

in Ceske Budejovice, October 20th, 2023

The disertation thesis reviewer assessment for Stefan Djukic

Stefan Djukic's PhD thesis, titled "Structure-assisted Design of Inhibitors Targeting Medicinally Relevant Enzymes," focuses on research that holds significant implications for understanding the concept of structure-assisted drug discovery. This approach relies on in-depth knowledge of 3D structures to design and enhance new inhibitors that target medically important enzymes. By using widely accepted X-ray crystallography, the research obtained a detailed view of the protein-inhibitor complex, allowing for a thorough analysis of how proteins and inhibitors interact.

Purine nucleoside phosphorylase (PNP), a key player in the purine metabolism salvage pathway, is essential in treating T-cell malignancies and parasitic infections. The thesis was dedicated to human and *M. tuberculosis* PNP, with a focus on creating highly selective and specific inhibitors. When short substituents like methoxy and bromide groups were introduced to the central phenyl component, they reduced the inhibitor's binding to human PNP but left the mycobacterial PNP unaffected. Conversely, larger substituents, like a fluorinated phenyl ring, weakened the interaction with human PNP while strengthening it with the mycobacterial enzyme. Although the differences in active sites did not provide a clear explanation, an analysis of the subunit-subunit interaction region suggested that this region is smaller and more flexible in mycobacterial PNP compared to human PNP.

The second part of the thesis investigated into the study of cyclin-dependent kinase 2 (CDK2), a cell cycle regulator crucial in treating various cancer types. Targeting CDK2, a member of the CDK kinase family, has been challenging due to the low selectivity and specificity of most inhibitors developed over the years. This thesis introduces three innovative series of ATP-competitive inhibitors, based on the pyrazolo-pyridine core and/or pyrazolo-quinoline core. Crystal structures of representative compounds from these series were utilized to enhance the inhibitors' affinity for CDK2. Furthermore, these structures played a critical role in developing novel inhibitors designed to target other members of the kinase family (e.g. FLT3, CDK12, and CDK7).

The thesis is comprised of 159 pages, excluding the References section. It begins with an Introduction that highlights the strength of structure-assisted drug discovery (SADD) as a method in pharmaceutical research for designing and optimizing drugs. The subsequent chapters deal with a complete examination of PNP structures, including their medical relevance, associated inhibitors, and the active and binding sites. Following this, there is a chapter dedicated to CDK2 kinase, which systematically explores its function,

regulatory mechanisms, structural attributes, inhibitors, and its significance in the field of biomedicine. The subsequent part of the thesis includes four peer-reviewed publications in high quality scientific journals, all of which Stefan Djukic is as a co-author. Eight structures, comprising five hPNP and three MtbPNP, were documented in the publications, and subsequently submitted to the Protein Data Bank (PDB). The inclusion of these publications highlights the significant contribution of the Stefan's work to the scientific community.

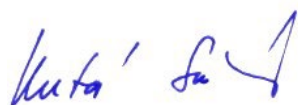
The thesis is characterized by its precision and clarity. It includes well-prepared, illustrative figures that help readers understand the scientific concepts. The writing is clear and contains very few typographical errors. It is clear that the author was highly committed to the scientific project and approached it with a clear sense of purpose.

In summary, the thesis successfully meets all the necessary requirements for a dissertation and offers an enjoyable reading experience.

Nevertheless, I have some more or less general questions for Stefan:

1. What specific criteria were employed in the design and selection of the individual inhibitors, and who was responsible for their preparation?
2. Is there ongoing consideration for clinical testing of these inhibitors, and what prerequisites need to be met before their implementation can be considered?
3. In terms of the inhibitors' specificity, are they intended for broad usage or do they exhibit a high level of selectivity? What factors cause this observed specificity?
4. CDK2 kinase inhibitors have been extensively investigated. What prompted your decision to conduct further research on them and on what previous studies is this decision based?

Sincerely,
Ivana Kuta Smatanova



Ivana Kuta Smatanova, Ph.D.
Professor and Teacher
Dept. of Chemistry, Faculty of Science
University of South Bohemia Ceske Budejovice
Branisovska 1760
CZ-370 05 Ceske Budejovice
CZECH REPUBLIC
Phone: +420 608 106 109
E-mail: ivanaks@seznam.cz (kuta@prf.jcu.cz)