# Structure Prediction and Molecular Dynamics of Open and Closed Conformations of PKMzeta for Neurotherapeutic Interventions

**SYNOPSIS**

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**PRIYANKA PURKAYASTHA**

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Under the supervision of

**Prof. D. Sriram**

**&**

co-supervision of

**Dr. Aruna Malapati**


BITS Pilani
Pilani | Dubai | Goa | Hyderabad

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## Introduction

Neurological disorders are a group of incurable, chronic disorders of central nervous system (CNS) and peripheral nervous system. Neurological disorders could affect an entire neurological pathway or a single neuron. Even a small disturbance to a neuron's structural pathway could result in improper function. Social security approves disability benefits for serious cases of epilepsy, cerebral palsy, Parkinson's disease, multiple sclerosis, ALS, and other nerve-based diseases. More than 36 million people worldwide suffer from Alzheimer's disease (AD) and Parkinson's disease (PD). The major symptoms of AD are dementia and aging. Kinases are well known enzymes involved in the regulation of eukaryotic cellular processes. Any perturbation from the normal condition causes human diseases or disorders. There are many types of kinases which have profound biological impact on the organism, and kinase classification offers less accuracy because of their fundamental classification algorithms. The assignments of relating unknown kinases with the known kinases could be encouraged by classification and by comprehension the kinase families and subfamilies.

Atypical PKC isoform, PKMzeta is one such kinase, which has been implicated for the hippocampus long term potentiation, synaptic plasticity and tau phosphorylation in Alzheimer's disease. PKMzeta was also reported to play a central role in neuropathic pain, drug addiction and cancer. With the experimentally solved structures of a known enzyme, the drug design and discovery process could be facilitated significantly. However, one of the most frequent situations faced for structure based drug designing would be when there are no experimentally solved structures available.

.Considering the gaps in research, we attempted to build the structure of PKMzeta and designed specific PKMzeta inhibitors to treat the population at the risk of developing cognitive deficit and combat neurodegenerative disorder symptoms. Thus, the main objectives of the current study are as follows:

- Classification of kinases and sequence identification
- Homology model and structure based drug designing of PKMzeta
- *In-vitro* enzyme inhibition assay
- *In vitro* screening of leads
- Effect of the lead on MeHg induced neurodegeneration
- Activity of the lead in neuropathic pain and neuroinflammatory models.

The plan of work has been categorized as follows:

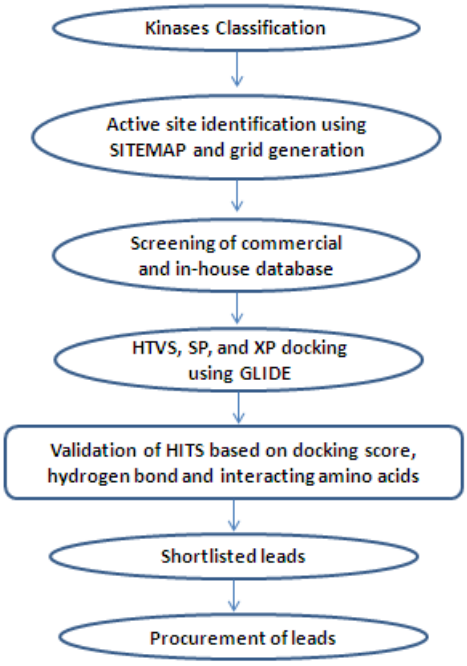


**Figure1:** Work for drug design and development

### *In vitro* enzyme inhibition assay

To check the inhibitory concentration of the identified inhibitors, the PKMzeta enzymatic assay was conducted at different concentrations and $IC_{50}$ values were calculated.

### *In vitro* toxicity studies

To evaluate the toxicity of the designed and synthesized compounds, HEK 293 cells were utilized and tested at five different concentrations using MTT assay to evaluate the lead cytotoxicity to normal cells.

### *In vitro* cell based screening of leads

Neuroblastoma cell lines (IMR-32) and glioblastoma cell lines (U87) were used to screen the potency of each leads towards neurodegenerative models, by employing chemical induction methods.

### *In vivo* screening of the lead

In vivo screening was done to find the effectiveness of the lead on motor behaviors and brain functionality in neurological conditions.

### Neuropathic pain and Neuroinflammatory model study

Effectiveness of PKMzeta inhibitor was evaluated for effectiveness of the test compound in neuropathic pain and also for anti-inflammatory effect.

### *In vivo* model to screen the lead on MeHg induced neurodegeneration

The compound’s effectiveness on motor behavior and brain functionality in MeHg induced neurodegeneration was evaluated.

**Measurement of *in vivo* gene expression levels of various key regulators NFκB, IL-1β, IL-6 and TNF-α using RT-qPCR.**

Inflammatory response was checked by measuring the gene expression level of various pro inflammatory mediators like NFκB, IL-1β, IL-6 and TNF-α.

**Results and Discussion**

To organize the kinases diversity and to compare distantly related sequences it is important to classify kinases with high precision. In this study we made an endeavor to classify kinases utilizing four diverse classification algorithms with three distinctive physiochemical features. Also, in search of novel PKMzeta inhibitors, we employed computational strategy for screening of large data sets of molecules, evaluated in-vitro and in vivo for identification of promising inhibitors. Virtual screening, a computational method where large libraries of compounds could be assessed for their potential to bind specific sites on target molecules such as proteins, was employed in the study.

**Classification of kinases**. To organize the kinases diversity and to compare distantly related sequences it is important to classify kinases with high precision. In this study we made an endeavor to classify kinases utilizing four diverse classification algorithms with three distinctive physiochemical features. Our results suggest that Random Forest gives an average precision of 0.99 for classification of kinases; and when amphiphilic pseudo amino acid composition was used as feature, the precision of the classifier was much higher than compared to amino acid composition and dipeptide composition. Hence, Random forest with amphiphilic pseudo amino acid composition is the best combination to achieve classification of kinases with high precision.

Further the same can be extended for subfamilies, which can give more insight into the predominant features specific to kinases subfamilies.

**Feature selection for classification of Kinases.**

We also checked the effect of feature selection on classification of kinases. Several algorithms exist for classification and most of them failed to classify when the dimension of the feature set large. Selecting the relevant features for classification is significant for a variety of reasons such as simplification of performance, computational efficiency and feature interpretability. Feature selection techniques are employed in such cases. However, there has been a limited study of feature selection techniques for classification of biological data. This work tries to determine the impact of feature selection algorithms for classification of kinases. We have used forward greedy feature selection algorithm along with the random forest classification algorithm. Classification models were built by considering one feature at a time. The model with best prediction precision was picked. Subsequently, the selected feature was joined with the remaining features one at a time. The model with the best prediction accuracy was picked further. This procedure was further rehashed until a model containing all features was built. The feature subset with most extreme prediction accuracy was picked as the best feature subset. The performance was evaluated by selecting the feature subset which maximizes Area Under the ROC Curve (AUC). The method identifies the feature subset from the datasets which contains the physiochemical properties of kinases like amino acid, dipeptide, and pseudo amino acid composition. An improvised performance of classification is noted for feature subset than with all the features. Thus, our method indicates that groups of kinases are classifiable with maximum AUC, if good subsets of features are used.

**Development of PKMzeta inhibitors.** We utilized the medicinal chemistry tools of structure molecular modeling, structure prediction and structure based drug design with two design strategies. Firstly, a study was performed by screening of commercial database, to identify new inhibitors. This strategy revealed new inhibitors with different scaffold and was exploited successfully in further drug development process. Secondly, we performed screening of in-house database, to identify and validate the potential targets for neurotherapeutric interventions.

**Design I: High throughput virtual screening of commercial database**

In the present study we attempted to build the open and closed models of the protein PKMzeta using homology modeling. The models were then used to identify PKMzeta inhibitors utilizing a high-throughput virtual screening protocol from a large commercial chemical database. Hit compounds were selected based on the binding interactions and Glide score. Compounds were subjected to in vitro luminescent based kinase assay for their inhibitory activity on targeted protein. Seven compounds exhibited IC50s less than or equal to 10 mM. Cell based assays revealed that the compounds **LeadC3** and **LeadC6** exhibited selectivity towards methylmercury treated neuroblastoma growth inhibition and suppressed reactive oxygen species with IC50s of 0.89 and 0.17 mM respectively. Furthermore, **LeadC3** exhibited attenuation of proinflammatory response with least energy in dynamic simulation studies and thus emerged as a prototypical lead for further development as novel inhibitor of PKMzeta for neurological implications.

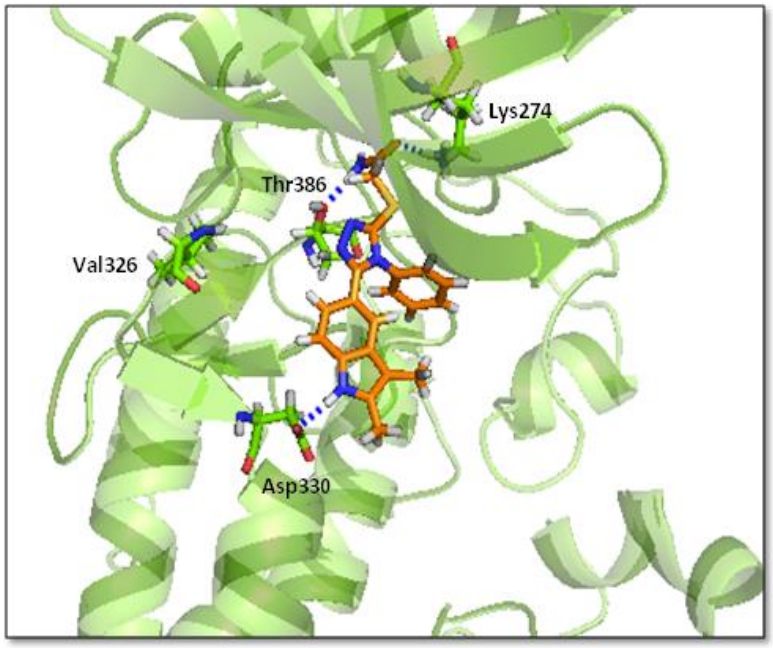


**Figure 2.** Interaction picture of LeadC3 with PKMzeta model.

**Design II: High throughput virtual screening of in-house database**

We took an effort to design non-peptidic inhibitors based on structure-based design techniques with biological proof of concept and pharmacological screening. In this study, small molecule inhibitors were designed and evaluated their potential to attenuate neuroinflammation. The inhibitors were screened based on virtual screening of our in-house compound library with the homology models of PKMzeta. Enzymatic and cell based assays revealed about five leads with good PKMzeta $IC_{50}$s less than 4 µM. Further, a compound (**BO5**) with highest selectivity index and ROS $IC_{50}$ 0.03 µM was selected for further neuropharmacological screening to check their neuroprotective effect in MeHg treated mouse and in chronic pain models. Lead BO5 was found to reverse spontaneous pain, cold allodynia and tactile allodynia with an $ED_{50}$ of 48.53 mg/kg, 9.25 mg/kg and 25.96 mg/kg, respectively in chronic pain studies. Thus the study revealed the importance of PKMzeta inhibition to be an attractive strategy to treat neuroinflammation related to neurodegeneration and neuropathic pain.

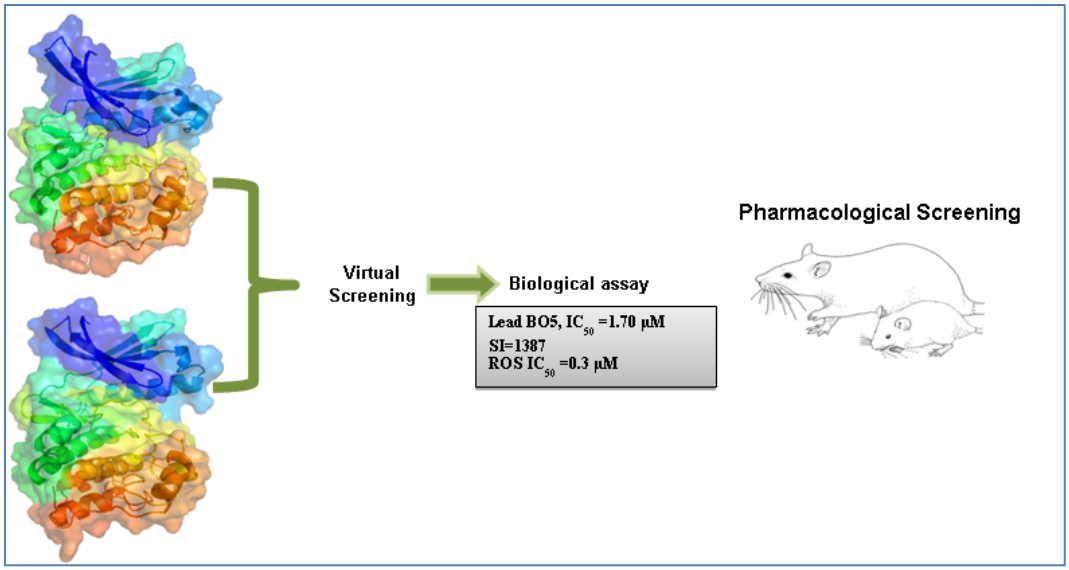


**Figure 3:** Showing the workflow for High throughput virtual screening of in-house database

**Recapitulation**

PKMzeta is the most important drug target for various neurological disorders including neurodegeneration, neuroinflammation and neuropathic pain. This work aimed at classification of kinases using machine learning approaches, building and validating homology models and identifying non-peptidic PKMzeta inhibitors by screening ASINEX and *in house* databases, which could be further evaluated and optimized as future prospective drug candidates. The biological evaluation of the designed compounds showed good inhibition with $IC_{50}$s less than 4 µM. Based on the $IC_{50}$, intracellular ROS estimation and cell based studies, **LeadBO5** was found promising with PKMzeta $IC_{50}$ of 1.7 µM, ROS $IC_{50}$ of 0.03 µM with highest SI of 1387 and was also found to suppress proinflammatory mediators. This compound in neuropharmacological screening showed good neuroprotective effect in MeHg induced neurodegeneration in mice. Along with the neuroprotective effect, the lead showed anti-inflammatory effect by significantly reducing the inflammatory mediators. **LeadBO5** reversed spontaneous pain, cold allodynia and

tactile allodynia with $ED_{50}$ of 48.53 mg/kg, 9.25 mg/kg and 25.96 mg/kg, respectively. Thus, it was evident from both the chronic pain and MeHg induced neurodegenerative studies, the effectiveness of a novel PKMzeta inhibitor **LeadBO5** in attenuating neuroinflammation and thus could be developed further as a prototype. However further studies need to be performed to assess the specificity of the lead compound with other kinase enzymes. In conclusion by employing the homology models of the target protein PKMzeta, we could successfully demonstrate the effectiveness of a small molecule inhibitor with promising attributes in attenuating neuroinflammation in chronic pain and neurodegeneration

**Future Perspective**

To fully utilize the unique potential of these identified hits against their respective targets, it was required to have the complete characterization of their modes of actions. The only way to make progress in this respect is to check the various factors in the molecular environments of these proteins, thus identifying the factors contributing towards the activity. Further extension of the pharmacological assays with large number of sample size and with other leads would be first required for drug development of PKMzeta. Second, to screen other databases or they could be optimized further to give better hits. Third, newly reported inhibitors could be utilized to generate better pharmacophoric models, or the collective structure-activity data could be utilized to develop QSAR models which would further add knowledge to the existing models. Fourth, molecular dynamics simulations of all the leads could be beneficial to track the movement of important amino acids interactions. Fifth, pharmacological evaluation of the leads could be done. The pain and MeHg model is complex, yet definitive test as it can give a lot of information about the role of protein kinase inhibitors in locomotor and cognitive behavioral aspects.