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Development of Chalcone-Aspirin Conjugates: A Novel Approach for Antimicrobial Potential

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Abstract

Traditional antibiotics are often restricted by resistance, adverse effects, and diminishing potency. Hybrid drug design, which combines two pharmacophores in a single molecule, offers a new alternative. Chalcones, flavonoid derivatives possessing α, β-unsaturated carbonyl system, exhibit antibacterial, anti-inflammatory, and antidiabetic properties. The Claisen-Schmidt condensation is used to synthesize the chalcones, which are then conjugated with aspirin fragments by esterification or amidation reactions. Spectroscopic methods including mass spectrometry, NMR, and infrared spectroscopy are used for structural analysis. The cup plate approach was used to assess the generated compounds' antibacterial activity against E. coli. Antimicrobial activity was determined using molecular docking experiments utilizing PyRx software. Using spectroscopic investigation, including IR, NMR, and mass spectroscopy, as well as preliminary testing, the chalcone-aspirin conjugate was produced and examined. Compound 17 was evaluated for antibacterial effectiveness using the cup plate method and showed a binding affinity of -7.7 kcal/mol against a microbial target by molecular docking. The synthesized chalcone-aspirin conjugates showed promising antibacterial activity, as evidenced by spectroscopic analysis and biological assessment. This work demonstrates the possibility of hybrid drug design for the development of innovative treatment medicines against resistant illnesses.

Keywords: Synthesis, Molecular docking, Aspirin, Chalcone, Derivatives, Antimicrobial

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Introduction

Rummage for new therapeutic agents with improved effectiveness and multifunctional qualities has been fueled by the rising incidence of metabolic diseases including diabetes and antibiotic resistance. Traditional antibiotics frequently have problems, such as decreased efficacy, drug resistance, and unfavorable side effects. This has brought attention to the necessity for creative methods in drug development. The synthesis of hybrid molecules, which integrate numerous pharmacophores into a single structure to boost potency and selectivity, is one potential method.

The varied pharmacological effects of chalcones, a subclass of flavonoids with a β-unsaturated ketone component, have garnered a lot of attention. These substances have strong antidiabetic, antioxidant, antibacterial, and anti-inflammatory effects. By interfering with microbial cell membranes and enzymatic processes, they can prevent the development of bacteria. The well-known non-steroidal anti-inflammatory drug (NSAID) aspirin (acetylsalicylic acid) is widely utilized due to its anti-inflammatory properties (Nordin *et al.,* 2020; Bandi *et al*., 2024; Belfiore *et al*., 2024; Sheshadri *et al*., 2024; Uneno *et al*., 2024).

Chalcone-aspirin conjugates hybrid molecules that combine the advantageous effects of both drugs within a single framework have been investigated as a way to improve the therapeutic potential of both chalcones and aspirin. This logical approach to medication design seeks to overcome the drawbacks of individual pharmacological regimens while achieving synergistic antibacterial action (Aloufi *et al*., 2022; Zhang *et al.,* 2025). The main aims of this project are to create, study, and test chalcone-aspirin conjugates as possible drugs that work in. Changes are made to the basic pharmacophores to make them more stable and better at targeting specific biological sites (Beibalaeva *et al*., 2022; Osipchuk *et al*., 2022; Col & Tunc, 2024). A panel of bacterial pathogens is used to assess these conjugates' antibacterial activity. Computational molecular docking studies also shed light on how they bind to targets of metabolic enzymes and microorganisms. Through the creation of these chalcone-aspirin hybrids, research advances the design of hybrid drugs and provides fresh approaches to treating metabolic and infectious illnesses (Mei & Jiang, 2022; Samaranayake *et al*., 2024; Ali *et al.,* 2025). In order to meet the pressing demand for safer and more efficient antimicrobial

treatments, the results of this study may open the door for future clinical uses (Rammohan *et al*., 2020; Alharbi *et al*., 2022; Chidambaranathan & Culathur, 2022; Patatou *et al*., 2022; You *et al*., 2023; Menhadji *et al*., 2024; Pavlova, 2024).

Materials and Methods

All reagents were commercially available, such as Sigma Aldrich, Fine Chem Lab, and Loba Chem, and were used with distillation purified solvents. Thin Layer Chromatography was used to monitor the reactions' development, using butanol and toluene as the mobile phases. The melting point was determined with an open capillary. The chemical was purified using the recrystallization technique. Spectral analysis methods such as FTIR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy were employed to characterize and identify the synthesized chemical (Dhanasekar *et al*., 2022; Saravanakumar *et al*., 2022; Ekpo *et al*., 2023; Eteng *et al*., 2023; El-Atawy *et al*., 2024). Virtual screening was carried out utilizing Autodock vina software. The cup plate method was used to conduct an antimicrobial research.

Experimental Procedure

Molecular Docking Study

Twenty aspirin-chalcone conjugates were prepared with help of chemsketch software. The Protein Data Bank provided the target protein crystal structure of E. coli PDB ID: 1KZN (DNA gyrase), whereas the PubChem database provided the standard ampicillin. To predict the binding relationship and score between the protein and ligand, a molecular simulation was conducted using AutoDock Vina in PyRx virtual screening, an open-source docking application. The protein-ligand docking process has been managed by Vina Wizard (Welday *et al.,* 2021; Arios-Caro *et al*., 2022; Rudayni *et al*., 2022). The grid's size can be changed based on the active site residues that are chosen for docking; moreover, AutoDock Vina is operated. Software called Discovery Studio Visualizer was utilized to examine the target-ligand interaction.

Synthesis of Hydroxy Chalcone

Mix the 4-hydroxy Benzaldehyde and Acetophenone in an appropriate solvent (such as ethanol). At room temperature or a little higher, whisk the mixture after adding a few drops of base (NaOH). Once the reaction hydroxy chalcone is complete, neutralize the mixture with dilute acid (HCl) to precipitate the chalcone (Basappa *et al.,* 2021). Filter and purify the resulting chalcone compound.

Synthesis of Aspirin

Put two grams of salicylic acid in a dry conical flask. 5 mL of acetic anhydride should be added. To catalyze the process, add three to four drops of concentrated sulfuric acid (H_2SO_4). To combine the solution, gently swirl. Utilize vacuum filtration to filter the precipitate. Use cold water to rinse the solid to get rid of any remaining acetic acid. Dissolve the crude aspirin in warm ethanol or a small amount of hot water. Cool the solution to recrystallize pure aspirin. Filter and dry the purified aspirin crystals (Salehi *et al.,* 2021).

Development Aspirin-Chalcone Conjugate

In a dry reaction flask, dissolve aspirin in dry DCM or THF under a cold atmosphere. Add DCC (1.2 equivalents) to activate the carboxyl group of aspirin. To create the active ester intermediate, stir the reaction for 30 minutes at 0°C. Chalcone should be dissolved in dry DCM in a different flask. To speed up the reaction, add a little quantity of DMAP and a few drops of TEA. While stirring, add the chalcone solution to the activated aspirin solution. For eight to twelve hours, let the reaction continue at room temperature while stirring constantly. After finishing, filter out (Lu *et al.,* 2018, Lodhi, 2021).

Identification and Characterization of Aspirin-Chalcone Conjugates

The aspirin-chalcone conjugate was successfully manufactured and studied using a variety of spectroscopic techniques, including mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and infrared (IR) spectroscopy.

Anti-Microbial Assay

The agar cup plate technique was used to assess antibacterial activity. Media such as nutrient broth culture are chosen for microbiological testing. To prevent aggregation, this broth was evenly distributed to petri dishes and then left to harden. Microbes were added to the broth and left at 25°C for 24 hours. Chalcone-aspirin conjugate was then diluted to 10 ug/ml, 20 ug/ml, 30 ug/ml, and 40 ug/ml. Additionally, their zone of inhibition was noted (Ardiansah *et al.,* 2019, Mohamed-Ezzat, 2024).

Molecular Dynamic Study

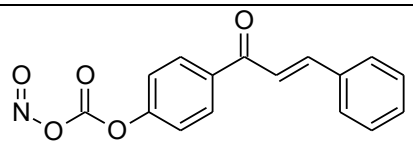
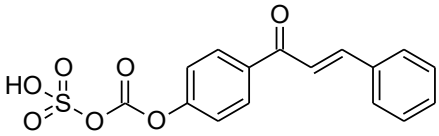
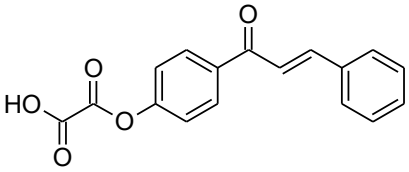
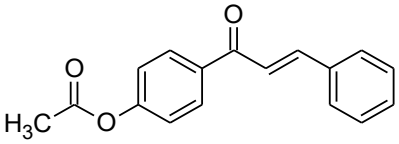
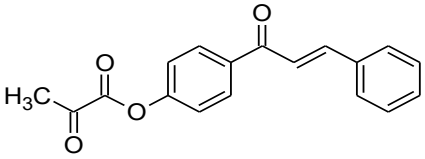
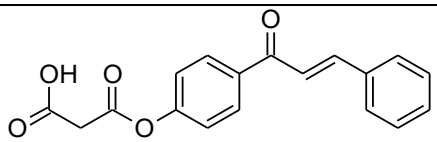
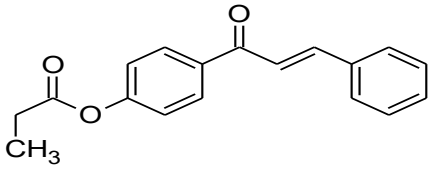
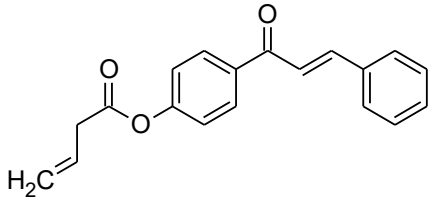
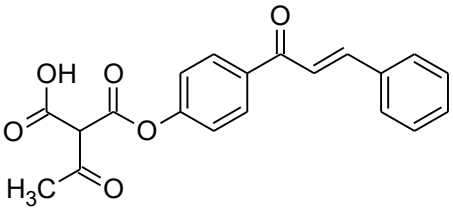
Molecular dynamics simulations of intricate receptor-ligand combinations are carried out using molecular dynamics models. The docked structure with the lowest docking energy underwent complex mobility and protein stability testing. Molecular dynamics simulations are available on the mods server (http://imods.chaconlab.org). By creating feasible transition paths between two homologous structures, iMODS makes it easier to examine such modes. The iMOD server determines the internal coordinates (NMA) of the protein and assesses its stability using normal mode analysis (Rani *et al.,* 2019). Protein stability is demonstrated using elastic network models, B-factor values, eigenvalues, covariance matrices, and backbone deformation diagrams.

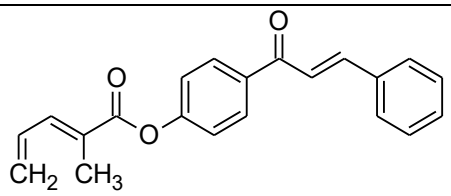
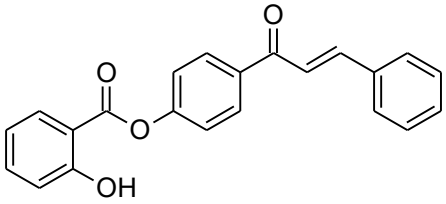
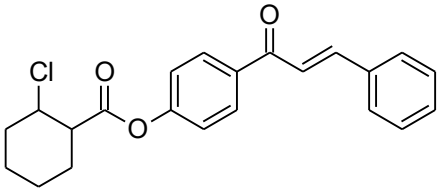
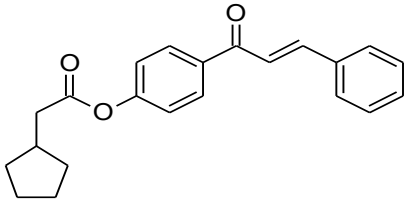
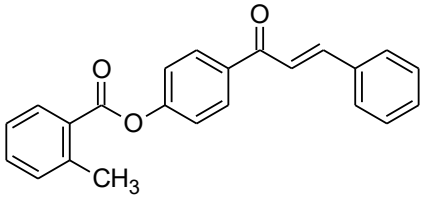
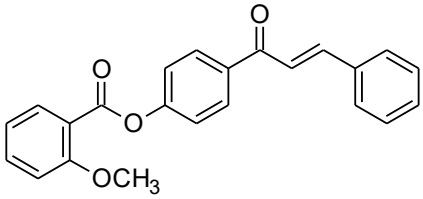
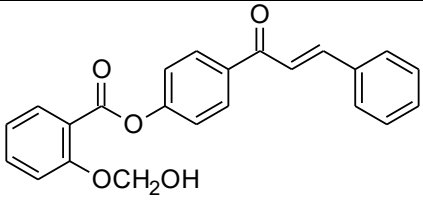
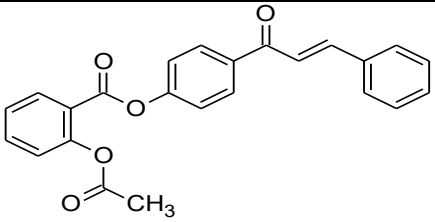
Results and Discussion

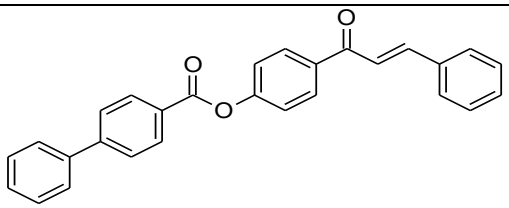
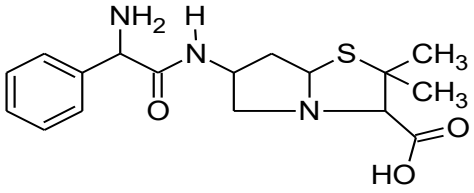
Docking Studies as PBP Interfere

For the produced compounds, Autodock Vina software was used to conduct molecular docking exploratory research. As a reference for penicillinase binding protein (PBP), which prevents the formation of bacterial cell walls, ampicillin was added. This investigation was conducted to determine the ligands' docking score with target protein 1KZN. The binding energies (kcal/mol) were presented in **Table 1**.

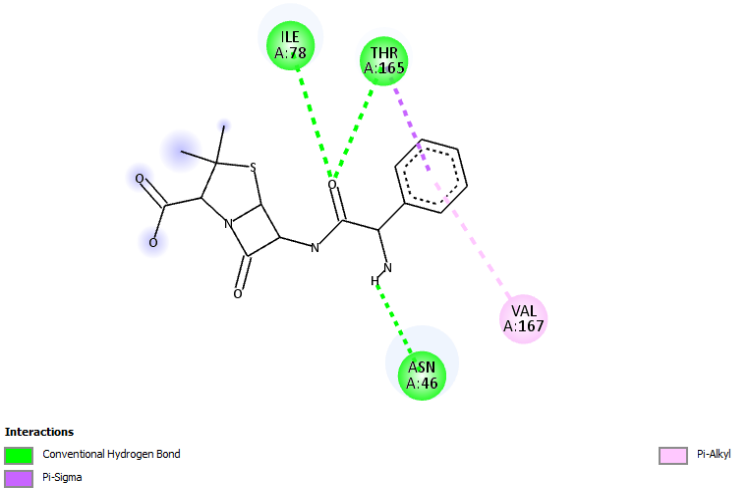
Table 1. Derivatives of Chalcone-Aspirin Conjugate

Compounds	Derivatives	Binding energies (kcal/mol)
1		-6.5
2		-6.7
3		-6.7
4		-6.3
5		-6.8
6		-6.7
7		-6.4
8		-6.3
9		-6.5

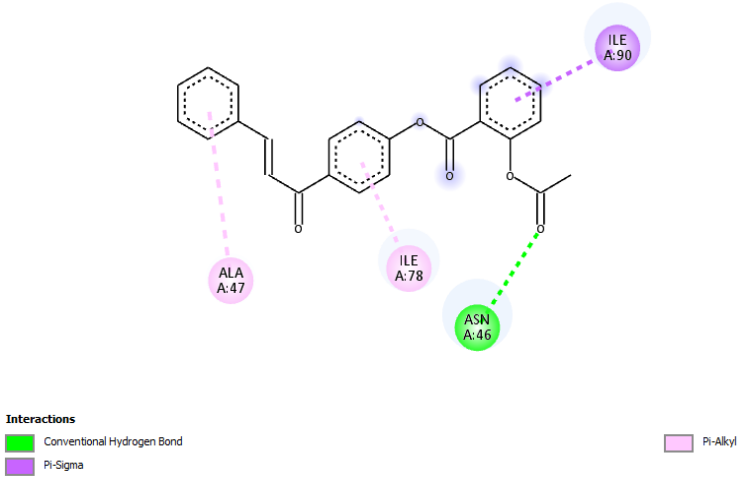
10		-6.7
11		-6.4
12		-6.5
13		-6.7
14		-6.8
15		-6.5
16		-5.9
17		-7.7

18		-6.0
19 (Standard: Ampicillin)		-7.9

Aspirin-Chalcone derivatives were prepared using Chemsketch software and evaluated for their binding affinity. Among the tested compounds, the Aspirin-conjugated chalcone derivative (Compound 17) exhibited the highest binding score of -6.9, for microbial target which is near to standard. Based on these findings, this derivative was selected for further synthesis and subsequently characterized. As depicted in **Figure 1**, the standard ampicillin and synthesized compound 17 has promptly fitted in to pocket of receptors. The manner of intraction of Compound 17 is near to same to standard. Therefore it concluded that compound 17 has prominent antimicrobial potential. Also, Compound 17 has huge crowded hydrogen bonding and hydrophobic bonding environment at the nucleus.



a)



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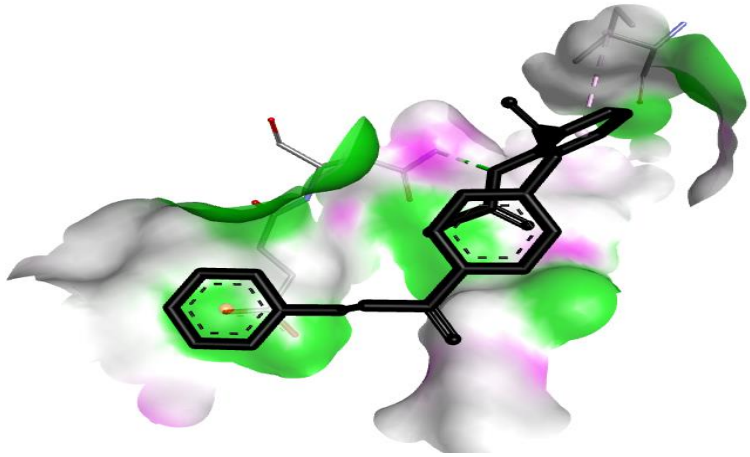
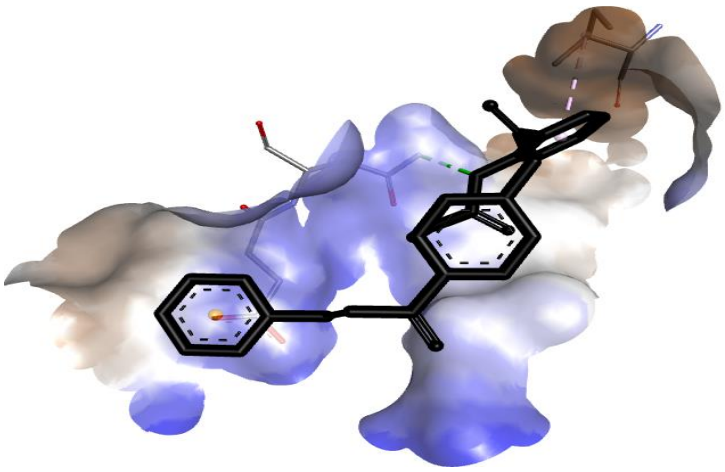


c)



d)

Figure 1. Interaction of Ampicilin and Conjugate Aspirin-Chalcone. a) Ampicillin with target protein, b) Aspirin-chalocone with target protein, c) Hydrogen surface of Aspirin-chalcone, d) Hydrophobic surface of Aspirin-chalcone.

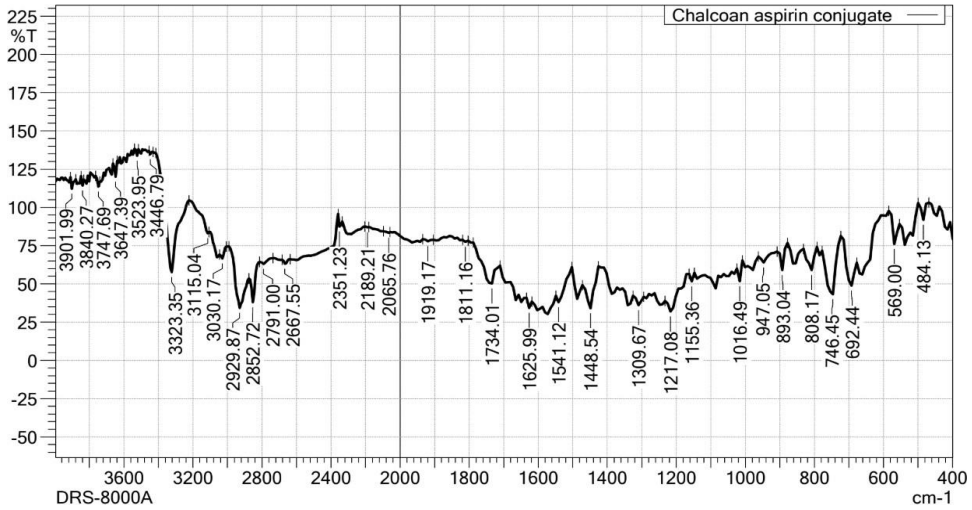
Experimental Study

Chemistry

A Biotech electric melting point device was used to measure and refine the melting point. A Shimadzu FTIR was used to record the infrared spectra using potassium bromide (KBr) disks. An Advance Neo NMR spectrometer (Bruker, U.S.A.) was used to perform the ^{1}H NMR spectra studies at 500 MHz using tetramethylsilane (TMS) as the internal standard (IS) in deuterated dimethyl sulfoxide (DMSO). Parts per million (ppm) were used to report the chemical changes (δ). 13C NMR spectra were recorded using a Bruker 100 MHz NMR spectrophotometer located in Bruker, USA. In the Thermo Scientific MS model, the mass spectra were recorded using a Shimadzu Synapt EX mass spectrometer (Shimadzu, Japan) equipped with an ionization energy of 70 eV, a source temperature of 200°C, an accelerative voltage of 8 kV, and a Direct Probe Controller Inlet component to a Single Quadruple mass analyzer. All of the elemental analysis and spectrum measurements were carried out. The elemental analysis of synthesized compound 17 was good. TLC was used to verify the synthetic chemicals' purity (Nainu *et al.,* 2025).

Chalcone-Aspirin Conjugate

The synthesized Compound 17 was yellowish solid phenolic odor compound having soluble in organic solvent like Ethanol, Methanol and Chloroform; yield 95%; m.p. 118^{0}-120^{0} C, R_f value – 0.75 (n- hexane and chloroform 1:1) IR(KBr) O-H, stretching (3323 cm^{-1}), C=O overtone (1741 cm^{-1}), and aromatic C=C stretching (1652 cm^{-1} and 1449 cm^{-1}), Additional bended C-O (1271 cm^{-1} and 1217 cm^{-1}), ^{1}H-NMR (DMSO, ppm) CHO 9.77, Ar-H 7.0-8.0, OH, CH 3.82, CH_2 1.3-2.0, CH_3 0.86; ^{13}C-NMR (DMSO-d6, 100MHz) CO 190.74, Ar-C 172.69-148.23, CH_3 132.00-106.64, EI-MS: Molecular formula: Em (Es, Positive mode) m/z (386.9728): Anal, Calc for ($C_{23}H_{16}O_5$): C, 74.22; H, 6.47; O, 19.87 Found: C, 74.64; H, 6.48; N, 20.90. **Figure 2** depicted the spectrum of synthesized hydroxy chalcone-aspirin conjugate FTIR, ^{1}H-NMR, ^{13}C-NMR and Mass spectrum.


Chalcoan aspirin conjugate
%T
DRS-8000A
cm-1
3901.99
3840.27
3747.69
3647.39
3523.95
3446.79
3323.35
3115.04
3030.17
2929.87
2852.72
2791.00
2667.55
2351.23
2189.21
2065.76
1919.17
1811.16
1734.01
1625.99
1541.12
1448.54
1309.67
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746.45
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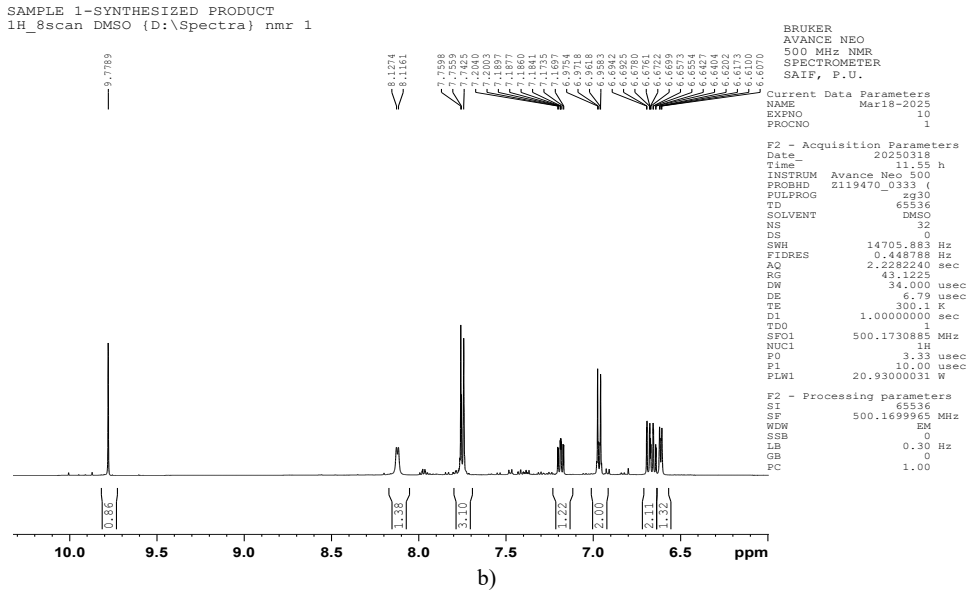
569.00
484.13

a)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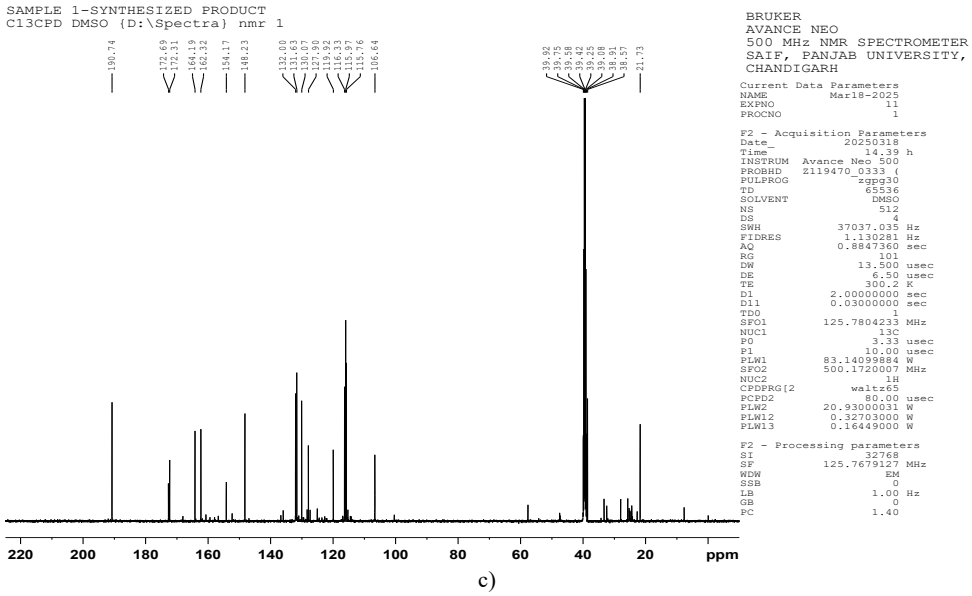
SPECTROMETER
SAIF, P.U.
ppm

b)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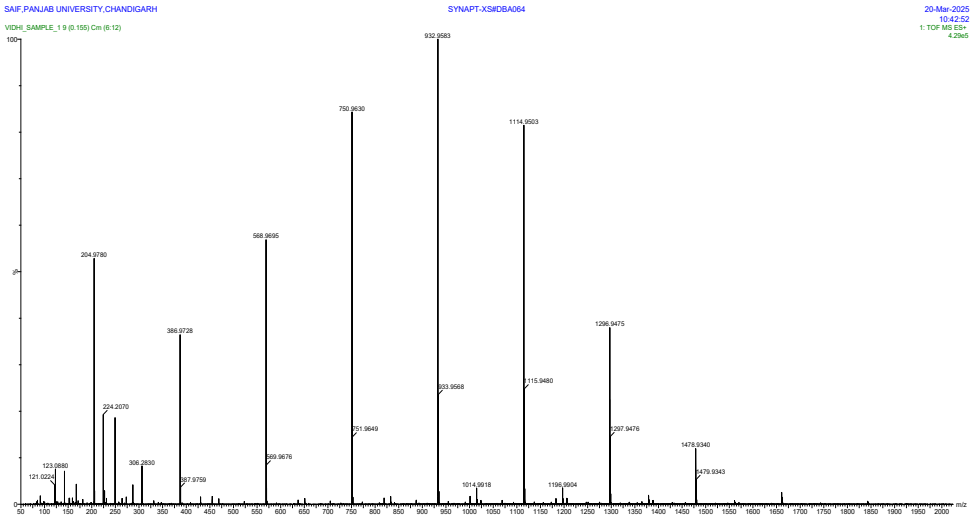
CHANDIGARH
ppm

c)



d)

Figure 2. Spectrum of synthesized hydroxy chalcone-aspirin conjugate A: FTIR B: ^{1}H-NMR C: ^{13}C-NMR D: Mass spectra

Structural Elucidation

The IR spectrum indicates the presence of functional groups such as O-H (3323 cm^{-1}), C=O (1741 cm^{-1}), and aromatic C=C (1652 cm^{-1} and 1449 cm^{-1}) stretches, suggesting a carboxylic acid or aromatic ketone. Additional C-O (1271 cm^{-1} and 1217 cm^{-1}) and fingerprint region peaks confirms functional groups (Zhang *et al.,* 2024).. At ^{1}H-NMR contains number of hydrogen atoms in the conjugate is 16, positioned at various locations in the molecular structure, specifically at 1, 2, 3, 4, 2', 3', 4', 5', 6', 2'', 3'', 4'', 5'', 6'', 7, and 12. Here, Total 23 carbon are present. The ^{13}C NMR spectrum suggests the presence of carbonyl (C=O) groups with a downfield peak at ~190.74 ppm, indicating an aldehyde, ketone, or carboxyl group. Aromatic and conjugated carbons (172.69 - 148.23 ppm) suggest a benzene or heterocyclic system. Aliphatic carbons (~39.92 - 38.57 ppm, 21.73 ppm) indicate alkyl chains or functionalized groups, supporting a complex aromatic-aliphatic framework. The position of carbon atom is Aromatic Carbon at 1,2,3,4,5,6,1',2',3',4',5',6',1'',2'',3'',4'',5'',6'', Aliphatic Carbon at 11, 12 and Carbonyl Carbon at 7,8,9. The results obtained from FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy, it is observed that the present structure is synthesized using hydroxy substituted chalcone and final structure is Aspirin-Chalcone Conjugate having Molecular Formula $C_{23}H_{16}O_5$ and Molecular weight is 372.37 g/mol. Identified structure by FTIR, NMR, and Mass Spectroscopy showed in **Figure 3.**

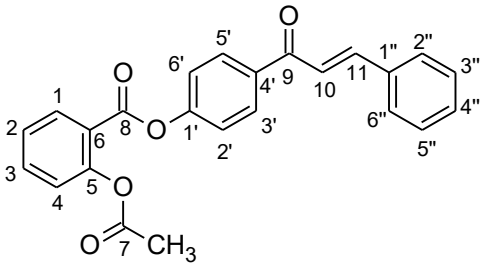
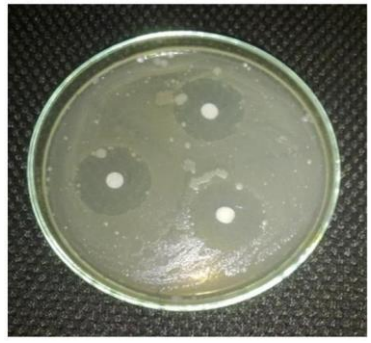
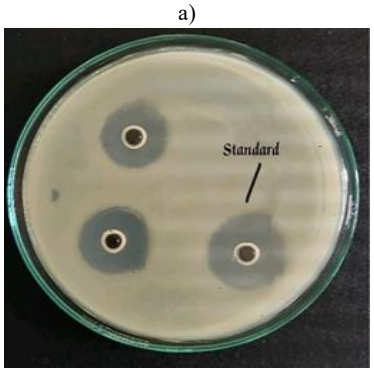


Figure 3. Identified structure by FTIR, NMR, and Mass Spectroscopy

Antimicrobial Activity of Synthesized Compound



a)



b)

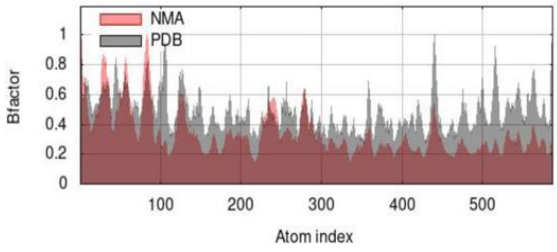
Figure 4. Zone of inhibition Cup Plate method, a) Concentration of compound 20 ug/ml, 40 ug/ml 60 ug/ml, b) Concentration of compound 80 ug/ml, 100 ug/ml, Standard (20 ug/ml)

As the concentration increases from 20 to 100 µg/ml, the zone of inhibition also increases, showing a dose-dependent antimicrobial

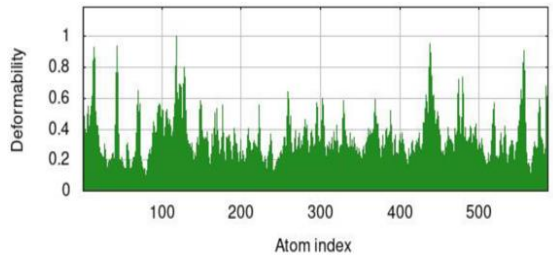
effect. The standard ampicillin (20 µg/ml) has the largest inhibition zone. The zone of inhibition depicted in **Figure 4**.

Molecular Dynamics (MD) Study

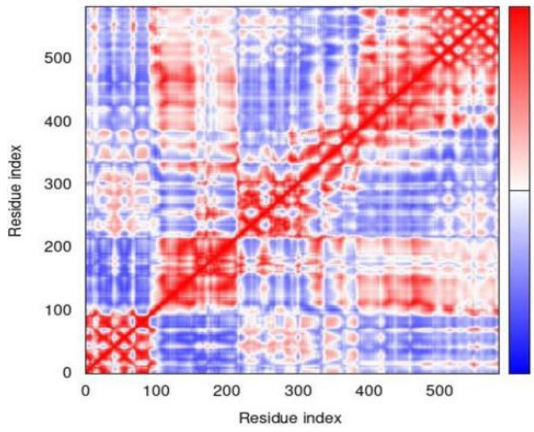
A significant and potent *in silico* method for elucidating the function of biological activity, macromolecular properties, and the dynamic nature of proteins at various periods is molecular dynamics simulation. MD is carried out to promote the target receptor's long-term molecular binding and interactions with ligands. **Figure 5** shows the three-dimensional interaction between the PBP enzyme and compound 17 inhibitor and **Figure 5** depicted deformability graph demonstrates that flexible hinge regions exhibit the highest deformability. The NMA B-factor describes the relative amplitude and mobility profiles of atomic displacements around the equilibrium conformation, as seen in **Figure 5**.



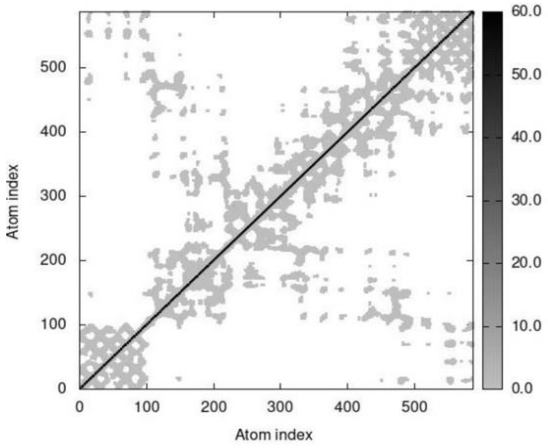
a)



b)



c)



d)

Figure 5. Molecular fluctuation plot of Compound 17 indicated by NMA.

Structure Activity Relationship (SAR) of Aspirin-Chalcone Conjugate

The synthesized Aspirin-chalcone conjugates has antimicrobial properties with their core structure. The SAR of synthesized compound Aspirin-chalcone conjugates are as follows:

1. Aspirin's efficacy against strains of both Gram-positive and Gram-negative bacteria is determined by its interaction with the chalcone scaffold.
2. Compound 17, which has a propanone linkage in its chalcone nucleus, has demonstrated remarkable effectiveness against Gram-negative bacteria. .
3. Additionally, compound 17 has rich with hydrogen and a hydrophobic environment showed encouraging action against gram-positive bacteria.
4. Compound 17 linked to the aspirin nucleus by the -$COCH_3$ group at the phenyl ring had excellent and encouraging antibacterial activity.

Conclusion

The study successfully synthesized and characterized chalcone-aspirin conjugates, demonstrating their potential as novel antimicrobial potential. Structural confirmation was achieved through IR, NMR, and mass spectrometry. *In-silico* molecular docking and MD simulation studies showed strong binding affinities, suggesting significant biological activity. Antimicrobial efficacy was confirmed using the cup plate method against *E. coli*. The combination of chalcone and aspirin pharmacophores presents a promising hybrid drug design approach for antimicrobial potential.

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