Synthetic and Antibacterial Studies of Quinolinylchalcones

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Abstract: A series of quinolinyl chalcones have been prepared by the condensation of N-substituted-3-acetyl-4-hydroxyquinolin-2(1H)-ones with different aromatic aldehydes using conventional heating and ultrasound-assisted methods. The percentage yields are considerably increased in ultrasound-assisted method. The prepared chalcone derivatives were assayed for antibacterial and cytotoxicity and were found to be active.

Key words: N-substituted-3-acetyl-4-hydroxyquinolin-2(1H)-one, chalcone, antibacterial, cytotoxicity, heterocyclic

INTRODUCTION

Quinolines (Meth-Cohn and Narine, 1978; Ali et al., 2001) are an important class of heterocyclic compounds and several of their derivatives have been used as bactericidal (Dube et al., 1998), anti-inflammatory (Chen et al., 2001) and antimalarial (Larsen et al., 1996) etc. Many of 4-hydroxy-1,2-dihydro-2-quinoliones have a wide range of applications in pharmacology, i.e., different quinoliones revealed antitumour (Hasegawa et al., 1990) anti HSV (Afonso et al., 1995), anticonvulsive (Rowly et al., 1993), anti-inflammatory (Ukrainets et al., 1996) and bactericidal, fungicidal and molluscicidal activity (Mohamed et al., 1994). Several 3-substituted quinolin-2(1H)-one bearing electron withdrawing groups have been reported to have pharmacological properties. These structures are present in various glycinine NMDA receptor antagonists (Hilts et al., 1999) and endothelin receptor antagonists (Mederski et al., 1997). A few quinolin-2(1H)-ones have been used as intermediate in the syntheses of HIV-1 reverse transcriptase (Patel et al., 2001) inhibitor, 5-HT1s receptor antagonists (Anzini et al., 1995) and AMPA/Kainate antagonists (Doses et al., 1996). Kappe et al. (1994) and Stadlbauer and Hojas (2004) have synthesized 3-acetyl-4-hydroxyquinolin-2(1H)-one and these compounds have been found to possess anticonvulsant properties (Rowly et al., 1993).

Chalcone derivatives belong to another important class of compounds. A large number of chalcone derivatives are found to exhibit antimalarial, antifungal and antibacterial (Katritzky, 1984) and anticancer properties (Rezig et al., 2006; Ducki et al., 1996). Sayed et al. (1976) and Ibrahim et al. (1996) have reported the synthesis of a few quinolinyl chalcones using secondary amines as catalyst and acetic acid as solvent. The use of ultrasonic bath in organic synthesis is well reported, however, the synthesis of chalcones using ultrasonic bath are scarcely reported. The present research involves the comparative study of the synthesis of quinolinyl chalcones under conventional and ultrasound-assisted methods. Antibacterial and cytotoxic study of the prepared chalcones is also the part of this research.

MATERIALS AND METHODS

N-ethylalanine, N-methylaniline, diphenylamine, diethylmalonate, 2-nitrobenzaldehyde, 4-chlorobenzaldehyde, benzaldehyde and p-anisaldehyde were purchased from Merck Company and were used as such. 1HNMR and 13CNMR spectra were recorded a Bruker ADVANCE 300 (300.13 MHz for 1H and 75.5 MHz for 13C). The NMR spectra were referenced with respect to the non-deuterated residual solvent in the sample. IR spectra were recorded on Perkin Elmer-spectrum. Mass spectra were recorded on a Bruker Esquire 3000+ ion trap with ESI ionization. Elemental analyses were performed using Perkin-Elmer 2400-CMN Analyzer. Melting points were recorded on Gallenkamp melting point apparatus and were uncorrected. Ultrasonic bath LC 30 H was used for ultrasonic irradiation. Aluminum coated TLC plates were purchased from Merck company and were used for monitoring of reaction and purity of compounds. N-Substituted-3-acetyl-4-hydroxyquinolin-2(1H)-ones are prepared by direct fusion of N-substituted anilines and diethyl malonate under Dean Stark trap and are confirmed by comparing the melting points.

General method

Conventional heating method: A mixture of N-substituted-3-acetyl-4-hydroxyquinolin-2(1H)-one (0.01 mol), aromatic
aldehyde (0.01 mol), 1-2 drop of piperidine and 1-butanol (25 mL) was refluxed for about 6 h. The precipitate were filtered under suction and washed with cold 1-butanol; dried and crude product was purified by column chromatography and determined the percentage yield.

**Ultrasound-assisted method:** A mixture of N-substituted-3-acetyl-4-hydroxyquinolin-2(1H)-one (0.01 mol), aromatic aldehyde, (0.01 mol), 1-2 drop of piperidine, 1-butanol (5 mL) and 1 g neutral alumina was stirred well for 5 min and removed the solvent under suction. This semi-dried material was heated and irradiated in ultrasonic bath at 60°C for time given in Table 1. TLC monitored the completion of the reaction. The reaction mixture was extracted with chloroform and dried over anhydrous magnesium sulfate and the crude product was purified by column chromatography and determined percentage yield.

### 4-hydroxy-3-[(E)-3-(4-methoxyphenyl)acyl]oyl-1-methylquinolin-2(1H)-one (2-a):

The title compound (2-a) was prepared by the described methods and purified by column with the mixture of solvents n-hexane: ethyl acetate (3:1) as eluents and mp 168-170°C (Lit. m.p. 172-3°C), IR (KBr, νmax in cm⁻¹) 1612 (C = O), 1532 (C = C), 1H NMR (300 MHz, CDCl₃) δ = 8.53 (d, J = 15.9 Hz, 1 H, H₂b), 8.16 (d, J = 7.5 Hz, 1 H, H₁), 7.87 (d, J = 15.9 Hz, 1 H, H₁), 7.59 (m, 3H, H₃, H₅, H₆), 7.19 (m, 2H, H₄', H₅'), 3.81 (s, 3H, OCH₃), 3.53 (s, 3H, N-CH₃).

**¹³C NMR (75.5 MHz) δ = 193.7 (CO), 176.2, 157.9, 134.5, 131.9, 129.2, 127.9, 126.1, 122.5, 114.2, 55.2, 28.9.**

Mass spectra (ESI) 336 (M+1, 60%) Anal. C₁₅H₁₂NO₃; (335.35): Calcd: C, 71.63; H, 5.11; 14.18; O, 19.08; found: C, 70.98; H, 4.98; N, 4.05%.

### 4-hydroxy-1-methyl-3-[{(E)-3-(4-phenylphenyl)acyl]oyl}quinolin-2(1H)-one (2-b):

The title compound (2-b) was prepared by the reported methods and purified by column with the mixture of solvents n-hexane: ethyl acetate (1:1) as eluent and mp 168-170°C (Lit. m.p. 170-1°C), IR (KBr, νmax in cm⁻¹) 1640 (C = O), 1536 (C = C), 1H NMR (300 MHz, CDCl₃) δ = 8.66 (d, J = 15.9 Hz, 1H, H₂b), 8.19 (d, J = 18 Hz, 1H, H₁), 7.86 (d, J = 15.9 Hz, 1H, H₁), 7.61 (m, 3H, H₃, H₅, H₆), 7.21 (m, 3H, H₄', H₅', H₆'), 7.16 (m, 2H, H₄', H₅'), 3.59 (s, 3H, N-CH₃).

**¹³C NMR (75.5 MHz) δ = 193.1 (CO), 176.0, 145.9, 141.6, 135.2, 134.8, 130.6, 129.1, 128.8, 126.3, 123.5, 123.1, 122.2, 114.2, 29.5.**

Mass spectra (ESI) 306 (M+1, 75%) Anal. C₁₅H₁₁NO₃; (305.33): Calcd: C, 74.74; H, 4.95; N, 4.59; O, 15.72; found: C, 73.9; H, 4.86; N, 4.35%.

### 3-[{(E)-3-(4-chlorophenyl)acyl]oyl}-4-hydroxy-1-methylquinolin-2(1H)-one (2-c):

The title compound (2-c) was prepared by the described methods and purified by the mixture of solvents n-hexane: ethyl acetate (3:1) as eluent and mp 182-184°C, IR (KBr, νmax in cm⁻¹) 1644.7 (C = O), 1536.6 (C = C), 1H NMR (300 MHz, CDCl₃) δ = 8.65 (d, J = 15.6 Hz, 1H, H₂b), 8.27 (d, J = 8.5 Hz, 1H, H₁), 7.8 (br-m, 4H, H₃, H₄, H₅, H₆), 7.5 (m, 4H, protons of chlorophenyl), 3.2 (s, 3H, N-CH₃). Mass spectra (ESI) 340 (M+1, 60%) Anal. C₁₅H₁₁NO₃; (339.77): Calcd: C, 67.16; H, 4.15; N, 14.2; Cl, 10.43; O, 14.13; found: C, 66.9; H, 4.00; N, 3.98%.

### 4-hydroxy-1-methyl-3-[(E)-3-(2-nitrophenyl)acryloyl]quinolin-2(1H)-one (2-d):

The title compound (2-d) was prepared by the reported methods and purified by column with the mixture of solvents n-hexane: ethyl acetate (1:1) as eluent and mp 202-204°C (Lit. m.p. 163°C), IR (KBr, νmax in cm⁻¹) 1632 (C = O), 1529.5 (C = C), 1H NMR (300 MHz, CDCl₃) δ = 8.58 (d, J = 15.6 Hz, 1H, H₂b), 8.24 (d, J = 7.8 Hz, 1H, H₁), 7.94 (d, J = 15 Hz, 1H, H₁), 7.67, (5H, H₃, H₄, H₅, H₆), 7.26 (m, 2H, H₄', H₅'), 6.92 (m, 2H, H₄', H₅'), 3.84 (s, 3H, N-CH₃).

**¹³C NMR (75.5 MHz) δ = 194.13 (CO), 176.2, 161.94, 161.4, 145.7, 140.72, 13.71, 131.0, 128.0, 126.4, 123.7, 121.8, 116.5, 114.3, 114.0, 105.4, 55.4 Mass spectra (ESI).** (351, M+1, 65%) Anal. C₁₅H₁₁NO₃; (350.32):

Calcd: C, 65.14; H, 4.03; N, 8.00; O, 22.84; found: C, 65.23; H, 3.96; N, 8.12%.

### 3-[{(E)-3-(4-chlorophenyl)acyl]oyl}-1-ethyl-4-hydroxyquinolin-2(1H)-one (2-e):

The title compound (2-e) was prepared by the described methods and purified by column with the mixture of solvents n-hexane: ethyl acetate (1:1) as eluent and mp 166-168°C, IR (KBr, νmax in cm⁻¹) 1641.2 (C = O), 1538.1 (C = C), 1H NMR (300 MHz, CDCl₃) δ = 8.57 (d, J = 15.6 Hz, 1H, H₂b), 8.17 (d, J = 8.1 Hz, 1H, H₁), 7.78 (d, J = 15.6 Hz, 1H, H₁), 7.6, (5H, H₃, H₄, H₅, H₆), 7.28 (br-m, 4H, protons of chlorophenyl), 4.2 (q, 2H, N-CH₂), 1.3 (t, 3H, proton of CH₃). **¹³C NMR (75.5 MHz) δ = 194.2 (CO), 176.0, 161.3, 145.2, 140.0, 136.4, 134.9, 133.7, 130.1, 129.1, 126.4, 125.8, 121.9, 116.2, 114.1, 105.6, 37.2, 12.8.**

Mass spectra (ESI) 354 (M+1, 100%), 356 (M+1+2, 33%) Anal. C₁₅H₁₁NO₃; (353.38): Calcd: C, 67.59; H, 4.56; N, 3.95; Cl, 10.02; O, 13.57; found: C, 67.54; H, 4.48; N, 3.92%.
1-ethyl-4-hydroxy-3-{(E)-3-phenylacryloyl}quinaldin-2(1H)-one (2-f): The title compound (2-f) was prepared by the described methods and purified by column with the mixture of solvents n-hexane: ethyl acetate (2:1) as eluent and mp 132-134°C (Litt. m.p. 135-136°C), IR (KBr, ηmax in cm⁻¹) 1615.2 (C = O), 1536.9 (C = C), 1HNMR (300 MHz, CDCl₃) δ = 8.6 (d, J = 15.6 Hz, 1H, H₂), 8.19 (d, J = 9 Hz, 1H, H₁), 7.9 (d, J = 15.6 Hz, 1H, H₃), 7.64 (br, m, 3H, H₁₃, H₁₃, H₁₅), 7.39 (m, 3H, H₇, H₂₄, H₂₅), 7.23 (m, 2H, H₆, H₈), 4.2 (q, 2H, N-CH₂). 1.34 (t, 3H, proton of CH₃)²⁷CNMR (75.5 MHZ) δ = 194.2 (CO), 176.2, 161.2, 144.9, 140.6, 135.2, 134.8, 130.6, 129.6, 129.0, 128.9, 128.6, 126.4, 125.2, 125.0, 121.9, 116.2, 114.0, 105.4, 37.2, 12.8 Mass spectra (ESI) 320 (M+1, 100%) Anal.C₂₅H₂₂N₂O₂: (319.35) Calculated: C, 75.22; H, 5.37; N, 4.39; O, 15.03; found: C, 74.95; H, 5.42; N, 4.23%.

4-hydroxy-3-{(E)-3-(4-methoxyphenyl)acryloyl}-1-phenylquinolin-2(1H)-one (2-g) was prepared by the described methods and purified by column with the mixture of solvents n-hexane: ethyl acetate (3:1) as eluent and mp 262-264°C (Litt. m.p. 260°C), IR (KBr, ηmax in cm⁻¹) 1648 (C = O), 1568 (C = C), 1HNMR (300 MHz, CDCl₃) δ = 8.52 (d, J = 15.6 Hz, 1H, H₂), 8.24 (d, J = 8.1 Hz, 1H, H₁), 7.94 (d, J = 15.6 Hz, 1H, H₃), 7.54 (br, m, 5H, H₄, H₅, H₆, H₂₄, H₂₅), 7.43 (br, m, 3H, H₂₁, H₂₂, H₂₃), 7.19 (m, 4H, protons of 4-methoxyphenyl), 3.8 (s, 3H, protons of OCH₃). Mass spectra (ESI) 398 (M+1, 65%) Anal.C₂₅H₂₂N₂O₄: (412.46) Calculated: C, 75.55; H, 4.82; N, 3.52; O, 16.10; found: C, 76.42; H, 4.68; N, 3.82%.

4-hydroxy-1-phenyl-3-{(E)-3-phenylacryloyl}quinolin-2(1H)-one (2-h): The title compound (2-h) was prepared by the described methods and purified by column with the mixture of solvents n-hexane: ethyl acetate (4:1) as eluent and mp 282-284°C (Litt. m.p. 306-30°C), IR (KBr, ηmax in cm⁻¹) 1660 (C = O), 1536.9 (C = C), 1HNMR (300 MHz, CDCl₃) δ = 8.3 (d, J = 15.6 Hz, 1H, H₂), 8.26 (d, J = 8.1 Hz, 1H, H₁), 7.96 (d, J = 15.6 Hz, 1H, H₃), 7.8-7.4 (br, m, 6H, H₄, H₅, H₂₄, H₂₅, H₂₆, H₂₇), 7.3-7.0 (br, m, 7H, H₂₈, H₂₉, and 5H of phenyl ring). Mass spectra (ESI) 368 (M+1, 100%) Anal.C₂₅H₂₂N₂O₂: (367.4) Calculated: C, 78.46; H, 4.66; N, 3.81; O, 13.06; found: C, 78.34; H, 4.48; N, 3.62%.

3-{(E)-3-(4-chlorophenyl)acryloyl}-4-hydroxy-1-phenylquinolin-2(1H)-one (2-i): The title compound (2-i) was prepared by the described methods and purified by column with the mixture of solvents n-hexane: ethyl acetate (3:1) as eluent and mp 240-242°C, IR (KBr, ηmax in cm⁻¹) 1648 (C = O), 1536.9 (C = C), 1HNMR (300 MHz, CDCl₃) δ = 8.62 (d, J = 15.6 Hz, 1H, H₂), 8.29 (d, J = 1.2 Hz, 1H, H₁), 7.89 (d, J = 15.6 Hz, 1H, H₃), 7.65 (m, 5H, protons of N-phenyl), 7.48 (m, 3H, H₁, H₂), 7.43 (m, 5H, H₈, 4 protons of p-chlorophenyl). Mass spectra (ESI) 402 (M+1, 100%) Anal.C₂₅H₂₀ClN₂O₄: (353.8) Calculated: C, 71.73; H, 4.01; N, 3.49; Cl, 8.82; O, 11.94; found: C, 72.06; H, 3.95; N, 3.32%.

RESULTS AND DISCUSSION

The present study was aimed to prepare the quinoline-based chalcone derivatives by using both conventional and ultrasound-assisted methods. Both the methods are compared with reference to reaction time, temperature and percentage yields. The chalcone derivatives are prepared by the condensation of N-substituted-3-acyetyl-4-hydroxyquinolin-2(1H)-ones with different aromatic aldehydes using piperidine as catalyst in 1-butanol, as there was no reaction in alcoholic sodium/potassium hydroxide (Scheme 1).

By the use of ultrasound-assisted method, reaction time is reduced marvelously from 6.0 h to 70-90 min and product yields are increased approximately two folds. Energetically preparation of quinolinyl chalcone derivatives by using ultrasound assisted method is more feasible than conventional heating (Table 1). Li et al. (2002) have reported similar results in the formation of simple chalcones by using pulverized KOH and KF-Al₂O₃. The product yields of chalcone derivatives probably increased due to decrease in Cannizzaro reaction. The optimum temperature in case of ultrasonic irradiation is found to be 60°C and results obtained are given in Table 1.

The structures of all the synthesized quinolinyl chalcone derivatives have been confirmed by their spectroscopic data such as IR, ¹HNMR, ¹³CNMR, Mass

![Scheme 1](image-url)
Table 2: Antibacterial activity of newly synthesized chalcones

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram positive</th>
<th>Gram negative</th>
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<td></td>
<td><em>Staphylococci</em></td>
<td><em>E. coli</em></td>
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<td>Conc. (mg mL⁻¹)</td>
<td>Conc. (mg mL⁻¹)</td>
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<td></td>
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<td>2-b</td>
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<td>2-I</td>
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<td>Strept.</td>
<td>18 18 20</td>
<td>15 16 18</td>
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</table>

and CHN analyses. It is remarkable to note that in ¹H NMR spectra of all the described compounds, α,β unsaturated enone system protons have appeared as two doublets around δ 8.6 ppm and δ 7.8 ppm for H₆ and H₇ respectively, with coupling constant between 1.5-16 Hz. The coupling constants predict that chalcone derivatives are trans isomers. The other peaks have appeared in the expected region and the numbers of protons are in accordance with the expected protons. Additional support to elucidate the structures is obtained from ³¹CNMR spectra of these compounds. The appearance of peak around δ 194 ppm indicates the α, β unsaturated carbonyl carbon present in the chalcones. M+1 peak in mass spectra (ESI) is the promising peak in most of the described compounds.

Some prepared compounds have long range of difference in their melting points with the literature melting points (Sayed et al., 1976; Ibrahim et al., 1996). The reported compounds have been supported only by CHN analyses. The compounds described in this study have been supported by spectroscopic data as well as CHN analyses.

Antibacterial activity of prepared quinolinyl chalcones:
All the prepared chalcones were screened for their antibacterial activities against the following four organisms viz., *Staphylococci, Bacillus megasestum-1, Salmonella typhi, Escherichia coli* and *proteus vulgaris*. For preliminary screening the antibacterial tests were carried out by using disc diffusion method (Karaman et al., 2003). One hundred microlitres of suspension containing 10⁵ colony forming unit (CFU mL⁻¹) of bacteria were spread on Mueller-Hinton agar (MHA) medium. The discs (6 mm in diameter), impregnated with 10 μL of the test compounds of different concentrations (33, 66 and 100 mg mL⁻¹) were placed on the inoculated discs. Negative controls were prepared by using the same solvent chloroform (CHCl₃), employed to dissolve the test compounds. Streptomycin 10 μL (33, 66 and 100 mg mL⁻¹) was used as positive reference standard to determine the sensitivity of each bacterial species tested. The discs were placed into the medium containing plates incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter (mm) of zone of inhibition around each disc and results were recorded in Table 2.

On the basis of the observed zone inhibition values, it is well understood that all the prepared chalcones have shown antibacterial activity against both the gram-positive and gram-negative bacterial strains. These prepared chalcone derivatives have inhibited the bacterial strains significantly.

Preliminary cytotoxic evaluation of chalcones: All the synthesized quinolinyl chalcone derivatives were evaluated in vitro against three-cell line panel consisting of MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). In this protocol, each cell line was inoculated and pre-incubated on a microtiter plate. Test agents were then added at a single concentration (100 μM) and the culture incubated for 48 h. End-point determinations were made with alamar blue (Gray and Wickstrom, 1996). Results for each test agent were recorded as the percent growth of the treated cells when compared to the untreated control. Compounds, which reduced the growth of any one of the cell lines to 32% or less, were considered to be cytotoxic.

According to the above-described criteria, chalcone derivatives 2-c, 2-e and 2-I are found to possess significantly high cytotoxicity, however, other chalcone derivatives have shown moderate cytotoxicity (Table 3).


Table 3: Cytotoxicity of quinolone chalcones

<table>
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<tr>
<th>Compound</th>
<th>MCF7 (Breast cancer)</th>
<th>NCI-H460 (Lung cancer)</th>
<th>SF-268 (CNS cancer)</th>
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<tr>
<td>2-i</td>
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