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THE SYNTHESIS AND MICROBIOLOGICAL ACTIVITY OF 2-MERCAPTO-4-(PYRROLIDIN-1-YL)PYRIDINE-3-CARBONITRILE DERIVATIVES

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Abstract – Synthesis of 2-mercapto-4-(pyrrolidin-1-yl)pyridine derivatives starting from 2-bromo-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**1**) is described. The desired derivatives of 2-(phenylthio)-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile were obtained in reaction of **1** with suitable thiophenoles, while reaction with benzyl mercaptan gave a related thiobenzyl derivative. Alternatively, compound **1** was transformed into 2-mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**). Reaction of **2** with chloroacetic acid or chloroacetone derivatives gave related products of mercapto group substitution. The latter compounds could be cyclized in Thorpe-Ziegler reaction to related 3-amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine derivatives. Reaction of **2** with sodium hydroxylamino-*O*-sulphonate gave the related aminosulfanyl derivative. Selected product were screened for bacteriostatic and antituberculosis activity, and some of them exhibited a significant activity.

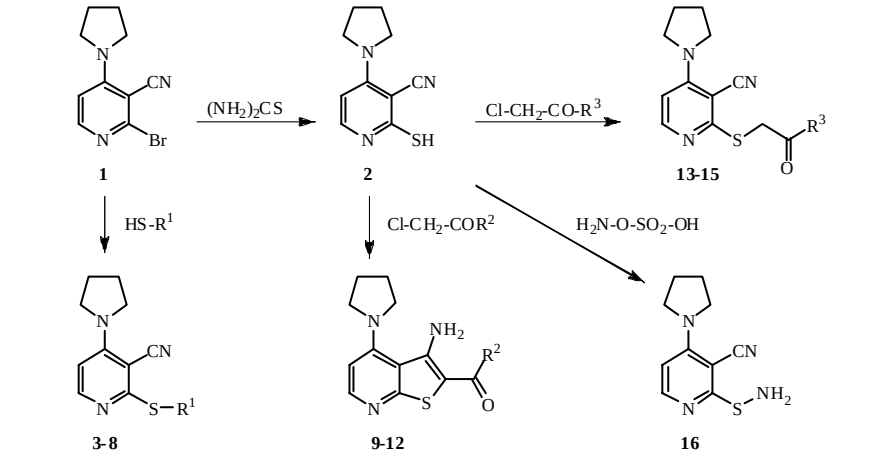
INTRODUCTION

Pyridine-3-carbonitrile derivatives exhibit a broad range of biological effects, the Beilstein Data Base lists over one thousand hundred biologically active compounds containing the pyridine-3-carbonitrile framework. A number of the compounds posses antimicrobial activity, so apparently the system constitute a promising lead in search for new antimicrobial agents. Over one hundred fifty of the

pyridine-3-carbonitriles is 4-amino substituted, and about two hundred forty bear a 2-thio substituents. Only four of the large set of pyridine-3-carbonitrile derivatives is both 4-amino and 2-thio substituted, and the compounds also posses interesting biological activities.[1-4] Surprisingly, biological activity of pyridine-3-carbonitriles bearing a cyclic amine at the position 4 was not studied yet, and for this reason we have decided to prepare and screen a series of 3-cyano-4-pyrrolidino-2-thiopyridines, including derivatives of thieno[2,3-*b*]pyridine, as several 4-aminothieno[2,3-*b*]pyridine derivatives are also known to be active.[5-7]

RESULTS AND DISCUSSION

The desired compounds were prepared starting from 2-bromo-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**1**),[8] which was found to be a very convenient reagent for synthesis of both linear (**3** - **8**) and cyclic (**9** - **12**) 2-mercaptopyridine (2-MP) derivatives (Scheme 1).



No	3	4	5	6	7	8
R^1	Ph	2-MeO-Ph	3-MeO-Ph	4-MeO-Ph	CH_2-Ph	4-F-Ph

No	9	10	11	12
R^2	NH_2	OEt	Ph	Me

No	13	14	15
R^3			OK

Scheme 1

Reaction of the bromo derivative **1** with thiourea resulted in formation of 2-mercapto derivative **2**. Similarly, heating of the compound **1** with thiophenol derivatives or with benzyl mercaptan, under alkaline conditions, gave related products of bromine substitution **3** - **8** with 15 - 96 % yield (Scheme 1). Reaction of the 2-mercapto compound **2** with chloromethylketones or chloroacetic acid derivatives gave, either products of substitution (**13** - **15**) or substitution and subsequent Thorpe-Ziegler cyclization (**9** - **12**). In spite of several attempts, compounds **13** - **14** could not be cyclized to related thienopyridines, even the unsubstituted amide, formed in reaction of **2** with chloroacetamide, cyclized *in situ* to the compound **9** under the influence of 1 equivalent of KOH. Interestingly, the methylketo derivative **12** was formed not

only in reaction of **2** with chloroacetone (method A) but also in reaction with 3-chloro-2,4-pentadione (method B) and the second approach gave a better yield of **12**. Finally, S-amino derivative **16** was obtained with 51 % yield, by reaction of the thiol **2** with sodium hydroxylamino-*O*-sulphonate.

MICROBIOLOGICAL ACTIVITY

The investigations included 27 strains of anaerobic bacteria and 26 strains of aerobic bacteria isolated from the oral cavity, respiratory system and abdominal cavity as well as 9 standard strains. The anaerobes belonged to the following genera: *Finegoldia* (2 strains), *Micromonas* (3 strains), *Propionibacterium* (4 strains), *Prevotella* (6 strains), *Porphyromonas* (2 strains), *Bacteroides* (4 strains) and standard strains: *Bacteroides fragilis* ATCC 25285, *Fusobacterium nucleatum* ATCC 25586, *Peptostreptococcus anaerobius* ATCC 27337 and *Propionibacterium acnes* ATCC 11827. There were also the following aerobes: *Staphylococcus* (4 strains), *Enterococcus* (3 strains), *Corynebacterium* (3 strains), *Acinetobacter* (4 strains), *Escherichia* (4 strains), *Klebsiella* (1 strain), *Pseudomonas* (7 strains) and 5 standard strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumonia* ATCC 13883, *Acinetobacter baumannii* ATCC 19606 and *Escherichia coli* ATCC 25922. The susceptibility of the anaerobic bacteria was determinated by means of the plate dilution technique in Brucella agar supplemented with 5% lamb blood.[9-11] For aerobic bacteria experiments agar dilution technique was with Miller-Hinton agar was used. The derivatives were dissolved in 1 mL of DMSO immediately before the experiment. Sterile distilled water was used for further dilutions. The following concentrations of derivatives were used: 200, 100, 50, 25, 12.5 and 6.2 μg/mL. The inoculums containing 10^6 CFU/spot applied to the agar plates with Steers replicator. For aerobes the inoculated agar plates without derivatives were incubated for 24 h at 37 ºC. For anaerobes agar plates were incubated in anaerobic jars for 48 h at 37 ºC in 10 % CO_2, 10 % H_2 and 80 % N_2 with palladium catalyst and indicator for anaerobiosis. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of derivative that inhibited growth of bacteria.

The investigation of aerobic and anaerobic bacteria susceptibility to the synthesized 2-mercaptopyridine derivatives are summarized in Table 1. The results have been compared with that obtained while testing the susceptibility of the same bacteria to metronidazole (for anaerobes) and amikacin (for aerobes). Activity against anaerobic bacteria exhibited 10 of 14 tested 2-mercaptopyridine derivatives: (**3** - **6, 11** - **16**). The anaerobes were the most susceptible at concentrations in range from ≤ 6.2 to 25 μg/mL to derivative (**11**) (22 % were susceptible) and to compound (**3**) (18% of susceptible strains). Moreover from 4 to 11 % of anaerobic strains were susceptible at concentration in range from ≤ 6.2 to 25 μg/mL to derivatives (**4** - **6** and **12** - **16**). Tested 2-mercaptopyridine derivatives at concentration in range from ≤ 6.2 to 100 μg/mL

inhibited growth of 15 to 70 % of anaerobic bacteria (**5, 11 - 14** and **16**). All derivatives active towards anaerobic bacteria were more effective to Gram-positive strains.

Aerobic bacteria were less susceptible to tested compounds than anaerobes. The aerobes were the most susceptible at concentrations in range from ≤ 6.2 to 25 μg/mL to derivative (**3**) (23 % were susceptible) and compound (**6**) (12 % of susceptible strains). Tested derivatives at concentration in range from ≤ 6.2 to 100 μg/mL inhibited growth of 4 to 27 % of aerobic bacteria (**3 - 8**, **11**, **12**). Other compounds did not inhibit the growth of aerobic bacteria in the range of tested concentration (≤ 6.2-200 μg/mL). The standard strains of aerobic types of bacteria exhibited rather high resistance towards tested compound (MIC ≥ 200 μg/mL), only of aerobic *Enterococcus faecalis* ATCC 29212 compounds (**11**) (MIC 100 μg/mL) and (**12**) (MIC 100 μg/mL) were active. In the case of anaerobic *Bacteroides fragilis* ATCC 25285 compounds (**14**) (MIC 50 μg/mL) and (**16**) (MIC 50 μg/mL) were active. Derivative (**14**) induced the growth inhibition of *Fusobacterium nucleatum* ATCC 25586 at concentration of 100 μg/mL. Compounds (**3**, **4**, **14**) inhibited the growth of *Peptostreptococus anaerobius* ATCC 27337 at 50 μg/mL and compounds (**5**, **6**, **15**) at 100 μg/mL. In the case of aerobic *Propionibacterium acnes* ATCC 11827 compounds (**12**) (MIC 25 μg/mL), (**3**, **11**, **16**) (MIC 50 μg/mL) and (**4 - 6**, **12 - 15**) (MIC 100 μg/mL) were active.

In summery, derivatives exhibited diversified activity against anaerobic bacteria. The anaerobes were the most susceptible at concentrations in ranges from 6.2 to 100 μg/ml to derivative (**14**) and (**16**) (70 %). The lowest susceptible in the same range of concentration to derivative (**11**) (30 %). The highest activity in the relation with investigated strains of aerobic bacteria showed derivatives (**3**) (27 % of strains were susceptible) and (**5**) (23 %), in range from (≤ 6.2 to 100 μg/mL).

Some of the newly obtained compounds were tested for their tuberculostatic activity towards the standard Mycobacterium tuberculosis $H_{37}Rv$ strain and two "wild" strains were isolated from the tuberculotic patients: Myc. Species 210 and Myc. Species 192. The tuberculostatic activity was determined *in vitro* by classical test tube method with Youman's liquid medium containing 10 % of bovine serum. In comparison with some of tuberculosis drugs: isonicotinic acid hydrazide (MIC 0.5 μg/mL), viomycin (MIC 6.2 μg/mL), cycloserine (MIC 5 μg/mL) and pyrazinamid (MIC 25 μg/mL), we concluded that some compounds (**3 - 10**) and (**14 - 16**) were worthy of notice, because their MIC exhibited 25 μg/mL (Table 2).

EXPERIMENTAL

All melting points were obtained with Boetius apparatus and are uncorrected. The obtained results of elemental analyses for C and H were in agreement with the calculated values within +/- 0,3% range. The IR spectra were taken using Thermo Mattson Satellite spectrophotometer, and the ^{1}H NMR spectra were obtained on Varian Gemini 200 MHz apparatus.

Table 1. Antibacterial Activity of tested compounds **3** - **16**

*Metronidazole (Sigma)

		MIC (μg/ml)													
Anaerobic bacteria	**Compound No**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Gram positive:	**Metronidazole***														
Finegoldia magna	≤0.4	≤6.2	≤6.2	≤6.2	25	≥200	≥200	≥200	≥200	12.5	50	≤6.2	≤6.2	≤6.2	100
Micromonas micros	≤0.4	≤6.2	100	25	≥200	≥200	≥200	≥200	≥200	≤6.2	25	100	50	≥200	100
Actinomyces israelii	1.6	≤6.2	≥200	100	≥200	≥200	≥200	≥200	≥200	≤6.2	12.5	12.5	100	≥200	100
Propionibacterium acnes	≥100	25	≤6.2	100	50	≥200	≥200	≥200	≥200	≤6.2	12.5	≤6.2	12.5	12.5	100
Propionibacterium granulosum	50	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Gram-negative:															
Prevotella bivia	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	100
Prevotella buccalis	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	100
Prevotella intermedia	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	100
Porphyromonas saccharolytica	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	100
Fusobacterium nucleatum	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	100
Fusobacterium necrophorum	1.6	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	≥200
Bacteroides fragilis	≤0.4	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	≥200	100
Bacteroides ureolyticus	3.1	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	25	100	≥200	100	≥200	100
Aerobic bacteria	**Amikacin****														
Gram positive:															
Staphylococcus aureus	≤6.2	25	50	≤6.2	≥200	100	100	≥200	≥200	≥200	25	≥200	≥200	≥200	≥200
Enterococcus faecalis	25	≤6.2	≥200	50	12.5	100	100	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Corynebacterium spp	25	≤6.2	≥200	50	≤6.2	≥200	≥200	≥200	≥200	50	100	≥200	≥200	≥200	≥200
Gram-negative:															
Acinetobacter baumannii	≤6.2	≥200	≥200	≥200	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Escherichia coli	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Klebsiella pneumoniae	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Pseudomonas aeruginosa	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Pseudomonas stutzeri	12.5	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200

**Amikacin sulfate salt (Sigma)

Table 2. Tuberculostatic Activity [μg/mL]

Compound no.	Myc.tbc $H_{37}Rv$	Myc.spec. 192	Myc.spec. 210
3	25	50	25
4	25	50	25
5	25	50	25
6	25	50	50
7	25	50	25
8	25	100	25
9	25	50	25
10	25	25	25
11	50	50	50
12	50	50	25
13	50	100	25
14	25	50	25
15	25	50	25
16	25	50	25

2-Mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (2)

Thiourea (0.61 g, 8 mmol) was added to a stirred solution of 2-bromo-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**1**) (1.00 g, 4 mmol) in MeOH (20 mL), the mixture was refluxed for 2 h and next cooled to rt. The precipitated solid was filtered off, dissolved in 10% aqueous NaOH (20 mL) and the solution was refluxed for 15 min. The mixture was cooled down, acidified with glacial acetic acid, the precipitate was filtered off, and crystallized from MeOH to give 2-mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (0.69 g, 84 %) as a white solid, mp > 360 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.95 (4H, s), 3.28 (1H, s), 3.67 (4H, s), 6.30 (1H, d, J = 6.3 Hz), 7.43 (1H, d, J = 6.3 Hz); IR (KBr) ν 2960, 2208, 1633, 1526, 1456, 1244, 1121, 1014, 758 cm^{-1}; Anal. Calcd for $C_{10}H_{11}N_3S$: C, 58.51; H, 5.40; N, 20.47. Found: C, 58,41; H, 5.38; N, 20.41.

2-Substituted-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (3-8)

General method:

A solution of 2-bromo-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**1**) (2 mmol) in MeOH (15 mL), was added to a solution of KOH (3.2 mmol) and a suitable thiophenole or benzyl mercaptan (2.7 mmol) in MeOH (10 mL) and the mixture was refluxed for 1 h. After cooling, the precipitated solid was filtered off and crystallized from MeOH.

2-(Phenylthio)-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (3)

Reaction with thiophenole. Product **3** was isolated as a white solid (yield 21 %), mp 194-195 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.60 (4H, m), 6.40 (1H, d, J = 6.3 Hz), 7.41-7.50 (5H, m), 7.84 (1H, d, J = 6.3 Hz); IR (KBr) ν 2873, 2201, 1577, 1497, 1329, 1268, 1133, 1002, 806, 756, 692, 507 cm^{-1}; Anal. Calcd for $C_{16}H_{15}N_3S$: C, 68.30; H, 5.37; N, 14.93. Found: C, 68.15; H, 5.36; N, 14.90.

2-(2-Methoxyphenylthio)-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (4)

Reaction with 2-methoxythiophenole. Product **4** was isolated as a white solid (yield 15 %), mp 168-170 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.64 (4H, m), 3.71 (3H, s), 6.41 (1H, d, *J* = 6.2 Hz), 6.94 (1H, t, *J* = 7.4 Hz), 7.12 (1H, d, *J* = 7.4 Hz), 7.45 (2H, t, *J* = 7.4 Hz), 7.79 (1H, d, *J* = 6.2 Hz); IR (KBr) ν 2964, 2202, 1584, 1495, 1332, 1255, 1004, 816, 754 cm^{-1}; Anal. Calcd for $C_{17}H_{17}N_3OS$: C, 65.57; H, 5.50; N, 13.49. Found: C, 65.44; H, 5.48; N, 13.46.

2-(3-Methoxyphenylthio)-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (5)

Reaction with 3-methoxythiophenole. Product **5** was isolated as a white solid (yield 96 %), mp 150-152 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.56 (4H, m), 3.75 (3H, s), 6.44 (1H, d, *J* = 6.2 Hz), 7.02 (2H, m), 7.30 (2H, m), 7.90 (1H, d, *J* = 6.2 Hz); IR (KBr) ν 2854, 2200, 1581, 1495, 1333, 1259, 1178, 1002, 832, 805, 526 cm^{-1}; Anal. Calcd for $C_{17}H_{17}N_3OS$: C, 65.57; H, 5.50; N, 13.49. Found: C, 65.46; H, 5.47; N, 13.45.

2-(4-Methoxyphenylthio)-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (6)

Reaction with 4-methoxythiophenole. Product **6** was isolated as a white solid (yield 40 %), mp 158-159 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.55 (4H, m), 3.79 (3H, s), 6.41 (1H, d, *J* = 6.2 Hz), 6.96 (2H, d, *J* = 6.9 Hz), 7.39 (2H, d, *J* = 6.9 Hz), 7.81 (1H, d, *J* = 6.2 Hz); IR (KBr) ν 2969, 2203, 1590, 1500, 1330, 1236, 1039, 1004, 815, 768, 691, 567 cm^{-1}; Anal. Calcd for $C_{17}H_{17}N_3OS$: C, 65.57; H, 5.50; N, 13.49. Found: C, 65.42; H, 5.47; N, 13.46

2-Benzylthio-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (7)

Reaction with benzyl mercaptan. Product **7** was isolated as a white solid (yield 40 %), mp 135-136 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.54 (4H, m), 4.42 (2H, s), 6.44 (1H, d, *J* = 6.3 Hz), 7.20-7.42 (5H, m), 8.05 (1H, d, *J* = 6.3 Hz); IR (KBr) ν 2868, 2199, 1578, 1497, 1330, 1272, 1139, 1079, 1007, 790, 713, 698 cm^{-1}; Anal. Calcd for $C_{17}H_{17}N_3S$: C, 69.12; H, 5.80; N, 14.22. Found: C, 68.98; H, 5.79; N, 14.19.

2-(4-Fluorophenylthio)-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (8)

Reaction with 4-fluorothiophenole. Product **8** was isolated as a white solid (yield 17 %), mp 177-179 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.59 (4H, m), 6.43 (1H, d, *J* = 6.2 Hz), 7.21 (2H, m), 7.51 (2H, m), 7.83 (1H, d, *J* = 6.2 Hz); IR (KBr) ν 2869, 2199, 1579, 1506, 1455, 1330, 1219, 1154, 1003, 829, 515 cm^{-1}; Anal. Calcd for $C_{16}H_{14}FN_3S$: C, 64.19; H, 4.71; N, 14.04. Found: C, 64.02; H, 4.70; N, 14.01.

3-Amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (9)

Method A:

2-Mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (410 mg, 2 mmol) followed by chloroacetamide (187 mg, 2 mmol) were added to a solution of MeONa obtained by dissolving of Na (92 mg, 4 mmol) in MeOH (25 mL) and the mixture was refluxed for 3 h. After cooling, the precipitate was filtered off and crystallized from EtOH/H_2O to give 3-amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (**9**) as a yellow solid (262 mg, 50 %), mp 264-266 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.29 (4H, m), 6.86 (1H, d, *J* = 5.8 Hz), 6.95 (2H, s), 7.10 (2H, s), 8.25 (1H, d, *J* = 5.8 Hz); IR (KBr) ν 3428, 3295, 3133, 1673,

1611, 1582, 1502, 1371, 1339, 1112, 1056, 1002, 617, 485 cm^{-1}. Anal. Calcd for $C_{12}H_{14}N_4OS$: C, 54.94; H, 5.38; N, 21.36. Found: C, 54.81; H, 5.37; 21.31.

Method B:

2-Mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (205 mg, 1 mmol) was dissolved in DMF (5 mL) and then KOH (56 mg, 1 mmol) in water (3 mL) was added. Next, chloroacetamide (93 mg, 1 mmol) was added and the mixture was stirred at rt for 20 min. The precipitated solid was filtered off and crystallized from EtOH/H_2O (1:1) to give 3-amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (**9**) as a yellow solid (146 mg, 56 %).

General procedure for synthesis of thieno[2,3-*b*]pyridine derivatives 10 and 11.

2-Mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (205 mg, 1 mmol) was dissolved in DMF (15 mL) and KOH (56 mg, 1 mmol) in water (3 mL) was added. Then ethyl chloroacetate (122 mg, 1 mmol) (for **10**) or 2-bromoacetophenone (199 mg, 1 mmol) (for **11**) was added and the mixture was stirred at rt for 20 min. Next, KOH (56 mg, 1 mmol) in water (3 mL) was added and the mixture was stirred for 20 min. The precipitated solid was filtered off and crystallized from MeOH/H_2O (1:1).

Ethyl 3-amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine-2-carboxylate (10)

Compound **10** was isolated as a yellow solid (72 mg, 25 %), mp 137-139 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 7.0 Hz), 1.90 (4H, m), 3.30 (4H, m), 4.20 (2H, q, J = 7.0 Hz), 6.82 (2H, s), 6.85 (1H, d, J = 5.6 Hz), 8.25 (1H, d, J = 5.6 Hz); IR (KBr) ν 3374, 3248, 1666, 1615, 1561, 1370, 1289, 1150, 1087, 1064, 1009, 806 cm^{-1}. Anal. Calcd for $C_{14}H_{17}N_3O_2S$: C, 57.71; H, 5.88; N, 14.42. Found: C, 57.59; H, 5.86; N, 14.39.

(3-Amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine-2-yl)phenylmethanone (11)

Compound **11** was isolated as a yellow solid (200 mg, 62 %), mp 249-252 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.37 (4H, m), 6.83 (1H, d, J = 5.7 Hz), 7.52-7.71 (5H, m), 8.09 (2H, s), 8.23 (1H, d, J = 5.7 Hz); (KBr) ν 1589, 1550, 1349, 1307, 1271, 1128, 1064, 1004, 927, 790, 732, 542 cm^{-1}. Anal. Calcd for $C_{18}H_{17}N_3OS$: C, 66.85; H, 5.30; N, 12.99. Found: C, 66.69; H, 5.29; N, 12.97.

1-(3-Amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine-2-yl)ethanone (12)

Method A:

2-Mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (205 mg, 1 mmol) was dissolved in DMF (15 mL), then KOH (56 mg, 1 mmol) and chloroacetone (92 mg, 1 mmol) was added. The mixture was stirred at rt for 20 min. Then a next portion of KOH (56 mg, 1 mmol) was added and the solution was stirred for 20 min. The mixture was evaporated, ice (10 g) was added to the residue, and the mixture was left for 12 h. The precipitated solid was filtered off and crystallized from MeOH/H_2O (1:1) to give 1-(3-Amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine-2-yl)ethanone (**12**) as a yellow solid (26 mg, 10 %),

mp 109-112 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.87 (4H, m), 2.48 (3H, s), 3.28 (4H, m), 6.83 (1H, d, J = 5.6 Hz), 7.63 (2H, s), 8.25 (1H, d, J = 5.6 Hz); (KBr) ν 3448, 3300, 1580, 1482, 1303, 1001, 474 cm^{-1}; Anal. Calcd for $C_{13}H_{15}N_3OS$: C, 59.75; H, 5.79; N, 16.08. Found: C, 59.63; H, 5.77; N, 16.04.

Method B:

2-Mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (205 mg, 1 mmol) was dissolved in DMF (10 mL). Then KOH (112 mg, 2 mmol) followed by 3-chloro-2,4-pentadione (134 mg, 1 mmol) were added. The mixture was refluxed for 2 h. Then the mixture was evaporated and ice (10 g) was added to the residue, and the mixture was left for 12 h. The precipitated solid was filtered off and crystallized from MeOH/H2O (1:1) to give 1-(3-amino-4-(pyrrolidin-1-yl)thieno[2,3-*b*]pyridine-2-yl)ethanone (**12**) as a yellow solid (91 mg, 35 %).

General procedure for the synthesis of 13 and 14.

2-Mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (1.03 g, 5 mmol) was dissolved in DMF (15 mL) and KOH (0.84 g, 15 mmol) was added to the solution. Then 1-(2-chloroacetyl)-4-(4-fluorophenyl)piperazine hydrochloride (1.47 g, 5 mmol) (for **13**) or 1-(2-chloroacetyl)-4-phenylpiperazine hydrochloride (1.38 g, 5 mmol) (for **14**) was added, and the mixture was stirred at rt for 30 min. The formed precipitate was filtered off and crystallized.

2-(2-(4-(4-Fluorophenyl)piperazin-1-yl)-2-oxoethylthio)-4-(pyrrolidin-1-yl))pyridine-3-carbonitrile (13)

The crude product was crystallized from EtOH to give a white solid (1.74 g, 82 %), mp 172-173 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.90 (4H, m), 3.05 (4H, m), 3.54-3.69 (8H, m), 4.22 (2H, s), 6.41 (1H, d, J = 6.3 Hz), 6.95 (4H, m), 7.97 (1H, d, J = 6.3 Hz). (KBr) ν 2917, 2206, 1635, 1576, 1496, 1443, 1220, 1006, 834, 801 cm^{-1}. Anal. Calcd for $C_{22}H_{24}FN_5OS$: C, 62.10; H, 5.68; N, 16.46. Found: C, 61.98; H, 5.66; N, 16.43.

2-(2-Oxo-2-(4-phenylpiperazin-1-yl)ethylthio)-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (14)

The crude product was crystallized from MeOH to give a white solid (0.83 g, 41 %), mp 178-180 ˚C; ^{1}H NMR (DMSO-d_6) δ 1.88 (4H, m), 3.12 (4H,m), 3.55 (4H, m), 3.70 (4H, m), 4.23 (2H, s), 6.42 (1H, d, J = 6.4 Hz), 6.76-6.97 (5H, m), 8.01 (1H, d, J = 6.4 Hz); (KBr) ν 2821, 2206, 1635, 1576, 1494, 1334, 1224, 1006, 803, 767, 699 cm^{-1}. Anal. Calcd for $C_{22}H_{25}N_5OS$: C, 64.84; H, 6.18; N, 17.18. Found: C, 64.70; H, 6.17; N, 17.14.

Potassium-2-(3-cyano-4-(pyrrolidin-1-yl)pyridine-2-ylthio)acetate (15)

2-Mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (205 mg, 1 mmol) was dissolved in DMF (15 mL) and then KOH (112 mg, 2 mmol) dissolved in water (3 mL) and chloroacetic acid (94 mg, 1 mmol) were added, and the mixture was stirred for 20 min. The precipitated solid was filtered off and crystallized from MeOH to give potassium-2-(3-cyano-4-(pyrrolidin-1-yl)pyridine-2-ylthio)acetate (**15**) as a white solid (123 mg, 41 %), mp 165-168 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.89 (4H, m), 3.56 (4H, m), 3.94 (2H, s), 6.42

(1H, d, *J* = 6.3 Hz), 8.97 (1H, d, *J* = 6.3 Hz); (KBr) ν 2199, 1721, 1574, 1499, 1334, 1006, 807, 591 cm^{-1}. Anal. Calcd for $C_{12}H_{12}KN_3O_2S$: C, 47.82; H, 4.01; N, 13.94. Found: C, 47.69; H, 4.00; N, 13.91.

2-Aminosulfanyl-4-(pyrrolidin-1-yl)-pyridine-3-carbonitrile (16)

Hydroxylamino-*O*-sulfonic acid (226 mg, 2 mmol) was dissolved in water (5 mL) and neutralized using anhydrous sodium carbonate (212 mg, 2 mmol). Then a solution of 2-mercapto-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile (**2**) (410 mg, 2 mmol) and NaOH (24 mg, 6 mmol) in MeOH (20 mL) was prepared, and both solutions were mixed and stirred for 15 min at rt. The precipitated solid was filtered off and crystallized from MeOH to give 2-aminosulfanyl-4-pyrrolidin-1-yl-nicotinonitrile (**16**) as a white solid (224 mg, 51 %), mp 160-161 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.91 (4H, m), 3.62 (4H, m), 3.87 (2H, s), 6.41 (1H, d, *J* = 6.3 Hz), 8.10 (1H, d, *J* = 6.3 Hz). (KBr) ν 3330, 3221, 2206, 1577, 1499, 1336, 1272, 1078, 1004, 797, 684, 455 cm^{-1}; Anal. Calcd for $C_{10}H_{12}N_4S$: C, 54.52; H, 5.49; N, 25.43. Found: C, 54.43; H, 5.48; N, 25.39.

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