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Nitriles in Heterocyclic Synthesis: Synthesis of Pyrido[3',2':4,5]Thieno[2,3-d] Pyrimidines Derivative

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Abstract

6-Amino-3,5-dicyano-4-methylpyridine-2(1*H*)-thione 1 reacted with *α*-haloketones to give the S-alkylated derivatives 2a-m. Compound 2a-m undergoes cyclization into thieno[2,3-d] pyridine derivatives 3a-m upon treatment with ethanolic sodium ethoxide. Saponification of 3a gave the amino acid 4 which afforded 5 when refluxed in Ac_2O. Treatment of 5 with $NH_4OAc/AcOH$ gave 6a. Compound 6a also was obtained when 3c was refluxed in Ac_2O. Reaction of 3a with formamide gave 7 and with hydrazine hydrate gave 8. The thiourea derivative 9 was obtained by reaction of 3a with benzoyl isothiocyanate. Compound 9 when refluxed in alcoholic KOH gave 10 and with 98% H_2SO_4 gave 12. Acetylation of 3a with Ac_2O gave the acetyl derivative 13 which on treatment with aniline afforded 14. Compound 14 was cyclized with H_2SO_4 to 15. Finally treatment of compound 5 with aniline in AcOH afforded 6b.

Keywords

Nitriles, Heterocyclic Synthesis

Subject Areas: **Analytical Chemistry, Organic Chemistry**

1. Introduction

Pyridines are among the most intensively studied heterocyclic compound and their chemistry has been reviewed frequently. Many of the pyridinethiones are biologically active as bactericides [1] [2] evaluated pharmacologically and have been found to show activity against diabetes mellitus, as analagesics and antiinflammants [3]-[6]. On the other hand, pyridothienopyrimidines have been the subject of chemical and biological studies on account of their interesting pharmacological properties. A number of syntheses for substituted derivatives of this trihetero-cyclic ring system, featuring a variety of pharmacological effects have been developed. Such derivatives have

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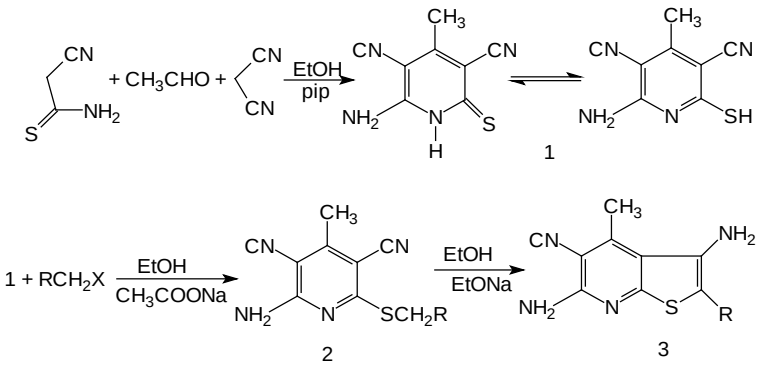
analgesic, [7] antipyretic, [8] antianaphilactic, [9] and anti-inflammatory [10] activity. Also, some are clinically effective antialergic [11] or potentially antineophilactic agent [12], and a few possess significant hypocholesteromic [13] activity. These assets promoted us to prepare new pyridothienopyrimidines with potential biological activity. So, it has been found that 6-amino-3,5-dicyano-4-methylpyridine-2(1*H*)-thione **1** [14] reacted with α-haloketones and α-halonitriles in ethanol and sodium acetate afforded the S-alkylated derivatives **2**. The structure of **2a-m** was confirmed by ^{1}H NMR which showed a singlet signal at δ 4.0 ppm corresponding to the active methylene group. Compound **2a-m** undergoes cyclization into thienopyridine derivatives **3a-m** upon treatment with ethanolic sodium ethoxide. The ^{1}H NMR of these compounds revealed the disappearance of the methyl group (**Scheme 1**).

Saponification of the amino ester **3a** using alcoholic sodium hydroxide gave the sodium salt of the amino acid **4**, which afforded 7-amino-2,9-dimethyl-4-oxo-3H-pyrido[3',2':4,5]thieno[3,2-d]oxazine-8-carbonitrile **5** when refluxed in acetic anhydride. Treatment of **5** with ammonium acetate in boiling acetic acid led to the formation of thienopyridopyrimidine derivative **6a**. Compound **6a** was also obtained by refluxing **3c** in acetic anhydride (**Scheme 2**).

On the other hand, when **3a** was treated with formamide afforded 7-amino-9-methyl-4-oxo-3H-pyrido [3',2': 4,5]thieno[3,2-d]pyrimidine-8-carbonitrile **7**. Also **3a** was treated with hydrazine hydrate to afford the hydrazide derivative **8**. The thiourea derivatives **9** was obtained by reaction of **3a** with benzoyl isothiocyante [15] [16] in anhydrous acetone solution. Compound **9** on alkaline cyclization with alcoholic sodium hydroxide give compound **10** instead of **11**. The ^{1}HNMR data of compound **10** revealed the absence of aromatic protons and the mass spectrum was compatible with the molecular formula $C_{11}H_7N_5OS_2$ (M^+ = 289). The cyclic amide structure of compound **10** furthermore was defined by comparison its cyclic thioester isomer **12** obtained by ring closure in 98% sulfuric acid at room temperature [17]-[20]. The two isomeric derivatives **10** and **12** are well differentiated according to the alkaline solubility and their ^{1}HNMR and IR spectra. Acetylation of **3a** with acetic anhydride gave the acetyl derivatives **13** that on treatment with aniline afforded **14**. Compound **14** was cyclized with 98% sulfuric acid to the pyridothienopyrimidine **15**. Treatment of compound **5** with aniline in acetic acid afforded compound **6**. The structure of these compounds was confirmed by ^{1}H NMR, mass, IR spectra and microanalysis (**Scheme 3**).

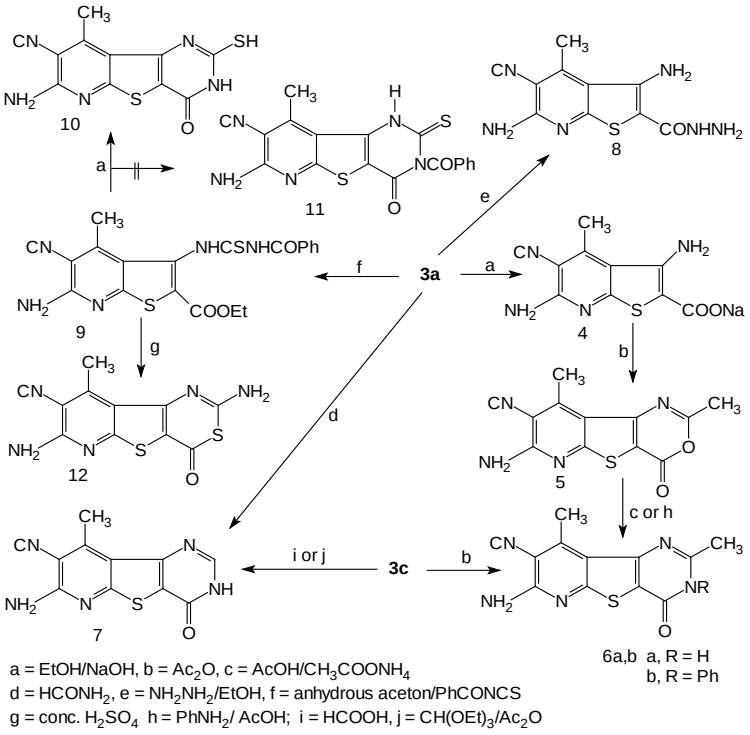
2. Biological Activities

Most of the synthesized compounds have been tested against four different kinds of bacteria. The result of the antimicrobial studies presented in **Table 1**. It has been found that the prepared compounds showed antimicrobial

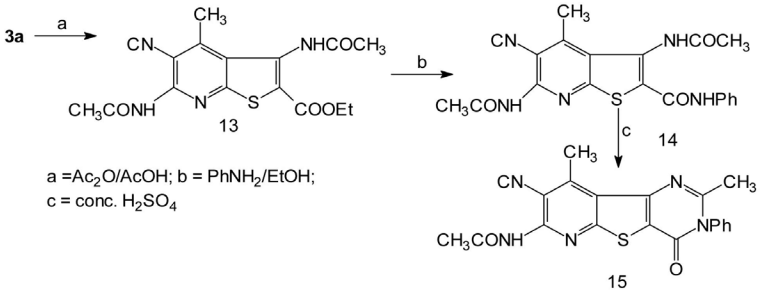


2,3,
a, R = COOEt
b, R = CN
c, R = $CONH_2$
d, R = $COC_6H_4CH_3$-p
e, R = CONHPh
f, R = $CONHC_6H_4CH_3$-p
g, R = $CONHC_6H_4OCH_3$-p
h, R = $CONHC_6H_4OCH_3$-m
i, R = $CONHC_6H_4COOEt$-p
j, R = CONH-2-pyridine
k, R = $CONHC_6H_4COCH_3$-p
l, R = $CONHC_6H_4Cl$-p
m, R = CONH-4-antipyrine

Scheme 1. Synthesis of pyridinethione and thienopyridine.



Scheme 2. Synthesis of fused pyridine.



Scheme 3. Synthesis of pyridothienoazines.

Table 1. Biological activity of some newly prepared compounds.

No of Compounds	A	B	C	D
3a	+	+	+	+
3b	+ + +	+ +	+	+ + + +
3c	+	+ +	+	+ + + +
3d	+	+++	+	+ +
3e	+ +	+ + +	+	+ + + +
3f	+	+	+	+ + + +
3g	+ + +	+ + +	+	+ + + +
3h	+ + + +	+ +	+	+ + + +
3I	+	+ +	+	+
3j	+	+	+	+ +
3k	+ +	+ + +	+ +	+ + +
15	+ + +	+ +	+ + + +	+ + +

Where: A = *Staphyllococcus aurous*; B = *Streptococcus mitor*; C = *Esherichia coli*; D = *Nisseria sica*; -- = Negative; + = Poor; + + = Fair; + + + = Good; + + + + = Very good.

activity against *Staphylococcus aurous*, *Streptococcus mitor*, *Esherichia coli* and *Nisseria sica*.

3. Experimental

All melting points are uncorrected and were determined on a Gellankamp apparatus, IR spectra were recorded on Schimadzu 470 spectrophotometer in potassium bromide discs; ^{1}H NMR spectra were recorded on a Varian EM-390 (90 Mhz) spectrophotometer using TMS as an internal standard, mass spectrometer MS 30 (AEL) at 70 ev. Analytical data were obtained from the microanalytical data center at Cairo University.

4. 6-Amino-3,5-Dicyano-4-Methylpyridine-2(1h)-Thione 1

It was prepared according to a liturature procedure [14].

5. 2-Substituted-Mercapto-6-Amino-4-Methylpyridine-3,5-Dicarbonitrile 2a-m. General Procedure

To a solution of mercaptopyridine **1** (0.01 mol) in ethanol (30 ml) and sodium acetate (0.01 mol), the appropriate halocompound (0.01 mol) was added. The reaction mixture was refluxed for 1 h. After cooling, the solid product formed was collected by filtration, washed with water several times and recrystallized from the appropriate solvent. The physical data (c.f. **Table 2** and **Table 3**).

6. 3,6-Diamino-4-Methyl-2-Substituted Carboxamidothieno[2,3-b] Pyridine-5-Carbonitrile 3a-m. General Procedure

To a solution of compound **2** (2 g) in absolute ethanol (30 ml), a few drops of sodium ethoxide was added and refluxed for 1 hour. After cooling the solid product formed was collected by filtration and recrystallized from the appropriate solvent.

7. Sodium-3,6-Diamino-5-Cano-4-Methylthieno[2,3-b] Pyridine-2-Carboxylate 4

The amino ester **3a** was refluxed for 3 h in ethanolic sodium hydroxide (30 ml 10%). The solid product obtained after cooling was collected by filtration, washed was ethanol and left to dry. This compound was used as such in the next procedure.

8. 7-Amino-2,9-Dimethyl-4-Oxo-3H-Pyridine[3',2':4,5]Thieno[3,2-d] Oxazine-8-Carbonitrile 5

The sodium salt **4** (0.5 g) was refluxed in acetic anhydride (30 ml) for 3 h. The reaction mixture was left to stand at room temperature and the solid product formed was filtered off and recrystallized from dioxan; mp 210˚C; yield 40%; IR ν cm^{-1} 3330 - 3200 (NH_2), 2190 (CN), 1700 (CO); MS, *m/z* = 272; Found: C, 53.0; H, 3.0; N, 20.8; S, 11.77; calcd for $C_{12}H_8N_4O_2S$: C, 52.94; H, 2.96; N, 20.58; S, 11.9%.

9. Preparation of 6a,b. General Procedure

A mixture of oxazine derivative **5** (0.01 mol) and ammonium acetate (0.02 mol) or aniline (0.01 mol) in acetic acid (30 mol) was heated under reflux for 3h. The solid product formed after cooling was collected by filtration and recrystallized from the appropriate solvent.

10. 7-Amino-2,9-Dimethyl-4-Oxo-3H-Pyrido[3',2':4,5]Thieno[3,2-d] Pyrimidine-8-Carbonitrile 6a

10.1. Method A

Compound **6a** was obtained as yellow crystals from DMF/Ethanol; yield 40%; mp 355˚C; IR ν cm^{-1} 3390 - 3225 (NH_2); 3225 - 3100 (NH); 2200 (CN); 1651 (CO); ^{1}H NMR (DMSO-d6) δ = 2.1 (s, 3H, CH_3); 2.3(s, 3H, CH_3); 6.4(5, 2H, NH_2); 12.2 (s, 1H, NH); Ms: *m/z* = 271; Found: C, 53.3; H, 3.0; N, 25.9; S, 12.0; calcd for $C_{12}H_9N_5OS$: C, 53.13; H, 3.34; N, 25.81; S, 11.82%.

Table 2. Physical and analytical data of all newly synthesized compounds 2a-m and 3a-m.

No	.mp˚C Solvent	Colour Yield %	M. Formula MS	Calcd/Found		
				C	H	N
2a	170 EtOH	White 70	$C_{12}H_{12}N_4O_2S$ 276	52.17 (52.3)	4.34 (4.5)	17.39 (17.4)
2b	275 DMF/EtOH	Green 45	$C_{10}H_7N_5S$ 229	52.39 (52.5)	3.08 (3.2)	30.55 (30.8)
2c	210 Dioxan	Grey 65	$C_{10}H_9N_5OS$ 247	48.78 (48.9)	3.65 (3.7)	28.45 (28.5)
2d	145 EtOH	Yellow 70	$C_{17}H_{14}N_4OS$ 322	63.35 (63.5)	3.54 (3.7)	17.39 (17.6)
2e	276 EtOH	Yellow 75	$C_{16}H_{13}N_5OS$ 323	59.43 (59.6)	4.05 (4.3)	21.66 (21.9)
2f	135 EtOH	White 70	$C_{17}H_{15}N_5OS$ 337	60.53 (60.7)	4.45 (4.7)	20.77 (20.9)
2g	140 EtOH	Green 68	$C_{17}H_{15}N_5O_2S$ 353	57.77 (57.9)	4.45 (4.5)	19.83 (20.1)
2h	165 EtOH	Green 60	$C_{17}H_{15}N_5O_2S$ 353	57.77 (58.0)	4.45 (4.7)	19.83 (19.9)
2I	120 EtOH	Black 70	$C_{19}H_{17}N_5O_3S$ 395	57.72 (57.9)	4.30 (4.6)	17.72 (17.9)
2j	320 DMF/EtOH	Grey 75	$C_{15}H_{12}N_6OS$ 324	55.55 (55.8)	3.70 (3.9)	26.25 (26.5)
2k	115 MeOH	White 60	$C_{18}H_{15}N_5O_2S$ 365	59.17 (59.4)	4.14 (4.4)	19.17 (19.3)
2l	176 EtOH	Green 60	$C_{16}H_{12}N_5OSCl$ 357.5	53.70 (53.9)	3.35 (3.5)	19.58 (19.8)
2m	175 EtOH	Yellow 70	$C_{21}H_{19}N_7O_2S$ 433	58.19 (58.4)	4.42 (4.5)	22.62 (22.9)
3a	270 EtOH	Yellow 30	$C_{12}H_{12}N_4O_2S$ 276	52.17 (52.4)	4.34 (4.4)	17.39 (17.5)
3b	>360 DMF/EtOH	Black 50	$C_{10}H_7N_5S$ 229	52.40 (52.6)	3.05 (3.3)	30.65 (30.9)
3c	275 DMF/EtOH	Orange 50	$C_{10}H_9N_5OS$ 247	48.78 (48.9)	3.65 (3.8)	28.45 (28.6)
3d	200 EtOH	Yellow 65	$C_{17}H_{14}N_4OS$ 322	63.35 (63.6)	3.54 (3.7)	17.39 (17.4)
3e	310 DMF/EtOH	Yellow 60	$C_{16}H_{12}N_5OS$ 323	59.44 (59.5)	4.02 (4.2)	21.16 (21.2)
3f	335 DMF/EtOH	Yellow 55	$C_{17}H_{15}N_5OS$ 337	60.53 (60.7)	4.45 (4.5)	20.77 (20.9)
3g	322 DMF/EtOH	Orange 60	$C_{17}H_{15}N_5O_2S$ 353	57.77 (57.8)	4.45 (4.7)	19.83 (20.0)
3h	326 DMF/EtOH	Brown 65	$C_{17}H_{15}N_5O_2S$ 353	57.77 (57.8)	4.45 (4.6)	19.83 (20.0)
3I	135 EtOH	Brown 30	$C_{19}H_{17}N_5O_3S$ 395	57.72 (57.9)	3.70 (3.9)	26.25 (26.4)
3j	255 dioxan	Grey 25	$C_{15}H_{12}N_6OS$ 324	55.55 (55.7)	3.70 (3.8)	26.25 (26.4)
3k	185 EtOH	White 45	$C_{18}H_{17}N_5O_2S$ 355	59.17 (59.3)	4.10 (4.4)	19.17 (19.3)
3l	299 dioxan	Yellow 40	$C_{16}H_{12}N_5OSCl$ 357.5	53.70 (53.9)	3.35 (3.4)	19.58 (19.7)
3m	>360 DMF/EtOH	Yellow 65	$C_{21}H_{19}N_9O_2S$ 443	54.19 (54.2)	4.08 (4.4)	21.07 (21.3)

Continued

3k	185 EtOH	White 45	$C_{18}H_{17}N_5O_2S$ 355	59.17 (59.3)	4.10 (4.4)	19.17 (19.3)
3l	299 Dioxan	Yellow 40	$C_{16}H_{12}N_5OSCl$ 357.5	53.70 (53.9)	3.35 (3.4)	19.58 (19.7)
3m	>360 DMF/EtOH	Yellow 65	$C_{21}H_{19}N_9O_2S$ 443	54.19 (54.2)	4.08 (4.4)	21.07 (21.3)

Table 3. IR, ^{1}HNMR for the new compounds.

No	IR ν cm^{-1}	^{1}H NMR (δ, DMSO-d$_6$)
2a	3330 - 3150 (NH_2); 2220 (CN); 1727 (ester CO).	1.1 (t, 3H, CH_3); 3.1; (s, 3H, CH_3); 4.0 (q, 2H, CH_2); 7.6 (s, 2H, NH_2).
2b	3415 - 3210 (NH_2); 2185 (CN).	2.6 (s, 2H, CH_2); 3.1 (s, 3H, CH_3); 6.8 (s, 2H, NH_2).
2c	3370 - 3160 (NH_2); 2190 (CN), 1667 (CO).	3.2 (s, 3H, CH_3); 4.0 (s, 2H, CH_2); 5.0 (s, 2H, NH_2); 9.8 (s, 2H, NH_2).
2d	3285 - 3200 (NH_2), 2190 (CN); 1660 (CO).	2.1 (s, 3H, CH_3); 3.2 (s, 3H, CH_3); 4.0 (s, 2H, CH_2); 6.8 - 7.8 (m, 6H, Ar-H and NH_2).
2e	3375 - 3165 (NH_2-NH); 2190 (CN) 1632 (CO).	2.1 (s, 3H, CH_3); 3.9 (s, 2H, CH_2); 6.8 - 7.8 (m, 6H, Ar-H and NH_2); 9.7 (s, 1H, NH).
2f	3295 - 3140 (NH_2-NH); 2190 (CN); 1650 (CO).	2.2 (s, 3H, CH_3); 3.2 (s, 3H, H_3); 4.0 (s, 2H, CH_2); 6.8 - 7.6 (m, 4H, Ar-H) 7.8 (s, 2H, NH_2); 9.8 (s, 1H, NH).
2g	3390 - 3200 (NH_2-NH); 2190 (CN); 1635 (CO).	2.3 (s, 3H, CH_3); 3.7 (s, 3H, OCH_3); 4.0 (s, 2H, CH_2); 6.8 - 7.9 (m, 4H, Ar-H and NH_2) 10.0 (s, 1H, NH).
2h	3295 - 3170 (NH_2); 2195 (CN); 1642 (CO).	2.0 (s, 3H, CH_3); 3.7 (s, 3H, OCH_3); 4.0 (s, 2H, CH_2); 7.0 - 7.9 (m, 4H, Ar-H and NH_2) 10.2 (s, 1H, NH).
2i	3400 - 3210 (NH_2-NH); 2195 (CN), 1690 (ester CO); 1630 (CO).	1.1 (t, 3H, CH_3); 2.3 (s, 3H, CH_3); 4.0 (s, 2H, CH_2); 4.2 (q, 2H, CH_2); 6.8 - 7.9 (m, 4H, Ar-H and NH_2); 9.5 (s, 1H, NH).
2j	3450 - 3110 (NH_2-NH); 2195 (CN); 1640 (CO).	3.4 (s, 3H, CH_3); 4.8 (s, 3H, CH_3); 4.0 (s, 2H, CH_2); 7.2 - 7.9 (m, 6H, pyrdine-H and NH_2); 9.9 (s, 1H, NH).
2k	3400 - 3250 (NH_2-NH); 2200 (CN); 1674 (CO); 1635 (CO).	2.3 (s, 3H, CH_3); 3.2 (s, 3H, CH_3); 4.0 (s, 2H, CH_2); 6.8 - 7.9 (m, 4H, Ar-H and NH_2) 10.0 (s, 1H, NH).
2l	3455 - 3125 (NH_2-NH); 2190 (CN), 1634 (CO).	2.3 (s, 3H, CH_3); 3.9 (s, 2H, CH_2); 6.8 - 7.9 (m, 4H, Ar-H and NH_2) 9.8 (s, 1H, NH).
2m	3370 - 3270 (NH2-NH); 2195 (CN); 1637 (CO).	
3a	3400 - 3145 (NH_2); 2190 (CN); 1727 (ester CO).	1.2 (t, 3H, CH_3); 2.3 (s, 3H, CH_3); 4.0 (q, 2H, CH_2); 3.5 (s, 2H, NH_2); 7.9 (s, 2H, NH_2).
3b	3260 - 3160 (NH_2); 2190 (CN).	2.2 (s, 3H, CH_3); 3.6 - 4.0 (br, 4H, 2NH_2).
3C	3440 - 3155 (NH_2); 2190 (CN); 1649 (CO).	2.8 (s, 3H, CH_3); 3.2 (s, 2H, NH_2); 5.5 (br, 4H, 2NH_2).
3d	3350 - 3175 (NH_2), 2190 (CN); 1660 (CO).	2.3 (s, 3H, CH_3); 3.8 (s, 3H, CH_3); 7 - 8 (m, 8H, Ar-H and 2NH_2).
3f	3395 - 3095 (NH_2-NH); 2210 (CN); 1646 (CO).	2.2 (s, 3H, CH_3); 3.1 (s, 3H, CH_3); 3.5 (b, 4H, NH_2); 6.8 - 7.5 (m, 7H, Ar-H and NH_2); 9.0 (s, 1H, NH).
3g	3485 - 3100 (NH_2-NH); 2215 (CN); 1682 (CO).	2.3 (s, 3H, CH_3); 3.4 (b, 4H, NH_2); 3.8 (s, 3H, OCH_3); 7.0 - 7.5 (m, 7H, Ar-H and NH_2); 9.2 (s, 1H, NH).
3i	3395 - 3190 (NH_2-NH); 190(CN); 1700 (ester CO); 1680 (CO).	1.2 (t, 3H, CH_3); 2.3 (s, 3H, H_3); 3.5 (b, 4H, 2CH_2); 4.0 (q, 2H, CH_2); 7.5 - 8.0 (m, 8H, Ar-H and 2NH_2); 10.4 (s, 1H, NH).
3j	3450 - 3185 (NH_2, NH); 2210 (CN); 1650 (CO).	3.2 (s, 3H, CH_3); 7.0 - 7.6 (m, 4H, Ar-H); 8.0 (s, 4H, 2NH_2); 10.0 (s, 2H, NH).
3k	3390 - 3185 (NH_2, NH); 2195 (CN); 1650 (CO).	3.0 (s, 3H, CH_3); 3.3 (s, 3H, CH_3); 7.1 - 7.6 (m, 4H, Ar-H); 8.0 (s, 4H, 2NH_2); 10.9 (s, 2H, NH).
3l	3430 - 3331(NH_2-NH); 2190 (CN); 1641 (CO).	2.9 (s, 3H, CH_3); 6.8 - 8.0 (m, 8H, Ar-H and 2NH_2); 9.0 (s, 1H, NH).
3m	3390 - 3015 (NH_2-NH); 2205 (CN); 1657 (CO).	

10.2. Method B for Preparation of 6a

A solution of **3c** (0.01 mol) in acetic anhydride (20 mol) was heated under refluxe for 5h. The solid product so formed after cooling was filtered off and recrystallized from DM/Ethanol as yellow crystals; yield 45%; m p and mixed m p as 6a.

11. 7-Amino-2,9-Dimethyl-4-OXO-3-Phenylpyrido[3',2':4,5]Thieno[3,2-d] Pyrimidine-8-Carbonitrile (6b)

Compound **6b** was crystallized from DMF/Dioxan as yellow crystals; yield 45%; mp > 360˚C; IR ν cm^{-1} 3355 - 3220 (NH_2); 3220 - 3150 (NH); 2200 (CN); 1655 (CO); Found: C, 62.5; H, 3.9: N, 20.3; calcd for $C_{18}H_{13}N_5OS$: C, 62.23; H, 3.77; N, 20.16%.

12. 7-Amino-9-Methyl-4-Oxo-3H-Pyrido[3',2':4,5]Thieno[3,2-d] Pyrimidine-8-Carbonitrile 7

12.1. Method A

A solution of **3a** (0.01mol) in formamide (10 mol) was heated under reflux for 2 h. The reaction mixture was poured on ice water. The solid product formed was filtered off, washed with water several times, dried and re-crystallized from ethanol as red crystals; yield 37%; mp 230˚C; IR ν cm^{-1} 3370 3220 (NH_2); 3220 - 3165 (NH); 2190 (CN); 1663 (CO) MS: *m/z* = 257; Found: C, 51.5; H, 2.8; N, 27.6; S, 12.7; calcd for $C_{12}H_7N_5OS$: 51.36; H, 2.72; N, 27.73; S, 12.46%.

12.2. Method B

A suspension of **3c** (0.01 mol) and triethylorthoformete (3 mol) in acetic anhydride (30 ml) was refluxed for 3 h. The reaction mixture was poured on water and left to stand overnight. The solid precipitate formed was filtered off and recrystallized from ethanol as red crystals; yield 40%; mp and mixed mp as 7.

12.3. Method C

Compound **3c** (2 g) was dissolved in formic acid (20 ml) and heated under refluxe for 3 h. The solid product thus formed on cooling was collected by filtration and recrystallized from ethanol as red crystals; yield 41%; mp and mixed mp as 7.

13. 3,6-Diamino-2-Carbohydrazido-4-Methylthieno[2,3-b] Pyridine-5-Carbonitrile 8

To a solution of **3a** (0.01 mol) in ethanol (30 ml), the hydrazine hydrate (0.02 mol) was added. The reaction mixture was refluxed for 3 h. The solid product formed was collected by filtration and recrystallized from DMF/Dioxan as white crystals; yield 66%; mp 295˚C; IR ν cm^{-1} 3400 - 3220 (NH_2); 3220 - 3100 (NH); 2195 (CN); 1650 (CO); MS: *m/z* = 262; Found: C, 45.9; H, 3.7; N, 32.5; S, 12.4; calcd for $C_{10}H_{10}N_6OS$: C, 54.79; H, 3.84; N, 32.04; S, 12.22%.

14. Ethyl-2-Amino-3-Cyano-4-Methyl-5-(Benzoylthiourea)Thieno[2,3-b] Pyridine-6-CArboxylate 9

To a solution of **3a** in anhydrous acetone, benzoyl isothiocyanate (prepared in situ by refluxed mixture of benzoyl chloride (0.1 mol) and ammonium thiocyanate (0.1 mol) in anhydrous acetone for ten minutes) was added. The reaction mixture was refluxed for 3 hours, then poured onto cold water. The precipitate was collected by filtration, repeatedly washed with cold water and recrystallized from ethanol as orange crystals; yield 50% mp 145˚C - 150˚C; IR ν cm^{-1} 3340 - 3200 (NH_2-NH); 2190 (CN); 1780 (CO) ester; 1650 (CO); MS: *m/z* = 439; Found: C, 55.9; H, 3.9; H, 15.0; S, 15.1; calcd for $C_{20}H_{17}N_5O_3S_2$: C, 54.66; H, 3.87; N, 15.94; S, 14.59%.

15. 7-Amino-9-Methyl-8-Cyano-4-Oxo-1,2,3,4-Tetrahydropyrido [3',2':4,5]Thieno[3,2-d]Pyrimidine-2-Thiol 10

A sample of compound **9** (1 g) was dissolved in 2N ethanolic sodium hydroxid solution (30 ml) and refluxed for 6 h. The reaction mixture was poured onto ice/water and acidified with 10% HCl. The solid formed was collected by filtration and recrystallized from DMF/water as brown crystals; yield 66%; mp > 350˚C; IR ν cm^{-1} 3300 - 3200 (NH_2); 2200 (CN); 1640 (CO); MS: *m/z* = 289; Found: C, 45.8; H, 2.5; N, 25.0; s, 22.4; calcd for $C_{11}H_7N_5OS_2$: C, 45.67; H, 2.42; N, 24.22; 22.16%.

16. Preparation of Compounds 12 and 15. General Procedure

A solution of compound 9 or 14 (1 g) in 98% sulfuric acid (5 ml) was stirred 1 h. then left at room temperature for 5 days. The solid product formed after pouring the clear solution in ice water (100 ml) was collected, wash with water, dried and recrystalized from the appropriate solvents.

17. 2,7-Diamino-9-Methyl-8-Cyano-4-Oxo-2,3,4-Trihydropyrido [3',2',4,5]Thieno[2,3-d]Thiazine 12

Compound **12** was obtained as brown crystals from DMF/water; mp > 350˚C; yield 30%; IR ν cm^{-1} 3375 - 3270 (NH_2), 2220 (CN), 1662 (CO); Found: C, 45.9; H, 2.6; N, 24.4; calcd for $C_{11}H_7N_5OS_2$: C, 45.67; H, 2.42; N, 24.22%.

18. 2,5-Diacetylamino-3-Cyano-4-Methylthieno[2,3-b]Pyridine-6-Carboxylate 13

To a solution of compound **9** (0.01 mol) in acetic acid (30 ml), the appropriate of acetic anhydride (3 ml) was added. The reaction mixture was heated under reflux for 3 h. The solid product formed after cooling was collected by filtration and recrystallization from methanol as orange crystal. mp 330˚C; yield 60%; IR ν cm^{-1} 3330 - 3150 (2NH), 2200 (CN), 1724 (CO ester), 1641(CO)); ^{1}H NMR (DMSO-d6) δ = 1.4 (s, 3H, CH_3); 2.4 (s, 3H, CH_3); 2.4 (br, 6H, $2CH_3$); 4.4 (q, 2H, CH_2); 8.4 (br, 2H, 2NH); Found: C, 53.5; H, 4.8; N, 15.8; S, 9.2; calcd for $C_{16}H_{16}N_4O_4S$: C, 53.32; H, 4.47; N, 15.55; S, 8.90%.

19. 2,5-Diacetylamino-3-Cyano-4-Methylthieno[2,3-b]Pyridine-6-Benzanilide 14

To a solution of compound **13** (0.01 mol) in ethanol (30 ml) the appropriate of aniline (0.01 mol) was added, the reaction mixture was heated under reflux for 3 h. The solid product formed after cooling was collected by filtration and recrystallized from acetic acid as yellow crystals; mp > 350˚C; yield 50%; IR ν cm^{-1} 3450 - 3195 (NH), 2220 (CN), 1670 (CO); ^{1}H NMR (DMSO-d6) δ = 2.3 (s, 3H, CH_3); 2.8 (d, 6H, $2CH_3$); 8.2 (s, 1H, NH); 4.2 - 4.6 (m, 5H, Ar-H); 8.4 (br, 1H, NH); 10.4 (s, 1H, NH); MS: *m/z* = 407; Found: 86.1; H, 4.4; N, 17.4: S, 8.0; calcd for $C_{20}H_{17}N_5O_3S$: C, 85.96; H, 4.21; N, 17.19; S, 7.87%.

20. 7-Acetylamino-2,9-Dimethyl-3-Phenyl-4-Oxo-Pyrido[3',2',4, 5]Thieno[3,2-d] Pyrimidine-8-Carbonitrile 15

Compound **15** was obtained as yellow crystals from dioxan; mp > 350˚C; yield 30%; IR ν cm^{-1} 3330 (NH), 2210 (CN), 1693 (2CO); MS: *m/z* = 389; Found: 61.9; H, 3.9; N, 18.1; S, 18.3; calcd for $C_{20}H_{15}N_5O_2S$: C, 61.68; H, 3.88; N, 17.98; S, 8.23%.

21. Biological Testing

The newly synthesized compounds were dissolved in propylene glycol (10 mg/20ml) and transferred to a filter paper disc (10 mm) diffusion plate method [18]. The bacterial suspension was prepared by adding 20 ml of distilled water to 10-d-old cultures of the test bacteria grown on a nutrient agar of NA. The spore suspension was prepared by adding 20 ml of distilled water to 10-d-od cultures of the test bacteria.

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