Molecular interaction studies of phosphatidylcholine as drug delivery substrate for asenapine maleate

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Phospholipid complexes have become promising delivery systems for delivery of drugs with poor bioavailability like asenapine maleate. To improve the bioavailability of asenapine maleate, phospholipid complex was chosen for the drug with Phospholipon 90 G (phosphatidylcholine). The automated molecular docking calculation for asenapine and maleate individually with phospholipid was performed by AutoGrid 4.2.6, a docking program. The van der Waals hydrogen bond, electrostatic potential energy and desolvation free energy grid maps were calculated by AutoDock parameter set- and distance-dependent dielectric functions respectively. The change in free energy was specifically seen for the complex between asenapine and phospholipid which exhibited least binding docking energy of –3.86 kcal/mol among the summary of 25 poses. Finally, molecular docking studies confirmed that the asenapine is able to make a complex with phosphatidylcholine, plausibly on account of its structural similarity with phospholipid in its physiochemical properties.

Keywords: Asenapine, binding docking energy, maleate, phosphatidylcholine.

PHOSPHOLIPID complexes have become promising delivery systems for delivery of drugs with poor bioavailability. Phospholipid complexation has evolved as a powerful technique to improve bioavailability and thus the therapeutic efficacy of several active constituents. The process involves incorporation of active constituents (synthetic drugs or herbal constituents) into their structure. The ampiphilic nature and unique structure of phospholipids help in improving permeability as well as solubility of the active ingredients[1]. The composition of phospholipids makes them compatible with components of cell membranes of humans[2]. The major lipid constituents present in the eukaryotic cell membranes include phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine and cardiolipin. Soyabean phospholipids are widely used because of high proportions of phosphatidylcholine (76%) with a high content of polyunsaturated fatty acids like linoleic acid (70%), linolenic acid and oleic acid[3]. The phospholipid complexation is a chemical interaction which involves establishment of a unique chemical bond between the lipid and drug molecules[4]. The thermal analysis study of the complex and the comparative study with pure drug, phospholipid and physical mixture establish the chemical bond formation in the complex. Various studies reported the formation of hydrogen bond or van der Waals forces as the reason behind the formation of the complex[5,6]. Figure 1 shows the phosphatidylcholine and asenapine maleate.

The drug polymer interactions are important in any pharmaceutical process. To improve the pharmacological efficiency of the drug molecules, existing drug candidates are designed to be delivered with pre-determined specificities. Carrier systems have become one of the best delivery systems for drug candidates serving the purposes of enhanced bioavailability and stability.

The choice of polymer is crucial in such instances. The best option would be biodegradable polymers, which have no toxicity[7–10]. Designing the delivery system to target the active constituent to the biological site was made successful by tailoring the carrier.

Computer molecular modelling is an important part of present-day research. The behaviour of carriers towards active ingredients can be more accurately examined by computer modelling. The number of experimental tests can also be reduced by using computer modelling. The polymer surfaces that interact with active ingredients are analysed to estimate the drug polymer complex formation. The properties of binding sites are studied in the form of physico-chemical character of the interface[11–13] like type of chemical groups, hydrophobicity, electrostatic interactions and hydrogen bonds. Efficient binding is contributed by hydrophobic interactions and electrostatic forces[14,15]. From the interface, drug association, solvent molecules are largely excluded (loss of receptor solvent). A large desolvation penalty introduced by the removal of water molecules (highly polarizable solvent to accommodate a nonpolarizable probe sphere) favours electrostatic interaction. Binding and offset by hydrophobic and attractive electrostatic contribution are needed to overcome desolvation penalty. This contribution can be calculated within the assumption of continuum electrostatics[16]. Solvent and solute regions are two partitions of the system that are assigned with two different dielectric constants. The electric displacement vectors of a location-dependent dielectric constant as an integral over three-dimensional space[17] are the terms to express electrostatic energy of the receptor in the solution. In the present study, docking study was employed for energy and structure characterization of formulation based on non-bond interaction between active ingredients and biodegradable polymeric carriers.

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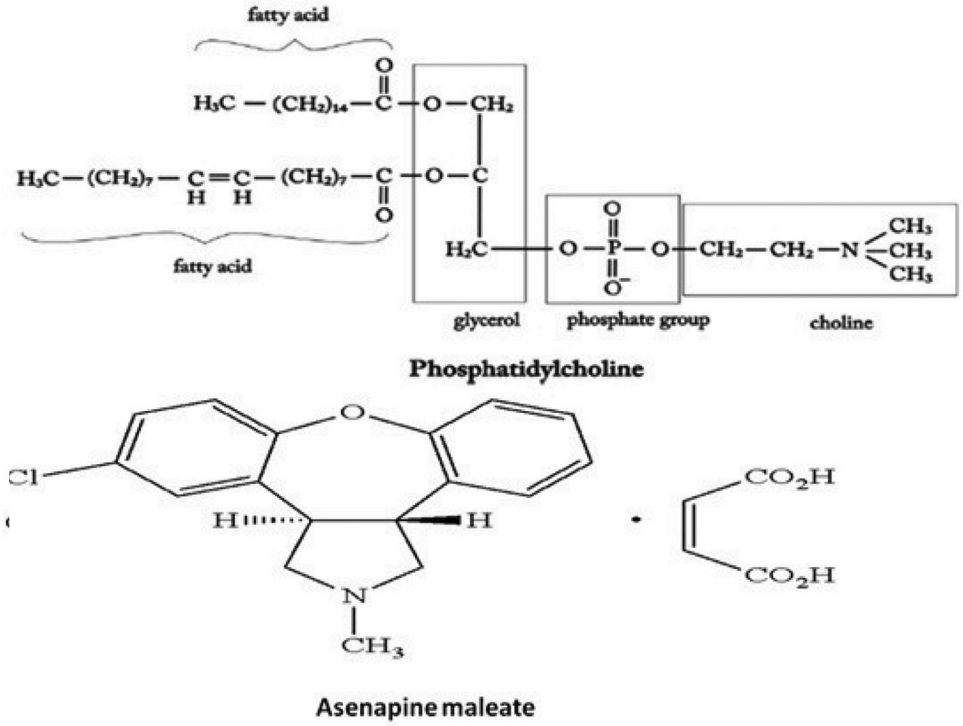

Figure 1. Structure of asenapine maleate and phosphotidylcholine.

Computer-assisted molecular modelling and simulation studies were employed for analysis, to enable interaction between the asenapine maleate and phosphatidylcholine during complexation process. The chemical structure of asenapine maleate was derived from the crystal structure (PubChem CID: 11954293). The molecular structure of the phosphatidyl choline (phospholipid) was derived from the crystal structure (PubChem CID: 5287971). Using Open Babel GUI software, the ligand and macromolecules were converted in the protein data bank file.

The molecular dynamics program Python 2.5.2 was used to calculate the energy minimization. The docking program AutoGrid 4.2.6 was employed for performing the automated molecular docking calculation. Affinity (grid) maps of 100 Å × 100 Å × 100 Å grid points and 0.336 Å spacing for asenapine and 0.403 Å spacing for maleate were created using the Autogrid program and was used to generate around the drug molecule for covering the entire molecule with phospholipids for calculating the bond individually. Essential hydrogen atoms, solvation parameters and Kollman united atom type charges were included with the support of AutoDock tools[18]. The van der Waals hydrogen bond, electrostatic potential energy and desolvation free energy grid maps were calculated by AutoDock parameter set- and distance-dependent dielectric functions respectively.

The grid box was occupied by one molecule of a macromolecule (phosphatidylcholine) with free 3D-movement of asenapine maleate and maleate individually for evaluating the interaction between them. Then Lamarckian genetic algorithm was used to find the different docked conformations of asenapine-macromolecules and maleate-macromolecules. Detailed analysis of the asenapine–phosphatidylcholine and maleate–phosphatidylcholine interactions was executed using AutoGrid 4.2.6. The final coordinates of the asenapine (free base), maleate and phospholipid were saved in the protein data bank (.pdb) file format to analyse the changes in energy related-parameters. Initial position, orientation and torsions of the ligand molecules were fixed randomly. During docking studies, all rotatable torsions were released.

Individual docking studies were performed for asenapine and maleate with phosphatidylcholine to study the region of interaction. The asenapine maleate is a salt of asenapine with maleate through ionic bonding which is evident in the structure.

The molecular docking and simulation studies identified possible site(s) of interaction between the asenapine (freebase)–phospholipid analogue (i.e. phosphatidylcholine) and maleate–phospholipid analogue. Mechanistically, the studies identified formation of weak intermolecular electrostatic interactions between the drug and lipid. Figure 2 shows the docking patterns of the asenapine, where the wired sphere represents the electrostatic interaction (ionic interaction) between the tertiary amine group of the asenapine with ether group of the macromolecule.

This can be attributed to electrostatic potential and desolvation potential in the activation energy of asenapine

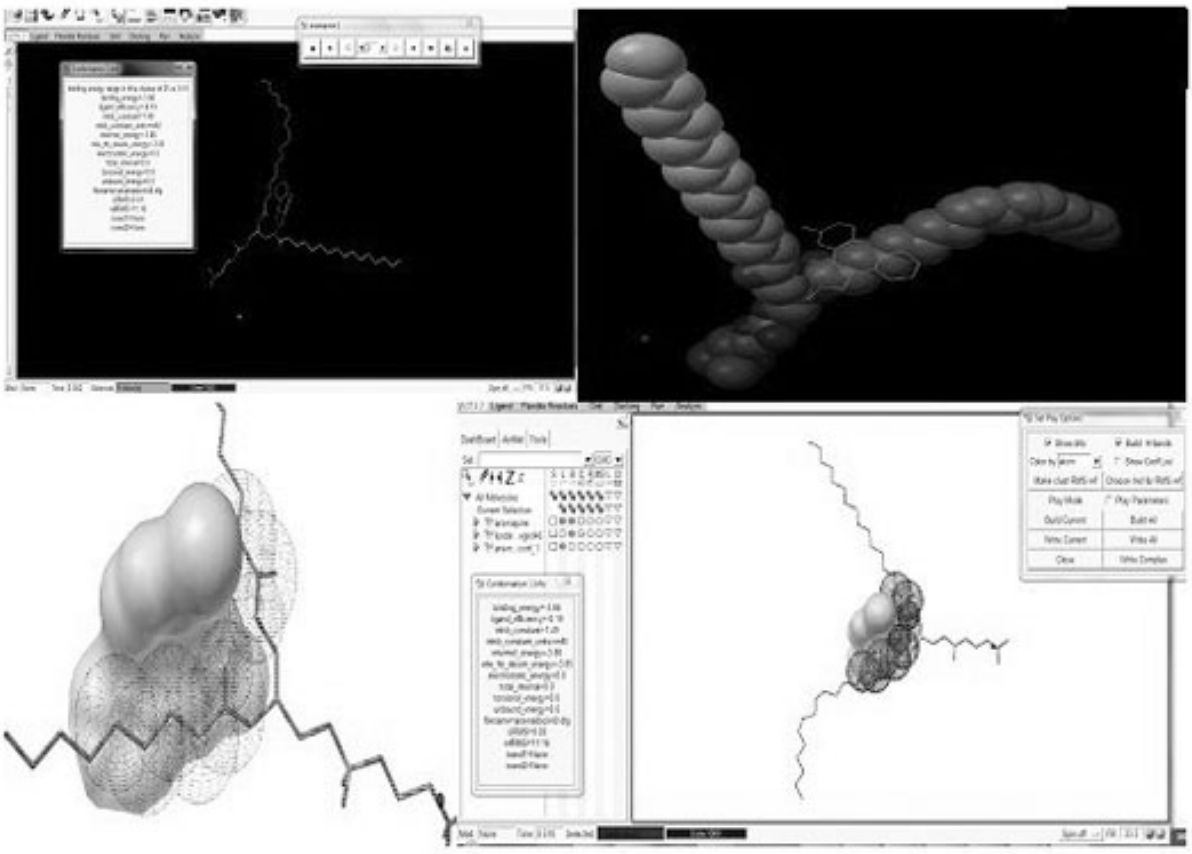

Figure 2. Molecular docking patterns of asenapine and phosphotidylcholine.

(freebase) upon complexation with macromolecule by forming an electrostatic ionic bond, leading eventually to minimization of the total free energy to attain thermodynamically stable confirmation.

Free energy changes were specifically observed for the complex exhibiting the least binding docking energy of –3.86 kcal/mol from the summary of 25 poses of asenapine–phosphatidylcholine (solid lump is asenapine). Comparison with finite difference solutions of the Poisson equation for a group of small molecules distributed over the binding site of macromolecules for a sum of 52 receptor-ligand complexes tested the accuracy of this approximation. Table 1 expressed the 25 poses of asenapine–phosphatidylcholine binding energy.

Mechanistically, studies between maleate and phosphatidylcholine revealed formation of intermolecular interactions between the drug and phospholipid. Figure 3 shows the docking patterns of the maleate from asenapine maleate, where the wired sphere represents the interaction between the carboxylic groups of the maleate with phosphate group of the macromolecule.

This can be attributed to the van der Waals hydrogen bonding, electrostatic potential and desolvation potential in the activation energy of the maleate upon complexation with the macromolecule leading eventually to minimization of the total free energy to attain thermodynamically stable confirmation. Free energy changes were specifically observed for the complex exhibiting the least binding docking energy of –1.61 kcal/mol from the summary of different poses of maleate–phosphatidylcholine (solid lump is maleate). The accuracy of this approximation was tested.

Results show that the asenapine interaction has the least binding energy than maleate interaction with macromolecules. It prefers more probability of free base complex with phosphatidylcholine.

Affinity (grid) maps of 101 Å × 101 Å × 101 Å grid points and 0.336 Å spacing for asenapine (free base) were generated using the Autogrid program for calculating covalent affinity of the ligand with macromolecules. Atom type number 6 (asenapine ligand types A, C, N, OA, Cl and Z respectively) was used to calculate a covalent affinity grid map. Maximum and minimum partial atomic charge was found to be +0.410 e and –0.624 e respectively. Affinity (grid) maps of 33.6 Å × 33.6 Å × 33.6 Å grid points and 0.5 Å spacing were generated using the Autogrid program for defining coordinates affinity of macromolecules with the ligand. Atom ID 4 (phospholipid macromolecule types C, N, OA and P respectively) was used to calculate a coordinate affinity grid map. Totally

Table 1. Asenapine–phosphotidylcholine binding energy

Poses/rank	1_1	1_2	1_3	1_4	1_5	1_6	1_7	1_8	
Binding energy	–3.86	–3.86	–3.86	–3.86	–3.86	–3.86	–3.86	–3.85	
kI (mM)	1.49	1.49	1.49	1.49	1.49	1.49	1.49	1.4	
Intermolecular energy	–3.86	–3.86	–3.86	–3.86	–3.86	–3.86	–3.86	–3.85	
Internal energy	0	0	0	0	0	0	0	0	
Torsional energy	0	0	0	0	0	0	0	0	
Unbound extended energy	0	0	0	0	0	0	0	0	
Cluster RMS	0	0.01	0.03	0.01	0.03	0.04	0.01	0.02	
Ref RMS	11.16	11.16	11.17	11.16	11.16	11.15	11.16	11.17	
Poses/rank	1_9	1_10	1_11	1_12	1_13	1_14	1_15	1_16	
Binding energy	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	
kI (mM)	1.49	1.49	1.49	1.5	1.5	1.5	1.5	1.51	
Intermolecular energy	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	
Internal energy	0	0	0	0	0	0	0	0	
Torsional energy	0	0	0	0	0	0	0	0	
Unbound extended energy	0	0	0	0	0	0	0	0	
Cluster RMS	0.07	0.03	0.05	0.06	0.07	0.08	0.07	0.63	
Ref RMS	11.14	11.16	11.15	11.16	11.18	11.14	11.18	11.09	
Poses/rank	1_17	1_18	1_19	1_20	1_21	1_22	1_23	1_24	1_25
Binding energy	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85
kI (mM)	1.51	1.51	1.51	1.51	1.51	1.51	1.51	1.51	1.51
Intermolecular energy	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85	–3.85
Internal energy	0	0	0	0	0	0	0	0	0
Torsional energy	0	0	0	0	0	0	0	0	0
Unbound extended energy	0	0	0	0	0	0	0	0	0
Cluster RMS	0.62	0.63	0.08	0.65	0.77	0.83	0.83	0.84	0.73
Ref RMS	11.09	11.09	11.18	11.08	11.04	11.01	11.01	11.01	11.06

42°C atom, 1 N atom, 8 OA atom and 1 P atom types were present in the receptor. Electrostatic potential and desolvation free energy were calculated. Covalent well's half-width was found to be 5 Å and covalent barrier energy was found to be 1000 kcal/mol. Based on this, covalent attachment point was positioned at 5.258, –4.652, 0.361 for *X*, *Y* and *Z* coordinates in the grid map.

The important parameter for solving a majority of the biological problems is the establishment of the influence of the ligand and macromolecule on pair-wise electrostatic interaction (PEI) at the interface. In the present study, the PEI energy was calculated at the interface of asenapine (ligand) and the macromolecule. The contribution of the macromolecule was evaluated by non-local electrostatics (interfacial electrochemical systems). The macromolecule orientation polarization was correlated by the network hydrogen bonds in asenapine and maleate. Coulomb's law was used for analytical expression of PEI. This uses the distance-dependent dielectric function. The asymptotic and numerical analysis performed, confirmed several features of dielectric heterogeneity at the asenapine (ligand) and macromolecule interface. The values of the dielectric function for charges located closely to the interface were determined to be smaller than those determined by the classical method. In the classical method, the macromolecule was considered as the uniform dielectric medium with high dielectric constant. The linear and nonlinear distance-dependent dielectric functions were used to consider the effect of the solvent and to evaluate the electrostatic interactions in biopolymers. Distance-dependent dielectric function of Mehler and Solmajer was calculated[19]. Table 2 showing the distance and its dielectric function value indicates the strong dependence of the dielectric function upon the distance *d* between two interacting charges. It can be proposed by the asymptotic and numerical analysis carried out in the study that low-dielectric boundary macromolecule layer is the major factor. It determines the values of the effective dielectric function for the short-distance electrostatic interactions in close proximity to the asenapine-macromolecule interface.

Lowest pairwise interaction energy within 0.5 Å ('smoothing') was calculated for A, C, N, OA, Cl and Z of ligand individually with C, N, OA and P of macromolecules (phospholipid). Over 1,030,301 elements, *r*-value of 0 to 5.8 grids for phospholipid macromolecule types C, N, OA and P with 52 receptor atoms were used for calculations. The high value of the PEI energy at the asenapine-macromolecule interface was estimated in the framework. In fact, our analysis showed that the PEI energy for the short-distance charges located in close proximity to the asenapine-macromolecule interface

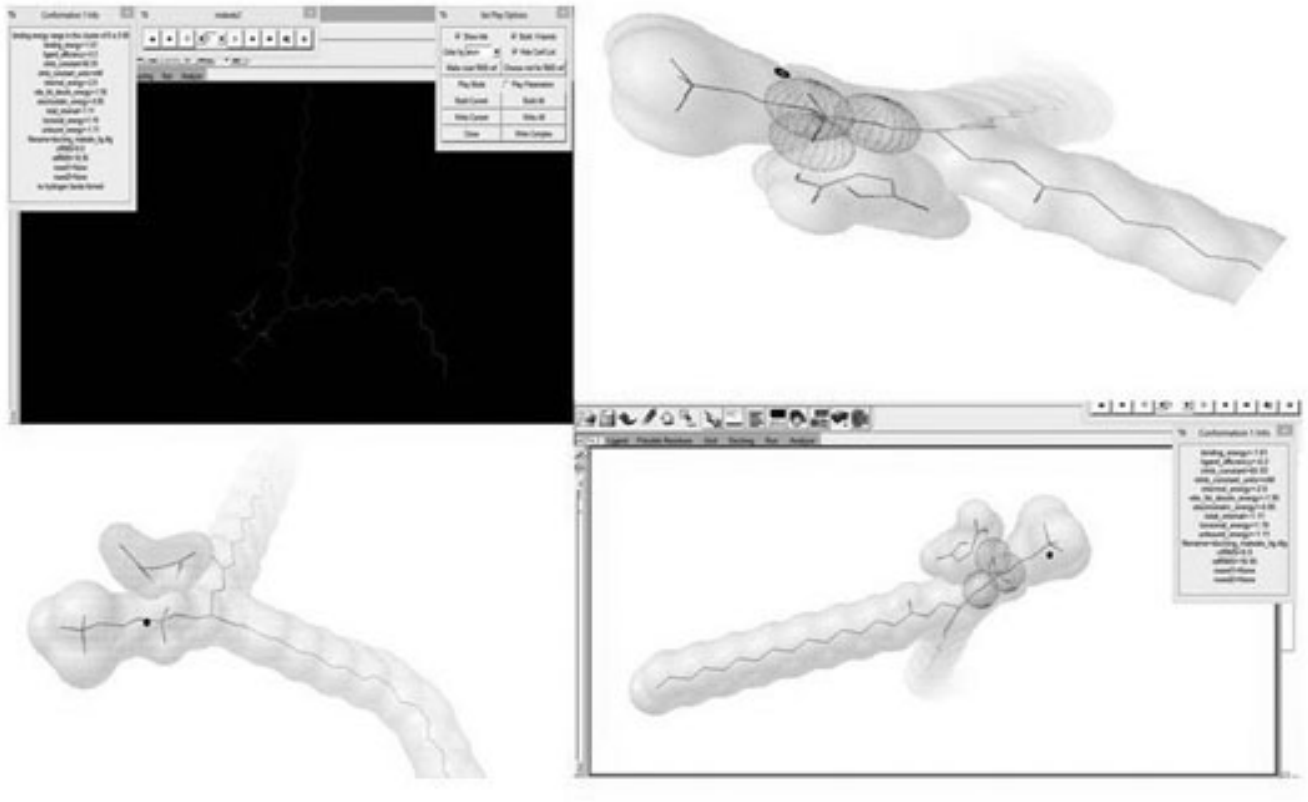

Figure 3. Molecular docking patterns of maleate and phosphotidylcholine.

Table 2. The distance dependent and dielectric function

Distance	Dielectric function	Distance	Dielectric function	Distance	Dielectric function	Distance	Dielectric function	Distance	Dielectric function
0	1	1	4.47	2	8.36	3	13.07	4	18.58
0.1	1.63	1.1	4.82	2.1	8.79	3.1	13.58	4.1	19.17
0.2	1.91	1.2	5.18	2.2	9.23	3.2	14.11	4.2	19.77
0.3	2.21	1.3	5.55	2.3	9.68	3.3	14.64	4.3	20.38
0.4	2.51	1.4	5.93	2.4	10.14	3.4	15.18	4.4	20.99
0.5	2.82	1.5	6.31	2.5	10.61	3.5	15.73	4.5	21.61
0.6	3.13	1.6	6.7	2.6	11.08	3.6	16.28	4.6	22.23
0.7	3.45	1.7	7.1	2.7	11.57	3.7	16.85	4.7	22.86
0.8	3.78	1.8	7.51	2.8	12.06	3.8	17.42	4.8	23.5
0.9	4.12	1.9	7.93	2.9	12.56	3.9	18	4.9	24.14
								5	24.78

Table 3. Asenapine different energy level

Grid map	Atom type in ligand	Minimum energy (kcal/mol)	Maximum energy (kcal/mol)
1	A	–0.32	200,000.00
2	C	–0.35	200,000.00
3	N	–0.35	200,000.00
4	OA	–0.41	200,000.00
5	Cl	–0.45	200,000.00
6	Z	0.17	1,000.00
7	E (electrostatic potential)	–31.23	15.30
8	D (desolvation potential)	0	0.45

adopts the values. Minimum and maximum energy were calculated for 8 grids which is tabulated in Table 3. Thus, it can be assumed that charged groups on the asenapine surface and their distribution determine a number of salt bridges. It is not random and is a result of the favourable electrostatic interactions associated with an additional driving force in asenapine folding.

Electrostatic energy is inversely proportional to the dielectric constant of the medium. Ionic interactions may take place between cationic groups, for example, between N-terminal amines and anionic groups like ester. As discussed earlier, these ionic interactions, and thereby electrostatic energy, are inversely proportional to the dielectric constant.

The results suggest that electrostatic interactions play a prominent role in phospholipid folding. This provides additional support to the assumption that specific distribution of the charged and amine residues along ester macromolecule may result in unique folding. When larger number of molecules of phospholipid interact with asenapine maleate then there may be chances of interaction between maleate group of the active ingredient and phospholipid. Successful drug delivery is based on special properties of asenapine–phosphatidylcholine tailored for a given biological target.

The molecular docking studies confirm that the asenapine is able to form a complex with phosphatidylcholine possibly owing to its structural similarity with phospholipid in its physiochemical properties.

Conflict of interest: The authors declare that there is no conflict of interest.

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