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Chalcones as effective Antimicrobials –a comparative *in silico* approach

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ABSTRACT

Microbial burden upon health of mankind is rapidly making the available therapeutics in practice ineffective. Our ultimate goal is to develop antimicrobials with potent broad spectrum activity. *Staphylococcus aureus* is a gram positive bacteria designated as superbug, challenging the scientific world for development of new therapeutics against multidrug resistance. Methicillin resistant and vancomycin susceptible forms of *Staphylococcus aureus* are emerging causes of many life threatening diseases like toxic shock syndrome etc., we have conducted an *in silico* comparative study of various metabolic pathways of human as well *Staphylococcus aureus* using database of Essential genes (DEG). Out of the identified targets we considered Penicillin binding protein (PBP2A) as potential drug target. According to the literature for effective antimicrobials we prepared libraries of various Chalcones, oxazolidinones and D-alanyl D-alanine like compounds etc., and docked against structure of PBP2A retrieved from protein data bank (PDB).

Key words: Resistance, DEG, PDB, PBP2A, Chalcones.

1. INTRODUCTION

Antibiotic resistance is a rapidly growing problem in treating infections caused by various bacteria, fungi and viruses. In exacting, resistance to antimicrobials has raised over past decades as a major health problem. All widely used antibiotics in practice are now subjected to bacterial resistance, forcing scientific research to find new drugs as alternatives [1]. This challenge can be achieved by two ways -through the discovery of completely novel antimicrobials and by the use of derivatives of existing antibiotics. An alternative to these two pathways towards new therapies as a response to antimicrobial resistance is development of inhibitors of resistance mechanisms [2]. *Staphylococcus aureus* is one of the gram positive bacteria seriously affecting the health of mankind by emergence of multidrug resistance. Many factors responsible for emergence of resistance in particular micro organism have been identified, which includes various metabolic enzymes, proteins and genes along with surface factors. Penicillin binding protein is a key protein involved in resistance mechanism particularly with Beta lactam antibiotics. Penicillin binding protein is regulated by *mec*A gene, which is considered as marker gene for Methicillin resistant *Staphylococcus aureus* (MRSA) [3]. The current *in silico* approach is based on docking study to identify the selected synthetic compounds to bind with Penicillin binding protein (PBP2A) depending on docking score. We are interested to go one step further in Insilco approach to understand the binding efficacy of various synthetic compounds like chalcones, Oxazolidinones and D-Alanyl D-Alanine like compounds. This Selection procedure was carried out by giving importance to the priority compounds for probable action against Methicillin resistant *Staphylococcus aureus*. Chalcones are significant antimicrobials by nature due to the presence of reactive α, β-unsaturated keto group [4, 5]. Oxazolidinones include Linezolid as effective drug for Anti MRSA therapy [6].Vancomycin represents D-Alanyl D-Alanine like compounds, which are significantly used by microorganisms for cell wall synthesis, which cannot be utilized by many human metabolic process [7].We used Accelry's Discovery studio 3.5 to understand the binding patterns of ligands on crystal structure of PBP2A. Based on the results obtained, we can compare the potency of selected compounds against PBP2A, which will provide insights in understanding the activities of compounds as inhibitors based on docking scores and potent compound can be brought to light for further trials[8].

2. MATERIALS AND METHODS

2.1. Materials

All the works were performed using HP Workstation Z220 with Next-generation 22nm

processors, including the Intel® Xeon® processor E3-1200v2 family with 16 Gb RAM, 1 TB Hard disk, NVIDIA Quadro 2000, Windows 7 Ultimate 64 bit. Software Used: Discovery Studio Client 3.5, Biosolve IT, CLC Genomic Workbench 5.1.

2.2. Methodology for Docking

2.2.1. Ligand

Ligand libraries of selected compounds like chalcones, Oxazolidinones and D-Alanyl D-Alanine like compounds were prepared based on various literatures and merged as a single library for the evaluation of effective group of compounds among them against target selected. 3D structure of the compounds have been downloaded from Pubchem compound and www.chemicalize.org. 3D structure optimized ligands has been employed for the docking study.

2.2.2. Drug likeliness evaluation

The drug likeliness property of the compounds was investigated with the help of Lipinski drug filter using Accelrys Discovery Studio 3.5. This filter predicts the Lipinski rule of 5 for the compounds based on its 2D structure and provides information regarding the utilization of compounds as a commercial drug [9].

2.2.3. ADME-Toxicity investigation

ADME-Toxicity studies were executed through Accelrys Discovery Studio 3.5 .The Absorption, Distribution, Metabolism and Excretion (ADME) studies provides insight into the pharmacokinetic property of the compounds. Aqueous solubility, Blood brain barrier level, CYP 2D6, Hepatoxicity and Plasma Protein Binding level were studied. Toxicity profile of the compounds are predicted using TOPKAT which uses a range of robust, cross-validated, Quantitative Structure-Toxicity Relationship (QSTR) models for assessing specific toxicological endpoints. Toxicity profile includes NTP carcinogenicity, mutagenicity, developmental toxicity and skin irritation test [10].

2.2.4. Molecular Simulation studies

Protein Minimization: PBP2A was further processed by applying CHARMm force field. Potential energy of a specified structure was evaluated by using calculate energy protocol of DS 3.5. The calculated energy protocol can be used to compare the relative stability of different configurations of the same structure; or as a prelude to lengthy simulations to confirm the availability of appropriate force field parameters.

Energy minimization of 3-D modeled protein structure was done with the help of standard dynamics cascade protocol of DS 3.5 which performs the following steps: minimization with steepest descent method, minimization with conjugate gradient, dynamics with heating, equilibration dynamics, and production dynamics. The minimization protocol minimizes the energy of a structure through geometry optimization. For the simulation cascade, following parameters are used: steepest descents minimization (500steps, RMS gradient 0.1) in first minimization step and in second steepest Descents minimization (500 steps, RMS gradient 0.0001), heating (2000 steps , initial temperature 50K, final temperature 300K), equilibration (120 ps, 1fs time step, coordinates saved every 1000 steps) and Production (120 ps, 1fs time step, 300 K, NVT ensemble, non-bond cutoff 14A, switching function applied between 10 and 12A, coordinates saved every 1000 steps)

2.2.5. Ligand Minimization

Ligand minimization was performed using CHARMm and MMF forcefield using Accelrys Discovery Studio 3.5. the minimization is accomplished using smart minimizer algorithm with parameter of 200 steps at RMS gradient 0.1. CHARMm energy of the ligands were calculated.

2.2.6. Target Protein and Active site Prediction

The structure of the target protein was retrieved from Protein Data Bank [PDB ID-1VQQ]. Most favored regions of the protein structure were evaluated through the literature and best site was selected with abundance of reactive amino acids among different active sites of protein.

2.2.7. Molecular Docking

The possible docking modes between the ligands and the target protein (1VQQ) were studied using Biosolve IT Flex. The docking algorithm in the LeadIT suite is the FlexX-docking approach. It flexibly places ligands into the active site with an incremental buildup algorithm. The active site of the target was loaded in the BioSolveIT-FlexX [11]. The active site amino acids were defined in the target molecule during the target preparation step of FlexX. A sphere of 10Å radius was defined as active site. The MOL2 file of 5 compounds was loaded in FlexX as docking library. The Protein Ligand clash was set to 2.9 Å and Intra Ligand clash was set to 0.6 in the docking. Maximum number of fragmentation and iterations were set to 200. This procedure was described in detail elsewhere (Rarey *et al*., 1996) [12]. The docked ligand-target complexes were analyzed carefully to identify the interactions and binding affinities.

The docking score was noted down and docking poses were saved for reference.

3. RESULTS AND DISCUSSION

3.1. Molecular simulation studies

CHARMm is a highly flexible molecular mechanics and dynamics program. It derives from the program CHARMM (Chemistry at HARvard Molecular Mechanics). CHARMm performs well over a broad range of calculations and Simulations, including calculation of geometries, interaction and conformation energies, local minima, barriers to rotation, time-dependent dynamic behavior, and free energy [13]. The CHARMm energy of the Ligands were also noted. Minimization values for the target protein PBP2A are shown in table-1.

Table-1: Energy minimization of PBP2A (1VQQ)

Name -1VQQ	Force field -1VQQ-charmm27	
ENERGY	INITIAL	FINAL
Potential Energy (kcal/mol)	-4845.75303	-30307.51397
Vander Waals Energy (kcal/mol)	-407.07832	-5163.35616
Electrostatic Energy (kcal/mol)	-18814.47842	-34291.02482
RMS Gradient (kcal/(mol x A),	33.28900	0.73133

Minimization Criteria -CONJUG> Minimization exiting with number of steps limit (200) exceeded.

Table-2: Comparison of the ADMET values of ligands

Descriptor	A	B	C	D	E
ADME.2D.FPSA	55.976	121.378	76.791	121.378	161.679
BBB LEV	1	4	2	4	4
CYP 2D6	-3.19655	-4.91542	-4.80694	-2.40873	-8.51737
HEPATOX	-1.12511	-3.63231	-3.63386	-3.72222	-5.33577
PPB LEV	4.3617	-0.110648	1.69232	-2.7844	-15.9399
HEPATOX	-1.12511	-3.63231	-3.63386	-3.72222	-5.33577
NTP Carcinogenicity Call (Female Rat) (v3.2)-TOPKAT	0.000	0.009	0.000	0.002	0.000
Ames Mutagenicity (v3.1)- TOPKAT	0.009	0.000	0.011	0.000	0.999
Developmental Toxicity Potential (DTP) (v3.1)- TOPKAT	0.998	0.979	0.998	0.990	0.998
Rat Oral LD50 (v3.1)- TOPKAT	6.0 g/kg	3.6 g/kg	6.5 g/kg	3.5 g/kg	1.8 g/kg
Skin Irritation (v6.1)- TOPKAT	0.712	0.013	0.996	0.642	0.394

NOTE:-

Probability values from 0.0 to 0.30 are considered low probabilities, and are likely to produce a negative response in an experimental assay; whereas probability values greater than 0.70 are considered high, and are likely to produce a positive response in an experimental assay. Probabilities greater than 0.30 but less than 0.70 are considered indeterminate.

A: (2E)-3-(2, 4-dimethoxyphenyl)-1-(2-hydroxy-5-methylphenyl) prop-2-en-1-one

B: Okanin - (2E)-3-(3, 4-dihydroxyphenyl)-1-(2, 3, 4-trihydroxyphenyl) prop-2-en-1-one

C: (2E)-1-(2, 4-dihydroxyphenyl)-3-(3, 4-dimethoxyphenyl) prop-2-en-1-one

D: (2E)-3-(3, 4-dihydroxyphenyl)-1-(2, 4, 6-trihydroxyphenyl) prop-2-en-1-one

E: Ophthalmic acid

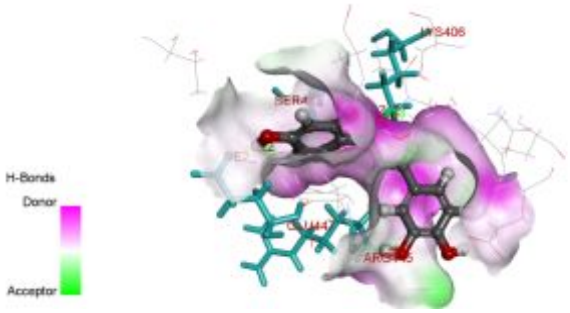
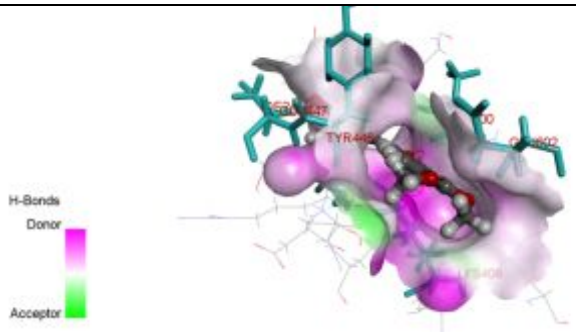
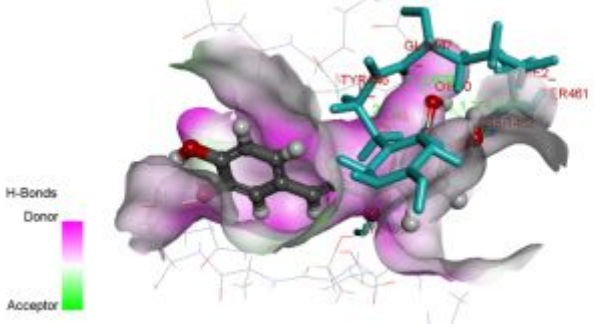
Table-3: Ligand-Protein interaction with docking scores

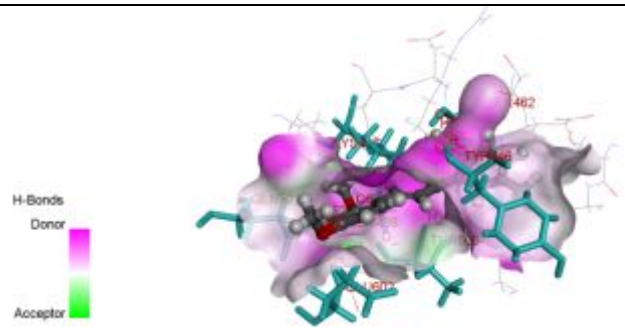
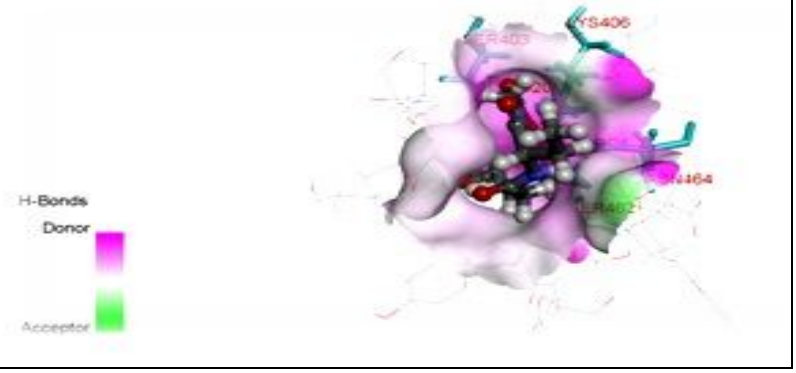
COMPOUND NAME	LEAD-IT					
	LEAD-IT SCORE	H-BOND	AMINO ACID	AMINO ACID ATOM	LIGAND ATOM	H-BOND LENGTH
2,4-dimethyl-2-hydroxy-5-methyl chalcone	-33.0036	10	SER403	HG_	O6	2.00086
			LYS406	HZ3_	O12	1.7426
			TYR446	HN_	O20	2.02202
			SER462	O_	O20	2.58679
			SER462	HG_	O12	2.48886
			GLN521	HE21	O6	2.05449
			THR600	O_	O6	2.54472
			THR600	HN_	O12	2.48478
			GLU602	HN_	O2	1.90777
			SER462	O_	H38	1.69822
Okanin	-32.8575	11	LYS406	HZ3_	O9	2.14452
			TYR446	HN_	O18	2.02951
			GLU447	OE2_	O14	2.94688
			GLU447	HN_	O16	2.07007
			SER461	O_	O16	2.62282
			SER462	O_	O18	2.64991
			THR600	HN_	O9	1.5668
			GLU602	HN_	O21	2.15088
			GLU447	OE2_	H29	2.26084
			SER461	O_	H30	1.76892
			SER462	O_	H31	1.69355
2,4-Dihydroxy-3,4-dimethoxy chalcone	-32.7776	8	LYS406	HZ3_	O12	2.08877
			TYR446	HN_	O20	2.09065
			GLU447	OE2_	O17	2.74063
			SER462	O_	O20	2.75062
			THR600	HN_	O12	1.76271
			GLU602	HN_	O5	2.09993
			GLU447	OE2_	H34	1.90308
			SER462	O_	H36	1.81472
3,4-pentahydroxy chalcone	-32.6151	10	LYS406	HZ3_	O8	2.21332
			ARG445	O_	O14	2.84506
			TYR446	HN_	O20	2.05645
			GLU447	OE2_	O1	2.7655
			SER462	O_	O20	2.67657
			THR600	HN_	O8	1.75559
			THR600	HG1_	O5	1.92996
			GLU447	OE2_	H22	1.88896

			ARG445	O_	H28	2.03025
			SER462	O_	H32	1.73272
Opthalmic acid	-31.9458	15	SER403	HG_	O20	1.92846
			LYS406	HZ3_	O12	1.49381
			LYS406	HZ1_	O19	1.80123
			SER462	HG_	O12	2.35925
			ASN464	OD1_	O19	2.66619
			GLN521	HE21	O20	2.16531
			THR600	O_	O20	2.89426
			THR600	HN_	O13	1.6923
			THR600	HG1_	O13	1.78968
			GLU602	HN_	O15	2.06561
			THR444	O_	H27	2.23466
			ASN464	ND2_	H27	2.14621
			ASN464	OD1_	H33	2.20875
			ASN464	OD1_	H34	2.3819
			SER462	O_	H38	1.99195
3,4-dimethoxy-2-hydroxy-5-methyl chalcone	-31.8505	6	LYS406	HZ3_	O12	2.12308
			TYR446	HN_	O20	1.85816
			SER462	O_	O20	2.74575
			THR600	HN_	O12	1.86858
			GLU602	HN_	O5	1.87927
			SER462	O_	H38	1.83611
3(S)-3-(valinyl)amino-4-oxobutanoic acid	-31.177	12	SER403	HG_	O12	2.15345
			LYS406	HZ1_	O13	2.14615
			ASN464	OD1_	O13	2.80028
			ASN464	HD22	O7	2.20384
			ASN464	HD22	O13	2.48532
			GLN521	HE21	O12	1.88988
			THR600	O_	O12	2.87626
			GLU602	HN_	O15	2.05577
			GLN613	OE1_	H24	1.70435
			THR600	OG1_	H25	1.66636
			GLU602	N_	H26	2.31753
			ALA601	N_	H31	2.45312
Butein	-31.0765	8	LYS406	HZ3_	O7	2.06442
			TYR446	HN_	O19	2.14769
			GLU447	OE2_	O1	2.69233
			SER462	O_	O19	2.69233
			THR600	HN_	O7	1.82777
			GLU602	HN_	O16	2.24988

			GLU447	OE2_	H21	1.86121
			SER462	O_	H31	1.7567
2-hydroxy-4-methoxy-5-methyl chalcone	-30.9954	6	LYS406	HZ3_	O10	1.75044
			TYR446	HN_	O18	2.08759
			SER462	O_	O18	2.61421
			SER462	HG_	O10	2.40135
			GLU602	HN_	O2	1.9714
			SER462	O_	H34	1.76136
2,4-hydroxy-2-methoxy-5-methyl chalcone	-30.9213	6	LYS406	HZ3_	O12	2.09678
			TYR446	HN_	O20	1.93752
			SER462	O_	O20	2.62536
			THR600	HN_	O12	1.78547
			GLU602	HN_	O2	1.86072
			SER462	O_	H36	1.71845

Figure-1: Binding interactions between ligands and target

Fig. No.	Compound Name	LEAD-IT SCORE	Binding Pose with Target protein
1	2,4-dimethyl-2-hydroxy-5-methyl chalcone	-33.0036	
2	Okanin	-32.8575	
3	2,4-Dihydroxy-3,4-dimethoxy chalcone	-32.7776	

4	3,4-pentahydroxy chalcone	-32.6151	
5	Opthalmic acid	-31.9458	

3.2. Drug likeness evaluation

The Lipinski rule of five for the compounds was predicted via Lipinski drug filter. The cut off values include:

- Molecular mass less than 500Da
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- High lipophilicity (expressed as Log P less than 5)

The results show that the compounds obey Lipinski rule of five and it can be strongly recommended as a drug.

3.3. ADMET investigation

The ADME (Absorption, Distribution, Excretion, Metabolism) properties and toxicity profile of the compounds are depicted in Table 2. These results show that the Lead compounds (1-5) possess good pharmacokinetic properties and it satisfies all the parameters to be taken over as a good drug. The toxicity profiles of the compounds were calculated using TOPKAT.

3.4. Molecular docking simulation

Molecular docking studies were performed using Lead IT. The results of interaction between Penicillin binding protein and ligands filtered after ADMET are shown in fig-1. The green dot lines denote the hydrogen (H) bonds. All the amino acid residues which involved in molecular interactions are displayed as lines and the ligands are displayed as ball and sticks. The results show that a good interaction occurs between the protein and the Ligand. Chalcones especially 2, 4-dimethyl-2-hydroxy-5-methyl chalcone showed more binding capacity based on Lead scores. However, ophthalmic acid showed 15 hydrogen bond interactions than the other compounds with target. LYS, SER, THR & GLU are the main amino acids involved in the interactions between target protein and Ligand. The details of the interaction such as number of hydrogen bonds involved, Amino acids , Ligands and their atoms involved and bond energy are tabulated in table 3.

Docking protocols are extensively used to predict the binding affinities for a number of ligands. The aim of our study was to analyze the probability of an existing protein under study and the docking score. Specifically five protein inhibitors were used for this study. The LeadIT software automatically proposes a reasonable protonation state of the active site amino acids. The build-in program ProToss [14] validates the hydrogen bond network of the active site and maximizes the number of hydrogen bonds with a scoring function. The LeadIT suite provides the FlexX-scoring function, which was used to find the initial best 200 poses. The best score from the best pose for each compound was taken and compared

to the scores of the other compounds. The compounds which shows highest negative LeadIT score shows that it has the capability to bind strongly with the protein and inhibit PBP2A. In the present study, 2,4-dimethyl-2-hydroxy-5-methyl chalcone showed greater binding energy (-33.0036) and the amino acid involved in interaction were SER 403,462 ,LYS 406 and TYR 446 followed by Okanin (-32.8575) with 11 hydrogen (H) bond interactions , 2,4-Dihydroxy-3,4-dimethoxy chalcone with 8 H bonds, 3,4-pentahydroxy chalcone with 10 H bonds and Ophthalmic acid with 15 H bonds respectively.

4. CONCLUSION

These results obtained reveal the significance of chalcones against the target with great binding capacity than others. Out of the best compounds with great binding affinity, chalcones exhibited majority contribution. This clearly gives a conclusion that chalcones are potent antimicrobials and synthesis of various novel substituted chalcones might be the way to decline the emerging resistance in micro organisms.

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