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NOVEL COUMARIN CHALCONE DERIVATIVES: SYNTHESIS, DOCKING, AND ANTIMICROBIAL EVALUATION

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ABSTRACT

Background: This study synthesized and evaluated a series of coumarin chalcones for their antimicrobial efficacy against microbial and fungal strains. **Methodology:** Ten new coumarin chalcones were prepared by Claisen- Schmidt condensation by using 4-hydroxy coumarin as a precursor and followed by refluxing obtained intermediate (3-(4-aminophenyl)-3-oxo prop-1-enyl)-4-hydroxy-2H-chromen-one) with substituted aromatic benzaldehyde in the presence of piperidine as a catalyst. IR, 1HNMR, 13CNMR, and GCMS characterized all synthesized compounds. The agar well diffusion method assessed these compounds for antimicrobial activity against various bacterial and fungal strains such as *E. coli*, *P. aeruginosa*, *B. subtills*, *S. aureus*, and C. albicans. Zone inhibition was measured for each compound (10µL) against all strains. **Results and Discussion:** The study showed that derivatives 4c, 4e, 4f, and 4g showed strong potential for inhibition towards various fungal and microbial strains. The inhibition zone for 4c and 4e was emerged as 5.48±0.448, 7.02±0.332, 5.62±0.321, 6.81±0.021, 7.72±0.421 and 5.13±0.179, 6.76±0.511, 4.24±0.273, 4.64±0.231, 5.48±0.049 while compound 4f and 4g showed 5.40±0.420, 6.69±0.168, 5.71±0.245, 5.28±0.042, 7.09±0.175, and 4.94±0.814, 6.58±.0160, 6.01±0.455, 6.61±0.021, 6.91±0.414 mm, respectively. Between -7.1 to -10.2Kcal/mol is the range of docking score of derivatives by interactions of DNA *gyrase* and compounds analyzed. Here, compound 4g exhibited the highest DNA *gyrase* inhibition, and compound 4c exhibited a strong inhibition with docking scores of -10.2 kcal/mol and -9.8 kcal/mol, respectively. **Conclusion:** The findings of this work contribute to a better understanding the potential of synthesised compounds as drug candidate against microbial infections through ADMET study.

INTRODUCTION

Humans are susceptible to infections and organ dysfunction frequently brought on by fungi and bacteria [1]. Some bacteria may resist current medication due to their excessive use, and infections may not be treated by it [2]. Furthermore, new strains of microbial diseases are emerging that harm humans. Antimicrobial medicines available in the market may or may not be able to control these infections [3]. Therefore, there is a huge opportunity to create novel antimicrobials to combat resistance to microbial infections. A unique field of chemistry, i.e.,

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heterocyclic chemistry, includes synthetic methods, physical and chemical properties, and various uses of heterocyclic compounds. Coumarin is a heterocyclic molecule considered the best for drug development [4]. The coumarin nucleus is a significant class of oxygen heterocycles widely found in the plant kingdoms. Due to their multifunctionality, a hybrid scaffold of coumarin and chalcone can be made with intriguing pharmacological properties. Researchers are motivated to create hybrid molecules with distinct biological functions and structures due to the pharmacological significance of naturally found substances [5,6].

The hybridization of chalcone in the coumarin network contributes to significant microbial inhibition [4,6,7]. The greatest class of phenolic compounds discovered in plants are coumarins (I) (2H-1-benzopyran-2-one), which belongs to the benzopyrone class, which includes more than 1300 secondary metabolites derived from fungi, bacteria, and plants [8]. Coumarins have also shown various pharmacological actions like anti-inflammatory, antifungal, antimicrobial [9,10] [11], antihyperlipidemic etc [12][13]. One major natural product of the flavonoid category is chalcone (II), having 1,3-diphenylprop-2-en-1-one. Because of the benefits of heterocyclic organic molecules, many researchers have put effort into their research on synthesizing heterocyclic platform coumarin chalcones. The distinct anti-inflammatory, anti-oedema, antimalarial [14], and anticancer properties of coumarin and chalcones have been described. Thus, all high-protein oedema or tumors can be treated with these coumarin and chalcones hybrid derivatives.[15]

Furthermore, it has been suggested that developing innovative derivatives of chalco-coumarin is imperative to combat upstream infection by microorganisms, particularly gram-negative and gram-positive bacteria. This is because coumarins and chalcone both have low toxicity profiles and increased significant value due to their beneficial health potential [16]. Many reports have been made by using different spacers /functional groups. The hybridization of two different moieties resulted in enhanced action of resulting components [17].

According to the reports, we planned to combine the nuclei of chalcone and coumarin to create novel conjugates from 4-hydroxycoumarin and assess the antimicrobial effect of the conjugates. Our study aims to replace the medications producing antibiotic resistance and develop new antimicrobial agents that could be made possible as efficient and cost-effective medication. Here, we focus on synthesis, characterization, ADMET study, and antimicrobial evaluation of coumarin chalcone derivatives.

MATERIAL AND METHOD

Reagents and instruments

All analytical grade reagents, catalysts, and solvents were used directly without further purification. The open capillary tubes were used for melting point determination, and the reported results were uncorrected. Thin layer chromatography was employed for reaction completion using glass plates (silica gel G coated) and visualized under a UV lamp/ iodine chamber. Bruker FTIR spectrophotometer used for infrared spectrum.

400 MHz JNM-ECZ600R/S1 spectrometer was used to get ^{1}H NMR and ^{13}C NMR spectra in $CDCL_3$ as solvent using TMS as an internal standard. Applied Biosystems 3200 Q-Trap Spectrometer used to get mass spectra. TLC was used to verify the purity of synthesized compounds. Microbial strains like *B. subtilis* (MTCC No. 441), *S. aureus* (MTCC No. 3161), *E. coli* (MTCC No. 1687), *P. aeruginosa* (MTCC No. 424), and fungal strains i.e., *Candida albicans* (ATCC No. 10231) were collected from Aakaar Biotechnologies Private Limited, Lucknow. In this study, Ciprofloxacin and fluconazole were employed as standard.

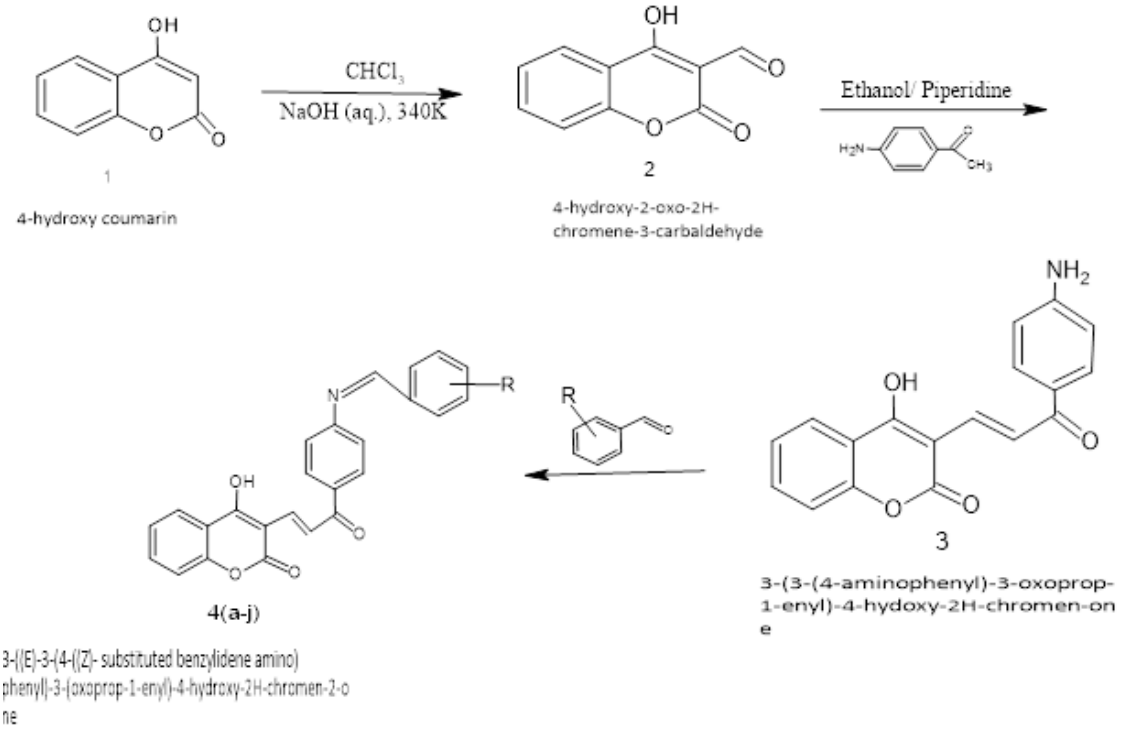
Chemistry

The synthesis process has three steps, as shown in Figure 1. A series of coumarin–chalcone derivatives were synthesized according to the reaction shown in Scheme 1. This scheme was modified from the one reported by Prasad et al [18].

Synthesis of 4-hydroxy-2-oxo-2H-chromene-3-carbaldehyde (2) 4-hydroxycoumarin (1) (5mmol) was added in cooled solution of chloroform (30ml) and aqueous sodium hydroxide(6ml) at 340k to create alkaline condition. The resultant mixture was mixed at room temperature for 3 hrs with continuous shaking. TLC technique monitored the reaction completion using a solvent system (acetone- chloroform 2:3); the reaction mixture was mixed with ice-treated cold water (40 mL). The obtained product was filtered, rinsed twice with 20 mL of water, and then dried. The obtained product is recrystallized with CH3OH and carbaldehyde [17].

Synthesis of (E)- 3-(3-(4-aminophenyl)-3-oxoprop-1-enyl)-4-hydoxy-2H-chromen-one (3)

Equimolar quantities (0.03 mmol) of 4-amino acetophenone and (0.031 mmol) 4-Hydoxy-2-oxo-2H-chromene-3-carbaldehyde (2) were dissolved in chloroform (40 mL). Piperidine was added as a catalyst (0.02 mmol) in the reaction mixture and refluxed for 4 hours. Obtained residue washed with methanol after chloroform removal from the mixture and pure chalcone (3) [16].



$^{R}_{(a\text{-}j)}$ = 4-OH, 4-NO_2,3-Cl, 2-Cl, 2,5-Dimethoxy, 4-H, 3-Br, 4-Dimethylamine, 4-OH-3-OCH_3, 2-OH

Figure 1: Scheme 1: Synthetic scheme of coumarin chalcone derivatives

General method of synthesis of 3-((E)-3-(4-((Z)- substituted benzylidene amino) phenyl)-3-(oxoprop-1-enyl)-4-hydroxy-2H-chromen-2-one (4a-j)

Compound 3 (0.033 mol) was poured into a round bottom flask containing 20 ml methanol. In a different beaker, 20 ml methanol was mixed with substituted benzaldehyde (0.033 mol). In solution of compound 3, benzaldehyde (substituted) was added dropwise with continuous stirring. Reflux of mixture was carried out for 3.5 hr. The resultant solution was taken into watch glass, and excess solvent was removed under reduced pressure. Recrystallization of the obtained solid was done with methanol[17]. Characterization of every derivative is provided below:

3-((E)-3-(4-((Z)-4-hydroxybenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4a

Yield: 70%; FTIR (KBr, cm^{-1}): 3420 (-OH str), 3422 (-OH str), 1654 (-C=O str, chalcone), 1710 (-C=O str, coumarin), 1469 (C=C str, aromatic), 3117 (-CH- str, aromatic), 1622 (-C=C str, alkenyl); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 6.7-7.9 (s,1H,Ar -H), 8.01 (s, H, imine), 4.9 (s,1H, OH), 7.24-7.74 (m, 12H,Ar); ^{13}CNMR($CDCl_3$, δ, ppm): 142.7 (C, Aromatic), 179.4 (CO, coumarin), 199.6 (CO, chalcone), 146.06 (CH), 67.1 (C-O-C), 131.8, 132.2 (C=C) ; MS (ESI) m/z- 414.42(M+ 1)

3-((E)-3-(4-((Z)-4-nitrobenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4b

Yield: 64%; FTIR (KBr, cm^{-1}): 1656 (-C=O str, chalcone), 1716 (-C=O str, coumarin), 1468 (C=C str, aromatic), 3424 (-OH str), 3066 (-CH- str, aromatic), 1625 (-C=C str, alkenyl), 1529 (-NO_2); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 7.3-7.7 (1H, Ar-H,s), 8.1 (imine, s, H), 9.23 (NO_2), 7.3-7.6 (s, 12H,Ar); ^{13}CNMR($CDCl_3$, δ, ppm): 123.69, 130.44, 136.05 (C, Aromatic), 168.2 (CO, coumarin), 196.9 (CO, chalcone), 67.01 (C-O-C), 137.1 (C=C); MS (ESI) m/z -442.15 (M +1)

3-((E)-3-(4-((Z)-2-chlorobenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4c
Yield: 60%; FTIR (KBr, cm^{-1}): 1654 (-C=O str, chalcone), 1712 (-C=O str, coumarin), 1468 (C=C str, aromatic), 3422 (-OH str), 3068 (-CH- str, aromatic), 1625 (-C=C str, alkenyl), 765 (C-Cl); ^{1}HNMR ($CDCl_3$, 600MHz,δ): 6.7-7.8 (Ar-H, s, 1H), 8.1 (H, imine, s), 6.89 for 3 protons (3H, m, Ar,CH-6,7,8 of coumarin ring), 6.73 (Ch- 2,3,4,6 of benzylidene ring); ^{13}CNMR ($CDCl_3$, δ, ppm): 124.4, 125.06, 128.05, 128.8, 132.8, 133.1, 133.9 (C, aromatic), 182.6 (CO, coumarin), 198.1 (CO, chalcone), ; MS (ESI) m/z - 432.09 (M +1)

3-((E)-3-(4-((Z)-3-chlorobenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4d
Yield: 66%; FTIR (KBr, cm^{-1}): 1654 (-C=O str, chalcone), 1712 (-C=O str, coumarin), 1469 (C=C str, aromatic), 3420 (-OH str), 3114 (-CH- str, aromatic), 1685 (-C=C str, alkenyl), 751 (C-Cl vibrations); ^{1}HNMR ($CDCl_3$, 600MHz,δ): 7.2-7.5 (Ar-H, s, 1H), 8.1 (imine, s, H), 7.3-7.9 (d, 1H), 6.89 (3H,s, Ar, CH-6,7,8 of coumarin); ^{13}CNMR($CDCl_3$, δ, ppm): 126.7, 128.05, 128.8, 131.1, 132.7 (C, Aromatic), 176.9 (CO, coumarin), 137.04 (C=C), 67.2 (C-O-C); MS (ESI) m/z -432.65 (M +1)

3-((E)-3-(4-((Z)-2,5-dimethoxybenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4e
Yield: 76%; FTIR (KBr, cm^{-1}): 1654 (-C=O str, chalcone), 1712 (-C=O str, coumarin), 1465 (C=C str, aromatic), 3420 (-OH str), 3066 (-CH- str, aromatic), 1130 (-OCH_3), 1639 (-C=C str, alkenyl), 1356 (-CH_3, str); ^{1}HNMR ($CDCl_3$, 600MHz δ): 7.1-7.7 (1H, Ar-H, s), 8.06 (imine, m, H), 2.5 (s, Ar-OCH_3,), ^{13}CNMR ($CDCl_3$, δ, ppm): 124.52, 125.06, 128.42, 131.78, 133.11, 134.53(C, Aromatic), 166.9, 169.2 (CO, coumarin), 116.7, 116.9 (CH), 198.6 (CO, chalcone), 55.4, 55.9 (OCH_3), 152.3 (C=C); MS (ESI) m/z - 457.06(M +1)

3-((E)-3-(4-((Z)-4-hydroxy-3-methoxybenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4f
Yield: 68%; FTIR (KBr, cm^{-1}): 3420 (-OH, str), 1650 (-C=O str, chalcone), 1712 (-C=O str, coumarin), 1465 (C=C str, aromatic), 3422 (-OH str), 3068 (-CH str, aromatic), 1688 (-C=C str, alkenyl), 1134 (-OCH_3, str); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 7.01-7.69 (1H, s, Ar-H), 8.01 (s, 1H, imine), 5.20 (OH, s, 1H), 2.05 (s,1H, methoxy), 7.64-7.99 (s, 12H, Ar) ^{13}CNMR ($CDCl_3$, δ, ppm): 57.8 (OCH_3), 133.7, 142.4, (C, Aromatic), 178.6 (CO, coumarin), 198.6 (CO, chalcone), 137.6 (C=C); MS (ESI) m/z - 443.43(M +1)

3-((E)-3-(4-((Z)-3-bromobenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4g
Yield: 78%; FTIR (KBr, cm^{-1}): 1650 (-C=O str, chalcone), 1717 (-C=O str, coumarin), 1465 (C=C str, aromatic), 3420 (-OH str), 3066 (-CH str, aromatic), 1622 (-C=C str, alkenyl), 632 (C-Br); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 6.1-7.7 (1H, Ar-H,s), 8.2 (H, imine), 7.2-7.6 (Ar-H ,d, 1H), 5.71-5.95 (s, 3H, CH-2,4,6 of benzylidene ring); ^{13}CNMR ($CDCl_3$, δ, ppm): 120.7, 124.4, 124.9, 128.3, 131.7, 133.03, 134.4 (C, Aromatic), 116.6 (C=C), 191.05 (CO, chalcone), 166.8, 169.1 (CO, coumarin); MS (ESI) m/z - 476.43(M +1)

3-((E)-3-(4-((Z)-4-dimethylaminebenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4h
Yield: 80%; FTIR (KBr, cm^{-1}): 1655 (-C=O str, chalcone), 1718 (-C=O str, coumarin), 1468 (C=C str, aromatic), 3424 (-OH str), 3108 (-CH str, aromatic), 1684 (-C=C str, alkenyl), 1356 (-CH_3 str); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 6.72-7.61 (s, 1H, Ar-H), 8.12 (s, H, imine), 2.65 (m, H, N-CH_3); ^{13}CNMR (CDCl3, δ, ppm): 124.4, 124.9, 127.5, 132.8, 135.2 (C, Aromatic), 178.8, 181.1 (CO, coumarin), 190.4 (CO, chalcone), 22.4, 22.64, 26.79, 26.9 (CH_3), 40.2, 41.9 (C-NH), 111.08, 111.3, 116.6, 117.1 (C=C); MS (ESI) m/z - 440.41(M +1)

3-((E)-3-(4-((Z)-benzylidene amino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4i
Yield: 79%; FTIR (KBr, cm^{-1}): 1656 (-C=O str, chalcone), 1712 (-C=O str, coumarin), 1468 (C=C str, aromatic), 3424 (-OH str), 3068 (-CH str, aromatic), 1632 (-C=C str, alkenyl); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 7.3-7.6 (1H, s, Ar-H), 8.05 (imine, s, 1H), 2.60 (s, 1H, OH); ^{13}CNMR ($CDCl_3$, δ ,ppm): 123.9, 128.4, 130.0, 133.2, 135.7, 136.7 (C, Aromatic), 169.2 (CO, coumarin), 168.4 (C=C), 196.93 (C, chalcone), 26.9 (CH_3); MS (ESI) m/z - 397.41(M +1)

3-((E)-3-(4-((Z)-2-hydroxybenzylideneamino) phenyl)-3-oxo-prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 4j
Yield: 74%; FTIR (KBr, cm^{-1}): 3420 (-OH str), 1654 (-C=O str, chalcone), 1718 (-C=O str, coumarin), 1468 (C=C str, aromatic), 3422 (-OH str), 3116 (-CH str, aromatic), 1685 (-C=C str, alkenyl); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 6.7-7.9 (s, 1H, Ar-H), 8.02 (s, H, imine), 4.9 (s,1H, OH), 7.3-7.6 (s,12H, Ar); ^{13}CNMR (CDCl3, δ, ppm): 123.5-132.2 (C, Aromatic), 174.62 (CO, coumarin), 143.5 (C=C), 206.12 (CO, chalcone), 149.01 (CH); MS (ESI) m/z - 414.12(M +1)

We get a yield of 60-80% for each compound. The chemical structures of derivatives characterized by IR, NMR, and MS analysis

Antimicrobial efficacy

The in vitro antimicrobial activity, i.e., antibacterial and antifungal, was determined for synthesized derivatives against various fungal, gram-positive, and gram-negative species (*C. albicans, P. aeruginosa, E. coli, B. subtills, S. aureus*) through the zone of inhibition method using the referenced protocol with some modification [19].

In short, a sterile cotton swab spread uniformly bacterial inoculum throughout the agar petri dish plate. After that, use a sterile tip to make 4 holes of 5mm in diameter. The drug solution with a concentration of 100 µg/ml (10µL) was added to 2 wells, and 10µL control (autoclaved double distilled water) was added to the remaining two wells. The plates were incubated at 37°C and aerobic conditions for 72 hours. After 72 hours in the agar plate clear zone was measured to determine antibacterial effect.

In silico screening (Molecular Docking)

The main goal of molecular docking is to obtain a complex of receptors and ligands with an optimized shape and less binding free energy. Autodock is a group of automated docking tool. Here we used Autodock vina an open-source tool for molecular docking study of coumarin chalcone derivatives (4(a-j)). Pymol version 4.6.0, employed to view molecular structures [20,21].

We used Intel Core i3-7100U CPU @ 2.40 GHz processor, 4 GB RAM memory, 64-bit operating system, Windows 10 as operating system. Four steps are taken into consideration for molecular docking:

1. Ligand preparation: 200 structures of coumarin chalcone hybrids were collected through a literature survey and databases and retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/).
   Drawing of 3D structure of ligands, canonical smiles of ligands were retrieved and converted to pdb. format after that pdbqt. and energy minimization was carried out via ChemDraw 12.0 software, which was then used for docking.
2. Protein preparation: 3D Crystal structure or sequence of enzyme DNA gyrase complexed with ciprofloxacin was obtained from the Protein Data Bank (PDB ID:2XCT) (https://www.rcsb.org/) for the process of molecular docking. Remove water molecules and heteroatoms. Added polar hydrogens, then saved as pdbqt.
3. Receptor grid preparation: This is the most important step in molecular docking. The co-crystallized ligand was used to find binding pockets via the discovery studio program. Various amino acid residues like LYS A: 501, TYR A: 434, ILE A: 487, ASN A: 498, SER A: 488, ASP A: 500, PHE A: 382, HIS A: 708, LUE A: 461 were found in the binding pocket.
   After identifying active site residues DNA gyrase in .pdb file, average coordinates were calculated and set values in grid box as GPF file format.
4. Protein ligand docking: Run Auto Dock and wait for docking to complete. The final DLG (docking log file) contained top ten binding free energy. The lowest binding energy complex was saved in pdb format.

After completion of docking run two steps carried out:

1. Interactions visualization: The optimum binding affinity of compounds was conducted using Discovery studio 2019 from Biovia and PyMol 2.3 were used to visualize 2D, 3D interactions of ligand with protein.
2. Docking Validation: Re-docking native ligand method used for docking procedure validation. Ciprofloxacin from DNA gyrase was removed re-docked into active site using Auto Dock.

The process was done manually by opening co-crystallized complex in notepad, extracting inhibitor heteroatoms from gyrase, pasting it into new notepad and saving as pdb file format. The grid parameters were unchanged by using same protocol in process. This was done to check that the inhibitor binds precisely to the active site and must exhibit less deviation than actual co-crystallized complex.

Then by using PyMol 2.3 superimposed the re-docked complex on to the reference co-crystallized complex. RMSD (root mean square deviation) was calculated [22].

RESULTS AND DISCUSSION

Thin-layer chromatography (TLC) was used characteristically during the synthesis process to monitor the chemical reaction. All synthesized compounds' melting points, Rf values, and percentage yield were successfully identified. The study's results are compiled in Table 1.

Table 1: Physical characteristics and substitutions of 4(a-j)

Compound	R group	Colour	Melting point	Yield (%)	Rf value
4a	4-hydroxy benzaldehyde	Pale yellow	149-152°C	70	0.87
4b	4-NO_2 benzaldehyde	Yellow	171-176°C	64	0.82
4c	2-Chloro benzaldehyde	Pale yellow	139-141°C	60	0.89
4d	3-Chloro benzaldehyde	Pale yellow	136-140°C	66	0.81
4e	2,5-Dimethoxy benzaldehyde	Yellowish brown	171-175°C	76	0.65
4f	4-hydroxy-3-methoxy benzaldehyde	Yellow	152-157°C	68	0.71
4g	3-Bromo benzaldehyde	Dark yellow	139-142°C	78	0.59
4h	4-Dimethylamine benzaldehyde	Dark Red	158-162°C	80	0.92
4i	Unsubstituted benzaldehyde	Pale yellow	161-164°C	79	0.84
4j	2-hydroxy benzaldehyde	Dark yellow	146-149°C	74	0.81

Antimicrobial effects of compounds

The agar well diffusion method, known as an assay of the zone of inhibition, is the most widely and affordable technique for regularly evaluating antimicrobial efficacy in clinical microbiology laboratories. By using this method, bacterial strain is inoculated onto agar plates, and the strength is tested into prepared plates by test sample diffusion. In addition, the inhibition zone is measured against the growth of bacteria after a period of incubation, and that assesses the efficacy of derived compounds [19]. In this research, synthesized coumarin chalcone derivatives were used for antimicrobial (in vitro) activity through the zone of inhibition/Agar well diffusion assay method against various bacterial and fungal strains such as (Gram +ve) *B. subtills*, *S. aureus*, (Gram -ve) *P. aeruginosa*, *E. coli* and *Candida albicans* (fungal) bacterial and fungal strains at a concentration of 100 µg/ml. In agar plates, the direct ratio of the zone obtained was used to calculate the inhibition effect against fungal and bacterial strains. Synthesized compounds against bacteria and fungi showed a variable degree of inhibitions. As per findings, the derivatives 4c, 4e, 4f, and 4g found a potential inhibitory effect (4.94±0.814 to 5.48±0.448) against *B. subtills*, while compounds 4c, 4e, 4f, and 4g have remarkable (6.58±1.60 to 7.02±0.332) potential inhibition for *S. aureus*. Furthermore, 4g, 4h, 4f, 4c, 4i, and 4e compounds

produced potential inhibitory action against *E. coli*, whereas 4c, 4f, 4h, and 4g showed inhibitory effects from 5.28±0.042 to 6.81±0.021 against the ability of survival for *P. aeruginosa*. Meanwhile, against *C. albicans* (fungal strain), compounds 4c, 4e, 4f, and 4g have potential inhibition. Only compound 4d showed no activity against *Candida albicans* at any concentration. Ciprofloxacin and fluconazole were employed as control (positive) to ascertain the correlational effect of obtained compounds. Compound 4c showed more inhibitory potential against *S. aureus* and similar effects against *P. aeruginosa* at a 100 µg/ml concentration compared to reference ciprofloxacin. Compound 4c is more effective against *C. albicans* than standard fluconazole. Among ten synthesized compounds, 4c, 4e, 4f, and 4g had notable inhibitory potential even against all microbial and fungal strains in the 100 µg/ml concentration. The average inhibitory impact of all 10 synthesized compounds and standard drugs is given in Table 2 and Figure 2.

Table 2: Antimicrobial effect of derivatives for *S. aureus, B. subtills, P. aeruginosa, E. coli,* and *Candida albicans* using Zone inhibition assay

S. No	Compounds	Zone of inhibitions (mm) with Standard deviation (±) #				
		Gram Positive Bacterial Strain		Gram Negative Bacterial Strain		Fungal strain
		B. subtills	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
1	4a	4.04±0.438	4.61±0.257	4.12±0.411	4.01±0.501	5.06±0.088
2	4b	4.79±0.541	6.56±0.236	5.21±0.247	4.84±0.371	3.94±0.424
3	4c	5.48±0.448	7.02±0.332	5.62±0.321	6.21±0.021	7.72±0.421
4	4d	4.20±0.222	5.31±0.361	4.21±0.612	4.28±0.112	---
5	4e	5.13±0.179	6.16±0.511	4.24±0.273	4.64±0.231	5.48±0.049
6	4f	5.40±0.42	6.69±0.168	5.71±0.245	5.28±0.042	7.09±0.175
7	4g	4.94±0.814	6.58±1.60	6.01±0.455	6.61±0.021	6.91±0.414
8	4h	4.75±0.314	4.33±0.0112	6.00±0.129	5.92±0.650	5.40±0.108
9	4i	4.20±0.241	5.89±0.078	4.89±0.012	4.80±0.186	4.85±0.088
10	4j	3.98±0.018	5.49±0.118	3.87±0.119	4.72±0.214	5.11±0.264
11	Ciprofloxacin	5.97±0.221	6.89±0.219	6.71±0.281	6.81±0.174	---*
12	Fluconazole	---*	---*	---*	---*	7.69±0.246

--- indicates no inhibition, # indicates average of triplicate, ---* indicates activity not performed

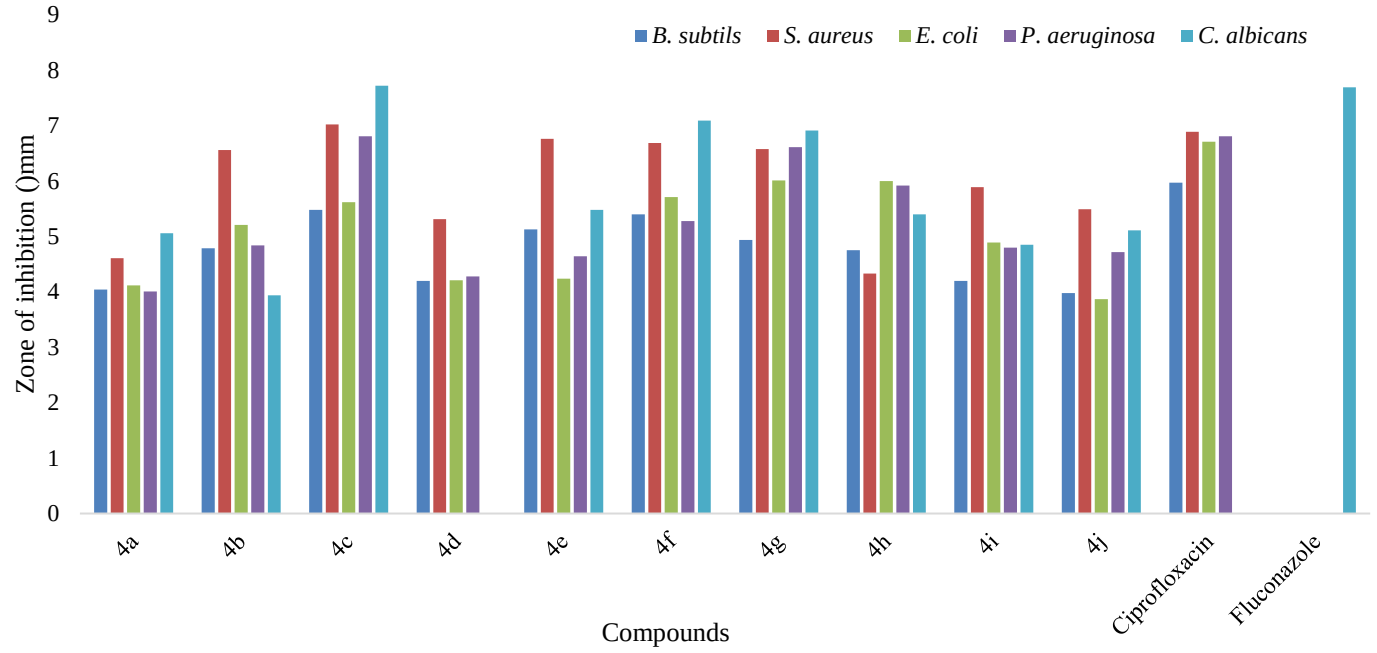


Figure 2: Antimicrobial profile of synthesized compounds

Molecular interactions of derivatives

The molecular docking study carried out the prediction of the binding interaction of protein receptors and ligands. The protein was derived from a PDB ID: 2XCT protein data bank. Our study employed synthesized derivatives for molecular docking to find binding capacity with DNA *gyrase* enzyme. In DNA synthesis, DNA *gyrase* (Bacterial) is the main component [23,24]. DNA replication, transcription, and relaxation are required, and inhibitors, affecting cell survival initiated this process. Here, we studied the amino acids involved, types of interactions, and energy scores for coumarin chalcone derivatives. The first step in the procedure was the preparation of ligand and protein structure. This required cleaning and optimizing the protein by minimizing its energy and removing unnecessary components. To fix an optimal conformation, the ligand was exposed to energy minimization. According to docking data, derivatives 4(a-j) fit into the target's active site pockets. Additionally, compounds had favorable energy scores (7.1 to 10.2 Kcal/mol). Docking results for compounds 4(a-j) are shown in Table 3. As a DNA *gyrase* inhibitor, derivative 4g with -10.2Kcal/mol docking score produced the best potency. This outstanding and remarkable binding energy points to a strong interaction between compound 4g and DNA gyrase, i.e., essential for transcription and replication of bacterial DNA. Our derivatives exhibited similar docking scores, such as from -10.1 kcal/mol to -9.1 kcal/mol in molecular docking of natural product-based coumarin-chalcones [7]. Standard Ciprofloxacin exhibited -8.3 Kcal/mol binding affinity [25]. Additionally, compound 4g showed interactions with different amino acids within the enzyme, including LYS(A):501, TYR(A): 434, TYR(A): 434, ILE(A): 487, ILE(A): 487, TYR(A): 463, ASN(A): 498, and LEU A): 461. The main interaction types were Pi-Alkyl, Pi-Sigma, Pi-Sigma, Pi-Pi stacked, Pi-Pi stacked, Conventional H-bond, C-H bond, and C-H bond. The 3D structure of DNA gyrase and Interactions of derivative 4g are shown in Figure 3 for derivative 4g. A comparison of the antimicrobial activity of synthesized derivatives with docking scores is provided in Figure 4.

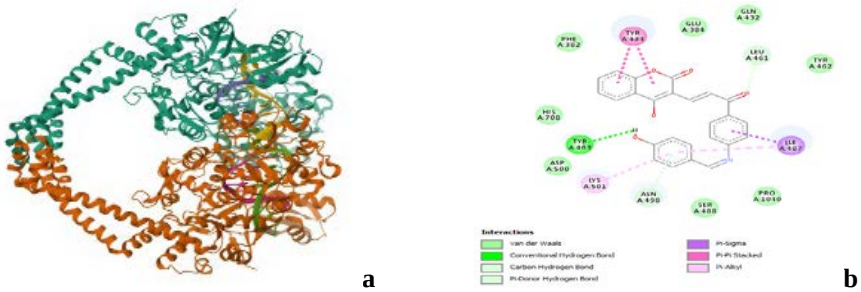


Figure 3: 3D structure of DNA gyrase (a) and molecular interaction of 4g.

Table 3. Docking scores of compounds 4(a-j)

Sr. no	Compounds	Binding energy (Kcal/mol)	Type of interaction	Amino acid involved
1.	4a	-8.6	Pi-Pi T shaped Pi-Pi T shaped Conventional H-bond Pi-Pi T shaped Pi-Pi T shaped Pi-alkyl Van der waals Van Der Waals	TYR A:434 TYR A:434 TYR A:463 ILE A:487 ILE A:487 LYS A:501 LEU A:461 ASN A:498
2.	4b	-7.3	Conventional H-bond Conventional H-bond Conventional H-bond Pi- alkyl Pi- alkyl Pi donor H-bond	LYS A:421 ALA A:528 LYS A:425 ILE A:469 PRO A:526 GLN A:601

3.	4c	-9.8	Conventional H-bond Conventional H-bond Conventional H-bond Conventional H-bond Amide-Pi stacked Pi- alkyl Pi-Pi stacked Pi- Pi stacked	ARG A:849 GLN A:291 LYS A:298 TYR A:210 ARG A:294 LEU A:657 PHE A:694 PHE A:694
4.	4d	-8.6	Pi-sigma Pi sigma C-H bond C-H bond Pi-Pi stacked Conventional H bond	ALA A:676 VAL A:645 ARG A:679 GLU A:852 PHE A:694 GLN A:291
5.	4e	-8.8	Pi-cation Pi-alkyl Pi-sigma Pi- sigma C-H bond	LYS A:683 ARG A:614 VAL A:645 ALA A:674 GLU A:638
6.	4f	-9.4	Pi- alkyl Pi- alkyl Pi- alkyl Pi- sigma Conventional H-bond Conventional H-bond Conventional H-bond Conventional H-bond Conventional H-bond	ILE A:831 ILE A:879 ILE A:963 TYR A:867 LYS A:807 LYS A:808 LYS A:833 SER A:806 VAL A:882
7.	4g	-10.2	Pi-alkyl Pi-sigma Pi-sigma Pi-Pi stacked Pi-Pi stacked Conventional H-bond C-H bond C-H bond	LYS A:501 ILE A: 487 ILE A:487 TYR A:434 TYR A:434 TYR A:464 ASN A:498 LEU A:461
8.	4h	-7.8	Conventional H-bond C-H bond Pi- alkyl C-H bond	TYR A:463 LYS A:501 ILE A:487 ASN A:498
9.	4i	-7.1	Conventional H-bond C-H bond Pi alkyl	SER A:488 PHE A:497 ALA A:642
10.	4j	-7.2	Pi- alkyl Pi- Pi stacked Pi- sigma Pi-Pi T shaped	ILE A:879 TYR A:434 MET A:953 ILE A:487

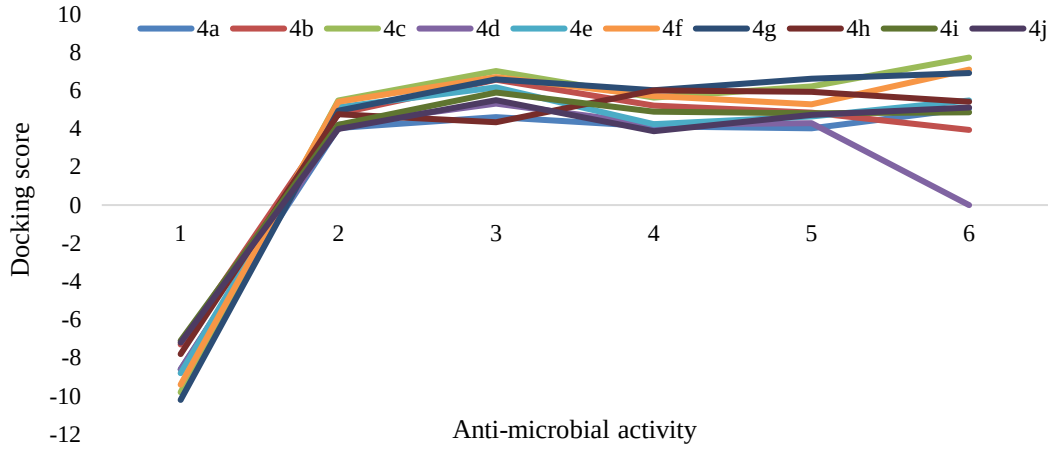


Figure 4: Comparision of docking score and antimicrobial activity

ADMET and drug likeness of the compounds

We have provided the expected ADMET values in Table 4 for coumarin chalcone derivatives. Evaluate every compound and support it as a potential drug. Consider different factors like interactions, molecular characteristics, and biological activities. The molecule's drug-like characteristics, such as lipophilicity, H-bond formation capacity, solubility, etc., are revealed by ADMET criteria [26][27]. It is significant to remember that these results or expectations are computational model-based and might not reflect in vivo behavior. Additional experimental investigations are required to verify these hypotheses and evaluate the drug's safety and pharmacokinetic characteristics. According to data, parameters like zone of inhibition and ADMET characteristics benefit drug candidates. Each of the derivatives showed a different level of antimicrobial activity. Their potential can be found as a drug candidate from their ADMET parameters.

Here is a summary of the ADMET analysis:

1. ADMET properties:

- Majority of compounds exhibit potential for favorable pharmacokinetics as indicated by their Log P values and moderate molecular weight, which ranges from 395.41 to 455.46 g/mol.
- Lipophilicity of molecule shown by Log P values; ranges between 2.85 to 3.87.
- A balanced hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) are present.
- Moderate lipophilicity of all compounds indicated by TPSA values.
- Consistent hydrogen bond acceptors and rotatable bonds are present.
- The ability of compound interaction shown by molar refractivity values ranges from 117.06 to 132.22°A.
- Not any compound was a P-gp substrate, but all were CYP450 enzyme inhibitors. Intestinal efflux is minimal; there is less first-pass metabolism and high bioavailability, and a small dose will be adequate to produce a therapeutic effect.

2. Drug like potential:

- For drug development, many molecules have shown advantageous ADMET features, such as suitable Log P values, molecular weight, and H-bonding capability.
- With potential antimicrobial activity and appropriate ADMET properties compounds 4c, 4e, 4g and 4i remarked as promising therapeutic candidates.
- Compounds with higher Log P values must be optimized to enhance drug-like properties.

3. Safety assessment

- None of the compounds violate safety assessment parameters like Brenk filters and PAINS indicate potential safety.
- To complete the evaluation of the safety profile of these derivatives, more in vivo and in vitro safety investigations would be needed.

In summary, derivatives 4c, 4e, 4f, and 4i have reasonable ADMET profiles and antimicrobial effects, which makes them viable candidates for drug development. Extensive experimental studies, such as pharmacokinetics and toxicity assessment, are required to ascertain their feasibility as secure and effective medicinal agents.

Table 4: ADMET (predicted) parameters of derivatives 4(a-j)

Compounds	Mol. Wt. (g/mol)	Log P	Log S	TPSA (°A)	MR	Log k_P (cm/S)	HBA	HBD	nAH	RB	PAINS	Brenk
4a	397.42	3.27	-4.86	79.12	117.06	-5.97	5	2	22	5	0	0
4b	440.40	2.98	-5.30	125.69	126.83	-6.02	7	1	22	00	0	3
4c	429.85	3.64	-5.24	79.87	123.02	-5.39	5	1	22	00	0	0
4d	429.85	3.43	-5.24	79.87	123.02	-5.39	5	1	22	00	0	0
4e	455.46	3.87	-5.38	98.33	130.09	-6.03	7	1	22	7	0	0
4f	395.41	2.96	-5.14	79.87	118.01	-5.62	5	1	22	5	0	0
4g	474.30	3.78	-4.15	79.87	125.71	-5.61	5	1	22	5	0	0
4h	438.47	3.34	-5.47	83.11	132.22	-5.80	5	1	22	6	0	0
4i	441.43	3.56	-5.18	109.33	126.52	-6.17	7	2	22	6	0	0
4j	411.41	2.85	-5.11	100.10	120.03	-5.97	6	2	22	5	0	0
Cipro	331.34	1.28	-1.85	74.57	89.39	-9.19	5	2	10	3	0	0

According to earlier research, coumarin chalcones and their derivatives are promising candidates with high antimicrobial action. A study by Moodley et al. (2016) reported that coumarinyl chalcones with hydroxy, methoxy, chloro and flouro substitutions showed effective antimicrobial efficacy against six microbial strains. 2-flouro derivative showed the most effective action against *C. albicans* and the inhibitory potential expressed in MBC (minimum bactericidal concentration) ranges from 0.0018 µg/L to 35 µg/L [28]. A study reported by Bensalah et al. (2023) observed the antimicrobial activity of coumarin chalcones and obtained a maximum of derivatives that showed inhibitory potential against bacterial and fungal strains tested [29]. Anti-microbial activity is only evaluated in vitro by susceptibility tests; it is not evaluated in patients. Effective therapy is not guaranteed by an antimicrobial drug's in vitro killing activity [30][31].

A further study revealed that molecular hybrids of chalcone and coumarin showed excellent antifungal and antibacterial properties [32][33]. Additionally, our research describes the effective antimicrobial substances that work against medications that seem susceptible to antibacterial resistance. Furthermore, the study shows a quiet response to the ongoing need for novel antimicrobials due to the persistent rise in strain resistance against existing antibiotics and the growing interest in synthetic antibiotic substitutions. This study's findings suggest adding these compounds in antibiotic formulation to boost their sensitivity.

CONCLUSION

In this study, 10 Coumarinyl chalcones were synthesised using Claisen- Schmidt condensation from 4-hydroxy coumarin and substituting aromatic aldehydes. IR, 1H and 13NMR, and GC-MS responses of all compounds revealed or provided structural determination. Molecular docking with DNA gyrase found insightful information for a mode of action of compounds. Compounds 4g and 4c have remarkable docking scores -10.2Kcal/mol and 9.8Kcal/mol, respectively, supposed as potential inhibitors of DNA gyrase. Antimicrobial activity was determined for all derivatives successfully. These compounds exhibited significant inhibitory potential against fungal and bacterial strains (Gram-positive and Gram-negative). One derivative, 4d, has negligible antifungal activity against C. albicans. In addition, affiliates 4c, 4e, 4f, and 4g have inhibitory potential even against all strains. Interestingly, the drug development potential of compounds supported by ADMET parameters provides a positive pharmacokinetic profile. Strong interaction with DNA gyrase, potent antimicrobial activity, and good ADMET profile highlight our study's importance in designing new drug affiliates against particular microbial strains. This study is noteworthy for coumarin-based drug development in the medicinal field.

ABBREVIATION

ATCC- American type culture collection, LYS- lysine, HIS- histidine, LEU- leucine, ILE- isoleucine, TYR- tyrosine, SER-

serine, PDB- protein data bank, RB- number of rotatable-bond, PAINS- presence of compounds flagged by the PAINS (Pan Assay Interference Compounds) filter, MR- molar refractivity, TPSA- topological polar surface area, ADMET- absorption, distribution, metabolism, excretion and toxicity.

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FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
All authors contributed to the study's conception and design. Sumita Kumar performed an analysis and completed all the evaluation parameters. Sonia Yadav collected and compiled the data, and Amit Sharma thoroughly evaluated the manuscript. All authors gave final approval.

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