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SYNTHESIS, AND BIOLOGICAL EVALUATION OF NEW PYRIMIDINE DERIVATIVES AS POTENTIAL ANTIBACTERIAL AGENTS

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ABSTRACT

Pyrimidine is the parent substance of a large group of heterocyclic compounds and plays a vital role in many biological processes, as found in nucleic acids, several vitamins, co-enzymes and purines**.** keeping this in mind new pyrimidines are synthesised by conventional method and the structures were confirmed by spectral evidence. synthesised compounds were screened for their antimicrobial activity. Compound **B_7P_7** and **B_8P_8** possessed maximum activity and this may be due to the presence of two electron withdrawing groups.

KEYWORDS: Pyrimidine, phenylhydrazine and antimicrobial activity.

INTRODUCTION

Pyrimidine is a six-membered heterocycle with two nitrogen atoms situated in a 1,3- arrangement. It is also known as *m*-diazine or 1,3-diazine. Both nitrogen atoms are like the pyridine nitrogen. Each has its lone pair of electrons in the sp^2 hybrid orbital in the plane of the aromatic ring. These lone pairs are not needed for the aromatic sextet, and they are basic, like the lone pair of pyridine.Pyrimidine is the parent substance of a large group of heterocyclic compounds and plays a vital role in many biological processes, as found in nucleic acids, several vitamins, co-enzymes and purines.

EXPERIMENTAL

General procedure for synthesis of Synthesis of 2-amino-4-(4'-chlorophenyl)-6-(3",4",5"-trimethoxyphenyl) pyrimidine (B_1P_1):1-(4'-chlorophenyl)-3-(3",4",5"-trimethoxyphenyl)-2-propen-1-one (B_1) (0.001 mol) was condensed with guanidine hydrochloride (0.001 mol) in the presence of potassium hydroxide (0.002 mol) in absolute ethanol (5 ml) at reflux temperature on a water bath for 3 hrs. The solvent was evaporated *in vacuo* and crushed ice was added to the residue while mixing thoroughly, whereupon a bright yellow solid separated out. This solid was filtered under vacuum, dried and purified by column chromatography to give pure pale yellow solid.physical characterization and spectral data are given below table 1-3.

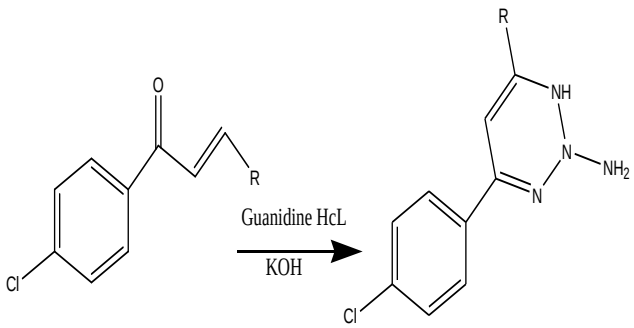
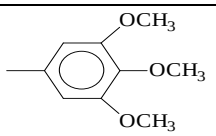
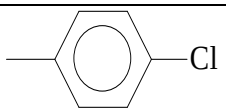


Table 1: Physical characterization data of 2,4,6-trisubstituted pyrimidines(B_1P_1-$B_{10}P_{10}$).

Compound	Ar	Molecular Formula	Relative Molecular Mass(RMM)	Melting Point (^oC)	Yield %
B_1P_1		$C_{19}H_{16}ClN_3O_3$	352	315-319	53
B_2P_2		$C_{16}H_9Cl_2N_3O$	312	250-254	65

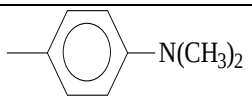
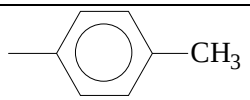
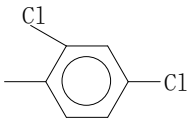
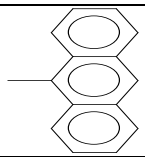
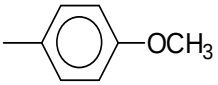
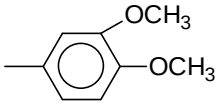
B_3P_3		$C_{18}H_{15}ClN_4$	322	178-182	58
B_4P_4		$C_{17}H_{12}ClN_3$	299	148-150	46
B_5P_5		$C_{16}H_8Cl_3N_3$	367	143-147	58
B_6P_6		$C_{24}H_{14}ClN_3$	379	238-240	61
B_7P_7		$C_{17}H_{12}ClN_3O$	309	313-317	63
B_8P_8		$C_{18}H_{14}ClN_3O_2$	349	223-225	66

Table 2: IR spectral data (KBr disc) of 2,4,6-trisubstituted pyrimidines (B_1P_1-$B_{10}P_{10}$).

Compound	Position of absoption band (cm^{-1})
B_1P_1	3414, 3380 (NH_2), 1591 (C=N), 1503 (C=C), 1387(C-N),1228 (C-O-C), 1178 (O-CH_3)
B_2P_2	3405, 3346 (NH_2), 1636 (C=N), 1578 (C=C),1383 (C-N), 858 (C-Cl)
B_3P_3	3410, 3332 (NH_2), 1610 (C=N), 1570 (C=C), 1391 (C-N),1178 (-N(CH_3)$_2$)
B_4P_4	3412, 3335 (NH_2), 1597 (C=N), 1520 (C=C), 1365 (C-N)
B_5P_5	,3410, 3326 (NH_2), 1605 (C=N), 1525 (C=C),1372 (C-N),892 (C-Cl)
B_6P_6	3413, 3328 (NH_2), 1632 (C=N), 1515 (C=C), 1375 (C-N)
B_7P_7	3414 (NH_2), 1598 (C=N), 1503 (C=C), 1366 (C-N),1225 (C-O-C)
B_8P_8	3320, 3187 (NH_2), 1597 (C=N), 1556 (C=C), 1354 (C-N), 1261(C-O-C)

Table 3: ^{1} H NMR spectral data (400MHz) of 2,4,6-trisubstituted pyrimidines (B_1P_1 – $B_{10}P_{10}$).

Compound	Chemical shift (δ) in ppm
B_1P_1	3.75-4.0 (9H, s, 3xOCH_3), 5.15 (2H, s, -NH_2), 6.45-6.60 (1H, m, C-4'-H) 7.38 (1H, d, J=6.0Hz, C-5'-H), 7.0 (1H, S,C-5-H) 6.40 (2H, S, C-2"-H and C-6"-H), 7.28 (1H, d, J=6.0Hz C-3'-H
B_2P_2	5.45 (2H, s, -NH_2), 6.60 (1H, m, C-4'-H)8.03 (2H, d, J=8.0Hz, C-3"-H and C-5"-H) 7.48 (2H, d, J=8.0Hz, C-2"-H and C-6"-H)7.62 (1H, d C-5'-H) 7.30 (1H, d, J=6.5Hz and C-3'-H), 7.40 (1H, S, C-5-H)
B_3P_3	3.10 (6H, s, -N(CH_3)$_2$), 5.20 (1H, s, -NH_2),6.61 (1H, m, C-4'-H), 7.36 (1H, s, C-5-H)8.12 (2H, d, J=8.5Hz, C-3"-H and C-5"-H) 6.78 (2H, d, J=8.5Hz, C-2"-H and C-6"-H)7.67 (2H, d, J=6.0Hz, C-3'-H and C-5'-H)
B_4P_4	2.46 (3H, s, Ar-CH_3), 5.25 (2H, s, -NH_2), 6.67 (1H, m, C-4'-H) 7.45 (1H, s, C-5-H), 8.06 (2H, d, J=8.0Hz, C-3"-H and C-5"-H) 7.36 (2H, d, J=8.0Hz, C-2"-H and C-6"-H) 7.71(1H, d, J=6.0Hz, C-5'),7.60 (1H, d, J=6.0Hz, C-3'H)
B_5P_5	5.78 (2H, s, -NH_2), 6.62 (1H, m, C-4'-H),7.62 (1H, s, -C-3"-H) 7.64 (1H, d, J=6.5Hz, C-5'-H), 7.54 (1H, d, J=8.5Hz, C-5"-H) 7.41 (1H, d, J=8.5Hz, C-6"-H), 7.39 (1H, d, J=6.5Hz, C-3'-H)7.35 (1H, s, C-5-H)
B_6P_6	5.85 (2H,s, -NH_2), 6.61 (1H,m, C-4'-H), 7.60 (1H,s, C-5-H) 8.06 (1H, d, J=6.0Hz, C-5'-H), 7.78 (1H, d, J=6.0Hz, C-3'-H) 7.22-7.55(9H, m, Ar-H)
B_7P_7	3.87 (3H, s, C-4"-OCH_3), 5.11 (2H, s, C-2-NH_2), 6.56 (2H, d, J=6.0 Hz, C-3' and 5'-H), 7.07 (2H, d, J=8.5 Hz, C-3"and 5"-H), 7.37 (1H, s, C-5-H), 7.58 (1H, s, C-2'-H), 8.05 (2H, d, J=8.5 Hz, C-2" and 6"-H)
B_8P_8	5.63 (2H, s, C-4'-NH_2), 5.21 (2H, s, C-2-NH_2), 6.64 (2H, d, J=6.5 Hz, C-3'), 7.19 (2H, dd, J=8.5 Hz, C-2" and 6"-H), 7.37 (1H, s, C-5-H),8.084 (2H, dd, J=8.5 Hz, J=8.5 Hz, C-3" and 5"-H)

Antibacterial activity

The antibacterial activity was tested by determining the minimum inhibitory concentration (MIC) for each compound using serial tube dilution technique. The following organisms were used.

Test organisms

Gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis.*

Gram negative bacteria: *Escherichia coli*, *Proteus vulgaris.*

The antibacterial activity of the chalcones (**B_1** to **B_8**) was assessed by determining the MIC, which is defined as the lowest concentration of the compound that completely inhibited the growth of each strain after overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the tube dilution and agar dilution methods.

Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

MATERIALS AND METHODS

In the present study, MIC was determined using serial tube dilution technique. In this technique the tubes of broth medium containing graded doses of compounds were inoculated with the test organisms. After suitable incubation, growth occurred in those tubes where the concentration of the compound was below the inhibitory level and the culture become turbid. No growth was noticed above the inhibitory level and the tubes remained clear.

1. **Preparation of the sample solution:** 2.048 mg of each test compound was taken in vials separately. Then 2 mL of methanol was added. Thus a solution with a concentration of 1.024 mg/mL was obtained.
2. **Preparation of the inoculums:** The test bacteria grown at 37^0C in nutrient agar medium was diluted in sterile nutrient broth medium in such a manner that the suspension contain about 10^7 cells/mL. This suspension was used as the inoculum.
3. **Procedure:**

a) 11 test tubes were taken, 9 of which were marked 1,2,3,4,5,6,7,8,9, and the rest two were assigned as T_M (medium), and T_{MI} (medium+inoculum).
b) 1 mL of nutrient broth medium was poured into each of the 11 test tubes.
c) These test tubes were cotton plugged and sterilized in an autoclave at 15 lbs/sq.inch pressure.
d) After cooling, 1 mL of the sample solution was added to the first test tube and mixed well and then 1 mL of this content was transferred to the second test tube.
e) The content of the second test tube was mixed well and again 1 mL of this mixture was transferred to the third test tube. This process of serial dilution was continued upto the ninth test tube.
f) 10 µL of properly diluted inoculum was added to each of the nine test tubes and mixed well.
g) 10 µL of the inoculum was added to the test tube T_{MI} to observe the growth of the organism in the medium used
h) The controlled test tube T_M containing only the medium was used to confirm the sterility of the medium.
i) All the test tubes were incubated at 37^0C for 18 h.

Table 4: Antibacterial activity of trisubstituted pyrimidines B_1P_1-B8P8

Compound	Ar	Zone of inhibition (in mm)									
		Quantity in µg/ml									
		B. subtilis		*B. pumilis*		*S. aureus*		*E. coli*		*P. vulgaris*	
		50	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**
B_1P_1	4-methylphenyl	24	27	24	27	21	26	20	23	26	27
B_2P_1	4-fluorophenyl	21	23	19	21	19	23	17	20	20	22
B_3P_3	4-chlorophenyl	20	22	19	22	18	22	18	20	20	23
B_4P_4	2-chlorophenyl	20	23	19	22	17	21	18	20	18	20
B_5P_5	2,4-difluorophenyl	18	20	17	20	18	20	15	17	13	15
B_6P_6	2,4-dichlorophenyl	15	17	17	19	15	19	14	16	12	14
B_7P_7	3-nitrophenyl	22	25	22	25	20	24	18	21	24	26
B_8P_8	3,4,5-trimethoxyphenyl	25	28	25	28	22	27	21	24	27	28
Ampicillin (standard)		28	33	31	32	27	30	25	27	28	31
Control		-	-	-	-	-	-	-	-	-	-

DISCUSSION ON RESULTS

All the pyrimidine derivatives **(B_1P_1-B_8P_8)** have been evaluated for their antibacterial activity against *Bacillus subtilis, Bacillus pumilis, Staphylococcus aureus* (Gram-positive) and *Escherichia Coli* and *Proteus vulgaris* (Gram-negative), by using cup-plate method. The results of this evaluation have been compared by taking benzyl penicillin as reference standard. vity data of pyrimidine derivatives **(B_1P_1-$B8P_8$, Table-4)** indicated that the compounds have some degree of inhibitory activity on all the bacteria at both 50 µg (0.05 mL) and 100 µg (0.1 mL) dose levels, when compared with the reference standard. It was also observed the antibacterial activity of the corresponding pyrimidines was found to more than the chalcones from which they are obtained.

CONCLUSION

All these observations were in agreement with the reports cited in the literature. Compounds having other heteroaromatic rings in the place of substituted thiophene ring can also be synthesized to get compounds with significant anti-inflammatory activity.

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