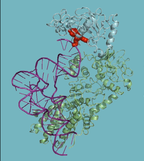

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Potency and Spectrum of Activity of AN3365, a Novel Boron-containing Protein Synthesis Inhibitor, Tested against Non-fermentative Gram-negative Bacilli

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Abstract

Background: AN3365, which has recently completed a phase I clinical trial, is a member of a novel class of boron-containing antibacterial protein synthesis inhibitors which inhibits leucyl-tRNA synthetase via an unique mechanism of action. The *in vitro* activity of AN3365 was assessed against wildtype and multidrug-resistant (MDR) strains of non-fermentative Gram-negative bacilli.

Methods: A total of 252 strains, including MDR subsets, collected from American and European hospitals were selected. Species consisted of *P.* aeruginosa (wildtype [50], MDR metallo-β-lactamase producers [MBL; 26] and MDR non-MBL producers [MDR MBL-negative; 25]); *A. baumannii* [wildtype; 25] and *Acinetobacter spp.* MDR [26]); *S. maltophilia* [50]; and *B. cepacia* [50]. AN3365 and nine comparators were tested for susceptibility by CLSI broth microdilution method (M07-A8) and interpretive criteria (M100-S20-U). The four most active comparators are shown alongside AN3365 (Table).

Results: AN3365 inhibited all tested *P. aeruginosa* at ≤16 μg/mL ($MIC_{50/90}$, 4/8 μg/mL), with slightly higher MIC_{50} values (4 μg/mL) for MDR MBL-negative and MBL subsets compared to wildtype (MIC_{50}, 2 μg/mL). AN3365 ($MIC_{50/90}$, 2/4 μg/mL) was very active against *S. maltophilia*, while less active against MDR *Acinetobacter* spp. ($MIC_{50/90}$, 8/16 μg/mL) and *B. cepacia* ($MIC_{50/90}$, 8/32 μg/mL).

Organism/group (No. tested)	MIC_{50} (Range) in μg/mL				
	AN 3365	Gentamicin	Imipenem	Levofloxacin	Tigecycline
P. aeruginosa (101)	8 (1-16)	>16 (≤0.5->16)	>64 (0.25->64)	>16 (≤0.5->16)	>16 (0.5->16)
WT (50)	8 (1-16)	4 (≤0.5->16)	2 (0.25-4)	2 (≤0.5->16)	16 (2->16)
MDR MBL-ve (25)	8 (1-16)	>16 (≤0.5->16)	32 (8-32)	>16 (≤0.5->16)	>16 (2->16)
MDR MBL (26)	8 (1-16)	>16 (8->16)	>64 (16->64)	>16 (≤0.5->16)	>16 (0.5->16)
A. baumannii WT (25)	8 (0.5-8)	2 (≤0.5->16)	0.25 (0.12-4)	4 (≤0.5->16)	1 (0.06-1)
Acinetobacter spp. MDR (26)	16 (4-32)	>16 (16->16)	>64 (8->64)	>16 (2->16)	8 (0.25-8)
S. maltophilia (50)	4 (0.12-8)	>16 (≤0.5->16)	>64 (16->64)	4 (≤0.5->16)	1 (0.12-2)
B. cepacia (50)	32 (0.5-128)	>16 (1->16)	32 (0.25->64)	8 (≤0.5-16)	4 (0.12-16)

WT, wildtype; MDR, multidrug-resistant; MBL-ve, metallo-β-lactamase-negative.

Conclusions: AN3365 was more potent than all nine comparators when tested against the MDR *P. aeruginosa* isolates, which warrants further development of AN3365 in managing difficult-to-treat infections caused by these MDR Gram-negative organisms.

Introduction

Opportunistic pathogens including *Pseudomonas aeruginosa, Acinetobacter* spp., *Burkholderia cepacia* and *Stenotrophomonas maltophilia* often infect seriously ill and/or immunocompromised patients. These species are clinically difficult to treat due to intrinsic resistance mechanisms, such as β-lactamase production, AmpC mediated resistance, efflux-pumps and outer membrane alterations (impermeability).

P. aeruginosa is the most commonly isolated non-fermentative species and can be implicated in numerous types of infection. Although strains harboring metallo-β-lactamase (MBL) enzymes remain rarely isolated in the United States (USA), *P. aeruginosa* with these enzymes have been documented worldwide and have become endemic in some countries. Class D β-lactamases (OXA) in *Acinetobacter baumannii* have emerged and disseminated globally. These isolates are often only susceptible to polymyxin B and tigecycline.

Trimethoprim/sulfamethoxazole is one of the only reliably active agents for the treatment of *S. maltophilia*, and unfortunately, resistance to this agent has now been detected on a global scale due to the acquisition of *sul* genes. Furthermore, multidrug resistance (MDR) is also common among *B. cepacia* and strains that exhibit resistance to essentially all commonly used antimicrobials have now been identified.

AN3365 (Figure 1) is a member of a new class of bacterial protein synthesis inhibitors, which inhibit leucyl-tRNA synthetase via a unique mechanism of action. This novel agent is being developed for the treatment of hospital/ventilator-associated pneumonia, complicated urinary tract and intra-abdominal infections. Herein, we report the potency and spectrum of activity for AN3365 against a large collection of contemporary Gram-negative non-fermentative bacilli associated with human infections. This collection was selected based upon susceptibility profiles (wildtype [WT] and MDR) and does not represent a prevalence mode design.

Materials and Methods

Bacterial isolates. The tested organisms were collected primarily from patients in USA and European medical centers during 2006-2008. A total of 72 medical centers contributed isolates to this investigation, which included those in the USA (30 sites), Canada (3 sites), Europe (31 sites) and Latin America (8 sites). Less commonly isolated species and those organisms with characterized resistance mechanisms were included from previous years. Nearly all of the tested isolates were from patients with bloodstream or respiratory tract infections.

Isolates included: *P. aeruginosa* (101 strains; including 51 carbapenem-resistant isolates [26 documented MBL producers]), *A. baumannii* (51; 26 MDR, ≥3 classes), *S. maltophilia* (50 WT) and *B. cepacia* (50 WT). All strains were identified by at least two laboratories including JMI Laboratories (North Liberty, Iowa, USA), which is a CLIA-certified and GLP-compliant reference laboratory.

Antimicrobial susceptibility testing. MIC values for AN3365 and comparator compounds were determined using the reference Clinical and Laboratory Standards Institute (CLSI) broth microdilution method as described in M07-A8 (2009). 96-well frozen-form assay panels were produced by JMI Laboratories using cation-adjusted Mueller-Hinton broth. AN3365 and the following comparator agents were tested: imipenem, piperacillin/tazobactam, ceftazidime, cefepime, levofloxacin, gentamicin and tigecycline (fresh frozen media). Quality control (QC) ranges and interpretive criteria for comparator compounds were as published in CLSI M100-S20-U (2010). Tigecycline breakpoints approved by the Food and Drug Administration (FDA) for Enterobacteriaceae (≤2 for susceptible; ≥8 for resistance) were applied for comparison purposes. Tested QC strains included *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

Results

- Overall, AN3365 exhibited a narrow MIC range when tested against *P. aeruginosa* (1 – 16 μg/mL; MIC_{50}, 2 – 4 μg/mL), wildtype *Acinetobacter* spp. (0.5 – 8 μg/mL; MIC_{50}, 2 μg/mL) and *S. maltophilia* (0.12 – 8 μg/mL; MIC_{50}, 2 μg/mL; Table 1). AN3365 MIC values were higher when tested against *B. cepacia* ($MIC_{50/90}$, 8/32 μg/mL) and MDR-*Acinetobacter* spp. ($MIC_{50/90}$, 8/16 μg/mL).
- AN3365 ($MIC_{50/90}$, 2/8 μg/mL) and cefepime ($MIC_{50/90}$, 2/8 μg/mL) were equally potent when tested against wildtype *P. aeruginosa* (Table 2). Resistance to the other comparison agents ranged from 2 to 8% and imipenem ($MIC_{50/90}$, 1/2 μg/mL) and levofloxacin ($MIC_{50/90}$, ≤0.5/2 μg/mL) were the most active compounds tested.
- AN3365 retained activity ($MIC_{50/90}$, 4/8 μg/mL) against the resistant subsets of *P. aeruginosa*, including carbapenem-resistant strains, regardless of MBL production (Table 2). Other comparison agents tested showed high resistance rates (≥44.0%).
- AN3365 ($MIC_{50/90}$, 2/8 μg/mL), piperacillin/tazobactam ($MIC_{50/90}$, ≤0.06/8 μg/mL; 100.0% susceptible) and cefepime ($MIC_{50/90}$, 2/8 μg/mL; 96.0% susceptible) displayed similar MIC_{90} values when tested against wildtype *Acinetobacter* spp. (Table 3). Whereas, imipenem ($MIC_{50/90}$, 0.25/0.25 μg/mL; 100.0% susceptible), tigecycline ($MIC_{50/90}$, 0.12/1 μg/mL) and gentamicin ($MIC_{50/90}$, ≤0.5/2 μg/mL; 92.0% susceptible) were also active.
- MDR *Acinetobacter* spp. had AN3365 MIC_{50} (8 μg/mL) and MIC_{90} (16 μg/mL) values two- to four-fold higher than wildtype strains ($MIC_{50/90}$, 2/8 μg/mL). Except for tigecycline ($MIC_{50/90}$, 2/8 μg/mL), comparator agents tested had limited activity against MDR *Acinetobacter* spp. (Table 3).
- Equivalent activities were demonstrated for AN3365 ($MIC_{50/90}$, 2/4 μg/mL) and levofloxacin ($MIC_{50/90}$, 1/4 μg/mL) when tested against *S. maltophilia*; while tigecycline exhibited ($MIC_{50/90}$, 0.5/1 μg/mL) potent activity when tested against this species (Table 4).
- A broader MIC range (0.5 – 128 μg/mL; $MIC_{50/90}$, 8/32 μg/mL) was noted for AN3365 when tested against *B. cepacia* isolates (Table 5). AN3365 activity was similar to those of cefepime ($MIC_{50/90}$, 8/32 μg/mL) and imipenem ($MIC_{50/90}$, 8/32 μg/mL).

Table 1. MIC frequency distribution of the investigational agent AN3365 tested against 252 isolates of non-enteric Gram-negative pathogens including strains with resistance phenotypes.

Organism (no. tested)[a]	Cumulative % of strains inhibited at each MIC (μg/mL)								
	≤0.5	1	2	4	8	16	32	64	128
P. aeruginosa (101)	0.0	5.9	33.7	74.3	95.0	100.0	–	–	–
WT (50)	0.0	8.0	56.0	88.0	98.0	100.0	–	–	–
MDR, MBL-negative (25)	0.0	4.0	8.0	52.0	92.0	100.0	–	–	–
MDR, MBL-positive (26)	0.0	3.9	15.4	69.2	92.3	100.0	–	–	–
Acinetobacter spp. (51)	2.0	3.9	27.5	58.8	92.2	98.0	100.0	–	–
WT (25)	4.0	8.0	56.0	88.0	100.0	–	–	–	–
MDR (26)	0.0	0.0	0.0	30.8	84.6	96.2	100.0	–	–
S. maltophilia (50)	8.0	38.0	66.0	96.0	100.0	–	–	–	–
B. cepacia (50)	2.0	4.0	12.0	42.0	66.0	82.0	96.0	98.0	100.0

a. WT, wildtype; MBL, metallo-β-lactamase; and MDR, multidrug-resistant.

Table 2. In vitro activity of AN3365 in comparison to selected antimicrobial agents tested against *P. aeruginosa*.

Microbial collection/ antimicrobial agent	MIC (μg/mL)			
	50%	90%	Range	% Susceptible/Resistant[a]
Wildtype (50)				
AN3365	2	8	1 – 16	– / –[b]
Piperacillin/tazobactam	4	32	0.5 – >128	94.0 / 6.0
Ceftazidime	4	16	≤1 – >32	88.0 / 8.0
Cefepime	2	8	≤1 – 32	92.0 / 2.0
Imipenem	1	2	0.25 – 4	100.0 / 0.0
Levofloxacin	≤0.5	2	≤0.5 – >16	92.0 / 6.0
Gentamicin	1	4	≤0.5 – >16	98.0 / 2.0
Tigecycline	4	16	2 – >16	– / –
MβL-negative (25)				
AN3365	4	8	1 – 16	– / –
Piperacillin/tazobactam	>128	>128	32 – >128	16.0 / 84.0
Ceftazidime	>32	>32	8 – >32	4.0 / 92.0
Cefepime	32	>32	8 – >32	4.0 / 72.0
Imipenem	16	32	8 – 32	0.0 / 84.0
Levofloxacin	>16	>16	1 – >16	8.0 / 84.0
Gentamicin	8	>16	≤0.5 – >16	32.0 / 44.0
Tigecycline	16	>16	2 – >16	– / –
MβL-positive (26)				
AN3365	4	8	1 – 16	– / –
Piperacillin/tazobactam	128	>128	32 – >128	26.9 / 73.1
Ceftazidime	>32	>32	32 – >32	0.0 / 100.0
Cefepime	>32	>32	16 – >32	0.0 / 88.5
Imipenem	>64	>64	16 – >64	0.0 / 100.0
Levofloxacin	16	>16	≤0.5 – >16	3.8 / 92.3
Gentamicin	>16	>16	8 – >16	0.0 / 96.2
Tigecycline	8	>16	0.5 – >16	– / –

a. Criteria as published by the CLSI [2010].
b. No breakpoint available.

Table 3. In vitro activity of AN3365 in comparison to selected antimicrobial agents tested against *A. baumannii*.

Microbial collection/ Antimicrobial agent	MIC (μg/mL)			
	50%	90%	Range	% Susceptible/Resistant[a]
Wildtype (25)				
AN3365	2	8	0.5 – 8	– / –[b]
Piperacillin/tazobactam	≤0.06	8	≤0.06 – 16	100.0 / 0.0
Ceftazidime	8	16	2 – 16	88.0 / 0.0
Cefepime	2	8	≤1 – 16	96.0 / 0.0
Imipenem	0.25	0.25	0.12 – 4	100.0 / 0.0
Levofloxacin	≤0.5	4	≤0.5 – >16	84.0 / 8.0
Gentamicin	≤0.5	2	≤0.5 – >16	92.0 / 8.0
Tigecycline[c]	0.12	1	0.06 – 1	100.0 / 0.0
Multidrug-resistant (26)				
AN3365	8	16	4 - 32	– / –
Piperacillin/tazobactam	>128	>128	≤0.06 – >128	3.8 / 96.2
Ceftazidime	>32	>32	32 – >32	0.0 / 100.0
Cefepime	32	>32	8 – >32	3.8 / 76.9
Imipenem	64	>64	8 – >64	0.0 / 92.3
Levofloxacin	16	>16	2 – >16	3.8 / 92.3
Gentamicin	>16	>16	16 – >16	0.0 / 100.0
Tigecycline[c]	2	8	0.25 – 8	76.9 / 11.5

a. Criteria as published by the CLSI [2010].
b. No breakpoint available.
c. Tigecycline breakpoints approved by the FDA for Enterobacteriaceae (≤2 for susceptible; ≥8 for resistance) were applied for comparison purposes.

Table 4. In vitro activity of AN3365 in comparison to selected antimicrobial agents tested against 50 wildtype isolates of *S. maltophilia*.

Microbial collection/ Antimicrobial agent	MIC (μg/mL)			
	50%	90%	Range	% Susceptible/Resistant[a]
AN3365	2	4	0.12 – 8	– / –[b]
Ceftazidime	32	>32	2 – >32	20.0 / 58.0
Imipenem	>64	>64	16 – >64	– / –
Levofloxacin	1	4	≤0.5 – >16	86.0 / 8.0
Gentamicin	16	>16	≤0.5 – >16	– / –
Tigecycline[c]	0.5	1	0.12 – 2	100.0 / 0.0

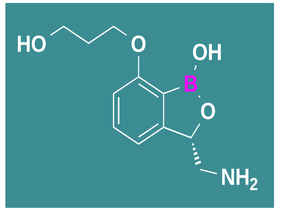
a. Criteria as published by the CLSI [2010].
b. No breakpoint available.
c. Tigecycline breakpoints approved by the FDA for Enterobacteriaceae (≤2 for susceptible; ≥8 for resistance) were applied for comparison purposes.

Table 5. In vitro activity of AN3365 in comparison to selected antimicrobial agents tested against 50 isolates of *B. cepacia*.

Microbial collection/ Antimicrobial agent	MIC (μg/mL)			
	50%	90%	Range	% Susceptible/Resistant[a]
AN3365	8	32	0.5 – 128	– / –[b]
Ceftazidime	4	16	≤1 – >32	86.0 / 6.0
Cefepime	8	32	≤1 – >32	– / –
Imipenem	8	32	0.25 – >64	– / –
Levofloxacin	1	8	≤0.5 – 16	70.0 / 20.0
Gentamicin	>16	>16	1 – >16	– / –
Tigecycline[c]	1	4	0.12 – 16	72.0 / 8.0

a. Criteria as published by the CLSI [2010].
b. No breakpoint available.
c. Tigecycline breakpoints approved by the FDA for Enterobacteriaceae (≤2 for susceptible; ≥8 for resistance) were applied for comparison purposes.

Figure 1. Chemical structure of AN3365 [GSK2251052].



Conclusions

- AN3365 sustained antimicrobial activity when tested against MDR *P. aeruginosa* ($MIC_{50/90}$, 4/8 μg/mL), exhibiting a significant potency advantage compared to other agents.
- AN3365 also demonstrated activity when tested against wildtype *Acinetobacter* spp. and *S. maltophilia*, while higher MIC values were noted against MDR *Acinetobacter* spp. and *B. cepacia.*
- These *in vitro* data warrant further development of AN3365 for potentially managing difficult-to-treat infections caused by these MDR Gram-negative organisms, especially *P. aeruginosa.*

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