Letter

Toward Orally Absorbed Prodrugs of the Antibiotic Aztreonam. Design of Novel Prodrugs of Sulfate Containing Drugs Part 2

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Toward Orally Absorbed Prodrugs of the Antibiotic Aztreonam. Design of Novel Prodrugs of Sulfate Containing Drugs Part 2

Eric M. Gordon,*[,a] Matthew A. J. Duncton,[a] Brian J. Wang,[b] Longwu Qi,[c] Dazhong Fan,[c] Xianfeng Li,[c] Zhi-Jie Ni,[c] Pingyu Ding,[b] Ruslan Grygorash,[b] Eddy Low,[b] Guijun Yu,[b] and Jiawei Sun[b]

[a]Arixa Pharmaceuticals, 525 University Avenue, Suite 1350, Palo Alto, CA 94301

[b]Synterys, Inc., 29540 Kohoutek Way, Union City, CA 94587

[c]Acme Bioscience, Inc., 3941 E. Bayshore Road, Palo Alto, CA 94303

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ABSTRACT: Aztreonam, first discovered in 1980, is an FDA approved, intravenous, monocyclic beta-lactam antibiotic. Aztreonam is active against gram-negative bacteria and is still used today. The oral bioavailability of aztreonam in human is less than 1%. Herein we describe the design and synthesis of potential oral prodrugs of aztreonam.

The inexorable rise of antibiotic resistance has forced clinical reliance onto a small group of drugs which themselves continue to lose efficacy. Oral antibiotics in particular, are urgently needed to safeguard future therapeutic options, including the ability to treat outside the hospital.[1,2] Aztreonam **3** is a totally synthetic antibiotic discovered by workers at E.R. Squibb & Sons in 1980.[3-6] It is the only monocyclic beta-lactam antibiotic approved by the FDA (1986). Aztreonam is scientifically significant chemically in validating the hypothesis that antimicrobial activity in beta-lactams was not rigidly dependent upon having a second ring fused to the monocycle as in penicillins and cephalosporins; and biologically aztreonam was the first of several examples of how a simple monocyclic beta-lactam when suitably equipped with electron with-drawing *N*-substituents and within the contraints imposed by other well documented binding interactions could possess high antibiotic activity.[7-10]

Aztreonam has potent activity against susceptible Gram-negative bacteria including *Pseudomonas aeruginosa*,[11] and although it is nearly 40 years old, aztreonam is still used clinically, and is noteworthy for being resistant to the growing problem of metallo beta-lactamases. However, the drug must be administered intravenously as the human bioavailability is <1%.[12]

Recently we have reported that the beta-lactamase inhibitor (BLI) avibactam **1**[13,14] which has poor oral bioavailability in man, could be converted into derivatives **2** which show high oral bioavailability in four (4) animal species including human.[15-18] With the aim of creating an orally absorbed prodrug of aztreonam we sought to apply the novel prodrug design strategy previously applied to oral avibactam prodrugs.[19] Both avibactam **1** and aztreonam **3** fall into a limited class of approved drugs containing an essential sulfate grouping. However, the chemistry of aztreonam and avibactam differ in that avibactam **1** has an *O*-sulfate group, while aztreonam **3** has an *N*-sulfate group.

Figure 1. Comparison of aztreonam and avibactam and respective prodrugs

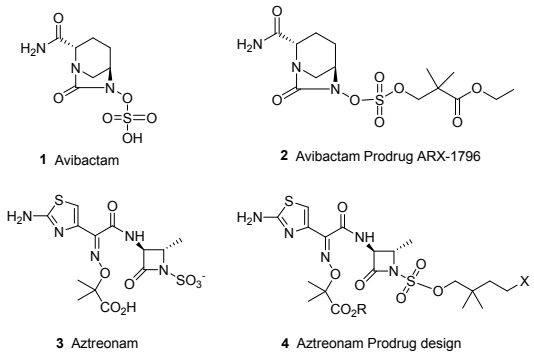
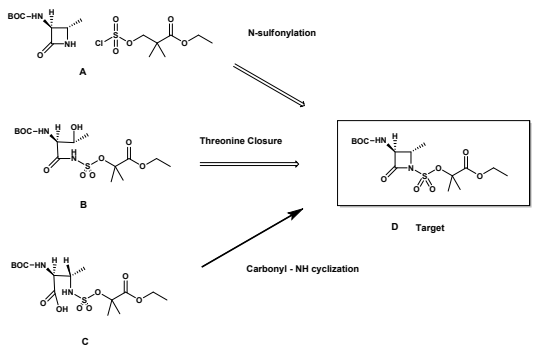


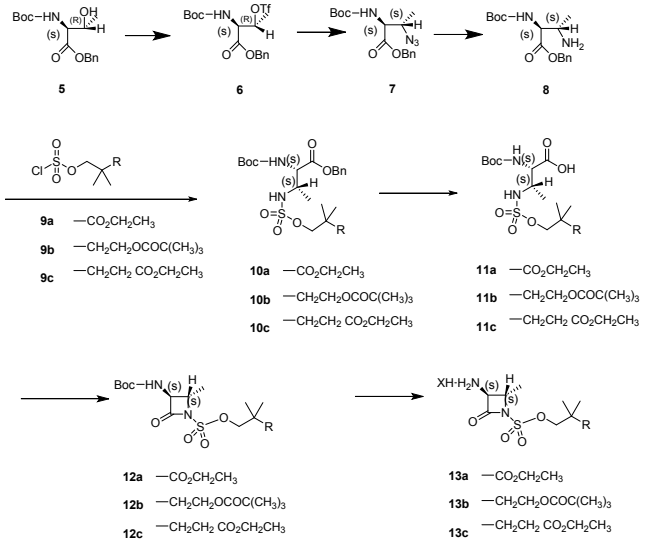
Figure 2 shows three potential routes to key intermediate D. Though various beta-lactam nitrogens have been sulfenylated and tosylated, considerable efforts to sulfamylate beta-lactam nitrogen (A) were unsuccessful.[20-23] The closure of the ring *via* an activated theonine hydroxyl (B), which is the method that aztreonam is actually synthesized, led mostly to beta-elimination to the corresponding dehydro-threonine.[24,25]

Figure 2. Potential routes to key intermediate D.



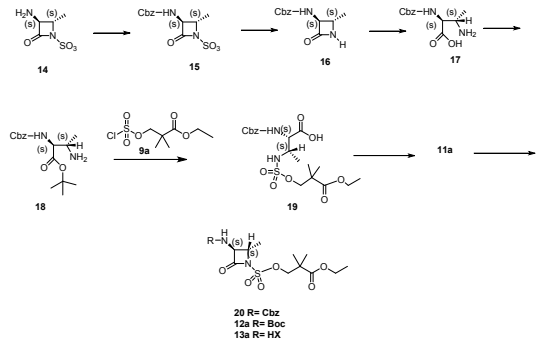
The following describes a successful target (D) synthesis (Figure 3). Starting with Boc-*O*-benzyl threonine **5**, treatment with triflic anhydride (to give **6**) followed by reaction with tetrabutylammonium azide gave **7**.[26] Trimethylphosphine reduction of the azide produced amino ester **8** in 77 % yield. *N*-sulfonylation with chlorosulfonate **9** to give **10** proved to be a difficult step and even after considerable optimization using organic solvents and bases only proceeded to sulfamate **10** in 33% yield. Subsequently, use of Schotten-Bauman conditions greatly improved the reaction to >70% yield. Quantitative hydrogenolysis of **10** afforded the sulfamate acid **11**, which smoothly underwent the critical cyclization with TCFH[27] (80-89%) to beta-lactam **12**, the key intermediate in the overall synthesis. As expected with an activated beta-lactam, **12a** displayed a strong 1813 cm^{-1} peak in the infrared spectrum. Beta-lactams **12a-c** were deprotected in high yield to give **13a-c**, which are one of the two acylation precursors needed to provide the desired target prodrugs **4**.

Figure 3. Synthesis of key intermediate **13**.



An alternative synthetic route to an analogous Cbz-protected version of **12** proceeded from the commercially available amino *N*-sulfate **14** (Figure 4). Following formation of the *N*-Cbz beta-lactam **15**, desulfation with TFA afforded **16**. Treatment of **16** with aqueous formic acid afforded crystalline amino acid **17**. It was necessary to protect the acid of **17** as *tert*-butyl ester to obtain good yields in the following *N*-sulfamation, which proceeds after deprotection to give acid **19**, **(**the Cbz analog of **11a**). Cyclization of **19** to **20** was carried out with TFCH as in Figure 3. Both synthetic routes to **13** were approximately equivalent in yield, and effort required, although the route in Figure 4 could be undertaken on a larger scale, since the starting material could be purchased on >100g amounts.

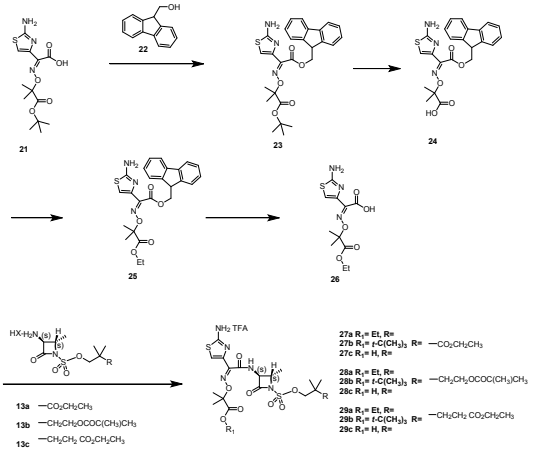
Figure 4. Synthesis of key intermediate **20** and **13**.



Though *N*-activated as beta-lactam sulfamates, both protected intermediates **12a-c** and **20** proved to be stable, well behaved compounds. Deprotection of **12a** (MsOH) led to a purer product **13a** than did hydrogenolysis of **20** (H_2/Pd/MeOH) and was generally used in the balance of the syntheses.[28] In order to prepare the target prodrug double esters, commercially available sidechain *tert*-butyl ester **21** was esterified on the free carboxyl with (9*H*-fluoren-9-yl)methanol to give **22**. The diester was treated with TFA and from the resulting neopentyl acid **24** the ethyl ester was prepared, and deprotected to afford **26** (Figure 5).

With the key components in hand (**21**, **26**, **13a-c**), efforts to convert these into the target compounds were undertaken.[29] To maintain stability of the final products, the coupled materials were kept as the TFA salts, as we observed the heteroaromatic amine is nucleophilic enough to attack the activated beta-lactam. Coupling of amines **13a-c** (EDCI) with the individual ester sidechains produced the final products **27-29.** The *tert*-butyl esters **27b**, **28b**, **29b** were deprotected with TFA to give free acid as TFA salts **27c**, **28c**, **29c** in 65-71% yields over three steps (Boc-deprotection, amide coupling and *tert*-butyl ester deprotection).

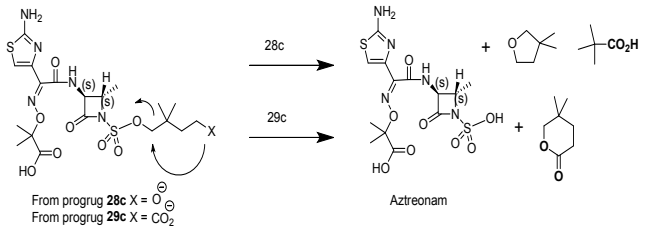
Figure 5. Preparation of sidechain esters and acylation



When the fully elaborated potential prodrugs **28c** and **29c** were treated with carboxyesterase 1 (CES1)[30,31] both rapidly and cleanly expelled aztreonam in high yields, with compound **28c** releasing aztreonam within 2 min when incubated with CES1 and compound **29c** requiring *ca*. 10-20 min for maximal release of aztreonam when incubated with CES1 (Table 1). Hence, a mechanism as depicted in Figure 6 is proposed for aztreonam release with both pro-moieties. In the case of **28c** the product of release besides aztreonam were 3,3-dimethyl tetrahydrofuran (identical to an authentic sample). Esterase cleavage of **29c** yielded aztreonam and 5,5-dimethyltetrahydro-2*H*-pyran-2-one / 5-hydroxy-4,4-dimethylpentanoic (identical to an authentic sample).[32]

Table 1.

Figure 6. Mechanism of prodrug release with CES1.



The rise in resistance to the antibiotics which for years were the standards of care, has by now compromised them severely. In recent years there have been no new safe and effective FDA-approved oral antibiotics with broad coverage for serious Gram-negative infections. Patients who in past years could have been treated with oral antibiotics now have to remain in the hospital and be treated with intravenous antibiotics. Aztreonam, a still effective Gram-negative antibiotic, has been used for 40 years, but its oral bioavailability of ~1% in human has restricted its use to hospital settings. Availability of an oral version of aztreonam could help fill an important medical need and also reduce the cost of treatment. The potential prodrugs of aztreonam reported herein are being evaluated for their oral bioavailability in animals. Results of these studies will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

Synthesis of intermediates and final prodrugs. Release of aztreonam from compounds **28c** and **29c** using CES1. ^{1}H-NMR studies detailing the release of 3,3-dimethyltetrahydrofuran from compound **28c** when treated with CES1, and release of 5,5-dimethyltetrahydro-2*H*-pyran-2-one / 5-hydroxy-4,4-dimethylpentanoic acid from compound **29c** when treated with CES1.

The Supporting Information is available free of charge on the ACS Publications website.

Supporting information (PDF).

AUTHOR INFORMATION

Corresponding Author

*Eric M. Gordon Arixa Pharmaceuticals, 525 University Avenue, Suite 1350, Palo Alto, CA 94301; emgordon@gmail.com; (650)-465-5783.

Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally. (match statement to author names with a symbol)

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ABBREVIATIONS

BQL, below limits of quantification; CES1, carboxyesterase 1; DCM, dichloromethane; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EtOAc, ethyl acetate; MsOH, methanesulfonic acid; TFA, trifluoroacetic acid; TCFH, *N*,*N*,*N*',*N*'-tetramethylchloroformamidinium hexafluorophosphate; rt, room temperature.

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[28] Intermediate **19** could also be converted in to **11a**. See Supporting Information.
[29] Our choice of target prodrug for aztreonam was influenced by our prior experience in designing oral prodrugs of avibactam. See reference 9 for details.
[30] See Supporting Information for experimental conditions.
[31] Fukami, T.; Yokoi, T. The Emerging Role of Human Esterases. *Drug Metab. Pharmacol.* **2012**, *27*, 466-477.
[32] See Supporting Information for experimental conditions. Several reactions are in play in Table 1. When CES1 esterase is in high concentrations, prodrugs are rapidly and cleanly converted to aztreonam. Less CES1 esterase under these conditions leads to a mixture of aztreonam and beta-lactam ring opened prodrug, which is formed by a non-enzymatic, time dependent hydrolysis reaction. In the control experiment, (no CES1 esterase), the products are ring opened beta-lactam and aztreonam. This result indicates that there is a relatively slow, non-enzymatic sulfate hydrolysis which occurs on the prodrug under these reaction conditions.

Table 1. Release of Aztreonam from Prodrugs using CES1.

Prodrug	Timepoint after treatment with CES1[a,b]						
	0 min	1 min	2 min	5 min	10 min	20 min	30 min
27c	BQL	BQL	4	8	14	23	28
28c	BQL	80	>95	>95	>95	>95	>95
29c	BQL	24	45	76	86	90	90

a) 0.5 mg of prodrug / mL of 2.5% acetonitrile in 0.05 M citrate buffer at pH 4.7 incubated at 37 °C with 150 Units / mL of CES1 enzyme. b) Release of aztreonam as monitored by HPLC reported at each timepoint.

TOC Graphic