FLAVONOID DERIVATIVES AS POTENTIAL INHIBITORS FOR HUMAN HSP70 USING IN SILICO APPROACHES

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Article History: Received: 03.07.2023 **Revised:** 02.08.2023 **Accepted:** 14.08.2023

Abstract

Background: Human Hsp70 exhibits significant potential as a target for treating a wide range of diseases, including cancer and dengue. Flavonoids have been selected as promising candidates for inhibiting Hsp70, with epigallocatechingallate and myricetin as positive controls. **Methods:**In this study, an in silico method involving docking ligands with the receptor was employed using AutoDockVina to identify flavonoid molecules capable of binding to the ATPase domain of Hsp70. **Results:** Molecular docking experiment results revealed that the two positive control molecules exhibited optimal binding energies of -9.0 kcal/mol and -10.2 kcal/mol, respectively. Seven of the eight other ligands tested demonstrated strong binding capabilities with the receptor. These findings suggested that various types of flavonoids hold potential as therapeutic agents in treating diseases involving Hsp70.

Keywords: Hsp70 ; Flavonoids ; In silico ; Molecular docking ; Therapeutic agents

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INTRODUCTION

The development of drugs to combat prevalent diseases like Parkinson's, Alzheimer's, HIV, dengue, and cancer typically centers around inhibiting specific proteins involved in the disease process[1, 2]. Designed drugs often target particular proteins in disease-causing components, such as viruses or cancer cells[3]. However, human diseases vary widely, from viral infections to genetic mutations, each with diverse biochemical pathways[4]. Consequently, drugs effective against one disease usually cannot be applied to other diseases, even those with similar characteristics.

Drug development should target components playing crucial roles in various ailments to create potential therapeutic agents capable of addressing a broad spectrum of diseases[5, 6]. One such protein is humanheat shock protein 70 (Hsp70) [7]. This protein functions as a mediator in the heat shock factor 1 (HSF1) stress response pathway, which is essential for protein folding and degradation, and regulates apoptosis [8], a significant process in the emergence of cancer cells[9]. Hsp70 also plays a role in flavivirus infections like the dengue virus; the protein is required in various stages of the flavivirus life cycle, including entry into host cells, RNA replication, and virion formation [10]. Therefore, inhibiting human Hsp70 is promising for treating various diseases, including cancer and flavivirus infections.

This study employs in silico methods, specifically docking ligands with proteins, to identify ligands that have the potential to inhibit Hsp70 activity. The selected ligands as candidates for Hsp70 inhibition in this research are flavonoids. Flavonoids are known for their bioactive properties with various medical benefits that can be derived from natural sources, making them suitable for testing as potential drugs. Two flavonoid molecules, epigallocatechingallate (EGCG) and myricetin, have already been proven to inhibit the ATPase domain of Hsp70[2] (this domain is catalytically active in various biochemical pathways). The chosen ligands come from different flavonoid classes, including flavone (luteolin and tangeretin), flavonol (quercetin, kaempferol, and myricetin), flavanone (eriodictyol), flavanone glycoside (hesperidin), flavanol (EGCG and (-)-epicatechin), flavanoneol (taxifolin), and isoflavonoid (genistein). The ligands were used to explore the different inhibitory capabilities of various flavonoid classes against Hsp70.

MATERIALS AND METHODS

Receptor and Ligand Coordinate Acquisition

The atom coordinates of the receptor molecule were obtained from the Protein Data Bank (PDB) with the .pdb file extension (http://www.rcsb.org/). Meanwhile, the coordinates for all ligands were obtained from PubChem with the .sdf file extension (https://pubchem.ncbi.nlm.nih.gov/). The utilized receptor was the ATPase domain of human Hsp70 (PDB ID: 2E88). The ligands for docking testing included luteolin, tangeretin, quercetin, kaempferol, eriodictyol, hesperidin, (-)-epicatechin, taxifolin, and genistein. EGCG and myricetin were used as positive controls.

Receptor Preparation

The ligand (Zn2+) and all water molecules in the 2E88 file obtained from PDB were removed initially to obtain a pure protein receptor file. Subsequently, refinement was performed on the protein receptor file using KoBaMIN[11]. The validation of the receptor structure before and after refinement using Protein Structure Validation Software (PSVS) version 1.5 [12].

Ligand Drug-Likeness Check

The drug-likeness of each selected ligand for docking experiments was computed using SwissADME[13]. Parameters checked for these ligands included water solubility, gastrointestinal absorption, and Lipinski's rule of five compliance. After suitable molecules were selected as potential drug candidates, their coordinate files were converted to .pdb format using OpenBabel[14, 15].

Receptor and Ligand Preparation for Docking Experiments

The preparation of molecules involved in docking was carried out using AutoDockTools4 (ADT) based on the protocol outlined by Garrett et al. [16]. The preparation of the receptor involved adding nonpolar hydrogen atoms to the molecule. The ligand molecules remained unchanged. After preparation, all molecules were saved with the .pdbqt extension.

Molecular Docking Experiments

Molecular docking experiments were performed using AutoDockVina[17]. All dockings were conducted with an exhaustiveness value of 24. To obtain the best interaction sites between the ligands and the receptor, blind docking experiments were carried out for all ligands with a search coordinate covering the entire receptor and the receptor as the center point. The optimal interaction sites were determined based on the ligand binding sites on the receptor with the lowest energy. In obtaining the optimal binding energy, docking experiments were repeated for all ligands with a smaller search area centered on the ligand. The docking results with the lowest energy for each ligand were visualized using ADT.

RESULTS

The Hsp70 protein file obtained from the PDB still contains atoms not part of the protein, namely Zn2+ and water molecules. To ensure that interactions in the docking experiment occur only between the test ligands and the receptor, the Zn^{2+} metal ion and all water molecules are removed from the protein coordinate file. Thus, the receptor used in the docking experiment is a pure protein.

However, due to these alterations from its original structure, refinement is necessary to observe any changes in the receptor's structure. Then, the original structure and the structure after refinement are validated to obtain the most suitable structure for use in docking. Based on Procheck analysis in PSVS, the Ramachandran plot of the receptor before and after refinement showed 91.7% and 96.2% of allowed regions for interactions, respectively, without any disallowed regions (Table 1). The higher percentage of allowed regions in the structure after refinement indicates that it is better suited for the docking experiment, making it the chosen structure.

Table 1: Summary of Ramachandran plot results for the protein receptor structure: before refinement and after refinement.

2D Protein State	Most favored regions	Additionally allowed regions	Generously allowed regions	Disallowed region
Before refinement	91.7%	8.3%	0.0%	0.0%
After refinement	96.2%	3.6%	0.3%	0.0%

Based on the SwissADME analysis, all ligands were found to be water-soluble (although tangeretin was categorized as moderately soluble). Out of the eleven ligands, three of them (EGCG, myricetin, and hesperidin) exhibited limited gastrointestinal absorption and violated one or more Lipinski's rules (Table 2).

Table 2: Ligand conformation results from molecular docking.

Compound	Solubility in water	Gastrointestinal absorption	Violation of Lipinski's rule
EGCG	Yes (log S = -3.56)	Low	2 (H-acceptor > 10; H-donor> 5)
Miricetin	Yes (log S = -3.01)	Low	1 (H-donor> 5)
Luteolin	Yes (log S = -3.71)	High	0
Tangeretin	Yes (log S = -4.11)	High	0

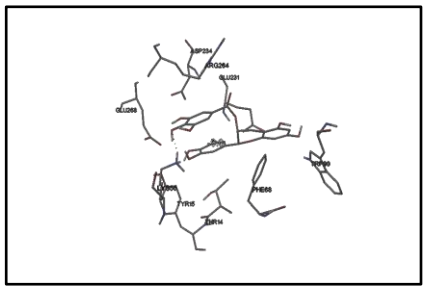
Quercetin	Yes (log S = -3.16)	High	0
Kaempferol	Yes (log S = -3.31)	High	0
Hesperidin	Yes (log S = -3.28)	Low	3 (molecular weight> 500; H-acceptor > 10; H-donor> 5)
Eriodictyol	Yes (log S = -3.26)	High	0
(-)-Epicatechin	Yes (log S = -2.22)	High	0
Taxifolin	Yes (log S = -2.66)	High	0
Genistein	Yes (log S = -3.72)	High	0

Based on the visualization of the docking results, it can be observed that all test ligands interact hydrophobically with 5-7 amino acid residues from the receptor (Table 3 and Figure 1).

Table 3: The optimal binding energies for each docking experiment and the interactions between the ligands and the receptor at the optimal interaction sites in docking.

Compound	**Structure-based docking**	**Site-specific docking**	
	Binding energy (kcal/mol)	**Binding energy (kcal/mol)**	**Chemical interaction with receptor**
EGCG	-9.1	-9.0	Hydrophobic interaction: Thr14, Tyr15, Phe68, Trp90, Glu231, Asp234, Arg264, Glu268 Hydrogen interaction: Lys56 (N−H···O)
Myricetin	-10.2	-10.2	Hydrophobic interaction: Tyr15, Thr37, Asp199, Gly201, Arg272, Glu339 Hydrogen interaction: Thr13 (O−H···O) Glu175 (N−H···O)
Luteolin	-8.8	-9.0	Hydrophobic interaction: Thr14, Tyr15, Thr37, Lys71, Glu175, Asp366 Hydrogen interaction: Thr13 (duaikatan N−H···O) Asp199 (O−H···O) Arg272 (N−H···O)
Tangeretin	-7.3	-7.4	Hydrophobic interaction: Thr14, Lys56, Val59, Arg72, Arg261, Arg264
Quercetin	-8.7	-8.7	Hydrophobic interaction: Tyr15, Thr37, Lys71, Asp199, Asp366 Hydrogen interaction: Thr13 (N−H···O dan O−H···O) Glu175 (O−H···O) Arg272 (N−H···O)
Kaempferol	-8.7	-8.7	Hydrophobic interaction: Tyr15, Thr37, Asp199, Asp366 Hydrogen interaction: Thr13 (N−H···O dan O−H···O) Arg272 (N−H···O)
Eriodictyol	-9.1	-9.0	Hydrophobic interaction:

			Thr13, Tyr15, Thr37, Glu175, Asp199, Gly202, Gly203 Hydrogen interaction: Arg272 (N−H···O)
(-)-Epicatechin	-10.0	-10.0	Hydrophobic interaction: Gly12, Tyr15, Thr37, Lys71, Glu175, Gly201, Gly202, Arg272, Asp366 Hydrogen interaction: Asp199 (O−H···O)
Taxifolin	-9.8	-9.8	Hydrophobic interaction: Thr13, Tyr15, Thr37, Glu175, Asp199, Val337, Val369 Hydrogen interaction: Thr14 (O−H···O) Arg272 (N−H···O)
Genistein	-9.0	-9.3	Hydrophobic interaction: Tyr15, Thr37, Glu175, Gly338, Gly339, Asp366, Val369 Hydrogen interaction: Asp199 (O−H···O) Gly201 (O−H···O) Arg272 (N−H···O)



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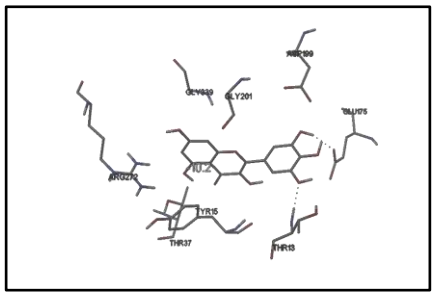

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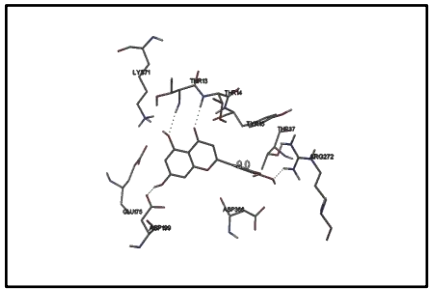

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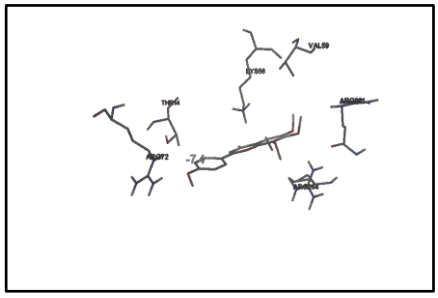

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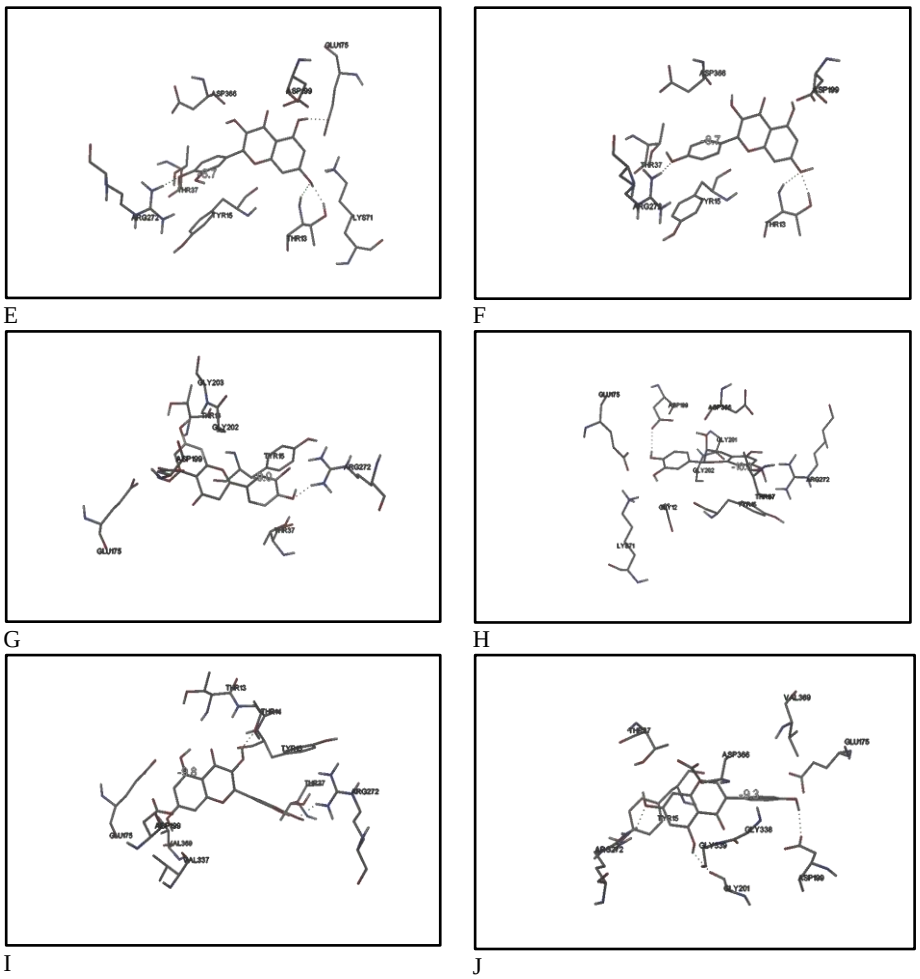

Figure 1: Visualization of molecular docking using AutoDockTools4: (A) EGCG, (B) myricetin, (C) luteolin, (D) tangeretin, (E) quercetin, (F) kaempferol, (G) hesperidin, (H) eriodictyol, (I) (-)-epicatechin, (J) taxifolin, and (K) genistein. Receptor amino acid residues indicate hydrophobic interactions, while dashed green lines depict hydrogen bonds.

DISCUSSION

Before being selected as candidates for the docking experiment, the ligands are checked for their similarity to drug molecules. SwissADME analysis shows all ligands are water-soluble (although tangeretin is moderately soluble). Three of the eleven ligands (EGCg, myricetin, and hesperidin) have low absorption through the digestive tract and violate one or more of Lipinski's rules (Table 1; complete SwissADME analysis results are shown in Figure S1). Therefore, these three molecules are less likely to be potent Hsp70 inhibitors. However, it's worth noting that EGCg and myricetin are still used in the docking experiment as positive controls due to their proven ability to inhibit Hsp70 activity. Hence, only hesperidin was not used in the experiment out of all the selected molecules.

In order to conduct docking, ligand coordinate files are first converted into PDB files. Prior to the docking process, the receptor file is modified in ADT by adding nonpolar hydrogen atoms to its carbon atoms. Subsequently, all files used in the docking are saved as PDBQT files. Once this stage is completed, the docking experiments are ready to proceed.

The first docking experiment aims to determine the optimal interaction sites between each ligand and the receptor (blind docking), covering the entire receptor for ligand binding. After identifying the optimal sites, docking is performed again for each ligand with a smaller search area centered on the ligand molecule. The lowest energy obtained from the second docking is the optimal binding energy between the ligand and the receptor.

Next, the optimal energies of each ligand are compared with the binding energy of the positive controls, EGCG, and myricetin. Ligands that can act as Hsp70 inhibitors are expected to have binding energies close to EGCG or myricetin, around -9.0 kcal/mol or -10.2 kcal/mol. This indicates that the ligand-receptor interaction is most stable at these energy levels, disrupting the protein's activity. According to the AutoDockVina docking results, seven of the

tested ligands (excluding the positive controls) have binding energies similar to the positive controls. (-)-Epicatechin exhibits the lowest energy among the eight tested ligands (-10.0 kcal/mol), followed by taxifolin (-9.8 kcal/mol). Tangeretin, on the other hand, has the highest energy and deviates significantly from the optimal energy of EGCG. It is evident that all flavonoids tested as potential drug candidates, except for tangeretin, have the potential to act as Hsp70 inhibitors.

The stability of the ligand-receptor binding can also be assessed by examining the interactions that occur at the binding site[18]. Stable ligand-receptor interactions are supported by hydrophobic and hydrogen bonding interactions[19]. Visualization of the docking results reveals that all tested ligands interact hydrophobically with 5-7 amino acid residues from the receptor. Except for tangeretin, these ligands also form at least one hydrogen bond, whether N−H•••O, O−H•••O, or both. From these results, it appears that within the ATPase domain of Hsp70, the amino acid residues most crucial for hydrophobic interactions with the ligands are Tyr15 and Thr37, which are involved in hydrophobic interactions with nine and eight ligands, respectively. Meanwhile, the O−H•••O bonds are mainly mediated by Asp199 and Thr13 (each interacting with three ligands), and N−H•••O bonds by Arg272 (interacting with six ligands). The increased number of interactions between ligands and the receptor tends to lower the binding energy, making the ligands more stable and potentially effective as inhibitors of the receptor[20, 21].

CONCLUSION

Our research demonstrated that flavonoids have considerable potential as drug candidates for cancer and other diseases due to their binding ability with Hsp70. The in silicoassay conducted in this study revealed that at least one molecule from each flavonoid class can bind to the human Hsp70 ATPase domain. This finding suggests that diverse flavonoids, spanning different classes, can be explored for their therapeutic properties as agents against dengue and cancer.

ACKNOWLEDGEMENT

We express our gratitude to the Institute for Research and Community Development UnikaAtma Jaya, the Directorate of Research and Development UI, and the Institute for Research and Community Service Unas for their unwavering support.

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