Bioorganic & Medicinal Chemistry Letters 20 (2010) 3416–3419

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structural study of phenyl boronic acid derivatives as AmpC β-lactamase inhibitors

Donatella Tondi [a,b,*], Samuele Calò [a], Brian K. Shoichet [b], Maria Paola Costi [a,*]

[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Modena e Reggio Emilia, Via Campi 183, 41126 Modena, Italy
[b] Department of Pharmaceutical Chemistry, University of California San Francisco, 600 16th Street San Francisco, CA 94143-2240, USA

ARTICLE INFO

Article history:
Received 26 January 2010
Revised 29 March 2010
Accepted 5 April 2010
Available online 9 April 2010

Keywords:
Drug design
Lead optimization
Organic synthesis
X-ray crystallography
Enzymology

ABSTRACT

A small set of boronic acids acting as low nanomolar inhibitors of AmpC β-lactamase were designed and synthesized in the effort to improve affinity, pharmacokinetic properties, and to provide a valid lead compound. X-ray crystallography revealed the binary complex of the best inhibitor bound to the enzyme, highlighting possibilities for its further rational derivatization and chemical optimization.

β-Lactamases are a major cause of antibiotic resistance to β-lactams such as penicillins and cephalosporins.[1] Class C β-lactamases, such as AmpC, are among the most problematic of these enzymes.[2] They are widely expressed among nosocomial pathogens and they are not inhibited by clinically used β-lactamase inhibitors, such as clavulanate. Moreover, they have a broad spectrum of action including their ability to hydrolyze 'β-lactamase resistant' β-lactams, such as the third-generation cephalosporins.[3–6] Indeed, β-lactam-based inhibitors and β-lactamase resistant β-lactams can upregulate the expression of class C β-lactamases. There is then a pressing need for novel, non-β-lactam-based inhibitors of these enzymes.[7]

Several X-ray crystal structures of class C β-lactamases have now been determined, making these enzymes attractive targets for novel inhibitor discovery using structure-based methods.[8–10] The boronic acids are an interesting class of non-β-lactam inhibitors of serine β-lactamases.[11–14] These molecules act competitively, forming reversible adducts with the catalytic serine of the enzymes, adopting a tetrahedral geometry resembling that of the high energy intermediate (Fig. 1). In our previous work, iterative cycles of rational design, parallel synthesis, and X-ray crystallography have led to inhibitors with nanomolar K_i values.[15] Among these designed and synthesized aryl-boronic derivatives, several functioned in the 100 nM range (lead **1**, K_i 83 nM, Table 1). Moreover, several of these inhibitors reversed the resistance of nosocomial Gram-positive bacteria, although they showed little activity against Gram-negative bacteria. This lack of activity reflects difficulties with outer membrane penetration, which is frequently a problem with antibiotics acting against Gram-negative bacteria.

Starting from this family of inhibitors, we were interested in obtaining a validated lead compound with higher affinity vs AmpC and improved pharmacokinetic (PK) properties. A possible way to improve properties such as solubility and Log *D* was the introduction of small polar and ionizable groups in the large and lipophilic structures characteristic of this class of boronic acids. To localize the more appropriate point of derivatization and to rationally select the chemical groups suitable for PK properties modulation without affecting the binding orientation adopted by the inhibitors in the active site, we referred to the X-ray structure of lead **1** in complex with AmpC β-lactamase and to four other X-ray binary complexes of AmpC bound to phenyl boronic acid derivatives (Table 1).

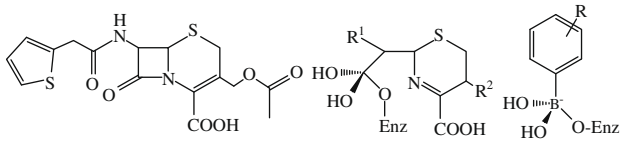
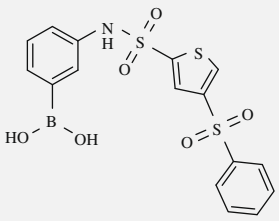
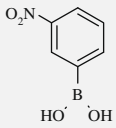
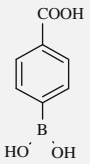
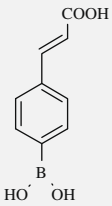
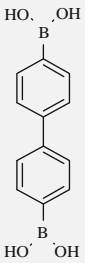


Figure 1. A common cephalosporin, cephalothin (A), its deacylation intermediate (B) and a transition-state analog of a generic phenylboronic acid derivative (C).

* Corresponding authors. Tel.: +39 0592055134; fax: +39 05920551531 (D.T.).
E-mail addresses: tondid@unimore.it (D. Tondi), costimp@unimore.it (M.P. Costi).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.04.007

Table 1
Phenyl boronic acid used for structural comparative analysis of AmpC β-lactamase binary complexes

1	2	3	4	5
				
K_i (μM) 0.083	K_i (μM) 1.7	K_i (μM) 2.9	K_i (μM) 4.2	K_i (μM) 0.20

Five X-ray crystallographic complexes were selected for comparison and analysis: AmpC_compound **1** (pdb code GA9), AmpC_3-nitrophenyl boronic acid, compound **2** (1KDS), AmpC_4-carboxyphenyl boronic acid, compound **3** (1KDW), AmpC_4-(2-carboxy-vinyl)-phenyl boronic acid, compound **4** (1KE0) and AmpC_biphenyl-4,4'-diboronic acid, compound **5** binary complexes (1KE3) (Table 1).[10]

When all five complexes were superimposed for comparison, conserved binding sites, already described as critical for the enzyme's molecular recognition, were clearly visible: the amide site, a hydroxyl site, a hydrophobic site, an aryl group site, a carboxylate site, and several conserved water sites (Fig. 2).[10] The unique functionalities that characterize each of the inhibitors make relatively few interactions with the protein. The nitro group of **2** does not make any interactions with AmpC. The carboxylate group of compound **3** is 3.3 Å from Nε2 of Gln120 while that in compound **2** is only 2.9 Å away due to the different conformation of Gln120. The carboxylate group in compound **4** interacts with a single ordered water molecule. In contrast, the structure of AmpC/compound **5** shows specific polar interactions between the inhibitor and the enzyme. One hydroxyl of the terminal boronic acid group interacts with both atom Nε2 of Gln120 and atom Oδ1 of Asp123 (2.5 and 2.9 Å, respectively). In the analyzed complex, the *para*-phenyl position is oriented towards the entrance of the AmpC binding site, in proximity to Gln120 (distances ranging from 3.05 and 5.35 Å). In particular, 1KDW, AmpC/**3** binary complex shows that the phenyl ring is oriented exactly as the phenyl ring of **1** while the 4-carboxy group is hydrogen bonded to Gln120 and to an ordered water molecule. Moreover, a polar interaction is acquired with Asn152 (Fig. 2). The other two complexes, one bearing a 4-boronic acid-phenyl group in the *para* position (**5**, IKE3) and the other with a propenoic acid (**4**, IKE0) group in the comparable site, show interactions with Gln120 accompanied by large conformational changes (Fig. 2).

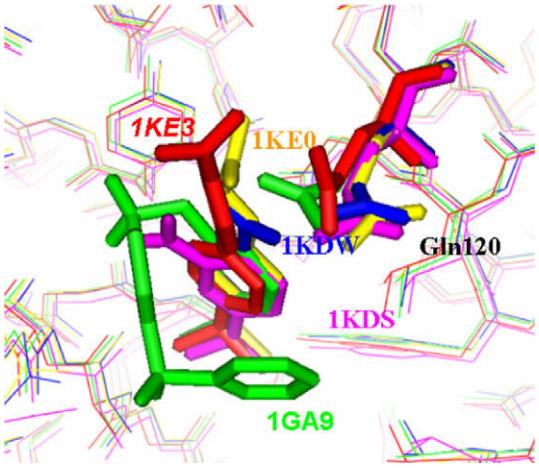


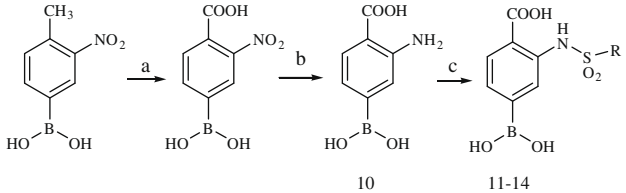
Figure 2. Superimposition of the five complexes used in the design of compound **1** follows up. The flexibility of Gln120 and then plasticity of AmpC in allocating functional groups different for chemistry and size is highlighted. Picture was generated using Pymol.[17]

The comparative analysis of the five selected crystal structures identified the *para*-phenyl position as the most convenient site to introduce appropriate chemical groups to modulate the PK properties of compound **1**. Our analysis highlighted Gln120 as having high conformational freedom which would allow the binding site to accommodate bulkier derivatives (Fig. 2). That said, a hydrophilic group introduced at the *para* position could interact with Gln120 and modulate the PK properties of the final designed molecule. We chose to derivatize lead **1** with a *para*-carboxylic group that could fit the site and has the optimal properties to improve PK properties; therefore a small set of compounds (**10**–**14**) was designed and synthesized as 4-carboxy-derivatives of **1**. We considered that moving from lead **1** to the corresponding carboxy derivative **11** would considerably decrease the lipophilicity (Log *D* = +2.32 for **1**; Log *D* = +0.026 for **11** at pH 7.4).[16]

Compounds **11**–**14** were synthesized using 3-amino-4-carboxyphenyl boronic acid (**10**) prepared in house as a starting scaffold as described in Scheme 1 (Table 2).[18]

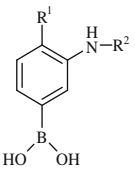
When compared to their non-carboxylate analogs (**6**–**9**), the affinities of the new derivatives **10**–**14** for AmpC increased between 2.6–4.5 fold, with the exception of **12** which performed worse with respect to its analog. For all new compounds, the predicted Log *D* improved because of the presence of the carboxylate group.

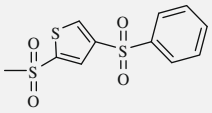
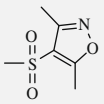
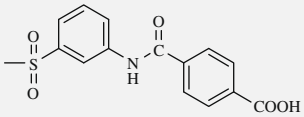
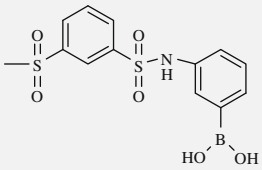
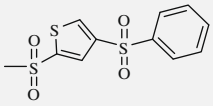
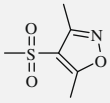
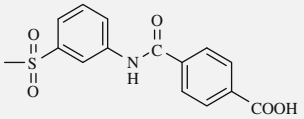
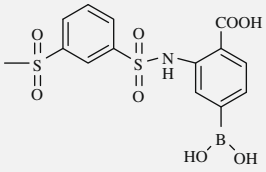
The X-ray crystallographic structure of **11** in complex with AmpC β-lactamase, determined to 2.1 Å resolution, was obtained. The coordinates and the structure factors have been deposited with the RCSB Protein Data Bank (PDB) database under accession code 3BM6.[18,19] X-ray analysis confirmed our design hypothesis and the predicted binding geometry (Fig. 3). Overall, the complex with **11** closely resembled the AmpC/**1** complex, with a few differences.



Scheme 1. Reagents and conditions: (a) $KMnO_4$, NaOH (0.26 M), 50 °C; (b) EtOH, MeOH, Pd/C 10%, H_2 (2 atm), rt; (c) $NaHCO_3$ (0.5 M), acetone, RSO_2Cl, rt, NaOH (2 N).

Table 2
Focused library based on crystallographic analysis. Inhibition activity of lead compounds and their corresponding 4-carboxy-analogues



Compound	R^1	R^2	K_i (nM)
1	H		83
6	H		100
7	H		450
8	H		60
9	H	H	7300
10	COOH	H	1800
11	COOH		26
12	COOH		350
13	COOH		100
14	COOH		23

Key hydrogen bond interactions are maintained. In the crystallographic complex, **11** adopts a jack-knife structure, folding back upon itself so that the last phenyl ring of the inhibitor overlies the first phenyl ring, reducing the exposure of both to solvent (Fig. 3A). The Oγ of Ser64 forms a dative covalent bond with the tetrahedral boronic acid. The O1 hydroxyl of the boronic acid is linked with the main chain nitrogens of Ser64 and Ala318 through hydrogen bonding, and also with the main chain carbonyl oxygen of Ala318. The O2 hydroxyl hydrogen bonds with the putative catalytic base of AmpC, Tyr150, and with a well-ordered water molecule (Wat400). The first phenyl ring (i.e., the ring closest to Ser64) of the inhibitor forms a dipole–quadrupole interaction with Nδ2 of the key catalytic residue Asn152.[20]

The sulfonamide nitrogen N1 of **11** forms a dipole–quadrupole interaction with the conserved Tyr221 aryl ring (Fig. 3A), similar

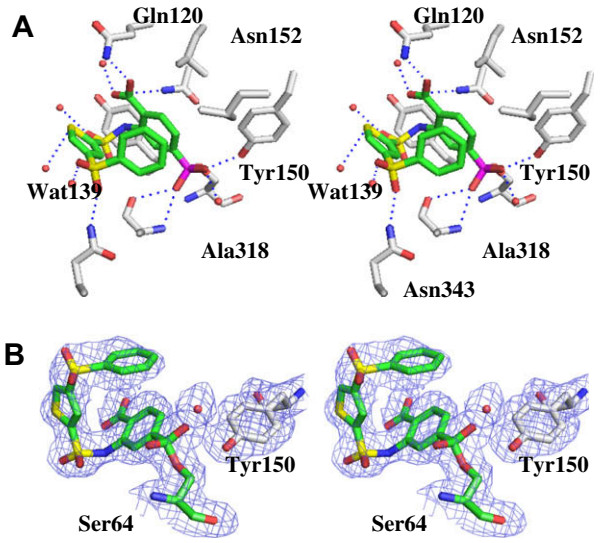


Figure 3. (A) Stereo view of key interactions observed in the complex of **11** with AmpC. The inhibitor is covalently attached to S64. Carbon atoms of AmpC are colored gray, carbon atoms for **11** are colored green. Nitrogen atoms are colored blue, oxygen atoms red, and sulfur atoms yellow. Red spheres represent water molecules. (B) Stereo view of electron density of compound **11** in complex with AmpC. The $2F_o3F_c$ electron density map is represented by the blue cage and is contoured at 1.0 σ. The inhibitor covalently attached to S64 is colored in green. Carbon atoms of AmpC are colored gray, carbon atoms of 11 green. Nitrogen atoms are colored blue, oxygen atoms red, and sulfur atoms yellow. This picture was generated using Pymol.[17]

to interactions observed in other complexes with AmpC.[21] An ordered water molecule (Wat325) interacts with a sulfonamide oxygen (O4, 2.9 Å), Tyr221OH (2.9 Å), the carboxylate group (O1, 3.6 Å), and Wat45 (3.0 Å). Up to this point, the crystallographic conformation of **11** closely resembles the binding orientation of compound **1** in the 1GA9 complex. The major difference between the two crystallographic structures can be observed from the rearrangement of several surrounding residues. These result in new interactions between the *para*-carboxylic group and Gln120. In 3BM6, the Cδ of Gln120 flips 180° from its original orientation in 1GA9 and shifts away by 1.9 Å, leaving space to accommodate the carboxylate group and, most importantly, attaining strong H-bonding with the carboxylate (Gln120Nε2-O2 2.8 Å). In the same X-ray structure, seven contiguous amino acid residues, 284–290, that normally represent a disordered region with very poor electron density in the complex with compound **1**, were well defined in their positioning.

The conformational change observed in the X-ray structure confirmed Gln120 as a highly flexible residue and accounted for the improved affinity of **11** with respect to compound **1** (K_i 26 nM vs K_i 83 nM). Compounds **12** and **14** showed the same improvement in binding affinity. This trend suggested that the presence of a *para*-carboxyl group generally favors the binding due to specific interaction with Gln120, with the only exception being compound **12** for which other factors may contribute to binding.

The validated lead compound **11** presents improved drug-like properties, strong binding to AmpC, and represents a promising starting candidate for further development of this series towards optimized derivatives with improved pharmacokinetic properties and, most importantly, with the ability to cross the outer membrane of Gram-negative bacteria. Moreover, the binding orientation adopted by compound **11** in AmpC suggests the gain of additional interactions with surrounding polar residues such as Asn289 and Asn343 through the introduction of functional groups at the distal phenyl ring of the inhibitors.

Acknowledgments

This work was supported by NIH Grant GM63815. We thank Professor Richard Bonnet at Service de Bactériologie, Faculté de Médecine, Université d'Auvergne, 63001 Clermont-Ferrand, France for microbiology testing and valuable suggestions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.007.

References and notes

1. Essack, S. *Pharm. Res.* **2001**, *18*, 1391.
2. Philippon, A.; Arlet, G.; Jacoby, G. A. *Antimicrob. Agents Chemother.* **2002**, *46*, 1.
3. Bradford, P. *Clin. Microbiol. Rev.* **2001**, *48*, 933.
4. Medeiros, A. *Clin. Microbiol. Infect.* **2000**, *6*, 27.
5. Meroueh, S. O.; Roblin, P.; Golemi, D.; Maveyraud, L.; Vakulenko, S. B.; Zhang, Y.; Samama, J. P.; Mobashery, S. *J. Am. Chem. Soc.* **2002**, *124*, 9422.
6. Sun, T.; Bethel, C. R.; Bonomo, R. A.; Knox, J. R. *Biochemistry* **2004**, *43*, 14111.
7. Jacobs, C.; Frere, J. M.; Normark, S. *Cell* **1997**, *88*, 823.
8. Usher, K.; Shoichet, B. K.; Blaszczak, L.; Weston, G. S.; Remington, J. R. *Biochemistry* **1998**, *37*, 16082.
9. Crichlow, G. V.; Kuzin, A. P.; Nukaga, M.; Mayama, K.; Sawai, T.; Knox, J. R. *Biochemistry* **1999**, *38*, 10256.
10. Powers, R. A.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 3222.
11. Weston, G. S.; Blazquez, J.; Baquero, F.; Shoichet, B. K. *J. Med. Chem.* **1998**, *41*, 4577.
12. Powers, R. A.; Blazquez, J.; Weston, G. S.; Morosini, M. I.; Baquero, F.; Shoichet, B. K. *Protein Sci.* **1999**, *8*, 2330.
13. Thomson, J. M.; Distler, A. M.; Prati, F.; Bonomo, R. A. *J. Biol. Chem.* **2006**, *28*, 26734.
14. Thomson, J. M.; Prati, F.; Bethel, C. R.; Bonomo, R. A. *Antimicrob. Agents Chemother.* **2007**, *51*, 1577.
15. Tondi, D.; Powers, R. A.; Caselli, E.; Negri, M. C.; Blazquez, J.; Costi, M. P.; Shoichet, B. K. *Chem. Biol.* **2001**, *8*, 593.
16. www.acdlabs.com.
17. DeLano, W. L. DeLano Scientific, San Carlos, CA, USA, 2002.
18. Additional information related to chemistry, enzymology, crystal growth, and structure determination is available as Supplementary data.
19. Resolution (Å) 2.10; Rmerge (%) 5.5; completeness (%) 92.6; <I>/<σ1>15.08; Rcryst (%) 19.87; Rfree (%) 25.49.
20. Dubus, A.; Normark, S.; Kania, M.; Page, M. G. *Biochemistry* **1994**, *33*, 8577.
21. Patera, A.; Blaszczak, L. C.; Shoichet, B. K. *J. Am. Chem. Soc.* **2000**, *122*, 10504.