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THE SYNTHESIS AND MICROBIOLOGICAL ACTIVITY OF 2-MERCAPTO-4-METHOXYPYRIDINE-3-CARBONITRILE DERIVATIVES

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Abstract – Synthesis of 2-(3-cyano-4-methoxypyridin-2-ylthio)acetic acid derivatives, starting either from 2-bromo-4-methoxypyridine-3-carbonitrile or 2-mercapto-4-methoxypyridine-3-carbonitrile is reported. The obtained products could be transformed by intramolecular Thorpe-Ziegler cyclization to related thieno[2,3-*b*]pyridines. Diazotization of 3-amino-*N*-(4-chlorophenyl)-4-methoxythieno[2,3-*b*]pyridine-2-carboxamide resulted in formation of a suitable pyridothienotriazine derivative. Some of the prepared compounds demonstrated noticeable bacteriostatic or tuberculostatic activity.

INTRODUCTION

Synthesis of substituted pyridine derivatives attracts much attention due to their interesting biological activity, such as bacteriostatic,[1] fungicidal[2] or insecticidal.[3] Derivatives of 4-substituted-2-thiopyridine have been reported as antibacterial agents,[4,5] what in connection with our previous results indicating their tuberculostatic activity[6] prompted us to synthesis of new compounds containing the 4-methoxy-2-thiopyridine framework, and evaluation of their antimicrobial activity, especially the tuberculostatic activity. Tuberculosis disease has staged a lethal comeback primarily because of resistance development by the causative organism, *Mycobacterium tuberculosis* against all major anti-tuberculosis drugs, and due to rising number of immuno-suppressed cases (through cancer chemotherapy, AIDS infection and transplantation).[7] For this reason new tuberculostatic drugs are urgently needed.

RESULTS AND DISCUSSION

The desired compounds were prepared starting either from 2-bromo-4-methoxypyridine-3-carbonitrile (**1**),[8] (Scheme 1), or from 2-mercapto-4-methoxypyridine-3-carbonitrile (**9**)[9] (Scheme 2). The original method of synthesis of the bromo derivative **1** was modified by replacement of gaseous HBr with the easily accessible 33% HBr in acetic acid.

Reaction of 2-bromo-4-methoxypyridine-3-carbonitrile (**1**) with methyl 2-mercaptoacetate and 1 eq of KOH at rt in DMF gave methyl 2-(3-cyano-4-methoxypyridin-2-ylthio)acetate (**2**). If an excess of KOH was used, the reaction resulted in formation of thieno[2,3-*b*]derivative (**3**), apparently through the Thorpe-Ziegler cyclization of the initially formed compound **2**. The ester **3** was hydrolyzed to 3-amino-4-methoxythieno[2,3-*b*]pyridine-2-carboxylic acid (**4**) by reflux in NaOH solution followed by acidification with acetic acid. The result stands in contradiction to Parnes report of unsuccessful attempt of hydrolysis of related methyl 3-amino-4-(dimethylamino)thieno[2,3-*b*]pyridine-2-carboxylate.[9]

Analogous reactions of the 2-bromopyridine **1** with mercapto-*N*-arylacetamides and an excess of KOH also gave products of substitution and subsequent Thorpe-Ziegler cyclization (**5**, **6**). Heating of the compound **5** in acetic anhydride lead to acylation of the amino group, accompanied, unexpectedly, by decomposition of the amido group, to give 3-acetamido-4-methoxythieno[2,3-*b*]pyridine-2-carboxylic acid (**7**). Diazotization of the amino derivative (**5**) with sodium nitrite in hydrochloric acid gave 3-(4-chlorophenyl)-9-methoxypyrido[3',2':4,5]thieno[3,2-*d*][1,2,3]triazin-4(3*H*)-one (**8**), apparently by cyclization of the initially formed diazonium salt (Scheme 1).

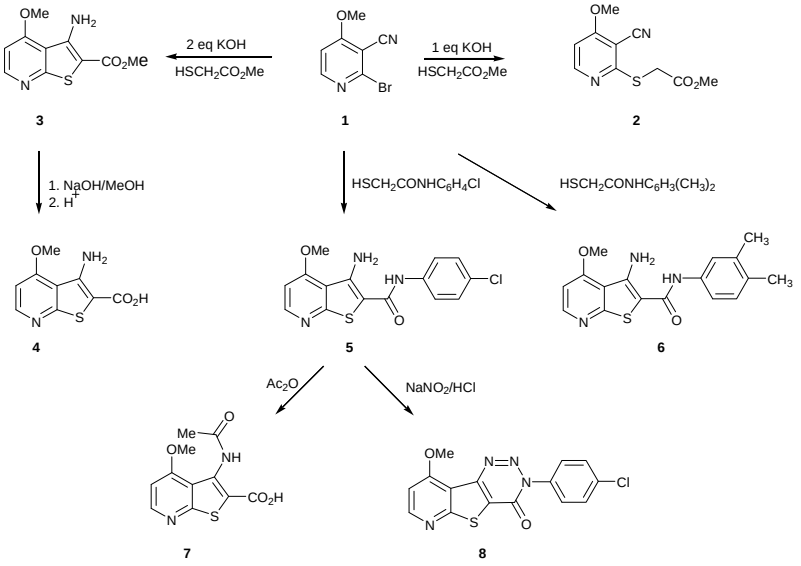
Alternatively to the approach presented on Scheme 1, the 2-thiopyridine derivatives were prepared starting from a suitable 2-mercaptopyridine (Scheme 2).

Reaction of 2-mercapto-4-methoxypyridine-3-carbonitrile (**9**) with chloroacetamides or methyl 3-bromopropanoate at rt in DMF containing equimolar amount of KOH gave *S*-substituted thiopyridines (**10-12**) with 20-30% yield. Similarly as for the reactions starting from 2-bromo derivative **1**, reaction of the 2-mercaptopyridine **9** with suitable halo compounds in the presence of excess of KOH gave directly products of substitution and subsequent Thorpe-Ziegler cyclization (**13** – **19**) (Scheme 2).

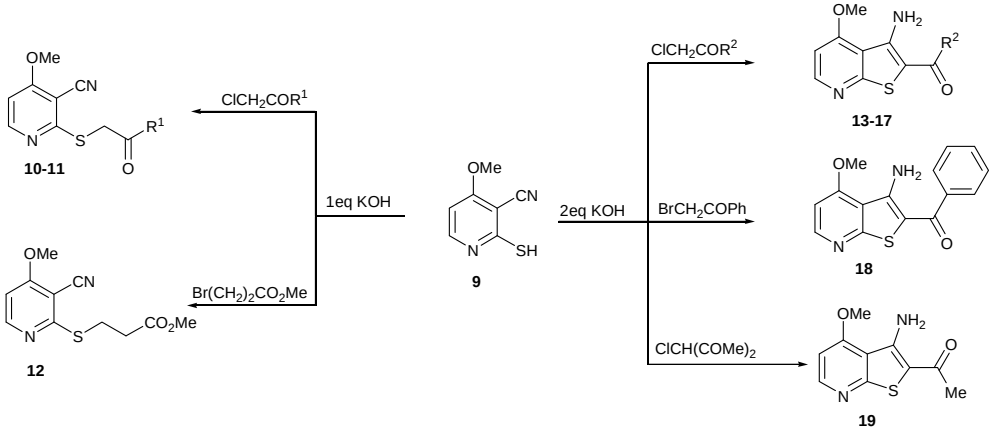
The structures of all new compounds were established based on elemental analysis and spectroscopic data. Some of the synthesized compounds were evaluated for their antimicrobiological activities against aerobic bacteria, anaerobic bacteria and *Mycobacterium tuberculosis* strains.

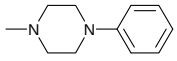
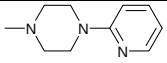
MICROBIOLOGICAL ACTIVITY

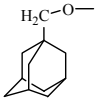
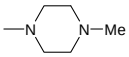
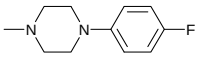
The investigations included 27 strains of anaerobic bacteria and 26 strains of aerobic bacteria isolated from the oral cavity, respiratory system and abdominal cavity as well as 9 standard strains. The anaerobes belonged to the following genera: *Finegoldia* (2 strains), *Micromonas* (3 strains), *Propionibacterium*



Scheme 1



No	10	11
R^1		

No	13	14	15	16	17
R^2	—OEt	—NH_2			

Scheme 2

(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 determined by means of the plate dilution technique in Brucella agar supplemented with 5% lamb blood.[10-12] For aerobic bacteria experiments agar dilution technique 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 were used: 200, 100, 50, 25, 12.5 and 6.2 μg/mL. The inoculums containing 10^6 CFU/spot was applied to the agar plates with Steers replicator. For aerobes the inoculated agar plates with derivatives were incubated for 24 h at 37 ºC. For anaerobes agar plates were incubated in anaerobic jars for 48 h at 37 ˚C 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. Metronidazole (for anaerobes) and Amikacin (for aerobes) were used as the reference substances.

The investigation of susceptibility of aerobic and anaerobic bacteria to the synthesized 2-mercaptopyridine derivatives are summarized in Table 1.

Of the tested 2-mercaptopyridine derivatives (16 samples) only compound **4** did not exhibit any activity against anaerobic bacteria. The anaerobes were most susceptible (at concentrations in the range from ≤ 6.2 to 25 μg/mL) to the compounds **12** (37% of strains were susceptible), **2** (30% were susceptible) and **14** (22% of susceptible strains). Moreover, from 4 to 15 % of the screened anaerobic strains were susceptible at concentration in the range from ≤ 6.2 to 25 μg/mL to derivatives **3, 5, 10, 11, 13, 15, 17** and **18**. Derivatives **2-3, 5-8, 10-15** and **17-19** inhibited growth of 8 to 35% of aerobic bacteria strains at concentration in the range from ≤ 6.2 to 100 μg/mL. All derivatives, which were active towards anaerobic bacteria, were more effective against Gram-positive strains.

Aerobic bacteria were less susceptible to the tested compounds than anaerobes. The aerobes were the most susceptible (at concentrations in the range from ≤ 6.2 to 25 μg/mL) to derivatives **2** and **19** (8% of susceptible strains) and compounds **3** and **12** (4% of susceptible strains). Derivatives **2, 3, 5, 12-14** and **19** inhibited growth of 8 to 35% of aerobic bacteria at concentration in the range from ≤ 6.2 to 100 μg/mL.

The standard strains of aerobic types of bacteria exhibited rather high resistance towards tested compound (MIC ≥ 200 μg/mL), only for the aerobic *Enterococcus faecalis* ATCC 29212, compounds **2, 12** and **13** (MIC 100 μg/mL) and **3** and **5** (MIC 50 μg/mL) were active.

In the case of aerobic *Staphylococcus aureus* ATCC 25923 compounds (**3, 5**) (MIC 100 μg/mL) were active. Derivative (**2**) induced the growth inhibition of *Klebsiella pneumonia* ATCC 13883 at concentration of 50 μg/mL. In the case of anaerobic *Bacteroides fragilis* ATCC 25285, compounds (**5, 11**) (MIC 100 μg/mL) were active. Derivative (**3, 5, 8, 10, 11, 12, 13**) induced the growth inhibition of *Fusobacterium nucleatum* ATCC 25586 at concentration of 100 μg/mL and compound (**2**) at

concentration of 50 μg/mL. Compound (**2**) inhibited the growth of *Peptostreptococus anaerobius* ATCC 27337 at ≤ 6.2 μg/mL, compounds (**10, 14**) at 12.5 μg/mL, derivatives (**3**, **5**, **11**, **13**, **18**) at 50 μg/mL and (**6**, **7**, **8**, **12**, **15**) at 100 μg/mL. In the case of aerobic *Propionibacterium acnes* ATCC 11827 compounds (**2**) (MIC ≤ 6.2 μg/mL), (**14**) (MIC 12.5 μg/mL), (**12**, **18**) (MIC 25 μg/mL), (**3**, **5, 10, 11, 13, 19**) (MIC 50 μg/mL) and (**6, 7, 8, 15**) (MIC 100 μg/mL) were active.

Selected compounds were tested for their tuberculostatic activity towards the standard Mycobacterium tuberculosis $H_{37}Rv$ strain and two wild strains 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. Antituberculosis 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) (towards Mycobacterium tuberculosis $H_{37}Rv$) were used as references. The results are given in Table 2. Of the tested group, only compounds **3, 13** and **17** exhibited activity at MIC 12.5 μg/mL. In summary, the tested 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 derivatives 8 (44%), 2 (41%) and 12 (41%). The lowest susceptible in the same range of concentration to derivative **3**, **13**, **14**, **15** (26%). The highest activity against the investigated strains of aerobic bacteria showed derivatives **2** and **3** in range from ≤ 6.2 to 100 μg/mL (35% of strains were susceptible).

EXPERIMENTAL

Melting points were obtained with a Boëtius apparatus and are uncorrected. Elemental analyses for C, H, N and S were performed on Carlo-Erba 1108 instrument. The IR spectra were taken with a Satellite spectrophotometer and the ^1H NMR spectra were taken with a Varian Gem 200 MHz apparatus.

2-Bromo-4-methoxypyridine-3-carbonitrile (1)[8]

To 1,1-dicyano-4-(*N*,*N*-dimethyloamino)-2-methoxy-1,3-butadiene (0.885 g, 5 mmol), 33% HBr/CH_3CO_2H (5 mL) was added and the mixture was left at rt for 48 h. Then ice (10 g) and concentrated ammonium hydroxide (10 mL) were added. The precipitated solid was filtered off and crystallized from MeOH to give 2-bromo-4-methoxypyridine-3-carbonitrile (**1**) (0.73 g, 69%) as a white solid.

Methyl 2-(3-cyano-4-methoxypyridin-2-ylthio)acetate (2)

Method A:

2-Bromo-4-methoxypyridine-3-carbonitrile (**1**) (0.639 g, 3 mmol) was dissolved in MeOH (20 mL), KOH (0.168 g, 3 mmol) in water (3 mL) followed by methyl 2-mercaptoacetate (0.318 g, 3 mmol) were added, and the mixture was refluxed for 2 h. Next, ice (20 g) was added, the precipitated solid was filtered off

Table 1. Antibacterial Activity of tested compounds 2-8, 10-15, 17-19

	MIC (μg/mL)																
Anaerobic bacteria	**Compound no**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**11**	**12**	**13**	**14**	**15**	**17**	**18**	**19**
Gram positive:	**Metronidazole***																
Finegoldia magna (2)	≤ 0.4	≤ 6.2	12.5	≥ 200	25	50	100	100	≤ 6.2	≤ 6.2	≤ 6.2	100	≤ 6.2	50	50	12.5	≥ 200
Micromonas micros (3)	≤ 0.4	≤ 6.2	≤6.2	≥ 200	≤ 6.2	50	≥ 200	≥ 200	≤ 6.2	50	≤ 6.2	25	≤ 6.2	25	25	12.5	≥ 200
Actinomyces israelii (2)	1.6	≤ 6.2	≤ 6.2	≥ 200	≥ 200	≥ 200	≥ 200	100	≥ 200	≥ 200	≤ 6.2	25	12.5	50	≥ 200	≥ 200	≥ 200
Propionibacterium acnes (2)	≥ 100	≤ 6.2	25	≥ 200	50	100	50	100	≤ 6.2	50	25	50	50	50	≥ 200	≥ 200	50
Propionibacterium granulosum	50	100	≥ 200	≥ 200	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≤ 6.2	≥200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Gram-negative:																	
Prevotella bivia (1)	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	50	≥ 200	≥ 200	25	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Prevotella buccalis (2)	≤ 0.4	50	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Prevotella intermedia (3)	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Porphyromonas saccharolytica	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Fusobacterium nucleatum (3)	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Fusobacterium necrophorum	1.6	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	50	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Bacteroides fragilis (2)	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Bacteroides ureolyticu (2)s	3.1	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	50	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥2 00
Aerobic bacteria	**Amikacin****																
Gram positive:																	
Staphylococcus aureus (4)	≤ 6.2	12.5	100	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Enterococcus faecalis (3)	25	50	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	25
Corynebacterium spp (3)	25	100	25	≥ 200	50	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	25	50	100	≥ 200	≥ 200	≥ 200	25
Gram-negative:																	
Acinetobacter baumanni (4)i	≤ 6.2	100	100	≥ 200	50	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Escherichia coli (4)	≤ 6.2	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Klebsiella pneumoniae (1)	≤ 6.2	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Pseudomonas aeruginosa (5)	≤ 6.2	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
Pseudomonas stutzeri (2)	12.5	100	50	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200

*Metronidazole (Sigma)

**Amikacin sulfate salt (Sigma)

Table 2. Tuberculostatic Activity [μg/mL]

Compound no.	Myc.tbc $H_{37}Rv$	Myc.spec. 192	Myc.spec. 210
2	25	50	25
3	12.5	50	25
4	25	25	25
5	25	50	25
6	25	50	25
7	50	50	25
8	100	100	100
10	25	50	25
11	25	50	25
12	25	50	25
13	25	25	12.5
14	25	50	25
15	50	50	25
17	25	25	12.5
18	25	25	25
19	25	50	25
Isonicotinic acid hydrazide	0.5	-	-
Cycloserine	5	-	-
Viomycin	6.2	-	-
Pyrazinamid	25	-	-

and crystallized from MeOH/H_2O (1:3) to give methyl 2-(3-cyano-4-methoxypyridin-2-ylthio)acetate (**2**) as a white solid (0.142 g, 20%), mp 129-131 ˚C. ^{1}H NMR (DMSO-d_6) δ 3.75 (3H, s), 4.00 (3H, s), 4.11 (2H, s), 6.68 (1H, d, J = 5.8 Hz), 8.43 (1H, d, J = 5.8 Hz); IR (KBr) ν_{max} 2225, 1735, 1559, 1475, 1303, 1163, 1042, 1025, 825 cm^{-1}. Anal. Calcd for $C_{10}H_{10}N_2O_3S$: C, 50.41; H, 4.23; N, 11.76; S, 13.46. Found: C, 50.29; H, 4.22; N, 11.73; S, 13.42.

Method B

2-Bromo-4-methoxypyridine-3-carbonitrile (**1**) (0.639 g, 3 mmol) was dissolved in DMF (20 mL), KOH (0.168 g, 3 mmol) in water (3 mL) followed by methyl 2-mercaptoacetate (0.318 g, 3 mmol) were added, and the mixture was stirred at rt for 15 min. Next, ice (20 g) was added, the precipitated solid was filtered off and crystallized from MeOH/H_2O (1:3) to give methyl 2-(3-cyano-4-methoxypyridin-2-ylthio)acetate (**2**) as a white solid (0.385 g, 54%).

General procedure for synthesis of thieno[2,3-*b*]pyridine derivatives 3, 5, and 6

2-Bromo-4-methoxypyridine-3-carbonitrile (**1**) (0.213 g, 1 mmol) was dissolved in DMF (15 mL), and KOH (0.056 g, 1 mmol) in water (3 mL) was added. Then methyl 2-mercaptoacetate (0.106 g, 1 mmol) (for **3**), *N*-(4-chlorophenyl)-2-mercaptoacetamide (0.201 g, 1 mmol) (for **5**) or *N*-(3,4-dimethylphenyl)-2-mercaptoacetamide (0.195 g, 1 mmol) (for **6**) was added and the mixture was stirred at rt for 15 min. Next, KOH (0,056 g, 1 mmol) in water (3 mL) was added and the mixture was stirred for additional 45 min. Then ice (10 g) was added and the precipitated solid was filtered off and crystallized from MeOH/H_2O (2:3).

Methyl 3-amino-4-methoxythieno[2,3-*b*]pyridine-2-carboxylate (3)

Reaction with methyl 2-mercaptoacetate. Product **3** was isolated as a white solid (yield 46%), mp 162-164 ˚C. ^{1}H NMR (DMSO-d_6) δ 3.76 (3H, s), 4.01 (3H, s), 6.95 (2H, s), 7.01 (1H, d, J = 5.6 Hz), 8.48 (1H, d, J = 5.6 Hz); IR (KBr) ν_{max} 3501, 3355, 1678, 1603, 1512, 1436, 1287, 1127, 1037, 842, 763, 573 cm^{-1}. Anal. Calcd for $C_{10}H_{10}N_2O_3S$: C, 50.41; H, 4.23; N, 11.76; S, 13.46. Found: C, 50.29; H, 4.22, N, 11.74; S, 13.42.

3-Amino-*N*-(4-chlorophenyl)-4-methoxythieno[2,3-*b*]pyridine-2-carboxamide (5)

Reaction with *N*-(4-chlorophenyl)-2-mercaptoacetamide. Product **5** was isolated as a yellow solid (yield 58%), mp 285-287 ˚C. ^{1}H NMR (DMSO-d_6) δ 4.01 (3H, s), 6.99 (1H, d, J = 5.6 Hz), 7.17 (2H, s), 7.33-7.37 (2H, m), 7.69-7.74 (2H, m), 8.48 (1H, d, J = 5.6 Hz), 9.47 (1H, s); IR (KBr) ν_{max} 3471, 3331, 1637, 1581, 1489, 1314, 1241, 1043, 841, 803 cm^{-1}. Anal. Calcd for $C_{15}H_{12}ClN_3O_2S$: C, 53.97; H, 3.62; N, 12.59; S; 9.61. Found: C, 53.86; H, 3.60; N, 12.56; S, 9.59.

3-Amino-4-methoxy-*N*-(3,4-dimethylphenyl)thieno[2,3-*b*]pyridine-2-carboxamide (6)

Reaction with *N*-(3,4-dimethylphenyl)-2-mercaptoacetamide. Product **6** was isolated as a yellow solid (yield 50%), mp 225-227 ˚C. ^{1}H NMR (DMSO-d_6) δ 2.17 (3H, s), 2.20 (3H, s), 4.02 (3H, s), 7.02 (1H, d, J = 5.8 Hz), 7.06 (1H, d, J = 7.9 Hz), 7.11 (2H, s), 7.36 (1H, d, J = 7.9 Hz), 7.46 (1H, s), 8.48 (1H, d, J = 5.8 Hz), 9.18 (1H, s); IR (KBr) ν_{max} 3462, 3325, 1636, 1588, 1500, 1313, 1258, 1046, 802, 756 cm^{-1}. Anal. Calcd for $C_{17}H_{17}N_3O_2S$: C, 62.36; H, 5.23; N, 12.83; S, 9.79. Found: C, 62.23, H, 5.21, N, 12.80; S, 9.77.

3-Amino-4-methoxythieno[2,3-*b*]pyridine-2-carboxylic acid (4)

Methyl 3-amino-4-methoxythieno[2,3-*b*]pyridine-2-carboxylate (**3**) (0.238 g, 1 mmol) was dissolved in MeOH (15 mL) and then NaOH (0.200 g, 5 mmol) was added. The mixture was refluxed for 1 h. Then the mixture was evaporated and ice (10 g) was added to the residue. Then glacial acetic acid was added, the precipitate was filtered off and crystallized from MeOH/H_2O (1:1) to give 3-amino-4-methoxythieno[2,3-*b*]pyridine-2-carboxylic acid (**4**) (0.103 g, 46%) as a white solid, mp 230-232 ˚C. ^{1}H NMR (DMSO-d_6) δ 4.15 (3H, s), 7.11 (1H, d, J = 5.3 Hz), 7.16 (2H, s), 8.46 (1H, d, J = 5.3 Hz), 9.46 (1H, s); IR (KBr) ν_{max} 3392, 1571, 1538, 1360, 1290, 1195, 1054, 799 cm^{-1}. Anal. Calcd for $C_9H_8N_2O_3S$: C, 48.21; H, 3.60; N, 12.49; S, 14.30. Found: C, 48.09; H, 3.59; N, 12.47; S, 14.26.

3-Acetamido-4-methoxythieno[2,3-*b*]pyridine-2-carboxylic acid (7)

3-Amino-*N*-(4-chlorophenyl)-4-methoxythieno[2,3-*b*]pyridine-2-carboxamide (**5**) (0.333 g, 1 mmol) was refluxed in acetic anhydride (15 mL) for 6 h. The mixture was evaporated, ice (10 g) was added to the residue and the precipitated solid was filtered off and crystallized from MeOH/H_2O (1:1) to give 3-acetamido-4-methoxythieno[2,3-*b*]pyridine-2-carboxylic acid (**7**) as a white solid (0.095 g, 36%), mp 240-242 ˚C. ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s), 3.92 (3H, s), 7.0 (1H, d, J = 5.5 Hz), 8.51 (1H, d, J = 5.5 Hz), 9.7 (1H, s); 13.20 (1H, bs); IR (KBr) ν_{max} 3359, 1698, 1518, 1476, 1282, 1211, 1056, 828, 752,

567 cm^{-1}. Anal. Calcd for $C_{11}H_{10}N_2O_4S$: C, 49.62; H, 3.79; N, 10.52; S, 12.04. Found: C, 49.52; H, 3.77; N, 10.49; S, 12.01.

3-(4-Chlorophenyl)-9-methoxypyrido[3',2':4,5]thieno[3,2-*d*][1,2,3]triazin-4(3*H*)-one (8)

3-Amino-*N*-(4-chlorophenyl)-4-methoxythieno[2,3-*b*]pyridine-2-carboxamide (**5**) (0.333 g, 1 mmol) was dissolved in hydrochloric acid (6 mL) and cooled down to 0 ºC. Then aqueous solution of $NaNO_2$ (0.138 g, 2 mmol) was added, and the mixture was stirred at 0 ºC for 10 min. The precipitated solid was filtered off and crystallized from acetic acid to give 3-(4-chlorophenyl)-9-methoxypyrido[3',2':4,5]thieno-[3,2-*d*][1,2,3]triazin-4(3*H*)-one (**8**) as a white solid (46%), mp 243-245 ˚C; ^{1}H NMR (DMSO-d_6) δ 4.16 (3H, s), 7.63 (1H, d, *J* = 5.7 Hz), 7.71-7.83 (4H, m), 8.77 (1H, d, *J* = 5.7 Hz); IR (KBr) ν_{max} 1677, 1562, 1488, 1290, 1037, 944, 828, 520 cm^{-1}. Anal. Calcd for $C_{15}H_9ClN_4O_2S$: C, 52.25; H, 2.63; N, 16.25; S, 9.30. Found: C, 52.11; H, 2.62; N, 16.21; S, 9.18.

2-Mercapto-4-methoxypyridine-3-carbonitrile (9)[9]

Thiourea (0.760 g, 10 mmol) was added to a stirred solution of 2-bromo-4-methoxypyridine-3-carbonitrile (**1**) (1.065 g, 5 mmol) in MeOH (25 mL), the mixture was refluxed for 3 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-methoxypyridine-3-carbonitrile (**9**) (0.639 g, 77%) as a white solid.

General procedure for the synthesis of 10 and 11

2-Mercapto-4- methoxypyridine-3-carbonitrile (**9**) (0.332 g, 2 mmol) was dissolved in DMF (15 mL) and KOH (0.224 g, 4 mmol) was added to the solution. Then 2-chloro-1-(4-phenylpiperazin-1-yl)ethanone hydrochloride (0.55 g, 2 mmol) (for **10**) or 2-chloro-1-(4-(pyridin-2-yl)piperazin-1-yl)ethanone hydrochloride (0.55 g, 2 mmol) (for **11**) was added, and the mixture was stirred at rt for 30 min. The formed precipitate was filtered off and crystallized from MeOH.

2-(2-Oxo-2-(4-phenylpiperazin-1-yl)ethylthio)-4-methoxypyridine-3-carbonitrile (10)

Reaction with 2-chloro-1-(4-phenylpiperazin-1-yl)ethanone hydrochloride. Product **10** was isolated as a white solid (26%), mp 188-190 ˚C. ^{1}H NMR (DMSO-d_6) δ 3.21-3.24 (4H, m), 3.84-3.92 (4H, m), 3.97 (3H, s), 4.23 (2H, s), 6.64 (1H, d, *J* = 5.9 Hz), 6.94-7.35 (5H, m), 8.37 (1H, d, *J* = 5.9 Hz); IR (KBr) ν_{max} 2828, 2218, 1629, 1559, 1472, 1309, 1233, 1153, 1043, 812, 761, 691 cm^{-1}. Anal. Calcd for $C_{19}H_{20}N_4O_2S$: C, 61.94; H, 5.47; N, 15.21; S, 8.70. Found: C, 61.82; H, 5.45; N, 15.17; S, 8.68.

2-(2-Oxo-2-(4-(pyridin-2-yl)piperazin-1-yl)ethylthio)-4-methoxypyridine-3-carbonitrile (11)

Reaction with 2-chloro-1-(4-(pyridin-2-yl)piperazin-1-yl)ethanone hydrochloride. Product **11** was isolated as a white solid (21%), mp 203-205 ˚C. ^{1}H NMR (DMSO-d_6) δ 3.45-3.65 (8H, m), 3.98 (3H, s), 4.36 (2H, s), 6.63-6.69 (1H, dd, J_1 = 4.8 Hz, J_2 = 6.6 Hz), 6.82 (1H, d, *J* = 8.5 Hz), 7.05 (1H, d, *J* = 6.1

Hz), 7.51-7.59 (1H, m), 8.10-8.14 (1H, dd, J_1 = 1.6 Hz, J_2 = 4.8 Hz), 8.49 (1H, d, J = 6.1 Hz); IR (KBr) ν_{max} 2846, 2217, 1628, 1560, 1474, 1306, 1236, 1158, 1046, 979, 810, 781, 735 cm^{-1}. Anal. Calcd for $C_{18}H_{19}N_5O_2S$: C, 58.52; H, 5.18, N, 18.96; S, 8.68. Found: C, 58.39; H, 5.16; N, 18.92; S, 8.66.

Methyl 3-(3-cyano-4-methoxypyridin-2-ylthio)propanoate (12)

2-Mercapto-4- methoxypyridine-3-carbonitrile (**9**) (0.332 g, 2 mmol) was dissolved in DMF (20 mL) and KOH (0.112 g, 2 mmol) was added to the solution. Then methyl 3-bromopropanoate (0.334 g, 2 mmol) was added, and the mixture was stirred at rt for 45 min. Then ice (10 g) was added to the mixture and the precipitated solid was filtered off and crystallized from MeOH/H_2O (2:3) to give methyl 3-(3-cyano-4-methoxypyridin-2-ylthio)propanoate (**12**) as a white solid (0.151 g, 30%), mp 102-104 ˚C. ^{1}H NMR (DMSO-d_6) δ 2.72 (2H, t, J = 6.9 Hz), 3.42 (2H, t, J = 6.9 Hz), 3.61 (3H, s), 3.97 (3H, s), 7.06 (1H, d, J = 5.8 Hz), 8.53 (1H, d, J = 5.8 Hz); IR (KBr) ν_{max} 2949, 2221, 1741, 1562, 1468, 1362, 1304, 1202, 1040, 814 cm^{-1}. Anal. Calcd for $C_{11}H_{12}N_2O_3S$: C, 52.37; H, 4.79; N, 11.10; S, 12.71. Found: C, 52.21; H, 4.57; N, 11.00; S, 12.68.

General procedure for the synthesis of compounds 13, 15-19

2-Mercapto-4-methoxypyridine-3-carbonitrile (**9**) (0.332 g, 2 mmol) was dissolved in DMF (15 mL) and KOH [(0.224 g, 4 mmol) or (0.336 g, 6 mmol for compound **16** and **17**)] was added. Then ethyl 2-chloroacetate (2 mmol) (for **13**), (adamantan-1-yl)methyl chloroacetate (for **15**), 2-chloro-1-(4-methylpiperazin-1-yl)ethanone hydrochloride (for **16**), 1-(2-chloroacetyl)-4-(4-fluorophenyl)piperazine hydrochloride (for **17**), 2-bromo-1-phenylethanone (for **18**) and 3-chloropentane-2,4-dione (for **19**) was added, and the mixture was stirred at rt for 30 min. The precipitated solid was filtered and recrystallized from MeOH/H_2O (1:1).

Ethyl 3-amino-4-methoxythieno[2,3-*b*]pyridine-2-carboxylate (13)

Reaction with ethyl 2-chloroacetate. Product **13** was isolated as a white solid (33%), mp 123-124 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.26 (3H, t, J = 7.3 Hz), 4.02 (3H, s), 4.22 (2H, q, J = 7.3 Hz), 6.94 (2H, s), 7.01 (1H, d, J = 5.3 Hz), 8.49 (1H, d, J = 5.3 Hz); IR (KBr) ν_{max} 3502, 3368, 1671, 1566, 1509, 1366, 1290, 1131, 1043, 972, 764, 571 cm^{-1}. Anal. Calcd for $C_{11}H_{12}N_2O_3S$: C, 52.37; H, 4.79; N, 11.10; S, 12.71. Found: C, 52.29; H, 4.77; N, 11.08; S, 12.68.

(Adamantan-1-yl)methyl 4-methoxythieno[2,3-*b*]pyridine-2-carboxylate (15)

Reaction with (adamantan-1-yl)methyl chloroacetate. Product **15** was isolated as a white solid (51%), mp 225-226 ˚C. ^{1}H NMR (DMSO-d_6) δ 1.52-1.91 (15H, m), 3.80 (2H, s), 4.02 (3H, s), 6.91 (2H, s), 7.04 (1H, d, J = 5.7 Hz), 8.48 (1H, d, J = 5.7 Hz); IR (KBr) ν_{max} 3438, 3322, 2904, 1675, 1600, 1510, 1294, 1051, 811, 762 cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_2O_3S$: C, 64.49; H, 6.49; N, 7.52; S, 8.61. Found: C, 64.32; H, 6.47; N, 7.50; S, 8.59.

(3-Amino-4-methoxythieno[2,3-*b*]pyridin-2-yl)(4-methylpiperazin-1-yl)methanone (16)

Reaction with 2-chloro-1-(4-methylpiperazin-1-yl)ethanone hydrochloride. Product **16** was isolated as a white solid (47%), mp 174-176 ˚C. ^{1}H NMR (DMSO-d_6) δ 2.18 (3H, s), 2.31 (4H, s), 3.60 (4H, s), 4.01 (3H, s), 6.24 (1H, s); 6.32 (1H, s), 6.93 (1H, d, *J* = 5.3 Hz), 8.38 (1H, d, *J* = 5.3 Hz); IR (KBr) ν_{max} 3481, 3346, 1617, 1591, 1499, 1424, 1295, 1141, 1050, 1002, 807, 752 cm^{-1}. Anal. Calcd for $C_{14}H_{18}N_4O_2S$: C, 54.88; H, 5.92; N, 18.29; S, 10.47. Found: C, 54.74; H, 5.91; N, 18.25; S, 10.44.

(3-Amino-4-methoxythieno[2,3-*b*]pyridin-2-yl)(4-(4-fluorophenyl)piperazin-1-yl)methanone (17)

Reaction with 1-(2-chloroacetyl)-4-(4-fluorophenyl)piperazine hydrochloride. Product **17** was isolated as a yellow solid (50%), mp 152-153 ˚C. ^{1}H NMR (DMSO-d_6) δ 3.11 (4H, m), 3.72 (4H, m), 4.00 (3H, s), 6.38 (2H, s), 6.97-7.06 (5H, m), 8.43 (1H, d, *J* = 5.8 Hz); IR (KBr) ν_{max} 3494, 3328, 1586, 1511, 1432, 1291, 1159, 1059, 1010, 823, 523 cm^{-1}. Anal. Calcd for $C_{19}H_{19}FN_4O_2S$: C, 59.05; H, 4.96; N, 14.50; S, 8.30. Found: C, 58.90; H, 4.94; N, 14.48; S, 8.28.

(3-Amino-4-methoxythieno[2,3-*b*]pyridin-2-yl)(phenyl)methanone (18)

Reaction with 2-bromo-1-phenylethanone. Product **18** was isolated as a yellow solid (52%), mp 145-147 ˚C. ^{1}H NMR (DMSO-d_6) δ 4.06 (3H, s), 7.04 (1H, d, *J* = 5.3 Hz), 7.51-7.59 (3H, m), 7.75 (2H, d, *J* = 7.3 Hz), 8.12 (2H, bs), 8.52 (1H, d, *J* = 5.3 Hz); IR (KBr) ν_{max} 3490, 3447, 3308, 1602, 1488, 1452, 1323, 1039, 809, 725, 693 cm^{-1}. Anal. Calcd for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85; S, 11.28. Found: C, 63.24; H, 4.33; N, 9.71; S, 11.11.

1-(3-Amino-4-methoxythieno[2,3-*b*]pyridin-2-yl)ethanone (19)

Reaction with 3-chloropentane-2,4-dione. Product **19** was isolated as a yellow solid (67%), mp 138-141 ˚C. ^{1}H NMR (DMSO-d_6) δ 2.30 (3H, s), 4.02 (3H, s), 7.02 (1H, d, *J* = 5.5 Hz), 7.49-7.81 (2H, bs), 8.53 (1H, d, *J* = 5.5 Hz); IR (KBr) ν_{max} 3326, 1606, 1579, 1496, 1460, 1367, 1295, 1050, 803, 720, 576 cm^{-1}. Anal. Calcd for $C_{10}H_{10}N_2O_2S$: C, 54.04; H, 4.53; N, 12.60; S, 14.43. Found: C, 53. 96; H, 4.51; N, 12.58; S, 14.39. **3-Amino-4-methoxythieno[2,3-*b*]pyridine-2-carboxamide (14)**

2-Mercapto-4-methoxypyridine-3-carbonitrile (**9**) was dissolved in MeOH (20 mL) and MeONa (92 mg, 4 mmol Na/MeOH 5 mL) was added. Then 2-chloroacetamide (0.35 mL, 2 mmol) was added and the mixture was refluxed for 3 h. The mixture was cooled down, the precipitated solid was filtered off and crystallized from MeOH/H_2O (1:1) to give 3-amino-4-methoxythieno[2,3-*b*]pyridine-2-carboxamide (**14**) as a white solid (14%), mp 238-240 ˚C. ^{1}H NMR (DMSO-d_6) δ 4.99 (3H, s), 6.94 (d, 1H, *J* = 5.7 Hz), 6.97 (2H, s) 7.05 (2H, s), 8.43 (1H, d, *J* = 5.7 Hz); IR (KBr) ν_{max} 3488, 3325, 1667, 1583, 1503, 1289, 1045, 743, 478 cm^{-1}. Anal. Calcd for $C_9H_9N_3O_2S$: C, 48.42; H, 4.06; N, 18.82; S, 14.36. Found: C, 48.32; H, 4.4; N, 18.79; S, 14.32.

REFERENCES

1. S. A. Armstrong, J. M. Berge, P. Brown, J. S. Elder, A. K. Forrest, O. W. Hamprecht, and R. L.

Jarrest, PCT Int. Appl. WO 0071, 524 (*Chem. Abstr*., **134**, 17496z, 2001).

2. Proctor and Gamble Ltd; U. S. Pat. 3236733 (*Chem. Abstr*., **64**, 17364, 1966).
3. A. B. Demilo, T. J. Kelly, and R. E. Redgern, *Chem. Week*, 1981, **129**, 89.
4. E. A. Bakhite, A. E. Abel-Rahman, O. S. Mohamed, and E. A. Thabet, *Bull. Korean Chem. Soc*., 2002, **23**, 1709.
5. R. Gilmour, J. E. Foster, Q. Sheng, J. R. McClain, A. Riley, P.-M. Sun, W.-L. Ng, D. Yan, T. I. Nicas, K. Henry, and M. E. Winkler, *J. Bacteriol.*, 2005, **187**, 8196.
6. A. Miszke and H. Foks, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2008, in print.
7. R. Jain, B. Vaitilingam, A. Nayyar, and P. B. Palde, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1051.
8. M. Mittelbach, G. Kastner, and H. Junek, *Arch. Pharm*., 1985, **318**, 481.
9. J. S. Parnes and M. Delgado, *Heterocycles*, 2004, **63**, 2199.
10. A. Balows, H. J. Hausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy, Manual of Clinical Microbiology (5^{th} ed). Am. Soc. Microbiol., Washington, 1991.
11. A. B. Forbes, D. F. Sahn, and A. S. Weissfeld, Bailey and Scott's Diagnostic Microbiology (12^{th} ed). Mosby Elsevier, St. Louis, 2007.
12. W. C. Winn, G. W. Allen, P. C. Schrekenberger, and G. L. Woods, Koneman's Color Atlas and Textbook of Diagnostic Microbiology (6^{th} ed). Williams and Wilkins., Philadelphia, 2006.