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Design and Development of Potential Flavonoid Moiety for Pbp2a Inhibition for Mrsa Therapy-A Computational Technique

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The antibiotic resistance is overwhelming at an alarming rate. These 'resistant bacteria' affect millions of people in their health care practices worldwide. The misuse of antibiotics and the overuse of antibiotics in humans, as well as animals may lead to accelerating the process. The 'Methicillin-Resistant Staphylococcus Aureus (MRSA) is the most common antibiotic-resistant bacterium in humans identified at present and is obtained through the mecA gene transcription. In spite of all modern strategies available to minimize the MRSA resistance, a new effective antimicrobial treatment is necessary to control infections. In this study, we designed and developed a new potential flavonoid moiety for MRSA therapy. Various computational methods are used for identifying the best compound for the treatment for this therapy including docking studies, ADEMTox analysis and the characterization of the respective mechanisms. The target protein molecules have been designed through homology modelling and potential flavonoid molecules have been suggested from PubChem database. Hesperetin ((S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one), has been identified as a potential flavonoid moiety suitable for MRSA.

Keywords: Antibiotic Resistance; Flavonoids; Hesperetin; Homology Modelling; MRSA.

Methicillin-Resistant Staphylococcus aureus is a commonly known bacterium that leads to several infections in humans. It is the major cause of multidrug-resistant infections with significant morbidity and mortality. Based on the reports of 'Centers for disease control and prevention (CDC)' in 2011, 80,000 invasive infections and 11,000 deaths were reported in the United States. An estimated 2 billion people carry staphylococcus aureus; about 53 million people are thought to carry MRSA[1].

The common mechanisms used by bacteria to minimize the effects of antibiotics include changes in the antibiotic molecule, reduced antibiotic penetration and efflux, changes in the target site and resistance due to global cell adaptations, antibiotic sequestration, etc. The most important mechanism behind the antibiotic resistance is often acquired by the transfer of resistance-conferring genes between bacteria facilitated by a conjugative plasmid[2,3].

Staphylococcus aureus is a gram-positive bacterium that commonly seen inside the nose and on human skin. These bacteria are resistant to numerous antibiotics. Methicillin is an antibiotic widely uses against Staphylococcus aureus. Once the bacteria develop a self-resistance to methicillin, the resistant bacteria is known as MRSA. Mainly all antibiotics are resistant to all microorganisms. The mecA gene, which is encoded with penicillin-binding protein (PBP2a) and is with reduced affinity for ß-lactam antibiotics, acquires methicillin resistance. The mecA gene, found in bacterial cells, is responsible for developing resistance to penicillin related antibiotics.

In the 'mec operator' is keeping mecA, mecI, mecRI and mecR2[4], out of which, the mecA gene, encoding 'penicillin-binding protein 2a (PBP2a)', acquires Methicillin-Resistant Staphylococcus Aureus (MRSA) infection. The inhibition of penicillin-binding protein 2a (PBP2a) has been identified as a potential therapeutic technique to overcome MRSA. This could be possible by using the antibiotic along with a supporting natural product such as flavonoid, which would inhibit the PBP2a activity[5].

The pathophysiology of methicillin resistance includes the transcription of the mecA gene into the cell wall producing resistance and leading into the degradation of cell membrane. So, the transcription of the mecA gene will produce resistance against the drug.

MRSA is the main carrier of the mecA gene, which can undergo horizontal gene transfer into the host species[6] through a mobile genetic element, Staphylococcal Cassette Chromosome (SCC) mec . The protein targets of PBP2a of Staphylococcus aureus does not have any crystalized structure in the database. However, the protein models can be generated through 'homology modeling' by taking one of the biosynthesized proteins as the template[7, 8]. The interaction between the target protein models and potential drug molecules can be studied through 'docking studies'[9-13]. Further, pharmacokinetic and pharmacodynamic parameters can also be predicted to make screening of the molecules identified.

In this work, the structural protein model molecules of the mecA gene and their interactions with selected flavonoids have been carried out. This would provide an insight into finding a suitable and potential flavonoid moiety for the treatment of MRSA infection.

MATERIALS AND METHODS

The mecA gene protein sequence has been identified using BLAST search and is further subject to homology modelling using 'Swiss Model' by taking 1MWT as the template[14-16]. The 'protein model' characterization has been carried out to study the quality of the models. The flavonoid molecules have been selected from PubChem as the ligand molecules.

The phytoconstituents like Epicatechin gallate, Dihydroquercetin, Fisetin, Myricetin, Farrerol, Peonidin, Quercetol, Hesperetin, Luteolin, Isorhamnetin, Epicatechin, Morin, Phloretin, Naringenin, Catechin, Kaempferol, Shogaol, Glycitein, Strobopinin, Apigenin, Genistein, Daidzein, Ellagic acid, Isoquercetin, etc. were selected. The model protein is further docked with the selected flavonoid molecules[17-18]. The drug-likeness has been further checked through ADMETox studies to identify the most suitable flavonoid to be used along with methicillin to arrest MRSA.

Fig. 1. (a) Predicted three dimensional structures of Penicillin-Binding Protein 2a(PBP2a) by using the template (b) 1MWT

RESULTS AND DISCUSSIONS

The protein models have been designed and developed using homology modelling using the biosynthetic protein, 1MWT as the template. The model protein showed, 96% sequence similarity towards the template protein. The structure of 1MWT and the model protein are shown in Fig 1.

An interaction study has been carried out with the ligand molecules and the modelled protein

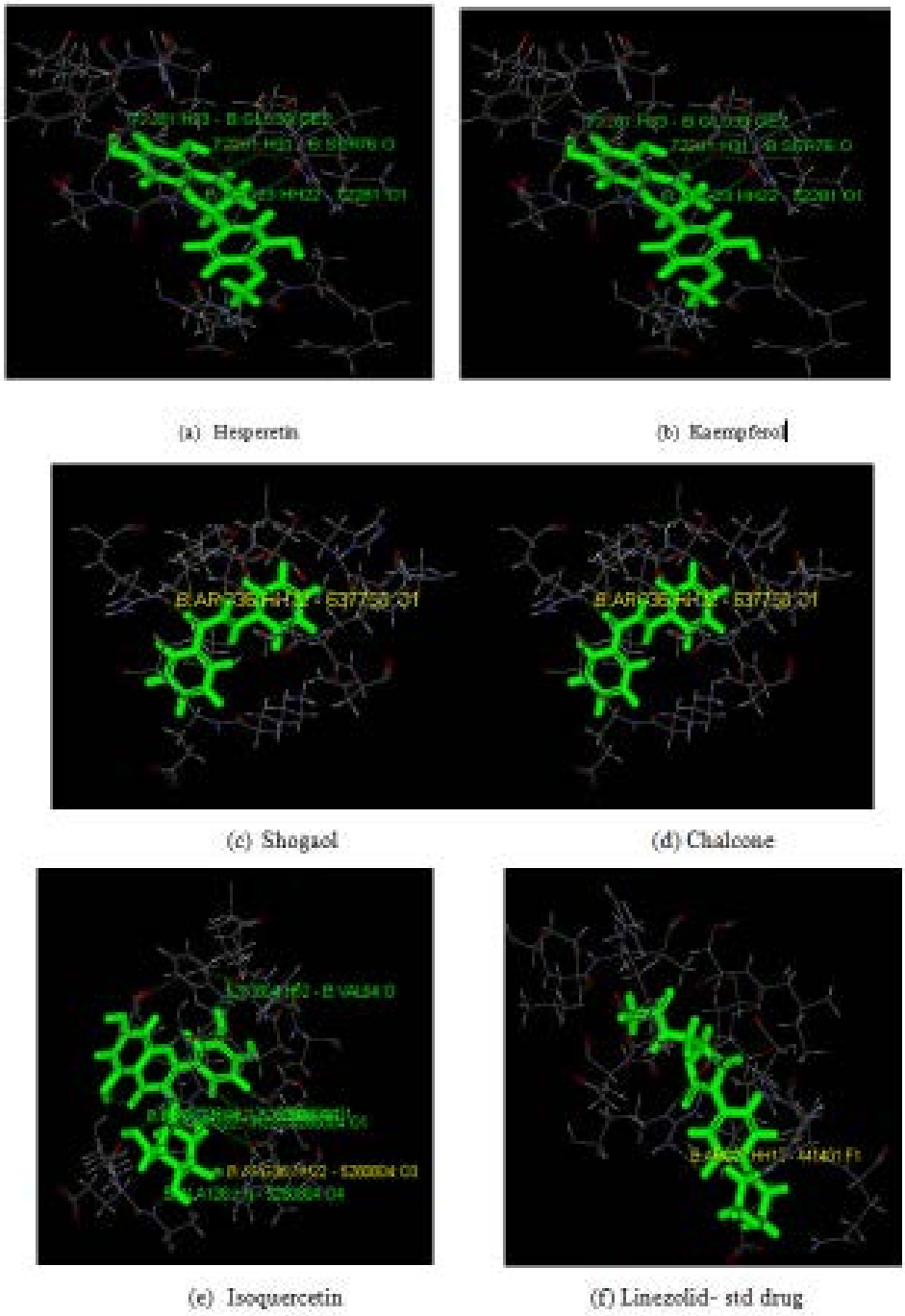

Fig. 2. Docking interaction of selected phytoconstituents in modeled protein. Docking energy and interaction energy of Hesperetin, Kaempferol, Shogaol, Chalcone, Isoquarcetin, and the standard drug Linezolid

structure. It has been observed that the amino acids GLU33, SER 76, ARG 36 and Val 64 were found to be interacting with the ligand molecules Fig 2 as expected through the mechanism of MRSA[19]. The phenolic hydroxyl groups in position 7 and the -OH group at position 3 on the C-ring of Hesperetin are found to be interacting site of the flavonoid moiety.

Among the flavonoid molecules used for the analysis, Hesperetin (Fig 3) is found to be most interacting with the model protein. In fact, this molecule has been identified as more interacting than the standard drug, linezolid used as the control molecule Table 1.

The drug-likeness studies suggest Kaempferol and Hesperetin as potential flavonoids with favourable number of 'hydrogen bonding acceptors and donors Table 2.

All the molecules are keeping good absorption excepting isoquercetin. Hesperain and Kaempferol showed good solubility and less blood-brain barrier penetration. However, these molecules are expected to be more hepatotoxic than the control drug (Table 3).

CONCLUSION

The extensive research into the interactions of flavonoid compounds with the penicillin-binding proteins of many species has shown the variety of the sensitivity of individual PBPs. The 'penicillin-

Table 1. Phytochemicals and their Interaction Energy and Docking score

Molecule	Docking score (kcal/mol)	Docking interaction (kcal/mol)
Chalcone	22.298	32.268
Shogaol	31.733	42.379
Hesperetin	34.041	42.560
Isoquercetin	15.027	50.301
Kaempferol	32.359	37.686
Linezolid –Std	28.623	41.820

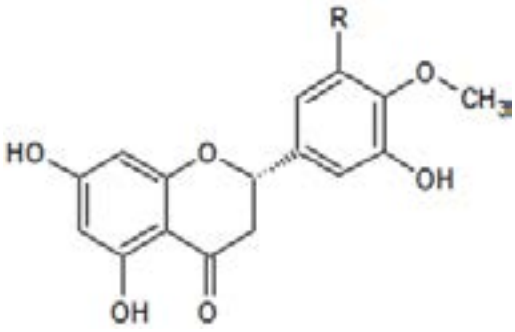

Fig. 3. Selected ligand: Hesperetin

Table 2. Drug-likeness prediction through Biovia Discovery Studio software

Sl.No.	Ligands	AlogP	MW	No. of HBA	No. of HBD
1	Chalcone	3.701	208.255	1	0
2	Shogaol	4.717	276.371	3	1
3	Hesperetin	2.357	302.279	6	3
4	Isoquercetin	-0.3	464.376	12	8
5	Kaempferol	1.872	286.236	6	4

Table 3. ADMET study of selected ligands

Sl. No.	Ligands	ADMET Solubility log (sw) – (S)	ADMET BBB ratio (R)	ADMET Hepatotoxic Probability	ADMET Absorption level (HIA)	ADMET AlogP 98
1	Chalcone	-6.00 S< -4.00	R>5:1	<0.5	<6.12	3.702
2	Shogaol	-6.00<S<-4.00	1:1<R< 5:1	<0.5	<6.12	4.717
3	Hesperetin	-4.00<S<-2.00	R<0.3:1	>0.5	<6.12	2.357
4	Isoquercetin	-6.00<S<-4.00	undefined	<0.5	>7.00	-0.3
5	Kaempferol	-4.00<S< -2.00	R<0.3:1	>0.5	<6.12	1.872

binding proteins (PBP)' can be potential drug targets. The protein models have been generated through homology modelling and are used for studying the possibility of using flavonoids along with Methicillin for controlling MRSA. While studying the interaction between these molecules and the model protein molecules, Hesperetin is found to be most favourable potential molecule. The interacting aminoacids present in the protein models are found to be in the expected binding site. Hence, Hesperetin ((S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one) can be tried as a flavonoid to be used along with methicillin for MRSA therapy subject to further invitro-invivo evaluations.

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