Boron Containing Compounds as Protease Inhibitors

ARTICLE *in* CHEMICAL REVIEWS · APRIL 2012

Impact Factor: 46.57 · DOI: 10.1021/cr608202m · Source: PubMed

4 AUTHORS, INCLUDING:

Reem Smoum
Hebrew University of Jerusalem
20 PUBLICATIONS **338** CITATIONS
SEE PROFILE

Abraham Rubinstein
Hebrew University of Jerusalem
105 PUBLICATIONS **2,630** CITATIONS
SEE PROFILE

Valery Dembitsky
Institute for Drug Discovery
332 PUBLICATIONS **4,183** CITATIONS
SEE PROFILE

CHEMICAL REVIEWS

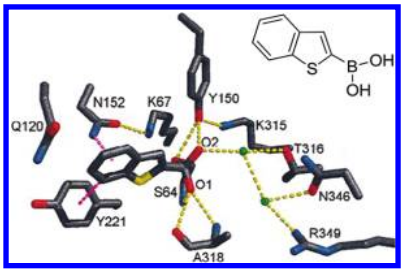
Review

pubs.acs.org/CR

Boron Containing Compounds as Protease Inhibitors

Reem Smoum,* Abraham Rubinstein,* Valery M. Dembitsky,* and Morris Srebnik†

The School of Pharmacy, Institute for Drug Research, The Hebrew University of Jerusalem, Faculty of Medicine, P.O. Box 12065, Jerusalem 91120, Israel



CONTENTS

1. INTRODUCTION

Proteases form one of the largest and more important groups of enzymes. They selectively catalyze the hydrolysis of peptide bonds and can be divided into four major classes: aspartic,[1a,b] serine,[1c−f] cysteine,[1g] and metalloproteases.[1h,i] Proteases are involved in numerous important physiological processes including protein turnover, digestion, blood coagulation and wound healing, fertilization, cell differentiation and growth, cell signaling, the immune response, and apoptosis.[1j,k] Proteolysis plays a key role in a number of pathological processes (diseases) such as rheumatoid arthritis, cardiovascular diseases, bacterial and viral infections, cancer, and Alzheimer's disease. Protease inhibitors thus have considerable potential utility for therapeutic intervention in a variety of disease states.[1l−p]

Boronic acids represent an exciting class of enzyme inhibitors.[2a,b] Most results were hitherto reported in the field of serine proteases.[2c,d] Their efficiency has been demonstrated with Sepharose-based arylboronic acid sorbent in the chromatographic purification of serine proteases.[3,4] Simple alkyl or aryl boronic acids were recognized as serine protease inhibitors already in the 1970s.[5−7] Since then, numerous boronic acid compounds with appropriate peptide sequences have been designed and synthesized to be used as more potent and selective inhibitors.[8] If boronic acid-based enzyme inhibitors are considered as drugs, their specificity is highly important to avoid adverse effects. For example, improved specificity of potent peptidylboronic acid analogues was accomplished by the development of the α-aminoalkylboronic acid analogues of α-amino acids. The most common inhibition mechanism is the formation of a tetracoordinate boronate complex by coordination of the side chain hydroxyl nucleophile of the active serine residue, thus mimicking the tetrahedral intermediate for amidolysis.[9] Other modes of inhibition have been identified, that is, the formation of covalent adducts with histidine residues in the active site.[10−12] 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 analogues.[13] However, the distribution of negative charge is very different than in an aldehyde inhibitor, which argues that boronic acids would be poorer mimics of the

Received: September 7, 2007
Published: April 20, 2012

   dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156–4220

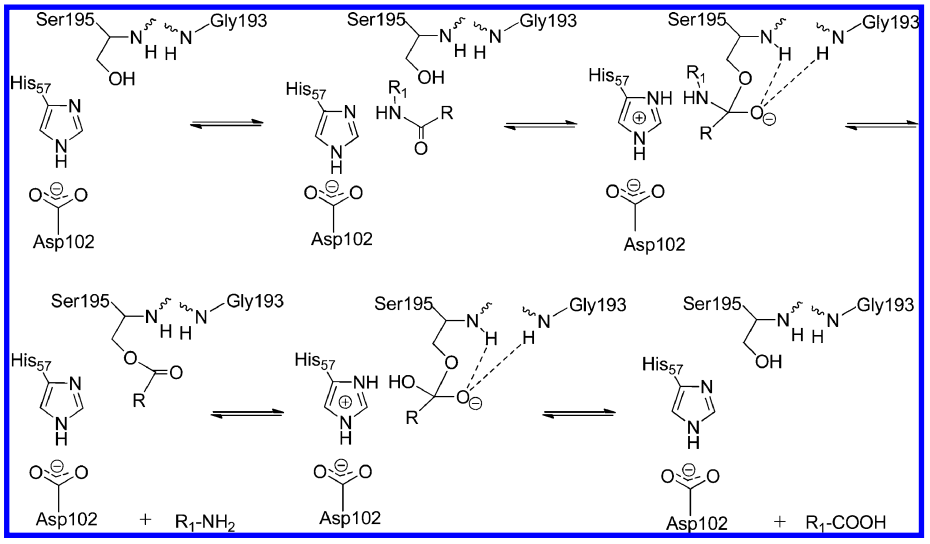


Figure 1. The generally accepted mechanism for serine proteases.

transition state. In fact, some of the increased potency arises from the histidine adducts. Matteson has established a general synthetic route to chiral α-aminoalkylboronic acid derivatives by stereoselective homologation of pinanediol boronic esters.[13,14] 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.[15−19]

The intensive research efforts in the medicinal chemistry of boron compounds and protease inhibition have recently culminated in the commercialization of the peptidyl boronic acid antineoplastic drug Velcade (also known as bortezomib, PS-341, Millennium Pharmaceuticals, and Cambridge, MA),[20,21] which has been approved by the FDA for the treatment of relapsed and refractory multiple myeloma.

This comprehensive review attempts to encapsulate and discuss the vast area of those boron containing compounds with the potential use as protease inhibitors.

2. SERINE PROTEASE INHIBITORS

Serine proteases[1,9,22−26] are one of the largest classes of proteases studied with almost 800 structures deposited in the Protein Data Bank (PDB) of which at least one-third are structures of thrombin and trypsin. Interest in this remarkable family of enzymes is generally due to their well-characterized, widespread, and spectacularly diverse role in a host of physiological and pathological processes. Many pathological disorders are caused by deficiencies in the normally exquisite regulation of the activity of proteolytic enzymes, resulting in abnormal tissue destruction and/or the abberant processing of other proteins and peptides. Most clotting abnormalities, and associated diseases, are mediated by inappropriate activity of these enzymes.[27] Additionally, the important role of these proteases has been elucidated in the pathology of viral infections, including hepatitis C and herpes.[28,29]

The active site of these enzymes consists of the catalytic triad of Ser195, His57, and Asp102 residues (chymotrypsin numbering system) and an oxyanion hole (Figure 1). The substrate binds in the active site forming a Michaelis complex, exposing the carbonyl group of the scissile amide bond to nucleophilic attack by the active site serine hydroxyl, under base catalysis by the imidazole side chain of His57. The resulting tetrahedral intermediate is stabilized by hydrogen bonding to the backbone NH of Ser195 and Gly193, which forms the oxyanion hole. Proton transfer from His57 to the amine of the tetrahedral intermediate facilitates expulsion of the amine fragment as a leaving group. The covalent acyl-enzyme complex is attacked by water with the formation of a new tetrahedral intermediate, which subsequently breaks down via acid-assisted catalysis by His57 to form the carboxyl fragment of the cleaved substrate and regenerate Ser195 (Figure 1).

Boronic acids, as protease inhibitors, appeared for the first time 40 years ago as chymotrypsin[30] and β-lactamase inhibitors.[5a,b] Since then, interest in these molecules increased continuously, and boronic acids with nanomolar affinity for proteases have been published[31] or patented.[32]

A boronic acid is an alkyl or aryl substituted boric acid containing a carbon to boron chemical bond belonging to the larger class of organoboranes.[33a−c] Boronic acids are strong Lewis acids because of the boron open shell. Their unique feature is that they are capable of forming reversible covalent complexes with sugars,[33d] amino acids, hydroxamic acids, etc. (i.e., molecules with vicinal, or occasionally substituted Lewis base donors (alcohol, amine, carboxylate)). They are occasionally used in the area of molecular recognition to bind to saccharides for fluorescent detection or selective transport of saccharides across membranes. Most phenyl boronic acids have a pK_a in the range of 4.5−8.8 depending upon the phenyl substitution.[2d] That means that with the appropriate substitution, boronic acids would have the right property for ready conversion from a neutral and triagonal planar sp^2 boron (B−O bond length of 1.361 Å) to an anionic tetrahedral sp^3 boron (B−O bond length of 1.469 Å)[33e] (Figure 2) under physiologic conditions. Therefore, boronic acid compounds would make transition state analogues for the inhibition of hydrolytic enzymes. The tetrahedral adduct generated from boronic acid

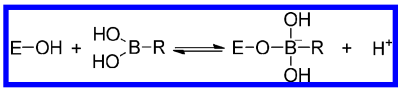


Figure 2. The reaction of the transition state analogue inhibitors (boronic acid derivative) with serine peptidases.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

derivatives bear a closer relationship to the structure of the true intermediate.[33]

2.1. Chymotrypsin and Subtilisin

Chymotrypsin (human and mammalian) is an active component in the digestive system of mammals, and it is present in the intestine as an inactive precursor.[34] Subtilisin is an extracellular serine endopeptidases produced by *Bacillus subtilis*. While chymotrypsin and subtilisin represent different structural families, both enzymes have highly specific cleavage sites, preferring large hydrophobic amino acids in the S_1 site [S_1 is the specificity subsite; P_1 is the amino acid side chain binding in S_1. The S_1, S_2, etc.. subsites of the protease recognize the P_1, P_2, etc.. residues of the substrate].[35] Furthermore, both are highly stereoselective, exhibiting a strong catalytic preference for L- over D-amino acid substrates, and both use the same chemical hydrolysis mechanism, involving the classical Ser-His-Asp triad.

Aryl- and arylalkylboronic acids were the first boronic acids reported to be strong competitive inhibitors of subtilisin and chymotrypsin,[5,6,36−38] and it was likely that these boronic acids act as "transition state" analogues by forming such tetrahedral enzyme complexes. Crystallographic,[39] nuclear magnetic resonance,[40] rapid temperature-jump,[41,42] and laser Raman[43] studies were consistent with this view. Early investigations showed that phenylethane boronic acid[5] and *trans*-β-styreneboronic acid[37] bound to the active site of α-chymotrypsin. ^{11}B NMR studies on phenylboronic acid in the presence of α-chymotrypsin confirmed a transition state like structure in solution.[44,45] Subtilisin was also strongly inhibited by phenylethane boronic acid,[38] and crystallographic studies of the enzyme−inhibitor adduct[7] showed that the aromatic side chain occupies the S_1 crevice.

Later, boronic acids possessing specific peptide moieties such as benzamidomethaneboronic acid,[46] and 1-acetamido-2-phenylethaneboronic acid[13,16] (Figure 3, **1** and **2**) were found

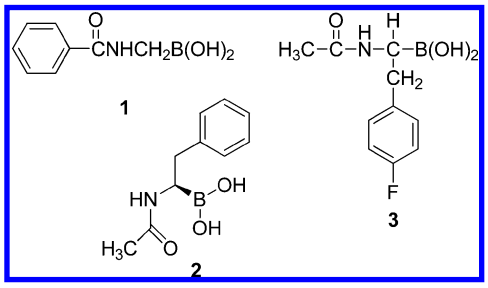


Figure 3. Structures of benzamidomethaneboronic acid (**1**), 1-acetamido-2-phenylethane-boronic acid (**2**), and (*R*)-1-acetamido-2-(4-fluorophenyl)ethane-1-boronic acid (**3**).

to be potent inhibitors of α-chymotrypsin. These boronic acids had dissociation constants of around 10^{-6} with chymotrypsin, and bound 10^3−10^4 times more tightly than the substrates which they resemble. Then, (*R*)-1-acetamido-2-(4-fluorophenyl)-ethane-1-boronic acid (Figure 3, **3**) was synthesized and was found to form a complex with 1:1 stoichoimetry with α-chymotrypsin.[47,48] ^{1}H NMR indicated that the boronic acid likely was coordinated to the Ser195 residue at the active site. Analysis of fluorine T_1 relaxation behavior, ^{19}F{^{1}H}NOE data, and two-dimensional ^{19}F{^{1}H}NOE experiments showed that the rate constant for the dissociation of the complex was faster at pH 7 than was observed at pH 4 and that a number of close contacts between the fluorine and hydrogen atoms of the protein were present in the complex; however, these contants were weaker at the higher pH value.

Bachovchin and co-workers had demonstrated that the binding mode used by a particular inhibitor appears to depend on how well the inhibitor matches the structure of the natural substrate(s) of the proteinase.[49] When a boronic acid derivative is a good analogue of the substrate, formation of a tetrahedral complex with the active-site serine is favored, while the boronic acids with structures not well-related to that of the substrate tend to coordinate with histidine or with both histidine and serine. Furthermore, and in order to probe the structural basis of stereoselectivity in the serine protease family, a series of novel L- and D-boronic acids $RCH_2CH(NHCOCH_3)B(OH)_2$ were synthesized and kinetically characterized as transition-state analogue inhibitors (Figure 4). When the R-substituent in

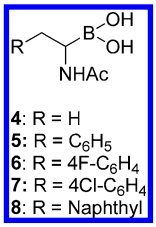


Figure 4. Enantiomeric 1-acetamido boronic acids analogues of alanine (**4**), phenylalanine (**5**), *p*-fluorophenylalanine (**6**), *p*-chlorophenylalanine (**7**), and 1-naphthylalanine (**8**).

this series was changed from a *p*-chlorophenyl to a 1-naphthyl group, the stereoselectivity of chymotrypsin switched from L for the chlorophenyl compounds to D for the naphthyl-substituted inhibitors.[50] Molecular modeling studies on the enzyme−inhibitor (EI) complexes pointed to variations in the hydrogen-bonding patterns and to a reversal of the orientation of the naphthyl ring as potential determinants of the switch in stereo preference for chymotrypsin. A limitation of this approach was the lack of proper potential functions which restricted calculations to covalent serine adducts. There was, however, evidence from NMR and X-ray crystallography that other covalent complexes were possible between serine proteases and boronic acid inhibitors.[12,51,52] Tsilikounas et al.[51] proposed that the properties of the specificity pockets were responsible for placing those inhibitors which closely mimic natural substrates into a position favoring a covalent bond between boron and the catalytic serine residue. Inhibitors that deviate from native substrate-like structures may be forced into a binding mode that could lead to the formation of histidine-boron adducts. Studies revealed that a covalent bond from the serine Oγ to the boron atom coexisted along with a coordinate-covalent bond between boron and histidine Nε2.[51] In a related study, X-ray crystallographic examination of subtilisin Carlsberg and γ-chymotrypsin complexes of the L- and D-enantiomers of *p*-chlorophenyl and 1-naphthyl boronic acid derivatives was used to determine the structural factors responsible for the differences in stereoselectivity between the two. In both enzymes, the L-isomers of the inhibitors, which were more closely related to the natural L-amino acid substrates, formed tetrahedral adducts, covalently linking the central boron atom and Oγ of the catalytic serine. The D-isomers, however, differed in the way they interact with subtilisin or γ-chymotrypsin. With subtilisin, both the D-*p*-chlorophenyl and D-1-naphthyl inhibitor complexes formed covalent Ser Oγ-to-boron bonds, but with γ-chymotrypsin, the same inhibitors lead to novel tetrahedral

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

adducts covalently linking both Ser195 Oγ and His57 Nε2 covalently via the boron atom.[53]

Peptidyl boronic acids are among the most potent inhibitors of serine proteases known,[54−60] achieving sub-nanomolar affinity from interaction with the S-subsites alone. For example, MeO-Suc-Ala-Ala-ProboroPhe-OH inhibited α-chymotrypsin with a K_i value of 0.16 nM.[55] Researchers were intrigued by the possibility of using a boronic acid to mimic the first tetrahedral intermediate in the enzymatic sequence in order to enhance binding affinity and specificity and to provide a structural model for this transition state. However, in view of the lability of boronate esters and amides in aqueous solution, the desired serine adduct required the formation of a ternary complex. Researchers sought to overcome the inherent disadvantage of ternary adduct formation by tethering the P′ components to the peptidyl boronic acid, making formation of the desired diester an intramolecular process. Tian et al.[61] described the design, synthesis, and evaluation of chymotrypsin inhibitors that embodied this concept (Figure 5). In **9**−**11**, the

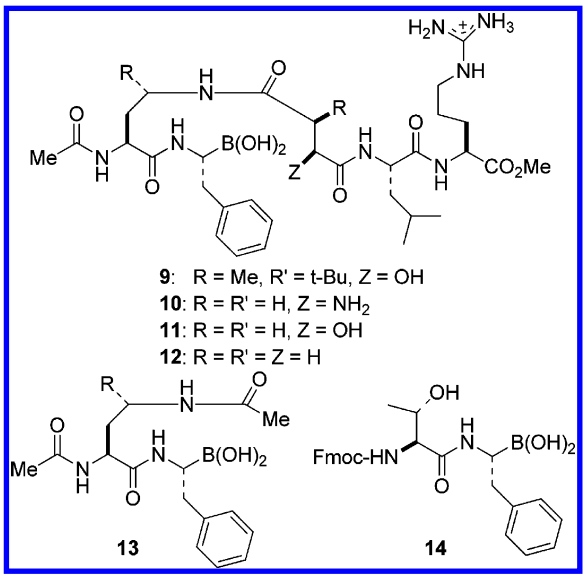


Figure 5. Peptidyl boronates as inhibitors of α-chymotrypsin.

P_1 and P_2 residues and the P_1'-P_3' residues were connected through the P_2 and P_1' side chains, to encourage formation of the diester or amide-ester adducts via macrocyclization. The complex peptidyl boronates (**9**, K_i = 26 nM) and (**11**, 68 nM) were potent inhibitors of α-chymotrypsin; however, the affinity of (**9**) was neither time- nor pH-dependent, and it was only moderately greater than that found for comparison compounds like (**12**, 114 nM), (**13**, 356 nM), and (**14**, 219 nM) that could not cyclize or form a diester adduct.

Recently, Hansen et al.[62] described the application of an on-bead, enzyme-catalyzed cyclization process as a way to screen for macrocyclic frameworks of potential relevance in the design of conformationally constrained peptidase inhibitors.[63] The synthesis and the evaluation of some transition state analogue inhibitors based on a macrocyclic motif identified in this manner were reported. The ring systems presented by the lactams (**15**) and (**16**) (Figure 6) was one of the simplest of those whose formation was readily catalyzed by trypsin and α-chymotrypsin. Thus, to assess the validity of this approach for the identification of effective inhibitor scaffolds, ketone (**17**) and boronic acid (**18**) were synthesized and evaluated as inhibitors of α-chymotrypsin. Ketone (**17**) was found to be a modest inhibitor of chymotrypsin (K_i = 220 μM). A precursor (**19**) to the amino boronic acid (**18**) was also prepared; although this derivative was a potent inhibitor of chymotrypsin (K_i = 130 nM) by virtue of the boronic acid moiety, it showed no advantage over the des-amino analogue (**20**) (K_i = 120 nM), which was not capable of cyclizing. In this context, the macrocyclic lactam proved to be a weak substrate of α-chymotrypsin, while the macrocyclic ketone was a modest inhibitor. Comparison of the cyclic ketone with an acyclic analogue showed that the macrocyclic structure was advantageous, but not to the extent seen in other cases. Although greater affinity would be anticipated on incorporation of a phenylalanine residue at P_1 or a more electrophilic difluoroketone moiety, the potential advantage of the macrocyclic framework did not appear to translate to the boronic acid class of inhibitors.

Among the first boron containing tetrahedral complexes of chymotrypsin studied were those arising in the reactions of boronate, benzene boronic acid, phenylethylboronic acid, and

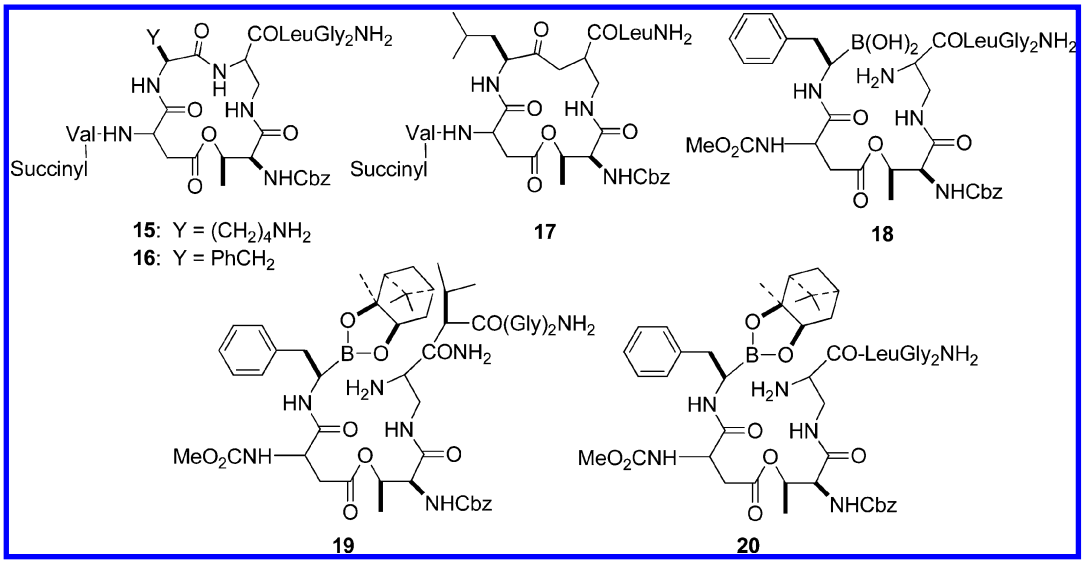


Figure 6. Transition state analogue inhibitors of α-chymotrypsin.

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156−4220

peptidyl boronic acids with chymotrypsin. The proton generated (Figure 2) was not released from the enzyme but was transferred to His57-Nϵ2. In these complexes, His57 was in its protonated state at neutral pH, but the NMR signal for the proton bridging His57-Nδ1 and Asp102-Oδ1 in boronate complexes was significantly upfield from that for free chymotrypsin at low pH. This may be because the boronate adducts were not strict analogues of the tetrahedral intermediate; the negative charge of the adduct resides on boron. A hydroxyl group on boron occupied the oxyanion site that bound the oxyanionic group of the tetrahedral intermediate in the catalytic mechanism. The electrostatic attraction between the boronide ion and the adjacent imidazolium ring of His57 may have weakened the hydrogen bond bridging His57-Nδ1 and Asp102-Oδ1, and this would have moved the NMR signal upfield.[64]

New research is aimed at controlling optically biological systems using photoswitchable enzyme inhibitors. Examples of inhibitors of serine proteases that combined an azobenzene photoswitch with a group that bound to the enzyme of interest were reported: for example, boronic acid (**21**) (Figure 7) was

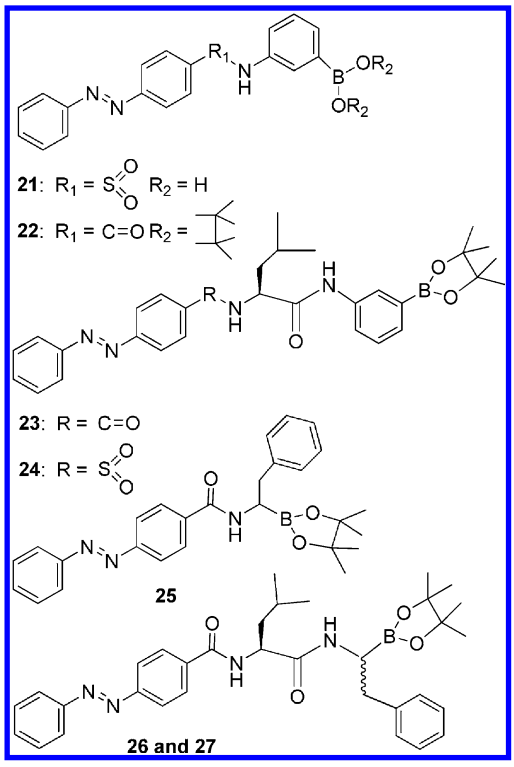


Figure 7. Monosubstituted azobenzenes.

found to be 3 times less active after UV irradiation (inhibition constant, K_i of 41 μM vs 11 μM) to give a predominance of the *cis* isomer.[65] This concept was extended with the preparation and testing of peptidomimetic-based photoswitchable inhibitors of proteases that were amenable to incorporation into a peptide sequence as a means to potentially increase potency and specificity for one protease over another. These systems were also amenable to attachment to other materials, such as metal surfaces, polymers, or nanoparticles, and this might form the basis for molecular computing or reversible biosensor technologies. A series of peptidomimetic boronate esters (Figures 7 and 8) containing the photoisomerizable azobenzene group were synthesized and assayed against α-chymotrypsin.

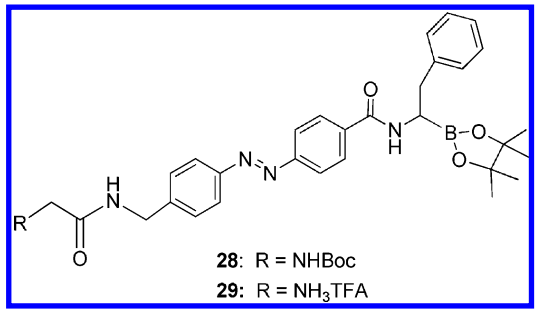


Figure 8. Disubstituted azobenzenes.

Compounds with borophenylalanine inhibition groups were found to be inhibitors with micromolar activity. Selected compounds were isomerized by UV or visible light to obtain enriched states of the (*Z*) or (*E*) isomers, respectively, and assayed. While the magnitude of photoswitching was not improved by increasing the substitution and peptidic character of inhibitors, it was possible to retain photoswitching in such extended chain inhibitors. The exact fit of the inhibitors into the active site appeared to be particularly sensitive to molecular shape or conformation. This was evidenced by the fact that in some cases (**21** and **25**) the (*E*) azobenzene was the more active isomer, while for compound **28** the (*Z*) isomer was more active. A direct correlation did not seem to exist between the nature of the warhead or linker, and the relative activities of the (*Z*) and (*E*) isomers. Rather, it was dependent upon the gross structure of the inhibitor and hence the nature of its overall "fit" in the active site.[66]

Recently, potassium organotrifluoroborates were found to be reversible competitive inhibitors of α-chymotrypsin (K_i values ranging from 2.84 to 0.14 mM). They increased inhibition by at least an order of magnitude over the corresponding boronates. The most potent among the compounds prepared was potassium 2-hydoxyphenyl-trifluoroborate that inhibited chymotrypsin with K_i of 0.14 mM.[67]

Kexin, the Kex-2 gene product of the yeast *Saccharomyces cerevisae*,[68−70] is a calcium-dependent serine proteinase of the subtilisin family. Kex2 is the prototype of a large family of eukaryotic pro-protein processing proteases that includes furin in mammals.[71] These serine proteases comprise a discrete branch of the subtilisin superfamily spanning eukaryotes from yeasts to humans.[72] Kex2 and furin[73] follow the classic serine protease mechanism.[74−76] Biochemical characterization of Kex2 suggests that the P_1 position is the primary specificity determinant.[73,77] Furin, however, seems to generate most of its selectivity through interactions with both P_1 and P_4.[73,78,79]

The first structure of a member of the Kex2/furin family of eukaryotic proprotein processing proteases, which cleaves sites consisting of pairs or clusters of basic residues, has been studied by Holyoak et al.[80] They reported the 2.4 Å resolution crystal structure of the two-domain protein secreted soluble Kex2 (ssKex2) in complex with an Ac-Ala-Lys-boroArg inhibitor (R = 20.9%, R_{free} = 24.5%) (Figure 9). The Kex2 proteolytic domain is similar in its global fold to the subtilisin-like superfamily of degradative proteases. The P-domain of the ssKex2, which does not participate in catalysis, has a novel jelly roll like fold consisting of nine β strands and may potentially be involved, along with the buried Ca^{2+} ion, in creating the highly determined binding site for P_1 arginine.

Another study of the 2.2 Å resolution crystal structure of ssKex2 in complex with an Ac-Arg-Glu-Lys-Arg peptidyl

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

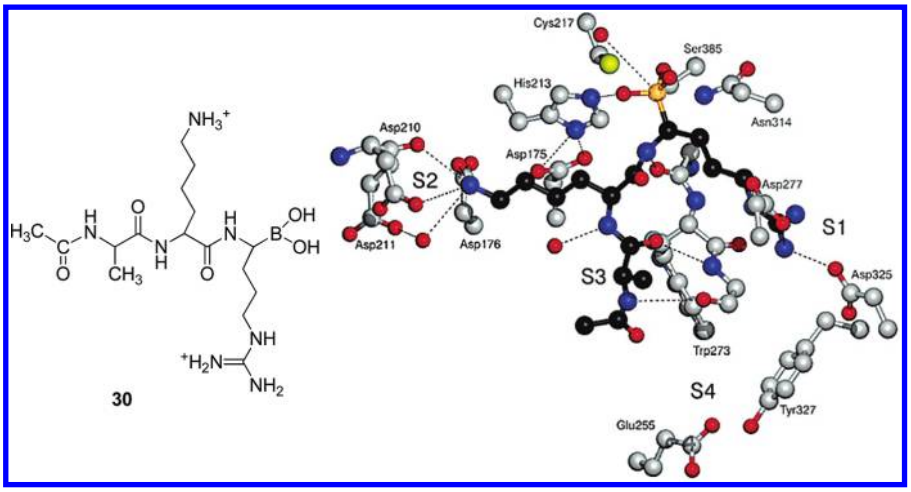


Figure 9. The Ac-Ala-Lys-boroArg inhibitor (**30**) and the overall view of the ssKex2 active site and S1−S4 subsites architecture illustrating the arrangement of the subsites. Those amino acids making contacts with the inhibitor and Ca^{2+} ion are shown, and their distances are indicated. The atoms are colored according to atom type, and the boron atom is rendered in gold. The catalytic triad D175, H213, and S385; the oxy-anion hole N314; the acyl-enzyme hydrolyzing water molecule; and the reactive C217 are also shown. Reprinted with permission from ref 80. Copyright 2003 American Chemical Society.

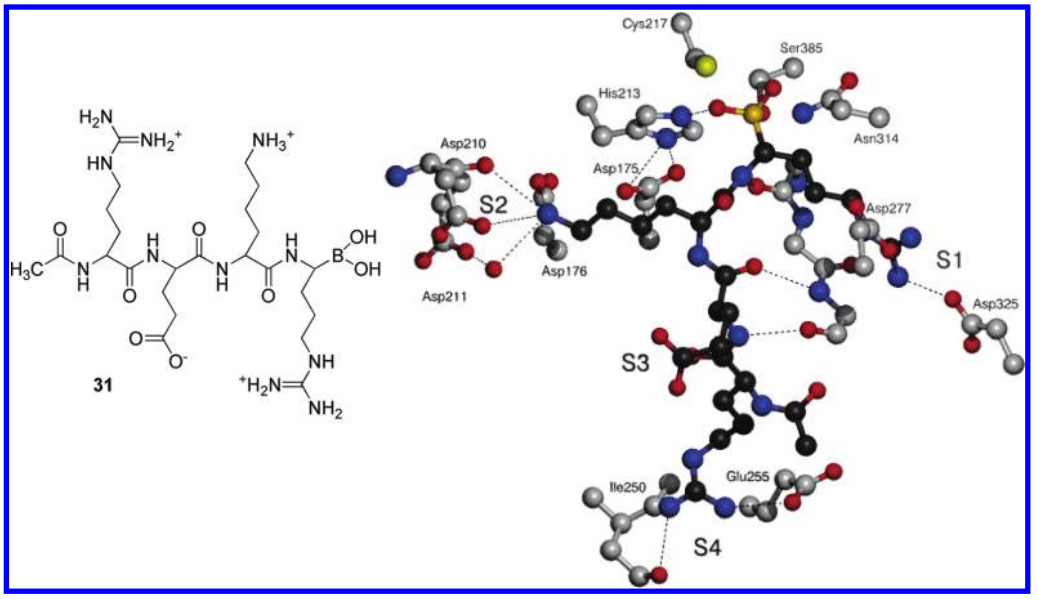


Figure 10. The Ac-Arg-Glu-Lys-boroArg inhibitor (**31**) and the overall view of the ssKex2 subsite architecture illustrating the arrangement of the S1−S4 subsites. Those amino acids making contacts with the inhibitor are shown. The atoms are colored according to atom type, and the boron atom is rendered in gold. The catalytic triad of D175, H213, and S385, the oxyanion hole N314, and the reactive C217 are also shown. Reprinted with permission from ref 82. Copyright 2004 American Chemical Society.

boronic acid inhibitor (R = 19.7, R_{free} = 23.4) was reported (Figure 10). By comparison of this structure with the structure of the mammalian homologue furin,[81] the authors suggested a structural basis for the differences in substrate recognition at the P_2 and P_4 positions between Kex2 and furin and provided a structural rationale for the lack of P6 recognition in Kex2. In addition, several monovalent cation binding sites were identified, and a mechanism of activation of Kex2 by potassium ion was proposed.[82]

Researchers have shown that three high-affinity inhibitors of furin efficiently blocked killing of murine J774A macrophages by recombinant protective antigen plus lethal factor: RRD-eglin and RRDG-eglin, developed by engineering the protein protease inhibitor eglin c, and the peptidyl boronic acid inhibitor acetyl-Arg-Glu-Lys-boroArg pinanediol. Inhibition of killing was dose dependent and correlated with prevention of protective antigen processing. However, Komiyama et al.[83] showed that combining furin inhibitors and chloroquine which neutralize acidic compartments and interfere with toxin-dependent killing, strongly augments the inhibition of toxin-dependent killing, suggesting that combined use of antifurin drugs and chloroquine might provide enhanced therapeutic benefits. Reversible furin inhibitors protected against anthrax toxin killing for at least 5 h, but by 8 h, toxin-dependent killing resumed even though furin inhibitors were still active.

2.2. α-Lytic Protease

α-Lytic protease (αLP), an extracellular bacterial protease secreted by *Lysobacter enzymogenes*, has for many decades served as a model representative in both mechanistic and structural studies for the chymotrypsin family of serine

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

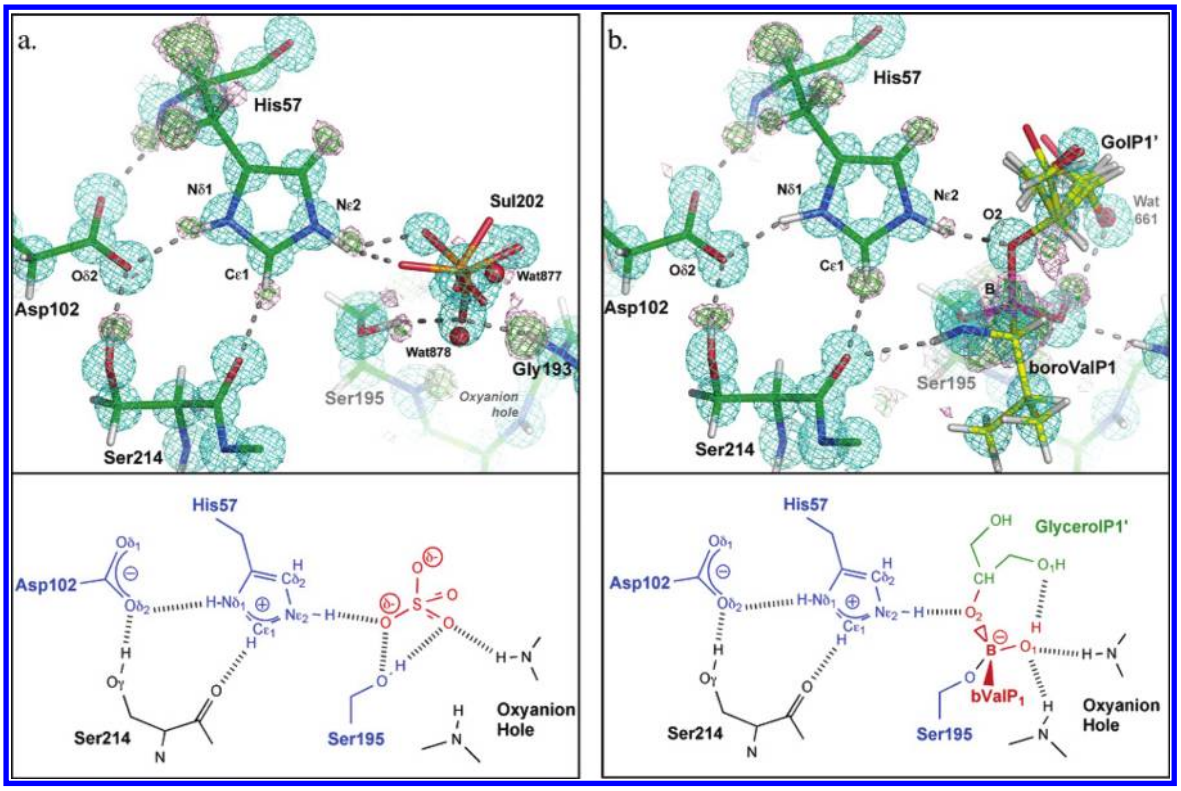


Figure 11. Experimentally observed hydrogen atoms in the active-site region. An illustrative schematic along with the refined model and electron density maps are shown for the active sites of (a) αLP$_{pH5}$ and (b) αLP+boroVal(gol). For both models, σA-weighted $2F_o - F_c$ electron density maps are drawn at 2σ (gold), and σ_A-weighted $F_o - F_c$ maps at 3σ (green) and 2.5σ (pink). $F_o - F_c$ difference electron density maps were calculated prior to addition of active-site hydrogens to the model. Sticks represent the final refined models, with the positions of key hydrogen atoms refined. Dashed lines illustrate hydrogen-bonding interactions. Reprinted with permission from ref 94. Copyright 2006 American Chemical Society.

proteases.[84] It is a bacterial protease which normally cleaves small hydrophobic amino acids. This extracellular serine protease produced by the soil microorganism *Lysobacter enzymogenes* apparently brings about the lysis of microbes and small organisms also found in soil.[85] Its crystal structure has been determined and refined to 0.8-Å resolution, demonstrating the presence of Asp, His, and Ser catalytic residues common to all serine proteases.[86,87] α-Lytic protease contains a single histidine residue located at the active site, and this has been a favorite for NMR studies.[88−90] It hydrolyzes substrates with either alanine or valine in the P_1 site and has a preference for a substrate with a P_1 alanine.

Peptide boronic acids are effective inhibitors of the α-lytic protease. They are tri- and tetrapeptide analogues[91] that have a boroVal residue in the P site. At pH 7.5, MeOSuc-Ala-Ala-Pro-boroVal has a K_i of 6.4 nM and Boc-Ala-Pro-boroVal has a K_i of 0.35 nM. Ac-boroVal-OH and Ac-Pro-boroVal-OH are 220000- and 500-fold less effective, respectively, than the tetrapeptide analogue. The kinetic properties of the tri- and tetrapeptide analogues are consistent with the mechanism for slow-binding inhibition, while the less effective inhibitors are simple competitive inhibitors. MeO-Suc-Ala-Ala-Pro-boroAla is a simple competitive inhibitor with a K_i of 67 nM at pH 7.5. Other peptide boronic acids, which are analogues of non-substrates, are less effective than substrate analogues but still are effective competitive inhibitors. For example, MeOSuc-Ala-Ala-Pro-boroPhe has a K_i of 0.54 pM, although substrates with a phenylalanine in the P position are not hydrolyzed.[92] ^{15}N NMR investigations of the MeOSuc-Ala-Ala-Pro-boroPhe peptide bound to α-lytic protease revealed that it forms a boron−nitrogen bond with the active site histidine.[49] The subsequent X-ray studies of several peptidyl boronic acid inhibitors bound to α-lytic protease confirmed that the binding modes of the boroVal and boroPhe inhibitors were different.[39h] The MeOSuc-Ala-Ala-Pro-boroVal inhibitor formed the expected tetrahedral adduct with the active site serine, while the MeOSuc-Ala-Ala-Pro-boroPhe was bound in an unusual bridging conformation, in which, in addition to a boron−serine bond, a boron−histidine bond was present. The geometry of the boron coordination appeared closer to triagonal pyramidal than to tetrahedral, with the active site histidine forming the axial bond to boron. This unusual coordination geometry was contested in a later ^{11}B solution NMR study, in which the ^{11}B chemical shift of the MeOSuc-Ala-Ala-Pro-boroVal and the MeOSuc-Ala-Ala-Pro-boroPhe inhibitors bound to α-lytic protease were found to be essentially the same.[91] The authors used a field-cycling NMR method to observe boron pure quadrupole resonance of two peptidyl boronic acid inhibitors bound to α-lytic protease. The method used a simple Hartmann−Hahn transfer from proton to ^{11}B before field cycle and direct ^{11}B observe after it. In the complex of the MeOSuc-Ala-Ala-Pro-boroVal inhibitor with the enzyme, the quadrupole resonance signal was observed at 600−650 kHz, which indicated tetrahedral boron coordination in the active site. The quadrupole frequency of the MeOSuc-Ala-Ala-Pro-boroPhe enzyme−inhibitor complex was found to be the same within experimental error as in the MeOSuc-Ala-Ala-Pro-boroVal enzyme−inhibitor adduct, suggesting that the boron coordination geometry in the enzyme-MeOSuc-Ala-Ala-Pro-boroPhe adduct is also close to tetrahedral.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

With knowing the structure of α-lytic protease at pH 8 at 0.83 Å resolution,[93] the mechanism of serine protease catalysis has been studied in greater depth. Researchers have solved two X-ray crystal structures of α-lytic protease that mimic aspects of the transition states: α-lytic protease at pH 5 (0.82 Å resolution) and α-lytic protease bound to the peptidyl boronic acid inhibitor, MeOSuc-Ala-Ala-Pro-boroVal (0.90 Å resolution) (Figure 11). The data suggested that upon protonation of His57, Ser195 undergoes a conformational change that destabilizes the His57-Ser195 hydrogen bond, preventing the back-reaction. In both structures, the His57-Asp102 hydrogen bond in the catalytic triad is a normal ionic hydrogen bond, and not a low-barrier hydrogen bond (LBHB) where the hydrogen bond is short and strong as previously hypothesized. Therefore, it was proposed that the enzyme has evolved a network of relatively short hydrogen bonds that collectively stabilize the transition states. In particular, a short ionic hydrogen bond (SIHB) between His57-Nε2 and the substrate's leaving group may promote forward progression of the metastable tetrahedral intermediate (TI_1)-to-acylenzyme reaction. Experimental evidence was provided that refutes use of either a short donor–acceptor distance or a downfield ^1H chemical shift as sole indicators of an LBHB.[94]

2.3. Trypsin

Trypsin[9,23,24] (human and mammalian) (~220 PDB structures) is the most widely studied serine protease. It is involved in the digestion of proteins in the digestive tract of mammals and is secreted in its precursor form in the pancreas. A key determinant of specificity involving the substrate side chain prior to the scissile bond cleaved by trypsin-like proteases is the P_1 pocket. In trypsin, the Asp189 side chain at the base of the P_1 pocket confers a preference for sequences containing Lys or Arg at S_1 of the substrate polypeptide chains, which form salt bridges with Asp189 upon binding.[95,96] However, mutagenesis to convert trypsin into chymotrypsin[97−100] demonstrates that the specificity features of each different serine protease also involve several (primarily three) loops that surround the P_1 binding pocket. These structural and mechanistic features have been exploited in the understanding and development of various classes of potent transition-state-like protease inhibitors including organofluorophosphates,[101] peptidyl chloromethyl ketones,[102] aldehydes,[103] phosphonate esters,[104] and boronic acids.[12,49,51,105]

In 1995, Katz et al.[54] developed a novel class of mechanism-based inhibitors of serine proteases using epitaxial selection (Figure 12). Tripeptide boronates esterified by an alcohol or alcohols at boron retained the tight binding to trypsin-like enzymes associated with transition-state analogues (K_i ~ 7 nM) and incorporated additional groups that could be utilized for selectivity between proteases. The most structurally compatible alcohol-derivatized inhibitors were either selected by binding to the enzyme (epitaxial selection) or assembled by epitaxial reaction on the enzyme surface. Trypsin was reacted with a peptidyl boronate pinanediol ester in the presence of an excess amount of various alcohols. Selection and incorporation of the alcohol derivative yielded mono- or disubstituted ternary boronate ester at the active site of the enzyme, with additional binding substituents.

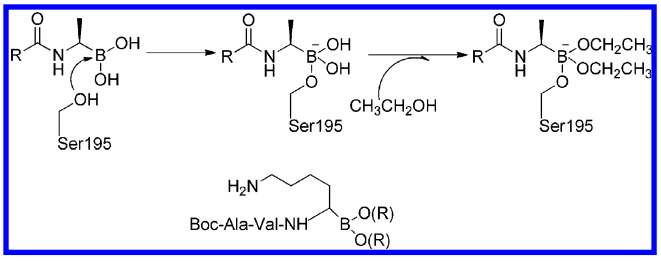


Figure 12. Epitaxial selection of enzyme-bound boronates.

Later, London et al.[106] observed the first ternary complex formed from 4-aminobutanol, borate, and trypsin. ^{11}B and ^1H NMR and spectrophotometric assays indicated a cooperative binding interaction in which the borate is esterified by the oxygen atoms of the 4-aminobutanol and trypsin residue Ser195. Two downfield-shifted proton resonances at 15.5 and 16.6 ppm were proposed to arise from the labile imidazolium protons on His57, indicating a salt bridge interaction with the negatively charged borate (Figure 13).

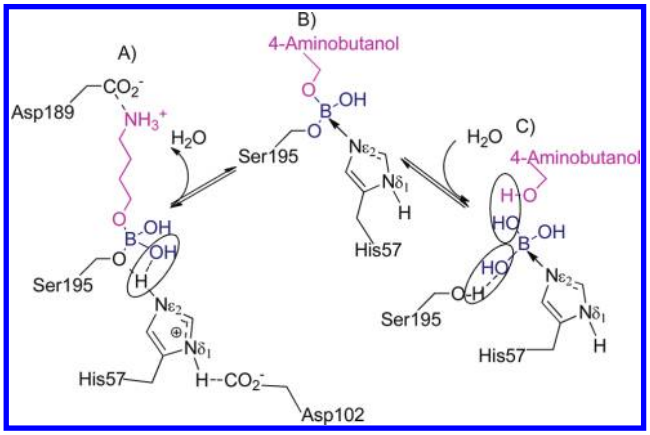


Figure 13. The downfield-shifted proton resonances arose from the labile imidazolium protons on His57, indicating a salt bridge interaction with the negatively charged borate.

In an extension of these NMR studies, Transue et al.[107] reported crystallographic and solution state NMR studies of trypsin in the presence of borate and either of three alcohols designed to bind to the S_1 affinity subsite: 4-aminobutanol, guanidine-3-propanol, and 4-hydroxymethylbenzamidine. Quaternary complexes of trypsin, borate, S_1-binding alcohol, and ethylene glycol (a cryoprotectant), as well as a ternary trypsin, borate, and ethylene glycol complex have been observed in the crystalline state (Figure 14). Borate formed ester bonds to Ser195, ethylene glycol (two bonds), and the S_1-binding alcohol (if present). ^1H and ^{11}B NMR studies confirmed that these complexes also exist in solution and also provided evidence for the formation of ternary trypsin, borate, and S_1-subsite alcohol complexes which were not observed in the crystals using the experimental protocols. Presumably, the inability to observe the ternary complexes in the crystalline state arose from the lower stability of these complexes and consequent inability to overcome the constraints imposed by the lattice contacts. It was suggested that the mechanism for the coupling of the lattice contacts with the active site involved a conformational rearrangement of Gln192.

^{11}B NMR thus far showed the formation of ternary complexes of trypsin, borate, and S_1-binding alcohols and revealed evidence for an additional binding interaction external to the enzyme active site. This binding interaction was explored as a prototypical interaction of borate and boronate ligands with residues on the protein surface. NMR studies of trypsin in

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156–4220

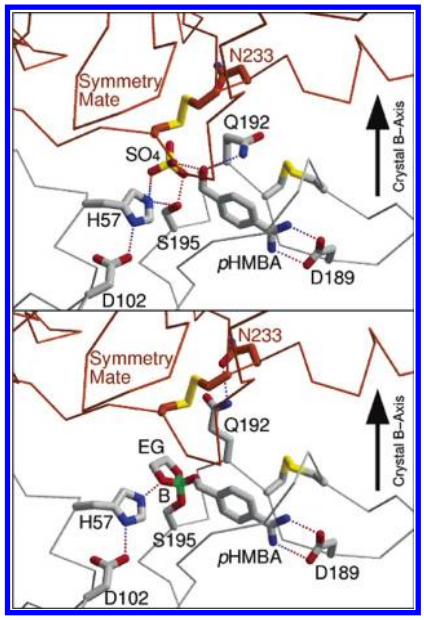


Figure 14. Examination of a series of eight trypsin structures indicates that formation of an active site borate−alcohol complex is accompanied by expansion of the crystal parallel to the *b*-axis of the unit cell. The figure illustrates an α-carbon trace corresponding to the active site of one trypsin molecule (shown in white) and the proximate residues of a symmetry-related molecule (shown in brown). Several of the side chains have been drawn in as well. In the upper trace, the 4-hydroxymethylbenzamidine (pHMBA) is bound to the S_1-subsite of the enzyme. Side chains corresponding to the residues of the catalytic triad (H57, D102, and S195), D189, Q192, and the disulfide bond connecting C191 and C220 are also indicated. In the lower trace, formation of the borate complex is shown to be accompanied by a significant change in the conformation of Q192, so that it extends toward the symmetry-related trypsin molecule in a direction that approximately parallels the crystal *b*-axis. Reprinted with permission from ref 107 Copyright 2004 American Chemical Society.

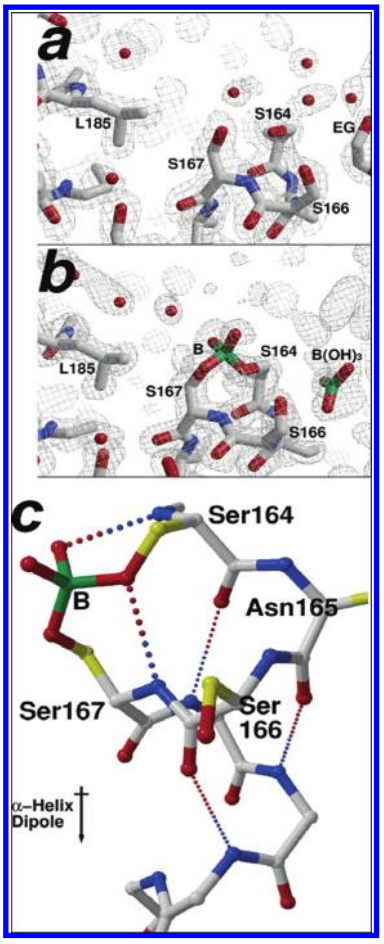


Figure 15. Crystallographic structure of the second borate binding site in trypsin: (a) X-ray structure of a serine-rich region of the trypsin surface obtained at pH 8.0 in the presence of 200 mM borate. PDB code: 1S6F; (b) structure of the same region of the protein crystallized at pH 10.6 in the presence of 200 mM borate, showing a borate ion covalently bound to the hydroxyl oxygens of Ser164 and Ser167; (c) covalent and hydrogen bonding interactions involving the borate anion bound at site 2. The boron is indicated in green and the β-carbons are in yellow. The two hydrogen bonding interactions involving the borate anion are indicated by large dotted lines. Small dotted lines indicate hydrogen bond characteristics of α-helical secondary structure. Reprinted with permission from ref 108. Copyright 2006 American Chemical Society.

which the active site was blocked with leupeptin or with the irreversible inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride indicated the existence of a low-affinity borate binding site with an apparent dissociation constant of 97 mM, measured at pH 8.0.[108] The field-dependent dynamic frequency shift of the ^{11}B resonance indicated that it corresponds to a complex for which $\omega\pi \gg 1$. The 0.120 ppm shift difference of the borate resonances measured at 11.75 and 7.05 T corresponded to a quadrupole coupling constant of 260 kHz. A much larger 2.00 ppm shift was observed in the ^{11}B NMR spectra of trypsin complexed with benzene boronic acid (BBA), leading to a calculated quadrupole coupling constant of 1.10 MHz for this complex. Crystallographic studies identified the second borate binding site as a serine-rich region on the surface of the molecule (Figure 15).[108] Specifically, a complex obtained at pH 10.6 showed a borate ion covalently bonded to the hydroxyl oxygen atoms of Ser164 and Ser167, with additional stabilization coming from two hydrogen-bonding interactions. A similar structure, although with low occupancy (30%), was observed for a trypsin−BBA complex. In this case, the BBA was also observed in the active site covalently bound in two different conformations to both His57-Nε and Ser195-Oγ. An analysis of pairwise hydroxyl oxygen distances was able to predict the secondary borate binding site in porcine trypsin. This approach is potentially useful for prediction of borate binding sites on the surfaces of other proteins. However, the distances between the Ser164/Ser167-Oγ atoms in all of the reported trypsin crystal structures was significantly greater than the Oγ distances of 2.2 and 1.9 Å observed in the trypsin complexes with borate and BBA, respectively. Thus, the ability of the hydroxyl oxygens to adopt a sufficiently close orientation to allow bidentate ligation is a critical limit on the borate binding affinity of surface-accessible serine/threonine/tyrosine residues.[108]

Potassium organotrifluoroborates were found to be reversible competitive inhibitors of trypsin (K_i values ranging from 1.50 to 0.26 mM). They increased inhibition by at least an order of magnitude over the corresponding boronates. The most potent was potassium 3-carboxyphenyltrifluoroborate that inhibited trypsin with K_i of 0.26 mM.[67]

Recently,[97] α-amino boronate complexes stabilized by amino cyanoborane complexation were prepared and found to moderately inhibit trypsin. The most efficient inhibition was for **32** (Figure 16) where the IC_{50} value was 0.08 mM.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

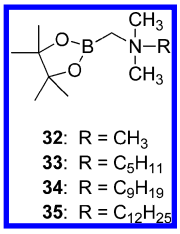


Figure 16. α-Amino boronate complexes.

2.4. Thrombin

Thrombin (human and mammalian) is a trypsin-like serine protease that plays a central role in hemostasis and induces platelet aggregation and secretion.[109,110] It is the final enzyme in the blood coagulation cascade that hydrolyses factors V, VIII, and XIII and fibrinogen, releasing fibrinopeptides A and B, which generate fibrin. The polymerization of fibrin produces the core of a blood clot. Thrombin cuts preferentially at Arg residues and the hydroxyl group of Ser195 attacks the reactive site P_1 residue of the substrate. Thrombin consists of two disulfide-linked polypeptide chains, A and B, which are folded into a trypsin-like protease. The thrombin B chain contains the active site residues Ser, His, and Asp, but also contains insertion loops that extend around the active site cleft, making it deeper and narrower than that of trypsin. The S_1 pocket of thrombin contains Asp189 at the bottom of the S_1 pocket which can form a salt bridge with basic side chains of substrates or inhibitors. However, there are two amino acid positions in close proximity to Asp189, namely, 190 and 213, which are different for the two enzymes: Ala/Val in thrombin and Ser/Val in trypsin; that is, this region is more hydrophobic in thrombin. In this context, the substrate pocket S_1 of thrombin is optimized to recognize an arginine side chain, while the S_2 and S_3 subsites are hydrophobic in contrast to trypsin.[111] The S_2 subsite is smaller than the S_3, typically accommodating a proline residue or a small hydrophobic unit, while the S_3 subsite can bind larger residues (e.g., D-Phe). Thrombin, however, is unlike trypsin in that it has extra surface structures that influence the interactions with macromolecular substrates and thus make it a more discriminating protease (Figure 17).[112,113] Thrombin cleaves selectively at specific Arg sites using ancillary interactions from "exosites" distinct from the active site. Exosite I is located "east" of the active site. It contains hydrophobic patches and numerous charged residues on its surface that provide electrostatic steering to fibrinogen on its approach to the active site of thrombin. This facilitated diffusion accounts for the fast rate of complex formation. Exosite I also provides the locale for the binding of thrombomodulin to thrombin. Exosite II is positioned "west" of the active site, opposite to exosite I. It features a conspicuous number of charged residues, but unlike exosite I has no hydrophobic patches on its surface. Exosite II is the locale for interaction with polyanionic ligands such as glycosaminoglycans and heparin.

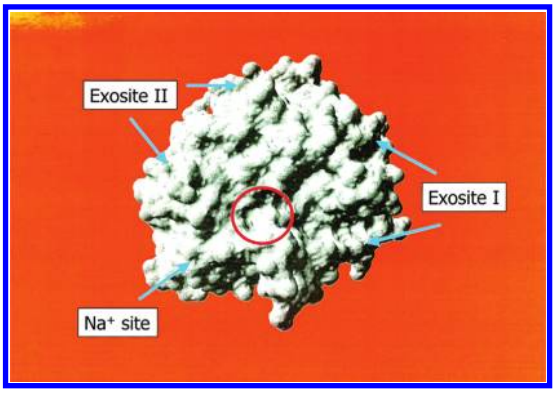


Figure 17. Schematic representation of the water-accessible surface of the B chain of thrombin.[113] The active-site region is circled. Note the location of exosite I (east), exosite II (west), and the Na^+ binding site (southwest) relative to the active site.[112] Reprinted with permission from ref 112. Copyright 2003 American Chemical Society.

Compounds that inhibit thrombin, such as the 65-residue protein hirudin isolated from the medicinal leech, are effective inhibitors of blood clotting. Clinical advantages exist for the use of low molecular weight thrombin inhibitors in place of the high molecular weight complex of antithrombin III and heparin because such molecules are effective in both blockage of arterial thrombosis[114] and venous blood clot accretion.[115,116] For these reasons, intense research was aimed at the discovery of safe, orally active, synthetic thrombin inhibitors.[117,118]

The α-aminoboronic acids with basic-3-guanidinopropyl side chain (boroArg) such as Ac-(D)Phe-Pro-boroArg-OH (DuP 714) (Figure 18), Boc-(D)Phe-Pro-boroArg-OH and H-(D)Phe-Pro-boroArg-OH were highly effective, slow-binding inhibitors of thrombin, with inhibition constants of 41, 3.6, and <1 pM, respectively.[58] These peptides were effective in inhibiting the action of thrombin in rabbit plasma against its physiological substrates. Activated partial thromboplastin time was significantly prolonged in vitro by all of the inhibitors at concentrations of 50–200 nM. Prolongation of activated partial thromboplastin time were also observed in rabbits after intravenous (40–80 μg/kg) or subcutaneous (0.2–2 mg/kg) injections of DuP 714. The in vivo pharmacology of DuP 714 (**36**)[119] indicated also that it was a highly effective anticoagulant.

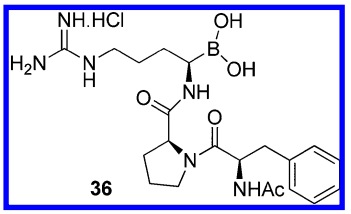


Figure 18. Thrombin inhibitor DuP 714 (**36**).

In subsequent studies, researchers[120] reported the thrombin inhibition constants and crystallographic complex structures of the lysine, homolysine, and ornithine analogues of DuP 714 and an amidine analogue where the NE nitrogen of arginine was changed to a carbon (Figure 19). The basic groups interact

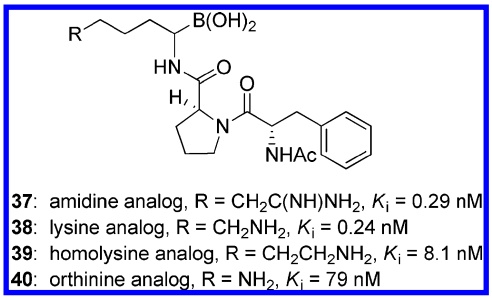


Figure 19. Chemical structures of thrombin inhibitor complexes.

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156–4220

with backbone carbonyl groups, water molecules, and an aspartic acid side chain (Asp189) located in the thrombin S_1 specificity pocket. The variation in inhibition constant by 3 orders of magnitude appears to reflect differences in the energetics of interactions made with thrombin and differences in ligand flexibility in solution. Comparison of the X-ray crystallographic structures of each of these inhibitors bound to thrombin revealed an overall similar mode of binding, especially for the Ac-(D)Phe-Pro-boronic acid portion. Therefore, despite their similarities in chemical structure and in many of their interactions with thrombin, the variation in inhibitor affinity was surprisingly wide and likely reflected energetic differences in the interactions made by the basic groups of these inhibitors, as well as differences in inhibitor flexibility.

Later, Galemmo et al.[121] discovered a novel class of inhibitors incorporating a less basic boroLys at P_1 with a binding motif differing from that reported for the D-Phe-Pro-AA class of inhibitors. Compared to boroArg analogues, boroLys analogues have a diminished tendency to produce elevations in serum transminase levels and hypertension in vivo.[122] A series of novel (*N*-acyl-*N*-alkyl)glycyl*boro*Lys thrombin inhibitors were synthesized. Structure−activity relationship studies for these inhibitors culminated in the discovery of (*N*-3-phenylpropanoyl-*N*-phenethyl)glycyl*boro*Lys (Figure 20), a potent orally active inhibitor (K_i = 0.42 nM) with

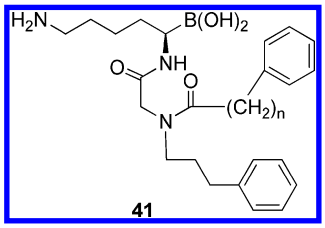


Figure 20. (*N*-3-phenylpropanoyl-*N*-phenethyl)glycyl*boro*Lys (**41**).

a binding conformation in which the N-phenethyl group occupies the aryl binding pocket of thrombin. The simplified structure lends itself to modification and served as the basis for a more selective class of enzyme inhibitors devoid of the hypotension and serum transminase enzyme elevation often associated with thrombin inhibitors.[122]

Although DuP 714 (**36**) proved to be a potent thrombin inhibitor, it had undesirable side effects. Therefore, new and selective boronic acid thrombin inhibitors were developed by replacing the guanidine of the boroArg side chain with various heterocycles ranging in size and basicity.[123] Also, the N-acetyl group of DuP 714 has been replaced by the *N*-butanesulfonamide group. As a result, potent and selective thrombin inhibitors (Figure 21) had been obtained. Although the affinity of these compounds was decreased compared to DuP 714, they were more selective for thrombin over the other serine proteases.

Animal studies indicated that DuP 714 (**36**) side effects appeared to be related to the undesirable inhibition of complement factor I. In this context, the difference in the binding requirement between factor I and thrombin was analyzed, and with no crystal structure of factor I available, the crystal structure of factor Xa (fXa) was used, working on the assumption that the overall conformation of factor I was similar to that of fXa. Crystal structural analysis of the inhibitor−enzyme complexes with different inhibitors showed that there were very noticeable differences in the P_2 pocket. Therefore, a

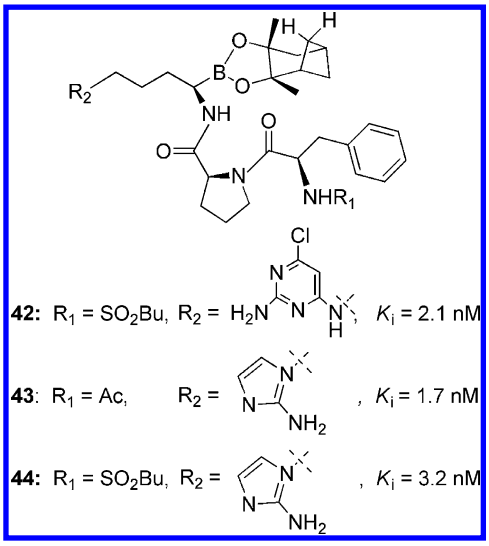


Figure 21. Examples of the potent five-membered S1 heterocyclic thrombin inhibitors.

series of β,β-dialkylphenethylglycine P_2 analogues of DuP 714 were designed and synthesized. These compounds (Figure 22) such as **45** and **46** had greater selectivity for thrombin over factor I and an improved safety profile.[124]

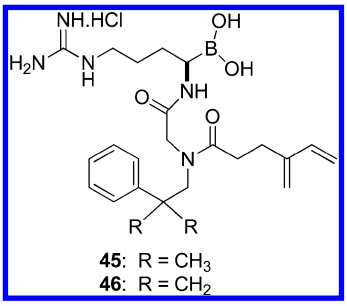


Figure 22. Compound **45** (K_i = 0.06 nM for thrombin, 99 nM for fXa) and compound **46** (K_i = 0.06 nM for thrombin, 46 nM for fXa).

With the knowledge that hirudin is an effective inhibitor of blood clotting, a novel peptide boronate thrombin inhibitor was designed and synthesized using solid phase chemistry and suitably protected aminoboronates.[125] The inhibitor consists of four parts: (i) an active site inhibitor, D-Phe-Pro-Boro(AA)-OPin, (ii) an anion binding exosite association moiety, hirudin, (iii) a spacer to link these components, and (iv) a novel "flexor" nonpeptide unit to allow correct orientation. The bivalent nature of the inhibitor [-D-Phe-Pro-BoroBpg-OPin]$COGly_2$-Hir (Bpg, 3-bromopropyl-glycine) (Figure 23) enhanced binding up to 10-fold greater than the corresponding native peptide *Z*-D-Phe-Pro-BoroBpg-OPin or the mixture of non-covalently linked units, and resulted in a potent and selective inhibitor of thrombin having a K_i of 0.6 nM.

Another related study showed that the activity of two active site directed thrombin inhibitors *Z*-D-Phe-Pro-boroIrg (Irg, 3-isothiouronuimpropylglycine, **48**) and *Z*-D-Phe-Pro-boroBpg (Bpg, 3-bromopropyl-glycine, **49**) was enhanced by attaching the hirudin segment for exosite 1 of thrombin via a novel reverse oriented linker to each of two tripeptide boronate inhibitors (Figure 24).[126] At P_1, compound **48** contained an arginine-like isothiouronium side chain, while compound **49** contained an uncharged bromopropyl residue. Inhibition of

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

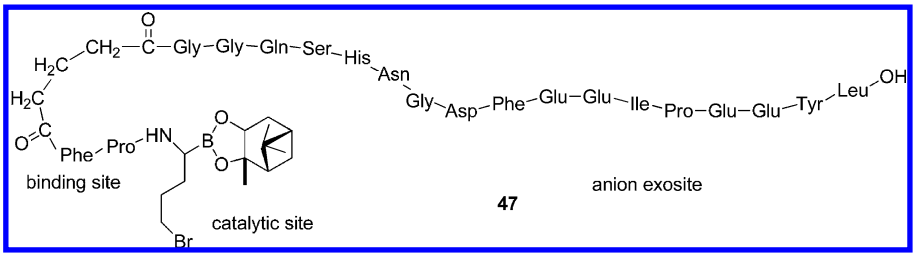


Figure 23. Schematic drawing of the hirulog [-D-Phe-Pro-BoroBpg-OPin]$COGly_2$-Hir.

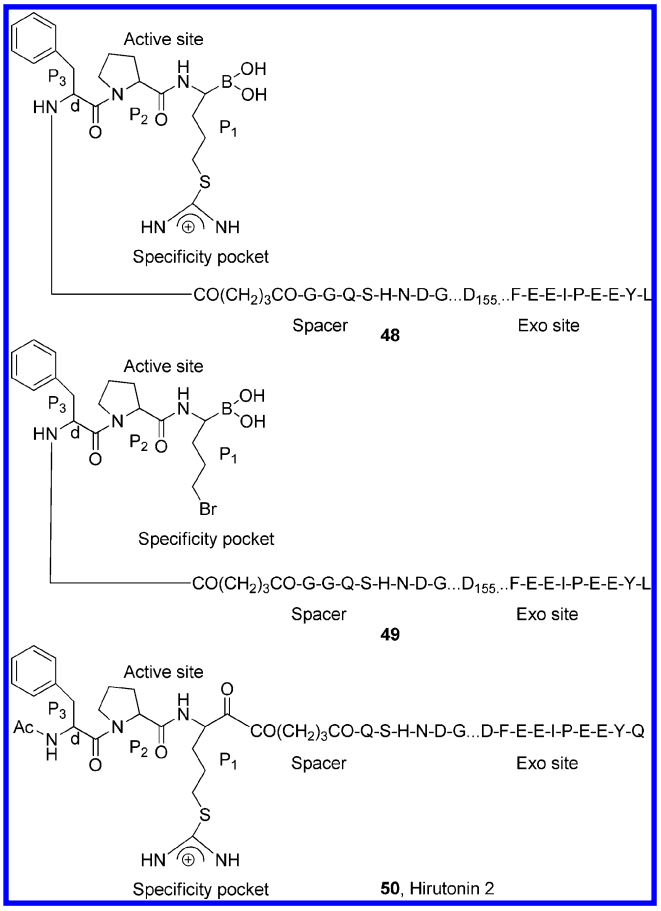
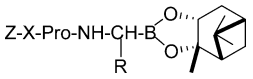


Figure 24. Schematic representation of the compounds **48** and **49** and hirutonin 2 (**50**). Amino acids are represented by single-letter codes for the spacer and exosite binding portions, while the active site binding unit is drawn in full. D-Enantiomers are represented by d, and numbering is based on the hirudin sequence. Drawings are annotated for the site of interaction of the inhibitor with thrombin.

human α-thrombin by compound **48** showed slow, tight-binding competitive kinetics (final K_i of 2.2 pM). The addition of hirudin peptide (20 μM) competed for exosite 1 binding and restored the k_1 and k_{-1} to that of the analogous tripeptide. Compound **48** had enhanced specificity for thrombin over trypsin with K_{iTry}/ K_{iThr} of ~900 compared to the analogous tripeptide, with K_{iTry}/K_{iThr} of ~4. Compound **49** acted as a competitive inhibitor (K_{iThr} of 0.6 nM) and was highly selective with no effect on trypsin. Crystallographic analysis of complexes of human α-thrombin with compounds **48** (1.8 Å) and **49** (1.85 Å) showed a covalent bond between the boron of the inhibitor and Ser195 (bond lengths B−O of 1.55 and 1.61 Å, respectively). The isothiouronium group of compound **48** formed bidentate interactions with Asp189. The P_2 and P_3 residues of the inhibitors formed interactions with the S_2 and S_3 sites of thrombin similar to other D-Phe-Pro-based inhibitors.[127] The linker exited the active site cleft of thrombin forming no interactions, while the binding of Hir49−64 segment to exosite 1 was similar to that previously described for hirudin.[128]

Researchers wanted to improve the specificity of action and to reduce toxicity of the peptidyl boroArg derivatives with a positive charge at the P_1 site over conventional, positively charged derivatives. Peptides with the sequence D-Phe-Pro-boroAla containing a P_1 aminoboronic acid with a neutral side chain were synthesized and showed good thrombin inhibition (Table 1) as well as selectivity for thrombin with no serious side effect on blood pressure.[129]

Table 1. K_i Value for Inhibition of Thrombin by Peptide Amino Boronic Esters



compound	R	K_i (nM), X = D-Phe	K_i (nM), X = D-Dpa[a]
51	NH_2═$C(NH_2)$-S-$(CH_2)_3$	1.0	2.0
52	$CH_3O(CH_2)_3$	7.0	14.0
53	$CH_3(CH_2)_4$	19.0	26.0
54	$CH_3CH_2C(CH_3)_2$	7.0	29.0

[a]Dpa = β,β-diphenylalanine.

Later, the peptide benzyloxycarbonyl-D-Phe-Pro-methoxypropylboroGly (Z-D-Phe-Pro-boroMpg$C_{10}H_{16}$) (**55**, Figure 25), with the side chain replaced by a neutral one, was

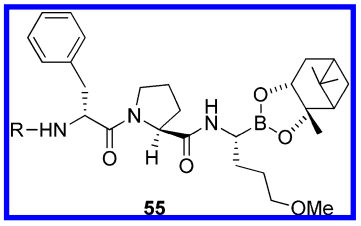


Figure 25. Benzyloxycarbonyl-D-Phe-Pro-methoxypropylboroGly, R = Z/ Boc.

synthesized.[130] This boronic acid derivative inhibited thrombin by a competitive mechanism with an inhibition constant (K_i) of 8.9 nM. In comparison to boroArg derivatives, Z-D-Phe-Pro-boroMpg$C_{10}H_{16}$ displayed higher selectivity for thrombin over trypsin (K_i = 1.1 μM) and plasmin (K_i = 15.7 μM). Prolongation of thrombin time and activated partial thromboplastin time were observed with micromolar concentrations of Z-D-Phe-Pro-boroMpg$C_{10}H_{16}$. In a thrombin-dependent in vitro aggregation assay with human platelets, Z-D-Phe-Pro-

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156−4220

boroMpgC$_{10}$H$_{16}$ inhibited aggregation with an IC$_{50}$ of 85 nM. When tested in a thrombin-dependent platelet accumulation model in the rat, a bolus injection of *Z*-D-Phe-Pro-boroMpgC$_{10}$H$_{16}$ (0.3−3 mg/kg) inhibited platelet accumulation. Thus, the substitution of the charged guanidino group in the P$_1$ side chain by the neutral methoxy group resulted in a potent and highly selective thrombin inhibitor with an interesting pharmacological profile with in vitro as well as in vivo models.[131]

Upon the basis of the *Z*-D-Phe-Pro-boroMpg-OPin compound, a range of compounds were synthesized by varying the neutral side chain at the P$_1$ site. Of the 20 examples based upon the structures at P$_2$ and P$_3$ of *Z*-D-X-Pro (X = Phe or *β*,*β*-diphenylalanine), all were found to be effective inhibitors of thrombin with very high specificity. As far as potency, compounds containing the methoxypropyl group at P$_1$ were most active (**56a**, Figure 26). The compounds displayed potent

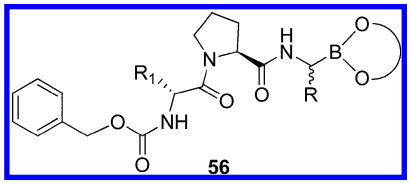


Figure 26. Peptide amino boronic compounds: (**56a**) boronic esters with pinacol, R = $(CH_2)_3OCH_3$, R_1 = $CHPhe_2$, (**56b**) boronic acid, R = $(CH_2)_3OCH_3$, R_1 = CH_2Phe.

anticoagulant activity with values for **56a** in thrombin time of 0.63 *μ*M and in activated partial thromboplastin time of 2.0 *μ*M. ^{11}B NMR confirmed the interaction of the boron atom with the active site.[132a] Another similar compound (**56b** (**TRI50c**), Figure 26) proved to be a highly potent (K_i = 22 nM) and selective competitive inhibitor of thrombin with high efficacy in animal models of venous and arterial thrombosis and minimal effect on bleeding.[132b] Trigen, a UK-based pharmaceutical company, has two compounds **TGN-255** (parenteral formulation) and **TGN-167** (oral formulation) in phase III clinical trials as anticoagulants. They are salts/esters of **TRI50c** which is the active principle.

The X-ray crystal structure of the boropeptide **57** (Figure 27) bound to thrombin showed that the 3-phenylpropionyl chain attached to the proline residue formed a favorable edge-to-face interaction with the Trp215 side chain located at the base of the S$_3$ specificity pocket of thrombin.[59] To maximize this edge-to-face interaction, rigid analogues of **57** and **58** were designed. In such a design, a cyclohexane ring (**59**) or a pyrrollidine ring (**60**) was used to hold the phenylpropionyl moiety in an orientation favorable for the interaction with the Trp215 residue as predicted by computer modeling studies based on the X-ray crystal structure. Both constrained analogues **59** and **60** showed a 2-fold increase in potency relative to their unconstrained counterparts **57** and **58**, respectively. In a related effort to maximize the edge-to-face interaction with the Trp215 side chain, the P$_3$ residue of **61**, a previously discovered inhibitor, was replaced by a benzoic acid-derived residues. This afforded an extremely potent thrombin inhibitor (**62**), which is approxiamately 3-fold more potent than the lead compound **61**.[60]

More efforts were undertaken in designing selective thrombin inhibitors by varying the P$_1$ position. These highly effective inhibitors have been obtained by preparing boronic acid analogues of *m*-cyano-substituted phenylalanine (Figure

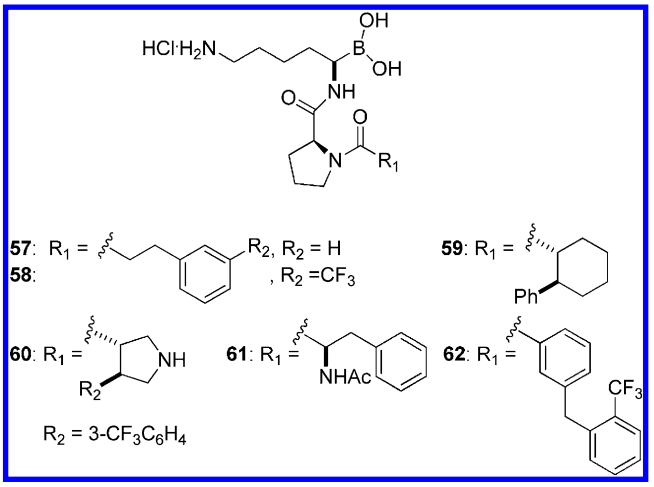


Figure 27. Thrombin inhibitors: (**57**, K_i = 0.80 nM); (**58**, K_i = 0.78 nM); (**59**, K_i = 0.46 nM), (**60**, K_i = 0.50 nM); (**61**, K_i = 0.24 nM); (**62**, K_i = 0.07 nM).

28) and its incorporation into peptides. The cyano group enhanced binding by several orders of magnitude. For example,

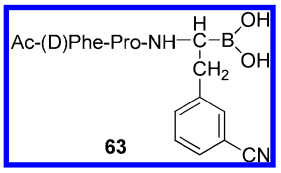


Figure 28. *m*-CyanoboroPheAla analogue.

Ac-(D)Phe-Pro-boroPheOH bound to thrombin with a K_i of 320 nM and the K_i of Ac-(D)Phe-Pro-boroPhe(*m*-CN)-OH was 0.79 nM (**63**). Trypsin exhibited similar enhanced affinity for the *m*-cyano substituted inhibitor. Protein crystal structure determination of trypsin complexed to H-(D)Phe-Pro-boroPhe(*m*-CN)-OH indicated that the aromatic side chain is bound in the P$_1$ binding site and that the cyano group can act as a H-bond acceptor for the amide proton of Gly219 (Figure 29).[133]

Thus far, it has been shown that peptidyl boronic acid inhibitors with nonbasic P$_1$ side chains could improve the selectivity for thrombin over trypsin and plasmin.[123,129,132−134] To further elaborate this hypothesis, Wienand et al.[135] described several boron containg thrombin inhibitors with nonbasic residues in P$_1$, which showed higher selectivity for thrombin over trypsin and plasmin. A series of Boc-D-trimethylsilylalanine-proline-boro-X pinanediol derivatives with boro-X being different amino acids were synthesized as inhibitors for thrombin (Figure 30). The influence of hydrogen donor/acceptor properties of different residues in the P$_1$ side chain of these inhibitors in the selectivity profile was investigated. The study confirmed the structure-based working hypothesis: The hydrophobic/hydrophilic character of amino acid residues 190 and 213 in the neighborhood of Asp189 in the S$_1$ pocket of thrombin (Ala/Val), trypsin (Ser/Val), and plasmin (Ser/Thr) define the specificity for the interaction with different P$_1$ residues of the inhibitors. Many of the synthesized compounds demonstrated antithrombin activity with Boc-D-trimethylsilylalanine-proline-boro-methoxypropylglycine pinandiol (**64**) being the most selective thrombin inhibitor.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

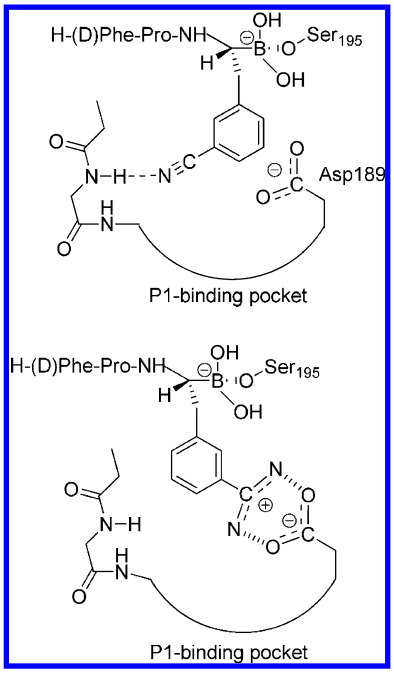


Figure 29. Illustration of the binding of −boroPhe(*m*CN)-OH to trypsin and comparison with the corresponding basic inhibitor.

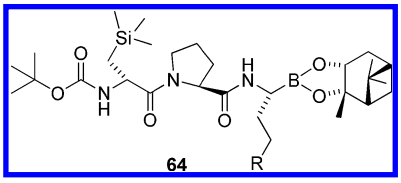


Figure 30. Boc-D-trimethylsilylalanine-proline-boro-X pinanediol derivatives with R = $-CH_2OCH_3$ for **64**.

Continuing the search for selective inhibitors of thrombin, and based on the structural comparison of the S_1 pocket in different trypsin-like serine proteases, a series of Boc-D-trimethylsilylalanine-proline-boro-X pinanediol derivatives with boro-X being different amino acids were synthesized as inhibitors of thrombin. Among the novel compounds, a number of derivatives were synthesized which appeared to have side-chain variants too bulky to fit into the S_1 pocket. Nevertheless, these compounds inhibited thrombin in the nanomolar range. The X-ray structure of one of these inhibitors (**65**, Figure 31)

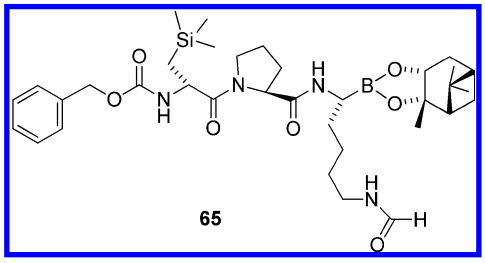


Figure 31. The tripeptide-boronate.

bound to the active site of thrombin revealed that a new binding mode is responsible for these surprising results (Figure 32). It was shown for the first time that the introduction of a butyl formamide as a P_1 side-chain in a tripeptide-boronate can force the thrombin/inhibitor complex to adopt a new binding mode in which the formamide group points out of the S_1 pocket and forms new hydrogen bonds with Gly219. The structure activity relationship of the other compounds under investigation indicated that these molecules show the same binding mode.[136]

In continuing studies, Spencer et al. embarked on a program aimed at synthesizing *ortho*-substituted arylboronic derivatives with potential heteroatom coordination or proximity to boron.[137] The compounds synthesized were 2-mercapto- and 2-piperazino-(methyl-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolanes, (**66a−i**) and (**67a−d**) (Figure 33). Several of these derivatives were studied in both solution and in the solid state, and displayed no S−B (sulfur−boron) coordination and only weak N−B (nitrogen−boron) coordination. Their inhibitory activity against serine proteases including thrombin was measured. There appeared to be a marked increase in potency toward thrombin on the *ortho*-modification of the pinacol ester of phenylboronic acid (PhBOPin) by certain mercaptomethyl substituents, whereas the aminomethyl substituents studied had a much less pronounced effect.

Boron containing compounds showed to be very potent inhibitors of thrombin. Therefore, over the next years, we will see the results of the some of these compounds currently in clinical trials, as well as more compounds entering clinical trials.

2.5. Factor Xa and Factor XIa

Factor Xa (human and mammalian) is another trypsin-like serine protease that plays a crucial role in the coagulation cascade by occupying the juncture of the intrinsic and extrinsic clotting pathways. The physiological role of fXa is the proteolytic cleavage of prothrombin to thrombin, which once generated can proceed to cleave fibrinogen to fibrin.[138] Fibrin can then proceed to cross-link with activated platelets to form a fibrin blood clot. Imbalances in the coagulation cascade can lead to excessive thrombotic conditions. Because of the central role of fXa in the coagulation cascade, its inhibition is an attractive method of thrombus prevention.[139]

During the screening of a series of conformationally restricted boropeptide thrombin inhibitors, a boroLys compound (**68**, Figure 34) containing a 2-(2-cyanophenylthio)-benzoyl in the P_3 position was found to be a potent fXa inhibitor (K_i = 1.1 nM). It had a 16-fold higher potency relative to the compound without the nitrile moiety (K_i = 17 nM).[139]

Recently, researchers began focusing on a relatively unexplored target in the blood coagulation cascade, namely, factor XIa (FXIa).[140] Factor XI is a serine protease expressed as a zymogen that is converted to its active form, FXIa, by factor XIIa and by thrombin. Upon activation, FXIa promotes coagulation by activating factor IX. There is evidence that a high level of FXIa increases the risk for venous thrombosis,[141] although currently there are few reports concerning FXIa as a potential target for the development of small molecule antithrombotic drugs.[142] Since FXIa plays a role in the amplification pathway in coagulation and not in initiation of clotting, an inhibitor specific for FXIa, may be effective at reducing the risk of thrombosis without a significant risk of bleeding. Indeed, in a recent publication, FXIa null mice (fXI−/−) were found to be resistant to clot induction by $FeCl_3$ in an arterial thrombosis model, but had normal bleeding times.[143]

In a new study,[144] researchers explored aryl boronic acids as potential inhibitors of FXIa. A series of functionalized aryl boronic acids were synthesized and evaluated as potential inhibitors of factor XIa (Figure 35). Crystal structures of the protein−inhibitor complexes (Figure 36) led to the design and

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

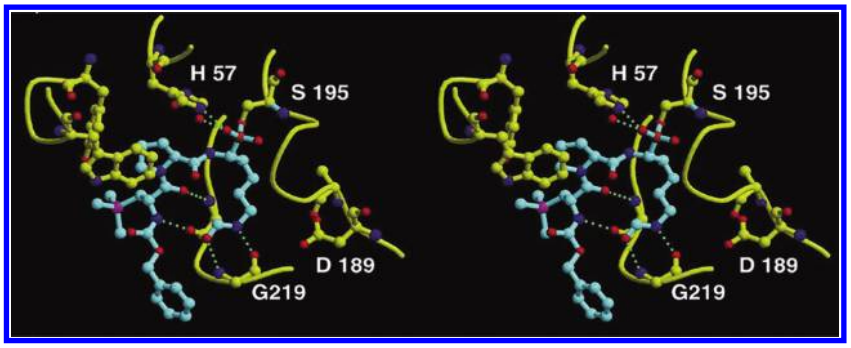


Figure 32. X-ray structure (stereoview) of human α-thrombin complexed with the inhibitor in Figure 31. Carbon atoms are colored in yellow for the protein atoms of thrombin. The boron atom is colored in orange and the silicium ion is colored in magenta. In thrombin, there is a water molecule hydrogen bonded to the side chain nitrogen of the lysine. Reprinted with permission from ref 136. Copyright 2000 Elsevier.

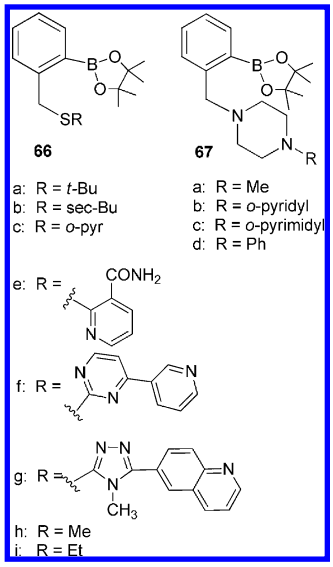


Figure 33. 2-Mercapto- and 2-piperazino-(methyl-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxa-borolanes.

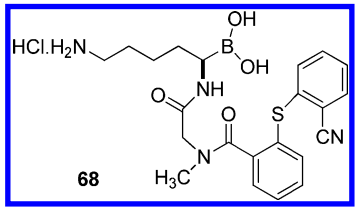


Figure 34. Boropeptide fXa inhibitor.

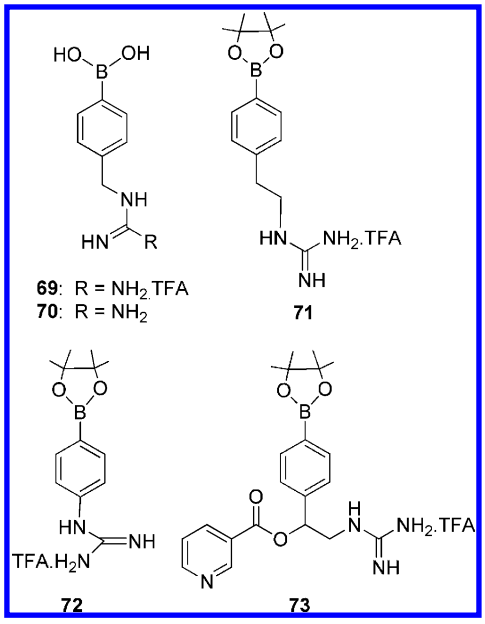


Figure 35. Aryl boronic acids as inhibitors for FXIa: (**69**, K_i = 22 μM); (**70**, K_i = 24.7 μM); (**71**, K_i = 7.3 μM); (**72**, K_i = 5.9 μM); (**73**, K_i = 1.4 μM).

synthesis of second generation compounds showing single digit micromolar inhibition against FXIa and selectivity against thrombin, trypsin, and FXa.

2.6. Dipeptidyl Peptidases (DPP)

Members of the DPP family include DPP4, DPP7, fibroblast activation protein a (FAP), DPP8, and DPP9.[145] Dipeptidyl peptidase IV (DP-IV; dipeptidyl peptide hydrolase; DPP4; CD26) is a membrane-bound serine protease with specificity for cleaving Xaa-pro dipeptides from polypeptides and proteins. It is found in a variety of mammalian cells tissues, including those of lyphoid origin where it is found specifically on the surface of $CD4^+$ T cells.[146−149] Although the functional significance of the enzyme has not been established, a role in T-cell activation and immune regulation has been proposed.[150] DPP7 is a 58 kDa glycoprotein with an enzymatic function similar to DPP4 but only distantly related by sequence homology.[151] The inhibition of DP-IV could cause the suppression of the T-cell mediated immune response both in vitro and in vivo. The inhibition of this enzyme has been shown to suppress IL-2 production[152] and antigen-induced T-cell proliferation[153] in vitro. Potentially therapeutic effects of inhibitors of DPP4 have been demonstrated using in vivo models of immunosuppression.[154] Furthermore, CD26 has been identified as the adenosine deaminase binding protein,[155,156] a molecule involved in the severe combined immunodeficiency disease (SCID), and has also been proposed to be a coreceptor on the surface of CD4+ cells for the human immunodeficiency virus (HIV),[157] the causative agent of acquired immune deficiency syndrome (AIDS).

Specific inhibitors of DP-IV are of some interest, both as tools to help elucidate the biological role or roles of DP-IV and as potential therapeutic agents. Bachovchin et al.[158] and Flentke et al.[159] reported the synthesis and a preliminary kinetic characterization of two potent inhibitors of DP-IV, Ala-boroPro (K_i = 2 nM) and Pro-boroPro (K_i = 3 nM) (boroPro refers to an analogue of proline in which the carboxylate group was replaced by a boronyl group). These inhibitors had an

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156−4220

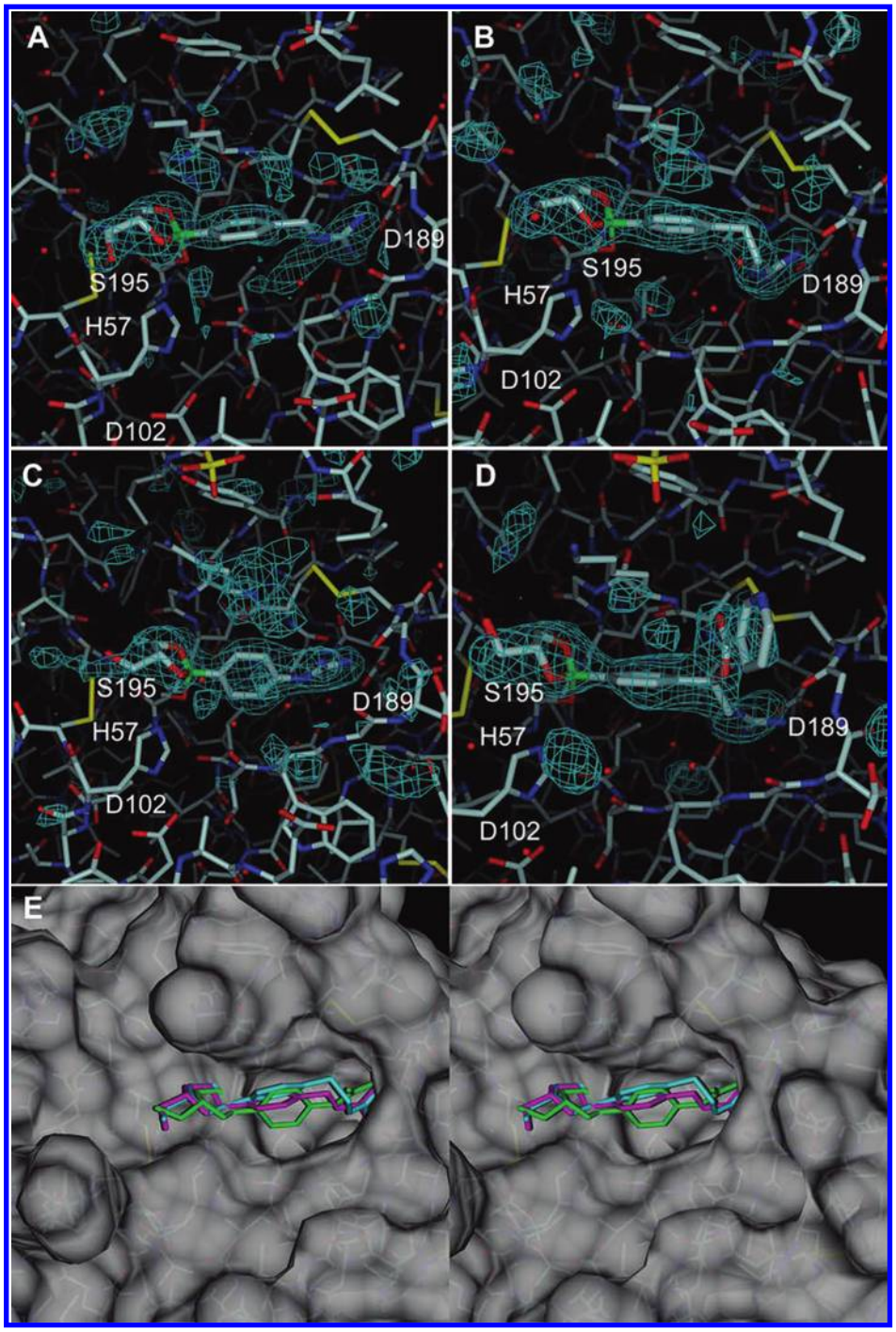
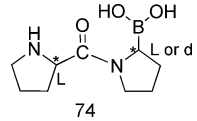


Figure 36. Panels A–D are electron density maps calculated using Fourier coefficients $F_{obs} - F_{calc}$ using phases from the final refined models omitting the atoms for the ligand and contoured at 2.5 sigma for FXIa complexes with compounds (a, **69**), (b, **71**), (c, **72**), and (d, **73**), respectively. Panel E is a wall-eyed stereodiagram of the superposition of the bound conformations of compounds (**69**, magenta), (**71**, cyan), and (**72**, green) in the active site of FXIa. Reprinted with permission from ref 144. Copyright 2006 Elsevier.

immunosuppressant activity, suppressing antigen-induced T-cell proliferation in T-cell culture systems[159] and antibody production in mice.[154] Later, the potent dipeptidyl peptidase (DP-IV) inhibitor [1-(2-pyrrolidinylcarbonyl)-2-pyrrolidinyl-boronic acid (L-Pro-DL-boroPro)[159] (Figure 37) was fractionated into its component L-L and L-D diastereomers by C18 HPLC. Inhibition kinetics confirmed that the L-L diastereomer was a potent inhibitor of DP-IV, having a K_i of 16 pM while the L-D isomer bound at least 1000-fold more weakly than the L-L, if it bound at all. The instability of Pro-boroPro, together with its



74

Figure 37. Structure of *trans*-Pro-boroPro (**74**) showing chiral centers. The coupling of L-Pro with racemic LD-boroPro was expected to yield a mixture of two diastereoisomers: L-Pro-L-boroPro and L-Pro-D-boroPro.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

very high affinity for DP-IV and the time dependence of the inhibition, made a rigorous kinetic analysis of its binding to DP-IV difficult. However, a method was developed which took advantage of the slow rate at which the inhibitor dissociates from the enzyme. Analysis of the slow-binding curves yielded a K_i value of 16 pM and a bimolecular rate constant of association, k_{on} of 5.0×10^6 M^{-1} s^{-1}. The experimentally determined k_{on} and K_i indicated that the dissociation rate constant, k_{off}, was 78×10^{-6} s^{-1} ($t_{1/2}$ = 150 min). The slow-binding curves were shown to fit a simple E + I ↔ E·I model, indicating that it was not necessary to invoke a two-step mechanism to explain the inhibition kinetics.[160]

In order to explore more fully the role of DPP4 in immunosuppression, Kelly et al.[161] examined the properties of the boroPro containing dipeptides **75** (Figure 38) (Xaa-boroPro). Literature on boronate ester polypeptides indicated that they gained activity upon exposure to aqueous buffer, presumably through hydrolysis to the active boronic acid (e.g., **76**).[55,162] In contrast, dipeptides (**75**) had been reported to lose activity in a time-dependent manner upon exposure to an aqueous medium,[159] suggesting a more complicated scenario. To simplify the analysis of this apparent contradiction, Kelly et al.[161] synthesized the dipeptide in the unprotected boronic acid form. Val-boroPro (**77**) was a remarkably potent inhibitor of DPP4 (IC_{50} = 16 nM).[163] However, the inhibitory activity of the material decreased rapidly upon standing in pH = 7.8 buffer, thus pointing to an intrinsic instability in the dipeptide motif. The authors hypothesized that the loss of inhibition was due to a cyclization taking place to generate a compound of structure (**78**) (eq 1). This compound was a boron analogue of a diketopiperazine, often a side product in peptide chemistry.

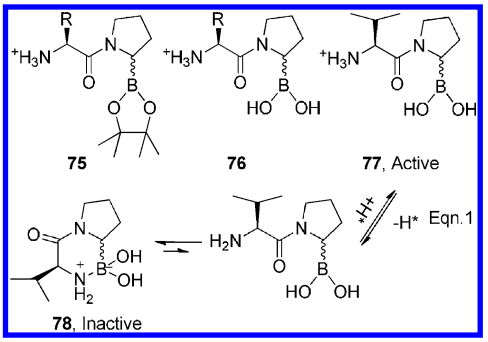


Figure 38. Val-boroPro (**77**) is a potent inhibitor of DPP4.

Later on, compound **78** was isolated and its cyclic structure was confirmed by ^{11}B-NMR and X-ray data for a protected analogue.[164] A ^{1}H NMR experiment was designed to follow the course of the reaction.[165] Compound **77** was dissolved in an aqueous buffer adjusted to pH = 7.8. Over time, increasing amounts of **78** were observed. Kinetic analysis of the data gave an observed first-order rate constant of $3.9 \times 10^{-4} \pm 0.7 \times 10^{-4}$ s^{-1} for the cyclization of **77** to **78**. A corresponding biochemical experiment was run measuring the time-dependent ability of the compound to inhibit DPP4 after also standing in an aqueous buffer at pH = 7.8. As expected, the compound became less active over time and the inactivation proceeded with a similar observed rate constant ($6.6 \times 10^{-4} \pm 0.6 \times 10^{-4}$ s^{-1}). The correlation demonstrated between the structural and enzymatic experiments established that the cyclization was indeed responsible for the loss in activity. The cyclization was also reversible due to the dative nature of the B−N bond in **78**. Researchers have observed that although activity against the enzyme diminished by 3 orders of magnitude, the final IC_{50} was still sub-micromolar, suggesting that at pH = 7.8, compound **78** was in equilibrium with a small amount of the open from **77**. In an acidic buffer (pH = 3.0), compound **78** reverted to its open form (**77**) and completely regained its activity against the enzyme. Because peptidyl boronic acids were known to display tight binding inhibition with a very slow off-rate,[160] the equilibrium had profound consequences for this series of immunosuppressants. When the enzyme was incubated in the presence of cyclized (i.e., inactive) inhibitor under standard (pH = 7.8 buffer) conditions and then assayed for the ability to hydrolyze substrate, a cumulative, time-dependent inhibition of the enzyme was observed. These data were consistent with the formation of the equilibrium between **77** and **78** being reestablished after the active open form **77** binds (and remains bound)[160] to the enzyme (Figure 39). ***Thus, it was the presence of the enzyme itself that drove the equilibrium toward the active form.***

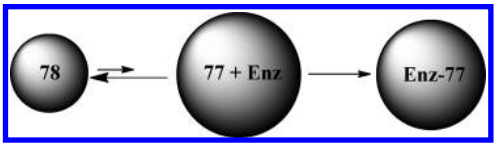


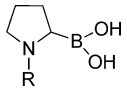
Figure 39. Formation of the equilibrium between **77** and **78**.

The results of these experiments showed a system in which the ability to inhibit a serine protease relied on the position of an equilibrium between two isolable and interchangeable conformations. They also showed that specific binding to an enzyme can drive equilibrium if the off-rate is such that the enzyme can entrain one of the components from the system.[161]

In this context, there were two complications which traditionally hampered the study of amino boronic acid dipeptides[161,166] as DP-IV inhibitors. The first was that these molecules were usually tested as their protected boronate esters which required that the compounds undergo hydrolysis in order to be activated.[166] Often this hydrolysis was neither rapid nor complete, which could affect the accurate determination of potency. The second concern was that boronic acid dipeptides lose activity in a time-dependent manner upon exposure to aqueous buffer. This loss in activity was correlated[161,166] to the position of a reversible intramolecular cyclization. In this context, Coutts et al.[167] detailed the structure−activity relationships associated with variations of the P_2 position of the dipeptide inhibitor as well as the synthetic protocols which generate the fully unprotected boronic acid dipeptides. A series of prolineboronic acid (boroPro) containing dipeptides were synthesized and assayed for their ability to inhibit DP-IV (Table 2). Inhibitory activity, which required the (*R*)-stereoisomer of boroPro in the P_1 position, appeared to tolerate a variety of L-amino acids in the P_2 position; however, substitution at the P_2 position which was not tolerated included the unnatural amino acids and R,R-disubstituted amino acids. These data were consistent with what was known from substrate studies.[168,169] The time course of the assay allowed for some equilibration of the active species to its inactive cyclic form, prohibiting a true ranking of the binding ability of the inhibitors. Regardless, the net inhibition of DP-IV by these compounds demonstrated a potent effect which was found to be an excellent predictor of

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

Table 2. Structure–Activity Relationships of the Dipeptides vs DP-IV[a]



compound	R = amino acid[b]	IC_{50}, nM
79	L-Val	26
80	L-Ala	15
81	L-Abu	11
82	L-Ile	25
83	L-Tyr	32
84	L-Pro	20

[a]Shown are the most potent derivatives. [b]Abbreviations: Abu, 2-aminobutyric acid; Ile, ioleucine.

the in vitro effects of these boronic acid dipeptides on immune function.

The HIV-1 Tat (an inhibitor of Ag-specific responsiveness of human peripheral T cells),[170] and the competitive inhibitor of DP-IV, ProboroPro,[159] which both had K_i values in the nanomolar range for binding to DP-IV, were utilized to study the mechanism of DP-IV-mediated suppression of T cell activation. The study showed that blocking DP-IV with either Tat or ProboroPro down-regulated TT, *Candida*, or anti-CD3-mediated responses in PBMC, with no impact on proliferation in response to mitogens or the mitogen pair of anti-CD2 mAb $T11_2$ and $T11_3$. The reduced Ag responsiveness induced by Tat or ProboroPro was neutralized by the addition of soluble DP-IV, indicating that the defect was caused either by blocking DP-IV or by the specific enzymatic inactivation of DP-IV by these agents. Addition of these two agents to T cell cultures resulted in substantially increased inhibition of Ag-specific T cell proliferation. The induced responsiveness was overcome by supplementing the cultures with rIL-2, suggesting that the lack of a functional DP-IV molecule had a profound impact on lymphokine production. This was further substantiated by the finding that costimulation of human PBMC via the CD28 molecule, which initiated a non-TCR-dependent signaling pathway, overcame the reduced Ag responsiveness induced by Tat and ProboroPro. The fact that ProboroPro had no impact on stimulation of T cells with PMA and ionomycin implied that blocking DP-IV was influencing events before the activation of protein kinase C and Ca^{2+} flux. These results suggested that DP-IV was necessary for amplification of signals generated by the engagement of the TCR-CD3 complex by nominal Ag.[171]

Huber and co-workers have reported that inhibition of DPP7 activity induces apoptosis in quiescent lymphocytes.[172] It was found that that DPP7 has an almost absolute specificity for proline in the P_1 position with the second most preferred P_1 residue determined to be norleucine (Nle). Furthermore, DPP7 was shown to have a preference in order at the P_2 position for Lys, Nle, Met, Ala, and Pro. In contrast, Pro and Ala, in that order, were found to be preferred P_1 residues for DPP4. In addition, the DPP4 S_2 site was found tolerant of a wide variety of P_2 residues. Therefore, there was a distinction between the S_1 and the S_2 subsites of these DPPs.

Recently,[173] dipeptide-based inhibitors with C-substituted (alkyl or aminoalkyl) α-amino acids in the P_2 position and boro-norleucine (boroNle) in the P_1 position were synthesized. Relative to boroPro, boroNle as a P_1 residue was shown able to significantly dial out DPP4, FAP, DPP8, and DPP9 activity. Dab-boroNle (Dab: (*S*)-2,4-diaminobutanoic acid, **85**) (Figure 40) proved to be the most selective and potent DPP7 inhibitor with a DPP7 IC_{50} value of 480 pM.

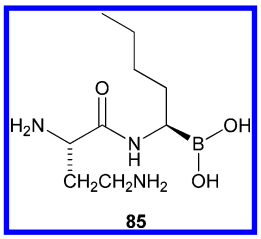


Figure 40. (*S*)-2,4-Diaminobutanoic acid is potent DPP7 inhibitor.

Fibroblast activation protein (FAB) is a type II integral membrane glycoprotein belonging to the serine protease family. It is a 95 kDa cell surface glycoprotein expressed by tumor stromal fibroblasts and has been shown to have dipeptidyl peptidase (DPP) and collagenase activity.[174,175] The catalytic site in human FAP contains the characteristic catalytic triad of Ser624, Asp702, His734, and the active serine is situated in a nucleophile elbow in the sequence Gly-Trp-Ser-Tyr-Gly.[176−178] This enzyme cleaves NH_2-terminal dipeptides from polypeptides with the sequence NH_2-Xaa-Pro/Ala, where Xaa can be any natural amino acid.[176,177,179] Fibroblast activation protein is well expressed by reactive stromal fibroblasts in >90% of human epithelial carcinomas. It was previously shown that forced fibroblast activation protein overexpression by tumor cells results in increased tumorigenicity and a significant enhancement in tumor growth.[178,179] Recently, these observations were extended by demonstrating that the enzymatic activity of fibroblast activation protein confers this tumor growth advantage. It was shown that enzymatic mutants of fibroblast activation protein that are devoid of fibroblast activation protein enzymatic activity, when xenografted into immunodeficient mice, resulted in attenuated tumor growth compared with the growth seen when tumors are transfected with wild-type fibroblast activation protein.

The dipeptidyl peptidase (DPP) inhibitor, PT-100 (Val-boroPro, **86**) (Figure 41), was shown to up-regulate gene

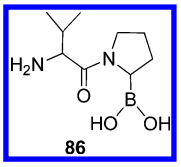


Figure 41. Structure of PT-100.

expression of certain cytokines in hematopoietic tissue via a high-affinity interaction, which appeared to involve fibroblast activation protein. PT-100 competitively inhibited the dipeptidyl peptidase (DPP) activity of FAP and there was a high-affinity interaction with the catalytic site due to the formation of a complex between Ser624 and the boron of PT-100.[180] PT-100, apparently through its interaction with FAP, could stimulate hematopoiesis via the increased production of cytokines both in vitro in stromal cell supported human bone marrow cultures and in vivo in mice.[167,180] The cytokines involved in the stimulation of hematopoiesis by PT-100 were also known to promote innate as well as T-cell-mediated antitumor responses. A subsequent study reported the potent antitumor effect of PT-100 in mice.[181] PT-100 abrogated tumor growth as a single agent and augmented antibody-dependent

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

cell-mediated cytotoxicity. The data suggested that via the up-regulation of cytokine and chemokine expression in both the tumor and the draining lymph nodes PT-100 could stimulate immune responses against tumors involving both the innate and adaptive branches of the immune system. Therefore, oral administration of PT-100 to mice slowed growth of syngeneic tumors derived from fibrosarcoma, lymphoma, melanoma, and mastocytoma cell lines. The treatment of mice with PT-100 resulted in tumor growth attenuation in a tumor model characterized by murine fibroblast activation protein expression in the surrounding tumor stromal fibroblasts (Figure 42).[182]

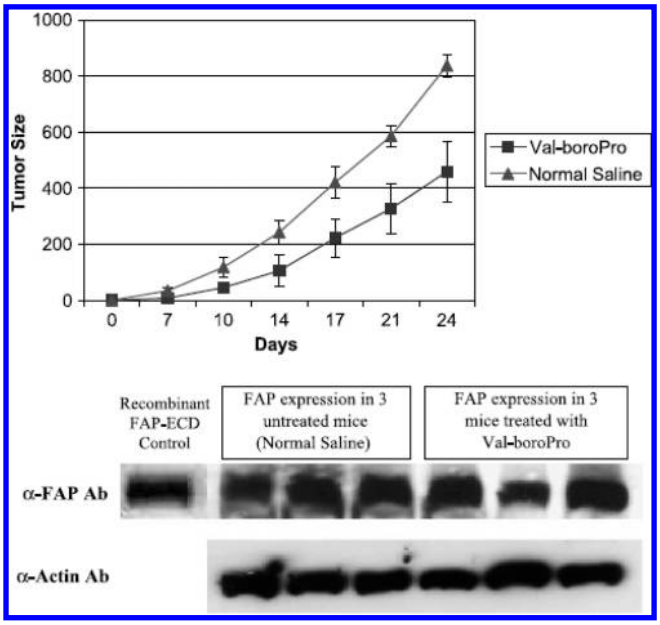


Figure 42. Attenuation of HT-29 xenograft tumor growth by small molecule inhibitor of fibroblast activation protein enzymatic activity. Tumor volumes of HT-29 colorectal cancer xenografts were assessed weekly in an animal model whereby fibroblast activation protein is induced in the mouse stroma as HT-29 cells themselves are negative for fibroblast activation protein expression. Tumor growth attenuation was seen in mice treated by gastric gavage with 50 μg of the fibroblast activation protein inhibitor Val-boroPro once daily for 21 consecutive days, P = 0.0312. Western analysis (bottom) of proteins extracted from HT-29 xenografts using rabbit polyclonal antifibroblast protein antibodies show similar levels of fibroblast activation protein expression in both treated and untreated tumors. A protein loading control as assessed by antiactin antibody was used. Reprinted with permission from ref 182. Copyright 2005 American Association for Cancer Research.

This established the proof-of-principle that the enzymatic activity of fibroblast activation protein played an important role in the promotion of tumor growth and that it was an attractive target for therapeutics designed to alter fibroblast activation protein-induced tumor growth by targeting its enzymatic activity.

As mentioned, FAB has been implicated in cancer; however, its specific role remains elusive because inhibitors that distinguish FAB from other prolyl peptidases like DPP-4 have not been developed. The dipeptidase inhibitor Val-boroPro that showed efficacy in tumor models[182,183] also inhibited DPP-4, -7, -8, and -9;[159,167,173] its mechanism of action remained unclear (Figure 43). To overcome this lack of selectivity, Edosada et al.[184a] sought to define the substrate specificity of FAB with the goal of identifying a FAB-selective motif for inhibitor design. To identify peptide motifs for FAB-selective inhibitor design, the P_2−Pro_1 and acetyl (Ac)-P_2−Pro_1 dipeptide substrate libraries have been used. P_2 was varied, and substrate hydrolysis occurred between Pro_1 and a fluorescent leaving group. With the P_2−Pro_1 library, FAB preferred Ile, Pro, or Arg at the P_2 residue; however, DPP-4 showed broad reactivity against this library, precluding selectivity. By contrast, with the (Ac)-P_2−Pro_1 library, FAB cleaved only Ac-Gly-Pro, whereas DPP-4 showed little reactivity with all substrates. FAB also cleaved formyl-, benzyloxycarbonyl-, biotinyl-, and peptidyl-Gly-Pro substrates, which DPP-4 cleaved poorly, suggesting an N-acyl-Gly-Pro motif for inhibitor design. Therefore, the compound Ac-Gly-boroPro (**87**) was synthesized and tested. This compound inhibited FAB with a K_i of 23 ± 3 nM, which is ∼9- to ∼5400-fold lower than the K_i values for other prolyl peptidases, including DPP-4, DPP-7, DPP-8, DPP-9, prolyl oligopeptidase, and acylpeptide hydrolase. These results identified Ac-Gly-boroPro as a FAB-selective inhibitor and suggested that *N*-acyl-Gly-Pro-based inhibitors will allow testing of FAB as a therapeutic target (Table 3).

Januvia is a selective DPP4 inhibitor approved by the FDA for the treatment of Type II diabetes. Dipeptide mimetics contain boroPro and are potent DPP4 inhibitors. They have the potential to be antidiabetic agents. While Val-boroPro proved to be a potent DPP4 inhibitor,[167] this compound is also in clinical trials for colorectal cancer as a fibroblast activation protein (FAP) inhibitor.[184b] The Gly-boroPro derivative DPP4 inhibitor PHX1149 (dutogliptin) is a low-molecular-weight, highly water-soluble, orally bioavailable selective DPP4 inhibitor, currently in phase III clinical trials. In phase I and II trials, PHX1149 was well tolerated up to 400 mg. The drug has a long half-life of 10−13 h and is well absorbed in humans.[184b]

2.7. IgA1 Protease

Immunity to bacterial infections in man involves many factors, one of which is antibacterial antibodies in both serum and secretions. IgA is the predominant form of antibody found in secretions bathing mucous membranes and in human milk and is widely regarded to comprise the first line of defense against infection.[151,185,186] Given this role of IgA in defending the sites of bacterial entry, it is significant that many species of human pathogens produce and release proteinases that cleave, with high specificity, a peptide bond in the hinge region of human IgA. Medically important IgA proteinase producing human pathogens include *Neisseria gonorrhoeae* and *Neisseria meningitidis*, which cause gonorrhea and meningitis, respectively; *Hemophilus influenzae* and *Streptococcus pneumoniae*, which cause bronchitis, pneumonia, otitis media, and meningitis; and *Streptococcus sanguis* and other *Streptococcus* and *Bacteroides* species which have been strongly implicated in periodontal diseases and dental cares.[187−190] The IgA proteinase-catalyzed cleavage of IgA separates the Fc from the antigen-binding Fab regions of the molecule. Such cleavage would be expected to impair or abolish its antimicrobial activity.[191] This fact coupled with the observation that non-IgA proteinase producing species of *Neisseria* and *Hemophilus* are not pathogenic, strongly implicates the IgA proteinases as a factor contributing to bacterial virulence. Researchers[192] reported that IgA proteinases may also cleave CD8, an important protein antigen found on the surface of certain T-cells. Thus, IgA proteinase-mediated virulence may involve attack on the cellular as well as on the

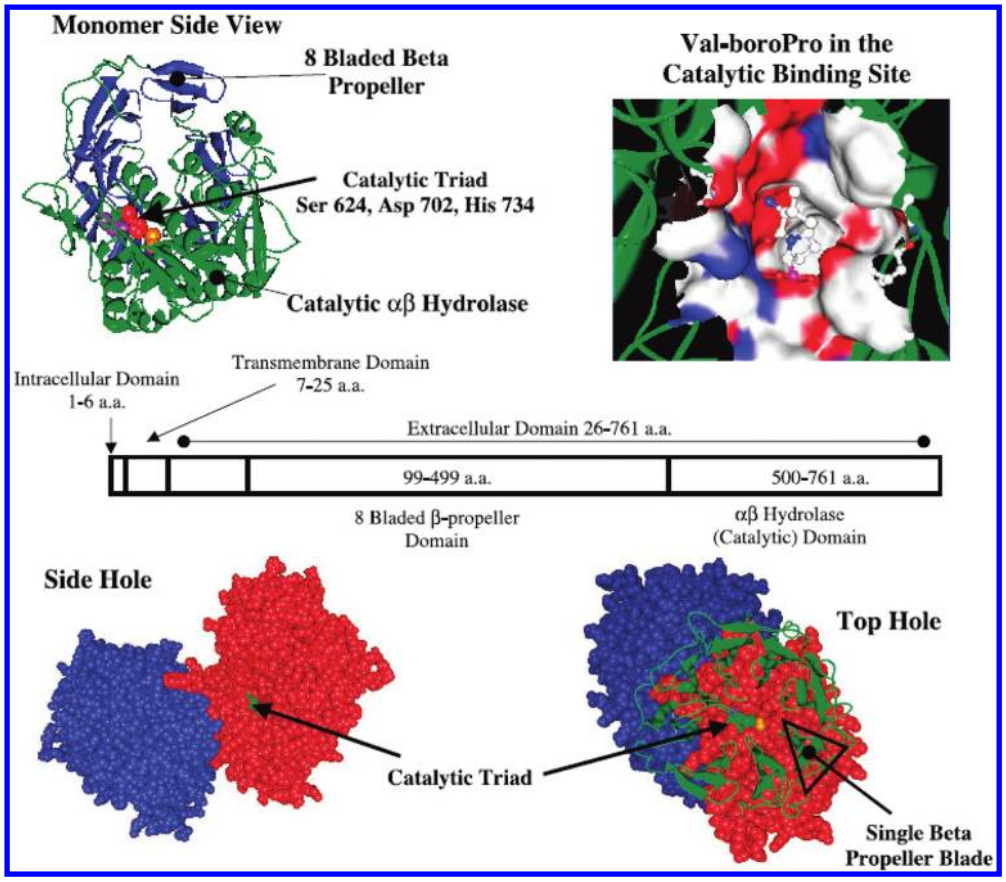
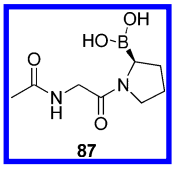


Figure 43. Predicted murine fibroblast activation protein structure. The modeled structure of fibroblast activation protein based on the crystal structure of DPPIV is hypothesized to consist of homodimers with a larger side hole of ~25 Å (bottom left, side hole), and a second opening in the h-propeller domain of ~10 Å (bottom right, top hole). The fibroblast activation protein inhibitor Val-boroPro is modeled interacting with Ser624 and His734 of the catalytic triad. Reprinted with permission from ref 182. Copyright 2005 American Association for Cancer Research.

Table 3. Inhibition of Prolyl Peptidases by Ac-Gly-boroPro[a]



87

protease	K_i (nM)	selectivity[b]
FAB	23	1
DPP-4	377	16.4
DPP-8	19100	830
DPP-9	8800	383
APH	575	25
POP	211	9.2
DPP-7	125000	5434

[a] K_i values were determined using the progress curves method. [b] Selectivity to FAB inhibition, K_i (protease)/K_i (FAB).

humoral immune system of the host. The IgA proteinases are therefore potentially valuable targets for antimicrobial agents. All IgA proteinases identified thus far cleave after a proline residue within the hinge region of human IgA.

Peptidyl prolyl boronic acids proved to be potent inhibitors of both the type 1 and type 2 IgA proteinases from *N. gonorrheae* and *H. influenzae*, but not of the functionally similar IgA proteinase from *S. sanguis*. The best inhibitors synthesized thus far, Ac-Ala-Pro-boroPro-OH, Boc-Ala-Pro-boroPro-OH, and MeOSuc-Ala-Ala-Pro-boroPro-OH, had K_i values in the nanomolar range (4.0−60 nM). These results indicated that the *N. gonorrheae* and the *H. influenzae* enzymes belong to the serine protease family of proteolytic enzymes while that from *S. sanguis* does not. As a group, the IgA proteinases have been noted for their remarkable specificity; thus, the peptidyl prolyl boronic acids reported were the first synthetic molecules to exhibit a relatively high affinity for the active site of an IgA proteinase and were therefore the first to yield some insight into the active site structure and specificity requirements of these enzymes.[158]

2.8. β-Lactamases

The bacterial β-lactamases catalyze the degradation of β-lactam antibiotics (such as penicillin and cephalosporin) through an incredibly efficient hydrolysis of the lactam bond, which lead to antibiotic resistance to the β-lactam family of antibiotics. With bacterial resistance to β-lactam antibiotics continuing to increase, identification of structural classes of β-lactamase inhibitors is of great clinical importance. TEM-1 is a representative member of the group 2b or class A β-lactamases that has achieved particular clinical notoriety.[193]

To combat these enzymes, agents that inhibit (e.g., clavulanic acid) or evade (e.g., aztreonam) β-lactamases have been developed. Both β-lactamase inhibitors and the β-lactamase-resistant antibiotics are themselves β-lactams, and bacteria have responded to these compounds by expressing variant enzymes resistant to inhibition (e.g, IRT-3) or that inactivate the β-lactamase-resistant antibiotic (e.g., TEM-10). Moreover, these

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156−4220

compounds have increased the frequency of bacteria with intrinsically resistant β-lactamases (e.g., AmpC). β-Lactamases resemble serine proteinases in the employment of an active-site serine residue, but differ in that the co-operating residues appear to be lysine and glutamic acid, rather than histidine and aspartic acid.[194] Again, β-lactamases resemble proteinases in catalyzing the hydrolysis of an amide bond, but differ in that the basic residue has to be deprotonated in proteinases but (apparently) protonated in β-lactamases.[195,196] Whereas deacylation is rate-limiting for good amide substrates of serine proteases, it is either rate limiting[195,196] or jointly rate limiting[197] for serine β-lactamases. These enzymes are expressed by a wide variety of bacteria. They have been grouped into four classes: A, B, C, and D, which differ from each other in mechanism, size, substrate preferences, and inhibition patterns.[198] Atomic resolution structures have been determined for enzymes from class A,[194,199−202] class C,[203,204] and class B;[205] the differences and similarities between these structures have been reviewed.[206,207]

Boronic acids β-lactamase inhibitors are transition-state analogues (Figure 44);[208−210] that is, the β-lactam recognition

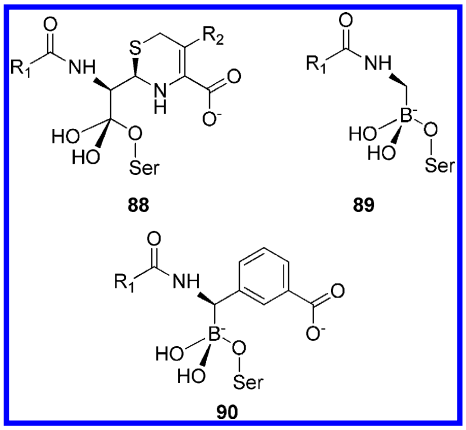


Figure 44. Comparison between the deacylation high energy intermediate of a cephalosporin in a serine β-lactamase (**88**), a transition-state analogue glycylboronic acid (**89**), and a transition-state analogue, *m*-carboxyphenyl-glycylboronic acid (**90**).

motif is replaced with a boronic acid, which makes a reversible, dative covalent bond with the active site serine residue forming a tetrahedral adduct (**88**, Figure 44). This replacement makes these inhibitors novel enough to evade many of the resistance mechanisms that jeopardize β-lactams.[211]

In 1978, Kiener and Waley found that boric acid, phenylboronic acid, and *m*-aminophenylboronic acid weakly inhibit a class A β-lactamase from *Bacillus cereus* (K_i range = 1.2−4.0 mM).[212] Later,[213] aromatic boronic acids (*ortho*-, *meta*-, and *para*-methyl-, hydroxymethyl- and formyl-phenylboronic acid) (Figure 45) were found to act as weak to moderate covalent, reversible inhibitors of class C β-lactamases from *Pseudomonas aeruginosa* and *E. coli* (K_i range = 2.4−920.0 μM). One of the more potent compounds reported, 3-iodoacetamidophenyl-boronic acid (K_i (*P. aeruginosa* β-lactamase) = 2.4 μM, K_i (*E. coli* AmpC) = 23 μM), was synergetic with cephalosporin C against *P. aeruginosa*, albeit at high (K_i = 1.64 mM and K_i = 0.5 mg/mL, respectively) concentrations.

The boronic acids that have hitherto been studied as β-lactamase inhibitors owe their affinity largely to the boronic

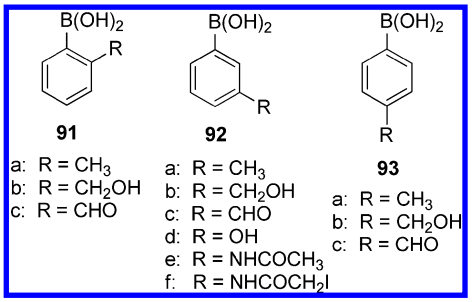


Figure 45. *Ortho*-, *meta*-, and *para*-methyl-, hydroxymethyl- and formyl-phenylboronic acid.

acid group, and lack any structural resemblance to the substrates of β-lactamases such as an appropriate side chain. Researchers[208] synthesized phenylacetamidomethaneboronic acid ($Ph-CH_2-CONH-CH_2-B(OH)_2$) and the related compounds trifluoroacetamidomethaneboronic acid ($CF_3-CH_2-CONH-CH_2-B(OH)_2$) and 2,6-dimethoxybenzamidomethaneboronic acid ($2,6-(CH_3O)_2C_6H_3-CONH-CH_2-B(OH)_2$) and reported their interaction with the class A β-lactamase I from *Bacillus cereus*. The first of these contains the side-chain moiety of penicillin G, and the last that of methicillin. The use of low temperature techniques, or rapid reaction methods at ordinary temperatures has provided evidence for a two-step mechanism of binding of the first two boronic acids to β-lactamase I, and for benzeneboronic acid to a β-lactamase from *Pseudomonas aeruginosa*. The slower step was probably associated with a change in enzyme conformation as well as the formation of an O−B bond between the active-site serine hydroxyl group and the boronic acid.

In another study to characterize the mechanism of the class C β-lactamases, the X-ray crystal structure of AmpC β-lactamase from *E. coli*, alone and in the presence of *m*-aminophenylboronic acid (MAPB) has been determined.[214] The X-ray crystal structure of the MAPB-AmpC complex showed that this compound formed a bond with the catalytic Ser64 of AmpC, consistent with the proposed mechanism of inhibition (Figure 46). However, MAPB made several

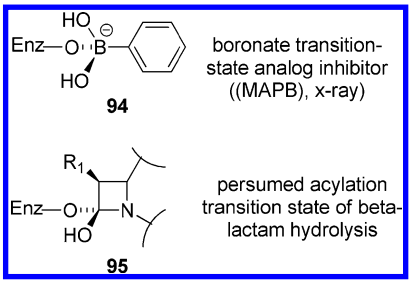


Figure 46. Comparison of a boronic acid-based transition-state analogue inhibitor (top) and a typical β-lactam antibiotic (bottom) in complex with a β-lactamase.

unexpected interactions and the most surprising was the observed interaction between the O1 oxygen of the boronic acid adduct and the carbonyl main-chain oxygen of Ala318. Such an interaction was observed in most other β-lactamase−inhibitor complexes. This interaction allowed for the possibility that in the tetrahedral high energy intermediates along the hydrolytic pathway what has until now been considered to be a substrate oxyanion may in fact be protonated. This crystallographic structure was used to suggest modifications that might

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

enhance the affinity of boronic acid-based inhibitors for class C β-lactamases.[39e] Several types of compounds were modeled into the AmpC binding site, and a total of 37 boronic acids were ultimately tested for β-lactamase inhibition. The most potent of these compounds, benzo[b]thiophene-2-boronic acid (BZBTH2B) (**99**, Figure 47), had an affinity for *Escherichia*

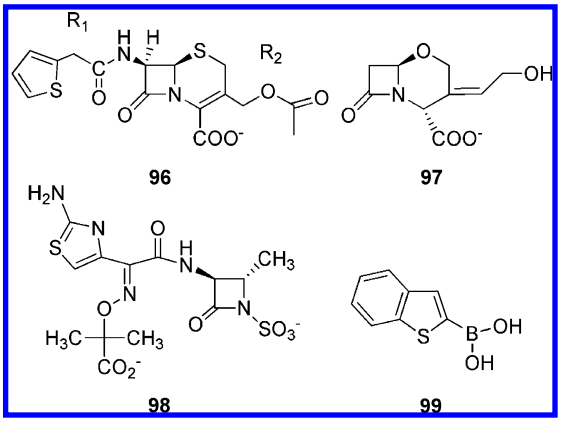


Figure 47. Comparison of β-lactamase ligands. (**96**): Cephalothin, a cephalosporin substrate. The R_1 and R_2 side chains are labeled. (**97**): Clavulanic acid, a clinically used β-lactamase inhibitor. (**98**): Aztreonam, a "β-lactamase stable" molecule. (**99**): benzo(b)-thiophene-2-boronic acid.

coli AmpC of 27 nM. This inhibitor was 100-fold more potent than boronic acid inhibitors previously described for this enzyme and was specific for AmpC over mechanistically related enzymes. BZBTH2B did not resemble β-lactams (Figure 47), but nevertheless inhibited AmpC β-lactamase potently and specifically. The X-ray crystallographic structure of BZBTH2B in complex with AmpC was determined to 2.25 Å resolution (Figure 48). The inhibitor appeared to complement the

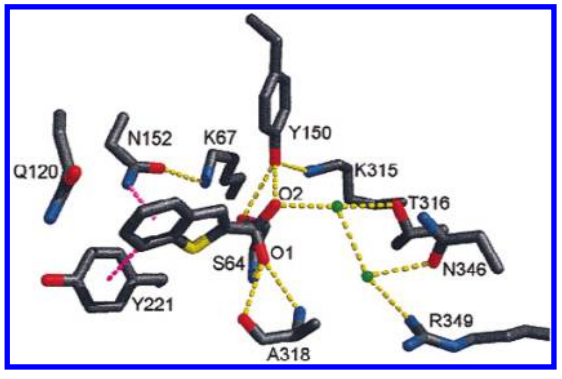


Figure 48. Key interactions observed in the structure of BZBTH2B in complex with AmpC. Dashed lines indicate hydrogen bonding (yellow) or quadrupole interactions (magneta). Green spheres represent ordered water molecules. The atoms are colored. The figure was generated with MidasPlus. Reprinted with permission from ref 215. Copyright 1999 Wiley.

observed, R1-amide binding region of AmpC, despite lacking an amide group. Interactions between one of the boronic acid oxygen atoms, Tyr150, and an ordered water molecule suggested a mechanism for acid/base catalysis and a direction for hydrolytic attack in the enzyme catalyzed reaction. In the antimicrobial assays, BZBTH2B significantly potentiated the activity of a third-generation cephalosporin against AmpC-producing resistant bacteria. This inhibitor was unaffected by two different mechanisms that often arose against β-lactams in conjugation with β-lactamases. Porin channel mutations did not decrease the efficacy of BZBTH2B against cells expressing AmpC. Also, this inhibitor did not induce expression of AmpC, a problem with many β-lactams. The structure of the BZBTH2B/AmpC complex provided a starting point for the structure-based elaboration of this class of non-β-lactam inhibitors.[215]

TEM-1 is a representative member of the group 2b or class A β-lactamases that has achieved particular clinical notoriety.[199] On the basis of the crystallographic structure of acyl-enzyme intermediate of TEM-1 bound to a substrate, penicillin G,[216] (1*R*)-1-acetamido-2-(3-carboxyphenyl)ethane boronic acid (**100**, Figure 49) was designed to mimic the critical interactions

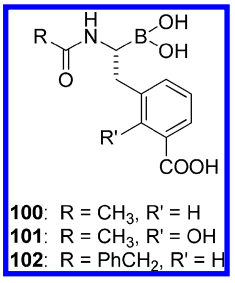


Figure 49. The boronate inhibitors of TEM-1 β-lactamase: (**100**, K_i = 110 nM), (**101**, K_i = 13 nM), (**102**, K_i = 5.9 nM).

observed in the penicillin G/TEM-1 complex and was found to be a potent TEM-1 inhibitor. The structure of the β-lactamase TEM-1 has been solved in a complex with boronic acid (**100**, Figure 50) at 1.7 Å resolution, which suggested a novel transition state of the deacylation step in the β-lactamase-catalyzed reaction pathway. Using the same strategy, further structure-guided incorporation of additional side chain functionalities (**101**, Figure 49) or hydrogen bonding groups (**102**, Figure 49) to maximize energetically favorable interactions of the boronate inhibitors and TEM-1 β-lactamase was investigated.[217] As designed, compounds **101** and **102** were highly effective deacylation transition state analogue inhibitors. The high-resolution crystallographic structures of these two inhibitors (Figure 50) covalently bound to TEM-1 showed interesting and unanticipated changes in the active site area, including strong hydrogen bond formation, water displacement, and rearrangement of side chains, which provided new insights for the further design of this class of inhibitors.

In continuing efforts to understand how β-lactamases recognize their substrates, it was helpful to know their binding energies. Eight acylglycineboronic acids that bear the side chains of characteristic penicillins and cephalosporins, as well as four other analogues, were synthesized to investigate the contribution to interaction energy of the key amide (R1) side chain of β-lactam antibiotics (Figure 51). These transition-state analogues form reversible adducts with serine β-lactamases. The binding energies were calculated directly from K_i values. The K_i values measured span 4 orders of magnitude against the Group I β-lactamase AmpC and 3 orders of magnitude against the Group II β-lactamase TEM-1. The acylglycineboronic acids have K_i values as low as 20 nM against AmpC and as low as 390 nM against TEM-1. R1 side chains characteristic of β-lactam inhibitors did not have better affinity for AmpC than did side chains characteristic of β-lactam substrates. Two of the

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

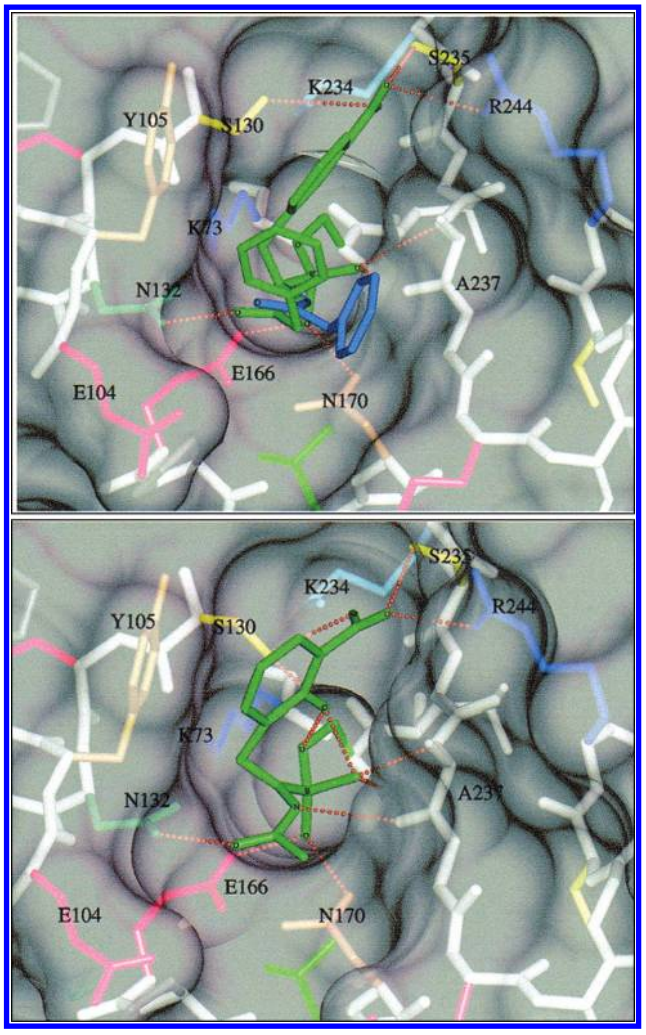


Figure 50. Combined transparent molecular surface and ball-and-stick representation of the active site of TEM-1 β-lactamase in complex with (up) (1*R*)-1-phenylacetamido-2-(3-carboxyphenyl)ethylboronic acid, and (down) (1*R*)-1-acetamido-2-(3-carboxyhyfroxyphenyl)-ethylboronic acid. The alternate conformation of the phenylacetamido group in (1*R*)-1-phenylacetamido-2-(3-carboxyphenyl)ethylboronic acid is shown in blue (up). Hydrogen bonds are shown as dotted lines. Main chain atoms are white, side chain atoms are colored differently according to type, and the substrate or inhibitor atoms are shown in green. The figure was generated with the software package PREPI (http://bonsai.lif.icnet.uk/people/-suhail/prepi.html). Reprinted with permission from ref 217. Copyright 2000 American Chemical Society.

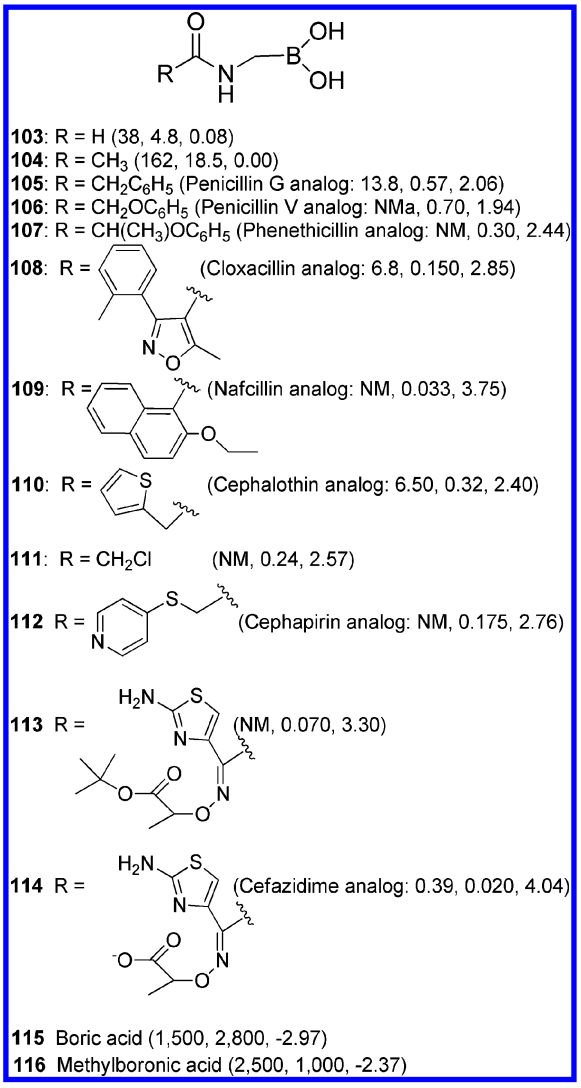


Figure 51. K_i (μM) values of acylglycineboronic acids against AmpC and TEM-1, the paranthesis include (β-lactam analogue, K_i (μM) vs TEM-1, K_i (μM) vs AmpC, $\Delta\Delta G^a$ from compound **104** (kcal/mol). [a]NM, not measured. [a]Differential free energy of binding relative to compound **104** at 298 K. Positive values indicate improved affinities.

inhibitors reversed the resistance of pathogenic bacteria to β-lactams in cell culture. Structures of two inhibitors in their complexes with AmpC were determined by X-ray crystallography to 1.90 Å and 1.75 Å resolution; these structures suggested interactions that are important to the affinity of the inhibitors (Figures 52 and 53). Therefore, acylglycineboronic acids allowed researchers to begin to dissect interaction energies between L-lactam side chains and L-lactamases. Surprisingly, there was little correlation between the affinity contributed by R1 side chains and their occurrence in β-lactam inhibitors or β-lactam substrates of serine L-lactamases.[211]

In continuing research to facilitate inhibitor design for the class C β-lactamase AmpC, binding site "hot spots" on the enzyme were identified using experimental and computational approaches. Experimentally, X-ray crystal structures of AmpC in complexes with four boronic acid inhibitors (Figure 54) and a higher resolution (1.72 Å) native apo structure were determined. Along with previously determined structures of AmpC in complexes with five other boronic acid inhibitors and four β-lactams, consensus binding sites were identified. Computationally, the programs GRID,[219] MCSS,[220] and X-SITE[221] were used to predict potential binding site hot spots on AmpC. Several consensus binding sites were identified from the crystal structures (Figure 55). An amide recognition site was identified by the interaction between the carbonyl oxygen in the R1 side chain of β-lactams and the atom Nδ2 of the conserved Asn152. Surprisingly, this site also recognized the aryl rings of arylboronic acids, appearing to form quadrupole–dipole interactions with Asn152. The highly conserved "oxyanion" hole defined a site that recognized both carbonyl and hydroxyl groups. A hydroxyl binding site was identified by the O2 hydroxyl in the boronic acids, which hydrogen bonds with Tyr150 and conserved water. A hydrophobic site was formed by Leu119 and Leu293. A carboxylate binding site was identified by the ubiquitous C3(4) carboxylate of the β-lactams,

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

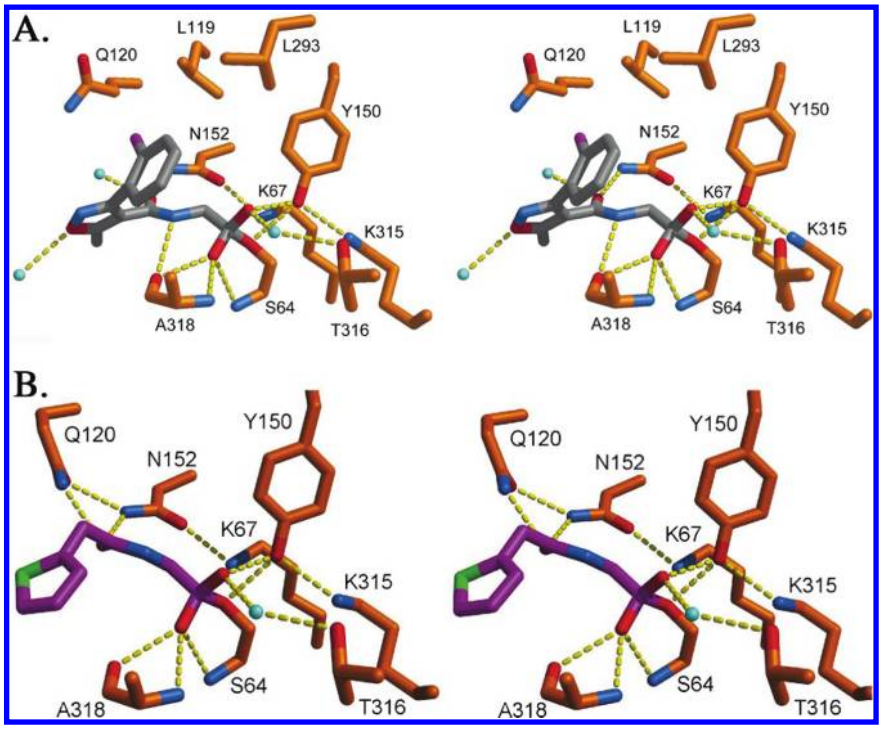


Figure 52. Key polar interactions observed between AmpC and (A) cloxacillin analogue and (B) cephalothin analogue. Dashed yellow lines indicate hydrogen bonds. Carbon atoms are colored orange, oxygen atoms red, nitrogen atoms blue, sulfur atoms green, chlorine atoms magenta. The carbon atoms and the boron atom in the inhibitors are colored gray in (A) and magenta in (B). Cyan spheres represent water molecules. These figures were generated with MidasPlus.[218] Reprinted with permission from ref 211. Copyright 2000 Elsevier.

which interacted with Asn346 and Arg349. Four water sites were identified by ordered waters observed in most of the structures; these waters form extensive hydrogen-bonding networks with AmpC and occasionally the ligand. Predictions by the computational programs showed some correlation with the experimentally observed binding sites. Several sites were not predicted, but novel binding sites were suggested. Taken together, a map of binding site hot spots found on AmpC, along with information on the functionality recognized at each site, was constructed. This map was useful for structure-based inhibitor design against AmpC.[222]

The glycylboronic acids resembled half of the β-lactam molecule, bearing the R1 side chain of substrates but lacking recognition elements corresponding to the thiazolidone or dihydrothiazine rings of penicillins or cephalosporins, respectively. The absence of a negatively charged group in a position corresponding to the C4′ position of the dihydrothiazine ring seemed particularly noteworthy. All β-lactams bear a carboxylic or sulfonic acid at this position. In class A β-lactamases, this group is a key recognition element.[210,217,223] In class C β-lactamases, the role of this group is less understood. Mutant and substrate analysis suggested that such a charged group is not key for recognition.[223] Conversely, structural analyses of the binding determinants ("hot spots") of AmpC suggested that there is a carboxylate binding site on the enzyme.[224] The contribution to binding energy that such a carboxylate might make, if any, remains in doubt, owing to the irreversible binding of β-lactams to β-lactamases, preventing equilibrium-based thermodynamic analyses.

In an effort to combat β-lactamases, a combination of stereoselective organic synthesis, enzymology, microbiology, and X-ray crystallography was used to design and evaluate new carboxyphenyl−glycylboronic acid transition-state analogue inhibitors of the class C β-lactamase AmpC.[225] The new compounds improved inhibition by over 2 orders of magnitude compared to analogous glycylboronic acids, with K_i values as low as 1 nM (Figure 56). On the basis of the differential binding of different analogues, the introduced carboxylate alone contributed about 2.1 kcal/mol, in affinity. This carboxylate corresponded to the ubiquitous C3(4)′ carboxylate of β-lactams, and this energy represented the first thermodynamic measurement of the importance of this group in molecular recognition by class C β-lactamases. The structures of AmpC in complex with two of these inhibitors were determined by X-ray crystallography at 1.72 and 1.83 Å resolution. These structures suggested a structural basis for the high affinity of the new compounds and provided templates for further design. The highest affinity inhibitor was 5 orders of magnitude more selective for AmpC than for characteristic serine proteases, such as chymotrypsin. This inhibitor reversed the resistance of clinical pathogens to the third generation cephalosporin ceftazidime.[225]

In another study, Wang et al.[226] tested transition state analogues, of greater or lesser similarity to substrates for inhibition of TEM-1 β-lactamase, the most widespread resistance enzyme to penicillin antibiotics and against four characteristic mutant enzymes: TEM-30, TEM-32, TEM-52, and TEM-64. The inhibitor most similar to the substrate (**135**, Figure 57) was the most potent inhibitor of the WT enzyme, with a K_i value of 64 nM. Conversely this inhibitor was the most potent susceptible to the TEM-30 (R244S) mutant, for which inhibition dropped by over 100-fold. The other inhibitors were relatively impervious to the TEM-30 mutant enzyme. The structures of four of these inhibitors in complex with TEM-1 were determined by X-ray crystallography. These structures suggested a structural basis for distinguishing

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

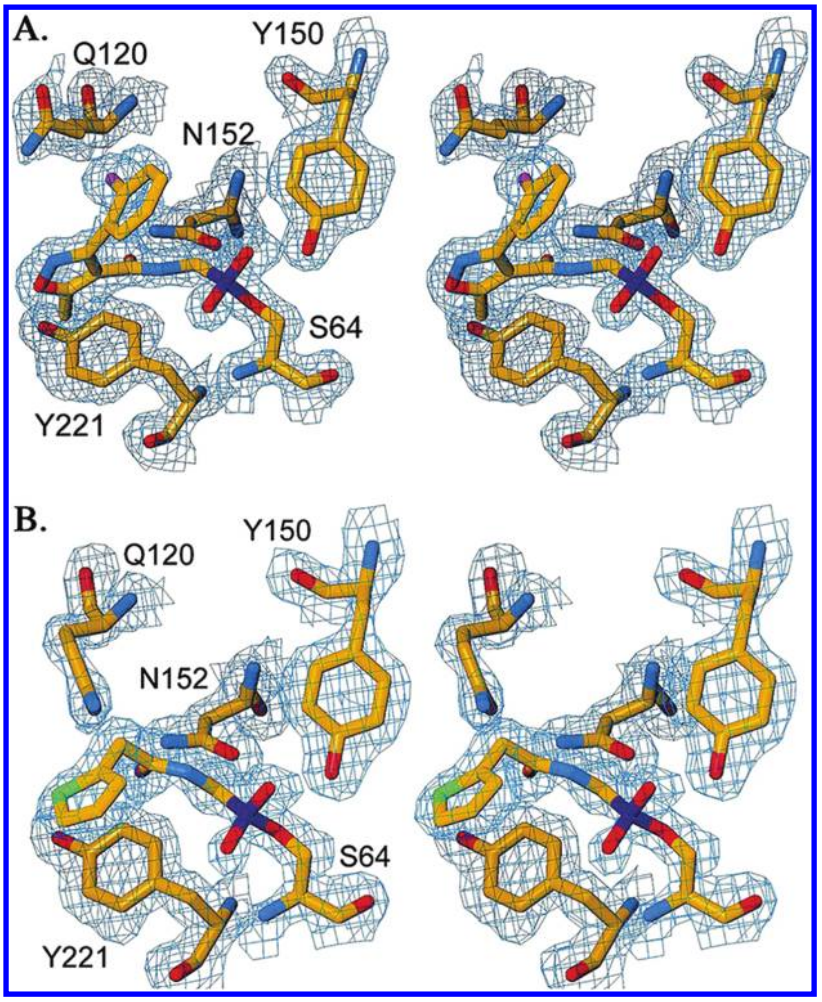


Figure 53. Stereoview of 2Fo3Fc electron density of the refined models for AmpC complexes of (A) compound **108** and (B) compound **110**. The density is contoured at 1s. Carbon atoms are colored orange, oxygen atoms red, nitrogen atoms blue, sulfur atoms green, chlorine atoms magenta, and boron atoms purple. Reprinted with permission from ref 211. Copyright 2000 Elsevier.

inhibitors that mimic the acylation transition state and those that mimic the deacylation transition state; they also suggested how TEM-30 reduces the affinity of the inhibitor (Figure 57). In cell culture, this inhibitor reversed the resistance of bacteria to ampicillin, reducing minimum inhibitory concentrations of this penicillin by between 4- and 64-fold, depending on the strain of bacteria. Notwithstanding this activity, the resistance of TEM-30, which is already extant in the clinic, suggested that there can be resistance liabilities with substrate-based design.

Later, Tafi et al.[227] embarked on advanced modeling of the inhibition of TEM-1 and of other serine β-lactamases by alkyl and aryl boronic acids, employing the software MacroModel[228] implemented with the AMBER* united atom force field.[229,230] X-ray crystal structures showed that boronic acids inhibit β-lactamases by the formation of tetrahedral adducts, analogous to the deacylation tetrahedral intermediate of these enzymes.[217,224] Similarly to other authors,[39e] therefore, Tafi et al. focused their attention on the interactions between inhibitors and enzymes as tetrahedral adducts formed after catalytic serine Oγ coordination, neglecting, for now, the influence of the recognition step, leading to the formation of the Michaelis−Menten complex.[227]

Recently, Chen et al.[231] investigated recognition of two classes of boronic acids and the β-lactam cefoxitin, each of which is known to inhibit classical class A β-lactamases, such as TEM-1. Glycylboronic acid bearing the R1 side chain of cephalosporins and penicillins, such as compounds **136** and **137** (Figure 58), bind to the class A β-lactamase TEM-1 as acylation transition-state analogues (step 2, Figure 59). Chiral glycylboronic acids, such as compound (**140**, Figure 58), bind to TEM as deacylation trasition state analogues (step 4, Figure 59). These boronic acids are especially useful for probing recognition in that they are reversible inhibitors, forming fast-on/fast-off Lewis acid adducts with serine β-lactamases, allowing K_i values to be converted into thermodynamic binding affinities. Cefoxitin, on the other hand, is an irreversible inhibitor of class A β-lactamases, but its enzyme adduct is long-lived enough to probe using reversible enzyme denaturation, which also may be interpreted thermodynamically (Table 4).

CTX-M enzymes (cefotaximases of the family, CTX-M-9 and CTX-M-14) are an emerging group of extended spectrum β-lactamases (ESBLs) that hydrolyze not only the penicillins but also the first-, second-, and third-generation cephalosporins. Researchers have described the X-ray crystal structures of CTX-M enzymes in complex with two glycylboronic acids, the β-lactam inhibitor cefoxitin and a chiral glycylboronic acid, representing the enzyme as it progresses from its acylation transition state to its acyl enzyme complex to the deacylation transition state. The structures, determined between 1.16 and 1.7 Å resolution, reveal conformational changes that active site

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

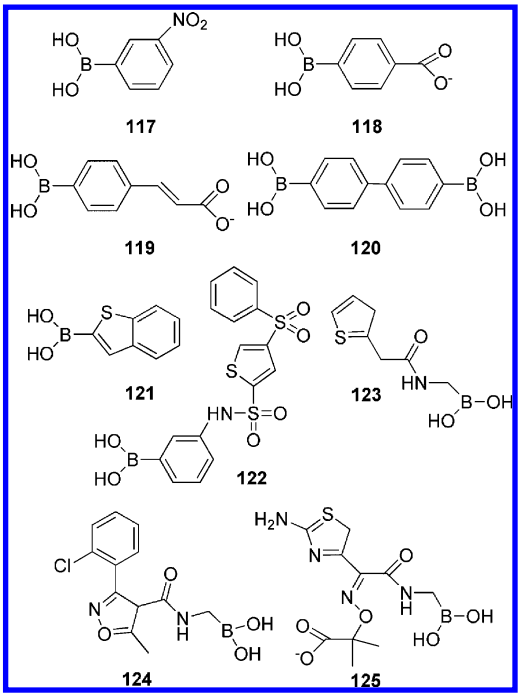


Figure 54. Ligands used to probe the active site of AmpC: (**117**, K_i = 1.7 μM), (**118**, K_i = 2.9 μM), (**119**, K_i = 4.2 μM), (**120**, K_i = 0.20 μM), (**121**, K_i = 0.027 μM), (**122**, K_i = 0.08 μM), (**123**, K_i = 0.15 μM), (**124**, K_i = 0.32 μM), and (**125**, K_i = 0.20 μM).

residue undergo along the reaction coordinate. As the enzyme moves along this reaction coordinate, two key catalytic residues, Lys73 and Glu166, change conformations, tracking the state of the reaction. Unexpectedly, the acyl enzyme complex with the β-lactam inhibitor cefoxitin still has the catalytic water bound; this water has been predicted to be displaced by the usual 7α-methoxy of the inhibitor. Instead, the 7α-group appears to inhibit by preventing the formation of the deacylation transition state through steric hindrance. From an inhibitor design standpoint, Chen et al.[231] noted that the best of the reversible inhibitors, a caftazidime-like boronic acid compound, binds to CTX-M-16 with a K_i value of 4 nM. When used together in cell culture, this inhibitor reversed cefotaxime resistance in CTX-M-producing bacteria. The structure of its complex with CTX-M enzyme and the structural view of the reaction coordinate described provide templates for inhibitor design and intervention to combat this family of antibiotic resistance enzymes.

Boronic acid compounds are virtually the only active-site-directed serine-type β-lactamase inhibitors that are not based on a β-lactam structure.[208] The inhibitory activity and the kinetic studies have shown that different boronic acid compounds reversibly and rapidly inhibit class C AmpC enzymes,[208] several class A β-lactamases, such as the chromosomal penicillinase of *Bacillus cereus*,[208,212] and some of the CTX-M-type ESBLs.[231] From an inhibitor design standpoint, the observation that a glycylboronic acid compound showed not only inhibition of AmpCs (K_i of 20 nM) but also an unexpected potent inhibition of the CTX-M-16 ESBL (K_i of 4 nM)[231] was intriguing, as compounds with good activity across β-lactamase classes are very rare and could have significant implications in designing global inhibitors for treating infections due to bacteria producing significant β-lactamases.

2.9. Carboxypeptidases

Penicillin-binding proteins (PBPs), which are lethal targets of β-lactam antibiotics, are ubiquitous bacterial enzymes that catalyze the final steps in cell wall biosynthesis.[232−234] PBP 5 from *E. coli* is a well-characterized D-alanine carboxypeptidase that serves as a prototypical enzyme to elucidate the structure, function, and catalytic mechanism of PBPs. Bacteria have multiple PBPs,[235] and these can be grouped into two classes: the high molecular mass (HMM) PBPs, which are essential for cell viability and catalyze transpeptidation and sometimes transglycosylation of dissacharide-pentapeptide chains during peptidoglycan synthesis, and low molecular mass (LMM) PBPs, which are not essential for cell viability and catalyze carboxypeptidase (CPase), transpeptidase, and/or endopeptidases reactions.[236] PBPs catalyze a two-step reaction: acylation, in which an active-site serine nucleophile on the PBP attacks the penultimate D-Ala residue to form an acyl−enzyme complex with the peptide chain, releasing the C-terminal D-Ala, and deacylation, which varies depending on the PBP. For HMM PBPs, deacylation occurs when an amino group on a second peptide substrate acts as an acceptor, resulting in a peptide cross-link between two adjacent peptidoglycan strands (transpeptidation). However, in most LMM PBPs, the acceptor is a water molecule and the outcome is hydrolysis (carboxypeptidation).

Boronyl compounds have proven to be very effective transition-state analogue of β-lactamases,[208,213,215,217,225] and the mechanistic similarity of these enzymes with PBPs suggested that a similar approach may be also effective with the PBPs.

Several potential transition state analogue inhibitors for the PBPs have been synthesized, including boronic acids. These agents were characterized chemically, stereochemically, and as inhibitors of a set of low molecular mass PBPs: *E. coli* (EC) PBP 5, *N. gonorrheae* (NG) PBP 3 and NG PBP 4. A peptidyl boronic acid was the most effective PBP inhibitor in the series, with a preference observed for a D-boroAla-based over an L-boroAla-based inhibitor, as expected given that physiological PBP substrates were based on D-Ala at the cleavage site. The lowest K_i of 370 nM was obtained for NG PBP 3 inhibition by Boc-L-Lys(Cbz)-D-boroAla. Competitive inhibition was observed for this enzyme−inhibitor pair, as expected for an active site-directed inhibitor. For the three PBPs included, an inverse correlation was observed between the values for log K_i with Boc-L-Lys(Cbz)-D-boroAla and the values for log(k_{cat}/K_m) for activity against the analogous substrate, and K_m/K_i ratios were 90, 1900, and 9600 for NG PBP 4, EC PBP 5, and NG PBP 3, respectively. These results demonstrated that peptidyl boronic acids could be effective transition state analogue inhibitors for the PBPs and provided a basis for the use of these agents as probes of PBP structure, function, and mechanism, as well as a possible basis for the development of new PBP-targeted antibacterial agents.[237]

In continuing studies, comprehensive understanding of the catalytic mechanism underlying D-alanine carboxypeptidation and antibiotic binding has proven elusive. Nicola et al.[238] reported recently the crystal structure at 1.6 Å resolution of PBP 5 in complex with a substrate-like peptidyl boronic acid (**145**, Figure 60), which was designed to resemble the transition state-intermediate during the deacylation step of the enzyme-catalyzed reaction with peptide substrates. In the structure of the complex, the boron atom was covalently attached to Ser44, which in turn was within hydrogen-bonding distance to Lys47.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

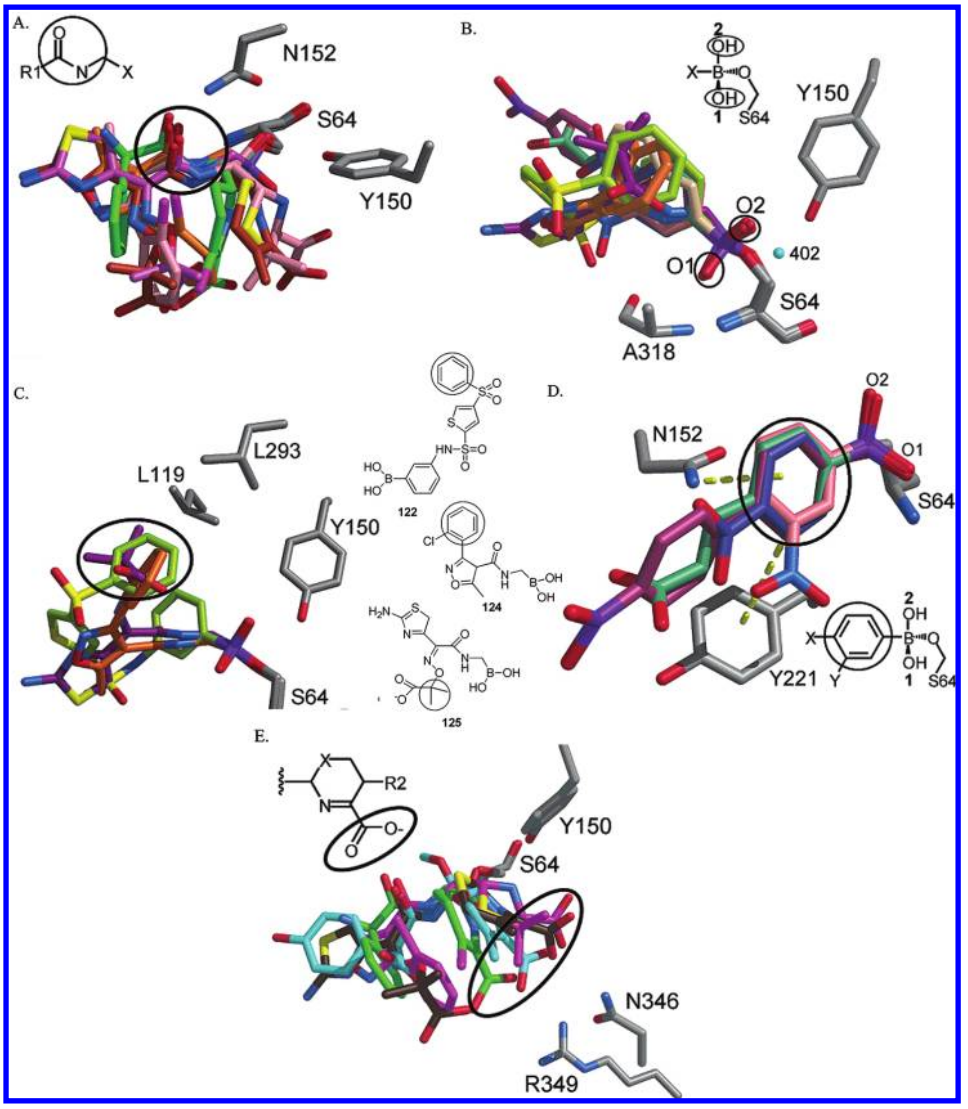


Figure 55. Binding sites identified experimentally on AmpC. (A) An amide binding site identified by superimposing all amide containing compounds from Figure 54. The amide group is circled in black. (B) Two hydroxyl binding sites identified by superimposing all boronic acid inhibitors. The hydroxyl sites are circled and labeled O1 and O2. (C) A hydrophobic binding site identified by superimposing the structures of **122**, **124**, and **125**. The hydrophobic binding site is composed of residues Leu119 and Leu293, and the hydrophobic portions of the compounds interacting with these residues are circled. (D) An aryl binding site identified by superimposing the structures of compounds **117**−**120**. Dashed yellow lines indicate the quadrupole interactions with Asn152 and Tyr221. (E) The carboxylate binding site identified by superimposing the structures of the *β*-lactams. The C3(4) carboxylate group is circled. Nitrogen atoms are colored blue, oxygens are colored red, sulfurs are colored yellow, chlorines are colored magenta, borons are colored purple, and carbons are colored gray, except for carbon atoms of the ligands, which are colored as follows: coral for **117**, indigo for **118**, sea green for **119**, violet for **120**, tan for **121**, lime green for **122**, orange for **123**, gold for **124**, magenta for **125**. Reprinted with permission from ref 222. Copyright 2002 American Chemical Society.

This arrangement further supported the assignment of Lys47 as the general base that activates Ser44 during acylation. One of the two hydroxyls in the boronyl center (O2) was held by the oxyanion hole comprising the amides of Ser44 and His216, while the other hydroxyl (O3), which was analogous to the nucleophilic water for hydrolysis of the acyl−enzyme intermediate, was solvated by a water molecule that bridges to Ser110. Lys47 was not well positioned to act as the catalytic base in the deacylation reaction. Instead, these data suggest a mechanism of catalysis for deacylation that uses a hydrogen-bonding network, involving Lys213, Ser110, and a bridging water molecule, to polarize the hydrolytic water molecule.

2.10. Lipases

Type 2 diabetes including the metabolic syndrome is a chronic multifactorial metabolic disease. One of the characteristics of the disease is elevated levels of fatty acids (FAs) in the circulation, which is widely considered a major pathogenetic factor. It has been shown that elevated FA levels are associated with peripheral insulin resistance, decreased glucose uptake by muscle tissue, and increased hepatic triglyceride (TG) release to the circulation.[239−241]

Hormone-sensitive lipase (HSL) is a multifunctional tissue lipase that plays a central role in the overall energy homeostasis as it is the rate limiting enzyme in the release of FA from adipocytes.[242] HSL catalyzes the first and second step in the breakdown of triglycerides, releasing two molecules of FA and monoacylglycerol, which is further hydrolyzed by monoacyl-

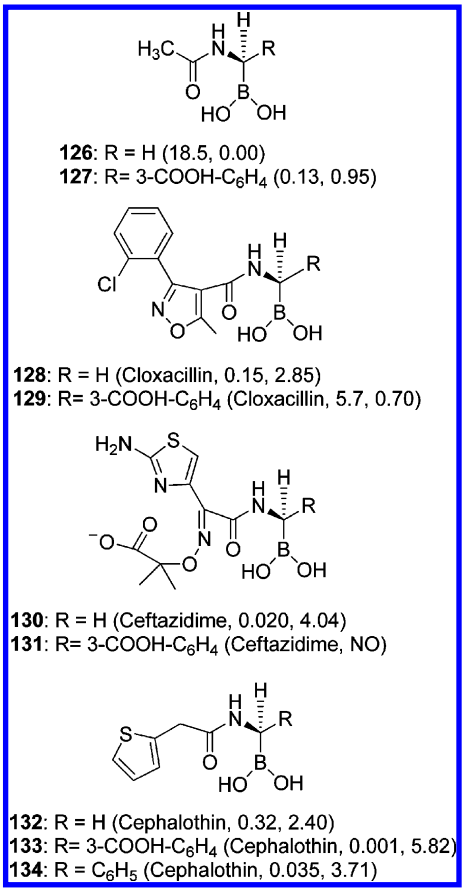


Figure 56. The values indicate the β-lactam analogue, K_i (μM), and $\Delta\Delta G$ from **126** (kcal/mol)). NO, not obtained.

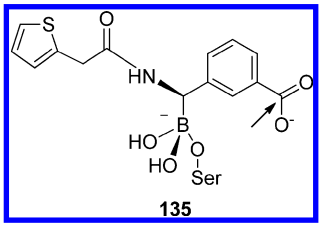


Figure 57. The deacylation transition-state analogue of the β-lactamase inhibitor. The *m*-carboxylate is identified by an arrow.

glycerol lipase.[243] It has broad substrate specificity as it catalyzes the hydrolysis of both cholesteryl esters and acylglycerides.[244] The activity of HSL is regulated via phosphorylation/dephosphorylation primarily controlled by catecholamines and insulin.[242] The enzyme belongs to a class of hydrolases that adapt the α/β hydrolase fold and contains a catalytic triad of serine, histidine and aspartic acid and an oxyanion hole.[245] Because of the disregulated metabolism of type 2 diabetic patients and the elevated level of FA, the interest in targeting HSL with the aim of reducing insulin resistance and dyslipidemia in obese, prediabetic, and diabetic individuals has recently gained considerable interest.

Reversible hydrolase inhibitors such as boronic acids have been used in the development of enzyme inhibitors of lipases.[246] Recently, a range of substituted phenyl and thiopheneboronic acids have been identified as hormone-sensitive lipase inhibitors. (2-Benzyloxy-5-fluorophenyl)boronic acid (**146**), (2-benzyloxy-5-chlorophenyl)-boronic acid (**147**) and 5-bromothiophene-2-boronic acid (**148**) (Figure 61) were found to be the most potent HSL inhibitors with IC_{50} values of 140, 17, and 350 nM, respectively. The two HSL inhibitors (2-benzyloxy-5-fluorophenyl)boronic acid and 5-bromo-thiophene-2-boronic acid were also tested for hepatic lipase (HL), lipoprotein lipase (LPL) and pancreatic lipase (PL) activity. None of the compounds inhibited HL and LPL, but unfortunately (2-benzyloxy-5-fluorophenyl)boronic acid inhibited PL with an IC_{50} value of 7 μM. Further investigations will be required with the aim of identifying selective HSL inhibitors and to evaluate the biological importance of targeting HSL. Inhibitors of HSL might have a future for the treatment of type 2 diabetes, the metabolic syndrome, and impaired glucose tolerance.[2]

2.11. HCV NS3 Protease

Chronic infection of the hepatitis C virus can lead to progressive liver injury, cirrhosis, and liver cancer.[247] The unsatisfactory efficacy and safety profile of current therapies, together with the highly valuble course of disease progression, makes clinical treatment of HCV infection difficult.[248] Therefore, there is a clear need for substantially improved antiviral therapies for HCV.[249]

HCV has a 9.5 kb RNA genome that encodes a polyprotein of 3010 to 3040 aa. Processing of the polyprotein by cellular and viral proteases generates at least 10 mature viral structural and nonstructural (NS) proteins. One of these is a serine protease known as NS3 protease, a flavivirus protease that is thought to be essential for viral replication and has become a target for anti-HCV drugs.[249] NS3 is responsible for cleaving the four carboxy terminal cleavage sites in the polyprotein. The X-ray crystal structure of the NS3 protease was shown to be monomeric with two domains: a trypsin-like fold and a structural zinc-binding site.[28,250,251] Its substrate specificity is however different from that of cellular serine proteases with a cysteine residue in the P_1 position of peptide substrates.[252,253] NS3 is well characterized biochemically[254] and has been crystallized alone,[255,256] in complex with its cofactor peptide NS4A,[28] and with peptide inhibitors.[255,256]

Starting from a hexapeptidyl boronic acid lead, 3-amino bicyclic pyrazinones as novel β-sheet dipeptide mimetics have been designed and synthesized.[257] Side-chain manipulation of this scaffold generated a series of potent, nonpeptidic inhibitors, for example, Figure 62 (**149**), of HCV NS3 protease.

Derived from the previously identified potent hexapeptidyl aldehyde inhibitors of the HCV proteinase,[258a] a set of homologous boronic acid inhibitors were designed and synthesized. A high throughput method of synthesizing such boronic acid compounds was developed by using a resin bound diol as a boronic acid protecting group and immobilization linking point. Therefore, a hexapeptidyl boronic acid library was established by parallel synthesis using an Advanced Chemtech 496 synthesizer, from which compounds (**150a**, K_i = 80 nM) and (**150b**, K_i = 80 nM) (Figure 63) were found to be highly potent inhibitors of the HCV NS3 protease.[258b]

Later on, Li et al.[259a,b] designed and synthesized a novel series of α-amino cyclic boronates (**151**−**153**, Figures 64 and 65) and incorporated them successfully in several acyclic templates at the P1 position. These compounds are inhibitors of the HCV NS3 serine protease, and structural studies show that they inhibit the NS3 protease by trapping the Ser-139 hydroxyl group in the active site. Synthesized α-amino oxaborole inhibitors were evaluated using FRET assay with HCV NS3/4A 1a protease domain. The IC_{50} value of VX-950

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

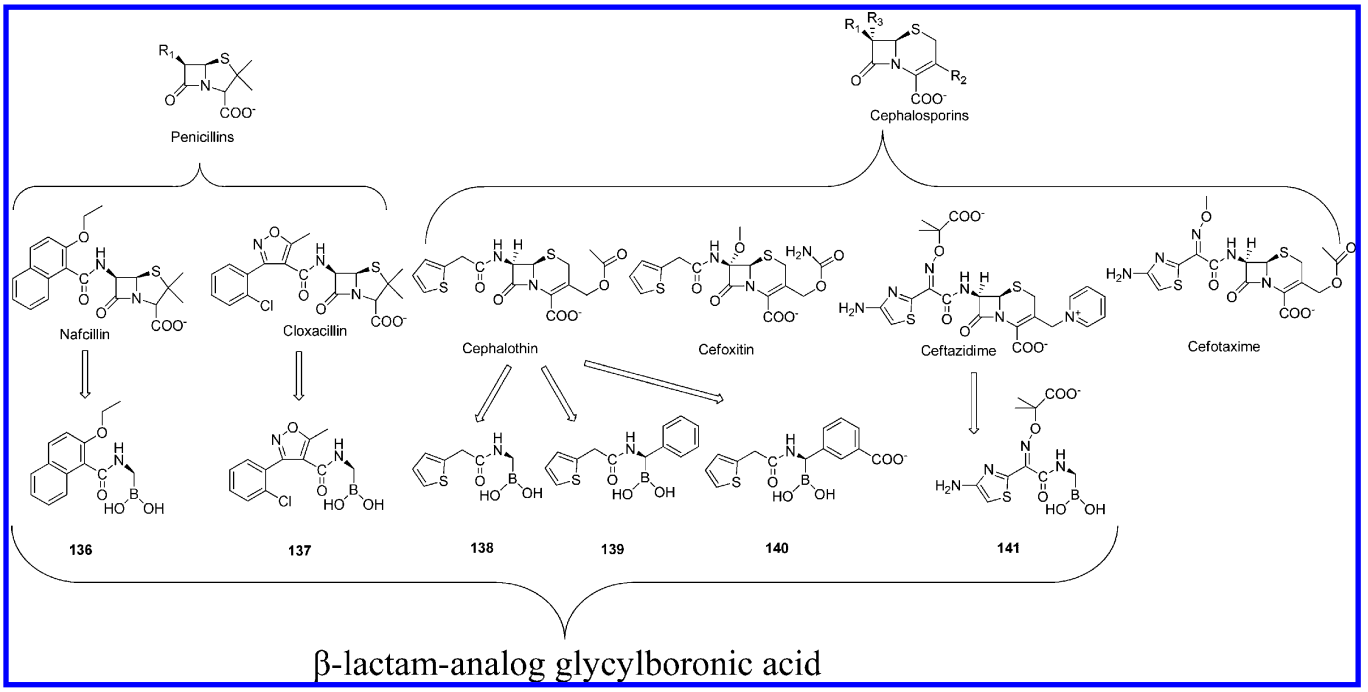


Figure 58. Characteristic β-lactam antibiotics and related β-lactamase inhibitors.

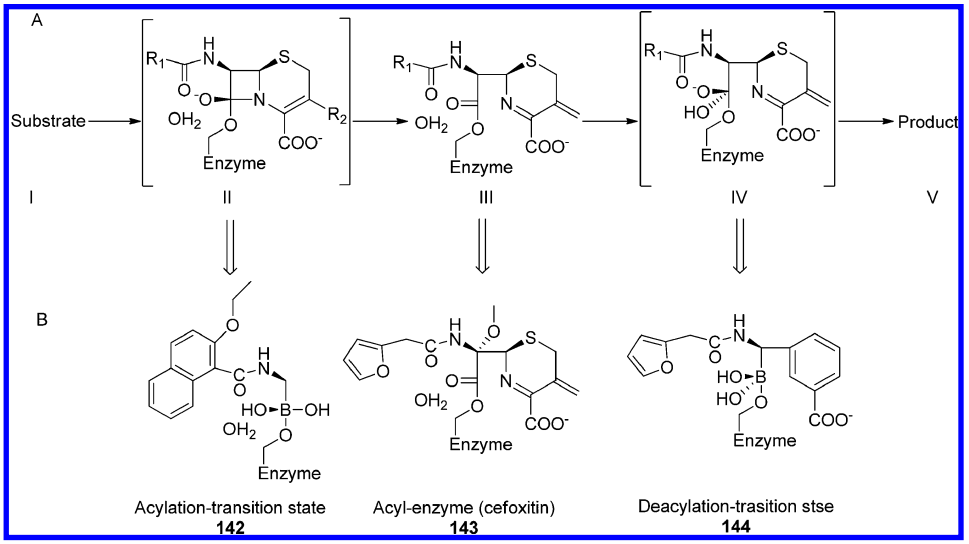


Figure 59. Reaction cycle of a serine β-lactamase. (A) The basic reaction pathway is shown with the acylation and deacylation tetrahedral intermediate. (B) Structural diagram of the reaction intermediates whose structures have been determined to understand each step.

Table 4. Inhibition Constants against CTX-M-9 and CTX-M-16

	K_i (μM) for	K_i (μM) for
compounds	CTX-M-9 (Asp240)	CTX-M-16 (Gly240)
136	1.2	3.0
137	51	
138	5.5	2.8
139	18.1	14
140	0.578	0.423
141	0.015	0.004
cefoxitin	14.0[a]	

[a] IC_{50}.

was found to be 0.10 μM in this assay. When the amino oxaborole was incorporated into the VX-950 template, the resulting compound (**151a**) exhibited good potency (IC_{50} = 0.12 μM). When the SCH-503034 template was used, the

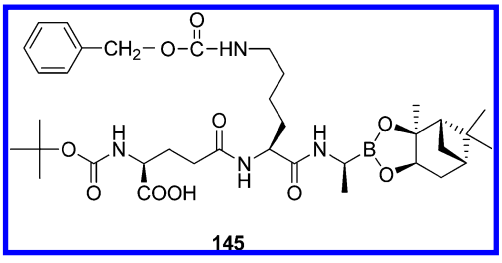


Figure 60. Peptidyl boronic acid.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

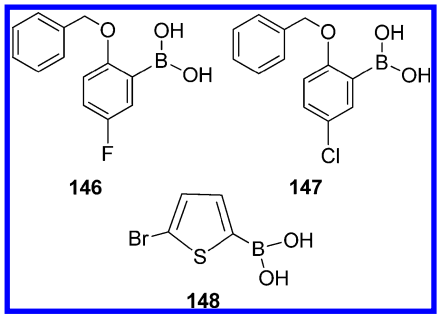


Figure 61. Hormone-sensitive lipase inhibitors.

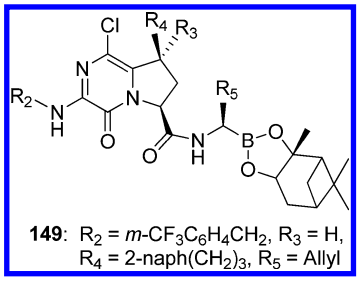


Figure 62. A nonpeptidic HCV NS3 protease inhibitor.

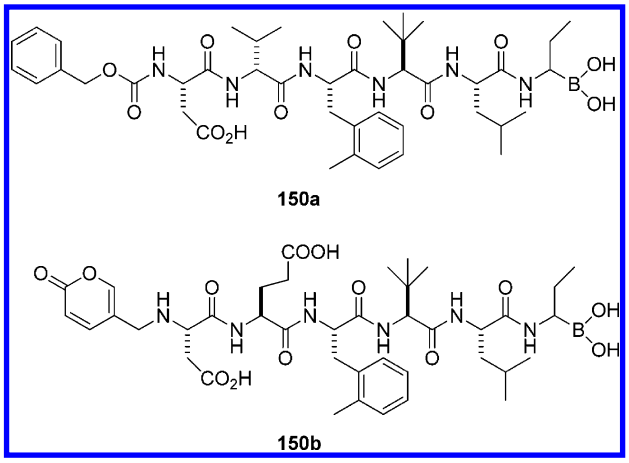


Figure 63. Hexapeptidyl boronic acids.

resulting compound (**151b**) was 3-fold less potent than **151a** (IC_{50} = 0.34 μM). On the other hand, incorporation of this α-amino oxaborole into the P2* isoindoline containing template c resulted in a very potent compound (**151c**) (IC_{50} = 0.023 μM). Replacement of the isoindoline with an isoquinoline at the P2* site led to compound (**151d**) with an IC_{50} of 0.12 μM.[259a]

In a related study, a novel series of P2−P4 macrocyclic HCV NS3/4A protease inhibitors with the α-amino cyclic boronates as warheads at the P_1 site were designed and synthesized (Figure 65). Compound **152a** inhibited NS3 1a enzyme with an IC_{50} of 0.043 μM in the sucrose assay, and it exhibited good replicon 1a and 1b potency with EC_{50} values of 0.78 and 0.52 μM, respectively. Synthesis of six- and seven-membered cyclic boronates (**153a**) and (**153b**) to explore the influence of ring size on potency has been studied. Compound **153b**, derived from seven-membered cyclic boronate, showed comparable enzymatic potency with an IC_{50} of 0.047 μM and was marginally more potent in replicon 1a and 1b assay with EC_{50} of 0.38 and 0.22 μM, respectively. Therefore, inhibitors **152a** and **153b** exhibited comparable replicon activity compared to two serine-trap inhibitors, VX-950 (replicon 1b, EC_{50} = 0.354 μM) and SCH-503034 (replicon 1b, EC_{50} = 0.20 μM).[259b] X-ray crystal structures of boronate inhibitor (**151d**) and of macrocyclic inhibitor (**152a**) complexed with HCV NS3 serine protease are shown in Figure 66.

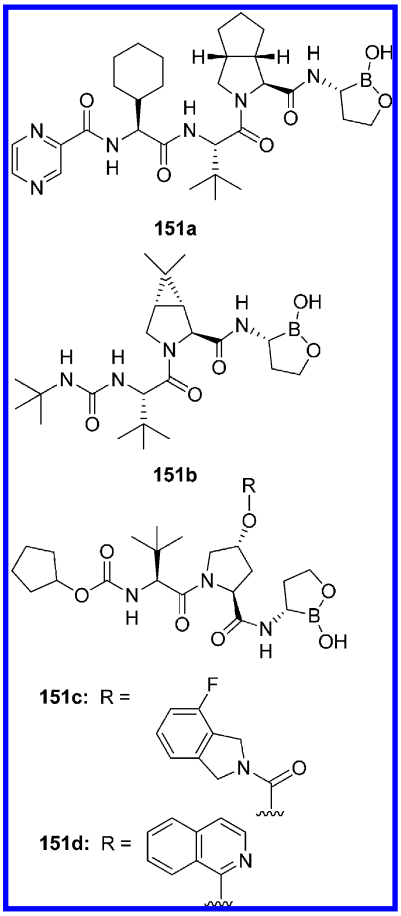


Figure 64. Novel NS3 protease peptidyl boronic inhibitors.

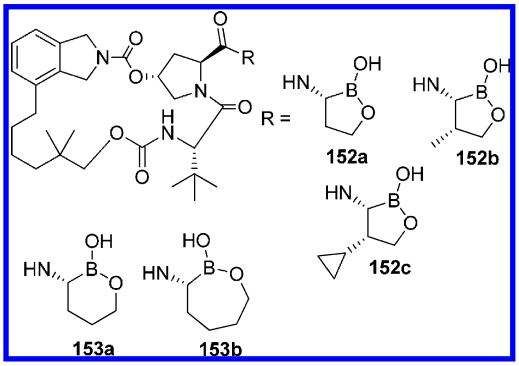


Figure 65. P2−P4 macrocyclic boronate inhibitors against HCV NS3/4A 1a: (**152a**, 0.043 μM; **152b**, 0.13 μM; **152c**, 0.11 μM; **153a**, 0.062 μM and **153b**, 0.047 μM).

Early in the evolution of the HCV NS3 protease medicinal chemistry program, Schechter et al.[35] focused on optimizing the P_1 residue in peptidyl boronic acid inhibitors.[55] Literature reports indicated that the protease prefers small, hydrophobic P_1 side chains (ethyl, allyl, CH_2SH) in peptide substrates[253,260] and inhibitors.[261−263] Inspection of the three-dimensional structure of NS3 protease shows that the S_1 site is very hydrophobic, as it is encompassed by the side chains of Phe154, Ala157, Lys136, Val132, and Leu135. Moreover, S_1 is not well-

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156−4220

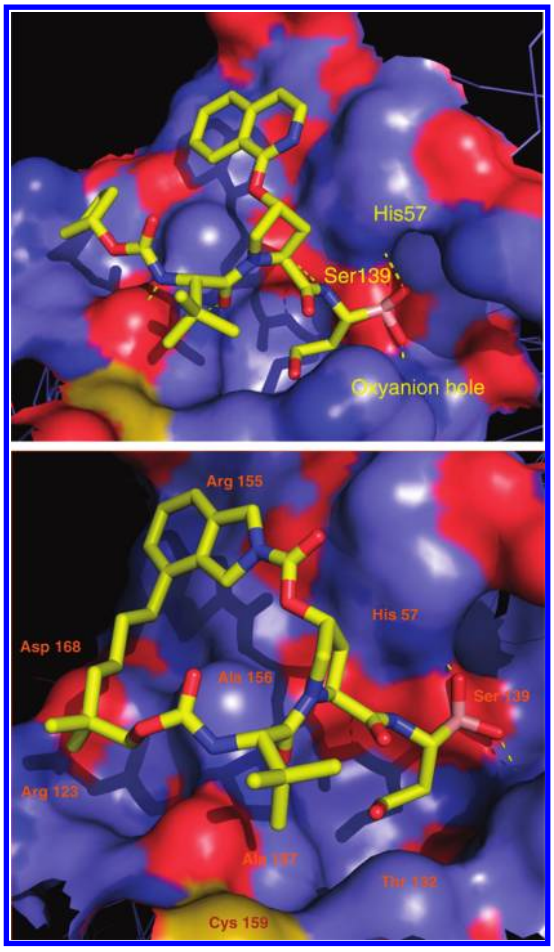


Figure 66. X-ray crystal structure (up) of boronate inhibitor (**151d**) complexed with HCV NS3 serine protease. X-ray crystal structure (down) of macrocyclic inhibitor (**152a**) complexed with HCV NS3 serine protease. Reprinted with permission from refs 259a and b. Copyright 2010 Elsevier.

defined, but rather exists as a broad surface extending to S_3. In order to further probe structure/activity relationships in this region of the protease active site, a series of extended boronic acids containing extended, hydrophobic P_1 residues was prepared to probe the shallow, hydrophobic S_1 region of HCV NS3 protease. The *p*-trifluoromethylphenethyl P_1 (**154**, Figure 67) substituent was identified as optimal with respect to

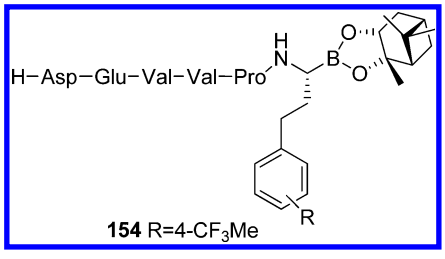


Figure 67. *p*-Trifluoromethylphenethyl P_1 substituent.

inhibitor potency for NS3 (K_i = 0.006 μM) and selectivity against elastase and chymotrypsin.[264]

In subsequent studies, reasearchers used NMR spectroscopy to characterize the hepatitis C virus (HCV) NS3 protease in a complex with the 24 residue peptide cofactor from NS4A and a boronic acid inhibitor, Ac-Asp-Glu-Val-Val-Pro-boroAlg–OH (boroAlg: boronic acid analogue of allylglycine). This inhibitor mimics the sequence of a good substrate for NS3 protease[265] and is expected to bind in the substrate P_6–P_1 binding sites. The allylic side chain is predicted to bind in a manner similar to the $-CH_2SH$ of Cys and the $-CH_2CHF_2$ side chain of the ketoacid inhibitors. The allylic proton is polarized and can potentially interact with the aromatic ring of Phe154 in the bottom of the P_1 pocket. Secondary structure information, NOE constraints between protease and cofactor, and hydrogen-deterium exchange rates revealed that the cofactor was an integral strand in the N-terminal β-sheet of the complex as observed in X-ray crystal structures. Upon the basis of chemical shift perturbations, inhibitor-protein NOEs, and the protonation state of the catalytic histidine, the boronic acid inhibitor was bound in the substrate binding site as a transition state mimic (Figure 68). In the absence of cofactor, the inhibitor had

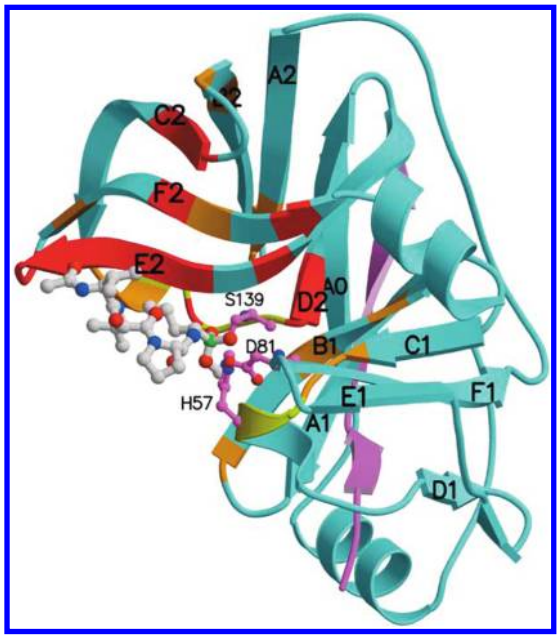


Figure 68. Chemical-shift perturbations mapped onto a model structure of NS3/NS4A/Inhl. The model was calculated from the crystal structure of NS3/NS4A. The inhibitor (Inhl) is depicted in stick representation (white C, blue N, red O, and green B) and NS4A (violet) and NS3 are shown as ribbons. The catalytic triad (His57, Asp81, and Ser139) are in stick representation (magenta C, blue N, and red O). Residues whose backbone amide resonances shift more than 0.2 and 0.1–0.2 ppm upon binding InhI are colored red and orange, respectively. Residues Tyr56, His57, Gly137, Ser138, and Ser139, which could not be identified in the absence of InhI, are colored yellow. This figure was generated with Molscript and Raster 3D. Reprinted with permission from ref 265. Copyright 2002 Elsevier.

a lower affinity for the protease. Although the inhibitor binds in the same location, differences were observed at the catalytic site of the protease.[265]

Bovine viral diarrhea virus (BVDV) is closely related to hepatitis C virus (HCV) and has been used as a surrogate virus in drug development for HCV infection. Similar to HCV, BVDV-encoded NS3 serine proteinase is responsible for multiple cleavages in the viral polyprotein, generating mature NS4A, NS4B, NS5A, and NS5B proteins. NS3-dependent cleavage sites of BVDV contain a strictly conserved leucine at P_1, and either serine or alanine at P_1'. The full length BVDV NS3/4A serine protease has been cloned and expressed in

bacterial cells. The enzyme has been purified from the soluble portion of *E. coli* via a two-step purification procedure employing chromatography on heparin resin and gel filtration. The protease activity was characterized using in vitro translated BVDV NS4A/B and NS5A/B polyprotein substrates. A boronic acid analogue of the BVDV NS4A/NS4B cleavage site was synthesized and shown to be an efficient inhibitor of the NS3 serine protease in vitro. The compound designated DPC-AB9144-00, inhibited approximately 75% of the NS3/4 activity at 10 μM with the NS4A/B substrate. However, no antiviral activity was detected with DPC-AB9144-00 in BVDV-infected Madin-Darby bovine kidney cells at concentrations as great as 90 μM, suggesting permeability or that other cellular-derived limitations were present.[266]

2.12. Dengue Virus NS3 Protease

The mosquito-borne dengue viruses are widespread human pathogens causing a self-limiting viral fever which can lead to life-threatening conditions such as dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). This virus is endemic to most tropical and subtropical regions throughout the world with an estimated 2.5 billion people living in areas at risk for epidemic transmission.[267−269] Like other viral proteases, its function perpetuates replication of the virus, and the inhibition of these proteases provides an ideal target for medical intervention.

The dengue virus genome contains a protease with a classical serine protease catalytic triad (His51, Asp75, and Ser135) which constitutes part of the nonstructural protein 3 (NS3). The enzymatic activity of NS3 protease is enhanced by interactions with the NS2B protein, which acts as an essential cofactor.[270−273] NS3 protease is vital for the post-translational proteolytic processing of the polyprotein precursor and is essential for viral replication and maturation of infectious dengue virons.[274] Therefore, dengue NS3 protease is an attractive therapeutic target for dengue virus infections.

The dengue protease has a marked cleavage site preference for dibasic residues. Two high affinity nonprime side substrates: Bz-Nle-Lys-Arg-Arg (S_1) (K_m = 12.42 μM) and Bz-Nle-Lys-Thr-Arg (S_2) (K_m = 33.9 μM), have been recently identified through substrate profiling of dengue protease using positional scanning tetrapeptides.[275] Researchers sought to capitalize on the substrate information of NS3 protease to develop potent small molecule inhibitors of the dengue serine protease. In order to rapidly map the active site, they decided, as a first step, to examine several well-established serine protease warheads. Therefore, substrate-based tetrapeptide inhibitors with various warheads were designed, synthesized, and evaluated against the dengue virus NS3 protease. Effective inhibition was achieved by peptide inhibitors with electrophilic warheads such as aldehyde, trifluoromethyl ketone, and boronic acid. A boronic acid has the highest affinity, exhibiting a K_i of 43 nM.

Yin et al.[276] examined the major requirements for nanomolar inhibition of the dengue NS3 protease. Starting with the best known substrate peptide sequence, they determined that, in contrast to HCV NS3 protease, cleavage products and their analogues do not appreciably inhibit the dengue NS3 protease. Peptide inhibitors with electrophilic warheads, such as aldehyde, trifluoromethyl ketone, and boronic acid, were needed to see effective inhibition of the enzyme activity. Among the examined warheads, tetrapeptidyl boronic acid has the highest affinity (**155**, Figure 69), exhibiting a K_i of 43 nM. These small molecule inhibitors offered valuable insights into the development of chemotherapeutics for the treatment of dengue infection in humans.

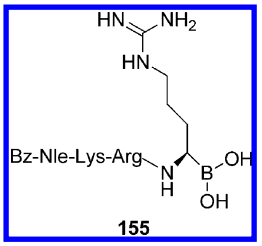


Figure 69. Tetrapeptidyl boronic acid.

2.13. Prostate-Specific Antigen

Prostate-specific antigen (PSA) is a human serine protease with chymotrypsin-like activity that is secreted almost exclusively by prostatic tissue into the prostatic fluid. Low concentrations of PSA are also found in blood, where it is bound to inhibitors and therefore enzymatically inactive, though still immunoreactive.[277] Most prostate cancers express PSA,[278] so that elevated levels of serum PSA are frequently associated with prostate carcinoma and benign prostatic hyperplasia. PSA is routinely used as a sensitive marker for monitoring prostate cancer progression and response to therapy.[279] The importance of PSA as a tumor marker may stem from its biological activity.[280] Reports in the literature indicate that insulin-like growth factor binding protein-3 (IGFBP-3) is cleaved by PSA, releasing IGF-1 and enhancing its mitogenic capabilities within the prostate.[281] Interest in the role of IGF in carcinogenesis has recently been increased by the finding of elevated serum levels of IGF-1 in association with prostate cancer.[282] PSA is also capable of degrading some extracellular matrix glycoproteins and in this way may facilitate invasion by, or escape of, prostate cancer cells.[283] Another possibility is the activation by PSA of single-chain urokinase-type plasminogen activator, which plays an important role in tumor invasion and metastasis.[284] Any of these mechanisms could imply that PSA could be an initiator of the protease cascade involved in prostate cancer. The multiple possible pathways for the involvement of PSA in early events leading to the development of malignant tumors have made it a target for prevention and intervention. It has also prompted some interest in the development of PSA-selective inhibitors as useful tools for the targeted treatment and imaging of prostate cancer.[285]

The regulation of relevant enzymatic activity pathways has been proposed as a role for dietary boron.[286] Because of their chemical structure, serine proteases are reversibly inhibited by simple and substituted boric acid compounds.[287] Simple boric acid compounds that contain a trigonal boron atom bind to the active site of serine proteases to form a reversible transition state analogue complex.[288] In vitro results show that low concentrations of boric acid can partially inhibit (30−40%) the proteolytic activity of purified PSA toward a synthetic substrate and also hinder the ability of PSA to degrade fibronectin.[289] In vivo results in nude mice showed that the size of tumors was decreased in mice exposed to the low and high dose of boric acid (1.7, 9.0 mgB/kg/day) by 38% and 25%, respectively.[290] Serum PSA levels decreased by 88.6% and 86.4%, respectively, as compared to the control group. There were morphological differences between the tumors in control and boron-dosed animals, including a significantly lower incidence of mitotic figures in the boron-supplemented groups. The expression of IGF-1 in the tumors was markedly reduced by boron treatment,

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

which has been shown by immunohistochemistry. These data indicate that low-level dietary boron supplementation reduced tumor size and content of a tumor trophic factor, IGF-1.

Later, it was found that selective peptidyl boronic acids were potent inhibitors of the PSA.[291] The best of these had the sequence Cbz-Ser-Ser-Lys-Leu-(boro)-Leu (**156**) (Figure 70),

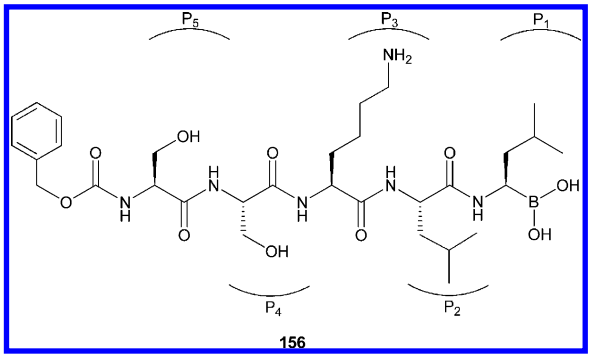


Figure 70. Chemical structure of compound Cbz-Ser-Ser-Lys-Leu-(boro)Leu used as the basis for the synthesis of the P2 and P3 inhibitor libraries. P5−P1 positions are indicated by brackets.

with a K_i of 65 nM. A validated model of PSA's catalytic site confirmed the critical interactions between the inhibitor and residues within the PSA enzyme.

More recently,[292] the same authors reported that the structural requirements in the P2 and P3 positions of peptide-based PSA inhibitors are explored through the substitution of a series of natural and unnatural amino acids in these positions. Reported data demonstrated a preference for hydrophobic residues in the P2 position and amino acids with the potential to hydrogen bond in the P3 position. Using this information, a peptidyl boronic acid inhibitor with the sequence Cbz-Ser-Ser-Gln-Nle-(boro)-Leu was identified with a K_i for PSA of 25 nM. The attachment of a bulky metal chelating group to the amino terminal of this peptide did not adversely affect PSA inhibition. Obtained result suggested that a platform of PSA inhibitor chelates could be developed as a PET-based imaging agents for prostate cancer.

2.14. Esterases

Esterases are hydrolases that split esters into an acid and an alcohol in a chemical reaction with water called hydrolysis. A wide range of different esterases exist that differ in their substrate specificity, their protein structure, and their biological function. For example, acetylcholinesterase inactivates the neurotransmitter acetylcholine.[293]

Acetylcholine was thought to induce a conformational change of the receptor protein in excitable membranes, thereby triggering a series of reactions resulting in increased ion permeability.[294,295] Much effort has been expended on isolating the biopolymer on which acetylcholine acts during activity.[296,297] Such work would be aided by highly specific reagents suitable for labeling of the macromolecules in question by formation of covalent bonds with groups at or near the active site. In the past, several such "active-site directed" labeling reagents have been reported.[298−301] Trimethylammonium diazonium fluoroborate (TDF) (**157**, Figure 71) had been used as an affinity-labeling reagent for anti-*p*-azophenyl trimethylammonium antibodies,[302] and was an irreversible inhibitor of the acetylcholine-receptor site of the electroplax at the level of the junctions. TDF, being a diazonium salt, readily

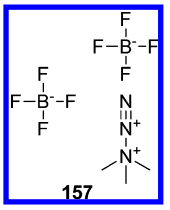


Figure 71. Trimethylammonium diazonium fluoroborate (TDF).

formed covalent bonds with suitable amino acid residues. The potent receptor inhibitors *d*-tubocurarine, dimethyl *d*-tubocurarine, and flaxedil protect against the irreversible inhibition by TDF, even when 10 times larger concentrations of TDF were used.[303]

Mautner et al.[303] tested TDF, its dimethyl analogue (DDF), its *p*-nitro analogue (NDF), and its *p*-acetoxy analogue (ADF). *p*-Nitrobenzene diazonium fluoroborate (NDF) was a potent inhibitor of the carbamylcholine-induced depolarization of the electroplax and of acetylcholinesterase. It probably formed covalent bonds with the acetylcholine receptor and esterase at the active site of the proteins. Its inhibitory strength was at least the same as that of TDF. After treatment of the electroplax preparation with dithiothreitol, NDF remained an irreversible receptor-inhibitor, while TDF became a potent reversible receptor activator. TDF was self-inhibitory: applied before reduction, it no longer depolarized. Although the first observations on TDF suggested that the compound labeled both proteins by virtue of the steric complementarity of its trimethylammonium group to a negative subsite in the proteins, the study by Bartels indicated that it is the positively charged diazonium group that reacted with the active sites of the proteins to form a covalent bond with an appropriate amino-acid residue.

In 1974, Koehler et al.[304] synthesized a powerful reversible bifunctional inhibitor of acetylcholinesterase. The inhibitor is an alkylborinic acid analogue of acetylcholine, *N*,*N*-trimethyl-propylammonium bromide methane boronic acid ($Br(CH_3)_3N$-$(CH_2)_3B(OH)(CH_3)$). The steady-state kinetic parameters of acetylcholinesterase hydrolysis of acetylcholine in the presence of this inhibitor were measured between pH 5.4 and pH 8.5 at 25 °C. The inhibition was competitive. The extent of inhibition depended on the state of ionization of a group of the enzyme with an apparent p*K* of ~6.7. Ionization of the boronic acid of the inhibitor interfered with the inhibition reaction and indicated that the acid pH form of the inhibitor binds preferentially to the enzyme. Inhibition of acetylcholinesterase by a number of related inhibitors was also measured for comparison. Electrophysiological experiments indicated that the inhibitor has an additional advantage over inhibitors used previously in that it interacted very poorly with membrane components which indicates changes in electrical potential of electroplax.

Pancreatic cholesterol esterase (CEase) is secreted into the small intestine in response to ingested fat, where it catalyzes the hydrolysis of cholesteryl esters, phospholipids, and acylglycerols.[305−307] CEase is necessary for the full absorption of cholesterol across the intestinal mucosa into the bloodstream.[308,309] Because high serum cholesterol is a primary risk factor for atherosclerosis and ischemic heart disease,[310] it was desirable to develop inhibitors of CEase. In vitro, CEase catalyzes the hydrolysis of the water-soluble substrate *p*-nitrophenyl butyrate (PNPB) via an acyl enzyme mechanism[311,312] that is reminiscent of the mechanisms of serine

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

proteinases.[313−315] Both the acylation and deacylation stages of CEase catalysis likely involve high energy tetrahedral intermediates that should bear some structural resemblance to the transition states for their formation and decomposition. Therefore, this intermediate serves as the focus for the design of transition state analogue inhibitors which bind to the enzyme's active site to form tetrahedral complexes. Boronic and borinic acids have been demonstrated as transition state analogue inhibitors of CEase-catalyzed reactions.[316]

In this context, Sutton et al.[317] synthesized a series of *n*-alkylboronic acid inhibitors of the CEase-catalyzed hydrolysis of PNPB. These compounds have been used to characterize the dimensional specificity of CEase for aliphatic straight chains. The most potent of these was *n*-hexaneboronic acid, with a K_i = 13 μM. The inhibitor potency declined for both longer and shorter boronic acids; that is, no inhibition was observed for methaneboronic acid and *n*-octaneboronic acid inhibits poorly, with a K_i of 7 mM. These results indicated that the ability of the enzyme to form tight complexes with boron containing transition state analogue inhibitors is sensitive to alkyl chain length. The trend in inhibitor potency was discussed in terms of substrate specificity and transition state stabilization by cholesterol esterase, and has important implications for the design of optimal reversible inhibitors of the enzyme.

3. ASPARTIC PROTEASE INHIBITORS

Aspartic proteases are a family of protease enzymes that use an aspartate residue for catalysis of their peptide substrates. In general, they have two highly conserved aspartates in the active site and are optimally active at acidic pH. Aspartyl proteases are highly specific — they tend to cleave dipeptide bonds that have hydrophobic residues as well as a beta-methylene group.[318] The general acid−base mechanism that is considered most likely for polypeptide hydrolysis catalyzed by aspartic proteases involves the attack of the scissile amide bond by a water molecule which is itself partially activated by a deprotonated catalytic aspartic acid residue. The protonated aspartic acid donates a proton to the amide bond nitrogen, generating a zwitterionic intermediate which collapses to the cleaved products. The water molecule that binds between the enzyme (Ile50 and Ile150) and inhibitor is thought to position a peptide substrate, stretching the peptide bond out of planarity toward a tetrahedral transition state that is stabilized by a second water molecule.[319] These proteases generally bind 6−10 amino acid regions of their polypeptide substrates which are typically processed with the aid of two catalytic aspartic acid residues in the active site. Thus, there is usually considerable scope for building inhibitor specificity for a particular aspartic protease by taking advantage of the collective interactions between a putative inhibitor, on both sides of its scissile amide bond, and a substantial portion of the substrate-binding groove of the enzyme. Some aspartic proteases also have one or more flaps that close down on top of the inhibitor further adding to inhibitor−protease interactions and increasing the basis for selectivity.[320]

Aspartic proteases play an important role in several aspects of our overall health and physiology, including blood pressure (renin), digestion (pepsin and chymosin), and in the maturation of the human immunodeficiency virus (HIV I protease).

3.1. HIV-1 Protease

The protease of the human immunodeficiency virus (HIV-1 PR) is a homodimeric enzyme with a molecular mass of 22 kDa that contains a single active site.[321] Inhibition of this enzyme can be achieved either by binding a substrate analogue to the enzyme active site (transition state inhibitors) or by interfering with the association of the two enzyme subunits (association inhibitors).

The HIV-1 PR has an essential role in the replicative cycle of HIV. The proteases encoded by the HIV viral genomes are responsible for processing the polyprotein precursors produced from the gag and pol genes into mature viral structural proteins (p6, p7, p17, and p24) required for replication and assembly of the mature virus[322] and replication enzymes in infected cells.[323−325] Inactivation of the HIV-1PR by mutation of the catalytic aspartyl residues at positions 25 and 125 thus yields noninfectious virions.[326] Therefore the key role played by the protease and the availability of high resolution crystallographic structures for HIV-lPR[327−329] have made this protease a highly attractive target for the design of anti-AIDS therapeutic agents.[330] The protease of the HIV-2 virus (HIV-2PR) plays a similar role in replication of the virus. Recently, the crystal structures of HIV-2PR complexed with two peptide inhibitors have been determined.[331] Intensive efforts over the past few years[332] have produced a variety of peptide inhibitors for both proteases with K_i values in the nanomolar to sub-nanomolar range, none of which has yet proven to be of clinical utility due to the low bioavailability and rapid degradation characteristic of peptidic agents. Several approaches for the therapy of AIDS involve a combination of two reverse transcriptase inhibitors with one protease inhibitor. Combination therapy can reduce viremia to unquantifiable levels.[333] However, 30−50% of patients fail antiviral therapy, presumably due to patient nonadherence and/or resistance development.

Curcumin (**158**, Figure 72), a natural product of low toxicity isolated from the rhizomes of *Curcuma longa*, has been shown

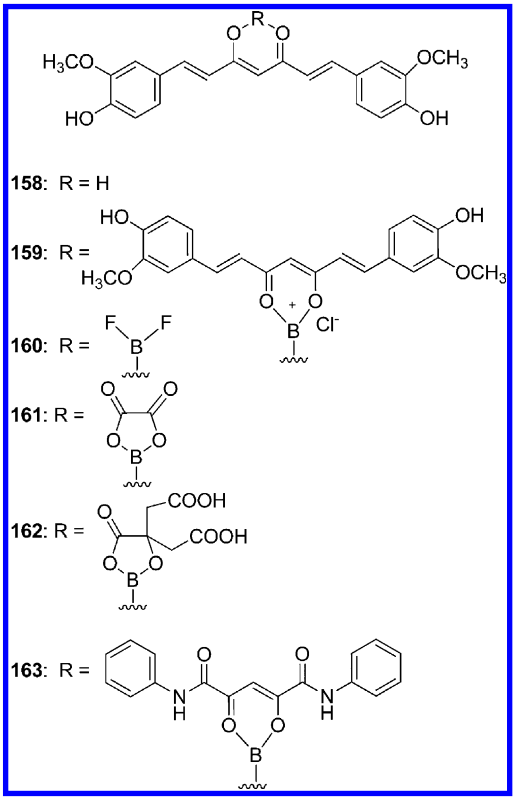


Figure 72. Curcumin and its boron containing derivatives.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

to have anti-inflammatory activity in both acute and chronic animal inflammation models. In animal studies, it was not toxic with oral LD_{50} in mice greater than 2.0 g/kg.[334] Sui et al.[335] reported the identification of curcumin as an inhibitor of HIV-1PR and HIV-2PR proteases (HIV-l (IC_{50} = 100 pM) and HIV-2 (IC_{50} = 250 pM)). Simple modifications of the curcumin structure raised the IC_{50} value but complexes of the central dihydroxy groups of curcumin with boron lowered the IC_{50} to a value as low as 6 μM. The boron complexes were also time-dependent inactivators of the HIV proteases. The increased affinity of the boron complexes may reflect binding of the orthogonal domains of the inhibitor in intersecting sites within the substrate-binding cavity of the enzyme, while activation of the α,β-unsaturated carbonyl group of curcumin by chelation to boron probably accounted for time-dependent inhibition of the enzyme. The modest activity of curcumin as an inhibitor of HIV-1PR and HIV-2PR was enhanced more than l0-fold when curcumin dimer (**159**, Figure 72) was formed by coordinating the central keto−enol groups of the two curcumin units to a boron atom. The activity of dimer (**159**) was 4−6 times greater than the activity of complexes in which curcumin is coordinated to a boron difluoride moiety (**160**) or to boron with small dioxygen ligands occupying the other two boron coordination sites (e.g., **161**−**163**, Figure 72). Complexation with boron therefore did not account for the higher inhibitory activity of **159**. It was therefore possible that the high affmity of **159** is due to simultaneous occupation of two binding sites in the proteases, although the decreases in the IC_{50} values were lower than might be expected for simultaneous occupancy of two binding sites. One possible explanation for this was that the inhibitor domains are truly orthogonal, whereas they were not truly orthogonal in the enzyme active sites. The inflexibility of the orientation of the two curcumin groups about the boron atom therefore might not permit optimal fit of the inhibitor in the substrate binding sites. On the other hand, binding to the boron activated the unsaturated carbonyl moiety of curcumin toward electrophilic additions and made the curcumin complexes time-dependent inhibitors of the two enzymes. Although the nature of the time-dependent inhibition was precisely determined, it was likely that it involved alkylation of one of the two active site aspartyls and/or one of the four cysteines of HIV-lPR, and one of the two active site aspartyls in HIV-2PR, by the boron-activated α,β-unsaturated carbonyl structure in the inhibitors.[336,337]

In continuing research, six boronated tetrapeptides, which were the substrate analogues of HIV-1 protease (Thr-Leu-Asn-Phe or Ser-Leu-Asn-Phe) with the carboxylic acid of phenylalanine replaced by boronic acid (**164**−**168**, Figure 73), were

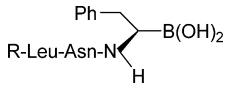


Figure 73. Chemical structures of boronated tetrapeptides: (**164**, R = Boc-Ser); (**165**, R = Boc-Thr); (**166**, R = Ac-Ser); (**167**, R = Cl^-H^+-Ser); (**168**, R = Cl^-H^+-Thr; Ph = phenyl).

synthesized, and their activities against human immunodeficiency virus 1 (HIV-1) protease was subsequently investigated. The sequences of these peptides were derived from HIV-1 protease substrates, which included the C-terminal part of the scissile bond (Phe-Pro) within the gag-pol polyprotein. Enzymatic studies showed that these compounds were competitive inhibitors of HIV-1 protease with K_i values ranging from 5 to 18 μM when experiments were performed at high enzyme concentrations (above 5×10^{-8} M); however, at low protease concentrations inhibition was due in part to an increase of the association constants of the protease subunits. Ac-Thr-Leu-Asn-PheB inhibited HIV-1 protease with a K_i of 5 μM, whereas the nonboronated parental compound was inactive at concentrations up to 400 μM, which indicated the significance of boronation in enzyme inhibition. The boronated tetrapetides were inhibitory to an HIV-1 protease variant that is resistant to several HIV-1 protease inhibitors. Fluorescence analysis showed that the interactions between the boronated peptide Ac-Thr-Leu-Asn-PheB and HIV-1 protease resulted in a rapid decrease of fluorescence emission at 360 nm, which suggested the formation of a compound/enzyme complex.[338]

Molecular modeling and/or random testing of compound libraries revealed several PR inhibitors with unexpected structures, for example, pseudopeptides, nonpeptidic structures, such as cyclic ureas, sulfonamides, etc.,[339] Nb containing polyoxometalates,[340] C_{60} fullerenes, hydrophobic and electrophilic spheric compounds.[341−344] Later, a group of inorganic compounds, icosahedral boranes, carboranes, and namely 12-vertex metal bis(dicarbollides), were identified as a promising framework for a novel class of nonpeptide PIs. These boron/carbon clusters were polyhedra based on a three-dimentional skeleton with triangular facets. Porphyrins substituted with dicarba-*closo*-dodecarboranes inhibited HIV-PR, with IC_{50} in the sub-micromolar range.[345] In a search for novel P_1 structures, a group of icosahedral metallocarboranes were investigated as a novel class of nonpeptidic PIs. Cigler et al.[346] reported the potent, specific, and selective competitive inhibition of HIV-PR by substituted metallocarboranes. The most active compound, sodium hydrogen butylamino bis-8,8-[5-(3-oxa-pentoxy)-3-cobalt bis(1,2-dicarbollide)]diate (**169**, Figure 74), exhibited a K_i value of 2.2 nM and a sub-

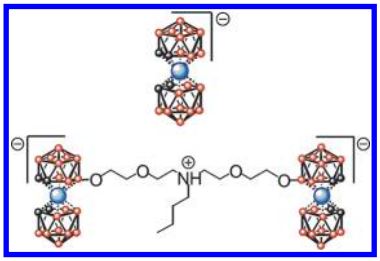


Figure 74. Structures and activities of metallacarborane inhibitors (**169**, up) and (**170**, down). Molecular weight of anion (**169**) 323.74; K_i, = 66 ± 30 nM; EC_{50} = 6 μM. Molecular weight of anion (**170**) 893.82; K_i, = 2.2 ± 1.2 nM; EC_{50} = 0.25 μM. Compounds were prepared as their sodium salts. Color coding: orange, BH groups; black, CH groups; blue, Co atom. Reprinted with permission from ref 346. Copyright 2005 National Academy of Sciences, U.S.A.

micromolar EC_{50} in antiviral tests, showed no toxicity in tissue culture, weakly inhibited human cathepsin D and pepsin, and was inactive against trypsin, papain, and amylase. The structure of the parent cobalt bis(1,2-dicarbollide) in complex with HIV-PR was determined at 2.15 Å resolution by protein crystallography and represents the first carborane−protein complex structure determined (Figure 75). It shows the following mode of inhibition: two molecules of the parent compound bind to the hydrophobic pockets in the flap-proximal region of the S_3 and S_3' subsites of PR. It was suggested, therefore, that these compounds flap closure in

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

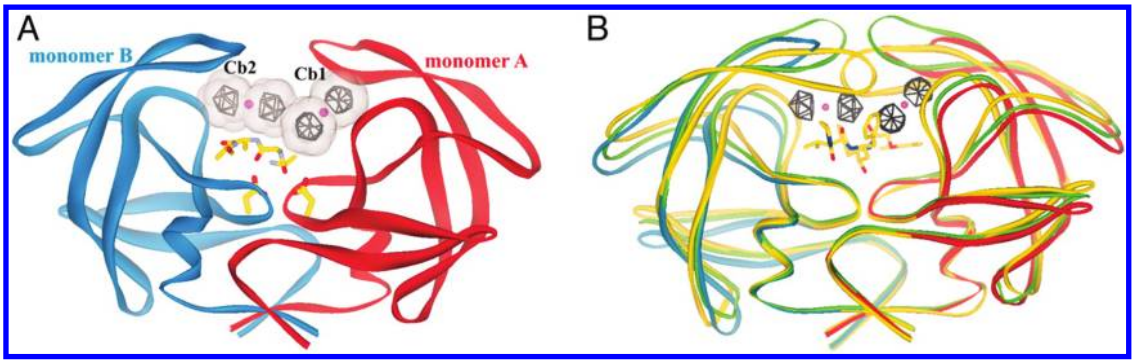


Figure 75. X-ray structure analysis of the binding of **169** to HIV-PR. (A) Overall structure of the HIV PR-(**169**) complex. The PR dimer is in ribbon representation with the two catalytic aspartates shown in sticks. Two **169** molecules are represented by their van der Waals surfaces and gray stick model, with cobalt ions shown as magenta spheres. Autoproteolytic peptide product is represented as a stick model. (B) Superposition of PR-compound (**169**) complex with PR-lopinavir complex and with the free PR structure. Protease complex with lopinavir (PDB ID code 1MUI) is represented in yellow ribbons, lopinavir is shown as a stick model, free PR structure (PDB ID code 1HHP) is shown in green ribbons, and color coding for PR-(**169**) complex is the same as in A. Reprinted with permission from ref 346. Copyright 2005 National Academy of Sciences, U.S.A.

addition to filling the corresponding binding pockets as conventional PIs. This type of binding and inhibition, chemical and biological stability, low toxicity, and the possibility to introduce various modifications make boron clusters attractive pharmacophores for potent and specific enzyme inhibition.

4. METALLOPROTEASE INHIBITORS

Hydrolases containing one or two metal ions connected by a bridging ligand catalyze diverse reactions as the degradation of DNA, RNA, phospholipids, and polypeptides.[347−350] They are, therefore, key players in carcinogenesis, tissue repair, protein maturation, hormone-level regulation, cell-cycle control, and protein degradation processes. The binuclear metallo-aminopeptidases catalyze the hydrolysis of the NH_2-terminal peptide bonds in polypeptides. All known metalloproteases[351−354] use a Zn^{2+} ion to catalyze the hydrolysis of a peptide bond. The metal is tetrahedrally coordinated to three donor groups from the enzyme and a water molecule that is also hydrogen bonded to the carboxylate side chain of a glutamic acid, which activates it for nucleophilic attack. Coordination by the carbonyl group of the scissile bond to zinc is presumably followed by nucleophilic attack of the zinc-bound water with simultaneous proton transfer to the carboxylate giving the zinc-complexed tetrahedral intermediate. Transfer of a proton from the glutamic acid to the amide nitrogen is followed by the collapse of the tetrahedral intermediate with the generation of a salt bridge between glutamic acid and free amine of the cleaved substrate.

4.1. Aminopeptidases

Aminopeptidases are a group of metalloenzymes that have been found in many tissues, including the lens, kidney, pancreas, muscle, and liver.[355,356] Studies on the mechanism of action have been reported.[357−362] Aminopeptidases participate in a wide range of biological processes. Some of them act as ectoenzymes that may participate in the degradation of extracellular matrices, and some act as intracellular enzymes that regulate the metabolism of bioactive molecules including hormones and neurotransmitters.[363] Effective inhibitors reported for aminopeptidases include amino ketones and derivatives,[364,365] small peptide analogues from microbial sources,[366−368] aliphatic and aromatic boronic acids,[358] amino acid hydroxamates,[358,369,370] and amino acid thiols.[371] Of these, aliphatic boronic acids and α-aminoaldehydes are thought to act as "transition-state analogues".

1-Butaneboronic acid (BuBA) was found to be an excellent inhibitor for *Aeromonas* aminopeptidase[372] because it reacted with the enzyme to form a transition-state analogue and was thus a marker for the catalytic subsites. 1-Butaneboronic acid was used as a marker for the catalytic subsite in multiple-inhibition studies and hydroxamate inhibitors of varying sizes were used as markers for the enzyme-bound zinc. Isoamyl alcohol, structurally similar to the side chain of preferred substrates, was chosen as a hydrophobic binding side marker. Mutual exclusivity of binding was observed between the following pairs of inhibitor: 1-butaneboronic acid and L-leucine hydroxamate; L-leucine hydroxamate and formyl hydroxamate; formyl hydroxamate and 1-butaneboronic acid; 1-butaneboronic acid and boric acid. The fact that binding of formyl hydroxamate completely blocked binding of both L-leucine hydroxamate and 1-butaneboronic acid demonstrated that the mutual exclusivity between the later two inhibitors was not due solely to competition between the hydrophobic side chains and that the dihydroxyboron group of 1-butaneboronic acid and the hydroxyamido group of the hydroxamates cannot bind simultaneously. Simultaneous binding was observed with the pairs isoamyl alcohol and formyl hydroxamate, isoamyl alcohol and hydroxylamine, and isoamyl alcohol and boric acid, but complete interference occurred between isoamyl alcohol and acetohydroxamic acid. The ability of formyl hydroxamate, but not acetohydroxamate, to bind simultaneously with the hydrophobic inhibitor implied that the hydrophobic pocket of the enzyme was separated from the binding sites for polar groups by no more than the dimensions of a methylene group. The interactions observed between the various inhibitors and the essential zinc ion of *Aeromonas* aminopeptidase was located within, or very near, the catalytic subsite that binds the substrate carboxamido group. This strongly suggested that the metal ion (or a water molecule/hydroxyl ion coordinated to it) is one of the enzyme functional groups that interact directly with the substrate atoms surrounding the scissile bond.[358]

In the course of synthesis of enzyme inhibitors, Shenvi et al.[162a] synthesized a number of α-aminoboronic acids and their derivatives (Figure 76). These compounds were effective inhibitors of human enkephalin aminopeptidase, microsomal leucine aminopeptidase, and cytosolic leucine aminopeptidase at micro- to nanomolar concentrations. The inhibition of

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

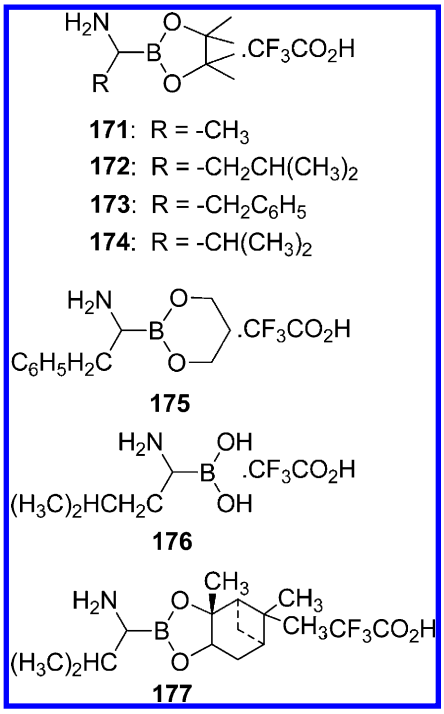


Figure 76. α-Aminoboronic acid inhibitors of aminopeptidases.

cytosolic leucine aminopeptidase has been studied in some detail. Kinetic data corresponded to the mechanism for biphasic slow-binding inhibition: E + I ⇌ E·I ⇌ E·I* binding was followed by a slow transformation to a stable enzyme inhibitor complex. The initial and final binding constants were dependent on the nature of the side chain at the α-carbon atom but were independent of the protecting group on the boronic acid moeity and followed the trend for the hydrolysis of the corresponding amino acids amides. The first-order rate constant for the transformation of E·I to E·I* was similar for all four compounds studied. These data suggested that the slow-binding step represented the formation of tetrahedral boronate species from trigonal boronic acid.[162a]

Later, Hussain et al.[373] demonstrated that boroalanine, boroleucine, and borovaline inhibitors that were effective in the inhibition of both cytosolic and microsomal aminopeptidases[162a] bound the nasal enzyme with K_i^{app} values of 0.009−0.02 μM. In 1995, borophenylalanine was also evaluated (K_i^{app} values of 0.004 μM). Comparison of this K_i value with those of cytosolic aminopeptidase and microsomal aminopeptidase derived from porcine kidney indicate that the nasal enzyme closely resembled the microsomal enzyme in properties. These studies suggested porcine microsomal aminopeptidase was a good model for the nasal aminopeptidase responsible for the hydrolysis of peptides containing neutral residues.[374]

An arginine aminopeptidase (RAP) of *Cryptosporidium parvum*, an intestinal protozoan parasite that can cause diarrhea in human immunodeficiency virus-infected patients,[375] children in day care centers,[376] and travelers to certain regions in the world[377,378] and is responsible for sporadic outbreaks of diarrhea worldwide,[377,378] has recently been described. *C. parvum* RAP preferentially cleaves synthetic substrates with arginine or alanine in the amino terminus. The enzyme is present on the surface membrane of freshly excysted sporozoites but not on the exterior of oocyst walls.[379] A study was made to test a series of new synthetic compounds for the ability to inhibit *C. parvum* RAP activity and to examine the effect of RAP inhibition during the in vivo excystation process. *C. parvum* arginine aminopeptidase (RAP) was studied during in vitro excystation. A series of α-aminoboronic acids blocked enzymatic activity. The H-boronoleucine (pinacol) inhibited excystation by 61% compared with solvent-treated control oocysts. Sporozoites remained viable within the oocyst as determined by propidium iodide and fluorescin diacetate dye uptake, suggesting that α-aminoboronic acids were not directly lethal to the parasite.[380]

The aminopeptidase from *Aeromonas proteolytica* serves as a paradigm for the study of bridged bimetallic proteases since its three-dimensional structure is known to very high resolution and its catalytic reaction is amenable to spectroscopic examination. In this context, De Paola et al.[381] reported the X-ray crystal structure at 1.9 Å resolution of AAP complexed with 1-butaneboronic acid (BuBA). This structure suggested that this complex represented a snapshot of the proteolytic reaction in an arrested form between the Michaelis complex and the transition state (Figure 77). Comparison of the

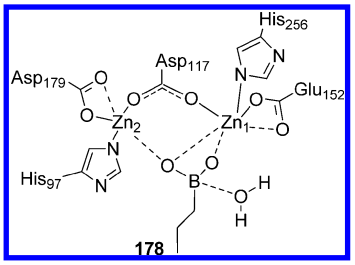


Figure 77. Schematic of BuBA bound to AAP.

structure with spectroscopic and other data allowed researchers to conclude that the apparently structurally symmetrical dizinc site was actually asymmetric electrostatistically.

4.2. Matrix Metalloproteases

The matrix metalloproteases (MMPs) (human and mammalian) are a large family of related proteases involved in extracellular matrix degradation.[352,354] This degradation is part of many pathological and physiological processes including the hydrolysis of collagen. Endogenous inhibitors maintain normal control of these processes. Many studies have been conducted on compounds that would accelerate the healing process. The huge number of products being recommended indicates that the problem is yet to be solved.

Blech et al.[382] demonstrated that a 3% boric acid solution improved dramatically the healing of deep wounds since it induced rapid wound granulation and angiogenesis. Later, Benderdour et al.[383,384] showed that boron affects the synthesis of the extracellular matrix (ECM), which plays an important role in the wound repair process by increasing the release of proteoglycans, collagen, and proteins. It also stimulates the synthesis and release of tumor necrosis factor (TNFα).

The usefulness of boric acid has led to the idea that boron derivatives might represent an alternative therapy for the repair of wounds. In this context, researchers[385] examined four boron derivatives (**179**−**182**, Figure 78) with different molecular structures. These derivatives exerted in vitro effects similar to those of boric acid. No relationship was established between structure and activity. Without affecting cell viability and proliferation, all boron derivatives appeared to modulate the

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

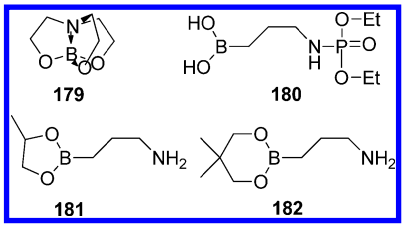


Figure 78. Structures of the four boron derivatives tested for repair of wounds: (**179**, triethanolamine borate), (**180**, *N*-diethyl-phosphoramidate propylboronique acid), (**181**, 2,2-dimethylhexyl-1,3-propanediolaminopropylboronate), and (**182**, 1,2-propanediol-amino-propylboronate).

turnover of extracellular matrix macromolecules (proteins and proteoglycans) by stimulating their release from the cells and decreasing their synthesis and by enhancing the activity of intra- and extracellular proteases.

Later, Nzietchueng et al.[386] reported that boron increased the turnover of the extracellular matrix and favored the protein phosphorylation (which could result from the activation of receptors by cytokines). The effect of boron may be due to its direct inhibitory effect on the activities of enzymes such as elastase or alkaline phosphatase, although this inhibition was not observed in living cells. This led to the hypothesis that part of the boron effect on wound healing was not direct, but indirect, via synthesis of cytokines (e.g., TNFα) involved in wound healing, or via generation of free radicals (or other compounds) and activation of transcription factors such as NFκB. It was also noteworthy that TNFα most likely included the production of VEGF, a strong angiogenic factor,[386] and that boron stimulated the production of VEGF.[387,388]

In continuing studies, series of novel phosphonoboronates consisting of PC_1B (C1-bridged phosphonoboronates), PC_nB (C_n-bridged phosphonoboronates), $PC(X)C_nB$ (heteroatom substituted phosphonoboronates), and PCC=CB (unsaturated phosphono-boronates) derivatives were evaluated as MMP-2 inhibitors. They have proven to have moderate inhibition properties toward MMP-2 with the alkenyl phosphonoboronate (**183**, Figure 79) showing significant activity against MMP-2 (75% inhibition at 50 mM).[389]

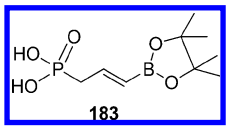


Figure 79. Alkenyl phosphonoboronate inhibitor of MMP-2.

Recently, Abu Ali et al.[390] showed that novel organoboronate derivatives consisting of BB (bis(pinacolato)diborane), BC(R_1R_2)B (C_1-bridged bis(pinacolato)diborane), BCCB ((pinacolato)$_2BCH_2CH_2B$), RBC=CBB (vinyl triboronates), and RBC=CBP (vinyl phosphonodiboronates) structures have significant activity against MMP-2. The trisboronate (**184**, Figure 80) proved to be a potent MMP-2 inhibitor at 5 and 10 μM. The exact mechanism of action of the tested organoboronate derivatives as MMP-2 inhibitors has not been determined. The authors suggested two modes of action: a zinc chelation mechanism and/or a transition state analogue mechanism.

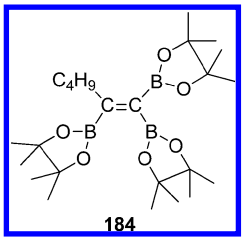


Figure 80. The trisboronate **184**, a potent MMP-2 inhibitor.

5. γ-GLUTAMYL TRANSPEPTIDASE INHIBITORS

γ-Glutamyl transpeptidase (γ-GT) is a membrane-associated enzyme that catalyzes the transfer of the γ-glutamyl group of glutathione to acceptor amino acids and peptides.[391,392] Its most well characterized in vivo function is in the metabolism and transport of glutathione and its derivatives.[393−395] The enzyme is expressed in response to stress or proliferative signals, for example, in regenerating liver,[396] and is also a marker for neoplasia[396] as well as transformation.[397−400]

Although the enzyme appears to function analogously to a serine protease, identification of the active site nucleophile has proven to be diffcult. Inoue et al.[401] have identifed the N-terminal threonine residue of the low-molecular-weight subunit as the active site nucleophile. This identification leads to the classifcation of γ-GT as an N-terminal threonine hydrolase. Such enzymes lack the Asp-His-Ser catalytic triad of traditional serine proteases such as chymotrypsin, utilizing the N-terminal α-amino group as a catalytic base.[402] Although a number of compounds were reported to inhibit γ-GT, no potent and specific inhibitors appear to have been reported. The alkylating agents 6-diazo-5-oxo-norleucine (DON), L-azaserine, and acivicin (AT-125) all inhibit γ-GT.[403,404] A particularly interesting sensitivity of the enzyme to the amino acid serine was discovered in borate buffer,[405] and this effect was subsequently proposed to result from the formation of a ternary enzyme-L-serine-borate complex, which functions as a transition state analogue.[403] Although this type of inhibition is apparently very specifc to γ-GT, its potency is relatively limited, requiring millimolar concentrations of serine and borate.

London et al.[406] investigated the inhibition of γ-GT by the amino acid analogue: L-2-amino-4-boronobutanoic acid (ABBA) (Figure 81). ABBA was found to be a potent inhibitor

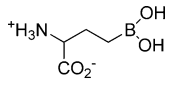


Figure 81. ABBA, L-2-amino-4-boronobutanoic acid.

of the enzyme, with K_i = 17 nM using typical assay conditions. It is a stable amino acid analogue with pK values determined from ^{13}C and ^{11}B NMR to be 2.30, 11.0 (amino titration), and 7.90 (boronate titration). The structural similarity to glutamate suggested that it might function as a glutamate analogue for some glutamate-dependent enzymes or receptors. This analogue effectively replaced the putative labile serine-borate oxygen ester linkage with a nonlabile methylene boronate linkage, resulting in a considerably more potent inhibitor of γ-GT. Effects of ABBA on the growth of cultured rat liver cell lines ARL-15C1 (nontumorigenic, low γ-GT activity) and ARL-16T2 (tumorigenic, high γ-GT activity) were also investigated, both in standard Williams media as well as in a low cysteine

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

growth medium. A high concentration (1 mM) of ABBA inhibited the growth of both cell lines in both media, with the degree of inhibition greater in the low cysteine medium. Alternatively, growth inhibition by 10 mM ABBA could be observed only in the low cysteine media. In general, there were no signifcant differences between the two cell lines in terms of sensitivity to ABBA.

A study following this finding, where the cellular effects of acivicin, an inhibitor of γ-glutamyl transpeptidase (γ-GT) that modulates cellular responses including growth, myeloid maturation, and apoptosis, against the more potent and specific inhibitor of γ-GT (L-2-amino-4-boronobutanoic acid, L-ABBA) in γ-GT-negative (B lymphoblastoid Ramos) and γ-GT-positive (myelomonocytic HL-60, γ-GT-transferred Ramos) cell lines were compared.[407] Under nonoxidative stress conditions, acivicin-induced cell growth arrest, apoptosis, and macrophage maturation occurred independent of γ-GT, while L-ABBA did not influence any of these processes. Acivicin triggered tyrosine phosphorylation and increased nuclear factor κB activity. In this context, growth inhibition, monocyte maturation, and apoptosis induced by acivicin could be mediated by mechanisms other than inhibition of γ-GT. L-ABBA is a potent inhibitor of γ-GT, yet did not cause any arrest or apoptosis in the cellular models.

6. THREONINE-BASED INHIBITORS

Threonine endoproteases are one of the new classes of proteases and use an N-terminal threonine residue as its catalytic machinery. The side-chain hydroxyl group acts as the proteolytic nucleophile activated by the N-terminal amine moiety.[408−410] The 20S proteaseome has been extensively characterized as the archetypal threonine protease along with the 26S proteasome.[411,412]

The ubiquitin-proteasome pathway is a major avenue for degradation of proteins in the cytosol and nucleus of eukaryotic cells.[413] Proteins are marked for degradation via the attachment of an ubiquitin group whereby the proteasome will recognize, unfold, and digests these proteins. The complete 26S proteasome incorporates the ATP-dependent 19S caps, which are understood to recognize and unfold substrates, which are in turn fed to the 20S proteasome, where proteolysis occurs.[413] The 20S proteasome structure is made up of 28 subunits arranged in four rings of seven, termed αββα with the interior of the β subunits responsible for proteolytic acitivity.[414]

6.1. 20S Proteasome

The 26S proteasome is a multifunctional ATP-dependent proteolytic system that is responsible for intracellular protein turnover.[415−420] The enzymatic acitivity of this protease complex occurs in a 700-kDa barrel-shaped core structure known as the 20S proteasome.[417] This protein consists of four stacked rings arrayed in a $\alpha_7\beta_7\beta_7\alpha_7$ manner.[421,422] Three β-subunits in each β-ring are catalytically active and have an N-terminal threonine as the active nucleophile involved in protein and peptide proteolysis.[423] The proteasome has at least three distinct peptidase activities and, by comparison with substrate specificities of known proteases, they are designated as chymotrypsin-like (β5-subunit), trypsin-like (β2-subunit), and peptidylglutamyl-peptide hydrolytic (PGPH) activities (β2-subunit). The involvement of the 20S proteasome in the ubiquitin (UB)-dependent and UB-independent degradation of critical regulatory proteins suggested the potential use of proteasome inhibitors as novel therapeutic agents being applicable in many different disease indications.[424−428] The specific target in the search for novel cytotoxic and antiproliferative agents is the chymotrypsin-like activity of the 20S proteasome. Modulation of this enzymatic activity by β5-subunit specific inhibitors may convey an antitumor effect by induction of cell cycle arrest and apoptosis in tumor cells.

Early synthetic proteasome inhibitors were relatively non-specific compounds but proved to be valuable molecular probes for improving the understanding of biological processes associated with the ubiquitin-proteasome pathway.[429] To overcome the inherent drawbacks of these molecular probes, new classes of inhibitors that target this proteolytic enzyme have emerged in the past few years by natural product screening and by combining traditional drug discovery approaches with new methods to fine and optimize lead structures.

6.1.1. Bortezomib. Bortezomib (**186**; PS-341, LDP-341, Velcade; Millennium Pharmaceuticals; Figure 82) has been the first and probably the only 20S proteasome inhibitor to enter the clinical trials thus far.[430] This dipeptidyl boronic acid derivative is a slow, tight-binding inhibitor of the chymotryptic activity of the 20S proteasome (K_i = 0.6 nM) and shows at least 500-fold selectivity over a series of enzymes (e.g., K_i = 2.3 μM, human leukocyte elastase; K_i = 630 nM, human cathepsin G; K_i = 320 nM, human chymotrypsin; and K_i = 13 μM, thrombin).[431,432] Preclinical studies showed that this compound had antitumor activity against a variety of solid tumors, including carcinomas of the breast,[433] lung,[434] colon,[435] bladder,[436] ovary,[437] pancreas,[438] prostate,[437] and glioblastoma multiforme (GBM).[439] Bortezomib prevented carcinoma metastases alone and in combination with other standard anticancer agents (e.g., fluorouracil, gemcitabine, etoposide) or radiation in a variety of hematological malignancies and solid tumor in mice models (e.g., prostate, colon, and lung human xenografts). All combination therapies were generally well tolerated. In the case of multiple myeloma — the indication in which the best results have so far been observed in the clinic — the compound abrogated TNF-α induced NFκB activation and IL-6 secretion, and decreased the binding of multiple myeloma cells to bone marrow stromal cells (Figure 82). The compound

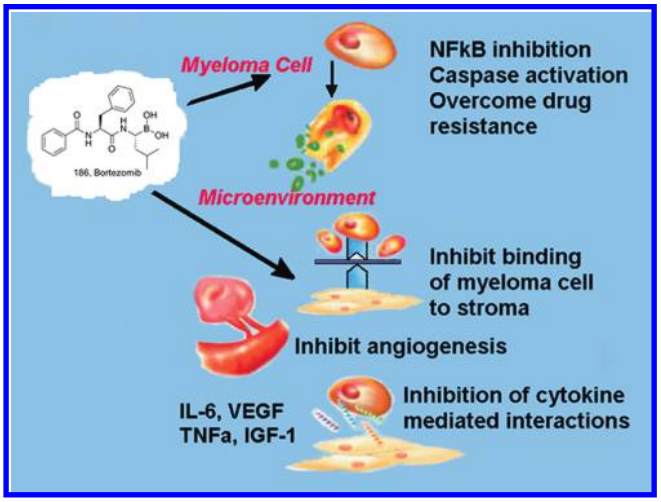


Figure 82. Mechanism of action of bortezomib in myeloma. Inhibition of the proteasome pathway by bortezomib has effects on both the myeloma cell and its microenvironment: NF-κB, nuclear factor B; IL-6, interleukin-6; VEGF, vascular endothelial growth factor; TNF-α, tumor necrosis factor alpha; IGF-1, insulin-like growth factor-1.[442]

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156−4220

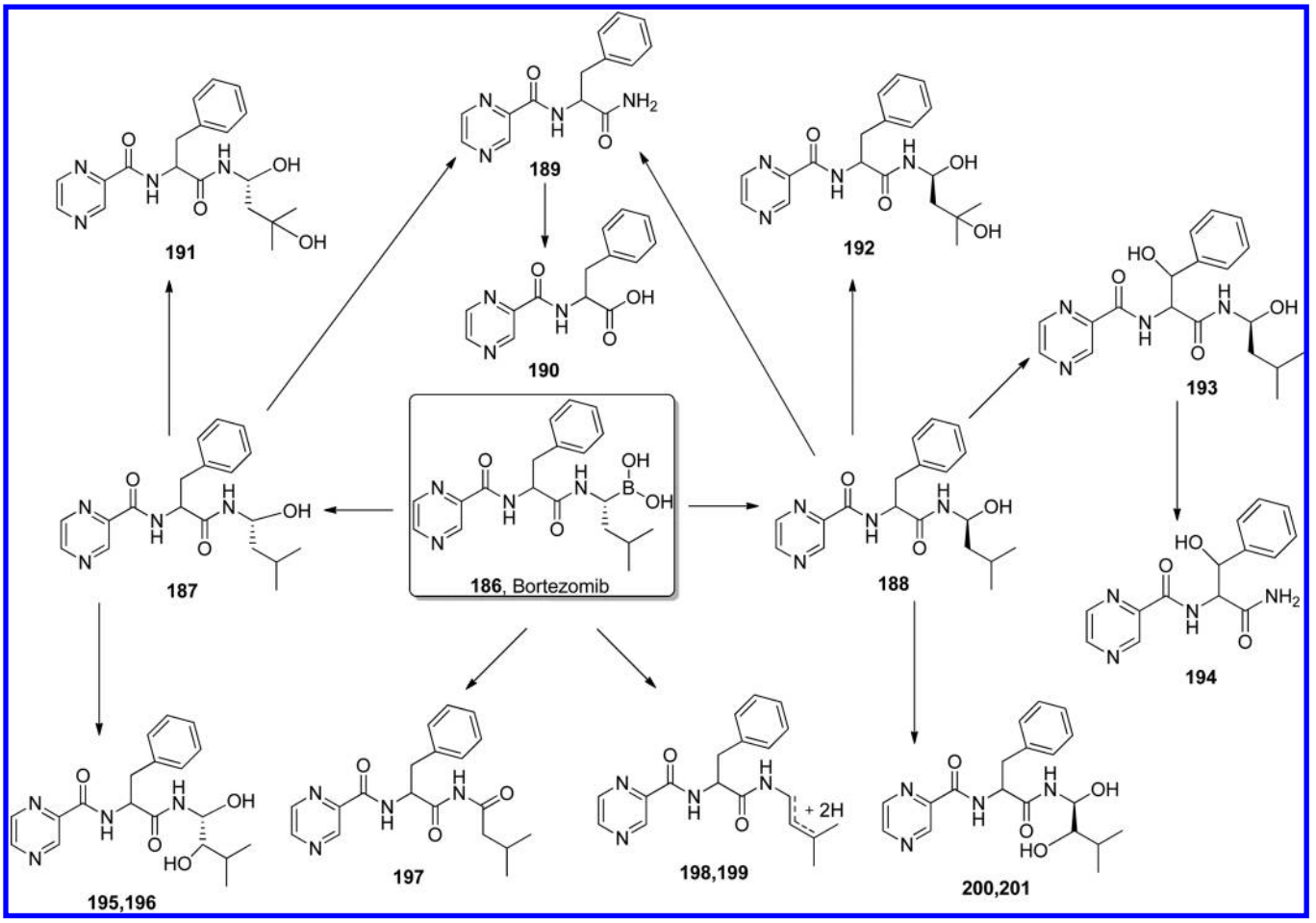


Figure 83. Proposed mechanism of bortezomib deboronation in humans.

passed all the preclinical requirements, and a broad phase I trial to assess its maximum tolerated dose in several tumors was initiated in 1998. Largely on the basis of the results obtained in Study 025 (response rate was 35%, including 10% positive complete responses), the compound, which was granted first track statues for the treatment of multiple myeloma in 2002, was approved in May 2003 by the U.S. FDA for the treatment of patients with multiple relapsed or refractory multiple myeloma — patients who have previously been treated with conventional chemotherapy alone or followed by high dose chemotherapy and stem cell transplant.[430] The most clinically significant toxicity was cumulative dose-related peripheral sensory neuropathy. Bortezomib has been investigated in lung cancer clinical trials, in which it has shown activity as a single agent and in combination regimens.[440] As of now, ongoing clinical trials are evaluating bortezomib in a variety of cancers, including refractory metastatic colorectal cancers, lymphomas, and leukemias. These trials are expected to provide critical data to confirm the activity of this agent in a variety of cancers and to clarify its potential adverse effect profile further.[441]

The exact mechanism of cytotoxicity and selectivity of bortezomib for transformed cells is known. A large number of proteins that are involved with carcinogenesis are known to be regulated by the ubiquitin−proteasome system of degradation, including transcription factors such as activator protein-1 and p53, signal transduction molecules such as Jak2 and cbl, the cell cycle regulators p21 and p27, and regulatory factors such as the nuclear factor-nB inhibitors InBa, h, q, and p100. Many laboratories have investigated these proteins as potential targets of bortezomib-mediated cytotoxicity, and these studies have clearly shown that bortezomib is a potent inhibitor of the 26S proteasome with effects that can be directly related to protein stabilization.[443−445] However, the molecular switch that initiates cell death in this pathway has not yet been identified. A recent study has shown that intracellular Ca^{2+} disregulation is a critical determinant of bortezomib cytotoxicity.[446]

Bortezomib (**186**) is cleared rapidly from the plasma compartment, with over 90% elimination within 15 min of intravenous administration. It is metabolized by cytochrome P450 liver microenzymes (3A4, 2D6, 2C19, 2C9, and 1A2) into several inactive deboronated hydroxylated metabolites.[447] On this basis, a bioassay that measures residual proteasome activity has been developed to enable accurate dosing of bortezomib in phase I trials. The bioassay can determine proteasome activity from whole blood or white blood cells and, by using biopsy samples, can also quantify the effect of the drug at the tumor site. Although the assay will likely not be required in clinical practice, it has been a critical tool for guiding dose escalation in phase I trials. Bioassay testing in volunteers indicated minimal intersubject and intrasubject variation in proteasome activity. The effects of the drug are clearly dose related, with the dose resulting in 80% proteasome inhibition estimated to be 1.96 mg/m^2. In rats, bioassay testing and whole-body autoradiography after administration of [^{14}C] bortezomib indicated that most organs received approximately the same amount of drug; bortezomib was not detected in the central nervous

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

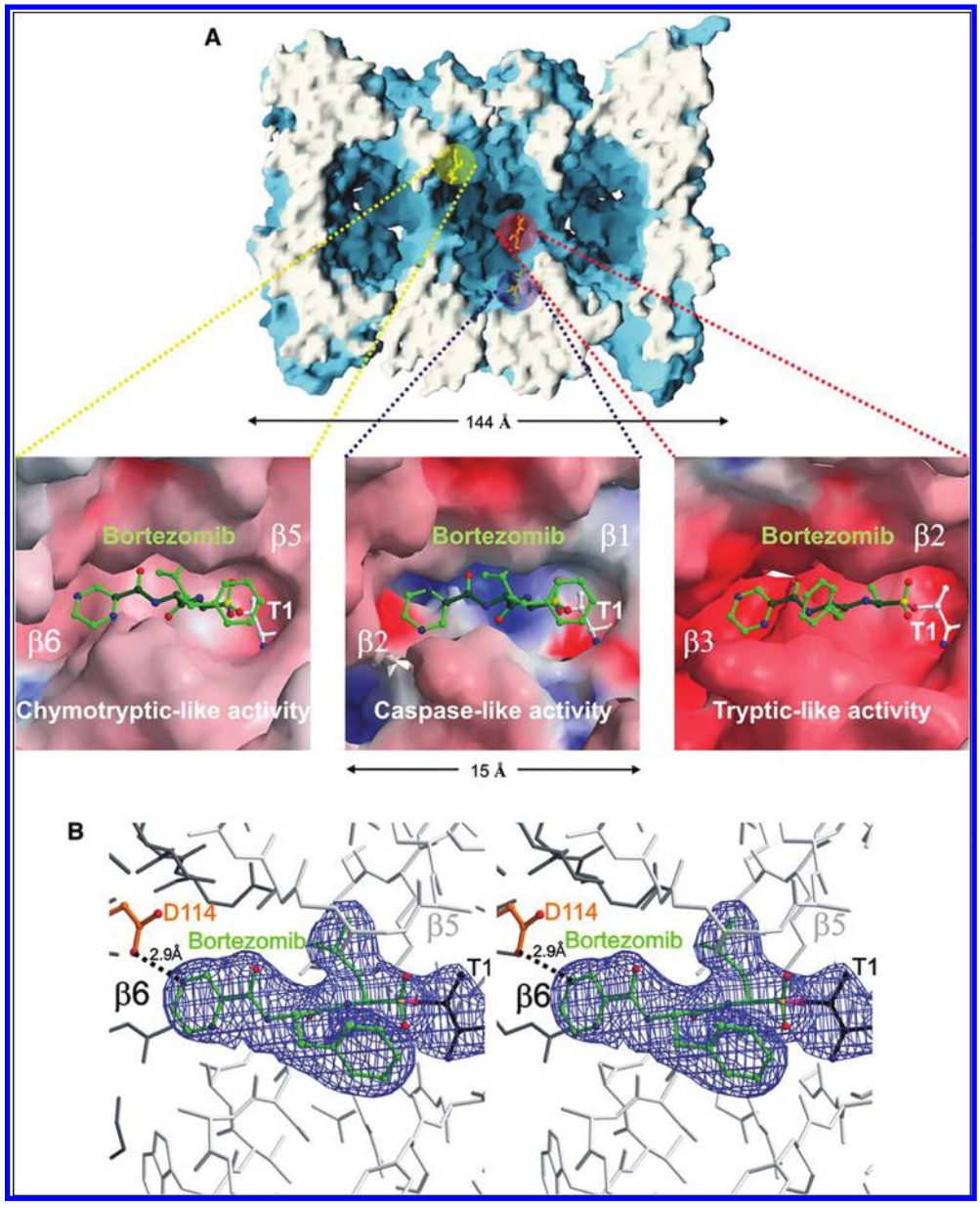


Figure 84. Structural representation of bortezomib bound to distinct active sites (A) Surface representation of the yeast 20S proteasome (blue) crystallized in the presence of bortezomib, clipped along the cylindrical pseudo-7-fold symmetry axis (white). The various proteolytic surfaces are marked by a specific color coding: red = subunit b1; blue = subunit b2; and green = subunit b5. Cleavage preferences, termed chymotryptic-like, caspase-like, and tryptic-like activity, are zoomed and illustrated in surface representations; the nucleophilic threonine and bortezomib are presented as a ball-and-stick model. Basic residues are colored blue, acidic residues are red, and hydrophobic residues are white (B) Stereorepresentation of the chymotryptic-like active site of the yeast 20S proteasome (white and gray) and bortezomib (green). Covalent linkage of the inhibitor with the active site Thr1 of subunit b5 is drawn in pink. The electron density map (blue) is contoured from 1.2s, with 2Fo-Fc coefficients after 2-fold averaging. Apart from the bound inhibitor, structural changes were noted only in the specificity pockets. Temperature factor refinement indicates full occupancy of bortezomib bound to the chymotryptic-like active site. Bortezomib has been omitted for phasing. Reprinted with permission from ref 455. Copyright 2006 Elsevier.

system, testes, or eyes.[448] The results of further animal studies indicated that baseline proteasome activity is restored 48−72 h after cessation of bortezomib treatment.

Toxicology studies in rodents and primates revealed that anorexia, vomiting, and diarrhea were the primary adverse events associated with bortezomib; bone marrow toxicity was not seen in primates. Gastrointestinal adverse events were dose related, with bortezomib being generally well tolerated until proteasome blockade exceeded 80%. Higher levels of blockade led to more pronounced gastrointestinal toxicity and changes in blood pressure and heart rate in primate models.[449]

Recent studies[450] found that vitamin C has a negative effect on bortezomib-mediated anticancer activity. It abrogated the ability of bortezomib to induce apoptosis in various human cancer cell lines, to induce G_2-M arrest, and to augment apoptosis induced by tumor necrosis factor-related apoptosis−inducing ligand. Moreover, vitamin C suppressed bortezomib-mediated inhibition of proteasome activity. Therefore, it was found that vitamin C directly binds to bortezomib, thus inactivating bortezomib independent of its antioxidant activity.

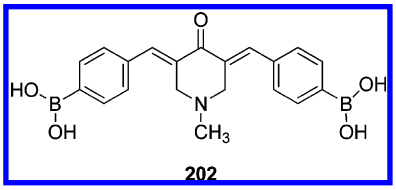
The inhibition of the 26S proteasome involves the formation of a dative bond between the N-terminal threonine residue of

the chymotryptic site and the boron atom of bortezomib.[451] Hence, deboronation represents a deactivation pathway for this chemotherapeutic agent. Researchers recently described the in vitro and in vivo metabolism of bortezomib in humans.[452] The identification and characterization of metabolites of bortezomib in humans indicated deboronation to be the principal route of metabolism, resulting in the formation of a pair of diasteriomeric carbinolamide metabolites, M1 and M2 (Figure 83). Secondary metabolites of M1 and M2 resulted in a number of metabolites, most of which carried an additional oxidation of the leucine moeity. It was also demonstrated that multiple cytochrome P450 enzymes contributed to the in vitro deboronation of bortezomib in humans, including P450s 1A2, 2C9, 2C19, 2D6, and 3A4.[452,453] However, the mechanism of P450-catalyzed oxidative deboronation of bortezomib resulting in the formation of diastereomeric carbinolamide metabolites M1 and M2 remained unknown.

In an effort to understand this mechanism, the in vitro metabolism of bortezomib in human liver microsomes, cDNA-expressed P450 enzymes, and chemical model reactions were examined. In vitro results from human liver microsomes and human cDNA-expressed cytochrome P450 enzymes (P450) indicated a role for P450 in the deboronation of bortezomib. Use of ^{18}O-labeled oxygen under controlled atmospheres confirmed an oxidative mechanism in the P450-mediated deboronation of bortezomib, as ^{18}O was found incorporated in both M1 and M2 (Figure 83). Chemically generated reactive oxygen species (ROS), such as those generated as byproducts during P450 catalysis, were also found to deboronate bortezomib resulting in the formation of M1 and M2. Known to undergo efficient redox cycling, P450 2E1 was found to catalyze the deboronation of bortezomib predominantly to the carbinolamide metabolites M1 and M2, as well as to a pair of peroxycarbinolamides, 2 and 3. The presence of superoxide dismutase (SOD) and catalase prevented the deboronation of bortezomib, thus supporting the involvement of ROS in the P450 2E1-catalyzed deboronation reaction. The presence of SOD and catalase also protected bortezomib against P450 3A4-catalyzed deboronation, albeit to a lesser extent. The remaining deboronation activity observed in the P450 3A4 reaction may suggest the involvement of the more conventional activated enzyme-oxidants previously described for P450. These findings indicated that the oxidase activity of P450 (i.e., formation of ROS) represents a mechanism of deboronation.[454]

In further trying to establish the bortezomib mode of action at the molecular level, the crystal structure of the yeast 20S proteasome in complex with bortezomib was determined[455] (Figure 84). The structural data for the bortezomib-proteasome complex in this study explained the different in vivo binding affinities of bortezomib for the individual subunits at atomic resolution. Subunit specificities can roughly be attributed to interactions of the leucine, pyrazine, and boronate moieties. The data immediately suggested possibilities for the design of bortezomib derivatives with superior inhibition characteristics, and for the design of inhibitors that will specifically bind to single proteolytically active sites. Thus, this crystal structure of this 20S-bortezomib complex might serve as a new lead for the rational design of selective boronic acid-based proteasome inhibitors.

6.1.2. Other Boron Containing Molecules That Inhibit the Proteasome. Chalcones and their derivatives are a group of compounds reported to exhibit promising anticancer activity. However, the exact mechanism of action of chalcones in tumor cells remains to be elucidated. On the basis of the structural and biochemical evidence that carboxylic chalcones disrupt the murine double minute 2 protein-p53 (MDM2-p53) interaction by binding the p53-binding domain of MDM2,[456] a series of boronic acid derivatives of chalcones were synthesized for further evaluation. Preliminary studies conducted with a primary series of boronic derivatives of chalcones demonstrated that these compounds show 5−10-fold greater growth inhibition in human breast cancer cell lines than in normal breast epithelial cells.[457] In addition, some of these compounds also induced accumulation of p53 and p21 proteins and showed significantly greater cytotoxicity in cells with p53 compared with p53 null cells. Later, a new series of boronic chalcones were evaluated for their anticancer activity and mechanisms of action. Among the eight chalcone derivatives tested, 3,5-bis-(4-boronic acid-benzylidene)-1-methyl-piperidin-4-one (AM114) (**202**, Figure 85) exhibited most potent growth inhibitory activity with IC_{50}



202

Figure 85. Chalcone derivative, 3,5-bis-(4-boronic acid-benzylidene)-1-methyl-piperidin-4-one known as AM 114.

values of 1.5 and 0.6 μM in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and colony formation assay, respectively. The cytotoxic activity of AM114 was shown to be associated with the accumulation of p53 and p21 proteins and induction of apoptosis. Mechanistic studies showed that AM114 treatment inhibited the chymotrypsin-like activity of the 20S proteasome in vitro, leading to a significant accumulation of ubiquitinated p53 and other cellular proteins in whole cells. In vitro studies showed that AM114 did not significantly disrupt the interaction of p53 and murine double minute 2 protein. It was noteworthy that AM114 as a single agent was preferentially toxic to cells with wild-type p53 expression, whereas combination of this compound with ionizing radiation (IR) significantly enhanced the cell-killing activity of IR in both wild-type p53 and p53-null cells. Together, these results indicated that the boronic chalcone derivative AM114 induced significant cytotoxic effect in cancer cells through the inhibition of the cellular proteasome and provided a rationale for the further development of this class of compounds as novel cancer chemotherapeutic agents.[458]

Further studies showed that different boronic acid-based compounds proved to be potent inhibitors of the proteasome. Of these, the borodipeptide *N*-(2,3-dihydro-1,4-benzodioxin-2-ylcarbonyl)-L-phenylalanyl-(*R*)-boropheneylalanine (**203**) (IC_{50} of 0.048, 0.34, 0.56, and 1.5 ng/mL against bovine spleen proteasome, bovine kidney proteasome, human immunotype proteasome, and human constitutive proteasome, respectively),[459] a lactam boronic acid-based compound (**204**) (IC_{50} = 0.031 μM against the 20S-proteasome),[460] and Ala-boroPro(thioxamide) (**205**)[461] (Figure 86).

More inhibitors based on a boronic electrophile have been reported.[462,463] For example, significant inhibition in B16 tumor growth was observed in female C57BL mice after i.p. administration of compound (**206**, Figure 87) at 10 mg kg^{-1} day^{-1}.[462] This compound is an analogue of CEP-1612,[464] a C-

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

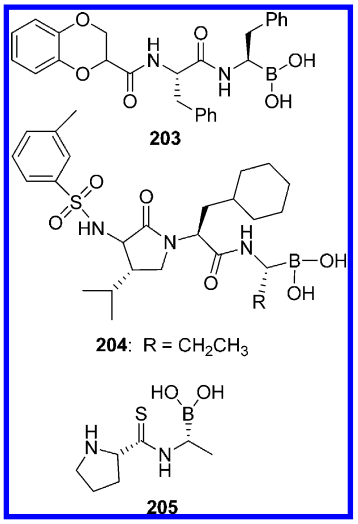


Figure 86. Boronic acid-based inhibitors of the proteasome.

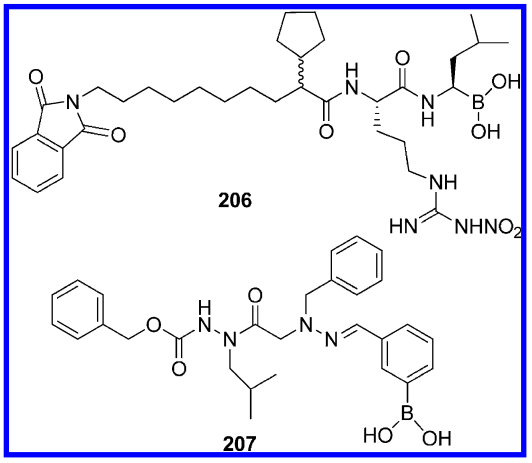


Figure 87. Peptidyl and peptoid boronic acids.

terminal aldehyde peptidomimetic. In addition, and to overcome some of the pharmaceutical limitations inherent in peptide, hydrazine-azapeptoids[465] derivatives containing a reactive boronic acid in a phenyl group were synthesized (e.g., compound (**207**), Figure 87; K_i = 53 μM, chymotrypsin-like activity). In spite of having the reactive "war-head" group, these peptidomimetics have in general weak activity in inhibiting the chymotrypsin-like activity of the 20S proteasome and show modest antiproliferative activity against a panel of tumor cells lines (IC_{50} ranging from 1 to 80 μM). Although not supported with structural data, the low affinity of these boronic acid derivatives for the proteasome was explained due to steric hindrance effects. But it is still unclear which of the structural features of the molecule are involved in these negative interactions.

Later, an extensive structure–activity relationship (SAR) study of 72 dipeptidyl boronic acid proteasome inhibitors constructed from β-amino acids was reported.[466] SAR analysis revealed that bicyclic groups at the R1 position, 3-F substituents at the R2 position, and bulky aliphatic groups at the R3 position were favorable to the activities. Enzymatic screening results showed that compound (**208**, Figure 88), comprising all of these features, was the most active inhibitor against the 20S human proteasome at less than a 2 nM level, as active as the marketed drug bortezomib. Cellular assays

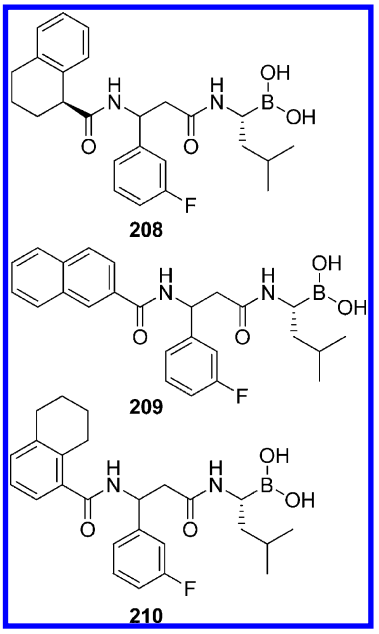


Figure 88. Dipeptidyl boronic acid proteasome inhibitors.

confirmed that compound (**208**) was the most potent against two hematologic and some solid tumor cells with IC_{50} values less than 1 nM. The compounds **209** and **210** have 3 and 3.9 nM of IC_{50}, respectively. Pharmacokinetic profiles suggested that **208** showed higher plasma exposure and a longer half-life than bortezomib.

In a related study, a series of novel dipeptidyl boronic acid proteasome inhibitors composed of β-amino acids were synthesized, in vitro and in vivo biologically evaluated, and theoretically modeled.[467] From the screened racemic compounds in enzyme, (*RS*,*R*)-(**211**) (Figure 89) was the most

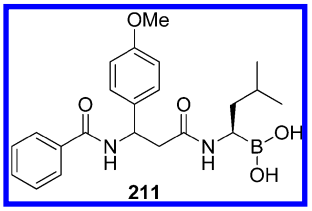


Figure 89. Structure of the (*R*,*R*)-**211**.

active. The IC_{50} value of its pure enantiomer (*R*,*R*)-**211** was 9.6 nM, 36-fold more active than its isomer (*S*,*R*)-**211** and as active as the marketed bortezomib in inhibiting human 20S proteasome. This candidate also showed good activities with IC_{50} values nearly less than 5 μM against several human solid and hematological tumor cell lines. Safety evaluation in vivo with zebrafish and Sprague–Dawley (SD) rats showed that the candidate (*R*,*R*)-**211** was less toxic than bortezomib and its pharmacokinetic profiles showed more plasma exposure and longer half-life than bortezomib. Docking results indicated that (*R*,*R*)-**211** nearly interacted with 20S proteasome in a similar way as bortezomib.

The bortezomib is remarkably effective against multiple myeloma (MM) but not against solid tumors. Dose-limiting adverse effects from "on target" inhibition of the 26S proteasome in normal cells and tissues appear to be a key obstacle. Achieving efficacy against solid tumors therefore is likely to require making the inhibitor more selective for tumor

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

tissue over normal tissues. A new study has shown that dipeptides of boroLeu can be sufficiently potent, cell penetrating, cytotoxic, resistant to degradation by cellular proteases and can serve as warheads in the construction of prodrugs designed to selectively target the proteasome in solid tumors.[468] The candidates that best fulfills all of the desired properties were **215**−**217**, and **212**−**214** (Figure 90) might

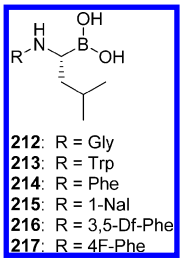


Figure 90. Dipeptides of boroLeu. Nal = naphthylalanyl; Df-Phe = difluorophenylalanyl.

also be suitable. The dipeptides of boroLeu can also undergo a pH-dependent cyclization, which, although relatively modest compared to that exhibited by dipeptides of boroPro, might in some cases be sufficient to provide additional tissue specificity by limiting systemic exposure to the active proteasome inhibitor following activation at the tumor. This phenomenom could make **212** one of the better candidates, as its true cytotoxicity could be 20-fold better than the values for a short time following release from a prodrug at the tumor.

6.2. *Mycobacterium tuberculosis* (Mtb) Proteasome

Mycobacterium tuberculosis (Mtb) has the remarkable ability to resist killing by human macrophages. The 750 kDa proteasome, not available in most eubacteria except *Actinomycetes*, appears to contribute to Mtb's resistance. The crystal structure of the Mtb proteasome at 3.0 Å resolution reveals a substrate-binding pocket with composite features of the distinct β1, β2, and β5 substrate binding sites of eukaryotic proteasomes, accounting for the broad specificity of the Mtb proteasome toward oligopeptides.[469] The substrate entrance at the end of the cylindrical proteasome appears open in the crystal structure due to partial disorder of the α-subunit N-terminal residues. However, cryo-electron microscopy of the core particle reveals a closed end, compatible with the density observed in negative-staining electron microscopy that depended on the presence of the N-terminal octapetides of the α-subunits in the companion article, suggesting that the Mtb proteasome has a gated structure.

Proteasome inhibition can induce cell-cycle arrest and apoptosis in cancer cells. *N*-(4-Morpholine)carbonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid (MLN-273) (Figure 91), an analogue of the antimyeloma drug bortezomib is a slow, tight-binding reversible inhibitor of the proteasome from *Rhodococcus* and Mtb,[451] and thus sensitize *M. tuberculosis* to reactive nitrogen intermediates and the host immune system.[470] The proteasomal inhibition mechanism of the dipeptidyl boronate was determined. The crystal structure reveals the inhibitor in the proteasomal substrate-binding pocket and elucidates its mechanism of action. X-ray crystallography revealed that Mtb proteasome has a chimeric substrate-binding surface that potentially explains its broad specificity toward oligopeptides. The structure improved prospects for designing Mtb-specific proteasomal inhibitors as a novel approach to chemotherapy of tuberculosis.[471]

6.3. The Plasmodium Parasites

Plasmodium falciparum, the causative agent of the most severe forms of malaria, is one of the leading causes of death in tropical Africa, Asia, and South America. Despite the fact that current antimalarial drugs are effective and well tolerated, there is a tendency toward the selection of drug-resistant parasites, the major example being resistance to chloroquine and its derivatives.[472]

Taking advantage of years of development and safety testing of bortezomib in humans and its known inhibitory activity on the 20S proteasome subunit, it was decided to investigate the antimalarial potential of these compounds. MLN-273 (morpholino-naphthylalanine-Leu-boronate) (Figure 92), a novel small molecule proteasome inhibitor with similar properties to bortezomib,[473] was chosen. MLN-273 has the advantage of a longer halflife than bortezomib and was hence a better therapeutic agent for infectious disease indications. A less frequent dosing regimen also meant fewer side effects. Recent findings that MLN-273 also acted on mycobacterial proteasomes have lent support for its further development in the treatment of diseases other than cancer. In the case of Plasmodium, the ability of the drug to completely arrest parasite growth made it a very interesting drug to be exploited as a new therapeutic agent against malarial infections.

Later, MLN-273 was tested on blood and liver stages of Plasmodium species, both of which undergo active replication, probably requiring extensive proteasome activity (Figures 92 and 93). The inhibitor blocked *Plasmodium falciparum* erythrocytic development at an early ring stage as well as *P. berghei* exoerythrocytic progression to schizonts. Importantly, neither uninfected erythrocytes nor hepatocytes were affected by the drug. MLN-273 caused an overall reduction in protein degradation in *P. falciparum*, as demonstrated by immunoblots using antiubiquitin antibodies to label ubiquitin-tagged protein conjugates. This led the authors to conclude that the target of the drug was the parasite proteasome. The fact that proteasome inhibitors are presently used as anticancer drugs in humans formed a solid basis for further development and made them potentially attractive drugs also for malaria chemotherapy.[474]

7. ARGINASE INHIBITORS

Arginase is a 105 kDa homotrimeric enzyme that requires manganese for the hydrolysis of L-arginine to form the L-ornithine and urea. Two genetically distinct isoenzymes have evolved with differing tissue distributions and subcellular locations in mammals.[475,476] Arginase I is found predominantly in the liver, where it catalyzes the final cytosolic step of the urea cycle.[477,478] Arginase II is a mitochondrial enzyme widely distributed in numerous tissues, for example, kidney, brain, skeletal muscle, and liver.[479,480] In addition, arginase activity has been detected in a number of nonhepatic tissues that lack a complete urea cycle, for example, in lactating mammary gland,[481] and recently, it has been detected in certain human colon cancer and human breast cancer cell lines.[482,483] The human type I and type II arginases are related by 58% sequence identity[484] and are immunologically distinct.[485−487] Genetic "knockout" experiments suggest that arginase II functions in L-arginine homeostasis by regulating L-arginine concentrations for cellular biosynthetic reactions such as nitric oxide (NO) biosynthesis.[488] Since L-arginine is the substrate of arginase and

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156−4220

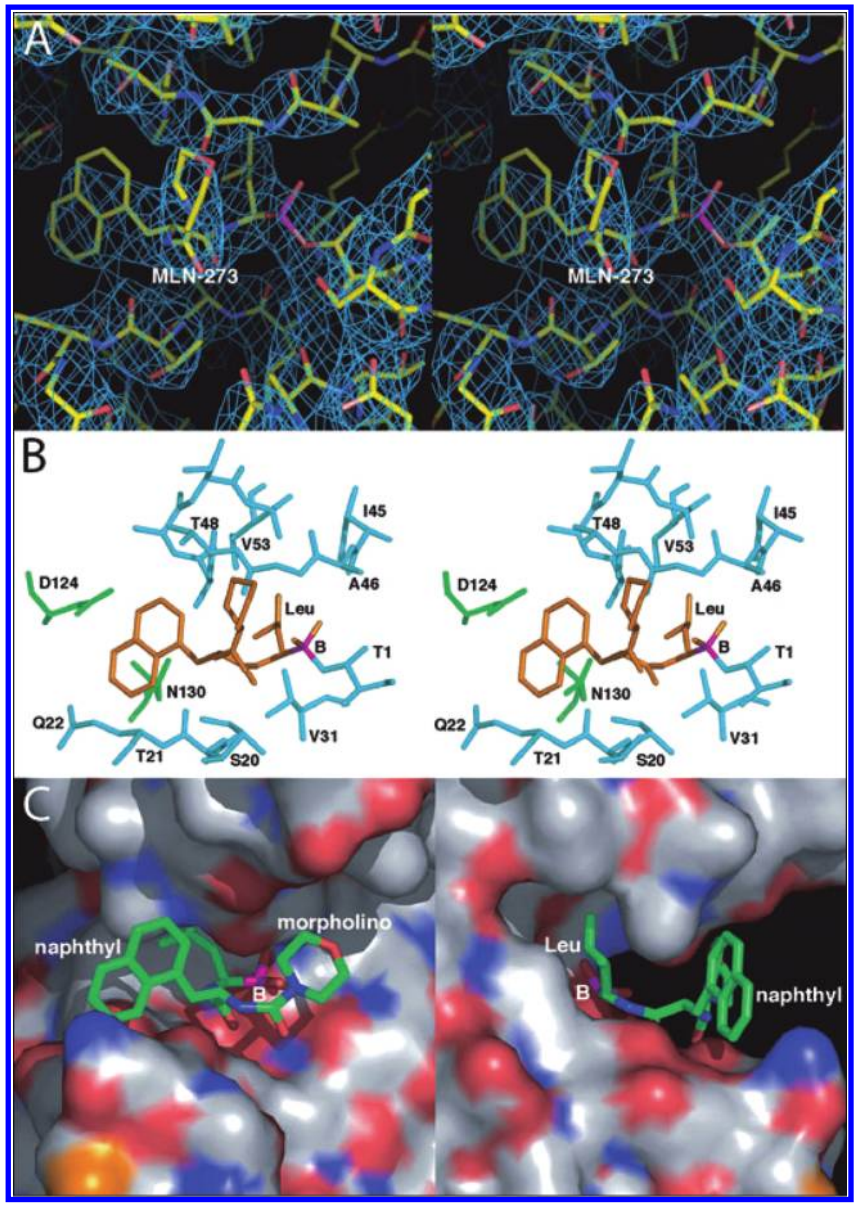


Figure 91. (A) Stereoview of $2F_o - F_c$ electron density map calculated from 50 to 3.0 Å, and contoured at 1.5σ. The map is drawn 8 Å around the active site Thr1 and the covalently bound MLN-273. The final model drawn as sticks is superimposed with the density map. The boron atom is colored magenta. (B) Stereoview of the active site structure drawn as sticks. The inhibitor MLN-273 is colored orange, except the boron atom in magenta. The molecule is oriented so that the residues above the inhibitor (Ile45, Ala46, Thr48, Ala49, and Val53, in blue) form the upper surface, and the residues below the inhibitors (Ser20, Thr21, Gln22, and Val31, colored blue) form the lower surface of the substrate-binding pocket, respectively. Residues from the adjacent β-subunit (Asp124 and Asn130, in green) form the back surface of the substrate-binding pocket. The Thr1O forms a tetrahedron with the boronate. The leucine side-chain of the MLN-273 faces a large hydrophobic pocket formed by the upper surface residues. (C) Two surface rendered near-orthogonal views of the substrate-binding pocket (C in white, N in blue, O in red, S in orange) with the inhibitor MLN-273 drawn as sticks (C in green, N in blue, O in red, B in magenta). The naphthyl and morpholino groups of MLN-273 occupy the large gap between the upper and the lower surface of the substrate-binding pocket. There is an extra space near the leucine side-chain of the MLN-273 in the substrate-binding pocket as revealed in the right panel. Reprinted with permission from ref 471. Copyright 2006 Wiley.

NO synthase, arginase activity can effectively inhibit NO-dependent processes by depleting the substrate pool available for NO biosynthesis.[489−493] Conversely, arginase inhibition can effectively enhance NO biosynthesis and NO-dependent processes by enhancing L-arginine bioavailability to NO synthase.[494−499] The arginase catalytic mechanism include (1) a precatalytic binding side chain for arginine in which the side chain of Glu277 plays a role in substrate recognition, (2) attack of a nucleophilic metal-bridging hydroxide ion, (3) formation of a neutral tetrahedral intermediate that is stabilized by the neutral Mn(II) center, and (4) a possible role for His141 as a proton shuttle in mediating proton transfer between the active site and bulk solvent.

The discovery NO synthase, which catalyzes the oxidation of arginine to form NO and citrulline, has generated significant interest in the interplay between the NO synthase and arginase pathways. A number of tissues express both type I and type II arginases as well as nitric oxide synthase, and because these enzymes compete for a common substrate, L-arginine, the coexpression of these enzymes raises interesting questions

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156−4220

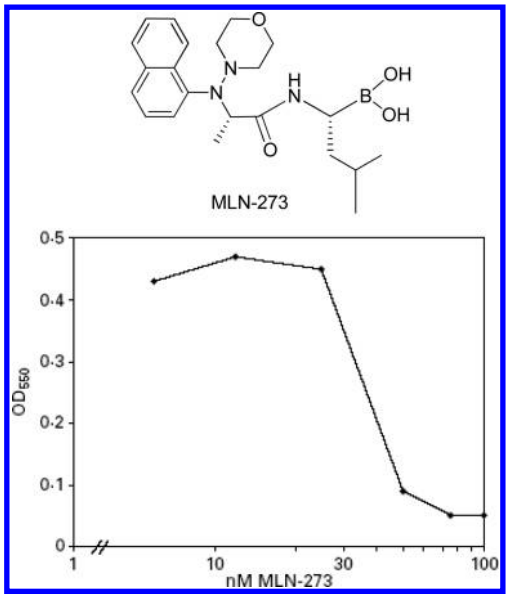


Figure 92. Inhibition of *Plasmodium falciparum* growth by MLN-273. *P. falciparum* strain Dd2 was incubated with 6, 12, 25, 50, 75, and 100 nM MLN-273 for 72 h and parasitaemia was determined by HRP2-ELISA. Each value on the curve is the average of two different experiments. Reprinted with permission from ref 474. Copyright 2005 Cambridge University Press.

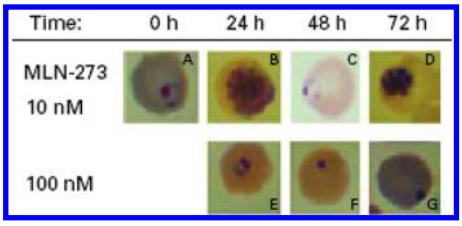


Figure 93. Cell cycle arrest of *Plasmodium falciparum* after treatment with MLN-273. Synchronized *P. falciparum* cultures (A) were treated with 0, 10 (B–D) or 100 nM (E–G) MLN-273 and grown for 24 h (B, E), 48 h (C, F), and 72 h (D, G). Giemsa-stained thin blood smears were then analyzed by light microscopy. The pictures observed in the absence or presence of 10 nM of the drug were very similar to each other and hence only the results using 10 nM of the drug are shown. Reprinted with permission from ref 474. Copyright 2005 Cambridge University Press.

concerning the regulation of arginine flux through the competing pathways. The reciprocal nature of the regulation of NOS and arginase activity has only been recently appreciated and, in part, stems from the development of potent boronic acid-based inhibitors of type I and type II arginases.[495−498,500]

Boronic acids are potent competitive inhibitors of both arginase I and arginase II.[496,500−502] In the boronic acid analogues *S*-(2-boronoethyl)-L-cysteine (BEC, **220**) and 2(*S*)-amino-6-boronohexanoic acid (ABH, **221**) (Figure 94), the trigonal planar boronic acid moiety replaces the trigonal planar guanidinium group of L-arginine.[495,496,500,501,503] The electron-deficient boron atom in each of these L-arginine analogues is particularly susceptible to attach by a solvent nucleophile, such as metal-bridging hydroxide ion, to yield the tetrahedral boronate anion — just as nucleophilic attack of hydroxide ion at the guanidinium group of L-arginine yields the tetrahedral intermediate in catalysis.

Structures have been determined for complexes of arginase I and arginase II with the boronic acid inhibitors.[496,498] The

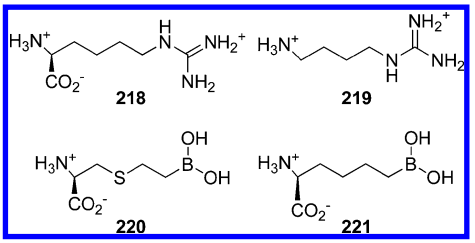


Figure 94. Selected substrates and inhibitors of rat arginase I: (**218**, L-homoarginine substrate, K_M (mM) = $K_{cat}/K_M \times 10^3$ (M^{-1} s^{-1}) = 2.8);[504] (**219**, agmatine substrate, K_M (mM) = 7.2, = 10.8, $K_{cat}/K_M \times 10^3$ (M^{-1} s^{-1}) = 0.048);[504] (**220**, BEC[496] inhibitor, K_i or K_d (μM) = 2.2, K_d = 0.27 μM against human arginase I); and (**221**, ABH[500,501,503] inhibitor, K_i or K_d (μM) = 0.11, K_d = 5 nM against human arginase I and K_i = 8.5 nM against human arginase II).

crystal structure of arginase I complexed with ABH (**221**, Figure 94) was the first to reveal molecular strategy for transition state stabilization in catalysis.[495] The tetrahedral boronate anion of ABH makes multiple interactions with the binuclear manganese cluster and neighboring protein residues: the boronate hydroxyl group O1 symmetrically bridges Mn^{2+}_A and Mn^{2+}_B (Mn^{2+}_A···O and Mn^{2+}_B···O separations = 2.2 Å) and donates a hydrogen bond to Oδ2 of D128; the boronate hydroxyl group O2 makes a 2.4 Å coordination interaction with Mn^{2+}_A and donates hydrogen bonds to Glu277 and the backbone carbonyl of His141; the boronate hydroxyl group O3 makes a water-mediated hydrogen bond interaction with Thr246 (Figure 95). The structures of rat and human arginases I and human arginase II complexed with BEC (**220**, Figure 94) reveal comparable intermolecular interactions.[496,498,505] The structure of the complex reveals that the boronic acid moiety undergoes nucleophilic attack by metal-bridging hydroxide ion to yield a tetrahedral boronate anion that bridges the binuclear manganese cluster, thereby mimicking the tetrahedral intermediate (and its flanking transition states) in the arginine hydrolysis reaction.

Given that ABH (**221**, Figure 94) binding mimics the transition state binding, it is clear that both Mn^{2+}_A and Mn^{2+}_B are critical for transition state stabilization: the metal-bridging hydroxyl group of the tetrahedral intermediate (corresponding to boronate hydroxyl group O1) remains close to its position as the former metal-bridging hydroxide ion of the native enzyme, and the vacant coordination site on Mn^{2+}_A in the native enzyme (Figure 96a)[506a] accommodates a sixth coordination interaction by the developing sp^3 lone electron pair on the former η2-NH_2 group of L-arginine (corresponding to boronate hydroxyl group O_2) (Figures 96b[506a] and 97[506b]).

Because the boronic acids ABH (**220**) and BEC (**221**) (Figure 94) are not inhibitors of NO synthase, they have been used to probe the role of arginase in the regulation of NO-mediated smooth muscle relaxation in the gastrointestinal tract,[500] as well as in the penile,[495,496] and clitoral[498] corpus cavernosum tissues. Both ABH and BEC enhance relaxation of these small muscle tissues in ex vivo organ bath experiments. Inhibitors of arginase activity thus enhance L-arginine concentration for NO biosynthesis and NO-dependent smooth muscle relaxation. Recent studies have shown that type I and type II arginases are located extrahepatically,[507] and therefore both enzymes may be involved in the regulation of NO production in mammals.

In other studies, enantiomerically pure ω-borono-α-amino acids of various chain lengths have been synthesized (**222**–**227**,

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

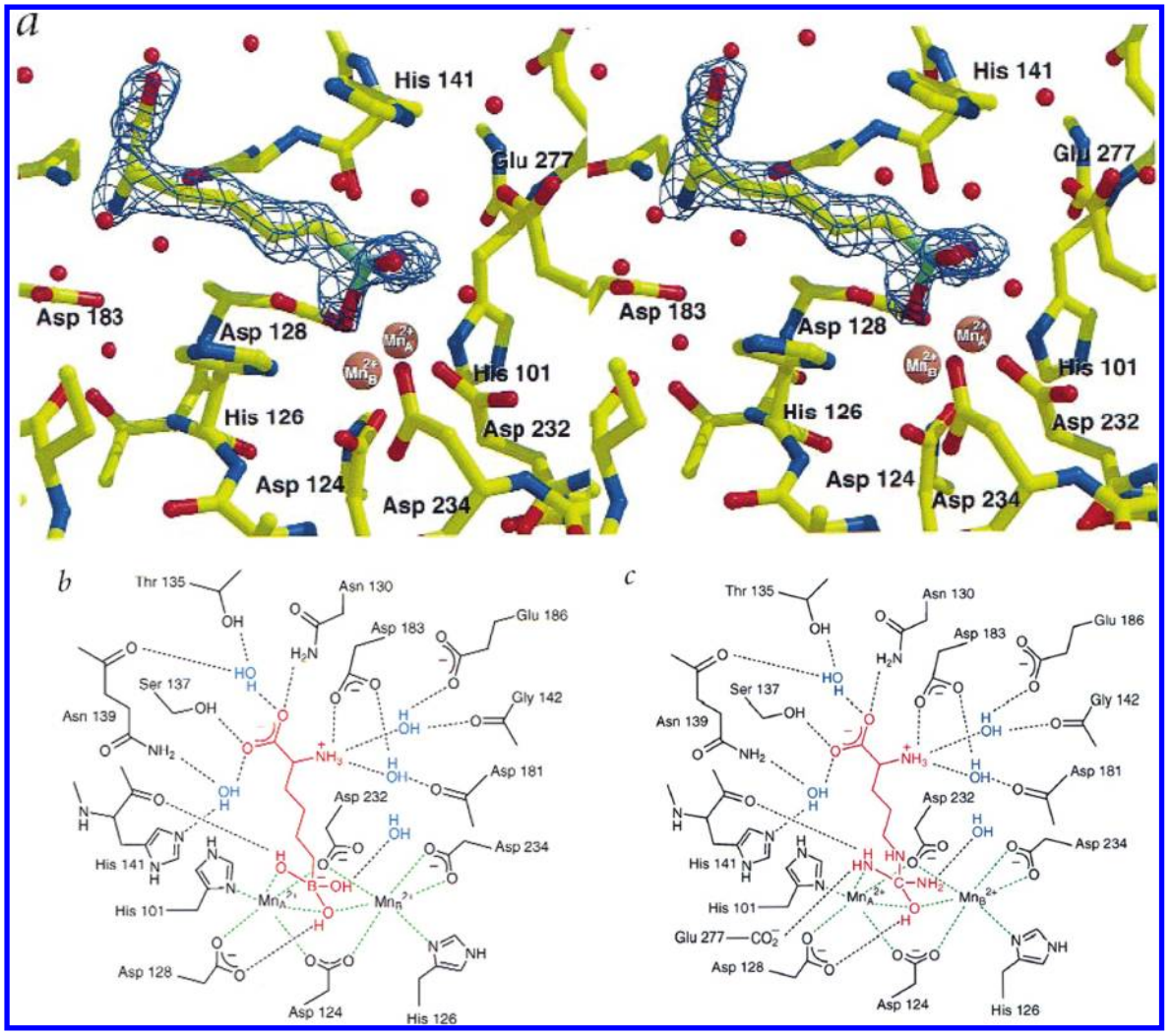


Figure 95. Arginase–ABH complex: (a) Omit electron density map of ABH in the arginase active site averaged over the two monomers in the asymmetric unit and averaged over the two twin domains A and B. The map is contoured at 7.7s and selected active site residues are indicated. Atoms are color-coded as follows: C = yellow, O = red, N = blue, B = pale green; water molecules appear as red spheres. This stereo drawing was generated with BOBSCRIPT and Raster3D. (b) Summary of arginase–ABH interactions; manganese coordination interactions are designated by green dashed lines, and hydrogen bonds are indicated by black dashed lines. (c) Stabilization of the tetrahedral intermediate (and flanking transition states) in the arginase mechanism based on the binding mode of ABH. Reprinted with permission from ref 495. Copyright 1999 Nature Publishing Group.

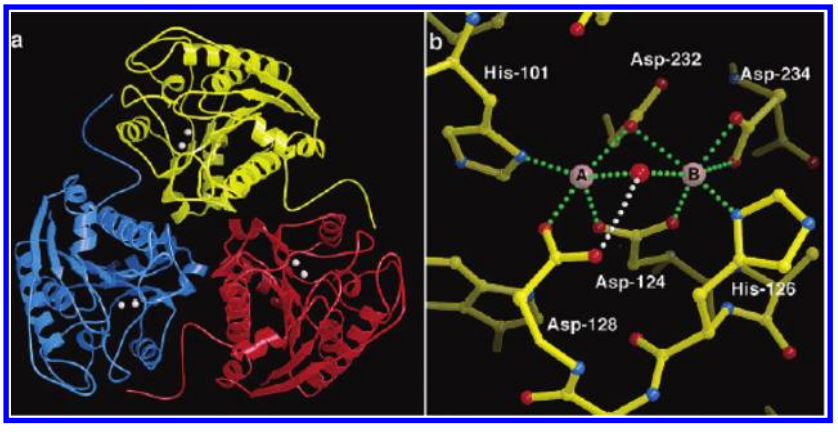


Figure 96. Panel (a) presents a ribbon plot of the arginase trimer. The binuclear manganese cluster is represented by a pair of spheres in each monomer. Panel (b) shows the binuclear manganese cluster of arginase. Metal coordination interactions are indicated by green dotted lines, and the hydrogen bond between the metal-bridging hydroxide ion (red sphere) and Asp128 is indicated by a white dotted line. Mn^{2+}_{A} is coordinated with square pyramidal geometry as a means of transition state stabilization in catalysis. Mn^{2+}_{B} is coordinated with octahedral geometry. Reprinted with permission from ref 506a. Copyright 1996 Macmillan Publishers Ltd.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

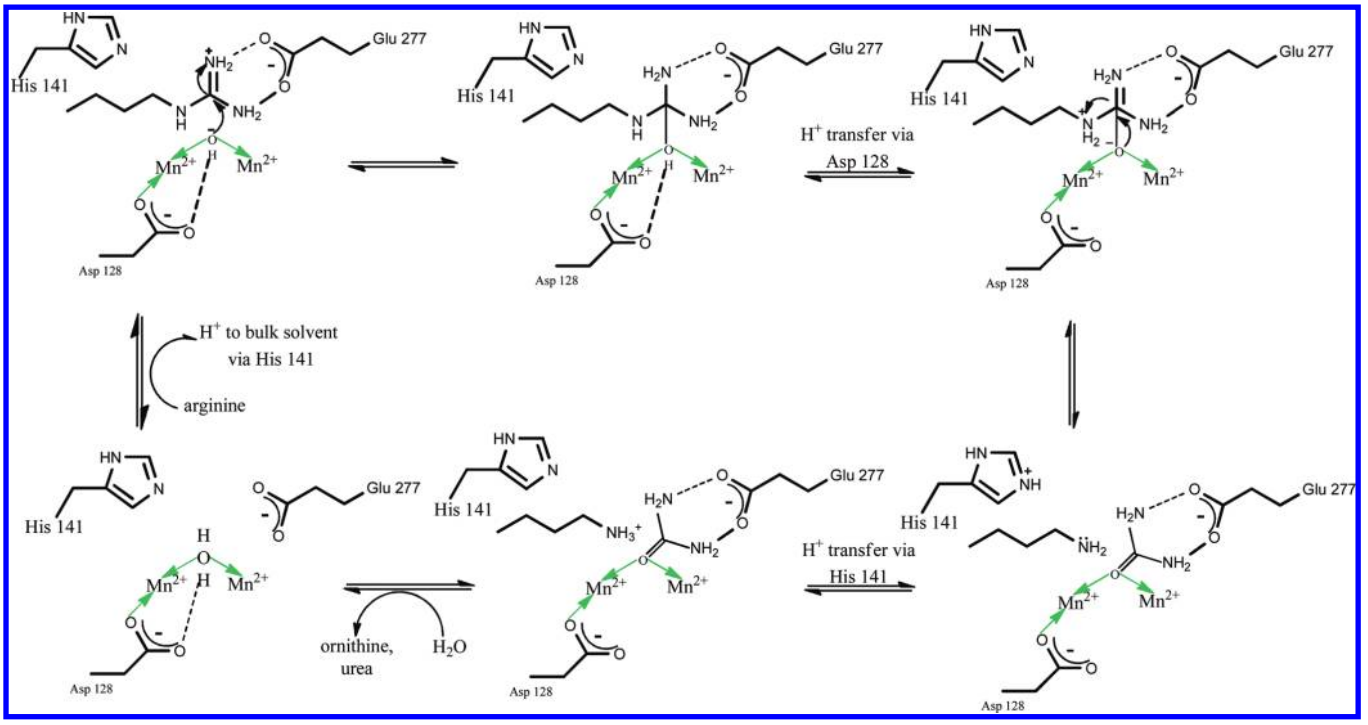


Figure 97. Structure-based mechanism of arginase,[506b] in which metal-activated hydroxide ion attacks the substrate guanidinium group to form a tetrahedral intermediate (for clarity, only the side chain atoms of substrate L-arginine are shown). Proton transfer mediated by Asp 128 facilitates collapse of this intermediate to form products L-ornithine and urea. Following product dissociation, a nucleophilic metal-bridging hydroxide ion bulk is regenerated from a metal-bridging water by proton transfer to bulk solvent. His 141 may function as a proton shuttle as indicated.

Figure 98). Some of these compounds act as potent inhibitors of rat liver and murine macrophage arginases, demonstrating

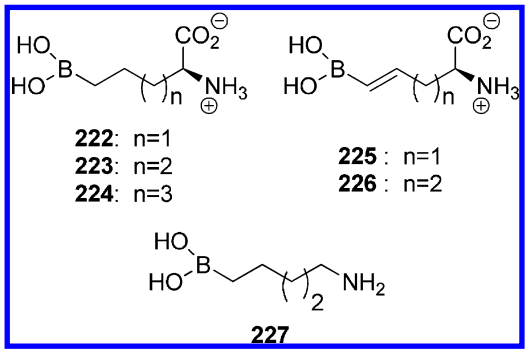


Figure 98. Structure of ω-borono-α-amino acids (**222**–**226**) and the decarboxylated analogue (**227**).

that distance between $B(OH)_2$ and α-amino acid groups is a key determinant for their interaction with arginase. In contrast, they are without effect on neuronal and inducible NO synthases (Table 5).[508]

8. SURFACTIN SYNTHETASE C-TERMINAL THIOESTERASE INHIBITORS

Macrocyclic natural products produced by nonribosomal peptide synthetases (NRPSs)[509,510] and polyketide synthases (PKSs), such as surfactin, display a wide variety of antibiotic and antitumor activities. The macrocyclic structure of these compounds reduces conformational flexibility, ordering the biologically active conformation, and contributes to stability against proteolytic enzymes, an important property for pharmacological applications.[509] NRPSs utilize various cyclization strategies to increase product diversity; that is, in surfactin, the cycle is a branched chain lipodepsipeptide. A β-hydroxyl fatty acid in surfactin acts as the nucleophile to form a lactone.[509,511] A peptide with high β-sheet content facilitates cyclization through substrate preorganization, and is also thought to be involved in target-ligand binding.[512,513] Surfactin is a lipoheptapeptide produced by the Gram positive bacterium *Bacillus subtilis*.[513] Its primary sequence is FA-Glu-Leu-D-Leu-Val-Asp-D-Leu-Leu, where FA is an α-hydroxy C13–C15 fatty acid. The molecule is cyclized through the formation of an ester bond between the hydroxyl group of the fatty acid and the carbonyl of the C-terminal Leu.[513] The biosynthesis of surfactin is achieved by the surfactin NRPS.[514,515] Cyclization and release of the surfactin lipoheptapeptide chain from the enzyme is mediated by a 28 kDa thioesterase (TE) domain (Srf TE) embedded at the downstream end of the final subunit, SrfC. Srf TE is active in vitro and accepts soluble peptidyl-S-*N*-acetylcysteamine (peptidyl-SNAC) thioester substrates as mimics for the natural interaction with the peptidyl-S-T domain.[509] Crystallographic data of Srf TE showed that it is a member of the *R*,α-hydrolase enzyme family, and structural similarity to serine esterases suggested that an active-site

Table 5. Inhibition of Rat Liver and Murine Macrophage Arginases by ω-Borono-α-amino Acids (222–227)

compound	IC_{50} (μM) pH 7.4	IC_{50} (μM) pH 9.0	mMA (murine macrophages)
222	5000	5000	1000
223	1.6 ± 8	18 ± 8	5 ± 1
224	300 ± 40	350 ± 50	5000
225	>5000	>5000	>10000
226	15 ± 5	65 ± 5	100 ± 20
227	3000	>5000	5000

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

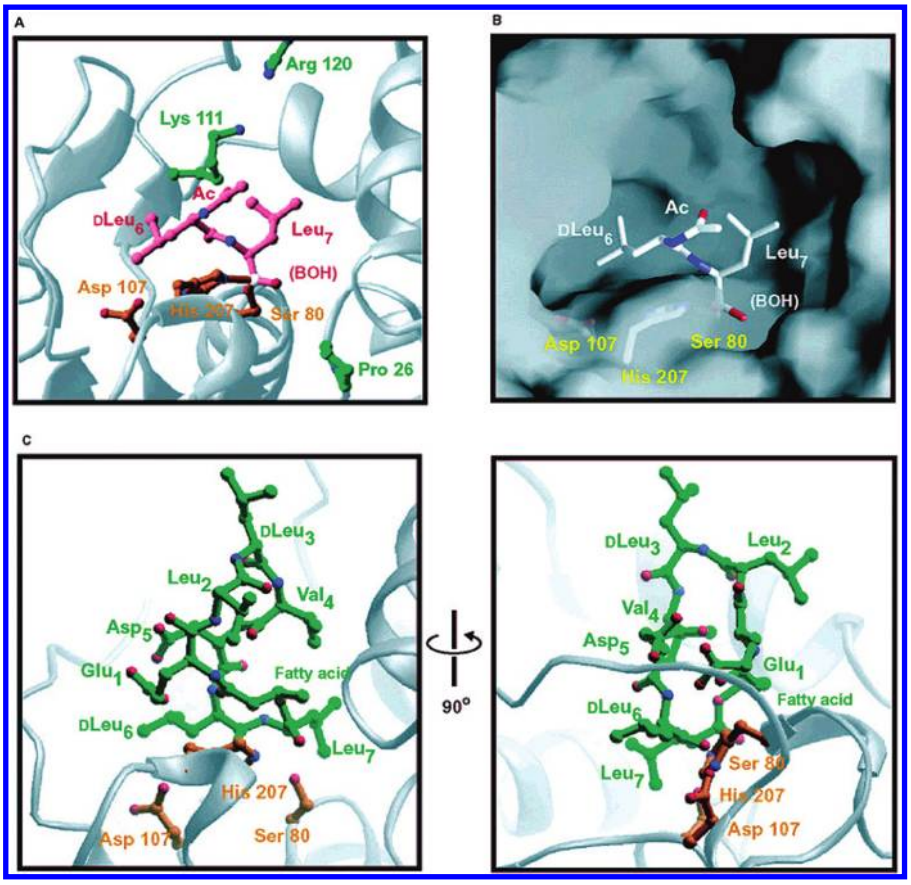


Figure 99. X-ray crystal structure of Srf TE bound to a dipeptidyl boronic acid analogue. (A) Structure of *N*-acetyl-D-Leu-Leu-B$(OH)_2$ complexed to Srf TE. The dipeptide analogue is shown in pink, the catalytic triad is in yellow, and additional active-site side chains are in green. (B) Surface representation of the active-site pocket in the same orientation as in panel A, with *N*-acetyl-D-Leu-Leu-B$(OH)_2$ and the catalytic triad in licorice representation. (C) Model of the cyclizing conformation of the surfactin linear peptide. Superposition of the NMR structure of surfactin onto the structure of *N*-acetyl-D-Leu-Leu-B$(OH)_2$ in the active site. The first panel is in the same orientation as in panels A and B; the second panel is rotated 90° to illustrate the β-sheet structure of the conformation. Reprinted with permission from ref 517. Copyright 2002 American Chemical Society.

catalytic triad is responsible for the enzyme's macrocyclizing chain termination activity.[516] According to a model based on the structure, the peptidyl chain bound to the adjacent T7 domain is directed through a cleft into the active site of the TE domain and transferred onto an invariant serine residue (Ser80), which is activated by histidine (His207) and aspartate (Asp107). The peptidyl chain of this peptidyl-O-TE acyl enzyme intermediate is accommodated in a predominantly hydrophobic binding pocket with two cationic residues (Lys111 and Arg120) predicted to direct cyclization through specific interactions with the substrate. In the deacylation half reaction, the β-hydroxyl group of the fatty acid moiety, activated by the same histidine and aspartate, forms the lactone by intramolecular nucleophilic attack on the acyl-enzyme ester bond. Alternatively, water can be a competing nucleophile, leading to linear acid products. It has remained unclear what features direct the acyl-OTE intermediates between cyclization and hydrolysis. Therefore, researchers used the Srf TE structural information to assess residues that may be involved in kinetic- and/or product-determining steps. In addition, they began an evaluation of the catalytic capacity of Srf TE to process surrogate substrates altered in the fatty acyl, peptidyl, or thioester leaving groups for both cyclization and hydrolysis.

In this context, the X-ray crystal structures of Srf TE bound to designed dipeptidyl boronate inhibitor molecules were examined (Figure 99).[517] A variety of linear peptides containing the surfactin heptapeptide sequence and a C-terminal boronic acid in place of the carboxyl group were synthesized using established procedures.[50,518] The peptides were soaked into crystals of Srf TE, and the structures were solved by molecular replacement using the structure of Srf TE.[516] Peptide electron density was only clearly present for D-Leu6, Leu7, and the boronic acid group, irrespective of the length of the peptide used in the soaking experiment. The remaining residues of the substrate peptide were not well ordered in the maps. Figure 99 shows the structure of the enzyme bound to the dipeptide analogue *N*-acetyl-D-Leu-Leu-B$(OH)_2$ (K_i = 50 μM) and is representative of the observed electron density with full-length heptapeptidyl boronic acid analogues. The structure shows a tetrahedral boron atom bound both to the hydroxyl side chain of Ser80 and N3 of His270, a bidentate binding mode sometimes observed in serine protease/boronic acid complexes.[515] The C-terminal Leu (corresponding to Leu7) is bound specifically in a pocket adjacent to the triad, forming contacts with residues Tyr156, Tyr159, and Leu129. The D-Leu residue (corresponding to D-Leu6) is also well ordered in the density maps, bound in a hydrophobic pocket consisting of Tyr109, Leu187, and Phe181. The N-acetyl group, corresponding to Asp5 of the linear surfactin peptide, points out of the active-site pocket into bulk solvent.

dx.doi.org/10.1021/cr608202m | *Chem. Rev.* 2012, 112, 4156–4220

9. CYSTEINE PROTEASE INHIBITORS

Cysteine (thio) proteases[23,519−521] exist in three structurally distinct classes which are papain-like, ICE-like, or picorna-viral. Cysteine proteases hydrolyze amide bonds in much the same manner as serine proteases. A noncovalent Michaelis complex is formed upon substrate binding. The thiolated anion then attacks the carbonyl carbon of the scissile amide bond. A tetrahedral intermediate is produced which is stabilized by the oxyanion hole. This is followed by the acylation of the enzyme and the liberation of the first product. Hydrolysis of the acyl-enzyme leads to the formation of the second tetrahedral intermediate. Following the collapse of the second intermediate, the product acid is released and the free enzyme is regenerated.

9.1. SARS Coronavirus Main Protease 3CL(pro)

Viral cysteine proteases[522,523] are commonly used as targets for the development of antiviral drugs. They are found in viruses of positive-strand RNA including the poliomyelitis virus and rhinovirus, coronavirus and hepacivirus.

Severe acute respiratory syndrome (SARS) is a typical pneumonial infection. It is caused by a novel human coronavirus (CoV) named SARS-CoV.[524−529] This virus belongs to the coronaviridae family[530−532] where the coronaviruses are large, enveloped, positive single stranded RNA viruses containing 27−31 kb genomes. The SARS-CoV genome comprises about 29 700 nucleotides which encode nonstructural proteins and structural proteins.[533,534] The nonstructural polyproteins are autocatalytically processed through the virally encoded main protease and papain-like protease to yield mature proteins including the RNA-dependent RNA polymerase (RdRp), the RNA helicase, and other proteins whose functions are not well characterized.[535] The main protease is called 3Clike protease (3CLpro) since it is analogous to the 3C proteases encoded by picornaviruses.[536] Because of its pivotal role in the SARS-CoV life cycle, the 3CLpro has been considered to be a promising target for anti-SARS drug discovery.

SARS-CoV 3CLpro is a chymotypsin-like protease but uses a Cys rather than a Ser residue as the active site nucleophile.[537] Moreover, the active site of the SARS protease comprises a catalytic dyad Cys145 and His41.[537,538] The protease contains three domains and the active site is located between domain I and II. Several crystal structures of coronavirus 3CLpro reported[537,539−541] reveals a common feature in 3CLpro: two chymotrypsin-like β-domains (residues 1−184) and one α-helical dimerization domain (residues 201−303). The additional helical C-terminal domain of about 100 residues is essential for dimerization of the 3CLpro and its enzymatic activity.[539,542] In addition to the C-terminus, the N-finger containing a number of N-terminal amino acids is important for enzyme activity of the main proteases from SARS-CoV since the deletion of the N-finger abolishes enzyme activity.[539,543]

Bacha et al.[544] proposed an attractive subsite for the design of inhibitors, which is a cluster of Ser residues (Ser139, Ser144, and Ser147) close to the catalytic residues. In fact, this Ser cluster is conserved in all known coronavirus proteases and may represent a common target of wide-spectrum coronavirus protease inhibitors. On the basis of the known reactivity of boronic acid compounds with the hydroxyl group in Ser residues, the inhibitory potency of bifunctional boronic acid compounds was evaluated. A chemical scaffold containing two phenyl boronic groups attached to a central aromatic ring by ester linkages was tested. This compound has an inhibition constant in the low micromolar range (K_i = 4.6 μM). Different variations of the compound were prepared, including several isomers with replacement of the central aromatic ring with shorter ester linkage, and different functionalities at the phenyl boronic rings. The highest improvement in affinity was observed when the ester linkage between the aromatic rings was replaced with an amide group, thereby resulting in nanomolar inhibition constants. The structures and K_i values of the five representative inhibitors are summarized in Figures 100 and101, and Table 6. Isothermal titration microcalorimetric

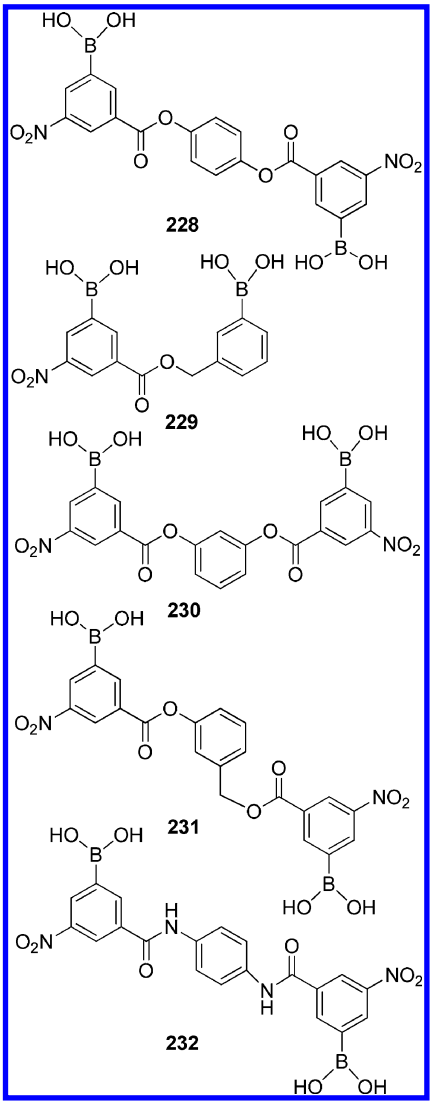


Figure 100. The structures of the five representative 3CLpro protease inhibitors.

experiments indicated that these inhibitors bind reversibly to 3CLpro in an enthalpically favorable fashion, implying that they establish strong interactions with the protease molecule, thus defining attractive molecular scaffolds for further optimization.[544]

10. EGFR AND VEGER-1 TYROSINE KINASE INHIBITORS

Researchers[545] focused in their search for boronic acid-based inhibitors of EGFR and VEGER-1 tyrosin kinases on the active

dx.doi.org/10.1021/cr608202m | Chem. Rev. 2012, 112, 4156−4220

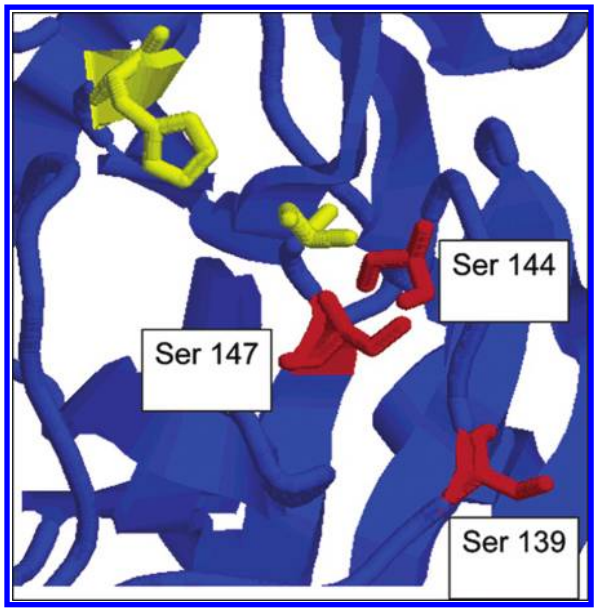


Figure 101. Catalytic residues His41 and Cys145 are located in a relatively shallow cavity between the two domains defining the catalytic site. Close to the catalytic residues is a cluster of serine residues (Ser139, Ser144, and Ser147) that define an attractive subsite for the design of high-affinity inhibitors. Ser144 and Ser147 are located in a cavity that can be targeted by low-molecular weight compounds. Catalytic site of $3CL^{pro}$ showing the location of Ser139, Ser144, and Ser147 (red). Catalytic residues His41 and Cys145 are shown in yellow. The serine cluster defines an attractive subsite for targeting by potential inhibitors. Reprinted with permission from ref 544. Copyright 2004 American Chemical Society.

Table 6. $3CL^{pro}$ Inhibition for Bifunctional Aryl Boronic Compounds

no. and name	MW	K_i μM	α	ΔH kcal/mol	$-T\Delta S$ kcal/mol
228 FL-078	496	4.5	3.3	−5 ± 1	−2
229 FL-101	344	16	4.2	−2 ± 1	−5
230 FL-106	496	6.0	5.6	−4 ± 2	−3
231 FL-136	524	6.0	2.5	−5 ± 2	−2
232 FL-166	494	0.04	1.8	−5 ± 2	−5

pharmacophore 1 of lavendustin A.[546−551] Lavendustin A is the EGFR protein tyrosine kinase (PTK) inhibitor isolated from a butyl acetate extraction of a *Streptomyces grisolovendus* culture filtrate.[546] The active pharmacophore 1 was a secondary amine containing three phenolic hydroxyl group and a carboxyl group. It was considered to interact with EGFR-PTK through hydrogen bonds formed by these functional groups. Inhibition activities would be expected to be higher by replacing those hydroxyl and carboxyl groups with boronic groups.

A series of aminoboronic acids was synthesized based on the structure of lavendustin (**233**) pharmacophore (**234**, Figure 102). Their inhibitory activity against the epidermal growth-factor receptor (EGFR) and vascular endothelial growth-factor receptor-1 (VEGFR-1, Flt-1) protein tyrosine kinases, and various protein kinases, PKA, PKC, PTK, and eEF2K was evaluated. Selective inhibition activities were observed in a series of aminoboronic acids. 4-Methoxy-3-((2-methoxyphenylamino)methyl)-phenylboronic acid (**235**), inhibited EGFR tyrosin kinase, whereas 4-(2,5-dihydroxybenzylamino) phenylboronic acid (**236**) inhibited Flt-1 protein kinase, although the lavendustin pharmacophore (**234**)

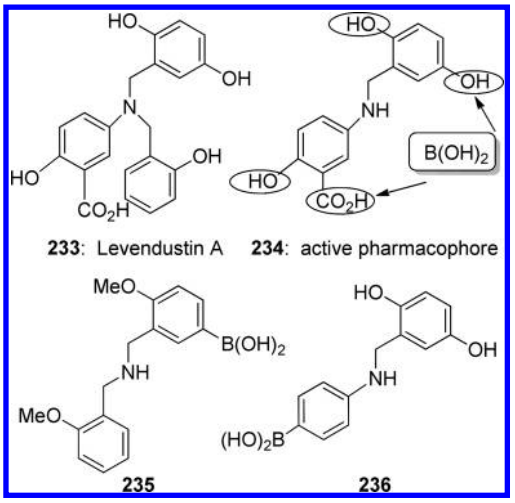


Figure 102. Introduction of a boronic acid group into the active pharmacophore of lavendustin A.

inhibited both EGFR and Flt-1 kinases at a compound concentration of 1 μg/mL. The selective inhibition of EGFR by b, (**235**), was considered to be due to the substitution of the dihydroxy groups on the benzyl moiety for a boronic acid group at the *para* position, whereas the selective inhibition of Flt-1 by c, (**236**), is due to the substitution of the carboxyl group on the aniline moiety in the lavenustin pharmacophore, (**234**), for a boronic acid group.

11. CONCLUSIONS AND PERSPECTIVES

The approval of the potent and selective proteasome inhibitor, bortezomib (VELCADE), in 2003, for the treatment of multiple myeloma and non-Hodgkin lymphoma triggered the search for boron compounds with protease inhibition properties that could be used in the clinic. Indeed some promising compounds have been developed and tested. Among those, noteworthy is PT-100 (**85**, Val-boroPro) that targets dipeptidyl peptidases, such as fibroblast activation protein (FAP). In 2004, Point Therapeutics, Inc. (OTCBB: POTP) announced that it had initiated a phase 2 clinical trial of the company's lead therapeutic compound, PT-100 (**85**), for the treatment of advanced nonsmall cell lung cancer (NSCLC). The study was designed to evaluate the antitumor and hematopoietic activity of PT-100 (**85**) in combination with Taxotere(R) in Stage IIIb/IV NSCLC patients.

In this context, the organoboron compounds are fast becoming compounds used for many things from starting materials in organic synthesis to approved drugs. Researchers are increasingly looking to boron containing molecules as protease inhibitors. Moreover, there are many interesting applications of some boronic acid compounds which target the HIV proteases. The elucidation of their mechanism of action as anti-HIV agents may lead to more biological targets for boronic acid compounds. An increasing research activity in this area is expected because of the enormous potential it presents.

AUTHOR INFORMATION

Corresponding Author

*E-mail: reems@ekmd.huji.ac.il (R.S.); avrir@ekmd.huji.ac.il (A.R.); dvalery@cc.huji.ac.il (V.M.D.).

Notes

The authors declare no competing financial interest.

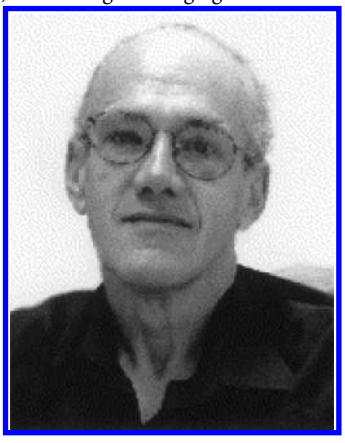
†Deceased.

Biographies

Reem Smoum was born in Jerusalem, in 1974. She received her B.Sc. in Chemistry from Birzeit University in 1996, and she pursued graduate work first at the University of Bergen, Norway, and then at the Hebrew University of Jerusalem, where she received a Ph.D. in Chemistry and Medicinal Chemistry in 2006. In her graduate research with Professor Morris Srebnik and Professor Abraham Rubinstein, she worked on new drug delivery systems based on novel boronated polysaccharide derivatives for the oral administration of peptide drugs. From 2007 until the present, she is a postdoctoral fellow in the laboratory of Professor Raphael Mechoulam at the Institute of Drug Research and Professor Itai Bab at the Bone Laboratory at the Hebrew University of Jerusalem, where she works with the chemistry and biological effects of natural products and drugs, specifically, the chemistry of endogenous cannabinoids, endocannabinoids, and endocannabinoids-like compounds, as well as synthesis of novel compounds to be tested as drugs against osteoporosis. The mechanism of action of these endogenous and synthetic drugs is investigated.

Abraham Rubinstein, Ph.D., is a Professor of Pharmaceutical Sciences at the School of Pharmacy, The Hebrew University of Jerusalem. His research is focused on site-specific therapy in the gastrointestinal (GI) tract. Employing modified polysaccharides as colon-specific drug vehicles, mucoadhesion techniques, and biological models, his work embraces topical, combinatorial treatment of colorectal cancer, targeted treatment of inflammatory bowel diseases (IBD), mechanistic understanding of affinity processes between sugar-containing polymers or particulate carriers and the intestinal epithelium, and the development of orally administered formulations for the delivery of drugs, susceptible to enzymatic degradation in the GI fluids. He is the author and coauthor of more than 100 publications and review articles.

Valery M. Dembitsky received his M.S. in Organic Synthesis from the Far East State University, Vladivostok, USSR, in 1973. He holds a Ph.D. degree in Biological Chemistry from USSR Academy of Sciences, Leningrad, in 1981, and received his D.Sc. degree in Bioorganic Chemistry and Chemistry of Natural Products from M.V. Lomonosov Moscow State Academy of Fine Chemical Technology, in 1997. From 1989 to 1991 he was Associate Professor at Organic Chemistry and Biochemistry Department, Samara State University. He also was a Visiting Professor at the Department of Scientific and Industrial Research, The Massey University, Palmerston North, New Zealand, 1990; Department of Organic and Biological Chemistry, Auckland University, Auckland, New Zealand, 1990; Department of Plant Chemistry, Institute of Organic Chemistry with Phytocentre, Bulgarian Academy of Science, 1990, 2006; Department of Natural Biogenesis, Institute of Microbiology, Czechoslovakia Academy of Science, Prague, 1989, 1990, 2002; Department of Marine Chemistry, Institute of Oceanology, Polish Academy of Science, Sopot, Poland, 1989; Nantes University, France, 2004; N.D. Zelinsky Institute of Organic Chemistry, Moscow, 2010. During 1991−1992 he held Guest Professorship at the School of Chemistry, Melbourne University, Australia, and from 1993 he started a collaboration with Professor Raphael Ikan, Department of Organic Chemistry, Hebrew University, Jerusalem. Since 2000 he joined the Institute for Drug Research, Hebrew University. He was awarded the 1st Prize of Russian Government in Nanotechnology and Metalonomics, 2009. He has published 4 books, 23 chapters in books, 81 review articles, and more than 150 research papers. His research interests are focused on the areas of organometallic chemistry, bioorganic chemistry, chemistry of natural products, and biological imaging.



Morris Srebnik received his Ph.D. in 1984 from the Hebrew University in Jerusalem under Professor Raphael Mechoulam. On a Lady Davis Fellowship, he joined Professor H. C. Brown's group at Purdue University, where he studied the applications of organoboranes to

synthesis, until 1986. After a short stint at the Sigma-Aldrich Corporation, he returned to Professor Brown's group. In 1990 he accepted a position at the Department of Chemistry, University of Toledo, USA. Since 1996, he was a Professor at School of Pharmacy, Hebrew University. His areas of interest included developing organometallic methodologies in synthesis centered around boron and zirconium and lately also titanium and investigating the potential uses of organoboranes in medicine. He also had an interest in isolating new sunscreen agents from natural sources such as cyanobacteria. He is the author and coauthor of more than 200 publications, one book, 8 chapters in books, and 43 review articles.

ACKNOWLEDGMENTS

The work was partially supported by Research Grant No. 1358/05 from the Israeli Science Foundation and a scholarship grant (S.R.) from the Ministry of Science and Technology.

DEDICATION

This article is dedicated to the late Professor Morris Srebnik, an outstanding scientist and a friend.

ABBREVIATIONS

Ala	alanine
AMBER	assisted model building and energy refinement
Arg	arginine
Asn	asparagine
Asp102	aspartic acid 102
C57BL	a common inbred strain of lab mouse
Cys	cysteine
EC_{50}	half maximal effective concentration
Gly193	glycine 193
His57	histidine 57
HPLC	high-performance liquid chromatography
IC_{50}	the half maximal inhibitory concentration
K_i	the dissociation constant for the enzyme–inhibitor complex
IGFBP	insulin-like growth factor binding protein 3
IGF-1	insulin-like growth factor 1
IL-2	interleukin-2
TNFα	tumor necrosis factor-alpha
Leu	leucine
LNCaP cells	androgen-sensitive human prostate carcinoma cells
Lys	lysine
mAb	monoclonal antibody
Met	methionine
NFκB	nuclear factor-kappa B
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
PBMC	peripheral blood mononuclear cell
PDB	Protein Data Bank
Phe	phenylalanine
PMA	paramethoxyamphetamine
Pro	proline
Ser195	serine 195
TCR	T cell receptor
Trp	tryptophan
Tyr	tyrosine
Val	valine
VEGF	vascular endothelial growth factor

REFERENCES

(1) (a) Wolfe, M. S. *Biol. Chem.* **2010**, *391*, 839. (b) Horimoto, Y.; Dee, D. R.; Yada, R. Y. *New Biotechnol.* **2009**, *25*, 318. (c) Heutinck, K. M.; ten Berge, I. J. M.; Hack, C. E.; Hamann, J.; Rowshani, A. T. *Mol. Immunol.* **2010**, *47*, 1943. (d) Bouteldja, N.; Timson, D. J. *Curr. Rheumat. Rev.* **2009**, *5*, 141. (e) Chen, K. X.; Njoroge, F. G. *Curr. Opin. Invest. Drugs* **2009**, *10*, 821. (f) Le Magueresse-Battistoni, B. *Adv. Exp. Med. Biol.* **2008**, *636*, 133. (g) Fricker, S. P. *Metallomics* **2010**, *2*, 366. (h) Ugalde, A. P.; Ordóñez, G. R.; Quiros, P. M.; Puente, X. S.; Lopez-Otin, C. *Methods Mol. Biol.* **2010**, *622*, 3. (i) Cruz-Munoz, W.; Khokha, R. *Crit. Rev. Clin. Lab. Sci.* **2008**, *45*, 291. (j) Moffitt, K. L.; Martin, S. L.; Walker, B. J. *Pharm. Pharmacol.* **2010**, *62*, 563. (k) Garrido, C.; Kroemer, G. *Curr. Opin. Cell Biol.* **2004**, *16*, 639. (l) Haq, S. K.; Rabbani, G.; Ahmad, E.; Atif, S. M.; Khan, R. H. J. *Biochem. Mol. Toxicol.* **2010**, *24*, 270. (m) Mejia, E. G.; Dia, V. P. *Cancer Metastasis Rev.* **2010**, *29*, 511. (n) Juillerat-Jeanneret, L. *Proteases Biol. Dis.* **2007**, *7*, 197. (o) Bernhard, E. J.; Brunner, T. B. *Cancer Biol. Ther.* **2008**, *7*, 636. (p) Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639.

(2) (a) Dembitsky, V. M.; Smoum, R.; Al Quntar, A. A.; Ali, H. A.; Pergament, I.; Srebnik, M. *Plant Sci.* **2002**, *163*, 931. (b) Baker, S. J.; Ding, C. Z.; Akama, T.; Zhang, Y. -K.; Hernandez, V.; Xia, Y. *Future Med. Chem.* **2009**, *1*, 1275. (c) Matteson, D. S. *Med. Res. Rev.* **2008**, *28*, 233. (d) Yang, W.; Gao, X.; Wang, B. *Med. Res. Rev.* **2003**, *23*, 346.

(3) Akparov, V. K.; Stepanov, V. M. *J. Chromatogr.* **1978**, *155*, 329.

(4) Cartwright, S. J.; Waley, S. G. *Biochem. J.* **1984**, *221*, 505.

(5) (a) Koehler, K. A.; Leinhard, G. E. *Biochemistry* **1971**, *10*, 2477. (b) Wolfende, R. *Acc. Chem. Res.* **1972**, *5*, 10. (c) Antonov, V. K.; Berezin, I. V.; Martinek, K.; Levashov, A. V.; Ivanina, T. V.; Ivanova, A. G. *FEBS Lett.* **1972**, *20*, 37.

(6) (a) Philipp, M.; Bender, M. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 478. (b) Rawn, J. D.; Lienhard, G. E. *Biochemistry* **1974**, *13*, 3124. (c) Bauer, C. A.; Petterss, G. *Eur. J. Biochem.* **1974**, *45*, 473.

(7) (a) Matthews, D. A.; Alden, R. A.; Birktoft, J. J.; Freer, S. T.; Kraut, J. *J. Biol. Chem.* **1975**, *250*, 7120. (b) Nakatani, H.; Uehara, Y.; Hiromi, K. *J. Biochem.* **1975**, *78*, 616.

(8) (a) Hiratake, J.; Oda, J. *Biosci. Biotech. Biochem.* **1997**, *61*, 211. (b) Dicko, A.; Bui-Van, T.; Baboulene, M.; Dousset, B. *Main Group Metal Chem.* **2001**, *24*, 15. (c) Knott, K.; Fishovitz, J.; Thorpe, S. B.; Lee, I.; Santos, W. L. *Org. Biomol. Chem.* **2010**, *8*, 3451.

(9) Powers, J. C.; Harper, J. W. In *Proteinase Inhibitors*; Barrett, A. J.; Salvesen, G., Eds; Elsevier: Amsterdam, 1986.

(10) Tsilikounas, E.; Kettner, C. A.; Bachovchin, W. W. *Biochemistry* **1993**, *32*, 12651.

(11) Stoll, V. S.; Eger, B. T.; Hynes, R. C.; Martichonok, V.; Jones, J. B.; Pai, E. F. *Biochemistry* **1998**, *37*, 451.

(12) Bone, R.; Shenvi, A. B.; Kettner, C. A.; Agard, D. A. *Biochemistry* **1987**, *26*, 7609.

(13) Matteson, D. S.; Sadhu, K. M.; Lienhard, G. E. *J. Am. Chem. Soc.* **1981**, *103*, 5241.

(14) Matteson, D. S.; Ray, R. *J. Am. Chem. Soc.* **1980**, *102*, 7590.

(15) Matteson, D. S.; Majumadar, D. *Organometallics* **1983**, *2*, 1529.

(16) Matteson, D. S.; Sadhu, K. M. *Organometallics* **1984**, *3*, 614.

(17) Matteson, D. S.; Jesthi, P. K.; Sadhu, K. M. *Organometallics* **1984**, *3*, 1284.

(18) Kinder, D. H.; Katzenellenbogen, J. A. *J. Med. Chem.* **1985**, *28*, 1917.

(19) Jagannathan, S.; Forsyth, T. P.; Kettner, C. A. *J. Org. Chem.* **2001**, *66*, 6375.

(20) (a) Baldock, C.; de Boer, G. -J.; Refferty, J. B.; Stuitje, R.; Rice, D. W. *Biochem. Pharmacol.* **1998**, *55*, 1541. (b) Huang, J.; Tsvetkov, L.; Qu, K.; Daniel-Issakani, S.; Payan, D. G. *Ernst Schering Found. Symp. Proc.* **2009**, 153. (c) Spizzo, G.; Mitterer, M.; Gunsilius, E. *Br. J. Hamaetol.* **2010**, *150*, 235. (d) Colland, F. *Biochem. Soc. Trans.* **2010**, *38*, 137. (e) McConkey, D. J. *Blood* **2009**, *114*, 931.

(21) (a) Paramore, A.; Frantz, S. *Nat. Rev.* **2003**, *2*, 611. (b) Curran, M. P.; McKeage, K. *Drugs* **2009**, *69*, 859. (c) Moore, B. S.; Eustaquio, A. S.; McGlinchey, R. P. *Curr. Opin. Chem. Biol.* **2008**, *12*, 434. (d) Richardson, P. G.; Mitsiades, C.; Schlossman, R.; Ghobrial, I.;

Hideshima, T.; Munshi, N.; Anderson, K. C. *Expert Rev. Anticancer Ther.* **2008**, *8*, 1053. (e) Fennell, D. A.; Chacko, A.; Mutti, L. *Oncogene* **2008**, *27*, 1189. (f) Daviet, L.; Colland, F. *Biochimie* **2008**, *90*, 270.
(22) (a) Steinmetzer, T.; Stuerzebecher, J. *Curr. Med. Chem.* **2004**, *11*, 2297. (b) Huntington, J. A.; Baglin, T. P. *Trends Pharmacol. Sci.* **2003**, *24*, 589. (c) Steinmetzer, T.; Hauptmann, J.; Sturzebecher, J. *Expert Opin. Invest. Drugs* **2001**, *10*, 845.
(23) (a) Rawlings, N. D.; Barrett, A. J. *Methods Enzymol.* **1994**, *244*, 19. (b) Tanaka, K. A.; Key, N. S.; Levy, J. H. *Anesth. Analg.* **2009**, *108*, 1433. (c) Liu, J.; Schuff-Werner, P.; Steiner, M. *Biomed. Rev.* **2003**, *14*, 9.
(24) (a) Barrett, A.; Rawlings, N. D. *Arch. Biochem. Biophys.* **1995**, *318*, 247. (b) Anderluh, M.; Dolenc, M. S. *Curr. Med. Chem.* **2002**, *9*, 1229. (c) Ohshima, T. *Chem. Pharm. Bull.* **2004**, *52*, 1031.
(25) (a) Krem, M. M.; Di Cera, E. *EMBO J.* **2001**, *20*, 3036. (b) Schoofs, L.; Clynen, E.; Salzet, M. *Curr. Pharm. Des.* **2002**, *8*, 483.
(26) Hedstrom, L. *Chem. Rev.* **2002**, *102*, 4501.
(27) (a) Walker, B.; Lynas, J. F. *Cell. Mol. Life Sci.* **2001**, *58*, 596. (b) Bouteldja, N.; Timson, D. J. *Curr. Rheum. Rev.* **2009**, *5*, 141. (c) Liu, W.; Hickey, E. R. *Annu. Rep. Med. Chem.* **2008**, *43*, 171. (d) Ovaere, P.; Lippens, S.; Vandenabeele, P.; Declercq, W. *Trends Biochem. Sci.* **2009**, *34*, 453. (e) Vande, W. L.; Lamkanfi, M.; Vandenabeele, P. *Cell Death Differ.* **2008**, *15*, 453.
(28) (a) Love, R. A.; Parge, H. E.; Wickersham, J. A.; Hostomsky, Z.; Habuka, N.; Moomaw, E. W.; Adachi, T.; Hostomska, Z. *Cell* **1996**, *87*, 331. (b) Chen, K. X.; Njoroge, F. G. *Curr. Opin. Invest. Drugs* **2009**, *10*, 821. (c) Mederacke, I.; Wedemeyer, H.; Manns, M. P. *Curr. Opin. Invest. Drugs* **2009**, *10*, 181. (d) Choi, S. -Y.; Bertram, S.; Glowacka, I.; Park, Y. W.; Poehlmann, S. *Trends Mol. Med.* **2009**, *15*, 303.
(29) (a) Tong, L.; Qian, C.; Massariol, M.-J.; Bonneau, P. R.; Cordingley, M. G.; Legace, M. G. *Nature* **1996**, *383*, 272. (b) Reiser, M.; Timm, J. *Expert Rev. Anti-Infect. Ther.* **2009**, *7*, 537. (c) Froelich, C. J.; Pardo, J.; Simon, M. M. *Trends Immunol.* **2009**, *30*, 117. (d) Lin, C.; Kwong, A. D.; Perni, R. B. *Infect. Disord. Drug Targets* **2006**, *6*, 3. (e) Fischmann, T. O.; Weber, P. C. *Curr. Pharm. Des.* **2002**, *8*, 2533.
(30) Dobozy, O.; Mite, I.; Ferencz, I.; Csanyl, V. *Acta Biochim. Biophys. Acad. Sci. Hung.* **1971**, *6*, 97.
(31) (a) Morandi, F.; Caselli, E.; Morandi, S.; Focia, P. J.; Blazquez, J.; Shoichet, B. K.; Prati, F. *J. Am. Chem. Soc.* **2003**, *125*, 685. (b) Eidam, O.; Romagnoli, C.; Caselli, E.; Babaoglu, K.; Pohlhaus, D. T.; Karpiak, J.; Bonnet, R.; Shoichet, B. K.; Prati, F. *J. Med. Chem.* **2010**, *53*, 7852. (c) Pournaras, S.; Poulou, A.; Tsakris, A. *J. Antimicrob. Chemother.* **2010**, *65*, 1319. (d) Tondi, D.; Calo, S.; Shoichet, B. K.; Costi, M. P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3416. (e) Doi, Y.; Paterson, D. L. *Int. J. Infect. Dis.* **2007**, *11*, 191.
(32) (a) Weston, G. S.; Shoichet, B. K. Inhibitors of β-lactamases and uses therefor. *PCT Int. Appl.*, WO 9856392 A1 19981217, 1998. (b) Burns, C. J.; Jackson, R. W.; Goswami, R.; Xu, H. Preparation of α-aminoboronic acids as β-lactamase inhibitors. U.S. Patent Appl. Publ., U.S. 20100120715 A1 20100513, 2010. (c) Rambach, A.; Le Coustumier, A.; Randall, L.; Clifton, H. F. A boronic acid-containing medium for selective enrichment of bacteria producing a broad-spectrum β-lactamase. *Fr. Demande* FR 2925070 A1 20090619, 2009. (d) Burns, C. J.; Jackson, R. W. β-Lactamase inhibitors. *PCT Int. Appl.*, WO 2009064413 A1 20090522, 2009. (e) Shoichet, B. K.; Prati, F. α-Boronated N-acyl-3-aminomethylbenzoates and N-benzylamides as β-lactamase inhibitors active in nanomolar concentrations. U.S. Patent, US 7271186 B1 20070918, 2007. (f) Freire, E.; Ross, P.; Xiao, Y.; Ottenbrite, R.; Luque, I. Boronic acid-based compound β-lactamase inhibitors, their preparation, and methods using these compounds for the treatment of bacterial infection. *PCT Int. Appl.*, WO 2005004799 A2 20050120, 2005; (g) Shoichet, B. K.; Weston, G. S. Boronic acid derivative inhibitors of β-lactamases and antibacterial use. U.S. Patent, US 6184363 B1 20010206, 2001. (h) Weston, G. S.; Shoichet, B. K. Inhibitors of β-lactamases and uses therefore. *PCT Int. Appl.*, WO 9856392 A1 19981217, 1998.
(33) (a) Dembitsky, V. M.; Abu Ali, H.; Srebnik, M. *Adv. Organomet. Chem.* **2004**, *51*, 193. (b) Dembitsky, V. M.; Abu Ali, H.; Srebnik, M. *Appl. Organomet. Chem.* **2003**, *17*, 327. (c) Dembitsky, V. M.; Srebnik, M. *Tetrahedron* **2003**, *59*, 579. (d) Polgar, L. *Cell. Mol. Life Sci.* **2005**, *62*, 2161. (e) Zhang, W.-J.; Liu, Z.-H. *Z. Kristallogr. NCS* **2006**, *221*, 189.
(34) MacSweeney, A.; Birrane, G.; Walsh, M. A.; O'Connell, T.; Malthouse, J. P.; Higgins, T. M. *Acta Crystallogr. Sect. D. Biol. Chem.* **2000**, *56* (Pt 3), 280.
(35) Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
(36) Antonov, V. K.; Ivanina, A. G.; Berezin, I. V.; Martinek, K. *FEBS* **1970**, *7*, 23.
(37) Rawn, J. D.; Lienhard, G. E. *Biochemistry* **1974**, *13*, 3124.
(38) Lindquist, R. N.; Terry, L. *Arch. Biochem. Biophys.* **1974**, *160*, 135.
(39) (a) Matteson, D. S. *Chem. Rev.* **1989**, *89*, 1535. (b) Blatch, A. J.; Chetina, O. V.; Howard, J. A. K.; Patrick, L. G. F.; Smethurst, C. A.; Whiting, A. *Org. Biomol. Chem.* **2006**, *4*, 3297. (c) Chen, Y.; Minasov, G.; Roth, T. A.; Prati, F.; Shoichet, B. K. *J. Am. Chem. Soc.* **2006**, *128*, 2970. (d) Kirchhoff, J. H.; Netherton, M. R.; Hills, I. D.; Fu, G. C. *J. Am. Chem. Soc.* **2002**, *124*, 13662. (e) Weston, G. S.; Blazquez, J.; Baquero, F.; Shoichet, B. K. *J. Med. Chem.* **1998**, *41*, 4577. (f) Skordalakes, E.; Tyrell, R.; Elgendy, S.; Goodwin, C. A.; Green, D.; Dodson, G.; Scully, M. F.; Freyssinet, J. -M. H.; Kakkar, V. V.; Deadman, J. J. *J. Am. Chem. Soc.* **1997**, *119*, 9935. (g) Nienaber, V. L.; Mersinger, L. J.; Kettner, C. A. *Biochemistry* **1996**, *35*, 9690. (h) Bone, R.; Frank, D.; Kettner, C. A.; Agard, D. A. *Biochemistry* **1989**, *28*, 7600.
(40) (a) Robillard, G.; Shulman., R. G. *J. Mol. Biol.* **1974**, *86*, 519 & 541. (b) Jabbour, A.; Smoum, R.; Takrouri, K.; Shalom, E.; Zaks, B.; Steinberg, D.; Rubinstein, A.; Goldberg, I.; Katzhendler, J.; Srebnik, M. *Pure Appl. Chem.* **2006**, *78*, 1425.
(41) Nakatani, H.; Hanai, K.; Uehara, Y.; Hiromi, K. *J. Biochemistry (Tokyo)* **1975**, *77*, 905.
(42) (a) Nakatani, H.; Hanai, K.; Uehara, Y.; Hiromi, K. *J. Biochemistry (Tokyo)* **1975**, *78*, 611. (b) London, R. E.; Gabel, S. A. *J. Am. Chem. Soc.* **1994**, *116*, 2570.
(43) Hess, G. P.; Seybert, D.; Lewis, A.; Spoonhower, J.; Cookingham, R. *Science* **1975**, *189*, 384.
(44) Kidd, G. R. *NMR of the Newly Accessible Nuclei*; Laszlo, P., Ed.; Academic Press: New York, 1983; p 49.
(45) Adebodun, F.; Jordan, F. *J. Am. Chem. Soc.* **1988**, *110*, 309.
(46) Lindquist, R. N.; Nguyen, A. C. *J. Am. Chem. Soc.* **1977**, *99*, 6435.
(47) Sylvia, L. A.; Gerig, J. T. *Biochim. Biophys. Acta* **1993**, *1163*, 321.
(48) Sylvia, L. A.; Gerig, J. T. *Biochim. Biophys. Acta* **1995**, *1252*, 225.
(49) Bachovchin, W. W.; Wong, W. Y. L.; Farr-Jones, S.; Shenvi, A. B.; Kettner, C. A. *Biochemistry* **1988**, *27*, 7689.
(50) Martichonok, V.; Jones, J. B. *J. Am. Chem. Soc.* **1996**, *118*, 950.
(51) Tsilikounas, E.; Kettner, C. A.; Bachovchin, W. W. *Biochemistry* **1992**, *31*, 12839.
(52) Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr. *Biochemistry* **1989**, *28*, 7610.
(53) Stoll, V. S.; Eger, B. T.; Hynes, R. C.; Martichonok, V.; Jones, J. B.; Pai, E. F. *Biochemistry* **1998**, *37*, 451.
(54) Katz, B. A.; Finer-Moore, J.; Mortezaei, R.; Rich, D. H.; Stroud, R. M. *Biochemistry* **1995**, *34*, 8264.
(55) Kettner, C.; Shenvi, A. *J. Biol. Chem.* **1984**, *259*, 15106.
(56) Webber, M. M.; Waghray, A.; Bello, D. *Clin. Cancer Res.* **1995**, *1*, 1089.
(57) Zhong, S.; Haghjoo, K.; Kettner, C.; Jordan, F. *J. Am. Chem. Soc.* **1995**, *117*, 7048.
(58) Kettner, C.; Mersinger, L.; Knabb, R. *J. Biol. Chem.* **1990**, *265*, 18289.
(59) Fevig, J. M.; Abelman, M.; Brittelli, D. R.; Kettner, C. A.; Knabb, R. M.; Weber, P. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 295.
(60) Cacciola, J.; Fevig, J. M.; Alexander, R. S.; Brittelli, D. R.; Kettner, C. A.; Knabb, R. M.; Weber, P. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 301.
(61) Tian, Z. Q.; Brown, B. B.; Mack, D. P.; Hutton, C. A.; Bartlett, P. *J. Org. Chem.* **1997**, *62*, 514.

(62) Hansen, K. K.; Grosch, B.; Greiveldinger-Poenaru, S.; Bartlett, P. A. *J. Org. Chem.* **2003**, *68*, 8465.
(63) Hansen, K. K.; Hansen, H. C.; Clark, R. C.; Barlett, P. A. *J. Org. Chem.* **2003**, *68*, 8459.
(64) Frey, P. A. *J. Phys. Org. Chem.* **2004**, *17*, 511.
(65) Westmark, P. R.; Kelly, J. P.; Smith, B. D. *J. Am. Chem. Soc.* **1993**, *115*, 3416.
(66) Pearson, D.; Abell, A. D. *Org. Biomol. Chem.* **2006**, *4*, 3618.
(67) Smoum, R.; Rubinstein, A.; Srebnik, M. *Org. Biomol. Chem.* **2005**, *3*, 941.
(68) (a) Julius, D.; Brake, A.; Blair, L.; Kunisawa, R.; Thorner, J. *Cell* **1984**, *37*, 1075. (b) Pozzuolo, S.; Breme, U.; Salis, B.; Taylor, G.; Tonon, G.; Orsini, G. *Protein Expres. Purific.* **2008**, *59*, 334.
(69) Fuller, R. S.; Brake, A. J.; Thorner, J. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1434.
(70) Fuller, R. S.; Brake, A. J.; Thorner, J. *Science* **1989**, *246*, 482.
(71) Zhou, A.; Webb, G.; Zhu, X.; Steiner, D. F. *J. Biol. Chem.* **1999**, *247*, 20745.
(72) Siezen, R. J.; Leunissen, J. A. *Protein Sci.* **1997**, *6*, 501.
(73) Rockwell, N. C.; Krysan, D. J.; Komiyama, T.; Fuller, R. S. *Chem. Rev.* **2002**, *102*, 4525.
(74) Perona, J. J.; Craik, C. *Protein Sci.* **1995**, *4*, 337.
(75) Brenner, C.; Bevan, A.; Fuller, R. S. *Curr. Biol.* **1993**, *3*, 498.
(76) Smeekens, S. P. *Bio/Technol.* **1993**, *11*, 182.
(77) Rockwell, N. C.; Fuller, R. S. *Biochemistry* **2001**, *40*, 3657.
(78) Rockwell, N. C.; Krysan, D. J.; Komiyama, T; Fuller, R. S. *Chem. Rev.* **2002**, *102*, 4525.
(79) Krysan, D. J.; Rockwell, N. C.; Fuller, R. S. *J. Biol. Chem.* **1999**, *274*, 23229.
(80) Holyoak, T.; Wilson, M. A.; Fenn, T. D.; Kettner, C. A.; Petsko, G. A.; Fuller, R. S.; Ringe, D. *Biochemistry* **2003**, *42*, 6709.
(81) Henrich, S.; Cameron, A.; Bourenkov, G. P.; Kiefersauer, R.; Huber, R.; Lindberg, I.; Bode, W.; Than, M. E. *Nat. Struct. Biol.* **2003**, *10*, 520.
(82) Holyoak, T.; Kettner, C. A.; Petsko, G. A.; Fuller, R. S.; Ringe, D. *Biochemistry* **2004**, *43*, 2412.
(83) Komiyama, T.; Swanson, J. A.; Fuller, R. S. *Antimicrob. Agents Chemother.* **2005**, *49*, 3875.
(84) Fuhrmann, C. N.; Ota, N.; Rader, S. D.; Agard, D. A. In *Handbook of Proteolytic Enzymes*, 2nd ed.; Barrett, A., Rawlings, N. D., Woessner, J. F., Eds.; Academic Press: Cambridge, 2004.
(85) (a) Whitaker, D. R. *Methods Enzymol.* **1970**, *19*, 599. (b) Serkina, A. V.; Shevelev, A. B.; Chestukhina, G. G. *Russian J. Bioorg. Chem* **2001**, *27*, 285. (c) Matselyukh, O. V.; Varbanets, L. D. *Mikrobiol. Zh. (Moscow)* **2008**, *70*, 54.
(86) Delbaere, L. T. J.; Brayer, G. D.; James, M. N. G. *Nature (London)* **1979**, *279*, 165.
(87) Fujinaga, M.; Delbaere, L. T. J.; Brayer, G. D.; James, M. N. G. *J. Mol. Biol.* **1985**, *183*, 479.
(88) Bachovchin, W. W. *Biochemistry* **1986**, *25*, 7751.
(89) Bachovchin, W. W.; Roberts, J. D. *J. Am. Chem. Soc.* **1978**, *100*, 8041.
(90) (a) Hunkapiller, M. W.; Smallcombe, S. H.; Whitaker, D. R.; Richards, J. H. *Biochemistry* **1973**, *12*, 4732. (b) Haddad, K. C.; Sudmeier, J. L.; Bachovchin, D. A.; Bachovchin, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1006.
(91) Ivanov, D.; Bachovchin, W. W.; Redfield, A. G. *Biochemistry* **2002**, *41*, 1587.
(92) Kettner, C.; Bone, R.; Agard, D. A.; Bachovchin, W. W. *Biochemistry* **1988**, *27*, 7682.
(93) Fuhrmann, C. N.; Kelch, B. A.; Ota, N.; Agard, D. A. *J. Mol. Biol.* **2004**, *338*, 999.
(94) Fuhrmann, C. N.; Daugherty, M. D.; Agard, D. A. *J. Am. Chem. Soc.* **2006**, *128*, 9086.
(95) Krieger, M.; Kay, L. M.; Stroud, R. M. *J. Mol. Biol.* **1974**, *83*, 209.
(96) Mangel, W. F.; Singer, P. T.; Cyr, D. M.; Umland, T. C.; Toledo, D. L.; Stroud, R. M.; Pflugrath, J. W.; Sweet, R. M. *Biochemistry* **1990**, *29*, 8351.
(97) Hedstrom, L.; Fan-Jones, S.; Kettner, C. A.; Rutter, W. J. *Biochemistry* **1994**, *33*, 8764.
(98) Hedstrom, L.; Perona, J. J.; Rutter, W. J. *Biochemistry* **1994**, *33*, 8757.
(99) Hedstrom, L.; Szilagyi, L.; Rutter, W. J. *Science* **1992**, *255*, 1249.
(100) Perona, J. J.; Hedstrom, L.; Wagner, R. L.; Rutter, W. J.; Craik, C. S.; Fletterick, R. J. *Biochemistry* **1994**, *33*, 3252.
(101) Stroud, R. M.; Kay, L. M.; Dickerson, R. E. *J. Mol. Biol.* **1974**, *83*, 185.
(102) James, M. N. G.; Brayer, G. D.; Delbaere, L. T. J.; Sielecki, A. R.; Gertler, A. *J. Mol. Biol.* **1980**, *139*, 423.
(103) Delbaere, L. T. J.; Brayer, G. D. *J. Mol. Biol.* **1985**, *183*, 89.
(104) Bone, R.; Sampson, N. S.; Bartlett, P. A.; Agard, D. A. *Biochemistry* **1991**, *30*, 2263.
(105) Bone, R.; Fujishige, A.; Kettner, C. A.; Agard, D. A. *Biochemistry* **1991**, *30*, 10388.
(106) London, R. E.; Gabel, S. A. *Biochemistry* **2002**, *41*, 5963.
(107) Transue, T. R.; Krahn, J. M.; Gabel, S. A.; DeRose, E. F.; London, R. E. *Biochemistry* **2004**, *43*, 2829.
(108) Transue, T. R.; Gabel, S. A.; London, R. E. *Bioconj. Chem.* **2006**, *17*, 300.
(109) (a) Verstraete, M. *Haemostasis* **1996**, *26*, 70. (b) Strukova, S. M. *Biochemistry (Moscow)* **2004**, *69*, 1067.
(110) (a) Sanderson, P. E. J.; NaylorØlsen, A. M. *Curr. Med. Chem.* **1998**, *5*, 289. (b) Stoltz, J. F.; Muller, S.; Labrador, V. *NATO Sci. Ser.* **1999**, *25*, 127.
(111) Wienand, A.; Ehrhardt, C.; Metternich, R.; Tapparelli, C. *Bioorg. Med. Chem. Lett.* **1999**, *7*, 1295.
(112) Di Cera, E. *Chest* **2003**, *124*, 11S.
(113) Bode, W.; Turk, D.; Karshikov, A. *Protein Sci.* **1992**, *1*, 426.
(114) (a) Hanson, S. R.; Harker, L. A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *27*, 1648. (b) Glusa, E.; Paintz, M. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1994**, *349*, 431. (c) Weitz, J. I.; Crowther, M. A. *Am. J. Cardiovasc. Drugs* **2003**, *3*, 201.
(115) (a) Jackson, C. V.; Wilson, H. C.; Growe, V. G.; Shuman, R. T.; Gesellchen, P. D. *J. Cardiovasc. Pharmacol.* **1993**, *21*, 587. (b) Rubens, F. D.; Weitz, J. I.; Brash, J. L.; Kinlough-Rathbone, R. L. *Thromb. Haemost.* **1993**, *69*, 130. (c) Romisch, J.; Stohr, H. A.; Stauss, H.; Koschinsky, R.; Stuber, W.; Paques, E. P. *Thromb. Haemost.* **1994**, *71*, 320.
(116) (a) Tapparelli, C.; Metternich, R.; Ehrhardt, C.; Cook, N. S. *Trends Pharmacol. Sci.* **1993**, *14*, 366. (b) McEvoy, F. J.; Webbon, P. M.; Edgell, T. A.; Gaffney, P. J. *Fibrinolysis* **1995**, *9*, 309.
(117) (a) Fenton, J. W.; Ofosu, F. A.; Moon, D. G.; Maraganore, J. M. *Blood Coagulation* **1991**, *2*, 69. (b) Callas, D. D.; Fareed, J. *Thromb. Res.* **1995**, *78*, 457. (c) Syed, S.; Kelly, K. E.; Sheffield, W. P. *Thromb. Res.* **1996**, *84*, 419. (d) Linder, R.; Frebelius, S.; Jansson, K.; Swedenborg, J. *Blood Coagul. Fibrinol.* **2003**, *14*, 139.
(118) (a) Doorey, A. J.; Michelson, E. L.; Topol, E. J. *J. Am. Med. Assoc.* **1992**, *268*, 3108. (b) Linder, R.; Blomback, M.; Egberg, N.; Grip, L. *Thromb. Res.* **1999**, *95*, 117. (c) Mattsson, C.; Menschik-Lundin, A.; Nylander, S.; Gyzander, E.; Deinum, J. *Thromb. Res.* **2001**, *104*, 475. (d) Kochanowski, R.; Kotlowski, R.; Szweda, P. *Appl. Biochem. Biotechnol.* **2007**, *141*, 321. (e) Peter, K.; Gupta, A.; Nordt, T.; Bauer, S.; Runge, M. S.; Bode, C. *J. Cardiovasc. Pharmacol.* **2003**, *42*, 237.
(119) Knabb, R. M.; Kettner, C. A.; Timmermans, P. B. M. W. M.; Reilly, T. M. *Thromb. Haemost.* **1992**, *67*, 56.
(120) Weber, P. C.; Lee, S. -L.; Lewandowski, F. A.; Schadt, M. C.; Chang, C. -H.; Kettner, C. A. *Biochemistry* **1995**, *34*, 3750.
(121) Galemmo, R. A., Jr.; Fevig, J. M.; Carini, D. J.; Cacciola, J.; Wells, B. L.; Hillyer, G. L.; Buriak, J., Jr.; Rossi, K. A.; Stouten, P. F. W.; Alexander, R. S.; Hilmer, R.; Bostrom, L.; Abelman, M. M.; Lee, S.-L.; Weber, P. C.; Kettner, C. A.; Knabb, R. M.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2913.
(122) Kettner, C.; Knabb, R.; Fevig, J.; Hugli, T.; Lee, S.; Mantri, P.; Pangbum, M.; Reilly, T.; Stouten, P.; Thoolen, M.; Weber, P.; Wexler, R. 212th American Chemical Society National Meeting, Orlando, FL, MEDI 0112, 1996.

(123) Dominguez, C.; Carini, D. J.; Weber, P. C.; Knabb, R. M.; Alexander, R. S.; Kettner, C. A.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 79.
(124) Fevig, J. M.; Buriak, J. J.; Cacciola, J.; Alexander, R. S.; Kettner, C. A.; Knabb, R. M.; Pruitt, J. R.; Weber, P. C.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 301.
(125) Elgendy, S.; Patel, G.; Green, D.; Goodwin, C. A.; Scully, M. F.; Husman, W.; Skordalakes, E.; Kakkar, V. V.; Deadman, J. J. *Tetrahedron Lett.* **1997**, *38*, 3305.
(126) Skordalakes, E.; Elgendy, S.; Goodwin, C. A.; Green, D.; Scully, M. F.; Kakkar, V. V.; Freyssinet, J. -M.; Dodson, G.; Deadman, J. J. *Biochemistry* **1998**, *37*, 14420.
(127) Bode, W.; Turk, D.; Karshikov, A. *Protein Sci.* **1992**, *1*, 426.
(128) Rydel, T. J.; Tulinsky, A.; Bode, W. *J. Mol. Biol.* **1991**, *221*, 583.
(129) Elgendy, S.; Deadman, J.; Patel, G.; Green, D.; Chino, N.; Goodmin, C. A.; Scully, M. F.; Kakkar, V. V.; Claeson, G. *Tetrahedron Lett.* **1992**, *33*, 4209.
(130) Claeson, G.; Philipp, M.; Agner, E.; Scully, M. F.; Metternich, R.; Kabbar, V. V. *Biochem. J.* **1993**, *290*, 309.
(131) Tapparelli, C.; Metternich, R.; Ehrhardt, C.; Zurini, M.; Claeson, G.; Scully, M. F.; Stone, S. R. *J. Biol. Chem.* **1993**, *268*, 4734.
(132) (a) Deadman, J. J.; Elgendy, S.; Goodwin, C. A.; Green, D.; Baban, J. A.; Patel, G.; Skordalakes, E.; Chino, N.; Claeson, G.; Kakkar, V. V.; Scully, M. F. *J. Med. Chem.* **1995**, *38*, 1511. (b) Abbenante, G.; Fairlie, D. P. *Med. Chem.* **2005**, *1*, 71.
(133) Lee, S.-H.; Alexander, R. S.; Smallwood, A.; Trievel, R.; Mersinger, L.; Weber, P. C.; Kettner, C. *Biochemistry* **1997**, *36*, 13180.
(134) Deadman, J. J.; Claeson, G.; Scully, M. F. *J. Enzyme Inhib.* **1995**, *9*, 29.
(135) Wienand, A.; Ehrhardt, C.; Metternich, R.; Tapparelli, C. *Bioorg. Med. Chem. Lett.* **1999**, *7*, 1295.
(136) von Matt, A.; Ehrhardt, C.; Burkhard, P.; Metternich, R.; Walkinshawc, M.; Tapparelli. *C. Bioorg. Med. Chem.* **2000**, *8*, 2291.
(137) Spencer, J.; Burd, A. P.; Goodwin, C. A.; Merette, S. A. M.; Scully, M. F.; Adatia, T.; Deadman, J. J. *Tetrahedron Lett.* **2002**, *58*, 1551.
(138) Kaiser, B. *Clin. Appl. Thromb./Hemostasis* **1997**, *3*, 16.
(139) Cacciola, J.; Fevig, J. M.; Stouten, P. F. W.; Alexander, R. S.; Knabb, R. M.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1253.
(140) Jin, L.; Pandey, P.; Babine, R. E.; Gorga, J. C.; Seidl, K. J.; Gelfand, E.; Weaver, D. T.; Abdel-Meguid, S. S.; Strickler, J. E. *J. Biol. Chem.* **2005**, *6*, 4704.
(141) Salomon, O.; Zivelin, A.; Livnat, T.; Dardik, R.; Loewenthal, R.; Avishal, O.; Steinberg, D. M.; Rosove, M. H.; O'Connell, N.; Lee, C. A.; Seligsohn, U. *Blood* **2003**, *101*, 4783.
(142) Ries, U. J.; Wienen, W. *Drugs Future* **2003**, *28*, 355.
(143) Wang, X.; Cheng, Q.; Xu, L.; Feuerstein, G. Z.; Hsu, M. Y.; Smith, P. L.; Seiffert, D. A.; Schumacher, W. A.; Ogletree, M. L. *J. Thromb. Haemostasis* **2005**, *3*, 695.
(144) Lazarova, T. I.; Jin, L.; Rynkiewicz, M.; Gorga, J. C.; Bibbins, F.; Meyers, H. V.; Babine, R.; Strickler, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5022.
(145) (a) Rosenblum, J. S.; Kozarich, J. W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 496. (b) De Mester, I.; Scharpe, S.; Lambeir, A.-M. *Clin. Chem. Lab. Med.* **2009**, *47*, 245. (c) Pitman, M. R.; Sulda, M. L.; Kuss, B.; Abbott, C. A. *Front. Biosci.* **2009**, *14*, 3619. (d) Maes, M. -B.; Scharpe, S.; De Meester, I. *Clin. Chim. Acta* **2007**, *380*, 31. (e) Abbott, C. A.; Yazbeck, R.; Geier, M. S.; Demuth, H. -U.; Howarth, G. S. *Adv. Exp. Med. Biol.* **2006**, *575*, 155. (f) Brandt, I.; Lambeir, A. -M.; Maes, M. -B.; Scharpe, S.; De Meester, I. *Adv. Exp. Med. Biol.* **2006**, *575*, 3. (g) Augustyns, K.; Van der Veken, P.; Haemers, A. *Expert Opin. Therap. Patents* **2005**, *15*, 1387. (h) Fujiwara, H. *Reprod. Med. Biol.* **2004**, *3*, 165. (i) Fujiwara, H.; Nakata, R.; Fujiwara, T.; Ueda, M.; Maeda, M. *Curr. Topics Biochem. Res.* **2004**, *6*, 1.
(146) Schön, E.; Ansorge, S. In *Dipeptidyl Peptidase IV-General and Applied Aspects*; Barth, A.; Schowen, R. L., Eds; Institat fur Pharmakologische Forschung: Berlin, 1990.
(147) Lolda, Z. *Histochemistry* **1977**, *54*, 299.
(148) Feller, A. C.; Heijnen, C. J.; Ballieux, R. E.; Parwaresch, M. R. *Br. J. Hamaetol.* **1982**, *51*, 227.
(149) Mentlein, R.; Heymann, E.; Scholz, W.; Feller, A. C.; Flad, H. D. *Cell. Immunol.* **1984**, *89*, 11.
(150) Schon, E.; Mansfeld, H. W.; Demuth, H. U.; Barth, A.; Ansorge, S.; Ekkehard, S. *Biomed. Biochim. Acta* **1985**, *44*, K9.
(151) Underwood, R.; Chiravuri, M.; Lee, H.; Schmitz, T.; Kabcenell, A. K.; Yardley, K.; Huber, B. T. *J. Biol. Chem.* **1999**, *274*, 34053.
(152) Petry, S.; Schoenafinger, K.; Mueler, G.; Baringhaus, K.-H. In vitro SAR of (5-(2H)-isoxazolonyl) ureas, potent inhibitors of hormone-sensitive lipase. PCT Int. Appl. WO 0117981, 2001; *Chem. Abstr.* **2001**, *134*, 207818.
(153) Schoen, E. E. E.; Grunow, R.; Jahn, S.; Kiessig, S. T.; Volk, H.-D.; Ansorge, S. *Biomed. Biochim. Acta* **1986**, *11*, 1523.
(154) Kubota, T.; Flentke, G. R.; Bachovchin, W. W.; Stollar, B. D. *Clin. Exp. Immunol.* **1992**, *89*, 192.
(155) Kameoka, J.; Tanaka, T.; Nojimi, Y.; Schlossman, S. F.; Morimoto, C. *Science* **1993**, *262*, 466.
(156) Morrison, M. E.; Vijayasaradhi, S.; Engelstein, D.; Albino, A. P.; Houghton, A. N. *J. Exp. Med.* **1993**, *177*, 1135.
(157) Callebaut, C.; Kurst, B.; Jacotot, E.; Hovanessian, A. G. *Science* **1993**, *262*, 2045.
(158) Bachovchin, W. W.; Plaut, A. G.; Flentke, G.; Lynch, M.; Kettner, C. A. *J. Biol. Chem.* **1990**, *265*, 3738.
(159) Flenkte, G. R.; Munoz, E.; Huber, B. T.; Plaut, A. G.; Kettner, C. A.; Bachovchin, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1556.
(160) Gutheil, W. G.; Bachovchin, W. W. *Biochemistry* **1993**, *32*, 8723.
(161) Kelly, T. A.; Adams, J.; Bachovchin, W. W.; Barton, R. W.; Campbel, S. J.; Coutts, S. J.; Kennedy, C. A.; Snow, R. J. *J. Am. Chem. Soc.* **1993**, *115*, 12637.
(162) (a) Shenvi, A. B. *Biochemistry* **1986**, *25*, 1286. (b) Shenvi, A. B. *Fed. Proc.* **1986**, *45*, 1859.
(163) Smith, R. E.; Van Frank, R. M. In *Lysosomes in Biology and Pathology*; Neuberger, A., Tatum, E. L., Eds; New Holland: Amsterdam, 1975; Vol. *43*, p 193.
(164) Snow, R. J.; Bachovchin, W. W.; Barton, R. W.; Campbell, S. J.; Coutts, S. J.; Freeman, D. M.; Gutheil, W. G.; Kelly, T. A.; Kennedy, C. A.; Krolikowski, D. A.; Leonard, S. F.; Pargellis, C. A.; Tong, L.; Adams, J. *J. Am. Chem. Soc.* **1994**, *116*, 10860.
(165) Bax, A. *J. Magn. Reson.* **1985**, *65*, 142.
(166) Snow, R. J.; Bachovchin, W. W.; Barton, R. W.; Campbell, S. J.; Coutts, S. J.; Freeman, D. M.; Gutheil, W. G.; Kelly, T. A.; Kennedy, C. A.; Krolikowski, D. A.; Leonard, S. F.; Pargellis, C. A.; Tong, L.; Adams, J. *J. Am. Chem. Soc.* **1994**, *116*, 10860.
(167) Coutts, S. J.; Kelly, T. A.; Snow, R. J.; Kennedy, C. A.; Barton, R. W.; Adams, J.; Krolikowski, D. A.; Freeman, D. M.; Cambell, S. J. *J. Med. Chem.* **1996**, *39*, 2087.
(168) Heins, J.; Welker, P.; Schonlein, C.; Born, I.; Hartrodt, B.; Neubert, K.; Tsuru, D.; Barth, A. *Biochim. Biophys. Acta* **1988**, *954*, 161.
(169) Harada, M.; Fukasawa, K.; Hiraoka, B. Y.; Mogi, M.; Barth, A.; Neubert, K. *Biochim. Biophys. Acta* **1985**, *830*, 341.
(170) Gutheil, W. G.; Subramanyam, M.; Flentke, G. R.; Sanford, D. G.; Munoz, E.; Huber, B. T.; Bachovchin, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6594.
(171) Subramanyam, M.; Gutheil, W. G.; Bachovchin, W. W.; Huber, B. T. *J. Immunol.* **1993**, *150*, 2544.
(172) Huber, R.; Kukla, D.; Bode, W.; Schwager, P.; Bartels, K.; Deisenhofer, J.; Steigemann, W. *J. Mol. Biol.* **1974**, *89*, 73.
(173) Shreder, K.; Wong, M.; Corral, S.; Yu, Z.; Winn, D.; Wu, M.; Hu, Y.; Nomanbhoy, T.; Alemayehu, S.; Fuller, S.; Rosenblum, J. S.; Kozarich, J. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4256.
(174) Park, J. E.; Lenter, M. C.; Zimmerman, R. N.; Garin-Chesa, P.; Old, L. J.; Rettig, W. J. *J. Biol. Chem.* **1999**, *274*, 36505.
(175) Scanlan, M. J.; Raj, B. K.; Calvo, B.; Garin-Chesa, P.; Sanz-Moncasi, M. P.; Healey, J. H.; Old, L. J.; Rettig, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5657.

(176) Goldstein, L. A.; Ghersi, G.; Piñeiro-Sánchez, M. L.; Salamone, M.; Yeh, Y.; Flessate, D.; Chen, W. T. *Biochim. Biophys. Acta* **1997**, *1361*, 11.
(177) Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. *Nat. Struct. Biol.* **2003**, *10*, 19.
(178) Cheng, J. D.; Dunbrack, R. I.; Valianou, M.; Rogatko, A.; Alpaugh, K.; Weiner, L. M. *Cancer Res.* **2002**, *62*, 4767.
(179) Niedermeyer, J.; Enenkel, B.; Park, J. E.; Lenter, M.; Rettig, W. J.; Damm, K.; Schnapp, A. *Eur. J. Biochem.* **1998**, *254*, 650.
(180) Jones, B.; Adams, S.; Miller, G. T.; Jesson, M. I.; Watanabe, T.; Wallner, B. P. *Blood* **2003**, *102*, 1641.
(181) Adams, S.; Miller, G. T.; Jesson, M. I.; Watanabe, T.; Jones, B.; Wallner, B. P. *Cancer Res.* **2004**, *64*, 5471.
(182) Cheng, J. D.; Valianou, M.; Canutescu, A. A.; Jaffe, E. K.; Lee, H.-O.; Wang, H.; Lai, J. H.; Bachovchin, W. W.; Weiner, L. M. *Mol. Cancer Ther.* **2005**, *4*, 351.
(183) Aertgeerts, K.; Ye, S.; Tennant, M. G.; Kraus, M. L.; Rogers, J.; Sang, B. -C.; Skene, R. J.; Webb, D. R.; Prasad, G. S. *Protein Sci.* **2004**, *13*, 1.
(184) (a) Edosada, C. Y.; Quan, C.; Wiesmann, C.; Tran, T.; Sutherlin, D; Reynolds, M.; Elliott, J. M.; Raab, H.; Fairbrother, W.; Wolf, B. B. *J. Biol. Chem.* **2006**, *281*, 7437. (b) Baker, S. J.; Ding, C. Z.; Akama, T.; Zhang, Y. -K.; Hernandez, V.; Xia, Y. *Future Med. Chem.* **2009**, *1*, 1275.
(185) Mestecky, J.; McGhee, J. R. *Adv. Immunol.* **1987**, *40*, 153.
(186) Underdown, B. J.; Schiff, J. M. *Annu. Rev. Immunol.* **1986**, *4*, 389.
(187) Plaut, A. G.; Gilbert, J.; Artenstein, M.; Capra, J. D. *Science* **1975**, *190*, 1103.
(188) Plaut, A. G. *Annu. Rev. Biochem.* **1983**, *37*, 603.
(189) Kilian, M.; Mestecky, J.; Schrohenloher, R. E. *Infect. Immun.* **1979**, *26*, 143.
(190) (a) Mulks, M. H. In *Bacterial Enzymes and Virulence*; Holder, I. A., Ed.; CRC Press: Boca Raton, FL, 1985; p 81. (b) Kennis, J. T. M.; Crosson, S. *Science* **2007**, *317*, 1041. (c) Sasindran, S. J.; Saikolappan, S.; Dhandayuthapani, S. *Future Microbiol.* **2007**, *2*, 619. (d) Lopez, R.; Garcia, E. *FEMS Microbiol. Rev.* **2004**, *28*, 553. (e) Mistry, D.; He, S.; Stockley, R. A. *Recent Res. Develop. Immunol* **2002**, *4*, 811. (f) Hirst, T. R.; Hillary, J. B.; Ruddock, L. W.; Yu, J. *Biochem. Soc. Trans.* **1995**, *23*, 985.
(191) Kilian, M.; Mestecky, J.; Russell, M. W. *Microbial. Rev.* **1988**, *52*, 296.
(192) Pohlner, J.; Halter, R.; Meyer, T. F. *Gonococci and Meningococci*; Poolman, J. T., Ed.; Kluwer Academic Publishers Group: Dordrecht, Netherlands, 1988; p 427.
(193) Bonomo, R. A.; Rice, L. B. *Front. Biosci.* **1999**, *4*, 34.
(194) Herzberg, O.; Moult, J. *Science* **1987**, *236*, 694.
(195) Pratt, R. F.; McConnell, T. S.; Murphy, S. J.; Kazumitsu, H. *Biochem. J.* **1988**, *254*, 919.
(196) Knott-Hunziker, V.; Petursson, S.; Waley, S. G.; Jaurin, B.; Grundstrom, T. T. *Biochem. J.* **1982**, *207*, 315.
(197) (a) Christensen, H.; Martin, M. T.; Waley, S. G. *Biochem. J.* **1990**, *266*, 853. (b) Hall, B. G.; Barlow, M. *Drug Resist. Updates* **2004**, *7*, 111.
(198) (a) Bush, K.; Jacoby, G. A.; Medeiros, A. A. *Antimicrob. Agents Chemother.* **1995**, *39*, 1211. (b) Hall, B. G.; Barlow, M. *J. Antimicrob. Chemother.* **2005**, *55*, 1050. (c) Bertoncheli, C. M.; Horner, R. *Revista Brasileira de Ciencias Farm* **2008**, *44*, 577. (d) Bebrone, C.; Lassaux, P.; Vercheval, L.; Sohier, J. -S.; Jehaes, A.; Sauvage, E.; Galleni, M. *Drugs* **2010**, *70*, 651.
(199) Strynadka, N. C. J.; Adachi, H.; Jensen, S. E.; Johns, K.; Sielecki, A.; Betzel, C.; Sutoh, K.; James, M. N. G. *Nature* **1992**, *359*, 700.
(200) Dideberg, O.; Charlier, P.; Wery, J. P.; Dehottay, P.; Dusart, J.; Erpicum, T.; Frere, J. M.; Ghuysen, J. M.; Kazumitsu, H. *Biochem. J.* **1987**, *245*, 911.
(201) (a) Knox, J. R.; Moews, P. C. *J. Mol. Biol.* **1991**, *220*, 435. (b) Carfi, A.; Duee, E.; Paul-Soto, R.; Galleni, M.; Frere, J. M.; Dideberg, O. *Acta Crystallogr.* **1998**, *D54*, 47.
(202) (a) Jelsch, C.; Mourey, L.; Masson, J. M.; Samama, J. P. *Proteins* **1993**, *16*, 364. (b) Nukaga, M.; Bethel, C. R.; Thomson, J. M.; Hujer, A. M.; Distler, A.; Anderson, V. E.; Knox, J. R.; Bonomo, R. A. *J. Am. Chem. Soc.* **2008**, *130*, 12656.
(203) Lobkovsky, E.; Moews, P. C.; Liu, H.; Zhao, H.; Frere, J. M.; Knox, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11257.
(204) Oefner, C. D.; Arcy, A.; Daly, J. J.; Gubernatro, K.; Charnas, R. L.; Winkler, F. K. *Nature* **1990**, *343*, 284.
(205) (a) Carfi, A.; Pares, S.; Euee, E.; Galleni, M.; Duez, C.; Frere, J. M.; Dideberg, O. *EMBO J.* **1995**, *14*, 4914. (b) Carfi, A.; Duee, E.; Galleni, M.; Frere, J. -M.; Dideberg, O. *Acta Crystallogr.* **1998**, *D54*, 313. (c) Garcia-Saez, I.; Hopkins, J.; Papamicael, C.; Franceschini, N.; Amicosante, G.; Rossolini, G. M.; Galleni, M.; Frere, J. -M.; Dideberg, O. *J. Biol. Chem.* **2003**, *278*, 23868.
(206) (a) Knox, J. R.; Moews, P. C.; Frere, J. M. *Chem. Biol.* **1996**, *3*, 937. (b) Vrielink, A.; Sampson, N. *Curr. Opin. Struct. Biol.* **2003**, *13*, 709.
(207) (a) Massova, I.; Mobashery, S. *Antimicrob. Agents Chem.* **1998**, *42*, 1. (b) Medeiros, A. A. *Clin. Microbiol. Infect.* **1997**, *3*, 4S2.
(208) Crompton, I. E.; Cuthbert, B. K.; Lowe, G.; Waley, S. G. *Biochem. J.* **1988**, *251*, 453.
(209) Chen, C. C.; Rahil, J.; Pratt, R. F.; Herzberg, O. *J. Mol. Biol.* **1993**, *234*, 165.
(210) Strynadka, N. C. J.; Martin, R.; Jensen, S. E.; Gold, M.; Johns, J. B. *Nat. Struct. Biol.* **1996**, *3*, 688.
(211) Caselli, E.; Powers, R. A.; Blasczcak, L. C.; Wu, C. Y.; Prati, F.; Shoichet, B. K. *Chem. Biol.* **2001**, *8*, 17.
(212) Kiener, P. A.; Waley, S. G. *Biochem. J.* **1978**, *169*, 197.
(213) Beesley, T.; Gascoyne, N.; Knott-Hunziker, V.; Petursson, S.; Waley, S. G.; Jaurin, B.; Grundtrom, T. *Biochem. J.* **1983**, *209*, 229.
(214) Usher, K. C.; Blaszczak, L. C.; Weston, G. S.; Shoichet, B. K.; Remington, S. J. *Biochemistry* **1998**, *37*, 16082.
(215) Powers, R. A.; Blázquez, J.; Weaton, G. S.; Morosini, M.-I.; Baquero, F.; Shoichet, B. K. *Protein Sci.* **1999**, *8*, 2330.
(216) Strynadka, N. C.; Jensen, S. E.; Alzari, P. M.; Johns, M. N. *Nat. Struct. Biol.* **1996**, *3*, 290.
(217) Ness, S.; Martin, R.; Kindler, A. M.; Paetzel, M.; Gold, M.; Jensen, S. E.; Jones, J. B.; Strynadka, N. C. *Biochemistry* **2000**, *39*, 5312.
(218) Ferrin, T. E.; Huang, C. C.; Jarvis, L. E.; Langridge, R. *J. Mol. Graph.* **1988**, *6*, 13.
(219) Goodford, P. J. *J. Med. Chem.* **1985**, *28*, 849.
(220) Miranker, A.; Karplus, M. *Proteins: Struct. Funct. Genet.* **1991**, *11*, 29.
(221) Laskowski, R. A.; Thornton, J. M.; Humblet, C.; Singh, J. *J. Mol. Biol.* **1996**, *259*, 175.
(222) Powers, J. C.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 3222.
(223) Ferre, J. M. *Mol. Microbiol.* **1995**, *16*, 385.
(224) Shoichet, B. K.; Weston, G. S. *J. Med. Chem.* **1998**, *41*, 4577.
(225) Morandi, F.; Caselli, E.; Morandi, S.; Focia, P. J.; Blazquez, J.; Shoichet, B. K.; Prati, F. *J. Am. Chem. Soc.* **2003**, *125*, 685.
(226) Wang, X.; Minasov, G.; Blázquez, J.; Caselli, E.; Prati, F.; Shoichet, B. K. *Biochemistry* **2003**, *42*, 8434.
(227) Tafi, A.; Agamennone, M.; Tortorella, P.; Alcaro, S.; Gallina, C.; Botto, M. *Eur. J. Med. Chem.* **2005**, *40*, 1134.
(228) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
(229) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230.
(230) McDonald, C. K.; Still, W. C. *Tetrahedron Lett.* **1992**, *33*, 7743.
(231) Chen, Y.; Shoichet, B.; Bonnet, R. *J. Am. Chem. Soc.* **2005**, *127*, 5423.
(232) (a) Waxman, D. J.; Strominger, J. L. *Annu. Rev. Biochem.* **1983**, *52*, 825. (b) Schneider, T.; Sahl, H. -G. *Int. J. Med. Microbiol.* **2010**, *300*, 161. (c) Sauvage, E.; Kerff, F.; Terrak, M.; Ayala, J. A.; Charlier, P. *FEMS Microbiol. Rev.* **2008**, *32*, 234. (d) Heisig, P. *Pharm. Unserer Zeit* **2006**, *35*, 400.
(233) (a) Ghuysen, J. M. *Annu. Rev. Microbiol.* **1991**, *45*, 37. (b) Toraskar, M.; Kulkarni, V.; Kadam, V. *J. Pharm. Res.* **2010**, *3*, 169.

(c) Di Guilmi, A. M. *Med. Chem. Rev.* **2005**, *2*, 265. (d) Katz, A. H.; Caufield, C. E. *Curr. Pharm. Des.* **2003**, *9*, 857.
(234) (a) Goffin, C.; Ghuysen, J. M. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 1079. (b) Pratt, R. F. *Cell. Mol. Life Sci.* **2008**, *65*, 2138. (c) Stewart, G. C. *Mol. Microbiol.* **2005**, *57*, 1177. (d) Di Guilmi, A. M.; Dessen, A.; Dideberg, O.; Vernet, T. *Curr. Pharm. Biotechnol.* **2002**, *3*, 63.
(235) (a) Ghuysen, J. M. *Trends Microbiol.* **1994**, *2*, 372. (b) Harris, F.; Phoenix, D. A. *Biol. Membr.* **1997**, *14*, 481. (c) Phoenix, D. A.; Harris, F. *Biochem. Soc. Trans.* **1995**, *23*, 976.
(236) (a) Holtje, J. V. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 181. (b) Van Heijenoort, J. *Glycobiology* **2001**, *11*, 25R. (c) Fisher, J. F.; Mobashery, S. *Curr. Protein Peptide Sci.* **2009**, *10*, 401.
(237) Pechenov, A.; Stefanova, M. E.; Nicholas, R. A.; Peddi, S.; Gutheil, W. G. *Biochemistry* **2003**, *42*, 579.
(238) Nicola, G.; Peddi, S.; Stefanova, M.; Nicholas, R. A.; Gutheil, W. G.; Davis, C. *Biochemistry* **2005**, *44*, 8207.
(239) (a) Unger, R. H. *Diabetes* **1995**, *44*, 863. (b) Franks, P. W. *Diabetes* **2010**, *59*, 2732. (c) Scheen, A. J.; Paquot, N. *Curr. Nutr. Food Sci.* **2007**, *3*, 123. (d) Shen, G. X. *Curr. Pharm. Anal.* **2007**, *3*, 17.
(240) (a) Boden, G. *Diabetes* **1997**, *46*, 3. (b) Van Linthout, S.; Spillmann, F.; Schultheiss, H.-P.; Tschoepe, C. *Curr. Pharm. Des.* **2010**, *16*, 1504. (c) Padwal, R. *Curr. Opin. Investig. Drugs* **2008**, *9*, 414. (d) Bays, H.; Ballantyne, C. *Future Lipidol.* **2006**, *1*, 389.
(241) (a) Frayn, K. N. *Diabetologia* **2002**, *45*, 1201. (b) Xie, C.; Wang, Z. C.; Liu, X. F.; Yang, M. S. *Eur. J. Hum. Gen.* **2010**, *18*, 3. (c) Franssen, R.; Visser, M. E.; Kuivenhoven, J. A.; Kastelein, J. J. P.; Dallinga-Thie, G. M.; Stroes, E. S. G. *Future Lipidol.* **2008**, *3*, 385. (d) Raz, I.; Eldor, R.; Cernea, S.; Shafrir, E. *Diabetes Metab. Res. Rev.* **2005**, *21*, 3. (e) Cheng, A. Y. Y.; Josse, R. G. *Drug Discovery Today: Ther. Strategies* **2004**, *1*, 201.
(242) (a) Raclot, T. *Prog. Lipid Res.* **2003**, *42*, 257. (b) Watt, M. J. *Appl. Physiol. Nutr. Metab.* **2009**, *34*, 340. (c) Bicknell, K. A.; Harmer, S. C.; Yiangson, S.; Lockwood, W.; Bicknell, A. B. *Mol. Cell. Endocrinol.* **2009**, *300*, 71. (d) Wang, S.; Soni, K. G.; Semache, M.; Casavant, S.; Fortier, M.; Pan, L.; Mitchell, G. A. *Mol. Gen. Metab.* **2008**, *95*, 117. (e) Fex, M.; Lucas, S.; Winzell, M. S.; Ahren, B.; Holm, C.; Mulder, H. *Diabetes* **2006**, *55*, S24.
(243) (a) Sztalryd, C.; Komaromy, M. C.; Kraemer, F. B. *J. Clin. Invest.* **1995**, *95*, 2652. (b) Labar, G.; Wouters, J.; Lambert, D. M. *Curr. Med. Chem.* **2010**, *17*, 2588. (c) Yecies, J. L.; Manning, B. D. *Cell* **2010**, *140*, 28. (d) Leah, E. *Nat. Rev. Cancer* **2010**, *10*, 160. (e) Guzman, M. *Chem. Biol.* **2010**, *17*, 4. (f) Kokotos, G. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 1081. (g) Guindon, J.; Hohmann, A. G. *Br. J. Pharmacol.* **2008**, *153*, 1341. (h) Vandevoorde, S. *Curr. Top. Med. Chem. (Sharjah, United Arab Emirates)* **2008**, *8*, 247. (i) Viso, A.; Cisneros, J. A.; Ortega-Gutierrez, S. *Curr. Top. Med. Chem. (Sharjah, United Arab Emirates)* **2008**, *8*, 231. (j) Imming, P. *Chem. Biol.* **2007**, *14*, 1311.
(244) (a) Langin, D.; Holm, C.; Lafontan, M. *Proc. Nutr. Soc.* **1996**, *55*, 93. (b) Zechner, R.; Kienesberger, P. C.; Haemmerle, G.; Zimmermann, R.; Lass, A. *J. Lipid Res.* **2009**, *50*, 3. (c) Dembitsky, V. M. *Chem. Biodiver* **2004**, *1*, 673. (d) Dembitsky, V. M. *Lipids* **2005**, *40*, 869. (e) Dembitsky, V. M. *Lipids* **2005**, *40*, 641. (f) Haemmerle, G.; Zimmermann, R.; Zechner, R. *Curr. Opin. Lipidol.* **2003**, *14*, 289.
(245) Contreras, J. A.; Karlsson, M.; Osterlund, T.; Laurell, H.; Svensson, A.; Holm, C. *J. Biol. Chem.* **1996**, *271*, 31426.
(246) Ebdrup, S.; Jacobsen, P.; Farrington, A. D.; Vedsø, P. *Bioorg. Med. Chem.* **2005**, *13*, 2305.
(247) (a) Lauer, G. M.; Walker, B. D. *N. Engl. J. Med.* **2001**, *345*, 41. (b) Avolio, S.; Summa, V. *Curr. Top. Med. Chem. (Sharjah, United Arab Emirates)* **2010**, *10*, 1403. (c) Campas, C.; Pandian, R.; Bolos, J.; Castaner, R. *Drugs Future* **2009**, *34*, 697. (d) Revill, P.; Serradell, N.; Bolos, J.; Rosa, E. *Drugs Future* **2007**, *32*, 788.
(248) (a) Patick, A. K.; Potts, K. E. *Clin. Microbiol. Rev.* **1998**, *11*, 614. (b) Perry, L.; Balfe, A. *Drugs* **2006**, *9*, 756. (c) Perni, R. B.; Almquist, S. J.; Byrn, R. A.; Chandorkar, G.; Chaturvedi, P. R.; Courtney, L. F.; Decker, C. J.; Dinehart, K.; Gates, C. A.; Harbeson, S. L. *Antimicrob. Agents Chemother.* **2006**, *50*, 899.
(249) (a) Manns, M. P.; Mchutchison, J. G.; Gorden, S. C.; Rustgi, V. K.; Shiffman, M.; Reindollar, R.; Goodman, Z. D.; Koury, K.; Ling, M. -H.; Albrecht, J. K. *Lancet* **2001**, *358*, 958. (b) Tsantrizos, Y. S. *Biopolymers* **2004**, *76*, 309. (c) De Francesco, R.; Carfi, A. *Adv. Drug Delivery Rev.* **2007**, *59*, 1242.
(250) Love, R. A.; Parge, H. E.; Wickersham, J. A.; Hostomsky, Z.; Habuka, N.; Moomaw, E. W.; Adachi, T.; Hostomska, Z. *Cell* **1996**, *87*, 331.
(251) Yan, N.; Li, Y.; Munshi, S.; Sardana, V.; Cole, J. L.; Sardana, M.; Steinkuehler, C.; Tomei, L.; De Francesco, R.; Kuo, L. C.; Chen, Z. *Protein Sci.* **1998**, *7*, 837.
(252) Koch, J. O.; Bartenschlager, R. *Virology* **1997**, *237*, 78.
(253) Zhang, R.; Durkin, J.; Windsor, W. T.; McNemar, C.; Ramanathan, L.; Le, H. V. *J. Virol.* **1997**, *71*, 6208.
(254) Bartenschlager, R. *J. Viral Hepat.* **1999**, *6*, 165.
(255) Kim, J. L.; Morgenstern, K. A.; Lin, C.; Fox, T.; Dwyer, M. D.; Landro, J. A.; Chambers, S. P.; Markland, W.; Lepre, C. A.; O'Malley, E. T.; Harbeson, S. L.; Rice, C. M.; Murko, M. A.; Caron, P. R.; Thomson, J. A. *Cell* **1996**, *87*, 343.
(256) Yao, N.; Reichert, P.; Taremi, S. S.; Prosise, W. W.; Weber, P. C. *Structure* **1999**, *7*, 1353.
(257) Zhang, X; Schmitt, A. C.; Jiang, W.; Wasserman, Z; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1157.
(258) (a) Attwood, M. R.; Bennett, J. M.; Campbell, A. D.; Canning, G. G. M.; Carr, M. G.; Dunsdon, R. M; Greening, J. R.; Jones, P. S.; Kay, P. B.; Handa, B. K.; Hurst, D. N.; Jennings, N. S.; Jordan, S.; Keech, E.; O'Brian, M. A.; Overton, N. H.; King-Underwood, J.; Raynham, T. M.; Stenson, K. P.; Wilkinson, C. S.; Wilkinson, T. C. I.; Wilson, F. X. *Antiviral Chem. Chemother.* **1999**, *10*, 259. (b) Dunsdon, R. M.; Greening, J. R.; Jones, P. S.; Jordan, S.; Wilson, F. X. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1577.
(259) (a) Li, X. F.; Zhang, Y. K.; Liu, Y.; Ding, C. Z.; Li, Q.; Zhou, Y.; Plattner, J. J.; Baker, S. J.; Qian, X. L.; Fan, D. Z.; Liao, L.; Ni, Z. J.; White, G. V.; Mordaunt, J. E.; Lazarides, L. X.; Slater, M. J.; Jarvest, R. L.; Thommes, P.; Ellis, M.; Edge, C. M.; Hubbard, J. A.; Somers, D.; Rowland, P.; Nassau, P.; McDowell, B.; Skarzynski, T. J.; Kazmierski, W. M.; Grimes, R. M.; Wright, L. L.; Smith, G. K.; Zou, W. X.; Wright, J.; Pennicott, L. E. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3550. (b) Li, X. F.; Zhang, Y. K.; Liu, Y.; Ding, C. Z.; Zhou, Y. S.; Li, Q.; Plattner, J. J.; Baker, S. J.; Zhang, S. M.; Kazmierski, W. M.; Wright, L. L.; Smith, G. K.; Grimes, R. M.; Crosby, R. M.; Creech, K. L.; Carballo, L. H.; Slater, M. J.; Jarvest, R. L.; Thommes, P.; Hubbard, J. A.; Convery, M. A.; Nassau, P. M.; McDowell, W.; Skarzynski, T. J.; Qian, X. L.; Fan, D. Z.; Liao, L. A.; Ni, Z. J.; Pennicott, L. E.; Zou, W. X.; Wright, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5695. (c) Perni, R. B.; Almquist, S. J.; Byrn, R. A.; Chandorkar, G.; Chaturvedi, P. R.; Courtney, L. F.; Decker, C. J.; Dinehart, K.; Gates, C. A.; Harbeson, S. L.; Heiser, A.; Kalkeri, G.; Kolaczkowski, E.; Lin, K.; Luong, Y. P.; Rao, B. G.; Taylor, W. P.; Thomson, J. A.; Tung, R. D.; Wei, Y.; Kwong, A. D.; Lin, C. *Antimicrob. Agents Chemother.* **2006**, *50*, 899.
(260) Urbani, A.; Bianchi, E.; Narjes, F.; Tramontano, A.; De Francesco, R.; Steinkuhler, C.; Pessi, A. *J. Biol. Chem.* **1997**, *272*, 9204.
(261) Llianas-Brunet, M.; Bailey, M.; Fazal, G.; Goulet, S.; Halmos, T.; Laplante, S.; Maurice, R.; Poirier, M.; Poupart, M. -A.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1713.
(262) Llianas-Brunet, M.; Bailey, M.; Deziel, R.; Fazal, G.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M. -A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2719.
(263) Igallinella, P.; Altamura, S.; Bianchi, E.; Taliani, M.; Ingenito, R.; Cortese, R.; De Francesco, R.; Steinkuhler, C.; Pessi, A. *Biochemistry* **1998**, *37*, 8906.
(264) Priestley, E. S.; De Lucca, I.; Ghavimi, B.; Erickson-Viitanen, S.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3199.
(265) Archer, S. J.; Camac, D. M.; Wu, Z. J.; Farrow, N. A.; Domaille, P. J.; Wasserman, Z. R.; Bukhtiyarova, M.; Rizzo, C.; Jagannathan, S.; Mersinger, L. J.; Kettner, C. A. *Chem. Biol.* **2002**, *9*, 79.
(266) Bukhtiyarova, M.; Rizzo, C. J.; Kettner, C. A.; Korant, B. D.; Scarnati, H. T.; King, R. W. *Antiviral Chem. Chemother.* **2001**, *12*, 367.

(267) (a) Chen, J. -C.; Chia, P. -Y.; Ng, M. M. -L.; Chu, J. J. H. *Immunol. Endocr. Metab. Agents Med. Chem.* **2010**, *10*, 31. (b) Rico-Hesse, R. *Future Virol.* **2009**, *4*, 581. (c) Coffey, L. L.; Mertens, E.; Brehin, A. -C.; Fernandez-Garcia, M. D.; Amara, A.; Despres, P.; Sakuntabhai, A. *Microb. Infect.* **2009**, *11*, 143. (d) Petersen, L. R.; Marfin, A. A. *J. Travel Med.* **2005**, *12*, S3. (e) Blair, C. D.; Adelman, Z. N.; Olson, K. E. *Clin. Microbiol. Rev.* **2000**, *13*, 651.
(268) (a) Jacobs, M. G.; Young, P. R. *Curr. Opin. Infect. Dis.* **1998**, *11*, 319. (b) Gubler, D. J. *J. Clin. Microbiol. Rev.* **1998**, *11*, 480. (c) Rico-Hesse, R. *Clin. Infect. Dis.* **2007**, *44*, 1462. (d) Delatte, H.; Paupy, C.; Dehecq, J. S.; Thiria, J.; Failloux, A. B.; Fontenille, D. *Parasite (Paris, France)* **2008**, *15*, 3. (e) Blair, C. D.; Adelman, Z. N.; Olson, K. E. *Clin. Microbiol. Rev.* **2000**, *13*, 651.
(269) (a) Monath, T. P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2395. (b) Olson, K. E.; Franz, A. W. E. *ACS Symp. Series* **2009**, *1014*, 123. (c) Wiwanitkit, V. J. *Infect. Develop. Count.* **2010**, *4*, 51.
(270) Clum, S.; Ebner, K. E.; Padmanabhan, R. *J. Biol. Chem.* **1997**, *272*, 30715.
(271) Falgout, B.; Miller, R. H.; Lai, C.-J. *J. Virol.* **1993**, *67*, 2034.
(272) Falgout, B.; Pethel, M.; Zhang, Y. M.; Lai, C.-J. *J. Virol.* **1992**, *65*, 2467.
(273) Zhang, L.; Mohan, P. M.; Padmanabhan, R. *J. Virol.* **1992**, *66*, 7549.
(274) Ramachandran, M.; Sasaguri, Y.; Nakano, R.; Padmanabhan, R. *Methods Enzymol.* **1996**, *275*, 168.
(275) Li, J.; Lim, S. P.; Beer, D.; Patel, V.; Wen, D. Y.; Tumanut, C.; Tullyl, D.; Williams, J. A.; Jiricek, J.; Priestle, J. P.; Harris, J. L.; Vasudevan, S. *J. Biol. Chem.* **2005**, *280*, 28766.
(276) Yin, Z.; Patel, S. J.; Wang, W. L.; Wang, G.; Chan, W. L.; Rao, K. R.; Alam, J.; Jeyaraj, D. A.; Ngew, X.; Patel, V.; Beer, D.; Lim, S. P.; Vasudevan, S. G.; Keller, T. H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 36.
(277) (a) Lilja, H.; Christensson, A.; Dahl'en, U.; Matikainen, M. T.; Nilsson, O.; Pettersson, K.; Lovgren, T. *Clin. Chem.* **1991**, *37*, 1618. (b) Suzuki, K.; Kise, H.; Nishioka, J.; Hayashi, T. *Semin. Thromb. Hemost* **2007**, *33*, 46. (c) Asatiani, E.; Gelmann, E. P. *Expert Opin. Therap. Targets* **2005**, *9*, 283. (d) Kim, J.; Coetzee, G. A. *J. Cell. Biochem.* **2004**, *93*, 233.
(278) (a) Karr, J. F.; Kantor, J. A.; Horan Hand, P.; Eggensperger, D. L.; Schlom, J. *Cancer Res.* **1995**, *55*, 2455. (b) Greene, K. L.; Albertsen, P. C.; Babaian, R. J.; Carter, H. B.; Gann, P. H.; Han, M.; Kuban, D. A.; Sartor, A. O.; Stanford, J. L.; Zietman, A. *J. Urol.* **2009**, *182*, 2232. (c) Patane, S.; Marte, F. *Eur. Heart J.* **2009**, *30*, 1169. (d) Thompson, I. M.; Ankerst, D. P.; Etzioni, R.; Wang, T. *J. Urol.* **2008**, *180*, 1219.
(279) (a) Oesterling, J. E. *J. Urol.* **1991**, *145*, 907. (b) Catalona, W. J.; Loeb, S. *J. Natl. Compr. Cancer Network* **2010**, *8*, 265. (c) Meany, D. L.; Sokoll, L. J.; Chan, D. W. *Expert Opin. Med. Diagn.* **2009**, *3*, 597. (d) Riedinger, J.-M.; Eche, N.; Bachaud, J. -M.; Crehange, G.; Fulla, Y.; Thuillier, F. *Ann. Biol. Clin.* **2009**, *67*, 395. (e) Schroeder, F. H. *Recent Results Cancer Res.* **2009**, *181*, 173. (f) Loeb, S.; Catalona, W. J. *Oncologist* **2008**, *13*, 299.
(280) Peehl, D. M. *Cancer Invest.* **1995**, *75* (Suppl), 2021.
(281) Cohen, P.; Graves, H. C. B.; Peehl, D. M.; Kamarei, M.; Giudice, L. C.; Rosenfeld, R. G. *J. Clin. Endocrinol. Metab.* **1992**, *75*, 1046.
(282) Grimberg, A.; Cohen, P. *J. Cell Physiol.* **2000**, *183*, 1.
(283) Yoshida, E.; Ohmura, S.; Suguki, M.; Maruyama, M.; Mihara, H. *Int. J. Cancer* **1995**, *63*, 863.
(284) Webber, M. M.; Waghray, A.; Bello, D. *Clin. Cancer Res.* **1995**, *1*, 1089.
(285) LeBeau, A. M.; Kostova, M.; Craik, C. S.; Denmeade, S. R. *Biol. Chem.* **2010**, *391*, 333.
(286) Hunt, C. D. *Biol. Trace Elem. Res.* **1998**, *66*, 205.
(287) Hunt, C. D.; Idso, J. P. *Trace Elem. Exp. Med.* **1999**, *12*, 221.
(288) Bauer, C. A.; Pettersson, G. *Eur. J. Biochem.* **1974**, *45*, 473.
(289) Gallardo-Williams, M. T.; Maronpot, R. R.; Wine, R. N.; Brunssen, S. N.; Chapin, R. E. *Prostate* **2003**, *54*, 44.
(290) (a) Gallardo-Williams, M. T.; Chapin, R. E.; King, P. E.; Moser, G. J.; Goldsworthy, T. L.; Morrison, J. P.; Maronpot, R. R. *Toxicol. Pathol.* **2004**, *32*, 73. (b) Barranco, W. T.; Eckhert, C. D. *Br. J. Cancer* **2006**, *94*, 884.
(291) LeBeau, A. M.; Singh, P.; Isaacs, J. T; Denmeade, S. R. *Chem. Biol.* **2008**, *15*, 665.
(292) LeBeau, A. M.; Banerjee, S. R.; Pomper, M. G.; Mease, R. C.; Denmeade, S. R. *Bioorg. Med. Chem.* **2009**, *17*, 4888.
(293) Purves, D.; Augustine, G. J.; Fitzpatrick, D.; Hall, W. C.; LaMantia, A. -S.; McNamara, J. O.; White, L. E. *Neuroscience*; Sinauer Associates, Inc.: Massachuusetts, USA, 2008; p 121.
(294) (a) Nachmansohn, D. *Chemical and Molecular Basis of Nerve Activity*; Academic Press: New York, 1959; p 235. (b) Grosman, C. *Biochemistry* **2003**, *42*, 14977. (c) Maciag, C. M.; Logue, A. R.; Tinker, W. J.; Saydoff, J. A.; Tam, S. W.; Zaczek, R. *J. Pharmacol. Exp. Therap* **1994**, *271*, 891.
(295) (a) Nachmansohn, D. *Science* **1970**, *168*, 1059. (b) Lear, J. D.; Wasserman, Z. R.; DeGrado, W. F. *Science* **1988**, *240*, 1177.
(296) O'Brien, R. D.; Gilmour, L. P. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *63*, 496.
(297) O'Brien, R. D.; Gilmour, L. P.; Eldefrawi, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *65*, 438.
(298) Takagi, K.; Ikao, M.; Takahashi, A. *Life Sci.* **1965**, *4*, 2165.
(299) Gill, E.; Rang, H. P. *Mol. Pharmacol.* **1966**, *2*, 284.
(300) Karlin, A.; Winnik, M. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *60*, 668.
(301) Changeux, J. -P.; Podleski, T.; Wofsy, L. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 2063.
(302) Fenton, J. W.; Singer, S. J. *Biochem. Biophys. Res. Commun.* **1965**, *20*, 315.
(303) Mautner, H. G.; Bartels, E. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 74.
(304) Koehler, K. A.; Hess, G. P. *Biochemistry* **1974**, *13*, 5345.
(305) (a) Brockerhoff, H.; Jensen, R. G. *Lipolytic Enzymes*; Academic Press: New York, 1974; p 176. (b) Lafontan, M.; Langin, D. *Prog. Lipid Res.* **2009**, *48*, 275. (c) Karabina, S.-A.; Gora, S.; Atout, R.; Ninio, E. *Biochimie* **2010**, *92*, 594. (d) Fickers, P.; Destain, J.; Thonart, P. *Biotechnol. Agronom. Soc. Environ.* **2008**, *12*, 119. (e) Mas, E.; Lombardo, D. *NATO ASI Ser.* **1994**, *266*, 149.
(306) (a) Kritchevsky, D.; Kothari, H. V. *Adv. Lipid Res.* **1978**, *16*, 221. (b) Doolittle, M. H.; Peterfy, M. *Clin. Lipidol.* **2010**, *5*, 117. (c) Karakas, M.; Koenig, W. *Clin. Lipidol.* **2010**, *5*, 43. (d) Wagner, K.; Brezesinski, G. *Curr. Opin. Colloid Interface Sci.* **2008**, *13*, 47. (e) Hasham, S. N.; Pillarisetti, S. *Clin. Chim. Acta* **2006**, *372*, 179.
(307) (a) Rudd, E. A.; Brockman, H. L. *Lipases*; Borgstrom, B.; Brockman, H. L., Eds.; Elsevier: Amsterdam, 1984; p 185. (b) Singh, H.; Ye, A.; Horne, D. *Prog. Lipid Res.* **2009**, *48*, 92. (c) Houde, A.; Kademi, A.; Leblanc, D. *Appl. Biochem. Biotechnol.* **2004**, *118*, 155. (d) Petkovic, M.; Schiller, J.; Mueller, J.; Lessig, J.; Fuchs, B.; Mueller, M.; Arnhold, J. *Recent Res. Develop. Lipids* **2005**, *8*, 53. (e) Wilton, D. C. *Eur. J. Lipid Sci. Technol.* **2005**, *107*, 193.
(308) (a) Bhat, S. G.; Brockman, H. L. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 486. (b) Aleman-Gomez, J. A.; Colwell, N. S.; Vyas, K.; Boreck, I.; Shonfeld, G.; Lange, L. G.; Kumar, V. B. *Life Sci.* **1999**, *64*, 2419. (c) Lopez-Candales, A.; Grosjlos, J.; Sasser, T.; Buddhiraju, C.; Scherrer, D.; Lange, L. G.; Kumar, V. B. *Biochem. Cell Biol.* **1996**, *74*, 257.
(309) (a) Gallo, L. L.; Clark, S. B.; Myers, S.; Vahouny, G. V. *J. Lipid Res.* **1984**, *25*, 604. (b) Krause, B. R.; Sliskovic, D. R.; Anderson, M.; Homan, R. *Lipids* **1998**, *33*, 489. (c) Lott, J. A.; Lu, C. J. *Clin. Chem.* **1991**, *37*, 361.
(310) (a) Humphries, J. *Internal Medicine*; Stein, J. H., Ed.; Little, Brown and Co.: Boston, 1983; p 519. (b) Subramanian, S.; Kalyanaraman, B.; Migrino, R. Q. *Recent Pat. Cardiovas. Drug Discovery* **2010**, *5*, 54. (c) Das, S.; Thakuria, N.; Kanodia, L. *J. Clin. Diagn. Res.* **2008**, *2*, 1215. (d) Bai, N.; Khazaei, M.; van Eeden, S. F.; Laher, I. *Pharm. Ther.* **2007**, *113*, 16.
(311) (a) Lombardo, D.; Guy, O. *Biochim. Biophys. Acta* **1981**, *657*, 425. (b) Lin, G.; Tsai, Y.-C.; Liu, H.-C.; Liao, W.-C.; Chang, C.-H. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1998**, *1388*, 161.

(c) Hosie, L.; Sutton, L. D.; Quinn, D. M. *J. Biol. Chem.* **1987**, *262*, 260.
(312) (a) Stout, J. S.; Sutton, L. D.; Quinn, D. M. *Biochim. Biophys. Acta* **1985**, *837*, 6. (b) Sohl, J.; Sutton, L. D.; Burton, D. J.; Quinn, D. M. *Biochem. Biophys. Res. Commun.* **1988**, *151*, 554. (c) Hosie, L; Sutton, L. D.; Quinn, D. M. *J. Biol. Chem.* **1987**, *262*, 260.
(313) (a) Stroud, R. M. *Sci. Am.* **1974**, *231*, 74. (b) Lin, G.; Chiou, S.-Y.; Hwu, B.-C.; Hsieh, C.-W. *Protein J.* **2006**, *25*, 33.
(314) (a) Blow, D. M. *Acc. Chem. Res.* **1976**, *9*, 145. (b) Segal, D.; Shalitin, C.; Shalitin, Y.; Fischer, D. R.; Stang, P. J. *FEBS Lett.* **1996**, *392*, 117.
(315) (a) Kraut, J. *Annu. Rev. Biochem.* **1977**, *46*, 331. (b) Hida, K.; Wada, J.; Zhang, H.; Hiragushi, K.; Tsuchiyama, Y.; Shikata, K.; Makino, H. *J. Lipid Res.* **2000**, *41*, 1615.
(316) (a) Sutton, L. D.; Stout, J. S.; Hosie, L.; Spencer, P. S.; Quinn, D. M. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 386. (b) Hall, I. H.; Spielvogel, B. F.; Griffin, T. S.; Docks, E. L.; Brotherton, R. J. *Res. Commun. Chem. Pathol. Pharm.* **1989**, *65*, 297.
(317) Sutton, L. D.; Lantz, J. L.; Eibes, T.; Quinn, D. M. *Biochim. Biophys. Acta* **1990**, *1041*, 79.
(318) Darke, P. L.; Nutt, R. F.; Brady, S. F.; Garsky, V.; Ciccarone, T.; Leu, C.; Lumma, P.; Freidinger, R.; Sigal., I. S. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 297.
(319) (a) Chatfield, D. C.; Brooks, B. R. *J. Am. Chem. Soc.* **1995**, *117*, 5561. (b) Boss, C.; Corminboeuf, O.; Grisostomi, C.; Weller, T. *Expert Opin. Ther. Pat.* **2006**, *16*, 295. (c) Kashparov, I. V.; Popov, M. E.; Popov, E. M. *Bioorg. Khim. (Moscow)* **1999**, *25*, 423.
(320) (a) James, M. N. G.; Sielecki, A. R. In *Biological Macromolecules and Assemblies: Active Sites of Enzymes*; Jurnak, F. A., McPherson, A., Eds.; John Wiley & Sons: New York, 1987; p 413. (b) Colbert, J. D.; Matthews, S. P.; Miller, G.; Watts, C. *Eur. J. Immunol.* **2009**, *39*, 2955. (c) Marks, N.; Berg, M. J. *Neurochem. Res.* **2010**, *35*, 181. (d) Eder, J.; Hommel, U.; Cumin, F.; Martoglio, B.; Gerhartz, B. *Curr. Pharm. Des.* **2007**, *13*, 271.
(321) Meek, T. D.; Dayton, B. D.; Metcalf, B. W.; Dreyer, G. B.; Strickler, J. E.; Gorniak, J. G.; Rosenberg, M.; Moore, M. L.; Magaard, V. W.; Debouck, C. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1841.
(322) (a) Farmerie, W. C.; Loeb, D. D.; Casavant, N. C.; Hutchison, C. A., III; Edgell, M. H.; Swanstrom, R. *Science* **1987**, *236*, 305. (b) Kogo, H.; Takeuchi, K.; Inoue, H.; Kihara, H.; Kojima, M.; Takahashi, K. *Biochim. Biophys. Acta, Proteins Proteom.* **2009**, *1794*, 70. (c) Kontijevskis, A.; Wikberg, J. E. S.; Komorowski, J. *Proteins Struct. Funct. Bioinform.* **2007**, *68*, 305.
(323) (a) Kohl, N. E.; Diehl, R. E.; Rands, E.; Davis, L. J.; Hanobik, M. G.; Wolanski, B.; Dixon, R. A. *J. Virol.* **1991**, *65*, 3007. (b) Law, S. K.-Y.; Wang, R.-R.; Mak, A. N.-S.; Wong, K.-B.; Zheng, Y.-T.; Shaw, P.-C. *Nucleic Acids Res.* **2010**, *38*, 6803.
(324) (a) Kramer, R. A.; Schaber, M. D.; Skalka, A. M.; Ganguly, K.; Wongstaal, F.; Reddy, E. P. *Science* **1989**, *231*, 1580. (b) Muratori, C.; Ruggiero, E.; Sistigu, A.; Bona, R.; Federico, M. *J. Gen. Virol.* **2009**, *90*, 2777.
(325) (a) Pettit, S. C.; Simsic, J.; Loeb, D. D.; Everitt, L.; Hutchison, C. A., III; Swanstrom, R. *J. Biol. Chem.* **1991**, *266*, 14539. (b) Budt, K.-H.; Peyman, A.; Hansen, J.; Knolle, J.; Meichsner, C.; Paessens, A.; Ruppert, D.; Stowasser, B. *Bioorg. Med. Chem.* **1995**, *3*, 559.
(326) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4686.
(327) Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schneider, J.; Kent, S. B. H. *Science* **1989**, *245*, 616.
(328) Navia, M. A.; Fitzgerald, P. M. D.; McKeever, B. M.; Leu, C.-T.; Haimbach, J. C.; Herber, W. K.; Sigal, I. S.; Darke, P. L.; Springer, J. P. *Nature* **1989**, *337*, 615.
(329) Lapatto, R.; Blundell, T.; Hemmings, A.; Overington, J.; Wilderspin, A.; Wood, S.; Merson., J. R.; Whittle, P. J.; Danley, D. E.; Geoghegan, K. F.; Hawrylik, S. J.; Lee, S. E.; Scheld, K. G.; Hobart, P. M. *Nature* **1989**, *342*, 299.
(330) (a) Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305. (b) Li, J.; Tang, S.; Hewlett, I.; Yang, M. *Infect. Disord. Drug Targets* **2007**, *7*, 238. (c) Cafaro, A.; Macchia, I.; Maggiorella, M. T.; Titti, F.; Ensoli, B. *Adv. Exp. Med. Biol.* **2009**, *655*, 189.
(331) Mulichak, A. M.; Hui, J. O.; Tomasselli, A. G.; Heinrikson, R. L.; Curry, K. A.; Tomich, C.-S.; Thaisrivongs, S.; Sawyer, T. K.; Watenpaugh, K. D. *J. Biol. Chem.* **1993**, *268*, 13103.
(332) (a) DesJarlais, R. L.; Seibel, G. L.; Kuntz, I. D.; Furth, P. S.; Alvaez, J. C.; Ortiz de Montellano, P. R.; DeCamp, D. L.; Babe, L. M.; Craik, C. S. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6644. (b) Lambert, D. M.; Petteway, S. R., Jr.; McDanal, C. E.; Hart, T. K.; Leary, J. J.; Dreyer, G. B.; Meek, T. D.; Bugelski, P. J.; Bolognesi, D. P. *Antimicrob. Agents Chemother.* **1992**, *36*, 982. (c) Vathipadiekal, V.; Umasankar, P. K.; Patole, M. S.; Rao, M. *Peptides* **2010**, *31*, 16.
(333) (a) Coffin, J. M. *Science* **1995**, *267*, 483. (b) Panter, G.; Kuznik, A.; Jerala, R. *Curr. Opin. Mol. Ther.* **2009**, *11*, 133. (c) Shen, L.; Siliciano, R. F. *J. Allergy Clin. Immunol.* **2008**, *122*, 22. (d) Clotet, B.; Raffi, F.; Cooper, D.; Delfraissy, J. -F.; Lazzarin, A.; Moyle, G.; Rockstroh, J.; Soriano, V.; Schapiro, J. *AIDS* **2004**, *18*, 1137.
(334) Scrimal, R. C.; Dhawan, B. N. *J. Pharm. Pharmacol.* **1973**, *25*, 447.
(335) Sui, Z.; Salto, R.; Li, J.; Craik, C.; Ortiz de Montellano, P. R. *Bioorg. Med. Chem.* **1993**, *1*, 415.
(336) De Voss, J.; Sui, Z.; DeCamp, D. L.; Salto, R.; Babe, L. M.; Craik, C. S.; Ortiz de Montellano, P. R. *J. Med. Chem.* **1994**, *37*, 665.
(337) Salto, R.; Babe, L. M.; Li., J.; Rose, J. R.; Yu, Z.; Burlingame, A.; De Voss, J. J.; Sui, Z.; Ortiz de Montellano, P. R.; Craik, C. S. *J. Biol. Chem.* **1994**, *269*, 10691.
(338) Pivazyan, A. D.; Matteson, D. S.; Fabry-Asztalos, L.; Singh, R. P.; Lin, P.; Blair, W.; Guo, K.; Robinson, B.; Prusoff, W. H. *Biochem. Pharmacol.* **2000**, *59*, 927.
(339) (a) Prejdova, J.; Soucek, M.; Konvalinka, J. *Curr. Drug Targets: Infect. Disord.* **2004**, *4*, 137. (b) Garrec, J.; Cascella, M.; Rothlisberger, U.; Fleurat-Lessard, P. *J. Chem. Theory Comput.* **2010**, *6*, 1369. (c) Holst, B.; Frimurer, T. M.; Mokrosinski, J.; Halkjaer, T.; Cullberg, K. B.; Underwood, C. R.; Schwartz, T. W. *Mol. Pharmacol.* **2009**, *75*, 44. (d) Zhou, D.; Chu, W.; Rothfuss, J.; Zeng, C.; Xu, J.; Jones, L.; Welch, M. J.; Mach, R. H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5041. (e) Chrusciel, R. A.; Strohbach, J. W. *Curr. Topics Med. Chem. (Sharjah, United Arab Emirates)* **2004**, *4*, 1097.
(340) Judd, D. A.; Nettles, J. H.; Nevins, N.; Snyder, J. P.; Liotta, D. C.; Tang, J.; Ermolieff, J.; Schinazi, R. F.; Hill, C. L. *J. Am. Chem. Soc.* **2001**, *123*, 886.
(341) Bosi, S.; Da Ros, T.; Spalluto, G.; Prato, M. *Eur. J. Med. Chem.* **2003**, *38*, 913.
(342) Friedman, S. H.; DeCamp, D. L.; Sijbesma, R. P.; Srdanov, G.; Wudl, F.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6506.
(343) Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; DeCamp, D. L.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510.
(344) (a) Zheng, Z., Juodawlkis, A. S., Wirtz, S. S., Schinazi, R. F., Zeng, H., Bellavia, C., Wudl, F., Hill, C. L. Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials; Electrochemical Society: San Diego, CA, 1998. (b) Toropova, A. P.; Toropov, A. A.; Benfenati, E.; Leszczynska, D.; Leszczynski, J. *J. Math. Chem.* **2010**, *48*, 959. (c) Toropov, A. A.; Toropova, A. P.; Benfenati, E.; Leszczynska, D.; Leszczynski, J. *Eur. J. Med. Chem.* **2010**, *45*, 1387. (d) Durdagi, S.; Mavromoustakos, T.; Chronakis, N.; Papadopoulos, M. G. *Bioorg. Med. Chem.* **2008**, *16*, 9957.
(345) DeCamp, D. L.; Babe, L. M.; Salto, R.; Lucich, J. L.; Koo, M. S.; Kahl, S. B.; Craik, C. S. *J. Med. Chem.* **1992**, *35*, 3426.
(346) Cigler, P.; Kozisek, M.; Rezacova, P.; Brynda, J.; Otwinowski, Z.; Pokorna, J.; Plesek, J.; Gruner, B.; Doleckova-Maresova, L.; Masa, M.; Sedlacek, J.; Bodem, J.; Krausslich, H. -G.; Kral, V.; Konvalinka, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15394.
(347) Strater, N.; Lipscomb, W. N.; Klabunde, T.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2024.

(348) (a) Wilcox, D. E. *Chem. Rev.* **1996**, *96*, 2435. (b) Dembitsky, V. M. *Tetrahedron* **2003**, *59*, 4701. (c) Dev, R.; Srivastava, P. K.; Iyer, J. P.; Dastidar, S. G.; Ray, A. *Expert Opin. Investig. Drugs* **2010**, *19*, 455. (d) Devel, L.; Czarny, B.; Beau, F.; Georgiadis, D.; Stura, E.; Dive, V. *Biochimie* **2010**, *92*, 1501.
(349) (a) Lipscomb, W. N.; Strater, N. *Chem. Rev.* **1996**, *96*, 2375. (b) Lichtenthaler, S. F. *J. Neurochem.* **2011**, *116*, 10. (c) Liebmann, C. *Mol. Cell. Endocrinol.* **2011**, *331*, 222. (d) Sharma, A.; Khuller, G. K.; Sharma, S. *Expert Opin. Ther. Targets* **2009**, *13*, 753.
(350) (a) Dismukes, G. C. *Chem. Rev.* **1996**, *96*, 2909. (b) Gomis-Ruth, X. F. *J. Biol. Chem.* **2009**, *284*, 15353. (c) Adekoya, O. A.; Sylte, I. *Chem. Biol. Drug Des.* **2009**, *73*, 7.
(351) (a) Pisabarro, M. T.; Ortiz, A. R.; Palomer, A.; Cabre, F.; Gracia, L.; Wade, R. C.; Gago, F.; Mauleon, D.; Carganico, G. *J. Med. Chem.* **1994**, *37*, 337. (b) Apte, S. S. *J. Biol. Chem.* **2009**, *284*, 31493. (c) Elias-Arnanz, M.; Padmanabhan, S.; Murillo, F. J. *FEMS Microbiol. Rev.* **2010**, *34*, 764. (d) Bromberg-White, J.; Lee, C. -S.; Duesbery, N. *Toxins* **2010**, *2*, 1038.
(352) (a) Bode, W.; Fernandez-Catalan, C.; Tschesche, H.; Grams, F.; Nagase, H.; Maskos, K. *Cell. Mol. Life Sci.* **1999**, *55*, 639. (b) Ji, R. -R.; Xu, Z. -Z.; Wang, X.; Lo, E. H. *Trends Pharmacol. Sci.* **2009**, *30*, 336. (c) Barrowman, J.; Michaelis, S. *Biol. Chem.* **2009**, *390*, 761. (d) Lebrun, I.; Marques-Porto, R.; Pereira, A. S.; Pereira, A.; Perpetuo, E. A. *Mini Rev. Med. Chem.* **2009**, *9*, 820. (e) Auld, D. S. *BioMetals* **2009**, *22*, 141.
(353) (a) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359. (b) Deitcher, S. R.; Funk, W. D.; Buchanan, J.; Liu, S.; Levy, M. D.; Toombs, C. F. *Expert Opin. Biol. Ther.* **2006**, *6*, 1361.
(354) (a) Massova, I.; Kotra, L. P.; Fridman, R.; Mobashery, S. *FASEB J.* **1998**, *12*, 1075. (b) Yiotakis, A.; Georgiadis, D.; Matziari, M.; Makaritis, A.; Dive, V. *Curr. Org. Chem.* **2004**, *8*, 1135.
(355) (a) Delange, R. J.; Smith, E. L. In *The Enzymes*, 3rd ed; Boyer, P. D., Ed.; Academic Press: New York, 1971. (b) Lisowska-Myjak, B. *Blood Purific.* **2010**, *29*, 357. (c) Cadel, S.; Piesse, C.; Gouzy-Darmon, C.; Cohen, P.; Foulon, T. *Proteases Biol. Dis.* **2004**, *2*, 113. (d) Toldra, F. *Trends Food Sci. Technol.* **2006**, *17*, 164. (e) Attaix, D.; Ventadour, S.; Codran, A.; Bechet, D.; Taillandier, D.; Combaret, L. *Essays Biochem.* **2005**, *41*, 173. (f) Hashimoto, Y. *Arch. Pharm. (Weinheim, Germany)* **2008**, *341*, 536.
(356) (a) Smith, E. L.; Hill, R. L. In *The Enzymes*, 2nd ed.; Boyer, P. D.; Lardy, H.; Myrback, K., Eds.; Academic Press: New York, 1960. (b) Cadel, S.; Foulon, T.; Balogh, A.; Gouzy, C.; Piesse, C.; Thoidis, G.; Kandror, K. V.; Cohen, P. *Int. Congress Ser.* **2001**, *1218*, 365. (c) Vlahovic, P.; Stefanovic, V. *Pathol. Biol.* **1998**, *46*, 779. (d) Nishimura, T. *ACS Symp. Ser.* **2003**, *851*, 419. (e) Little, G. H.; Starnes, W. L.; Behal, F. J. *Methods Enzymol.* **1976**, *45*, 495. (f) Kim, H.; Lipscomb, W. N. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1994**, *68*, 153. (g) Taylor, A. *FASEB J.* **1993**, *7*, 290. (h) Bretzel, R. G.; Schneider, J.; Zimmermann, I.; Kueppers, B.; Weise, M.; Federlin, K. *Contrib. Nephrol.* **1981**, *24*, 153.
(357) (a) Allen, M. P.; Yamada, A. H.; Carpenter, F. H. *Biochemistry* **1983**, *22*, 3778. (b) Evnouchidou, I.; Papakyriakou, A.; Stratikos, E. *Curr. Pharm. Des.* **2009**, *15*, 3656.
(358) (a) Baker, J. O.; Wilkes, S. H.; Bayliss, M. E.; Prescott, J. M. *Biochemistry* **1983**, *22*, 2098. (b) Weston, J. *Chem. Rev.* **2005**, *105*, 2151.
(359) (a) Mucha, A.; Drag, M.; Dalton, J. P.; Kafarski, P. *Biochimie* **2010**, *92*, 1509. (b) Li, X.; Hu, Y. Z. *Curr. Med. Chem.* **2010**, *17*, 3030. (c) Zhang, X. P.; Xu, W. F. *Curr. Med. Chem.* **2008**, *15*, 2850. (d) Luan, Y. P.; Xu, W. F. *Curr. Med. Chem.* **2007**, *14*, 639. (e) Taylor, A. *Trends Biochem. Sci.* **1993**, *18*, 167.
(360) (a) Lin, S. H.; Van Wart, H. E. *J. Biol. Chem.* **1982**, *21*, 5528. (b) Roques, B. P. *Biochem. Soc. Trans.* **1993**, *21*, 678. (c) Chai, S. Y.; Yeatman, H. R.; Parker, M. W.; Ascher, D. B.; Thompson, P. E.; Mulvey, H. T.; Albiston, A. L. *BMC Neurosci.* **2008**, *9*, S14.
(361) (a) Makinen, K. K.; Makinen, P. -L.; Wilkes, S. H.; Bayliss, M.; Prescott, J. M. *Eur. J. Biochem.* **1982**, *128*, 257. (b) Banegas, I.; Ramirez, M.; Alba, F.; Vives, F.; De Gasparo, M.; Segarra, A. B.; Hermoso, F.; Duran, R.; Prieto, E. I. *Endocrinol. Nutr.* **2008**, *55*, 402. (c) Hui, K.-S. *Neurochem. Res.* **2007**, *32*, 2062.
(362) (a) Taylor, A.; Sawan, S.; James, T. L. *J. Biol. Chem.* **1982**, *257*, 11571. (b) Geueke, B.; Kohler, H.-P. E. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 1197. (c) Toldra, F. *Trends Food Sci. Technol.* **2006**, *17*, 164.
(363) Lowther, W. T.; Matthews, B. W. *Chem. Rev.* **2002**, *102*, 4581.
(364) (a) Birch, P. L.; El-Obeid, H. A.; Akhtar, M. *Arch. Biochem. Biophys.* **1972**, *148*, 447. (b) Tarnus, C.; Remy, J.-M.; d'Orchymont, H. *Bioorg. Med. Chem.* **1996**, *4*, 1287. (c) Fittkau, S. *J. Prakt. Chem. (Leipzig)* **1973**, *315*, 1037.
(365) (a) Kettner, C.; Glover, G. I.; Prescott, J. M. *Arch. Biochem. Biophys.* **1974**, *165*, 739. (b) Jahreis, G.; Fittkau, S.; Aurich, H. *Biomed. Biochim. Acta* **1987**, *46*, 683. (c) Fittkau, S.; Foerster, U.; Pascual, C.; Schunck, W. H. *Wissenschaftliche Beitraege (Martin Luther Universitaet Halle-Wittenberg)* **1974**, *6*, 480.
(366) (a) Umezawa, H.; Aoyagi, T.; Suda, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1976**, *29*, 97. (b) Andersson, H.; Demaegdt, H.; Vauquelin, G.; Lindeberg, G.; Karlen, A.; Hallberg, M.; Erdelyi, M.; Hallberg, A. *J. Med. Chem.* **2010**, *53*, 8059.
(367) (a) Rich, D. H.; Moon, B. J.; Harbeson, S. *J. Med. Chem.* **1984**, *27*, 417. (b) Perlikowska, R.; Fichna, J.; Wyrebska, A.; Poels, J.; Vanden, B. J.; Toth, G.; Storr, M.; do Rego, J.-C.; Janecka, A. *Basic Clin. Pharmacol. Toxicol.* **2010**, *106*, 106.
(368) (a) Aoyagi, T.; Tobe, H.; Kojima, F.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 636. (b) V'yunova, T. V.; Shevchenko, K. V.; Shevchenko, V. P.; Bezuglov, V. V.; Myasoedov, N. F. *Radiochemistry (Moscow)* **2009**, *51*, 183.
(369) Chan, W. W. -C.; Dennis, P.; Demmer, W.; Brand, K. *J. Biol. Chem.* **1982**, *257*, 7955.
(370) Wilkes, S. H.; Prescott, J. M. *J. Biol. Chem.* **1983**, *258*, 13517.
(371) (a) Chan, W. W.-C. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 297. (b) Tishinov, K.; Stambolieva, N.; Petrova, S.; Galunsky, B.; Nedkov, P. *Acta Physiol. Plant.* **2009**, *31*, 199. (c) Wilk, S.; Thurston, L. S. *Neuropeptides* **1990**, *16*, 163.
(372) Baker, J. O.; Prescott, J. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1980**, *39*, 1686.
(373) Hussain, M. A.; Shenvi, A. B.; Rowe, S. M.; Shefter, E. *Pharm. Res.* **1989**, *6*, 186.
(374) Hussain, M. A.; Mersinger, L.; Maurin, M. B.; Kettner, C. *Int. J. Pharm.* **1995**, *117*, 181.
(375) Navin, T. R.; Hardy, A. M. *J. Infect. Dis.* **1987**, *155*, 150.
(376) Alpert, G.; Bell, L. M.; Kirkpatrick, C. E.; Budnik, L. D.; Campos, J. M.; Friedman, H. M.; Poltkin, S. A. *New Engl. J. Med.* **1984**, *311*, 860.
(377) Jokipii, L.; Pohjola, S. S.; Jokipii, A. M. *Gastroenterology* **1985**, *89*, 838.
(378) Soave, R.; Ma, P. *Arch. Intern. Med.* **1985**, *145*, 70.
(379) Okhuysen, P. C.; Chappell, C. I.; DuPont, H. L.; Sterling, C. R. *Infect. Immun.* **1994**, *62*, 4667.
(380) Okhuysen, P. C.; Chappell, C. L.; Kettner, C.; Sterling, C. R. *Antimicrob. Agents Chemother.* **1996**, *40*, 2781.
(381) De Paola, C. C.; Bennett, B.; Holz, R. C.; Ringe, D.; Petsko, G. A. *Biochemistry* **1999**, *38*, 9048.
(382) Blech, M. F.; Martin, M.; Pichon, C.; Borrelly, J.; Hartemann, P. *J. Orthop. Surg. B* **1990**, *4*, 123.
(383) Benderdour, M.; Hess, K.; Gadet, M. D.; Dousset, B.; Nabet, P.; Belleville, F. *Biochem. Biophys. Res. Commun.* **1997**, *234*, 263.
(384) Benderdour, M.; Hess, K.; Dzondo-Gadet, M.; Nabet, P.; Belleville, F.; Dousset, B. *Biochem. Biophys. Res. Commun.* **1998**, *246*, 746.
(385) Benderdour, M.; Van Bui, T.; Hess, K.; Dicko, A.; Belleville, F.; Dousset, B. *J. Trace Elem. Med. Biol.* **2000**, *14*, 168.
(386) Nzietchueng, R. M.; Dousset, B.; Frank, P.; Benderdour, M.; Nabet, P.; Hess, K. *J. Trace Elem. Med. Biol.* **2002**, *16*, 239.
(387) Dzondo-Gadet, M.; Nzietchueng, R. M.; Hess, K.; Nabet, P.; Belleville, F.; Dousset, B. *Biol. Trace. Elem. Res.* **2002**, *85*, 23.
(388) Dousset, B.; Benderdour, M.; Hess, K.; Nzietchueng, R. M.; Belleville, F.; Duprez, A. In *Trace Elements in Man and Animals 10*;

Rousse, R. M.; Anderson, R. A.; Tavier, A. E., Eds.; Kluwer Academic/Plenum Publishers: New York, 2000; p 1061.
(389) Pergament, I.; Reich, R.; Srebnik, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1215.
(390) Abu Ali, H.; Berkovitz, R.; Reich, R.; Srebnik, M. *Arch. Pharm. Med. Chem.* **2004**, *337*, 183.
(391) (a) Taniguchi, N.; Ikeda, Y. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1998**, *72*, 239. (b) Limon-Pacheco, J. H.; Gonsebatt, M. E. *Centr. Nerv. Syst. Agents Med. Chem.* **2010**, *10*, 287. (c) Estrela, J.; Ortega, A.; Obrador, E. *Crit. Rev. Clin. Lab. Sci.* **2006**, *43*, 143. (d) Stark, A.-A.; Porat, N.; Volohonsky, G.; Komlosh, A.; Bluvshtein, E.; Tuby, C.; Steinberg, P. *BioFactors* **2003**, *17*, 139.
(392) (a) Hanigan, M. H. *Chem. Biol. Interact.* **1998**, *111*, 333. (b) Zhang, H.; Forman, H. J. *Am. J. Respirat. Cell Mol. Biol.* **2009**, *41*, 509. (c) Burg, D.; Mulder, G. J. *Drug Metab. Rev.* **2002**, *34*, 821. (d) McLellan, L. I.; Wolf, C. R. *Drug Resist. Updates* **1999**, *2*, 153.
(393) Hahn, R.; Wendel, A.; Flohr, L. *Biochim. Biophys. Acta* **1978**, *539*, 324.
(394) Griffith, O. W.; Bridges, R. J.; Meister, A. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 6319.
(395) Griffith, O. W.; Meister, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3384.
(396) Bone, S. N.; Michalopoulos, G.; Jirtle, R. J. *Cancer Res.* **1985**, *45*, 1222.
(397) Huberman, E.; Montesano, R.; Drevon, C.; Kuroke, T.; Saint Vincent, L.; Pugh, T. D.; Goldfarb, S. *Cancer Res.* **1979**, *39*, 269.
(398) Sinha, S.; Hockin, L. J.; Neal, G. E. *Cancer Lett.* **1987**, *35*, 215.
(399) Caspers, M. L.; Diglio, C. A. *Biochim. Biophys. Acta* **1984**, *803*, 1.
(400) Hanigan, M. H.; Frierson, H. F., Jr.; Brown, J. E.; Lovell, M. A.; Taylor, P. T. *Cancer Res.* **1994**, *54*, 286.
(401) Inoue, M.; Hiratkae, J.; Suzuki, H.; Kumagai, H.; Sakata, K. *Biochemistry* **2000**, *39*, 7764.
(402) Paetzel, M.; Dalbey, R. E. *Trends Biochem. Sci.* **1997**, *22*, 28.
(403) Tate, S. S.; Meister, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4806.
(404) Allen, L.; Meck, R.; Yunis, A. *Res. Commun. Chem. Pathol. Pharmacol.* **1980**, *27*, 175.
(405) Revel, J. P.; Ball, E. G. *J. Biol. Chem.* **1959**, *234*, 577.
(406) London, R. E.; Gabel, S. A. *Arch. Biochem. Biophys.* **2001**, *385*, 250.
(407) Antczak, C.; Karp, D. R.; London, R. E.; Bauvois, B. R. *FEBS Lett.* **2001**, *508*, 226.
(408) (a) Stock, D.; Ditzel, L.; Baumeister, W.; Huber, R.; Lowe, J. *Cold Spring Harbor Symp. Quant. Biol* **1995**, *60*, 525. (b) Flotho, C.; Kratz, C.; Niemeyer, C. M. *Curr. Drug Targets* **2007**, *8*, 715.
(409) Groll, M.; Kim, K. B.; Kairies, N.; Huber, R.; Crews, C. M. *J. Am. Chem. Soc.* **2000**, *122*, 1237.
(410) Kisselev, A. F.; Songyang, Z.; Goldberg, A. L. *J. Biol. Chem.* **2000**, *275*, 14831.
(411) Sousa, M. C.; Kessler, B. M.; Overkleeft, H. S.; McKay, D. B. *J. Mol. Biol.* **2002**, *318*, 779.
(412) Pickart, C. M.; Cohen, R. E. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 177.
(413) Bochtler, M.; Ditzel, L.; Groll, M.; Hartmann, C.; Huber, R. *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 295.
(414) Groll, M.; Brandsletter, H.; Bartunik, H.; Bourenkow, G.; Huber, R. *J. Mol. Biol.* **2003**, *327*, 75.
(415) (a) Baumeister, W.; Lupas, A. *Curr. Opin. Struct. Biol.* **1997**, *7*, 273. (b) Foerster, F.; Lasker, K.; Nickel, S.; Sali, A.; Baumeister, W. *Mol. Cell. Proteomics* **2010**, *9*, 1666.
(416) (a) Baumeister, W.; Walz, J.; Zuhl, F.; Seemuller, E. *Cell* **1998**, *92*, 367. (b) Rockel, B.; Baumeister, W. *Ernst Schering Found. Symp. Proc.* **2009**, *1*, 17.
(417) (a) Coux, O.; Tanaka, K.; Goldberg, A. L. *Annu. Rev. Biochem.* **1996**, *65*, 801. (b) Kraft, C.; Peter, M.; Hofmann, K. *Nat. Cell Biol.* **2010**, *12*, 836.
(418) (a) Goldberg, A. L.; Stein, R.; Adams, J. *Chem. Biol.* **1995**, *2*, 503. (b) Bedford, L.; Paine, S.; Sheppard, P. W.; Mayer, R. J.; Roelofs, J. *Trends Cell Biol.* **2010**, *20*, 391.
(419) (a) Hochstrasser, M. *Curr. Opin. Cell Biol.* **1995**, *7*, 215. (b) Moiseeva, T. N.; Mittenberg, A. G.; Barlev, N. A. *Tsitologiya (Russia)* **2010**, *52*, 195.
(420) (a) Voges, D.; Zwickl, P.; Baumeister, W. *Annu. Rev. Biochem.* **1999**, *68*, 1015. (b) Jo, Y.; De Bose-Boyd, R. A. *Crit. Rev. Biochem. Mol. Biol.* **2010**, *45*, 185.
(421) Groll, M.; Ditzel, L.; Loewe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463.
(422) Tanaka, K. *J. Biochem.* **1998**, *123*, 195.
(423) Loewe, J.; Stock, D.; Jap, B.; Zwickl, P.; Baumeister, W.; Huber, R. *Science* **1995**, *268*, 533.
(424) (a) Checler, F.; da Costa, C. A.; Ancolio, K.; Chevallier, N.; Lopez Perez, E.; Marambaud, P. *Biochim. Biophys. Acta* **2000**, *1502*, 133. (b) Colland, F. *Biochem. Soc. Trans.* **2010**, *38*, 137. (c) Berkers, C. R.; Ovaa, H. *Biochem. Trans* **2010**, *38*, 14.
(425) (a) Elliott, P. J.; Pien, C. S.; McCormack, T. A.; Chapman, I. D.; Adams, J. *J. Allergy Clin. Immunol.* **1999**, *104*, 294. (b) Koreth, J.; Alyea, E. P.; Murphy, W. J.; Welniak, L. A. *Biol. Blood Marrow Transpl* **2009**, *15*, 1502. (c) Gerecitano, J.; O'Connor, O. A. *Cancer Treat. Res.* **2006**, *131*, 413.
(426) (a) Dou, Q. P.; Nam, S. *Expert Opin. Ther. Pat.* **2000**, *10*, 1263. (b) Ostrowska, H. *Cell. Mol. Biol. Lett.* **2008**, *13*, 353. (c) Bennett, M. K.; Kirk, C. J. *Curr. Opin. Drug Discovery Dev.* **2008**, *11*, 616.
(427) (a) Myung, J.; Kim, K. B.; Crews, C. M. *Med. Res. Rev.* **2001**, *21*, 245. (b) Orlowski, R. Z.; Kuhn, D. J. *Clin. Cancer Res.* **2008**, *14*, 1649. (c) Terpos, E.; Sezer, O.; Croucher, P.; Dimopoulos, M.-A. *Blood* **2007**, *110*, 1098. (d) Mattingly, L. H.; Gault, R. A.; Murphy, W. J. *Endocr. Metab. Immun. Disord.: Drug Targets* **2007**, *7*, 29.
(428) (a) Adams, J. *Trends Mol. Med.* **2002**, *8*, S49. (b) Breen, H. B.; Espat, N. J. *JPEN: J. Parent. Enteral Nutr.* **2004**, *28*, 272. (c) Delcros, J. G.; Floc'h, M. B.; Prigent, C.; Arlot-Bonnemains, Y. *Curr. Med. Chem.* **2003**, *10*, 479.
(429) Garcia-Echeverria, C. *Mini-Rev. Med. Chem.* **2002**, *2*, 247.
(430) (a) Chauhan, D.; Hideshima, T.; Mitsiades, C.; Richardson, P.; Anderson, K. C. *Mol. Cancer Ther.* **2005**, *4*, 686. (b) Chari, A.; Mazumder, A.; Jagannath, S. *Biol. Targets Ther.* **2010**, *4*, 273. (c) Wright, J. J. *Clin. Cancer Res.* **2010**, *16*, 4094. (d) Cavaletti, G.; Jakubowiak, A. J. *Leukem. Lymph.* **2010**, *51*, 1178. (e) Dimopoulos, M. A.; Chen, C.; Kastritis, E.; Gavriatopoulou, M.; Treon, S. P. *Clin. Lymph. Myeloma Leukem.* **2010**, *10*, 110. (f) Russo, A.; Bronte, G.; Fulfaro, F.; Cicero, G.; Adamo, V.; Gebbia, N.; Rizzo, S. *Curr. Cancer Drug Targets* **2010**, *10*, 55.
(431) Adams, J.; Behnke, M.; Chen, S.; Cruickshank, A. A.; Dick, L. R.; Grenier, L.; Klunder, J. M.; Ma, Y. T.; Plamondon, I.; Stein, R. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333.
(432) Adams, J.; Ma, Y. T.; Stein, R. L.; Baevsky, M.; Grenier, L.; Plamondon, L. Boronic ester and acid compound, synthesis and uses. U.S. Patent 6,083,903, 2000.
(433) (a) Teicher, B. A.; Ara, G.; Herbst, R.; Palombella, V. J.; Adams, J. *Clin. Cancer Res.* **1999**, *5*, 2638. (b) Ludwig, H.; Khayat, D.; Giaccone, G.; Facon, T. *Cancer* **2005**, *104*, 1794. (c) Owen, R. T.; Castaner, R.; Bolos, J. *Drugs Future* **2009**, *34*, 115.
(434) (a) Ling, Y. H.; Liebes, L.; Jiang, J. D.; Holland, J. F.; Elliott, P. J.; Adams, J.; Muggia, F. M.; Perez-Soler, R. *Clin. Cancer Res.* **2003**, *9*, 1145. (b) Davies, A. M.; Lara, P. N., Jr.; Mack, P. C.; Gandara, D. R. *Clin. Cancer Res.* **2007**, *13*, 4647. (c) Board, R. E.; Thatcher, N.; Lorigan, P. *Drugs* **2006**, *66*, 1919. (d) Davies, A. M.; Lara, P. N., Jr.; Mack, P. C.; Gumerlock, P. H.; Bold, R. J.; Gandara, D. R. *Clin. Lung Cancer* **2005**, *7*, S59.
(435) (a) Cusack, J. C. J.; Liu, R.; Houston, M.; Abendroth, K.; Elliott, P. J.; Adams, J.; Baldwin, A. S. J. *Cancer Res.* **2001**, *61*, 3535. (b) Lee, C.; Waldman, T. *Cancer Biol. Ther.* **2003**, *2*, 700.
(436) Johnson, T. R; Stone, K.; Nikrad, M.; Yeh, T.; Zong, W. X.; Thompson, C. B.; Nesterov, A.; Kraft., A. S. *Oncogene* **2003**, *22*, 4953.
(437) (a) Frankel, A.; Man, S.; Elliott, P.; Adams, J.; Kerbel, R. S. *Clin. Cancer Res.* **2000**, *6*, 3719. (b) Piwowar, K. R.; Milacic, V.; Chen,

D.; Yang, H.; Zhao, Y.; Chan, T. H.; Yan, B.; Dou, Q. P. *Drug Resist. Updates* **2006**, *9*, 263. (c) Gadducci, A.; Cosio, S.; Genazzani, A. R. *Expert Rev. Endocrin. Metab.* **2007**, *2*, 225. (d) Aghajanian, C. *Semin. Oncol.* **2004**, *S31*, 22.
(438) (a) Nawrocki, S. T.; Bruns, C. J.; Harbison, M. T.; Bold, R. J.; Gotsch, B. S.; Abbruzzese, J. L.; Elliott, P.; Adams, J.; McConkey, D. J. *Mol. Cancer Ther.* **2002**, *1*, 1243. (b) Sebens, S.; Arlt, A.; Schaefer, H. *Recent Results Cancer Res.* **2008**, *177*, 151. (c) Wadler, S. *Rev. Recent Clin. Trials* **2007**, *2*, 69.
(439) Yin, D.; Zhou, H.; Kumagai, T.; Liu, G.; Ong, J. M.; Black, K. L.; Koeffler., H. P. *Oncogene* **2005**, *24*, 344.
(440) Scagliotti, G. *Crit. Rev. Oncol./Hemat.* **2006**, *58*, 177.
(441) Gracía-Echeverría, C. *Int. J. Pep. Res. Ther.* **2006**, *12*, 49.
(442) Rajkumar, S. V.; Richardson, P. G.; Hideshima, T.; Anderson, K. C. *J. Clin. Oncol.* **2005**, *23*, 630.
(443) An, W. G.; Hwang, S. G.; Trepel, J. B.; Blagosklonny, M. V. *Leukemia* **2000**, *14*, 1276.
(444) Hideshima, T.; Mitsiades, C.; Akiyama, M.; Hayashi, T.; Chauhan, D.; Richardson, P.; Schlossman, R.; Podar, K.; Munshi, N. C.; Mitsiades, N.; Anderson, K. C. *Blood* **2003**, *101*, 1530.
(445) Ling, Y. H.; Liebes, L.; Jiang, J. D.; Holland, J. F.; Elliott, P. J.; Adams, J.; Muggia, F. M.; Perez-Soler, R. *Clin. Cancer Res.* **2003**, *9*, 1145.
(446) Landowski, T. H.; Megli, C. J.; Nullmeyer, K. D.; Lynch, R. M.; Dorr, R. T. *Cancer Res.* **2005**, *65*, 3828.
(447) (a) Carrier, L. *Cardiovasc. Res.* **2010**, *88*, 389. (b) Schubert, U.; Zinser, E.; Steinkasserer, A. Method for inhibiting the maturation of dendritic cells especially the treatment of autoimmune diseases with velcade. *PCT Int. Appl.*, WO 2011006980 A1 20110120, 2011. (c) Zhu, Y.; Zhao, X.; Zhu, X.; Hu, Y.; Wu, G. Preparation of peptidyl boronic acid and its ester derivatives as antitumor agents. *PCT Int. Appl.*, WO 2010012222 A1 20100204, 2010.
(448) Adams, J.; Palombella, V. J.; Sausville, E. A.; Johnson, J.; Destree, A.; Lazarus, D. D.; Maas, J.; Pien, C. S.; Prakash, S.; Elliott, P. J. *Cancer Res.* **1999**, *59*, 2615.
(449) Adams, J.; Kauffman, M. *Cancer Invest.* **2004**, *22*, 304.
(450) Zou, W.; Yue, P.; Lin, N.; Me, M.; Zhou, Z.; Lonial, S.; Khuri, F.; Wang, B.; Sun, S. -Y. *Clin. Cancer Res.* **2006**, *12*, 273.
(451) McCormack, T.; Baumeister, W.; Grenier, L.; Moomaw, C.; Plamondon, L.; Pramanik, B.; Slaughter, C.; Soucy, F.; Stein, R.; Zuhl, F.; Dick, L. *J. Biol. Chem.* **1997**, *272*, 26103.
(452) Pekol, T.; Daniels, J. S.; Labutti, J.; Parsons, I.; Nix, D.; Baronas, E.; Hsieh, F.; Gan, L.-S.; Miwa, G. *Drug Metab. Dispos.* **2005**, *33*, 771.
(453) Uttamsigh, V.; Lu, C.; Miwa, G.; Gan, L.-S. *Drug Metab. Dispos.* **2005**, *33*, 1.
(454) Labutti, J.; Parsons, I.; Huang, R.; Miwa, G.; Gang, L.-S.; Daniels, J. S. *Chem. Res. Toxicol.* **2006**, *19*, 539.
(455) Groll, M.; Berkers, C. R.; Ploegh, H. L.; Ovaa, H. *Structure* **2006**, *14*, 451.
(456) (a) Batovska, D. I.; Todorova, I. T. *Curr. Clin. Pharmacol.* **2010**, *5*, 1. (b) Elgorashi, E. E.; van Staden, J. *ACS Symp. Ser.* **2009**, *1021*, 151. (c) Gupta, D.; Jain, D. K.; Trivedi, P. *Int. J. Chem. Sci.* **2010**, *8*, 649. (d) Prasad, S.; Phromnoi, K.; Yadav, V. R.; Chaturvedi, M. M.; Aggarwal, B. B. *Planta Med.* **2010**, *76*, 1044. (e) Liu, H. L.; Jiang, W. B.; Xie, M. X. *Recent Patents Anti-Cancer Drug Discovery* **2010**, *5*, 152. (f) Rios, J. L.; Recio, M. C.; Escandell, J. M.; Andujar, I. *Curr. Pharm. Des.* **2009**, *15*, 1212. (g) Stoll, R.; Renner, C.; Hansen, S.; Palme, S.; Klein, C.; Belling, A.; Zeslawski, W.; Kamionka, M.; Rehm, T.; Muhlhahn, P.; Schumacher, R.; Hesse, F.; Kaluza, B.; Voelter, W.; Engh, R. A.; Holak, T. A. *Biochemistry* **2001**, *40*, 336.
(457) Kumar, S. K.; Hager, E.; Pettit, C.; Gurulingappa, H.; Davidson, N. E.; Khan, S. R. *J. Med. Chem.* **2003**, *46*, 2813.
(458) (a) Achanta, G.; Modzelewska, A.; Feng, L.; Khan, S. R.; Huang, P. *Mol. Pharmacol.* **2006**, *70*, 426. (b) Sasayama, T.; Tanaka, K.; Mizukawa, K.; Kawamura, A.; Kondoh, T.; Hosoda, K.; Kohmura, E. *J. Neuro-Oncol.* **2007**, *85*, 123.
(459) Satoh, H.; Ueno, M.; Tachibana, Y.; Nakamaru, K.; Kojima, R. Aminoborane acid derivative and proteasome inhibittory drug containing the same. PCT Int. Appl., WO 2003033506 A1, 2003.
(460) Purandare, A. V.; Wan, H.; Laing, N.; Benbatoul, K.; Vaccaro, W.; Poss, M. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4701.
(461) Lai, H. -S; Wu, W; Bachovchin, W. W. Soft protease inhibitors and pro-soft forms thereof, and their therapeutic use. *PCT Int. Appl.*, WO 2007100374 A2, 2007
(462) Siman, R.; Jani, J.; Goldfarb, R.; Dou, Q. *Multicatalytic protease inhibitors for use as antitumor agents*. U.S. Patent 9930707, 1999.
(463) Gardner, R. C.; Assinder, S. J.; Christie, G.; Mason, G. G. F.; Markwell, R.; Wadsworth, H.; Mclaughlin, M.; King, R.; Ghabot-Fletcher, M. C.; Breton, J. J.; Allsop, D.; Rivett, A. J. *Biochem. J.* **2000**, *346*, 447.
(464) Iqbal, M.; Chatterjee, S.; Kauer, J. C.; Das, M.; Messina, P.; Freed, B.; Biazzo, W.; Siman, R. *J. Med. Chem.* **1995**, *38*, 2276.
(465) Aubin, S.; Martin, B.; Delcros, J. -G.; Arlot-Bonnermains, Y.; Baudy-Floch, M. *J. Med. Chem.* **2005**, *48*, 330.
(466) Zhu, Y. -Q.; Wu, G.; Zhu, X. -R.; Ma, Y. -H.; Zhao, X.; Li, Y. -J.; Yuan, Y. -X.; Yang, J.; Yu, S.; Shao, F.; Lei, M. *J. Med. Chem.* **2010**, *53*, 8619.
(467) Zhu, Y.; Zhu, X.; Wu, G.; Ma, Y.; Li, Y.; Zhao, X.; Yuan, Y.; Yang, J.; Yu, S.; Shao, F.; Li, R.; Ke, Y.; Lu, A.; Liu, Z.; Zhang, L. *J. Med. Chem.* **2010**, *53*, 1990.
(468) Milo, L. J.; Lai, J. H.; Wu, W.; Liu, Y.; Maw, H.; Li, Y.; Jin, Z.; Shu, Y.; Poplawski, S. E.; Wu, Y.; Sanford, D. G.; Sudmeier, J. L.; Bachovchin, W. W. *J. Med. Chem.* **2011**, *54*, 4365.
(469) (a) Behar, S. M.; Divangahi, M.; Remold, H. G. *Nat. Rev. Microbiol.* **2010**, *8*, 668. (b) Rachel, M.; Lucinda, R.; Paul, E. *J. Infection* **2010**, *61*, 518. (c) Shiloh, M. U.; Champion, P. A. D. *Curr. Opin. Microbiol.* **2010**, *13*, 86. (d) Russell, D. G.; Cardona, P. J.; Kim, M. J. *Nat. Immunol.* **2010**, *10*, 943. (e) Lin, G.; Hu, G.; Tsu, C.; Kunes, Y. Z.; Li, H.; Dick, L.; Parsons, T.; Li, P.; Chen, Z.; Zwickl, P.; Weich, N.; Nathan, C. *Mol. Microbiol.* **2006**, *59*, 1405.
(470) Darwin, K. H.; Ehrt, S.; Gutierrez-Ramos, J.-C.; Weich, N.; Nathan, C. F. *Science* **2003**, *302*, 1963.
(471) Hu, G.; Lin, G.; Wang, M.; Dick, L.; Xu, R. -M.; Nathan, C.; Li, H. *Mol. Micobiol.* **2006**, *59*, 1417.
(472) (a) Garcia, I.; Fall, Y.; Gomez, G. *Curr. Pharm. Des.* **2010**, *16*, 2666. (b) John, C. C.; Kutamba, E.; Mugarura, K.; Opoka, R. O. *Expert Rev. Anti-Infect. Ther.* **2010**, *8*, 997. (c) Besteiro, S.; Duy, S. V.; Perigaud, C.; Lefebvre-Tournier, I.; Vial, H. J. *Parasitology* **2010**, *137*, 1343. (d) Bloland, P. B. *Drug Resistance in Malaria*: World Health Organization Publishers: Geneva, Switzerland, 2001.
(473) Kisselev, A. F.; Goldberg, A. L. *Chem. Biol.* **2001**, *8*, 739.
(474) Lindenthal, C.; Weich, N.; Chia, Y. -S.; Heussler, V.; Klinkert, M.-Q. *Parasitology* **2005**, *131*, 37.
(475) Ash, D. E.; Cox, J. D.; Christianson, D. W. In *Manganese and Its Role in Biological Processes*; Sigel, A., Sigel, H., Eds.; M. Dekker: New York, 1999; Metal Ions in Biological Systems, Vol. *37*, p 407.
(476) (a) Wu, G.; Morris, S. M., Jr. *Biochem. J.* **1998**, *336*, 1. (b) North, M. L.; Meurs, H.; Zaagsma, J.; Scott, J. A.; Maarsingh, H. *Open Nitric Oxide J.* **2010**, *2*, 20. (c) Kepka-Lenhart, D.; Ash, D. E.; Morris, S. M. *Methods Enzymol.* **2008**, *440*, 221. (d) Hrabak, A. *Br. J. Pharmacol.* **2006**, *147*, 835.
(477) Krebs, H. A.; Henseleit, K. *Hoppe-Seyler's Z. Physiol. Chem.* **1932**, *210*, 33.
(478) Herzfeld, A.; Raper, S. M. *Biochem. J.* **1976**, *153*, 469.
(479) (a) Morris, S. M., Jr. *Ann. Rev. Nutr.* **2002**, *22*, 87. (b) Nelin, L. D.; Stenger, M. R.; Malleske, D. T.; Chicoine, L. G. *Curr. Hypertens. Rev.* **2007**, *3*, 242. (c) Zimmermann, N.; Rothenberg, M. E. *Eur. J. Pharmacol.* **2006**, *533*, 253.
(480) (a) Miyamoto, Y.; Ganapathy, V.; Barlas, A.; Neuber, K.; Barth, A.; Leibach, F. H. *Am. J. Physiol.* **1987**, *252*, F670. (b) Mori, M. *J. Nutr.* **2007**, *137*, 1616S. (c) King, N. E.; Rothenberg, M. E.; Zimmermann, N. *J. Nutr.* **2004**, *134*, 2830S.
(481) Yip, M. C. M.; Knox, W. E. *Biochem. J.* **1972**, *127*, 893.
(482) Buga, G. M.; Wei, L. H.; Bauer, P. M.; Fukuto, J. M.; Ignarro, L. J. *Am. J. Physiol.* **1998**, *275*, R1256.

(483) Singh, R.; Pervin, S.; Karimi, A.; Cederbaum, S.; Chaudhuri, G. *Cancer Res.* **2000**, *60*, 3305.
(484) Morris, S. M., Jr.; Bhamidipati, D.; Kepka-Lenhart, D. *Gene* **1997**, *193*, 157.
(485) Jenkinson, C. P.; Grody, W. W.; Cederbaum, S. D. *Comp. Biochem. Physiol.* **1996**, *114B*, 107.
(486) Lyer, R.; Jenkinson, C. P.; Vockley, J. G.; Kern, R. M.; Grody, W. W.; Cederbaum, S. *J. Inher. Metab. Dis.* **1998**, *21* (Suppl. 1), 81.
(487) Perozich, J.; Hempel, J.; Morris, S. M., Jr. *Biochim. Biophys. Acta* **1998**, *1382*, 23.
(488) Shi, O.; Morris, S. M., Jr.; Zoghbi, H.; Porter, C. W.; O'Brien, W. E. *Mol. Cell. Biol.* **2001**, *21*, 811.
(489) Albina, J. E.; Mills, C. D.; Henry, W. L., Jr.; Caldwell, M. D. *J. Immunol.* **1990**, *144*, 3877.
(490) Wang, W. W.; Jenkinson, C. P.; Griscavage, J. M.; Kern, R. M.; Arabolos, N. S.; Byrnes, R. E.; Cederbaum, S. D.; Ignarro, L. J. *Biochem. Biophys. Res. Commun.* **1995**, *210*, 1009.
(491) Modolell, M.; Corraliza, I. M.; Link, F.; Solar, G.; Eichmann, K. *Eur. Immunol.* **1995**, *25*, 1101.
(492) Sonoki, T.; Nagasaki, A.; Gotoh, T.; Takiguchi, M.; Takeya, M.; Matsuzaki, H.; Mori, M. *J. Biol. Chem.* **1997**, *272*, 3689.
(493) Chakder, S.; Rattan, S. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 378.
(494) Baggio, R.; Emig, F. E.; Christianson, D. W.; Ash, D. E.; Chakder, S.; Rattan, S. *J. Pharm. Exp. Ther.* **1999**, *290*, 1409.
(495) Cox, J. D.; Kim, N. N.; Traish, A. M.; Christianson, D. W. *Nat. Struct. Biol.* **1999**, *6*, 1043.
(496) Kim, N. N.; Cox, J. D.; Baggio, R. F.; Emig, F. A.; Mistry, S. K.; Harper, S. L.; Speicher, D. W.; Morris, S. M., Jr.; Ash, D. E.; Traish, A.; Christianson, D. W. *Biochemistry* **2001**, *40*, 2678.
(497) Christianson, D. W. *Acc. Chem. Res.* **2005**, *38*, 191.
(498) Cama, E.; Colleluori, D. M.; Emig, F. A.; Shin, H.; Kim, S. W.; Kim, N. N.; Traish, A. M.; Ash, D. E.; Christianson, D. W. *Biochemistry* **2003**, *42*, 8445.
(499) Xu, L.; Hillard, B.; Carmody, R. J.; Tsabary, G.; Shin, H.; Christianson, D. W.; Chen, Y. H. *Immunology* **2003**, *110*, 141.
(500) Baggio, R.; Cox, J. D.; Harper, S. L.; Speicher, D. W.; Christianson, D. W. *Anal. Biochem.* **1999**, *276*, 251.
(501) (a) Colleluori, D. M.; Ash, D. E. *Biochemistry* **2001**, *40*, 9356. (b) Cox, J. D.; Christianson, D. W. *ACA Transact.* **2001**, *35*, 85.
(502) Gevorkyan, M. L.; Davtyan, M. A. *Hayastani Kensabanakan Handes* **2008**, *60*, 16.
(503) Baggio, R.; Elbaum, D.; Kanyo, Z. F.; Carroll, P. J.; Cavalli, R. C.; Ash, D. E.; Christianson, D. W. *J. Am. Chem. Soc.* **1997**, *119*, 8107.
(504) Reczkowski, R. S.; Ash, D. *Arch. Biochem. Biophys.* **1994**, *312*, 31.
(505) Di Constanzo, L.; Sabio, G.; Mora, A.; Rodriguez, P. C.; Ochoa, A. C.; Centeno, F.; Christianson, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *63*, 13058.
(506) (a) Kanyo, Z. F.; Scolnick, L. R.; Ash, D. E.; Christianson, D. W. *Nature* **1996**, *383*, 554. (b) Marmorstein, R. *Structure* **2001**, *9*, 1127.
(507) Mori, M.; Gotoh, M. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 715.
(508) Collet, S.; Carreaux, F.; Boucher, J. -L.; Pethe, S.; Lepoivre, M.; Danion-Bougot, R.; Danion, D. *J. Chem. Soc., Perkin Trans 1* **2000**, *2*, 177.
(509) (a) Kohli, R. M.; Trauger, J. W.; Schwarzer, D.; Marahiel, M. A.; Walsh, C. T. *Biochemistry* **2001**, *40*, 7099. (b) Strieker, M.; Tanovic, A.; Marahiel, M. A. *Curr. Opin. Struct. Biol.* **2010**, *20*, 234. (c) Felnagle, E. A.; Jackson, E. E.; Chan, Y. A.; Podevels, A. M.; Berti, A. D.; McMahon, M. D.; Thomas, M. G. *Mol. Pharm.* **2008**, *5*, 191. (d) Tiburzi, F.; Visca, P.; Imperi, F. *IUBMB Life* **2007**, *59*, 730.
(510) (a) Schwarzer, D.; Marahiel, M. A. *Naturwissenschaften* **2001**, *88*, 93. (b) Marahiel, M. A.; Essen, L.-O. *Methods Enzymol.* **2009**, *458*, 337. (c) Wenzel, S. C.; Mueller, R. *Nat. Prod. Rep.* **2007**, *24*, 1211. (d) Haynes, S. W.; Challis, G. L. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 203.
(511) Trauger, J. W.; Kohli, R. M.; Walsh, C. T. *Biochemistry* **2001**, *40*, 7092.
(512) Skelton, N. J.; Harding, M. M.; Mortishire-Smith, R. J.; Rahman, S. K.; Williams, D. H.; Rance, M. J.; Ruddock, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7522.
(513) Peypoux, F.; Bonmatin, J. M.; Wallach, J. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 553.
(514) Cosmina, P.; Rodriguez, F.; de Ferra, F.; Grandi, G.; Perego, M.; Venema, G.; van Sinderen, D. *Mol. Microbiol.* **1993**, *8*, 821.
(515) Stein, T.; Vater, J.; Kruft, V.; Otto, A.; Wittmann-Liebold, B.; Franke, P.; Panico, M.; McDowell, R.; Morris, H. R. *J. Biol. Chem.* **1996**, *271*, 15428.
(516) Bruner, S. D.; Weber, T.; Kohli, R. M.; Schwarzer, D.; Marahiel, M. A.; Walsh, C. T.; Stubbs, M. T. *Structure* **2002**, *10*, 301.
(517) Tseng, C. C.; Bruner, S. D.; Kohli, R. M.; Marahiel, M. A.; Walsh, C. T.; Sieber, S. A. *Biochemistry* **2002**, *41*, 13350.
(518) Coutts, S. J.; Adams, J.; Krolikowski, D.; Snow, R. J. *Tetrahedron Lett.* **1994**, *35*, 5109.
(519) (a) Shaw, E. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1990**, *63*, 271. (b) Fricker, S. P. *Metallomics* **2010**, *2*, 366. (c) Zavasnik-Bergant, T.; Turk, B. *Biol. Chem.* **2007**, *388*, 1141.
(520) (a) Otto, H. -H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133. (b) Ettari, R.; Bova, F.; Zappala, M.; Grasso, S.; Micale, N. *Med. Res. Rev.* **2010**, *30*, 136. (c) Dubey, V. K.; Pande, M.; Singh, B. K.; Jagannadham, M. V. *Afr. J. Biotechnol.* **2007**, *6*, 1077.
(521) (a) Rasnick, D. *Prospect Drug Discovery Des.* **1996**, *6*, 47. (b) Juncker, T.; Schumacher, M.; Dicato, M.; Diederich, M. *Biochem. Pharmacol.* **2009**, *78*, 1. (c) Abbenante, G.; Fairlie, D. P. *Med. Chem.* **2005**, *1*, 71.
(522) (a) Maliar, T.; Balaz, S.; Tandlich, R.; Sturdik, E. *Acta Virol.* **2002**, *46*, 131. (b) Curry, S.; Roque-Rosell, N.; Sweeney, T. R.; Zunszain, P. A.; Leatherbarrow, R. J. *Biochem. Soc. Trans.* **2007**, *35*, 594.
(523) (a) Ryan, M. D.; Flint, M. *J. Gen. Virol.* **1997**, *78*, 699. (b) Schirmeister, T.; Welker, A. *Pharm. Unserer Zeit* **2009**, *38*, 564. (c) Greber, U. F. *Rev. Med. Virol.* **1998**, *8*, 213.
(524) (a) Rota, P. A.; Oberste, M. S.; Monroe, S. S.; Nix, W. A.; Campagnoli, R.; Icenogle, J. P.; Penaranda, S.; Bankamp, B.; Maher, K.; Chen, M. -H.; Tong, S.; Tamin, A.; Lowe, L.; Frace, M.; Derisi, J. L.; Chen, Q.; Wang, D.; Erdman, D. D.; Peret, T. C. T.; Burns, C.; Ksiazek, T. G.; Rollin, P. E.; Sanchez, A.; Liffick, S.; Holloway, B.; Limor, J.; McCaustland, K.; Olsen-Rasmussen, M.; Fouchier, R.; Gunther, S.; Osterhaus, A. D. M. E.; Drosten, C.; Pallansch, M. A.; Anderson, L. J.; Bellini, W. J. *Science* **2003**, *300*, 1394. (b) Shah, F.; Mukherjee, P.; Desai, P.; Avery, M. *Curr. Comp.-Aided Drug Des.* **2010**, *6*, 1. (c) Santos, M. M. M.; Moreira, R. *Mini Rev. Med. Chem.* **2007**, *7*, 1040. (d) Sirois, S.; Zhang, R.; Gao, W.; Gao, H.; Li, Y.; Zheng, H.; Wei, D.-Q. *Curr. Comp.-Aided Drug Des.* **2007**, *3*, 191. (e) De Clercq, E. *Expert Rev. Anti-Infective Ther.* **2006**, *4*, 291.
(525) Peiris, J. S.; Lai, S. T.; Poon, L. L.; Guan, Y.; Yam, L. Y.; Lim, W.; Nicholls, J.; Yee, W. K.; Yan, W. W.; Cheung, M. T.; Cheng, V. C.; Chan, K. H.; Tsang, D. N.; Yung, R. W.; Ng, T. K.; Yuen, K. Y. *Lancet* **2003**, *361*, 1319.
(526) Ksiazek, T. G.; Erdman, D.; Goldsmith, C. S.; Zaki, S. R.; Peret, T.; Emery, S.; Tong, S.; Urbani, C.; Comer, J. A.; Lim, W.; Rollin, P. E.; Dowell, S. F.; Ling, A. E.; Humphrey, C. D.; Shieh, W. J.; Guarner, J.; Paddock, C. D.; Rota, P.; Fields, B.; DeRisis, J.; Yang, J. Y.; Cox, N.; Hughes, J. M.; LeDue, J. W.; Bellini, W. J.; Anderson, L. J. A. *New Eng. J. Med.* **2003**, *348*, 1953.
(527) Fouchier, R. A.; Kuiken, T.; Schutten, M.; van Amerongen, G.; van Doornum, G. J.; van den Hoogen, B. G.; Peiris, M.; Lim, W.; Stohr, K.; Osterhaus, A. D. *Nature* **2003**, *423*, 240.
(528) Drosten, C.; Gunther, S.; Preiser, W.; van der Werf, S; Brodt, H. R.; Becker, S.; Rabenau, H.; Panning, M.; Kolesnikova, L.; Fouchier, R. A.; Berger, A.; Burguiere, A. M.; Cinatl, J.; Eickmann, M.; Escriou, N.; Grywna, K.; Kramme, S.; Manuguerra, J. C.; Muller, S.; Rickerts, V.; Sturmer, M.; Vieth, S.; Klenk, H. D.; Osterhaus, A. D.; Schmitz, H.; Doerr, H. W. *New Eng. J. Med.* **2003**, *348*, 1967.
(529) Kuiken, T.; Fouchier, R. A.; Schutten, M.; Rimmelzwaan, G. F.; van Amerongen, G.; van Riel, D.; Laman, J. D.; de Jong, T.; van Doornum, G.; Lim, W.; Ling, A. E.; Chan, P. K.; Tam, J. S.; Zambon,

M. C.; Gopal, R.; Drosten, C.; van der Werf, S.; Escriou, N.; Manuguerra, J. C.; Stohr, K.; Peiris, J. S.; Osterhaus, A. D. *Lancet* **2003**, *362*, 263.
(530) Marra, M. A.; Jones, S. J.; Astell, C. R.; Holt, R. A.; Brooks-Wilson, A.; Butterfield, Y. S.; Khattra, J.; Asano, J. K.; Barber, S. A.; Chan, S. Y.; Cloutier, A.; Coughlin, S. M.; Freeman, D.; Girn, N.; Griffith, O. L.; Leach, S. R.; Mayo, M.; McDonald, H.; Montgomery, S. B.; Pandoh, P. K.; Petrescu, A. S.; Robertson, G.; Schein, J. E.; Siddiqui, A.; Smailus, D. E.; Stott, J. M.; Yang, G. S.; Plummer, F.; Andonov, A.; Artsob, H.; Bastien, N.; Bernard, K.; Booth, T. F.; Bowness, D.; Czub, M.; Drebot, M.; Fernando, L.; Flick, R.; Garbutt, M.; Gray, M.; Grolla, A.; Jones, S.; Feldmann, H.; Meyers, A.; Kabani, A.; Li, Y.; Normand, S.; Stroher, U.; Tipples, G. A.; Tyler, S.; Vogrig, R.; Ward, D.; Watson, B.; Brunham, R. C.; Krajden, M.; Petric, M.; Skowronski, D.; Upton, C.; Roper, R. *Science* **2003**, *300*, 1399.
(531) Ruan, Y. J.; Wei, C. L.; Ee, A. L.; Vega, V. B.; Thoreau, H.; Su, S. T.; Chia, J. M.; Ng, P.; Chiu, K. P.; Lim, L.; Zhang, T.; Peng, C. K.; Lin, E. O.; Lee, N. M.; Yee, S. L.; Ng, L. F.; Chee, R. E.; Stanto, L. W.; Long, P. M.; Liu, E. T. *Lancet* **2003**, *361*, 1779.
(532) Yeh, S. H.; Wang, H. Y.; Tsai, C. Y.; Kao, C. L.; Yang, J. Y.; Liu, H. W.; Su, I. J.; Tsai, S. F.; Chen, D. S.; Chen, P. J.; Chen, D. S.; Lee, Y. T.; Teng, C. M.; Yang, P. C.; Ho, H. N.; Chen, P. J.; Chang, M. F.; Wang, J. T.; Chang, S. C.; Kao, C. L.; Wang, W. K.; Hsiao, C. H.; Hsueh, P. R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2542.
(533) Snijder, E. J.; Bredenbeek, P. J.; Dobbe, J. C.; Thiel, V.; Ziebuhr, J.; Poon, L. L. M.; Guan, Y.; Rozanov, M.; Spaan, W. J. M.; Gorbalenya, A. E. *J. Mol. Biol.* **2003**, *331*, 991.
(534) Thiel, V.; Ivanov, K. A.; Putics, A.; Hertzig, T.; Schelle, B.; Bayer, S.; Weissbrich, B.; Snijder, E. J.; Rabenau, H.; Wilhelm, D.; Gorbalenya, A. E.; Ziebuhr, J. *J. Virol.* **2003**, *84*, 2305.
(535) Gao, F.; Ou, H. Y.; Chen, L. L.; Zheng, W. X.; Zhang, C. T. *FEBS Lett.* **2003**, *553*, 451.
(536) Allaire, M.; Chernaia, M. M.; Malcolm, B. A.; James, M. N. *Nature* **1994**, *369*, 72.
(537) (a) Anand, K.; Ziebuhr, J.; Wadhwani, P.; Mesters, J. R.; Hilgenfeld, R. *Science* **2003**, *300*, 1763. (b) Wojdyla, J. A.; Manolaridis, I.; van Kasteren, P. B.; Kikkert, M.; Snijder, E. J.; Gorbalenya, A. E.; Tucker, P. A. *J. Virol.* **2010**, *84*, 10063. (c) Piotrowski, Y.; Hansen, G.; Boomaars-van der Zanden, A. L.; Snijder, E. J.; Gorbalenya, A. E.; Hilgenfeld, R. *Protein Sci.* **2009**, *18*, 6.
(538) Huang, C.; Wei, P.; Fan, K.; Liu, Y.; Lai, L. *Biochemistry* **2004**, *43*, 4568.
(539) (a) Anard, K.; Palm, G. J.; Mesters, J. R.; Siddell, S. G.; Ziebuhr, J.; Hilgenfeld, R. *EMBO J.* **2002**, *21*, 3213. (b) Chen, I. J.; Chou, C. C.; Liu, C. L.; Lee, C. C.; Kan, L. S.; Hou, M. H. *Acta Crystallogr.* **2010**, *F66*, 815. (c) Xu, Y.; Cong, L.; Chen, C.; Wei, L.; Zhao, Q.; Xu, X.; Ma, Y.; Bartlam, M.; Rao, Z. *J. Virol.* **2009**, *83*, 1083.
(540) (a) Yang, H.; Yang, M.; Ding, Y.; Liu, Y.; Lou, Z.; Zhou, Z.; Sun, L.; Mo, L.; Ye, S.; Pang, H.; Gao, G. F.; Anand, K.; Bartlam, M.; Hilgenfeld, R.; Rao, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13190. (b) Wu, K.; Li, W.; Peng, G.; Li, F. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 19970. (c) Manolaridis, I.; Wojdyla, J. A.; Panjikar, S.; Snijder, E. J.; Gorbalenya, A. E.; Berglind, H.; Nordlund, P.; Coutard, B.; Tucker, P. A. *Acta Crystallogr.* **2009**, *D65*, 839.
(541) (a) Hsu, M. F.; Kuo, C. J.; Chang, K. T.; Chang, H. C.; Chou, C. C.; Ko, T. P.; Shr, H. L.; Chang, G. G.; Wang, A. H . -J.; Liang, P. H. *J. Biol. Chem.* **2005**, *280*, 31257. (b) Wojdyla, J. A.; Manolaridis, I.; Snijder, E. J.; Gorbalenya, A. E.; Coutard, B.; Piotrowski, Y.; Hilgenfeld, R.; Tucker, P. A. *Acta Crystallogr.* **2009**, *D65*, 1292. (c) Wu, K.; Li, W.; Peng, G.; Li, F. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 1.
(542) Shi, J. H.; Wei, Z.; Song, J. X. *J. Biol. Chem.* **2004**, *279*, 24765.
(543) Hsu, W. C.; Chang, H. C.; Chou, C. Y.; Tsai, P. J.; Lin, P. I.; Chang, G. G. J. *Biol. Chem.* **2005**, *280*, 22741.
(544) Bacha, U.; Barrila, J.; Velazquez-Campoy, A.; Leavitt, S. A.; Freire, E. *Biochemistry* **2004**, *43*, 4906.
(545) Asano, T.; Nakamura, H.; Uehara, Y.; Yamamoto, Y. *ChemBioChem* **2004**, *5*, 483.
(546) Onoda, T.; Linuma, H.; Sasaki, Y.; Hamada, M.; Isshiki, K.; Nakagawa, H.; Takeuchi, T. *J. Nat. Prod.* **1989**, *52*, 1252.
(547) Chen, H.; Boriziau, J.; Parker, F.; Maroun, R.; Tocque, B.; Roques, B. P. *J. Med. Chem.* **1993**, *36*, 4094.
(548) Chen, H.; Boriziau, J.; Parker, F.; Mailliet, P.; Commercon, A.; Tocque, B.; Le Pecq, J.-B.; Roques, B. P.; Garbay, C. *J. Med. Chem.* **1994**, *37*, 845.
(549) Nussbaumer, P.; Winiski, A. P.; Cammisuli, S.; Hiestand, P.; Weckbecker, G.; Stuts, A. *J. Med. Chem.* **1994**, *37*, 4079.
(550) Mu, F.; Coffing, S. L.; Riese, D. J., II; Geahlen, R. L.; Verdier-Pinard, P.; Hamel, E.; Johnson, J.; Cushman, M. *J. Med. Chem.* **2001**, *44*, 441.
(551) (a) Mu, F.; Lee, D. J.; Pryor, D. E.; Hamel, E.; Cushman, M. J. *Med. Chem.* **2002**, *45*, 4774. (b) Umezawa, K. *Stud. Nat. Prod. Chem.* **1995**, *15*, 439. (c) Lepple-Wienhues, A.; Szabo, I.; Wieland, U.; Heil, L.; Gulbins, E.; Lang, F. *Cell. Physiol. Biochem.* **2000**, *10*, 307. (d) Henley, D. V.; Korach, K. S. *Endocrinol.* **2006**, *147*, S25.