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Discovery of novel ureas and thioureas of 3-decladinosyl-3-hydroxy 15-membered azalides active against efflux-mediated resistant *Streptococcus pneumoniae*

Mirjana Bukvić Krajačić [a,*,†], Miljenko Dumić [b], Predrag Novak [c], Mario Cindrić [d], Sanja Koštrun [a,†], Andrea Fajdetić [a,†], Sulejman Alihodžić [a,†], Karmen Brajša [a,†], Nedjeljko Kujundžić [a,†]

[a] GlaxoSmithKline Research Centre Zagreb, Prilaz baruna Filipovića 29, HR-10000 Zagreb, Croatia
[b] Department of Biotechnology, University of Rijeka, S. Krautzeka bb, HR-51000 Rijeka, Croatia
[c] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb, Croatia
[d] Institute 'Ruđer Bošković', Bijenička cesta 54, HR-10000 Zagreb, Croatia

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ABSTRACT

A series of novel ureas and thioureas of 3-decladinosyl-3-hydroxy 15-membered azalides, were discovered, structurally characterized and biologically evaluated. They have shown good antibacterial activity against selected Gram-positive and Gram-negative bacterial strains. These include N″ substituted 9a-(*N*′-carbamoyl-γ-aminopropyl)- (**6a,c**), 9a-(*N*′-thiocarbamoyl-γ-aminopropyl)- (**7a,e**), 9a-[*N*′-(β-cyanoethyl)-*N*′-(carbamoyl-γ-aminopropyl)]- (**9a–c, 9g**) 9a-[*N*′-(β-cyanoethyl)-*N*′-(thiocarbamoyl-γ-aminopropyl)]-derivatives (**10d–f**) of 5-*O*-desosaminyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythronolide A (**3**).

Among the synthesized compounds thiourea **7a** and urea **9b** have shown substantially improved activity comparable to azithromycin (**1**) and significantly better activity than the 3-decladinosyl-azithromycin (**2**) and the parent 3-cladinosyl analogues against efflux-mediated resistant *S. pneumoniae*.

In spite of a numerous existing macrolide antibiotics, such as azithromycin (**1**)[1–5], the emerging multi-drug resistant microbial pathogens present serious and challenging problems in medical treatment which demand novel and more effective antimicrobial agents to be discovered. The key bacterial pathogens involved in community acquired respiratory tract infections (CARTI) included *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*.[6–9] Active efflux has been recognized as one of the most frequent mechanism in developing antibiotic resistance.[10–13]

The discovery of highly potent 3-*O*-decladinosyl derivatives, for example, ketolides,[14] acylides,[15,16] anhydrolides,[17] etc., was a step forward to tackle the efflux problems.[18–24] However, some serious drawbacks have been observed for those compound classes: the emergence of resistance developed shortly after their introduction[24] and rare but serious side effects which lead to restrictions and withdrawal[24] as seen recently with telithromycin.[25]

Thus, in order to prepare active compounds less prone to the above mentioned difficulties we developed a strategy aiming at the synthesis of azithromycin analogues involving 3-keto- and 3-*O*-acyl-azalides,[26] 9a-carbamoyl-, 9a-thiocarbamoyl- and sulfonylcarbamoyl-3-decladinosyl-derivatives of 15-membered azalides.[26–30] As expected, 3-hydroxy-3-decladinosyl macrolides and azalides lacked any significant antimicrobial activity[27,29,30,33,34] being consistent with the role cladinose was found to play in antimicrobial activity.[3–5,15,18] This is supported by recently published NMR binding studies showing that the absence of cladinose sugar has been found to be the main cause of their inability to bind to their target ribosome.[31] Recently[32] we reported the two 3-decladinosyl-3-hydroxy derivatives **6a** and **9a** (Scheme 1) tested only against panel of *S. pneumoniae* strains. As we expected decladinosyl urea derivative **9a** did not show activity against tested strains. However, decladinosyl urea derivative **6a** showed significant activity against erythromycin-susceptible *S. pneumoniae* strain (1 μg/ml), as well as efflux-mediated resistant *S. pneumoniae* strain (8 μg/ml) comparable to azithromycin. Those results encouraged us to further investigate decladinosyl macrolide compounds. Hence, as a continuation of our research in this field we describe here the synthesis of a small library of ureas and thioureas of 3-decladinosyl-3-hydroxy 15-membered azalides and their activity

* Corresponding author. Tel.: +385 1 8886346.
E-mail address: mirjana.bukvic@gmail.com (M. Bukvić Krajačić).
† Present address: Galapagos Research Centre, Prilaz baruna Filipovića 29, 10000 Zagreb, Croatia.

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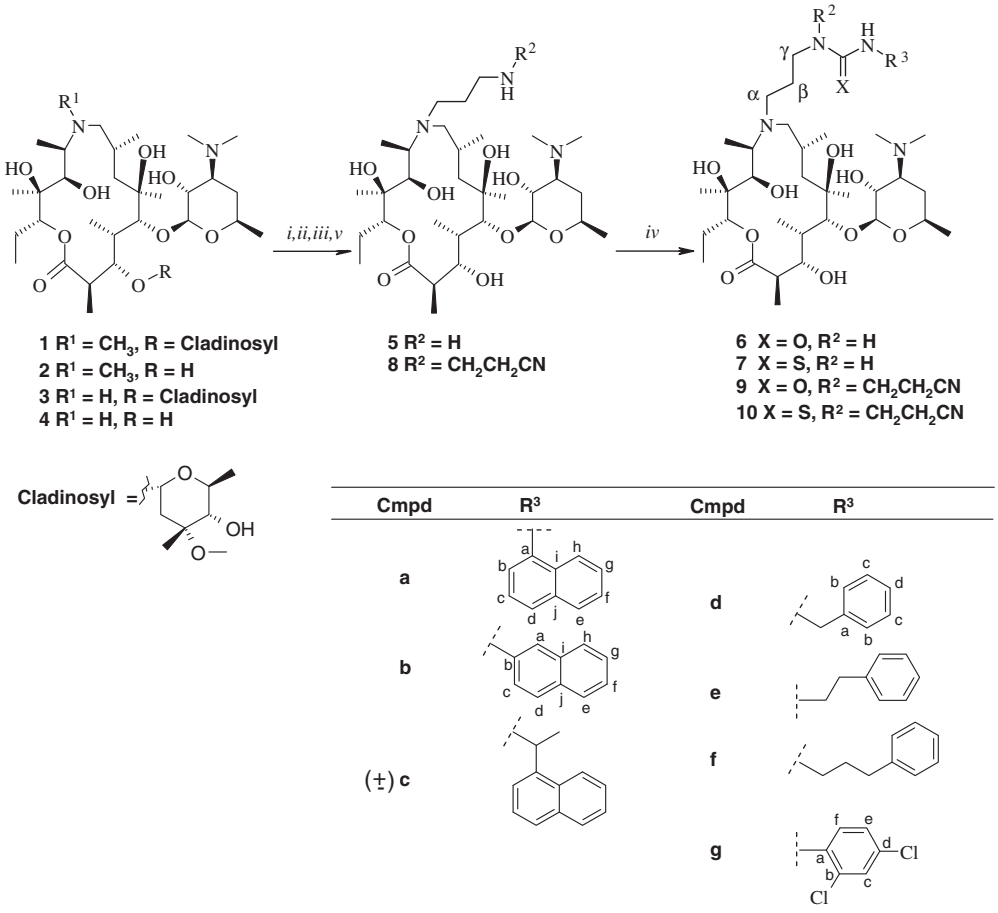

Scheme 1. Synthesis of novel decladinosyl ureas and thioureas of 15-membered azalides. Reagents and conditions: (i) 6 M hydrochloric acid, rt, (ii) acrylonitrile, 60 °C, 10 h; (iii) H_2/5% Pt/C, 5% hydrochloric acid, 4 bar, rt, 40 h; (iv) alkyl or aryl isocyanate/isothiocyanate, CH_2Cl_2, room temperature, 1 h; (v) 1 equiv acrylonitrile, methanol, reflux, 10 h.

against key respiratory Gram-positive and Gram-negative pathogens including efflux-mediated resistant strains.

The first samples of 3-decladinosyl ureas **6a** and **9a** were obtained by hydrolytic degradation of their 3-cladinosyl analogues.[32] High reactivity of secondary and primary amino groups of 3-decladinosyl-derivatives **5** and **8** obtained according to procedure described in the previous papers[27,32] toward isocyanates and isothiocyanates assured highly site-selective introduction of carbamoyl and thiocarbamoyl groups[27,28,32] and preparation of ureas **6, 9** and thioureas **7, 10** in high yield (Scheme 1).

Structures of all synthesized compounds were determined by IR and NMR spectroscopies and mass spectrometry. Assignments of proton and carbon chemical shifts were made by the combined use of one- (^{1}H and APT) and two-dimensional (gCOSY, gHSQC and gHMBC) NMR spectra. N″ substituted 9a-(*N*′-carbamoyl-γ-aminopropyl)- (**6a,c**), 9a-(*N*′-thiocarbamoyl-γ-aminopropyl)- (**7a,e**), 9a-[*N*′-(β-cyanoethyl)-*N*′-(carbamoyl-γ-aminopropyl)]- (**9a–c, 9g**) 9a-[*N*′-(β-cyanoethyl)-*N*′-(thiocarbamoyl-γ-aminopropyl)]-derivatives (**10d–f**) are new and their structures were supported by spectral data. Physical and spectral data of the compounds are in agreement with the proposed structures.

The in vitro minimum inhibitory concentrations (MICs) of 3-decladinosyl ureas **6, 9**, and thioureas **7**, **10** against a panel of erythromycin-susceptible and erythromycin-resistant Gram-positive and Gram-negative bacterial strains in comparison to azithromycin **1** and 3-decladinosyl azithromycin (**2**)[35] as standards are presented in Table 1. Most of the synthesized 3-decladinosyl derivatives showed moderate to high activity against efflux-mediated resistant *S. pneumoniae* and moderate activity against susceptible *S. pneumoniae* and *Streptococcus pyogenes* strains. Against efflux-mediated resistant *S. pneumoniae* thiourea **7a** and urea **9b**, had the lowest MIC values of 2 and 4 μg/ml, respectively, and comparable to azithromycin (**1**) but significantly better in comparison to the 3-decladinosyl azithromycin (**2**) and their parent 3-cladinosyl analogues (Fig. 1).[32] The racemic mixture of **6c** showed the highest activity against susceptible *S. pneumoniae* and *S. pyogenes* strains (<0.125 μg/ml) and the same activity as its 3-cladinosyl analogue[32] and azithromycin. Interestingly, some of discovered 3-decladinosyl-3-hydroxy ureas **6, 9**, and thioureas **7**, **10**, maintain antibacterial activity against Gram-negative pathogens *H. influenzae* and *M. catarrhalis* in comparison to their parent 3-cladinosyl derivatives,[32] and demonstrate large improvement in comparison to the inactive 3-decladinosyl sulfonylureas[27] and 3-decladinosyl azithromycin-sulfonamide conjugates.[29] Activity of **6a** and **7a** against *H. influenzae* (MIC 2 μg/ml) is only one dilution lower than the corresponding MIC of azithromycin. Urea **6c** was more potent (MIC 8 μg/ml) than its 3-cladinosyl analogue (MIC 16 μg/ml)[32] against *Enterococcus faecalis* and **6a** showed the same activity (MIC 8 μg/ml) against *Escherichia coli* in comparison to its cladinosyl analogue.

Thus, it seems that appropriate linked urea or thiourea moiety at 9a nitrogen atom of 3-decladinosyl azalides might interact with particular ribosome binding sites and substitute the cladinose sugar interaction. In order to gain more information about that we carried out a conformational analysis of compound **7a** by using systematic conformational search around flexible propyl linker. Macrolactone

Table 1
Antibacterial activity (MIC/μg ml^{-1}) of 3-decladinosyl ureas **6a**, (±)-**6c** and **9a-c**, **9g** and thioureas **7a**, **7e** and **10d–10f** in comparison to azithromycin (**1**)

Compounds	**6a**	(±)-**6c**	**7a**	**7e**	**9a**	**9b**	(±)-**9c**	**9g**	**10d**	**10e**	**10f**	**2**	**1**
X	O	O	S	S	O	O	O	O	S	S	S	-	
R	H	H	H	H	H	H	H	H	H	H	H	H	
R^3													
S. aureus ATCC 29213	>64	16	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	0.5
S. pneumonia-eryS	1	<0.125	1	4	16	NT	4	NT	NT	NT	NT	>64	<0.125
S. pneumoniae-M	8	16	2	16	64	4	8	8	16	16	16	>64	8
S. pyogenes-eryS	2	<0.125	8	32	16	8	4	>64	>64	>64	32	>64	<0.125
S. pyogenes-M	16	16	64	64	64	16	>64	>64	>64	>64	64	>64	4
M. catarrhalis ATCC 23246	2	4	0.5	4	8	2	4	8	2	8	8	NT	<0.125
H. influenzae ATCC 49247	2	4	4	16	32	2	16	16	8	32	8	16	1
E. faecalis ATCC 29212	32	8	32	64	>64	32	64	>64	>64	>64	>64	NT	8
E. coli ATCC 25922	8	16	16	64	64	16	>64	>64	>64	>64	>64	16	2

eryS: erythromycin-susceptible, M: efflux-mediated macrolide resistance, NT: not tested.

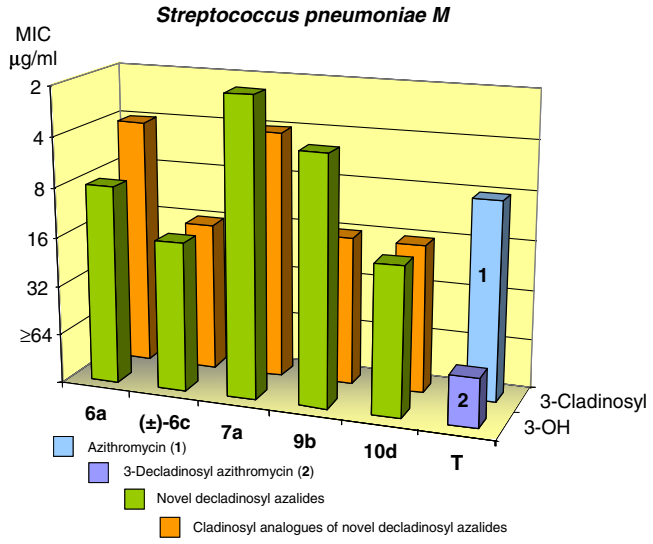

Figure 1. Antibacterial activity of 3-decladinosyl ureas **6a**, (±)-**6c** and **9b** and thioureas **7a** and **10d** on *S. pneumoniae* efflux-mediated resistant strain in comparison to their parent 3-cladinosyl analogues[32] and test standards azithromycin (**1**) and 3-decladinosyl azithromycin (**2**).

and desosamine rings were not optimized as the available force fields do not reproduce reliably macrolactone conformations. A crystal structure data of azithromycin was used as a template.[36] The sugar conformation is not expected to change significantly due to the substitutions at 9a-position of **7a**. Analysis of NOE cross peaks in the NOESY spectrum indicated that there is no strong interaction between macrolactone ring and the substituent at 9a-position of **7a** pointing to the stretched conformations that were also found to be most stable ones in the conformational analysis. Figure 2 shows superposed X-ray conformations for ABT-773,[37] azithromycin,[38,40] two bound conformations of telithromycin from *Deinococcus radiodurans*[39] and *Haloarcula marismortui*[40] and the lowest conformation for compound **7a**. It is clear that substituents at different positions have different spatial arrangements with respect to macrolactone. Until now there is a number of evidence including here mentioned ketolides,[37–40] that high structural diversity is tolerated within the flexible macrolide-binding site of ribosome. In spite of the knowledge gained so far on macrolide-binding,[31,37–41] an understanding of the mode of their interactions with ribosome still remain incomplete with many issues unresolved. Therefore, it can only be speculated about the possible binding mode of the compound **7a** but it is likely that the additional

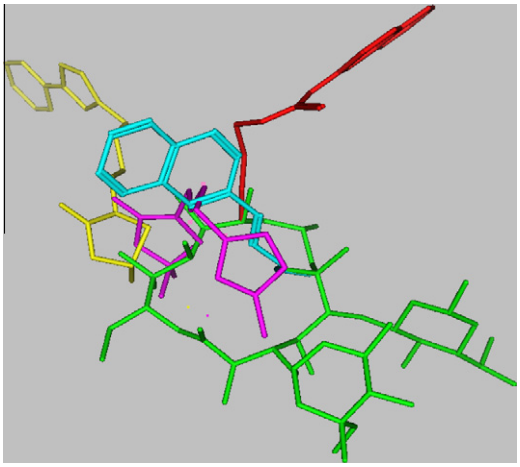

Figure 2. Superposed X-ray conformations for azithromycin (green),[38,40] ABT-773 (cyan),[37] two conformations of telithromycin from *Deinococcus radiodurans* (magenta)[39] and *Haloarcula marismortui* (yellow)[40] complexes and most stable conformation for compound **7a** (red).

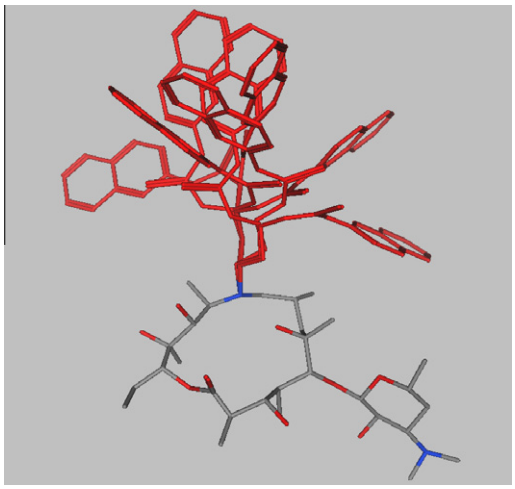

Figure 3. First 10 most stable conformations of compound **7a**.

interaction involving 1-naphthyl-propyl-side-chain might lead to a further stabilization of a complex with ribosome. In Figure 3, first ten most stable conformations of **7a** are shown ranging from stretched most stable conformation to conformation where the naphthyl

substituent of **7a** is eclipsed with macrolactone ring. Since energy difference between first ten conformations is only several kcal/mol (3 kcal/mol at MMFF94x as well as at the B3LYP/6-31G** level), any of these conformations can be adopted depending on the entropic and enthalpic effect within the active site.

In conclusion, it was shown here that urea and thiourea derivatives of 3-decladinosyl-3-hydroxy azalides, although lacking a cladinose sugar, showed noticeable antibacterial activity. The compound **6a** was found to be significantly active against erythromycin-susceptible *S. pneumoniae* strain as well as efflux-mediated resistant *S. pneumoniae* strain. Compound **6c** showed the same antibacterial activity as a control drug azithromycin against susceptible *S. pneumoniae* and *S. pyogenes* strains. Also some 3-decladinosyl-3-hydroxy ureas **6, 9**, and thioureas **7**, **10**, maintain antibacterial activity against Gram-negative pathogens *H. influenzae* and *M. catarrhalis* in comparison to their parent 3-cladinosyl derivatives,[32] and comparable to azithromycin, but demonstrate a large improvement in comparison with inactive 3-decladinosyl azithromycin **2**[35] and other 3-decladinosyl derivatives reported in the literature.[27,29] The results presented here can be a further step in the development of new decladinosyl azalides. Although the limited number of compounds studied here can not allow for a comprehensive SAR analysis, they can serve as a good platform to explore the nature of bacterial resistance. Thus, this novel class of 3-decladinosyl-3-hydroxy azalides represents the promising hit compounds, which can be a basis for further modifications and development of novel potent antibacterials, especially against efflux-mediated resistant *S. pneumoniae*.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.079.

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