Abstract

More than 20 years after its discovery HIV protease still remains one of the primary targets in HIV treatment. Currently there are 9 approved protease inhibitors on the market. However, due to immense replication rate and the high error prone nature of reverse transcriptase, resistance to each of them has already been described. Therefore, the search for new protease inhibitors with different binding mode is still active. A novel type of protease inhibitors (1, 4-benzodiazepine analogs) was recently discovered in our laboratory. Even though this new class of inhibitors is highly potent ($K_i$ in range of $10^{-9}$), it also has several undesirable qualities, such as low solubility and a high number of stereogenic centers. Primary objective of this study was to try to prepare more soluble compounds with lower number of possible stereoisomers, enzymologically characterize its binding to the wild-type and mutated HIV protease and to determine its structure in the complex with the enzyme.

A small library of 1, 4-benzodiazepine inhibitors of HIV protease was synthesized and fully characterized using NMR spectroscopy and mass spectroscopy. The number of stereogenic centers was successfully reduced from 4 to 2 without loosing activity of the inhibitor. The improvement in solubility was always associated with a dramatic decrease in activity. Therefore, all structural and kinetic studies were performed with the most potent inhibitor with limited solubility. The mechanism of inhibition of HIV protease was determined kinetically and the specificity of action was confirmed using two other proteases. After several steps of optimization, well diffracting crystals of HIV protease-inhibitor complex were obtained. The effort to determine the 3D structure of the complex is going on.

Key words: HIV protease, 1, 4-benzodiazepines, rational drug design, protein crystallization