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Article

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Click Chemistry in Lead Optimization of Boronic Acids as β-Lactamase Inhibitors

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KEYWORDS Boronic acid transition state inhibitor, β-lactamase, click chemistry, triazole, cephalosporinase, penicillinase

ABSTRACT

Boronic acid transition state inhibitors (BATSIs) represent one of the most promising class of β-lactamase inhibitors. Here we describe a new class of BATSIs, namely 1-amido-2-triazolylethaneboronic acids, which were synthesized combining the asymmetric homologation of boronates with Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) for the stereoselective insertion of the amido group and the regioselective formation of the 1,4-disubstituted triazole, respectively. This synthetic pathway, which avoids intermediate purifications, proved to be flexible and efficient, affording in good yields a panel of fourteen BATSIs bearing three different R1 amide side chains (acetamido, benzylamido and 2-thienylacetamido) and several R substituents on the triazole. This small library was tested against two clinically relevant class C β-lactamases from *Enterobacter* spp. and *Pseudomonas aeruginosa*. The K_i value of the best compound (**13a**) was as low as 4 nM with significant reduction of bacterial resistance to the combination of cefotaxime/**13a**.

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INTRODUCTION

The World Health Organization recently declared that antimicrobial resistance is one of the three greatest threats to human health. This current alarming public health crisis is driven by two key factors: the emergence of antibacterial resistance in important pathogens and a lack of development of new antibacterial agents.[1] Commercial, developmental, and regulatory hurdles challenge industry in identifying new antibacterial agents. Since antibiotics are usually prescribed as short-duration therapies (as brief as a single dose), the emergence of resistance compromises their commercial life span, and the cost of carrying out the large studies that are required for approval are significant.

Until now, the main approach to discovering new antibacterial agents has been to modify existing classes of drugs that inhibit validated bacterial targets. Yet, how many more generations of "modified molecules" can be obtained on known scaffolds that will avert resistance? In addition, circumventing pre-existing resistance mechanisms that have arisen in the clinic also challenges us. Recently, efforts are underway to develop drug libraries based on novel chemistries. A premier example of this is the emerging interest in boron chemistry.[2] Boron-based compounds demonstrate ideal drug-like properties and possess specific geometries and reactivities that enhance their capacity to interact with select biological targets. Presently, boron containing molecules are directed against new targets, such bacterial leucyl tRNA synthetase inhibitors (GSK2251052, under Clinical Phase II), or as 'potentiators' that when administered together with antibiotics enhance their effect. In the latter case the development of efflux-pump[3] and β-lactamase inhibitors (BLIs)[4] are the main thrusts of this research.

Bacterial production of β-lactamases represents the most clinically concerning mechanism of resistance to β-lactam antibiotics in Gram negative bacteria. The emergence of many recent β-lactamase variants (> 1600 new enzymes) jeopardizes the efficacy of both the latest developed

antibiotic and the combination of β-lactam-BLI (e.g., the formulation ampicillin and clavulanic acid are ineffective against bacteria expressing inhibitor resistant TEM and SHV β-lactamases). At the moment, boronic acids transition state inhibitors (BATSIs) represent one of the most promising and exciting class of BLIs in development, as testified by the advancement of a combination of a boronic acid inhibitor (RPX7009 currently developed by the Medicines Company) with meropenem (a carbapenem), in clinical trials (Clinical Trials Phase 1 registration number NCT01897779, RPX7009 is joined with RPX2014). Carbavance™ (meropenem/RPX7009) is particularly targeted against KPC (*Klebsiella pneumoniae* carbapenemase)-producing carbapenem-resistant *Enterobacteriaceae* (CRE).

Independently, we[5a-c,6,7] and others[5d] previously demonstrated that the design and synthesis of several new BATSIs, whose structures resembled more and more the parental β-lactams, is an innovative strategy in novel BLI discovery.[5,6,7] The rationale behind the design of these potent BATSIs relies on the bioisoster identification of the boronic moiety replacing the highly reactive β-lactam ring, and on the mimicking of the natural substrates. The more sophisticated structures, in particular the 1-amido-2-(*meta*-carboxyphenyl)ethaneboronic acids, incorporate different features that make them similar to the parental β-lactam during the hydrolytic process: a R1 amide group on the boron-bearing carbon atom C1 mimicking the β-lactams R1 side chain, an aromatic ring stereoselectively inserted in C2 to resemble the ring adjacent to the β-lactam, and the presence of a carboxylate on the phenyl important for recognition and cell penetration (Figure 1).[6]

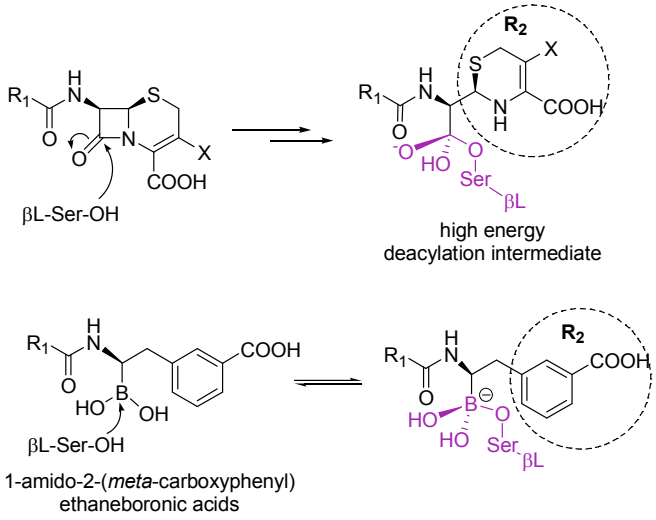
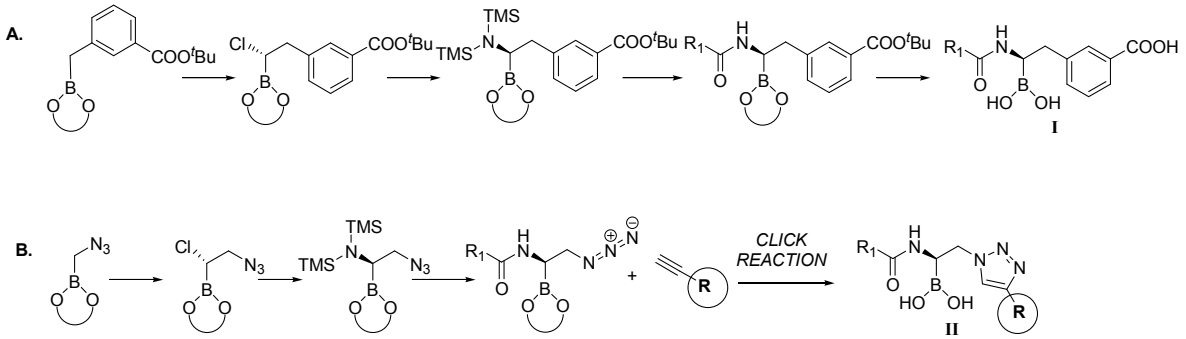


Figure 1. Mechanism of action of a β-lactamase (βL) with a β-lactam and with a boronic acid.

This strategy has been very successful, since the activities of 1-amido-2-phenylethaneboronic acids against different enzymes (as measured by K_is) and against specific strains (minimum inhibitory concentrations, MICs) are encouraging. Nevertheless the synthetic pathway leading to these structures is substantially rigid, allowing the insertion of different R1 side chains, but forcing the choice of the R2 group from the beginning with precise constraints given by the conditions of the homologation reaction that stereospecifically introduces a halogenated carbon atom. (Scheme 1, **A**.).



Scheme 1. Stereosective synthesis of 1-amido-2-phenylethaneboronic acids **I** and of the newly designed α-amido-β-triazolylethanboronic acids **II**.

Here we report our attempts to simplify this synthesis and improve the lead optimization process in order to obtain a new class of BATSIs with more potent biochemical affinity, increased accessibility to the target, and chemical diversity. The approach undertaken (Scheme 1, **B**) relies on the "click chemistry" reaction, in particular the well-known Copper Catalyzed Azide-Alkyne Cycloaddition (CuAAC).[8] This can be performed in the last step on a chiral boronic scaffold bearing both the R_1 amide side chain and a properly inserted azido group with mono-substituted alkynes, thus affording highly substituted α-amido-β-triazolylethanboronic acids **II**. Whereas the 1,4-disubstituted 1,2,3-triazole ring replaces the *m*-carboxybenzyl group preserving its aromatic character, it exploits a stronger dipole moment, thus potentially acting as an active linker, and allows a large and easy variability of the R groups (Scheme 1, **B**).[9] The novel synthetic pathway proves to be more flexible, efficient, with higher yields and avoids the laborious process required to purify intermediates. The development of this original class of potent BLIs is based both on bioisoster identification, and on rational scaffold hopping of the R2 ring.[10]

In this report a panel of fourteen 1-amido-2-triazolylethaneboronic acids compounds bearing three different R1 amide side chains (acetamido, benzylamido and 2-thienylacetamido) and at least three R substituents for each series, was synthesized and tested against two clinically relevant class C β-lactamases, P99 and PDC-3. To assess the activity of our compounds, we targeted representative class C cephalosporinases that are often responsible for high-level resistance to β-lactam antibiotics among lactose fermenting (e.g., *Enterobacter cloacae)* and non-lactose fermenting (*Pseudomonas aeruginosa*) strains.[11-13] The atomic structures of these β-lactamases were previously determined (4HEF and 1XX2 respectively) and served as models to help us understand why each compound was so effective. As expected, the BATSIs synthesized by click chemistry showed potent activity against these enzymes, spanning from low micromolar to low nanomolar range inhibition constants.

We advance that this activity was primarily modulated by the identity of the R1 acetylamino side chain, while the possibility of using the click chemistry to introduce different R chemical groups on the triazole ring allowed a first exploration of the recognition of the R2 side of the molecules. Comparison of the K_is permitted an evaluation of the energetic contribution to molecular recognition due to the best R substituents and to the triazole ring by itself. The synergistic effect with β-lactams to reverse antibiotic resistance in cell culture assays was explored.

RESULTS

Design

The previous studies that focused on the activity of a series of 1-amido-2-phenylethaneboronic acids **I** (Scheme 1) against class C (AmpC) β-lactamases, demonstrated that the presence of a phenyl side chain on the stereogenic carbon atom improved activity when compared to the achiral compounds, in particular with small R1, such as for side chains typical of penicillin G.[6] Since P99 and PDC-3 β-lactamases are structurally similar to AmpC β-lactamase (Figure 2) and share 62.4% similarity (Fig S1**,** supporting materials), we chose the acetyl, phenylacetyl and 2-thienyl-2-acetyl side chains as R1 groups.

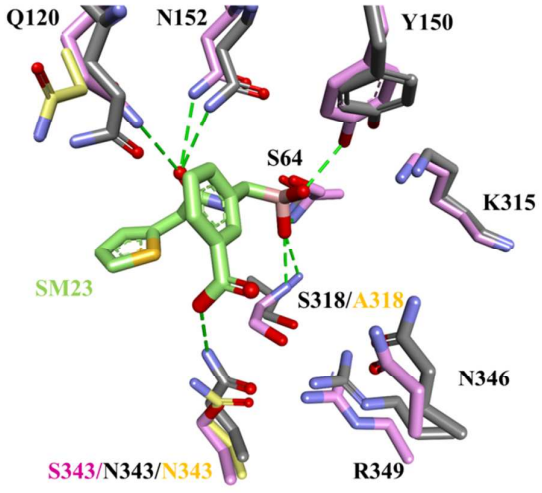


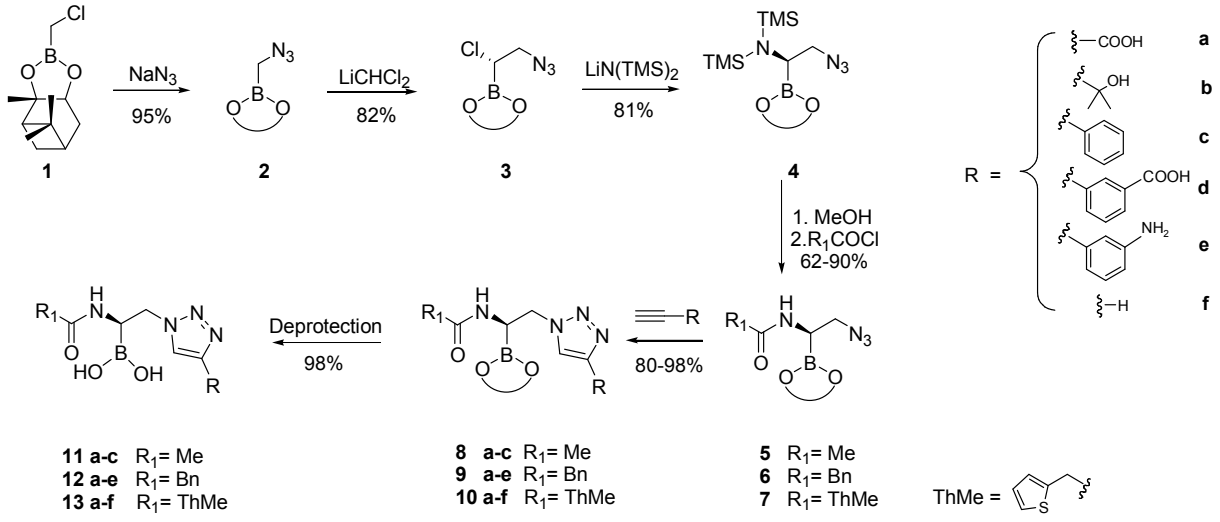
Figure 2. Superimposition of the active site of class C β-lactamases: PDC-3 (PDB_4HEF) colored by atoms, P99 (PDB_1XX2) carbons magenta, and AmpC active site (yellow) in complex with a boronic inhibitor, (PDB_1MXO).

We reasoned that the triazole ring could replace the aromatic phenyl ring, allowing the introduction of different R groups to explore the topology of the β-lactamase active site. As shown in Figure 2 the active site pocket is very similar in all three β-lactamases; the only notable differences are at position 343 where 1) asparagine is replaced by serine for P99 enzyme and 2) the oxyanion hole Ser318 in P99 and PDC-3 is replaced by alanine in AmpC enzyme. The Arg residue at position 349 and Asn343 and 346 respectively are conserved as well.

As a first approach, we chose to use simple alkynes to assess the best conditions for the click reaction, bearing different polarities and hydrophilicities which might take advantage of the active site electrostatic potential (Figure S2 supporting materials).

Synthesis of α-amido-β-triazolylethaneboronic acids.

The synthesis of the new BATSIs is depicted in Scheme 2; the stereoselective synthesis of these inhibitors is based on Matteson' homologation of (+)-pinanediol azidomethaneboronate **2**,[14] a strategy that allow to introduce a stereogenic carbon in α position of boronic esters.[15]



Scheme 2. General synthesis of α-amido-β-triazolylethaneboronic acids

Compound **2** was obtained (97%) through chlorine substitution of **1** with sodium azide in a biphasic system (water/ethyl acetate), in the presence of tetrabutylammonium iodide as phase transfer catalyst.[14] Subsequent treatment of **2** with *in situ* generated dichloromethyl lithium at –100 °C allowed the insertion of an halogenated carbon on the carbon-boron bond; the use of (+)-pinanediol as chiral auxiliary agent afforded the expected α-chloroboronate (*S*)-**3** with high diastereoselectivity (d.e.>98%, 96% yield). Treatment with lithium bis(trimethylsilyl)amide, performed at –100 °C to minimize the elimination reaction, produced the α-aminoboronate **4** (80%), the key intermediate for the synthesis of all BATSIs. *In situ* deprotection of the amino-group with a stoichiometric amount of methanol followed by coupling with the selected acyl-chloride afforded compounds **5-7**, which were recovered as pure solids (62-90% yield) by fractional crystallization of the crude. These compounds were stable at 4 °C for months without any appreciable degradation.

With the α-acylamido-β-azido derivatives **5-7** in hand for the assembling of the right part of the inhibitors we resorted to the Huisgen's 1,3-dipolar azide-alkyne cycloaddition exploiting copper catalysis (CuAAC) to gain 1,4-regioselectivity in the formation of the triazole. Among the wide

variety of conditions described in the literature for this robust reaction, we adopted the most common one, employing water/*t*.butanol as solvent and generating the Cu(I) catalyst *in situ* from $CuSO_4$ in the presence of an excess of ascorbate as reducing agent (5% catalyst, 20% ascorbate).[8] In these conditions the three azides **5-7** were allowed to react at 60 °C with the terminal alkynes **a-f**; complete conversions were reached in 0.5-18 hours and the expected 1,4-disubstituted triazoles **8-10** were recovered in good to excellent yields (80-98%) by extraction with ethyl acetate and crystallization of the crude. The formation of triazoles **8-10** was confirmed by the presence in the 1H NMR spectra of a singlet downfield at 7.6-8.7 ppm which correlates (HSQC experiment) with a signal at 119-130 ppm of the ^{13}C-NMR spectra, as expected for a triazolic CH. Moreover, the 1,4-disubstitution was confirmed by the long-range correlation (HMBC experiment). The 4-unsubstituted triazole **10f** was obtained using trimethylsilylacetylene as alkyne counterpart; in this case, the 4-trimethylsilylsubstitued triazole formed by CuCAAC undergoes spontaneous proto-desilylation to **10f** in the reaction conditions partially and was isolated by chromatography. Finally, deprotection of the boronic moiety was accomplished by transesterification with phenylboronic acid in a biphasic system acetonitrile/*n*-hexane,[16] allowing the removal of the chiral auxiliary (+)-pinanediol and to obtain the final products **11a-c, 12a-e, 13a-f** (80-100% yield). Except for the starting compound **1**, purified by chromatography, intermediates **2**, **3** and **4** didn't required any purification, while intermediate **8a-c**, **9a-e**,**10a-e**, as well as the final product **11a-c, 12a-e, 13a-f** could be purified by crystallization of the crude material. NMR spectra of all BATSIs are reported in the Supporting Information.

Inhibition Kinetics.

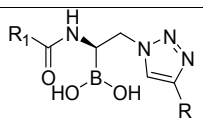
As a result of the synthesis above, fourteen new chiral boronic acid inhibitors were tested against the representative class C β-lactamases, P99 and PDC-3. All compounds were first screened

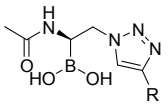
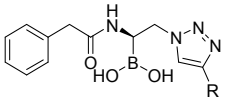
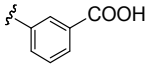
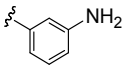
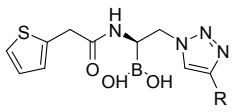
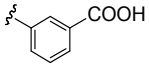
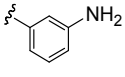
with a 5 min pre-incubation between the β-lactamase and BATSI, and the reaction initiated with nitrocefin (NCF). Activity was also measured at different time points (0 to 10 min) to assess the pre-incubation effect on inhibitor affinity.

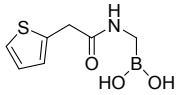
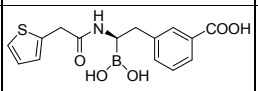
As expected, a time-dependence effect on inhibition was observed, in particular, the most potent inhibitor, compound **13a** showing the greater inhibition (Figure S3, supporting materials). Three compounds **11a-c** bearing the R1 acetamido side chain demonstrated an IC_{50} in the micromolar range (5-27 μM), while activity significantly improved for inhibitors bearing the phenylacetyl or 2-thienyl-acetyl side chains the (IC_{50}s reduced to the nanomolar range). These results are consistent with previous data obtained for achiral α-acylaminoboronic acids against class C AmpC beta-lactamase, where the simplest acetamido side chain performed worse than more elaborate side chains.[7]

Consequently, five alkynes in both series **12** and **13** were used to further investigate recognition for R1 amide side chain and R2 groups. We determined that IC_{50}s (after 5 minute incubation) were in general better for BATSI bearing the R1 typical of cephalothin (series **13**) than of penicillin G (series **12**), with the most notable difference being compounds **12c** vs compound **13c** against P99. Amongst the R groups introduced through click chemistry, very low IC50s were obtained for boronic acids bearing the R carboxy sidechain as in **13a** (35 ± 2 nM vs. PDC-3 and 140 ± 20 nM vs. P99) and the 3-carboxyphenyl sidechain as in **13d** (67 ± 2 nM vs. PDC-3 and 84 ± 10 nM vs. P99), supporting our early findings that a carboxylate on the R2 side chain improves recognition.[6]

Table 1: IC_{50}s values of compounds **11-13** versus class C β-lactamases PDC-3 and P99.



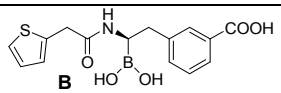
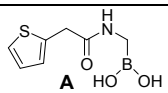
Entries	Structures	Cmp	R	IC_{50}[μM]	
				PDC-3	P99
1		**11a**		5±1	27.1±3
2		**11b**		18.4±0.3	71±5
3		**11c**		9.3±1	13.2±2
4		**12a**		0.23±0.01	0.11±0.01
5		**12b**		1.18±0.2	0.66±0.01
6		**12c**		0.3±0.02	0.15±0.02
7		**12d**		0.12±0.01	0.11±0.01
8		**12e**		0.4±0.05	0.6±0.02
9		**13a**		0.035±0.002	0.14±0.02
10		**13b**		0.25±0.05	0.42±0.01
11		**13c**		0.3±0.02	0.32±0.02
12		**13d**		0.067±0.002	0.084±0.01
13		**13e**		0.28±0.02	0.36±0.02

14		**13f**		1.3±0.2	0.8±0.02
15		**A**		3.3±0.2	2.7±0.2
16		**B**		0.15±0.01	0.1±0.02

Antimicrobial Activity and Binding Energies.

MIC values (μg/mL) of *E. coli* DH10B expressing PDC-3 and P99 class C β-lactamases were determined by the agar dilution method to assess potency in whole cell assays. Based upon previous studies, we chose cefotaxime (TAX) as a partner β-lactam. These results are summarized in Table 2. The best compounds of the newly synthesized series, **13a** and **13d,** were compared to previously described BATSIs: the achiral thienylacetamidomethane boronic acid (reference compound **A**) and the correspondent chiral *meta*-carboxybenzyl (reference compound **B**). Boronic acids bearing the triazole mojety show ability to penetrate *E. coli* DH10B and inhibit both P99 and PDC-3 in whole cell assays with MICs lowered into the susceptible range.

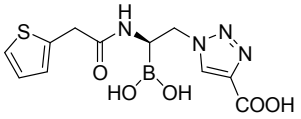
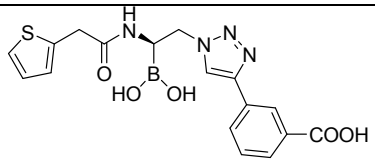
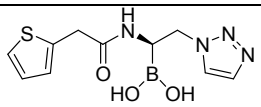
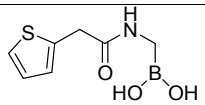
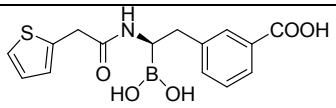
Table 2: Synergy of compounds **13a**, **13d** and reference compounds **A** and **B** with cefotaxime against *E.coli* bacteria producing PDC-3 and P99. The boronate were tested at a constant concentration of 4μg mL^{-1}

Strain	**β-lactamase**	**TAX**	**13a + TAX**	**13d + TAX**	 **B** **+ TAX**	 **A** + **TAX**
E.coli DH10B	none	**<0.125**	**<0.125**	**<0.125**	**<0.125**	**<0.125**
E.coli DH10B	PDC-3	**16**	**0.5**	**1**	**2**	**4-8**

pBCSK-						
E.coli DH10B Pcs900	P99	**16**	**2**	**4**	**2**	**4**

To evaluate the energetic contribution of different groups to molecular recognition in the newly synthesized inhibitors, K_is of the best inhibitors as well as of the two reference compounds **A** and **B** were determined taking in consideration the substrate nitrocefin contribution (Table 3).

Table 3: Kis (μM) values of compounds **13a**, **13d**, **13f** and of reference compounds **A** and **B**.

Cmp	**Structure**	**K_i (μM) vs. PDC-3**	**K_i (μM) vs. P99**
13a		0.004±0.001	0.023±0.002
13d		0.008±0.001	0.014±0.001
13f		0.164±0.008	0.134±0.006
Ref cmp **A**		0.411±0.001	0.451±0.002
Ref cmp **B**		0.013±0.006	0.015±0.005

Boronates **13a** and **13d** bearing a negative charge on the triazole substituent, have K_is of 4 ± 1 and 8 ± 1 nM respectively against PDC-3 and of 23 ± 2 and 14 ± 1 nM against P99. These results are amongst the best obtained against class C β-lactamases. Since these compounds bind covalently and reversibly to the enzyme, differential binding energies can be calculated directly from K_i values. For example, the contribution to binding of the simple carboxylate in **13a** against PDC-3, can be calculated by comparing the affinities of compound **13f** (K_i = 164 ± 8 nM) and compound **13a** (Ki = 4 ± 1 nM), thus obtaining an energetic contribution of 2.2 Kcal/mol (using $\Delta\Delta G_{bind} = -RT\ln K_{i\ ref}/K_i$), a value consistent with the 2.1 Kcal/mol previously calculated for chiral acylaminophenylmethaneboronic acids *vs.* AmpC.

DISCUSSION AND CONCLUSIONS

In this study we developed a flexible synthetic pathway to obtain a series of chiral boronic acids that exhibit potent activity against representative class C serine β-lactamases, P99 and PDC-3. The BATSIs designed were highly active microbiologically against *E. coli* harboring the tested cephalosporinases and biochemically as they mimicked the natural substrate of these enzymes, the β-lactams, and exploited the chemical properties that facilitate cell entry.

The synthetic pathway depicted in Scheme 2 illustrates that the key intermediate **4**, the (+)-pinanediol 1-(*N,N*-bis-trimethylsilyl)-amino-2-azidoethaneboronate, is able to exploit the presence of two orthogonally reactive groups for in parallel synthesis: the bis-trimethylsilylamino group as precursor for amide formation in R1, and the azido group for triazole formation in R2 through the click reaction (CuAAC). Three acylamido groups are introduced on **4** as R1 amide side chains: the acetamido as reference compound (compound **5**), the benzylacetamido typical of the antibiotic penicillin G (compound **6**) and the 2-thienylacetamido typical of cephalothin (compound **7**).

Three terminal alkynes bearing a carboxy, a hydroxymethyl, and a phenyl group are next used in click reaction with each β-azidoboronate **5-7**. Triazole formation is obtained through the

well-known click reaction catalyzed by Copper (I), that represent a rapid, selective and efficient strategy to synthesize a library of compounds either during early stage drug discovery and lead optimization process. The strengths of this intrinsically convergent reaction resides on wide functional groups compatibility, mild reaction conditions, regioselective formation of the 1,4-disubstituted isomer, high yields and ease of product purification.[8,17] Indeed click reaction on compounds **5-7** proceeds easily in each case, employing water/*t*.butanol as solvent and generating the Cu(I) catalyst *in situ* by reduction of copper sulfate. Products **8a-c**, **9a-c**, **10a-c** are obtained in high yields (80-98%) after simple crystallization of the crude material. Upon removal of the pinanediol chiral auxiliary, nine boronic acids **11a-c**, **12a-c** and **13a-c** are obtained and tested against two class C beta-lactamases, PDC-3 and P99, two common cephalosporinases responsible for resistance against the most potent cephalosporin (eg. cefotaxime).

Boronates **11a-c** bearing the acetamido side chain exhibit IC_{50} values in the micromolar range (entries 1-3, 5-71 μM), proving that the simple methyl group does not enhance active site recognition. In contrast, introduction of the PenG side chain as in **12a-c** improves IC_{50}s up to two orders of magnitude (entries 4-6, 0.11-1.18 μM). As expected, the cephalothin side chain demonstrates the highest inhibition, with IC_{50} values that are reduced of one more orders of magnitude for compound **13a** compared to **12a** against PDC-3 (entry 9, 0.035 ± 0.002 μM vs. entry 4, 0.23 ± 0.01 μM). This trend closely resembles data obtained with chiral acylaminoboronic acids vs AmpC,[6] thus suggesting a similar spatial arrangement in the catalytic pocket.

To help understand the activity of these compounds we resorted to molecular models (Figure 2). Our analysis shows that most of the residues presented in the active site of AmpC are preserved in P99 and PDC-3 and maintain similar conformations (less than 1Å difference in side chain conformation). We observe that the flexibility of Q120 (showed to have multiple conformations in different crystallographic structures of class C beta lactamases) along with N152 allows H-bond interactions with acylamino part of the R1 side of new BATSI series. The other residues which can be part of the inhibitor-enzyme interaction (N346, R349, S318, K315, and Y150

or K67) are preserved. Furthermore, the minimized structure of PDC-3 and P99 in complex with **13a** (Figure **3a** and **b**) suggest that boronic moiety hydroxyl group makes H-bonds with S318, Y150 and possible K67. The R1 amide recognition pattern is highly conserved (Q120 and N152) for both class C enzymes.

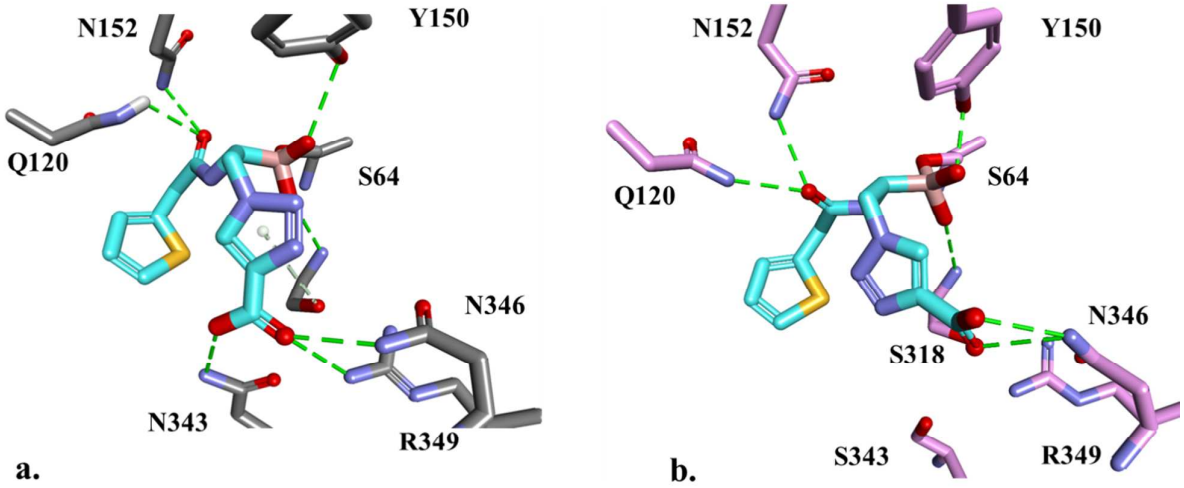


Figure 3a. PDC-3 and b. P99 with 13a docked in the active site. The molecular docking revealed key interactions between conserved residues in class C β-lactamase and R1 and R2 groups of BATSI.

To assess the potential of the click chemistry approach to better explore the R2 binding site of the catalytic pocket, we focused our attention on the R triazole substituent, obtaining two more compounds of series **12**, inserting a *meta*-carboxy- and a *meta*-amino-phenyl group (compounds **12d** and **12e**), and three more compounds of series **13**, inserting the same groups (compounds **13d** and **13e**), as well as the simple hydrogen atom (compound **13f**). All these compounds prove to be very good β-lactamase inhibitors with IC_{50s} in the low micromolar and nanomolar range (entries 7-14, 1.3 μM to 67 nM), affirming the best inhibitors as those bearing the R1 amide side chain typical of cephalothin vs that of Penicillin G (series **13** *vs* **12**), with the exception of compound **13c** *vs* **12c** in the assays against P99. Furthermore compounds bearing a carboxylate in the triazole R

substituent exhibit the best inhibition values. In particular compound **13d** bearing the *meta*-carboxyphenyl R group, has IC_{50} slightly worse than compound **13a** against PDC-3 (entry 12 *vs* 9, 67 *vs* 35 nM), but even better against P99 (entry 12 *vs* 9, 84 *vs* 140 nM), suggesting of quite flexible pocket able to accommodate differently hindered R groups bearing a negative charge.[18] Lastly, our models show that the carboxyl of the BATSI is positioned in a favorable conformation to make H-bonds with N343, and a longer R2 substituent of **13a** introduced by the methyltriazole can facilitate the interaction with R349 and/or N346 in PDC-3 and P99 (Figure 3a and b).

We next explored the contribution of the triazole ring to active site binding. Comparing the affinity of the unsubstituted triazole **13f** (K_i = 164 ± 8 nM *vs* PDC-3) to the achiral cephalothin analogue Ref Compound **A** (K_i = 411 ± 0.001nM) suggests that the methyltriazole itself contributes 0.5 Kcal/mol to binding. Even if at a first examination this might not appear a significant interaction energy (especially when compared to the contribution of 1.3 Kcal/mol of the simple phenyl ring for chiral acylaminophenylmethaneboronic acids)[6] we stress that the methylene bridge between the triazole and the α carbon atom increases the entropic factor negatively affecting the overall free energy contribution of the triazole *(ΔΔG)*. Another comparison that might further assist in evaluating the contribution of the triazole to binding is when Reference compound **B**, bearing the *meta*-carboxybenzyl moiety, is compared to **13a**, bearing the 4-carboxytriazolylmethyl moiety, and an improvement of 0.7 Kcal/mol in recognition caused by ring hopping is obtained. Thus, the triazole not only reveals to be a phenyl bioisoster and a valuable solution for an easier synthetic feasibility, but also behaves as an active linker partly for its high dipole moment (about 5 D).[19]

Another avenue explored is the role of the triazole in cell penetration. It is recognized that 1,4-disubstituted triazoles are not a novelty in medicinal chemistry. Indeed, more than seven thousand 1,4-disubstituted 1-H-1,2,3-triazole compounds had been reported before the discovery of the copper catalyzed click reaction. Triazoles are in fact known to possess a number of desirable features in the context of medicinal chemistry. For example, triazoles are stable to acid and base hydrolysis and reductive and oxidative conditions, as well as metabolic degradation.[9]

One of the three commercially available β-lactamase inhibitors, tazobactam, is among the best-known examples of triazole-containing pharmaceutically active compounds. Clinical experience has shown that tazobactam coupled to piperacillin is a potent combination with greater potency and more broad spectrum activity than amoxicillin-clavulanic acid or ampicillin-sulbactam, and part of this potency is ascribed to the presence of the triazole ring.[20] In order to evaluate the role of the triazole in the new BATSI we tested the best inhibitors **13a** and **13d** and reference compounds **A** and **B** as well against *E. coli* strains expressing either PDC-3 and P99 (Table 2).

The addition of each of these BATSI (**13a**, **13d**, **A** and **B**) results in a significant lowering of MICs when coupled to cefotaxime. Without a BATSI, MICs are in the resistant range. In the best case, the addition of the **13a** BATSI results in a five dilution (2 log) reduction in MIC against *E. coli* possessing the *P. aeruginosa* AmpC and **13d** results in a similar lowering. Since **13a** differs from **B** only for the phenyl-triazole ring hopping, the significant improvement in biological activity can be ascribed to the triazole ring.

In conclusion, this work describes the design, synthesis and biological properties of a new class of chiral boronic acids that effectively target representative β-lactamase that are found in problematic hospital pathogens. Most notably, two out of fourteen compounds (**13a** and **13d**) demonstrate a β-lactamase inhibitory profile (K_is) similar to a chiral boronate previously synthesized (reference compound **B**), with improved *in vitro* activity (MICs, see Table 2). Furthermore, the improved synthetic accessibility of these boronic acids, which relies on the click chemistry reaction (CuAAC), opens up the possibility of obtaining a large number of compounds with a wider range of substituents that could better explore the β-lactamase binding site. This synthetic approach promises to further expand the repertoire of compounds to be explored against β-lactamases that have defied inhibition and have contributed to the current crisis in antimicrobial chemotherapy. Extension of the studies herein against different microbiological targets of importance and diverse β-lactamases are in progress.

METHODS

Synthesis

All reactions were performed under argon using oven-dried glassware and dry solvents. Dry tetrahydrofuran (THF) and diethyl ether were obtained by standard methods and freshly distilled under argon from sodium benzophenone ketyl prior to use. The –100 °C bath was prepared by addition of liquid nitrogen to a pre-cooled (–78 °C) mixture of ethanol/methanol (1:1). Preloaded (0.25 mm) glass supported silica gel plates (Kieselgel 60, Merck) were used for TLC analysis, and compounds were visualized by exposure to UV light and by dipping the plates in 1 % $Ce(SO_4)\cdot 4H_2O$, 2.5% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 10% sulfuric acid followed by heating on a hot plate. Chromatographic purification of the compounds was performed on silica gel (particle size 0.05-0.20 mm). Melting points were measured in open capillary tubes on a Stuart SMP30 Melting Point apparatus. Optical rotations were determined at +20 °C on a Perkin-Elmer 241 polarimeter and are expressed in 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance-400 MHz spectrometer. Chemical shifts (δ) are reported in ppm and were calibrated to the residual signals of the deuterated solvent.[21] Multiplicity is given as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal; coupling constants (J) are given in Hz. Two-dimensional NMR techniques (COSY, HMBC, HSQC) were used to aid in the assignment of signals in ^1H and ^{13}C spectra. Particularly, in the ^{13}C spectra, the signal of the boron-bearing carbon atom, which tend to be broadened, and the signal of the quaternary triazole carbon are often beyond the detection limit, but their resonances were unambiguously determined by HSQC. Mass spectra were determined on an Agilent Technologies LC-MS (n) Ion Trap 6310A (ESI, 70 eV). High-resolution mass spectra were recorded on an Agilent Technologies 6520 Accurate-Mass Q-TOF LC/MS.

The purity of all tested compounds was above 95%, determined by elemental analysis performed on a Carlo Erba elemental analyzer 1110; results from elemental analyses for the compounds were within (0.3% of the theoretical values). For these BATSIs the presence of residual traces of copper

was checked on a Quadrupole Inductively Coupled Plasma Mass Spectrometer ICP-MS X Series II, indicating the presence of copper ≤ 0.5 ppm. Data from MS fragmentation and elemental analyses of free boronic acids were not obtainable because of the formation of dehydration products. Nevertheless, these boronic acids could be converted into analytically pure pinacol/pinanediol esters by exposure to an equimolar amount of pinacol/pinanediol in anhydrous THF.

(+)-**pinanediol chloromethaneboronate 1** was synthesized according to the literature.[22]

(+)-**pinanediol azidomethaneboronate 2**: was synthesized according to the literature.[14]

(+)-Pinanediol (1*S*)-chloro-2-azidoethaneboronate (3): was synthesized according to the literature.[14]

(+)-pinanediol (1*R*)-2-azido-1-(N-bis(trimethylsilyl)amino)ethaneboronate (4): Lithium bis(trimethylsilyl)amide (1 M solution in THF, 9.87 mL, 9.87 mmol) was added dropwise to a solution of **3** (2.80 g, 9.87 mmol) in anhydrous THF (25 mL) at –100 °C under argon flow. The mixture was allowed to warm to room temperature overnight. The resulting solution was concentrated under reduced pressure, and the crude was treated with light petroleum (150 mL). The white inorganic precipitate (LiCl) was filtered off on a $MgSO_4$ pad and washed with abundant light petroleum. The solvent was evaporated *in vacuo* to afford **4** as a pale yellow oil (3.23 g, 80%), used as such for the subsequent reaction. $[\alpha]_D$ = +4.2 (*c* 1.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.15 (18H, s, $[Si(CH)_3]_2$), 0.83 (3H, s, pynanyl CH_3), 1.10 (1H, d, J = 10.9, pinanyl H_{endo}), 1.28 (3H, s, pinanyl CH_3), 1.39 (3H, s, pinanyl CH_3), 1.84- 2.31 (5H, m, pinanyl protons), 2.85 (1H, dd, J = 8.5, 5.9, BC*H*), 3.29 (1H, dd, J = 12.3, 8.5, BCH-CH_2), 3.37 (1H, dd, J = 12.3, 5.9, BCH-CH_2), 4.31 (1H, dd, J = 8.7, 1.7, C*H*OB). ^{13}C NMR (100 MHz, $CDCl_3$): δ 2.5, 2.7, 24.0, 26.3, 27.0, 28.3, 35.2, 38.2, 39.4, 43.4 (br, *C*B), 51.4, 56.1, 78.6, 86.1.

(+)-Pinanediol (1*R*)-acetylamino-2-azidoethaneboronate (5). A solution of dried methanol (0.05 mL, 1.2 mmol) in anhydrous THF (1.5 mL) was added to a solution of **4** (0.5 g, 1.2 mmol) in anhydrous THF (4 mL) at -10 °C under argon flow. After being stirred for 10 min at -10 °C, the cooling bath was removed. The reaction mixture was stirred for 1 hr at rt. Thereafter the reaction mixture was cooled again at -10 °C and a solution of acetylchloride (0.09 mL, 1.2 mmol) was slowly added. The resulting mixture was allowed to react for one hour, and thereafter partitioned between EtOAc (80 mL) and H_2O (20 mL). The aqueous phase was extracted with EtOAc (2 x 25 mL) and the combined organic phases were washed with saturated $NaHCO_3$ and concentrated *under vacuum* to afford **5** as a beige solid (0.337, 90.1%), mp 44-46 °C. $[\alpha]_D$ – 66.9 (*c*, 2.2 , $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, s, pynanyl CH_3), 1.30 (3 H, s, pinanyl CH_3), 1.40 (3H, s, pinanyl CH_3), 1.42 (1H, d, J = 7.1, pinanyl H_{endo}), 1.77-2.37 (5H, m, pinanyl protons), 2.15 (3H, s, CH_3CONH), 2.81 (1H, d, J = 9.5, BC*H*), 3.35 (1H, dd, J = 13.0,11.0, BCH-CH_2), 3.67 (1H, dd, J = 13.0, 3.6, BCH-CH_2), 4.21 (1H, dd, J = 8.6, 2.0, C*H*OB), 8.43 (1H, b, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.1, 24.2, 26.7, 27.4, 29.3, 36.8, 38.1, 40.1, 43.5 (*C*B), 52.4, 54.2, 76.4, 83.6, 175.8. MS (ESI, Ion Trap): 307 $[M + H]^+$; MS/MS 307, *m/z* (%): 279 (4), 263 (10) , 250 (59), 237 (7), 208 (100), 155 (61), 130 (3), 100 (8). Anal. Calcd for $C_{14}H_{23}BN_4O_3$: C, 54,92; H, 7,57; N, 18,30. Found: C, 54,73; H, 7,88; N, 18,050

(+)-Pinanediol (1*R*)-phenylacetylamino-2-azidoethaneboronate (6). According to the procedure described above, phenylacetylchloride was added to a solution of deprotected **4** (2.10 g, 5.14 mmol) to afford **6** as a brownish solid, which was crystallized from *n*-hexane and diethylether (75% yield), mp 130-132°C. $[\alpha]_D$ – 64.9 (*c*, 1.2 , $CHCl_3$) . ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, pynanyl CH_3), 1.31 (3 H, s, pinanyl CH_3), 1.40 (1H, d, J = 10.4, pinanyl H_{endo}), 1.43 (3H, s, pinanyl CH_3), 1.78- 2.39 (5H, m, pinanyl protons), 2.88 (1H, m, BC*H*CH_2), 3.41 (1H, dd, J = 13.0, 10.7, BCHCH_2), 3.64 (1H, dd, J = 13.0, 3.6, BCHCH_2), 3.75 (2H, s, CH_2CO), 4.26 (1H, dd, J = 8.7, 2.2,

C*H*OB), 6.65 (1H, b, N*H*), 7.26-7.45 (5H, m, H_{ar}). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.2, 26.6, 27.3, 29.1, 36.5, 38.2, 39.5, 40.0, 42.5 (*C*B), 52.2, 54.2, 76.7, 84.1, 128.2, 129.4, 129.5, 132.1, 176.3. MS (ESI, Ion Trap): 383 $[M^+ + H]^+$; MS/MS 383, *m/z* (%): 355 (5), 326 (100), 231 (45), 208 (33), 176 (5), 159 (10). Anal. Calcd for $C_{20}H_{27}BN_4O_3$: C, 62,84; H, 7,12; N, 14,66. Found: C, 62,50; H, 7,36; N, 14,43.

(+)-Pinanediol (1*R*)-2-azido-1-(2-thienylacetylamino)ethaneboronate (7). According to the procedure described above, 2-thienylacetylchloride was added to a solution of deprotected **4** (3.32 g, 7.91 mmol) to afford **7** as a brownish solid, which was crystallized from diethyl ether and *n*-hexane (62% yield), mp 139-140°C. $[\alpha]_D$ – 61.5 (*c*, 1.0 , $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, s, pynanyl C*H*$_3$), 1.28 (3 H, s, pinanyl C*H*$_3$), 1.34 (1H, d, *J* = 10.5, pinanyl H_{endo}), 1.39 (3H, s, pinanyl C*H*$_3$), 1.77-2.36 (5H, m, pinanyl protons), 2.95 (1H, dt, *J* = 10.1, 3.4, BC*H*), 3.42 (1H, dd, *J* = 12.9, 10.1, BCHC*H*$_2$), 3.60 (1H, dd, *J* = 12.9, 3.4, BCHC*H*$_2$), 3.91 (2H, s, C*H*$_2$CO), 4.24 (1H, dd, *J* = 8.7, 2.1, C*H*OB), 6.59 (1H, br, N*H*), 6.96-6.97 (1H, m, H_{ar}), 7.02 (1H, dd, *J* = 5.2, 3.5, H_{ar}), 7.30 (1H, dd, *J* = 5.2, 1.1, H_{ar}). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.2, 26.6, 27.3, 28.9, 34.0, 36.3, 38.2, 39.9, 41.4 (br, *C*B), 52.0, 53.9, 77.3, 84.6, 126.3, 127.6, 128.2, 133.4, 174.4. MS (ESI, Ion Trap): 389 $[M^+ + H]^+$; MS/MS 389, *m/z* (%): 355 (5), 326 (100), 231 (45), 208 (33), 176 (5), 159 (10). Anal. Calcd for $C_{18}H_{25}BN_4O_3S$: C, 55,68; H, 6,49; N, 14,43; S, 8,26. Found: C, 55,59; H, 6,41; N, 14,28; S, 8,02.

General Procedure for Cu/C-catalyzed "click" reaction:

In a glass vial the starting compound (0.6 mmol) and the proper alkyne (1.5 mmol) were dissolved in *tert*-BuOH (3 mL) and $CuSO_4$ (0.06 mmol), sodium ascorbate (0.12 mmol) and water (3 mL) were added. The vessel was sealed and temperature raised at 60 °C. The reaction was monitored through TLC until disappearance of the azidoboronate. After the proper reaction time, that spanned from 0.5 hour for more activated alkynes to 2 hours for less activated alkynes, the reaction mixture

was partitioned between EtOAc (30 mL), H_2O (12 mL) and saturated NaCl (7 mL). The aqueous phase was extracted with EtOAc (2 x 20 mL) and the combined organic phases were washed with saturated NaCl, dried over Na_2SO_4, and concentrated *under vacuum* to afford the crude product which was thereafter purified as described.

(+)-Pinanediol (1*R*)-2-(4-carboxy-[1,2,3]triazol-1-yl)-1-acetylaminoethaneboronate (8a). The reaction was terminated after 30 min. The crude residue was crystallized from CH_2Cl_2 and *n*-hexane to afford **5a** as a beige solid (173 mg, 80% yield), mp 177-179°C. $[\alpha]_D$ – 71.9 (*c* 1.3 , DMSO). ^{1}H NMR (400 MHz, DMSO): δ 0.82 (3H, s, pynanyl C*H*$_3$), 1.23 (3H, s, pynanyl C*H*$_3$), 1.24 (3H, s, pynanyl C*H*$_3$), 1.39 (1H, d, J = 9.7, pynanyl H_{endo}), 1.63-2.21 (5H, m, pynanyl protons), 2.05 (3H, s, C*H*$_3$CONH), 2.95-2.98 (1H, m, BC*H*CH$_2$), 4.00 (1H, d, J = 7.1, C*H*OB), 4.30-4.38 (2H, m, BCHC*H*$_2$), 8.70 (1H, s, C*H*$_{triazole}$), 9.81 (1H, s, N*H*). ^{13}C NMR (100 MHz, DMSO): δ 17.4, 24.5, 26.8, 27.8, 30.0, 37.4, 38.1, 40.0, 44.2 (br, *C*B), 52.7, 53.3, 75.8, 82.3, 129.7 (*C*H$_{triazole}$), 140.0, 162.4,177.0. MS (ESI, Ion Trap): 377 $[M + H]^+$, MS/MS 377, *m/z* (%): 359 (3), 264 (96), 225 (100),140 (4%), 130 (4%). Anal. Calcd for $C_{17}H_{25}BN_4O_5$: C, 54,27; H, 6,70; N, 14,89. Found: C, 53,99; H, 6,91; N, 14,53.

(+)-Pinanediol (1*R*)-2-[4-(1-hydroxy-1-methylethyl)-[1,2,3]triazol-1-yl)-1-acetylaminoethaneboronate (8b). The reaction was terminated after 30 min. The crude residue was triturated from *n*-hexane to afford **8b** as a whitish solid (172 mg, 79% yield), mp 75-77°C. $[\alpha]_D$ – 58.5 (*c*, 1.3 , $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s, pynanyl C*H*$_3$), 1.32 (3H, s, pynanyl C*H*$_3$), 1.44 (3H, s, pynanyl C*H*$_3$), 1.48 (1H, d, J = 10.1, pynanyl H_{endo}), 1.53 (6H, s, (C*H*$_3$)$_2$COH), 1.81-2.42 (5H, m, pynanyl protons), 2.20 (3H, s, C*H*$_3$CONH), 3.15-3.18 (1H, m, BC*H*), 4.24 (1H, d, J = 6.9, C*H*OB), 4.43-4.51 (2H, m, BCHC*H*$_2$), 7.65 (1H, s, C*H*$_{triazole}$), 9.25 (1H, br, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.6, 24.3, 27.0, 27.5, 29.4, 29.7, 37.1, 38.2, 40.2, 45.5 (br, *C*B), 52.5, 53.3, 76.3, 83.2,

119.6 (CH$_{triazole}$), 176.8, C-4 triazole ring not seen. MS (ESI, Ion Trap): 391.3 $[M + H]^+$, MS/MS 391, *m/z* (%): 373 (67), 345 (23), 317 (10), 264 (100), 222 (7), 193 (10), 135 (9), 130 (7), 112 (6). Anal. Calcd for $C_{19}H_{31}BN_4O_4$: C, 58,47; H, 8,01; N, 14,36. Found: C, 58,18; H, 7.75; N, 14,08.

(+)-Pinanediol (1*R*)-2-[4phenyl-[1,2,3]triazol-1-yl)-1-acetylaminoethaneboronate (8c). The reaction was terminated after 2 hours. The crude residue was triturated from *n*-hexane to afford **8c** as a whitish solid (117 mg, 86% yield), mp 77-77°C. $[\alpha]_D$ – 60.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, s, pynanyl CH_3), 1.34 (3H, s, pynanyl CH_3), 1.47 (3H, s, pynanyl CH_3), 1.54 (1H, d, J = 10.2, pynanyl H_{endo}), 1.85 – 2.45 (5H, m, pynanyl protons), 2.26 (3H, s, CH_3CONH), 3.29 (1H, d, J = 7.3, BCH), 4.27 (1H, d, J =7.4, CHOB), 4.45 (1H, dd, J = 14.7, 2.0, BCHCH_2), 4.62 (1H, dd, J = 14.7, 12.4, BCHCH_2), 7.30 – 7.45 (5H, m, H_{ar}), 7.69 (1H, s, C$H_{triazole}$), 9.43 (1H, br, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.8, 24.3, 27.0, 27.5, 29.4, 37.1, 38.2, 40.2, 45.0 (br, CB), 52.6, 53.4, 76.4, 83.2, 119.4 (CH$_{triazole}$), 125.2, 128.2, 128.8, 129.8, 147.6, 176.9. MS (ESI, Ion Trap): 409 $[M + H]^+$, MS/MS 409, *m/z* (%): 324 (26), 264 (100), (75), 187 (7), 135 (5). Anal. Calcd for $C_{22}H_{29}BN_4O_3$: C, 64,72; H, 7,16; N, 13,72. Found: C, 64,58; H, 7,28; N, 13,54.

(+)-Pinanediol (1*R*)-2-(4-carboxy-[1,2,3]triazol-1-yl)-1-phenylacetylaminoethaneboronate (9a). The reaction was terminated after 30 min. The crude residue was crystallized from CH_2Cl_2 and *n*-hexane to afford **9a** as a beige solid (277 mg, 80% yield), mp 130-132°C. $[\alpha]_D$ – 87.5 (*c* 1.1, DMSO). ^{1}H NMR (400 MHz, DMSO): δ 0.81 (3H, s, pynanyl CH_3), 1.21 (3H, s, pynanyl CH_3), 1.22 (3H, s, pynanyl CH_3), 1.34 (1H, d, J = 10.0, H_{endo}), 1.62-2.22 (5H, m, pynanyl protons), 3.01-3.03 (1H, m, BCH), 3.667 (1H, d, J = 14.8, PhCH_2), 3.675 (1H, d, J = 14.8, PhCH_2), 4.01 (1H, d, J =6.8, CHOB), 4.35 (1H, dd, J = 14.4, 10.5, BCHCH_2), 4.43 (1H, dd, J = 14.4, 3.9, BCHCH_2) 7.23-7.35 (5H, m, H_{ar}), 8.71 (1H, s, C$H_{triazole}$), 9.89 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO): δ 24.5, 26.7, 27.7, 29.8, 37.2, 37.1, 38.0, 40.3, 43.4 (br, CB), 52.5, 52.9, 75.9, 82.6, 127.6, 129.0, 129.4,

129.8 (C$H_{triazole}$), 134.1, 140.0, 162.3,177.0. MS (ESI, Ion Trap): 453 $[M + H]^+$, MS/MS 453, *m/z* (%): 340 (75), 301 (100), 206 (8). Anal. Calcd for $C_{23}H_{29}BN_4O_5$: C, 61,07; H, 6,46; N, 12,39. Found: C, 60,86; H, 6,63; N, 12,10.

(+)-Pinanediol (1*R*)-2-[4-(1-hydroxy-1-methylethyl)-[1,2,3]triazol-1-yl)-1-phenylacetylaminoethaneboronate (9b). The reaction was terminated after 30 min. The crude residue was triturated from *n*-hexane to afford **9b** as a beige solid (288 mg, 98% yield), mp 78-80°C. $[\alpha]_D$ – 73.9 (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s, pynanyl CH_3), 1.32 (3H, s, pynanyl CH_3), 1.44 (3H, s, pynanyl CH_3), 1.45 (1H, d, *J* = 9.5, H_{endo}), 1.49 (6H, s, (CH_3)$_2$COH), 1.82-2.53 (5H, m, pynanyl protons), 3.14-3.17 (1H, m, BC*H*), 3.77 (2H, s, PhCH_2), 4.25 (1H, dd, *J* = 6.6, 1.9, C*H*OB), 4.41-4.49 (2H, m, BCHCH_2), 7.25-7.35 (5H, m, H_{ar}), 7.65 (1H, s, C$H_{triazole}$), 9.16 (1H, br, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.3, 26.9, 27.4, 29.3, 30.9, 31.3, 37.0, 38.1, 38.2, 40.2, 44.9 (br, *C*B), 52.4, 53.0, 76.4, 83.4, 119.8 (*C*H$_{triazole}$), 127.8, 129.0, 129.3, 132.3, 155.5, 177.4. MS (ESI, Ion Trap): 467 $[M + H]^+$, MS/MS 467, *m/z* (%): 449 (53), 421 (23), 340 (100), 297 (16), 269 (7). Anal. Calcd for $C_{25}H_{35}BN_4O_4$: C, 64,38; H, 7,56; N, 12,01. Found: C, 64,30; H, 7,65; N, 11,93.

(+)-Pinanediol (1*R*)-2-(4-phenyl-[1,2,3]triazol-1-yl)-1-phenylacetylaminoethaneboronate (9c). The reaction was terminated after 2 hours. The crude residue was triturated from *n*-hexane to afford **9c** as a beige solid (303 mg, 85% yield), mp 94-96 °C. $[\alpha]_D$ – 68.9 (*c* 1.0 , $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, s, pynanyl CH_3), 1.33 (3H, s, pynanyl CH_3), 1.46 (3H, s, pynanyl CH_3), 1.52 (1H, d, *J* = 10.1, H_{endo}), 1.85 – 2.41 (5H, m, pynanyl protons), 3.26-3.29 (1H, m, BC*H*), 3.80 (2H, s, PhCH_2), 4.29 (1H, d, *J* = 8.1, C*H*OB), 4.46-4.60 (2H, m, BCHCH_2), 7.29-7.48 (10H, m, H_{ar}), 7.67 (1H, s, C$H_{triazole}$), 8.89 (1H, br, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.3, 26.9, 27.4, 29.3, 37.0, 38.2, 38.5, 40.2, 44.2 (br, *C*B), 52.4, 52.9, 76.6, 83.6, 119.8 (*C*H$_{triazole}$), 125.3, 127.9, 128.2, 128.8, 129.1, 129.4, 130.0, 132.1, 147.5, 177.4. MS (ESI, Ion Trap): 485 $[M + H]^+$, MS/MS

485, *m/z* (%): 340 (100), 333 (65), 324 (10), 188 (3.5). Anal. Calcd for $C_{28}H_{33}BN_4O_3$: C, 69,43; H, 6,87; N, 11,57. Found: C, 69,59; H, 6,70; N, 11,31.

(+)-Pinanediol (1*R*)-2-[4-(3-carboxy-phenyl)-[1,2,3]triazol-1-yl]-1-phenylacetylaminoethaneboronate (9d). The reaction was terminated after 2.5 hours. The crude residue was repeatedly washed with diethyl ether to afford **9d** as a white solid (248 mg, 89% yield), mp 202-205 °C (dec). $[\alpha]_D$ – 94.3 (*c* 1.0 , CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 0.88 (3H, s, pynanyl C*H*$_3$), 1.28 (3H, s, pynanyl C*H*$_3$), 1.35 (3H, s, pynanyl C*H*$_3$), 1.42 (1H, d, J = 10.4, H_{endo}), 1.70 – 2.40 (5H, m, pynanyl protons), 3.21 (1H, dd, J = 4.1, 10.5, BC*H*), 3.31 (2H, s, PhCH_2), 4.12 (1H, dd, J = 2.3, 8.6, C*H*OB), 4.22 (1H, dd, J = 14.5, 10.5, BCHCH_2), 4.50 (1H, dd, J = 4.2, 14.5, BCHCH_2), 7.20-7.35 (5H, m, H_{ar}), 7.56 (1H, t, J = 7.7, H_{ar}), 8.03 (2H, m, H_{ar}) 8.45 (1H, s, C$H_{triazole}$), 8.48 (1H, s, H_{ar}). ^{13}C NMR (100 MHz, CD_3OD): δ 24.6, 27.6, 27.8, 29.7, 37.8, 38.1, 39.1, 41.4, 46.1 (br, *C*B), 53.7, 53.8, 77.5, 84.4, 123.2 (*C*H$_{triazole}$), 127.9, 128.7, 129.9, 130.2, 130.3, 130.4, 131.0, 132.4,134.1, 173.0 179.9. MS (ESI, Ion Trap): 529 $[M + H]^+$, MSMS 529, *m/z* (%): 377 (26), 340 (100), 222 (8), 188 (14). Anal. Calcd for $C_{29}H_{33}BN_4O_5$: C, 65,92; H, 6,29; N, 10,60. Found: C, 65,77; H, 6,50; N, 10,43.

(+)-Pinanediol (1*R*)-2-[4-(3-amino-phenyl)-[1,2,3]triazol-1-yl]-1-(2-phenylacetylamino) ethaneboronate (9e). The reaction was terminated after 2 hours. The crude residue was triturated from diethylether/*n.*hexane (1 : 1) to afford **9e** as light brown solid (250 mg, 96% yield), mp 125-128 °C. $[\alpha]_D$ – 83.9 (*c* 0.45 , MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 0.81 (3H, s, pynanyl C*H*$_3$), 1.21 (3 H, s, pinanyl C*H*$_3$), 1.28 (3H, s, pinanyl C*H*$_3$), 1.34 (1H, d, J = 10.3, pinanyl H_{endo}), 1.85-2.35 (5H, m, pinanyl protons), 3.14 (1H, dd, J = 4.2, 10.5, BC*H*), 3.67 (2H, s, CH_2CO), 4.12 (1H, dd, J = 2.3, 8.7, C*H*OB), 4.32 (1H, dd, J = 14.5, 10.5 , BCHCH_2), 4. 46 (1H, dd, J = 14.5, 4.2, BCHCH_2), 6.64 (1H, d, J = 6.6, H_{ar}), 7.00-7.29 (8H, m, H_{ar}), 8.20 (1H, s, C$H_{triazole}$). ^{13}C NMR (100 MHz, CD_3OD): δ 24.5, 27.6, 27.8, 29.8, 32.8, 37.8, 38.0, 39.2, 41.4 (*C*B), 53.69, 53.74, 77.4, 84.4,

113.4, 116.6, 115.9, 122.5 (CH$_{triazole}$), 128.7, 129.9, 130.3, 130.7, 132.4, 134.1, , 149.3(CH$_{triazole}$) 179.8. MS (ESI, Ion Trap): 500 $[M + H]^+$, MSMS 500, *m/z* (%): 347 (21), 340 (100), 222 (7), 206 (11), 188 (19), 144 (23). Anal. Calcd for $C_{28}H_{34}BN_5O_3$: C, 67,34; H, 6,86; N, 14,02. Found: C, C, 67,18; H, 6,71; N, 13,89.

(+)-Pinanediol (1*R*)-2-(4-carboxy-[1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)ethaneboronate (10a). The reaction was terminated after 30 min. The crude residue was crystallized from diethyl ether and *n*-hexane to afford **10a** as a beige solid (150 mg, 82% yield), mp 112-114°C. $[\alpha]_D$ –71.6 (*c* 1.3, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.82 (3H, s, pinanyl CH_3), 1.23 (3H, s, pinanyl CH_3), 1.24 (3H, s, pinanyl CH_3), 1.37 (1H, d, J = 9.9, H_{endo}), 1.64-2.23 (5H, m, pinanyl protons), 3.05 (1H, d, J = 8.6, BCH), 3.89 (2H, s, CH_2CO), 4.06 (1H, d, J = 7.1, CHOB), 4.35 (1H, dd, J = 14.4, 10.6, BCHCH_2), 4.43 (1H, dd, J = 14.4, 3.9, BCHCH_2), 6.95-6.99 (2H, m, H_{ar}), 7.43 (1H, d, J = 4.9, H_{ar}), 8.69 (1H, s, C$H_{triazole}$), 9.76 (1H, s, NH), 13.06 (1H, br, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.0, 26.2, 27.2, 29.2, 31.6, 36.5, 37.6, 39.3, 42.5 (br, CB), 52.0, 52.3, 75.5, 82.4, 125.7, 126.8, 126.9, 129.3 (CH$_{triazole}$), 134.6, 139.5, 161.9, 175.1. MS (ESI, Ion Trap): 471 $[M + Na]^+$, 459 $[M + H]^+$, MS/MS 459, *m/z* (%): 346 (44), 307 (100), 212 (6). HRMS (ESI-TOF) *m/z*: $[M–H]^-$ Calcd for $C_{21}H_{26}BN_4O_5S$ 457.1726; Found 457.1747. Anal. Calcd for $C_{21}H_{27}BN_4O_5S$: C, 55,03; H, 5,94; N, 12,22; S, 7,00. Found: C, 54.92; H, 6,05; N, 12,06; S, 6,81.

(+)-Pinanediol (1*R*)-2-[4-(1-hydroxy-1-methylethyl)-[1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)ethaneboronate (10b). The reaction was terminated after 30 min. The crude residue was triturated from *n*-hexane to afford the compound **10b** as pale yellow solid (217 mg, 92% yield), mp 76-78 °C. $[\alpha]_D$ – 54.9 (*c*, 0.90 , MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (3H, s, pinanyl CH_3), 1.32 (3 H, s, pinanyl CH_3), 1.43 (6H, s, (CH_3)$_2$COH), 1.47 (1H, d, J = 10.0, H_{endo}), 1.49 (3H, s, pinanyl CH_3), 1.82- 2.41 (5H, m, pinanyl protons), 3.18-3.200 (1H, m, BCH), 3.95 (2H, s, CH_2CO), 4.26 (1H, dd, J = 8.6, 2.1, CHOB), 4.43 (1H, dd, J = 14.6, 3.4, BCHCH_2), 4.51 (1H, dd,

J = 14.6, 11.0, BCHCH_2), 6.93-6.97 (2H, m, H_{ar}), 7.22 (1H, dd, *J* = 6.5, 3.2, H_{ar}), 7.66 (1H, s, C$H_{triazole}$) 9.29 (1H, br, N*H*) . ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.2, 26.8, 27.4, 29.2, 29.5, 30.9, 32.6, 36.8, 38.0, 38.2, 40.1, 44.3 (*C*B), 52.3, 52.8, 76.5, 83.7, 120.3 (*C*H$_{triazole}$), 125.8, 127.2, 127.7, 133.2, 155.5, 175.9. MS (ESI, Ion Trap): 473 $[M + H]^+$, MS/MS 473, *m/z* (%): 455 (70), 427 (20), 346 (100). Anal. Calcd for $C_{23}H_{33}BN_4O_4S$: C, 58,48; H, 7,04; N, 11,86; S, 6,79. Found: C, 58,59; H, 7,16; N, 11,59; S, 6,53.

(+)-Pinanediol (1*R*)-2-(4-Phenyl-[1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)-ethaneboronate (10c). The reaction was terminated after 2 hours. The crude residue was triturated from diethylether to afford **10c** as a beige solid (205 mg, 82 % yield), mp 115-117 °C. $[\alpha]_D$ –63.1 (*c*, 1.03, $CDCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (3H, s, pinanyl CH_3), 1.29 (3 H, s, pinanyl CH_3), 1.40 (1H, d, *J* = 11.7, pinanyl H_{endo}), 1.47 (3H, s, pinanyl CH_3), 1.52- 2.57 (5H, m, pinanyl protons), 3.32-3.35 (1H, m, BC*H*), 3.98 (2H, s, CH_2CO), 4.32 (1H, dd, *J* = 8.7, 2.2, C*H*OB), 4.51-4.55 (2H, m, BCHCH_2), 6.95-7.99 (2H, m, H_{ar}), 7.23 (1H, dd, *J* = 2.4, 4.1, H_{ar}), 7.35-7.44 (3H, m, H_{ar}), 7.62-7.67 (2H, m, H_{ar}), 7.75 (1H, s, CH_{triaz}), 8.13 (1H, br, N*H*) . ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.2, 26.8, 27.4, 29.2, 33.0, 38.2, 39.9, 43.0 (*C*B), 52.2, 54.0, 69.2, 76.9, 84.2, 120.3 (*C*H$_{triazole}$), 125.5, 126.1, 127.5, 128.0, 128.2, 128.8, 130.2, 132.9, 147.5, 175.4. MS (ESI, Ion Trap): 513 $[M + Na]^+$, 491 $[MH]^+$. MSMS 491, *m/z* (%): 346 (100), 339 (91), 324 (11), 194 (9). Anal. Calcd for $C_{26}H_{31}BN_4O_3S$: C, 63,67; H, 6,37; N, 11,42; S, 6,54. Found: C, 63,50; H, 6,58; N, 11,19; S, 6,33.

(+)-Pinanediol (1*R*)-2-[4-(3-carboxy-phenyl)-[1,2,3]triazol-1-yl]-1-(2-thienylacetylamino)-ethaneboronate (10d). The reaction was terminated after 2 hours. The crude residue was triturated from diethylether to afford **10d** as white solid (216 mg, 79 % yield), mp 228°C dec. $[\alpha]_D$ – 81.5 (*c* 0.7 , MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.81 (3H, s, pinanyl CH_3), 1.22 (3 H, s, pinanyl CH_3), 1.25 (3H, s, pinanyl CH_3), 1.38 (1H, d, *J* = 10.0, pinanyl H_{endo}), 1.65- 2.21 (5H, m, pinanyl

protons), 3.07-3.09 (1H, m, BC*H*), 3.91 (2H, s, CH_2CO), 4.73 (1H, d, *J* = 6.9, C*H*OB), 4.32 (1H, dd, *J* = 14.4, 11.0, BCHCH_2), 4.30 (1H, dd, *J* = 14.4, 3.8, BCHCH_2), 6.96-6.99 (2H, m, H_{ar}), 7.42 (1H, dd, *J* = 4.9, 1.0, H_{ar}), 7.60 (1H, t, *J* = 7.7, H_{ar}), 7.90 (1H, d, *J* = 7.7), 8.07 (1H, d, *J* = 7.7, H_{ar}), 8.42 (1H, s, H_{ar}), 8.71 (1H, s, C$H_{triazole}$), 9.81 (1H, br, N*H*), 13.1 (1H, br, COO*H*) . ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.9, 26.2, 27.2, 29.2, 31.6, 36.5, 37.6, 39.7, 42.5 (*C*B), 51.9, 52.3, 75.6, 82.5, 122.3 (*C*$H_{triazole}$), 125.7, 125.8, 126.8, 126.9, 128.5, 129.22, 129.27, 131.39, 131.45, 134.7, 145.4, 167.1, 175.0. MS (ESI, Ion Trap): 557 $[M + Na]^+$, 535 $[M + H]^+$. MSMS 535, *m/z* (%): 383 (100), 368 (6), 346 (71). Anal. Calcd for $C_{27}H_{31}BN_4O_5S$: C, 60,68; H, 5,85; N, 10,48; S, 6,00. Found: C, 60,99; H, 6,13; N, 10,25; S, 5,73.

(+)-Pinanediol (1*R*)-2-[4-(3-amino-phenyl)-[1,2,3]triazol-1-yl]-1-(2-thienylacetylamino)ethaneboronate (10e). The reaction was terminated after 2 hours. The crude residue was triturated from diethylether to afford **10e** as white solid (185 mg, 74% yield), mp 87-90 °C. $[\alpha]_D$ – 47.8 (*c* 0.96 , $CDCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s, pynanyl CH_3), 1.32 (3 H, s, pinanyl CH_3), 1.45 (3H, s, pinanyl CH_3), 1.50 (1H, d, *J* = 10.3, pinanyl H_{endo}), 1.84- 2.39 (5H, m, pinanyl protons), 3.28-3.31 (1H, m, BC*H*), 3.50 (2H, b, NH_2), 3.96 (2H, s, CH_2CO), 4.30 (1H, d, *J* = 8.0, C*H*OB), 4.47 (1H, dd, *J* = 14.6, 3.7 , BCHCH_2), 4. 54 (1H, dd, *J* = 14.6, 10.6, BCHCH_2), 6.65 (1H, d, *J* = 7.8, H_{ar}), 7.35-7.44 (4H, m, H_{ar}), 7.14 (1H, t, *J* = 7.9, H_{ar}), 7.20 (1H, d, *J* = 4.9, $H_{ar\ tioph}$), 7.61 (1H, s, C$H_{triazole}$), 8.89 (1H, s, N*H*) . ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.2, 26.8, 27.4, 29.2, 33.0, 36.7, 38.2, 40.1, 43.4 (*C*B), 52.3, 52.6, 76.8, 84.0, 112.2, 115.1, 115.9, 120.2 (*C*$H_{triazole}$), 125.9, 127.3, 127.9, 129.7, 131.0, 133.1, 146.7, 147.6, 175.7. MS (ESI, Ion Trap): 528 $[M + Na]^+$, 506 $[M + H]^+$. MSMS 506, *m/z* (%): 354 (79), 346 (100), 339 (18). Anal. Calcd for $C_{26}H_{32}BN_5O_3S$: C, 61,78; H, 6,38; N, 13,86; S, 6,34. Found: C, 61,50; H, 6,11; N, 13,57; S, 6,15.

(+)-Pinanediol (1*R*)-2-([1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)ethaneboronate (10f). In a glass vial the compound **7** (50 mg, 0.13 mmol) and trimethylsilylacetylene (28 mg, 0.2 mmol) were

dissolved in *tert*-BuOH (1 mL) and $CuSO_4$ (40 μL of a solution 50 mg/mL, 0.013 mmol), sodium ascorbate (5 mg, 0.026) and water (1 mL) were added. The vessel was sealed, temperature was raised up to 60 °C and reaction was monitored through TLC. After 2 hours, starting material was still prominent, therefore the same amount of alkyne, $CuSO_4$ and ascorbate were added. The same procedure was repeated twice, until no more starting material was evident. The reaction mixture was partitioned between EtOAc (20 mL), H_2O (8 mL) and saturated NaCl (5 mL). The aqueous phase was extracted with EtOAc (2 x 20 mL) and the combined organic phases were washed with saturated NaCl, dried over Na_2SO_4, and concentrated *under vacuum.* The crude residue was purified through gel chromatography (SiO_2, EtOAc), and a fraction of the protected silylated compound (20 mg, 31%) was recovered together with another fraction of compound **10f** as a brownish oil (17 mg, 31%). $[\alpha]_D$ –27.1 (*c* 1.4, $CDCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s, pynanyl CH_3), 1.33 (3H, s, pynanyl CH_3), 1.44 (3H, s, pynanyl CH_3), 1.45 (1H, d, J = 10.1 H_{endo}), 1.70-2.43 (5H, m, pynanyl protons), 3.27-3.30 (1H, m, BC*H*), 3.95 (2H, s, CH_2CONH), 4.31 (1H, dd, J = 8.6, 1.9, C*H*OB), 4.48 (1H, dd, J = 14.5, 9.8, BCHCH_2), 4.53 (1H, dd, J = 14.5, 3.6, BCHCH_2), 6.97-7.03 (2H, m, H_{ar}), 7.29 (1H, d, J = 5.2, H_{ar}), 7.57-7.66 (3H, m, CON*H*, C$H_{triazole}$). ^{13}C NMR (100 MHz, CDCl3): δ 24.2, 26.7, 27.3, 29.1, 29.7, 36.6, 38.2, 40.0, 42.7 (br, *C*B), 52.1, 52.2, 76.7, 84.3, 124.1 (*C*H_{triaz}), 126.2, 127.6, 128.1, 132.8, 133.1 (*C*H_{triaz}), 175.2. MS (ESI, Ion Trap): 415 $[M + H]^+$, MS/MS 415, *m/z* (%): 346.1 (100), 263.0 (50), 194.0 (11). Anal. Calcd for $C_{20}H_{27}BN_4O_3S$: C, 57,98; H, 6,57; N, 13,52; S, 7,74. Found: C, 57,82; H, 6,71; N, 13,34; S, 7,60.

General Procedure for pinanediol removal.

The pinanediol esters **8a-c**, **9a-c,e** and **10a-c,e,f** (0.3 mmol) were dissolved in CH_3CN (3 mL) and HCl (0.3 mmol of a 1M solution in degassed H_2O), phenylboronic acid (0.28 mmol) and *n*-hexane (3 mL) were sequentially added and the resulting biphasic solution was vigorously stirred. In case of pinanediol esters **9d** and **10d**, MeOH was used instead of CH_3CN. After 30 min the *n*-hexane solution (containing the pinanediol phenylboronate) was removed and fresh *n*-hexane (3 mL) added.

This last procedure was repeated several times until a TLC analysis of the *n*-hexane layer not revealed anymore phenylboronate production (total reaction time 3 hours). The acetonitrile phase was concentrated, and the crude recrystallized from acetonitrile, affording the desired compounds **11a-c**, **12a-e**, **13a-f**. Copper (II) concentration in these products was found below 0.5 ppm through plasma analysis on a ICP-MS.

The enantiomeric purity of chiral boronic acids was checked by reconversion into their pinanediol esters. In particular final compounds **11a-c**, **12a-e** and **13a-f** were allowed to react with an equimolar amount of (+)-pinanediol in anhydrous THF: the NMR spectra of the crude products displayed the presence of a single diastereoisomer, proving that no racemization occurred during transesterification.

(1*R*)-2-[4-Carboxy-[1,2,3]triazol-1-yl]-1-acetylaminoethaneboronic acid 11a. Compound **11a** was obtained as a yellow solid (62 mg, 85% yield), mp 96-97 °C. $[\alpha]_D$ – 101.1 (*c* 0.8 , MeOH). ^{1}H NMR (400 MHz, MeOD): δ 2.20 (3H, s, C*H*$_3$CONH), 3.20 (1H, dd, *J* = 10.6, 3.7, BC*H*CH$_2$), 4.43 (1H, dd, *J* = 14.5, 10.6, BCHC*H*$_2$), 4.54 (1H, dd, *J* = 14.5, 3.7, BCHC*H*$_2$), 8.63 (1H, s, C*H*$_{triazole}$). ^{13}C NMR (100 MHz, MeOD): δ 15.1, 49.4 (br, *C*B), 52.6, 128.8 (*C*H$_{triazole}$), 139.1, 161.1, 178.4. HRMS (ESI-TOF) m/z: $[M–H]^-$ Calcd for $C_7H_{10}BN_4O_5$ 241.0751; Found 241.0757. EI-MS and elemental analysis results were not obtainable, but exposure of **11a** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **8a** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{17}H_{25}BN_4O_5$: C, 54,27; H, 6,70; N, 14,89. Found: C, 54,06; H, 6,88; N, 14,61.

(1*R*)-2-[4-(1-hydroxy-1-methylethyl)-[1,2,3]triazol-1-yl]-1-acetylaminoethaneboronic acid 11b. Compound **11b** was obtained as a yellow solid (70 mg, 91% yield), mp 88-90°C. $[\alpha]_D$ – 86.4 (*c* 1.1 , MeOH). ^{1}H NMR (400 MHz, MeOD): δ 1.67 (3H, s, C*H*$_3$COH), 1.69 (3H, s, C*H*$_3$COH), 2.23 (3H, s, C*H*$_3$CONH), 3.26 (1H, dd, *J* = 10.3, 3.5, BC*H*CH$_2$), 4.55 (1H, dd, *J* = 14.4, 10.3, BCHC*H*$_2$), 4.67

(1H, dd, J = 14.4, 3.5, BCHCH_2), 8.73 (1H, s, C$H_{triazole}$). ^{13}C NMR (100 MHz, MeOD): δ 18.9, 24.9, 25.0, 45.2 (br, *C*B), 55.6, 71.9, 117.6 (*C*H$_{triazole}$), 144.6, 178.6. HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_9H_{18}BN_4O_4$ 257.1417; Found 217.1409. EI-MS and elemental analysis results were not obtainable, but exposure of **11b** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **8b** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{18}H_{31}BN_4O_4$: C, 58,47; H, 8,01; N, 14,36. Found: C, 58,59; H, 7.82; N, 14,15.

(1*R*)-2-[4-Phenyl-[1,2,3]triazol-1-yl]-1-acetylaminoethaneboronic acid 11c. Compound **11c** was recovered as a yellow solid (66 mg, 83% yield), mp 176-178°C. $[\alpha]_D$ – 91.8 (*c* 0.9 , MeOH). ^{1}H NMR (400 MHz, MeOD): δ 2.24 (3H, s, CH_3CONH), 3.34-3.35 (1H, m, BC*H*CH$_2$), 4.60 (1H, dd, J = 14.5, 11.1, BCHCH_2), 4.72 (1H, dd, J = 14.5, 3.5, BCHCH_2), 7.55-7.67 (3H, m, H_{ar}), 9.01 (1H, s, C$H_{triazole}$). ^{13}C NMR (100 MHz, MeOD): δ 15.3, 45.4 (br, B*C*HCH$_2$), 55.3, 124.87 (*C*H$_{triazole}$), 124.94, 126.3, 129.3, 130.6, 144.2, 178.7. HRMS (ESI-TOF) m/z: [M+H]$^+$ Calcd for $C_{12}H_{16}BN_4O_3$ 275.1312; Found 275.1305. EI-MS and elemental analysis results were not obtainable, but exposure of **11c** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **8c** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{22}H_{29}BN_4O_3$: C, 64,72; H, 7,16; N, 13,72. Found: C, 64,75; H, 7,06; N, 13,49.

(1*R*)-2-[4-Carboxy-[1,2,3]triazol-1-yl]-1-phenylacetylaminoethaneboronic acid 12a. Compound **12a** was obtained as a yellow solid (86 mg, 90% yield), mp 214-216°C. $[\alpha]_D$ – 104.4 (*c* 0.9, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 3.19 (1H, dd, J = 10.2, 4.0, BC*H*CH$_2$), 3.78 (2H, s, PhCH_2) 4.42 (1H, dd, J = 14.5, 10.2, BCHCH_2), 4.54 (1H, dd, J = 14.5, 4.0, BCHCH_2), 7.29-7.38 (5H, m, H_{ar}) 8.56 (1H, s, C$H_{triazole}$). ^{13}C NMR (100 MHz, MeOD): δ 36.3, 46.4 (br, *C*B), 52.5, 127.4, 128.54, 128.93, 129.6 (*C*H$_{triazole}$), 132.6, 140.3, 161.8, 179.1. HRMS (ESI-TOF) m/z: [M–H]$^-$ Calcd for $C_{13}H_{14}BN_4O_5$ 317.1065; Found 317.1073. EI-MS and elemental analysis results were

not obtainable, but exposure of **12a** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **9a** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{23}H_{29}BN_4O_5$: C, 61,07; H, 6,46; N, 12,39. Found: C, 60,90; H, 6,54; N, 12,22.

(1*R*)-2-[4-(1-Hydroxy-1-methylethyl)-[1,2,3]triazol-1-yl]-1-phenylacetylaminoethaneboronic acid 12b. Compound **12b** was obtained as a yellow solid (95 mg, 95% yield), mp 95-97°C. $[\alpha]_D$ – 75.9 (*c* 0.9 , MeOH). ^{1}H NMR (400 MHz, MeOD): δ 1.67 (3H, s, C*H*$_3$COH), 1.72 (3H, s, C*H*$_3$COH), 3.27-3.29 (1H, m, BC*H*CH$_2$), 3.84 (2H, s, PhC*H*$_2$), 4.50-4.70 (2H, m, BCHC*H*$_2$), 8.70 (1H, s, C*H*$_{triazole}$). ^{13}C NMR (100 MHz, MeOD): δ 25.1, 36.5, 44.8 (br, *C*B), 55.8, 65.5, 124.8 (*C*H$_{triazole}$, not seen), 127.5, 128.6, 129.0, 132.6, 145.1, 179.2. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{22}BN_4O_4$ 333.1731; Found 333.1733. EI-MS and elemental analysis results were not obtainable, but exposure of **12b** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **9b** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{25}H_{35}BN_4O_4$: C, 64,38; H, 7,56; N, 12,01. Found: C, 64,17; H, 7,44; N, 11,83.

(1*R*)-2-[4-Phenyl-[1,2,3]triazol-1-yl]-1-phenylacetylaminoethaneboronic acid (12c). Compound **12c** was obtained as a yellow solid (101 mg, 96% yield), mp 152-154°C. $[\alpha]_D$ – 91.4 (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 3.32-3.34 (1H, m, BC*H*CH$_2$), 3.83 (2H, s, PhC*H*$_2$), 4.58 (1H, dd, *J* = 14.3, 10.5, BCHC*H*$_2$), 4.69 (1H, dd, *J* = 14.3, 2.9, BCHC*H*$_2$), 7.23-7.88 (10H, m, *H*$_{ar}$), 8.93 (1H, s, C*H*$_{triazole}$). ^{13}C NMR (100 MHz, MeOD): δ 36.5, 45.5 (br, *C*B), 54.8, 124.8 (*C*H$_{triazole}$), 125.5, 126.2, 127.5, 128.6, 129.0, 129.3, 130.3, 132.6, 145.1, 179.2. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{20}BN_4O_3$ 351.1626; Found 351.1615. EI-MS and elemental analysis results were not obtainable, but exposure of **12c** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **9c** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{28}H_{33}BN_4O_3$: C, 69,43; H, 6,87; N, 11,57. Found: C, 69,50; H, 6,66; N, 11,39.

(1*R*)-2-[4-(3-Carboxyphenyl)-[1,2,3]triazol-1-yl]-1-phenylacetylaminoethaneboronic acid (12d). Compound **12d** was obtained as white solid (114 mg, 97% yield), mp 194-197 °C dec. $[\alpha]_D$ = –80.7 (*c* 0.27, MeOH). ^{1}H NMR (600 MHz, MeOD): δ 3.29 (1H, dd, *J* = 10.6, 3.8, BC*H*CH$_2$), 3.77 (2H, s, C*H*$_2$CONH), 4.52 (1H, dd, *J* = 14.5, 10.6, BCHC*H*$_2$), 4.66 (1H, dd, *J* = 14.5, 3.9, BCHC*H*$_2$), 7.25-7.36 (5H, m, *H*$_{ar}$), 7.66 (1H, t, *J* = 7.8, *H*$_{ar}$), 8.07 (1H, dt, *J* = 7.8, 1.5, *H*$_{ar}$), 8.13 (1H, dt, *J* = 7.8, 1.5, *H*$_{ar}$), 8.52 (1H, t, *J* = 1.5, *H*$_{ar}$), 8.78 (1H, s, C*H*$_{triaz}$). ^{13}C NMR (150 MHz, MeOD): δ 37.9, 47.1 (br, *C*B), 55.3, 125.1 (*C*H$_{triaz}$), 128.3, 128.9, 129.3, 130.0, 130.3, 130.7, 131.5, 131.7, 133.3, 134.0, 146.2, 168.9, 180.6. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{19}BN_4O_5$ 394.1558; Found 394.1553. EI-MS and elemental analysis results were not obtainable, but exposure of **12d** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **9d** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{29}H_{33}BN_4O$: C, 65,92; H, 6,29; N, 10,60. Found: C, 65,68; H, 6,46; N, 10,39.

(1*R*)-2-[4-(3-Amino-phenyl)-[1,2,3]triazol-1-yl]- 1-phenylacetylaminoethaneboronic acid (12e). Compound **12e** was obtained as white solid (100 mg, 92% yield), mp 188-192 °C. $[\alpha]_D$ = –84.8 (*c* 0.36, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 3.26 (1H, dd, *J* = 10.1, 3.7, BC*H*CH2), 4.04 (2H, s, C*H*$_2$CONH), 4.46 (1H, dd, *J* = 14.4, 10.3, BCHC*H*$_2$), 4.57 (1H, dd, *J* = 14.5, 3.9, BCHC*H*$_2$), 7.15-7.35 (5H, m, *H*$_{ar}$), 7.45 (1H, d, *J* = 8.4, H$_{ar}$), 7.65 (1H, t, *J* = 7.9, *H*$_{ar}$), 7.91-7.97 (2H, m, H$_{ar}$), 8.65 (1H, s, C*H*$_{triaz}$). ^{13}C NMR (100 MHz, MeOD): δ 37.8, 47.3 (br, *C*B), 54.2, 121.4 (*C*H$_{triaz}$), 124.2, 124.5, 127.5, 128.8, 129.9, 130.3, 132.2, 132.8, 133.2, 134.1, 146.7, 180.5. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{20}BN_5O_3$ 365.1768; Found 365.1764. EI-MS and elemental analysis results were not obtainable, but exposure of **12e** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **9e** in quantitative yield and satisfactory elemental analysis

results. Anal. Calcd for $C_{28}H_{34}BN_5O_3$: C, 67,34; H, 6,86; N, 14,02. Found: C, 67,13; H, 6,69; N, 13,77.

(1*R*)-2-(4-Carboxy-[1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)ethaneboronic acid (13a). Compound **13a** was obtained as beige solid (95 mg, 98% yield), mp 89-91 °C. $[\alpha]_D$ = –85.0 (*c* 0.9, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 3.25 (1H, dd, *J* = 10.3, 3.8, BC*H*), 4.04 (2H, s, CH_2CO), 4.44 (1H, dd, *J* = 14.5, 10.3, BCHCH_2), 4.54 (1H, dd, *J* = 14.5, 3.8, BCHCH_2), 6.97 (1H, dd, *J* 5.0, 3.6, H_{ar}), 7.01-7.02 (1H, m, H_{ar}), 7.33 (1H, d, *J* = 5.0, H_{ar}), 8.53 (1H, s, CH_{triaz}). ^{13}C NMR (100 MHz, MeOD): δ 32.1, 47.2 (br, *C*B), 53.7, 126.9 (*C*H$_{triazole}$), 128.2, 128.9, 130.2, 134.5 (CH triaz), 141.0, 163.2, 179.2. HRMS (ESI-TOF) m/z: $[M–H]^-$ Calcd for $C_{11}H_{12}BN_4O_5S$ 323.0629; Found 323.0619. EI-MS and elemental analysis results were not obtainable, but exposure of **13a** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **10a** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{21}H_{27}BN_4O_5S$: C, 55,03; H, 5,94; N, 12,22; S, 7,00. Found: C, 54,88; H, 5,80; N, 12,02; S, 6,76.

(1*R*)-2-[4-(1-Hydroxy-1-methylethyl)-[1,2,3]triazol-1-yl]-1-(2-thienylacetylamino)ethaneboronic acid (13b). Compound **13b** was obtained as white solid (101 mg, 100% yield), mp 107-110°C. $[\alpha]_D$ = –68.5 (*c* 0.9, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 1.63 (3H, s, CCH_3), 1.64 (3H, s, CCH_3), 3.22 (1H, dd, *J* = 10.2, 3.9, BC*H*CH$_2$), 4.02 (2H, s, CH_2CONH), 4.43 (1H, dd, *J* = 14.4, 10.2, BCHCH_2), 4.55 (1H, dd, *J* = 14.4, 3.9), 6.98-7.04 (2H, m, H_{ar}), 7.36 (1H, dd, *J* = 5.1, 1.1, H_{ar}), 8.54 (1H, s, H_{triaz}). ^{13}C NMR (100 MHz, MeOD): δ 24.98, 25.03, 30.9, 44.7 (br, *C*B), 55.3, 71.9, 124.9 (*C*H$_{triaz}$), 125.6, 126.8, 127.6, 133.1, 144.6, 178.0. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{20}BN_4O_4S$ 339.1295; Found 339.1298. EI-MS and elemental analysis results were not obtainable, but exposure of **13b** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **10b** in quantitative yield and satisfactory elemental analysis

results. Anal. Calcd for $C_{23}H_{33}BN_4O_4S$: C, 58,48; H, 7,04; N, 11,86; S, 6,79. Found: C, 58,77; H, 7,28; N, 11,81; S, 6,56.

(1*R*)-2-(4-Phenyl-[1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)ethaneboronic acid (13c). Compound **13c** was obtained as white solid (91 mg, 85% yield), mp 159-163 °C dec. $[\alpha]_D$ = –96.5 (*c* 1.1; MeOH). ^{1}H NMR (400 MHz, MeOD): δ 3.26 (1H, d, *J* = 8.5, BC*H*CH$_2$), 4.02 (2H, s, C*H*$_2$CONH), 4.33-4.65 (2H, m, BCHC*H*$_2$), 6.96-7.03 (2H, m, *H*$_{ar}$), 7.33 (1H, d, *J* = 5.0, *H*$_{ar}$), 7.38 (1H, d, *J* = 7.2, *H*$_{ar}$), 7.45 (1H, t, *J* = 7.4, *H*$_{ar}$), 7.82 (1H, s, *H*$_{ar}$), 7.84 (1H, s, *H*$_{ar}$), 8.40 (1H, s, C*H*$_{triaz}$). ^{13}C NMR (100 MHz, MeOD): δ 30.7, 45.7 (br, *C*B), 52.5, 121.6 (*C*H$_{triaz}$), 125.38, 125.45, 126.7, 127.4, 128.1, 128.6, 130.0, 133.2, 147.2, 177.7. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{18}BN_4O_3S$ 357.1190; Found 357.1197. EI-MS and elemental analysis results were not obtainable, but exposure of **13c** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **10c** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{26}H_{31}BN_4O_3S$: C, 63,67; H, 6,37; N, 11,42; S, 6,54. Found: C, 63,62; H, 6,40; N, 11,13; S, 6,23.

(1*R*)-2-[4-(3-Carboxyphenyl)-[1,2,3]triazol-1-yl]-1-(2-thienylacetylamino)ethaneboronic acid (13d). Compound **13d** was obtained as white solid (112 mg, 93% yield), mp 175-180 °C dec. $[\alpha]_D$ = –71.4 (*c* 0.94, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 3.27 (1H, dd, *J* = 10.5, 3.9, BC*H*CH$_2$), 4.03 (2H, s, C*H*$_2$CONH), 4.48 (1H, dd, *J* = 14.6, 10.5, BCHC*H*$_2$), 4.62 (1H, dd, *J* = 14.6, 3.9, BCHC*H*$_2$), 6.97 (1H, dd, *J* = 5.1, 3.6, *H*$_{ar}$), 7.03 (1H, d, *J* = 3,6, *H*$_{ar}$), 7.33 (1H, d, *J* = 5.1, *H*$_{ar}$), 7.62 (1H, t, *J* = 7.8, *H*$_{ar}$), 8.04-8.09 (2H, m, *H*$_{ar}$), 8.50 (1H, d, *J* = 8.2, *H*$_{ar}$), 8.69 (1H, s, C*H*$_{triaz}$). ^{13}C NMR (100 MHz, MeOD): δ 30.8, 45.3 (br, *C*B), 53.5, 123.3 (*C*H$_{triaz}$), 125.5, 126.79, 126.83, 127.5, 128.5, 129.2, 129.9, 130.0, 131.7, 133.1, 145.1, 167.5, 177.9. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{18}BN_4O_5S$ 401.1089; Found 401.1103. EI-MS and elemental analysis results were

not obtainable, but exposure of **13d** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **10d** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{27}H_{31}BN_4O_5S$: C, 60,68; H, 5,85; N, 10,48; S, 6,00. Found: C, 60,42; H, 5,69; N, 10,31; S, 6,25.

(1*R*)-2-[4-(3-Amino-phenyl)-[1,2,3]triazol-1-yl]-1-(2-thienylacetylamino)ethaneboronic acid (13e). Compound **13e** was obtained as white solid (77 mg, 98% yield), mp 215-217 °C. $[\alpha]_D$ = –77.3 (*c* 0.73, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 3.25 (1H, dd, *J* = 10.0, 4.2, BC*H*CH2), 4.03 (2H, s, CH_2CONH), 4.46 (1H, dd, *J* = 14.5, 10.0, BCHCH_2), 4.56 (1H, dd, *J* = 14.5, 4.2, BCHCH_2), 6.99 (1H, dd, *J* = 5.1, 3.5, H_{ar}), 7.038-7.045 (1H, m, H_{ar}), 7.35 (1H, dd, *J* = 5.1, 1.2, H_{ar}), 7.39-7.42 (1H, m, H_{ar}), 7.66 (1H, t, *J* = 8.2, H_{ar}), 7.92-7.94 (2H, m, H_{ar}), 8.49 (1H, s, CH_{triaz}). ^{13}C NMR (100 MHz, MeOD): δ 30.6, 44.5 (br, *C*B), 52.1, 113.5, 116.5, 117.3, 121.3 (*C*H_{triaz}), 125.4, 126.7, 127.4, 129.5, 131.4, 133.2, 144.8, 147.5, 177.5. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{19}BN_5O_3S$ 372.1299; Found 372.1284. EI-MS and elemental analysis results were not obtainable, but exposure of **13e** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **10e** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{26}H_{32}BN_5O_3S$: C, 61,78; H, 6,38; N, 13,86; S, 6,34. Found: C, 61,45; H, 6,59; N, 14,07; S, 6,11.

(1R)-2-([1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)ethaneboronic acid (13f). Compound **13f** was obtained as white solid (37 mg, 100% yield), mp 148-150°C. $[\alpha]_D$ = –78.8 (*c* 1.3, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 3.29 (1H, dd, *J* = 10.0, 4.1, BC*H*CH2), 4.05 (2H, s, CH_2CONH), 4.62 (1H, dd, *J* = 14.4, 10.0, BCHCH_2), 4.75 (1H, dd, *J* = 14.4, 4.1, BCHCH_2), 7.01 (1H, dd, *J* = 5.1, 4.3, H_{ar}), 7.06 (1H, d, *J* = 3.3., H_{ar}), 7.38 (1H dd, *J* = 5.1, 1.1, H_{ar}), 8.55 (1H, s, C$H_{triazole}$), 8.67 (1H, s, C$H_{triazole}$). ^{13}C NMR (100 MHz, MeOD): δ 30.9, 44.7 (br, *C*B), 55.0, 125.5, 126.8, 127.6, 129.0 (*C*H_{triaz}), 129.3 (*C*H_{triaz}), 133.1, 178.0. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{10}H_{14}BN_4O_3S$

281.0876; Found 281.0888. EI-MS and elemental analysis results were not obtainable, but exposure of **13f** to an equimolar amount of (+)-pinanediol in anhydrous THF afforded compound **10f** in quantitative yield and satisfactory elemental analysis results. Anal. Calcd for $C_{20}H_{27}BN_4O_3S$: C, 57,98; H, 6,57; N, 13,52; S, 7,74. Found: C, 58,14; H, 6,81; N, 13,28; S, 7,51.

Molecular modeling simulation and inhibitor docking.

For the PDC-3 enzyme the model used was the crystal structure of *Pseudomonas aeruginosa* PAO1 (PDB: 4HEF). The difference between PAO1 and PDC-3 is the mutation at position 79 (105) from Ala to Thr. Using Build and Edit Protein module of Discovery Studio 4.1 (Accelrys, San Diego, CA) molecular modeling software, the PDC-3 model was created by changing Thr to Ala. For P99 β-lactamase enzyme was used the crystal structure (PDB: 1XX2) from *Enterobacter cloacae*.

The generated PDC-3 model and P99 crystal structure were optimized by energy minimization using DS 4.1 software as previously described[18]. Briefly, the minimization was performed in several steps, using Steepest Descent and Conjugate Gradient algorithms to reach the minimum convergence (0.002 kcal mol^{-1}*Å). The protein was immersed in a water box, 7 Å from any face of the box, and the solvation model used was with periodic boundary conditions. The force-field parameters of CHARMm were used for minimization and the Particle Mesh Ewald method addressed long-range electrostatics. The bonds that involved hydrogen atoms were constrained with the SHAKE algorithm.

The minimized and equilibrated PDC-3 and P99 structures were used for constructing the complexes of the β-lactamases and the **13a** boronic acid inhibitor. The ligand structure was built using DS 4.1 Fragment Builder tools. The molecule was solvated with periodic boundary conditions and minimized using a Standard Dynamics Cascade protocol (one minimization using Steepest Descent algorithm, followed by Adopted Basis Newton-Raphson algorithm and three subsequent dynamics stages at NPT and 300 K).

The minimized ligand was docked in the active site of the enzyme using CDOCKER.[23] The generated conformations (234 poses) were analyzed based on the scoring function and most favorable in terms of energetics and binding pattern were chosen. The complex between the ligand and the enzymes was created, solvated, and energy minimized. The acyl-enzyme complex was created by making a bond with Ser64, and the assembly was further minimized using conjugate gradient algorithm with periodic boundary conditions to 0.001 minimum derivatives. To reach the minimum energetics and to assess the stability of the system in time, the best conformations were further analyzed during 130ps MDS (two separate 10 ps MD simulations (heating/cooling and equilibration) at constant pressure and temperature (300 K) were carried followed by 110ps production. The trajectories were saved every 2ps and analyzed.

Enzymology and Microbiology

Antimicrobial activity.

Antimicrobial susceptibility profiles (MICs) against *E. coli* DH10B expresing bla$_{PDC\text{-}3}$ and *bla*$_{P99}$ (Table 3) were determined using cation-adjusted Muller-Hinton agar dilution according to the Clinical and Laboratory Standard Institute (CLSI) standards.[24] For the cefotaxime/BATSI combination, the cefotaxime concentrations were varied from 0 to 64μg/ml where the boronate inhibitors were maintained at a constant concentration of 4 μg/ml.

Enzymes expression and purification.

P99 *Enterobacter cloacae* and PDC-3 *P. aeruginosa* were expresed and purified as previously described.[13,18]

Briefly, the *P. aeruginosa* 18SH *bla*$_{PDC\text{-}3}$ gene used in these studies was directionally subcloned into pBC SK(-) vector for MIC's and into the pET 24a(-) vector (Novagen, Madison, WI) for protein expression from *E. coli* BL21 (DE3) RP CodonPlus cells (Stratragene, La Jolla,

CA). Preparation of purified P99 was carried out using *E. coli* AS226-51 and the P99 expressing pCS980 vector described by Nukaga, et al.[12]

E. coli AS226-51 containing the pCS980 were grown in superoptimal broth (SOB) containing 50 μg/ml kanamycin. The PDC-3 and P99 β-lactamase expressed in *E. coli* cells were grown at 37^0C and were induced with 0.5mM isopropyl-β-D-thiogalactopyranoside (IPTG: Sigma) when the cells absorbance at 600nm was 0.6. The cell cultures were grew for additional 2-3 hours, were pelleted, resuspended in 50 mM Tris (pH 7.4), and purified in several steps as previously described by preparative isoelectric focusing and fast protein liquid chromotography with a Sephadex Hi Load 16/60 column and a HiTrap strong cation exchanger (Pharmacia, Uppsala, Sweden).[25,26]

The protein concentration was determined using the absorbance of enzyme measured at 280nm with an extinction coefficient of 54320 M-1cm-1 for PDC-3 and 84340 M-1cm-1 for P99 as calculated by ProtParam from Expasy bioinformatics portal.[27]

Steady-state and inhibitor kinetics was performed on an Agilent 8453 diode array spectrophotometer (Palo Alto, CA). Each assay was performed in 10 mM phosphate-buffered saline at pH 7.4 at room temperature in a quartz cuvette with a 1-cm pathlength. Measurements were obtained using nitrocefin (Δε482 =17,400 M-1cm-1). The kinetic parameters, V_{max} and K_m, were obtained with non-linear least squares fit of the data (Henri Michaelis-Menten equation 1) using Origin 7.5VR (OriginLab, Northampton, MA) as previously described.[18]

$$v = \frac{V\max * [S]}{Km + [S]}$$

For this reversible boronic acid inhibitors, K_i values were calculated as previously described by measuring the initial velocity (0–10 s) in the presence of a constant concentration of enzyme (7 and respectively 3 nM) and increasing concentrations of the inhibitors against the fix concentration (100μM) of the indicator substrate, nitrocefin.[28,29,31]

Briefly, K_i's were determined from the initial velocity equation

$$v0 = \frac{V \max[S]}{Km[1 + I / \mathrm{IC}_{50}] + [S]}$$

The K_i values were corected for the nitrocefin activity ($K_m^{PDC\text{-}3}$=14.56 ± 2.2 μM and K_m^{P99}=20.03 ± 2.05 μM) using equation:

$$Ki = \frac{IC_{50}}{(1 + [S] / Km)}$$

The IC_{50} is the concentration of inhibitor that reduce the velocity by 50% and was determined as previosly described.[18] Because of the time-dependent inhibition observed previously with some of the chiral boronates, all BAI were preincubated with enzyme for 5 min in phosphate-buffered saline before initiating the reaction with the addition of substrate.[6,18,31]

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ABBREVIATIONS. BATSIs, Boronic Acids Transition State Inhibitors; CuAAC, Copper-Catalyzed Azide-Alkyne Cycloaddition; BLIs, β-Lactamase Inhibitors; MICs, Minimum Inhibitory Concentration; HSQC, Heteronuclear Single Quantum Coherence spectroscopy; HMBC, Heteronuclear Multiple Bond Correlation; NCF, nitrocefin; TAX, Cefotaxime; THF, tetrahydrofuran; LC/MS, Liquid Chromatography/Mass Spectrometry; DMSO, dimethylsulfoxyde; ESI, Electrospray Ionization; MeOH, methanol.

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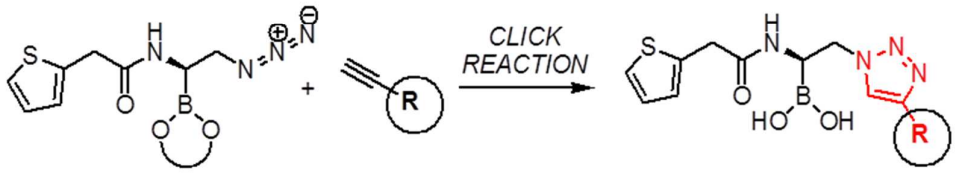

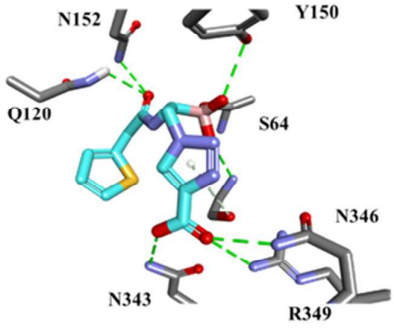

1-amido-2-triazolylethaneboronic acids

✓**Potent β-lactamase inihibitors**

✓**Easy synthetic accessibility (CuAAC)**

✓**High *in vivo* synergistic effect with β-lactams**