# *Insilico* Drug Designing and Molecular Docking Analysis of Novel Drug Analogues Targeting Pde4a Protein in Non-alcoholic Steatohepatitis

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**Abstract: The development of therapeutics for Nonalcoholic Steatohepatitis (NASH) is of significant importance due to the disease's rising prevalence. Utilizing bioinformatics databases and tools, this study aimed to design a novel drug by exploring chemical protein interactions and conducting in-silico tests to assess its toxicity and efficacy. Through bioinformatics analysis, Phosphodiesterase 4A (PDE4A) was identified as a crucial target protein involved in NASH. The active site of PDE4A was detected, and a drug analogue was subsequently designed to bind effectively to this site. In-silico toxicity analyses indicated a reduction in active toxic sites from three to one, with the remaining activity related to blood-brain barrier penetration. Molecular docking studies confirmed a strong binding affinity between the drug and the active site of PDE4A, suggesting promising therapeutic potential. These results highlight the effectiveness of using computational approaches in drug design and pave the way for further experimental validation of the analogue drug for treating NASH.**

**Keywords: Drug Design, Molecular Docking, Nonalcoholic Steatohepatitis**

## I.INTRODUCTION

The alarming rise in Nonalcoholic Steatohepatitis (NASH) has created an urgent demand for effective therapeutic solutions. NASH, characterized by the accumulation of fat, inflammation, and liver damage not related to alcohol use, poses significant health risks[1]. If left untreated, NASH can progress to severe conditions such as cirrhosis, liver failure, and hepatocellular carcinoma, contributing markedly to global morbidity and mortality rates[13].

Despite its critical impact, the therapeutic landscape for NASH remains sparse, underscoring a vital need for innovative research and novel treatment strategies[2]. This study embarks on a pioneering journey to design a novel therapeutic agent for NASH, leveraging state-of-the-art bioinformatics tools and databases. By delving into the complex interactions between chemicals and proteins, we aim to identify a highly effective drug candidate with a strong safety and efficacy profile.

Our research spotlights Phosphodiesterase 4A (PDE4A), a protein identified through rigorous bioinformatics analysis as a pivotal contributor to the pathogenesis of NASH. PDE4A's involvement in modulating inflammatory responses and metabolic pathways renders it a compelling target for therapeutic intervention[27]. Extensive bioinformatics analyses have revealed the active site of PDE4A, paving the way for the strategic design of a drug analogue specifically tailored to this target[6].

Employing advanced structure-based techniques, our drug design process integrates molecular docking and virtual screening to meticulously assess the binding affinity and stability of potential candidates. Through several iterations of optimization, the designed analogue has shown improved binding efficiency and minimized off target effects. Molecular docking studies have validated a moderate binding affinity between the drug and PDE4A's active site, indicating promising therapeutic potential[24]. These studies harness advanced docking algorithms to simulate the drug protein interaction at an atomic level, offering valuable insights into binding orientation and interaction forces. *In-silico* toxicity analyses have been instrumental in evaluating the safety profile of the designed drug analogue. These computational tools predict critical toxicological endpoints, including neurotoxicity,

respiratory toxicity, and blood-brain barrier penetration. A key achievement of these analyses is the reduction of active toxic sites from three to one, emphasizing the need for further optimization to mitigate potential risks. The findings of this research not only demonstrate the efficacy of computational approaches in drug design but also lay the groundwork for the experimental validation of the drug analogue as a treatment for NASH.

## II. LITERATURE REVIEW

Nonalcoholic Steatohepatitis (NASH) is a progressive liver disease characterized by inflammation, fat accumulation, and liver cell damage. It is part of the spectrum of nonalcoholic fatty liver disease (NAFLD) and has been increasingly recognized as a major global health concern due to its rising prevalence. The development of effective therapeutics for NASH is crucial, given the limited treatment options currently available[10]. This review explores the role of bioinformatics and in-silico approaches in designing novel therapeutics for NASH, with a focus on the identification and targeting of Phosphodiesterase 4A (PDE4A).

Phosphodiesterase 4A (PDE4A) has been identified as a critical target protein involved in the pathogenesis of NASH. PDE4A is an enzyme that hydrolyzes cyclic AMP (cAMP), a second messenger important for cellular signaling. Dysregulation of cAMP levels has been implicated in various metabolic and inflammatory processes associated with NASH. The identification of PDE4A as a target for therapeutic intervention is supported by bioinformatics analysis, which highlights its crucial role in the disease's molecular pathways

Using bioinformatics tools like ChemSketch and MarvinSketch, modifications were made to pentoxifylline to enhance its safety and binding affinity to PDE4A. In-silico analyses reduced neurotoxicity, respiratory toxicity, and BBB penetration, showing a strong binding affinity and promising therapeutic potential.

The findings from this study underscore the effectiveness of using computational approaches in drug design. The identification of PDE4A as a target and the subsequent modification of pentoxifylline to reduce its toxicity while retaining its efficacy represent significant advancements in the development of therapeutics for NASH. These results pave the way for further experimental validation and hold potential for the future treatment of NASH.

## III. MATERIALS & METHODS

The first step in this study involved identifying a suitable target protein associated with Nonalcoholic Steatohepatitis (NASH). We utilized various bioinformatics tools and databases to achieve this. Opentargets.org was employed to screen potential target proteins linked to NASH pathogenesis. This database integrates data from multiple sources, including genetic, transcriptomic, and proteomic data, to provide comprehensive insights into disease-associated targets[22]. Through this analysis, Phosphodiesterase 4A (PDE4A) was identified as a critical protein involved in NASH. PDE4A was chosen due to its significant role in modulating inflammatory responses and metabolic pathways, which are central to NASH development and progression.

To design an effective drug, it was crucial to identify the active site of the target protein, PDE4A. Active site prediction tools, PRANK WEB, was employed to accurately determine the binding pockets of PDE4A[18],[19]. These tools use computational algorithms to predict the most favorable binding sites based on the protein's structural conformation. The active site was identified by analyzing the protein's three-dimensional structure, obtained from the Protein Data Bank[3]. This step was critical in ensuring the designed drug could effectively bind to PDE4A and exert its therapeutic effects.

Following the identification of PDE4A's active site, the next phase involved screening potential drugs that could bind to this site. Databases such as DrugBank, PubChem, and OpenTarget were utilized for this purpose[17],[20],[32]. These databases provide extensive libraries of chemical compounds, including their structural and pharmacological properties. Pentoxifylline was identified as a potential drug candidate due to its known pharmacological properties and previous research indicating its efficacy in related pathways. The selection process involved virtual screening techniques to evaluate the binding affinity

and stability of various compounds with PDE4A.

Given the potential neurotoxicity, respiratory toxicity, and blood-brain barrier penetration of pentoxifylline, modifications were necessary to enhance its safety profile. ChemSketch and MarvinSketch, molecular modeling tools, was used to modify the chemical structure of pentoxifylline. The modifications aimed to reduce its toxicity while maintaining or enhancing its binding affinity to PDE4A. The structure-based drug design approach was employed, where specific alterations were made to the drug's functional groups to achieve the desired pharmacological properties. Each modification was iteratively tested using in-silico tools to ensure it met the criteria for efficacy and safety.To assess the safety profile of the modified drug, extensive in silico toxicity analyses were conducted. Tool such as ProTox employed to predict the potential toxicological endpoints of the modified drug. The primary objective was to ensure the drug exhibited minimal toxic effects while retaining its therapeutic potential. The in-silico analysis indicated a reduction in active toxic sites from three to one, with the remaining activity related to blood-brain barrier penetration, thus confirming the improved safety profile of the modified drug.Molecular docking studies were conducted to validate the binding affinity and interaction between the modified drug and the active site of PDE4A. Autodock, a widely used molecular docking software, was employed for this purpose[24]. The software uses a scoring algorithm to predict the binding affinity and orientation of the drug within the active site of the protein. The docking studies provided insights into the atomic-level interactions between the drug and PDE4A, confirming a strong binding affinity. The results indicated that the modified drug could effectively bind to the active site, suggesting its potential as a viable therapeutic agent for NASH.In conclusion, the methodology employed in this study demonstrates the power of bioinformatics tools and computational approaches in designing novel therapeutics for complex diseases like NASH. The identification of PDE4A as a critical target protein and the subsequent design and validation of a modified drug highlight the potential for developing effective treatments through integrated computational and experimental strategies. The findings from this study pave the way for further research and development, ultimately contributing to the advancement of therapeutic options for NASH.

## IV. RESULTS

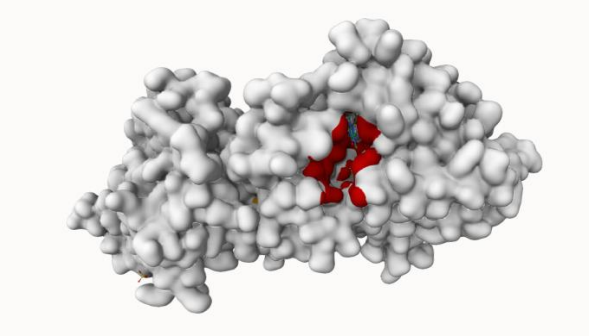


Fig 1.The three-dimensional structure of the PDE4A protein showcases its predicted active sites, highlighted in distinct colors: red, yellow, and blue. The regions marked in red denote high-confidence active sites, which are crucial for ligand binding. These areas play a significant role in the protein's function and interactions.

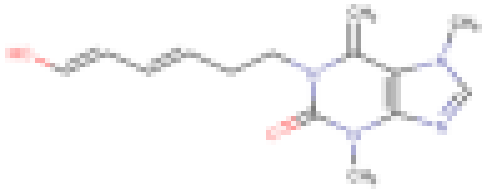


Fig 2. Pentoxifylline drug analogue after structural modification

Table 1. Toxicity analysis of pentoxifylline drug analogue before and after modification

| | LD50 value (mg/kg) | Toxicity Class | Toxic effects |
|---|---|---|---|
| **Before modification** | 780 | 4 | Neurotoxicity, Respiratory toxicity and BBB-barrier |
| **After modification** | 3000 | 5 | BBB-barrier |

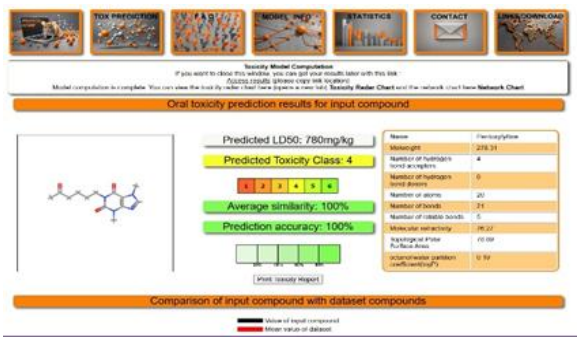
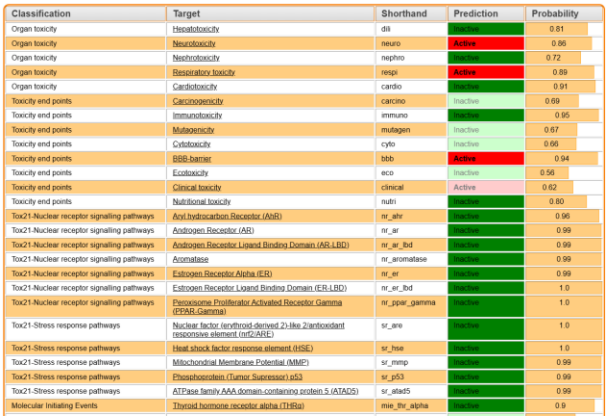


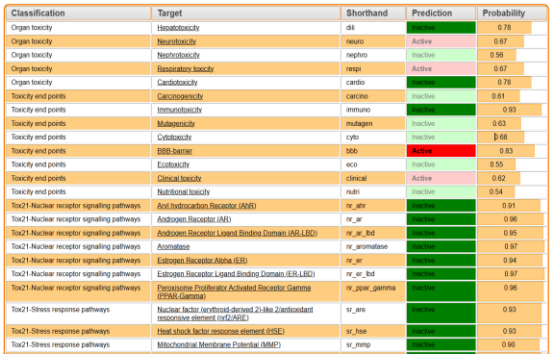
Fig 3. This figure illustrates the toxicity prediction results for the pentoxifylline drug analogue prior to modification. It indicates a predicted LD50 of 780mg/kg, categorizing the drug within toxicity class 4 with a prediction accuracy of 100%



| Classification | Target | Shorthand | Prediction | Probability |
|---|---|---|---|---|
| Organ toxicity | Hepatotoxicity | dili | Inactive | 0.81 |
| Organ toxicity | Neurotoxicity | neuro | Active | 0.86 |
| Organ toxicity | Nephrotoxicity | nephro | Inactive | 0.72 |
| Organ toxicity | Respiratory toxicity | respi | Active | 0.89 |
| Organ toxicity | Cardiotoxicity | cardio | Inactive | 0.91 |
| Toxicity end points | Carcinogenicity | carcino | Inactive | 0.69 |
| Toxicity end points | Immunotoxicity | immuno | Inactive | 0.95 |
| Toxicity end points | Mutagenicity | mutagen | Inactive | 0.67 |
| Toxicity end points | Cytotoxicity | cyto | Inactive | 0.66 |
| Toxicity end points | BBB-barrier | bbb | Active | 0.94 |
| Toxicity end points | Ecotoxicity | eco | Inactive | 0.56 |
| Toxicity end points | Clinical toxicity | clinical | Active | 0.62 |
| Toxicity end points | Nutritional toxicity | nutri | Inactive | 0.80 |
| Tox21-Nuclear receptor signalling pathways | Aryl hydrocarbon Receptor (AhR) | nr_ahr | Inactive | 0.96 |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor (AR) | nr_ar | Inactive | 0.99 |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor Ligand Binding Domain (AR-LBD) | nr_ar_lbd | Inactive | 0.99 |
| Tox21-Nuclear receptor signalling pathways | Aromatase | nr_aromatase | Inactive | 0.99 |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Alpha (ER) | nr_er | Inactive | 0.99 |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Ligand Binding Domain (ER-LBD) | nr_er_lbd | Inactive | 1.0 |
| Tox21-Nuclear receptor signalling pathways | Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma) | nr_ppar_gamma | Inactive | 1.0 |
| Tox21-Stress response pathways | Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) | sr_are | Inactive | 1.0 |
| Tox21-Stress response pathways | Heat shock factor response element (HSE) | sr_hse | Inactive | 1.0 |
| Tox21-Stress response pathways | Mitochondrial Membrane Potential (MMP) | sr_mmp | Inactive | 0.99 |
| Tox21-Stress response pathways | Phosphoprotein (Tumor Suppressor) p53 | sr_p53 | Inactive | 0.99 |
| Tox21-Stress response pathways | ATPase family AAA domain-containing protein 5 (ATAD5) | sr_atad5 | Inactive | 0.99 |
| Molecular Initiating Events | Thyroid hormone receptor alpha (THRα) | mie_thr_alpha | Inactive | 0.9 |

Fig 4. This figure presents a comprehensive toxicity model report, detailing both active and inactive toxicity targets across various biological systems for the pentoxifylline drug analogue prior to modification.

Fig 5. This figure illustrates the toxicity prediction results for the pentoxifylline drug analogue after modification. It indicates a predicted LD50 of 3000mg/kg, categorizing the drug within Toxicity Class 5.



| Classification | Target | Shorthand | Prediction | Probability |
|---|---|---|---|---|
| Organ toxicity | Hepatotoxicity | dili | Inactive | 0.78 |
| Organ toxicity | Neurotoxicity | neuro | Active | 0.67 |
| Organ toxicity | Nephrotoxicity | nephro | Inactive | 0.56 |
| Organ toxicity | Respiratory toxicity | respi | Active | 0.67 |
| Organ toxicity | Cardiotoxicity | cardio | Inactive | 0.78 |
| Toxicity end points | Carcinogenicity | carcino | Inactive | 0.61 |
| Toxicity end points | Immunotoxicity | immuno | Inactive | 0.93 |
| Toxicity end points | Mutagenicity | mutagen | Inactive | 0.63 |
| Toxicity end points | Cytotoxicity | cyto | Inactive | 0.68 |
| Toxicity end points | BBB-barrier | bbb | Active | 0.83 |
| Toxicity end points | Ecotoxicity | eco | Inactive | 0.55 |
| Toxicity end points | Clinical toxicity | clinical | Active | 0.62 |
| Toxicity end points | Nutritional toxicity | nutri | Inactive | 0.54 |
| Tox21-Nuclear receptor signalling pathways | Aryl hydrocarbon Receptor (AhR) | nr_ahr | Inactive | 0.91 |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor (AR) | nr_ar | Inactive | 0.96 |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor Ligand Binding Domain (AR-LBD) | nr_ar_lbd | Inactive | 0.95 |
| Tox21-Nuclear receptor signalling pathways | Aromatase | nr_aromatase | Inactive | 0.97 |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Alpha (ER) | nr_er | Inactive | 0.94 |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Ligand Binding Domain (ER-LBD) | nr_er_lbd | Inactive | 0.97 |
| Tox21-Nuclear receptor signalling pathways | Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma) | nr_ppar_gamma | Inactive | 0.96 |
| Tox21-Stress response pathways | Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) | sr_are | Inactive | 0.93 |
| Tox21-Stress response pathways | Heat shock factor response element (HSE) | sr_hse | Inactive | 0.93 |
| Tox21-Stress response pathways | Mitochondrial Membrane Potential (MMP) | sr_mmp | Inactive | 0.90 |

Fig 6. This figure presents a comprehensive toxicity model report, detailing both active and inactive toxicity targets acrosss various biological systems for the pentoxifylline drug analogue after modification.

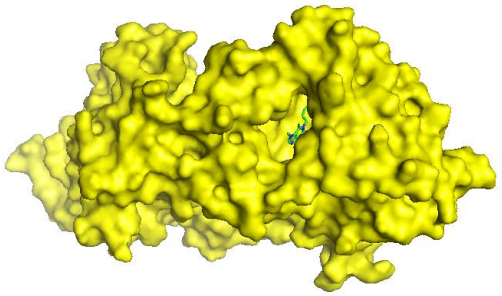


Fig 7. This image illustrates yellow coloured protein and blue coloured ligand.

## V. CONCLUSION

This study underscores the promising potential of targeting the PDE4A protein, for developing therapeutic strategies against Nonalcoholic Steatohepatitis (NASH). Employing a systematic in silico approach that integrates structural modeling, toxicity analysis, and molecular docking, we assessed and optimized pentoxifylline drug analogue for improved safety and efficacy. Through this detailed computational analysis, significant advancements were made in enhancing the therapeutic profiles of the evaluated drug analogues. The modifications aimed at reducing toxicity levels, improving LD50 values, and minimizing adverse effects such as neurotoxicity, respiratory toxicity, and blood-brain barrier penetration. The screening process, conducted using Open Targets and PRANKWEB, identified PDE4A crucial protein target, with active sites precisely predicted and protein quality verified using SAVEServer. The initial screening of the pentoxifylline drug analogue against PDE4A showed promise, but the toxicity testing via ProTox revealed significant risks, categorizing the compound in Class 4 toxicity with an LD50 of 780 mg/kg, alongside neurotoxicity, respiratory toxicity, and BBB-barrier toxicity.

To address these issues, the analogue was modified using the MarvinSketch and ChemSketch tool, resulting in a markedly improved toxicity profile. Post-modification, the analogue exhibited only BB barrier toxicity, with an LD50 value of 3000 mg/kg, and was reclassified into Class 5 toxicity, indicating a safer drug profile. Protein-ligand binding studies using AutoDock and visualized with PyMol demonstrated a moderate

binding affinity of -6.7, suggesting effective interaction with the PDE4A protein.

Future research should focus on conducting comprehensive in vitro and in vivo studies to validate the therapeutic potential and safety of the modified pentoxifylline drug analogue. Additionally, exploring the pharmacokinetics and pharmacodynamics of the analogue will be crucial in determining its long-term efficacy and suitability for clinical application in treating NASH.

## VI. FUTURE PROSPECTS

Future research should focus on conducting comprehensive in vitro and in vivo studies to validate the therapeutic potential and safety of the modified pentoxifylline drug analogue. In vitro assays will be essential to assess the neurotoxicity, respiratory toxicity, and blood-brain barrier and efficacy of the drug in liver cell lines, providing preliminary data on its potential therapeutic effects. These studies will offer insights into the drug's mechanisms of action and its impact on cellular processes relevant to NASH.

In vivo studies will further evaluate the pharmacokinetics and therapeutic potential of the modified drug in animal models of NASH. These studies will help determine the drug's absorption, distribution, metabolism, and excretion (ADME) properties, as well as its overall safety and efficacy in a complex biological system. The results from in vivo studies will be crucial in assessing the drug's potential to treat NASH effectively and safely in a living organism. Pending successful preclinical results, clinical trials should be initiated to determine the drug's safety and efficacy in humans. These trials will involve multiple phases, starting with small-scale studies to assess safety and dosage, followed by larger trials to evaluate efficacy and monitor side effects. Successful clinical trials will provide the necessary data to support the regulatory approval of the drug for treating NASH.

Advancing personalized medicine approaches by tailoring therapies based on individual genetic and molecular profiles could enhance treatment outcomes. By identifying specific biomarkers and genetic variations associated with NASH, researchers can develop targeted therapies that are more effective for individual patients. This personalized approach could improve the efficacy of treatments and reduce the risk of adverse effects.

Collaborative efforts and open-access sharing of findings will further accelerate advancements in developing effective NASH therapies. By fostering collaboration between researchers, institutions, and industry partners, the scientific community can leverage diverse expertise and resources to expedite the drug discovery process. Open-access platforms can facilitate the dissemination of research findings, enabling researchers to build on each other's work and drive innovation in NASH therapy development.

The demonstrated improvements in safety and efficacy reinforce the transformative potential of computational methods in drug discovery. This study lays a solid foundation for further exploration and validation, paving the way for novel therapeutic interventions for NASH and potentially other related conditions. The continued integration of computational and experimental approaches will be key to advancing our understanding of NASH and developing effective treatments for this challenging disease.

## VII. APPENDIX

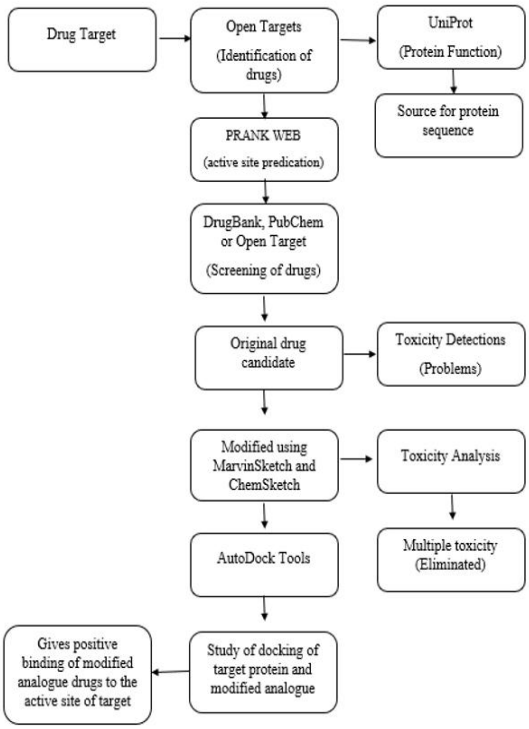



Fig A1. This systematic workflow illustrates the application of bioinformatics tools and software to

identify, design and validate drugs targeting specific proteins, ensuring their binding efficacy and safety through detailed computational analysis.

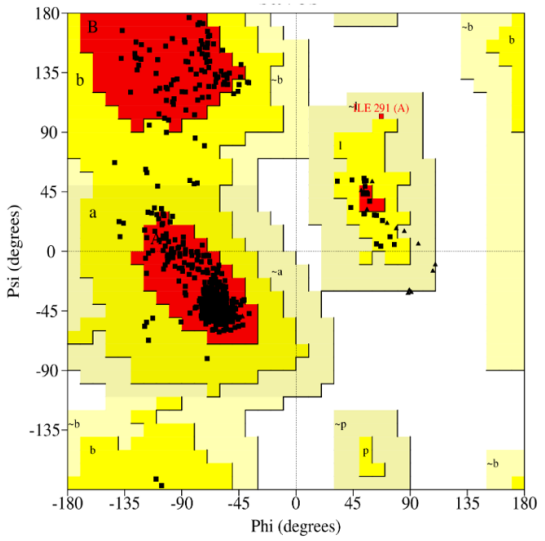



Fig A2. Ramachandran plot for PDE4A protein, which illustrates the residues are in allowed regions.

## VIII. ACKNOWLEDGMENT

F.A. thanks God for providing the strength, wisdom, and perseverance to complete this work. Deep gratitude is extended to those who have supported and guided throughout this research journey. First and foremost, thanks to my parents for their unconditional love, encouragement, and unwavering belief in my abilities. Their support has been the cornerstone of my academic pursuits.

Special thanks to my guide for their insightful guidance, patience, and invaluable feedback. Their expertise and mentorship have been instrumental in shaping the direction and quality of this research. I also thank the coordinator, Mrs. Ruchita Dalvi, for giving us this opportunity and for their continuous support.

Appreciation goes to my friends, whose companionship and moral support have made this journey enjoyable and fulfilling. Your encouragement and camaraderie have been a source of great strength. Thank you all for your invaluable contributions and support.

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