Microwave assisted synthesis, characterization and antibacterial activity of quinoxaline derivatives

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ABSTRACT
An efficient microwave assisted synthesis of substituted quinoxalines in presence of MgBr$_2$OEt$_2$ catalyst. Recycling of the catalyst has been efficiently achieved using a simple procedure. All the compounds are characterized by $^1$H NMR and Mass spectral studies. All the synthesized compounds were screened for four different bacterial strains (Escherichia coli and Proteus mirabilis are gram (-) ve strains, Staphylococcus aureus and Bacillus subtilis are gram (+) ve strains).

INTRODUCTION
Quinoxaline derivatives display a broad spectrum of biological activities[1-4]. Quinoxalines play an important role in many pharmacologically and biologically active compounds such as insecticides, fungicides, herbicides and antihelminitics[5-6]. Quinoxaline derivatives have also found its application in dyes[7], efficient electron luminescent material[8], organic semiconductors[9], chemically controllable switches[10], building blocks for the synthesis of anion receptor[11], cavitands[12], dehydroannulenes[13], DNA cleaving agents[14] and they also serve as useful rigid subunits in macro cyclic receptor or molecular recognition[15]. For instance quinoxaline derivative, 6,7-dimethyl-2-phenylquinoxaline and 6,7-dimethoxy-2-phenylquinoxaline exhibited Potent Inhibitors of PDGFR (Platelet-Derived Growth Factor Receptor) Tyrosine Kinase activity[16]. Literature survey revealed that the synthesis of title compounds quinoxalines have been reported so far, and also to know the effect of quinoxalines moiety on biological activity, We have taken up the synthesis of some quinoxalines.

EXPERIMENTAL SECTION
All the chemicals were purchased from Aldrich and Fluka. All the reactions carried out under the multisyn microwave oven. Melting points were determined in open capillary tubes. IR (KBr) spectra were recorded in a Perkin-Elmer spectrum BX series FT-IR spectrophotometer $^1$H-NMR and $^{13}$C-NMR were recordedon Bruker Avance II 400 MHz instrument using tetramethylsilane as an internal standard. Mass spectra were measured on a GCMS-QP 1000 EX mass spectrometer.
General procedure for the synthesis of substituted quinoxalines (3a-l)

a) Conventional heating method
A mixture of substituted-o-Phenylenediamines (0.001mol), 1,2-diketones (0.001mol) and MgBr₂.OEt₂ (0.001mol) were refluxed in acetonitrile for 1-3 hours. The progress of the reaction was monitored by TLC. After completion of the reaction the reaction mixture was cooled, solvent removed under reduced pressure, extracted with ethyl acetate (2 x 20 ml) and washed with water (2 x 10 ml). The two layers were separated, the organic layer was dried over sodium sulfate and solvent removed under reduced pressure, recrystallized from chloroform gave quinoxalines.

b) Microwave irradiation method
Substituted-o-Phenylenediamines (0.001mol), 1,2-diketones (0.001 mol) and MgBr₂.OEt₂ (0.004mol) was taken in a quartz tube and inserted into teflon vial with screw capped and then subjected to microwave irradiation for 1-2.5 min. (5 x 30 sec. interval time). Progress of the reaction was monitored by TLC. After completion of the reaction the reaction mixture was cooled, extracted with ethyl acetate (2 x 20 ml) and washed with water (2 x 10 ml). The two layers were separated, the organic layer was dried over sodium sulfate and solvent removed under reduced pressure, recrystallized from chloroform. Employing the above procedure as mentioned for (3a), the other heterocyclic compounds (3b-l) using substituted-o-Phenylenediamines (1a-h), 1,2-diketones (2a-d) and MgBr₂.OEt₂ were prepared.

Spectral data of Substituted Quinoxalines

(i) 2-methyl-3-phenylpyrido[2,3-b]pyrazine (3a)
1H NMR (200 MHz, CDCl₃): δ 2.88 (s, 3H, CH₂), 7.50-7.60 (m, 3H, Ar-H), 7.62-7.86 (m, 3H, Ar-H), 8.40-8.52 (m, 3H, Ar-H), 9.14 (d, 1H, Het-Ar-H), MS: m/z 222 (base peak) (M+H)+.

(ii) 2,3-di(furan-2-yl)-6,7-dimethylquinoxaline (3b)
1H NMR (300 MHz, CDCl₃): δ 2.58 (s, 6H, 2CH₂), 6.15 (s, 2H, furyl-H), 6.58 (d, 3H, Ar-H), 7.45-7.60 (m, 3H, Ar-H), 7.62-7.86 (m, 3H, Ar-H), 8.40-8.52 (m, 3H, Ar-H), MS: m/z 291 (base peak) (M+H)+.

(iii) 6-chloro-2-methyl-3-phenylquinazoline (3c)
1H NMR (200 MHz, CDCl₃): δ 2.79 (s, 3H, CH₃), 7.45-7.60 (m, 3H, Ar-H), 7.62-7.70 (m, 3H, Ar-H), 7.96-8.00 (m, 3H, Ar-H), MS: m/z 255 (base peak) (M+H)+.

(iv) (2,3-di(furan-2-yl)quinazolin-6-yl)(phenyl)methanone (3d)
1H NMR (200 MHz, CDCl₃): δ 6.56-6.66 (d, 2H, Ar-H), 7.00-7.09 (d, 2H, Ar-H), 7.45-7.50 (m, 5H, Ar-H), 8.20-8.35 (m, 2H, Ar-H), MS: m/z 367 (base peak) (M+H)+.

(v) (3-methyl-2-phenylquinazolin-6-yl)(phenyl)methanone (3e)
1H NMR (200 MHz, CDCl₃): δ 2.80 (s, 3H, CH₃), 7.45-7.50 (m, 9H, Ar-H), 7.85-7.92 (d, 2H, Ar-H), 8.10-8.25 (m, 2H, Ar-H), 8.42 (d, 1H, Ar-H), MS: m/z 325 (base peak) (M+H)+.

Table-3: Antibacterial activity of substituted quinoxalines (3a-l)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>E. coli</th>
<th>P. mirabilis</th>
<th>B. subtilis</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>100</td>
<td>50</td>
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<tr>
<td></td>
<td>200</td>
<td>100</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

* = Concentration expressed as µg/mL dissolved in DMSO, + = No growth observed (i.e., compound is active against organism), - = Growth observed (i.e., compound has no antibacterial activity)
( ) = Inhibition zone’s radius given in mm.

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(vi) 7-methyl-2-phenylquinoxaline (3f)
$^1$H NMR (200 MHz, CDCl$_3$): 2.75 (s, 3H, CH$_3$), 7.30-7.62 (m, 5H, Ar-H), 7.63-7.68 (m, 2H, Ar-H) 7.78-8.00 (m, 2H, Ar-H). MS: m/z (MM-ES): m/z 221 (base peak) (M+H)$^+$.  
(vii) (4-methoxyphenyl)(3-methyl-2-phenylquinoxalin-6-yl)methanone (3g)
$^1$H NMR (300 MHz, CDCl$_3$): 2.8 (s, 3H, CH$_3$), 3.65 (s, 3H, OCH$_3$), 7.45-7.65 (m, 7H, Ar-H), 7.98-8.15 (dd, 2H, Ar-H), 8.25-8.60 (d, 1H, Ar-H). MS: m/z (LCMS): m/z 355 (base peak) (M+H)$^+$.  
(viii) 5,6-diphenyl-2,3-dihydropyrazine (3h)
$^1$H NMR (200 MHz, CDCl$_3$): 3.16 (s, 4H, CH$_2$), 7.18-7.32 (m, 6H, Ar-H), 7.38-7.42 (m, 4H, Ar-H). MS: m/z (MM-ES): m/z 235 (base peak) (M+H)$^+$.  
(ix) 2,3-di(furan-2-yl)pyrido[2,3-b]pyrazine (3i)
$^1$H NMR (200 MHz, CDCl$_3$): 6.54-6.64 (d, 2H, Ar-H), 6.67-6.74 (d, 1H, Ar-H), 7.02-7.12 (s, 1H, Ar-H), 7.55-7.74 (m, 3H, Ar-H), 8.40-8.48 (d, 1H, Ar-H), 9.05-9.15 (d, 1H, Ar-H). MS: m/z (MM-ES): m/z 264 (base peak) (M+H)$^+$.  
(x) 2,3-di(furan-3-yl)-5,6-dihydropyrazine (3j)
$^1$H NMR (300 MHz, CDCl$_3$): 1.55-1.8 (m, 4H, aliphatic-H) 7.2 (m, 6H, Ar-H). MS: m/z (LCMS): m/z 215 (base peak) (M+H)$^+$.  
(xi) 2-(furan-3-yl)-3-methylbenzo[g]quinoxaline (3k)
$^1$H NMR (300 MHz, CDCl$_3$): 2.85 (s, 3H, CH$_3$), 7.4-7.7 (m, 5H, Ar-H), 7.95 (s, 1H, Ar-H), 8.25-8.40 (m, 2H, Ar-H), 8.60 (d, 3H, Ar-H). MS: m/z (LCMS): m/z 261 (base peak) (M+H)$^+$.  
(xii) 2,3-di(furan-3-yl)benzo[g]quinoxaline (3l)
$^1$H NMR (300 MHz, CDCl$_3$): 7.2-7.4 (m, 4H, Ar-H), 7.65 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H), 8.15 (d, 2H, Ar-H), 8.42 (d, 2H, Ar-H). MS: m/z (LCMS): m/z 313 (base peak) (M+H)$^+$.  

RESULTS AND DISCUSSION

The present study towards the synthesis of substituted quinoxaline derivatives (3a-l) with 1,2-diamines (1a-h) with 1,2-dicarbonyl compounds (2a-d) in presence of MgBr$_2$OEt$_2$ catalyst. Initially the reaction of o-phenylenediamines with benzil was carried out at refluxing conditions with MgBr$_2$OEt$_2$ (Magnesium bromide etherate) in presence of methanol gave the corresponding quinoxaline in 80-85% yield. The reaction of other substituted-o-phenylenediamines was carried out at room temperature, where as the reaction is preceded at room temperature with lower yields, in the place of microwave irradiation gave good yields and reaction proceeds in minutes to give corresponding quinoxalines (3a-l). Based on this 2-Methyl-3-phenyl-pyrido[2,3-b]pyrazine (3a) was synthesized from Pyridine-2,3-diamine (1a) on condensation with 1-Phenyl-propane-1,2-dione (2a) in the presence of MgBr$_2$OEt$_2$ (Magnesium bromide etherate) under microwave irradiation with 94% yield. The 1H-NMR spectrum singlet at δ 2.88 is due to methyl protons present at C-2 position of the quinoxaline moiety formed. The doublet at δ 9.14, quartet at δ 8.51 and doublet at δ 7.62 integrating for one proton each correspond to pyridine moiety of quinoxalines. In mass spectrum of 2-Methyl-3-phenyl-pyrido[2,3-b]pyrazine (3a) show the molecular ion peak [M+H]$^+$ at 222 matches with the desired product. Basis of the above analytical data the base peak in mass spectra and $^1$H NMR spectra are strong evidence for the formation of 2-Methyl-3-phenyl-pyrido[2,3-b]pyrazine (3a). Similarly all the substituted quinoxalines (3a-l) compounds are characterized.
Scheme-1: Synthesis of substituted quinoxalines using MgBr$_2$.OEt$_2$ (3 a-g)

\[ \text{R}_3 \text{R}_4 \text{X} \text{NH}_2 + \text{O} \text{R}_1 \text{R}_2 \xrightarrow{\text{MgBr}_2 \cdot \text{OEt}_2} \text{Acetonitrile, R. T.} \]

\[ \text{MWI, 160W} \rightarrow \text{R}_3 \text{R}_4 \text{X} \text{N} \text{R}_1 \text{R}_2 \]

(1a-f) (2a-c)

\[ \text{NH}_2 + \text{O} \text{R}_1 \text{R}_2 \xrightarrow{\text{MgBr}_2 \cdot \text{OEt}_2} \text{Acetonitrile, R. T.} \]

\[ \text{MWI, 160W} \rightarrow \text{N} \text{R}_1 \text{R}_2 \]

(1g) (2a-c)

(3a-g)

\[ \text{NH}_2 + \text{O} \text{R}_1 \text{R}_2 \xrightarrow{\text{MgBr}_2 \cdot \text{OEt}_2} \text{Acetonitrile, R. T.} \]

\[ \text{MWI, 160W} \rightarrow \text{N} \text{R}_1 \text{R}_2 \]

(1h) (2a-b)

(3h-j)

(3k-l)

Table-1: List of various substituted quinoxalines (3a-l)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mol. Formula</th>
<th>M.P. (°C)</th>
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<tbody>
<tr>
<td>3a</td>
<td>C$_7$H$_8$N$_3$</td>
<td>153</td>
</tr>
<tr>
<td>3b</td>
<td>C$_8$H$_7$N$_2$O$_2$</td>
<td>112</td>
</tr>
<tr>
<td>3c</td>
<td>C$_8$H$_6$N$_2$Cl</td>
<td>123</td>
</tr>
<tr>
<td>3d</td>
<td>C$_9$H$_6$N$_2$O$_2$</td>
<td>143</td>
</tr>
<tr>
<td>3e</td>
<td>C$_9$H$_6$N$_2$O$_2$</td>
<td>94</td>
</tr>
<tr>
<td>3f</td>
<td>C$_{10}$H$_8$N$_2$</td>
<td>84</td>
</tr>
<tr>
<td>3g</td>
<td>C$_{10}$H$_8$N$_2$O$_2$</td>
<td>161</td>
</tr>
<tr>
<td>3h</td>
<td>C$_{10}$H$_8$N$_2$</td>
<td>103</td>
</tr>
<tr>
<td>3i</td>
<td>C$_{10}$H$_8$N$_2$O$_2$</td>
<td>84</td>
</tr>
<tr>
<td>3j</td>
<td>C$_{10}$H$_8$N$_2$O$_2$</td>
<td>102</td>
</tr>
<tr>
<td>3k</td>
<td>C$_{10}$H$_8$N$_2$O$_2$</td>
<td>152</td>
</tr>
<tr>
<td>3l</td>
<td>C$_{10}$H$_8$N$_2$O$_2$</td>
<td>134</td>
</tr>
</tbody>
</table>

Table-2: Physical and analytical data of substituted quinoxalines (3a-l)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compounds</th>
<th>Mol. Formula</th>
<th>M.P. (°C)</th>
<th>Comparative study</th>
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<td>Conventional Yield (%)</td>
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<tr>
<td>1</td>
<td>3a</td>
<td>C$_7$H$_8$N$_3$</td>
<td>153</td>
<td>2.0 90 1.5 94</td>
</tr>
<tr>
<td>2</td>
<td>3b</td>
<td>C$_8$H$_7$N$_2$O$_2$</td>
<td>112</td>
<td>2.0 85 2.0 95</td>
</tr>
<tr>
<td>3</td>
<td>3c</td>
<td>C$_8$H$_6$N$_2$Cl</td>
<td>123</td>
<td>1.5 87 1.0 93</td>
</tr>
<tr>
<td>4</td>
<td>3d</td>
<td>C$_9$H$_6$N$_2$O$_2$</td>
<td>143</td>
<td>1.0 87 1.0 94</td>
</tr>
<tr>
<td>5</td>
<td>3e</td>
<td>C$_9$H$_6$N$_2$O$_2$</td>
<td>94</td>
<td>2.5 85 2.0 94</td>
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<tr>
<td>6</td>
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<td>84</td>
<td>3.0 85 2.3 90</td>
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<tr>
<td>7</td>
<td>3g</td>
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<td>161</td>
<td>2.0 84 1.5 92</td>
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<tr>
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<td>103</td>
<td>2.0 83 2.0 91</td>
</tr>
<tr>
<td>9</td>
<td>3i</td>
<td>C$_{10}$H$_8$N$_2$O$_2$</td>
<td>84</td>
<td>2.0 85 2.5 93</td>
</tr>
<tr>
<td>10</td>
<td>3j</td>
<td>C$_{10}$H$_8$N$_2$O$_2$</td>
<td>102</td>
<td>1.3 89 1.5 94</td>
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<tr>
<td>11</td>
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<td>152</td>
<td>1.0 90 1.3 95</td>
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<tr>
<td>12</td>
<td>3l</td>
<td>C$_{10}$H$_8$N$_2$O$_2$</td>
<td>134</td>
<td>1.5 82 2.0 90</td>
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</table>

Biological properties:  

The newly synthesized substituted quinoxalines were screened for their antibacterial activity against gram negative bacteria viz. Escherichia coli, Proteus mirabilis strains and gram-positive bacteria viz. Staphylococcus aureus, Bacillus subtilis strains at three concentrations i.e. 200, 100 and 50 µg using ditch dilution methods. The test organism was a two hour culture of Escherichia coli, Proteus mirabilis, Staphylococcus aureus and Bacillus subtilis incubated and grown in peptone-water medium (temp-37°C). DMSO was used as solvent control which did not show any zone of inhibition. Muller-Hilton agar medium was used as culture medium. The culture plates were
incubated at 37°C for 24 hrs. The newly synthesized compounds were screened for their antibacterial activity against gram negative bacteria viz. Escherichia coli, Proteus mirabilis strains and gram-positive bacteria viz. Staphylococcus aureus, Bacillus subtilis with three concentrations i.e. 200, 100 and 50 µg. Out of these concentrations chosen the best result was obtained 200 µg and hence this was optimum concentration.

Compounds 3a and 3i were exhibited maximum activity against E. coli at 200µg/disc. 3d, 3e, 3f and 3j showed moderate activity and 3b, 3c, 3g, 3h, 3k and 3l did not exhibit significant activity against E. coli. In case of P. mirabilis compound 3d and 3f showed maximum activity at 200µg/disc. 3c, 3e and 3k exhibited moderate activity, where as 3a, 3b, 3g, 3h, 3i and 3l were found to be inactive. In case of S. aureus compound 3e, 3j and 3i showed maximum activity at 200µg/disc, 3a, 3b, 3c, 3d, 3f, 3g, 3h, 3i and 3l were found to be inactive as shown in Table-3.

CONCLUSION

In conclusion we describe a simple, easy, high yielding, convenient and green methods for the synthesis of substituted quinoxalines under microwave irradiation using MgBr₂·OEt₂ catalyst. The process proved to be a simple, environmentally friendly technique with high yields and high rate of acceleration was achieved in performing the reaction in microwave irradiation technique. A series of quinoxalines screened for antibacterial activity among them compounds 3a and 3i was activity against E. coli, compounds 3d and 3f against P. mirabilis, compounds 3c and 3j against B. subtilis and compound 3e, 3j and 3i against S. aureus shows maximum activity at 200µg/disc.

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REFERENCES