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Research Article

# 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 $^1$HNMR 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 anticancer[3], antiviral[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. Conventional methods for various chemical syntheses is very well documented and practiced. The methods for synthesis of organic compounds has continuously modified from the decade. This method has drawback i.e. lower yield and longer reaction time. Microwave assisted synthesis which has emerged as a new lead in organic synthesis. This technique offers clean, fast, efficient, and economic for large number of organic molecules and reduction in reaction time with improvement in the yield and quality of the product. The attempt was done to synthesize and antibacterial activity studies of pyrimidine derivatives via microwave irradiation.

### Chemistry

The melting points were recorded in melting point apparatus and were uncorrected. IR spectra were recorded using Perkin Elmer FT-IR (89258) spectrophotometer. A $^1$HNMR spectrum was recorded using DMSO on Bruker Avance (400 MHz) and their chemical shifts are recorded in δ (parts per million) units with respect to tetramethylsilane (TMS) as internal standard. All the reagents and solvents used were of analytical grade and were used as supplied unless otherwise stated.

## MATERIALS AND METHODS

The synthesis of pyrimidine derivatives is divided into two parts:

**Step I**. Synthesis of chalcones.

**Step II**. Synthesis and characterization of pyrimidine derivatives.

### Step I: Synthesis of chalcones

Chalcones were synthesized by base catalyzed Claisen - Schmidt condensation [12-13] reaction of appropriately substituted acetophenones and aldehydes. A mixture of benzaldehyde derivatives (0.01 mol) and acetophenone 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 $H_2O$) 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 (0.01mol) 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 2-3hours and resulted formation of pyrimidine derivatives (fig 1.) The chemical profile of the compounds is as shown in Table 1.

### Spectral data

**SB1: 2-(4-choloro phenyl)-4-(phenyl-6-amino pyrimidine)**

**IR (nujol):** (C-H): 2672.24 cm $^{-1}$( Stretch); (C = C) : 1658.41 cm $^{-1}$ (stretch); (C – N): 1169 cm $^{-1}$ (stretch); (C – C): 1078 cm $^{-1}$ (stretch); ( C- Cl): 524.27 cm $^{-1}$ (stretch); (C – NH 2):1566 cm $^{-1}$ (stretch); (N-H): 3401 cm $^{-1}$ (stretch);. (C-H): 2672.24 cm $^{-1}$( Stretch); (C = C) : 1658.41cm $^{-1}$ (stretch); (C – N): 1169 cm $^{-1}$ (stretch); (C – C): 1078 cm $^{-1}$ (stretch);(C- Cl): 524.27 cm $^{-1}$ (stretch); (C – NH 2):1566 cm $^{-1}$ (stretch); (N-H): 3401 cm $^{-1}$ (stretch). **$^1$HNMR Spectra**: H$^1$NMR DMSO 8.11 (d, J = 9Hz, H, Ar 6') 7.85(d, J = 8Hz, 2H, Ar 2''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-choloro phenyl)-4-(amino phenyl-6-amino pyrimidine)**

**IR (nujol):(**C-H): 2672.24 cm $^{-1}$( Stretch); (C = C) : 1658.41 cm $^{-1}$ (stretch); (C – N): 1169 cm $^{-1}$ (stretch); (C – C): 1064 cm $^{-1}$ (stretch); ( C- Cl): 522.27 cm $^{-1}$ (stretch); (C – NH 2):1107 cm $^{-1}$ (Bend) ;(N-H): 3419 cm $^{-1}$ (stretch). $^{1}$**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 = C) : 1658.41 cm $^{-1}$ (stretch); (C – N): 1306.82 cm $^{-1}$ (stretch); (C – C): 1064 cm $^{-1}$ (stretch); (C – NH 2):1559 cm $^{-1}$ (Bend);(N-H): 3649 cm $^{-1}$ (stretch);(C = N): 1597 cm $^{-1}$ (stretch). $^{1}$**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).

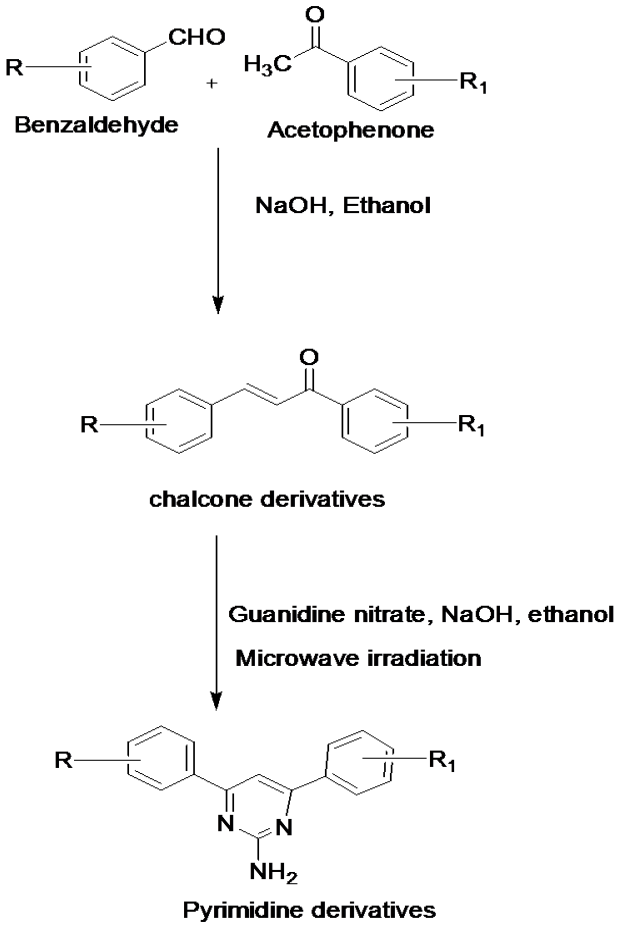
**SB4: 2-(4-dimethylaminophenyl) -4- (aminophenyl-6-amino pyrimidine)**

**IR (nujol)** (C-H): 2672 cm $^{-1}$( Bend); (C = C) : 1882 cm $^{-1}$ (stretch);(C – N): 1256.61 cm $^{-1}$ (stretch);(C – C): 945.61 cm $^{-1}$ (stretch);(C – NH 2):1523 cm $^{-1}$ (Bend) (N-H): 3649 cm $^{-1}$ (stretch);(C = N): 1597 cm $^{-1}$ (stretch); $^{1}$**HNMR DMSO**:8.06 (dd, J = 8Hz, 1H, Ar 2'6')7.60(d, J = 4Hz, 1H, Ar 3'5')7.53(d, J = 8Hz, 2H, Ar 3)7.48(d, J = 12Hz, 1H, Ar 2''6'')7.51(d, J = 8Hz, 2H, Ar 3'5') 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$^{0}$- 37$^{0}$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.

$$\% \text{ inhibition} = \frac{\text{Zone of inhibition of test compound (in diameter) X100}}{\text{Zone of inhibition of standard drug (in diameter)}}$$




**R= Cl, N(CH$_3$)$_2$, Cl, N(CH$_3$)$_2$; R1=H, NH$_2$, H, NH$_2$**

**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.

**Table 1: The chemical profile of pyrimidine derivatives:**

| Compound code | R | R1 | Mol. Formula | Melting point$^0$C | % Yield | Mol. Wt. |
|---|---|---|---|---|---|---|
| SB 1 | Cl | H | $C_{16}H_{12}ClN_3$ | 238-42 $^0$C | 55.5% | 281.74 |
| SB 2 | Cl | $NH_2$ | $C_{16}H_{13}ClN_4$ | 198-200 $^0$C | 58.06% | 296.75 |
| SB 3 | $N(CH_3)_2$ | H | $C_{18}H_{18}N_4$ | 244-246 $^0$C | 53.1% | 290.36 |
| SB 4 | $N(CH_3)_2$ | $NH_2$ | $C_{18}H_{19}N_5$ | 186-188 $^0$C | 64.51% | 305.37 |

**Characterization of pyrimidine derivatives**:

Infra red spectroscopy, can be used to identify and study chemicals. The absorption bands for C=C 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-X 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 (2'6'), 7.85 (2''6''), 7.52(3'5') and 7.50(3''5'') 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 500µg/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 2: Results of Antibacterial activity of pyrimidine derivatives:**

| Compound code | Pseudomonas aeruginosa | | | | E. Coli | | | |
|---|---|---|---|---|---|---|---|---|
| | 500 µg/ ml | % Inhib*. | 1000 µg/ ml | % Inhib*. | 500 µg/ ml | % Inhib*. | 1000 µg/ ml | % Inhib*. |
| SB1 | 3mm | 25% | 6mm | 46.15% | 8 mm | 33.3% | 12 mm | 46.1% |
| SB2 | 4mm | 33.3% | 7mm | 53.8% | 6 mm | 25% | 14mm | 53.8% |
| SB3 | 4mm | 33.3% | 7mm | 53.8% | 6 mm | 25% | 12mm | 46.15% |
| SB4 | 5mm | 41.6% | 9mm | 69.23% | 8 mm | 33.3% | 12mm | 46.15% |
| AMOXICILLIN | 12mm | | 13mm | | 24 mm | | 26mm | |

*Inhibition

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**REFERENCES**

1. Varma RS. The chemistry of Heterocycles: Structure, Reactions, Syntheses and Applications.2nd ed. WileyVCH, Weinheim; 1999.p.1565.
2. Eicher T, Hauptmann S. The chemistry of Heterocycles:Structure, Reactions, Syntheses and Applications. 2nd ed. WileyVCH, Weinheim;2003.p.572.
3. Abdel Mohsen HT, Ragab FAF, Ramla MM, El Diwani HI: Novel benzimidazole–pyrimidine conjugates as potent antitumor agents. Eur J Med Chem 2010:2336-2344.
4. Pontikis R, Benhida R, Aubertin AH, Grieson DS, Monneret C: Synthesis and anti-HIV activity of novel N-1 side chain-modified analogs of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT). J Med Chem 1997:1845-1854.
5. Deshmukh MB, Salunkhe SM, Patil DR, Anbhule PV. A novel and efficient one step synthesis of 2-amino-5-cyano-6-hydroxy-4-arylpyrimidines and their anti bacterial activity. Eur J Med Chem 2009: 2651–2654.
6. Mai A, Rotili D, Massa S, Brosch G, Simonetti G, Passariello C, Palamara AT. Discovery of uracil-based histone deacetylase inhibitors able to reduce acquired antifungal resistance and trailing growth in *Candida albicans.* Bioorg Med Chem Lett.2007:1221-1225.
7. McCarthy O, Musso-Buendia A, Kaiser M, Brun R, Ruiz-Perez LM, Johansson NG, Pacanowska DG, Gilbert IH. Design, Synthesis and evaluation of novel uracil acetamide derivatives as potential inhibitors of Plasmodium falciparum dUTPnucleotidohydrolase. Eur J Med Chem 2009:678-688.
8. Amin KM, Awadalla FM, Eissa AAM, Abou-Seri SM, Hassan GS. Design, synthesis and vasorelaxant evaluation of novel coumarin–pyrimidine hybrids. Bioorg and Med Chem 2010:6087-6097.
9. Rahaman SA, Pasad YR , Kumar P , Kumar B. Synthesis and anti-histaminic activity of some novel pyrimidines. Saudi Pharmaceutical Journal 2009: 255-258.
10. Da S Falcao EP, De Melo SJ, Srivastava RM, De A.Catanh MTJ, Nascimento SCD. Synthesis and anti-inflammatory activity of 4-amino-2-aryl-5-cyano-6-{3- and 4-(*N*-phthalimidophenyl)} pyrimidines. Eur J Med Chem 2006:276-282.
11. Chetana B Patil, S. K. Mahajan, Suvarna A. Katti. Chalcone: A Versatile Molecule. J Pharm Sci & Res 2009; 1 Suppl 3:11-22.
12. Balaji PN, Sai Sreevani M, Harini P , Rani Johnsi P, Prathusha K, Chandu T. J J Chem Pharm Res 2010:754-758.
13. Patil Chetana B , Mahajan SK, Suvarna A. Katti. Chalcone: A Versatile Molecule. J Pharm Sci & Res. 2009; 1 Suppl 3:11-22.
14. Ahmed MG, Romman UKR, Ahmed SM, Akhter K. Synthesis and Correlation of Spectral Properties of Some Substituted 1,3-Diphenyl-2-Propen-1-Ones. Bangladesh J Sci Ind Res.2007;1 Suppl 3:45-52.
15. Berry A. Procedure and theoretical considerations for testing antimicrobial agent in agar media. In: Corian V (Ed.) Antibiotics in Laboratory Medicine. Williams and Wilkins, Baltimore.5th ed.1991.p.1-16.