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NOVEL BORONIC ACID TRANSITION STATE INHIBITOR (BATSI) ANALOGS WITH *IN VITRO* INHIBITORY ACTIVITY AGAINST CLASS A, B AND C β-LACTAMASES

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ABSTRACT (modified)

BACKGROUND: Catalytic mechanisms of serine β-lactamases (SBL; classes A, C and D) and metallo-β-lactamases (MBLs) have directed divergent strategies towards inhibitor design. SBL inhibitors act as high affinity substrates that -as in BATSIs- form a reversible, dative covalent bond with the conserved active site Ser. MBL inhibitors bind the active-site Zn^{2+} ions and displace the nucleophilic OH^-. Herein, we explore the efficacy of a series of BATSI compounds at inhibiting both SBL and MBL.

METHODS: Exploratory compounds were synthesized using stereoselective homologation of (+) pinandiol boronates to introduce the amino group on the boron-bearing carbon atom, which was subsequently acylated with mercaptopropanoic acid. Representative SBL (KPC-2, ADC-7, PDC-3 and OXA-23) and MBL (IMP-1, NDM-1 and VIM-2) were purified and used for the kinetic characterization of the BATSIs. *In vitro* activity was evaluated by a modified time-kill curve assay, using SBL and MBL-producing strains.

RESULTS: Kinetic assays revealed that IC_{50} values ranged from 1.3 μM to >100 μM for this series. The best compound, s08033, which have a free-thiol group, demonstrated inhibitory activity against KPC-2, VIM-2, ADC-7 and PDC-3, with IC50 in the low μM range. Reduction of at least 1.5 log_{10}-fold of viable cell counts upon exposure to sub-lethal concentrations of antibiotics (AB) + s08033, compared to the cells exposed to AB alone, demonstrated the microbiological activity of this novel compound against SBL- and MBL-producing *E. coli*.

CONCLUSION: Addition of a free-thiol group to the BATSI scaffold increases the range of these compounds resulting in a broad-spectrum inhibitor toward clinically important carbapenemases and cephalosporinases.

BACKGROUND

- The effectiveness of β-lactams is continually threatened by the emergence and dissemination of β-lactamases, the most common cause of β-lactam resistance in Gram-negative bacteria [1]. Based on amino acid sequence homology, these enzymes are classified into four classes A, B, C and D [2]
- Fundamental differences in the catalytic mechanism between serine β-lactamases (SBL; classes A, C and D) and MBLs (class B) has directed divergent strategies towards inhibitor design (**Fig 1;** [1])

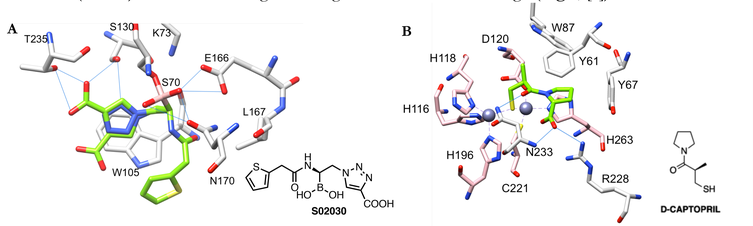


Fig. 1. Interactions of mechanism-based inhibitors with SBL and MBLs. A. SBLs inhibitors like BATSIs are intended to form a reversible, dative covalent bond with the catalytic Ser70. KPC-2 in complex with S02030 (PDB: 5EEC; [3]). Only residues interacting with the inhibitor are shown in light gray; Ser70 is shown in pink covalently bonded to the boronate of the inhibitor, displayed in green. **B.** MBL inhibitors can be designed to bridge the Zn^{2+} ions and displace the nucleophilic hydroxide. VIM-2 in complex with D-captopril (PDB: 4C1E). Only residues interacting with the inhibitor are shown in light gray; Zn-binding residues are in pink; inhibitor is displayed in green; atoms are represented by gray spheres; H-bonds are shown as blue lines.

We hypothesized that combining both features (high affinity dative covalent bond and nucleophile displacement of OH by Zn binding chemical groups) would result in broad class inhibition.

METHODS

Synthesis
- Stereoselective homologation of (+) pinandiol boronates with subsequent acylation with mercaptopropanoic acid
- A Cu-catalyzed azide-alkyne cycloaddition was performed on the suitable β-azido-boronate [4]

Steady state kinetics
- IC_{50} values were calculated using the indicator substrate nitrocefin
- 5 minutes of pre-incubation

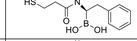
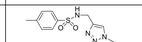
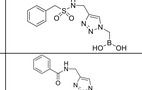
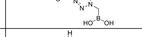
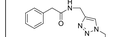
Antimicrobial activity
- Modified time-kill curves assay performed using ≈10^6 CFU/ml *E. coli* DH10B cells grown at 37°C at a sub-lethal concentrations of antibiotic supplemented with 100 μg/ml s08033
- Viable cells after 5 hrs of incubation were determined serial dilutions on Muller Hinton Agar

Molecular modeling
- Molecular simulation and docking (MD) were performed using CDOKER module of Discovery Studio

RESULTS

- As a proof of principle, we designed an achiral compound with a thiol group at the R1 and a R2 phenyl group and a series of sulfonyl-triazoles, that where inspired on a previous publication [5]
- As shown in Table 1, only the achiral compound **s08033** demonstrated broad spectrum activity, inhibiting class A (KPC-2), class B MBLs (IMP-1, VIM-2, and VIM-24), and class C (ADC-7 and PDC-3)
- None of the tested compounds inhibited OXA-23

Table 1. Kinetic constants of inhibition (**IC50's)** of various β-lactamases by BATSI's [a]

Compound	Structure	IC_{50} (μM)						
		IMP-1 [a]	NDM-1 [a]	VIM-2 [a]	VIM-24 [a]	KPC-2 [b]	ADC-7 [b]	PDC-3 [b]
s08033		32 ± 3	>100	2.8 ± 0.3	30 ± 4	16.5 ± 1.7	13.4 ± 1.7	4.4 ± 0.4
s08024		>100	>100	>100	>100	1.3 ± 0.1	24 ± 3	9 ± 1
s10046		>100	>100	51 ± 5	>100	4.2 ± 0.8	14.3 ± 1.4	4.5 ± 0.5
s10151		>100	>100	55 ± 5	>100	10.8 ± 1.5	23 ± 3	26 ± 3
s10080		>100	>100	92 ± 9	>100	12 ± 1	39 ± 4	23 ± 3
s10092		>100	>100	39 ± 4	>100	5.2 ± 0.2	14 ± 2	10 ± 1

[a] Reaction performed in 10mM HEPES pH 7.5, 200mM NaCl, 50uM Zn_2SO_4, and 50ug/ml BSA.
[b] 10mM phosphate-buffered saline, pH 7.4
[c] 50mM Na Phosphate buffer, pH 7.2 (supplemented with 20mM Na bicarbonate)
Values reported are the averages from triplicate experiments

- Results of three biological replicates confirmed that s08033 is able to inhibit β-lactamases within the bacterial cells, as demonstrated by a reduction of at least 1.5 log 10-fold of viable cell counts after 5 hours of incubation. s08033 does not have antibacterial effect of its own (**Figure 2**)

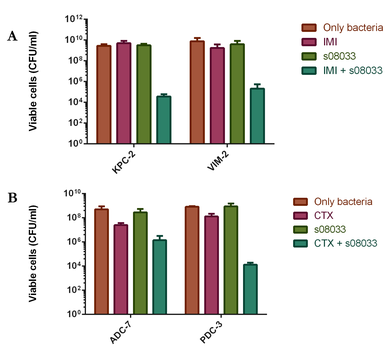


Figure 2. s08033 restores the susceptibility of *E. coli* DH10B cells expressing KPC-2, VIM-2, ADC-7 and PDC-3 to different antibiotics. Modified time-kill curve assays were performed by growing bacterial suspension of ≈10^6 CFU/ml at 37°C in Mueller Hinton broth supplemented with sub-lethal concentrations of antibiotic (imipenem at 2 μg/ml and 4 μg/ml for KPC-2 and VIM-2; and cefotaxime at 16 μg/ml and 2 μg/ml for ADC-7 and PDC-3, respectively) plus 100 μg/ml s08033. Samples were removed after 5 hours of treatment. The number of viable cells was determined by plating serial dilutions on Muller Hinton Agar. The plates were incubated at 37°C overnight, and the number of colonies counted.
Results shown are the average of three biological replicates.

RESULTS

- Docking of s08033 into the active site of VIM-2, KPC-2 and OXA-23 shed some light on key structural features that could explain the kinetic results (Fig. 3)

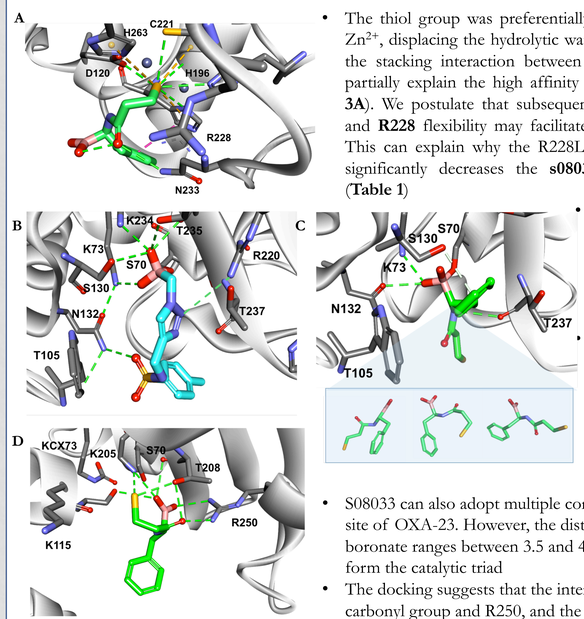


Figure 3. Molecular docking of s08033 into the active site of VIM-2 (A), KPC-2 (C), OXA-23 (D), and of s08024 into the active site of KPC-2 (B).

- The thiol group was preferentially positioned in between the Zn^{2+}, displacing the hydrolytic water. H-bonds with N233 and the stacking interaction between the phenyl ring and W87, partially explain the high affinity of **s08033** for VIM-2 (**Fig. 3A**). We postulate that subsequent rearrangement of **s08033** and **R228** flexibility may facilitate the interaction with R228. This can explain why the R228L substitution in VIM-24 [6] significantly decreases the **s08033** inhibition (higher IC_{50}) (**Table 1**)
- MD revealed that **s08024** works best for KPC-2- due to H-bonds interactions between R220, N132 and the sulpha-triazole groups (**Fig. 3B**).
- **s08033** is more flexible and can adopt multiple conformations into the active site of KPC-2, leading to less interactions than with **s08024** and lower affinity (**Fig. 3C**)
- S08033 can also adopt multiple conformations into the active site of OXA-23. However, the distance between the S70 and boronate ranges between 3.5 and 4.5 Å, and it is not prone to form the catalytic triad
- The docking suggests that the interactions between S08033 carbonyl group and R250, and the thiol group and K205 or K115 backbone, might hold the compound before its boronate can reach the catalytic S70 (**Fig 3D**)

CONCLUSIONS

- Exploratory compounds confirmed that BATSI compounds can be designed to covalently and reversibly link the catalytic serine by the boron atom, while addition of a thiol group interacts with active-site Zn^{2+} ions, yielding to a broad-spectrum inhibition
- Further structural and quantum mechanics/molecular mechanics (QM/MM) studies are warranted to fully explain the behavior of these compounds and to improve their activity against all β-lactamase classes

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