Streszczenie pracy doktorskiej w języku angielskim

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‘Thiosemicarbazones as inhibitors of tyrosinase’

Tyrosinase is an enzyme catalysing two different reactions: the oxidation of monophenols to *o*-quinones (monophenolase activity) and the oxidation of *o*-diphenols to corresponding *o*-quinones (diphenolase activity). When L-tyrosine or L-dopa are the substrates, the product of the reaction catalysed by tyrosinase is dopaquinone, the intermediate in melanin biosynthesis pathway. Melanin is the pigment responsible for skin and hair colour in mammals. Excess of the pigment which is accumulated in the skin leads to numerous skin disorders including freckles, age spots, melasma and one of the most dangerous skin cancer, malignant melanoma. Unfortunately, because of toxicity and instability most of tyrosinase inhibitors described in the literature is unable to be used orally or applied on the skin.

Thiosemicarbazones (TSCs) represent an interesting class of ligands with numerous pharmacological and biological properties.

The main aim of the study was to investigate the interactions between tyrosinase and a group of its potential strong inhibitors – aryl thiosemicarbazone derivatives. In the frame of doctoral dissertation an enzyme from *Agaricus bisporus* was isolated and purified. Then the effect of a group of 53 thiosemicarbazone derivatives was tested on mushroom tyrosinase using spectrophotometric enzymatic assay (determination of IC50). Kinetic studies (mechanism and type of inhibition, and determination of inhibitory constants) were performed for selected thiosemicarbazones. Moreover, the nature of enzyme-inhibitor interactions was explained using molecular docking. The relationship between the thiosemicarbazones structure and their biological activity towards tyrosinase was determined (SAR). Additionally, the results for *in vitro* enzyme research were compared to the results for the inhibition studies of the melanogenesis pathway in B16F10 cell line.

Almost all investigated compounds revealed inhibitory activity towards tyrosinase. IC50 of inhibitors varied from 0.3 to 800 µM. Seven out of investigated compounds achieved IC50 lower than 1 µM what locates them among the best tyrosinase inhibitors.

SAR analysis revealed that derivatives of acetophenone with bromine, chloride, fluorine atom or amino or hydroxyl group at *para* position in the phenyl ring provide IC50 below 1 µM.

Thiosemicarbazones are reversible inhibitors of tyrosinase. Most of them showed mixed type of inhibition what indicates their affinity to both: free enzyme and substrate-enzyme complex. Few compounds revealed pure competitive inhibition.

Molecular docking studies show that investigated TSCs penetrate active site of tyrosinase via thiourea moiety and the sulfur atom interact with copper ions in the active site.

All investigated thiosemicarbazones inhibit melanogenesis in B16F10 cells on the micromolar level.

4-fluoroacetophenone thiosemicarbazone possessing IC50 of 0.8 µM for tyrosinase inhibition and 1.7 µM for melanogenesis inhibition seems to be the most promising thiosemicarbazone in context of anti-pigmentation.