CHAPTER IV

BIOLOGICAL ACTIVITIES OF SOME NOVEL PYRAZOLINES

4.1 INTRODUCTION:

Heterocyclic compounds are a group of organic compounds containing rings in which one or more of the carbon atom is replaced by an atom other than carbon, usually nitrogen, oxygen, sulphur or other hetero atoms. Heterocyclic compounds containing nitrogen are most abundant with great biological applicability than those containing oxygen or sulphur. Pyrazolines as a class of nitrogen containing heterocyclic compounds have many medicinal applications.

Pyrazoline derivatives constitute an interesting class of organic compounds with diverse chemical and pharmacological applications. Several pyrazoline derivatives possess important pharmacological activities and therefore they are useful materials in drug research.

The pharmacological and antitumor activities of many compounds containing pyrazoline rings have been reviewed. They are reported to be potential extractants and powerful drugs.

It is also reported that many pyrazoline derivatives have found applications in both pharmaceutical and agrochemical fields. Pyrazolines used as pesticide coating material agents. Pyrazoline derivatives are also found useful for prevention and treatment or ischemic heart disease, angina, migraine and Parkinson’s disease. So pyrazolines have played a crucial part in the development or heterocyclic chemistry.

Pyrazolines have played an avoidable part in the development of theory in heterocyclic chemistry. They are useful as biodegradable agrochemicals.
Pyrazoline derivatives such as antipyrine, aminopyrine, and dipyrone are known as antipyretic and analgesic substances and their pharmacological action have been widely surveyed. They have are reported to their clinical application as NSAIDs\(^9\).

The discovery of this class of drugs provides an outstanding case history of modern drug development and also points out the unpredictability of biological activity from structural modification of a prototype drug molecule.

Survey of literature in the recent past reveals that some pyrazoline derivatives possess cerebroprotective effect\(^10\).

As evident from the literature, in recent years a significant portion of research work in heterocyclic chemistry has been devoted to pyrazolines containing different aryl groups as substituents. Among a wide variety of heterocyclic compounds that have been explored for developing biologically active molecules pyrazolines have played an important role in medicinal chemistry.

Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. They have several prominent effects, such as antibacterial\(^11-12\), antifungal\(^13-14\), antihistaminic\(^15\), antimycobacterial\(^16\), antinociceptive activities\(^17\), anticancer\(^18\), antitumor\(^19\), analgesic\(^20\), antihapatotoxic activity\(^21\), anticonvulsant\(^22\), Cholesterol inhibitory activity\(^23\), antiproliferative activity\(^24\), antitubercular\(^25\), antiamoebic\(^26\), cardiovascular activity\(^27\), anthelmentic\(^28\), antioxidant\(^29\), antidepressant\(^30\), immunosuppressive\(^31\), antidiabetic\(^32\), anti-inflammatory\(^33\), tranquilising\(^34\), musclerelaxant\(^35\), psychoanaleptic\(^36\), herbicidal\(^37\), insecticidal\(^38\). Recently these classes of compounds are reported to possess potential antiviral activity against flavivirus\(^39\) and HIV\(^40\).
Vaidya et al.\textsuperscript{41} synthesized several 3-substituted aryl-5-(3-substituted-2-naphthofuryl)-2-pyrazolines (1) and screened their antimicrobial activities.

\begin{center}
\includegraphics[width=0.5\textwidth]{1.png}
\end{center}

Hawaiz et al.\textsuperscript{42} synthesized a ten 3-[4-(benzyloxy)-3-(2-chlorophenylazo)-phenyl]-5-(substituted-phenyl)-1-substituted-2-pyrazolines. The pyrazoline derivatives were evaluated for their anti-bacterial activity. Baluja et al.\textsuperscript{43} synthesized ten 2-chloro-6-fluoro-3-[3-(4-substituedphenyl)-4, 5-dihydro-1H-pyrazol-5-yl] quinolones and evaluated their antibacterial activity. Shinde et al.\textsuperscript{44} synthesized a new series of 3, 5-diphenyl-2-pyrazolines. Sachchar et al.\textsuperscript{45} synthesized certain substituted pyrazoline derivatives and evaluated their antifungal activity.

Sorthiya et al.\textsuperscript{46} have synthesized and tested the antimicrobial activity of p-(2',5'-Dibromobenzenesulphonamido)-phenyl-5-aryl-1H/acetyl/phenyl-2-pyrazolines. Parekh et al.\textsuperscript{47} have prepared 1-acetyl-5-aryl-3-[3-(3,4-dihydro-2-methyl-4-one-3-quinazolinyl)-phenyl]-2-pyrazolines which possess antimicrobial activity. Sonarc et al.\textsuperscript{48} have synthesised-3-(2-acetoxy-4-methoxy phenyl)-5-(substituted phenyl)-pyrazolines and tested their antimicrobial activity. Dobaria and Co-workers\textsuperscript{49} has discovered pyrazolines bearing chloroquinoline nucleus which used as antimicrobial agents.

**Antidepressant activity**

Palaska et al.\textsuperscript{50} synthesized ten new 3, 5-Diphenyl-2-pyrazoline derivatives and evaluated their antidepressant activities.
Analgesic and Anti-inflammatory activity

Sahu et al.\textsuperscript{51} synthesized some novel pyrazoline derivatives and tested for their analgesic, anti-inflammatory and antimicrobial activity. Hiremath et al.\textsuperscript{52} have synthesized pyrazolines as analgesic, anti-inflammatory and antimicrobial agents. Adnan and Tarek\textsuperscript{53} have synthesized pyrazoline derivatives as anti-inflammatory and antimicrobial agents. Moreover, F.Manna and coworkers\textsuperscript{54} have described 1-acetyl-5-(2'-bromophenyl)-4,5-dihydro-3-(2'-hydroxyphenyl)-1H-pyrazolines and its derivatives which acts as potent anti-inflammatory, analgesic and antipyretic agents.

Antitumor activity

Recently Brozowski et al.\textsuperscript{55} have synthesised some pyrazolines and reported them as antitumor agents.

Cholesterol inhibitory activity

Jeong et al.\textsuperscript{56} synthesized a series of 3-(3,5-di-tert-butyl-4-hydroxyphenyl)-5-(di- or tri-substituted 4-hydroxyphenyl)-2-pyrazolines(2) and evaluated their inhibitory action on acyl-CoA: cholesterol acyltransferase.

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\end{center}

Cytotoxic activity

Bhat and co-workers\textsuperscript{57} synthesized pyrazoline derivatives, for their cytotoxic activity.
Anti convulsant activity

Sowmya et al. synthesized a series of substituted pyrazoline compounds. The synthesized compounds were screened for anti convulsant activity.

Antitubercular and Antimycobacterial activity

Chovatia et al. synthesized some 1-acetyl-3, 5-diphenyl-4, 5-dihydro-(1H)-pyrazoles derivatives and evaluated for their antitubercular and antimicrobial activities. Nimavat have synthesized 1-substituted 3-aryl-5-(3'-bromophenyl)-pyrazolines which shows anticancer, antitubercular and antimicrobial activity

Analgesic activity

Velmurugan et al. synthesized pyrazoline derivatives and screened for their analgesic activity by tail-flick method.

Antiepileptic activity

Ozdemir et al. synthesized twelve 1-N-substituted thiocarbamoyl-3-(2-furyl)-5-phenyl-2-pyrazoline derivatives and studied their antiepileptic activity.

Anticonvulsant agents

Guniz Kucukguzel et al. have synthesized pyrazolines as a anticonvulsant agents.

Hypotensive activity

Turan-Zitouni et al. synthesized some 1-(4-Arylthiazol-2-yl)-3, 5-diaryl-2-pyrazoline derivatives and investigated their hypotensive activity.
**Antioxidant activity**

Babu et al.\textsuperscript{65} synthesized a series of pyrazoline derivatives and evaluated for antioxidant activity.

**Antiamoebic activity**

Abid et al.\textsuperscript{66} synthesized new 1-N-substituted thiocarbamoyl-3-phenyl-2-pyrazoline derivatives and evaluated their in vitro antiamoebic activities.

**Steroidal Activity**

Zhang et al.\textsuperscript{67} designed and synthesized a novel series of pyrazolines and evaluated by in vivo screening as tissue selective androgen receptor modulators (SARMs).

**Antiandrogenic activity**

Amr et al.\textsuperscript{68} have screened some new 3-substituted pyrazolines and reported their antiandrogenic activity.

**Antidiabetic agents & DP-IV inhibitor activity**

Ahn et al.\textsuperscript{69} have described the synthesis of cyano-pyrazoline derivatives as potent antidiabetic agents and DP-IV inhibitor activity. Fuche Rainer et al.\textsuperscript{70} have prepared some new 1H-pyrazoline derivatives and reported them as pesticides. Furthermore, Tsubai et al.\textsuperscript{71} have synthesized some new (phenylcarbamoyl) pyrazolines as an insecticide. Nisha Singh and co-workers\textsuperscript{72} have synthesized 1-acetyl pyrazolines and reported them as potent pesticides and fungicides. Nesrin Gokhan et al.\textsuperscript{73} have synthesised new 1-N-substituted thiocarbomoyl-3-phenyl-5-thienyl-2-pyrazoline derivatives and evaluated their antidepressant, antiogenic and mammalian monoamine oxidase (MAO) - A & B inhibitory activities.

Novel 3, 5-diaryl pyrazolines have been discovered as low density lipoprotein (LDL) oxidation inhibitor by Tae-Sock et al.\textsuperscript{74}.
Thus interesting biological activities of a novel heterocyclic compounds like pyrazolines have stimulated considerable research work in recent years leading to the synthetic utility of the derivatives of this ring system. In our search for new potential antimicrobial compounds, the reaction of series chalcones with hydrazine hydrate under different conditions has been investigated and the pharmacological profiles of the compounds have been studied.

4.1.1 Antimicrobial activity

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoan. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microstatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body. Antimicrobials include not just antibiotics but synthetically formed compounds as well.

The discovery of antimicrobials like penicillin and tetracycline paved way for better health for millions around the world. Before penicillin became a viable medical treatment in early 1940’s, no true cure for gonorrhea, strep throat or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

However, with the development of antimicrobials, microorganisms have adopted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbes survive, change and become resistant to the poisons and / or heavy metals.
Antimicrobial nanotechnology is a recent addition to the fight against disease causing organisms, replacing heavy metals and toxins and may someday be a viable alternative.

### 4.1.2 Characteristics of microorganisms

#### Bacterial species:

**Gram positive Bacteria**
- (a) *Bacillus subtilis*
- (b) *Micrococcus luteus*
- (c) *Staphylococcus aureus*

**Gram negative Bacteria**
- (a) *Escherichia coli*
- (b) *Klebsiella pneumoniae*

#### Fungal species:
- (a) *Aspergillus niger*
- (b) *Mucor species*
- (c) *Trichoderma viride*

The characteristics of microorganisms used in the present investigation have been consolidated in chapter-II of this thesis.

### 4.2 MATERIALS AND METHODS

The chemicals namely nutrient broth, Mueller Hinton agar, potato dextrose agar, Tween-80 solution and other materials required have been procured from Hi media, Mumbai.

#### 4.2.1 Collection of Microorganisms

*Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Micrococcus luteus, Staphylococcus aureus, Aspergillus niger, Mucor species* and *Trichoderma viride* were procured from the Research department of Microbiology, Sengunthar Arts and Science College, Thiruchengode, Namakkal Dt., Tamilnadu. The stock cultures have been stored in the refrigerator for further studies.
4.2.2 Innoculum preparation

The nutrient broth was procured from Himedia, Mumbai. The nutrient broth was prepared by weighing 1.3 gram of the broth and dissolved it in 100 ml of sterile distilled water. The flask was swirled gently while adding the nutrient broth and the pH of the medium was adjusted to 7.0. The Erlemeyer flask was plugged with non-adsorbent cotton and sterilized in an autoclave at 121°C and 15 lbs/\text{inc}^2 pressure for 15 minutes. After cooling inside a laminar flow, a loopful of fresh bacterial sample was inoculated and incubated in an orbital shaker at 37°C for 24 hours. Then the cultures were diluted 1:50 with sterile physiological saline and 0.5 ml of the inoculum was used for the preparation of the spread plate. The same procedure has been adopted for all test bacterial samples.

4.2.3 Preparation of agar slants

Nutrients agar medium was prepared and sterilized in an autoclave at 121°C and 15 lbs/\text{inc}^2 pressure for 15 minutes. After sterilization the medium was dispensed into the test tubes. The test tubes were kept in the slanting position on a support. After complete solidification of the medium, streaking of the microorganism was done in the slant area using sterile inoculation loop. After the streaking the test tubes were incubated at 37°C for 24 hours. After good growth, the slants have been stored in a deep freezer (2°C) for further studies.

4.2.4 Preparation of Mueller Hinton agar plates

The Mueller Hinton agar of weight 38 gram was dissolved in 1000 ml of sterile distilled water. The pH of the medium was adjusted to 7.0. The flask was plugged with cotton and sterilized at 121°C and 15 lbs/\text{inc}^2 pressure for 15 minutes. After sterilization, the medium was cooled to 45-47°C, poured 15 ml of it in each sterile Petri-plate and allowed to solidify.
4.2.5 Preparation of test compound

The newly synthesized Chalcone compounds of weight 15 mg of each was dissolved in 1 ml of DMSO solvent. Using 100 µml solution, the discs were impregnated and placed on the Mueller Hinton solidified Agar medium to find out the antimicrobial activity of the compounds on each organism.

By adapting the above procedure, the antimicrobial activity of the five series of chalcones has been studied on six microorganisms and the results have been discussed.

4.2.6 Antibacterial sensitivity assay

Antibacterial sensitivity assay was performed using Kirby-Bauer disc diffusion technique. In each Petri plate about 0.5 ml of the test bacterial sample was spread uniformly over the solidified Mueller Hinton agar using sterile glass spreader. Then the discs with 5mm diameter made up of Whatman No.1 filter paper, impregnated with the solution of the compound were placed on the medium using sterile forceps. The plates were incubated for 24 hours at 37°C by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24 hours, the plates were visually examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

4.2.7 Preparation of the Potato dextrose agar medium

PDA agar medium was prepared in a conical flask by dissolving 3.9gram of the agar in 100ml distilled water. It was sterilized in the autoclave for 15min. at 121°C and 15 lbs/inch² pressure. Then the medium was allowed for solidification for an hour. After that the fungal species was inoculated in the medium and kept for 5 to 7 days at room temperature.
4.2.8 Preparation of the fungal inoculam

About 20 to 25 ml of sterile water (after cooling) is mixed with the medium. The water over the medium is swirled and decanted with the fungal species. Tween-80 (1 to 2 ml) may be added with this solution for uniform growth.

4.2.9 Antifungal sensitivity assay

Antifungal sensitivity assay was performed using Kirby-Bauer disc diffusion technique. PDA medium was prepared and sterilized as above. It was poured (ear being heated condition) in the Petri-plate which was already filled with 1 ml of the fungal species. The plate was rotated clockwise and counter clock-wise for uniform spreading of the species. The discs were impregnated with the test solution. The test solution was prepared by dissolving 15mg of the Chalcone in 1ml of DMSO solvent. The medium was allowed to solidify and kept for 24 hours. Then the plates were visually examined and the diameter values of zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

4.3 RESULTS AND DISCUSSION

4.3.1 Antibacterial Activity

The antibacterial effect of the pyrazolines in series-D is given in Fig- 4.1 (Plates 4.1-4.10). The observed zones of inhibition are given in Table 4.1 and the corresponding clustered column chart is given in Fig- 4.2. The pyrazolines with 4-F, 2-CH$_3$ and 4-NO$_2$ substituents have shown moderate activity against all the microorganism in the present investigation. The rest of the compounds in series-D have shown weak activity against all the microorganism in the present investigation.
The antibacterial screening effect of the pyrazolines in series-E is given in Fig-4.3(Plates 4.11- 4.20). The observed zones of inhibition are given in Table 4.2 and the corresponding clustered column chart is given in Fig- 4.4. All the pyrazolines in series-E have shown moderate activity against all the five microorganisms used in the present investigation. The compounds with 4-Br, 4-CH$_3$ and 4-NO$_2$ substituents have shown moderate activity against all the microorganism in the present investigation. The pyrazolines compounds with 4-CH$_3$ and 4-NO$_2$ substituents have shown equal effect against $E$.coli in series-F.

The antibacterial effect of the substituted pyrazolines in Series-F is given in Fig-4.5(Plates 4.21-4.30) and the corresponding clustered column chart is given in Fig- 4.6. It reveals that the pyrazolines with 3-OH and 2-OCH$_3$ substituents have shown improved activity against the all the five microorganisms in the present investigation. This pyrazolines with 2-Cl and 4-CH$_3$ substituents have shown very good activities against $S$.aureus.
Fig-4.1: Antibacterial activity of 3-(5-chlorothiophen-2-yl)-4,5-dihydro-5-substituedphenyl-1H-pyrazoles: Series-D
### Table 4.1

Zone of inhibition (nm) values of antibacterial activity of 3-(5-chlorothiophen-2-yl)-4,5-dihydro-5-substituedphenyl-1H-pyrazoles: Series-D

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<th>Substituent</th>
<th>Zone of Inhibition (mm)</th>
<th>Gram positive Bacteria</th>
<th>Gram negative Bacteria</th>
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<td>M.luteus</td>
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Sereies-D
Antibacterial activity of 3-(5-chlorothiophen-2-yl)-4,5-dihydro-5-substituedphenyl-1H-pyrazoles

Fig-4.2

1. H
2. 3-Br
3. 3-Cl
4. 2-F
5. 4-F
6. 4-OH
7. 2-OCH₃
8. 4-OCH₃
9. 2-CH₃
10. 4-CH₃
11. 4-NO₂
12. 3-OC₆H₅

Zone of inhibition (mm)

B.subtilis
M.luteus
S.aureus
E.coli
K.pneumoniae
Fig-4.3: Antibacterial activity of 1-(4-(4,5-dihydro-5-substitutedphenyl-1H-pyrazol-3-yl)phenyl)piperidines: Series-E
Table 4.2

Zone of inhibition (nm) values of antibacterial activity of 1-(4-(4,5-dihydro-5-substitutedphenyl-1H-pyrazol-3-yl)phenyl)piperidines: Series -E

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Series-E
Antibacterial activity of 1-(4-(4,5-dihydro-5-substitutedphenyl-1H-pyrazol-3-yl)phenyl)piperidines

Zone of Inhibition (mm)

1. H
2. 3-Br
3. 4-Br
4. 4-Cl
5. 2-F
6. 4-F
7. 3-OH
8. 2-OCH₃
9. 4-CH₃
10. 3-NO₂
11. 4-NO₂
12. 3-OC₆H₅

1-4(4,5-dihydro-5-substitutedphenyl-1H-pyrazol-3-yl)phenyl)piperidines

Fig 4.4
Fig-4.5: Antibacterial activity of 2-(5-substituedphenyl-4,5-dihydro-1H-pyrazol-3-yl)-10H-phenothiazines: Series-F
Table 4.3

Zone of inhibition (nm) values of antibacterial activity of 2-(5-substituedphenyl-4,5-dihydro-1H-pyrazol-3-yl)-10H-phenothiazines: Series-F

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<td>B.subtilis</td>
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<tr>
<td></td>
<td>Control DMSO</td>
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328
Series-F
Antibacterial activity of 2-(5-substituedphenyl-4,5-dihydro-1H-pyrazol-3-yl)-10H-phenothiazines

Diagram showing the antibacterial activity of different compounds with varying substituents. The compounds are numbered 1 to 12, and the substituents include H, Br, Cl, F, OH, OCH₃, CH₃, NO₂, and 3-NO₂.

Fig 4.6

Zone of Inhibition (mm)
4.3.2 Antifungal Activity:

The antifungal activity of substituted pyrazolines in series-D is given in Fig.- 4.7 (Plates 4.31- 3.36). The observed zones of inhibition are given in Table-4.4 and corresponding clustered column chart is given in Fig-4.8. It reveals that all the pyrazoline compounds in series-D have shown moderate antifungal activity against *A. niger*, *Mucor species* and *T. viride*. The Pyrazolines with 3-Cl, 2-OCH$_3$ and 2-OCH$_3$ substituents have shown greater antifungal activity when compared to other compounds in the series-D.

The antifungal activity of substituted pyrazolines in the series-E is given in Fig-9 (Plates 4.37- 4.42). The observed zones of inhibition are given in Table-4.9 and corresponding clustered column chart is given in Fig-4.10. It reveals that all the compounds in series-E have shown moderate activity against all the three fungal species. The pyrazoline with H, 3-Br, 2-OCH$_3$ and 3-OC$_6$H$_5$ substituents have shown greater antifungal activity when compared to other compounds in the series-E.

The antifungal activity of substituted pyrazolines in the series-F is shown in Fig-10 (Plates 4.43- 4.48). The observed zones of inhibition are given in Table- 4.11 and corresponding clustered column chart is given in Fig-4.12. It reveals that all the compounds have shown moderate antifungal activity against *A. niger*, *Mucor species*, *T. viride*. The Pyrazolines with 2-OCH$_3$ and 4OCH$_3$ substituents have shown greater antifungal activity when compared to other compounds in the series-F.
Fig-4.7: Antifungal activity of 3-(5-chlorothiophen-2-yl)-4,5-dihydro-5-substituedphenyl-1H-pyrazoles: Series-D
Table-4.4
Zone of inhibition (nm) values of antibacterial activity of
3-(5-chlorothiophen-2-yl)-4,5-dihydro-5-substituedphenyl-1H-pyrazoles:
Series-D

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Substituent</th>
<th>Zone of Inhibition (mm)</th>
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<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A.niger</td>
<td>M.spp.</td>
<td>T.viride</td>
</tr>
<tr>
<td>1</td>
<td>H</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>3-Br</td>
<td>-</td>
<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3-Cl</td>
<td>-</td>
<td>6</td>
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</tr>
<tr>
<td>4</td>
<td>2-F</td>
<td>7</td>
<td>10</td>
<td>9</td>
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</tr>
<tr>
<td>5</td>
<td>4-F</td>
<td>7</td>
<td>12</td>
<td>10</td>
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</tr>
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<td>4-OH</td>
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<td>9</td>
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<td>-</td>
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<td>2-CH₃</td>
<td>7</td>
<td>9</td>
<td>-</td>
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</tr>
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<td>10</td>
<td>4-CH₃</td>
<td>-</td>
<td>8</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>12</td>
<td>3-OC₆H₅</td>
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</tr>
<tr>
<td>Standard</td>
<td>Miconazole</td>
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<td>18</td>
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<td>Control</td>
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Series-D
Antifungal activity of 3-(5-chlorothiophen-2-yI)-4,5-dihydro-5-substituedphenyl-1H-pyrazoles

Fig-4.8
Fig-4.9: Antifungal activity of 1-(4-(4,5-dihydro-5-substituted phenyl-1H-pyrazol-3-yl)phenyl)piperidines: Series-E
Table-4.5
Zone of inhibition (nm) values of antifungal activity of of 1-(4-(4, 5-dihydro-5-substitutedphenyl-1H-pyrazol-3-yl)phenyl)piperidines: Series-E

<table>
<thead>
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<th>Substituent</th>
<th>Zone of Inhibition (mm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>A.niger</strong></td>
</tr>
<tr>
<td>1</td>
<td>H</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>3-Br</td>
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<td>4-Br</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>4-Cl</td>
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</tr>
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<tr>
<td>7</td>
<td>3-OH</td>
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<td>8</td>
<td>2-OCH₃</td>
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<td>4-CH₃</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>3-NO₂</td>
<td>5</td>
</tr>
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<td>11</td>
<td>4-NO₂</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td>3-OC₆H₅</td>
<td>8</td>
</tr>
<tr>
<td>Standard</td>
<td>Miconazole</td>
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<tr>
<td>Control</td>
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</table>
Series-E
Antifungal activity of 1-(4-(4,5-dihydro-5-substitutedphenyl-1H-pyrazol-3-yl)phenyl)piperidines

1. H
2. 3-Br
3. 4-Br
4. 4-Cl
5. 2-F
6. 4-F
7. 3-OH
8. 2-OCH₃
9. 4-CH₃
10. 3-NO₂
11. 4-NO₂
12. 3-OC₆H₅

Zone of inhibition (mm)

1-(4-(4,5-dihydro-5-substitutedphenyl-1H-pyrazol-3-yl)phenyl)piperidines
Fig 4.10
Fig. 4.11: Antifungal activity of 2-(5-substitutedphenyl-4,5-dihydro-1H-pyrazol-3-yl)-10H-phenothiazines: Series-F
Table 4.6
Zone of inhibition (nm) values of antibacterial activity of 2-(5-substituedphenyl-4, 5-dihydro-1H-pyrazol-3-yl)-10H-phenothiazines: Series-F

<table>
<thead>
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<th>S.NO.</th>
<th>Substituent</th>
<th>Zone of Inhibition (mm)</th>
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</thead>
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<td></td>
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<td>A.niger</td>
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<td>8</td>
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</tr>
<tr>
<td>11</td>
<td>2-NO₂</td>
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</tr>
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<td>Standard</td>
<td>Miconazole</td>
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</table>
Series-F
Antifungal activity of 2-(5-substitutedphenyl-4,5-dihydro-1H-pyrazol-3-yl)-10H-phenothiazines

Fig-4.12
4.4 SCOPE OF THE PRESENT WORK

Palaska et al.\textsuperscript{50} synthesized ten new 3, 5-Diphenyl-2-pyrazoline derivatives and evaluated their antidepressant activities by the ‘Porsolt Behavioral Despair Test’ on Swiss-Webster mice.

Chovatia et al.\textsuperscript{59} synthesized some 1-acetyl-3,5-diphenyl-4,5-dihydro-(1\textsubscript{H})-pyrazoles derivatives and evaluated for their antitubercular and antimicrobial activities. Nimavat\textsuperscript{60} have synthesized 1-substituted 3-aryl-5-(3’-bromophenyl)-pyrazolines which shows anticancer, antitubercular and antimicrobial activity.

Jeong et al.\textsuperscript{56} synthesized a series of 3-(3,5-di-tert-butyl-4-hydroxyphenyl)-5-(di-or tri-substituted 4-hydroxyphenyl)-2-pyrazolines and evaluated their inhibitory action on acyl-CoA: cholesterol acyltransferase.

Sowmya et al.\textsuperscript{58} synthesized a series of substituted pyrazoline compounds. The synthesized compounds were screened for anti convulsant activity.

Ahn et al.\textsuperscript{69} have described the synthesis of cyano-pyrazoline derivatives as potent antidiabetic agents and DP-IV inhibitor activity.

Nesrin Gokhan et al.\textsuperscript{73} have synthesised new 1-N-substituted thiocarbomoyl-3-phenyl-5-thienyl-2-pyrazoline derivatives and evaluated their antidepressant, antiogenic and mammalian monoamine oxidase (MAO) - A & B inhibitory activities.

Some of the synthesized substituted pyrazolines may be studied for such activities and thereby they may be utilized in drug chemistry.
4.5 CONCLUSION:

All the synthesized pyrazolines (series-D, E and F) have been screened for antibacterial activity against certain bacteria namely *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* and antifungal activity against certain fungi namely *Aspergillus niger*, *Mucor species* and *Trichoderma viride*.

**Antibacterial activity:**

In series-D, the pyrazolines with substituents 4-F, 2-CH$_3$ and 4-NO$_2$ have moderate activity against the all the microorganisms used in the present investigation. The rest of the compounds have shown weak activity against them. However the activities of the compounds in series-D are found to be lesser than that of the standard antibacterial agent used.

In series-E, pyrazolines with 4-Br, 4-CH$_3$ and 4-NO$_2$ substituents exhibit moderate inhibition against the all the microorganisms in the present investigation. The pyrazolines with 4-CH$_3$ and 4-NO$_2$ substituents have equal effect against *E.coli* in series-E.

In series-F, all the pyrazolines compounds with 3-OH, 2-OCH$_3$ substituents have excellent and improved antibacterial activity against the all the five microorganisms used in the present investigations. The pyrazolines with 2-Cl and 4-CH$_3$ showed very good activities against *S.aureus* in series-F.

In the present study of the antibacterial activity, the compounds with methyl and nitro substituent have shown greater significance in all pyrazolines (series-D, E and F).
Antifungal activity:

In series-D, the pyrazolines with 3-Cl, 2-OCH₃ and 2-OCH₃ substituents have shown greater antifungal activity against all the fungal species A.niger, Mucor species and T. viride.

In series-E, the pyrazolines with H, 3-Br, 2-OCH₃ and 3-OC₆H₅ substituents have shown greater antifungal activity when compared to other compounds present in the series.

In series-F, the pyrazolines with 2-OCH₃ and 4OCH₃ substituents have shown greater antifungal activity when compared to other compounds present in the series.

In the present study it is observed that the antifungal activity of pyrazolines with chloro, bromo and methoxy substituents have shown greater significance in all the series (series-D, E and F).
4.6 REFERENCES:


7. Maurer, Fricz, Rainer; Erdelen, Christoph, Turberg, and Andreas; *PCT Int. Appl. 2002, WO 03 59 887 (Cl. C07 D231/28); Chem. Abstr., 2003*, 139(8), 861, 117441z.


58. H. G. Sowmya and Dr. Chandrashekar javali, *IJPWR VOL 3 ISSUE 2 (Mar-June) - 2012*.


