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Sub-Picomolar Inhibition of HIV-1 Protease with a Boronic Acid

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Abstract

Boronic acids have been typecast as moieties for covalent complexation and are employed only rarely as agents for noncovalent recognition. By exploiting the profuse ability of a boronic acid group to form hydrogen bonds, we have developed an inhibitor of HIV-1 protease with extraordinary affinity. Specifically, we find that replacing an aniline moiety in darunavir with a phenylboronic acid leads to 20-fold greater affinity for the protease. X-Ray crystallography demonstrates that the boronic acid group participates in three hydrogen bonds, exceeding that of the amino group of darunavir or any other analog. Importantly, the boronic acid maintains its hydrogen bonds and its affinity for the drug-resistant D30N variant of HIV-1 protease. The BOH \cdots OC hydrogen bonds between the boronic acid hydroxy group and Asp30 (or Asn30) of the protease are short ($r_{O\cdots O} = 2.2 \text{ \AA}$), and density functional theory analysis reveals a high degree of covalency. These data highlight the utility of boronic acids as versatile functional groups in the design of small-molecule ligands.

Graphical Abstract

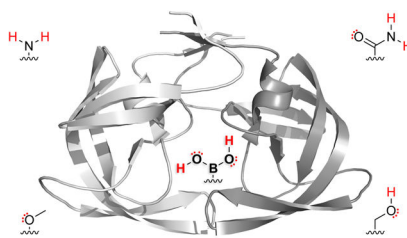
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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.xxxxxxx.

Experimental protocols and analytical data (PDF)

The authors declare no competing financial interest.



Clinical inhibitors of HIV-1 protease are quintessential triumphs of structure-based drug design.¹ The protease cleaves diverse sequences that connect individual domains of viral polyproteins, recognizing four substrate residues on each side of the scissile bond.² The components of most effective inhibitors—a tetrahedral-intermediate mimetic flanked by subsite-targeting groups—have undergone iterative optimization for 30 years.³ The discovery of the bis-THF moiety of darunavir, which targets the enzymic S2 subsite, was a major breakthrough.⁴ Its two bis-THF oxygen atoms accept hydrogen bonds from the main-chain amides of Asp29 and Asp30, leading to low picomolar affinity (Table 1).⁵ Mutations that overcome such main-chain interactions are rare,⁶ and darunavir is among the most resilient of protease inhibitors.⁷

Despite countless attempts at optimization, an ideal functional group for the S2' subsite has been elusive. Inspection of structures of complexes between substrates and darunavir analogs (Table 1, Figure 1) in conjunction with biochemical characterization revealed opportunities to us. Half of endogenous substrates occupy the S2' subsite with a glutamine or glutamic acid residue.^{8,2} These side-chains have been observed to form hydrogen bonds with both the main-chain N-H and the side-chain carboxylate group of Asp30 (Figure 1A). The aniline nitrogen of darunavir and the methoxy group of an anisole analog form only a single hydrogen bond (Figures 1B and 1C). Benzyl alcohol and cyclopropyl-amino-benzothiazole groups can form two hydrogen bonds with Asp30, one with the main-chain N-H and another with the side-chain (either via a water-bridge or directly), but provide <2-fold increases in affinity (Table 1, Figures 1D and 1E). Other aryl sulfonamide substituents, including benzoic acid and benzamide, form a water-bridge with Gly48 in addition to accepting a hydrogen bond from the main chain of Asp30, but again exhibit a <2-fold increase in affinity (Table 1, Figure 1F). This water-bridge with Gly48 is another interaction that can be exploited to recognize the main chain. Yet, no extant protease inhibitor interacts with all three of these targets: main chain and side chain of Asp30, and a water molecule that bridges to the main chain of Gly48.

We reasoned that an optimal functional group for targeting the S2' subsite would serve as *both* a donor and an acceptor of hydrogen bonds. We were aware that the two hydroxy groups presented by boronic acids are versatile in this manner.¹² These hydroxy groups display four lone pairs and two hydrogen-bond donors. No other functional group provides six opportunities to form hydrogen bonds so economically. We anticipated that one hydroxy group of a boronic acid could form both interactions with Asp30 while allowing the other hydroxy group to form a water-bridge with Gly48. Accordingly, we synthesized boronic acid **1**, in which the 4-sulfonylaniline moiety of darunavir is replaced with a 4-sulfonylphenylboronic acid (Table 1, Scheme S1).

Boronic acid **1** is a competitive inhibitor of catalysis by HIV-1 protease. By using a hypersensitive assay of catalytic activity,⁹ we found its inhibition constant (K_i) to be 0.5 ± 0.3 pM, which is indicative of 20-fold greater affinity compared to darunavir itself (Table 1, Figure S1D). Because the boronic acid moiety of **1** is anticipated to interact with Asp30, we suspected that D30N HIV-1 protease, which is a common variant that endows resistance, could compromise the affinity of boronic acid **1**. For example, the D30N substitution entices darunavir to form a water-bridge between its aniline nitrogen and the nascent asparagine, diminishing affinity by 30-fold.¹³ Remarkably, the affinity of boronic acid **1** for the D30N variant ($K_i = 0.4 \pm 0.3$ pM) is indistinguishable from that for wild-type HIV-1 protease.

To understand the basis for the extraordinary affinity and resiliency of boronic acid **1**, we determined the X-ray crystal structures of its complexes with both wild-type HIV-1 protease and the D30N variant. The two structures were solved at resolutions of 1.60 Å ($R_{\text{free}} = 0.1967$) and 1.94 Å ($R_{\text{free}} = 0.2203$), respectively (Table S1, Figure S2). True to its design, the boronic acid participated in all three hydrogen-bonding interactions (Figures 1G and 1H). Of special note are BOH \cdots OC hydrogen bonds observed in both structures (Figures 1G and 1H). The interatomic distance of 2.2 Å between the boronate oxygen and side-chain O $^{\delta}$ of residue 30 is reminiscent of a low-barrier hydrogen bond (LBHB).¹⁴

We analyzed the atypically short hydrogen bonds between boronic acid **1** and HIV-1 protease with computational methods. First, we optimized the hydrogen atoms by applying density functional theory (DFT) to a simple model extracted from the crystal structure. We examined the electronic structure by using Natural Bonding Orbital (NBO) analysis.¹⁵ NBO analysis revealed an interaction energy of 69.8 kcal/mol between boronic acid **1** and the wild-type protease. The typically non-hybridized p -type lone pair of the carboxylate oxygen hybridizes to sp^3 .⁹⁹ in the hydrogen-bonded complex. This large interaction energy and hybridization suggest a large degree of covalency in the BOH \cdots OC hydrogen bond. Next, we assessed the covalency of the short hydrogen bond between boronic acid **1** and the wild-type protease with quantum theory of atoms in molecules (AIM).¹⁶ AIM calculations—specifically, structural elements at the bond critical point (BCP)—enable quantification of the covalency between neighboring atoms. At the BOH \cdots OC BCP, we calculated an electron density (ρ) of $0.174 \text{ e}\text{\AA}^{-3}$, a Laplacian ($\nabla^2\rho$) of $-0.08 \text{ e}\text{\AA}^{-5}$, and a bond index of 0.22. Typical OH \cdots OC hydrogen bonds display $\rho < 0.2 \text{ e}\text{\AA}^{-3}$, positive $\nabla^2\rho$ values, and a bond index < 0.1 .¹⁷ Instead, the attributes of BOH \cdots OC are consistent with the attributes of an LBHB.¹⁴

An LBHB arises from functional groups with closely matched pK_a values.¹⁴ This requirement can be met by a carboxylic acid and a boronic acid,¹⁸ which are isoelectronic. In the enzyme-inhibitor complex (Figure 1G), the boronic acid group of **1** displays an $n_{\text{O},p} \rightarrow p_{\text{B}}$ interaction (*i.e.*, resonance) of 89.1 kcal/mol, and the carboxylic acid group of Asp30 in HIV-1 protease displays a comparable $n_{\text{O},p} \rightarrow \pi^*_{\text{C}=\text{O}}$ interaction of 87.9 kcal/mol. The ensuing hyperconjugative interaction between a boronic acid and carboxyl acid is reminiscent of a resonance-assisted hydrogen bond.¹⁹ Such hyperconjugation is absent in other inhibitors, such as the benzyl alcohol analog of darunavir (Figure 1D).

Boronic acids possess attractive properties beyond their versatile hydrogen bonding. Boronic acid **1**, like darunavir, is not toxic to human cells at concentrations up to 1 mM (Figure S3). *In vivo*, aniline moieties can exhibit problematic genotoxicity as a result of metabolic activation.²⁰ In contrast, the major metabolite of boronic acids is the oxidative deboronation product, an alcohol, which is modified further in phase II metabolism for efficient excretion.^{21,22}

We conclude that a boronic acid group in a ligand can be profuse and versatile in forming hydrogen bonds with a protein. These attributes are especially valuable in the design of ligands for proteins that are under the selective pressure of drug resistance. In those instances, the ability of boronic acids to form multiple hydrogen bonds enhances affinity, and the admixture of hydrogen-bond acceptors and donors enables adaption to mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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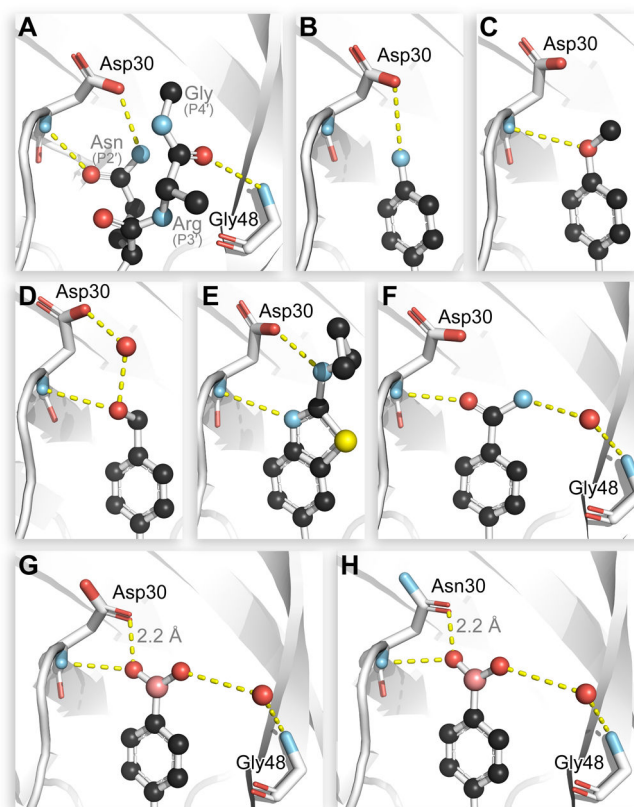


Figure 1. Interactions with a substrate, darunavir, or its analogs and the S2' subsite of HIV-1 protease. (A) A substrate (PDB entry 1kj7). (B) Darunavir (4hla). (C) Anisole analog (2i4u). (D) Benzyl alcohol analog (3o9g). (E) Cyclopropyl-amino-benzothiazole analog (5tyr). (F) Benzamide analog (4i8z). (G) Boronic acid **1** bound to wild-type HIV-1 protease (6c8x). (H) Boronic acid **1** bound to D30N HIV-1 protease (6c8y). Major conformers are shown for inhibitors that bound in non-symmetry-related conformations.

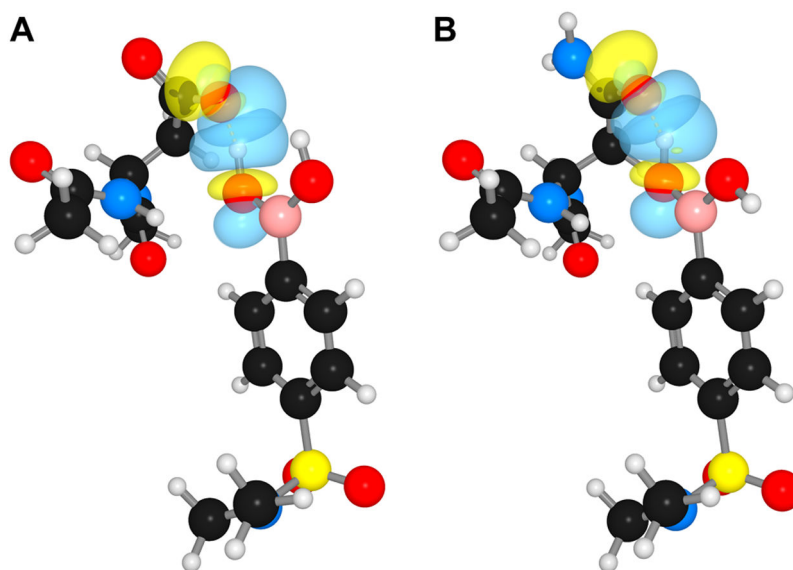
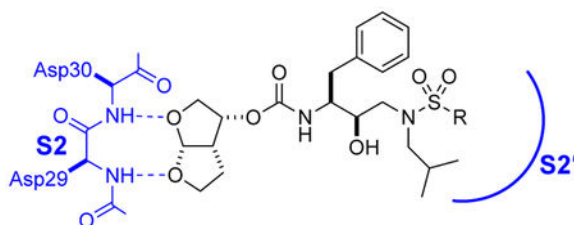
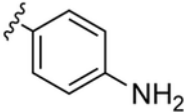
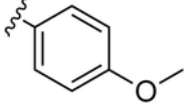
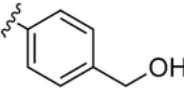
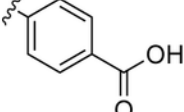
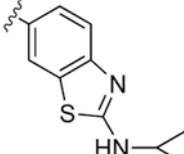
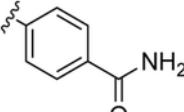
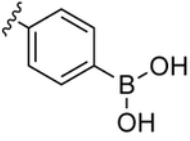


Figure 2. Orbital interactions in a model of boronic acid **1** and residue 30 of HIV-1 protease derived from X-ray crystal structures (PDB entries 6c8x and 6c8y). NBO rendering of the hydrogen bond between a boronic acid hydroxy group and O^δ of Asp30 (A) and Asn30 (B) with hydrogen atoms optimized at the M06-2X/6-311+G(d,p) level of theory employing the IEFPCM solvation model.

Table 1.Values of K_i for Inhibition of HIV-1 Protease

R	K_i (pM)	Relative Affinity ^a	Ref.
	10 ± 1	—	9
darunavir			
	14	1.0	1b
	12	1.2	1b
	12.7	1.3	10
	10	1.6	11
	8.9	1.8	10

R	K_i (pM)	Relative Affinity ^a	Ref.
	0.5 ± 0.3	20	This work
1			

^a Values of K_i can depend on assay conditions. Here, values are compared by using darunavir as a benchmark with Relative Affinity = $K_{i,darunavir} / K_{i,analog}$ as reported in the indicated reference.

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