ABSTRACT IN ENGLISH

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Biological and Medical Sciences

Supervisors: prof. Dr. Michael Gütschow

RNDr. Klára Konečná, Ph.D.

Candidate: Michaela Hympánová

Title of the diploma thesis: Enzyme kinetic evaluation of several potential inhibitors of certain human cysteine and serine proteases

Background

Cysteine and serine proteases are enzymes involved in many physiological processes. The imbalance between them and their endogenous inhibitors is associated with various diseases such as cancer and osteoporosis. Synthetic inactivators could be useful in the treatment of these enzyme-mediated pathological conditions. Therefore, there are ongoing attempts to develop low-molecular weight inactivators for therapeutically relevant cysteine and serine proteases. In the course of this thesis, compounds synthesized in prof. Gütschow's group were investigated as potential inhibitors of selected human proteases. They belong to imidazole compounds derived from *N*-protected cyclohexylalanine, 2-phenyl-7,8-dihydroimidazo[1,2-*a*]pyrazin-6(5*H*)-one derivatives, α,β-unsaturated peptidomimetic compounds, carbamates, an *N,N*-dibenzylcrotonamide derivatives and peptoides.

Aims

This diploma thesis has been focused on the evaluation of new potential inhibitors against human cathepsins B, L, S and K, which belong to cysteine proteases, as well as representatives of serine proteases, human leukocyte elastase and human thrombin. Furthermore, the structure-activity relationships of a small series of low molecular weight compounds were investigated.

Methods

In the experimental part, enzyme inhibition assays and their corresponding kinetic evaluation was introduced. In the course of this project, a human leukocyte elastase inhibition assay was modified and the determination of the Michaelis-Menten constant (K_M) was performed. Based on the obtained data, the inhibitory potency of the tested inhibitors was characterized by methods of linear and non-linear regression analysis to calculate different inhibition parameters.

Results

Most of the imidazole derivatives have shown significant inhibition against human leukocyte elastase. The best inhibitor from this group has been compound **3161** ($IC_{50}/(1 + [S]/K_M)$ = 0.60 ± 0.03 μM) containing a methyl- and phenyl-substituted imidazole moiety. Carbamate **3167** (k_{inac}/K_i = 12.44 $\mu M^{-1}s^{-1}$) was considered as an irreversible cathepsin B inhibitor. The *N,N*-dibenzylcrotonamide derivative, compound **3110** (k_{inac}/K_i = 2292.8 ± 240.74 $\mu M^{-1}s^{-1}$) containing an α,β-unsaturated Michael acceptor substructure, was considered as an irreversible inhibitor of cathepsin K. All other investigated compounds did not show any inhibitory potency.

Conclusion

In summary, some potent inhibitors of some tested enzymes have been found. The imidazole derivatives proved reversible inhibitory potential against human leukocyte elastase. Results with compound **3167** confirmed that the introduction of a carbamate structure is a possible way for the development of new cathepsin B inhibitors. The *N,N*-dibenzylcrotonamide derivative (compound **3110**) demonstrated the potential of Michael acceptors as irreversible cathepsin K inhibitors. Further analogous compounds need to be investigated in the future.

Key words: cathepsins, cysteine proteases, serine proteases, enzyme kinetics, enzyme inhibition