ABSTRACT

Protein kinases are the most studied targets in modern drug discovery majorly for cancer due to their key role in significant cell signaling pathways. In this thesis, we emphasize some of the chemical biology approaches across the two diverse chemical scaffolds, Myo-inositols, and phenothiazine. The first part of the thesis consists of the design and synthesis of the above scaffolds. These molecules are also studied for their novel structures using Single-crystal X-ray diffraction analysis. In the second part, the biological evaluation of these small molecules based on *in vitro* studies such as cell viability assay, cell death analysis, cell cycle arrest, and western blotting using specific antibodies of its downstream signaling protein.

In the second chapter, we have studied the Holy Grail of cancer called "RAS". RAS gene family is involved in multiple cellular functions consist of cell proliferation, survival, and gene expression is directly related to cancer pathogenesis, and often mutated in most of the human cancers. They have highly conserved G-domain (GTP/GDP binding regions) and share almost analogous activation/deactivation processes. In this prospect, *in silico* method has been used to recognize potential binding sites on RAS as a preliminary study to synthesize small molecules. In brief, the first objective of this chapter was to design and synthesize phosphate derivatives of Myo-inositol molecules as RAS inhibitors and designing of small molecules based on the recently reported inhibitors. We have also done biological studies on breast cancer cell lines of synthesized small molecule inhibitors for RAS inhibition. Our first-stage *in-vitro* screening of the in-house synthesized inhibitors showed that the compound **1b** (C2-O-phosphate derivative of Myo-inositol 1,3,5-orthobenzoate) inhibited the RAS-RAF-MEK-ERK signaling pathway. Besides, we also found that this compound induced cell death and causes cell cycle arrest at S phase. This class of molecules may work as a potential inhibitor of breast cancer caused by a mutation in KRAS and its downstream proteins. Though the efficacy of the molecules is in the micromolar scale, they have not been explored previously for RAS inhibition. This result could be helpful further to explore its detailed biological studies to get better candidates as RAS inhibitors and advance the hunt of the small molecule in the mutant KRAS driven cancers.

In the third chapter, we have studied new kinase inhibitors for a novel kinase called TLK (Tousled-like Kinase, TLK1/1B). The prominent expression of TLK1B was observed in approximately ~30% of breast cancer antigens. Targeting TLK1/1B, which is S-phase active

kinases and its connection to these functions, can cause effective and selective druggable targets for cancer therapy. Thioridazine (THD) is known as a lead compound for anticancer drug discovery in the field of TLK1/1B, and it has been researched extensively in preclinical studies. However, THD has not been successful further in clinical studies. The primary objective of this chapter was the design and synthesis of the THD based scaffolds, which have phenothiazine as parent molecule by modifying the substituents at C2, N10 positions, and also changing the functional group at the C5 position. **J3-54** showed promising results against prostate cancer in the combinatorial approach. Further, SAR studies based on the selectivity or potential of the **J3-54** would be proposed to improve the therapeutic properties.

In the last chapter, we have explored the packing features of the novel small molecules, which are presented in previous chapters with the help of single-crystal crystallography with supporting theoretical studies. Several small molecules were crystallized, and their structures have been solved by diffracted using single-crystal X-ray diffraction. The main motive is to identify the various molecular interactions generated among the molecules were studied using Hirshfeld surfaces (HS), and 2D fingerprint plots. The Hirshfeld surfaces of all the crystallized compounds have been mapped over a d_{norm}, shape index and curvedness to visualize the significant intermolecular interactions. These plots are mostly accountable for the distribution of strong and weak intermolecular interactions in the crystal packing of solid networks of the compound. From the experimental studies of the crystal structures, it is found that C–H···O, P–O···H, C–H···C, and π–π interactions play an important role in the stabilization of the crystal packing. The intermolecular charge transfer property of these compounds was studied by the density functional theory (DFT) calculations (B3LYP/6-31G* level). The theoretical values of bond length and bond angle were compared to the experimental ones obtained by single-crystal X-ray diffraction. This investigation may lead to the categorization of the promising active conformation of inhibitor and its intermediates additionally to the understanding of their structural packing.

In brief, the summary of the thesis is conceptualized around the designing, synthesis, structural and biological studies of new molecules to target kinases responsible for the growth of cancer cells. The studies reported here would lead to the pathway for advancing the drug-like properties of small molecules further.