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SYNTHESIS, REACTIONS AND BIOLOGICAL ACTIVITY OF 2-SUBSTITUTED 3-CYANO-4,6-DIMETHYLPYRIDINE DERIVATIVES

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The reaction of 2-chloro-4,6-dimethylpyridine-3-carbonitrile with hydrazine hydrate, hydroxylamine, and anthranilic acid afforded the corresponding pyrazolo, isoxazolo, and pyridoquinazoline derivatives. Alkylation of 2-mercapto-4,6-dimethylpyridine-3-carbonitrile with ethyl chloroacetate or phenacyl bromide followed by cyclization in NaOH gave thienopyridine derivatives. Diazotization of ethyl 3-amino-4,6-dimethylthieno[2,3-b]pyridine-2-carboxylate followed by the reaction with thiourea, guanidine carbonate, and hydroxylamine hydrochloride gave the corresponding thienopyridine derivatives. The biological activity of some new compounds has been discussed.

Keywords: isoxazolopyridine, pyrazolopyridine, pyridine-3-carbonitrile, pyridoquinazoline, antibacterial activity.

In continuation of our previous works [1, 2] on sulfur-containing compounds annulated with various five- and six-membered heterocyclic compounds and the considerable biological activity of pyridine derivatives as fungicidal, antibacterial, antifungal [3, 4], antimycotic [5], and antidepressant agents [6], as well as thienopyridines as antithrombotic agents [7, 8] against the platelet aggregation, we study the synthesis of several new pyridine derivatives.

In the present investigation 2-hydroxy-4,6-dimethylpyridine-3-carbonitrile (**1**) has been prepared *via* the reaction of cyanoacetamide and acetylacetone in boiling *n*-butanol in the presence of a few drops of piperidine. The treatment of compound **1** with $POCl_3$ gave 2-chloro-4,6-dimethylpyridine-3-carbonitrile (**2**). IR spectrum of compound **2** showed an absorption band at 2240 cm^{-1} (CN) with the absence of on OH group absorption band. ^1H NMR spectrum of compound **2** showed signals at 2.3 (3H, s, CH_3); 2.5 (3H, s, H_3C–C=N), and 7.7 ppm (1H, s, H-5).

The reaction of compound **2** with hydrazine hydrate in boiling *n*-butanol [9] gave 3-amino-4,6-dimethylpyrazolo[3,4-*b*]pyridine (**3**). The structure of compound **3** was confirmed by its analytical and spectral data. IR spectrum of compound **3** showed absorption bands at 1620 (C=N), 3300 (NH), and 3470 cm^{-1} (NH_2), while ^1H NMR spectrum showed signals at 2.3 (3H, s, CH_3); 2.5 (3H, s, H_3C–C=N); 4.0 (1H, s, NH); 7.1 (1H, s, H-5), and 12.5 ppm (2H, s, NH_2).

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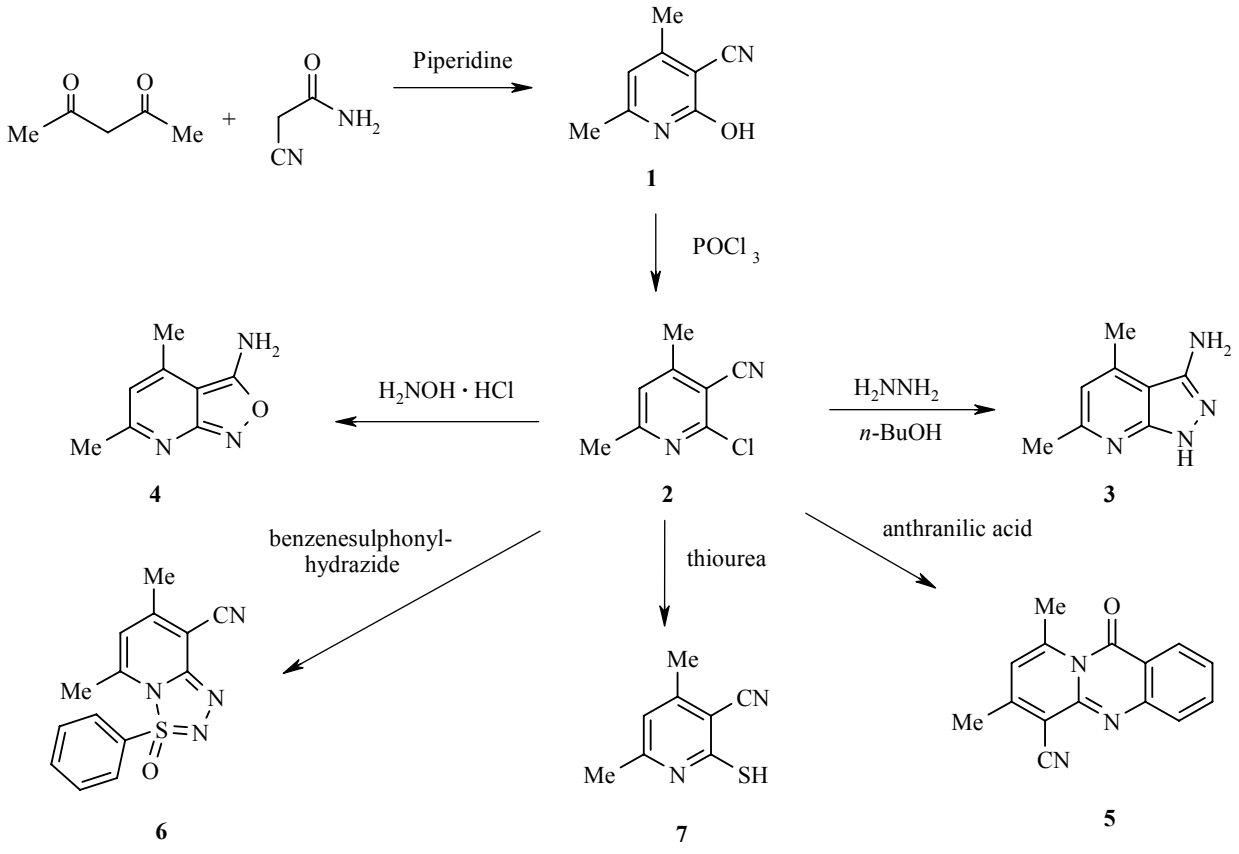
The treatment of 2-chloro-4,6-dimethylpyridine-3-carbonitrile **2** with hydroxylamine hydrochloride in refluxing dry toluene in the presence of a few drops of triethylamine afforded 3-amino-4,6-dimethylisoxazolo-[3,4-*b*]pyridine (**4**). IR spectrum of compound **4** showed absorption bands at 3300–3430 (NH_2) and 1620 cm^{-1} (C=N). ^{1}H NMR spectrum of compound **4** showed signals at 2.3 (3H, s, CH_3); 2.5 (3H, s, H_3C–C=N); 4.1 (2H, s, NH_2), and 7.1 ppm (1H, s, H-5). On the other hand, when compound **2** was allowed to react with anthranilic acid it gave 1-cyano-2,4-dimethylpyrido[2,1-*b*]quinazolin-5-one (**5**). The structure of compound **5** was elucidated by its correct analytical and spectral data. IR spectrum showed absorption bands in the regions of 2220 (CN), 1667 (C=O), and 1618 cm^{-1} (C=N), respectively. ^{1}H NMR spectrum of compound **5** showed signals at 2.3 (3H, s, CH_3); 2.5 (3H, s, H_3C–C=N); 7.3 (1H, s, H-3); and 7.1–7.5 ppm (4H, m, arom). Also, when compound **2** was allowed to react with benzenesulfonylhydrazide in refluxing *n*-butanol [10] it yielded the corresponding 7-cyano-4,6-dimethyl(3-oxo-3-phenyl)thia[1,2,4]triazolo[4,5-*a*]pyridine (**6**) (Scheme 1).

Scheme 1



The structure of compound **6** was confirmed by its analytical and spectral data. IR spectrum of compound **6** showed absorption bands at 2220 (CN), 1610 (C=N), 1330 (S=O), and 1370 cm^{-1} (S=N), while its ^{1}H NMR spectrum showed signals at 2.4 (3H, s, CH_3); 2.7 (3H, s, H_3C–C=N); 7.3 (1H, s, H-5); and 7.5-8.0 ppm (5H, m, arom).

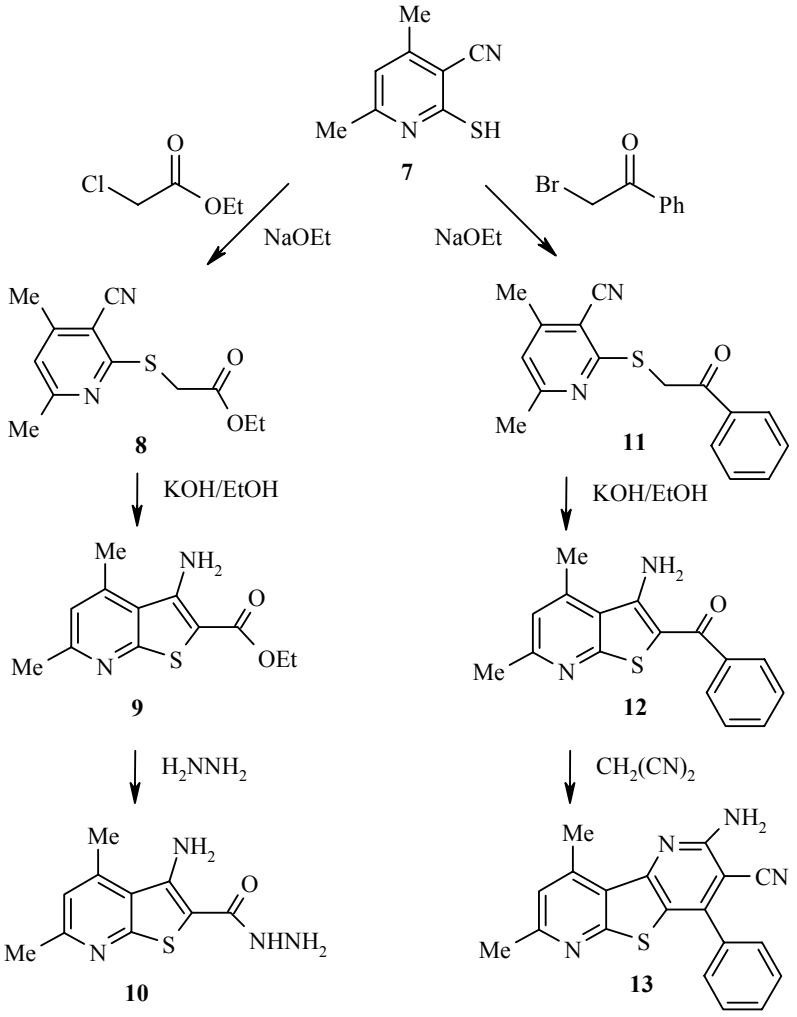
The reaction of 2-chloro-4,6-dimethylpyridine-3-carbonitrile **2** with thiourea in *n*-butanol gave the corresponding 2-mercapto-4,6-dimethylpyridine-3-carbonitrile (**7**). When compound **7** was allowed to react with ethyl chloroacetate in the presence of sodium ethoxide [11] it yielded ethyl 2-(3-cyano-4,6-dimethylpyrid-2-ylthio)acetate (**8**), which on cyclization in 10% ethanolic KOH afforded 3-amino-2-ethoxy-carbonyl-4,6-dimethylthieno[2,3-*b*]pyridine (**9**). The structures of compounds **8** and **9** were confirmed by their correct

analytical and spectral data. IR spectrum of compound **8** showed absorption bands at 1620 (C=N), 1720 (C=O of ester), and 2220 cm^{-1} (CN), while ^1H NMR spectrum of compound **8** showed signals at 1.5 (3H, t, $CH_2C\underline{H}_3$); 2.5 (3H, s, CH_3); 2.9 (3H, s, $H_3C–C=N$); 3.6 (2H, q, $C\underline{H}_2CH_3$), and 7.2 ppm (1H, s, H-5). IR spectrum of compound **9** showed the disappearance of (CN) band, while ^1H NMR spectrum showed signals at 1.3 (3H, t, $CH_2C\underline{H}_3$); 2.3 (3H, s, CH_3); 2.7 (3H, s, $H_3C–C=N$); 4.1 (2H, q, $C\underline{H}_2CH_3$), and 7.2 (1H, s, H-5). Chemically the structure of compound **9** was confirmed through its reaction with hydrazine hydrate in refluxing ethanol to give the corresponding 3-amino-2-(hydrazinocarbonyl)-4,6-dimethylthieno[2,3-*b*]pyridine (**10).**

IR spectrum of compound **10** showed absorption bands at 1620 (C=N), 1630 (CO, hydrazide), and 3470–3140 cm^{-1} (NH and two NH_2), while its ^1H NMR spectrum showed signals at 2.3 (3H, s, CH_3); 2.7 (3H, s, $H_3C–C=N$); 3.6 (2H, s (br), $CONHN\underline{H}_2$); 5.5 (1H, s (br), $CON\underline{H}NH_2$), and 7.3 ppm (1H, H-5).

Analogously, alkylation of pyridinethione **7** with phenacyl bromide in the presence of sodium ethoxide yielded 4,6-dimethyl-2-[(2-oxo-2-phenylethyl)thio]nicotinonitrile (**11**), which on cyclization in 10% KOH gave 3-amino-2-benzoyl-4,6-dimethylthieno[2,3-*b*]pyridine (**12**). IR spectrum of compound **11** showed absorption bands at 1620 (C=N) and 2220 cm^{-1} (CN), while ^1H NMR sprectrum of compound **11** showed signals at 2.3 (3H, s, CH_3); 2.7 (3H, s, $H_3C–C=N$); 4.1 (2H, s, CH_2S); and 7.3 ppm (6H, m, arom). When compound **12** was allowed to react with malononitrile in refluxing dimethylformamide in the presence of anhydrous potassium carbonate, it yielded 2-amino-3-cyano-7,9-dimethyl-4-phenylpyrido[5,4-*b*]thieno[2,3-*b*]pyridine (**13**). IR spectra

Scheme 2



of compounds **9** and **12** were found free from the nitrile function and instead the bands of the newly born NH_2 group were found. Moreover, the signals of methylene CH_2 protons were not revealed in ^1H NMR spectrum, proving that they were involved in the cyclization step through addition to the nitrile group (Scheme 2).

Diazotization of compound **9** by adding sodium nitrite solution in water afforded the corresponding 3-diazo-2-ethylcarbonyl-4,6-dimethylthieno[2,3-*b*]pyridine (**14**), which on reaction with thiourea, guanidine carbonate, and hydroxylamine hydrochloride gave the corresponding 7,9-dimethyl-2-thioxo-1,3-dihydropyrido[5,4-*b*]thieno[3,2-*d*]pyrimidin-4-one (**15**), 2-amino-7,9-dimethylpyrido[5,4-*b*]thieno[3,2-*d*]pyrimidin-4-one (**16**), and 6,8-dimethylisoxazolo[4,3-*b*]thieno[5,4-*b*]pyridin-3-one (**17**), respectively.

The structure of the tricyclic compounds **15**, **16**, and **17** was elucidated by their correct analytical and spectral data. IR spectrum of compound **15** showed absorption bands at 3323 (NH–CO), 3188 (NH), and 1638 cm^{-1} (C=O). ^1H NMR of compound **15** showed signals at 2.4 (3H, s, CH_3); 2.6 (3H, s, H_3C–C=N); 5.4 (1H, s, HN–C=S); 7.1 (1H, s, H-8), and 10.6 (s, CONH), while IR spectrum of compound **16** showed absorption bands at 3300–3160 (NH_2), 3473 (NH), 1676 (C=O), and 1599 $^{-1}$ (C=N). ^1H NMR spectrum of compound **16** showed signals at 2.3 (3H, s, CH_3); 2.5 (3H, s, H_3C–C=N); 7.2 (1H, s, arom); 8.2–8.8 (3H, br, NH and NH_2). IR spectrum of compound **17** showed absorption bands in the regions of 3150 (NH), 1710 (C=O), 1593 $^{-1}$ (C=N). ^1H NMR spectrum of compound **17** showed signals at 2.4 (3H, s, CH_3); 2.6 (3H, s, H_3C–C=N); 5.1 (1H, s, NH), and 7.1 (1H, s, arom) (Scheme 3). Characterization and physical data of compounds **1–17** are listed in Table 1.

TABLE 1. Characterization and Physical Data of Compounds **1–17**

Compound	Empirical formula	Found, % / Calculated, %				mp, °C*	Yield, %
		C	H	N	S		
1	$C_8H_8N_2O$	64.54 / 64.58	5.40 / 5.44	18.78 / 18.91		288	85
2	$C_8H_7ClN_2$	57.60 / 57.64	4.02 / 4.03	16.76 / 16.81		90	82
3	$C_8H_{10}N_4$	59.01 / 59.24	6.11 / 6.21	34.35 / 34.54		280	80
4	$C_8H_9N_3O$	58.79 / 58.88	5.53 / 5.56	25.59 / 25.75		252	73
5	$C_{15}H_{11}N_3O$	72.26 / 72.05	4.43 / 4.45	16.79 / 16.86		230	70
6	$C_{14}H_{12}N_4OS$	59.11 / 59.19	4.20 / 4.22	19.69 / 19.72	11.23 / 11.26	198	75
7	$C_8H_8N_2S$	58.44 / 58.51	4.87 / 4.91	17.00 / 17.06	19.48 / 19.52	180	59
8	$C_{12}H_{14}N_2O_2S$	57.54 / 57.58	5.60 / 5.64	11.00 / 11.19	12.77 / 12.81	90	80
9	$C_{12}H_{14}N_2O_2S$	57.55 / 57.58	5.58 / 5.64	11.11 / 11.19	12.75 / 12.81	135	63
10	$C_{10}H_{12}N_4OS$	50.22 / 50.38	5.04 / 5.12	23.66 / 23.71	13.46 / 13.57	198	65
11	$C_{16}H_{14}N_2OS$	68.00 / 68.06	5.00 / 5.00	9.82 / 9.92	11.32 / 11.36	140	62
12	$C_{16}H_{14}N_2OS$	68.02 / 68.06	5.00 / 5.00	9.85 / 9.92	11.31 / 11.36	200	72
13	$C_{19}H_{14}N_4S$	69.85 / 69.90	4.17 / 4.20	16.92 / 16.96	50.14 / 50.17	286	78
15	$C_{11}H_9N_3OS_2$	50.14 / 50.17	3.39 / 3.44	15.90 / 15.96	24.29 / 24.35	270	59
16	$C_{11}H_{10}N_4OS$	53.58 / 53.64	4.07 / 4.09	22.69 / 22.75	13.00 / 13.02	230	66
17	$C_{10}H_8N_2O_2S$	54.47 / 54.53	3.61 / 3.66	12.69 / 12.72	14.51 / 14.56	280	77

* Solvent: EtOH (compounds **1, 3, 7, 9, 10, 17**), MeOH (compound **2**), xylene (compounds **4–6**), benzene (compounds **8, 16**), AcOH (compounds **11, 12**), $CHCl_3$ (compound **13**), BuOH (compound **15**).

Scheme 3

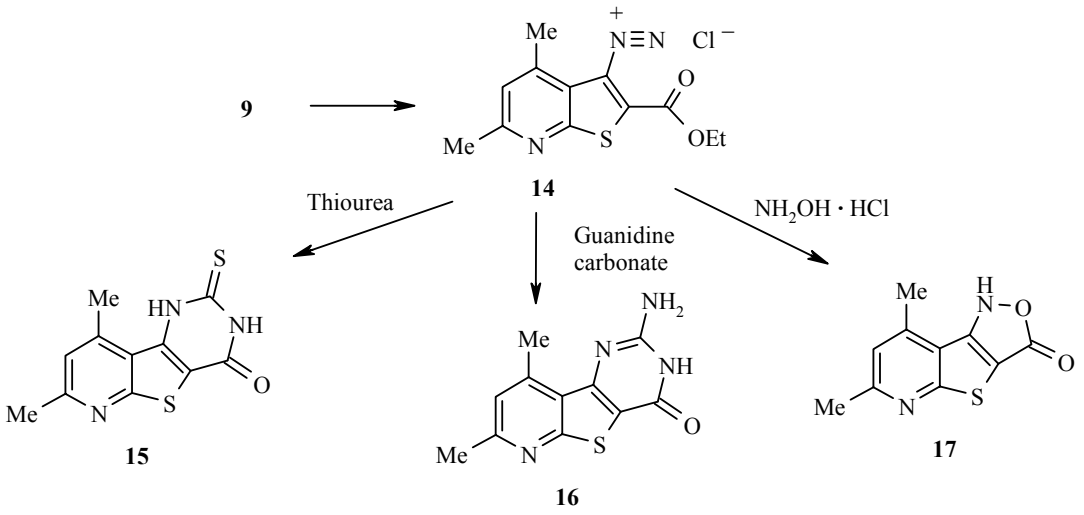


TABLE 2. Antimicrobial Activity of Compounds **3–5, 9–13, 15**

Compound	Solvent	Inhibition zone diameter, mm				
		Staphylococcus aureus	*Proteus vulgaris*	*Klebsiella Spp*	*Pseudomonas aeruginosa*	*Escherichia coli*
3	EtOH	8	12	12	12	12
4	EtOH	12	12	12	18	12
5	EtOH	8	—	18	8	12
9	EtOH	8	8	18	18	12
10	Acetone	8	8	18	12	8
11	Acetone	8	—	18	12	12
12	Acetone	12	8	18	18	12
13	Acetone	8	—	12	8	18
15	AcOEt	8	—	18	18	12

The biological effect of compounds **3**–**5**, **9**–**13**, and **15** against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella Spp*, and *Proteus vulgaris*) has been studied. The antimicrobial activity results are listed in Table 2.

EXPERIMENTAL

All melting points were uncorrected. IR spectra were measured in KBr on a Bruker FT-IR ISS 25 spectrophotometer (ν_{max}, cm^{-1}). ^1H NMR spectra (DMSO-d_6 and $CDCl_3$) were determined on a Bruker Avance (300 MHz) spectrometer using TMS as an internal reference.

2-Hydroxy-4,6-dimethylpyridine-3-carbonitrile (1). A mixture of cyanoacetamide (2 g, 0.02 mol), acetyl acetone (1.7 ml, 0.02 mol), and piperidine (3 ml) was refluxed for 6 h in *n*-butanol (20 ml) and the solid precipitate was collected and recrystallized from ethanol to give a white crystalline solid.

2-Chloro-4,6-dimethylpyridine-3-carbonitrile (2). A mixture of compound **1** (0.01 mol) with excess phosphorous oxychloride (30 ml) was refluxed for 4 h. The reaction mixture was left to cool; the excess $POCl_3$ was evaporated under vacuo, the precipitate was washed several times with water, filtered off, and recrystallized from methanol to give a white crystalline solid.

3-Amino-4,6-dimethylpyrazolo[3,4-*b*]pyridine (3). A mixture of compound **2** (0.01 mol) and hydrazine hydrate (0.03 mol) was refluxed for 6 h in *n*-butanol (20 ml). The reaction mixture was left to cool, and the precipitate was collected and recrystallized from ethanol to give a yellow crystalline solid**.**

3-Amino-4,6-dimethylisoxazolo[3,4-*b*]pyridine (4). A mixture of compound **4** (0.01 mol) and hydroxylamine hydrochloride (0.01 mol) was refluxed for 6 h in dry toluene (20 ml) in the presence of triethylamine (TEA) (3 ml). The reaction mixture was left to cool, and the precipitate was collected and recrystallized from xylene to give white crystals.

1-Cyano-2,4-dimethylpyrido[2,1-*b*]quinazolin-5-one (5). A mixture of compound **2** (0.01 mol) and anthranilic acid (0.01 mol) was refluxed for 10 h in *n*-butanol (30 ml). The reaction mixture was left to cool, and the precipitate was collected and crystallized from xylene to give a brown crystalline solid.

7-Cyano-4,6-dimethyl(3-oxo-3-phenyl)thia[1,2,4]triazolo[4,5-*a*]pyridine (6). A mixture of compound **2** (0.01 mol) and benzenesulfonylhydrazide (0.01 mol) was refluxed for 10 h in *n*-butanol (30 ml). The reaction mixture was left to cool, and the precipitate was collected and recrystallized from xylene to give a yellow solid.

2-Mercapto-4,6-dimethylpyridine-3-carbonitrile (7). A mixture of compound **2** (0.01 mol) and thiourea 0.5 g (0.01 mol) in *n*-butanol (20 ml) was refluxed for 6 h. The precipitate was washed with *n*-butanol and recrystallized from ethanol.

Ethyl 2-(3-Cyano-4,6-dimethylpyridine-2-ylthio)acetate (8). A mixture of compound **7** (0.01 mol) and ethyl chloroacetate (0.01 mol) was treated with sodium metal (0.01 mol) in ethanol (30 ml) and stirred for 2 h, then poured gradually with stirring into ice-cold water; the solid formed was collected and recrystallized from benzene to give compound **8**.

3-Amino-2-ethoxycarbonyl-4,6-dimethylthieno[2,3-*b*]pyridine (9). Compound **8** (0.01 mol) was treated with 10% KOH (0.01 mol) in ethanol (30 ml), stirred for 2 h, and neutralized with diluted HCl; the precipitated solid formed after neutralization was collected by filteration, dried, and recrystallized from ethanol to give compound **9**.

3-Amino 2-(Hydrazinocarbonyl)-4,6-dimethylthieno[2,3-*b*]pyridine (10). A mixture of compound **9** (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (30 ml) was heated under reflux for 5 h, and the reaction mixture was left to cool. The precipitate was collected and recrystallized from ethanol to give compound **10**.

4,6-Dimethyl-2-[(2-oxo-2-phenylethyl)thio]nicotinonitrile (11). A mixture of compound **7** (0.01 mol) and phenacyl chloride (0.01 mol) was treated with sodium metal (0.01 mol) in ethanol (30 ml) and stirred for 2 h, then poured gradually with stirring into ice-cold water. The precipitate was collected and crystallized from acetic acid to give compound **11**.

3-Amino-2-benzoyl-4,6-dimethylthieno[2,3-*b*]pyridine (12). Compound **11** (0.10 mol) was treated with 10% KOH in ethanol (30 ml) and stirred for 2 h, and neutralized with diluted HCl; the precipitated solid formed after neutralization was collected by filteration, dried, and recrystallized from acetic acid to give compound **12** in good yield.

2-Amino-3-cyano-7,9-dimethyl-4-phenylpyrido[5,4-*b*]thieno[2,3-*b*]pyridine (13). A mixture of compound **12 (**2.8 g, 0.01 mol) and malononitrile (0.5 ml, 0.01 mol) was heated under reflux for 9 h in dimethylformamide (20 ml) and anhydrous potassium carbonate (1 g). The reaction mixture was left to cool and then poured into ice-cold water. The precipitate was collected and crystallized from chloroform to give brown crystals of compound **13**.

3-Diazo-2-ethoxycarbonyl-4,6-dimethylthieno[2,3-*b*]pyridine (14). A solution of sodium nitrite (4 g) in water (20 ml) was added slowly to an ice-cold solution of compound **9** (0.01 mol) in diluted hydrochloric acid (25 ml). The resulting diazonium salt solution was stirred at 0°C for 15 min and the diazonium salt solution formed was used *in situ*.

7,9-Dimethyl-2-thioxo-1,3-dihydropyrido[5,4-*b*]thieno[3,2-*d*]pyrimidin-4-one (15). A solution of thiourea (0.5 g, 0.01 mol) was added to diazonium salt solution **14**, and the mixture was stirred for 5 h, then

concentrated by evaporation. The separated solid produced was collected by filteration, dried, and crystallized from *n*-butanol to give compound **15**.

2-Amino-7,9-dimethylpyrido[5,4-*b*]thieno[3,2-*d*]pyrimidin-4-one (16). Diazonium salt solution **14** was added to the solution of guanidine carbonate (0.15 g, 0.01 mol) in water (10 ml), and the reaction mixture was stirred for 5 h; the obtained solid was collected by filtration, dried, and recrystallized from benzene to give a yellow crystalline solid.

6,8-Dimethylisoxazolo[4,3-*b*]thieno[5,4-*b*]pyridin-3-one (17). Hydroxylamine hydrochloride (1 g, 0.03 mol) was added to diazonium salt solution **14**, and the reaction mixture was stirred for 5 h; the solid produced was collected and recrystallized from ethanol to give a white crystalline solid.

Method for Testing of Biological Activity.

The biological activity of the tested compounds has been evaluated using filter paper disk method after dissolving the substances in the appropriate solvent indicated in Table 2. The inhibition zones of microbial growth surrounding the paper disk were measured in millimeters at the end of the incubation period [12] (18–24 h at 27^{o}C).

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