The disordering effect of SARMs on a biomembrane model

Alma Jessica Díaz-Salazar[a], Arián Espinosa-Roa[b], Enrique Saldívar-Guerra[c], Rosendo Pérez-Isidoro[c]

[a]Laboratorio de Bio-fisicoquímica. Departamento de Fisicoquímica, Facultad de Química, Universidad Nacional Autónoma de México, México City 04510, México.

[b]CONAHCyT-Centro de Investigación en Química Aplicada (CIQA), unidad Monterrey. Alianza Sur no.204, Parque de Investigación en Innovación Tecnológica (PIIT), km 10 autopista internacional Mariano Escobedo, C.P. 66628, Apodaca, Nuevo León, México.

[c]Centro de Investigación en Química Aplicada (CIQA), Enrique Reyna, 140, 25294 Saltillo Coahuila, México.

Email addresses: jessica.diaz@quimica.unam.mx (A. J. Díaz-Salazar); arian.espinosa@ciqa.edu.mx (A. Espinosa-Roa); enrique.saldivar@ciqa.edu.mx (E. Saldívar-Guerra); rosendo.perez@ciqa.edu.mx (R. Pérez-Isidoro),

1. ^{1}H NMR spectrum for SARMs

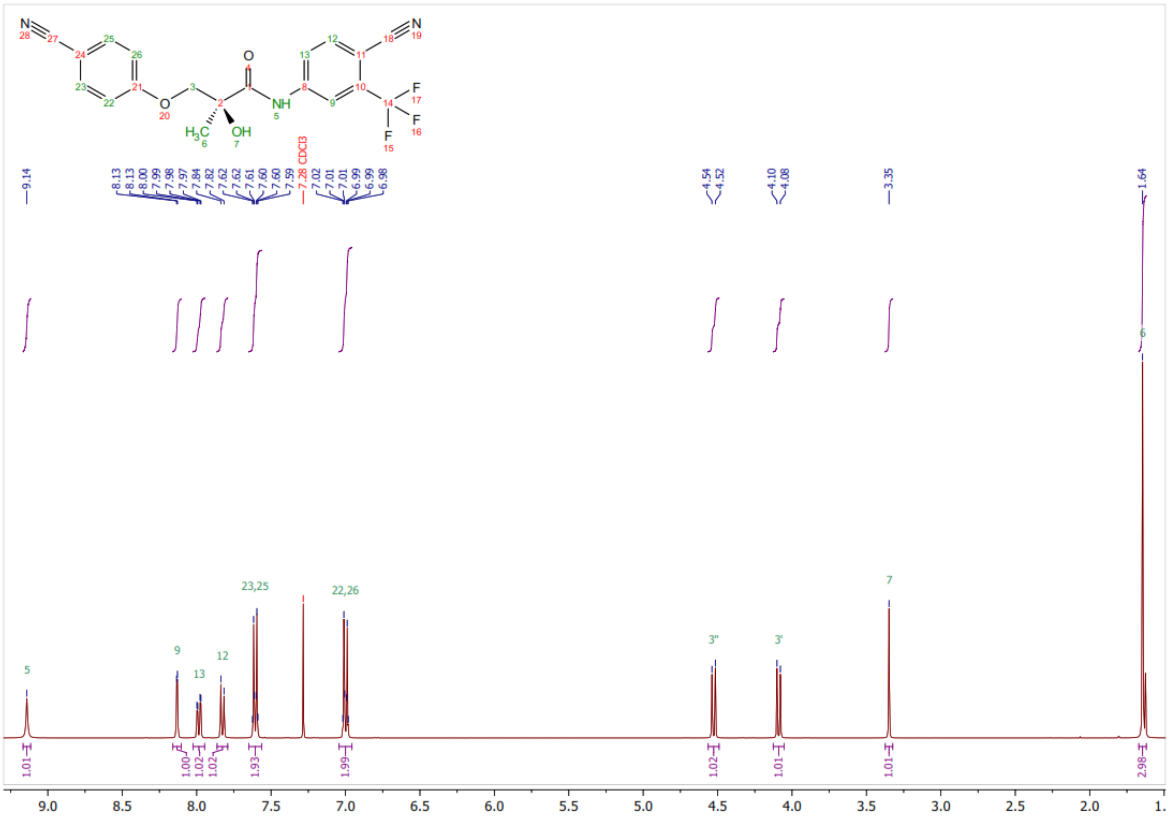

Figure S1. ^{1}H RMN of ostarine. $CDCl_3$, 500MHz.

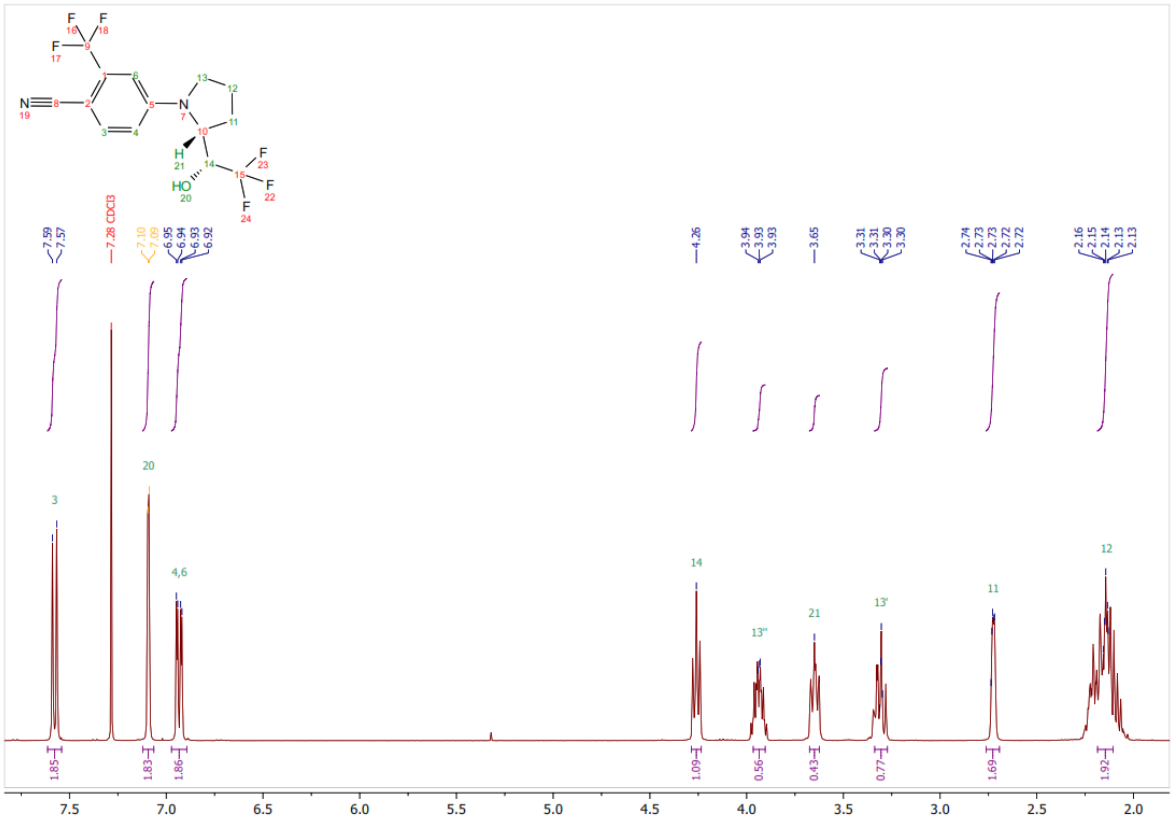

Figure S2. ^{1}H RMN of ligandrol. $CDCl_3$, 500MHz.

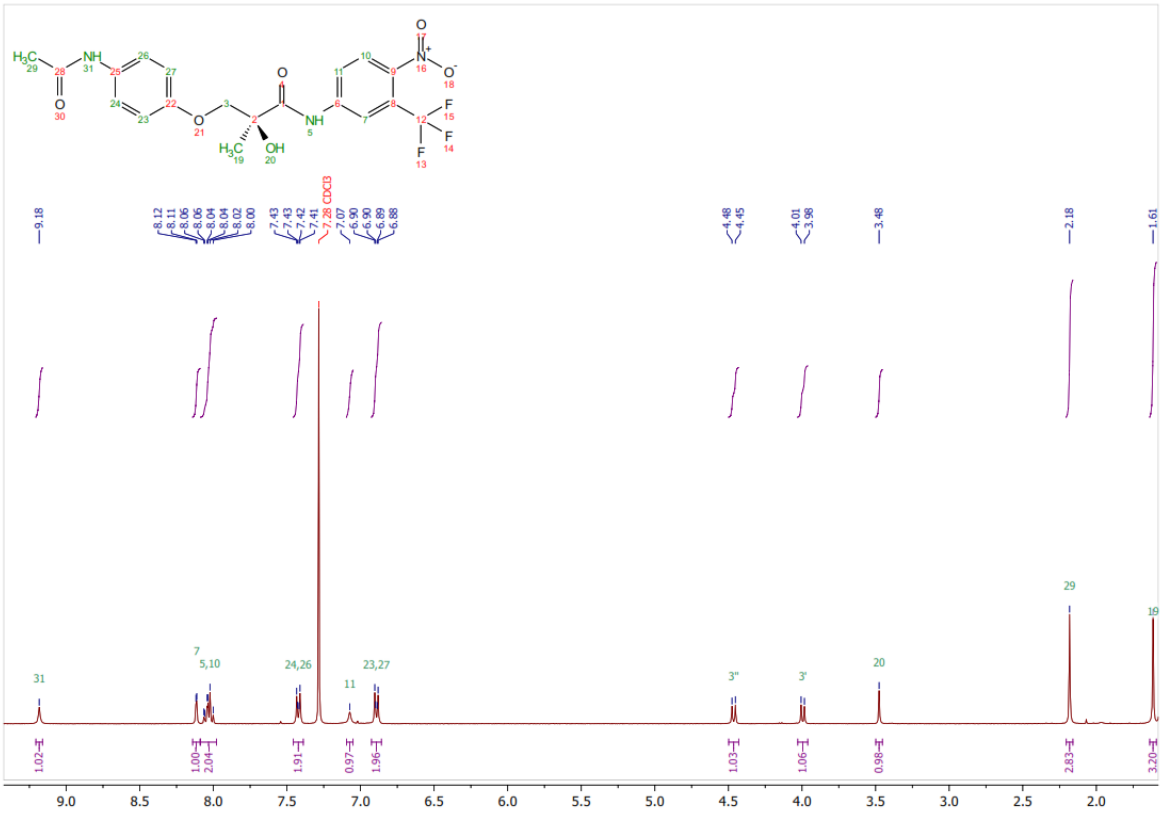

Figure S3. ^{1}H RMN of andarine. $CDCl_3$, 500MHz.

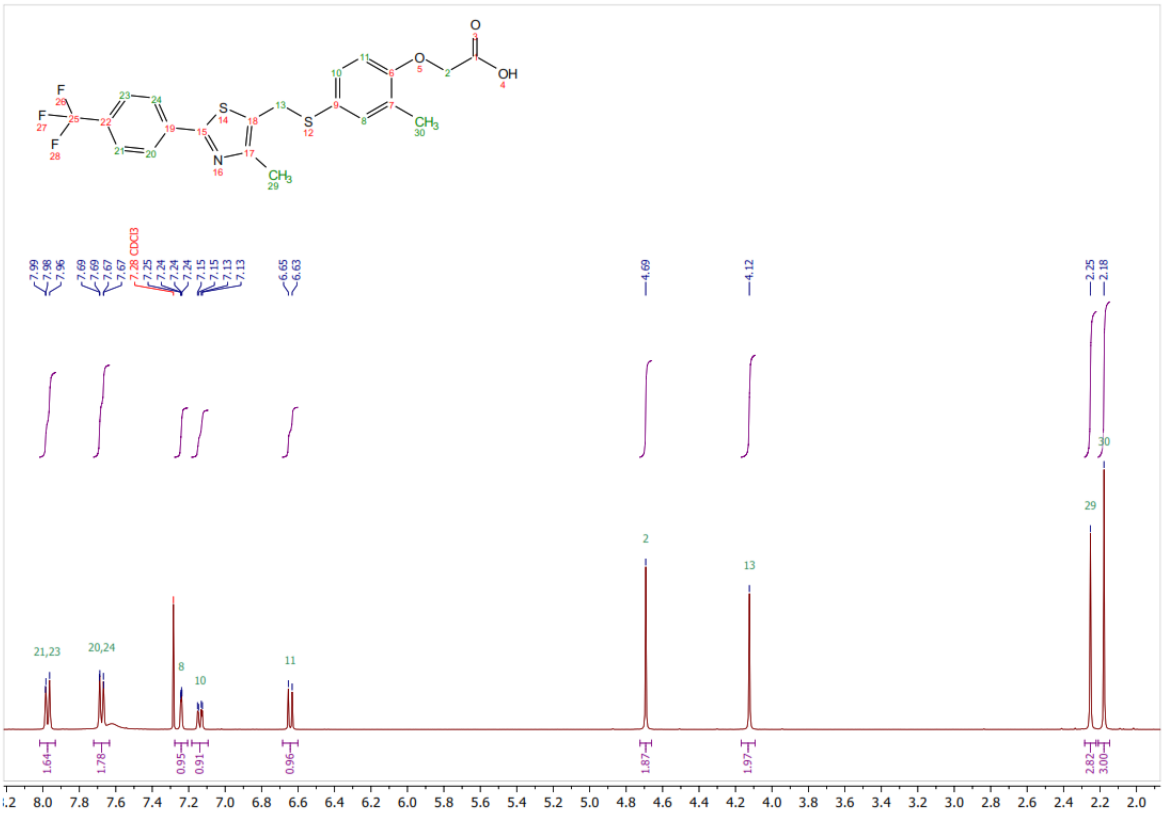

Figure S4. ^{1}H RMN of cardarine. $CDCl_3$, 500MHz.

2. DSC analysis for DMPC liposomes under the effect of SARMs

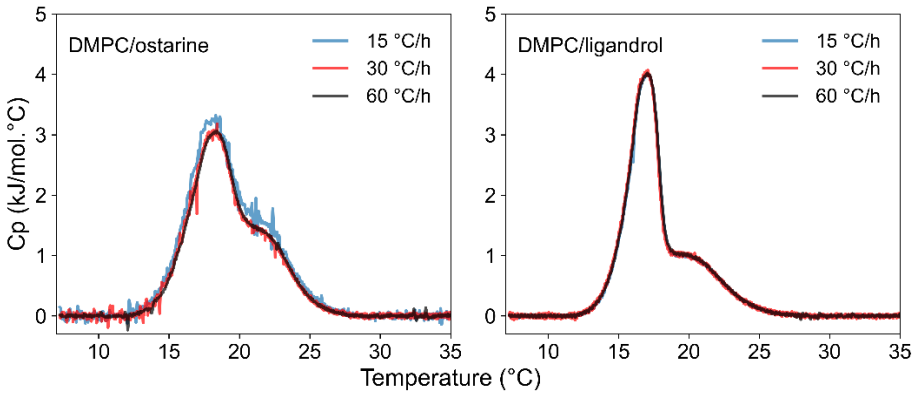

Figure S5. Calorimetric profiles of DMPC LUVs under the effect of ostarine and ligandrol dispersed in HEPES/NaCl buffer at pH 7.4 and obtained at three different heating scan rates, 15, 30, and 60 °C/h.

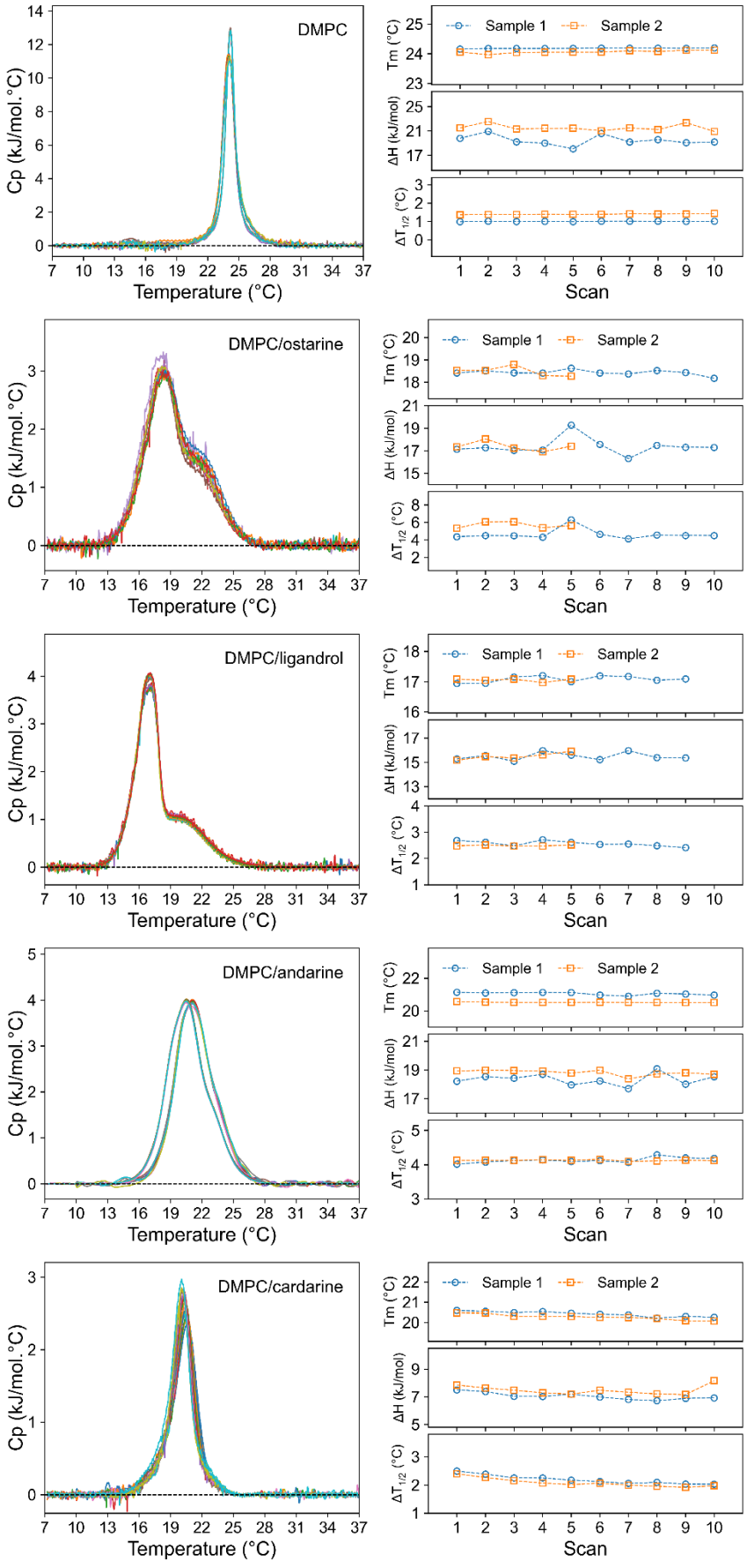
DMPC
DMPC/ostarine
DMPC/ligandrol
DMPC/andarine
DMPC/cardarine
Cp (kJ/mol.°C)
Temperature (°C)
Sample 1
Sample 2
Tm (°C)
ΔH (kJ/mol)
ΔT1/2 (°C)
Scan

Figure S6. Experimental DSC profiles (left panel) and the calorimetric parameters: T_m, ΔH and $\Delta T_{1/2}$ (right panel) for DMPC LUVs under the effect of ostarine, ligandrol, andarine, and cardarine dispersed in HEPES/NaCl buffer at pH 7.4. DSC scans were performed in heating mode. The system was measured in duplicate form, considering two independent sample preparations. All scans (at least fourteen) from the two experiments are plotted and processed for estimating calorimetric parameters.

3. Intermolecular interactions DMPC/SARMs by DFT

Intermolecular interactions are a fundamental phenomenon in chemical reactivity and play a crucial role in a wide range of chemical and biological processes. These interactions, which occur between molecules without a covalent chemical bond, are responsible for the physical and chemical properties of organic and inorganic compounds. The study of these intermolecular forces has been a central objective of biophysics since their manipulation has a direct impact on the understanding of biological phenomena.

In this context, the present study also attempts to investigate and analyze the intermolecular interactions in the DMPC/SARM system using theoretical methods. Through this approach, we seek to shed light on the underlying forces that govern these interactions, understanding their impact at the initial stages of intermolecular contacts.

First, a model was generated in which the DMPC has 5-carbon alkyl chains to simplify the calculation while retaining part of the hydrophobic chains. Thereafter, the geometries of ostarine, ligandrol, andarine, and cardarine were optimized, and the molecules were positioned near the R-phosphocholine model using HF/6-31g(d) calculations. Figure S7 displays the structures of R-phosphocholine/drugs under interactions.

This theoretical approach allows us to explore the electrostatic interactions between the headgroup of a simplified DMPC model and the drugs. These calculations inform us about the possible initial electrostatic interactions between the headgroup of DMPC and the drugs, which can be correlated with the experimental observations. However, it is important to note that these interactions do not represent the full complexity of interactions within the lipid bilayer, particularly the interactions with the hydrophobic core.

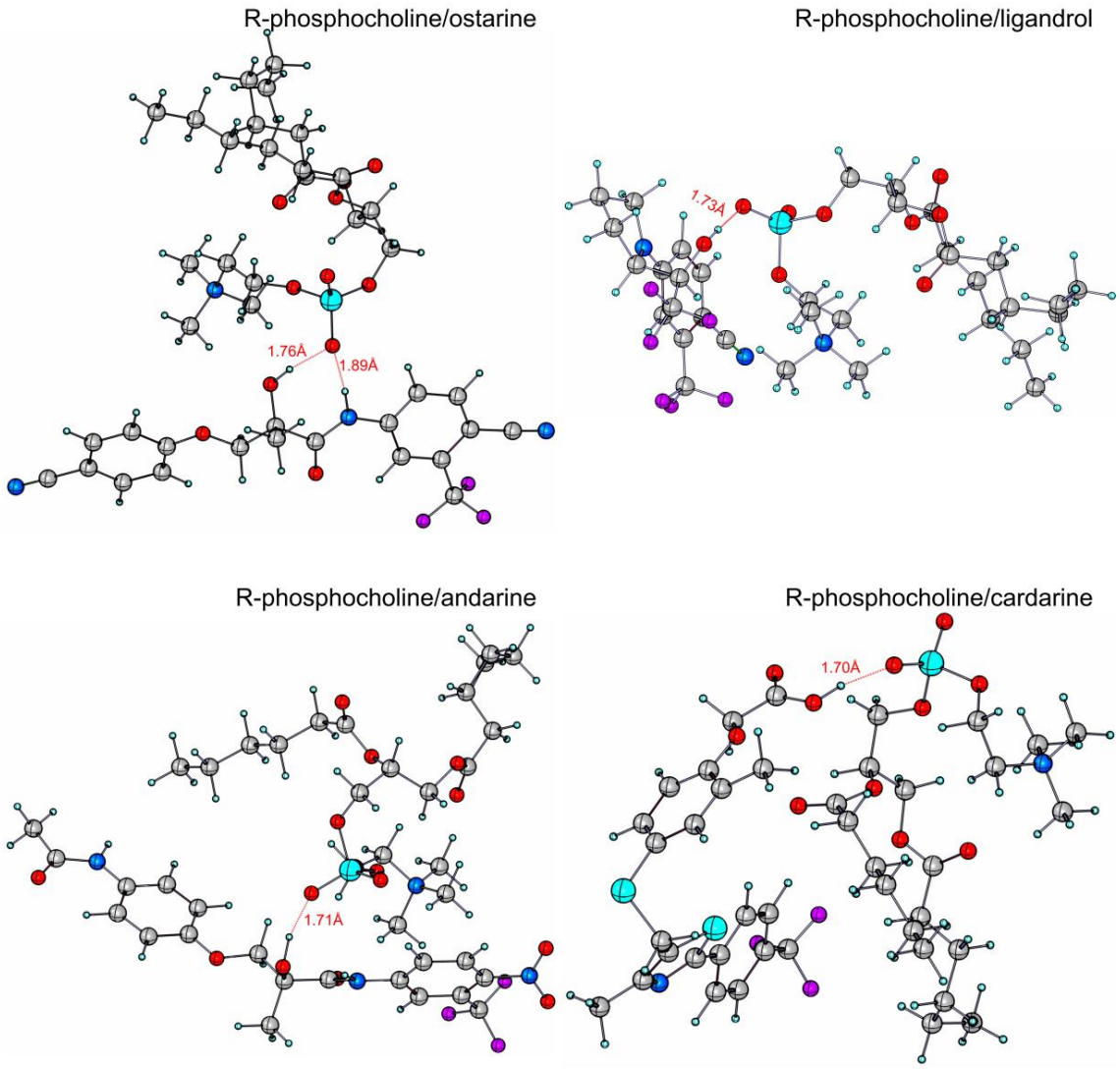

Figure S7. Estimated interactions (red dot lines) of R-phosphocholine/ostarine, R-phosphocholine/ligandrol, R-phosphocholine/andarine, and R-phosphocholine/cardarine by HF/6-31g(d) calculations.

We identified the formation of hydrogen bonds between hydroxyls of SARMs (and cardarine) and O=P belonging to the lipid choline (polar heads of the lipid). These hydrogen bond interactions contribute to the stabilization of the initial relative conformation between the investigated drugs and the lipid model. For the case of the R-phosphocholine/ostarine system, there is a double interaction between the proton of the secondary amine and the same

oxygen of the choline (Figure S7). It is remarkable that in the case of the MEP calculated for ostarine, two zones of low electron density can be observed, indicating the presence of protons that in fact enable the interaction via hydrogen bonds in the R-phosphocholine/ostarine system. The distances of these hydrogen bonds are 1.76 Å for O-H...O=P, and 1.89 Å for N-H...O=P. For the R-phosphocholine/ligandrol system (Figure S7), an interaction between the hydroxyl (-OH) and the phosphate of R-phosphocholine of 1.73 Å was calculated. This interaction is likely promoted by an electron density in the ligandrol hydroxyl. For the case of R-phosphocholine/andarine, the hydroxyl positioned on the chiral carbon of andarine, which in the MEP shows a deficiency in electron density, forms a hydrogen bond with the oxygen belonging to the R-phosphocholine (Figure S7). The distance of such interaction is 1.71 Å. It should be noted, as expected, that the geometry of the molecule is changed by this interaction with respect to that previously described (Figure S7).

Finally, as we can see from the MEP for the case of R-phosphocholine/cardarine, the calculated interaction is 1.70 Å between the carboxylic acid proton and the choline group of R-phosphocholine, which represents an electron density deficiency (Figure S7). In all cases, we noticed a slight reorganization in the local lipid structure in the presence of SARMs (and cardarine). This behavior could correlate with the changes in compactness and organization that SARMs could produce in more complex biological systems such as the plasma membrane.