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Synthesis, ribosomal selectivity, and antibacterial activity of netilmicin 4'-derivatives†

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Halogenation of a suitably protected netilmicin derivative enables preparation of 4'-chloro-, bromo-, and iodo derivatives of netilmicin after deprotection. Suzuki coupling of a protected 4'-bromo derivative with phenylboronic acid or butyltrifluoroborate affords the corresponding 4'-phenyl and 4'-butyl derivatives of netilmicin. Sulfenylation of suitably protected netilmicin derivative with ethanesulfonyl chloride followed by deprotection affords 4'-ethylsulfanyl netilmicin. All netilmicin 4'-derivatives displayed reduced levels of inhibition for prokaryotic ribosomes and reduced antibacterial activity against typical Gram-positive and Gram-negative strains. None of the derivatives displayed enhanced target selectivity.

Introduction

Substitution in ring I of the 4,5-disubstituted 2-deoxystreptamine (DOS) class of aminoglycosides (AGAs) at the 4'-position is known to increase selectivity for the bacterial ribosomal decoding A site over the corresponding site in the eukaryotic ribosomes.¹ In particular, the introduction of a simple linear two or three carbon ether to O4' of the parent AGA paromomycin 1, as in compounds 2 or 3, gave increased ribosomal selectivity and reduced ototoxicity as determined by shifts in threshold levels of auditory brain stem responses in guinea pigs and by estimation of the loss of inner and outer cochlea hair cells.² Interestingly, however, comparable modifications in the kanamycin series of 4,6-DOS AGAs did not result in significantly increased ribosomal selectivity.³ More recently, we reported that replacement of the 4'-hydroxy group of paromomycin by an ethylthio group or by a propyl group, as in 4 or 5, resulted in marked increases in ribosomal selectivity, borne out by *in vivo* measurements in the guinea pig model, without the reduction in antibacterial activity attendant on formation of the ethers 2 and 3.⁴ Thus prompted, we turned to the 4,6-DOS class of AGAs, and the installation of ethylthio and alkyl chains at the 4'-position of netilmicin 6, reputedly the least ototoxic of its class.^{5–10} Accordingly, we re-

port on the synthesis of a small series of 4'-derivatives of the 4,6-DOS AGA 6, and on their ribosomal selectivity and antibacterial activity against a set of representative Gram-negative and Gram-positive bacterial pathogens.

Results and discussion

Chemical synthesis

Treatment of the readily prepared netilmicin derivative 7,¹¹ in which the phenyltriazene group is the preferred protecting group for the two secondary amino groups for reasons of ease of installation and cleavage, and especially the sharpness of the NMR spectra, with acetic anhydride and pyridine gave 8 in 75% yield. Treatment of 8 with *N*-iodosuccinimide and silver nitrate in acetonitrile at 80 °C for 2.5 h afforded the 4'-iodide 9 in 45% yield. Comparable reaction with *N*-bromosuccinimide gave the 4'-bromide 10 in 50% yield. The corresponding 4'-chloroalkene 11 was obtained by treatment of 8 with iodobenzene dichloride and potassium

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carbonate in acetonitrile at 0 °C in 30% yield (Scheme 1). All attempts at the formation and/or isolation of the analogous 4'-fluoro derivative were unsuccessful, because of the apparent instability of 4'-fluoronetilmicin itself toward degradation with loss of ring I.

Suzuki coupling of **10** with phenylboronic acid was best achieved with 10 mol% of Pd(dppf)₂Cl₂ as catalyst in the presence of potassium carbonate in hot dioxane when a 23% yield of the 4'-phenyl derivative **12** was isolated (Scheme 1). B-Alkyl Suzuki coupling¹² of **10** with potassium butyltrifluoroborate in the presence of the same catalyst and base at 90 °C, albeit in toluene, gave the corresponding 4'-butyl derivative **13** in a disappointing but sufficient yield of 8% in spite of all attempts at optimization, including the variation of base, temperature, solvent, and catalyst (Scheme 2).¹³

Deprotection of the 4'-halo and 4'-phenyl and butyl derivatives of netilmicin **9–13** was achieved by sequential saponification of the esters and Staudinger reduction of the azides¹⁴ giving **14–18**. Treatment with trifluoroacetic acid then cleaved the triazene functionality¹⁵ giving the netilmicin derivatives **19–23**, which were isolated in the form of their peracetate salts (Scheme 3).

All attempts at the sulfonylation of the bis(triazene) **8** were fruitless, but the 5,2'' di-*O*-acetyl penta-*N*-trichloroethylcarbamate derivative **25**, obtained in excellent yield *via* **24**, was amenable to the reaction conditions and gave the 4'-ethylthio derivative **26** in 43% yield on exposure to ethanesulfonyl chloride and then diazabicycloundecene. Treatment of **26** with 6*N* sodium hydroxide then afforded the desired 4'-ethylthio netilmicin derivative **27** in 15% yield, isolated in the form of its peracetate salt (Scheme 4).

Scheme 1 Synthesis of the protected 4'-halo netilmicin derivatives **9–11**.

Scheme 2 Carbon-carbon bond formation at the netilmicin 4'-position.

Ribosomal selectivity

The 4'-derivatives of netilmicin **19–23** and **27** were screened for their ability to inhibit translation by the wild-type *M. smegmatis* ribosome and humanized versions of it (Fig. 1) carrying the decoding A sites of the human mitochondrial ribosome (Mit 13), the A1555G mutant of the human mitochondrial ribosome (A1555G), and the human cytoplasmic ribosome (Cyt 14).¹⁶ Inhibition of the human mitochondrial ribosome and slow clearance of AGAs from the cochlea combined together are the root cause of AGA-induced ototoxicity

Scheme 3 Deprotection of the netilmicin derivatives **19–23**.

Scheme 4 Preparation of 4'-ethylthio netilmicin 27.

Fig. 1 Decoding A sites of the bacterial and human ribosomes. The AGA binding pocket is boxed. The prokaryotic numbering scheme is illustrated for the AGA binding pocket. Changes in the binding pocket from the bacterial ribosome are coloured red. The A1555G mutant conferring hypersusceptibility to aminoglycoside ototoxicity is coloured green.

with the mitochondrial A1555G mutation being associated with a subset of the population that is hypersusceptible to ototoxicity.^{17–19} Inhibition of the cytoplasmic ribosome leads to broad systemic toxicity.

Table 1 reports the IC_{50} values for the inhibition of the various ribosomes by netilmicin and its 4'-derivatives together, for comparison purposes, with those of the 4,5-AGA paromomycin 1 and its 4'-deoxy-4'-ethylthio and 4'-propyl derivatives 4 and 5. It is apparent that modification of netilmicin at the 4'-position whether by halogenation, arylation, alkylation, or alkylsulfenylation results in a significant loss of antibacterioribosomal activity. These losses in activity are also accompanied by reduced selectivity for the bacterial over the eukaryotic ribosomes. This pattern of reduced activity and selectivity contrasts with that seen in the 4,5-series, where the introduction of the 4'-deoxy-4'-ethylthio and especially the 4'-deoxy-4'-propyl modification have little effect on activity against the bacterial ribosome and result in increased selectivity (Table 1).⁴ The pattern of reduced antibacterioribosomal activity on substitution at the 4'-position is, however, reminiscent of that seen in the kanamycin series of 4,6-AGAs and does not encourage further work in this direction.³

Antibacterial activity

All compounds were screened against a panel of three clinical isolates each of the Gram-positive Methicillin-resistant *S. aureus* (MRSA), and of the Gram-negative *E. coli* and *P. aeruginosa* (Table 2) from the Diagnostics Department of the University of Zurich, Institute of Medical Microbiology. The observed activities largely mirror the antiribosomal activities in so far as all modifications at the 4'-position of netilmicin result in a net reduction of antibacterial activity. The greatest losses in activity are observed with the 4'-butyl and 4'-ethylthio derivatives 23 and 27. This pattern is distinctly different to that observed in the paromomycin series of 4,5-AGAs where the 4'-deoxy-4'-ethylthio and 4'-deoxy-4'-ethylthio derivatives retain comparable activity to the parent.

Conclusions

We have developed methods for the introduction of substituents at the 4'-position of the 4',5'-unsaturated 4,6-AGA

Table 1 Ribosomal inhibition and selectivity of netilmicin, its 4'-derivatives, and comparators (IC_{50} , μM)

Cmpd	4'-Subs	IC_{50}				Selectivity		
		wt	Mit 13	A1555G	Cyt 14	Mit 13	A1555G	Cyt 14
6	H	0.02	28	0.7	69	1400	35	3450
19	I	0.1	49	1.5	221	490	15	2210
20	Br	0.1	52	1.2	146	520	12	1460
21	Cl	0.1	58	1.5	>500	580	15	>5000
22	Ph	0.27	29	3.5	81	106	13	299
23	Bu	0.58	408	22	940	699	37	1612
27	SEt	0.67	256	46	783	381	69	1165
Paromomycin	OH	0.038	142	12	31	3737	315	816
4'-EtSparom 4	SEt	0.053	166	76	104	3132	1434	1962
4'-Pr parom 5	Pr	0.031	167	52	64	5387	1677	2065

Table 2 Antibacterial activity of netilmicin and its 4'-derivatives (MIC, $\mu\text{g mL}^{-1}$)

Cmpd	4'-Subs	MRSA			<i>Escherichia coli</i>			<i>Pseudomonas aeruginosa</i>		
		AG038	AG039	AG042 ^a	AG001	AG055	AG003	AG031	AG032	AG033
6	H	0.5	0.5	2	2	2	8	2	2	>128
19	I	4–8	16	64	2	1–2	16	4–8	4–8	>128
20	Br	4	8–16	64	2	1–2	16	8	8	64
21	Cl	4	8	32	4	2–4	16–32	4	64	>128
22	Ph	8	16	64	4	2	64	16–32	16–32	>64
23	Bu	16	16	64	8–16	5	8–16	64	64	>128
27	SEt	32	32	128	16	8–16	8–16	>128	128	>128

^a Strain AG042 carries the ANT4',4'' gene.

netilmicin suitable for application to cognate compounds. Unfortunately, all such modifications resulted in reductions in antibacterioribosomal activity and in decreased antibacterial activity as tested against a small panel of Gram-positive and Gram-negative bacteria. This pattern of reduced activity upon 4'-substitution corroborates our earlier observations on the functionalization of the 4'-hydroxy group in the kanamycin series of 4,6-AGAs,³ but is in stark contrast to the 4,5-series of AGAs.⁴ The reduced activity seen on modification of netilmicin at the 4'-position is accompanied by reduced target selectivity, again in contrast to the effects of comparable modifications in the 4,5-series of AGAs.^{1,2,4} When combined with recent indications from resistome analysis that a much broader range of AMEs act on the 4,6- than the 4,5-series of AGAs,²⁰ these observations suggest that developmental programs for next generation AGAs should favour the 4,5-over the 4,6-series.

Conflicts of interest

ECB, DC, and AV are co-founders of and maintain an equity interest in Juvabis AG a biotech start-up working in the aminoglycoside field. All other authors declare no conflicts of interest.

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