**Name of the Research Scholar: Huma Naz**
**Name of the Supervisor: Dr. Md. Imtaiyaz Hassan**
**Name of the Co-Supervisor: Prof. Faizan Ahmad**
**Name of the Centre: Centre for Interdisciplinary Research in Basic Sciences (CIRBSc), Jamia Millia Islamia,**
**Title of PhD thesis: Structural and Biophysical Studies on Human Protein Kinase: The Major Therapeutic Targets for the Cancer**

Protein kinases are the group of enzymes which play a significant role in the biological system. Functional processes of the cell such as cell signalling, signal transduction, cell differentiation and cell proliferation are regulated through phosphorylation. The entire protein kinase functioning processes is depend on its binding to the phosphorylating distinct residues like serine and threonine of the target protein. We have successfully cloned (in pET 28a (+)) and expressed human CAMKIV (15-340aa) in the BL21 (λE3) strain of *E. coli* and purified by Ni-NTA chromatography. The yield of purified protein was found to be approximately 5 mg/liter of *E. coli* culture. Our described purification protocol is quick, simple and produces protein in plenty, with high purity in limited steps.

To see the effect of different pH on the structural and functional properties of CAMKIV, we performed CD, absorption spectroscopy and fluorescence measurements on CAMKIV. We have observed that within of the pH ranges from 5.0 - 11.5, the CAMKIV maintains its both secondary and tertiary structures along with its function, whereas, significant aggregation was observed at acidic pH (2.0 to 4.5). We have also performed the ATPase activity assay in different pH conditions and found a significant correlation between structure and enzymatic activities of CAMKIV. This biophysical study further complemented by MD simulations.

We further carried out urea-induced denaturation of CAMKIV at pH 7.4 and 25 °C, using three different probes, far-UV CD, near-UV absorption, and tryptophan fluorescence. A

coincidence of normalized denaturation curves of these optical properties suggests that urea-induced denaturation is a two-state process.

To find the potential ligands of CAMKIV, we studied the interaction of curcumin with the human CAMKIV, using molecular docking, molecular dynamics (MD) simulations, fluorescence binding, and SPR methods. We performed MD simulations for both neutral and anionic forms of CAMKIV-curcumin complexes for 150 ns to see the overall stability of the protein–ligand complex. Molecular docking studies revealed that the curcumin binds in the large hydrophobic cavity of kinase domain of CAMKIV through several hydrophobic and hydrogen-bonded interactions. A high binding affinity ($K_D$ = 3.7 × 10−8 ± .03 M) of curcumin for the CAMKIV was measured by SPR further indicating curcumin as a potential ligand for the CAMKIV. After the successful binding studies of curcumin to the active site of kinase CAMKIV, we synthesized pyrimidine-based small molecules as inhibitor of CAMKIV. Finally, 10 active pyrimidine substituted compounds **(Molecules 1–10)** have been successfully synthesized and characterized. Binding affinities were estimated by Fluorescence emission spectroscopy and binding constant was calculated by using modified Stern-Volmer equation. Molecule 10 is showing comparatively very high binding-affinity for the CAMKIV ($9.2X10^{10}$, $M^{-1}$(±0.18)). To observe the viability, cytotoxicity and apoptotic potential of these chemically synthesized compounds, MTT & PI assay have been performed on HEK293 (normal cell lines) and HuH7 (cancerous cell lines). However among these 10 inhibitory molecules, molecule 10 shows the best cell viability, anticancer activity and apoptotic potential .So we can conclude over here that molecule 10 might be used for further studies. This result shows an excellent agreement with the docking and fluorescence binding studies.