In vitro antibacterial activity of different adenosine analogues

Luca A. Vitali,¹ Dezemona Petrelli,² Catia Lambertucci,³ Manuela Prenna,² Rosaria Volpini³ and Gloria Cristalli³

Correspondence

Luca A. Vitali

luca.vitali@unicam.it

¹School of Pharmacy, Microbiology Unit, University of Camerino, via Gentile III da Varano, 62032 Camerino (MC), Italy

²School of Biosciences and Biotechnology, University of Camerino, via Gentile III da Varano, 62032 Camerino (MC), Italy

³School of Pharmacy, Medicinal Chemistry Unit, University of Camerino, via S. Agostino 1, 62032 Camerino (MC), Italy

Nucleoside analogues may represent good candidates for the discovery of new antibacterial agents, therefore, a library of adenosine analogues was assessed for their antibacterial activity, and the relationship between the structure and activity of these molecules was outlined. Antibacterial activity was evaluated against that of reference strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. We tested 54 adenosine analogues, modified both at ribose and base moieties, including adenine and 1/3-deazaadenine derivatives substituted in the 2- and/or *N*⁶-positions and bearing *N*-9 sugar moieties, such as ribose, 2'-deoxyribose, 3'-deoxyribose, 2',3'-dideoxyribose or cycloalkyl groups like cyclopentane. The data obtained, MIC and minimal bactericidal concentrations demonstrated that the presence of bulky substituents such as cycloheptyl and cyclooctyl rings on the *N*⁶-amino, together with a chlorine atom in the 2-position, conferred antibacterial activity against the Gram-positive group with MIC values ranging from 16 to 128 mg l⁻¹. The intact sugar moiety seemed to be not essential for antimicrobial activity and nucleosides bearing deoxyribose or cyclopentyl groups associated with bulky substituents in *N*⁶-position showed good antimicrobial properties. Furthermore, *N*-1 proved to be non-crucial and the 2-chloro-*N*⁶-cyclooctyl-1-deaza-3'-deoxyadenosine and 2',3'-dideoxyadenosine compounds were among the more active in the series with an MIC of 32 mg l⁻¹ against *Staph. aureus* and *Strep. pneumoniae*. None of the analogues was active against the two Gram-negative species tested. Hence, adenosine derivatives bearing bulky substituents in the *N*⁶-position may represent good lead compounds for the future discovery of a novel series of antibacterial agents.

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INTRODUCTION

Antimicrobial resistance is growing at a fast rate and micro-organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), staphylococci with decreased susceptibility to vancomycin, multi-drug resistant Gram-negative species and *Streptococcus pneumoniae* with decreased susceptibility to penicillin and other antibacterials are frequently isolated in both hospital and community settings (ECDC, 2010). Therefore, there is a growing need for novel antimicrobial agents with new mechanisms of action.

Nucleosides and their analogues are one class of compounds that are largely studied and used as antiviral and anti-tumour agents (Isono, 1988). Some derivatives show moderate to good activity against specific bacterial strains,

although, in the last two decades, studies on antibacterial properties have often been neglected in favour of those relating to antiviral and anti-tumour activity. Some recent experiences, however, have proven the great value of this field of research. For example, complex nucleosides exhibit antibacterial activity by specific inhibition of cell-wall peptidoglycan biosynthesis (Kimura & Bugg, 2003), certain bacterial enzymes, such as purine nucleoside phosphorylases (Bzowska *et al.*, 2000), and DNA ligases (Mills *et al.*, 2011). In particular, adenosine analogues such as cordycepin (Ahn *et al.*, 2000), 2'-amino-2'-deoxyadenosine and oxetanocin (Shimada *et al.*, 1986), formycins (Bzowska *et al.*, 2000), toyocamycin and its derivatives (Kimura & Bugg, 2003) and some purine derivatives (Tunçbilek *et al.*, 2009) showed biological activity including antibiotic properties. More detailed investigations on the antimicrobial activity of various classes of still untested nucleoside derivatives need

Abbreviation: MBC, minimal bactericidal concentrations.

to be conducted in order to maximize the potential for developing nucleoside analogues for the treatment of bacterial infections. In this study we present the antibacterial activity of a library of adenosine analogues.

METHODS

MICs and minimum bactericidal concentrations (MBCs) were determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2009) and expressed in mg l^{-1} . The adenosine analogues tested are shown in Table

1. Their synthesis and chemical data were previously described (Cristalli *et al.*, 1995; Salvatori *et al.*, 2002; Vittori *et al.*, 2000, 2006; Volpini *et al.*, 1998, 1999), while compounds 53 and 54 will be described elsewhere. Stock solutions of the compounds were prepared in DMSO.

The panel of micro-organisms used in this study included *Staph. aureus* RN4200, *Staph. epidermidis* ATCC 35984, *Strep. pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

A test with DMSO alone was included as a negative control and one with the known antibiotic vancomycin against *Staph. aureus* was used as a positive control. Each experiment was repeated three times.

Table 1. Adenosine analogues used in this study

Cpd, compound; R, replacement/substituent group.

Cpd	R	R ₁	R ₂	R ₃	Cpd	R	R ₁	R ₂	R ₃	Cpd	R	R ₁	R ₂	R ₃
1	H	Cl	OH	OH	32	CH ₃	Cl	OH	OH	50	H	Cl	OH	OH
2	H	I	OH	OH	33	cC ₃ H ₅	Cl	OH	OH	51	H	Cl	H	OH
3	H	SH	OH	OH	34	cC ₄ H ₇	Cl	OH	OH	52	cC ₆ H ₁₁	Cl	OH	H
4	H	S-(CH ₂) ₄ CH ₃	OH	OH	35	cC ₆ H ₁₁	Cl	OH	OH
5	H	S-(CH ₂) ₂ -Ph	OH	OH	36	cC ₈ H ₁₅	Cl	OH	OH					
6	H	S-cC ₅ H ₉	OH	OH	37	H	Cl	H	OH					
7	H	C≡C-nC ₄ H ₉	OH	OH	38	CH ₃	Cl	H	OH					
8	H	C≡C-Ph	OH	OH	39	cC ₃ H ₅	Cl	H	OH					
9	H	C≡C-CH(OH)Ph	OH	OH	40	cC ₆ H ₁₁	Cl	H	OH					
10	CH ₃	C≡C-nC ₄ H ₉	OH	OH	41	cC ₇ H ₁₃	H	OH	H					
11	CH ₃	C≡C-Ph	OH	OH	42	cC ₇ H ₁₃	Cl	OH	H					
12	cC ₃ H ₅	Cl	OH	OH	43	cC ₈ H ₁₅	H	OH	H					
13	cC ₅ H ₉	Cl	OH	OH	44	cC ₈ H ₁₅	Cl	OH	H					
14	cC ₇ H ₁₃	H	OH	OH	45	H	Cl	H	H					
15	cC ₇ H ₁₃	Cl	OH	OH	46	CH ₃	Cl	H	H					
16	CH ₂ CH(Ph) ₂	H	OH	OH	47	cC ₆ H ₁₁	Cl	H	H					
17	CH ₂ CH(Ph)Ph-(4-OH)	Cl	OH	OH	48	cC ₇ H ₁₃	Cl	H	H					
18	CH ₂ CH(Ph)cC ₆ H ₁₁	H	OH	OH	49	cC ₈ H ₁₅	H	H	H					
19	H	Cl	H	OH										
20	cC ₅ H ₉	Cl	H	OH										
21	cC ₇ H ₁₃	Cl	H	OH										
22	cC ₈ H ₁₅	H	H	OH										
23	cC ₈ H ₁₅	Cl	H	OH										
24	H	Cl	OH	H										
25	cC ₅ H ₉	H	OH	H										
26	cC ₅ H ₉	Cl	OH	H										
27	cC ₅ H ₈ -(2-OH)	Cl	OH	H										
28	3-THF	Cl	OH	H										
29	cC ₃ H ₅	H	H	H										
30	cC ₅ H ₉	Cl	H	H										
31	cC ₇ H ₁₃	Cl	H	H										

Table 2. MIC values (mg l⁻¹) for adenosine analogues showing reliable antimicrobial activity

Compound	<i>Staph. aureus</i>	<i>Staph. epidermidis</i>	<i>Strep. pneumoniae</i>
4	>128	>128	128
6	32–64	>128	128
15	>128	>128	128
16	>128	>128	>128
17	64	128	128
18	>128	>128	128
21	>128	>128	128
23	>128	>128	>128
31	>128	>128	128
35	>128	>128	128
36	64	64	64–128
41	>128	>128	128
42	64	128	64
43	32–64	>128	128
44	32	128	32
47	>128	>128	128
48	32	64–128	32
49	>128	>128	64
53	>128	>128	16
54	>128	>128	16

Statistical analysis was performed on the antimicrobial activity MIC values by using the Kruskal–Wallis Test and multifactor analysis of variance in the STATGRAPHICS Centurion XV software package. The tested compounds were grouped and ranked based on the various substitutions at different sites. The N⁶-substituted molecules were ranked according to size of the substituent and three main groups were identified: no substituent, substituents with <5 carbons, and substituents with ≥5 carbons (bulky). The analogues substituted at the 2-position in the adenine moiety were grouped as halogenated, thioalkyl/cycloalkyl-, or alkynyl-substituted compounds. In addition, five groups were ranked based on variations in the sugar moiety: ribose, 2'-deoxyribose, 3'-deoxyribose, 2'-3'-deoxyribose and analogues with the cyclopentyl group in place of the ribose.

RESULTS AND DISCUSSION

The data demonstrated that some of the compounds showed good antibacterial activity against all or some of the Gram-positives with MIC values ranging from 16 to 128 mg l⁻¹ (Table 2). Among the adenosine derivatives 1–18, the presence of a 2-thioalkyl/cycloalkyl group (compounds 4 and 6) conferred antibacterial activity and, in particular, compound 6 proved to be one of the most active towards *Staph. aureus* (MIC 32–64 mg l⁻¹). The antibacterial activity of several 2-substituted adenosine analogues has recently been reported by Mills *et al.* (2011). This study showed that 2-thio-n-butyl adenosine was an inhibitor of the bacterial NAD⁺-dependent DNA ligase. In the present work, among the 2-substituted adenosine derivatives, the most active agents of the series were 2-thio-n-pentyladenosine (compound 4) and 2-thio-cyclopentyladenosine (compound 6). The structure of these

compounds is very similar to that of the previously reported NAD⁺-dependent DNA ligase inhibitors. Hence, it is possible to hypothesize that a similar mechanism of action is responsible for their antimicrobial activity.

On the other hand, the presence of halogens (compounds 1 and 2) or of a lipophilic alkynyl chain (compounds 7–11) in the same position led to inactive compounds. Moreover, compounds bearing bulky alkyl or aralkyl chains on the N⁶ amino (compounds 15–18) showed antibacterial activity against *Strep. pneumoniae* ($P < 0.05$). The presence of a chlorine atom in the 2-position led to a derivative that was also active towards *Staph. aureus* (compound 17, MIC 64 mg l⁻¹).

The intact sugar moiety seemed to be non-essential for activity against *Strep. pneumoniae*, and nucleosides bearing deoxyribose groups associated with bulky substituents in the N⁶-position showed good antimicrobial properties. In particular, the 2'-deoxy derivative 21 and the 2',3'-dideoxy derivative 31, bearing a 2-chlorine atom and an N⁶-cycloheptyl ring, showed the highest activity in the series. On the other hand, the 3'-hydroxy group seemed to be more important for antimicrobial activity; in fact, none of the 3'-deoxy derivatives (compounds 24–28) displayed antimicrobial properties.

Furthermore, N-1 proved to be non-crucial as the 2-chloro-N⁶-cyclooctyl-1-deaza-3'-deoxyadenosine and 2',3'-dideoxyadenosine were among the most active compounds (compounds 44 and 48, MIC 32 mg l⁻¹ against *Staph. aureus* and *Strep. pneumoniae*). On the contrary, none of the tested 3-deazaadenosine derivatives showed antibacterial activity, although general conclusions cannot be drawn for this subset of compounds given the limited number available for testing (compounds 50–52, Table 1).

When the ribose was replaced by a cyclopentyl group and an N⁶-cyclooctyl substituent was introduced, compounds 53 (R=H) and 54 (R=Cl) were obtained (Table 1), which showed the highest antibacterial activity against *Strep. pneumoniae* (MIC 16 mg l⁻¹, Table 2).

In conclusion, the presence of bulky substituents at N⁶-position of the tested compounds led to molecules that showed, in general, good antimicrobial properties ($P < 0.01$). The simultaneous presence of a chlorine atom in the

Table 3. MBC values (mg l⁻¹) for adenosine analogues showing the most reliable MIC values

ND, Not determined (because of an MIC ≥ 64 mg l⁻¹).

Compound	<i>Staph. aureus</i>	<i>Strep. pneumoniae</i>
6	>128	ND
44	>128	32
48	>128	32
53	ND	>128
54	ND	16

2-position conferred greater antibacterial activity in comparison with the unsubstituted compounds (e.g. compound 44, MIC 32 mg l⁻¹ versus compound 43, MIC 128 mg l⁻¹ against *Strep. pneumoniae*). The isosteric substitution of the nitrogen in the 1-position with a CH group resulted in nucleosides with improved antimicrobial activity against both *Staph. aureus* and *Strep. pneumoniae* (e.g. compound 31, MIC=128 mg l⁻¹, versus compound 48, MIC=32 mg l⁻¹). Table 3 shows the MBCs determined for compounds that had MICs <64 mg l⁻¹. Three of these compounds, 6, 44, and 48, were bactericidal against *Staph. aureus* and three out of the five compounds with MICs <64 mg l⁻¹, namely 44, 48, and 54, when assayed with *Strep. pneumoniae*, had matching MBC and MIC values, indicating bactericidal activity. Interestingly, compound 53, which was inhibitory at a concentration of 16 mg l⁻¹ as with compound 54, was not bactericidal against the *Strep. pneumoniae*. This finding suggests that the chlorine in the 2-position is sufficient to convert the analogue from bacteriostatic to bactericidal.

On the other hand, none of the analogues was active against the two Gram-negative species tested (*P. aeruginosa* and *E. coli*), with the exception of the well-known derivative 19 (cladribine, 2-chloro-2'-deoxyadenosine), which had an MIC of 64 mg l⁻¹ against the *E. coli* strain.

Adenosine and 1-deazaadenosine derivatives bearing bulky substituents in the N⁶-position may represent good lead compounds for the future discovery of a novel series of antibacterial agents. A combination of substituents in the N⁶-position with selected 2-thioalkyl/cycloalkyl chains could be a strategy to improve the antibacterial activity of this class of nucleosides.

Further studies to determine the mechanism of action of these compounds are necessary to identify their cellular target and to design and synthesize more potent analogues.

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