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Synthesis and Antibacterial Activity of Isomeric 15-Membered Azalides

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Abstract A series of 3-keto and 3-*O*-acyl derivatives of both 6-*O*-alkyl-8a-aza-8a-homoerythromycin A and 6-*O*-alkyl-9a-aza-9a-homo-erythromycin A were synthesised and tested against Gram-positive and Gram-negative bacteria. Derivatives of 8a-aza-8a-homoerythromycin A have potent antibacterial activity against not only azithromycin-susceptible strains, but also efflux (M) and inducible macrolide-lincosamide-streptogramin (iMLS$_B$) resistant Gram-positive pathogens, while the corresponding 9a-isomers were less active. Introduction of an additional ring such as 11,12-cyclic carbonate reduced antibacterial activity of both series. 3-Keto and 3-*O*-(4-nitrophenyl)-acetyl derivatives of 6-*O*-methyl-8a-aza-8a-homo-erythromycin A show typical macrolide pharmacokinetics in preliminary *in vivo* studies in mice, and their *in vivo* efficacy is demonstrated.

Keywords azalide, ketolide, acylide, antibacterial activity, structure-activity relationship

Introduction

Second-generation macrolide antibiotics such as clarithromycin [1] (6-*O*-methylerythromycin A) and azithromycin [2, 3] (15-membered azalide) have been widely prescribed for upper and lower respiratory tract infections because of their superior antibacterial activity, pharmacokinetic properties and fewer gastrointestinal side effects compared to erythromycin A. However, the therapeutic utility of these macrolides has been severely compromised by the emergence of resistant pathogens [4].

The discovery that the cladinose moiety was not absolutely necessary for good antibacterial activity [5] has opened up new areas on the macrolactone ring for SAR exploration. The next generation of macrolide antibiotics, *e.g.* ketolides, anhydrolides and acylides, all derived from 3-*O*-decladinosylerythromycin A, were identified over the past decade. The ketolide telithromycin (HMR 3647) [6] has gained market approval in EU and USA and cethromycin (ABT-773) [7] is in clinical development.

In this report, we describe a novel series of 3-keto and 3-*O*-acyl derivatives of both 8a-aza-8a-homoerythromycin A (8a-lactam) and 9a-aza-9a-homoerythromycin A (9a-lactam), that showed potent antibacterial properties against susceptible pathogens and improved activity against several species of efflux (M) and inducible (iMLS$_B$) macrolide resistant Gram-positive pathogens.

Chemistry

The synthetic route to 3-keto derivatives of 9a- and 8a-lactams is presented in Fig. 1. Starting compounds for the synthesis were 6-*O*-alkylerythromycin A 9(*E*)- and 9(*Z*)-oximes [8, 9]. The Beckmann rearrangement of both oxime isomers was carried out with 4-toluenesulfonyl chloride in acetone-water solution at 0~5°C to give corresponding 9a- (**1a**~**c**) and 8a- (**2a**~**c**) lactams. Subsequent removal of L-cladinose by treatment with aqueous HCl provided desired decladinosyl compounds (**3a**~**c** and **4a**~**c**). A series of 3-ketolides were prepared by

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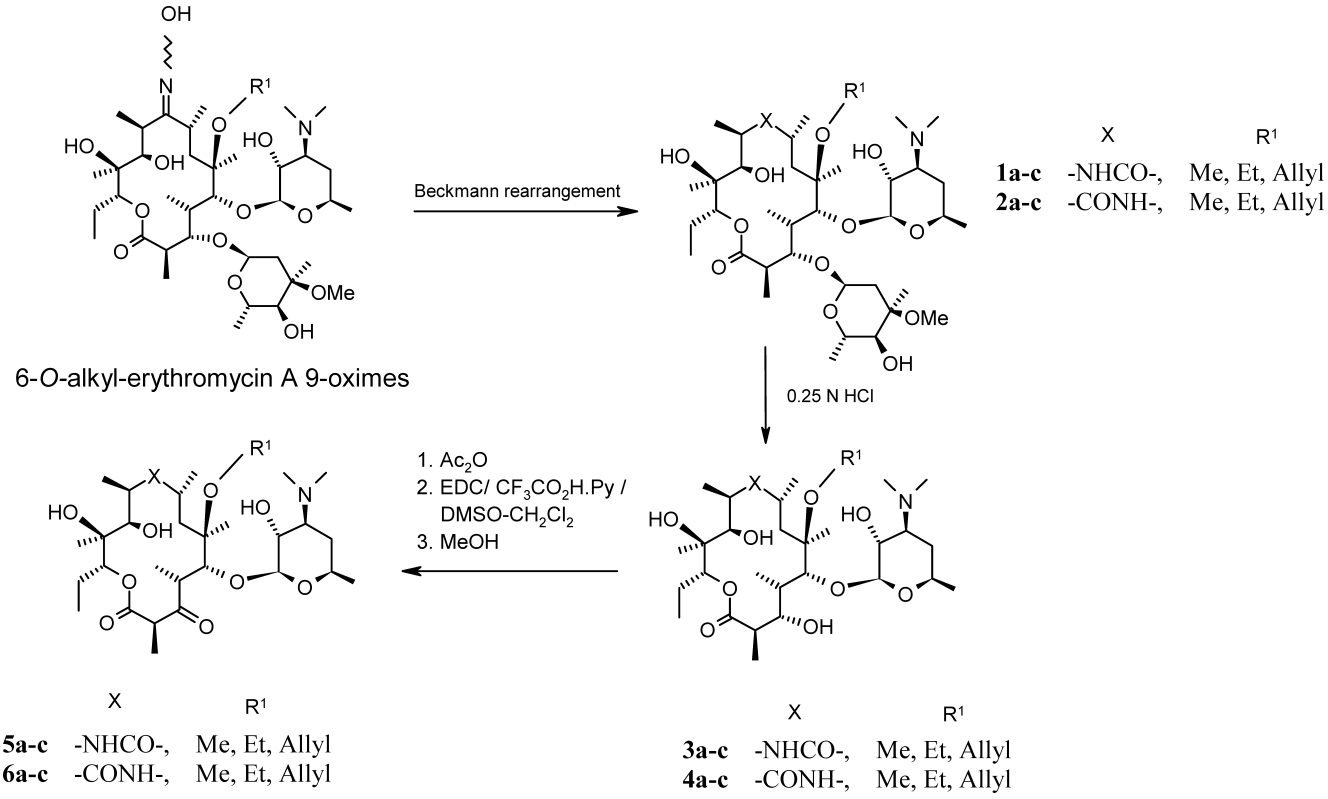


Fig. 1 Preparation of 3-keto derivatives of 8a-aza-8a- and 9a-aza-9a-homoerythromycin A.

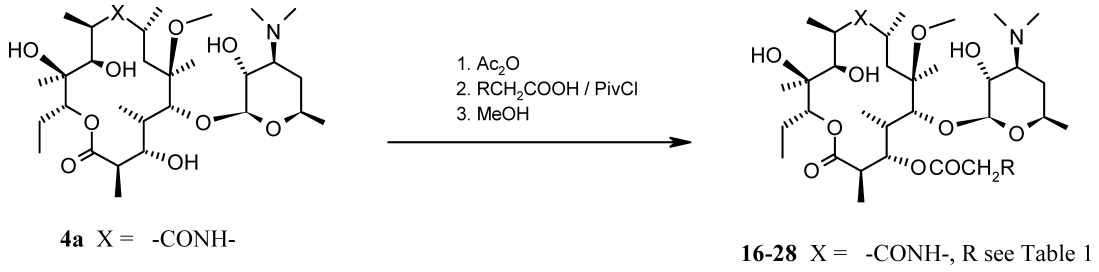


Fig. 2a Preparation of 3-*O*-acyl derivatives of 6-*O*-methyl-8a-aza-8a-homoerythromycin A.

Fig. 2b Preparation of 6-*O*-alkyl-3-*O*-(4-nitrophenyl)acetyl derivatives of 8a-aza-8a- and 9a-aza-9a-homoerythromycin A.

oxidation of 3-hydroxy group of **3a**∼**c** and **4a**∼**c**, using acetyl as 2′-hydroxy-protecting group. The oxidation was performed with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) in the presence of pyridine trifluoroacetate. Subsequent deprotection and purification by column chromatography gave desired 3-ketolides **5a**∼**c** and **6a**∼**c**.

Decladinosyl derivatives **3** and **4** having 2′-hydroxy-protecting group were also starting compounds for the preparation of 3-*O*-acyl derivatives. To prepare compounds **16** and **18**∼**32** mixed anhydrides, prepared from the corresponding carboxylic acid and pivaloyl chloride, were used followed by deprotection in methanol and purification by column chromatography, as shown in Fig. 2a for examples **16** and **18**∼**28** and Figure 2b for examples **29**∼**32**. The only exception was synthesis of carbamate compound **17** that was prepared by reaction of **4a** with 4-aminophenyl isocyanate.

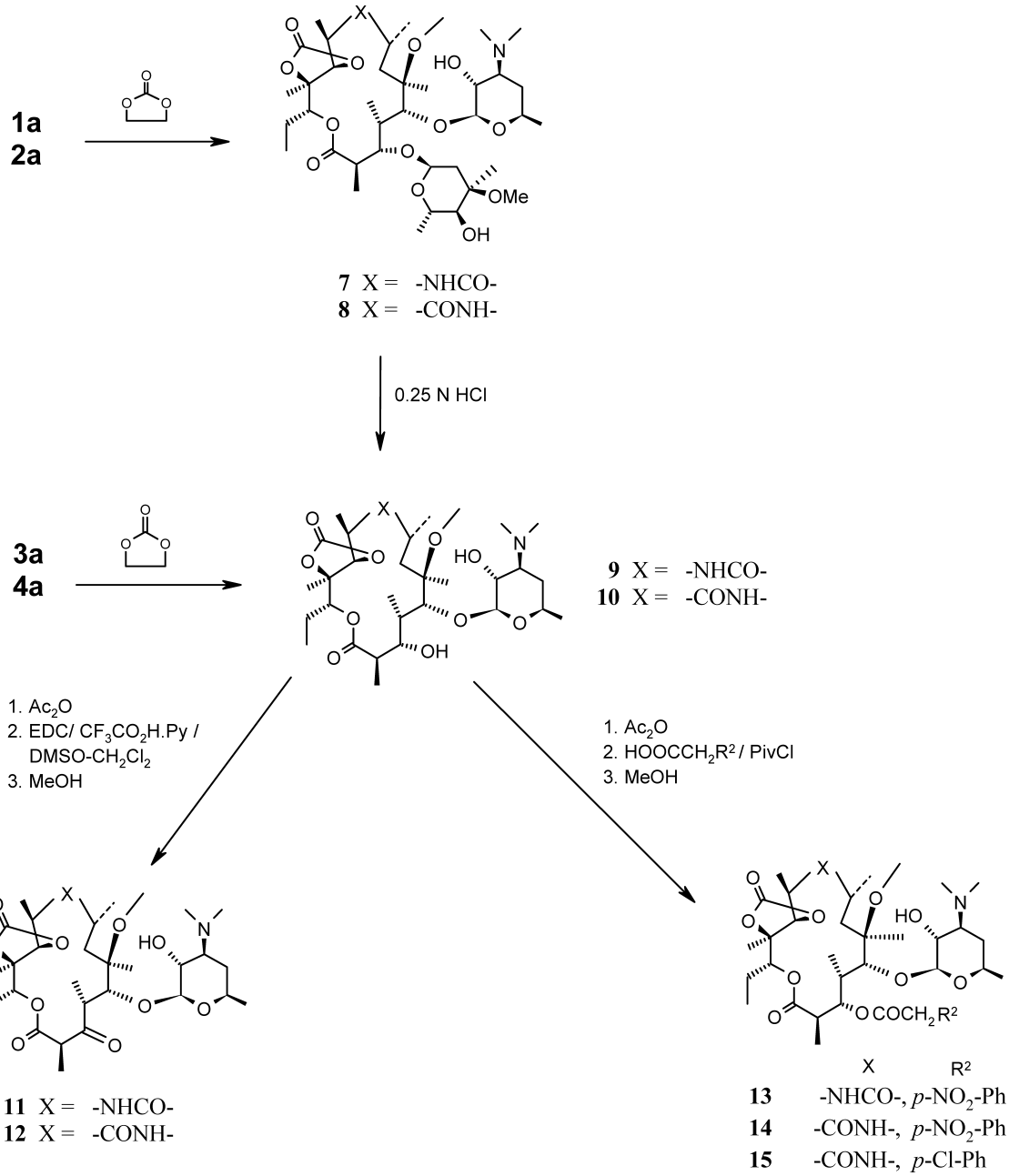
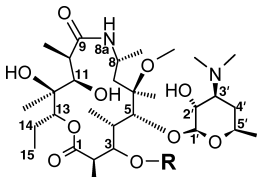


Fig. 3 Preparation of 3-keto and 3-*O*-acyl derivatives of 6-*O*-methyl-8a-aza-8a- and 9a-aza-9a-homoerythromycin A 11,12-cyclic carbonates.

Table 1 ^{13}C NMR data for 3-*O*-acyl-6-*O*-methyl-8a-lactams

Compound



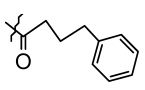
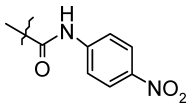
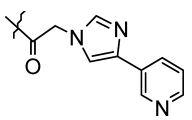
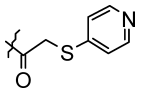
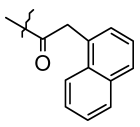
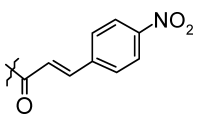
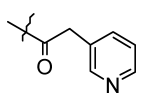
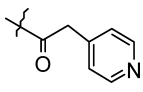
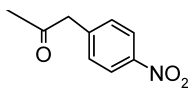
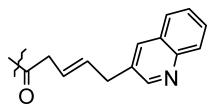
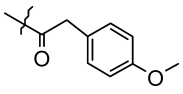
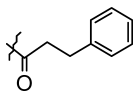
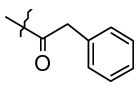
R	δ
 **16**	175.4 (C-1), 175.1 (C-9), 173.1 (3-OCO), 141.1, 128.5, 128.4, 126.1 (Ph), 103.3 (C-1′), 81.8 (C-5), 78.6 (C-6), 77.6 (C-13), 77.2 (C-3), 75.0 (C-12), 70.8 (C-11), 70.9 (C-9), 70.5 (C-2′), 69.5 (C-5′), 65.9 (C-3′), 51.2 (6-OCH_3), 43.1, 42.9 (C-2, C-8, C-10), 42.1 (C-7), 40.4 [3′$N(CH_3)_2$], 37.0 (C-4), 35.2, 33.9, 26.4 (4xCH_2), 28.8 (C-4′), 23.1 (8-CH_3), 21.4 (C-14), 21.1 (5′-CH_3), 20.8 (6-CH_3), 16.4 (12-CH_3), 15.9 (2-CH_3), 10.9 (14-CH_3), 10.3 (10-CH_3), 9.5 (4-CH_3).
 **17**	175.9 (C-1), 175.7 (C-9), 153.6 (3-OCONH), 144.9, 143.3, 125.6, 118.0 (Ph), 103.9 (C-1′), 84.0 (C-5), 80.2 (C-3), 78.9 (C-6), 77.9 (C-13), 75.7 (C-12), 71.3 (C-11), 71.0 (C-2′), 69.4 (C-5′), 66.4 (C-3′), 51.4 (6-OCH_3), 43.8, (C-8, C-10), 43.5 (C-2), 42.2 (C-7), 40.6 [3′$N(CH_3)_2$], 36.5 (C-4), 28.9 (C-4′), 23.3 (8-CH_3), 21.8 (C-14), 21.3 (5′-CH_3), 21.0 (6-CH_3), 16.9 (12-CH_3), 16.1 (2-CH_3), 11.3 (14-CH_3), 10.9 (10-CH_3), 9.8 (4-CH_3).
 **18**	175.2 (C-1), 174.9 (C-9), 167.7 (3-OCO), 148.5, 146.9, 132.5, 130.2, 124.0 (Py), 139.9, 139.1, 117.0 (Im), 105.1 (C-1′), 85.4 (C-5), 80.8 (C-3), 77.1 (C-6), 78.0 (C-13), 75.1 (C-12), 71.1 (C-11), 70.7 (C-2′), 69.8 (C-5′), 66.9 (C-3′), 51.4 (6-OCH_3), 49.9 (CH_2), 43.7 (C-2), 43.4 (C-10), 43.1 (C-7), 42.8 (C-8), 40.7 [3′$N(CH_3)_2$], 37.6 (C-4), 28.8 (C-4′), 23.6 (8-CH_3), 21.8 (C-14), 21.4 (5′-CH_3), 21.2 (6-CH_3), 16.7 (12-CH_3), 16.1 (2-CH_3), 11.3 (14-CH_3), 10.4 (10-CH_3), 10.0 (4-CH_3).
 **19**	174.9 (C-1), 174.7 (C-9), 168.6 (3-OCO), 149.4, 147.1, 120.6, (Py), 104.1 (C-1′), 83.9 (C-5), 79.7 (C-3), 78.4 (C-6), 77.4 (C-13), 74.8 (C-12), 70.7 (C-11), 70.2 (C-2′), 69.4 (C-5′), 66.0 (C-3′), 50.9 (6-OCH_3), 43.1, (C-2), 42.9 C-10), 42.5 (C-8), 42.1 (C-7), 40.2 [3′$N(CH_3)_2$], 36.9 (C-4), 33.5 (CH_2), 28.6 (C-4′), 23.0 (8-CH_3), 21.3 (C-14), 21.0 (5′-CH_3), 20.7 (6-CH_3), 16.3 (12-CH_3), 15.6 (2-CH_3), 10.8 (14-CH_3), 10.0 (10-CH_3), 9.4 (4-CH_3).
 **20**	175.0 (C-1), 174.9 (C-9), 170.8 (3-OCO), 133.7, 131.9, 130.1, 128.5, 128.5, 128.0, 126.3, 125.8, 125.3, 124.1 (naphthyl), 103.5 (C-1′), 82.4 (C-5), 78.4 (C-6), 77.7 (C-3), 77.2 (C-13), 74.7 (C-12), 70.6 (C-11), 70.4 (C-2′), 69.2 (C-5′), 65.7 (C-3′), 50.9 (6-OCH_3), 43.1 (C-2), 42.7 (C-10), 42.9 (C-7), 42.6 (C-8), 40.3 [3′$N(CH_3)_2$], 39.4 (CH_2), 37.1 (C-4), 28.6 (C-4′), 22.9 (8-CH_3), 21.2 (C-14), 21.0 (5′-CH_3), 20.9 (6-CH_3), 16.2 (12-CH_3), 15.2 (2-CH_3), 10.7 (14-CH_3), 10.1 (10-CH_3), 9.4 (4-CH_3).
 **21**	175.2 (C-1), 174.9 (C-9), 165.4 (3-OCO), 148.6, 140.0, 128.8, 124.2 (Ph), 142.6, 122.3 (C=C), 103.1 (C-1′), 82.3 (C-5), 78.4 (C-6), 78.4 (C-3), 77.4 (C-13), 75.0 (C-12), 70.7 (C-11), 70.2 (C-2′), 69.2 (C-5′), 65.8 (C-3′), 50.9 (6-OCH_3), 43.1 (C-10), 42.9 (C-2), 42.9 (C-8), 41.9 (C-7), 40.0 [3′$N(CH_3)_2$], 36.7 (C-4), 28.5 (C-4′), 22.9 (8-CH_3), 21.3 (C-14), 20.9 (5′-CH_3), 20.4 (6-CH_3), 16.3 (12-CH_3), 15.7 (2-CH_3), 10.8 (14-CH_3), 10.3 (10-CH_3), 9.4 (4-CH_3).
 **22**	175.3 (C-1), 175.3 (C-9), 170.8 (3-OCO), 150.7, 149.1, 137.5, 130.0, 123.8 (Py), 104.3 (C-1′), 83.3 (C-5), 79.1 (C-3), 78.9 (C-6), 77.8 (C-13), 75.2 (C-12), 71.2 (C-11), 70.7 (C-2′), 69.8 (C-5′), 66.5 (C-3′), 51.5 (6-OCH_3), 43.6, (C-2), 43.3 C-8), 43.0 (C-10), 42.6 (C-7), 40.7 [3′$N(CH_3)_2$], 39.1 (CH_2), 37.6 (C-4), 28.9 (C-4′), 23.5 (8-CH_3), 21.7 (C-14), 21.5 (5′-CH_3), 21.3 (6-CH_3), 16.7 (12-CH_3), 16.1 (2-CH_3), 11.3 (14-CH_3), 10.6 (10-CH_3), 9.9 (4-CH_3).
 **23**	174.8 (C-1), 174.7 (C-9), 169.5 (3-OCO), 149.9, 142.5, 124.6 (Py), 103.9 (C-1′), 83.0 (C-5), 78.5 (C-6), 78.4 (C-3), 77.3 (C-13), 74.7 (C-12), 70.6 (C-11), 70.3 (C-2′), 69.4 (C-5′), 65.8 (C-3′), 50.9 (6-OCH_3), 43.2, (C-2), 42.9 C-8), 42.5 (C-10), 42.1 (C-7), 40.8 (CH_2), 40.2 [3′$N(CH_3)_2$], 37.1 (C-4), 28.2 (C-4′), 21.2 (C-14).

Table 1 (Continued)

R	δ
 **24**	174.9 (C-9), 174.7 (C-1), 169.7 (3-OCO), 147.2, 141.1, 130.4, 123.7 (Ph), 104.1 (C-1′), 83.3 (C-5), 78.7 (C-3), 78.6 (C-13), 77.5 (C-6), 74.8 (C-12), 70.5 (C-11), 70.4 (C-2′), 69.6 (C-5′), 66.1 (C-3′), 51.0 (6-OCH_3), 43.2 (C-2), 42.9 (C-10), 42.6 (C-8), 42.2 (C-7), 41.3 (CH_2), 40.3 [3′$N(CH_3)_2$], 37.1 (C-4), 28.3 (C-4′), 21.3 (C-14).
 **25**	175.3 (C-1), 175.0 (C-9), 171.3 (3-OCO), 151.8, 147.0, 132.5, 132.3, 129.2, 128.9, 128.1, 127.4, 126.8 (Q), 134.6 (CH_2CH=<u>CH</u>CH_2-Q), 124.2 (CH_2<u>CH</u>=CHCH_2-Q), 103.5 (C-1′), 82.3 (C-5), 78.6 (C-6), 77.9 (C-3), 77.5 (C-13), 75.0 (C-12), 70.8 (C-11), 70.4 (C-2′), 69.4 (C-5′), 66.0 (C-3′), 51.1 (6-OCH_3), 43.1, 42.8 (C-2, C-8, C-10), 42.1 (C-7), 40.3 [3′$N(CH_3)_2$], 38.0 (<u>CH_2</u>CH=CHCH_2-Q), 36.9 (C-4), 36.2 (CH_2CH=CH<u>CH_2</u>-Q), 28.7 (C-4′), 23.1 (8-CH_3), 21.4 (C-14), 21.1 (5′-CH_3), 20.6 (6-CH_3), 16.4 (12-CH_3), 15.9 (2-CH_3), 10.9 (14-CH_3), 10.3 (10-CH_3), 9.4 (4-CH_3).
 **26**	175.2 (C-1), 175.2 (C-9), 171.4 (3-OCO), 158.8, 130.4, 125.6, 113.8 (Ph), 103.4 (C-1′), 81.9 (C-5), 78.4 (C-6), 77.5 (C-3), 77.3 (C-13), 74.8 (C-12), 70.6 (C-11), 70.3 (C-2′), 69.2 (C-5′), 65.6 (C-3′), 55.0 (Ph-OCH_3), 50.9 (6-OCH_3), 43.0, (C-8), 42.7 (C-2), 42.6 (C-10), 41.8 (C-7), 40.6 (CH_2), 40.1 [3′$N(CH_3)_2$], 36.9 (C-4), 28.3 (C-4′), 22.8 (8-CH_3), 21.1 (C-14), 20.8 (5′-CH_3), 20.6 (6-CH_3), 16.1 (12-CH_3), 15.3 (2-CH_3), 10.6 (10-CH_3), 10.0 (14-CH_3), 9.2 (4-CH_3).
 **27**	175.4 (C-1), 175.2 (C-9), 172.5 (3-OCO), 140.1, 128.5, 128.3, 126.3, (Ph), 102.7 (C-1′), 82.0 (C-5), 78.4 (C-6), 77.4 (C-13, C-13), 75.0 (C-12), 70.7 (C-11), 70.1 (C-2′), 68.7 (C-5′), 65.7 (C-3′), 51.0 (6-OCH_3), 43.0, (C-8, C-10)), 42.8 C-2), 41.6 (C-7), 40.2 [3′$N(CH_3)_2$], 36.6 (C-4), 35.9 (CH_2), 30.5 (CH_2), 30.3 (C-4′), 22.8 (8-CH_3), 21.2 (C-14), 20.8 (5′-CH_3), 20.5 (6-CH_3), 16.3 (12-CH_3), 15.6 (2-CH_3), 10.8 (14-CH_3), 10.3 (10-CH_3), 9.4 (4-CH_3).
 **28**	175.2 (C-1, C-9), 171.1 (3-OCO), 133.5, 129.4, 128.5 127.2 (Ph), 102.7 (C-1′), 81.5 (C-5), 78.4 (C-6), 77.6 (C-3), 77.4 (C-13), 74.9 (C-12), 70.7 (C-11), 70.2 (C-2′), 68.7 (C-5′), 66.5 (C-3′), 51.0 (6-OCH_3), 43.0, (C-2), 42.9 C-8, C-10), 41.7 (C-7), 41.5 CH_2), 40.1 [3′$N(CH_3)_2$], 36.9 (C-4), 29.8 (C-4′), 22.8 (8-CH_3), 21.2 (C-14), 20.8 (5′-CH_3), 20.6 (6-CH_3), 16.3 (12-CH_3), 15.5 (2-CH_3), 10.8 (14-CH_3), 10.3 (10-CH_3), 9.4 (4-CH_3).

All spectra were taken in $CDCl_3$ at 125 MHz and chemical shifts are reported in ppm relative to TMS.

On the basis of previous reports [10] that the introductions of cyclic carbonate to the 11,12-position of macrolide derivatives enhanced the antibacterial activity, the 11,12-cyclic carbonate analogues were synthesized by known method [11, 12]. Key intermediates for compounds **11** and **12**, 3-*O*-decladinosyl-carbonates **9** and **10**, were obtained either from **1a** and **2a**, with hydrolytic cleavage of cladinose in the final step, or from 3-*O*-decladinosyl precursors **3a** and **4a**, which reacted with ethylene carbonate in the final step. Oxidation of **9** and **10** under condition described above for **5** and **6** provided 3-keto-11,12-carbonates **11** and **12**, while acylation afforded 3-*O*-acyl-11,12-carbonates **13**∼**15**, Fig. 3.

MS and NMR spectra confirmed the structures of all compounds. For example, the ^{13}C NMR spectra of 3-hydroxy and 3-keto compounds revealed the shift of the C-3 signal in 3-hydroxy compounds **3a**∼**c**, **4a**∼**c**, **9** and **10** from around 78 ppm to the carbonyl signal around 206 ppm in compounds **5a**∼**c**, **6a**∼**c**, **11** and **12**. The long-range coupling of H-2 and H-4 to this carbonyl group provided evidence that a new-formed carbonyl group was at C-3 position of the azalide ring.

Similar, acylation of 3-hydroxy group was indicated by appearance of new ^{13}C carboxylic signal around 170 ppm. The connectivity between this signal and H-3 in HMBC spectrum confirmed the position of acyl group in compounds **13**∼**32**.

The ^{13}C NMR data for 3-keto **5a**∼**c**, **6a**∼**c** and 3-*O*-acyl-8a-lactam derivatives **16**∼**28** are summarized in Tables 1, 2.

Results and Discussion

In vitro antibacterial activity of all compounds was determined against the panel of diverse bacterial stains, covering most important Gram-positive (*i.e. Streptococcus pneumoniae*, *S. pyogenes*) and Gram-negative (*i.e. Haemophilus influenzae*, *Moraxella catarrhalis*) respiratory tract pathogens and different mechanisms of macrolide

Table 2 ^{13}C NMR data for 3-keto derivatives of 9a- and 8a-lactams

Compound	δ
5a	206.8 (C-3), 177.3 (C-1), 173.8 (C-9), 102.6 (C-1′), 79.3 (C-13), 78.4 (C-6), 74.4 (C-5), 73.9 (C-12), 73.1 (C-11), 70.0 (C-2′), 69.1 (C-5′), 65.5 (C-3′), 50.1 (6-OCH_3), 49.0 (C-2), 46.2 (C-4), 45.3 (C-10), 40.3 (C-7), 40.0 [3′-$N(CH_3)_2$], 34.6 (C-8), 28.3 (C-4′), 20.7 (C-14), 10.7 (15-CH_3).
5b	206.1 (C-3), 177.0 (C-9), 174.8 (C-1), 102.5 (C-1′), 80.3 (C-13), 79.3 (C-6), 74.2 (C-11, C-5), 70.2 (C-2′), 69.4 (C-5′), 65.8 (C-3′), 57.8 (6-$O\underline{CH_2}CH_3$), 49.1 (C-2), 46.0 (C-4), 45.8 (C-10), 41.4 (C-7), 40.3 [3′-$N(CH_3)_2$], 33.6 (C-8), 28.6 (C-4′), 21.2 (C-14), 16.3 (6-$OCH_2\underline{CH_3}$) 11.1 (14-CH_3).
5c	206.7 (C-3), 176.9 (C-9), 174.1 (C-1), 136.1 (6-$OCH_2\underline{CH}{=}CH_2$), 117.2 (6-$OCH_2CH{=}\underline{CH_2}$), 101.8 (C-1′), 79.8 (C-13), 79.5 (C-6), 74.9 (C-5), 74.2 (C-12), 73.4 (C-11), 70.0 (C-2′), 68.7 (C-5′), 66.0 (C-3′), 63.9 (6-$O\underline{CH_2}CH{=}CH_2$), 49.2 (C-2), 46.0 (C-4), 44.9 (C-10), 41.1 (C-7), 40.5 [3′-$N(CH_3)_2$], 34.2 (C-8), 30.7 (C-4′), 20.8 (C-14), 11.1 (14- CH_3).
6a	206.2 ((C-3), 170.0 (C-9), 174.6 (C-1), 103.1 (C-1′), 78.2 (C-6), 77.9 (C-5), 77.5 (C-13), 74.1 (C-12), 70.6 (C-11), 70.0 (C-2′), 69.1 (C-5′), 65.5 (C-3′), 50.5 (6-OCH_3), 50.4 (C-2), 47.6 (C-4), 42.2 (C-10), 42.1 (C-7), 41.6 (C-8), 39.9 [3′-$N(CH_3)_2$], 28.0 (C-4′), 22.8 (8-CH_3), 21.2 (C-14), 10.5 (15-CH_3).
6b	205.9 (C-3), 175.5 (C-9), 170.9 (C-1), 103.8 (C-1′), 81.4 (C-5), 78.4 (C-13), 78.3 (C-6), 70.4 (C-2′), 71.3 (C-11), 69.7 (C-5′), 65.9 (C-3′), 58.3 (6-$O\underline{CH_2}CH_3$), 50.3 (C-2), 47.4 (C-4), 42.6 (C-10), 41.9 (C-7), 40.4 [3′-$N(CH_3)_2$], 28.6 (C-4′), 21.5 (C-14), 15.6 (6-$OCH_2\underline{CH_3}$) 10.8 (14-CH_3).
6c	206.2 (C-3), 174.4 (C-9), 170.5 (C-1), 136.8 (6-$OCH_2\underline{CH}{=}CH_2$), 115.6 (6-$OCH_2CH{=}\underline{CH_2}$), 102.6 (C-1′), 78.8 (C-6), 77.8 (C-13), 77.6 (C-5), 74.3 (C-12), 70.7 (C-11), 69.9 (C-2′), 69.0 (C-5′), 65.6 (C-3′), 64.0 (6-$O\underline{CH_2}CH{=}CH_2$), 49.9 (C-2), 46.9 (C-4), 42.5 (C-10), 42.2 (C-8), 41.9 (C-7), 40.0 [3′-$N(CH_3)_2$], 28.6 (C-4′), 21.2 (C-14), 10.5 (14-CH_3).

All spectra were taken in $CDCl_3$ at 125 MHz and chemical shifts are reported in ppm relative to TMS.

resistance. Efflux mediated resistance gives rise to M phenotype and is characterized by expression of genes encoding efflux pumps, such as mef in streptococci and msr in staphylococci, that actively extrude macrolide antibiotics from bacterial cells. MLSb phenotype occurs due to expression of erm genes that encode ribosomal methyltransferases, a family of enzymes that specifically methylate ribosomal RNA and therefore prevent binding of macrolide, lincosamide and streptogramin B group of antibiotics.

In all *in vitro* experiments we used azithromycin as reference compound to novel derivatives, being a "golden standard" in macrolide therapy, due to its superior stability, pharmacokinetic and safety properties over erythromycin A.

In Vitro Evaluation

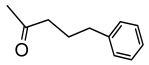
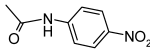
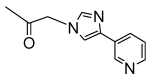
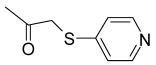
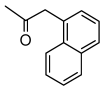
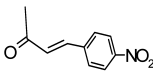
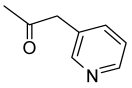
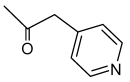
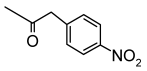
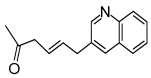
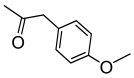
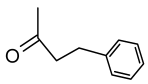
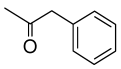
The activities are reported in Tables 3∼5 as minimum inhibitory concentrations (MIC). First we studied a variety of 3-*O*-acyl 8-a-lactams (compounds **16**∼**28**) and their activity is given in Table 3. To probe the effect of the phenyl group on antibacterial activity we examined unsubstituted (**28**), 4-nitro substituted (**24**) and 4-methoxy substituted (**26**) phenyl derivatives. Compound **24**, with electron withdrawing 4-nitro group, demonstrated the best antibacterial activity compared to unsubstituted phenyl ring, or phenyl ring substituted with electron-donating 4-methoxy group, with significant improvement over azithromycin against macrolide resistant strains (*i.e.* 2 μg/ml *vs.* >64 against MLSb *S. aureus*, =0.013 μg/ml *vs.* 4 μg/ml against M *S. pneumoniae*, respectively).

Further we examined the effect of the chain length between macrolide skeleton and aryl ring, by introducing C1∼C3 linkers (**28**, **27** and **16**, respectively). Linker elongation decreased the antibacterial activity and the effect was most pronounced against *S. aureus* strains. Insertion of sulphur into the linker (**19**) did not influence activity substantially, while the presence of nitrogen atom in **17** resulted with diminished activity, especially against macrolide resistant strains.

Introduction of pyridyl ring (**22** and **23**), compared to phenyl ring (**28**), enhanced activity against macrolide sensitive strains, but decreases activity against macrolide resistant pathogens. The presence of naphthyl ring in **20** resulted in similar antimicrobial spectrum as **28**, but increased activity against susceptible *S. pyogenes*.

As 4-nitrophenylacetyl derivative **24** was the most active 3-*O*-acyl derivative, we continued further chemical modification focusing on macrolide skeleton while maintaining this group at C-3 position. Consequently, 8a

Table 3 Antibacterial activity of 3-*O*-decladinosyl-3-*O*-acyl-6-*O*-methyl-8a-aza-8a-homoerythromycins

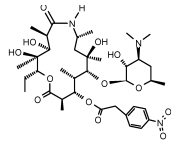
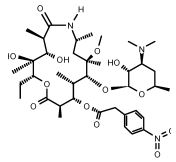
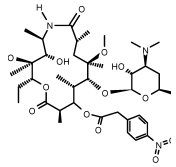
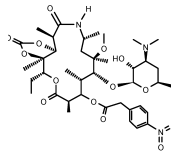
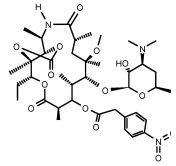
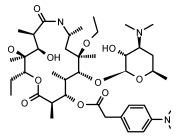
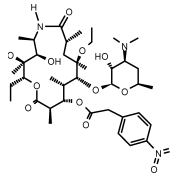
Compound	R	*Sau*			*Spn*		*Spy*			*Mca*	*Hin*	*Efa*
		eryS ATCC 13709	$iMLS_B$ PSCB* 0538	M PSCB 0331	eryS PSCB 0541	M PSCB 0326	eryS PSCB 0542	$iMLS_B$ PSCB 0543	M PSCB 0545	ATCC 23246	ATCC 49247	ATCC 29212
16		64	>64	64	2	1	4	>64	1	8	32	2
17		16	>64	16	2	4	0.5	>64	16	4	64	4
18		32	>64	32	1	4	1	>64	4	1	16	8
19		4	8	2	≤0.13	0.5	1	32	0.5	≤0.13	8	0.5
20		4	4	1	≤0.13	0.5	≤0.13	16	0.5	0.5	4	2
21		8	64	16	2	8	4	64	8	1	32	2
22		1	>64	4	≤0.13	≤0.13	≤0.13	8	2	≤0.13	1	0.5
23		2	>64	8	≤0.13	<0.13	≤0.13	64	4	≤0.13	8	1
24		1	1	1	≤0.13	≤0.13	≤0.13	1	≤0.13	0.25	8	1
25		32	>64	32	0.25	2	16	>64	>64	1	32	8
26		8	>64	4	≤0.13	0.5	0.25	64	1	0.25	16	4
27		16	>64	32	0.25	1	2	>64	2	0.5	16	2
28		8	8	4	≤0.13	0.5	1	8	0.5	≤0.13	16	0.5
	Azithromycin	0.5	>64	64	≤0.13	4	≤0.13	8	2	≤0.13	0.5	4

Sau, Staphylococcus aures; Spn, Streptococcus pneumoniae; Spy, Streptococcus pyogenes; Mca, Moraxella catarrhalis; Hin, Haemophilus influenzae; Efa, Enterococcus faecalis.

* Bacterial strains are clinical isolates from various clinical institutions, stored and characterised in GlaxoSmithKline Research Centre Zagreb (formerly Pliva Research Institute) Microbial strain collection.

and 9a-lactams, with or without 11,12-cyclic carbonate, having 6-OH or 6-*O*-$C_{1\sim2}$ alkyl group were prepared, Table 4. All 9a-lactams (**29**, **13**, **30**) showed inferior activity compared to their 8a-lactam counterparts (**24**, **14**, **31**). Compared to the parent 11,12-diol acylides **24** and **29**, their 11,12-carbonate derivatives **14** and **13**, respectively, exhibit reduced activity against both macrolide sensitive and efflux resistant Streptococci strains. This result contrasted with the results for the 14-membered macrolides in which introduction of 11,12-cyclic carbonate group led to

Table 4 Antibacterial activities of 3-*O*-(4-nitrophenyl)acetyl-8a- and 9a-lactams

	Compound	*Sau*			*Spn*		*Spy*			*Mca*	*Hin*	*Efa*
		eryS	$iMLS_B$	M	eryS	M	eryS	$iMLS_B$	M			
32		2	>128	1	≤0.25	≤0.25	≤0.25	16	≤0.25	≤1	2	≤1
24		1	1	1	≤0.13	≤0.13	≤0.13	1	≤0.13	0.25	8	1
29		8	8	16	≤0.13	≤0.13	≤0.13	1	0.25	0.25	16	4
14		4	4	4	≤0.13	≤0.13	≤0.13	1	0.5	1	8	1
13		>64	>64	>64	0.5	1	1	>64	4	4	32	32
31		4	>64	4	≤0.13	≤0.13	≤0.13	>64	≤0.13	≤0.13	4	8
30		>64	>64	>64	8	16	8	>64	8	2	>64	64
	Azithromycin	0.5	>64	64	=0.13	4	=0.13	8	2	=0.13	0.5	4

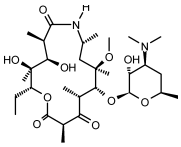
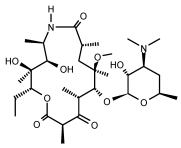
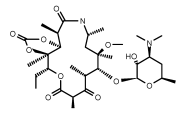
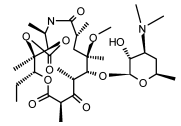
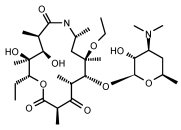
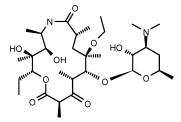
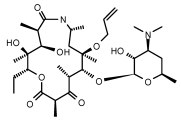
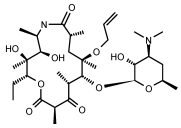
Sau, Staphylococcus aures; Spn, Streptococcus pneumoniae; Spy, Streptococcus pyogenes; Mca, Moraxella catarrhalis; Hin, Haemophilus influenzae; Efa, Enterococcus faecalis.

improved activity [10, 13]. Regarding alkylation at the 6-hydroxyl group, the best activity was shown by the compound with 6-*O*-methyl group (**24**). Overall, 6-*O*-methyl-3-*O*-acyl-8a-lactam (**24**) was found to be the most effective against all the pathogens tested.

Furthermore, we examined compounds having 3-keto instead of 3-*O*-acyl group, with varying macrolide skeleton, Table 5, and found that SAR is similar to 3-*O*-acyl series. The 6-*O*-methyl-8a-lactam (**6a**), which is a 3-keto counterpart of **24**, displayed the best antibacterial activity.

Based on the above results of *in vitro* antibacterial activity, compounds **6a** and **24** were selected for further pharmacokinetic and *in vivo* efficacy evaluation.

Table 5 Antibacterial activities of 3-keto-8a- and 9a-lactams

Compound		*Sau*			*Span*		*Spy*			*Mca*	*Hin*	*Efa*
		eryS	$iMLS_B$	M	eryS	M	eryS	$iMLS_B$	M			
6a		1	4	2	=0.13	2	0.25	4	4	=0.13	2	1
5a		>64	>64	>64	64	>64	>64	>64	>64	4	>64	64
12		16	>64	32	1	4	4	>64	8	≤0.13	32	8
11		>64	>64	>64	8	32	>64	>64	>64	1	>64	>64
6b		8	>64	8	0.5	4	1	>64	2	0.25	8	1
5b		>64	>64	>64	>64	>64	>64	>64	>64	4	>64	>64
6c		8	>64	16	2	4	2	>64	8	=0.13	16	4
5c		>64	>64	>64	>64	>64	>64	>64	>64	16	>64	>64
	Azithromycin	0.5	>64	64	=0.13	4	=0.13	8	2	=0.13	0.5	4

Sau, Staphylococcus aures; Spn, Streptococcus pneumoniae; Spy, Streptococcus pyogenes; Mca, Moraxella catarrhalis; Hin, Haemophilus influenzae; Efa, Enterococcus faecalis.

In Vivo Evaluation

Pharmacokinetic parameters in mice were determined in plasma and different tissues (liver, kidney, brain, spleen and lungs) for both selected derivatives and are given in Table 6.

The 3-keto compound **6a** was very well and rapidly absorbed after oral administration in dose of 100 mg/kg. It achieved peak concentration 30 minutes after administration and showed high tissue accumulation with $AUC_{(0\sim4)}$ levels relative to plasma $AUC_{(0\sim4)}$ (AUC_{rel}) values ranging from 5.7 to 34.6. The only exception to this was brain level with AUC_{rel} of 0.31. This is in accordance with behaviour of macrolides in general, as they are known to penetrate into cerebrospinal fluid very poorly [14]. High accumulation principally in liver tissue could be attributed to the first pass effect after oral administration, as all the blood from gut circulates through liver before going towards heart and other organs. Compound **6a** has relatively short half-life around 2 hours, but its $t_{1/2}$ in plasma is prolonged (more than 5 hours), which might

Table 6 Oral pharmacokinetic parameters of compounds **6a** and **24** in mice

a) Compound **6a** following a single dose of 100 mg/kg

Tissue	C_{max} (μg/g)	DNC_{max} (μg/g)	t_{max} (h)	$AUC_{(0\sim4)}$ (h*μg/g)	$DNAUC_{(0\sim4)}$ (h*μg/g)	AUC_{rel}	$t_{1/2}$ (h)
Liver	987.57	9.876	0.5	798.78	7.988	34.61	1.72
Kidney	148.89	1.489	0.5	149.99	1.500	6.49	2.57
Brain	5.26	0.053	0.5	7.16	0.072	0.31	n.d.
Spleen	218.66	2.187	0.5	223.43	2.234	9.68	2.16
Lungs	165.20	1.652	0.5	131.60	1.316	5.70	1.66
Plasma	12.45	0.125	0.5	23.08	0.231	1	5.29

b) Compound **24** following a single dose of 50 mg/kg

Tissue	C_{max} (μg/g)	DNC_{max} (μg/g)	t_{max} (h)	$AUC_{(0\sim4)}$ (h*μg/g)	$DNAUC_{(0\sim4)}$ (h*μg/g)	AUC_{rel}	$t_{1/2}$ (h)
Liver	19.567	0.391	0.75	19.451	0.389	2.509	0.60
Kidney	16.04	0.321	0.75	10.759	0.215	1.390	0.62
Brain	1.794	0.036	0.75	2.201	0.044	0.284	n.d.
Spleen	24.933	0.499	0.75	24.016	0.480	3.103	2.39
Lungs	22.696	0.454	0.75	28.228	0.565	3.647	1.04
Plasma	4.83	0.097	0.75	7.738	0.155	1	1.20

n.d.: not determined. DNC_{max}, DNAUC: dose-normalized C_{max} and AUC, respectively. AUC_{rel}: $AUC_{tissue}/AUC_{plasma}$.

Table 7 *In vivo* efficacy of compounds **6a** and **24** in comparison with azithromycin against susceptible and inducible-resistant *Staphylococcus aureus*

Microorganism	Compound	ED_{50} (mg/kg)	95% Confidence interval	MIC (μg/ml)
S. aureus ery-S ATCC 29213	**6a**	48.6	33.8~71.34	1
	24	29.7	12.40~70.97	2
	Azithromycin	17.6	10.57~29.17	0.5
S. aureus $iMLS_B$ PSCB 0009	**6a**	61.7	32.99~115.36	2
	24	74.7	57.21~97.52	4
	Azithromycin	261.0	86.12~791.73	>64

indicate high plasma protein binding. Due to low concentrations, $t_{1/2}$ for brain tissue could not be determined.

The 3-*O*-acyl compound **24** was given orally at 50 mg/kg. It was well absorbed, but displayed moderate and more even tissue distribution than **6a**, with AUC_{rel} ranging from 1.4 to 3.6, with the exception of brain tissue where AUC_{rel} was 0.28. Peak concentrations were reached 45 minutes after administration. Half-life was short, approximately one hour, with the exception of spleen, where it was prolonged to 2.4 hours.

Overall, both derivatives were well absorbed after p.o. administration in mice, had high peak concentrations, pronounced tissue accumulation and relatively short half-life. The 3-keto compound **6a** was better absorbed and achieved significantly higher levels than the 3-*O*-acyl compound **24** in all organs examined, most markedly in liver where $DNAUC_{(0\sim4)}$ was 20 times higher for **6a** than for **24**, and kidney, where **6a** $DNAUC_{(0\sim4)}$ was 7 times higher.

The *in vivo* efficacy of **6a**, **24** and azithromycin was

assessed in lethal septicaemia model in mice, using erythromycin susceptible and iMLS$_b$ resistant strains of *Staphylococcus aureus*. Mice were infected by single i.v. administration of bacterial suspension and compounds were dosed orally every 12 hours for three days, starting 30 minutes post infection. The efficacy of each compound is reported as the effective drug dosage (ED$_{50}$) that resulted in 50% survival rate, Table 7. Compounds **6a** and **24** where effective against ery-S *S. aureus* strains, but less than azithromycin, which could be correlated to corresponding *in vitro* activities, as well as differences in plasma half-lives (AZM $t_{1/2}$ being >12 hours [15]). When tested against iMLS$_b$ resistant *S. aureus* strain, **6a** and **24** were four times more effective than azithromycin.

Conclusion

In summary, we have identified a combination of structural factors that contribute to enhancement of antibacterial activity for novel 3-keto- and 3-*O*-acyl-azalides. These factors are 3-keto group or 3-*O*-acetyl group having the phenyl unit. In particular, phenyl unit bearing electron-withdrawing *p*-nitro group enhanced activity. Introduction of 11,12-cyclic carbonate substituent decreases activity of all tested compounds, as well as elongation of alkyl chain at 6-position of the lactam ring. Derivatives of 8a-lactam showed superior activity as compared to 9a-analogues.

The encouraging *in vivo* results for **6a** and **24** warrant further investigation to overcome MLS$_B$-resistant pathogens and to develop potential next-generation azalides.

Experimental

General

NMR spectra were recorded on a Bruker Avance DRX500 or Bruker Avance DPX300 spectrometer in $CDCl_3$ and chemical shifts are reported in ppm using TMS as an internal standard. Mass spectra were obtained on a Waters Micromass ZQ mass spectrometer for ES$^+$-MS. Silica gel chromatography was performed on Merck Kieselgel 60 and Merck TLC 60F$_{254}$, respectively.

In general, organic layer was dried with anhydrous Na_2SO_4 or K_2CO_3, evaporation and concentration were carried out under reduced pressure below 40°C, unless otherwise noted.

6-*O*-Ethyl- and 6-*O*-allylerythromycin 9-oxime derivatives were prepared from erythromycin 9(*E*)-oxime [9] according to the published procedure [8] which include protection, alkylation, deprotection and separation of obtained 9(*E*)- and 9(*Z*)-oxime isomers. 2′-*O*-Acetyl-protected derivatives were prepared by the known procedure [3, 16].

Antibacterial Activity *in Vitro*

Strains were cultured on MH agar (Merck, Germany) except strains belonging to genus *Streptococcus* and *Haemophilus*, which were cultured on blood agar plates (Biomerioux, France) and chocolate agar plates (Biomerioux, France), respectively. Minimum inhibitory concentrations (MICs) were determined by the microtitre liquid dilution method as described by NCCLS [17] except that for *Streptococcus* medium, blood was substituted with 5% horse serum.

Pharmacokinetics in Mice

Experiments were carried out on male Balb/c mice (Charles River, Germany), weighing 20~25 g, divided into groups, 6 mice each. Substances were administered *p.o.* to fasted mice as suspension in 0.5% carboxy - methylcellulose (w/v) (SIGMA) saline solution (0.9%) (PLIVA) at 100 mg/kg (**6a**) and 50 mg/kg (**24**). Blood and tissue samples (liver, kidney, lung, brain and spleen) were taken at 0.25, 0.5, 1, 2, 4, 6, 12, 24 and 48 hours after administration of **6a** and at 0.25, 0.75, 1.5, 2, 3, 4, 6, 12, 24, 48 and 72 hours in case **24**. The concentrations of the compounds were measured by microbiological agar diffusion method using *Micrococcus luteus* as test microorganism.

In Vivo Efficacy

In vivo efficacy of selected compounds was tested in *S. aureus* lethal septicaemia mouse model using Balb/c mice (Charles River, Germany), weighing 20~25 g, divided into groups consisting of 10 mice each. Strains were grown overnight in MHB (Merck, Germany) at 37°C. Cells were suspended in fresh medium and the inoculum size (CFU/mouse) was adjusted to 1.0×10^7 for both eryS (ATCC 29213) and iMLS$_B$ strains (Pliva Strain Collection Bacteria, PSCB 0009). Mice were infected by single i.v. administration. Therapy started 30 minutes after infection and daily p.o. doses were administered in two portions every 12 hours for 3 days. Compounds were dissolved in DMF (Merck, Germany) given as 0.5% carboxy - methylcellulose (w/v) (SIGMA) saline solution (0.9%) (PLIVA) and 3% with respect to DMF. Survival was measured after 6 days. ED$_{50}$ values were calculated by in-house log-probit method.

6-*O*-Methyl-9a-aza-9a-homoerythromycin A (**1a**)

6-*O*-Methylerythromycin A 9(*E*)-oxime (4.0 g, 0.005 mol) was dissolved in acetone (130 ml) and the solution was cooled to 0~5°C. Subsequently, 4-toluenesulfonyl chloride

(2.6 g, 0.01 mol) in acetone (40 ml) and sodium hydrogen carbonate (0.83 g, 0.01 mol) in water (130 ml) were added dropwise thereto within 1 hour under stirring. After stirring at room temperature for 8 hours, acetone was evaporated and aqueous solution was extracted with $CHCl_3$ by gradient extraction at pH 5 and 9. The combined organic layers at pH 9 were evaporated to give **1a** (2.8 g, 70%) as a white solid.

^{13}C NMR (75 MHz, $CDCl_3$) δ 179.5 (C-1), 177.3 (C-9), 102.5 (C-1′), 94.9 (C-1″), 79.4 (C-6), 78.5 (C-5), 77.7 (C-4″), 77.7 (C-13), 75.9 (C-3), 73.9 (C-12), 72.6 (C-11), 72.5 (C-3″), 70.7 (C-2′), 68.2 (C-5′), 65.3 (C-5″), 65.1 (C-3′), 51.0 (6-OCH_3), 49.1 (3″-OCH_3), 45.1 (C-10), 44.5 (C-2), 41.3 (C-4), 40.0 [3′-$N(CH_3)_2$], 39.6 (C-7), 35.4 (C-8), 34.4 (C-2″), 28.8 (C-4′), 21.1 (5′-CH_3), 21.0 (3″-CH_3), 20.3 (C-14), 20.2 (6-CH_3), 19.1 (8-CH_3), 18.1 (5″-CH_3), 15.9 (12-CH_3), 14.6 (2-CH_3), 13.4 (10-CH_3), 10.7 (14-CH_3), 8.7 (4-CH_3).

ES^+-MS *m*/*z* 763.6 $(M+H)^+$

6-*O*-Ethyl-9a-aza-9a-homoerythromycin A (**1b**)

Reaction of 6-*O*-ethylerythromycin A 9(*E*)-oxime with 4-toluenesulfonyl chloride gave **1b** as a colourless solid in 85% yield by a similar procedure to **1a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 179.3 (C-1), 177.1 (C-9), 102.1 (C-1′), 96.0 (C-1″), 80.1 (C-6), 79.0 (C-13),78.8 (C-5), 77.8 (C-3), 77.7 (C-4″), 74.2 (C-12), 73.7 (C-11), 73.0 (C-3″), 71.1 (C-2′), 68.4 (C-5′), 65.5 (C-3′), 66.4 (C-5″), 58.8 (6-O$\underline{CH_2}CH_3$), 49.4 (3″-OCH_3), 45.5 (C-10), 44.7 (C-2), 40.4 (C-7), 40.1 (C-4), 34.4 (C-8), 40.5 [3′-$N(CH_3)_2$], 35.1 (C-2″), 29.3 (C-4′), 21.2 (6-$OCH_2\underline{CH_3}$) 20.8 (C-14), 11.2 (14-CH_3).

ES^+-MS *m*/*z* 777.5 $(M+H)^+$

6-*O*-Allyl-9a-aza-9a-homoerythromycin A (**1c**)

The title compound was prepared from 6-*O*-allylerythromycin A 9(*E*)-oxime and 4-toluenesulfonyl chloride in 10.7% yield according to the procedure used to prepare **1a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 179.3 (C-1), 177.1 (C-9), 136.6 (6-$OCH_2\underline{CH}=CH_2$), 118.0 (6-$OCH_2CH=\underline{CH_2}$), 102.5 (C-1′), 95.6 (C-1″), 80.6 (C-6), 78.9 (C-5), 78.4 (C-13), 77.9 (C-4″), 77.2 (C-3), 74.3 (C-12), 73.1 (C-11), 72.9 (C-3″), 71.1 (C-2′), 68.5 (C-5′), 66.1 (C-5″), 65.5 (C-3′), 65.3 (6-O$\underline{CH_2}CH=CH_2$), 49.4 (3″-OCH_3), 45.6 (C-10), 44.8 (C-2), 41.0 (C-4), 40.2 (C-7), 40.5 [3′-$N(CH_3)_2$], 35.2 (C-8), 35.0 (C-2″), 29.5 (C-4′), 20.8 (C-14), 11.1 (14-CH_3).

ES^+-MS *m*/*z* 789.8 $(M+H)^+$

6-*O*-Methyl-8a-aza-8a-homoerythromycin A (**2a**)

Reaction of 6-*O*-methylerythromycin A 9(*Z*)-oxime with 4-toluenesulfonyl chloride gave **2a** as a colourless solid in 58% yield by a similar procedure to **1a**.

^{13}C NMR (75 MHz, $CDCl_3$) δ 177.0 (C-1), 174.3 (C-9), 102.9 (C-1′), 95.1 (C-1″), 80.1 (C-5), 78.6 (C-6), 77.9 (C-4″), 77.2 (C-3), 76.7 (C-13), 74.0 (C-12), 72.6 (C-3″), 70.4 (C-2′), 70.1 (C-11), 68.7 (C-5′), 65.4 (C-3′), 65.2 (C-5″), 51.5 (6-OCH_3), 49.1 (3″-OCH_3), 45.4 (C-2), 42.6 (C-7), 42.1 (C-4), 41.8 (C-10), 40.6 (C-8), 40.0 [3′-$N(CH_3)_2$], 34.5 (C-2″), 28.3 (C-4′), 23.5 (6-CH_3), 21.3 (C-14), 21.2 (12-CH_3), 21.1 (5′-CH_3), 21.1 (3″-CH_3), 17.9 (5″-CH_3), 15.8 (8-CH_3), 14.8 (2-CH_3), 10.8 (14- CH_3), 9.2 (10- CH_3), 9.1 (4-CH_3).

ES^+-MS *m*/*z* 763.6 $(M+H)^+$

6-*O*-Ethyl-8a-aza-8a-homoerythromycin A (**2b**)

Reaction of 6-*O*-ethylerythromycin A 9(*Z*)-oxime with 4-toluenesulfonyl chloride gave **2b** as a colourless solid in 89% yield by a similar procedure to **1a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0 (C-9), 174.1 (C-1), 103.3 (C-1′), 97.3 (C-1″), 82.6 (C-5), 81.9 (C-3), 78.5 (C-6), 77.7 (C-4″), 77.0 (C-13),73.5 (C-12), 72.4 (C-3″), 70.7 (C-11), 70.4 (C-2′), 68.8 (C-5′), 65.2 (C-3′), 65.2 (C-5″), 60.0 (6-O$\underline{CH_2}CH_3$), 48.9 (3″-OCH_3), 45.2 (C-2), 42.0 (C-7), 41.4 (C-8, C-10), 40.2 (C-4), 40.0 [3′-$N(CH_3)_2$], 35.2 (C-2″), 28.7 (C-4′), 21.0 (C-14), 10.2 (14-CH_3).

ES^+-MS *m*/*z* 777.6 $(M+H)^+$

6-*O*-Allyl-8a-aza-8a-homoerythromycin A (**2c**)

The title compound was prepared from 6-*O*-allylerythromycin A 9(*Z*)-oxime and 4-toluenesulfonyl chloride in 25% yield according to the procedure used to prepare **1a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8 (C-1), 174.5 (C-9), 137.6 (6-$OCH_2\underline{CH}=CH_2$), 115.7 (6-$OCH_2CH=\underline{CH_2}$), 103.3 (C-1′), 95.6 (C-1″), 81.4 (C-5), 80.2 (C-6), 79.9 (C-3), 78.1 (C-4″), 77.2 (C-13), 74.2 (C-12), 72.9 (C-3″), 70.9 (C-11), 70.8 (C-2′), 69.0 (C-5′), 65.9 (6-O$\underline{CH_2}CH=CH_2$), 65.7 (C-5″), 65.6 (C-3′), 49.4 (3″-OCH_3), 45.6 (C-2), 42.8 (C-7), 42.2 (C-10), 41.6 (C-8), 40.4 [3′-$N(CH_3)_2$], 35.4 (C-2″), 28.9 (C-4′), 201.5 (C-14), 10.9 (14-CH_3).

ES^+-MS *m*/*z* 789.7 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-9a-aza-9a-homoerythromycin A (**3a**)

Compound **1a** (2.8 g, 3.7 mmol) was dissolved in 0.25 N HCl (100 ml) and stirred at room temperature for 24 hours. To the reaction mixture CH_2Cl_2 (50 ml) was added, pH was adjusted to 9.0 by adding conc. NH_4OH and extracted with CH_2Cl_2 (50 ml×3). The combined organic layers were washed with 10% aq. $NaHCO_3$ and water and evaporated under reduced pressure to give **3a** (2.05 g, 92.3%).

^{13}C NMR (75 MHz, $CDCl_3$) δ 179.3 (C-1), 176.9 (C-9), 106.4 (C-1′), 88.1 (C-5), 79.1 (C-6), 78.7 (C-13), 78.0 (C-3), 73.8 (C-12), 73.9 (C-11), 70.2 (C-2′), 69.7 (C-5′), 65.4 (C-3′), 49.9 (6-OCH_3), 45.6 (C-10), 43.9 (C-2), 40.8 (C-7), 39.9 [3′-$N(CH_3)_2$], 35.6 (C-4), 32.8 (C-8), 27.8 (C-4′), 20.5 (C-14), 10.7 (14-CH_3).

ES^+-MS *m/z* 605.5 $(M+H)^+$

6-*O*-Ethyl-3-*O*-decladinosyl-9a-aza-9a-homoerythromycin A (**3b**)

The title compound was prepared from **1b** in 95.2% yield according to the procedure used to prepare **3a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 179.3 (C-1), 177.2 (C-9), 107.0 (C-1′), 88.9 (C-5), 79.7 (C-6), 79.3 (C-13), 78.5 (C-3), 74.5 (C-11), 74.3 (C-12), 70.5 (C-2′), 70.2 (C-5′), 65.8 (C-3′), 58.1 (6-O$\underline{CH_2}CH_3$), 46.0 (C-10), 44.4 (C-2), 41.7 (C-7), 40.3 [3′-$N(CH_3)_2$], 36.0 (C-4), 32.9 (C-8), 28.0 (C-4′), 21.0 (C-14), 16.0 (6-$OCH_2\underline{CH_3}$) 11.1 (14-CH_3).

ES^+-MS *m/z* 619.6 $(M+H)^+$

6-*O*-Allyl-3-*O*-decladinosyl-9a-aza-9a-homoerythromycin A (**3c**)

The title compound was prepared from **1c** in 32.8% yield according to the procedure used to prepare **3a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 179.0 (C-1), 177.0 (C-9), 135.2 (6-$OCH_2\underline{CH}=CH_2$), 117.7 (6-$OCH_2CH=\underline{CH_2}$), 106.1 (C-1′), 88.8 (C-5), 80.1 (C-6), 79.0 (C-13), 78.3 (C-3), 74.2 (C-11), 70.3 (C-2′), 69.5 (C-5′), 66.1 (C-3′), 64.2 (6-O$\underline{CH_2}CH=CH_2$), 46.1 (C-10), 44.3 (C-2), 41.6 (C-7), 40.3 [3′-$N(CH_3)_2$], 35.9 (C-4), 33.0 (C-8), 28.9 (C-4′), 20.9 (C-14), 11.1 (14- CH_3).

ES^+-MS *m/z* 631.6 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-8a-aza-8a-homoerythromycin A (**4a**)

The title compound was prepared from **2a** in 82.7% yield according to the procedure used to prepare **3a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 176.5 (C-1), 174.9 (C-9), 106.6 (C-1′), 90.2 (C-5), 78.9 (C-3), 77.9 (C-6), 76.6 (C-13), 75.0 (C-12), 71.0 (C-11), 70.5 (C-2′), 70.1 (C-5′), 65.7 (C-3′), 50.5 (6-OCH_3), 44.5 (C-2), 43.2 (C-10), 42.8 (C-8), 42.5 (C-7), 40.3 [3′-$N(CH_3)_2$], 35.9 (C-4), 28.2 (C-4′), 21.7 (C-14), 10.9 (14-CH_3).

ES^+-MS *m/z* 605.6 $(M+H)^+$

6-*O*-Ethyl-3-*O*-decladinosyl-8a-aza-8a-homoerythromycin A (**4b**)

The title compound was prepared from **2b** in 89.3% yield according to the procedure used to prepare **3a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 176.5 (C-1), 175.2 (C-9), 106.5 (C-1′), 89.0 (C-5), 77.9 (C-3), 78.0 (C-6), 77.0 (C-13), 75.2 (C-12), 71.2 (C-11), 70.6 (C-2′), 70.1 (C-5′), 65.7 (C-3′), 57.8 (6-O$\underline{CH_2}CH_3$), 44.4 (C-2), 43.3 (C-8), 43.1 (C-10), 42.1 (C-7), 40.3 [3′-$N(CH_3)_2$], 28.1 (C-4′), 15.9 (6-$OCH_2\underline{CH_3}$) 21.5 (C-14), 10.8 (14-CH_3).

ES^+-MS *m/z* 619.5 $(M+H)^+$

6-*O*-Allyl-3-*O*-decladinosyl-8a-aza-8a-homoerythromycin A (**4c**)

The title compound was prepared from **2c** in 43.8% yield according to the procedure used to prepare **3a**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 179.0 (C-1), 177.1 (C-9), 135.2 (6-$OCH_2\underline{CH}=CH_2$), 117.7 (6-$OCH_2CH=\underline{CH_2}$), 106.5 (C-1′), 88.7 (C-5), 81.8 (C-6), 79.0 (C-13), 78.4 (C-3), 74.3 (C-11), 74.2 (C-12), 70.5 (C-2′), 69.9 (C-5′), 65.8 (C-3′), 63.8 (6-O$\underline{CH_2}CH=CH_2$), 46.1 (C-8), 44.3 (C-2), 41.6 (C-7), 40.3 [3′-$N(CH_3)_2$], 36.0 (C-4), 33.0 (C-10), 28.3 (C-4′), 20.9 (C-14), 10.9 (14-CH_3).

ES^+-MS *m/z* 631.7 $(M+H)^+$

6-*O*-Methyl-3-oxo-9a-aza-9a-homoerythromycin A (**5a**)

To a solution of 2′-*O*-acetyl-6-*O*-methyl-3-decladinosyl-9a-aza-9a-homoerythromycin A (**3a**) (0.760 g, 1.2 mmol) in CH_2Cl_2 (15 ml), DMSO (1.27 ml) and EDC (1.34 g, 7 mmol) were added. A solution of pyridine trifluoroacetate (1.37 g, 7 mmol) in CH_2Cl_2 (5 ml) was added dropwise within 30 minutes at 15°C. After being stirred at room temperature for 3 hours, the reaction mixture was partitioned between CH_2Cl_2 and brine and extracted at pH 9.5 with CH_2Cl_2 (3×20 ml). The organic extract was washed with brine and water, dried over K_2CO_3 and concentrated under reduced pressure. The oily residue was dissolved in MeOH (30 ml) and stirred at room temperature for 24 hours. After evaporation of MeOH, the residue was purified by column chromatography (CH_2Cl_2 - MeOH - NH_4OH, 90 : 9 : 0.5) to give **5a** in 83.1% yield.

ES^+-MS *m/z* 603.3 $(M+H)^+$

By using this procedure (2′-*O*-acetylation, oxidation of 3-OH and 2′-deacetylation), the compounds **5b**～**c** and **6a**～**c** were prepared.

6-*O*-Ethyl-3-oxo-9a-aza-9a-homoerythromycin A (**5b**)

The title compound was prepared starting from **3b** in 31.4% yield.

ES^+-MS *m/z* 617.4 $(M+H)^+$

6-*O*-Allyl-3-oxo-9a-aza-9a-homoerythromycin A (**5c**)

The title compound was prepared starting from **3c** in 20.6% yield.

ES^+-MS *m/z* 629.4 $(M+H)^+$

6-*O*-Methyl-3-oxo-8a-aza-8a-homoerythromycin A (**6a**)
The title compound was prepared starting from **4a** in 82.9% yield.

ES^+-MS *m*/*z* 603.4 $(M+H)^+$

6-*O*-Ethyl-3-oxo-8a-aza-8a-homoerythromycin A (**6b**)
The title compound was prepared starting from **4b** in 34.3% yield.

ES^+-MS *m*/*z* 617.5 $(M+H)^+$

6-*O*-Allyl-3-oxo-8a-aza-8a-homoerythromycin A (**6c**)
The title compound was prepared starting from **4c** in 30.3% yield.

ES^+-MS *m*/*z* 629.6 $(M+H)^+$

6-*O*-Methyl-9a-aza-9a-homoerythromycin A 11,12-cyclic Carbonate (**7**)
To a solution of **1a** (3.0 g, 0.004 mol) in EtOAc (80 ml) ethylene carbonate (9.0 g, 0.102 mol) and K_2CO_3 (9.0 g, 0.065 mol) were added. After stirring at reflux temperature for 12 hours, the reaction mixture was diluted with EtOAC (100 ml), washed with brine (100 ml) and then with H_2O (100 ml). The evaporation of the organic solvent gave an oily residue, which was purified by silica gel column chromatography (CH_2Cl_2 - MeOH - conc. NH_4OH, 90 : 9 : 1.5) to afford **7** (2 g, 65%).

^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4 (C-9), 177.2 (C-1), 153.9 (C=O cyclic carbonate), 102.7 (C-1′), 94.0 (C-1″), 84.7 (C-12), 83.6 (C-11), 79.1 (C-5), 78.7 (C-6), 77.9 (C-4″), 75.5 (C-3), 75.2 (C-13), 72.6 (C-3″), 70.7 (C-2′), 68.5 (C-5′), 65.3 (C-5″), 65.1 (C-3′), 51.0 (6-OCH_3), 49.2 (3″-OCH_3), 44.9 (C-2), 44.3 (C-10), 42.0 (C-4), 40.1 [3′-$N(CH_3)_2$], 39.4 (C-7), 36.2 (C-8), 34.3 (C-2″), 28.6 (C-4′), 21.8 (C-14), 10.2 (14-CH_3).

ES^+-MS *m*/*z* 789.8 $(M+H)^+$

6-*O*-Methyl-8a-aza-8a-homoerythromycin A 11,12-cyclic Carbonate (**8**)
The title compound was prepared from **2a** in 64.3% yield according to the procedure used to prepare **7**.

^{13}C NMR (75 MHz, $CDCl_3$) δ 176.4 (C-1), 170.3 (C-9), 153.4 (C=O cyclic carbonate), 103.2 (C-1′), 96.0 (C-1″), 85.6 (C-12), 82.2 (C-11), 80.2 (C-5), 79.8 (C-6), 78.0 (C-4″), 77.0 (C-3), 75.7 (C-13), 72.8 (C-3″), 70.7 (C-2′), 69.1 (C-5′), 65.6 (C-5″), 65.5 (C-3′), 51.1 (6-OCH_3), 49.4 (3″-OCH_3), 42.5 (C-8), 42.4 (C-10), 42.3 (C-7), 42.3 (C-4), 40.3 [3′-$N(CH_3)_2$], 34.9 (C-2″), 28.8 (C-4′), 22.5 (C-14), 10.5 (14-CH_3).

ES^+-MS *m*/*z* 789.7 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-9a-aza-9a-homoerythromycin A 11,12-cyclic Carbonate (**9**)
The title compound was prepared from **7** in 87.2% yield according to the procedure used to prepare **3a**.

The title compound was also prepared from **3a** in 66.5% yield according to the procedure used to prepare **7**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.0 (C-9), 176.2 (C-1), 153.4 (C=O carbonate), 106.3 (C-1′), 87.9 (C-5), 85.4 (C-12), 84.1 (C-11), 79.3 (C-6), 78.3 (C-3), 76.4 (C-13), 70.4 (C-2′), 69.8 (C-5′), 65.9 (C-3′), 50.2 (6-OCH_3), 44.5 (C-2), 44.5 (C-10), 40.3 [3′-$N(CH_3)_2$], 39.8 (C-7), 36.8 (C-4), 34.8 (C-8), 28.7 (C-4′), 22.0 (C-14), 10.3 (14-CH_3). IR ν_{max} (KBr) cm^{-1} 3440, 2974, 2939, 1822, 1729, 1650, 1525, 1457, 1380, 1241, 1167, 1113, 1073, 1047, 983.

ES^+-MS *m*/*z* 631.4 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-8a-aza-8a-homoerythromycin A 11,12-cyclic Carbonate (**10**)
The title compound was prepared from **8** in 85.5% yield according to the procedure used to prepare **3a**.

The title compound was also prepared from **4a** in 51.2% yield according to the procedure used to prepare **7**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 175.5 (C-1), 170.4 (C-9), 153.1 (C=O carbonate), 106.9 (C-1′), 91.7 (C-5), 86.8 (C-12), 82.9 (C-11), 79.1 (C-6), 76.8 (C-3), 74.8 (C-13), 70.4 (C-2′), 69.8 (C-5′), 65.5 (C-3′), 49.3 (6-OCH_3), 43.9 (C-2), 43.8 (C-8), 42.5 (C-10), 41.1 (C-7), 40.2 [3′-$N(CH_3)_2$], 37.1 (C-4), 28.1 (C-4′), 22.2 (C-14), 10.2 (14-CH_3).

ES^+-MS *m*/*z* 631.4 $(M+H)^+$

6-*O*-Methyl-3-oxo-9a-aza-9a-homoerythromycin A 11,12-cyclic Carbonate (**11**)
The title compound was prepared starting from **9** in 21.3% yield according to the procedure used to prepare **5a**.

^{13}C NMR (75 MHz, $CDCl_3$) δ 206.7 (C-3), 177.0 (C-9), 170.1 (C-1), 153.6 (C=O carbonate), 103.4 (C-1′), 84.4 (C-12), 84.1 (C-11), 78.5 (C-5), 78.1 (C-6), 75.7 (C-13), 70.1 (C-2′), 69.2 (C-5′), 65.4 (C-3′), 50.1 (6-OCH_3), 50.0 (C-2), 47.7 (C-4), 44.2 (C-10), 39.9 [3′-$N(CH_3)_2$], 39.1 (C-7), 36.2 (C-8), 28.0 (C-4′), 21.7 (C-14), 10.0 (14-CH_3).

ES^+-MS *m*/*z* 629.5 $(M+H)^+$

6-*O*-Methyl-3-oxo-8a-aza-8a-homoerythromycin A 11,12-cyclic Carbonate (**12**)
The title compound was prepared starting from **10** in 56.6% yield according to the procedure used to prepare **5a**.

^{13}C NMR (75 MHz, $CDCl_3$) δ 203.0 (C-3), 171.1 (C-9), 169.6 (C-1), 152.4 (C=O carbonate), 104.8 (C-1′), 85.2 (C-12), 82.6 (C-11), 82.6 (C-5), 81.1 (C-6), 75.9 (C-13), 70.2 (C-2′), 69.8 (C-5′), 65.9 (C-3′), 52.7 (C-2), 49.1 (6-OCH_3), 48.3 (C-4), 44.8 (C-8), 43.6 (C-10), 40.3 [3′-

$N(CH_3)_2$], 40.2 (C-7), 28.4 (C-4′), 22.7 (C-14), 10.5 (14-CH_3). IR ν_{max} (KBr) cm^{-1} 3379, 2976, 1814, 1755, 1713, 1668, 1539, 1457, 1381, 1243, 1166, 1110, 1062, 995.

ES^+-MS *m/z* 629.6 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-nitrophenyl)acetyl-9a-aza-9a-homoerythromycin A 11,12-cyclic Carbonate (**13**)

To a solution of 4-nitrophenylacetic acid (0.263 g, 1.5 mmol) in dry CH_2Cl_2 (5 ml) and Et_3N (0.20 ml, 1.5 mmol) pivaloyl chloride (0.18 ml, 1.5 mmol) was added at 0°C. After being stirred for 30 minutes, a solution of 2′-acetyl protected **9** (0.269 g, 0.4 mmol) in CH_2Cl_2 (2 ml) and pyridine (0.4 ml) were added and the mixture was stirred for another 3 hours. The reaction mixture was partitioned between CH_2Cl_2 and brine, the layers were separated and the aqueous one extracted with CH_2Cl_2 (20 ml×3). Combined organic extracts were dried, the solvent removed under reduced pressure and the residue left in MeOH (30 ml) at room temperature overnight. Methanol was removed and the residue was purified by chromatography on silica gel, eluting with CH_2Cl_2 - MeOH - conc. NH_4OH, 90 : 9 : 0.5 to give 0.142 g (44.7%) of title compound **13**.

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.1 (C-9), 174.8 (C-1), 170.0 (3-OCO), 153.5 (C=O carbonate), 147.3, 141.1, 130.5, 123.7 (Ph), 103.9 (C-1′), 85.1 (C-12), 84.0 (C-11), 81.3 (C-5), 79.3 (C-6), 77.8 (C-3), 76.3 (C-13), 70.4 (C-2′), 69.5 (C-5′), 66.2 (C-3′), 50.9 (6-OCH_3), 44.4 (C-10), 43.3 (C-2), 41.2 (CH_2-Ph), 40.4 [3′-$N(CH_3)_2$], 39.4 (C-7), 37.8 (C-4), 35.4 (C-8), 28.7 (C-4′), 22.0 (C-14), 10.4 (14- CH_3). IR ν_{max} (KBr) cm^{-1} 3417, 3380, 2975, 2939, 1813, 1750, 1669, 1524, 1526, 1458, 1348, 1167, 1076, 1046.

ES^+-MS *m/z* 794.3 $(M+H)^+$

By using this procedure (2′-*O*-acetylation, 3-*O*-acylation with the corresponding carboxylic acid and pivaloyl chloride and 2′-deacetylation), compounds **14**～**16** and **18**～**32** were prepared.

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-nitrophenyl)acetyl-8a-aza-8a-homoerythromycin A 11,12-cyclic Carbonate (**14**)

Reaction of **10** with 4-nitrophenylacetic acid gave the title compound in 75.3% yield.

^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5 (C-1), 170.8 (C-9), 169.8 (3-OCO), 153.1 (C=O carbonate), 147.2, 141.2, 130.5, 123.7 (Ph), 103.9 (C-1′), 88.9 (C-12), 82.4 (C-5), 82.0 (C-11), 79.6 (C-6), 76.7 (C-3), 76.0 (C-13), 70.3 (C-2′), 69.6 (C-5′), 66.9 (C-3′), 50.7 (6-OCH_3), 43.8 (C-2), 43.1 (C-10), 43.0 (C-8), 41.7 (C-7), 41.2 (CH_2-Ph), 40.3 [3′-$N(CH_3)_2$], 38.4 (C-4), 28.3 (C-4′), 22.3 (C-14), 10.2 (14-CH_3).

ES^+-MS *m/z* 794.4 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-chlorophenyl)acetyl-8a-aza-8a-homoerythromycin A 11,12-cyclic Carbonate (**15**)

Reaction of **10** with 4-chlorophenylacetic acid gave the title compound in 68.5% yield.

^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5 (C-1), 171.1 (3-OCO), 170.6 (C-9), 153.2 (C=O carbonate), 133.2, 132.3, 131.0, 128.8 (Ph), 102.7 (C-1′), 86.3 (C-12), 82.3 (C-5), 82.3 (C-11), 78.6 (C-6), 77.3 (C-3), 76.1 (C-13), 69.8 (C-2′), 68.4 (C-5′), 66.4 (C-3′), 50.7 (6-OCH_3), 43.7 (C-2), 42.8 (C-10), 42.8 (C-8), 41.8 (C-7), 40.8 (CH_2-Ph), 40.5 [3′-$N(CH_3)_2$], 38.1 (C-4), 30.8 (C-4′), 22.3 (C-14), 10.2 (14-CH_3). IR ν_{max} (KBr) cm^{-1} 3388, 2976, 2941, 2883, 2787, 1812, 1744, 1667, 1541, 1493, 1458, 1380, 1357, 1332, 1234, 1165, 1111, 1051, 1017, 981.

ES^+-MS *m/z* 783.3 $(M+H)^+$

^{13}C NMR data for the compounds **16**～**28** are presented in the Table 1.

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-phenylbutyryl)-8a-aza-8a-homoerythromycin A (**16**)

Reaction of **4a** with 4-phenylbutyric acid gave the title compound in 25.0% yield.

ES^+-MS *m/z* 751.4 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-nitrophenylcarbamoyl)-8a-aza-8a-homoerythromycin A (**17**)

To a solution of 2′-acetyl protected **4a** (90 mg, 0.14 mmol) in DMF (0.5 ml) and toluene (0.5 ml) 4-aminophenyl isocyanate (70 mg, 4.2 mmol) was added. The solution was stirred under argon at 50°C for 4.5 hours and then at room temperature overnight. Water (10 ml) was added and the product extracted with CH_2Cl_2 (2×30 ml). Combined organic extracts were washed with brine (15 ml), dried over K_2CO_3 and evaporated to dryness. The residue was purified by column chromatography (EtOAc - hexane - Et_2N, 60 : 30 : 0.2) yielding 95 mg of 2′-acetyl protected **17** that after deprotection in MeOH (15 ml) at room temperature overnight gave the title compound (81 mg, 71.2%).

ES^+-MS *m/z* 769.5 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-pyridin-3-yl-imidazol-1-yl)acetyl-8a-aza-8a-homoerythromycin A (**18**)

Reaction of **4a** with (4-pyridin-3-yl-imidazol-1-yl)acetic acid gave the title compound in 45.1% yield.

ES^+-MS *m/z* 790.4 $(M+H)^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(pyridin-4yl-sulfanyl)-acetyl-8a-aza-8a-homo-erythromycin A (**19**)
Reaction of **4a** with (pyridin-4-yl)thioacetic acid gave the title compound in 30.0% yield.
ES$^+$-MS *m*/*z* 756.5 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(naphthalene-1-yl)-acetyl-8a-aza-8a-homo-erythromycin A (**20**)
Reaction of **4a** with (naphthalen-1-yl)acetic acid gave the title compound in 57.6% yield.
ES$^+$-MS *m*/*z* 773.6 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-nitrophenyl)acryloyl-8a-aza-8a-homo-erythromycin A (**21**)
Reaction of **4a** with 3-(4-nitrophenyl)acrylic acid gave the title compound in 22.0% yield.
ES$^+$-MS *m*/*z* 780.6 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(pyridin-3-yl)acetyl-8a-aza-8a-homoerythromycin A (**22**)
Reaction of **4a** with (pyridin-3-yl)acetic acid gave the title compound in 66.4% yield.
ES$^+$-MS *m*/*z* 724.6 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(pyridin-4-yl)acetyl-8a-aza-8a-homoerythromycin A (**23**)
Reaction of **4a** with (pyridin-4-yl)acetic acid gave the title compound in 60.4% yield.
ES$^+$-MS *m*/*z* 724.5 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-nitrophenyl)acetyl-8a-aza-8a-homoerythromycin A (**24**)
Reaction of **4a** with 4-nitrophenylacetic acid gave the title compound in 47.8% yield.
ES$^+$-MS *m*/*z* 768.4 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-[(5-quinolin-3-yl)pent-3-enoyl]-8a-aza-8a-homo-erythromycin A (**25**)
Reaction of **4a** with (5-quinolin-3-yl)pent-3-enoic acid gave the title compound in 60.1% yield.
ES$^+$-MS *m*/*z* 814.7 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-methoxyphenyl)-acetyl-8a-aza-8a-homo-erythromycin A (**26**)
Reaction of **4a** with 4-methoxyphenylacetic acid gave the title compound in 51.4% yield.
ES$^+$-MS *m*/*z* 753.7 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(3-phenylpropionyl)-8a-aza-8a-homoerythromycin A (**27**)
Reaction of **4a** with 3-phenylpropionic acid gave the title compound in 61.5% yield.
ES$^+$-MS *m*/*z* 737.5 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-phenylacetyl-8a-aza-8a-homoerythromycin A (**28**)
Reaction of **4a** with phenylacetic acid gave the title compound in 46.6% yield.
ES$^+$-MS *m*/*z* 723.4 (M+H)$^+$

6-*O*-Methyl-3-*O*-decladinosyl-3-*O*-(4-nitrophenyl)acetyl-9a-aza-9a-homoerythromycin A (**29**)
Reaction of **3a** with 4-nitrophenylacetic acid gave the title compound in 80.1% yield.
IR ν_{max} (KBr) cm^{-1} 3396, 2976, 2941, 2879, 1732, 1698, 1669, 1601, 1521, 1456, 1380, 1346, 1232, 1182, 1111, 1073, 1051, 983.
ES$^+$-MS *m*/*z* 768.4 (M+H)$^+$

6-*O*-Ethyl-3-*O*-decladinosyl-3-*O*-(4-nitrophenyl)acetyl-9a-aza-9a-homoerythromycin A (**30**)
Reaction of **3b** with 4-nitrophenylacetic acid gave the title compound in 63.3% yield.
^{13}C NMR (125 MHz, $CDCl_3$) δ 177.4 (C-1), 170.2 (3-OCO), 170.0 (C-9), 147.0, 140.8, 130.5, 123.8 (Ph), 103.7 (C-1′), 81.8 (C-5), 80.1 (C-13), 80.0 (C-6), 78.8 (C-3), 74.4 (C-11), 74.1 (C-12), 70.5 (C-2′), 69.6 (C-5′), 66.1 (C-3′), 58.3 (6-O$\underline{CH_2}CH_3$), 45.8 (C-10), 42.7 (C-2), 41.4 (CH_2-Ph), 41.2 (C-7), 40.4 [3′-N$(CH_3)_2$], 36.5 (C-4), 33.2 (C-8), 28.8 (C-4′), 15.8 (6-OCH$_2\underline{CH_3}$), 20.9 (C-14), 11.2 (14-CH_3).
ES$^+$-MS *m*/*z* 782.5 (M+H)$^+$

6-*O*-Ethyl-3-*O*-decladinosyl-3-*O*-(4-nitrophenyl)acetyl-8a-aza-8a-homoerythromycin A (**31**)
Reaction of **4b** with 4-nitrophenylacetic acid gave the title compound in 39.7% yield.
^{13}C NMR (125 MHz, $CDCl_3$) δ 175.6 (C-9), 174.0 (C-1), 169.6 (3-OCO), 147.3, 141.2, 130.4, 123.8 (Ph), 104.1 (C-1′), 83.9 (C-5), 78.4 (C-6), 77.8 (C-3), 77.8 (C-13), 74.6 (C-12), 71.0 (C-11), 70.5 (C-2′), 69.8 (C-5′), 65.9 (C-3′), 58.4 (6-O$\underline{CH_2}CH_3$), 42.8 (C-2), 42.4 (C-10), 41.8 (C-7), 41.6 (C-8, CH_2-Ph), 40.4 [3′-N$(CH_3)_2$], 37.6 (C-4), 28.6 (C-4′), 15.8 (6-OCH$_2\underline{CH_3}$), 21.3 (C-14), 10.4 (14-CH_3).
ES$^+$-MS *m*/*z* 782.5 (M+H)$^+$

3-*O*-Decladinosyl-3-*O*-(4-nitrophenyl)acetyl-8a-aza-8a-homo-erythromycin A (**32**)
Hydrolysis of 8a-aza-8a-homoerythromycin [18], according to the procedure for preparation of **3a**, resulted in 3-*O*-decladinosyl-8a-aza-8a-homoerythromycin [19] which reacted with 4-nitrophenyl acetic acid to give the title

compound in 80.3% yield.

^{13}C NMR (125 MHz, $CDCl_3$) δ 175.7 (C-9), 175.1 (C-1), 169.5 (3-OCO), 147.2, 141.4, 130.6, 123.7 (Ph), 104.2 (C-1′), 87.4 (C-5), 79.3 (C-3), 77.8 (C-13), 75.1 (C-12), 74.0 (C-6), 70.6 (C-11), 70.4 (C-2′), 69.6 (C-5′), 65.2 (C-3′), 43.7 (C-2), 42.3 (C-10), 42.2 (C-7), 41.3 (C-8, $\underline{CH_2}$-Ph), 40.4 [3′-N$(CH_3)_2$], 37.9 (C-4), 28.3 (C-4′), 21.4 (C-14), 11.0 (14- CH_3).

ES$^+$-MS *m*/*z* 754.4 $(M+H)^+$

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