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MONOGRAPH

PLAZOMICIN

Aminoglycoside antibiotic

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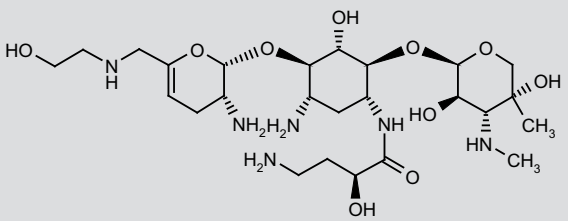

ACHN-490

4-Amino-*N*-[(1*R*,2*S*,3*S*,4*R*,5*S*)-5-amino-4-[3(*R*)-amino-6-(2-hydroxyethylaminomethyl)-3,4-dihydro-2*H*-pyran 2(*S*)-yloxy]-2-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyloxy]-3-hydroxycyclohexyl]-2(*S*)-hydroxybutyramide

1-[4-Amino-2(*S*)-hydroxybutyryl]-6'-(2-hydroxyethyl)sisomicin

InChI = 1S/C25H48N6O10/c1-25(37)11-38-24(18(35)21(25)29-2)41-20-15(31-22(36)16(33)5-6-26)9-14(28)19(17(20)34)40-23-13(27)4-3-12(39-23)10-30-7-8-32/h3,13-21,23-24,29-30,32-35,37H,4-11,26-28H2,1-2H3,(H,31,36)/t13-,14+,15-,16+,17+,18-,19-,20+,21-,23-,24-,25+/m1/s1

$C_{25}H_{48}N_6O_{10}$; Mol wt: 592.6828
CAS RN®: 1154757-24-0
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CONTENTS

SUMMARY

Plazomicin (formerly ACHN-490) is a next-generation aminoglycoside, a semi-synthetic derivative of sisomicin. Plazomicin has completed four phase I clinical trials in which the drug was well tolerated and no ototoxicity or nephrotoxicity was reported, as well as a phase II trial for the treatment of complicated urinary tract infections. In December 2013 plazomicin is expected to enter a phase II trial which will compare the efficacy and safety of plazomicin with colistin in patients with bloodstream infections and nosocomial pneumonia caused by carbapenem-resistant Enterobacteriaceae (NCT01970371). Plazomicin has in vitro antibacterial activity against many multidrug-resistant Gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae, Staphylococcus aureus (including methicillin-resistant S. aureus) and Acinetobacter spp. Plazomicin is not affected by any of the known aminoglycoside-modifying enzymes, except AAC(2')-Ia, -Ib and -Ic (only found in Providencia spp.), but it is not effective against Gram-negative strains that carry 16S rRNA methylases, enzymes found mostly in Asia, but less in Europe and the U.S.

Key words: Next-generation aminoglycoside – RNA methylase – Infections – Plazomicin – ACHN-490

SYNTHESIS*

Plazomicin was prepared from sisomicin by two related processes:

1) Selective *N*-protection of sisomicin (I) with HONB-pNZ (prepared in situ by reaction of *N*-hydroxy-5-norbornene-2,3-dicarboximide [HONB] with *p*-nitrobenzylchloroformate [pNZCl] and Et_3N in THF) by means of $Zn(OAc)_2$ in MeOH/CH_2Cl_2 gives 6'-pNZ-sisomicin (II), which is selectively protected at the glycosidyl amino groups with HONB-Boc (prepared by reacting HONB with Boc_2O and Et_3N in THF) by means of $Zn(OAc)_2$ and Et_3N in MeOH/THF to yield 6'-pNZ-2',3-diBoc-sisomicin (III). Protection of the remaining amino groups of compound (III) with HONB-Fmoc (prepared by reacting HONB with Fmoc-Cl by means of NMM in THF) and Boc_2O in the presence of NMM in THF provides 6'-pNZ-2',3,3''-triBoc-1-Fmoc-sisomicin (IV), which is selectively deprotected by removing the Fmoc-group by means of $N(CH_2CH_2NH_2)_3$ in CH_2Cl_2 to afford 6'-pNZ-2',3,3''-triBoc-sisomicin (V). Condensation of protected sisomicin (V) with *N*-Boc-4-amino-2(*S*)-hydroxybutyric acid (VI) [prepared by *N*-protection of 4-amino-2(*S*)-hydroxybutyric acid (VII) with Boc_2O and K_2CO_3 in diox-

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*Synthesis prepared by J. Bolòs, R. Castañer, Thomson Reuters, Barcelona, Spain.

ane/H_2O] in the presence of HONB and EDC in DMF generates 6′-pNZ-2′,3,3″-triBoc-1-(*N*-Boc-4-amino-2(*S*)-hydroxybutyryl)sisomicin (VIII). Basic hydrolysis of the 6′-pNZ group of compound (VIII) by means of NaOH and $Na_2S_2O_4$ in EtOH/H_2O at 70 °C results in 2′,3,3″-triBoc-1-(*N*-Boc-4-amino-2(*S*)-hydroxybutryl)sisomicin (IX), which then undergoes reductive alkylation with *tert*-butyldimethylsilyloxy acetaldehyde (X) in the presence of silica-supported cyanoborohydride (Si-CBH) in MeOH at 100 °C (microwave) to give the 6′-[2-(*tert*-butyldimethylsilyloxy)ethyl]sisomicin derivative (XI). Finally, compound (IX) is completely deprotected by means of TFA in CH_2Cl_2 (1). Scheme 1.

2) *N*-Acylation of sisomicin (I) with ethyl trifluorothioacetate (XII) in MeOH gives 6-trifluoroacetyl-sisomicin (XIII) (2-4), which by selective *N*-protection with CbzOSu in the presence of $Zn(OAc)_2$ and Et_3N in MeOH/THF yields 2′,3-diCbz-sisomicin (XIV). *N*-Acylation of compound (XIV) with the activated carboxylic acid (XV) provides 6′-(trifluoroacetamido)-2′,3-diCbz-1-[4-amino-2(*S*)-hydroxybutryl]sisomicin (XVI). Hydrolysis of the trifluoroacetamide group in compound (XVI) with NH_4OH in acetonitrile, followed by reductive alkylation with *O*-benzoylglycoaldehyde (XVII) in the presence of $NaBH_3CN$ in MeOH affords the secondary amino alcohol (XVIII). Finally, compound (XVIII) is deprotected by removal the *O*-benzoyl group by means of NaOH in MeOH/H_2O, followed by catalytic hydrogenolysis with H_2 over Pd/C in AcOH (3, 4). Scheme 2.

BACKGROUND

Infections caused by antibiotic-resistant bacteria, especially the "ESKAPE" pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.), cause significant morbidity and mortality (5, 6). During the last 20 years, the efforts to combat multidrug-resistant microorganisms mainly focused on Gram-positive bacteria and drug companies have developed several novel antimicrobial agents to fight these bacteria. Unfortunately, the growing problem of multidrug-resistance in Gram-negative bacteria was not paralleled with the development of novel antimicrobials.

As a result, there are now a growing number of reports on infections caused by Gram-negative microorganisms for which no adequate therapeutic options exist. As we are approaching the "end of antibiotics", a concerted action by industry, government and academia is urgently required. Furthermore, the withdrawal of several large pharmaceutical companies from antibacterial research and development has compromised the infrastructure of discovery and development of new antimicrobials.

The Infectious Diseases Society of America (IDSA) identified in a new report, published as part of the 10 x '20 Initiative (http://www.idsociety.org/10x20), only seven new drugs in development for the treatment of infections caused by multidrug-resistant (MDR) Gram-negative bacteria (7). Their survey results demonstrated some tangible progress in the clinical development of new antibacterial drugs that target infections caused by drug-resistant Gram-negative bacteria. However, progress remains alarmingly slow. The prognosis for sustainable research and development infrastructure depends on clarification of FDA regulatory clinical trial guidance, as well as on fair and appropriate economic incentives for small and large pharmaceutical companies.

Achaogen is a biopharmaceutical company focused on the discovery and development of new antibiotics for the treatment of serious MDR Gram-negative bacterial infections. Plazomicin (ACHN-490), Achaogen's lead compound, is a next-generation "neoglycoside" which demonstrates in vitro potency and in vivo activity against extended-spectrum beta-lactamase (ESBL)-producing pathogens, fluoroquinolone-resistant and aminoglycoside-resistant Gram-negative bacteria, and Gram-negative bacteria expressing AmpC cephalosporinases, carbapenemases and metallo-β-lactamases, but not *Proteus* species or strains with aminoglycoside-resistant methylase genes (e.g., *ArmA*, *RmtC*) (8). Activity against *P. aeruginosa* and *A. baumannii* remains limited.

Data from a phase II study of intravenous (i.v.) plazomicin versus levofloxacin for the treatment of urinary tract infection were reported in September 2012 (9). In September 2013, Achaogen, Inc. announced that it had reached an agreement with the U.S. FDA on a Special Protocol Assessment (SPA) for a phase III clinical trial of plazomicin in patients with serious MDR Gram-negative bacterial infections. This phase III trial is designed as a superiority study to evaluate the efficacy and safety of plazomicin in comparison to colistin in patients with bloodstream infections and nosocomial pneumonia caused by carbapenem-resistant Enterobacteriaceae.

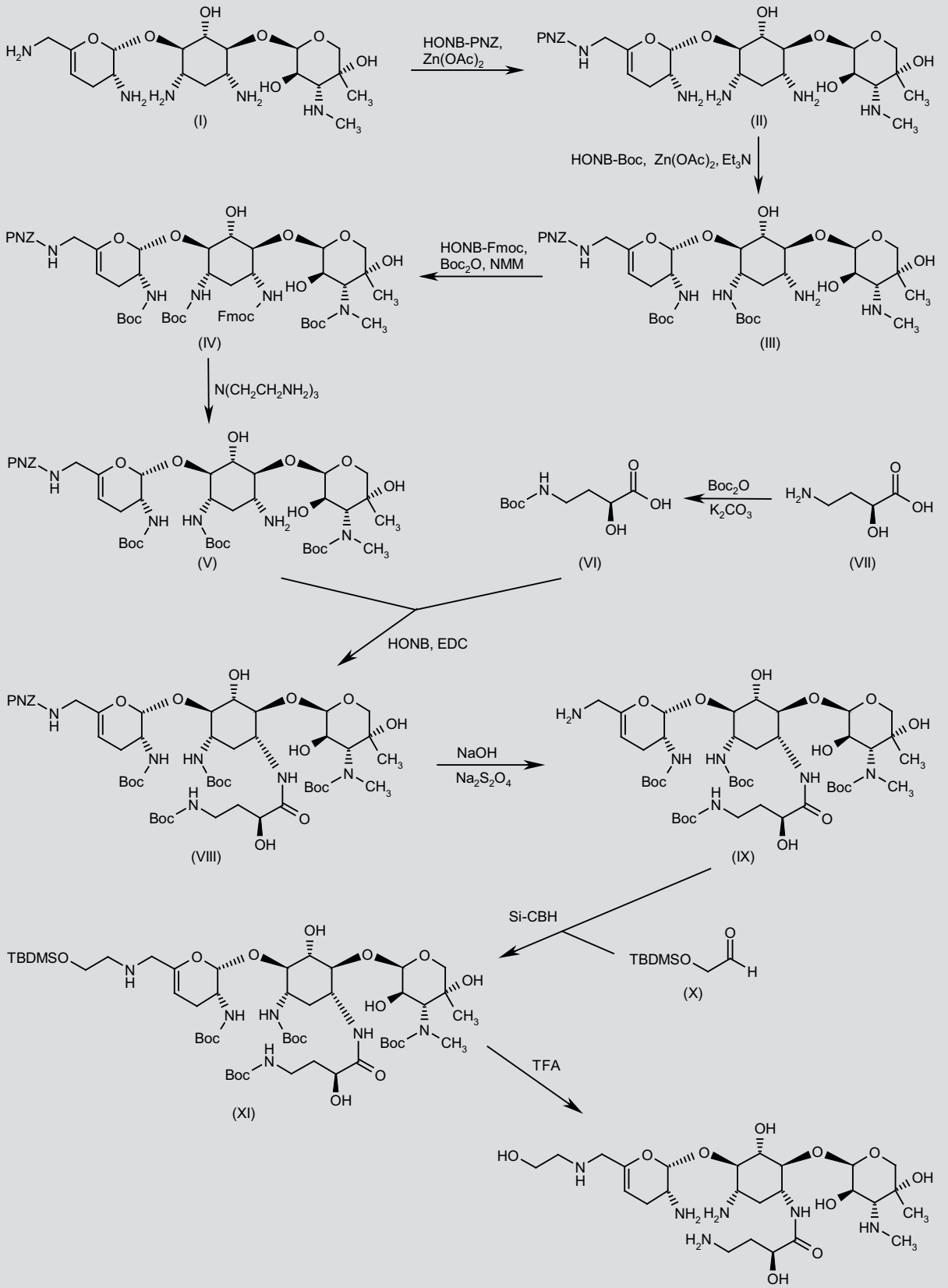
Aminoglycosides have broad-spectrum activity and have historically been useful in serious, life-threatening infections. As with other classes of antibiotics, many mechanisms of resistance to aminoglycosides have developed in pathogens. Primarily these consist of the aminoglycoside-modifying enzymes *N*-acetyltransferases, *O*-nucleotidyltransferases and *O*-phosphotransferases (10), which inactivate aminoglycosides by covalently modifying specific amino or hydroxyl moieties on the drugs. Another mechanism of resistance is the upregulation of efflux pumps and reductions in membrane permeability developed by bacteria to affect the transport of hydrophilic aminoglycosides across cell membranes. Recently, methyltransferases that modify bacterial rRNA, the molecular target of aminoglycosides, have been proven to occur at a low incidence in clinical isolates (11) and confer high-level resistance to all widely used aminoglycosides.

PRECLINICAL PHARMACOLOGY

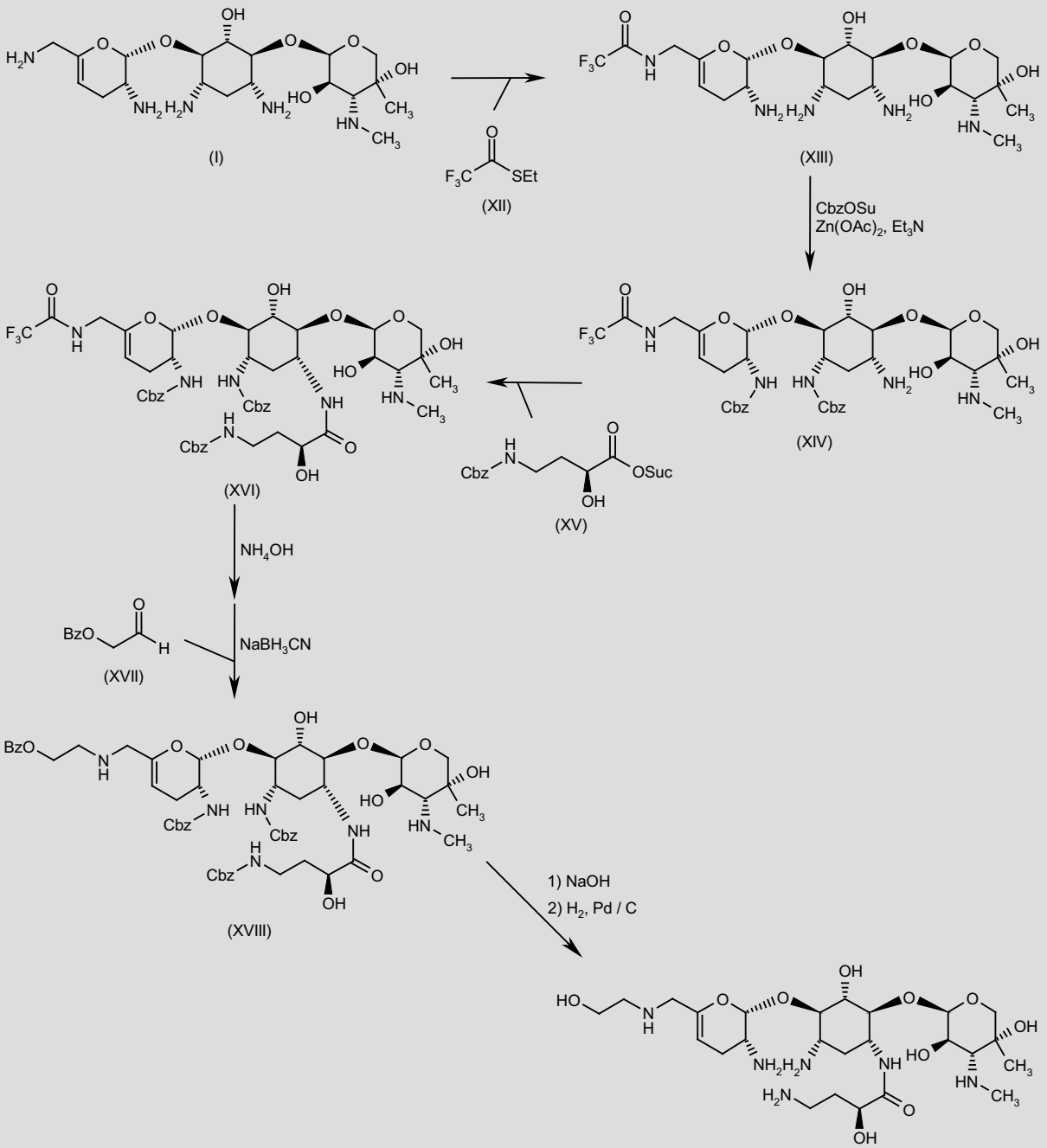
Plazomicin is not affected by any known aminoglycoside-modifying enzymes (AMEs), except *N*-acetyltransferases (AACs) AAC(2′)-Ia, -Ib and -Ic (only found in *Providencia* spp.) (12). As a sisomicin derivative, it lacks the 3′- and 4′-OH groups. As such, plazomicin and sisomicin are protected from the *O*-phosphotransferase (APH) APH (3′) and *O*-adenyltransferase (ANT) ANT (4′) enzymes that generate resistance to amikacin. The hydroxy-aminobutyric acid substitute introduced at the N^1 position of sisomicin provides protection from the AAC(3), ANT(2″) and APH(2″) AMEs, while the hydroxyethyl substitute at the 6′ position blocks the multitude of AAC(6′) AMEs, without reducing potency, as occurred in previous efforts to shield this position (13, 14).

Plazomicin demonstrates activity against both Gram-negative and Gram-positive bacterial pathogens (Table I), including isolates harboring any of the clinically relevant aminoglycoside-modifying enzymes. However, like older parenteral aminoglycosides, plazomicin is not active against bacterial isolates expressing ribosomal methyltransferases conferring aminoglycoside resistance.

Scheme 1. Synthesis of Plazomicin



Scheme 2. Synthesis of Plazomicin



The MIC_{50} and MIC_{90} values for plazomicin were 0.5 and 1 mg/L against 102 MDR *K. pneumoniae* isolates, including 25 carbapenem-resistant isolates containing the serine carbapenemase bla_{KPC} (15). The $MIC_{50/90}$ values against a contemporary collection of *A. baumannii* and *P. aeruginosa* from 16 hospitals in New York were 8 of 16 and 8 of 32 mg/L respectively (16), while against isolates from lower res-

Table I. *In vitro activity of plazomicin and comparator aminoglycosides against Gram-negative and Gram-positive clinical isolates.*

		Plazomicin		Amikacin	Gentamicin	Tobramycin	
Microorganism	No. of isolates	Range (mg/L)	$MIC_{50/90}$ (mg/L)		$MIC_{50/90}$ (mg/L)		Ref.
Escherichia coli	33	≤ 0.25-2	1/2	≤ 8/> 32	8/> 8	32/> 32	17
	214	ND	1/2	4/8	1/> 32	1/16	13
Enterobacter spp.	26	≤ 0.5-2	1/1	16/> 32	2/4	32/32	17
E. aerogenes	37	ND	0.5/1	1/2	≤ 0.25/0.5	0.5/1	13
E. cloacae	64	ND	≤ 0.25/0.5	1/4	≤ 0.25/8	0.5/16	13
Klebsiella pneumoniae	241	≤ 0.5-4	1/2	32/> 32	4/> 8	32/> 32	17
	102	≤ 0.125-4	0.5/1	2/32	8/> 32	8/16	11
	210	ND	≤ 0.25/0.5	1/16	≤ 0.25/> 32	0.5/32	13
	32	0.12-4	0.25	ND	ND	ND	24
KPC	25	0.25-1	0.5/1	32/32	32/> 32	32/> 32	11
NDM	17	0.25-> 128	128/> 128	> 128/> 128	> 128/> 128	64/64	15
Enterobacteriaceae*	102	ND-16	1	4	ND	ND	16
Morganella spp.	17	ND-128	8	64	ND	ND	16
Acinetobacter baumannii	407	0.12-> 64	8/16	32/> 64	64/> 64	32/> 64	12
	201	ND	4/64	32/> 128	32/> 32	4/> 32	13
	17	ND-> 128	16	> 128	ND	ND	16
Pseudomonas aeruginosa	679	0.12-> 64	8/32	8/16	2/> 64	1/64	12
	200	ND	8/16	4/64	2/> 32	0.5/> 32	13
	22	ND> 128	0.5	8	ND	ND	16
Staphylococcus aureus MRSA	493	≤ 0.12–8	1/2	8/32	0.5/1	2/> 16	14
	50	ND	1/2	16/128	0.5/> 32	> 32/> 32	13
	22	< 0.25	< 0.25	< 0.25	ND	ND	16
Staphylococcus aureus MSSA	50	ND	1/2	4/8	0.5/1	0.5/16	13
	22	< 0.25	< 0.25	< 0.25	ND	ND	16

**E. coli* (n = 18), *K. pneumoniae* (n = 20), *Enterobacter* spp. (n = 17), *Citrobacter* spp. (n = 8), *Serratia* spp. (n = 15), *Proteae* (n = 16), *Providencia* spp. (n = 8).

piratory tract infections they were 4 of 64 and 8 of 16 mg/L (17). The activity of plazomicin was also evaluated against 493 methicillin-resistant *S. aureus* isolates collected in 2009 and 2010 from 23 U.S. hospitals. The MIC_{50} and MIC_{90} values were 1 and 2 mg/L for plazomicin, 8 and 32 mg/L for amikacin, 0.5 and 1 mg/L for gentamicin and 2 and > 16 mg/L for tobramycin (18). In a study of 65 isolates with carbapenem resistance mechanisms other than the New Delhi metallo-β-lactamase-1 (NDM-1) enzyme, all exhibited MICs to plazomicin were ≤ 0.12-2 mg/L, while 16 of the 17 isolates with the NDM-1 enzyme were resistant, with MICs ≥ 256 mg/L (20). The latter were nonsusceptible to plazomicin due to the presence of either an *ArmA* or *RmtC* 16S rRNA methyltransferase, which is often present in strains carrying the NDM-1 carbapenemase, and these were cross-resistant to all other human use aminoglycosides tested.

Another study conducted in Tel Aviv, where 67% of the ICU-acquired infections are MDR and 33% are extremely drug resistant, with 202 randomly selected intensive care unit (ICU) isolates from three hospitals showed MIC_{90} values of ≤ 0.25, 4, 64, 8 and > 128 mg/L against MRSA or methicillin-susceptible *S. aureus* (MSSA), Enterobacteriaceae, *Morganella* spp., *P. aeruginosa* and *A. baumannii* isolates, respectively (20). Finally, the in vitro activity of plazomicin was evaluated against 300 MDR (carbapenemase and/or ESBL-producing) isolates from four hospitals in Athens, Greece, where carbapenemase-producing organisms are endemic. Most of the isolates were also resistant to the legacy aminoglycosides, with the MIC_{50}/MIC_{90} values for tobramycin, amikacin and gentamicin being 32/> 32, 32/> 32 and 4/> 8 mg/L, respectively. Plazomicin retained activity (MIC ≤ 4 mg/L) against all isolates of *K. pneumoniae, Escherichia coli* and *Enterobacter* spp. tested, with MIC_{50} and MIC_{90} values of of 1 and 2 mg/L, respectively, irrespective of their MDR phenotype (21).

Using an in vitro model with bovine-derived lung extract to mimic the lung environment, no significant MIC changes were seen when plazomicin was tested against 10 isolates of *S. aureus*, 5 isolates of *P. aeruginosa* and 5 isolates of *K. pneumoniae* in the presence of 1-10% bovine pulmonary surfactant (22). Plazomicin has also been evaluated against *Yersinia pestis* and *Francisella tularensis*, with $MIC_{50/90}$ values of 0.5/1 mg/L for about 30 isolates of each species (23).

Plazomicin is bactericidal in a concentration-dependent manner against isolates with and without aminoglycoside modifying enzymes (24). In time kill assays, plazomicin was rapidly bactericidal, achieving a 3 log decrease in CFU/mL within the first 2 hours of exposure against three *E. coli*, two *K. pneumoniae*, two *Enterobacter aerogenes* and one *S. aureus* isolates tested. At 24 hours there was sporadic evidence of regrowth at concentrations two- and fourfold above the MIC with either plazomicin or the aminoglycoside comparator. Represen-

tative plazomicin regrowth colonies from exposures of fourfold the MIC or greater demonstrated a four- to eightfold increase in the MICs of all aminoglycosides tested, characteristic of changes in membrane permeability (25) rather than a plazomicin-specific mechanism.

Aminoglycosides are often employed in combination with other agents. Plazomicin has been reported to demonstrate in vitro synergistic activity when combined with daptomycin, ceftobiprole or linezolid in 91.5%, 36.2% and 12.8% of MRSA (26). All 47 clinical MRSA isolates tested had plazomicin MICs of 4 mg/L, including those with decreased susceptibility to vancomycin. Synergy time-kill analysis of plazomicin combined with cefepime, doripenem, imipenem or piperacillin/tazobactam has also been reported against 25 *P. aeruginosa* strains with different resistance phenotypes, yielding synergies in > 70% and > 80% of strains at 6 and 12 hours, respectively, and in > 68% at 24 hours (27).

In a more recent study, presented at the 53rd ICAAC 2013, plazomicin was active against 32 KPC-producing *K. pneumoniae,* with both MIC_{50} and MIC_{90} values of 0.5 mg/L (28). Isolates varied in their overall susceptibility to the other evaluated agents. No apparent synergy or antagonism was observed for plazomicin in combination with piperacillin/tazobactam, ceftazidime, meropenem, tigecycline, rifampin or phosphomycin, as shown by the mean fractional inhibitory concentration index (0.53-2.38), with the exception of two isolates, for which a mean fractional inhibitory concentration index indicative of synergy (0.29 and 0.49) was observed for plazomicin in combination with ceftazidime and meropenem, respectively.

Plazomicin shows potent activity in murine models of urinary tract infections (UTIs), septicemia, thigh infection and pneumonia. Plazomicin demonstrated dose-dependent bactericidal activity in animal models and efficacy similar to or better than other aminoglycosides used as comparators.

Plazomicin was first evaluated against the CO92 strain of *Y. pestis* and the Schu S4 strain of *F. tularensis* in murine inhalational challenge models of infection. Ten female BALB/c mice per cohort were inoculated with aerosol doses of the two strains. Twenty-four hours after challenge, animals were treated i.v. with plazomicin at 48 mg/kg once per day (3 or 5 days) for *Y. pestis*, and either 48 mg/kg once per day or 60 mg/kg twice per day (5, 7, 10 or 14 days) for *F. tularensis*. In the *Y. pestis* model, there was significant survival (90% 20 days post-aerosolization) over the controls in plazomicin-treated mice for 5-day treatments. In the *F. tularensis* efficacy model, plazomicin provided 90-100% survival 35 days post-inoculation when dosed at 60 mg/kg every 12 hours for 7 or 10 days. While both of these bacterial agents have intracellular phases during infection, *F. tularensis* has more prominent intracellular requirements, and this may partially account for the higher dose and prolonged treatment needed. Gentamicin, used as comparator in both studies, was administered at 48 mg/kg every 24 hours for 5 and 14 days and provided 90% and 100% survival 20 and 35 days postinoculation, respectively (23).

In a murine UTI model with uropathogenic *E. coli*, infected transurethrally with 8.8 $\log_{10}$ colony-forming units (CFUs) per animal and compounds dosed twice daily for three days post-infection, plazomicin (0.25-16 mg/kg/day) reduced kidney bacterial count by 2.4-4.5 mean $\log_{10}$ CFU compared to a 3.7-4.4 mean $\log_{10}$ CFU reduction for gentamicin (0.25-4 mg/kg/day) and 2.1 and 3.7 mean $\log_{10}$ CFU reduction for 0.25 and 1 mg/kg/day levofloxacin. Plazomicin, gentamicin and levofloxacin MICs for the *E. coli* isolate were 0.5, 0.5 and 0.06 mg/L respectively. Reductions in *E. coli* counts in the bladders and urine were also observed with plazomicin, and were especially notable at doses ≥ 0.5 mg/kg/day (29).

Plazomicin also demonstrated potent in vivo efficacy against a variety of drug-resistant and -susceptible pathogens in an experimental model of septicemia and in a mouse neutropenic thigh model (30). When its activity against a gentamicin-susceptible strain of *E. coli* was tested in the septicemia model, plazomicin improved 7-day survival, with a dose–response profile similar to that of gentamicin, with 100% survival seen at doses of 1.6 mg/kg and above. In animals infected with a gentamicin-susceptible strain of *P. aeruginosa*, treatment with either plazomicin or gentamicin led to 100% survival at doses of 16 mg/kg and above in the septicemia model. Plazomicin was also effective in the neutropenic thigh model, reducing the bacterial load of MDR Enterobacteriaceae and MRSA strains, as well as broadly susceptible strains, to static levels with dose-dependent activity. Against gentamicin-sensitive Enterobacteriaceae and MRSA, the efficacy of plazomicin was comparable to that of gentamicin. However, gentamicin-resistant Enterobacteriaceae strains and those harboring the *K. pneumoniae* carbapenemase responded to plazomicin but not gentamicin, with static doses ranging from 12 mg/kg to 64 mg/kg for plazomicin.

Plazomicin had potent activity in vivo that generally correlated with the MIC. In a murine neutropenic lung model, animals were inoculated intranasally with 10^5-10^6 CFU/mouse of *S. aureus* (three strains), *P. aeruginosa* (four strains), *A. baumannii* (four strains) and *K. pneumoniae* (one isolate) (31). After subcutaneous dosing of plazomicin (4 mg/kg 2 hours post-infection, up to 4 times at 6-hour dosing intervals) 1- or 2-log bacterial reductions were attained in the lung for two of three *S. aureus* isolates, two of four *P. aeruginosa*, all four *A. baumannii* and one *K. pneumoniae* isolate, while for the remaining strains only stasis could be observed at the doses tested (up to 256 mg/kg/day).

PHARMACOKINETICS AND METABOLISM

Pharmacokinetic (PK) properties of plazomicin were linear and similar to other aminoglycosides. The PK of plazomicin in the plasma and in the epithelial lining fluid (ELF) was measured in a murine neutropenic lung model and static AUC_{ELF}/MIC ratios averaged 13 ± 8, 4.7, 52 ± 36 and 8.8 ± 6.6 for *A. baumannii*, *K. pneumoniae* (n = 1), *P. aeruginosa* and *S. aureus*, respectively (31). The exposure in ELF was generally twice the exposure in plasma in this model. In the mouse neutropenic thigh model, static doses for *E. coli* (n = 2), *K. pneumoniae* (n = 3), *Serratia marcescens* (n = 1) and MRSA (n = 1) were 11-25, 7.8-64, 37 and 54 mg/kg/day, similar to or better than gentamicin (30).

Cass et al. examined the PK of plazomicin in mice, rats and dogs and found it to be similar to gentamicin (32). The AUC_{inf} values for plazomicin were similar in rats and mice after a single i.v. dose of 10 mg/kg, providing 16-19 mg·h/L, but the AUC_{inf} was about fivefold higher in dogs. Maximum concentration (C_{max}) was lower in rats (38 mg/L) than mice (88 mg/L) and higher in dogs (120 mg/L), likely corresponding to the reduced clearance (CL) and lower volume of distribution at steady-state (V_{ss}) in dogs. The 24-hour cumulative urinary recovery of plazomicin postdose was 59 ± 13% and 119 ± 22% in rats

and dogs, respectively. There was 38% less plazomicin in canine kidneys in comparison to gentamicin after a dose of 30 mg/kg/day for 14 days. The half-life of plazomicin in all species was ≤ 1.4 hours. Based on allometric scaling, the predicted human PK parameters are similar to the predicted gentamicin PK parameters.

The PK of plazomicin injection in healthy subjects was first investigated in two randomized, double-blind, placebo-controlled clinical studies (33). In the initial study (study 1), healthy male and female volunteers received single 10-minute i.v. infusions (1-15 mg/kg), followed by a ≥ 7-day washout and then crossed-over to multiple dose regimens with a therapeutic duration of 3-10 days. Consistent with a linear PK profile, the mean C_{max} was dose-proportional and ranged from 8.1 mg/L for a 1 mg/kg dose to 144 mg/L for a 15 mg/kg dose, and the mean $AUC_{0-\infty}$ ranged from 14.5 mg·h/L for a 1 mg/kg dose to 246 mg·h/L for a 15 mg/kg dose. In study 2, healthy volunteers received the highest dose (15 mg/kg intravenously once daily as a 10-minute infusion) for five days. The C_{max} was 113 mg/L, the AUC_{0-24} was 235 mg·h/L, the half-life was 4.0 hours and the V_{ss} was 0.25 L/kg (33). In a more recent study by Cass et al. (34), the plasma PK profile of plazomicin after a 10-minute i.v. infusion as a single dose and after 5 days of once-daily dosing was similar to that observed in the previous study (Table II) (34). Lung penetration of plazomicin, calculated as the ratio of ELF to plasma AUC, was approximately 13% in these healthy subjects after a single dose.

When plazomicin is administered to subjects with varying degrees of renal impairment, dose adjustments will be required to achieve a target AUC. In a phase I open-label study following a single 30-minute i.v. infusion at a dose of 7.5 mg/kg, subjects with mild renal dysfunction had similar PK parameters compared to subjects with normal renal function, whereas subjects with moderate and severe renal dysfunction had significantly reduced total clearance of plazomicin and significantly increased exposure to the drug based on $AUC_{0-\infty}$ (Table III) (35). These PK results indicate that when plazomicin is administered to subjects with moderate or severe renal dysfunction, dose adjustments will be required to achieve a target AUC.

A population PK model developed by Van Wart et al. using data obtained from subjects in phase I and II trials, showed that a three-compartment model with zero-order i.v. input and first-order elimination best described the PK of plazomicin in both healthy subjects and patients with complicated urinary tract infections (cUTI) or acute pyelonephritis (AP) (cUTI/AP) (36-38). The covariate analysis identified creatinine clearance (CL_{cr}) and height as the major covariates which impact plazomicin PK. For a typical subject with normal renal function (120 mL/min/1.73 m^2), plazomicin CL was estimated to be 4.81 L/h and V_{ss} 19.8 L; alpha-, beta- and gamma-phase elimination half-lives ($t_{1/2}$) were 0.223, 2.33 and 18.9 hours, respectively (36). The beta-phase $t_{1/2}$ was reported to best represent the effective half-life of plazomicin, and this value corresponds well with the known values for the comparator aminoglycosides (36, 37).

Plazomicin $t_{1/2}$ (β) was reported to be 4.0 ± 1.0 hours (Table II), while sisomicin, amikacin, gentamicin and tobramycin $t_{1/2}$ (β) have been reported to be 2.03 ± 0.45, 1.90 ± 0.41, 1.6 ± 0.4 and 2.01 ± 0.30 hours, respectively. Plazomicin CL was 3.99, 2.77 and 1.11 L/h for a typical subject with renal impairment and a CrCL of 90, 60 and 30 mL/min/1.73 m^2, respectively (38). Plazomicin V_{ss} was predicted to be higher in both acute pyelonephritis and cUTI patients relative to healthy subjects. No difference in plazomicin CL was detected between infected patients and healthy subjects (39).

Table II. *Plazomicin pharmacokinetics in healthy subjects (33, 34).*

Plazomicin dose	Single dose 10.7 mg/kg	Single dose 15 mg/kg	Multiple dose 15 mg/kg QD x 5 days
Number of subjects	N = 9	N = 15	N = 5
C_{max} (mg/L), mean (SD)	133 (21.9)	161 (30.7)	113 (17.3)
AUC_{0-24} (mg·h/L), mean (SD)	224 (36.6)	NA	235 (43.9)
C_{min} (24 h) (mg/L), mean (SD)	NA	NA	0.375 (0.115)
$t_{1/2}$ (β) (h), mean (SD)	3.65 (0.333)	2.75 (0.562)	3.97 (0.983)
V_{ss}, weight (L/kg), mean (SD)	0.184 (0.0162)	0.161 (0.0203)	0.248 (0.0398)
CL, weight (mL/min/kg), mean (SD)	0.806 (0.132)	0.824 (0.116)	1.09 (0.194)

NA, not applicable; QD, once daily; SD, standard deviation.

Table III. *Plazomicin pharmacokinetics in subjects with varying degrees of renal impairment receiving a single dose of 7.5 mg/kg (35).*

Parameter	Normal renal function (N = 6)	Mild renal dysfunction (N = 6)	Moderate renal dysfunction (N = 6)	Severe renal dysfunction (N = 6)
C_{max} (mg/L)	37.9 (5.01)	32.8 (4.30)	39.2 (6.43)	41.4 (7.83)
AUC_{0-24} (mg·h/L)	136 (17.2)	138 (23.7)	281 (96.0)	647 (259)
$t_{1/2}$ (γ) (h), median (min, max)	33.8 (28.1, 62.4)	26.6 (22.9, 32.7)	20.3 (16.3, 32.4)	24.7 (15.6, 33.5)
V_{SS} (L), mean (SD)	36.0 (7.76)	28.5 (2.17)	25.8 (6.96)	25.1 (7.89)
CL_T (L/h), mean (SD)	4.64 (1.17)	3.98 (0.481)	2.25 (0.685)	0.96 (0.379)

Plazomicin dosing regimens, based on CL_{cr}, were designed to provide a mean steady-state target AUC_{0-24} of 262 mg.L/h, comparable to the average AUC associated with plazomicin 15 mg/kg/day, which was generally well tolerated in subjects with normal renal function at a dosing duration of up to 5 days (Table IV) (38).

The overall probability of patients achieving a plasma AUC:MIC ratio target across renal function groups was high for carbapenem-resistant *K. pneumoniae* (≥ 98.1%), suggesting that the proposed plazomicin dosing regimens will provide plasma exposures consistent with efficacy in the majority of patients with bacteremia caused by carbapenem-resistant *K. pneumoniae* (38).

Plazomicin does not appear to be metabolized by liver microsomes or hepatocytes, as found in a study performed in male CD-1 mice, Sprague-Dawley rats and beagle dogs (32). Plazomicin was renally cleared rapidly in both rats and dogs ($t_{1/2}$ = 1 hour) and was distributed to rat and canine kidneys, with the volume of distribution closely matched to extracellular fluid volumes. Plazomicin had low plasma protein binding (< 20%), with the percentage of bound drug independent of concentration and not species-specific.

SAFETY

Aminoglycosides as a class are known for their ototoxic and nephrotoxic adverse effects. No evidence of nephrotoxicity or ototoxicity was observed in any clinical trial of plazomicin and all adverse events were reported as mild to moderate (9, 33, 34, 35, 40).

A 14-day rat model was used to assess nephrotoxicity and evaluate dosing regimens of plazomicin (41). Neomycin (3-100 mg/kg), gentamicin (3-100 mg/kg), apramycin (3-100 mg/kg), tobramycin (10-100 mg/kg), paromomycin (30-300 mg/kg) and amikacin (10-300 mg/kg) were dosed s.c. once daily, as well as twice or three times daily for the same total daily dose for gentamicin for 14 days. Kidney function was evaluated by blood urea nitrogen, serum creatinine and histopathology. This rat model confirmed that once-daily dosing of aminoglycosides is significantly less toxic than twice- or three-times daily dosing on the same total daily dose. It was also found that limiting the duration of dosing may allow higher dosing for improved efficacy, without an increase in toxicity. No ototoxicity, a known side effect of aminoglycosides, was observed for plazomicin compared to gentamicin and amikacin when assessed in a 28-day guinea pig model with doses that provided 1.5 times the exposure with the highest plazomicin dose used in phase I studies (370 mg·h/L in guinea pigs versus 240 mg·h/L in humans) (42). Sisomicin has previously been proved to cause both cochlear and vestibular audiotoxicity in this model (43); thus, the modifications of sisomicin yielding plazomicin significantly reduced the ototoxic potential.

Plazomicin sulfate injection administered at doses of up to 20 mg/kg daily for 1 or 5 days was generally safe and well tolerated in healthy subjects (33, 34, 40). A single i.v. infusion of plazomicin at a dose of 7.5 mg/kg, administered over 30 minutes, in healthy or medically stable volunteers with varying degrees of renal dysfunction was also well tolerated (35). Although the intended therapeutic dose of plazomicin is 15 mg/kg, a dose of 7.5 mg/kg was chosen in order to reduce the risk of nephrotoxicity and ototoxicity, since plazomicin exposure was expected to increase in case of renal impairment.

The most common adverse effects (AEs) reported in the plazomicin-injected subjects (healthy volunteers or patients with cUTI/AP) were ear discomfort, tinnitus, nausea, vomiting, dizziness, somnolence and anxiety. Transient mild to moderate hypotension was reported in 5 of the 15 subjects that received a single dose of 15 mg/kg of plazomicin sulfate injection (34). Serious AEs were experienced by one patient in the plazomicin 15 mg/kg group (spontaneous abortion, with no temporal association to study drug administration) in the phase II study (9).

Analyses of blood urea nitrogen and serum creatinine, as well as calculated creatinine clearance, revealed no overall effects on renal function after plazomicin injection (9, 33). No clinically significant effects on vestibular, cochlear or renal function were observed in the clinical trials performed with plazomicin (9, 33, 34, 35, 40). AEs (mild vertigo and mild unilateral permanent tinnitus) possibly associated with vestibular and cochlear function occurred in two patients in the plazomicin 15 mg/kg group with complicated urinary tract infections or acute pyelonephritis (9). No clinically relevant differences in laboratory values, ECG results or physician examination results were observed between plazomicin injection and placebo (33, 35).

Small increases in the QTc interval associated with plazomicin, observed at a single time point in the plazomicin 15 mg/kg dose group and at two time points in the plazomicin 20 mg/kg dose group of healthy male and female subjects, were not considered to be clinically meaningful (40). No clinically meaningful QTc interval prolongations were observed associated with plazomicin.

Table IV. *Plazomicin dosing regimens based on CL_{cr}* (38).*

CL_{cr} (mL/min/1.73 m^2)	Plazomicin i.v. dose (mg/kg)	Frequency of administration
> 70	15	q24h
> 60 to 70	14	q24h
> 50 to 60	12	q24h
> 40 to 50	10	q24h
> 30 to 40	8	q24h
> 25 to 30	12	q48h
> 20 to 25	10	q48h
> 15 to 20	8	q48h

*Represents initial plazomicin dosing regimens which would be administered to patients but subsequently adjusted based on therapeutic drug management applied as early in therapy as possible.

CLINICAL STUDIES

Achaogen has successfully completed five clinical trials with plazomicin and plans to start a phase III clinical trial of plazomicin in patients with serious MDR Gram-negative bacterial infections in 2014.

A double-blind, randomized, placebo-controlled, parallel-group, single- and multiple-dose escalation study was conducted during 2009 in order to assess the safety, tolerability and PK of plazomicin injection, administered i.v. in healthy volunteers. It was the first-in-human phase I study to assess if plazomicin injection was safe for people. Plazomicin was evaluated in healthy humans (N = 39), of which 30 subjects received the study medication (plazomicin) and 9 placebo (normal saline). Thirty subjects completed the treatment period and 7 were withdrawn (34). Subjects received single 10-minute i.v. infu-

sions (1-15 mg/kg), followed by a ≥ 7 day washout and then crossed over to multiple-dose regimens with a therapy duration of 3-10 days. In a second part of the study, the highest dose, 15 mg/kg, was given to five subjects for five days; one subject received only three days of dosing. Safety was monitored by the usual clinical laboratory tests, but a cochlear function analysis was also included at baseline, end of treatment, 3 and 6 months posttreatment. Plazomicin exhibited a linear and dose-proportional PK profile after single and multiple doses of the drug injection up to a 15-fold dose range, and there was no evidence of treatment-related side effects or effects on renal, cochlear or vestibular functions that would preclude further clinical development (33).

A randomized, double-blind, placebo- and positive-controlled, crossover study was conducted in the end of 2011 to evaluate the effect of i.v. plazomicin injection on the QT/QTc interval in healthy volunteers. The hypothesis was that the drug does not cause an increase in the QT interval. Results of this study were presented by Riddle et al. (40) in the form of a poster (A-017f) at the 53rd ICAAC meeting held in Denver, Colorado in September 2013, showing that single i.v. infusions of plazomicin at doses of 15 and 20 mg/kg were generally well tolerated by the healthy male and female subjects. The majority of the AEs were mild, with hypoesthesia being the most common. Small, statistically significant increases in QTc were observed at a single timepoint for the plazomicin 15 mg/kg dose group and at two timepoints in the plazomicin 20 mg/kg dose group. At all timepoints, the upper bounds of the 90% two-sided confidence intervals were less than 10 ms. Therefore, the small increases in the QTc interval associated with plazomicin were not considered to be clinically relevant. The QTc–plasma concentration linear regression models predicted slightly negative but not statistically significant slopes, with no clear plasma concentration effect on QTc. In general, there were no clinically relevant trends in laboratory parameters, vital signs, ECG results or audiometry test results.

In 2010, a double-blind, randomized, placebo-controlled phase I trial was conducted to assess the safety, tolerability, plasma PK and lung penetration of i.v. plazomicin in healthy subjects. Plazomicin was administered as an i.v. infusion over 10 minutes and subjects were randomized 3:1 to plazomicin or placebo (saline). Results were presented by Cass et al. (34), showing that plazomicin sulfate injection administered at doses of up to 15 mg/kg daily for 1 or 5 days was well tolerated. No effects on renal or VIIIth cranial nerve function were observed. Plasma levels of plazomicin were high, with good intersubject and intrasubject reproducibility. No drug accumulation was observed with repeated dosing. The lung penetration of plazomicin into ELF in normal healthy volunteers with non-inflamed lung following a single i.v. dose of 15 mg/kg was similar (13% by AUC) to the lung penetration reported for amikacin in bronchial secretions of normal subjects (14% by AUC) (44).

A phase I trial was conducted in September 2011 to assess the PK, safety and tolerability of i.v. plazomicin injection in volunteers with varying degrees of renal dysfunction compared to healthy volunteers, and results were presented at the 23rd European Congress of Clinical Microbiology and Infectious Diseases, held in Berlin in 2013. In this study, 24 subjects were enrolled, 6 in each of 4 groups, with normal renal function (CL_{cr} ≥ 90 mL/min) and mild, moderate or severe impairment (CL_{cr} = 60-89, 30-59 and 15-29 mL/min, respectively). All subjects received a single 7.5 mg/kg i.v. infusion of plazomicin administered over 30 minutes. Blood and urine samples were collected to determine plazomicin plasma concentrations and renal clearance. Compared to subjects with normal renal function, subjects with mild renal dysfunction had similar PK parameters, whereas subjects with moderate and severe renal dysfunction had significantly reduced total renal clearance and therefore significantly increased exposure to plazomicin (based on $AUC_{0-\infty}$). These PK results indicate that when plazomicin is administered to subjects with moderate or severe renal dysfunction, dose adjustments will be required to achieve a target AUC. No significant trends were observed in the nature and frequency of AEs and laboratory or clinical parameters evaluating renal function. A single 7.5 mg/kg dose of plazomicin was well tolerated by subjects in this study (35).

A fifth study was commenced to assess the safety, efficacy and PK of plazomicin injection administered i.v. in patients with complicated urinary tract infections or acute pyelonephritis. This multicenter, multinational, double-blind, randomized, comparator-controlled phase II study was conducted in 27 centers in the U.S., India, Colombia and Chile between July 2010 and April 2012 and compared plazomicin with levofloxacin, a standard approved i.v. therapy for cUTI/AP. Patients were randomized 2:1 to i.v. plazomicin (10 or 15 mg/kg) or i.v. levofloxacin (750 mg), administered once daily for 5 days. A total of 98 and 47 patients were randomized. Plazomicin was well tolerated, with no unexpected toxicities. The efficacy of plazomicin was similar to levofloxacin, as determined by microbiological and clinical outcomes (9).

Finally, a study comparing plazomicin with colistin in patients with carbapenem-resistant Enterobacteriaceae infection is planned to start in December 2013. This will be a multicenter, randomized, open-label superiority phase III study that will compare the efficacy and safety of plazomicin to that of colistin, with both combined with a second antibiotic (either meropenem or tigecycline) for the treatment of patients with bloodstream infection or nosocomial pneumonia due to carbapenem-resistant Enterobacteriaceae. Therapeutic drug monitoring will be used to help ensure that plazomicin exposure lies within an acceptable range of the target mean steady-state AUC (http://clinicaltrials.gov).

DRUG INTERACTIONS

Plazomicin is extensively renally cleared, with a very low risk of drug–drug interactions resulting from cytochrome P450 inhibition (31). Plazomicin at 25 μM did not inhibit the human cytochrome P450 isoforms 1A2, 2C9, 2C19, 2D6 or 3A4 in vitro. Therefore, it is unlikely that plazomicin exhibits drug–drug interactions with compounds that are cytochrome P450 inhibitors, inducers or substrates. The product has a relatively low molecular weight and is highly polar, so it would not be expected to be metabolized or interact with cytochromes.

ORGANIZATION

Achaogen, Inc. (US).

DISCLOSURES

The author states no conflicts of interest.

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