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Research Article

COMPUTER ASSISTED FRAGMENT BASED ANALYSIS OF PREGNENOLONE ANALOGUES AGAINST CYP17A1 RECEPTOR: A DOCKING APPROACH

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ABSTRACT

Cortisol, Dehydroepiandrosterone (DHEA) and Pregnenolone are the most clinically significant adrenal hormones. Among them, Pregnenolone is a precursor for sex steroidal hormones and also acts as a neuroprotective agent. Dis-regulation of their balance is observed at the stress conditions which result in the pathological state, pregnenolone steal. In this study we have computationally investigated the key enzyme CYP17A1 interaction with pregnenolone and its structural analogues. We first explored the active sites of the enzyme (PDBid: 3RUK) using CASTp and POCKET FINDER and was found to be consistent with the interaction residues. Next, fragment based search was performed to generate pregnenolone like compounds through Enchanced NCI server. Then the most potential competitive ligands were filtered by applying PASS Prediction tool, Lipinsky rule and ADME properties. The array of ligand along with pregnenolone was docked to enzyme structure using AUTODOCK 4.0 software. The prospective ligands were ranked according to the highest binding energy as the best conformers. The compounds such as pregnenolone-3acetate, 16-Dehydropregnenolone and Pregnenolone 3-methyl ether were identified as the significant ligands that can compete with pregnenolone in maintaining the balance during the stressed conditions. These compounds can be tried for such pathological states as a competitive binder for endogenous ligand, pregnenolone. However, further experimental evidence is required to support this study.

Keywords: SPregnenolone - Cytochrome CPY17A1 - Stress - Cortisol - Molecular docking - Active binding site - Binding energy

INTRODUCTION

Pregnenolone known as 3α, 5β-tetrahydroprogesterone is a naturally occurring major endogenous steroid synthesized in the central and peripheral nervous system, in the part independent of steroidogenic gland secretion [1]. It is a precursor to other steroid hormones, such as progesterone, DHEA (Dehydroepiandrosterone), mineralocorticoids (regulate electrolyte balance), corticosteroids that influence inflammation and metabolism, estrogens, and androgens [2]. It is also involved in the "neurosteroid" possessing intrinsic behavioral and brain effects in animals, affecting the $GABA_a$ and other receptors [3]. Low level of pregnenolone is observed during aging and is responsible for cancer growth. The immediate precursor of pregnenolone is reported in behavioral disorders in individuals receiving drugs that block synthesis of cholesterol. Additionally the lower level of precursor is observed in generalized anxiety disorder[4], dementia and patients with major depression suggesting pregnenolone's clinical utility[5]. At present pregnenolone has been suggested as an important nutritional supplement by the US Food and Drug Administration. However, high dose of pregnenolone can lead to over-stimulation, insomnia, irritability, anger and anxiety as well as to headaches, scalp hair loss, acne and rarely an irregular cardiac rhythm [6]. Human studies suggested that enhancement of performance by pregnenolone is seen under taxing conditions, such as stress [3]. Stressed state harmful effects are usually regarded as an inevitable long-term outcome of responses that are evolved from short-term adaptive benefits [7]. Pregnenolone plays a major role in stress condition by getting converted into cortisol, a major stress hormone via cytochrome P450 17a-hydroxylase (CYP17A1).

Human CYP17A1 is a single gene-encoded multifunctional enzyme with two activities: 17a-hydroxylase and 17, 20-lyase [8] that lies at the crossroads of androgen and corticoid biosynthesis protein. In general, CYP17A1 catalyses a hydroxylation reaction common to both pathways, and also the cleavage of a carbon-carbon bond required only for the formation of androgens [9]. 17a-hydroxylase needed for glucocorticoid production acts by inserting the oxygen atom into a C-H bond and the 17, 20-lyase activity is needed for sex steroid production in which 21-carbon 17a-hydroxysteroids are cleaved to 19-carbon, 17-ketosteroids, and acetic acid (Figure 1). CYP17A1 can also catalyse a modest degree of 16a-hydroxylase activity but does not hepatic 16a-hydroxylase. Thus, CYP17A1 catalyses two fundamentally different reactions on a single active site both of which are crucial for human physiology [10]. The dual activity of this enzyme is important for controlling the gonadal steroid genesis in patho-physiological condition and the mechanism of maturation of adrenal gland. Mutation is considered to be one of the major causes of deficiency of this protein. Initially the F417C mutation was reported in 17, 20-lyase deficiency case and represented the first phosphorylation CYP17A1-deficient mutant [8]. Later the mutations at different sites such as, A174E, V178D, and L465P and R440C were found to be the prominent cause for congenital adrenal hyperplasia, also known as 17OHD [11].

The excess release of cortisol after prolonged stressed state upsets the rhythmicity of Hypothalamus-Pituitary-Adrenal (HPA) axis, figure 2 [12]. This in turn is directly involved in the course of depression state [13]. In this study, cortisol was chosen as a major stress hormone over adrenaline since cortisol is a relatively long term effector. It has a half-life measured in hours, and its effects last longer. Adrenaline (epinephrine) is an immediate-response hormone having a low half-life with its effects disappearing rapidly. Cortisol increases (or decreases) the amount of a given enzyme whereas, adrenaline acts by modulating the activity of existing enzymes. Cortisol also potentiates epinephrine effects by increasing epinephrine synthesis and inhibiting catecholamine breakdown, and has permissive effects in many tissues [14].

The idea in this work was to computationally find novel ligands that can compete with the natural ligand, pregnenolone for CYP17A1 active site, whereby controlling the prolonged over production of cortisol in stress conditions. Initially the binding sites of CYP17A1 were explored through computer assisted investigation. The active site residues of the receptor protein were explored by CASTp and Pocket Finder program. Then the prospective ligands were extracted through fragment based search through online NCI server. The activities of the obtained ligands were ranked in order by filtering those using LIPIN SKI values and ADME properties. Finally, docking analysis was performed for the ligands against the predicted binding site of CYP17A1 using AutoDock4 software. The docked molecules were ranked based on the values of binding energy to predict the most competitive binding molecules within the selected dataset of selected ligands.

MATERIALS AND METHODS

Protein structural data

The structural data for the respective enzyme protein and the ligand was collected from the PDB and PUBMED database. The target enzyme taken in this study comes under the CypX/450 super family. Recently the human CPY17A1 protein was crystallized and its structure is available as homotetrameric structure complex with abiraterone drug (PDBid: 3RUK) [15]. Then the 3D structure data file format of the natural endogenous ligand, pregnenolone (CID: 8955) was obtained from Pubmed database for further investigation.

Binding site prediction in CPY17A1 protein

The active ligand binding site or the binding pocket residues were predicted using online CASTp [16] server. It uses the pre-calculated PDB surface features such as void volume and accessible surface of the pocket. In addition to the above tool, a different algorithm was used to validate the binding sites using the Pocket-Finder [17]. Pocket-Finder works by scanning probe radius 1.6 angstroms along all gridlines of grid resolution 0.9 angstroms surrounding the protein. The probe also scans cubic diagonals. The validation of the result from these 2 servers was done by identifying the common active site residues reported by these tools. Again it was cross verified with the abiraterone binding residues of CYP17A1 protein as observed using Pymol [18]. Within the pocket we further probed the exact amino acids using the Conseq program version 1.1[19] to determine the most conserved and exposed for ligand binding. Conseq works by identifying the functional and structural residues based on the multiple alignment of homologous sequence. The program indicates the highly conserved functional residues with its surface exposure.

Screening of similar compound

A fragment based search for the pregnenolone compound was conducted using the Enhanced NCI Database Browser2.1 [20] in order to identify similar structural compounds. Enchanced NCI Database is part of NCI/CADD group, a research unit within the Chemical Biology Laboratory, which is part of the Molecular Discovery Program at the National Cancer Institute. It a web service to the open NCI database compounds (>250,000 structures). Here we queried the database by fixing the field specific parameter in search of the novel ligands by using name and fragments option while keeping all the other parameters as default. A total of 100 hits were taken further along with the query molecule.

Chemo-informatics analysis

The properties of biologically active substance from the above compounds were tested using the PASS prediction online tool [21]. The PASS software predicts more than 300 pharmacological effects and biochemical mechanisms on the basis of the structural formula of a substance. The tool uses the descriptors to predict the activity of a substance. This information can be efficiently used to find novel ligands for CYP17A1 receptor targets and LIPINSKI rule [22]. Lipinski's Rule of five is a rule of thumb to evaluate drug likeliness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion (ADME). These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures. These compounds were then filtered based on the glucocorticoid agonist and antagonist activity. Also the number of catalytic conformers was taken in consideration and the ligands were ranked more appropriately.

Docking and scoring

A rigid docking methodology present in the AUTODOCK 4.0 software [23] was followed while docking the filtered compounds against the CYP17A1 target protein. Autodock consist of two main programs, (1) autogrid, pre-calculates these grids and (2) autodock performs the docking of the ligand to a set of grids describing the target protein. In addition to using them for docking, the atomic affinity grids can be visualized. A graphical user interface called Auto Dock Tools or ADT was utilized to generate grids, calculate dock score and evaluate the conformers. The rigid docking is blindingly fast and at the same time allows side chains in the macromolecule to be flexible. The free-energy scoring function is based on a linear regression analysis, the AMBER force field, and an even larger set of diverse protein-ligand complexes with known inhibition constant.

RESULT AND DISCUSSION

Active site binding pocket of cytochrome CYP17A1 molecule

The active site residues that form the core cavity will accommodate and interact with the ligands. However the functionally important residues within the ligand binding pocket should be accessible for the ligand to interact. Hence these functional residues are expected to be highly conserved and exposed. The functional sites that were predicted by CASTp and Pocket Finder were shown in Table.1. From this table the CASTp and Pocket Finder showed several common residues. Moreover these amino acids were also shown to be active site residue of the native complex (Figure 3). The residues incorporated for docking are Asn202 as it was the only residue the formed the hydrogen bond with abiraterone in the original bound state, Arg239 and Thr306. Val483 not a highly conserved residue was selected for docking due to its critical positioning in the binding pocket [9].

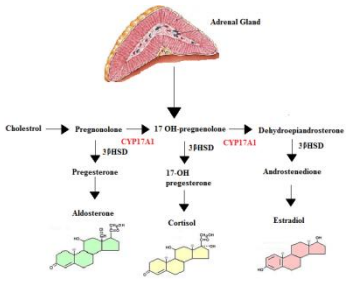


FIGURE1: THE COMPLETE INTERCONNECTED BIOLOGICAL PATHWAY FOR THE FORMATION OF CORTISOL AND THE ROLE PLAYED BY CYTOCHROME CPY17A1.

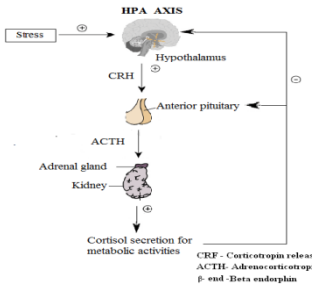


FIGURE 2: FLOWCHART REPRESENTATION OF HPA (HYPOTHALAMUS-PITUITARY-ADRENAL) AXIS AND ITS ROLE IN CORTISOL SYNTHESIS.

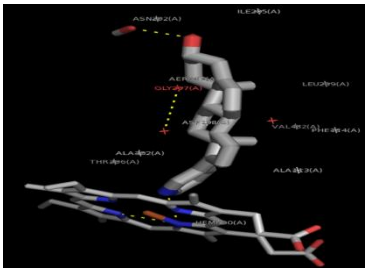


FIGURE 3:INTERACTING RESIDUES INVOLVED IN CYTOCHROME CYP17A1 PROTEIN WITH ABIRATERONE MOLECULE, VISUALIZED IN PYMOL VIEWER

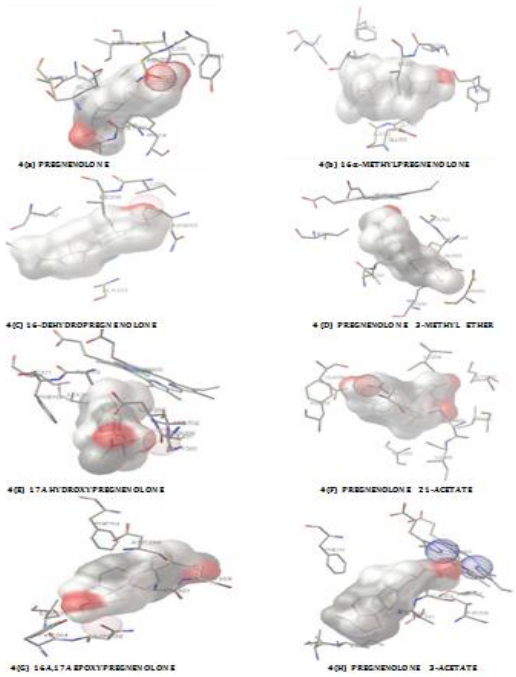


FIGURE 4:RIGID LIGAND INTERACTION OF CYTOCHROME CYP17A1WITH PROSPECTIVE LIGANDS ALONG WITH THE BINDING RESIDUES AS BY AUTODOCK4

Ranking of the pregnenolone analogues for docking

The first 10 analogues from Enhanced NCI were chosen such that they show maximum competitive binding and antagonistic activity on binding with CYP17A1 molecule. The molecules were prioritized based on the parameters 1) Lipinski rule of 5, 2) Drug Likeliness

(ADME) and 3) PASS prediction of glucocorticoid agonistic and antagonistic activity. Based on the above property prediction it was found that three analogues namely, Pregnenolone acetate semicarbazone, 16α,17α Epoxypregnenolone acetate and Dehydropregnenolone acetate were not included because they did not obeyed the Lipinski rule of 5(Table 2). Moreover the PASS predicted was applied to the remaining seven ligands in which 17α Hydroxy-pregnenolone was found to have the highest glucocorticoid antagonistic activity (P(a)=0.309) while Pregnenolone 3-acetate was next with the P(a)=0.187 and the lowest activity was shown by 16α-Methylpregnenolone (P(a)=0.116) but greater than pregnenolone (P(a)=0.086).

Protein-Ligand docking studies on Cytochrome CYP17A1The above obtained analogues were subjected to docking with the crystallized structure of cytochrome CYP17A1 by the widely used docking program AUTODOCK 4.0. Docking was performed for all four molecules by keeping the ligand interaction as rigid. The grid of 50*50*50 was placed around the selected conserved functional residues as stated above, centering all the residues. Genetic algorithm (GA) docking was applied for 100 docking poses. Figure 4(A-H) shows the best configuration of binding along with the interacting amino acid residues of CYP17A1 with the respective docked molecules. The bound analogues and pregnenolone were examined for their binding energies, inhibitory constant and hydrogen bonding. The conformations with the highest binding energy, greater number of hydrogen bonds, and interaction with the heme atom in all the ligands were taken in consideration for ranking of the analogues as a prospective competitor of pregnenolone and is represented in table 3. Heme is considered as an important factor for consideration as from the previous experimental studies done by DeVore and Scott[24], it is found that both the prostate cancer drugs abiraterone and tok-001 bound with the heme iron forming a 60° angle above the heme plane, packing against the central I helix of CYP17A1[24].

TABLE 1: THE FUNCTIONAL AND EXPOSED RESIDUES OF THE ACTIVE BINDING POCKET OF CYP17A1

S.NO.	POCKET FINDING SERVER/ SOFWARE	HIGHLY CONSERVED AND EXPOSED RESIDUES OF POCKET	Predicted in this work
1	CASTp	Gly95, Arg96, Pro97, Gly111, Asp116, Arg125, Asn261, Thr306, Gly436, Arg440, Val483	
2	Pocket Finder	Gly111, Asp116, Arg125, Glu143, Try250, Asn261, Gly436, Arg440, Thr306, Val483	
3	Experimental	Asn202, Arg239, Glu305, Thr306, Val483	Devore and Scott 2012

TABLE 2:THE PROSPECTIVE SUBSTRATES FOR CYP12A1 INTERACTION WITH THEIR RESPECTIVE CHEMICAL PROPERTIES AND CHEMICAL STRUCTURE

SN	MOLECULE NAME	LIPINSKI RULE OF 5				GLUCO-CORTICOID ACTIVITY (ACTIVITY PREDICTION BY PASS)		2D-STRUCTURE
		H-bond doner <5	H-bond acceptor<10	Mol Mass <500 Dalton	Log P<5	Agonist P(a)/P(i)	Antagonist P(a)/P(i)	
1	Pregnenolone	1	2	316.48	4	0.374/0.006	0.086/0.007	
2	16-Dehydropregnenolone	1	2	314.47	4.1	0.049/0.011	0.149/0.019	
3	Pregnenolone 3-methyl ether	0	2	330.51	4.6	0.069/0.008	0.174/0.014	
4	16α-Methylpregnenolone	1	2	330.51	4.3	0.234/0.011	0.116/0.005	
5	16α,17α Epoxypregnenolone acetate	0	4	372.5	4.9	0.126/0.264	0.122/0.115	
6	Dehydropregnenolone acetate	0	3	356.5	5.1	0.042/0.013	0.144/0.020	

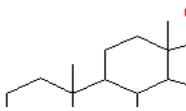
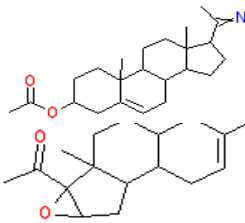
7	17α Hydroxypregnenolone	2	3	332.48	3.7	0.157/0.004	0.309/0.005	
8	Pregnenolone 3-acetate	0	3	358.52	5	0.071/0.008	0.187/0.013	
9	Pregnenolone 21 acetate	1	4	374.52	3.9	0.121/0.005	0.182/0.013	
10	Pregnenolone acetate semicarbazone	2	5	415.57	6.1	0.112/0.035	0.274/0.229	
11	16α,17α Epoxypregnenolone	1	3	330.47	3.9	0.142/0.057	0.135/0.004	

TABLE 3:RIGID LIGAND INTERACTION STUDIES: CONFORMATIONAL PROPERTIES OF THE LIGANDS SHOWING THE BES ENERGIES SELECTED AND PLOTED AS BY AUTODOCK VERSION 4.0

SN	PROPERTY	PREG *	PREG 3-METHYL ETHER	16α-METHYL-PREG	16-DEHYDRO-PREG	PREG 21-ACETATE	PREG 3-ACETATE	16α17α-EPOXYPREG	PREG
1	Total Binding energy	-9.88	-7.3	-7.63	-8.27	-7.42	-6.77	-9.55	-10.33
2	Inhibition constant(nM)	57.7	4480	2540	868.24	3630	10810	99.14	31.49
3	Intermolecular energy	-10.56	-7.89	-8.33	-8.86	-8.41	-7.68	-10.15	-0.42
4	Total internal energy	-0.06	-0.27	-0.09	-0.19	-0.8	-0.34	-0.19	-0.42
5	Torsion	0.6	0.6	0.6	0.6	1.49	0.89	0.6	0.89
6	Unbound energy	-0.15	-0.26	-0.01	-0.19	-0.3	-0.36	-0.19	-0.17
7	Hydrogen bonds	1	0	0	1	1	2	1	1
8	Heme 600 interaction	NO	YES	NO	NO	NO	YES	NO	YES

PREG*- Pregnenolone
Unit of energies is kcal/mol

TABLE 4: RIGID LIGAND INTERACTION STUDY: LIGANDS RANKED BY THE BEST BINDING ENERGIES ALONG WITH THEIR INTERACTING RESIDUES

RANK	ANTAGONIST	ENERGY (Kcal/mol)	INTERACTING RESIDUES
1	Pregnenolone 3-acetate	-6.77	Phe114, Ile209, Ile266, Gly301, Ala302, Thr306, Val 366, Val483, **Hem600**
2	Pregnenolone 3-methyl ether	-7.3	Asn202, Ile206, Gly301, Ala302, Glu305, Ile371, Val482, Val483, **Heme600**
3	16α-Methylpregnenolone	-7.36	Phe114, Try201, Ile205, Ile206, Gly301, Ala302, Glu305, Ile371, Val482
4	Pregnenolone 21-acetate	-7.42	Phe172, Gln199, Ile208, Glu305, Ile439, Lys481, 485, Ile486, Ser488
5	16-Dehydropregnenolone	-8.27	Asn202, Ile205, Ile206, Ala302, Ile371, Val482
6	16α, 17α-Epoxypregnenolone	-9.55	Phe114, Ala298,Try201, Asn202, Gly301, Ala302, Ile235
7	17α-Hydroxypregnenolone	-10.88	Ala113, Phe114, Gly297, Ala298, Gly301, Ala302, Thr306, Ala367, Ile371, **Hem600**

CONCLUSION

Structure based drug designing is a wide area in rational drug designing that offers a valuable alternative to the costly and time consuming process of random screening [25]. Among them, fragment based approach is one of the promising area for identifying the promiscuous ligands. Our study has explored active sites of CYP17A1 protein that can be further utilized for future aspect of drug designing. CASTp and Pocket Finder tools have been benchmarked tools for predicting active site amino acid in a protein with high accuracy. Here these tools were employed to decipher the binding residues present in the CYP17A1 protein which were most consistent with the previous experimental results from another study. In this approach, docking protocol was applied which ensure us to find the prospective ligands that can bind to the active sites of the CYP17A1 protein for that may control the prolonged cortisol biosynthesis. The scoring functions were based on protein-ligand energy terms, namely Van der Waals energy, dissolving energy, electrostatic energy, torsion free energy and unbound system energy. According to the binding energy, inhibitory constant, hydrogen binding and heme binding the top scoring energy ligand is considered best with highest rank. Table 4 shows that the most

appropriate competitor for pregnenolone can be pregnenolone 3-acetate with highest binding energy of -6.77 kcal/mol, inhibitory constant 10,810 nM along with Heme600 binding and 2 hydrogen bonds, both with heme. The next promising compound predicted is Pregnenolone 3-methyl ether, even though it failed to form hydrogen bond possessed binding energy of -7.3 kcal/mol and inhibitory constant 4480nM. 17α-Hydroxypregnenolone even though bonded with Heme600 and one hydrogen bond with Asp289 was positioned last as the binding energy was -10.88 kcal/mole and a very low inhibition constant of 31.49 nM. The functional region of the receptor molecule was contributed by charged residues for effective antagonistic binding. In the docking analysis, the high scoring ligands were found to interact with the amino acid residues in the binding pocket namely, Phe114, Try201, Asn202, Ile205, Ile206, Gly301, Ala302, Glu305, Thr306, Ile371, Val482 and Val483. The physiochemical properties of these amino acids are predominantly hydrophobic and aliphatic residues. Thus we strongly hope that this fragment based approach will lead to novel insight into these classes of ligand molecules, which could further

enhance our understanding to design novel compound for the stress conditions.

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