

Synthesis and Anti-Microbial Activity of Novel Substituted Chalcone Derivatives

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Conflict of interest: Nil

Abstract

Chalcone derivatives have attracted considerable research interest due to their potential antimicrobial, antifungal, and insecticidal activities. The present study aimed to synthesize chalcone derivatives and evaluate their antimicrobial activity using in vitro methods, supported by molecular docking studies. All solvents were redistilled before use, and reaction progress was monitored by thin-layer chromatography (TLC), with spots visualized under ultraviolet light or iodine vapour. The synthesized compounds were purified by recrystallization, and their melting points were determined using the open-capillary method. During synthesis, finely powdered zinc chloride (8.25 g) was dissolved in glacial acetic acid (18 mL) by heating, followed by the addition of dry resorcinol (approximately 5.5 g) with continuous stirring. The reaction mixture was maintained at elevated temperatures under the specified experimental conditions. The antimicrobial activity of compounds C1–C6 was evaluated against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* using the cup-plate method. Dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) were used as controls. The results indicated that compounds C3 and C4 exhibited promising antibacterial activity, approaching that of the standard compound amoxicillin. Molecular docking studies revealed that compound C1 showed the maximum negative contribution, with a score of -8.03006 , whereas compound C6 exhibited the lowest negative contribution, at -7.71101 . These findings suggest that the synthesized derivatives may serve as promising candidates for further antimicrobial investigation.

Keywords: chalcones, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, antimicrobial, 2K35, Docking.

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Introduction

A microorganism or microbe is an organism that is microscopic (usually too small to be seen by the naked human eye). Microorganisms are very diverse; they include bacteria, fungi, archaea, and protists; microscopic plants (green algae) and animals such as plankton the planarian and the amoeba. Robert Koch (1843-1910) defined the principles of infectious diseases, namely, that a microbe causing disease in one animal when transferred to another animal produces the same disease (Koch's postulate). Koch also identified *Mycobacterium tuberculosis* and *Vibrio cholera* [1-2]. Broadly, all microbes that can grow in the absence of oxygen are called anaerobic bacteria. They include clostridia, a spore-bearing anaerobe, and Gram-negative bacteria like bacteroides and fusobacteria. Microbes that require

oxygen to grow are called aerobic bacteria. Those that grow in the presence of some oxygen (but not a lot) are called microaerophilic; they include *E. coli*, *neisseria*, *haemophilus* and others. Gram (1853-1938) classified all bacteria by the colour they take with the Gram's stain. Those that take a blue colour (Gentian violet) are called Gram-positive, and those that take the red stain (eosin) are called Gram-negative [3-4]. Some chalcones derivatives show antimicrobial, anti-fungal and insecticidal activity. Some of chalcones derivatives are isolated with the anti-cancer activity [5, 6]. Moreover, chalcones derivatives are also regarded as a new class of effective anti-TB candidates owing to their potential anti-TB activities.

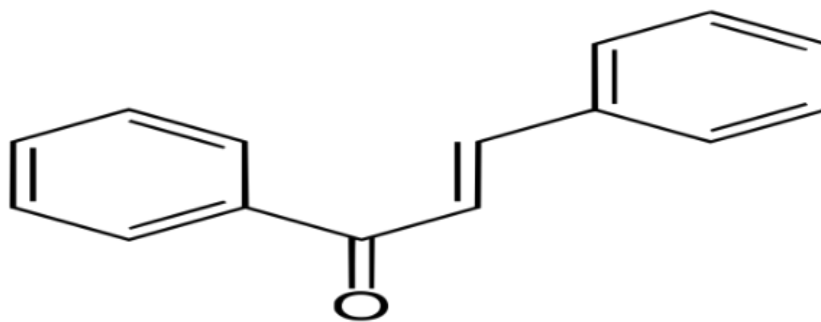
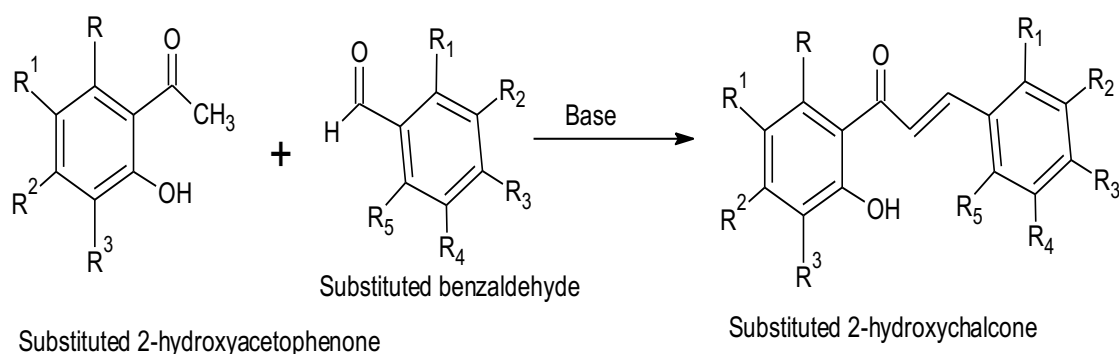


Figure 1: structure of chalcone

Materials And Methods:

All chemicals were provided from our college. All solvents were redistilled before use. Reactions were routinely monitored by thin layer chromatography and spots were visualized by exposure to iodine vapour or UV light. All the synthesized compounds were purified by recrystallization. Melting points were determined by using open capillary method. Fourier Transform Infra-Red spectra (FTIR) were recorded on Shimadzu FTIR-8400S spectrophotometer using potassium bromide pellets and sodium

chloride cell. Nuclear Magnetic Resonance spectra ($^1\text{H-NMR}$) were recorded on JEOL-300 MHz spectrophotometer in CDCl_3 using TMS as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm). Mass spectra were recorded on HEWLETT 180017, PACKARD GCD System mass spectrophotometer using electron ionization detector and anticonvulsant activity checked by electroconvulsometer.

Synthetic Scheme:**Methods:****Synthesis of 2, 4-dihydroxyacetophenone:**

Fine powdered zinc chloride (8.25) was dissolved in glacial acetic acid (18ml) by heating on sand bath then dry resorcinol (appx.5.5 gm) was added with continuous stirring to the mixture at 140°C . The

solution was heated until the solution just begins boil and kept it for 20 min at 150°C temperatures. Dilute HCl was added to mixture and cooled at the temperature of 5°C then filter & washed with dil. Hydrochloric acid (1:3) and crystallized from hot water containing a little HCl [7].

Table 1: Physicochemical characterization of starting materials

Sr. No.	Compound	Mol. Formula	Mol. Weight	M.P./ B.P.
1	2-Hydroxyacetophenone	$\text{C}_8\text{H}_8\text{O}_2$	136.14	213°C
2	2,4-Dihydroxyacetophenone	$\text{C}_8\text{H}_8\text{O}_3$	152.14	143°C
3	Salicylaldehyde	$\text{C}_7\text{H}_6\text{O}_2$	122.12	-
4	4-Methylbenzaldehyde	$\text{C}_8\text{H}_8\text{O}_2$	120.15	-
5	4-Isopropylbenzaldehyde	$\text{C}_{10}\text{H}_{12}\text{O}$	148.20	235°C

General procedure for synthesis of chalcone derivatives:

2.5 mmol of the benzaldehyde derivative and 2.5 mmol of the acetophenone derivative were measured accurately and placed in a mortar. To this added 3 pellets of NaOH (7.5 mmole). Mixture was ground

for about 5–10 min. The mixture was turned yellow and pasty after a few times of grinding. Grinding was continued until the mixture turn into a solid mass and the solid became powdery. 10 mL of water added to the mortar at the last minute of the grinding period. Product was mixed properly with the water

using the spatula solid product was dislodged from the mortar's wall and filtered [8].

Anti-Bacterial Activity:

All the compounds synthesized in the present investigation were screened for their anti-bacterial activity by Cup plate Method [9]. Antibacterial activities were tested on nutrient medium against, *Staphylococcus aureus* and *Escherichia coli* which are representative types of gram positive and gram negative organisms respectively. The antibacterial activities of the compounds were assessed by disc-diffusion method.

Preparation of nutrient agar media:

Media Composition and Procedure:

The nutrient agar media were prepared by using the following ingredients.

- | | |
|-----------------------------------|---------|
| 1) Peptone (Bacteriological) | 20 gm |
| 2) Beef extract (Bacteriological) | 5 gm |
| 3) Sodium chloride | 5 gm |
| 4) Agar | 20 gm |
| 5) Distilled water up to | 1000 ml |

Weighed quantities of peptone and beef extract were dissolved in distilled water by gentle warming and then specified amount of agar were dissolve by heating on water bath. Then the pH of the solution was adjusted to 7.2 to 7.4 by adding the sodium chloride and the volume of the final solution was made up to 1000 ml with distilled water. Then it was transferred into a suitable container, plugged with non-adsorbent cotton and the media were sterilized in autoclave at 121°C for 20 minutes at 15 lbs pressure [10].

Preparation of test solutions:

10 mg of the compound was dissolved in 10 ml of DMF. From this 1 ml of solution was taken and diluted up to 10 ml with DMF. Now the concentration of the test solution was 100 µg/ml. From the stock solution 1ml of solution was taken and diluted with 1ml of DMF now the concentration was 50µg/ml [11].

Preparation of Standard Antibiotic Solution:

Amoxicillin was used as standard antibiotic for comparison and solution was prepared by using sterile water, as it is water-soluble. The solution was diluted by using sterile water so that the concentrations of the solution were 100 µg/ml and 50 µg/ml [12].

Preparation of Discs:

Discs of 6-7 mm in diameter were punch from No. 1 Whatmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C for 60 minutes. Then standard and test solutions were added to each disc and discs were air-dried.

Method of Testing:

The sterilize media was cooled to 45°C with gentle shaking to bring about uniform cooling and then inoculate with 18-24 hrs old culture under aseptic conditions, mix well by gentle shaking. This was poured in to sterile Petri dishes (properly labeled) and allowed the medium to set. After solidification all the Petri dishes were transfer to laminar flow unit. Then the discs, which were previously prepared were carefully kept on the solidified media by using sterilized forceps. These Petri dishes were kept as it is for one-hour diffusion at room temperature and then for incubation at 37°C for 24 hours in an incubator [13].

The diameter of inhibition after 24 hours was measured as the zone of inhibition in milli meters.

Docking Analysis:

Docking analysis is important to study the ligand-enzyme interaction. For Docking analysis all compounds were selected to evaluated anticancer activity as series under consideration. Docking was done using to PDB code 2K35, using Maestro 9.0. The molecular docking tool, GLIDE was used for ligand docking studies into the EGFR-TK pocket. All designed compounds have good docking score on the PDB as shown in table.

PDB Description: 2K35

The crystal structure of the kinase domain from the epidermal growth factor receptor (EGFRK) including forty amino acids from the carboxyl-terminal tail has been determined to 2.6-Å resolution. The EGFR family members are distinguished from all other known receptor tyrosine kinases in possessing constitutive kinase activity without a phosphorylation event within their kinase domains. Despite its lack of phosphorylation, we find that the EGFRK activation loop adopts a conformation similar to that of the phosphorylated active form of the kinase domain from the insulin receptor. Surprisingly, key residues of a putative dimerization motif lying between the EGFRK domain and carboxyl-terminal substrate docking sites are found in close contact with the kinase domain. Significant intermolecular contacts involving the carboxyl-terminal tail are discussed with respect to receptor oligomerization.

Results and Discussion

The physical characteristics of six substituted chalcone derivatives (C1–C6) were evaluated based on melting point, percentage yield, TLC R_f value, molecular formula, and molecular weight. The melting

points ranged from 89°C to 190°C, while the percentage yields varied from 80.4% to 94.20%. Compound C3 exhibited the highest yield (94.20%), whereas C4 showed the highest melting point

(190°C). The R_f values ranged from 0.59 to 0.80, indicating differences in chromatographic behavior among the synthesized compounds.

Table 2: Physical characteristics of substituted chalcone derivatives

Property	Results					
Comp. Code	C1	C2	C3	C4	C5	C6
Melting point	89 ⁰ C	92 ⁰ C	160 ⁰ C	190 ⁰ C	150 ⁰ C	150 ⁰ C
Yield	80.4 %	91%	94.20%	90.03%	88.8%	88.8%
R _f value	0.69	0.8	0.73	0.59	0.69	0.69
Molecular formula	C ₁₅ H ₁₂ O ₃	C ₁₈ H ₁₈ O ₂	C ₁₆ H ₁₄ O ₂	C ₁₅ H ₁₂ O ₄	C ₁₈ H ₁₈ O ₃	C ₁₆ H ₁₄ O ₃
Molecular weight	240	266	238	256	282	254

Screening of anti-microbial activity:

Table no. 3: Zone of inhibition of synthesized compounds

Sr.No	Compound code	Concentration µg/ml	E.coli	S.Aureus
1	C1	50	11	10
2		100	12	11
3	C2	50	11	9
4		100	13	11
5	C3	50	9	10
6		100	11	12
7	C4	50	10	11
8		100	12	14
9	C5	50	12	13
10		100	14	14
11	C6	50	10	9
12		100	12	10
13	Amoxicilline	50	14	13
14		100	15	14

Note: Range of Zone of inhibition of synthesized compounds: * 6-8 mm poor activity, 9-11 mm moderate activity, 12-15 above good.

Strain of E.Coli Strain of S. Aureus
Conc. 50µg/ml

Strain of E.Coli Strain of S. Aureus
Conc. 100µg/ml

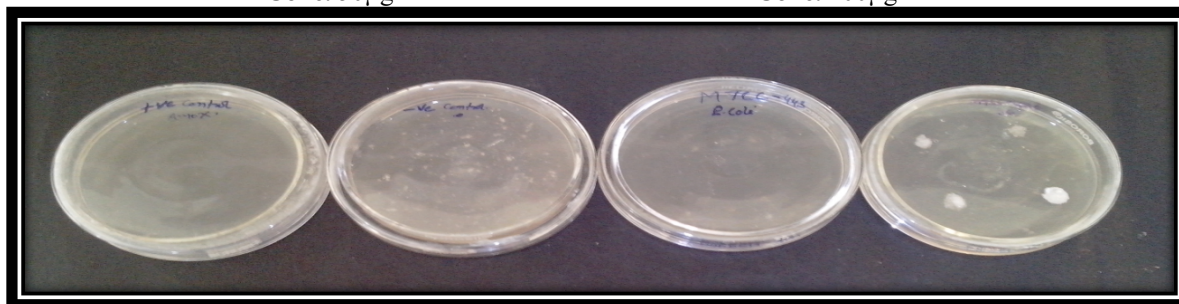


Fig 2: Zone of inhibition of comp C1

Strain of E. Coli Strain of S. Aureus
Conc. 50µg/ml

Strain of E. Coli Strain of S. Aureus
Conc. 100µg/ml

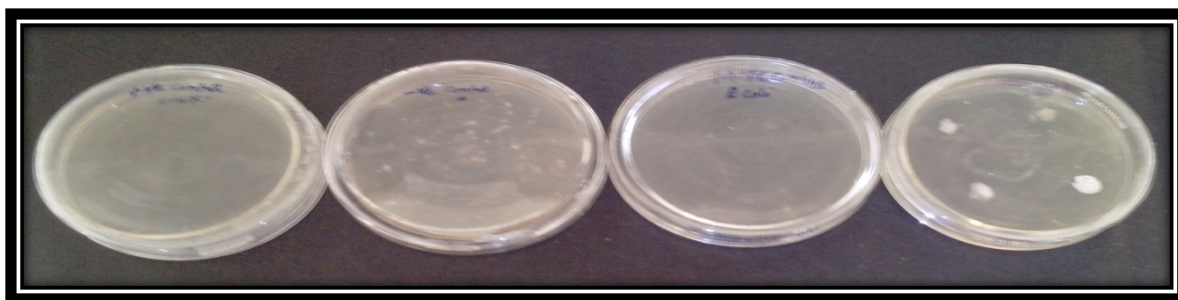


Fig 3: Zone of inhibition of comp C2

Strain of E.Coli Strain of S. Aureus
Conc. 50µg/ml

Strain of E.Coli Strain of S. Aureus
Conc. 100µg/ml

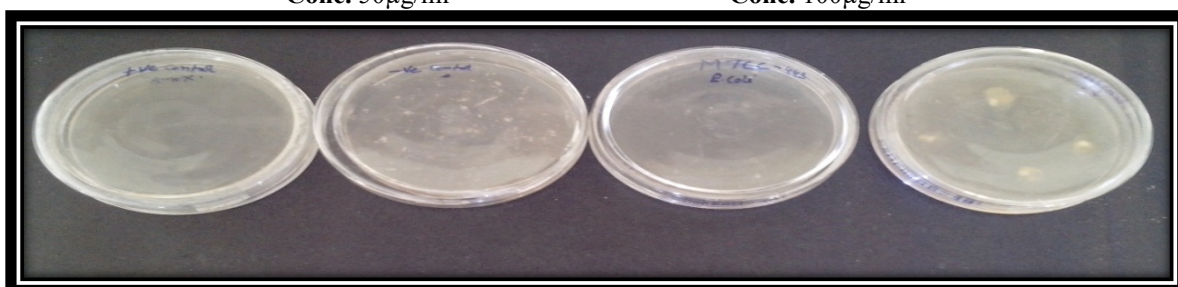


Fig 4: Zone of inhibition of comp C3

Strain of E.Coli Strain of S. Aureus
Conc. 50µg/ml

Strain of E.Coli Strain of S. Aureus
Conc. 100µg/ml

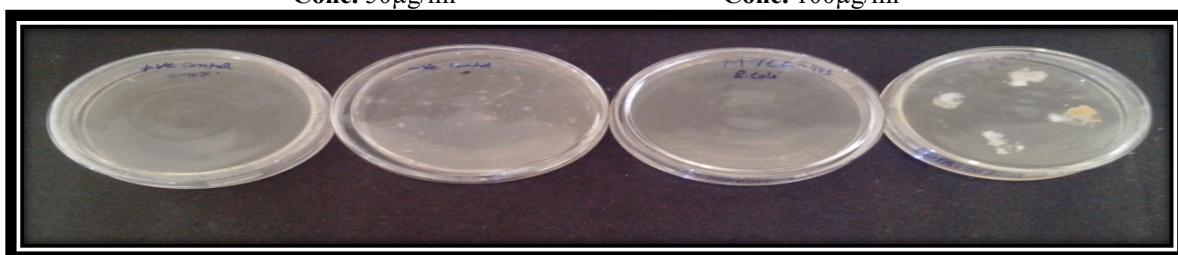


Fig 5: Zone of inhibition of comp C4

Strain of E. Coli Strain of S. Aureus
Conc. 50µg/ml

Strain of E. Coli Strain of S. Aureus
Conc. 100µg/ml

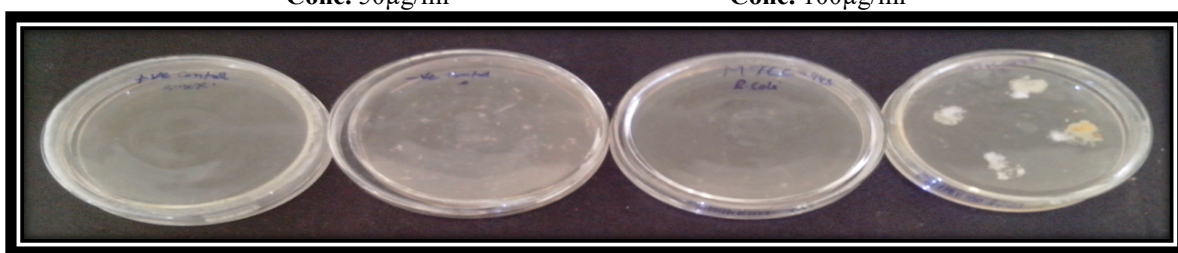


Fig 6: Zone of inhibition of comp C5

Strain of E. Coli Strain of S. Aureus
Conc. 50µg/ml

Strain of E. Coli Strain of S. Aureus
Conc. 100µg/ml

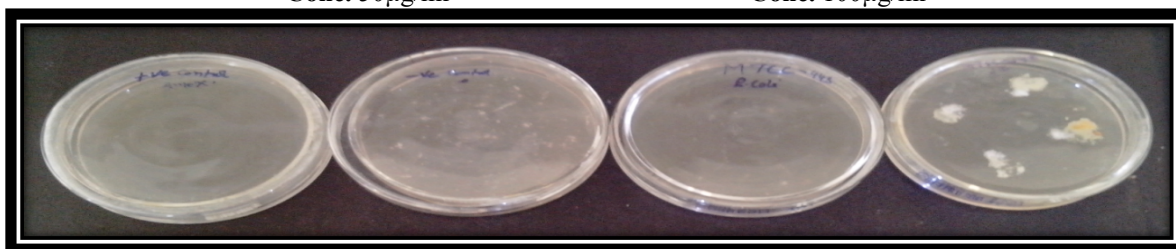


Fig 7: Zone of inhibition of comp C6

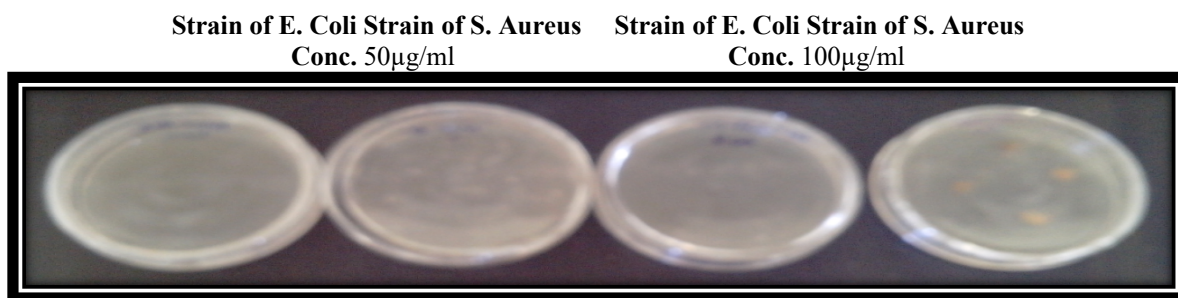


Fig 8: Zone of inhibition of Amoxicillin (Standard)

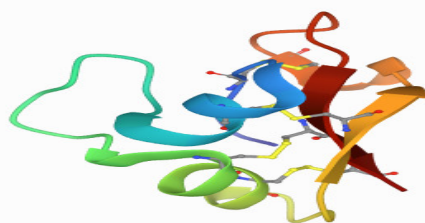
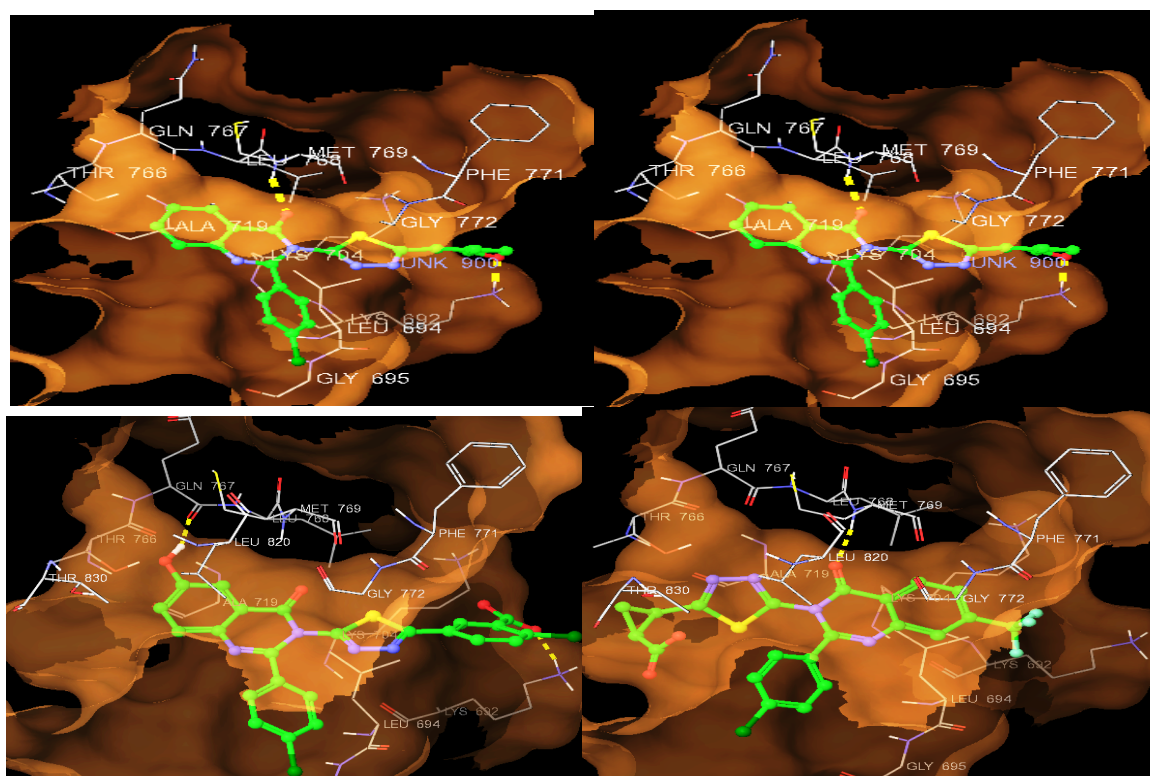


Fig 9: PDB View 2K35



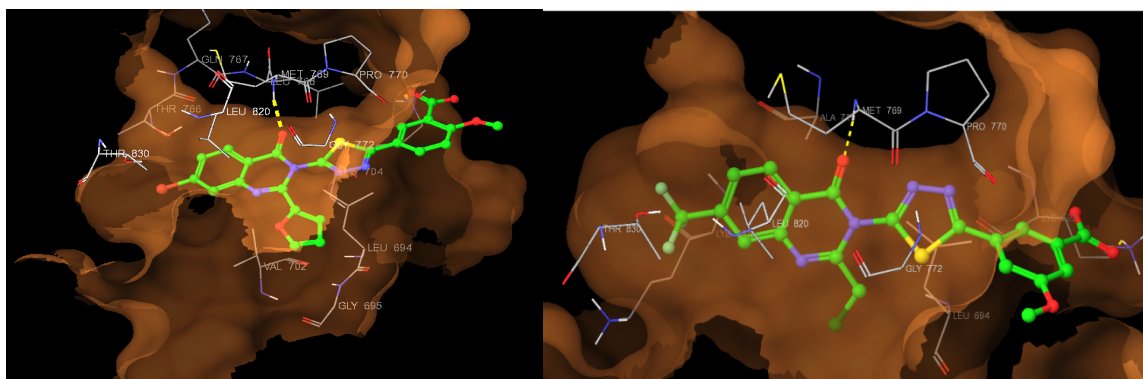


Fig 10: Docking pose of synthesized compounds C1-C6

Table 4. Molecular Docking and ADMET Prediction of Substituted Chalcone Derivatives (C1–C6)

T i t l e	R M S D	Doc kin g sco re	Gli de ev dw	Gli de em ode l	Gli de en erg y	Glid e ein ter nal	Do no rH B	Ac cp th B	QP log Po/ w	Q Pl o g S	QP log HE RG	QP s PC ac o	QP P M DC K	QP log Kh sa	Percent Human Oral Ab sorption	Rule of Fiv e
C 1	0.028859	-8.03006	-39.9366	-82.9757	-53.7226	8.343221	2	9.5	2.211	-5.292	-4.123	10.832	13.242	-0.221	58.413	0
C 2	0.009363	-7.81076	-37.1224	-75.1048	-50.252	3.499143	2	8.75	3.771	-6.706	-4.954	21.093	33.858	0.25	59.768	1
C 3	0.008341	-7.80312	-41.4639	-78.679	-51.6437	5.531269	1	8.75	4.541	-7.19	-5.12	59.252	190.55	0.295	72.303	1
C 4	0.011714	-7.79771	-37.8445	-77.5613	-50.3613	4.783609	2	8.75	3.895	-6.914	-5.03	19.237	64.199	0.216	59.778	1
C 5	0.010529	-7.7112	-43.1603	-80.5693	-52.8868	6.257923	1	8.75	5.263	-8.252	-5.182	57.141	441.692	0.504	63.291	2
C 6	0.013159	-7.71107	-44.58	-81.6336	-54.0733	3.10326	1	8	5.153	-7.965	-5.133	60.794	583.095	0.473	63.126	2

The molecular docking study evaluated six substituted chalcone derivatives (C1–C6) to investigate their potential interactions with the selected biological target. Compound C1 exhibited the most favorable docking score of -8.03006 , indicating the strongest predicted binding among the tested compounds. Compounds C2, C3, and C4 showed comparable docking scores of -7.81076 , -7.80312 , and -7.79771 , respectively. Compounds C5 and C6 demonstrated slightly less favorable scores of

-7.71120 and -7.71107 . Compound C1 also showed a QPlogPo/w value of 2.211 and predicted human oral absorption of 58.413%. Compound C3 displayed higher predicted oral absorption (72.303%) and a favorable QPlogHERG value of -7.19 . However, compounds C5 and C6 showed two Lipinski rule violations, suggesting possible limitations in their drug-like properties. Overall, compound C1 emerged as the strongest docking candidate, while C3 showed promising predicted oral absorption.

These computational findings require experimental validation to establish antimicrobial efficacy and pharmacological potential.

Conclusion:

All the compounds C1-C6 were evaluated for antimicrobial activity against gram positive bacteria, *Staphylococcus aureus*, gram negative bacteria *Escherichia coli*, and fungi *Candida albicans*. The results suggested that in the compound C3 presence of electron withdrawing group -CHO, and compound C4 having methyl group -CH₃ is important for the antibacterial activity. All three compounds C3 and C4 have near to activity significant with standard compound amoxicilline, other synthesized compounds were found to be less active than amoxicilline as standard drugs. Compound C1, C3, C5 shown growth of fungus in petridish, other compounds having shown no growth so they possess a capacity of antifungal activity. According to docking studies compound C1 shown as maximum negative contribution -8.03006 and C6 shown less contribution -7.71101, so binding affinity of ligand with protein was highest found in Compound C1.

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