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Binding of Ceftobiprole and Comparators to the Penicillin-Binding Proteins of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*▽

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Ceftobiprole exhibited tight binding to PBP2a in methicillin-resistant *Staphylococcus aureus*, PBP2x in penicillin-resistant *Streptococcus pneumoniae*, and PBP3 and other essential penicillin-binding proteins in methicillin-susceptible *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Ceftobiprole also bound well to PBP2 in the latter organisms, contributing to the broad-spectrum antibacterial activity against gram-negative and gram-positive bacteria.

Ceftobiprole, an investigational parenteral cephalosporin in phase 3 clinical trials, exhibits a broad spectrum of activity against many clinically important gram-negative and gram-positive bacteria (3, 4, 7, 18, 20, 21, 29). Ceftobiprole is distinguished from other marketed β-lactams by its increased binding to penicillin-binding protein (PBP) 2a (PBP2a) from methicillin-resistant staphylococci (18, 24).

PBPs, the targets of β-lactam antibiotics, are membrane-associated enzymes involved in the last steps of peptidoglycan biosynthesis. The affinities of ceftobiprole for PBPs from *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were determined.

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MICs were determined according to CLSI methods (5) using Trek Diagnostic Systems (Cleveland, OH) panels, except for ceftobiprole, which was prepared fresh for each assay. *E. coli*, *P. aeruginosa*, or *S. aureus* PBPs were isolated as described previously (18, 30). For *S. pneumoniae*, whole cells were used. PBPs were labeled with Bocillin FL (Invitrogen, Carlsbad, CA) as described previously (26). *S. aureus* OC 3726 membranes were preincubated with 1 mg/ml clavulanic acid (USP, Rockville, MD) to saturate all PBPs except PBP2a. PBPs were visualized using a LumiImager (Roche, Indianapolis, IN), and 50% inhibitory concentration (IC_{50}) values were determined using Quantity One software (Bio-Rad, Hercules, CA). *P. aeruginosa* cell morphology after ceftobiprole exposure was monitored by microscopic examination at a magnification of ×1,000. *S. pneumoniae pbp1a*, *pbp2x*, and *pbp2b* were amplified by PCR as described previously (23) and sequenced by ACGT (Wheeling, IL).

For *E. coli* MC4100, all drugs had good affinity for PBP3, the primary target for monobactams and most cephalosporins (10, 12) (Table 1). Ceftobiprole, ceftriaxone, and cefepime also had good affinity for the essential PBP2 (IC_{50}s of ≤0.6 μg/ml) (Table 1). However, they were at least 20-fold less potent than imipenem (Table 1), for which the primary target is PBP2 in gram-negative bacteria (28). Ceftobiprole and ceftriaxone had IC_{50}s for PBP1a and PBP4 that were approximately 5- to 10-fold lower than those for cefepime and ceftazidime (Table 1). Ceftriaxone and imipenem had greater affinity for PBP1b than the other drugs (Table 1). Only imipenem had high affinity for the nonessential PBP5 and PBP6 (Table 1). Cephalosporins like cefotaxime and ceftazidime target primarily PBP3 and have at least a 50-fold-lower affinity for PBP2 (6, 17, 25). Conversely, the increased affinity of ceftriaxone and cefepime for PBP2 compared to those of other cephalosporins (9, 14, 25) was confirmed in this study and also observed for ceftobiprole (Table 1).

For *P. aeruginosa* PAO1, the cephalosporins had the greatest affinity for PBP1a and PBP3 (Table 1). Ceftobiprole and cefepime had approximately a 10-fold-higher affinity for PBP4 than ceftazidime (Table 1). Ceftobiprole and imipenem had the lowest IC_{50} values for PBP1b. Imipenem had the highest affinity for PBP2 (IC_{50} of 0.1 μg/ml), with ceftobiprole having the greatest affinity (IC_{50} of 3 μg/ml) among the cephalosporins (Table 1). Cefepime and ceftazidime bound PBP2 with at least an 80-fold-lower affinity than that for PBP3, similar to previously reported data (17, 25). Conversely, ceftobiprole had measurable affinity for PBP2, with an IC_{50} value 30-fold higher than that for PBP3. The cephalosporins did not bind to PBP5/6 at concentrations as high as 32 μg/ml (Table 1). Aztreonam had highest affinity for PBP3 (Table 1). *P. aeruginosa* cells grown in the presence of ceftobiprole produced filamentation (Fig. 1), suggesting that PBP3 was the primary target.

In methicillin-susceptible *S. aureus* ATCC 29213, ceftobiprole had good affinity (IC_{50} of ≤1 μg/ml) for all four PBPs (Table

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TABLE 1. Binding of selected β-lactams to PBPs from gram-negative bacteria

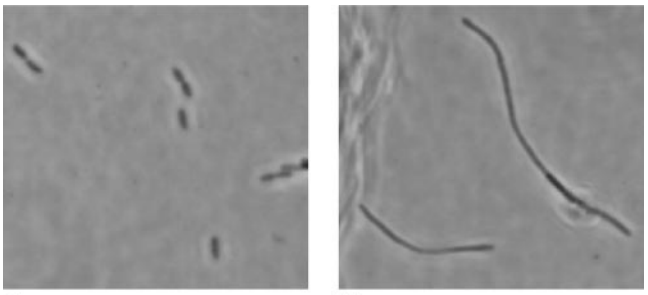
Organism	PBP	IC_{50} (μg/ml)[a]					
		Ceftobiprole	Ceftriaxone	Ceftazidime	Cefepime	Imipenem	Aztreonam
E. coli MC4100	1a	0.3	0.2	1.1	2	0.5	>8
	1b	8	0.5	1.1	3.7	0.5	>8
	2	0.2	0.2	4	0.6	0.01	>8
	3	0.2	<0.01	0.07	0.1	8	0.03
	4	0.5	0.4	>4	>4	0.01	>8
	5	>8	6	>4	>4	0.5	>8
	6	3	>8	>4	>4	0.1	>8
MIC[b]		0.03	0.06	0.12	0.015	0.12	0.12
P. aeruginosa PAO1	1a	0.1	ND[c]	0.2	0.1	0.5	2
	1b	0.5	ND	5	2	0.5	2
	2	3	ND	>32	8	0.1	16
	3	0.1	ND	0.1	0.1	0.1	0.03
	4	0.2	ND	2	0.3	0.01	16
	5/6	>32	ND	>32	>32	2	>16
MIC[b]		1	ND	1	2	1	4

[a] Concentration of β-lactam that inhibits 50% of Bocillin FL binding to that PBP compared to a no-drug control. For *E. coli* MC4100, 10 concentrations of each drug ranging from 0.016 μg/ml to 8 μg/ml were used in the competition assay, and for *P. aeruginosa*, 10 concentrations ranging from 0.06 μg/ml to 32 μg/ml were used.
[b] MIC in μg/ml.
[c] ND, not determined.

2). Ceftobiprole had the greatest affinity for PBP3, with an IC_{50} that was 20-fold lower than that of ceftriaxone. Inhibition of this PBP leads to cell enlargement and the termination of septation (11). Ceftobiprole had better affinity than ceftriaxone for all PBPs except PBP2 (Table 2), whose inhibition leads to cell lysis (11).

PBP2a from methicillin-resistant *S. aureus* strain OC 3726 (Table 2 and Fig. 2) had high affinity for ceftobiprole, unlike ceftriaxone and ceftazidime. Entenza et al. previously reported low ceftobiprole IC_{50}s for PBP2a (≤0.47 μg/ml), which were >100 times lower than those for methicillin (8). Hebeisen et al. also reported potent binding of ceftobiprole to PBP2a from *S. epidermidis* (18).

S. pneumoniae OC 8865 was penicillin susceptible and had no *pbp1a*, *pbp2b*, and *pbp2x* mutations. Ceftobiprole and ceftriaxone PBP-binding profiles were similar (Table 2), having high affinity for PBP1a and PBP2x, the primary cephalosporin targets (13, 22). Unexpectedly, ceftobiprole had good affinity for PBP2b (IC_{50} value of 0.06 μg/ml), unlike ceftriaxone, which had an IC_{50} of >1 μg/ml (Table 2). Studies have shown that



A B

FIG. 1. *P. aeruginosa* PAO1 grown for 1.5 h in (A) nutrient broth alone (control) or (B) nutrient broth containing 1 μg/ml ceftobiprole (1× MIC). Magnification, ×1,000.

TABLE 2. Binding of ceftobiprole, ceftriaxone, and ceftazidime to PBPs from gram-positive cocci

Organism	PBP	IC_{50} (μg/ml)[a]		
		Ceftobiprole	Ceftriaxone	Ceftazidime
S. aureus ATCC 29213 (methicillin susceptible)	1	0.1	0.5	ND[c]
	2	0.5	0.1	ND
	3	0.05	1	ND
	4	1	10	ND
MIC[b]		0.25	2	ND
S. aureus OC 3726 (methicillin resistant)	2a	0.9	>50	>50
MIC[b]		2	>64	>128
S. pneumoniae OC 8865 (penicillin susceptible)	1a	0.03	0.01	ND
	1b	0.05	0.03	ND
	2x	0.01	0.03	ND
	2a	0.03	0.1	ND
	2b	0.06	>1	ND
	3	0.02	0.02	ND
MIC[b]		0.008	0.03	ND
S. pneumoniae OC 8819 (penicillin resistant)	1a	0.1	0.02	ND
	1b	>8	0.02	ND
	2x	1	8	ND
	2a	0.1	0.5	ND
	2b	>8	>8	ND
	3	0.01	0.01	ND
MIC[b]		1	8	ND

[a] Concentration of β-lactam that inhibits 50% of Bocillin FL binding to that PBP compared to a no-drug control. The following concentration ranges of each drug were used in the competition assays for the following organisms: *S aureus* ATCC 29213, 0.001 μg/ml to 50 μg/ml; *S. aureus* OC 3726, 0.16 μg/ml to 50 μg/ml; *S. pneumoniae* OC 8865, 0.004 μg/ml to 1 μg/ml; and *S. pneumoniae* OC 8819, 0.06 μg/ml to 8 μg/ml.
[b] MIC in μg/ml.
[c] ND, not determined.

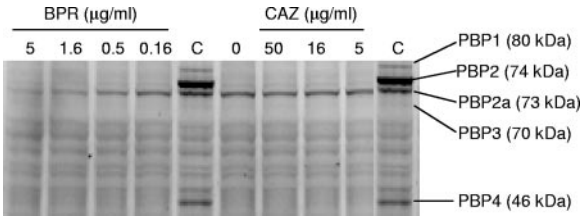


FIG. 2. Affinity of PBP2a from *S. aureus* 3726 for ceftobiprole (BPR) or ceftazidime (CAZ). All samples except "C" were initially preincubated with 1 mg/ml of clavulanic acid to saturate all PBPs except PBP2a. For competition assays, ceftobiprole or ceftazidime was added at the indicated concentrations, followed by labeling with Bocillin FL. IC_{50} values were calculated by 50% inhibition of Bocillin FL binding compared to the no-drug (0 μg/ml) sample. Lane "C" contains PBPs labeled only with Bocillin FL in order to visualize all PBPs.

cefotaxime, ceftriaxone, cefuroxime, and ceftazidime have poor affinity for PBP2b (13, 16, 22). The ceftobiprole affinity for PBP2b may be due to improved kinetic interactions as seen with the methicillin-resistant staphylococcus PBP2a (18).

In pneumococcal clinical isolates, β-lactam resistance is caused primarily by alterations in PBP1a, PBP2x, and PBP2b (1, 2, 15, 27). Penicillin- and ceftriaxone-resistant *S. pneumoniae* OC 8819 had the following substitutions: T371S to S and P432 to T in PBP1a; T338 to A, M339 to F, I371 to T, R384 to G, M400 to T, and L546 to V in PBP2x; and T446 to A and A619 to G in PBP2b. For PBP2x, ceftobiprole had an eightfold-higher binding affinity than ceftriaxone (Table 2). Heinze-Krauss et al. similarly reported that a ceftobiprole analog had IC_{50} values that were approximately sixfold lower than those of ceftriaxone against purified PBP2x from two cefotaxime-resistant isolates (19). Neither ceftobiprole nor ceftriaxone bound to PBP2b at concentrations of ≤8 μg/ml (Table 2), which was probably due to the PBP2b T446-to-A substitution known to contribute to penicillin resistance (13). Affinities for PBP1a, PBP2a, and PBP3 were similar for both drugs, but ceftriaxone had a higher affinity for PBP1b than did ceftobiprole (Table 2).

In summary, ceftobiprole demonstrated potent binding to PBPs from gram-positive bacteria, including those with decreased β-lactam sensitivity, such as PBP2a in MRSA and PBP2x in a penicillin-resistant *S. pneumoniae* strain, in contrast to ceftriaxone. In *E. coli*, ceftobiprole exhibited strong binding to the essential PBPs PBP2 and PBP3. Ceftobiprole exhibited a binding profile similar to those of cefepime and ceftazidime in *P. aeruginosa* but with enhanced binding to PBP2. These binding profiles explain the broad-spectrum activity for ceftobiprole that includes gram-negative bacteria and many β-lactam-resistant gram-positive cocci, including MRSA.

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REFERENCES

1. **Asahi, Y., Y. Takeuchi, and K. Ubukata.** 1999. Diversity of substitutions within or adjacent to conserved amino acid motifs of penicillin-binding protein 2X in cephalosporin-resistant *Streptococcus pneumoniae* isolates. Antimicrob. Agents Chemother. **43:**1252–1255.
2. **Asahi, Y., and K. Ubukata.** 1998. Association of a Thr-371 substitution in a conserved amino acid motif of penicillin-binding protein 1A with penicillin resistance of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **42:**2267–2273.
3. **Bogdanovich, T., C. Clark, L. Ednie, G. Lin, K. Smith, S. Shapiro, and P. C. Appelbaum.** 2006. Activities of ceftobiprole, a novel broad-spectrum cephalosporin, against *Haemophilus influenzae* and *Moraxella catarrhalis*. Antimicrob. Agents Chemother. **50:**2050–2057.
4. **Bogdanovich, T., L. M. Ednie, S. Shapiro, and P. C. Appelbaum.** 2005. Antistaphylococcal activity of ceftobiprole, a new broad-spectrum cephalosporin. Antimicrob. Agents Chemother. **49:**4210–4219.
5. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 7th ed. M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **Curtis, N. A. C., D. Orr, G. W. Ross, and M. G. Boulton.** 1979. Affinities of penicillins and cephalosporins for the penicillin-binding proteins of *Escherichia coli* K-12 and their antibacterial activity. Antimicrob. Agents Chemother. **16:**533–539.
7. **Davies, T. A., W. Shang, and K. Bush.** 2006. Activities of ceftobiprole and other β-lactams against *Streptococcus pneumoniae* clinical isolates from the United States with defined substitutions in penicillin-binding proteins PBP1a, PBP2b, and PBP2x. Antimicrob. Agents Chemother. **50:**2530–2532.
8. **Entenza, J. M., P. Hohl, I. Heinze-Krauss, M. P. Glauser, and P. Moreillon.** 2002. BAL9141, a novel extended-spectrum cephalosporin active against methicillin-resistant *Staphylococcus aureus* in treatment of experimental endocarditis. Antimicrob. Agents Chemother. **46:**171–177.
9. **Fontana, R., M. Aldegheri, M. Ligozzi, G. Lo Cascio, and G. Cornaglia.** 1998. Interaction of ceftriaxone with penicillin-binding proteins of *Escherichia coli* in the presence of human serum albumin. J. Antimicrob. Chemother. **42:**95–98.
10. **Georgopapadakou, N. H.** 1993. Penicillin-binding proteins and bacterial resistance to β-lactams. Antimicrob. Agents Chemother. **37:**2045–2053.
11. **Georgopapadakou, N. H., B. A. Dix, and Y. R. Mauriz.** 1986. Possible physiological functions of penicillin-binding proteins in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29:**333–336.
12. **Georgopapadakou, N. H., S. A. Smith, and R. B. Sykes.** 1982. Mode of action of azthreonam. Antimicrob. Agents Chemother. **21:**950–956.
13. **Grebe, T., and R. Hakenbeck.** 1996. Penicillin-binding proteins 2b and 2x of *Streptococcus pneumoniae* are primary resistance determinants for different classes of β-lactam antibiotics. Antimicrob. Agents Chemother. **40:**829–834.
14. **Gutmann, L., S. Vincent, D. Billot-Klein, J. F. Acar, E. Mrena, and R. Williamson.** 1986. Involvement of penicillin-binding protein 2 with other penicillin-binding proteins in lysis of *Escherichia coli* by some β-lactam antibiotics alone and the synergistic lytic effect of amdinocillin (mecillinam). Antimicrob. Agents Chemother. **30:**906–912.
15. **Hakenbeck, R.** 1999. β-Lactam-resistant *Streptococcus pneumoniae*. Epidemiology and evolutionary mechanism. Chemotherapy (Basel) **45:**83–94.
16. **Hakenbeck, R., S. Tornette, and N. F. Adkinson.** 1987. Interaction of nonlytic β-lactams with penicillin-binding proteins in *Streptococcus pneumoniae*. J. Gen. Microbiol. **133:**755–760.
17. **Hayes, M. V., and D. C. Orr.** 1983. Mode of action of ceftazidime: affinity for the penicillin-binding proteins of *Escherichia coli* K12, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. J. Antimicrob. Chemother. **12:**119–126.
18. **Hebeisen, P., I. Heinze-Krauss, P. Angehrn, P. Hohl, M. G. P. Page, and R. L. Then.** 2001. In vitro and in vivo properties of Ro 63-9141, a novel broad-spectrum cephalosporin with activity against methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **45:**825–836.
19. **Heinze-Krauss, I., P. Angehrn, P. Guerry, P. Hebeisen, C. Hubschwerlen, I. Kompis, M. G. P. Page, H. G. F. Richter, V. Runtz, et al.** 1996. Synthesis and structure-activity relationship of (lactamylvinyl)cephalosporins exhibiting activity against staphylococci, pneumococci, and enterococci. J. Med. Chem. **39:**1864–1871.
20. **Jones, R. N., L. M. Deshpande, A. H. Mutnick, and D. J. Biedenbach.** 2002. In vitro evaluation of BAL9141, a novel parenteral cephalosporin active against oxacillin-resistant staphylococci. J. Antimicrob. Chemother. **50:**915–932.
21. **Kosowska, K., D. B. Hoellman, G. Lin, C. Clark, K. Credito, P. McGhee, B. Dewasse, B. Bozdogan, S. Shapiro, and P. C. Appelbaum.** 2005. Antipneumococcal activity of ceftobiprole, a novel broad-spectrum cephalosporin. Antimicrob. Agents Chemother. **49:**1932–1942.
22. **Munoz, R., C. G. Dowson, M. Daniels, T. J. Coffey, C. Martin, R. Hakenbeck, and B. G. Spratt.** 1992. Genetics of resistance to third-generation cephalosporins in clinical isolates of *Streptococcus pneumoniae*. Mol. Microbiol. **6:**2461–2465.
23. **Nichol, K. A., G. G. Zhanel, and D. J. Hoban.** 2002. Penicillin-binding protein 1A, 2B, and 2X alterations in Canadian isolates of penicillin-resistant *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **46:**3261–3264.
24. **Page, M. G. P., P. Caspers, and M. Kania.** 2005. Interaction of ceftobiprole with a purified soluble form of *Staphylococcus epidermidis* PBP2′. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1157.
25. **Pucci, M. J., J. Boice-Sowek, R. E. Kessler, and T. J. Dougherty.** 1991. Comparison of cefepime, cefpirome, and cefaclidine binding affinities for penicillin-binding proteins in *Escherichia coli* K-12 and *Pseudomonas aeruginosa* SC8329. Antimicrob. Agents Chemother. **35:**2312–2317.
26. **Sifaoui, F., M.-D. Kitzis, and L. Gutmann.** 1996. In vitro selection of one-

step mutants of *Streptococcus pneumoniae* resistant to different oral β-lactam antibiotics is associated with alterations of PBP2x. Antimicrob. Agents Chemother. **40:**152–156.
27. **Smith, A. M., and K. P. Klugman.** 1995. Alterations in penicillin-binding protein 2B from penicillin-resistant wild-type strains of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **39:**859–867.
28. **Yang, Y., N. Bhachech, and K. Bush.** 1995. Biochemical comparison of imipenem, meropenem and biapenem: permeability, binding to penicillin-binding proteins, and stability to hydrolysis by β-lactamases. J. Antimicrob. Chemother. **35:**75–84.
29. **Zbinden, R., V. Punter, and A. Von Graevenitz.** 2002. In vitro activities of BAL9141, a novel broad-spectrum pyrrolidinone cephalosporin, against gram-negative nonfermenters. Antimicrob. Agents Chemother. **46:**871–874.
30. **Zhao, G., T. I. Meier, S. D. Kahl, K. R. Gee, and L. C. Blaszczak.** 1999. BOCILLIN FL, a sensitive and commercially available reagent for detection of penicillin-binding proteins. Antimicrob. Agents Chemother. **43:**1124–1128.