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Original Article

IN SILICO DOCKING OF QUERCETIN COMPOUND AGAINST THE HELA CELL LINE PROTEINS

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ABSTRACT

The present molecular docking study can be useful for the design and development of novel compound having better inhibitory activity against human cervical cancer cell line proteins. The docking scores were highest for cellular tumor antigen p53 with -4.52 kcal/mol with the stronger interaction followed by Caspase-3 (-4.09 kcal/mol.), Mucosal address in cell adhesion molecule 1(-4.0 kcal/mol) and the last score was found in the NF-kappaB (-2.83 kcal/mol) and the LogP, lower hydrogen bond counts, confirming the capability of the Quercetin for binding at the active site of the receptor. These potential drug candidates can further be validated in wet lab studies for its proper function.

Keywords: In silico docking, Quercetin, Auto Dock.

INTRODUCTION

Cancer is a major cause of death and the number of new cases, as well as the number of individuals living with cancer, is expanding continuously. Cervical cancer is one of the most common cancers among women worldwide; its mortality exemplifies health inequity, as its rates are higher in low & middle income countries [1], and in low socio-economic groups within countries [2]. Around 80% of global cervical cancer cases are in low & middle income countries [3]. The human papilloma virus (HPV) is the main causative agent for cervical cancer. The viral DNA from specific group of HPV can be detected in 90% of all cervical cancer [4]. High-risk HPV encode two major oncoproteins termed as E6 and E7, and the respective genes are the only viral genes that are generally retained and ex-pressed in cervical cancer tissues [5].

Flavonoids are polyphenolic compounds that occur in foods of plant origin. The average daily intake of the flavonoid subclasses of flavonols and flavones in the Netherlands is 23 mg (expressed as aglycones) of which quercetin supplies 16 mg [6]. Quercetin is an antioxidant in vitro because it can scavenge radicals, inhibit lipid peroxidation and chelate metals [7]. Quercetin was able to inhibit oxidation of LDL in vitro at a concentration as low as 0.25 µmol/L, which is in the physiological range [8-9]. Therefore quercetin might contribute to the prevention of cardiovascular disease [10]. However, to induce these health effects in humans, quercetin must enter the systemic circulation. Quercetin in foods is bound to sugars, mainly as β-glycosides, and the bioavailability of these various quercetin glycosides is affected by their sugar moiety [11-13]. Quercetin-3-rutinoside and quercetin-4′-glucoside are important forms of quercetin in foods. Quercetin-3-rutinoside accounts for ~40% of quercetin in black tea[14], and consumption of black tea contributes about 48% to the total flavonol and flavone intake in The Netherlands[6]. Quercetin-4′-glucoside accounts for ~ 45% of quercetin in onions [15], and consumption of onions contributes another 29% to the total flavonol and flavone intake [6]. Although intake of quercetin-3-rutinoside is twice that of quercetin-4′-glucoside, the absorption of quercetin-3-rutinoside is only 17% of ingested dose, whereas the absorption of quercetin-4′-glucoside is 52% of ingested dose [16]. Furthermore, the bioavailability of quercetin-3-rutinoside is only 20% of that of quercetin-4′-glucoside [13]. Therefore it would be interesting to attempt to increase the bioavailability of quercetin-3-rutinoside. Rutinose is a dimer of glucose and rhamnose; therefore quercetin-3-rutinoside can be transformed into quercetin-3-glucoside by splitting of the rhamnose molecule with the enzyme alpha-L-rhamnosidase [17-19]. The resulting quercetin-3-glucoside differs only from the highly bioavailable quercetin-4′-glucoside in the position of the glucose moiety on the quercetin aglycone. However, the bioavailability of quercetin-3-glucoside is unknown. Therefore we tested whether the position of the glucose moiety affected the bioavailability of quercetin glucosides in humans [20].

The objective of the study is to identify the proteins present in the Hela cell line, to analyze the domain and active sites, to assess the chemical and physical properties of the protein, to analyze the potentiality of the therapeutic agents in terms of their properties, to perform Docking of the proteins with a compound Quercetin and to evaluate the compound docking and active site binding.

MATERIALS AND METHODS

Preparation of protein structure

Protein structures of Hela cell line protein **were obtained from** RCSB Protein Data Bank (http: //www. pdb. org). All water molecules were removed and on the final stage hydrogen atoms were added to the target protein molecule.

Preparation of ligand structure

Quercetin compound used for docking study was selected from the literature [21]. ChemSketch, chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http: //www. acdlabs. com) was used to construct the structure of the ligands. Using draw mode of Chemsketch, the ligands were generated and three dimensional optimizations were done and then saved in. mol file and TORSDOF is used in calculating the change in free energy caused by the loss of torsional degrees of freedom upon binding. After al the above conditions are set the ligand is saved in "pdbq" format.

Preparation of macromolecule

The receptor file used by AutoDock must be in "pdbqs" format which is pdb plus 'q' charge and 's' solvation parameters: AtVol, the atomic fragmental volume, and AtSolPar, the atomic solvation parameter which are used to calculate the energy contributions of desolvation of the macromolecule by ligand binding.

Preparation of grid parameter file

The grid parameter file tells AutoGrid the types of maps to compute, the location and extent of those maps and specifies pair-wise potential energy parameters. In general, one map is calculated for each element in the ligand plus an electrostatics map. Self-consistent 12-6 Lennard- Jones energy parameters - Rij, equilibrium internuclear separation and epsij, energy well depth - are specified for each map based on types of atoms in the macromolecule. If you want to model hydrogen bonding, this is done by specifying 12-10 instead of 12-6 parameters in the "gpf" format.

Starting auto grid

Auto Grid (and AutoDock) must be run in the directories where the macromolecule, ligand and parameter files are to be found.

Preparation of docking parameter file

The docking parameter file tells AutoDock which map files to use, the ligand molecule to move, what its center and number of torsions are, where to start the ligand, which docking algorithm to use and how many runs to do. It usually has the file extension, ". dpf". Four different docking algorithms are currently available in AutoDock: SA, the original Monte Carlo simulated annealing; GA, a traditional Darwinian genetic algorithm; LS, local search; and GA-LS, which is a hybrid genetic algorithm with local search. The GA-LS is also known as a Larmarckian genetic algorithm, or LGA, because children are allowed to inherit the local search adaptations of their parents.

Starting auto dock

Auto Grid and AutoDock must be run in the directories where the macromolecule, ligand, gpf and dpf files are to be found.

Analyzing the docking results

The key results in a docking log are the docked structures found at the end of each run, the energies of these docked structures and their similarities to each other.

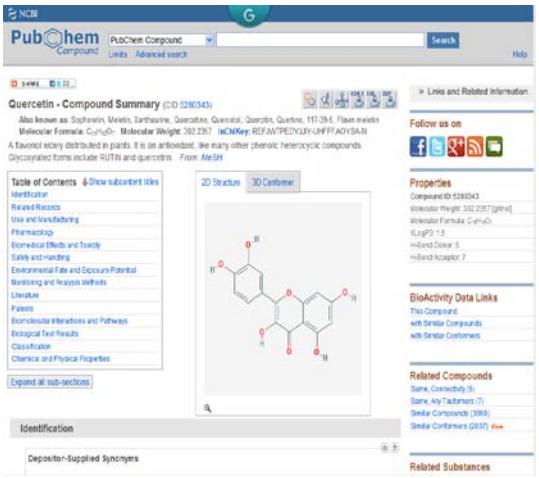

Fig. 1: Pub chem image of Quercetin

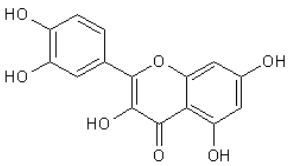

Fig. 2: 2D Structure of Quercetin

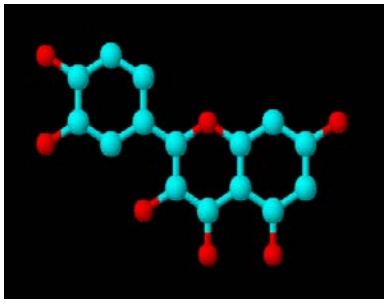

Fig. 3: 3D Structure of Quercetin

The similarity of docked structures is measured by computing the root-mean-square-deviation, rmsd, between the coordinates of the atoms. The docking results consist of the PDBQ of the Cartesian coordinates of the atoms in the docked molecule, along with the state variables that describe this docked conformation and position.

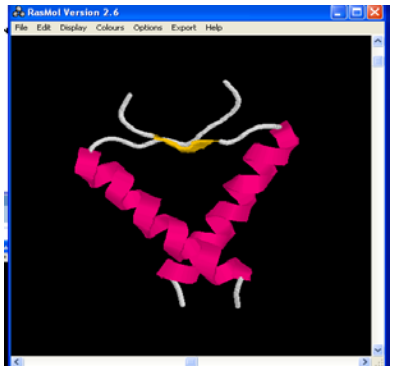

Fig. 4: Active site residues of Cellular tumor antigen p53

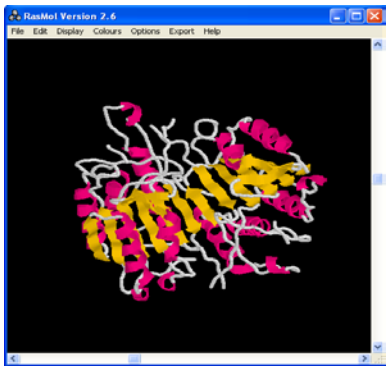

Fig. 5: Active site residues of Caspase-3

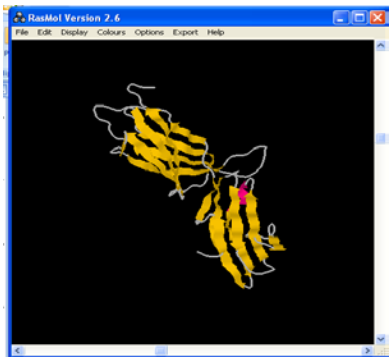

Fig. 6: Active site residues of mucosal addressin cell adhesion molecule 1

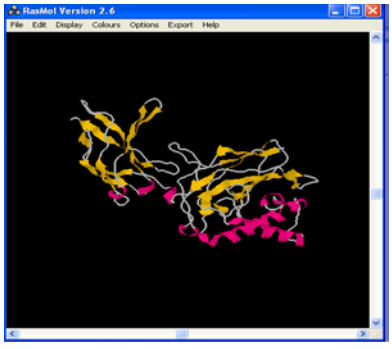

Fig. 7: Active site residues of NF-kappa-B

Table 1: Quercetin compound details

Name of the compound	Alternative name	Molecular weight	Molecular formula	LogP3	H-Bond Donor	H-Bond Acceptor	Description
Quercetin	Sophoretin, Meletin, Xanthaurine, Quercetine, Quercetol, Quercitin, Quertine	302.2357 g/mol	$C_{15}H_{10}O_7$	1.5	5	7	A flavonol widely distributed in plants. It is an antioxidant, like many other phenolic heterocyclic compounds. Glycosylated forms include rutin and quercetrin.

Table 2: Docking Score and Number of Hydrogen Bonds formed between the proteins and Quercetin compound

S. No.	Proteins	Quercetin	
		Docking score (KCal/mol)	H-BOND
1	Cellular tumor antigen p53	-4.52	3
2	Caspase-3	-4.09	5
3	Mucosal addressin cell adhesion molecule 1	-4.0	2
4	Nuclear factor NF-kappa-B p105 subunitS	-2.83	1

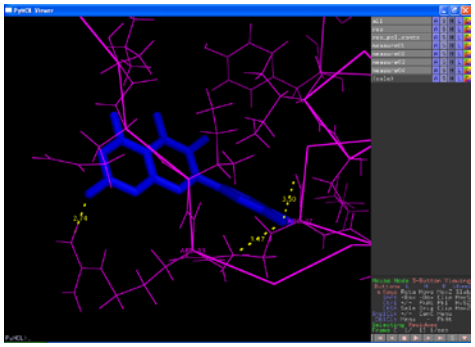

Fig. 8: Crucial Interaction between Quercetin (blue) and Cellular tumor antigen p53 (rose)

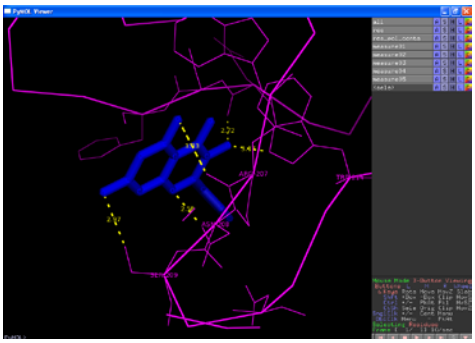

Fig. 9: Crucial Interaction between Quercetin (blue) and Caspase 3 (rose)

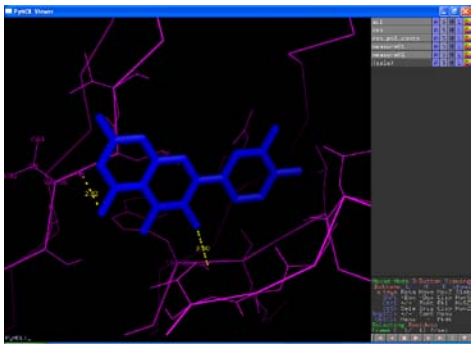

Fig. 10: Crucial Interaction between Quercetin (blue) and Mucosal address in cell adhesion molecule 1 (rose)

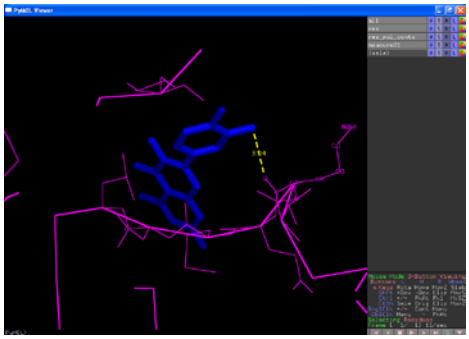

Fig. 11: Crucial Interaction between Quercetin (blue) and NF kappa-B (rose)

RESULTS AND DISCUSSION

In the present study, to understand the interactions between the ligands and Hela cell line proteins (Cellular tumor antigen p53, Caspase-3, Mucosal address in cell adhesion molecule 1 and Nuclear factor NF-kappa-B) and to explore their binding mode, docking study was performed using Auto Dock

Hela cell line protein structures were derived from PDB and used as a target for docking simulation. The compound selected from the literature was listed in the table 1. Ligands were created and prepared for the docking procedure using ChemSketch. The structures of the ligands obtained from the ChemSketch were shown in the figure1, 2and 3.

Binding site of the protein

The detection of ligand-binding sites is often the starting point for protein function identification and drug discovery. In our study, Q-site Finder predicted active site of the Hela cell line proteins (Cellular tumor antigen p53, Caspase-3, Mucosal addressin cell adhesion molecule 1 and NF-kappa-B) with a higher average precision as showed in the Fig. 4, 5,6 and 7.

The active sites of Hela cell protein comprises of amino acid residues are as follows:

Cellular tumor antigen p53

LEU30,GLN31,ILE32,ARG37,PHE38,GLU39,LYS40,ILE41,ARG42,TYR 44,ASN45.

Caspase-3

ARG64,SER120,HIS121,GLY122,GLN161,ALA162,CYS163,SER205,TR P206,ARG207,ASN208,SER209,TRP214,MET222,GLN225,TYR226,A RG238,ARG241,LYS242,THR245,GLU246,PHE247,GLU248,SER249,P HE250,SER251,PHE256. s

Mucosal addressin cell adhesion molecule 1

LEU17,GLY18,TRP38,ARG39,GLY40,LEU41,ASP42,LEU45,GLY46,ALA47,VAL48,LEU57,ASN61,ALA62,SER63,THR69,ARG70,PHE127,SER128,LEU129,LEU130,GLU135,LEU136,GLU137,GLY138,ALA139,ALA141,ASP156,GLU157,ASP158,TRP167.

NF- kappa B

THR102,ASN103,GLY104,LYS105,ASN106,HIS108,LEU109,HIS110,LEU154,GLN204,LYS206,THR205,GLU207,MET208,ASP209,VAL212,VAL213.

As most of the amino acid residues present in the Hela cell line proteins hydrophobic so they are the main contributors to the receptor-ligand interaction.

Interaction studies

The goal of ligand-protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structure [22].

To study the binding mode of Quercetin compound in the binding site of Hela cell line protein**,** intermolecular flexible docking simulations were performed and.

Energy values were calculated from the docked conformations of the Hela cell protein-inhibitor complexes. Docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein. Several potential inhibitors have been identified through the docking simulation. The binding affinity of the Hela cell line proteins with the Quercetin compound were measured by kcal/mol.

The docking scores were highest for Cellular tumor antigen p53 with -4.52 kcal/mol with the stronger interaction followed by Caspase-3 (-4.09 kcal/mol.), Mucosal address in cell adhesion molecule 1(-4.0 kcal/mol) and the least score was found in the NF-kappa-B (-2.83 kcal/mol) as showed in the table 2 and Fig. 8, 9, 10 and 11

Analysis of ligand binding interaction with the Hela cell line protein can be useful for new preventive and therapeutic drug for cancer. The results obtained from this study would be useful in both understanding the inhibitory mode as well as in rapidly and accurately predicting the activities of new inhibitors on the basis of docking scores.

CONCLUSION

In this study, the molecular docking was applied to explore the binding mechanism and to correlate its docking score with the activity of Quercetin compound. The results of our present study can be useful for the design and development of novel compound having better inhibitory activity against several type of cancer. These potential drug candidates can further be validated in wet lab studies for its proper function.

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