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Potent activity of BAY 73-7388, a novel aminomethylcycline, against susceptible and resistant Gram-positive and Gram-negative organisms

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Objectives: BAY 73-7388 is the first of a new class of antibiotics, the aminomethylcyclines, which evolved from the tetracycline (TET) family. BAY 73-7388 has potent activity against antibiotic susceptible and resistant Gram-positive and Gram-negative pathogens. The present study compared the activity *in vitro* of BAY 73-7388 and 10 other agents including vancomycin (VAN), linezolid (LIN), levofloxacin (LVX) and TET against recent clinical isolates including MRSA, VAN-resistant *Enterococcus faecium* (Efa VRE), *Enterococcus faecalis* (Ef), penicillin-resistant *Streptococcus pneumoniae* (Spn PENR), Groups A and B beta-haemolytic streptococci (BHS), *Escherichia coli* (Ec), and other pathogens. Potential microbiological interactions between BAY 73-7388 and other antibiotics were also assessed.

Methods: Microdilution MIC tests were performed according to NCCLS guidelines. The activity of BAY 73-7388 in the presence of other antibacterial agents was assessed using standard chequerboard MIC methods. TET-resistance determinants were identified using multiplex PCR.

Results: Susceptibility *in vitro* (MIC₉₀ mg/L) for selected agents is shown in the table below.

| Strains | MIC 90 (mg/L) | | | |
|---------------------------|---------------|-------|------|-------|
| | BA Y 73-7388 | VA N | LI N | LV X |
| MRSA (<i>n</i> = 39) | 0.5 | 1.0 | 2.0 | 32.0 |
| Efa VRE (<i>n</i> = 19) | 0.5 | >64.0 | 2.0 | >64.0 |
| Ef (<i>n</i> = 31) | 0.5 | 2.0 | 2.0 | 32.0 |
| Spn PENR (<i>n</i> = 23) | 0.06 | 0.25 | 1.0 | 1.0 |
| BHS (<i>n</i> = 48) | 0.25 | 0.5 | 1.0 | 0.5 |
| Ec (<i>n</i> = 23) | 2.0 | NA | NA | 4.0 |
| | | | | |

Conclusions: BAY 73-7388 has potent activity *in vitro* against a range of common pathogens, including those resistant to currently available antibiotics: most notably MRSA, VRE and penicillin-resistant *S. pneumoniae*. Chequerboard studies *in vitro* demonstrate BAY 73-7388 does not affect, and is not affected by, the activity of other antibiotics.

(BAY 73-7388 was discovered by Paratek Pharmaceuticals Inc., Boston, MA, and designated PTK 0796.)

POTENT ACTIVITY OF BAY 73-7388, A NOVEL AMINOMETHYL-CYCLINE, AGAINST SUSCEPTIBLE AND RESISTANT GRAM-POSITIVE AND GRAM-NEGATIVE ORGANISMS

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Abstract

Objectives:

BAY 73-7388 is the lead compound of a new class of antibiotics, the aminomethylcyclines, which evolved from the tetracycline (TET) family. BAY 73-7388 has potent activity against antibiotic-susceptible and resistant gram-positive and gram-negative pathogens. The present study compared the activity *in vitro* of BAY 73-7388 and 10 other agents including vancomycin (VAN), linezolid (LZD), levofloxacin (LVX) and TET against recent clinical isolates including methicillin-resistant *Staphylococcus aureus* (MRSA), VAN-resistant *Enterococcus faecium* (Efa VRE), *Enterococcus faecalis* (Ef), penicillin-resistant *Streptococcus pneumoniae* (Spn PENR), Groups A and B beta-haemolytic streptococci (BHS), *Escherichia coli* (Ec), and other pathogens. Potential microbiological interactions between BAY 73-7388 and other antibiotics were also assessed.

Methods:

Microdilution MIC tests were performed according to NCCLS guidelines. The activity of BAY 73-7388 in the presence of other antibacterial agents was assessed using standard checkerboard MIC methods. TEI-resistance determinants were identified using multiplex polymerase chain reaction (PCR).

Results:

Susceptibility *in vitro* (MIC 90 mg/L) for selected agents is shown in the Table below.

Conclusions:

BAY 73-7388 has potent activity *in vitro* against a range of common pathogens, including those resistant to currently available antibiotics: most notably MRSA, VRE and penicillin-resistant *S. pneumoniae*. Checkerboard studies *in vitro* demonstrate BAY 73-7388 does not affect, and is not affected by, the activity of other antibiotics.

(BAY 73-7388 was discovered by Paratek Pharmaceuticals Inc., Boston, MA, and designated PTK 0796.)

Introduction

- BAY 73-7388, 7-dimethylamino-9-(2,2-dimethylpropyl)-aminomethylcycline is the first of a new class of antibiotic, the aminomethylcyclines (Figure 1)
- In this study, the activities of BAY 73-7388 and comparator compounds were determined against a variety of aerobic gram-positive and gram-negative bacteria including strains resistant to currently available classes of antibiotics
- The activity of BAY 73-7388 was determined alone and in combination with antibiotics having differing mechanisms of action

Methods

Strains

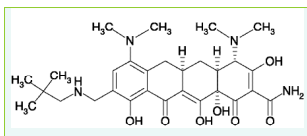
- Recent clinical isolates obtained from 7 laboratories within the United States
- Appropriate quality control strains were included in each test

MIC Determination

- By current NCCLS-recommended microdilution method¹
- Broth

- Cation-adjusted Mueller Hinton broth for staphylococci, enterococci, and gram-negative rods
- Cation-adjusted Mueller Hinton broth supplemented with 5% horse blood for streptococci
- Haemophilus test medium for *Haemophilus*

Figure 1. Structure of BAY 73-7388.



- Inoculum: Organisms were grown (or direct inoculum for fastidious organisms) to a 0.5 McFarland standard. Turbidity was measured using a Microscan turbidity meter.
- Incubation at 35°C for 18 to 24 hours depending on the organisms tested

Chequerboard MIC

- By standard broth microdilution method²
- Fractional inhibitory concentration index was calculated as FIC_A + FIC_B where FIC represents the minimum concentrations that inhibited growth for compounds A and B/ the MIC of A or B alone.

Table 1. Comparative *in vitro* activity of BAY 73-7388 vs aerobic gram-positive bacteria

| Organism | N | Compound | MIC (mg/L) | | | Organism | N | Compound | MIC (mg/L) | | |
|----------------------------------|-------------|--------------|-------------|-------------------|-------------------|-------------------------------------|-----|--------------|------------|-------------------|-------------------|
| | | | Range | MIC ₅₀ | MIC ₉₀ | | | | Range | MIC ₅₀ | MIC ₉₀ |
| S. aureus | 55 | BAY 73-7388 | <0.06-1.0 | 0.125 | 0.5 | E. faecalis | 31 | BAY 73-7388 | 0.125-5.0 | 0.25 | 0.5 |
| | | Vancomycin | 0.25-2 | 0.5 | 1 | | | Linezolid | 0.5-4 | 1 | 2 |
| | | Linezolid | 0.5-2.0 | 2 | 2 | | | Linezolid | 1.0-4.0 | 1 | 2 |
| | | Levofloxacin | <0.06-64.0 | 4 | 32 | | | Levofloxacin | 0.5-4 | 1 | 32 |
| | | Azithromycin | 0.25-64.0 | >64.0 | >64.0 | | | Azithromycin | 0.125-64.0 | 8 | >64.0 |
| | | Clindamycin | <0.06-64.0 | 0.125 | >64.0 | | | Clindamycin | 2.0-64.0 | 32 | >64.0 |
| | | Cefotaxime | 1.0-64.0 | 32 | >64.0 | | | Tetracycline | 0.125-64.0 | 32 | 64 |
| | | Tetracycline | <0.06-64.0 | 0.125 | 64 | | | Minoocycline | 0.125-16 | 8 | 16 |
| | | Doxycycline | <0.06-16.0 | 0.125 | 8 | | | Doxycycline | <0.06-16.0 | 4 | 16 |
| | | Doxycycline | <0.06-8.0 | <0.06 | 8 | | | | | | |
| S. aureus: Methicillin-resistant | 39 | BAY 73-7388 | 0.125-1.0 | 0.25 | 0.5 | E. faecalis: Multiresistant | 3 | BAY 73-7388 | 0.25-0.5 | NA | NA |
| | | Vancomycin | 0.25-2.0 | 0.5 | 1 | | | Vancomycin | 0.5-4.0 | NA | NA |
| | | Linezolid | 0.25-2.0 | 0.5 | 1 | | | Linezolid | 1 | NA | NA |
| | | Levofloxacin | 0.5-64.0 | 8 | 32 | | | Levofloxacin | 16.0-64.0 | NA | NA |
| | | Azithromycin | 0.5-64.0 | >64.0 | >64.0 | | | Azithromycin | >64.0 | NA | NA |
| | | Clindamycin | <0.06-64.0 | >64.0 | >64.0 | | | Clindamycin | >64.0 | NA | NA |
| | | Cefotaxime | <0.06-64.0 | >64.0 | >64.0 | | | Tetracycline | 32.0-64.0 | NA | NA |
| | | Tetracycline | <0.06-64.0 | 0.25 | 64 | | | Minoocycline | 8.0-16.0 | NA | NA |
| | | Minoocycline | <0.06-16.0 | 0.25 | 8 | | | Doxycycline | 4 | NA | NA |
| | | Doxycycline | <0.06-8.0 | 0.125 | 8 | | | | | | |
| S. aureus: Methicillin-sensitive | 16 | BAY 73-7388 | <0.06-0.25 | 0.125 | 0.125 | S. pneumoniae | 41 | BAY 73-7388 | <0.06-0.25 | <0.06 | 0.125 |
| | | Vancomycin | 0.25-0.5 | 0.5 | 0.5 | | | Vancomycin | <0.06-0.5 | 0.06 | 0.125 |
| | | Linezolid | 1.0-2.0 | 1 | 2 | | | Linezolid | 0.25-2.0 | 1.0 | 1.0 |
| | | Levofloxacin | 0.5-64.0 | 0.125 | 0.125 | | | Levofloxacin | 0.25-1.0 | 0.5 | 1.0 |
| | | Azithromycin | 0.5-64.0 | 0.125 | 0.125 | | | Azithromycin | <0.06-64.0 | 2.0 | >64.0 |
| | | Clindamycin | <0.06-0.25 | 0.5 | 0.5 | | | Clindamycin | <0.06-64.0 | <0.06 | >64.0 |
| | | Cefotaxime | 0.25-32.0 | 0.5 | 0.5 | | | Cefotaxime | <0.06-8.0 | 1.0 | 2.0 |
| | | Tetracycline | <0.06-0.125 | <0.06 | 0.125 | | | Penicillin | 0.06-0.125 | 2.0 | 4.0 |
| | | Minoocycline | 1.0-2.0 | 2 | 2 | | | Tetracycline | <0.06-64.0 | 16.0 | 32.0 |
| | | Doxycycline | <0.06-16.0 | <0.06 | 0.125 | | | Minoocycline | <0.06-8.0 | 2.0 | 8.0 |
| Doxycycline | <0.06-0.125 | <0.06 | 0.125 | Doxycycline | <0.06-4.0 | 2.0 | 4.0 | | | | |
| S. aureus: Multiresistant | 10 | BAY 73-7388 | 0.25-0.5 | 0.5 | 0.5 | S. pneumoniae: Penicillin-resistant | 23 | BAY 73-7388 | <0.06 | <0.06 | <0.06 |
| | | Vancomycin | 0.5-1.0 | 1 | 1 | | | Vancomycin | 0.125-0.25 | 0.25 | 0.25 |
| | | Linezolid | 0.5-2.0 | 1 | 1 | | | Linezolid | 0.5-2.0 | 1.0 | 1.0 |
| | | Levofloxacin | 6.0-32.0 | 8 | 32 | | | Levofloxacin | 0.5-1.0 | 0.5 | 1.0 |
| | | Azithromycin | >64.0 | >64.0 | >64.0 | | | Azithromycin | <0.06-64.0 | 4.0 | >64.0 |
| | | Clindamycin | >64.0 | >64.0 | >64.0 | | | Clindamycin | <0.06-64.0 | <0.06 | >64.0 |
| | | Cefotaxime | 32.0-64.0 | >64.0 | >64.0 | | | Penicillin | 2.0-8.0 | 4.0 | 8.0 |
| | | Tetracycline | 32.0-64.0 | >64.0 | >64.0 | | | Tetracycline | <0.06-64.0 | 32.0 | 2.0 |
| | | Minoocycline | 2.0-16.0 | 8 | 8 | | | Minoocycline | 0.125-8.0 | 8.0 | 8.0 |
| | | Doxycycline | 2.0-8.0 | 8 | 8 | | | Doxycycline | <0.06-4.0 | 4.0 | 4.0 |
| E. faecium | 24 | BAY 73-7388 | 0.125-0.5 | 0.25 | 0.5 | S. pneumoniae: Multiresistant | 18 | BAY 73-7388 | <0.06 | <0.06 | <0.06 |
| | | Vancomycin | 0.5-64.0 | >64.0 | >64.0 | | | Vancomycin | 0.125-0.25 | 0.125 | 0.25 |
| | | Linezolid | 0.5-1.0 | 1 | 1 | | | Linezolid | 0.5-1.0 | 1.0 | 1.0 |
| | | Levofloxacin | 1.0-64.0 | 64 | >64.0 | | | Levofloxacin | 0.5-1.0 | 0.5 | 1.0 |
| | | Azithromycin | <0.06-64.0 | >64.0 | >64.0 | | | Azithromycin | 2.0-64.0 | >64.0 | >64.0 |
| | | Clindamycin | <0.06-64.0 | >64.0 | >64.0 | | | Clindamycin | <0.06-64.0 | >64.0 | >64.0 |
| | | Cefotaxime | 0.125-64.0 | 32 | 64 | | | Cefotaxime | 0.5-8.0 | 1.0 | 8.0 |
| | | Tetracycline | 0.125-32.0 | 8 | 16 | | | Penicillin | 2.0-8.0 | 4.0 | 8.0 |
| | | Minoocycline | 0.25-32.0 | 2 | 8 | | | Minoocycline | 16.0-64.0 | 32.0 | 32.0 |
| | | Doxycycline | <0.06-16.0 | 2 | 8 | | | Doxycycline | 4.0-8.0 | 8.0 | 8.0 |
| E. faecium: Vancomycin-resistant | 16 | BAY 73-7388 | 0.125-0.5 | 0.25 | 0.5 | S. pyogenes | 30 | BAY 73-7388 | <0.06-0.5 | 0.125 | 0.25 |
| | | Vancomycin | 64.0-64.0 | >64.0 | >64.0 | | | Vancomycin | 0.25 | 0.25 | 0.25 |
| | | Linezolid | 0.5-4.0 | 2 | 2 | | | Linezolid | 0.5 | 1.0 | 1.0 |
| | | Levofloxacin | 1.0-64.0 | 64 | >64.0 | | | Levofloxacin | 0.25-1.0 | 0.25 | 1.0 |
| | | Azithromycin | >64.0 | >64.0 | >64.0 | | | Azithromycin | <0.06-64.0 | <0.06 | >64.0 |
| | | Clindamycin | >64.0 | >64.0 | >64.0 | | | Clindamycin | <0.06-64.0 | <0.06 | >64.0 |
| | | Cefotaxime | 0.125-64.0 | 32 | 64 | | | Cefotaxime | 0.5-8.0 | 1.0 | 8.0 |
| | | Tetracycline | 0.125-32.0 | 8 | 16 | | | Penicillin | 2.0-8.0 | 4.0 | 8.0 |
| | | Minoocycline | 0.25-32.0 | 2 | 8 | | | Minoocycline | 16.0-64.0 | 32.0 | 32.0 |
| | | Doxycycline | <0.06-8.0 | 2 | 8 | | | Doxycycline | 4.0-8.0 | 8.0 | 8.0 |
| E. faecium | 12 | BAY 73-7388 | 0.125-0.5 | 0.25 | 0.5 | S. agalactiae | 18 | BAY 73-7388 | <0.06-0.25 | 0.125 | 0.125 |
| | | Vancomycin | >64.0 | >64.0 | >64.0 | | | Vancomycin | 0.125-0.25 | 0.25 | 0.5 |
| | | Linezolid | 0.5-2.0 | 1 | 2 | | | Linezolid | 1.0-1.0 | 1.0 | 1.0 |
| | | Levofloxacin | 8.0-64.0 | 32 | >64.0 | | | Levofloxacin | 0.125-0.5 | 0.5 | 0.5 |
| | | Azithromycin | >64.0 | >64.0 | >64.0 | | | Azithromycin | 0.125-0.5 | 0.5 | 0.5 |
| | | Clindamycin | >64.0 | >64.0 | >64.0 | | | Clindamycin | <0.06-8.0 | <0.06 | 0.125 |
| | | Tetracycline | 32.0-64.0 | 32 | >64.0 | | | Tetracycline | <0.06-8.0 | <0.06 | 0.125 |
| | | Minoocycline | 4.0-16.0 | 8 | 16 | | | Minoocycline | <0.06-8.0 | <0.06 | 0.125 |
| | | Doxycycline | 2.0-8.0 | 2 | 4 | | | Doxycycline | <0.06-8.0 | <0.06 | 0.125 |

Results

Gram-Positive Aerobic Bacteria (Table 1)

- BAY 73-7388 inhibited all strains of
 - Methicillin-sensitive *Staphylococcus aureus* (MIC₉₀ = 0.125 mg/L)
 - Methicillin-resistant *Staphylococcus aureus* (MIC₉₀ = 0.5 mg/L)
 - Methicillin-resistant and multiresistant (multiR) *Staphylococcus aureus* (MIC₉₀ = 0.5 mg/L)
 - Enterococcus faecium*, including vancomycin-resistant and vancomycin-resistant, multiR strains (MIC₉₀ = 0.5 mg/L)
 - Enterococcus faecalis*, including vancomycin-resistant and vancomycin-resistant, multiR strains (MIC₉₀ = 0.5 mg/L)
 - Streptococcus pneumoniae* including penicillin-resistant and penicillin-resistant, multiR strains (MIC₉₀ ≤ 0.06 mg/L)
 - Streptococcus pyogenes* (MIC₉₀ = 0.25 mg/L)
 - Streptococcus agalactiae* (MIC₉₀ = 0.125 mg/L)
- BAY 73-7388 was the most active compound tested against all gram-positive bacteria

Select Gram-Negative Aerobic Bacteria (Table 2)

- BAY 73-7388 inhibited all strains of
 - Escherichia coli* (MIC₉₀ = 2.0 mg/L)
 - Klebsiella pneumoniae* (MIC₉₀ = 4.0 mg/L)
 - Haemophilus influenzae* (MIC₉₀ = 2.0 mg/L)

Table 2. Comparative *in vitro* activity of BAY 73-7388 vs *Escherichia coli*, *Klebsiella pneumoniae*, and *Haemophilus influenzae*

| Organism | N | Compound | MIC (mg/L) | | | | |
|---------------|-----------|---------------|------------|-------------------|-------------------|------|-------|
| | | | Range | MIC ₅₀ | MIC ₉₀ | | |
| E. coli | 23 | BAY 73-7388 | 0.5-2.0 | 1.0 | 2.0 | | |
| | | Ampicillin | 2.0-64.0 | >64.0 | >64.0 | | |
| | | Gentamicin | <0.06-0.5 | <0.06 | 0.125 | | |
| | | Clindamycin | 0.25-64.0 | 1.0 | 8.0 | | |
| | | Ciprofloxacin | <0.06-32.0 | <0.06 | 8.0 | | |
| | | Levofloxacin | <0.06-16.0 | <0.06 | 4.0 | | |
| | | Tetracycline | 0.5-8.0 | 2.0 | >64.0 | | |
| | | Minoocycline | 0.5-16.0 | 1.0 | 8.0 | | |
| | | Doxycycline | 0.5-16.0 | 1.0 | 8.0 | | |
| | | | | | | | |
| K. pneumoniae | 14 | BAY 73-7388 | 1.0-8.0 | 2.0 | 4.0 | | |
| | | Cefotaxime | <0.06-64.0 | <0.06 | 32.0 | | |
| | | Gentamicin | 0.5-32.0 | 0.5 | 32.0 | | |
| | | Ciprofloxacin | <0.06-64.0 | <0.06 | >64.0 | | |
| | | Levofloxacin | <0.06-8.0 | <0.06 | 32.0 | | |
| | | Minoocycline | 0.5-8.0 | 2.0 | >64.0 | | |
| | | Doxycycline | 1.0-16.0 | 2.0 | 32.0 | | |
| | | | | | | | |
| | | H. influenzae | 53 | BAY 73-7388 | 0.5-8.0 | 1.0 | 2.0 |
| | | | | Ampicillin | <0.06-64.0 | 64.0 | >64.0 |
| Cefotaxime | <0.06-1.0 | | | <0.06 | <0.06 | | |
| Azithromycin | 1.0-2.0 | | | 1.0 | 2.0 | | |
| Levofloxacin | <0.06-8.0 | | | <0.06 | <0.06 | | |
| Minoocycline | 0.125-8.0 | | | 2.0 | 32.0 | | |
| Doxycycline | 0.125-8.0 | | | 0.5 | 32.0 | | |
| | | | | | | | |

Chequerboard (Table 3)

- No interactions were observed between BAY 73-7388 and any of the antibiotics tested

Conclusions

- BAY 73-7388 is highly active *in vitro* against a broad spectrum of pathogens, most notably:
 - Methicillin-resistant *Staphylococcus aureus*
 - Vancomycin-resistant *Enterococcus*
 - Penicillin-resistant *Streptococcus pneumoniae*
 - Escherichia coli*
 - Klebsiella pneumoniae*
 - Haemophilus influenzae*

BAY 73-7388 is active *in vitro* against organisms which are resistant to multiple classes of antibiotics including:

- Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* also resistant to tetracyclines, levofloxacin, azithromycin, and clindamycin
- Penicillin-resistant *Streptococcus pneumoniae* also resistant to tetracyclines, cefotaxime, azithromycin,