SYNTHESIS AND EVALUATION OF PYRAZOLINE DERIVATIVES AS ANTIBACTERIAL AGENTS

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ABSTRACT
Pyrazoline derivatives were found to exhibit broad spectrum of biological activity. Among all the pyrazolines, 2-pyrazoline has gained attraction and reported to possess wide range biological activities including antitumor, antibacterial, antifungal, antiviral, antiparasitic, anti-tubercular, anti-inflammatory, anti-diabetic, anesthetic, analgesic, insecticidal and potent selective activity such as nitric oxide synthase (NOS) inhibitors and cannabinoid CB1 receptor antagonistic activity. Due to its wide range of biological activity, pyrazolines have received a considerable interest in the field of medicinal chemistry and drug discovery.

KEY WORDS
Pyrazoline derivatives, Antiparasitic, anti-tubercular.

INTRODUCTION
The Dihydro derivative of pyrazole is known as pyrazoline. It is having two adjacent nitrogen atoms, one endocyclic bond within the ring and basic in nature. The aromatic nature arises from the four electrons and the unshared pair of electrons on the â€”NH nitrogen. Pyrazolines are play important role in medicinal chemistry and also used as useful synthones in the field of organic, pharmaceutical and medicinal chemistry.

Three of them are possible structures depending on the position of double bond. These are 1-pyrazoline, 2-pyrazoline, 1, 3-pyrazoline out of these structures 1, 3-pyrazoline is most common.
**Scheme:**

Reagent and conditions:
(a) 40% NaOH, EtOH, 6-8h stirring,
(b) NH₃·NH₂H₂O, EtOH, 75°C, 6-8 hrs reflux,
(c) Et₃N, Ethylchloroformate, EtOH, 1-3 hrs reflux,
(d) Hydroxyl amine HCl, KOH, CH₃OH, 70°C, 8-12 h, reflux.

**EXPERIMENTAL:**

**Chemistry:**

**Materials**

Chemicals used in synthetic work were Acetophenone, p-chloroacetophenone, p-methoxy acetophenone, p-nitro-acetophenone, Benzaldehyde, p-Chloro-benzaldehyde, p-methoxy-benzaldehyde, p-nitro-benzaldehyde, hydrazine hydrate (80%), ethyl chloroformate, tri ethyl amine, potassium hydroxide, Hydroyl amine, ethanol, methanol, sodium hydroxide, chloroform, Hexane and ethyl acetate.

Chemicals were purchased from HIMEDIA Laboratories Pvt Ltd, Mumbai. All the solvents used were Analytical grades were obtained from FINAR Chemicals Ltd Ahmedabad.

**Instruments and apparatus**

➢ All the reactions were performed in dried Borosil glass beakers, round bottom flasks, conical flasks.
➢ Pre-coated silica gel plates (MERCK) was used for TLC (Silica gel 60 F₂₅₄.)
➢ Compounds melting points were determined by open capillary method.
➢ JASCO UV Chamber was used for detection of spots in TLC.
➢ IR Spectra were recorded on BRUKER FTIR Spectrophotometer.
H^1^NMR spectra were recorded on BRUKER SPECTROSPIN-400MHz. Spectrometer using DMSO as solvent and TMS as an internal standard. The chemical shift data were expressed as values relative to TMS in ppm.

MS data reports were recorded on GCMS–QP5050 SHIMADZU instrument.

General procedure for the synthesis of chalcones (A1–A6):
To a cold solution of solution of ethanol & sodium hydroxide (40%) was placed in a conical flask provided with a mechanical stirrer. Acetophenone (0.01M) was poured with constant stirring, then benzaldehyde (0.01M) was added drop wise to the solution. The progress of the reaction was monitored by TLC. The reaction mixture was kept at refrigerator overnight. Filter the product & washed with cold water until the washings were neutral to litmus and then with ice cold ethanol. The crude product was recrystallized from ethanol.

The physical properties of prepared chalcones(A1–A6) given in Table 1.

Table: 1. The physical data for chalcone derivatives (A1–A6)

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<thead>
<tr>
<th>Code</th>
<th>R</th>
<th>R1</th>
<th>MF</th>
<th>M.W</th>
<th>% Yield</th>
<th>*Rf</th>
<th>M.P°C</th>
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<td>-H</td>
<td>C_{15}H_{12}ClO</td>
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<td>-Cl</td>
<td>C_{15}H_{10}Cl_2O</td>
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<td>-H</td>
<td>-OCH3</td>
<td>C_{15}H_{11}O_2</td>
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<td>-OCH3</td>
<td>C_{17}H_{16}O_3</td>
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<td>A5</td>
<td>-NO_2</td>
<td>-H</td>
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<td>253</td>
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<td>0.46</td>
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<tr>
<td>A6</td>
<td>-Cl</td>
<td>-NO_2</td>
<td>C_{15}H_{10}ClNO_3</td>
<td>287</td>
<td>78.6</td>
<td>0.58</td>
<td>123-125</td>
</tr>
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</table>

*Solvent system: Hexane: Ethylacetate (2:1)

Synthesis of Pyrazoline derivatives (B1–B6):
To excess quantity of hydrazine hydrate added chalcone derivatives (A1–A6, 0.01mol) and refluxed for 6-8hrs. The progress of the reaction was monitored by TLC. The mixture were poured into crushed ice and the solid mass which separated out was filtered dried and recrystallized from appropriate solvents.

The physical properties of pyrazoline derivatives(B1–B6) given in Table 2.
Synthesis of ethyl 3,5-substituted-diphenyl-4,5-dihydro-pyrazole-1-carboxylate derivatives (C₁-C₆):
The pyrazoline derivatives (B₁-B₆, 0.01mol) were added to ethyl chloroformate (0.02mol), triethylamine (0.02mol) taken in methanol and stirred for 3-6 hours. The progress of the reaction was monitored by TLC. The resulting solid products were filtered dried and recrystallized from appropriate solvents. The products obtained from ethyl chloroformate are named as C₁-C₆.

The physical properties of ethyl 3, 5-substituted-diphenyl-4, 5-dihydro-pyrazole - 1 - carboxylate derivatives (C₁-C₆) given in Table 3.

Table 3. The physical data of ethyl 3, 5-substituted-diphenyl-4, 5-dihydro-pyrazole-1-carboxylate derivatives (C₁-C₆)
**Synthesis of substituted Hydroxyl amine derivatives (D₁-D₃):**

The ethylchloroformate derivatives (0.001 mol) were dissolved in methanol and to that equimolar quantity of Hydroxyl amine (0.001 mol) and potassium hydroxide (0.001 mol) were added and refluxed for overnight. The progress of the reaction was monitored by TLC. After completion of reaction, mixture was evaporated, solid obtained was washed with water to get the product and recrystallized from ethanol.

**4-(4-chlorophenyl)-4,5-dihydro-N-hydroxy-3-phenylpyrazole-1-carboxamide (D₁)**

Molecular formula: C₁₆H₁₄N₃ClO₂

- Molecular weight: 315
- Solubility: Chloroform, C₂H₅OH
- Percentage yield: 54.6
- Melting Point: 175-178°C
- Rf value: 0.42 (Pet ether: Ethylacetate : Chloroform- 2:1:2)

**3,4-bis(4-chlorophenyl)-4,5-dihydro-N-hydroxypyrrole-1-carboxamide (D₂)**

Molecular formula: C₁₆H₁₃N₃ClO₂

- Molecular weight: 350
- Solubility: Chloroform, C₂H₅OH
- Percentage yield: 52.4
- Melting Point: 182-186°C
- Rf value: 0.42 (Hexane: Ethylacetate: Chloroform- 2:1:2)

**3,4-bis(4-methoxyphenyl)-4,5-dihydro-N-hydroxy-3-phenylpyrazol-1-carboxamide (D₃)**

Molecular formula: C₁₇H₁₇N₃O₃

- Molecular weight: 311
- Solubility: Chloroform, C₂H₅OH
- Percentage yield: 60
- Melting Point: 190-194°C

IR spectrum (KBr, cm⁻¹): N-H stretch amide (3397 cm⁻¹), Ar-H stretch (3069 cm⁻¹), (C-H stretch in CH₂ (2346 cm⁻¹), C=O stretch in amides (1746 cm⁻¹), C=N stretch (1561 cm⁻¹), N-O def (1476 cm⁻¹), C-C def parade substituted (802 cm⁻¹).

¹H NMR (DMSO, δ, ppm): 8.4(d,1H,pyri-H), 7.8(d,1H,Ar-H), 7.41(d,1H,Ar-H), 7.42(d,1H,pyri-H), 7.20(d,1H,Ar-H), 7.06(d,1H,Ar-H), 6.50(s,1H,NH), 4.90(t,1H,pyr-H), 3.83(s,3H,CH₃).
The compounds were synthesized as shown in Scheme. The chalcones (A₁-A₆) were prepared through Claisen-Schmidt condensation of substituted acetophenones with substituted benzaldehydes in alcoholic sodium hydroxide by conventional method. Among the synthesized chalcones, A₂ has given high yield (82.8%) and A₄ given low yield (72.3%). The pyrazoline derivatives (B₂-B₆) were synthesized by the reaction of excess hydrazine hydrate (80%) with A₁-A₆ in ethanol by conventional method. Out of synthesized compounds B₂ & B₅ has given high yield (76.0%) and B₆ has given low yield (60.0%). The synthesized Pyrazolines derivatives were treated with ethylchloroformate and the resulted compounds (C₁-C₆) were then reacted with...
Hydroxylamine yielding pyrazoline derivatives (D1-D6). All the newly synthesized final products were characterized based on their physical and spectral data.

**Characterization data**
The purified final compounds were characterized as pyrazoline derivatives on the basis of their spectral data (IR, $^1$H NMR, and Mass).

**IR spectrum** of the respective compound D10 has shown characteristic peak of N-H stretch (3397 cm$^{-1}$), aromatic C-H stretch (3069 cm$^{-1}$), C-O stretch in amide (1746 cm$^{-1}$), C=N stretch (1576 cm$^{-1}$) and N-O bending (1476 cm$^{-1}$).

**$^1$H NMR spectrum** (DMSO, δ ppm) of the respective compound D10 shows a specific pattern of signals. It shows singlet at 6.1 which is corresponds to the one proton of amine group, seven doublets at 8.5 which corresponds to the one proton of pyridine, 8.4, 8.1, 7.9 corresponds to three protons on aromatic ring, 7.4 corresponds to one proton on pyridine ring, 3.94 corresponds at one proton on pyridine ring and 3.7 corresponds to the one proton on pyrazole ring. One triplet at 4.9 corresponds to one proton on pyrazole ring.

Further, presence of molecular ion peak [M+2] at m/e 424 with 40% abundance in mass spectrum confirms the structure of D10.

The peaks obtained in the IR $^1$NMR and Mass spectra confirmed the structure of compound D10 as 3,5-bis(4-nitrophenyl)-N-(pyridin-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide.

**ANTIBACTERIAL ACTIVITY**
All the six derivatives (D1-D6) were screened for their antibacterial activity by following standard protocol against different pathogenic bacterial organisms and compared with the standard (given in Table 4). Results of the study indicated that all compounds exhibited mild to moderate antibacterial activity against the test organisms. The degree of inhibition varied with test compound and test bacterial.

All the compounds showed mild activity against B. subtilis, K. pneumonia and D6, D10 compounds showed moderate activity against S. aureus, D6, D10 compounds showed moderate activity against E. Coli. Among all derivatives (D1-D10), D10 compound is having potent activity against bacteria, the increased potency may be the presence electron withdrawing group (-NO$_2$) on R$_1$, R$_2$ position of the phenyl ring. Apart from nitro groups, the mild electron withdrawing group (-Cl) also influenced and showed significant activity but the phenyl ring substituted with electron donating groups (-OCH$_3$) showed decrease in activity.

**ANTIFUNGAL ACTIVITY**
All the six derivatives (D1-D6) were screened for their antifungal activity by following standard protocol against different pathogenic fungal organisms and compared with the standard (given in Table 5). Results of antifungal study indicated that all compounds exhibited mild to moderate antifungal activity against the test organisms. The degree of inhibition varied with test compound and test fungal.

All the compounds showed mild activity against B. subtilis, K. pneumonia and D9, D10 compounds showed moderate activity against S. aureus, D6, D10 showed moderate activity against E. Coli. Among all derivatives (D1-D6), compound D10 were exhibited potent activity against bacteria, the increased potency may be the presence electron withdrawing group (-NO$_2$) on R$_1$, R$_2$ position of the phenyl ring. Apart from nitro groups, the mild electron withdrawing group (-Cl) also influenced and showed significant activity but the phenyl ring substituted with electron donating groups (-OCH$_3$) showed decrease in activity.

All the compounds mild activity against albicans, Malassezia furfur and D6, D10 showed moderate activity against A. niger. Among all the derivatives (D1-D6), compound D10 were shown potent activity, the increased potency may be the presence electron withdrawing group (-NO$_2$) on R$_1$, R$_2$ position of the phenyl ring. Apart from nitro groups, the mild electron withdrawing group (-Cl) also influenced and showed significant activity but the phenyl ring substituted with electron donating groups (-OCH$_3$) showed decrease in activity.
### Table 4: Antibacterial activity of compounds D₁-D₆ (MIC)

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<tr>
<th>Code</th>
<th>R₁</th>
<th>R₂</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>K. pneumonia</th>
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<tr>
<td></td>
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<td>µg/ml</td>
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<td>µg/ml</td>
<td>µM</td>
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<tr>
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<tr>
<td>D₃</td>
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<td>-H</td>
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<td>0.38</td>
<td>125</td>
<td>0.32</td>
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<tr>
<td>D₄</td>
<td>-OCH₃</td>
<td>-OCH₃</td>
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<td>0.45</td>
<td>150</td>
<td>0.37</td>
</tr>
<tr>
<td>D₅</td>
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<td>0.27</td>
<td>125</td>
<td>0.37</td>
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<tr>
<td>D₇</td>
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<td>-NO₂</td>
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<tr>
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<tr>
<td>Std</td>
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<td>0.03</td>
<td>15</td>
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### Table 4: Antibacterial activity of compounds D₁-D₆ (MIC)

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<td>D₃</td>
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<td>0.45</td>
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