P925

Activity of BAY 73-7388, a novel aminomethylcycline, and other novel antibiotic classes against resistant bacteria *in vitro*

B. Bhatia, T. Bowser, J. Chen, M. Ismail, L. Honeyman, R. Mechiche, M. Nelson, K. Ohemeng, A. Verma, K. Tanaka, A. Macone
Boston, USA

Objectives: The emergence of antibiotic resistance among Gram-positive pathogens has impacted the clinical management of these infections. Paratek Pharmaceuticals initiated a programme to apply medicinal chemistry to the core structure of tetracycline (TET) with the goal of creating novel classes of proprietary antibiotics that would (a) be unaffected by the known TET resistance mechanisms and (b) retain the safety and tolerability profile of the TET family. Since there is no cross-resistance between the TETs and other antibiotics, such new agents would be expected to be active against isolates resistant to all other currently available classes. The aim of the programme was to synthesise new agents active against Gram-positive, common Gram-negative, atypical and anaerobic bacteria.

Methods: A series of 7-position and 7,9-position derivatives of sancycline were synthesised and tested for activity *in vitro* against MRSA, VRE, *Enterococcus faecalis* and *Streptococcus pneumoniae* by microdilution. The presence of TET-resistance determinants was assessed by PCR and confirmed by resistance to currently available TETs.

Results: A number of 7-dimethylamino-9-aminomethylcyclines (AMC) and 7-aryl or heteroaryl sancyclines with potent activity *in vitro* (MIC range less than or equal to 0.06–2.0 mg/L) were identified. Both novel series were more potent against one or more of the resistant strains than currently available antibiotics tested (MIC range 16–64 mg/L). The AMC derivatives were active against bacteria resistant to TET by both efflux and ribosome-protection mechanisms.

Conclusions: This study identified the AMCs as a novel class of antibiotics evolved from TET that exhibit potent activity *in vitro* against TET-resistant bacteria, including Gram-positive bacteria resistant to currently available antibiotics. One agent of this class, BAY 73-7388 (discovered by Paratek Pharmaceuticals, Inc., Boston, MA, and designated PTK 0796) has been chosen for development.

ACTIVITY OF BAY 73-7388, A NOVEL AMINOMETHYLCYCLINE, AND OTHER NOVEL ANTIBIOTIC CLASSES AGAINST RESISTANT BACTERIA *IN VITRO*

B. Bhatia, T. Bowser, J. Chen, M. Ismail, L. McIntyre, R. Mechiche, M. Nelson, K. Ohemeng, A. Verma, S.K. Tanaka* and A. Macone

Paratek Pharmaceuticals, Inc., Boston, MA.

Abstract

Objectives:

The emergence of antibiotic resistance among gram-positive pathogens has impacted the clinical management of these infections. Paratek Pharmaceuticals initiated a programme to apply medicinal chemistry to the core structure of tetracycline (TET) with the goal of creating novel classes of proprietary antibiotics that would (a) be unaffected by the known TET resistance mechanisms and (b) retain the safety and tolerability profile of the TET family. Since there is no cross-resistance between the TETs and other antibiotics, such new agents would be expected to be active against isolates resistant to all other currently available classes.The aim of the programme was to synthesize new agents active against gram-positive, common gram-negative, atypical, and anaerobic bacteria.

Methods:

A series of 7-position and 7,9-position derivatives of sancycline were synthesized and tested for activity *in vitro* against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Enterococcus faecalis*, and *Streptococcus pneumoniae* by microdilution.The presence of TET-resistance determinants was assessed by polymerase chain reaction (PCR) and confirmed by resistance to currently available tetracyclines.

Results:

A number of 7-dimethylamino-9-aminomethylcyclines (AMC) and 7-aryl or heteroaryl sancyclines with potent activity *in vitro* (MIC range, ≤0.06-2.0 mg/L) were identified. Both novel series were more potent against one or more of the resistant strains than currently available antibiotics tested (MIC range, 16-64 mg/L).The AMC derivatives were active against bacteria resistant to TET by both efflux and ribosome-protection mechanisms.

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This study identified the AMCs as a novel class of antibiotics evolved from TET that exhibit potent activity *in vitro* against TET-resistant bacteria, including gram-positive bacteria resistant to currently available antibiotics. One agent of this class, BAY 73-7388 (discovered by Paratek Pharmaceuticals, Inc., Boston, MA, and designated PTK 0796) has been chosen for development.

Introduction

The emergence of antibiotic resistance among bacterial pathogens responsible for serious infections is impacting clinical management strategies, and there is an urgent need for novel classes of antibiotics not affected by resistance to current agents. A number of approaches have been developed in the search for new classes of antibiotics, including applying novel chemistry techniques to known core structures.The tetracycline class of natural products has been used for more than 50 years as broad-spectrum antibiotics (Figure 1).[1] The mechanism of action of tetracyclines is distinct from all other classes of antibiotics, meaning that they do not exhibit cross-resistance. In order to take advantage of the low toxicity and broad spectrum of activity typically displayed by the tetracyclines, Paratek Pharmaceuticals, Inc. has developed a medicinal chemistry approach to create new classes of antibiotics. The novel classes designed are active against the tetracycline resistance mechanisms of efflux and/or ribosome protection and exhibit no cross resistance to other classes of antibiotics.

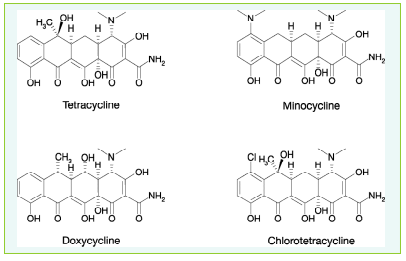
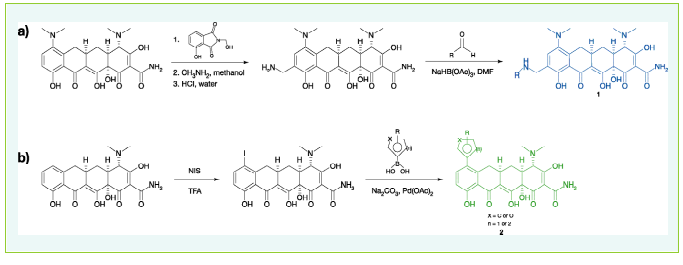


Figure 1. Tetracycline structures.

Methods

- Novel 7-dimethylamino-9-aminomethylcyclines (AMC, 1) and 7-aryl and heteroaryl sancyclines (2) were synthesized as outlined in Scheme 1
- MIC values were obtained for each new analog against a broad range of tetracycline-resistant and -susceptible strains including gram-positive strains which were demonstrated by PCR to contain ribosome protection resistance (Tet M) and/or efflux resistance (Tet K, Tet L). Microdilution MIC assays were performed following NCCLS guidelines
- The MIC values obtained for the novel classes were compared with the values of representative members of other classes: tetracyclines (tetracycline and minocycline), glycopeptides (vancomycin), and quinolones (ciprofloxacin)

Scheme 1. Synthesis of novel classes of antibiotics (aminomethylcyclines and sancyclines)
a) 7-dimethylamino-9-aminomethylcyclines (BAY 73-7388, 1a-c);
b) 7-aryl and heteroaryl sancyclines (2a-d).[2]



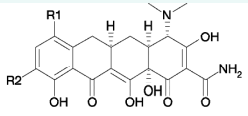
Results

- The novel AMCs (BAY 73-7388, 1a-c) and 7-aryl and heteroaryl sancyclines (2a-d) were active *in vitro* against all resistant strains tested (MICs 0.06-1 µg/mL) (Table 1)
- All analogs tested were active against both ribosome protection (TetM) resistance and efflux resistance (Tet K, Tet L)
- Both series of novel classes (AMCs and sancyclines) were more potent than the representatives of currently available classes (MIC range 16-64) against ≥1 of the resistant strains tested (Table 1)

Conclusions

- The novel classes of aminomethylcyclines and sancyclines designed by medicinal chemistry demonstrate potent activity *in vitro* against gram-positive bacteria
- Aminomethylcyclines and sancyclines are not affected by resistance to currently available classes of antibiotics including quinolones, glycopeptides, and tetracyclines (both ribosome protection and efflux resistance mechanisms)

Table 1. MIC values (mg/L) for aminomethylcyclines (BAY 73-7388, 1a-c), 7-aryl and heteroaryl sancyclines (2a-d) and comparators against strains with known tetracycline resistance mechanisms (Tet K = efflux, Tet M = ribosome protection, Tet L = efflux). Values in RED indicate resistance



Compound	R1	R2	*S. aureus* RN4250 (Tet K)	*S. aureus* MRSA5 (Tet M)	*E. faecalis* JH2-2 + pAM211 (Tet M)	*E. faecalis* JH2-2 + pMV158 (Tet L)	*E. faecium* 494 (Tet L + Tet M)	*S. pneu.* 700905 (Tet M)
BAY 73-7388	$N(CH_3)_2$		0.25	0.25	0.5	0.5	0.5	≤0.06
1a	$N(CH_3)_2$		0.5	0.5	0.5	0.25	0.25	0.125
1b	$N(CH_3)_2$		1	1	1	1	1	0.5
1c	$N(CH_3)_2$		1	1	1	1	1	≤0.06
2a		H	0.25	1	1	0.25	1	0.25
2b		H	≤0.06	0.5	1	≤0.06	1	1
2c		H	0.5	1	0.25	0.25	0.5	0.25
2d		H	0.25	1	1	≤0.06	1	0.5
Vancomycin			0.25	1	1	1	>64	0.25
Ciprofloxacin			0.5	16	1	1	1	1
Tetracycline			>64	32	>64	32	>64	32
Minocycline			0.5	2	16	0.5	16	8

References

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2.Nelson ML,Ismail MY, McIntyre L,et al. Versatile and facile synthesis of diverse semisynthetic tetracycline derivatives via Pd-catalyzed reactions. *J Org Chem*. 2003;68:5838-5851.