Supporting Information

Probing the Interactions of Macrolide Antibiotics with Membrane-Mimetics by NMR Spectroscopy

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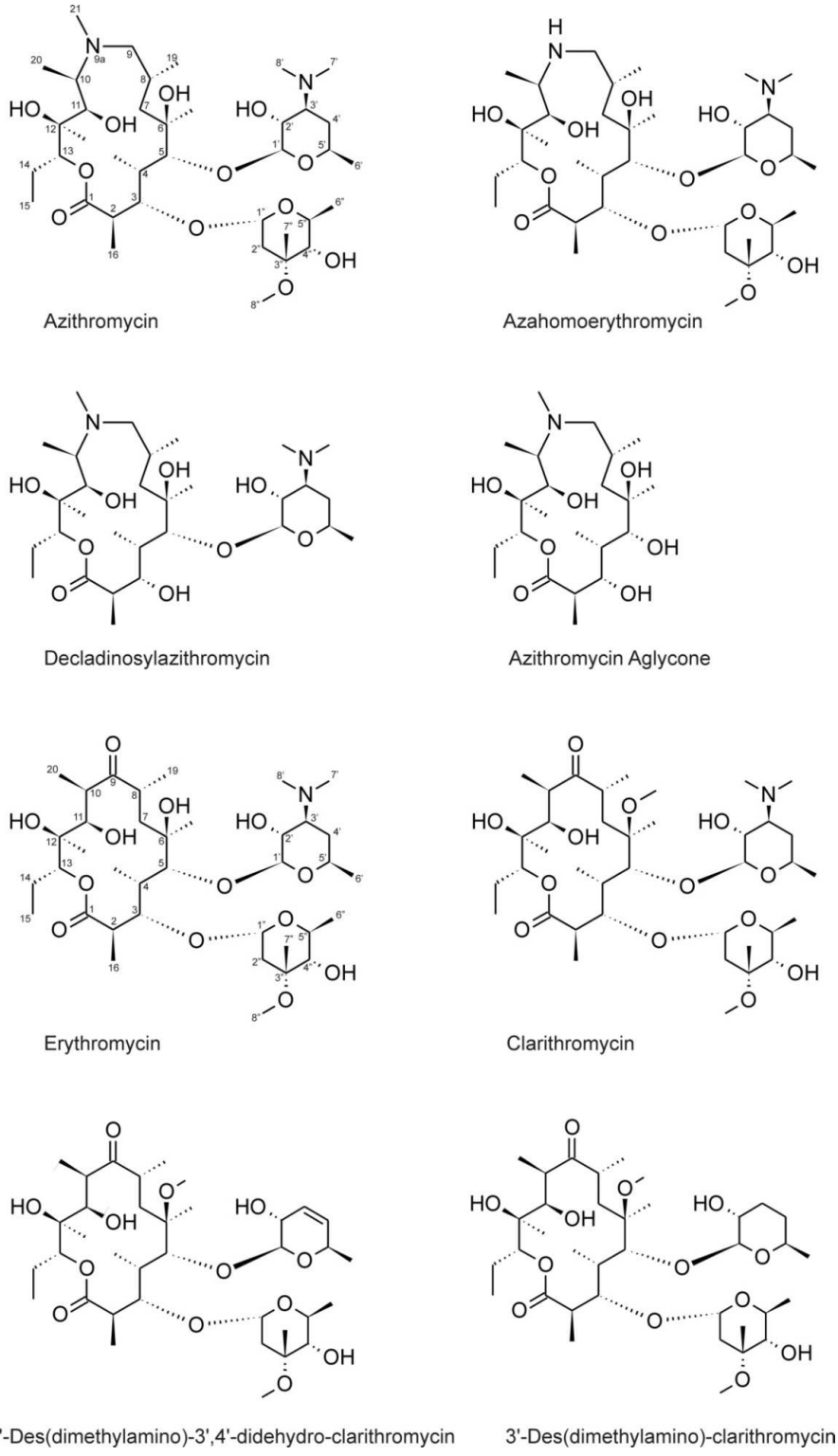
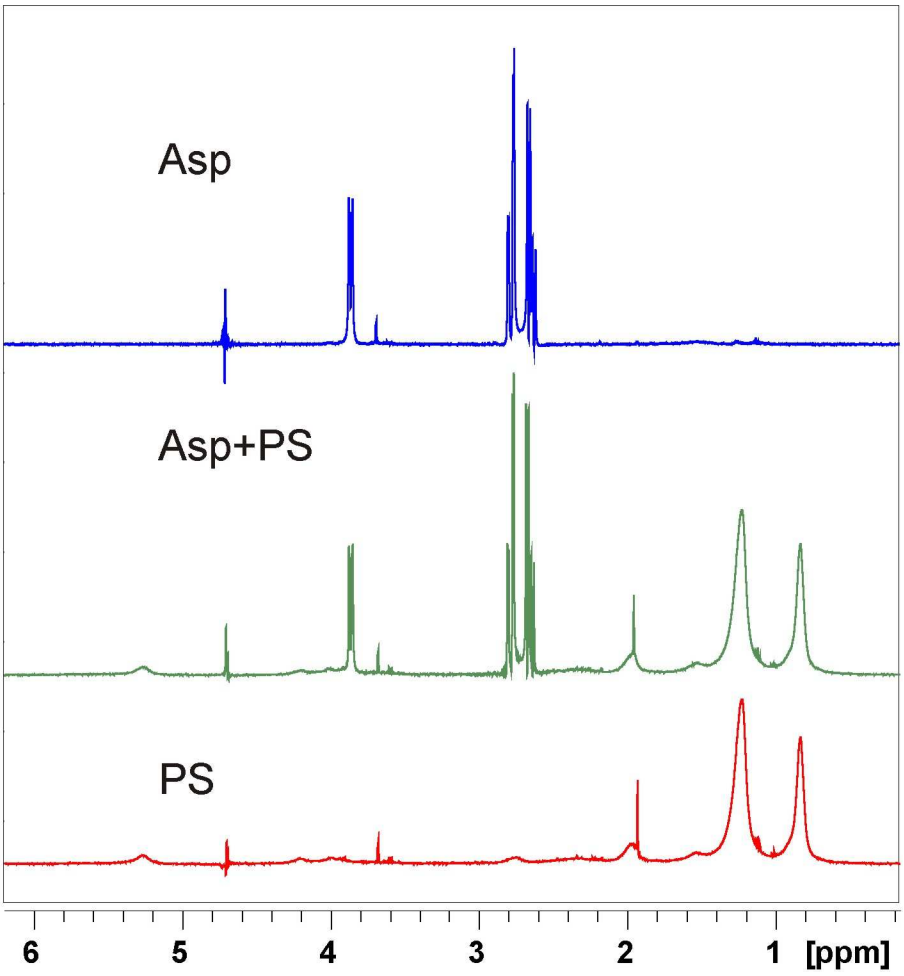
Supporting Figure 1:



Supporting Figure 1:

Structures of all macrolides used with the numbering scheme given for erythromycin and azithromycin as representatives of 14- and 15-membered macrolides, respectively.

Supporting Figure 2:



Supporting Figure 2:

One-dimensional ^{1}H NMR spectra of phosphatidylserine (PS), PS incubated with aspartate (ASP) and free Asp. The absence of signal changes of aspartate upon the addition of PS confirms that broadening of spectral lines of azithromycin is a result of binding and not simple mixing.

Supporting Table 1:

Self-diffusion coefficients of free macrolides, DPC micelles, SDS micelles and macrolides bound to DPC or SDS micelles as well as mole fraction partition coefficients (K_p).

DPC:

	D_{free} [$*10^{-10}$ m²/s]	D_{DPC} [$*10^{-10}$ m²/s]	$D_{DPC\text{-}Mac}$ [$*10^{-10}$ m²/s]	K_p
Aglycone	14,58 ± 0,08	2,97 ± 0,08	8,92 ± 0,27	0,95
Azithromycin	11,46 ± 0,11	2,97 ± 0,08	4,62 ± 0,12	4,17
Azahomoerythromycin	11,88 ± 0,17	2,97 ± 0,08	3,79 ± 0,15	9,89
Clarithromycin	11,68 ± 0,17	2,97 ± 0,08	3,27 ± 0,04	28,10
Decladinosylazithromycin	12,76 ± 0,47	2,97 ± 0,08	11,43 ± 0,10	0,16
Erythromycin	12,24 ± 0,11	2,97 ± 0,08	3,74 ± 0,02	10,99

SDS:

	D_{free} [$*10^{-10}$ m²/s]	D_{SDS} [$*10^{-10}$ m²/s]	$D_{SDS\text{-}Mac}$ [$*10^{-10}$ m²/s]	K_p
Aglycone	14,58 ± 0,08	0,92 ± 0,29	1,81 ± 0,08	14,37
Azithromycin	11,46 ± 0,11	0,92 ± 0,29	0,81 ± 0,05	n.a.[1]
Azahomoerythromycin	11,88 ± 0,17	0,92 ± 0,29	0,70 ± 0,04	n.a.[1]
Clarithromycin	11,68 ± 0,17	0,92 ± 0,29	0,71 ± 0,02	n.a.[1]
Decladinosylazithromycin	12,76 ± 0,47	0,92 ± 0,29	0,69 ± 0,01	n.a.[1]
Erythromycin	12,24 ± 0,11	0,92 ± 0,29	1,04 ± 0,05	93,00

[1] not available: the binding of the macrolide to SDS micelles is too strong. Therefore, the difference between the diffusion coefficient of the macrolide-SDS complex and free SDS micelles is within the experimental uncertainty of the diffusion coefficient of free SDS micelles.

Hemolysis

All investigated soluble macrolides gave negative results in the hemolysis test as expected. This is in accordance with earlier findings that macrolide antibiotics do not affect the cellular membranes but only transverse them and may stick to them to gain easier access to their targets, the ribosomes. The negative results also corroborate a rather shallow immersion depth of the antibiotics in membranous environment as they are therefore not able to penetrate and destroy the bilayer.

LC-MS

The main component in the phosphatidyl (PS) solution was PS 36:1 (m/z 788.513, RT 18.8 min). Low abundant components were PS 36:2 (m/z 786.529, RT 17.3 min), PS 38:1 (m/z 816.575, RT 20.7 min) and PS 38:2 (m/z 814.560, RT 19 min). After addition of the lipase, the corresponding Lyso-PS and the free fatty acid could be observed: LysoPS 18:0 (m/z 522.283, RT 4.7 min) and C18:1 (m/z 281.248, RT 9.5 min).

Experimental

Hemolysis test

Human erythrocytes were used to analyze the hemolytic activity of the macrolide antibiotics with intact cell-membranes. Fresh blood (donated by S.K.; a 30 year old healthy female) was centrifuged and washed four times with 0.9% NaCl solution to remove the plasma. Azithromycin, erythromycin A, clarithromycin, azahomoerythromycin, decladinosylazithromycin and azithromycin aglycone were dissolved in PBS (pH 7.4) and added to the erythrocyte suspension ($1 * 10^8$ cells/ ml in 0.9% NaCl). The suspension was incubated for 2 h at 37 °C and then centrifuged. The extent of hemolysis was determined by measuring the optical density of the supernatant at 451 nm. Hypotonically lyzed erythrocytes were used as a standard for 100% hemolysis. The experiment was repeated three times

including positive (Triton X100) and negative controls (PBS buffer without any macrolides).

LC-MS

In order to study the degradation of phosphatidylserine by phospholipase A_1 mixtures of phosphatidylserine (from bovine brain) and phospholipase A_1 (from *Thermomyces lanuginosus*) were mixed as described in the experimental part of the main text and then extracted using a modified Folch protocol. In brief, 2 ml of the aqueous solution were mixed with 20 ml of chloroform : methanol (2 : 1). After vortexing, 1 ml 0.9% NaCl solution was added to separate the aqueous from the organic phase. This mixture was then centrifuged at 4000 rpm and the aqueous phase taken off. The organic phase was evaporated to dryness. PS, LysoPS and free fatty acids were analyzed using an Aquity UPLC system coupled to a Synapt HDMS Q-TOF mass spectrometer (Waters, Manchester, UK). Chromatographic separation was done using a BEH C18 column (1,7 µm; 2.1 x 150mm) (Waters) and a gradient from methanol/water (1/1, v/v) to 2-propanol (both containing 1% NH_4Ac (1M)). Electrospray ionization was performed in negative ion mode with 2.1 kV capillary voltage. The QTOF was operated in V-mode with Leucine-Enkephaline as lock mass.

Determining mole fraction partition coefficients from self-diffusion measurements

The experimental diffusion coefficient D_{exp} depends on the concentrations of free $[A_{free}]$ and bound $[A_{bound}]$ antibiotic according to

$$D_{exp} = D_{free}\frac{[A_{free}]}{[A_{tot}]} + D_{bound}\frac{[A_{bound}]}{[A_{tot}]}, \qquad \text{Eq.1}$$

Where $[A_{tot}]=[A_{free}]+[A_{bound}]$ is the total macrolide concentration, D_{free} and D_{bound} are the self-diffusion coefficients of free and bound antibiotics, respectively. The diffusion coefficient in the bound state is assumed to be the one for the micelle which was obtained independently. Therefore, the mole fraction partition coefficient ($K_p=X_A/Y_A$, where X_A is the mole fraction of the macrolide in the lipid

phase and Y_A is the mole fraction in the aqueous phase) can be obtained from the diffusion measurements according to

$$K_p = \frac{[A_{bound}]}{[A_{free}]} = \frac{\frac{(D_{exp}-D_{free})[A_{tot}]}{D_{bound}-D_{free}}}{[A_{tot}]-\frac{(D_{exp}-D_{free})[A_{tot}]}{D_{bound}-D_{free}}} \quad \text{Eq.2}$$