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# In Vitro Evaluation of BRL 42715, a Novel β-Lactamase Inhibitor

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**The penem BRL 42715, C6-(*N*1-methyl-1,2,3-triazolylmethylene)penem, is a potent inhibitor of a broad range of bacterial β-lactamases, including the plasmid-mediated TEM, SHV, OXA, and staphylococcal enzymes, as well as the chromosomally mediated enzymes of *Bacteroides*, *Enterobacter*, *Citrobacter*, *Serratia*, *Morganella*, *Escherichia*, *Klebsiella*, and *Proteus* species. The concentration of BRL 42715 needed to reduce the initial rate of hydrolysis of most β-lactamase enzymes by 50% was <0.01 μg/ml, which was 10- to 100-fold lower than for other β-lactamase inhibitors. These potent inhibitory activities were reflected in the low concentrations of BRL 42715 needed to potentiate the antibacterial activity of β-lactamase-susceptible β-lactams. Concentrations of 0.25 μg/ml or less considerably enhanced the activity of amoxicillin against many β-lactamase-producing strains. The $MIC_{50}$ (MIC for 50% of strains tested) of amoxicillin for 412 β-lactamase-producing members of the family *Enterobacteriaceae* fell from >128 to 2 μg/ml in the presence of 1 μg of BRL 42715 per ml, whereas 5 μg of clavulanic acid per ml brought the $MIC_{50}$ down to 8 μg/ml. Among these 412 strains were 73 *Citrobacter* and *Enterobacter* strains, and 1 μg of BRL 42715 per ml reduced the $MIC_{50}$ of amoxicillin from >128 to 2 μg/ml for the 48 cefotaxime-susceptible strains and from >128 to 8 μg/ml for the 25 cefotaxime-resistant strains.**

The β-lactamase enzymes form a large and diverse group (3, 19) and are recognized as a major cause of bacterial resistance to β-lactam antibiotics (14). This resistance can often be overcome either by using β-lactam antibiotics that are stable to hydrolysis by β-lactamases or by combining labile β-lactams with enzyme inhibitors which may not themselves be useful antibacterial agents but which have the ability to inactivate β-lactamases. The first β-lactamase inhibitor to have clinical application was clavulanic acid (8, 16), and formulations of amoxicillin plus clavulanic acid and of ticarcillin plus clavulanic acid are available which protect the antibiotics amoxicillin and ticarcillin from hydrolysis by many β-lactamase-producing organisms. More recently described β-lactamase inhibitors include sulbactam (5) and tazobactam (YTR 830; 1). Clavulanic acid is highly active against a broad range of β-lactamases, including the Ic enzymes produced by *Proteus vulgaris* and *Bacteroides fragilis*, but is only weakly active against other class I enzymes. Sulbactam is generally less potent than clavulanic acid (9), although it does have some activity against most class I enzymes, while tazobactam is similar in potency to clavulanic acid and also has moderate activity against the class I enzymes.

Studies done in our laboratories on the structural modification of the penem nucleus culminated in the synthesis of compounds containing an alkylidene moiety at the C6 position of the penem ring system (I. S. Bennett, G. Brooks, N. J. P. Broom, K. Coleman, S. Coulton, R. A. Edmundson, D. R. J. Griffin, J. B. Harbridge, N. F. Osborne, I. Stirling-Francois, and G. Walker, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 118, 1988). This series lacked any clinically useful antibacterial activity but proved to have β-lactamase inhibitory activity of a degree and spectrum not seen with any previous agent. This paper describes the preliminary evaluation of BRL 42715 (Fig. 1), one of the most active members of this series (15; Bennett et al., 28th ICAAC).

## MATERIALS AND METHODS

**Antibacterial agents.** Sodium amoxicillin, potassium clavulanate, sodium sulbactam, nitrocefin, tazobactam and BRL 42715 were prepared by Beecham Pharmaceuticals; all data on these compounds are expressed in terms of pure free acid. Other antibacterial agents were obtained as commercial preparations.

**Enzyme inhibition studies.** The concentration of inhibitor needed to reduce the initial rate of hydrolysis of nitrocefin by 50% ($I_{50}$ value) was recorded for a range of β-lactamases. The enzymes were crude cell extracts prepared by ultrasonication, as described by Reading and Cole (16). Nitrocefin at 250 μg/ml was added to the reaction mixture, either immediately or after preincubating the enzyme with the test inhibitor for 5 min at 37°C. The reaction rate was subsequently measured spectrophotometrically at 482 nm (18).

**Organisms.** Most of the bacterial strains used in this study were clinical isolates collected from various sources around the world. The β-lactamases produced by the reference strains in Tables 1 and 2 were identified by substrate profile and isoelectric focusing by the methods described by Matthew et al. (12). All amoxicillin-resistant strains of *Esche-*

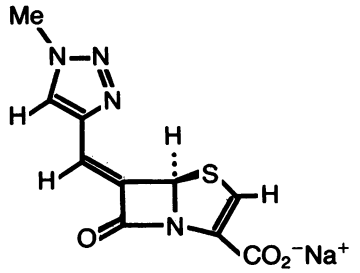


FIG. 1. Chemical structure of BRL 42715.

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TABLE 1. Intrinsic β-lactamase inhibitory activity of BRL 42715 compared with those of other β-lactamase inhibitors

| Organism[a] | Enzyme class[b] | $I_{50}$ values (μg/ml) for enzyme with (+) or without (−) 5 min preincubation with: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | BRL 42715 | | Clavulanic acid | | Sulbactam | | Tazobactam | |
| | | − | + | − | + | − | + | − | + |
| *Enterobacter cloacae* P 99 | Ia | 0.069 | 0.002 | >50 | >50 | >50 | 5.0 | >50 | 0.93 |
| *Escherichia coli* JT 410 | Ib | 0.013 | 0.001 | >50 | >50 | >50 | 7.6 | >50 | 2.9 |
| *Bacteroides fragilis* 11295/BC 4 | Ic | 1.2 | 0.005 | 1.4 | 0.006 | 4.8 | 0.041 | 6.3 | 0.03 |
| *Proteus vulgaris* H | Ic | 0.009 | 0.003 | 0.84 | 0.017 | 1.8 | 0.12 | 0.32 | 0.006 |
| *Pseudomonas aeruginosa* A | Id | 0.13 | 0.002 | >50 | >50 | >50 | 2.9 | 21.0 | 0.97 |
| *Proteus mirabilis* C 889 | II | 1.4 | 0.009 | 3.6 | 0.021 | 2.9 | 0.057 | 1.0 | 0.006 |
| *Escherichia coli* JT 4 (TEM-1) | III | 0.044 | 0.002 | 0.88 | 0.055 | 3.0 | 1.7 | 0.12 | 0.028 |
| *Escherichia coli* K-12 R1010 (SHV-1) | III | 0.037 | 0.001 | 2.4 | 0.035 | 29.5 | 13.0 | 0.68 | 0.14 |
| *Klebsiella pneumoniae* E 70 | IV | 0.036 | 0.001 | 1.0 | 0.011 | 15.7 | 3.8 | 0.68 | 0.047 |
| *Klebsiella oxytoca* 1082 (K1) | IV | 0.093 | 0.019 | 3.2 | 0.047 | 27.5 | 4.5 | 0.71 | 0.038 |
| *Escherichia coli* K-12 RGN238 (OXA-1) | V | 0.29 | 0.001 | >50 | 0.71 | >50 | 2.2 | >50 | 1.1 |
| *Escherichia coli* K-12 pMG19 (PSE-4) | V | 12.5 | 0.13 | 2.0 | 0.022 | 3.6 | 0.29 | 0.42 | 0.025 |
| *Staphylococcus aureus* NCTC 11561 | | 3.3 | 0.016 | >50 | 0.063 | >50 | 1.4 | >50 | 0.27 |

[a] Enzymes in parentheses were produced particularly by the strains shown.
[b] Class based on the classification of Richmond and Sykes (19).

*richia coli* and *Klebsiella pneumoniae* in Table 3 were examined by isoelectric focusing and, when necessary, substrate profile to determine the type(s) of β-lactamase produced.

For a number of the class I β-lactamase-producing organisms, enzyme activity was determined by measuring the amount of cephaloridine hydrolyzed per minute per milligram (dry weight) of cells. This reaction was carried out at 37°C, pH 7.3, and monitored at 299 nm.

**Susceptibility testing.** MICs were determined by twofold serial dilution of the antibiotic either alone or in the presence of a fixed concentration of β-lactamase inhibitor, as described in guidelines for the National Committee for Clinical Laboratory Standards (21). Mueller-Hinton agar (Difco Laboratories) was used for all organisms except the following: *B. fragilis* (Wilkins-Chalgren agar; Oxoid Ltd.), *Haemophilus influenzae* (nutrient agar plus 5% Fildes extract; Oxoid), and *Branhamella catarrhalis* and *Neisseria gonorrhoeae* (Mueller-Hinton agar plus 5% defibrinated horse blood; Difco).

Inocula were prepared by diluting overnight broth cultures of all organisms to a final concentration of approximately $10^7$ CFU/ml, except for *Staphylococcus aureus*, which was used undiluted. The overnight cultures were in brain heart infusion broth, with the following exceptions: *B. fragilis* (BACTO cooked meat medium [Difco]), *H. influenzae* (Oxoid nutrient broth plus 5% Fildes extract), *Branhamella catarrhalis*, and *N. gonorrhoeae* (nutrient agar plus 5% horse blood). Surface growth was removed and suspended in broth to a final concentration of approximately $10^7$ CFU/ml.

Volumes (1 μl) of bacterial suspensions were spotted onto the surfaces of agar plates with a multipoint inoculator and were incubated for 18 to 24 h at 37°C aerobically. *B. fragilis* was incubated in an anaerobic cabinet in an atmosphere of $N_2$-$H_2$-$CO_2$ (80:10:10). *N. gonorrhoeae* and *Branhamella catarrhalis* were incubated in a 5% $CO_2$ atmosphere. The MIC was determined as the lowest concentration that completely inhibited growth, disregarding a single colony or faint haze.

MIC determinations in liquid media were carried out in microdilution plates by serial dilution of antibiotics in tryptone soy broth (Oxoid), followed by addition of 1 μg of the inhibitor per ml. The test organisms were then added to a final concentration of approximately $2 \times 10^6$ CFU/ml. The total volume per well was 100 μl. The MIC was recorded after aerobic incubation at 37°C for 18 h as the lowest concentration to inhibit visible growth.

TABLE 2. Protective effect of various concentrations of BRL 42715 on amoxicillin activity against bacteria producing a range of different β-lactamases

| Organism[a] | Enzyme class[b] | Amoxicillin MIC (μg/ml) in the presence of a BRL 42715 concn (μg/ml) of: | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4 | 1 | 0.25 | 0.06 | 0.016 | 0 |
| *Escherichia coli* JT410 | Ib | 2 | 2 | 2 | 4 | 16 | 256 |
| *Proteus vulgaris* Q3618 | Ic | 1 | 1 | 1 | 1 | 1 | >256 |
| *Proteus mirabilis* C889 | II | 1 | 2 | 8 | 32 | 64 | >256 |
| *Escherichia coli* JT39 (TEM-1) | III | 0.5 | 1 | 2 | 4 | 16 | 128 |
| *Klebsiella pneumoniae* E70 | IV | 2 | 2 | 2 | 4 | 8 | 256 |
| *Escherichia coli* P91 (OXA-1) | V | 2 | 4 | 2 | 16 | 64 | >256 |

[a] Enzymes in parentheses were produced particularly by the strains shown.
[b] Classification based on that of Richmond and Sykes (19).

## RESULTS

**β-Lactamase inhibitory activity.** The penem BRL 42715 showed potent and progressive inhibitory activity against a wide range of β-lactamases (Table 1). In most cases the $I_{50}$ values, both with and without preincubation, were at least 1 to 2 orders of magnitude lower than those observed for other β-lactamase inhibitors.

Both clavulanic acid and tazobactam were good inhibitors of the plasmid-mediated class III enzymes TEM-1 and SHV-1, but BRL 42715 was a better inhibitor, with $I_{50}$ values 30- to 140-fold lower against these two enzymes. BRL 42715 also proved a potent inhibitor of the class V plasmid-mediated enzyme OXA-1, against which the other inhibitors had poor activity, and was slightly more potent than the

TABLE 3. Antibacterial activity of amoxicillin alone and in combination with BRL 42715 and clavulanic acid

| Organism (no. of strains) | Test agent[a] | MIC (μg/ml)[b] | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Citrobacter freundii* (25)[c] | Amx | 16–>128 | 128 | >128 |
| | Amx + PE (1)[d] | 0.25–64 | 1 | 2 |
| | Amx + PE (5) | 0.25–8 | 1 | 4 |
| | Amx + CA (5) | 8–>128 | 128 | >128 |
| | CA | 8–64 | 16 | 16 |
| | PE | >64 | >64 | >64 |
| | Cefotaxime | 0.03–2 | 0.25 | 1 |
| *Citrobacter freundii* (11)[e] | Amx | >128 | >128 | >128 |
| | Amx + PE (1) | 1–16 | 2 | 4 |
| | Amx + PE (5) | 0.5–2 | 1 | 2 |
| | Amx + CA (5) | >128 | >128 | >128 |
| | CA | 8–32 | 16 | 32 |
| | PE | >64 | >64 | >64 |
| | Cefotaxime | 8–32 | 16 | 32 |
| *Enterobacter aerogenes* (7)[c] | Amx | 32–>128 | >128 | |
| | Amx + PE (1) | 1–8 | 4 | |
| | Amx + PE (5) | 1–8 | 4 | |
| | Amx + CA (5) | 32–>128 | >128 | |
| | CA | 16–32 | 32 | |
| | PE | 64–>64 | >64 | |
| | Cefotaxime | 0.06–0.5 | 0.25 | |
| *Enterobacter aerogenes* (5)[e] | Amx | >128 | >128 | |
| | Amx + PE (1) | 2–16 | 4 | |
| | Amx + PE (5) | 0.5–8 | 2 | |
| | Amx + CA (5) | >128 | >128 | |
| | CA | 16–32 | 16 | |
| | PE | 64–>64 | 64 | |
| | Cefotaxime | 8–32 | 16 | |
| *Enterobacter cloacae* (16)[c] | Amx | 16–>128 | >128 | >128 |
| | Amx + PE (1) | 0.5–64 | 4 | 8 |
| | Amx + PE (5) | 1–16 | 4 | 8 |
| | Amx + CA (5) | 32–>128 | >128 | >128 |
| | CA | 32–64 | 32 | 64 |
| | PE | 64–128 | >64 | >64 |
| | Cefotaxime | 0.06–1 | 0.25 | 0.5 |
| *Enterobacter cloacae* (9)[e] | Amx | >128 | >128 | |
| | Amx + PE (1) | 2–>128 | 16 | |
| | Amx + PE (5) | 1–64 | 4 | |
| | Amx + CA (5) | >128 | >128 | |
| | CA | 32–64 | 32 | |
| | PE | 32–>64 | >64 | |
| | Cefotaxime | 8–32 | 32 | |
| *Escherichia coli* (10) | Amx | 4–16 | 8 | 8 |
| | Amx + PE (1) | 1–2 | 1 | 2 |
| | Amx + PE (5) | 0.5–2 | 1 | 2 |
| | Amx + CA (1) | 2–8 | 4 | 8 |
| | Amx + CA (5) | 2–4 | 2 | 4 |
| | CA | 8–16 | 16 | 16 |
| | PE | 16–64 | 32 | 32 |
| *Escherichia coli* (104)[f] | Amx | >128 | >128 | >128 |
| | Amx + PE (1) | 1–>128 | 2 | 8 |
| | Amx + PE (5) | 0.25–128 | 1 | 2 |
| | Amx + CA (1) | 8–>128 | 32 | 128 |
| | Amx + CA (5) | 1–128 | 4 | 16 |
| | CA | 8–32 | 16 | 16 |
| | PE | 8–64 | 32 | 32 |
| *Escherichia coli* (32)[g] | Amx | 64–>128 | >128 | >128 |
| | Amx + PE (1) | 0.5–16 | 1 | 4 |

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TABLE 3—*Continued*

| Organism (no. of strains) | Test agent[a] | MIC (μg/ml)[b] | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| | Amx + PE (5) | 0.5–4 | 1 | 4 |
| | Amx + CA (5) | 8–>128 | 128 | >128 |
| | CA | 8–32 | 16 | 16 |
| | PE | 16–32 | 32 | 32 |
| *Haemophilus influenzae* (11) | Amx | 8–>64 | >64 | >64 |
| | Amx + PE (0.05) | 0.25–16 | 0.5 | 1 |
| | Amx + CA (0.05) | 1–32 | 2 | 8 |
| | CA | 2–>64 | 32 | 32 |
| | PE | 6–64 | 32 | 32 |
| *Klebsiella pneumoniae* (56)[f] | Amx | 32–>128 | >128 | >128 |
| | Amx + PE (1) | 0.5–>128 | 2 | 32 |
| | Amx + PE (5) | 0.25–16 | 2 | 8 |
| | Amx + CA (1) | 1–>128 | 32 | >128 |
| | Amx + CA (5) | 0.5–128 | 2 | 64 |
| | CA | 16–32 | 32 | 32 |
| | PE | 16–64 | 64 | 64 |
| *Klebsiella pneumoniae* (20) | Amx | 4–>128 | 64 | 128 |
| | Amx + PE (1) | 0.5–2 | 1 | 2 |
| | Amx + PE (5) | 0.25–4 | 1 | 2 |
| | Amx + CA (1) | 1–4 | 2 | 2 |
| | Amx + CA (5) | 0.5–2 | 1 | 2 |
| | CA | 16–64 | 32 | 32 |
| | PE | 16–128 | 64 | 64 |
| *Klebsiella oxytoca* (16) | Amx | >128 | >128 | >128 |
| | Amx + PE (1) | 1–>128 | 8 | >128 |
| | Amx + PE (5) | 0.5–256 | 2 | 32 |
| | Amx + CA (1) | 32–>128 | 128 | >128 |
| | Amx + CA (5) | 8–128 | 16 | 64 |
| | CA | 16–32 | 32 | 32 |
| | PE | 16–64 | 32 | 64 |
| *Morganella morganii* (16) | Amx | 64–>128 | 128 | >128 |
| | Amx + PE (1) | 0.5–4 | 0.5 | 1 |
| | Amx + PE (5) | 0.5–2 | 0.5 | 1 |
| | Amx + CA (5) | 64–>128 | 128 | >128 |
| | CA | 64–>64 | >64 | >64 |
| | PE | 16–32 | 32 | 32 |
| *Proteus mirabilis* (27) | Amx | 64–>128 | >128 | >128 |
| | Amx + PE (1) | 0.5–128 | 4 | 128 |
| | Amx + PE (5) | 0.5–16 | 2 | 16 |
| | Amx + CA (1) | 1–128 | 64 | 128 |
| | Amx + CA (5) | 0.5–64 | 16 | 64 |
| | CA | 16–32 | 32 | 32 |
| | PE | 8–16 | 16 | 16 |
| *Proteus vulgaris* (27) | Amx | 8–>128 | 8 | >128 |
| | Amx + PE (1) | 0.5–128 | 1 | 8 |
| | Amx + PE (5) | 0.25–64 | 1 | 2 |
| | Amx + CA (1) | 0.5–512 | 2 | 16 |
| | Amx + CA (5) | 0.25–64 | 1 | 4 |
| | CA | 32–128 | 64 | 64 |
| | PE | 16–128 | 32 | 64 |
| *Providencia alcalifaciens* (7) | Amx | 64–>128 | >128 | |
| | Amx + PE (1) | 1–>128 | 4 | |
| | Amx + PE (5) | 0.5–>128 | 4 | |
| | Amx + CA (5) | 16–256 | 128 | |
| | CA | 64–>64 | >64 | |
| | PE | 16–>64 | 64 | |
| *Providencia rettgeri* (14) | Amx | 16–>128 | 128 | >128 |
| | Amx + PE (1) | 0.5–2 | 1 | 1 |
| | Amx + PE (5) | 0.5–2 | 0.5 | 1 |
| | Amx + CA (5) | 32–>128 | 64 | >128 |

*Continued on following page*

TABLE 3—*Continued*

| Organism (no. of strains) | Test agent[a] | MIC (μg/ml)[b] | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| | CA | 64–>64 | >64 | >64 |
| | PE | 32–>64 | 64 | >64 |
| *Providencia stuartii* (14) | Amx | 32–>128 | 128 | 128 |
| | Amx + PE (1) | 0.5–4 | 1 | 4 |
| | Amx + PE (5) | 0.25–4 | 0.5 | 4 |
| | Amx + CA (5) | 32–>128 | 128 | >128 |
| | CA | 32–>64 | 64 | >64 |
| | PE | 8–64 | 32 | 32 |
| *Pseudomonas aeruginosa* (37) | Amx | >512 | >512 | >512 |
| | Amx + PE (5) | 0.5–512 | 32 | 128 |
| | Amx + PE (20) | 0.25–128 | 16 | 64 |
| | Amx + CA (20) | 32–>512 | >512 | >512 |
| | CA | 64–>64 | >64 | >64 |
| | PE | 64–>64 | >64 | >64 |
| | Ticarcillin | 1–128 | 16 | 32 |
| *Serratia marcescens* (21) | Amx | 8–>128 | 64 | >128 |
| | Amx + PE (1) | 1–2 | 2 | 2 |
| | Amx + PE (5) | 1–2 | 1 | 2 |
| | Amx + CA (5) | 16–>128 | 64 | 128 |
| | CA | 64–>64 | 64 | 64 |
| | PE | >64 | >64 | >64 |
| *Branhamella catarrhalis* (12) | Amx | 8–32 | 16 | 32 |
| | Amx + PE (0.05) | 0.12–0.5 | 0.5 | 0.5 |
| | Amx + CA (0.05) | 0.12–1 | 0.25 | 0.5 |
| | CA | 8–32 | 16 | 16 |
| | PE | 8–32 | 16 | 16 |
| *Neisseria gonorrhoeae* (4) | Amx | 2–32 | 2 | |
| | Amx + PE (0.05) | 0.06–0.25 | 0.06 | |
| | Amx + CA (0.05) | 0.5–1 | 0.5 | |
| | CA | 2–4 | 2 | |
| | PE | 2–4 | 4 | |
| *Bacteroides fragilis* (30) | Amx | 16–128 | >128 | >128 |
| | Amx + PE (0.2) | 0.25–32 | 1 | 8 |
| | Amx + PE (1) | 0.06–8 | 0.25 | 2 |
| | Amx + CA (0.2) | 0.25–>128 | 1 | 32 |
| | Amx + CA (1) | 0.25–64 | 0.5 | 4 |
| | CA | 8–64 | 8 | 16 |
| | PE | 4–16 | 4 | 16 |
| | Metronidazole | 0.25–4 | 0.5 | 1 |
| *Staphylococcus aureus* (21) | Amx | 64–>128 | >128 | >128 |
| | Amx + PE (0.05) | 0.25–4 | 1 | 2 |
| | Amx + PE (0.2) | 0.06–0.5 | 0.12 | 0.25 |
| | Amx + CA (0.05) | 2–64 | 16 | 32 |
| | Amx + CA (0.2) | 0.5–8 | 2 | 4 |
| | CA | 8–32 | 16 | 32 |
| | PE | 0.25–1 | 1 | 1 |
| | Methicillin | 1–4 | 2 | 4 |
| *Staphylococcus aureus* (8) | Amx | 32–>128 | >128 | >128 |
| | Amx + PE (0.1) | 32–128 | 32 | 64 |
| | Amx + PE (0.5) | 2–64 | 32 | 64 |
| | Amx + CA (0.1) | 32–>128 | 128 | >128 |
| | Amx + CA (0.5) | 32–128 | 64 | 128 |
| | CA | >64 | >64 | >64 |
| | PE | 32–>64 | >64 | >64 |
| | Methicillin | 16–>128 | >64 | >128 |

[a] Amx, Amoxicillin; PE, BRL 42715; CA, clavulanic acid.
[b] 50% and 90%, MIC for 50% and 90% of strains tested, respectively.
[c] Susceptible to cefotaxime (MIC, <4 μg/ml).
[d] Inhibitor concentration (μg/ml).
[e] Resistant to cefotaxime (MIC, >4 μg/ml).
[f] Strains known to produce a plasmid-mediated β-lactamase.
[g] Strains producing AmpC chromosomal β-lactamase.

TABLE 4. Antibacterial activity of BRL 42715 and clavulanic acid

| Organism[a] | MIC (μg/ml) | |
|---|---|---|
| | BRL 42715 | Clavulanic acid |
| *Citrobacter freundii* E 8 | 32 | 32 |
| *Enterobacter aerogenes* T 765 | 64 | 64 |
| *Enterobacter cloacae* T 626 | 64 | 64 |
| *Escherichia coli* NCTC 10418 | 32 | 32 |
| *Escherichia coli* E 96 (TEM-1) | 16 | 32 |
| *Escherichia coli* JT 450 (AmpC) | 16 | 32 |
| *Haemophilus influenzae* NCTC 11931 | 4 | 64 |
| *Klebsiella pneumoniae* Ba 95 (TEM-1) | 64 | 32 |
| *Klebsiella pneumoniae* E 70 | 64 | 64 |
| *Proteus mirabilis* C 889 | 16 | 16 |
| *Proteus vulgaris* Q 3618 | 16 | 32 |
| *Pseudomonas aeruginosa* NCTC 10662 | >64 | >64 |
| *Bacteroides fragilis* NCTC 10581 | 4 | 8 |
| *Branhamella catarrhalis* Ravasio | 16 | 16 |
| *Neisseria gonorrhoeae* F81 | 4 | 4 |
| *Corynebacterium xerosis* NCTC 2086 | 1 | 1 |
| *Enterobacter faecalis* ATCC 29212 | >16 | >64 |
| *Staphylococcus aureus* NCTC 6571 | 0.5 | 8 |
| *Staphylococcus aureus* NCTC 11561 | 1 | 16 |
| *Staphylococcus aureus* SH-1-CM (MRSA) | 16 | >64 |
| *Staphylococcus epidermidis* 54813 | 2 | 64 |
| *Streptococcus pneumoniae* CT 7 | 4 | 32 |
| *Streptococcus pyogenes* B 9 | 8 | 64 |

[a] Enzymes in parentheses were produced particularly by the strains shown.

other inhibitors against staphylococcal β-lactamase. Clavulanic acid and tazobactam had slightly better activity than BRL 42715 against the PSE-4 enzyme.

BRL 42715 proved the most effective inhibitor of all classes of chromosomal β-lactamase except for the class II enzyme produced by *Proteus mirabilis* and the class Ic enzyme produced by *B. fragilis*, against which all four inhibitors had similar activities. The chromosomal class I cephalosporinases, with the exception of the Ic enzymes, were poorly inhibited by clavulanic acid and only moderately inhibited by sulbactam and tazobactam. In contrast, BRL 42715 showed extremely good inhibitory activity against a broad range of these β-lactamases.

**Antibacterial activity.** BRL 42715 at MICs of 16 to 64 μg/ml showed poor antibacterial activity against the majority of organisms (Table 4), but was somewhat more active against *Streptococcus pyogenes* and *Streptococcus pneumoniae* (4 to 8 μg/ml), *B. fragilis* and *N. gonorrhoeae* (4 μg/ml), and *Corynebacterium xerosis* (1 μg/ml). Notable antibacterial activity was seen against methicillin-susceptible strains of *S. aureus* (0.5 to 1 μg/ml), although MICs for methicillin-resistant strains were generally in excess of 8 μg/ml.

**Potentiation of antibacterial activity in vitro.** Table 2 shows the protective effect of various concentrations of BRL 42715 on amoxicillin activity against a variety of β-lactamase-producing bacteria. An inhibitor concentration of 0.25 μg/ml proved sufficient to render all seven organisms susceptible to amoxicillin (MIC, <16 μg/ml), and some remained susceptible even at a BRL 42715 concentration as low as 0.016 μg/ml.

In Table 3, the activity of amoxicillin alone and in combination with BRL 42715 or clavulanic acid at concentrations of 1 and 5 μg/ml has been determined for a large number of bacteria. Both inhibitors showed good potentiation of amoxicillin activity against *E. coli*, *K. pneumoniae*, *H. influenzae*, *S. aureus*, and *N. gonorrhoeae* producing plasmid-mediated β-lactamases and against *K. pneumoniae*, *Klebsiella oxytoca*, *P. vulgaris*, *P. mirabilis*, and *Branhamella catarrhalis* producing chromosomally mediated enzymes. BRL 42715 gave better protection than clavulanic acid against all of these organisms except for *K. pneumoniae* producing a chromosomally mediated class IV enzyme and *Branhamella catarrhalis*, for which both inhibitors were equally active. The improved activity seen with BRL 42715 was particularly striking against strains of *E. coli* and *K. pneumoniae* producing plasmid-mediated enzymes, for which a level of 1 μg of penem per ml was at least as effective as 5 μg of clavulanic acid per ml. We have examined the enzymes produced by these organisms and found that most produced TEM or SHV enzymes and that very few produced an OXA-type enzyme.

Clavulanic acid failed to enhance the activity of amoxicillin against all class I β-lactamase-producing organisms except *P. vulgaris* and *B. fragilis*. In contrast, BRL 42715 was a very broad-spectrum inhibitor, and 1 μg/ml was sufficient to protect amoxicillin from hydrolysis by virtually all class I β-lactamase-producing species of *Enterobacteriaceae*, including *E. coli* and *Enterobacter*, *Citrobacter*, *Morganella*, *Providentia*, and *Serratia* spp. A penem concentration of 20 μg/ml reduced the amoxicillin MIC for 90% of the class Id β-lactamase-producing *Pseudomonas aeruginosa* strains from >512 to 64 μg/ml.

Some cefotaxime-resistant *Enterobacter* and *Citrobacter* strains which, cephaloridine hydrolysis studies indicated, produce higher levels of enzyme than do wild-type strains were included in this study (data not shown). Many of these high-level producers were susceptible to amoxicillin in the presence of 1 μg of BRL 42715 per ml, and virtually all were susceptible to amoxicillin plus 5 μg of BRL 42715 per ml.

From the data in Table 5, it can be seen that the protective effect of BRL 42715 was not restricted to amoxicillin. Both cefotaxime-susceptible and cefotaxime-resistant class I β-lactamase-producing strains were resistant to cefazolin alone but susceptible to cefazolin plus BRL 42715. Likewise, piperacillin, cefotaxime, and ceftazidime were all ineffective against the high-level class I β-lactamase-producing strains when tested alone, but when tested in combination with 1 μg of BRL 42715 per ml, the MICs obtained were generally much closer to those achieved against cefotaxime-susceptible strains.

## DISCUSSION

The improvement seen in $I_{50}$ values with preincubation suggests that BRL 42715, like clavulanic acid (17), is a suicide or active-site-directed inhibitor of β-lactamases. The $I_{50}$ values obtained against most β-lactamases after preincubation are from 1.0 to 0.1 μg/ml for sulbactam (17) and from 0.1 to 0.01 μg/ml for clavulanic acid (17) and tazobactam. In all but one case, the $I_{50}$ values obtained with BRL 42715 were <0.02 μg/ml, the exception being the PSE-4 enzyme, for which the $I_{50}$ value of 0.13 μg/ml was somewhat higher than that seen with clavulanic acid or tazobactam. These low inhibitory concentrations are reflected in the in vitro activity seen against a number of β-lactamase-producing organisms; BRL 42715 levels of 0.06 to 0.25 μg/ml are sufficient to render them susceptible to amoxicillin. A similar effect is seen with a 1- to 2-μg/ml level of clavulanic acid (9) or tazobactam.

BRL 42715 is a potent inhibitor of a broad range of plasmid-mediated β-lactamases, including the class V OXA

TABLE 5. Comparative synergistic activities of BRL 42715 and tazobactam with a range of β-lactams against cefotaxime-susceptible and cefotaxime-resistant bacteria

| Inhibitor | Concn (μg/ml) of inhibitor | MIC (μg/ml) for: | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Citrobacter freundii* | | *Enterobacter aerogenes* | | *Enterobacter cloacae* | |
| | | E 8 | T 1739[a] | T 660 | 53[a] | T 626 | P 99[a] |
| Amoxicillin | | 256 | >512 | 128 | >512 | 128 | >512 |
| Amoxicillin + tazobactam | 4 | 16 | 512 | 64 | >512 | 128 | >512 |
| Amoxicillin + BRL 42715 | 1 | 1 | 1 | 1 | 2 | 2 | 64 |
| Piperacillin | | 4 | 512 | 32 | >512 | 4 | >512 |
| Piperacillin + tazobactam | 4 | 4 | 128 | 16 | 512 | 4 | 512 |
| Piperacillin + BRL 42715 | 1 | 2 | 4 | 8 | 16 | 1 | 2 |
| Cefazolin | | >512 | >512 | >512 | >512 | >512 | >512 |
| Cefazolin + tazobactam | 4 | 16 | >512 | 128 | >512 | 512 | >512 |
| Cefazolin + BRL 42715 | 1 | 2 | 4 | 8 | 2 | 4 | 16 |
| Cefotaxime | | 1 | 64 | 0.5 | 64 | 0.25 | >64 |
| Cefotaxime + tazobactam | 4 | 1 | 32 | 1 | >64 | 0.5 | 64 |
| Cefotaxime + BRL 42715 | 1 | <0.06 | 0.5 | 0.5 | 2 | 0.13 | 2 |
| Ceftazidime | | 16 | >64 | 2 | >64 | 0.5 | 64 |
| Ceftazidime + tazobactam | 4 | 1 | >64 | 1 | >64 | 0.25 | 64 |
| Ceftazidime + BRL 42715 | 1 | 0.25 | 0.5 | 0.5 | 32 | 0.25 | 0.5 |
| Tazobactam | | 32 | 64 | >128 | 64 | 128 | >128 |
| BRL 42715 | | 128 | >128 | >128 | >128 | >128 | 128 |

[a] Cefotaxime-resistant strain.

group of enzymes, against which the other three inhibitors have poor activity. Both class III (TEM) and class V (OXA) enzymes occur commonly in β-lactam-resistant strains of *E. coli* and *K. pneumoniae* (11), and the high inhibitory activity of BRL 42715 makes it a potentially useful agent against these two clinically important species.

Many clinical isolates of *S. aureus* produce a plasmid-mediated β-lactamase, and both BRL 42715 and clavulanic acid are good inhibitors of this enzyme. These β-lactamase-producing strains are resistant to amoxicillin, but in the presence of clavulanic acid they become susceptible (8). BRL 42715 reduces the amoxicillin MIC for all of these β-lactamase-producing strains to a value about 16-fold lower than an equivalent concentration of clavulanic acid, because of slightly better inhibitory activity of BRL 42715, although the good antistaphylococcal activity of this compound may be a contributory factor. Both BRL 42715 and clavulanic acid give some limited protection to amoxicillin against β-lactamase-producing, methicillin-resistant strains of *S. aureus*.

All four of the β-lactamase inhibitors included in this study showed good activity against the class Ic cefuroximase enzymes of *P. vulgaris* and *B. fragilis*, but against the remaining class I enzymes clavulanic acid is known to be inactive (16), and sulbactam (5, 6) and tazobactam (10) have only limited inhibitory activity. On the other hand, the inhibitory activity of BRL 42715 extends to all of the class I cephalosporinase enzymes. Many of the organisms which produce a class I β-lactamase show inducible expression of the enzyme (20), but there are a growing number of reports of isolates which have high-level constitutive expression of the enzyme (4, 7, 22). Such overproducers are resistant to broad-spectrum cephalosporins, and one interesting property of BRL 42715 is its ability to neutralize this resistance and render many of these organisms fully susceptible. This protection extends to the ureidopenicillins and to more labile compounds, such as amoxicillin and cefazolin, so that they too become effective agents against these very resistant organisms.

Potentiation of amoxicillin by BRL 42715 could also be seen against ticarcillin-susceptible strains of *Pseudomonas aeruginosa* which produce the class Id Sabbath and Abraham enzyme. Although the penem is a very potent inhibitor of this enzyme, potentiation can only be demonstrated with high inhibitor levels. The outer membrane of *Pseudomonas aeruginosa* is a very effective barrier to the majority of β-lactams (13, 23), and this is possibly the reason why such high levels of BRL 42715 are required.

It has long been known that amoxicillin-susceptible strains of *E. coli* produce a class I noninducible β-lactamase (the AmpC enzyme; 2) but in levels so low that amoxicillin MICs are only slightly elevated. BRL 42715 is an effective inhibitor of the AmpC enzyme, and potentiation of amoxicillin activity can be seen quite clearly against these amoxicillin-susceptible strains. Most amoxicillin-resistant strains of *E. coli* produce plasmid-mediated β-lactamases, but some produce elevated levels of this chromosomally mediated AmpC enzyme (2), and BRL 42715 affords good protection to amoxicillin against all such strains.

In conclusion, BRL 42715, a C6-triazolylmethylene penem, is an extremely potent β-lactamase inhibitor. The spectrum and degree of activity observed with this novel agent represent a significant improvement over other available β-lactamase inhibitors.

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