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In-silico SCREENING OF FLAVONOIDS AS AgrA INHIBITORS BY MOLECULAR DOCKING, NORMAL MODE ANALYSIS, AND ADMET STUDY

Hriday Kumar Basak, Uttam Paswan, Sujoy Karmakar and Abhik Chatterjee✉

Design, Synthesis and Simulation Laboratory, Department of Chemistry, Raiganj University, Raiganj -733134, West Bengal, India.

✉Corresponding Author: chemistryabhik@gmail.com

ABSTRACT

Excessive and prolonged intake of antibiotics results in the emergence of antibiotic-resistant pathogenic bacterial strains. As a consequence, managing bacterial infections with currently available antibiotics is extremely challenging. In order to tackle the bacterial resistance, bacterial communication mechanism, especially bacterial quorum sensing (QS) is targeted. In *agr* QS system, the various gene expression involved in the secretion of virulence factors can be attenuated by inhibiting the DNA-AgrA interactions. Here, we docked twenty flavonoids with previously homology modeled AgrA proteins together with PDB ID: 4G4K (template protein of the homology models) using the AutoDock tool. Out of twenty flavonoids, three flavonoids (dracorhodin, acacetin, and oroxylin A) exhibited promising binding free energies (<-9.00 kcal/mol). We also performed a normal mode analysis to check the stability of docked complexes. The best-docked complexes are stable as indicated by normal mode analyses. The best three flavonoids (dracorhodin, acacetin, and oroxylin A) possess desirable drug-likeness attributes. Hence, these compounds can act as novel AgrA inhibitors

Keywords: AgrA Protein, Bacterial Resistance, Docking, Normal Mode, Quorum Sensing

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INTRODUCTION

Antibiotics, since their discovery, are in use for the treatment of bacterial infections by inhibiting the growth of the bacteria.[1] Prolonged and uncontrolled use of antibiotics has accelerated the emergence of antimicrobial-resistant bacteria, resulting in troubles for the management of bacterial infections.[2] In order to overcome the issue of bacterial resistance, the bacterial communication mechanism is targeted.[3] Quorum sensing (QS) is a bacterial signaling mechanism that is targeted for the development of antibacterial drugs.[4] QS controls a number of biological processes including virulence factor production and biofilm formation.[5] *agr* QS system found in many Gram-positive bacteria is responsible for various virulence factor production. In *agr* QS system, AgrA which is a response regulator binds with the DNA through its LytTR domain (from amino acid residue138 to 238).[6] This LytTR domain is widespread among bacteria but absent in humans and other higher organisms.[7] The expression of various virulence genes in *agr* QS system is controlled by the LytTR domain containing transcriptional regulators.[8] This LytTR domain is a well-known therapeutic target for novel antibacterial drug research. It is evident that the LytTR domain of AgrA protein when binds with DNA, a conformational change takes place at the residues 168, 170, 183, 187, 202, 218, and 234 which interact with the backbone of the DNA and also at the residues 169, 201 and 233 which involved direct base-specific interactions with DNA.[9,10] Thus blocking the LytTR domain with small molecules could serve as an effective way of combating bacterial infections. In recent times, natural sources have attracted more attention for the development of drugs as they are less toxic, better bioavailable, biodegradable, and have therapeutic applications. The plant kingdom is in use as an important source of medicines for infectious diseases since the ancient period.[11] In that context, flavonoids, a class of secondary metabolites of a wide variety of plants, have been found to inhibit biofilm formation and the virulence factors production in some pathogens by disrupting the QS mechanisms.[12] To develop better anti-QS drugs, 3D structural information of the LytTR domain is

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necessary, so we modeled LytTR domains of different pathogenic bacteria in our earlier study.[13] Our aim of this investigation is to find out potential inhibitors for the template protein (PDB ID: 4G4K) and the modeled proteins from selected natural flavonoids. Twenty natural flavonoids (Table-1) having QS inhibiting activity [14] were docked with the modeled proteins along with the template protein to study drug-receptor interaction using the AutoDock program and also perform the normal mode analyses (NMA) to investigate the firmness of the docked adducts. Drug-likeness of the flavonoids was assessed by performing ADMET properties calculations.

EXPERIMENTAL

Receptors and Ligands Selection

PDB of the template protein (4G4K) used for homology modeling was retrieved from Protein Data Bank powered by RCSB.[15-17] Homology modeled 3D structures of LytTR domains of five pathogenic bacteria, *Chlamydia trachomatis, Streptococcus pyogenes*, *Enterococcus faecalis*, *Listeria monocytogenes,* and *Macrococcus canis* obtained in our earlier investigation were used in this study.[13] From the literature, twenty natural flavonoids (Table-1) having QS inhibitory potential were selected.[14] ChemSketch (http://www.acdlabs.com/resources/freeware/chemsketch/) was used to draw the two-dimensional structures of these flavonoids. Geometry optimization of these compounds was performed using MOPAC2016 (http://openmopac.net/). Optimized geometries of these compounds were then used in molecular docking studies.

Molecular Docking Simulations

AutoDock 4.2 was used to dock the flavonoids with the AgrA proteins. All the proteins were prepared by checking, repairing missing atoms, and adding all hydrogen with the aid of the AutoDock Tools (ADT) which is a freely accessible graphical user interface (GUI) for the AutoDock 4.2.[18] ADT was used to create a grid box that enclosed the DNA interacting residues (residues 138-238) and the grid dimension was X= 82, Y= 72, and Z= 88 (x, y, and z) with 0.375 Å grid separation and the grid center was specified at X= 32.489, Y= 30.909, and Z= 39.574. The rigid-Docking approach (both the ligand and receptor are rigid) was used in the docking simulation and the ligand conformations were optimized using Lamarckian genetic algorithm 4.2.

Normal Mode Analysis

iMods online server (http://imods.chaconlab.org/) was used to analyze the normal mode of the protein-ligand complexes.[19] From the output, different parameters like deformability, B-factor, etc. were obtained.

Drug Likeness and ADMET Properties

The drug-likeness of the compounds was analyzed on the SwissADME website (http://swissadme.ch) to calculate different ADMET properties including the number of Lipinski's rule violations.[20]

RESULTS AND DISCUSSION

Docking Simulation

In computer-aided drug design processes, the molecular docking tool is widely used. The LytTR domain of AgrA protein binds with DNA and the transcription factors with it regulate the expression of virulence genes, hence, this domain is a potential target for the identification of potential inhibitors against *agr* QS system. Molecular docking studies of the flavonoids under study were performed with LytTR domain of five modeled AgrA proteins associated with the bacterial quorum sensing(QS) of five pathogens, *S. pyogenes*, *L. monocytogenes*, *E. faecalis*, *C. trachomatis* and *M. canis*, and also with their template protein (Protein Data Bank ID: 4G4K) of *S. aureus*. Binding free energies are shown in Table-1.

Among the twenty inhibitors, dracorhodin is the best inhibitor for the AgrA protein of *C. trachomatis* (B.E = -9.79 kcal/mol). A close analysis of the docked complex of dracorhodin and AgrA protein of *C. trachomatis* reveals that the major interactions are characterized by two hydrogen bonds: O atom of OCH_3 group of dracorhodin formed a H-bond with the -OH group of the residue Thr164 (H-bond distance, 2.13 Å), and another H-bond between the O atom of C=O group of dracorhodin and NH group of Ser187 which is a hotspot residue for DNA binding at a distance of 2.28 Å (Fig.-1A). Docking results

showed that out of twenty flavonoids, acacetin binds most effectively with the AgrA protein of *E. faecalis* (B.E = -9.66 kcal/mol). Such a low binding free energy can be explained by a strong H-bond formed between the hydroxylic O atom of the ligand and the hydroxylic O atom of the COOH group of the residue Glu163 with a distance of 1.77 Å (Fig.-1B). Docking results reveal that dracorhodin is the best ligand among the ligands under study for the AgrA protein of *L. monocytogenes* (B.E = -9.87 kcal/mol). In this docked complex, a hydrogen bond is found to form between the O atom of the C=O group of dracorhodin and the NH_2 group of Arg198 with a distance of 3.03 Å (Fig.-1C). Among the twenty flavonoids, dracorhodin is the best inhibitor for *M. canis* (B.E = -9.44 kcal/mol).

Table-1: Binding Free Energy (kcal/mol) of the flavonoids with AgrA Proteins

Molecules	*C. trachomatis*	*E. faecalis*	*L. monocytogenes*	*M. canis*	*S. pyogenes*	Template (4G4K)
Acacetin	-8.21	-9.66	-9.2	-8.81	-7.75	-7.45
Apigenin	-8.11	-7.6	-8.87	-8.57	-7.66	-7.7
Apigenin-7-O-glucoside	-6.81	-6.71	-6.74	-7.2	-7.04	-7.55
Catechin 7-xyloside	-6.27	-4.93	-6.29	-5.49	-6.53	-5.82
Diosmetin	-7.68	-8.35	-7.61	-8.28	-7.49	-7.34
Dracorhodin	-9.79	-9.43	-9.87	-9.44	-9.88	-9.38
Epicatechin-3-gallate	-8.00	-7.36	-8.51	-7.53	-8.85	-7.96
Epigallocatechin	-6.2	-5.62	-7.15	-6.43	-6.13	-6.03
Epigallocatechingallate	-7.25	-5.51	-8.3	-6.86	-8.07	-7.6
Farrerol	-8.16	-8.06	-8.26	-7.79	-7.72	-7.8
Isorhamnetin	-6.65	-7.03	-7.87	-6.78	-6.96	-6.57
Isovitexin	-8.49	-6.73	-7.08	-7.28	-7.47	-7.33
Kaempferol	-7.38	-6.85	-8.11	-7.67	-7.65	-7.04
Kaempferol-7-O-glucoside	-6.69	-6.16	-6.42	-6.13	-6.5	-6.92
Lysionotin	-7.36	-6.99	-7.94	-8.69	-7.21	-7.19
Morin	-7.49	-6.87	-8.12	-6.54	-6.99	-7.23
myricetin	-6.86	-7.05	-6.07	-6.52	-6.55	-6.5
Oroxylin A	-9.12	-8.29	-8.62	-8.83	-9.06	-8.64
Quercetin	-7.6	-6.90	-7.3	-6.55	-6.81	-6.53
Silibinin	-8.17	-8.05	-8.17	-8.37	-8.32	-8.77

A close analysis of the docked complex of dracorhodin and AgrA protein of *M. canis* reveals that the ligand forms two hydrogen bonds: a hydrogen bond between the O atom of the C=O of the ligand and NH_2 group of Lys187 with a distance of 3.00 Å and another H-bond between the O atom (OCH_3 group) of the ligand and NH_2 group of Lys237 with a distance of 2.47 Å. A π- π T shaped interaction between the π electron of the ligand and π electron of the His200 at a distance of 4.26 Å is found to exist in this complex (Fig.-1D). Docking results reveal that dracorhodin is the best ligand among all the ligands under study for the AgrA protein of *S. pyogenes* (B.E = -9.88 kcal/mol). In the docked complex, a hydrogen bond is found to form between the O atom of the C=O group of dracorhodin and -NH group of Ser187 with a distance of 2.24 Å (Fig.-1E). Among the twenty flavonoids, dracorhodin is the best inhibitor for the template protein of *S. aureus* (B.E = -9.38 kcal/mol). Dracorhodin on docking with the template protein makes a π-donor interaction with Gly184, a π-lone pair interaction with His169, a π-sigma interaction with Leu171, and three π-alkyl interactions withArg170, Leu171, and Leu186 respectively (Fig.-1F). From the above discussion, it is clear that ligands interact with the DNA binding hotspot residues, so they may possess the *agr* QS inhibiting activity.

Normal Mode Analysis

The stability and mobility of the docked complexes were studied by analyzing the normal mode. The deformability graph of the best complex of all the proteins is illustrated in Fig.-2A. The peaks in the deformability graph curve suggest the amino acids in the receptor with deformability. From the

deformability curve, it is clear that *E. faecalis*- acacetin, *L. monocytogenes*-dracorhodin, *M.* canis-dracorhodin, 4G4K- dracorhodin complexes showed a larger amount of deformability compared to *C. trachomatis*- dracorhodin and *S. pyogenes*- dracorhodin. B-factor plots showed the comparative insight of normal mode analysis of the docked complexes with respect to the PDB field. In the B-factor test, *C. trachomatis*- dracorhodin, *L. monocytogenes*-dracorhodin, and *S. pyogenes*- dracorhodincomplexes showed the better result among the complexes as these showed smaller differences between the normal mode analysis and PDB fields (Fig.-2B).

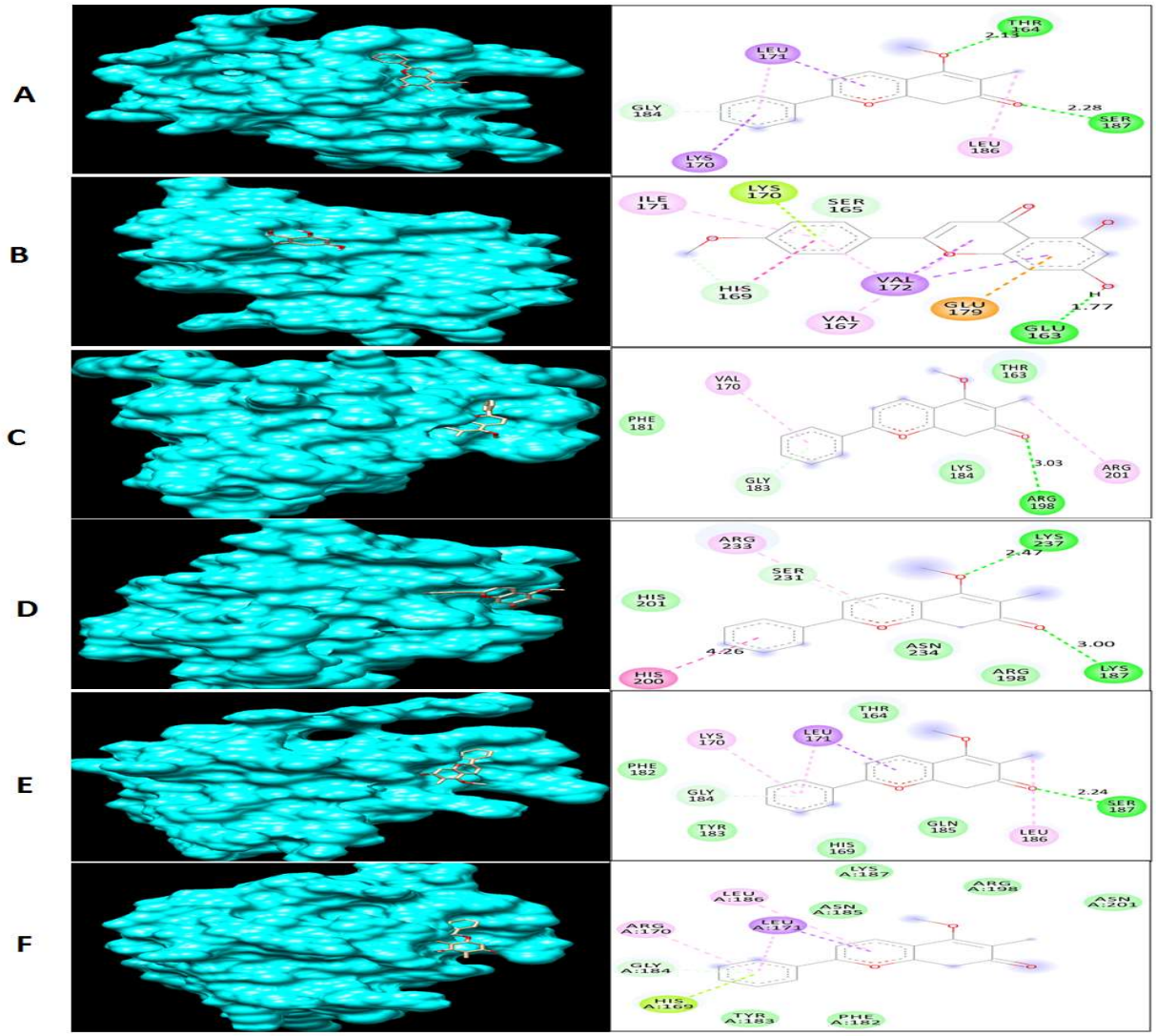

Fig.-1: Docking interactions: (A) *C. trachomatis*- dracorhodin, (B) *E. faecalis*- acacetin, (C*) L. monocytogenes*-dracorhodin, (D) *M. canis*- dracorhodin, (E) *S. pyogenes*- dracorhodin and (F) 4G4K- dracorhodin

Drug Likeness and ADMET Properties

Apart from the promising binding scores of these compounds against AgrA proteins, their pharmacokinetics and bioavailability data are necessary to assure safety. All the flavonoids have a consensus Log p<5 suggesting that the absorption and permeation of these compounds in the living system are in an acceptable range.[21] According to Lipinski's Rule of Five, the qualifying ranges of drug-likeness constraints are as follows: MW less than 500 g/mol, log P less than 5, hydrogen bond donor atoms less than 5, hydrogen bond acceptor atoms less than 10.[22] Among the flavonoids, apigenin-7-O-glucoside, catechin7-xyloside, epicatechin-3-gallate, epigallocatechin, isovitexin, and myricetin show one Lipinski violation and epigallocatechin gallate and kaempferol-7-O-glucoside show two Lipinski violations (Table-2).[23-27]

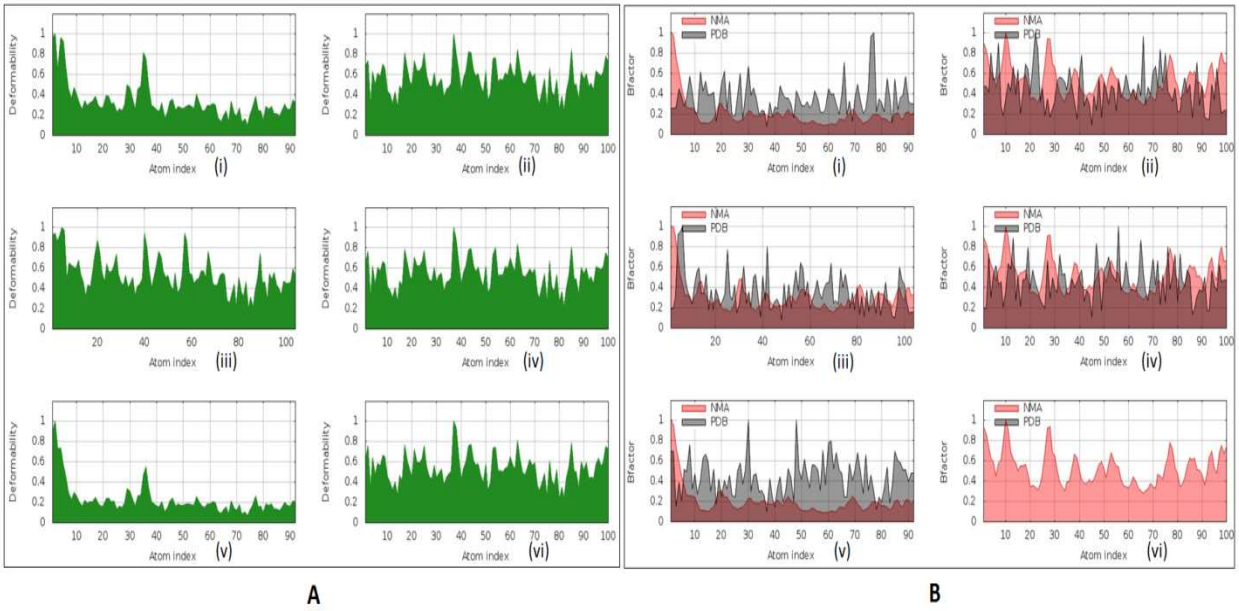

Fig.-2: (A) Deformability and (B) B-factor graph of the docked complexes: (i) *C. trachomatis*- dracorhodin, (ii) *E. faecalis*- acacetin, (iii*) L. monocytogenes*- dracorhodin, (iv) *M. canis*- dracorhodin, (v) *S. pyogenes*- dracorhodin and (vi) 4G4K- dracorhodin

Table-2: Physicochemical Properties, Lipophilicity Indexes, Water Solubility, Pharmacokinetics, and Drug-likeness of the flavonoids

Molecule	MW	Heavy atoms	Aromatic heavy atoms	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	Consensus Log P	ESOL Class	GI absorption	BBB permeant	Lipinski violations
Acacetin	284.26	21	16	2	5	2	79.90	2.52	Moderately soluble	High	No	0
Apigenin	270.24	20	16	1	5	3	90.90	2.11	Soluble	High	No	0
Apigenin-7-O-glucoside	432.38	31	16	4	10	6	170.05	0.52	Soluble	Low	No	1
Catechin7-xyloside	422.38	30	12	4	10	7	169.30	-0.31	Very soluble	Low	No	1
Diosmetin	300.26	22	16	2	6	3	100.13	2.19	Moderately soluble	High	No	0
Dracorhodin	266.29	20	16	2	3	0	39.44	3.18	Soluble	High	Yes	0
Epicatechin-3-gallate	442.37	32	18	4	10	7	177.14	1.34	Soluble	Low	No	1
Epigallocatechin	306.27	22	12	1	7	6	130.61	0.42	Soluble	High	No	1
Epigallocatechingallate	458.37	33	18	4	11	8	197.37	1.07	Soluble	Low	No	2
Farrerol	298.33	22	12	1	4	2	66.76	3.20	Moderately soluble	High	Yes	0
Isorhamnetin	316.26	23	16	2	7	4	120.36	1.65	Soluble	High	No	0
Isovitexin	418.35	30	16	2	10	7	181.05	-0.25	Soluble	Low	No	1
Kaempferol	286.24	21	16	1	6	4	111.13	1.58	Soluble	High	No	0
Kaempferol-7-O-glucoside	448.38	32	16	4	11	7	190.28	0.10	Soluble	Low	No	2
Lysionotin	344.32	25	16	4	7	2	98.36	2.42	Moderately soluble	High	No	0
Morin	302.24	22	16	1	7	5	131.36	1.20	Soluble	High	No	0

Myricetin	318.24	23	16	1	8	6	151.59	0.79	Soluble	Low	No	1
Oroxylin A	284.26	21	16	2	5	2	79.90	2.56	Moderately soluble	High	No	0
Quercetin	302.24	22	16	1	7	5	131.36	1.23	Soluble	High	No	0
Silibinin	482.44	35	18	4	10	5	155.14	1.51	Moderately soluble	Low	No	0

CONCLUSION

The best three flavonoids, dracorhodin, acacetin, and oroxylin A showed an excellent docking score with all the proteins and possess very good drug likeness and pharmacokinetic properties. Hence, these three compounds can be used as AgrA inhibitors. However, more *in vivo* and *in vitro* studies should be done on these flavonoids to confirm their potentiality as QS inhibitors.

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