Design of boron-based peptidomimetics leads to potent inhibitors of human ClpP and ClpXP

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Abstract

Boronic acids have attracted the attention of synthetic and medicinal chemists due to boron’s ability to modulate enzyme function. Recently, we demonstrated that boron-containing amphoteric building blocks facilitate the discovery of bioactive aminoboronic acids. Herein, we have augmented this capability with a de novo library design and virtual screening platform modified for covalent ligands. This technique has
allowed us to rapidly design and identify a series of α-aminoboronic acids as the first inhibitors of human ClpXP, which is responsible for the degradation of misfolded proteins.

**Introduction**

Boron-containing molecules (BCMs) have garnered much attention over the years due to their recent successes as chemical probes and therapeutic agents.\(^1\)–\(^7\) The FDA approval of the multiple myeloma drug bortezomib (Velcade)\(^8\) in 2008 led synthetic and medicinal chemists to reconsider the significance of boron in therapeutics. The empty \(p\) orbital of boronic acids can interact with nucleophilic amino acid residues forming a tetracoordinate “ate” complex. In addition, tricoordinate boron can adopt various coordination modes upon biological target engagement.\(^9\) This stands in contrast to other electrophiles such as epoxides, aziridines, and Michael acceptors, which display a singular type of interaction with active site nucleophiles. Despite the versatility and recent successes of BCM-driven medicinal chemistry, there are still few examples of boron-containing therapeutic agents. This can be partially explained by the fact that synthetic technologies to site-selectively introduce boron into heteroatom-rich environments remain underdeveloped. The thermodynamic preference of boron to migrate from carbon to oxygen or nitrogen, further aggravated by the low kinetic barrier for these transformations, also accounts for the dearth of available methods.\(^10\),\(^11\)

We recently developed a series of amphoteric molecules such as boryl aldehydes,\(^12\),\(^13\) acyl boronates\(^14\) and boryl isocyanides\(^15\) and applied them for rapid construction of bioactive BCMs that contain numerous heteroatoms in the vicinity of boron. For instance, we have reported the synthesis of structurally novel boromorpholinone scaffolds from boryl isocyanides and demonstrated their role as potent inhibitors of the 20S proteasome.\(^15\) We have also identified a novel β-aminoboronic acid chemotype as a cell-active inhibitor of the serine hydrolase, (ox)lipid-metabolizing enzyme α/β-hydrolyase domain 3 (ABHD3), where the \(N\)-methyliminodiacetic acid (MIDA) ligand increased the cellular permeability of
the inhibitor. To fully leverage the synthetic prowess of boron-based amphoteric reagents, we now report on the integration of this chemistry with a de novo library design and virtual screening program and detail the discovery of bioactive inhibitors of human caseinolytic protease P.

Caseinolytic protease P (ClpP) is a highly conserved serine protease present in both bacteria and eukaryotes. Its primary role is to maintain cellular homeostasis by working together with its AAA+ chaperones such as ClpA, ClpC and ClpX, to recognize, unfold, and translocate substrates into the proteolytic chamber of ClpP for protein degradation. In the absence of its chaperones, ClpP can only degrade small peptides. Thus far, research efforts in small molecule modulation of ClpP have been primarily directed towards bacteria. This has led to the development of both activators and inhibitors of bacterial ClpP which can cause bactericidal inhibition, and impact bacterial virulence. Human ClpP (hClpP) is overexpressed in the mitochondria of a subset of acute myeloid leukemia (AML) cells and stem cells. Therefore, inhibition or activation of hClpP has the potential to be an effective strategy for therapeutic intervention of AML. Thus far, the only reported activator of hClpP is D9 which was discovered by Sieber and coworkers earlier this year. In 2015, Sieber and coworkers identified phenyl ester AV167 as a low micromolar inhibitor of hClpP (IC$_{50}$ = 1.54 μM). More recently, Sieber has shown that TG42, an analogue of AV167, is a more potent and selective inhibitor of hClpP (IC$_{50}$ = 0.39 μM) compared to AV167. Additionally, TG42 was shown to inhibit the hClpP in the presence of Escherichia coli ClpX (EcClpP) complex in a FITC-caesin degradation assay and GFP degradation assay. Despite inhibiting ClpP in the presence of its chaperone for the first time, the hClpP-EcClpX complex exhibits bacterial ClpXP substrate specificity. Currently, there are no reported small molecule inhibitors of hClpXP which may be attributed to conformational changes in ClpP upon ClpX binding.

In order to develop boron-containing inhibitors of hClpP, we opted to utilize a de novo design approach. Small molecule virtual screening has become a valuable tool in drug discovery. The benefit of this approach is that it negates the need to manually synthesize libraries of structurally diverse molecules,
thus saving time and resources. For the docking of boronic acid-based ligands with hClpP, an important part of the screening process is to select only compounds which bind in a manner that allows for formation of a covalent bond between the boronic acid and the hydroxyl group of the catalytically active serine residue. A number of different software solutions exist for performing covalent docking,\textsuperscript{25–28} however, none of them are optimally suited for screening a large user-designed library of covalent ligands, such as those synthesized using our amphoteric boron reagents. For many programs, only a small set of reactions can be performed, which limits their applicability. In addition, the side chain conformation of the reactive residue is often not optimized during the calculations, but instead treated as static, which means that the covalent docking results are heavily dependent on the chosen conformation of the residue. Furthermore, the majority of covalent docking methods are not designed for screening large libraries, and therefore become prohibitively slow when it comes to screening thousands of compounds. In 2014, the Shoichet lab developed the webserver DOCKovalent as a covalent docking program made for screening large libraries.\textsuperscript{26} However, while it is well-suited for screening predetermined libraries of commercially available compounds, it cannot readily be used for screening user-designed libraries, and is thus not suitable for our chemistry. In light of these limitations of covalent docking programs, we developed a protocol for screening covalent inhibitors using a standard non-covalent docking method.

Herein, we show that chemistry derived from our boryl isocyanide building blocks combined with a \textit{de novo} library design and virtual screening leads to peptidomimetic boron-based inhibitors of hClpP with low micromolar potency. The boron-based inhibitors are the first reported inhibitors of the more therapeutically relevant hClpXP protease.

\textbf{Results and Discussion}
Substrate-based hClpP inhibitor. The structures of the most celebrated boron-containing chemotherapeutics currently on the market are based on the α-aminoboronic acid motif. Therefore, we hypothesized that α-aminoboronic acids may also covalently inhibit the activity of hClpP. We synthesized compound 1, which is a boronic acid analogue of the ClpP substrate Phe-Ala-Pro-His-Phe (Figure 1). Due to the substrate-like nature of the compound it is predicted to bind in the active site of ClpP, followed by an addition of the catalytic serine (Ser97) to the boronic acid. To synthesize boropeptide 1, we first prepared benzyl boryl amine. Starting from the N-Cbz-protected benzyl-α-amino(MIDA)boronate, which was prepared according to literature procedures, a hydrogenation with Pd/C was performed to obtain the benzyl-α-amino(MIDA)boronate (See Supporting Information). Boropeptide 1 was then prepared by coupling benzyl-α-amino(MIDA)boronate and the tetrapeptide Ac-Phe-Ala-Pro-His. Testing of 1 in a fluorescence-based Ac-Trp-Leu-Ala-AMC (WLA-AMC) assay confirmed that the compound is capable of inhibiting hClpP with an IC₅₀ value of 27.27 ± 2.07 μM.

![Boronic acid analog of ClpP substrate Phe-Ala-Pro-His-Phe.](image)

Library enumeration. Powered with the knowledge that α-aminoboronic acids display weak inhibitory activity towards hClpP, we realized that our amphoteric boron building blocks enabled us to synthesize peptidomimetic α-aminoboronic acids. Using our boryl isocyanide reagents, we performed an Ugi 4-component reaction (U4CR) with an aldehyde, amine and carboxylic acid. The resulting compounds
contain a similar molecular backbone to ClpP’s natural peptide substrates. We constructed a virtual library of all possible compounds that could potentially be made using the combinatorial U4CR reaction with the starting materials available in-house using BIOVIA Pipeline Pilot. This afforded a virtual library of ~85,000 compounds. In order to determine which of these compounds would fit into the active site of hClpP, a virtual screen of the compound library was performed. As previously mentioned, none of the available covalent docking programs are well-suited for screening our custom library of α-aminoboronic acids. Therefore, we developed a new protocol for screening covalent inhibitors using a standard non-covalent docking method using Glide from Schrodinger, Inc. The protocol involves three changes as compared to a regular virtual screening with Glide (Figure 2). For covalent ligands, it is essential that the docking procedure produces complexes where the reactive group on the ligand is in close proximity of the reactive residue in the target. However, complexes in which the reactive residue sidechain is as close to the ligand as it would be in a covalent complex will invariably lead to steric clashes, and thus an unfavorable docking score. To solve this, the reactive residue, Ser97, is mutated to a glycine prior to the docking calculation. Second, a distance constraint between the Cα atom of the reactive residue and the reactive group on the ligand is applied. This allows the reactive group in the ligand to be positioned in close contact with the reactive residue without the introduction of steric clashes. Lastly, for addition reactions, such as the reaction between a serine residue and a boronic acid, the geometry of the attachment atom on the ligand changes. In the case of a boronic acid addition, the boron changes from a trigonal to a tetrahedral geometry. To mimic this, an artificial conversion of each ligand is performed prior to the docking, which consists of adding a hydrogen atom to the boron atom. This allows the docking procedure to test whether the tetrahedral boronates that are formed upon interact with Ser97 will able to fit into the binding pocket. In combination, the three described deviations from a standard docking protocol; mutation of reactive residue, distance constraint, and compound conversion, provide the basis for using a standard non-covalent docking program to screen covalent ligands.
Figure 2: Depiction of the three changes made to a standard covalent docking protocol that makes it possible to use non-covalent docking to virtually screen large libraries of covalent ligands within a reasonable time.

The main caveat of this approach is that although the resulting poses will have the reacting atoms in the vicinity of each other, they will not necessarily be in an optimal position for covalent bond formation. Therefore, additional visual inspection of the results is needed for determining the most promising hits. Alternatively, geometric filters can be applied to the docking results, to only select poses that would allow the reactive residue to adopt a favorable conformer and at the same time keep the distance between the two reacting atoms close to the value of the equilibrium bond length for such an interaction. Ai et al. have also published a method for using non-covalent docking for screening covalent ligands, known as Steric-Clashes Alleviating Receptor (SCAR). In SCAR, the reactive residue is also mutated to glycine, however there is no distance constraint between the reactive groups, and the geometry of the ligands is not changed prior to the docking calculation. Instead, the docking poses are tested after the calculation and only ligands which have the two reactive groups in close vicinity are selected. This means that if the top-scoring poses of a given ligand does not fulfill the distance criteria it is discarding even though it might also be able to
adopt a slightly less favorable mode in which the two groups do come close together. In our approach, we avoid such issues, since the distance constraint is applied during the actual docking.

**Virtual screening and compound selection.** The method described above was used to screen the U4CR library against the active site of hClpP. The top 1,000 highest-scoring compounds based on Glide SP docking score were subjected to further analysis in order to select compounds for synthesis. Due to the combinatorial nature of the library, it is possible to separate the analysis into three parts, focusing at finding the most favorable groups at the either the R\(^1\), R\(^2\) or R\(^3\) position (See Supporting Information, Figure S1). Thus, an analysis was performed to determine the substituents that appeared most frequently at each of the three positions. The results of this analysis, combined with a visual inspection of the top-scoring poses to ensure that bond formation to Ser97 was possible for the chosen R\(^1\), R\(^2\) and R\(^3\) substituents, was used to select a final set of substituents predicted to be favorable for each position (See Supporting Information, Figure S1). Based on the observed binding mode of the docked compounds, the R\(^1\) group was expected to impact the hClpP potency the most, followed by the group at the R\(^3\) position. For the R\(^1\) position, aliphatic groups such as isobutyl and \(n\)-butyl appeared to be well suited for filling out the S1 pocket. Phenyl and benzyl groups were predicted to be less favorable at the R\(^1\) position compared to aliphatic groups. However, a compound containing a benzylic group at R\(^1\) was still selected for synthesis in order to validate this hypothesis. For the R\(^2\) position, aliphatic and phenyl groups were selected. For the R\(^3\) position, aromatic groups dominated the top-scoring compounds, including phenylic compounds and bicyclic ring systems. Overall, these results are consistent with Sieber’s findings on hClpP specificity derived from a fluorogenic library screening.\(^{33}\) The molecules that were selected from this virtual library for synthesis were chosen based on this analysis as well as synthetic feasibility.
**Synthesis of α-aminoboronic acids.** We began our investigation by subjecting isobutyl- or benzyl-substituted boryl isocyanide 2a and 2b, respectively, to an U4CR with an aldehyde, carboxylic acid and ammonia in TFE (Scheme 1). From this reaction, we obtained a 1:1 diastereomeric mixture of the desired α-amino(MIDA)boronates 3 which could be separated by flash normal-phase column chromatography. The compounds that were below 95% purity as determined by $^1$H NMR were purified again by reverse-phase column chromatography. In order to test the substrate tolerance of the P1, P2 and P3 sites of hClpP, we tried to synthesize α-amino(MIDA)boronates with various aldehydes, carboxylic acids and isocyanides. The reaction proceeded smoothly with both isobutyl- and benzyl-substituted boryl isocyanides. Isovaleraldehyde and 3,5-dichlorosalicyaldehyde performed well in the reaction, however, the diastereomers afforded from 3,5-dichlorosalicyaldehyde were inseparable by normal-phase and reverse-phase chromatography. Cyclopropanaldehyde failed to react which we presume to be a result of steric hinderance. We also attempted to synthesize the histidine-containing aldehyde through Dess-Martin Periodinane oxidation of its alcohol precursor but were unsuccessful. We were able to modify the carboxylic acid component with aromatic and alkyl carboxylic acids. Phenol-based carboxylic acids were tolerated in the reaction as well. No derivatizations were performed on the amine component since the resulting amide is expected to be participate in hydrogen bonding with the backbone carbonyl of Ser125 in hClpP.

MIDA-protected boronates are inactive as serine hydrolase inhibitors due to the tetracoordinate environment around boron. Thus, MIDA is typically released *in situ* under the assays conditions or as a separate synthetic step prior to assay submission. Although α-aminoboronic acids possess the ability to inhibit serine hydrolases, they are susceptible to degradation through a 1,2-boryl migration. However, acylation at this nitrogen atom engages the lone pair in an amide bond which eliminates the decomposition pathway. Thus, α-amino(MIDA)boronates 3 should be stable upon MIDA hydrolysis. We began our experiments by attempting to hydrolyze MIDA under basic conditions. We were pleased to see formation
of free boronic acids 4, however, we also saw evidence of boric acid. On the other hand, the hydrolysis went smoothly with acid to solely provide the free boronic acid in quantitative yields. It is important to mention that this deprotection only proceeded at reasonable rates in alcoholic solvents and when using other solvents, degradation was observed over time. Unfortunately, the second diastereomer of compound 4d did not readily undergo MIDA hydrolysis due to poor solubility.

Scheme 1: Synthesis of α-aminoboronic acids from an U4CR reaction with our boryl isocyanide building blocks. “Unable to access the boronic acid through MIDA hydrolysis due to poor solubility of the starting material.

Initial testing for the inhibition of human ClpP. The racemic α-aminoboronic acids 4 were screened for hClpP inhibition in a fluorescence-based WLA-AMC assay in triplicate (Table 1). Ac-WLA-AMC has been recently reported as a substrate for human ClpP that is used for measuring its peptidase activity by monitoring release of fluorescent amino-methylcoumarin.34 The syn and anti configurations of compounds 4 were assigned based on the relative positioning of the R¹ and R² side chains. The relative stereochemistry was assigned after a crystal structure of compound anti-4a bound to Staphylococcus aureus ClpP (SaClpP)
was obtained as described in the next paragraph (Figure 3). Interestingly, only the second diastereomer anti-4 of this series of compounds exhibited activity towards hClpP whereas the syn-4 diastereomer was inactive. This suggests that stereochemistry plays an important role in binding. Compounds anti-4a, anti-4b, anti-4c and anti-4e displayed IC_{50} values of 41.9 ± 2.8, 18.4 ± 5.5, 28.9 ± 5.0 and 34.27 ± 7.84 μM, respectively. Compound anti-4f, which was synthesized to probe the P1 pocket of hClpP, was significantly less potent than anti-4a (IC_{50} = 103.0 ± 31.1 μM). This result confirms that aliphatic groups are more favorable than benzylic groups at the R^1 position as predicted from the virtual screening results.

Table 1: IC_{50} values for the inhibition of hClpP by compounds 4 in WLA-AMC assay.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>hClpP IC_{50} (μM)</th>
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<tbody>
<tr>
<td></td>
<td>syn-4 (Diastereomer 1)</td>
</tr>
<tr>
<td>4a</td>
<td>No inhibition</td>
</tr>
<tr>
<td>4b</td>
<td>No inhibition</td>
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<tr>
<td>4c</td>
<td>No inhibition</td>
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<td>4d</td>
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<td>4e</td>
<td>No inhibition</td>
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<tr>
<td>4f</td>
<td>No inhibition</td>
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<tr>
<td>AV167^b</td>
<td>4.44 ± 0.18</td>
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^aUnable to synthesize boronic acid. ^bAV167 was tested as a positive control.

Crystal structure of ligand-bound bacterial ClpP. Crystallization trials to produce structural data depicting compound anti-4a bound to hClpP were undertaken but ultimately failed to produce data. S. aureus ClpP (SaClpP) and hClpP display high protein sequence homology (See Supporting Information, Figure S2). As an alternative we utilized previously established SaClpP conditions to co-crystalize anti-4a with SaClpP to a resolution of 2.0 Å (PDB 6N80; See Supporting Information, Table S2). The structure displayed clear electron density for anti-4a confirming its binding mode (See Supporting Information, Figure S3). A comparison of the X-ray structure of anti-4a bound to SaClpP with the docking pose of anti-
4a in hClpP from the non-covalent virtual screening reveals striking similarities (Figure 3). The predicted hydrogen bonds between the amide group in the compound and the backbone atoms in the protein are also observed in the X-ray structure. There is also excellent agreement between the predicted and observed interactions of the boronic acid group with the protein. The main difference is observed at the R³ position where the rotation angle of the phenyl ring differs by ~90°. This change is likely due to residue differences between the SaClpP and hClpP in this part of the binding site, including Thr146/Leu145 and His142/Glu141 (See Supporting Information, Figure S2, black). Overall, the similarity in binding mode for the docking pose and the X-ray structure show that our non-covalent protocol for docking covalent compounds is a valuable tool for predicting protein-ligand interactions and design of covalent inhibitors.

**Figure 3:** Binding modes of *anti-4a*. Left: X-ray structure of *anti-4a* (purple) bound to the active site of SaClpP (yellow). Right: Predicted binding mode of *anti-4a* (purple) in the active site of hClpP. The compound was docked using the non-covalent docking protocol described in this paper. The dashed lines represent hydrogen bonds between the ligand and the protein.
Synthesis of enantiomerically pure α-aminoboronates. Based on the results of the initial hClpP screening, compounds *anti-4a, anti-4b* and *anti-4c* were chosen for follow up studies. We decided to pursue the synthesis of the enantiopure variants given that it is likely we will see improvements in the potency. The crystal structure of compound *anti-4a* bound to *SaClpP* revealed to us that the stereochemistry of the active α-aminoboronic acid is (*R*,*S*) (Figure 3). We began the synthesis with the protection of isobutyl boronic acid with (+)-pinanediol to afford the boronic ester (See Supporting Information). Next, the Matteson homologation was performed using dichloromethane and *n*-butyllithium followed by zinc chloride addition to obtain the (*S*)-chloroboronate ester in high diastereoselectivity (>95:5 based on HPLC of compounds 7). The (*S*)-chloroboronate ester was then converted to (*R*)-aminoboronate ester through an *S*$_{N}$2 reaction with LHMDS. The trimethylsilyl groups were removed using excess trifluoroacetic acid to provide (*R*)-aminoboronate ester 5 as the TFA salt. At this point, we presumed there was no erosion of the enantiopurity and carried on to the next steps. The corresponding carboxylic acids 6 were prepared using standard Fmoc solid-phase synthesis with 2-chlorotrityl resin, HATU and DIPEA for the coupling, 30% piperidine in DMF for the Fmoc removal, and 25% HFIP in DCM for the resin cleavage. Carboxylic acids 6 were coupled to (*R*)-aminoboronate ester 5 using TBTU and DIPEA in DCM to yield α-aminoboronates 7 (Scheme 2). The diastereoselectivity of α-aminoboronates 7 was analyzed by HPLC to be >95:5 which indicates there was no epimerization during the coupling step. Lastly, the α-aminoboronates 7 were deprotected with 10 equivalents of aqueous HCl and phenylboronic acid for effective pinanediol transfer in hexanes:MeOH (1:1) at room temperature to provide the α-aminoboronic acids 8 in excellent yields.
Scheme 2: Synthesis of enantiopure $\alpha$-aminoboronates and boronic acids. $^a$Yield is reported over two steps from 5. $^b$er is based on the dr of 7 from the HPLC trace.

**Testing of enantio- and diastereomerically pure $\alpha$-aminoboronic acids for inhibition of human ClpP.**

With the enantio- and diastereomerically pure $\alpha$-aminoboronic acids 8 in hand, we began to screen them along with the corresponding racemate in the WLA-AMC assay. The IC$_{50}$ values of compounds 8a, 8b and 8c were $10.2 \pm 3.7$, $8.4 \pm 2.6$ and $12.4 \pm 0.1$ μM, respectively (Figure 4a). Therefore, the enantiomerically pure $\alpha$-aminoboronic acids are up to four times more potent than the racemate.

**Testing for inhibition of hClpXP.** ClpXP performs protein degradation in cells$^{18}$ whereas ClpP can only degrade small peptides.$^{17}$ In 2015, Sieber and coworkers showed that AV167 was a potent inhibitor of hClpP; however, it was unable to inhibit hClpXP proteolysis.$^{20}$ Thus, the development of small molecule inhibitors of hClpXP is important in order to study the therapeutic relevance of hClpXP. We began by subjecting the enantiopure compounds 8a, 8b and 8c and racemates anti-4a, anti-4b and anti-4c to a hClpXP fluorescein-isothiocyanate-labelled casein (FITC-casein) assay in triplicate. ClpXP-induced proteolysis occurs through binding of a chaperone such as ClpX to the proteolytic component ClpP whereupon a functional ClpXP protease complex is formed. This assay is aimed at detecting the presence
of protease activity of ClpXP by using casein labeled with fluorescein isothiocyanate (FITC) as a substrate. The fluorescein label on the FITC-casein is highly quenched until it is digested into smaller peptides by ClpXP. The IC$_{50}$ values for inhibition of hClpXP protease activity of the enantiopure $\alpha$-aminoboronic acids 8a, 8b and 8c are $0.8 \pm 0.3$ $\mu$M, $0.9 \pm 0.4$ $\mu$M and $1.0 \pm 0.3$ $\mu$M, respectively (Figure 4b). These results indicate that $\alpha$-aminoboronic acids are potent inhibitors of hClpXP and are unique in their ability to inhibit the holoenzyme.

![Graph](image)

**Figure 4:** a) Inhibition of hClpP by racemic and enantiopure $\alpha$-aminoboronic acids *anti-4* and 8, respectively, in a WLA-AMC assay. The assay was performed in triplicate. b) Inhibition of hClpXP by racemic and enantiopure $\alpha$-aminoboronic acids *anti-4* and 8, respectively, in a FITC-casein assay. The assay was performed in triplicate.

**Conclusions**

For the first time, we have exemplified the power of combining our boron-based amphoteric reagents with a *de novo* library design and virtual screening in order to rapidly identify and generate bioactive boron-containing molecules for human ClpP and ClpXP. More specifically, we utilized our boryl isocyanides in a multicomponent Ugi reaction to synthesize peptidomimetic $\alpha$-aminoboronic acids while the *de novo*
virtual screening effectively analyzed and filtered all possible $\alpha$-aminoboronic acid candidates. By integrating these two capabilities, we have successfully identified three $\alpha$-aminoboronic acids as potent inhibitors of hClpP and hClpXP with IC$_{50}$ values in the low micromolar range. These IC$_{50}$ values were comparable to AV167 in our testing against hClpP but as opposed to AV167 and TG42 the $\alpha$-aminoboronic acids also inhibited hClpXP. To the best of our knowledge, these $\alpha$-aminoboronic acids are the first reported inhibitors of hClpXP. This work serves as a starting point for the development of more synthetically complex $\alpha$-aminoboronic acid inhibitors of hClpXP.

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24. In reference 20 only compounds with an IC$_{50}$ value lower than 2 μM were considered inhibitors. The following reference described a molecule that weakly binds to hClpXP with an IC$_{50}$ value of 29 μM: Knott, K., Fishovitz, J., Thorpe, S. B., Lee, I. & Santos, W. L. N-Terminal peptidic boronic acids selectively inhibit human ClpXP. *Org. Biomol. Chem.* **8**, 3451–3456 (2010).


