Novel protease inhibitors markedly adapting to the structural plasticity of HIV-1 protease exert extreme potency with high genetic barrier

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Over the last 3 decades, HIV-1 caused a devastating pandemic with ~80 million people infected worldwide and nearly half of them died from the disease, AIDS. Antiretroviral therapy (ART) has proven to suppress virus replication and significantly elongate the survival of people with HIV-1. The inhibitors of HIV-1 protease (PIs), an essential enzyme that cleaves gag-pol polyproteins into mature functional proteins, are the key element of ART. Blocking the activity of protease (PR) leaves daughter virions replication-incompetent, enabling the immune system of the hosts restored. However, HIV-1 often acquires resistance to PIs during long-term therapy. Upon comparative analyses of X-ray structures of wild type PR and mutated PR variants such as PRs of $HIV_{DRV}{}^{R}{}_{P30}$ and $HIV_{DRV}{}^{R}{}_{P51}$ (*in vitro* selected with darunavir, DRV, over 30 or 51 weeks, respectively), we identified multiple critical residues (F33, M45, R20, and D35) that induce the structural deformation in PR structure. In order to overcome the reduced affinity as a result of structural plasticity of PR, we systematically modified the chemical moieties around the scaffold of DRV and synthesized various novel PIs. In particular, we introduced a P2' cyclopropyl-amino-benzothiazole moiety (Cp-Abt) or P2' isopropyl-amino-benzoxazole (Ip-Abo) at the S2' sub-pocket and a benzene ring located in the S1 sub-pocket with fluorine atoms at meta or para positions, and examined their anti-HIV-1 potency and cell permeability. Although the meta- and para-positioned fluorines had similarly facilitated cell permeability, we observed ~30-fold increase in cell permeability with P1 *bis*-fluorobenzene in the meta positions (*bis*-Fbz). Overall, the new set of PIs exerted significantly greater anti-HIV-1 potency (up to ~10,000-folds) and much higher genetic barriers to the emergence of resistant variants as compared to DRV. Particularly, GRL-063 containing *bis*-Fbz and Ip-Abo showed extremely potent inhibition against wild-type HIV-1 with IC_{50}s of ~25 attoM; however it turned out to be significantly less potent against resistant variants as compared to GRL-142 that contains *bis*-Fbz and Cp-Abt. Even upon 1-year *in vitro* selection with GRL-142, no HIV-1 variants acquired resistance to GRL-142. The extreme potency of GRL-142 is expected to reduce the dose to be administered, to have lesser or least side effects, and to have very high genetic barrier, making it the most promising PI for potential clinical development.