ORIGINAL ARTICLE

Synthesis and biological properties of 4''-O-acyl derivatives of 8a-Aza-8a-homoerythromycin

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A series of 4''-O-acyl derivatives of 8a-aza-8a-homoerythromycins A were synthesized and tested against Gram-positive and Gram-negative bacteria. Derivatives of 8a-aza-8a-homoerythromycin A have potent anti-bacterial activity against not only azithromycin-susceptible strains, but also efflux (M) and inducible macrolide–lincosamide–streptogramin-resistant Gram-positive pathogens. These compounds show moderate to high clearance and low oral bioavailability in preliminary *in vivo* pharmacokinetic studies in rat.

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INTRODUCTION

Second-generation macrolide anti-biotics, such as clarithromycin¹ (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 anti-bacterial activity, pharmacokinetic properties and fewer gastrointestinal side effects as compared with erythromycin A. However, the therapeutic utility of these macrolides has been severely compromised by the emergence of resistant pathogens.⁴

We recently reported on the synthesis of 3-keto and 3-O-acyl derivatives of 9a- and 8a-aza-8a-homoerythromycins A having anti-bacterial activity against sensitive pathogens as well as against macrolide-resistant Gram-positive pathogens.⁵

In this report we describe a novel series of 4''-O-acyl derivatives of 8a-aza-8a-homoerythromycin A (8a-lactams), having (hetero)arylalkyl side chain on 4''-O atom, which showed potent anti-bacterial properties against sensitive pathogens and improved activity against several species of efflux (M) and inducible (iMLS_B) macrolide-resistant Gram-positive pathogens.

Chemistry

In an attempt to define the structure-activity relationship, 4''-O-acyl derivatives of 8a-lactams have been prepared by modifying several structural units in the molecule. Thus, the length of a linker connecting macrolide and aromatic unit varied from one to five carbon atoms. Unsaturated and saturated analogs were synthesized. Furthermore, 4-nitrophenyl, pyridyl or quinolyl groups were introduced as aromatic substituents, whereas the macrolide moiety had either a free hydroxyl or a free C₁–C₃-alkoxy group at position C-6.

The synthetic route to the derivatives is presented in Scheme 1. Starting compounds for the synthesis were 6-O-alkyl-8a-aza-8a-homoerythromycins A⁵ (1–5) having acetyl as 2'-hydroxy-protecting group. Compounds 6, 13–18, 22, 24, 27 and 29 were prepared by condensation of 2'-protected compounds 1–5 with the corresponding (hetero)arylalkyl-carboxylic acid using 1-[(3-(dimethyl-amino)propyl]-3-ethyl-carbodiimide hydrochloride (EDC) in the presence of 4-(dimethyl-amino)pyridine (DMAP) in dichloromethane. To synthesize compounds 7–9, 11, 23, 25, 26 and 28 mixed anhydrides, prepared from the corresponding arylalkyl-carboxylic acid and pivaloyl chloride, were used instead. Subsequent deprotection by stirring overnight in methanol and purification by column chromatography gave the desired 4''-O-acyl derivatives.

Compounds 10, 12 and 19–21 were obtained by catalytic hydrogenation of the corresponding compounds having a double bond in the linker. Thus, compound 10 was prepared from 9, compounds 19 and 20 from 16, compound 12 from 11 and compound 21 from 15. Although the reaction conditions for compounds 11 and 9 were mild, in addition to reduction of the double bond in the linker, reduction of *p*-nitro to the *p*-amino group occurred. Using more vigorous conditions, the quinoline in 15 or 16 can also be reduced to the 1,2,3,4-tetrahydroquinolines 20 and 21.

Previous reports⁶ indicated that conversion of the macrolide 11,12-diol to the corresponding cyclic carbonate may enhance the anti-bacterial activity. The 11,12-carbonate 5 was synthesized by treating the 6-O-methyl-8a-aza-8a-homoerythromycin A with ethylene carbonate.⁷ Acylation of 5 in the presence of EDC under condition described above provided 4''-O-acyl-11,12-carbonate 29.

The mass spectra for all new compounds were in agreement with the proposed structure. These results were further confirmed by the

Scheme 1.

NMR spectra of all compounds, which showed two sets of signals, arising from the presence of macrolide and aromatic moieties, respectively, as well as signals reflecting the number of methylene groups in the linker.

In the ^{13}C NMR spectrum, the newly formed ester carbonyl resonates around 170 p.p.m. The significant deshielding of the 4''-H signal in the ^1H NMR, together with its long-range coupling to the new ester carbonyl signal (HSQC), provided evidence that the esterification occurred at the 4''-OH group of the macrolide scaffold.

The *E*-configuration of the double bond was assigned on the basis of the vicinal coupling constant between the olefinic proton pair (for example, $^3J = 16.05$ Hz in **16**).⁸

Hydrogenation of the double bond in the linker was also confirmed by NMR spectroscopy. For example, in the ^{13}C NMR spectrum of compound **19**, two signals corresponding to the olefinic carbon of the double bond have disappeared and two new methylene signals appeared at δ 35.3 and 28.1 p.p.m. These signals correlated with the proton signals at δ 2.89, 2.66 and 3.18 p.p.m. in the ^1H - ^{13}C COSY spectra.

RESULTS AND DISCUSSION

In vitro anti-bacterial activity of all compounds was determined against a panel of diverse bacterial strains, covering most important Gram-positive (that is, *Streptococcus pneumoniae*, *S. pyogenes*) and Gram-negative (that is, *Haemophilus influenzae*, *Moraxella catarrhalis*) respiratory tract pathogens and different mechanisms of macrolide 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 transport

macrolide anti-biotics out of 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 anti-biotics. This phenotype is either inducible (giving iMLS phenotype) or constitutive (cMLS).

In all *in vitro* experiments, in addition to starting compounds, azithromycin was used as the reference compound, as it is the 'Gold standard' in macrolide therapy.

Anti-bacterial activities are reported in Tables 1 and 2 as minimum inhibitory concentrations (MIC, in $\mu\text{g ml}^{-1}$).

In vitro evaluation

Comparing 4-substituted phenyl derivatives (**6–12**) with 4''-O-non-functionalized compounds (**1–4**), an encouraging increase was observed in the level of activity against macrolide-resistant efflux mutants and *E. faecalis*, especially analogs **9** and **11**. However, this was offset by a significant decrease in potency against the Gram-negative organisms *M. catarrhalis* and *H. influenzae*. There appears to be little or no effect on the potency against erythromycin-sensitive strains or constitutively resistant *S. pneumoniae*. The effect of 6-substituent is marginal, although the 6-O-methyl scaffold tends to have a better overall spectrum of activity and potency.

Reduction of the linker double bond and concomitant reduction of the nitro to an amino group (**10**, **12**) diminished potency of compounds compared with their counterparts with unsaturated linker (**9**, **11**), but they are still more active than the starting compounds (**1**, **2**).

Replacement of the nitrophenyl ring by pyridine (**13**) markedly improved the activity against *Streptococcus* efflux mutants (M) but showed no improvement against *Staphylococcus* organisms. Potency

against other strains in the assay is broadly similar in both compounds.

Quinolyl derivatives **14–29** retained similar potency to the nitrophenyl substituent against erythromycin-sensitive strains but showed considerable improvement against the efflux strains (**15** vs **9** and **16** vs **11**). The improvement was most marked in the 6-hydroxy scaffold (**15** vs **9**), where there was also an increase in potency against the inducibly resistant *Streptococcus* strain.

However, activity against the Gram-negative organisms remained weak. Increasing the size of the substituent on 6-hydroxyl was detrimental to potency on the resistant, but not on the sensitive strains (H > Me > Et = Allyl).

Saturation of the linker double bond (**19** vs **16**) reduces potency against the resistant mutants, as does saturation of hetrocyclic ring (**20**, **21**). There is a minimal difference between 6-hydroxy and 6-methoxy substituents for these changes.

Table 1 Anti-bacterial activity of 4''-O-acyl-6-O-alkyl-8a-aza-8a-homoerythromycins (MIC, $\mu\text{g ml}^{-1}$)

Strain Phenotype	Compound	<i>Staphylococcus aureus</i> ; ATCC13709 eryS	<i>Streptococcus pneumoniae</i> eryS	<i>Streptococcus pyogenes</i> eryS	<i>Staphylococcus aureus</i> M	<i>Streptococcus pneumoniae</i> M	<i>Streptococcus pyogenes</i> M	<i>Staphylococcus aureus</i> iMLS	<i>Streptococcus pyogenes</i> iMLS	<i>Streptococcus pneumoniae</i> cMLS	<i>Moraxella catarrhalis</i> ; ATCC23246	<i>Haemophilus influenzae</i> ; ATCC49247	<i>Enterococcus faecalis</i> ; ATCC29212
	Azithromycin	0.5	<0.13	<0.13	>64	8	4	>64	8	>64	<0.13	1	8
1	H, H	1	<0.13	<0.13	>64	32	32	>64	16	>64	0.25	2	8
2	Me, H	0.5	<0.13	<0.13	>64	16	16	>64	8	>64	<0.13	4	4
3	Et, H	0.25	<0.13	<0.13	>64	32	16	>64	16	>64	<0.13	1	8
4	Allyl, H	1	<0.13	<0.13	64	32	16	>64	16	>64	<0.13	4	8
6	H,	8	<0.13	0.25	64	8	32	64	>64	>64	4	16	8
7	Me,	4	<0.13	<0.13	16	2	32	>64	16	64	8	8	4
8	Et,	2	<0.13	<0.13	8	4	16	>64	16	>64	4	4	4
9	H,	0.5	<0.13	<0.13	8	0.25	2	1	4	>64	8	8	<0.13
10	H,	4	<0.13	0.25	32	4	4	>64	8	>64	4	16	2
11	Me,	1	<0.13	<0.13	1	<0.13	2	16	8	64	8	32	<0.13
12	Me,	4	0.5	1	16	2	4	64	16	>64	8	8	8
13	Me,	8	<0.13	<0.13	>64	0.5	0.5	>64	64	>64	2	16	32
14	Me,	1	<0.13	<0.13	4	4	32	>64	8	>64	8	8	2

Table 1 Continued

15	H,		0.5	<0.13	<0.13	0.5	<0.13	0.5	1	0.25	>64	4	8	<0.13
16	Me,		1	<0.13	<0.13	2	<0.13	<0.13	8	2	64	8	16	0.5
17	Et,		2	<0.13	0.25	8	4	8	>64	32	>64	2	8	4
18	Allyl,		1	<0.13	<0.13	8	16	16	16	4	32	4	16	4
19	Me,		2	<0.13	0.5	4	4	64	>64	16	>64	8	16	4
20	Me,		1	<0.13	<0.13	4	<0.13	8	64	8	64	8	16	0.5
21	H,		1	<0.13	<0.13	2	0.5	32	64	4	>64	8	8	2
22	Me,		2	<0.13	<0.13	16	0.25	<0.13	64	4	16	8	8	2
23	Me,		1	<0.13	<0.13	4	<0.13	0.25	>64	2	16	2	8	2
24	Me,		2	<0.13	<0.13	4	<0.13	0.5	8	0.5	16	4	4	0.25
25	H,		<0.13	<0.13	<0.13	1	<0.13	2	>64	8	64	2	-	1
26	Me,		1	<0.13	<0.13	8	0.25	<0.13	8	8	16	8	4	1
27	Me,		4	<0.13	0.25	8	2	8	>64	8	>64	8	2	2
28	Me,		2	<0.13	<0.13	8	4	8	>64	16	>64	4	2	2

In vitro susceptibility testing was carried out by standard microdilution broth assay as recommended by NCCLS M7-A5 (2001).

The compound with a quinolyl group attached directly to the 4''-hydroxyl through carbonyl (**14**) had lower potency than corresponding compounds attached by longer linkers.

Extension of the linker by one carbon (**23**, **24** vs **16**) was detrimental to the level of activity against inducible organisms. However, some of the potency can be regained by saturation of linker double bond (**24** vs **23**). In both compounds, there is the first indication of activity against the constitutively resistant *S. pneumoniae*. Extension of the linker by two carbons, in the case of compounds **25** vs **15**, decreased activity against inducible strains. Movement of the double bond out of conjugation with the quinoline (**27** vs **26**) or reduction of the double bond (**28** vs **26**) was detrimental to potency in these examples.

Attachment of the linker on the 4-position of the quinoline (**22**), compared with the position 3 (**16**), retained activity against all

sensitive and efflux *Streptococcus* strains, but was weaker against inducible organisms.

Introduction of a 11,12-cyclic carbonate group (**29**) reduced overall activity compared with the 11,12-diol derivative (**16**). A similar slight diminution in overall profile for the 11,12-carbonate was also observed in the case of the 4''-hydroxy analog **5** vs **2**.

In vivo evaluation

Pharmacokinetic studies were carried out in rats with several compounds, chosen based on specific substituents on the macrolide moiety in position 6-O and position 4''-O. Compound **2** was screened as a representative of the 4''-hydroxy 8a-aza-8a-homoerythromycin A analogs, and compounds **6**, **7**, **9**, **11** (as diacetate salts), **15** and **16** where selected as analogs with similar substituents in either the 6-O and/or 4''-O positions. Summarized

Table 2 Anti-bacterial activity of 4''-O-acyl-6-O-alkyl-8a-aza-8a-homoerythromycins 11,12-cyclic carbonate derivatives (MIC, $\mu\text{g ml}^{-1}$)

	Strain Phenotype	Compound	R¹,	R²	<i>Staphylococcus aureus</i> ; ATCC13709 eryS	<i>Streptococcus pneumoniae</i> eryS	<i>Streptococcus pyogenes</i> eryS	<i>Staphylococcus aureus</i> M	<i>Streptococcus pneumoniae</i> M	<i>Streptococcus pyogenes</i> M	<i>Staphylococcus aureus</i> iMLS	<i>Streptococcus pyogenes</i> iMLS	<i>Streptococcus pneumoniae</i> cMLS	<i>Moraxella catarrhalis</i> ; ATCC23246	<i>Haemophilus influenzae</i> ; ATCC49247	<i>Enterococcus faecalis</i> ; ATCC29212
5			Me,	H	2	<0.13	0.25	64	16	16	>64	4	>64	0.25	8	4
29			Me,		8	<0.13	0.25	16	16	16	32	32	>64	8	8	4

Table 3 Summarized pharmacokinetic parameters after ¹intravenous and oral administration to rats, expressed as mean $\pm$ s.d.

Compound no.		2	6	7	9	11	15	16
Target dose intravenously	mg kg ⁻¹	10	5.7	10	10	10	10	10
n ^a		4	3	5	3	5	5	5
¹ CL	ml min ⁻¹ kg ⁻¹	48.0 $\pm$ 11.7	87.6 $\pm$ 34.2	25.3 $\pm$ 7.0	121.0 $\pm$ 86.0	143.8 $\pm$ 22.0	140 $\pm$ 60.0	59.7 $\pm$ 37.2
¹ Vss	l kg ⁻¹	3.6 $\pm$ 1.0	2.8 $\pm$ 1.7	4.1 $\pm$ 1.5	53.8 $\pm$ 36.6	47.3 $\pm$ 11.8	41.4 $\pm$ 27.2	10.3 $\pm$ 8.1
¹ t _{1/2}	Hr	1.5 $\pm$ 0.1	1.2 $\pm$ 0.4	3.8 $\pm$ 0.8	13.4 $\pm$ 11.9	3.0 $\pm$ 0.19	6.7 $\pm$ 3.6	3.8 $\pm$ 1.7
Oral F ^b	%	<1	<1	27.2 $\pm$ 11.7	<1	9.4 $\pm$ 2.3	3.6 $\pm$ 3.3	12.0 $\pm$ 7.9

^an=number of rats included to express mean $\pm$ s.d. values.^bOral F determined based on area under the curve (0–t); compounds administered as salts at target doses of 30 mg kg⁻¹ orally.

pharmacokinetic parameters after intravenous and oral dosing are presented in Table 3.

Compound **2**, lacking the substitution on position 4''-O, has a moderate systemic clearance (Cl_s) of approximately 56% of liver blood flow (LBF), moderate volume of distribution (V_{ss}) (3.6 l kg⁻¹), short half-life (1.5 h) and oral bioavailability of less than 1%. The lack of systemic availability is most likely due to extrahepatic extraction and/or poor gastrointestinal absorption.

Introduction of a (4-nitrophenyl)acetyl substituent on position 4''-O (compound **6**) resulted in a substantial increase in Cl_s ($\approx$ LBF), a moderate volume of distribution and no improvement in oral bioavailability (<1%). However, methylation of the same analog at position 6-O (compound **7**) gave a lower Cl_s ($\approx$ 30% LBF), moderate V_{ss}, longer t_{1/2} (3.3 h) and variable, but substantial increase in oral bioavailability (27%). Lengthening the linker by introducing a double bond between the carbonyl and aromatic moiety (3-(4-nitrophenyl)-2-propenoyl) (compounds **9** and **11**) increased the Cl_s (> LBF) in both cases, with compound **11** having a variable, but higher oral bioavailability in comparison with compound **9** ($\approx$ 9 vs <1%). This low bioavailability is most likely due to extensive extrahepatic clearance, in addition to limited absorption. Both compounds had a several fold higher volume of distribution, in comparison with compounds **6** and **7**, and methylation at position 6-O did not improve the oral bioavailability of compound **11** to the extent that was observed in the previous case.

Replacement of the nitrophenyl moiety with a 3-quinolinyl substituent (compounds **15** (6-hydroxy) and **16** (6-methoxy)) resulted in a high Cl_s, exceeding the LBF in case of compound **15** and $\approx$ 70% of LBF with compound **16**. Both compounds had a large V_{ss}, moderate-long t_{1/2} and variable and low bioavailability (3.6 vs 12%, respectively). Yet again, methylation at position 6-O resulted in a slightly higher oral bioavailability. Both compounds showed a delayed absorption, with maximum levels at 8 (compound **15**) and 3 h (compound **16**) upon oral dosing.

In general, the analogs of 4''-O-acyl derivatives of 8a-aza-8a-homoerythromycins tested *in vivo* can be characterized by a moderate (**2**, **7**) to high Cl_s (**6**, **9**, **11**, **15**, **16**), exceeding the LBF in some cases (**9**, **11**, **15**), suggesting extrahepatic clearance. This is consistent with *in vitro* microsomal CL (in house data), which is low for compounds **7** (0.3 ml min⁻¹ g⁻¹) and **16** (0.8 ml min⁻¹ g⁻¹), and moderate for compound **11** (1.9 ml min⁻¹ g⁻¹) predicting low *in vivo* hepatic metabolism. These derivatives have a moderate (**2**, **6**, **7**) to very high (**9**, **11**, **15**, **16**) volume of distribution, as well as a moderate to long half-life. The oral bioavailability for these compounds is variable and low to moderate, ranging from <1 to 27%, most likely due to extrahepatic clearance, as well as limited absorption. Methylation at position 6-O increased the oral bioavailability in all three cases relative to the non-methylated analog, possibly due to improved absorption or decreased extrahepatic clearance.

Conclusions

A series of 4''-O-acyl derivatives of 8a-aza-8a-homoerythromycins A have been prepared by varying several structural units on the macrolide scaffold. Thus, 4-nitrophenyl, pyridyl or quinolyl groups were introduced as aromatic substituents linked by an ester bond at the 4''-position of the macrolide scaffold by a spacer of different length. Position 6 of the macrolide moiety was either a free hydroxyl or a C₁-C₃-alkoxy group.

The results indicate improvements in the *in vitro* anti-bacterial activity in comparison with 4''-unsubstituted compounds against efflux or inducibly resistant strains of Gram-positive organisms. Furthermore, activity against the constitutively resistant *S. pneumoniae* was observed.

The compounds evaluated *in vivo* show moderate to high clearances and low oral bioavailabilities in preliminary *in vivo* studies in rat, possibly most likely due to extrahepatic clearance as well as limited absorption.

The overall profile of the reported compounds justifies further structure-based optimization of this series to additionally increase potency against macrolide-resistant strains and to improve pharmacokinetic parameters.

EXPERIMENTAL SECTION

General

All commercial reagents (Merck, Hohenbrunn, Germany; Sigma-Aldrich, Steinheim, Germany) were used as provided unless otherwise indicated.

NMR spectra were recorded on a Bruker Avance DRX500 or Bruker Avance DPX300 spectrometer in CDCl₃ or dimethylsulfoxide (DMSO), and chemical shifts are reported in p.p.m. using tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a Waters Micromass ZQ mass spectrometer for ES⁺-MS. Electrospray-positive ion mass spectra were acquired using a Micromass Q-ToF2 hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray interface, over a mass range of 100–1500 Da, with a scan time of 1.5 s and an interscan delay of 0.1 s in a continuum mode. Reserpine was used as the external mass calibrant lock mass ([M+H]⁺=609.2812 Da). The elemental composition was calculated using a MassLynx v4.1 for the [M+H]⁺ and the mass error quoted within ± 5 p.p.m. range. In synthetic procedures, column chromatography was carried out over Merck Kieselgel 60 (230–400 mesh) and thin layer chromatography on 0.24 mm silica gel plates Merck TLC 60F₂₅₄. The eluent used was indicated and solvent ratios refer to volume.

In general, organic solutions were dried with anhydrous Na₂SO₄ or K₂CO₃, evaporation and concentration were carried out under reduced pressure below 40 °C, unless otherwise noted.

8a-Aza-8a-homoerythromycin A, 6-O-methyl-8a-aza-8a-homoerythromycin A, 6-O-ethyl-8a-aza-8a-homoerythromycin A and 6-O-allyl-8a-aza-8a-homoerythromycin A were prepared by Beckmann rearrangement of the corresponding oxime derivatives according to the published procedures.^{5,9} 2'-O-Acetyl-protected derivatives were prepared by the known procedure.¹⁰

Anti-bacterial 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 (Biomerieux, Crajonne, France) and chocolate agar plates (Biomerieux), respectively. MICs were determined by the microtitre liquid dilution method as described by NCCLS¹¹ except for *Streptococcus* medium, where blood was substituted by 5% horse serum.

In vivo pharmacokinetic studies

Male Wistar Han rats, weighing 300–350 g, were purchased from IFFA CREADO, Lyon, France. The rats were maintained in an air-conditioned animal quarter at a temperature of 22 $\pm$ 2 °C and a relative humidity of 50 $\pm$ 10%. Water and food (Laboratory Rodent Chow, Nanjing, China) were allowed *ad libitum*. Rats were divided into groups of five and the pharmaco-

kinetic study was performed in a crossover design including a 2-day washout period. Each compound was administered intravenously at 10 mg kg⁻¹, followed by a 2-day washout period and orally at 30 mg kg⁻¹ free base equivalent. Compound 6 (as acetate salt) was administered at an intravenous dose of 5.7 mg kg⁻¹. For intravenous dosing, compounds were formulated in 1% dimethyl formamide (DMF)/phosphate buffer, and blood samples (50 μ l) were collected at 0.17, 0.33, 0.67, 1, 2, 4, 6, 8, 12 and 24 h. For oral administration, compounds were dissolved in 1% DMF/20% Encapsin (w/v), and saline and blood samples (50 μ l) were collected at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h. Blood samples were collected serially from the tail vein, hemolyzed with deionized water in a 1:2 ratio and frozen at -20 °C until analysis.

Sample preparation and bioanalysis

Hemolyzed blood samples (150 μ l) in Eppendorf test tubes were treated by protein precipitation with the addition of two volumes of a mixture MeCN/MeOH (1:2), containing an internal standard. A 1 mg ml⁻¹ stock solution of each compound was prepared in DMSO, diluted in water and spiked into rat blank blood to prepare duplicate standards ranging from 5 to 25 000 ng ml⁻¹. One set of standards was analyzed at the beginning and one set at the end of each sample batch. Quality control samples (QCs) were prepared from separate stock solutions and analyzed at three concentrations (low, medium, high). The mixtures were centrifuged at 4000 r.p.m. at 4 °C for 10 min and aliquots (0.01 ml) of the resulting supernatant fractions were transferred to a 96-well plate. Samples were analyzed with either a Sciex API 3000 or Sciex API 2000 Triple Quadrupole Mass Spectrometer (Sciex, Division of MDS Inc., Toronto, Canada) coupled to an HP HPLC System (HP1100, Hewlett-Packard, Palo Alto, CA, USA) and an HTS PAL CTC Autosampler (CTC). Samples (5 μ l) were injected onto an HPLC column (3 μ m Phenomenex Luna C18(2), 2.0 $\times$ 50 mm) and eluted with a gradient at room temperature. The chromatographic conditions consisted of mobile phase A (1000:1 MeCN/formic acid, v/v) and mobile phase B (1000:1 water/formic acid, v/v) that was run over a 6 min gradient at a flow rate of 0.3 ml min⁻¹. A positive ion mode with turbo spray, an ion source temperature of 400–450 °C and a dwell time of 300–400 ms were utilized for mass spectrometric detection. Quantification was performed using multiple reaction monitoring (MRM) at the following transitions for each particular compound: 2 (*m/z* 763.8 to *m/z* 606.3), 15 (*m/z* 930.8 to *m/z* 591.2), 16 (*m/z* 945.3 to *m/z* 606.7), 6 (*m/z* 912.8 to *m/z* 591.7), 7 (*m/z* 926.8 to *m/z* 605.5), 9, 11 (*m/z* 938.6 to *m/z* 605.4) and internal standard (*m/z* 837.6 to *m/z* 158.4). Linear regression plots of compounds to internal standard peak area ratios versus drug concentrations were derived with 1/*x* or 1/*x*² weighting. The dynamic range for the blood assay ranged from 0.005 to 10 μ g ml⁻¹. Accuracy of the QCs used during the analysis was below 24% (judged by percent deviation from nominal value) for at least two of three concentrations, with a precision of $\leq$ 20% (as judged by relative standard deviation).

Pharmacokinetic analysis

Noncompartmental analysis for all compounds was performed using WinNonlin Professional, version 4.0.1 (Pharsight, Mountain View, CA, USA). Individual blood concentrations and sample times for each animal were used in the analysis. Following intravenous bolus administration, the terminal elimination half-life (*t*), total area under the curve, concentration-time curve extrapolated to infinity, systemic blood clearance and steady-state volume of distribution were calculated by standard methods. After oral administration, the peak plasma concentration (*C*_{max}) and the time to *C*_{max} (*T*_{max}) were taken directly from individual profiles and the areas under the curve and oral bioavailabilities were determined. Summarized pharmacokinetic parameters were reported as mean values $\pm$ s.d. both after intravenous and oral administration.

4''-O-[(4-nitrophenyl)acetyl]-8a-aza-8a-homoerythromycin A (6)

To a solution of 2'-O-acetyl-8a-aza-8a-homoerythromycin A (0.50 g, 0.63 mmol) in CH₂Cl₂ (25 ml) and DMF (5 ml), 4-(nitrophenyl)acetic acid (0.57 g, 3.15 mmol) was added, and the resulting white suspension was cooled to 0 °C under argon. EDC hydrochloride (0.50 g, 2.62 mmol) was added in one portion, followed by addition of DMAP (0.075 g, 0.62 mmol). The reaction mixture was stirred at room temperature for 5 h. The reaction was quenched by

addition of saturated aqueous NaHCO_3 solution (30 ml) and extracted with CH_2Cl_2 (3×20 ml) at pH 9.5. Combined organic extracts were washed with brine (20 ml), dried over K_2CO_3 and concentrated *in vacuo*. The brown residue was dissolved in MeOH (30 ml), stirred at room temperature overnight and concentrated to dryness. The residue was purified by flash column chromatography (eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1) to obtain the title compound **6** (88 mg, 15.3%).

MS (ES) m/z : $[\text{M}+\text{H}]^+$ 912.9.

HRMS (ES positive): calculated for $\text{C}_{45}\text{H}_{74}\text{N}_3\text{O}_{16}$ $[\text{M}+\text{H}]^+$ 912.5069 found 912.5060.

^1H NMR (500 MHz, CDCl_3) δ 8.20 (Ph-2H), 7.49 (Ph-2H), 5.94 (8a-NH), 5.13 (1''-H), 4.92 (13-H), 4.74 (4''-H), 4.57 (1'-H), 4.38 (5''-H), 4.33 (3-H), 4.20 (8-H), 4.78 (Ph-CH₂, 5'-H), 3.57 (5-H), 3.50 (11-H), 3.32 (2'-H), 3.31 (3''-OCH₃), 2.83 (3'-H), 2.64 (2-H), 2.52 [3'-N(CH₃)₂], 2.38 (2''a-H, 10-H), 1.94 (4-H), 1.90 (14a-H), 1.77 (4'a-H), 1.62 (2''b-H), 1.55 (7a-H), 1.45 (14b-H), 1.41 (7b-H), 1.39 (6-CH₃), 1.21 (8-CH₃, 5'-CH₃), 1.19 (10-CH₃), 1.18 (2-CH₃), 1.14 (5''-CH₃), 1.10 (12-CH₃), 1.05 (4-CH₃), 1.04 (3''-CH₃), 0.89 (14-CH₃).

^{13}C NMR (125 MHz, DMSO) δ 177.8 (C-1), 176.5 (C-9), 169.9 (4''-OCO), 147.3, 140.9, 130.3, 123.8 (Ph), 102.0 (C-1'), 94.6 (C-1''), 82.9 (C-5), 79.9 (C-4''), 77.2 (C-3), 76.9 (C-13), 74.8 (C-12), 73.9 (C-6), 73.2 (C-3''), 70.6 (C-2'), 70.1 (C-11), 67.7 (C-5'), 65.7 (C-3'), 62.6 (C-5''), 49.5 (3''-OCH₃), 45.5 (C-2), 42.9 (C-4), 42.3 (C-7), 41.9 (C-10), 40.7 (CH₂), 40.6 (C-8), 40.4 [3'-N(CH₃)₂], 34.9 (C-2''), 30.3 (C-4'), 21.7 (C-14), 11.3 (14-CH₃).

4'-O-[(4-nitrophenyl)acetyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (7)

To a solution of (4-nitrophenyl)acetic acid (0.25 g, 1.4 mmol) in CH_2Cl_2 (3 ml) triethylamine (TEA) was added (0.13 ml), and the resulting solution was cooled to 0 °C in an ice-bath under argon. Pivaloyl chloride (0.115 ml) was added, the resulting mixture was stirred for 30 min and then pyridine (0.3 ml) and 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.25 g, 0.31 mmol) were added. The reaction was stirred for 6 h and during that time it was allowed to warm to room temperature. The reaction was quenched with saturated aqueous NaHCO_3 solution (20 ml). CH_2Cl_2 (20 ml) was added and aqueous layer was extracted with CH_2Cl_2 (2×10 ml). Combined organic extracts were washed with brine (20 ml), dried over K_2CO_3 and concentrated *in vacuo*. The brown foamy residue was dissolved in MeOH (15 ml), stirred at room temperature overnight and concentrated to dryness. Column chromatography (eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ 90:5:0.5) afforded the title compound **7** (0.12 g, 41.8%) as a brown-yellow solid.

MS (ES) m/z : $[\text{M}+\text{H}]^+$ 926.9.

HRMS (ES positive): calculated for $\text{C}_{46}\text{H}_{76}\text{N}_3\text{O}_{16}$ $[\text{M}+\text{H}]^+$ 926.5226 found 926.5222.

^1H NMR (500 MHz, CDCl_3) δ 8.20 (Ph-H), 7.49 (Ph-H), 5.69 (8a-CONH), 5.07 (1''-H), 4.95 (13-H), 4.71 (4''-H), 4.55 (1'-H), 4.38 (5''-H), 4.17 (8-H), 3.99 (3-H), 3.83 (PhCH₂-1H), 3.74 (5'-H), 3.73 (PhCH₂-1H), 3.68 (5-H), 3.49 (11-H), 3.31 (3''-OCH₃), 3.28 (2'-H), 3.16 (6-OCH₃), 2.72 (3'-H), 2.70 (2-H), 2.48 [3'-N(CH₃)₂], 2.39 (2''a-H), 2.29 (10-H), 1.93 (14a-H), 1.93 (4-H), 1.91 (4'a-H), 1.66 (7a-H), 1.63 (2''b-H), 1.54 (7b-H), 1.46 (14b-H), 1.36 (6-CH₃), 1.31 (4'b-H), 1.24 (2-CH₃), 1.20 (5'-CH₃), 1.18 (10-CH₃, 5''-CH₃), 1.14 (8-CH₃), 1.12 (3''-CH₃), 1.06 (4-CH₃), 1.04 (12-CH₃), 0.90 (14-CH₃).

^{13}C NMR (125 MHz, DMSO) δ 177.2 (C-1), 174.4 (C-9), 169.9 (4''-OCO), 147.3, 140.9, 130.3, 123.8 (Ph), 102.3 (C-1'), 95.4 (C-1''), 80.3 (C-5), 79.8 (C-4''), 78.8 (C-6), 77.4 (C-3), 77.2 (C-13), 74.3 (C-12), 73.0 (C-3''), 70.8 (C-2'), 70.4 (C-11), 67.9 (C-5'), 65.5 (C-3'), 62.8 (C-5''), 51.8 (6-OCH₃), 49.5 (3''-OCH₃), 45.4 (C-2), 42.9 (C-7), 42.4 (C-4), 42.2 (C-10), 41.0 (C-8), 41.0 [3'-N(CH₃)₂], 40.8 (CH₂), 35.1 (C-2''), 21.6 (C-14), 11.2 (14-CH₃).

4'-O-[(4-nitrophenyl)acetyl]-6-O-ethyl-8a-aza-8a-homoerythromycin A (8)

A solution of (4-nitrophenyl)acetic acid (0.110 g, 0.61 mmol) and TEA (0.086 ml, 0.63 mmol) in CH_2Cl_2 (5 ml) was cooled to 0 °C under nitrogen. Pivaloyl chloride (0.075 ml, 0.62 mmol) was added and the reaction mixture was stirred for 0.5 h. To a solution of 2'-O-acetyl-6-O-ethyl-8a-aza-8a-homoerythromycin A (0.100 g, 0.12 mmol) in CH_2Cl_2 (4 ml), pyridine (0.108 ml,

1.34 mmol) and DMAP (0.015 g, 0.12 mmol) were added. The reaction mixture was allowed to slowly warm to room temperature and was stirred for 20 h, then additional amounts of (4-nitrophenyl)acetic acid (0.110 g, 0.61 mmol), TEA (0.086 ml, 0.63 mmol) and pivaloyl chloride (0.075 ml, 0.62 mmol) were added and the mixture was stirred for additional 20 h. The reaction was quenched by addition of saturated aqueous NaHCO_3 solution and extracted with CH_2Cl_2 . Combined organic extracts were washed with brine and dried over K_2CO_3 . The solution was concentrated to form a solid that after deprotection in MeOH at room temperature for 48 h gave the crude product. After chromatography on a silica gel column (eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ 90:5:0.5), the title compound **8** was obtained (0.055 g, 48.7%).

MS (ES) m/z : $[\text{M}+\text{H}]^+$ 940.4.

HRMS (ES positive): calculated for $\text{C}_{47}\text{H}_{78}\text{N}_3\text{O}_{16}$ $[\text{M}+\text{H}]^+$ 940.5382 found 940.5355.

^1H NMR (500 MHz, CDCl_3) δ 8.20 (Ph-2H), 7.51 (Ph-2H), 6.93 (8a-NH), 5.01 (13-H), 4.91 (1''-H), 4.70 (4''-H), 4.44 (1'-H), 4.38 (5''-H), 3.93 (8-H), 3.90 (3-H), 3.82 (Ph-CH₂-), 3.64 (5-H), 3.52 (5'-H), 3.50 (11-H), 3.43 (6-OCH₂-), 3.30 (3''-OCH₃), 3.25 (2'-H), 2.79 (2-H), 2.68 (3'-H), 2.44 [3'-N(CH₃)₂], 2.40 (2''a-H), 2.35 (10-H), 2.16 (7a-H), 2.10 (4-H), 1.93 (14a-H), 1.86 (4'a-H), 1.65 (7b-H), 1.62 (2''b-H), 1.49 (14b-H), 1.27 (4'b-H), 0.85 (14-CH₃).

^{13}C NMR (125 MHz, DMSO) δ 175.3 (C-9), 174.7 (C-1), 170.0 (4''-OCO), 147.3, 141.0, 130.4, 123.8 (Ph), 103.2 (C-1'), 97.6 (C-1''), 82.6 (C-5), 82.1 (C-3), 79.7 (C-4''), 78.9 (C-6), 77.5 (C-13), 74.1 (C-12), 73.1 (C-3''), 71.1 (C-11), 70.9 (C-2'), 68.7 (C-5'), 65.5 (C-3'), 62.8 (C-5''), 58.9 (6-OCH₂-CH₃), 49.5 (3''-OCH₃), 45.4 (C-2), 42.4 (C-7), 41.9 (C-8, C-10), 40.9 (CH₂), 40.6 [3'-N(CH₃)₂], 40.4 (C-4), 35.9 (C-2''), 29.7 (C-4'), 21.4 (C-14), 10.8 (14-CH₃).

4'-O-[3-(4-nitrophenyl)-2-propenoyl]-8a-aza-8a-homoerythromycin A (9)

The same method was followed as for the synthesis of compound **8** but starting from 2'-O-acetyl-8a-aza-8a-homoerythromycin A (0.5 g, 0.63 mmol) and 4-nitrocinnamic acid (0.61 g, 3.2 mmol) to obtain the title compound **9** (0.190 g, 32.6%) as a white foam.

MS (ES) m/z : $[\text{M}+\text{H}]^+$ 924.4.

HRMS (ES positive): calculated for $\text{C}_{46}\text{H}_{74}\text{N}_3\text{O}_{16}$ $[\text{M}+\text{H}]^+$ 924.5069 found 924.5034.

^1H NMR (500 MHz, CDCl_3) δ 8.27 (Ph-2H), 7.79 (Ph-CH=CH), 7.70 (Ph-2H), 6.59 (Ph-CH=CH), 5.83 (8a-NH), 5.17 (1''-H), 4.94 (13-H), 4.84 (4''-H), 4.58 (1'-H), 4.42 (5''-H), 4.35 (3-H), 4.20 (8-H), 3.82 (5'-H), 3.58 (5-H), 3.52 (11-H), 3.36 (3''-OCH₃, 2'-H), 3.03 (3'-H), 2.65 (2-H), 2.58 [3'-N(CH₃)₂], 2.43 (2''a-H), 2.37 (10-H), 1.97 (4'a-H), 1.94 (14a-H), 1.91 (4-H), 1.68 (2''b-H), 1.54 (7-2H), 1.45 (14b-H), 1.40 (6-CH₃), 0.90 (14-CH₃).

^{13}C NMR (125 MHz, DMSO) δ 177.8 (C-1), 176.4 (C-9), 165.8 (4''-OCO), 148.7, 140.4, 128.6, 124.3 (Ph), 142.7, 121.9 (CH=CH), 102.6 (C-1'), 94.8 (C-1''), 82.6 (C-5), 79.6 (C-4''), 76.9 (C-3, C-13), 74.9 (C-12), 74.1 (C-6), 73.3 (C-3''), 70.8 (C-2'), 70.2 (C-11), 68.0 (C-5'), 65.5 (C-3'), 62.9 (C-5''), 49.6 (3''-OCH₃), 45.7 (C-2), 43.1 (C-4), 42.5 (C-7), 42.0 (C-10), 40.8 (C-8), 40.2 [3'-N(CH₃)₂], 35.1 (C-2''), 29.5 (C-4'), 21.8 (C-14), 11.3 (14-CH₃).

4'-O-[3-(4-aminophenyl)propanoyl]-8a-aza-8a-homoerythromycin A (10)

Compound **9** (0.17 g, 0.18 mmol) was dissolved in MeOH (9 ml), 10% Pd/C catalyst (0.017 g) was added and the reaction mixture was stirred at room temperature under H_2 (balloon) for 3 h. The mixture was filtered and MeOH was evaporated under reduced pressure yielding the title compound (0.14 g, 87%) as white foam.

After chromatography on a silica gel column (eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ 90:9:0.5) and precipitation from EtOAc/n-hexane, the title compound **10** was obtained (0.078 g, 55.7%) as a white powder.

MS (ES) m/z : $[\text{M}+\text{H}]^+$ 896.4.

HRMS (ES positive): calculated for $\text{C}_{46}\text{H}_{78}\text{N}_3\text{O}_{14}$ $[\text{M}+\text{H}]^+$ 896.5484 found 896.5487.

^1H NMR (500 MHz, CDCl_3) δ 6.97 (Ph-2H), 6.61 (Ph-2H), 6.23 (8a-NH), 5.13 (1''-H), 4.92 (13-H), 4.68 (4''-H), 4.56 (1'-H), 4.34 (5''-H), 4.33 (3-H),

4.18 (8-H), 3.74 (5'-H), 3.56 (5-H), 3.50 (11-H), 3.37 (3''-OCH₃), 3.17 (2'-H), 2.85 (Ph-CH₂CH₂), 2.66-2.55 (Ph-CH₂CH₂, 3'-H), 2.35 (2''a-H, 10-H), 2.30 [3'-N(CH₃)₂], 1.92 (4-H), 1.88 (14a-H), 1.67 (4'a-H), 1.60 (2''b-H), 1.53 (7-2H), 1.42 (14b-H), 1.39 (6-CH₃), 0.89 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 177.3 (C-1), 176.0 (C-9), 172.1 (4''-OCO), 144.2, 129.7, 128.5, 114.7 (Ph), 101.8 (C-1'), 94.1 (C-1''), 82.3 (C-5), 78.3 (C-4''), 76.5 (C-3), 76.2 (C-13), 74.2 (C-12), 73.3 (C-6), 72.5 (C-3''), 70.0 (C-2'), 69.4 (C-11), 67.5 (C-5'), 65.1 (C-3'), 62.3 (C-5''), 48.8 (3''-OCH₃), 45.1 (C-2), 42.6 (C-4), 41.9 (C-7), 41.0 (C-10), 40.1 (C-8), 39.7 [3'-N(CH₃)₂], 35.7 (CH₂), 34.4 (C-2''), 29.5 (CH₂), 28.3 (C-4'), 21.2 (C-14), 10.8 (14-CH₃).

4''-O-[3-(4-nitrophenyl)-2-propenoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (11)

The same method was followed as for the synthesis of compound **8** but starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.5 g, 0.62 mmol) and 3-(4-nitrophenyl)-2-propenoic acid (0.60 g, 3.1 mmol) to obtain the title compound **11** (36 mg, 6.2%) as a white powder.

MS (ES) *m/z*: [M+H]⁺ 938.4.

HRMS (ES positive): calculated for C₄₇H₇₆N₃O₁₆ [M+H]⁺ 938.5226 found 938.5199.

¹H NMR (500 MHz, CDCl₃) δ 8.27 (Ph-2H), 7.77 (Ph-CH=CH), 7.67 (Ph-2H), 6.59 (Ph-CH=CH), 5.71 (8a-NH), 5.11 (1''-H), 4.96 (13-H), 4.83 (4''-H), 4.54 (1'-H), 4.45 (5''-H), 4.19 (8-H), 4.00 (3-H), 3.75 (5'-H), 3.70 (5-H), 3.51 (11-H), 3.36 (3''-OCH₃), 3.21 (2'-H), 3.17 (6-OCH₃), 2.71 (2-H), 2.61 (3'-H), 2.44 (2''a-H), 2.36 [3'-N(CH₃)₂], 2.28 (10-H), 1.94 (14a-H), 1.92 (4-H), 1.72 (4'a-H), 1.69 (7a-H), 1.67 (2''b-H), 1.58 (7b-H), 1.47 (14b-H), 1.37 (6-CH₃), 1.27 (4'b-H), 0.90 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 177.1 (C-1), 174.4 (C-9), 165.8 (4''-OCO), 148.7, 140.3, 128.7, 124.3 (Ph), 142.8, 121.7 (CH=CH), 102.7 (C-1'), 95.6 (C-1''), 80.3 (C-5), 79.4 (C-4''), 78.9 (C-6), 77.8 (C-3), 77.2 (C-13), 74.3 (C-12), 73.1 (C-3''), 70.9 (C-2'), 70.4 (C-11), 68.2 (C-5'), 65.5 (C-3'), 63.0 (C-5''), 51.8 (6-OCH₃), 49.6 (3''-OCH₃), 45.6 (C-2), 43.0 (C-7), 42.4 (C-10), 42.3 (C-4), 41.0 (C-8), 40.4 [3'-N(CH₃)₂], 35.2 (C-2''), 29.4 (C-4'), 21.6 (C-14), 11.2 (14-CH₃).

4''-O-[3-(4-aminophenyl)propanoyl]-8a-aza-8a-homoerythromycin A (12)

The same method was followed as for the synthesis of compound **10** but starting from compound **11** (0.25 g, 0.27 mmol) to obtain the title compound **12** (0.203 g, 81.5%) as a white powder.

MS (ES) *m/z*: [M+H]⁺ 910.5.

HRMS (ES positive): calculated for C₄₇H₈₀N₃O₁₄ [M+H]⁺ 910.5640 found 910.5636.

¹H NMR (500 MHz, CDCl₃) δ 6.98 (Ph-2H), 6.61 (Ph-2H), 5.59 (8a-NH), 5.05 (1''-H), 4.94 (13-H), 4.68 (4''-H), 4.52 (1'-H), 4.34 (5''-H), 4.17 (8-H), 3.97 (3-H), 3.91 (12-OH), 3.71 (5'-H), 3.67 (5-H), 3.50 (11-H), 3.29 (3''-OCH₃), 3.19 (2'-H), 3.16 (6-OCH₃), 2.85 (Ph-CH₂CH₂), 2.69-2.55 (Ph-CH₂CH₂, 2-H), 2.37 (2''a-H), 2.34 [3'-N(CH₃)₂], 2.27 (10-H), 1.93 (14a-H), 1.88 (4-H), 1.71 (4'a-H), 1.62 (7a-H), 1.57 (7b-H), 1.44 (14b-H), 1.36 (6-CH₃), 0.89 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 177.2 (C-1), 174.4 (C-9), 172.7 (4''-OCO), 144.8, 130.2, 129.1, 115.3 (Ph), 102.6 (C-1'), 95.5 (C-1''), 80.2 (C-5), 78.9 (C-6), 78.8 (C-4''), 77.2 (C-3), 77.2 (C-13), 74.4 (C-12), 73.0 (C-3''), 71.0 (C-2'), 70.5 (C-11), 68.1 (C-5'), 65.5 (C-3'), 63.0 (C-5''), 51.8 (6-OCH₃), 49.4 (3''-OCH₃), 45.6 (C-2), 43.1 (C-7), 42.5 (C-10), 42.3 (C-4), 41.1 (C-8), 40.4 [3'-N(CH₃)₂], 36.3 (CH₂), 35.2 (C-2''), 30.1 (CH₂), 29.4 (C-4'), 21.7 (C-14), 11.2 (14-CH₃).

4''-O-(3-pyridinyl)acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (13)

The same method was followed as for the synthesis of compound **6** starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (1.0 g, 1.24 mmol) and 3-pyridylacetic acid hydrochloride (0.646 g, 3.72 mmol). Crude product was purified by column chromatography (eluent CH₂Cl₂/

MeOH/NH₄OH 90:3:0.3) to obtain the title compound **13** (0.085 g, 7.9%) as a white solid.

MS (ES) *m/z*: [M+H]⁺ 883.0.

HRMS (ES positive): calculated for C₄₅H₇₆N₃O₁₄ [M+H]⁺ 882.5327 found 882.5348.

¹H NMR (500 MHz, CDCl₃) δ 8.51, 8.49, 7.65, 7.26 (Py), 5.85 (8a-NH), 5.04 (1''-H), 4.92 (13-H), 4.67 (4''-H), 4.49 (1'-H), 4.37 (5''-H), 4.17 (8-H), 3.96 (3-H), 3.70 (5'-H), 3.65 (5-H), 3.62 (Py-CH₂-), 3.49 (11-H), 3.27 (3''-OCH₃), 3.15 (2'-H), 3.13 (6-OCH₃), 2.66 (2-H), 2.52 (3'-H), 2.34 (2''a-H), 2.29 [3'-N(CH₃)₂], 2.25 (10-H), 1.91 (4-H, 14a-H), 1.68 (7a-H, 4'a-H), 1.62 (2''b-H), 1.55 (7b-H), 1.47 (14b-H), 1.34 (6-CH₃), 1.31 (5''-CH₃), 1.18 (5'-CH₃, 2-CH₃, 4'b-H), 1.16 (10-CH₃), 1.14 (12-CH₃), 1.09 (8-CH₃), 1.06 (4-CH₃), 0.98 (3''-CH₃), 0.86 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 176.8 (C-1), 174.0 (C-9), 170.0 (4''-OCO), 148.5, 148.4, 136.3, 129.0, 123.4 (Py), 102.3 (C-1'), 95.0 (C-1''), 79.8 (C-5), 79.3 (C-4''), 78.5 (C-6), 77.2 (C-3), 76.7 (C-13), 73.9 (C-12), 72.6 (C-3''), 70.4 (C-2'), 70.0 (C-11), 67.8 (C-5'), 65.1 (C-3'), 62.4 (C-5''), 51.4 (6-OCH₃), 49.1 (3''-OCH₃), 45.2 (C-2), 42.6 (C-7), 42.0 (C-4), 41.9 (C-10), 40.5 (C-8), 40.0 [3'-N(CH₃)₂], 38.0 (CH₂), 34.8 (C-2''), 28.5 (C-4'), 21.3 (C-14), 10.28 (14-CH₃).

4''-O-(3-quinolinyl)carbonyl-6-O-methyl-8a-aza-8a-homoerythromycin A (14)

The same method was followed as for the synthesis of compound **6** starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.09 g, 0.11 mmol) and 3-quinolinecarboxylic acid (0.095 g, 0.55 mmol). Crude product was purified by column chromatography (eluent CH₂Cl₂/MeOH/NH₄OH 90:5:0.5) to obtain the title compound **14** (0.088 g, 87.1%) as a white powder.

MS (ES) *m/z*: [M+H]⁺ 918.4.

HRMS (ES positive): calculated for C₄₈H₇₆N₃O₁₄ [M+H]⁺ 918.5327 found 918.5319.

¹H NMR (500 MHz, CDCl₃) δ 9.41 (Q2-H), 8.83 (Q8-H), 8.19 (Q4-H), 7.94 (Q5-H), 7.87 (Q7-H), 7.66 (Q6-H), 5.85 (8a-NH), 5.16 (1''-H), 5.01 (4''-H), 4.96 (13-H), 4.65 (1'-H), 4.57 (5''-H), 4.20 (8-H), 4.04 (3-H), 3.83 (5'-H), 3.71 (5-H), 3.53 (11-H), 3.41 (3''-OCH₃), 3.21 (2'-H), 3.18 (6-OCH₃), 2.74 (2-H), 2.67 (3'-H), 2.48 (2''a-H), 2.32 (10-H), 1.96 (14a-H), 1.93 (4-H), 1.78 (4'a-H), 1.73 (2''b-H), 1.62 (7a-H), 1.57 (7b-H), 1.47 (14b-H), 1.37 (6-CH₃), 1.25 (2-CH₃, 5''-CH₃), 1.22 (4'b-H), 1.20 (3''-CH₃), 1.18 (10-CH₃), 1.13 (12-CH₃, 8-CH₃, 4-CH₃), 0.94 (5'-CH₃), 0.91 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 177.3 (C-1), 174.1 (C-9), 165.0 (4''-OCO), 149.9, 149.7, 138.7, 131.9, 129.5, 128.8, 127.5, 126.6, 122.6 (Q), 102.6 (C-1'), 95.1 (C-1''), 79.9 (C-5), 79.8 (C-4''), 78.6 (C-6), 77.1 (C-3), 77.0 (C-13), 74.1 (C-12), 72.9 (C-3''), 70.7 (C-2'), 70.2 (C-11), 68.1 (C-5'), 65.5 (C-3'), 63.0 (C-5''), 51.7 (6-OCH₃), 49.4 (3''-OCH₃), 45.4 (C-2), 42.8 (C-7), 42.4 (C-4), 42.1 (C-10), 40.7 (C-8), 40.3 [3'-N(CH₃)₂], 34.9 (C-2''), 28.9 (C-4'), 21.5 (C-14), 11.1 (14-CH₃).

4''-O-[3-(3-quinolinyl)-2-propenoyl]-8a-aza-8a-homoerythromycin A (15)

The same method was followed as for the synthesis of compound **6** starting from 2'-O-acetyl-8a-aza-8a-homoerythromycin A (0.498 g, 0.63 mmol) and 3-(3-quinolinyl)-2-propenoic acid (0.627 g, 3.15 mmol). Crude product was purified by column chromatography (eluent CH₂Cl₂/MeOH/NH₄OH 90:5:0.5) to obtain the title compound **15** (0.250 g, 42.7%) as a yellow solid.

MS (ES) *m/z*: [M+H]⁺ 930.6.

HRMS (ES positive): calculated for C₄₉H₇₆N₃O₁₄ [M+H]⁺ 930.5327 found 930.5315.

¹H NMR (500 MHz, CDCl₃) δ 9.10 (Q2), 8.25 (Q4), 8.11 (Q8), 7.90 (Q5), 7.89 (Q-CH=CH), 7.78 (Q7), 7.61 (Q6), 6.68 (Q-CH=CH), 6.06 (8a-NH), 5.18 (1''-H), 4.94 (13-H), 4.86 (4''-H), 4.61 (1'-H), 4.45 (5''-H), 4.38 (3-H), 4.20 (8-H), 3.87 (5'-H), 3.62 (5-H), 3.52 (11-H), 3.35 (3''-OCH₃), 3.24 (2'-H), 2.68 (3'-H), 2.66 (2-H), 2.65 (6-OCH₃), 2.43 (2''a-H), 2.41 [3'-N(CH₃)₂], 2.37 (10-H), 1.94 (4-H), 1.91 (14a-H), 1.83 (4'a-H), 1.69 (2''b-H), 1.60 (7a-H), 1.53 (7b-H), 1.44 (14b-H), 1.41 (6-CH₃), 1.29 (4'b-H), 1.22-1.18 (8-CH₃,

5'-CH₃, 5''-CH₃, 2-CH₃, 3''-CH₃ and 10-CH₃), 1.11 (4-CH₃), 1.10 (12-CH₃), 0.91 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 177.4 (C-1), 176.2 (C-9), 165.9 (4''-OCO), 148.3, 148.3, 141.9, 135.7, 130.5, 129.0, 127.3, 127.3, 126.8 (Q), 128.2, 118.9 (-CH=CH-), 102.0 (C-1'), 94.3 (C-1''), 82.4 (C-5), 78.9 (C-4''), 76.9 (C-3), 76.5 (C-13), 74.4 (C-12), 73.6 (C-6), 72.9 (C-3''), 70.2 (C-2'), 69.7 (C-11), 67.7 (C-5'), 65.4 (C-3'), 62.6 (C-5''), 49.2 (3''-OCH₃), 45.3 (C-2), 42.7 (C-4), 42.1 (C-7), 41.4 (C-10), 40.3 [3'-N(CH₃)₂], 40.0 (C-8), 35.7 (C-2''), 29.0 (C-4'), 21.4 (C-14), 11.0 (14-CH₃).

4''-O-[3-(3-quinolinyl)-2-propenoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (16)

The same method was followed as for the synthesis of compound 6 starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.500 g, 0.62 mmol) and 3-(3-quinolinyl)-2-propenoic acid (0.740 g, 3.72 mmol). Crude product was purified by column chromatography (eluent CH₂Cl₂/MeOH/NH₄OH 90:5:0.5) to obtain the title compound 16 (0.420 g, 71.8%) as a yellow solid.

MS (ES) *m/z*: [M+H]⁺ 945.1.

HRMS (ES positive): calculated for C₅₀H₇₈N₃O₁₄ [M+H]⁺ 944.5484 found 944.5480.

¹H NMR (500 MHz, CDCl₃) δ 9.11 (Q2-H), 8.24 (Q4-H), 8.12 (Q8-H), 7.89 (Q-CH=CH), 7.87 (Q5-H), 7.78 (Q7-H), 7.61 (Q6-H), 6.71 (Q-CH=CH), 5.69 (8a-NH), 5.11 (1''-H), 4.97 (13-H), 4.85 (4''-H), 4.56 (1'-H), 4.47 (5''-H), 4.19 (8-H), 4.01 (3-H), 3.79 (5'-H), 3.71 (5-H), 3.52 (11-H), 3.37 (3''-OCH₃), 3.20 (2'-H), 3.18 (6-OCH₃), 2.71 (2-H), 2.61 (3'-H), 2.45 (2''a-H), 2.35 [3'-N(CH₃)₂], 2.27 (10-H), 1.93 (14a-H), 1.74 (4'a-H), 1.69 (7a-H), 1.69 (2''b-H), 1.59 (7b-H), 1.47 (14b-H), 1.38 (6-CH₃), 1.29 (4'b-H), 1.24 (2-CH₃), 1.23 (5'-CH₃), 1.20 (5''-CH₃), 1.19 (3''-CH₃), 1.18 (10-CH₃), 1.13 (12-CH₃), 1.13 (8-CH₃), 1.11 (4-CH₃), 0.90 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 177.1 (C-1), 174.4 (C-9), 166.2 (4''-OCO), 148.7, 148.7, 136.1, 130.9, 129.5, 128.4, 127.7, 127.6, 127.2 (Q), 142.4, 119.3 (-CH=CH-), 102.8 (C-1'), 95.6 (C-1''), 80.3 (C-5), 79.2 (C-4''), 78.9 (C-6), 77.8 (C-3), 77.2 (C-13), 74.3 (C-12), 73.1 (C-3''), 70.9 (C-2'), 70.4 (C-11), 68.3 (C-5'), 65.6 (C-3'), 63.1 (C-5''), 51.8 (6-OCH₃), 49.6 (3''-OCH₃), 45.6 (C-2), 43.0 (C-7), 42.4 (C-4, C-10), 41.0 (C-8), 40.4 [3'-N(CH₃)₂], 35.2 (C-2''), 29.4 (C-4'), 21.4 (C-14), 11.2 (14-CH₃).

4''-O-[3-(3-quinolinyl)-2-propenoyl]-6-O-ethyl-8a-aza-8a-homoerythromycin A (17)

The same method was followed as for the synthesis of compound 6 starting from 2'-O-acetyl-6-O-ethyl-8a-aza-8a-homoerythromycin A (0.100 g, 0.12 mmol) and 3-(3-quinolinyl)-2-propenoic acid (0.146 g, 0.73 mmol). Crude product was purified by column chromatography (eluent CH₂Cl₂/MeOH/NH₄OH 90:5:0.5) to obtain the title compound 17 (0.044 g, 38.3%).

MS (ES) *m/z*: [M+H]⁺ 958.5.

HRMS (ES positive): calculated for C₅₁H₈₀N₃O₁₄ [M+H]⁺ 958.5640 found 958.5671.

¹H NMR (500 MHz, CDCl₃) δ 9.11 (Q2-H), 8.31 (Q4-H), 8.11 (Q8-H), 7.94 (Q-CH=CH), 7.91 (Q5-H), 7.78 (Q7-H), 7.61 (Q6-H), 6.78 (Q-CH=CH), 5.01 (13-H), 4.96 (1''-H), 4.87 (4''-H), 4.49 (5''-H), 4.47 (1'-H), 3.93 (3-H, 8-H), 3.69 (5'-H), 3.51 (11-H), 3.41 (6-OCH₂-), 3.35 (3''-OCH₃), 3.24 (2'-H), 2.82 (2-H), 2.47 (2''a-H), 1.93 (14a-H), 1.70 (2''b-H), 1.48 (14b-H), 0.86 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 175.3 (C-1), 175.2 (C-9), 166.4 (4''-OCO), 149.0, 148.7, 136.0, 130.9, 129.4, 128.6, 127.6, 127.6, 127.1 (Q), 146.7, 119.0 (CH=CH), 103.1 (C-1'), 97.6 (C-1''), 82.5 (C-5), 82.1 (C-3), 79.0 (C-4''), 78.9 (C-6), 77.5 (C-13), 74.1 (C-12), 73.3 (C-3''), 71.0 (C-2'), 70.9 (C-11), 68.7 (C-5'), 65.4 (C-3'), 63.1 (C-5''), 53.4 (6-OCH₂CH₃), 49.6 (3''-OCH₃), 45.4 (C-2), 42.4 (C-7), 42.0 [3'-N(CH₃)₂], 41.9 (C-8), 40.6 (C-4, C-10), 35.9 (C-2''), 29.7 (C-4'), 21.4 (C-14), 15.4 (6-OCH₂CH₃), 10.8 (14-CH₃).

4''-O-[3-(3-quinolinyl)-2-propenoyl]-6-O-allyl-8a-aza-8a-homoerythromycin A (18)

The same method was followed as for the synthesis of compound 6 starting from 2'-O-acetyl-6-O-allyl-8a-aza-8a-homoerythromycin A (0.320 g, 0.39 mmol) and 3-(3-quinolinyl)-2-propenoic acid (0.260 g, 1.31 mmol). Crude product

was purified by column chromatography (eluent CH₂Cl₂/MeOH/NH₄OH 90:5:0.5) to obtain the title compound 18 (0.230 g, 61.6%) as a yellowish solid. MS (ES) *m/z*: [M+H]⁺ 970.7.

HRMS (ES positive): calculated for C₅₂H₇₉N₃O₁₄ [M+H]⁺ 970.7813 found 970.7842.

¹H NMR (500 MHz, DMSO) δ 9.28 (s, Q2-H), 8.73 (s, Q4-H), 8.05 (d, Q8-H), 8.00 (d, Q5-H), 7.89 (d, Q-CH=CH), 7.84 (t, Q7-H), 7.68 (t, Q6-H), 6.94 (d, Q-CH=CH), 6.05 (m, 6-OCH₂CHCH₂), 5.77 (s, 8a-NH), 4.98 (d, 1''-H), 4.91 (m, 13-H, 6-OCH₂CHCH₂), 4.78 (d, 4''-H), 4.48 (d, 1'-H), 4.45 (q, 5''-H), 4.02 (m, 8-H), 3.94 (m, 3-H, 6-OCH₂CHCH₂), 3.80 (m, 11-H), 3.62 (d, 5-H), 3.39 (s, 5'-H), 3.35 (s, 3''-OCH₃), 3.08 (t, 2'-H), 2.72 (m, 2-H), 2.61 (m, 3'-H), 2.44 (d, 2''a-H), 2.42 (d, 10-H), 2.29 [s, 3'-N(CH₃)₂], 2.03 (m, 4-H), 1.83 (m, 14a-H), 1.82 (d, 2''b-H), 1.78 (d, 4'a-H), 1.75 (d, 7a-H), 1.40 (m, 14b-H), 1.22 (s, 6-CH₃), 1.13 (5''-CH₃, 3''-CH₃), 1.12 (5'-CH₃), 1.11 (2-CH₃), 1.06 (12-CH₃), 1.02 (4-CH₃), 1.00 (8-CH₃), 0.98 (10-CH₃), 0.83 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 177.2 (C-1), 172.7 (C-9), 165.8 (4''-OCO), 149.6, 147.9, 136.0, 130.8, 128.8, 128.7, 127.4, 127.2, 126.9 (Q), 142.2, 119.3 (-CH=CH-), 140.0 (6-OCH₂CH=CH₂), 113.2 (6-OCH₂CH=CH₂), 102.2 (C-1'), 94.5 (C-1''), 79.4 (C-6), 78.5 (C-5), 78.3 (C-4''), 75.9 (C-3), 75.8 (C-13), 74.1 (C-12), 72.4 (C-3''), 71.3 (C-5'), 70.6 (C-2'), 67.0 (C-11), 65.3 (6-OCH₂CH=CH₂), 64.6 (C-3'), 62.5 (C-5''), 49.0 (3''-OCH₃), 44.7 (C-2), 41.7 (C-7), 41.6 (C-4), 40.4 [3'-N(CH₃)₂], C-8, C-10], 34.1 (C-2''), 30.5 (C-4'), 21.2 (C-14), 11.1 (14-CH₃).

4''-O-[3-(3-quinolinyl)propanoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (19)

4''-O-[3-(1,2,3,4-tetrahydro-3-quinolinyl)propanoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (20)

To a solution of compound 16 (0.061 g, 0.065 mmol) in EtOH (8 ml) HOAc-AcONa buffer (pH 5) was added dropwise to a pH value of 6.5. Then catalyst 10% Pd/C (0.025 g) was added and the reaction mixture was stirred under hydrogen pressure of 1.4 bar for 4 h. The catalyst was filtered off and the filtrate was evaporated under reduced pressure. The residue was partition between water (10 ml) and CH₂Cl₂ (5 ml), the organic layer was separated and the aqueous was extracted with a fresh aliquot of CH₂Cl₂. The combined organic extracts were dried over Na₂SO₄ and evaporated to yield a mixture of the title compounds (0.054 g).

Purification by column chromatography (eluent CH₂Cl₂/MeOH/NH₄OH 90:9:0.5) afforded the title compounds 19 (0.014 g, 23.1%) and 20 (0.031 g, 50.8%).

Compound 19

TLC *R*_f=0.69 (eluent CH₂Cl₂/MeOH/NH₄OH 90:9:1.5).

MS (ES) *m/z*: [M+H]⁺ 946.6.

HRMS (ES positive): calculated for C₅₀H₈₀N₃O₁₄ [M+H]⁺ 946.5640 found 946.5630.

¹H NMR (500 MHz, CDCl₃) δ 8.80 (Q2-H), 8.07 (Q8-H), 7.99 (Q4-H), 7.76 (Q5-H), 7.68 (Q7-H), 7.54 (Q6-H), 5.62 (8a-NH), 5.05 (1''-H), 4.94 (13-H), 4.69 (4''-H), 4.49 (1'-H), 4.34 (5''-H), 4.17 (8-H), 3.96 (3-H), 3.93 (12-OH), 3.67 (5'-H), 3.65 (5''-H), 3.50 (11-H), 3.27 (3''-OCH₃), 3.18 (2'-H), 3.15 (Q-CH₂CH₂-), 2.79 (Q-CH₂CH₂-), 2.68 (2-H), 2.61 (3'-H), 2.36 (2''a-H), 2.35 [3'-N(CH₃)₂], 2.26 (10-H), 1.93 (14a-H), 1.90 (4-H), 1.71 (4'a-H), 1.58 (2''b-H), 1.44 (14b-H), 1.27 (4'b-H), 1.18 (2-CH₃, 10-CH₃), 1.13 (5'-CH₃), 1.12 (8-CH₃), 1.05 (5''-CH₃, 4-CH₃), 0.88 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 176.1 (C-1), 173.3 (C-9), 171.0 (4''-OCO), 150.4, 146.0, 133.6, 131.9, 128.2, 128.0, 127.0, 126.4, 125.9 (Q), 101.5 (C-1'), 94.4 (C-1''), 79.1 (C-5), 78.2 (C-4''), 77.8 (C-6), 76.5 (C-3), 76.1 (C-13), 73.3 (C-12), 71.9 (C-3''), 69.8 (C-2'), 69.4 (C-11), 67.0 (C-5'), 64.4 (C-3'), 61.8 (C-5''), 50.8 (6-OCH₃), 48.5 (3''-OCH₃), 44.5 (C-2), 42.0 (C-7), 41.4 (C-10), 41.2 (C-4), 40.0 (C-8), 39.3 [3'-N(CH₃)₂], 34.3 (CH₂), 34.1 (C-2''), 28.7 (C-4'), 27.2 (CH₂), 20.6 (C-14), 10.1 (14-CH₃).

Compound 20

TLC *R*_f=0.81 (eluent CH₂Cl₂/MeOH/NH₄OH 90:9:1.5).

MS (ES) *m/z*: [M+H]⁺ 950.7.

HRMS (ES positive): calculated for $C_{50}H_{84}N_3O_{14}$ $[M+H]^+$ 950.5954 found 950.5949.

1H NMR (500 MHz, $CDCl_3$) δ 6.96 (THQ6-H), 6.92 (THQ8-H), 6.60 (THQ7-H), 6.49 (THQ5-H), 5.60 (8a-NH), 5.06 (1''-H), 4.94 (13-H), 4.70 (4''-H), 4.55 (1'-H), 4.35 (5''-H), 4.17 (8-H), 3.99 (3-H), 3.73 (5'-H), 3.68 (5-H), 3.50 (11-H), 3.34 (THQ-CH₂CH₂-), 3.31 (3''-OCH₃), 3.26 (2'-H), 3.16 (6-OCH₃), 2.97 (THQ-CH₂CH₂-), 2.84 (THQ3-H), 2.69 (2-H), 2.48 (THQ2-2H), 2.38 (2''a-H), 2.27 (10-H), 1.93 (14a-H), 1.91 (4-H), 1.73 (THQ4-2H, 4'a-H), 1.65 (2''b-H), 1.62 (7a-H), 1.56 (7b-H), 1.45 (14b-H), 1.27 (4'b-H), 0.89 (14-CH₃).

^{13}C NMR (125 MHz, DMSO) δ 177.1 (C-1), 174.3 (C-9), 173.1 (4''-OCO), 144.2, 129.5, 126.8, 120.0, 117.0, 113.9 (THQ), 102.2 (C-1'), 95.3 (C-1''), 80.1 (C-5), 78.7 (C-6), 78.6 (C-4''), 76.9 (C-3, C-13), 74.2 (C-12), 72.9 (C-3''), 70.8 (C-2'), 70.3 (C-11), 67.8 (C-5') 65.3 (C-3'), 62.9 (C-5''), 51.8 (6-OCH₃), 49.4 (3''-OCH₃), 46.6 (CH₂), 46.5 (CH₂), 45.4 (C-2), 42.9 (C-7), 42.4 (C-10), 42.1 (C-4), 40.9 (C-8), 40.9 [3'-N(CH₃)₂], 35.0 (C-2''), 33.3 (THQ), 31.8 (THQ, C-4'), 28.4 (THQ), 21.5 (C-14), 11.1 (14-CH₃).

4''-O-[3-(1,2,3,4-tetrahydro-3-quinolinyl)propanoyl]-8a-aza-8a-homoerythromycin A (21)

A similar method was followed as for the synthesis of compound 10 but starting from compound 15 (0.06 g, 0.065 mmol) and at 2 bar pressure to obtain the title product 21 (0.027 g, 44.6%).

MS (ES) m/z : $[M+H]^+$ 936.4.

HRMS (ES positive): calculated for $C_{49}H_{82}N_3O_{14}$ $[M+H]^+$ 936.5797 found 936.5782.

1H NMR (500 MHz, DMSO) δ 7.80 (8a-NH), 6.83 (THQ6-H), 6.80 (THQ8-H), 6.41 (THQ7-H), 6.39 (THQ5-H), 5.66 (THQ1-NH), 4.96 (1''-H), 4.84 (13-H), 4.52 (4''-H), 4.38 (1'-H), 4.34 (5''-H), 4.14 (3-H), 4.10 (2'-OH), 3.97 (12-OH), 3.92 (8-H), 3.66 (5'-H), 3.49 (11-H), 3.38 (5-H), 3.24 (3''-OCH₃), 3.20 (THQ4-2H), 3.05 (2'-H), 2.79 (THQ2a-H), 2.72 (THQ2b-H), 2.62 (2-H), 2.42 (THQ-CH₂CH₂-), 2.41 (3'-H), 2.35 (THQ3-H), 2.30 (2''a-H), 2.23 [3'-N(CH₃)₂], 1.97 (4-H), 1.78 (14a-H), 1.68 (2''b-H), 1.62 (4'a-H), 1.57 (THQ-CH₂CH₂-), 1.54 (7a-H), 1.34 (14b-H), 1.27 (7b-H), 1.24 (6-CH₃), 0.80 (14-CH₃).

^{13}C NMR (125 MHz, DMSO) δ 177.7 (C-1), 175.0 (C-9), 173.2 (4''-OCO), 145.3, 129.4, 126.8, 119.4, 115.6, 113.5 (THQ), 102.7 (C-1'), 94.3 (C-1''), 82.4 (C-5), 78.6 (C-4''), 77.0 (C-3), 76.2 (C-13), 75.1 (C-12), 73.8 (C-6), 72.9 (C-3''), 71.2 (C-11), 70.9 (C-2'), 67.4 (C-5'), 65.2 (C-3'), 62.6 (C-5''), 49.4 (3''-OCH₃), 46.1 (THQ), 45.2 (C-2), 42.7 (C-4), 41.2 (C-7), 40.9 [3'-N(CH₃)₂], 39.6 (C-8), 34.7 (C-2''), 33.3 (THQ), 31.8 (CH₂), 31.3 (THQ), 30.7 (C-4'), 28.6 (CH₂), 21.9 (C-14), 11.7 (14-CH₃).

4''-O-[3-(4-quinolinyl)-2-propenoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (22)

The same method was followed as for the synthesis of compound 6 starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.050 g, 0.062 mmol) and 3-(4-quinolinyl)-2-propenoic acid (0.062 g, 0.31 mmol). Crude product was purified by column chromatography (eluent CH_2Cl_2 /MeOH/NH₄OH 90:5:0.5) to obtain the title compound 22 (0.030 g, 51.3%) as a yellow solid.

MS (ES) m/z : $[M+H]^+$ 944.7.

HRMS (ES positive): calculated for $C_{50}H_{78}N_3O_{14}$ $[M+H]^+$ 944.5484 found 944.5475.

1H NMR (500 MHz, $CDCl_3$) δ 8.97 (Q2-H), 8.50 (Q-CH=CH), 8.17 (Q8-H), 8.16 (Q7-H), 7.79 (Q7-H), 7.65 (Q6-H), 7.54 (Q3-H), 6.67 (Q-CH=CH), 5.66 (8a-NH), 5.12 (1''-H), 4.96 (13-H), 4.87 (4''-H), 4.55 (1'-H), 4.46 (5''-H), 4.17 (8-H), 4.02 (3-H), 3.78 (5'-H), 3.71 (5-H), 3.51 (11-H), 3.37 (3''-OCH₃), 3.23 (2'-H), 3.18 (6-OCH₃), 2.71 (2-H), 2.68 (3'-H), 2.46 (2''a-H), 2.37 [3'-N(CH₃)₂], 2.27 (10-H), 1.93 (4-H), 1.93 (14a-H), 1.76 (4'a-H), 1.70 (2''b-H), 1.67 (7a-H), 1.56 (7b-H), 1.47 (14b-H), 1.37 (6-CH₃), 1.29 (4'b-H), 1.23 (5'-CH₃), 1.20 (2-CH₃, 3''-CH₃, 5''-CH₃), 1.19 (10-CH₃), 1.13 (8-CH₃, 12-CH₃), 1.10 (4-CH₃), 0.90 (14-CH₃).

^{13}C NMR (125 MHz, DMSO) δ 177.4 (C-1), 174.4 (C-9), 166.3 (4''-OCO), 149.1, 148.7, 135.9, 130.9, 129.3, 128.9, 127.7, 127.6, 127.0 (Q), 142.7, 118.9 (CH=CH), 101.8 (C-1'), 95.3 (C-1''), 80.2 (C-5), 79.0 (C-4''), 78.7 (C-6), 77.3

(C-13), 77.0 (C-3), 74.4 (C-12), 73.2 (C-3''), 70.9 (C-2'), 70.4 (C-11), 67.4 (C-5'), 65.6 (C-3'), 63.2 (C-5''), 51.9 (6-OCH₃), 49.6 (3''-OCH₃), 45.4 (C-2), 42.8 (C-7), 42.6 (C-10), 42.1 (C-4), 41.1 (C-8), 40.5 [3'-N(CH₃)₂], 35.0 (C-2''), 29.7 (C-4'), 21.7 (C-14), 11.2 (14-CH₃).

4''-O-[4-(3-quinolinyl)-3-butenoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (23)

The same method was followed as for the synthesis of compound 8 but starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.07 g, 0.09 mmol) and 4-(3-quinolinyl)-3-butenic acid (0.132 g, 0.62 mmol). Crude product was purified by column chromatography (eluent EtOAc/MeOH 7:3) to obtain the title compound 23 (0.038 g, 44.1%) as a white solid.

MS (ES) m/z : $[M+H]^+$ 958.5.

HRMS (ES positive): calculated for $C_{51}H_{80}N_3O_{14}$ $[M+H]^+$ 958.5640 found 958.5640.

1H NMR (500 MHz, DMSO) δ 8.99 (Q2-H), 8.10 (Q8-H), 8.07 (Q4-H), 7.81 (Q5-H), 7.70 (Q7-H), 7.55 (Q6-H), 6.72 (Q-CH=CH), 6.55 (Q-CH=CH), 5.60 (8a-NH), 5.08 (1''-H), 4.96 (13-H), 4.77 (4''-H), 4.58 (1'-H), 4.40 (5''-H), 4.17 (8-H), 4.01 (3-H), 3.78 (5'-H), 3.71 (5-H), 3.50 (11-H), 3.38 (OCOCH₂), 3.36 (3''-OCH₃), 3.19 (6-OCH₃), 2.71 (2-H), 2.48 [3'-N(CH₃)₂], 2.43 (2''a-H), 2.29 (10-H), 1.95 (14a-H), 1.93 (4-H), 1.67 (2''b-H), 1.62 (7a-H), 1.54 (7b-H), 1.47 (14b-H), 1.38 (6-CH₃), 0.91 (14-CH₃).

^{13}C NMR (125 MHz, DMSO) δ 177.1 (C-1), 174.3 (C-9), 170.9 (4''-OCO), 148.9, 147.4, 132.4, 129.5, 129.3, 129.1, 127.9, 127.8, 127.0 (Q), 130.7, 123.8 (-CH=CH-) 102.6 (C-1'), 95.3 (C-1''), 80.1 (C-5), 79.2 (C-4''), 78.7 (C-6), 76.9 (C-3, C-13), 74.2 (C-12), 73.0 (C-3''), 70.8 (C-2'), 70.3 (C-11), 67.8 (C-5'), 65.4 (C-3'), 62.8 (C-5''), 51.8 (6-OCH₃), 49.4 (3''-OCH₃), 45.3 (C-2), 42.8 (C-7), 42.4 (C-10), 42.0 (C-4), 40.9 (C-8), 40.4 [3'-N(CH₃)₂], 38.1 (CH₂), 35.0 (C-2''), 29.6 (C-4'), 21.5 (C-14), 11.1 (14-CH₃).

4''-O-[4-(3-quinolinyl)butanoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (24)

The same method was followed as for the synthesis of compound 6 starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.10 g, 0.12 mmol) and 4-(3-quinolinyl)butanoic acid (0.13 g, 0.60 mmol). The crude product was purified by column chromatography (eluent EtOAc/MeOH 8:2) to give the title compound 24 (0.028 g, 38.3%) as a yellow solid.

MS (ES) m/z : $[M+H]^+$ 960.5.

HRMS (ES positive): calculated for $C_{51}H_{82}N_3O_{14}$ $[M+H]^+$ 960.5796 found 960.5784.

1H NMR (500 MHz, $CDCl_3$) δ 8.78 (Q2, s, 1H), 8.07 (Q8, d, 1H), 7.97 (Q4, d, 1H), 7.81 (Q5, d, 1H), 7.67 (Q7, m, 1H), 7.54 (Q6, m, 1H), 5.51 (8a-NH, d, 1H), 5.07 (1-H, d, 1H), 4.93 (13-H, dd, 1H), 4.70 (4-H, d, 1H), 4.56 (1'-H, d, 1H), 4.27 (5-H, dq, 1H), 4.13 (8-H, m, 1H), 3.99 (3-H, dd, 1H), 3.70 (5'-H, m, 1H), 3.67 (5-H, d, 1H), 3.46 (11-H, s, 1H), 3.38 (2'-H, m, 1H), 3.30 (3-OCH₃, s, 3H), 3.16 (6-OCH₃, s, 3H), 2.89 (CH₂, m, 2H), 2.68 (2-H, dq, 1H), 2.50, 2.37 (CH₂, m, 2H), 2.39 (2a-H, d, 1H), 2.28 (10-H, q, 1H), 2.09 (CH₂, m, 2H), 1.96-1.88 (14a-H, 4-H, m, 2H), 1.64 (2b-H, dd, 1H), 1.57 (7a-H, t, 1H), 1.49-1.43 (7b-H, 14b-H, m, 2H), 0.89 (14-CH₃, t, 3H).

4''-O-[5-(3-quinolinyl)-4-pentenoyl]-8a-aza-8a-homoerythromycin A (25)

A solution of 4-pentenoic acid (0.25 ml, 2.4 mmol) and TEA (0.7 ml, 5.1 mmol) in CH_2Cl_2 (20 ml) was cooled to 0 °C under argon. Pivaloyl chloride (0.6 ml, 4.9 mmol) was added and the reaction mixture was stirred for 0.5 h. To a solution of 2'-O-acetyl-8a-aza-8a-homoerythromycin A (0.5 g, 0.63 mmol) in CH_2Cl_2 (4 ml), pyridine (0.80 ml, 9.90 mmol) and DMAP (0.08 g, 0.63 mmol) were added. The reaction mixture was allowed to slowly warm to room temperature and then stirred for 2 h. Water (30 ml) was added and pH was adjusted to 4 using 2 M HCl. The residue was partitioned between water and CH_2Cl_2 , and the pH was adjusted to 9 using 2 M NaOH. The organic phase was concentrated to yield an oily residue, which was dissolved in MeOH (110 ml) and stirred at room temperature for 30 h. The MeOH was evaporated and the residue was purified by column chromatography on a silica gel (eluent CH_2Cl_2 /MeOH/NH₄OH 90:9:0.5). The 4''-O-(4-pentenoyl)-8a-aza-8a-homoerythromycin A (0.125 g, 0.15 mmol) that was obtained was dissolved in DMF

(1.5 ml). Into this solution Pd(II), acetate (2.4 mg) and tri-*o*-tolylphosphine (6.4 mg) were added and the mixture stirred under argon for 20 min. To the solution, 3-bromoquinoline (0.022 ml, 0.15 mmol) and TEA (0.029 ml, 2.1 mmol) were added and the reaction mixture was stirred at 80 °C for 38 h. The mixture was partitioned between water and EtOAc, and evaporation of the organic phase resulted in solid product. Column chromatography (eluent EtOAc/*n*-hexane/diethylamine 5:5:1) afforded the title compound **25** (0.097 g, 67.4%).

MS (ES) *m/z*: [M+H]⁺ 958.0.

HRMS (ES positive): calculated for C₅₁H₈₀N₃O₁₄ [M+H]⁺ 958.5640 found 958.5606.

¹H NMR (500 MHz, CDCl₃) δ 8.94 (Q2-H), 8.06 (Q8-H), 8.00 (Q4-H), 7.78 (Q5-H), 7.67 (Q7-H), 7.54 (Q6-H), 6.60 (Q-CH=CH), 6.48 (Q-CH=CH), 6.18 (8a-NH), 5.14 (1''-H), 4.92 (13-H), 4.73 (4''-H), 4.57 (1'-H), 4.37 (5''-H), 4.35 (3-H), 4.19 (8-H), 3.87 (11-OH), 3.79 (5'-H), 3.58 (5-H), 3.50 (11-H), 3.30 (3''-OCH₃), 2.66-2.55 (2xCH₂), 2.39 (2''a-H), 2.37 (10-H), 1.92 (4-H), 1.62 (2''b-H), 1.57 (7a-H), 1.53 (7b-H), 0.90 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 177.8 (C-1), 176.6 (C-9), 172.4 (4''-OCO), 149.1, 147.3, 132.0, 130.0, 129.2, 129.1, 127.9, 127.8, 126.8 (Q), 130.8, 128.0 (CH=CH), 102.1 (C-1'), 94.6 (C-1''), 82.8 (C-5), 79.1 (C-4''), 77.2 (C-3), 77.0 (C-13), 74.7 (C-12), 73.9 (C-6), 73.1 (C-3''), 70.5 (C-2'), 70.0 (C-11), 67.9 (C-5'), 65.7 (C-3'), 62.7 (C-5''), 49.4 (3''-OCH₃), 45.6 (C-2), 43.0 (C-4), 42.4 (C-7), 41.7 (C-10), 40.6 (C-8), 40.3 [3'-N(CH₃)₂], 35.0 (C-2''), 33.8, 28.5 (CH₂-CH₂), 29.7 (C-4'), 21.8 (C-14), 11.3 (14-CH₃).

4''-O-[5-(3-quinolinyl)-4-pentenyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (26)

The same method was followed for the synthesis of compound **25** but starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.300 g, 0.36 mmol). Column chromatography (eluent EtOAc/MeOH 7:3) afforded the title compound **26** (0.060 g, 17.1%) as a white solid.

MS (ES) *m/z*: [M+H]⁺ 972.0.

HRMS (ES positive): calculated for C₅₂H₈₂N₃O₁₄ [M+H]⁺ 972.5797 found 972.5793.

¹H NMR (500 MHz, CDCl₃) δ 8.94 (Q, d, 1H), 8.06 (Q, d, 1H), 8.00 (Q, s, 1H), 7.78 (Q, d, 1H), 7.67 (Q, t, 1H), 7.53 (Q, t, 1H), 6.61 (Q-CH=CH, d, 1H), 6.47 (Q-CH=CH, m, 1H), 5.64 (8a-NH, d, 1H), 5.08 (1-H, d, 1H), 4.94 (13-H, dd, 1H), 4.73 (4-H, d, 1H), 4.55 (1'-H, d, 1H), 4.36 (5-H, dq, 1H), 4.17 (8-H, m, 1H), 3.99 (3-H, d, 1H), 3.74 (5'-H, m, 1H), 3.69 (5-H, d, 1H), 3.49 (11-H, s, 1H), 3.31 (3-OCH₃, s, 3H), 3.24 (2'-H, dd, 1H), 3.16 (6-OCH₃, s, 3H), 2.70-2.53 (2-H, 3'-H, 2xCH₂, ov, 6H), 2.40 [3'-N(CH₃)₂, s, 6H] 2.38 (2a-H, d, 1H), 2.28 (10-H, q, 1H), 1.95-1.89 (14a-H, 4-H, 4'a-H, ov, 3H), 1.63 (7a-H, 2b-H, ov, 2H), 1.54 (7b-H, d, 1H), 1.45 (14b-H, m, 1H), 0.89 (14-CH₃, t, 3H).

¹³C NMR (125 MHz, DMSO) δ 177.3 (C-1), 174.3 (C-9), 172.4 (4''-OCO), 149.1, 147.3, 132.1, 130.1, 129.2, 129.1, 128.0, 127.8, 127.0 (Q), 130.7, 128.3 (CH=CH-), 102.3 (C-1'), 95.4 (C-1''), 80.1 (C-5), 78.9 (C-4''), 78.8 (C-6), 77.3 (C-3), 77.2 (C-13), 74.3 (C-12), 73.0 (C-3''), 70.9 (C-2'), 70.4 (C-11), 67.9 (C-5'), 65.4 (C-3'), 62.9 (C-5''), 51.9 (6-OCH₃), 49.5 (3''-OCH₃), 45.5 (C-2), 43.0 (C-7), 42.4 (C-10), 42.3 (C-4), 41.0 (C-8), 40.4 [3'-N(CH₃)₂], 35.1 (C-2''), 33.8, 29.4 (-CH₂CH=CHCH₂-), 30.0 (C-4'), 21.7 (C-14), 11.2 (14-CH₃).

4''-O-[5-(3-quinolinyl)-3-pentenyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (27)

The same method was followed for the synthesis of compound **6** starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.050 g, 0.06 mmol) and 5-(3-quinolinyl)-3-pentenoic acid (0.042 g, 0.18 mmol). The crude product was purified by column chromatography (eluent EtOAc/MeOH 7:3) to give the title compound **27** (0.027 g, 46.2%) as a white powder.

MS (ES) *m/z*: [M+H]⁺ 972.0.

HRMS (ES positive): calculated for C₅₂H₈₂N₃O₁₄ [M+H]⁺ 972.5797 found 972.5792.

¹H NMR (500 MHz, CDCl₃) δ 8.77 (Q, s, 1H), 8.08 (Q, d, 1H), 7.94 (Q, d, 1H), 7.77 (Q, d, 1H), 7.68 (Q, m, 1H), 7.54 (Q, m, 1H), 5.82, 5.72 (CH=CH, m, 2H) 5.62 (8a-NH, d, 1H), 5.07 (1-H, d, 1H), 4.94 (13-H, dd, 1H), 4.69 (4-H, d,

1H), 4.53 (1'-H, d, 1H), 4.33 (5-H, dq, 1H), 4.15 (8-H, m, 1H), 3.98 (3-H, br d, 1H), 3.73 (5'-H, m, 1H), 3.62 (5-H, d, 1H), 3.59 (CH₂, d, 2H) 3.49 (11-H, s, 1H), 3.28 (2'-H, ov, 1H), 3.28 (3-OCH₃, s, 3H), 3.22, 3.10 (CH₂, m, 2H), 3.16 (6-OCH₃, s, 3H), 2.68 (2-H, dq, 1H), 2.40 [3'-N(CH₃)₂, s, 6H] 2.38 (2a-H, d, 1H), 2.28 (10-H, q, 1H), 1.95-1.90 (14a-H, 4-H, 4'a-H, ov, 3H), 1.65-1.61 (7a-H, 2b-H, ov, 2H), 1.54 (7b-H, d, 1H), 1.47 (14b-H, m, 1H), 0.89 (14-CH₃, t, 3H).

¹³C NMR (125 MHz, DMSO) δ 177.3 (C-1), 174.3 (C-9), 171.4 (4''-OCO), 151.8, 146.9, 134.7, 132.5, 129.1, 128.9, 128.1, 127.4, 126.7 (Q), 132.4, 124.0 (-CH₂CH=CHCH₂-Q), 102.1 (C-1'), 95.3 (C-1''), 80.0 (C-5), 79.2 (C-4''), 79.0 (C-6), 77.2 (C-3, C-13), 74.3 (C-12), 73.0 (C-3''), 70.8 (C-2'), 70.4 (C-11), 67.7 (C-5'), 65.4 (C-3'), 62.9 (C-5''), 51.9 (6-OCH₃), 49.5 (3''-OCH₃), 45.4 (C-2), 42.9 (C-7), 42.4 (C-4), 42.2 (C-10), 41.0 (C-8), 40.3 [3'-N(CH₃)₂], 37.6, 36.1 (-CH₂CH=CHCH₂-Q), 35.0 (C-2''), 31.0 (C-4'), 21.6 (C-14), 11.2 (14-CH₃).

4''-O-[5-(3-quinolinyl)pentanoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A (28)

The same method was followed as for the synthesis of compound **8** but starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A (0.100 g, 0.12 mmol) and 5-(3-quinolinyl)pentanoic acid (0.141 g, 0.6 mmol). The crude product was purified by column chromatography (eluent EtOAc/MeOH 7:3) to obtain the title compound **28** (0.022 g, 18.8%) as a white solid.

MS (ES) *m/z*: [M+H]⁺ 974.0.

HRMS (ES positive): calculated for C₅₂H₈₄N₃O₁₄ [M+H]⁺ 974.5953 found 974.5956.

¹H NMR (500 MHz, CDCl₃) δ 8.77 (Q, d, 1H), 8.07 (Q, d, 1H), 7.94 (Q, s, 1H), 7.78 (Q, d, 1H), 7.66 (Q, m, 1H), 7.53 (Q, m, 1H), 5.51 (8a-NH, d, 1H), 5.06 (1-H, d, 1H), 4.92 (13-H, dd, 1H), 4.69 (4-H, d, 1H), 4.59 (1'-H, d, 1H), 4.25 (5-H, m, 1H), 4.12 (8-H, m, 1H), 4.00 (3-H, d, 1H), 3.78 (5'-H, m, 1H), 3.69 (5-H, d, 1H), 3.45 (11-H, s, 1H), 3.41 (2'-H, m, 1H), 3.28 (3-OCH₃, s, 3H), 3.16 (6-OCH₃, s, 3H), 2.84 (CH₂, t, 2H), 2.67 (2-H, m, 1H), 2.47, 2.30 (CH₂, m, 2H), 2.38 (2a-H, d, 1H), 2.27 (10-H, q, 1H), 1.95-1.88 (14a-H, 4-H, m, 2H), 1.78 (2x CH₂, m, 4H), 1.63 (2b-H, dd, 1H), 1.57 (7a-H, t, 1H), 1.49-1.40 (7b-H, 14b-H, m, 2H), 0.88 (14-CH₃, t, 3H).

4''-O-[3-(3-quinolinyl)-2-propenoyl]-6-O-methyl-8a-aza-8a-homoerythromycin A 11,12-cyclic carbonate (29)

The same method was followed as for the synthesis of compound **6** starting from 2'-O-acetyl-6-O-methyl-8a-aza-8a-homoerythromycin A 11,12-cyclic carbonate (0.950 g, 1.14 mmol) and 3-(3-quinolinyl)-2-propenoic acid (1.36 g, 6.8 mmol). Crude product was purified by column chromatography (eluent CH₂Cl₂/MeOH/NH₄OH 90:3:0.3) to obtain the title compound **29** (0.850 g, 76.8%) as a pale yellow solid.

MS (ES) *m/z*: [M+H]⁺ 970.9.

HRMS (ES positive): calculated for C₅₁H₇₆N₃O₁₅ [M+H]⁺ 970.5277 found 970.5264.

¹H NMR (500 MHz, CDCl₃) δ 9.11, 8.26, 8.12, 7.92, 7.78, 7.61 (Q), 7.89 (Q-CH=CH-), 6.75 (Q-CH=CH-), 5.04 (1''-H), 4.96 (13-H), 4.87 (4''-H), 4.59 (1'-H), 4.45 (11-H), 4.42 (5''-H), 4.02 (3-H), 4.07 (8-H), 3.67 (5'-H), 3.63 (5-H), 3.38 (3''-OCH₃), 3.18 (6-OCH₃), 2.76 (2-H), 2.46 (2''a-H), 2.39 (10-H), 2.39 [3'-N(CH₃)₂], 1.92 (4-H), 1.87 (14a-H), 1.85 (4'a-H), 1.85 (7a-H), 1.71 (2''b-H), 1.60 (14b-H), 1.55 (7b-H), 1.45 (12-CH₃), 1.36 (4'b-H), 1.35 (6-CH₃), 1.30 (10-CH₃), 1.26 (2-CH₃), 1.22 (5''-CH₃), 1.21 (5'-CH₃), 1.15 (8-CH₃, 3''-CH₃), 1.01 (4-CH₃), 0.92 (14-CH₃).

¹³C NMR (125 MHz, DMSO) δ 176.1 (C-1), 169.8 (C-9), 165.7 (4''-OCO), 152.9 (C=O cyclic carbonate), 148.2, 148.1, 135.5, 130.3, 128.8, 127.8, 127.1, 127.0, 126.6 (Q), 141.9, 118.7 (CH=CH), 102.3 (C-1'), 95.4 (C-1''), 85.1 (C-12), 81.7 (C-11), 79.6 (C-5), 79.0 (C-6), 78.5 (C-4''), 76.7 (C-3), 75.2 (C-13), 76.3 (C-3), 72.5 (C-3''), 70.3 (C-2'), 68.1 (C-5'), 64.8 (C-3'), 62.6 (C-5''), 51.3 (6-OCH₃), 49.1 (3''-OCH₃), 45.1 (C-2), 41.7 (C-7), 41.6 (C-8), 41.5 (C-4), 41.5 (C-10), 39.7 [3'-N(CH₃)₂], 34.7 (C-2''), 28.4 (C-4'), 22.0 (C-14), 10.0 (14-CH₃).

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