ABSTRACT OF PHD THESIS

Title:

A LOW MOLECULAR WEIGHT TYROSINASE INHIBITORS – DESIGN AND INTERACTIONS WITH THE ENZYME.

Author: Ewa Wolińska

Supervisor: Rafał Latajka, Ph. D, D. Sc.

Tyrosinase (EC 1.14.18.1) is an enzyme widely distributed in the natural environment, performing many biologically important functions in all organisms. It is the key enzyme of melanin biosynthesis, which are responsible for the color of human skin, eyes and hair. Melanin is a protective barrier against the harmful effects of UV radiation and oxidative stress, which may result in mutations in cellular DNA and initiation of the carcinogenesis process. It is believed that UV radiation is the main factor influencing the initiation of the development of skin cancers, such as malignant melanoma. Increased tyrosinase activity is related to occuring many diseases such as hyperpigmentation and melasma. Tyrosinase inhibitors are found as therapeutic agents in the treatment of pigmentation disorders.

The aim of the research presented in this PhD thesis was to develop new and highly active classes of a low molecular weight tyrosinase inhibitors from *Agaricus bisporus*, which could be used in the prevention and / or treatment of diseases caused by the abnormal activity of this enzyme, which is manifested by disorders of skin pigmentation in humans.

The implementation of the doctoral dissertation consisted of several stages: obtaining an enzyme preparation, designing new classes of strong tyrosinase inhibitors, determining the type and mechanism of inhibition mode using spectrophotometric methods, determining the inhibition parameters of the tested compounds and comparing their potency in relation to the known tyrosinase inhibitor – kojic acid that was used as a positive control. The last stage was an attempt to explain the correlation between the structure of the tested compounds and their inhibitory activity against tyrosinase.

There were examined **51** compounds, belonging to 4 different groups. Compounds **1-30** were derived from cromone, coumarin, pyridine and 2-azanorbornane. They showed different types of inhibition against to tyrosinase (competitive, non-competitive, mixed). It was not possible to clearly determine the relationship between the structures of the molecules and the type of inhibition they exhibited. All compounds were characterized by a reversible enzyme inhibition mechanism. Inhibition constants (K_I and K_{IS}) were at the millimolar level.

The most numerous group of tested compounds (**21-51**) were aryl thiosemicarbazone derivatives. Thiosemicarbazone derivatives were synthesized, in which the substituents R_1 and R_2 were modified, and the thiosemicarbazide moiety remained unchanged. Compounds were analyzed for their inhibitory activity against tyrosinase, SAR (*Structure Activity-Relationship*) analysis, and studies on melanogenesis inhibition and B16 cell proliferation. Studies have led to the discovery of a new and very potent inhibitor – compound **32** - of tyrosinase activity, for which the inhibition constant K_I was **0.17μM**. It completely inhibits the melanin synthesis process, however, it also has high cytotoxic effect on live cells and prevents their proliferation.

Other compounds also proved to be potent enzyme inhibitors, with inhibition constants at the micromolar level. Aryl thiosemicarbazone derivatives exhibited different types of inhibition against tyrosinase: competitive, non-competitive, mixed and competitive. However, no direct relationship was found between the structure of the compound and the type of inhibition it had on the enzyme's activity. All thiosemicarbazone compounds were a reversible inhibitors. In general, it can be seen that thiosemicarbazones substituted with a halogen atom in the aromatic ring in the *para*- and *meta*-position inhibited tyrosinase activity in comparison to compounds in which the halogen atom was in the *ortho*- position.

Compounds **22-26** and **36-45** are characterized by the moderate cytotoxicity and a high degree of inhibition of the melanogenesis process. These compounds can be taken into account at later stages of the work, taking into account their use as biologically active agents in cosmetic and pharmaceutical preparations preventing the occurrence and threatment of changes caused by abnormal melanization. Thiosemicarbazone compounds are characterized by high biological activity and pharmacological properties. Due to uncomplicated and low-cost synthesis, thiosemicarbazones can become a major focus in the design of new, effective tyrosinase inhibitors.