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Molecular modelling of Hydroxypropyl Alpha Cyclodextrin inclusion complex with stigmasterol

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ABSTRACT

The geometry and structural features of the inclusion complex of HPα-cyclodextrin (HPα-CD) with the stigmasterol are studied by molecular modeling method. Cyclodextrins are effective host compounds in molecular recognition. To evaluate the complexation role of HPα-CD toward stigmasterol in an attempt to assess their potential as new formulation additives for more efficient drug formulation and delivery is the aim of this study. Cyclodextrin complex was prepared by solvent evaporation method and the formation of inclusion complex was confirmed by NMR spectroscopy. Docking studies generated the most stable complex, demonstrating the aliphatic tail of the guest enters inside the cavity of HPα-CD and the aromatic rings are outside the cavity. The binding energies were essentially due to hydrogen-bonded structures involving the aliphatic chain of the guest. The optimized structures and conformations of HPα-CD and its inclusion compound showed acceptable general agreement with NMR studies. Stoichiometry in the complex formation is 1:1.

Keywords: *Hydroxypropyl Cyclodextrin, Stigmasterol, inclusion complex, molecular modelling, NMR spectroscopy*

1. Introduction

Cyclodextrins are widely utilized as complexing agents to enhance the solubility of poorly water-soluble drugs, to increase their bioavailability and stability, to prevent gastrointestinal or ocular irritation, to eliminate the unpleasant smells or tastes and to prevent drug-drug or drug-additive interactions [1]. Cyclodextrins present a three-dimensional shape similar to a hollow torus and present different polarities in their interior and exterior surfaces. Their primary and secondary hydroxyl groups are placed on the narrow and the wider rim, respectively. Due to this particular structure, CDs can encapsulate guest molecules leading to the formation of inclusion complexes. Computational chemistry paves the way to understand the mode of interaction between the guest and the host. It also provides the possible orientation of the guest molecule into the cavity of the host molecule. Molecular modelling is a method which predicts the preferred orientation of one molecule to a second when bound to

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each other to form a stable complex [2]. Knowledge of the preferred orientation is used to predict the strength of association or binding affinity between two molecules using scoring functions. Hence molecular modelling plays an important role in the rational design of drugs [3]. The aim of molecular modelling is to achieve an optimized confirmation for both the host and the guest, so that the free energy of the overall system is minimized. This research focuses on the inclusion complexation between hydroxypropyl alpha cyclodextrin (HPα-CD) and stigmasterol, lead compound for the formulation of cervical cancer drugs. HPα-CD behaves as host and stigmasterol as guest molecule.

2. Materials and Methods

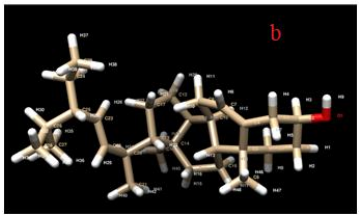
The molecular modelling of the host-guest interaction was performed as follows: Molecules required (stigmasterol, HPα-CD) for the molecular docking studies were retrieved in the pubchem database and drawn using chem sketch [4]. Before the analysis, molecules were prepared and hydrogen atoms were added by chimera software. Then it was converted as pdb format to molecular docking and inclusion. Initially, the Patchdock server was utilized to process the docking and reveal the grid values (25×25×25). Further, Autodock vina was used to study the host-guest interaction. The grid values were adjusted and executed for molecular docking between stigmasterol and HPα-cyclodextrin. Complex files were analyzed and modeled for major forces by PyMOL and chimera tools. Solid inclusion complex was synthesized by solvent evaporation method [5]. ^{1}H NMR spectroscopy studies of the solid inclusion complex were recorded in Bruker 400MHz FT-NMR spectrometer. $CDCl_3$ was used as solvent and Tetramethylsilane (TMS) as internal reference. The chemical shifts (δ) were reported in ppm relative to TMS at 298 K.

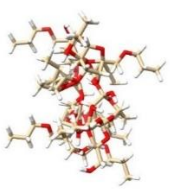
3. Results and Discussions

3.1 Molecular Docking Studies

Docking study has been utilized to perform virtual screening of compounds and propose structural hypotheses of how the drug binds with the cyclodextrin for lead optimization. Stigmasterol is docked with HPα-CD after optimizing their structures. The optimized structures of both HPα-CD and stigmasterol are shown in figure 1a and 1b respectively.

a

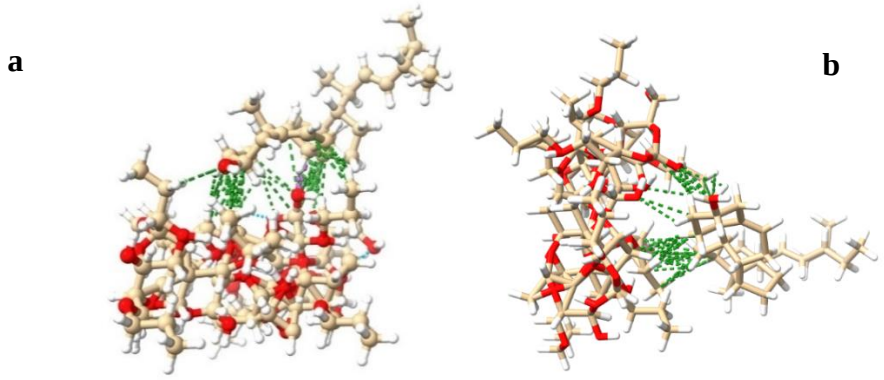




b

Fig. (1a) Optimised structure of stigmasterol (1b) Optimised structure of HPα-CD

From the figures 1a and 1b it is clear that the aliphatic hydrocarbon tail of stigmasterol has entered through the wider rim of the HPα-CD cavity and the stigmasterol is bound to HPα-CD. The steroid moiety of stigmasterol protrudes on the wider rim of the HPα-CD cavity, which is supported experimentally by NMR studies. Docking studies support the data obtained experimentally. The best binding affinity score of stigmasterol and HPα-CD is -2.36Kcal. Hydrophobic effect is predominant in the HP α-CD inclusion complex, as indicated by green doted bonds in the figure 2a and 2b. As the spectroscopic data obtained experimentally confirm the formation of inclusion, docking studies also serve as anevidence. Thus, HP α-CD form stable inclusion complex with Stigmasterol.



-2.36 Kcal/mol **-2.26 Kcal/mol**

Fig. 2 The most stable optimized geometry of the inclusion complex (stigmasterol: HPα-CD) at different positions

3.2 Nuclear Magnetic Resonance (NMR) Spectroscopic Studies

Chemical shift assignments of ^{1}H NMR spectrum of stigmasterol, HP α-CD and stigmasterol: HPα-CD is listed in tables. 1. From the chemical shift values, it is clear that in the presence of HPα-CD, aliphatic hydrocarbon tail of stigmasterol undergoes changes in their chemical shift indicating the complex formation between HPα-CD and stigmasterol.

Table 1: Chemical shifts of α-CD and the HPα-CD:S inclusion complex

H	$\delta_{HP\ \alpha\text{-}CD}$	$\delta_{HP\ \alpha\text{-}CD:S}$	Δδ
1	5.360	5.360	0
2	3.80	3.78	-0.02
3	3.35	3.23	-0.13
4	3.97	3.99	0.02
5	3.70	3.61	-0.09
6	5.027	5.027	0

The unaltered ^{1}H chemical shift due to cyclic hydrocarbons after complex formation demonstrates that cyclic rings are not incorporated into the cavity of HP α-CD. This is because in the cyclodextrin complexes, the mode of binding involves the insertion of the less polar part of the guest into the CD cavity, while the more polar groups remain with the bulk solvent outside [6]. The aliphatic chain would be expected to enter into the cavity due to their hydrophobic nature. Internal protons of HP α-CD are shielded and experience an upfield shift, indicating the presence of electron releasing groups such as the aliphatic end of stigmasterol within the CD cavity. Since the upfield shift of H_3(-0.13) is greater in magnitude than that of H_5(-0.09) the inclusion is partial. Thus, NMR spectra serve as good evidence for the formation of an inclusion complex between HPα-CD and stigmasterol.

4. Conclusion

The inclusion complex of stigmasterol with HPα-CD is synthesized by solvent evaporation method. The stoichiometry of the inclusion complexes is determined to be 1:1. Stigmasterol forms a more stable complex with HPα-CD. This may be due to the presence of substituent hydroxypropyl groups in HPα-CD which reduces the interactions of stigmasterol with the aqueous region and enlarges the hydrophobic environment. This enhances the binding of stigmasterol via hydrophobic effect which is confirmed by molecular modelling. The best binding affinity score of stigmasterol and HPα-CD is -2.36 Kcal. The preferred orientation is energetically favorable. ^{1}H-NMR studies serve as supporting evidence for the formation of an inclusion complex. The formation of strong inclusion could increase solubility and hence, facilitate the delivery of stigmasterol preventing the undesired properties of stigmasterol.

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