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Thiophenyl oxime-derived phosphonates as nano-molar class C beta-lactamase inhibitors reducing MIC of imipenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

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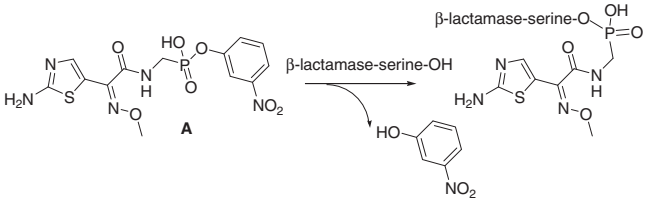
ABSTRACT

The preparation and characterization of a series of thiophenyl oxime phosphonate beta-lactamase inhibitors is described. A number of these analogs were potent and selective inhibitors of class C beta-lactamases from *Pseudomonas aeruginosa* and *Enterobacter cloacae*. Compounds **3b** and **7** reduced the MIC of imipenem against an AmpC expressing strain of imipenem-resistant *P. aeruginosa.* A number of the title compounds retained micromolar potency against the class D OXA-40 beta-lactamase from *Acinetobacter baumannii* and at high concentrations compound **3b** was shown to reduce the MIC of imipenem against a highly imipenem-resistant strain of *A. baumanii* expressing the OXA-40 beta-lactamase. In mice compound **3b** exhibited phamacokinetics similar to imipenem.

Beta-lactamases, enzymes that catalyze the chemical degradation of beta-lactam antibiotics, are a leading cause of the fading efficacy of beta-lactam antibiotics, particularly in problematic Gram-negative organisms.[1] Co-administration of beta-lactam antibiotics with beta-lactamase inhibitors (BLI's) has been a time honored strategy to restore the clinical efficacy. Beta-lactamases have been categorized into four classes:[2] classes A, C and D are serine-based hydrolases; class B comprises zinc–metallo hydrolases. Unfortunately, the currently marketed beta-lactamase inhibitors, such as clavulanate, tazobactam and sulbactam, are only effective on a subset of class A beta-lactamases. Thus there is an important medical need to develop new beta-lactamase inhibitors to combat the growing threat of antibiotic resistance.

Recently phosphonate **A** was reported as a class C beta-lactamase inhibitor. Incubation of compound A with a susceptible beta-lactamase results in an irreversible phosphorylation of the active site serine.[3] While compound A is a valuable proof-of-principle



compound, it possesses a number of liabilities among which are moderate potency, and a problematic nitro-phenol leaving group. Moreover, it has not been reported whether compound A improves the efficacy of beta-lactam antibiotics against resistant bacterial strains.

We selected **1**, a close analog of compound **A**, as an initial target for our studies, and tested it against class C as well as class A and D beta-lactamases. Similar to compound **A**, **1** carries a good phenolic leaving group; but unlike compound **A**, **1** does not have a nitrophenol as a leaving group. As Table 1 shows, **1** is a selective class C beta-lactamase inhibtor, with a low μM IC_{50} for AmpC and P99. Initial SAR by capping the amino group as trichloroacetamide (**2a** & **2b**) did not improve potency.

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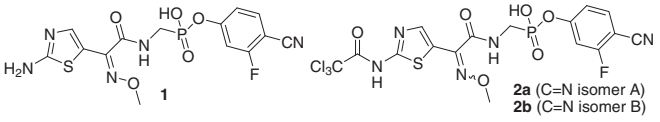
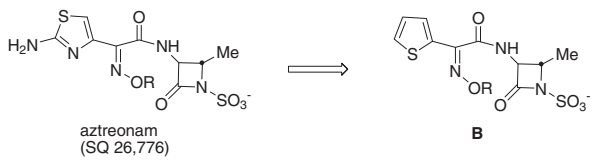
Table 1
IC_{50} (μM) against different classes (bold letter in parenthesis) of beta-lactamases

Compound	TEM-1 (**A**) (*Pseudomonas aeruginosa*)	SHV-5 (**A**) (*Klebsiella pneumoniae*)	AmpC (**C**) (*Pseudomonas aeruginosa*)	P99 (**C**) (*Enterobacte cloacae*)	OXA-40 (**D**) (*Acinetobacter baumannii*)
1	>50	>50	1.5	0.80	>50
2a	>50	25	3.0	0.34	>12.5
2b	>50	15	3.0	0.71	>12.5
3	>50	>50	0.0096	0.039	>50
3a	>50	>12.5	0.046	0.10	26.
3b	28	22	0.0075	0.032	17
4	23	27	0.20	0.10	21
5a	>50	>50	6.5	21	>50
5b	>50	>50	3.2	3.0	>50
6a	17	>50	0.021	0.087	27
6b	>50	>50	0.087	0.17	>50
7	16	-	0.0050	0.019	31



A carries an amino-thiazole oxime moiety common to many 2nd and 3rd generation Cephlosporins (Fig. 1), a design[3] intended to reduce recognition by beta-lactamases. This perhaps explains its moderate potency. Thus to improve potency we replaced the amino-thiazole with a thiophene (**3** ∼ **7**), a design inspired by the following:



Aztreonam, a narrow spectrum monobactam antibiotic, carries a similar amino-thaizole oxime moiety. However, it has been reported that certain thiophene analogs such as compound **B** were significantly better than aztreonam in acting as a beta-lactamase inhibitor and synergizing the antibacterial activity of ceftazidime.[4]

Syntheses of thiophene analogs (**3** ∼ **7**, Fig. 2) are straightforward. The published general chemistry[3] gave access to both oxime isomers **14** (Scheme 1). A slight modification as shown in Scheme 2

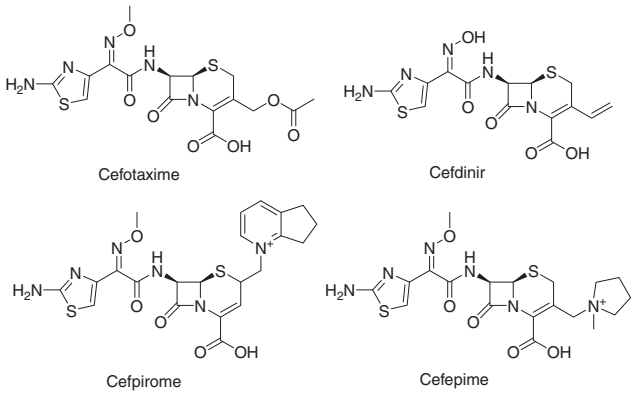


Figure 1. Representative cephalosporins carrying amino-thiazole oxime moiety.

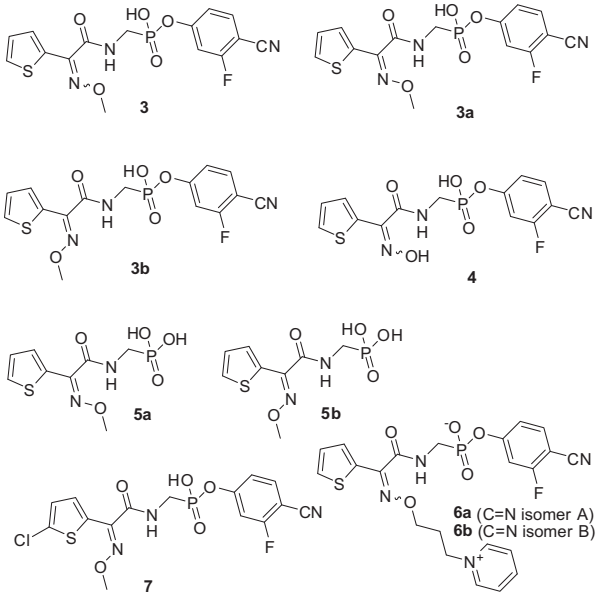


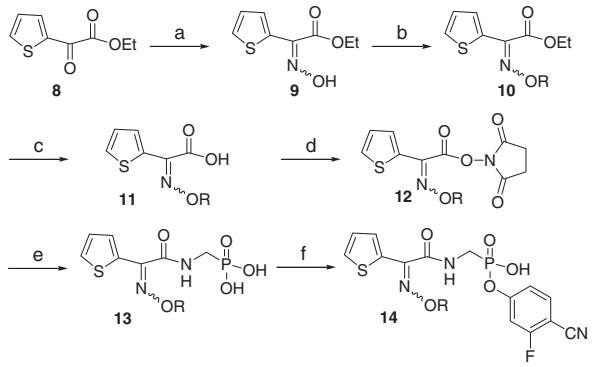
Figure 2. Thiophenyl oxime-derived phosphonate as novel BLI's.

provided isomer **3b** stereoselectively, employing oxime C=N bond isomerization to yield a single isomer **17** during TMSBr-induced ester cleavage of isomer mixture **16**.[5] Conveniently, as shown next, **3b** is the more potent isomer.

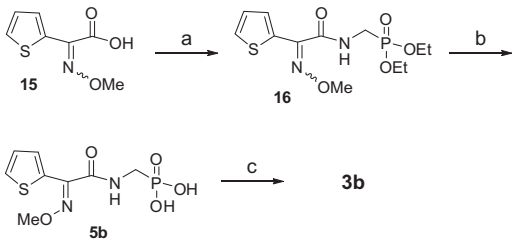
As shown in Table 1 replacement of the amino-thiazole with a thiophene resulted in a significant improvement in potency against the two class C b-lactamases tested. The Z oxime stereoisomer **3b** is more potent than the E isomer, **3a**. The desmethyl analog **4** is slightly less potent, as are **6a** & **6b**, compounds designed to modify the overall molecular charge. Compounds **5a** & **5b** confirm the presence of the phenolic leaving group is necessary for inhibitory activity, consistent with the mechanism of action. The chloro thiophene **7** exhibited potency similar to **3b**.

All of these compounds are much less potent on class A and D enzymes, with double-digit μM or more IC_{50}. Noteworthy is that the best class C compounds, **3b** & **7**, also possesses the best class A and class D activities, suggesting that it might be possible to design an optimal compound with broad spectrum inhibitory activity.

Compounds **3b** and **7** were evaluated for their abilty to synergize the antimicrobial activity of imipenem against an AmpC producing strain of imipenem-resistant *Pseudomonas aeruginosa*. As shown in Table 2 co-administration with up-to-100 μM **3b** or **7**



Scheme 1. Reagents and conditions: (a) hydroxyamine hydrochloride, pyridine, EtOH; gave ca. 1:1 mixture of oxime isomers; (b) cesium carbonate, DMF, RBr or RI; (c) KOH, MeOH, THF, water; (d) EDC, hydroxysuccinimide, dioxane, DMF; (e) (aminomethyl)phosphonic acid, sodium bicarbonate, water, DMF, EtOH, THF; (f) ArOH, trichloroacetonitrile, pyridine, 80 °C.



Scheme 2. Reagents and conditions: (a) HATU, Hunig's base, diethyl (aminomethyl)phosphonate hydrochloride, DMF; (b) TMSBr, DCM; (c) ArOH, trichloroacetonitrile, pyridine, 80 °C.

Table 2
MIC of Imipenem against *Pseudomonas aeruginosa* (CL5701) in the presence of BLI

Imipenem MIC (μg/mL)	With **3b** (μM)	With **7** (μM)
20	0	0
10	25	12.5
5	50	25
2.5	>100	100

reduced the imipenem MIC against *P. aeruginosa* in a dose-dependent fashion. In terms of both dose-response and maximum MIC reduction, **7** is slightly more potent than **3b** in this assay (Table 1). In a control experiment, neither compound **3b** or **7** alone displayed antibiotic activity at maximum dose of 100 μM.

Highly resistant *Acinetobacter baumannii* strains are known to resist the activity of beta-lactam antibiotics by a variety of mechanisms including the high level expression of both class C and class D beta-lactamases. As a proof of principle experiment we evaluated the ability of compounds **3b** and **7** to synergize the activity of imipenem against the highly imipenem-resistant strain of *A. baumannii* (CL6188).[6] *A. baumannii* (CL6188) expresses multiple beta-lactamases of class A, C and D. For this strain, compound **3b** was able to significantly lower the MIC of impenem from 128 to 16 μg/mL. Interestingly, compound **7** was ineffective. No antibiotic activity was observed with either inhibitor alone at 100 μM.

Taking all the data together we decided to take a further look at **3b**. This compound in mice at 2.5 mg/kg i.v. dosing gave the following pharmacokinetics:

Half life 0.54 h; AUC(Norm) 0.98 μM/h/kg/mg; Clearance 43 mL/min/kg; volume of distribution 1 L/kg.

Its half life matches or exceeds the imipenem mouse half life;[7] together with the MIC reduction data, **3b** warrants further study as a potential beta-lactamase inhibitor in combination with imipenem.

In summary, we discovered a series of novel thiophenyl oxime-derived phosphonates as potent and selective class C beta-lactamase inhibitors, with weak class A and class D activities. The most potent compounds displayed suitable in vitro and PK profile suggesting further studies of their potential to enhance the efficacy of beta-lactam antibiotics, in particular imipenem, against resistant bacterial strains. These studies and further SAR exploration will be reported in due course.

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5. ^{1}H NMR (CD_3OD, 500 MHz) δ (ppm): Compound **5b** 7.91 (dd, J = 4.0, 1.1 Hz, 1H), 7.68 (dd, J = 5.1, 1.1 Hz, 1H), 7.11 (dd, J = 5.1, 4.0 Hz, 1H), 4.12 (s, 3H), 3.73 (d, J = 12.6 Hz, 2H). NOE: irradiation of –OMe protons at 4.12 ppm gave NOE signal at 7.91 ppm corresponding to proton at thiophene C-3. Compound **3b** 7.95 (d, J = 3.4 Hz, 1H), 7.70 (d, J = 4.3 Hz, 1H), 7.54 (t, J = 8.2 Hz, 1H), 7.14 (t, J = 4.5 Hz, 1H), 6.74 (dd, J = 8.5, 2.2 Hz, 1H), 6.70 (dd, J = 11.3, 2.2 Hz, 1H), 4.15 (s, 3H), 3.77 (d, J = 12.6 Hz, 2H).
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