Inhibition of HIV-1 Protease by a Boronic Acid with High Oxidative Stability

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ABSTRACT: HIV-1 protease is an important target for pharmaceutical intervention in HIV infection. Extensive structure-based drug design led to darunavir becoming a key chemotherapeutic agent. We replaced the aniline group of darunavir with a benzoxaborolone to form BOL-darunavir. This analogue has the same potency as darunavir as an inhibitor of catalysis by wild-type HIV-1 protease and, unlike darunavir, does not lose potency as an inhibitor of the common D30N variant. Moreover, BOL-darunavir is much more stable to oxidation than is a simple phenylboronic acid analogue of darunavir. X-ray crystallography revealed an extensive network of hydrogen bonds between the enzyme and benzoxaborolone moiety, including a novel direct hydrogen bond from a main-chain nitrogen to the carbonyl oxygen of the benzoxaborolone moiety that displaces a water molecule. These data highlight the utility of benzoxaborolone as a pharmacophore.

KEYWORDS: Benzoxaborolone, boronic acid, darunavir, HIV-1 protease, oxidative stability, pharmacophore

The human immunodeficiency virus (HIV) remains a global health scourge. Inhibitors of HIV protease are an important tool in the management of HIV infection. Years of drug-design have led to highly potent inhibitors such as darunavir (1), which is among the most resilient of protease inhibitors. Recently, we reported on a boronic acid derivative of darunavir (B-darunavir; 2) with subpicomolar affinity toward the protease, 20-fold greater than darunavir itself. Despite its high affinity, phenylboronic acid 2 proved to be less efficient than expected at inhibiting the reproduction of HIV in human cells. The low activity is likely due to oxidative deboration, as has been seen with other boronic acid-based pharmaceuticals. The phenol metabolite of B-darunavir has substantially reduced affinity for the protease and will be excreted readily by phenol conjugation through phase II metabolism. Thus, the high affinity of B-darunavir is outweighed by its oxidative instability.

Recently, we reported that benzoxaborolone exhibits dramatically improved stability toward oxidation compared to phenylboronic acid while retaining its desirable attributes as a ligand. Moreover, the oxygen-rich benzoxaborolone scaffold can provide additional opportunities for hydrogen bonding. Thus, we reasoned that BOL-darunavir (3), which is the benzoxaborolone analogue of B-darunavir (Figure 1), could display improved oxidative stability while maintaining high affinity for HIV-1 protease. Here, we describe the synthesis and analysis of BOL-darunavir.

SYNTHESIS OF BOL-DARUNAVIR

Initially, we attempted to synthesize benzoxaborolone 3 by modification of our synthetic route to phenylboronic acid using a carboxy-modified phenyl bromide. Unfortunately, the catalytic borylation and subsequent deprotection of the boronate and carboxy esters produced no isolable product.

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Attempts with other halides and more sterically bulky carboxy esters did not increase the yield. A synthetic route relying on magnesium−halogen exchange proved successful on a model compound but incompatible with the full molecule. Similarly, attempts to use other metal-catalyzed reactions and more reactive electrophiles were unsuccessful. The synthesis of a boronated sulfonyl chloride succumbed to protodeboronation of the boronic acid moiety.

We reasoned that the failure of the catalytic borylation reaction could be due to a competitive protodeboronation or protodehalogenation reaction. If so, then stoichiometric palladium could decrease the reaction time, allowing borylation to outcompete degradation. By using stoichiometric palladium, we were indeed able to elicit C−B bond formation. Borylation was followed by mild deprotection of the pinacol ester through its potassium trifluoroborate salt (which allowed for purification of the borylated product), and subsequent hydrolysis to boronic acid using chlorotrimethylsilane and water.

Hydrolysis of the methyl ester in a mild sodium bicarbonate solution resulted in benzoxaborolone (Scheme 1).

**OXIDATIVE STABILITY OF B-DARUNAVIR AND BOL-DARUNAVIR**

As a proxy for in vivo oxidation of the boronic acid derivatives, we used 1H NMR spectroscopy to obtain rate constants for the oxidation of compounds 2 and 3 by hydrogen peroxide, which is the major reactive oxygen species in humans. As expected, the use of the benzoxaborolone moiety resisted oxidation, with benzoxaborolone 3 oxidizing ~50-fold more slowly than phenylboronic acid 2 (Figure S1; Table 1).

Table 1. Parameters for the Oxidation of and HIV-1 Protease Inhibition by Darunavir (1), B-Darunavir (2), and BOL-Darunavir (3)

<table>
<thead>
<tr>
<th>compound</th>
<th>( k_{\text{obs}} ) (M⁻¹ s⁻¹)</th>
<th>( k_{\text{red}} )</th>
<th>( K_i ) (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.12 ± 0.01</td>
<td>1</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>0.026 ± 0.0004</td>
<td>0.021</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For oxidation by hydrogen peroxide in 1:1 50 mM sodium phosphate buffer in D₂O, pH 7.4/CD3CN. For inhibition of the cleavage of RE(EDANS)SGIFLETSK(DABCYL)R in sodium acetate buffer, pH 5.0, containing NaCl (0.10 M), DMF (2% v/v). Values are from ref 13.

The strongly electron-withdrawing sulfonamide group differentially affects the oxidative stability of compounds 2 and 3. Compound 2 is more oxidatively stable than phenylboronic acid itself (which has \( k_{\text{obs}} \approx 2.4 \text{ M}^{-1} \text{s}^{-1} \); ref 8), presumably because of the stabilization of its anionic form. By contrast, compound 3 is less stable than benzoxaborolone itself (which has \( k_{\text{obs}} = 0.00015 \text{ M}^{-1} \text{s}^{-1} \); ref 8). This decrease could reflect an increase in the stability of the dianion that is formed upon hydrolysis of the boralactone ring of compound 3 due to the electron-withdrawing nature of its sulfonamide group. Indeed, DFT calculations of a model compound indicate that the dianion has a much faster oxidation rate than does the intact boralactone (Table S1). Regardless, substitution of the phenylboronic acid with a benzoxaborolone enhances the oxidative stability of benzoxaborolone 3 to be well beyond that of typical boronic acids.
INTERACTION OF BOL-DARUNAVIR WITH HIV-1 PROTEASE

Benzoazaborolone 3 proved to be an effective inhibitor of catalysis by HIV protease. Using the hypersensitive assay developed in our laboratory,13 we obtained an inhibitory constant (Kᵢ) of 10 ± 2 pM with the wild-type enzyme. This value indicates a loss of affinity relative to phenylboronic acid 2 but is indistinguishable from the inhibitory constant observed for darunavir itself (Figure S1; Table 1).13

D30N is a common substitution in clinical isolates of HIV-1 protease.14 The ensuing loss of a hydrogen bond between Asp30 and the aniline moiety of darunavir leads to a drop in potency of 30-fold.15 In contrast, we did not observe a decrease in affinity of BOL-darunavir to the D30N variant (Kᵢ = 7 ± 5 pM; Figure S2).

To characterize the interaction of benzoazaborolone 3 with HIV-1 protease further, we obtained an X-ray crystal structure of the enzyme-inhibitor complex. In previous work, we found that a benzoazaborolone moiety in a transthyretin ligand forms a covalent B–O bond with a serine side chain.8 In that protein-ligand complex, the boron adopts a trigonal geometry. In contrast, BOL-darunavir binds to HIV-1 protease in a noncovalent manner, and its boron adopts a tetrahedral geometry (Figure 2). Both covalent16−18 and noncovalent19 binding modes have been observed in FDA-approved and investigational pharmaceutical agents that contain boronic acid moieties.20−22 These differences showcase the high versatility of the benzoazaborolone pharmacophore and indicate its adaptability to local environments.

The benzoazaborolone moiety of BOL-darunavir forms an extensive network of intermolecular hydrogen bonds with HIV-1 protease (Figure 2). Of particular note is the replacement of a canonical bridging water molecule with a hydrogen bond directly with the main-chain nitrogen of Gly48 (Figure 2A,B). Gly48 is in one of the two flexible flaps that closes to bind an enzymic substrate (Figure 2C). Highly mutated proteases exhibit a resistance mechanism wherein the closed state of the flaps is destabilized, allowing transient closure for substrate hydrolysis but weakening the binding of known inhibitors.23 To our knowledge, no other hydroxyethylamine sulfonamide accepts a hydrogen bond from the main-chain nitrogen of Gly48; importantly, such an interaction cannot be averted simply by a mutation that installs a new side chain.24−26 Overall, two hydrogen bonds are formed by the benzoazaborolone moiety with the protease main chain (Asp29 and Gly48), one with a side chain (Asp30) and two with bridging water molecules. The extent of this hydrogen-bonding network is unique and could offer affinity to protease variants that resist darunavir or other drugs.3,4,27−29

CONCLUSION

BOL-darunavir is a 10 pM inhibitor of HIV-1 protease. This darunavir analogue is equipotent with darunavir itself and avoids the potential for genotoxic metabolites that can arise from aniline moieties.5,31 This darunavir analogue is equipotent with darunavir itself and displays significantly enhanced oxidative stability while affording a unique pattern of hydrogen-bond donors and acceptors. The dense hydrogen-bond network could be less sensitive to point mutations than with other pharmacophores. We suspect that the benefits of a benzoazaborolone moiety could be generalizable to other targets of pharmacological importance.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00464.
Experimental procedures, computational data, and NMR spectra (PDF)

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Notes
The authors declare the following competing financial interest(s): The Massachusetts Institute of Technology has applied for a patent on the use of benzoxaborolone as a pharmacophore.

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