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4,7-Dichloro benzothien-2-yl sulfonylaminomethyl boronic acid: First boronic acid-derived beta-lactamase inhibitor with class A, C, and D activity

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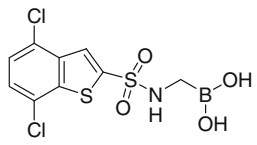
ABSTRACT

4,7-Dichloro-1-benzothien-2-yl sulfonylaminomethyl boronic acid (DSABA, Compound **I**) was discovered as the first boronic acid-based class D beta-lactamase inhibitor. It exhibited an IC_{50} of 5.6 μM against OXA-40. The compound also inhibited class A and C beta-lactamases with sub to low μM IC_{50}, and synergized with imipenem against *Acinetobacter baumannii*.

Drug resistance arising from beta-lactamases has been a leading cause of the fading efficacy of beta-lactam based antibiotics.[1] Beta-lactamases are divided into four classes:[2] classes A, C, D are serine-based hydrolases; class B is zinc-metallo hydrolases. These enzymes catalyze the chemical degradation of beta-lactam antibiotics, rendering them inactive. In particular the recent increase of class D beta-lactamase-expressing bacterium strains such as *Acinetobacter baumannii*[3] has become an emerging multidrug-resistant threat. *A. baumannii* strains express A, C, and D classes of serine-based beta-lactamases. The class D beta-lactamases such as OXA families are particularly effective at destroying newer generation of carbapenem type beta-lactam antibiotics. This has imposed a pressing threat to drugs in that category, which includes imipenem, the active carbapenem component of Merck's Primaxin®. An effective way to restore the efficacy of beta-lactam antibiotics is to co-dose with beta-lactamase inhibitors. However, the currently marketed beta-lactamase inhibitors, such as clavulanates, tazobactam, and sulbactam, are only effective on a subset of class A beta-lactamases.

In an effort of searching for novel class C beta-lactamase inhibitors, we encountered a group of aryl sulfonylaminomethyl derived carboxylic acids such as **1a**–**e**, that show moderate inhibition on AmpC (*Pseudomonas aeruginosa*) (Fig. 1). N-methylated compound **1f** lost activity. To follow up, we proposed to replace the carboxylic acid with a boronic acid on the following rationale. First, boronic acid may pick up polar interaction within the enzyme binding pocket, similar as a carboxylic acid. Moreover, boronic acids provide a unique mechanism to inhibit serine-based hydrolases. The concept of using boronic acids to dock into the catalytic pocket of serine hydrolases and form a transition-state tetrahedral complex with the serine hydroxyl group, thereby shutting down the hydrolytic cycle of the enzyme, has been widely studied in general[4] and specifically on beta-lactamases.[5] Indeed, in our assay phenyl sulfonylaminomethyl boronic acid displayed an improved potency of 29% inhibition against AmpC with 0.8 μM compound. In addition, since for compounds **1a**–**e** greasy group at the aryl moiety seems to be helpful, we synthesized 4,7-dichloro-1-benzothien-2-yl sulfonylaminomethyl boronic acid (DSABA, Compound **I**). DSABA showed a further improved potency of 38% inhibition against AmpC with 0.8 μM compound.



DSABA (**I**)

The synthesis of DSABA (**I**) was straightforward (Scheme 1). Bromochloromethane was treated with *n*-BuLi, followed by quenching with mixed boronic ester to give chloromethyl boronic

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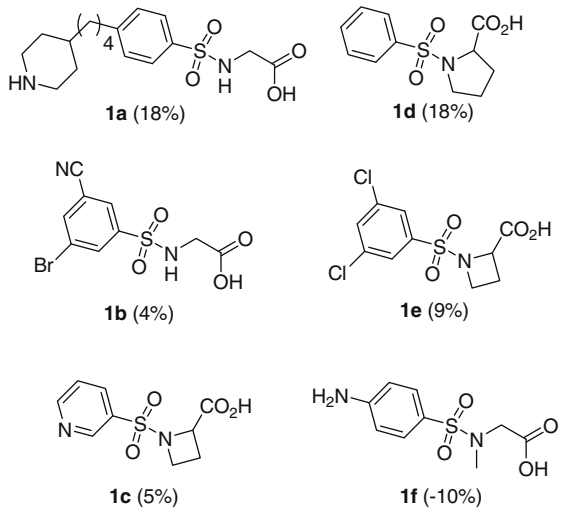
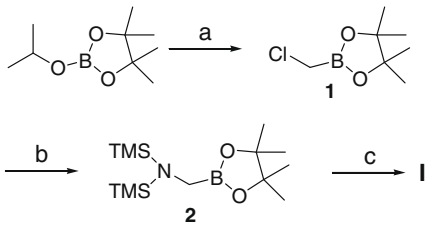


Figure 1. In parentheses are levels of inhibition against AmpC (*Pseudomonas aeruginosa*) in the presence of 2 μM compound.



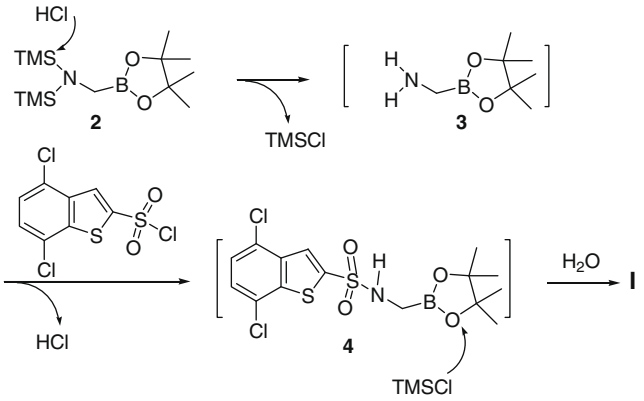
Scheme 1. Synthesis of DSABA (**I**). Reagents and conditions: (a) (i) CH_2BrCl, BuLi, THF, −78 °C; (ii) added 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, then rt 1 h; (b) $LiN(TMS)_2$, THF; (c) (i) 4,7-dichloro-1-benzothiophene-2-sulfonyl chloride, DCM, rt, 3 days; (ii) aqueous workup, RP-HPLC. **1** and **2** were carried on as crude, Overall yield 9.2%.

ester **1**. Displacement of Cl by $LiN(TMS)_2$ gave crude bis-TMS protected amine **2**. The latter was found to be very labile and the TMS group can be easily removed by treatment with MeOH. We further noticed that the resulting TMS-free aminomethyl boronic ester is very unstable. It underwent decomposition at room temperature and its reaction with sulfonyl chloride was quite messy. We suspected the instability may arise from the free nitrogen through its interaction with the boron. On the other hand, we hypothesized that we could take advantage of the instability of TMS group. Therefore we opted to directly combine a crude preparation of **2** with the aryl sulfonyl chloride to afford, after aqueous workup and RP-HPLC purification, the desired final product **I**.[6]

We believe this is the result of a cascade of events. One possible mechanism is proposed in Scheme 2. First there is some amount of HCl present from hydrolysis of sulfonyl chloride. HCl leads to the deprotection of TMS groups from nitrogen with the release of TMSCl. The free amine **3** reacts with 4,7-dichloro-1-benzothiophene-2-sulfonyl chloride to give sulfonamide **4** with regeneration of HCl. TMSCl further facilitates the cleavage of boronic ester bond

Table 1
IC_{50} (μM) of DSABA against different classes (bold letter in parenthesis) of beta-lactamases

TEM-1 (**A**) (*Pseudomonas aeruginosa*)	SHV-5 (**A**) (*Klebsiella pneumoniae*)	AmpC (**C**) (*Pseudomonas aeruginosa*)	P99 (**C**) (*Enterobacte cloacae*)	OXA-40 (**D**) (*Acinetobacter baumannii*)
1.1	0.57	1.2	0.62	5.6



Scheme 2. Proposed mechanism of cascade reactions.

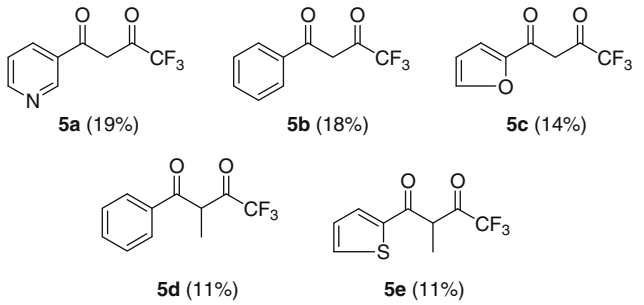


Figure 2. In parentheses are levels of inhibition against AmpC (*Pseudomonas aeruginosa*) in the presence of 2 μM compound.

driven by formation of stronger Si–O bond, and upon aqueous workup gave the desired boronic acid **I**.

As show in Table 1, in full titration, DSABA **I** in fact exhibited low μM IC_{50} against class A, C, and D beta-lactamases. To our knowledge no boronic acid has been reported to exhibit inhibition on class D beta-lactamases. Phenyl sulfonylaminomethyl boronic acid, despite its class C activity (measured IC_{50}: AmpC 1.8 μM; P99 1.9 μM), is not effective against class D OXA-40, as well as class A TEM-1 and SHV-5 (IC_{50} >50 μM).

Now that we established that DSABA is active in enzymatic assays, the next step was to evaluate it in presence of a carbapenem on bacterial strains carrying all class A, C, and D beta-lactamases. Indeed, co-dosing DSABA (100 μM) with imipenem against *A. baumannii* (CL6188) attenuated imipenem MIC from 256 μM by half to 128 μM.

Similar to boronic acid, trifluoromethyl ketone can also form a tetrahedral complex with hydroxyl group to inhibit serine hydrolase.[7] However replacing the boronic acid moiety in compound **I** with a trifluoromethyl ketone led to loss of activity in enzymatic assays on all A, C, and D beta-lactamases. Interestingly, structurally related aryl trifluoromethyl diketones (**5a**–**e**) showed modest activities against AmpC (Fig. 2).

In summary, we have discovered the first boronic acid-based beta-lactamase inhibitor which is able to inhibit A, C, and D classes of beta-lactamses, and reduce MIC of imipenem against *A. baumannii* (CL6188), a bacterium strain expressed with A, C, and D classes of those enzymes. This is a significant result since no boronic acid has been reported to inhibit class D beta-lactamase, while bacteria carrying class D beta-lactamases have emerged as a new threat. Further studies on the scope and SAR of aryl sulfonamide derived boronic acids and the related aryl trifluoromethyl diketones as novel beta-lactamase inhibitors will be reported in due course.

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5. For examples, see: (a) Morandi, S.; Morandi, F.; Caselli, E.; Shoichet, B. K.; Prati, F. *Bioorg. Med. Chem. Lett.* **2008**, *16*, 1195; (b) Shoichet, B. K.; Prati, F. U.S. patent 7271186 B1.; (c) Freire, E.; Ross, P.; Xiao, Y.; Ottenbrite, R.; Luque, I. Int. Patent WO 2005004799 A2.; (d) Beesley, T.; Gascoyne, N.; Knott-Hunziker, V.; Petursson, S.; Waley, S. G.; Jaurin, B.; Grundstroem, T. *Biochem. J* **1983**, *209*, 229; (e) Amicosante, G.; Felici, A.; Segatore, B.; Di Marzio, L.; Franceschini, N.; Di Girolamo, M. *J. Chemother.* **1989**, *1*, 394; (f) Martin, R.; Gold, M.; Jones, J. B. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1229; (g) Shoichet, B. K.; Prati, F. *JACS* **2003**, *125*, 685.
6. ^{1}H NMR (CD_3OD, 500 MHz) for **I**: δ (ppm) 7.90 (s, 1H), 7.37 (s, 2H), 2.69 (s, 2H).
7. For examples, see: Seierstad, M.; Breitenbucher, J. G. *J. Med. Chem.* **2008**, *51*, 7327.