SUMMARY OF DOCTORAL THESIS

In recent years, there has been a significant rise in interest in cancer treatment methods that are characterised by high selectivity and minimal side effects. One of the most promising approaches is photodynamic therapy (PDT), which utilises light, a photosensitiser, and molecular oxygen to generate reactive oxygen species (ROS) that destroy cancer cells. Of particular interest is the therapy based on 5-aminolaevulinic acid (ALA-PDT), where 5-ALA acts as a precursor to the natural, endogenous photosensitiser – protoporphyrin IX (PPIX). Despite the potential of ALA-PDT, a key challenge remains the limited accumulation of PPIX in cancer cells, significantly reducing the treatment's effectiveness.

The aim of this study was to investigate the impact of novel iron chelators from the thiosemicarbazone (TSC) group on enhancing the efficacy of ALA-PDT by increasing the accumulation of protoporphyrin IX (PPIX) in cancer cells. The selected TSC derivatives were characterised by their lack of dark cytotoxicity and demonstrated the ability to chelate iron ions. Since iron chelators in ALA-PDT have not been widely studied, an analysis of their physicochemical properties using spectroscopic methods was conducted, along with an examination of the expression of genes related to iron metabolism and haem biosynthesis, in order to identify the molecular mechanisms responsible for improving the efficacy of this therapy. Additionally, studies were carried out to compare the effectiveness of the selected TSC derivatives with the reference chelator Cp94, which is currently commonly used in ALA-PDT.

During the research, two particularly promising thiosemicarbazone derivatives – TSC-34 and TSC-113 – were identified. Both compounds exhibited high iron chelation capacity, as confirmed by UV-VIS spectroscopy. The mechanism based on metal ion complexation is key to increasing the accumulation of protoporphyrin IX (PPIX) and inducing a phototoxic effect. The application of interdisciplinary biophysical methods allowed for the investigation of the complex actions of compounds with significant potential for use in medicine. The combination of molecular interactions with gene expression analysis clarified the mechanism of action of the TSC derivatives, including the regulation of gene expression related to haem biosynthesis and iron metabolism, such as the downregulation of ferrochelatase (FECH) and haem oxygenase 1 (HO-1). Each derivative acted in different ways, affecting the availability of iron for FECH through changes in the expression of mitoferrin and frataxin, further enhancing the effectiveness of the treatment. In the case of the Hs683 cell line, an atypical response to TSC-34 was observed, suggesting the existence of more complex mechanisms of action in this particular cell line. Compared to the TSC derivatives, the chelator Cp94 was found to be ineffective in increasing PPIX accumulation, confirming the greater potential of the new TSC derivatives as more effective iron chelators in ALA-PDT. The compounds TSC-34 and TSC-113 are promising candidates for further studies aimed at optimising 5-aminolaevulinic acid-based photodynamic therapy.