Synthesis and antibacterial activity of hydroxy and chloro-substituted chalcone derivatives

Abstract `

Chalcones are a class of natural products reported with a wide range of biological activities. Among them antibacterial is much promising and many potent chalcones have been emerged as useful antibacterial agents. In view of this, we synthesized 15 chalcones (**3a-3o**) containing both hydroxyl and chlorine substituents and studied them by using spectroscopic methods. The compounds were tested for antibacterial efficacy against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Proteus vulgaris*, among other harmful microorganisms. The compounds have moderate to high antibacterial activity, among them heteroaromatic ring containing compounds **(3m, 3n,** and **3o**) elicited higher activity than the standard drug benzyl penicillin. The compound 3m having the pyridinyl compound displayed the maximum activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Proteus vulgaris*, with zone of inhibition (in mm) values of 27.52±0.16, 28.85±0.11, 22.05±0.16, and 23.18±0.17, respectively. The synthesized compounds could be used as lead molecules in the development of novel antibacterial medicines.

Key Words: Chalcone; Spectroscopic methods; Antibacterial activity; Heteroaromatic; Benzyl penicillin.

Introduction

Natural products contain a diverse range of secondary metabolites, including flavonoids and isoflavonoids, which have been linked to a significant number of medications used in the treatment of different diseases including microbial infections and cancer [1]. Chalcone is a chemically open-chain flavonoid with two aromatic rings linked by α, β-unsaturated propenone [2]. Plants with high in flavonoid derivatives should be included in our diet on a regular basis as such practices will have considerable health benefits. A part from that, chalcones are important in the treatment of a variety of disorders [3]. Chalcone's chemical template has the ability to participate in a variety of metabolic reactions and physiological processes that provide good impact on our health [4-6]. Chalcones possess different activities like antibacterial [7-10], antifungal [11-13], anticancer [14-16], anti-inflammatory [17-19], antioxidant [20-22], cytotoxic [23-24], antimalarial [25-27] etc.

Furthermore, the structures of these molecules are straightforward, and they can be easily produced in the laboratory. In response to these two characteristics of chalcones, academic and industry researchers have been working hard to develop, manufacture and test chalcones with a variety of substituents and changed versions in order to produce novel compounds with good biological functions. Based on the foregoing, we present the synthesis and antibacterial evaluation of 15 chalcone derivatives (**3a-3o**) containing chlorine and hydroxyl substituents on one phenyl ring portion (ring-A) and another phenyl ring portion (ring-B) replaced with either a bioisosteric heteroaryl ring or a phenyl ring containing electron withdrawing or releasing substituents in order to assess the influence of the chalcone on antibacterial activity (Figure 1).

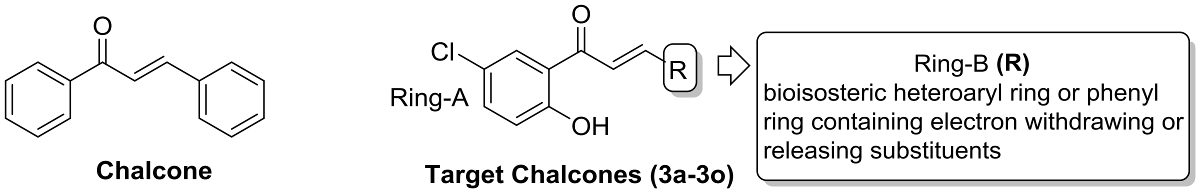


Figure 1. General structure of chalcone and the structure of target chalcones (**3a-3o**)

Materials and Methods

General

All the chemicals including the ketone and aromatic aldehydes, reagents and soliutions used in the study were procured from Sigma Aldrich and S.D. Fine Chemicals. The melting points of all 15 target compounds were determined using a Boetius melting point apparatus, and the 1H NMR and 13C-NMR spectra were acquired using Bruker Avance 400 NMR spectrophotometers (Bruker Switzerland AG) at 400 and 100 MHz for the 1H and 13C nuclei, respectively, and the results were reported as chemical shifts for all 15 target compounds (ppm). The FT-IR was scanned on a Bruker alpha-T (BRUKER biospin International AG., Zug, Switzerland) and the wave numbers were reported in cm^{-1}. The mass spectra were scanned using an Agilent LC-MS spectrometer (Agilent technologies, USA). To monitor the chemical reactions and determine the purity of the compounds, a precoated silica gel-G TLC (Merck) with a 20-30 percent ethyl acetate-hexane mobile phase was employed in conjunction with a precoated silica gel-G TLC (Merck). A UV light was used to watch the TLC plate in action.

Synthetic protocol: Equimolar concentrations of the ketone i.e., 5'-chloro-2'-hydroxy acetophenone (1 mmol) and substituted aromatic aldehydes (1 mmol) were dissolved in 7.5 mL of ethanol. To the above mixture, 7.5 mL of 50 percent alcoholic KOH was added dropwise and the reaction mixture was allowed to react for 24 h at room temperature. At the end of the reaction (monitored by TLC), the reaction mixture was neutralized with 1:1

solution of hydrochloric acid and water for the precipitation of the target chalcones (**3a-3o**). The chalcones formed were filtered using vacuum filtration and then washed in cold water, dried, and recrystallized in either ethanol or chloroform to complete the process (Scheme 1).

Table 1: The physicochemical and spectral features of the compounds are reported

(*E*)-1-(5'-Chloro-2'-hydroxyphenyl)-3-(2''-fluorophenyl)prop-2-ene-1-one (**3a**): Yellow color solid; Yield: 74%. Recryastallized from ethanol. m.p: 160 ^{0}C; **FT-IR** (KBr ν_{max} cm^{-1}): 668 (C-Cl), 1215 (C-F), 1610 (str, CH=CH, conjugated), 1717 (intense conjugated C=O band), 3344 (Ar-OH); **^{1}HNMR** (CDCl$_3$ 400 MHz) δ (ppm): 7.17 (d, 1H, H$_\alpha$, J = 16.1 Hz), 7.09 (d, 1H, H$_\beta$, J = 16 Hz), 7.24-7.68 (m, 7H, Ar-H), 11.96 (s, Ar-OH); LC-MS: m/z 276.69 (M$^+$, 99.06), 278.69 (M+2, 33.02).

(*E*)-(1-(5'-Chloro-2'-hydroxyphenyl)-3-(3''-fluorophenyl)prop-2-ene-1-one (**3b**):Yellow color solid; Yield:84%. Recryastallized from ethanol. m.p: 151^{0}C; **FT-IR** (KBr ν_{max} cm^{-1}): 771 (C-Cl), 1214 (C-F), 1611 (str, CH=CH, conjugated), 1717 (intense conjugated C=O band), 3344 (Ar-OH); **^{1} HNMR** (CDCl$_3$ 400MHz) δ (ppm): 7.17 (d, 1H, H$_{\alpha,}$ J = 16.0 Hz), 7.10 (d, 1H, H$_\beta$, J = 16 Hz), 7.24-7.69 (m, 7H, Ar-H), 11.96 (s, Ar-OH); LC-MS: m/z 276.69 (M$^+$, 99.06), 278.69 (M+2, 33.02).

(*E*)-(1-(5'-Chloro-2'-hydroxyphenyl)-3-(4''-fluorophenyl)prop-2-ene-1-one (**3c**), yellow color solid, yeild 85%. Recryastallized from ethanol. m.p: 160^{0}C; **FT- IR** (KBr ν_{max}cm^{-1}): 771 (C-Cl),1215 (C-F), 1623 (str, CH=CH, conjugated), 1720 (intense conjugated C=O band), 3200 (Ar-OH); **^{1} HNMR** (CDCl$_3$ 400MHz) δ (ppm): 7.26 (d, 1H, H$_{\alpha\ ,}$ J=16.0 Hz), 7.52 (d, 1H, H$_{\beta,}$ J=16.0Hz), 7.24-7.69 (m, 7H, Ar-H), 11.89 (s, Ar-OH); LCMS: m/z 276.69 (M$^+$, 99.08), 278.69(M+2, 33.02).

(*E*)-1-(5'-Chloro-2'-hydroxyphenyl)-3-(2''-chlorophenyl)prop-2-ene-1-one(**3d**): Yellow color solid, Yield: 75%. Recrystallized from ethanol (Sawant and Nirwan., 2013). **FT-IR** (KBr ν_{max} cm^{-1}): 706 (C-Cl), 794 (C-Cl), 1626 (str, CH=CH, conjugated), 1721 (intense conjugated C=O band), 3349 (Ar -OH); **^{1}H NMR** (CDCl$_3$ 400 MHz) δ (ppm): 7.43 (d, 1H, H$_{\alpha,}$ J=16.1 Hz), 7.92 (d, 1H, H$_{\beta,}$ J=16.3 Hz), 7.04-8.93 (m, 7H, Ar-H), 12.36 (s, Ar-OH); LC-MS: m/z 293.14 (M$^+$, 99.06), 295.14 (M+2, 33.01).(literature (28)).

(*E*)-(1-(5'-Chloro-2'-hydroxyphenyl)-3-(3''-chlorophenyl)prop-2-ene-1-one (**3e**), yellow color solid, yeild 80%. Recryastallized from ethanol. m.p: 161^{0}C; **FT-IR** (KBr ν_{max} cm^{-1}), 699 (C-Cl), 780 (C-Cl), 1622 (str, CH=CH, conjugated), 1718 (intense conjugated C=O band), 3344 (Ar -OH); **^{1} HNMR** (CDCl$_3$ 400MHz) δ (ppm): 7.44 (s, Ar-OH), 7.48 (d, 1H, H$_{\alpha,}$ J=16.2 Hz), 7.98 (d, 1H, H$_{\beta,}$ J=16.0 Hz), 7.08-8.98 (m, 7H, Ar H); LCMS: m/z 293.14 (M$^+$, 99.02), 295.14 (M+2, 33.00).

(*E*)-1-(5'-Chloro-2'-hydroxyphenyl)-3-(4''-chlorophenyl)prop-2-ene-1-one (**3f**): Yellow color solid, Yield: 82%. Recryastallized from ethanol. m.p: 161^{0}C; **FT-IR** (KBr ν_{max} cm^{-1}): 786 (C-Cl), 1667 (str, CH=CH conjugated), 1751 (intense conjugated C=O band), 3253 (Ar-OH); **^{1}H NMR** (CDCl$_3$ 400 MHz) δ (ppm): 7.42 (d, 1H, H$_{\alpha,}$ J=16.2 Hz), 7.86 (d, 1H, H$_{\beta,}$ J=16.0 Hz), 7.09-8.91 (m, 7H, Ar H), 12.31 (s, Ar-OH); LC-MS: m/z 293.14 (M$^+$, 99.09), 295.14 (M+2, 33.04). (literature (29)).

(*E*)-(1-(5'-Chloro-2'-hydroxyphenyl)-3-(2"-hydroxyphenyl)prop-2-ene-1-one (**3g**), yellow color solid, yeild 80%. Recrystallized from chloroform. m.p: 200 ^{0}C; **FT-IR** (KBr v_{max} cm^{-1}), 795 (C-Cl), 1665 (str, CH=CH conjugated), 1756 (intense conjugated C=O band), 2800 (Ar-OH), 3230 (Ar -OH); **^{1} HNMR** (CDCl$_3$ 400MHz) δ (ppm): 6.94 (Ar-OH), 6.97 (s, Ar-OH), 7.57 (d, 1H, $H_{\alpha,}$ *J*=16.5Hz), 7.84 (d, 1H, $H_{\beta,}$ *J*=16.0Hz), 6.72-8.08 (m, 7H, Ar-H); **LC-MS**: m/z 274.70 (M$^+$, 99.09), 276.70(M+2, 33.03);

(*E*)-1-(5'-Chloro-2'-hydroxyphenyl)-3-(3"-hydroxyphenyl)prop-2-ene-1-one (**3h**): Yellow color solid, Yield: 75%. Recrystallized from chloroform. Mp:200^{0}C; **FT-IR** (KBr v_{max} cm^{-1}): 795 (C-Cl), 1665 (str, CH=CH conjugated), 1756 (intense conjugated C=O band), 3255 (Ar-OH); **^{1}H NMR** (CDCl$_3$ 400 MHz) δ (ppm): 5.41 (s, Ar-OH), 7.49 (d, 1H, $H_{\alpha,}$ *J* = 16.2 Hz), 7.81 (d, 1H, $H_{\beta,}$ *J* = 16.2 Hz), 6.89-8.16 (m, 7H, Ar-H), 12.24 (s, Ar-OH); **LC-MS**: m/z 274.70 (M$^+$, 99.02), 276.70 (M+2, 33.00). (literature (30)).

(*E*)-1-(5'-Chloro-2'-hydroxyphenyl)-3-(4"-hydroxyphenyl)prop-2-ene-1-one(**3i**):Yellow color solid, Yield: 70%. Recrystallized from chloroform. m.p: 200 ^{0}C; **FT-IR** (KBr v_{max} cm^{-1}): 799 (C-Cl), 1672 (str, CH=CH conjugated), 1759 (intense conjugated C=O band), 3258 (Ar-OH); **^{1}H NMR** (CDCl$_3$ 400 MHz) δ (ppm): 5.34 (s, Ar-OH), 7.53 (d, 1H, $H_{\alpha,}$ *J* = 16.2 Hz), 7.86 (d, 1H, $H_{\beta,}$ *J* = 16.2 Hz), 6.96-8.33 (m, 7H, Ar-H), 12.30 (s, Ar-OH); LC-MS: m/z 274.70 (M$^+$, 99.07), 276.70 (M+2, 33.04). (literature (31)).

(*E*)-1-(5'-Chloro-2'-hydroxyphenyl)-3-(2"-methoxyphenyl)prop-2-ene-1-one(**3j**):Yellow color solid, Yield: 85%. Recrystallized from chloroform. **FT-IR** (KBr v_{max} cm^{-1}): 778 (C-Cl), 1681 (str, CH=CH conjugated), 1782 (intense conjugated C=O band), 2831 (-OCH$_3$), 3516 (Ar -OH); **^{1}H NMR** (CDCl$_3$ 400 MHz) δ (ppm): 2.41 (Ar-OCH$_3$), 7.78 (d, 1H, $H_{\beta,}$ *J* = 16.3 Hz), 7.89 (d, 1H, $H_{\alpha,}$ *J* = 16.2 Hz), 6.61-8.17 (m, 7H, Ar- H), 12.39 (s, Ar-OH); LC-MS: m/z 288.06 (M$^+$, 99.05), 290.06 (M+2, 33.04). (literature (31)).

(*E*)-(1-(5'-Chloro-2'-hydroxyphenyl)-3-(3"-methoxyphenyl)prop-2-ene-1-one (**3k**), yellow color solid, yeild 80%. Recrystallized from chloroform. m.p: 150^{0}C; FT-IR (KBr v_{max} cm^{-1}), 775 (C-Cl), 1685 (str,CH=CH conjugated), 1780 (intense conjugated C=O band), 2823 (C-OCH$_3$), 3500 (Ar -OH); ^{1} HNMR (CDCl$_3$ 400MHz) δ (ppm): 6.95 (Ar-OCH$_3$), 7.03 (s, Ar-OH), 7.75 (d, 1H, $H_{\beta,}$ *J*=16.2Hz), 7.85 (d, 1H, $H_{\alpha,}$ *J*=16.75Hz), 6.56-8.08 (m, 7H, Ar- H); LCMS: m/z 288.06(M$^+$, 99.08), 290.06 (M+2, 33.0).

(*E*)-1-(5'-Chloro-2'-hydroxyphenyl)-3-(2"-methoxyphenyl)prop-2-ene-1-one (**3l**): Yellow color solid, Yield: 85%. Recrystallized from chloroform. **FT-IR** (KBr v_{max} cm^{-1}): 778 (C-Cl), 1681 (str, CH=CH conjugated), 1782 (intense conjugated C=O band), 2831 (-OCH$_3$), 3516 (Ar -OH); **^{1}H NMR** (CDCl$_3$ 400 MHz) δ (ppm): 2.41 (Ar-OCH$_3$), 7.78 (d, 1H, $H_{\beta,}$ *J* = 16.3 Hz), 7.89 (d, 1H, $H_{\alpha,}$ *J* = 16.2 Hz), 6.61-8.17 (m, 7H, Ar- H), 12.39 (s, Ar-OH); LC-MS: m/z 288.06 (M$^+$, 99.05), 290.06 (M+2, 33.04). (literature (32)).

(*E*)-(1-(5'-Chloro-2'-hydroxyphenyl)-3-(pyridin-4"-yl)prop-2-ene-1-one (**3m**), cream color solid, yeild 95%. Recrystallized from ethanol. m.p: 150^{0}C; FT- IR (KBr v_{max} cm^{-1}), 775 (C-Cl), 1258 (str, C=N conjugated), 1684 (str,CH=CH conjugated), 1787 (intense conjugated C=O band), 3221 (Ar C-OH); ^{1} HNMR (CDCl$_3$ 400MHz) δ (ppm): 6.09 (s, Ar-OH), 6.96 (d, 1H, $H_{\alpha,}$ *J*=16 Hz), 8.06 (d, 1H, $H_{\beta,}$ *J*=16.8 Hz), 7.08-8.84 (m, 7H, Ar- H); LCMS: m/z 259.69 (M$^+$, 99.06), 261.69 (M+2, 33.02).

(*E*)-(1-(5'-Chloro-2'-hydroxyphenyl)-3-(thiophen-2"-yl)prop-2-ene-1-one (**3n**): Yellow color solid; Yield: 95%. Recrystallized from ethanol. **FT-IR** (KBr v_{max}cm^{-1}): 856 (C-S), 771 (C-Cl), 1688 (str, CH=CH conjugated), 1779 (intense conjugated C=O band), 3228 (Ar-OH); **^{1}H NMR** ($CDCl_3$ 400 MHz) δ (ppm): 7.38 (d, 1H, $H_{\alpha,}$ J = 16.3 Hz), 7.59 (d, 1H, $H_{\beta,}$ J = 16.0 Hz), 6.94-8.32 (m, 6H, Ar H), 12.46 (s, Ar-OH); LC-MS: m/z 264.72 (M^+, 99.09), 266.72 (M^+, 33.07). (literature (33)).

(E)-(1-(5'-Chloro-2'-hydroxyphenyl)-3-(furan-2''-yl)prop-2-ene-1-one (**3o**), yellow color solid, yeild 95%. Recrystallized from ethanol. m.p: 190^{0}C;FT-IR (KBr ν_{max}cm^{-1}), 744 (furon) , 775 (C-Cl), 1685 (str, CH=CH conjugated), 1776 (intense conjugated C=O band), 3230 (Ar -OH); ^{1} HNMR ($CDCl_3$ 400MHz) δ (ppm): 6.98 (s, Ar-OH), 7.34 (d, 1H, $H_{\alpha,}$ J=16.0Hz), 7.54 (d, 1H, $H_{\beta,}$ J=16.5Hz), 6.87-8.17 (m, 7H, Ar H); LCMS: m/z 248.66 (M^+, 99.03), 250.66(M^+, 33.01).

Scheme 1. Synthesis of chalcones (3a-3o). Reagents and conditions: (a) ethanol, KOH, and room temperature; (1) 5-chloro-2-hydroxy acetophenone; R”-CHO aryl or heteroaryl aldehydes. R” = ring B; 3a: 2’’-fluorophenyl; 3b: 3”-fluorophenyl; 3c: 4”-fluorophenyl; 3d: 2”-chlorophenyl; 3e: 3”-chlorophenyl; 3f: 4”-chlorophenyl; 3g: 2”-methoxyphenyl; 3h: 3”-methoxyphenyl; 3i: 4”-methoxyphenyl; 3j: 2”-hydroxyphenyl; 3k: 3”-hydroxyphenyl; 3l: 4”-hydroxyphenyl; 3m: 4”-pyridinyl; 3n: 2”-thienyl; 3o:2”-furfuryl.

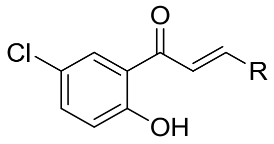
Antibacterial evaluation: Antibacterial activity was evaluated against four clinically significant bacterial strains, including the Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, and the Gram-negative *Escherichia coli* and *Proteus vulgaris*. Benzyl penicillin was used as the reference standard by following the previously reported technique [34]. The glass ware was sterilized at 160 ^{o}C for 2 hours in a hot air oven. The medium was sterilized, and then standard drug solutions (benzyl penicillin) as well as the target compounds (**3a-3o**) were prepared. In the meantime, a nutritional agar medium was prepared (composition: peptone 0.5 percent, meat extract 0.3 percent, sodium chloride 0.5 percent, agar 2 percent, distilled water to make up to 100 mL, and pH was adjusted to a value of 7.2). In 1000 mL of distilled water add measure amount of peptone, meat extract, and sodium chloride were dissolved to maintain pH of 7.2. As soon as the agar was dissolved, the medium was transferred into 25 mL conical flasks and placed in the refrigerator. The nutrient medium used in the study was sterilized using an autoclave at 121°C and 15 lbs/sq. inch pressure. Sterilization of the petri plates, test tubes, pipettes, and borers required for the experiment was accomplished using dry heat sterilization using a hot air oven. Cultures of the various organisms (18 hours old) were collected, and sterile water was used to form a suspension of the microorganisms in order to test their viability. This solution was used as an inoculum later on the amount of bacteria present in each sample was determined using the pour plate method. It was necessary to place the inoculated agar media in sterile petri dishes with a diameter of 10 cm and allow it to solidify before continuing. In DMSO, solutions of test substances at concentrations of 0.1µg/mL were generated. Borer in the suitable media was utilized to manufacture the 5 mm diameter cups. Five wells were formed on each plate. Three wells were used for testing substances: one for standard compounds, one for control compounds, and one for a combination of both. It was necessary to place sample into each well before placing the plates in the refrigerated for 45 minutes to allow diffusion to take place. After an 18-hour incubation period at 37°C, the plates were examined for the presence of inhibitory zones. In order to decrease the possibility of experimental errors, the experiments were carried out in triplicate on the same day and under the same conditions. In order to determine the values of the zone of inhibition, a vernier was used, and the results were presented as a mean of three values with standard deviation.

Results and discussions

Chemistry: Chalcones were produced by the Claisen-Schmidt condensation of 5'-chloro-2'-hydroxy acetophenone with substituted aryl aldehydes and unsubstituted heteroaryl aldehydes, which were then purified (3a-3o). Recrystallization was used to purify all of the compounds, with either ethanol or chloroform being used as the recrystallizing solvent to achieve maximum purity. The structures of the compounds were investigated by using FT-IR, 1H NMR, and mass spectroscopy techniques, among others. Two characteristic absorption bands with wave numbers of 1610-1685 cm^{-1} and 1704-1787 cm^{-1} respectively, corresponding to -C=C- and -C=O, respectively were seen in their FT-IR spectrum. On the other hand, the vinylic protons (H and H) of chalcones revealed two distinct doublet peaks in their 1H NMR spectra, with chemical shift values of 6.96-7.95 and 7.09-8.06 ppm, respectively. Multiple peaks were observed for the other aromatic protons, with chemical shift values ranging from 6.72 to 8.06 ppm, while a singlet peak was observed for the -OH proton, with a chemical shift value of more than 12 ppm. M+ peaks were found in the mass spectra of all the compounds, which matched to their molecular weights, as well as an isotopic M+2 peak, which corresponded to the chlorine isotope (^{37}Cl) atom present in these molecules.

Evaluating antibacterial activity: The antibacterial activity of all the synthesized compounds was tested against four bacterial species, including the Gram-positive *Staphylococcus aureus* and *Bacillus subtilis* and the Gram-negative , *Escherichia coli* and *Proteus vulgaris*, respectively. The results indicate that 2'-hydroxy-5'-chlorophenyl chalcones possess considerable antibacterial activity. The nature of ring-B, on the other hand, is critical for the intensity of the activity (Table 2).

Table 2. Antibacterial and antifungal activity results of compounds **3a-3o** (Mean±SD)*

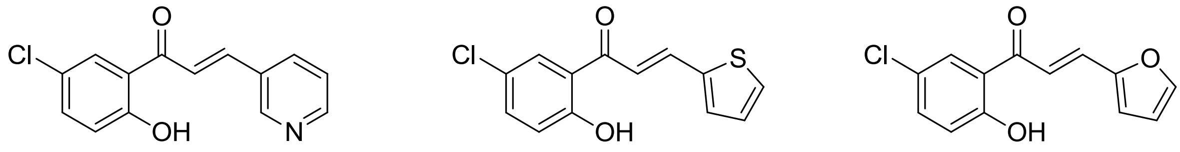


Entry	***R***	**Microorganisms**			
Compound code		***S.aureus***	***B.subtilis***	***E. coli***	***P.vulgaris***
3a	2-fluorophenyl	21.56±0.45	22.83±0.61	12.45±0.34	16.30±0.29
3b	3-fluorophenyl	20.13±0.29	23.02±0.19	11.02±0.39	15.43±0.61
3c	4-fluorophenyl	22.03±0.24	21.02±0.34	12.32±0.43	14.78±0.65

3d	2-chlorophenyl	17.87±0.54	19.22±0.31	11.56±0.90	12.32±0.43
3e	3-chlorophenyl	20.12±0.67	23.22±0.89	11.67±0.33	14.23±0.19
3f	4-chlorophenyl	19.54±0.32	23.87±0.12	12.33±0.57	15.22±0.43
3g	2-methoxyphenyl	18.14±0.54	18.19±0.23	10.14±0.75	12.43±0.76
3h	3-methoxyphenyl	19.55±0.65	18.06±0.22	11.54±0.12	13.53±0.21
3i	4-methoxyphenyl	19.12±0.42	19.16±0.54	11.14±0.16	12.55±0.65
3j	2-hydroxyphenyl	23.11±0.34	24.12±0.18	14.65±0.76	20.54±0.76
3k	3-hydroxyphenyl	20.32±0.55	21.51±0.23	13.93±0.65	18.19±0.43
3l	4-hydroxyphenyl	23.14±0.18	22.11±0.73	14.12±0.92	19.55±0.32
3m	3-pyridinyl	27.52±0.16	28.85±0.11	22.05±0.16	23.18±0.17
3n	2-thienyl	26.56±0.21	27.09±0.22	21.14±0.21	22.14±0.12
3o	2-furyl	26.12±0.52	27.05±0.19	18.12±0.52	19.67±0.19
Benzyl penicillin	-	24.06±0.05	27.02±0.02	14.05±0.05	19.04±0.03

*Results are mean of three experiments±Standard Deviation

We found that just three of the chalcones examined had potential antibacterial action against all the bacterial strains tested: 3m, 3n, and 3o, which all had the heteroaryl ring as a ring-B component. The activity of these compounds exceeds that of the ordinary benzyl penicillin by a significant margin. The bioisosteric pyridinyl scaffold in compound **3m** demonstrated the greatest activity, with an inhibitory zone (in mm) of 27.52±0.16, 28.85±0.11, 22.05±0.16, and 23.18±0.17 against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Proteus vulgaris*, respectively, among the compounds tested. In a similar way, the compounds **3n** and **3o**, which included thienyl and furyl moieties, that shown approximately equivalent activity against *Staphylococcus aureus* and *Bacillus subtilis*, respectively. To the contrary, 3n was more effective than **3o** against *Escherichia coli* and *Proteus vulgaris*, with zone of inhibition values of 21.14±0.21 and 22.14±0.12 for each pathogen, respectively. This could be owing to the presence of a sulfur atom within the thienyl ring. Antibacterial activity of the compounds **3j** and **3l**, which contain the electron-releasing -OH group at the ortho and para-positions of the phenyl rings at the ring-B portion, was moderate against all the tested bacterial species, with zone of inhibition values that were comparable to those of benzyl penicillin in all cases. The rest of the compounds, which contained electron-releasing methoxy groups as well as halogen atoms, exhibited only moderate activity. The findings reveal that chalcones with heteroaryl rings are more effective in inhibiting bacterial growth than standard chalcones with two phenyl rings connected to the ketovinyl component of chalcones in terms of antibacterial activity. The structures of the most potent compounds were represented in figure 2.



3m 3n 3o

Figure 2. Structures of the most potent antibacterial chalcones **3m, 3n** and **3o**

Conclusion

We have synthesized and tested the antibacterial activity of fifteen chalcones bearing chlorine and hydroxyl groups on the ring-A moiety. All of the compounds were purified and characterized. It was discovered that the compounds possessing a heteroaryl scaffold at the ring-B region of chalcones had good antibacterial activity against all of the strains tested, with activity greater than that of the standard benzyl penicillin. The most potent antibacterial compounds **3m, 3n,** and **3o**, can be considered as potential lead compounds for the design and development of improved antibacterial agents. As part of our ongoing research, we are testing these compounds against methicillin-resistant *Staphylococcus aureus* (MRSA) strains to determine their probable mode of action for the proposed activity.

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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