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Silibinin-saturated fatty acid bioconjugate / natural cyclodextrin interactions: molecular modeling, docking and QSPR experiments

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Abstract

Silibins (silybins) are well known flavonolignans from milk thistle (*Silybum marianum* L.) having valuable biological activities such as hepatoprotective and effects against liver disorders, as well as anti-inflammatory, antifibrotic, immunomodulating, and anticancer activities. Due to their low water solubility, they have low bioavailability.

In this study, the molecular encapsulation of the main isomer silibinin A and its saturated fatty acid (from caprylic to behenic acid) bioconjugates by natural α-, β- and γ-cyclodextrin (α-, β-, and γ-CD) have been theoretically investigated. The most stable conformers of silibinin were docked in CDs using various starting orientations. Molecular mechanics method (*MM+*) from *HyperChem* molecular modeling package, with a Polak-Ribiere optimization algorithm were applied. Better interactions were obtained for long-chain silibinin-fatty acid bioconjugates with β- and γ-CD (15.6-25.2 and 25.6-36.8 kcal/mol, respectively) in comparison with α-CD (11.3-22.7 kcal/mol). Furthermore, the hydrophobicity of bioconjugates well correlates with the guest-host interaction energy, suggesting the possibility to enhance the bioavailability by two mechanisms: increasing the hydrophobicity of silibinin by bioconjugation, followed by CD nanoencapsulation for better water solubility.

Keywords: silibinin, silybin, silymarin, saturated fatty acids, bioconjugates, cyclodextrins, molecular modeling, guest-host supramolecular systems, docking experiments, QSPR relationships

1. Introduction

Silibinins (silybins) are natural structures having flavonolignan skeleton. They occurs in *Silybum marianum* L. (milk thistle). *S. marianum* L. extracts are known as silymarin. They especially consist of silibinins (silybins) A and B, isosilibinins (isosilybins) A and B, silychristins A and B, and silydianin (Figure 1) [1]. Extracts are generally obtained from *S. marianum* degreased fruits (by petroleum ether or hexane) using percolation method with methanol, ethanol, ethyl acetate, or acetone. Moreover, optically pure silibinins can be separated using immobilized *Candida antarctica* lipase B (Novozym 435) [2]. The pharmacological activities of silibinins or silymarin related to the treatment of various liver and gastrointestinal diseases (as well as problems related to alcohol abuse and toxin exposure) is well known from ancient time. They also have hypocholesterolemic, chemopreventive, cardioprotective, and neuroprotective effects [3-5]. On the other hand, silibinin structures contain OH-phenolic groups that provide antioxidant activity [6-8]. Due to their very low water solubility and bioavailability, various methods for enhancing these properties were used.

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Thus, derivatization of silibinin isomers to esters at alcoholic [9-13] or phenolic OH-groups [10] have been performed.

Cyclodextrin complexation of silibinins is another method that enhances the apparent water solubility and bioavailability [14-16]. The presence of hydroxyl groups on silibinin structures reduces the molecular encapsulation capability of cyclodextrins. Consequently, the "hydrophobization" of silibinin structure, without affecting the biologically active moiety of this valuable compounds (e.g. the phenolic OH-groups having antioxidant activity) by fatty acid esterification of aliphatic OH-groups provides appropriate biologically active structures for cyclodextrin encapsulation [8,11-13].

The study is focused on the theoretical evaluation of natural cyclodextrin molecular encapsulation efficiency for silibinins and their bioconjugates with saturated fatty acid derivatives. Moreover, possible bioconjugate-cyclodextrin interaction energy / chemical structure property relationships (QSPRs) have been investigated.

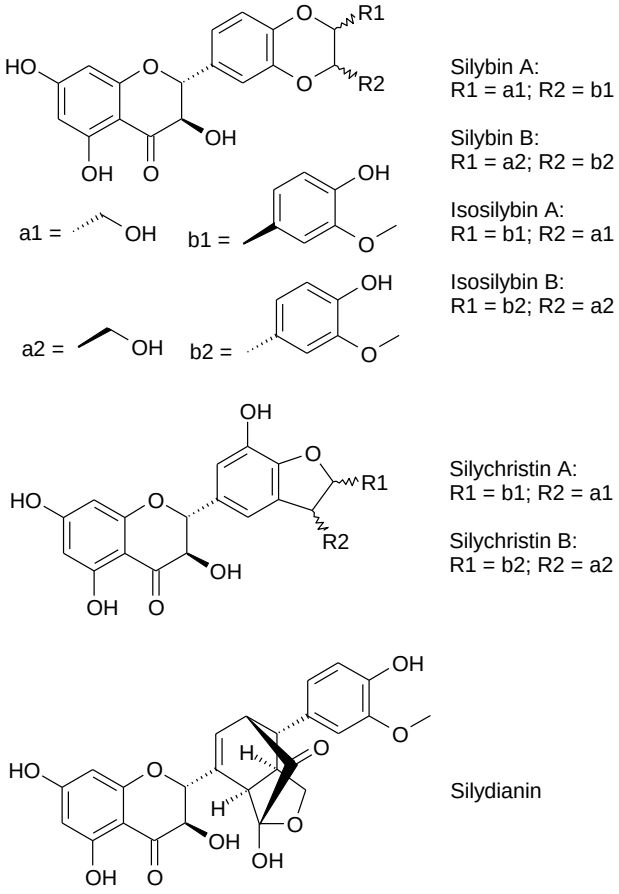


Figure 1. The main compounds from silymarin (the standardized extract of *S. marianum* L.)

2. Methods

2.1. Compound selection

Silibinin A and its saturated fatty acid (caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, and behenic acids) ester bioconjugates have been included in this study. The host compounds were α-, β-, and γ-cyclodextrin, which are natural complexation compounds. Consequently, 24 bioconjugate-cyclodextrin complexes have been investigated.

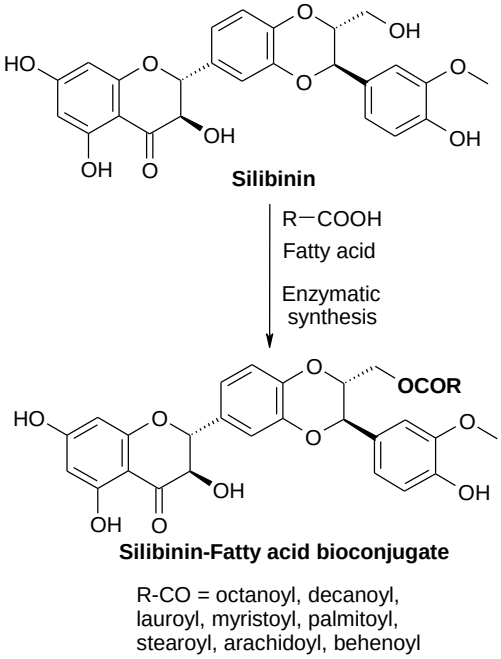


Figure 2. Schematic enzymatic synthesis of silibinin-fatty acid bioconjugates included in this study

2.2. Molecular modeling and conformational analysis

Silibinin A and the corresponding saturated fatty acid bioconjugates, as well as α-, β- and γ-cyclodextrins were built using *HyperChem 5.1* molecular modeling package (HyperCube). Geometry optimization have been performed using *Molecular Mechanics MM+* program, with a RMS gradient of 0.01 kcal/mol and the Polak-Ribiere conjugate gradient algorithm. Conformational analysis was performed by *Conformational Search* program. The following conditions were set up: range for acyclic torsion variation ±60 - ±180°, range for ring torsion flexing ±30 - ±120°, acceptance energy criterion maximum 4 kcal/mol above best, pre-optimization checks for skipping the conformation if the corresponding atoms are closer than 0.5 Å or the corresponding torsion angles are

closer than 15°, considering structures to be duplicates if energy difference was lower than 0.05 kcal/mol. All flexible bonds and rings were considered for conformational analysis of bioconjugates and cyclodextrins. The optimization program was *MM+* and Polak-Ribiere conjugate gradient as algorithm. The RMS gradient was set at 0.01 kcal/mol and the maximum number of iterations and optimizations was set to 500. A maximum number of 20 most stable conformations were retained. However, the minimum energy conformations for both bioconjugate and cyclodextrin structures were used in the guest-host docking experiments.

2.3. Molecular docking of silibinin bioconjugates and cyclodextrins

The guest-host interaction of silibinin bioconjugates and cyclodextrins have been investigated by molecular docking of 1:1 stoichiometry. Minimum energy conformations of silibinin or silibinin bioconjugate and cyclodextrin were oriented along *OZ* axis at ~8 Å distance between their gravity centers. The best interaction was observed if the hydrophobic moiety of bioconjugate was oriented to the secondary face of cyclodextrin. Consequently, only this type of complexes were considered in this study. Complexes were optimized using the *Molecular Mechanics MM+* program from the *HyperChem* package. The optimization algorithm was Polak-Ribiere and the RMS optimization gradient was 0.01 kcal/mol. The guest-host interaction energy, E_{int} (kcal/mol), was determined as the difference between the sum of internal energies for separated compounds and the energy of the complex, in vacuum.

2.4. Structural parameters of silibinin and its saturated fatty acid bioconjugates

The following structural descriptors for silibinin and its bioconjugates in minimum energy conformations were determined using *QSAR Properties* program from *HyperChem* package: van der Waals molecular surface, calculated by two methods (*Approximate* method by van der Waals parameters but without explicit H-atoms, S_{app} (Å^2) and *Grid* method, S_{grid} (Å^2)), van der Waals molecular volume, V_{vdW} (Å^3), hydration energy, E_{hydr} (kcal/mol), which is calculated according to solvent accessible surface and the type of atoms exposed to the solvent, the logarithm of octanol/water partition coefficient, log*P*, based on atomic contribution, refractivity (Å^3), also based on atomic contributions, and polarizability (Å^3), based on increments associated to various types of atoms.

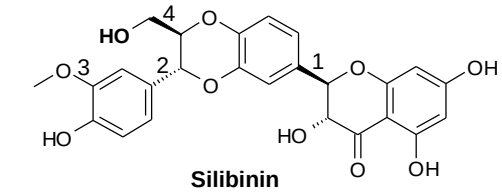
2.5. Regression analysis

Quantitative structure – property relationships (QSPRs) were obtained using guest-host interaction energy ($E_{int,}$ kcal/mol) as dependent parameter and structural descriptors as independent variables. Linear and parabolic models have been used. The statistically significance of the models was evaluated by *Pearson* correlational coefficient, *r*, *Fisher* distribution, *F*, *p*-values, and standard deviations for coefficients and equations.

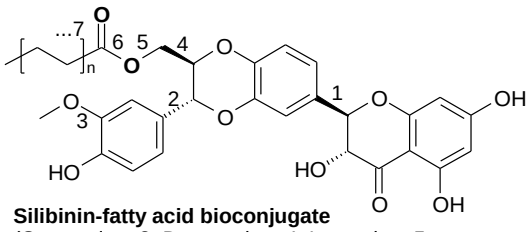
3. Results and discussion

3.1. Molecular modeling of silibinin, bioconjugates and cyclodextrins

Four flexible bonds and two flexible rings (pyrane and dioxane moieties) were identified for silibinin A structure (Figure 3). On the other hand, silibinin-fatty acid bioconjugates having significantly higher number of flexible bonds, which allows to have many stable conformations. Thus, the number of flexible bonds increases from 12 for octanoyl derivative to 26 flexible bonds for behenoyl-silibinin bioconjugate (Figure 3).



Silibinin



Silibinin-fatty acid bioconjugate
(Octanoyl: n=3; Decanoyl: n=4; Lauroyl: n=5; Myristoyl: n=6; Palmitoyl: n=7; Stearoyl: n=8; Arachidoyl: n=9; Behenoyl: n=10)

Figure 3. Flexible bonds of silibinin and its saturated fatty acid bioconjugates

In all cases of the most stable conformations of bioconjugates, fatty acid moiety is oriented to the pseudoplanar 2-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-chroman-4-one moiety (Figure 4, middle). If the fatty acid moiety is too long, such as in the case of behenic acid derivative, the hydrophobic part take a spiral-like conformation close to the dihydrobenzo[1,4]dioxine moiety (Figure 4, bottom). On the other hand, silibinin most stable conformer has a pseudoplanar aspect (especially on the 2-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-chroman-4-one moiety (Figure 4, top).

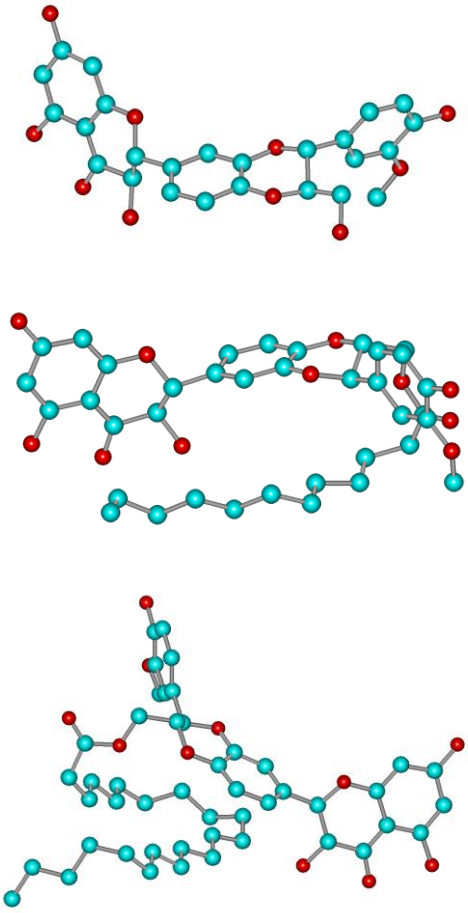
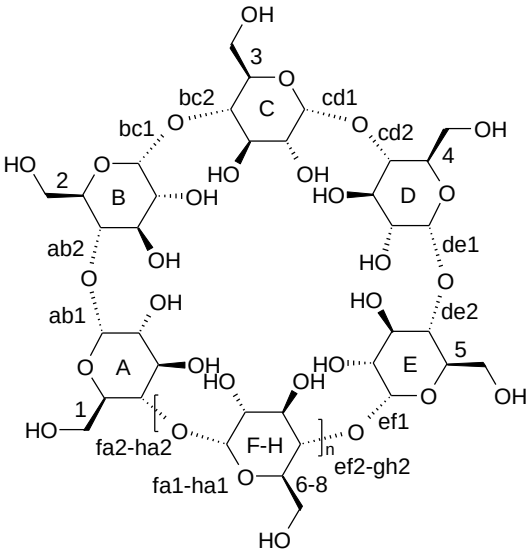


Figure 4. Minimum energy conformations for silibinin (top) and two representative bioconjugates (silibinin-lauric acid (middle) and silibinin-behenic acid (bottom) bioconjugates)

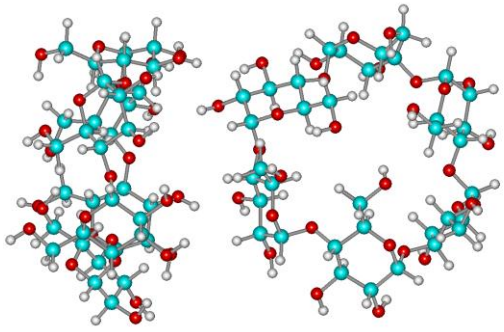
The cyclodextrin structure is more complex because it is a cyclic oligosaccharide that contains six to eight α-(1→4) linked D-glucopyranose units for α-, β- and γ-cyclodextrin, respectively. All glucopyranose units have flexible rings. Consequently, they were considered for conformational analysis. Regarding the flexible bonds, all bonds from hydroxymethyl (numbers 1-8) and inter-glucopyranose etheric moieties (labels from "ab1" to "ha2") have been considered (Figure 5).



α-Cyclodextrin: n = 1
β-Cyclodextrin: n = 2
γ-Cyclodextrin: n = 3

Figure 5. Flexible bonds and rings in α-, β-, and γ-cyclodextrin structures

The most stable conformers for cyclodextrins have architectures such as truncated cones, with the primary and secondary hydroxyl groups oriented to the exterior and forming H-bonds each other (or with water, if this solvent is considered; not presented here). The inner cavity is formed by tetrahydropyrane moieties (6 to 8 corresponding to glucopyranose units). This structure provides hydrophobic properties to the cavity and possibility to well interact with geometrically compatible hydrophobic molecules or hydrophobic moieties such as silibinin-fatty acid bioconjugates (Figure 6).



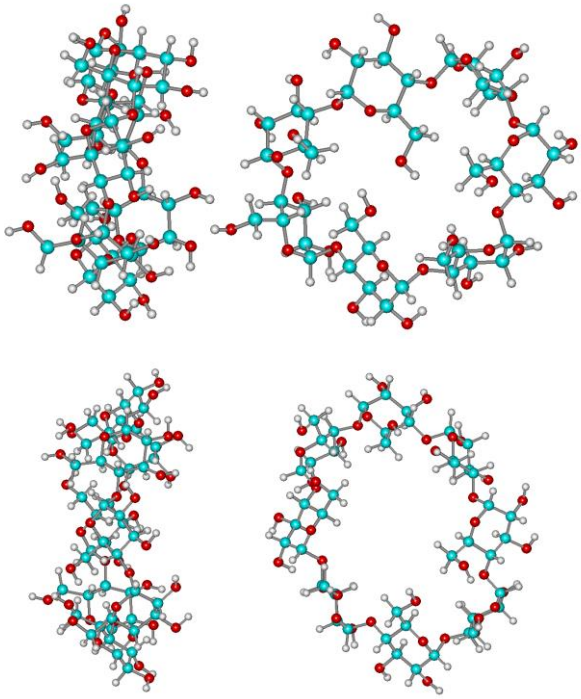


Figure 6. Minimum energy conformations for α-cyclodextrin (top), β-cyclodextrin (middle) and γ-cyclodextrin (bottom)

3.2. Molecular docking and optimization of silibinin-saturated fatty acid bioconjugate/cyclodextrin complexes

Equimolecular silibinin or silibinin bioconjugate / cyclodextrin interaction was evaluated by molecular mechanics and the starting orientation was to the secondary face of cyclodextrin along *OZ* axis against the hydrophobic fatty acid moiety of bioconjugate (or the phenyl group for silibinin) to a distance of ~8 Å between the gravity centers of those two molecules (Figure 7).

In almost all cases the optimization of silibinin-fatty acid bioconjugate/cyclodextrin complexes take more than one thousand cycles, especially for long-chain bioconjugates complexed by β- and γ-cyclodextrin. It can be seen from Figure 8 that a fast interaction occurs up to ~600 cycles and the refinement of the complex is very slow and take sometimes more than 1500 cycles.

The best interaction of silibinin was observed for γ-cyclodextrin. The phenyl group from the 2-position of the dihydrobenzo[1,4]dioxin moiety is completely encapsulated, while the chromanone moiety bearing hydroxyl and carbonyl groups could interact with the secondary hydroxyl groups of γ-cyclodextrin by H-bonds (Figure 9). On the other hand, there was no significant interaction for silibinin with α-cyclodextrin. In the case of silibinin bioconjugates, they well interact with all cyclodextrins by hydrophobic fatty acid moieties, especially with β- and γ-cyclodextrin (Figure 9). However, the inclusion of fatty acid moiety into the α-cyclodextrin cavity is poor in comparison with the β- and γ-cyclodextrin cases.

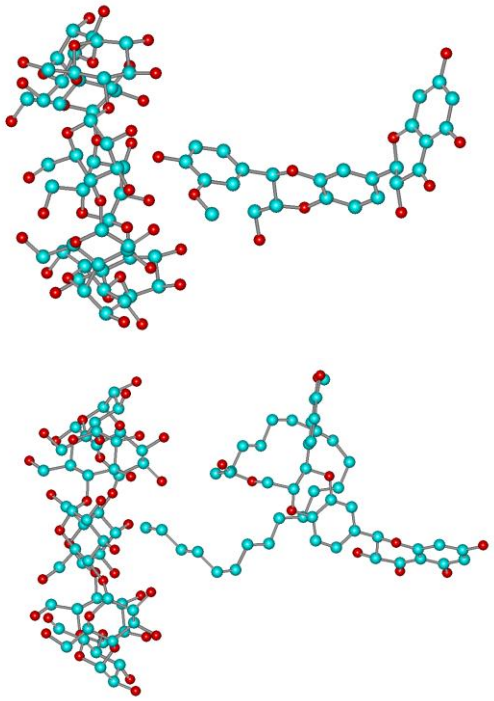


Figure 7. Starting positions of the guest and host molecules in molecular docking experiments (silibinin/γ-cyclodextrin – top, silibinin-behenic acid bioconjugate/γ-cyclodextrin – bottom)

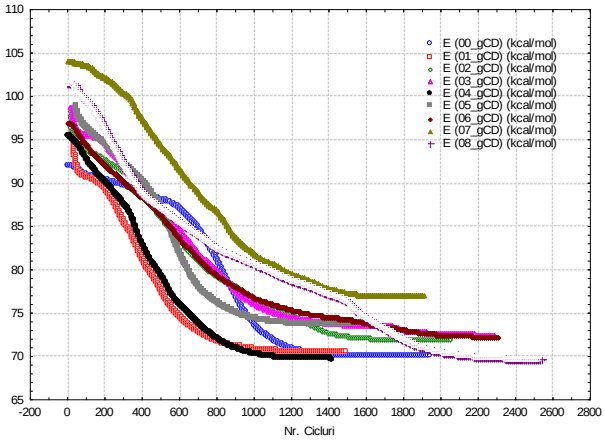


Figure 8. Energy variation during the docking/optimization process of silibinin or silibinin-fatty acid bioconjugate/γ-cyclodextrin complexes

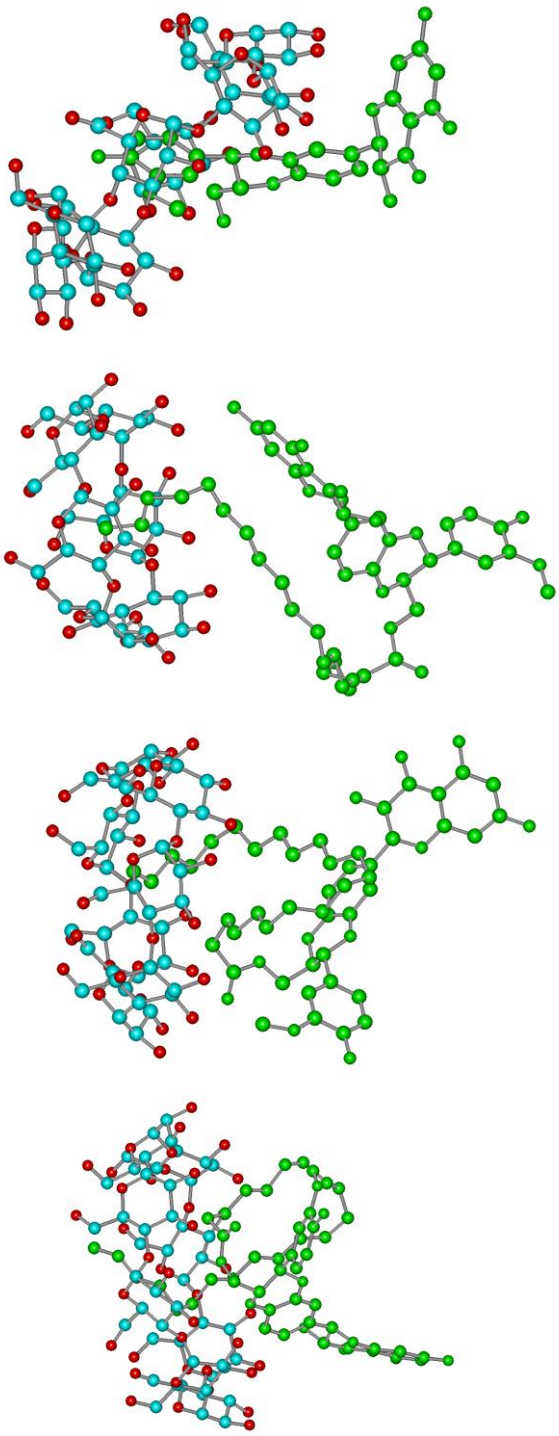


Figura 9. The final silibinin or silibinin-fatty acid bioconjugate (green)/cyclodextrin supramolecular system after docking experiments (silibinin/γ-cyclodextrin complex – top, silibinin-arachidic acid bioconjugate/α-cyclodextrin complex – top/middle, silibinin-behenic acid bioconjugate/β-cyclodextrin complex – top/bottom, and silibinin-behenic acid bioconjugate/γ-cyclodextrin complex – bottom)

Quantification of the guest-host interaction is revealed by the value of the interaction energy, determined as the difference between the sum of internal energies of separated compounds (silibinin or bioconjugate and cyclodextrin) and the energy of the complex. These values were in the range of 11.3-22.7 kcal/mol for α-cyclodextrin complexes, 15.6-27.4 kcal/mol for β-cyclodextrin complexes and 25.6-36.8 kcal/mol for the corresponding γ-cyclodextrin complexes (Table 1). In all cyclodextrin subsets, the interaction energy was at minimum for silibinin complexation (only for silibinin/γ-cyclodextrin complexes the value of interaction energy was very close to that of silibinin-octanoic acid bioconjugate/γ-cyclodextrin complexes of ~26 kcal/mol. Shorter hydrophobic chain of silibinin-fatty acid bioconjugates better interact with α-cyclodextrin than long-chain derivatives (interaction energy >22 kcal/mol for decanoic, lauric and myristic acid derivatives in comparison with values <21.1 kcal/mol for the case of palmitic, stearic, arachidic and behenic acid derivatives. On the other hand, increasing of the number of C-atoms in the fatty acid chain of bioconjugates increases the interaction energy with β- and especially γ-cyclodextrin (a maximum value of 27.4 kcal/mol for silibinin-arachidic acid bioconjugate/β-cyclodextrin complex and a best interaction for the γ-cyclodextrin complex of silibinin bioconjugate having the highest number of C-atoms, 36.8 kcal/mol).

Table 1. Values of the interaction energy, E_{int} (kcal/mol) of silibinin (code *Sb00*) or silibinin-fatty acid bioconjugates (codes from *Sb08* for octanoic acid derivative to *Sb22* for the corresponding behenic acid bioconjugate)/α- (*aCD*), β- (*bCD*) and γ-cyclodextrin (*gCD*) complexes

Code	E_{int} (kcal/mol)	Code	E_{int} (kcal/mol)	Code	E_{int} (kcal/mol)
Sb00/aCD	11.3	*Sb00/bCD*	15.6	*Sb00/gCD*	26.0
Sb08/aCD	15.0	*Sb08/bCD*	25.0	*Sb08/gCD*	25.6
Sb10/aCD	22.0	*Sb10/bCD*	25.9	*Sb10/gCD*	27.4
Sb12/aCD	22.4	*Sb12/bCD*	26.7	*Sb12/gCD*	27.4
Sb14/aCD	22.7	*Sb14/bCD*	24.9	*Sb14/gCD*	32.0
Sb16/aCD	19.4	*Sb16/bCD*	23.7	*Sb16/gCD*	28.4
Sb18/aCD	19.3	*Sb18/bCD*	25.2	*Sb18/gCD*	29.1
Sb20/aCD	17.7	*Sb20/bCD*	27.4	*Sb20/gCD*	30.7
Sb22/aCD	21.1	*Sb22/bCD*	27.1	*Sb22/gCD*	36.8

3.3. Quantitative structure – property relationships (QSPRs) for silibinin or silibinin-fatty acid bioconjugates/cyclodextrin complexes

Interesting variation of the guest-host interaction energy for silibinin or silibinin-saturated fatty acid bioconjugate/cyclodextrin complexes was observed. Consequently, the main structural descriptors, such as molecular surface and volume, hydration energy or hydrophobicity, were calculated for guest molecules (silibinin and its bioconjugates) and correlated with the interaction energy in order to identify and quantify these interactions by means of QSARs. In Table 2, values of the main descriptors for silibinin and bioconjugates are presented.

Table 2. Values of the main descriptors (van der Waals molecular surface, S_{app} (Å^2), and molecular volume, V (Å^3), hydration energy, E_{hydr} (kcal/mol), and the logarithm of octanol/water partition coefficient, log$P$) of silibinin A (*Sb00*) and corresponding silibinin-saturated fatty acid bioconjugates (codes *Sb08*-*Sb22* for the silibinin esters with C_8-C_{22} saturated acyclic fatty acids)

Code	S_{app} (Å^2)	V_{vdW} (Å^3)	E_{hydr} (kcal/mol)	log$P$
Sb00	441.9	394.0	-34.05	2.10
Sb08	615.0	532.1	-27.56	4.84
Sb10	655.4	566.5	-26.44	5.63
Sb12	697.1	600.9	-24.43	6.42
Sb14	734.3	634.3	-23.99	7.21
Sb16	778.2	668.4	-22.33	8.01
Sb18	815.8	702.2	-22.07	8.80
Sb20	859.7	736.6	-21.18	9.59
Sb22	900.1	769.9	-22.47	10.38

Statistically significant QSPRs were obtained for all subsets of silibinin or silibinin-fatty acid bioconjugate/cyclodextrin complexes (subsets corresponding to α-, β- and γ-cyclodextrin) if molecular surface or hydrophobicity were considered. However, these descriptors intercorrelates (r = 0.99). Second order polynomial equations were obtained for α- and β-cyclodextrin complexes (Eqs. 1 and 2), while γ-cyclodextrin complexes provide monolinear correlations between interaction energy and silibinin or silibinin bioconjugate molecular surface or hydrophobicity (Eqs. 3 and 4).

$$E_{int.(Sb-aCD)} = 2.262 + \\ +4.847(\pm 1.682)\cdot logP - \\ -0.313(\pm 0.129)\cdot (logP)^2$$

n = 9, r = 0.816, F = 5.98, p = 0.037 [Eq. 1]

$$E_{int.(Sb-bCD)} = 9.103(\pm 3.787) + \\ +4.801(\pm 1.244)\cdot logP - \\ -0.238(\pm 0.096)\cdot (logP)^2$$

n = 9, r = 0.891; F = 11.44; p = 0.009 [Eq. 2]

$$E_{int.(Sb-gCD)} = 21.903(\pm 2.412) + \\ +1.054(\pm 0.326)\cdot logP$$

n = 9, r = 0.774; F = 10.48; p < 0.014 [Eq. 3]

$$E_{int.(Sb-gCD)} = 15.591(\pm 4.532) + \\ +0.019(\pm 0.006)\cdot S_{app}$$

n = 9, r = 0.758; F = 9.43; p < 0.018 [Eq. 4]

4. Conclusion

Bioconjugation of silibinin by enzymatic esterification of saturated fatty acids enhances the interaction with natural cyclodextrins, as is revealed by theoretical molecular modeling and docking experiments in vacuum for equimolecular guest-host supramolecular systems.

The best interaction of silibinin bioconjugates was observed for the case of γ-cyclodextrin complexes. Furthermore, statistically significant monoparametrial models were obtained for the interaction energy and hydrophobicity of the guest compound.

These findings suggest that bioconjugation of silibinin to more hydrophobic derivatives enhances the cyclodextrin complexation and bioavailability of such compounds with antioxidant and hepatoprotective effects.

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