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EVALUATION OF NOVEL CURCUMIN DERIVATIVES AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS (MRSA)*

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ABSTRACT: Methicillin resistance mediated by PBP2a protein is a serious issue limiting treatment options and necessitating the search of newer safe and effective alternative treatment regimens. The Multiple Drug Resistance in MRSA (Methicillin Resistant *Staphylococcus aureus*) has become a major clinical problem worldwide. This study is an attempt to evaluate the potential of the plant product 'Curcumin' and its derivatives as effective antibacterial agents against MRSA by means of *In-silico* and *In-vitro* studies. A series of 25 derivatives of Curcumin were constructed and optimized using Chemsketch Software. Molecular docking was performed using the GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA), to study the binding orientation of these derivatives into the PBP2a Protein structure. Among the Curcumin Derivatives tested, Derivatives No 11 and 16 showed better docking fitness values compared to other Derivatives and also Curcumin. The Molecular, Physicochemical, and Biological properties were determined using Molinspiration Cheminformatics softwares for compounds showing better docking scores. These compounds were further subjected to Toxicity Predictions using the Osiris Software. The anti MRSA activity was further confirmed by *In-vitro* studies using Muller-Hinton Agar well diffusion method. The inhibitory activity of the selected derivatives was compared with Parent compound Curcumin and Vancomycin. The synthesized Compounds showed effective and comparable inhibitory activity to Curcumin and vancomycin on MRSA.

Key words: MRSA, Curcumin, Docking studies, Molinspiration, Osiris software

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INTRODUCTION

Microbial Resistance to Antibiotics causing increasing rates of morbidity and mortality across populations worldwide a serious issue concerning Medical community and other institutions involved in Healthcare management (Nimmo et al., 2006). Resistance in microbes is induced through random mutations from improper and excessive use of antibiotics and also through uptake of plasmid DNA from other microbes. Methicillin resistant *S. aureus* is an example of antibiotic Resistance by uptake of plasmid DNA. The resistance in MRSA results when a plasmid encoding for mecA gene is incorporated into its genome. The MecA gene is responsible for the expression of Penicillin Binding Protein 2a (PBP2a) which inactivates the molecules of methicillin or any other β-lactum antibiotic (Katayama et al., 2000). MRSA has now become resistant to a wide number of antibiotics including Methicillin, Oxicillin, Penicillin and Amoxicillin (CDC, Center for Disease Control and Prevention, 2009).

Spread of MRSA has been associated with Hospital and Healthcare acquired infections (HA MRSA) and Community Acquired infections (CA MRSA) (Klevens et al 2007). MRSA is becoming increasingly difficult to be controlled because of its resistance to variable normal of drugs. Our fight against MRSA has come down to the availability of limited number of exotic drugs like Vancomycin, Teicoplanin etc.It is thus evident that there is an urgent need for development of novel antibiotic drugs with broad spectrum of activity including activity against resistant microorganisms.

Since it is the difference between Methicillin Sensitive *S. aureus* (MRSA) and MRSA is the presence of mecA gene studies are directed towards finding suitable inhibitors of PBP2a to counter the resistance of MRSA to antibiotics. Attempts are now being made to evaluate active metabolites present in medicinal plants as therapeutic alternatives for MRSA infections.

Curcumin and other curcuminoid constitute the phytochemicals of the rhizome *Curcuma longa* belonging to Zingibiraceae family with the common name of Turmeric extensively used in India as a spice in Indian kitchen. Curcumin or diferuloyl methane, a polyphenolic compound with a chemical formula of (1,7-dis,4hydroxy-3-methoxy phenyl-1,6-heptadine) has been found to contain a variety of biological activities in recent times and has gained significant attention of researchers all over the world (Moghadamtousi et al., 2014; Anand et al 2008). Curcumin has been used in traditional Indian medicine for bilary disorders, anorexia, cough, regular and diabetic wounds as anticeptic and in rheumatoid arthritis (Othman et al., 2013**).** Several other studies have shown that curcumin has a variety of pharmacological action including anti-inflammatory, antioxidant, antiviral and against different forms of cancer (Agarwal et al., 2003 and Prusty and Das, 2005). You et al. (2003) and Masroor et al. (2015**) .**

In the present study new derivatives of Curcumin are synthesized and their activity against MRSA evaluated and compared to the parent compound curcumin and the available anti-MRSA antibiotic Vancomycin. The study involved docking and evaluation of molecular properties, bioactivity, drug likeness and toxicity prediction of the derivatives using bioinformatic tools in In-Silico. The studies were also extended to confiormation of the anti-MRSA activity in-Vitro using the standard well diffusion method in Muller Hinton Agar media.

METHODOLOGY

Selection and Preparation of the Target

The crystal structure of Penicillin Binding Protein 2a (PBP2a) was taken from the Protein Data Bank (PDB_ID:1VQQ**)** (Figure 1). After removing the statins from the binding site, the chain A was selected for docking studies. Hydrogen atoms were added to the PBP2a.

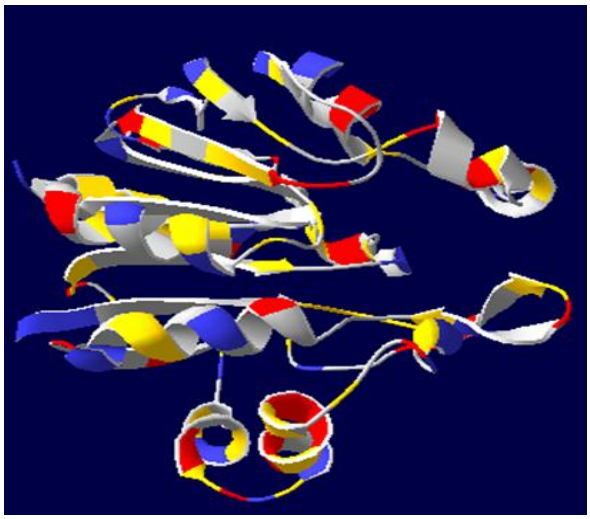

Figure 1: Structure of PBP2a

Designing of New Derivatives for Curcumin

Docking studies by modifying the structure of Curcumin (Figure 2), 25 derivatives were designed. The structures of these derivatives were constructed and optimized using ACD labs Chemsketch v 12.0. The chemical names of all these derivatives are provided in Table 1.

Structures of Curcumin and its Derivatives (Figures 2-5):

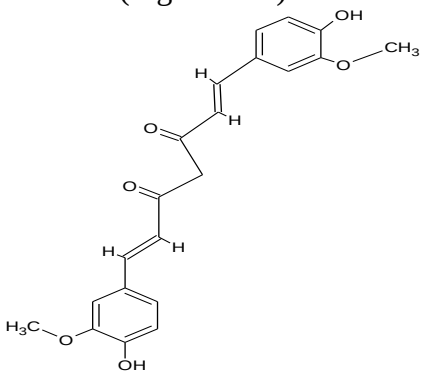

Fig.2: Curcumin (Original plant compound)

(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

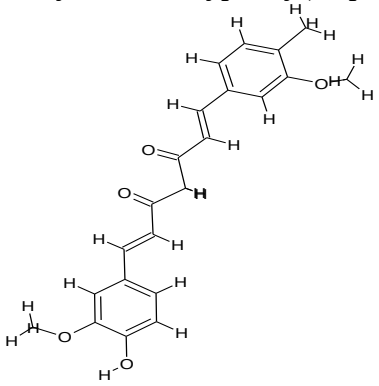

Fig. 3: curcumin derivative 11

(1*E*,6*E*)-1-(4-hydroxy-2-cholorophenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

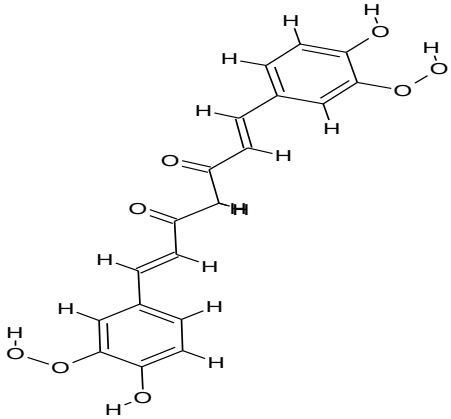

Fig. 4: Curcumin derivative 16

(1*E*,6*E*)-1-(3-hydroperoxy-4-hydroxyphenyl)-7-(3-hydroperoxyphenyl)hepta-1,6-diene-3,5-dione

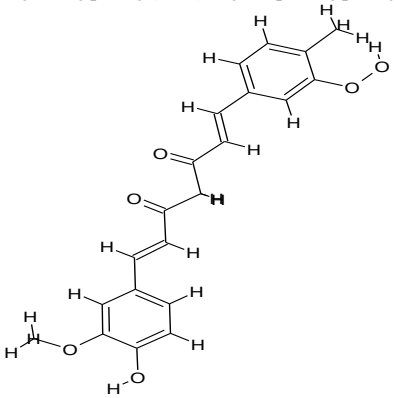

Fig. 5: Curcumin derivative 20

(1*E*,6*E*)-1-(3-hydroperoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

Table 1.Chemical names of synthetic Curcumin derivatives

S.No.	Derivative no	Chemical name
1.	Curcumin (original) or 1	(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
2.	Curcumin 2	(1*E*,6*E*)-1-(4-hydroxy-3-methoxyphenyl)-7-(3-methoxyphenyl)hepta-1,6-diene-3,5-dione
3.	Curcumin 3	(1*E*,6*E*)-1-(3-hydroxy-4-ethoxylphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
4.	Curcumin 4	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
5.	Curcumin 5	(1*E*,6*E*)-1-(3-hydrophenyl-4-ethyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
6.	Curcumin 6	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
7.	Curcumin 7	(1*E*,6*E*)-1-(3-hydroperoxy-4-methylphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
8.	Curcumin 8	(1*E*,6*E*)-1-(3-hydroperoxy-4-hydroxyphenyl)-7-(3-methoxy-4-methylphenyl)hepta-1,6-diene-3,5-dione
9.	Curcumin 9	1*E*,6*E*)-1-(4-hydroxy-3-methoxyphenyl)-7-(3-methoxy-4-methylphenyl)hepta-1,6-diene-3,5-dione
10.	Curcumin 10	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(3-hydroperoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione
11.	Curcumin 11	(1*E*,6*E*)-1-(4-hydroxy-2-cholorophenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
12.	Curcumin 12	(1*E*,6*E*)-1-(3-hydroperoxy-4-methylphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
13.	Curcumin 13	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(3-hydroperoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione
14.	Curcumin 14	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(5-hydroxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione
15.	Curcumin15	(1E,6E)-1,7-bis(3-hydroperoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione
16.	Curcumin 16	(1*E*,6*E*)-1-(3-hydroperoxy-4-hydroxyphenyl)-7-(3-hydroperoxyphenyl)hepta-1,6-diene-3,5-dione
17.	Curcumin 17	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(3-hydroxyphenyl)hepta-1,6-diene-3,5-dione
18.	Curcumin 18	1*E*,6*E*)-1-(3-hydroperoxy-4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
19.	Curcumin 19	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(3-methoxyphenyl)hepta-1,6-diene-3,5-dione
20.	Curcumin 20	(1*E*,6*E*)-1-(3-hydroperoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
21.	Curcumin 21	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
22.	Curcumin 22	(1*E*,6*E*)-1-(2,4-dihydroxyphenyl)-7-(2-hydroperoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione
23.	Curcumin 23	(1*E*,6*E*)-1-(3,6-dihydroxy)-7-(3-hydroperoxy-3-hydroxyphenyl)hepta-1,6-diene-3,5-dione
24.	Curcumin 24	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(3-hydroxy-6-hydroxyphenyl)hepta-1,6-diene-3,5-dione
25.	Curcumin 25	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(3-hydroperoxy-4-methylphenyl)hepta-1,6-diene-3,5-dione

Docking Method

The binding site of PBP2a was identified using CASTp (Computed Atlas of Surface Topography of proteins) server. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

GOLD version 3.0.1 (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA) was used for molecular docking to study binding orientation of compounds into the PBP2a structure. This method allows full flexibility of compounds and partial flexibility of protein .The designed derivatives were docked to the active site of the PBP2a. The interaction of these Curcumin analogues with the active site residues are studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of islands (1) and niche size (2).

Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0 A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the binding site in the PBP2a was defined within a 10 A° radius with the centroid as CE atom of GLN207. The number of poses for each inhibitor was set at 100, and early termination was allowed if the top three bound conformations of a compound were within 1.5A° RMSD (Root-Mean-Square Deviation). After docking, the individual binding poses of each compound were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each compound was selected.

Gold Score fitness function

Gold Score performs a force field based scoring function. It is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vanderwaals energy (external vdw); 3.Ligand internal vanderwaals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand vander waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

Hence, the docking of compounds into the active site of PBP2a was performed using the GOLD software and the docking evaluations were made on the basis of GoldScore fitness functions. We preferred Gold fitness score than Chemscore fitness as Gold fitness score is marginally better than Chemscore fitness function.

ADMET Studies

The Curcumin derivatives showing the best docking scores, derivatives no. 11, 16 and 20 (Figures 3-5) were used for ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) studies and physico-chemical parameters were predicted insilico by Molinspiration and Osiris software.

Smilies notations of the selected compounds were fed in the online molinspiration software (version-2014) for calculation of molecular properties (such as Log P, Total polar surface area, Number of hydrogen bond donors and acceptors, Molecular weight, Number of atoms, Number of violations, Number of rotatable bonds and Volume) and prediction of bioactivity score for drug targets (GPCR ligands, Ion channel modulators, Kinase inhibitors, Nuclear receptor ligand, Enzyme and Protease inhibitors). The toxicity parameters predicted were Mutagenicity, Tumorogenicity, Skin Irritation, and Reproductive Effect. Physico-chemical parameters like C log P, Solubility, Drug Likeness, Molecular Weight, and Drug Score were also predicted using Osiris property explorer software.

In vitro studies

The results obtained from *Insilico* studies were tested experimentally for further confirmation by *In-Vitro* studies.

The pure culture of MRSA was grown on agar media. The compounds were added into wells bored in the media (well diffusion method) and the plates were incubated at 37^{o}C for 24 hours. The antimicrobial activity was determined by measuring the inhibitory zone. The efficiency of Vancomycin, Curcumin and its three best docked derivatives 11, 16 and 20 were compared. The antibiotic susceptibility patterns were carried out by disc diffusion method. The sensitivity patterns of each antibiotic were confirmed by measuring the zone of inhibition and compared with standard antibiotic susceptibility chart.

RESULTS AND DISCUSSION

After constructing the derivatives of Curcumin, optimizing them using chemsketch software , and having searched for the crystal model and the possible binding sites of PBP2a with CASTp server, we identified from the binding site analysis of PBP2a that, the binding pockets are identical in all chains and the largest binding pocket was taken for further docking studies.

Docking Images Of Curcumin And Its Derivatives (Figure 6-9)

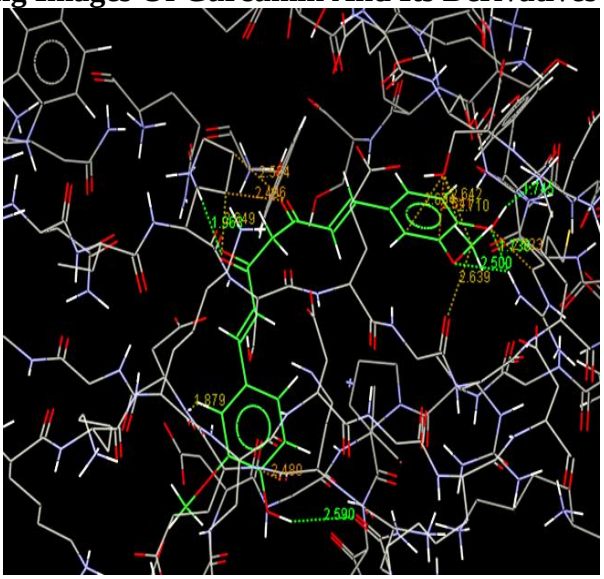

Fig. 6: Docking of Curcumin

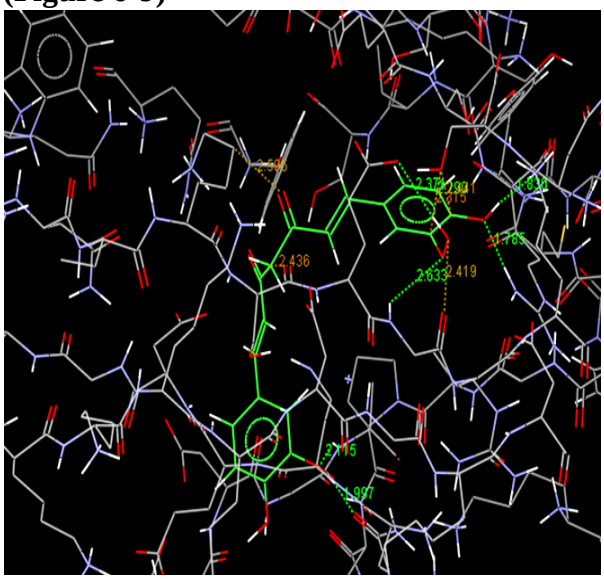

Fig. 7: Docking Image Of Derivative11

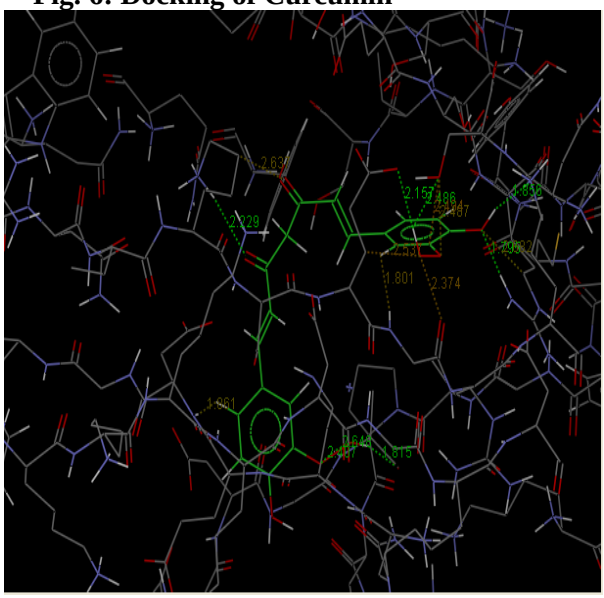

Fig. 8: Docking image of Derivative 16

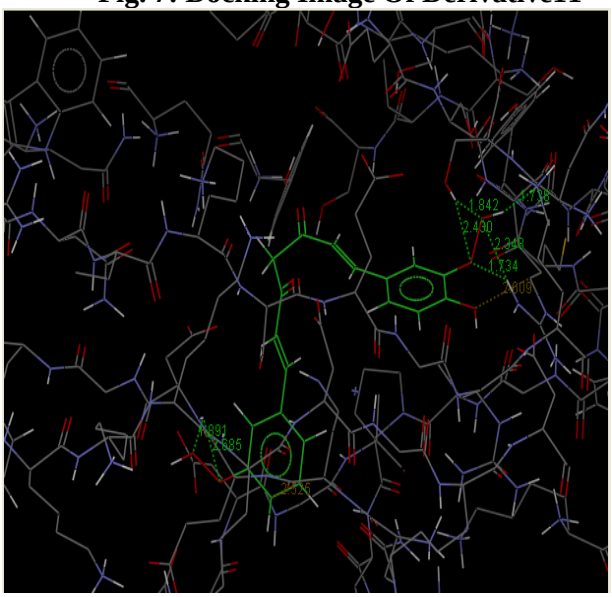

Fig.9: Docking image of Derivative 20

The crystal structures of PBP2a are similar and we have therefore taken 1VQQ (chain A) as representative structure for docking studies. The selected docked conformations of the PBP2a binding site are shown in Figure 6-9. The docked conformations revealed that all derivatives were located in the hydrophobic binding pocket surrounding the binuclear copper active site. In this study, all docked curcumin analogues were found to have some interaction between an oxygen atom of the compound and PBP2a. Moreover, these docked conformations also formed an H-bonding interaction with in the active site. In the binding pocket, common H-bonding interactions were formed between all docked compounds and GLY 135, GLN 137, GLN 140, HIS 143, GLU 145, GLN 145, GLN 207, ASP 209, HIS 232, THR 300, and HIS 311. In order to explain the binding of these compounds, the H-bonding interactions with the other surrounding residues in the hydrophobic binding pocket were also investigated. The docking results showed that Curcumin derivates have more affinity towards the protein than the molecule (Table 2).

Table 2: Docking scores of Curcumin derivatives with PBP2a

Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(in_t)	Ligand name
49.94	12.67	41.58	0.00	-19.91	**Curcumin**
47.54	7.84	38.73	0.00	-13.55	Curcumin10
58.42	**22.08**	**32.85**	**0.00**	**-12.84**	**Curcumin11**
44.79	7.02	36.00	0.00	-11.72	Curcumin18
49.06	18.30	38.51	0.00	-22.19	Curcumin12
45.96	7.66	38.27	0.00	-14.32	Circumin13
49.02	21.16	29.76	0.00	-13.06	Curcumin14
45.55	10.32	36.55	0.00	-15.03	Curcumin15
48.96	19.97	31.41	0.00	-14.21	Curcumin16
48.95	7.45	40.03	0.00	-13.54	Curcumin17
45.74	6.75	38.05	0.00	-13.32	Curcumin2
50.60	11.25	37.21	0.00	-11.81	Curcumin3
47.67	3.13	40.63	0.00	-11.33	Curcumin4
54.48	**11.16**	**39.26**	**0.00**	**-12.66**	**Curcumin16**
50.73	8.01	43.05	0.00	-16.47	Curcumin6
53.95	**15.32**	**37.47**	**0.00**	**-14.89**	**Curcumin20**
40.50	14.67	38.33	0.00	-16.87	Curcumin8
51.25	20.84	31.33	0.00	-12.67	Curcumin19

Curcumin derivative 11 **(1*E*,6*E*)-1-(4-hydroxy-2-cholorophenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione** showed best fitness value of 58.42, when compared to Curcumin and was higher than those of other derivatives, closely followed by derivative 16 and 20 (54.48 and 53.95, respectively). Out of the 25 derivatives, 3 derivatives showed better docking than the parent compound Curcumin. The structure of these compounds is provided in Figure 3-5. The chemical properties are tabulated in Table 3a.

Table 3a: Chemical Properties Of Derivatives

Active Plant product and derivatives	MF	FW	COMPOSI-TION	MR Cm^3	MV ($Cm^{3)}$	PARA CHOR ($Cm^{3)}$	RI	ST (Dyne/ cm)	DENSITY ($g/cm^{3)}$	P ($Cm^{3)}$	MI (Da)	NM (Da)	AM (Da)
Curcumn	$C_{21}H_{20}O_6$	368.3799	C(68.47%) H(5.47%) O(26.06%)	104.04 ±0.3	287.8 ±3.0	781.5 ±4.0	1.642 ±0.02	54.3 ±3.0	1.279 ±0.06	41.24± 0.5 10^{-24}	368.125988	368	368.3799
Derivative-11	$C_{19}H_{16}O_8$	372.32554	C(61.29%) H(4.33%) O(34.38%)	97.85 ± 0.3	249.8 ± 3.0	735.4 ±4.0	1.711 ± 0.02	75.0 ± 3.0	1.489 ± 0.06	38.79 ± 0.5 $10^{-24}cm^3$	372.084517	372	372.3255 Da
Derivative- 16	$C_{21}H_{20}O_6$	368.3799	C(68.47%) H(5.47%) O(26.06%)	103.89 ± 0.3	286.7 ± 3.0	781.1 ± 4.0	1.644 ± 0.02	55.0 ± 3.0	1.284 ± 0.06	41.18 ± 0.5 $10^{-24}cm^3$	368.125988	368	368.3799 Da
Derivative -20	$C_{22}H_{22}O_5$	366.40708	C(72.12%) H(6.05%) O(21.83%)	106.99 ± 0.3 cm^3	305.7 ± 3.0 cm^3	804.2 ± 4.0	1.617 ± 0.02	47.8 ± 3.0	1.198 ± 0.06	42.41 ± 0.5 $10^{-24}cm^3$	366.146724	366	366.4071 Da

AP= Active Plant Products, MF= Molecular Formula, FW= Formula Weight, MR= Molar Refractivity, MV = Molar Volume, RI=Refraction Index, ST = Surface Tension, DC = Dielectric Constant,MI= Monoisotropic Mass, NM = Nomimal Mass, AM Average Mass , P= Polalarizibility.

The compounds fulfilled Lipinski's rule and showed good drug likeness score (Table 3b). Lipinski's rule is widely used to determine molecular properties that are important for drug's pharmacokinetic profile *in vivo*.

Table 3b: Drug Likeness Score For Derivatives

COMPOUNDS	MI LOG P	TPSA	NATOMS	MW	N ON	NOHNH	NVIOLATIONS	NROTB	VOLUME
1.Curcumin	2.303	93.066	27.0	368.385	6	2	0	8	332.182
2.Derivative-11	2.063	133.522	27.0	372.329	8	4	0	8	315.095
3.Derivative-16	3.08	93.066	27.0	368.385	6	2	0	8	332.182
4.Derivative-20	3.397	72.838	27.0	366.413	5	1	0	8	340.725

According to Lipinski's rule of five (Lipinski *et.al.* 1997), a candidate molecule is more likely to be orally active if: a) the molecular weight(MW) is under 500(are easily transported, diffuse and absorbed as compared to heavy molecules), b) the calculated octanol/water partition coefficient (log P) is less than 5 (show good permeability across cell membrane.), (c) there are not more than 5 hydrogen bond donors (N 'OH' and 'NH' groups), (d) there are not more than 10 hydrogen bond acceptors (notably Oxygen and Nitrogen) and (e) No more than one number of violation (NViolations).

Total polar surface area (TPSA) was below 160 Å2 (means compound can easily bind to receptor). Numbers of rotatable bonds (NROTB) were acceptable and it is a simple topological parameter that measures molecular flexibility and is considered to be a good descriptor of oral bioavailability of drugs**.** (Veber *et.al*.2002).

These compounds were then taken for further calculation of bioactivity score (Table 3c).

Table 3c: Bioactivity Score Of The Derivative

COMPOUNDS	MOLINSPI-RATION BIOACTI-VITY SCORE	GPCR LIGAND	ION CHANNEL MODU-LATOR	KINASE INHIBITOR	NUCLEAR RECEPTOR LIGAND	PROTE-ASE INHI-BITOR	ENZYME INHI-BITOR
1.Curcumin	v2011.06	-0.06	-0.20	-0.26	0.12	-0.14	0.08
2.Derivative-11	v2011.06	0.05	-0.14	-0.24	0.19	-0.06	0.16
3.Derivative-16	v2011.06	0.02	-0.27	-0.28	0.13	-0.19	0.07
4.Derivative-20	v2011.06	-0.10	-0.28	-0.29	0.15	-0.21	0.02

The compounds under investigation showed to be biologically active molecules and can produce the physiological actions by interacting with GPCR(G Protein Coupled Receptors) ligands, nuclear receptor ligands, and inhibit protease and other enzymes.

According to the Osiris ADMET molecular property prediction results (Table 3d) though all the compounds have a good physiochemical profile, derivative 7 with high drug score and poor toxic effects qualified as a potent candidate for drug development.

Table 3d: OSIRIS ADMET Molecular Prediction Results Of the Derivatives

COMPOUNDS	MUTAGENIC	TUMORO-GENIC	IRRIT-ANT	REPRODUC-TIVE EFFECTS	C log P	SOLU-BILITY	MOL. WT	TPSA	DRUG LIKELI-NESS	DRUG SCORE
Curcumin	-	-	-	+	3.17	-3.35	382.0	93.06	-5.6	0.23
Derivative11	+	+	+	+	2.36	-5.83	386.0	133.5	-4.05	0.07
.Derivative-16	+	+	+	+	3.52	-5.54	382.0	93.06	-5.13	0.06
Derivative-20	-	-	-	-	3.71	-4.29	366.0	83.83	-5.02	0.34

In vitro studies

The *In vitro* studies of Vancomycin, curcumin and its derivatives were well aggregated with insilico stuides (Figure 10). The antibiotics suseptibility was studied and compared with Vancomycin. When compared to antibiotic Vancomycin, derivatives showed less inhibitory activity on MRSA but showed higher activity than curcumin.

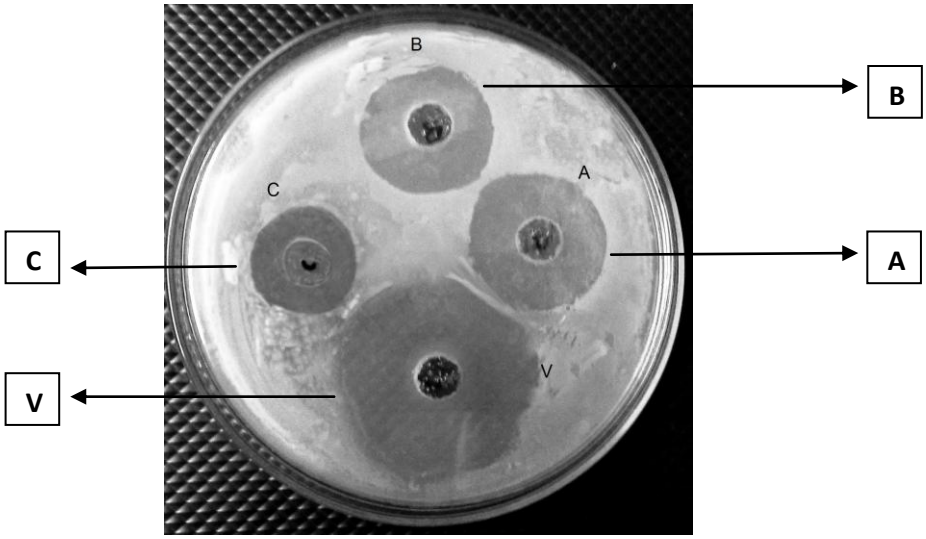

Fig 10: *Invitro* studies: A- derivative 11; B-derivative 16; C-Curcumin; V-Vancomycin

CONCLUSIONS

Comparing the docking values, drug-likeliness, ADME profile and toxicity analysis of the derivatives to the parent compound Curcumin, the derivatives are found to have favourable scores thus suggesting that the problem of poor bioavailability, pharmacokinetics and solubility can be overcome by structural changes, and serve as promising lead candidates for alternative anti MRSA therapy.

The docking results showed that Derivative 11 : 1*E*,6*E*)-1-(4-hydroxy-2-cholorophenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione showed best docking fitness value than other derivatives including Curcumin. The study also revealed that the orientation of compounds in the PBP2a binding pocket surrounding the active site resulted in inhibition of enzyme activity. From the docking results we can conclude that these Curcumin derivatives can act as inhibitory compounds of PBP2a protein and exhibit a promising future to be developed into potent antimicrobial drugs. In silico approaches in the form of molecular, physicochemical, biological properties and toxicity analysis further increase our ability to predict and model the most relevant pharmacokinetic, metabolic and toxicity end points, helping to choose the most appropriate compound thereby accelerating the process of drug discovery. Synthesis of these Curcumin derivatives and invitro confirmation showed the inhibitory activity of the derivatives on MRSA.

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