Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 641-644

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Synthesis and Antimicrobial Activity of Some New 2,4,6-Trisubstituted Pyrimidines

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(*Received*: 26 February 2010; *Accepted*: 24 September 2010) AJC-9128

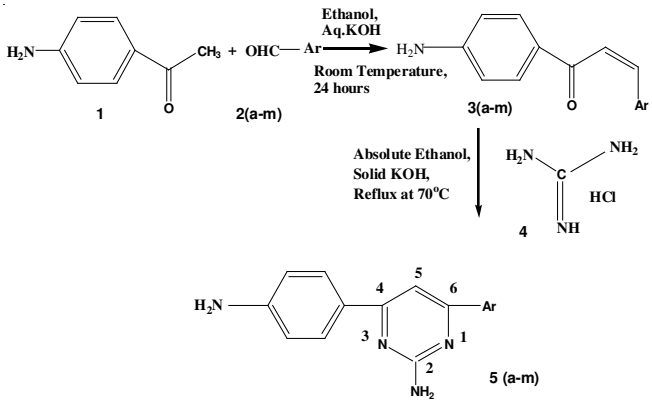
Chalcone derivatives **[3(a-m)]** were prepared by condensing 4-aminoacetophenone with various substituted aromatic and hetero aromatic aldehydes in dilute ethanolic potassium hydroxide solution at room temperature according to Claisen-Schmidt condensation. These chalcones react with guanidine hydrochloride in a basic alcoholic media to give 2,4,6-trisubstituted pyrimidines **[5(a-m)]**. All these pyrimidines were characterized by means of their IR, ^{1}H NMR and elemental analyses and screened for their antimicrobial activity. Some of these compounds showed significant antimicrobial activity.

Key Words: Chalcone, Pyrimidine, Antimicrobial activity.

INTRODUCTION

Chalcones have been very attractive starting materials in medicinal chemistry from the begining. They are easy to prepare with large variability at the two aromatic rings and the enone provides a bifunctional site for 1,3-dinucleophiles affording several heterocyclic ring systems while incorporating other diversity elements[1]. Pyrimidines have been the subject of substantial attention by synthetic and medicinal chemists because of the role of this heteroaromatic ring in many biological activities such as anticancer[2], antiviral[3], antitumor[4], antiinflammatory[5], antimicrobial[6], antifungal[7], antihistaminic[8] and analgesic[9] properties were widely cited.

Folate metabolism has long been recognized as an attractive target for chemotherapy because of its crucial role in the biosynthesis of nucleic acid precursors[10]. Inhibitors of folate-dependent enzymes in cancer, microbial and protozoan cells provide compounds that have found clinical utility as antitumor, antimicrobial and antiprotozoal agents[11]. In view of these reports, the synthesis of a new series of 2,4,6-trisubstituted pyrimidines is now reported. The desired target compounds (**5a-m**) were prepared from the 1,3-diaryl-propenones (**3a-m**)[12,13] and guanidine (**4**) by refluxing them together in a basic alcoholic media (**Scheme-I**). All the synthesized compounds (Table-1) were tested for their antimicrobial activity. Some authors gave different mechanistic suggestions of their experimental findings, where *e.g.*, either hydrogen evolution[14] or hydride ion migration[15] was considered for pyrimidine ring formation.



Scheme-I

TABLE-1
2,4,6-TRISUBSTITUTED PYRIMIDINES

Comp.	Ar	Comp.	Ar
5a	4-chlorophenyl	**5h**	4-methylphenyl
5b	2,4-dichlorophenyl	**5i**	4-dimethylaminophenyl
5c	4-fluorophenyl	**5j**	9-anthracenyl
5d	3-bromophenyl	**5k**	2-pyridinyl
5e	4-methoxyphenyl	**5l**	4-pyridinyl
5f	3,4-dimethoxyphenyl	**5m**	3-pyridinyl
5g	3,4,5-trimethoxyphenyl	–	–

EXPERIMENTAL

Melting points were determined on a standard Boetius apparatus and are uncorrected. The IR spectra were recorded

in Perkin-Elmer BXF1 FT-IR spectrophotometer using KBr disc method. ^{1}H NMR spectra were recorded in the indicated solvent on a Bruker AMX (400 MHz) with tetramethylsilane (TMS) as internal standard. The elemental analyses of the synthesized compounds were recorded on Carlo Erba 1108 elemental analyzer and were within ± 0.4 % of the theoretical values. Analytical TLC was performed on Silica Gel F_{254} plates (Merck) with visualization by UV (254 nm) chamber with protective filters. All the pyrimidines have been purified by column chromatography performed on silica gel (100-200 mesh, Merck).

General procedure for the synthesis of 2,4,6-trisubstituted pyrimidines [5(a-m)][16]: To a reaction vial containing 50 µmol of corresponding chalcone (**3**)[12,13] and 50 µmol of KOH as solid was mixed in 400 µL absolute ethanol. To the reaction mixture was added 200 µL of a 0.25 M solution of guanidine hydrochloride (**4**) in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to reflux at 70 ºC for 2-6 h. Reaction completion was identified by TLC. Upon completion, the reaction mixture were cooled to room temperature and quenched with 100 µL of a 0.5 M solution of HCl in water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vacuuo* to afford the product as a solid. It was purified by column chromatography, using ethyl acetate and hexane mixture as mobile phase obtained pure pyrimidine derivatives. The physical and spectral data of these pyrimidines are shown in Tables 2 and 3, respectively.

Antimicrobial activity: Cup plate method[17,18] using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of pyrimidines **5(a-m)** against 3 gram positive bacteria *viz*., *B. pumilis*, *B. subtilis* and *S. aureus* and 2 gram negative bacteria *viz*., *E. coli* and *P. vulgaris*. The agar medium was purchased from HI-media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water were done as per the standard procedure. Each test compound (5 mg) was dissolved in DMSO (5 mL) (1000 µg/mL). Amikacin and penicillin G were employed as reference standards (1000 µg/mL of each) to compare the results. All the compounds were tested at a concentration of 0.05 mL (50 µg) and 0.1 mL (100 µg) level and DMSO as a control did not show any inhibition.

Same cup plate method using potato dextrose agar (PDA) medium was employed to study the preliminary antifungal activity of these pyrimidines against *A. niger*, *C. albican* and *R. oryzae*. The PDA medium was purchased from HI-media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium and PDA medium were done as per the standard procedure. Each test compound (5 mg) was dissolved in DMSO (5 mL) (1000 µg/mL). Fluconazole employed as reference standard (1000 µg/mL) to compare the results. All the compounds were tested at a concentration of 0.05 mL (50 µg) and 0.1 mL (100 µg) level and DMSO as a control did not show any inhibition.

The cups each of 8 mm diameter were made by scooping out medium with a sterilized cork borer in a Petri dish which was inoculated with the organisms. The solutions of each test compound, control and reference standard(s) (0.05 and 0.1 mL) were added separately in the cups and Petri dishes were subsequently incubated at 37 ± 1 ºC for 24 h for antibacterial activity and kept aside at room temperature for 48 h for antifungal activity. Zone of inhibition produced by each compound was measured in mm and the results are presented in Table-4 for antibacterial and in Table-5 for antifungal activity.

RESULTS AND DISCUSSION

Pyrimidines **5(a-m)**, showed significant antibacterial activity at both 50 and 100 µg concentration level when compared with reference standard amikacin and pencillin G used in the study. Among all the compounds tested, compounds **5a**, **5d**, **5k** and **5l** possessed maximum activity which may be due to the presence of 4-chlorophenyl, 3-bromophenyl, 2-pyridinyl and 4-pyridinyl chemical moiety at C-6 position of pyrimidine nucleus, respectively. In addition, it has been found that compounds **5a**, **5d**, **5e**, **5k**, **5l** and **5m** were effective against *Bacillus pumilis* and *Escherichia coli*.

The results pertaining to the antifungal activity data of pyrimidine derivatives **5(a-m)** revealed that all the compounds in this series have been found to be effective against all fungi at both 50 and 100 µg concentration level when compared with reference standard fluconazole. Compounds **5b**, **5d**, **5h** and **5i** which carries 2,4-dichlorophenyl, 3-bromophenyl, 4-methylphenyl and 4-dimethylaminophenyl substituents at C-6 position of pyrimidine ring exhibited maximum activity.

TABLE-2
PHYSICAL DATA OF COMPOUNDS **[5(a-m)]**

Compound	m.f. (m.w.)	m.p. (ºC)	Yield (%)	Elemental analyses (%) calcd. (found)		
				C	H	N
5a	$C_{16}H_{13}N_4Cl$ (296.5)	297	32.65	64.81 (64.75)	4.41 (4.38)	18.89 (18.88)
5b	$C_{16}H_{12}N_4Cl_2$ (331)	305	15.87	58.05 (58.00)	3.65 (3.62)	16.92 (16.91)
5c	$C_{16}H_{13}N_4F$ (280)	234	18.98	68.63 (68.57)	4.67 (4.64)	20.01 (20.00)
5d	$C_{16}H_{13}N_4Br$ (341)	230	45.53	56.35 (56.30)	3.84 (3.81)	16.42 (16.42)
5e	$C_{17}H_{16}N_4O$ (292)	248	37.64	69.92 (69.86)	5.52 (5.47)	19.18 (19.17)
5f	$C_{18}H_{18}N_4O_2$ (322)	225	31.43	67.14 (67.08)	5.66 (5.59)	17.38 (17.39)
5g	$C_{19}H_{20}N_4O_3$ (352)	215	22.12	64.83 (64.77)	5.72 (5.68)	15.89 (15.90)
5h	$C_{17}H_{16}N_4$ (276)	276	40.97	73.98 (73.91)	5.84 (5.79)	20.29 (20.28)
5i	$C_{18}H_{19}N_5$ (305)	243	48.75	70.88 (70.82)	6.27 (6.22)	22.96 (22.95)
5j	$C_{24}H_{18}N_4$ (362)	296	23.68	79.63 (79.56)	5.01 (4.97)	15.47 (15.46)
5k	$C_{15}H_{13}N_5$ (263)	205	33.56	68.50 (68.44)	4.98 (4.94)	26.63 (26.61)
5l	$C_{15}H_{13}N_5$ (263)	232	37.03	68.50 (68.45)	4.98 (4.94)	26.63 (26.61)
5m	$C_{15}H_{13}N_5$ (263)	260	23.77	68.50 (68.44)	4.98 (4.94)	26.63 (26.61)

TABLE-3
SPECTRAL DATA OF THE PYRIMIDINES **[5(a-m)]**

Comp.	Ar	IR (KBr, ν_{max}, cm^{-1})	^{1}H NMR (δ, ppm) (DMSO-d_6)
5a	4-Chlorophenyl	3415, 3308 (NH_2), 1632 (C=N), 1579 (C=C), 1358 (C-N), 814 (C-Cl)	5.64 (2H, s, C-4'-NH_2), 6.51 (2H, s, C-2-NH_2), 6.65 (2H, d, *J* = 8.4 Hz, C-3' and 5'-H), 7.54 (1H, s, C-5-H), 7.56 (2H, d, *J* = 8.4 Hz, C-2' and C-6'-H), 7.97 (2H, d, *J* = 8.8 Hz, C-2" and 6"-H), 8.22 (2H, d, *J* = 10.0 Hz, C-3" and 5"-H)
5b	2,4-Dichloro phenyl	3445, 3327 (NH_2), 1645 (C=N), 1588 (C=C), 1358 (C-N), 824 (C-Cl)	5.66 (2H, s, C-4'-NH_2), 6.60 (2H, s, C-2-NH_2), 6.62 (2H, d, *J* = 8.8 Hz, C-3' and 5'-H), 7.13 (1H, s, C-5-H), 7.55 (1H, d, *J* = 6.0 Hz, C-6"-H), 7.60 (1H, s, C-3"-H), 7.74 (1H, d, *J* = 7.8 Hz, C-5"-H), 7.85 (2H, d, *J* = 8.2 Hz, C-2' and 6'-H)
5c	4-Fluorophenyl	3405, 3308 (NH_2), 1632 (C=N), 1576 (C=C), 1361 (C-N), 1227 (C-F)	5.63 (2H, s, C-4'-NH_2), 6.48 (2H, s, C-2-NH_2), 6.64 (2H, d, *J* = 8.4 Hz, C-3' and 5'-H), 7.33 (2H, dd, *J* = 9.2 Hz, *J* = 8.8 Hz, C-2" and 6"-H), 7.52 (1H, s,C-5-H), 7.97 (2H, d, *J* = 8.8 Hz, C-2' and 6'-H), 8.24 (2H, dd, *J* = 8.8 Hz, *J* = 8.6 Hz, C-3" and 5"-H)
5d	3-Bromophenyl	3401, 3305 (NH_2), 1635 (C=N), 1575 (C=C), 1362 (C-N), 578 (C-Br)	5.65 (2H, s, C-4'-NH_2), 6.55 (2H, s, C-2-NH_2), 6.65 (2H, d, *J* = 8.4 Hz, C-3' and 5'-H), 7.49-7.45 (1H, t, C-5"-H), 7.58 (1H, s, C-5-H), 7.69 (1H, d, *J* = 8.8 Hz, C-6"-H), 7.99 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H), 8.20 (1H, d, *J* = 8.0 Hz, C-4"-H), 8.41 (1H, s, C-2"-H)
5e	4-Methoxy phenyl	3440, 3309 (NH_2), 1614 (C=N), 1575 (C=C), 1364 (C-N), 1238 (C-O-C)	3.84 (3H, s, C-4"-OCH_3), 5.60 (2H, s, C-4'-NH_2), 6.39 (2H, s, C-2-NH_2), 6.64 (2H, d, *J* = 8.4 Hz, C-3' and 5'-H), 7.04 (2H, d, *J* = 8.8 Hz, C-3" and 5"-H), 7.46 (1H, s, C-5-H), 7.95 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H), 8.15 (2H, d, *J* = 8.8 Hz, C-2" and 6"-H)
5f	3,4-Dimethoxy phenyl	3450, 3362 (NH_2), 1623 (C=N), 1568 (C=C), 1358 (C-N), 1260 (C-O-C)	3.83 (3H, s, C-3"-OCH_3), 3.87 (3H, s, C-4"-OCH_3), 5.61 (2H, s, C-4'-NH_2), 6.39 (2H, s, C-2-NH_2), 6.64 (2H, d, *J* = 8.4 Hz, C-3' and 5'-H), 7.06 (1H, d, *J* = 8.4 Hz, C-5"-H), 7.47 (1H, s, C-5-H), 7.75 (1H, s, C-2"-H), 7.80 (1H, d, *J* = 10.4 Hz, C-6"-H), 7.97 (2H, d, *J* = 8.8 Hz, C-2' and 6'-H)
5g	3,4,5-Trimethoxyl phenyl	3446, 3358 (NH_2), 1655 (C=N), 1583 (C=C), 1328 (C-N), 1263 (C-O-C)	3.78 (3H, s, C-4"-OCH_3), 3.95 (6H, s, C-3" and 5"-OCH_3), 6.77 (2H, d, *J* = 8.8 Hz, C-3' and 5'-H), 7.58 (2H, s, C-2" and 6"-H), 7.78 (1H, s, C-5-H), 8.19 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H), 8.49 (2H, s, C-2-NH_2)
5h	4-Methyl phenyl	3443, 3337 (NH_2), 1575 (C=N), 1522 (C=C), 1359 (C-N)	2.38 (3H, s, C-4"-CH_3), 5.61 (2H, s, C-4'-NH_2), 6.42 (2H, s, C-2-NH_2), 6.64 (2H, d, *J* = 8.8 Hz, C-3' and 5'-H), 7.31 (2H, d, *J* = 8.0 Hz, C-3" and 5"-H), 7.48 (1H, s, C-5-H), 7.95 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H), 8.08 (2H, d, *J* = 8.4 Hz, C-2" and 6"-H)
5i	4-Dimethyl-amino phenyl	3444, 3335 (NH_2), 1614 (C=N), 1567 (C=C), 1366 (C-N)	2.99 {6H, s, C-4"-N(CH_3)$_2$}, 5.56 (2H, s, C-4'-NH_2), 6.27 (2H, s, C-2-NH_2), 6.64 (2H, d, *J* = 8.4 Hz, C-3' and 5'-H), 6.78 (2H, d, *J* = 8.8 Hz, C-3" and 5"-H), 7.38 (1H, s, C-5-H), 7.93 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H), 8.05 (2H, d, *J* = 8.8 Hz, C-2" and 6"-H)
5j	9-Anthracenyl	3444, 3357 (NH_2), 1615 (C=N), 1576 (C=C), 1375 (C-N)	5.65 (2H, s, C-4'-NH_2), 6.60 (2H, s, C-2-NH_2), 6.63 (2H, d, *J* = 7.6 Hz, C-3' and 5'-H), 7.14 (1H, s, C-10"-H), 7.56-7.45 (4H, m, anthracenyl-H), 7.74 (2H, d, *J* = 9.2 Hz, anthracenyl-H), 7.92 (2H, d, *J* = 8.8 Hz, C-2' and 6'-H), 8.15 (2H, d, *J* = 8.4 Hz, anthracenyl-H), 8.69 (1H, s, C-5-H)
5k	2-Pyridinyl	3338 (NH_2), 1646 (C=N), 1567 (C=C), 1363 (C-N)	5.66 (2H, s, C-4'-NH_2), 6.55 (2H, s, C-2-NH_2), 6.66 (2H, d, *J* = 8.8 Hz, C-3' and 5'-H), 7.53-7.50 (1H, m, C-5"-H), 7.89 (2H, d, *J* = 8.6 Hz, C-2' and 6'-H), 7.90 (1H, s, C-5-H), 7.99-7.95 (1H, m, C-3"-H), 8.33 (1H, d, *J* = 7.6 Hz, C-4"-H), 8.73 (1H, d, *J* = 4 Hz, C-6"-H)
5l	4-Pyridinyl	3442, 3355 (NH_2), 1575 (C=N), 1526 (C=C), 1365 (C-N)	6.18 (2H, s, C-4'-NH_2), 7.06 (2H, d, *J* = 8.8 Hz, C-3' and 5'-H), 7.21 (2H, s, C-2-NH_2), 7.38 (1H, s, C-5-H), 8.16 (2H, d, *J* = 6.0 Hz, C-3" and 5"-H), 8.35 (2H, d, *J* = 8.8 Hz, C-2' and 6'-H), 8.90 (2H, d, *J* = 6.0 Hz, C-2" and 6"-H)
5m	3-Pyridinyl	3332, 3207 (NH_2), 1645 (C=N), 1566 (C=C), 1359 (C-N)	6.16 (2H, s, C-4'-NH_2), 7.06 (2H, d, *J* = 8.4 Hz, C-3' and 5'-H), 7.20 (2H, s, C-2-NH_2), 7.34 (1H, s, C-5-H), 7.37-7.35, 8.53-8.50, 8.81-8.79 (3H, m, pyridinyl-H), 8.35 (2H, d, *J* = 8.8 Hz, C-2' and 6'-H), 9.72 (1H, s, C-2"-H)

TABLE-4
ANTIBACTERIAL ACTIVITY OF 2,4,6-TRISUBSTITUTED PYRIMIDINES, **5(a-m)**

Compound	Ar	Zone of inhibition (mm) Quantity (μg)									
		B. pumilis		*B. subtilis*		*S. aureus*		*E. coli*		*P. vulgaris*	
		50	100	50	100	50	100	50	100	50	100
5a	4-Chlorophenyl	16	21	17	21	17	19	20	21	16	16
5b	2,4-Dichlorophenyl	16	20	18	21	17	17	18	21	16	16
5c	4-Fluorophenyl	16	19	16	19	16	16	19	22	17	17
5d	3-Bromophenyl	17	20	18	21	16	17	19	21	18	20
5e	4-Methoxyphenyl	15	19	17	20	17	19	19	23	16	18
5f	3,4-Dimethoxy phenyl	12	14	12	12	15	15	17	18	14	14
5g	3,4,5-Trimethoxy phenyl	14	16	14	14	17	17	18	21	15	15
5h	4-Methylphenyl	15	19	17	18	17	18	18	20	14	16
5i	4-Dimethylamino phenyl	16	18	16	16	17	17	18	20	12	12
5j	9-Anthracenyl	16	18	17	18	17	18	17	20	14	14
5k	2-Pyridinyl	16	20	15	18	16	16	19	20	16	18
5l	4-Pyridinyl	22	26	16	20	17	19	21	24	20	24
5m	3-Pyridinyl	14	16	15	16	17	17	21	24	21	23
	Amikacin	28	33	31	33	24	26	25	28	28	32
	Pencillin G	11	11	8	8	8	8	7	8	7	8

TABLE-5
ANTIFUNGAL ACTIVITY OF 2,4,6-TRISUBSTITUTED PYRIMIDINES, **5(a-m)**

Compound	Ar	Zone of inhibition (mm), Quantity (µg)					
		A. niger		*C. albicans*		*R. oryzae*	
		50	100	50	100	50	100
5a	4-Chlorophenyl	14	18	17	17	15	19
5b	2,4-Dichlorophenyl	13	15	13	15	12	18
5c	4-Fluorophenyl	17	23	20	24	22	25
5d	3-Bromophenyl	18	22	18	21	18	21
5e	4-Methoxyphenyl	12	16	13	19	14	18
5f	3,4-Dimethoxyphenyl	10	13	9	12	8	13
5g	3,4,5-Trimethoxyphenyl	10	14	12	14	11	14
5h	4-Methylphenyl	16	20	18	21	20	23
5i	4-Dimethylaminophenyl	20	23	18	20	20	22
5j	9-Anthracenyl	11	13	11	14	11	13
5k	2-Pyridinyl	12	16	14	17	12	18
5l	4-Pyridinyl	11	18	12	15	12	18
5m	3-Pyridinyl	12	16	13	18	13	18
	Fluconazole	25	28	24	29	22	28

It is interestingly to note that compound **5d** carrying bromine at 3-position on the aromatic ring which is attached at C-6 position of pyrimidine ring exhibited maximum activity among the all compounds tested for antimicrobial activity.

Conclusion

The structure activity relationship suggested that pyrimidines containing electron releasing groups like amino, methyl, dimethylamino and halogens like chlorine and bromine *etc*., showed higher antibacterial and antifungal activity than the corresponding chalcones because pyrimidines directly inhibits folate metabolism which plays a crucial role in the biosynthesis of nucleic acid precursors in cancer, microbial and protozoan cells. These findings encourage us to explore other molecules by introducing these potent moieties into other fused heterocycles such as pyridopyrimidines and tetrahydro pyrimidines. Our prediction is that these compounds with new ring systems may show even better antimicrobial activities.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. T. Prabhakar, Department of Biotechnology, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, for his cooperation in the screening of antimicrobial activity.

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