CHAPTER 3

STUDIES ON PYRAZOLINES
Introduction:

Like the discovery of so many interesting occurrences in the world of science, the discovery of pyrazoline was accidental. In the course of their study of the behavior of phenyl hydrazine towards aldehyde and ketones, Fischer and Knoevenagel in 1877 attempted to condense acrolein with phenyl hydrazine, and they obtained 1-phenyl pyrazoline instead of expected hydrazone.

Pyrazolines are 1, 2-diazacyclopent-2-one. They may be regarded as being formally derived from five member ring system of pyrrole by replacement of the 2- or 5- annular carbon by a nitrogen atom and saturation of the 4:5 double bond.

![Pyrazoline structures](image)

Saturation of the 4:5 double bond of pyrazole destroys the virtual equivalence of the 3- and 5- positions characteristic of N-unsubstituted pyrazoles and also destroys the pyrazole aromaticity.

Because of the close structural relationship, it has been a practice to treat pyrazolines as a chemical sub-unit of the pyrazoles. As dihydropyrazoles, the development of pyrazoline chemistry has closely paralleled that of the pyrazoles. Examples of each of the three tautomeric pyrazoline structures are well known, the 2-pyrazolines being by far most common.

![Pyrazoline structures](image)

General Heterocyclic nomenclature is applied to 2-pyrazolines requires that the two nitrogen atoms as numbered 1 and 2 beginning with the amino nitrogen.

According to the present Chemical Abstract Nomenclature 2-pyrazolines are referred to as 4, 5-dihydro (1H) pyrazoles.
Therapeutic approach shown by Pyrazoline:

Chimenti F. et al. [1] have synthesized a series of N-substituted 3, 5-diphenyl pyrazoline and evaluated for their antibacterial activity against Helicobacter pylori. Among them, compounds (I) showed the best activity against H.pylori metronidazole resistant strain in the 1-4 µg/ml MIC range.

(I) Where, R= alkyl, halo

Liu Xin-Hua and co workers [2] have synthesized series of pyrazoline derivatives and evaluated antibacterial activities. Among all the compound, (II) and (III) have shown strong activity against S. aureus and E. coli. Nassar Ibrahim F. et al. [3] synthesized new C-furyl glycosides pyrazoline and evaluated antibacterial activity against different bacterial species.

Munawar A. et al. [4] have synthesized quinoline-based 2-pyrazoline (IV) and evaluated their anti bacterial activity against gram positive and gram negative...
bacterial strains and mentioned that compounds with a chloro group as a substituent have shown excellent activity.

Where, R = Phenyl (IV)

Bajia Birbal et al. [5] have synthesized 3-(2’-Hydroxyphenyl)-5-(substituted aryl) -2-pyrazoline-N1-caboxaldehyde (V) via irradiated under microwave and evaluated their anti bacterial activity.

(V)

Rao Sudheendra et al. [6] have synthesized a series of new pyrazoline derivatives (VI) and screened for their antifungal activity. Holla Bantwal Shivarama et al. [7] have synthesized thioanisol containing pyrazolines (VII) and tested for their antifungal activity.
Graziia Momolo et al. [8] have synthesized 5-aryl-1-isonicotinoyl-3-(pyridine-2-yl)-4, 5-dihydro-1H-pyrazole ((VIII), (IX)) derivatives and reported as antituberculosis agent.

![Structure of (VIII)](image)

![Structure of (IX)](image)

Where, \( R_1, R_2, R_3 = \text{OH, OMe, Cl} \)

Ozdemir Ahmet et al. [9] have synthesized 1-[(N, N-di substituted thiocarbamoyl thio) acetyl]-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives and evaluated for \textit{in vitro} antimycobacterial activity against \textit{Mycobacterium tuberculosis} \( H_{37}Rv \).

Shekarchi M. et al. [10] have synthesized a series of 3-aryl-5-(pyridin-3-yl)-1-thiocarbamoyl-2-pyrazoline derivatives and evaluated for antituberculosis activity against \textit{Mycobacterium tuberculosis} \( H_{37}Rv \). The preliminary results showed that compound (X) and (XI) have 87%, 93% inhibitory effect respectively.

![Structure of (X)](image)

![Structure of (XI)](image)

Bhat Abdul R. et al. [11] have synthesized thiocarbamoyl bis-pyrazoline derivatives and screened for antiamoebic activity against \textit{HM1:1MSS} strain of \textit{Entamoeba histolytica}. Compound (XII) and (XIII) were highly active.
Mohammad Abid et al. [12] have synthesized pyrazoline containing N-4 substituted thiosemicarbazide derivatives and evaluated their *in vitro* antiamoebic activity against *HM1:1MSS* strain of *Entamoeba histolytica* and compared with the standard drug metronidazole. It was concluded that 3-chloro (XIV) and 3-bromo (XV) substituent on the phenyl ring at position 3 of the pyrazoline ring enhanced the antiamoebic activity. Compounds showed less IC$_{50}$ value than metronidazole.

Uhledorf and co-workers [14] have studied 3-N-aryl amino-1-(4’, 5’, 6’, 7’-tetrahydro-2’-benzothiazolyl)-2-pyrazolines (XVII) and evaluated their antiinflammatory activity.

\[ \text{(XVII)} \]

Nimavat K. S., Popat K. H. et al. [15] have synthesized 1-substituted 3-aryl-5-(3’-bromo phenyl) pyrazolines and compound (XVIII) was undertaken for the primary anticancer screening against batteries of cell line derived from human solid tumors of the cell NCL-H 460 (Lung), MCF-7 (Breast), and SF-268 (CNS).

\[ \text{(XVIII)} \]

Dmytro Havrylyuk and co-workers [16] have synthesized thiazolone based pyrazoline and evaluated their \textit{in vitro} anticancer activity. Most of them displayed anticancer activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancer cell lines. Among them the most efficient anticancer compound (XIX) have been found to be active with selective influence on colon cancer cell lines, especially on HT29.

\[ \text{(XIX)} \]
N-acetylated and non-acetylated 3, 4, 5-trimethoxy or 2, 5-dimethoxy pyrazolines have synthesized by Johnson Marlie and co-workers [17]. A non-acetylated derivative (XX) have the most active compound in the series, with IC$_{50}$ values of 2.1 and 0.5 µM in B16 and L1210 cell lines respectively. In contrast, a similar compound with an N-acetyl pyrazoline ring showed poor activity in the cell lines studies.

![Chemical Structure of XX](image)

Omar Mahmoud T. et al. [18] have synthesized pyrazoline 9H-thioxanthon-9-ones containing N-acetyl pyrazoline (XXI) and evaluated their antitumor activity activity against leukemia in mice.

![Chemical Structure of XXI](image)

Siddiqui A. A. et al. [19] have synthesized substituted phenoxy acetic acid containing pyrazolines and tested for their in vitro cytotoxicity. Among them compound (XXII) have shown strong cytotoxic activity in human embryonic lung (HEL) cells.

![Chemical Structure of XXII](image)

Yar M. Shahar et al. [20] have synthesized substituted phenoxy acetic acid containing pyrazolines and tested for their in vitro cytotoxicity and antiviral activity.
Among them compound (XXIII) showed most cytotoxic activity with a minimum cytotoxic concentration.

\[
\text{\text{(XXIII)}}
\]

Ahasan Nusrat Binta et al. [21] have synthesized derivatives of 3-methyl-1-phenyl-2-pyrazoline-5-one and among them the compound (XXIV) was highly active.

\[
\text{\text{(XXIV)}}
\]

Palska E. et al. [22] have prepared series of 3,5 diphenyl 2- pyrazolines (XXV) and evaluated their antidepressant activity.

\[
\text{\text{(XXV)}}
\]

Where, \( R_1 = H, \text{Cl,Br, Me, OMe} \quad R_2 = H,\text{Cl.} \)

[1, 10B-dihydro-2-(amino carbonyl-phenyl)-5H-pyrazolo[1, 5-C][1, 3]benzoxazine-5-yl]phenyl methenone derivatives were patented [23] Some of the compounds (XXVI) and (XXVII) are useful for preventing or treating AIDS.
Yar M. S. et al. [24] have synthesized a new series of N’-nicotinoyl-3-(4'-hydroxy-3'-methylphenyl)-5-(substitutedphenyl)-2-pyrazolines (XXVIII). The synthesized compounds were tested for their anti-HIV activity.

Tengli Anand Kumar et al. [25] have synthesized N-phenyl and acetyl pyrazoline (XXIX) by microwave method and studied their antioxidant activity by DPPH method.

Osama I. El-Sabbagh et al. [26] have synthesized new N-acetyl and N-thiocarbamoyl-derivatives of 4, 5-dihydropyrazole and evaluated their antiviral activity against a broad panel of viruses in different cell cultures. Among them N-acetyl 4, 5-dihydropyrazole have the only active one at subtoxic concentrations against vaccinia virus (Lederle strain) in HEL cell cultures.
Ali, Mohamed Ashraf. et al. [27] have synthesized N-1-nicotinoyl-3- (4-hydroxy-3-Me phenyl)-5-(substituted phenyl)-2-pyrazolines and tested for their \textit{in vitro} anti-viral activity. Among the synthesized compounds, (XXX) with halogeno group have shown the most promising antiviral activity.

\[
\begin{align*}
&\text{XXX} \\
&\text{Where, } R = \text{Cl, F}
\end{align*}
\]

Rao B.Sooryanarayana et al. [28] have synthesized a series of 1-aryloyl-3-aryl-5-hydroxy-5-(2, 4-dichloro-5-fluro phenyl) pyrazoline (XXXI) and screened for their antiproliferative activity.

\[
\begin{align*}
&\text{XXXI} \\
&\text{Kumar Ashok et al. [29] have synthesized pyrazolines as anticonvulsant agent. 3-(2', 3', 4'- tri hydroxyl phenyl)-1, 5-diphenyl pyrazoline (XXXII) have been found to be potent anticonvulsant agent.}
\end{align*}
\]
Gineinah et al. [30] have synthesized pyrazoline derivatives and evaluated their anticonvulsant activity.

Malhotra V., Pathak S. et al. [31] have synthesized different pyrazoline derivatives substituted at 1- position and evaluated cardiovascular activity. It was found that compounds (XXXIII) and (XXXIV) containing - Cl group have shown better response with maximum activity.

\[
\text{Cl-}\begin{array}{c}
\text{N} \\
\text{S} \\
\text{Cl}
\end{array}
\begin{array}{c}
\text{NH} \\
\text{S} \\
\text{OH}
\end{array}
\begin{array}{c}
\text{Cl}
\end{array}
\] (XXXIII)

\[
\text{Cl-}\begin{array}{c}
\text{N} \\
\text{S} \\
\text{Cl}
\end{array}
\begin{array}{c}
\text{NH} \\
\text{S}
\end{array}
\begin{array}{c}
\text{Cl}
\end{array}
\] (XXXIV)

Srivastava Brijesh Kumar et al. [32] have demonstrated that analogues of diaryl dihydropyrazole-3-carboxamides as antiobesity agent. Activities are evaluated for appetite suppression and body weight reduction in animal models. Compound (XXXV) showed significant body weight reduction \textit{in vivo}, which was attributed to their CB\textsubscript{1} antagonistic activity and exhibited a favorable pharmacokinetic profile.

\[
\text{Cl-}\begin{array}{c}
\text{N} \\
\text{O} \\
\text{N}
\end{array}
\begin{array}{c}
\text{NH} \\
\text{N} \\
\text{O}
\end{array}
\begin{array}{c}
\text{Cl}
\end{array}
\] (XXXV)

Tsuboi-Shinichiwada et al. [33] have reported phenyl aminocarbonyl pyrazolines (XXXVI) with good insecticidal activity. Hes Rolf van, Wellinga Kobus et al. [34] have synthesized 3, 5-diphenyl-1-phenylcarbamoyl-2-pyrazolines and evaluated their insecticidal properties. From the economic and practical aspects compound (XXXVII) appeared as one of the most promising compound in these series.
Stavenson T. M., Piotrowski D W et al.\textsuperscript{[35]} have reported pyrazolines and evaluated their insecticidal activity.

Chimenti Franco et al.\textsuperscript{[36]} have synthesized series of 1-thiocarbamoyl-3, 5-diaryl-4, 5-dihydro-(1H)-pyrazole derivatives and investigated for the ability to inhibit selective activity of the A and B isoforms of monoamine oxidase (MAO). Among the compounds (XXXVIII) and (XXXIX) most potent against MAO-A and MAO-B respectively.

Chimenti. Franco et al.\textsuperscript{[37]} have synthesized series of 1-acetyl-3-(4-hydroxy- and 2, 4-dihydroxyphenyl)-5-phenyl-4, 5-dihydro-(1H)-pyrazole derivatives. Among them compounds (XL) and (XLI) have most potent inhibitors of MAO-A and MAO-B.

Several Co-workers have reported pyrazoline as Antidiabetic agents\textsuperscript{[38]}, Hypotensive\textsuperscript{[39]}, Antinoceptive effect\textsuperscript{[40]}. 
Section : 2.1

Synthesis, spectral studies and biological evaluation of 1- 3-(2, 5-dimethoxy -4-methylphenyl)-5-substitutedphenyl-4, 5-dihydro-1H-pyrazole (2.1 a-f):

Introduction:

3, 5-diarylpyrazolines have received considerable interests from the fields of medicinal and agricultural chemistry due to their broad spectrum of biological activities. The considerable attention has been focused on the development and synthesis of pyrazoline for obtaining biological potent molecules, which have been prepared by the condensation of chalcone with hydrazine in ethanol.

Reaction Scheme:

Synthesis of 1- 3-(2, 5-dimethoxy -4-methylphenyl)-5-substitutedphenyl-4, 5-dihydro-1H-pyrazole (2.1 a-f):
Step-1: Preparation of 1-(2, 5-dimethoxyphenyl)ethanone:

Step-2: Preparation of 2-methyl-1, 4-dimethoxybenzene:
Step-3: Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)ethanone:

\[
\begin{align*}
\text{2-methyl-1,4-dimethoxybenzene} & \quad \text{Anhy.AlCl}_3, \quad 0-5 ^\circ C \quad \text{CH}_3\text{COCl, EDC} \\
\end{align*}
\]

1-(4-methyl-2, 5-dimethoxyphenyl)ethanone

Step-5: Preparation of 1-3-(2, 5-dimethoxy-4-methylphenyl)-5-substitutedphenyl-4, 5-dihydro-1H-pyrazole (2.1 a-f):

\[
\begin{align*}
\text{1-(4-methyl-2, 5-dimethoxyphenyl)ethanone} + \text{Substituted Benzaldehyde} & \quad 40\% \text{ KOH,} \quad 35-40 ^\circ C, \text{ Et-Oh} \\
\end{align*}
\]

The constitutions of the synthesized compounds have been characterized by using elemental analysis, IR, $^1$H and $^{13}$C NMR and LC Mass spectroscopy. The synthesized compounds have been screened for their in vitro biological assay and evaluated MIC against gram-positive and gram-negative bacterial strains and evaluated MIC against fungal strains C.albicans at a concentration of 6.25 µg/ml. The MIC values of synthesized compounds were compared with standard drugs like Gentamycin and K.Nystatin.
Experimental:

Synthesis of 1-3-(2, 5-dimethoxy-4-methylphenyl)-5-phenyl-4, 5-dihydro-1H-pyrazole (2.1 a-f):

**Step-1:**
Preparation of 1-(2, 5-dimethoxyphenyl)ethanone:
Please refer reaction scheme page No:

**Step-2:**
Preparation of 2-methyl-1, 4-dimethoxybenzene:
Please refer Reaction scheme page No:

**Step-3:**
Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl) ethanone:
Please refer Reaction scheme page No:

**Step-4:**
Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one (1a-f):
Please refer Reaction scheme page No:
Step-5:

Preparation of 1-3-(2, 5-dimethoxy-4-methylphenyl)-5-(2-chloroxyphenyl) -4, 5-dihydro-1H-pyrazole (2.1d):

To a solution of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(2-chlorophenyl) prop-2-en-1-one (0.01mol) in 50 ml ethanol was stirred at room temperature. Hydrazine (99%) (0.05mol) was added drop wise at room temperature then reaction mixture was refluxed for 8-10 hours. The reaction was monitored by continuous TLC method. After reaction complies, reaction mixture was poured in to water and it was kept for 24 hours. The resulting solid obtained was filtered, washed with water, dried and crystallized from methanol to give white needles. **Rf value:** 0.69, **Yield:** 58%, **M.P.:** 83 °C.

Analysis: C_{18}H_{19}ClN_{2}O_{2}

**Calculated:** C: 65.35 %, H: 5.79%,
N: 8.47 %, X: 10.72 %,

**Found:** C: 65.37 %, H: 5.78%,
N: 8.46 %, X: 10.74 %,

Similarly other 3-(2, 5-dimethoxy-4-methylphenyl)-5-(substituted phenyl) -4, 5-dihydro-1H-pyrazole (2.1 a-f) were prepared. The physical data are recorded in **Table No: 2.1.**
Table 2.1

Physical data of 3-(2, 5-dimethoxy-4-methylphenyl)-5-substitutedphenyl-4, 5-dihydro-1H-pyrazole (2.1 a-f)

<table>
<thead>
<tr>
<th>No.</th>
<th>-R</th>
<th>Molecular Formula (M.W)</th>
<th>Mp °c</th>
<th>Rf</th>
<th>% of Yield</th>
<th>% of C (Calc.) Found</th>
<th>% of H (Calc.) Found</th>
<th>% of N (Calc.) Found</th>
<th>% of X (Calc.) Found</th>
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</thead>
<tbody>
<tr>
<td>2.1a</td>
<td></td>
<td>C_{21}H_{25}ClN_{2}O_{4} (404)</td>
<td>118</td>
<td>0.62</td>
<td>70</td>
<td>62.30</td>
<td>6.22</td>
<td>6.92</td>
<td>8.76</td>
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<tr>
<td>2.1b</td>
<td>Cl</td>
<td>C_{18}H_{13}ClN_{2}O_{2} (365)</td>
<td>154</td>
<td>0.56</td>
<td>59</td>
<td>59.19</td>
<td>4.97</td>
<td>7.67</td>
<td>19.41</td>
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<td>2.1c</td>
<td>Cl</td>
<td>C_{20}H_{23}ClN_{2}O_{4} (390)</td>
<td>170</td>
<td>0.59</td>
<td>72</td>
<td>61.46</td>
<td>5.93</td>
<td>7.17</td>
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<td>2.1d</td>
<td>Cl</td>
<td>C_{18}H_{19}ClN_{2}O_{2} (330)</td>
<td>83</td>
<td>0.69</td>
<td>58</td>
<td>65.35</td>
<td>5.79</td>
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<tr>
<td>2.1e</td>
<td>OH</td>
<td>C_{19}H_{21}ClN_{2}O_{4} (376)</td>
<td>108</td>
<td>0.80</td>
<td>62</td>
<td>60.56</td>
<td>5.62</td>
<td>7.43</td>
<td>9.41</td>
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<tr>
<td>2.1f</td>
<td>Br</td>
<td>C_{19}H_{17}BrN_{2}O_{4} (421)</td>
<td>112</td>
<td>0.67</td>
<td>72</td>
<td>54.17</td>
<td>5.02</td>
<td>6.65</td>
<td>18.97</td>
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Spectroscopic evaluation:

- **IR Spectra**

  The IR spectra of pyrazoline revealed the absence of α, β-unsaturated carbonyl group, indicating involvement of this functionality in the formation of pyrazoline ring which is characterized by several intense absorptions bands.

  IR spectra of 1-3-(2, 5-dimethoxy-4-methylphenyl)-5-(3-chloro, 4-hydroxy-5-ethoxyphenyl)-4, 5-dihydro-1H-pyrazole (2.1a) showed absorption peak at 1516 cm\(^{-1}\), which is a characteristics of -C=N. Pyrazoline showed sharp band at 1222 cm\(^{-1}\) due to C-N stretching vibration. The IR spectra of compound showed sharp absorption band appearing at 3414 cm\(^{-1}\) which is attributed to stretching vibration of free -NH group. Appearance of stretching band at 2839 cm\(^{-1}\) have been assigned to –CH\(_2\) ring of the pyrazoline ring. It was also observed -CH deformation of pyrazoline at 638 cm\(^{-1}\). The ether linkage (C-O-C) stretching bands appeared in range of 1275-1200 cm\(^{-1}\) (symmetric) and 1089-1020 cm\(^{-1}\) (asymmetric). The aromatic in plane bending was observed at 1135 cm\(^{-1}\) and out of plane bending was observed at 867 cm\(^{-1}\). C-H asymmetric vibration was found in the range of 2975-2950 cm\(^{-1}\) while C-H asymmetric deformation observed at 1470-1435 cm\(^{-1}\). Multisubstituted benzene ring gives out of plane deformation in the range of 900-860 cm\(^{-1}\) [41-43].

  In addition to above mentioned peaks, spectrum of pyrazoline consists other common stretching and bending vibrations of compound under study. The IR spectra of the compound (2.1a) is given on page no.77.

- **\(^1\)H NMR Spectra**

  The presence of two diastereotopic protons H\(_A\) and H\(_B\) at C-4 showed two doublet of doublet and one single proton Hx at the C-5 positions of pyrazoline ring showed as triplet in \(^1\)HNMR spectrum is the most convincing proof of an ABX pattern exhibited due the resonance in pyrazoline structure.

  \(^1\)H NMR Spectrum of 1-3-(2, 5-dimethoxy-4-methylphenyl)-5-(3-chloro-4-hydroxy-5-ethoxyphenyl)-4, 5-dihydro-1H-pyrazole (2.1c) exhibited H\(_A\) proton which is cis to H\(_X\) resonated at 3.249-3.330 δ ppm as a doublet of doublet and H\(_B\), the other proton which is trans to Hx resonated at 3.774 – 3.991 δ ppm as a doublet of doublet.
The $H_X$ proton which is vicinal to methylene protons $H_A$ and $H_B$ resonated as doubledoublet in the range of $5.33\delta$ ppm. A downfield singlet at $7.50\delta$ ppm revealed the presence of -NH at pyrazoline ring. Singlets of -OCH$_3$ merged with signals of pyrazoline double doublet signal and appeared as multiplate. Singlet of -CH$_3$ appeared at $2.37\delta$ ppm. Triplet of three protons of ethoxy groups displayed at $1.429$-$1.464\delta$ ppm and Quartet of two protons of ethoxy group showed at $4.074$-$4.096\delta$ ppm. Hydroxy group displayed as singlet at $11.01\delta$ ppm.

The aromatic region of the spectrum exhibited as multiplet in the region at $6.73$-$7.16\delta$ ppm. The $^1$H NMR spectra of the compound (2.1c) is given on page no.78.

**$^{13}$C NMR Spectra**

In $^{13}$C NMR spectra of the compound (2.1c), three carbons of the pyrazoline ring were resonated at $158.77$ $\delta$ ppm (C-7), $42.94$ $\delta$ ppm (C-8), $47.92$ $\delta$ ppm (C-9), and the values were in close agreement with the reported values for pyrazoline carbons C-7, C-8 and C-9.

The signal of carbon detected at $56.05$ $\delta$ ppm (C-18), $56.11$ $\delta$ ppm (C-19), were appeared due to the –OCH$_3$. The signal appeared at $13.82\delta$ ppm (C-20) was assignable to methyl group at phenyl ring.

The aromatic carbons showed signals at $149.44$(C-1), $116.32$(C-2), $127.95$(C-3), $152.46$(C-4), $112.99$(C-5), $127.08$(C-6), $120.95$(C-10), $110.54$(C-11), $147.83$(C-12), $138.39$(C-13), $124.27$(C-14), $123.52$(C-15), $66.12$(C-16), $14.23$(C-17). The $^{13}$C NMR spectrum of the compound (2.1b) is given on page no.79.
IR spectra of 1-3-(2,5-dimethoxy-4-methylphenyl)-5-(2-chloro 3-hydroxy-4-ethoxyphenyl)-4,5-dihydro-1H-pyrazole (2.1a):

**IR (cm⁻¹):**
- 2964 (C-H str (asym) alkyl)
- 2910 (C-H str (sym) alkyl)
- 1458 (C-H def (asym) alkyl)
- 1319 (C-H def (sym) alkyl)
- 3000 (C-H str. arom.)
- 1590 (C=C str arom.)
- 1135 (C-H i.p.def arom.)
- 867 (C-H o.o.p.def.arom.)
- 1254 (C-O-C (sym) ether)
- 1222 (C-N str., Pyrazoline)
- 1089 (C-O-C (asym) ether)
- 1516 (C=N, Pyrazoline)
- 2839 (C-H ring str., Pyrazoline)
- 638 (C-H def of –CH₂, Pyrazoline)
- 1222 (C-N str., Pyrazoline)
- 3414 (N-H str., Pyrazoline)
- 3554 (O-H str.)
- 595 (C-Cl str.)
$^1$HNMR spectra of 1- 3-(2, 5-dimethoxy-4-methylphenyl)-5-(3-chloro-4-hydroxy-5-ethoxyphenyl)-4, 5-dihydro-$^1$H-pyrazole (2.1c):

![Chemical Structure](image)

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 1.429- 1.464 (3H, trip), 2.37(s, 3H), 3.249-3.330 (dd, 1H pyrazoline),3.774-3.991 (m, 6H, OCH$_3$+ 1H pyrazoline), 4.074-4.096(qur, 2H), 5.33(dd, 1H, pyrazoline), 6.73(d, 2H), 6.85(d, 1H), 7.16(s, 1H), 7.609(s, 1H, NH), 11.01(s, OH).
$^{13}$C NMR spectra of 1- 3-(2, 5-dimethoxy-4-methylphenyl)-5-(3-chloro-4-hydroxy-5-ethoxyphenyl)-4, 5-dihydro-1H-pyrazole (2.1c):

$^{13}$CNMR spectra of 1- 3-(2, 5-dimethoxy-4-methylphenyl)-5-(3-chloro-4-hydroxy-5-ethoxyphenyl)-4, 5-dihydro-1H-pyrazole (2.1c):

$^{13}$C NMR (CDCl$_3$) $\delta$ppm: 149.44(C-1), 116.32(C-2), 127.95(C-3), 152.46(C-4), 112.99(C-5), 127.08(C-6), 158.77(C-7), 42.94(C-8), 47.92(C-9), 120.95(C-10), 110.54(C-11), 147.83(C-12), 138.39(C-13), 124.27(C-14), 123.52(C-15), 66.12(C-16), 14.23(C-17), 56.058(C-18), 56.11(C-19), 13.82(C-20).
Biological Evaluation

Antibacterial and Antifungal Activities:

**Method**: Broth dilution method [44]

**Gram-positive bacteria**
- Staphylococcus aureus (Gram-positive) MTCC-96
- Streptococcus pyogenes (Gram-positive) MTCC-442

**Gram-negative bacteria**
- Pseudomonas aeruginosa (Gram-negative) MTCC-1688

**Interpretation of Results**
- Escherichia coli (Gram-negative) MTCC-443
  - 6.25, 12.5 = excellent active, 25=good active,
  - 50=moderate active, 100=poor active, 100< inactive

**Fungi**
- Candida albicans MTCC-227

**Interpretation of Results**
- 100= excellent active, 125=good active,
  - 200=poor active, 200< inactive

**Concentration**: 6.25 µg/ml

**Solvent**: DMSO

**Standard drugs**: Gentamycin and K.Nystatin
Table - 2.1: Antibacterial and Antifungal activities of 1-3-(2, 5-dimethoxy-4-methylphenyl)-5-phenyl-4, 5-dihydro-1H-pyrazole (2.1 a-f):

All these synthesized compounds were screened for their antimicrobial potential against different panel of organisms, i.e., *E.coli*, *P.aeruginosa*, *S.aureus*, *S. pyogenes*. and antifungal strains *C. albican*. The results of antimicrobial activities are depicted in following Table-2.1.1

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>-R</th>
<th>Bacterial activity</th>
<th>Fungal activity</th>
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<tr>
<td></td>
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<td>Minimal Inhibition Concentrations (MIC) in µg/ml</td>
<td>Minimal Inhibition Concentration (MIC) in µg/ml</td>
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<tr>
<td></td>
<td></td>
<td>S. aureus MTCC 96</td>
<td>S. pyogenes MTCC 442</td>
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<tr>
<td>2.1a</td>
<td></td>
<td>250</td>
<td>250</td>
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<tr>
<td>2.1b</td>
<td></td>
<td>100</td>
<td>50</td>
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<tr>
<td>2.1e</td>
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<td>250</td>
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<tr>
<td>2.1f</td>
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<td>25</td>
<td>25</td>
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</tbody>
</table>

**Interpretation of results**

<table>
<thead>
<tr>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25, 12.5=excellent, 25=good, 50=moderate, 100=poor active, 100&lt; inactive.</td>
<td>100=excellent, 125=good, 200=poor active 200&lt; inactive.</td>
</tr>
</tbody>
</table>
Result and discussion:

This series contains N- linkage to the basic scaffold di-substituted pyrazoline moiety.

All the synthesized compounds have been screened for their antimicrobial activity against different panel of organisms, i.e., *E.coli*, *P.aeruginosa*, *S.aureus*, *S.pyogenes* and antifungal strains *C. albicans*. The results of antimicrobial activities are depicted in following Table-2.1.1

**Anti bacterial activity**

Among all the synthesized compounds, compound 2.1a with ethoxy & methoxy group at phenyl nucleus exhibited excellent antibacterial activity against *E.coli* comparable to reference agent Gentamycin.

Compound 2.1f have showed good anti bacterial activity against both the gram positive bacterial strains. Introduction of bromo, hydroxyl and alkyl group at phenyl nucleus showed an improvement in activity. Compound 2.1b exhibited moderate activity against the gram negative strain *P.aeruginosa* and gram positive bacterial strains *S.pyogenes*. While the other compounds showed poor or no activity even at concentration of 100 µg/ml. The results of antibacterial activity are mentioned in Table 2.1.1

**Anti fungal activity**

All the synthesized compounds (2.1a-f) were screened for their antifungal activity. The MIC value of the test compounds 2.1b have showed excellent activity against the fungal strain *C.albicans* comparable to reference agent K Nystatin.

No significant or comparable activities have been observed for rest of other compounds up to concentration of 200 µg/ml. The overall results of antifungal activity are mentioned in Table-2.1.1
Section : 2.2

Synthesis and Spectral studies and biological evaluation of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted-phenyl-4, 5-dihydropyrazol-1-yl] propan-1-one (2.2 a-f):

❖ Introduction:

Due to the interesting activity of N-substituted pyrazolines as biological agents considerable attention has been focused on this class. The pharmaceutical importance of these compounds lies in the fact that they can be effectively utilized as antibacterial, antifungal, anti tubercular and insecticidal agents. In addition, pyrazolines have played a crucial part in the development of theory in heterocyclic chemistry and also used extensively in organic synthesis. To further assess the potential of such a class of compounds as antimycobacterial, antibacterial and antifungal agents, a series of propionyl pyrazoline have been synthesized by condensation of chalcone with hydrazine in propanoic acid.

❖ Reaction Scheme:

Synthesis of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one (1 a-f):

Step-1: Preparation of 1-(2, 5-dimethoxyphenyl)ethanone:

\[
\begin{align*}
\text{1,4-dimethoxybenzene} & \quad \text{Anhy. AlCl}_3, \quad \text{CH}_3\text{COCl, EDC} & \quad \text{0-5 °C} \quad \text{1-(2,5-dimethoxyphenyl)ethanone}
\end{align*}
\]

Step-2: Preparation of 2-methyl-1, 4-dimethoxybenzene:

\[
\begin{align*}
\text{1-(2,5-dimethoxyphenyl)ethanone} & \quad \text{KOH, 205-210 °C} \quad \text{NHNH}_2 \text{H}_2\text{O} & \quad \text{2-methyl-1,4-dimethoxybenzene}
\end{align*}
\]
SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME HETEROCYCLIC DERIVATIVES

**Step-3: Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)ethanone:**

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{OCH}_3 \\
\text{H}_3\text{C} & \quad \text{OCH}_3 \\
\text{H}_3\text{CO} & \quad \text{CH}_3 \\
\end{align*}
\]

2-methyl-1,4-dimethoxybenzene

\[
\begin{align*}
\text{Anhy.AlCl}_3, & \quad 0-5^\circ \text{C} \\
\text{CH}_3\text{COCl}, & \quad \text{EDC} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{OCH}_3 \\
\text{H}_3\text{CO} & \quad \text{CH}_3 \\
\end{align*}
\]

1-(4-methyl-2,5-dimethoxyphenyl)ethanone

**Step-4: Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl)prop-2-en-1-one (1a-f):**

\[
\begin{align*}
\text{HO} & \quad \text{R} \\
\text{Substituted Benzaldehyde} & \quad + \\
\text{Et-OH} & \quad \text{40% KOH,} \\
35-40^\circ \text{C.} & \\
\end{align*}
\]

1-(4-methyl-2,5-dimethoxyphenyl)ethanone

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{OCH}_3 \\
\text{H}_3\text{CO} & \quad \text{CH}_2\text{COOH} \\
\end{align*}
\]

1-(4-methyl-2,5-dimethoxyphenyl)-3-(substituted-phenyl)prop-2-en-1-one

**Step-5: 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted-phenyl-4, 5-dihydropyrazol-1-yl]propan-1-one (2.2 a-f):**

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{OCH}_3 \\
\text{H}_3\text{CO} & \quad \text{CH}_2\text{COOH} \\
\end{align*}
\]

1-(4-methyl-2, 5-dimethoxyphenyl)

\[
\begin{align*}
\text{NH}_2\text{NH}_2 & \quad 100-110^\circ \text{C.} \\
\text{CH}_3\text{CH}_2\text{COOH} & \\
\end{align*}
\]

1-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted-3-(substituted-phenyl-4, 5-dihydropyrazol-1-yl]propan-1-one

The constitutions of the synthesized compounds have been characterized by using elemental analysis, IR, \(^1\)H NMR, \(^{13}\)C NMR spectroscopy and LC Mass spectroscopy.

The synthesized compounds have been screened for their *in vitro* biological assay and evaluated MIC against gram-positive and gram-negative bacterial strains and evaluated MIC against fungal strains *C.albicans* at a concentration of 6.25 µg/ml. The MIC of synthesized compounds was compared with standard drugs like Gentamycin and K.Nystatin.
SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME HETEROCYCLIC DERIVATIVES

❖ Experimental:

Synthesis of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted-phenyl-4, 5-dihydropyrazol-1-yl)propan-1-one (2.2 a-f)

Step-1:
Preparation of 1-(2, 5-dimethoxyphenyl)ethanone:
Please refer Reaction scheme page No:

Step-2:
Preparation of 2-methyl-1, 4-dimethoxybenzene:
Please refer Reaction scheme page No:

Step-3:
Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)ethanone:
Please refer Reaction scheme page No:

Step-4:
Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one (1a-f):
Please refer Reaction scheme page No:
Step-5:
Preparation of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(3-dichloro-4-hydroxy-5-ethoxy phenyl-4, 5-dihydropyrazol-1-yl]propan-1-one (2.2c) :

To a solution of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(3-chloro-4-hydroxy-5-ethoxy phenyl) prop-2-en-1-one (0.01 mol) in 50 ml of propanoic acid was stirred at room temperature. Hydrazine (99%) [0.05 mol] was added drop wise at room temperature then reaction mixture was refluxed for 8-10 hours. The reaction was monitored by TLC. After reaction complies, reaction mixture was poured in water and it was kept for 24 hours. The resulting solid obtained was filtered, washed with water, dried and crystallized from methanol to give white needles. **Rf value:** 0.66, **Yield:** 55 %, **M.P.:** 108 °C.

**Analysis:** C₂₃H₂₇ClN₂O₅

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<tr>
<th>Calculated</th>
<th>Found</th>
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<tr>
<td>C: 61.84%, H: 6.07%,</td>
<td>C: 61.81%, H: 6.09%,</td>
</tr>
<tr>
<td>N: 6.25%, X: 7.94 %</td>
<td>N: 6.27%, X: 7.93 %</td>
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</tbody>
</table>

Similarly other 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one (2.2 a-f) were prepared. The physical data are recorded in **Table No:** 2.2.
Table: 2.2

Physical data of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted-phenyl-4, 5-dihydropyrazol-1-yl] propan-1-one (2.2 a-f)

<table>
<thead>
<tr>
<th>No.</th>
<th>-R</th>
<th>Molecular Formula (M.W)</th>
<th>Mp °C</th>
<th>Rf</th>
<th>% of Yield</th>
<th>% of C (Cal)</th>
<th>% of C (Found)</th>
<th>% of H (Cal)</th>
<th>% of H (Found)</th>
<th>% of N (Cal)</th>
<th>% of N (Found)</th>
<th>% of X (Cal)</th>
<th>% of X (Found)</th>
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<td>C_{24}H_{29}ClN_{2}O_{3} (460)</td>
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<tr>
<td>2.2b</td>
<td></td>
<td>C_{21}H_{25}ClN_{2}O_{3} (421)</td>
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<td>65</td>
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<td>6.65</td>
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<td>59.89</td>
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<td>6.66</td>
<td>16.85</td>
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<td>2.2c</td>
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<td>C_{23}H_{27}ClN_{2}O_{3} (446)</td>
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<td>55</td>
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<td>2.2d</td>
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<td>2.2e</td>
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<td>C_{22}H_{25}ClN_{2}O_{3} (432)</td>
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<td>2.2f</td>
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<td>C_{23}H_{25}BrN_{2} (477)</td>
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<td>55.34</td>
<td>5.29</td>
<td>5.88</td>
<td>16.76</td>
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</tbody>
</table>

+ TLC Solvent System: - Benzene: Carbon Tetra Chloride: Ethyl acetate (5:4:1)
SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME HETEROCYCLIC DERIVATIVES

❖ Spectroscopic evaluation:

❖ IR spectra

IR spectra of compound 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(2-chlorophenyl-4, 5-dihydropyrazol-1-yl)propan-1-one (2.2d) showed stretching band at 1635 cm\(^{-1}\) due to C=O of -N-CO-CH\(_2\)-CH\(_3\) group and -CH\(_3\) deformation at 1349 cm\(^{-1}\). Spectra also showed absorption band at 1610 cm\(^{-1}\), which is a characteristic of -C=N. IR spectrum of compound exhibits an intense sharp band at 1231 cm\(^{-1}\) due to C-N stretch vibration. It was also observed that -CH\(_2\) ring stretching at 2942 cm\(^{-1}\). Due to ether linkage two bands appeared in the range of 1275-1200 cm\(^{-1}\) and 1089-1020 cm\(^{-1}\). Multisubstituted benzene ring gave out of plane deformation in the range of 900-860 cm\(^{-1}\).[41-43]

In addition to above mentioned peaks, spectrum of propionyl pyrazoline consists other stretching and bending vibration common to all compound under study. The IR spectra of the compound 2.2b is given on page no.90.

❖ \(^1\)H NMR Spectra

Absence of doublets of (-CH=CH-) vinyl protons and presence of the three protons at C-4 and C-5 of the pyrazoline ring are represented by a typical (ABX) system. Each of the two diastereotopic protons H\(_A\) and H\(_B\) at C-4, and one single proton H\(_X\) at the C-5, are represented by doublet of doublet which is most convincing proof of pyrazoline derivatives.

The spectra of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(2, 4-dichlorophenyl-4, 5-dihydropyrazol-1-yl)propan-1-one (2.2b) showed the first doublet of doublet at 3.245-3.303 \(\delta\)ppm and it was assigned to H\(_A\) at C-4 carbon, signal due to H\(_B\) also appeared as doublet of doublet at around at 3.797-3.847 \(\delta\)ppm but this proton signal is merged with the signal of -OCH\(_3\) groups. Third proton H\(_X\), at C-5 carbon of pyrazoline ring appeared as doublet of doublet at 5.390-5.431\(\delta\)ppm. The N-COCH\(_2\)CH\(_3\) group showed as one triplet at 1.186-1.255 \(\delta\)ppm and one quartet at
2.779-2.799 δppm to provide additional proof of propionyl group attached to pyrazoline ring. The three protons of -CH$_3$ group appeared as a singlet at 2.26 δppm. The three protons of -OCH$_3$ group appeared as a singlet at 3.772 and 3.872 δppm, respectively.

The protons of aromatic region appeared as multiplet of four protons in the range of 6.752-7.518 δppm. In addition to above mentioned peaks remaining protons of the compound are given in detail analysis. The $^1$H NMR spectra of the compound (2.2b) is given on page no.91.

**LC Mass Spectra**

The mass spectra of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(3-chloro-4-hydroxy-5-ethoxyphenyl-4, 5-dihydropyrazol-1-yl]propan-1-one (2.2c) showed strong molecular ion peak at 446 m/e. The LC mass spectra of compound (2.2c) is given on page no.92.
IR spectra of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(2-chlorophenyl-4, 5-dihydropyrazol-1-yl)propan-1-one (2,2 d):

IR (cm\(^{-1}\)) : 3006 (C-H str (asym) alkyl), 2810 (C-H str (sym) alkyl), 1466 (C-H def (asym) alkyl), 1349 (C-H def (sym) alkyl), 3037 (C-H str.arom.), 1544 (C=C str arom.), 1149 (C-H i.p.def arom.), 879 (C-H o.o.p.def.arom.), 1276 (C-O-C (sym) ether), 1060 (C-O-C (asym) ether), 1544 (C=N, Pyrazoline), 2942(C-H ring str., Pyrazoline), 621(C-H defof –CH\(_2\), Pyrazoline), 1231(C-N str., Pyrazoline), 621(C-Clstr.).
$^1$HNMR spectra of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(3-chloro-4-hydroxyphenyl-4, 5-dihydropyrazol-1-yl]propan-1-one (2.2b):

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 1.186- 1.255 (3H, trip), 2.26(s, 3H), 2.612(qur, 2 H), 3.245-3.303 (dd, 1H, pyrazoline), 3.772(s, OCH$_3$), 3.797-3.84 (dd, 1H, pyrazoline), 3.872(s, OCH$_3$), 5.41(dd, 1H, pyrazoline), 6.752 (s, 1H), 6.86(d, 1H), 7.96(d, 1H), 7.172(d, 1H), 7.404-7.518(dd, 1H).
LC MASS spectra of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(3-chloro-4-hydroxy-5-ethoxyphenyl-4, 5-dihydropyrazol-1-yl)propan-1-one (2.2 c):
BIOLOGICAL EVALUATION

Antibacterial and Antifungal Activities:

Method: Broth dilution method [44]

Gram-positive bacteria: *Staphylococcus aureus* (Gram-positive) MTCC-96
   *Streptococcus pyogenes* (Gram-positive) MTCC-442

Gram-negative bacteria: *Pseudomonas aeruginosa* (Gram-negative) MTCC-1688

Interpretation of Results: *Escherichia coli* (Gram-negative) MTCC-443
   6.25, 12.5 = excellent active, 25=good active,
   50=moderate active, 100=poor active, 100< inactive

Fungi: *Candida albicans* MTCC-227

Interpretation of Results: 100= excellent active, 125=good active,
   200=poor active, 200< inactive

Concentration: 6.25 µg/ml

Solvent: DMSO

Standard drugs: Gentamycin and K.Nystatin
SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME HETEROCYCLIC DERIVATIVES

Table – 2.2: Antibacterial and antifungal activities of 1-[3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted-phenyl-4, 5-dihdropyrazol-1-yl]propan-1-one (2.2 a-f):

Six compounds have been synthesized based on N-proponyl pyrazoline of 4- methyl -2, 5-di-methoxy phenyl nucleus.

All these synthesized compounds were screened for their antimicrobial potential against different panel of organisms, i.e., E.coli, P.aeruginosa, S.aureus, S. pyogenes, and antifungal strains C. albican. The results of antimicrobial activities are depicted in following Table-2.2.1

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>-R</th>
<th>Bacterial activity Minimal Inhibition Concentrations (MIC) in µg/ml</th>
<th>Fungal activity Minimal Inhibition Concentration (MIC) in µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S. aureus MTCC 96</td>
<td>S. pyogenes MTCC 442</td>
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<tr>
<td>2.2a</td>
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<td>500</td>
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<tr>
<td>2.2b</td>
<td><img src="2.2b" alt="Image" /></td>
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<td>250</td>
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<tr>
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<td><img src="2.2c" alt="Image" /></td>
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<td>50</td>
</tr>
<tr>
<td>2.2d</td>
<td><img src="2.2d" alt="Image" /></td>
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<td>250</td>
</tr>
<tr>
<td>2.2e</td>
<td><img src="2.2e" alt="Image" /></td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>2.2f</td>
<td><img src="2.2f" alt="Image" /></td>
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<td>250</td>
</tr>
</tbody>
</table>

Interpretation of results

<table>
<thead>
<tr>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25, 12.5=excellent, 25=good, 50=moderate, 100=poor active, 100&lt; inactive.</td>
<td>100=excellent, 125=good, 200=poor active 200&lt; inactive.</td>
</tr>
</tbody>
</table>
**Result and discussion:**

- **Antibacterial activity**
  
  The MIC values which have been observed of test compounds (2.2a-f) exhibited significant antibacterial activity however with a degree of variation.

  The observation of inhibition data suggest that the tested compound 2.2f substituted with alkoxy, bromo and hydroxyl group at phenyl nucleus exhibited good activity against gram-negative bacterial strains *E.coli* and *P.aeruginosa*, comparable to reference agent Gentamycin, respectively.

  Amongst the synthesized compounds 2.2c and 2.2e chloro, hydroxy y and alkoxy substituent at phenyl nucleus appeared with moderate antibacterial activity profile against *S. aureus* and *S. pyogenus*. whereas no significant activities was observed against *E.coli* and *P.aeruginosa*. No significant or comparable activities have been observed for rest of other compounds up to concentration 100 µg/ml. The results of antibacterial activity are mentioned in Table 2.2.1.

- **Antifungal activity**
  
  All the synthesized compounds were screened for antifungal activity. The MIC values of screened compounds suggest that the test compound 2.2b showed good activity against fungal strain *C. albicans* comparable to reference agent K Nystatin.

  While other compounds showed poor or no activity even at concentration of 200 µg/ml. The overall results of antifungal activity are mentioned in Table-2.1.1
Section : 2.3

Synthesis and Spectral studies and biological evaluation of 1- (3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted phenyl)- (4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3 a-f):

❖ **Introduction:**
Variously substituted pyrazolines and their derivatives are important biological agents and a significant amount of research activity has been directed towards this class. The pyrazoline skeletal is quite stable and can be effectively used to prepare novel heterocyclic compounds in a large number with biological activities. This inspired us to prepare a new pyrazolines prepared by condensation of chalcones with 4-chlorobenzohydrazide in ethanol.

❖ **Reaction Scheme:**

**Step-1: Preparation of 1-(2, 5-dimethoxyphenyl)ethanone:**

\[
\begin{align*}
\text{1,4-dimethoxybenzene} & \xrightleftharpoons[0-5 \ ^\circ \text{C}]{\text{Anhy. AlCl}_3, \ \text{CH}_3\text{COCl}, \ \text{EDC}} \text{1-(2,5-dimethoxyphenyl)ethanone} \\
\end{align*}
\]

**Step-2: Preparation of 2-methyl-1, 4-dimethoxybenzene:**

\[
\begin{align*}
\text{1-(2,5-dimethoxyphenyl)ethanone} & \xrightleftharpoons[205-210 \ ^\circ \text{C}]{\text{KOH, NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}} \text{2-methyl-1,4-dimethoxybenzene} \\
\end{align*}
\]

**Step-3: Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)ethanone:**

\[
\begin{align*}
\text{2-methyl-1,4-dimethoxybenzene} & \xrightleftharpoons[0-5 \ ^\circ \text{C}]{\text{Anhy. AlCl}_3, \ \text{CH}_3\text{COCl}, \ \text{EDC}} \text{1-(4-methyl-2,5-dimethoxyphenyl)ethanone} \\
\end{align*}
\]
Step-4: Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one (1a-f) :

\[
\begin{align*}
1\text{-}(4\text{-methyl-2, 5-dimethoxyphenyl})\text{ethanone} & \quad \text{Substituted Benzaldehyde} \\
\text{Et-OH, } 35-40^\circ \text{C.} & \quad 40\% \text{ KOH, Ethanol} \quad \text{1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one}
\end{align*}
\]

Step-5: 1- (3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted phenyl)- (4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl)methanone (2.3 a-f):

\[
\begin{align*}
\text{1-(4-methyl-2, 5-dimethoxyphenyl) 4- chloro benzo hydrazide 1- (3-(4-methyl-2, 5-dimethoxyphenyl) -3-(substituted-phenyl) prop-2-en-1-one hydrochloride -5-(substituted phenyl) - 4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone}
\end{align*}
\]

The constitutions of the synthesized compounds have been characterized by using elemental analysis, IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and LC Mass spectroscopy. The synthesized compounds have been screened for their \textit{in vitro} biological assay and evaluated MIC against gram-positive and gram-negative bacterial strains and evaluated MIC against fungal strains \textit{C.albicans} at a concentration of 6.25 \(\mu\text{g/ml}\). The MIC of synthesized compounds were compared with standard drugs like Gentamycin and K.Nystatin.
SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME HETERO CYCLIC DERIVATIVES

Experimental:

Synthesis of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)-4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3 a-f):

Step-1:
Preparation of 1-(2, 5-dimethoxyphenyl)ethanone:
Please refer Reaction scheme page No:

Step-2:
Preparation of 2-methyl-1, 4-dimethoxybenzene:
Please refer Reaction scheme page No:

Step-3:
Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)ethanone:
Please refer Reaction scheme page No:

Step-4:
Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one (2.3a-f):
Please refer Reaction scheme page No:
Step-5:

Preparation of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)-4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3 a-f):

To a solution of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(4-chlorophenyl) prop-2-en-1-one (0.01 mol) in 50 ml of ethanol was stirred at room temperature. 4-chlorobenzohydrazide (99%) [0.05 mol] was added drop wise at room temperature then reaction mixture was refluxed for 8-10 hours. The reaction was monitored by TLC. After reaction complies, reaction mixture was poured in water and it was kept for 24 hours. The resulting solid obtained was filtered, washed with water, dried and crystallized from methanol to give white needles. **Rf value:** 0.74, **Yield:** 56%, **M.P.:** 155°C.

**Analysis:** C$_{25}$H$_{22}$Cl$_2$N$_2$O$_3$

| Calculated: | C: 63.97%, H: 4.72%, N: 5.97%, X: 15.11 % |
| Found :     | C: 63.99%, H: 4.74%, N: 5.99%, X: 15.07 % |

Similarly other 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)-4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3 a-f) were prepared. The physical data are recorded in **Table: 2.3.**
Table: 2.3

Physical data 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3 a-f):

| No. | -R | Molecular Formula (M.W) | Mp °c | % of Yield | % of C (Calc.) | % of C (Found) | % of H (Calc.) | % of H (Found) | % of N (Calc.) | % of N (Found) | % of X (Calc.) | % of X (Found) |
|-----|----|-------------------------|-------|------------|---------------|----------------|---------------|----------------|---------------|---------------|---------------|----------------|----------------|
| 2.3a |  | C_{28}H_{28}Cl_{2}N_{2}O_{5} (543.5) | 198   | 0.56       | 61.88         | 5.19           | 5.15          | 13.05          |               |               |               |               |
|      |  |                         |       |            | 61.83         | 5.22           | 5.18          | 13.07          |               |               |               |               |
| 2.3b |  | C_{25}H_{21}Cl_{3}N_{2}O_{3} (504.5) | 183   | 0.63       | 59.60         | 4.20           | 5.56          | 21.11          |               |               |               |               |
|      |  |                         |       |            | 59.62         | 4.18           | 5.57          | 21.13          |               |               |               |               |
| 2.3c |  | C_{27}H_{26}Cl_{2}N_{2}O_{5} (529.5) | 161   | 0.74       | 61.25         | 4.95           | 5.29          | 13.39          |               |               |               |               |
|      |  |                         |       |            | 61.27         | 4.92           | 5.32          | 13.35          |               |               |               |               |
| 2.3d |  | C_{25}H_{22}Cl_{2}N_{2}O_{3} (470) | 155   | 0.52       | 63.97         | 4.72           | 5.97          | 15.11          |               |               |               |               |
|      |  |                         |       |            | 63.95         | 4.73           | 5.92          | 15.14          |               |               |               |               |
| 2.3e |  | C_{26}H_{23}BrCl_{2}N_{2}O_{5} (515) | 235   | 0.49       | 6.59          | 4.69           | 5.44          | 13.76          |               |               |               |               |
|      |  |                         |       |            | 6.56          | 4.72           | 5.43          | 13.74          |               |               |               |               |
| 2.3f |  | C_{26}H_{24}BrCl_{2}N_{2}O_{5} (560.5) | 207   | 0.38       | 55.78         | 4.32           | 5.00          | 6.33           |               |               |               |               |
|      |  |                         |       |            | 55.80         | 4.31           | 5.03          | 6.30           |               |               |               |               |

TLC Solvent System: - Benzene: Carbon Tetra Chloride: Ethyl acetate (5:4:1)
Spectroscopic evaluation:

Spectroscopic analysis of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3 a-f)::

The IR spectra of pyrazoline revealed the absence of α, β-unsaturated carbonyl group, indicating involvement of this functionality in the formation of pyrazoline ring.

The IR spectra of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(2-chloro-3-ethoxy-4-methoxy phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3 a) was confirmed by absorption of C=N, C-N and –CH$_2$-bands of pyrazoline ring and presence of -N-CO-C$_6$H$_5$-Cl group.

On other hand, it contained characteristics absorption band at 1500 cm$^{-1}$ due to C=N of pyrazoline ring. It was also observed that -CH$_2$ ring stretching at 2838 cm$^{-1}$. The IR spectra of pyrazoline exhibited peak at around 1205 cm$^{-1}$ indicating the presence to C-N stretch vibration of the pyrazoline ring.IR spectra of butryl pyrazoline showed C=O stretching vibration at 1614 cm$^{-1}$. Due to ether linkage(C-O-C), two bands appeared in the range of 1275-1200 cm$^{-1}$ and 1089-1020 cm$^{-1}$ and multi substituted benzene ring gives out of plane deformation at 900-860 cm$^{-1}$ range. Hydroxyl group showed at 3649 cm$^{-1}$C-Cl stretching observed at 532 cm$^{-1}$.[41-43].

In addition to above mentioned peaks, spectrum of (2.3a) consists other stretching and bending vibration common to all compound under study. The IR spectrum of the compound is given on page no.103.

$^1$H NMR Spectra

The PMR spectrum of pyrazoline derivative compound 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(2-chloro-4-hydroxy-4-methoxy)- 4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3e) showed resonance due to two magnetically non-equivalent geminal protons attached at C$_4$ and one vicinal proton at C$_5$ showed up in the form of three triplet exhibiting a typical splitting pattern of ABX system.

The first triplet of H$_A$ at C$_4$ was discernible at 2.520-2.531 δppm, signal due to H$_B$ also resonated as triplet at around at 3.171-3.192 δppm.The signal of –OCH$_3$ protons showed at 3.84 and 3.901 δppm.as singlet.Signal showed up as triplet at 5.59 δppm attributed H$_X$. The three protons of -CH$_3$ group appeared as a singlet at 6.83 δppm.
The aromatic region has eight protons of phenyl ring, which showed as multiplet 7.113- 8.012. The $^1$H NMR of the compound (2.3d) is given on page no.104.

**$^{13}$C NMR Spectra**

In $^{13}$C NMR spectra of the compound 2.3b, carbons of the pyrazoline ring were resonated at 151.54 δppm (C-7), 41.32 δppm (C-8), 59.43 δppm (C-9) and the values were in close agreement with the reported values for pyrazoline carbons.

The signals of two carbon atoms detected at 17.64 (CH$_3$) and 171.27 (C=O) δppm. Confirms the presence of N-CO- group. The carbons of two –OCH$_3$ group resonated at 56.99 δppm.

The aromatic carbon showed sharp signals at 152.55 (C-1), 116.87(C-2), 127.01(C-3), 154.55(C-4), 116.878(C-5), 111.57(C-6), 130.31(C-11), 127.47(C-12, 16), 129.27(C-13, 15), 140.12(C-14), 137.52(C-17), 131.75(C-18), 134.69(C-20), 124.85(C-21), 126.06(C-22), 17.64(C-23), δppm. The $^{13}$C NMR spectra of the compound (2.3b) is given on page no.105.
IR spectra of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(2-chloro-3-ethoxy-4-methoxy phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3 a)::

IR (cm$^{-1}$) : 2937 (C-H str (asym) alkyl), 1447 (C-H def (asym) alkyl), 1383 (C-H def (sym) alkyl), 3063 (C-H str.arom.), 1179 (C-H i.p.def arom.), 854 (C-H o.o.p.def.arom.), 1262 (C-O-C (sym) ether), 1080 (C-O-C (asym) ether), 1500(C=N, Pyrazoline), 1614(C=Ostr.)2838(C-H ring str., Pyrazoline), 697(C-H defof –CH$_2$, Pyrazoline), 1205(C-N str., Pyrazoline), 3649(O-H str.), 532(C-Clstr.).
$^1$HNMR spectra of 1-(3-(4-methyl-2,5-dimethoxy-phenyl)-5-(3-chloro-4-hydroxy-5-methoxy phenyl)-4,5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3e):

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 2.28 (s, 3H), 3.84 (s, 3H, OCH$_3$), 3.865 (s, 3H, OCH$_3$), 3.901 (s, 3H, OCH$_3$), 2.520-2.531 (trip, 1H, pyrazoline), 3.171-3.192 (trip, 1H, pyrazoline), 6.830 (s, OH), 5.59 (trip, 1H, pyrazoline), 7.113-8.012 (m, 8H, Ar-H).
$^{13}$CNMR spectra of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(2, 4 dichloro phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-chlorophenyl) methanone (2.3b)::

$^{13}$C NMR (CDCl$_3$) $\delta$ ppm: 152.55 (C-1), 116.87(C-2), 127.01(C-3), 154.55(C-4), 116.878(C-5), 111.57(C-6), 151.54(C-7), 41.32(C-8), 59.43(C-9), 171.27(C-10), 130.31(C-11), 127.47(C-12, 16), 129.27(C-13, 15), 140.12(C-14), 137.52(C-17), 131.75(C-18), 134.69(C-20), 124.85(C-21), 126.06(C-22), 17.64(C-23), 56.99(C-24, 25).
Biological Evaluation

Antibacterial and Antifungal Activities:

Method : Broth dilution method [44]

Gram-positive bacteria : *Staphylococcus aureus* (Gram-positive) MTCC-96
                      : *Streptococcus pyogenes* (Gram-positive) MTCC-442

Gram-negative bacteria : *Pseudomonas aeruginosa* (Gram-negative) MTCC-1688

Interpretation of Results : *Escherichia coli* (Gram-negative) MTCC-443
                            : 6.25, 12.5 = excellent active, 25=good active,
                            : 50=moderate active, 100=poor active, 100< inactive

Fungi : *Candida albicans* MTCC-227

Interpretation of Results : 100= excellent active, 125=good active,
                            : 200=poor active, 200<inactive

Concentration : 6.25 µg/ml

Solvent : DMSO

Standard drugs : Gentamycin and K.Nystatin
Six compounds have been synthesized based on N-proponyl pyrazoline of 4-methyl-2, 5-di-methoxy phenyl nucleus. All these synthesized compounds were screened for their antimicrobial potential against different panel of organisms, i.e., *E.coli, P.aeruginosa, S.aureus, S. pyogenes,* and antifungal strains *C. albican.* The results of antimicrobial activities are depicted in following Table-2.3.1

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>-R</th>
<th>Bacterial activity (MIC) in µg/ml</th>
<th>Fungal activity (MIC) in µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em> MTCC 96</td>
<td><em>S. pyogenes</em> MTCC 442</td>
</tr>
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<td>250</td>
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<tr>
<td>2.3b</td>
<td><img src="image2" alt="Structure" /></td>
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<tr>
<td>2.3c</td>
<td><img src="image3" alt="Structure" /></td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>2.3d</td>
<td><img src="image4" alt="Structure" /></td>
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<td>250</td>
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<tr>
<td>2.3e</td>
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<td>2.3f</td>
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<td>50</td>
</tr>
</tbody>
</table>

**Interpretation of results**

<table>
<thead>
<tr>
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<td>100=excellent, 125=good, 200=poor active, 200&lt; inactive.</td>
</tr>
</tbody>
</table>
Result and discussion:

The synthesized a series of (3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted phenyl-1H-pyrazol-1-yl)(4-chlorophenyl)methanone (2.3 a-f) and evaluated their MIC of anti bacterial, antifungal activity.

**Anti bacterial activity**

The MIC values of anti bacterial activity suggest that the test compound 2.3(a-f) with chloro substituent at N-CO-C\textsubscript{6}H\textsubscript{5} pharmacophore appeared with promising activity against both the bacterial strains.

Compound 2.3 b, d have showed excellent anti bacterial activity against gram negative strain bacterial strain *E.coli* and gram positive bacterial strains *S.aureus*, introduction of halogen group at phenyl nucleus showed an improvement in activity.

Compound 2.3f showed moderate activity against gram negative bacterial strain *E.coli* and both gram positive bacterial strain.

Compound 2.3e with hydroxyl and methoxy substituent are found to be good active against gram negative bacterial strain *P. aeruginosa*.

While the other compounds showed poor or no activity even at concentration of 100 \(\mu g/ml\). The results of antibacterial activity are mentioned in Table -2.3.1

**Anti fungal activity**

All synthesized compounds (2.3 a-f) were screened for their antifungal activity. The MIC value of the test compounds 2.3 f have showed good activity against the fungal strain comparable to reference agent K Nystatin.

No significant or comparable activities have been observed for rest of other compounds up to concentration of 200 \(\mu g/ml\). The overall results of antifungal activity are mentioned in Table-2.3.1
Synthesis and Spectral studies and biological evaluation of 1- (3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted phenyl)- (4, 5-dihydropyrazol-1-yl)-(4-methyl phenyl)methanone (2.4 a-f):

Introduction:
Variously substituted pyrazolines and their derivatives are important biological agents and a significant amount of research activity has been directed towards this class. The pyrazoline skeletal is quite stable and can be effectively used to prepare novel heterocyclic compounds in a large number with biological activities. This inspired us to prepare a new pyrazolines prepared by condensation of chalcones with 4-methylbenzohydrazide in ethanol.

Reaction Scheme:
Step-1: Preparation of 1-(2, 5-dimethoxyphenyl)ethanone:

\[
\begin{align*}
\text{1,4-dimethoxybenzene} & \xrightarrow{\text{Anhy.AlCl}_3, \text{CH}_3\text{COCl, EDC}} \text{1-(2,5-dimethoxyphenyl)ethanone} \\
\end{align*}
\]

Step-2: Preparation of 2-methyl-1, 4-dimethoxybenzene:

\[
\begin{align*}
\text{1-(2,5-dimethoxyphenyl)ethanone} & \xrightarrow{\text{KOH, 205-210 }^\circ\text{C}} \text{2-methyl-1,4-dimethoxybenzene} \\
\end{align*}
\]

Step-3: Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)ethanone:

\[
\begin{align*}
\text{2-methyl-1,4-dimethoxybenzene} & \xrightarrow{\text{Anhy.AlCl}_3, \text{CH}_3\text{COCl, EDC}} \text{1-(4-methyl-2,5-dimethoxyphenyl)ethanone} \\
\end{align*}
\]
Step-4: Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one (1a-f):

\[
\text{HO}
\]
\[
\text{R}
\]
\[
\text{Substituted Benzaldehyde}
\]
\[
\text{Et-OH}
\]
\[
\text{40\% KOH, 35-40\textdegree C.}
\]

1-(4-methyl-2,5-dimethoxyphenyl)ethanone

Step-5 : 1- (3-(4-methyl-2, 5-dimethoxyphenyl)-5-(substituted phenyl)- (4, 5-dihydropyrazol-1-yl)-(4-methylphenyl)methanone (2.4 a-f):

\[
\text{OOCH}_3
\]
\[
\text{OCH}_3
\]
\[
\text{R}
\]
\[
\text{HCl}
\]
\[
\text{12 - 14 hours}
\]
\[
\text{Ethanol}
\]

1-(4-methyl-2, 5-dimethoxyphenyl) 4- methyl benzo hydrazide 1- (3-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl)- prop-2-en-1-one hydrochlo ride -5-(substituted phenyl) - 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone

The constitutions of the synthesized compounds have been characterized by using elemental analysis, IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and LC Mass spectroscopy.

The synthesized compounds have been screened for their \textit{in vitro} biological assay and evaluated MIC against gram-positive and gram-negative bacterial strains and evaluated MIC against fungal strains \textit{C.albicans}. at a concentration of 6.25 \textmu g/ml. The MIC of synthesized compounds were compared with standard drugs like Gentamycin and K.Nystatin.
Experimental:

Synthesis of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone (2.4 a-f):

Step-1:
Preparation of 1-(2, 5-dimethoxyphenyl)ethanone:
Please refer Reaction scheme page No:

Step-2:
Preparation of 2-methyl-1, 4-dimethoxybenzene:
Please refer Reaction scheme page No:

Step-3:
Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)ethanone:
Please refer Reaction scheme page No:

Step-4:
Preparation of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one (1a-f):
Please refer Reaction scheme page No:
Step-5:

Preparation of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(2-choro phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone (2.4d):

To a solution of 1-(4-methyl-2, 5-dimethoxyphenyl)-3-(2-chlorophenyl) prop-2-en-1-one (0.01 mol) in 50 ml of ethanol was stirred at room temperature. 4-methylbenzohydrazide (99%) [0.05 mol] was added drop wise at room temperature then reaction mixture was refluxed for 8-10 hours. The reaction was monitored by TLC. After reaction complies, reaction mixture was poured in water and it was kept for 24 hours. The resulting solid obtained was filtered, washed with water, dried and crystallized from methanol to give white needles. \textbf{Rf value:} 0.74, \textbf{Yield:} 56\%, \textbf{M.P.-} 155^\circ C.

\textbf{Analysis: }\text{C}_26\text{H}_25\text{ClN}_2\text{O}_3

<table>
<thead>
<tr>
<th>Calculated</th>
<th>C: 69.56%, H: 5.61%, N: 6.24%, X: 7.90 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found</td>
<td>C: 69.55%, H: 5.66%, N: 6.21%, X: 7.93 %</td>
</tr>
</tbody>
</table>

Similarly other 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone (2.4a-f) were prepared. The physical data are recorded in \textbf{Table: 2.4}.
Table : 2.4

Physical data 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone (2.4 a-f):

<table>
<thead>
<tr>
<th>No.</th>
<th>-R</th>
<th>Molecular Formula (M.W)</th>
<th>Mp °C</th>
<th>Rf</th>
<th>% of Yield</th>
<th>% of C (Calc.) Found</th>
<th>% of C (Found)</th>
<th>% of H (Calc.) Found</th>
<th>% of H (Found)</th>
<th>% of N (Calc.) Found</th>
<th>% of N (Found)</th>
<th>% of X (Calc.) Found</th>
<th>% of X (Found)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4a</td>
<td>[OCH₃]</td>
<td>C₂₀H₁₃ClN₂O₅ (523.5)</td>
<td>138</td>
<td>0.54</td>
<td>60</td>
<td>66.60</td>
<td>5.97</td>
<td>5.36</td>
<td>6.78</td>
<td></td>
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<td></td>
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<tr>
<td>2.4b</td>
<td>[Cl]</td>
<td>C₂₀H₁₃Cl₂N₂O₃ (483)</td>
<td>98</td>
<td>0.48</td>
<td>62</td>
<td>64.60</td>
<td>5.00</td>
<td>5.80</td>
<td>14.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4c</td>
<td>[Cl]</td>
<td>C₂₁H₂₅Cl₃N₃O₅ (509)</td>
<td>142</td>
<td>0.61</td>
<td>55</td>
<td>66.07</td>
<td>5.74</td>
<td>5.50</td>
<td>6.97</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2.4d</td>
<td>[Cl]</td>
<td>C₂₁H₂₅Cl₃N₃O₅ (449.5)</td>
<td>155</td>
<td>0.74</td>
<td>56</td>
<td>69.56</td>
<td>5.61</td>
<td>6.24</td>
<td>7.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4e</td>
<td>[Cl]</td>
<td>C₂₁H₂₅Cl₃N₃O₅ (495.5)</td>
<td>160</td>
<td>0.67</td>
<td>59</td>
<td>65.52</td>
<td>5.50</td>
<td>50.66</td>
<td>7.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4f</td>
<td>[Br]</td>
<td>C₂₁H₂₅BrN₂O₅ (540)</td>
<td>120</td>
<td>0.38</td>
<td>56</td>
<td>60.12</td>
<td>5.05</td>
<td>5.19</td>
<td>14.81</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* TLC Solvent System: - Benzene: Carbon Tetra Chloride: Ethyl acetate (5:4:1)
SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME HETEROCYCLIC DERIVATIVES

❖ Spectroscopic evaluation:

Spectroscopic analysis of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(substituted phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone (2.4 a-f):

❖ IR Spectra

The IR spectra of pyrazoline revealed the absence of $\alpha$, $\beta$-unsaturated carbonyl group, indicating involvement of this functionality in the formation of pyrazoline ring.

The IR spectra of compound 1- (3-(4-methyl-2, 5-dimethoxyphenyl)-5-(3-bromo-4-hydroxy-5-methoxyphenyl) - (4, 5-dihydropyrazol-1-yl) - (4-methylphenyl) methanone (2.4f) was confirmed by absorption of C=N, C-N and $\text{-CH}_2$-bands of pyrazoline ring.

On other hand, it contained characteristics absorption band at 1517 cm$^{-1}$ due to C=N of pyrazoline ring. It was also observed that $\text{-CH}_2$ ring stretching at 2839 cm$^{-1}$. The IR spectra of pyrazoline exhibited peak at around 1227 cm$^{-1}$ indicating the presence to C-N stretch vibration of the pyrazoline ring.IR spectra of pyrazoline showed N- C=O stretching vibration at 1608 cm$^{-1}$. Band of halogen displayed at 457 cm$^{-1}$.Due to ether linkage(C-O-C), two band appeared in the range of 1275-1200 cm$^{-1}$ and 1089-1020 cm$^{-1}$ and multi substituted benzene ring gives out of plane deformation at 900-860 cm$^{-1}$ range. Hydroxyl group showed absorption band at 3747 cm$^{-1}$[41-43].

In addition to above mentioned peaks, spectrum of (2.4f) consists other stretching and bending vibration common to all compound under study. The IR spectrum of the compound is given on page no.116.

❖ NMR Spectra

The PMR spectrum of butyryl pyrazoline derivative compound 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(2-chloro-4-hydroxy-5-methoxy phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone (2.3d)showed resonance due to two magnetically non–equivalent geminal protons attached at C$_4$ and one vicinal
proton at C₅ showed up in the form of three triplet exhibiting a typical splitting pattern of ABX system.

The first triplet of Hₐ at C₄ was discernible at 3.067-3.126 δppm, signal due to Hₐ also resonated as double doublet at around at 3.816-3.941 δppm. and the signal of –OCH₃ are merged with this protons. Singlet of –OCH₃ showed at 3.752 δppm. Signal showed up as double doublet at 5.68 δppm attributed Hₓ. The three protons of -CH₃ group appeared as a singlet at 2.26 and 2.34 δppm.respectivevly.

The aromatic region has eight protons of phenyl ring, which showed as multiplet 6.5662-7.557 The ¹H NMR of the compound (2.4d) is given on page no.117.
IR spectra of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(3-bromo-4-hydroxy-5-methoxy phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone (2.4f):

IR ($\text{cm}^{-1}$) : 2973 (C-H str (asym) alkyl), 1459 (C-H def (asym) alkyl), 1313 (C-H def (sym) alkyl), 3033 (C-H str.arom.), 1183 (C-H i.p.def arom.), 1591 (C=C str arom.), 878 (C-H o.o.p.def.arom.), 1263 (C-O-C (sym) ether), 1088 (C-O-C (asym) ether), 1517(C=N, Pyrazoline), 1608(C=Ostr.), 2839(C-H ring str., Pyrazoline), 639(C-H def of –CH$_2$, Pyrazoline), 1227(C-N str., Pyrazoline), 3647(O-H str.), 457(C-Brstr.).
$^1$HNMR spectra of 1- (3-(4-methyl-2, 5-dimethoxy-phenyl)-5-(3-chloro-4-hydroxy-5-methoxy phenyl)- 4, 5-dihydropyrazol-1-yl)-(4-methylphenyl) methanone (2.4d):

$^1$H NMR (CDCl$_3$) $\delta$ppm: 2.26(s, 3H), 2.34(s, 3H), 3.067-3.126 (dd, 1H, pyrazoline), 3.752(s, OCH$_3$), 3.816-3.941 (dd, 1H, pyrazoline+ 6H, OCH$_3$), 5.04(s, OH), 5.68(dd, 1H, pyrazoline), 6.566 (s, 1H), 6.74(s, 1H), 7.04(d, 1H), 7.225-7.377(m, 5H, Ar-H).
Biological Evaluation

Antibacterial and Antifungal Activities:

Method: Broth dilution method [44]

Gram-positive bacteria:
- *Staphylococcus aureus* (Gram-positive) MTCC-96
- *Streptococcus pyogenes* (Gram-positive) MTCC-442

Gram-negative bacteria:
- *Pseudomonas aeruginosa* (Gram-negative) MTCC-1688

Interpretation of Results:
- *Escherichia coli* (Gram-negative) MTCC-443
  - 6.25, 12.5 = excellent active, 25 = good active,
  - 50 = moderate active, 100 = poor active, 100< inactive

Fungi:
- *Candida albicans* MTCC-227

Interpretation of Results:
- 100 = excellent active, 125 = good active,
- 200 = poor active, 200< inactive

Concentration: 6.25 μg/ml

Solvent: DMSO

Standard drugs: Gentamycin and K.Nystatin
Six compounds have been synthesized in this series, this series contain 4-methyl, 2, 5di-methoxyphenyl pyrozoline nucleus as a basic pharmacofore. Variations was made to the phenyl nucleus, with various substituent attached to position-2 to the basic pharmacofore. Modification was made at N-1 position with N-CO-C₆H₅4-methyl group.

All these synthesized compounds were screened for their antimicrobial potential against different panel of organisms, i.e., *E.coli, P.aeruginosa, S.aureus, S. pyogenes* and antifungal strains *C. albicans*. The results of antimicrobial activities are depicted in following Table-2.4.1

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>-R</th>
<th>Bacterial activity</th>
<th>Fungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimal Inhibition Concentrations (MIC) in µg/ml</td>
<td>Minimal Inhibition Concentration (MIC) in µg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus MTCC 96</td>
<td>S. pyogenes MTCC 442</td>
</tr>
<tr>
<td>2.4a</td>
<td>OCH₃ OCH₂ CH₃ Cl</td>
<td>250 500 250 500</td>
<td>1000</td>
</tr>
<tr>
<td>2.4b</td>
<td>Cl Cl</td>
<td>12.5 500 250 500</td>
<td>100</td>
</tr>
<tr>
<td>2.4c</td>
<td>Cl OH OCH₃ CH₃</td>
<td>50 50 25 25</td>
<td>1000</td>
</tr>
<tr>
<td>2.4d</td>
<td>Cl Cl</td>
<td>500 250 500 250</td>
<td>125</td>
</tr>
<tr>
<td>2.4e</td>
<td>Cl OH OCH₃ CH₃</td>
<td>50 50 25 25</td>
<td>1000</td>
</tr>
<tr>
<td>2.4f</td>
<td>Br OH OCH₃ CH₃</td>
<td>50 50 25 25</td>
<td>1000</td>
</tr>
</tbody>
</table>

Interpretation of results

<table>
<thead>
<tr>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25, 12.5=excellent, 25=good, 50=mode-rate, 100=poor active, 100&lt; inactive.</td>
<td>100=excellent, 125=good, 200=poor active 200&lt; inactive.</td>
</tr>
</tbody>
</table>
Result and discussion:

Anti bacterial activity

The MIC value of anti bacterial activity revealed that they showed varying degrees of inhibition against the tested microorganisms.

The in vitro antibacterial evaluation of the tested compounds revealed that, compounds 2.4 c, e and 2.4 f exhibited a good antibacterial activity against both gram negative strains and showed moderate activity against both gram positive strains. Compound 2.4b showed excellent activity against gram positive bacterial stains S.aureus. In addition, compounds 2.4a, b and d showed moderate to poor activity against all bacterial strains.

No significant or comparable activity results have been observed for rest of other compounds up to concentration of 100 µg/ml. The overall results of antibacterial activity are mentioned in Table-2.4.1

Anti fungal activity

All the synthesized compounds were screened for their anti fungal activity. The MIC value of the test compound 2.4b showed excellent activity and 2.4d displayed good activity against C.albicans comparable to reference agents K Nystatin.

While the other compounds showed poor or no activity even at concentration of 200 µg/ml. The overall results of antifungal activity are mentioned in Table-2.4.1
References:


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