Supporting Information

Substituted Bis-THF Protease Inhibitors with Improved Potency against Highly Resistant Mature HIV-1 Protease PR20

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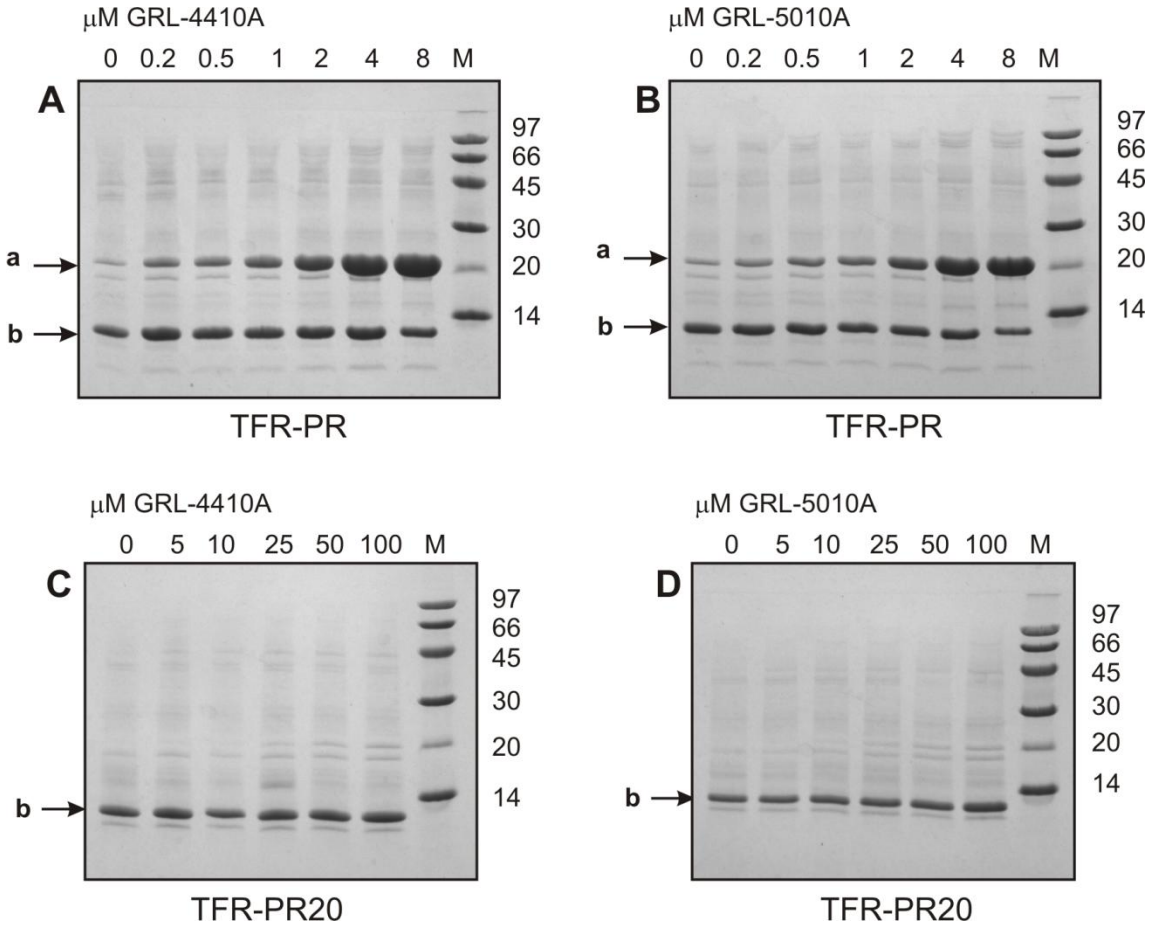
Figure S1. Dose-response for the inhibition of autocatalytic processing of wild-type TFR-PR and TFR-PR20 precursors in E. coli by GRL-4410A and GRL-5010A provided in the culture medium as described previously [1-2]. Final concentrations (μM) of inhibitor added to the medium are indicated above the lanes. Maximum accumulation of TFR-PR precursor is observed with 4 μM PI with an IC50 value of 1-2 μM similar to earlier studies with DRV under identical conditions. In contrast, no inhibition of the autoprocessing of TFR-PR20 is observed with GRL-4410A (C) or GRL-5010A (D) indicated by the absence of any accumulation of precursor. Bands indicated by arrows denote full length TFR-PR precursor (a) and mature PR or PR20 (b). Lane M denotes molecular weight standards in kDa. Proteins were subjected to 20% homogeneous PhastGel SDS-PAGE and visualized by PhastGel blue R staining.



(1) Louis, J. M., Aniana, A., Weber, I. T., and Sayer, J. M. (2011) Inhibition of autoprocessing of natural variants and multidrug resistant mutant precursors of HIV-1 protease by clinical inhibitors. *Proc Natl Acad Sci U S A 108*, 9072-9077.

(2) Agniswamy, J., Shen, C. H., Wang, Y. F., Ghosh, A. K., Rao, K. V., Xu, C. X., Sayer, J. M., Louis, J. M., and Weber, I. T. (2013) Extreme multidrug resistant HIV-1 protease with 20 mutations is resistant to novel protease inhibitors with P1'-pyrrolidinone or P2-tris-tetrahydrofuran. *J Med Chem 56*, 4017-4027.