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VIP Cephem-Pyrazinoic Acid Conjugates: Circumventing Resistance in *Mycobacterium tuberculosis*

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Abstract: Tuberculosis (TB) is a leading source of infectious disease mortality globally. Antibiotic-resistant strains comprise an estimated 10% of new TB cases and present an urgent need for novel therapeutics. β-lactam antibiotics have traditionally been ineffective against *M. tuberculosis* (Mtb), the causative agent of TB, due to the organism's inherent expression of β-lactamases that destroy the electrophilic β-lactam warhead. We have developed novel β-lactam conjugates, which exploit this inherent β-lactamase activity to achieve selective release of pyrazinoic acid (POA), the active form of a first-line TB drug. These conjugates are selectively active against *M. tuberculosis* and related mycobacteria, and activity is retained or even potentiated in multiple resistant strains and models. Preliminary mechanistic investigations suggest that both the POA "warhead" as well as the β-lactam "promoiety" contribute to the observed activity, demonstrating a codrug strategy with important implications for future TB therapy.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis (Mtb)*, has killed a staggering 30 million people in the 21st century and infects more than one billion people worldwide.[1] The dual HIV and diabetes pandemics have amplified the impact of this major global health threat, as HIV co-infection and co-morbidity with diabetes greatly accelerates progression to active TB disease. While the World Health Organization (www.who.int) has set an objective of eliminating TB as a global health problem by 2050, achieving this goal will require innovative treatment strategies that address the growing problem of drug resistance and other limitations of current treatment regimens. Pyrazinamide (PZA) is a first-line antitubercular used under WHO standard treatment guidelines for the first two months of TB therapy. Because of its tolerability and unique sterilizing effect, PZA is arguably the most important therapeutic among the current first-line agents, and also the most likely to be used in future treatment regimens (as demonstrated by its synergy with novel antituberculars such as bedaquiline and pretomanid).[2] PZA requires activation within *Mtb* via pyrazinamidase, an enzyme within the NAD^+ salvage pathway encoded by *pncA*, to the carboxylic acid form pyrazinoic acid (POA). Given its importance to current and future TB therapy, resistance to PZA is an emerging cause for concern. PZA resistance appears to be on the rise clinically, including within a large proportion of multidrug-resistant (MDR-TB) patients.[3] Among these cases, the most commonly observed resistance mechanism is point mutations in *pncA* and its promoter region, which prevent activation of PZA (Scheme 1A). A number of options have previously been explored to overcome these resistance mechanisms, including direct treatment with POA or alternative POA prodrugs; however, no treatment has yet been successful in vivo, in part due to lack of a TB-selective delivery mechanism that results in rapid clearance of POA and simple POA prodrugs.[4]

In pursuit of this goal, we envisioned using a β-lactam as a delivery vehicle for the POA "warhead", taking advantage of the natively expressed BlaC, a Class A β-lactamase chromosomally encoded within the *Mtb* genome. Cleavage of the β-lactam bond liberates the lone pair on the nitrogen, which can then donate into a leaving group at the C-3′ position, triggering elimination of the POA moiety (Scheme 1B); this strategy has

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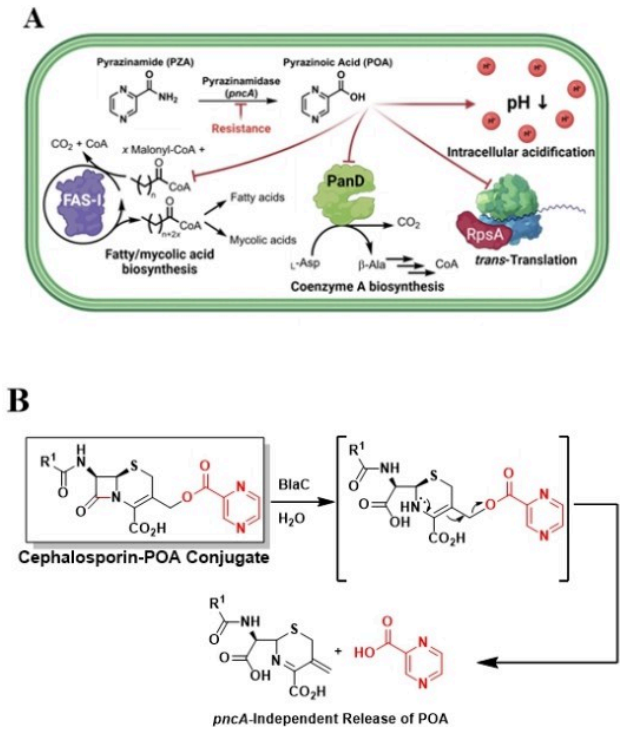


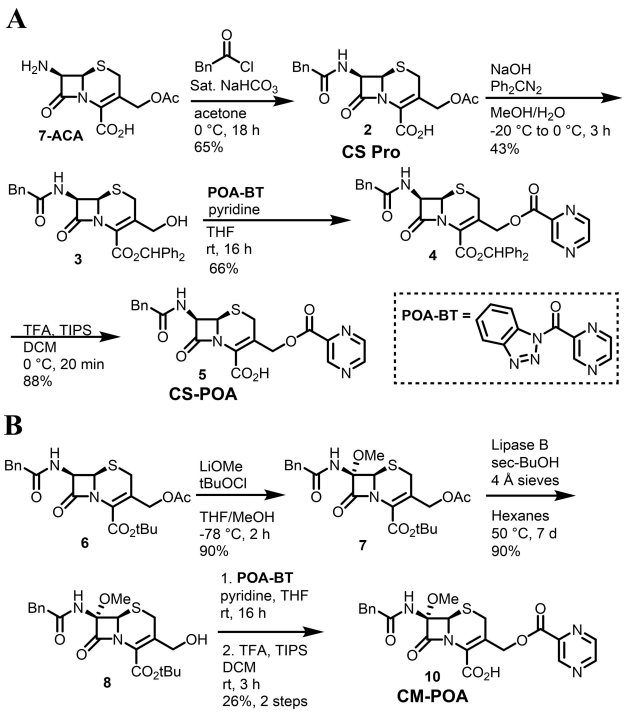
Scheme 1. A. PZA activation and proposed mechanisms of POA activity (as reviewed by Baughn et al., 2020).[7] **B**. *pncA*-independent release strategy employing a β-lactamase-labile cephalosporin promoiety.

substantial precedent, but has only recently been employed for the development of antituberculars.[5] This prodrug approach allows for *pncA*-independent POA delivery and circumvents the most common resistance mechanism to this important TB drug. In addition, the inherent β-lactam activity of the cephalosporin scaffold should render resistance development difficult, since mechanisms that reduce POA release, such as mutations that attenuate BlaC expression, will confer increased β-lactam susceptibility. Moreover, *pncA*-independent resistance mechanisms, such as mutations to PanD, should retain or even potentiate the β-lactam activity.[6] In this report, we describe our efforts towards the development of cephem-POA conjugates as novel antituberculars.

Results and Discussion

The synthesis of the first-generation POA conjugate began from the bulk chemical 7-aminocephalosporanic acid (7-ACA), that was elaborated to **3**[8] by *N*-acylation and concomitant protection of the carboxylic acid as a diphenylmethyl ester and hydrolysis of the C-3 acetate. We explored a variety of methods for attachment of POA to the primary allylic alcohol of **3**, and ultimately discovered the Katritzky benzotriazole activated POA ester (**POA-BT**) provided the highest and most consistent yield of **4**.[9] Subsequent deprotection of the C-4 carboxylate of **4** by trifluoroacetic acid (TFA) in the presence of triisopropylsilane (TIPS) afforded the desired conjugate **CS-POA** in 88% yield (Scheme 2A). We were also interested in exploring different classes of β-lactam scaffolds, such as the cephamycins, which contain a C-7 methoxy group neighboring the amide sidechain. Rao and coworkers have previously designed chromo- and fluorogenic cephamycin probes demonstrating exquisite selectivity for BlaC over similar class A β-lactamases, due to significantly increased flexibility of its active site.[10] In the context of our prodrug approach, we envisioned a cephamycin scaffold might impart desirable selectivity and limit off-target degradation/release triggered from β-lactamases expressed by commensal organisms. Our synthetic route to these analogues began by C-7 oxidative methoxylation of **6** using optimized conditions reported by Rao and co-workers (Scheme 2B).[9,11] Regioselective hydrolysis of the C-3′ acetate moiety of **7** proved extremely challenging, but was eventually realized using Lipase B with a *tert*-butyl protecting group at C-4 to furnish **8** in 90% yield.[11a] Conjugation of POA on **8** to afford **CM-POA** was accomplished analogously as for **CS-POA**, the lower yield for the last step being attributed to longer reaction times required for removal of the *tert*-butyl ester.

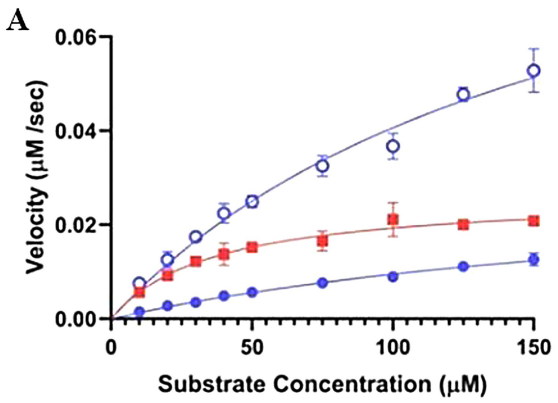
We performed several experiments to assess the potential viability of our conjugates as therapeutic candidates. Stability assays were performed with cephalosporin conjugate **CS-POA** in HEPES buffer (pH 7.4), simulated gastric fluid (SGF, pH 1.2) and in mouse, rat and human serum. Gratifyingly, **CS-POA** showed no observable breakdown in serum from all three species and was stable at pH 7.4, although 50% hydrolysis in SGF suggests moderate acid instability (Table S1).[12] Next, we performed a simple enzymatic assay to verify POA release upon



Scheme 2. Synthesis of (**A**) cephalosporin- and (**B**) cephamycin-pyrazinoic acid conjugates. Abbreviations: POA-BT = 1*H*–1,2,3-Benzotriazol-1-yl(2-pyrazinyl)methanone.[9]

β-lactamase exposure. Recombinant BlaC was overexpressed in transformed *E. coli* BL21/DE3 cells and purified as described.[13] BlaC was incubated with **CS-POA**, and removed aliquots were quenched at the indicated time points and analyzed by HPLC. Time- and enzyme concentration-dependent release of POA were observed, confirming **CS-POA** is a substrate for BlaC and validating our strategy as a *pncA*-independent, TB-selective POA delivery vehicle (Figure 1).

We next sought to further characterize POA release through spectrophotometry-based continuous kinetic assays. Analogues **CS-POA**, **CM-POA**, and **CSPro** (**2**, Scheme 2A), a cephalosporin "promoiety" lacking the C-3′ POA, were assessed against BlaC, and the steady-state rate of substrate hydrolysis was quantified by a decrease in absorbance at 263–270 nm (Figure 2, Table S2).[14] All three β-lactams are substrates for BlaC (displaying specificity constants comparable to classical cephalosporins),[15] and thus compatible with a BlaC-targeted prodrug strategy. We also evaluated the conjugates against CTX–M-1, a representative Ambler class A extended spectrum β-lactamase (ESBL) capable of hydrolyzing many β-lactam classes, including oxyimino-cephalosporins.[16] CTX–M-1 retains activity against **CS-POA** and **CSPro** but is incapable of hydrolyzing the bulkier cephamycin substrate **CM-POA** (Figure 2B), validating our hypothesis that this scaffold could provide enhanced TB selectivity and avoid undesired release by



B

Substrate	*Mtb* BlaC	CTX-M-1
	k_{cat}/K_M (M^{-1} s^{-1})	
CS-POA	(6.77±1.27) ×10^4	(6.93±1.19) ×10^5
CS Pro	(5.47±0.65)×10^4	(5.92±0.38) ×10^5
CM-POA	(1.45±0.04) ×10^4	<5 ×10^1 [a]

Figure 2. A. Michaelis-Menten curves for hydrolysis of cephem-POA substrates; ● = **CS-POA** + BlaC, ■ = **CM-POA** + BlaC, ○ = **CS-POA** + CTX–M-1; **B**. Kinetic parameters determined for each substrate and enzyme. [a] **CM-POA** was not a substrate for CTX–M-1; specificity constant estimated using k_{cat} derived from 1 mM enzyme and maximal substrate concentration.

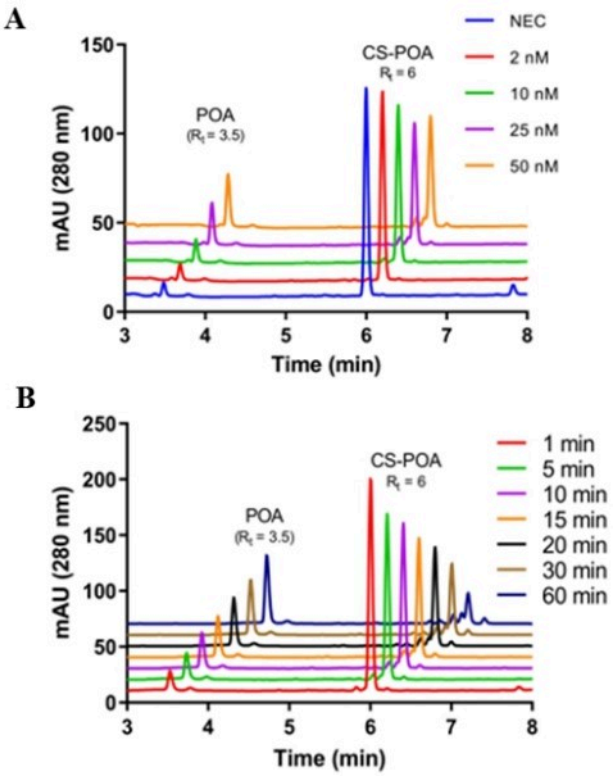


Figure 1. A. BlaC concentration-dependence release assay. Replicates were prepared as described with the indicated enzyme concentration (2–50 nM) and quenched at 10 min with TCA. 25 nM was chosen for the time-dependence assay. NEC = no enzyme control. **B**. Time-dependence release assay. Replicates were prepared as described with 25 nM BlaC and aliquots were quenched at the indicated timepoint with TCA.

commensal β-lactamases. To our knowledge, this study represents the first application of this paradigm for the design of an antitubercular.

Compounds were evaluated against a panel of organisms, including *M. tuberculosis* H37Rv and *M. bovis* BCG (an attenuated strain lacking pyrazinamidase activity), along with several non-mycobacterial pathogens, to determine the minimum inhibitory concentrations resulting in 90% growth inhibition (MIC_{90}, Table S1). The POA conjugates **CS-POA** and **CM-POA** exhibited activity commensurate with POA against *M. tuberculosis* H37Rv (Table 1) suggesting POA is successfully released by BlaC. More significantly, the POA conjugates were active against *M. bovis* BCG, which is completely resistant to PZA. Of particular interest is the selective antimycobacterial activity observed for **CS-POA** and **CM-POA**, a feature shared by PZA/POA (Table S1). This selectivity would prove particularly useful in avoiding nonspecific killing of commensal organisms and related gastrointestinal dysbiosis, a common cause for antibacterial side effects in vivo. To probe the antimicrobial mechanism as well as to assess their potential therapeutic utility against drug-resistant strains, a number of *Mtb* mutants were evaluated (Table 2; Figure 3). Validating results against *M. bovis*, we found that a *Mtb pncA* knockout mutant fully resistant to PZA also retained susceptibility to **CS-POA** and **CM-POA**. To confirm the role of *blaC* in bioactivation of the POA conjugates, we prepared a *Mtb blaC* deletion strain *(Mtb ΔblaC)* as well as a complemented strain (*Mtb ΔblaC::blaC*). Contrary to our initial expectations, the conjugates were significantly more active

Table 1. MIC_{90} data for select *Mycobacterium* strains and mutants (μg/mL).[a]

Compound	*Mtb* (H37Rv)	*M. bovis* (BCG)	*Mtb ΔpncA*	*Mtb ΔblaC*	*Mtb ΔblaC::blaC*	*Mtb panD::Tn*
PZA	25–50	>800	>800	50	50	>800
POA	100	100	100	100	100	>800
CS-POA	100	100	100	25	100	200
CS Pro	200	50	100–200	12.5	50	100
CM-POA	100	200	200	100	100	(n.d.)[b]

[a] Assays were performed in Middlebrook 7H9 liquid medium at pH 5.8, as described in the Supporting Information; [b] not determined.

Table 2. Frequency of Resistance Assays for PZA/POA and cephalosporins.

Compound	MIC_{90} [μg/mL]	FoR[a]
PZA	25–50[b]	1.2×10^{-5}
POA	100	2.9×10^{-7}
CS-POA	100	$<5\times10^{-8}$
CS Pro	200	$<5\times10^{-8}$

[a] FoR determined against *Mtb* H37Rv at pH 5.8 at concentrations 4× MIC_{90} of test compounds. [b] The upper bound (50 μg/mL) was used to determine the 4× MIC_{90} concentration.

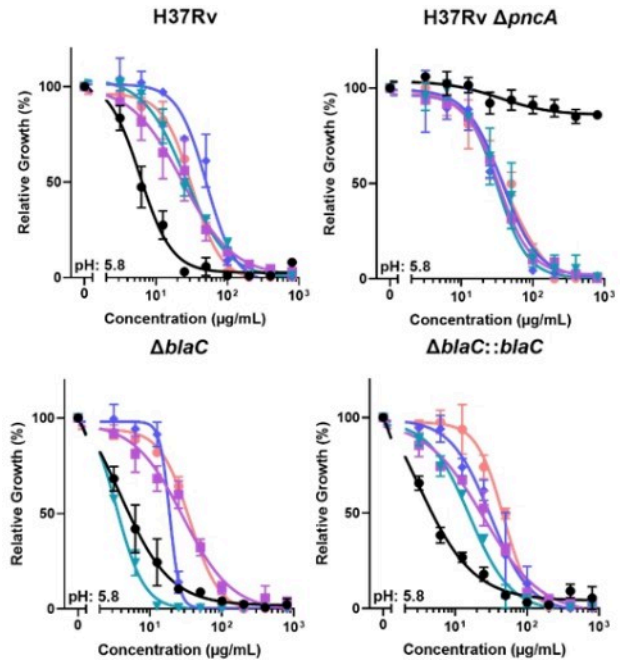


Figure 3. Antibacterial evaluation of POA conjugates against *Mycobacterium* strains. Conjugates retain activity against resistant mutants. ● = PZA; ■ = POA; ♦ = **CS-POA**; ▼ = **CS Pro**; ● = **CM-POA**.

against *Mtb ΔblaC*, while complementation in *Mtb ΔblaC::blaC* restored close to wild-type susceptibility. These results highlight the role of the β-lactam promoiety, which in the absence of BlaC is able to exert anti-mycobacterial activity, likely through on-target inhibition of penicillin-binding proteins (PBPs); this suggests development of resistance through loss-of-function mutations in BlaC will not be possible, since the promoiety renders BlaC conditionally essential. Lastly, to demonstrate on-target activity, we evaluated the compounds against *Mtb panD::Tn*, a strain where the proposed molecular target PanD was inactivated by transposon-insertion, conferring resistance to POA (Table 1).[17] The finding that **CS-POA** retains some activity against *Mtb ΔpanD* highlights the moderate intrinsic activity of the β-lactam promoiety, which is further evidenced by its weak activity against wild-type *Mtb* H37Rv.

The cephalosporins were also assessed in IFN-γ activated THP-1 macrophages, a model for in vivo *Mtb* infection (Figure 4).[18] Cephalosporins (and most β-lactams) are typically thwarted by phenotypic resistance in non-replicating infection models, as demonstrated by the lack of activity for **CSPro**.[19] Conversely, the efficacy of PZA in vivo is largely due to its sterilizing activity against non-replicating *Mtb*, presenting in this study as a tenfold reduction in bacterial load by day 8.[20] Interestingly, **CS-POA** also appears capable of achieving moderate killing in this infection model, demonstrating a fourfold reduction in colony-forming units (CFU) at the same endpoint. Other β-lactams active in slow/non-replicating models have only recently been identified by Aube et al. through a high-throughput screening campaign.[21] Our conjugates, like reported second-generation pyrithione conjugates,[5f] also possess activity against actively replicating organisms, providing additional evidence of a potential advantage of this strategy against *Mtb*. Most importantly, this sterilizing activity demonstrates the benefit of the POA warhead, which appears to impart the traditional cephalosporin scaffold with novel activity against intracellular *Mtb*.

In our initial experimental design, we had conceived of a cephalosporin-POA conjugate as a prodrug strategy, with the β-lactam merely imparting a mechanism for selective drug delivery. However, the activity of **CS-POA** suggests a potential codrug effect, wherein both components of the molecule play a role in its observed activity. In order to further investigate this hypothesis, we assessed intracellular POA accumulation in response to conjugate treatment. We incubated *M. bovis* BCG cells with **CS-POA** and, following cell lysis and metabolite extraction, analyzed samples using LC-MS/MS.[22] After 24 h incubation, conjugate-treated cells displayed comparable intracellular POA concentrations when compared to POA-treated cells (Figure S3). Having verified intracellular delivery of POA, we next sought to investigate the effect of pantothenate supplementation on conjugate activity. Previous work by Baughn et al. has demonstrated that pantothenate, a CoA pathway metabolite downstream of PanD, is able to strongly antagonize POA antibacterial activity in vitro.[23] We grew *M. bovis* BCG cultures in the presence of 100 μM pantothenate and discovered that, while POA was strongly antagonized, **CS-POA**

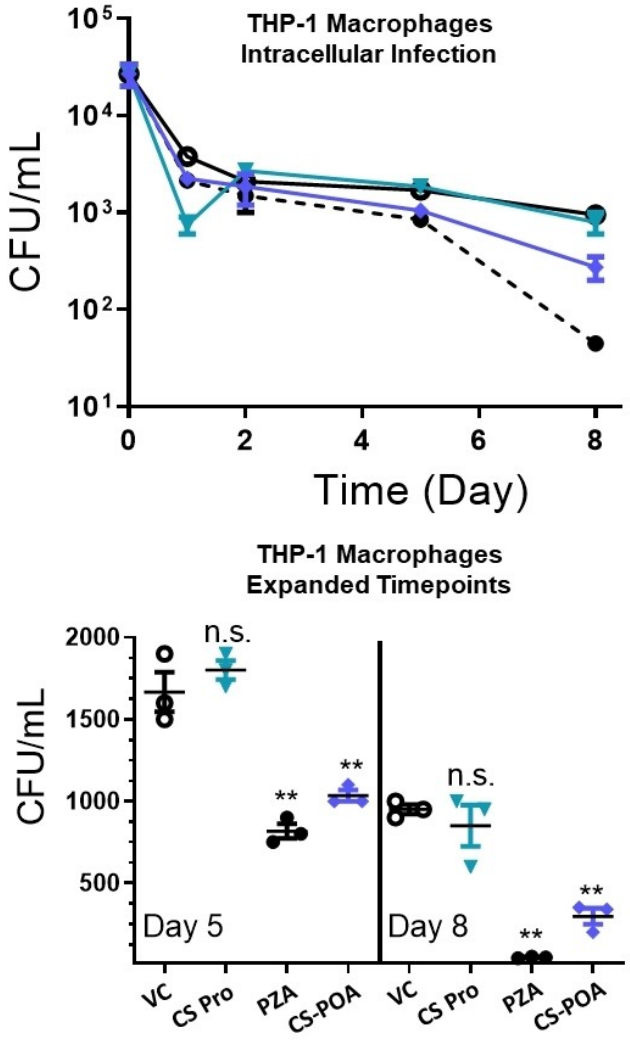


Figure 4. Activity of PZA and cephalosporins at 1.6 mM against *Mtb* in IFN-γ activated THP-1 macrophages. **CS-POA** and PZA retain activity, while CS Pro is inactive in this nonreplicating model. Abbreviations: VC = vehicle control (DMSO). ○ = VC; ● = PZA; ♦ = **CS-POA**; ▼ = **CS Pro**.

was completely agnostic to pantothenate supplementation (Figure S4), further suggesting that the β-lactam scaffold drives conjugate activity in vitro. This result provides further evidence of the nuanced interplay between the conjugates' dual therapeutic modalities, where the β-lactam and POA components confer activity under conditions where the other warhead is inactive (*panD* disruption/pantothenate supplementation and intracellular infection, respectively). Finally, in order to evaluate the ability of our conjugates to circumvent resistance development, we performed assays to determine the frequency of resistance (FoR) for our compounds against *Mtb* H37Rv. Cultures were challenged with superinhibitory concentrations of PZA, POA, **CS-POA** and **CSPro**. High levels of resistance were observed for PZA; POA resistance was less frequent, but a few colonies were identified (Table 2). Gratifyingly, no resistant colonies were observed for POA conjugate **CS-POA**, suggesting this strategy is less susceptible to resistance development than the first line monotherapy. Resistant colonies were also not observed for the cephalosporin promoiety **CSPro**, providing limited evidence that the decreased FoR for the POA conjugate may result from the biological effect of the β-lactam scaffold.

Conclusions

We have presented a novel strategy for targeted delivery of POA which circumvents the most common resistance mechanism blocking the parent drug. A cephalosporin-based POA conjugate (**CS-POA**) displays comparable activity to PZA/POA and retains activity against resistant mutants and in a macrophage infection model. A cephamycin-based approach (**CM-POA**) achieves selective release by the mycobacterial β-lactamase, a new proof of concept for the therapeutic potential of this strategy. Mechanistic studies have suggested **CS-POA** acts as a co-drug, with the β-lactam scaffold and pyrazinoic acid warhead both contributing to the observed activity. This dual approach is superior to either class alone, conferring both reduced resistance susceptibility and improved activity against non-replicating organisms. This study contributes to a growing trend demonstrating the utility of the previously neglected β-lactams as a viable treatment option against the ongoing threat of drug-resistant TB.[15,19b,24]

Experimental Section

Detailed experimental procedures and additional data can be found in the Supporting Information.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: beta-lactamase · cephalosporin · *mycobacterium tuberculosis* · new antibiotics · pyrazinamide

[1] WHO in Global tuberculosis report 2019. World Health Organization, Geneva, Switzerland, 2019.
[2] A. H. Diacon, R. Dawson, F. von Groote-Bidlingmaier, G. Symons, A. Venter, P. R. Donald, C. van Niekerk, D. Everitt, H. Winter, P. Becker, C. M. Mendel, M. K. Spigelman, *Lancet* **2012**, *380*, 986–993.
[3] a) A. H. Diacon, P. R. Donald, A. Pym, M. Grobusch, R. F. Patientia, R. Mahanyele, N. Bantubani, R. Narasimooloo, T. De Marez, R. van Heeswijk, N. Lounis, P. Meyvisch, K. Andries, D. F. McNeeley, *Antimicrob. Agents Chemother.* **2012**, *56*, 3271–3276; b) M. G. Whitfield, H. M. Soeters, R. M. Warren, T. York, S. L. Sampson, E. M. Streicher, P. D. van Helden, A. van Rie, *PLoS One* **2015**, *10*, e0133869.
[4] a) J. P. Lanoix, R. Tasneen, P. O'Brien, J. Sarathy, H. Safi, M. Pinn, D. Alland, V. Dartois, E. Nuermberger, *Antimicrob. Agents Chemother.* **2016**, *60*, 4197–4205; b) M. H. Cynamon, S. P. Klemens, T. S. Chou, R. H. Gimi, J. T. Welch, *J. Med. Chem.* **1992**, *35*, 1212–1215; c) M. H. Cynamon, R. Gimi, F. Gyenes, C. A. Sharpe, K. E. Bergmann, H. J. Han, L. B. Gregor, R. Rapolu, G. Luciano, J. T. Welch, *J. Med. Chem.* **1995**, *38*, 3902–3907.
[5] a) S. Mobashery, S. A. Lerner, M. Johnston, *J. Am. Chem. Soc.* **1986**, *108*, 1685–1686; b) H. A. Albrecht, G. Beskid, K. K. Chan, J. G. Christenson, R. Cleeland, K. H. Deitcher, N. H. Georgopapadakou, D. D. Keith, D. L. Pruess, J. Sepinwall, A. C. Specian Jr., R. L. Then, M. Weigele, K. F. West, R. Yang, *J. Med. Chem.* **1990**, *33*, 77–86; c) A. J. Corraz, S. L. Dax, N. K. Dunlap, N. H. Georgopapadakou, D. D. Keith, D. L. Pruess, P. L. Rossman, R. Then, J. Unowsky, C. C. Wei, *J. Med. Chem.* **1992**, *35*, 1828–1839; d) G. H. Hakimelahi, K. S. Shia, C. Xue, S. Hakimelahi, A. A. Moosavi-Movahedi, A. A. Saboury, A. Khalafi-Nezhad, M. N. Soltani-Rad, V. Osyetrov, K. Wang, J. H. Liao, F. T. Luo, *Bioorg. Med. Chem.* **2002**, *10*, 3489–3498; e) S. Yan, M. J. Miller, T. A. Wencewicz, U. Moollmann, *MedChemComm.* **2010**, *1*, 145–148; f) L. L. Quezada, K. Li, S. L. McDonald, Q. Nguyen, A. J. Perkowski, C. W. Pharr, B. Gold, J. Roberts, K. McAulay, K. Saito, S. S. Karakaya, P. E. Javidnia, E. Porras de Francisco, M. M. Amieva, S. P. Díaz, A. M. Losana, M. Zimmerman, H. H. Liang, J. Zhang, V. Dartois, S. Sans, S. Lagrange, L. Goullieux, C. Roubert, C. Nathan, J. Aubé, *ACS Infect. Dis.* **2019**, *5*, 1433–1445.
[6] J. M. Thiede, N. A. Dillon, M. D. Howe, R. Aflakpui, S. J. Modlin, S. E. Hoffner, F. Valafar, Y. Minato, A. D. Baughn, *mBio.* **2022**, *13*, e00439-21.
[7] E. A. Lamont, N. A. Dillon, A. D. Baughn, *Microbiol. Mol. Biol. Rev.* **2020**, *84*, e00070–00019.
[8] K. Yu, N. Sun, S. Fang, W. Mo, B. Hu, Z. Shen, X. Hu, *Org. Process Res. Dev.* **2009**, *13*, 815–819.
[9] S. S. Panda, O. S. Detistov, A. S. Girgis, P. P. Mohapatra, A. Samir, A. R. Katritzky, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2198–2205.
[10] a) H. Xie, J. Mire, Y. Kong, M. Chang, H. A. Hassounah, C. N. Thornton, J. C. Sacchettini, J. D. Cirillo, J. Rao, *Nat. Chem.* **2012**, *4*, 802–809; b) Y. Cheng, H. Xie, P. Sule, H. Hassounah, E. A. Graviss, Y. Kong, J. D. Cirillo, J. Rao, *Angew. Chem. Int. Ed.* **2014**, *53*, 9360–9364.
[11] a) L. D. Patterson, M. J. Miller, *J. Org. Chem.* **2010**, *75*, 1289–1292; b) G. Koppel, R. Koehler, *J. Am. Chem. Soc.* **1973**, *95*, 2403–2404.
[12] S. Dawadi, S. Kawamura, A. Rubenstein, R. Remmel, C. C. Aldrich, *Bioorg. Med. Chem.* **2016**, *24*, 1314–1321.
[13] S. Hazra, S. G. Kurz, K. Wolff, L. Nguyen, R. A. Bonomo, J. S. Blanchard, *Biochemistry* **2015**, *54*, 5657–5664.
[14] A. Samuni, *Anal. Biochem.* **1975**, *63*, 17–26.
[15] J.-E. Hugonnet, J. S. Blanchard, *Biochemistry* **2007**, *46*, 11998–12004.
[16] K. Bush, G. A. Jacoby, *Antimicrob. Agents Chemother.* **2010**, *54*, 969–976.
[17] P. Gopal, W. Nartey, P. Ragunathan, J. Sarathy, F. Kaya, M. Yee, C. Setzer, M. S. S. Manimekalai, V. Dartois, G. Gruber, T. Dick, *ACS Infect. Dis.* **2017**, *3*, 807–819.
[18] S. A. Theus, M. D. Cave, K. D. Eisenach, *Infect. Immun.* **2004**, *72*, 1169–1173.
[19] a) E. Tuomanen, *Rev. Infect. Dis.* **1986**, *8*, S279–S291; b) J.-E. Hugonnet, L. W. Tremblay, H. I. Boshoff, C. E. Barry, J. S. Blanchard, *Science* **2009**, *323*, 1215.
[20] a) R. M. McCune Jr., R. Tompsett, *J. Exp. Med.* **1956**, *104*, 737–762; b) E. A. Lamont, A. D. Baughn, *EBioMedicine* **2019**, *49*, 374–380.
[21] B. Gold, R. Smith, Q. Nguyen, J. Roberts, Y. Ling, L. Lopez Quezada, S. Somersan, T. Warrier, D. Little, M. Pingle, D. Zhang, E. Ballinger, M. Zimmerman, V. Dartois, P. Hanson, L. A. Mitscher, P. Porubsky, S. Rogers, F. J. Schoenen, C. Nathan, J. Aubé, *J. Med. Chem.* **2016**, *59*, 6027–6044.
[22] a) S. Chakraborty, T. Gruber, C. E. Barry, 3rd, H. I. Boshoff, K. Y. Rhee, *Science* **2013**, *339*, 88–91; b) M. D. Howe, S. L. Kordus, M. S. Cole, A. A. Bauman, C. C. Aldrich, A. D. Baughn, Y. Minato, *Front. Cell. Infect. Microbiol.* **2018**, *8*, 399.
[23] N. A. Dillon, N. D. Peterson, B. C. Rosen, A. D. Baughn, *Antimicrob. Agents Chemother.* **2014**, *58*, 7258–7263.
[24] a) N. Veziris, C. Truffot, J.-L. Mainardi, V. Jarlier, *Antimicrob. Agents Chemother.* **2011**, *55*, 2597–2600; b) M. Gurumurthy, R. Verma, C. Naftalin, K. Hee, Q. Lu, K. Tan, S. Issac, W. Lin, A. Tan, K.-Y. Seng, L. Lee, N. Paton, *J. Antimicrob. Chemother.* **2017**, *72*; c) K. A. Cohen, T. El-Hay, K. L. Wyres, O. Weissbrod, V. Munsamy, C. Yanover, R. Aharonov, O. Shaham, T. C. Conway, Y. Goldschmidt, W. R. Bishai, A. S. Pym, *EBioMedicine* **2016**, *9*, 170–179; d) S. Solapure, N. Dinesh, R. Shandil, V. Ramachandran, S. Sharma, D. Bhattacharjee, S. Ganguly, J. Reddy, V. Ahuja, V. Panduga, M. Parab, K. G. Vishwas, N. Kumar, M. Balganesh, V. Balasubramanian, *Antimicrob. Agents Chemother.* **2013**, *57*, 2506–2510.

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