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CONFORMATIONAL ANALYSIS OF SULOCTIDIL AND DERIVATIVES INSERTED IN LIPID LAYERS

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Abstract—Interaction between suloctidil (CP 556 S) and lipids (phosphatidylcholine, phosphatidylserine) is studied using a new conformational analysis procedure. This analysis is extended to two compounds related to suloctidil but bearing no protonable group (CP 894 S) or of different hydrophobicity (CP 1136 S). It gives a molecular description of the mode of insertion of the drugs into the lipid layer. The influence of the calculated lipid–drug interaction and area occupied per drug molecule in the lipid layer is tentatively related to the effect on the lipid dynamics. For a given conformer, an effect on the lipid dynamics is expected only if (a) the area occupied per conformer is similar to that of the lipid and/or (b) the drug–lipid interaction energy is equal or superior to that of the lipid–lipid interaction. These predictions are analyzed in terms of the available experimental data.

It is widely accepted that the membrane is the site of action of a large variety of molecules. Some molecules act through specific protein receptors, enzymes, located in the membranes. For a large number of other molecules, due to the heterogeneity of their chemical structures, the precise mode of interaction with the membrane components (both lipid or proteins) remains a matter of debate. In the case of anaesthetics, however, a clear relationship has been established [1, 2] between the anaesthetic potency of a compound and its lipid solubility. This has led to several works on the effects of anaesthetics on the structure of the lipid bilayer [2–7]. It has been shown that anaesthetics are able to induce membrane expansion [2], lipid disorder [3, 4] and are able to modify the lipid phase transition [5–7]. The exact implications of these effects on protein structures and functions are still questioned [8]. Suloctidil is a drug directed towards the overall management of atherosclerosis and its complications [9, 10]. Its mode of action is unknown but might be related to membrane effects. Indeed, suloctidil protects the erythrocyte from haemolysis [11]; it inhibits Na^+/K^+ ATPase of rat brain synaptosome [12] and plasma membrane ATPase of yeast *Schizosaccharomyces pombe* [13]; in the latter it also inhibits the phosphorylation of a plasma membrane polypeptide [14]. In addition to its effects on membrane enzymes, suloctidil alters the lipid dynamics in erythrocytes [15], platelets [15], synaptosomes [12] and liposomes [16]. In all natural and artificial membranes tested, suloctidil fluidified the lipid matrix.

In this communication, the interaction between suloctidil and lipids is investigated further using a new procedure of conformational analysis [17, 18]. The analysis was extended to protonated and deprotonated forms of suloctidil and to the interaction with acidic lipids. Two compounds related to suloctidil, but bearing no protonable group or of different hydrophobicity, were considered. This approach allows a molecular description of the orientation of the drugs in the lipid matrix to be obtained.

MATERIALS AND METHODS

The procedure of conformational analysis is based on a strategy described elsewhere [17, 18]. The method currently used for the study of polypeptides [19, 20] was modified to take into account variations in dielectric constant and energy of transfer when the molecule moves from one environment to another at the simulated lipid–water interface.

The strategy supposes a two-step procedure: (a) conformation and orientation of the isolated molecule; (b) conformation of the molecules inserted in a lipid monolayer. Briefly, the total conformational energy of the interfacial isolated molecule was empirically calculated as the sum of all contributions resulting from local interactions, i.e. Van der Waals energy, torsional potential, electrostatic interaction and transfer energy. The electrostatic energy was calculated as a function of the dielectric constant. To simulate a lipid–water interface, the dielectric constants of the hydrophobic and hydrophilic media were taken as 3 and 30, respectively [21–23]. Changes of 60° were imposed on each torsional angle. The internal energy was calculated for each of these conformers. The most probable configurations were taken as those yielding the lowest internal energy. After a simplex minimization procedure [24], the most probable conformers were orientated at a simulated membrane interface taking into account the values of the hydrophilic and hydrophobic gravity centres calculated as described [23, 25]. The total conformational energy of the monolayer was empirically calculated as the sum of all contributions resulting from interaction between molecules, i.e. Van der Waals energy, electrostatic interaction and transfer

energy. The procedure of assemblage can be summarized as follows [17]. Molecule A was fixed and the position of molecule B was modified along the x-axis by steps of 0.5 Å. For each distance, a rotation angle of 30° was imposed to molecule B around its own Z-axis and around molecule A. Only the structure of minimum energy was considered. Molecule B was allowed to move along the Z-axis perpendicular to the lipid–water interface. Again, only the structure of minimum energy was considered. Molecule B has the possibility of changing its orientation around the Z-axis, compared to molecule A. This procedure allows the most probable packing of the two molecules to be defined. The procedure was repeated further until the drug molecule was completely surrounded by phospholipids.

The configuration of the final mixed monolayer was projected onto the interface plane (X,Y) and the areas occupied per molecule were estimated. The mean interaction energy between drug and lipid is equal to the total lipid–drug interaction energy divided by the number of surrounding lipids [22]. Calculations were performed at 25° on a CDC Cyber 170 Computer coupled to a Calcomp 1051 drawing table.

RESULTS

Conformational analysis of isolated molecules

The chemical structures of suloctidil, CP 556 S [1-(4-isopropylthiophenyl)-2-*n*-octylamino-1-propranol], CP 894 S [1-(4-isopropylthiophenyl)-2*n*-octylthio-1-propanol] and CP 1136 S [1-(4-isopropylthiophenyl)-2-amino-1-propanol] are illustrated in Fig. 1, which indicates the numbering of all torsional angles for each molecule. The values used for the valence angles, bond lengths, and atomic charges were generated using standard values [26] or were those currently used in conformational analysis [19, 20].

In a first systematic study, the torsional angles α_3, $\alpha_4,\alpha_5,\alpha_6,\alpha_7$ for sulocticil, CP 894 S and the torsional angles α_3,α_4 for CP 1136 S underwent changes of 60° yielding 6^5 conformers for suloctidil and CP 894 S, and 6^2 conformers for CP 1136 S. In a medium with a dielectric constant equal to 3 (chosen as representative of a bulk hydrophobic phase), the conformers selected have an individual probability in excess of 5%. The characteristics of these conformers for each of the three compounds studied are listed in Table 1. Values of the torsional angles of the most probable conformers obtained after application of the simplex minimization procedure and orientation at the simulated membrane–water interface are listed in Table 2. Conformers A and B as well as C and D of the neutral form of suloctidil are equivalent. Thus minimization yields two conformers of this compound with a probability of 68.2% (A + B) and 21% (C + D). Values of the torsional angles of conformers B and C of CP 894 S are equivalent and yield the same conformers with a probability of 36.8%. All conformers of CP 1136 S are equivalent and thus reduce to one conformer with a probability of 42.7%. For the most probable conformers of each compound (suloctidil, CP 894 S and CP 1136 S) in either the neutral or protonated form, the hydrophilic and hydrophobic transfer energies were calculated (Table 2) in order to localize the hydrophilic and hydrophobic gravity centres. These centres determine the position of the conformer at the interface. The distances between these centres, which are critical for a possible re-orientation at the interface [22], are listed in Table 2. The stereo-view of the most probable conformers of suloctidil and CP 894 S are shown in Fig. 2. The most probable conformers of the neutral and protonated forms of suloctidil are either bent or elongated. The probability of existence of the two conformers is not identical. The bent conformer of the neutral form and the elongated conformer of the charged form are the most probable. The two most probable conformers of CP 894 S are also either bent or elongated.

Conformational analysis of molecules inserted in lipid monolayers

The conformation, position and orientation of DL-α-dipalmitoylphosphatidylcholine (DPPC) at the interface have been calculated previously [17] according to the procedure described in Materials and Methods. Because of the excellent agreement with

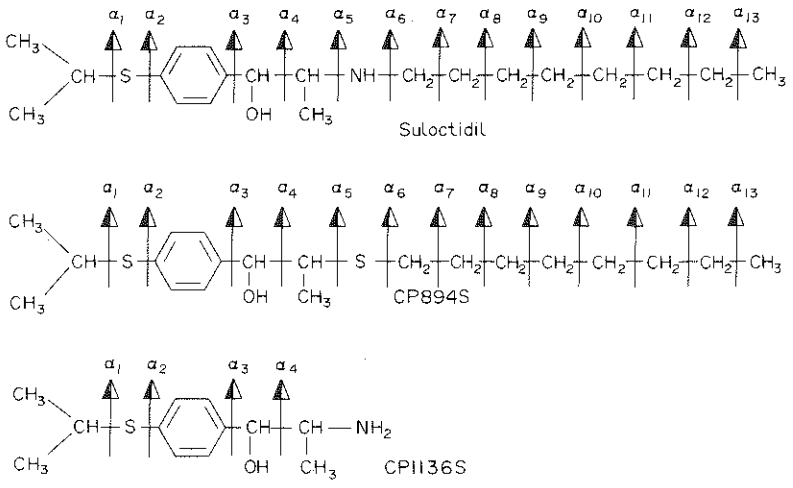

Fig. 1. Chemical structure and torsional angles of suloctidil and derivatives.

Table 1. Torsional angles of the most probable conformers after systematic analysis

Compound		Angle α_3	α_4	α_5	α_6	α_7	Probability (%)	Energy above minimal values (kcal/mole)
Suloctidil (neutral)	A	240	120	240	120	240	43.9	0
	B	240	120	240	240	120	14.3	0.660
	C	240	120	240	120	120	10.7	0.837
	D	240	120	240	120	180	10.3	0.855
Suloctidil (protonated)	A	240	120	300	180	240	57.0	0
	B	240	120	300	180	120	43.0	0.167
CP 894 S	A	240	120	0	120	180	40.9	0
	B	240	120	0	240	180	28.8	0.441
	C	240	120	180	240	180	8.0	0.922
CP 1136 S (neutral)	A	180	120	—	—	—	26.0	0
	B	180	180	—	—	—	8.7	0.321
	C	300	120	—	—	—	8.0	0.381
CP 1136 S (protonated)	A	180	120	—	—	—	26.0	0
	B	180	180	—	—	—	8.7	0.321
	C	300	120	—	—	—	8.0	0.381

experimental data for rigid DPPC [17], the conformational analysis was extended to fluid DPPC and dipalmitoylphosphatidylserine (DPPS). The interaction energies between homologous phospholipid molecules were −13, −11 and −11 kcal/mole for rigid DPPC, fluid DPPC and DPPS, respectively. The corresponding mean molecular areas were 59, 76 and 77 Å^2/molecule. Details of the conformation obtained for fluid DPPC and DPPS will be published elsewhere (R. Brasseur, manuscript in preparation).

The values of some of the parameters characterizing the drug–lipid interactions are shown in Table 3. These values were obtained by inserting all the most probable conformers in each phospholipid matrix. Only the assembling modes corresponding to the minimal energy were retained. Some of the structures obtained after insertion of a conformer in the lipid layer are shown in Figs. 3 and 4.

Figure 3 gives the configuration of protonated suloctidil interacting with fluid DPPC. Interestingly, it must be noted that the amine residue of suloctidil is located in the immediate neighbourhood of the DPPC phosphate group. The orientation of the two most probable conformers differs markedly; the elongated conformer is orientated parallelly to the lipid–water interface (Fig. 3b) whereas in the bent conformer the *n*-octyl chain is orientated parallelly to the acyl chains of the lipid (Fig. 3a). Figure 4 shows a comparison of the configuration of the neutral (right) and protonated (left) form of CP 1136 S inserted into a DPPS monolayer. For the protonated form the amino group is located near the negative charge of the DPPS carboxyl group. The neutral form is more deeply inserted into the lipid layer than the protonated form and interacts mainly with the hydrocarbon chain. Protonation of CP 1136 S clearly modulates the drug–lipid interaction. This effect is not observed with neutral lipids.

Table 2. Torsional angles of the most probable conformers after minimization and orientation at the simulated membrane–water interface

		Torsional angle α_1	α_2	α_3	α_4	α_5	α_6	α_7	α_8	α_9	α_{10}	α_{11}	α_{12}	Probability*	E_{tr}^{phi}†	E_{tr}^{pho}†	Δ‡
Suloctidil (neutral)	A	189	144	247	76	245	103	214	175	188	177	183	177	43.9	43.12	6.86	4.11
	B	189	148	247	78	245	108	214	175	189	178	184	179	14.3			4.08
	C	191	129	104	153	225	176	292	181	177	179	181	179	10.7			2.08
	D	192	129	106	157	227	172	300	188	178	180	178	178	10.3			2.12
Suloctidil (protonated)	A	189	117	104	152	283	179	178	180	178	177	178	181	57.0	43.12	7.89	2.16
	B	167	231	265	155	283	179	276	177	178	179	179	179	43.0			4.52
CP 894 S	A	335	200	180	159	32	70	192	187	186	184	186	185	40.9	43.12	3.86	2.12
	B	345	186	175	150	354	182	178	184	183	183	186	181	36.8			1.63
CP 1136 S (neutral)	A	330	183	170	160	—	—	—	—	—	—	—	—	26.0	23.92	7.89	1.50
	B	332	183	175	162	—	—	—	—	—	—	—	—	8.7			
	C	331	182	173	165	—	—	—	—	—	—	—	—	8.0			
CP 1136 S (protonated)	A	330	183	171	162	—	—	—	—	—	—	—	—	26.0	23.42	8.92	1.60
	B	332	184	174	160	—	—	—	—	—	—	—	—	8.7			
	C	332	181	173	167	—	—	—	—	—	—	—	—	8.0			

* The probability of existence or statistical weight is expressed as a percentage by application of the Boltzmann distribution.

† Hydrophilic (E_{tr}^{phi}) and hydrophobic (E_{tr}^{pho}) transfer energies (kcal/mole).

‡ Distance (Δ) between the hydrophilic and hydrophobic gravity centre (Å).

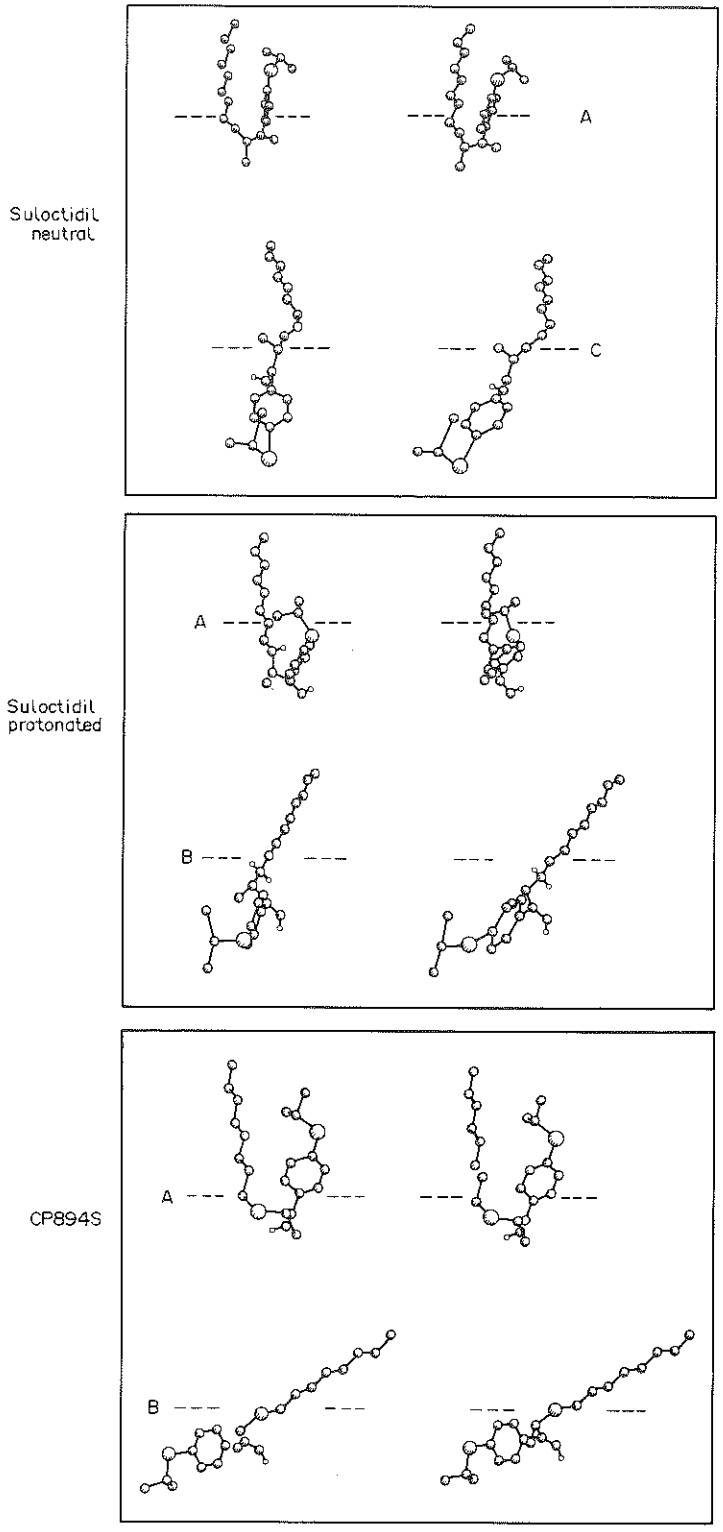

Fig. 2. Stereo-view of most probable conformers after the minimization procedure and orientation at the simulated membrane–water interface. The torsional angles are given in Table 2. Suloctidil, neutral: 43.9%, A; 10.7%, C. Suloctidil, protonated: 57.0%, A; 43.9%, B. CP 894 S: 40.9%, A; 28.8%, B.

Table 3. Parameters of the drug–lipid interaction in the phospholipid monolayer

		Interaction energy (kcal/mole) between lipid and drug			Mean area (Å²/molecule) occupied per molecule in lipid monolayer		
		Rigid DPPC	Fluid DPPC	DPPS	Rigid DPPC	Fluid DPPC	DPPS
CP 556 S (neutral)	A	−9	−8	−9	48	47	49
	C	−4	−5	−5	82	81	80
CP 556 S (protonated)	A	−10	−10	−16	42	43	43
	B	−6	−8	−12	78	77	77
CP 894 S	A	−15	−14	−14	40	39	43
	B	−15	−14	−14	70	71	72
CP 1136 S (neutral)	A	−14	−12	−14	44	43	48
CP 1136 S (protonated)	A	−16	−13	−20	48	49	42

Values of the interaction between lipid and drug, and of the mean area occupied by the drug in the monolayer were obtained as described in Materials and Methods using the most probable conformers calculated in Table 2 for the drugs, and those of ref. [17] for the lipids.

DISCUSSION

Table 2 indicates changes in the $\alpha_3, \alpha_4, \alpha_6$ and α_7 values for the neutral conformers of suloctidil whereas only α_2, α_3 and α_7 were modified in the protonated conformers. Because α_3 and α_4 have little influence on the final structure, it is suggested that α_7 depends on the charge of the amino group. For the conformers of CP 894 S, only α_5 and α_6 vary significantly. As already mentioned, application of the simplex minimization procedure reduces the number of conformers (Table 2). The three compounds studied share a common region (Fig. 1). The increase in hydrophobic transfer energy observed in suloctidil and CP 894 S is related to the presence of the *n*-octyl chain. Protonation of the amine residue increases the hydrophilic transfer energy by 1.03 kcal/mole in both suloctidil and CP 1136 S, and produces a different conformer. This change in transfer energy modifies the distance between the hydrophilic and hydrophobic gravity centres, and consequently the position of the drug molecule at the interface and the lipid–drug interaction (Table 3).

For a given conformer, the interaction energy with fluid and solid DPPC is equal. When the conformer is in the neutral form, the same interaction energy with the different lipids is obtained. The interaction

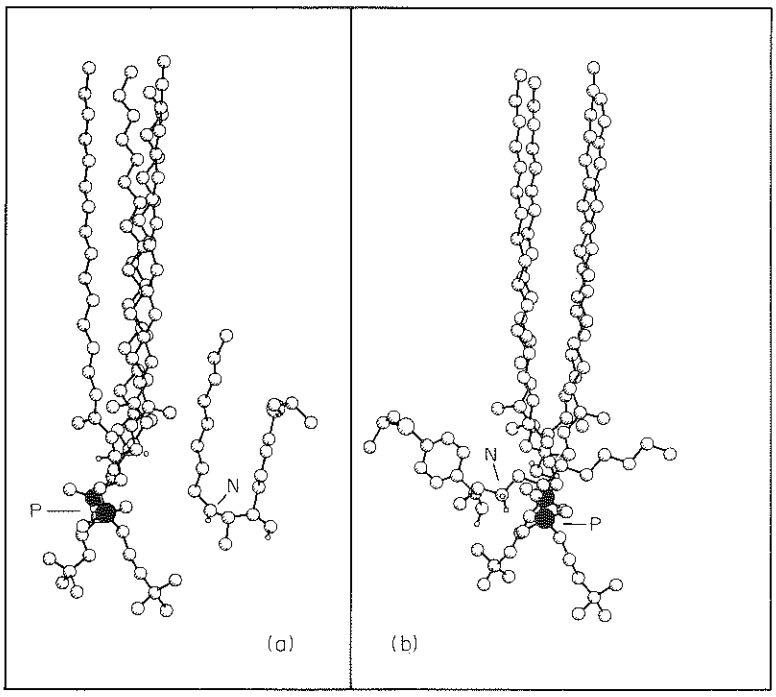

Fig. 3. Configuration of suloctidil interacting with fluid DPPC. (a) Suloctidil (A); (b) suloctidil (C). Open circles refer to carbon, oxygen and nitrogen atoms; black circles represent phosphorus atoms, and S sulphur atoms.

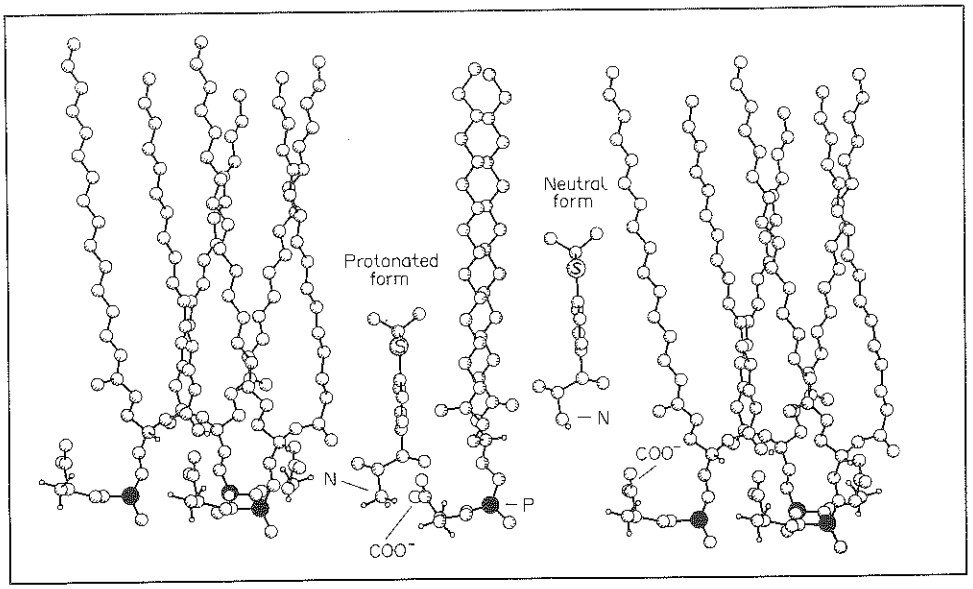

Fig. 4. Configuration of CP 1136 S interacting with DPPS. Left: protonated form (A form); right: neutral form (A form). Symbols for the various atoms have been defined in Fig. 3.

energy of a charged conformer and DPPS is 4–6 kcal/mole larger than the interaction energy of the same conformer with the neutral phospholipids, reflecting the importance of the electrostatic interaction. The interaction energy with DPPS of the most probable conformers of suloctidil in both the neutral (A and C) and protonated (A and B) forms differs by 4 kcal/mole, independent of the phospholipid. No variation of interaction energy is observed for CP 894 S. The reasons for the variation of the interaction energy for the different conformers of suloctidil are unclear, but they do not seem to be related to the conformation of the drug and/or the protonation of the amino group. The mean molecular area occupied by the different conformers in the lipid matrix are either smaller than 49 $Å^2$/molecule or greater than 70 $Å^2$/molecule. The mean molecular area of a given conformer depends mainly on the conformation of the isolated molecules and is only slightly influenced by the nature of the lipids. The perturbation of the lipid matrix induced by a given conformer is related, among other factors, to its molecular area [22]. CP 1136 S has only a marginal effect on lipid dynamics when incubated at 10^{-4} M with DPPC liposomes [16]. It may be suggested that the mean molecular area calculated for this compound is too small to perturb the lipid matrix in the case of a neutral lipid. By contrast, suloctidil and CP 894 S produce a dose-dependent downward shift of the transition temperature of DPPC liposomes, as well as fluidization of both the solid and fluid lipid phases [16]. It is tempting to suggest that the perturbation of the lipid matrix occurs when these compounds are in the extended form. Finally, the importance of both the interaction energy and the mean molecular area occupied per drug molecule can be stressed in the case of CP 1136 S. As long as the interaction energies between drug and lipid are of the same order, no perturbation of the lipid dynamics is expected for this compound, due to its small mean molecular area. However, increasing the interaction energy between drug and lipid above the lipid–lipid value should lead to a perturbation of the lipid dynamics by promoting a complex between the drug and the lipid. This has been demonstrated for CP 1136 S in experiments where the incorporation of phosphatidylserine in the DPPC matrix induced fluidization [16].

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