

Note

Pyrido[2,3-*d*]pyrimidines and their ribofuranosides: Synthesis and antimicrobial investigations

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4-Amino-5,7-disubstituted pyrido[2,3-*d*]pyrimidines **3**, 4-amino-5,7-disubstituted pyrido[2,3-*d*]pyrimidine-2(1*H*)-thiones **4**, and 4-amino-3,5,7-trisubstituted pyrido[2,3-*d*]pyrimidines-2,4-(1*H*, 3*H*)-dione **5** have been synthesized by condensation of 2-amino-3-cyano-4,6-disubstituted pyridines **2** and formamide, thiourea and aryl isocyanate respectively. Compounds **2** have been prepared by the treatment of chalcones **1** with malononitrile in presence of ammonium acetate in quantitative yield. The heterocyclic bases *viz.* **4** and **5** have been treated with hexamethyl disilazane to give S/O-trimethylsilyl derivative, *in situ*, which on condensation with β -D-ribofuranosyl-1-acetate-2,3,5-tribenzoate yield the corresponding nucleoside, *i.e.* 4-amino-5,7-disubstituted-1-(β -D-ribofuranosyl) pyrido[2,3-*d*]-2(1*H*)-thiones **6** and 4-imino-3,5,7-trisubstituted-1-(2',3',5'-tri-O-benzoyl- β -D-ribofuranosyl)-pyrido[2,3-*d*]pyrimidin-2-ones **7**, respectively (**Scheme I**).

by the treatment of 2-amino-3-cyano 4,6-disubstituted pyrimidines **2** with formamide, thiourea and arylisocyanate respectively.

Compounds **4** and **5** were then transferred to their respective nucleosides by treating with hexamethyl disilazane, which converted to O/S-hexamethylsilyl derivative, *in situ*. To it, when β -D-ribofuranosyl-1-acetate-2,3,5-tribenzoate was added and reaction was carried out *in vacuo* at 155-160°C for 10 hr, afforded the 4-amino-5,7-disubstituted-1-(β -D-ribofuranosyl) pyrido[2,3-*d*]pyrimidin-2(1*H*)-thiones **6** and 4-imino-3,5,7-trisubstituted-1-(2',3',5'-tri-O-benzoyl- β -D-ribofuranosyl) pyrido[2,3-*d*]-2-ones, **7**, respectively (**Scheme I**).

These compounds have been characterized by elemental analysis, IR and ¹H NMR studies. All these data have been summarized in **Table I**.

IR-data

Compound **2** showed a sharp band in the region 2240-2210 cm^{-1} due to the presence of $\text{C}\equiv\text{N}$ group. The $-\text{NH}_2$ group was observed at 3450-3350 cm^{-1} and 1545-1510 cm^{-1} due to the stretching and bending vibrations, respectively. The $-\text{C}\equiv\text{N}$ band appeared in compound **2** has been found to disappear in compound **3** and **4**. The latter compounds showed IR bands at 3180-3150, 1680-1650, 3500-3400 and 1210-1190 cm^{-1} regions, which were attributed to $>\text{NH}$, $>\text{C}=\text{O}$, $-\text{NH}_2$ and $>\text{C}=\text{S}$ respectively. Absorption due to $>\text{C}=\text{NH}$ group has appeared in the region 3180-3150 cm^{-1} in compound **5**.

¹H NMR data

¹H NMR spectra of synthesized compounds **3-7** revealed a multiplet of aromatic protons in the region δ 6.4-7.8. In compounds having the 5,7-disubstituted pyrido[2,3-*d*]pyrimidines the appearance of singlet at δ 6.5-7.5 and at δ 7.6 were due to $-\text{NH}_2$ group and $>\text{NH}$ group respectively. 3,5,7-trisubstituted pyrido[2,3-*d*]pyrimidine **5** showed singlet at δ 8.2 due to $>\text{NH}$ proton attached to pyrimidine ring and a singlet at δ 8.4 due to $>\text{C}=\text{NH}$ proton.

Antimicrobial Activity

The compound **3-7** were screened for antimicrobial activity according to paper disc method by Varma and

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A survey of the pertinent literature has revealed versatile applications of pyrido[2,3-*d*]pyrimidines *viz.* as antibacterial¹⁻³, antifungal^{4,5} and antiallergic⁶ *etc.* The nucleosides of pyrido[2,3-*d*]pyrimidines, which improve their solubility and enhance their biological activity, have been reported as promising antileukemic⁷, anti-AIDS agents⁸, antifungal^{9,10}, antibacterial¹¹, hypnotic activity¹², anticancer^{13,14}, antiherpes¹⁵ *etc.* The many-fold importance of these derivatives and their nucleosides and the interest in this area has attracted attention to explore some new pyrido[2,3-*d*]pyrimidine derivatives and their nucleosides with the goal to achieve some novel biologically effective compounds.

4-Amino-5,7-disubstituted pyrido[2,3-*d*]pyrimidines **3**, 4-amino-5,7-disubstituted pyrido[2,3-*d*]pyrimidine-2(1*H*)-thiones **4** and 4-imino-3,5,7-trisubstituted pyrido[2,3-*d*]pyrimidine **5** have been prepared

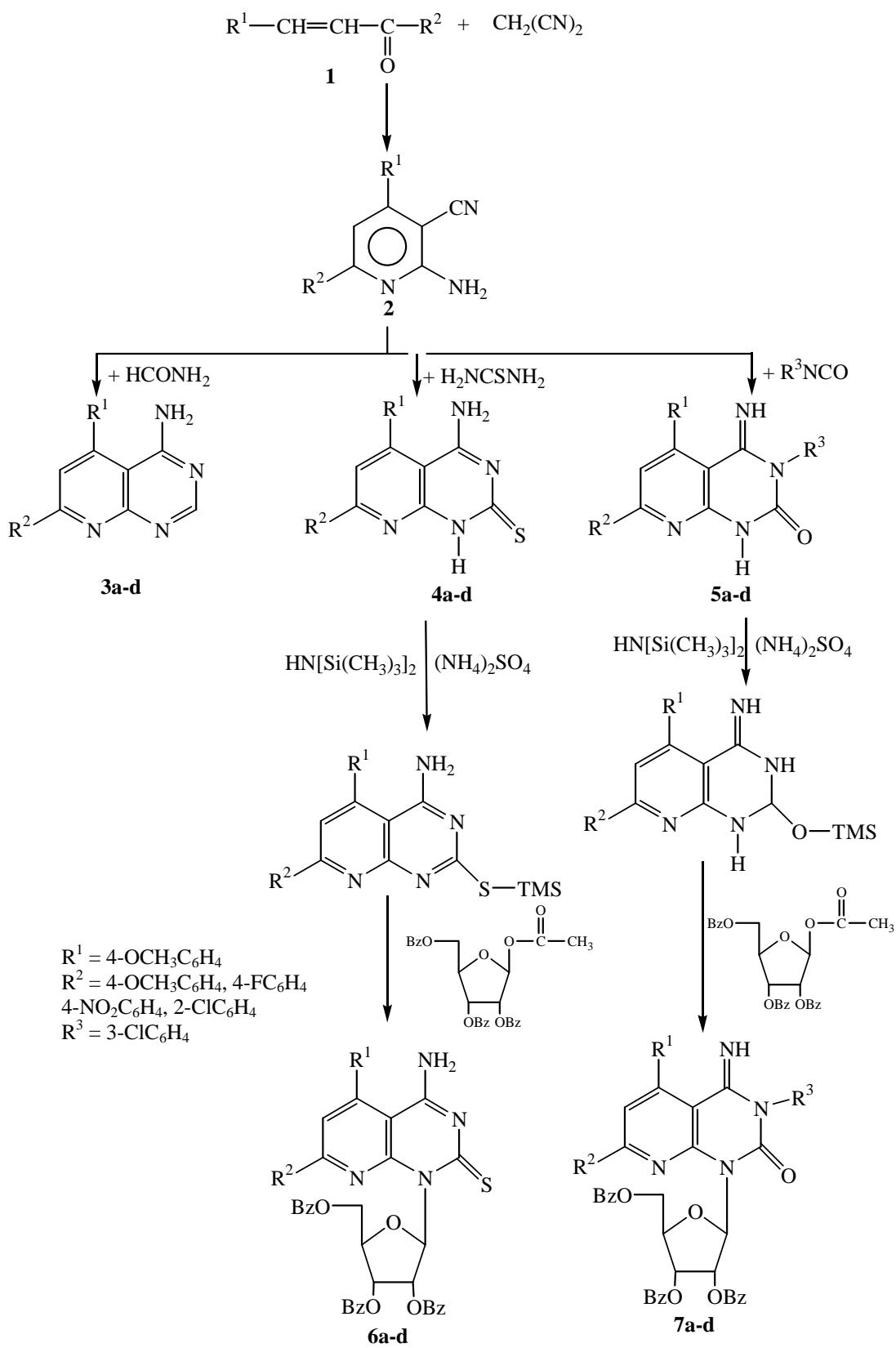
**Scheme I**

Table I — The physical and ^1H NMR spectral data of synthesized compounds

Compd	R ¹	R ²	R ³	m.p. (°C)	Yield	^1H NMR (δ , ppm)
3 a	4-OCH ₃ C ₆ H ₄	4-OCH ₃ C ₆ H ₄	—	110	78	6.85-7.72(10H, m, Ph-H), 6.50-7.50(2H, s, -NH ₂), 3.85(6H, s, -OCH ₃)
3 b	4-OCH ₃ C ₆ H ₄	4-NO ₂ C ₆ H ₄	—	142	80	6.89-7.54(10H, m, Ph-H), 6.45-7.45(2H, s, -NH ₂), 3.72(3H, s, -OCH ₃)
3 c	4-OCH ₃ C ₆ H ₄	4-FC ₆ H ₄	—	140	72	6.90-7.64(10H, m, Ph-H), 6.50-7.55(2H, s, -NH ₂), 3.80(3H, s, -OCH ₃)
3 d	4-OCH ₃ C ₆ H ₄	2-ClC ₆ H ₄	—	189	70	6.60-7.75(10H, m, Ph-H), 6.3-7.42(2H, s, -NH ₂), 3.82(3H, s, -OCH ₃)
4 a	4-OCH ₃ C ₆ H ₄	4-OCH ₃ C ₆ H ₄	—	160	82	6.3-8.2(9H, m, Ph-H), 6.2-7.63(2H, s, -NH ₂), 3.86(3H, s, -OCH ₃)
4 b	4-OCH ₃ C ₆ H ₄	4-NO ₂ C ₆ H ₄	—	182	73	6.25-7.90(9H, m, Ph-H), 6.35-7.65(2H, s, -NH ₂), 3.83(3H, s, -OCH ₃)
4 c	4-OCH ₃ C ₆ H ₄	4-FC ₆ H ₄	—	165	65	6.42-7.53(9H, m, Ph-H), 6.42-7.63(2H, s, -NH ₂), 3.78(3H, s, -OCH ₃)
4 d	4-OCH ₃ C ₆ H ₄	2-ClC ₆ H ₄	—	185	71	6.34-7.62(10H, m, Ph-H), 6.48-7.67(2H, s, -NH ₂), 3.82(3H, s, -OCH ₃)
5 a	4-OCH ₃ C ₆ H ₄	4-OCH ₃ C ₆ H ₄	3ClC ₆ H ₄	165	79	6.22-7.28(13H, m, Ph-H), 7.92 (1H, s, >NH), 3.90(6H, s, -OCH ₃)
5 b	4-OCH ₃ C ₆ H ₄	4-NO ₂ C ₆ H ₄	3ClC ₆ H ₄	110	68	6.26-7.32(13H, m, Ph-H), 7.80(1H, s, >NH), 3.92(3H, s, -OCH ₃)
5 c	4-OCH ₃ C ₆ H ₄	4-FC ₆ H ₄	3ClC ₆ H ₄	123	69	6.32-7.43(13H, m, Ph-H), 7.78(1H, s, >NH), 3.96(3H, s, -OCH ₃)
5 d	4-OCH ₃ C ₆ H ₄	2-ClC ₆ H ₄	3ClC ₆ H ₄	125	78	6.38-7.49(13H, m, Ph-H), 7.73(1H, s, >NH), 3.87(3H, s, -OCH ₃)
6 a	4-OCH ₃ C ₆ H ₄	4-OCH ₃ C ₆ H ₄	—	105	71	6.47-8.30(20H, m, Ph-H), 6.22-7.53(2H, s, -NH ₂), 3.72(6H, s, -OCH ₃)
6 b	4-OCH ₃ C ₆ H ₄	4-NO ₂ C ₆ H ₄	—	130	75	6.46-8.15(20H, m, Ph-H), 6.31-7.67(2H, s, -NH ₂), 3.69(3H, s, -OCH ₃)
6 c	4-OCH ₃ C ₆ H ₄	4-FC ₆ H ₄	—	137	67	6.42-8.22(20H, m, Ph-H), 6.33-7.62(2H, s, -NH ₂), 3.62(3H, s, -OCH ₃)
6 d	4-OCH ₃ C ₆ H ₄	2-ClC ₆ H ₄	—	105	76	6.40-8.20(20H, m, Ph-H), 6.27-7.71(2H, s, -NH ₂), 3.60(3H, s, -OCH ₃)
7 a	4-OCH ₃ C ₆ H ₄	4-OCH ₃ C ₆ H ₄	3ClC ₆ H ₄	117	72	6.82-8.42(24H, m, Ph-H), 6.32-7.52(1H, s, =NH), 3.82(6H, s, -OCH ₃)
7 b	4-OCH ₃ C ₆ H ₄	4-NO ₂ C ₆ H ₄	3ClC ₆ H ₄	179	77	6.83-8.32(24H, m, Ph-H), 6.34-7.46(1H, s, =NH), 3.87(3H, s, -OCH ₃)
7 c	4-OCH ₃ C ₆ H ₄	4-FC ₆ H ₄	3ClC ₆ H ₄	153	70	6.79-8.30(24H, m, Ph-H), 6.36-7.48(1H, s, =NH), 3.69(3H, s, -OCH ₃)
7 d	4-OCH ₃ C ₆ H ₄	2-ClC ₆ H ₄	3ClC ₆ H ₄	172	75	6.83-8.29(24H, m, Ph-H), 6.38-7.51(1H, s, =NH), 3.7(3H, s, -OCH ₃)

Nobles¹⁶ against bacteria *Escherichia coli* (gram-negative), and *Staphylococcus aureus* (gram-positive), and fungi *Aspergillus niger* and *Aspergillus flavus* at the concentration of 100 $\mu\text{g}/\text{disc}$ using mycostatin and streptomycin drugs, as reference compounds. A closer look at the activity indices reveals that the ribofuranosides are better antimicrobial agents than their precursors (**Table II**).

Experimental Section

All melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded on a Shimadzu FT-IR spectrometer using KBr disc and ^1H NMR spectra on JEOL AL-300 NMR spectrometer in $\text{CDCl}_3/\text{DMSO}-d_6$ using TMS as an internal standard. The homogeneity of the synthesized compounds were checked by TLC using

Table II — Antimicrobial activity of compounds **3a-d** to **7a-d**
zone of growth inhibition (mm) (activity index)

Compd	Bacteria		Fungi	
	<i>E. coli</i>	<i>S. aureus</i>	<i>A. niger</i>	<i>A. flavus</i>
3 a	7.8(0.86)	7.6(0.84)	7.7(0.87)	7.4(0.84)
3 b	8.4(0.93)	8.3(0.92)	8.5(0.96)	8.1(0.92)
3 c	12.2(1.35)	12.0(1.33)	12.4(1.40)	12.2(1.38)
3 d	7.9(0.87)	7.7(0.85)	7.6(0.86)	7.5(0.85)
4 a	7.6(0.84)	7.5(0.83)	7.7(0.87)	7.4(0.84)
4 b	9.2(1.02)	9.0(1.00)	9.3(1.05)	9.1(1.03)
4 c	11.8(1.31)	11.6(1.28)	11.7(1.32)	11.5(1.30)
4 d	7.5(0.83)	7.4(0.82)	7.8(0.88)	7.6(0.86)
5 a	8.2(0.91)	8.1(0.9)	8.0(0.90)	7.8(0.88)
5 b	7.8(0.86)	7.7(0.85)	7.9(0.89)	7.8(0.88)
5 c	12.4(1.37)	12.1(1.34)	12.2(1.38)	12.0(1.36)
5 d	8.1(0.90)	8.0(0.88)	8.2(0.93)	7.9(0.89)
6 a	8.0(0.88)	7.9(0.87)	7.9(0.89)	7.6(0.86)
6 b	8.8(0.97)	8.5(0.94)	8.7(0.98)	8.3(0.94)
6 c	12.4(1.37)	12.2(1.35)	12.6(1.43)	12.4(1.40)
6 d	8.2(0.91)	8.1(0.9)	8.0(0.90)	7.8(0.88)
7 a	8.4(0.93)	8.3(0.92)	8.2(0.93)	8.0(0.90)
7 b	8.1(0.9)	7.9(0.87)	8.0(0.90)	7.8(0.80)
7 c	12.6(1.4)	12.4(1.37)	12.4(1.40)	12.2(1.38)
7 d	8.4(0.93)	8.2(0.9)	8.1(0.92)	8.0(0.90)

Activity Index = inhibition area of the sample/inhibition area of the standard

silica gel as adsorbent and visualization was accomplished by UV light or iodine.

Synthesis of 2-amino-3-cyano-4,6-disubstituted pyridines, **2a-d**

A mixture of chalcone **1** (0.05 mole), ammonium acetate (0.4 mole) and malononitrile (0.05 mol) in ethyl alcohol (50 mL) was refluxed on a water bath for 18-22 hr. After cooling, the contents were poured onto crushed ice with stirring. The solid so obtained was washed with water and purified by recrystallization from ethyl alcohol.

Synthesis of 4-amino-5,7-disubstituted pyrido[2,3-*d*]pyrimidine, **3a-d**

A mixture of compound **2** (0.01 mole) and formamide (0.04 mole) was refluxed on an oil bath for 14 to 18 hr. The contents were cooled and then poured into crushed ice. The solid so obtained, was washed with water, dried and purified by recrystallization from ethyl alcohol.

Synthesis of 4-amino-5,7-disubstitutedpyrido[2,3-*d*]pyrimidines-2(1*H*)-thiones, **4a-d**

A mixture of compound **2** (0.01 mole) and thiourea (0.02 mole) was heated on an oil bath at 120-130°C for 2-3 hr with stirring. The temperature was raised to 180°C and finally the reaction mixture was heated at 230°C for 2-3 hr. The residue was washed with water, saturated NaHCO₃ solution and finally with ethanol. The crude product so obtained was purified by recrystallization from DMF-EtOH mixture (1:2).

Synthesis of 4-imino-3,5,7-trisubstitutedpyrido[2,3-*d*]pyrimidines-2(1*H*)-ones, **5a-d**

A mixture of compound **2** (0.01 mole), 3-chlorophenylisocyanate (0.01 mole) and diphenyl ether (30 mL) were refluxed for 8-9 hr. After cooling, the reaction mixture was added to ethanol and the separated solid was filtered, washed with water and purified by recrystallization from DMF-EtOH mixture (1:2).

Synthesis of 5,7-disubstituted-1-(β -D-ribofuranosyl)pyrido[2,3-*d*]pyrimidines and 3,5,7-trisubstituted-1-(β -D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine, **6a-d,7a-d**

To a concentration solution of pyrido[2,3-*d*]pyrimidines **4** and **5** (0.002 mole) in toluene was added hexamethyldisilazane (0.0124 mole) together with a few crystals of ammonium sulphate. After 4 hr of refluxing, a clear coloured solution was obtained. It was filtered and the solvent was removed *in vacuo* at 100°C. The sugar (β -D-ribofuranosyl-1-acetate-2,3,5-tribenzoate) (0.002 mole) was added to the above pasty mixture and the mixture was stirred at 155-160°C under vacuum for 15 min in absence of moisture. The reaction mixture was stirred for 10 hr. The vacuum was continuously applied for 5 min at the end of every hour. The melt was boiled in methanol for 10 min, cooled and filtered. The viscous mass of nucleoside thus obtained was purified by recrystallization from diethyl ether.

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