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Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW 2,4,6-TRISUBSTITUTED PYRIMIDINES

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ABSTRACT

A variety of 2-amino-4-(substituted)-6-(3″-thienyl) pyrimidines (3a-o) were synthesized by reacting various chalcones with guanidine hydrochloride. The required chalcones were prepared by condensation of 3-acetylthiophene with various substituted aromatic / hetero-aromatic aldehydes in the presence of alkali. All these compounds were characterized by IR, ^{1}H NMR and elemental analyses. The newly synthesized compounds were evaluated for their antimicrobial activity and some of them have shown significant activity when compared with the standard.

Keywords: 2-amino-4-(substituted)-6-(3″-thienyl) pyrimidines, Antimicrobial activity.

INTRODUCTION

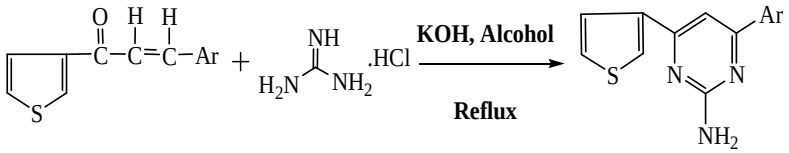
Among a wide variety of heterocycles that have been explored for developing medicinally important molecules, pyrimidine derivatives occupy an important place in the present day therapeutics. They were reported to possess a broad spectrum of biological activities such as antimicrobial[1,2], anti-inflammatory[3], anticancer[4], antiviral[5], antitubercular[6]and antimalarial[7] properties. These observations prompted the authors to carryout the synthesis of some new 2-amino-4-(substituted)-6-(3″-thienyl) pyrimidines (3a-o) which were also evaluated for their antimicrobial activity.

EXPERIMENTAL

Chemicals and solvents were of 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. IR 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 PYRIMIDINES

A mixture of chalcones (3a-o) of 3-acetylthiophene (1 mmole), guanidine hydrochloride (500 mg) in absolute ethanol (10 mL) and potassium hydroxide (5 mmole) were refluxed on a water bath for 6 h. 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-o) (Scheme 1). The chemical and spectral data of the compounds (3a-o) are given in Tables 1 and 2.



1 2 3a-o

Scheme 1

Where **Ar**

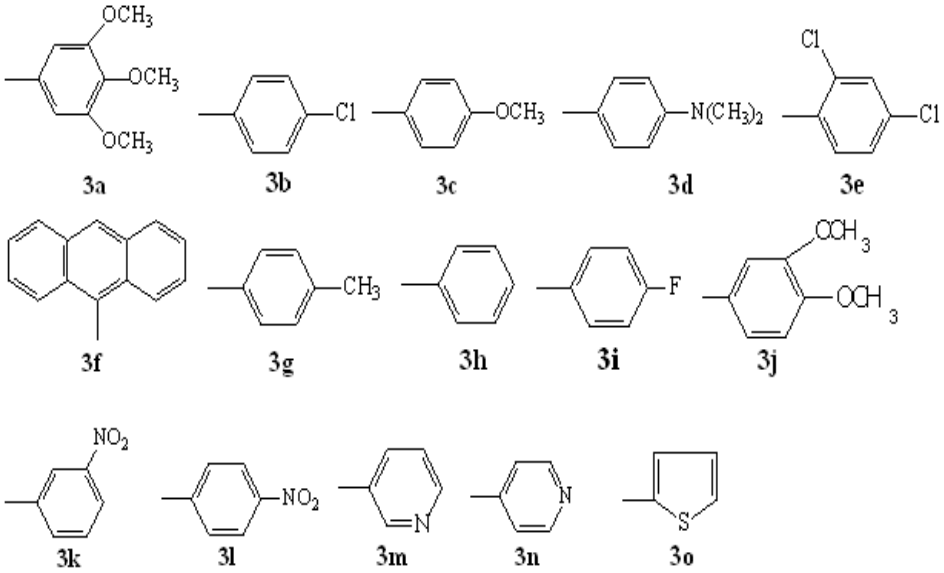


Table 1: Physical characterization data of the compounds (3a-o)

Compound	Ar	Molecular formula	M.p (°C)	Yield (%)
3a	3',4',5'-Trimethoxyphenyl	$C_{17}H_{17}N_3O_3S$ (C,H,N) [a]	150	72
3b	4'-chlorophenyl	$C_{14}H_{10}$ Cl N_3 S (C,H,N) [a]	132	81
3c	4'-methoxyphenyl	$C_{15}H_{13}OSN_3$ (C,H,N) [a]	160	78
3d	4'-dimethylaminophenyl	$C_{16}H_{16}N_4S$ (C,H,N) [a]	120	76
3e	2',4'-dichlorophenyl	$C_{14}H_9Cl_2N_3$ S (C,H,N) [a]	220	83
3f	9'-anthracenyl	$C_{22}H_{15}N_3S$ (C,H,N) [a]	155	79
3g	4'-methylphenyl	$C_{15}H_{13}N_3$ S (C,H,N) [a]	95	77
3h	Phenyl	$C_{14}H_{11}N_3S$ (C,H,N) [a]	115	86
3i	4'-fluorophenyl	$C_{14}H_{10}FN_3S$ (C,H,N) [a]	109	72
3j	3',4'-dimethoxyphenyl	$C_{16}H_{15}N_3O_2S$ (C,H,N) [a]	143	82
3k	3'-nitrophenyl	$C_{14}H_{10}N_4O_2S$ (C,H,N) [a]	181	62
3l	4'-nitrophenyl	$C_{14}H_{10}N_4O_2S$ (C,H,N) [a]	232	76
3m	3'-pyridinyl	$C_{13}H_{10}N_4S$ (C,H,N) [a]	172	66
3n	4'-pyridinyl	$C_{13}H_{10}N_4S$ (C,H,N) [a]	198	64
3o	2'-thienyl	$C_{12}H_9N_3S_2$(C,H,N) [a]	202	90

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

Table 2: Spectral data of the compounds (3a-o)

Compound	IR (KBr, cm^{-1})	^1H NMR ($CDCl_3$, ppm)*
3a	3361(NH_2), 1602(C=N), 1572 (C=C), 1120 (-O-CH_3) and 704 (C-S)	3.91-3.99 (9H, s, 3 X Ar-OCH_3), 5.22 (2H, s, -NH_2), 7.17 (1H, dd, Ar-H), 7.29 (2H, s, Ar-H), 7.30 (1H, s, Ar-H), 7.50 (1H, d, J=6.3 Hz, Ar-H) and 7.80 (1H, d, J=6.3 Hz, Ar-H)
3b	3342 (NH_2), 1628 (C=N), 1580 (C=C), 856 (C-Cl) and 652 (C-S)	5.35 (2H, s, -NH_2), 7.16 (1H, dd, Ar-H), 7.31 (1H, s, Ar-H), 7.45 (2H, d, J=7Hz, Ar-H), 7.51 (1H, d, J=6.5Hz, Ar-H), 7.79 (1H, d, J=6.5Hz, Ar-H) and 7.99 (2H, d, J=7Hz, Ar-H)
3c	3345 (NH_2), 1625 (C=N), 1590 (C=C), 1165 (OCH_3) and 646 (C-S)	3.89 (3H, s, -OCH_3), 4.90 (1H, s, -NH_2), 7.03 (2H, d, J=7Hz, Ar-H), 7.19 (1H, dd, Ar-H), 7.59 (1H, d, J=6.5Hz, Ar-H), 7.69 (1H, s, Ar-H), 7.89 (1H, d, J=6.5Hz, Ar-H) and 8.01 (2H, d, J=7Hz, Ar-H)
3d	3338 (NH_2), 1633 (C=N), 1588 (C=C), 1185 ($N(CH_3)_2$) and 660 (C-S)	3.10 (6H, s, -$N(CH_3)_2$), 5.40 (2H, s, -NH_2), 6.75 (2H, d, Ar-H), 7.15 (1H, dd, Ar-H), 7.28 (1H, s, Ar-H), 7.48(1H, d, J=6.5Hz, Ar-H), 7.79 (1H, d, J=6.5Hz, Ar-H) and 8.01 (2H, d, Ar-H)
3e	3348 (NH_2), 1635 (C=N), 1582 (C=C), 850 (C-Cl) and 676 (C-S)	5.48 (2H, s, -NH_2), 7.08 (1H, s, Ar-H), 7.12 (1H, dd, Ar-H), 7.69 (1H, d, J=6.5Hz, Ar-H), 7.81(1H, d, J=6.5Hz, Ar-H), 8.08 (1H, d, J=7Hz, Ar-H), 8.56 (1H, d, J=2Hz, Ar-H) and 8.78 (1H, d, J=7Hz, Ar-H)
3f	3356 (NH_2), 1636 (C=N), 1582 (C=C) and 676 (C-S)	5.70 (2H, s, -NH_2), 7.15-7.60 (9H, m, Ar-H), 7.18 (1H, dd, Ar-H), 7.28 (1H, s, Ar-H), 7.63 (1H, d, J=6.5Hz, Ar-H) and 7.82 (1H, d, J=6.5Hz, Ar-H).
3g	3350 (NH_2), 1630 (C=N), 1580 (C=C) and 662 (C-S)	2.15 (3H, s, -CH_3), 5.35 (2H, s, -NH_2), 7.15 (1H, dd, Ar-H), 7.32 (1H, s, Ar-H), 7.45 (2H, d, J=7Hz, Ar-H), 7.51 (1H, d, J=6.5Hz, Ar-H), 7.99 (2H, d, J=7Hz, Ar-H) and 7.80 (1H, d, J=6.5Hz, Ar-H)
3h	3340 (NH_2), 1630 (C=N), 1575 (C=C) and 682 (C-S)	5.36 (2H, s, -NH_2), 7.14 (1H, dd, Ar-H), 7.35 (1H, s, Ar-H), 7.45-7.53 (3H, m, Ar-H), 7.63 (2H, m, Ar-H), 8.02 (1H, d, J=6.5Hz, Ar-H) and 8.05 (1H, d, J=6.5Hz, Ar-H)
3i	3335 (NH_2), 1630 (C=N), 1575 (C=C), 1120 (C-F) and 655 (C-S)	5.18 (2H, s, -NH_2), 7.15 (1H, dd, Ar-H), 7.31 (1H, s, Ar-H), 7.48 (2H, d, J=7Hz, Ar-H), 7.63 (2H, d, J=7Hz, Ar-H), 7.75 (1H, d, J=6.3Hz, Ar-H) and 8.02 (1H, d, J=6.3Hz, Ar-H)
3j	3414 (NH_2), 1641 (C=N), 1519 (C=C), 1145 (-O-CH_3) and 735 (C-S)	3.94 (3H, s, -OCH_3), 3.99 (3H, s, -OCH_3), 5.23 (2H, s, - NH_2), 6.95 (1H, d, J=8Hz, Ar-H), 7.05 (1H, s, Ar-H), 7.14 (1H, dd, Ar-H), 7.31 (1H, s, Ar-H), 7.47 (1H, d, J=8Hz, Ar-H), 7.60 (1H, d, J=6.5Hz, Ar-H) and 7.68 (1H, d, J=6.5Hz, Ar-H)
3k	3335(NH_2), 1635 (C=N), 1575 (C=O), 1510 (N=O, asymmetric) and 1330 (N=O, symmetric)	5.19 (2H, s, -NH_2), 7.17 (1H, dd, Ar-H), 7.27 (1H, d, J=6Hz, Ar-H), 7.40 (1H, s, Ar-H), 7.52 (1H, d, J=6Hz, Ar-H), 7.66 (1H, dd, Ar-H), 8.35 (1H, d, J=8Hz, Ar-H), 8.5 (1H, m, Ar-H) and 8.91 (1H, d, J=2Hz, Ar-H)
3l	3413 (NH_2), 1512 (N=O, asymmetric), 1335 (N=O, symmetric) and 1605 (C=N), 688 (C-S)	5.26 (2H, s, -NH_2), 7.17 (1H,dd, Ar-H), 7.38 (1H, s, Ar-H), 7.52 (1H, d, J=6Hz, Ar-H), 7.81 (1H, d, J=6Hz, Ar-H), 8.20 (2H, d, J=8Hz, Ar-H) and 8.33 (2H, d, J=8Hz, Ar-H)
3m	3335 (NH_2), 1635 (C=N), 1570 (C=C) and 680 (C-S)	5.22 (2H, s , -NH_2), 7.16 (1H, dd, Ar-H), 7.36 (1H, s, Ar-H), 7.42 (1H, d, J=6Hz, Ar-H), 7.50 (1H, d, J=6Hz, Ar-H), 8.33 (1H, dd, Ar-H), 8.72 (1H, d, J=8Hz,Ar-H) and 9.25 (2H, d, Ar-H)
3n	3335 (NH_2), 1635 (C=N), 1570 (C=C) and 680 (C-S)	5.22 (2H, s, -NH_2), 7.16 (1H, dd, Ar-H), 7.38 (1H, s, Ar-H), 7.51 (1H, d, J=6Hz, Ar-H), 7.80 (1H, d, J=6Hz, Ar-H), 7.90 (2 H, d, J=8Hz, Ar-H) and 8.76 (2H, d, J=8Hz, Ar-H)
3o	3335 (NH_2), 1635 (C=N), 1570 (C=C) and 680 (C-S)	5.10 (1H, s, -NH_2), 7.14 (2H, dd, Ar-H), 7.38 (1H, s, Ar-H), 7.47 (2H, d, J=6Hz, Ar-H) and 7.76 (2H, d, J=6Hz, Ar-H)
* s, singlet; dd, double doublet; 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-o) against *B. pumilis*, *B. subtilis*, *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 waster was done as per the standard procedure. Each test compound (5mg) was dissolved in 5 mL of dimethyl sulfoxide (1000 µg/mL). Volumes of 0.05 mL and 0.1 mL of each compound were used for testing.

Same cup plate method using PDA medium was employed to study the preliminary antifungal activity of (3a-o) 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 0.05 mL (50 µg) and 0.1 mL (100 µg) of each compound were used for testing.

The cups each of 9 mm diameter were made by scooping out the medium with a sterilized cork borer in a petri dish, which was streaked with the organisms. The solutions of each test compound (0.05 mL and 0.1 mL) were added separately in the cups and petri dishes were subsequently incubated. Benzyl penicillin and Fluconazole were used as standard (reference) drugs and dimethyl sulfoxide 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.

Table 3: Antibacterial activity of pyrimidine derivatives (3a-o)

Compound		Zone of inhibition (in mm)			
		B.subtilis	***B.pumilis***	***E.coli***	***P.vulgaris***
3a	A	13	14	11	11
	B	20	15	20	15
3b	A	14	13	14	13
	B	18	13	16	17
3c	A	10	11	10	12
	B	19	12	13	18
3d	A	15	12	11	12
	B	20	14	20	18
3e	A	16	13	12	15
	B	20	15	21	21
3f	A	15	12	13	12
	B	16	14	20	20
3g	A	14	14	11	14
	B	19	13	20	19
3h	A	15	13	12	12
	B	17	14	20	18
3i	A	17	14	13	14
	B	20	15	21	20
3j	A	14	14	13	14
	B	20	13	19	20
3k	A	13	13	11	11
	B	20	15	18	17
3l	A	13	13	14	13
	B	17	13	17	18
3m	A	12	11	10	12
	B	19	12	13	18
3n	A	11	10	9	12
	B	18	11	13	18
3o	A	11	10	10	12
	B	17	10	13	17
C		-	-	-	-
S (0.1 mL)	A	25	29	26	28
	B	30	31	29	31

A: 0.05 mL (50µg); B: 0.1 mL (100µg);
C: Control (DMSO); S: Standard (Benzyl penicillin)

Table 4: Antlfungal activity of pyrimidine derivatives (3a-o)

Compound		Zone of inhibition (in mm)	
		A.niger	*P.crysogenium*
3a	A	10	11
	B	13	13
3b	A	11	14
	B	13	16
3c	A	10	10
	B	11	15
3d	A	11	12
	B	13	14
3e	A	16	15
	B	18	18
3f	A	12	11
	B	14	13
3g	A	10	12
	B	12	16
3h	A	10	11
	B	13	13
3i	A	15	14
	B	17	17
3j	A	10	10
	B	11	15
3k	A	11	12
	B	13	14
3l	A	13	13
	B	14	14
3m	A	12	11
	B	14	13
3n	A	13	13
	B	13	11
3o	A	12	12
	B	14	14
C		-	-
S (0.1 mL)	A	23	24
	B	28	27

A: 0.05 mL (50µg); B: 0.1 mL (100µg);
C: Control (DMSO); S: Standard (Fluconazole)

RESULTS AND DISCUSSION

From the above results, it is evident that compounds **3a** to **3o** showed antibacterial activity at both 50µg and 100µg dose levels, but less than that of the benzyl penicillin used as standard. Among the compounds tested, **3e**, **3i** and **3d** were found to be more potent against all the organisms tested. Compounds **3b**, **3f**, **3j**, **3k** and **3l** exhibited similar degree of antibacterial activity. Other compounds, although showed zones of inhibition, not much potent when compared to the above compounds. Structure-Activity-Relationship studies (SAR) based on the above results indicate that the compound having fluorine substituent on the phenyl ring at position 4 (**3i**) enhanced the activity. This reveals the importance of synthesis of compounds having fluorine at different positions of the phenyl ring or synthesis of the compounds with more than one fluorine group on the phenyl ring and such compounds are likely to possess significant antibacterial activity. Compounds having other halogens at different positions on the phenyl ring can also be synthesized and screened for better antibacterial activity, since compound **3e** having dichlorophenyl moiety showed more antibacterial activity. **3d** having dimethylamino group on the phenyl ring at position 4 also found to be potent and this is consistent with the several observations reported earlier in literature and now calls for synthesis of such compounds having more than one such group on the phenyl ring in order to improve the potency further. The results also indicate that the introductions of groups like nitro and methoxy at different positions of the phenyl ring contribute favorably to the antibacterial activity.

Among the compounds tested for antifungal activity, **3e** exhibited the highest activity followed by **3i** and **3l**. In general, many of these compounds have not showed much antifungal activity when compared to fluconazole used as the standard. From the results it is evident that the compounds having

halogens like chlorine (**3e**) and fluorine (**3i**) found to be more potent and hence compounds having number of these substituents on different positions of the phenyl ring can be synthesized in order to improve the activity further. Since, no studies in the present case were carried out with bromine as a substituent, such compounds having bromine at one or more positions of the phenyl ring can be synthesized to enhance the antifungal activity. Introduction of nitro group also contributed favorably to the antifungal activity (**3l**) and hence compounds with more nitro groups at different positions of the phenyl ring, if synthesized, may exhibit significant antifungal activity. Attempts can also be made to have the above substituents on the thiophene ring also in order to have a cumulative positive effect on the antifungal activity of the pyrimidines.

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