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# Novel chalcones of 1-(4-(4-((6-((7chloroquinolin-4-yl) amino) hexyl) amino)-6 ((4-nitrophenyl) amino)-1,3,5-triazin-2-yl) amino) phenyl) ethanone for Biological Applications: Synthesis, Characterization and Antimicrobial Studies)

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## Abstract

**A series of biologically active chalcones are prepared using 1-(4-(4-((6-((7chloroquinolin-4-yl)amino)hexyl)amino)-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)phenyl)ethanone and different substituted aromatic aldehyde under bio-friendly method. Prepared chalcones are characterized by IR, 1H-NMR and tested for their antimicrobial activity against a gram positive and gram negative bacteria's are using Minimum Inhibitory Concentration (MIC) method. Chlorine containing chalcones shows high antibacterial activity against tested bacteria, among the synthesized chalcones.**

***Keywords:*** ***Bacteria; Chlorine; Chalcone; MIC; Antimicrobial activity***

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## Introduction

Chalcone are well identified intermediates for the synthesis of various hetero cyclic compounds. The chalcones and its derivatives have been reported by many researchers [1-7] over a decade due to its excellent activity especially in the field of medicine, synthetic chalcone containing electron releasing group exhibited wonderful activity against the cancer cell [8]. Rodrigo [9] synthesized novel quinoline-2-one based chalcones for potential anti-tumor activity, in his paper he concluded that vinylic ketone joined by two quinoline molecule shows highest activity against the tested cancer cell. Muhammad Azad [10] prepared a series of quinolone based chalcones using Schmith condensation method by using

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quinolone-3-carbaldehydes with acetophenone and N-substituted-3-acetyl-4-hydroxy-2-quinolines with hetrocyclic aldehydes. Siva Kumar [11] was reported heterocyclic chalcones having antioxidant activities. Here they prepared chalcones using thiophene derivatives with different aldehydes by Schmitt condensation method. In this paper we have prepared chalcones using cynuric chloride and quinoline based novel molecules and tested their antibacterial activity against a gram +ve and a –ve bacteria's.

## Experimental Methods

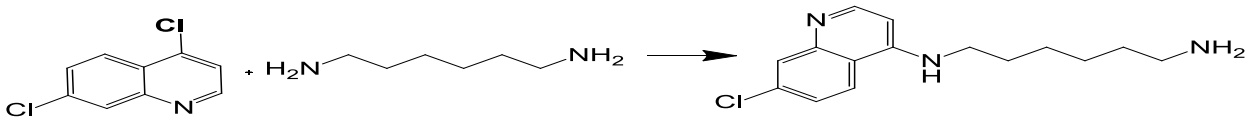
### Materials

4-nitrobenzaldehyde,4-bromobenzaldehyde,4-chlorobenzaldehyde,4-flurobenzldehyde,3-ethoxy-4 hydroxybenzaldhyde,4-hydroxybenaldehyde,3-fluloro-4-hydroxybenzaldehyde,3-bromo-2 hydroxybenzaldehyde,2-chloro-5-nitrobenzaldehyde,5-hydroxy-2-4-nitrobenzaldehyde,1,6 hexamethyldiamine and paraaminoacetophenone were got from sigma-Aldrich and used as such.

### Synthesis of 7-chloroquinolin (4-yl)-hexane-1,6-diamine (CHD) (SCHEME 1)

Cyanuric chloride (6g, 0.03mol) dissolved in acetone and to this 4-nitroaniline(4.2g, 0.04mol) was added, taken in a R.B flask and stirred for 3hrs at $0^0$C. The PH of the reaction mixture was maintained at 6-7 by the adding of aqueous Na2CO3 (10%) after completion of the reaction, the reaction mixture was transferred into ice-cold water, filtered and washed with water. The obtained CNA must be dried in vacuum oven at 60ºC and finally recrystallized from ethanol. The molecular weight of the CNA was 286.07 and melting point was 272- 275 0C. The FT-IR ($cm^{-1}$), the peak at 3050 (aromatic CH-stretching), 1555 (C-NO2) and 3560 (-NH). 1H NMR (500 MHz, DMSO-d6, δ): 7.3-8.1 (m, 4HAr H) and 4.6 (s, 1H NH).

Scheme 1: **7-chloroquinolin (4-yl)-hexane-1,6-diamine (CHD)**




$N^1$-(7-chloroquinolin-4-yl)hexane-1,6-diamine

### Synthesis of 4, 6-dichloro- N-(4-nitrophenyl)-1,3,5-triazine-2-amine (CNA)(SCHEME 2)

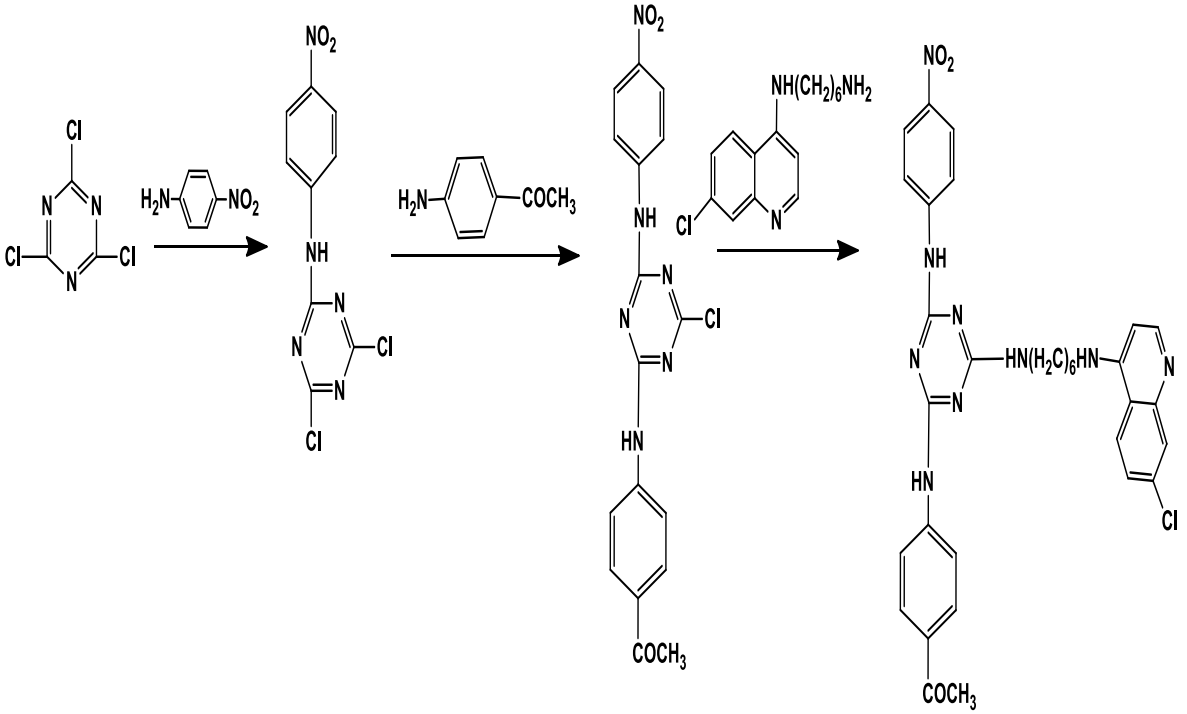
1,6-hexmethyldiamine (4.5g, 0.03mol) dissolved in acetone was added to CNA (3.03g 0.02 mol in 40mL acetone) and stirred for 3hrs at 45 $^0$C. To this 10% $Na_2CO_3$solution was added by drop by drop. On completion of this reaction, the reaction mixture was transferred into ice-cold water, the precipitate were separated by filtration and washed with cold- water and dried in vacuum oven at 60 $^0$C. 5.4 g of CNA was obtained (85%), Mol. Wt. of CAN was 542.76 and melting point was 188-190$^0$C. FT-IR ($cm^{-1}$), the peak at 3360 is due to N-H stretching, at 3090 is due to the aromatic CH stretching, at 1693 is outstanding to the occurrence of C=O stretching, at 1650 and 1600 is corresponding to C=C and Ar-$NO_2$ stretching frequency respectively. The peak at 1070 is shown to the C-Cl stretching, at 1050 is shown to C-O-C stretching and the peak at 850 is

due to C-N stretching region of *s*-triazine compound. $^1$H NMR (500 MHz, DMSO-$d_6$, δ): the peak at 7.3-8 is owing to aromatic protons (10.8 H), at 6.7-6.9 is due to the -CO-CH=CH proton besides the peak at 4.3 is due to the occurrence of Ar-NH compound.

**Synthesis of 1-(4-(4-((6-((7chloroquinolin-4-yl) amino) hexyl) amino)-6-((4-nitrophenyl) amino)-1,3,5-triazin-2-yl) amino) phenyl) ethanone (QCP)( SCHEME 2 )**

3.60 gm, 0.006 mol of 1,6-hexamethyldiamine is dissolved in acetone and it is added slowly to 4-aminoacetophenone (0.78g, 0.007mol in acetone) in RB flask and stirred this for 3hrs at the temperature of 60$^0$C. The P$^H$ of the reaction mixture maintained at 6-7 by the adding of aqueous $Na_2CO_3$ (10%). Finally the contents were poured into crushed ice. The solid separated out and filtered and washed with water, dried and recrystallized from ethanol. The molecular weight of QCP is 590. Yield: 2.5g (79%) and the melting point is 180-182$^o$C. FT-IR (cm$^{-1}$), the peak appear at 3305 is due to N-H stretching, at 3100 is showing for aromatic C-H stretching, at 1665 is due to the presence of the free and conjugated C=O stretching frequency, at 1693 showing C=C stretching, at 1035 for the C-O-C stretching, and at 1665 showing the Ar-$NO_2$ stretching. The peak at 1093 is due to C-Cl stretching. UV (nm) spectrum reveals two characteristic peak, one at 254 nm another at 336 corresponding aromatic double bond and $\pi$-$\pi^*$ transition of CH=CH respectively. $^1$H NMR (δ, ppm), the peak at 6.7 to 7.8 showing for vinylic (2H) and aromatic protons (15H) and also the peak at 4.5 are present for Ar-NH proton. The peak appear at 3.76 is owing to -CO-$CH_3$ in the QCP system.

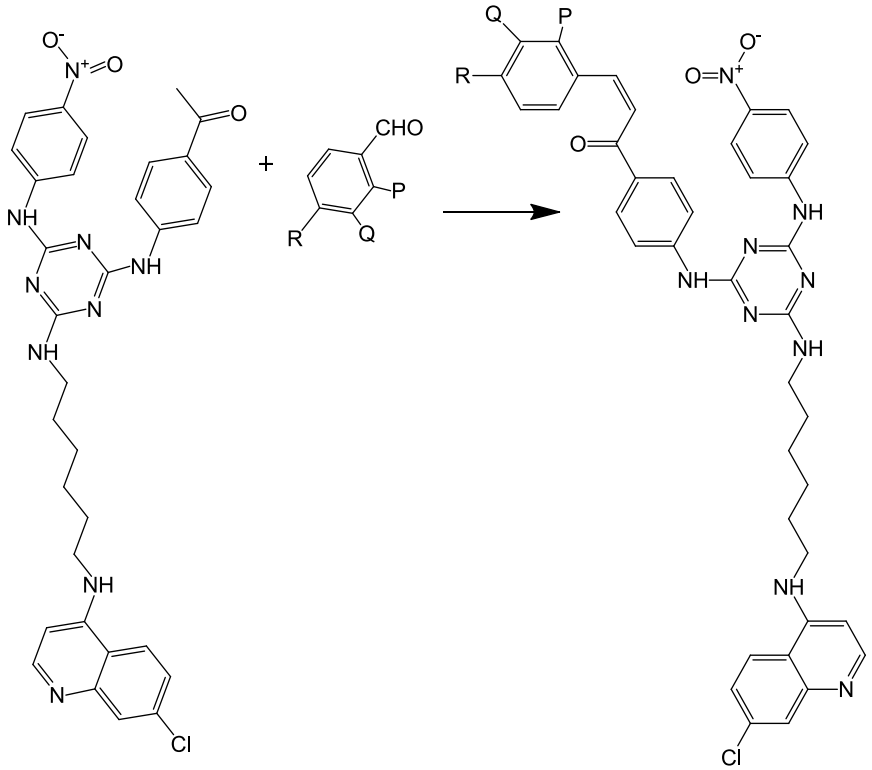
SCHEME 2. **Synthesis of 3-(4-((4-(4-acetylphenoxy)-6-((4-nitrophenyl) amino)-1,3,5-triazin-2-yl)oxy)phenyl)-1-(2,4-dichlorophenyl)prop-2-en-1-one (QCP)**



**Synthesis of triazine containing novel chalcones (SCHEME 3) Grindstone technique**

Novel chalcones were prepared by grinding together the equivalent amount of QCP and substituted benzaldehydes in presence of KOH in a porcelain mortar under solvent free conditions for 4-8 mins. On completion of reaction, the mixture was diluted with cold water neutralized by dilute HCl and recrystallized from acetic acid.

SCHEME 3: **Synthesis of Chalcones QBC, QBHC, QCC, QFC, QFHC and QEHC.**




**R** = Br or Cl or F or OH
**P** = OH
**Q**= F or Br or $C_2H_5O$

**Synthesis of 3-(4-bromophenyl)1-1-(4-((4-((E)-3-(2,4-dichlorophenyl)-3-oxoprop -1-en-1-yl) phenoxy)-6-((4-nitrophenyl) amino)-1,3,5-triazin-2-yl)oxy) phenyl) prop-2-en- 1-one (QBC)**

QBC, Mol. wt. 793.17 and m.p. is206-208$^0$C.**IR** (cm-$^1$), 3030, (C-H)Ar)), 1643 (CO), 1597 (CH=CH–, str.),1550 (N-O ), 811 (C–N, s- triazine), 845 (C-Br) and786 (C-Cl). **$^1$H-NMR** (δ, ppm): 6.8-6.9 (d, 2H, –CO–CH=CH]),7.15–7.8 (m, 19H, Ar–H).**UV**(nm): aromatic (CH=CH) 224 and vinylic (CH=CH) 328. Calculated Elemental analysis for Molecular Formula, $C_{39}H_{35}BrClN_9O_3$ (%): C, 59.06; H, 4.45; Cl,4.47; Found: C, 57.02; H, 4.04; Cl, 3.33.

**Synthesis of 3-(2-bromo-3-hydroxyphenyl)-1-(4-((4-(4-((E)-3-(2,4-dichloro phenyl) 3-oxoprop-1-en-1-yl)phenoxy)-6-((4-nitrophenyl)amino)-1,3,5triazin-2-yl)oxy) phenyl)prop-2-en-1-one (QBHC)**

QBHC, M.wt. 809.11 and m.p. 211-213$^0$C.**IR** (cm-$^1$), 3520 (O-H), 3040, (C-H)Ar)), 1640 (CO), 1590 (CH=CH–, str.),1545 (N-O ), 806 (C–N, s- triazine)770 (C-Cl).. **$^1$H-NMR** (δ, ppm) 6.5-6.7 (d, 4H, –CO–CH=CH]),7.2–8.1 (m, 18H,Ar–H), **UV**(nm) aromatic (CH=CH) 230 and vinylic (CH=CH) 332. Elemental analysis Calculated for the Molecular Formula, $C_{39}H_{35}BrClN_9O_4$ (%): C, 57.89.75; H, 4.36; Cl, 4.38; Found: C, 56.07; H, 4.02; Cl, 3.10.

**Synthesis of 3-(4-chlorophenyl)-1-(4-((4-(4-((E)-3-(2,4-dichlorophenyl) -3-oxoprop-1-en-1-yl)phenoxy)-6-((4-nitrophenyl)amino)-1,3,5triazin-2-yl)oxy)phenyl)prop-2-en-1-one (QCC)**

QCC, M.wt. 748.66 and m.p. 180-181$^0$C.**IR** (cm-$^1$), 3024, (C-H)Ar)), 1643 (CO), 1597 (CH=CH–, str.),1550 (N-O ), 811 (C–N, s- triazine)786 (C-Cl). 1640 (CO), 1591 (CH=CH–, str.),1337 (C–N), 813 (C–N, s- triazine)760 (C-Cl). **$^1$H-NMR** (δ, ppm) 6.6-6.9 (d, 4H, –CO–CH=CH]),7.15–7.80 (m, 19H,Ar–H), **UV**(nm) aromatic (CH=CH) 235 and vinylic (CH=CH) 330. Elemental analysis Calculated for the Molecular Formula, $C_{39}H_{35}Cl_2N_9O_3$ (%): C, 62.57; H, 4.71; Cl, 9.47;. Found: C, 60.08; H, 3.91; Cl, 7.50.

**Synthesis of (2E)-1-(2,4-dichloroyphenyl)-3-(4-((4-(4-(3-(3-ethoxy-4-hydroxyphenyl) acryloyl)phenoxy)-6-((4-nitrophenyl)amino)-1,3,5triazin-2-yl)oxy)phenyl)prop-2-en- 1-one (QEHC)**

QEHC, M.wt. 774.27 and m.p. 215-216$^0$C.**IR** (cm-$^1$), 3030, (C-H)Ar)), 2870 (C-H) Aliphatic) 1643 (CO), 1585 (CH=CH–, str.),1545 (N-O ), 801 (C–N, s- triazine)780 (C-Cl). **$^1$H-NMR** (δ, ppm) 6.8-6.9 (d, 4H, –CO–CH=CH]),7.15–7.80 (m, 18H,Ar–H), **UV**(nm) aromatic (CH=CH) 229 and vinylic (CH=CH) 336. Elemental analysis Calculated for the Molecular Formula, $C_{41}H_{40}ClN_2O_5$ (%): C, 63.60; H, 5.21; Cl, 4.58:, Found: C, 61.20; H, 3.91; Cl, 3.28.

**Synthesis of (2E)-1-(2,4-dichlorophenyl)-3-(4-((4-(4-(3-(4-fluorophenyl)acryloyl) phenoxy)-6-((4-nitrophenyl)amino)-1,3,5triazin-2-yl)oxy)phenyl)prop- 2-en-1- one (QFC)**

QFC, M.wt. 731.25 and m.p. 204$^0$C.**IR** (cm-$^1$), 3040, (C-H)Ar)), 1649 (CO), 1590 (CH=CH–, str.),1552 (N-O ), 1120 (C-F), 811 (C–N, s- triazine)782 (C-Cl). **$^1$H-NMR** (δ, ppm) 6.8-6.9 (d, 4H, –CO–CH=CH]),7.3–7.8.4 (m, 19H,Ar–H), **UV**(nm) aromatic (CH=CH) 232and vinylic (CH=CH) 337. Elemental analysis Calculated for the Molecular Formula, $C_{39}H_{35}FClN_9O_5$ (%): C, 63.97; H, 4.82; Cl, 4.84; Found: C, 61.57; H, 3.56; Cl, 3.77.

**Synthesis of (2E)-1-(2,4-dichlorophenyl)-3-(4-((4-(3-(3-fluro-4- hydroxyl phenyl) acryloyl)phenyl)-6-((4-nitrophenyl)amino)-1,3,5triazin -2-yl)oxy) phenyl)prop-2-en-1-one (QFHC)**

QFHC, M.wt. 748.20 and m.p. 182-183$^0$C.**IR** (cm-$^1$), 3340 (OH), 3037, (C-H)Ar)), 1650 (CO), 1590 (CH=CH–, str.),1545 (N-O ), 818 (C–N, s- triazine)770 (C-Cl). **$^1$H-NMR** (δ, ppm) 6.8-6.9 (d, 4H, –CO–CH=CH]),7.4–8.6 (m, 18H,Ar–H),

**UV**(nm) aromatic (CH=CH) 241 and vinylic (CH=CH) 332. Elemental analysis Calculated for the Molecular Formula, $C_{39}H_{35}ClFN_9O_4$ (%): C, 62.61; H, 4.72; Cl, 4.74; Found: C, 59.78; H, 3.55; Cl, 3.54.

**Antibacterial activity (Bacterial strains)**

Bacteria chosen for antibacterial activities of synthesized compounds were Gram positive; *Staphylococcus aureus* MTCC 29213, Gram negative; *Pseudomonas aeruginosa* MTCC 2488. Everything bacterial strains stayed on the process since Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh-160036, India. Completely bacterial strains are located sub cultured proceeding nutrient agar medium, protected at 37°C for 24 hrs and kept at 4°C in refrigerator to keep stock culture (12).

**Media used**

**Nutrient Agar (NA) (gram/litre)**

| | | |
|---|---|---|
| Peptone | - | 5.0g |
| Yeast extract | - 2.0g | |
| NaCl | - | 5.0g |
| Agar | - | 18.0g |
| Distilled water | - | 1000ml |
| PH | - | 7.0 |

**Nutrient broth (NB) (gram/litre)**

| | | |
|---|---|---|
| Peptone | - | 5.0g |
| Yeast extract | - 2.0g | |
| NaCl | - | 5.0g |
| Distilled water | - | 1000ml |
| PH | - | 7.0 |

**Muller Hilton agar (gram/litre)**

| | | |
|---|---|---|
| Beef Extract Powder | - | 2.0 g |
| Acid Digest of Casein | - | 17.5 g |
| Starch | - | 1.5 g |
| Agar | - | 17.0 g |
| PH | - | 7.0 |

**Muller Hilton broth (gram/litre)**

| | | |
|---|---|---|
| Beef Extract Powder | - | 2.0 g |
| Acid Digest of Casein | - | 17.5 g |
| Starch | - | 1.5 g |
| PH | - | 7.0 |

## Result and Discussion

Novel bioactive chalcones were prepared under bio friendly conditions. Synthesized chalcones were characterized by UV, IR and $^{1}$H NMR to confirm the structure. UV, proton NMR and FT-IR spectrum QBC was depicted in FIG. 1-3 respectively for the representative of the chalcone series. The presence of vinylic ketone in chalcone was confirmed by FT-IR ($cm^{-1}$), the peak appear at 1597 and 1643 confirms the presence of CH=CH and C-O of QBC. In proton NMR (δ, ppm), the peak at 6.8-6.9 and 7.1-7.8 confirms the presence of CH=CH and aromatic hydrogen's of QBC respectively. UV spectrum of QBC reveals the two characteristic peak one at 224 was due the aromatic $\pi$-$\pi^*$ transition and around 328 nm was due to the $\pi$-$\pi^*$ transition of CH=CH. Melting points of the synthesized compounds were measured using open capillary method and were incorrect. The antimicrobial activities of the synthesized compounds were evaluated using minimum inhibitory concentration (MIC) method and the obtained MIC values were presented in the TABLE 1 and 2.

### 3.1. Minimum Inhibitory Concentrations (MICS)

The antibacterial activity of the synthesized chalcone QBC, QCC, QFC, QBHC, QFHC and QEHC were tested by MIC methods. The synthesized chalcones showed excellent activity against tested gram negative bacteria's than gram positive bacteria. Among the synthesized compounds chalcone containing fluorine, chlorine and bromine moiety showed excellent activity against tested gram positive; *Staphylococcus aureus* MTCC 29213, gram negative; *Pseudomonas aeruginosa* MTCC 2488. QFHC showed better activity against tested *Staphylococcus aureus* MTCC 29213 with the MIC values of 0.072 at 125 µg/mL. The obtained antibacterial activities of synthesized chalcones at the concentration of 7.81 µg/mL were presented as the bar graph (Fig. 4). In this concentration QEHC showed good activity against *Pseudomonas aeruginosa (0.229* µg/mL) and QBCHC had the superior activity on *Staphylococcus aureus* the obtained value was *0.228* µg/mL. All the synthesized quinoline based chalcones revealed good activity on both tested bacteria's and the obtained MIC values at various concentrations are given in the TABLE 1 and 2.

### 3.2. Solubility of the synthesized compounds

The solubility data of the synthesized compounds were presented in the TABLE 3. The chalcone QBC, QCC, QFC, QBHC, QFHC and QEHC are almost soluble in all the solvents used for solubility test except water, benzene and n-hexane but freely soluble in methanol, ethanol, dimethylsulfoxide, dimethylformamide and tetrahydrofuran.

TABLE 1. **MIC values of synthesized compounds on *P.aeruginosa*.**

| | Control | 250µl | 125µl | 62.5µl | 31.25µl | 15.62µl | 7.81µl |
|---|---|---|---|---|---|---|---|
| QBC | 0.314 | 0.062 | 0.077 | 0.096 | 0.141 | 0.188 | 0.241 |
| QBHC | 0.314 | 0.046 | 0.068 | 0.081 | 0.14 | 0.166 | 0.234 |
| QCC | 0.314 | 0.057 | 0.063 | 0.088 | 0.139 | 0.186 | 0.256 |
| QFC | 0.314 | 0.59 | 0.082 | 0.101 | 0.166 | 0.21 | 0.286 |
| QFHC | 0.314 | 0.053 | 0.072 | 0.086 | 0.149 | 0.193 | 0.261 |
| QEHC | 0.314 | 0.053 | 0.067 | 0.082 | 0.12 | 0.167 | 0.229 |

TABLE 2**. MIC values of synthesized compounds on *S.aureus.***

| | Control | 250µl | 125µl | 62.5µl | 31.25µl | 15.62µl | 7.81µl |
|---|---|---|---|---|---|---|---|
| QBC | 0.369 | 0.07 | 0.093 | 0.128 | 0.148 | 0.198 | 0.304 |
| QBHC | 0.369 | 0.049 | 0.068 | 0.097 | 0.12 | 0.179 | 0.228 |
| QCC | 0.369 | 0.065 | 0.087 | 0.117 | 0.134 | 0.227 | 0.337 |
| QFC | 0.369 | 0.073 | 0.099 | 0.137 | 0.167 | 0.269 | 0.371 |
| QFHC | 0.369 | 0.058 | 0.072 | 0.129 | 0.158 | 0.25 | 0.342 |
| QEHC | 0.369 | 0.066 | 0.093 | 0.139 | 0.167 | 0.231 | 0.31 |

TABLE 3**. Solubility Data of the novel chalcones at 30°C**

| Chalcones | $H_2O$ | MeOH | EtOH | $CHCl_3$ | DMSO | DMF | Acetone | $C_6H_6$ | THF | n-Hexane |
|---|---|---|---|---|---|---|---|---|---|---|
| QBC | _ | + | + | + | + | + | + | _ | + | _ |
| QBHC | _ | + | + | + | + | + | + | _ | + | _ |
| QCC | _ | + | + | + | + | + | + | _ | + | _ |
| QFC | _ | ± | ± | ± | + | ± | ± | _ | ± | _ |
| QFHC | _ | + | ± | ± | + | + | + | _ | + | _ |
| QEHC | _ | + | ± | ± | + | + | + | _ | + | _ |
| + = Soluble, - = Insoluble and ± = partially soluble. | | | | | | | | | | |

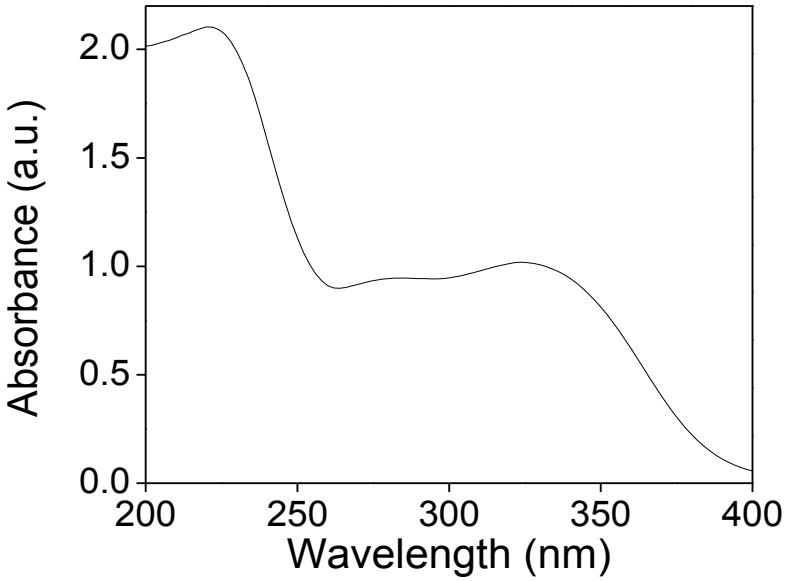



FIG. 1**. UV spectrum of QBC**

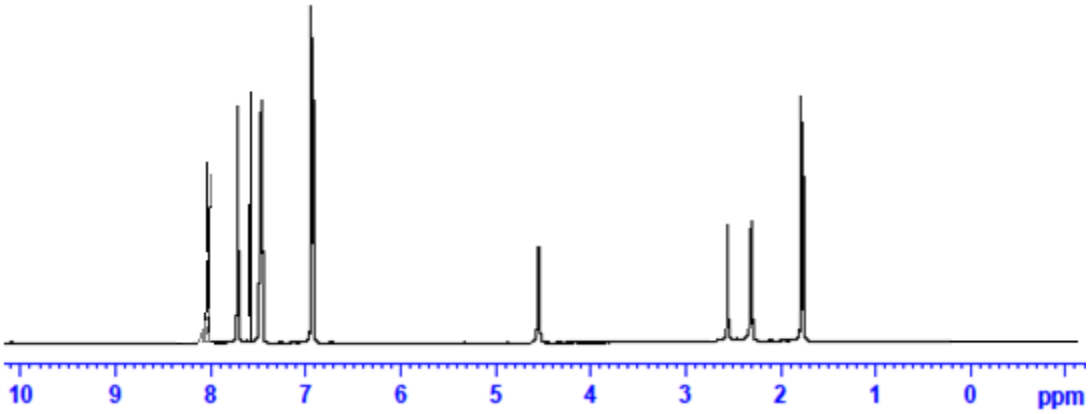



FIG. 2. **$^{1}$H NMR spectrum of QBC**

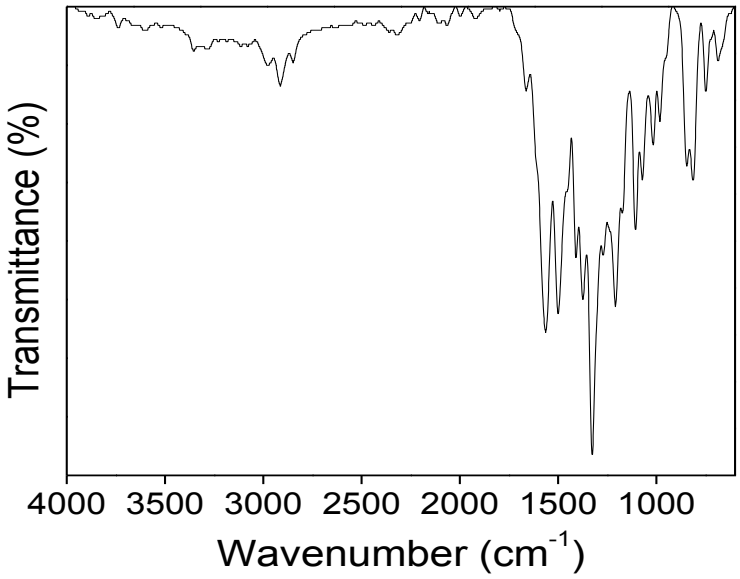



FIG. 3. **FT-IR spectrum of QBC**

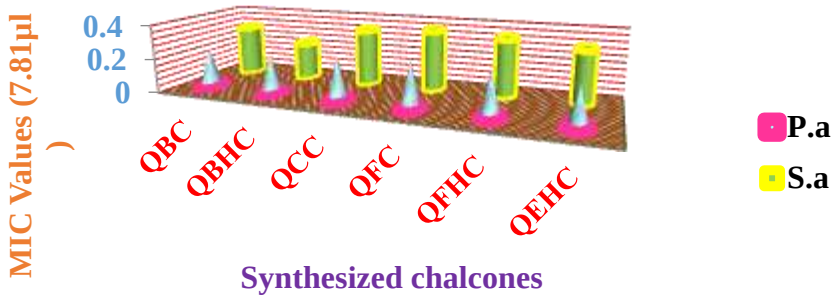



FIG. 4. **MIC value bar graph of QBC, QBHC, QCC, QCHC, QFC, QFHC and QEHC**

## Conclusion

There are six novel chalcones were synthesized using quinoline derivatives of acetophenone and substituted aldehydes. All the synthesized compounds were characterized by FT-IR, NMR and UV spectroscopy techniques. All the synthesized chalcones were completely soluble in polar protic solvents like acetone, alcohol, DMSO, THF, ether but insoluble in water, hexane and benzene. Melting points of all the synthesized compounds were determined in an open capillary and found to be dependent on the molecular weight of the compounds. Antimicrobial activity of chalcone QBC, QCC, QFC, QBHC, QFHC and QEHC were tested on *Staphylococcus aureus* and *Pseudomonas aeruginos* QBHC showed more inhibitory activity at the lowest dilutions (MIC 7.8µg/ml) than other compounds (MIC 7.8 µg/ml) against Gram-negative pathogens such as *Pseudomonas aeruginos* (0.229).Whereas QEHC showed excellent activity on tested gram positive *Staphylococcus aureus* bacteria's with the MIC value of 0.228.

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