Molecular Docking Analysis of Apigenin against Antiglaucoma Property- An in-silico Approach

Dr. N. Deepa, Dr. A. R. Vijayakumar, Uzair Ahmad Dar, S.Predisha, Mu.Akshya, S. Vasantha Ganesh

ABSTRACT:
Glaucoma is a complicated eye disease that causes progressive optic nerve damage and is frequently associated with elevated intraocular pressure (IOP).Apigenin is a naturally occurring flavonoid found in fruits and vegetables has received interest for its possible medicinal characteristics including anti-inflammatory and antioxidant activities. In this study we use an in-silico approach to investigate apigenin's apigenin molecular docking studies against antiglaucoma targets. Apigenin may offer promise as a potential candidate for the treatment of glaucoma by targeting critical proteins involved in the disease's pathogenesis according to the findings

I. INTRODUCTION :

GLAUCOMA

Glaucoma is a group of progressive eye diseases which can lead to irreversible vision loss and blindness if it is untreated .It is described by damage of optic nerve that is responsible for visual transmitting signals from eye to the brain.

The utmost modifiable risk factors for glaucoma is intraocular pressure (IOP) elevation, which results in imbalance between the production and drainage of aqueous humor which is defined as a clear fluid that fills the front part of eye.

The key components for management of glaucoma are antiglaucoma therapies. Which aims to reduce the intraocular pressure and slow down or prevents the damage progression of optic nerve and visual field loss.

Antiglaucoma treatments may include different approaches such as medication (topical or systematic), laser therapies and surgical interventions . These treatments work by either reducing the production of aqueous humor, increasing its outflow or drainage or both.

APIGENIN:

A naturally occurring flavonoid chemical called apigenin belongs the flavone subclass. It is a plant derived substance that has anti-oxidant ,anti-inflammatory and anticancer qualities. Apigenin is well known for its capacity to neutralize free radicals and lessen oxidative stress ,cardiovascular disease and neurological disorder.

Additionally apigenin has neuroprotective properties and has demonstrated promisein the treatment and prevention of neurodegenerative illness including Alzheimer's and Parkinson's disease. It might lessen neuroinflammation ,protect neurons from oxidative damage and regulate signaling pathway important for neuron survival and performance.

II. METHODOLOGY

Software and Modules

Schrodinger software was used to obtain the necessary knowledge about the interactions at the atomic level. The modules, namely, protein preparation, Grid generation, Site map prediction, Ligprep, and glide docking are used to study molecular mechanical interactions of the protein 7QK9, 2ESM, IGSN, 4QJO, 2C7W, and 5OYJ   between the apigenin.

Protocol for preparing the Ligand and the Protein

Interaction with six proteins structure of the human 7 human glucocorticoid receptor, Rho associated coiled containing protein kines, Vascular endothelial growth factor (VEGF), VEGFR tyrosine kinase complex with apigenin energy was reduced to a local energy level that ensures its stability. The protein was preprocessed by enabling Assign bond order using the CCD database together with the hydrogens substituted . In the preparation method, the PDB file 7QK9, 2ESM, IGSN, 4QJO, 2C7W, and 5OYJ   was used as a source entry.

The metal and disulfide linkages are made using zero-order. The Epik module generates the pH levels of 7.0 +/- 2.0.This preprocessed protein was assigned an optimised H-Bond using the PROPKA optimisation technique and sample water orientation. Finally, the protein was minimised using the OPLS4 force field computation, with the heavy atom RMSD coverage set to 0.30 A. The apigenin was prepared for docking using the LigPrep technique. By enabling the OPLS4 force field parameters, the Epik algorithm was utilised to desalt the system.

Analysis of the binding pocket by the Grid generation method

The receptor grid-generating procedure used the ligand position in the PDB protein 7QK9, 2ESM, IGSN, 4QJO, 2C7W, and 5OYJ   as a binding pocket for the apigenin . The VdW radius scaling factor is set to 1.0, with a cutoff partial charge of 0.25. The enclosing box was sized to fit the workspace ligand's centroid, and the dock ligands were set to be 12 inches long.

SET UP FOR DOCKING LIGANDS

The scaling factor approach was set to 0.80 for ligand docking, and the partial charge cutoff (Van Der Waals Radii) was set to 0.15. Using the extra precision technique, the flexible ligand sampling setup was used in conjunction with the imported grid. For bias sampling of torsions, all predefined functional groups were used, as well as sample nitrogen inversion, ring conformations, and sample nitrogen inversion. Finally, Epik state penalties raised the docking score. The ring sampling energy window was set at 2.5 kcal/mol for the generation of conformers, and the dielectric constant value as a function of distance was chosen at 2.0 for the purpose of minimization.

The binding pocket was fixed based on the PDB binding site for the protein 7QK9, 2ESM, IGSN, 4QJO, 2C7W, and 5OYJ respectively record with coordinates of X-39.436882, Y-7.043081, Z- 13.931808 and radius of 9.700000 Å (red circle) (Figure 1), X -0.335365, Y 133.983178, Z 25.142164, and radius of 9.400000 Å (red circle) (Figure 2), X - 65.094274, Y-20.883514, Z 57.398147, and radius of, 8.200000 Å (red circle) (Figure 3), X 24.052585, Y-1.413131, Z 36.730050, and radius of, 26.400000Å (red circle) (Figure 4), X - 44.152341, Y -25.766460, Z-0.187103, and radius of, 9.700000Å (red circle) (Figure 5), X -31.528968, Y - 33.348304, Z- -39.630804, and radius of, 16.900000 Å (red circle) (Figure 6). CHARMm-based CDOCKER protocol (Table 1) performed the docking of molecules inside the above said coordinates. The complete protocol shown in Table 1 includes the simulated annealing with the Heating Steps of 2000, Heating Target Temperature of 700, Cooling Steps of 5000, and Cooling Target Temperature 300.

**Table 1:** Parameter setup for docking study.

Name of the Parameter	Setup value
Input Receptor	7QK9.dsv
Input Ligands	APIGENIN.dsv
Input Site Sphere	39.436882, -7.043081, -13.931808, 09.700000
Pose Cluster Radius	0.1
Random Conformations	10
Dynamics Steps	1000
Dynamics Target Temperature	1000
Include Electrostatic Interactions	YES
Orientation vdW Energy Threshold	300
Simulated Annealing	True
Grid Extension	8.0
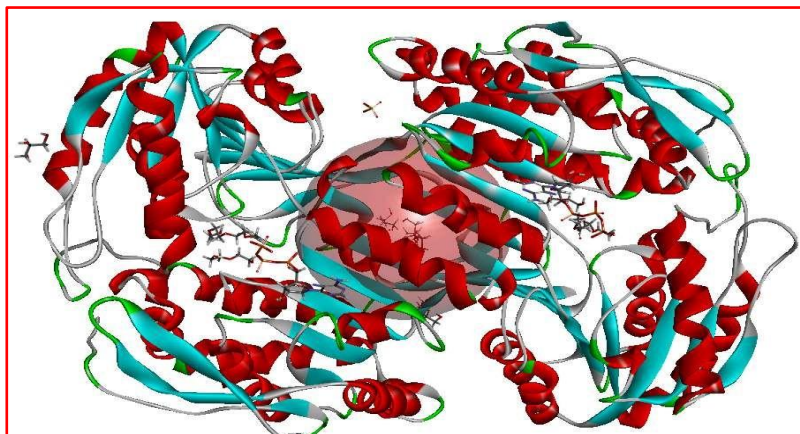
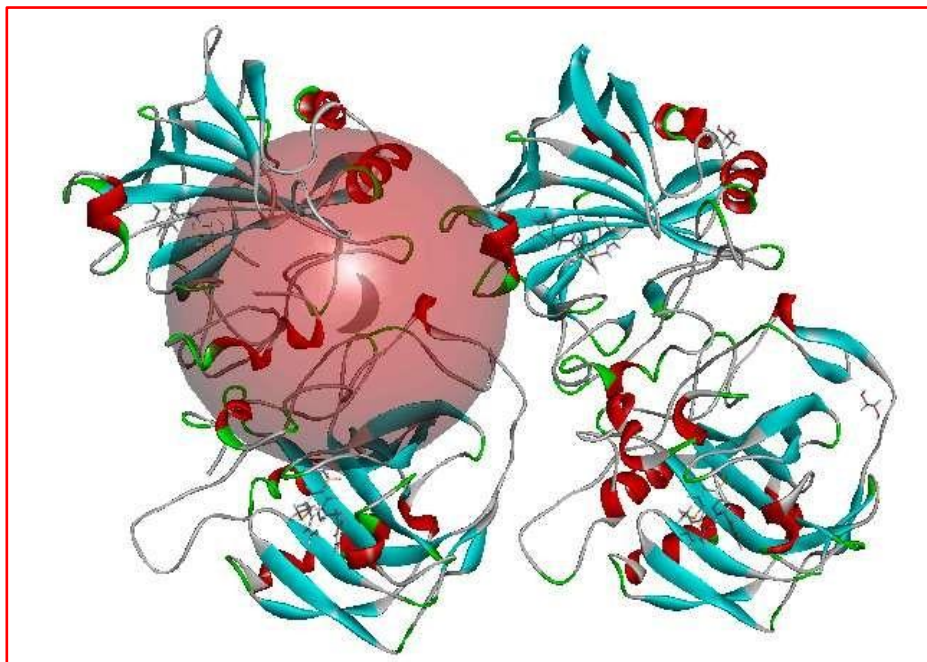**Figure 3:** Secondary Crystal structure Adenosine A3 receptor (A3AR) of (PDB ID: 7QK9).
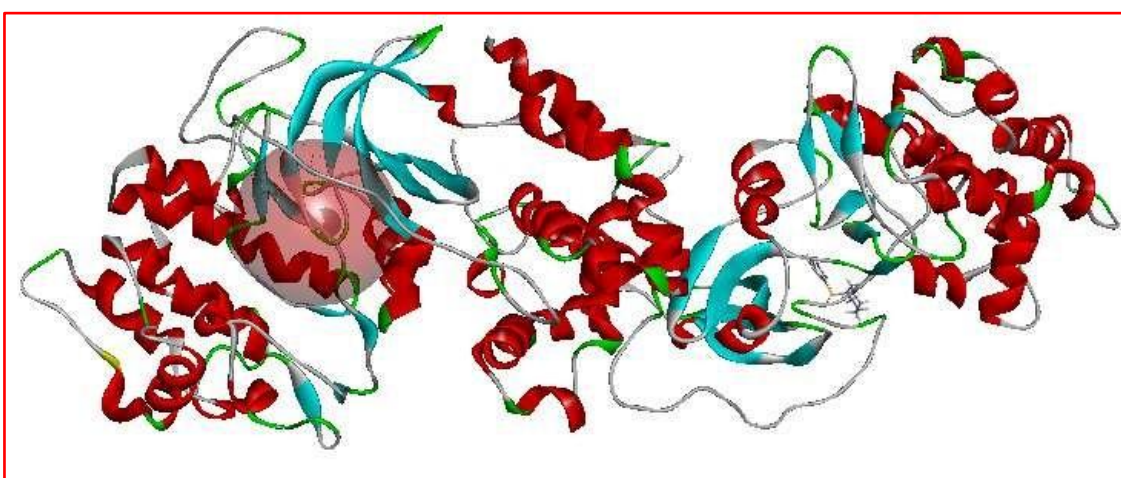
Figure 4: Secondary structure of Crystal structureRho-associated coiled-containing protein kinase (ROCK), (PDB ID: 2ESM).
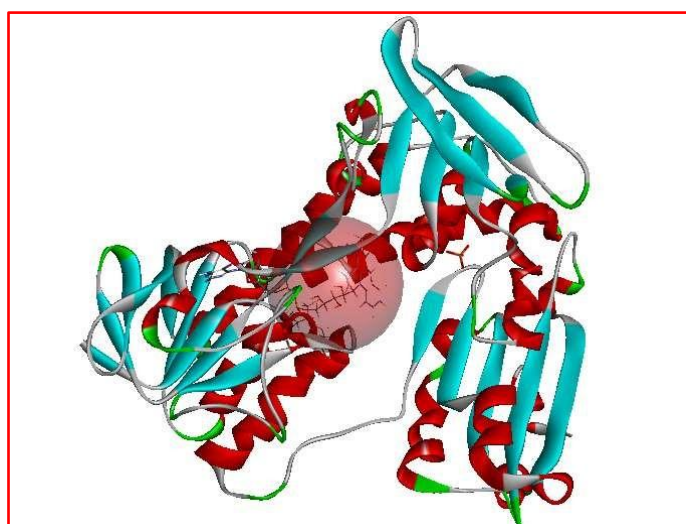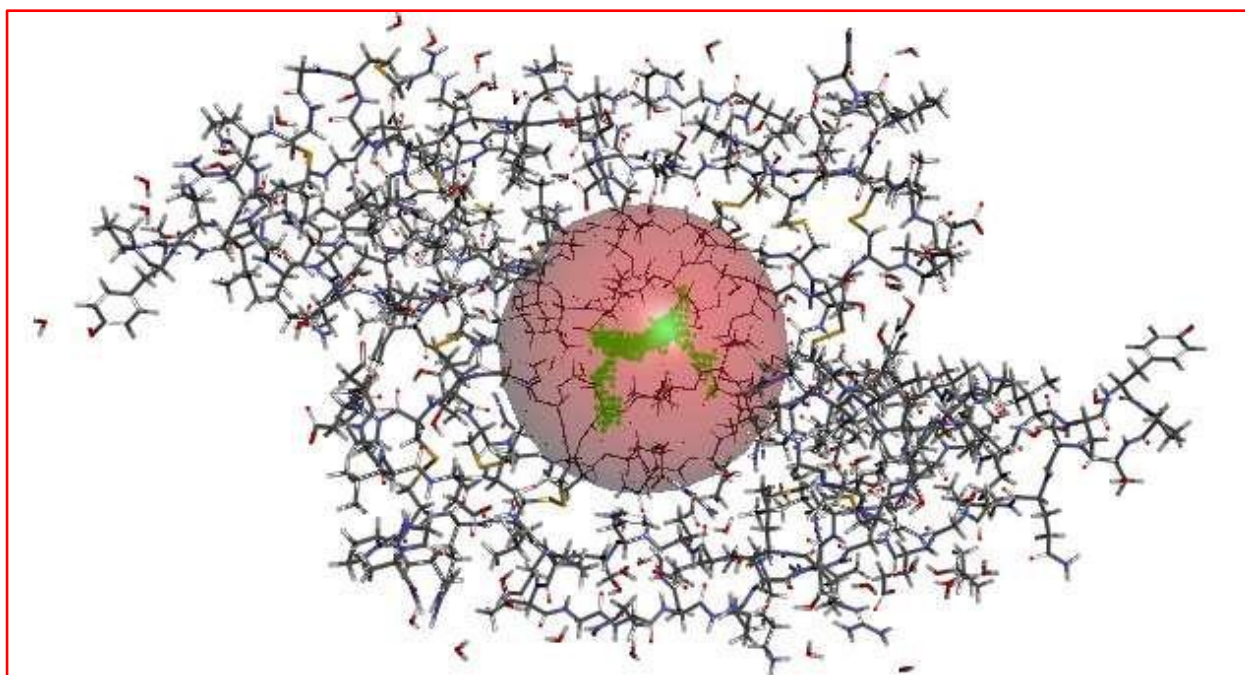

Figure 5: Secondary structure of Crystal structure. 7Human glucocorticoid receptor (hGR) (PDB ID: IGSN).
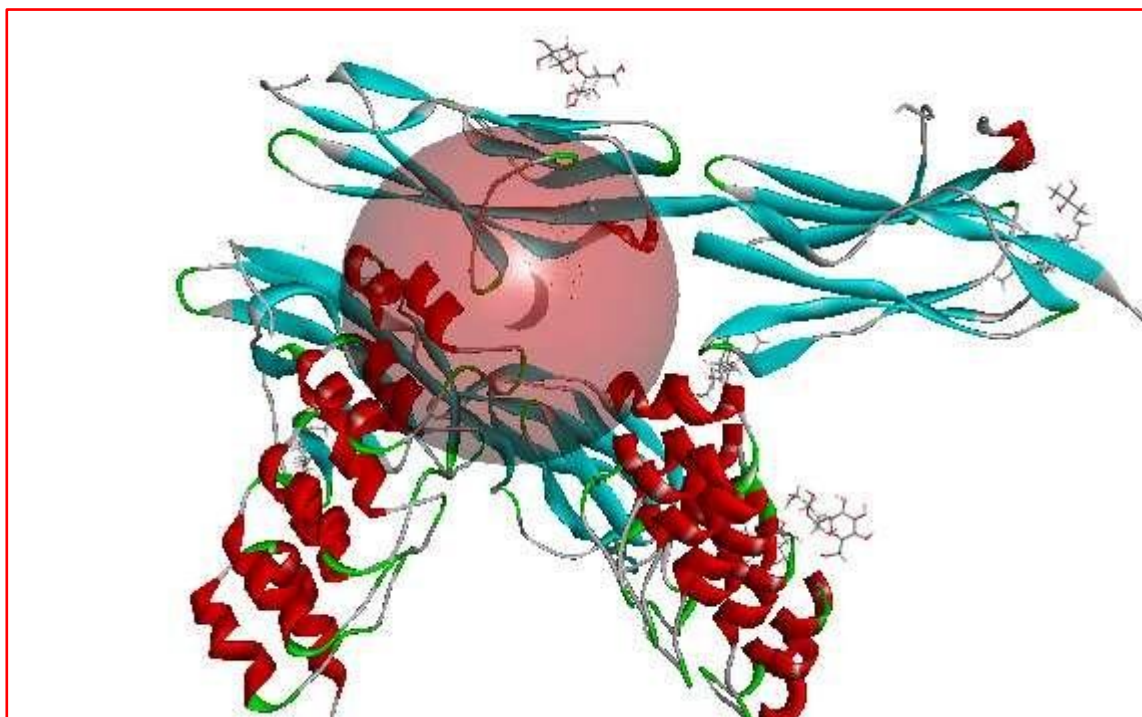
Figure 6: Secondary structure of Crystal structure. 7Human glucocorticoid receptor (hGR) (PDB ID:4QJO).

Figure 7: Secondary structure of Crystal structure Vascular endothelial growth factor (VEGF) (PDB ID:2C7W).

Figure 8: Secondary structure of Crystal structure VEGFR tyrosine kinase (PDB ID:5OYJ).

III.   RESULT AND DISCUSSION

Results and Discussion:

Molecule Apigenin formed H-bond interaction with GluB:199, Hydrophobic interaction shaped by Ala B:495, ValB:500, Ile B:171, TyrA492, and AlaA: 495, Positively charged interaction generated by Arg B:276, Arg A:487, and Arg A:276. ThrB:498 and Asn B:485 made polar interactions, Glu B:496, 499, A:496. Gly A:486 and B486 also aid the orientation fix of the Apigenin molecule. These interactions made the Apigenin binding affinity of -35.5181kcal/mol with A3AR protein (Table 2).
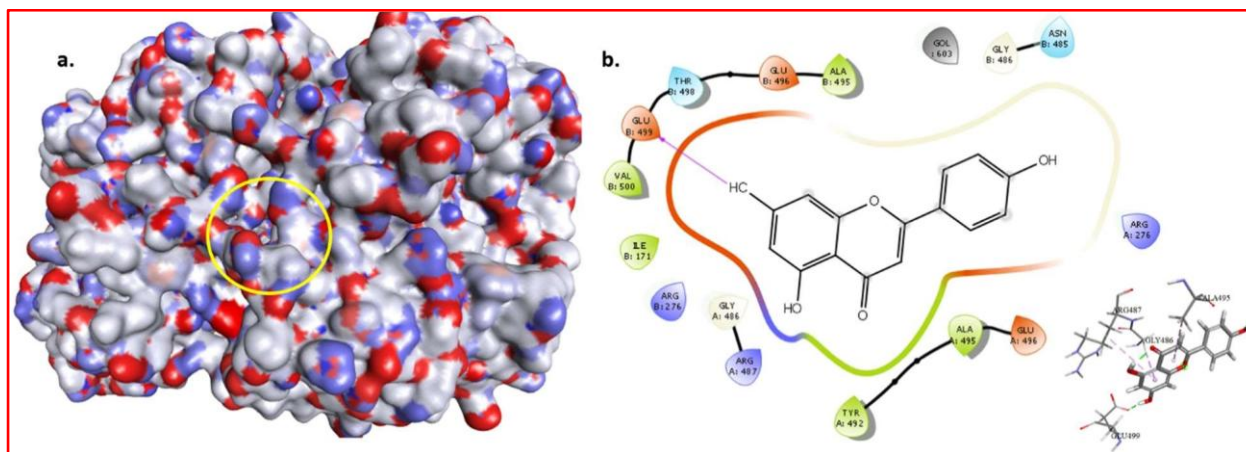
Figure 9: a. Surface binding of molecule with A3AR protein. b. Binding pocket amino acids interaction with Apigenin.

Table 2: Energy table of different Proteins and Apigenin molecule.

Name	Initial Potential Energy	Initial RMS gradient	Final Potential Energy	vdW energy	Final RMS gradient	C Docker energy
7QK9	116105	8958.62	-65219.2	-7013.92	1.16059	
Apigenin	63.4678	41.4807	24.3032	4.567	0.00995	-35.5181
2ESM	-24284.3	151.338	-53564.4	-5538.43	0.99725	
Apigenin	63.4678	41.4807	24.3032	4.567	0.00995	-28.5734
IGSN	-14077.2	36.6764	-29198.9	-3261.8	0.97455	
Apigenin	63.4678	41.4807	24.3032	4.567	0.00995	-23.7251
4QJO	-17790.2	482.628	-67484.2	-7866.87	1.09903	
Apigenin	63.4678	41.4807	24.3032	4.567	0.00995	-42.4898
2C7W	-1842.6	160.162	-12259.9	-1257.83	1.2633	
Apigenin	63.4678	41.4807	24.3032	4.567	0.00995	-17.5521
5OYJ	-18806.9	154.663	-49516.8	-5222.66	1.10295	
Apigenin	63.4678	41.4807	24.3032	4.567	0.00995	-23.1493

Molecule Apigenin bonded inside the binding cavity of protein with the energy of -28.5734 kcal/mol. This affinity produced by 16 amino acids such as formed H-bond interaction with Gly85, Ala86, Phe87 (Hydrogen bond), Gly88, Glu89, Phe120 (π-π stacking), Asp117 (H-bond), Asn203 (Hbond), Asp202, Lys200, Asp198, Leu107,Lys105,Asp216, Gly218, Thr219. These interactions confirmed the binding of all the fragments of apigenin with a correct orientation Figure 10.
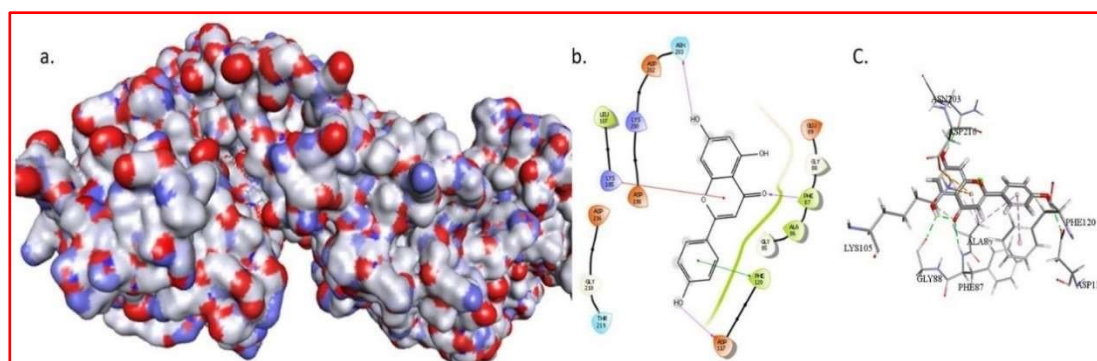
Figure 10. a. Surface binding of Crystal structure Rho-associated coiled - containing protein kinase (ROCK). b. Binding of active site amino acids of hGR with Apigenin molecule. c. 3D binding of amino acids.

Molecule Apigenin formed only one H-bond interaction with PO4 human glucocorticoid receptor (hGR) of and other very weak interaction such as Leu337, Ile198, Tyr197, Gly196, Ala195, Phe226 and Leu223. Two positive interactions with Arg291 and Arg224 fold the phenol ring system and chromone moiety of the molecules figure 11.

Figure 11: a. Surface binding of human glucocorticoid receptor (hGR). b. Binding of active site amino acids of hGR with Apigenin molecule. c. 3D binding of amino acids.

The figure 10 illustrate the binding of Apigenin with Carbonic anhydrase protein. Totally, 7 types of interactions such as Hydrogen bonds by AsnD244, AspD99, GlnD248 and GlnD110. The π-π stacking interactions (PheD246, HisC33 and HisD111) aid the fixing of three ring system of the molecules inside the binding pocket of Carbonic anhydrase protein. Additional polar, negative and positive Interactions strengthen the binding of the molecule with Carbonic anhydrase protein with the affinity of -42.4898 kcal/mol.

Figure 12. a. Surface binding view of Apigenin in Carbonic Anhydrase. b. Binding of active site amino acids of Carbonic Anhydrase with Apigenin molecule. c. 3D binding of amino acids.

The molecule Apigenin binds to vascular endothelial growth factor (VEGF) with an energy of 17.5521 kcal/mol. Only three types of interactions are involved in the interaction generation. Specifically, one A chain amino acid Glu30 formed a phenolic OH group of molecules and 5 amino acids (ValA: 31,32 and ValB:31,32, ProB:34) only alter the angle of one fragment of a molecule.
Additionally, 2 negative and 2 positive charged interaction are generated by the molecule.

Figure 13. a. Surface binding view of Apigenin in Vascular endothelial growth factor (VEGF). b. Binding of active site amino acids of Vascular endothelial growth factor (VEGF) with Apigenin molecule. c. 3D binding of amino acids.

Apigenin and VEGFR tyrosine kinase bind with the energy of -23.1493 kcal/mol. This energy was generated due to the four types of interactions, first, three hydrogen bonds formed with ArgC:391, AsnA:140, and sodium ion. Further, the π-cationic bond formed with LysC:366 and additional affinity produced by AspC392, LeuC:371, IleC369, and AsnC:367 (Figure 14).
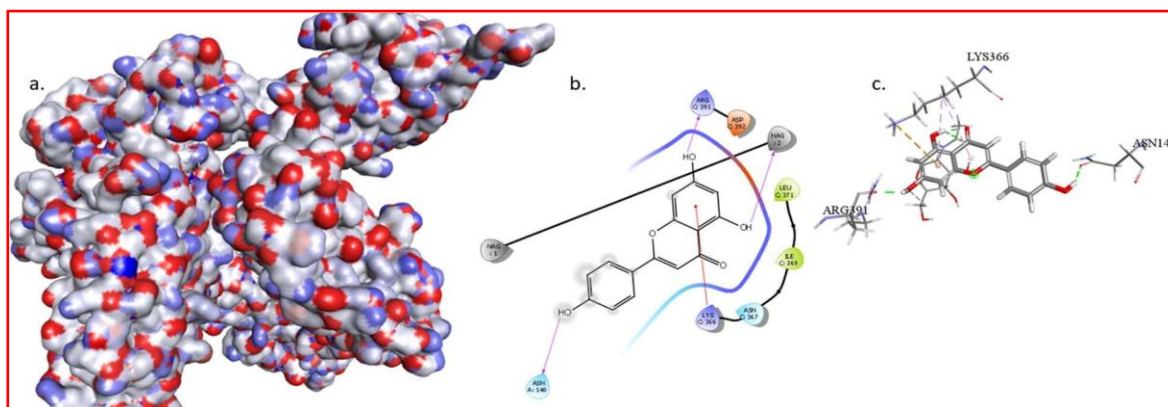

Figure 14. a. Surface binding view of Apigenin in VEGFR tyrosine kinase. b. Binding of active site amino acids of VEGFR tyrosine kinase with Apigenin molecule. c. 3D binding of amino acids.

Evaluating the anti-glaucoma activity of apigenin using an in-silico model can provide valuable insights for present work. By employing computational methods, you can assess the potential of apigenin as an effective treatment for glaucoma, a condition characterized by increased intraocular pressure and optic nerve damage. One of the key advantages of using an in-silico model is the ability to predict the binding affinity of apigenin with target proteins involved in glaucoma pathogenesis. By conducting virtual screening and molecular docking studies, it imply identify potential protein targets and understand the interactions between apigenin and these targets. This information can help elucidate the mechanism of action of apigenin and provide insights into its efficacy in combating glaucoma.

Additionally, in-silico models enable the evaluation of apigenin's pharmacokinetic properties. By predicting its absorption, distribution, metabolism, and excretion (ADME), you can estimate its bioavailability and potential for reaching the target tissues in the eye. This information is crucial for understanding the optimal dosing and administration of apigenin. To ensure the safety of apigenin as a potential anti-glaucoma agent, it can employ toxicity prediction models to assess its potential adverse effects. This helps in identifying any potential concerns or limitations associated with its use.

Furthermore, in-silico models can facilitate the identification of relevant signaling pathways or biological processes modulated by apigenin. Pathway analysis can provide a comprehensive understanding of the molecular mechanisms underlying its anti-glaucoma activity, potentially revealing new therapeutic targets for the treatment of glaucoma.

It is important to note that while in-silico models can provide valuable predictions and insights, they should be complemented with experimental validation to confirm the efficacy and safety of apigenin as an anti-glaucoma agent. Combining computational and experimental approaches can provide a comprehensive assessment and strengthen the reliability of present work.

IV. CONCLUSION

In conclusion, the evaluation of the anti-glaucoma activity of apigenin using an in-silico model holds significant potential for present work. By employing computational methods, such as virtual screening, molecular docking, pharmacophore modelling, and pathway analysis, important insights can be gained regarding the effectiveness, mechanisms of action, pharmacokinetic properties, and safety profile of apigenin as a potential treatment for glaucoma.

The in-silico model allows for the prediction of the binding affinity of apigenin with target proteins involved in glaucoma pathogenesis. This knowledge aids in understanding the interactions between apigenin and these proteins, shedding light on its potential therapeutic efficacy. Additionally, by assessing the ADME properties of apigenin, such as its absorption, distribution, metabolism, and excretion, the model helps estimate its bioavailability and potential for reaching the target tissues in the eye.

Toxicity prediction models provide an important aspect of safety evaluation, offering insights into any potential adverse effects associated with apigenin.

By considering the signalling pathways and biological processes modulated by apigenin, the in-silico model contributes to a comprehensive understanding of its molecular mechanisms and potential therapeutic targets.

However, it is difficult to note that while the in-silico model provides valuable predictions, experimental validation is essential to confirm the efficacy and safety of apigenin as an antiglaucoma agent. Integrating computational and experimental approaches ensures a more reliable assessment.

In summary, leveraging an in-silico model for the evaluation of the anti-glaucoma activity of apigenin offers a promising avenue for ophthalmic research. Further studies need to understand the molecular interactions, pharmacokinetics, and mechanisms of action of apigenin, which is contributing to the development of effective and safety of treatments for glaucoma in the near future.

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