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MOLECULAR DOCKING EVALUATION OF GLYCYRRHIZIN AS ANTI-STRESS AND ANTI-DIABETIC AGENT

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Abstract. Glycyrrhizin and its aglycon, glycyrrhetinic acid, an active metabolite found in licorice, exhibit inhibitory properties on the metabolism of cortisol, the stress hormone, that increases glucose level in the bloodstream. Our work represents the molecular modeling of glycyrrhizin (ChemSpider ID: 14263) complexes with anti-cortisol (17) Fab fragment, both in the absence of cortisol (PDB ID: 8CBX) and the presence of cortisol (PDB ID: 8CBY). The molecular docking software AutoDock Vina was used for these studies. It was found that glycyrrhizin forms more effective interactions with the active site of the anti-cortisol (17) Fab fragment in the presence of cortisol (affinity value -7.4 kcal/mol), than in its absence (affinity value -4.3 kcal/mol), i.e. glycyrrhizin is a competitive inhibitor of the target enzyme. These results suggest that glycyrrhizin may inhibitory influence on the metabolism of cortisol, the mechanism of which is more effective interactions of this drug with both anti-cortisol (17) Fab and cortisol itself, stabilizing its position in the receptor pocket. Based on the calculated results and SAR data analysis, the pharmacophore model of glycyrrhizin for its interaction with the (17) Fab was proposed. The results obtained are of interest for further studies on glycyrrhizin as a potential anti-stress and anti-diabetic agent.

Keywords: *Glycyrrhizin, cortisol, anti-cortisol (17) Fab fragment, molecular docking, pharmacophore model.*

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1. Introduction

Extracted from the root of the Glycyrrhiza glabra plant, commonly known as licorice, glycyrrhizin is primarily known for its sweet flavor and various therapeutic properties. Understanding the mechanisms through which glycyrrhizin exerts its effects can provide valuable insights into its potential applications in modern medicine. Glycyrrhizin and its active metabolite in licorice metabolite glycyrrhetinic acid, influence various physiological processes through multiple mechanisms (Yonekura-Sakakibara & Saito, 2009; Wahab *et al*., 2021; Wang *et al*., 2015; Graebin, 2018; Kim & Kim, 2016; Khavkin *et al*., 2024; Schröfelbauer *et al*., 2009; Li *et al*., 2014; Yuan *et al*., 2019; Li *et al*., 2019; Ma *et al*., 2020; Ojima *et al*., 1990). It has been found that glycyrrhizin and glycyrrhetinic acid have inhibitory properties on the metabolism of cortisol and prednisolone both in vivo and in vitro (Kageyama *et al*., 1992; Soma *et al*., 1994; Armanini *et al*., 1996; Watson *et al*., 2024; Thompson, 2018; Imamovic *et al*., 2023). Cortisol is a steroid hormone that is produced by the adrenal gland. It is a primary stress hormone that increases glucose levels in the bloodstream (Yaribeygi *et al*., 2017). Patients with postural hypotension caused by diabetic autonomic neuropathy have also shown

improvement with licorice ingestion (Basso *et al*., 1994). High concentrations of cortisol in the body can be used as a biomarker for acute and chronic stress, as well as related mental and physiological disorders such as Cushing's disease, chronic fatigue and Addison's disease (Steckl & Ray, 2018). Recently, a high cortisol level have been suggested as a potential biomarker for the severity of SARS-CoV-2 infection (Tan *et al*., 2020). Understanding the mechanism of action of licorice has promoted its therapeutic benefit in several groups of patients. The binding of licorice to the mineralocorticoid receptor (MR) explains its utility in patients with Addison's disease. These effects are due to the affinity of glycyrrhetinic acid for MR in addition to its plasma concentration being more than 5000 times that of aldosterone (Omar *et al*., 2012). It was found glycyrrhizin inhibits the activity of 11β-HSD2, which is responsible for converting active cortisol to its inactive form, cortisone (Tanahashi *et al*., 2002). That's why by inhibiting this enzyme, glycyrrhizin increases the local concentration of cortisol, a potent anti-inflammatory agent. This aldosterone-like action is the fundamental basis for understanding its health benefits and the wide spectrum of adverse effects.

It is known that the biological properties of peptide molecules are determined by their chemical, spatial and electronic structure. Glycyrrhizin is a triterpenoid saponin glycoside, which means it is composed of a triterpene aglycone (glycyrrhetinic acid) linked to two glucuronic acid molecules. This unique structure is essential for its biological activity. Therefore, it seems relevant to apply a theoretical approach to study of the structural-functional relationships of this molecule. In connection with the above, to elucidate the mechanism of action of the studied drug - glycyrrhizin, we decided to conduct sequential molecular docking: docking of glycyrrhizin into anti-cortisol (17) Fab in the absence of cortisol at the first stage, then in the presence of cortisol. To achieve this goal, we studied topography of the anti-cortisol (17) Fab active center. Subsequently, glycirrhizin complexes with this receptor were studied and at the final stage of this study a model of the pharmacophore of the glycyrrhizin molecule was constructed. It should be noted that the results of the studies hold significant practical importance in medicine and pharmacology as they open avenues for exploring structural and functional relationships and developing analogs of glycyrrhizin with high stability and efficiency. The data obtained form the basis for the development of effective anti-stress and anti-diabetic drugs for medical purposes, which may also be important for the treatment of patients with a severe related mental and physiological diseases.

2. Calculation methods

Molecular docking is an effective method for drug design and discovery, as it the reveals biomolecular interactions between the target protein and the docked ligand. In this work, molecular docking was used to examine the interaction mechanism of the glycyrrhizin molecule and anti-cortisol (17) Fab fragment, both in the absence and presence of cortisol, using AutoDock Vina software (Trott & Olson, 2010). PyMol and Discovery Studio 3.0 programs (DeLano, 2010; Discovery Studio Visualization, 2016) were used for the generating 2D diagrams, as well as for 3D molecular visualization and analysis of the receptor-ligand interactions.

The structure of the glycyrrhizin (ChemSpider ID: 14263) was retrieved from the chemical structure database at https://www.chemspider.com/Chemical-Structure.14263.html. Ball-and-stick model of glycyrrhizin molecule with atomic numbering is shown in Figure 1. The 3D crystal structures of the anti-cortisol (17) Fab

fragment in the absence (PDB ID: 8CBX) and presence (PDB ID: 8CBY) of cortisol were retrieved from the protein data bank at http://www.rcsb.org (Eronen *et al*., 2023). Water and ligand molecules in the receptor were removed and the receptor was prepared for docking by adding only polar hydrogens. Kollman charges of the receptor were also calculated, while the partial charges of ligand were calculated using the Geistenger method. The target protein was kept rigid and the ligand was maintained flexible during the docking procedure.

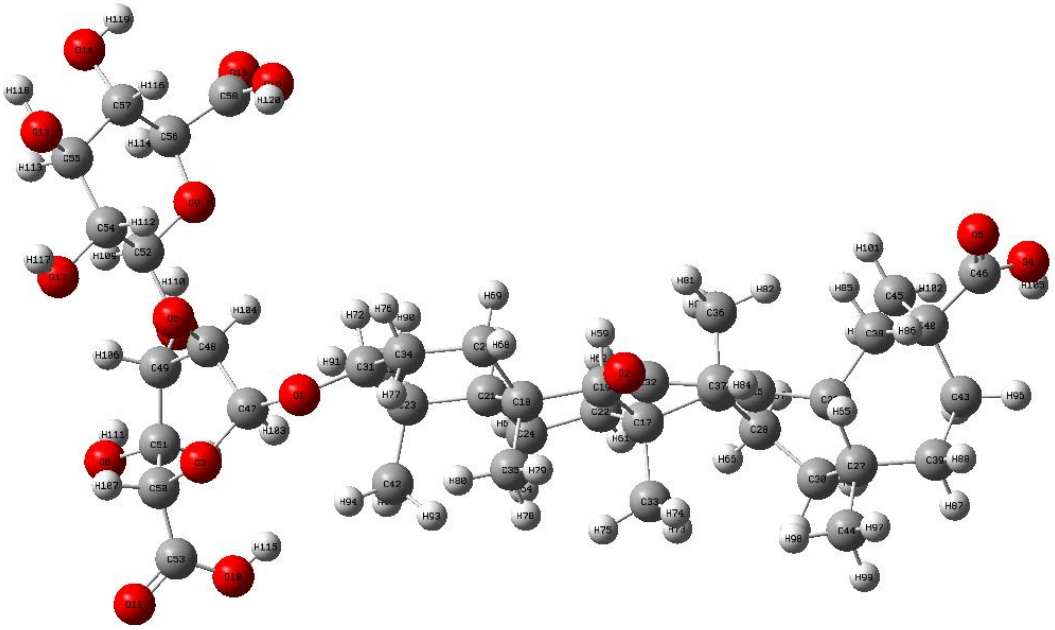


Figure 1. The molecular structure of glycyrrhizin with atomic numbering scheme

3. Results and discussion

3.1. Study topography of anti-cortisol (17) Fab active center

(17) Fab fragment is recombinant anti-cortisol antibody with high affinity for cortisol. The high-resolution crystal structures of this protein in the absence of cortisol (2.00 Å) and presence of cortisol (2.26 Å) were determined and are represented in (Eronen *et al*., 2023). According to this work the overall protein structure of (17) Fab consists of two polypeptide chains (L and H, with each chain containing one constant domain (C_L and C_H) and one variable domain (V_L and V_H) (Figure 2). Each domain is folded into a sandwich-like structure formed by two sheets of antiparallel beta strands commonly known as the immunoglobulin fold. To study the topography of the anti-cortisol (17) Fab active center, we analyzed the cortisol binding site of this protein.

The chemical structure of cortisol is represented in Figure 3. According to the work Eronen et al. (2023). data cortisol binds in such a way that the edges of the B and D steroid rings are positioned at the bottom of the cavity. In this binding, oxygen atoms of cortisol are on the outer surface of the binding cavity, making them accessible to the solvent. Due to the shape of the binding site, the core steroid structure of cortisol is stacked between the aromatic residues. We constructed a 2D diagram of the interactions of cortisol with anti-cortisol (17) Fab using Discovery Studio 3.0 program as shown in Figure 4.

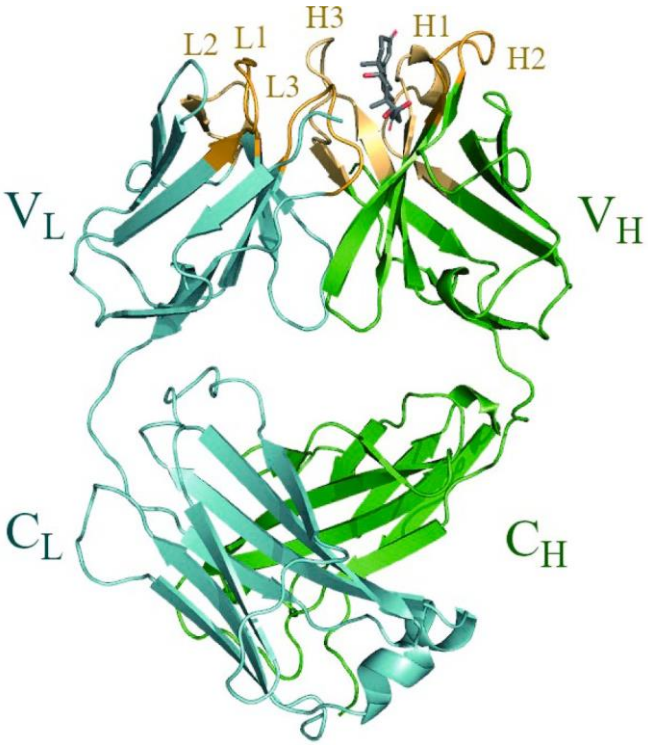


Figure 2. The overall 3D structure of the anti-cortisol (17) Fab

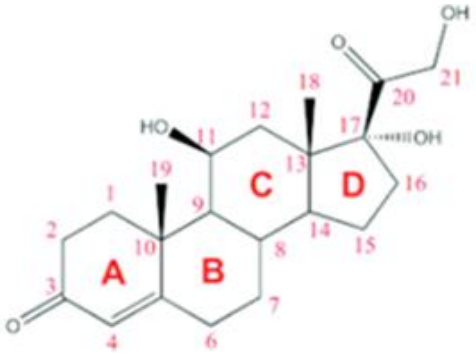


Figure 3. The chemical structure of cortisol with numbering of atoms

It has been shown that cortisol forms four strong conventional hydrogen bonds with the antibody residues Asp62, Tyr60, Arg57 of the H-chain, Arg90 of the L-chain and two carbon hydrogen bonds with the residues Gly61 of the H-chain and Ser91of the L-chain. The H-chain aromatic residues Trp47, Phe50 and Tyr104 of the active site are involved in the extensive Pi-Alkyl interactions. Tyr59 of the H-chain takes part in the hydrophobic Pi-Sigma contacts. The L-chain reseptor residue Phe93, along with one water molecule are also involved in van der Waals interactions with cortisol. The restricted conformational freedom of cortisol favors binding, which explains the high affinity of (17) Fab for cortisol.

Thus, the active site residues Trp47, Phe50, Arg57, Tyr59, Tyr60, Gly61, Asp62, Tyr104 of H-chain and Arg90, Ser91, Phe93 of L-chain of (17) Fab are important for the ligand binding. The information obtained from the topography of the anti-cortisol (17) Fab active center served as the basis for subsequent docking of glycyrrhizin to 8CBX and 8CBY.

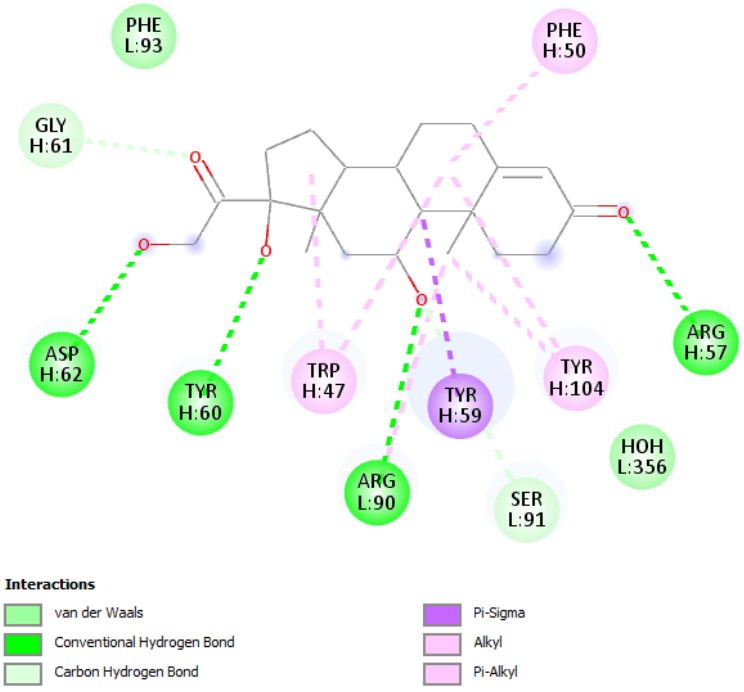


Figure 4. 2D diagram of the cortisol - anti-cortisol (17) Fab interactions

3.2. The molecular docking of glycyrrhizin into 8CBX

Molecular docking of glycyrrhizin into the anti-cortisol (17) Fab fragment in the absence of cortisol (8CBX) was carried out. The binding energies for the nine poses of the ligand are given in Table 1. 3D (a) visualization and 2D diagram (b) of the receptor-ligand interactions are shown in Figure 5. The most important contacts are shown in dotted lines.

Table 1. Comparison of the binding energies of glycyrrhizin with 8CBX

Mode	Affinity (kcal/mol)	Distances from best mode (Å)	
		Rmsd l.b.	Rmsd u.b.
1	-4.3	0.000	0.000
2	-4.1	1.405	1.806
3	-3.6	5.548	9.443
4	-3.4	5.986	9.204
5	-3.4	5.938	12.946
6	-3.2	4.968	7.353
7	-3.1	5.526	9.237
8	-3.1	5.683	11.152
9	-3.0	5.761	12.400

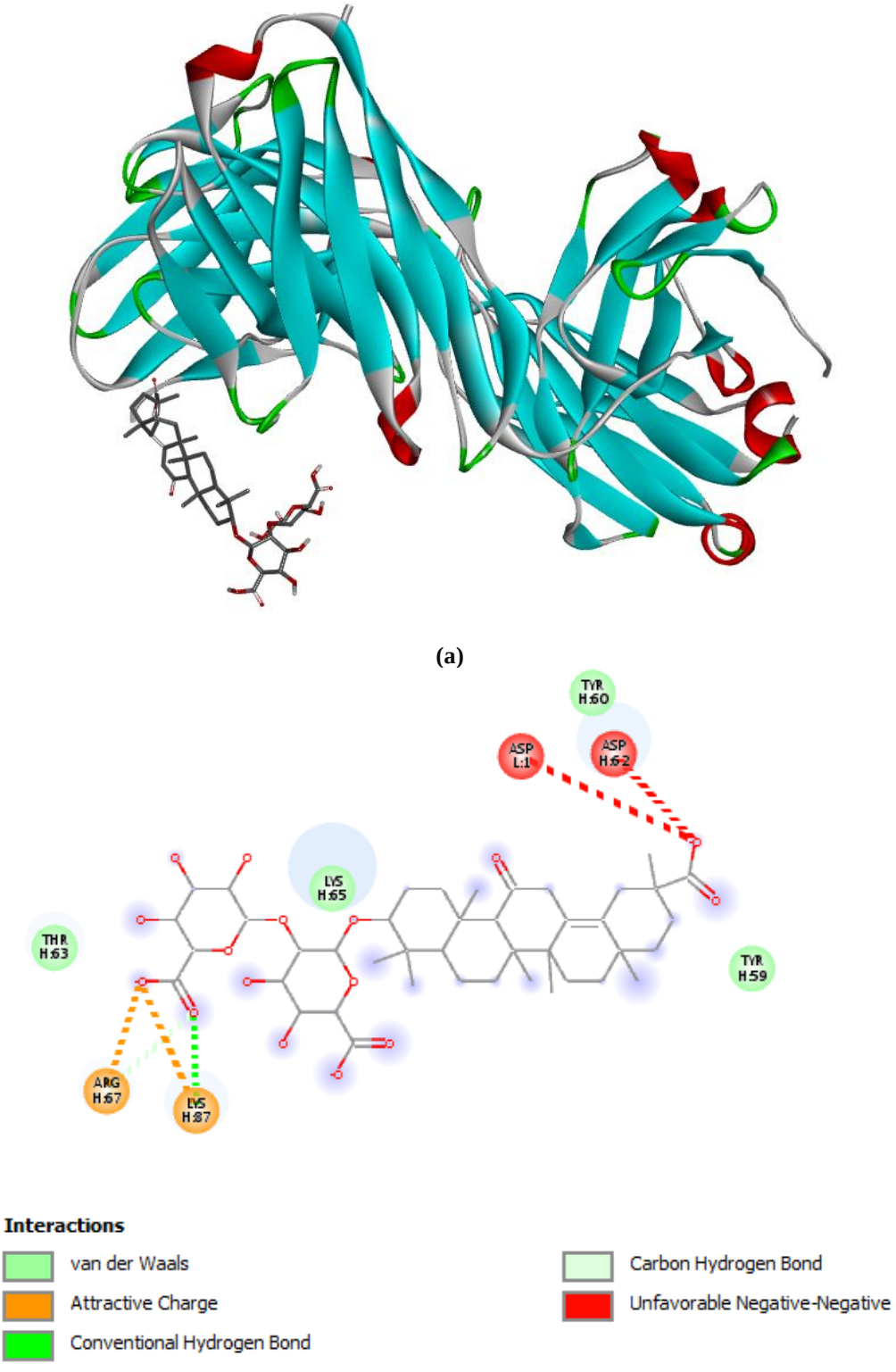


Figure 5. The molecular docking results of glycyrrhizin into 8CBX: 3D visualization (a) and 2D diagram (b) of the receptor-ligand interactions

The attributes of the intermolecular interactions of glycyrrhizin and 8CBX are given in Table 2. As seen from these results the ligand weakly binds with the active site of the

receptor: the binding affinity (ΔG) for the best binding pose between glycyrrhizin and reseptor was calculated as - 4.3 kcal/mol. With such ligand binding the terminal oxygen atoms of its aliphatic part participate in the formation of the hydrogen bonding and attractive charge interactions with the positive charged receptor residues Arg67 and Lys87 of the H-chain, but the terminal carboxyl group of the hydrophobic part is involved in the unfavorable negative-negative interactions with the Asp1 residue of L-chain and Asp62 residue of H-chain of the receptor. Glycyrrhizin also participates in the van der Waals interactions with the H-chain residues Tyr59, Tyr60, Thr63, Lys65 and Tyr104 of the receptor pocket.

Table 2. The attributes of the intermolecular interactions of glycyrrhizin with 8CBX

No	Contacts	Types interactions	Distances (Å)	∠DHA (degree)	∠HAY (degree)
1	L:Asp1:OD2 – glycyrrhizin:O4	Unfavorable N-N	4.24		
2	H:Asp62:OD1 – glycyrrhizin:O4	Unfavorable N-N	2.88		
3	H:Arg67:HD1 – glycyrrhizin:O16	Carbon HB	2.90	115.64	128.02
4	H:Arg67:NH2 – glycyrrhizin:O15	Attractive Charge	3.22		
5	H:Lys87:HZ2 – glycyrrhizin:O16	Conventional HB	2.18	150.54	110.31
6	H:Lys87:NZ – glycyrrhizin:O15	Attractive Charge	4.31		
7	H:Tyr59 – glycyrrhizin	Van der Waals			
8	H:Tyr60 – glycyrrhizin	Van der Waals			
9	H:Thr63 – glycyrrhizin	Van der Waals			
10	H:Lys65 – glycyrrhizin	Van der Waals			

Note: The numbering of the ligand atoms is given in accordance with Figure 1;
HB - Hydrogen Bond, N-negative atom, in the D-H-A/H-A-Y angles: D is the donor atom covalently bound to the hydrogen; H is a hydrogen atom; A is an acceptor atom; Y is a heavy covalently bound neighbor of the acceptor atom

As observed, despite the absence of cortisol, the glycyrrhizin molecule is weakly bound to the anti-cortisol (17) Fab active center.

3.3. The molecular docking of glycyrrhizin into 8CBY

The next stage of the study involved molecular docking of glycyrrhizin into anti-cortisol (17) Fab fragment in the presece of cortisol (8CBY). As a result the binding energies for the nine poses of the ligand are given in Table 3. Tthe binding affinity (ΔG) for the best binding pose between glycyrrhizin and reseptor was calculated as - 7.4 kcal/mol.

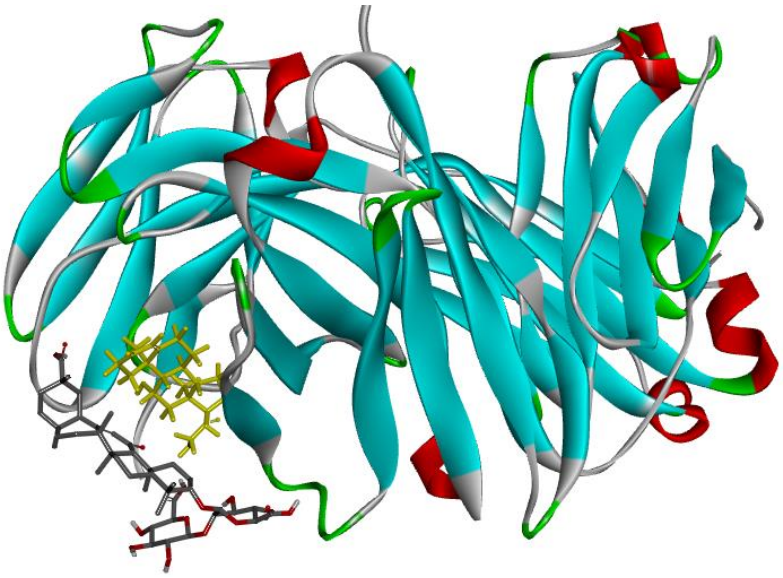
Table 3. Comparison of the binding energies of glycyrrhizin with 8CBY

Mode	Affinity (kcal/mol)	Distances from best mode (Å)	
		Rmsd l.b.	Rmsd u.b.
1	-7.4	0.000	0.000
2	-7.1	3.000	12.224
3	-7.1	3.025	12.265
4	-7.0	1.131	1.307
5	-6.9	5.165	12.970
6	-6.7	2.808	11.326
7	-6.7	3.991	12.897
8	-6.6	2.868	12.184
9	-6.6	2.864	4.497

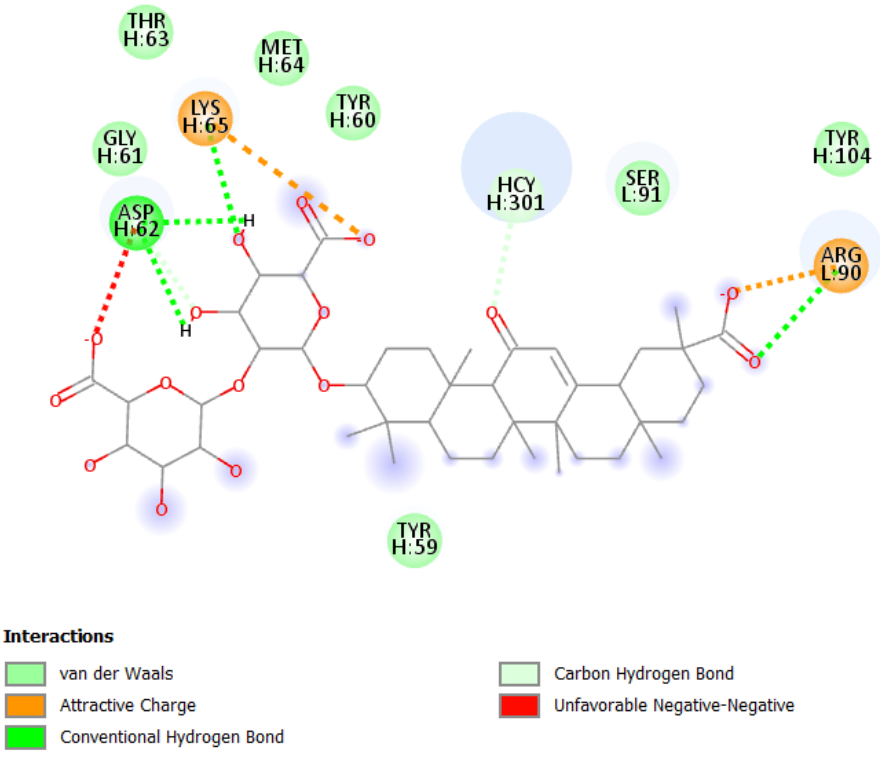
The molecular docking results are represented in Figure 6, where the 3D (a) visualization and the 2D diagram (b) of the receptor-ligand interactions are shown. The attributes of the intermolecular interactions of glycyrrhizin and the receptor are given in Table 4. It was revealed that the amino acid residues Asp62, Lys65 of the H-chain and Arg90 of the L-chain, serve as anchoring amino acid residues of the 8CBY receptor for the ligand-bonding. The H-bonding is significant for the stabilization of the glycyrrhizin complex with anti-cortisol (17) Fab in the presence of cortisol. Thus, glycyrrhizin forms four hydrogen bonds with the antibody residues and one with cortisol. It was found that the hydrophilic part of the ligand is at the center of a network of polar and nonpolar contacts with receptor residues. The distances between ligand moieties and the interacting receptor residues in the minimized complex were calculated. The Asp62 amino acid residue of the H-chain of the receptor is involved in intermolecular interactions with hydrogen atoms of the intermediate glucuronic acid ring through two conventional hydrogen bonds with two ligand hydrogen atoms at distances of 2.68 and 2.19 Å and one carbon hydrogen bond with the oxygen atom of this ligand moiety at a distance of 1.81 Å. However, this residue is also involved also in an unfavorable negative-negative electrostatic interaction with oxygen atom of the carboxyl group of the terminal glucuronic acid at distance of 2.89 Å. Lys65 of the receptor forms one conventional hydrogen bond at a distance of 2.29 Å with the oxygen atom of the intermediate glucuronic acid ring and attractive charge interactions at a distance of 4.99 Å with the oxygen atom of the intermediate glucuronic acid carboxyl group. It was revealed that the receptor L-chain residue Arg90 forms both two conventional hydrogen bonds and one attractive charge interaction with the oxygen atoms of the terminal carboxyl group of the hydrophobic fragment of glycyrrhizin. At distances of 2.01, 2.68 and 3.97 Å, respectively. As seen from the results, H-bonding is important for the stabilizing the interactions between the glycyrrhizin and anti-cortisol Fab complex in the presence of cortisol. However, the pose of the ligand in the receptor pocket is also formed by van der Waals interactions with the H-chain receptor residues Tyr59, Tyr60, Gly61, Thr63, Met64, Tyr104 and with the L-chain receptor residue Ser91. The residues mentioned likely affect the overall conformation of the receptor cavity and the stability of the entire complex. It should be noted that glycyrrhizin takes part also in the carbon hydrogen bonding with cortisol.

As seen in the represented results, glycyrrhizin forms more effective interactions with the active site of the anti-cortisol (17) Fab, in the presence of cortisol (affinity value

-7.4 kcal/mol), than in its absence (affinity value -4.3 kcal/mol). This indicates glycyrrhizin behaves as a competitive agonist for the anti-cortisol (17) Fab inhibition in the presence of cortisol.



(a)



(b)

Figure 6. The molecular docking results of glycyrrhizin into 8CBY: 3D visualization (a) and 2D diagram (b) of the receptor-ligand interactions

Table 4. The attributes of the intermolecular interactions of glycyrrhizin with 8CBY

No	Contacts	Types interactions	Distances (Å)	∠DHA (degree)	∠HAY (degree)
1	glycyrrhizin:H110 – H:Asp62:OD1	Conventional HB	2.68	94.52	122.02
2	glycyrrhizin:H111–H:Asp62:O	Conventional HB	2.19	135.18	106.37
3	H:Asp62:HA - : glycyrrhizin:O7	Carbon HB	1.81	168.58	123.75
4	H:Asp62:OD1 - : glycyrrhizin:O15	Unfavorable N-N	2.89		
5	H:Lys65:HN - : glycyrrhizin:O8	Conventional HB	2.29	157.44	113.94
6	H:Lys65:NZ - : glycyrrhizin:O10	Attractive Charge	4.99		
7	L:Arg90:HE - : glycyrrhizin:O5	Conventional HB	2.01	153.71	111.83
8	L:Arg90:HH22 - : glycyrrhizin:O5	Conventional HB	2.68	118.33	115.80
9	L:Arg90:NH2 - : glycyrrhizin:O4	Attractive Charge	3.97		
10	H:Tyr59 - : glycyrrhizin	Van der Waals			
11	H:Tyr60 - : glycyrrhizin	Van der Waals			
12	H:Gly61 - : glycyrrhizin	Van der Waals			
13	H:Thr63 - : glycyrrhizin	Van der Waals			
14	H:Met64 - : glycyrrhizin	Van der Waals			
15	H:Tyr104 - : glycyrrhizin	Van der Waals			
16	L:Ser91 - : glycyrrhizin	Van der Waals			
17	HCY H:301 - : glycyrrhizin:O2	Carbon HB	3.36	142.05	119.11

Note: The numbering of the glycirrhizin atoms is given in accordance with Figure 1; HCY H:301 is cortisol, located in the H-chain of anti-cortisol (17) Fab; HB - Hydrogen Bond, N-negative atom; in the D-H-A/H-A-Y angles: D is the donor atom covalently bound to the hydrogen; H is a hydrogen atom; A is an acceptor atom; Y is a heavy covalently bound neighbor of the acceptor atom

The important intermolecular interactions of cortisol itself with anti-cortisol (17) Fab in the gycirrhizin - 8CBY complex according to our caculated results also were analysed. In this case, the 2D diagram of the cortisol - (17) Fab interactions is represented in Figure 7. The most important contacts are indicated by dotted lines. The attributes of the cortisol - (17) Fab interactions in this complex are given in Table 5.

As seen in the represented results, cortisol binds to the active site binding pocket of the 8CBY receptor primarily through polar and Pi-contacts. It was revealed that cortisol forms two conventional hydrogen bonds with the target protein residues Arg57, Tyr60 of H-chain and three carbon hydrogen bonds with Arg57, Gly61 of the H-chain and Phe93 of the L-chain. The H-chain aromatic residues Trp47, Phe50 and Tyr104 of the active site are involved in the extensive Pi-Alkyl interactions. Especially, the indole ring of Trp47 is at the center of a network of intermolecular contacts. Tyr59 of H-chain and Arg90 of the L-chain form the hydrophobic Pi-Sigma and Alkyl contacts, respectively. However, Asp62 of the H-chain participates in the Unfavorable Acceptor-Acceptor interactions with the terminal group of cortisol. The L-chain reseptor residues Ser91 and Ile92 are involved in van der Waals interactions with cortisol.

The compilation of the docking results of the glycyrrhizin molecule into 8CBY, along with data on the interaction of cortisol with the anti-cortisol (17) Fab, revealed that the H-chain residues Tyr59, Tyr60, Gly61, Asp62, Tyr104 and the L-chain residues Arg90, Ser91 of the (17) Fab are important for the interaction with both cortisol and

glycyrrhizin. It is worth noting that the H-chain residue Asp62 and L-chain residue Arg90 break conventional hydrogen bonds with cortisol and enter more favorable interactions with the glycyrrhizin molecule. These results suggest that glycyrrhizin may inhibitory influence on the metabolism of cortisol, the mechanism of which is more effective interactions of this drug with both anti-cortisol (17) Fab and cortisol itself, stabilizing its position in the receptor pocket. It can be stated that glycyrrhizin acts as a competitive inhibitor of enzyme (17) Fab.

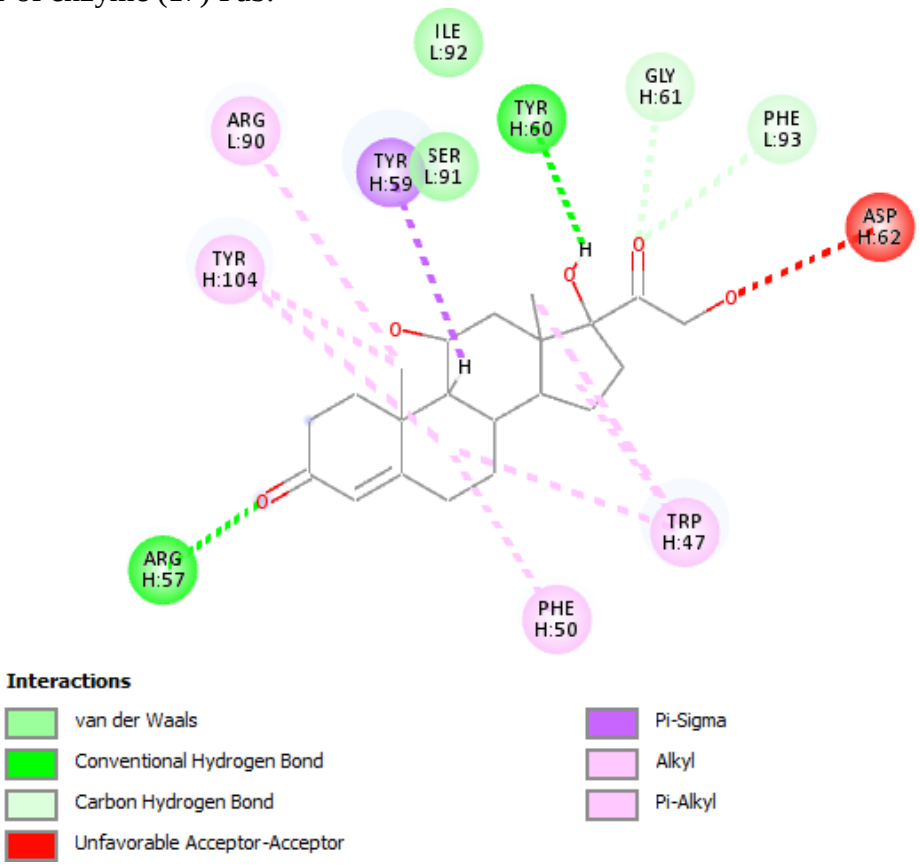


Figure 7. 2D diagram of the cortisol - anti-cortisol (17) Fab interactions in the glycyrrhizin - 8CBY complex

Table 5. The attributes of the intermolecular interactions of cortisol with anti-cortisol (17) Fab in the glycyrrhizin - 8CBY complex

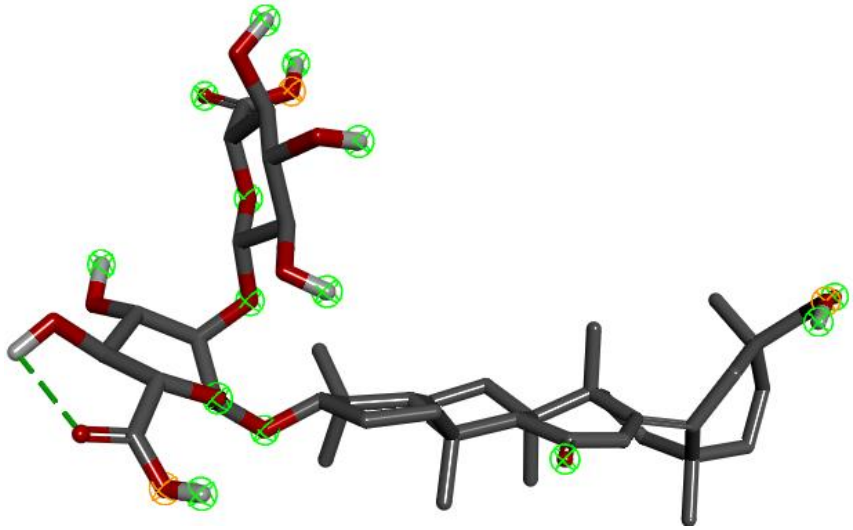
No	Contacts	Types interactions	Distances (Å)	∠DHA (degree)	∠HAY (degree)
1	H:cortisol:H3 - H:Tyr60:O5	Conventional HB	1.92	163.78	135.00
2	H:Arg57:HH11 - H: cortisol:O1	Conventional HB	2.03	155.70	111.05
3	H:Arg57:HD1 - H: cortisol:O1	Carbon HB	2.39	147.83	123.62
4	L:Phe93:HA - H: cortisol:O4	Carbon HB	3.03	117.36	144.04
5	H:Gly61:HA1 - H: cortisol:O4	Carbon HB	2.63	178.04	110.61
6	H:Asp62:OD1 - H: cortisol:O5	Unfavorable A-A	2.73		
7	H: cortisol:H9 - H:Tyr59	Hydrophobic Pi-Sigma	2.88		
8	H: cortisol:C19 - L:Arg90	Alkyl	4.32		

9	H:Trp47 - H: cortisol	Pi-Alkyl	5.07		
10	H:Trp47 - H: cortisol	Pi-Alkyl	4.25		
11	H:Trp47 - H: cortisol	Pi-Alkyl	4.73		
12	H:Trp47 - H: cortisol:C18	Pi-Alkyl	4.43		
13	H:Phe50 - H: cortisol	Pi-Alkyl	4.73		
14	H:Tyr104 - H: cortisol	Pi-Alkyl	5.00		
15	H:Tyr104 - H: cortisol:C19	Pi-Alkyl	4.27		
16	L:Ser91 - H: cortisol	Van der Waals			
17	L:Ile92 - H: cortisol	Van der Waals			

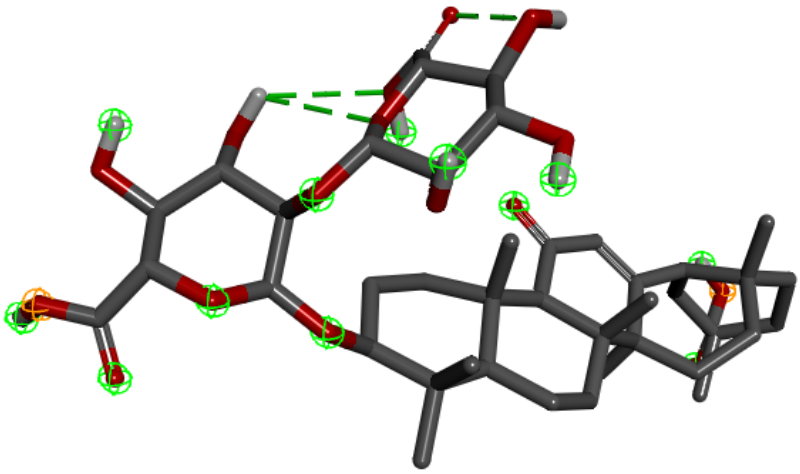
Note: HB - Hydrogen Bond, A - acceptor atom; in the D-H-A/H-A-Y angles: D is the donor atom covalently bound to the hydrogen; H is a hydrogen atom; A is an acceptor atom; Y is a heavy covalently bound neighbor of the acceptor atom, N-negative

We conducted a comparative analysis of the ligand structures in the minimized complexes of glycyrrhizin with anti-cortisol (17) Fab in the absence and presence of cortisol. It was found in the absence of cortisol, glycyrrhizin adopts a structure (Figure 8a), where thee terminal glucuronic acid is positioned perpendicularly to the rest of the molecule. Notably, in this case, only one intramolecular strong hydrogen bond (H111···O11) is formed between the atoms of the second glucuronic acid of the aliphatic part of the glycyrrhizin molecule at a distance of 2.74 Å. The parameters of this bond are as follows: DHA= 90.28º and HAY= 98.05º.

In contrast, in the presence of cortisol, three intramolecular strong hydrogen bonds are formed in glycyrrhizin: one between the atoms of the terminal glucuronic acid of the aliphatic part (H119···O16) at a distance of 2.82 Å, with the parameters DHA= 93.39º and HAY= 93.86º and two between the atoms of both glucuronic acids of the aliphatic part – (H110···O15) at a distance of 3.05 Å, with the parameters DHA= 113.67º and HAY= 102.99º and (H110···O9) at a distance of 2.80 Å, with the parameters DHA= 112.90º and HAY= 109.75º. The binding pocket of anti-cortisol (17) Fab is a groove so the ligand adopts a more compact structure in the presence of cortisol, than in its absence (Figure 8b). In this conformation, the terminal glucuronic acid of the ligand molecule, bend approaches its hydrophobic part in space.



(a)



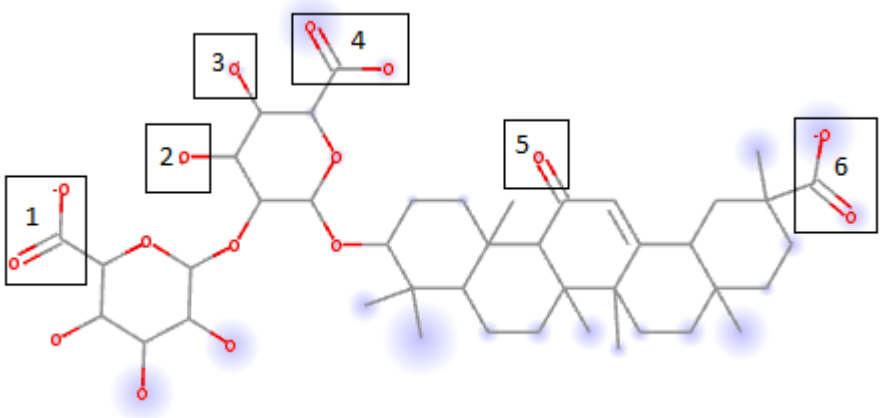
(b)

Figure 8. Spatial structure of glycyrrhizin in the minimized complexes with 8CBX (a) and 8CBY (b)

As a result of the molecular docking simulation, it was found that the conformation of glycyrrhizin is automatically selected based on the shape of the receptor pocket in which it is placed. The receptor-ligand complex exhibits perfect complementary between the pharmacophore moieties of glycyrrhizin and receptor binding cavity pockets of the anti-cortisol (17) Fab in the presence of cortisol.

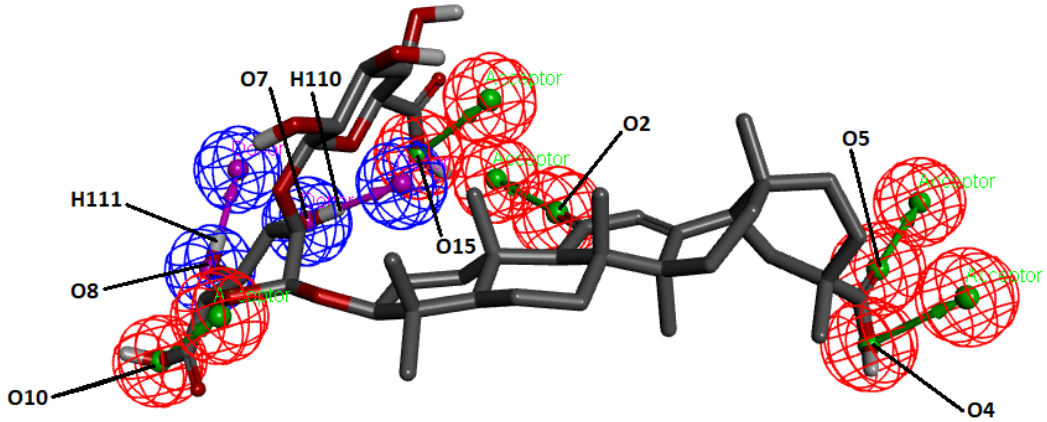
3.4. Pharmacophore model of glycyrrhizin for the interaction with 8CBY

Based on the calculated results and SAR (Structure-Activity Relationship) data, we proposed a pharmacophore model of glycyrrhizin for its interaction with 8CBY (Figure 9). It was established that six pharmacophore moieties - three carboxyl groups (-COOH), two hydroxyl radicals (-OH) and a ketone group (=O) of glycyrrhizin are predicted to play key rolse in the interactions with amino acid residues of the active site of the 8CBY target protein (Figure 9a).



Pharmacophore moieties:
1, 4, 6 – carboxyl groups (COOH)
2, 3 - hydroxyl radicals (OH)
5 – ketone group (=O)

(a)



(b)

Figure 9. Pharmacophore model of glycyrrhizin for its interaction with 8CBY / the pharmacophore moieties 1 - 6 (a) are shown: H-acceptors are in red, H-donors are in blue (b); the pharmacophore elements are indicated by the atomic labels and symbols

In this model, the O15 atom of the carboxyl group from the terminal glucuronic acid, O7 and H110 atoms, O8 and H111 atoms from two hydroxyl radicals of the intermediate glucuronic acid, O10 atom from the carboxyl group of the intermediate glucuronic acid, O2 atom from the ketone group of the hydrophobic fragment, O4 and O5 atoms from the terminal carboxyl group of the hydrophobic fragment are the glycyrrhizin pharmacophore elements involved in the intermolecular interactions with the 8CBY (Figure 9b). The relative positions of these pharmacophore elements of glycyrrhizin are characterized by the sets of distances and angles, as depicted in Table 6. The proposed pharmacophore model may be used for designing new glycyrrhizin derivatives as potential anti-stress and anti-diabetic agents.

Table 6. The respective geometrical arrangement of the glycyrrhizin pharmacophore elements involved in the intermolecular interactions with 8CBY

Distances	In Å	Angles	In degrees
O2 – O5	8.42	O10 O2 O15	46.14
O2 - O7	6.93	O10 O2 O5	136.61
O2 – O10	11.12	O8 O15 O4	137.24
O2 – O15	4.22	O10 O8 O7	130.09
O4 – O10	18.48	O10 O7 O15	155.37
O4 – O15	12.90	O10 O8 O15	130.57
O5 – O8	17.78	O7 O2 O4	162.87
O5 – O10	18.87	O15 O2 O5	167.34
O5 – O15	12.44	O8 O2 O5	147.98
O8 – O15	6.51	H110 O2 O5	161.86
O10 – O15	8.74	H111 O2 O4	163.08
H110 – O2	6.84	O10 H110 O2	117.22
H111 – O15	6.51	O10 H111 O2	103.40
H110 – O10	3.88	H110 O15O2	125.92
H111 – O4	17.82	H111 O15 O2	127.30

4. Conclusion

This work represents the molecular docking studies of glycyrrhizin with 8CBX and 8CBYanti-cortisol (17) Fab. It was found that the ligand had better binding affinity to the anti-cortisol (17) Fab in the presence of cortisol than in its absence, i.e. glycyrrhizin is a competitive inhibitor for this antibody. It was found that the receptor-ligand complex exhibits a perfect complementary between the pharmacophore moieties of glycyrrhizin and receptor binding cavity pocket in the presence of cortisol: glycyrrhizin is decently bound to (17) Fab via hydrogen bonds and charge interactions with Asp62, Lys65 residues of H-chain and Arg90 residue of L-chain of receptor, leading to the increased anti-stress and anti-diabetic activities of this compound. The relative positions of pharmacophore moieties of glycyrrhizin for its interaction with 8CBY were characterized by a set of distances and angles. The proposed pharmacophore model is the basis for the development of effective glycyrrhizin derivatives, which can be more specific concerning their putative target - anti-cortisol (17) Fab.

References

Armanini, D., Lewicka, S., Pratesi, C., Scali, M., Zennaro, M.C., Zovato, S., ... & Zampollo, V. (1996). Further studies on the mechanism of the mineralocorticoid action of licorice in humans. *Journal of Endocrinological Investigation*, 19, 624-629. https://doi.org/10.1007/BF03349029

Basso, A., Paola, L.D., Erle, G., Boscaro, M. & Armanini, D. (1994). Licorice ameliorates postural hypotension caused by diabetic autonomic neuropathy. *Diabetes Care*, *17*(11), 1356-1356. https://doi.org/10.2337/diacare.17.11.1356

ChemSpider: ID 14263. https://www.chemspider.com/Chemical-Structure.14263.html

DeLano, W.L. (2010). *The PyMOL Molecular Graphics System*. San Carlos. https://www.ccp4.ac.uk/newsletters/newsletter40/11_pymol.pdf

Discovery Studio Visualization (2016). Discovery studio 3.1. http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php

Eronen, V., Tullila, A., Iljin, K., Rouvinen, J., Nevanen, T.K. & Hakulinen, N. (2023). Structural insight to elucidate the binding specificity of the anti-cortisol fab fragment with glucocorticoids. *Journal of Structural Biology*, *215*(2), 107966. https://doi.org/10.1016/j.jsb.2023.107966

Graebin, C.S. (2018). The pharmacological activities of glycyrrhizinic acid (glycyrrhizin) and glycyrrhetinic acid. In *Sweeteners*, 245-261. https://doi.org/10.1007/978-3-319-27027-2_15

Imamovic, M., Bäcklund, N., Lundstedt, S., Brattsand, G., Aardal, E., Olsson, T. & Dahlqvist, P. (2023). Confounding effects of liquorice, hydrocortisone and blood contamination on salivary cortisol but not cortisone. *Endocrine Connections*, *12*(1). https://doi.org/10.1530/EC-22-0324

Kageyama, Y., Suzuki, H. & Saruta, T. (1992). Glycyrrhizin induces mineralocorticoid activity through alterations in cortisol metabolism in the human kidney. *Journal of Endocrinology*, *135*(1), 147-152. https://doi.org/10.1677/joe.0.1350147

Khavkin, A.I., Nikolaychuk, K.M., Shreiner, E.V., Novikova, M.F., Veremenko, A.S., Levchenko, I.D., … & Pokushalov, E.A. (2024). Anti-inflammatory and antimicrobial properties of some licorice metabolites. *Experimental and Clinical Gastroenterology*, *222*(2), 75-88. https://doi.org/10.31146/1682-8658-ecg-222-2-75-88 (In Russian).

Kim, J.H., Kim, H. (2016). Combination treatment with herbal medicines and Western medicines in atopic dermatitis: Benefits and considerations. *Chinese Journal of Integrative Medicine*, *22*(5), 323-327. https://doi.org/10.1007/s11655-016-2099-0

Li, J.Y., Cao, H.Y., Liu, P., Cheng, G.H. & Sun, M.Y. (2014). Glycyrrhizic acid in the treatment of liver diseases: literature review. *BioMed Research International*, 1, 872139. https://doi.org/10.1155/2014/872139

Li, X., Sun, R. & Liu, R. (2019). Natural products in licorice for the therapy of liver diseases: Progress and future opportunities. *Pharmacological Research*, 144, 210-226. https://doi.org/10.1016/j.phrs.2019.04.025.

Ma, X., Jiang, Y., Wen, J., Zhao, Y., Zeng, J. & Guo, Y. (2020). A comprehensive review of natural products to fight liver fibrosis: Alkaloids, terpenoids, glycosides, coumarins and other compounds. *European Journal of Pharmacology*, 888, 173578. https://doi.org/10.1016/j.ejphar.173578

Ojima, M., Satoh, K., Gomibuchi, T., Itoh, N., Kin, S., Fukuchi, S. & Miyachi, Y. (1990). The inhibitory effects of glycyrrhizin and glycyrrhetinic acid on the metabolism of cortisol and prednisolone-in vivo and in vitro studies. *Nihon Naibunpi Gakkai Zasshi*, *66*(5), 584-596. https://doi.org/10.1507/endocrine1927.66.5_584

Omar, H.R., Komarova, I., El-Ghonemi, M., Fathy, A., Rashad, R., Abdelmalak, H.D., ... & Camporesi, E.M. (2012). Licorice abuse: Time to send a warning message. *Therapeutic Advances in Endocrinology and Metabolism*, *3*(4), 125-138. https://doi.org/10.1177/2042018812454322

Schröfelbauer B., Raffetseder J., Hauner M., Wolkerstorfer A., Ernst W. & Szolar O.H. (2009). Glycyrrhizin, the main active compound in liquorice, attenuates pro-inflammatory responses by interfering with membrane-dependent receptor signalling. *Biochemical Journal*, *421*(3), 473-482. https://doi.org/10.1042/BJ20082416

Soma, R., Ikeda, M., Morise, T., Miyamori, I. & Takeda, R. (1994). Effect of glycyrrhizin on cortisol metabolism in humans. *Endocrine Regulations*, *28*(1), 31-34.

Steckl, A.J., Ray, P. (2018). Stress biomarkers in biological fluids and their point-of-use detection. *ACS Sensors*, *3*(10), 2025-2044. https://doi.org/10.1021/acssensors.8b00726

Tan, B.Y., Chew, N.W., Lee, G.K., Jing, M., Goh, Y., Yeo, L.L., ... & Sharma, V.K. (2020). Psychological impact of the COVID-19 pandemic on health care workers in Singapore. *Annals of Internal Medicine*, *173*(4), 317-320. https://doi.org/10.7326/M20-1083

Tanahashi, T., Mune, T., Morita, H., Tanahashi, H., Isomura, Y., Suwa, T., ... & Yasuda, K. (2002). Glycyrrhizic acid suppresses type 2 11β-hydroxysteroid dehydrogenase expression in vivo. *The Journal of Steroid Biochemistry and Molecular Biology*, *80*(4-5), 441-447. https://doi.org/10.1016/S0960-0760(02)00033-X

Thompson, S. (2018). Elevation of cortisol levels by ingesting liquorice. *Clinical Research in Neurology*, *1*(1), 1-4.

Trott, O., Olson, A.J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *Journal of Computational Chemistry*, *31*(2), 455-461. https://doi.org/10.1002/jcc.21334

Wahab, S., Annadurai, S., Abullais, S.S., Das, G., Ahmad, W., Ahmad, M.F., ... & Amir, M. (2021). Glycyrrhiza glabra (Licorice): A comprehensive review on its phytochemistry, biological activities, clinical evidence and toxicology. *Plants*, *10*(12), 2751. https://doi.org/10.3390/plants10122751

Wang, L., Yang, R., Yuan, B., Liu, Y. & Liu, C. (2015). The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb. *Acta Pharmaceutica Sinica B*, *5*(4), 310-315. https://doi.org/10.1016/j.apsb.2015.05.005

Watson, A., Fuess, E., Laxalde, J. & Mitchell, D. (2024). Glycyrrhizin intake higher than the current international guidelines has no detectable hypermineralocorticoid-like effect in dogs. *Journal of Animal Physiology and Animal Nutrition*, *108*(6), 1906-1912. https://doi.org/10.1111/jpn.14030

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T.P. & Sahebkar, A. (2017). The impact of stress on body function: A review. *EXCLI Journal*, 16, 1057. https://doi.org/10.17179/excli2017-480

Yonekura-Sakakibara, K., Saito, K. (2009). Functional genomics for plant natural product biosynthesis. *Natural Product Reports*, *26*(11), 1466-1487. https://doi.org/10.1039/B817077K

Yuan, T., Wang, J., Chen, L., Shan, J. & Di, L. (2019). Glycyrrhizic acid improving the liver protective effect by restoring the composition of Lactobacillus. *Journal of Functional Foods*, 52, 219-227. https://doi.org/10.1016/j.jff.2018.11.001