A ONE POT SYNTHESIS OF PYRANO(C)CHROMENES AS ANTIMICROBIAL AGENTS.

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ABSTRACT
The 4H-pyran nucleus is a fertile source of biologically important molecule possessing a wide spectrum of pharmacological activities. The advantage of one pot multicomponent reactions are synthetic efficiency, high atom-economy, structural diversity, operational simplicity and to avoid waste product formed in multi-step reaction. The synthesis of dihydropyrano[c]chromene derivatives have been undertaken by the condensation of 4-hydroxycoumarin and 4-hydroxy-8-isopropyl-5-methylcoumarin with malononitrile and aromaticaldehyde using diammonium hydrogen phosphate as a catalyst and alcohol as a solvent. The products obtained are to be characterized using spectral techniques like IR, NMR and mass spectrometry. The synthesised products are to be tested for antimicrobial activity against Gram-positive and Gram-negative bacterial strains, antifungal activity.

KEYWORDS: Lornoxicam Multicomponent reaction, dihydropyrano[c]chromene, diammonium hydrogen phosphate[DAHP], Antibacterial, Antifungal.

INTRODUCTION
The advantage of one pot multicomponent reactions are synthetic efficiency, high atom-economy, structural diversity, operational simplicity and to avoid waste product formed in multi-step reaction.

Dihydropyrano[c]chromene and their derivatives are of considerable interest as they possess wide range of biological activities such as anticoagulant, antimicrobial, antitumor, antibacterial and antidepressant. Looking to wide variety of pharmacological significance of compounds inspired us to continue working on the synthesis of new dihydropyrano[c]chromenes derivatives.

3,4-Dihydro(c)chromenes were prepared by condensation of 4-hydroxycoumarin with malononitrile and mono substituted aromaticaldehyde in the presence of catalyst. Varieties of catalysts are used by different workers i.e., TEBA, piperidine, aqueous potassiumcarbonate, diammonium hydrogen phosphate (DAHP), Hexamethlenetetraamine.
We have prepared some novel pyrano(c)chromenes derivatives by condensation of 4-hydroxycoumarin or 4-hydroxy-8-isopropyl-5-methylcoumarin (0.01 M) with malononitrile and aromaticaldehyde using diammonium hydrogen phosphate (DAHP) as a catalyst, and alcohol as a solvent.

Synthesis of 4-Hydroxycoumarin and 4-hydroxy-8-isopropyl-5-methylcoumarin

4-Hydroxycoumarin and 4-hydroxy-8-isopropyl-5-methylcoumarin was prepared by the condensation of phenol/thymol(1 mol) with malonic acid(1 mol) in the presence of phosphorous oxychloride (80 ml) and zinc chloride(60 g). The mixture was heated at 60°C for 12-15 hr. Product was isolated and crystallized.

MATERIALS AND METHODS

Materials: The chemicals and reagents used in the project work were of AR and LR grade, procured from Purvi chemicals, Ahmedabad and they are used as they obtained.

Analytical Techniques:

Melting points were determined in open capillary tubes and are uncorrected. Compounds were checked for their purity by TLC on silica gel GF-254 and the spots resolved were visualized under ultraviolet light.

Instruments:
The IR spectra of synthesized compounds were recorded on FTIR DRS 8400, Shimadzu. Mass spectra were recorded on ITMS - c ESI Full ms and ESI method. $^1$H NMR spectra were obtained in DMSOd6 on BRUKER 400MHz instrument.

**Synthesis of 2-amino-4-(aryl)-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile.**

4-Hydroxycoumarin or 4-hydroxy-8-isopropyl-5-methylcoumarin (0.01mol) was stirred for four hrs with aromatic aldehyde(0.01 mol) and malononitrile (0.012mol) at room temperature using di-ammoniumhydrogen phosphate as a catalyst and ethanol and water as a solvent. The product was isolated by pouring in crushed ice. The product was treated with diluted sodium carbonate to remove unreacted 8-Hydroxyquinoline. The product was crystallized using methanol to remove undesirable product. The residue was crystallized with 1,4-dioxane.

![Scheme for the synthesis of 2-amino-4-(aryl)-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile](image)

**Scheme for the synthesis of 2-amino-4-(aryl)-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile**

![Scheme for the synthesis of 2-amino-4-(aryl)-7-isopropyl-10-methyl-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile](image)
TABLE (1) 2-amino-4-(aryl)-5-oxo-4H,5H-pyrano-[3,2-c]chromene-3-carbonitrile.

<table>
<thead>
<tr>
<th>Sr no</th>
<th>Ar</th>
<th>Molecular formula</th>
<th>Molecular weight (gm/mol)</th>
<th>Melting Point °C</th>
<th>% Yield</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-C₄H₃S</td>
<td>C₁₇H₁₀N₂O₅S</td>
<td>322.3</td>
<td>226-228 °C</td>
<td>87</td>
<td>0.60</td>
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<tr>
<td>2</td>
<td>-3,4-(OCH₃)₂C₆H₃</td>
<td>C₂₁H₁₉N₂O₅</td>
<td>376.4</td>
<td>228-230 °C</td>
<td>83</td>
<td>0.59</td>
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<tr>
<td>3</td>
<td>-2,3,4-(OCH₃)₂C₆H₂</td>
<td>C₂₂H₁₈N₂O₆</td>
<td>406.4</td>
<td>236-238 °C</td>
<td>85</td>
<td>0.67</td>
</tr>
</tbody>
</table>

TABLE (2) 2-amino-4-(aryl)-7-isopropyl-10-methyl-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile

<table>
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<tr>
<th>Sr no</th>
<th>Ar</th>
<th>Molecular formula</th>
<th>Molecular weight (gm/mol)</th>
<th>Melting Point °C</th>
<th>% Yield</th>
<th>Rf value</th>
</tr>
</thead>
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<td>1</td>
<td>-C₆H₅</td>
<td>C₂₃H₂₀N₂O₃</td>
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<tr>
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<td>-4-Cl C₆H₄</td>
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<td>240-242 °C</td>
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<tr>
<td>3</td>
<td>-4-FC₆H₄</td>
<td>C₂₃H₁₉FN₂O₃</td>
<td>390.1</td>
<td>228-230 °C</td>
<td>91</td>
<td>0.47</td>
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<tr>
<td>4</td>
<td>-4-OCH₃C₆H₄</td>
<td>C₂₄H₂₂N₂O₄</td>
<td>402.4</td>
<td>255-257 °C</td>
<td>87</td>
<td>0.57</td>
</tr>
<tr>
<td>5</td>
<td>-4-OH-3-OCH₃C₆H₃</td>
<td>C₂₄H₂₂N₂O₅</td>
<td>418.1</td>
<td>221-223 °C</td>
<td>93</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Spectral data of pyrano(c)chromenes compounds

M1 2-amino-4-(2-thienyl)-5-oxo-4H,5H-pyrano-[3,2-c]chromene-3-carbonitrile
IR(v, cm⁻¹): 702.09(C-S str.), 1049.28(C-O-C assym.), 1218(C-O-C sym.), 1705.07(C=O), 2198.85 (C=N), 3310, 3363.85 (N-H), ¹H NMR(δ, ppm): 7.8920-7.8720(d, 1H, ArH), 7.750-7.6820(t, 1H, ArH), 7.5713(s, 2H, NH₂) 7.5174-7.4485(dd, 2H, ArH), 7.4143-7.4016(dd, 2H, ArH), 7.045-7.0164(d, 1H, ArH), 6.9809-6.9595(d, 1H, ArH), 4.8298(s, 1H, =CH).

M2 2-amino-4-(3,4-dimethoxyphenyl)-5-oxo-4H,5H-pyrano-[3,2-c]chromene-3-carbonitrile
IR(v, cm⁻¹): 702.09(C-S str.), 1029.13(C-O-C assym.), 1218(C-O-C sym.), 1705.07(C=O), 2210.28 (C=N), 3310, 3363.85 (N-H); Mass (m/z) 376.7 (m).
M3 2-amino-4-(2,3,4-trimethoxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile

IR (υ, cm⁻¹): 1029.13 (C=O asym.), 1218 (C=O sym.), 1702 (C=O), 2198.85 (C≡N), 3302 (N-H), 2947 (C-H str. asym.), 2839 (C-H str. sym.); Mass (m/z) 406.8 (m⁺+1); ¹H NMR (δ, ppm): 7.85 (d, 1H, ArH), 7.62 (d, 1H, ArH), 7.38-7.29 (d, 2H, ArH), 6.94 (d, 1H, ArH), 6.62 (d, 1H, ArH), 4.82 (s, 2H, NH₂), 4.77 (s, 1H, =CH), 3.88-3.73 (s, 9H, CH₃).

M4 2-amino-7-isopropyl-4-(phenyl)-10-methyl-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile

IR (υ, cm⁻¹): 1026 (C=O asym.), 1238 (C=O sym.), 1705.07 (C=O), 2198.85 (C≡N), 3317.56, 3394.72 (N-H), 2970 (C-H str. asym.), 2839.22 (C-H str. sym.).

M5 2-amino-4-(4-chlorophenyl)-7-isopropyl-10-methyl-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile

IR (υ, cm⁻¹): 779.24 (C-Cl), 1018.41 (C=O asym.), 1228.45 (C=O sym.), 1705.07 (C=O), 2198.85 (C-N), 3325.28, 3410.15 (N-H), 2910 (C-H str. asym.), 2870 (C-H str. sym.).

M6 2-amino-7-isopropyl-4-(4-methoxyphenyl)-10-methyl-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile

IR (υ, cm⁻¹): 1026.13 (C=O asym.), 1258.59 (C=O sym.), 1718 (C=O), 2198.28 (C-N), 3394.14 (N-H), 3533.59 (O-H str.), 2980 (C-H str. asym.), 2839.22 (C-H str. sym.).

M7 2-amino-4-(4-hydroxy-3-methoxyphenyl)-7-isopropyl-10-methyl-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile

IR (υ, cm⁻¹): 1026.13 (C=O asym.), 1265.3 (C=O sym.), 1718 (C=O), 2206.57 (C-N), 3317.56, 3394.14 (N-H), 3533.59 (O-H str.), 2980 (C-H str. asym.), 2839.22 (C-H str. sym.); Mass (m/z) = 418.95 (M⁺+1); ¹H NMR (δ, ppm): 8.96 (s, 1H, -OH), 7.46 (d, 1H, ArH), 7.21-7.18 (d, 2H, ArH), 6.845-6.841 (d, 1H, ArH), 6.72-6.70 (d, 1H, ArH), 6.63-6.60 (d, 1H, ArH), 4.36 (s, 1H, =CH), 3.74 (s, 3H, -OCH₃), 3.38-3.31 (m, 1H, -CHO of isopropyl), 2.50 (s, 3H, -CH₃ of methyl), 1.22-1.17 (dd, 6H, -CH₃ of isopropyl).

M8 2-amino-4-(4-fluorophenyl)-7-isopropyl-10-methyl-5-oxo-4H,5H-pyrano[3,2-c]chromene-3-carbonitrile

IR (υ, cm⁻¹): 779.24 (C-Cl), 1018.41 (C=O asym.), 1228.45 (C=O sym.), 1705.07 (C=O), 2198.85 (C-N), 3325.28, 3410.15 (N-H), 2910 (C-H str. asym.), 2870 (C-H str. sym.).
7.174(d,1H,-ArH),7.14-7.16(d,1H,-ArH),7.12(d,1H,-ArH),4.48(s,1H,=CH),3.32-3.35(m,1H,-CH of isopropyl) ,2.50(s,3H,-CH$_3$ of methyl),1.215-1.168(dd,6H,-CH$_3$ of isopropyl).

Pharmacological study:

It was carried out by cup-plate method using gram positive bacteria *S.aureus* ATCC 25923, gram negative bacteria *Escherichia coli* ATCC 25922 and fungal culture *Aspergillus niger* ATCC. Starndard drug used are, ciprofloxacin, streptomycin and trimethoprime(for bacterial activity) and amphotericin B(for fungal activity) at concentrate 100 µg and 200 µg.

<table>
<thead>
<tr>
<th>Organism</th>
<th>S. aureus (ATCC 25923) Zone of Inhibition in (mm)</th>
<th>E. coli (ATCC 25922) Zone of Inhibition in (mm)</th>
<th>Aspergillus niger (ATCC 16404) Zone of Inhibition in (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration Compounds</td>
<td>100 µg  200 µg</td>
<td>100 µg  200 µg</td>
<td>100 µg  200 µg</td>
</tr>
<tr>
<td>M1</td>
<td>6        6</td>
<td>9        12</td>
<td>6        6</td>
</tr>
<tr>
<td>M2</td>
<td>6        6</td>
<td>7        11</td>
<td>6        6</td>
</tr>
<tr>
<td>M3</td>
<td>6        6</td>
<td>10       12</td>
<td>6        6</td>
</tr>
<tr>
<td>M4</td>
<td>6        6</td>
<td>7        10</td>
<td>6        6</td>
</tr>
<tr>
<td>M5</td>
<td>6        6</td>
<td>12       14</td>
<td>6        6</td>
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<tr>
<td>M6</td>
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<tr>
<td>M8</td>
<td>6        6</td>
<td>13       16</td>
<td>6        6</td>
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<tr>
<td>Dimethylsulphoxide(DMSO) as a solvent does not show zone of inhibition</td>
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</table>

TABLE Zone of inhibition against different strains of bacteria and fungi

DISCUSSION

This presented research work was carried out to synthesize, purify, characterize the Pyrano(c)chromenes derivatives and all these synthesized compounds were evaluated for their antibacterial and antifungal activity. The all synthesized compound, most of them showed the significant to good antibacterial activity against Gram –ve bacteria (*E.coli*) as compared to trimethoprim as standard drug.
REFERENCES


