INVITRO AND IN SILICO STUDIES OF CERTAIN CURCUMIN ANALOGUES' ANTIBACTERIAL EFFECT

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ABSTRACT

Curcumin analogues are widely used in numerous biological disciplines. They are widely employed in the pharmaceutical sector and offer good pharmacology application prospects in the present era. In the current study, two curcumin analogues—(1E,6E)-1,7-diphenylhepta-1,6-diene-3,5-dione (DPHDD) and (1E,6E)-1,7-bis(3,4-dimethoxyphenyl) hepta-1,6-diene-3,5-dione(BDMPHDD)—were tested in vitro against the bacterial strain *Staphylococcus aureus*.The testing demonstrated that the ligands may have antibacterial potential. The Lipinski rule of five was used to pre-filter the compounds' drug-like characteristics prior to computer analysis. Then, to determine the mechanism by which the compounds limit the growth of S. aureus, molecular docking research was carried out using the AutoDock 4.2 tool.Six distinct target proteins from S. aureus were chosen for this purpose (PDB ID: 1T2P, 3U2D, 2W9S, 1N67, 2ZCO, and 4H8E). The target protein *Dihydrofolate reductase* enzyme (PDB ID: 2W9S) and *Staphylococcus aureus sortase-A* (PDB ID: 1T2P) demonstrated a good binding affinity for the two analogues, (BDMPHDD) & (DPHDD) respectively. Due to the inactivation of these enzymes, the substances(1E,6E)-1,7-diphenylhepta-1,6-diene-3,5-dione (DPHDD) and (1E,6E)-1,7-bis(3,4- dimethoxyphenyl) hepta-1, 6-diene-3,5-dione (BDMPHDD) exhibit significant growth-inhibitory potential against *S. aureus*.

Key Words: Curcumin analogue, Antibacterial study, Molecular docking.

INTRODUCTION

Therapeutic research is crucial for lowering the incidence of human diseases and improving human quality of life. Pathogenic organisms are responsible for many diseases. One of these multi-drug-resistant bacteria is *S. aureus*. This bacterium is naturally present on the skin and in the nasopharynx of humans. Infections of the nose, skin, vagina, urethra and digestive system can be brought on by *S. aureus*[1,2]. Although there are many different antibiotics and chemotherapeutic drugs available to treat these bacteria, due to their high cost, only individuals with severely resistant strains should use them. The development of innovative and potent chemotherapy medications is therefore essential for the medical sector.

MATERIALS AND METHODS

1.1 . In silico molecular docking studies

ChemSketch software was used to determine the structure of the curcumin analogues in MOL format, and open babel software was used to transfer the structure to PDB format. Protein structures were retrieved from RCSB PDB in PDB format. Hydrogen atoms were added and the proteins' existing ligands and water molecules were removed using Pymol software before being saved in PDB format.

1.1.1 Lipinski rule of five: According to the Lipinski rule, an orally active medication will be tiny and somewhat lipophilic. According to this criterion, which illustrates molecular characteristics rather than pharmacological

action, a medicine has strong oral activity if it meets the five requirements.

1.1.2 Molecular docking: To determine the mechanism through which the curcumin analogues inhibit bacterial growth, docking tests were conducted. Docking studies were used to determine the binding affinities and interactions of these drugs with various target proteins in *S. aureus*. The PDB IDs for the chosen target molecules were 1T2P, 3U2D, 2W9S, 1N67, 2ZCO, and 4H8E. The protein—curcumin analogue adducts' binding energies were learned using molecular docking calculations using the Auto Dock 4.2 programme [3]. The 3D and 2D interaction graphs of the protein-ligand complexes were created using the software BIOVIA Discovery Studio.

1.2 . Preparation and Characterization of Curcumin Analogues

In an ethyl acetate medium with tributyl borate and n-butyl amine, aldehydes (benzaldehyde and 3,4- dimethoxy benzaldehyde) were coupled with an acetylacetone-boric oxide complex to create curcuminoid mimics. The products were refined using a 5:1 (v/v) chloroform: acetone mixture as the eluent in column chromatography over silica gel (60-120 mesh) and recrystallized twice from hot benzene in order to get pure crystalline content.IR, ^{13}C NMR, ^{1}H NMR, and mass spectrum methods are used to characterize the ligands.

1.3 . In vitro Antibacterial Studies

The Agar well diffusion method is frequently used to assess the test sample's antibacterial activity. Mueller-Hinton agar (15–20 mL) was put onto identical-sized glass petri plates and allowed to set. Using a sterile cotton swab, a standardised inoculum of the test organism was evenly distributed across the surface of the plates. A sterile cork borer was used to aseptically punch four 8 mm-diameter wells spaced 20 mm apart into each plate. The test sample (40 and 80 L) from the 10 mg/ml stock was added into wells T1 and T2. As a positive and negative control, gentamycin (40 l from a 4 mg/ml stock) and the solvent used for sample dilution, respectively, were added. The plates were incubated for 24 h at 36ºC ± 1ºC, under aerobic conditions. After incubation, the plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm.

RESULT AND DISCUSSION

To assess for drug-like qualities, the compounds were first pre-filtered using Lipinski's rule of five. The properties of the two analogues, including their masses, hydrogen bond donors and acceptors, log P (the octanol-water partition coefficient), and molar refractivity were assessed using the rule and are shown in Table 1. According to this rule,an orally active drug is must have less than two violations[4]. Results showed that both the compounds have no violations and hence they obey the Lipinski rule, suggesting that these compounds have the potential to behave as orally active drugs.

It is now crucial to comprehend the process by which the chemicals prevent bacterial development. In order to determine which protein target in bacteria the ligands have the strongest binding affinity for, molecular docking studies were carried out. The greatest binding energy and the number of interactions between the ligand and the active site

residues were used to determine the stability of the protein-ligand complex .Hydrogen bond interactions such as conventional and non-conventional H-bonds, hydrophobic interactions such as pi-sigma, alkyl and pi-alkyl interactions, electrostatic interactions such as pi-anion interactions, van der Waals interaction, and unfavourable pi-donor interactions are commonly seen between protein and ligand. The binding affinity of the compound with the target protein is the result of all the interactions and binding energy existing between them. The highest binding energies (BE) and the number of interactions of the ligands with protein models under study were enlisted in Table 2.

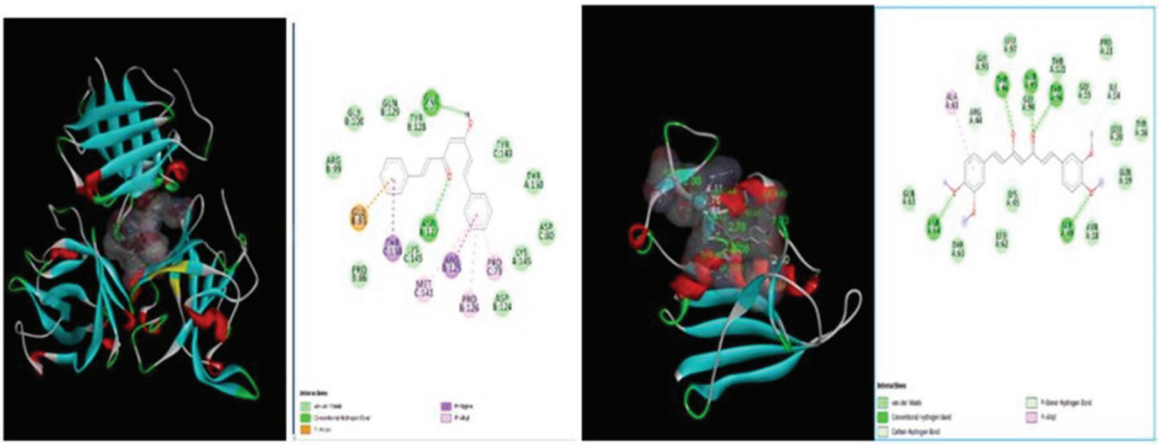
Table 1Lipinski Rule of Five

Lipinski rule of five			
Parameters	**Ligands**	**Conditions for Druglike property**	
	dphdd	**bdmphdd**	
Molecular weight g/mol	276.33	396.43	< 500
Hydrogen Bond Donor	1	1	< 5
Hydrogen Bond Acceptor	2	6	< 10
log P	3.96	3.91	< 5
Molar Refractivity	86.67	112.64	40–130

Table 2 Docking scores and No.of Interactions

Active Target	2W9S		1N67		1T2P		2ZCO		3U2D		4H8E	
Ligands	dphdd	bdmphdd	dphdd	bdmphdd	dphdd	bdmphdd	dphdd	bdmphdd	dphdd	bdmphdd	dphdd	bdmphdd
Active Site and Run	9	1	2	1	7	3	3	1	2	9	3	6
BE(kcal/mol)	-8.7	-9.4	-8.5	-8.96	-9.8	-7.48	-7.62	-6.73	-7.89	-7.3	-9.4	-8.4
Ligand efficiency	-0.4	-0.3	-0.4	-0.17	-0.5	-0.26	-0.36	-0.23	-0.38	-0.3	-0.45	-0.3
inhib constant (nM)	394	120	758.6	231.2	69.5	3.27	2.6	11.6	1.65	4.66	128.6	692
intermolar energy	-11	-12	-10.1	-7.94	-12	-10.5	-9.41	-9.71	-9.68	-10	-11.2	-11
vdw hb disolv energy	-10	-12	-10.1	-7.97	-12	-9.92	-9.39	-9.78	-9.73	-10	-11.1	-11
electrostatic energy	-0.1	-0.1	-0.07	0.02	-0	-0.54	-0.02	0.07	0.05	-0.1	-0.07	-0
total internal	-0.9	-1.2	-1.18	-1.78	-1.2	-1.85	-1.31	-1.94	-1.41	-1.9	-0.7	-1.7
torsional energy	1.79	2.98	1.79	2.98	1.79	2.98	1.79	2.98	1.79	2.98	1.79	2.98
unbound energy	-0.9	-1.2	-1.18	-1.78	-1.2	-1.85	-1.31	-1.94	-1.41	-1.9	-0.7	-1.7
cRMS	0	0	0	0	0	0	0	0	0	0	0	0
refRMS	40.8	39	81.12	86.97	24.4	39.1	78.49	57.6	25	24	26.29	23.7
rseed1	None	None	None	None	None	None	None	None	None	None	None	None
rseed2	None	None	None	None	None	None	None	None	None	None	None	None
Van der Waals	13	14	12	13	11	4	11	8	6	7	11	12
Hydrogen bond	1	5	2	2	2	2	0	1	2	2	2	3
Others	5	3	3	5	6	13	7	6	6	10	5	14
Total Interactions	19	22	17	20	19	19	18	15	14	19	18	29

The following Figures showing the Binding pockets and Protein Ligand Interactions.



The curcumin analogues were synthesized in the wet lab by Pabon's Method and the characterization by various spectral analyses was conducted. Spectral Data are shown in Tables 3,4,5 and 6.

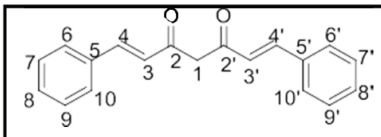
Table 3 IR Data

DPHDD	BDMPHDD	Probable IR Assaignments
3040	2929	ν Enolic
1622	1620	ν (C=O)Chelated
1581	1585	ν (C=C)Phenyl
1512	1507	ν (C-C)Alkenyl
1456	1466	ν as(C-C-C)Chelate ring
1426	1423	ν s(C-C-C)Chelate ring
1145	1121	ν β(C-H)Chelate ring
968	958	ν (CH=CH)trans

Table 4 ^{1}H NMR and Mass Spectral Data

Ligands	Chemical shift(δ) in ppm					Mass spectral data (m/z)
	Enolic	Methine	Alkenyl	Phenyl	Substituent	
DPHDD	10.014	6.78	6.98-7.79	7.31-7.60	---	276,199,173,145,131,103,90,77
BDMPHDD	9.84	6.75	6.81-7.61	7.06-7.14	3.85 (methoxy)	396,259,233,205,191,137,163

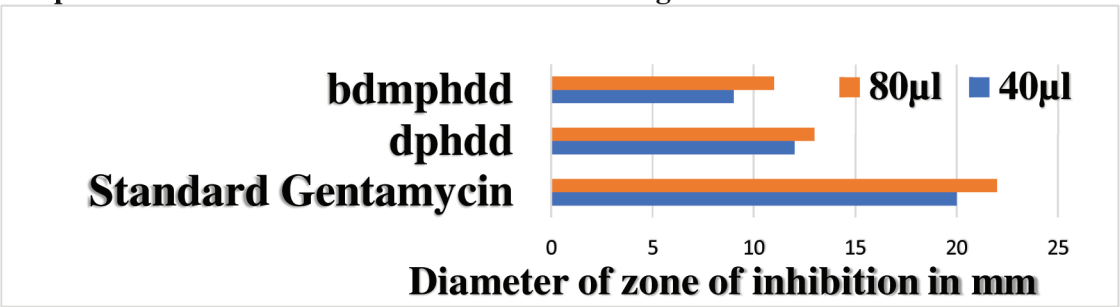
Table 5 ^{13}C NMR Data of DPHDD



C1	C2,C2'	C3,C	C4,C4'	C5,C5'
98.63	200.99	130.26	130.26	133.76
C6,C6'	**C7,C7'**	**C8,C8'**	**C9,C9'**	**C10,C10'**
128.57	128.57	128.57	128.57	128.57

The ligands BDMPHDD & DPHDD have the potential to function as effective antibacterial agents, according to an in vitro antibacterial investigation (Graph 1). Despite having less action than the common antibiotic gentamycin, both BDMPHDD and DPHDD have noticeable growth-inhibitory potential. At an 80 l well-1 concentration, gentamycin's zone of inhibition on S. aureus measured 22 mm in diameter, whereas the analogues BDMPHDD & DPHDD measured 11 mm & 13 mm, respectively. It was discovered that the zone of inhibition grew larger as the chemical concentration did. Thus, they can be consideredgoodantibacterialagentsfor*S.aureus.*

Graph 1 Antibacterial Effects of Curcumin Analogues



CONCLUSION

The two curcumin analogues (1E,6E)-1,7-diphenylhepta-1,6-diene-3,5-dione (DPHDD) and (1E,6E)-1,7-bis(3,4-dimethoxyphenyl) hepta-1,6-diene-3,5-dione (BDMPHDD) exhibit significant growth-inhibitory potential on comparing with the inhibitory power of the standard antibiotic gentamycin against the pathogenic bacteria *S.aureus*. Maximum zone of inhibition of about 13mm and 11mm were shown by DPHDD and BDMPHDD, respectively, at a concentration of 80ìlwell[1]. Both obeyed Lipinski's rule of five and possess drug- like properties. Among the target proteins selected for study, the target protein *Dihydrofolate reductase* enzyme (PDB ID: 2W9S) and *Staphylococcus aureus sortase-A* (PDB ID: 1T2P) demonstrated a good binding affinity for the two analogues, (BDMPHDD) & (DPHDD) respectively which clearly establish that the appreciable growth-inhibitory power of these curcumin analogues against the pathogenic bacteria *S. aureus* is mainly due to deactivation of the enzymes, *Dihydrofolate reductase* and *Staphylococcus aureus sortase-A*.

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