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Original Research Article

In-Silico Validation of Glycyrrhizin against Proinflammatory Mediator COX-2: Anti-Proliferative Potential

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Abstract: ***Background*:** Licorice's scientific name is Glycyrrhiza glabra. It is a well-known medicinal plant that grows in numerous locations throughout the world. It is one of the most ancient and widely utilised plants and has been used for a very long period in both western and eastern nations. The primary source of the triterpenoid saponin, glycyrrhizic acid (also known as glycyrrhizin), which is a sweeter component and around 50 times sweeter than sugar, was the Glycyrrhiza glabra (Fabaceae) root. Glycyrrhetinic acid has been found as the chemical constituent of glycyrrhizin. ***Aim*:** The current work sought to elucidate the molecular basis for glycyrrhizin's antiproliferative activity against the COX-2 enzyme, which functions as a proinflammatory factor in proliferation. ***Method*:** A molecular docking method was employed in the current work to look for COX 2 protein inhibitors. The binding was determined by the Auto Dock software utilising a grid-based docking method. Compounds' 2D structures were constructed using the Merck Molecular Force Field, converted to 3D, and then energetically reduced up to an arms gradient of 0.01. (MMFF). ***Results*:** The molecular docking result revealed that glycyrrhizin showed encouraging docking score. The docking score found to be -7.7 kcal mol^{-1}. ***Conclusion*:** The interaction of ligand hits to targeted site and docking score finding it can be predicted that glycyrrhizin found in the plants *G.glabra* exhibited good inhibitor of *COX 2* protein inhibitors.

Keywords: Glycyrrhizin, COX 2, in-silico molecular docking & Anti proliferative activity.

INTRODUCTION

Drug designing is a technique for finding and creating novel compounds that have a particular effect on people [1]. A new class of antimicrobial medications must be developed because the number of microbial illnesses that are multidrug resistant is increasing daily [2]. A serious health problem and the second most common cause of death worldwide is cancer. Deregulation of the cell cycle, which impairs cellular differentiation and promotes unchecked cellular proliferation, is the root cause [3, 4]. Therefore, it is essential to create new bioactive compounds whose chemical makeup and mechanisms of action are notably different from those of currently accessible drugs [5]. Discovery of drug is a sluggish, lengthy costly and interdisciplinary approach but the recent discoveries have revolutionised the methods by which researchers manufacture novel therapeutic compounds e.g. The CADD technology reduces the cost of drug design by up to 50%. The I drug-receptor interaction (ii) binding affinity (iii) orientation and approach of drug molecules to the target site are all understood using the molecular docking technique [6]. The major goals of docking studies are accurate structural modelling and accurate activity prediction. It creates a new logical approach to drug design and offers the most optimistic image of drug-receptor interaction.

The sweet flavour of licorice root is primarily due to the triterpenoid glycyrrhizin. This chemical is a part of a combination of potassium, calcium, and magnesium salts of glycyrrhizic acid [7].

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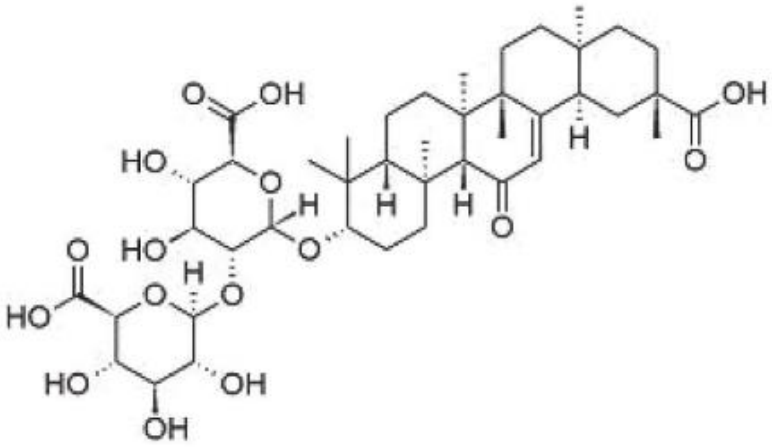


Structure of Glycyrrhizin

Description of Glycyrrhizin [8, 9]

S. No	Glycyrrhizin Description	
1.	**Mol. Formula**	**$C_{42}H_{62}O_{16}$**
2.	**Average Molecular Weight**	822.9 g/mol
3.	**IUPAC Name**	S,3S,4S,5R,6R)-6-[(2R,3R,4S,5S,6S)-2-[[(3S,4aR,6aR,6bS,8aS,11S,12aR,14aR,14bS)-11-carboxy-4,4,6a,6b,8a,11,14b-heptamethyl-14-oxo-2,3,4a,5,6,7,8,9,10,12,12a,14a-dodecahydro-1H-picen-3-yl]oxy]-6-carboxy-4,5-dihydroxyoxan-3-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid.
4.	**Class**	Triterpene glycoside
5.	**M.P.**	220 °C
6.	**Partition coefficient**	2.80
7.	**Pharmacology**	It possesses expectorant (antitussive) characteristics in addition to being useful in the treatment of peptic ulcers.
8.	**Mechanism of Action**	Inhibition of Hepatic Apoptosis and Necrosis Anti-Inflammation and Immunity Regulation Antiviral Effects Antitumor Effects Inductive Effect of Liver Enzyme Activity

Experimental work

Ligand Preparation:

2D Structure of ligand (glycyrrhizin) was drawn using ChemSketch [10], the two-dimensional structure of was converted into 3-D structure and optimized with 3D geometry. The optimized structure was saved in PDB format for AutoDock compatibility. The basic structure of ligand (glycyrrhizin) is given below:

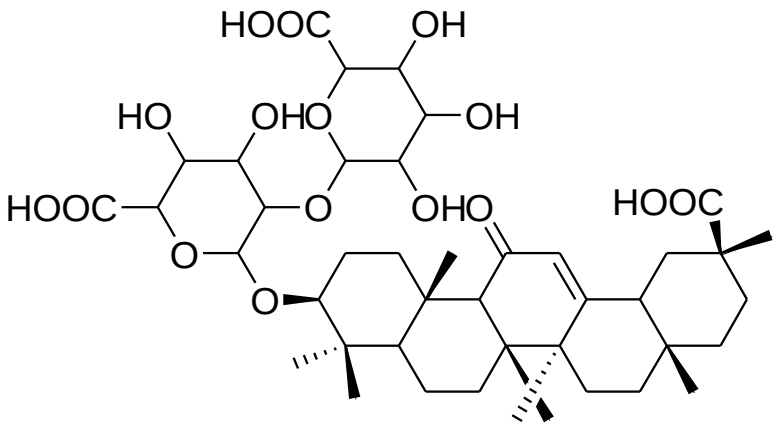


Figure 1: 2D conformer of glycyrrhizin

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.375 Å and No. of points considered are 46, 44 and 46 points in the x, y, and z dimensions and 38.042, 2.131and 61.28 as x, y, z centers [11, 12].

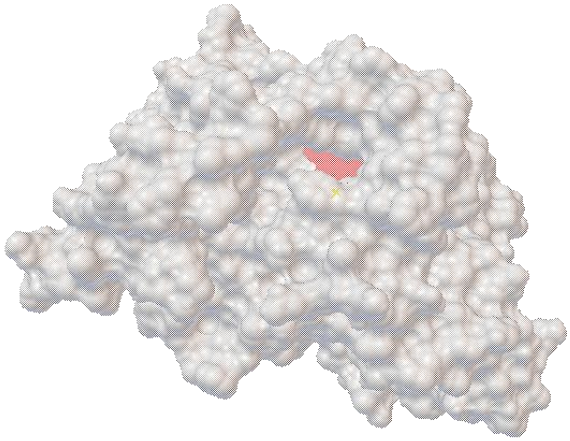


Figure 2: Grid box covering all active sites in receptor

Preparation of the docking file

All the calculations were carried out by using Autodock4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [13-15].

Docking of COX2 with Glycyrrhizin

Crystal structure

The crystal structure of the protein consisting of receptor was downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (5ikr.pdb) registered in the Protein data bank was used [16].

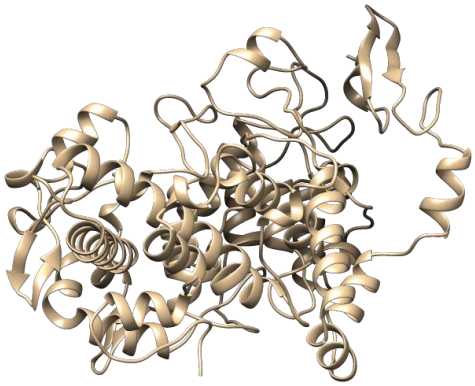


Figure 3: Crystal structure of COX2 protein (PDB ID-5ikr)

Processing of Protein

The downloaded receptor protein is having two chains A, and B, out of which two chain A were selected for the experimental purpose. There was mefenamic acid ligand was present within the macromolecular complex [17].

Molecular Docking Simulation Studies

Docking of glycyrrhizin ligand on COX 2 protein was performed by Autodock. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible [18].

Figure 4: Binding mode of glycyrrhizin within the active site of COX2 protein receptor

Toxicity & ADME-T Studies

The modified lead molecules are studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [19].

RESULTS AND DISCUSSION

Nature has always been a bizarre supply of medical ingredients, providing us with several medicinal plants that yield beneficial phytochemicals. Licorice is a member of the Leguminosae family and has the scientific name *Glycyrrhiza glabra*. The ayurvedic herb G. glabra is commonly used. Numerous licorice chemical components have undergone extensive pharmacological research to determine their potent anticancer, antibacterial, anti-inflammatory, cardioprotective, hepatoprotective, and other pharmacological effects. Gastric and duodenal ulcers have traditionally been treated with liquorice as a prophylactic measure. It is used as an anti-inflammatory medication in dyspepsia during allergic responses. The stereoisomer forms of glycyrrhizin are 18α and 18 β. Due to its anti-inflammatory effects on neutrophil functions, especially the production of ROS, glycyrrhizin is regarded to be the most popular folk remedy (reactive oxygen species).

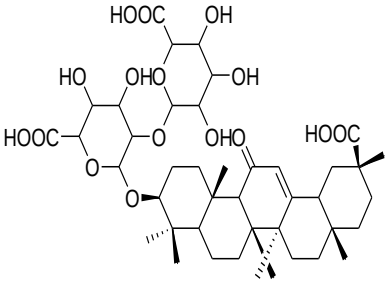
The majority of cancer treatment plans in use today focus primarily on surgical excision of tumour masses. Chemo- and radiotherapies, among other chemical and physical therapies, have significantly slowed the spread of malignant cells. Additionally, these strategies are frequently coupled to improve treatment indices. It is well known that normal cell growth is also inhibited by surgery, chemotherapy, and radiotherapy. A bad quality of life is also a result of the severe side effects and high toxicity of various therapeutic techniques.

The *in-silico* molecular docking investigation was done using autodock in order to clarify the suggested mechanism of glycyrrhizin as an antiproliferative drug. First, based on a review of the literature, a target protein was selected for the molecular modelling investigation. According to a simple definition, molecular docking techniques are used to determine the ideal orientation of a ligand to its molecular target with the least amount of free energy in the formation of a stable complex. When compared to other conventional cancer therapy methods, this computational drug design approach can be claimed to be more thorough, time- and money-efficient, and complete. The possibility for improved output and quality in pharmaceutical research is another advantage of adopting molecular modelling.

The binding energy between the protein and ligand is a crucial metric generated by molecular docking. This provides information on the binding affinity and efficacy of the protein and ligand-receptor docking. The lower the binding energy value, the higher the binding affinity and docking. This work demonstrates how molecular docking analysis was used to calculate the binding energies of the selected protein COX-2 with glycyrrhizin and amino acid residues.

The bioactive compound glycyrrhizin which exhibits the best binding affinity with Cox 2 having binding energy -7.7 kcal/mol. As per outcome of investigation, glycyrrhizin reduces the multiplication of malignant cells which also results in the death of cancerous cells. At growth phase 1 (G1), it induces apoptosis, and at growth phase 2 (G2), or mitotic phase, it induces cell arrest (M). The docking results were tabulated in table 1. The binding mode of glycyrrhizin within the active site of COX 2 protein receptor showed in figure 4-5. The glycyrrhizin interacts with the Tyr385, and Tyr348 residues of COX2 protein to form a complex structure. Glycyrrhizin showed the good binding affinity with COX-2. Cyclooxygenase-2 (COX-2) is liked with breast cancer. Therefore, it is of interest to design and develop new yet effective compounds against COX-2 from medicinal plants. COX-2 is released by cancer-associated fibroblasts (CAFs), macrophage type 2 (M2) cells, and cancer cells to the tumor microenvironment (TME). COX-2 induces cancer stem cell (CSC)-like activity, and promotes apoptotic resistance, proliferation, angiogenesis, inflammation, invasion, and metastasis of cancer cells. The docking results were produced and shown using pymol. As shown by the test, the docking score for the selected compound is similar enough and thus reflects their maximal activity against COX-2. The findings showed that the investigative molecules had higher energy values on the COX-2 protein, which means that glycyrrhizin have greater affinity and steric compatibility with COX-2 protein. The pharmacokinetic profile of glycyrrhizin reveals that it is having good pharmacokinetic profile without presence of any major toxic effects. The pharmacokinetic and toxicity profiling results of glycyrrhizin were shown in figure 6.

Table 1: Results of docking of glycyrrhizin against COX2 protein

S. No	Compound Name	Structure	Binding Energy (Kcal/mole)	Ki (µM)
1	Glycyrrhizin		-7.7	6.82

Interactions

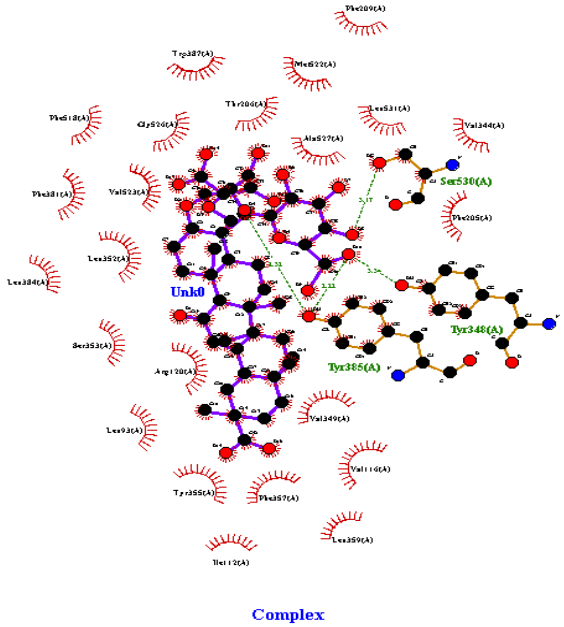


Figure 5: Binding interaction of glycyrrhizin with COX2 protein

Toxicity & ADME-T Studies

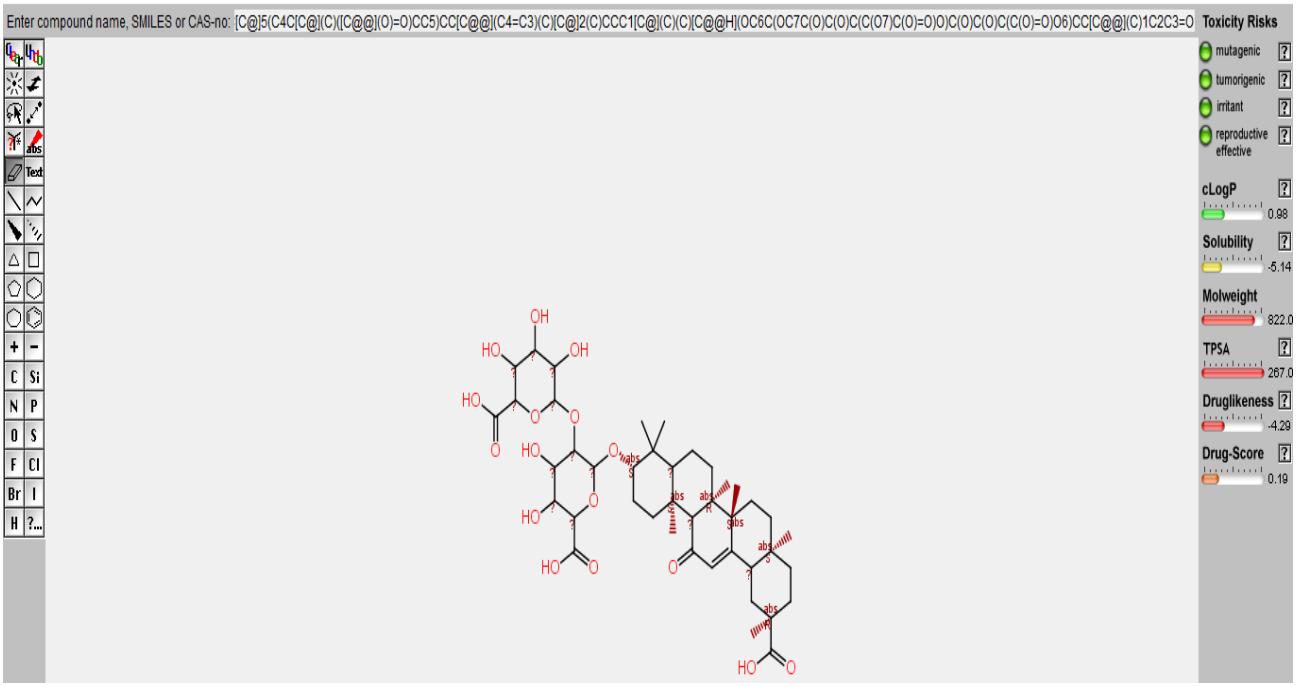


Figure 6: Pharmacokinetic and toxicity profiling of glycyrrhizin

CONCLUSION

The molecular docking of glycyrrhizin with COX2 protein revealed that, it has exhibited the chemical interaction with the amino acids in the active pockets which is showed in Figure.5. Theoretically, the ligand molecule has shown encouraging docking score. The docking result of glycyrrhizin revealed that their docking scores was -7.7 kcal mol^{-1}, and it can be predicted as good inhibitor of COX 2 protein.

REFERENCES

1. Taft, C. A., & Da Silva, V. B. (2008). Current topics in computer-aided drug design. *Journal of pharmaceutical sciences*, *97*(3), 1089-1098.
2. Kakkar, S., Kumar, S., Narasimhan, B., Lim, S. M., Ramasamy, K., Mani, V., & Shah, S. A. A. (2018). Design, synthesis and biological potential of heterocyclic benzoxazole scaffolds as promising antimicrobial and anticancer agents. *Chemistry Central Journal*, *12*, 1-11.
3. Rani, J., Saini, M., Kumar, S., & Verma, P. K. (2017). Design, synthesis and biological potentials of novel tetrahydroimidazo [1, 2-a] pyrimidine derivatives. *Chemistry Central Journal*, *11*(1), 1-11.
4. Hu, Y., & Fu, L. (2012). Targeting cancer stem cells: a new therapy to cure cancer patients. *American journal of cancer research*, *2*(3), 340-356.
5. Kassab, A. E., & Gedawy, E. M. (2013). Synthesis and anticancer activity of novel 2-pyridyl hexahyrocyclooctathieno [2, 3-d] pyrimidine derivatives. *European Journal of Medicinal Chemistry*, *63*, 224-230.
6. Kumar, S., Lim, S. M., Ramasamy, K., Vasudevan, M., Shah, S. A. A., Selvaraj, M., & Narasimhan, B. (2017). Synthesis, molecular docking and biological evaluation of bis-pyrimidine Schiff base derivatives. *Chemistry Central Journal*, *11*(1), 1-16.
7. Pizzorno, J. E., & Murray, M. T. (1999). Textbook of Natural Medicine. 2nd edition. J. Harcourt, eds. Publishers Limited, 768.
8. Chandler, R. F. (1985). Licorice, more than just a flavour. *Canadian Pharmaceutical Journal*, 118, 420-424.
9. Mukharjee, P. K. (2002). Quality control of herbal drugs- an approach to evaluation of botanicals 31st edition. New Delhi, Business Horizons Pharmaceuticals. Publications; pp 183-197.
10. ACD/Structure Elucidator, version 2018. 1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2019.
11. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*, *30*(16), 2785-2791.
12. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as Potent Inhibitor of COVID-19 Main Protease: In-Silico Docking Approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, 1-7.

13. Malik, J. K., Soni, H., Sharma, S., & Sarankar, S. (2020). Hydroxychloroquine as potent inhibitor of COVID-19 main protease: Grid based docking approach. *Eurasian Journal of Medicine and Oncology*, *4*(3), 219-226.
14. Soni, H., Gautam, D., Sharma, S., & Malik, J. (2020). Rifampicin as potent inhibitor of COVID-19 main protease: In-silico docking approach. *Saudi Journal of Medical and Pharmaceutical Sciences*, *6*(9), 588-593.
15. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as Potent Inhibitor of COVID-19 Main Protease: In-Silico Docking Approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, 1-7.
16. Soni, S., Malik, J. K., Sarankar, S. K., & Soni, H. (2019). Rutin as a potent inhibitor of dihydrofolate reductase: A computational design and docking. *EAS J. Pharm. Pharmacol*, *1*, 130-134.
17. Soni, N. U. P. U. R., Pardasani, K. R., & Mujwar, S. O. M. D. U. T. T. (2015). Insilico analysis of dietary agents as anticancer inhibitors of insulin like growth factor 1 receptor (IGF1R). *J Pharm Pharm Sci*, *7*(9), 191-196.
18. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, *25*(13), 1605-1612.
19. Thomas, S. Idorsia Pharmaceuticals Ltd, Hegenheimermattweg 91, 4123 Allschwil, Switzerland, Email: thomas.sanderidorsia.com