Faculty of Science
CHARLES UNIVERSITY

Department of
ORGANIC CHEMISTRY

Peer review for Doctoral thesis

Name of the thesis: Desing, synthesis and evaluation of novel inhibitors of class II PI4Ks and RIPK2/3 kinases

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The doctoral thesis of Mbilo Misehe aims for the design and synthesis of new inhibitors of PI4K and RIPK2/3 kinases which are valuable chemical tools to uncover novel cellular roles of these enzymes and could be converted in the future into potential drugs. Taken together the thesis covers one of the important topics in medicinal chemistry.

The thesis is divided into several chapters where the particular sub-projects are described together with their theoretical background and results.
Chapter 1, the introductory part of this thesis offers general insight into human kinases, a description of kinase's catalytic domains, and a mechanistic classification of inhibitors.
Chapter 2, follows with a detailed introduction to PI4Ks, implications towards human diseases, and various types of inhibitors. In this part, novel 4-aminoquinazoline inhibitors of PI4K class II were rationally designed and prepared, based on the previous knowledge of the kinase binding site and structure of inhibitor **MD59**. Some of the prepared compounds showed micromolar potency against PI4K2A and can serve as a good point for further development.
Chapter 3, aims for the design and synthesis of novel RIPK inhibitors. Employing quinazoline scaffold and structure of known compounds a new series of RIP2/3 kinase inhibitors was prepared showing nanomolar activities against isoform selective RIPK2 and dual-target RIP2/3 kinases.
Chapter 4 contains the experimental part and references.

Overall, the thesis is well-written and offers pleasant reading. I appreciate the separation of particular topics into chapters, and specifying the particular aims to avoid confusion. The applicant showed good synthetic skill in both chapters 2 and 3, presenting de novo synthesis of the quinazoline core employing microwave-assisted cyclization 2-aminobenzonitrile **28** or Dimroth rearrangement of formamidine **81**, as well as employment Suzuki or Stille coupling

for later decorations of quinazolines scaffold. In general, the author provided good knowledge of organic chemistry and the given topic. However, there are a couple of issues that deserve some criticism such as the formatting of the text and caption below schemes. Very often the captions are indented on the other page which does not look great. Schemes themselves do not have the same size e.g. pages 58 and 59. The biggest problem in my opinion is missing yields – the exact mass of the isolated compounds, in the experimental part. Apart from that the experimental part contains all the necessary data.
Regarding this thesis, I have several remarks and questions.

Notes:
There are a couple of text and graphic issues e.g., schemes 11 and 12 do on have the same size; compounds in scheme 16 do not have the same order as the text above; the abbreviation DCM does not probably stand for methylene dichloride but dichloromethane. Page 37, a symbol for percentage in the case of compound **88** is shifted to another row.

Questions:
1) Pages 22-23. What is the function of cysteine (target for covalent inhibitors) in the EGRF kinase domain?
2) Pages 37-39. Was there any optimization of the reaction conditions for Suzuki coupling? Using 4 equivalents of boronic acid and 3 equivalents of ligand does not seem to be optimized.
3) Page 44. Are you planning any further optimization of compound **49** which was among the best in the series?
4) Page 56. Dimroth rearrangement was shown to be very useful for the preparation of quinazolines. Is there any other potential application of Dimroth rearrangement in the synthesis of heterocyclic compounds? Did you observe the formation of any interesting impurities in the reaction?
5) Pages 58-61. Diversification of positions 6 and 7 on the quinazoline core *via* Suzuki and Stille coupling. What was the reasoning behind selecting either Suzuki or Stille coupling for particular modifications (e.g., source of boronic acids, boronates, or stannanes, the reactivity of quinazoline starting compounds, early/later synthetic stage, larger synthetic scales, etc.? Was there any optimization of coupling conditions?
6) What is so special about the GSK872 (compound **66**) in comparison with your compounds?
7) Who performed the docking studies, and created the pictures for most of the figures in the thesis?
8) What was the solubility of your final compounds?

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In conclusion, I would like to say that the doctoral thesis by Mbilo Misehe represents a solid piece of work in medicinal chemistry and fulfills all the necessary criteria for this type of work. Moreover, this work contains high-quality results useful for further potential development of drugs. I recommend this work for further defense and other processes leading to the Ph.D. title.

21. 01. 2024 in Vyšní Lhoty v Beskydech

Ing. Ondřej Baszczyński, PhD.