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Synthesis and biological activity of new chalcone scaffolds as prospective antimicrobial agents

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Abstract

Chalcones are open-chain flavonoids which contains two aromatic rings are joined by 3-carbons α-, β-unsaturated carbonyl chain. The, β-unsaturated ketonic group which is liable for the antimicrobial activity of the chalcone is additionally of vast use in further chemical modification into a variety of heterocyclic compounds. A new series of chalcone derivatives was synthesized and characterized by spectral analysis (IR, 1H-NMR, 13C-NMR, MS and elemental analysis) and evaluated for its in vitro antimicrobial activity against bacterial (Gram negative and Gram positive) and fungal strains using tube dilution method. The antimicrobial screening results revealed that some compounds of the series 1 (MICpa = 1.16 µM), 3 (MICbs = 1.82 µM), 6 (MICan = 2.09 µM), 8 (MICec and se = 0.94 and 1.88 µM) and 17 (MICsa and ca = 0.91 and 1.81 µM) showed the most promising antimicrobial activity against both Gram-positive as well as Gram-negative bacterial and fungal strains, and comparable to the standard drugs used as positive control (cefadroxil and fluconazole).

Keywords Chalcone derivatives · Antimicrobial activity · Spectral analysis · SAR study

Abbreviations

MIC	Minimum Inhibitory Concentration
Mµ	Micro mole
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration

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B. subtilis	*Bacillus subtilis*
S. aureus	*Staphylococcus aureus*
E. coli	*Escherichia coli*
S. enterica	*Salmonella enterica*
P. aeruginosa	*Pseudomonas neruginosa*
C. *albicans*	*Candida albicans*
A.niger	*Aspergillus niger*
IR	Infrared spectroscopy
NMR	Nuclear magnetic resonance
DMSO	Dimethyl sulfoxide
CDCl3	Deuterated chloroform
FT-IR	Fourier-transform infrared spectroscopy

Introduction

Chalcones, one of the major classes of natural products, are open-chain flavanoids in which two aromatic rings are linked by a 3-carbon *α, β*-unsaturated/saturated carbonyl system [1]. Various types of antimicrobial drugs are already present inside the market but thanks to the arbitrary use of antimicrobial drugs often followed the experience of resistant strains of microorganism [2]. Mutation, phenotypic change, gene transfer and selective pressure also are significant causes behind antimicrobial resistance [3]. So, there is an urgent need to discover new antimicrobial agent which have an extensive spectrum of activity against the resistant micro-organism [4, 5]. *Staphylococcus aureus* and other microorganisms are the major human pathogen cause's mild superficial infections to severe life-threatening invasive infections [6, 7]. An important quality for an antimicrobial drug is selective toxicity, meaning that it selectively kills or inhibits the growth of microbial targets while causing minimal or no harm to the host [8].

The chemistry of chalcone has been generated intensive scientific interest due to their industrial and biological application. Chalcone is natural biocides and are eminent intermediates in the synthesis of heterocyclic moieties exhibiting diverse biological activities. Chalcone is the core substructure of some natural compounds and important precursor for the synthesis of various heterocyclic compounds like pyrimidines, pyrazolines, pyrazoles, flavonoids, isoflavonoids [9], benzoyl coumarones, deoxybenzoins, hydantoins and aurones containing benzylidene acetophenone, having some therapeutic value, where the two aromatic rings are joined by a 3 carbon *α, β*-unsaturated carbonyl system [10]. Generally, they consist of polyhydroxy groups in their aromatic nuclei and exist as either trans (E, 1) or cis (Z, 2) isomeric forms (Fig. 1); whereas, trans isomer being thermodynamically more stable than cis form [11]. Chalcones contain conjugated double bonds and a totally delocalized π-electron system on both benzene rings. The non-toxic concentrations of all compounds were determined on the HEK-293 cell line. Then, anti-proliferative activity studies were performed at these non-toxic concentrations [1].

The radical-quenching property of these phenolic groups augment the interest within the use of these compounds as food preservatives or chalcone-rich plant

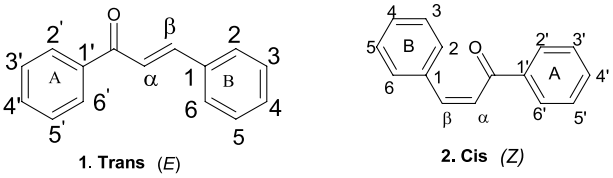


Fig. 1 Trans (E, 1) or Cis (Z, 2) isomers of chalcone

extracts as drugs [12]. Chalcones are generally known by different names i.e. benzylidene acetophenone, phenyl styryl ketone, *β*-phenylacrylophenone *α*-phenyl-*β*-benzoylethylene etc. these names are given by Kostanecki and Tambor [4]. Chalcones have been shown potent pharmacological profile and have received a great deal of attention due to their relatively simple structures, ease of hydrogen atom manipulation, simple chemistry, ease of synthesis, diversity of substituent's and are associated with a plethora of biological actions like antifungal, antimalarial [13, 14], anticancer, antidiabetic, antituberculor, antioxidant, neuroprotective, antiviral [15], anti-Alzheimer, anti-influenza [16], anti-inflammatory, anti-ulcerative, gastric protectants [10], anticonvulsant, anti-inflammatory, antileishmanial [11], antiallergic, antispasmodic, fungicidal, antibacterial [5], germicidal, herbicidal and insecticidal, [17] immunosuppressive pharmacological activity [18]. Chemical modification of chalcone is one of the most universal approaches in drug discovery with improved therapeutic effect and the wide occurrence of chalcone in bioactive natural products [10]. Marketed drugs of chalcone used in clinical practice, selected drugs are shown in Fig. 2

Stereochemically, chalcone can exist both cis (Z) and trans (E) isomeric forms, but the Z isomer is most unstable because of steric effect of ring A with group. In chalcones, two aromatic nuclei are present therefore the electrophilic *α, β*-unsaturated carbonyl system is in continuous conjugation. It may be the reason for their stability, electron transfer reactions and low redox potential, more significant for its potent biological activities [9].

Recently, it has been reported that *para*-hydroxy phenyl nucleus present in chalcone derivative **(I)** [19] improved the antidiabetic activity. *ortho*-Hydroxy and *para*-methoxy substitution on phenyl ring enhanced the antimicrobial potential of the chalcone compounds (**II**) [20], tri-Methoxy at 2nd, 4th and 5th position of the phenyl ring of chalcone derivatives improved the antioxidant activity (**III**) [21]. Bromo group at *meta*-position of the phenyl nucleus of chalcone derivative (**IV**) [22] improved the antiviral activity. Any substitutions on chalcone and heterocyclic derivatives (pyrimidine moiety) have shown antitubercular activity [23]. The di- and tri-acetylbenzyl derivatives showed the good anti-neurotoxicity activity [24]. The presence of electron releasing (-OH and -OCH_3) and electron withdrawing (-Br) functional groups on various position of phenyl ring (I, II, III and IV) of the chalcone derivatives enhanced their biological activity [25], from these information we have reported a design of different biologically active agents (Fig. 3).

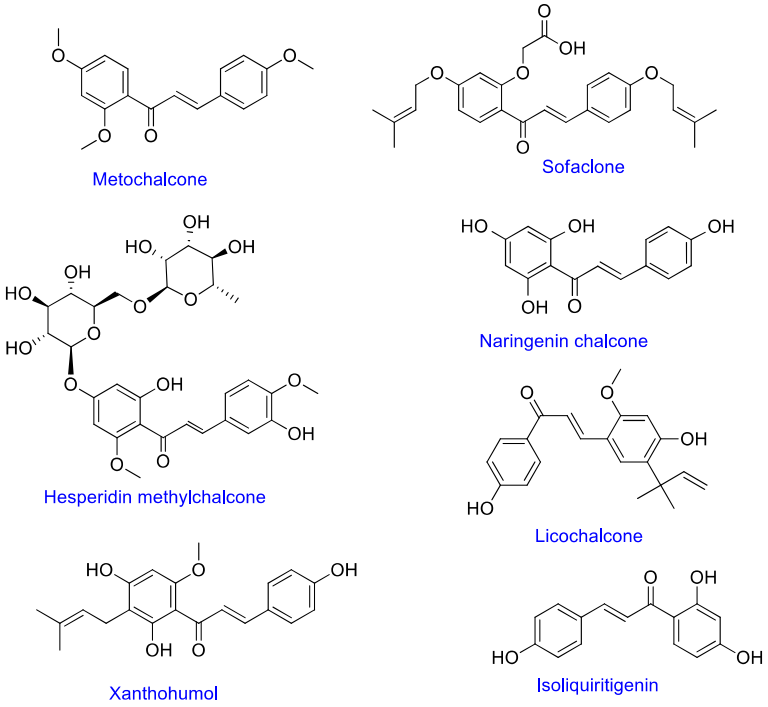


Fig. 2 Marketed drugs of chalcone

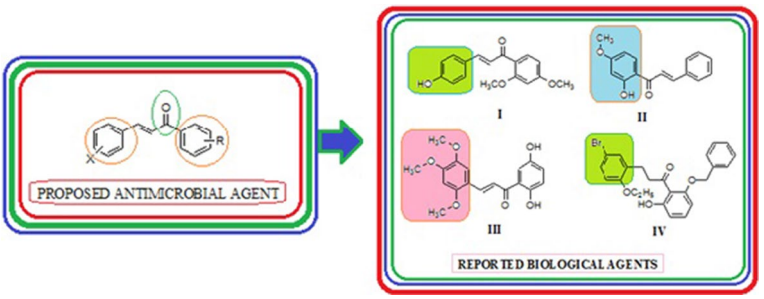


Fig. 3 Literature based design of the proposed chalcone derivatives

In light of above-mentioned details, we hereby enlighten design, synthesis and evaluate antimicrobial activity of 3-(substituted aldehyde)-1-(4-substituted phenyl)prop-2-en-1-one derivatives.

Experimental

All other chemicals and reagents obtained from marketable sources and these are used without further purification. Confirmation of the final product formed in the reaction done by thin layer chromatography technique (TLC plate), using silica gel G as stationary phase and benzene as mobile phase. Melting points of final derivatives were obtained by open capillary tube method. ^{13}C-NMR and ^{1}H-NMR of the synthesized compounds were recorded at 150 MHz and 600 MHz, respectively on Bruker Avance III 600 NMR spectrometer by taking suitable deuterated solvent and the results were expressed in parts per million (δ, ppm) downfield from internal standard (tetramethylesilane). ^{1}H-NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. An infrared (IR) spectrum were recorded on Bruker 12,060,280, Software: OPUS 7.2.139.1294 spectrometer in the sort of 400–4000 by making pellets with KBr using FT-IR Spectrophotometer and the value of λ max were found in cm^{-1} [26]. Elemental analysis was performed on PerkinElmer 2400 C, H and N analyzer and all synthesized chalcone derivatives gave C, H and N analysis within ± 0.4% of the theoretical results. Mass spectra of synthesized compounds were taken on Waters Micromass Q-ToF Micro instrument.

Synthetic procedure

The reaction mixture of *para*-substituted acetophenone (0.01 M) and subsequent aldehyde (0.01 M) was stirred for 2–3 h in methanol (30 ml) in a round bottom flask. NaOH solution (10 ml sodium hydroxide solution 40%) was added drop wise and continuous stirring at room temperature. The reaction mixture was placed at room temperature overnight and then it was poured into ice cold water after that the solution was acidified by dilute HCl (hydrochloric acid). Then the precipitated of final product was filtered, desiccated and recrystallized from ethanol (Rectified spirit) [27].

*Antimicrobial evaluation (*In Vitro*)*

The antimicrobial potential of the synthesized new derivatives of chalcone was evaluated against microorganisms, i.e., Gram-positive bacterial strains (*S. aureus* MTCC3160 [2, 6], *B. subtilis* MTCC441), Gram-negative bacterial strains (*E. coli* MTCC443*, S. enterica* MTCC116 and *P. aeruginosa* MTCC3542) and fungal strains (*C. albicans* MTCC227, A. niger MTCC281) using tube dilution method [28].

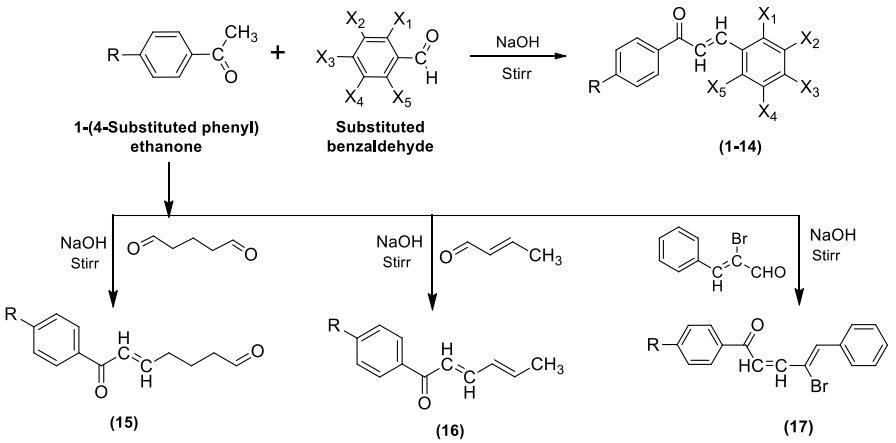
For the synthesized derivatives and standard drugs i.e. cefadroxil and fluconazole stock solution (100 µg/mL in DMSO) was prepared [29]. Here, two types of broth were used for antimicrobial study, i.e., Sabouraud dextrose broth-I.P for antifungal study and double strength nutrient broth-I.P for antibacterial study. The synthesized chalcone compounds and reference drugs were incubated at different temperature i.e. Gram-positive and Gram-negative bacterial strains at 37 ± 1 °C for 24 h, fungal

strains- *A. niger* at 25 ± 1 °C for 7 days and *C. albicans* at 37 ± 1 °C for 48 h. The antimicrobial screening result, i.e., minimum inhibitory concentration was recorded in (MIC = μM).

Results and discussion

Chemistry

The chemistry of chalcone moiety has generated intensive scientific interest due to their industrial and biological application. Chalcone is natural biocides and are eminent intermediates in the synthesis of heterocyclic moieties exhibiting diverse biological activities. The synthesis of allylic chalcone derivatives by Claisen–Schimdt condensation reaction followed various steps discussed in synthetic Scheme 1. Here, the reaction of various *p*-substituted acetophenone with substituted aldehyde in the presence of sodium hydroxide solution in methanolic solvent at room



1. R= NO_2; X_1, X_2, X_4, X_5 = H; X_3= OH
2. R= NO_2; X_1, X_3, X_4, X_5 = H; X_2= NO_2
3. R= NO_2; X_1, X_5, = H; X_2, X_3, X_4= OCH_3
4. R= NO_2; X_2, X_3, X_4, X_5 = H; X_1= NO_2
5. R= NO_2; X_1, X_4, X_5 = H; X_2= OC_2H_5; X_3=OH
6. R= NO_2; X_1, X_4, X_5 = H; X_2= OCH_3; X_3=OH
7. R= NO_2; X_1, X_2, X_4, X_5 = H; X_3= $N(C_2H_5)_2$
8. R= NO_2; X_1, X_2, X_4, X_5 = H; X_3= Br
9. R= NO_2; X_1, X_2, X_4, X_5 = H; X_3= NO_2
10. R= NO_2; X_1, X_4, X_5 = H; X_2, X_3= OCH_3
11. R= NO_2; X_2, X_3, X_4, X_5 = H; X_1= OCH_3
12. R= NO_2; X_2, X_3, X_4, X_5 = H; X_1= OH
13. R= NH_2; X_1, X_2, X_4, X_5 = H; X_3= Cl
14. R= OH; X_1, X_5, = H; X_2, X_3, X_4= OCH_3
15. R= NO_2
16. R= NO_2
17. R= NO_2

Reaction condition:
p-Substituted acetophenone, Substituted aldehyde, NaOH, methanol, stirred 2-3 h, at room temp

Scheme 1 Synthetic scheme of 3–(substituted aldehyde)–1–(4–substituted phenyl) prop–2–en–1-one derivatives (1–17)

temperature resulted in the formation of the various biologically active compounds (1–17). The molecular structures of synthesized compounds were characterized by physicochemical properties and spectral characteristics are presented Table 1 and Table 2, respectively. The phenyl nucleus exhibited the C–H stretching in range of 3183–2854 cm^{-1}. Peak range at 1390–1331 cm^{-1} showed the –C–N str. of the compounds. Peak range 1691–1523 cm^{-1} indicates the presence of –C = O stretch in the compound [30]. Here, also the appearance of $–NO_2$ asymmetric stretches in a scale of 1524–1253 cm^{-1} of the synthesized compounds. The compound having peak range 1692–1519 cm^{-1} indicates the presence of –C = C– group. The compounds showed the Ar-NO_2 stretch (C–N str.) in the range of 854–724 cm^{-1}. ^{1}H–NMR showed the presence of OCH_3 group at 3.73 δ ppm. The presence of singlet signal of OH group at 5.35–5.00 δ ppm and showed multiplet protons of aromatic ring at 6.26–8.45 δ ppm [10, 31]. In addition, 2 doublet signal around at 7.39–7.59 δ ppm confirm the presence of protons of O = CH = CH– groups. ^{13}C–NMR δ showed the different carbon signal of the compound around at 121.4 (–CH), 68.0 (–OCH), 145.0, 148.1 (phenyl), 115.8, 127.8, 130.8 (Ar–C), 157.7 (–NH–C = 0), 189.7(C = O), 14.8 (–OCH_2CH_3) [31] and synthesized chalcone compounds gave C, H and N analysis within. ± 0.4% of the theoretical values.

In vitro antimicrobial activity

The newly synthesized chalcone derivatives were evaluated for their *in vitro* antimicrobial potential against Gram–positive bacterial strains (*S. aureus* and *B. subtilis*),

Table 1 The physicochemical properties of synthesized chalcone derivatives

Comp	M. Formula	M. Wt	m.pt. (˚C)	R_f Value*	% Yield
1	$C_{15}H_{11}NO_4$	269	67–69	0.58	70.97
2	$C_{15}H_{10}N_2O_5$	298	123–125	0.31	75.08
3	$C_{18}H_{17}NO_6$	343	> 200	0.53	84.68
4	$C_{15}H_{10}N_2O_5$	298	208–210	0.68	76.69
5	$C_{17}H_{15}NO_5$	313	61–63	0.63	65.49
6	$C_{16}H_{13}NO_5$	299	73–75	0.51	72.56
7	$C_{19}H_{20}N_2O_3$	324	68–70	0.56	72.56
8	$C_{15}H_{10}BrNO_3$	331	139–141	0.61	75.08
9	$C_{15}H_{10}N_2O_5$	298	95–97	0.41	71.45
10	$C_{17}H_{15}NO_5$	313	68–70	0.42	69.32
11	$C_{16}H_{13}NO_4$	283	82–84	0.45	72.59
12	$C_{15}H_{11}NO_4$	269	77–79	0.62	68.34
13	$C_{15}H_{12}ClNO$	257	119–121	0.39	69.56
14	$C_{18}H_{18}O_5$	314	> 200	0.38	76.45
15	$C_{13}H_{13}NO_4$	247	71–73	0.62	75.00
16	$C_{12}H_{11}NO_3$	217	55–57	0.47	84.72
17	$C_{17}H_{12}BrNO_3$	358	56–58	0.35	78.78

*TLC mobile phase: Benzene

Table 2 Molecular structures and spectral characteristics of the synthesized chalcone derivatives

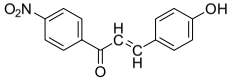
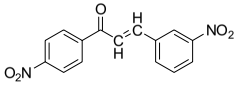
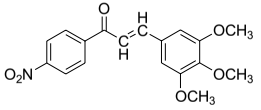
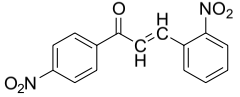
S. No	Molecular Structures	Spectral characteristics [IR (KBr pellets, cm^{-1}) and ^{1}H–NMR/^{13}H–NMR ($CDCl_3$, ppm) δ; Mass: *m/z*; CHN analysis]
1	 *(E)–3–(4–Hydroxyphenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 3106 (C–H str.), 1603 (C = C str.), 742 (C–C str.), 1355 (C–N str.), 3369 (OH str.), 1396 (C–O str., and O–H in plane bending), 1691 (C = O str.), 1524 (NO_2 asym str.), 854 (C–N str., Ar–NO_2); ^{1}H–NMR: 6.65–8.45 (m, 8H, ArH), 7.59 (d, 1H, CH), 5.35 (s, 1H, OH); ^{13}H–NMR: 115.8, 121.3, 124.4, 127.8, 130.6, 130.8, 144.0, 145.1, 153.7, 157.7, 189.7; CHN analysis Calc: C, 66.91; H, 4.42; N, 5.42; Found: C, 66.95; H, 4.48; N, 5.46; Mass: *m/z* 270 [$M^+ + 1$]
2	 *(E)–3–(3–Nitrophenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 2932 (C–H str.), 1597 (C = C str.), 692 (C–C str.), 1349 (C–N str.), 1684 (C = O str.), 1518 (NO_2 asym str.), 843 (C–N str., Ar–NO_2); ^{1}H–NMR: 7.47–8.38 (m, 8H, ArH), 7.81 (d, 1H, CH); ^{13}H–NMR: 120.3, 121.3, 121.6, 129.6, 130.8, 132.5, 136.1, 144.0, 145.2, 148.3, 154.2, 189.7; CHN analysis Cal: C, 60.41; H, 4.12; N, 9.42; Found: C, 60.45; H, 4.18; N, 9.46; Mass: *m/z* 299 [$M^+ + 1$]
3	 *(E)–3–(3,4,5–Trimethoxyphenyl)–1–(4–Nitrophenyl)Prop–2–en–1–one*	IR: 3101 (C–H str.), 1600 (C = C str.), 695 (C–C str.), 1350 (C–N str.), 1684 (C = O str.), 1518 (NO_2 asym str.), 843 (C–N str., Ar–NO_2), 1112 (C–O–C str., –OCH_3); ^{1}H–NMR: 6.26–8.38 (m, 6H, ArH), 7.56 (d, 1H, CH), 3.73 (s, 3H, OCH_3); ^{13}H–NMR: 56.2, 103.9, 121.4, 129.5, 130.8, 138.4, 145.2, 150.7, 154.2, 189.7; CHN analysis Calc: C, 62.97; H, 4.99; N, 4.08; Found: C, 62.95; H, 4.95; N, 4.12; Mass: *m/z* 344 [$M^+ + 1$]
4	 *(E)–3–(2–Nitrophenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 3041 (C–H str., phenyl nucleus), 1610 (C = C str.), 661(C–C str.), 1356 (C–N str.), 1672 (C = O str.), 1529 (NO_2 asym str.), 724 (C–N str., Ar–NO_2); ^{1}H–NMR: 7.40–8.38 (m, 8H, ArH), 7.63 (d, 1H, CH); ^{13}H–NMR: 121.0, 121.4, 127.3, 128.9, 130.8, 134.8, 144.0, 145.2, 154.2, 189.7; CHN analysis Calc: C, 60.41; H, 3.38; N, 9.39; Found: C, 60.45; H, 3.42; N, 9.43; Mass: *m/z* 299 [$M^+ + 1$]

Table 2 (continued)

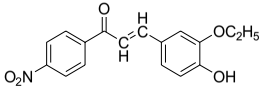
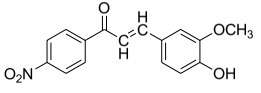
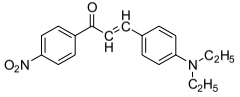
S. No	Molecular Structures	Spectral characteristics [IR (KBr pellets, cm^{-1}) and ^{1}H–NMR/^{13}H–NMR ($CDCl_3$, ppm) δ; Mass: *m/z*; CHN analysis]
5	 *(E)–3–(3–Ethoxy–4–hydroxyphenyl)–1–(4–nitrophenyl)prop–2–en–1–one*	IR: 3104 (C–H str.), 1602 (C=C str.), 691 (C–C str.), 1351 (C–N str.), 3362 (OH str., phenyl ring), 1430 (C–O str., and O–H in plane bending), 1692 (C=O str.), 1522 (NO_2 asym str.), 1112 (C–O–C str., $–OC_2H_5$); ^{1}H–NMR: 6.57–8.38 (m, 7H, ArH), 7.56 (d, 1H, CH), 5.00 (s, 1H, OH), 4.04 (q, 2H, CH_2), 1.32 (q, 3H, CH_3); ^{13}H–NMR: 14.8, 65.0, 112.1, 119.4, 121.4, 121.6, 128.4,130.8, 144, 145.2, 154.2,189.7; CHN analysis Calc: C, 65.17; H, 4.83; N, 4.47; Found: C, 65.22; H, 4.83; N, 4.52; Mass: *m/z* 314 [$M^+ + 1$]
6	 *(E)–3–(4–Hydroxy–3–methoxyphenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 3002 (C–H str.), 1601 (C=C str.), 740 (C–C str.), 1351 (C–N str., phenyl ring), 3358 (OH str.), 1430 (C–O str., and O–H in plane bending), 1692 (C=O str.), 1523 (NO_2 asym str.), 1112 (C–O–C str., $–OCH_3$), 852 (C–N str., Ar–NO_2); ^{1}H–NMR: 6.57–8.36 (m, 7H, Ar–H), 7.56 (d, 1H, CH), 3.73 (s, 3H, OCH_3), 5.00 (s, 1H, OH); ^{13}H–NMR: 56.2, 112.0, 116.8, 120.1, 121.4, 121.6, 130.8, 144.0, 145.2, 154.2, 189.7; CHN analysis Calc: C, 64.21; H, 4.38; N, 4.68; Found: C, 64.25; H, 4.42; N, 4.71; Mass: *m/z* 300 [$M^+ + 1$]
7	 *(E)–3–(4–(Diethylamino)phenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 3103 (C–H str.), 1519 (C=C str.), 694 (C–C str.), 1349 (C–N str.), 1691 (C=O str.), 1519 (NO_2 asym str.), 738 (C–N str., Ar–NO_2); ^{1}H–NMR: 6.54–8.38 (m, 8H, ArH), 7.56 (d, 1H, CH); ^{13}H–NMR: 13.0, 44.7, 114.2, 121.4, 124.7, 127.3, 130.8, 144.0, 145.2, 154.2, 189.7; CHN analysis Calc: C, 70.35; H, 6.21; N, 8.64; Found: C, 70.31; H, 6.25; N, 8.68; Mass: *m/z* 325 [$M^+ + 1$]

Table 2 (continued)

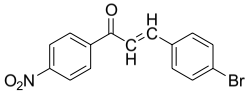
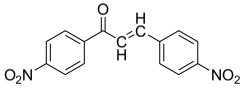
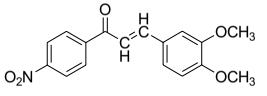
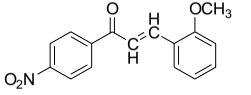
S. No	Molecular Structures	Spectral characteristics [IR (KBr pellets, cm^{-1}) and ^1H–NMR/^{13}H–NMR ($CDCl_3$, ppm) δ; Mass: *m/z*; CHN analysis]
8	 *(E)–3–(4–Bromophenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 3083 (C–H str.), 1593 (C=C str.), 702 (C–C str.), 1331 (C–N str., phenyl ring), 1653 (C=O str.), 1517 (NO_2 asym str.), 672 (C–Br str.), 756 (C–N str., Ar–NO_2); ^1H–NMR: 7.60–8.40 (m, 8H, Ar–H), 7.56 (d, 1H, CH), 8.10 (d, 1H, CH); ^{13}H–NMR: 121.4, 121.6, 122.3, 128.6, 130.8, 134.2 144.0, 145.2, 154.2, 189.7; CHN analysis Calc: C, 54.24; H, 3.03; N, 4.22; Found: C, 54.29; H, 3.07; N, 4.26; Mass: *m/z* 332 [$M^+ + 1$]
9	 *(E)–1,3–Bis(4–nitrophenyl) Prop–2–en–1–one*	IR: 3075 (C–H str.), 1592 (C=C str.), 702 (C–C str.), 1343 (C–N str., phenyl ring), 1687 (C=O str.), 1520 (NO_2 asym str., phenyl ring), 740 (C–N str., Ar–NO_2); ^1H–NMR: 7.56–8.38 (m, 8H, ArH), 7.85 (d, 1H, CH), 8.04 (d, 1H, CH); ^{13}H–NMR: 121.0, 121.4, 121.6, 127.3, 130.8, 141.3, 144.0, 145.2, 147.6, 154.2, 189.7; CHN analysis Calc: C, 60.41; H, 3.38; N, 9.39; Found: C, 60.45; H, 3.42; N, 9.42; Mass: *m/z* 299 [$M^+ + 1$]
10	 *(E)–3–(3,4–Dimethoxyphenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 3104 (C–H str.), 1595 (C=C str.), 693 (C–C str.), 1353 (C–N str., phenyl ring), 1691 (C=O str.), 1527 (NO_2 asym str., phenyl ring), 743 (C–N str., Ar–NO_2), 1263 (C–O–C str.); ^1H–NMR: 6.61–8.38 (m, 7H, ArH), 7.56 (d, 1H, CH), 8.12 (d, 1H, CH), 3.73 (s, 6H, $(OCH_3)_2$); ^{13}H–NMR: 56.2, 111.6, 119.7, 121.4, 121.6, 128.5, 130.8, 144.0, 145.2,154.2, 189.7; CHN analysis Calc: C, 65.17; H, 4.83; N, 4.47; Found: C, 62.75; H, 4.88; N, 4.52; Mass: *m/z* 314 [$M^+ + 1$]
11	 *(E)–3–(2–Methoxyphenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 3104 (C–H str.), 1595 (C=C str.), 693 (C–C str.), 1351 (C–N str.), 1690 (C=O str.), 1521 (NO_2 asym str., phenyl ring), 745 (C–N str., Ar–NO_2), 1263 (C–O–C str.); ^1H–NMR: 6.72–8.38 (m, 8H, ArH), 7.39 (d, 1H, CH), 8.17 (d, 1H, CH), 3.73 (s, 3H, OCH_3); ^{13}H–NMR: 56.3, 114.2,115,121.0, 121.4, 121.6, 127.4, 130.8, 144.0, 145.2 154.3, 189.7; CHN analysis Calc: C, 67.84; H, 4.63; N, 4.94; Found: C, 67.88; H, 4.65; N, 4.98; Mass: *m/z* 284 [$M^+ + 1$]

Table 2 (continued)

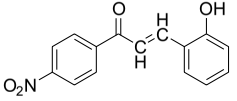
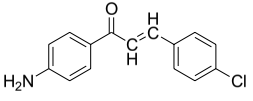
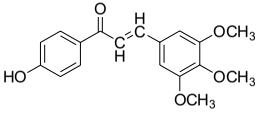
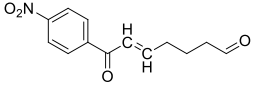
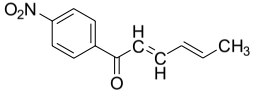
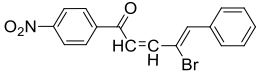
S. No	Molecular Structures	Spectral characteristics [IR (KBr pellets, cm^{-1}) and ^{1}H–NMR/^{13}H–NMR ($CDCl_3$, ppm) δ; Mass: *m/z*; CHN analysis]
12	 *(E)–3–(2–Hydroxyphenyl)–1–(4–Nitrophenyl)prop–2–en–1–one*	IR: 3106 (C–H str.), 1596 (C=C str.), 691 (C–C str.), 1349 (C–N str., phenyl ring), 3356 (OH str.), 1517 (C–O str., and O–H in plane bending vib.), 1691 (C=O str.), 1429 (NO_2 asym str.), 816 (C–N str., Ar–NO_2), 1596 (C=C str., R–CH=CH_2); ^{1}H–NMR: 6.68–8.38 (m, 8H, ArH), 7.39 (d, 1H, CH), 8.17 (d, 1H, CH), 5.0 (s, 1H, OH); ^{13}H–NMR: 115.8, 116.6 121.3, 121.4, 121.6, 127.8, 129.4, 130.8, 144.0, 145.1, 154.2, 158.3, 189.7; CHN analysis Calc: C, 66.91; H, 4.12; N, 5.20; Found: C, 66.95; H, 4.16; N, 5.26; Mass: *m/z* 270 [$M^+ + 1$]
13	 *(E)–1–(4–Aminophenyl)–3–(4–Chlorophenyl)prop–2–en–1–one*	IR: 2854 (C–H str.), 1599 (C=C str.), 1411 (C–N str., phenyl ring), 3351 (N–H asym str.), 1599 (C=O str.), 672 (C–Cl str.), 1599 (C=C str., –CH=CH–), 1272 (C–N str., 1° NH_2); ^{1}H–NMR: 6.65–8.38 (m, 8H, ArH), 7.39 (d, 1H, CH), 8.17 (d, 1H, CH), 5.0 (s, 1H, OH); ^{13}H–NMR: 116.8, 121.4, 127.9, 128.8, 130.7, 133.3, 133.5, 145.2, 154.2, 189.7; CHN analysis Calc: C, 69.91; H, 4.69; N, 5.43; Found: C, 69.95; H, 4.72; N, 5.47; Mass: *m/z* 258 [$M^+ + 1$]
14	 *(E)–1–(4–Hydroxyphenyl)–3–(3,4,5–Trimethoxyphenyl)prop–2–en–1–one*	IR: 3061 (C–H str.), 1588 (C=C str.), 623 (C–C str.), 1390 (C–N str., phenyl ring), 3354 (OH str., phenyl ring), 1464 (C–O str. and O–H in plane bending vib), 1588 (C=O str.), 1239 (C–O–C str.), 1588 (C=C str., –CH=CH–); ^{1}H–NMR: 6.26–7.64 (m, 6H, ArH), 7.56 (d, 1H, CH), 7.90 (d, 1H, CH) 3.73 (s, 3H, OCH_3), 5.0 (s, 1H, OH); ^{13}H–NMR: 56.2, 56.5, 103.9, 121.4, 129.4, 130.5, 131.3, 138.4, 145.2,150.7, 164.3, 189.7; Mass: *m/z* 315 [$M^+ + 1$]

Table 2 (continued)

S. No	Molecular Structures	Spectral characteristics [IR (KBr pellets, cm^{-1}) and ^{1}H-NMR/^{13}H-NMR ($CDCl_3$, ppm) δ; Mass: *m/z*; CHN analysis]
15	 *(E)-7-(4-Nitrophenyl)-7-oxohept-5-enal*	IR: 3106 (C-H str.), 1522 (C = C str.), 690 (C–C str.), 1343 (C-N str., phenyl ring), 1522 (C = O str.), 851 (C-N str., Ar-NO_2), 2851 (C-H str., R-CH_3), 1253 (NO_2 asym str., phenyl ring), 1601 (C = C str., R-CH = CH_2); ^{1}H-NMR: 8.07–8.38 (q, 4H, ArH), 2.12 (t, 2H, CH_2), 1.73 (m, 2H, CH_2), 2.42 (q, 2H,CH_2); ^{13}H-NMR: 28.8, 32.7, 43.7, 121.6, 130.8, 131.1, 144.0, 147.1, 154.2, 189.7; CHN analysis Calc: C, 63.15; H, 5.30; N, 5.67; Found: C, 63.19; H, 5.32; N, 5.72; Mass: *m/z* 248 [$M^+ + 1$]
16	 *(2E,4E)-1-(4-Nitrophenyl) hexa-2,4-dien-1-one*	IR: 3103 (C-H str.), 1523 (C = C str.), 693 (C–C str.), 1341 (C-N str., phenyl ring), 1523 (C = O str.), 1253 (NO_2 asym str., phenyl ring), 2851 (C-H str., R-CH_3), 1253 (NO_2 asym str.), 741 (C-N str., Ar-NO_2), 1600 (C = C str., -CH = CH-); ^{1}H-NMR: 8.07–8.38 (q, 4H, ArH), 7.21 (d, 1H, CH), 7.61 (t, 1H, CH), 6.16 (t, 1H, CH), 5.71 (m, 1H, CH), 2.04 (q, 3H, CH_3); ^{13}H-NMR: 19.3, 121.6, 129.0, 130.3, 130.4, 130.8,144.0,154.2, 189.7; CHN analysis Calc: C, 66.35; H, 5.10; N, 6.45; Found: C, 66.38; H, 5.12; N, 6.48; Mass: *m/z* 218 [$M^+ + 1$]
17	 *(4Z)-4-Bromo-1-(4-nitrophenyl)-5-phenylpenta-2,4-dien-1-one*	IR: 3104 (C-H str.), 1692 (C = C str.), 690 (C–C str.), 1352 (C-N str., phenyl ring), 1523 (C = O str.), 615 (C–Br str.), 1250 (NO_2 asym str.), 741 (C-N str., Ar-NO_2), 1598 (C = C str., R-CH = CH_2); ^{1}H-NMR: 7.21–8.38 (m, 9H, ArH), 7.23 (d, 1H, CH), 7.76 (d, 1H, CH), 7.21 (s, 1H,CH); ^{13}H-NMR: 116.3, 121.6, 126.4, 127.9, 128.0, 128.7, 129.0, 135.2, 141.1, 152.2, 189.7; CHN analysis Calc: C, 57.00; H, 3.38; N, 3.91; Found: C, 57.04; H, 3.42; N, 3.95; Mass: *m/z* 359 [$M^+ + 1$]

Gram–negative bacterial strains (*E. coli, S. enterica* and *P. aeruginosa)* and fungal strains (*C. albicans, A. niger*) using tube dilution method.

The minimum inhibitory concentration (MIC, i.e., lowest conc. required of test substance to finish or abolish the bacterial/Fungal growth) values of the synthesized compounds are presented in Table 3. From the results of antimicrobial screening (Fig. 4) it has been observed that the whole synthesized compounds showed appreciable antimicrobial activity. An active compound was found to be active against specific microorganisms. In case of Gram + ve bacterial strain, compound **3** ($MIC_{bs} = 1.82$ µM) showed significant antibacterial activity against *B. subtilis* and compound **17** ($MIC_{sa} = 0.91$ µM) exhibited promising antibacterial activity against *S. aureus* [32]. In case of Gram − ve bacterial strains, compound 1 ($MIC_{pa} = 1.16$ µM) showed appreciable activity against *P. aeruginosa* and

Table 3 Antimicrobial screening results of the synthesized compounds

Comp	Minimum inhibitory concentration (MIC = µM/ml)						
	Bacterial strains					Fungal strains	
	Gram–positive bacteria		Gram-negative bacteria				
	S.A	*B.S*	*E.C*	*P.A*	*S.E*	*C.A*	*A.N*
1	4.64	4.64	2.32	1.16	2.32	4.64	4.64
2	16.76	4.19	1.05	2.10	4.19	8.38	2.10
3	3.64	1.82	1.82	1.82	3.64	1.82	3.64
4	2.10	4.19	2.10	2.10	4.19	4.19	4.19
5	1.00	3.99	3.99	1.99	1.99	3.99	3.99
6	4.18	2.09	2.09	2.09	4.18	4.18	2.09
7	3.85	3.85	1.93	1.93	1.93	1.93	3.85
8	3.76	3.76	0.94	1.88	1.88	3.76	3.76
9	4.19	4.19	2.10	2.10	4.19	4.19	4.19
10	1.00	1.99	1.00	1.99	3.99	3.99	3.99
11	4.41	2.21	4.41	2.21	2.21	4.41	4.41
12	2.32	4.64	4.64	2.32	4.64	4.64	9.29
13	2.43	4.85	4.85	2.43	2.43	4.85	4.85
14	1.99	3.98	3.98	1.99	3.98	3.98	3.98
15	2.53	5.06	2.53	2.53	5.06	5.06	5.06
16	1.44	5.75	5.75	2.88	5.75	5.75	5.75
17	0.91	3.62	1.81	1.81	3.62	1.81	3.62
Std. Drugs	1.72[a]	1.72[a]	1.72[a]	1.72[a]	1.72[a]	2.04[b]	2.04[b]
DMSO	NA	NA	NA	NA	NA	NA	NA
Broth Control	NG	NG	NG	NG	NG	NG	NG

Std. Drugs (+ Ve Control): [a]Cefadroxil, [b]Fluconazole; *DMSO* Dimethyl sulfoxide; Microbial species- B.S.: *Bacillus subtilis*; *S.A.: Staphylococcus aureus*; *E.C.: Escherichia coli*; *C.A.: Candida albicans*; *A.N.: Aspergillus niger*; P.A.: *Pseudomonas aeruginosa*; *S.E.:* Salmonella enterica; *N.A:* No activity; *N.G:* No growth

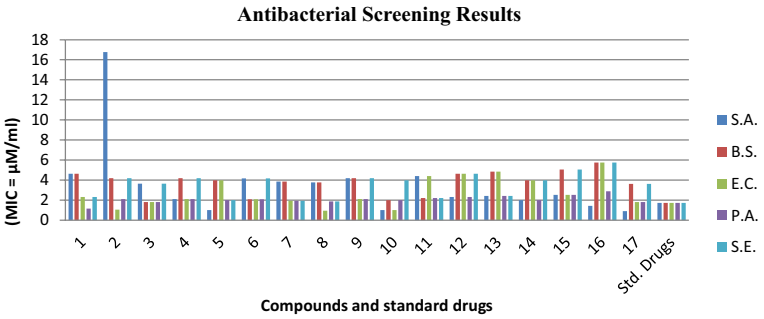


Fig. 4 Antibacterial screening results

compound 8 ($MIC_{ec \text{ and } se}$ = 0.94 and 1.88 µM) displayed good antibacterial activity against *E. coli* and *S. enterica.* In case of antifungal screening results (Fig. 5), Compound 6 (MIC_{an} = 2.09 µM) showed significant activity against *A. niger* and compound 17 (MIC_{ca} = 1.81 µM) showed promising antifungal activity against *C. albicans* [5]. The antimicrobial screening results were comparable to the reference drugs (Cefadroxil and Fluconazole) used as positive control and DMSO used as negative control [33].

Examination of MBC/MFC study

On the basis of minimum inhibitory concentration results of the synthesized derivatives against different microbial strains, i.e., Gram-positive (*S. aureus* and *B. subtilis*) [34], Gram-negative (*E. coli, P. aeruginosa* and *S. enterica*) [8] and fungal strains.

(*C. albicans* and *A. niger*) after that we determine their MBC and MFC by Agar and Sabouraud dextrose media, respectively, and shows results within the range of 95–97% end point reduction of the used bacterial and fungal strains (used as test) [35, 36].

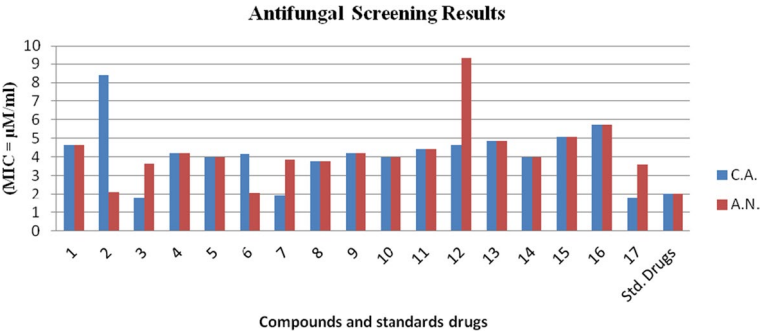


Fig. 5 Antifungal screening results

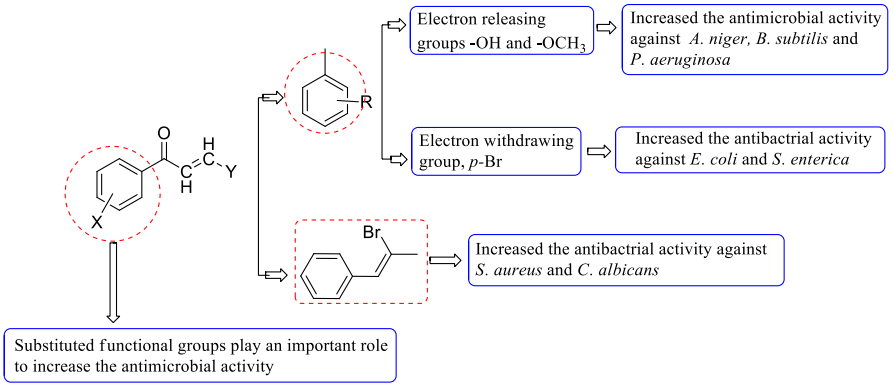


Fig. 6 Structure activity relationship study of synthesized chalcone derivatives

SAR (Structure activity relationship)

From the above mentioned antimicrobial results of the synthesized chalcone derivatives, the subsequent structure activity relationship can be derived in Fig. 6.

(a) In the synthesized derivatives, the electron releasing groups, i.e., –OH and –OCH_3 are present on benzylidene portion of the synthesized compounds **1**, **3** and **6** improved the antibacterial activity against *P. aeruginosa, B. subtilis* and antifungal activity against *A. niger*.
(b) In, Compound **8**, presence of electron withdrawing group (–Br) on benzylidene portion improved the antibacterial activity against *E. coli* and *S. enterica*.
(c) Alpha–Br–cinnamaldehyde (Compound **17**) improves the antimicrobial activity against *S. aureus* and *C. albicans.*
(d) Presence the NO_2, NH_2 and OH substitution groups on benzylidene portion (*para*–position) of acetophenone play a crucial role to improves the antibacterial and antifungal activity against the microorganisms [37].

Conclusion

In this study, we concluded that the newly prepared chalcone compounds 1, 3, 6, 8 and 17 displayed appreciable antimicrobial activity against Gram-positive bacterial strains (*S. aureus*, *B. subtilis*), Gram-negative bacterial strains (*E. coli, S. enterica* and *P. aeruginosa*) and fungal strains (*C. albicans*, *A. niger*). The antimicrobial screening results of synthesized compounds were found to be more potent than reference drugs (Cefadroxil and Fluconazole). So, the newly synthesized derivatives may be used as lead molecules for the development of novel therapeutic agents.

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Author contributions PKV - designed research work; SN - performed research work and SK- analyzed the spectral data, biological screening results and wrote the manuscript. All authors read and approved the final manuscript.

Funding Not applicable.

Data availability We have presented all our main data in the form of tables and figures.

Compliance with ethical standards

Conflict of interest The author(s) confirms that this article content has no conflict of interest.

References

1. S. Burmaoglu, O. Algul, D.A. Anıl, A. Gobek, G.G. Duran, R.H. Ersan, N. Duran, Bioorg. Med. Chem. Lett. **26**(13), 3172 (2016)
2. X. Zhang, H.M. Manukumar, K.P. Rakesh, C.S. Karthik, H.N. Prasad, S.N. Swamy, P. Mallu, Y.H. Mohammed, H.L. Qin, Microb. Pathog. **123**, 275 (2018)
3. S. Tekale, S. Mashele, O. Pooe, S. Thore, P. Kendrekar, R. Pawar (2020) https://doi.org/10.5772/intechopen.91626
4. P.J. Chen, A. Yang, Y. Gu, X.S. Zhang, K.P. Shao, D.Q. Xue, P. He, T.F. Jiang, Q.R. Zhang, H.M. Liu, Bioorg. Med. Chem. Lett. **24**, 2741 (2014)
5. K.P. Rakesh, H.K. Kumara, B.J. Ullas, J. Shivakumara, D.C. Gowda, Bioorg. Chem. **90**, 103093 (2019)
6. Y.H. Mohammed, H.M. Manukumar, K.P. Rakesh, C.S. Karthik, P. Mallu, H.L. Qin, Microb. Pathog. **123**, 339 (2018)
7. X. Zhang, M.H. Marichannegowda, K.P. Rakesh, H.L. Qin, Microbiol. Res. **212**, 59 (2018)
8. K.P. Rakesh, H.K. Vivek, H.M. Manukumar, C.S. Shantharam, S.N. Bukhari, H.L. Qin, M.B. Sridhara, RSC advances. **8**(10), 5473 (2018)
9. A. Rammohan, J.S. Reddy, G. Sravya, C.N. Rao, G.V. Zyryanov, Environ. Chem. Lett. **18**, 433 (2020)
10. H. Iqbal, V. Prabhakar, A. Sangith, B. Chandrika, R. Balasubramanian, Med. Chem. Res. **23**, 4383 (2014)
11. D. Gupta, D.K. Jain, P. Trivedi, Int. J. Chem. Sci. **8**(1), 649 (2010)
12. X. Fang, B. Yang, Z. Cheng, P. Zhang, M. Yang, Res. Chem. Intermed. **40**, 1715 (2014)
13. V. Tomar, G. Bhattacharjee, S. Rajakumar, K. Srivastava, S.K. Puri, Eur. J. Med. Chem. **45**(2), 745 (2010)
14. C. Diaz-Tielas, E. Grana, M.J. Reigosa, A.M. Sanchez-Moreiras, Planta Daninha **34**(3), 1806 (2016)
15. C. Zhuang, W. Zhang, C. Sheng, W. Zhang, C. Xing, Z. Miao, Chem. Rev. **117**(12), 7762 (2017)
16. A. Rani, A. Anand, K. Kumar, V. Kumar, Taylor and Francis **14**(3), 249 (2019)
17. D.I. Ugwu, B.E. Ezema, U.C. Okoro, F.U. Eze, O.C. Ekoh, M.C. Egbujor, D.I. Ugwuja, Int. J. Chem. Sci. **13**(1), 459 (2015)
18. U. Sahu, N.C. Panda, B.V.V. Ravikumar, A. Kumar, Pharma. Tutor. **2**(1), 62 (2014)
19. D.K. Mahapatra, V. Asati, S.K. Bharti, Eur. J. Med. Chem. **92**, 839 (2015)
20. Y.H. Wang, H.H. Dong, F. Zhao, J. Wang, F. Yan, Y.Y. Jiang, Y.S. Jin, Bioorg. Med. Chem. Lett. **26**, 3098 (2016)
21. S. Shenvi, K. Kumar, K.S. Hatti, K. Rijesh, L. Diwakar, G.C. Reddy, Eur. J. Med. Chem. **30**(62), 435 (2013)
22. A.L. Cole, S. Hossain, A.M. Cole, O. Phanstiel, Bioorg. Med. Chem. **24**, 2768 (2016)
23. P.K. Jayaramu, R.R. Maralihallia, Der. Pharma. Chemica. **7**(8), 30 (2015)
24. J.C. Jung, Y. Lee, D. Min, M. Jung, S. Oh, Molecules **22**, 1871 (2017)
25. H.L. Qin, J. Liu, W.Y. Fang, L. Ravindar, K.P. Rakesh, Eur. J. Med. Chem. **20**, 112245 (2020)

26. B.J. Ullas, K.P. Rakesh, J. Shivakumar, D.C. Gowda, P.G. Chandrashekara, Results in Chemistry. **2**, 100067 (2020)
27. M. Kachroo, R. Panda, Y. Yadav, Pharm. Chem. **6**(2), 352 (2014)
28. J.G. Cappuccino, N. Sherman, *In Microbiology-a Laboratory Manual*, 4th edn. (Addison Wesley Longman, California, 1999), p. 263
29. Pharmacopoeia of India, vol. I (2007) Controller of Publication, Ministry of Health Department, Govt. of India, New Delhi, pp.37
30. R.A. Gupta, S.G. Kaskhedikar, Med Chem Res. **22**(8), 3863 (2013)
31. A.D. Panchal, P.D. Kunjadia, P.M. Patel, Int J Pharm Sci Drug Res. **3**, 331 (2011)
32. K.P. Rakesh, M.H. Marichannegowda, S. Srivastava, X. Chen, S. Long, C.S. Karthik, P. Mallu, H.L. Qin, ACS combinatorial science. **20**(12), 681 (2018)
33. H. Liu, S. Long, K.P. Rakesh, G.F. Zha, Eur. J. Med. Chem. **185**, 111804 (2020)
34. G.F. Zha, S.M. Wang, K.P. Rakesh, S.N. Bukhari, H.M. Manukumar, H.K. Vivek, N. Mallesha, H.L. Qin, Eur. J. Med. Chem. **162**, 364 (2019)
35. K.P. Rakesh, S. Ramesh, D.C. Gowda, Russ. J. Bioorg. Chem. **44**(2), 158 (2018)
36. D. Sharma, B. Narasimhan, P. Kumar, V. Judge, R. Narang, E.D. Clercq, J. Balzarini, J. Enzyme Inhib. Med. Chem. **24**(5), 1161 (2009)
37. H.L. Qin, Z.W. Zhang, R. Lekkala, H. Alsulami, K.P. Rakesh, Eur. J. Med. Chem. **9**, 112215 (2020)