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INHIBITION OF THE RTEM-1 β-LACTAMASE BY BORONIC ACIDS

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Abstract: Inhibition constants for substituted phenyl and phenylethyl boronic acids have been determined. The trends and factors involved in their binding are discussed.

Introduction

The antibiotic properties of penicillins and cephalosporins are due to their abilities to inhibit bacterial growth by acylation of the active site serine residues of DD-carboxypeptidases involved in cell wall synthesis.[1] β-Lactamases defend growing bacteria against the lethal effect of β-lactam antibiotics by catalyzing the hydrolysis of the β-lactam ring, thus rendering them inactive. Application of β-lactamase inhibitors represents one strategy for combating the β-lactam-deactivating capacities of these enzymes.[2] The most effective β-lactamase inhibitors described so far are themselves β-lactams, but only one of these, clavulanic acid, is employed clinically.[3] With bacterial resistance to β-lactamases continuing to increase, identification of new structural classes of inhibitors is of interest for clinical reasons. Furthermore, the additional information that inhibitor studies provide about active site binding requirements can identify new lead structures for potential drugs.

Boronic acid derivatives represent one inhibitor group that has already provided useful information in this regard, on class A and C β-lactamases.[4,5] Boronic acids have been shown by ^{11}B NMR spectroscopy to be reversible transition state-analog inhibitors that form tetrahedral adducts with the active site serine of β-lactamases.[6] Recently, a breakthrough in understanding the β-lactamase mechanism was reported[7], based on the 1.7 Å resolution X-ray structure of the class A RTEM-1 β-lactamase from *Escherichia coli*. This prompted us to examine the capacities of boronic acids to inhibit the RTEM-1 β-lactamase, beginning with compounds **1-22** prepared for previous serine protease inhibitor studies.[8a]

$R{-}B(OH)_2$

Phenylethyl series

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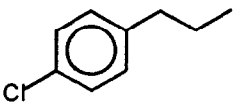

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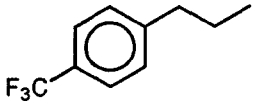

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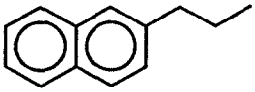

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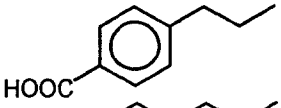

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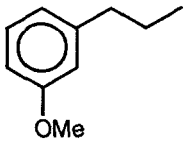

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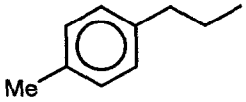

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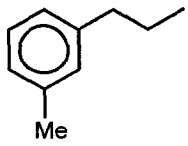

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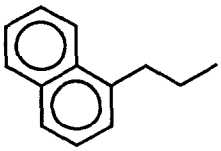

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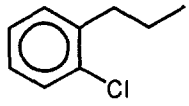

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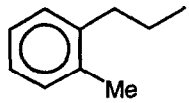

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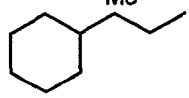

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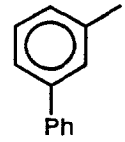

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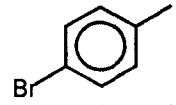

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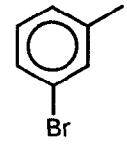

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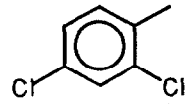

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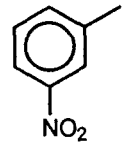

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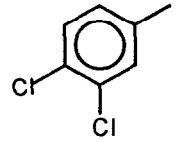

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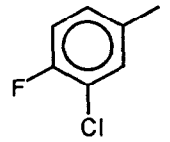

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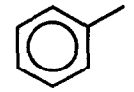

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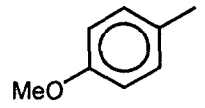

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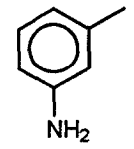


Results and Discussion

Boronic acids **1-22** were obtained as described previously[8a] and their inhibition constants determined using the method of Waley.[9] The assays were carried out with penicillin G (at λ = 232 nm),[10] 6-aminopenicillanic acid (at λ = 220-262 nm)[10] or nitrocefin (at λ = 482 nm)[11] as substrates, with the substrate selection being determined by the constraints imposed by the absorption spectra of the individual boronic acids. Competitive inhibition behavior was observed in each case, and the K_I's, each determined in duplicate, were calculated using the Grafit program. The boronic acid structures **1-22** are conveniently identifiable as being of the phenylethyl (**1-12**) or phenyl (**13-22**) family and the K_I data are recorded on this basis in Tables 1 and 2 respectively.

Table 1: Inhibition Constants for Phenylethyl Boronic Acids **1-12**

Inhibitor	K_I (μM)[a]
2-Phenylethyl boronic acid (**1**)	29.8 ± 0.7 [b]
2-(4-Chlorophenyl)ethyl boronic acid (**2**)	41 ± 3 [c]
2-(4-Trifluoromethylphenyl)ethyl boronic acid (**3**)	43 ± 3 [c]
2-(2-Naphthyl)ethyl boronic acid (**4**)	44 ± 3 [c]
2-(4-Carboxyphenyl)ethyl boronic acid (**5**)	49 ± 1 [b]
2-(3-Methoxyphenyl)ethyl boronic acid (**6**)	50 ± 3 [c]
2-(4-Methylphenyl)ethyl boronic acid (**7**)	58 ± 3 [d]
2-(3-Methylphenyl)ethyl boronic acid (**8**)	83 ± 4 [d]
2-(1-Naphthyl)ethyl boronic acid (**9**)	101 ± 6 [c]
2-(2-Chlorophenyl)ethyl boronic acid (**10**)	106 ± 7 [c]
2-(2-Methylphenyl)ethyl boronic acid (**11**)	141 ± 7 [d]
2-Cyclohexylethyl boronic acid (**12**)	276 ± 13 [c]

[a] Determined[9] in duplicate in NaH_2PO_4 (50mM) buffer, pH 7.0, 25°C
Substrates: [b] nitrocefin 0.1 mM, 0.5% DMSO, K_M = 25.5±0.5 μM
[c] 6-aminopenicillanic acid 2.5-5 mM, K_M = 160±10 μM
[d] penicillin G potassium salt 1 mM, K_M = 20±1 μM

As Table 1 shows, all of the phenylethyl boronic acids **1-12** are good inhibitors, with the best being the parent member of the series, phenylethyl boronic acid (**1**) itself. Competitive inhibition was observed for all the Table 1 inhibitors with the exception of 2-(4-trifluoromethylphenyl)ethyl boronic acid (**3**), for which slow-binding kinetics were manifest. For substituted phenylethyl boronic acids, a general trend in the potencies of inhibition is that, for a given function, the K_I increases for para, meta, and ortho substitution respectively. This is most clearly seen for the methyl-substituted inhibitors **7,8,** and **11**. This trend is clearly structurally dictated, but graphic analyses did not permit the specific interactions responsible to be identified.

The inhibition constants, all competitive, observed for the phenyl boronic acids **13-22** are shown in Table 2. The range of K_I values for these inhibitors is seen to be much broader than for their phenylethyl counterparts, with this series exhibiting both the best (**13**) and worst (**22**) inhibitors of the current study. The wider range of K_I values reflects the greater sensitivity of the inhibition potency of phenyl boronic acids to the nature of the ring substituent.

Table 2: Inhibition Constants for Phenyl Boronic Acids **13-22**

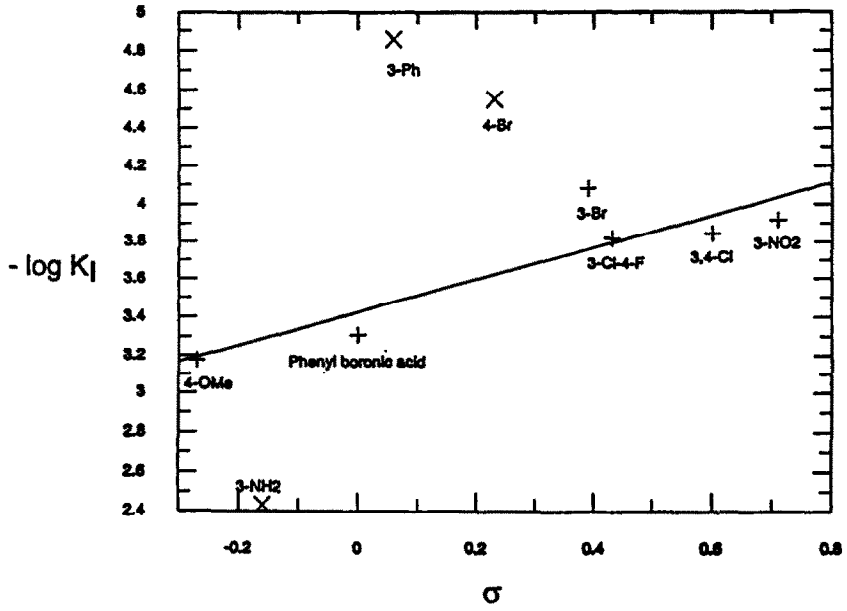
Inhibitor	Ki (μM)
3-Biphenyl boronic acid (**13**)	13.9 ± 0.4 [a]
4-Bromophenyl boronic acid (**14**)	28 ± 2 [b]
3-Bromophenyl boronic acid (**15**)	83 ± 2 [a]
2,4-Dichlorophenyl boronic acid (**16**)	94 ± 6 [b]
3-Nitrophenyl boronic acid (**17**)	121 ± 3 [a]
3,4-Dichlorophenyl boronic acid (**18**)	143 ± 9 [b]
3-Chloro-4-fluorophenyl boronic acid (**19**)	152 ± 10 [b]
Phenyl boronic acid (**20**)	491 ± 27 [c]
4-Methoxyphenyl boronic acid (**21**)	670 ± 20 [a]
3-Aminophenyl boronic acid (**22**)	3700 ± 100 [a]

Substrate: [a] nitrocefin, [b] 6-aminopenicillanic acid, [c] penicillin G potassium salt. Assay conditions as for Table 1.

In previous subtilisin Carlsberg studies with the Table 2 inhibitors,[8a] it was noted that the effects of the ring substituents on K_I's were proportional to the electrophilicity of the boron atom, and hence its susceptibility to nucleophilic attack by the active site serine hydroxyl group. Accordingly, the validity of this concept was also analyzed for RTEM-1 β-lactamase inhibition by the substituted phenyl series inhibitors **13-22**. The Hammett-type plot of K_I versus σ is shown in Figure 1. For most inhibitors there is a linear correlation between the K_I's with σ, confirming that their potency is generally proportional to the electrophilicity of their respective boron atoms for the RTEM 1 β-lactamase also. However, there are three deviations from linearity in the Figure 1 plot. The point for 3-aminophenyl boronic acid (**22**) falls well below the line while we are unable to account for this deviation, since **22**, with its K_I of 3700 μM, is by far the poorest inhibitor of the current study, other unfavorable factors that we cannot identify at this time are clearly strongly overriding the contribution of the electrophilicity of its boron atom to EI binding. In contrast, the points for the excellent inhibitors, 4-bromophenyl boronic acid (**14**) and 3-biphenyl boronic acid (**13**), lie well above the Hammett-plot line. In both these cases, the favorable contributions to binding conferred by their respective substituents are rationalizable by graphics analysis with the Insight II program[12] (version 2.2). For **14**, calculations using the Delphi program[12] (version 2.4) identified a favorable binding contribution between the

electronegative *para*-Br-substituent and the strongly electropositive region of the guanidinium residue of Arg 244 while molecular mechanics and dynamics calculations with the Discover program[12] (version 2.9) revealed phenyl stacking between the 3-phenyl substituent of the inhibitor and the hydroxyphenyl residue of Tyr 105 as a source of additional binding power.

Figure 1: Hammett Plot of Phenyl Boronic acids 13-22



In interpreting K_I values of competitive inhibitors, the contribution of desolvation energy[8b,c,d] as an inhibitor is transferred from aqueous solution to the enzyme's active site must be considered, with desolvation- driven EI formation being most favored for hydrophobic inhibitors. On this basis, binding of the cyclohexyl derivative **12** should be facilitated over that of phenylethyl boronic acid (**1**), whereas the K_I data show that the converse is the case. The reason for the superior inhibiting power of **1** was explained by graphics analysis, which revealed that, as for **13** above, a likely explanation was advantageous phenyl stacking of the aromatic group of **1** with the side chain of Tyr 105. In considering desolvation effects, it should be noted that the highest single desolvation cost for inhibitors **1-22** is incurred in removing the carboxylate function of **5** from aqueous solution, Normally this barrier results in poor enzyme-inhibitor binding.[8b] However, with its K_I value of 49 μM, the carboxyphenylethyl inhibitor **5** is in fact one of the better inhibitors of the current study. Delphi[12] calculations showed that this unexpectedly strong binding is attributable to a strong electrostatic interaction between the carboxylate ion and the guanidinium function of Arg 244 that overrides the unfavorable desolvation factor opposing EI formation.

Acknowledgments

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