

Synthesis and Biological Evaluation of Some Novel Thienopyridines

Abd El-Badih A. G. Ghattas, Ahmed. Khodairy, Hassan M. Moustafa and Bahgat R. M. Hussein*.

Chemistry Department, Faculty of Science, Sohag University, Sohag 82524, Egypt.

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Abstract: Alkylation of 4,6-diamino-3-cyanopyridine-2(1*H*)-thione (**1**) with active halo compounds namely; ethyl chloroacetate, chloroacetic acid, 2-chloro-*N*-phenylacetamide, chloroacetonitrile, or chloroacetamide gave *S*-alkyl derivatives **2-6**, respectively. Thienopyridine derivatives **7-10** were furnished by heating of *S*-alkyl derivatives **2-6** in hot KOH solution or by reaction of compound **1** with appropriate halo compound directly in hot KOH solution. The reaction of compound **1** with 3-bromopentane-2,4-dione or ethyl 2-bromo-3-oxobutanoate afforded 2-acetylthienopyridine **11**. Cyanoethylthienopyridine **12** yielded *via* the addition of acrylonitrile with compound **1**. The potency of the results as antibacterial and antifungal agents has been evaluated. The compounds have been characterized based on their IR, ¹H NMR, ¹³C NMR and elemental analysis.

Keywords: Thienopyridines, *S*-alkylpyridines, Antibacterial agents, Antifungal agents.

1 Introduction

Pyridine derivatives one of the most important organic compounds used greatly in the pharmaceutical industry, forms the nucleus of over 7000 existing drugs^[1] and are reported as antiviral,^[2] antifungal,^[3] antibacterial,^[4,5] anti-inflammatory,^[6,7] antimicrobial,^[8] anticancer,^[8,9] antichagasic,^[10] antioxidant,^[11] antidote^[12] and antidiabetic activity.^[13] Also, thienopyridines one of the important fused heterocyclic compounds which are reported as drugs namely: Prasugrel,^[14] Ticlopidine and Clopidogrel,^[15] to irreversible ADP receptor/P2Y₁₂ inhibitors which are used for their antiplatelet activity.^[16] Thienopyridines are reported as anticancer,^[17] antibacterial,^[18] antifungal,^[19] anti-inflammatory,^[20] and antimicrobial.^[21] In view of these findings and in continuation of our previous work,^[22-25] we report herein synthesis of some new thienopyridines and study their antibacterial and antifungal activities.

2 Results and Discussion

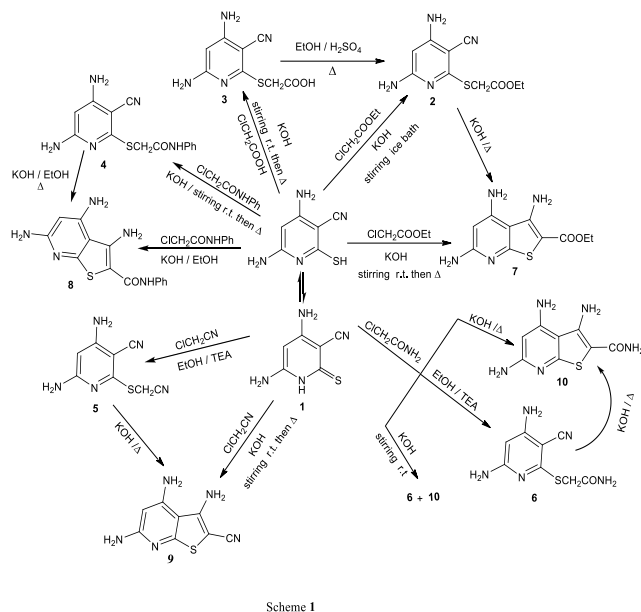
2.1 Chemistry

4,6-Diamino-3-cyanopyridine-2(1*H*)-thione(**1**)^[26,27] reacted with active halo compounds namely; ethyl chloroacetate, chloroacetic acid, 2-chloro-*N*-phenylacetamide in aqueous KOH; chloroacetonitrile or chloroacetamide in EtOH and TEA as catalyst to give the corresponding *S*-alkyl derivatives **2-6**, respectively (cf. Scheme 1). Compound **2** was synthesized, *via* esterification of

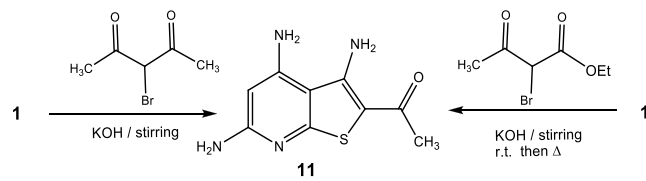
compound **3**. The structure of these compounds was characterized on the basis of their spectroscopic and elemental analyses (cf. Experimental section). IR spectrum (KBr, cm⁻¹) of compound **2** showed the absence of absorption band corresponding to (NH) group in pyridine ring, while exhibiting characteristic new absorption bands corresponding to CH_{aliphatic} at 2985, 2926 and C=O_{ester} at 1723. Its ¹H NMR spectrum (DMSO-*d*₆, δ) showed the absence of absorption signal corresponding to NH group in pyridine ring, while exhibiting characteristic absorption signals corresponding to quartet CH_{2 ester} at 4.13-4.08, singlet SCH₂ at 4.01 and triplet CH₃ at 1.21-1.17 respectively. Its ¹³C NMR spectrum (DMSO-*d*₆, δ) showed a new signals corresponding to C=O_{ester} 169.45, CH₂ groups at 61.42, 31.59 and CH₃ group at 14.46. Thienopyridine derivatives **7-10** were synthesized *via* the reaction of compound **1** with respective halo compound in hot KOH or *via* treatment of *S*-alkyl derivatives **2-6** with hot KOH solution (cf. Scheme 1). IR spectrum (KBr, cm⁻¹) of compound **7** showed disappearance of absorption band corresponding to C≡N group. Its ¹H NMR spectrum (DMSO-*d*₆, δ) showed disappearance of S-CH₂ signal and appeared new signal corresponding to the NH₂ group at 6.79 ppm. The structure of compound **8** was corroborated by X-ray crystallography.²⁸ Mixture of compounds **6** (75%), **10** (25%) was formed when compound **1** reacted with chloroacetamide in aqueous KOH solution.

Treatment of compound **1** with 3-bromopentane-2,4-dione or 2-bromo-3-oxobutanoate afforded the same product 2-acetylthienopyridine **11**. The reaction took place *via*

deacetylation or dealkoxycarbonylation reaction^[29-32] (cf. Scheme 2). Its ¹H NMR spectrum (DMSO-*d*₆, δ) showed absorption signal corresponding to the NH₂ group at 7.82 and a singlet signal at 2.16 for (CH₃ group).

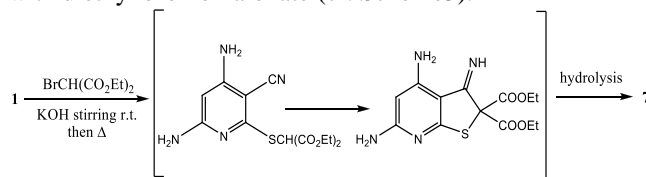


Scheme 1. Reaction of 4,6-diamino-3-cyanopyridine-2(1H)-thione with ethyl chloroacetate, chloroacetic acid, 2-chloro-N-phenylacetamide, chloroacetonitrile, and chloroacetamide.



Scheme 2. Reaction of 4,6-diamino-3-cyanopyridine-2(1H)-thione with 3-bromopentane-2,4-dione or 2-bromo-3-oxobutanoate.

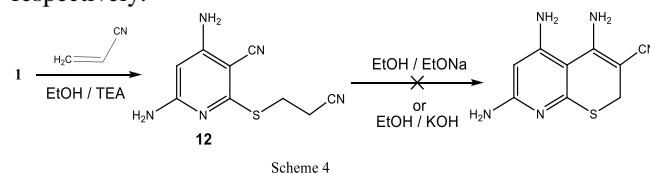
Compound **7** was obtained also by reaction of compound **1** with diethyl bromomalonate (cf. Scheme3).



Scheme 3. Reaction of 4,6-diamino-3-cyanopyridine-2(1H)-thione with diethyl bromomalonate.

Treatment of compound **1** with acrylonitrile afforded S-alkyl derivative **12**. Attempts to cyclize compound **12** in

hot sod. ethoxide or pot. hydroxide solution were failed (cf. Scheme 4). ¹H NMR spectrum (DMSO-*d*₆, δ) of compound **12** showed new absorption signals corresponding to S-CH₂ and CH₂-CN as doublet at 3.34-3.32 and 2.95-2.92, respectively.



Scheme 4. Reaction of 4,6-diamino-3-cyanopyridine-2(1H)-thione with acrylonitrile.

2.2 Antimicrobial Studies

All synthesized new substituted pyridines and fused thienopyridines were screened for antibacterial and antifungal activity. The study is carried out by cup plate method to determine the zone of inhibition against four strains of bacteria and four strains of fungi.

2.3 Anti-bacterial Activity

The compounds were dissolved in dimethyl sulfoxide, in order to ensure that the solvent had no effect on bacterial growth or enzymatic activity, negative control tests were performed using DMSO at the same concentrations. The inhibitory effect of compounds **2**, **3**, **5-11** and **12** *in vitro* growth of broad spectrum of bacteria representing two species of Gram positive bacteria, namely *Bacillus cereus* and *Micrococcus latus* and two species of Gram negative bacteria, namely *Pseudomonas aeruginosa* and *Escherichia coli* was evaluated using the agar diffusion method (cup and plate method)^[33] by measuring the zone of inhibition on agar plates at three different concentrations 10,000 ppm, 30,000 ppm and 50,000 ppm. DMSO was used as solvent control. All plates were incubated at 37±0.5 °C for 24h. The zone of inhibition of compounds was measured using mm scale. The results are summarized in Table 1.

The zone of inhibition of all compounds was fixed or increased by increasing the concentrations. Thienopyridines **7-11** display good activities against all types of bacteria but possess poor activities against *Pseudomonas aeruginosa*, except compound **8** showed the highest inhibitory effect against it. Compound **7** showed the highest inhibitory effect against *Escherichia coli*. S-Alkyl derivatives **2**, **3**, **5**, **6** and **12** showed more activities against Gram negative bacteria, but possess moderate to poor activities against Gram negative bacteria. Compounds **3** and **12** showed the highest inhibitory effect against *Micrococcus latus* and *Bacillus cereus*, respectively. Compounds **5**, **8** and **12** showed the highest inhibitory effect against *Bacillus cereus* and *Micrococcus latus* at high concentration (cf. Table 1).

Table 1. Antibacterial activity of the tested pyridine derivatives **2**, **3**, **5-11** and **12** by measuring inhibition zone (mm).

Type	<i>Bacillus cereus</i>			<i>Micrococcus latus</i>			<i>Pseudomonas aeruginosa</i>			<i>Escherichia coli</i>		
	Concentrations			Concentrations			Concentrations			Concentrations		
	A	B	C	A	B	C	A	B	C	A	B	C
Comp.	A	B	C	A	B	C	A	B	C	A	B	C
2	3	5	14	3	4	6	-	-	-	2	7	14
3	3	5	10	10	11	24	-	-	12	2	5	10
5	2	4	9	-	-	3	-	-	-	-	-	-
6	5	6	9	2	4	8	-	-	-	-	-	-
7	7	12	13	14	18	20	-	-	-	9	16	19
8	2	4	9	-	-	3	-	4	25	5	10	13
9	15	17	19	3	6	12	-	-	-	4	5	8
10	8	10	13	5	7	9	-	-	-	3	5	8
11	9	15	18	5	7	13	-	2	4	4	6	8
12	11	14	22	-	4	10	-	-	-	5	9	17

A= concentration of comp. = 10000 ppm.
 B= concentration of comp. = 30000 ppm.
 C= concentration of comp. = 50000 ppm.

2.4 Anti-fungal Activity

The compounds were dissolved in dimethyl sulfoxide, in order to ensure that the solvent had no effect on fungicidal growth or enzymatic activity, negative control tests were performed using DMSO at the same concentrations. The inhibitory effect of compounds **2**, **3**, **5-11** and **12** *in vitro* growth of broad spectrum of fungi representing four species of fungi, namely *Aspergillus plavus*, *Pichia anomola*, *Penicillium griseoflvum* and *Aspergills ochraceous* and was evaluated using the agar diffusion method (cup and plate method)^[33] by measuring the zone of inhibition on Czapek's agar plates at three different concentrations 10,000 ppm, 30,000 ppm and 50,000 ppm. DMSO was used as solvent control. All plates were incubated at 28±0.5 °C for 72h. The zone of inhibition of compounds was measured using mm scale. The results are summarized in Table 2.

Most of compounds possess moderate to poor activities against different type of fungi except thienopyridine **7** display good activities against all types of fungi. S-alkylderivative **2** and thienopyridine **8** showed the highest inhibitory effect against *Aspergills ochraceous* and *Pichia anomola* respectively. Compounds **2**, **3**, **5**, **6**, **9**, **10** and **12** showed the highest inhibitory effect at high concentration (cf. Table 2).

Table 2. Antifungal activity of the tested pyridine derivatives **2**, **3**, **5-11** and **12** by measuring inhibition zone (mm).

Type	<i>Aspergillus plavus</i>			<i>Pichia anomola</i>			<i>Penicillium griseoflvum</i>			<i>Aspergills ochraceous</i>		
	Concentrations			Concentrations			Concentrations			Concentrations		
	A	B	C	A	B	C	A	B	C	A	B	C
Comp.	A	B	C	A	B	C	A	B	C	A	B	C
2	-	-	-	-	-	-	-	-	6	9	10	20
3	-	-	-	-	-	-	-	-	-	-	4	8
5	-	-	-	-	-	-	-	-	-	-	-	13
6	-	-	-	-	-	-	-	-	-	-	2	7
7	4	7	10	4	12	14	7	12	19	7	16	18
8	-	-	-	6	12	17	8	11	14	-	5	12
9	-	-	-	-	-	-	-	-	-	-	5	9
10	-	-	-	-	-	-	-	-	7	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	4	-	-	25	-	-	10	-	4	7

A= concentration of comp. = 10000 ppm.
 B= concentration of comp. = 30000 ppm.
 C= concentration of comp. = 50000 ppm.

4 Experimental

All melting points were recorded by Kofeler melting point apparatus and uncorrected. IR (cm⁻¹) spectra were recorded (KBr disc) on a Shimadzu FT-IR spectrophotometer. ¹H NMR and ¹³C NMR (DMSO-*d*₆) spectra were recorded at 400 MHz on Bruker Bio Spin AG at Sohag University, the chemical shift is expressed in δ value (ppm) using TMS as an internal reference. Elemental analyses were carried out on an elemental analyzer 240°C.

General procedure for synthesis of compounds 2-4:

Compound **1** (0.5 g, 3 mmol) in aqueous solution of KOH (0.17 g, in 30 mL H₂O); 3 mmol of appropriate halo compounds; ethyl chloroacetate (0.36 g), chloroacetic acid (0.28 g), or 2-chloro-N-phenylacetamide (0.5 g) in 5 mL ethanol was added with stirring in ice bath for 1 h, then the reaction mixture was stirred at room temperature for 1 h. The formed precipitate was collected, washed with water and crystallized from the appropriate solvent.

Ethyl [(4,6-diamino-3-cyanopyridin-2-yl)thio]acetate (**2**).

Colourless crystals (benzene), yield 0.63 g (83%), mp. 125-127 °C; IR: 3483, 3429, 3369, 3258 (2NH₂), 2985, 2926 (CH_{aliphatic}), 2199 (C≡N), 1723 (C=O), 1605 (C=N) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 6.30 (s, 2H, NH₂ exchanged by D₂O), 6.26 (s, 2H, NH₂ exchanged by D₂O), 5.45 (s, 1H, CH_{pyridyl}), 4.13-4.08 (q, 2H, *J* = 8 Hz, CH₂), 4.01 (s, 2H, S-CH₂), 1.21-1.17 (t, 3H, *J* = 8 Hz, CH₃) ppm; ¹³C NMR

(DMSO-*d*₆): δ 169.45, 160.69, 160.63, 157.28, 117.01, 85.31, 80.06, 61.42, 31.59, 14.46 ppm. *Anal.* Calcd. For C₁₀H₁₂N₄O₂S (252): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.81; H, 4.52; N, 22.34.

[(4,6-Diamino-3-cyanopyridin-2-yl)thio]acetic acid (3). Pale green powder (ethanol), yield 0.5 g (75%), mp. 235-237 °C; IR: 3412, 3315, 3209 (2NH₂+OH), 2910 (CH_{aliphatic}), 2210 (C≡N), 1637 (C=O) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 12.69 (br, 1H, OH exchanged by D₂O), 6.31 (s, 2H, NH₂ exchanged by D₂O), 6.30 (s, 2H, NH₂ exchanged by D₂O), 5.46 (s, 1H, CH_{pyridyl}), 3.96 (s, 2H, S-CH₂) ppm. *Anal.* Calcd. for C₈H₈N₄O₂S (224): C, 42.85; H, 3.60; N, 24.99. Found: C, 42.67; H, 3.73; N, 24.79.

2-[(4,6-Diamino-3-cyanopyridin-2-yl)thio]-N-phenylacetamide (4). This compound was synthesized previously by stirring of compound **1** with 2-chloro-N-phenylacetamide in DMf and 10% aqueous potassium hydroxide yield (78%), mp. 165-168 °C.^[34]

General procedure for synthesis of compounds 5 and 6.

A mixture of compound **1** (0.5 g, 3 mmol) in ethanol (30 mL), triethylamine (0.3 g, 3 mmol) and chloroacetonitrile (0.22 g, 3 mmol), or chloroacetamide (0.28 g, 3 mmol) was added. The reaction mixture was refluxed for 3 h. and the solvent was evaporated under vacuum, the resulting solid product was filtered off, washed with ether and crystallized from ethanol.

4,6-Diamino-2-[(cyanomethyl)thio]nicotinonitrile (5). Pale brown crystals, yield 0.54 g (88%), mp. 148-150 °C; IR: 3415, 3354, 3225 (2NH₂), 2934 (CH_{aliphatic}), 2199 (C≡N) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 6.51 (s, 2H, NH₂ exchanged by D₂O), 6.43 (s, 2H, NH₂ exchanged by D₂O), 5.51 (s, 1H, CH_{pyridyl}), 4.21 (s, 2H, CH₂) ppm; *Anal.* Calcd. for C₈H₇N₅S (205): C, 46.82; H, 3.44; N, 34.12. Found: C, 46.95; H, 3.48; N, 34.25.

2-[(4,6-Diamino-3-cyanopyridin-2-yl)thio]acetamide (6). White crystals, yield 0.61 g (92%), mp. 223-225 °C; IR: 3458, 3356, 3235 (3NH₂), 2901 (CH_{aliphatic}), 2206 (C≡N), 1650 (br, C=O) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 7.30, 6.98 (s, s, 2H, CONH₂) exchanged by D₂O), 6.27 (s, 2H, NH₂ exchanged by D₂O), 6.17 (s, 2H, NH₂ exchanged by D₂O), 5.51 (s, 1H, CH_{pyridyl}), 3.76 (s, 2H, CH₂) ppm. *Anal.* Calcd. for C₈H₉N₅OS (223): C, 43.04; H, 4.06; N, 31.37. Found: C, 43.11; H, 4.22; N, 31.24.

Synthesis of compound 7:

Method A: A solution of compound **1** (0.5 g, 3 mmol) in (30 mL) of aqueous solution of KOH (0.17 g, 3 mmol), ethyl chloroacetate (0.36 g, 3 mmol) or diethyl bromomalonate (0.71 g, 3 mmol) was added drop by drop for 10 min. with stirring at room temperature for 1 h. The reaction mixture was warmed for 20 min. and was allowed to cool. The formed precipitate was collected, washed with water and crystallized from ethanol.

Method B: Compound **2** (0.75 g, 3 mmol) in aqueous solution of KOH (0.17 g, 3 mmol in 30 mL H₂O) was warmed for 20 min. and the reaction mixture was allowed to cool. The formed precipitate was collected, washed with water and crystallized from ethanol.

Ethyl 3,4,6-triaminothieno[2,3-*b*]pyridine-2-carboxylate (7). Colourless crystals, yield 0.64 g (85%), mp. 244-246 °C; IR: 3445, 3355, 3159 (3NH₂), 2977 (CH_{aliphatic}), 2210 (C≡N), 1710 (C=O) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 6.79 (s, 2H, NH₂ exchanged by D₂O), 6.10 (s, 2H, NH₂ exchanged by D₂O), 6.01 (s, 2H, NH₂ exchanged by D₂O), 5.59 (s, 1H, CH_{pyridyl}), 4.20-4.15 (q, 2H, *J* = 8 Hz, CH₂), 1.26-1.23 (t, 3H, *J* = 8 Hz, CH₃) ppm; ¹³C NMR (DMSO-*d*₆): δ 165.32, 163.81, 161.57, 152.97, 151.77, 106.34, 88.30, 86.90, 59.46, 15.03 ppm. *Anal.* Calcd. for C₁₀H₁₂N₄O₂S (252): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.55; H, 4.91; N, 22.48.

Synthesis of compound 8:

Method A: To a solution of compound **1** (0.44 g, 2.7 mmol) in ethanol (30 ml), pot. hydroxide (0.15 g, 2.7 mmol) and 2-chloro-N-phenylacetamide (0.45 g, 2.7 mmol) were added. The reaction mixture was refluxed for 3 h. The solvent was evaporated under vacuum and the resulting solid product was filtered off, washed with water and crystallized from ethanol.

Method B: To a solution of compound **4** (0.5 g, 1.6 mmol) in ethanol (30 ml), pot. hydroxide (0.09 g, 1.6 mmol) was added. The reaction mixture was refluxed for 3 h., the excess solvent was evaporated under vacuum and the resulting solid product was filtered off, washed with ethanol and crystallized from ethanol.

3,4,6-Triamino-N-phenylthieno[2,3-*b*]pyridine-2-carboxamide (8). Colourless crystals, yield 0.42 g (85%), mp. 258-260 °C; IR: 3462, 3402, 3352, 3213 (3NH₂+NH), 1645 (C=O), 1614 (C=N) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 8.88 (s, 1H, NH exchanged by D₂O), 7.65-7.63 (d, 2H, *J* = 8 Hz, H_{arom}), 7.29-7.25 (t, 2H, *J* = 8 Hz, H_{arom}), 7.02-6.99 (m, 3H, H_{arom}+ NH₂), 6.11 (s, 2H, NH₂ exchanged by D₂O), 6.02 (s, 2H, NH₂ exchanged by D₂O), 5.59 (s, 1H, CH_{pyridyl}) ppm. *Anal.* Calcd. for C₁₄H₁₃N₅OS (299): C, 56.17; H, 4.38; N, 23.40. Found: C, 56.25; H, 4.51; N, 23.36.

General procedure for synthesis of compounds 9- 11:

Method A: Dissolve compound **1** (0.5 g, 3 mmol) in (30 mL) of aqueous solution of KOH (0.17 g, 3 mmol); 3 mmol of chloroacetonitrile (0.61 g), chloroacetamide (0.67 g), or [3-bromopentane-2,4-dione (0.53 g) or 2-bromo-3-oxobutanoate (0.62 g)] was added respectively drop by drop for 10 min. with stirring at room temperature for 1 h. The reaction mixture was warmed for 20 min. and the reaction mixture was allowed to cool. The formed precipitate was collected, washed with water and crystallized from the appropriate solvent.

Method B for synthesis of compounds 9 and 10: To a solution of pot. hydroxide (0.17 g, 3 mmol in 30 mL H₂O), compound **5** (0.61 g, 3 mmol), or **6** (0.67 g, 3 mmol) was added. The reaction mixture was warmed for 20 min. and was allowed to cool. The formed precipitate was collected, washed with water and crystallized from the appropriate solvent.

3,4,6-Triaminothieno[2,3-b]pyridine-2-carbonitrile (9). Pale brown crystals (ethanol), yield 0.58 g (95%), mp. 248-250 °C; IR: 3414, 3341, 3155 (3NH₂), 2181 (C≡N), cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 6.20 (s, 2H, NH₂ exchanged by D₂O) 6.17 (s, 2H, NH₂ exchanged by D₂O), 6.03 (s, 2H, NH₂ exchanged by D₂O), 5.61 (s, 1H, CH_{pyridyl}) ppm. *Anal.* Calcd. for C₈H₇N₅S (205): C, 46.82; H, 3.44; N, 34.12. Found: C, 46.79; H, 3.66; N, 34.21.

3,4,6-Triaminothieno[2,3-b]pyridine-2-carboxamide (10). Pale gray crystals (benzene), yield 0.6 g (90%), mp. 255-257 °C; IR: 3402, 3358, 3221 (4NH₂), 1648 (C=O) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 6.85 (s, 2H, NH₂ exchanged by D₂O), 6.67 (s, 2H, NH₂ exchanged by D₂O), 6.04 (s, 2H, NH₂ exchanged by D₂O), 5.93 (s, 2H, NH₂ exchanged by D₂O), 5.59 (s, 1H, CH_{pyridyl}) ppm. *Anal.* Calcd. for C₈H₉N₅OS (223): C, 43.04; H, 4.06; N, 31.37. Found: C, 42.95; H, 4.21; N, 31.19.

1-(3,4,6-Triaminothieno[2,3-b]pyridin-2-yl)ethanone (11). Brown crystals (ethanol), yield 0.64 g (85%), mp. 235-237 °C; IR: 3424, 3346, 3171 (3NH₂), 2926 (CH_{aliphatic}), 1700 (C=O) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 7.82 (s, 2H, NH₂ exchanged by D₂O), 6.24 (s, 2H, NH₂ exchanged by D₂O), 6.18 (s, 2H, NH₂ exchanged by D₂O), 5.58 (s, 1H, CH_{pyridyl}), 2.16 (s, 3H, CH₃) ppm; ¹³C NMR (DMSO-*d*₆): δ 188.90, 164.17, 162.00, 153.40, 152.43, 105.69, 98.66, 88.11, 28.95 ppm. *Anal.* Calcd. for C₉H₁₀N₄OS (222): C, 48.63; H, 4.53; N, 25.21. Found: C, 48.43; H, 4.68; N, 25.18.

Synthesis of compound 12:

A mixture of compound **1** (0.5 g, 3 mmol) in ethanol (30 mL), triethylamine (0.3 g, 3 mmol) and acrylonitrile (0.16 g, 3 mmol) was added. The reaction mixture was refluxed for 3 h. The excess solvent was evaporated under vacuum and the resulting solid product was collected, washed with ether and crystallized from ethanol.

3-[(4,6-diamino-3-cyanopyridin-2-yl)thio]propanenitrile (12). White crystals, yield 0.60 g (91%), mp. 185-187 °C; IR: 3395, 3305, 3209 (2NH₂), 2930 (CH_{aliphatic}), 2203 (C≡N), cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 6.41 (s, 2H, NH₂ exchanged by D₂O), 6.30 (s, 2H, NH₂ exchanged by D₂O), 5.47 (s, 1H, CH_{pyridyl}), 3.34-3.32 (d, 2H, *J* = 8 Hz, CH₂), 2.95-2.92 (d, 2H, *J* = 8 Hz, CH₂) ppm. *Anal.* Calcd. for C₉H₉N₅S (219): C, 49.30; H, 4.14; N, 31.94. Found: C, 49.44; H, 4.03; N, 31.76.

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