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Synthesis and Evaluation of Coumarin Chalcone Derivatives as DNA Gyrase Inhibitors

SUMITA KUMARI[1]*, AMIT SHARMA[1] and SONIA YADAV[2]

[1*]Jagannath University, Jaipur, Rajasthan-302022, India.
[1]Department of Pharmacy, Jagannath University, Jaipur, Rajasthan-302022, India.
[2]SGT College of Pharmacy, SGT University, Gurugram, Haryana-122505, India.
*Corresponding author E-mail: sumitabajia87@gmail.com

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ABSTRACT

The purpose of this work was assessing *in vitro* antimicrobial effects of recently synthesised coumarin chalcone derivatives against various microbial strains. A scheme of coumarin chalcone hybrids were designed, synthesised and characterised their structures by spectral studies such as infrared, nuclear magnetic resonance and mass spectrometry and evaluated for their antimicrobial potential against different strains. *In silico* designing carried out by molecular docking technique by targeting DNA gyrase protein receptor with PDB ID: 6m1j. All the compounds showed greater binding energy range from -8.4Kcal/mol to -8.9kcal/mol than reference drug ciprofloxacin. Microbial resistance is most challenging issue for all world. To defeat this problem the well diffusion assay method used for assessing antimicrobial profile, demonstrated that most of compounds exhibited remarkable antibacterial and antifungal activity with zones of inhibition in mm. Potential candidates as antimicrobials are 5c, 5e, 5g and 5h with zones of inhibition against *B. Subtills, S. aureus*, *E. coli* and *P. aeruginosa* (5.56±0.179, 5.58±0.449, 4.94±0.811, 4.82±0.378; 7.25±0.191, 6.14±0.496, 5.55±0.496, 5.41±0.421; 6.36±0.024, 6.27±0.029, 5.99±0.666, 6.04±0.432; 5.93±0.118, 4.94±0.016, 6.58±0.029, 5.94±0.119) respectively. Compound 3d exhibited highest antimicrobial potential against *C. albicans* (7.92±0.389) and *S. aureus* (7.25±0.191). It is concluded that the potential of synthesised derivatives could be more effective in microbial resistance.

Keywords: Antimicrobial, Coumarin chalcone hybrids, DNA gyrase, Microbial resistance, Molecular docking.

INTRODUCTION

Microbial resistance is becoming a big concern and a difficult challenge for researchers worldwide since it poses a substantial threat to human health. The WHO estimates, 50,000 population including men, women and also children dying per day due to infection of microbes.[1] Due to microbial resistance, a high level of toxicity, inadequate antimicrobial action, the current target is creation of antimicrobial agents has failed to reach expectations, prompting a quest for new antimicrobial agents.[2] According to the reports, drug resistance is more likely to occur with single (solo) targeting agent which defeats the expected successful drug compound.[3] It is commonly accepted that,

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drugs which affect many sites of single target or many more targets are thought to be more potent and less resistant than single targeting drugs.[4,5,6] Pharmacologically significant heterocyclics are essential in the fight against illness that impact living things, including animals, humans and plants. They also give fresh findings on novel molecules that may have biological effects.[7] The lack of new antifungal medications, the rise of infectious diseases, various infection's resurgence, growing fungal resistance to present chemotherapeutic drugs are main problems associated in drug design and development. Due to this, analysts are searching for new affiliates that can fight against organisms which are resistant to multidrug therapy.[8] Because the field of chemistry is developing steadily, novel compounds are created in laboratories to find leads with target-specific action.[9] The molecular hybridization approach is the only way to get this. Molecular hybridization is a drug development technique to develop new drug entities by joining two distinct active pharmacophores with or without a linker. Nowadays, this technique is most widely used in drug development.[7,10,11] Thus, hybrid compounds may help human, fight against microbial resistance by decreasing both drug-drug interaction and multiple drug resistance. Chalcones, coumarins, heterocyclic molecules, and their derivatives can be found in both natural and synthetic sources. Because of their diverse range of pharmacological effects, these compounds have attracted the most attention in current research in drug development. Coumarins are classified as heterocyclic compounds belonging to the benzopyrone family, which has a 6-membered α-pyrone ring fused with a benzene ring. As a benzo derivative these compounds found in natural substances.[12] Coumarins have variety of therapeutic uses including anti-inflammatory[13], antifungal[14], antibacterial[15], antiviral[16] and antioxidant.[17] Coumarins also possess anticancer properties against various malignancies.[18-20]

Throughout the entire kingdom of plants, chalcones are one of the most prominent types of flavonoids.[21] 'Chalcone' word comes from "chalcos" a Greek word which means "bronze." Chemistry of chalcones has developed extensive scientific research throughout the world.[22] As benzyl acetophenone, chalcones are also known. Chalcones are α and β unsaturated ketones with two aromatic rings and various substituents arrangement. In chalcones three carbon aliphatic chain act as linker between two aromatic rings.[23]

We attempted to create new substituted coumarinyl chalcone as heterocyclic compounds with heteroatom oxygen, which has been noted as a common denominator of pharmacological and biochemical activities. We then examined them like antimicrobials.[24] There is more literature on hybrid molecules based on coumarin moiety showed their antimicrobial potential such as coumarin-benzimidazole hybrid (1), coumarin-thiazolyl hybrids (2), sulfonamide-coumarin hybrids (3), hybrids of coumarin-imidazole (4), coumarin-chalcone hybrids (5,6), coumarin-thiosemicarbazones[25-30] (Figure 1).

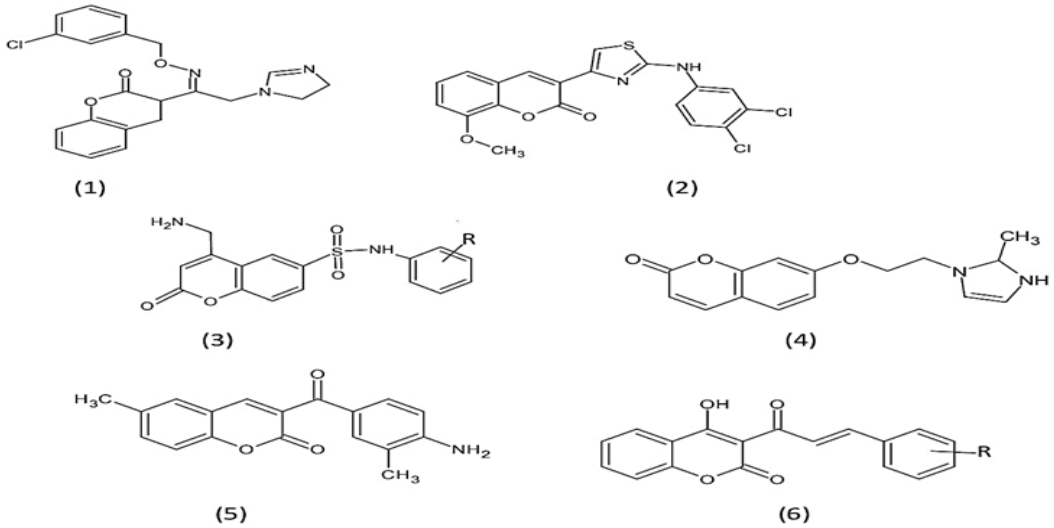


Fig. 1. Hybrid compounds as antibacterial agents[25-30] (Recently reported) (Chem Draw Pro 12.0)

Additionally, the findings were consistent with another published studies which demonstrated that antimicrobial potential of coumarin chalcone hybrids are summarised in vast area of pharmaceutical chemistry. Study by Wei *et al.,* 2016 coumarin chalcone hybrids assessed for antibacterial activity and displayed significant inhibition zones.[31] A study carried by Vazquez-Rodriguez *et al.,*2015 found that methoxy bearing coumarin moiety of chalcone coumarin hybrids exhibited zones of inhibition ranges 16.1 to 41.4mm as compared to reference drug enrofloxacin.[32] A study conducted by Hamdi *et al.,* evaluated that presence of electron withdrawing and releasing groups on chalcone moiety of hybrids possess moderate antibacterial activity *S. aureus* with 8mm to 16mm zone of inhibition by disk diffusion method while gentamycin as reference has 15-20mm.[33] A study conducted by Wang *et al.,* 2021 observed design, synthesis, characterization, remarkable antibacterial profile of chalcone derivatives bearing coumarin moiety with EC50 value ranges from 49.77 µg/mL to 162.48 µg/mL.[34]

These important findings offer a potential favour of combining two moieties into a single molecular structure that may have substantial antibacterial action and low toxicity. The aim of current study to synthesize coumarin chalcone hybrids using a click chemistry approach (Fig. 2) and evaluate them against different fungal and bacterial strains, keeping in mind the issue of antimicrobial resistance as a major limitation of currently available antimicrobial drugs. After the determination of zone of inhibition for each derivative, their MIC (minimum inhibitory concentration) were calculated for selection of potent compound. Additionally, using molecular modelling technique different binding interactions of the most potent compound was find out. Synthetic route shown in scheme 1 (Figure 3).

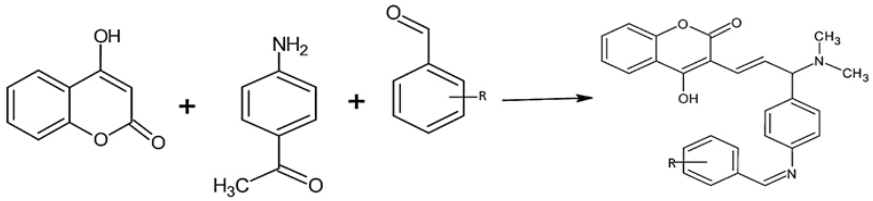
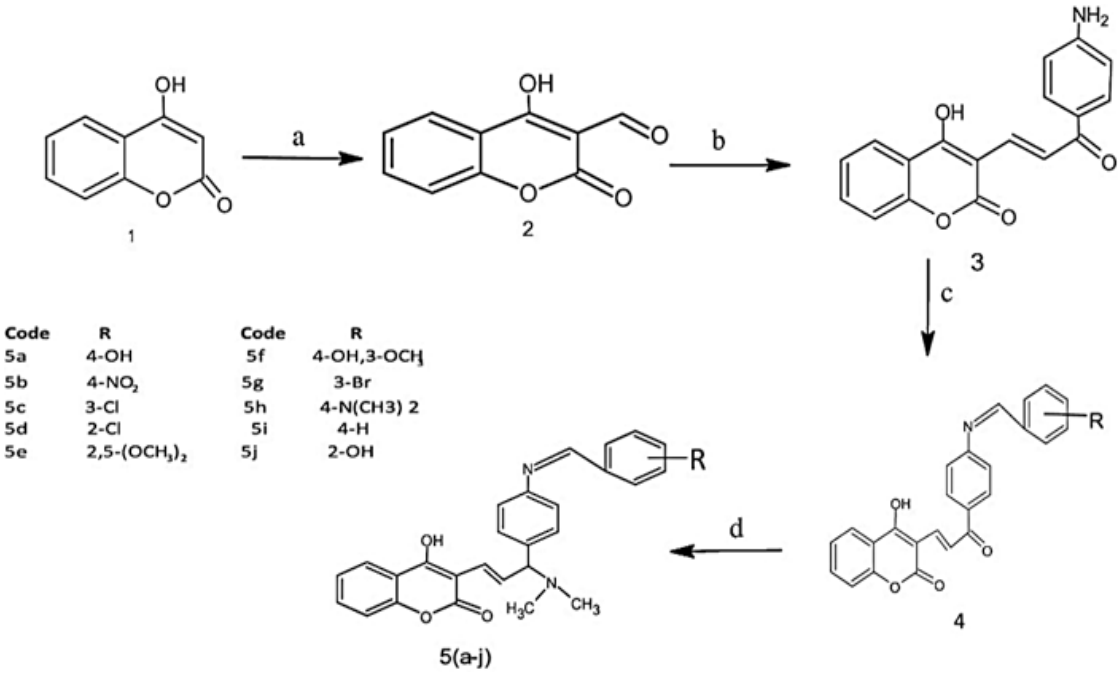


Fig. 2. Design of hybrids[6] (Chem Draw Pro software 12.0)

Scheme 1. Synthesis of coumarin-chalcone hybridsa



Code	R	Code	R
5a	4-OH	5f	4-OH,3-OCH$_3$
5b	4-NO$_2$	5g	3-Br
5c	3-Cl	5h	4-N(CH3) 2
5d	2-Cl	5i	4-H
5e	2,5-(OCH$_3$)$_2$	5j	2-OH

Fig. 3. Synthetic route for derivatives[35-37] (Chem Draw Pro 12.0 software)

EXPERIMENTAL

Methodology

We used all the reagent chemicals that were procured from Loba Chemie Pvt. India, sigma Aldrich, Qualigens, Thermo-Fisher Scientific India Pvt. Ltd. and CDH. Aromatic benzaldehydes, acetic acid, dioxane etc. These chemicals included sodium hydroxide, 4-hydroxy coumarin, piperidine, dimethylamine, chloroform, methanol. Using spectroscopic methods, such as ^{1}H NMR and ^{13}CNMR compounds were characterized. Bruker FTIR and JNM-ECZ600R/S1 600MHz instruments were used to capture IR and NMR spectra respectively. Spectra obtained after dissolving in DMSO-d6 and $CDCl_3$ in relation with TMS. In Nuclear Magnetic Resonance spectra chemical shifts shown as ppm values using the internal standard tetramithysilane (TMS) with a number of protons, coupling constants (J) in hertz (Hz), multiplicities (singlet, doublet, triplicate). Open capillaries were used to measure melting point and were uncorrected.

General procedure for synthesis of 4 hydroxy-2 oxo 2H chromene 3 carbaldehyde (2)

In 250 mL RBF, 4-hydroxy coumarin (20 g) was added alongwith 80 mL of chloroform and aqueous sodium hydroxide to create alkaline condition. Obtained mixture was mixed with continuous shaking at room temperature for 3 h, complete reaction was tracked/assessed by thin layer chromatography technique using solvent system in ratio 2:3 (chloroform: acetone). When reaction completed 40 mL ice water added into solution. Resulting carbaldehyde coumarin was filtered, rinsed with water and allowed to air dry.[35]

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

In small amount of obtained carbaldehyde (2) 4-aminoacetophenone (5 g) was added with 40 mL of chloroform. Piperidine used as catalyst and reaction mixture refluxed for 4 hours. Resultant solid rinsed with chloroform after removal of chloroform and obtained pure chalcone (3).[36]

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

Obtained chalcone in 2nd step was poured into RBF with methanol (40 mL). Separately, aromatic substituted benzaldehyde mixed with methanol. Solution of benzaldehyde added dropwise in above solution. Refluxed the mixture for 4 hours. Excess of solvent removed under pressure from resultant mixture and recrystallization done with methanol.[35]

General method of synthesis of 3-((E)-3-(4-((Z)-benzylideneamino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one (5a-j)

Mixes of compound 4 and acetic acid (20 mL) was added with dimethylamine (10 mL) and continuous shaking was done for 30 min at room temperature. Resultant mixture was poured onto ice cold water. Obtained product was filtered, rinsed with aqueous acetic acid in three portion and kept dried. Recrystallization done with methanol to get pure desired product (5a-j).[36]

3-((1E,3E)-3-(4-(((Z)-4-hydroxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5a

Yield: 69; m.p.t: 179^{0}C-182^{0}C; R_f value: 0.89; Colour: Pale Yellow; FTIR (KBr, cm^{-1}): 3422 cm^{-1}, 3424 cm^{-1}(OH), 1654 cm^{-1}(C=O), 1716 cm^{-1} (C=C), 1468 cm^{-1} (C-C, Ar), 3069 cm^{-1} (C-H, Ar), 1638 cm^{-1} (C=C), 1359 cm^{-1}(CH_3); ^{1}H NMR ($CDCl_3$, 600 MHz, ppm, TMS=0): 6.8-7.99 (s,1H, Ar-H), 8.01 (s, 1H, Imine), 3.76 (s, 1H, OH), 1.25-1.30 (d,3H, CH_3); ^{13}CNMR ($CDCl_3$, 100 MHz, ppm): 29.7(CH_3), 37.1(CH_3), 67.1(C-O-C), 76.8-77.2(CO), 115.7, 119.9-127.5(C=C), 146.1-149.1, 132.2, 152.8, 168.0 and 171.62(CO, coumarin). Mass (ESI) m/z: 440.49; found: 442.24 (M+2).

3-((1E,3E)-3-(4-(((Z)-4-nitrobenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5b

Yield: 61; m.p.t: 185^{0}C-189^{0}C; R_f: 0.84; Colour: Yellow; FTIR (KBr, cm^{-1}): 3422 cm^{-1} (OH), 1656 cm^{-1}(C=O), 1714 cm^{-1} (C=C), 1468 cm^{-1} (C-C), 3114 cm^{-1}(C-H, Ar), 868 cm^{-1} (NO_2, str), 1685 cm^{-1} (C=C, alkenyl); ^{1}H NMR ($CDCl_3$, 600MHz, ppm, TMA=0): 6.7-7.9 (s,1H, Ar -H), 8.01 (s, H, imine), 9.18 (No_2), 4.9 (s,1H, OH), 7.4-7.7 (m, 12H, Ar), 1.7-1.9 (s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm, TMS=0): 77.2-77.6(CO), 123.6(C=C), 130.4, 136.8, 151.2(C, aromatic),178.60(CO, coumarin), 129.3(C=C), 25.9(CH_3), 26.01(CH_3). Mass (ESI) m/z: 469.49 found 471.08 (M+2).

3-((1E,3E)-3-(4-(((Z)-3-chlorobenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5c

Yield: 59; m. p. t:159^{0}C-161^{0}C; R_f: 0.79; Colour: Pale Yellow; FTIR (KBr, cm^{-1}): 3420 cm^{-1} (OH), 1657 cm^{-1}, 1649 cm^{-1} (C=O), 1727 cm^{-1} and 1465 cm^{-1} (C=C, Ar) 3068 cm^{-1} (C-H, Ar), 1359 cm^{-1} (CH_3), 1688 cm^{-1} (C=C, alkenyl), 1219 cm^{-1} (C-O-C) and 755 cm^{-1} (C-Cl) respectively; ^{1}HNMR ($CDCl_3$, 600MHz, ppm, TMS=0): 7.15-7.19 (s,1H,Ar), 8.06 (s, imine), 3.78 (s, OH), 7.2-7.9 (s, 12H, Ar), 2.09 (s,CH_3); ^{13}C NMR ($CDCl_3$, 100MHz, ppm, TMS=0): 20.8, 24.8, 26.1 (CH_3), 35.8-36.3 (CH_3),171.6 (CO, coumarin), 103.1 (C, aromatic), 115.7-125.4 (C, aromatic),128.1-133.04 (C, aromatic), 67.3 (C-O-C), 152.7 and 168.5 (C=C). Mass (ESI) m/z: 458.94 found: 460.21 (M+2).

3-((1E,3E)-3-(4-(((Z)-2-chlorobenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5d

Yield: 71; m. p. t:149^{0}C-152^{0}C; R_f: 0.78; Colour: Dark red; FTIR (KBr, cm^{-1}): 3420 cm^{-1}(OH), 1656 cm^{-1} (C=O), 1464 cm^{-1}, 1685 cm^{-1}, and 1717 cm^{-1} (C=C), 3114 cm^{-1} (C-H, Ar), 1356 cm^{-1} (CH_3), 763 cm^{-1} (C-Cl); ^{1}H NMR ($CDCl_3$, 600MHz, ppm, TMS=0): 7.1-7.4 (m,1H,Ar), 8.06(d,H,imine), 3.78 (s,H, OH), 7.6-7.9(s,12H,Ar), 1.98(s,CH_3); ^{13}C NMR ($CDCl_3$, ppm, 100 MHz): 22.4(CH_3), 22.5(CH_3), 26.4(CH_3), 34.8, 76.9-77.3(CO), 103.1(C,aromatic), 126.7-129.0(C, aromatic), 168.5(CO, coumarin), 151.4(C=C). Mass (ESI) m/z: 458.94 found 460.29 (M+2).

3-((1E,3E)-3-(4-(((Z)-2,5-dimethoxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5e

Yield: 66; m.p.t.:191^{0}C-196^{0}C; R_f: 0.76; Colour: Dark Yellow; FTIR (KBr, cm^{-1}): 3424 cm^{-1} (OH), 1654 cm^{-1} (C=O), 1714 cm^{-1}(C=C), 1469 cm^{-1} (C-C), 3066 cm^{-1} (C-H, Ar, str), 1688 cm^{-1} (C=C, alkenyl), 1134 cm^{-1} (OCH_3); ^{1}H NMR ($CDCl_3$, 600MHz, ppm, TMS=0): 7.2-7.4(m, 1H, Ar), 8.08-8.09 (s, H, imine), 3.70 (H,OH), 7.10-7.46(m,12H,Ar) 2.84(s,OCH_3), 3.4-3.7(s,OCH_3), 1.38(s,CH_3); ^{13}C NMR ($CDCl_3$, ppm, 100 MHz, TMs=0): 55.5(OCH_3), 56.5(OCH_3), 115.4(CH), 164.7-169.2(CO, coumarin), 171.2(CO, coumarin), 103.7 and 105.2(C, aromatic), 110.8-116.9(C, aromatic), 120.8 and 128.4 (C, aromatic), 131.7 and 133.1(C, aromatic)), 33.3(CH_3), 35.9(CH_3). Mass (ESI) m/z: 480.54 found 481.74 (M+1).

3-((1E,3E)-3-(4-(((Z)-benzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5f

Yield: 52; m. p. t: 167^{0}C-171^{0}C; R_f: 0.69; Colour: Yellow; FTIR (KBr, cm^{-1}): 3426 cm^{-1} (O-H), 1654 cm^{-1}(C=O), C=C and alkenyl C=C observed at 1712 cm^{-1}, 1468 cm^{-1} and 1632 cm^{-1} respectively. A stretching peak for aromatic C-H was found to be at 3068 cm^{-1}. At 1356 cm^{-1} peak showed in spectrum due to CH_3.; ^{1}H NMR ($CDCl_3$, 600MHz, ppm, TMS=0): 3.15 (s, H, OH), 1.6-1.8 (s, CH_3), 7.3-7.4(m, 12H, Ar), 10.18(H, imine); ^{13}C NMR (CDCL3, 100MHz, ppm, TMS=0): 22.4(CH_3), 22.5(CH_3), 44.6, 196.5(CO, coumarin), 113.7-130.8(C, aromatic), 147.4(C=-phenyl). Mass (ESI) m/z: 424.49 found: 426.44 (M+2).

3-((1E,3E)-3-(4-(((Z)-3-bromoxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5g

Yield: 80; m. p.t:163^{0}C-168^{0}C; R_f: 0.62; Colour: Pale Yellow; FTIR (KBr, cm^{-1}): 3422 cm^{-1} (OH), 1641 cm^{-1} (C=O), 1712 cm^{-1} and 1454 cm^{-1} (C=C, Ar), 3065 cm^{-1} (CH), 1628 cm^{-1} (C=C, alkenyl), and 1358 cm^{-1} (CH_3) respectively, 753 cm^{-1} (C-Br, str); ^{1}H NMR ($CDCl_3$, 600MHz, ppm, TMS=0): 1.67, 3.17, 7.3, 9.30; ^{13}C NMR ($CDCl_3$, 100MHz, ppm, TMS=0): 22.4(CH_3), 26.5(CH_3), 119.9-133.2(C, aromatic), 141.8(CH), 196.7(CO, coumarin). Mass (ESI) m/z: 503.39 found: 503.42 (M+H).

3-((1E,3E)-3-(4-(((Z)-4-dimethylaminebenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5h

Yield: 73; m. p. t.:173^{0}C-178^{0}C; R_f: 0.89; Colour: Red; FTIR (KBr, cm^{-1}): 3424 cm^{-1}(OH), 1460 cm^{-1} (C=C), 1716 cm^{-1} and 3048 cm^{-1} (CH, str) cm^{-1}, 1674 cm^{-1} (C=O), 1346 cm^{-1} (CH_3), 1602 cm^{-1} (C=C, alkenyl); ^{1}H NMR ($CDCl_3$, 600MHz, ppm, TMS=0): 7.2-7.9(m,1H,aromatic), 3.82(H,OH),1.25-1.60 (d,3H,CH_3), 2.81-2.90(m, CH_3), 8.05(H,imine); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm, TMS=0): 168.52 (CO, coumarin), 112.8(C, aromatic), 123.7-127.3(C, aromatic), 131.3, 132.5(C, aromatic), 67.18(C-O-C), 115.6(CH), 116.6(CH), 152.6(C=C), 168.5(C=C).; Mass (ESI) m/z: 425.09 found: 425.13 (M+1).

3-((1E,3E)-3-(4-(((Z)-4-hydroxy-3-methoxy-benzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5i

Yield: 66; m. p.t:175^{0}C-178^{0}C; R_f: 0.76;

Colour: Pale Yellow; FTIR (KBr, cm^{-1}): 3424 cm^{-1}, 3426 cm^{-1} (OH), 1656 cm^{-1} (C=O), 1718 cm^{-1} (C=C), 1456 cm^{-1} and 3068 cm^{-1} (CH, Ar), 1354 cm^{-1} (CH_3) and 1688 cm^{-1} (C=C, alkenyl) respectively.; ^{1}H NMR ($CDCl_3$, 600MHz, ppm, TMS=0): 4.05 (H, OH), 2.45(s, CH_3), 3.15(s, OCH_3), 7.2-7.6(H, Ar), 11.03(H, imine); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm, TMS=0): 55.8(OCH_3), 27.4(CH_3), 27.7(CH_3), 114.6-119.6(C, aromatic), 122.01-122.8(C, aromatic), 131.6-133.4(C, aromatic), 167.2 (CO, coumarin). Mass (ESI) m/z: 470.52 found: 471.81 (M+1).

3-((1E,3E)-3-(4-(((Z)-2-hydroxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5j

Yield: 66; m. p. t.:153^{0}C-155^{0}C; R_f: 0.87; Colour: Dark Yellow; FTIR (KBr, cm^{-1}): 3420 cm^{-1} and 3424 cm^{-1} (OH), 1654 cm^{-1} (C=O), 1465 cm^{-1} and 1712 cm^{-1} (C=C, Ar), 3066 cm^{-1} (CH, Ar) , 1648 cm^{-1} (C=C, alkenyl), 1359 cm^{-1} 9CH_3); ^{1}H NMR ($CDCl_3$, 600MHz, ppm, TMS=0): 4.25(H, OH), 2.45(s,CH_3), 7.24-7.79(12H, Ar), 9.82(H, imine), 3.70(s, H, OH); ^{13}C NMR ($CDCl_3$, 100MHz, ppm, TMS=0): 131.4, 133.04, 141.4(C, aromatic), 26.8(CH_3), 24.7(CH_3), 33.3, 35.7(CH_3), 55.5(OCH_3), 56.5(OCH_3), 110.8(C, aromatic), 151.4-153.6(C=C), 178.4(CO, coumarin). Mass (ESI) m/z: 440.49 found: 442.53 (M+2).

***In silico* studies**

Auto Dock software was used for computational studies like molecular docking. 3D structures were drawn by Chem Sketch 12.0 software for both ligand (compounds) and reference. From RSCB protein data bank 3D Protein DNA gyrase (PDB ID: 6m1j) pdb file was downloaded and saved as pdbqt. Input configuration files were prepared in PDBQT format prior to docking.[38] Run the docking procedure after removal of water molecules and heteroatoms. Added polar hydrogens. Results and images of docking were analysed by Biovia Discovery studio and docking score expressed in terms of Kcal/mol. The potential of the ligands as DNA gyrase inhibitors was assessed by the interactions between the gyrase and ligands and identified the main residues involved in interactions. Physicochemical properties *(in silico)* of designed compounds were analysed by Swiss ADME web tool to evaluate their drug likenes[39] (https:/www.swissadme.ch/). SMILES formats of designed compounds were used for prediction.

Antimicrobial activity *(in vitro)*

The agar well diffusion method was used to check antimicrobial activity of synthesised hybrids against various strains such as *E. coli, P. aeruginosa, S. aureus, B. subtills*, and *C. albicans* in accordance with the NCCLS, 2002.[40] Target compound's zone of inhibition was calculated and compared with standard drugs like ciprofloxacin and fluconazole. For their antifungal and antibacterial, the microbial strains were sub cultured in potato dextrose agar and pre-sterilised nutrient agar media, respectively. After that, pre-inoculated media was aseptically moved into 4-inch diameter sterilised petri plates. Once the medium solidified, well was made with a sterile cork borer and labelled it. DMSO was used to create the standard compound's solution of 50 µg/mL concentration, while DMSO was used to dissolve the test compounds and dilute them to dose level of 100 µg/mL. After that, under sterilized conditions these concentrations were added to the petri plate bores then incubated for 48 hours. As a negative control DMSO has no effect. For every test and standard compound, zone of inhibition was measured and also compared against negative control. Almost all of the hybrids were effective against the tested microorganisms.[41]

RESULTS AND DISCUSSION

Chemistry

Synthesis of coumarin chalcone hybrids was carried out by following Scheme 1. (Fig. 3) First, 4-hydroxy coumarin was mixed with $CHCl_3$ by using solution of sodium hydroxide at room temperature, resultant mixer was stirred continuously on magnetic stirrer for 3 hours. After 3 h of stirring, ice cold water was added to the solution, filtered, rinsed with water twice then allowed to dry. Methanol was used for recrystallization and get carbaldehyde (2). Obtained product mixed with 4-aminoacetophenone by using chloroform as solvent and added piperidine as catalyst, refluxed mixture for 4 h to get chalcone (3). Obtained residue recrystalised with methanol after removal of chloroform.

Separately, aromatic substituted benzaldehyde dissolved in methanol and this solution poured into RBF containing compound 3. Refluxed the mixture for 3 h at 50^0-70^0-C. Recrystallization of obtained solid was carried with methanol (4).

Mixture of compound 4 further reacted with dimethylamine in the presence of CH_3COOH for 30 min at room temperature, shaken, filtered and rinsed with aqueous CH_3COOH thrice then kept dried. 1,4-dioxane used for recrystallization to obtain desired hybrid compounds (5(a-j)). Spectroscopic technique (^1H NMR, ^{13}C NMR and GC-MS(ESI)) were used for characterization of hybrids and spectral data were found accordingly as assumed structures. Characterization already discussed under experimental section.

Molecular docking

Autodock vina was used to analyse the targeted compounds' inclusive docking interaction and evaluate their binding activity relationship with protein. Docking demonstrated the interaction of targeted molecules (ligand) with binding sites of receptor (protein), termed as binding affinity and which observed through various interactions like Van der waal interactions, hydrogen bonding, carbon hydrogen bonding, pi sigma, amide-pi stacked, halogen bonding and hydrophobic π alkyl, $\pi-\pi$ interactions as well as electrostatic pi-anion, pi-cation interactions etc. Therefore, the strongest inhibitory action of compounds is due to hydrogen bonds and hydrophobic interactions. Whereas salt bridge also enhances inhibitory action.[42] DNA gyrase protein (PDB ID:6m1j) was selected as receptor to find binding interactions. The DNA binding protein gyrase is a potential target for the ligands in our study.[43] Molecular docking results observed that coumarin chalcone hybrids had high binding affinity towards DNA gyrase as compared to ciprofloxacin and ranges between -8.4 kcal/mol to -8.9 kcal/mol. Generally, binding affinity showed the intensity of interaction and high ligand binding affinity resulting from a stronger intermolecular force between ligand and receptor. Binding pose with main active residues ILE A:80, ASP A:75, ILE A:96, ASN A:48 and GLU A:52 showed hydrogen bonding as well as hydrophobic interactions. These solid interactions suggested the effective inhibitory activity against DNA gyrase enzyme. The molecular docking interactions and binding affinities of derivatives with targeted protein displayed in Table 1 and Figure 4.

Table 1: Binding interactions and docking score of compounds

Compounds	Binding Affinity (Kcal/mol)	Interaction Types	Amino Acid Residues
5a	-8.5	Conventional hydrogen bond, Carbon hydrogen bond, Pi alkyl, Pi sigma, Salt bridge	ASN B:48, VAL B:122, GLU B:52, GLY B:121, VAL B:169, ILE B:80, ILE B:96, THR B:167, ASP B:75
5b	-8.4	Conventional hydrogen bond, Carbon hydrogen bond, Pi alkyl, Pi sigma, Pi-amide stacked, Salt bridge	VAL B:122, GLU B:54, GLY B:121, ASN B:48, ASP B:51, GLU B:52, VAL B:169, ILE B:80, THR B:167, ASP B:75
5c	-8.8	Carbon hydrogen bond, Pi alkyl, Pi sigma, Salt bridge, Pi-amide stacked, Halogen bond (chloro), Pi-donor hydrogen bond	THR A:167, ILE A:80, VAL A:169, LUE A:198, ILE A:96, VAL A:169, ASP A:75, ASN A:48, VAL A:99, GLU A:52
5d	-8.6	Hydrogen bond, Pi alkyl, Pi-amide stacked, Salt bridge (attractive charge)	PRO A:81, THR A:167, ALA A:102, ILE A:96, ILE A:80, ASN A:48, GLU A:52
5e	-8.8	Carbon hydrogen bond, Pi sigma, Pi alkyl, Salt bridge, Amide-pi stacked	SER B:200, GLU A:52, ILE A:80, VAL A:122, ILE A:96, LUE A:198, PRO A:80, ILE A:80, ASP A:75, ASN A:48
5f	-8.9	Van der waals, Pi-alkyl, Amide pi stacked, Pi-Pi T shaped, Pi sigma, Salt bridge, Carbon hydrogen bond	GLU A:52, VAL A:122, ILE A:80, PRO A:81, ASN A:48, PHE A:197, ILE A:80, ASP A:75, GLU A:52
5g	-8.7	Conventional hydrogen bond, Carbon hydrogen bond, Pi sigma, Halogen bond, Pi cation	GLY B:121, PRO A:121, GLY B:221, ILE A:96, VAL A:122, ARG A:78, GLU A:52
5h	-8.7	Carbon hydrogen bond, Pi donor H-bond, Amide pi stacked, Pi-Pi T shaped, Pi alkyl, Pi sigma, Van der waals	ASN A:48, THR A:167, GLU A:52, PHE A:197, ASN A:48, ILE A:96, VAL A:169, VAL A:45, ILE A:80, VAL A:45, GLY A:221
5i	-8.5	Conventional H-bond, Pi-Pi stacked, Pi alkyl, Pi anion, Van der waals, Pi sigma	ILE A:96, PHE A:197, ALA A:102, ILE A:80, VAL A:122, LUE B:198, GLU A:52, THR A:167, LUE B:198
5j	-8.4	Carbon hydrogen bond, Attractive charge, Amide pi stacked, Pi alkyl, Conventional H-bond, Van der waals	THR A:167, PRO A:81, GLU A:52, ASN A:48, ILE A:96, ALA A:102, ILE A:80, LUE A:100, GLY A:121
Ciprofloxacin	-8.3	Conventional H-bond, Pi anion, Pi alkyl, Alkyl bond, Van der waals	GLU B:44, ARG A:191, LYS A:190, GLU B:194, ARG B:191, VAL A:90, HIS B:40, ILE B:187, PHE B:43

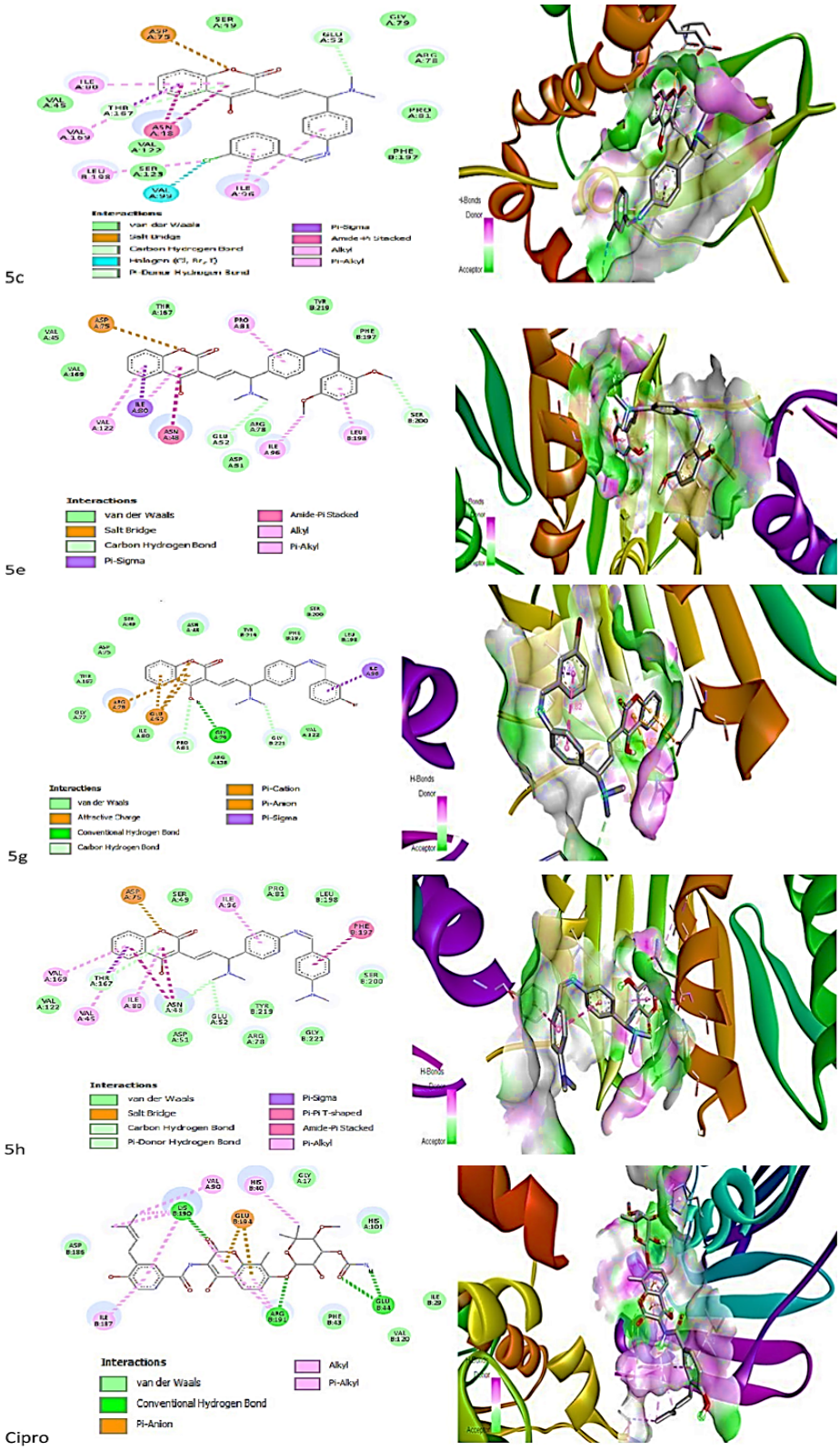


Fig. 4. 2D and 3D images of docking interactions. (Obtained from Auto Dock Vina software)

ADMET parameters

The Swiss ADME web-based program was used to assess the ADMET profile of designed compounds 5(a-j) and predict their physicochemical properties. All the properties fall within desired pink area of bioavailability radar (Fig. 5), indicating that the derivatives have favourable drug-like properties. ADMET profile provided in Table 2. All the compounds have reasonable ADMET properties.[44]

Table 2: ADMET properties of compounds as per LRO5.[44]

Compounds	Mol. Wt.	Lipophilicity	TPSA(A^0)$_2$	RB	HBD	HBA	MR	Water solubility	G. I. absorption	B.B.B. Permeant	PAINS	BRENK
5a	440.49	2.84	86.27	6	2	6	132.12	MS	High	No	0	0
5b	469.49	3.28	111.86	7	1	7	138.92	PS	High	No	0	3
5c	458.94	3.85	66.04	6	1	5	135.11	MS	High	No	0	0
5d	458.94	3.85	66.04	6	1	5	135.11	MS	High	No	0	3
5e	484.54	2.70	84.50	8	1	7	143.08	MS	High	No	0	0
5f	424.49	3.38	66.04	6	1	5	130.10	PS	High	No	0	0
5g	503.39	3.94	66.04	6	1	5	137.80	MS	High	No	0	0
5h	424.49	3.38	66.04	6	1	5	130.10	MS	High	No	0	0
5i	470.52	2.50	95.50	7	2	7	138.61	MS	High	No	0	0
5j	440.49	2.84	86.27	6	2	6	132.12	MS	High	No	0	0

MS: Moderately soluble, RB: Rotatable bond, MR: Molar refractivity, TPSA: Topological polar surface area

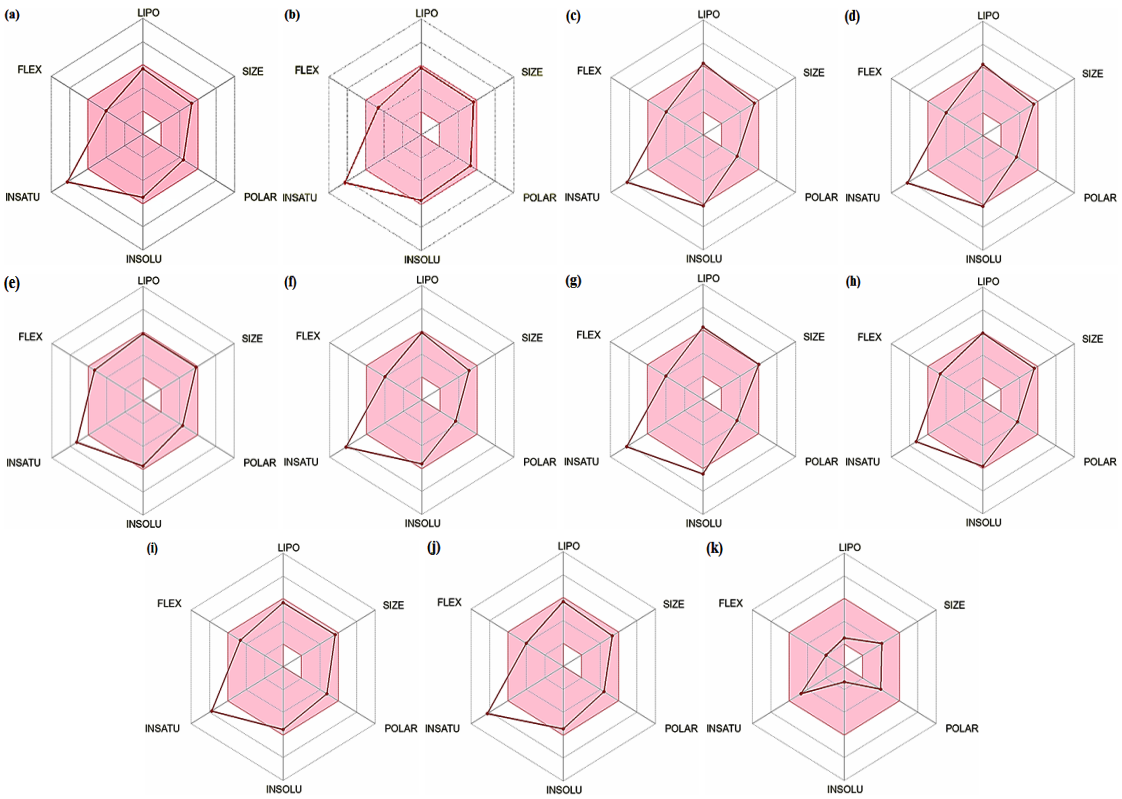


Fig. 5. Bioavailability radar for compounds (5a-j) and ciprofloxacin (designed by Swiss ADME Swiss drug design software)

Antimicrobial Evaluation *(In vitro)*

Using agar well diffusion method,[45] the antimicrobial properties of synthesised derivatives assessed against *P. aeruginosa, E. coli, B. subtills, S. aureus* and *C. albicans*. MHA (Muller Hinton Agar) was used to culture the inoculum bacteria overnight at 37^{0}C at 200 rpm with constant shaking. To create an even lawn, sterilised cotton buds were used to inoculate the cultured bacteria on MHA surface. A sterile paper disc was placed on the

agar plate's surface using sterile forceps after being impregnated with compound (100 ppm) in DMSO. The inhibitory zones were measured in mm after incubation of plates 24 h at 37^{0}C to estimate efficacy of tested derivatives.[46,47] Compounds 5c, 5e, 5g and 5h demonstrated significant inhibitory effect against the targeted strains of bacteria and fungi, according to the results whereas other compounds showed reduced activity. For the potent compounds zones of inhibition were observed as follows: 5.56±0.179, 5.58±0.449, 4.94±0.811, 4.82±0.378 against *B. subtills*; 7.25±0.191, 6.11±0.496, 5.55±0.496, 5.41±0.421 against *S. aureus*; 6.36±0.024, 6.27±0.029, 5.99±0.666, 6.04±0.432 against *E. coli*; 5.93±0.118, 4.94±0.016, 6.58±0.079, 5.94±0.119 against *P. aeruginosa*; 7.92±0.389, 7.12±0.401, 7.10±0.171, 6.96±0.331mm against *C. albicans*, respectively. Compound 5c exhibited strong potential as both antibacterial and antifungal against *S. aureus* and *C. albicans* more than reference drugs ciprofloxacin and fluconazole respectively. The obtained results of the selected compounds may be effective agents for antimicrobial efficacy. Table 3 and Fig. 6 and 7 lists the results of both antibacterial and antifungal activities.

Table 3: Antimicrobial profile of derivatives (5a-j)

Compounds	Zone of inhibitions (mm)#				
	Bacterial strains				Fungal strain
	Gram+ve		*Gram-ve*		
	B. subtills	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
5a	4.06±0.396	4.24±0.152	4.04±0.024	4.14±0.411	5.16±0.267
5b	4.80±0.361	4.89±0.014	4.58±0.262	4.88±0.368	3.99±0.421
5c	5.56±0.179	7.25±0.191	6.36±0.024	5.93±0.118	7.92±0.389
5d	4.14±0.410	5.01±0.086	4.71±0.314	4.80±0.543	4.96±0.811
5e	5.58±0.449	6.14±0.496	6.27±0.029	4.94±0.016	7.12±0.401
5f	5.48±0.446	4.98±0.368	5.91±0.189	5.49±0.106	7.10±0.171
5g	4.94±0.811	5.55±0.496	5.99±0.666	6.58±0.029	6.96±0.331
5h	4.82±0.378	5.41±0.421	6.04±0.432	5.94±0.119	5.95±0.648
5i	4.36±0.126	4.89±0.376	4.64±0.261	4.93±0.810	4.87±0.081
5j	3.99±0.020	4.16±0.401	3.92±0.421	4.80±0.364	4.74±0.211
Ciprofloxacin	5.97±0.221	6.89±0.219	6.71±0.281	6.81±0.174	**
Fluconazole	**	**	**	**	7.69±0.246

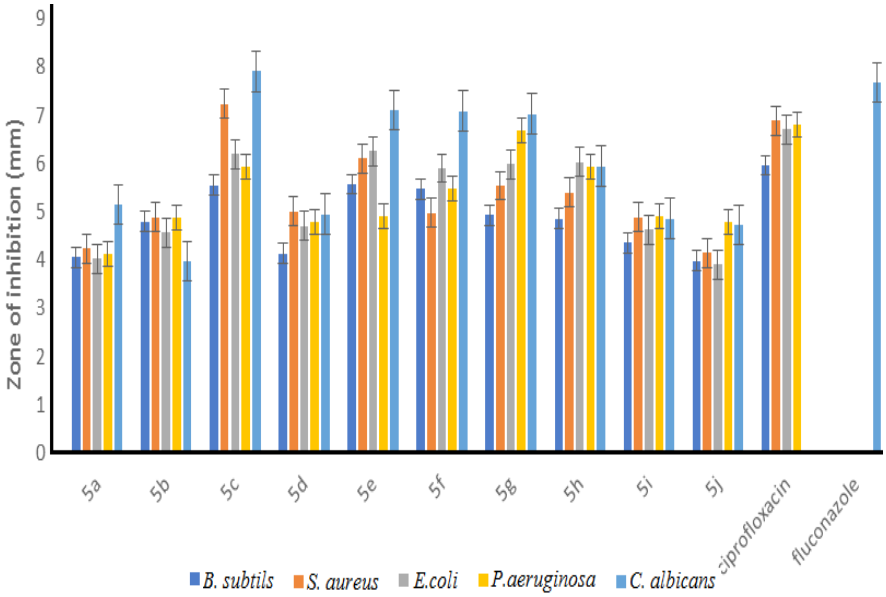


Fig. 6. Graphical representation of antimicrobial activity of synthesised derivatives and reference drugs against different strains. (created by raw data obtained by well diffusion assay)

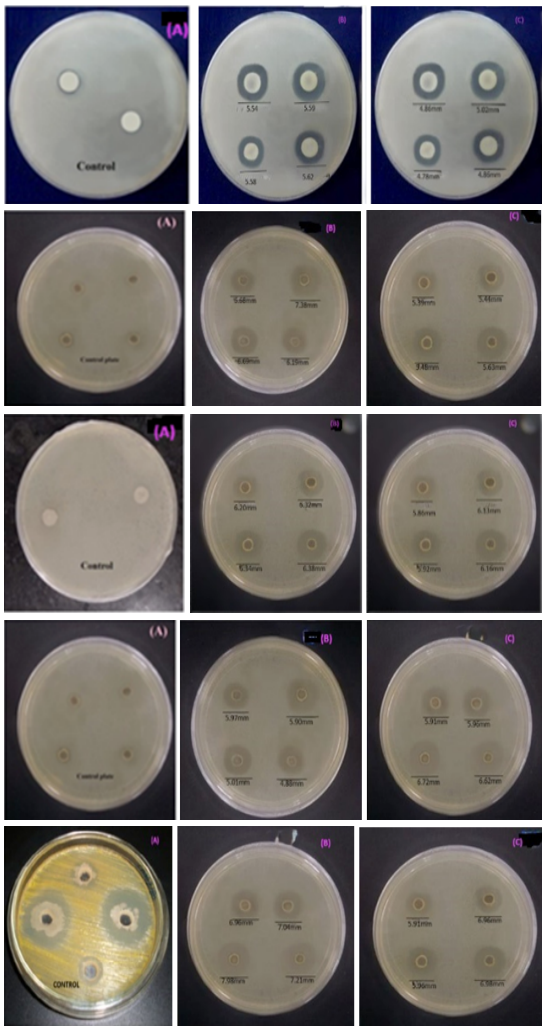


Fig. 7. Antimicrobial activity of potential compounds 5c, 5e, 5g and 5h against various microbial strains such as *B. subtills, S. aureus, E. coli, P. aeruginosa* and *C. albicans*. A1 represent plates for *B. subtills*; B1 represents *S. aureus*; C1 represents *E. coli*; D1 represents *P. aeruginosa*; E1 represents *C. albicans* (In each A plate for control, B plate for 5c and 5e, C plate for 5g and 5h). (obtained through well diffusion assay)

CONCLUSION

In medicinal chemistry, chalcones are useful lead compounds for development of novel candidates together with coumarin. These derivatives designed by in silico technique particularly molecular docking. All derivatives exhibited greater binding energy than ciprofloxacin (standard) in range -8.3 kcal/mol to -8.9 kcal/mol. In this study, chalcone coumarin hybrids were synthesised by base catalysed condensation reaction and synthesis performed by click chemistry approach. Characteristically the response was observed by TLC for purity determination of derivatives. Spectral analysis done for characterization of compounds and subjected for antimicrobial activity against DNA gyrase for the survivability of different strains such as *B. subtills, S. aureus, E. coli, P. aeruginosa* and *C. albicans* as potential antibacterial agents. However, in silico ADME analysis found that most of compounds fits in Lipinski's rule of five of drug likeness and showed moderate water solubility that indicates synthesised compounds could be applicable for orally administration. Results found that derivatives 5c, 5e, 5g and 5h possess promising antimicrobial activity. Therefore, it is most evident that hybrids of coumarin and chalcone are most prominent candidate for antimicrobial action. The results of present study showed that coumarin chalcone derivatives provide an additional option for antimicrobial action.

Thus, this work opens the door for further research focussed on creating novel therapeutic candidates against microbial resistance.

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Conflict of Interest

Authors have no conflict of interest.

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