Selective and reversible cathepsin X inhibitors: development and structure-activity relationships study

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Cathepsin X is a carboxymonopeptidase found mainly in immune cells, where it regulates migration, adhesion, proliferation, maturation, phagocytosis and signal transduction through cleaving different substrates. Cathepsin X is highly elevated in various types of cancer, neurodegenerative disorders, inflammatory and other diseases and became an object of interest as a possible therapeutic target. Till recently, an epoxysuccinyl-based inhibitor AMS36 was the only one that showed certain selectivity toward cathepsin X. It is an irreversible inhibitor that also partially inhibits cathepsin B. However, for treatment of pathologic conditions associated with excessive proteolytic cleavage, reversible small molecular inhibitors could be a preferred option. In our study 579 compounds from the in-house library were tested for the relative inhibition of cathepsin X, following further characterization. A reversible and specific inhibitor Z9 (1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-2-((4-isopropyl-4*H*-1,2,4-triazol-3-yl)thio)ethan-1-one) with K_i 2.45 ± 0.05 μM was further validated on PC-3 prostate cancer cells and PC-12 pheochromocytoma cells revealing substantial inhibition of PC-3 migration and PC-12 neurite outgrowth (previously published in: Pečar Fonović U et al. (2017) Sci Rep 7(1): 11459). Both processes are under the control of cathepsin X carboxypeptidase activity. Next, a concise series of inhibitor Z9 modifications on its benzodioxine or triazol moieties and ketomethylenethio linker were synthesized to study the structure-activity relationships. Several of the most promising candidates were further characterized and tested in cell-based assays.