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Abstract of the doctoral dissertation

Title: “Urease inhibitors limiting the growth of pathogenic, ureolytically active strains”

Gram-negative bacteria with ureolytic activity belong to the most common human pathogens. Urease, which catalyses the hydrolysis of urea that results in the formation of carbamate and ammonia, is a significant virulence factor, responsible for both bacterial development in human body and pH-dependent pathological changes. *Helicobacter pylori* and *Proteus mirabilis* are the most common pathogens with ureolytic activity.

The goal of this doctoral dissertation was defined as the presentation of the full antibacterial and antiureolytic characteristics of 3 groups of compounds: 20 phosphoric derivatives (**1a**-**20a**), 27 selenium derivatives (**1s**-**27s**) and 60 flavonoids (**1f**-**60f**). Compounds of known effectiveness against purified bacterial ureases (**1a**-**20a**, **1s**-**27s**) were examined in the whole-cell studies, mainly concerning *H. pylori* i *P. mirabilis* strains. Flavonoids of unknown antiureolytic properties (**1f**-**60f**) were tested in both purified enzyme as well as whole cell studies.

In the presented research both standardized, international protocols (e.g. minimal inhibitory concentration (MIC)) and experiments requiring the optimization step were used. The aspects of MTT assay incorporation into antibacterial studies were vastly discussed. The optimized MTT assay protocol was used in both screening tests and final evaluation of bacteria viability. In most cases, the obtained results were verified with the plate counts and/or fluorescent staining. Unfortunately, most of examined compounds was described with negligible antibacterial properties. None of phosphonic derivatives limited the growth of bacteria with MIC < 1 mM. In contrast, most of them had permeabilizing activity against bacterial outer membrane. Further experiments concerning the antibacterial activity of selenium-containing compounds and flavonoids resulted in the identification of bacteriostatic agents, namely **1s** (MIC = 25 μM), **2s** (MIC = 100 μM), **4f** (MIC = 100 μg/mL) and **5f** (MIC = 100 μg/mL).

Compounds **1f**-**60f** were weaker inhibitors of native bacterial urease than other flavonoids, known from the literature. The most active compounds of **1f**-**60f** group were characterized with K_i values of 100-200 μg/mL and described as weak urease inhibitors. Most of active flavonoids was characterized with the mixed model of inhibition. Only 5 out of 60 examined flavonoids were active against whole bacterial cells. The strongest antiureolytic activity against *P. mirabilis* was observed for 2'-hydroxychalcone **2f** (IC_{50} = 190 μg/mL) and isoxanthohumol **53f** (IC_{50} = 174 μg/mL). Under applied incubation conditions these inhibitors were more effective against whole bacterial cells than quercetin – flavonoid described in the scientific literature as the most active against *H. pylori* urease.

Phosphonic derivatives had stronger antiureolytic activity against whole *P. mirabilis* cells than against *H. pylori*. *N*,*N*-dimethylaminomethyl-*P*-methylphosphinic acid **3a** (IC_{50} =10 μM) was the strongest of examined phosphonic inhibitors. Additionally, selenium-containing compounds limited the urease activity with exceptionally high effectiveness. 3,69 μM **2s**, 3,91 μM **12s**, 3,31 μM **14s** and 3,14 μM **15s** limited the activity of *H. pylori* by 50%. Diselenides were significantly less effective than monoselenium derivatives.

Presented experiments allowed to achieve the target of the research: the antibacterial and antiureolytic characteristics of 107 compounds was obtained.