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Synthesis, Characterization and Biological Evaluation of Some New Pyrimidine Derivatives as Anti-Inflammatory and Cytotoxic Agents

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ABSTRACT

Pyrimidine derivatives are a group of heterocyclic compounds which are associated with a wide variety of biological activities. Broad spectrum of biological activities of pyrimidine derivatives elated to synthesize a series of some new different pyrimidine derivatives by condensation of different chalcones with guanidine hydrochloride in the presence of potassium hydroxide as catalyst. The synthesized pyrimidine derivatives were purified by either recrystallization or column chromatographic techniques. The characterization of the purified pyrimidine derivatives was done by IR, ^{1}H NMR spectral data and elemental analysis. These compounds were further screened for anti-inflammatory and cytotoxic activities using standard protocols. When these pyrimidine derivatives were evaluated for anti-inflammatory and cytotoxic activities some of them found to possess significant biological activity when compared to standard drugs.

Keywords: Chalcone, Pyrimidine, Anti-inflammatory, Cytotoxic.

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INTRODUCTION

Pyrimidine **(Fig 1)** 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. The most common pyrimidine synthesis [1] belong to the (3 + 3) or NCN = CCC route in which one component synthon is an amine and the other is a 1, 3-dipolar component. The amine component mostly used is urea, thiourea, guanidine, amidines, imidines and substituted urea and amines. A large variety of compounds like 1,3-diketones, β-keto aldehydes, carboxylic acids, esters, α,β-unsaturated carbonyl compounds are used in the condensation reactions. Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms. Purines, uric acid, alloxan, barbituric acid and a group of antimalarial and antibacterials also contain the pyrimidine ring. Pyrimidine derivatives are associated with diverse spectrum of biological activities like antimicrobial [2], antifungal [3], anticancer [4], anti-inflammatory [5], herbicidal [6], antiviral [7], antitubercular [8], antimalarial [9-13], antileishmanial [14], neuroprotective [15] and antihyperlipidemic [16] activities.

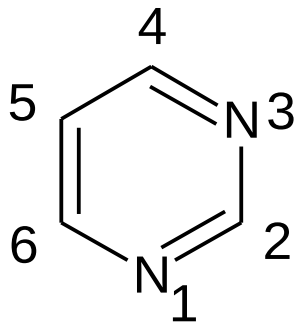

Figure 1: General structure of Pyrimidine

In the current work, we report the condensation of different chalcones with guanidine hydrochloride in the presence of pyridine as catalyst and ethanol as solvent to form different pyrimidine derivatives (P-1 to P-15). The structures of various synthesized pyrimidine derivatives were characterized on the basis of elemental analyses, IR and ^{1}H NMR spectral data. The compounds were evaluated for their anti-inflammatory activity by carrageenan induced rat paw edema method and cytotoxic activity by MTT assay methods.

MATERIALS AND METHODS

Melting points were determined in an open capillary melting point apparatus and are uncorrected. ^{1}H NMR was recorded in $CDCl_3$ on Bruker WM 400 MHz spectrometer with TMS as internal standard. Infrared spectra were recorded (KBr) on Perkin-Elmer AC-1 spectrophotometer. Microanalyses were performed on Carloerba EA-1108 element analyzer and were within the ± 0.4 % of the theoretical values. Reaction completion was identified by TLC using Silica gel-G for TLC (Merck). All the substituted pyrimidines have been purified by column chromatography performed on Silica gel (100-200 mesh, Merck).

Experimental:

General procedure for the preparation of Pyrimidines:

To a mixture of chalcones of 2-acetyl-5-bromothiophene (0.005mol) and guanidine hydrochloride (0.005 mol) in absolute ethanol (10 ml) few drops of potassium hydroxide was added and refluxed on a water bath for6 hours.Thesolventwascompletelyevaporatedandtheresiduewaspouredintoicecold water, the precipitated solid was collected by filtration and crystallized from a suitable solvent to give the desired substituted pyrimidine **(Scheme 1)**. Physical characterization data of the synthesized compounds is presented in table-1, elemental analysis data in table-2, IR and ^{1}H NMR data in tables-3 and 4.

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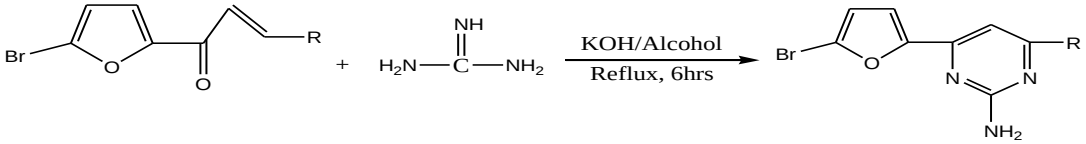

Substituted chalcone Guanidine hydrochloride Substituted pyrimidine

Scheme 1: Synthesis of different pyrimidine derivatives

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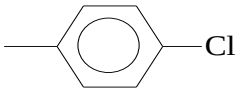

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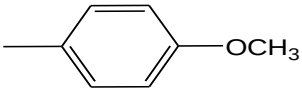

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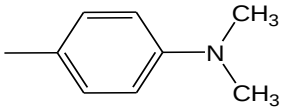

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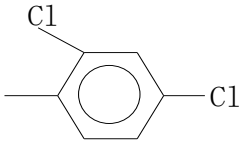

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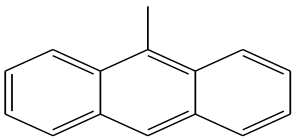

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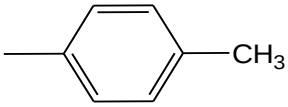

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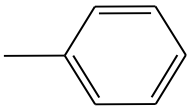

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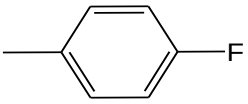

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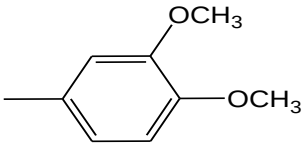

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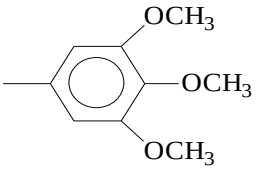

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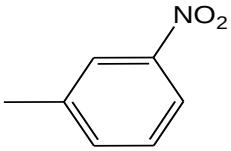

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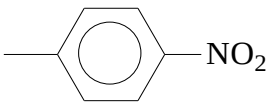

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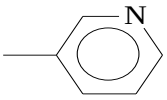

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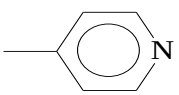

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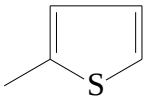

P-15

The List of New Pyrimidines Synthesized are:

1. 2-amino-4-(4''-chlorophenyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-1)
2. 2-amino-4-(4''-methoxyphenyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-2)
3. 2-amino-4-(4''-dimethylaminophenyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-3)
4. 2-amino-4-(2'',4''-dichlorophenyl)-6-(5'-bromo-2'- furyl) pyrimidine(P-4)
5. 2-amino-4-anthryl-6-(5'-bromo-2'- furyl) pyrimidine(P-5)
6. 2-amino-4-(4''-methylphenyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-6)
7. 2-amino-4-phenyl-6-(5'-bromo-2'-furyl) pyrimidine(P-7)
8. 2-amino-4-(4''-fluorophenyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-8)
9. 2-amino-4-(3'',4''-dimethoxyphenyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-9)
10. 2-amino-4-(3'',4'',5''-trimethoxyphenyl)-6-(5'-bromo-2'-furyl) pyrimidine (P-10)
11. 2-amino-4-(3''-nitrophenyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-11)
12. 2-amino-4-(4''-nitrophenyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-12)
13. 2-amino-4-(3''-pyridinyl)-6-(5'-bromo-2'-furyl) pyrimidine (P-13)
14. 2-amino-4-(4''-pyridinyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-14)
15. 2-amino-4-(2''-thienyl)-6-(5'-bromo-2'-furyl) pyrimidine(P-15)

Characterization of new pyrimidines:

Table 1: Physical characterization data of pyrimidines (P-1 toP-15)

Compound	Molecular formula	Relative Molecular Mass(RMM)	Melting point	Yield (%)
P-1	$C_{14}H_9BrClN_3O$	350	178	96
P-2	$C_{15}H_{12}BrN_3O_2$	346	175	53
P-3	$C_{16}H_{15}BrN_4O$	359	155	68
P-4	$C_{14}H_8BrCl_2N_3O$	385	167	70
P-5	$C_{22}H_{14}BrN_3O$	416	188	91
P-6	$C_{15}H_{12}BrN_3O_2$	330	151	85
P-7	$C_{14}H_{10}BrN_3O$	316	152	59
P-8	$C_{14}H_9BrFN_3O$	334	149	81
P-9	$C_{16}H_{14}BrN_3O_3$	376	129	65
P-10	$C_{17}H_{16}BrN_3O_4$	406	194	79
P-11	$C_{14}H_9BrN_4O_3$	361	189	69
P-12	$C_{14}H_9BrN_4O_3$	361	231	72
P-13	$C_{13}H_9BrN_4O$	316	231	64
P-14	$C_{13}H_9BrN_4O$	316	212	71
P-15	$C_{12}H_8BrN_3OS$	322	144	80

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Table 2: Elemental analysis data of pyrimidines (P-1 toP-15):

Compound	(%)Calculated			(%)Found		
	C	H	N	C	H	N
P-1	47.96	2.59	11.99	48.01	2.61	12.00
P-2	52.04	3.49	12.14	52.09	3.50	12.17
P-3	53.50	4.21	15.60	53.52	4.24	15.64
P-4	43.67	2.09	10.91	43.69	2.11	10.95
P-5	63.48	3.39	10.09	63.52	3.41	10.10
P-6	54.56	3.66	12.73	54.59	3.69	12.74
P-7	53.19	3.19	13.29	53.21	3.22	13.30
P-8	50.32	2.71	12.58	50.38	2.75	12.61
P-9	51.08	3.75	11.17	51.11	3.77	11.21
P-10	50.26	3.97	10.34	50.29	3.98	10.36
P-11	46.56	2.51	15.51	46.60	2.52	15.54
P-12	46.56	2.51	15.51	46.60	2.52	15.54
P-13	49.23	2.86	17.67	49.25	2.88	17.69
P-14	49.23	2.86	17.67	49.25	2.88	17.69
P-15	44.74	2.50	13.04	44.77	2.52	13.05

Table 3: IR (KBr disc) spectral data of pyrimidines (P-1 toP-15):

Compound	Position of absorption band(cm^{-1})
P-1	3342 (NH_2), 1628 (C=N), 1580 (C=C), 856 (C-Cl), 1052 (C-O), 672(C-Br)
P-2	3345 (NH_2), 1625 (C=N), 1590 (C=C),1165 (OCH_3), 1142 (C-O), 664(C-Br)
P-3	3338 (NH_2), 1633 (C=N), 1588 (C=C), 1185 ($N(CH_3)_2$), 1160 (C-O),670 (C-Br)
P-4	3348 (NH_2), 1635 (C=N), 1582 (C=C), 850 (C-Cl), 1067 (C-O), 680(C-Br)
P-5	3356 (NH_2), 1636 (C=N), 1582 (C=C), 1099 (C-O), 682(C-Br)
P-6	3350 (NH_2), 1630 (C=N), 1580 (C=C), 1077 (C-O), 670(C-Br)
P-7	3340 (NH_2), 1630 (C=N), 1575 (C=C), 1082 (C-O),686(C-Br)
P-8	3335 (NH_2), 1630 (C=N), 1575 (C=C), 1120 (C-F), 1055 (C-O),661(C-Br)
P-9	3414 (NH_2), 1641 (C=N), 1519 (C=C), 1145 (-O-CH_3), 1073 (C-O),686(C-Br)
P-10	3361(NH_2), 1602(C=N), 1572 (C=C), 1120 (-O-CH_3), 1070 (C-O), 684(C-Br)
P-11	3335(NH_2), 1635 (C=N), 1575 (C=O), 1510 (N=O, asymmetric), 1330(N=O,
P-12	3413 (NH_2), 1512 (N=O, asymmetric), 1335 (N=O, symmetric), 1605 (C=N), 1083 (C-O), 672(C-Br)
P-13	3335 (NH_2), 1635 (C=N), 1570 (C=C) , 1122 (C-O), 688(C-Br)
P-14	3335 (NH_2), 1635 (C=N), 1570 (C=C), 1101 (C-O) ,684(C-Br)
P-15	3335 (NH_2), 1635 (C=N), 1570 (C=C), 1093 (C-O) ,686(C-Br)

ANTI-INFLAMMATORYACTIVITY:

The compounds were tested for anti-inflammatory activity by carrageenan induced rat paw oedema model employing Zeitlin's apparatus to measure the paw thickness.

Materials:

All the materials used for this experiment were of analytical grade. Carrageenan was procured from Hi-media. Sodium carboxymethyl cellulose (Sodium CMC, E. Merck), Saline (Core Health Care) were purchased from the local supplier. Phenylbutazone sample was the gift sample from Jagsonpal, New Delhi.

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Preparation of sodium CMC suspension:

Stock suspension of sodium CMC was prepared by triturating 1gm of Sodium CMC in 100mL of distilled water and used for suspending the test compounds and standard drug.

Table 4. ^{1}H-NMR ($CDCl_3$) spectral data (400MHz) of pyrimidines (P-1 toP-15)

Compound	Chemical shift(δ) in ppm
P-1	7.30 (1H, s, C-5-H), 5.34 (2H,s,C-2-NH_2),7.98 (2H, d, J=7 Hz, C-2''-H andC-6'-H), 7.44 (2H, d, J=7 Hz, C-3''-H andC-5''-H),7.50 (1H, d, J=6.5 Hz, C-3'-H), 7.15 (1H, d, J=7.0 Hz, C-4'-H)
P-2	7.68 (1H, s, C-5-H) , 4.90 (2H, s, C-2-NH_2) , 3.88 (3H,s,C-4''-OCH_3),8.00 (2H, d, J=7 Hz, C-2''-H andC-6''-H), 7.02 (2H, d, J=7 Hz, C-3''-HandC-5''-H),7.58 (1H, d, J=6.5 Hz, C-3'-H), 7.18 (1H, d, J=7.0 Hz,C-4'-H)
P-3	7.27 (1H, s, C-5-H), 5.39 (2H, brs,C-2-NH_2),3.09 (6H, s,C-4''-$N(CH_3)_2$), 8.00 (2H, d,C-2''-H andC-6''-H),6.74 (2H, d, C-3''-H and C-5''-H), 7.47 (1H, d, J=6.5 Hz, C-3'-H),7.14 (1H, d, J=7.0 Hz,C-4'-H)
P-4	7.07 (1H, s, C-5-H), 5.47 (2H, s, C-2-NH_2), 8.55 (1H, d, J=2 Hz,C-3''-H), 8.07 (1H, d, J=7 Hz, C-5''-H), 8.77 (1H, d, J=7 Hz,C-6''-H),7.80 (1H, d, J=6.5 Hz, C-3'-H), 7.11 (1H, d, J=7.0 Hz,C-4'-H)
P-5	7.27 (1H, s, C-5-H), 5.69 (2H, s,C-2-NH_2), 7.14-7.59 (9H, m, Ar-H),7.62 (1H, d, J=6.5Hz, C-3'-H), 7.17 (1H, d, J=7.0 Hz,C-4'-H)
P-6	7.31 (1H, s, C-5-H), 5.34 (2H,s,C-2-NH_2),2.14 (3H, s,C-4''-CH_3), 7.98 (2H, d, J=7 Hz, C-2''-H andC-6''-H),7.44 (2H, d, J=7 Hz, C-3''-H and C-5''-H), 7.50 (1H, d, J=6.5 Hz,C-3'-H),7.14 (1H, d, J=7.0 Hz,C-4'-H)
P-7	7.34 (1H, s, C-5-H), 5.35 (2H, s,C-2-NH_2),7.62 (2H, m, C-2'' and 6''-H), 7.44-7.52 (3H, m, C-3'', 4'' and5''-H),8.04 (1H, d, J=6.5 Hz, C-3'-H), 7.13 (1H, d, J=7.0 Hz,C-4'-H)
P-8	7.30 (1H, s, C-5-H), 5.17 (2H, s,C-2-NH_2),7.62 (2H, d, J=7 Hz, C-2'' and C-6'' H), 7.47 (2H, d, J=7 Hz, C-3'' and C-5'' H),7.74 (1H, d, J=6.3 Hz, C-3'H), 7.14 (1H, d, J=7.0 Hz,C-4'-H)
P-9	7.30 (1H, s, C-5-H), 5.22 (2H, s, C-2- NH_2), 3.93 (3H, s,-OCH_3), 3.98 (3H, s, - OCH_3), 7.04 (1H, s, C-2''H), 6.94 (1H, d, J=8 Hz,C-5''H),7.46 (1H, d, J=8 Hz, C-6''H), 7.59 (1H, d, J=6.5 Hz,C-3'H),7.13 (1H, d, J=7.0 Hz,C-4'H)
P-10	7.29 (1H, s, C-5-H), 5.21 (2H, s, -NH_2), 3.90-3.98 (9H, s,=3X-OCH_3),7.28 (2H, s, C-2''-H and C-6''-H), 7.16 (1H, d, J=7.0 Hz,C-4'-H),7.49 (1H, d, J=6.3 Hz,C-3'-H)
P-11	7.39 (1H, s, C-5-H), 5.18 (2H, s, C-2-NH_2), 8.90 (1H, d, J=2 Hz, C-2''-H), 8.49 (1H, m, C-4''-H), 7.65 (1H, d, J=7.0 Hz, C-5''-H), 8.34 (1H, d, J=8 Hz,C-6''-H), 7.26 (1H, d, J=6 Hz, C-3'-H), 7.16 (1H, d, J=7.0 Hz,C-4'-H)
P-12	7.37 (1H, s, C-5-H), 5.25 (2H, s,C-2-NH_2),8.32 (2H, d, J=8 Hz, C-2'' and 6''-H), 8.19 (2H, d, J=8 Hz, C-3'' and 5''-H),7.51 (1H, d, J=6 Hz, C-3'-H), 7.16 (1H, d, J=7.0 Hz,C-4'-H)
P-13	7.35 (1H, s, C-5-H), 5.21 (2H, s , C-2-NH_2), 9.24 (2H, C-2'' andC-6''H), 8.71 (1H, d, J=8 Hz,C-4''-H), 8.32 (1H, d, J=7.0 Hz,C-5''-H),7.41 (1H, d, J=6 Hz, C-3'-H), 7.15 (1H, d, J=7.0 Hz,C-4'-H)
P-14	7.37 (1H, s, C-5-H), 5.21 (2H, s, C-2-NH_2),7.89 (2 H, d, J=8 Hz, C-2'' and 6''H), 8.75 (2H, d, J=8 Hz, C-3'' and5''-H),7.50 (1H, d, J=6 Hz, C-3'-H), 7.15 (1H, d, J=7.0 Hz,C-4'-H)
P-15	7.37 (1H, s, C-5-H), 5.09 (2H, s, C-2-NH_2),7.46 (2H, d, J=6 Hz, C-3' and 3''-H), 7.13 (2H, d, J=7.0 Hz, C-4' and4''-H), 7.75 (1H, d, J=6 Hz, C- 5''-H)

Preparation of carrageenan suspension:

A 1 % suspension of carrageenan sodium salt was prepared by sprinkling 100 mg of carrageenan powder in 10 mL of saline (0.9% NaCl) solution and set aside to soak for 1 hr. A homogenous suspension was then obtained by thorough mixing with a magnetic stirrer.

Experimental procedure:

Albino rats (M/S Ghosh Enterprises, Calcutta, West Bengal) of either sex, weighing between 200-250 gm were used in the experiment. They were divided into nineteen groups of five animals each for chalcones .All groups were fasted for overnight and allowed water *ad libitum*. The animals were given following treatment.

Inflammation was induced by injecting 0.05 ml of 1 %carrageen an subcutaneously into the subplant ar region of the right hind paw and 0.05ml of saline was injected into the sub plantar region of the left hind paw for all groups. One hour prior to carrageen an injection, the groups III-XIX treated with compounds 1-15 (10mg/kg). 1 % Sodium CMC gel (1 ml/kg), was given to group-I used as carrageenan treated control and the standard drug aceclofenac (2 mg/kg) was administered to group-II. All the doses were administered orally. Anti-inflammatory activity was evaluated by measuring carrageenan induced paw oedema [17].

Measurement of paw thickness:

The thickness of the both paws of each rat, lower and upper surface was measured using Zeitlin's constant load lever method [18]consisting of a graduated micrometer combined with a constant loaded lever system to magnify the small changes in paw thickness during the course of the experiment. The percent increase of paw oedema thickness was determined at 0.5, 1, 2, 3, 4 and 6 hrs after induction of inflammation.

$$\text{Percentage increase in paw thickness} = \frac{Y_t - Y_0}{Y_0} \times 100$$

Y_t = paw thickness at the time 't' hours (After injection)
Y_O = paw thickness at the time '0' hours (Before injection)

The percent increase in paw thickness during 6hrs was determined. The percent inhibition of paw oedema thickness is calculated using the formula

$$\text{Percentage inhibition} = 100 \left[1 - \frac{Y_t}{Y_c}\right]$$

Y_t= Average increase in paw thickness in groups tested with test compounds
Y_C = Average increase in paw thickness in control

RESULTS, DISCUSSION AND SAR

ANTI-INFLAMMATORYACTIVITY

All the pyrimidines tested for anti-inflammatory activity, showed significant percent inhibition in paw thickness at 4th and 6th hour and is more or less comparable to that produced by the standard drug aceclof-enac, but not at an identical dose levels in the compounds were tested at 10 mg/Kg, while the drug tested at 2 mg/ Kg body weight dose levels. Among the pyrimidines tested, P-1, P-4, P-5, P-9, P-10, P-12, P-13, and P-14exhibited highest percent inhibition. All these compounds also possessed more anti-inflammatory activity even at 3rd hour (Table 5).

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Based on the above results, it is evident that electron withdrawing substituents like 4-chloro **(P-1)**, 2,4-dichloro **(P-4)** and 4-nitro **(P-12)** groups when present on the phenyl ring enhanced the anti-inflammatory activity and hence attempts can be made to have these substituents at other positions of the phenyl ring or on the furan ring, either separately or simultaneously, in order to have significant activity.

Table 5: Anti-inflammatory activity of compounds P- 1 to P- 15

Compound code	±% inhibition in paw thickness at various time intervals					
	0.5hr	1hr	2hr	3hr	4hr	6hr
Standard	23 ±1	26 ±1	61 ±2	67±2	96±2	98±2
P-1	19 ±2	28 ±3*	58 ±4**	79 ±3	90 ±3**	94 ±2
P-2	26 ±1	26 ±1	59 ±3*	78 ±4**	84 ±3	89 ±2
P-3	25 ±1	28 ±2*	45 ±1	65 ±2	84 ±4**	87 ±1
P-4	20 ±1	27 ±2	43 ±1	64 ±2	82 ±2*	99 ±2
P-5	22 ±2	34 ±3**	62 ±4***	80 ±3**	90 ±2	97 ±1
P-6	21 ±1	29 ±2*	54 ±4**	79 ±2	89 ±2*	90 ±1
P-7	18 ±1	26 ±3*	57 ±2	70 ±2	87 ±2*	90 ±1
P-8	20 ±1	29 ±2*	54 ±4**	79 ±2	84 ±2*	85 ±1
P-9	19 ±1	29 ±2*	58 ±4**	79 ±2	89 ±2*	93 ±1
P-10	19 ±1	28 ±1	59 ±3*	79 ±3**	90 ±3	94 ±1
P-11	27 ±1	27 ±2*	60 ±1	79 ±2	89 ±4**	89 ±1
P-12	21 ±1	34 ±3*	62 ±4**	80 ±3	89 ±3**	97 ±1
P-13	21 ±2	36 ±3**	60 ±4***	81 ±3**	87 ±2	97 ±1
P-14	21 ±1	30 ±2*	54 ±4**	79 ±2	89 ±2*	93 ±1
P-15	18 ±1	26 ±3*	57 ±2	70 ±2	87 ±2*	90 ±1

Values are expressed as mean SEM(n=5). *p<0.05; **p<0.01; ***p<0.001 compared to controls. Students t-test

The results also revealed the favorable effect of electron releasing substituents like 3,4,-dimethoxyl **(P-9)** and 3,4,5-trimethoxyl**(P-10) (Table 6)**.on anti-inflammatory activity and hence more number of compounds containing different types of electron releasing substituents at different positions of the phenyl ring can be synthesized in order identify compounds with significant anti-inflammatory activity.

Compounds having 9-anthracenyl **(P-5)**, 3-pyridyl **(P-13)** or 4-pyridyl **(P-14)** were also found to possess significant anti-inflammatory activity. This shows that compounds having polycyclic and hetero-aromatic rings in the place of phenyl or substituted phenyl may enhance anti- inflammatory activity.

CYTOTOXICITY STUDIES

The *in vitro* cytotoxicity of the test compounds (**P-1 to P-15**) was evaluated by the MTT assay. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (**1**) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitrochondria where it is reduced to an insoluble, colored (dark purple) formazan product **(2)**. The cells are then solubilized with DMSO and the released, solubilized formazan reagent is measured spectro-photometrically at 570 nm. Since reduction of MTT can only occur in metabolically active cells, the level of

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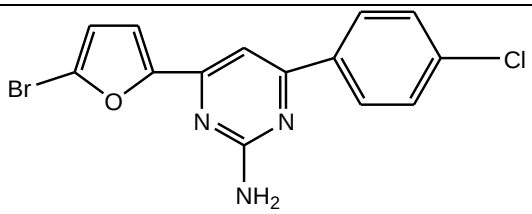
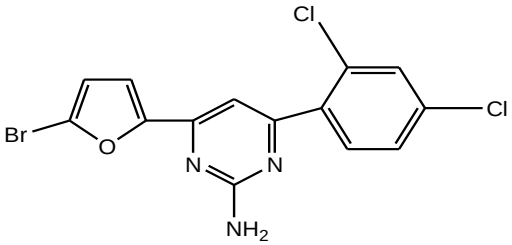
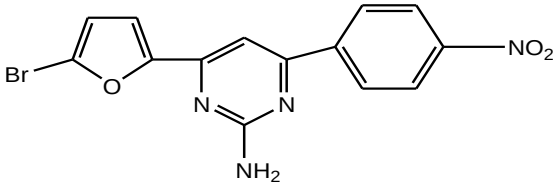
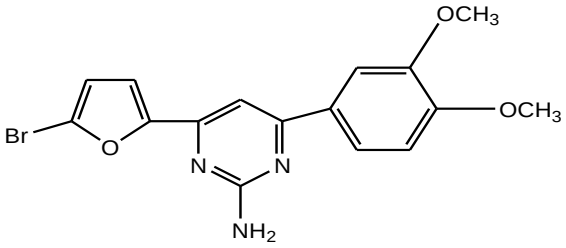
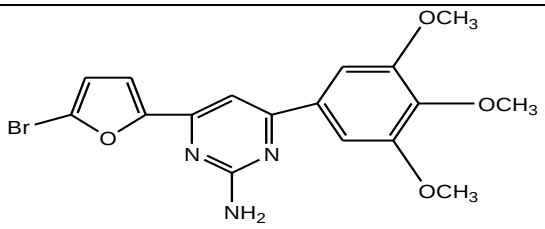
activity is a measure of the viability of the cells. When the amount of dark purple formazan produced by the cells is treated with an agent compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced through the production of a dose-response curve [19-21].

Table 6: Prominent compounds identified with potential anti-inflammatory activity

Compound	Structure	Nature of Substituents
P-1		Electron withdrawing substituents
P-4		
P-12		
P-9		Electron releasing substituents
P-10		

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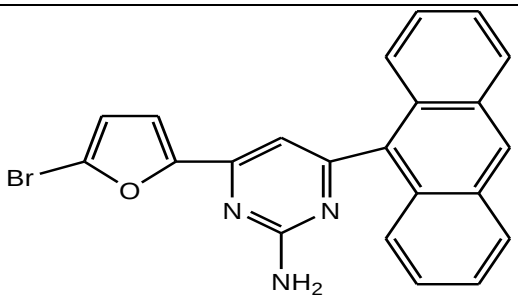
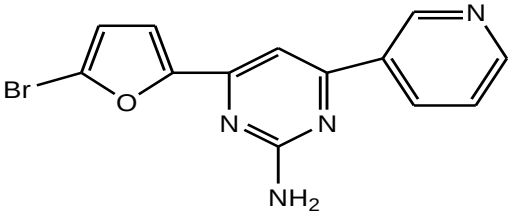
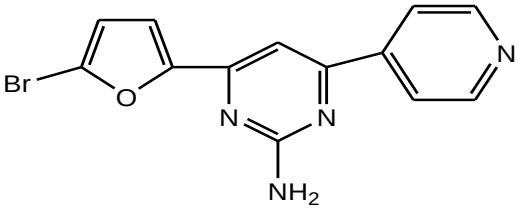
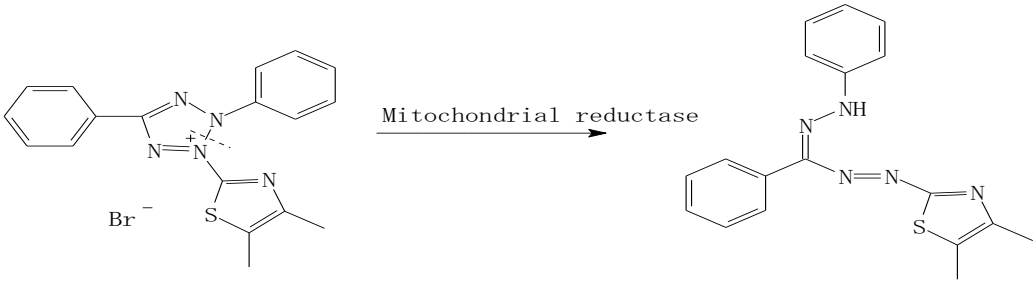
P-5		Unsubstituted polycyclic aromatic substituent
P-13		Unsubstituted heteroaromatic substituents
P-14		



MTT
(1)

Purple colored complex
(2)

Scheme 2.Reduction of MTT:

Materials:

DU-145 (prostate cancer) cell line was obtained from National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagels Medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, EDTA were purchased from Sigma chemicals (St.Louis,MO).Fetal bovine serum (FBS) was purchased from Arrow Labs,96 well flat bottom tissue culture plates were purchased from Tarson.

Method:

Maintainence of cell lines:

DU-145cell line was grown as adherent in DMEM media. The culture was maintained in a humidified atmosphere with 5% CO_2.

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Preparation of samples for cytotoxicity:

Stock solutions of test compounds (**P-1 to P-15)** were prepared (10 mg/mL) in DMSO and from them various dilutions were made with sterile water to get the final drug concentrations of 10, 50, 100 and 200 mg/mL.

Cytotoxicity evaluation:

The cells were seeded in 96 well plates at a density of $1x10^4$ (counted by Tryphan blue exclusion dye method) per well and were incubated for 24 h to recover. After incubation the medium was replaced with fresh media containing different dilutions of the test compounds. Then the plated were incubated for additional 48 h at 37^0C in DMEM/MEM with 10% FBS medium. Following incubation, the medium was removed and replaced with 90 µl of fresh DMEM without FBS. To the above wells, 10 µl of MTT reagent (5 mg/mL of stock solution in DMEM without FBS) was added and incubated at 37^0C for 3-4 h, there after the above media was replaced by adding 200 µl of DMSO to each well (to dissolve the blue formazan crystals) and incubated at 37^0C for 10 min. The absorbance at 570 nm was measured on a spectrophotometer.

Methotrexate was used as reference drug for comparison. Assay was performed in triplicate for three independent determinations. The cytotoxicity was expressed as IC_{50} (µg/mL) which is the concentration of the compound that inhibited proliferation rate of the tumor cells by 50% as compared to the control untreated cells. IC_{50} values were determined from the plot: % inhibition versus concentration.

Table 7: Cytotoxicity of the new pyrimidines derivatives (P-1 to P-15)

S.No.	CompoundCode	DU-145
Standard	**Methotrexate**	5 ± 1
1.	**P-1**	38 ± 2
2.	**P-2**	82 ± 2
3.	**P-3**	174 ± 2
4.	**P-4**	23 ± 2
5.	**P-5**	105 ± 2
6.	**P-6**	74 ± 2
7.	**P-7**	107 ± 2
8.	**P-8**	123 ± 2
9.	**P-9**	75 ± 1
10.	**P-10**	70 ± 1
11.	**P-11**	48 ± 1
12.	**P-12**	43 ± 2
13.	**P-13**	65 ± 2
14.	**P-14**	68 ± 2
15.	**P-15**	61± 2

Data presented as mean ± SD (n=3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1% DMSO. The control cells were treated with culture medium containing 0.1% DMSO.

RESULTS, DISCUSSIONS AND SAR OF CYTOTOXIC ACTIVITY

Among the compounds tested for cytotoxicity on DU-145 cell lines, it is interesting to note that the compound with electron withdrawing substituents like 2,4-chloro **(P-4)** showed maximum activity (IC_{50} 23 µg/mL) which is followed by compounds containing 4-chloro **(P-1)** (IC_{50} 38 µg/mL)**,** 4-nitro **(P-12)** (IC_{50} 43 µg/mL) and 3-nitro **(P-11)** (IC_{50} 48 µg/mL).The compounds with heteroaromatic rings are next in their cytotoxic activity as seen with compound **P-15** containing thienyl moiety (IC_{50} 61 µg/mL), **P-13** with 3-pyridinyl ring (IC_{50} 65 µg/mL) and **P-14** containing 4-pyridinyl ring (IC_{50} 68 µg/mL). Some of the compounds containing electron releasing substituents such as **P-9** containing 3,4,-dimethoxyl substituent (IC_{50} 75 µg/mL) and **P-10** 3,4,5-trimethoxy substituent (IC_{50} 70 µg/mL) also exhibited significant cytotoxic activity.

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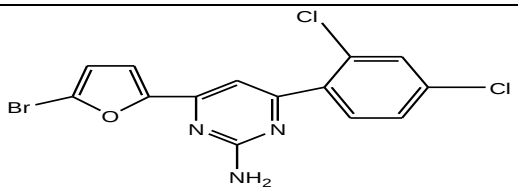
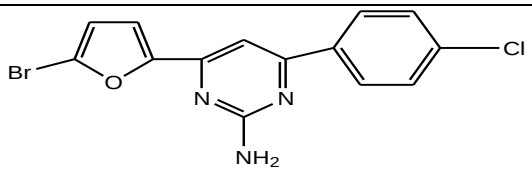
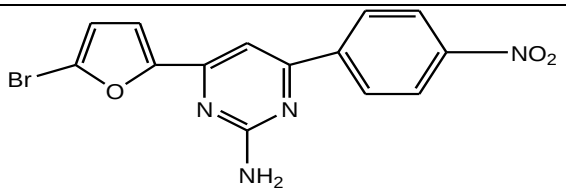
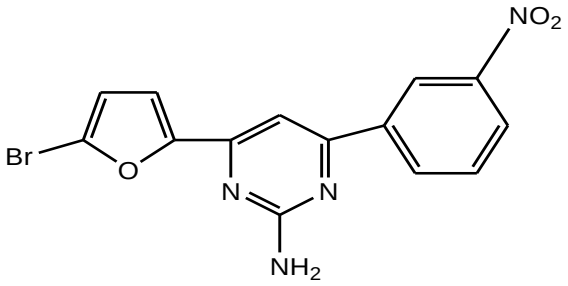
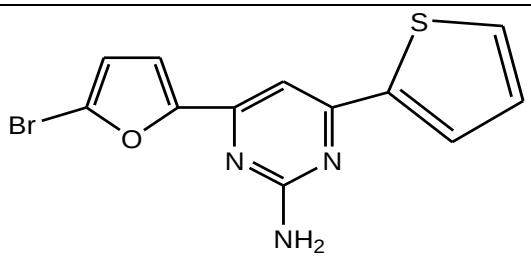
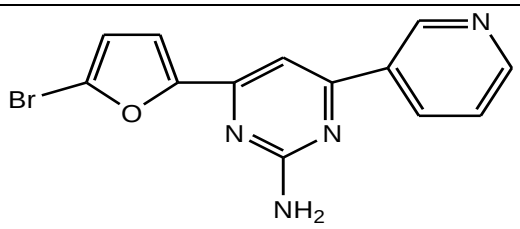
The above results indicate that molecules containing electron withdrawing substituents and heteroaromatic ring are more promising compounds for cytotoxic activity than the compounds containing electron withdrawing substituents. Consequently other compounds containing different types of electron withdrawing substituents at different positions of phenyl ring and heteroaromatic rings can be synthesized to develop more effective cytotoxic agents.

Table 7: Prominent compounds identified with potential cytotoxic activity

Compound	Structure	Nature of Substituents
P-4		Electron withdrawing substituents
P-1		
P-12		
P-11		
P-10		Unsubstituted heteroaromatic substituents
P-13		

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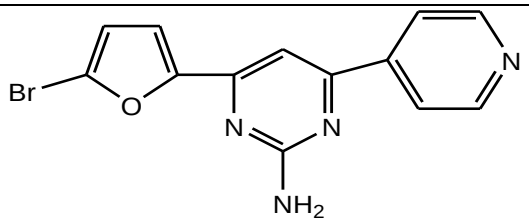
P-14		

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