

# Cathepsin X inhibition – new approach for improvement of cathepsin B directed antipeptidase therapy

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A. Mitrović<sup>I</sup>, J. Završnik<sup>II</sup>, G. Mikhaylov<sup>II</sup>, U. Pečar Fonovič<sup>III</sup>, D. Knez<sup>III</sup>, M. Butinar<sup>II</sup>, S. Gobec<sup>III</sup>, B. Turk<sup>III,IV,V</sup>, J. Kos<sup>I,III</sup>

<sup>I</sup>Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia, <sup>II</sup>Department of Biochemistry and molecular biology, Jožef Stefan Institute, Ljubljana, Slovenia, <sup>III</sup>University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia, <sup>IV</sup>Center of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Ljubljana, Slovenia, <sup>V</sup>University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia

Cathepsin B is lysosomal cysteine peptidase that is one of the crucial peptidases in cancer. It is unique among cysteine cathepsins due to its dual endo- and exopeptidase activity that is a consequence of the presence of extra structural element the occluding loop. Cathepsin B has been validated as a promising target for antitumor therapy. However, decrease in the effectiveness of cathepsin B inhibition has been observed over time. This can be attributed to the compensation of its activity by increased expression and activity of cathepsin X, another lysosomal cysteine carboxypeptidase that is also involved in progression of cancer. Here we showed that triazole-based selective reversible inhibitor of cathepsin X (1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-((4-isopropyl-4H-1,2,4-triazol-3-yl)thio)ethan-1-one) (Compound 22) significantly reduces tumor growth and lung metastasis formation *in vivo* in transgenic FVB/PyMT breast cancer mouse model and in orthotopic MMTV-PyMT mouse breast cancer model. Therefore, the use of cathepsin X inhibitors presents novel strategy for improvement of cathepsin B directed therapy. Addition of cathepsin X inhibitors to the cathepsin B directed antipeptidase therapy, additionally decreased tumor invasion and migration *in vitro* on multiple cell-based models of tumor invasion and migration both in two- and three-dimensional settings and *in vivo* on mouse model. Taken together, our data show that potent selective reversible inhibitors of cathepsin X impair tumor progression both *in vitro* and *in vivo* and can be used in combination with other antipeptidase inhibitors as an innovative approach for overcoming resistance to the antipeptidase therapy.