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Original article

Molecular docking of γ-sitosterol with some targets related to diabetes

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ABSTRACT

γ-sitosterol isolated from *Lippia nodiflora* was taken as ligand for molecular docking. The molecular targets, glucokinase, Fructose 1, 6- bisphosphatase 1, Human multidrug resistance protein 1 and Cytochromes P450 whose crystallographic structures are available on the PDB database as 1V4S, 2JJK, 3LC4, 2CBZ respectively, were used for the docking analysis using the Autodock tool v 4.2 and ADT v1.5.4 programs. The docking studies of the ligand γ- sitosterol with four different target proteins showed that this is a good molecule which docks well with various targets related to diabetes mellitus. Hence γ-sitosterol can be considered for developing into a potent antidiabetic drug.

1. Introduction

Diabetes mellitus refers to a group of disorders with different etiologies. It is characterized by derangements in carbohydrates, proteins and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action [1]. To date, there are different groups of oral hypoglycemic agents for clinical use, having characteristic profiles of side effects [2,3]. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with antidiabetic activity with less side effects. Indian traditional systems of medicine are having many plants to treat diabetes one of them is *Lippia nodiflora* L. Several compounds have been isolated from *L. nodiflora* such as resin, stigmastrol, β- sitosterol, sugars, as well as essential oil constituents such as monoterpenes and sesquiterpenes, Lippiflorin A and Lippiflorin B, flavonoids like nepetin, jaceosidin, hispidulin, flavone monosulfates and flavones disulfates [4]. For the first time, we have isolated γ-sitosterol from this plant and established its antidiabetic property [5]. It has been previously isolated from soya and its antihyperlipidemic activity has been described [6]. The same compound has also been reported to occur in *Abelmoschus manihot* [7] and *Polygonum bistorta* [8].

Bioinformatics tools have become very important to pinpoint the targets for different ligands. Using bioinformatics tools we tried to evaluate whether γ-sitosterol is a good ligand to some of the target proteins related to diabetes such as glucokinase, fructose 1,6 bis phosphatase, human multidrug resistance protein and cytochrome P450.

Glucokinase (PDB ID: 1V4S) is a monomeric cytoplasmic enzyme found in the liver and pancreas. Its main function is regulation of glucose levels in these organs. Through phosporylation glucokinase is able to increase the metabolism of glucose. In the liver it increases the synthesis of glycogen and it is the first step in glycolysis, the main producer of ATP in the body. Due to the importance of glucose regulation, individuals who display an irregularity in glucokinase are often afflicted with Type-II diabetes, hypoglycemia, or hyperglycemia [9].

Fructose 1,6- bisphosphatase 1 (PDB ID-2JJK) [10] is a rate controlling enzyme in the pathway of gluconeogenesis [11] that has been targeted by the pharmaceutical industry since 1970s. Inhibitors of fructose 1, 6-bisphosphatase (FBPase) represent a new strategy for direct inhibition of endogenous glucose production (EGP) [12].

Abbreviations: ADME/T, Absorption, distribution, metabolism, excretion and toxicity; HTS, High-throughput screening; ADT, Autodock tool; STZ, Streptozotocin; AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, alkaline phosphatase; ACP, Acid phosphatase; NADPH, Nicotinamide adenine dinucleotide phosphate; HDL, High density lipoprotein.

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Human multidrug resistance protein 1(MRP-1) (PDB ID:2CBZ) [13] is a member of the ATP- binding cassette (ABC) super family which is involved in a variety of physiological processes such as lipid metabolism. A large number of this protein has been causatively linked to rare and common human genetic diseases including diabetes, HDL- lipoprotein deficiency [14].

Cytochromes P450 (PDB ID: 3LC4) [15] are a group of heme-thiolate monooxygenases. In liver microsomes, this enzyme is involved in a NADPH-dependent electron transport pathway [16,17]. Polymorphic alteration on the metabolic activities of cytochrome P450 dependent monooxygenase enzyme system will be caused during diabetes condition due to oxygen free radicals [18].

The above mentioned targets were subjected to *in silico* docking with a view to identify whether γ-sitosterol could be a good molecule to treat diabetes.

2. Experimental

2.1. Isolation and identification of γ-sitosterol

Shade dried and coarsely powdered plant metrial (whole plant, 3 kg) was extracted with methanol in the cold for 72 h. The extract was filtered through Whatman No:1 filter paper and concentrated in rotary evaporator at 40 °C to get a syrupy mass (97 gm).The extract was diluted with water and extracted with equal volume of diethyl ether in a separating funnel. The ether extract was dried over anhydrous Na_2SO_4 and concentrated to get a light green residue (54 gm). γ-sitosterol was obtained by column chromatography over silica-gel (100–200 mesh) and elution with hexane and ethyl acetate (25:75). The structure of the γ-sitosterol was determined on the basis of FT-IR, 1H NMR, 13C NMR and MS.

2.2. Docking analysis

The structures of the target receptor binding sites of human glucokinase (PDB ID: 1V4S), human fructose 1, 6-bisphosphatase (PDB ID: 2JJK), human multidrug resistance protein 1 (PDB ID: 2CBZ) and human Cytochrome P450 Cyp2E1 (PDB ID: 3LC4) were obtained from the RCSB Protein Data Bank, http://www.rcsb.org/pdb. Then the possible binding sites of selected target receptors were searched using Q- site Finder to predict the ligand binding site and also whole protein structure assumed as a binding site. It works by binding hydrophobic probes to the protein and finding clusters of probes with the most favorable binding energy. [www.bioinformatics.leeds.ac.uk/qsitefinder]. These include active sites located on protein surfaces and voids buried in the interior of proteins. Q-site Finder includes a graphical user interface, flexible interactive visualization, as well as on-the fly calculation for user uploader structures. The characteristics of the γ-sitosterol were retrieved from pubchem data base. http://pubchem.ncbi.nlm.nih.gov/search/search.cgi.

The docking analyses of γ-sitosterol were carried out by means of the Autodock tools [19] (ADT) v1.5.4 and Autodock v4.2 program; (Autodock, Autogrid, Autotors, Copyright-1991e2000) from the Scripps Research Institute, http://www.scripps.edu/mb/olson/doc/autodock To run autodock, we used a searching grid extended over the selected target proteins; polar hydrogens were added to the ligand moieties. Kollman charges were assigned and atomic solvation parameters were added. Polar hydrogen charges of the Gasteiger-type were assigned and the nonpolar hydrogens were merged with the carbons and the internal degrees of freedom and torsions were set. γ-sitosterol was docked to all the target protein complexes with the molecule considered as a rigid body and the ligands being flexible. The search was extended over the whole receptor protein used as blind docking. Affinity maps for all the atom types present, as well as an electrostatic map, were computed with a grid spacing of 0.375 E. The search was carried out with the Lamarckian Genetic Algorithm [20]; populations of 150 individuals with a mutation rate of 0.02 have been evolved for 10 generations. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy. A cluster analysis based on root mean square deviation values, with reference to the starting geometry, was subsequently performed and the lowest energy conformation of the more populated cluster was considered as the most trustable solution. The hydrophobic effect of ligand was retrieved by ALOGPS 2.1. This Applet provides interactive online prediction of logP, water solubility and pKa(s) of compounds for drug design (ADME/T and HTS) and environmental chemistry studies [21].

3. Results and discussion

3.1. γ-Sitosterol and its antidiabetic activity

Elution of the column with Hexane : ethyl acetate (25:75) gave γ-sitosterol which was crystallized from ethanol as colourless crystal (mp – 147° lit. mp – 147°–148°, Yield 600 mg) $C_{29}H_{50}O$ (M^+, *m*/*z* 414). The GC–MS spectrum revealed the compound to be the γ-sitosterol 100% pure (Fig. 1(a,b). $[\alpha]_D$ – 45 ($CHCl_3$). γ- sitosterol (Fig. 2). The mass spectral values corresponded to literature [7].

IR ν $KBr_{(max)}$: 3418[hydroxyl], 2932, 2854, 1637(trisub. double bond), 1462, 1379, 1254, 1164, 1024, 839, 801 (trisub. double bond).

^{1}H NMR (δ, $CDCl_3$, 400 mhz): 0.68 (3H, S, H-18), 1.02 (3H, S, H-19), 0.82 (3H, d, J = 6.5 Hz, H-26) 0.84 (3H, d, J = 6.5 Hz, H-27), 0.85 (3H, t, J = 7.0 Hz, H-29), 0.92 (3H, d, J = 6.5 Hz, H-21), 3.50 (1H, m, H-3), 5.35 (1H, m, H-6).

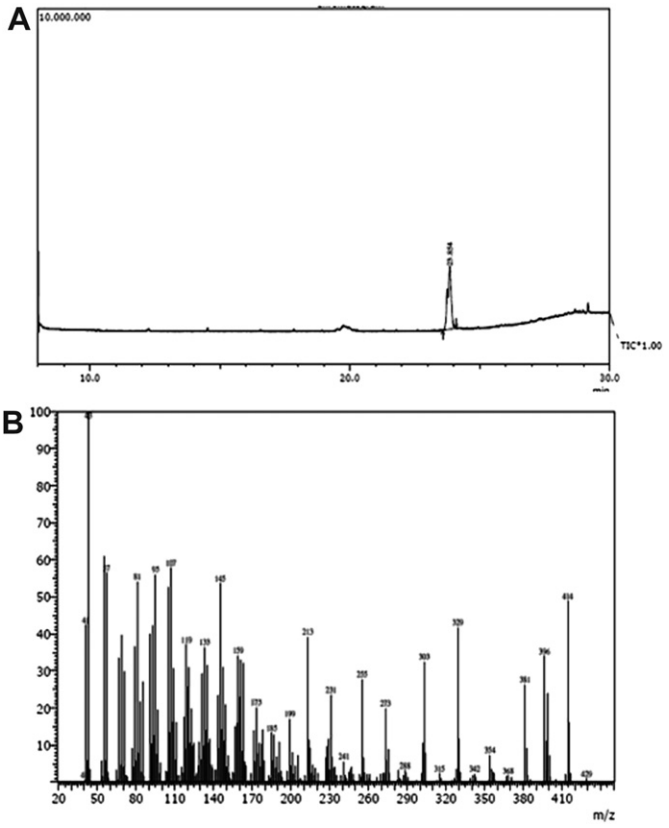

Fig. 1. GC–MS spectral analysis of γ- sitosterol isolated from *L. nodiflora*. A - GC–MS chromatograph of γ- sitosterol, B- mass spectrum of γ- sitosterol.

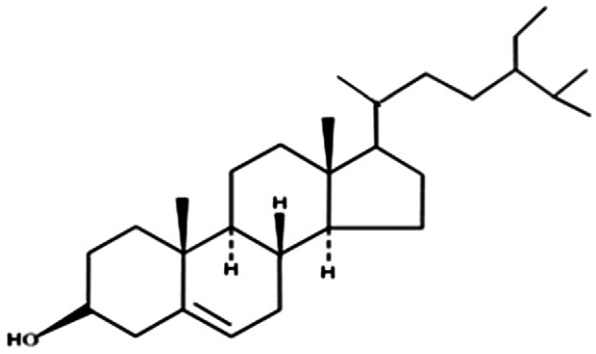

Fig. 2. Structure of γ-sitosterol.

^{13}C NMR (δ, $CDCl_3$, 100 MHz): 37.25 (C-1), 31.899 (C-2), 71.79 (C-3), 42.29 (C-4), 140.74 (C-5), 121.70 (C-6), 31.65 (C-7), 31.65 (C-8), 50.12 (C-9), 36.13 (C-10), 21.07 (C-11), 39.77 (C-12), 42.29 (C-13), 56.759C-14), 26.07 (C-15), 28.23 (C-16), 56.05 (C-17), 11.84 (C-18), 19.38 (C-19), 36.49 (C-20), 19.02 (C-21), 33.93 (C-22), 26.07 (C-23), 45.82 (C-24), 29.14 (C-25), 18.77 (C-26), 19.81 (C-27), 23.05 (C-26), 12.13 (C-29).

EI-MS m/z (rel. int %): 414 (M^+, $C_29H_{50}O$, 48.9), 396 (34.0), 381 (26.3), 329 (41.7), 303 (32.4), 275 (8.8), 255 (25.6), 213 (39.2), 199 (17.0), 163 (31.1), 161 (32.8), 159 (34.2), 147 (30.9), 145 (53.6), 135 (31.5), 131 (23.3), 121 (31.0), 119 (36.9), 109(30.8), 107(57.8), 105 (52.5), 95 (56.0), 93 (42.3), 91 (39.9), 81 (54.0), 79 (34.4), 71 (29.9), 69 (39.7), 67 (33.4), 57 (56.4), 55 (60.8), 43 (100).

The above physical and spectroscopic data are comparable with those reported in literature [7,8].

It should be pointed out that γ-sitosterol has the same structure as beta sitosterol. In beta sitosterol it is known that C-24 has beta -ethyl group (24S) and the side chain at C-20 is attached with beta configuration while C-21 methyl is attached with alpha configuration. The steriochemical details for γ-sitosterol are not yet established.

The antidiabetic effect of the γ-sitosterol was evaluated using the STZ-induced diabetic rat model in vet lab. Diabetic rats treated with 20 mg/kg γ-sitosterol significantly reduced fasting blood glucose level and decreased glycosylated haemoglobin (66.96%), glucose-6-phosphatase (40.07%), decreased liver marker enzymes such as AST, ALT, ALP and ACP along with lipid profile and significantly increased plasma insulin (69.05%), liver glycogen (77.15%) and muscle glycogen content (76.86%) and glucose 6 phosphate dehydrogenase (38.52%) [5].

3.2. Chemical characteristic of γ-sitosterol

Chemical characteristics of γ-sitosterol were retrieved from pubchem database http://pubchem.ncbi.nlm.nih.gov/, Molecular Weight 414.7067 (g/mol), Molecular Formula $C_29H_{50}O$, XLogP3-AA9.3, H-Bond Donor1, H-Bond Acceptor1, Rotatable Bond Count6, Exact Mass 414.386166, IUPACName: (3S, 8R, 9R, 10S, 13R, 14S, 17R)-17-((2R,5S)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16, 17-dodecahydro-1H-cyclopenta (a)phenanthren-3-ol.SMILES:CCC (CCC(C)C1CCC2C1(CCC3C2CC = C4C3(CCC (C4)O)C)C)C(C)C. The Chemical properties were also checked in the ALOGPS 2.1. Chemical Formula: $C_29H_{50}O$,MW:414.79,SMILES:C1C(CH)(CC2 = CC(CH) 3(CH)((C)12C)CC(C)1((CH)3CC(CH)1(CH)(CC(CH)(C(C)C)CC)C)C)O, ALOGPs:7.27, ALOGpS:-7.35, AC_logP: 8.24, AC_logS: −6.67, miLogP: 8.62, Average logs:-7.01, ALOGP:8.08, MLOGP:6.79, KOWWIN:9.65, XLOGP2:9.06, XLOGP3: 9.34, Average logP: 8.38 [22].

3.3. Docking analysis

The docking simulations in the active sites of 1V4S, 2JJK, 2CBZ and 3LC4 were performed by the Auto dock program, which has been shown to successfully reproduce experimentally observed binding modes in terms of lowest docking energy. The target protein structure of 1V4S, 2JJK, 2CBZ and 3LC4 were docked with γ-sitosterol which provided excellent results as were seen by the least values of the binding energy.

The best possible binding modes of the γ-sitosterol at four targeted protein's active sites are displayed in Fig. 3 a, b, c and d and their corresponding energy values are listed in Table 1 by using PYMOL tool v 1.1 [23].

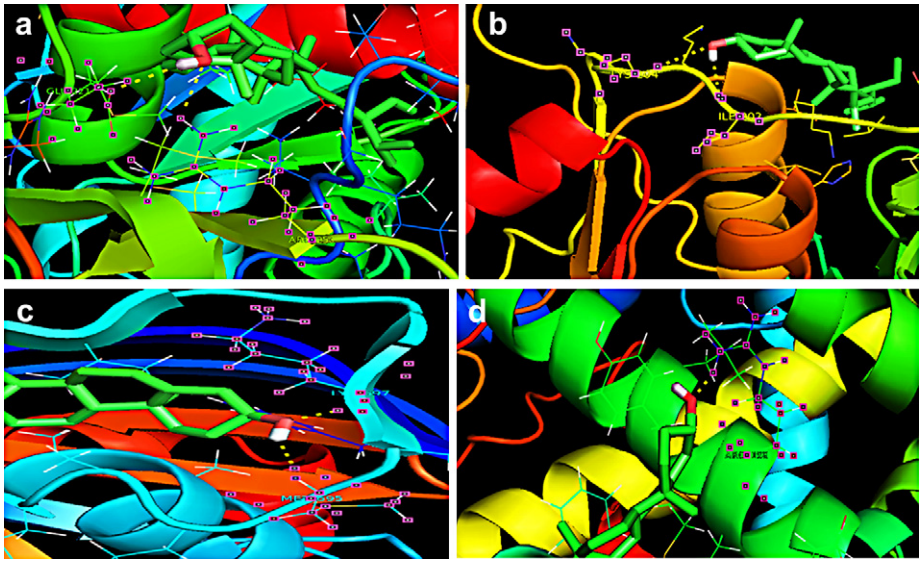

Fig. 3. a. Docked orientations of gamma sitosteriol with additional depiction of selected aminoacid residues of 1V4S and active site. Hydrogen bonds and polar interactions are shown as dotted lines, b. Docked orientations of gamma sitosteriol with additional depiction of selected aminoacid residues of 2JJK and active site. Hydrogen bonds and polar interactions are shown as dotted lines, c. Docked orientations of gamma sitosteriol with additional depiction of selected aminoacid residues of 2CBZ and active site. Hydrogen bonds and polar interactions are shown as dotted lines, d. Docked orientations of gamma sitosteriol with additional depiction of selected aminoacid residues of 3LC4 and active site. Hydrogen bonds and polar interactions are shown as dotted lines.

Table 1
Inhibitory activity of γ-sitosterol on 1V4S, 2JJK, 1ZNQ and 3LC4 target proteins.

S. no	Target proteins [PDB ID]	Ligand	No of H-bonds	Docked residues	Binding energy [kcal/mol]
1	1V4S	γ-sitosterol	2	GLU221/OE2 with 15 atoms, ARG'250/2HH1 with 39 atoms	−7.49
2	2JJK		2	[LYS204/N] with 17 atoms, ILE- 202/O with 8 atoms	−7.23
3	2CBZ		2	MET695/O with 17 atoms, LYS697/HN with 39 atoms	−8.59
4	3LC4		1	ARG198/HH1 with 24 atoms	−6.64.

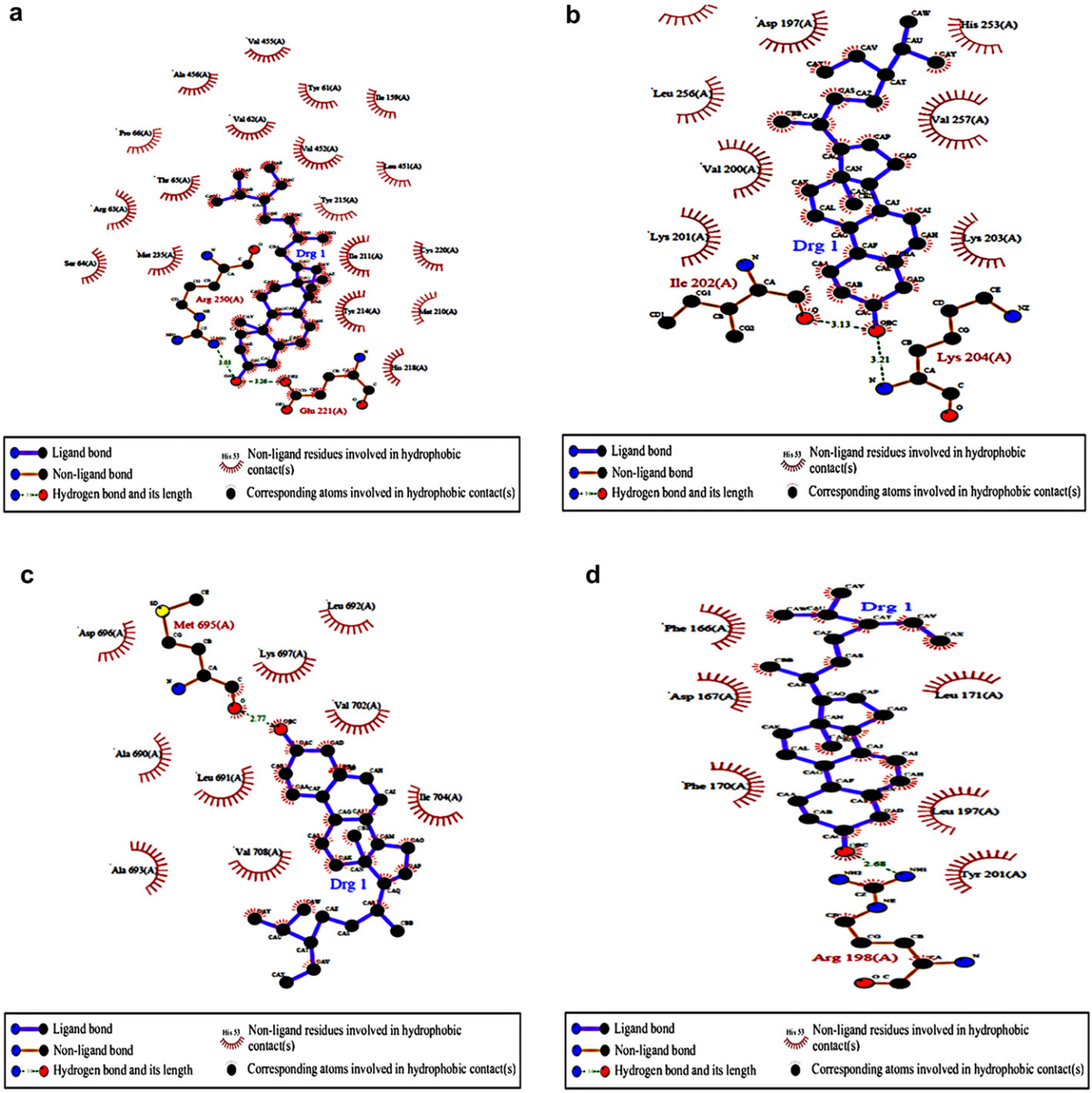

Fig. 4. a. Main hydrogen bonds between γ-sitosterol and (A)- human glucokinase (1V4S), b. Main hydrogen bonds between γ-sitosterol and fructose 1,6-bisphosphatase 1 (2JJK), c. Main hydrogen bonds between γ-sitosterol and (C)-human multidrug resistance protein 1 (2CBZ), d. Main hydrogen bonds between γ-sitosterol and human Cytochrome P450 CYP2E1 (3LC4).

Fig. 3(a) shows the result of docking analysis of human glukokinase (1V4S) with γ- sitosterol; it showed the binding site of the protein and ligand GLU'221/OE2 with 15 atoms and ARG'250/2HH1 with 39 atoms. Glucokinase is expressed only in liver and pancreatic beta cells and plays a key role in the regulation of glucose homeostatis. In the hepatocyte, the phosphorylation of glucose by glucokinase facilitates the uptake and metabolism of glucose by maintaining a gradient for glucose transport into these cells thereby regulating hepatic glucose disposal. In the beta cells, glucokinase is believed to be part of the glucose – sensing mechanism and to be involved in the regulation of insulin release [24]. During diabetes condition total or partial deficiency of insulin causes derangements in carbohydrate metabolism that decreases activity of several key enzymes including glucokinase [25] resulting in the impaired glucose utilization and augmented hepatic glucose production. Chandramohan et al. (2008) [26] reported that diabetic rats treated with 3-HMX active principle from plant increased glucokinase activity. In the same way γ-sitosterol increases glucokinase activity by binding with GLU221/OE2 and ARG 250/2HHI, thereby increasing the utilization of glucose leading to decreased blood sugar level.

Fructose 1, 6-bisphosphatase 1 (2JJK) showed binding interaction of protein –ligand LYS-204 N with 17 atoms and ILE- 202/O with 8 atoms when docked with lead molecule γ- sitosterol Fig. 3(b). Fructose 1, 6 bis phosphate catalyses the hydrolysis of fructose 1, 6-bisphosphate to fructose 6-phosphate and Pi [27] leading to glucose production in gluconeogenesis process during diabetes condition. Normally insulin inhibits the hepatic glucose production by suppressing G6Pase and fructose 1, 6-bisphosphatase activity [28,29]. In our study, docking of γ-sitosterol with 2JJK showed binding interaction with two amino acid residues, LYS-204N with 17 atoms and ILE-202/O with 8 atoms respectively. This may be the specific target for γ-sitosterol where it inhibits the Fructose 1, 6- bisphosphatase activity.

Fig. 3(c) illustrates the docking analysis of the human multidrug resistance protein 1 (2CBZ); it showed binding interaction of protein-ligand MET695/O with 17 atoms and LYS697/HN with 22 atoms. The multidrug resistance associated protein-1 (MRP-1) is the main transporter of oxidized glutathione in endothelial cells and blocking of MRP-1 prevents endothelial cell dysfunction induced by reactive oxygen species (ROS). MRP-1 therefore may represent a therapeutic target in treatment of diabetes induced vascular dysfunction [30].

Fig. 3(d) depicts the docking analysis of Human Cytochrome P450 CYP2E1 (3LC4); it showed binding interaction of protein – ligand ARG198/HH1 with 24 atoms. This enzyme plays an important role in drug metabolism. CYP2E1 expression has been linked to the generation of specific pathological condition including alcohol and non-alcohol induced liver disease [31,32]. This link stems from the unusually high capacity of CYP2E1 to generate free radicals due to low degree of coupling of the enzyme turnover and substrate binding. The free radicals produced by CYP2E1 are thought to result in lipid peroxidation and thus contribute to liver disease. Diabetes is commonly associated with development of fatty liver disease such as non alcoholic steatohepatitis (NASH). Ketones and other small organic molecules are both substrate and inducer of CYP2E1 [33,34]. Production of ketone bodies by diabetes would result in increased expression and catalytic activity of CYP2E1 [35]. Numerous studies in animal models have confirmed the induction of CYP2E1 in diabetes, but this was reversed by insulin treatment. In our study, γ-sitosterol docked with human cytochrome P450 2E1 protein indicating good protein–ligand interaction. Gupta et al. (2011) [36] reported the antioxidant property of β-sitosterol.

PDB SUM is a web based database providing largely a pictorial summary of the key information such as images of the structure and protein-ligand interaction using the LIGPLOT program [37]. In our study the targeted proteins 1V4S, 2JJK, 2CBZ and 3LC4 were subjected to secondary structure elucidation between ligand and protein. The ligand γ-sitosterol interacted mainly with target proteins through the formation of hydrogen bonds between the C-3 beta hydroxyl groups of A ring. γ-sitosterol interacted with target residues such as Glutamine Q221 (A) and Arginine R250 (A) of 1V4S, Lysine K204 (A) and Isoleusine I202 (A) of 2JJK, Methionine M695 (A) and Lysine K697 (A) of 2CBZ, Argnine R198 (A) of 3CL4 (A) (Fig.4a, b, c and d).

4. Conclusion

Docking studies of the ligand γ-sitosterol with four different target proteins showed that this is a good molecule which docks well with various targets related to diabetes mellitus. Thus γ-sitosterol can be considered for developing into a potent antidiabetic drug.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.10.007.

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