MICROWAVE ASSISTED SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF PYRIMIDINE DERIVATIVES

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ABSTRACT
Pyrimidine, one of the bases of hydrolysed product of nucleosides is an interesting subject to medicinal chemist by virtue of its diverse biological activities. In the present scheme attempt has been made to synthesize pyrimidine derivatives.

The synthesis of pyrimidine derivatives is based on condensation of chalcones with guanidine nitrate in the presence of sodium hydroxide and ethanol. Furthermore, the synthesis of chalcone derivatives is based on Claisen – Schmidt condensation. All the new title compounds were characterized by IR and 1H NMR spectroscopy and then screened for antibacterial activity against Pseudomonas aeruginosa and Escherichia coli using amoxicillin as standard by filter paper disc method.

Keywords: Synthesis, Pyrimidine derivatives, Antibacterial activity: Filter paper disc method.

INTRODUCTION
Heterocyclic compounds hold a special place among pharmaceutically significant natural products and synthetic compounds. The remarkable ability of heterocyclic nuclei to serve both as biometrics and reactive pharmacophores has largely contributed to their unique value as traditional key elements of numerous drugs [1]. Conventional methods of organic synthesis are orders of magnitude too slow to satisfy the demand for generation of such compounds. The synthetic chemical community has been under increased pressure to produce, in an environmentally benign fashion, the myriad of substances required by society in short periods of time, and the best option to accelerate these synthetic processes is to use microwave (MW) technology. The efficiency of MW flash-heating has resulted in dramatic reductions in reaction times (reduced from days and hours to minutes and seconds [2]). The time saved by using MW heating approach is potentially important in traditional organic synthesis and assembly of heterocyclic systems.

“Pyrimidine” and their derivatives are popular inorganic synthetic chemistry. Pyrimidine does not exist in nature but in the form of its different derivatives, it is found as a part of more complex system and are widely distributed. Pyrimidine derivatives are of interest because of their pharmacological properties. These properties include antitumour[3], antivirus[4], antibacterial[5], antifungal[6] antiprotozoal[7], antihypertensive[8], antihistaminic[9], anti-inflammatory[10] and central nervous activities[11]. Synthesis of new chemical entities is major bottleneck in drug discovery.

Step I: Synthesis of chalcones

Chalcones were synthesized by base catalyzed Claisen–Schmidt condensation [12-13]. Reaction of appropriately substituted acetoephones and aldehydes. A mixture of benzaldehyde derivatives (0.01 mol) and acetoephone derivatives (0.01 mol) was dissolved in 30 ml ethanol in a 250 ml round-bottomed flask equipped with a magnetic stirrer. Then 10 ml NaOH solution (1g in 10ml H2O) was added drop wise to the reaction mixture on vigorous stirring for 30 minutes when solution became turbid. The reaction temperature was maintained between 20-25°C using a cold water bath on the magnetic stirrer. After vigorous stirring for 4-5 hours the reaction mixture was neutralized by 0.1-0.2N HCl whereby the precipitation occurred. On filtering off, the crude chalcones were dried in air and recrystallized by rectified ethanol (fig 1).

Step II: Synthesis of pyrimidine derivatives

A mixture (0.01 mol) of chalcone derivative and guanidine nitrate in alkaline medium viz. in potassium hydroxide (0.003mol) in the presence of ethanol (10ml). The entire reaction mixture was microwave irradiated at 180 watts for 2-16 minutes and then kept aside for 2-hours and resulted formation of pyrimidine derivatives (fig 1). The chemical profile of the compounds is as shown in Table 1.

Spectral data

<table>
<thead>
<tr>
<th>SB1: 2-(4-chloro phenyl)-4-(phenyl-6-amino pyrimidine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR (nujol): (C-H): 2672.24 cm -1 (Stretch)</td>
</tr>
<tr>
<td>(C = C): 524.27 cm -1 (stretch)</td>
</tr>
<tr>
<td>(C-H): 2672.24 cm -1 (Stretch)</td>
</tr>
<tr>
<td>(C = C): 524.27 cm -1 (stretch)</td>
</tr>
</tbody>
</table>

HNMR Spectra: H-NMR DMSO 8.11 (d, J = 9Hz, H, Ar C’7.85(d, J = 8Hz, 2H, Ar C’6”) 7.82(d, J = 8Hz, 1H, Ar 3”5”) 7.70(d, J = 16Hz, 1H, Ar 4”) 7.52(m, J = 16Hz, 2H, Ar 3”5”) 7.44(d, J = 8Hz, 2H, Ar 6).
SB2: 2-(4-chloro phenyl)-4-(amino phenyl)-6-amino pyrimidine

IR (nujol) (C-H): 2672 cm⁻¹ (Stretch); (C=O): 1658 cm⁻¹ (Stretch); (C=N): 1644 cm⁻¹ (Stretch); (C-Cl): 522.27 cm⁻¹ (Stretch); (C=NH 2): 1107 cm⁻¹ (Bend); (N-H): 3419 cm⁻¹ (Stretch).  

HNMR DMSO: 8.03 (d, J = 4Hz, 2H, Ar 3'6'); 7.68 (d, J = 16Hz, 1H, Ar 2''6''); 7.57 (d, J = 8Hz, 1H, Ar 3'5'); 7.50 (d, J = 4Hz, 1H, Ar 3''5''); 7.49 (d, J = 4Hz, 2H, Ar 4''); 6.66 (d, J = 8Hz, 2H, Ar 6').

SB3: 2-(4-dimethylaminophenyl)-4-(phenyl)-6-amino pyrimidine

IR (nujol) (C-H): 1376 cm⁻¹ (Bend); (C=O): 1658 cm⁻¹ (Stretch); (C=N): 1306.82 cm⁻¹ (Stretch); (C–N): 1256 cm⁻¹ (Stretch); (C–Cl): 1559 cm⁻¹ (Bend); (C=O): 1597 cm⁻¹ (Stretch); (C–NH₂): 1559 cm⁻¹ (Bend); (N–H): 3649 cm⁻¹ (Stretch); (C=O): 1597 cm⁻¹ (Stretch); (C–NH₂): 1559 cm⁻¹ (Bend); (N–H): 3649 cm⁻¹ (Stretch).

HNMR DMSO: 7.52 (d, J = 8Hz, 2H, Ar 2''6''); 7.53 (d, J = 4Hz, 1H, Ar 3'5'); 7.32 (d, J = 8Hz, 1H, Ar 3'5'); 7.23 (d, J = 4Hz, 2H, Ar 2''6'); 5.07 (d, J = 4Hz, 1H, Ar 4''); 6.91 (d, J = 12Hz, 2H, Ar 6').

SB4: 2-(4-dimethylaminophenyl)-4-(aminophenyl)-6-amino pyrimidine

IR (nujol) (C-H): 2672 cm⁻¹ (Stretch); (C=O): 1658 cm⁻¹ (Stretch); (C=N): 1256.61 cm⁻¹ (Stretch); (C–N): 945.61 cm⁻¹ (Stretch); (C–Cl): 1532 cm⁻¹ (Bend); (C=O): 1597 cm⁻¹ (Stretch); (C–NH₂): 1523 cm⁻¹ (Bend); (N–H): 3649 cm⁻¹ (Stretch); (C=O): 1597 cm⁻¹ (Stretch); (C–NH₂): 1523 cm⁻¹ (Bend); (N–H): 3649 cm⁻¹ (Stretch).

HNMR DMSO: 8.06 (dd, J = 4Hz, 1H, Ar 2'6'); 7.60 (d, J = 4Hz, 1H, Ar 3'5'); 7.57 (d, J = 8Hz, 1H, Ar 3'5'); 7.48 (d, J = 12Hz, 1H, Ar 2''6'); 7.36 (d, J = 8Hz, 2H, Ar 6').

Antibacterial Activity

The antibacterial activity of all synthesized compounds was determined by disc diffusion method [15]. All human pathogenic bacteria viz, Escherichia coli, Pseudomonas aeruginosa were procured from SGRRITS Dehradun. The nutrient agar medium was prepared. Preparation of nutrient broth, subculture, base layer medium and peptone water was done as per the standard procedure. The disc measuring 6.25mm in diameter was punched from whatmann No.1 filter paper. Stock solution of synthesized compounds diluted in dimethylsulphoxide (1% DMSO) to give final concentration of 500µg/ml and 1000µg/ml. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of amoxicillin (500µg/ml, 1000µg/ml) respectively in sterile distil water separately. The incubation was carried at 33–37°C for 48 hours. All the experiment was carried out in triplicate. Simultaneously, controls were maintained by employing 0.1ml of DMSO which did not reveal any inhibition. A zone of inhibition produced by each compound was measured in mm and antibacterial activity (% inhibition) was calculated by using this formula. The results of antibacterial activity are shown in table 2.

% inhibition = Zone of inhibition of test compound (in diameter) X100 / Zone of inhibition of standard drug (in diameter)

Fig. 1: Synthesis of the different derivatives of Pyrimidine derivatives

RESULTS AND DISCUSSION

The synthesis of pyrimidine derivatives is divided into two steps.

- Synthesis and characterization of pyrimidine derivatives.
- Biological evaluation of pyrimidine derivatives.

Synthesis and characterization of pyrimidine derivatives:

The synthesis of chalcones is based on claisen Schmidt reaction, which is condensation reaction of substituted benzaldehyde with substituted acetophenone in the presence sodium hydroxide and ethanol. The starting material required for the synthesis of
pyrimidine derivatives is chalcones derivatives. Then mixture of chalcone derivatives and guanidine nitrate in alkaline medium viz. in potassium hydroxide in the presence of ethanol. Then entire mixture is microwave irradiated at 180 watts for 2-16 minutes and resulted formation of pyrimidine derivatives. The chemical profile of pyrimidine derivatives is shown in table 1.

Characterization of pyrimidine derivatives:

Infra red spectroscopy, can be used to identify and study chemicals. The absorption bands for C=O of aromatic ring system in the compounds SB1 – SB4 were in good agreement with standard values reported in the literature for these types of structure.

The absorption bands for N-H stretching were observed between 3450-3600 cm⁻¹. Furthermore, in compounds SB1 and SB2 chloride were characterized by bands and 524-27 cm⁻¹ for aryl C=O stretching vibrations. In Nuclear magnetic resonance spectroscopy, the aromatic protons of ring A and ring B appeared either as a doublet or doublet of doublet at 8.06 (2''), 7.85 (2''). 

7.52 (3'') and 7.50 (3'') showing effective coupling of adjacent protons.

Biological evaluation of synthesized pyrimidine derivatives:

The antibacterial activity of all synthesized compounds was determined by disc diffusion method. The results of compounds of preliminary antibacterial testing are shown in table 2. The screening results revealed that the compounds (SB1-SB4) showed significant antibacterial activity at both 500 µg/ml and 1000 µg/ml concentration levels when compared with amoxicillin drug. It was found that compound SB4 showed maximum activity and compound SB1 showed least activity.

Table 1: The chemical profile of pyrimidine derivatives:

<table>
<thead>
<tr>
<th>Compound code</th>
<th>R</th>
<th>R1</th>
<th>Mol. Formula</th>
<th>Melting point°C</th>
<th>% Yield</th>
<th>Mol. Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB 1</td>
<td>Cl</td>
<td>H</td>
<td>C₃H₁₁ClN₅</td>
<td>238-42</td>
<td>53.5%</td>
<td>281.74</td>
</tr>
<tr>
<td>SB 2</td>
<td>Cl</td>
<td>NH₂</td>
<td>C₃H₁₂ClN₄</td>
<td>190-200</td>
<td>58.06%</td>
<td>296.75</td>
</tr>
<tr>
<td>SB 3</td>
<td>N(CH₃)₂</td>
<td>H</td>
<td>C₄H₁₃N₄</td>
<td>244-246</td>
<td>53.1%</td>
<td>290.36</td>
</tr>
<tr>
<td>SB 4</td>
<td>N(CH₃)₂</td>
<td>NH₂</td>
<td>C₄H₁₄N₅</td>
<td>186-188</td>
<td>64.51%</td>
<td>305.37</td>
</tr>
</tbody>
</table>

Table 2: Results of Antibacterial activity of pyrimidine derivatives:

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Pseudomonas aeruginosa</th>
<th>E. Coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 µg/ml % Inhib*</td>
<td>1000 µg/ml % Inhib*</td>
</tr>
<tr>
<td>SB1</td>
<td>3 mm 25% 6 mm 46.15%</td>
<td>8 mm 33.3% 6 mm 25%</td>
</tr>
<tr>
<td>SB2</td>
<td>4 mm 33.3% 7 mm 53.8%</td>
<td>6 mm 25% 6 mm 25%</td>
</tr>
<tr>
<td>SB3</td>
<td>4 mm 33.3% 7 mm 53.8%</td>
<td>6 mm 25% 6 mm 25%</td>
</tr>
<tr>
<td>SB4</td>
<td>5 mm 41.6% 9 mm 69.23%</td>
<td>8 mm 33.3% 12 mm 46.15%</td>
</tr>
<tr>
<td>AMOXICILLIN</td>
<td>12 mm 13 mm</td>
<td>24 mm 26 mm</td>
</tr>
</tbody>
</table>

*Inhibition

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REFERENCES