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Streszczenie rozprawy doktorskiej:

Designing and chemical syntheses of selective matriptase-2 inhibitors based on trypsin inhibitor SFTI-1 isolated from sunflower seeds.

Serine proteases are the largest family of proteases, they compose over one-third of all known proteolytic enzymes. The name comes from the unique nucleophilicity of the hydroxyl group of serine residue in enzyme's active site. According to recent data collected in MEROPS database [1] there are over 50 families of serine peptidases that are grouped into 14 clans by comparing the tertiary structures and the order of the catalytic residues in the sequences [1].The main interest of my work were serine proteases of chymotrypsin fold, belonging to clan PA and S1 family. Recently, a novel group belonging to S1 family was discovered. It is called transmembrane serine proteasesand they are synthesized with extensions that serve to attach them to cell membrane so the catalytic domain is directed extracellularly. Localization at the cell surface, makes these enzymes perfectly positioned to interact with other proteins on the cell surface, soluble proteins, matrix components and proteins on neighboring cells. What is more, these enzymes posses intracellular, cytoplasmic domain, what gives an excellent opportunity to mediate signal transduction between the cell and its extracellular environment and to regulate various cellular responses [2].

The aim of my work was to obtain potent and selective inhibitors of matriptase-2, member of type II transmembrane serine proteases (TTSPs), that was first identified in 2002 [3]. TTSPs are in general composed of a single-pass transmembrane domain located near the *N*-terminus of the protein that separates a short, cytoplasmic domain from a larger extracellular part of the protease that contains a stem region and a *C*-terminal serine protease domain of the chymotrypsin (S1) fold. Recent studies have shown [4] that expression of matriptase-2 correlates with suppression of the migration and invasiveness of breast and prostate cancer cells. However, what focused my attention is matriptase-2 role in

keeping iron concentrations in a narrow physiological range. In short: matriptase-2 degrades hemojuvelin in cell membranes and subsequently inhibits hepcidin expression leading to high iron concentrations [5].

Matriptase-2 is closely related to another member of TTSPs – matriptase-1, which is found in the epithelial compartments of most embryonic and adult tissues. In contrast to matriptase-2, matriptase-1 possesses potent oncogenic activity. In breast cancer, overexpression of matriptase-1 is connected with tumor grade and stage and high enzyme expression is predictive factor of poor survival [6]. Because of so distinct, but important physiological functions of both enzymesit is important to find compounds, that are able to selectively inhibit matriptase-2 without influencing activity of structurally homological matriptase-1.

Taking into consideration results of previous studies [7] that shows that trypsin inhibitor SFTI-1 isolated from sunflower seeds suppresses activity matriptase-1,the work on low molecular weight inhibitors of matriptase-2 [8] and results on matriptase-2 substrates [9], I designed compounds with potential inhibitory activity towards matriptase-2.

I synthesized and examined enzymatic activity of 40 SFTI-1 analogues – with modifications mainly in 1, 4, 5, 10 and 12 positions, all possessed disulfide bridge and different combinations of additional backbone cyclizations (‘head to tail’ , ‘said-chain to tail’)or were just linear peptides. Series of peptidic and peptomericSFTI-1 analogues was synthesized by the solid-phase method. After synthesis, purification and MS and HPLC analysis, all peptides were examined for their inhibitory activity against matriptase-2 and homological protease matriptase-1 and three other serine proteases with similar specificity- thrombin, trypsin and plazmin.

In effect, I proved that both monocyclic SFTI-1 and native SFTI-1 are able to inhibit matriptase-2 (inhibition constant (K_i) values 1.365 μM and 0.218 μM, respectively) and that SFTI-1 is good lead structure for designing potential inhibitors of this protease. I obtained two inhibitors of matriptase-

2 with the highest inhibitory potency described to date: with K_i = 19 nM and K_i = 15 nM and five compounds with selectivity towards matriptase-2 over matriptase-1, plasmin and thrombin. Two of them:

- Gly-D-Arg-Cys(&)-Thr-Arg-Ser-Ile-Pro-Pro-Ile-Cys(&)-Phe-Pro-Asp& (**7**), K_i = 0.433 μM
- &[1]Gly-D-Arg-Cys(&[2])-Thr-Arg-Ser-Ile-Pro-Pro-Ile-Cys(&[2])-Phe-Pro-Asp&[1] (**12**), K_i = 0.278 μM

demonstrated significantly high selectivity towards matriptase-2. Analogues **7** was176 timesstronger inhibitor of matriptase-2 than matriptase-1 and **12** was 228 times stronger. Their inhibitory potency towards plasmin was marginal and they activated thrombin. Additionally, I obtained potent inhibitors of matriptase-1 with single-digit nanomolar K_i values, that were 40 to 100-fold weaker inhibitors of matriptase-2, over 1000-fold less potent inhibitors of plasmin and did not influence activity of thrombin.

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