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Synthesis and Biological Activities of Some New Pyrimidine Derivatives from Chalcones

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ABSTRACT

Chalcones have been reported to present various biological activities such as anti-inflammatory, antioxidant, antitubercular, antibacterial activities. It is a basic moiety of many heterocyclic systems containing oxygen, sulphur and nitrogen. Nitrogen containing heterocyclic derivatives synthesized from Chalcones have exhibited anti-inflammatory, anticancer and antimicrobial activities. An attempt has been made to synthesize Chalcones by the reaction of 3-acetyl-2,5-dimethylfuran with various aromatic and heteroaromatic aldehydes. Further, Chalcones derivatives were cyclised to pyrimidine analogs by using guanidine hydrochloride. The newly synthesized pyrimidine derivatives have been characterized by IR, ^{1}HNMR, ^{13}CNMR, Mass spectra and elemental analysis and evaluated for their anti-inflammatory, anticancer, antifungal and antibacterial activities. It was found that 2-amino pyrimidine analog bearing 4-chloro substitution on phenyl ring has exhibited excellent anticancer activity at lowest concentration in the series moreover it has also exhibited good anti-inflammatory and antibacterial activities.

Keywords: Chalcones, pyrimidine, anticancer activity, antifungal activity, antibacterial activity.

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INTRODUCTION

Heterocyclic compounds are abundant in nature and having a great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics etc. A practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry [1]. Nitrogen containing heterocyclic play an important role in medicinal chemistry. Pyrimidine is a six-member heterocyclic compound that contains two nitrogen atoms at positions 1 and 3. The structure of the pyrimidine ring is similar to benzene and pyridine [2]. The key role pyrimidine play in cellular processes has made them valuable leads for drug discovery [3]. Pyrimidine derivatives are known to be biologically active compounds and substituted pyrimidines have shown wide range of biological activities like anticancer [4-9], antibacterial [4,7, 9-11], antifungal[4,8], anti-inflammatory [12] activity.

MATERIALS AND METHOD

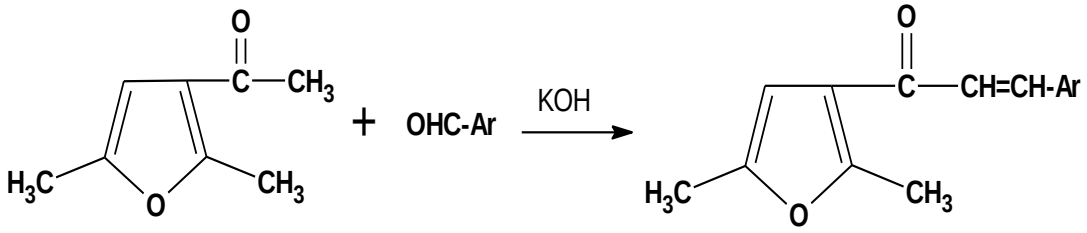
All the melting points were determined in a Boitus melting point apparatus and are uncorrected. The ^{1}H NMR spectra of the compounds were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

The ^{13}C NMR spectra of the compounds were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. The mass spectra of the compounds were recorded either on on Agilent 1100 ESI-Mass (Turbo Spray) Spectrophotometer or API-ES mass spectrometer using positive mode ionization method. Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography. The column was subjected to gradient elution using n-hexane, mixtures of hexane and ethyl acetate (5%, 10%, 15 %, 25%, 50% and 75% hexane in ethyl acetate), ethyl acetate and mixtures of ethyl acetate and methanol (1%, 2%, 5% and 10% ethyl acetate in methanol). Fractions each of 100 mL were collected. The separations of the compounds were checked on TLC under UV lamp and also by spraying the plates with 10% sulphuric acid. Elemental analyses were carried out with a Perkin-Elmer model 2400 series II apparatus. The results of elemental analyses (C,H,N) were within ± 0.4 % of the calculated values.

General procedure for the synthesis of chalcones by Claisen-Schmidt condensation:

Equimolar quantities (0.005 mol) of 3-acetyl-2,5-dimethylfuran and respective aldehydes were mixed and dissolved in minimum amount of alcohol. To this, aqueous potassium hydroxide solution (50%, 7.5 mL) was added slowly and mixed occasionally for 24 h, at room temperature. Completion of the reaction was identified by TLC using silica gel-G. After

completion of the reaction, the mixture was poured onto crushed ice, acidified if necessary with dilute hydrochloric acid, and the solid that separated was isolated by filtration, dried and purified by column chromatography on silica gel with a mixture of ethyl acetate and hexane as the mobile phase. The overall reaction involving the formation of chalcones are shown in **Scheme 1**



A **B** **C**

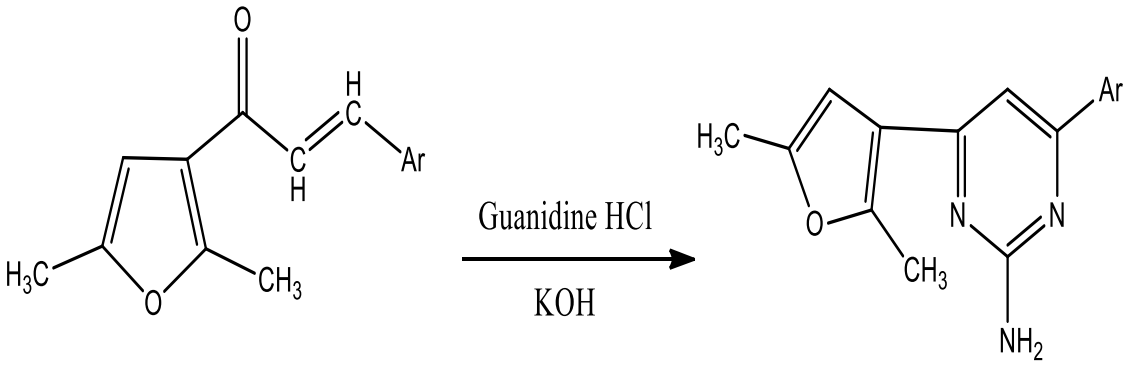
A = 3-acetyl-2,5-dimethylfuran; B = aldehydes;

C = 1-(2',5'-dimethyl-3'-furyl)-3-(aryl)-2-propen-1-one

General procedure for the synthesis of pyrimidines:

The condensation of the chalcones with guanidine hydrochloride in an alkaline medium using potassium hydroxide in the presence of ethanol, at reflux temperatures (2 to 6 h) resulted in the formation of corresponding pyrimidines (**Scheme 2**). Completion of the reaction was established by TLC using silica gel-G. After completion of the reaction, the reaction mixture was poured onto crushed ice with constant stirring. The solid that separated was filtered, dried and purified by column chromatography on silica gel, using a mixture of ethyl acetate and hexane as the mobile phase. The purified pyrimidine derivatives were obtained as light to bright yellow fine powders.

Scheme 2:



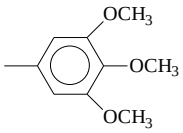
C **(Scheme 2)** **PM**

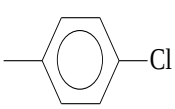
C = 1-(2',5'-dimethyl-3'-furyl)-3-(aryl)-2-propen-1-one;

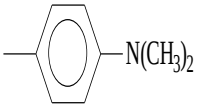
PM = 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(aryl)-pyrimidine.

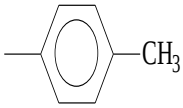
Ar

PM_1 PM_2 PM_3 PM_4

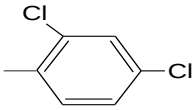


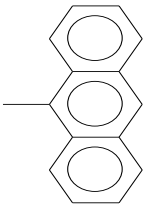


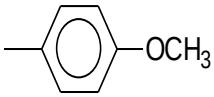


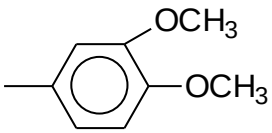


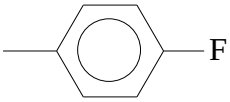
PM_5



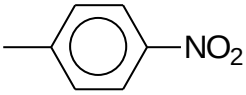


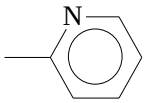


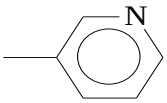


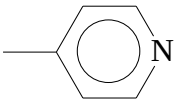


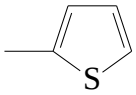
PM_6 PM_7 PM_8 PM_9











PM_{10} PM_{11} PM_{12} PM_{13} PM_{14}

List of synthesized 2,4,6-trisubstituted pyrimidine compounds:

1. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(3",4",5"-trimethoxyphenyl) pyrimidine (PM_1)

2. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(4"-chlorophenyl)pyrimidine(PM_2)

3. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(4"-dimethylaminophenyl)pyrimidine (PM_3)

4. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(4"-methylphenyl)pyrimidine (PM_4)

5. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(2",4"dichlorophenyl)pyrimidine (PM_5)

6. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(9"-anthracenyl)pyrimidine (PM_6)

7. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(4"-methoxyphenyl)pyrimidine (PM_7)

8. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(3",4"-dimethoxyphenyl)pyrimidine (PM_8)

9. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(4"-flurophenyl)pyrimidine (PM_9)

10. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(4"-nitrophenyl)pyrimidine (PM_{10})

11. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(2"-pyridinyl)pyrimidine (PM_{11})

12. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(3"-pyridinyl)pyrimidine (PM_{12})

13. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(4"-pyridinyl)pyrimidine (PM_{13})

14. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(2"-thienyl)pyrimidine (PM_{14})

RESULTS AND DISCUSSION:

Spectral properties of pyrimidines:

The 2,4,6-trisubstituted pyrimidines showed the C-5-H proton as singlet around δ 7.0 -7.35 and a broad signal at δ 5.15-5.25 due to the amino protons and another two singlets at δ 2.2 and 2.9 each integrating for three protons attributed to aromatic methyl groups. The spectrums also accounted for the other three aromatic protons of the furan and the phenyl rings in between δ 6.45-7.40.

Table 1: Physical characterization data of synthesized 2,4,6-trisubstituted pyrimidine compounds(PM_1-PM_{14})

Compound	Molecular formula	Relative molecular mass (RMM)	Melting point(^oC)	Yield (%)
PM_1	$C_{19}H_{21}N_3O_4$	355	312-314	63
PM_2	$C_{16}H_{14}ClN_3O$	299	241-245	57
PM_3	$C_{18}H_{20}N_4O$	308	164-165	64
PM_4	$C_{17}H_{17}N_3O$	279	120-122	54
PM_5	$C_{16}H_{13}Cl_2N_3O$	333	132-134	60
PM_6	$C_{24}H_{19}N_3O$	365	223-225	72
PM7	C17H17N3O2	295	302-305	68
PM8	C18H19 N3O3	325	220-221	70
PM9	C16H14FN3O	283	233-235	53
PM10	C16H14N4O3	310	260-262	65
PM11	C15H14N4O	266	194-196	56
PM12	C15H14N4O	266	212-213	46
PM13	C15H14N4O	266	233-235	48
PM14	C14H13N3OS	271	240-241	55

Table 2: Elemental Analysis data of 2,4,6-trisubstituted pyrimidines(PM_1-PM_{14})

Compound	(% Calculated value)			(% practically found)		
	C	H	N	C	H	N
PM_1	64.22	5.91	11.83	64.24	5.92	11.84
PM_2	64.21	4.68	14.01	64.24	4.69	14.11
PM_3	70.12	6.49	18.18	70.14	6.50	18.16
PM_4	73.11	5.01	15.05	73.14	5.03	15.15
PM_5	57.65	3.90	12.61	57.62	3.87	12.64
PM_6	78.90	5.20	11.50	78.92	5.21	11.54
PM_7	69.15	5.76	14.23	69.14	5.75	14.22
PM_8	66.46	5.84	12.92	66.47	5.83	12.93
PM_9	67.84	4.94	14.84	67.83	4.92	14.82
PM_{10}	61.93	4.51	18.06	61.92	4.54	18.04
PM_{11}	67.66	5.26	21.05	67.62	5.23	21.04
PM_{12}	67.66	5.26	21.05	67.63	5.22	21.03
PM_{13}	67.66	5.26	21.05	67.64	5.25	21.06
PM_{14}	61.99	4.79	15.49	61.98	4.78	15.51

Table 3: IR spectral data (KBr disc) of 2,4,6-trisubstituted pyrimidines(PM_1-PM_{14})

Comp.	Position of absorption band (cm^{-1})
PM_1	3414, 3380 (NH_2), 1591 (C=N), 1502 (C=C), 1387(C-N),1228 (C-O-C), 1178 (O-CH_3)
PM_2	3405, 3346 (NH_2), 1636 (C=N), 1578 (C=C),1383 (C-N), 858 (C-Cl)
PM_3	3410, 3332 (NH_2), 1610 (C=N), 1570 (C=C), 1391 (C-N),1178 (-N-(CH_3)$_2$)
PM_4	3412, 3335 (NH_2), 1597 (C=N), 1520 (C=C), 1365 (C-N)
PM_5	3410, 3326 (NH_2), 1605 (C=N), 1525 (C=C),1372 (C-N),892 (C-Cl)
PM_6	3413, 3328 (NH_2), 1632 (C=N), 1515 (C=C), 1375 (C-N)
PM_7	3414 (NH_2), 1598 (C=N), 1503 (C=C), 1366 (C-N),1225 (C-O-C)
PM_8	3320, 3187 (NH_2), 1597 (C=N), 1556 (C=C), 1354 (C-N), 1261(C-O-C)
PM_9	3468, 3318(NH_2), 1599 (C=N), 1510 (C=C), 1350(C-N), 1219 (C-F)
PM_{10}	3370 (NH_2), 1645 (C=N), 1557 (N=O, asymmetric), 1406 (C-N), 1350 (N=O, symmetric)
PM_{11}	3425, 3238 (NH_2), 1656 (C=N), 1510 (C=C), 1380 (C-N)
PM_{12}	3415, 3332 (NH_2), 1645 (C=N), 1512 (C=C), 1359 (C-N)
PM_{13}	3418, 3355 (NH_2), 1575 (C=N), 1526 (C=C), 1365 (C-N)
PM_{14}	3405, 3325 (NH_2), 1565 (C=N), 1516 (C-C), 1360 (C-N), 670 (C-S)

Table 4: ^{1} H NMR spectral data (400MHz) of 2,4,6-trisubstituted pyrimidines (PM_1-PM_{14})

Compound	Chemical shift (δ) in ppm
PM_1	3.75-4.0 (9H, s, 3xOCH_3), 5.15 (2H, s, -NH_2), 6.45-6.60 (1H, s, C-4'-H), 7.45 (1H, s, C-5-H), 6.40 (2H, S , C-2"-H and C-6"-H), 2.4(3H,s,Ar-CH_3), 2.9(3H,s, Ar-CH_3).
PM_2	5.45 (2H, s, -NH_2), 6.60 (1H, s , C-4'-H), 7.35 (1H, s, C-5-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), 2.2(3H,s, Ar-CH_3), 2.6(3H,s, Ar-CH_3).
PM_3	3.10 (6H, s, -N(CH_3)$_2$), 5.20 (2H, s , -NH_2), 7.2 (1H, s, C-5-H), 6.61 (1H, s, C-4'-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), 2.65(3H,s, Ar-CH_3), 2.9(3H,s, Ar-CH_3).
PM_4	2.46 (3H, s, Ar-CH_3), 5.25 (2H, s, -NH_2), 6.67 (1H, s, 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), 2.15(3H,s, Ar-CH_3), 2.25(3H,s, Ar-CH_3).
PM_5	5.78 (2H, s, -NH_2), 6.62 (1H, s, C-4'-H),7.62 (1H, s , -C-3"-H), 7.54 (1H, d, J=8.5Hz, C-5"-H)7.41 (1H, d, J=8.5Hz, C-6"-H), 7.35 (1H, s, C-5-H), 2.4(3H,s, Ar-CH_3), 2.9(3H,s, Ar-CH_3).
PM_6	5.85 (2H,s, -NH_2), 6.61 (1H,s, C-4'-H), 7.60 (1H,s, C-5-H) 7.22-7.55(9H, m, Ar-H), 2.2(3H,s, Ar-CH_3), 2.7(3H,s, Ar-CH_3).
PM_7	3.87 (3H, s, C-4"-OCH_3), 5.11 (2H, s, C-2-NH_2), 7.07 (2H, d, J=8.5 Hz, C-3"and 5"-H), 7.37 (1H, s, C-5-H), 6.51 (1H, s , C-4'-H), 8.05 (2H, d, J=8.5 Hz, C-2" and 6"-H), 2.35(3H,s, Ar-CH_3), 2.7(3H,s, Ar-CH_3).
PM8	5.21 (2H, s, C-2-NH2), 3.75-4.0 (6H, s, 2xOCH3), 7.19 (1H, S, C-2"-H), 7.94 (2H, dd, J=8.5 Hz, J=8.5 Hz, C-3" and 5"-H), 6.63 (1H, s , C-4'-H), 7.0 (1H, s, C-5-H), 2.35(3H,s, Ar-CH3), 2.7(3H,s, Ar-CH3).
PM9	5.21 (2H, s, C-2-NH2), 7.19 (2H, dd, J=8.5 Hz, C-2" and 6"-H), 6.60 (1H, s, C-4'-H), 8.2 (2H, dd, J=8.5 Hz, C-3" and 5"-H), 7.25 (1H, s, C-5-H), 2.4(3H,s, Ar-CH3), 2.8(3H,s, Ar-CH3).
PM10	5.22 (2H, s, C-2-NH2), 6.64-6.65 (1H, s, C-4'-H), 7.35 (1H, s, C-5-H), 7.79 (2H, d, J=8.0Hz, C-2″ and 6″-H), 8.34 (2H, d, J=8.0Hz, C-3″and 5″-H), 2.2(3H,s, Ar-CH3), 2.6(3H,s, Ar-CH3).
PM11	5.22 (2H, s, C-2-NH2), 7.53-7.50 (1H, m, C-5"-H), 7.99-7.95 (1H, d, J=8.5 Hz ,C-3"-H), 8.33 (1H, m, C-4"-H), 8.73 (1H, d, J=8.5 Hz, C-6"-H), 7.25 1H, s, C-

	5-H), 6.60 (3H, s, C-4'-H), 2.4(3H,s, Ar-CH3), 2.8(3H,s, Ar-CH3).
PM12	5.3 (2H, s, C-2-NH2), 7.53-7.50 (1H, m, C-5"-H), 6.62 (1H, s, C-4'-H), 7.25 (1H, s, C-5-H), 8.33 (1H, d, J=8.0 Hz, C-4"-H), 7.4(1H,s,C-2"-H), 8.73 (1H, d, J=8.0 Hz, C-6"-H), 2.4(3H,s, Ar-CH3), 2.8(3H,s, Ar-CH3).
PM13	5.32 (2H, s, C-2-NH2), 6.55-6.54 (1H, s, C-4'H), 7.25 (1H, s, C-5-H), 7.46 (2H,d, J=8.5Hz, C-3"H and 5"H), 7.58 (2H, d,J=8.2Hz C-2"H and 6"H), 2.4(3H,s, Ar-CH3), 2.7(3H,s Ar-CH3).
PM14	5.3 (2H, s, C-2-NH2), 6.55-6.58 (1H, s,C-4'H), 7.32(1H,s,C-5-H), 7.1-7.12 (1H, t, C-4"H), 7.26 (1H, d, J=6Hz,C-3"H), 7.46 (1H, d , J=8Hz, C- 5"H), 2.3(3H,s, Ar-CH3), 2.5(3H,s, Ar-CH3).

Antimicrobial studies of pyrimidines:

Antibacterial activity:

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5mL) to give a concentration of 1000 μg/mL.Benzyl penicillin solution was prepared to give a concentration of 1000 μg/mL in sterilized distilled water. All the compounds were tested at dose levels of 50 μg (0.05 mL) and 100 μg (0.1mL) and DMSO used as a control. The solutions of each test compound, control and reference standards (0.05 and 0.1 mL) were added separately in the cups and the plates were kept undisturbed for at least 2 h in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37±1 ^{0}C for 24 h. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

Compound	**Ar**	**Zone of inhibition (in mm) Quantity in μg/mL**									
		B.nsubtilis		***B.npumilis***		***S.aureus***		***E. coli***		***P. vulgaris***	
		50	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**
PM_1	3″,4″,5″-trimethoxyphenyl	12	11	09	10	08	11	11	10	11	08
PM_2	4″-chlorophenyl	15	21	17	20	18	23	13	16	14	18
PM_3	4″-dimethylaminophenyl	16	18	18	23	20	23	18	23	21	26
PM_4	4″-methylphenyl	20	23	19	18	11	19	18	19	17	18
PM_5	2″,4″-dichlorophenyl	11	13	14	14	17	21	12	16	11	14
PM_6	9″-anthracenyl	14	18	18	23	14	21	15	17	16	20
PM_7	4″-methoxyphenyl	19	22	20	24	18	22	16	17	13	16
PM_8	3″,4″-dimethoxyphenyl	19	20	18	21	17	17	18	24	25	23
PM_9	4″-fluorophenyl	19	21	19	16	21	24	19	21	19	20
PM_{10}	4″-nitrophenyl	19	21	20	24	21	26	21	23	20	25
PM_{11}	2″-pyridinyl	18	20	17	20	16	21	16	18	115	20
PM_{12}	3″-pyridinyl	16	20	15	19	15	20	16	18	14	16
PM_{13}	4″-pyridinyl	14	16	17	20	15	20	18	20	16	18
PM_{14}	2”thienyl	13	16	15	19	18	19	15	18	20	19
Benzylpenicillin (standard)		28	33	31	32	27	30	25	27	28	31
Control		-	-	-	-	-	-	-	-	-	-

Among all the compounds tested, compounds **PM_2, PM_5, PM_{11}** and **PM_{13}** produced maximum inhibitory zones. All these compounds possessed the electron withdrawing substituent's on the aromatic ring, except in the case of last two compounds, where the aromatic ring is replaced by hetero aryl rings.

Antifungal activity of pyrimidines:

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 mL) to give a concentration of 1000 μg/mL. Fluconazole solution was also prepared at a concentration of 1000 μg/mL in sterilized distilled water. All the compounds were tested at dose levels of 50 μg (0.05 mL) and 100 μg (0.1 mL) and DMSO used as a control. The solutions of each test compound, control and reference standards (0.05 and 0.1 mL) were added separately in the cups and the plates were kept undisturbed for at least 2 hr in a refrigerator to allow diffusion of the solution properly into the PDA medium. Petri dishes were subsequently kept at room temperature for 48 h. After that, the diameter of zone of inhibition in mm surrounding each of the cups was measured with the help of an antibiotic zone reader.

Compound	**Ar**	**Zone of inhibition (in mm) Quantity in μg/mL**					
		A. niger		**C. albicans**		**R. oryzae**	
		50	**100**	**50**	**100**	**50**	**100**
PM_1	3″,4″,5″-trimethoxyphenyl	16	20	17	21	17	19
PM_2	4″-chlorophenyl	17	21	15	20	15	18
PM_3	4″-dimethylaminophenyl	17	23	24	25	16	18
PM_4	4″-methylphenyl	14	17	16	21	13	18
PM_5	2″,4″-dichlorophenyl	15	17	21	22	14	16
PM_6	9″-anthracenyl	18	20	22	20	14	19
PM_7	4″-methoxyphenyl	17	20	21	22	15	18
PM_8	3″,4″-dimethoxyphenyl	17	18	20	19	16	18
PM_9	4″-fluorophenyl	16	19	21	23	15	19
PM_{10}	4″-nitrophenyl	14	18	19	22	18	21
PM_{11}	2″-pyridinyl	16	20	22	23	15	18
PM_{12}	3″-pyridinyl	15	18	19	21	11	16
PM_{13}	4″-pyridinyl	10	12	12	14	10	15
PM_{14}	2”thienyl	15	18	18	20	11	17
Fluconazole (standard)		24	28	24	28	22	27

Among all the compounds **PM_3, PM_5** and **PM_9**, which carries 4-dimethylaminophenyl, 2,4-dichlorophenyl and 4-fluorophenyl substituent's at C-6 position of pyrimidine ring exhibited maximum activity. The results clearly revealed the contribution of electron withdrawing groups and electron releasing groups on the aromatic ring in enhancing the antifungal activity.

Anticancer activity of pyrimidines

The synthesized pyrimidines have been screened for anticancer activity on prostate cancer

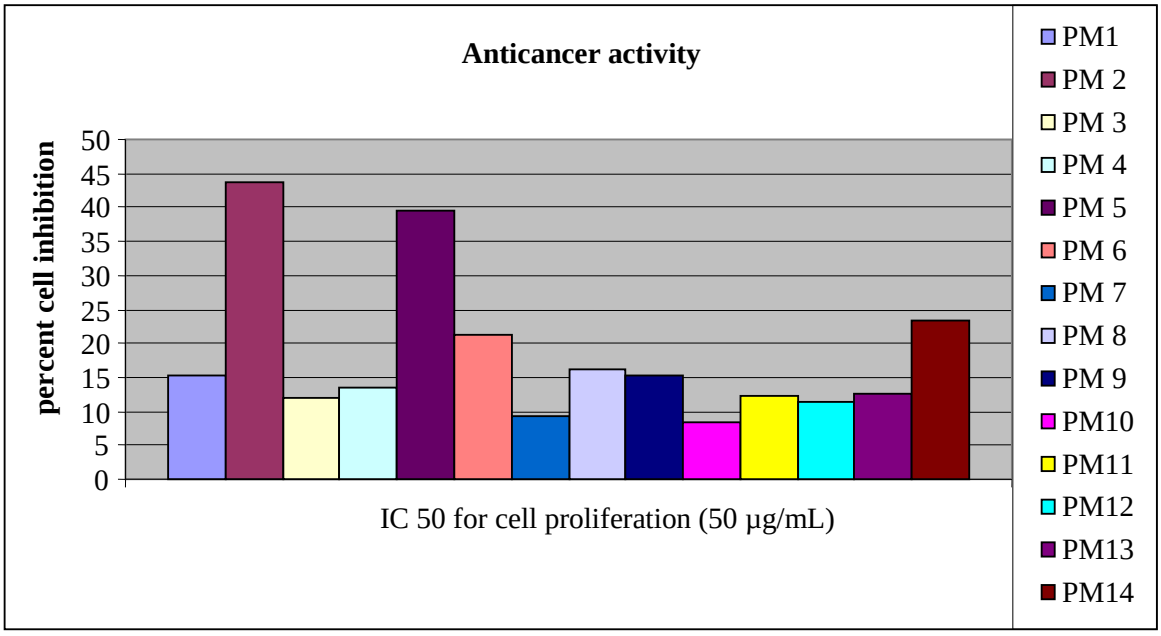
cell lines (DU-145) using MTT based cytotoxicity assay. DMEM (Dulbecco's Modified Eagles Medium),10% Fetal bovine serum (FBS),MTT reagent : 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide,Cell lines: DU-145 cell lines, were obtained from the National Centre for Cell Science (NCCS), pune (India) were used for this study.

This method is based on a colorimetric assay which takes into account the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The level of formazan created is a reflection of the number of surviving cells and shows a proportionality relationship between them.The required cell proliferation assay kit was obtained from Roche Applied Sciences, Germany The procedure consists of seeding an equal number of DU-145 cells in each well of a 96- well microplate and incubating at 37°C in the presence of 5% CO_2. Various concentrations of the test substances were added to the cells. For every 24 h the culture medium was renewed with the test substances. 0.5% DMSO was added into the vehicle control culture wells. After 72 h treatment, 5 µL of MTT reagent (R&D systems USA) along with 45µL of phenol red and FBS free DMEM (Sigma Life Science, USA) was added to each well and incubated for 4 h at 37°C in presence of 5% CO_2. Then 50 µL of solublization buffer (R&D systems, USA) was added to each well to solubilize the coloured formazan crystals produced by the reduction of MTT. After 24 h the optical density was measured at 550 nm using a microplate reader (BioRad, USA). The results (mean O.D.± SD) obtained from quadruplicate wells were used in calculation to determine the IC_{50} of the test compounds.

The percent inhibition is then calculated from the formula:

$$\% \text{ inhibition} = \text{Control O.D.} - \text{Sample O.D}/\text{Control O.D.} \times 100$$

The above IC-50 values for pyrimidines revealed that some of the compounds have significant anticancer activity against the cell line (DU-145) tested. Out of all the compound **PM_2** showed maximum activity, closely followed by **PM_5**. A chloro group on the phenyl ring enhanced the anticancer activity. Compound **PM_{14}** also showed considerable anticancer activity. The thiophene ring contributed favorably to the observed anticancer activity, which is consistent with the literature reports.



CONCLUSION

Chalcones derivatives were cyclised using guanidine hydrochloride, potassium hydroxide and ethanol to obtain pyrimidine derivatives. All the pyrimidine derivatives were evaluated for anticancer, antibacterial and anti fungal activities. 2-amino-4-(2',5'-dimethyl-3'-furyl)-6-(4''-chlorophenyl)pyrimidine(PM_2)has exhibited excellent anticancer activity at the concentration of 50 µg/mL. Compounds which carries 4-dimethylaminophenyl, 2,4-dichlorophenyl and 4-fluorophenyl substituent's at C-6 position of pyrimidine ring exhibited maximum antifungal activity. Compounds possessed the electron withdrawing substituent's on the aromatic ring exhibited maximum antibacterial activity.

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