studies demonstrate that the PLA/mPEG<sub>75</sub>/L61<sub>25</sub> block copolymeric NPs are nontoxic to healthy cells (HEK-293 and L929) and red blood cells at a concentration of up to 1 mg/ml and 7 mg/ml, respectively. Furthermore, in-vivo biodistribution studies of indocyanine green (ICG) dye encapsulated NPs have showed higher tumor accumulation as compared to free ICG after intravenous administration into mice tail vein.

These NPs were used to encapsulate and deliver novel dual targeted PI3-K $\delta$ /HDAC6 inhibitor for cancer therapy. The prepared PI3-K $\delta$ /HDAC6-NPs showed an average diameter of  $96 \pm 3$  nm, a zeta potential of  $-17 \pm 2$  mV, and PDI of  $<0.2$  with a slow and sustained release profile of PI3-K $\delta$ /HDAC6 inhibitors in phosphate buffer saline (pH 7.4). In-vitro studies with PI3-

K $\delta$ /HDAC6-NPs have demonstrated substantial growth inhibition of breast cancer cell lines, MDA-MB-468, SUM-149, and Ehrlich ascites carcinoma (EAC), as well as downregulation of phospho-AKT, phospho-ERK, and c-Myc levels. Importantly, bi-weekly treatment of Balb/c wild-type mice harboring EAC cells with PI3-K $\delta$ /HDAC6-NPs at a 25 mg/kg dose resulted in significant tumor growth inhibition. The treatment with PI3-K $\delta$ /HDAC6-NPs had no significant effect on the mice's body weights. The comparative in-vitro and in-vivo studies of PI3-K $\delta$ /HDAC6-NPs resulted in better therapeutic efficacy than SAHA-NPs and IDL-NPs. These results demonstrate that a novel PI3-K $\delta$ /HDAC6 dual inhibitor encapsulated PLA/L61<sub>25</sub>/mPEG<sub>75</sub> block copolymeric nanoparticles (HSB-510) represents as a promising approach for breast cancer therapy.

A combination of PI3-K $\delta$ /HDAC6 dual inhibitor with BCL-2/BCL-xL inhibitor (Navitoclax) was also evaluated for breast cancer therapy. The prepared dual drug loaded PI3-K $\delta$ /HDAC6-NAV-NPs have shown high encapsulation efficiency, size, and polydispersity of ~93%, 159 $\pm$ 2.6 nm, and 0.19 $\pm$ 0.03, respectively. These PI3-K $\delta$ /HDAC6-NAV-NPs exhibited slow and sustained release profiles of PI3-K $\delta$ /HDAC6 inhibitor and Navitoclax in PBS (pH 7.4). The in-vitro cell proliferation inhibition studies done with PI3-K $\delta$ /HDAC6-NAV-NPs in ER<sup>+</sup> breast cancer cell lines have shown a synergistic effect with lower IC<sub>50</sub> values as compared to individual PI3-K $\delta$ /HDAC6-NPs and NAV-NPs. PI3-K $\delta$ /HDAC6-NAV-NPs of 1:3 weight ratio (1:3-NPs) were selected based on their lower combination index value for in-vivo studies. The therapeutic potential of PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) was assessed in ER<sup>+</sup> breast cancer syngeneic mice tumor model (EAC), which resulted in complete tumor eradication after twice a week I.V. dosing for three weeks at a dose of 4 mg/kg. The treatment with PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) at a dose of 4 mg/kg did not show any significant body weight change of mice, confirming no overt toxicity. These results demonstrate that a unique

nanoformulation of a novel PI3-K $\delta$ /HDAC6 dual inhibitor in combination with Navitoclax represents an alternative approach for an efficient treatment option for ER<sup>+</sup> breast cancer.

Another combination of novel PI3-K $\delta$ /HDAC6 dual inhibitor with commonly used anti-cancer agent Epirubicin was also evaluated for breast cancer therapy. The co-encapsulated NPs, PI3-K $\delta$ /HDAC6-Epi-NPs, showed size of 99 $\pm$ 3 nm and PDI of 0.18 $\pm$ 0.07 with a sustained and slow-release profile in PBS (pH 7.4). The in-vitro cell proliferation inhibition studies done in MCF-7, SUM149, and EAC cells confirmed the synergistic effect of PI3-K $\delta$ /HDAC6-Epi-NPs (1:3-NPs) with lower IC<sub>50</sub> and combination index value. Additionally, twice a week treatment (I.V.) for three weeks with PI3-K $\delta$ /HDAC6-Epi-NPs (4 mg/kg) resulted in complete tumor eradication in EAC tumor bearing Balb/c mice. In contrast, the PI3-K $\delta$ /HDAC6-NPs and Epi-NPs at the same dose resulted in tumor growth inhibition of 15.86% and 81.59%, respectively. These studies further predict that the clinical use of PI3-K $\delta$ /HDAC6-Epi-NPs may be effective in breast cancer treatment.

In addition to the above research work, we also explored a novel PLA-PEG-PPG-PEG-PLA biomimetic nanoparticles modified with cell membranes of cancer cells and macrophage cells for tumor targeted delivery of anti-cancer agents i.e., PI3-K $\delta$ /HDAC6 dual inhibitor, Epirubicin, and Navitoclax. The prepared biomimetic NPs of PI3-K $\delta$ /HDAC6 dual inhibitor, Navitoclax, and Epirubicin showed an average diameter of 169 $\pm$ 4 nm, 191 $\pm$ 9 nm, and 163 $\pm$ 4 nm, respectively. The in-vitro drug release profile of anti-cancer agent encapsulated biomimetic NPs showed slow and sustained cumulative release of about 15-24% over a period of 7 days in PBS (pH 7.4). These biomimetic NPs have shown faster internalization to source cancer cell lines, confirming the tumor targeting. Furthermore, in-vivo biodistribution studies confirmed the higher accumulation of biomimetic nanoparticles at the tumor site as compared to untargeted NPs. Targeted delivery of anti-cancer agents using biomimetic nanoparticles improved their therapeutic efficacy. Among all biomimetic nanoformulations, CMP-Epi-NPs

at a dose of 3 mg/kg twice a week for three weeks showed 100% tumor regression with 100% survival. Altogether, these studies predict the applicability of CMP coated Epi-NPs for the treatment of breast cancer with higher therapeutic efficacy.

## सार

कैंसर दुनिया भर में मौत का एक प्रमुख कारण है। सर्जरी, कीमोथेरेपी, हार्मोनल थेरेपी और इम्यूनोथेरेपी के उपलब्ध पारंपरिक उपचार विकल्पों ने समग्र कैंसर रोगी की उत्तरजीविता में वृद्धि की है। उनमें से, सभी प्रकार के कैंसर के इलाज के लिए कीमोथेरेपी प्रमुख उपचार विकल्प है। कैंसर की शुरुआत और प्रगति कई रिसेप्टर्स या सिग्नलिंग कैस्केड पर निर्भर करती है। फॉस्फॉइनोसाइटाइड-3-किनासेस (PI3K) लिपिड किनेज एंजाइमों का एक परिवार है जो कोशिका की सतह के रिसेप्टर्स से संकेतों को जीवित रहने, प्रसार, विभेदन, चयापचय और एंजियोजेनेसिस सहित विभिन्न कोशिकीय प्रक्रियाओं के डाउनस्ट्रीम इफेक्टर्स में ट्रांसड्यूस करता है। कई कैंसर उपचारों में विभिन्न PI3K अवरोधकों का मूल्यांकन किया गया है। हालांकि, एकल PI3K अवरोधक आधारित उपचार की चिकित्सीय प्रभावकारिता सीमित है। हिस्टोन डीएसेटाइलेज़ (एचडीएसी) अवरोधक एपिजेनेटिक एंजाइमों के परिवार से संबंधित हैं जो पी53 और पी21 सहित विभिन्न कैंसर संबंधी प्रोटीन की अभिव्यक्ति को नियंत्रित करते हैं। हाल के अध्ययनों से पता चला है कि दोहरे लक्षित PI3K/HDAC अवरोधकों में फॉस्फो-एकेटी और सी-माइसी स्तरों को एक साथ रोककर कैंसर कोशिका वृद्धि को रोकने के लिए सहक्रियात्मक तंत्र हैं। इसके अतिरिक्त, फॉस्फो-एकेटी और सी-माइसी स्तरों का अवरोध डीएनए को नुकसान पहुंचाने वाले कैंसर रोधी एजेंटों (एंथ्रासाइक्लिन) और बीसीएल2 परिवार के प्रोटीन के अवरोधकों के साथ तालमेल बिठाता है। कैंसर का कई दवाओं के साथ मिश्रित उपचार कम जैवउपलब्धता, अतुल्यकालिक फार्माकोकाइनेटिक्स, लक्षित विषाक्तता और दवा प्रतिरोध से ग्रस्त है, जो कैंसर चिकित्सा में उनकी चिकित्सीय प्रभावकारिता को सीमित करता है। नैनोपार्टिकल (एनपी) आधारित दवा वितरण प्रणाली कैंसर रोधी दवा उपचार से जुड़ी लगभग सभी सीमाओं को पार कर सकती है। एनपी कैंसर कोशिकाओं को निष्क्रिय और सक्रिय रूप से लक्षित कर सकते हैं, इस प्रकार ट्यूमर में दवा संचय को बढ़ाते हैं और स्वस्थ कोशिकाओं पर कैंसर विरोधी एजेंटों के विषाक्त प्रभाव को कम करते हैं। इसके अलावा, दवा वितरण के माध्यम से शामिल कैंसर रोधी एजेंटों की निरंतर रिहाई और बढ़ी हुई जैवउपलब्धता प्राप्त की जा सकती है।

वर्तमान कार्य कैंसर चिकित्सा के लिए दोहरे लक्षित PI3-K $\delta$ /HDAC6 अवरोधक, BCL-2/BCLxL अवरोधक, और एंथ्रासाइक्लिन के संयोजन की डिलीवरी के लिए प्लूरोनिक® संशोधित पॉलीलैक्टिक एसिड ब्लॉक कोपॉलीमरिक नैनोकणों के विकास पर केंद्रित है। इसके अलावा, कैंसर रोधी एजेंटों के कैंसर लक्षित

वितरण का मूल्यांकन उनकी विषाक्तता को कम करने और चिकित्सीय प्रभावकारिता को अधिकतम करने के लिए किया गया है। कैंसर चिकित्सा के लिए विभिन्न हाइड्रोफिलिक और हाइड्रोफोबिक ब्लॉक अनुपात वाले प्लूरोनिक® संशोधित पीएलए ब्लॉक कॉपोलिमर को उनके संभावित दवा वाहक के लिए संश्लेषित, विशेषता और जैविक रूप से मूल्यांकन किया गया है। संश्लेषित हाइब्रिड ब्लॉक कॉपोलिमर MPEG-PLA और PLA-PEG-PPG-PEG-PLA को जेल परमीशन क्रोमेटोग्राफी (GPC), न्यूक्लियर मैग्नेटिक रेजोनेंस (NMR) और फूरियर ट्रांसफॉर्म इंफ्रारेड स्पेक्ट्रोस्कोपी (FT-IR) का उपयोग करके लक्षणों को वर्णित किया गया है। इन ब्लॉक कॉपोलिमर का उपयोग नैनोप्रिसिपिटेशन विधि के माध्यम से एनपी तैयार करने के लिए किया गया, और आकार और स्थिरता के आधार पर सर्वश्रेष्ठ ब्लॉक कोपॉलीमरिक नैनोपार्टिकल्स (PLA/L61<sub>25</sub>/mPEG<sub>75</sub> NPs) को आगे कैंसर रोधी एजेंटों के एनकैप्सुलेशन के लिए चुना गया। कॉन्फोकल माइक्रोस्कोपी अध्ययनों ने रोडामाइन-बी एनकैप्सुलेटेड PLA/L61<sub>25</sub>/mPEG<sub>75</sub> ब्लॉक कोपॉलीमरिक एनपी के सेलुलर आंतरिककरण की पुष्टि की। साइटोकम्पैटिबिलिटी और हीमोकम्पैटिबिलिटी अध्ययनों से पता चलता है कि PLA/L61<sub>25</sub>/mPEG<sub>75</sub> ब्लॉक कोपॉलीमरिक एनपी स्वस्थ कोशिकाओं (HEK-293 और L929) और लाल रक्त कोशिकाओं के लिए क्रमशः 1 mg/ml और 7 mg/ml तक की सांद्रता पर गैर विषैले होते हैं। इसके अलावा, इंडोसायनिन ग्रीन (आईसीजी) डाई एनकैप्सुलेटेड एनपी के इन-विवो जैव वितरण अध्ययन ने मुक्त आईसीजी की तुलना में उच्च ट्यूमर संचय दिखाया है।

इन एनपी का उपयोग कैंसर चिकित्सा के लिए दोहरे लक्षित PI3-K $\delta$ /HDAC6 अवरोधक को एनकैप्सुलेट और वितरित करने के लिए किया गया था। तैयार PI3-K $\delta$ /HDAC6-NPs ने फॉस्फेट बफर (पीबीएस पीएच 7.4) में PI3-K $\delta$ /HDAC6 अवरोधकों की धीमी और निरंतर रिलीज़ प्रोफ़ाइल के साथ 96 $\pm$ 3 एनएम का औसत व्यास, -17 $\pm$ 2 mV का ज़ीटा पोटेंशियल और ~0.2 का PDI दिखाया। PI3-K $\delta$ /HDAC6-NPs के साथ इन-विट्रो अध्ययनों ने स्तन कैंसर सेल लाइन्स, MDA-MB-468, SUM-149, और EAC के पर्याप्त विकास निषेध, साथ ही फॉस्फो-एकेटी, फॉस्फो-ईआरके और सी-माइसी स्तरों का डाउनरेगुलेशन का प्रदर्शन किया है। महत्वपूर्ण रूप से, 25 मिलीग्राम/किग्रा खुराक पर PI3-K $\delta$ /HDAC6-NPs के द्वि-साप्ताहिक उपचार के परिणामस्वरूप EAC कैंसर कोशिकाओं वाले Balb/c जंगली-प्रकार के चूहों अभिप्रायपूर्ण ट्यूमर वृद्धि अवरोध हुआ। PI3-K $\delta$ /HDAC6-NPs के साथ उपचार का चूहों के शरीर के वजन पर कोई महत्वपूर्ण प्रभाव नहीं पड़ा। PI3-K $\delta$ /HDAC6-NPs, SAHA-NPs और IDL-NPs के तुलनात्मक इन-विट्रो और

इन-विवो अध्ययनों के परिणामस्वरूप PI3-K $\delta$ /HDAC6-NPs में बेहतर चिकित्सीय प्रभावकारिता प्राप्त हुई । इन परिणामों से पता चलता है कि PI3-K $\delta$ /HDAC6 दोहरे अवरोधक एनकैप्सुलेटेड PLA/L61<sub>25</sub>/mPEG<sub>75</sub> ब्लॉक कोपॉलीमरिक नैनोपार्टिकल्स (HSB-510) स्तन कैंसर चिकित्सा के लिए एक आशाजनक दृष्टिकोण के रूप में प्रतिनिधित्व करता है ।

स्तन कैंसर चिकित्सा के लिए PI3-K $\delta$ /HDAC6 दोहरे अवरोधक के साथ BCL-2/BCL-xL अवरोधक (नेविटोक्वैक्स) के संयोजन का भी मूल्यांकन किया गया । तैयार डुअल ड्रग लोडेड PI3-K $\delta$ /HDAC6-NAV-NPs ने क्रमशः ~93%, 159 $\pm$ 2.6 एनएम, और 0.19 $\pm$ 0.03 की उच्च एनकैप्सुलेशन दक्षता, आकार और पॉलीडिस्पेरीटी दिखाई है । इन PI3-K $\delta$ /HDAC6-NAV-NPs ने पीबीएस (पीएच 7.4) में PI3-K $\delta$ /HDAC6 दोहरे अवरोधक और नेविटोक्वैक्स के धीमे और निरंतर रिलीज़ प्रोफाइल प्रदर्शित किए । ER+ स्तन कैंसर कोशिकाओं में PI3-K $\delta$ /HDAC6-NAV-NPs के साथ किए गए इन-विट्रो कोशिका प्रसार निषेध अध्ययनों ने व्यक्तिगत PI3-K $\delta$ /HDAC6-NPs और NAV-NPs की तुलना में निम्न IC<sub>50</sub> मानों के साथ सहक्रियात्मक प्रभाव दिखाया है । PI3-K $\delta$ /HDAC6-NAV-NPs 1:3 वजन अनुपात (1:3-NPs) को इन-विवो अध्ययनों के लिए उनके निम्न संयोजन सूचकांक मूल्य के आधार पर चुना गया था । PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) की चिकित्सीय क्षमता का मूल्यांकन ER+ स्तन कैंसर सिन्जेनिक चूहों के ट्यूमर मॉडल (EAC) में किया गया था, जिसके परिणामस्वरूप 4 मिलीग्राम/किग्रा की खुराक (सप्ताह में दो बार तीन सप्ताह के लिए) के बाद पूर्ण ट्यूमर उन्मूलन हुआ । PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) के साथ 4 mg/kg की खुराक पर किए गए उपचार में चूहों के शरीर के वजन में कोई महत्वपूर्ण परिवर्तन नहीं दिखा, जिससे कोई प्रत्यक्ष विषाक्तता की पुष्टि नहीं हुई । इन परिणामों से पता चलता है कि नेविटोक्वैक्स के साथ संयोजन में PI3-K $\delta$ /HDAC6 दोहरे अवरोधक का एक अनूठा नैनोफॉर्म्यूलेशन ER+ स्तन कैंसर के लिए एक कुशल उपचार विकल्प के लिए एक वैकल्पिक दृष्टिकोण का प्रतिनिधित्व करता है ।

आमतौर पर इस्तेमाल किए जाने वाले एंटी-कैंसर एजेंट एपिरुबिसिन के साथ नए PI3-K $\delta$ /HDAC6 दोहरे अवरोधक के एक अन्य संयोजन का भी स्तन कैंसर चिकित्सा के लिए मूल्यांकन किया गया था । सह-एनकैप्सुलेटेड एनपी, PI3-K $\delta$ /HDAC6-Epi-NPs, ने पीबीएस (पीएच 7.4) में एक निरंतर और धीमी-रिलीज़ प्रोफाइल के साथ 99 $\pm$ 3 एनएम का आकार दिखाया । MCF-7, SUM149, और EAC कोशिकाओं में किए गए इन-विट्रो कोशिका प्रसार निषेध अध्ययनों ने कम IC<sub>50</sub> और संयोजन सूचकांक मूल्य के साथ PI3-

K $\delta$ /HDAC6-Epi-NPs (1:3-NPs) के सहक्रियात्मक प्रभाव की पुष्टि की। इसके अतिरिक्त, PI3-K $\delta$ /HDAC6-Epi-NPs (4 mg/kg) के साथ तीन सप्ताह के लिए सप्ताह में दो बार उपचार के परिणामस्वरूप EAC ट्यूमर वाले Balb/c चूहों में पूर्ण ट्यूमर उन्मूलन हुआ। इसके विपरीत, समान खुराक पर PI3-K $\delta$ /HDAC6-NPs और Epi-NPs के परिणामस्वरूप ट्यूमर का विकास क्रमशः 15.86% और 81.59% हुआ। ये अध्ययन आगे भविष्यवाणी करते हैं कि स्तन कैंसर के उपचार में PI3-K $\delta$ /HDAC6-Epi-NPs का नैदानिक उपयोग प्रभावी हो सकता है।

उपरोक्त शोध कार्य के अलावा, हमने PLA-PEG-PPG-PEG-PLA बायोमिमेटिक नैनोकणों का भी अध्ययन किया, जो कैंसर कोशिकाओं और मैक्रोफेज कोशिकाओं की कोशिका झिल्लियों के साथ संशोधित होकर एंटी-कैंसर एजेंटों के ट्यूमर लक्षित वितरण के लिए हैं। PI3-K $\delta$ /HDAC6 दोहरे अवरोधक, नेविटोक्वैक्स और एपिरुबिसिन के तैयार बायोमिमेटिक एनपी ने क्रमशः 169 $\pm$ 4 एनएम, 191 $\pm$ 9 एनएम, और 163 $\pm$ 4 एनएम का औसत व्यास दिखाया। कैंसर रोधी एजेंट एनकैप्सुलेटेड बायोमिमेटिक एनपी ने पीबीएस (पीएच 7.4) में 7 दिनों की अवधि में लगभग 15-24% की धीमी और निरंतर संचयी रिलीज दिखाई। इन बायोमिमेटिक एनपी ने ट्यूमर लक्ष्यीकरण की पुष्टि करते हुए स्रोत कैंसर कोशिकाओं में तेजी से आंतरिककरण दिखाया है। इसके अलावा, इन-विवो जैव वितरण अध्ययनों ने अलक्षित एनपी की तुलना में ट्यूमर साइट पर बायोमिमेटिक नैनोकणों के उच्च संचय की पुष्टि की। बायोमिमेटिक नैनोकणों का उपयोग कर एंटी-कैंसर एजेंटों की लक्षित वितरण ने उनकी चिकित्सीय प्रभावकारिता में सुधार किया। सभी बायोमिमेटिक नैनोफॉर्म्यूलेशन में, तीन सप्ताह के लिए सप्ताह में दो बार 3 मिलीग्राम/किग्रा की खुराक पर सीएमपी-एपी-एनपी ने 100% जीवित रहने के साथ 100% ट्यूमर प्रतिगमन दिखाया। कुल मिलाकर, ये अध्ययन उच्च चिकित्सीय प्रभावकारिता के साथ स्तन कैंसर के उपचार के लिए सीएमपी-एपी-एनपी की प्रयोज्यता की भविष्यवाणी करते हैं।

# Table of Contents

List of figures.....	I
List of tables.....	XI
List of Abbreviations.....	XIV

## Chapter I: Introduction and literature review

---

<b>1.1. Introduction.....</b>	<b>2</b>
<b>1.2. Signaling pathways of cancer progression .....</b>	<b>3</b>
1.2.1. PI-3K signaling pathway.....	4
1.2.1.1. Activation of PI-3K signaling pathway.....	5
1.2.1.2. PI-3K signaling in cancer progression.....	8
<b>1.3. Cancer treatment strategies.....</b>	<b>11</b>
1.3.1. Surgery.....	11
1.3.2. Radiation Therapy.....	11
1.3.3. Chemotherapy.....	12
1.3.4. Immunotherapy.....	13
1.3.5. Hormonal therapy.....	13
1.3.6. Targeted therapy.....	13
1.3.7. Combinatorial treatment strategies.....	15
<b>1.4. Limitations of free drug treatment in cancer therapy.....</b>	<b>19</b>
<b>1.5. Significance of nano drug delivery system in cancer therapy.....</b>	<b>20</b>
1.5.1. Types of nanocarriers in cancer therapy.....	21
1.5.1.1. Liposomes.....	21
1.5.1.2. Polymeric micelles.....	21
1.5.1.3. Polymeric nanoparticles.....	22
1.5.2. Overcoming multidrug resistance using polymeric nanocarriers.....	24
1.5.3. Tumor targeting with polymeric nanocarriers.....	26
1.5.3.1. Passive targeting.....	26
1.5.3.2. Active targeting.....	26
<b>1.6. Rationale of the work.....</b>	<b>27</b>
<b>References.....</b>	<b>32</b>

## Chapter II: Synthesis, characterization, and biological evaluation of Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles

---

<b>2.1 Introduction</b> .....	55
<b>2.2 Materials and methods</b> .....	57
<b>2.2.1 Materials</b> .....	57
<b>2.2.2 Methods</b> .....	58
<b>2.2.2.1 Synthesis of Pluronic<sup>®</sup> modified polylactic acid block copolymers</b> .....	58
<b>2.2.2.2 Characterization of synthesized block copolymer</b> .....	59
<b>2.2.2.2.1 Gel permeation chromatography (GPC)</b> .....	59
<b>2.2.2.2.2 Nuclear magnetic resonance (NMR)</b> .....	59
<b>2.2.2.2.3 Fourier transform infrared spectroscopy (FTIR)</b> .....	59
<b>2.2.2.3 Preparation and characterization of nanoparticles</b> .....	59
<b>2.2.2.4 Nanoparticle stability studies</b> .....	60
<b>2.2.2.5 Preparation and characterization of dye encapsulated nanoparticles</b> .....	60
<b>2.2.3 In-vitro studies</b> .....	61
<b>2.2.3.1 Cytocompatibility studies</b> .....	61
<b>2.2.3.2 Hemocompatibility studies</b> .....	62
<b>2.2.3.3 Cellular internalization studies</b> .....	62
<b>2.2.4 In-vivo biodistribution studies</b> .....	63
<b>2.3 Results</b> .....	64
<b>2.3.1 Synthesis of Pluronic<sup>®</sup> modified PLA block copolymers</b> .....	64
<b>2.3.2 Characterization of Pluronic<sup>®</sup> modified PLA block copolymers</b> .....	65
<b>2.3.2.1 GPC</b> .....	65
<b>2.3.2.2 NMR</b> .....	65
<b>2.3.2.3 FTIR</b> .....	69
<b>2.3.3 Preparation and characterization of nanoparticles</b> .....	70
<b>2.3.4 In-vitro studies</b> .....	71
<b>2.3.4.1 Cytocompatibility studies</b> .....	71
<b>2.3.4.2 Hemocompatibility studies</b> .....	71
<b>2.3.4.3 Cellular internalization studies</b> .....	74
<b>2.3.5 In-vivo biodistribution studies</b> .....	74
<b>2.4 Discussion</b> .....	76
<b>2.5 Conclusion</b> .....	78

References.....	79
-----------------	----

### **Chapter III: Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles mediated delivery of PI3-K $\delta$ /HDAC6 dual inhibitor for cancer therapy**

---

<b>3.1 Introduction.....</b>	<b>83</b>
<b>3.2 Materials and methods.....</b>	<b>86</b>
3.2.1 Materials.....	86
3.2.2 Methods.....	86
3.2.2.1 Preparation and characterization of nanoparticles.....	86
3.2.2.2 Quantification of PI3-K $\delta$ /HDAC6 dual inhibitor .....	88
3.2.2.3 In-vitro drug release studies.....	89
3.2.2.4 Cell culture preparation and maintenance .....	89
3.2.2.5 Cytotoxicity studies.....	89
3.2.2.6 Western blot studies.....	90
3.2.2.7 In-vivo tumor growth inhibition and toxicity studies.....	90
3.2.2.8 Statistical analysis.....	92
<b>3.3 Results.....</b>	<b>92</b>
3.3.1 Characterization of nanoparticles.....	92
3.3.2 In-vitro drug release studies.....	94
3.3.3 Cytotoxicity studies.....	96
3.3.4 Western blot studies.....	100
3.3.5 In-vivo tumor growth inhibition studies.....	102
3.3.6 In-vivo hepatotoxicity and nephrotoxicity studies.....	110
3.3.7 Histopathological studies.....	110
<b>3.4 Discussion.....</b>	<b>110</b>
<b>3.5 Conclusion.....</b>	<b>112</b>
References.....	114

**Chapter IV: Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles mediated co-delivery of PI3-K $\delta$ /HDAC6 dual inhibitor and Navitoclax for ER+ breast cancer therapy**

---

<b>4.1</b>	<b>Introduction</b> .....	120
<b>4.2</b>	<b>Materials and methods</b> .....	123
<b>4.2.1</b>	Materials.....	123
<b>4.2.2</b>	Methods.....	123
<b>4.2.2.1</b>	Preparation and characterization of nanoparticles.....	123
<b>4.2.2.2</b>	In-vitro drug release studies.....	125
<b>4.2.2.3</b>	Cytotoxicity studies.....	126
<b>4.2.2.4</b>	Combination index analysis.....	126
<b>4.2.2.5</b>	In-vivo therapeutic, survival and toxicity studies.....	126
<b>4.2.2.6</b>	Statistical analysis.....	127
<b>4.3</b>	<b>Results</b> .....	128
<b>4.3.1</b>	Characterization of nanoparticles.....	128
<b>4.3.2</b>	In-vitro drug release studies.....	131
<b>4.3.3</b>	Cytotoxicity studies.....	131
<b>4.3.4</b>	Combination index analysis.....	132
<b>4.3.5</b>	In-vivo therapeutic, survival and toxicity studies.....	136
<b>4.4</b>	<b>Discussion</b> .....	141
<b>4.5</b>	<b>Conclusion</b> .....	143
	<b>References</b> .....	145

**Chapter V: Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles mediated co-delivery of PI3-K $\delta$ /HDAC6 dual inhibitor and Epirubicin for breast cancer therapy**

---

<b>5.1</b>	<b>Introduction</b> .....	151
<b>5.2</b>	<b>Materials and methods</b> .....	153
<b>5.2.1</b>	Materials.....	153
<b>5.2.2</b>	Methods.....	155
<b>5.2.2.1</b>	Preparation and characterization of nanoparticles.....	155
<b>5.2.2.2</b>	In-vitro drug release studies.....	156
<b>5.2.2.3</b>	Cell culture and in-vitro cellular internalization studies.....	156

5.2.2.4	Cytotoxicity studies.....	157
5.2.2.5	Combination index analysis.....	157
5.2.2.6	In-vivo therapeutic, survival and toxicity studies.....	157
5.2.2.7	Statistical analysis.....	158
<b>5.3</b>	<b>Results.....</b>	<b>158</b>
5.3.1	Characterization of nanoparticles.....	158
5.3.2	In-vitro drug release studies.....	159
5.3.3	In-vitro cellular internalization studies of Epi-NPs.....	163
5.3.4	Cytotoxicity studies.....	163
5.3.5	Combination index analysis.....	165
5.3.6	In-vivo therapeutic, survival and toxicity studies.....	170
<b>5.4</b>	<b>Discussion.....</b>	<b>174</b>
<b>5.5</b>	<b>Conclusion.....</b>	<b>176</b>
	<b>References.....</b>	<b>177</b>

**Chapter VI: Tumor targeted delivery of anti-cancer agents using Pluronic® modified polylactic acid block copolymeric nanoparticles for breast cancer therapy**

---

<b>6.1</b>	<b>Introduction.....</b>	<b>182</b>
<b>6.2</b>	<b>Materials and methods.....</b>	<b>185</b>
6.2.1	Materials.....	185
6.2.2	Methods.....	185
6.2.2.1	Preparation and characterization of nanoparticles.....	185
6.2.2.2	Cell membrane protein extraction and characterization.....	187
6.2.2.3	Preparation and characterization of tumor targeted nanoparticles.....	188
6.2.2.4	In-vitro drug release studies.....	189
6.2.2.5	Cellular internalization studies.....	189
6.2.2.6	Cytotoxicity studies.....	189
6.2.2.7	In-vivo biodistribution studies.....	190
6.2.2.8	In-vivo therapeutic and survival studies.....	190
6.2.2.9	Statistical analysis.....	191
<b>6.3</b>	<b>Results.....</b>	<b>191</b>
6.3.1	Characterization of nanoparticles.....	191
6.3.2	Cell membrane protein characterization.....	195

<b>6.3.3</b>	In-vitro drug release studies.....	196
<b>6.3.4</b>	Cellular internalization studies.....	198
<b>6.3.5</b>	Cytotoxicity studies.....	199
<b>6.3.6</b>	In-vivo biodistribution studies.....	201
<b>6.3.7</b>	In-vivo therapeutic and survival studies.....	203
<b>6.4</b>	<b>Discussion</b> .....	208
<b>6.5</b>	<b>Conclusion</b> .....	210
	<b>References</b> .....	211

## **Chapter VII: Summary and future perspectives of the research work**

---

<b>7.1</b>	<b>Summary and conclusion</b> .....	217
<b>7.2</b>	<b>Future perspectives of the research work</b> .....	219
	<b>Publications and patents from this thesis</b> .....	221
	<b>Curriculum Vitae</b> .....	222

## List of Figures

<b>Chapter I</b>	<b>Introduction &amp; literature review</b>	<b>Page no.</b>
Figure 1.1	The overview of PI-3K signaling pathway.	7
Figure 1.2	Downstream effectors of the PI3-K/AKT signaling pathway and their cellular functions.	10
<b>Chapter II</b>	<b>Synthesis, characterization, and biological evaluation of Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles</b>	
Figure 2.1	Schematic representation of Pluronic <sup>®</sup> modified PLA block copolymer synthesis	64
Figure 2.2	GPC chromatograms of synthesized Pluronic <sup>®</sup> modified PLA block copolymers.	66
Figure 2.3	<sup>1</sup> H NMR of Pluronic <sup>®</sup> modified PLA block copolymers	67-68
Figure 2.4	Comparative FTIR spectra of Pluronic <sup>®</sup> L61, mPEG and Pluronic <sup>®</sup> modified PLA block copolymers.	69
Figure 2.5	In-vitro cytocompatibility study of PLA/L61 <sub>25</sub> /mPEG <sub>75</sub> block copolymeric blank NPs on healthy cell lines (HEK-293 and L-929), (n = 3).	72
Figure 2.6	Hemocompatibility study of PLA/L61 <sub>25</sub> /mPEG <sub>75</sub> block copolymeric blank NPs.	72
Figure 2.7	Cellular internalization studies: <b>(A)</b> Confocal laser scanning microscopic images Rho-B-NPs internalization assessed on EAC cells (2D culture); <b>(B)</b> Confocal laser scanning microscopic images Rho-B-NPs internalization assessed on SUM149 cells (3D culture).	73
Figure 2.8	<b>(A and B)</b> In-vivo biodistribution of free ICG and ICG-NPs in EAC tumor bearing mice; <b>(C)</b> Ex-vivo biodistribution of ICG-NPs	75

in mice organs including heart, lungs, liver, spleen, kidneys, and tumor.

Figure 2.9 Quantitative biodistribution of ICG and ICG-NPs in vital organs and tumors, taken at different time points, fluorescence represents the radiant efficiency expressed as (photon/s/cm<sup>2</sup>/steradian). 76

### **Chapter III Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles mediated delivery of PI3-K $\delta$ /HDAC6 dual inhibitor for cancer therapy**

Figure 3.1 Chemical name and structure of **(A)** PI3-K $\delta$ /HDAC6 dual Inhibitor; **(B)** Vorinostat (SAHA, HDAC inhibitor); **(C)** Idelalisib (PI3K $\delta$  inhibitor). 87

Figure 3.2 **(A)** Schematic of nanoprecipitation method for the preparation of PI3-K $\delta$ /HDAC6 dual inhibitor encapsulated NPs (HSB-510); **(B)** DLS particle size distribution of HSB-510; **(C)** TEM image of HSB-510 nanoformulation. 93

Figure 3.3 In-vitro release studies of anti-cancer agent encapsulated PLA block copolymeric NPs; **(A)** Per day and **(B)** cumulative percent release of PI3-K $\delta$ /HDAC6 dual inhibitor from HSB-510; **(C)** Per day and **(D)** cumulative percent release of Idelalisib from IDL-NPs; **(E)** Per day and **(F)** cumulative percent release of SAHA (Vorinostat) from SAHA-NPs. 95

Figure 3.4 Comparative IC<sub>50</sub> values of free PI3-K $\delta$ /HDAC6 dual inhibitor and HSB-510 nanoformulation on various cancer cell lines. 97

Figure 3.5 **(A)** IC<sub>50</sub> plot of HSB-510 treated SUM149 mammospheres; **(B)** Representative confocal images of SUM149 mammospheres; untreated and HSB-510 treated. Live cells were stained with calcein-AM dye and dead cells are stained with propidium iodide dye 98

Figure 3.6	Cytotoxicity of PI3-K $\delta$ /HDAC6 dual inhibitor, SAHA, IDL and their encapsulated polymeric nanoparticles (HSB-510, SAHA-NPs, and IDL-NPs) in <b>(A)</b> MDA-MB468 cell line; <b>(B)</b> SUM-149 cell line; and <b>(C)</b> EAC cell line.	99
Figure 3.7	Western blots phospho-ERK, phospho-AKT and c-Myc; SUM-149 cells were treated with 1 $\mu$ M concentration of HSB-510 nanoformulation; 6h treatment was given for phospho-ERK and phospho-AKT analysis, 24h treatment was given for c-Myc analysis.	101
Figure 3.8	Bar graph representation of western blot analysis plotted using Image-J software; SUM-149 cells were treated with 1 $\mu$ M concentration of HSB-510 nanoformulation; 6h treatment was given for phospho-ERK and phospho-AKT analysis, 24h treatment was given for c-Myc analysis.	101
Figure 3.9	Dosing routine of anti-cancer agent encapsulated NPs.	103
Figure 3.10	Relative tumor growth inhibition recorded for untreated and treated mice groups; <b>(A)</b> relative EAC tumor growth inhibition of PI3-K $\delta$ /HDAC6 dual inhibitor (●, 12.5 mg/kg), HSB-510 (●, 12.5 mg/kg), and HSB-510 (●, 25 mg/kg) as compared to PBS treated mice group (○); <b>(B)</b> Relative tumor volume of PI3-K $\delta$ /HDAC6 dual inhibitor (●, 12.5 mg/kg), HSB-510 (●, 12.5 mg/kg), and HSB-510 (●, 25 mg/kg) as compared to PBS treated mice group (○) on day 29.	104
Figure 3.11	Relative tumor growth inhibition recorded for untreated and treated mice groups; <b>(A)</b> relative EAC tumor growth inhibition of IDL-NPs (▲, 25 mg/kg), SAHA-NPs (■, 25 mg/kg) and HSB-510 (●, 25 mg/kg); <b>(B)</b> Relative tumor volume of IDL-NPs (▲, 25 mg/kg), SAHA-NPs (■, 25 mg/kg) and HSB-510 (●, 25 mg/kg) as compared to PBS treated mice group (○) on day 29. All doses were given intravenously twice a week for three weeks in lateral tail vein of mice.	105

- Figure 3.12 Toxicity and survival studies of untreated and treated mice groups; 106  
**(A)** average mice body weight plots of PI3-K $\delta$ /HDAC6 dual inhibitor (●, 12.5 mg/kg), HSB-510 (●, 25 mg/kg) as compared to PBS treated mice group (○); **(B)** mice survival proportions of PI3-K $\delta$ /HDAC6 dual inhibitor (green, 12.5 mg/kg), HSB-510 (blue, 12.5 mg/kg), and HSB-510 (red, 25 mg/kg) as compared to PBS treated mice group (black). The doses were given intravenously in lateral tail vein of mice bearing EAC breast cancer tumor.
- Figure 3.13 Toxicity and survival studies of untreated and treated mice groups; 107  
**(A)** average mice body weight plots of IDL-NPs (▲, 25 mg/kg), SAHA-NPs (■, 25 mg/kg) and HSB-510 (●, 25 mg/kg); **(B)** mice survival proportions of IDL-NPs (yellow, 25 mg/kg), SAHA-NPs (purple, 25 mg/kg) and HSB-510 (red, 25 mg/kg); ) as compared to PBS treated mice group (black). The doses were given intravenously in lateral tail vein of mice bearing EAC breast cancer tumor.
- Figure 3.14 Toxicity studies of HSB-510 25 mg/kg twice a week for three 108  
weeks; **(A, B, and C)** Hepatotoxicity studies; **(D and E)** Nephrotoxicity studies
- Figure 3.15 H&E stained vital organ (heart, lung, liver, spleen and kidney) 109  
sections of untreated and HSB-510 (25 mg/kg) treated mice.
- Chapter IV Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles mediated co-delivery of PI3-K $\delta$ /HDAC6 dual inhibitor and Navitoclax for ER+ breast cancer therapy**
- Figure 4.1 Chemical name and structure of **(A)** PI3-K $\delta$ /HDAC6 dual 124  
inhibitor and **(B)** Navitoclax (BCL-2/BCL-xL inhibitor).

- Figure 4.2 Characterization and in-vitro release studies of NPs. (A) Hydrodynamic size of drug encapsulated NPs (n=3); (B) and (C) TEM image of PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs); (D) In-vitro release kinetics of PI3-K $\delta$ /HDAC6-NPs (n=3),  $\square$  shows percent release per day,  $\blacksquare$  shows percent cumulative release; (E) In-vitro release kinetics of NAV- NPs (n=3),  $\circ$  shows percent release per day,  $\bullet$  shows percent cumulative release; (F) In-vitro release kinetics of PI3-K $\delta$ /HDAC6-NAV-NPs (n=3),  $\blacktriangledown$  shows cumulative release of PI3-K $\delta$ /HDAC6 dual inhibitor and  $\blacktriangle$  shows cumulative release of Navitoclax. 130
- Figure 4.3 In-vitro cytotoxicity studies (n=3). (A, B and C) Comparative percent cell growth inhibition plots of NAV-NPs ( $\bullet$ ), PI3-K $\delta$ /HDAC6-NPs ( $\square$ ), and PI3-K $\delta$ /HDAC6-NAV-NPs {1:1-NPs ( $\blacklozenge$ ), 3:1-NPs ( $\blacktriangle$ ), and 1:3-NPs ( $\blacktriangledown$ )}; (D, E and F) Comparative percent cell viability bar graphs of drug encapsulated NPs at 10 $\mu$ M concentration. 134
- Figure 4.4 Combination index analysis (n=3). Combination index value vs Fraction affected plots of PI3-K $\delta$ /HDAC6-Nav-NPs {1:1-NPs ( $\blacklozenge$ ), 3:1-NPs ( $\blacktriangle$ ), and 1:3-NPs ( $\blacktriangledown$ )} in MCF7 cells (A), ZR-75-1 cells (B), and EAC cells (C). 135
- Figure 4.5 In-vivo therapeutic efficacy of Nav-NPs (n=5), PI3-K $\delta$ /HDAC6-NPs (n=5), and PI3-K $\delta$ /HDAC6-Nav-NPs (1:3-NPs) (n=5) in EAC syngeneic mice ER<sup>+</sup> breast cancer model. (A) Relative tumor volume percentage of untreated group ( $\bullet$ ), PI3-K $\delta$ /HDAC6-NPs treated group ( $\blacksquare$ ), NAV-NPs treated group ( $\blacktriangle$ ) and PI3-K $\delta$ /HDAC6-Nav-NPs (1:3-NPs) treated group ( $\blacktriangledown$ ); (B) Relative tumor volume percentage of independent mice from each group, PBS ( $\bullet$ ), PI3-K $\delta$ /HDAC6-NPs ( $\blacksquare$ ), Nav-NPs ( $\blacktriangle$ ) and PI3-K $\delta$ /HDAC6-Nav-NPs (1:3-NPs) group ( $\blacktriangledown$ ). 137

Figure 4.6	In-vivo therapeutic efficacy of Nav-NPs (n=5), PI3-K $\delta$ /HDAC6-NPs (n=5), and PI3-K $\delta$ /HDAC6-Nav-NPs (1:3-NPs) (n=5) in EAC syngeneic mice ER <sup>+</sup> breast cancer model. <b>(A)</b> Average body weight of mice treated with NAV-NPs ( $\blacktriangle$ ), PI3-K $\delta$ /HDAC6-NPs ( $\blacksquare$ ), and PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) group ( $\blacktriangledown$ ); <b>(B)</b> Kaplan Meir survival curves of EAC cell line-derived syngeneic breast cancer tumor bearing mice upon intravenous administration of PBS (black), PI3-K $\delta$ /HDAC6-NPs (blue), NAV-NPs (green) and PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) group (red). Mice were treated twice a week for three weeks.	138
Figure 4.7	Toxicity evaluation of PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) on EAC tumor bearing mice (n=3). <b>(A)</b> Hepatotoxicity and <b>(B)</b> nephrotoxicity of healthy, untreated and PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) treated mice.	139
Figure 4.8	Toxicity evaluation of PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) on EAC tumor bearing mice. H&E stained images of heart, lung, liver, spleen and kidney of untreated and PI3-K $\delta$ /HDAC6-NAV-NPs (1:3-NPs) treated mice.	140
<b>Chapter V</b>	<b>Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles mediated co-delivery of PI3-K<math>\delta</math>/HDAC6 dual inhibitor and Epirubicin for breast cancer therapy</b>	
Figure 5.1	Chemical name and structure of <b>(A)</b> PI3-K $\delta$ /HDAC6 dual inhibitor and <b>(B)</b> Epirubicin (Anthracycline).	154
Figure 5.2	Characterization and in-vitro studies of NPs. <b>(A)</b> Comparative hydrodynamic size distribution profiles of single and dual drug-NPs; <b>(B)</b> and <b>(C)</b> TEM image of PI3-K $\delta$ /HDAC6-Epi-NPs (1:3-NPs); <b>(D)</b> Release kinetics of PI3-K $\delta$ /HDAC6-NPs, $\blacksquare$ shows percent cumulative release; <b>(E)</b> Release kinetics of Epi-NPs, $\blacksquare$ shows percent cumulative release; <b>(F)</b> In-vitro release profile of PI3-K $\delta$ /HDAC6-Epi-NPs (1:3-NPs), $\blacksquare$ shows cumulative release	161

of Epirubicin and ■ shows cumulative release of PI3-K $\delta$ /HDAC6 dual inhibitor.

- Figure 5.3 Cellular internalization of Epi-NPs. **A-** CLSM images of cellular internalized Epi-NPs in 2D SUM149 cells; **B-** CLSM images of cellular internalized Epi-NPs in 3D SUM149 mammosphere. 162
- Figure 5.4 In-vitro cytotoxicity studies. Comparative % cell inhibition plots of PI3-K $\delta$ /HDAC6-NPs (■), Epi-NPs (●), and PI3-K $\delta$ /HDAC6-Epi-NPs {1:3-NPs (▼), 3:1-NPs (▲), and 1:1-NPs (◆)} in MCF7 cells (**A**), SUM149 cells (**B**), and EAC cells (**C**). 166
- Figure 5.5 In-vitro cytotoxicity studies. Comparative % cell viability bar graphs of drug-NPs at 1 $\mu$ M concentration of PI3-K $\delta$ /HDAC6-NPs (■), Epi-NPs (●), and PI3-K $\delta$ /HDAC6-Epi-NPs {1:3-NPs (▼), 3:1-NPs (▲), and 1:1-NPs (◆)} in MCF7 cells (**A**), SUM149 cells (**B**), and EAC cells (**C**). 167
- Figure 5.6 In-vitro cytotoxicity studies. (**A**) IC<sub>50</sub> plot of PI3-K $\delta$ /HDAC6-NPs, Epi-NPs and 1:3-NPs on SUM149 mammospheres. (**B**) Activity of PI3-K $\delta$ /HDAC6-NPs, Epi-NPs and 1:3-NPs on SUM149 mammospheres at 10 $\mu$ M concentration. 168
- Figure 5.7 In-vitro cytotoxicity studies. Combination index (CI) value vs Fraction affected (FA) plots of PI3-K $\delta$ /HDAC6-Epi-NPs {1:3-NPs (▼), 3:1-NPs (▲), and 1:1-NPs (◆) in MCF7 cells (**A**), SUM149 cells (**B**), and EAC cells (**C**). 169
- Figure 5.8 In-vivo therapeutic efficacy of PI3-K $\delta$ /HDAC6-NPs, Epi-NPs and PI3-K $\delta$ /HDAC6-Epi-NPs in EAC syngeneic mice breast cancer model. (**A**) Relative tumor volume % of untreated group (●), PI3-K $\delta$ /HDAC6-NPs treated group (■), Epi-NPs treated group (●) and PI3-K $\delta$ /HDAC6-Epi-NPs treated group (▼); (**B**) Ex-vivo images of tumors from each group taken on day 20. 171

- Figure 5.9 In-vivo therapeutic efficacy of PI3-K $\delta$ /HDAC6-NPs, Epi-NPs and PI3-K $\delta$ /HDAC6-Epi-NPs in EAC syngeneic mice breast cancer model. (A) Relative tumor volume % of independent mice from each group, PBS (●), PI3-K $\delta$ /HDAC6-NPs (■), Epi-NPs (●) and PI3-K $\delta$ /HDAC6-Epi-NPs group (▼) on day 20; (B) Average body weight of mice treated with PI3-K $\delta$ /HDAC6-NPs (■), Epi-NPs (●), and PI3-K $\delta$ /HDAC6-Epi-NPs (▼); (C) Kaplan Meir survival curves of EAC syngeneic breast cancer tumor bearing mice upon intravenous administration of PBS (black), PI3-K $\delta$ /HDAC6-NPs (blue), Epi-NPs (green) and PI3-K $\delta$ /HDAC6-Epi-NPs (red). Mice were treated twice a week for three weeks. 172
- Figure 5.10 Toxicity evaluation of PI3-K $\delta$ /HDAC6-Epi-NPs on EAC tumor bearing mice. (A) Hepatotoxicity and (B) Nephrotoxicity of healthy, untreated and PI3-K $\delta$ /HDAC6-Epi-NPs treated mice; (C) H&E stained images of heart tissue section of healthy and PI3-K $\delta$ /HDAC6-Epi-NPs treated mice. 173
- Chapter VI Tumor targeted delivery of anti-cancer agents using Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles for breast cancer therapy**
- Figure 6.1 Chemical name and structure of (A) PI3-K $\delta$ /HDAC6 dual inhibitor; (B) Navitoclax (BCL-2/BCL-xL inhibitor) and (C) Epirubicin (Anthracycline). 186
- Figure 6.2 Encapsulation efficiency of anti-cancer agents into Pluronic modified PLA block copolymeric nanoparticles. 192
- Figure 6.3 Hydrodynamic diameter and zeta potential of prepared nanoparticles. Comparative hydrodynamic diameter of PI3-K $\delta$ /HDAC6 encapsulated NPs (A), Navitoclax encapsulated NPs (B), and Epirubicin encapsulated NPs (C). Comparative zeta potential of PI3-K $\delta$ /HDAC6-NPs (D), Navitoclax-NPs (E), and Epirubicin-NPs (F). 193

Figure 6.4	TEM images of prepared nanoparticles. <b>(A and B)</b> TEM images of uncoated nanoparticles; <b>(C and D)</b> TEM images of CMP-Epi-NPs; <b>(E)</b> TEM image of CMP-Nav-NPs; <b>(F)</b> TEM image of CMP-PI3-K $\delta$ /HDAC6-NPs.	194
Figure 6.5	Characterization of cell membrane proteins. <b>(A)</b> SDS-PAGE protein analysis of cell membrane proteins (CMPs) of J774A.1 cells, EAC cells and CMP-NPs (A- ~10 $\mu$ g CMP and B- ~20 $\mu$ g CMP); <b>(B)</b> Macrophage and cancer cell membrane specific markers characterization by western blot analysis.	196
Figure. 6.6	In-vitro release studies of anti-cancer agent encapsulated NPs; <b>(A)</b> Per day and <b>(B)</b> cumulative percent release of PI3-K $\delta$ /HDAC6 dual inhibitor from PI3-K $\delta$ /HDAC6-NPs and CMP-PI3-K $\delta$ /HDAC6-NPs; <b>(C)</b> Per day and <b>(D)</b> cumulative percent release of Navitoclax from Nav-NPs and CMP-Nav-NPs; <b>(E)</b> Per day and <b>(F)</b> cumulative percent release of Epirubicin from Epi-NPs and CMP-Epi-NPs.	197
Figure 6.7	Intracellular uptake of Epi-NPs and CMP-Epi-NPs in J774A.1 and EAC cells. Cells were imaged after 45 min incubation.	198
Figure 6.8	Quantitative analysis of Epi fluorescence from Epi and CMP-Epi-NPs.	199
Figure 6.9	IC <sub>50</sub> plots of anti-cancer agent encapsulated nanoparticles in EAC cell line. <b>(A)</b> PI3-K $\delta$ /HDAC6 dual inhibitor encapsulated NPs; <b>(B)</b> Navitoclax encapsulated NPs; <b>(C)</b> Epirubicin encapsulated NPs.	200
Figure 6.10	Biodistribution studies of CMP-ICG-NPs in EAC tumor bearing mice. <b>(A and B)</b> In-vivo distribution of ICG, ICG-NPs and CMP-ICG-NPs; <b>(C)</b> Quantitative distribution of ICG, ICG-NPs and CMP-ICG-NPs.	202

- Figure 6.11 Relative tumor growth inhibition recorded for untreated and treated mice groups; **(A)** relative EAC tumor growth inhibition of PI3-K $\delta$ /HDAC6-NPs (●, 12.5 mg/kg), CMP-PI3-K $\delta$ /HDAC6-NPs (●, 12.5 mg/kg) as compared to PBS treated mice group (○); **(B)** Relative tumor volume of PI3-K $\delta$ /HDAC6-NPs (●, 12.5 mg/kg), CMP-PI3-K $\delta$ /HDAC6-NPs (●, 12.5 mg/kg) as compared to PBS treated mice group (○) on day 32. 204
- Figure 6.12 Relative tumor growth inhibition recorded for untreated and treated mice groups; **(A)** relative EAC tumor growth inhibition of Nav-NPs (●, 4 mg/kg), CMP-Nav-NPs (●, 4 mg/kg) as compared to PBS treated mice group (○); **(B)** Relative tumor volume of Nav-NPs (●, 4 mg/kg), CMP-Nav-NPs (●, 4 mg/kg) as compared to PBS treated mice group (○) on day 32. 205
- Figure 6.13 Relative tumor growth inhibition recorded for untreated and treated mice groups; **(A)** relative EAC tumor growth inhibition of Epi-NPs (●, 3 mg/kg), CMP-Epi-NPs (●, 3 mg/kg) as compared to PBS treated mice group (○); **(B)** Relative tumor volume of Epi-NPs (●, 3 mg/kg), CMP-Epi-NPs (●, 3 mg/kg) as compared to PBS treated mice group (○) on day 32. 206
- Figure 6.14 Survival studies of untreated and treated mice groups; **(A)** mice survival proportions of PI3-K $\delta$ /HDAC6 encapsulated NPs as compared to PBS treated mice group (black); **(B)** mice survival proportions of Navitoclax encapsulated NPs as compared to PBS treated mice group (black); **(C)** mice survival proportions of Epirubicin encapsulated NPs as compared to PBS treated mice group (black). The doses were given intravenously in lateral tail vein of mice bearing EAC breast cancer tumor. 207

## List of Tables

<b>Chapter I</b>	<b>Introduction &amp; literature review</b>	<b>Page no.</b>
Table 1.1	Combination therapies comprising two or more anti-cancer agents in clinical practice or in clinical development.	16
Table 1.2	Dual targeted inhibitors of cell signaling pathways in clinical development.	18
Table 1.3	Liposomal nanocarriers in market or in clinical development.	22
Table 1.4	Polymeric nanocarriers in market or in clinical development.	24
<b>Chapter II</b>	<b>Synthesis, characterization, and biological evaluation of Pluronic<sup>®</sup> modified poly(lactic acid) block copolymeric nanoparticles</b>	
Table 2.1	Characterization of Pluronic <sup>®</sup> modified PLA block copolymers	65
Table 2.2	Characterization of nanoparticles prepared using Pluronic <sup>®</sup> modified PLA block copolymers. Size and zeta potential were measured using DLS instrument.	71
<b>Chapter III</b>	<b>Pluronic<sup>®</sup> modified poly(lactic acid) block copolymeric nanoparticles mediated delivery of PI3-K<math>\delta</math>/HDAC6 dual inhibitor for cancer therapy</b>	
Table 3.1	Characterization of anti-cancer agent encapsulated Pluronic <sup>®</sup> modified PLA block copolymeric nanoparticles.	94
Table 3.2	IC <sub>50</sub> values of anti-cancer agent encapsulated NPs in breast cancer cell lines.	100

**Chapter IV Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles mediated co-delivery of PI3-K $\delta$ /HDAC6 dual inhibitor and Navitoclax for ER+ breast cancer therapy**

Table 4.1	Characterization of drug encapsulated Pluronic <sup>®</sup> modified PLA block copolymeric (quatramer) nanoparticles	129
Table 4.2	IC <sub>50</sub> values of free PI3-K $\delta$ /HDAC6 dual inhibitor, Navitoclax and their combinations in ER <sup>+</sup> breast cancer cell lines	132
Table 4.3	IC <sub>50</sub> values of drug encapsulated nanoparticles in ER <sup>+</sup> breast cancer cell lines	132
Table 4.4	CI value of free PI3-K $\delta$ /HDAC6 dual inhibitor and Navitoclax combinations in ER+ breast cancer cell lines	133
Table 4.5	CI value of PI3-K $\delta$ /HDAC6-NAV-NPs in ER+ breast cancer cell lines	133

**Chapter V Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles mediated co-delivery of PI3-K $\delta$ /HDAC6 dual inhibitor and Epirubicin for breast cancer therapy**

Table 5.1	Characterization of drug encapsulated Pluronic <sup>®</sup> modified PLA block copolymeric (quatramer) nanoparticles	160
Table 5.2	IC <sub>50</sub> values of free PI3-K $\delta$ /HDAC6, Epirubicin and their combinations in breast cancer cell lines.	164
Table 5.3	IC <sub>50</sub> values of drug encapsulated nanoparticles in breast cancer cell lines	164
Table 5.4	CI value of free PI3-K $\delta$ /HDAC6 dual inhibitor and Epirubicin combinations in breast cancer cell lines	165
Table 5.5	CI value of PI3-K $\delta$ /HDAC6-Epi-NPs in breast cancer cell lines	165

**Chapter VI Tumor targeted delivery of anti-cancer agents using Pluronic<sup>®</sup> modified polylactic acid block copolymeric nanoparticles for breast cancer therapy**

Table 6.1	Characterization of drug encapsulated Pluronic <sup>®</sup> modified PLA block copolymeric nanoparticles	192
Table 6.2	IC <sub>50</sub> value of free drug and drug encapsulated nanoparticles in EAC cell line	201

## List of Abbreviations

<b>ACN</b>	Acetonitrile
<b>AKT</b>	Ak strain transforming
<b>ALT</b>	Alanine transaminase
<b>AML</b>	Acute myeloid leukemia
<b>ANOVA</b>	Analysis of variance
<b>AST</b>	Aspartate transaminase
<b>BCA</b>	Bicinchoninic acid
<b>BCL-2</b>	B-cell lymphoma 2
<b>BCL-xL</b>	B-cell lymphoma-extra large
<b>BCR</b>	B cell receptor
<b>CLSM</b>	Confocal laser scanning microscope
<b>CMC</b>	Critical micelle concentration
<b>CMP</b>	Cell membrane protein
<b>c-Myc</b>	Cytosolic-Myelocytomatosis
<b>CPCSEA</b>	Committee for the Purpose of control and supervision of experiments on animals
<b>DAPI</b>	4',6-diamidino-2-phenylindole
<b>DCM</b>	Dichloromethane
<b>DLS</b>	Dynamic light scattering
<b>DMEM</b>	Dulbecco's modified eagle medium
<b>DMSO</b>	Dimethyl sulfoxide

<b>DNA</b>	Deoxyribonucleic acid
<b>Dox</b>	Doxorubicin
<b>EAC</b>	Ehrlich ascites carcinoma
<b>EDTA</b>	Ethylenediamine tetra acetic acid
<b>EE%</b>	Encapsulation efficiency percentage
<b>EGFR</b>	Epidermal growth factor receptor
<b>Epi</b>	Epirubicin
<b>EPR</b>	Enhanced permeability and retention effect
<b>ER+</b>	Estrogen receptor positive
<b>ERK</b>	Extracellular signal-regulated kinase
<b>FA</b>	Fraction affected
<b>FACS</b>	Fluorescence activated cell sorting
<b>FBS</b>	Fetal bovine serum
<b>FDA</b>	Food and drug administration
<b>FOXO</b>	Forkhead box O
<b>GFR</b>	Growth factor receptor
<b>gm</b>	Gram
<b>GPC</b>	Gel permeation chromatography
<b>GPCR</b>	G-protein coupled receptor
<b>GSK-3</b>	Glycogen synthase kinase 3
<b>h</b>	Hour
<b>H&amp;E</b>	Hematoxylin and eosin

<b>HDAC</b>	Histone deacetylase
<b>HDACi</b>	Histone deacetylase inhibitor
<b>HER-2</b>	Human epidermal growth factor receptor 2
<b>HPLC</b>	High performance liquid chromatography
<b>IC50</b>	Half-maximal inhibitory concentration
<b>ICG</b>	Indocyanine green
<b>IDL</b>	Idelalisib
<b>IV</b>	Intravenous
<b>kDa</b>	Kilodalton
<b>Kg</b>	Kilogram
<b>LALS</b>	Low-angle light scattering
<b>MAP</b>	Mitogen activated protein
<b>MAPK</b>	Mitogen activated protein kinase
<b>MDR</b>	Multidrug resistance
<b>MeOH</b>	Methanol
<b>Mg</b>	Milligram
<b>Min</b>	Minutes
<b>mM</b>	Millimolar
<b>MM</b>	Multiple myeloma
<b>mm</b>	Millimetre
<b>mTOR</b>	Mammalian target of rapamycin
<b>MTT</b>	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

<b>mV</b>	Millivolt
<b>n</b>	Number
<b>NAV</b>	Navitoclax
<b>NCATS</b>	National Centre for Advancing Translational Sciences
<b>NCCS</b>	National Centre for Cell Science
<b>NHL</b>	Non-Hodgkin lymphoma
<b>NIH</b>	National institute of Health
<b>nm</b>	Nanometre
<b>NMR</b>	Nuclear magnetic resonance
<b>NPs</b>	Nanoparticles
<b>NSCLC</b>	Non-small cell lung carcinoma
<b>PAM</b>	PI3K/AKT/mTOR pathway
<b>PBS</b>	Phosphate buffer saline
<b>PCL</b>	Polycaprolactone
<b>PDI</b>	Polydispersity index
<b>PEG</b>	Polyethylene glycol
<b>PGA</b>	Polyglutamic acid
<b>Pgp</b>	P-glycoprotein
<b>pH</b>	Potential of hydrogen
<b>PI</b>	Propidium iodide
<b>PI3K</b>	Phosphoinositide 3-kinase
<b>PK</b>	Pharmacokinetics

<b>PLA</b>	Polylactic acid
<b>PLGA</b>	Polylactic-co-glycolic acid
<b>PPG</b>	Polypropylene glycol
<b>PTEN</b>	Phosphatase and TENsin
<b>RALS</b>	Right angle light scattering
<b>RBC</b>	Red blood cells
<b>Rho-B</b>	Rhodamine B
<b>RI</b>	Refractive index
<b>RNA</b>	Ribonucleic acid
<b>ROI</b>	Region of interest
<b>rpm</b>	Rotation per minute
<b>RT</b>	Room temperature
<b>RTGI</b>	Relative tumor growth inhibition
<b>RTK</b>	Receptor tyrosine kinase
<b>SD</b>	Standard deviation
<b>TCR</b>	T cell receptor
<b>TEM</b>	Transmission electron microscope
<b>THF</b>	Tetrahydrofuran
<b>TLR</b>	Toll like receptor
<b>WHO</b>	World health organisation
<b>μg</b>	Microgram
<b>μl</b>	Microlitre
<b>μM</b>	Micromolar