Boronic Acid Compounds as Potential Pharmaceutical Agents

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Abstract: Boronic acid compounds have been used, because of their unique structural features, for the development of potent enzyme inhibitors, boron neutron capture agents for cancer therapy, and as antibody mimics that recognize biologically important saccharides. Consequently, there has been a surge of interests in boronic acid compounds. This study reviews the recent development in this area during the last six years. © 2003 Wiley Periodicals, Inc. Med Res Rev, 23 No. 3, 346–368, 2003

Key words: boronic acid; enzyme inhibitor; boron neutron; capture agent; antibody mimics; drug delivery

1. INTRODUCTION

Recently, there is an increasing interest in boronic acid compounds. Such an interest stems from the tremendous importance of boronic acids in the synthesis of biologically active compounds and the use of boronic acid themselves as pharmaceutical agents. In the area of synthetic medicinal chemistry, boronic acids are important intermediates that have been widely used in Suzuki cross-coupling reactions,[1] protection of diols,[2] Diels-Alder reactions,[3] asymmetric synthesis of amino acids,[4] selective reduction of aldehydes,[5] carboxylic acid activation,[6,7] and as a template in organic synthesis.[8] As potential pharmaceutical agents, boronic acids have been used for the development of enzyme inhibitors;[9] boron neutron capture therapy (BNCT) agents,[10] feedback controlled drug delivery polymers,[11] saccharide sensors,[12–14] and the antibody mimics for cell-surface polysaccharides.[15,16] This review will focus on the recent development in the last six years in the development of enzyme inhibitors, BNCT agents, and polymers used for feedback controlled delivery insulin. There have been several reviews published in the area of saccharide sensors.[13,14] Therefore, this part will not be duplicated here.

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2. GENERAL PROPERTIES OF BORONIC ACID COMPOUNDS AND THEIR IMPLICATIONS IN BIOLOGICAL APPLICATIONS

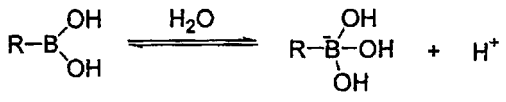
The utility of boronic acid compounds as pharmaceutical agents is directly related to their unique electronic and physicochemical properties. Boron occupies a special place in the periodic table. It is in the same period as carbon, but has one less electron. Therefore, it has many similarities with carbon in terms of structural features, which make it very useful in the world of carbon in organic and medicinal chemistry. The fact that there are many boron-based reagents in organic synthesis reflects this structural similarity.[17] In medicinal chemistry, the use of boronic acids as enzyme inhibitors to a large degree reflects the usefulness of boron as a carbon analog in the binding process, but not in terms of reactions, which is the essence of a good enzyme inhibitor. Boronic acids have been used for the development of enzyme inhibitors of peptidases/proteases, proteasomes, arginase, nitric oxide synthase (NOS), as well as transpeptidases.

One unique property of boronic acid is that it is a strong Lewis acid because of the boron open shell. Most phenylboronic acids have a pKa in the range of 4.5–8.8[18] depending upon the phenyl substitution.[19,20] This means that with the appropriate substitution, boronic acids would have the right property for ready conversion from a neutral and trigonal planar sp^2 boron to an anionic tetrahedral sp^3 boron (Scheme 1) under physiological conditions. Realizing that the process of cleaving an amide bond also requires the conversion of an sp^2 carbonyl carbon to a tetrahedral sp^3 carbon, it is easy to understand that boronic acid compounds would make good transition state analogs for the inhibition of hydrolytic enzymes. This is indeed the case. Some 20 years ago, simple alkyl or arylboronic acids were recognized as serine protease inhibitors.[21–23] Since then, many boronic acid compounds with an appropriate peptide sequences have been designed and synthesized for the development of more potent and selective inhibitors.[24] When compared with aldehyde-based inhibitors of hydrolytic enzymes, the ready conversion of boronic acids to their anionic sp^3 form seems to make them better transition state analogs.[25] Although not the emphasis of this review, it needs to be noted that Matteson et al. has established a general synthetic route to chiral α-aminoalkylboronic acid derivatives by stereoselective homologation of pinanediol boronic esters.[25,26] This enabled the synthesis of many potent boronic acid-based enzyme inhibitors. Thereafter, several variations of the general route have been developed and used for the synthesis of different kinds of enzyme inhibitors.[27–31]

In addition to being developed as enzyme inhibitors, boron-based compounds (not limited to boronic acid compounds although that is the focus of this review) are also being studied for their utility as BNCT agents.[10,32,33] Such applications are based on the unique property of boron-10, which emit α particles upon irradiation with neutron. Since α particles do not travel a long distance (a few millimeter), they are ideal for localized radiation therapy. Therefore, targeted delivery of high concentrations of boron agents can be used for BNCT of certain tumor.

The third potential application of boronic acid compounds to be discussed is the development of feedback controlled delivery systems for insulin. For such an application, the ideal controlling signal is glucose concentration. Boronic acids have the unique properties of forming reversible complexes with diol-containing compounds such as sugars.[19,34] Therefore, efforts have been made to prepare polymers that can respond to glucose concentration variation with permeability changes. Such permeability changes can in turn be used for controlling the release of insulin from the polymer encapsulation.

Again, this review will focus on using boronic acid compounds for the development of enzyme inhibitors, BNCT agents, and feedback controlled delivery systems for insulin.

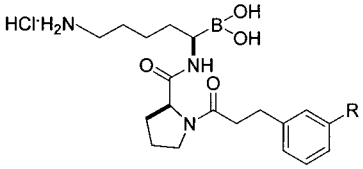


3. BORONIC ACID COMPOUNDS AS ENZYME INHIBITORS

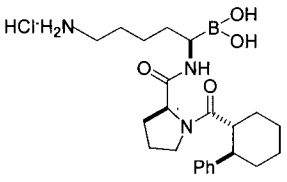
As discussed in the Introduction, the use of boronic acid compounds as enzyme inhibitors is mostly based on their easy conversion between the trigonal and tetrahedral forms (Scheme 1), which make them ideal transition state analogs in hydrolytic processes. Therefore, various boronic acid compounds have been widely studied for their inhibition of hydrolytic enzymes such as proteases. The following discussion is divided based on the target enzymes.

A. Serine Protease Inhibitors

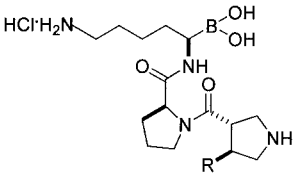
Thrombin, as the final serine protease in the blood coagulation cascade, is a promising target for the development of an anticoagulant agent. Therefore, there is a great deal of interest in the development of thrombin inhibitors. Boronic acid compounds were found to exhibit potent inhibition activities.[35] Recently, through the examination of the X-ray crystal structure of boropeptide (**1**) bound to thrombin, it was found that the 3-phenylpropionyl chain attached to the proline residue forms a favorable edge-to-face interaction with the Trp-215 side chain located at the base of the S3 specificity pocket of thrombin[36] (Figs. 1 and 2). To maximize this edge-to-face interaction, rigidified analogs of **1** and **2** were designed. In such a design, a cyclohexane ring (**3**) or a pyrrolidine ring (**4**) was used to hold the phenylpropionyl moiety in an orientation favorable for the interaction with the Trp-215 residue as predicted by computer modeling studies based on the X-ray crystal structure. Both constrained analogs **3** and **4** showed a twofold increase in potency relative to their unconstrained counterparts **1** and **2**, respectively. In a related effort to maximize the edge-to-face interaction with the Trp-215 side chain, the P3 residue of **5**, a previously discovered inhibitor, was replaced by benzoic acid-derived residues. This afforded an extremely potent thrombin inhibitor, compound **6**, which is approximately threefold more potent than the lead compound (**5**).[37]



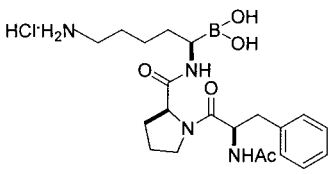
1 R = H (K_i = 0.80 nM)
2 R = CF_3 (K_i = 0.78 nM)



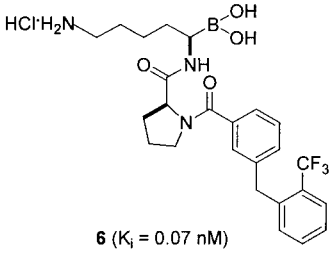
3 (K_i = 0.46 nM)



4 R = 3-$CF_3C_6H_4$ (K_i = 0.50 nM)



5 (K_i = 0.24 nM)



6 (K_i = 0.07 nM)

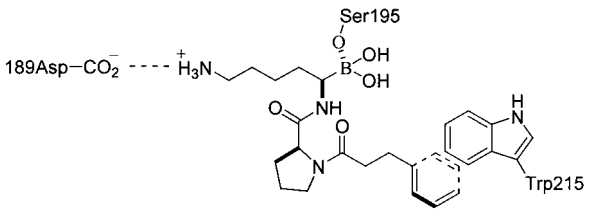
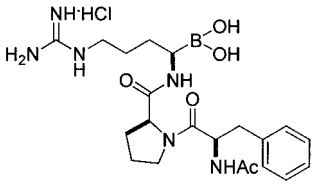


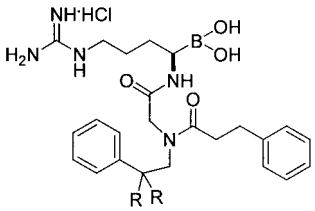
Figure 2. The binding of compound **1** to thrombin. Three expected interactions are shown: (**a**) interaction of the amino side chain with Asp-189 in the S1 specificity pocket; (**b**) tetrahedral complex between the hydroxyl of Ser-195 and the boron of the inhibitor; (**c**) the edge-to-face interaction of the 3-phenylpropionyl (P3) residue with Trp-215 located at the base of the S3 specificity pocket.

One concern with the use of thrombin inhibitors as anti-coagulants is the non-specific inhibition of other related enzymes. Earlier studies from DuPont Pharmaceuticals identified DuP-714 as a very potent thrombin inhibitor with a K_i of 0.07 nM. However, animal studies indicated that DuP-714 caused side effect that appears to be related to the undesirable inhibition of complement factor I. To design inhibitors with minimal interaction with factor I, it was important to analyze the difference in the binding requirements between factor I and thrombin. However, the crystal structure of factor I was not available. Therefore, the crystal structure of factor Xa (fXa) was used, working on the assumption that the overall conformation of factor I is similar to that of fXa. Crystal structural analyses of the inhibitor–enzyme complexes with different inhibitors showed that there were very noticeable differences in the P2 pocket. Therefore, a series of β,β-dialkylphenethylglycine P2 analogs of DuP-714 were designed and synthesized. These compounds, such as **7** and **8**, have greater selectivity for thrombin over factor I and improved safety profile.[38]

There have also been efforts in designing selective thrombin inhibitors by varying the P1 position. For example, incorporation of m-cyano-substituted phenylalanine boronic acid analogues into R-(D)Phe-Pro-OH dipeptides produced several highly effective thrombin inhibitors such as H-(D)Phe-Pro-boroPhe(m-CN)-OH.[39] The cyano group enhances binding by several orders of magnitude. Because of its structural and functional similarities with thrombin, trypsin was used as a surrogate in the crystal structural studies. The trypsin-H-(D)Phe-Pro-boroPhe(m-CN)-OH (K_i = 0.48 nM) complex showed that the aromatic side chain was bound in the P1 binding site and that the cynao group acted as a H-bond acceptor for the amide proton of the Gly-219.

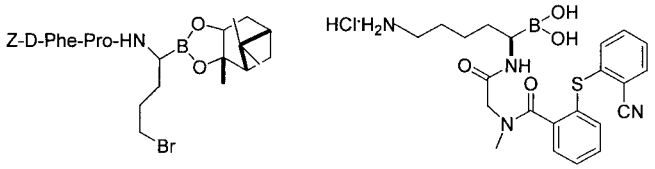


DuP 714
(K_i = 0.04 nM for thrombin, 9 nM for fXa)



7 R = Me (K_i = 0.06 nM for thrombin, 99 nM for fXa)

8 R = -CH_2- (K_i =0.06 nM for thrombin, 46 nM for fXa)



9 (K_i = 8 nM)

10 (K_i = 1.1 nM)
11 (K_i = 17 nM)

Figure 4

In a separate study, based on the known potent inhibition effect of hirudin on thrombin, a novel peptide boronate as thrombin inhibitor was designed and synthesized using solid phase chemistry and suitably protected aminoboronates.[40] By conjugating a boronic acid moiety with a hirudin-based recognition moiety, [-D-PheProBoroBpgOPin]-$CO(CH_2)_3COCly_2$Hir was synthesized and shown to have a very high affinity for the target enzyme (K_i = 0.6 nM). It has a 10-fold higher potency relative to the corresponding non-hirudin-containing portion Z-D-PheProBoroBpgOPin (**9**) or the mixture of non-covalently linked units.

Factor Xa (fXa) is another important protease in the coagulation cascade, which occupies the juncture of the intrinsic and extrinsic clotting pathways. The physiological role of fXa is the proteolytic cleavage of prothrombin to thrombin. Therefore, development of inhibitors against fXa should be an attractive method of thrombosis prevention. During the screening of a series of conformationally restricted boropeptide thrombin inhibitors, a borolysine compound (**10**) containing a 2-(2-cyanophenylthio)benzoyl in the P3 position was found to be a potent fXa inhibitor.[41] It has a 16-fold higher potency relative to the corresponding compound **11** without the nitrile moiety.

The serine proteases subtilisin Carlsberg and α-chymotrypsin are commercially available enzymes for which high-resolution X-ray crystal structures are known. Therefore, they are good targets for probing the factors responsible for determining the structural and stereospecificity of enzymes toward unnatural substrates and inhibitors. Enantiomeric 1-acetomido boronic acid analogs of the L- and D-forms of alanine, phenylalanine, p-fluorophenyalanine, *p*-chlorophenylalanine, and 1-naphthyanaline were synthesized and evaluated as inhibitors of the serine proteases subtilisin Carlsberg and α-chymotrypsin.[42] All of the boronic acids examined are powerful competitive inhibitors of both enzymes. The L-enantiomers are generally more potent than the D-enantiomers. However, [1-acetamido-2-(1-naphthyl)ethyl]boronic acid showed a dramatic reversal of the normal stereoselectivity preference with the D-enantiomer being 25-fold more potent than the L-enantiomer. Molecular modeling analyses of the possible binding modes of the inhibitors indicate that the stereoselectivity reversal is because of S1-pocket orientation differences between the naphthyl group and the aromatic chains of the phenylalanine analogs.

To explore the possibility of forming a peptide boronate adduct in the serine protease active site that mimics the first tetrahedral intermediate in the peptide hydrolysis mechanism, peptidyl boronic acids (**12**), (**13**), and (**14**) were designed and synthesized.[43] This design intended to take advantage of an intramolecular process hoping to overcome the inherent disadvantage of ternary adduct formation (Fig. 5a) by tethering P′ components to the peptidyl boronic acid (Fig. 5b). The complex boronates thus prepared are potent inhibitors of α-chymotrypsin. However, the affinity of **12** is neither time- nor pH-dependent, which would be expected for a covalent inhibitor, and it only shows a moderately increase in affinity compared to compounds **15**, **16**, and **17** that can not form a diester adduct. These results do not follow the predictions. The authors suggested that either boronate ester formation was not occurring, or that the energy derived from binding of the S′ binding fragment and boronate ester formation was not sufficient to offset the flexibility or binding characteristics of the linking group. They thus believed that a more detailed structural investigation was needed to confirm this interpretation.

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