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Research Article

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An *in silico* study on the interaction between RU486, a glucocorticoid antagonist and some regulatory proteins of lipid metabolism

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ABSTRACT

Antagonism of the glucocorticoid receptor is used to reduce the detrimental effects of elevated glucocorticoid levels and associated metabolic abnormalities. RU486, also referred as mifepristone, is a known antiprogestin that also competitively blocks the glucocorticoid receptor. Pharmacological inhibition of glucocorticoid action using RU486 improves insulin intolerance and obesity. However, the effect of RU486 on lipid metabolism has not been studied. The present study investigates the interaction of RU486 with proteins involved in lipid metabolism through in silico approach using Accelrys Discovery Studio software. We observed that RU486 interacts with the proteins involved in lipogenesis such as hairy enhancer of split -1, fatty acid synthase, liver X receptor β and phosphoenolpyruvate carboxykinase. Also, RU486 binds to phosphodiesterase 3B, a protein engaged in lipolysis. Thus, RU486 exerts a direct effect on lipid metabolism. These results may have implications in the treatment of disorders associated with lipid metabolism.

Keywords: Insulin resistance, RU486, glucocorticoid receptor, lipogenesis, lipolysis

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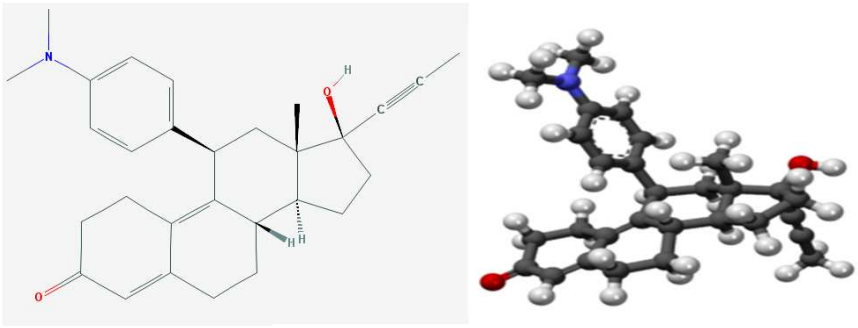
INTRODUCTION

Glucocorticoid excess in patients suffering from hypercortisolism (Cushing's syndrome) results in whole body insulin resistance, increased glucose levels, and central obesity and conversely, circulating/cellular glucocorticoid levels are high in patients with type 2 diabetes (T2D) [1]. High levels of glucocorticoid increases the amount of fatty acids in circulation, which then induces ectopic lipid accumulation in liver, skeletal muscle, and central adipocytes [2]. Also, excess glucocorticoid increases the fatty acid synthase (FASN) activity resulting in hepatic *de novo* lipid production [3]. These evidences suggest that subtle abnormalities in lipid metabolism induced during hypercortisolism have a significant role in the pathogenesis of the complications of metabolic disorders like insulin resistance, hyperglycemia, dyslipidemia and hypertension. Glucocorticoid antagonism has become an active research area as the antagonists are potential candidates for treating metabolic disorders.

The drug RU486 (Figure 1), also known as mifepristone, is a glucocorticoid antagonist which has been approved by FDA (*Korlym*™, 2012) for the treatment of patients with Cushing's syndrome [4]. RU486 also blocks the progesterone receptor and is clinically used as an agent to terminate pregnancy [5]. Clinical studies show that RU486 inhibits the hypercortisolemia-related hyperglycemia and insulin resistance and improves glucose tolerance in *db/db* mice as well as patients with Cushing's syndrome [6, 7]. Treatment with RU486 has been shown to decrease white adipose tissue (WAT) lipolysis by attenuating the expression of angiopoietin-like 4 (Angptl4), a lipolytic gene [8].



PubChem CID: 55245
Molecular Formula: $C_{29}H_{35}NO_2$

Figure 1. Mifepristone (RU486)

These studies point out the possibility of RU486 as an effective target in treating lipid disorders associated with insulin resistance. The objective of this study is to investigate the binding characteristics of RU486 with the glucocorticoid - targeted lipogenic and lipolytic proteins through *in silico* analysis.

EXPERIMENTAL SECTION

The glucocorticoid targeted lipogenic proteins namely hairy enhancer of split -1 (HES-1), peroxisome proliferator-activated receptor γ (PPARγ), sterol regulatory element-binding protein 1c (SREBP-1c), cluster differentiation 36 (CD36), acetyl-CoA Carboxylase (ACC), FASN, stearoyl-CoA desaturase-1 (SCD-1), liver X receptor β (LXRβ) and phosphoenolpyruvate carboxykinase (PEPCK) and the lipolysis regulatory proteins namely hormone-sensitive lipase (Lipe), monoglyceride lipase (Mgll), phosphodiesterase 3B (PDE3B) and Angptl4 were analyzed in this study. The 3D structure of target proteins involved in the regulation of lipid homeostasis were obtained from Protein Data Bank (PDB) database and the drug was downloaded from chemical database. The interaction was analyzed using Discovery Studio software (Accelrys, version 4.0, San-Diego, CA, USA).

Target Preparation

For docking analysis the target proteins were obtained from databases namely PDB, National Centre for Biotechnology Information (NCBI) and DeepView (Swiss PDB - Viewer) application.

Ligand Preparation and modification

The ligand RU486 was obtained and modified using PUBMED and PUBCHEM databases and CHEMSKETCH, respectively [PubChem CID: 55245].

Docking Steps

Docking analysis of the protein molecules and the ligand were carried out according to the Discovery Studio Module. The protein molecule was imported and prepared by deleting the ligand and the water molecules and by adding the hydrogen atoms. The force field was applied to the protein molecule and the binding sites were detected. The ligand molecule was imported and optimized. Docking was performed by selecting the ligand against the available receptor sites.

The best docked poses at receptor sites were observed and the best poses were analyzed using the dock score and the Potential of Mean Force (PMF). The docking results were saved for future reference.

RESULTS AND DISCUSSION

The present study verified the interaction of RU486 with glucocorticoid targeted lipogenic and lipolytic proteins using Accelrys Discovery Studio 4.0 [@]. RU486 is well known to have a good binding affinity to glucocorticoid receptor [9]. Our docking results showed that among all the target proteins, RU486 interacted with four lipogenic proteins namely HES-1, FASN, LXRβ and PEPCK and a lipolysis regulatory protein PDE3B (Table 1).

RU486 docked with HES-1 protein at three binding cavities S1 (dock score 42.87 and ligand internal energy 93.56), S3 (dock score 35.092 and ligand internal energy 90.44) and S6 (dock score 42.314 and ligand internal energy 70.87) (Figures 2B, 2C and 2D, respectively). RU486 showed significantly higher interaction with FASN protein than all of the proteins and displayed interaction at three binding sites S1 (dock score 51.689 and ligand internal

energy 98.55), S2 (dock score 44.486and ligand internal energy 100.68) and S3 (dock score 45.274 and ligand internal energy 121.29) (Figures 3B, 3C and 3D, respectively). Similarly, RU486 showed interaction with LXRβ protein at two binding sites S8 with dock score 17.015 and ligand energy 42.29 and, S10 with dock score 2.454 and ligand energy 88.08 (Figures 4B and 4C, respectively). RU486 docked with PEPCK protein and showed binding affinity at four binding sites S4 (dock score 44.866 and ligand internal energy 106.78), S8 (dock score 18.932 and ligand internal energy 52.09), S10 (dock score 31.061 and ligand internal energy 54.80) and S13 (dock score 15.561 and ligand internal energy 55.82) (Figures 5B, 5C, 5D and 5E, respectively). Among the lipolysis regulatory proteins, RU486 showed interaction only with PDE3B protein at four binding cavities S3 (dock score 44.501 and ligand internal energy 109.99), S4 (dock score 69.148 and ligand internal energy 161.04), S5 (dock score 37.509 and ligand internal energy 47.39), S9 (dock score 6.671 and ligand internal energy 25.23) (Figures 6B, 6C, 6D and 6E, respectively).

FASN, a rate limiting lipogenic enzyme, catalyzes the conversion of malonyl-CoA to fatty acyl-CoA which is then directed towards triglyceride (TG) synthesis. An important lipogenic transcription factor LXRβ upregulates PEPCK which inturn promotes hepatic gluconeogenesis and fatty acid production in adipose tissue. Interaction of RU486 with FASN and LXRβ may lead to reduced lipid synthesis through inhibiting the actions of FASN and LXRβ. HES-1, a anti-lipogenic transcription factor gets downregulated during metabolic disorders like insulin resistance and obesity leading to the increased expression of lipogenic proteins PPARγ, SREBP-1c and CD36 leading to decreased

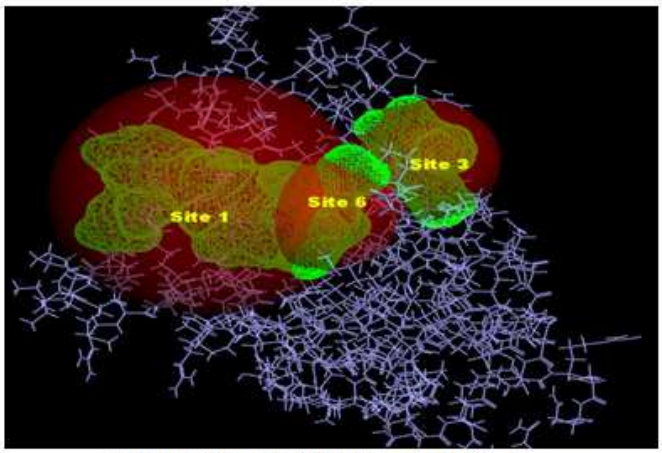


Figure 2A. Binding sites of HES-1

TG hydrolysis and lipid accumulation. The interaction of RU486 with HES-1 may regulate lipid accumulation through activating the transcription of HES-1 and thus, increasing the TG hydrolysis and fatty oxidation. Another important regulator of lipid metabolism which was found to bind with RU486 was PDE3B. Interaction of RU486 with PDE3B may have an effect on lipolysis and FFA production. Thus, interaction of RU486 with these molecules may have potential regulatory effects on lipid metabolism.

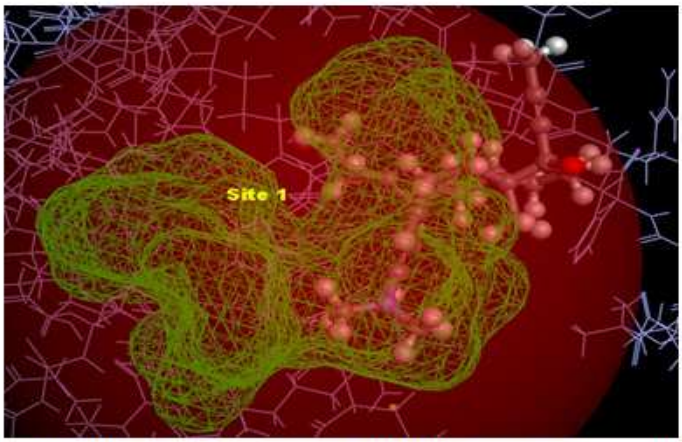


Figure 2B. Interaction of RU486 with HES-1 at binding site 1 (S1)

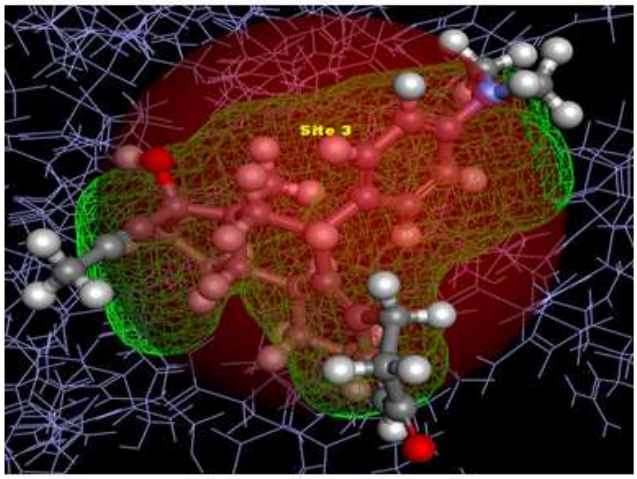


Figure 2C. Interaction of RU486 with HES-1 at binding site 3 (S3)

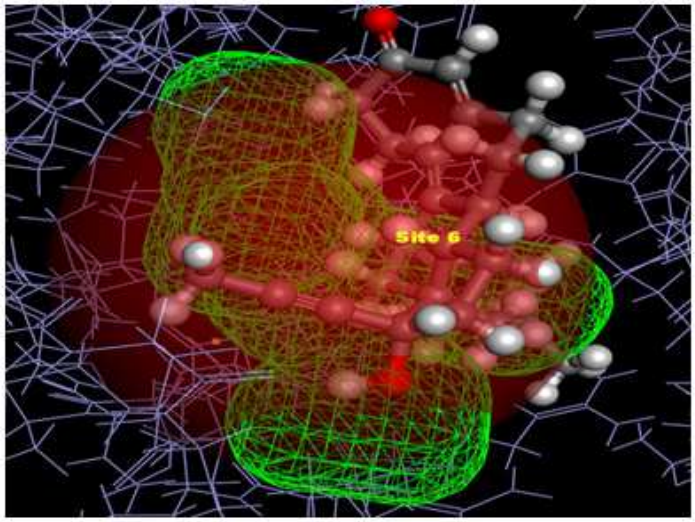


Figure 2D. Interaction of RU486 with HES-1 at binding site 6 (S6)

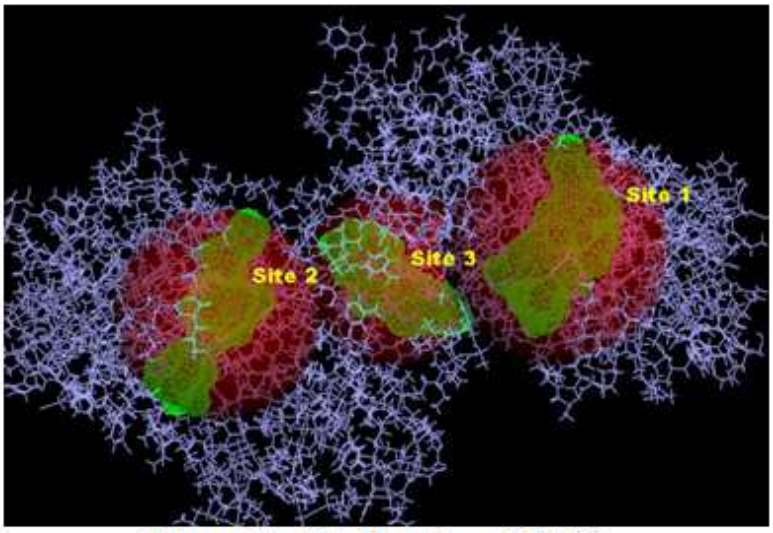


Figure 3A. Binding sites of FASN

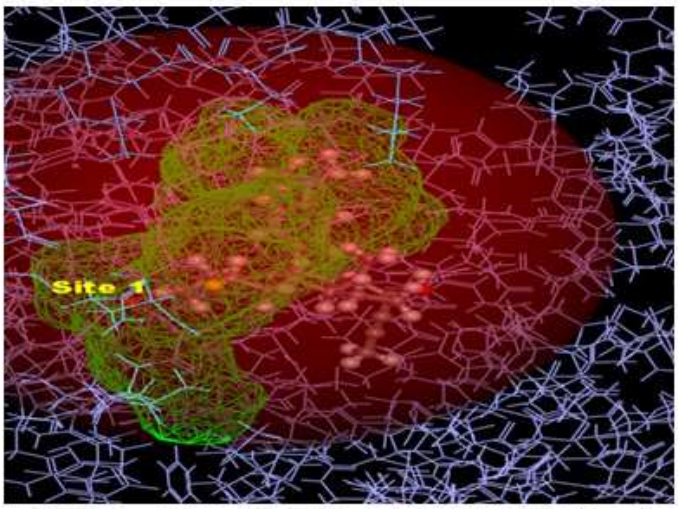


Figure 3B. Interaction of RU486 with FASN at binding site 1 (S1)

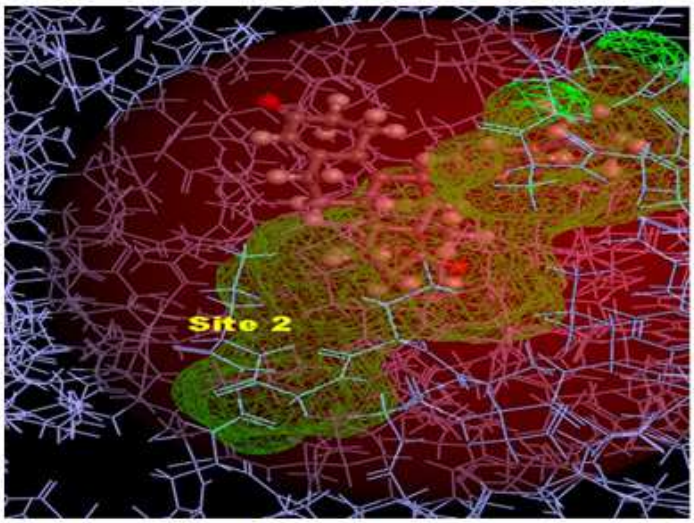


Figure 3C. Interaction of RU486 with FASN at binding site 2 (S2)

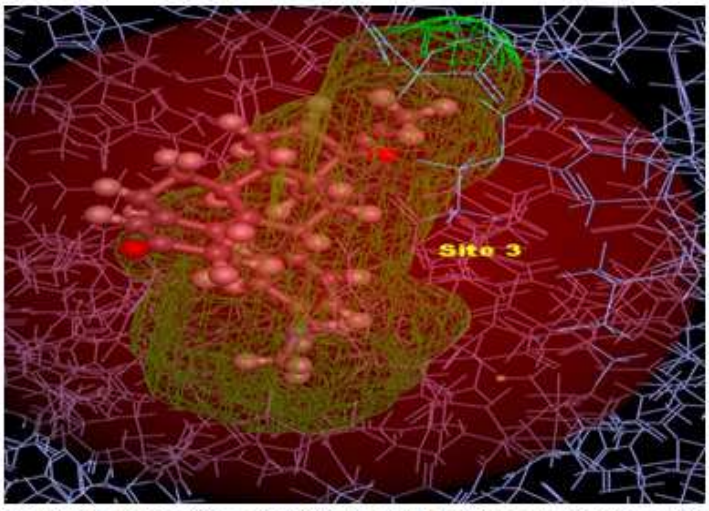


Figure 3D. Interaction of RU486 with FASN at binding site 3 (S3)

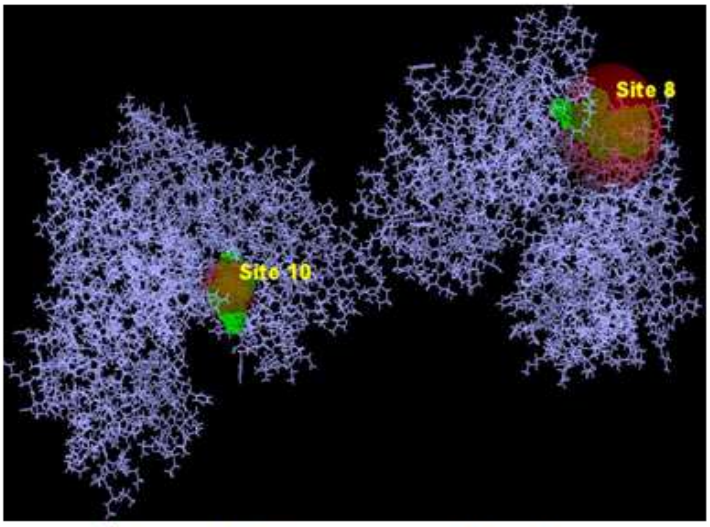


Figure 4A. Binding sites of LXRβ

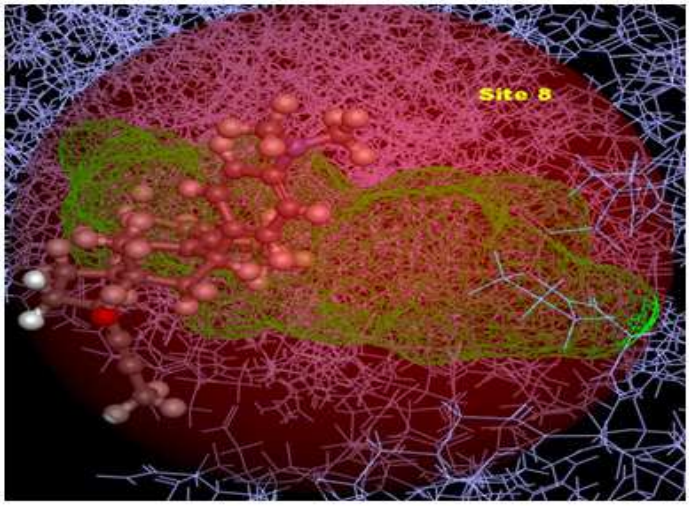


Figure 4B. Interaction of RU486 with LXRβ at binding site 8 (S8)

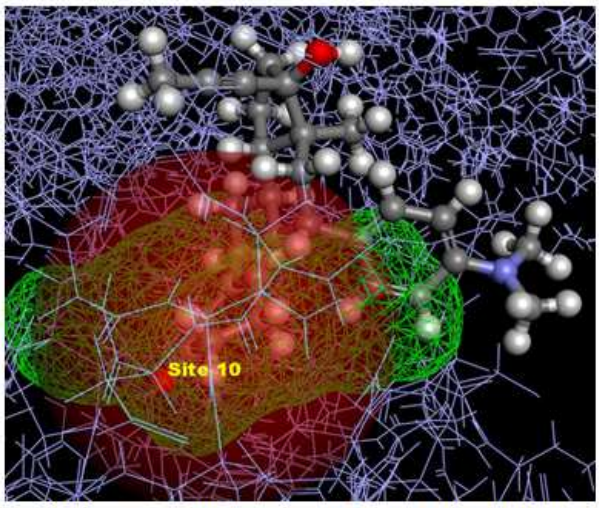


Figure 4C. Interaction of RU486 with LXRβ at binding site 10 (S10)

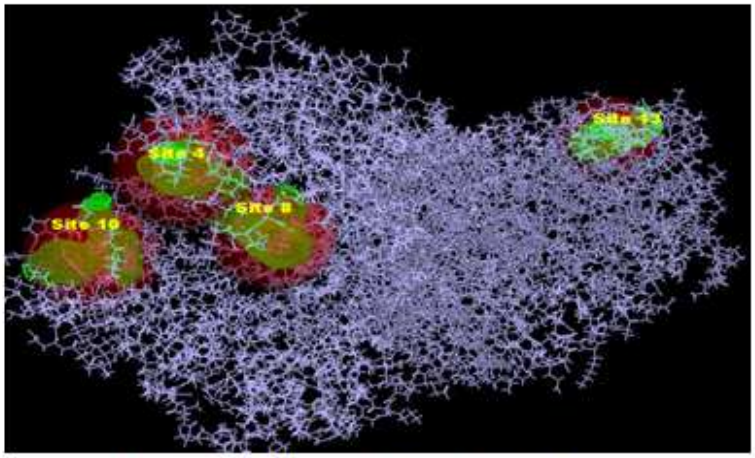


Figure 5A. Binding sites of PEPCK

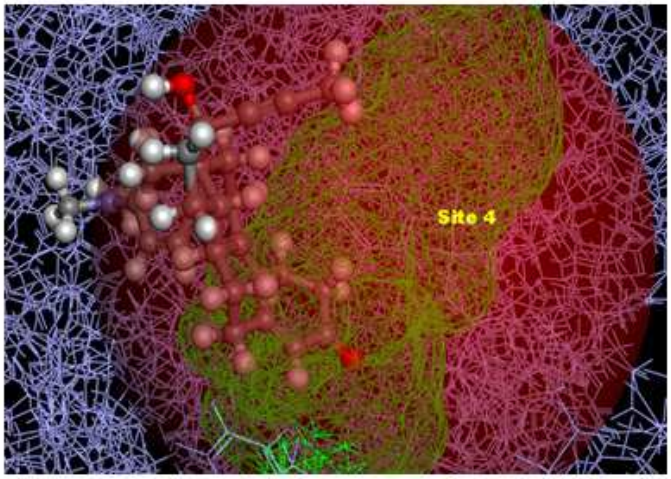


Figure 5B. Interaction of RU486 with PEPCK at binding site 4 (S4)

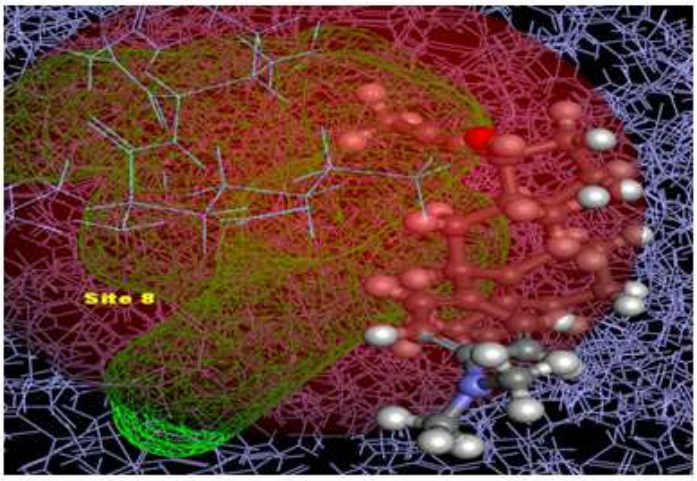


Figure 5C. Interaction of RU486 with PEPCK at binding site 8 (S8)

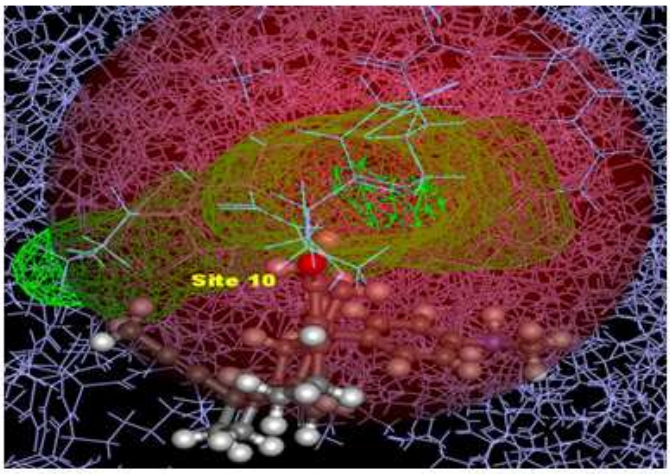


Figure 5D. Interaction of RU486 with PEPCK at binding site 10 (S10)

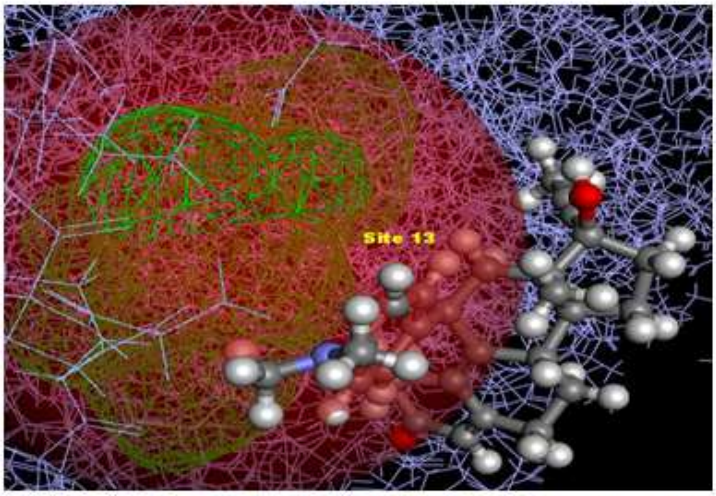


Figure 5E. Interaction of RU486 with PEPCK at binding site 13 (S13)

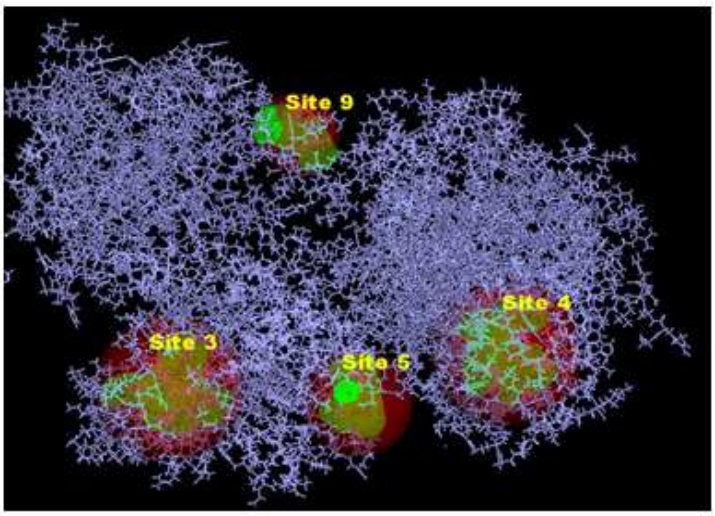


Figure 6A. Binding sites of PDE3B

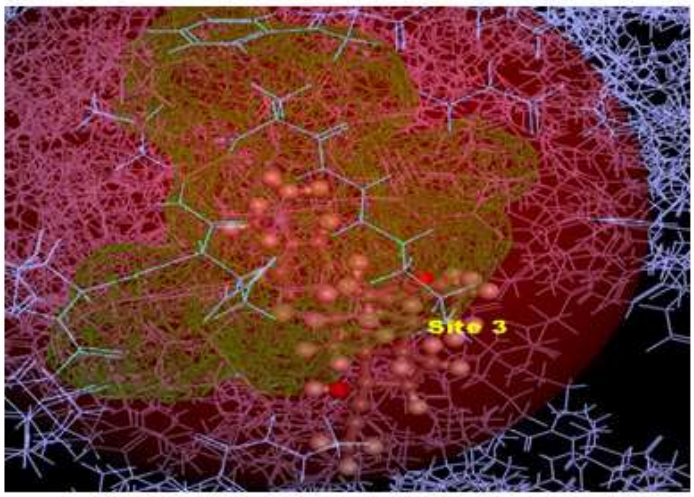


Figure 6B. Interaction of RU486 with PDE3B at binding site 3 (S3)

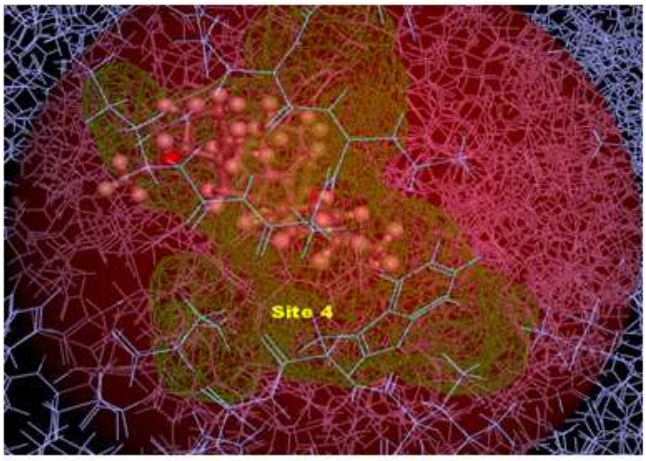


Figure 6C. Interaction of RU486 with PDE3B at binding site 4 (S4)

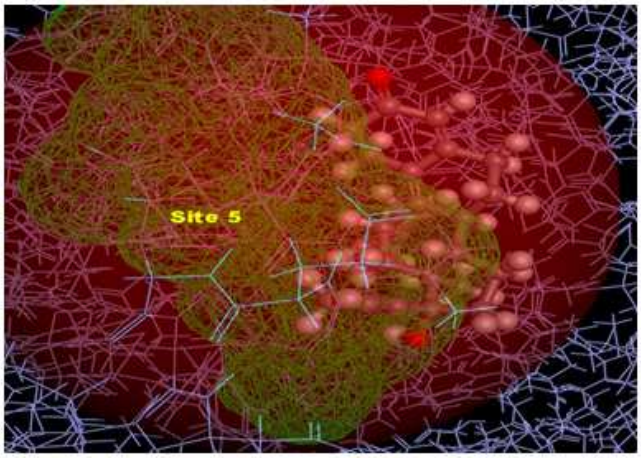


Figure 6D. Interaction of RU486 with PDE3B at binding site 5 (S5)

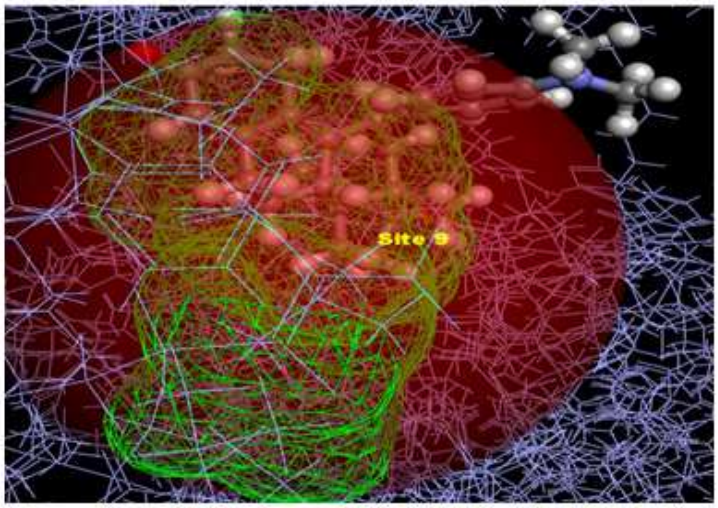


Figure 6E. Interaction of RU486 with PDE3B at binding site 9 (S9)

Table 1. Binding site, ligand internal energy and dock score of interaction of RU486 with lipogenic and lipolytic proteins

S.No.	Protein	PDB ID/ UniPort Entry ID	Binding Site	-PMF	Dock Score
1.	HES-1	2MH3	1	93.56	42.87
			3	90.44	35.092
			6	70.87	42.314
2.	FASN	4W82	1	98.55	51.689
			2	100.68	44.486
			3	121.29	45.274
3.	LXRβ	1PQ9	8	42.29	17.015
			10	88.08	2.454
4.	PEPCK	2GMV	4	106.78	44.866
			8	52.09	18.932
			10	54.80	31.061
			13	55.82	15.561
5.	PDE3B	1SO2	3	109.99	44.501
			4	161.04	69.148
			5	47.39	37.509
			9	25.23	6.671

CONCLUSION

The results of the present study show that RU486 can interact with some glucocorticoid - targeted lipid regulatory proteins and suggest a possible therapeutic role of RU486 in treating insulin resistance associated lipid abnormalities. A deeper understanding on the interaction of RU486 on proteins involved in lipid metabolism through other approaches (*in vivo* and *in vitro*) may lead to the identification of novel actions of RU486 on metabolic functions.

Acknowledgement
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