Synthesis and Biological Evaluation of some Chalcone Derivatives

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Abstract: The compound Pyrazole derivatives, is prepared from \( p \)-Amino benzoic acid and ethanol. Chalcone has been prepared by the condensation reaction of Ethyl-4-acetamido benzoate and different ten aldehydes. These chalcones are cyclized with hydrazine hydrate and glacial acetic acid under reflux condition give pyrazole derivatives. These compounds have been characterized by detailed spectral analysis and have been screened for their antimicrobial activity against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, and Staphylococcus aureus, Aspergillus Niger and Anti inflammatory activity (by invitro)

Key words: Antimicrobial activity, Anti inflammatory activity, chalcones, pyrazole.

INTRODUCTION

The chemistry of chalcones has generated intensive scientific interest due to their biological and industrial applications. Chalcones are natural biocides and are well known intermediates in the synthesis of heterocyclic compounds exhibiting various biological activities. Chalcones and their derivatives possess some interesting biological properties such as antibacterial, antifungal, insecticidal, anesthetic, anti-inflammatory, analgesic etc. Pyrazole is a class of compounds, which has many applications in different field. One of the methods for the synthesis of such compound is from unsaturated carbonyls (chalcone) by the cyclization with hydrazine and substituted hydrazine. Pyrazole and their derivatives are considered to be important for drugs and agricultural chemicals. Some substituted pyrazoles and their derivatives have been reported to possess several interesting biological activities such as hypnotic properties, antimicrobial, antitumor and antifungal. Many pyrazoles are used for the treatment of thyroid and leukemia. It has incidental antiviral activity against Herpes infections.

MATERIAL METHODS

SYNTHETIC METHOD

STEP 1: Synthesis of Ethyl-4-amino benzoate

To \( p \)-Amino benzoic acid 12 gms (0.088 moles) add 80 mL of 95% v/v ethanol in a round bottom flask and 4 mL of concentrated sulphuric acid add slowly. Refluxed for 2 hour at 60°C on water bath. Then cooled the flask for several minutes. Then added 150 mL of 10 % sodium carbonate solution, which resulted in the evolution of considerable gas until the solution is neutralized. Filtered the solution and collect the precipitate. Recrystallized from rectified spirit and drying in desiccators under vacuum. (% yield 68 % w/w: m.pt: 91°C – 92°C)

STEP 2: Synthesis of Ethyl-4-acetamido benzoate

To 6.6 g of Ethyl-4-amino benzoate (0.045 moles) added 12 mL of acetic anhydride and few mgs of zinc dust. Refluxed for 1 hour. Cooled the content
and filtered. Washed the precipitate with cold water and filter it. Recrystallized from rectified spirit and drying in desiccators. (% yield 64 % w/w: m.pt: 104°C –105°C)

**STEP 3: Synthesis of Chalcones**

To Ethyl-4-acetamido benzoate (2.07 g, 0.01 moles) added aromatic aldehydes (0.01 moles) in ethanol (20 mL) and catalytic quantity of sodium hydroxide. The mixture was stirred for 2 to 3 hours at room temperature using magnetic stirrer. The reaction was monitored by TLC and it was kept at room temperature and then cooled in an ice bath. After filtration, the product was washed with ethanol (5 mL) followed by distilled water, dried and crystallized from ethanol to yield a pure chalcones. (14)

**STEP 4: Synthesis of Chalcone derivatives (pyrazolines) 12**

To the ten different Chalcones (0.01 moles) added glacial acetic acid (10 mL) and hydrazine hydrate 99 % (0.01 mole). Refluxed for 8 hour on water bath at 80°C and cool it. The resulting solid was filtered, washed with distilled water. Recrystallized from ethanol and drying in desiccators.

**Biological Assay 13**

**Bacterial stains**

Strains of *Pseudomonas aeruginosa* (resistant to ceftazidime, cefepime), *Staphylococcus aureus* (resistant to oxacilline), *Aspergillus niger*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, and *Salmonella typhimurium* were used. The bacterial strains were clinical isolates from the SRM School of Biotechnology, Kancheepuram, Tamil Nadu, India. Bacterial strains were maintained on Mueller Hinton Agar. For inoculums preparations, bacteria were sub culture in peptone water, at 37°C for 18 hrs. The total viable count of culture was average 1 x 10^12/mL.

**Assay of Inhibition of bacterial growth**

The antimicrobial activities of compounds C_1 to C_10 were determined by the cup plate method. The tested bacterial suspension was homogeneously seeded onto petri dishes containing 15 mL of the MH agar medium. Holes were aseptically bores into the agar with a hallow punch and 25 μL aliquots of the extract were placed into wells with sterile pipette. The plate was kept for 2 hrs at room temperature for the diffusion of the extract into the agar. Bacterial growth inhibition was determined as the diameter of the inhibition zone around the holes. Ethyl acetate was used as a solvent for dilution of the extracts.

**SCHEME C 1-C10**

Determination of Minimum Inhibitory Concentration (MIC)

The plates were incubated at 37°C for 18 hrs. The MIC was considered the lowest concentration of the samples that prevents visible growth.

In-vitro anti-inflammatory activity

All the newly synthesized compounds were tested for Anti-inflammatory activity by In-Vitro (HRBC) Human Red Blood Cell Membrane Stabilization method. The reaction mixtures (4.5 ml) consisted of 2 ml hypotonic saline solution, phosphate buffer (P10 7.4) and 1 ml test solution in normal saline. 0.5 ml of 10 % rabbit RBC in normal saline was added. For control tests, 1 ml of isotonic solution was used instead of test solution while product control tests lacked RBC. The mixtures were incubated at 56°C for 30 min, cooled under running water and centrifuged and the absorbances of the supernatants were read at 560 nm. Percentage membrane stabilizing activity was calculated as follows,

\[
\text{Percentage stabilization} = \frac{\text{O. D. Of Test} - \text{O.D. of control}}{\text{O. D. of control}} \times 100
\]

The control represents 100 % lysis. The result was compared with STD (100μg/ml) treated samples.

Result and Discussion

All the synthesized compounds were characterized by TLC, Melting point, elemental analysis, IR, Mass, and 1H NMR. Analysis indicated by the symbols of the elements is very close to the theoretical values. The compounds were evaluated for their anti microbial activity by cup-plate method against various Gram positive, Gram negative bacteria and fungal stains. Many of the compounds show comparable activity with that of standard (Ampicillin and Ketonazole). The compounds were also evaluated for their in-vitro Anti-inflammatory activity by HRBC membrane stabilization method. All the compounds have highly significant activity when compared with standard drug Ibuprofen, with percentage of inhibition to the inflammatory response ranging from 62% to 75%.

Compound C1

Ethyl-4-(1-acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-ylamino) enzoate, a white crystalline solid, was purified by crystallization using ethanol; m.pt. 234°C; IR (KBr, cm\textsuperscript{-1}) : 3305.23 (-NH), 3029.14 (=C-H), 1681.70 (Acetyl carbonyl), 1607.76 (α, β - unsaturated double bond), 1514.52 (Aromatic region), 1264.57, 1178.9 (-C-O), 834.79 (Para disubstituted), 867.94 (C-N). \textsuperscript{1}H NMR (CDCl\textsubscript{3}): δ 7.72 – 6.57 (m,9H,Ar-H), δ 4.9 (d,1H,CH), δ 4.29 (d,2H,CH\textsubscript{2}), δ 4.0 (s,1H,NH), δ 2.02 (t,3H,CH\textsubscript{3}), δ 2.0 - 1.8 (d,2H,CH\textsubscript{2}), δ 1.30 (t,3H,CH\textsubscript{3}); MS: Mol.Wt: 351.4, m/e: 351.16 (100.0%), 352.16 (23.1%), 353.17 (2.3%). \textsuperscript{13}C NMR spectrum of compound showed 20 carbon atoms. δ 14.1 (CH\textsubscript{3}), 23.1 (CH\textsubscript{3}), 39.3 (CH\textsubscript{2}), 56.2 (CH), 60.1 (CH\textsubscript{3}), 116.1 (CH), 116.0 (CH), 120.6 (C), 121.4 (CH), 126.1 (CH), 127.6 (CH), 127.0 (CH), 128.4 (CH), 130.0 (CH), 131.1 (CH), 143.2 (C), 148.1 (C), 155.8(C), 166.2 (C), 168.8 (C). Anal.Caled for C\textsubscript{20}H\textsubscript{22}N\textsubscript{2}O\textsubscript{2}: C, 68.36; H, 6.02; N, 11.96; O, 13.66. Found C, 68.31; H, 5.80; N, 11.28; O, 13.42; Conformed the product formed.

Compound C2

Ethyl-4-(1-acetyl-5-(2-hydroxyphenyl) - 4, 5-dihydro-1H-pyrazol-3-ylamino) benzoate, a white crystalline solid, was purified by crystallization using ethanol; m.pt. 221°C; IR (KBr, cm\textsuperscript{-1}): 3305.54 (-NH), 3029.14 (=C-H), 1681.62 (Acetyl carbonyl), 1607.93 (α, β-unsaturated double bond), 1519.82 (Aromatic region), 1264.57, 1178.9 (-C-O), 834.72(Para di substituted), 867.72 (C-N). \textsuperscript{1}H NMR (CDCl\textsubscript{3}): δ 7.72 - 6.57 (m,8H,Ar-H), δ 5.0 (s,1H,OH), δ 4.9 (d,1H,CH), δ 4.29 (m,2H,CH\textsubscript{2}), δ 4.0 (s,1H,NH), δ 2.0 (t,3H,CH\textsubscript{3}), δ 2.0 - 1.8 (d,2H,CH\textsubscript{2}), δ 3.0 (t,3H,CH\textsubscript{3}); MS: Mol.Wt: 351.4, m/e: 351.16 (100.0%), 352.16 (23.1%), 353.17 (2.3%). \textsuperscript{13}C NMR spectrum of compound showed 20 carbon atoms. δ 14.1 (CH\textsubscript{3}), 23.1 (CH\textsubscript{3}), 39.3 (CH\textsubscript{2}), 56.2 (CH), 60.1 (CH\textsubscript{3}), 116.1 (CH), 116.0 (CH), 120.6 (C), 121.4 (CH), 126.1 (CH), 127.6 (CH), 127.0 (CH), 128.4 (CH), 130.0 (CH), 131.1 (CH), 143.2 (C), 148.1 (C), 155.8(C), 166.2 (C), 168.8 (C). Anal.Caled for C\textsubscript{20}H\textsubscript{22}N\textsubscript{2}O\textsubscript{2}: C, 68.36; H, 6.02; N, 11.96; O, 13.66. Found C, 68.31; H, 5.80; N, 11.28; O, 13.42; Conformed the product formed.

Compound C3

Ethyl-4-(1-acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-amino)benzoate, a white crystalline solid, was purified by crystallization using ethanol; m.pt. 230°C; IR (KBr, cm\textsuperscript{-1}): 3305.35 (-NH), 3029.39 (=C-H), 1681.96 (Acetyl carbonyl), 1607.97 (α, β-unsaturated double bond), 1519.87 (Aromatic region), 1264.44, 1178.91 (-C-O), 834.49 (Para di substituted), 867.94 (C-N). \textsuperscript{1}H NMR (CDCl\textsubscript{3}): δ 7.72
- 6.57 (m, 8H, Ar-H), δ 4.9 (s, 1H, CH), δ 4.29 (d, 2H, CH₂), δ 4.0 (m, 1H, NH), δ 2.0 ~ 1.8 (d, 2H, CH₂), δ 1.30 (t, 3H, CH₃). MS: Mol. Wt.: 385.844; m/e: 385.119 (100.0%), 387.116 (32.0%), 386.123 (21.6%), 388.120 (6.9%), 387.126 (2.2%), 386.116 (1.1%). Anal. Caled for C₂₀H₂₃ClN₃O₅: C, 70.62; H, 5.22; N, 11.14. Found C, 70.29; H, 5.01; N, 11.04. Compounds C₄

**Ethyl-4-(1-acetyl-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-ylamino) benzamide**

The compound was synthesized as a white crystalline solid, with a melting point of 178.5°C. The IR spectrum showed strong absorption bands at 3305.88 (-NH), 3029.78 (-CH), 1681.54 (Acetyl carbonyl), 1607.98 (α, β-unsaturated double bond), 1519.68 (Aromatic region), 1264.57, 1178.91 (-C-O), 834.77 (Para substituted). IR spectrum of compound showed 20 carbon atoms. δ 14.1 (CH₃), 24.3 (CH₃), 38.3 (CH₃), 47.6 (CH), 60.9 (CH₂), 116.1 (CH), 116.2 (CH), 120.2 (C), 128.4 (CH), 128.7 (CH), 130.7 (CH), 130.7 (CH), 143.5 (C), 148.8 (C), 155.0 (C), 166.0 (C), 168.2 (C); Conformed the product formed.

**Compound C₅**

Ethyl-4-(1-acetyl-5-(4-hydroxy phenyl)-4,5-dihydro-1H-pyrazol-3-ylamino) benzamide was purified by crystallization using ethanol; m.p: 226°C; IR (KBr, cm⁻¹): 3305.88 (s, -NH), 3029.78 (s, =C-H), 1681.54 (Acetyl carbonyl), 1607.98 (α, β-unsaturated double bond), 1519.68 (Aromatic region), 1264.35, 1178.91 (-C-O), 834.61 (Para substituted), 867.90 (C-N). IR spectrum of compound showed 20 carbon atoms. δ 14.1 (CH₃), 23.4 (CH₃), 38.3 (CH₃), 47.6 (CH), 60.9 (CH₂), 116.1 (CH), 116.2 (CH), 120.2 (C), 128.4 (CH), 128.7 (CH), 130.7 (CH), 130.7 (CH), 143.5 (C), 148.8 (C), 155.0 (C), 166.0 (C), 168.2 (C); Conformed the product formed.
Compounds C₈
Ethyl-4-(1-acetyl-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-3-ylamino) benzene, a white brown crystalline solid, was purified by crystallization using ethanol; m.p.t. 237°C; IR (KBr, cm⁻¹): 3305.55 (-NH), 3029.14 (=C-H), 1681.01 (Acetyl carbonyl), 1607.01 (α, β-unsaturated double bond), 1519.79 (Aromatic region), 1264.57, 1178.91 (-C-O), 834.26 (Para di substituted), 867.53 (C-N). §H NMR (CDCl₃): δ 7.72 – 6.54 (m, 8H, Ar-H), δ 4.9 (s, 1H, CH), δ 4.29 (m, 2H, CH₂), δ 4.0 (d, 1H, NH), δ 2.85 (m, 6H, CH₂), δ 2.02 (t, 2H, CH₂), δ 2.0 - 1.8 (d, 3H, CH₃), δ 1.30 (t, 3H, CH₂). MS: Mol. Wt: 394.47; m/e: 394.20 (100.0%), 395.20 (25.0%), 396.21 (2.8%). Anal. Caled for C₂₉H₂₆N₆O₅: C, 66.13; H, 6.08; N, 11.02; O, 16.74. ¹³C NMR spectrum of compound showed 20 carbon atoms. δ 14.1 (CH₃), 23.4 (CH₃), 39.3 (CH₂), 40.3 (CH₃), 40.3 (CH₃), 56.7 (CH), 60.9 (CH₂), 114.1 (CH), 114.1 (CH), 116.2 (CH), 116.2 (CH), 120.2 (C), 127.9 (CH), 127.9 (CH), 130.7 (CH), 130.7 (CH), 133.1 (C), 147.6 (C), 148.7 (C), 155.1 (C), 166.0 (C), 168.3 (C); Conformed the product formed.

Compounds C₉
Ethyl-4-(1-acetyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-ylamino) benzene, a white brown crystalline solid, was purified by crystallization using ethanol; m.p.t. 237°C; IR (KBr, cm⁻¹): 3305.55 (-NH), 3029.14 (=C-H), 1681.01 (Acetyl carbonyl), 1607.01 (α, β-unsaturated double bond), 1519.79 (Aromatic region), 1264.57, 1178.91 (-C-O), 834.26 (Para di substituted), 867.53 (C-N). §H NMR (CDCl₃): δ 7.72 – 6.54 (m, 8H, Ar-H), δ 4.9 (s, 1H, CH), δ 4.29 (d, 2H, CH₂), δ 4.0 (d, 1H, NH), δ 2.02 (t, 2H, CH₂), δ 2.0 - 1.8 (d, 3H, CH₃), δ 1.30 (t, 3H, CH₂). MS: Mol. Wt: 394.47; m/e: 394.20 (100.0%), 395.20 (25.0%), 396.21 (2.8%). Anal. Caled for C₂₉H₂₆N₆O₅: C, 66.13; H, 6.08; N, 11.02; O, 16.74. ¹³C NMR spectrum of compound showed 20 carbon atoms. δ 14.1 (CH₃), 23.4 (CH₃), 39.3 (CH₂), 40.3 (CH₃), 40.3 (CH₃), 56.7 (CH), 60.9 (CH₂), 114.1 (CH), 114.1 (CH), 116.2 (CH), 116.2 (CH), 120.2 (C), 127.9 (CH), 127.9 (CH), 130.7 (CH), 130.7 (CH), 133.1 (C), 147.6 (C), 148.7 (C), 155.1 (C), 166.0 (C), 168.3 (C); Conformed the product formed.

Compounds C₁₀
Ethyl-4-(1-acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-ylamino) benzene, a white crystalline solid, was purified by crystallization using ethanol; m.p.t. 234°C; IR (KBr, cm⁻¹): 3305.76 (-NH), 3029.01 (=C-H), 1681.76 (Acetyl carbonyl), 1607.97 (α, β-unsaturated double bond), 1519.62 (Aromatic region), 1264.19, 1178.91 (-C-O), 834.25 (Para di substituted), 867.89 (C-N). §H NMR (CDCl₃): δ 7.72 – 6.57 (m, 8H, Ar-H), δ 4.9 (s, 1H, CH), δ 4.29 (d, 2H, CH₂), δ 4.0 (d, 1H, NH), δ 3.73 (m, 3H, CH₃), δ 2.02 (t, 2H, CH₂), δ 2.0 - 1.8 (d, 3H, CH₃), δ 1.30 (t, 3H, CH₂); MS m/z (M⁺) 369.08, m/z (B⁻) 269.16. MS: Mol. Wt: 381.43; m/e: 381.17 (100.0%), 382.17 (24.0%), 383.18 (2.6%), 383.17 (1.1%). Anal. Caled for C₁₀H₁₀O₅N: C, 66.13; H, 6.08; N, 11.02; O, 16.78. Found C, 66.21; H, 6.40; N, 11.20; O, 16.74. ¹³C NMR spectrum of compound showed 20 carbon atoms. δ 14.1 (CH₃), 23.4 (CH₃), 39.3 (CH₂), 55.3 (CH), 56.7 (CH₂), 60.9 (CH₂), 114.1 (CH), 114.1 (CH), 116.2 (CH), 116.2 (CH), 120.2 (C), 128.9 (CH), 128.9 (CH), 130.7 (CH), 130.7 (CH), 135.8 (C), 148.7 (C), 155 (C), 158.1 (C), 166.0 (C), 168.3 (C); Conformed the product formed.

Biological activity
The Compounds C₁ to C₁₀ were tested for antibacterial activity against Pseudomonas aeroginosa, Staphylococcus aureus, Aspergillus niger, Escherichia coli, Proteus vulgaris, Bacillus subtilis, and Salmonella typhimurium. Many of the compounds show comparable activity with that of standard (Ampicillin and Ketoconazole). The result revealed that the growth of bacterial strains against Escherichia coli, Salmonella typhimurium and Proteus vulgaris get affected by compounds C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, and C₁₀ which is seen by inhibition of 5, 7, 6, 5, 6, 5, 7, 7, 8, and 6 mm respectively (Table 1).

The compounds were also evaluated for their Anti-inflammatory activity invitro by HRBC membrane stabilization method. All the compounds have highly significant activity when compared with standard drug Ibuprofen, with percentage of inhibition to the inflammatory response ranging from 64% to 75%.
Table 1: Antimicrobial activity of compounds C_1 to C_{10}

<table>
<thead>
<tr>
<th>Compds</th>
<th>Concentration (mg/mL)</th>
<th>M.I.C.-Zone (mm)</th>
<th>Active Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_1</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>5mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_2</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>7mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_3</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>6mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_4</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>5mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_5</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>6mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_6</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>5mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_7</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>8mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_8</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>7mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_9</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>8mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>C_{10}</td>
<td>2.00, 4.00, 6.00, 8.00 and 10.00</td>
<td>6mm for 10.00 mg/mL</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em>, and <em>Proteus vulgaris</em></td>
</tr>
</tbody>
</table>

The values for zone diameter are given in bracket and are measured excluding the diameter of the disc.

**CONCLUSION**

Chalcones and Pyrazole derivatives were synthesized. Compounds with electron releasing groups such as methoxy and hydroxyl showed better antibacterial activity than other not having such groups. Compounds having pharmacophore, such as chloro group have exhibit more antifungal activity than the other. These results suggest that the chalcone derivatives have excellent scope for further development as commercial antimicrobial agents. Further experiments were needed to elucidate their mechanism of action.

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