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## **ROZPRAWA DOKTORSKA**

**Synteza pochodnych aldehydów  
hydroksybenzoesowych o potencjalnych  
właściwościach inhibitorów ludzkiej heksokinazy 2  
(HK2)**

**PROMOTOR: prof. dr hab. inż. Krzysztof Walczak**

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## ABSTRACT

### **“Synthesis of hydroxybenzoic aldehyde derivatives with potential inhibitory properties against human hexokinase 2 (HK2)”**

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Glucose metabolism is one of the most important metabolic processes occurring in human cells. As a result of glycolysis, pyruvate is produced, which when introduced into the mitochondrial matrix undergoes a series of redox reactions. The end result of these transformations is mitochondrial oxidative phosphorylation and the formation of adenosine triphosphate (ATP). ATP is a donor of phosphate groups and a source of energy necessary for cellular processes. Cancer cells are characterized by a different glucose metabolism, in which glycolysis is accompanied by lactate synthesis, even in the presence of sufficient oxygen. The first step in glycolysis is the phosphorylation of glucose to glucose-6-phosphate, catalyzed by hexokinase 2 (HK2). In cancer cells, HK2 is overexpressed due to the increased demand for glucose caused by the low efficiency of glycolysis in generating ATP compared to oxidative phosphorylation. The overexpressed HK2 in cancer cells is currently being considered as a promising molecular target in anticancer therapies. In the conducted research, a number of compounds that could act as inhibitors of the enzymatic activity of HK2 were synthesized.

The aim of the work was to develop effective methods for the synthesis of derivatives from the group of hydrazones and N-acylated hydrazines of mono- and polyhydroxybenzoic aldehydes, in which the N-acyl fragment consisted of aromatic acids with various molecular regions (benzene, naphthalene, anthracene), 4-derivatives of benzoic acid, propionic acid and endogenous amino acids. These are multi-step syntheses that require an individual synthesis strategy due to the different chemical properties of the substrates used. The basic steps of the synthesis were the synthesis of esters of individual carboxylic acids, their transformation into hydrazides and condensation with hydroxybenzaldehyde derivatives to the corresponding N'-acylated hydrazones. When amino acids were used as acyl donors, it was necessary to protect the amino group in them. For selected hydrazones, the imine moiety was reduced by selecting appropriate reduction methods, ultimately obtaining derivatives of N-substituted hydrazides of mono- and polyhydroxybenzoic aldehydes. The physicochemical properties of the obtained compounds were examined, and the structures were confirmed using <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy and HRMS mass spectrometry.

For the obtained derivatives, the inhibitory activity with respect to hexokinase 2 was determined. Comparison of the inhibitory activity with the structure of the obtained derivatives allowed to determine the necessary structural fragments that a potential HK2 inhibitor should have. Among the synthesized compounds, the most promising derivative was (*E*)-4-fluoro-N'-(2,3,4-trihydroxybenzylidene)benzhydrazide, which showed higher in vitro inhibitory activity against HK2 than benserazide and benitrobenrazide described in previous studies.

These research results presented in the doctoral dissertation thesis are a starting point in designing effective HK2 inhibitors with potential use in anticancer therapies.