Novel O-Alkylated Chromones as Antimicrobial Agents: Ultrasound Mediated Synthesis, Molecular Docking and ADME Prediction

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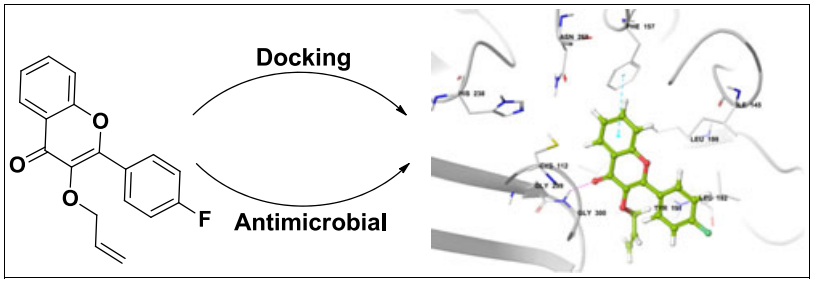


In the development of novel antimicrobial agents, we synthesized novel O-alkylated chromones **4a–f** by ultrasound-assisted method. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. All compounds were assessed *in vitro* for their efficacy as antimicrobial agents against four bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*) and three fungi (*Candida albicans*, *Candida glabrata*, *Candida tropicalis*). In particular, compounds **4a**, **4b**, **4d**, **4e**, and **4f** exhibited potent antimicrobial activity. Molecular docking study was used to rationalize binding interaction at the active site, and the result showed good binding interaction. The compounds were also processed for *in silico* ADME prediction, and the result showed that compounds could be exploited as an oral drug candidate.

INTRODUCTION

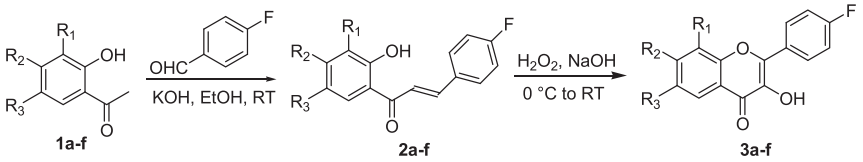
Ultrasound-mediated organic synthesis has attracted much attention during the past few years. Ultrasound-assisted synthesis has been considered as a green and efficient technique used to accelerate organic reactions proceed via acoustic cavitations. Cavitation induces in a liquid because of the compression and rarefaction of cycles of the propagating sound waves [1]. Under ultrasound irradiation, a large number of organic reactions can be carried out with improved yield, shorter reaction time, and milder reaction condition [2,3].

Infections triggered by the rapid development of multidrug resistant pathogens have reached an alarming level and became a complex and challenging health problem. In order to prevent this serious problem, there is an urgent need for the development of novel antimicrobial agents [4]. Therefore, in recent years, the researchers have been focused on the synthesis of new antimicrobial agents. Inclusion of fluorine atom into an organic molecule may enhance biological and physical properties of compounds such as metabolic stability, solubility, bioavailability, and lipophilicity [5,6]. Inherent electronic properties of fluorine such as small size, high electronegativity, and strong C─F bond can dramatically alter the biological response of the compound [7]. Fluorine substitution remains a striking aspect in the development of more active and selective drug candidate.

Chromone and its analogs are important pharmacophore in the medicinal chemistry and have featured in some clinically used drugs. Chromone (benzopyran-4-one) derivatives have exhibited an interesting biological activity such as antitumor, anti-diabetes [8], antifungal [9], anti-HIV [10], antioxidant [11], antiallergic [12], anti-inflammatory [13], and neuro protective activity [14]. It was reported that the 3-substituted chromone such as Ipriflavone possesses antiosteoporosis activity [15].

Many synthetic methods are reported for the synthesis of alkylated chromone derivatives [16,17]. The present method is advantageous with respect to the green protocol of synthesis (ultrasound method). In our ongoing research work, focusing on chromone as a scaffold for

Scheme 1. Synthesis of 3-hydroxychromones **3a–f.**



the development of novel antimicrobial agent [18], we designed and synthesized fluorine containing O-alkylated chromone derivatives under ultrasound method and evaluated for antimicrobial activity. We also investigated the binding interaction of the synthesized compounds at the active site of *Staphylococcus aureus* KAS IIIenzyme using molecular docking studies and also performed ADME prediction of the synthesized compounds.

RESULTS AND DISCUSSION

Chemistry. We have synthesized novel O-alkylated chromone derivatives **4a–f** under conventional method as well as ultrasound irradiation method. The key starting material, 3-hydroxy chromones **3a–f** was synthesized in our previous report [19] (Scheme 1). 3-Hydroxy chromones **3a–f** have been alkylated with allyl bromide in the presence of K_2CO_3 in *N*,*N*′-DMF at the room temperature to furnish the desired product **4a–f** (Scheme 2).

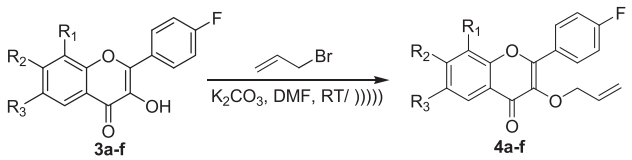
Considering the significance of ultrasound irradiation, we next attempted to investigate the effect of ultrasonication on this synthesis using optimized reaction conditions. The results are summarized in (Table 1). It was observed that ultrasonic irradiation led to relatively higher yield and reduced reaction time as compared with the conventional method. Thus, ultrasonic irradiation was found to be beneficial for the synthesis of O-alkylated chromone derivatives **4a–f**.

The compounds **4a–f** was confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. The IR spectrum of **4a** showed the significant alkene CH-stretching frequency υ at 3075 cm^{-1} and υ at 1606 cm^{-1} have confirmed the (C═C). In the ^{1}H NMR spectrum of compound **4a**, the methylene group attached to oxygen showed doublet at δ 4.65 ppm, two germinal olefinic protons shows doublet of doublets at δ 5.16 and 5.28 ppm, and one olefinic proton adjacent to a methylene group shows doublet of doublet of triplets at δ 5.93 ppm. In the ^{13}C NMR spectrum of compound **4a**, the signal at 73.21 ppm indicates the presence of methylene group attached to oxygen. The mass spectrum of **4a** showed $[M+Na]^+$ at 319.0. Likewise, **4b–f** derivative were in good conformity with the spectroscopic data.

Antimicrobial activity. In the screening assay studies, all the compounds were evaluated for antibacterial activity against Gram-positive bacteria viz. *Staphylococcus aureus* NCIM 2178, *Bacillus subtilis* NCIM 2250, and Gram-negative bacteria viz. *Escherichia coli* NCIM 2137, *Pseudomonas aeruginosa* NCIM 2036, and antifungal activity against *Candida albicans* MTCC 277, *Candida glabrata* NCIM 3236, and *Candida tropicalis* NCIM 3110 (Table 2). The minimum inhibitory concentration (MIC) values were determined by micro-broth dilution method. The results of antibacterial screening were compared with the standard antibacterial drug streptomycin. DMSO was used as solvent control.

From the antimicrobial screening of newly synthesized compounds, compound **4d** showed equipotent activity compared with streptomycin MIC 400 μg/mL against *E. coli*, and compounds **4b**, **4d**, and **4e** showed equipotent activity against *P. aeruginosa* as compared with standard streptomycin MIC 200 μg/mL. Compounds

Scheme 2. Synthesis of O-alkylated chromone derivatives **4a–f.**



a R_1=H R_2=H R_3=H
b R_1=H R_2=H R_3=Cl
c R_1=CH_3 R_2=H R_3=H
d R_1=H R_2=H R_3=CH_3
e R_1=Cl R_2=H R_3=Cl
f R_1=H R_2=CH_3 R_3=Cl

Table 1

Ultrasound-promoted synthesis of O-alkylated chromone derivatives **4a–f.**

Entry	Ultrasound method		Conventional method		Melting point (°C)
	Time (min)	Yield[a] (%)	Time (min)	Yield[a] (%)	
4a	40	83	110	75	55–57
4b	30	90	95	78	79–81
4c	35	80	95	69	80–82
4d	35	82	100	74	53–55
4e	32	86	95	80	90–92
4f	35	92	90	87	113–115

[a]Isolated yields.

4a and **4c** with MIC 50 μg/mL showed considerable activity against *S. aureus* compared with standard streptomycin MIC 12.5 μg/mL. Compound **4b** has a *chloro* group on chromone ring with MIC 50 μg/mL showed potent activity against *B. subtilis.* Compounds **4a** and **4f** with MIC 100 μg/mL exhibited highly potent activity against *E. coli* and *P. aeruginosa*.

Remarkably, allyl derivative of fluorinated chromone are more susceptible to fungal strains. Compounds **4a**, **4d**, and **4f** showed equipotent activity with miconazole MIC 12.5 μg/mL against *C. albicans*. Compound **4a** has no substituent, **4d** has *methyl* substituent, and **4f** has *chloro*, *methyl* substituent on chromone ring, with MIC 12.5 μg/mL exhibited highly potent antifungal activity against *C. glabrata* and *C. tropicalis*.

Molecular docking study. In order to understand activity against *S. aureus*, we performed the docking study of the most potent compounds to visualize and compare their binding pose with literature data into the active site of *S. aureus* KAS III. β-Ketoacyl-acyl carrier protein (ACP) synthase III catalyses the initial condensation of acetyl-CoA to malonyl-ACP and plays an essential role in the biosynthesis of bacterial fatty acids. The enzyme is characterized by having a Cys-His-Asn catalytic triad, located at the bottom of a hydrophobic tunnel. The docking pose of compounds for *S. aureus* KAS III was compared with the standard streptomycin.

The docking studies showed that the primer site of *S. aureus* KAS III enzyme is associated with key amino acid residues Cys112, His238, Asn268, Phe298, Phe157, Leu190, and Gly300. In spite of the identical sequence for all amino acids, the sifting of the side chain and the movement of some key amino acids makes the primer binding site of *S. aureus* KAS III enzyme somewhat larger [20]. Thus, compounds from series **4a–f** bind more perfectly in the primer binding site of *S. aureus* KAS III enzyme and showed higher activity against *S. aureus.* Compound **4a** acquire the binding pose in the active site of *S. aureus* KAS III enzyme (Fig. 1). Chromone group of compound **4a** spans near the catalytic tried and forms hydrogen bond with Gly300. It is also observed that all the docked compounds are stabilized in the prime site of *S. aureus* KAS III enzymes mainly through hydrophobic interaction with surrounding amino acid residues.

***In silico* ADME prediction.** The drug achievement is determined by good efficacy and also through the pharmacokinetics filters. ADME properties such as absorption, distribution, metabolism, and excretion are important to determine the efficacy of the molecule. Molecular volume, MW, logarithm of partition coefficient (milog*P*), number of hydrogen bond acceptors (n-ON), number of hydrogen bond donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB), and Lipinski's rule of five [21] were calculated using the Molinspiration online property calculation

Table 2

Antimicrobial screening data of the compounds **4a–f.**

Entry	Antimicrobial activity[a] (μg/mL)						
	Antibacterial activity				Antifungal activity		
	Sa	*Bs*	*Ec*	*Pa*	*Ca*	*Cg*	*Ct*
4a	50	100	100	100	12.5	12.5	12.5
4b	100	50	200	200	25	25	25
4c	50	100	800	400	50	50	50
4d	400	800	400	200	12.5	12.5	12.5
4e	100	200	200	200	50	25	50
4f	100	100	100	100	12.5	12.5	12.5
Streptomycin	12.5	400	400	200	—	—	—
Miconazole	—	—	—	—	12.5	400	800

Sa, *Staphylococcus aureus*; *Bs*, *Bacillus subtilis; Ec*, *Escherichia coli*; *Pa*, *Pseudomonas aeruginosa*; *Ca*, *Candida albicans*; *Cg*, *Candida glabrata*; *Ct*, *Candida tropicalis.*

[a]Minimum inhibitory concentration (μg/mL) against the pathological strains based on micro-broth dilution method.

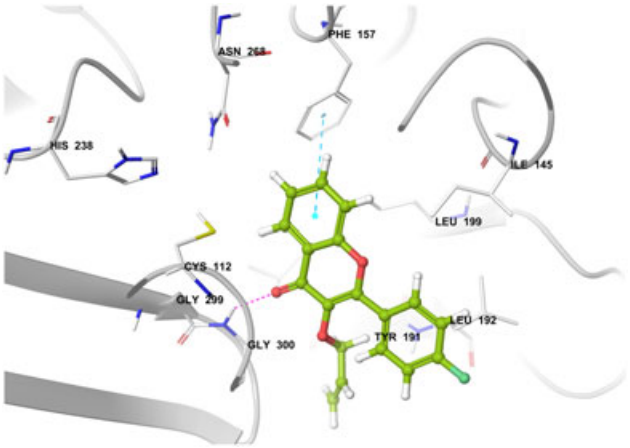


Figure 1. Binding pose of compound **4a** in the prime site of *Staphylococcus aureus* KAS III. Pink dotted line indicated hydrogen bonds and light blue dotted line indicate Pi-Pi stacking between ligand and enzyme. [Color figure can be viewed at wileyonlinelibrary.com]

toolkit [22]. Absorption was calculated by percentage (%) ABS = 109 – (0.345 × TPSA) [23]. Drug-likeness model score was computed by MolSoft software [24].

Some important chemical descriptors correlate with ADME properties are such as TPSA, low MW, and octanol–water partition coefficient (milog*P*). TPSA analysis is the primary determinant of fractional absorption through which bioavailability of compounds was accessed. It was observed that the passively absorbed compounds with PSA > 140 Å^2 has low oral bioavailability and low MW has high oral absorption. The excretion process that eliminates the compound from the body depends on the MW and octanol–water partition coefficient (milog*P*). A molecule likely to be developed as an orally active drug candidate should be evidence for no more than one violation of the following four criteria: MW $\leq$ 500, number of hydrogen bond acceptors $\leq$10, number of hydrogen bond donors $\leq$5, and milog*P* (octanol–water partition coefficient) $\leq$ 5 [25].

A computational study of compounds was performed for assessment of ADME properties. The obtained values are presented in (Table 3). From all these parameters, it was observed that the compounds exhibited good % ABS (% absorption) ranging from 91.60 to 95.39%. Compounds obey Lipinski's rule of five except compounds **4b**, **4e**, and **4f**, which violated the Lipinski's rule of five (milog*P* $\leq$ 5). The larger the value of the drug-likeness model score, the higher will be the probability of a particular molecule to be active. All tested compounds followed the criteria for orally active drug. As a result, these compounds may have good potential for eventual development as oral agents.

CONCLUSIONS

Ultrasound method was used to promote the synthesis of O-alkylated chromone derivatives **4a–f** in good yields with more purity in short reaction time. The synthesized compounds were evaluated for antimicrobial activity. It can be concluded that the compounds **4a**, **4b**, **4d**, **4e**, and **4f** were identified as potent antimicrobial agents. Binding of the most active compound against *S. aureus* KAS III was studied using molecular docking studies. In addition, ADME analysis has shown that compounds have good drug-like properties and can be developed as oral drug candidates.

Table 3

Pharmacokinetic parameters important for good bioavailability.

Entry	% ABS	TPSA (Å^2)	n-ROTB	MV	MW	milog*P*	n-ON	n-OHNH	Lipinski's violations	Drug-likeness model score
Rule	—	—	—	—	<500	≤5	<10	<5	≤1	—
4a	95.39	39.45	4	258.44	296.30	4.53	3	0	0	0.89
4b	95.39	39.45	4	271.98	330.74	5.18	3	0	1	0.48
4c	95.39	39.45	4	275.00	310.32	4.93	3	0	0	0.26
4d	95.39	39.45	4	275.00	310.32	4.95	3	0	0	0.41
4e	95.39	39.45	4	285.52	365.19	5.79	3	0	1	-0.07
4f	95.39	39.45	4	288.54	344.77	5.56	3	0	1	0.28

EXPERIMENTAL

All the solvents and reagents used in synthesis were obtained from commercial sources and were used without further purification. Melting points were recorded by the open tube capillary method and are uncorrected. The purity of compounds and completion of the reaction was checked by TLC. Infrared spectra were recorded on Carry 600 Series FT-IR spectrophotometer using KBr pellets. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Bruker AVANCE II 400 NMR spectrometer in $CDCl_3$/DMSO-d_6 solution. Tetramethylsilane was used as an internal standard. Chemical shift values are given in ppm relative to TMS as internal reference and the coupling constant (J) in Hertz. The splitting pattern abbreviations are assigned as singlet (s), doublet (d), triplet (t), broad singlet (brs), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of doublet of triplets (ddt), and multiplet (m). Mass spectra were recorded on an WATERS, Q-TOF micro mass equipped with an electron spin impact source. Elemental analysis was performed on a Perkin-Elmer EAL-240 elemental analyzer. For ultrasonic irradiation, Bandelin Sonorex (frequency 40 MHz, power 100 W) ultrasound bath was used, and the reaction flask was located in the ultrasonic bath containing water.

General procedure for the synthesis of compounds 3a–f. The mixture of (*E*)-3-(4-fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one **2a–f** (1 mmol), ethanol (15 mL), NaOH (10%, 5 mL), and hydrogen peroxide (30%, 1.5 mL) was stirred vigorously for 30 min and kept for 2–3 h at ice cold condition. The progress of reaction was observed by TLC using ethyl acetate:hexane as a solvent system. After completion of the reaction, the reaction mixture was poured into ice cold water and acidified with 1 M HCl. The precipitate was collected by filtration, washed with water and crystallized from chloroform-ethanol to afford the pure product.

2-(4-Fluorophenyl)-3-hydroxy-4H-chromen-4-one (3a). The compound was obtained from **(***E*)-3-(4-fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**2a**) as yellow solid, yield 74%, mp: 152–154°C, IR (KBr, cm^{-1}): 3297, 2925, 1618, 1572; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (brs, 1H, ArH), 7.22 (t, 2H, J = 8.6 Hz, ArH), 7.42 (ddd, 1H, J = 7.6, 7.5, 1.1 Hz, ArH), 7.58 (d, 1H, J = 8.4 Hz, ArH), 7.71 (ddd, 1H, J = 8.5, 7.1, 1.7 Hz, ArH), 8.24 (d, 1H, J = 1.8 Hz, ArH), 8.26 (s, 1H, OH), 8.27–8.30 (m, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 115.7, 115.9, 118.2, 120.7, 124.6, 125.5, 127.3, 129.9, 133.7, 138.2, 144.1, 155.3, 162.3, 164.8, 173.4; LCMS calculated for $C_{15}H_9FO_3$ $[M+H]^+$: 257.0, found: 257.0. *Anal*. Calcd for $C_{15}H_9FO_3$: C, 70.31; H, 3.54; found: C, 70.34; H, 3.57.

6-Chloro-2-(4-fluorophenyl)-3-hydroxy-4H-chromen-4-one (3b). The compound was obtained from (*E*)-1-(5-chloro-2-hydroxyphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**2b**) as yellow solid, yield 77%, mp: 213–215°C, IR (KBr, cm^{-1}): 3303, 3028, 1622, 1575; ^{1}H NMR (400 MHz, DMSO): δ 7.08–7.25 (m, 2H, ArH), 7.68–7.82 (m, 2H, ArH), 8.12–8.23 (m, 2H, ArH), 8.51 (s, 1H, ArH), 9.08 (brs, 1H, OH); ^{13}C NMR (100 MHz, DMSO): δ 114.1, 117.5, 120.6, 125.4, 127.4, 128.4, 129.7, 130.2, 133.6, 148.5, 155.4, 157.6, 160.8, 162.5, 172.9; LCMS calculated for $C_{15}H_8ClFO_3$ $[M+H]^+$: 291.0224, found: 291.2223. *Anal*. Calcd for $C_{15}H_8ClFO_3$: C, 61.98; H, 2.77; found: C, 61.72; H, 2.94.

2-(4-Fluorophenyl)-3-hydroxy-8-methyl-4H-chromen-4-one (3c). The compound was obtained from (*E*)-3-(4-fluorophenyl)-1-(2-hydroxy-3-methylphenyl)prop-2-en-1-one (**2c**) as yellow solid, yield 69%, mp: 160–162°C, IR (KBr, cm^{-1}): 3218, 2915, 1604, 1562; ^{1}H NMR (400 MHz, DMSO): δ 2.54 (s, 3H, CH_3), 7.24–7.29 (m, 3H, ArH), 7.53 (d, 1H, J = 7.0 Hz, ArH), 7.93 (d, 1*H*, J = 7.7 Hz, ArH), 8.26 (dd, 2H, J = 8.1, 5.5 Hz, ArH), 9.38 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO): δ 15.3, 115.1, 115.3, 120, 122.3, 123.6, 127.1, 127.9, 129.6, 133.7, 138.7, 143.5, 152.8, 161.3, 163.7, 173; LCMS calculated for $C_{16}H_{11}FO_3$ $[M+H]^+$: 271.077, found: 271.2154. *Anal*. Calcd for $C_{16}H_{11}FO_3$: C, 71.11; H, 4.10; found: C, 71.23; H, 4.16.

2-(4-Fluorophenyl)-3-hydroxy-6-methyl-4H-chromen-4-one (3d). The compound was obtained from (*E*)-3-(4-fluorophenyl)-1-(2-hydroxy-5-methylphenyl)prop-2-en-1-one (**2d**) as yellow solid, yield 73%, mp: 171–173°C, IR (KBr, cm^{-1}): 3239, 2983, 1628, 1504; ^{1}H NMR (400 MHz, DMSO): δ 2.46 (s, 3H, CH_3), 7.27 (t, 2H, J = 8.6 Hz, ArH), 7.54 (s, 2H, ArH), 7.91 (s, 1H, ArH), 8.27–8.30 (m, 2H, ArH), 9.31 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO): δ 20.5, 115, 115.2, 117.7, 120.9, 123.8, 127.7, 129.8, 130.6, 134.5, 138.7, 143.9, 152.8, 161.3, 163.8, 172.8; LCMS calculated for $C_{16}H_{11}FO_3$ $[M+H]^+$: 271.077, found: 271.2234. *Anal*. Calcd for $C_{16}H_{11}FO_3$: C, 71.11; H, 4.10; found: C, 71.24; H, 4.11.

6,8-Dichloro-2-(4-fluorophenyl)-3-hydroxy-4H-chromen-4-one (3e). The compound was obtained from (*E*)-1-(3,5-dichloro-2-hydroxyphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**2e**) as yellow solid, yield 75%, mp: 203–205°C, IR (KBr, cm^{-1}): 3245, 2916, 1625, 1502; ^{1}H NMR (400 MHz, DMSO): δ 7.25 (t, 2H, J = 8.8 Hz, ArH), 7.82 (d, 1H, J = 2.6 Hz, ArH), 7.96 (d, 1H, J = 2.2 Hz, ArH), 8.29 (dd, 2H, J = 8.8, 5.5 Hz, ArH), 9.81 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO): δ 115.2, 115.4, 122.8, 122, 123.7, 127.1, 128.8, 129.8, 132.6, 139.2, 144.5, 148.4, 161.5, 164, 171.3; LCMS calculated for $C_{15}H_7Cl_2FO_3$ $[M+H]^+$: 324.9835, found: 325.1267. *Anal*. Calcd for $C_{15}H_7Cl_2FO_3$: C, 55.41; H, 2.17; found: C, 55.49; H, 2.15.

6-Chloro-2-(4-fluorophenyl)-3-hydroxy-7-methyl-4H-chromen-4-one (3f). The compound was obtained from (*E*)-1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**2f**) as yellow solid, yield 79%, mp: 222–224°C, IR

(KBr, cm^{-1}): 3238, 2919, 1628, 1503; ^1H NMR (400 MHz, DMSO): δ 2.5 (s, 3H, CH_3), 7.28 (t, 2H, J = 8.3 Hz, ArH), 7.64 (brs, 1H, ArH), 8.03 (s, 1H, ArH), 8.27 (brs, 2H, ArH), 9.54 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO): δ 20.3, 115.1, 115.3, 120.1, 120.5, 124, 127.5, 129.9, 130.1, 138.8, 141.8, 144.3, 152.8, 161.4, 163.9, 171.8; LCMS calculated for $C_{16}H_{10}ClFO_3$ $[M+H]^+$: 305.0381, found: 305.2251. *Anal*. Calcd for $C_{16}H_{10}ClFO_3$: C, 63.07; H, 3.31; found: C, 63.13; H, 3.38.

General procedure for synthesis of compounds 4a–f. ***Conventional method.*** To the stirred solution of substituted 2-(4-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one **3a–f** (1 mmol) in DMF, potassium carbonate (2 mmol) and allyl bromide (1 mmol) were added at room temperature for the period of time indicated in Table 1. The progress of reaction was observed by TLC using ethyl acetate:hexane as a solvent system. After completion of the reaction, ice cold water was added to it. Precipitated solid was collected by filtration and recrystallized from ethanol to afford the pure product.

Ultrasound method. To the solution of substituted 2-(4-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one **3a–f** (1 mmol) in DMF, potassium carbonate (2 mmol) and allyl bromide (1 mmol) were added into 50-mL round-bottomed flask. The reaction flask was placed in the ultrasonic cleaner bath with the surface of reactants slightly lower than the water level and irradiated at 25–30°C for the period of time indicated in Table 1. The progress of reaction was observed by TLC using ethyl acetate:hexane as a solvent system. After completion of reaction indicated by TLC, ice cold water added to it, and precipitated solid were collected by filtration and recrystallized from ethanol to afford pure product.

3-(Allyloxy)-2-(4-fluorophenyl)-4H-chromen-4-one (4a). The compound was obtained from 2-(4-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (**3a**) and allyl bromide as yellow solid, IR (KBr, cm^{-1}): 3075, 2945, 1639, 1606; ^1H NMR (400 MHz, $CDCl_3$): δ 4.65 (d, 2H, J = 5.9 Hz, OCH_2), 5.16 (dd, 1H, J = 10.3, 1.1 Hz, olefinic proton), 5.28 (dd, 1H, J = 17.2, 1.5 Hz, olefinic proton), 5.93 (ddt, 1H, J = 17.0, 10.5, 6.1 Hz, olefinic proton), 7.20 (t, 2H, J = 8.8 Hz, ArH), 7.38–7.42 (m, 1H, ArH), 7.53 (d, 1H, J = 8.1 Hz, ArH), 7.68 (ddd, 1H, J = 8.6, 7.0, 1.7 Hz, ArH), 8.15 (dd, 2H, J = 9.0, 5.3 Hz, ArH), 8.26 (dd, 1H, J = 8.1, 1.5 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 73.2, 115.5, 115.7, 117.9, 118.7, 124, 124.7, 125.8, 127.2, 130.9, 131, 133.5, 139.7, 154.9, 155.1, 162.6, 165.2, 175; LCMS calculated for $C_{18}H_{13}FO_3$ $[M+Na]^+$: 319.0, found: 319.0. *Anal*. Calcd for $C_{18}H_{13}FO_3$: C, 72.97; H, 4.42; found: C, 72.76; H, 4.58.

3-(Allyloxy)-6-chloro-2-(4-fluorophenyl)-4H-chromen-4-one (4b). The compound was obtained from 6-chloro-2-(4-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (**3b**) and allyl bromide as yellow solid, IR (KBr, cm^{-1}): 3089, 2937, 1639, 1605; ^1H NMR (400 MHz, $CDCl_3$): δ 4.57 (d, 2H, J = 5.9 Hz, OCH_2), 5.09 (dd, 1H, J = 10.2, 1.1 Hz, olefinic proton), 5.20 (dd, 1H, J = 17.2, 1.5 Hz, olefinic proton), 5.83 (ddt, 1H, J = 16.9, 10.5, 6.1 Hz, olefinic proton), 7.13 (t, 2H, J = 8.8 Hz, ArH), 7.42 (d, 1H, J = 9.2 Hz, ArH), 7.54 (d, 1H, J = 8.8 Hz, ArH), 8.06 (dd, 2H, J = 9.0, 5.3 Hz, ArH), 8.14 (d, 1H, J = 2.6 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 73.3, 115.6, 115.8, 119, 119.7, 125.2, 126.8, 130.8, 131, 131.1, 133.2, 133.7, 139.7, 153.5, 155.3, 162.8, 165.3, 173.9; LCMS calculated for $C_{18}H_{12}ClFO_3$ $[M+H]^+$: 331.1, found: 331.0. $C_{18}H_{12}ClFO_3$ $[M+Na]^+$: 353.0, found: 353.0. *Anal*. Calcd for $C_{18}H_{12}ClFO_3$: C, 65.37; H, 3.66; found: C, 65.39; H, 3.74.

3-(Allyloxy)-2-(4-fluorophenyl)-8-methyl-4H-chromen-4-one (4c). The compound was obtained from 2-(4-fluorophenyl)-3-hydroxy-8-methyl-4*H*-chromen-4-one (**3c**) and allyl bromide as yellow solid, IR (KBr, cm^{-1}): 3073, 2942, 1637, 1605; ^1H NMR (400 MHz, $CDCl_3$): δ 2.56 (s, 3H, CH_3), 4.66 (d, 2H, J = 5.9 Hz, OCH_2), 5.16 (dd, 1H, J = 10.3, 1.5 Hz, olefinic proton), 5.29 (dd, 1H, J = 17.2, 1.5 Hz, olefinic proton), 5.95 (ddt, 1H, J = 17.0, 10.5, 6.1 Hz, olefinic proton),7.20 (t, 2H, J = 8.8 Hz, ArH), 7.27 (t, 1H, J = 7.5 Hz, ArH), 7.50 (d, 1H, J = 6.6 Hz, ArH), 8.08 (dd, 1H, J = 7.9, 1.1 Hz, ArH), 8.17 (dd, 2H, J = 9.0, 5.3 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.8, 73.2, 115.6, 115.8, 118.7, 123.5, 124, 124.4, 127.4, 127.6, 130.8, 133.5, 134.3, 139.7, 153.7, 154.4, 162.6 165.1, 175.3; ESI-MS calculated for $C_{19}H_{15}FO_3$ $[M+H]^+$: 311.1083, found: 311.2619. $C_{19}H_{15}FO_3$ $[M+Na]^+$: 333.0903, found: 333.2385. *Anal*. Calcd for $C_{19}H_{15}FO_3$: C, 73.54; H, 4.87; found: C, 73.61; H, 4.92.

3-(Allyloxy)-2-(4-fluorophenyl)-6-methyl-4H-chromen-4-one (4d). The compound was obtained from 2-(4-fluorophenyl)-3-hydroxy-6-methyl-4*H*-chromen-4-one (**3d**) and allyl bromide as yellow solid, IR (KBr, cm^{-1}): 3077, 2924, 1638, 1618; ^1H NMR (400 MHz, $CDCl_3$): δ 2.46 (s, 3H, CH_3), 4.64 (d, 2H, J = 5.9 Hz, OCH_2), 5.15 (dd, 1H, J = 10.3, 1.5 Hz, olefinic proton), 5.27 (dd, 1H, J = 17.2, 1.5 Hz, olefinic proton), 5.93 (ddt, 1H, J = 17.0, 10.5, 6.1 Hz, olefinic proton), 7.18 (t, 2H, J = 8.6 Hz, ArH), 7.41 (d, 1H, J = 8.4 Hz, ArH), 7.48 (dd, 1H, J = 8.8, 1.2 Hz, ArH), 8.02 (brs, 1H, ArH), 8.14 (dd, 2H, J = 9.2, 5.5 Hz,ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9, 73.2, 115.4, 115.6, 117.7, 118.7, 123.8, 125, 127.3, 130.9, 133.5, 134.7, 134.8, 139.7, 153.5, 154.8, 162.6, 165.1, 175; ESI-MS calculated for $C_{19}H_{15}FO_3$ $[M+H]^+$: 311.1083, found: 311.1058. $C_{19}H_{15}FO_3$ $[M+Na]^+$: 333.0903, found: 333.0783. *Anal*. Calcd for $C_{19}H_{15}FO_3$: C, 73.54; H, 4.87; found: C, 73.33; H, 4.96.

3-(Allyloxy)-6,8-dichloro-2-(4-fluorophenyl)-4H-chromen-4-one (4e). The compound was obtained from 6,8-dichloro-2-(4-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (**3e**) and

allyl bromide as yellow solid, IR (KBr, cm^{-1}): 3012, 2967, 1634, 1600; ^1H NMR (400 MHz, $CDCl_3$): δ 4.68 (d, 2H, J = 6.2 Hz, OCH_2), 5.19 (dd, 1H, J = 10.3, 1.1 Hz, olefinic proton), 5.30 (dd, 1H, J = 17.1, 1.3 Hz, olefinic proton), 5.94 (ddt, 1H, J = 17.0, 10.5, 6.1 Hz, olefinic proton), 7.22 (t, 2H, J = 8.8 Hz, ArH), 7.72 (d, 1H, J = 2.6 Hz, ArH), 8.11 (d, 1H, J = 2.6 Hz, ArH), 8.26 (dd, 2H, J = 9.0, 5.3 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 72.4, 115.7, 115.9, 118.4, 123, 123.7, 125.2, 126.3, 129.4, 130.8, 130.9, 133.2, 139.3, 148.8, 154.1, 162.2, 164.7, 172.2; LCMS calculated for $C_{18}H_{11}Cl_2FO_3$ $[M+H]^+$: 365.0, found: 365.1. $C_{18}H_{11}Cl_2FO_3$ $[M+Na]^+$: 387.0, found: 387.1. *Anal*. Calcd for $C_{18}H_{11}Cl_2FO_3$: C, 59.20; H, 3.04; found: C, 59.24; H, 3.12.

3-(Allyloxy)-6-chloro-2-(4-fluorophenyl)-7-methyl-4H-chromen-4-one (4f). The compound was obtained from 6-chloro-2-(4-fluorophenyl)-3-hydroxy-7-methyl-4*H*-chromen-4-one (**3f**) and allyl bromide as yellow solid,IR (KBr, cm^{-1}): 3069, 2952, 1628, 1600; ^1H NMR (400 MHz, $CDCl_3$): δ 2.5 (s, 3H, CH_3), 4.63 (d, 2H, J = 6.2 Hz, OCH_2), 5.16 (dd, 1H, J = 10.3, 1.5 Hz, olefinic proton), 5.26 (dd, 1H, J = 17.2, 1.5 Hz, olefinic proton), 5.91 (ddt, 1H, J = 17.0, 10.5, 6.2 Hz, olefinic proton), 7.18 (t, 2H, J = 8.6 Hz, ArH), 7.40 (s, 1H, ArH), 8.11 (dd, 2H, J = 9.0, 5.3 Hz, ArH), 8.17 (s, 1H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8, 73.3, 115.5, 115.7, 118.8, 119.7, 123.1, 125.4, 127, 130.9, 131.6, 133.3, 139.6, 142.8, 153.4, 154.9, 162.7, 165.2, 173.9; ESI-MS calculated for $C_{19}H_{14}ClFO_3$ $[M+H]^+$: 345.0694, found: 345.2405. $C_{19}H_{14}ClFO_3$ $[M+Na]^+$: 367.0513, found: 367.2264. *Anal*. Calcd for $C_{19}H_{14}ClFO_3$: C, 66.19; H, 4.09; found: C, 66.31; H, 4.01.

EXPERIMENTAL PROTOCOL FOR BIOLOGICAL ACTIVITY

Antimicrobial activity. *In vitro* antibacterial activity of the synthesized compounds was tested against Gram-positive bacteria viz. *Staphylococcus aureus* (NCIM 2178), *B. subtilis* (NCIM 2250), and Gram-negative bacteria *E. coli* (NCIM 2137), *P. aeruginosa* (NCIM 2036). The compounds were also screened for antifungal activity against *C. albicans* (MTCC 277), *C. glabrata* (NCIM 3236), and *C. tropicalis* (NCIM 3110). Compounds were diluted in DMSO with 1 μg/mL concentrations for bioassay. Micro-broth dilution method [26] used to determined *in vitro* MICs of compounds in 96-well micro titre plates. National Committee for Clinical Laboratory Standards defined the method against a panel of human pathogenic strains. Test compounds were serially double diluted in growth medium. Plates were incubated at 30°C for fungi and 37°C for bacteria for 24 h. All experiments were carried out in triplicates, and mean values are represented.

Molecular docking study. Glide v6.2 (Schrodinger, LLC) software was used for molecular docking studies. The procedure was followed as per the literature [27–30]. For the said work, X ray crystal structure of *S. aureus* KAS III was procured from PDB entry 1ZOW [31] and prepared for docking using protein preparation wizard. The termini were covered with the help of ACE and NMA residue. From the structure, water molecules were removed, and to all atoms, hydrogen was added. For the hetero groups, formal charges and bond orders were added. Side chains, which were not near to the binding cavity and do not contribute in salt bridges, were neutralized. After the preparation, the structures were refined to optimize the hydrogen bond network using OPLS_2005 force field. This helps in reorientation of the side chain hydroxyl group. When the energy converged or the RMSD reached a maximum cut off of 0.30 A, the minimization was terminated.

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