CHAPTER-IV

Synthesis of Pyrazoles by oxidative aromatization using SiO$_2$-HNO$_3$ and their antimicrobial activity

- Introduction
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- Experimental
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4.1 Introduction

Pyrazoles are extensively found as core motifs in a variety of compounds, having diverse applications such as pharmaceutical agents, agrochemicals, pesticides, dyestuffs and building blocks of other compounds. The attractiveness of pyrazoles and their derivatives is due to their versatility that allows for synthesis of a series of analogues with different moieties in them. The description of pyrazole 4.2 was first made by Buchner in 1889 and it was synthesized by the decarboxylation of pyrazole-3,4,5-tricarboxylic acid\(^1\) 4.1.

\[
\begin{align*}
\text{HOOC} & \quad \text{N} \quad \text{COOH} \\
\text{HOOC} & \quad \text{N} \quad \text{COOH} \quad \text{Heat} \quad \rightarrow \\
\text{N} & \quad \text{COOH} \\
\text{N} & \quad \text{COOH} \\
\text{3CO}_2 & 
\end{align*}
\]

However, in 1954, Kosuge and Okeda\(^2\) extracted the first natural pyrazole derivative 3(5)-nonylpyrazole 4.3 from a plant which is called as *Houttuynia cordata*. The importance of this pyrazole derivative is due to its antimicrobial activity. Another natural pyrazole derivative which is *levo-\(\beta\)-1-pyrazolyl)alanine* 4.4 (an isomer of histidine) was isolated from *Citrus vulgaris* (watermelon seeds) (Figure 4.1). It is the first example of pyrazole containing natural product which possesses antidiabetic activity\(^4\). Examples of some other pyrazole containing natural products\(^5\) are *L-\(\alpha\)-amino-\(\beta\)-(pyrazolyl-N)-propanoic acid* 4.5, pyrazofurin 4.6, pyrazofurin B 4.7, pyrazole-3(5)carboxylic acid 4.8, 4-methyl-3(5)carboxylic acid 4.9 etc.

![Figure 1: Pyrazole containing natural products](image-url)
Appearance of large number of research articles during last decade further shows the keen interest of synthetic as well as medicinal chemists in pyrazole related compounds. Pyrazole is found as a pharmacophore in many bioactive molecules which act as anti-inflammatory, anticancer, anticoagulant, antimicrobial, analgesic, antidiabetic, hypoglycaemic, anticonvulsant, psychoanaleptic, antibacterial, antiallergic, antiangiogenic and antimitotic agents. Examples of leading commercial drugs based on pyrazole scaffold are selective COX-2 inhibitor, non-steroidal anti-inflammatory drugs, celecoxib; deracoxib; rimonabant and tepoxalin; anorectic antiobesity drug-lonazolac; anxiolytic drug-mepiprazole and cardiovascular drug zoniporide; anticancer drug-crizotinib; inverse antagonist on serotonin receptor-nelotanserin and temanogrel.
The pyrazole moiety is also present in many important agrochemicals, such as insecticides\textsuperscript{26-28}–fenpyroximate 4.20, fipronil 4.21 and tebufenpyrad 4.22; pesticides\textsuperscript{29} cyenopyrafen 4.23. Some pyrazoles display herbicidal and fungicidal activity\textsuperscript{30} such as azimsulfuron 4.24 and RPA406194 4.25, respectively.

\textbf{Figure 2:} Commercially leading drugs based on pyrazole

\textbf{Figure 3:} Agrochemically significant compounds with pyrazole moiety
Pyrazoles are also found as a key constituent of ligands for transition metals, receptor for supramolecular chemistry & liquid crystals and cotton azo dyes\textsuperscript{31-34}. 4.26.

Due to the wide range of applications of pyrazole in medicinal, agriculture and industry, the search of new synthetic routes have been received much attention. A number of methods for the synthesis of pyrazoles and its derivatives have been reported in literature. A few of them are mentioned below;

1. **From Diketones**: Heller \textit{et al}\textsuperscript{35} reported the synthesis of pyrazoles from hydrazine and 1,3-diketones 4.29. These diketones were synthesized from ketones and acid chlorides.

\begin{align*}
\text{R}^1\text{O}\text{Li} + \text{R}^2\text{OCl} & \rightarrow \text{R}^1\text{O} \text{R}^2 \text{O} \\
\text{4.27} + \text{4.28} & \rightarrow \text{4.29} \\
\text{R}^1\text{O} \text{R}^2 \text{O} & \rightarrow \text{R}^1\text{N} \text{N} \text{H} \text{N} \text{H} \text{R}^4 \\
\text{CH}_3\text{COOH} & \rightarrow \text{4.30} \\
\end{align*}

\text{MeO}\text{O} \text{O} \text{Me} \text{O} \text{Me} \text{MeO} \text{Me} \\
\text{4.31} \text{Allyltrimethylsilane} \text{CAN, MeCN} \\
\text{MeO}\text{O} \text{Me} \text{MeO} \text{Me} \text{4.32} \\
\text{4.32} \text{CAN, MeCN} \text{p-O\text{Me}C}_6\text{H}_4\text{N} \text{H} \text{N} \text{H} \text{R}^4 \\
\text{MeO}\text{O} \text{O} \text{Me} \text{MeO} \text{Me} \text{4.33}
Devery Iii et al\textsuperscript{36} also reported a good method which involves treatment of 1,3-diketone 4.31 and allyltrimethylsilane using ceric ammonium nitrate (CAN) followed by addition of hydrazine catalysed by cerium to synthesize pyrazole 4.33.

2. From Hydrazones: One of the reported methods for synthesis of pyrazoles 4.36 involves oxidative cyclization of hydrazones 4.35 of chalcones 4.34 in presence of DDQ\textsuperscript{37}, thianthrene cation radical\textsuperscript{38}, t-BuOK\textsuperscript{39}, iodobenzenediacetate\textsuperscript{40}, magnesium dioxide\textsuperscript{41}, lead tetraacetate\textsuperscript{42} and anodic oxidation\textsuperscript{43}.

![Chemical Reaction](image1)

3. By dipolar cycloaddition reaction: A number of methods have been reported for the synthesis of pyrazoles by dipolar cycloaddition reactions using diazo compounds. In 1979, Fields et al\textsuperscript{44} carried out 1,3-dipolar cycloaddition of 2,2,2-trifluorodiazoethane 4.37 to a series of alkynes to obtain good yields of corresponding pyrazoles 4.39 and 4.40.

![Chemical Reaction](image2)

Perez-Aguilar et al\textsuperscript{45} reported reaction of tosylhydrazone 4.41 and phenylacetylene 4.42 in 1,4-dioxane to obtain pyrazoles 4.43. Qi et al\textsuperscript{46} carried out copper promoted cycloaddition of diazocarbonyl compounds and acetylenes.
4. From pyrazolines: The another important approach for the synthesis of pyrazoles 4.48 involves initial condensation of hydrazines with chalcones 4.46 or synthetic equivalents to get pyrazolines 4.47 followed by oxidation in presence of various reagents.

For the oxidative aromatization of 2-pyrazolines, a plethora of reagents have been reported such as Zr(NO₃)₄, Bi(NO₃)₃, Pd/C, AgNO₃, iodobenzene diacetate, Pb(OAc)₄, Co(II) and O₂, MnO₂, KMnO₄, HgO, I₂O₅/KBr, HAuCl₄, CAN, Fe(NO₃)₃, SiO₂-H₂SO₄.

All of these methods suffer from a number of limitations such as the use of toxic organic solvents, reagents embedded with toxic metal ions and expensive catalyst, moderate to reduced yields, drastic reaction conditions like prolonged reaction time and high reaction temperature, tedious and multistep procedure. Furthermore, these reactions are of less importance for synthesis of pyrazoles of the hydrazines with electron-withdrawing groups such as 4-nitrophenyl and 2,4-dinitrophenyl, which do not cyclize to 4,5-dihydropyrazoles and very few examples of 1,3,5-triphenylpyrazoles with nitro substituent on N-phenyl ring are known. Therefore, this transformation still has challenge for organic chemist to improve the reaction time and yield, to reduce the use of toxic organic solvents and reagents and to establish an economically benign procedure. In the previous chapter we studied the economic, simple and convenient synthesis of 2-aryl/heteroaryl/styryl/alkyl benzothiazoles using SiO₂-HNO₃.

Recently in our lab, synthesis of substituted diethyl 4-(2-chlorquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates from substituted diethyl-4-(2-chlorquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylates (1,4-DHPs) has been carried out by
using SiO$_2$–HNO$_3$ as oxidising agent. In continuation of our ongoing research to explore the applications of supported reagent SiO$_2$-HNO$_3$ which is cheap, eco-friendly, easy to prepare, we have planned to carry out the oxidative aromatization of 1,3,5-trisubstituted 2-pyrazolines 4.52(a-i) using this reagent.

![Chemical structure](image)

4.52(a-i) 4.53(a,c,e-i) 4.54(a-i)

a. $R^1 = H$, $R^2 = H$  
   f. $R^1 = OCH_3$, $R^2 = OH$

b. $R^1 = H$, $R^2 = CH_3$  
   g. $R^1 = OCH_3$, $R^2 = Cl$

c. $R^1 = H$, $R^2 = OCH_3$  
   h. $R^1 = Br$, $R^2 = Cl$

d. $R^1 = H$, $R^2 = 3,4,5-(OCH_3)_3$  
   i. $R^1 = Br$, $R^2 = Br$

e. $R^1 = H$, $R^2 = Cl$
4.2 Result and discussion

The target compounds 4,5-dihydro-1,3,5-triaryl-1H-pyrazole 4.52(a-i) were synthesized by the following synthetic protocol. Initially, the reaction of acetophenones 4.49 with aromatic aldehydes 4.50 in presence of KOH was carried out to obtain chalcones 4.51(a-i).

These compounds 4.51(a-i) on condensation with phenylhydrazine in acetic acid gave 4,5-dihydro-1,3,5-triaryl-1H-pyrazoles 4.52(a-i). The structures of these compounds were ascertained through their spectral parameters. The IR \textsuperscript{1}H-NMR spectral data of 4,5-dihydro-1,3,5-triaryl-1H-pyrazoles 4.52(a-c, g, h) overlapped with data reported in the literature while structures of other substrates 4.52(d, f, i) were arrived at by interpreting their NMR data. As a representative example, the formation of 5-(4-chlorophenyl)-4,5-dihydro-1,3-diphenyl-1H-pyrazole 4.52e was confirmed by appearance of three dd at \( \delta \) 3.12 (\( J = 7.2 \text{ Hz}, J = 17.1 \text{ Hz} \)), 3.86 (\( J = 12.3 \text{ Hz}, J = 17.1 \text{ Hz} \)) and 5.27 (\( J = 7.2 \text{ Hz}, J = 12.3 \text{ Hz} \)) due to H-4a, H-4b and H-5 protons of 4,5-dihydro pyrazole ring. The fourteen aromatic protons gave signals at \( \delta \) 7.74 (2H, d, \( J = 7.2 \text{ Hz} \)), 7.44-7.24 (7H, m), 7.20 (2H, d, \( J = 7.2 \text{ Hz} \)), 7.09 (2H, d, \( J = 7.8 \text{ Hz} \)), 6.82 (1H, d, \( J = 7.8 \text{ Hz} \)).

Silica supported nitric acid SiO\textsubscript{2}-HNO\textsubscript{3} is generally used as an oxidising agent and to check the oxidative potential of this reagent here we have tried the oxidation of 4,5-dihydro-1H-pyrazoles 4.52(a-i) under different sets of conditions (Scheme 2).
Scheme 2: Transfromation of 5-(4-chlorophenyl)-4,5-dihydro-1,3-diphenyl-1H-pyrazole into pyrazoles

Initially, 5-(4-chlorophenyl)-4,5-dihydro-1,3-diphenyl-1H-pyrazole 4.52e was chosen as model substrate. In this attempt, reaction of 5-(4-chlorophenyl)-4,5-dihydro-1,3-diphenyl-1H-pyrazole (1 mmol) in dichloromethane with SiO\(_2\)-HNO\(_3\) (300 wt%, 3 times wt of pyrazoline) was performed at room temperatures (Table 1, entry 7). The reaction ensued with appearance of wine red colour which changed to yellow colour with the progress of reaction. The progress of reaction was monitored on TLC and the starting material disappeared within five minute. The reaction was quenched with ice cold 5% aqueous solution of NaHCO\(_3\) and extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel using pet.ether-ethylacetate mixture (as eluent) with increasing proportions of ethylacetate. Serendipitously, two oxidized products (in 93% yield)-5-(4''-chlorophenyl)-1,3-diphenylpyrazole 4.53e (12% yield, a minor product) and 5-(4''-chlorophenyl)-1-(4-nitrophenyl)-3-phenylpyrazole 4.54e (80% yield, a major product) were produced. To study the effect of temperature, the reaction was performed at 0 °C. But both products 5-(4''-chlorophenyl)-1,3-diphenylpyrazole 4.53e (9% yield) and 5-(4''-chlorophenyl)-1-(4-nitrophenyl)-3-phenylpyrazole 4.54e (75% yield) was formed 30 min (on TLC). The lower catalytic loading of the catalyst resulted in lower yields with no effect on the ratio of % yield of 4.53e to 4.54e. Even on refluxing the reaction mixture no improvement in time of reaction and ratio of % of yield (Table 1, entry 8-9) was observed. Increasing the catalytic loading and temperature didn’t show any improvement in time of reaction, overall % yield and ratio of % yield of 4.53e to 4.54e. After this, we screened some more solvents (ethanol and acetonitrile) vis-a-vis reaction conditions (room temperature as well as refluxing) and it was found that highest yields and ratio of 4.53e to 4.54e were obtained at room temperature with dichloromethane as the solvent.
Synthesis of Pyrazoles

Table 1: Optimization of reaction conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Wt%</th>
<th>Solvent</th>
<th>Temperature(°C)</th>
<th>Yield (%)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>CH₂Cl₂</td>
<td>Rt</td>
<td>-b</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reflux</td>
<td>-</td>
<td>180</td>
</tr>
<tr>
<td>2</td>
<td>HNO₃ᵃ</td>
<td>-</td>
<td>CH₂Cl₂</td>
<td>Rt</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reflux</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>SiO₂-HNO₃</td>
<td>300</td>
<td>EtOH</td>
<td>Rt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reflux</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>SiO₂-HNO₃</td>
<td>300</td>
<td>CH₃CN</td>
<td>Rt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reflux</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>SiO₂-HNO₃</td>
<td>300</td>
<td>CH₂Cl₂</td>
<td>Zero</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>SiO₂-HNO₃</td>
<td>400</td>
<td>CH₂Cl₂</td>
<td>Zero</td>
<td>10</td>
<td>82</td>
</tr>
<tr>
<td>7</td>
<td>SiO₂-HNO₃</td>
<td>300</td>
<td>CH₂Cl₂</td>
<td>Rt</td>
<td>10</td>
<td>82</td>
</tr>
<tr>
<td>8</td>
<td>SiO₂-HNO₃</td>
<td>200</td>
<td>CH₂Cl₂</td>
<td>Rt</td>
<td>9.8</td>
<td>79</td>
</tr>
<tr>
<td>9</td>
<td>SiO₂-HNO₃</td>
<td>100</td>
<td>CH₂Cl₂</td>
<td>Rt</td>
<td>9.5</td>
<td>77.5</td>
</tr>
</tbody>
</table>

ᵃ 10 mol%, ᵇ no reaction

The success of the protocol developed with SiO₂-HNO₃ was compared with some other protocols reported in literature for commercially available oxidising reagents (Table 2). The results indicated that the oxidative cyclization (of course along with the nitration of 5-(4-chlorophenyl)-4,5-dihydro-1,3-(diphenyl)-1H-pyrazole 4.52e regioselectively at p-position of N-phenyl ring with no nitration at o-position of the ring owing to the steric hindrance) was achieved more efficiently with SiO₂-HNO₃. This is the first case in which SiO₂-HNO₃ acted as a nitrating agent along with oxidising property. Formation of
the nitro products \textit{4.54(a-i)} by this protocol is a new finding and is of considerable importance because literature survey reveals that the synthesis of 4-nitrophenylhydrazone from chalcones is very tedious and the oxidation of these hydrazones is not a fruitful protocol. Moreover, limited reports are available for the synthesis of 3,5-diaryl-1-(\textit{p}-nitrophenyl) pyrazoles from chalcones and 4-nitrophenylhydrazine. In 1987, Kovelesky \textit{et al}\textsuperscript{38} reported the synthesis of 1-(4-nitrophenyl)-3,5-diphenylpyrazole oxidative cyclization of arylhydrazones of chalcones to pyrazoles by thianthrene cation radical with 60\% yield. In 2009, Aggarwal \textit{et al} reported\textsuperscript{40} only one example i.e. 3-phenyl-1-(4-nitrophenyl)-5-(\textit{p}-fluorophenyl)pyrazole from 4-nitrophenyl hydrazone of 1-(phenyl)-3-(\textit{p}-fluorophenyl)propenone using IBD/CH\textsubscript{2}Cl\textsubscript{2} with 69\% yield after 6h. Hu \textit{et al}\textsuperscript{64} reported synthesis of 5-(3-chlorophenyl)-3-(5-methoxyphenyl)-1-(4-nitrophenyl)pyrazole with [RuCl\textsubscript{2}(p-cymene)]\textsubscript{2} in dimethylsulfoxide (DMSO) in 5-6h with 83\% yield.

\textbf{Table 2: Oxidative aromatisation of 5-(4-chlorophenyl)-4,5-dihydro-1,3-diphenyl-1\textit{H}-pyrazole 4.52e}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Yield (%)</th>
<th>Time(min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IBD\textsuperscript{40}</td>
<td>60</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>Zr(NO\textsubscript{3})\textsubscript{4}\textsuperscript{47}</td>
<td>75</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Fe(NO\textsubscript{3})\textsubscript{4}\textsuperscript{60}</td>
<td>65</td>
<td>180</td>
</tr>
<tr>
<td>4</td>
<td>CAN\textsuperscript{59}</td>
<td>68</td>
<td>180</td>
</tr>
<tr>
<td>5</td>
<td>AgNO\textsubscript{3}\textsuperscript{50}</td>
<td>90</td>
<td>360</td>
</tr>
<tr>
<td>6</td>
<td>SiO\textsubscript{2}-HNO\textsubscript{3}</td>
<td>92 (82+10)</td>
<td>5</td>
</tr>
</tbody>
</table>

Under the optimized reaction conditions, a wide range of pyrazolines bearing electron donating and electron withdrawing groups can be oxidised with SiO\textsubscript{2}-HNO\textsubscript{3} (300 wt\%) at room temperature in dichloromethane (\textbf{Scheme 3}).
Synthesis of 1,3,5-triarylsubstituted pyrazoles

Scheme 3: Transformation of 4,5-dihydro-1,3,5-(aryl)-1H-pyrazoles

Table 3: Synthesis of 1,3,5-triarylsubstituted pyrazoles 4.53(a,c,e-i) and 4.54(a-i)

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Product 4.53</th>
<th>Product 4.54</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mp °C</td>
<td>Yield (%)</td>
<td>Mp °C (lit mp)</td>
<td>Yield (%)</td>
</tr>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>136-138(140)&lt;sup&gt;26&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>traces</td>
<td>166-168</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>76-78(79-80)&lt;sup&gt;26&lt;/sup&gt;</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>3,4,5-(OCH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>traces</td>
<td>132-134</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>Cl</td>
<td>112-114 (115)&lt;sup&gt;27&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>OH</td>
<td>122-124</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Cl</td>
<td>98-100(101-103)&lt;sup&gt;26&lt;/sup&gt;</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>Br</td>
<td>Cl</td>
<td>130-132(131-132)&lt;sup&gt;37&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>Br</td>
<td>Br</td>
<td>140-142</td>
<td>10</td>
</tr>
</tbody>
</table>

The formation of products 4.53 and 4.54 were confirmed by using various spectroscopic techniques like <sup>1</sup>H/<sup>13</sup>C NMR and mass. In <sup>1</sup>H NMR, disappearance of signal at δ 5.27 which was observed due to benzylic proton H-5 in 4.52e and appearance of a signal as singlet at δ 6.71 confirms the dehydrogenation of 4.52e which itself confirms the formation of 4.53e. Resonance due to fourteen aromatic protons appeared at δ 7.83 (m, 2H), 7.33 (2H, m), 7.27-7.23 (6H, m), 7.19 (2H, dd, J = 6.4 Hz, J = 1.9 Hz), 7.10 (2H, dd, J = 6.4 Hz, J = 2.0 Hz). In proton decoupled <sup>13</sup>C NMR spectrum fifteen signals were obtained due to fifteen non-equivalent carbon atoms. Further in mass spectrum of 4.53e a
molecular ion peak was observed at m/z 331.3 and a peak at m/z 333.4 due to its isotopes in ratio of (3:1).

Similarly, the structure of compound 4.54e was also confirmed by using $^1$H/$^{13}$C NMR and mass. In $^1$H NMR, disappearance of signal at $\delta$ 5.27 which was observed due to benzylic proton H-5 in 4.54e and appearance of a signal as singlet at $\delta$ 6.77 confirms the dehydrogenation of 4.52e which indicates the oxidative aromatization of 5-(4-chlorophenyl)-4,5-dihydro-3-(4-methoxyphenyl)-1-phenyl-$^1$H-pyrazole. Decrease in one aromatic proton in this case and appearance of para substitution pattern indicates para substitution along with oxidative aromatization in case of 4.54e. Resonance due to thirteen aromatic protons appeared at $\delta$ 8.13 (2H, dd, $J = 7.0$ Hz, $J = 1.7$ Hz), 7.83 (m, 2H), 7.45 (2H, dd, $J = 7.0$ Hz, $J = 1.7$ Hz), 7.37 (m, 2H), 7.33-7.28 (3H, m) and 7.16 (m, 2H). In proton decoupled $^{13}$C NMR spectrum fifteen signals were observed due to fifteen non-equivalent carbon present in 4.54e. Further, in mass spectrum of 4.54e a molecular ion peak was observed at m/z 376.3 and a peak at m/z 378.4 due to its isotope in ratio of (3:1). Furthermore, the structure of product 4.54e was confirmed by correlation with 2D-spectra.

![2D-COSY spectra of 4.54e](image)

**Figure 4: 2D-COSY spectra of 4.54e**
Figure 5: $^1$H NMR spectra of (a) 4.53e (b) 4.54e (c) mix spectra of 4.53e and 4.54e
Mechanistic aspects:

The formation of the oxidation products \textbf{4.53} and \textbf{4.54} (Scheme 4) may be envisioned to occur via two mechanisms-

(i) The imine nitrogen of the pyrazoline nucleus may attack first on the nitrate ion of SiO\textsubscript{2}-\textsubscript{HNO\textsubscript{3}} to trigger the oxidative aromatization (Scheme 4, path A) resulting into the formation of 3,5-diaryl-1-phenyl pyrazoles \textbf{4.53}. This pyrazole may furnish the nitro product on further reaction and/or

(ii) Firstly the nitration occurs on the \textit{p}-position of N-phenyl ring (more active) followed by the oxidative aromatization (Scheme 4, path B) to form the 3,5-diaryl-1-(\textit{p}-nitrophenyl) pyrazoles \textbf{4.54}.

\begin{center}
\textbf{Scheme 4: Oxidative aromatization of pyrazolines 4.52 to pyrazoles 4.53 and 4.54.}
\end{center}
To establish the exact mechanistic pathway(s), we performed an experiment of the nitration (Scheme 5) of 1,3,5-triphenylpyrazole 4.53a with SiO$_2$-HNO$_3$ under the optimised conditions. But, no formation of the nitro derivative 4.54a was observed with SiO$_2$-HNO$_3$. Therefore, the formation of 4.53 and 4.54 takes place via two independent routes. Hence, it may be concluded that second mechanism i.e. the nitration followed by oxidative aromatization is the most plausible mechanism (Scheme 4, path B) for the formation of nitration product 4.54 while the first mechanism is operative (Scheme 4, path A) for the formation of simple pyrazole 4.53.

![Scheme 5: Nitration of 1,3,5-triarylpyrazoles 4.53a](image)

**Biological assay:**

All the synthesized compounds 4.53(a,c,e-i) and 4.54(a-i) were evaluated in vitro for their antimicrobial activity against two gram positive bacterium strains i.e. *Bacillus subtilis* and *Staphylococcus aureus*, two gram negative bacterium strains i.e. *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against two yeasts i.e. *Candida albicans* and *Saccharomyces cerevisiae* by agar well diffusion method$^{65}$. Among all the tested compounds, many compounds revealed moderate to good activity. As compared to reference drug ciprofloxacin, the pyrazoles 4.54h (ZOI = 21 mm, MIC = 25 µg/ml), 4.53h (ZOI = 24 mm, MIC = 25 µg/ml) and 4.54i (ZOI = 22 mm, MIC = 25 µg/ml) revealed very good activity against *Bacillus subtilis*. The compounds 4.54e (ZOI = 21 mm, 25 µg/ml), 4.54h (ZOI = 21 mm, 25 µg/ml), 4.53h (ZOI = 23 mm, MIC = 25 µg/ml) and 4.54i (ZOI = 21 mm, MIC = 25 µg/ml) showed very good activity against *Staphylococcus aureus*. Compounds 4.54b (ZOI = 21 mm, 25 µg/ml) and 4.53h (ZOI = 21 mm, 25 µg/ml) showed good activity against *Escherichia coli* and the compounds 4.53c (ZOI = 21 mm, 25 µg/ml), 4.54h (ZOI = 22 mm, 25 µg/ml), 4.53h (ZOI = 23 mm, 25 µg/ml) and 4.54i (ZOI = 22 mm, 25 µg/ml) showed excellent activity against *Pseudomonas aeruginosa*. As compared to reference drug Amphotericin-B, the compounds 4.54b (ZOI = 22 mm, 25 µg/ml)
Syntese of Pyrazoles

µg/ml), 4.53c (ZOI = 22 mm, 25 µg/ml), 4.53e (ZOI = 23 mm, 25 µg/ml), 4.54f (ZOI = 24 mm, 25 µg/ml), 4.54g (ZOI = 22 mm, 25 µg/ml), 4.54h (ZOI = 21 mm, 25 µg/ml) and 4.54i (ZOI = 22 mm, 25 µg/ml) and 4.53j (ZOI = 22 mm, 25 µg/ml) showed good activity against Candida albicans and compounds 4.54b (ZOI = 24 mm, 25 µg/ml) and 4.54h (ZOI = 23 mm, 25 µg/ml) showed good whereas compound 4.53h (ZOI = 26 mm, 12.5 µg/ml) revealed excellent activity against Saccharomyces cerevisiae.

Table 4: In vitro antimicrobial activity of synthesised compounds 4.53 and 4.54 through agar well diffusion method

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of growth of inhibition zone (mm)(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>4.53a</td>
<td>11</td>
</tr>
<tr>
<td>4.53c</td>
<td>16</td>
</tr>
<tr>
<td>4.53e</td>
<td>10</td>
</tr>
<tr>
<td>4.53f</td>
<td>16</td>
</tr>
<tr>
<td>4.53g</td>
<td>12</td>
</tr>
<tr>
<td>4.53h</td>
<td>24</td>
</tr>
<tr>
<td>4.53i</td>
<td>19</td>
</tr>
<tr>
<td>4.54a</td>
<td>15</td>
</tr>
<tr>
<td>4.54b</td>
<td>18</td>
</tr>
<tr>
<td>4.54c</td>
<td>16</td>
</tr>
<tr>
<td>4.54d</td>
<td>12</td>
</tr>
<tr>
<td>4.54e</td>
<td>16</td>
</tr>
<tr>
<td>4.54f</td>
<td>18</td>
</tr>
<tr>
<td>4.54g</td>
<td>14</td>
</tr>
<tr>
<td>4.54h</td>
<td>21</td>
</tr>
<tr>
<td>4.54i</td>
<td>22</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>24.0</td>
</tr>
<tr>
<td>Amphotericin-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(a\) Values, including diameter of the well (8mm), are means of three replicate set
### Table 5: Minimum inhibitory concentration (MIC) (in µg/ml) of compounds

<table>
<thead>
<tr>
<th>Product</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
<td>S. aureus</td>
<td>E. coli</td>
</tr>
<tr>
<td>4.53a</td>
<td>100</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>4.53c</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>4.53e</td>
<td>50</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>4.53f</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>4.53g</td>
<td>100</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>4.53h</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>4.53i</td>
<td>25</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4.54a</td>
<td>100</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>4.54b</td>
<td>50</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>4.54c</td>
<td>50</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>4.54d</td>
<td>200</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>4.54e</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>4.54f</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>4.54g</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>4.54h</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>4.54i</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Amphotericin-B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
**Figure 6:** Zone of inhibition of pyrazoles (a) 4.53(a,c,e-i) and (b) 4.54(a-i)
Conclusion

An oxidative aromatization of 4,5-dihydro-1,3,5-triaryl-1H-pyrazoles to 3,5-diaryl-1-(p-nitrophenyl)pyrazoles (major) and 3,5-diaryl-1-phenylpyrazoles (minor) has been executed by developing a new protocol using SiO$_2$-HNO$_3$ under mild conditions. This synthetic approach appears to be convenient and useful due to its operational simplicity, practical application, ecofriendly nature, mild reaction conditions, easy workup and high yield. All the synthesized compounds have been evaluated in vitro for their antimicrobial activity against two gram positive bacterium strains i.e. *Bacillus substillis* and *Staphylococcus aureus*, two gram negative bacterium strains i.e. *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against two yeasts i.e. *Candida albicans* and *Saccharomyces cerevisiae*. 
4.3 Experimental:

Preparation of catalyst

5g silica gel (Acme’s; 100-200 mesh size) was added to a 100 ml conical flask containing 10 ml conc. HNO₃ was added and stirred well for 10 min. Filtered the residue under reduced pressure to obtain white solid i.e. SiO₂-HNO₃ which was kept in oven for 10 min at 120 °C to activate the catalyst⁶⁴.

Preparation of chalcones:

A general procedure- Acetophenone 4.49 (1.20g, 0.01 mol) and benzaldehyde 4.50 (1.06g, 0.01 mol) were added to stirring solution of 20% aqueous KOH (10 ml) in ethanol in 100 ml conical flask. The reaction mixture was allowed to stir for 4h. The progress of reaction was monitored on TLC. After the completion of reaction, it was poured into ice-cold water and neutralised with 5% HCl. Filtered and dried the crude product and crystallized with Ethanol to obtain crystals of pure product 4.51a. The other chalcones 4.51(b-e) were obtained by taking the respective aldehydes (b: 1.20g; c: 1.36g; d: 1.96g; e: 1.40g) likewise. The chalcones 4.51f and 4.51g were prepared by reacting p-methoxycacetophenone (1.50g, 0.01 mol) with p-hydroxy benzaldehyde (1.22g, 0.01 mol) and p-chlorobenzaldehyde (1.40g, 0.01 mol) respectively. And the chalcones 4.51h and 4.51i were realised by interacting p-bromoacetophenone (1.99g, 0.01 mol) with p-chlorobenzaldehyde (1.40g, 0.01 mol) and p-bromobenzaldehyde (1.85g, 0.01 mol).
Preparation of 1,3,5-trisubstituted-4,5-dihydro-1H pyrazoles from chalcone 4.52(a-i):

To solution of respective chalcone (0.01 mol, 4.51a-2.08g, 4.51b-2.22g, 4.51c-2.38g, 4.51d-2.98g, 4.51e-2.42g, 4.51f-2.54g, 4.51g-2.72g, 4.51h-3.21g, 4.51i-3.66g) in acetic acid, added phenyl hydrazine (1.15g, 0.015 mol) and the reaction mixture was refluxed for 6-8h. The progress of reaction was monitored on TLC. After completion of reaction, the reaction mixture was poured into ice-cold water to obtain crude product. Filtered, dried the crude product and recrystallized with ethanol to obtain pure product dihydropyrazole.

![Reaction Scheme]

4.5-Dihydro-1,3,5-(triphenyl)-1H-pyrazole 4.52a:

Yield 84%, off white solid, mp 132-133 °C (Lit mp 134-135 °C)\(^\text{46}\);

\(^1\)H NMR (300 MHz, δ (ppm), CDCl\(_3\)) : 7.75 (2H, d, J = 7.2 Hz, ArH), 7.46-7.29 (8H, m, ArH), 7.20 (2H, t, J = 8.1 Hz, ArH), 7.10 (2H, d, J = 7.8 Hz, ArH), 6.80 (1H, d, J = 7.8 Hz, ArH), 5.32 (1H, dd, J = 7.2 Hz, J = 12.3 Hz, H\(_6\)), 3.87 (1H, dd, J = 12.3 Hz, J = 17.1 Hz, H\(_5\)), 3.17 (1H, dd, J = 7.2 Hz, J = 17.1 Hz, H\(_6\)).

4.5-Dihydro-1,3-diphenyl-(p-tolyl)-1H-pyrazole 4.52b:

Yield 83%, off white solid, mp 130-132 °C (Lit mp128-130 °C)\(^\text{46}\);

\(^1\)H NMR (300 MHz, δ (ppm), CDCl\(_3\)) : 7.76 (2H, d, J = 8.4 Hz, ArH), 7.44-7.20 (6H, m, ArH), 7.16 (2H, d, J = 9.0 Hz, ArH), 7.11 (3H, d, J = 7.8 Hz, ArH), 6.80 (1H, t, J = 7.2 Hz, ArH), 5.27 (1H, dd, J = 7.2 Hz, J = 12.3 Hz, H\(_5\)), 3.84 (1H, dd, J = 12.3 Hz, J = 17.1 Hz, H\(_5\)), 3.15 (1H, dd, J = 7.2 Hz, J = 17.1 Hz, H\(_6\)), 2.35 (3H, s, CH\(_3\)).

4.5-Dihydro-5-(4-methoxyphenyl)- 1,3-diphenyl-1H-pyrazole 4.52c:

Yield 85%, off white solid, mp 110-112 °C (Lit mp 110-112 °C)\(^\text{46}\);

\(^1\)H NMR (300 MHz, δ (ppm), CDCl\(_3\)) : 7.75 (2H, d, J = 7.5 Hz, ArH), 7.43-7.32 (4H, m, ArH), 7.24 (1H, d, J = 8.1 Hz, ArH), 7.19 (2H, d, J = 7.5 Hz, ArH), 7.10 (2H, d, J = 8.1 Hz, ArH), 6.86 (2H, d, J = 7.2 Hz, ArH), 3.84 (3H, s, CH\(_3\)), 3.76 (3H, s, OCH\(_3\)).
Hz, ArH), 6.88 (2H, d, $J = 8.1$ Hz, ArH), 6.80 (1H, t, $J = 7.2$ Hz, ArH), 5.75 (1H, dd, $J = 7.2$ Hz, $J = 12.3$ Hz, H$_c$), 3.82 (1H, dd, $J = 12.3$ Hz, $J = 17.1$ Hz, H$_b$), 3.14 (1H, dd, $J = 7.2$ Hz, $J = 17.1$ Hz, H$_a$), 3.80 (3H, s, OCH$_3$).

**4,5-Dihydro-5-(3,4,5-trimethoxyphenyl)-1,3-diphenyl-1H-pyrazole 4.52d:**

Yield 88%, off white solid, mp 142-144 °C;

$^1$H NMR (300 MHz, $\delta$ (ppm), CDCl$_3$) : 7.75 (2H, d, $J = 7.2$ Hz, ArH), 7.42-7.21 (4H, m, ArH), 7.12 (2H, d, $J = 6.9$ Hz, ArH), 6.84 (2H, d, $J = 7.8$ Hz, ArH), 6.57 (2H, s, ArH), 5.15 (1H, dd, $J = 7.2$ Hz, $J = 12.3$ Hz, H$_c$), 3.83 (9H, s, OCH$_3$), 3.34 (1H, dd, $J = 7.2$ Hz, $J = 12.3$ Hz, H$_a$), 3.16 (1H, dd, $J = 7.2$ Hz, $J = 17.1$ Hz, H$_b$).

**5-(4-Chlorophenyl)-4,5-dihydro-1,3-diphenyl-1H-pyrazole 4.52e:**

Yield 86%, off white solid, mp 143-144 °C (Lit mp 144-145 °C);

$^1$H NMR (300 MHz, $\delta$ (ppm), CDCl$_3$) : 7.74 (2H, d, $J = 7.2$ Hz, ArH), 7.44-7.24 (7H, m, ArH), 7.20 (2H, d, $J = 7.2$ Hz, ArH), 7.09 (2H, d, $J = 7.8$ Hz, ArH), 6.82 (1H, d, $J = 7.8$ Hz, ArH), 5.27 (1H, dd, $J = 7.2$ Hz, $J = 12.3$ Hz, H$_c$), 3.86 (1H, dd, $J = 12.3$ Hz, $J = 17.1$ Hz, H$_a$), 3.12 (1H, dd, $J = 7.2$ Hz, $J = 17.1$ Hz, H$_b$).

**4-(4,5-Dihydro-3-(4-methoxyphenyl)-3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazole 4.52f.**

Yield 84%, off white solid, mp 124-126 °C;

$^1$H NMR (300 MHz, $\delta$ (ppm), CDCl$_3$) : 7.80 (2H, d, $J = 7.8$ Hz, ArH), 7.44-7.27 (5H, m, ArH), 7.10 (2H, d, $J = 8.1$ Hz, ArH), 7.07 (2H, d, $J = 8.1$ Hz, ArH), 6.80 (2H, t, $J = 6.6$ Hz, ArH), 5.24 (1H, dd, $J = 7.2$ Hz, $J = 12.3$ Hz, H$_c$), 3.84 (1H, dd, $J = 12.3$ Hz, $J = 17.1$ Hz, H$_a$), 3.81 (3H, s, OCH$_3$), 3.10 (1H, dd, $J = 7.2$ Hz, $J = 17.1$ Hz, H$_b$).

**5-(4-Chlorophenyl)-4,5-dihydro-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazole 4.52g:**

Yield 87%, off white solid, mp 146-148 °C (Lit mp 148-150 °C);

$^1$H NMR (300 MHz, $\delta$ (ppm), CDCl$_3$) : 8.08 (2H, d, $J = 9.0$ Hz, ArH), 7.71 (2H, d, $J = 8.7$ Hz, ArH), 7.47 (2H, d, $J = 7.2$ Hz, ArH), 7.37 (2H, d, $J = 7.2$ Hz, ArH), 7.20 (2H, d, $J = 8.7$ Hz, ArH), 6.98 (3H, dd, $J = 9.0$Hz, ArH), 5.38 (1H, dd, $J = 7.2$ Hz, $J = 12.3$ Hz, H$_c$), 3.92 (1H, dd, $J = 12.3$ Hz, $J = 17.1$ Hz, H$_a$), 3.88 (3H, s, OCH$_3$), 3.20 (1H, dd, $J = 7.2$ Hz, $J = 17.1$ Hz, H$_b$).
3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole 4.52h:

Yield 84%, off white solid, mp 135-136 °C (Lit mp 136-138 °C)⁴⁶;

$^1$H NMR (300 MHz, δ (ppm), CDCl$_3$): 7.81 (2H, d, $J = 8.1$ Hz, ArH), 7.42-7.20 (7H, m, ArH), 7.08 (2H d, $J = 8.1$ Hz, ArH), 6.83 (2H, t, $J = 6.6$ Hz, ArH), 5.29 (1H, dd, $J = 7.2$ Hz, $J = 12.3$ Hz, H$_c$), 3.83 (1H, dd, $J = 12.3$ Hz, $J = 17.1$ Hz, H$_a$), 3.09 (1H, dd, $J = 7.2$ Hz, $J = 16.8$ Hz, H$_b$).

3,5-(4-Bromophenyl)-4,5-dihydro-1-phenyl-1H-pyrazole 4.52i:

Yield 86%, off white solid, mp 128-130 °C (Lit mp 132-134 °C)⁴⁶;

$^1$H NMR (300 MHz, δ (ppm), CDCl$_3$): 7.60 (6H, m, ArH), 7.37-7.19 (4H, m, ArH), 7.05 (2H, d, $J = 7.8$ Hz, ArH), 6.84 (1H, t, $J = 6.9$ Hz, ArH), 5.27 (1H, dd, $J = 6.9$ Hz, $J = 12.3$ Hz, H$_c$), 3.82 (1H, dd, $J = 12.3$ Hz, $J = 17.1$ Hz, H$_a$), 3.08 (1H, dd, $J = 6.9$ Hz, $J = 17.1$ Hz, H$_b$).

Oxidative aromatisation of 1,3,5-trisubstituted-4,5-dihydro-1H-pyrazoles to 1,3,5-trisubstituted-pyrazole 4.53(a,c,e-i) and 4.54(a-i):

Optimization of reaction conditions: To optimise the reaction conditions, the many experiments were conducted. Firstly, to the stirring solution of 5-(4-chlorophenyl)-4,5-dihydro-1,3-(triphenyl)-1H-pyrazole 4.52e (0.33g, 1mmol) in dichloromethane was added SiO$_2$-HNO$_3$ (300% wt of pyrazoline) and the reaction mixture was stirred at room temperature. The reaction ensued with appearance of wine red color which changed to yellow color with the progress of reaction. The progress of reaction mixture was monitored on TLC and starting material disappeared within five minutes indicating the completion of reaction. The reaction mixture was quenched with ice-cold 5% aqueous solution of NaHCO$_3$ and extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulphate and solvent was distilled off under reduced pressure. The residue was purified by column chromatography by using pet.ether-ethylacetate mixture (as eluent) on silica gel with increasing proportions of ethylacetate to obtain two oxidation products 5-(4"-chlorophenyl)-1,3-diphenylpyrazole 4.53e (minor) & 5-(4"-chlorophenyl)-1-(4-nitrophenyl)-3-phenylpyrazole 4.54e. This reaction procedure was adopted for oxidation of 5-(4-chlorophenyl)-4,5-dihydro-1,3-(triphenyl)-1H-pyrazole 4.52e (0.33g, 1mmol) in dichloromethane with variation of catalytic loading of SiO$_2$-HNO$_3$ where the reaction completed in varying times furnishing the same two products 4.53e (minor) and 4.54e (major) (Table 1). To see the effect of solvent on reaction and to find out best
reaction condition the oxidation reaction of 5-(4-chlorophenyl)-4,5-dihydro-1,3-(triphenyl)-1H-pyrazole 4.52e (0.33g, 1mmol) with SiO₂-HNO₃ (300% wt of pyrazoline) was carried out in different solvents (ethanol, acetonitrile, chloroform) at different temperatures (Table 1) (vide result and discussion).

Perusal of the results presented in Table 1 above indicates that the oxidative aromatization of 5-(4-chlorophenyl)-4,5-dihydro-1,3-(triphenyl)-1H-pyrazole 4.52e (0.33g, 1mmol) with SiO₂-HNO₃ (300% wt of pyrazoline) in dichloromethane at room temperature is the best optimized condition (Table 1, entry 3-4).

**Comparison of the above protocol with some other reported protocols:** To compare the results of SiO₂-HNO₃ with some other reported protocols, the oxidation of 5-(4-chlorophenyl)-4,5-dihydro-1,3-(triphenyl)-1H-pyrazole 4.52e was carried out with IBD, Zr(NO₃)₄, Fe(NO₃)₄, CAN and AgNO₃. The observations have been recorded in Table 2 (vide result and discussion).

So, the reaction condition (300 wt% SiO₂-HNO₃ at room temperature in dichloromethane) was best optimized condition and the reaction of pyrazolines bearing electron donating and withdrawing groups carried out under optimized conditions.

**Conversion of 1,3,5-trisubstituted-4,5-dihydro-1H-pyrazoles to pyrazole 4.53(a,c,e-i) and 4.54(a-i):**

SiO₂-HNO₃ (300% wt of pyrazoline) was added to a magnetically stirred solution of pyrazoline 4.52e (0.33g, 1 mmol) in dichloromethane and the reaction mixture was stirred at room temperature. The reaction started with appearance of wine red colour which changed to yellow colour with the progress of reaction. The progress of reaction was monitored on TLC. The starting material disappeared within five minute (TLC). The reaction was quenched with ice-cold 5% aqueous solution of NaHCO₃ and extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel using pet.ether-ethylacetate mixture (as an eluent) with increasing proportions of ethylacetate. The two products 5-(4"-chlorophenyl)-1,3-diphenylpyrazole 4.53e (minor) and 5-(4"-chlorophenyl)-1-(4-nitrophenyl)-3-phenylpyrazole 4.54e (major) were obtained. Similar procedure was adopted for the oxidative aromatization of all other 1,3,5-trisubstituted-4,5-dihydro-1H-pyrazoles (4.52a-0.29g, 4.52b-0.31g, 4.52c-0.32g, 4.52d-0.33g, 4.52f-0.34g, 4.52g-0.36g, 4.52h-0.44g, 4.52i-0.45g).
Synthesis of Pyrazoles

Characterization of 1-phenyl-2,5-diarylpyrazoles 4.53(a,c,e-i):

**1,3,5-Triphenylpyrazole 4.53a:**

Yield 10%, white solid, mp 136-138 °C (lit mp 140)\(^2\);

\(\nu_{\text{max}}\) (cm\(^{-1}\)): 3050, 1651, 1597;

\(^1\)H NMR (400 MHz, \(\delta\) (ppm), CDCl\(_3\)): 7.93 (2H, dd, \(J = 7.2\) Hz, \(J = 1.3\) Hz, ArH), 7.42 (2H, td, \(J = 7.2\) Hz, \(J = 1.7\) Hz, ArH), 7.38-7.24 (11H, m, ArH), 6.82 (1H, s, ArH);

\(^{13}\)C NMR (CDCl\(_3\), \(\delta\) (ppm)): 151.99, 144.41, 140.16, 133.06, 130.60, 128.94, 128.77, 128.67, 128.50, 128.32, 128.02, 127.46, 125.84, 125.33, 105.23;

Mass (m/z): 296.1 (M+1, 100%).

**5-(4''-Methoxyphenyl)-1,3-diphenylpyrazole 4.53c:**

Yield 6%, white solid, mp 76-78 °C (lit mp 79-80)\(^2\);

\(\nu_{\text{max}}\) (cm\(^{-1}\)): 3001, 2970, 1682, 1582;

\(^1\)H NMR (300 MHz, \(\delta\) (ppm), CDCl\(_3\) : 7.94 (2H, d, \(J = 7.5\) Hz, ArH), 7.46 (3H, m, ArH), 7.41 (3H, m, ArH), 7.37-7.30 (2H, m, ArH), 7.22 (2H, d, \(J = 8.7\) Hz, ArH), 6.86 (2H, d, \(J = 8.4\) Hz, ArH), 6.79 (1H, s, ArH), 3.83 (3H, s, OCH\(_3\));

\(^{13}\)C NMR (CDCl\(_3\), \(\delta\) (ppm)): 160.71, 153.79, 144.47, 139.62, 133.14, 129.98, 129.87, 129.54, 129.14, 128.54, 128.44, 127.72, 127.62, 126.31, 125.49, 120.92, 120.62, 114.87, 107.09, 56.02;

Mass (m/z): 327.3 (M+1, 100%).
5-(4''-Chlorophenyl)-1,3-diphenylpyrazole 4.53e:

Yield 12%, white solid, mp 112-114 °C (lit mp 115)\(^{27}\);

\(v_{\text{max}}\) (cm\(^{-1}\)): 3055, 1651, 1597;

\(^1\)H NMR (300 MHz, δ (ppm), CDCl\(_3\)): 7.83 (2H, m, ArH), 7.33 (2H, m, ArH), 7.27-7.23 (6H, m, ArH), 7.19 (2H, dd, \(J = 6.4 \text{ Hz}, J = 1.9 \text{ Hz}, \text{ ArH}\)), 7.10 (2H, dd, \(J = 6.4 \text{ Hz}, J = 2.0 \text{ Hz}, \text{ ArH}\)), 6.71 (1H, s, ArH);

\(^{13}\)C NMR (CDCl\(_3\), δ (ppm)): 152.11, 143.20, 139.92, 134.41, 132.89, 129.99, 129.10, 129.04, 128.82, 128.72, 128.15, 127.71, 125.84, 125.36, 105.32;

Mass (m/z): 331.3/333.4 (M+1, M+3).

5-(4''-Hydroxyphenyl)-3-(4'-methoxyphenyl)-1-phenylpyrazole 4.53f:

Yield 12%, white solid, mp 122-124 °C;

\(v_{\text{max}}\) (cm\(^{-1}\)): 3405 (OH), 3011, 1682, 1605, 1597;

\(^1\)H NMR (300 MHz, δ (ppm), CDCl\(_3\)): 7.73 (2H, d, \(J = 8.4 \text{ Hz}, \text{ ArH}\)), 7.45-7.27 (6H, m, ArH), 7.23 (3H, m, ArH), 7.00 (2H, d, \(J = 8.7 \text{ Hz}, \text{ ArH}\)), 6.78 (1H, s, ArH), 3.89 (3H, s, OCH\(_3\));

\(^{13}\)C NMR (CDCl\(_3\), δ (ppm)): 160.11, 153.77, 145.98, 145.86, 145.31, 139.77, 138.00, 129.80, 129.57, 128.66, 127.87, 126.73, 126.03, 124.78, 121.09, 114.53, 106.09, 55.36;

Mass (m/z): 343.4 (M+1, 100%).

5-(4''-Chlorophenyl)-3-(4'-methoxyphenyl)-1-phenylpyrazole 4.53g:

Yield 11%, white solid, mp 98-100 °C (lit mp 101-103)\(^{26}\);

\(v_{\text{max}}\) (cm\(^{-1}\)): 3001, 1680, 1589;

\(^1\)H NMR (300 MHz, δ (ppm), CDCl\(_3\)): 7.99 (2H, d, \(J = 8.4 \text{ Hz}, \text{ ArH}\)), 7.93 (2H, d, \(J = 8.4 \text{ Hz}, \text{ ArH}\)), 7.47 (2H, d, \(J = 8.1 \text{ Hz}, \text{ ArH}\)), 7.33-7.21 (5H, m, ArH), 7.00 (2H, d, \(J = 8.7 \text{ Hz}, \text{ ArH}\)), 6.77 (1H, s, ArH), 3.88 (3H, s, OCH\(_3\));

\(^{13}\)C NMR (CDCl\(_3\), δ (ppm)): 160.09, 153.17, 145.88, 145.56, 145.35, 139.38, 138.65, 129.91, 129.77, 128.64, 127.89, 126.76, 126.13, 124.58, 121.19, 114.53, 106.11, 55.31;

Mass (m/z): 361.1/363.2 (M+1, M+3).
Synthesis of Pyrazoles

3-(4'-Bromophenyl)-5-(4''-chlorophenyl)-1-phenylpyrazole 4.53h:
Yield 12%, white solid, mp 130-132 °C (lit mp 131-132)\textsuperscript{26};

ν\textsubscript{\text{max}} (cm\textsuperscript{-1}): 2924, 2854, 1744, 1651, 1582;

\textsuperscript{1}H NMR (300 MHz, δ (ppm), CDCl\textsubscript{3}): 7.90 (2H, d, J = 8.4 Hz, ArH), 7.80 (2H, d, J = 6.9 Hz, ArH), 7.67 (2H, d, J = 8.4 Hz, ArH), 7.59 (2H, d, J = 8.1 Hz, ArH), 7.49-7.30 (4H, m, ArH), 7.21 (1H, d, J = 8.4 Hz, ArH), 6.80 (1H, s, ArH);

\textsuperscript{13}C NMR (CDCl\textsubscript{3}, δ (ppm)): 153.31, 145.81, 139.70, 134.33, 132.72, 132.47, 131.21, 130.02, 129.78, 129.67, 129.41, 128.97, 123.12, 120.41, 106.03;

Mass (m/z): 410.6/412.7 (M+1, M+3).

3-(4'-Bromophenyl)-5-(4''-bromophenyl)-1-phenylpyrazole 4.53i:
Yield 10%, white solid, mp 140-142 °C;

ν\textsubscript{\text{max}} (cm\textsuperscript{-1}): 2926, 1682, 1651, 1582;

\textsuperscript{1}H NMR (300 MHz, δ (ppm), CDCl\textsubscript{3}): 7.80 (2H, d, J = 8.4 Hz, ArH), 7.57 (2H, d, J = 8.4 Hz, ArH), 7.47 (2H, d, J = 8.4 Hz, ArH), 7.40-7.33 (5H, m, ArH), 7.15 (2H, d, J = 8.4 Hz, ArH), 6.81 (1H, s, ArH);

\textsuperscript{13}C NMR (CDCl\textsubscript{3}, δ (ppm)): 151.40, 148.36, 144.25, 138.67, 135.32, 133.97, 132.45, 128.70, 128.82, 128.57, 127.26, 124.54, 120.15, 106.02;

Mass (m/z): 455.1 (M+1, M+3).

Characterization of 1,3-diaryl-1-(nitrophenyl)pyrazoles 4.54(a-i):

1-(4-Nitrophenyl)-3,5-diphenylpyrazole 4.54a:
Yield 82%, yellow solid, mp 118-119 °C;

ν\textsubscript{\text{max}} (cm\textsuperscript{-1}): 3091, 3055, 1589, 1512 & 1335 (NO\textsubscript{2});

\textsuperscript{1}H NMR (400 MHz, δ (ppm), CDCl\textsubscript{3}): 8.17 (2H, dd, J = 7.0 Hz, J = 2.1 Hz, ArH), 7.92 (2H, dd, J = 7.0 Hz, J = 1.4 Hz, ArH), 7.54 (2H, m, ArH), 7.47-7.35 (6H, m, ArH), 7.31-7.24 (2H, m, ArH), 6.85 (1H, s, ArH);

\textsuperscript{13}C NMR (CDCl\textsubscript{3}, δ (ppm)): 151.88, 144.39, 140.02, 132.94, 130.47, 128.82, 128.71, 128.65, 128.44, 128.20, 127.91, 127.38, 125.76, 125.29, 106.45;

Mass (m/z): 342.1 (M+1, 100%)
5-(4''-Methylphenyl)-1-(4-nitrophenyl)-3-phenylpyrazole 4.54b:

Yield 89%, yellow solid, mp 166-168 °C;

ν\text{max} (\text{cm}^{-1}): 3140, 3040, 1589, 1520 & 1335 (NO\textsubscript{2});

\textsuperscript{1}H NMR (300 MHz, δ (ppm), CDCl\textsubscript{3}): 8.21 (2H, d, J = 9.0 Hz, ArH), 7.95 (2H, d, J = 8.4 Hz, ArH), 7.57 (3H, d, J = 9.0 Hz, ArH), 7.48-7.34 (5H, m, ArH), 7.22 (1H, s, ArH), 6.84 (1H, s, ArH), 2.46 (3H, s, CH\textsubscript{3});

\textsuperscript{13}C NMR (100.6 MHz, δ (ppm), CDCl\textsubscript{3}): 154.48, 146.76, 146.07, 139.84, 133.49, 129.97, 129.09, 128.58, 128.13, 127.62, 127.56, 127.19, 126.03, 125.37, 124.71, 107.97, 21.36;

Mass (m/z): 356.3 (M\textsuperscript{+}, 100%).

5-(4''-Methoxyphenyl)-1-(4-nitrophenyl)-3-phenylpyrazole 4.54c:

Yield 85%, yellow solid, mp 92-94 °C;

ν\text{max} (\text{cm}^{-1}): 3140, 3055, 1589, 1528 & 1330 (NO\textsubscript{2});

\textsuperscript{1}H NMR (300 MHz, δ (ppm), CDCl\textsubscript{3}): 8.21 (2H, d, J = 8.7 Hz, ArH), 7.93 (2H, d, J = 7.2 Hz, ArH), 7.57 (2H, d, J = 9.0 Hz, ArH), 7.47 (2H, t, J = 7.5 Hz, ArH), 7.41 (1H, d, J = 6.9 Hz, ArH), 7.24 (2H, d, J = 8.7 Hz, ArH), 6.94 (2H, J = 7.8 Hz, ArH), 6.82 (1H, s, ArH), 3.87 (3H, s, CH\textsubscript{3});

\textsuperscript{13}C NMR (CDCl\textsubscript{3}, δ (ppm)): 160.90, 153.77, 145.94, 145.83, 144.45, 133.19, 129.32, 129.12, 128.84, 128.53, 128.23, 127.54, 127.43, 125.44, 121.78, 121.65, 121.14, 121.02, 114.94, 114.71, 106.02, 55.09.

Mass (m/z): 372.3 (M\textsuperscript{+}, 100%).

5-(3''',4''',5'''-Trimethoxyphenyl)-1-(4-nitrophenyl)-3-phenylpyrazole 4.54d:

Yield 91%, yellow solid, mp 132-134 °C;

ν\text{max} (\text{cm}^{-1}): 3140, 3055, 1682, 1528 & 1335 (NO\textsubscript{2});

\textsuperscript{1}H NMR (300 MHz, δ (ppm), CDCl\textsubscript{3}): 8.21 (2H, dd, J = 7.0 Hz, J = 2.0 Hz, ArH), 7.92 (2H, dd, J = 7.0 Hz, J = 1.4 Hz, ArH), 7.60 (2H, dd, J = 7.0 Hz, J = 2.0 Hz, ArH), 7.45 (2H, t, J = 7.3 Hz, ArH), 7.39 (1H, d, J = 7.3 Hz, ArH), 6.84 (1H, s, ArH), 6.50 (2H, s, ArH), 3.90 (3H, s, OCH\textsubscript{3}), 3.74 (6H, s, OCH\textsubscript{3});
5-(4''-Chlorophenyl)-1-(2,4-nitrophenyl)-3-phenylpyrazole 4.54e:

Yield 80%, yellow solid, mp 182-184 °C;

νmax (cm⁻¹): 3094, 3063, 1589, 1512 & 1335 (NO₂);

1H NMR (400 MHz, δ (ppm), CDCl₃): 8.13 (2H, d, J = 7.0 Hz, J = 2.0 Hz, ArH), 7.83 (2H, dt, J = 8.4 Hz, J = 1.4 Hz, ArH), 7.45 (2H, dt, J = 7.0 Hz, J = 1.7 Hz, ArH), 7.37 (2H, td, J = 8.4 Hz, J = 1.5 Hz, ArH), 7.33-7.28 (3H, m, ArH), 7.17 (2H, td, J = 8.4 Hz, J = 1.5 Hz, ArH), 6.77 (1H, s, ArH).

13C NMR (CDCl₃, δ (ppm)): 153.43, 145.98, 144.65, 143.70, 135.35, 132.11, 130.08, 129.31, 128.85, 128.75, 128.49, 125.93, 124.58, 124.57, 107.35;

Mass (m/z): 376.3/378.4 (M+1, M+3).

5-(4''-Hydroxyphenyl)-3-(4'-methoxyphenyl)-1-(4-nitrophenyl)pyrazole 4.54f:

Yield 81%, yellow solid, mp 138-140 °C;

νmax (cm⁻¹): 3117, 3016, 1690, 1605, 1535 & 1342 (NO₂);

1H NMR (300 MHz, δ (ppm), CDCl₃): 8.73 (1H, s, ArH), 8.36 (2H, d, J = 7.8 Hz, ArH), 7.79 (2H, d, J = 8.4 Hz, ArH), 7.48 (2H, d, J = 7.8 Hz, ArH), 7.18 (5H, m, ArH), 6.97 (2H, d, 8.4 Hz, ArH), 6.81 (1H, s, ArH), 3.87 (3H, s, OCH₃);

13C NMR (CDCl₃, δ (ppm)): 160.25, 154.47, 145.91, 145.72, 145.14, 139.60, 138.20, 129.81, 129.45, 128.53, 127.37, 126.93, 125.85, 124.56, 120.87, 114.18, 106.13, 55.34;

Mass (m/z): 388.3 (M+1, 100%).

5-(4''-Chlorophenyl)-3-(4'-methoxyphenyl)-1-(4-nitrophenyl)pyrazole 4.54g:

Yield 83%, yellow solid, mp 136-138 °C;

νmax (cm⁻¹): 3140, 3055, 1728, 1520 & 1335 (NO₂);

1H NMR (300 MHz, δ (ppm), CDCl₃): 8.23 (2H, d, J = 8.7 Hz, ArH), 7.86 (2H, d, J = 8.7 Hz, ArH), 7.54 (2H, d, J = 9.0 Hz, ArH), 7.39 (2H, d, J = 8.4 Hz, ArH), 7.26 (2H, d, J = 9.0 Hz, ArH), 7.01 (2H, d, J = 8.1 Hz, ArH), 6.80 (1H, s, ArH), 3.88 (3H, s, OCH₃);
Synthesis of Pyrazoles

$^{13}$C NMR (CDCl$_3$, $\delta$ (ppm)): 160.16, 153.28, 145.88, 144.74, 143.62, 135.30, 130.07, 129.28, 128.63, 127.25, 124.82, 124.56, 124.46, 114.25, 107.06, 55.36;

Mass (m/z): 406.1/408.3 (M+1, M+3).

3-(4'-Bromophenyl)-5-(4''-chlorophenyl)-1-(4-nitrophenyl)pyrazole 4.54h:

Yield 80%, yellow solid, mp 146-148 °C;

$\nu_{\text{max}}$ (cm$^{-1}$): 3124, 3094, 1659, 1597, 1520 & 1335 (NO$_2$);

$^1$H NMR (300 MHz, $\delta$ (ppm), CDCl$_3$): 8.23 (2H, d, $J = 9.0$ Hz, ArH), 7.83 (2H, d, $J = 9.6$ Hz, ArH), 7.61-7.53 (4H, m, ArH), 7.40 (2H, d, $J = 8.4$ Hz, ArH), 6.84 (1H, s, ArH);

$^{13}$C NMR (CDCl$_3$, $\delta$ (ppm)): 152.30, 146.11, 144.50, 135.47, 132.29, 131.98, 130.28, 130.06, 129.34, 129.03, 128.29, 127.45, 124.62, 122.74, 107.15;

Mass (m/z): 455.3/457.3 (M+1, M+3).

3-(4'-Bromophenyl)-5-(4''-bromophenyl)-1-(4-nitrophenyl)pyrazole 4.54i:

Yield 81%, yellow solid, mp 176-178 °C;

$\nu_{\text{max}}$ (cm$^{-1}$): 3144, 3047, 1651, 1597, 1520 & 1335 (NO$_2$);

$^1$H NMR (300 MHz, $\delta$ (ppm), CDCl$_3$): 8.23 (2H, d, $J = 8.7$ Hz, ArH), 7.79 (2H, d, $J = 8.1$ Hz, ArH), 7.60-7.45 (6H, m, ArH), 7.18 (2H, d, $J = 8.1$ Hz, ArH), 6.81 (1H, s, ArH);

$^{13}$C NMR (CDCl$_3$, $\delta$ (ppm)): 152.47, 148.56, 144.45, 138.73, 135.35, 134.04, 132.60, 128.99, 128.72, 128.52, 127.19, 124.45, 120.23, 105.95;

Mass (m/z): 500.3 (M+1, 100%).

Test microorganisms

Total six microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (Bacillus subtilis MTCC 121) and (Staphylococcus aureus MTCC 96); two Gram-negative bacteria (Escherichia coli MTCC 1652 and Pseudomonas aeruginosa MTCC 741) and two yeast, Candida albicans (MTCC 3017) and Saccharomyces cerevisiae (MTCC 170) were screened for evaluation of antibacterial and antifungal activities of the synthesized pyrazoles. All the microbial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH,
Chandigarh. The bacteria were subcultured on nutrient agar whereas yeast on malt yeast agar.

**In-vitro antibacterial activity**

The antimicrobial activity of pyrazoles 4.53(a,c,e-i) and 4.54(a-i) was evaluated by the agar-well diffusion method (Table 4) (vide result and discussion). All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately $1.5 \times 10^8$ cfu/ml. Agar medium (20 ml) was poured into each Petri plate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were created into the seeded agar plates which were loaded with a 100 µl volume with concentration of 8.0mg/ml of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 hrs. Antimicrobial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as positive control for bacteria and amphotericin-B for fungi. This procedure was performed in triplicates for each organism.

**Determination of Minimum Inhibitory Concentration of benzothiazoles**

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after incubation. MIC of the various compounds against bacterial and yeast strains was tested through a modified agar well diffusion method (Aneja et al., 2011) (Table 5) (vide result and discussion). In this method, a two-fold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 50 to 0.39µg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100µl of standardized inoculum ($10^6$ cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 hrs and observed for the inhibition zones. MIC, taken as the concentration of the chemical compound that completely inhibited the growth of the microbe, showed by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin and amphotericin B was used as positive control while DMSO as negative control.
References: