Chapter 11
Section A
Synthesis and assessment of the effect of ring substituent’s on the antimicrobial activity of imidazole analogues

INTRODUCTION

The imidazole ring display a wide array of activities in many pharmacologically important molecules. The imidazole derivatives are found naturally, in the amino acid histidine, Vitamin B₁₂, a component of DNA base structure. Imidazole is an important class of heterocyclic molecule reported for activities like anticancer, antifungal, antibacterial, antioxidant. These diversified activity of the imidazole analogues lured us in developing the molecules as part of our ongoing research on discovering chemical entities that are effective as antimicrobial agents. The development of imidazole analogues by simple and practical synthetic routes is one of the major challenges before the synthetic chemists. The 2,4,5-trisubstituted imidazoles are generally synthesized by three component condensation of a 1,2-diketone, α-hydroxyketone with an aldehydes and ammonium acetate, which comprise refluxing in acetic acid, the use of microwaves, ionic liquids, silica sulfuric acid, NiCl₂·6H₂O/Al₂O₃, iodine and acidic Al₂O₃.

The applications of multicomponent reactions (MCRs) in synthesis can be very helpful in developing libraries of diversified products, in minimal efforts. MCRs have gain huge importance and are widely explored as they save time. In continuation of our work on the development of synthetic methodologies and screening of the analogues, we present a method for synthesis of some new 2,4,5-trisubstituted imidazole analogues and their antimicrobial activity (Scheme 1). The imidazole analogues (4a-l) were prepared by reactions of substituted aldehydes, ammonium acetate and benzil, using resin bound propyl sulphonic acid (RPSA) as a catalyst in water. The reaction was performed conventionally (thermal heating) and under microwave irradiation.

Scheme 1. Synthesis of the imidazole analogues (4a-l).
RESULT AND DISCUSSION

Imidazole and its analogues, is the centre of attraction for researchers around the globe due to their biodiversity. Thus, establishing practical synthetic method for imidazole analogues is the challenge before the synthetic chemist. This problem can be easily overcome by the employment of MCRs in synthesis. The 2,4,5-trisubstituted imidazoles is pharmaceutically important owing to the diversified biologically activity. The literature study\textsuperscript{7-13} revealed some reported methods have the potential to be exploited on large scale. But still there is scope for improving the existing methods, as these reactions suffer from certain drawbacks like low yields, longer reaction times for completion, use of expensive reagents, use of strong corrosive acid, requirement of high temperature for the completion of these reactions. Hence, it is desirable to develop mild, environment friendly protocol for the synthesis of highly substituted imidazoles and screen them for their antimicrobial activity. Initially, our investigation began for the mild and facile synthesis of 2,4,5-trisubstituted imidazoles at room temperature by employing the model reaction of benzil (1 mmol), benzaldehyde (1 mmol), ammonium acetate (4 mmol) with different sets of catalysts (Table 1).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Condition</th>
<th>Time (min)</th>
<th>Yield\textsuperscript{b} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>No catalyst</td>
<td>Reflux in EtOH</td>
<td>600</td>
<td>Traces</td>
</tr>
<tr>
<td>2.</td>
<td>p-toluene sulphonic acid</td>
<td>Reflux in EtOH</td>
<td>120</td>
<td>68</td>
</tr>
<tr>
<td>3.</td>
<td>Sulphamic Acid</td>
<td>Reflux in EtOH</td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>4.</td>
<td>Sulphanilic Acid</td>
<td>Reflux in EtOH</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>5.</td>
<td>5 wt % RPSA</td>
<td>Reflux in EtOH</td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>6.</td>
<td>10 wt % RPSA</td>
<td>Reflux in EtOH</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>7.</td>
<td>15 wt % RPSA</td>
<td>Reflux in EtOH</td>
<td>20</td>
<td>98</td>
</tr>
<tr>
<td>8.</td>
<td>20 wt % RPSA</td>
<td>Reflux in EtOH</td>
<td>15</td>
<td>98</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction condition: Benzil (1 mmol), benzaldehyde (1 mmol), ammonium acetate (4 mmol), and various catalysts (10 mol %, entry 2-4) and varied concentration of RPSA carried out in ethanol (5 mL) at reflux temperature.

\textsuperscript{b} Isolated yields.

The reaction was very sluggish in case of absence of catalyst (Table 1, entry 1). Considering the data of model reaction (Table 1, entry 7) in terms of reaction time and yield, RPSA was found to be superior catalyst for this reaction. The model reaction conditions were optimized by varying the catalyst loadings to increase the yield of the product, results of which are summarized in (Table 2). The results
suggest that 15 wt % of the RPSA catalyst (Table 2, entry 3) was best suited for the reaction. However, when the loading of catalyst was raised to 20 and 25 wt %, surprisingly it did not appreciably alter the time or the yield (98 %) of the reaction (Table 2, entry 4, 5).

Table 2. The effect of catalyst loading on model reaction (4a).a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Time (min)</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5 wt % RPSA</td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>2.</td>
<td>10 wt % RPSA</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>3.</td>
<td>15 wt % RPSA</td>
<td>20</td>
<td>98</td>
</tr>
<tr>
<td>4.</td>
<td>20 wt % RPSA</td>
<td>15</td>
<td>98</td>
</tr>
<tr>
<td>5.</td>
<td>25 wt % RPSA</td>
<td>15</td>
<td>98</td>
</tr>
</tbody>
</table>

a. Reaction condition: Benzil (1 mmol), benzoaldehyde (1 mmol), ammonium acetate (4 mmol), and various concentration of RPSA catalyst carried out in ethanol (5 mL) at reflux temperature.
b. Isolated yields.

The model reaction was further carried out by employing range of polar and non polar solvents such as ethanol, methanol, isopropanol, acetonitrile, dichloromethane and chloroform to study the effects of solvents on the reaction. Ethanol was found to be far more effective solvent as compared to methanol, isopropanol, and acetonitrile as solvents (Table 3, entry 2). The results signify that the polarity of the solvent is also very crucial for the reaction as gradual decrease in yield was observed with decrease in polarity of solvents.

Table 3. Screening of solvents for model reaction (4a).a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>60</td>
<td>85</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>20</td>
<td>98</td>
</tr>
<tr>
<td>3.</td>
<td>Isopropanol</td>
<td>120</td>
<td>85</td>
</tr>
<tr>
<td>4.</td>
<td>Acetonitrile</td>
<td>120</td>
<td>92</td>
</tr>
<tr>
<td>5.</td>
<td>Dichloromethane</td>
<td>120</td>
<td>90</td>
</tr>
<tr>
<td>6.</td>
<td>Chloroform</td>
<td>120</td>
<td>87</td>
</tr>
</tbody>
</table>

a. Reaction condition: Benzil (1 mmol), benzoaldehyde (1 mmol), ammonium acetate (4 mmol), and 15 wt % RPSA, carried out at reflux temperatures in various solvents (5 mL).
b. Isolated yields.

The main advantage of using RPSA as catalyst, is that it could be reused making it economical and eco-friendly. The reusability of RPSA was also examined (Table 4). After the reaction, water was added and the reaction mixture was filtered. The residue (RPSA catalyst) was washed with ethanol, dried and reused for
further reactions. The RPSA catalyst was found to be reusable, although with a gradual decline in activity.

### Table 4. Effect of reusability of RPSA acid on model reaction (4a).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cycle</th>
<th>Time (min)</th>
<th>Yield(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>20</td>
<td>98</td>
</tr>
<tr>
<td>2.</td>
<td>1</td>
<td>20</td>
<td>98</td>
</tr>
<tr>
<td>3.</td>
<td>2</td>
<td>25</td>
<td>95</td>
</tr>
<tr>
<td>4.</td>
<td>3</td>
<td>25</td>
<td>92</td>
</tr>
<tr>
<td>5.</td>
<td>4</td>
<td>30</td>
<td>90</td>
</tr>
</tbody>
</table>

\( ^a \) Reaction condition: Benzil (1 mmol), benzaldehyde (1 mmol), ammonium acetate (4 mmol), and 15 wt % RPSA in ethanol (5 mL) at reflux temperature.

\( ^b \) Isolated yields.

Additionally, the effect of RPSA catalyst under the influence of microwave irradiation was also studied. The model reaction (4a) were carried out under microwave irradiation in ethanol with the accelerated the rate of transformation due to the effect of high energetic microwave irradiation.

### Table 5. Synthesis of 2,4,5 trisubstituted imidazoles (4a-l) catalyzed by RPSA.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Aldehyde</th>
<th>Time(^c) (min)</th>
<th>Yield(^d) (%)</th>
<th>Melting Point (°C)</th>
<th>Observed</th>
<th>Reported(^24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>Benzaldehyde</td>
<td>20/2</td>
<td>98/99</td>
<td>269-271</td>
<td>270</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>2-naphthaldehyde</td>
<td>40/5</td>
<td>97/98</td>
<td>240-242</td>
<td>242-243</td>
<td></td>
</tr>
<tr>
<td>4c</td>
<td>4-nitro benzaldehyde</td>
<td>20/2</td>
<td>96/98</td>
<td>241-243</td>
<td>242-243</td>
<td></td>
</tr>
<tr>
<td>4d</td>
<td>4-fluoro benzaldehyde</td>
<td>20/2</td>
<td>97/97</td>
<td>189-191</td>
<td>189-191</td>
<td></td>
</tr>
<tr>
<td>4e</td>
<td>4-Chloro benzaldehyde</td>
<td>25/2</td>
<td>97/98</td>
<td>261-263</td>
<td>263</td>
<td></td>
</tr>
<tr>
<td>4f</td>
<td>2,4- dichloro benzaldehyde</td>
<td>25/2</td>
<td>98/98</td>
<td>177-179</td>
<td>176-178</td>
<td></td>
</tr>
<tr>
<td>4g</td>
<td>2,4- dihydroxy benzaldehyde</td>
<td>25/2</td>
<td>96/97</td>
<td>270-272</td>
<td>270-273</td>
<td></td>
</tr>
<tr>
<td>4h</td>
<td>4-methyl benzaldehyde</td>
<td>30/5</td>
<td>98/98</td>
<td>232-234</td>
<td>233-236</td>
<td></td>
</tr>
<tr>
<td>4i</td>
<td>4-methoxy benzaldehyde</td>
<td>30/2</td>
<td>96/98</td>
<td>230-232</td>
<td>231-233</td>
<td></td>
</tr>
<tr>
<td>4j</td>
<td>thiophene-2-carbaldehyde</td>
<td>25/2</td>
<td>98/98</td>
<td>261-263</td>
<td>260-262</td>
<td></td>
</tr>
<tr>
<td>4k</td>
<td>furan-2-carbaldehyde</td>
<td>25/2</td>
<td>95/97</td>
<td>199-201</td>
<td>200-201</td>
<td></td>
</tr>
<tr>
<td>4l</td>
<td>6-chloro-2-hydroxy nicotinaldehyde</td>
<td>30/2</td>
<td>94/95</td>
<td>235-237</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) Reaction condition: Benzil (1 mmol), substituted aldehydes (1 mmol), ammonium acetate (4 mmol), and 15 wt % RPSA carried out in ethanol (5 mL) at reflux temperature.

\( ^b \) Reaction condition: Benzil (1 mmol), substituted aldehydes (1 mmol), ammonium acetate (4 mmol), and 15 wt % RPSA carried out at 55°C under microwave irradiation in ethanol (5 mL).

\( ^c \) Time required by conventional and microwave irradiation respectively.

\( ^d \) Isolated yields by conventional and microwave irradiation respectively.
The robustness of the method was evaluated by using a wide variety groups on aldehyde (both aromatic and heteroaromatic) and 1,2-diketones successfully, giving respective products in excellent yields (Table 5). As the developed protocol is mild, side product formation is avoided which is generally major drawback where strong acids are employed.

ANTIMICROBIAL ACTIVITY

The synthesized imidazole analogues (4a-4l) were screened for their antimicrobial activity against two gram positive bacteria; Bacillus subtilis (NCIM-2063) and Staphylococcus aureus (NCIM-2901), one gram negative bacteria; Escherichia coli (NCIM-2256) and three fungal strains; Candida albicans (NCIM-3471), Aspergillus flavus (NCIM-539) and Aspergillus niger (NCIM-1196).

Table 6. Antimicrobial activity of the synthesized compounds (4a-4l).

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC Values (μg/mL) *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>4a</td>
<td>60</td>
</tr>
<tr>
<td>4b</td>
<td>80</td>
</tr>
<tr>
<td>4c</td>
<td>60</td>
</tr>
<tr>
<td>4d</td>
<td>35</td>
</tr>
<tr>
<td>4e</td>
<td>50</td>
</tr>
<tr>
<td>4f</td>
<td>40</td>
</tr>
<tr>
<td>4g</td>
<td>80</td>
</tr>
<tr>
<td>4h</td>
<td>90</td>
</tr>
<tr>
<td>4i</td>
<td>80</td>
</tr>
<tr>
<td>4j</td>
<td>35</td>
</tr>
<tr>
<td>4k</td>
<td>45</td>
</tr>
<tr>
<td>4l</td>
<td>15</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>50</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
</tr>
<tr>
<td>Miconazole</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values are the average of three readings.
Activity of compounds was monitored by observing their minimum inhibitory concentration (MIC) by broth dilution method as per CLSI guidelines $^{20-23}$ parallel with Ciprofloxacin and Ampicillin as control drugs in antibacterial study (Table 6), while antifungal study by standard agar dilution method as per CLSI guidelines $^{20-23}$ were Fluconazole and Miconazole were used as control drugs. Dimethyl sulfoxide was used as solvent control. From antimicrobial data represented in (Table 6), it is observed that all the screened compounds shows good to moderate antimicrobial activity against the bacterial and fungal strains. The important observation from the results of activity is that among all the synthesized compounds (4I) is most active having a broad spectrum, which is found to be slightly more potent than the control drugs, inhibiting the growth of *Escherichia coli* and *Bacillus subtilis* completely at 15 $\mu$g/mL of concentration and that of *Staphylococcus aureus* at 20 $\mu$g/mL. It is also active against fungal strain *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*, inhibiting growth of each of them at 20 $\mu$g/mL. Amongst the other compounds only (4e) and (4f) shows definite class specificity, as observed (4e) is more effective as antifungal agent having MIC values ranging from 35 to 50 $\mu$g/mL (35 $\mu$g/mL for *Candida albicans* and 50 $\mu$g/mL for *Aspergillus niger*), as compared to its bacterial MIC value which is 50 to 70 $\mu$g/mL (50 $\mu$g/mL for *Bacillus subtilis* and 70 $\mu$g/mL for *Escherichia coli*).

On a another side (4f) has predominant antibacterial property consisting MIC values between 35 to 40 $\mu$g/mL, as compared to its fungal MIC values, 60 to 75 $\mu$g/mL. Compounds (4d, 4j and 4k) also have the broad spectrum, affecting the bacterial and fungal strains that were tested, exhibiting better activity than the control drug ampicillin. Their activity shows variation in magnitude in order (4d > 4j > 4k) in both strains. The remaining compounds (4a, 4b, 4c, 4g, 4h and 4i) are also promising in their antimicrobial activity and require further structural modification for improvement of their activity.

**STRUCTURE ACTIVITY RELATIONSHIP**

The main objective of this work was to study the effect of different substituent’s to the imidazole ring on their antimicrobial property. The highlighting part of the antimicrobial study of these molecules has been summarized below,

**1. Effect of ring size:** When the 2$^{nd}$ position of the imidazole ring is occupied by the phenyl ring (4a) the compound shows moderate activity. But similarly when the
2-naphthalene (4b) occupies the 2\textsuperscript{nd} position of the imidazole ring it shows the lowest activity amongst all the synthesized compounds (4a-4l). These results indicate that as the ring size increase, it leads in decreasing the antimicrobial activity.

2. Effect of substituent’s on Phenyl ring: The Imidazole analogues (4a, 4c-4i) having various substituents on phenyl ring (4c-4i). These analogues showed most diversified action and possessed interesting variations, which were largely affected by the substituent’s attached to the phenyl ring.

- The compound (4d) having a \textit{p-}fluoro substituent enhances the antimicrobial activity to a great extent and was the most suitable analogue of the phenyl substituent possessing both the antibacterial and antifungal activity.
- The compound (4e) having a \textit{p-}chloro substituent, has selectively high antifungal property but has a low antibacterial property. The results indicate that as the substituent is changed from the \textit{p-}fluoro to \textit{p-}chloro there is selectively rise in antifungal property. In other words \textit{chloro} substituent is favorable for the antifungal activity.
- The compound (4f) having \textit{o, p-}dichloro substituent, shows high antibacterial activity but it shows low antifungal activity. This indicates that as the number of chlorine atoms in the molecules increases it results in rise of the antibacterial activity.
- The compound (4g, 4h and 4i) having \textit{o, p-}dihydroxy, \textit{p-}methyl, \textit{p-}methoxy, strongly inhibit the antimicrobial activity, are have lowest activity amongst the various substituent’s on phenyl ring. This indicates that if electron donating groups like \textit{methyl, methoxy} are present as the substituent’s it results in diminishing the antimicrobial activity of the compounds.
- The compound (4c) having a \textit{p-}nitro substituent reduces the antimicrobial activity to moderate level, indicating that the strong electron withdrawing group reduces the antimicrobial activity of the compounds.

3. Effect of ring: The compounds (4j-4l) having heterocyclic substituents on the 2\textsuperscript{nd} position of the imidazole ring, showed increase in antimicrobial activity compared to plain phenyl ring (4a). This indicated that when the phenyl ring is replaced by heterocyclic ring there is rise in antimicrobial properties of the compounds.

4. Effect of heterocyclic ring: The compounds (4j-4l) having heterocyclic substituent’s were evaluated for their antimicrobial properties which are as
summarized below,

- The compound (4j) having a thiophene ring substituent, has increased activity in both antibacterial and antifungal property.
- The compound (4k) having a furan ring substituent has lower activity in both antibacterial and antifungal property. It indicates that the substitution of thiophene ring by furan will reduce the potency of the analogues.
- The compound (4l) having a pyridine ring substituent is the most potent analogue of this series and has slightly more activity compared to the control drug. This indicates that the heterocyclic substituent favor the antimicrobial activity. It indicate that Nitrogen containing heterocycles are the most likely substituent’s to raise the activity compared to the sulphur and oxygen containing heterocycles.

**Highlight of synthesis of 2,4,5-trisubstituted imidazole analogues:**

The novelty and highlight in synthesis of 2,4,5-trisubstituted imidazoles analogues is (i) the first report of 2,4,5-trisubstituted imidazoles analogues, catalyzed by RPSA; (ii) good yields in shorter reaction time (iii) synthesis of series of 2,4,5-trisubstituted imidazoles analogues having antibacterial and antifungal activity (iv) the systematic evaluation, of the structural variation on the antimicrobial activity of the imidazole analogues.

In conclusion, we report a convenient and facile approach for the synthesis of 2,4,5-trisubstituted imidazoles analogues catalyzed by RPSA. The mild reaction conditions, shorter reaction time and promising antibacterial and antifungal activity of (4d, 4e, 4f and 4l) the compounds compared to standard are the advantages of the present method. The in depth study of the effect of structural variation on the antimicrobial activity of the analogues will help the researchers in developing more potent molecules in the future.

**EXPERIMENTAL**

The substituted aldehydes, ammonium acetate and RPSA used were commercially available. Melting points were obtained with an SRS Optimelt melting point apparatus and are uncorrected. Microwave reactions were carried out in Ethusi Milestone (MicroSynth) Labstation with temperature control. $^1$H NMR spectra were recorded on a 400 MHz Bruker spectrometer and $^{13}$C NMR spectra were recorded on a 100 MHz Bruker spectrometer are reported as parts per million (ppm) downfield from a tetramethylsilane internal standard. The following abbreviations are used,
singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

**General procedure for the synthesis of 2,4,5-trisubstituted imidazoles (4a-l) by conventional method:**

In a 50 ml RBF, benzil (1 mmol), substituted aldehydes (1 mmol) and ammonium acetate (4 mmol) were stirred in the presence of 15 wt % of RPSA in ethanol (5 mL) at reflux temperature for 20-40 min (Table 5). After completion (monitored by TLC, 20 % Ethyl acetate: n-hexane), the reaction mixture was diluted with water (10 mL), filtered to remove the insoluble catalyst (obtained as residue) and filtrate was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, concentrated and recrystallized from ethanol to afford pure product in good yields (94-98%). The catalyst which was obtained as residue was washed with ethanol, dried and reused for further reactions.

**General procedure for the synthesis of 2,4,5-trisubstituted imidazoles (4a-l) under microwave irradiation:**

In a 50 ml RBF, benzil (1 mmol), substituted aldehydes (1 mmol) and ammonium acetate (4 mmol) were stirred in the presence of 15 wt % of RPSA in ethanol (5 mL) at 55 °C for 2-5 min (Table 5) under microwave irradiation. After completion (monitored by TLC, 20 % Ethyl acetate: n-hexane), the reaction mixture was diluted with water (10 mL), filtered to remove the insoluble catalyst (obtained as residue) and filtrate was and extracted with ethyl acetate (3 x 10 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, concentrated and recrystallized from ethanol to afford pure product in good yields (95-99%). The catalyst which was obtained as residue was washed with ethanol, dried and reused for further reactions.

**Spectral characterization**

**2,4,5-triphenyl-1H-imidazole (4a):**

White Solid, ES-MS m/z (%): 297 (M+H).

$^1$H NMR (400 MHz, CDCl$_3$): δ ppm 7.25-7.48 (m, 5H), 7.52-7.70 (m, 5H), 7.80-7.94 (m, 5H), 12.70 (s, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 124.7, 125.3, 126.0, 126.6, 127.2, 127.8, 128.0, 128.3, 128.5, 128.9, 129.2, 129.8, 130.2, 130.7, 131.2, 131.9, 132.5, 135.2, 137.2, 145.6, 174.1.

**2-(naphthalen-2-yl)-4,5-diphenyl-1H-imidazole (4b):**

White Solid, ES-MS m/z (%): 347 (M+H).

$^1$H NMR (400 MHz, CDCl$_3$): δ ppm 7.18-7.30(m, 5H), 7.52(t, 2H), 7.58-7.76(m, 5H),
7.85 (d, 1H), 7.97 (d, 2H), 8.47 (d, 1H), 8.86 (s, 1H), 12.78 (s, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 122.3, 122.9, 123.6, 124.1, 124.8, 125.4, 126.1, 126.7, 127.2, 127.8, 128.2, 128.5, 128.9, 129.3, 129.8, 130.3, 130.8, 131.4, 131.9, 132.6, 133.1, 135.4, 137.3, 142.3, 173.4.

2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazole (4c):
White Solid, ES-MS m/z (%): 297 (M+H).

$^1$H NMR (400 MHz, CDCl$_3$): δ ppm 7.30-7.45 (m, 5H), 7.62-7.80 (m, 5H), 8.05-8.24 (m, 4H), 12.73 (s, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 118.5, 122.2, 122.9, 124.3, 124.8, 125.4, 126.1, 126.9, 127.4, 127.8, 128.4, 128.9, 129.3, 129.7, 130.6, 130.8, 131.3, 131.9, 143.7, 148.9, 173.4.

2-(4-fluorophenyl)-4,5-diphenyl-1H-imidazole (4d):
White Solid, ES-MS m/z (%): 315 (M+H).

$^1$H NMR (400 MHz, CDCl$_3$): δ ppm 7.20 (d, 2H), 7.35-7.48 (m, 5H), 7.56 (d, 2H), 7.70-7.85 (m, 5H), 12.65 (s, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 119.7, 121.8, 122.5, 123.2, 123.8, 124.1, 124.8, 125.3, 125.7, 126.2, 126.9, 127.3, 128.2, 128.9, 130.2, 131.0, 131.8, 133.2, 146.8, 165.1, 173.4.

2-(2,4-dichlorophenyl)-4,5-diphenyl-1H-imidazole (4f):
White Solid, ES-MS m/z (%): 366 (M+H).

$^1$H NMR (400 MHz, CDCl$_3$): δ ppm 7.24-7.40 (m, 5H), 7.62 (d, 2H), 7.75-7.85 (m, 5H), 7.95 (s, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 125.5, 126.1, 127.0, 127.9, 128.3, 128.9, 129.3, 129.9, 130.4, 130.9, 131.7, 132.1, 132.7, 133.2, 133.9, 135.8, 136.4, 137.0, 137.5, 142.7, 151.4.

4-(4,5-diphenyl-1H-imidazol-2-yl)benzene-1,3-diol (4g):
White Solid, ES-MS m/z (%): 329 (M+H).

$^1$H NMR (400 MHz, CDCl$_3$): δ ppm 5.40 (s, 1H), 5.73 (s, 1H), 6.37 (s, 1H), 7.27-7.44 (m, 5H), 7.52 (d, 2H), 7.61-7.74 (m, 5H), 12.81 (s, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 107.3, 112.3, 113.0, 115.4, 121.6, 125.7, 126.4, 127.0, 127.3, 127.9, 128.5, 129.2, 131.3, 135.4, 136.6, 137.4, 139.1, 146.1, 150.2, 157.7, 158.5.

4,5-diphenyl-2-(p-tolyl)-1H-imidazole (4h):
White Solid, ES-MS m/z (%): 311 (M+H).

$^1$H NMR (400 MHz, CDCl$_3$): δ ppm 2.67 (s, 3H), 7.12 (d, 2H), 7.32-7.45 (m, 5H),
Chapter II  Section A  Synthesis and Screening of Some Bioactive Heterocycles

7.67-7.84 (m, 5H), 8.24 (d, 2H), 12.71 (s, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ ppm 20.5, 121.4, 122.3, 123.1, 123.9, 125.1, 126.5, 127.0, 127.8, 128.1, 128.5, 128.9, 129.4, 130.2, 131.1, 132.5, 133.1, 135.2, 136.9, 137.6, 145.6, 172.1.

2-(4-methoxyphenyl)-4,5-diphenyl-1H-imazole (4i):
White Solid, ES-MS m/z (%): 327 (M+H).
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ ppm 3.81 (s, 3H), 6.96 (d, 2H), 7.21-7.37 (m, 5H), 7.55-7.69 (m, 5H), 7.90 (d, 2H), 12.62 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ ppm 20.5, 121.4, 122.3, 123.1, 123.9, 125.1, 126.5, 127.0, 127.8, 128.1, 128.5, 128.9, 129.4, 130.2, 131.1, 132.5, 133.1, 135.2, 136.9, 137.6, 145.6, 172.1.

4,5-diphenyl-2-(thiophen-2-yl)-1H-imazole (4j):
Pale Yellow Solid, ES-MS m/z (%): 303 (M+H).
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ ppm 7.12 (t, 1H), 7.28-7.41 (m, 5H), 7.50 (d, 1H), 7.63-7.75 (m, 5H), 7.85 (d, 1H), 12.71 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ ppm 123.5, 124.3, 125.2, 126.1, 127.0, 127.5, 128.0, 128.5, 128.7, 129.0, 129.4, 129.9, 130.3, 130.9, 131.5, 132.4, 135.3, 137.2, 143.7.

2-(furan-2-yl)-4,5-diphenyl-1H-imazole (4k):
Pale Yellow Solid, ES-MS m/z (%): 287 (M+H).
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ ppm 7.08 (t, 1H), 7.21-7.38 (m, 5H), 7.45 (d, 1H), 7.58-7.77 (m, 5H), 7.81 (d, 1H), 12.67 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ ppm 109.6, 115.3, 123.6, 125.5, 126.2, 127.1, 127.6, 128.0, 128.8, 129.5, 129.9, 130.3, 130.8, 131.6, 132.2, 137.4, 140.7, 143.3, 152.1.

6-chloro-3-(4,5-diphenyl-1H-imidazol-2-yl)pyridin-2-ol (4l):
Yellow Solid, ES-MS m/z (%): 349 (M+H).
$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ ppm 6.76 (d, 1H), 7.25-7.40 (m, 5H), 7.60-7.75 (m, 5H), 7.91 (d, 1H), 11.07 (s, 1H), 12.70 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ ppm 104.5, 124.4, 125.5, 126.1, 126.9, 127.5, 128.1, 128.7, 129.3, 130.0, 130.8, 131.6, 132.7, 134.2, 139.2, 143.6, 149.5, 165.7.
$^1$H NMR spectrum of compound (4I):

$^{13}$C NMR spectrum of compound (4I):
Mass spectrum of compound (4l):
Chapter II  Section A  Synthesis and Screening of Some Bioactive Heterocycles

REFERENCES

Chapter II
Section B
Synthesis and screening of new 6-chloro-5-(2,3-dichlorophenoxy)-2-phenyl-1H-benzo[d]imidazole analogues

INTRODUCTION

The benzimidazole and their analogues are considered as important bioactive heterocycles. The benzimidazole analogues are widely explored in the past decade for their interesting biological activities. Amongst the various Nitrogen heterocycles, benzimidazoles are considered of prime importance owing to their diversified activities. Benzimidazole derivatives have been found to possess a wide range of biological activities, such as anticancer,\(^1\) antiHIV,\(^1\) antiulcer,\(^2\) antihypertensive,\(^3\) antibacterial,\(^4\) enzyme inhibition,\(^5\) antiinflammatory,\(^6\) antihistamine,\(^7\) anti-ulcerative,\(^8\) antioxidant,\(^9\) antiproliferative,\(^10\) antiinflammatory,\(^11\) and antikinase.\(^13\)

The benzimidazole derivatives are generally prepared by the reaction of 1,2-phenylenediamines either with carboxylic acids\(^14\) under strongly acidic conditions and sometimes combined with very high temperatures. These derivatives are often generated from the condensation of phenylenediamines with aldehydes.\(^15\) In recent times variety of catalyst were employed for the synthesis of benzimidazole like, Zn-proline,\(^16\) heteropolyacids,\(^17\) sulfamic acid,\(^18\) ionic liquids,\(^19\) p-TSA,\(^20\) ZrOCl\(_2\).8H\(_2\)O,\(^21\) boron trifluoride etherate,\(^21\) VO(acac)\(_2\)-CeCl\(_3\) combo catalyst\(^22\) and gold/CeO\(_2\).\(^23\) Although these reactions were satisfactorily carried out by the above conditions, they were often associated with several drawbacks like high cost of the catalysts, prolonged reaction times at elevated temperatures, occurrence of several side reactions due to strongly acidic conditions and difficulty in isolation of products from the reaction mixture. Therefore, the attention of researchers is on developing mild, ecofriendly, feasible and stable reaction conditions. In continuation of our work on developing effective protocol for the synthesis and screening of heterocycles,\(^24\) herein we would like to report the synthesis of substituted benzimidazole catalyzed by resin bound propylsulphonic acid (RPSA) which is recyclable and ecofriendly (Scheme 1).

RESULT AND DISCUSSION

Initially, we attempted the model reaction (3a) by condensation of 4-chloro-5-(2,3-dichlorophenoxy)benzene-1,2-diamine (1 mmol) with benzaldehyde (1 mmol) in ethanol at reflux temperature for 10 hr, in absence of catalyst and we observed about 13 % of product was formed (Table 1, entry 1). The yield of the
product formed was much less than we had anticipated. To improve the yield of the reaction we employed RPSA as a catalyst for the model reaction. The model reaction of 4-chloro-5-(2,3-dichlorophenoxy)benzene-1,2-diamine (1 mmol) with (1 mmol) of benzaldehyde in ethanol at reflux temperature, catalyzed by 5 wt % RPSA gave 62 % yield in 4 hr (Table 1, entry 2).


In order to yield of the reaction we decided to carry out the reaction using 10 wt % of catalyst. To our delight the reaction got to completion within 3 hr. and the yield of the product also rose to 85% (Table 1, entry 3). Encouraged by these results we decided to carry out the reaction using different concentration of the catalysts (Table 1, entry 4-6). The data obtained from (Table 1), indicated that the amount of catalyst plays a significant role in progress of the reaction.

Table 1. The effect of catalyst on model reaction (3a).a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Time (min)</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>No catalyst</td>
<td>600</td>
<td>13</td>
</tr>
<tr>
<td>2.</td>
<td>5 wt % RPSA</td>
<td>240</td>
<td>62</td>
</tr>
<tr>
<td>3.</td>
<td>10 wt % RPSA</td>
<td>150</td>
<td>85</td>
</tr>
<tr>
<td>4.</td>
<td>15 wt % RPSA</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>5.</td>
<td>20 wt % RPSA</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>6.</td>
<td>25 wt % RPSA</td>
<td>100</td>
<td>95</td>
</tr>
</tbody>
</table>

a. Reaction condition: 4-chloro-5-(2,3-dichlorophenoxy)benzene-1,2-diamine (1 mmol), benzaldehyde (1 mmol) carried out in ethanol (5mL) at reflux temperature using different concentrations of RPSA.
b. Isolated yields.

Among the various concentration of catalyst studied, 15 wt % of the catalyst was found to be suitable for the model reaction (3a), yield 95% (Table 1, entry 4). The 20 and 25 wt % of the catalyst gave 95% yield and the time required was comparable to that of 15 wt %. The effect of solvent (Table 2) was also studied on the model reaction and it was observed that the reaction proceeds smoothly in polar solvents like ethanol, methanol, isopropanol, but was sluggish in non polar
solvents (Table 2, entry 5-6). Ethanol was the most suitable solvent (Table 2, entry 2) of the tested solvents.

Table 2. Screening of solvent for model reaction (3a). a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>150</td>
<td>85</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>3.</td>
<td>Isopropanol</td>
<td>180</td>
<td>82</td>
</tr>
<tr>
<td>4.</td>
<td>Acetonitrile</td>
<td>180</td>
<td>90</td>
</tr>
<tr>
<td>5.</td>
<td>Dichloromethane</td>
<td>245</td>
<td>90</td>
</tr>
<tr>
<td>6.</td>
<td>Chloroform</td>
<td>240</td>
<td>85</td>
</tr>
</tbody>
</table>

a. Reaction condition: 4-chloro-5-(2,3-dichlorophenoxy)benzene-1,2-diamine (1 mmol), benzaldehyde (1 mmol) and 15 wt % RPSA carried out at reflux temperatures in various solvents (5mL).

b. Isolated yields.

The main advantage of the RPSA was its thermal and chemical stability at elevated temperature without much loss in its reactivity. The RPSA was evaluated on the parameter of reusability (Table 3, entry 1-5). The catalyst that was recovered by filtration of reaction mixture was washed with ethanol, dried and reused for further reactions.

Table 3. Effect of reusability of RPSA on model reaction (3a). a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cycle</th>
<th>Time (min)</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>2.</td>
<td>1</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>3.</td>
<td>2</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>4.</td>
<td>3</td>
<td>120</td>
<td>90</td>
</tr>
<tr>
<td>5.</td>
<td>4</td>
<td>150</td>
<td>90</td>
</tr>
</tbody>
</table>

a. Reaction condition: 4-chloro-5-(2,3-dichlorophenoxy)benzene-1,2-diamine (1 mmol), benzaldehyde (1 mmol) and 15 wt % RPSA carried out in ethanol (5mL) at reflux temperature.

b. Isolated yields.

The data obtained from (Table 3), indicated that the catalyst can be successfully employed in cycles making it an economical and ecofriendly. But as the recycles increased it resulted in slight decrease in its reactivity. The practical feasibility of the method was evaluated by using series of electron donating and electron withdrawing groups on the aldehydes, heterocyclic aldehydes were also used to determine the robustness of the method (Table 4). The method was found to suit the variety of substituent’s on the rings giving excellent yields.
Table 4. Synthesis of substituted benzimidazoles (3a-1) catalyzed by RPSA.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Aldehyde</th>
<th>Time (min)</th>
<th>Yield\textsuperscript{b} (%)</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>Benzaldehyde</td>
<td>100</td>
<td>95</td>
<td>259-261</td>
</tr>
<tr>
<td>3b</td>
<td>p-fluoro benzaldehyde</td>
<td>105</td>
<td>94</td>
<td>263-265</td>
</tr>
<tr>
<td>3c</td>
<td>p-Chloro benzaldehyde</td>
<td>105</td>
<td>96</td>
<td>227-229</td>
</tr>
<tr>
<td>3d</td>
<td>o,p- dichloro benzaldehyde</td>
<td>110</td>
<td>93</td>
<td>254-256</td>
</tr>
<tr>
<td>3e</td>
<td>p-nitro benzaldehyde</td>
<td>100</td>
<td>95</td>
<td>277-279</td>
</tr>
<tr>
<td>3f</td>
<td>p-methyl benzaldehyde</td>
<td>115</td>
<td>94</td>
<td>192-194</td>
</tr>
<tr>
<td>3g</td>
<td>p-methoxy benzaldehyde</td>
<td>120</td>
<td>95</td>
<td>256-258</td>
</tr>
<tr>
<td>3h</td>
<td>p-hydroxy benzaldehyde</td>
<td>110</td>
<td>94</td>
<td>217-219</td>
</tr>
<tr>
<td>3i</td>
<td>o,p- dihydroxy benzaldehyde</td>
<td>105</td>
<td>96</td>
<td>241-243</td>
</tr>
<tr>
<td>3j</td>
<td>thiophene-2-carbaldehyde</td>
<td>110</td>
<td>94</td>
<td>231-233</td>
</tr>
<tr>
<td>3k</td>
<td>furan-2-carbaldehyde</td>
<td>105</td>
<td>93</td>
<td>239-241</td>
</tr>
<tr>
<td>3l</td>
<td>6-chloro-2-hydroxy nicotinaldehyde</td>
<td>100</td>
<td>92</td>
<td>265-267</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction condition: 4-chloro-5-(2,3-dichlorophenoxy)benzene-1,2-diamine (1 mmol), substituted aldehydes (1 mmol) and 15 wt % RPSA carried out in ethanol (5mL) at reflux temperature.

\textsuperscript{b} Isolated yields.

ANTIMICROBIAL ACTIVITY

The synthesized benzimidazole analogues (3a-3l) were screened for their antimicrobial activity against two gram positive bacteria; Bacillus subtilis (NCIM-2063) and Staphylococcus aureus (NCIM-2901), one gram negative bacteria; Escherichia coli (NCIM-2256) and three fungal strains; Candida albicans (NCIM-3471), Aspergillus flavus (NCIM-539) and Aspergillus niger (NCIM-1196).

Activity of compounds was monitored by observing their minimum inhibitory concentration (MIC) by broth dilution method as per CLSI guidelines\textsuperscript{25-28} parallel with Ciprofloxacin and Ampicillin as control drugs in antibacterial study (Table 5), while antifungal study was carried by standard agar dilution method as per CLSI guidelines\textsuperscript{25-28} were Fluconazole and Miconazole were used as control drugs (Table 5). Dimethyl sulfoxide was used as solvent control.

From the antimicrobial data, represented in (Table 5), it was observed that synthesized compounds showed good to moderate antimicrobial activity against tested bacterial and fungal strains.
Table 5. Antimicrobial activity of the synthesized compounds (3a-l).

<table>
<thead>
<tr>
<th>Compound</th>
<th>B. subtilus</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>C. Albicans</th>
<th>A. Flavus</th>
<th>A. Niger</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>40</td>
<td>60</td>
<td>35</td>
<td>50</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>3b</td>
<td>25</td>
<td>20</td>
<td>12.5</td>
<td>20</td>
<td>15</td>
<td>12.5</td>
</tr>
<tr>
<td>3c</td>
<td>60</td>
<td>90</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>3d</td>
<td>25</td>
<td>30</td>
<td>25</td>
<td>30</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>3e</td>
<td>40</td>
<td>100</td>
<td>80</td>
<td>35</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>3f</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>60</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>3g</td>
<td>40</td>
<td>90</td>
<td>60</td>
<td>50</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>3h</td>
<td>50</td>
<td>100</td>
<td>60</td>
<td>70</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>3i</td>
<td>80</td>
<td>90</td>
<td>80</td>
<td>60</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>3j</td>
<td>25</td>
<td>30</td>
<td>20</td>
<td>30</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>3k</td>
<td>30</td>
<td>35</td>
<td>30</td>
<td>35</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>3l</td>
<td>15</td>
<td>20</td>
<td>12.5</td>
<td>20</td>
<td>12.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Ciprofloxacin | 25 | 25 | 15 | - | - | - |
Ampicillin     | 50 | 50 | 50 | - | - | - |
Fluconazole    | -  | -  | -  | 40| 25| 25 |
Miconazole     | -  | -  | -  | 12.5| 12.5| 12.5 |

a. Values are the average of three readings.

The results show a common trend in antibacterial and antifungal activity, which can drawn in descending order of (3l, 3b > 3d, 3j, 3k, > 3a > 3c, 3g, 3i, 3e). The compounds (3f and 3h) are more specific as antifungal agents (average MIC is 32.5 μg/mL) and have very poor antibacterial activity (average MIC is 68.33 μg/mL). The results indicate that among the all compounds (3l and 3b) are the most active and broad spectrum molecule that were more potent than the control drugs, both of which inhibited the growth of Escherichia coli and Aspergillus niger completely at concentration of 12.5 μg/mL and of Staphylococcus aureus and Candida albicans at 20 μg/mL, while the MIC value of both for Bacillus subtilis and Aspergillus flavus ranges from 12.5 to 25 μg/mL. The compounds (3d, 3j and 3k) showed broad spectrum and good activity against all the tested strains with MIC value...
varying in narrow range of 25 to 35 μg/mL which is slightly lower than Ciprofloxacin (21.60 μg/mL) and Miconazole (16.66 μg/mL) but better than Ampicillin (50 μg/mL) and Fluconazole (30 μg/mL). The compounds (3c, 3g, 3i, and 3e) were least active with MIC values of 50 to 100 μg/mL, among which (3e) was moderately active against fungi at MIC of 31.66 μg/mL.

**STRUCTURAL ACTIVITY RELATIONSHIP**

The main aim of this work was to synthesize the new benzimidazole analogues and study their antimicrobial activity. As this work is the first report of synthesis of such analogues it would be of immense help to the researchers around the globe to plan their future work based on these studies. The highlighting aspects of the variation in structure on the antimicrobial activity is as summarized below,

1. **Effect of substituent’s on Phenyl ring:** The benzimidazole analogues (3a-3i) having substituent’s on phenyl ring exhibited most diversified action and possessed interesting variations, which were largely affected by the various substituent’s attached to the phenyl ring.
   a. The compound (3a) having a plain phenyl ring attached on the 2\textsuperscript{nd} position of the benzimidazole analogue, showed moderate antibacterial and antifungal properties without side chains (substituent’s). It has high potential to be developed as antimicrobial moiety but itself it is not sufficient as antimicrobial agent. The compounds (3b-3i) showed interesting variations, depending upon the substituent’s attached to phenyl ring. The substituent’s (side chains) attached not only altered the degree of activity but also its specificity as antibacterial or antifungal property.
   b. The compound (3b) having a \textit{p-fluoro} substituent enhances both the antimicrobial activity to a great extent and was the most suitable analogue of the phenyl substituent possessing both the antibacterial and antifungal activity.
   c. The compound (3c and 3e) having a \textit{p-chloro} substituent and \textit{p-nitro} substituent make the molecule fungal specific, having good antifungal activity but it has reduced antibacterial activity. This indicate that the presence of strong electron group make the molecule specifically potent for antifungal activity. It is the most appropriate agent among the synthesized compounds for the study and development as antifungal drug.
   d. The compound (3d) having \textit{o, p-chloro} substituent’s, have enhanced the antibacterial and antifungal property to a moderate level against the tested fungal and
bacterial strains. But it is not as specific as compared to (3c).

e. The compounds (3f, 3g, 3h and 3i) having p-methyl, p-methoxy, p – hydroxy and o, p – dihydroxy compounds respectively to great extent showed reduced antibacterial and antifungal properties, making them amongst the lowest active molecules in the series. It would further suggest that with the electron donating nature of the substituents results in decreasing antimicrobial activity.

2. Effect of ring: The compounds (3j-3l) having heterocyclic substituent’s on the 2\textsuperscript{nd} position of the benzimidazole ring, showed increase in antimicrobial activity compared to plain phenyl ring (3a). This indicated that when the phenyl ring is replaced by heterocyclic ring there is rise in antimicrobial properties of the compounds.

3. Effect of heterocyclic ring: The compounds (3j-3l) having heterocyclic substituent’s were evaluated for their antimicrobial properties which are summarized as below,

a. The compound (3j) having a thiophene ring substituent, has increased activity in both antibacterial and antifungal property compared to (3a).

b. The compound (3k) having a furan ring substituent has lower activity in both antibacterial and antifungal property compared to the thiophene analogue (3j). It indicates that the substitution of thiophene ring to furan will reduce the potency of the analogues.

c. The compound (3l) having a pyridine ring substituent is the most potent analogue of this series and has slightly more activity compared to the control drug, this might be due to the additional chloro and hydroxyl substituent’s on the pyridine ring which needs to be explored further. In general it indicates that the heterocyclic substituent’s favor the antimicrobial activity.

**Highlight of synthesis of 6-chloro-5-(2,3-dichlorophenoxy)-2-phenyl-1H-benzo[d]imidazole derivatives:**

The remarkable highlight of synthesis of 6-chloro-5-(2,3-dichlorophenoxy)-2-phenyl-1H-benzo[d]imidazole analogues is (i) the first report for the synthesis of series of 6-chloro-5-(2,3-dichlorophenoxy)-2-phenyl-1H-benzo[d]imidazole; (ii) the present work is the first report of 6-chloro-5-(2,3-dichlorophenoxy)-2-phenyl-1H-benzo[d]imidazole analogues, which has been synthesized and evaluated for antimicrobial activity; (iii) the first report of use of RPSA as a catalyst for synthesis of substituted benzimidazole; (iv) recyclability of the
RPSA making it environment friendly and cost effective; (v) the noticeable chemical, mechanical and thermal stability of the RPSA would make it ideal catalyst for the synthesis; (vi) shorter reaction time with excellent yields make the process lucrative; (vii) that these analogues may be evaluated for different array of activities in future.

In conclusion, it is the first report for the synthesis of series of 6-chloro-5-(2,3-dichlorophenoxy)-2-phenyl-1H-benzo[d]imidazole analogues which are not explored. It is also the first report on use of novel and recyclable RPSA as a catalyst for this reaction. The mild reaction conditions, shorter reaction time and promising antibacterial and antifungal activity of compounds (3b, 3j, 3k and 3l) compared to standard are the advantages of the present method. Additionally, in future other biological evaluation of this series is going to be carried out.

**EXPERIMENTAL**

The substituted aldehydes and RPSA used were commercially available. The 4-chloro-5-(2,3-dichlorophenoxy)benzene-1,2-diamine was obtained as a gift sample from Molecules Ltd. Melting points were recorded on SRS Optimelt melting point apparatus and are uncorrected. \(^1\)H NMR spectra were recorded on a 400 MHz Bruker spectrometer and \(^1^3\)C NMR spectra were recorded on a 100 MHz Bruker spectrometer are reported as parts per million (ppm) downfield from a tetramethylsilane internal standard. The following abbreviations are used, singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

**General procedure for the synthesis of substituted benzimidazoles (3a-l):**

In a 50 ml RBF, 4-chloro-5-(2,3-dichlorophenoxy)benzene-1,2-diamine (1 mmol), substituted aldehydes (1 mmol) and were stirred in the presence of 15 wt % of RPSA in ethanol (5 mL) at reflux temperature for 100-120 min (Table 3). After completion (monitored by TLC, 30 % Ethyl acetate: n-hexane), the reaction mixture was diluted with water (10 mL), filtered to remove the insoluble catalyst (obtained as residue) and the filtrate was extracted with ethyl acetate (3 x 10 mL). The organic layer were combined and dried over anhydrous Na\(_2\)SO\(_4\), concentrated in-vacuo and recrystallized from ethanol to afford pure product in good yields (92-95 %). The catalyst which was obtained as residue by filtration of reaction mixture was washed with ethanol, dried and reused for further reactions.
Chapter II  Section B  Synthesis and Screening of Some Bioactive Heterocycles

Spectral characterization

6-chloro-5-(2,3-dichlorophenoxy)-2-phenyl-1H-benzo[d]imidazole (3a):
Yellow Solid, ES-MS m/z (%): 391 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.95 (s, 1H), 7.02-7.22 (m, 3H), 7.47 (s, 1H), 7.61-7.90 (m, 5H), 8.17 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 107.4, 113.8, 117.1, 119.4, 121.1, 123.7, 124.3, 128.4, 129.2, 129.7, 130.2, 131.4, 133.7, 135.0, 135.6, 137.2, 150.1, 153.3, 154.8.

6-chloro-5-(2,3-dichlorophenoxy)-2-(4-fluorophenyl)-1H-benzo[d]imidazole (3b):
Yellow Solid, ES-MS m/z (%): 409 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.90 (s, 1H), 7.05-7.18 (m, 3H), 7.41 (s, 1H), 7.55 (d, 2H), 7.71 (d, 2H), 8.10 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 107.1, 114.3, 116.7, 117.2, 118.1, 119.7, 121.7, 124.7, 125.7, 128.2, 129.0, 129.8, 131.4, 135.1, 137.2, 148.7, 153.3, 155.4, 165.5.

6-chloro-2-(4-chlorophenyl)-5-(2,3-dichlorophenoxy)-1H-benzo[d]imidazole (3c):
Yellow Solid, ES-MS m/z (%): 425 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.96 (s, 1H), 7.10-7.24 (m, 3H), 7.35 (s, 1H), 7.51 (d, 2H), 7.84 (d, 2H), 8.03 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 106.8, 114.7, 116.5, 117.8, 122.6, 125.1, 126.0, 128.2, 128.9, 129.6, 130.2, 131.1, 132.7, 134.2, 135.1, 137.0, 149.4, 153.1, 155.4.

6-chloro-5-(2,3-dichlorophenoxy)-2-(2,4-dichlorophenyl)-1H-benzo[d]imidazole (3d):
Yellow Solid, ES-MS m/z (%): 459 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.91 (s, 1H), 7.12-7.27 (m, 3H), 7.39 (s, 1H), 7.51 (d, 1H), 7.72 (d, 1H), 7.85 (s, 1H), 8.16 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 107.3, 114.9, 116.9, 117.5, 121.8, 125.4, 126.1, 127.3, 128.0, 129.4, 130.5, 131.7, 132.9, 134.0, 135.4, 137.1, 151.4, 153.7, 155.0.

6-chloro-5-(2,3-dichlorophenoxy)-2-(4-nitrophenyl)-1H-benzo[d]imidazole (3e):
Yellow Solid, ES-MS m/z (%): 436 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.91 (s, 1H), 7.06-7.19 (m, 3H), 7.30 (s, 1H), 7.89 (d, 2H), 8.03 (s, 1H), 8.14 (d, 2H).
13C NMR (100 MHz, CDCl3): δ ppm 107.0, 115.2, 117.2, 118.2, 122.8, 124.7, 125.1, 126.9, 128.0, 128.7, 129.5, 131.7, 133.6, 134.4, 138.1, 149.0, 152.4, 153.8, 155.7.

6-chloro-5-(2,3-dichlorophenoxy)-2-p-tolyl-1H-benzo[d]imidazole (3f):
Yellow Solid, ES-MS m/z (%): 405 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 2.45 (s, 3H), 4.95 (s, 1H), 7.06-7.15 (m, 3H), 7.31 (s, 1H), 7.45 (d, 2H), 8.10 (s, 1H), 8.24 (d, 2H).
13C NMR (100 MHz, CDCl3): δ ppm 30.4, 107.4, 114.2, 117.0, 118.2, 122.9, 124.7, 125.4, 127.9, 129.0, 129.9, 130.5, 131.1, 131.9, 133.6, 135.2, 138.1, 149.7, 153.8, 155.3.

6-chloro-5-(2,3-dichlorophenoxy)-2-(4-methoxyphenyl)-1H-benzo[d]imidazole (3g):
Yellow Solid, ES-MS m/z (%): 421 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 3.85 (s, 3H), 4.95 (s, 1H), 7.01-7.17 (m, 3H), 7.30 (s, 1H), 7.40 (d, 2H), 7.74 (d, 2H), 8.10 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 61.2, 107.1, 110.3, 114.4, 115.0, 116.3, 117.4,
Chapter II  Section B  Synthesis and Screening of Some Bioactive Heterocycles

118.7, 123.2, 124.5, 125.8, 127.6, 131.7, 132.4, 135.6, 138.5, 149.5, 153.2, 155.1, 162.5.

4-(6-chloro-5-(2,3-dichlorophenoxy)-IH-benzo[d]imidazol-2-yl)phenol (3h):
Yellow Solid, ES-MS m/z (%): 407 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.95 (s, 1H), 5.65 (s, 1H), 7.03-7.14 (m, 3H), 7.21 (d, 2H), 7.33 (s, 1H), 7.82 (d, 2H), 8.10 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 106.9, 114.2, 115.7, 116.7, 117.7, 118.5, 119.2, 123.2, 124.5, 125.1, 127.6, 129.4, 130.6, 134.0, 138.6, 150.1, 152.3, 156.1, 160.5.

4-(6-chloro-5-(2,3-dichlorophenoxy)-IH-benzo[d]imidazol-2-yl)benzene-1,3-diol (3i):
Yellow Solid, ES-MS m/z (%): 423 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.97 (s, 1H), 5.53 (s, 1H), 5.71 (s, 1H), 6.74 (s, 1H), 6.86 (d, 1H), 7.08-7.20 (m, 3H), 7.39 (s, 1H), 7.55 (d, 1H), 8.21 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 104.7, 107.1, 114.0, 109.2, 111.4, 117.4, 118.5, 122.6, 124.8, 125.7, 127.8, 131.8, 135.2, 138.5, 149.7, 153.8, 157.1, 155.3, 162.7.

6-chloro-5-(2,3-dichlorophenoxy)-2-(thiophen-2-yl)-IH-benzo[d]imidazole (3j):
Yellow Solid, ES-MS m/z (%): 397 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.95 (s, 1H), 7.09-7.22 (m, 3H), 7.30 (s, 1H), 7.70-7.82 (m, 3H), 8.15 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 107.1, 114.5, 117.2, 118.7, 122.7, 124.4, 125.3, 127.0, 127.5, 128.1, 129.7, 135.4, 138.7, 142.7, 145.6, 149.9, 153.6.

6-chloro-5-(2,3-dichlorophenoxy)-2-(furan-2-yl)-IH-benzo[d]imidazole (3k):
Yellow Solid, ES-MS m/z (%): 381 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.98 (s, 1H), 7.12-7.25 (m, 3H), 7.38 (s, 1H), 7.78-7.91 (m, 3H), 8.12 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 107.5, 109.4, 111.7, 114.0, 117.4, 118.6, 122.5, 124.7, 125.6, 127.5, 135.7, 138.4, 142.9, 145.2, 149.9, 153.7, 156.1.

6-chloro-3-(6-chloro-5-(2,3-dichlorophenoxy)-IH-benzo[d]imidazol-2-yl)pyridin-2-ol (3l):
Yellow Solid, ES-MS m/z (%): 442 (M+H).
1H NMR (400 MHz, CDCl3): δ ppm 4.90 (s, 1H), 6.92-7.17 (m, 3H), 7.22 (d, 1H), 7.40 (s, 1H), 7.61 (d, 1H), 8.10 (s, 1H), 12.15 (s, 1H).
13C NMR (100 MHz, CDCl3): δ ppm 105.1, 107.7, 114.1, 117.4, 119.4, 124.1, 125.3, 126.7, 128.1, 129.0, 134.4, 135.2, 135.8, 142.0, 150.3, 153.6, 154.7, 167.5.
\[ ^{1}H \text{NMR spectrum of compound (3l)}: \]

![Image of 1H NMR spectrum](image)

\[ ^{13}C \text{NMR spectrum of compound (3l)}: \]

![Image of 13C NMR spectrum](image)
Mass spectrum of compound (3l):
REFERENCES

17. Heravi, M. M.; Sadjadi, S.; Oskooie, H. A.; Shoar, R. H. Bamoharram, F. F. 


28. a) Duraiswamy, B.; Mishra, S. K.; Subhashini, V.; Dhanraj, S. A.; Suresh, B. 
Chapter II
Section C
Resin bound propylsulphonic acid as a pioneering novel catalyst for the synthesis of tetrasubstituted imidazoles

INTRODUCTION

The heterocyclic ring systems containing imidazoles are explored because of their broad and rewarding pharmacological properties. The predominance of imidazoles in natural products and pharmacologically active compounds has attracted a diverse research group to develop new and better methods for synthesis of these heterocycles.\textsuperscript{1,2} Tetrasubstituted imidazole is the core of active pharmaceutical ingredient such as Losartan and Olmesartan. The survey of the literature revealed, lack of general methodologies even for the practical synthesis of these types of heterocycles. For the past decade multicomponent reactions are employed in order to overcome these hurdles. The versatile applicability of these multicomponent reactions has given them an outstanding success in organic and medicinal chemistry with high yield and operational simplicity.

The efforts to develop synthetic protocols for imidazole derivative are widely under study around the globe. Literature reveals number of methods have been developed for the synthesis of 1,2,4,5-tetrasubstituted imidazoles. On the other hand, the synthesis of 1,2,4,5-tetrasubstituted imidazoles are mainly carried out by four-component condensation of a 1,2-diketone, α -hydroxyketone with an aldehyde, primary amine and ammonium acetate using heteropolyacid,\textsuperscript{3a} silica gel/NaHSO\textsubscript{4} \textsuperscript{3b} HClO\textsubscript{4}–SiO\textsubscript{2}.\textsuperscript{3c} and microwaves.\textsuperscript{3d} In addition, also by the reaction of mesoionic 1,3-oxazolium-5-olates with \textit{N}-(arylmethylene)- benzenesulfonamides,\textsuperscript{4} hetero-Cope rearrangement,\textsuperscript{5} condensation of a 1,2-diketone with an aryl nitrile and primary amine under microwave irradiation\textsuperscript{6} and by \textit{N}-alkylation of trisubstituted imidazoles.\textsuperscript{7}

Although the synthetic methods employed above are effective, but they still experience one or more serious drawbacks, as they i) require elevated refluxing temperatures, ii) require laborious and complex work-up and purification procedures, iii) produce sizeable amounts of byproduct, iv) require harshly acidic conditions, v) are associated with occurrence of side reactions resulting in low yields and vi) use expensive reagents which increases the cost of synthesis.

Therefore, the organic chemists have a challenge to develop a new catalytic system \textsuperscript{8-12} which will overcome these inadequacies and accomplish the criteria of a mild, environmentally benevolent and effectual protocol for the synthesis
of highly substituted imidazoles. In past decade, vast interest is bestowed in mild lewis acid to create various organic transformations. In pursuing our efforts at developing environmental friendly methodologies\textsuperscript{13} for the synthesis of heterocycles, we tried to employ use of resin bound propylsulphonic acid (RPSA) as a catalyst (Scheme 1).

Scheme 1. Synthesis of tetrasubstituted imidazoles (5a-l).

![Scheme 1](image)

The use of RPSA offers a new and environmentally benign catalyst for modern synthesis having low toxicity and being easy to handle. RPSA have interesting advantages such as, excellent chemical, mechanical and thermal stability making it ideal catalyst for the organic transformation. The other major advantage of the RPSA is its recyclability, where it can be reused. The insolubility of RPSA in water and other organic solvent helps in recovering of catalyst by simple filtration, which in turn can be effectively used for further reactions. The RPSA thus can be successfully employed as lewis acid catalyst for organic transformations. In continuation of our effort to develop ecofriendly synthetic methodologies, we report herein, for the first time, a simple, mild and rapid synthesis of 1,2,4,5-tetrasubstituted imidazoles in high yields using RPSA as a heterogeneous catalyst.

**RESULTS AND DISCUSSION**

The past decade has observed development of synthetic methods for the synthesis of 1,2,4,5-tetrasubstituted imidazoles owing to the biological importance of these molecules. The literature study as mentioned above revealed although these methods have potential, the reactions suffer from fewer shortcomings like low yields, longer reaction times for completion, use of expensive reagents, use of strong corrosive acid, requirement of high temperature for the completion of these reactions. These highlighting points make, it is desirable for developing mild, environment friendly protocol for the synthesis of highly substituted imidazoles. These factors and according to our interest in development of synthetic methodologies\textsuperscript{13} propelled us to employ use of RPSA as a catalyst. Initially, our investigation began for the mild and
facile synthesis of tetra substituted imidazoles at room temperature in the absence of the catalyst, the reaction proceeded very sluggishly (Table 1, entry 1) highlighting the importance of the catalyst.

**Table 1. The effect of catalyst on model reaction (5a).**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Conditions</th>
<th>Time (min)</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>No catalyst</td>
<td>Reflux in EtOH</td>
<td>600</td>
<td>Traces</td>
</tr>
<tr>
<td>2.</td>
<td>p-toluene sulphonic acid</td>
<td>Reflux in EtOH</td>
<td>120</td>
<td>63</td>
</tr>
<tr>
<td>3.</td>
<td>Sulphamic Acid</td>
<td>Reflux in EtOH</td>
<td>90</td>
<td>85</td>
</tr>
<tr>
<td>4.</td>
<td>Sulphanilic Acid</td>
<td>Reflux in EtOH</td>
<td>75</td>
<td>83</td>
</tr>
<tr>
<td>5.</td>
<td>Oxalic Acid</td>
<td>Reflux in EtOH</td>
<td>65</td>
<td>89</td>
</tr>
<tr>
<td>6.</td>
<td>L-proline</td>
<td>MeOH/R.T</td>
<td>540</td>
<td>888</td>
</tr>
<tr>
<td>7.</td>
<td>InCl3.3H2O</td>
<td>140°C</td>
<td>440</td>
<td>799</td>
</tr>
<tr>
<td>8.</td>
<td>BF3/SiO2</td>
<td>140°C</td>
<td>120</td>
<td>9212</td>
</tr>
<tr>
<td>9.</td>
<td>AlCl3</td>
<td>MeOH/60°C</td>
<td>120</td>
<td>5312</td>
</tr>
<tr>
<td>10.</td>
<td>5 wt % RPSA</td>
<td>Reflux in EtOH</td>
<td>100</td>
<td>84</td>
</tr>
<tr>
<td>11.</td>
<td>10 wt % RPSA</td>
<td>Reflux in EtOH</td>
<td>80</td>
<td>89</td>
</tr>
<tr>
<td>12.</td>
<td>15 wt % RPSA</td>
<td>Reflux in EtOH</td>
<td>50</td>
<td>98</td>
</tr>
<tr>
<td>13.</td>
<td>20 wt % RPSA</td>
<td>Reflux in EtOH</td>
<td>45</td>
<td>98</td>
</tr>
<tr>
<td>14.</td>
<td>15 wt % RPSA(1st Cycle)</td>
<td>Reflux in EtOH</td>
<td>50</td>
<td>98</td>
</tr>
<tr>
<td>15.</td>
<td>15 wt % RPSA(2nd Cycle)</td>
<td>Reflux in EtOH</td>
<td>50</td>
<td>95</td>
</tr>
<tr>
<td>16.</td>
<td>15 wt % RPSA(3rd Cycle)</td>
<td>Reflux in EtOH</td>
<td>55</td>
<td>92</td>
</tr>
<tr>
<td>17.</td>
<td>15 wt % RPSA(4th Cycle)</td>
<td>Reflux in EtOH</td>
<td>60</td>
<td>92</td>
</tr>
</tbody>
</table>

a. Reaction condition: Benzil (1 mmol), benzaldehyde (1 mmol), aniline (1 mmol), ammonium acetate (1 mmol), and various catalyst (10 mol%) and various concentration of RPSA carried out in ethanol (5 mL) at reflux temperature.
b. Isolated yields.

Thus we decided to employ RPSA as a catalyst for the model reaction (4a). The model reaction of benzil (1 mmol), benzaldehyde (1 mmol), primary amine (1 mmol), ammonium acetate (1 mmol) and 5 wt % of RPSA in ethanol afforded 84% yield (Table 1, entry 10) of the desired product. The reaction conditions were optimized by varying the catalyst and their loadings as summarized in (Table 1). The yield was increased to 89 % using 10 wt % of catalyst while 15 wt % of the catalyst afforded 98 % yield (Table 1, entry 12). However, 20 wt % of the catalyst did not appreciably alter the time or the yield (98 %) of the reaction (Table 1, entry 13) as compared to the 15 wt % of the catalyst. The other lewis acid catalysts like p-TSA, sulphamic acid, sulphanilic acid and oxalic acid (Table 1, entry 2-5) were also examined. All of these catalysts gave comparable yield, but RPSA was favored due to its reusability. The reusability of RPSA was also examined for model reaction (5a). The RPSA catalyst that was recovered by filtration of reaction mixture was washed.
with ethanol, dried and reused for further reactions, and it was observed that the catalyst can be effectively reused in cycles (Table 1, entry 14-17). The slight decrease in reactivity of catalyst was observed with the increase in the number of recycles. The catalyst required slightly more time in the recycles compared to the first cycle (Table 1, entry 12), but the yields of the product obtained remained comparable.

Then the focus of model reaction was shifted to the choice of solvent, the reaction was carried out by employing variety of polar and non polar solvents such as ethanol, methanol, isopropanol, tert-butanol, acetonitrile, dichloromethane, chloroform, and toluene to study the effects of solvents on the reaction (Table 2).

**Table 2. Screening of solvent for model reaction (5a).**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethanol</td>
<td>20</td>
<td>97</td>
</tr>
<tr>
<td>2.</td>
<td>Methanol</td>
<td>30</td>
<td>93</td>
</tr>
<tr>
<td>3.</td>
<td>Isopropanol</td>
<td>100</td>
<td>78</td>
</tr>
<tr>
<td>4.</td>
<td>tert-Butanol</td>
<td>120</td>
<td>69</td>
</tr>
<tr>
<td>5.</td>
<td>Acetonitrile</td>
<td>60</td>
<td>75</td>
</tr>
<tr>
<td>6.</td>
<td>Dichloromethane</td>
<td>120</td>
<td>70</td>
</tr>
<tr>
<td>7.</td>
<td>Chloroform</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>8.</td>
<td>Toluene</td>
<td>180</td>
<td>54</td>
</tr>
</tbody>
</table>

*a. Reaction condition: Benzil (1 mmol), benzaldehyde (1 mmol), aniline (1 mmol), ammonium acetate (1 mmol), and carried out in various solvents (5 mL) at reflux temperatures.

b. Isolated yields.

Ethanol was found to be far more effective solvent (Table 2, entry 1) as compared to methanol, isopropanol, tert-butanol and acetonitrile, as solvents (Table 2, entries 2-5). The results indicate gradual decrease in yield as we moved from highly polar to less polar alcoholic solvents. Other non polar solvents, such as dichloromethane, chloroform, and toluene (Table 2, entries 6-8) were found to be ineffective for this transformation. This suggests the importance of polar solvents, for these types of reactions. The robustness of this reaction was then evaluated using a variety of diverse aldehydes and primary amines (Table 3). This method is suitable for both aliphatic and aromatic aldehydes and primary amines. The data from (Table 3) reveals that aromatic aldehydes produced 1,2,4,5-tetrasubstituted imidazoles in high yields. Additionally, the effect of RPSA catalyst under the influence of ultrasound irradiation on the tetra-substituted imidazoles was also studied. The model reaction was carried out under ultrasound irradiation in ethanol at milder conditions,
with accelerated rate of transformation. The obvious reason for the acceleration may be attributed to the phenomenon of cavitation which is produced by ultrasound.\textsuperscript{11,14}

### Table 3. Synthesis of 1,2,4,5-tetrasubstituted imidazoles (5a-l) catalyzed by RPSA.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Aldehyde</th>
<th>Amine</th>
<th>Time\textsuperscript{c} (min)</th>
<th>Yield\textsuperscript{d} (%)</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Benzoil</td>
<td>Benzoin</td>
<td>Benzoil</td>
</tr>
<tr>
<td>5a</td>
<td>Benzaldehyde</td>
<td>Aniline</td>
<td>20/2</td>
<td>25/5</td>
<td>97/98</td>
</tr>
<tr>
<td>5b</td>
<td>Benzaldehyde</td>
<td>Benzylamine</td>
<td>20/2</td>
<td>25/8</td>
<td>95/97</td>
</tr>
<tr>
<td>5c</td>
<td>Benzaldehyde</td>
<td>Methyl amine</td>
<td>15/1</td>
<td>20/3</td>
<td>95/95</td>
</tr>
<tr>
<td>5d</td>
<td>Benzaldehyde</td>
<td>Ethyl amine</td>
<td>15/2</td>
<td>25/5</td>
<td>94/96</td>
</tr>
<tr>
<td>5e</td>
<td>4-methyl benzaldehyde</td>
<td>Aniline</td>
<td>25/5</td>
<td>25/3</td>
<td>95/96</td>
</tr>
<tr>
<td>5f</td>
<td>4-methyl benzaldehyde</td>
<td>Benzyl amine</td>
<td>25/3</td>
<td>30/8</td>
<td>94/97</td>
</tr>
<tr>
<td>5g</td>
<td>4-chloro benzaldehyde</td>
<td>Aniline</td>
<td>25/5</td>
<td>25/5</td>
<td>95/98</td>
</tr>
<tr>
<td>5h</td>
<td>4-chloro benzaldehyde</td>
<td>Benzyl amine</td>
<td>25/6</td>
<td>30/6</td>
<td>95/96</td>
</tr>
<tr>
<td>5i</td>
<td>4-chloro benzaldehyde</td>
<td>4-chloro Aniline</td>
<td>30/5</td>
<td>35/8</td>
<td>97/98</td>
</tr>
<tr>
<td>5j</td>
<td>4-hydroxy benzaldehyde</td>
<td>Aniline</td>
<td>25/2</td>
<td>30/10</td>
<td>94/95</td>
</tr>
<tr>
<td>5k</td>
<td>4-methoxy benzaldehyde</td>
<td>Benzyl amine</td>
<td>20/5</td>
<td>30/5</td>
<td>94/96</td>
</tr>
<tr>
<td>5l</td>
<td>Thiophene-2-carbaldehyde</td>
<td>4-amino phenol</td>
<td>25/5</td>
<td>30/8</td>
<td>94/95</td>
</tr>
</tbody>
</table>

\textsuperscript{a.} Reaction condition: Benzil/ benzoin (1 mmol), substituted aldehydes (1 mmol), substituted amine (1 mmol), ammonium acetate (1 mmol), and 15 wt % RPSA carried out in ethanol (5 mL) at reflux temperatures.

\textsuperscript{b.} Reaction condition: Benzil/ benzoin (1 mmol), substituted aldehydes (1 mmol), substituted amine (1 mmol), ammonium acetate (1 mmol), and 15 wt % RPSA carried out in ethanol (5 mL) at 25°C under ultra sound irradiation.

\textsuperscript{c.} Time required by conventional and ultra sound assisted method respectively.

\textsuperscript{d.} Isolated yields by conventional and ultra sound assisted method respectively.

As the developed protocol is mild, side product formation is avoided which is generally major drawback where strong acids are employed. In conclusion, an efficient environmentally friendly multicomponent methodology has been developed for the synthesis of 1,2,4,5-tetrasubstituted imidazoles which offers several merits like milder reaction conditions, good yields, no side product formation and facile work-up. The use of RPSA as a recoverable, reusable and nontoxic catalyst is the other remarkable feature of this method.
EXPERIMENTAL

The benzil, benzoin, ammonium acetate, substituted amines, substituted aldehydes and RPSA used were commercially available. Melting points were recorded on SRS Optimelt melting point apparatus and are uncorrected. Ultrasonication was performed in an Ultrasonic Bath Sonicator of PCI Analytics,® having ultrasound cleaner with a frequency of 35 kHz and a nominal power of 200 W. The reaction flask was located in close proximity of the maximum energy area in the cleaner such that the reaction vessel was slightly lower than the water level and the temperature of the water bath was controlled at 25°C. 1H NMR spectra were recorded on a 400 MHz Varian-Gemini spectrometer and are reported as parts per million (ppm) downfield from a tetramethylsilane internal standard. The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

**General procedure of conventional method for the synthesis of 1,2,4,5-tetrasubstituted imidazoles (5a-l):**

In a 50 ml RBF, benzil/benzoin (1 mmol), substituted aldehydes (1 mmol), substituted amine (1 mmol) and ammonium acetate (1 mmol) were stirred in the presence of 15 wt % of RPSA in ethanol (5 mL) at reflux temperature for 25-45 min (Table 3). The reaction mixture (monitored by TLC, 20 % Ethyl acetate: n-hexane), after completion was diluted with water (10 mL), filtered to remove the insoluble catalyst (obtained as residue) and filtrate was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated in-vacuo. The crude product was recrystallized from ethanol to afford pure product in good yields (Table 3). The catalyst which was obtained as residue by filtration of reaction mixture was washed with ethanol, dried and reused for further reactions.

**General procedure of ultrasound assisted method for the synthesis of 1,2,4,5-tetrasubstituted imidazoles (5a-l):**

In a 50 ml round-bottom flask, benzil/benzoin (1 mmol), substituted aldehydes (1 mmol), primary amine (1 mmol) and ammonium acetate (1 mmol) were stirred in the presence of 15 wt % of RPSA in ethanol (5 mL) at 25°C for 5-10 min under ultrasound irradiation (Table 3). The reaction mixture (monitored by TLC, 20 % Ethyl acetate: n-hexane), after completion was diluted with water (10 mL), filtered to
remove the insoluble catalyst (obtained as residue) and filtrate was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated in-vacuo. The crude product was recrystallized from ethanol to afford pure product in good yields (Table 3). The catalyst which was obtained as residue by filtration of reaction mixture was washed with ethanol, dried and reused for further reactions.

**Spectral characterization**

**1,2,4,5-tetraphenyl-1H-imidazole (5a):**
White Solid, ES-MS m/z (%): 373 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 7.17-7.32 (m, 5H), 7.40-7.55 (m, 5H), 7.62-7.86 (m, 10H).

13C NMR (100 MHz, CDCl₃): δ ppm 121.2, 124.3, 125.0, 125.8, 126.2, 128.3, 128.9, 129.1, 129.8, 130.2, 131.4, 131.9, 132.8, 137.2, 139.1, 142.7, 145.5.

**1-benzyl-2,4,5-triphenyl-1H-imidazole (5b):**
White Solid, ES-MS m/z (%): 387 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 6.20(s, 2H), 7.20-7.35 (m, 5H), 7.42-7.60 (m, 5H), 7.70-7.95 (m, 10H).

13C NMR (100 MHz, CDCl₃): δ ppm 51.3, 123.7, 124.0, 125.5, 126.3, 128.1, 128.9, 129.2, 129.7, 130.3, 131.2, 132.5, 137.0, 137.5, 139.0.

**1-methyl-2,4,5-triphenyl-1H-imidazole (5c):**
White Solid, ES-MS m/z (%): 311 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 4.12(s, 3H), 7.12-7.24 (m, 5H), 7.35-7.52 (m, 5H), 7.76-7.90 (m, 5H).

13C NMR (100 MHz, CDCl₃): δ ppm 15.2, 124.5, 125.5, 125.9, 126.5, 128.1, 129.1, 129.3, 129.9, 130.2, 130.9, 131.6, 132.3, 137.1, 142.3, 151.4.

**1-ethyl-2,4,5-triphenyl-1H-imidazole (5d):**
White Solid, ES-MS m/z (%): 325 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 1.45 (t, 3H), 4.15(q, 2H), 7.08-7.21 (m, 5H), 7.32-7.47 (m, 5H), 7.76-7.91 (m, 5H).

13C NMR (100 MHz, CDCl₃): δ ppm 15.2, 34.7, 123.9, 124.3, 125.2, 126.4, 128.2, 129.0, 129.7, 130.2, 130.9, 131.6, 132.3, 137.1, 142.3, 153.7.

**1,4,5-triphenyl-2-(p-tolyl)-1H-imidazole (5e):**
White Solid, ES-MS m/z (%): 387 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 2.42 (s, 3H), 7.05-7.20 (m, 5H), 7.30 (d, 2H), 7.45-7.58 (m, 5H), 7.75-7.89 (m, 5H), 8.15(d, 2H).

13C NMR (100 MHz, CDCl₃): δ ppm 23.6, 121.3, 124.3, 125.1, 125.9, 126.2, 127.8, 128.7, 129.1, 129.8, 130.2, 130.6, 131.5, 132.5, 137.1, 139.3, 142.5, 145.7.

**1-benzyl-4,5-diphenyl-2-(p-tolyl)-1H-imidazole (5f):**
White Solid, ES-MS m/z (%): 401 (M+H).
H NMR (400 MHz, CDCl₃): δ ppm 2.54 (s, 3H), 6.25 (s, 2H), 7.04-7.18 (m, 5H), 7.28 (d, 2H), 7.47-7.61 (m, 5H), 7.75-7.86 (m, 5H), 8.05 (d, 2H).

13C NMR (100 MHz, CDCl₃): δ ppm 21.5, 51.5, 122.6, 123.6, 124.0, 124.9, 125.3, 126.3, 128.0, 128.9, 129.3, 129.9, 130.3, 131.1, 131.9, 132.2, 133.3, 137.6, 139.3.

2-(4-chlorophenyl)-1,4,5-triphenyl-1H-imidazole (5g):
White Solid, ES-MS m/z (%): 408 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 7.10-7.22 (m, 5H), 7.35 (d, 2H), 7.52-7.65 (m, 5H), 7.80-7.92 (m, 5H), 8.10 (d, 2H).

13C NMR (100 MHz, CDCl₃): δ ppm 121.5, 121.9, 124.1, 124.5, 124.9, 125.3, 125.8, 126.3, 128.0, 128.8, 129.0, 129.2, 129.4, 129.7, 129.9, 130.3, 130.5, 130.9, 131.4, 131.9, 132.8, 137.1, 137.9, 139.5, 142.2, 144.3.

1-benzyl-2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole (5h):
White Solid, ES-MS m/z (%): 422 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 6.34 (s, 2H), 7.05-7.17 (m, 5H), 7.32 (d, 2H), 7.45-7.60 (m, 5H), 7.74-7.88 (m, 5H), 8.12 (d, 2H).

13C NMR (100 MHz, CDCl₃): δ ppm 48.1, 122.0, 123.8, 124.7, 125.2, 126.5, 127.8, 128.6, 129.3, 129.9, 130.3, 131.1, 132.2, 137.1, 139.3, 142.6, 145.0.

1,2-bis(4-chlorophenyl)-4,5-diphenyl-1H-imidazole (5i):
White Solid, ES-MS m/z (%): 442 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 7.05-7.15 (m, 5H), 7.28 (d, 2H), 7.42-7.58 (m, 4H), 7.65-7.79 (m, 5H), 8.14 (d, 2H).

13C NMR (100 MHz, CDCl₃): δ ppm 121.0, 123.6, 124.1, 125.4, 126.5, 127.2, 128.5, 128.9, 129.2, 129.7, 130.0, 130.9, 131.4, 132.2, 137.0, 139.3, 141.2, 143.8.

4-(1,4,5-triphenyl-1H-imidazol-2-yl)phenol (5j):
White Solid, ES-MS m/z (%): 389 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 5.75 (s, 1H), 7.15-7.30 (m, 5H), 7.37 (d, 2H), 7.45-7.62 (m, 5H), 7.70-7.85 (m, 5H), 8.05 (d, 2H).

13C NMR (100 MHz, CDCl₃): δ ppm 121.7, 122.2, 124.2, 125.3, 126.1, 128.2, 129.1, 129.9, 130.2, 131.0, 131.4, 132.8, 136.6, 137.2, 139.3, 142.2, 156.1.

4-(1-benzyl-4,5-diphenyl-1H-imidazol-2-yl)phenol (5k):
White Solid, ES-MS m/z (%): 403 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 5.75 (s, 1H), 6.30 (s, 2H), 7.10-7.25 (m, 5H), 7.34 (d, 2H), 7.42-7.60 (m, 5H), 7.75-7.90 (m, 5H), 8.05 (d, 2H).

13C NMR (100 MHz, CDCl₃): δ ppm 51.3, 123.7, 124.3, 124.6, 125.0, 125.9, 126.4, 127.5, 128.1, 129.0, 129.7, 130.2, 130.9, 131.4, 132.7, 137.1, 139.3, 156.8.

4-(4,5-diphenyl-2-(thiophen-2-yl)-1H-imidazol-1-yl)phenol (5l):
White Solid, ES-MS m/z (%): 395 (M+H).

1H NMR (400 MHz, CDCl₃): δ ppm 5.78 (s, 1H), 6.95 (d, 2H), 7.08 (t, 1H), 7.15-7.30 (m, 5H), 7.40 (d, 2H), 7.50-7.65 (m, 5H), 7.85 (d, 2H), 8.14 (d, 2H).

13C NMR (100 MHz, CDCl₃): δ ppm 119.2, 124.0, 125.0, 125.8, 126.1, 128.2, 128.9, 129.1, 129.8, 130.2, 130.8, 131.4, 132.5, 137.1, 139.4, 142.5, 156.7.
$^1$H NMR spectrum of compound (5a):

$^{13}$C NMR spectrum of compound (5a):
Mass spectrum of compound (5a):
REFERENCES


