

Molecular Docking Of Cyclosenegalina As Anticancer

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ABSTRACT

Cancer is recognized as a leading cause of death globally, responsible for approximately 14.5% of all deaths. Cyclosenegalina A showed activity against DU-145 human prostate cancer cell line with IC₅₀ of 54.92.35 μM. This study aims to investigate whether the cyclosenegalina A compound can interact with the target receptors 4IEH and potentially act as an anticancer candidate. The results of the study show that the docking of cyclosenegalina A with the 4IEH receptor yielded the best results, with an affinity value of -8.88 kcal/mol. The hydrogen bonding at the GLY 104, ASP 70, VAL 92, and GLU 95 amino acids was identical to that observed in the standard ligand, n-heteroarilsulfonamides. The interaction between cyclosenegalina A and the target receptor is effective, indicating that cyclosenegalina A holds potential as an anti-cancer candidate.

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Introduction

Cancer is recognized as a leading cause of death globally, responsible for approximately 14.5% of all deaths, with expectations that this figure will double by 2030. The "Hallmarks of Cancer" concept serves as a valuable framework for research focused on enhancing and creating more targeted cancer therapies. One of the key hallmarks of cancer is the ability of cancer cells to resist cell death and evade apoptosis, enabling continuous proliferation. As a result, this characteristic has become a primary focus in the development of targeted cancer treatments[1]

Cancer cells can evade apoptosis through various mechanisms. In human cancer cells, the downregulation of pro-apoptotic proteins (such as Bax, Bak, Bad, Bim) and the upregulation of anti-apoptotic proteins (like

BCL-2, BCL-xl, MCL-1) prevent the release of cytochrome c from the mitochondria, contributing to the immortality of the cancer cells. In cancer cell lines, the expression of apoptotic inhibitors and activators is often abnormal. For example, BCL-2 overexpression has been observed in nearly half of all human cancers.[1]

Cyclosenegalina A is a cyclopeptide, isolated from *Annona senegalensis* and *Annona scleroderma*. The peptide, cyclo-(Pro1-Gly2-Leu3-Ser4-Ala5-Val6-Thr7-) was isolated from the methanolic extract of both plants. Cyclosenegalina A showed activity against DU-145 human prostate cancer cell line with IC₅₀ of 54.92.35 μM. Cyclosenegalina A is a proline-glycine cyclopeptide with two β-turns conformations, which are type I and type II, incorporating a β-bulge [2]

Molecular docking is a computational technique designed to simulate the interaction

between a ligand molecule and a targeted protein in an in vitro setting. Through molecular docking, we can forecast the binding affinity and interactions of Cyclosporin A with key receptor proteins involved in the development of anticancer properties [3]

Cancer is a disease marked by the uncontrolled growth of cells that extends beyond normal boundaries, allowing them to invade nearby tissues and spread to other organs. Cancer is also referred to as malignant tumors or neoplasms. A malignant tumor, or cancer, invades surrounding tissues and has the potential to spread to distant organs through metastasis.[4]

Materials and Methods

This study was conducted on a computer with an Intel Core i5 10400F processor and a GEFORCE GTX 1650 Super GPU. The receptor structures were obtained from the Protein Data Bank (PDB), then separated from their original ligands, non-standard residues, water molecules, and additional charges using the BIOVIA Discovery Studio Visualizer 2017 software. The design aimed to create space (a pocket or cavity) to define the pocket shape and coordinates for the docking process. The receptors used were n-heteroarilsulfonamides, downloaded from the PDB website [5]

These files were then opened with AutoDockTools-1.5.6, where water molecules were removed, and hydrogen atoms were added. If the receptor was solvated, one of the atomic chains was removed, and the ligand was separated from the protein, with files named accordingly. Test ligands were prepared by aligning their coordinates with those of the standard ligand using BIOVIA Discovery Studio.

The downloaded ligand and brazilin files were selected, with the ligand structure being copied into the brazilin structure document and saved under the ligand's name. To optimize the 3D geometric structure of the ligands, the "clean geometry" option and the Dreiding-like forcefield were applied using BIOVIA Discovery Studio. The receptor setup was completed in AutoDockTools by selecting the ligand.pdb and saving it in pdbqt format.

The parameter file for GridBox was then created to define the binding site coordinates and size of the receptor, and saved as grid.gpf. Finally, the docking parameters were prepared by selecting receptor.pdbqt [6]

A series of molecular docking experiments were carried out using the AutoDockTools software to ensure binding efficiency through the interaction of the Cyclosporin A with the selected target.

The results of the molecular restraint simulation were clustered using an RMSD tolerance of 2.0 Å. The ligand conformation with the lowest binding energy (ΔG) from the cluster was selected as the best for the analysis phase using BIOVIA Discovery Studio 2017 software. The free-binding energy of the docking results was analyzed from the output in AutoDockTools. The ligand-protein complex conformation with the lowest free binding energy value was chosen for further analysis. The docking results were visualized using the BIOVIA Discovery Studio program[7]

Results and Discussion

The preparation of the receptor and ligand was carried out using the BIOVIA Discovery Studio program. This preparation aimed to create space for the test compound to bind to the protein. It was a crucial step in the docking process, as it directly influences the quality of the docking results.

During the docking process, receptor preparation involves preparing the 3D structure of the target protein or receptor. This includes cleaning the residues and removing water molecules and metal ions. The removal of water molecules is essential to prevent interference with the docking process and the interaction formed, ensuring that only the receptors and ligands interact [8]

Method validation was carried out using the AutoDockTools program by redocking the respective ligands with the prepared receptors. The key factor in validating the molecular construction method was defining the grid box was created to represent the area where the interaction between the ligand and the amino acids of the target compound would produce a pharmacological

effect. The grid box size is 50 x 40 x 40 0.375Å° space

This research was using 4ieh_protein as the receptors and 4ieh_ligand as a ligand. (Figure 1)

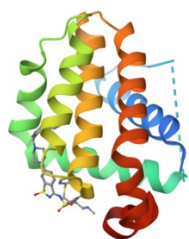


Figure 1. 4IEH Receptor [9]

Root Mean Square Deviation (RMSD) is a parameter used in the validation of molecular restraints. A validation result with an RMSD value below 2.0 Å is considered acceptable and validated. The validation results for the 4IEH receptors is 0,2026 Å. Based on the RMSD result, the validation outcomes can be considered to meet the criteria and have been successfully validated. The receptors are now ready for the next stage of docking with the brazilin target compound.

The cycloenegalin A compound was initially prepared using the AutoDockTools application, followed by the docking process with the receptors that had successfully passed the validation.

The molecular docking results of cycloenegalin A and n-heteroarilsulfonamids, used as a reference ligand, against the 4IEH receptors are shown in Table 1.

Table1. The results of molecular docking were obtained from the interaction between cycloenegalin A and the receptors

Receptor	ΔG Result	
	References	cycloenegalin A
4IEH	-9,63 kcal/mol	-8,88 kcal/mol

The more negative or lower the value of binding-free energy (ΔG) value indicates a good level of stability of the ligand and receptor bond so that the bond formed is stronger and the inhibitory activity is maximum [10]

An interaction that occurs can enhance the binding affinity between the ligand and brazilin, making it important to understand the intermolecular interactions involved (Table 2). By default, AutoDockTools software classifies hydrogen bonds into categories such as classical, non-classical, water, and salt bridges. It also provides information on the bond length, angle, and atom type. If these criteria are not met, the software will not classify the interaction as a hydrogen bond.[11]

Based on the AutoDock results, there are 9 types of interactions between the ligand and receptor, as shown in Table 2 - 11

Table 2. Molecular docking results of the interaction between n-heteroarilsulfonamids and receptors

n-heteroarilsulfonamids		
Receptors	Number of Bond	Amino Acid
4IEH	13	ALA A:59
		TRP A:103
		PHE A:157
		GLU A:95
		VAL A:92
		PHE A:112
		GLU A:111
		ASP A:70
		TYR A:161
		ASP A:62
		ASN A:102
		GLY A:104
		ARG A:66

Table 3. Molecular docking results 1 of the interaction between cycloenegalin A and receptors

Cycloenegalin A		
Receptors	Number of Bond	Amino Acid
	12	ARG A:105
		GLY A:104
		ALA A:108
		PHE A:63
		ASP A:70
		MET A:74

4IEH	VAL A:92
	PHE A:97
	LEU A:96
	GLU A:95
	ARG A:98
	ASP A:99

Tabel 4. Molecular docking results 2 of the interaction between cyclosenegalina A and receptors

Cyclosenegalina A		
Receptors	Number of Bond	Amino Acid
4IEH	9	TYR A:67
		ASN A:102
		ARG A:105
		LEU A:96
		PHE A:63
		TRP A:103
		GLY A:104
		ARG A:66
		PHE A:157

Tabel 5. Molecular docking results 3 of the interaction between cyclosenegalina A and receptors

Cyclosenegalina A		
Receptors	Number of Bond	Amino Acid
4IEH	10	ASN A:102
		TRP A:103
		LEU A:160
		GLY A:104
		ARG A:66
		TYR A:161
		PHE A:63
		ALA A:108
		ARG A:105
		ASP A:70

Tabel 6. Molecular docking results 4 of the interaction between cyclosenegalina A and receptors

Cyclosenegalina A		
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Receptors	Number of Bond	Amino Acid
4IEH	9	ASN A:102
		ARG A:98
		ASP A:99
		GLU A:95
		MET A:74
		VAL A:92
		ASP A:70
		GLU A:73
		GLN A:77

Tabel 7. Molecular docking results 5 of the interaction between cyclosenegalina A and receptors

Cyclosenegalina A		
Receptors	Number of Bond	Amino Acid
4IEH	10	ARG A:105
		ASN A:102
		ASP A:70
		GLY A:104
		ARG A:66
		TYR A:67
		PHE A:63
		ALA A:108
		LEU A:96
		GLU A:95

Tabel 8. Molecular docking results 6 of the interaction between cyclosenegalina A and receptors

Cyclosenegalina A		
Receptors	Number of Bond	Amino Acid
4IEH	9	VAL A:101
		ASP A:99
		ARG A:105
		ASN A:102
		GLY A:104
		TRP A:103
		LEU A:160
		TYR A:161
		ASN A:151

Tabel 9. Molecular docking results 7 of the interaction between cyclosenegalinal A and receptors

Cyclosenegalinal A		
Receptors	Number of Bond	Amino Acid
4IEH	8	VAL A:115
		SER A:75
		GLU A:119
		ASN A:122
		ARG A:26
		GLN A:25
		PHE A:71
		GLY A:27

Tabel 10. Molecular docking results 8 of the interaction between cyclosenegalinal A and receptors

Cyclosenegalinal A		
Receptors	Number of Bond	Amino Acid
4IEH	8	TYR A:161
		LEU A:160
		GLY A:162
		PRO A:163
		GLN A:58
		ARG A:65
		ALA A:59
		ARG A:66

Tabel 11. Molecular docking results 9 of the interaction between cyclosenegalinal A and receptors

Cyclosenegalinal A		
Receptors	Number of Bond	Amino Acid
4IEH	7	TYR A:67
		ARG A:66
		ARG A:105
		PHE A:63
		ASN A:102
		TRP A:147
		GLY A:104

Based on the analysis results, the compound cyclosenegalinal A can interact with

the 4IEH receptor by forming hydrogen bonds with the GLY 104, ASP 70, VAL 92, and GLU 95 amino acids. These hydrogen bonds are identical to those formed between the ligand and the 4IEH receptor.

This indicates that the active site where the ligand binds to cyclosenegalinal A in the 4IEH receptor is compatible, leading to a similar affinity as the standard ligand in inhibiting the 4IEH [12]. The two-dimensional interactions of receptors can be seen in Figure 2. The hydrogen bonding interaction between a small molecule and a protein determines the effectiveness of the molecule in inhibiting the protein[13]

Molecular docking simulations may not fully reflect the complexities of real-world interactions. To validate these findings and assess the safety and efficacy of cyclosenegalinal A in vivo studies are crucial. To calculate the affinity, or fitness, of the protein-ligand interaction, an empirical scoring function sums up the contributions of various individual components. The scoring function in AutoDock Vina is based on the contributions of intermolecular interactions such as steric, hydrophobic, and hydrogen, as well as the number of rotatable bond interactions. Protein-ligand binding can only take place spontaneously when the system's free energy is negative. The strength of the negative ΔG determines the degree of protein-ligand stability; the more negative the ΔG , the more stable the proteinligand complex. A drug's capacity to bind to a receptor is measured by its binding affinity. The stronger receptor-ligand interactions are correlated with lower binding affinities [14]. Computational molecular docking is routinely performed at various stages of the drug discovery process. It is often used to predict the binding orientation of drug candidates to protein targets to predict the affinity and activity of small molecules [15]

The interaction between cyclosenegalinal A and the target receptor is effective, and it can be identified that brazilin has potential as a candidate for anticancer treatment



Figure 2. The two-dimensional interaction between cyclosporin A and the target receptor (a)Result 1,(b) Result 2,(c) Result 3,(d) Result 4,(e) Result 5,(f) Result 6,(g) Result 7,(h) Result 8, (i) Result 9

Conclusion

The results of the study show that the docking of cyclosenegalinal A with the 4IEH receptor yielded the best results, with

an affinity value of -8.88 kcal/mol. The hydrogen bonding at the GLY 104, ASP 70, VAL 92, and GLU 95 amino acids was identical to that observed in the standard ligand, n-heteroarilsulfonamides. The interaction between cyclosenegalinal A and the target receptor is effective, indicating that cyclosenegalinal A holds potential as an anti-cancer candidate.

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