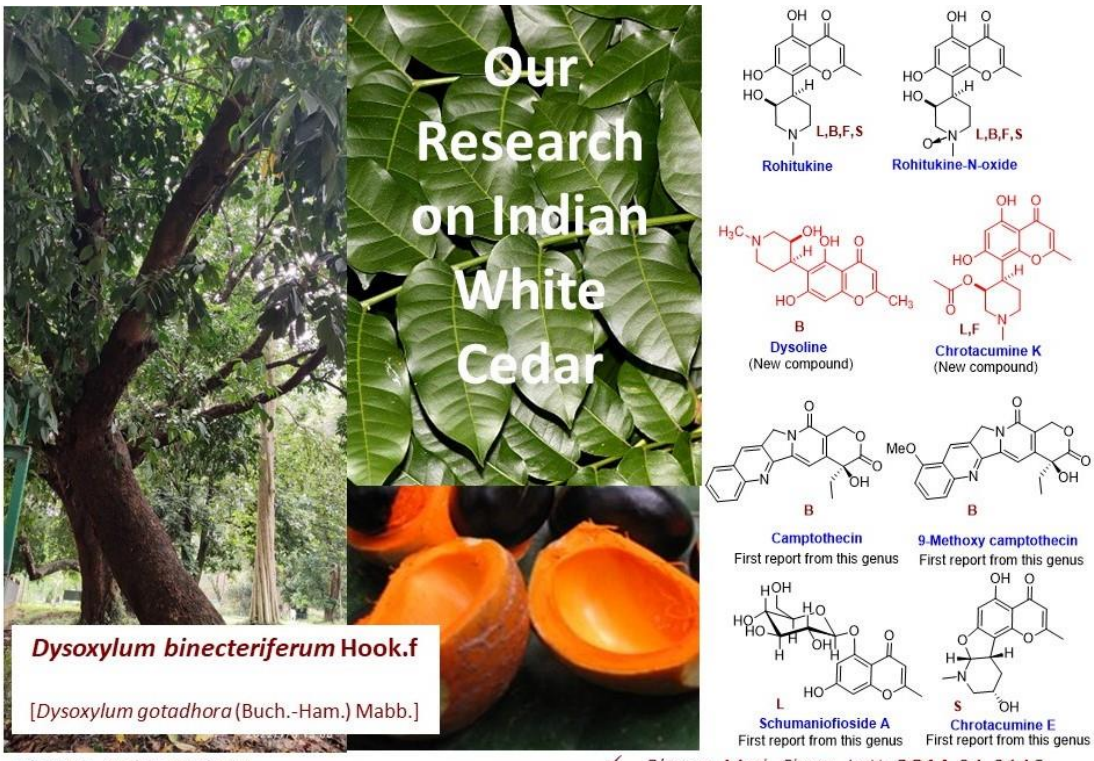
d) Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

Two key areas of research – Anticancer & Anti-Alzheimer's drug discovery

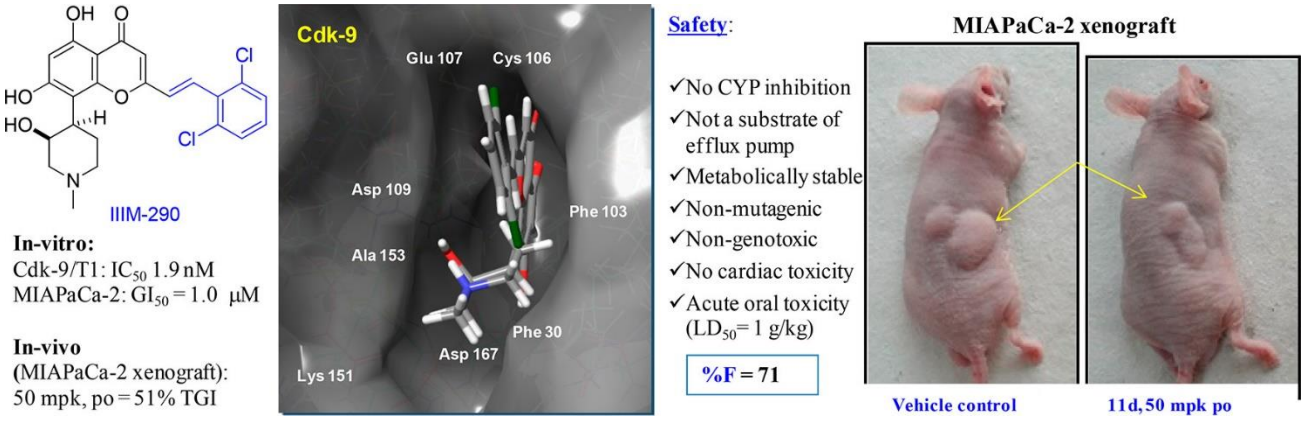
A. Discovery of small molecule kinase inhibitors as potential cancer therapeutics. The primary research focus of Dr. Sandip Bharate's research group is medicinal chemistry targeted at kinases as molecular targets to discover anticancer leads. Over the past decade, his group has significantly contributed to the discovery of small-molecule kinase inhibitors. One of the most advanced discoveries from his research group is IIIM-290 which has reached to the clinical trials. It is an outcome of his research on a medicinal plant, Indian white cedar. The phytochemical investigation of the Indian white cedar (*Dysoxylum binecteriferum*) provided various secondary metabolites, including rohitukine, rohitukine *N*-oxide, dysoline, schumaniofioside A, chrotacumine K, and camptothecin.



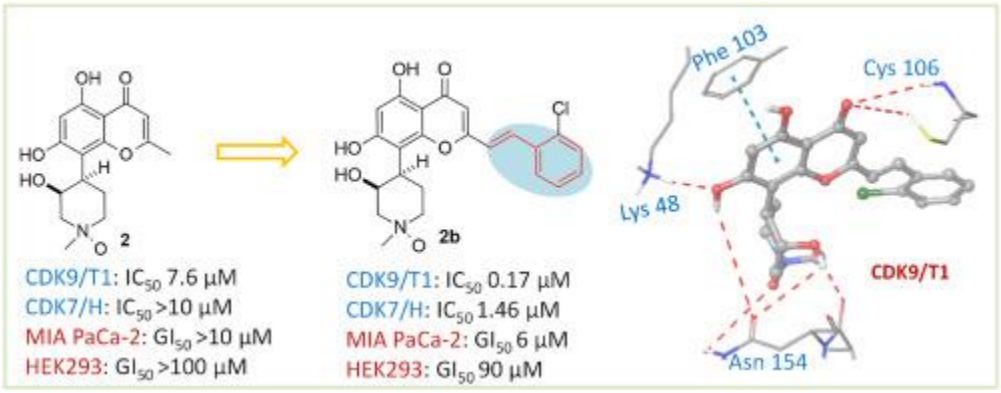
Bharate and coworkers:

- ✓ *Tetrahedron Lett.* **2013**, 54, 7140
- ✓ IN **2013**DE01077A; WO2014167580A1
- ✓ *Bioorg. Med. Chem. Lett.* **2014**, 24, 3146
- ✓ *Bioorg. Med. Chem. Lett.* **2016**, 26, 3457
- ✓ *Tetrahedron Lett.* **2017**, *58*, 3974

The major constituent, rohitukine, inhibits CDK9/cyclin T1 with an IC_{50} value of 0.3 μM. His group isolated rohitukine in multi-gram quantities from the leaves of the White Cedar tree and pursued synthetic modifications on it. The medicinal chemistry efforts on rohitukine yielded a potent CDK9 inhibitor, IIIM-290 which is effective in human xenograft models of pancreatic cancer (MIA PaCa-2) [WO2014170914A1- IP granted in US, EP, IN-US9932327B2(April 2018); EP2986605 (Nov 2017); GB2986605 (Nov 2017); IN322330 (Oct 2019)]. The lead is orally bioavailable and displayed excellent safety profile. The work is published as: *J. Med. Chem.* **2018**, 61, 1664-1687.[1] Recently another orally bioavailable CDK9 inhibitor has been discovered (*Eur. J. Med. Chem.* **2023**, *258, 115533*).[3]

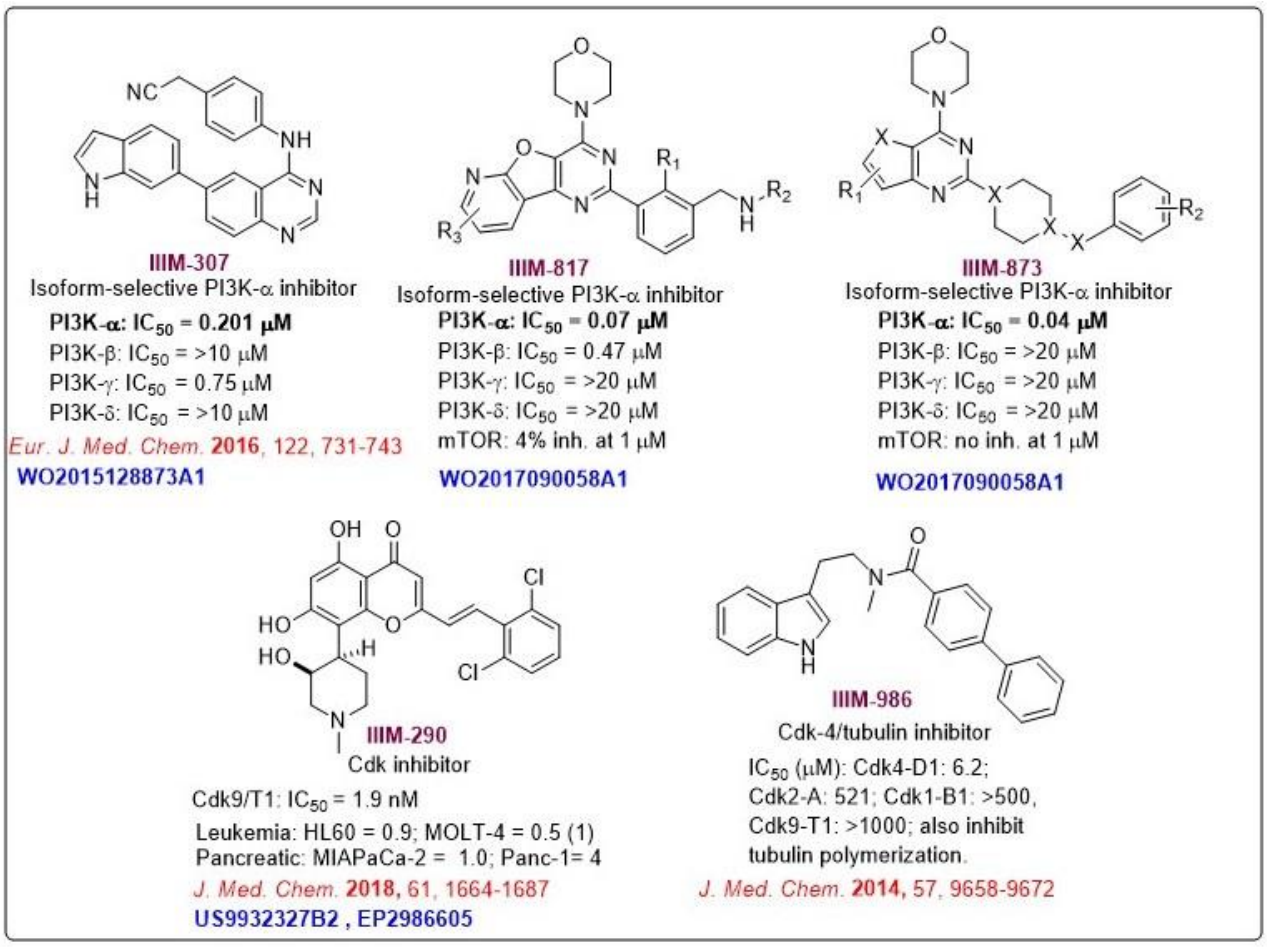


(*J. Med. Chem.* **2018**, 61, 1664-1687)



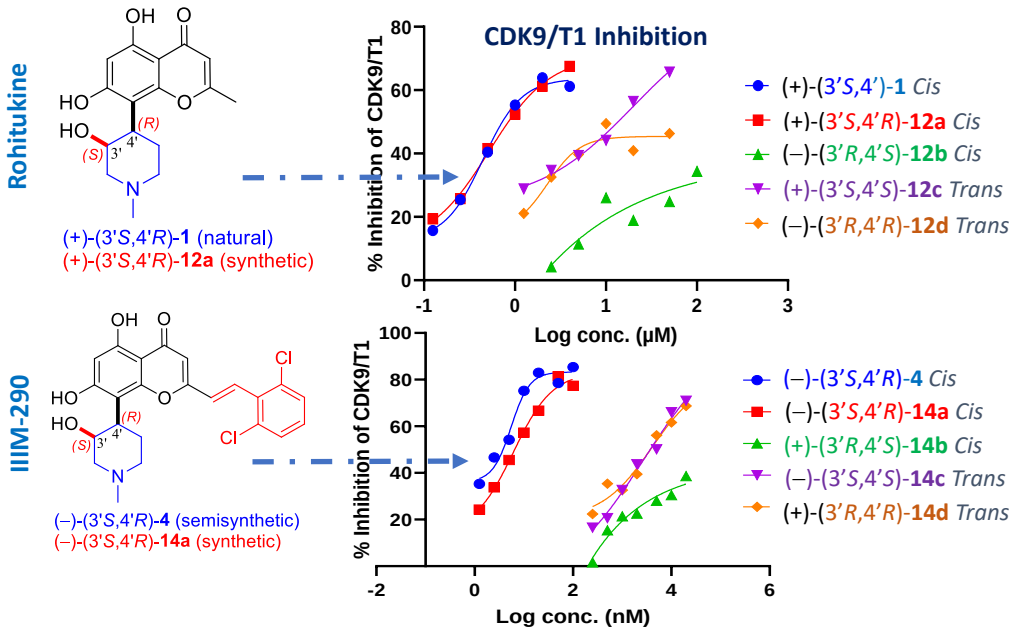
(*Eur. J. Med. Chem.* **2023**, *258, 115533*).

The IND-enabling studies, including GLP-regulatory toxicology and CMC documentation is completed. The IND application for a small molecule, IIIM-290 was filed in January 2020; which has been granted by CDSCO, and gave the permission to conduct phase I/II clinical trial in metastatic pancreatic cancer patients.



(Kinase inhibitors discovered from Dr. Bharate's group)

To take care of the future need of the API and to address the commercial feasibility aspect, recently the total synthesis of IIIM-290 has been accomplished by Dr Bharate's group. His group has synthesized IIIM-290 along with its other three stereoisomers to understand the impact of stereochemistry on the biological activity. The work is under consideration for publication (revision is submitted).

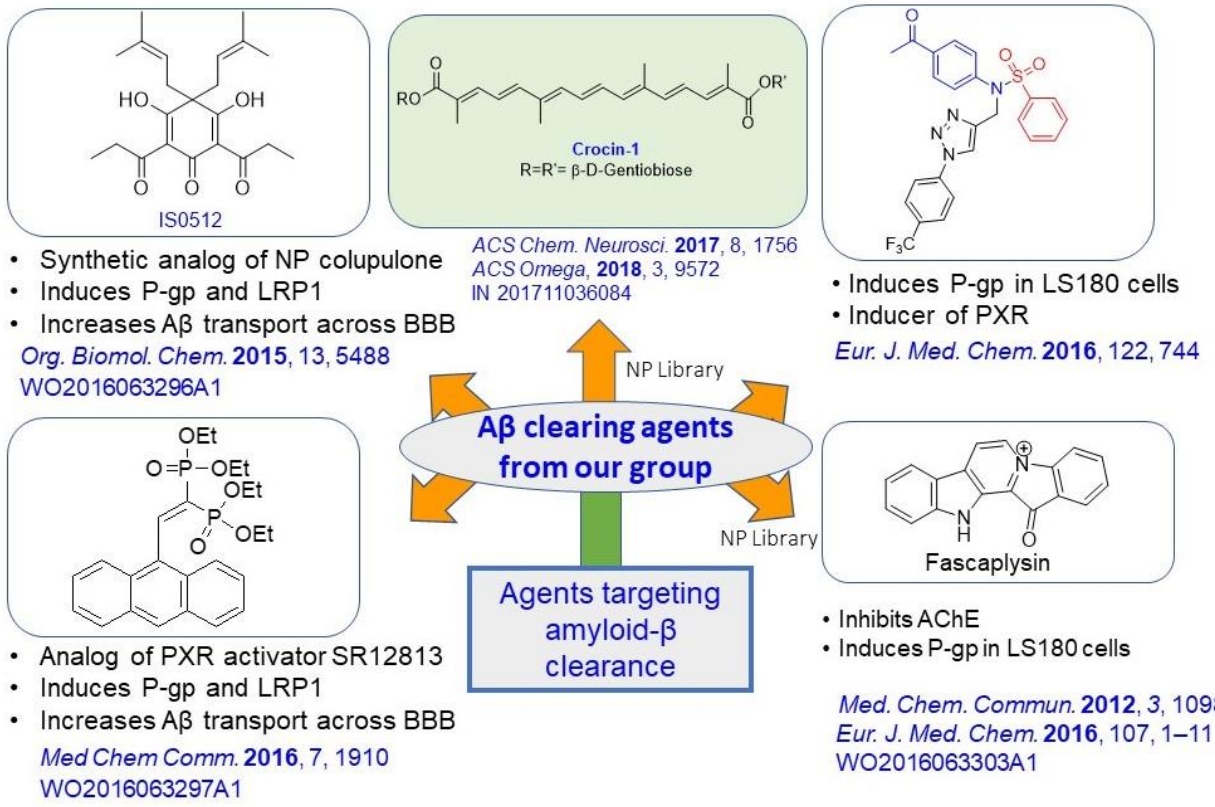


Besides, his group has discovered isoform-selective PI3K-alpha inhibitors, that are effective in in-vivo tumor models of breast cancer. The alpha-isoform-specific inhibitor, IIIM-873, inhibits PI3K-alpha with IC_{50} value of 40 nM and does not inhibit other three isoforms (beta, gamma and delta) of PI3K (IC_{50} > 20 μM) [IP: WO2017090058A1; granted in US, EP and IN - US10696688 (June 2020), EP 3380476 (Sept 2020), GB 3380476 (Sept 2020), IN359878 (March 2021)]. This lead is currently under IND-enabling studies for triple-negative breast cancer.

The group's current focus is finding selective CDK7/9 inhibitor leads as anticancer agents with better safety profile.

B. Discovery of Small Molecules as disease-modifying agents for Alzheimer's disease (AD). "Alzheimer's disease (AD)" is the most common form of senile dementia and the fourth highest cause of disability and death in the elderly. It is characterized by the presence of three main brain hallmarks viz. diffuse neuronal loss with a particular involvement of the cholinergic system, extracellular protein deposits (amyloid plaques) and intracellular protein deposits (neurofibrillary tangles, NFTs). All current therapies are based on the cholinergic hypothesis and demonstrate only symptomatic treatment. Progression of the disease is not slowed or halted, with symptoms continuing to deteriorate over time. The amyloid-beta clearance

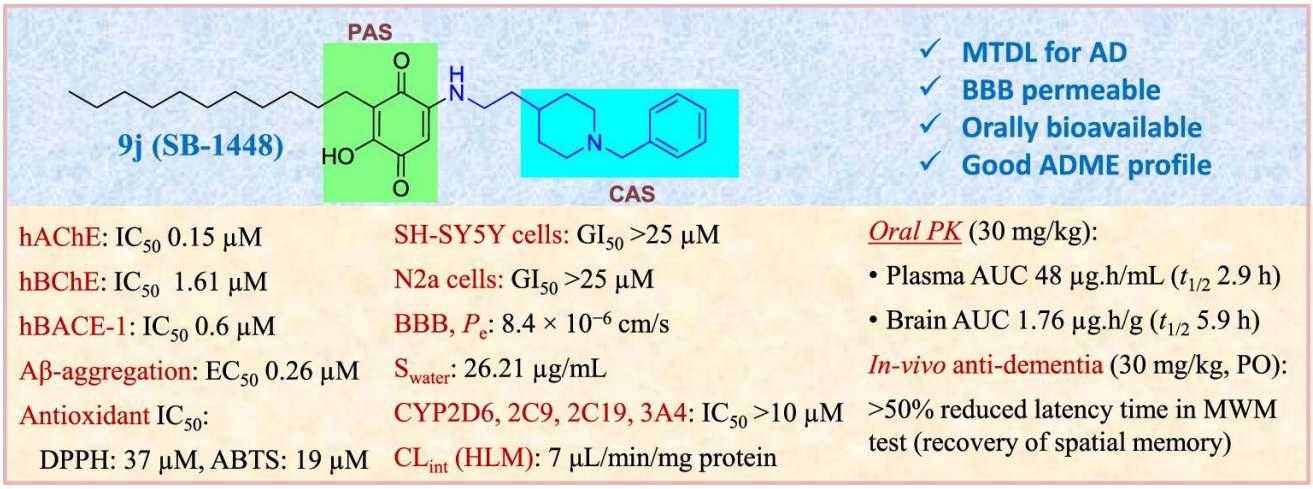
occurs primarily via p-glycoprotein efflux pump and several studies have shown that there exists a direct link between P-gp expression and amyloid-beta clearance from brain. Clinical data strongly supported these studies (Science 2010, 330, 1774), stating that decreased clearance of CNS amyloid-beta is the prime cause of AD. Thus, drugs with the ability to induce the expression of P-gp (and other transporters located at the BBB) have a great potential to emerge as novel AD therapeutics. In this direction, his group has made significant efforts to discover new Pgp inducers that can increase amyloid-beta clearance from AD brains. His medicinal chemistry efforts over the past decade, has resulted in the discovery of several small molecule Pgp inducers viz. phenyl benzene sulfonamides (Eur. J. Med. Chem. **2016**, 122, 744-755), aryl phosphonate esters (WO2016063297A1; Med. Chem. Commun. **2016**, 7, 1910-1915), fascaplysin and its derivatives (WO2016063303A1; Eur. J. Med. Chem. **2016**, 107, 1–11), colupulone derivatives (WO2016063296A1; Org. Biomol. Chem., **2015**, 13, 5488-5496), 4-arylquinoline-2-carboxylates (Org. Biomol. Chem. **2014**, 12, 6267-6277) and a botanical lead IIIM-141/17. The botanical lead was found to be the most promising P-gp inducer which displayed an ability to enhance amyloid-beta clearance both in in-vitro as well as in-vivo models. The in vivo studies confirmed the effect of this lead (50 mg/kg/day, added to mice diet) on the BBB tightness and function that was associated with reduced Aβ load and related pathological changes in 5XFAD mice used as an AD model (ACS Chem. Neurosci. **2017**, 8, 1756-1766).



Crocin and the crocin-rich extract of Saffron (IIIM-141) increased the Aβ-clearance in the 5XFAD transgenic AD mice model. Crocin hydrolyzes to its aglycone, crocetin in GIT, which reaches the blood and finally to the brain. Crocetin is the bioactive metabolite responsible for Saffron's biological effects. In 2018, the IIIM-141 was licensed to the industry for launching in the market as a nutraceutical product. Its preclinical development under a drug route is currently ongoing under CSIR Phytopharmaceutical Mission.

The current research focus of Dr Bharate's group is to discover a "small molecule" which can engage multiple (more than 2) molecular targets of the disease. The proposed multi-targeted agents are aimed to re-establish cholinergic machinery (by inhibiting either AChE, BChE, or MAO-B), stop the formation of amyloid plaques (by inhibiting BACE-1), and stop the hyperphosphorylation of tau (by inhibiting GSK-3β). A single lead compound acting against these targets belonging to different hallmarks of the disease, would be an excellent strategy to tackle the complex pathology of AD. In this direction, a library of pure natural products was screened for their effect on "multiple targets" available in the laboratory. His group has

screened 100 NPs for inhibition of AChE, BChE and BACE-1 and also for P-gp induction in cellular assay, to discover new multi-targeted agents. These efforts have resulted in the identification of two important scaffolds viz. fused indole alkaloids [Bioorg. Chem. 2019, 90, 103062] and 1,4-benzoquinone "embelin" [Drug Dev. Res., **2019**, 80, 655-665], which showed multi-targeted activity. Further, the lead optimization of the identified natural product hit, yielded an orally bioavailable, blood-brain barrier permeable small molecule, **SB-1448** that shows in vivo anti-dementia activity in mice model [ACS Chem Neurosci. **2023**, 14, 6, 1193–1219].[2] Besides this, recently, his group also discovered a few NLRP3 inflammasome inhibitors for Alzheimer's disease. The current research focus is the medicinal chemistry/ optimization of identified NP leads to discover potential disease-modifying anti-Alzheimer agents.



Key Publications:

1. **Bharate SB;*** Kumar V; Jain SK; Mintoo NJ; Guru SK; Nuthakki VK; Sharma M; Bharate SS; Gandhi SG; Mondhe DM; Bhushan S; Vishwakarma RA. Discovery and Preclinical Development of IIIM-290, an Orally Active Potent Cyclin-dependent Kinase Inhibitor. *J. Med. Chem.* **2018**, 61, 1664-1687.
2. Nuthakki VK, Choudhary S, Reddy CN, Bhatt S, Jamwal A, Jotshi A, Raghuvanshi, R, Sharma A, Thakur S, Jadhav H, Bharate SS, Nandi U, Kumar A, **Bharate SB*** Design, Synthesis, and

Pharmacological Evaluation of Embelin-Aryl/alkyl amine Hybrids as Orally Bioavailable Blood-Brain Barrier Permeable Multitargeted Agents with Therapeutic Potential in Alzheimer's Disease: Discovery of SB-1448. *ACS Chem Neurosci.* **2023**, *14, 6, 1193–1219.*

3. Bhurta D, Hossain MM, Bhardwaj M, Showket F, Nandi U, Dar MJ, **Bharate SB***. Orally Bioavailable Styryl Derivative of Rohitukine-N-oxide Inhibits CDK9/T1 and the Growth of Pancreatic Cancer cells. *Eur. J. Med. Chem.* **2023**, *258, 115533.*

(Dr. Sandip B. Bharate)