

POSTER FI-840

ABSTRACT

Background: The rapid emergence of drug resistance in Gram-negative bacteria is causing alarm in hospitals and health care facilities around the world. With few new agents in the pipeline to address this growing threat, new antibiotics effective against drug resistant (R) strains of Gram-negative bacteria are desperately needed. A focused medicinal chemistry campaign identified the neoglycoside, a next-generation aminoglycoside (AG), ACHN-490 as a potentially useful agent. ACHN-490 is a broad-spectrum, rapidly bactericidal agent with excellent potency against Gram-negative and select Gram-positive bacteria. Unlike other AGs, ACHN-490 retains effectiveness against widespread and increasing resistance from AG-modifying enzymes (AMEs).

Methods: ACHN-490 was synthesized in 8 steps from sisomicin. ACHN-490 was tested for antibacterial activity using the CLSI microbroth dilution method against a panel of 26 organisms with characterized AG resistance mechanisms (AGRM). Activity was confirmed against a broader panel of 461 Gram-negative and Gram-positive clinical isolates. The AG-R organisms in this collection were selected to represent the most clinically relevant AGRM. AMEs in the collection were confirmed by colony PCR.

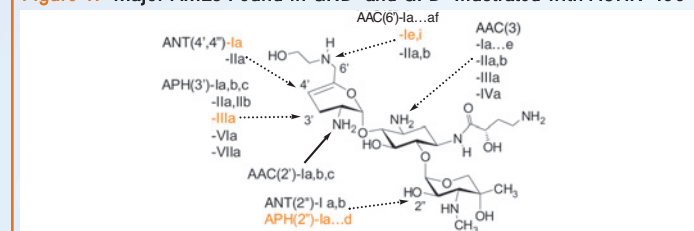
Results: The MIC₉₀s of ACHN-490 against AG-R Enterobacteriaceae were all ≤4 µg/mL with the exception of *Proteus mirabilis* and indole positive Proteae (MIC₉₀s 8 and 16 µg/mL respectively). The MIC₉₀ of ACHN-490 against AG-R staphylococci was 2 µg/mL. ACHN-490 was less active against AG-R *Pseudomonas aeruginosa* and *Acinetobacter baumannii* with changes in permeability/efflux.

Conclusion: ACHN-490 has emerged as a promising new antibacterial agent with potential to rejuvenate the AG class of antibiotics, and thus has been advanced into early clinical development.

INTRODUCTION

Aminoglycosides (AGs) are a well-established class of antibacterials that act by binding to the A-site of the bacterial ribosome and interfering with normal protein synthesis. Since their introduction 50 years ago, resistance to AGs has emerged. At present, the most significant contribution to clinical AG resistance is represented by the diversity of AG-modifying enzymes (AMEs; Figure 1) that inactivate compounds by N-acetylation (AAC), O-adenylylation (ANT), or O-phosphorylation (APH).¹ Numerous enzymes in each class have been identified, often occurring in combinations that can impart broad AG-resistance. As increasing resistance

Figure 1: Major AMEs Found in GNB^a and GPB^b Illustrated with ACHN-490^c



^a AMEs found in Gram-negative bacteria (GNB) shown in black
^b AMEs found in Gram-positive bacteria (GPB) shown in orange
^c Dashed arrows indicate AMEs that do not alter ACHN-490 activity

SYNTHESIS, STRUCTURE, AND *IN VITRO* ACTIVITY OF THE NEOGLYCOSIDE ACHN-490

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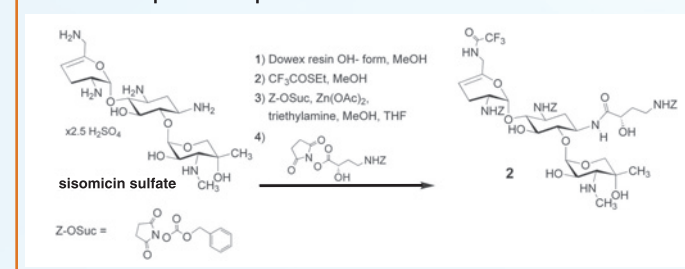
renders current AGs and other broad-spectrum antibiotics including the cephalosporins, carbapenems, tetracyclines, and fluoroquinolones ineffective, new AGs are an attractive option for the treatment of serious infections, especially if developed with improved dosing regimens to optimally balance safety and efficacy.² In the present study, we focused on the modification of sisomicin in search of substituents that gave broad improvements in *in vitro* potency against AG-resistant (AG-R) strains. The novel compound ACHN-490 showed promise in a screening panel, and thus was further challenged with a set of 461 recent clinical isolates. The majority of these carried AG-resistance mechanisms (AGRM), and many were also resistant to other classes of antibiotics.

METHODS

Synthesis of ACHN-490

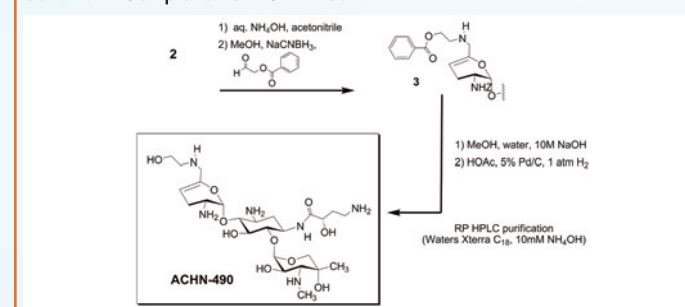
Sisomicin sulfate (SIS) was rendered basic by treatment with ion-exchange resin, followed by reaction with ethyl trifluoroacetate to selectively form the 6'-trifluoroacetamide (Scheme 1).³ Treatment of the 6'-trifluoroacetamide with Zn (II) acetate and Z-succinimide resulted in selective Z-blocking of 2 additional positions.^{4,5} Further reaction with the active ester of N-Z-(S)-Haba resulted in selective N-1 acylation to give compound 2.

Scheme 1: Sequential Tri-protection of SIS



Treatment of compound 2 with concentrated ammonia, followed by reductive alkylation with O-benzoyl glycolaldehyde appended the hydroxyethyl group to give compound 3 (Scheme 2). Finally, basic hydrolysis of the O-benzoyl group, followed by catalytic hydrogenolysis of the Z-groups and HPLC purification gave ACHN-490. Key structural assumptions were confirmed by LCMS fragmentation.

Scheme 2: Completion of ACHN-490



In vitro activity of ACHN-490

The antibacterial activity of ACHN-490 was determined using the CLSI broth microdilution method against:

- 26 strain screening panel with diverse AGRM (Table 1)
- 461 isolates from diverse geographic regions isolated 2004-2006 (Tables 2 and 3)
- GNB: Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.
- GPB: *Staphylococcus aureus* and *Staphylococcus epidermidis*
- AG-susceptible isolates (AG-S; amikacin [AMK] MICs of ≤16 µg/mL, gentamicin [GEN] and tobramycin [TOB] MICs of ≤4 µg/mL)
- AG-R isolates (AMK MIC >16 µg/mL and/or GEN MIC >4 µg/mL and/or TOB MIC >4 µg/mL)

The AMEs in the larger collection were assigned based on antibiograms using standard AGs.⁶ The genes for 6 of the most common AMEs in GNB and 7 in GPB were confirmed by colony PCR. The distribution of AGRM in this collection was similar to that observed in 2 surveys of the SENTRY collection in 2005 and 2007.⁷

RESULTS

Table 1: ACHN-490 Shows Improved Activity over SIS, AMK, and GEN against Strains with Defined AGRMs

Species	AGRM	MIC (µg/mL)			
		ACHN-490	SIS	AMK	GEN
<i>E. coli</i>	ATCC 25922 (QC)	1	1	2	0.5
	ANT(2')-I	0.5	32	4	64
	AAC(6')-I	0.25	16	64	2
	AAC(3)-II	1	>64	8	>64
	APH(3')-Ib	0.25	0.5	0.5	0.25
	AAC(3)-IVa	0.5	64	2	64
	armA methylase	>64	>64	>64	>64
<i>K. pneumoniae</i>	ATCC 10031	0.125	2	1	1
<i>P. stuartii</i>	AAC(2')-I	>64	64	8	64
<i>S. marcescens</i>	ATCC 27853 (QC)	2	1	4	2
	Wild-type pump	8	1	4	4
	Efflux pumps	0.125	0.5	0.5	0.125
	MexXY up	8	1	8	4
	Permeability	>64	8	>64	64
<i>P. aeruginosa</i>	ANT(4')-II	4	1	>64	2
	AAC(3)-I	8	16	8	64
	AAC(6')-II	1	32	4	64
	Susceptible	0.5	1	2	2
	ATCC 19606	32	8	32	32
<i>Acinetobacter</i> spp.	APH(3')-VI	0.5	1	>64	1
<i>A. calcoaceticus</i>	AAC(6')-I	1	32	>64	4
<i>S. aureus</i>	ATCC 29213 (QC)	0.5	1	16	0.5
	ANT(4')-I	1	1	>64	1
	APH(3')-III	0.25	0.5	2	1
	APH(2') + AAC(6')	4	>64	>64	>64
	ATCC 29713 (QC)	0.5	1	16	0.5

Orange shading indicates ACHN-490 MICs that are improved (>4-fold) relative to SIS

Table 2: MIC₉₀s of ACHN-490 and Comparators against AG-R Isolates. ACHN-490 Retains Activity in the Presence of AGRM that Inactivate AMK and GEN, and in the Presence of Resistance to Several Other Antibiotic Classes

	MIC ₉₀	MIC ₅₀	Range	
				ACHN-490
<i>Citrobacter</i> spp. n=9	ACHN-490	0.5	1	0.25 – 1
	AMK	32	64	2 – 64
	GEN	>64	>64	2 – >64
	BPR	1	>32	≤0.03 – >32
	CIP	8	>16	0.015 – >16
	IMI	0.5	1	0.125 – 4
<i>E. coli</i> n=24	ACHN-490	1	2	0.5 – 16
	AMK	16	32	2 – 64
	GEN	64	>64	1 – >64
	BPR	>32	>32	≤0.03 – >32
	CIP	>16	>16	<0.015 – >16
	IMI	0.25	0.25	0.06 – 0.5
<i>Enterobacter</i> spp. n=20	ACHN-490	0.5	1	0.25 – 8
	AMK	8	64	1 – >64
	GEN	32	>64	0.5 – >64
	BPR	>32	>32	0.06 – >32
	CIP	1	>16	≤0.015 – >16
	IMI	1	4	0.125 – >16
<i>Klebsiella</i> spp. n=45	ACHN-490	0.5	1	0.25 – 8
	AMK	32	>64	2 – >64
	GEN	32	>64	0.5 – >64
	BPR	>32	>32	0.125 – >32
	CIP	16	>16	≤0.015 – >16
	IMI	0.25	>16	0.06 – >16
<i>P. mirabilis</i> n=16	ACHN-490	4	8	1 – 16
	AMK	8	64	8 – >64
	GEN	32	>64	1 – >64
	BPR	0.125	>32	≤0.03 – >32
	CIP	2	>16	0.03 – >16
	IMI	4	8	0.25 – >16
Proteae, Indole+ n=8	ACHN-490	0.5	1	0.25 – 8
	AMK	32	>64	2 – >64
	GEN	32	>64	0.5 – >64
	BPR	>32	>32	0.06 – >32
	CIP	16	>16	1 – >16
	IMI	1	2	1 – 2
<i>Serratia</i> spp. n=9	ACHN-490	1	4	0.5 – 4
	AMK	8	>64	0.5 – >64
	GEN	64	>64	1 – >64
	BPR	>32	>32	0.5 – >32
	CIP	4	16	0.06 – 16
	IMI	1	2	0.125 – 2
<i>Acinetobacter</i> spp. n=67	ACHN-490	16	32	1 – >64
	AMK	>64	>64	4 – >64
	GEN	>64	>64	2 – >64
	BPR	>32	>32	0.5 – >32
	CIP	>16	>16	0.25 – >16
	IMI	2	>16	0.125 – >16
<i>P. aeruginosa</i> n=51	ACHN-490	8	64	0.5 – >64
	AMK	16	>64	1 – >64
	GEN	>64	>64	8 – >64
	BPR	16	>32	4 – >32
	CIP	8	>16	0.03 – >16
	IMI	4	16	0.125 – >16
<i>Staphylococcus</i> spp. n=49	ACHN-490	1	2	0.25 – 4
	AMK	16	>64	0.5 – >64
	GEN	32	>64	0.25 – >64
	BPR	ND	ND	ND
	CIP	16	>16	0.125 – >16
	IMI	ND	ND	ND
<i>S. aureus</i> n=49	ACHN-490	1	2	0.25 – 4
	AMK	16	>64	0.5 – >64
	GEN	32	>64	0.25 – >64
	BPR	ND	ND	ND
	CIP	16	>16	0.125 – >16
	IMI	ND	ND	ND

IMI = imipenem; CIP = ciprofloxacin; BPR = ceftiprole; TGC = tigecycline

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Table 3: Activity of ACHN-490 against 461 AG-S and AG-R Isolates. ACHN-490 Activity Is Not Altered by the Presence of AMEs. Changes in Permeability/Efflux in *P. aeruginosa* and *Acinetobacter* spp. that Cause Resistance to AMK, GEN, and TOB Also Affect ACHN-490

	AG-R (n)	MIC distribution (µg/mL)										
		≤0.25	0.5	1	2	4	8	16	32	64	>64	
<i>Citrobacter</i> spp.	S (15)				3	10	2					
	R (9)	3	5	1								
<i>E. coli</i>	S (15)		1	5	6	3						
	R (24)		3	14	6			1				
<i>Enterobacter</i> spp.	S (15)	2	6	5	1	1						
	R (20)	2	10	6	1		1					
<i>Klebsiella</i> spp.	S (19)	1	12	2								
	R (45)	1	23	18	2		1					
<i>P. mirabilis</i>	S (7)				3	2	1	1				
	R (16)			1	7	7	1					
Proteae, indole+	S (23)			2	12	7	1	1				
	R (8)				2	4	2					
<i>Salmonella</i> & <i>Shigella</i>	S (13)		1		8	2	2					
	R (1)											
<i>Serratia</i> spp.	S (20)			5	14	1						
	R (8)		2	4	1	1						
<i>Staphylococcus</i> spp.	S (10)	2	5	2	1							
	R (49)	3	21	18	6	1						
<i>Acinetobacter</i> spp.	S (15)		1	3	10	1		19	15	5	1	
	R (67)			1	2	16	8					
<i>P. aeruginosa</i>	S (15)				3	11	1					
	R (51)		1		3	3	19	10	8	3	4	

CONCLUSIONS

- The specific structural modifications incorporated into ACHN-490 resulted in improved activity against all of the AMEs tested, with the exception of AAC(2')-I (a chromosomal enzyme restricted to *Providencia stuartii*).
- ACHN-490 was equally active against AG-S and AG-R isolates of Enterobacteriaceae and *Staphylococcus* spp.
- Improved activity against isolates with AMEs was also observed in *P. aeruginosa* and *Acinetobacter* spp. but, as with currently marketed AGs, ACHN-490 showed reduced potency against isolates with changes in permeability/efflux.
- ACHN-490 has emerged as a promising new antibacterial agent with the potential to rejuvenate the AG class of antibiotics, and thus has been advanced into early clinical development.

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