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Structural studies of pravastatin and simvastatin and their complexes with SDS micelles by NMR spectroscopy

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ABSTRACT

Conformational features of pravastatin and simvastatin molecules in solution and in their complexes with sodium dodecyl sulfate micelles (SDS) were studied by 2D NOESY NMR spectroscopy. On the basis of the nuclear magnetic resonance experiments it was established that pravastatin and simvastatin can form molecular complex with SDS micelles which were considered as the model of cell membrane. In addition, interatomic distances for studied compounds were calculated based on 2D NOESY NMR experiments. It was shown that pravastatin interacts only with a surface of model membrane. However, in contrast to pravastatin, simvastatin penetrates into the inner part of SDS micelles. Observed distinctions in the mechanisms of interaction of pravastatin and simvastatin with models of cell membranes could explain the differences in their pharmacological properties.

1. Introduction

Cholesterol-lowering agents, such as pravastatin and simvastatin, participate in the treatment of hypercholesterolemia because of their ability to regulate cholesterol synthesis [1]. These compounds also have profitable actions in many other diseases, such as osteoporosis, cardiac and neurological sicknesses [2]. It is known that the efficacy, metabolism, and safety of statins depend on their location in molecular membrane [3]. The knowledge about mechanisms of interaction of these drugs with cellular membranes can provide a way to explain an origin of their pharmacologic characteristics.

NMR spectroscopy is productive instrument for structural studies of biomolecules [4–10]. Particularly, one of the most effective methods for conformational structure investigations of statins and their complexes with different compounds is nuclear Overhauser effect spectroscopy (NOESY) [2,11–15]. However, there is a disadvantage in applying this technique for studies of interactions in phospholipid membrane because transverse proton relaxation time of phospholipid aggregates is too short relative to the NMR time-scale. Nevertheless, interactions of different drugs with phospholipid bilayers can be effectively investigated by NMR using model membranes. Sodium dodecyl sulfate (SDS) micelles is one of the commonly used membrane models in NMR studies [16–22]. Head polar groups of SDS can be used to physically mimic a surface of biological membrane. Furthermore, SDS micelles are the most convenient model for NMR studies due to their smaller size and larger correlation times relative to the NMR time-scale [23,24]. As a consequence, NOESY experiments provide many information about the mechanisms of interactions of statins with SDS micelles. That is why this model of cell membranes was used in this work.

The aim of our investigation was to study the conformational features of pravastatin and simvastatin molecules and the mechanisms of interaction between statins and model membranes by NMR spectroscopy. We hope that the results presented in this article will help to shed some light on the origin of the physicochemical and pharmacological distinctions of different statins.

2. Experimental section

Pravastatin, simvastatin and SDS were purchased from Sigma–Aldrich Rus (Moscow, Russia) and used without further purification. Pravastatin was dissolved in SDS + D_2O with concentration of 6 g/l. Simvastatin was dissolved in DMSO and SDS + D_2O with concentration of 6 g/l. The concentration of SDS in D_2O solution was greater than critical micelle concentration (8.2 mM) and was equal to 23 mM, diameter of micelle – 5 nm. Solution volume was 0.6 ml,

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pH = 6.0 (for the experiments in water solution).

All NMR experiments were performed on a Bruker Avance II-500 NMR spectrometer equipped by 5 mm probe using standard Bruker TOPSPIN software. Temperature control was performed using a Bruker variable temperature unit (BVT-2000) in combination with a Bruker cooling unit (BCU-05). Experiments were performed at 303 K without sample spinning. Chemical shifts are given in values of ppm, referenced to residual solvent signals (4.72 ppm for ^{1}H in D_2O; 2.50 ppm for ^{1}H in DMSO). ^{1}H NMR data were collected with 32k complex data points.

Assignments of ^{1}H and ^{13}C NMR signals of compounds were achieved from signal multiplicities, integral values and characteristic chemical shifts from the through–bond correlations in 2D COSY spectra, through–space correlations in 2D NOESY spectra as well as from ^{1}H–^{13}C heteronuclear correlations in 2D HSQC and HMBC spectra.

All two-dimensional experiments were performed with 2k × 512 data points; the number of transients (96 scans) and the sweep widths were optimized individually. In the homonuclear ^{1}H–^{1}H COSY (Bruker pulse program *cosygpqf*) and 2D ge-NOESY [25], experiments were performed with pulsed filtered gradient techniques [26], the relaxation delay was set to 2 s and the 90° pulse length to 7.5 μs. Mixing time values were 0.5, 0.4, 0.3, 0.2 and 0.1 s. The resulting FIDs were zero-filled to a 2k × 1k data matrix and apodized with a sine function for COSY and a shifted sine function for NOESY in both the ω1 and ω2 dimensions prior to Fourier transformation. Heteronuclear spectra were recorded with 2k × 512 data points, zero-filled in F1 to a 2k × 512 data matrix, and apodized in both dimensions with a shifted sine function. HSQC experiments (*hsqcetgpsp*) were acquired using adiabatic pulses for inversion of ^{13}C and GARP-sequence for broadband ^{13}C-decoupling, optimized for ^{1}J (CH) = 135 Hz. ^{1}H–^{13}C long-range spectra HMBC (*hmbcgplpndqf*) were performed with ^{n}J(CH) set to 7 Hz.

To determine interproton distances the standard method based on the analysis of cross-peak integrals in 2D NOESY spectra at different mixing times was used. Integration of cross-peaks was performed in the program “Sparky” [27]. The interproton distances (r_{ij}) were calculated by the formula [28]:

$$r_{ij} = r_{cal}\left(\frac{\sigma_{cal}}{\sigma_{ij}}\right)^{1/6},$$

where r_{cal} – calibration distance, σ_{cal} – calibration cross-relaxation rate.

The cross-relaxation rate σ_{ij} can be determined from the linear dependence of intensity ratios of cross peaks (a_{ij}, a_{ji}) to the diagonal peaks (a_{ii}, a_{jj}) from mixing time τ_m:

$$\frac{1}{2}\left(\frac{1}{n_i}\frac{|a_{ij}(\tau_m)|}{|a_{jj}(\tau_m)|}+\frac{1}{n_j}\frac{|a_{ji}(\tau_m)|}{|a_{ii}(\tau_m)|}\right)\approx\sigma_{ij}\tau_m,$$

where n_i, n_j – relative proportions of the respective nuclei for certain resonance line. Calibration distances were r_{cal} = 2,45 Å (σ_{cal} = 0.047) for C<u>H</u>-8 … C<u>H</u>-9 in simvastatin and C<u>H</u>-3 … C<u>H</u>-4 in pravastatin.

3. Results and discussion

3.1. NMR study of pravastatin and simvastatin

Chemical and spatial structure of pravastatin (Fig. 1a) in a presence of SDS micelles was studied in our previous paper [14]. NMR spectra of simvastatin were assigned analogously to this work. It is known that as most of statins simvastatin has low aqueous solubility. Therefore, simvastatin was initially studied in DMSO solvent.

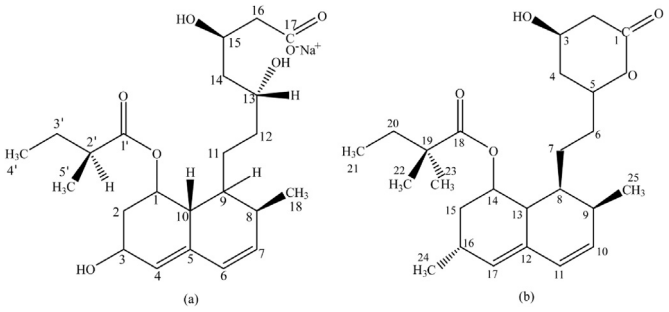

Fig. 1. Chemical structures of pravastatin (a) and simvastatin (b).

Chemical structure of the simvastatin (Fig. 1b) dissolved in DMSO was confirmed by the results of 1D ^{1}H and ^{13}C and 2D NMR experiments. The signals in the ^{1}H NMR spectrum (Fig. 2) were assigned using 2D NMR techniques, including ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC. Chemical shifts are shown in a Table 2(a).

The signals of methyl protons C<u>H</u>$_3$-22, 23, 24, 25 and 21 are observed in typical high-field region at 1.03, 1.03, 0.99, 0.80 and 0.74 ppm respectively in the ^{1}H NMR spectrum (Fig. 2).

The protons C<u>H</u>-11, 10, 17 resonate in the low-field region at 5.94, 5.78, 5.48 ppm respectively.

The signals of the protons near the electronegative oxygen C<u>H</u>-26, 14, 5 and 3 are also well resolved in the low-field region of spectrum. Other signals of C<u>H</u> and C<u>H</u>$_2$ groups with insignificant overlapping are located in a region of 1.10–2.70 ppm. The 2D^{1}H-^{1}H COSY spectrum clearly reveals all spin systems of the compound.

To define the conformational structure of simvastatin molecule in DMSO solution 2D NOESY NMR experiments with different mixing times were carried out (Fig. 3).

There are several nontrivial cross peaks observed in the spectrum. Nondiagonal signals. C<u>H</u>-5/C<u>H</u>$_3$-21 and C<u>H</u>-5/C<u>H</u>$_2$-20 indicate proximity of different cyclic parts of the molecule. So, these cross peaks are the evidence of the spatial proximity of two aliphatic chains of the simvastatin. Spatial structure of simvastatin is shown schematically in the Fig. 4.

3.2. NMR study of complexes of pravastatin and simvastatin with model membrane

Study of the molecular complex formed by simvastatin and pravastatin with model membrane can be useful for understanding of the basic principles of interaction between statins and cell membrane.

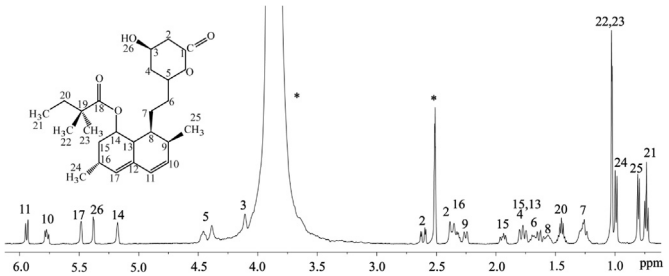

Fig. 2. The ^{1}H NMR spectrum of the simvastatin in DMSO at 303 K. The signals of solvent-DMSO (2.5 ppm) and residual D_2O (3.7 ppm) are marked by asterisks.

Table 1
The values of internuclear distances for pravastatin in: D_2O and SDS + D_2O solutions calculated by using 2D NOESY NMR spectra with different mixing times. * – calibration distance.

Pravastatin + D_2O		Pravastatin + SDS + D_2O	
Protons	Distance (Å)	Protons	Distance (Å)
CH-3 … CH-4*	2.45*	CH-3 … CH-4*	2.45*
CH-1 … CH_2-2	2.23	CH-1 … CH_2-2	2.35
CH_2-2 … CH-3	2.35	CH_2-2 … CH-3	2.23
CH_2-2 … CH-10	2.67	CH_2-2 … CH-10	2.75
CH-2′ … CH_2-3′	2.80	CH-2′ … CH_2-3′	2.85
CH-2′ … CH_3-5′	2.72	CH-2′ … CH_3-5′	2.68
CH-6 … CH-8	3.91	CH-6 … CH-8	3.91
CH_2-11 … CH-13	2.48	CH_2-11 … CH-13	3.11
CH-4 … CH-6	2.36	CH-4 … CH-6	2.35
CH-13 … CH-14	2.51	CH-13 … CH-14	2.63
CH_3-4′ … CH-13	4.87	CH-1 … CH_2-11	3.50
CH-6 … CH-10	3.93	CH-1 … CH_2-12	2.61
CH-13 … CH_2-16	4.69	CH_2-2 … CH_2-12	4.57
CH_2-14 … CH-15	3.23	CH_3-4′ … CH_2-11	3.65
CH_3-4′ … CH-15	4.23	CH-1 … CH-10	2.33
CH_2-11 … CH-15	4.18	CH-3 … CH-10	4.30
CH_2-2 … CH_2-11	4.67	CH-1 … CH-3	2.68
CH-13 … CH-15	3.15	CH-7 … CH-8	2.59
		CH-7 … CH_3-18	2.32
		CH-1 … CH_2-12^{SDS}	**3.69**
		CH-2′ … CH_2-12^{SDS}	**3.71**

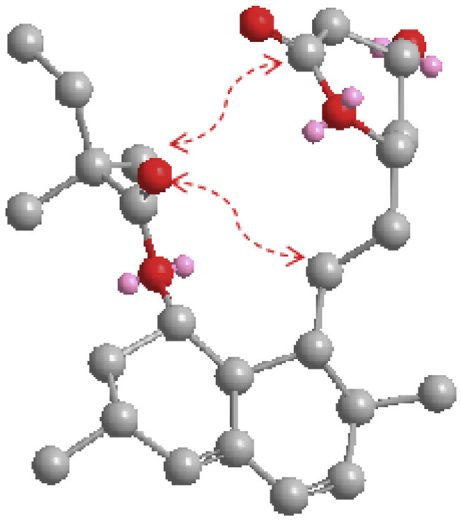

Fig. 4. Schematic presentation of the conformational structure of simvastatin and observed NOE (dashed arrows).

Table 2
^{1}H NMR chemical shifts (δ, ppm) of simvastatin in: DMSO (a); D_2O + SDS (b) at 303 K.

	CH_3-21	CH_3-25	CH_3-24	CH_3-22,23	CH_2-7	CH_2-20	CH-8	CH_2-6	CH-13
a)	0.74	0.80	0.99	1.03	1.27	1.45	1.56	1.68	1.71
b)	0.87	0.94	1.11	1.17	1.35	1.60	1.71	1.84	1.96
	CH_2-4	CH_2-15	CH-9	CH-16	CH_2-2	CH-3	CH-5	CH-14	OH-26
a)	1.79	1.79; 1.94	2.25	2.32	2.38; 2.61	4.11	4.42	5.18	5.38
b)	1.96; 2.08	2.08; 2.44	2.41	2.49	2.64; 2.82	4.41	4.71	5.39	–
	CH-17	CH-10	CH-11	1^{SDS}	2^{SDS}	3–11^{SDS}	12^{SDS}		
a)	5.48	5.78	5.94	–	–	–	–		
b)	5.48	5.77	5.95	0.87	1.30	1.60	4.00		

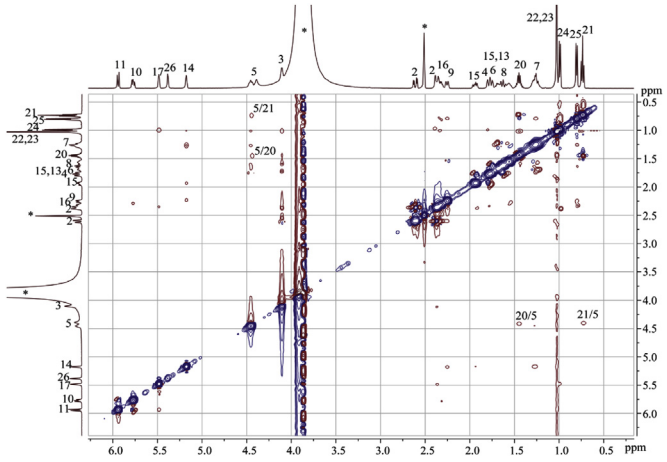

Fig. 3. 2D NOESY NMR spectrum of simvastatin in DMSO solution at 303 K. Mixing time (τ_m) is 100 ms.

3.2.1. NMR study of pravastatin + the model of cell membrane

In our recent article we studied the complex formation between pravastatin and SDS micelles [14]. It was proved that CH-1, CH_2-2′ groups of pravastatin and CH_2O protons («head » groups) of SDS (marked as 12 in Fig. 5) are spatially close in the complex. So, it was concluded that pravastatin is located on the polar surface of the micelles which were used as model membranes (Fig. 8a). In continuation of this work we are publishing the data about the internuclear distances in pravastatin-D_2O and pravastatin + SDS + D_2O systems (Table 1) calculated from the analysis of a set of 2D NOESY spectra with different mixing times.

It can be seen from the Table 1 that adding of the micelles in D_2O solution of pravastatin lead to some changes in its conformation. Aliphatic « tales » C1′-C4′ and C11–C17 come closer to each other because of the additional interaction with the surface of mimetic membrane. This interaction is confirmed by the intermolecular NOEs between CH-1/CH_2-12^{SDS} and CH-2′/CH_2-12^{SDS} in D_2O + SDS solution. Besides there are some differences in distances for the same groups of cyclic part depending on the type of model membranes: CH-4 … CH-6 (2.35 Å), CH-1 … CH-3 (2.68 Å). These changes can be explained by reorientation of corresponding protons relative to the cycles and to each other. Besides, there are more cross peaks between the protons of aliphatic chains in 2D NOESY spectrum of pravastatin in the presence of micelles if compared

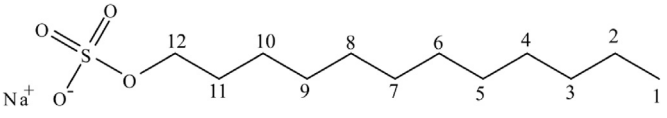

Fig. 5. Chemical structures of sodium dodecyl sulfate (SDS).

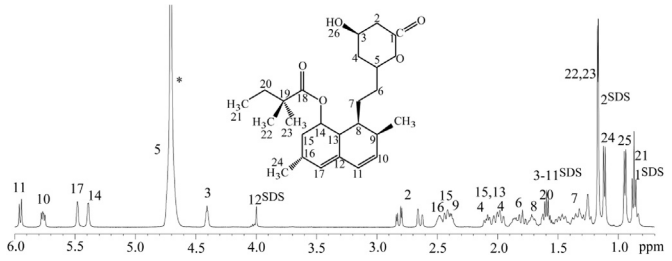

Fig. 6. ^{1}H NMR spectra of the simvastatin + SDS in D_2O at 303 K. The signals of solvents are marked by *.

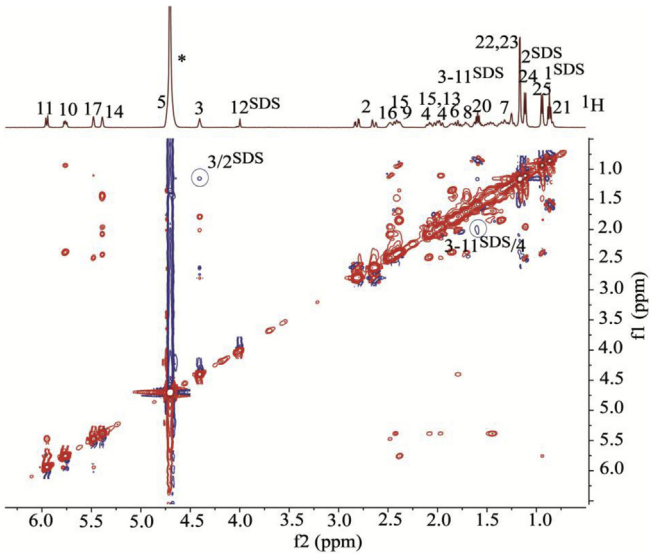

Fig. 7. 2D NOESY NMR spectrum of simvastatin + SDS mixture in D_2O solution at 303 K. Mixing time is τ_M = 100 ms.

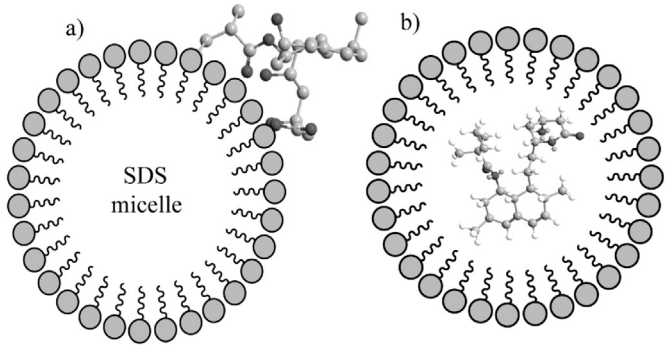

Fig. 8. Schematic presentation of the complexes: a) pravastatin + SDS micelle; b) simvastatin + SDS micelle.

with the spectrum of pure pravastatin. Therefore, it can be concluded that pravastatin binded to the surface of the model membrane with its flexible part containing partially negatively charged oxygens which interact with Na^+ ions and with a polar « head » groups of SDS and DMPC/DHPG.

3.2.2. NMR study of simvastatin + the model of cell membrane

Adding of the sodium dodecyl sulfate to D_2O leads to possibility of simvastatin dissolution in water. Solubilization phenomenon here is the dissolution in micellar solutions of substances that are usually does not dissolve in water [29]. This observation is an indirect proof that simvastatin molecules are involved in some additional intermolecular interactions with SDS micelles in D_2O solution.

^{1}H NMR spectrum of simvastatin dissolved in D_2O solution with SDS micelles is shown in Fig. 6. The signals in ^{1}H NMR spectrum were assigned using 2D NMR technique, including ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC spectra. Chemical shifts are shown in a Table 2(b). The signals of OH group do not appear in the ^{1}H NMR spectrum because they are involved in a fast relative to the NMR time scale exchange with protons of D_2O.

Lineshape of the signal CH_2-4 changes significantly from one multiplet in DMSO (Fig. 2) to two multiplets in SDS + D_2O solution (Fig. 6). This indicates a non-equivalence of CH_2-4 protons. It means that in the presence of the micelles flexible part of simvastatin become rigid due to some additional intermolecular interactions with mimetic membrane.

In order to investigate the mechanisms of complex formation between simvastatin and SDS micelles 2D NOESY experiments were carried out (Fig. 7). The cross peaks between CH-3, CH_2-4 groups of simvastatin and «tail » protons of the SDS (CH_2-2,3–11) (Fig. 5) are observed in the spectrum. There are also cross peaks between aliphatic chains and rigid parts of the molecule, as it were observed in DMSO solution. The values of internuclear distances in simvastatin and simvastatin + SDS are shown in the Table 3.

Thereby, it can be concluded that simvastatin is located inside the micelles which were used as a model membrane (Fig. 8b). This conclusion does not contradict the known observations of Mason et al. [3], that simvastatin molecule intercalates into the upper hydrocarbon core of the membrane lipid bilayer adjacent to the glycerol backbone. Thus, SDS micelles can be used as model membranes for investigations of interaction between simvastatin and cell membranes.

It is known that drug–membrane interactions are highly influenced by changes in membrane cholesterol content and microdomain formation, as observed during hypercholesterolemia [30–32]. The results of NMR experiments showed that even minor differences in chemical structure of pravastatin and simvastatin lead to crucial changes in the nature of their interaction with the models of cellular membranes and, therefore, in their pharmacologic properties. Pravastatin interacts only with the hydrated surface of the model membrane in water solution (Fig. 8a). However,

Table 3
The values of internuclear distances for simvastatin in DMSO and SDS + D_2O solutions, calculated from 2D NOESY NMR spectra with different mixing times. * – calibration distances.

Simvastatin + DMSO		Simvastatin + SDS + D_2O	
Protons	Distance (Å)	Protons	Distance (Å)
CH-8 … CH-9	2.22*	CH-8 … CH-9	2.22*
CH-3 … CH_2-4	2.49	CH-8 … CH_3-25	3.55
CH-3 … CH_2-6	4.43	CH_2-7 … CH-9	2.88
CH_2-4 … OH-26	3.18	CH_2-4 … CH-8	2.44
CH_2-6 … CH-9	4.48	CH_2-4 … CH-14	4.10
CH-9 … CH-10	2.78	CH-8 … CH-17	4.43
CH-9 … CH_3-25	2.32	CH-5 … CH_2-6	2.18
CH-10 … CH-11	2.39	CH-8 … CH_2-15	4.75
CH-11 … CH-17	2.46	CH-14 … CH_2-15	2.33
CH-14 … CH_2-15	2.73	CH-8 … CH-14	2.91
CH-11 … CH_3-24	4.78	**CH-3 … 2^{SDS}**	**2.69**
CH-8 … CH-14	3.95	**CH_2-4 … 3–11^{SDS}**	**3.88**
CH-14 … CH-16	4.32		
CH-16 … CH-17	2.45		
CH_2-20 … CH_3-21	2.57		
CH-17 … CH_3-24	3.08		
CH-13 … CH-14	3.18		

more lipophilic simvastatin is associated with the hydrocarbon core of the model membranes (Fig. 8b). The membrane locations correlated with differences in their metabolism and antioxidant effects [3]. In particular, simvastatin had overlapping locations in the membrane hydrocarbon core. This agent is oxidized by cytochrome P450 enzyme. Its location is different from pravastatin (located on the membrane surface). This is one of the reasons why pravastatin has separate metabolic pathway in comparison with simvastatin.

4. Conclusions

The results of NMR experiments showed that pravastatin and simvastatin can form molecular complexes with models of cell membrane in D_2O solution (SDS micelles). It was shown that even minor differences in chemical structure of pravastatin and simvastatin leads to different nature of their interactions with model membranes. Pravastatin is associated with a polar surface of mimetic membranes while simvastatin penetrates into a hydrocarbon core of SDS micelles. These distinctions can explain some differences in pharmacological properties of these compounds. Quantitative analysis of 2D NOESY spectra allowed determining of conformational features of pravastatin and simvastatin in pure D_2O solution and in the presence of SDS micelles.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molstruc.2015.10.059.

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