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Report on the PhD Thesis: "Novel approaches for diabetes management by inhibition of DYRK1A kinase" by Agata Barzowska

The PhD thesis entitled "Novel approaches for diabetes management by inhibition of DYRK1A kinase" by Agata Barzowska, presented to the Jagiellonian University, constitutes a significant contribution to the field of diabetes research. Diabetes, type 1 and in final stage also type 2, are ultimately characterized by loss of functional beta cell mass, leading ultimately to inability to produce insulin. The development of therapeutic options to restore beta cell loss or prevent beta cell failure is thus of utmost importance for development of a potential future curative treatment.

This dissertation explores innovative strategies for managing diabetes by mainly focusing on the inhibition of DYRK1A kinase, a promising target in diabetes therapy. The research undertaken in this dissertation is thorough, methodologically sound, and presents novel insights that could pave the way for new therapeutic approaches in diabetes management. The following discussion will evaluate the scientific merits of the work, examining the robustness of the methodologies, the significance of the findings, and the overall impact on the field of diabetes research.

Scientific merit of the thesis

The identification and careful characterization of small-molecule inhibitors of DYRK1A, including the novel compound AC27, is a significant achievement. The dissertation demonstrates that AC27 not only inhibits DYRK1A activity but also selectively targets this kinase, offering advantages over existing inhibitors like harmine. This selectivity is crucial for reducing off-target effects and enhancing therapeutic efficacy.

Ms. Barzowska's findings on the impact of DYRK1A inhibitors on β-cell proliferation and insulin secretion are groundbreaking. The dissertation provides compelling evidence that AC27 enhances β-cell growth and insulin production, both in cell lines and in more complex organoid

models. This dual effect is particularly promising for diabetes therapy, as it addresses both the loss of β-cell mass and the functional impairment of existing β-cells. The comparative analysis with harmine, a well-documented DYRK1A inhibitor, underscores the superior performance of AC27. The dissertation demonstrates that AC27 not only promotes β-cell proliferation more effectively but also shows better kinase selectivity. This comparison is crucial for positioning AC27 as a potential therapeutic candidate in diabetes management.

Lastly, the *in vivo* studies conducted in diabetic mice models provide strong evidence for the therapeutic potential of DYRK1A inhibitors. The dissertation shows that AC27, especially when encapsulated in Pluronic P123, maintains long-lasting normoglycemia and enhances β-cell function *in vivo*. These findings are significant as they translate the *in vitro* and *ex vivo* results to a living organism, reinforcing the potential clinical applicability of the research.

In another aspect of the thesis, an interesting class of natural products, the so-called leucettines, are explored with respect to their DYRK1A inhibitory properties. Specifically, in addition with the TGFß-receptor kinase I inhibitor LY364947, one representative of this series, L41, significantly increased insulin secretion.

The last chapter of the work presented explores potential relevance of SPOP in the pathogenesis of diabetes. SPOP is an underexplored E3 ligase, which is involved in the degradation of PDX1. Ms. Barzowska's work is to my knowledge the first exploration of SPOP's role in diabetes.

To summarize this section, Ms. Barzowska's dissertation makes substantial contributions to the field of diabetes research. The identification of AC27 as a novel, selective DYRK1A inhibitor with dual effects on β-cell proliferation and insulin secretion is a significant advancement. This work not only enhances our understanding of DYRK1A's role in diabetes but also opens new avenues for therapeutic intervention. The comprehensive methodological approach and the robust experimental data presented in the dissertation set a high standard for future research in this area. Also, the results on SPOP will lay the ground for further research in the Czarna lab, thus providing a highly important contribution.

Substantial merit of the thesis

The literature review in the dissertation is comprehensive, offering a detailed background on diabetes mellitus (DM), its types, and the current therapeutic approaches. Ms. Barzowska effectively contextualizes the need for new diabetes treatments by discussing the limitations of existing therapies. The inclusion of sections on insulin homeostasis and the molecular mechanisms underlying diabetes is particularly valuable, providing a clear rationale for targeting DYRK1A kinase. The review of the DYRK family of kinases and their role in various physiological processes sets a solid foundation for understanding the significance of DYRK1A in diabetes.

The dissertation clearly outlines its aims and objectives, focusing on the identification and characterization of small-molecule inhibitors of DYRK1A, their efficacy in promoting β-cell proliferation, and their potential therapeutic effects in different diabetes models. These objectives are well-defined and address critical gaps in current diabetes research, specifically the need for therapies that can restore β-cell function and insulin homeostasis.

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Ms. Barzowska employs a very robust methodological approach to achieve the dissertation's objectives. The use of various *in vitro* and *in vivo* models, including pancreatic β-cell organoids, INS-1E and MIN6 cell lines, and mouse islets, is a notable strength. These models provide a comprehensive platform for evaluating the efficacy of DYRK1A inhibitors. The application of advanced techniques such as fluorescence microscopy, flow cytometry, and Western blot analysis further enhances the rigor of the research. The systematic evaluation of the inhibitors' effects on β-cell proliferation, insulin secretion, and glucose homeostasis is methodologically sound and thorough.

The discussion section of the dissertation is insightful, linking the experimental findings to the broader context of diabetes research and therapy. Ms. Barzowska critically evaluates the limitations of her study, such as the need for further long-term *in vivo* studies and the exploration of potential side effects. The dissertation also highlights future research directions, including the development of more potent and selective DYRK1A inhibitors and the investigation of combination therapies involving stem cells and organoids.

The references in the dissertation are extensive and relevant, reflecting a thorough engagement with the existing literature. The referencing style is consistent and professional, adhering to academic standards.

Layout and register

The language is always scientific, very clear and precise. The results are discussed in a well-structured manner and illustrated by very well chosen figures. New chapters are introduced by a colorful, well designed entry-figure. Overall, the layout is very well done and lives up to the beautiful scientific results generated in this work.

Critical notes

I have no critical comments to make that would impair the overwhelmingly positive impression of this work. A few minor typographical errors should be removed. Also, the conclusion contains a sentence on GLP-1 targeting, which should be omitted – not for originality, but for intellectual property protection reasons. However, these remarks do not by any means compromise the very positive assessment of this dissertation

Final assessment

In conclusion, Agata Barzowska's PhD thesis is a commendable piece of scientific work that addresses critical challenges in diabetes management. The research is characterized by its methodological rigor, comprehensive approach, and significant findings. The identification and characterization of DYRK1A inhibitors, particularly AC27, represent a promising step forward in the development of new diabetes therapies. Ms. Barzowska's work is poised to have a lasting impact on the field, offering new hope for patients with diabetes. This dissertation is highly recommended for its scientific merit and its potential to drive future research and therapeutic innovations in diabetes management.

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To summarize, the dissertation presented stands out as a substantial achievement in the field of diabetes research, offering new insights and therapeutic possibilities for the management of this chronic disease. Multiple compound classes were explored in an absolutely state-of-the-art profiling panel for diabetes research; *in vitro*, *in cellulo* and *in vivo*.

The significant achievements are also documented by an impressive publication record in renowned international peer-reviewed journals like Scientific reports, Plos one, Cells and the International Journal of Molecular Sciences, at least one other publication is currently in the final stage of preparation.

I thus request that this dissertation deserves a distinction.

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