1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: Bioanalytical approach to metabolomic analysis of cyclitols: advanced chemical techniques in the study of molecular mechanisms

1.1. Project goals

The aim of this project is a comprehensive chemical and biochemical characterization of the modulatory effects of selected cyclitols—myo-inositol, D-chiro-inositol, and pinitol—on pancreatic function. The research focuses on analyzing their impact on metabolic pathways and enzymatic activity at the cellular level, utilizing advanced analytical chemistry methods in both in vitro and in vivo models under conditions of disturbed glucose–insulin homeostasis. Chromatographic techniques (HPLC, UPLC, GC) coupled with high-resolution mass spectrometry (HR-MS/MS) will be employed for qualitative and quantitative analysis of metabolites, including sugar polyols and phosphorylated inositol derivatives. High-field NMR spectroscopy will be used to determine the structure and stereochemical configuration of the metabolites, while MALDI-TOF imaging will enable the localization of compounds within pancreatic tissue.

The project includes the development and validation of analytical protocols using isotope-labeled standards to ensure high quality and reproducibility of measurements. Metabolomic data will be subjected to advanced statistical and chemometric analyses, allowing for the identification of key biomarkers and the reconstruction of metabolic networks. The results will be correlated with enzymatic assays and histological analyses, enabling the verification of molecular mechanisms by which cyclitols influence pancreatic metabolism.

1.2. Outline

The pancreas plays a pivotal role in systemic carbohydrate metabolism through its endocrine function and supports digestive processes via its exocrine function. Dysfunction of pancreatic β-cells and impaired secretion of digestive enzymes are central features in the pathophysiology of various metabolic disorders, including type 2 diabetes mellitus, metabolic syndrome, and chronic pancreatitis. Given the increasing

prevalence of these conditions and the limited efficacy of current therapies, there is an urgent need to explore novel, safe, and effective therapeutic strategies based on natural bioactive compounds.

Cyclitols—such as myo-inositol, D-chiro-inositol, and pinitol—are naturally occurring cyclic polyols with promising therapeutic potential in the management of metabolic dysfunctions, particularly type 2 diabetes and metabolic syndrome. These compounds have demonstrated modulatory effects on glucose and lipid metabolism, insulin signaling pathways, and oxidative stress responses. However, their direct biochemical and molecular effects on pancreatic endocrine and exocrine functions remain insufficiently characterized.

This project aims to deliver a comprehensive chemical and biochemical evaluation of the modulatory effects of selected cyclitols on pancreatic function using cutting-edge metabolomic and molecular biology approaches. The experimental plan includes *in vitro* studies utilizing pancreatic β-cell lines (e.g., MIN6, INS-1) and *in vivo* investigations in C57BL/6 mice subjected to metabolic challenges (high-fat diet combined with streptozotocin administration).

Advanced analytical chemistry techniques—including liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography-mass spectrometry (GC-MS), gas chromatography-flame ionization detection (GC-FID), matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), and nuclear magnetic resonance (NMR) spectroscopy—will be employed for detailed qualitative and quantitative metabolite profiling in cellular, tissue, and biofluid samples. Complementary histological assessments and enzymatic activity assays will support the biochemical data.

By integrating metabolomic data with molecular and histopathological analyses, the project seeks to identify novel biomarkers and elucidate the molecular mechanisms through which cyclitols modulate pancreatic metabolism and function. These insights may pave the way for innovative therapeutic strategies aimed at improving pancreatic health and metabolic homeostasis. Ultimately, the findings could have significant translational implications for precision medicine and clinical nutrition in managing metabolic diseases.

1.3. Work plan

Stage 1: In vitro investigations employing pancreatic cell cultures to assess biochemical and cellular responses to cyclitols (Months 1–9).
Stage 2: Development and optimization of advanced chromatographic separation protocols coupled with high-resolution mass spectrometry (LC-MS/MS, GC-MS) for comprehensive metabolomic profiling of in vitro samples (Months 10–15).
Stage 3: Computational chemometric and bioinformatic processing of metabolomic datasets to identify and characterize key bioactive metabolites critical for pancreatic function (Months 16–18).
Stage 4: Design and execution of pharmacokinetic and pharmacodynamic studies in animal models to evaluate in vivo biochemical effects of cyclitols (Months 19–24).

Stage 5: Integrated metabolomic analysis of in vivo biological matrices using multimodal analytical platforms: chromatographic-MS techniques, nuclear magnetic resonance (NMR) spectroscopy, and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, with method validation (Months 25–30).
Stage 6: Histopathological examination complemented by chemical imaging techniques to correlate morphological changes with molecular alterations (Months 31–33).
Stage 7: Advanced bioinformatics analysis focusing on pathway elucidation and molecular interaction networks derived from multi-omics data (Months 34–36).
Stage 8: Application of chemometric tools including multivariate statistical analysis and pattern recognition for the interpretation of complex metabolomic and biochemical datasets (Months 37–42).
Stage 9: Preparation of publications and doctoral thesis (42–48 months)

1.4. Literature *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

1. Nicholson, J., Lindon, J. Metabonomics. Nature 2008
2. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica. 1999
3. Croze ML, Soulage CO. Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie. 2013
4. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. Mass Spectrom Rev. 2007.
5. Croze ML, Soulage CO. Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie. 2013.
6. Zhang A, Sun H, Wang P, et al. Recent and potential developments of metabolomics in the field of diabetic complications. Clin Chim Acta. 2014.

1.5. Required initial knowledge and skills of the PhD candidate

The project requires a solid knowledge of the basics of analytical chemistry and biochemistry. Candidates with practical laboratory experience, including sample preparation methods and separation techniques such as LC-MS and GC-MS or spectroscopy (MALDI, NMR), will be preferred. The candidate should

demonstrate an interest in metabolomics and a willingness to learn advanced analytical and statistical methods (multivariate analysis, metabolic bioinformatics). Proficiency in English, both spoken and written, is required, at a level enabling work with scientific literature, preparation of publications, and presentation of results. Independence in carrying out research tasks, teamwork skills, and a willingness for further scientific development, including scientific mobility, are also expected.

1.6. Expected development of the PhD candidate's knowledge and skills

During the project, the PhD candidate will acquire advanced knowledge in analytical chemistry, metabolomics, and bioorganic chemistry, with particular emphasis on analytical techniques used for characterizing metabolites and biomarkers. They will develop practical skills in designing and conducting in vitro experiments (pancreatic cell cultures) and in vivo studies (animal models), focusing on sample quality control and optimization of extraction and biological material preparation protocols.

The candidate will master advanced instrumental techniques such as high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy, including their applications in the identification and quantification of biologically active compounds. Moreover, they will learn to apply chemometric and bioinformatic methods for analyzing multidimensional metabolomic data, enabling reconstruction of metabolic pathways and identification of key molecular mechanisms regulating pancreatic function. The candidate will also develop skills in critical scientific data analysis, independent formulation of research hypotheses, and management of interdisciplinary projects