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Original Article

STUDY ON DIOSMETIN DERIVATIVES AS THE INHIBITORS OF FIMH OF UROPATHOGENIC *ESCHERICHIA COLI* BY MOLECULAR DOCKING WITH HEX

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ABSTRACT

The uropathogenic *E. coli* (UPEC) is one of the causative agents of urinary tract infections (UTI). Like any other bacterial diseases, the UTI by UPEC is treated by antibiotics since many years. However the increase in antibiotic resistance among UPEC has created a great concern in the treatment of UTI. Hence we are in need of new and effective anti-microbial strategies. One such strategy is inhibition of virulence factor of bacteria and thereby reduction of its virulence. The important prerequisite of the UPEC's pathogenecity is its adhesion and colonisation. FimH in the type 1 pili is responsible for the adherence of bacteria with the host cell. Currently the FimH antoganists are tried as new therapeutics for the treatment and prevention of urinary tract infections. The three dimensional crystal structure of FimH was obtained from RCSB database. Its PDB code is 4AUU. The polyphenol diosmetin present in the tea is selected for the present study. The derivatives of the diosmetin were prepared in ACD chemsketch software. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0. The selected ligands were then analyzed for drug- relevant properties based on "Lipinski's rule of five" and other drug like properties using ACD/iLab web portal. The protein – ligand docking was performed by Hex version 8.0.0. Four disometin derivatives that have shown excellent drug properties against UPEC. These drug candidates have been validated through all *insilico* methods and have been proved to have excellent inhibitory effect against FimH of UPEC with good drug like properties.

Key words: Uropathogenic *Escherichia coli*, FimH, diosmetin derivatives, molecular docking.

INTRODUCTION

Escherichia coli is one of the important bacterial species that is responsible for many serious infections. The uropathogenic *E. coli* (UPEC) is one such bacterium, which is one of the causative agents of urinary tract infections (UTI). Like any other bacterial diseases, the UTI by UPEC is treated by antibiotics since many years. However the increase in antibiotic resistance among UPEC has created a great concern in the treatment of UTI. Hence we are in

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need of new and effective anti-microbial strategies. One such strategy is inhibition of virulence factor of bacteria and thereby reduction of its virulence.

In general the bacterial pathogenesis is multi-factorial process that is medicated by various virulence factors which helps bacteria to cause various diseases. The important prerequisite of the UPEC's pathogenecity is its adhesion and colonisation[1,2]. The adhesion to the host cell is done by a hair like projection called as fimbriae. It was found that it is type 1 fimbriae, which are 1 to 2 um long and less than 7 nm wide[3,4]. It is a well established fact that type 1 fimbriae are expressed in all uropathogenic strains of E. coli and is the main virulence factor of the bacteria. These type 1 fimbriae helps the bacteria to attach in specific regions of urinary tract, thus causing urinary tract infection[5]. The type 1 fimbriae is one of the well characterised adhesion molecule[6-8]. The type 1 fimbriae is a linear string of repeating, non-covalently linked, immunoglobulin like FimA subunits wound into a rigid right handed helical rod, followed by a short and stubby tip fibrillum composed of the FimF and FimG adaptor subunits and the FimH adhesion[9].

FimH is a two-domain adhesin protein at the end of the tip fibrillum, responsible for the mannose-sensitive bacterial adhesion[10, 11]. The primary receptor for FimH in the urinary tract is the glycoprotein uroplakin Ia that is abundantly present on differentiated uroepithelial cells[12, 13]. However Fim H binds with a wide range of glycoproteins carrying one or more N-linked high-mannose structures[14].

The blocking of the FimH receptor interaction has been shown to prevent bacterial adhesion to the bladder uroepithelium and therby the infection[15-18]. However, relatively recently, it has been found that FimH is a lectin that can function according to a catch bond mechanism[19]. Tensile forces, flow, or shear force, respectively, induce an allosteric switch, that also involves the carbohydrate-binding site, which is rearranged to a conformation, which binds D-mannosides more strongly[20]. Thus, FimH can be considered as an especially intriguing lectin, with the potential to structurally rearrange its carbohydrate-binding site. Currently the FimH antoganists are tried as new therapeutics for the treatment and prevention of urinary tract infections[21].

The use of plant and plant product in the treatment of UTI is not much observed in the literature. However Cranberry (*Vaccinium macrocarpon*) fruits and leaves have a long history of traditional use in folk medicine for the prevention and treatment of urinary tract infections[22]. Cranberry juice irreversibly inhibits p-fimbriae preventing attachment of to the uroepithelium[23]. However cranberry is not the native of India. There are many medicinal plants in India that are traditionally used as the treatment for UTI[24]. However there is no information regarding its anti adhesion properties in the literature.

The bioinformatics tool has been utilised to find out effective inhibitors that can inhibit FimH of UPEC. The molecular docking is the technique that gives the best fit between the protein and the ligand molecules. This technique has been widely used to find inhibitors for various virulence factors of bacteria and other microbes. In the present study molecular docking technique has been utilised to find inhibitors from various derivates of diosmetin, a polyphenol present in various medicinal plants.

MATERIALS AND METHODS

Protein preparation

The three dimensional crystal structure of FimH was obtained from RCSB database (http://www.rcsb.org/pdb/explore/explore.do?structureId=4AUU)[25]. Its PDB code is 4AUU. Using Pymol software the water molecules were removed and the hydrogen atoms were added to the protein.

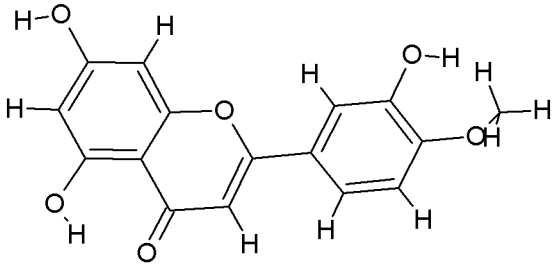

Figure 1: Diosmetin

Generation and optimization of Ligand

The polyphenol diosmetin present in the tea is selected for the present study. Its structure (Figure 1) in SDF format was obtained from Pubchem database. Its IUPAC name is 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-chromen-4-one and molecular weight is 300.36. The structure was converted to MDL format in Open Babel software. The derivatives of the diosmetin were prepared in ACD chemsketch software[26]. ACD/ChemSketch is the powerful all-purpose chemical drawing and graphics package from ACD/Labs developed to draw the desired molecules and to store it in various desired formats. It also helps to generate IUPAC names and to calculate certain chemical properties of the chemicals. All the prepared compounds were saved in MDL format. Finally all the compounds were converted to PDB format by Open Babel software.

Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0[27]. A population size of 150 is set with 70 generation and one solution for quick docking. The ligands with low binding energy were selected for the further study. The selected ligands were then analyzed for drug- relevant properties based on "Lipinski's rule of five" and other drug like properties using ACD/iLab web portal.

Lipinski Rule of 5 (Ro5), also known as Lipinski Alert Index, is a filter that identifies compounds with low probability of useful oral activity because of poor absorption or permeation[28, 29].

In the discovery setting the Lipinski rule of 5 predicts that poor absorption or permeation is more likely when:

- there are more than 5 H-bond donors (nHDon)
- there are more than 10 H-bond acceptors (N + O)
- molecular weight (MW) is over 500
- Moriguchi's logP (MLogP) is over 4.15

Other important drug like properties that were checked was lead like scores and Ghose filter. Lead-like Scores (LLS) are defined as the ratio between the numbers of satisfied conditions over the total number of conditions.

Eight drug-like indices were proposed by Ghose-Viswanadhan-Wendoloski[30] in order to help to streamline the design of combinatorial chemistry libraries for drug design and develop guidelines for prioritizing large sets of compounds for biological testing. They are based on a consensus definition and have been derived from analysis

of the distribution of some physicochemical properties (logP, molar refractivity, molecular weight, and number of atoms) and chemical constitutions of known drug molecules available in the Comprehensive Medicinal Chemistry (CMC) database and seven drug classes defined by disease state.

Figure 2: The 3D structure of FimH protein viewed with Rasmol structure colour scheme

Protein – Ligand docking

The protein – ligand docking was performed by Hex version 8.0.0. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate Protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes[31]. It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result[32].

The parameters used for the docking process were:

1. Correlation type – Shape + Electrostatics
2. FFT Mode - 3D
3. Post Processing- MM Energies
4. Grid Dimension - 0.6
5. Receptor range – 180
6. Ligand range – 180
7. Twist range – 360
8. Distance Range – 40

RESULTS AND DISCUSSION

Protein preparation

The 3D structure of FimH protein is shown Figure 2. Alpha helices are coloured magenta, beta sheets are coloured yellow, turns are coloured pale blue, and all other residues are coloured white. It is the mannose binding domain consisting of 300 aminoacids. The aminoacids 1 to 20 acts as signal peptide and 21 to 300 contributes the binding domain. Its primary structure in FASTA format is given in Figure 2.

>tr|C9QSZ7|C9QSZ7_ECOD1 FimH mannose-binding domain protein OS=Escherichia coli (strain ATCC 33849 / DSM 4235 / NCIB 12045 / K12 / DH1) GN=fimH PE=4 SV=1
MKRVITLFAVLLMGWSVNAWSFACKTANGTAIPIGG
GSANVYVNLAPVVNVGQNLVVDLSTQIFCHNDYP
ETITDYVTLQRGSAYGGVLSNFSGTVKYSGSSYPFPT
TSETPRVVYNSRTDKPWPVALYLTPVSSAGGVAIKA
GSLIAVLILRQTNNYNSDDFQFVWNIYANNDVVVP
TGGCDVSARDVTVTLPDYPGSVPIPLTVYCAKSQNL
GYYLSGTTADAGNSIFTNTASFSPAQGVGVQLTRN
GTIIPANNTVSLGAVGTSAVSLGLTANYARTGGQVT
AGNVQSIIGVTFVYQ

Figure 2: Primary structure of mannose binding domain of FimH protein Screening and selection of ligands

Table 1: The results of iGEMDOCK showing binding energies of the four diosmetin derivatives.

S.No.	Ligand	Total binding Energy (kcal/mol)	Vanderwaals force	H Bond
1.	2-(4-ethoxy-3-hydroxyphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	-139.042	-109.212	-29.8295
2.	2-(4-ethoxy-5-hydroxy-2,3-dimethylphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	-132.306	-105.098	-27.2078
3.	3,5-dihydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	-120.684	-110.73	-9.95413
4.	5-hydroperoxy-3-hydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	-118.323	-107.387	-10.9984

Two hundred derivatives were prepared from diosmetin using ACD chemsketch software. They were converted to pdb format using OPEN BABEL software. On virtual rapid screening of disometin derivatives with iGEMDOCK software, four compounds were found to have good fit with a low binding energy. The Table 1 shows the energy values of the four compounds generated from diosmetin.

The Table 2 shows the Lipinski's rule of five and Table 3 and 4 shows other drug likeness properties and general chemical properties of the selected three compounds. From the tables it is evident that all the four derivatives have good drug like properties.

Table 2: Lipinski's rule of five for diosmetin derivatives

S. No.	Ligand	Molecular weight	Xlog p	H bond donor	H bond acceptor
1.	2-(4-ethoxy-3-hydroxyphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	388.37	2.14	2	8
2.	2-(4-ethoxy-5-hydroxy-2,3-dimethylphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	416.2	3.17	2	8
3.	3,5-dihydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	418.39	2.70	4	9
4.	5-hydroperoxy-3-hydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	434.39	1.91	4	10

Table 3: Important drug like properties of diosmetin derivatives.

S. No.	Ligand	Bioavailability	Ghose filter	Lead likeness
1.	2-(4-ethoxy-3-hydroxyphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	Yes	Yes	Yes
2.	2-(4-ethoxy-5-hydroxy-2,3-dimethylphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	Yes	Yes	Yes
3.	3,5-dihydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	Yes	Yes	No
4.	5-hydroperoxy-3-hydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	Yes	Yes	No

Table 4: Basic chemical properties of diosmetin derivatives

S. No.	Ligand	Chemical formula	Composition	Rotatable bond count
1.	2-(4-ethoxy-3-hydroxyphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	$C_{20}H_{20}O_8$	C (61.85%), H (5.19%), O (32.96%)	6
2.	2-(4-ethoxy-5-hydroxy-2,3-dimethylphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	$C_{22}H_{24}O_8$	C (63.45%), H (5.81%), O (30.74%)	6
3.	3,5-dihydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	$C_{21}H_{22}O_9$	C (60.28%), H (5.3%), O (34.42%)	5
4.	5-hydroperoxy-3-hydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	$C_{21}H_{22}O_{10}$	C (58.06%), H (5.1%), O (36.83%)	6

Docking with Hex

All the selected ligands were then subjected to accurate docking with Hex version 8.0.0 to analyse its binding energies.

The Table 5 shows the Energy values of the diosmetin derivatives. From the table it is clear that all the derivatives show high energy values. The Figure 3 displays the docking pose of the four diosmetin derivatives.

Table 5: E-values of diosmetin derivatives obtained by docking in Hex.

S.No.	Ligand	E-value
	2-(4-ethoxy-3-hydroxyphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	-271.65
2.	2-(4-ethoxy-5-hydroxy-2,3-dimethylphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one	-278.53
3.	3,5-dihydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	-286.61
4.	5-hydroperoxy-3-hydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one	-284.21

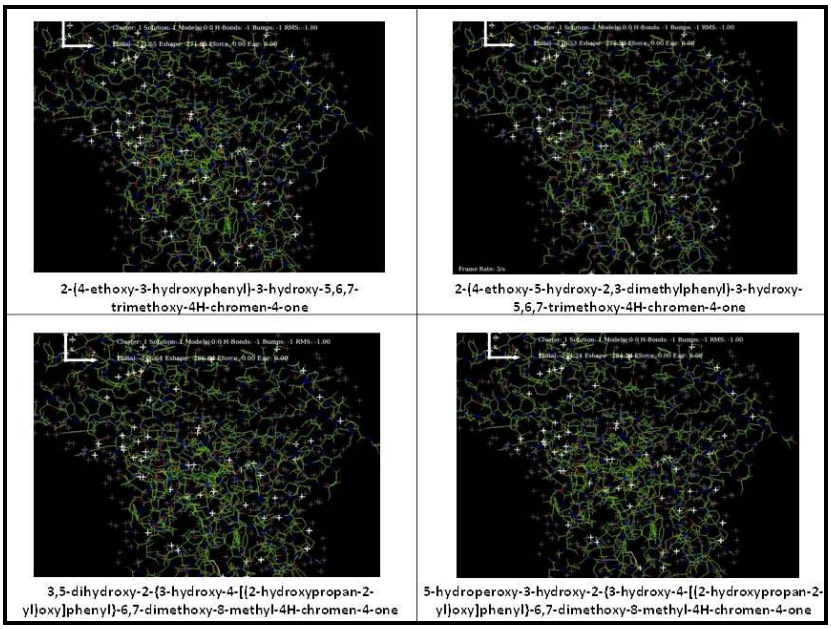

Figure 3: Docking pose of diosmetin derivatives in Hex.

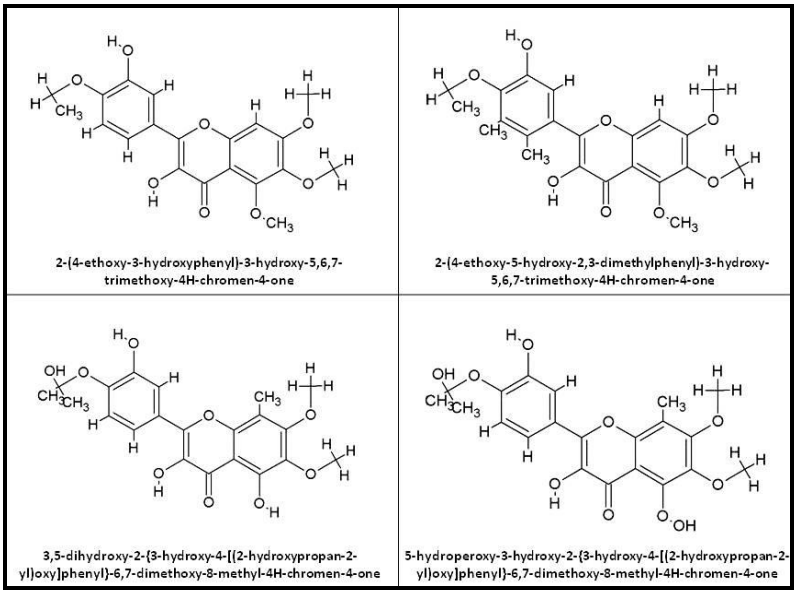

Figure 4: The drug candidates for the inhibition of FimH of UPEC

The Figure 4 shows the four diosmetin derivatives that have shown excellent drug properties against UPEC. These drug candidates have been validated through all *insilico* methods and have been proved to have excellent inhibitory effect against FimH of UPEC with good drug like properties.

CONCLUSION

From the present study, by *in silico* molecular docking, four possible FimH inhibitors have been derived. They are 2-(4-ethoxy-3-hydroxyphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one, 2-(4-ethoxy-5-hydroxy-2,3-dimethylphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-chromen-4-one, 3,5-dihydroxy-2-{3-hydroxy-4-[(2-

hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one and 5-hydroperoxy-3-hydroxy-2-{3-hydroxy-4-[(2-hydroxypropan-2-yl)oxy]phenyl}-6,7-dimethoxy-8-methyl-4H-chromen-4-one. These inhibitors can be excellent drug candidate in the treatment of the cases of UTI. These drug candidates are anti-bacterial agents, simply act as anti-virulent agent of UPEC. Hence they are target specific unlike the antibiotics and can be active against all UPEC that have type I pili.

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