SYNTHESIS OF 1-((1-(5-PHENYL-1,3,4-OXADIAZOL-2-YL)METHYL)-1H-PYRAZOL-3-YL)METHYL)PIPERAZINE

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ABSTRACT
Synthesis of 1-((1-(5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-pyrazol-3-yl)methyl)piperazine were synthesis by condensation of ethyl 2-(3-((piperazin-1-yl)methyl)-1H-pyrazol-1-yl)acetate with hydrazine hydrate and polyposable acid to obtain 2-(3-((piperazin-1-yl)methyl)-1H-pyrazol-1-yl)acetohydrazide in excellent yield. The structure of these newly synthesis compound were characterised by H\textsuperscript{1}-NMR, C\textsuperscript{13}-NMR, Mass and IR elemental analysis.

KEYWORD: Pyrazole, Manich bases, 1,3,4 oxadiazole.

INTRODUCTION
Heterocyclic compounds are acquiring more importance in recent years because of their immense biological and pharmacological potency. Various biologically active synthetic compounds have five membered nitrogen containing heterocyclic ring in their structures. Many compounds bearing pyrazoles and their reduced forms pyrazolines constitute an interesting class of heterocycles due to their synthetic versatility and effective biological activities such as antimicrobial\textsuperscript{[1,2]}, antiviral\textsuperscript{[3]}, anti-inflammatory\textsuperscript{[4,5]}, antidepressant\textsuperscript{[6]}, antitubercular\textsuperscript{[7]}, antiamoebic\textsuperscript{[8]}, analgesic\textsuperscript{[9]} activities. Literature survey reveals several synthetic protocols for the synthesis of these compounds and the presence of this core in any molecule plays a key role in enhancing the activity. On the other hand, coumarins and its derivatives represent one of the important class of heterocyclic compounds possessing a wide range of biological activities. These include antibacterial\textsuperscript{[10]}, antifungal\textsuperscript{[11,12]}, antitumor\textsuperscript{[13,14]}, herbicidal, antiinflammatory\textsuperscript{[15]} activities. Coumarins are oxygen containing heterocycles widely distributed in nature. They are also used as additives in food, perfumes, agrochemicals, pharmaceuticals and in the preparation of insecticides, optical brighteners, dispersed fluorescent and dye lasers. Chalcones are 1,3-diaryl-propan-1-ones are natural or synthetic compounds prepared by claisen-schmidt condensation of aromatic aldehydes with acetophenones in presence of base and alcohol as solvent medium.\textsuperscript{[16,17]} These compounds found application in the synthesis of various heterocyclic compounds. Keeping in view of the above interesting pharmacological features, we hereby report the synthesis and antimicrobial activity of a series of new pyrazoline derivatives.

MATERIALS AND METHODS
All the chemicals were used as received without further purification. Melting points were determined in open capillary tubes in Buchi 530 circulating oil apparatus and are not corrected. Reactions were carried out using household micro oven (power consumption 1200 W, microwave frequency 2450 MHz) and monitored by thin layer chromatography (TLC) on silica gel plates (60 F254) visualizing with ultraviolet light or iodine spray. 1H NMR spectra were determined in DMSO-d\textsubscript{6} solution on JOEL AL300 Spectrometers. Proton chemical shifts are relative to tetramethylsilane as internal standard and expressed in ppm.

<table>
<thead>
<tr>
<th>R</th>
<th>H</th>
<th>H</th>
<th>H</th>
<th>H</th>
<th>H</th>
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</tr>
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<tr>
<td>R'</td>
<td>C\textsubscript{6}H\textsubscript{5}</td>
<td>C\textsubscript{6}H\textsubscript{5}CH\textsubscript{3}</td>
<td>C\textsubscript{6}H\textsubscript{5}C\textsubscript{2}H\textsubscript{5}</td>
<td>C\textsubscript{6}H\textsubscript{5}Cl</td>
<td>C\textsubscript{6}H\textsubscript{5}Br</td>
<td>C\textsubscript{6}H\textsubscript{5}NO\textsubscript{2}</td>
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</tbody>
</table>
Experimental section

Chemicals and reagents used in the current study were of analytical grade. The reactions were monitored by thinlinear chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points were determined using a Mettler Toledo FP62 capillary melting point apparatus (Mettler-Toledo, Greifensee, Switzerland) and were uncorrected. Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Perkin Elmer, Norwalk, CT, USA), using potassium bromide pellets; the frequencies were expressed in cm⁻¹. The ¹H- and ¹³C-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian, Palo Alto, CA, USA), using tetramethylsilane as the internal reference, with chloroform-CDCl₃ as solvent, the chemical shifts were reported in parts per million (ppm) and coupling constants (J) were given in hertz (Hz). Elemental analyses were performed on a LECO 932 CHNS instrument (Leco-932, St. Joseph, MI, USA) and analyses for C, H, and N were within 0.4% of the theoretical values.

General procedure for the synthesis of compounds (3)

Pyrazole (1) (2 mmol, 235 mg) was dissolved in 20 mL of ethanol-water (1:1) solution and formaldehyde 37% (3mmol) and substituted piperazine (2) (2 mmol) were added. The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene: methanol (9:1) and toluene: ethyl acetate (75:25:1). At the end of the reaction, the precipitate was filtered, dried and recrystallized using an appropriate solvent.

Yield: 45%: mp 179.7⁰C. IR (KBr) ν in cm⁻¹: 3130 (N-H), 3095-2756 (C-H). 1H-NMR (CDCl₃): δ 7.45-6.15 (bs, 2H, pyrazole C-H), 3.79 (s, 2H, C-CH₂-N), 3.20 (t, 4H, piperazine H₃, H₅, J = 4.8), 2.68(t, 4H, piperazine H₂, H₆, J = 4.8). Anal. Calc. for C₈H₁₄N₂O: C, 75.81; H, 8.45; N, 23.65%. found: C, 75.81; H, 8.49; N, 23.71%, m/z: 166.5

ethy1 2-(3-((piperazin-1-yl)methyl)-1H-pyrazol-1-yl)acetate (4)

An equimolar mixture of 1-(1H-pyrazol-3-yl)methyl piperazine (3) and chloro ethyl acetate were dissolved in dimethyl formamide solvent and to this reaction mixture anhydrous K₂CO₃ was added and the reaction mixture was stirred at room temperature (35⁰C) for 8 hours and the progress of the reaction was monitored by TLC using cyclohexane and ethylacetate solvent mixture (7:3) as eluent the reaction mixture was kept over night. After completion of the reaction the solvent was evaporated on rota-evaporator. The gummy solid was seperated and it was recrystallised from -2-propanol-petroleum ether (80°c) solvent mixture. The crystalline solid was found to be 1-(1H-pyrazol-3-yl)methyl piperazine. with a yield of 75% and mp 143-145⁰C.

Yield: 55%: mp 185.7⁰C. IR (KBr) ν in cm⁻¹: 3150 (N-H), 3095-2782 (C-H). 1H-NMR (CDCl₃): δ 6.45-7.65 (m, 2H, pyrazole), 3.85 (s, 2H, C-CH₂-N), 3.25 (t, 4H, piperazine H₃, H₅, J = 4.8), 2.70(t, 4H, piperazine H₂, H₆, J = 4.8), 1.29 (t,3H, J=13.2Hz, CH₃ of ethyl group), 4.13 (q, 2H, J=13.2Hz, CH₂ of ethyl group). Anal. Calc. for C₁₅H₂₀N₂O: C, 57.12; H, 7.99; N, 22.21; O, 12.68%. found: C, 57.51; H, 7.85; N, 22.50; O, 12.55%, m/z: 252238.

2-(3-((piperazin-1-yl)methyl)-1H-pyrazol-1-yl)acetohydrazide (5)

A solution of 4 (0.01mol) and hydrize hydhrade (0.015) in ethanol (20ml) was refluxed for 5 hours. The reaction mixture was cooled and poured in to icecold water with stirring. The seperated solid was filtered, washed with water and recrystallised from ethanol.

Yield: 50%: mp 180.7⁰C. IR (KBr) ν in cm⁻¹: 3160 (N-H), 3070-2780 (C-H). 1H-NMR (CDCl₃): δ 6.25-7.50 (m, 2H, pyrazole), 3.80 (s, 2H, C-CH₂-N), 3.25 (t, 4H, piperazine H₃, H₅, J = 4.8), 2.70 (t, 4H, piperazine H₂, H₆, J = 4.8), 4.28 (s, 2H, NH₂), 4.36 (s,2H N-CH₂-C =O), 4.98 (s,1 H, N-NH). Anal. Calc. for C₁₅H₂₀N₃O: C, 50.40; H, 7.61; N, 35.27; O, 6.71%. found: C, 50.75; H, 6.94; N, 34.95O; 6.71%. m/z: 238.36.

1-(1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-pyrazol-3-yl)methyl)piperazine 6(a)

A mixture of 2-(3-((piperazin-1-yl)methyl)-1H-pyrazol-1-yl)acetohydrazide (5) (0.01 mol) and substituted carboxilic acid (0.01 mol) was heated at 100–120°C in presence of excess phosphoric acid (PPA) for 4–5 h. After cooling, the mixture was poured into crushed ice and neutralized with 5% aq. NaHCO₃ solution. The precipitated solid was filtered and purified using column chromatography (petroleum ether: ethyl acetate, 9:1).

Yield: 60%: mp 190.7⁰C. IR (KBr) ν in cm⁻¹: 3150 (N-H), 3050-2750 (C-H). 1H-NMR (CDCl₃): δ 6.25-7.80 (m, 2H, pyrazole), 7.35-7.45(m, 5H, phenyl group), 3.80 (s, 2H, C-CH₂ -N), 3.25 (t, 4H, piperazine H₃, H₅, J = 4.8), 2.70(t, 4H, piperazine H₂, H₆, J = 4.8), Anal. Calc. for C₁₅H₂₀N₃O: C, 62.95%; H, 6.21%; N, 25.91%. found: C, 62.93; H, 6.15; N, 25.50; O: 4.93%. m/z: 373.45

Anti-Bacterial Activity

The anti bacterial activity of synthesized compounds was studied by the disc diffusion method against the following pathogenic organisms. The gram-positive bacteria screened were staphylococcus aureus NCCLS 2079. The gram negative bacteria screened were Escherichia coli NCCLS 2065 and pseudomonas aeruginosa NCCLS 2200.

The synthesized compounds were used at the concentration of 250 µg/ml and 500µg/ml using DMSO as a solvent the Cefaclor 10µg/ml disc was used as a standard. (Himedia, Laboratories Ltd, Mumbai). The test
results presented in the table -1. suggest that 6d,6e,6f exhibit high activity against the tested bacteria, the rest of the compounds were found to be moderate active against the tested microorganisms.

**Table 1: Antibacterial activity by disc diffusion method of pyrazole linked 1,3,4 oxadiazole 6(a-f).**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Staphylococcus aureus</th>
<th>Bacillus cereus</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
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<tbody>
<tr>
<td>6a</td>
<td>16</td>
<td>18</td>
<td>13</td>
<td>12</td>
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<td>6b</td>
<td>14</td>
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<td>Cefaclor</td>
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</tbody>
</table>

**Table 2: Antifungal activity by disc diffusion method for pyrazole linked 1,3,4 oxadiazole 6(a-f).**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aspergillus niger</th>
<th>Candida albicans</th>
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<tbody>
<tr>
<td>6a</td>
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</tr>
<tr>
<td>6f</td>
<td>15</td>
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</tr>
<tr>
<td>Clotrimazole</td>
<td>25-30</td>
<td>25-30</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

1. Further more the substitution of mannich bases showed better activities.
2. pyrazole linked mannich bases showed better antibacterial activity.
3. 1,3,4 oxadiazole and its derivatives were found to play an important role in medicinal chemistry as herbicidal, fungicidal, bacterial, anti-inflammatory.

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**REFERENCES**

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