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Synthesis and bioassay studies of 7-substituted pyrido[2,3-d]pyrimidines

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Abstract

4-Aminopyrimidine-5-carbaldehyde **1** underwent facile condensation with various aromatic ketone derivatives in the presence of K_2CO_3 and small catalytic amount of KI in acetone was afforded corresponding 7-Substituted pyrido[2,3-d]pyrimidine derivatives **3a-j.** Their chemical structures were characterized using IR, H^1 NMR and Mass spectral studies. All the above compounds were screened for anti-microbial activity, anti-oxidant activity and their bioassay showed them to possess significant antimicrobial activity and anti-oxidant activity.

Keywords: 7-Substituted pyrido[2,3-d]pyrimidine, spectral analysis, anti-microbial activity, anti oxidant.

Introduction

For small organic molecules, simple nitrogen-containing heterocycles receive a large amount of attention in the literature, of these heterocycles, the synthesis, reactions and biological activities of pyridine containing molecules stands as an ever expanding area of research in hetero aromatic chemistry. pyrido[2,3-*d*]pyrimidine heterocycles have received much less attention in the literature, in spite of their structural relationship to pyridines. Interest in pyrido[2,3-*d*]pyrimidine derivatives has increased dramatically in recent years, based upon a diverse range of biological properties[1-4] as antitumour[5-8], antibacterial[9-11], anti-inflammatory[12], insecticidal agents[13], diuretics properties and activity against platelet aggregation[14]. To continue our interest in the synthesis of simple nitrogen-containing heterocycles[15-16]. We set out to develop a new method for the synthesis of highly-functionalised, pyrido [2,3-*d*] pyrimidine heterocycles. Central to our approach was the need to develop a novel method, using readily available starting materials and simple experimental procedures and very good yields. This paper describes a new

and highly efficient method for the preparation of pyrido[2,3-*d*]pyrimidines that exhibits all of these features.

Materials and Methods

Experimental Section

The IR spectra were recorded in KBr discs (υ_{max} in cm^{-1}) on Perkin-Elmer FT-IR spectrophotometer. The ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker Avance DPX 300 instrument. Mass spectra under electron impact conditions (EI) were recorded at 70 ev ionizing voltage with a VG Prospec instrument and the presented as m/z (% rel int.). Elemental analyses (C, N, H) results were found to be in good agreement with the calculated values. Melting points were determined with Capillaries Thomas Hoover melting point apparatus and are uncorrected. TLC monitored all reactions and purity of the synthesized compounds.

General procedure for the preparation of 7-Substituted pyrido[2,3-d]pyrimidine derivatives (3a-j):

A Solution of respective aromatic substituted ketones **2a-j** (1.2mmol) in dry acetone was added to a stirred solution of 4-Aminopyrimidine-5-carbaldehyde (**1**) (1mmol) in the presence of K_2CO_3 (2.5mmol) and catalytic amount of KI in dry acetone. After completion of addition, the stirring was continued for 4-6 h. Progress of the reaction was monitored by TLC. The reaction mixture was filtered and evaporation of the filtrate under reduced pressure to got the solid. The solid was recrystallization from ethanol.

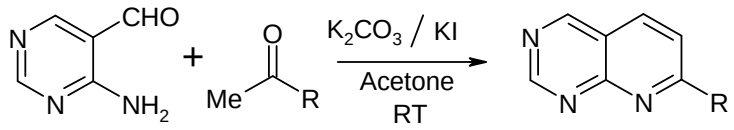
7-Phenylpyrido[2,3-d]pyrimidine ***3a*****:** Light Yellow solid: Yield was found to be 83%, mp 165-166°C. IR (KBr) cm^{-1}: 3026 (C-H), 1599 (C=N), 1467 (C=C). ^{1}H NMR (DMSO-d_6), δ, ppm: 7.55-9.55 (m, 9H). Mass spectrum, m/z: 208 $(M+1)^+$. *Anal.* Calculated. For $C_{13}H_9N_3$: C, 75.35; H, 4.38; N, 20.28. Found, %: C, 75.33; H, 4.35; N, 20.25.

7-(4-Chlorophenyl)pyrido[2,3-d]pyrimidine ***3b*****:** Yellow solid: Yield was found to be 80%, mp 203-204°C. IR (KBr) cm^{-1}: 3022(C-H), 1603 (C=N), 1459 (C=C). ^{1}H NMR (DMSO-d_6), δ, ppm: 7.45-9.29 (m, 8H). Mass spectrum, m/z: 242 $(M+1)^+$. *Anal.* Calculated. For $C_{13}H_8ClN_3$: C, 64.61; H, 3.34; N, 17.39. Found, %: C, 64.59; H, 3.37; N, 17.36.

7-(4-Methoxyphenyl)pyrido[2,3-d]pyrimidine ***3c****:* Pale Yellow solid: Yield was found to be 84%, mp 251-252°C. IR (KBr) cm^{-1}: 3021 (C-H), 1598 (C=N), 1459 (C=C). ^{1}H NMR (DMSO-d_6), δ, ppm: 3.15 (s, 3H), 7.05-9.55 (m, 8H). Mass spectrum, m/z: 237 (M^+). *Anal.* Calculated. For $C_{14}H_{11}N_3O$: C, 70.87; H, 4.67; N, 17.71. Found, %: C, 70.85; H, 4.69; N, 17.74.

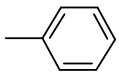
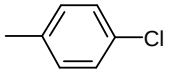
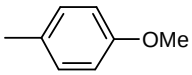
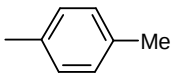
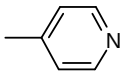
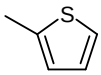
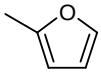
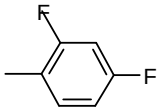
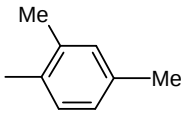
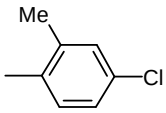
7-p-Tolylpyrido[2,3-d]pyrimidine ***3d*****:** Yellow solid: Yield was found to be 79%, mp 225-226°C. IR (KBr) cm^{-1}: 3022 (C-H), 1611 (C=N), 1463 (C=C). ^{1}H NMR ($CDCl_3$), δ, ppm: 2.2 (s, 3H), 7.3-9.58 (m, 8H). Mass spectrum, m/z: 221 (M^+). *Anal.* Calculated. For $C_{14}H_{11}N_3$: C, 76.01; H, 5.01; N, 18.99. Found, %: C, 76.04; H, 5.05; N, 18.97.

SCHEME



1 **2 a-j** **3 a-j**

Synthetic Scheme for 3a-j

Compound	R
3a	
3b	
3c	
3d	
3e	
3f	
3g	
3h	
3i	
3j	

7-(Pyridin-3-yl)pyrido[2,3-d]pyrimidine ***3e*****:** Light Yellow solid: Yield was found to be 81%, mp 238-239°C. IR (KBr) cm^{-1}: 3042 (C-H), 1600 (C=N), 1465 (C=C). ^1H NMR (DMSO-d_6), δ, ppm:

7.25-9.15 (m, 8H). Mass spectrum, m/z: 209 $(M+1)^+$. *Anal.* Calculated. For $C_{12}H_8N_4$: C, 69.22; H, 3.87; N, 26.91. Found, %: C, 69.19; H, 3.85; N, 26.89.

7-(Thiophen-2-yl)pyrido[2,3-d]pyrimidine ***3f*****:** Light Yellow solid: Yield was found to be 85%, mp 186-187°C. IR (KBr) cm^{-1}: 3026 (C-H), 1599 (C=N), 1466 (C=C). ^1H NMR ($CDCl_3$), δ, ppm: 7.2-9.6 (m, 7H). Mass spectrum, m/z: 213 (M^+). *Anal.* Calculated. For $C_{11}H_7N_3S$: C, 61.95; H, 3.31; N, 19.70. Found, %: C, 61.92; H, 3.30; N, 19.72.

7-(Furan-2-yl)pyrido[2,3-d]pyrimidine ***3g*****:** Brown solid: Yield was found to be 78%, mp 164-165°C. IR (KBr) cm^{-1}: 3025 (C-H), 1605 (C=N), 1460 (C=C). ^1H NMR (DMSO-d_6), δ, ppm: 6.52-6.60 (t, 3H), 8.0-9.6 (m, 4H). Mass spectrum, m/z: 198 $(M+1)^+$. *Anal.* Calculated. For $C_{11}H_7N_3O$: C, 67.00; H, 3.58; N, 21.31. Found, %: C, 66.98; H, 3.56; N, 21.35.

7-(2,4-Diflorophenyl)pyrido[2,3-d]pyrimidine ***3h*****:** Brown solid: Yield was found to be 78%, mp 144-145°C. IR (KBr) cm^{-1}: 3016 (C-H), 1599 (C=N), 1466 (C=C). ^1H NMR ($CDCl_3$), δ, ppm: 6.95-9.6 (m, 7H). Mass spectrum, m/z: 243 (M^+). *Anal.* Calculated. For $C_{13}H_7F_2N_3$: C, 64.20; H, 2.90; N, 17.28. Found, %: C, 64.23; H, 2.93; N, 17.30.

7-(2,4-Dimethylphenyl)pyrido[2,3-d]pyrimidine ***3i*****:** Yellow solid: Yield was found to be 78%, mp 202-203°C. IR (KBr) cm^{-1}: 3014 (C-H), 1611 (C=N), 1458 (C=C). ^1H NMR ($CDCl_3$), δ, ppm: 2.23 (s, 6H), 7.28-9.55 (m, 7H). Mass spectrum, m/z: 238 $(M+1)^+$. *Anal.* Calculated. For $C_{15}H_{15}N_3$: C, 75.92; H, 6.37; N, 17.71. Found, %: C, 75.89; H, 6.35; N, 17.74.

7-(4-Chloro-2-methylphenyl)pyrido[2,3-d]pyrimidine ***3j*****:** Light Yellow solid: Yield was found to be 80%, mp 173-174°C. IR (KBr) cm^{-1}: 3019 (C-H), 1609 (C=N), 1465 (C=C). ^1H NMR ($CDCl_3$), δ, ppm: 2.45 (s, 3H), 7.10-9.45 (m, 7H). Mass spectrum, m/z: 258 $(M+1)^+$. *Anal.* Calculated. For $C_{14}H_{12}ClN_3$: C, 65.25; H, 4.69; N, 16.30. Found, %: C, 65.31; H, 4.69; N, 16.33.

Antimicrobial Testing

The compound 3**a-j** was tested for in vitro antimicrobial activity at two different concentrations 100 and 200µg per disc. The antibacterial activity was screened against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria) and *Proteus vulgaris, Klebsiella pneumoniae* (Gram-nagative bacteria) on nutrient agar plates at 37 ^{0}C for 24 hrs using chloramphenicol as reference during. The compounds were also evaluated for their antifungal activity against *Aspergillus niger* and *Pencillium chrysogenium* using fluconazole as standard drug. Fungi cultures were grown on potato dextrose agar (PDA) medium at 25 ^{0}C. The spore suspension was adjusted to 10^6 pores ml^{-1} at an mg ml^{-1} concentration by the Vincent and Vincent method.

TABLE 1. Antibacterial Activity* of the Target Compounds 3a-j

Compound	Concentration	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
	(µg)	*S.aureus*	*B.subtilis*	*P.vulgaris*	*K.pneumoniae*
3a	100	14	12	17	16
	200	17	15	19	19
3b	100	29	30	30	34
	200	34	38	39	39
3c	100	26	24	21	23
	200	32	26	25	28
3d	100	12	11	14	13
	200	15	13	17	16
3e	100	32	27	24	26
	200	35	30	28	27
3f	100	26	26	20	21
	200	32	30	24	24
3g	100	12	13	12	14
	200	14	12	15	17
3h	100	11	12	11	11
	200	14	16	14	13
3i	100	28	31	22	21
	200	33	35	26	25
3j	100	25	24	20	21
	200	31	28	23	24
Chloramphenicol	100	35	37	40	42
	200	40	40	45	45

* c = 100 µg / ml.; * c = 200 µg / ml.

Antioxidant Testing

The compounds 3**a-j** is tested for antioxidant property by Nitric Oxide and DPPH methods.

Assay for Nitric Oxide (NO) Scavenging Activity Sodium nitroprusside (5µM) in phosphate buffer pH 7.4 was incubated with 100 µM concentration of test compounds dissolved in a suitable solvent (methanol) and tubes were incubated at 25^0C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals 0.5 ml of incubation solution was taken and diluted with 0.5 ml of griess reagent (1% sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was read at 546 nm.

TABLE 2. Antifungal Activity* of the Target Compounds 3a-j

Compound	Concentration	Zone of Inhibition (mm)	
	(µg/ml)	*A.niger*	*P.chrysogenium*
3a	100	24	16
	200	27	18
3b	100	14	14
	200	19	17
3c	100	26	26
	200	30	29
3d	100	31	18
	200	38	19
3e	100	15	25
	200	18	28
3f	100	24	31
	200	27	32
3g	100	30	26
	200	33	28
3h	100	17	16
	200	22	18
3i	100	16	28
	200	20	34
3j	100	33	34
	200	35	36
Fluconazole	100	38	40
	200	42	44

* c = 100 µg / ml.; * c = 200 µg / ml.

Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free Radical (DPPH Method) The nitrogen centered stable free radical DPPH has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm, which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties. The solutions of test compounds (100 µM) were added to DPPH (100 µM) in ethanol. The tubes were kept at an ambient temperature for 25 minutes and the absorbance was measured at 517 nm. The difference between the test and the control experiments was taken and expressed as the percentage scavenging of the DPPH radical.

TABLE 3: Antioxidant Activity* of the Target Compounds 3i-j

Compound	% Inhibition at 100 µM	
	Nitric oxide method	DPPH method
3a	34.55	38.45
3b	82.25	84.24
3c	91.18	93.65
3d	29.21	27.75
3e	70.23	72.25
3f	79.10	73.85
3g	24.16	35.22
3h	24.78	26.15
3i	72.14	74.15
3j	95.18	94.11

* c = 100 µM.

Results and Discussion

The results of the compounds of preliminary antimicrobial testing are shown in Tables-1 and 2. The results revealed that the inhibitory activity against Gram-positive bacteria was higher than Gram-negative bacteria. The imidazole derivatives **3a, 3d, 3g,** and 3**h** were displayed least activity. The compounds **3b, 3c, 3e, 3f, 3i**, and 3**j** showed excellent activity against Gram-positive bacteria (inhibitory zone>25mm) and good activity against Gram negative bacteria (inhibitory zone>20mm). All the test compounds **3a, 3c, 3d, 3f, 3g** and **3j** excellent activity and compounds 3**b, 3e,** 3**h and 3i** exhibited moderate activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Aspergillus niger*. Compounds **3c, 3e, 3f, 3g, 3i and 3j** excellent activity and compounds **3a, 3b, 3d** and 3**h** exhibited moderate activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Pencillium chrysogenium* (Table 1 & 2). The compounds **3b, 3c, 3e, 3f, 3i,** and 3**j** exhibited high antioxidant property in both **Nitric Oxide** and **DPPH** methods at 100 µM concentration (Table 3).

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