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TMC114 BINDS WITHIN THE SUBSTRATE ENVELOPE OF HIV-1 PROTEASE, WHICH COULD ACCOUNT FOR ITS EFFICACY AGAINST MULTI-PROTEASE INHIBITOR-RESISTANT VIRUS

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INTRODUCTION: HIV-1 protease is the target of very potent antiviral drugs for the treatment of HIV-1 infection. All current protease inhibitors (PIs) are the successful result of structure-based drug design. Unfortunately, as the viral reverse transcriptase is highly error prone, and under the selective pressure of drug therapy, many viable drug-resistant variants of HIV-1 protease have emerged. These PI-resistance mutations occur mostly at positions in the protease that will compromise inhibitor binding whilst retaining substrate specificity. We have determined from crystal structures of substrate complexes with HIV protease that the current PIs protrude beyond the substrate envelope, this may explain why resistance mutations constrain inhibitor binding. TMC114 is a next generation PI: recent virological and clinical results indicate that it is effective against known multi-PI-resistant variants of HIV-1. Furthermore, *in vitro* selection of TMC114-resistant variants from wild-type HIV-1 has proven difficult. In the present study we determined and compared the high-resolution crystal structure and thermodynamics of TMC114 or substrate binding to wild type HIV-1 protease.

METHODS: TMC114 was crystallized in complex with wild-type HIV-1 protease, and X-ray diffraction data was collected, processed and refined, using standard crystallographic techniques. The structure of the inhibitor complex was compared graphically with substrate complexes previously determined in our laboratory. The thermodynamics of inhibitor binding was determined using isothermal titration calorimetry (ITC) at 25°C and compared with other inhibitors binding.

RESULTS: The structure of TMC114 in complex with wild-type protease was determined to 1.93Å (P212121; R=19.7; Rf=22.2). In addition, the binding constant determined by ITC showed that the interaction of the inhibitor with the enzyme is very tight (K_d=10–12 M) and is extremely enthalpically driven. When the structure was

compared with the substrate complexes of HIV-1 protease, it was found that TMC114 occupies a volume that is contained within the substrate envelope, unlike most of the currently approved PIs.

CONCLUSIONS: Many drug-resistant variants of HIV protease evolve to maintain substrate recognition while compromising inhibitor binding, especially when the inhibitors extend beyond the substrate envelope. The fact that TMC114 fits well within the substrate envelope, associated with its tight binding to the enzyme, therefore, could account for why TMC114 remains active against most multi-PI-resistant variants. Hence, a mutation that affects TMC114 binding will likely cause a dramatic change in the ability of HIV-1 protease to recognize its substrates. This may also explain why selection of TMC114-resistant virus in vitro has proven difficult, as this might require changes beyond the protease gene, most probably in the cleavage sites. These results support our previous hypothesis that inhibitors that fit within the substrate envelope of HIV-1 protease may be more effective and less susceptible to drug resistance mutations.

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