J. Antibiot. 58(6): 380–389, 2005

ORIGINAL ARTICLE

THE JOURNAL OF ANTIBIOTICS

Synthesis, Characterization and *In Vitro* Antimicrobial Activity of Novel Sulfonylureas of 15-Membered Azalides

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Received: February 25, 2005 / Accepted: June 3, 2005

Abstract Three series of the novel sulfonylurea derivatives of 15-membered azalides, *i.e.* 9a-*N*-[*N'*-(aryl)sulfonylcarbamoyl] (**4a**∼**4f**, **5a**∼**5f**), 9a-*N*-{*N'*-[(aryl)sulfonylcarbamoyl-γ-aminopropyl]} (**10a**∼**10f**, **11a**, **11c**) and 9a-*N*-{*N'*-(β-cyanoethyl)-*N'*-[(aryl)sulfonylcarabamoyl-γ-aminopropyl]} (**14a**∼**14f**, **15a**, **15b**, **15f**) derivatives of 9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A (**2**) and 5-*O*-desosaminyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythronolide A (**3**) were prepared and their structures elucidated by NMR and IR spectroscopic methods and mass spectrometry. Minimal inhibitory concentration (MIC) of these compounds was determined on a panel of sensitive and resistant Gram-positive and Gram-negative bacterial strains. Several compounds of the series of 9a-*N*-[*N'*-(aryl)sulfonylcarbamoyl] derivatives that showed significant improvements in activity against inducible resistant *Streptococcus pyogenes* strain were suggested for further optimization.

Keywords azalides, sulfonylurea, synthesis, NMR and MS, antimicrobial activity

Introduction

Macrolide antibiotic azithromycin (**1**) was shown to have good activity against all key respiratory pathogens [1]. It also possesses excellent safety and tolerability profiles and is widely prescribed for the treatment of upper and lower respiratory tract infections [2∼5]. However, the growing resistance to antibiotics conferred by microorganisms commonly involved in respiratory tract infections has become a serious clinical problem [6]. *Streptoccoccus pyogenes* is the most common bacterial strain implicated in acute pharyngitis, skin and soft tissue infections and also one of the most problematic respiratory pathogen [7]. The widespread use of macrolides has contributed to the increase of resistance within *S. pyogenes* strains and its level varies worldwide, with an alarming upper rate of 25% in some European countries [8∼11].

It has been shown that the resistance to macrolide antibiotics in *S. pyogenes* can be attributed to two main mechanisms: target site modification and active efflux [12]. It is known that macrolides exert their activity by binding to the large 50S ribosomal subunit. They inhibit bacterial

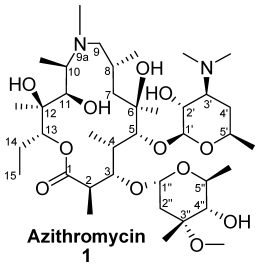

Fig. 1 The chemical structure of Azithromycin (**1**) and the atom numbering.

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protein synthesis at peptidyl transferase center by blocking the nascent peptide exit tunnel [13]. The modification of specific rRNA bases can prevent macrolides to bind. This may be due to the action of methylases encoded either by *erm*(B) or *erm*(A) genes [14]. The methylases are responsible for developing macrolide, lincosamide and streptogramin (MLS) resistance; inducible-(iMLS) or constitutive (cMLS). The active drug efflux is another common type of resistance developed by bacteria and is mediated by the membrane-associated pump encoded by the *mef*(A) gene [15]. In order to overcome the resistance problems, lots of efforts have been made to search for novel and more potent agents with all of the desirable features of the earlier generation of macrolides. So far, several attempts [16∼21] were aimed at the modification of the substituents at the azalide nitrogen atom and some of the synthesized compounds showed antibacterial activity. In that respect, the observed activity [20] of initially prepared 9a-*N*-carbamoyl and *N*-thiocarbamoyl derivatives of **2** encouraged us to extend our study in this direction.

This paper deals with the synthesis, structure elucidation and antibacterial *in vitro* evaluation of a series of new sulfonylurea derivatives of 15-membered azalides, *i.e.* 9a-*N*-[*N′*-(aryl)sulfonylcarbamoyl], 9a-*N*-{*N′*-[(aryl)sulfonylcarbamoyl-γ-aminopropyl]} and 9a-*N*-{*N′*-(β-cyanoethyl)-*N′*-[(aryl)sulfonyl-carabamoyl-γ-aminopropyl]} derivatives of 9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A (**2**) and 5-*O*-desosaminyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythronolide A (**3**) shown in Scheme 1. New derivatives of **2** were prepared in order to study whether antibacterial activity toward resistant strains would be achieved by replacing the carbamoyl moiety with an arylsulfonylcarbamoyl group into the azalide molecule and how the activity would be affected by nature and position of the substituents in the phenyl ring. Of particular interest was to study the influence of the linker between sulfonylcarbamoyl-group and aglycon moiety on the antibacterial activity. A special attention was paid to achieving the activity against *S. pyogenes* resistant strains.

Results and Discussion

Synthesis

The starting 9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A (**2**) is the last intermediate in the azithromycin (**1**) synthesis [16, 17]. Its hydrolysis in acidic media led to the formation of decladinosyl derivative **3** [22]. Both **2** and **3** smoothly reacted with substituted benzensulfonyl isocyanate in aprotic solvent at 0∼5°C to form a less polar 9a-*N*-[*N′*-(aryl)sulfonylcarbamoyl] derivatives, **4a**∼**4f** or **5a**∼**5f** in high yields, respectively (Scheme 1). Alternatively, compound **5a** was prepared by acidic hydrolysis of **4a**. All structural data were identical to previously synthesized **5a**.

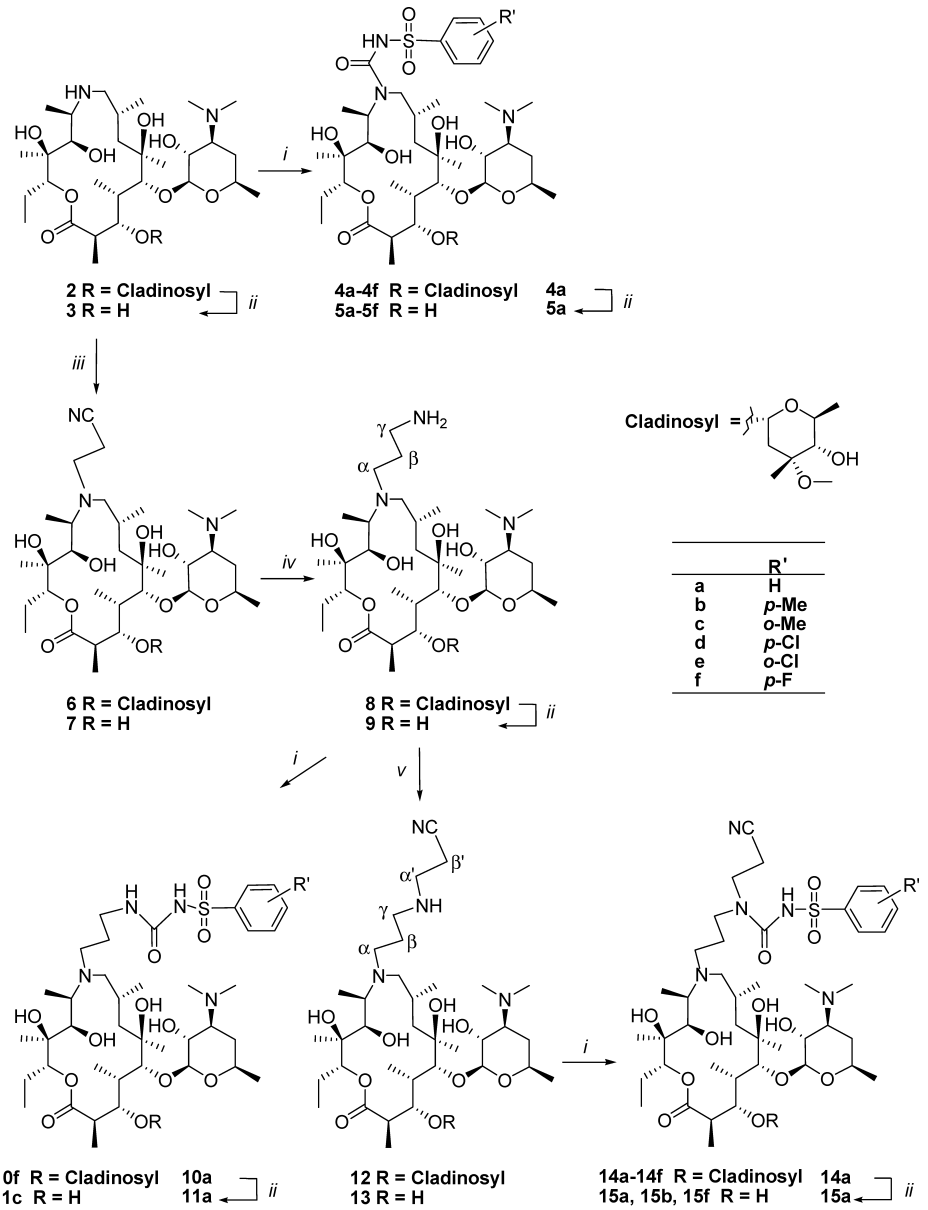
The key intermediates, 9a-*N*-(γ-aminopropyl) derivatives **8** and **9** were prepared in moderate yields by standard Michael addition of acrylonitrile to the starting amine **2**, followed by catalytic hydrogenation with PtO_2 as a catalyst of so obtained 9a-*N*-(β-cyanoethyl) derivatives **6** [19] and **7**. The preparation of **8** as described here differs from the procedure already reported in the literature [19]. Alternative synthetic route for the preparation of compound **9** was carried out by hydrolysis of **8** in acidic conditions. Structural data of so prepared **9** were the same as those of **9** prepared by catalytic hydrogenation. 9a-*N*-{*N′*-[(Aryl)sulfonylcarbamoyl-γ-aminopropyl]}-derivatives **10a**∼**10f**, **11a** and **11c** were obtained by addition of substituted benzensulfonyl isocyanate to the amines **8** and **9** under the same reaction conditions as used in preparation of **4a**∼**4f** and **5a**∼**5f** (Scheme 1). 9a-*N*-{*N′*-(β-Cyanoethyl)-*N′*-[(aryl)sulfonylcarabamoyl-γ-aminopropyl]}derivatives **14a**∼**14f**, **15a**, **15b** and **15f** were prepared by the selective cyanoethylation of amines **8** or **9** with equivalent amounts of acrylonitrile, followed by the addition of the substituted benzensulfonyl isocyanate to the intermediate 9a-*N*-[*N′*-(β-cyanoethyl)-γ-aminopropyl)]-derivatives **12** and **13** (Scheme 1). The parallel synthetic pathway for the preparation of compounds **11a** and **15a** was also carried out by hydrolysis of **10a** and **14a** in acidic conditions.

The general synthetic procedures for the selected representatives of each series are given in the experimental, while all the others are given as supplementary material.

Structural Characterization

The structures of all the synthesized compounds were determined by IR and NMR spectroscopies and mass spectrometry. In the case of compounds **5a**, **9**, **11a** and **15a**, chemical structures were also confirmed by the parallel synthetic pathways. Carbon chemical shifts of the starting compound **2** [20], and the selected representatives of each series, *e.g.* **4a**, **10a** and **14a** are displayed in Table 1. Carbon and proton chemical shifts of all the other compounds are given in experimental or in supplementary material.

The 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. Some resonances could not be unambiguously assigned due to a severe peak overlap. In order to overcome the problems connected with low solubility of some



Scheme 1 Synthesis of novel sulfonylureas of 15-membered azalides.

Reagents and conditions: *i*, substituted benzensulfonyl isocyanate, toluene, 0~5°C, 1 hour; *ii*, 6 M hydrochloric acid, r.t., 24 hours; *iii*, acrylonitrile, 60°C, 12 hours; *iv*, H_2/PtO_2, ethanol, 20 bar, r.t., 72 hours; *v*, 1 eq acrylonitrile, methanol, reflux, 7 hours.

compounds, different solvents were used.

As seen in the Table 1, chemical shifts of the carbon atoms in the lower part of the lactone ring and sugar moieties of the synthesized molecules resemble to those of **2**. However, changes in chemical shifts of H-9ab, H-8 and H-10 protons (see experimental) indicated structural modifications at 9a position. Furthermore, new resonances were observed in NMR spectra of **4a**~**4f**, **5a**~**5f**, **10a**~**10f**, **11a**, **11c**, **14a**~**14f**, **15a**, **15b** and **15f**. With respect to **2**, an additional signal at approximately 160 ppm in the ^{13}C-NMR spectra of arylsulfonylcarbamoyl derivatives was observed which confirmed the presence of the carbamoyl group. The proton and carbon chemical shifts in the regions 7.1~7.7 ppm (DMSO-d_6), 7.1~8.4 ppm (pyridine-d_5) and 7.36~8.16 ($CDCl_3$) for protons and 125~135 ppm, for carbons, respectively, were diagnostic for substituted benzene rings.

Two-dimensional COSY, HSQC and HMBC spectra showed correlation peaks characteristic for structures depicted in the Scheme 1. The HMBC correlation peaks

Table 1 ^{13}C-NMR chemical shifts (δ/ppm) of compounds **2**, **4a**, **10a** and **14a**

Position	^{13}C NMR			
	2[a]	**4a**[a]	**10a**[a]	**14a**[b]
1	178.5	178.6	177.7	177.5
2	46.0	46.1	45.5	45.0
3	78.5	79.0	79.5	79
4	43.5	43.4	41.8	42.4
5	83.6	83.8	84.2	84
6	74.5	73.8	74.7	75.0
7	43.4	43.4	41.8	na
8	30.4	28.0	28.7	29
9	57.5	57.7	64	na
10	57.0	57	61	na
11	74.2	71.7	75.9	71
12	74.1	73.6	75.4	75.2
13	78.2	78.0	77.9	77
14	22.1	22.6	22.4	21.2
15	11.6	11.5	11.5	11.0
1′	103.6	103.6	103.5	103
2′	71.7	71.9	71.6	70.4
3′	66.0	65.9	66.0	65.6
3′NMe_2	40.6	40.6	40.5	40.6
4′	30.5	29.6	30.4	29.7
5′	68.2	68.2	68.2	68.5
1″	95.4	95.6	96.2	95
2″	35.5	35.5	35.6	34.9
3″	73.8	74.5	73.7	72.8
3″OMe	49.7	49.7	49.7	49.5
4″	79.0	78.7	78.8	77.8
5″	65.9	66.1	66.2	66.4
2Me	15.3	15.4	15.7	15.6
4Me	10.1	10.1	10.4	8
6Me	28.4	28.0	29.2	28
8Me	22.6	21.6	22.7	22.4
10Me	14.9	14	9.5	na
12Me	17.9	18.0	18.4	18.2
5′Me	22.0	22.0	22.0	21.4
3″Me	19.4	19.3	21.6	21
5″Me	21.7	21.6	19.5	21.2
NHCONH		160	na	na
Phenyl		132.0	132.7	130.4
Phenyl		128.6	129.3	128.1
Phenyl		127.0	127.6	126.5
Phenyl		na	134.9	128.2
α-CH_2			49.7	49
β-CH_2			30.7	29.4
γ-CH_2			39.1	51.9
α'-CH_2				45
β'-CH_2				18.2
–CN				119.7

na=not assigned due to peak overlap. [a] Recorded in pyridine-d_5. [b] Recorded in $CDCl_3$.

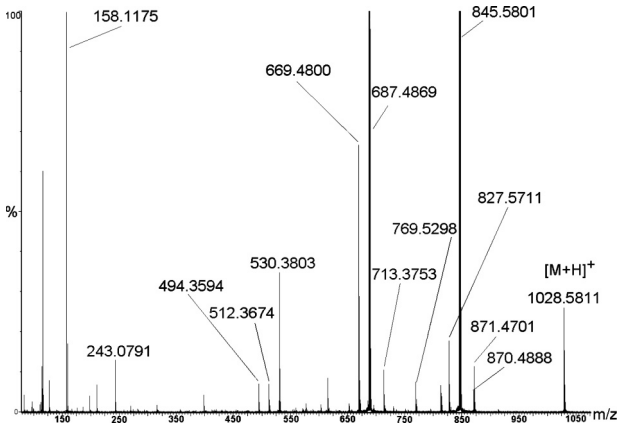


Fig. 2 The ESI-MS/MS spectrum of compound **14a**. Precursor ion $[M+H]^+$ at *m/z* 1028.5811 and all fragment ions were accurately measured with mass accuracy better than 5 ppm.

between atoms H-9 and C-α corroborated the position of the propyl chain on the N-9a atom of the aglycon ring. In the ^{13}C NMR spectra of the compounds **12**, **13**, **14a**∼**14f**, **15a**, **15b** and **15f** a cyano group was observed at approximately 120 ppm.

The IR spectra of the novel sulfonylcarbamoyl derivatives **4a**∼**4f**, **5a**∼**5f**, **10a**∼**10f**, **11a**, **11c**, **14a**∼**14f**, **15a**, **15b** and **15f** differ from the spectra of their parent compounds in the new bands observed at approximately 1580 cm^{-1} which correspond to a carbamoyl group C=O stretching vibrations. Bands observed at 600∼800 cm^{-1}, reflect mono and di-substituted benzene rings. IR bands in the region 1644∼1572 cm^{-1} which are common to carbamoyl group vibrations confirmed that the substitutions occurred at NH- amino group of the parent cyclic amine **2** and not at some of hydroxyl groups present in the molecule. This is also in agreement with NMR data. IR absorption bands observed at approximately 2249 cm^{-1} were assigned to C≡N stretching vibrations.

The mass spectra exhibited precursor ions that are consistent with masses of the respective compounds as shown in Scheme 1. Accurately measured mass spectra of compounds **4a**, **10a** and **14a** (Fig. 2) were recorded with internal calibrant (leucine-enkephaline) and they revealed masses of positive charged ions at *m/z* 918.4955, 975.5531 and 1028.5811 with mass accuracy better than 5 ppm. MS/MS spectra of the above compounds were also recorded with an internal calibrant, and thus obtained accurately measured masses together with calculated molecular formulas of the fragments obtained in spectra, led to the proposed fragmentation pattern in Fig. 3. A leaving of the cladinose sugar was found to be the first

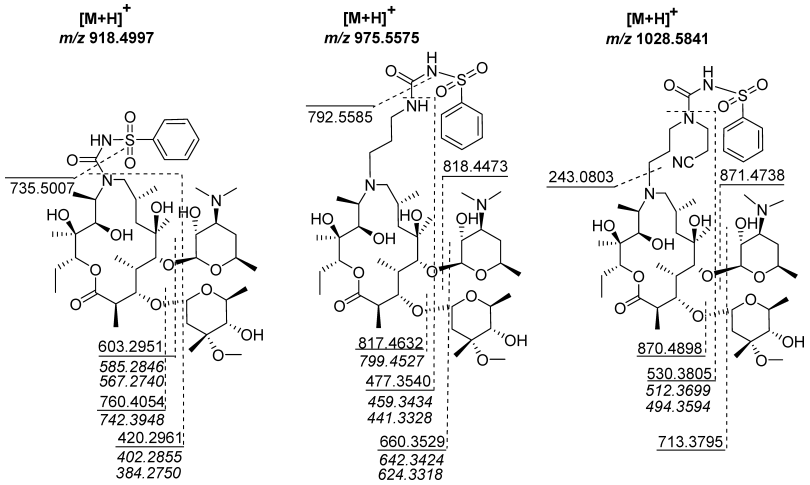


Fig. 3 Proposed fragmentation pattern based on accurate mass MS/MS experiments of compounds **4a**, **10a** and **14a** with theoretically calculated masses.

common precursor ion for all the three classes of compounds. Next, the fragmentation pathway included precursor ions at *m*/*z* 157.1110 which corresponded to a loss of the desosamine sugar, subsequently followed by the loss of one or two water molecules. Fragments produced by loss of the arylsulfonylcarbamoyl group (Fig. 3) were also found to be common for these classes of compounds.

In Vitro Activity

The preliminary antibacterial screening of the novel sulfonylcarbamoyl derivatives **4a**∼**4f**, **5a**∼**5f**, **10a**∼**10f**, **11a**, **11c**, **14a**∼**14f**, **15a**, **15b** and **15f** was performed by a standard dilution assay for the determination of minimal inhibitory concentrations (MICs).

The MICs against a panel of Gram-positive and Gram-negative bacteria determined in comparison with azithromycin (**1**) and 9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A (**2**) are presented in Table 2. MIC levels for all compounds were determined on a panel of sensitive and resistant Gram-positive bacterial strains (*Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*) and on Gram-negative strains (*Escherichia coli*, *Haemophilus influenzae* and *Moraxella catarrhalis*).

For the sulfonylureas directly linked to macrocyclic ring **4a**∼**4f** it was observed that compounds with methyl group and chlorine in *p*-**4b**, **4d** and *o*-**4c**, **4e** positions and fluorine in *p*-position **4f** showed significantly improved activity against iMLS resistant *S. pyogenes* strain when compared with both azithromycin (**1**) and starting cyclic amine **2**. Also, these compounds exhibited two times better activity than **2** and similar activity to **1** against sensitive *S. pneumoniae*. However, the activities against Gram-negative bacteria were all lower than those for **1** and **2**.

The potency of the majority of compounds **10a**∼**10f** decreased in comparison with compounds **4a**∼**4f** having sulfonylurea group directly linked to the macrocyclic ring. Among the tested compounds, only **10e** with chlorine in *o*-position showed comparable antibacterial activity to **1** against sensitive *S. pneumoniae* and *S. pyogenes* (Table 2). For the class of sulfonylureas **14a**∼**14f** the results showed lower activity against *S. pneumoniae* and *S. pyogenes*, when compared to **1** and **2**. In general, it was observed that antibacterial activity decreased with the introduction of a propyl side chain and an additional cyanoethyl chain on the nitrogen atom.

Compounds without the cladinose sugar at position 3 of macrocyclic ring, **5a**∼**5f**, **11a**, **11c**, **15a**, **15b** and **15f** did not show any antibacterial activity against tested bacterial strains as already observed for the related macrolides.

Generally, it was observed that antibacterial activity of the novel arylsulfonylcarbamoyl derivatives **4a**∼**4f**, **10a**∼**10f** and **14a**∼**14f** against all the tested erythromycin sensitive (Ery-S) Gram-positive strains decreased in the series **4a**∼**4f**>**10a**∼**10f**>**14a**∼**14f** with the introduction of a propyl side chain and additional cyanoethyl chain on the nitrogen atom.

Based on the results presented in this paper, in order to improve the acivity against cMLS resistant and efflux mediated resistant *S. pyogenes* strains, we are considering further derivatisation of arylsulfonylcarbamoyl derivatives of 15-membered azalides, with sulfonylcarbamoyl group directly linked at the N-9a position of the macrocyclic ring.

Table 2 *In vitro* antibacterial activity of novel sulfonylureas **4a**~**4f**, **10a**~**10f** and **14a**~**14f** against selected pathogenic bacteria

Compd.	Strain/MIC (μg/ml)								
	Staphylococcus aureus Ery-S	*Streptococcus pneumoniae* Ery-S	*Streptococcus pyogenes* Ery-S	*S. pyogenes* iMLS *erm*TR	*S. pyogenes* cMLS	*S. pyogenes* M *mef*A	*Moraxella catarrhalis* ATCC-0324	*Haemophilus influenzae* ATCC-0529	*Escherichia coli* ATCC-0001
2	2	0.25	0.25	16	>64	16	NT	1	4
1	0.5	≤0.125	≤0.125	8	64	2	>0.125	0.25	1
4a	64	1	4	8	>64	>64	>64	64	>64
4b	8	≤0.125	0.5	1	>64	64	16	16	32
4c	8	≤0.125	0.25	0.5	>64	32	8	8	16
4d	8	≤0.125	0.5	1	>64	32	16	16	32
4e	32	1	2	2	>64	>64	64	32	>64
4f	32	1	2	2	>64	>64	>64	>64	>64
10a	32	0.5	2	>64	>64	>64	8	64	>64
10b	16	0.25	1	64	>64	64	64	2	>64
10c	16	0.25	1	64	>64	>64	4	32	>64
10d	32	0.5	1	>64	>64	>64	>64	8	>64
10e	8	≤0.125	≤0.125	32	>64	32	2	32	>64
10f	>64	32	16	>64	>64	>64	>64	>64	>64
14a	64	1	2	>64	>64	>64	2	>64	>64
14b	>64	8	4	>64	>64	>64	32	>64	>64
14c	>64	4	4	>64	>64	>64	32	>64	>64
14d	>64	4	2	>64	>64	>64	16	>64	>64
14e	>64	4	4	>64	>64	>64	32	>64	>64
14f	>64	4	8	>64	>64	>64	32	>64	>64

iMLS: inducible resistance to macrolide, lincosamide and streptogramin (MLS) antibiotics, cMLS: constitutive MLS resistance, M: efflux mediated macrolide resistance, NT: not tested

Experimental

General Methods

Melting points were measured with a Büchi B-540 melting point apparatus and are uncorrected. TLC were performed on Merck 60 F254 plates using $CH_2Cl_2 : CH_3OH : NH_3(25\%) = 90 : 9 : 1.5$ as eluents. Column chromatography was performed on Merck Silica gel 60 (0.043~0.060 mm).

MS and MS/MS Measurements

MS/MS analyses were made using a Q-TOF Micromass mass spectrometer (Micromass, UK) with an electrospray source operating in the positive-ion mode with an additional Harvard Pump 11, syringe pump used for direct infusion with a flow of 5 μl/minute (for measuring accurate mass with Lock spray option). Samples were dissolved in solution of acetonitrile and water (1 : 1, v : v) at concentration of 0.1 μg/ml. The flow rate of the mass spectrometer and additional syringe pump was the same. Capillary voltage was 3000 V and sample cone voltage was 40 V. Source and desolvation temperatures were 80 and 150°C, respectively. Collision energy was 10 V for MS measurement and 30~40 V for MS/MS measurement. The lock-mass reagent was leucine-enkephaline with an exact mass of 556.2771 u. For accurate mass measurements the direct infusion method was used. Half of the scans were generated by the lock-mass reagent. The flow remained unchanged for both lock-mass reagent and analyte at all times. One thousand scans and resolution better than 5000 were minimally required. MassLynx 4 (Micromass, UK) software was used to calculate molecular formulas of product and precursor ions according to the accurate mass data.

Spectroscopic Measurements

NMR experiments were carried out on a Bruker Avance DRX500 spectrometer operating at 500.13 MHz for ^{1}H, and

125.77 MHz, for ^{13}C, respectively, with a 5 mm diameter inverse detection probe and a z-axis gradient coil. Standard spectral conditions were used. The solvents were pyridine-d_5, DMSO-d_6 and $CDCl_3$ and all experiments were performed at ambient temperature. TMS was used as the internal standard and the sample concentration was 30 mg/ml.

IR spectra were recorded in KBr pellets on a Nicolet Magna 760 FT-IR spectrometer. The number of scans was 16 and the resolution was 4 cm^{-1}.

***In Vitro* Evaluation**

Antibacterial activity data given in Table 2 were obtained by microdilution test in Mueller-Hinton media. Test substances and standards are dissolved in DMF (Merck) at concentration 5 mg/ml. Solutions of the substances were prepared in Mueller-Hinton broth media, final concentration from 64 to 0.125 μg/ml. After 24 hours incubation, optical density was detected by measuring absorbance at 600 nm. MIC is defined as the concentration that shows 90% growth inhibition, and was determined by broth dilution methods, National Committee for Clinical Laboratory Standards (NCCLS, M7-A2 protocols). All screening procedures were done on Tecan Genesis 150 robot unit.

General procedure for the preparation of compounds **4a**∼**4f**: To a solution of **2** [17] (5.5 mmol) in toluene (40 ml), substituted benzensulfonyl isocyanate (5.5 mmol) was added dropwise at 0∼5°C temperature. Reaction mixture was stirred for 1 hour and white crystalline solid was filtered off to give a crude compound **4a**∼**4f**. Column chromatography of crystalline solid on silica gel with $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 90 : 9 : 1.5 as the mobile phase, afforded amorphous compounds **4a**∼**4f**.

9-Deoxo-9-dihydro-9a-*N*-[*N′*-(phenylsulfonyl)carbamoyl]-9a-aza-9a-homoerythromycin A (4a)

mp 148∼149°C. ^1H NMR (500 MHz, pyridine-d_5) δ 3.18 (1H, H-2), 4.80 (1H, H-3), 1.87 (1H, H-4), 4.12 (1H, H-5), 2.06, 1.86 (2H, H-7), 1.96 (1H, H-8), 3.31, 2.54 (2H, H-9), 3.20 (1H, H-10), 3.65 (1H, H-11), 5.78 (1H, H-13), 2.15; 1.72 (2H, H-14), 0.93 (3H, H-15), 5.01 (1H, H-1′), 3.67 (1H, H-2′), 2.77 (1H, H-3′), 2.41 (6H, 3′NMe_2), 1.45; 1.20 (2H, H-4′), 4.08 (1H, H-5′), 5.15 (1H, H-1″), 2.38; 1.47 (2H, H-2″), 3.46 (3H, 3″OMe), 3.25 (1H, H-4″), 4.55 (1H, H-5″), 1.39 (3H, 2Me), 1.63 (3H, 4Me), 1.87 (3H, 6Me), 1.02 (3H, H-8Me), 1.59 (3H, 10Me), 1.53 (3H, 12Me), 1.35 (3H, 5′Me), 1.93 (3H, 3″Me) 1.30 (3H, 5″Me), 7.40 (1H, phenyl), 8.34 (1H, phenyl), 7.38 (1H, phenyl). ^{13}C NMR (500 MHz, pyridine-d_5) δ see Table 1. MS *m*/*z* 931 (M, $C_{45}H_{77}N_3O_{15}S$).

Procedure A for the preparation of compounds **5a**∼**5f**: Compound **3** (1.73 mmol) was dissolved in toluene (25 ml) and substituted benzensulfonyl isocyanate (1.73 mmol) was added dropwise at a temperature from 0°C to 5°C. After stirring the reaction mixture for one hour at the same temperature, the formed crystals of the crude product were sucked off. The isolation of the pure **5a**∼**5f** was performed by chromatography on a silica gel column in a solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 90 : 9 : 1.5.

5-*O*-Desosaminyl-9-deoxo-9-dihydro-9a-*N*-[*N′*-(phenylsulfonyl)carbamoyl]-9a-aza-9a-homoerythronolide A (5a)

mp 172°C. ^1H NMR (500 MHz, pyridine-d_5) δ 8.3 (1H, phenyl), 7.34 (1H, phenyl), 7.38 (1H, phenyl). ^{13}C NMR (500 MHz, pyridine-d_5) δ 163.4 (NCONH), 131.3 (phenyl), 128.6 (phenyl), 127.5 (phenyl). MS *m*/*z* 759 (M, $C_{36}H_{62}N_3O_{12}S$).

Procedure B: The suspension of **4a** (0.5 g, 0.55 mmol) in 6 M hydrochloric acid (5 ml) was stirred for 24 hours at room temperature, the pH was adjusted to 9.5∼10 by adding 5 M NaOH and was extracted with CH_2Cl_2 (3×4 ml). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, evaporated to dryness under reduced pressure to give crude product wherefrom by chromatography on silica gel column pure **5a** was obtained. The spectroscopic data of so obtained sample was identical to the spectroscopic data of the **5a**, obtained by the Procedure A.

5-*O*-Desosaminyl-9-deoxo-9-dihydro-9a-*N*-(β-cyanoethyl)-9a-aza-9a-homoerythronolide A (7)

The solution of **3** (20.0 g, 0.0 mol) in acrylonitrile (100 ml) was heated under reflux for 10 hours. The residue obtained after evaporation *in vacuo* was chromatographed on silica gel in a solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 80 : 20 : 2 to give **7**. ^1H NMR (500 MHz, $CDCl_3$) δ 2.56; 2.44 (β-CH_2). ^{13}C NMR (500 MHz, $CDCl_3$) δ 119.1 (CN), 16.3 (β-CH_2). MS *m*/*z* 629 (M, $C_{32}H_{59}N_3O_9$).

9-Deoxo-9-dihydro-9a-*N*-(γ-aminopropyl)-9a-aza-9a-homoerythromycin A (8)

The solution of **6** (10.0 g, 12.6 mmol) was hydrogenated for 72 hours under pressure of 20 bar in ethanol (100 ml) with PtO_2 (3.0 g). The catalyst was filtered and solvent was removed *in vacuo* which afforded colorless foam. Pure **8** was obtained by column chromatography using solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 90 : 9 : 1.5. ^1H NMR (500 MHz, pyridine-d_5) δ 1.83, 1.34 (2H, α-CH_2), 3.10; 2.76 (2H, γ-CH_2). ^{13}C NMR (500 MHz, pyridine-d_5) δ 38.5

(α-CH_2), 29.8 (β-CH_2). MS *m/z* 792 (M, $C_{40}H_{77}N_3O_{12}$).

5-*O*-Desosaminyl-9-deoxo-9-dihydro-9a-*N*-(γ-aminopropyl)-9a-aza-9a-homoerythronolide A (9)

Procedure A: The solution of **7** (20.0 g, 0.031 mol) was hydrogenated for 40 hours under pressure of 4 bar in 5% hydrochloric acid (80 ml) with 5% Pt/C (4.0 g). The catalyst was filtered and diluted sodium hydroxide was added to filtrate until pH 11. The formed precipitate was collected by filtration, washed by water (100 ml) and dried to give **9** (12.5 g, 84%). Pure **9** was obtained by column chromatography using solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 80 : 20 : 2. ^{1}H NMR (500 MHz, pyridine-d_5) δ 1.83, 1.34 (α-CH_2), 3.10; 2.76 (γ-CH_2). ^{13}C NMR (500 MHz, pyridine-d_5) δ 38.5 (α-CH_2), 29.8 (β-CH_2). MS *m/z* 633 (M, $C_{32}H_{63}N_3O_9$).

Procedure B: procedure was the same as described for the preparation of compound **5a**. Spectroscopic data of so obtained sample were identical to the spectroscopic data of the **9**, obtained by the Procedure A.

General procedure for the preparation compounds **10a**～**10f**: Compound **8** (1.26 mmol) was dissolved in CH_2Cl_2 (25 ml) and substituted benzensulfonyl isocyanate (1.26 mol) was added dropwise at a temperature from 0°C to 5°C. After stirring for one hour at the same temperature the solvent was removed under reduced pressure. The isolation of the pure **10a**～**10f** were performed by chromatography on a silica gel column in a solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 90 : 9 : 1.5.

9-Deoxo-9-dihydro-9a-*N*-[*N*′-(phenylsulfonyl)carbamoyl-γ-aminopropyl]-9a-aza-9a-homoerythromycin A (10a)

mp 151～155°C. ^{1}H NMR (500 MHz, pyridine-d_5) δ 3.15 (1H, H-2), 4.75 (1H, H-3), 1.90 (1H, H-4), 4.17 (1H, H-5), 2.51, 1.93 (2H, H-7), 1.77 (1H, H-8), 2.66; 2.26 (2H, H-9), 3.08 (1H, H-10), 4.19 (1H, H-11), 5.45 (1H, H-13), 2.19; 1.74 (2H, H-14), 0.95 (3H, H-15), 5.00 (1H, H-1′), 3.67 (1H, H-2′), 2.74 (1H, H-3′), 2.22 (6H, 3′NMe$_2$), 1.53; 1.16 (2H, H-4′), 4.02 (1H, H-5′), 5.28 (1H, H-1″), 2.45; 1.52 (2H, H-2″), 3.52 (3H, 3″OMe), 3.32 (1H, H-4″), 4.66 (1H, H-5″), 1.36 (3H, 2Me), 1.61 (3H, 4Me), 1.33 (3H, 6Me), 0.97 (1H, H-8Me), 1.35 (3H, 10Me), 1.41 (3H, 12Me), 1.37 (3H, 5′Me), 1.37 (3H, 3″Me), 1.70 (3H, 5″Me), 7.46 (1H, phenyl), 7.45 (1H, phenyl), 8.35 (1H, phenyl), 2.70 (2H, α-CH_2), 2.22 (2H, β-CH_2), 3.37 (2H, γ-CH_2). ^{13}C NMR (500 MHz, pyridine-d_5) δ See Table 1. MS *m/z* 974 (M, $C_{47}H_{82}N_4O_{15}S$).

General procedure A for the preparation of compounds **11a** and **11c**: Compound **9** (1.58 mmol)) was dissolved in CH_2Cl_2 (25 ml) and substituted benzensulfonyl isocyanate (1.58 mmol) was added dropwise at a temperature from 0°C to 5°C. After stirring for one hour at the same temperature the solvent was removed under reduced pressure. The isolation of the pure **11a** and **11c** were performed by chromatography on a silica gel column in a solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 75 : 25 : 1.5.

5-*O*-Desosaminyl-9-deoxo-9-dihydro-9a-*N*-[*N*′-(phenylsulfonyl)carbamoyl-γ-aminopropyl]-9a-aza-9a-homoerythronolide A (11a)

mp 151～153°C. ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.40 (1H, phenyl), 7.51 (1H, phenyl), 7.48 (1H, phenyl), 3.38 (2H, γ-CH_2), 2.04; 1.99 (2H, α-CH_2). ^{13}C NMR (500 MHz, pyridine-d_5) δ 158.8 (NHCONH), 131.5 (phenyl), 127.6 (phenyl), 126.1 (phenyl), 37.5 (α-CH_2), 28.2 (β-CH_2). MS *m/z* 816 (M, $C_{39}H_{68}N_4O_{12}S$).

Procedure B: procedure was the same as described for the preparation of compound **5a**. Spectroscopic data of so obtained sample were identical to the spectroscopic data of the **11a**, obtained by the Procedure A.

9-Deoxo-9-dihydro-9a-*N*-[*N*′-(β-cyanoethyl)-γ-aminopropyl]-9a-aza-9a-homoerythromycin A (12)

The solution of **8** (10.0 g, 15.7 mmol) and acrylonitrile (1.0 ml, 18.0 mmol) in methanol (200 ml) was heated at the boiling temperature for 10 hours and evaporated to dryness and the crude product was obtained. Pure **12** was afforded by chromatography on silica gel column using the solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 90 : 9 : 1.5 as white powder mp 145～147°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.91, 2.56 (2H, γ-CH_2), 3.22, 2.33 (2H, α-CH_2), 2.97; 2.91 (2H, α'-CH_2), 2.69 (2H, β'-CH_2). ^{13}C NMR (500 MHz, $CDCl_3$) δ 48.3 (γ-CH_2), 50.0 (α-CH_2), 29.2 (β-CH_2), 119.2 (CN), 17.4 (β'-CH_2), 45.3 (α'-CH_2). MS *m/z* 844 (M, $C_{43}H_{80}N_4O_{12}$).

5-*O*-Desosaminyl-9-deoxo-9-dihydro-9a-*N*-[*N*′-(β-cyanoethyl)-γ-aminopropyl]-9a-aza-9a-homoerythronolide A (13)

The solution of **9** (10.0 g, 0.0158 mol) and acrylonitrile (0.8 ml, 0.0158 mol) in methanol (200 ml) was heated at the boiling temperature for 10 hours and evaporated to dryness. Crude product was obtained wherefrom by chromatography on silica gel column using the solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 90 : 9 : 1.5 pure **13** was obtained as white powder mp 145～147°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.5 (2H, γ-CH_2); 1.6 (2H, α-CH_2), 3.5 (2H, α'-CH_2), 2.6 (2H, β'-CH_2). ^{13}C NMR (500 MHz, $CDCl_3$) δ 50.9 (γ-CH_2), 38.7 (α-CH_2), 119.7 (CN), 17.5 (β'-CH_2), 44.7 (α'-CH_2). MS *m/z* 686 (M, $C_{35}H_{66}N_4O_9$).

General procedure for the preparation of compounds

14a～**14f**: To a solution of **12** (1.18 mmol) in toluene (5 ml), substituted benzensulfonyl isocyanate (1.18 mmol) was added dropwise at 0～5°C temperature. Reaction mixture was stirred for 1 hour and white crystalline solid was filtered off to give crude compounds **14a**～**14f**. Column chromatography of crystalline solid on silica gel with $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 90 : 9 : 1.5 as the mobile phase afforded amorphous compounds **14a**～**14f**.

9-Deoxo-9-dihydro-9a-*N*-[*N′*-(*β*-cyanoethyl)-*N′*-(phenylsulfonyl)carbamoyl-*γ*-aminopropyl]-9a-aza-9a-homoerythromycin A (14a)

mp 148°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.70 (1H, H-2), 4.11 (1H, H-3), 1.92 (1H, H-4), 3.54 (1H, H-5), 1.9 (1H, H-8), 3.80 (1H, H-11), 5.01 (1H, H-13), 1.91; 1.48 (2H, H-14), 0.89 (3H, H-15), 4.49 (1H, H-1′), 3.38 (1H, H-2′), 2.56 (1H, H-3′), 2.55 (6H, 3′NMe$_2$), 1.75; 1.24 (2H, H-4′), 3.67 (1H, H-5′), 4.90 (1H, H-1″), 2.27; 1.57 (2H, H-2″), 3.29 (3H, 3″OMe), 3.03 (1H, H-4″), 4.04 (1H, H-5″), 1.24 (3H, 2Me), 1.09 (3H, 4Me), 1.39 (3H, 6Me), 1.02 (1H, H-8Me), 1.30 (3H, 12Me), 1.24 (3H, 5′Me), 1.24 (3H, 3″Me), 1.30 (3H, 5″Me), 7.94 (1H, phenyl), 7.39 (1H, phenyl), 7.39 (1H, phenyl), 2,87, 2.4 (2H, γ-CH$_2$), 2.56 (2H, β-CH$_2$), 2,97 (α'-CH$_2$), 2.70 (β'-CH$_2$). ^{13}C NMR (500 MHz, $CDCl_3$) δ See Table 1. MS *m*/*z* 1028 (M, $C_{50}H_{85}N_5O_{15}S$).

General procedure A for the preparation compounds **15a**, **15b** and **15f**: Compound **13** (1.46 mmol) was dissolved in CH_2Cl_2 (25 ml) and substituted benzensulfonyl isocyanate (1.46 mmol) was added dropwise at a temperature from 0°C to 5°C. After stirring for one hour at the same temperature the solvent was removed under reduced pressure. The isolation of the pure **15a**, **15b** and **15f** were performed by chromatography on a silica gel column in a solvent system $CH_2Cl_2 : CH_3OH : NH_3$ (25%) = 75 : 25 : 1.5.

5-*O*-Desosaminyl-9-deoxo-9-dihydro-9a-*N*-[*N′*-(*β*-cyanoethyl)-*N′*-(phenylsulfonyl)carbamoyl-*γ*-aminopropyl]-9a-aza-9a-homoerythronolide A (15a)

mp 151～152°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (1H, phenyl), 7.31 (1H, phenyl), 7.35 (1H, phenyl), 3.47, 2.52 (2H, γ-CH$_2$); 1.63, 1.34 (2H, α-CH$_2$), 3.45 (2H, α'-CH$_2$), 2.62 (2H, β'-CH$_2$). ^{13}C NMR (500 MHz, $CDCl_3$) δ 167.8 (NCONH), 145.2 (phenyl), 130.4 (phenyl), 128.2 (phenyl), 126.5 (phenyl), 50.9 (γ-CH$_2$), 38.7 (α-CH$_2$), 119.7 (CN), 17.5 (β'-CH$_2$), 44.7 (α'-CH$_2$). MS *m*/*z* 869 (M, $C_{42}H_{71}N_5O_{12}S$).

Procedure B: procedure was the same as described for the preparation of compound **5a**. Spectroscopic data of so obtained sample were identical to the spectroscopic data of the **15a**, obtained by the Procedure A.

Acknowledgments We acknowledge to Prof. Vitomir Šunjić and Prof. Marija Šindler-Kulyk for helpful comments and suggestions.

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