ABSTRACT

Human cathepsins K and S are members of the protein family of cysteine cathepsins with very similar tertiary structures. They have numerous physiological roles in human body, particularly the most important biological role of cathepsin K is degradation of collagen fibre in bone tissue, whereas cathepsin S plays a very important physiological role in regulating antigen presentation. Cathepsins K and S have to be sufficiently regulated since their increased enzyme activities could contribute to the progression of numerous diseases. Considering certain inhibitors of individual cathepsins K and S have reached clinical trials, these two enzymes represent important potential therapeutic targets for drug design. Targeting evolutionary less conserved allosteric sites could enable more selective inhibition, which could cause less side effects and could therefore represent an alternative approach for therapeutic purposes.

After detailed characterization of hyperbolic inhibitor of cathepsin K, methyl [(3*RS*)-2,5-dioxopyrrolidin-3-yl]glycinate (**3a**), it was shown that it is selective against cathepsin K over human cathepsins B, L, S in V. Since **3a** displayed weak inhibitory effect for cathepsin S, as well, we designed and prepared potential inhibitors of cathepsins K and S based on **3a**, which would bind with higher affinities and more selectively to individual enzyme. For the most potent inhibitors, more accurate values of affinities and mechanisms of action were determined. The aim of the research work was also to design and prepare mutant forms of enzymes based on superposition of tertiary structures of human cathepsins K and S and based on potential allosteric pathways of human cathepsin K, predicted by molecular dynamic simulations, as well, and to identify residues important for their allosteric regulation.

We designed and prepared compounds with substituents at two sites for diversification of the succinimide scaffold of compound **3a**. Among them, methyl [(3*R*)-2,5-dioxopyrrolidin-3-yl]-*L*-threoninate (***R*-3c**) was characterized as a hyperbolic inhibitor of cathepsin S and its absolute (2*S*,3*R,*3'*R*)-configuration was determined by X-ray structural analysis. Then, we designed and prepared cyclic derivatives of the compound ***R*-3c** and among four derivatives, (3'*RS*)-3-{[(1*S*,2*R*)-2-hydroxycyclohexyl]amino}pyrrolidine-2,5-dione (**(1*S*,2*R*)-7**) was identified and characterized as the most potent hyperbolic selective inhibitor of cathepsin S. It was shown by enzyme kinetics that allosteric modifiers of cathepsin K or S, compounds **3a**, ***R*-3c** and **(1*S*,2*R*)-7**, act via similar mechanisms as allosteric effectors of cathepsin K NSC13345 in NSC94914. Therefore, the first small-molecule allosteric effectors of cathepsin S (***R*-3c** and **(1*S*,2*R*)-7**) were identified and characterized. Apart from that, one part of the hypotheses of our research work was confirmed since it was shown that the compound **(1*S*,2*R*)-7** selectively inhibits cathepsin S over cathepsin K. The mechanisms of action, which were determined for the compounds, are consistent with the results of compound screening on degradation of macromolecular substrates via cathepsins K and S. Hyperbolic inhibitors **3a**, ***R*-3c** in **(1*S*,2*R*)-7** have the potential to be used for further development to lead compounds, which could be used for research purposes.

Mutant forms of cathepsins K and S with residues substituted by alanine residue in predicted allosteric pathway and allosteric site of cathepsin K as well as its homologous site of cathepsin S were designed and prepared. We have shown that residues in predicted allosteric pathway N202 and N208 are important for allosteric communication in cathepsin K between allosteric site and site S.