Journal of Molecular Structure 1125 (2016) 193–203

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Combined spectroscopic and quantum chemical studies of ezetimibe

Preeti Prajapati [a], Jaya Pandey [a], Manishkumar R. Shimpi [b], Anubha Srivastava [a], Poonam Tandon [a, *], Sitaram P. Velaga [b], Kirti Sinha [a]

[a] Department of Physics, University of Lucknow, Lucknow 226 007, India
[b] Department of Health Sciences, Luleå University of Technology, S-971 87 Luleå, Sweden

ARTICLE INFO

Article history:
Received 3 May 2016
Received in revised form
27 June 2016
Accepted 27 June 2016
Available online 29 June 2016

Keywords:
Ezetimibe
Conformational studies
FT-IR
FT-Raman
UV–Visible
Molecular docking

ABSTRACT

Ezetimibe (EZT) is a hypocholesterolemic agent used for the treatment of elevated blood cholesterol levels as it lowers the blood cholesterol by blocking the absorption of cholesterol in intestine. Study aims to combine experimental and computational methods to provide insights into the structural and vibrational spectroscopic properties of EZT which is important for explaining drug substance physical and biological properties. Computational study on molecular properties of ezetimibe is presented using density functional theory (DFT) with B3LYP functional and 6-311++G(d,p) basis set. A detailed vibrational assignment has been done for the observed IR and Raman spectra of EZT. In addition to the conformational study, hydrogen bonding and molecular docking studies have been also performed. For conformational studies, the double well potential energy curves have been plotted for the rotation around the six flexible bonds of the molecule. UV absorption spectrum was examined in methanol solvent and compared with calculated one in solvent environment (IEF-PCM) using TD-DFT/6-31G basis set. HOMO-LUMO energy gap of both the conformers have also been calculated in order to predict its chemical reactivity and stability. The stability of the molecule was also examined by means of natural bond analysis (NBO) analysis. To account for the chemical reactivity and site selectivity of the molecules, molecular electrostatic potential (MEPS) map has been plotted. The combination of experimental and calculated results provide an insight into the structural and vibrational spectroscopic properties of EZT. In order to give an insight for the biological activity of EZT, molecular docking of EZT with protein NPC1L1 has been done.

1. Introduction

Ezetimibe (EZT) belongs to a class of lipid-lowering compounds that acts at the brush border of the small intestine and inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. It is also used for the treatment of atherosclerosis, which causes the absorption of plasma cholesterol in the arteries. Atherosclerosis is the formation of plaque in the arteries which can lead to serious problems like heart attack, stroke and even death. Exact mechanism of action of EZT is not yet known. However, it is proposed that Niemann-Pick C1 Like1 (NPC1L1) protein mediates the intestinal absorption of cholesterol, EZT inhibits this protein resulting in reduction of intestinal cholesterol absorption leading to reduction of plasma cholesterol levels (Kwon et al. 2011, Phan et al., 2012) [1]. EZT exists as monohydrate and anhydrate crystalline form with quite similar conformation except for different orientation of the propyl group (Scmidt et al. 2010, Ravikumar & Sridhar 2005). However, anhydrate form has different hydrogen bonding network because of fewer donor and acceptor atoms [2,3].

Density functional theory (DFT) is widely used to study molecular structure, electronic properties, hydrogen bonding and chemical reactivity of pharmaceutical compounds [4–6]. In continuation to our work on study of hydrogen bonding, conformation, chemical and biological activity of active pharmaceutical ingredients [Eram et al. 2015, Anuradha et al. 2015, Anubha et al.] [7,8] in the present communication, we report combined spectroscopic (solid-state FT-IR, Raman and UV–visible spectra) and quantum chemical studies of EZT.

Geometry optimization, vibrational and conformational analysis, electronic structure calculations have been performed using DFT. Further, chemical activity and reactivity of EZT have been

* Corresponding author.
E-mail addresses: poonam_tandon@yahoo.co.uk, poonam_tandon@hotmail.com (P. Tandon), sitaram.velaga@ltu.se (S.P. Velaga).

http://dx.doi.org/10.1016/j.molstruc.2016.06.070
0022-2860/

studied by calculating molecular electrostatic potential surfaces (MEPS), frontier orbitals and, global and local reactivity descriptors, along with molar refractivity, at the same level of theory. Natural bond analysis (NBO) has been used to study the stability of the molecule arising from hyperconjugative and charge transfer interaction. The quantum theory of atoms in molecule (QTAIM) has been used for better understanding of the nature and strength of H-bonding. Molecular docking studies were performed for understanding the mechanism of action and biological activity of EZT.

2. Experimental details

Ezetimibe, (1-(4-fluorophenyl)-3-[3-4-(fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone, was (purchase from Tecoland batch number 20120615) generously gifted by Dr. Scott Child (Renovo research, Atlanta, USA).

2.1. Fourier transform–infrared spectroscopy

Infrared spectrum was recorded on a Bruker Vertex 80v FTIR spectrometer equipped with a DTGS detector and a platinum-ATR accessory with a diamond crystal as ATR element. Both a single beam background without sample and single beam spectra of the powered sample was obtained by averaging 128 scans with an optical resolution of 4 cm^{-1}.The resulting interferograms were Fourier transformed using the Mertz phase correction mode, a Blackman-Harris 3-termapodization function, and a zero filling factor of 2. All spectra were recorded under vacuum using the double-side forward–backward acquisition mode [9].

2.2. Fourier transform–Raman spectroscopy

The Raman spectrum was recorded on a Chromex Sentinel dispersive Raman unit equipped with a 785 nm, 70 mW excitation laser and a TE cooled CCD. The spectrum is a result of twenty co-added 20 s scans. The unit has continuous automatic calibration using an internal standard. The data was collected by Sentinel Soft data acquisition software and processed in GRAMS AI [9].

2.3. UV–Visible spectroscopy

The UV absorption spectrum of EZT was recorded in the range 200–800 nm using a Varian cary 50, UV–visible Spectrophotometer, the UV pattern is taken from a 1×10^{-5} M solution of EZT dissolved in methanol solvent.

3. Computational details

The calculation of optimized geometry, potential energy scan (PES), vibrational frequencies, electronic structure, MEPS and NBO analysis were performed by DFT [10] at B3LYP/6-311++G(d,p) [11–13] level of theory using Gaussian09 software [14]. GaussView [15] and ChemCraft [16] software were used for visualization of the calculated data. The vibrational assignments of the normal modes were established on the basis of the potential energy distribution (PED) calculated by using the program Gar2Ped [17,18]. AIMALL software [19] employing Quantum theory of Atoms in Molecules (QTAIM) [20–23], was used for the analysis of hydrogen bonding in molecule. To test the biological activity of the title molecule, molecular docking (ligand-protein) simulations have been performed using AutoDock1.5.4 software [24]. The active site was examined for detailed interactions in Discovery Studio Visualizer 4.5 software [25].

4. Results and discussion

4.1. Geometry optimization and conformational analysis

Initial geometry of EZT, was taken from crystal data by Manish et al. [9]. The ground state optimized energy was −883324.0417 kcal/mol (conformer I) and the molecular structure of EZT is shown in Fig. 1. The conformational study using one-dimensional potential energy surface (PES) scans was also performed to find out possible conformers and the most stable structure having minimum energy. PES scan was done by scanning the dihedral angles φ1, φ2, φ3, φ4, φ5 and φ6, using B3LYP/6-31G(d,p) basis set. The variation of torsion angles were carried out at a step of 10° in the range of 0–360° rotation around the bond. The graphs obtained by varying the potential energy as a function of dihedral angles for all the bonds are given in Fig. 2. Total nine conformers were obtained and optimized structure of all the conformers along with their relative energies are presented in the Fig. S1. The optimized energy and the energy difference of all the conformers with respect to the most stable conformer is given in Table S1. Among these, conformer II was found to be the most stable with energy −883324.8268 kcal/mol. The initially optimized structure (conformer I) is the next most stable conformation with energy −883324.0417 kcal/mol. However, conformer I matches well with the experimental values, as such, the present work is based on these two conformers. The experimental and optimized structural parameters (bond lengths, bond angles, dihedral angles) of conformers I and II are illustrated in the Table S2. It is observed that optimized geometrical parameters that the bond length of C–H, C=O, C–C, O–H, C–N are nearly similar in both the conformers. The difference is not more than 0.15 Å. The large variation was found around the bond angles C12–C14–C19 and O3–C19–H20; 0.92°/3.32° and 3.27°/3.2° in conformer I and II, respectively. The difference between experimental and theoretical values of bond angles was found to be less in conformer I than conformer II as shown in Table S2. The optimized and experimental structures of the molecule were compared by superimposing them using a least squares algorithm that minimizes the distances of the corresponding non hydrogen atoms as shown in Fig. S2. Although both are in good agreement with each other, there are certain discrepancies around Ring 1, Ring 2 and attached C=O group. These deviations occur due to the intermolecular interactions between the OH and C=O group of EZT molecule and its neighbouring moieties which can be seen from Fig. S3.

4.2. Vibrational assignment

The EZT molecule contain 51 atoms, hence it gives 147 (3 N−6)

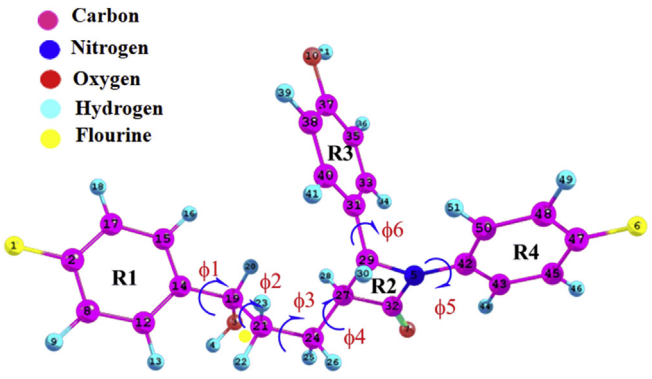

Fig. 1. Optimized structure for EZT (conformer I) with the atoms numbering scheme adopted in this study.