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Synthesis and antibacterial activity of desosamine-modified macrolide derivatives

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ABSTRACT

Structural factors behind erm macrolide resistance were studied through synthesis of new macrolide derivates possessing truncated desosamine sugar moieties and subsequent determination of their antibacterial activity. Synthesized compounds with 2′-deoxy and 3′-desmethyl desosamine rings demonstrated decreased antibacterial activity on the native *Staphylococcus aureus* strain and were inactive against constitutively resistance *S. aureus*. The obtained results indicate that steric repulsion between the dimethylated A2058 and desosamine ring cannot be considered as a primary reason for erm-resistance.

Macrolide antibiotics of the first (erythromycin **1a**) and second (clarithromycin **1b**, azithromycin **1c**) generations are commonly used for the treatment of infectious diseases.[1] More than 50 years of macrolide antibiotics research have revealed both their strengths—low toxicity, high efficacy, and the rare occurrence of allergic reactions—as well as their drawbacks—poor bioavailability and rapidly spread resistance.[2] Ketolides such as telithromycin[3] **2d**, cethromycin[4] **2e**, and solithromycin[5] **2f** constitute the third generation of macrolide antibiotics that demonstrate improved activity against a number of resistant isolates and represent successful examples of chemical modifications of the macrolide platform which results in superior antibacterial agents.

Macrolide antibiotics inhibit protein synthesis by blocking the progression of nascent proteins through the exit tunnel in the 50S ribosomal subunit preventing the elongation of the polypeptide chain.[6,7] All macrolide antibiotics exhibit remarkable consistency in their binding modes to the binding pocket in the exit tunnel. Their high affinities originate mainly from hydrophobic interactions of their macrocyclic lactone rings and hydrogen bonding of their desosamine sugars with rRNA. While Van der Waals interactions between rRNA and the macrolactone ring are important for the drug binding, a substantial part of the binding energy comes from contacts that involve C5-desosamine that approaches adenines residues 2057, 2058, and 2059 of 23S rRNA (Fig. 1).[8] Two major components dominate the binding of desosamine: (a) hydrogen bond between the 2′ hydroxy group and the N-1 of A2058, and (b) electrostatic interaction between the protonated 3′-dimethylamino group with neighboring phosphate groups (4.5 Å distance, Fig. 1). The placement of desosamine in the ribosome is essentially identical for all studied macrolide antibiotics. Acylation of 2′-OH group yields inactive but labile 2′-esters that are hydrolyzed by water or alcohols to regenerate initial macrolides.[9] Other chemical modifications of desosamine such as replacement of the 3′-dimethylamino group with acylamino groups results in complete loss of antibacterial activity.[10]

Dimethylation of N6 of A2058 residue by *Erm*-type methyltransferases dramatically decreases affinity of macrolides to ribosome rendering bacteria that produce these enzymes resistant to all known types of macrolide antibiotics.[11] Despite significant effort invested in structural modifications of macrolides and ketolides,[12] there have been no reports of successful macrolide antibiotics capable of binding to A2058 dimethylated ribosomes. Although structural reasons for *Erm*-mediated resistance have been proposed on the basis of crystallographic analysis of native ribosome complexed with macrolide antibiotics, the Erm-modified ribosome has never been investigated crystallographically. As a result, predictions about the interactions of macrolide antibiotics with A2058 dimethylated ribosomes are currently based solely on theoretical considerations.[13] A graphic representation based on the crystal structure of ketolide telithromycin **2d** bound to native *Escherichia coli* ribosome[13b] is shown in Figure 1. Three possible factors could contribute to a sharply decreased affinity of macrolides to the A2058 dimethylated ribosome:

(a) Introduction of methyl groups on N-6 of A2058 can disrupt the hydrogen bonding between 2′-OH of macrolides and N-1 of A2058;

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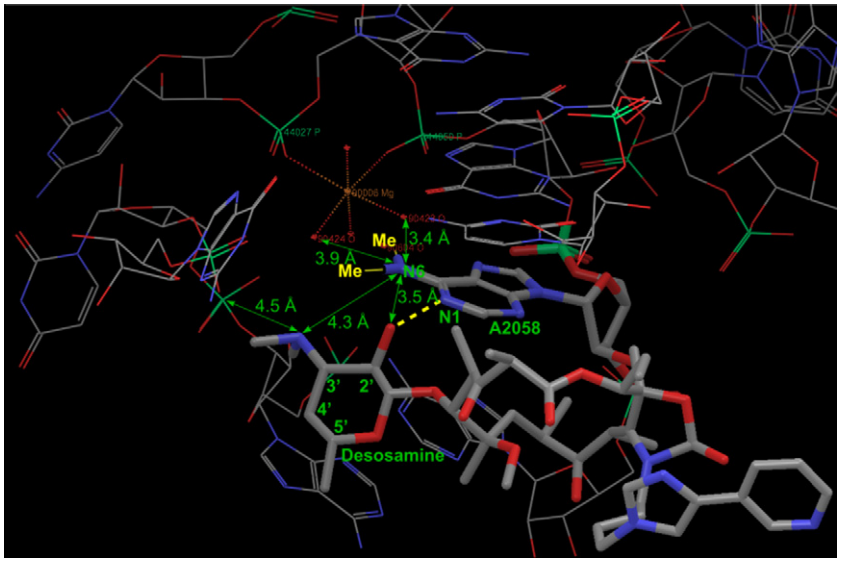


Figure 1. Fragment of crystal structure of telithromycin–*E. coli* 50S ribosome subunit complex (PDB 3OAT) with measured distances between N6 of A2058 residue and nearby 3′-NMe_2 (4.3 Å) and 2′-OH (3.5 Å) groups in desosamine sugar of telithromycin, and between N6 of A2058 residue and water molecules (3.4 and 3.9 Å) coordinated to magnesium cation in 23S RNA. Two yellow methyl groups attached to N6 were manually added to the crystal structure to illustrate possible non-bonding interactions arising in A2058 dimethylated ribosome.

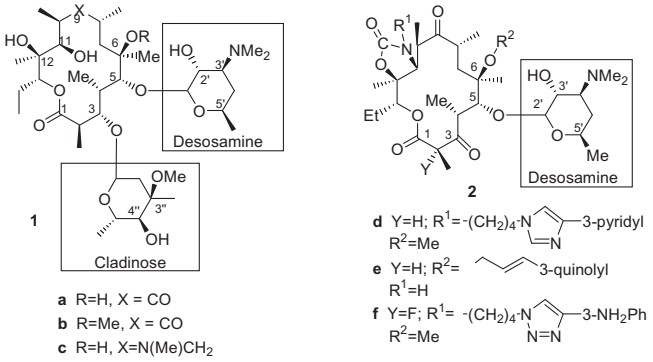
(b) The methyl group on N-6 of A2058 may sterically clash with 2′-OH and/or 3′-dimethylammonium groups of the desosamine. Due to the rigidity of the six-membered desosamine scaffold the clash may result in squeezing the desosamine sugar out of its binding pocket.

(c) The N-6 methyl group(s) on A2058 may clash with neighboring water molecules (3.9 Å, 3.4 Å, Fig. 1) bound to the Mg^{2+} cation that is chelated by phosphate groups of G2056 and G2057. In this case, positioning of 23S rRNA residues in the whole macrolide binding pocket may be altered.

Discrimination between these possibilities can be done through synthesis of macrolide derivatives containing a modified desosamine cycle and examining their antibacterial activity on native and *Erm*-modified strains. Particularly, the contribution of hydrogen bonding between N-1 of A2058 and 2′-OH can be tested by comparing the antibacterial activity of macrolide antibiotics with their 2′-deoxy derivatives in which 2′-OH of desosamine is replaced with a hydrogen atom. In this Letter we report on synthesis of new macrolide derivatives possessing desosamine sugar modified in positions 2′ and 3′, and their corresponding antibacterial activity on macrolide susceptible and macrolide resistant strains of *Staphylococcus aureus*.

Synthesis of all new macrolide compounds was done starting from the available erythromycin, clarithromycin, telithromycin, and cethromycin (ABT-773) antibiotics. *N*-Desmethyl erythromycin **3a** and clarithromycin **3b** were prepared by reaction of corresponding macrolides **1a**,**b** with iodine and sodium acetate in methanol.[14] *N*-Desmethyl clarithromycin **2b** was used for preparation of 3′-(1-methylguanidino) clarithromycin by refluxing with excess of cyanamide in ethanol. *O*-Deoxygenation[15] of erythromycin and clarithromycin was performed by free radical reduction using treatment with 2 equiv of PhOC(S)Cl/triethylamine to afford corresponding thionocarbonates that were converted into deoxygenated macrolides **5a**,**b** through subsequent reaction with Bu_3SnH/AIBN (see Schemes 1 and 2).

Modification of the desosamine ring in ketolides **2d**,**e** required different set of reaction conditions (Scheme 3). *N*-Desmethyl telithromycin **6d** was prepared through treatment of telithromycin with *N*-iodosuccinimide in acetonitrile.[16] To study the effect of

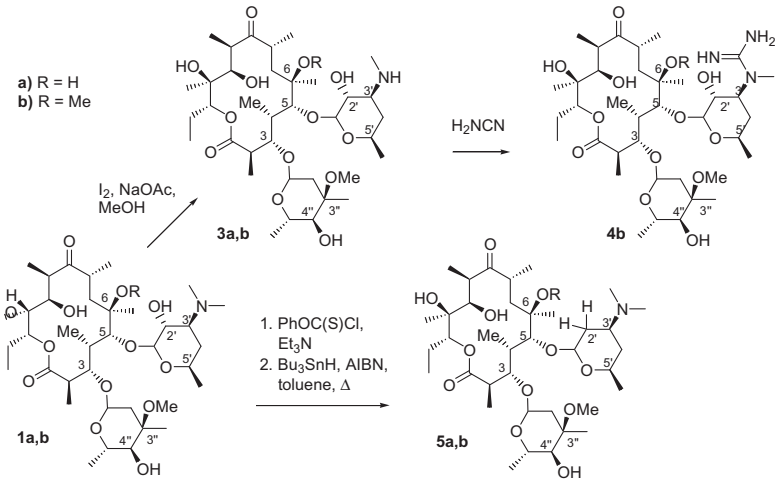


Scheme 1. Macrolide antibiotics erythromycin **1a**, clarithromycin **1b**, azithromycin **1c**, and ketolide telithromycin **2d**, cethromycin **2e**, solithromycin **2f**.

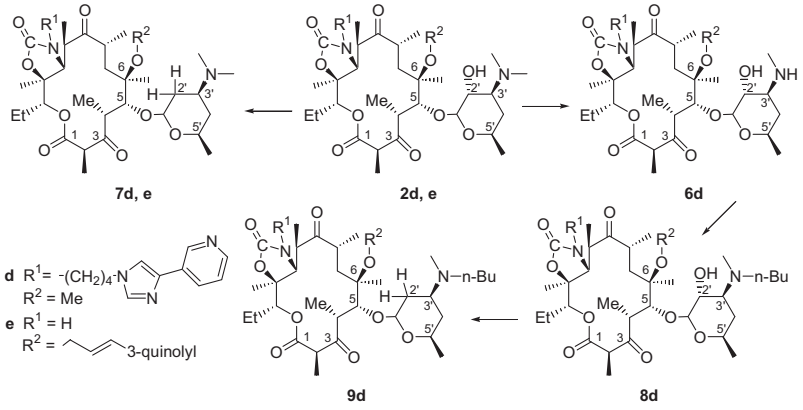
replacement of additional groups in the desosamine ring we also introduced *n*-Bu chain in 3′-desmethyltelithromycin **6d** by reductive alkylation with excess of sodium cyanoborohydride and butyraldehyde to give ketolide **8d**.

Initial attempts for deoxygenation of telithromycin **2d** and cethromycin **2e** through preparation of corresponding 2′-thionocarbonate with PhOC(S)Cl/triethylamine were not successful due to formation of complex mixture of products during the reaction. Negative results were also obtained by using other reagents including PhOC(S)Cl/pyridine, PhOC(S)Cl/ dimethylaminopyridine, and $C_6F_5OC(S)Cl$/dimethylamino-pyridine. The deoxygenation of telithromycin was eventually achieved through its prolonged treatment with an excess of *N*,*N*-thiocarbonyldiimidazole at room temperature followed by one pot reaction of the reaction mixture with Bu_3SnH/AIBN to give compound **7d**. The same procedure was used for preparation of 2′-deoxycethromycin **7d**, and 2′-deoxy-3′-(*n*-butylmethylamino) telithromycin **9d**.

All new macrolide derivatives possessing a modified desosamine function showed moderate antibacterial activity on native, antibiotic-sensitive *S. aureus* strain ATCC29213 (Table 1). Five new 2′-deoxymacrolide derivatives **5a**, **5b**, **7d**, **7e**, and **9d** showed MIC values ranging between 4 and 64 μg/mL which were substantially higher than those of parent macrolides. These results are



Scheme 2. Synthesis of *N*-desmethyl macrolides **3a**,**b**, 3′-(1-methylguanidino)clarithromycin **4b**, and 2′-deoxymacrolides **5a**,**b**.



Scheme 3. Preparation of 3′-desmethyltelithromycin **6d**, 2′-deoxy-3′-(n-butylmethylamino)telithromycin **9d** and 2′-deoxyketolides **7d**,**e**, **9d**.

Table 1
Antibacterial activity of macrolide and ketolide derivatives

Compound	MIC for *S. aureus* ATCC29213 (wild type), μg/mL	MIC for *S. aureus* ATCC33591 (*erm*A, constitutive), μg/mL
3a	8	>256
3b	4	>256
4b	32	>256
5a	64	>256
5b	64	>256
6d	16	>256
7d	8	>256
7e	4	>256
Erythromycin (**1a**)	0.5	>256
Telithromycin (**2d**)	0.08	>256
Cethromycin (**2e**)	0.015	>256
8d	0.5	>256
9d	64	>256

consistent with the important role of hydrogen bond between 2′-OH in macrolides and N-1 in A2058. However, these macrolides retained moderate antibacterial activities, and even the biggest difference (~500-fold) observed for 2′-deoxycethromycin **7e** (MIC50 4 μg/mL) versus cethromycin **2e** (0.0075 μg/mL) was still much smaller than the $>10^4$ increase in MIC for **2e** in ATCC33591 strain of *S. aureus* that carries the constitutively-expressed *erm*A gene to macrolides in comparison to the native ATCC29213 strain. This comparison clearly indicates that the far more dramatic decrease in antibacterial activity observed in *erm*-positive resistant bacteria (see Table 1, ermA-positive strain ATCC33591) cannot be attributed solely to the loss of the hydrogen bond between 2′-OH of macrolide antibiotics and A2058.

All new 2′-deoxy macrolide derivatives were completely inactive against *erm*A-positive strain ATCC33591. This result argues against any considerable contribution of a steric clash between N-6 methyl groups of A2058 and 2′-OH of macrolide derivative to *erm*-mediated resistance.

Similarly to 2′-deoxygenated macrolide derivatives; 3′-desmethyl macrolide derivatives **3a**,**b** and **6d** showed lower antibacterial activity in the native strain of *S. aureus*. A moderate decrease in antibacterial activity was observed in ketolide **8d** bearing *n*-BuNMe group in 3′-position instead of dimethylamino group. Replacement of 3-methylamino group in clarithromycin analog **5b** with 1-methylguanidino group resulted in a significant drop in antibacterial activity with MIC rising from 4 to 32 μg/mL. Nevertheless, the compounds retained the general ability to inhibit bacterial growth thereby demonstrating for the first time, the antibacterial activity of macrolide derivatives possessing an sp^2 nitrogen function at the position 3′. These results provide incentive for further investigation of possible substitutions at this position. Despite the removal of one methyl group at the 3′ position of desosamine ring which was expected to decrease the possible steric clash between a *N*-6 methyl group of dimethylated A2058 and desosamine residue, all synthesized derivatives were completely inac-

tive against erm-positive ATCC33591 *S. aureus* strain. However, change in steric volume by removal of one methyl group may be not sufficient to completely rule out this possibility.

Based on our results, it can be concluded that the loss of the hydrogen bond between 2′-OH and *N*-1 A2058 residue as well as the possible steric clash between 2′-OH of desosamine sugar and N-6 methyl groups in A2058-dimethylated bacterial ribosome are not major factors that account for resistance of A2058-dimethylated strains. It can be further hypothesized that the possible structural factors for the dramatically decreased binding of macrolides to A2058 dimethylated ribosome involve non-bonding interactions between N-6 methyl group(s) of A2058 and water molecules coordinated to the Mg^{2+} cation bound to phosphate groups of G2056 and G2057 residues of 23S RNA. If this hypothesis is correct, the conformation of rRNA in A2058 dimethylated ribosome may be different from that seen in the native ribosome.

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