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SYNTHESIS, ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES OF SOME NEW 2, 4, 6-TRISUBSTITUTED PYRIMIDINES

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ABSTRACT

A variety of 2-amino-4-(substituted phenyl/anthryl)-6-(2'-furyl) pyrimidines **(3a-f)** were synthesized by reacting 1-(2'-furyl)-3- (substituted phenyl/anthryl)-2-propene-1-ones **(2a-f)** with guanidine hydrochloride. The required 1-(2'-furyl)-3- (substituted phenyl/anthryl)-2-propene-1-ones **(2a-f)** were prepared by the condensation of 2-acetyl furan with different aldehydes according to Claisen-Schmidt condensation. All these compounds were characterised by means of their IR, ^{1}H NMR spectroscopic data and microanalyses. The newly synthesized compounds were also evaluated for antimicrobial and anti-inflammatory activities and some of these compounds have shown significant activities.

Key words: Synthesis, Pyrimidines, Antimicrobial activity, Anti-inflammatory activity.

INTRODUCTION

Among a wide variety of heterocycles that have been explored for developing pharmaceutically important molecules such as pyrimidine derivatives have played an important role in medicinal chemistry. They are reported to possess a broad spectrum of biological activities such as antimicrobial[1,2], antiinflammatory[3], anticancer[4], antiviral[5], antitubercular[6] as well as antimalarial[7] properties.

These observations led to the authors to undertake the synthesis of some new 2-amino-4-(substituted phenyl)-6-(2'-furyl) pyrimidines **(3a-f)** and to evaluate their antimicrobial and anti-inflammatory activities.

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EXPERIMENTAL

Chemicals and solvents were reagent grade and used without further purification. Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded in the indicated solvent on Bruker WM 400 MHz spectrometer with TMS as internal standard. Infrared spectra were recorded in KBr on Perkin-Elmer AC-1 spectrophotometer. Microanalyses were performed on Carlo Erba EA-1108 element analyzer and were within the ± 0.4% of the theoretical values. Column chromatography was performed on silica gel (Merck, 60-120 mesh).

General procedure for the preparation of 2,4,6-trisubstitutrd pyrimidine(3a-f):

A mixture of chalcones **(2a-f)** of 2-acetyl furan (1 mmole), guanidine hydrochloride (500 mg) in absolute ethanol (10 mL) and potassium hydroxide (5 mmoles) were refluxed on a water bath for 6 hours. The solvent was completely evaporated and the residue was poured into ice cold water, the precipitated solid was collected by filtration, purified by column chromatography and crystallized from suitable solvent to give pyrimidine derivatives **(3a-f) (Scheme 1)**. The chemical and spectral data of the compounds **(3a-f)** are given in Tables 1 and 2.

Table 1. Physical data of the compounds (3a-f)

Compd.	R	Formula	M.P. (°C)	Yield (%)
(3a)	3,4,5- Trimethoxy phenyl	$C_{17}H_{17}O_4N_3$ (C,H,N)[a]	210	68
(3b)	4-Chloro phenyl	$C_{14}H_{10}ON_3Cl$ (C,H,N)	215	76
(3c)	4-Dimethylamino phenyl	$C_{16}H_{16}ON_4$ (C,H,N)	180	70
(3d)	4-Methyl phenyl	$C_{15}H_{13}ON_3$ (C,H,N)	150	72
(3e)	2,4-Dichloro phenyl	$C_{14}H_9ON_3Cl_2$ (C,H,N)	145	78
(3f)	Anthryl	$C_{22}H_{15}ON_3$ (C,H,N)	240	66

[a] Elemental analyses for C, H, N are within ± 0.4% of the theoretical values.

Table 2. Spectral data of the compounds (3a-f)

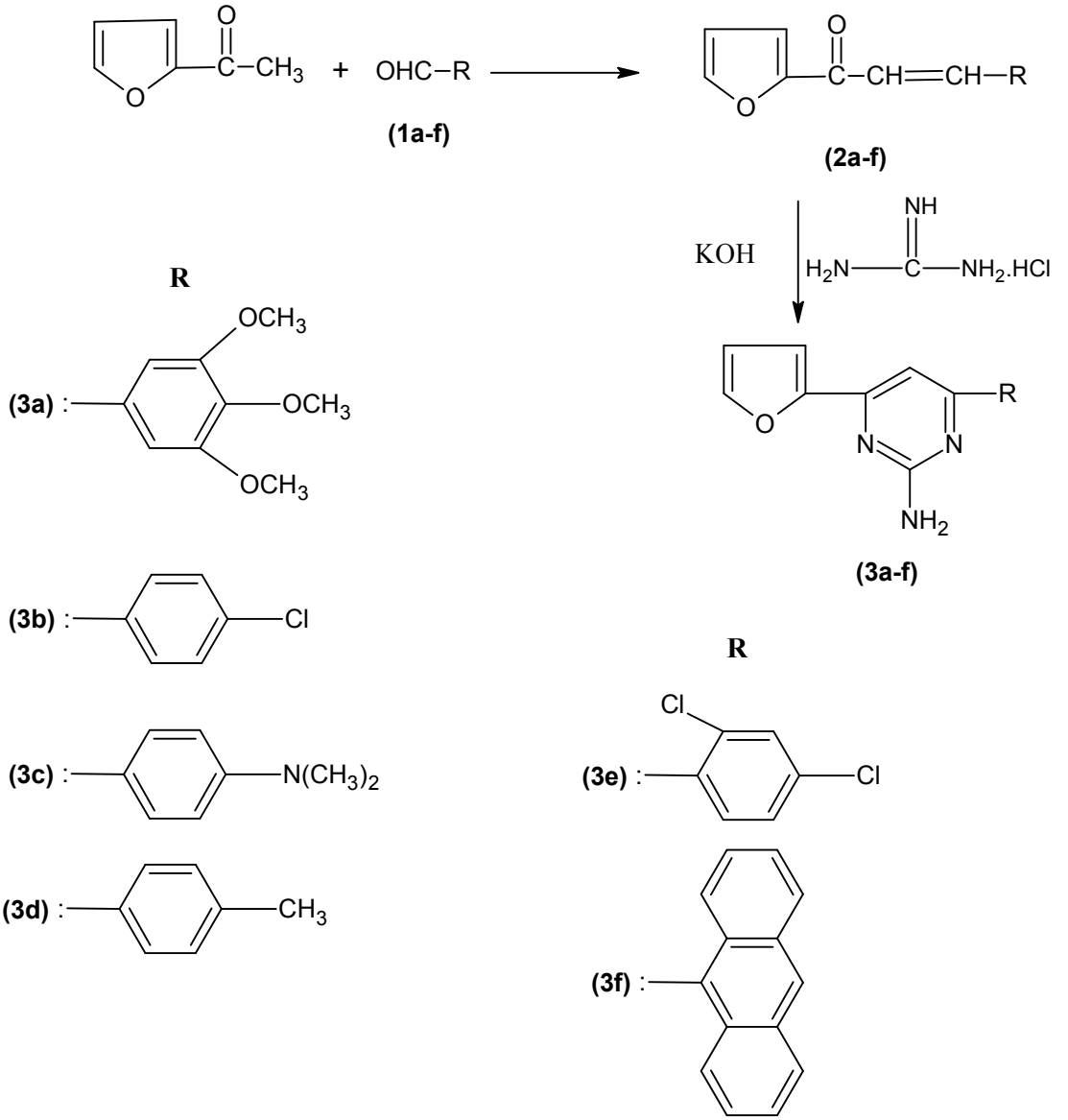
Compd.	IR (KBr, cm^{-1})	^1H NMR ($CDCl_3$, ppm) [a]
(3a)	3338(-NH_2),1628(C=N), 1572(C=C), 1168(-OCH_3)	3.75-4.0 (9H, s, 3X-OCH_3), 5.15 (2H, s, -NH_2), 6.40 (2H, s, C-2''-H,C-6''-H), 6.45-6.60 (1H, m, C-4'-H), 7.00 (1H, s, C-5-H), 7.28 (1H, d, C-3'-H), 7.38 (1H, d, C-5'-H)
(3b)	3346(-NH_2), 1636(C=N), 1578(C=C), 858(C-Cl)	5.45 (2H, s, -NH_2),6.60 (1H, m, C-4'-H), 7.30 (1H, d, C-3'-H), 7.40 (1H, s, C-5-H), 7.48 (2H, d, C-2''-H, C-6''-H), 7.62 (1H, d, C-5'-H), 8.03 (2H, d, C-3''-H, C-5''-H)
(3c)	3332(-NH_2), 1630(C=N), 1570(C=C),1178($N(CH_3)_2$)	3.10 (6H, s, -$N(CH_3)_2$), 5.20 (1H, s, -NH_2), 6.61 (1H, m, C-4'-H), 6.78 (2H, d, C-2''-H, C-6''-H), 7.36 (1H, s, C-5-H), 7.67 (2H, d, C-3'-H, C-5'-H), 8.12 (2H, d, C-3''-H, C-5''-H)
(3d)	3335(-NH_2), 1632(C=N), 1576(C=C)	2.46 (3H, s, Ar-CH_3), 5.25 (2H, s, -NH_2), 6.67 (1H, m, C-4'-H) 7.36 (2H, d, C-2''-H, C-6''-H), 7.45 (1H, s, C-5-H), 7.60 (1H, d, C-3'-H), 7.71 (1H, d, C-5'-H), 8.06 (2H, d, C-3''-H, C-5''-H)
(3e)	3326(-NH_2), 1638(C=N), 1578(C=C), 892(C-Cl)	5.78 (2H, s, -NH_2), 6.62 (1H, m, C-4'-H), 7.35 (1H, s, C-5-H), 7.39 (1H, d, C-3'-H), 7.41 (1H, d, C-6''-H), 7.54 (1H, d, C-5''-H), 7.62 (1H, s, -C-3''-H), 7.64 (1H, d, C-5'-H)
(3f)	3328(-NH_2), 1642(C=N), 1587(C=C)	5.85 (2H, s, -NH_2), 6.61 (1H, m, C-4'-H), 7.22-7.55 (9H, m, Ar-H), 7.60 (1H, s, C-5-H), 7.78 (1H, d, C-3'-H), 8.06 (1H, d, C-5'-H)

[a]s, singlet; d, doublet; m, multiplet

Antimicrobial activity

Cup plate method[8,9] using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of **(3a-f)** against *B. pumilis B. substilis and E. coli* and *P. vulgaris*. The agar medium was purchased from Hi media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subsculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (5 mg) was

dissolved in 5 mL of dimethyl sulfoxide (1000 µg/mL). Volumes of 0.1 mL and 0.2 mL of each compound were used for testing.



Scheme 1. Synthesis of 2, 4, 6-trisubstituted pyrimidines (3a-f)

Same cup plate method using PDA medium was employed to study the preliminary antifungal activity of **(3a-f)** against *A. niger* and *P. crysogenium*. The PDA medium was purchased from Hi media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium and PDA medium was done as per the standard procedure. Each test compound (5 mg) was dissolved in 5 mL of dimethyl sulfoxide (1000 μg/mL). Volumes of 0.1 mL and 0.2 mL of each compound were used for testing.

Table 3. Antibacterial activity of pyrimidine derivatives (3a-f)

Organisms	(3a)		(3b)		(3c)		(3d)		(3e)		(3f)		C	S
	0.1 mL	0.2 mL	0.1 mL	0.2 mL	0.1 mL	0.2 mL	0.1 mL	0.2 mL	0.1 mL	0.2 mL	0.1 mL	0.2 mL		0.1 mL
B. pumilis	13	15	9	11	13	15	9	11	13	15	13	14	--	17
B. substilis	12	14	9	11	12	15	9	10	12	15	12	15	--	18
E. coli	7	9	10	12	13	16	10	12	14	16	13	16	--	20
P. vulgaris	9	11	11	12	14	16	11	12	15	17	14	16	--	19

C: Control (DMSO); S: Standard (Benzyl penicillin)

Table 4. Antifungal activity of pyrimidine derivatives (3a-f)

organisms	(3a)		(3b)		(3c)		(3d)		(3e)		(3f)		C	S
	0.1 mL	0.2 mL	0.1 mL	0.2 mL	0.1 mL	0.2 mL	0.1 mL	0.2 mL	0.1 mL	0.2 mL	0.1 mL	0.2 mL		0.1 mL
A. niger	12	14	--	11	15	18	--	12	--	10	12	13	--	18
Pencillium crysogenium	13	16	--	13	16	19	--	11	--	11	10	13	--	20

C: Control (DMSO); S: Standard (Fluconazole)

The cups each of 9 mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish, which was streaked with the organisms. The solutions of each test compound (0.1 and 0.2 mL) were added separately in the cups and petri dishes were subsequently incubated. Benzyl penicillin and fluconazole were used as standard reference drugs (100 μg/mL) and dimethyl sulphoxide as a control which did not reveal any inhibition. Zone of inhibition produced by each compound was measured in mm and the results are presented in Tables 3 and 4.

Anti-inflammatory activity

The anti-inflammatory activity of pyrimidine derivatives was evaluated by using carrageenan-induced rat paw oedema method[10]. Sprague-Dawley rats of either sex (180–200 g) procured from National Institute of Nutrition, Hyderabad, India were used in the study. The animals were housed under standard environmental conditions (temperature of 22 ± 1 °C with an alternating 12-h light–dark cycle and relative humidity of 60 ± 5%), fed with standard diet and water *ad libitum*.

Rats were divided into eight groups of 5 animals each. The control group I was injected saline solution into the sub-plantar region of the right hind paw and given saline (1 mL/kg). Inflammation was induced by injecting carrageenan (Sigma) (100 μg/rat) subcutaneously into the sub-plantar region of the right hind paw. One hour prior to carrageenan injection, the groups III-VIII treated with compounds **(3a-f)** at the dose level of 100 mg/kg respectively. Saline solution (1 mL/kg) was given to group II used as carrageenan treated control and the standard drug ibuprofen (100 mg/kg) was administered to group VIII rats. All the doses were administered orally. Anti-inflammatory activity was evaluated by measuring carrageenan-induced paw oedema. The thickness of right paw was measured before carrageenan injection and after carrageenan injection at time intervals 1, 2, 3, 4, 5 and 6 h using the Zeitlin's constant loaded lever method[11]. The percent increase in paw oedema thickness was calculated.

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

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 6 hrs was determined. The percent inhibition of paw oedema thickness was 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

Table 5. Anti-inflammatory activity of compounds (3a-f)

Compd.	Dose (mg/kg)	Percent inhibition ± SEM at various time intervals					
		1hr	2hr	3hr	4hr	5hr	6hr
(3a)	100	2.16 ± 1.17	16.75 ± 2.62	27.48 ± 2.07	27.5 ± 2.19	66.89 ± 1.91**	60.56 ± 3.33**
(3b)	100	7.22 ± 1.66	8.61 ± 2.91	10.62 ± 1.97	48.23 ± 1.45*	71.00 ± 0.91***	63.80 ± 1.48**
(3c)	100	4.96 ± 1.75	12.41 ± 0.75	15.49 ± 2.16	52.20 ± 1.01**	72.40 ± 2.21***	60.23 ± 2.91**
(3d)	100	9.77 ± 2.18	15.28 ± 1.58	28.36 ± 1.96	59.78 ± 2.63**	68.59 ± 1.19**	61.51 ± 1.67**
(3e)	100	6.88 ± 3.15	13.94 ± 1.46	20.65 ± 1.23	52.60 ± 2.12**	70.82 ± 1.19***	59.71 ± 1.16**
(3f)	100	6.68 ± 1.78	13.70 ± 1.92	36.84 ± 0.96*	48.78 ± 1.33*	68.11 ± 1.92**	57.01 ± 1.26**
Ibuprofen	100	2.46 ± 1.67	16.75 ± 1.91	36.96 ± 2.06*	59.35 ± 2.16**	89.11 ± 2.11***	67.39 ± 1.17**

Values are expressed as mean ± SEM (n = 5)

*p < 0.05; **p < 0.01; ***p < 0.001 compared to controls. Students's t-test.

RESULTS AND DISCUSSION

From the results, it is evident that the compounds **(3a), (3c), (3e)** and **(3f)** exhibited moderate antibacterial activity, at a concentration of 200 μg/mL (0.2 mL dose level) and is comparable to that of standard drug, benzyl penicillin at a concentration of 100 μg/mL (0.1 mL dose level). The other compounds **(3b)** and **(3d)** also showed antibacterial activity less when compared to other pyrimidine derivatives. Compounds **(3a), (3b), (3d), (3e)** and **(3f)** exhibited moderate antifungal activity and it is comparable to that of fluconazole, whereas compound (3c) exhibited antifungal activity more than that of the other compounds.

The results of anti-inflammatory activity of pyrimidine derivatives revealed that compounds **(3b), (3c)** and **(3e)** (100 mg/kg) exhibited significant anti-inflammatory activity. Maximum inhibitions was observed with the compounds **(3b), (3c)** and **(3e)** are 71.00%, 72.40% and 70.82%, respectively during 5^{th} hour. The standard drug ibuprofen, 100 mg/kg) showed maximum inhibition (89.11%) of paw oedema during 5^{th} hour. The other compounds also showed anti-inflammatory activity, but less when compared to compounds **(3b), (3c)** and **(3e)** Further studies have to be conducted on other models to establish the mechanism of action for these compounds.

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