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Inhibitors of HIV Proteinase

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Hydrolytic cleavage of the human immunodeficiency virus (HIV) encoded polyproteins 'gag' and 'gag-pol' is an essential process in the maturation of new virus particles. This processing is brought about by a proteolytic enzyme, itself virus encoded, which has been characterised as an aspartic proteinase. Site-specific mutation in the active site of this enzyme results in the production of immature and therefore uninfectious virus particles. This suggests that inhibitors of HIV proteinase should display antiviral activity but high specificity of action would be required in order to achieve therapeutic utility. We have designed and synthesised a range of novel compounds based on the 'pol' fragment Leu^{165}-Val^{170} which contain the transition-state moiety PheΨ[CH(OH)CH_2N]Pro in place of the Phe^{167}-Pro^{168} scissile bond. Optimisation of subsite specificity has afforded potent (IC_{50} 10^{-8} M) and highly selective inhibitors of HIV proteinase with good antiviral activity (IC_{50} $<10^{-6}$ M). Structure-activity relationships and some biological properties of these compounds will be discussed.

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X-ray Structures Of The HIV-1 Protease Complexed With Statine-Containing Peptide Inhibitors - Implications For Structure Based Drug Design

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A synthetic gene encoding the HIV-1 protease was expressed in E.coli from a fusion with the E. coli dihydrofolate reductase gene. Insoluble fusion protein was purified in the presence of 6 M urea and release of the native protease by autoproteolysis was facilitated by slow removal of urea at pH 7.0. The protease was further purified and concentrated on FPLC Mono-S column. Cleavage of a synthetic deca-peptide (TVSFNFPQIT) followed by separation of the products on HPLC was used to determine kinetic constants. A Michaelis constant of 1.9 ± .2 mM, kcat = 198 ± 9 s^{-1}, and Vmax = 212 ± 18 nM per min per ug of protease correspond well to those published by other groups for other substrates of the HIV-1 protease. Several statine containing peptides representing specific sequences recognized by the protease have been synthesized by solid state methodology. The inhibition constants were quantified by a peptidolytic assay using variable concentrations of substrate and inhibitor. Depending on the length and sequence of the inhibitors, the Ki's vary between 4uM and .6uM. The sequence specific inhibitors and Pepstatin A (Sigma) have been used for extensive co-crystallization studies. Several crystals of inhibitory complexes have been grown. The complexes have crystallized in one of two different space groups and the structures were solved by the molecular replacement method using published coordinates of the HIV-1 protease and refined at 2.5 Å resolution. The atomic interactions of different inhibitors with the protease's active site are being carefully examined in view of pursuing protein structure based inhibitor/drug design.