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Synthesis and antimicrobial evaluation of some new thienopyridine, pyrazolopyridine and pyridothienopyrimidine derivatives

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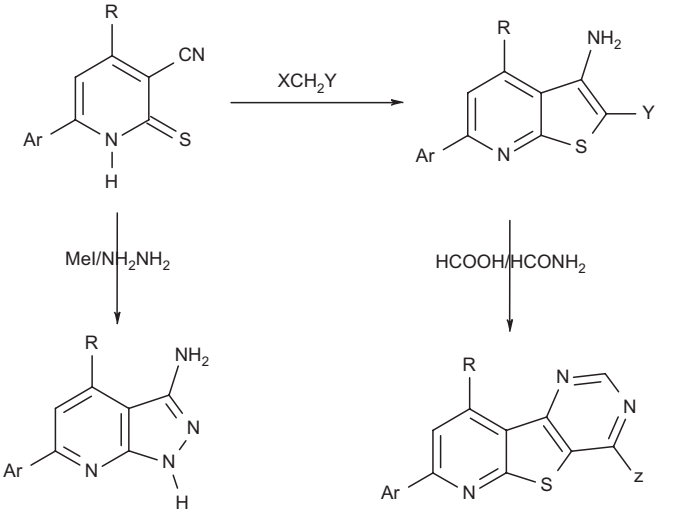
Thieno[2,3-b]pyridines **7**, **8**, **9** were obtained via the S-alkylation of **3** with a variety of alkylating agents followed by cyclization in a basic medium. On the other hand, compound **3** reacted with methyl iodide to give compound **10** which was converted to pyrazolo[3,4-b]pyridine derivative **13** by reaction with hydrazine hydrate. Pyrido[3′, 2′:4,5]thieno[3,2-d]pyrimidines derivatives **14**, **16** were obtained by reaction of **7** with each of formic acid and formic/formamide, respectively. Furthermore, compound **14** was obtained by the reaction of **8** with formamide. Structures of the newly synthesized products were determined by elemental analysis and spectral data.



Keywords: cyanopyridinethiones; thienopyridine; polycyclic pyridines; cyanothioacetamide; pyridothienopyrimidine; enaminonitrile

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1. Introduction

3-Cyano-2(1H)-pyridinethiones are of interest because of their use as intermediates for the synthesis of biologically active polycyclic compounds (*1*, *2*). Numerous thieno[2,3-b]pyridines have been investigated in relation to their biological and pharmacological activities and some of them proved to possess antibacterial (*3*, *4*), antiviral (*5*), antihypertensive (*6*) and immunostimulating (*7*) activities. Others are characterized by a broad spectrum of biological activities such as anticancer (*8*), antidepressant (*9*) and neurotropic activities (*10*). Also, the biological activities of condensed pyridines and pyrimidines as antimicrobial (*11–13*), sedative and antimalarial agents are well documented (*14*, *15*). On the other hand, pyridothienopyrimidine derivatives have found applications as analgesics (*16*), antipyretics (*17*), anti-inflammatories (*18*) and selective Cdc7 kinase inhibitors (*19*). In view of the above facts, it was of interest to synthesize the ring system combining both the pyrimidine and the thienopyridine moieties which might have good biological and medicinal applications.

2. Results and discussion

2.1. *Chemistry*

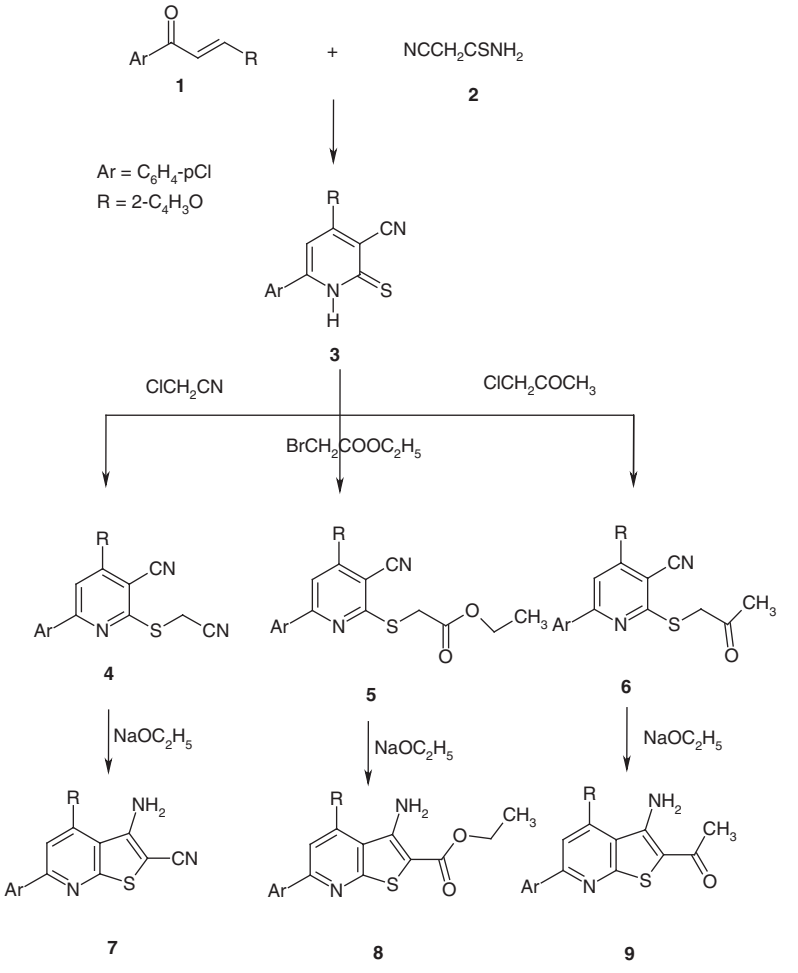
Treatment of (2*E*)-1-(4-chlorophenyl)-3-(2-furyl)prop-2-en-1-one (**1**) with 2-cyanothioacetamide (**2**) in ethanolic sodium ethoxide solution afforded 6-(4-chlorophenyl)-4-(2-furyl)-2-thioxopyridine-1,2-dihydro-3-carbonitrile (**3**) in moderate yield. The structure of the product was supported by its elemental analysis and spectral data. Compound **3** was alkylated with chloroacetonitrile, ethyl bromoacetate and chloroacetone in dimethylformamide (DMF)/KOH to give **4**, **5** and **6** derivatives, respectively (Scheme 1).

The IR spectrum of compound **4** revealed the presence of two absorption bands at 2193 and 2215 cm^{-1} for the two cyano groups. ^1H NMR spectrum showed a signal at δ 4.48 (s, 2H, CH_2), signal at δ 6.80 (s, 1H, 5-H of the pyridinethione ring) and a broad signal at δ 7.60–8.35 (m, 7H, Ar-H).

The IR spectrum of compound **5** revealed the presence of absorption bands at 2210 and 1736 cm^{-1} for the cyano group and the CO ester group, respectively. Its ^1H NMR spectrum showed signal at δ 1.55 (t, 3H, CH_3), δ 4.08 (s, 2H, CH_2), δ 4.23 (q, 2H, CH_2), δ 6.68 (s, 1H, 5-H of the pyridinethione ring) and a broad signal at δ 7.27–8.06 (m, 7H, Ar-H).

Both structures **4** and **5** were confirmed by their mass spectra which showed peaks corresponding to their molecular ion at m/z 351 and m/z 398, respectively. Similarly, the structure of compound **6** was confirmed based on elemental analysis and spectral data. Compounds **4**, **5** and **6** were converted to the corresponding thienopyridine derivatives by refluxing in sodium ethoxide solution for 3–6 h to give **7**, **8** and **9**, respectively (Scheme 1). The reaction seemed to proceed via intramolecular cyclocondensation to give the substituted thieno[2,3-b]pyridine derivatives in good yield. The absence of cyano group absorption in the IR spectrum of compound **9** and the appearance of absorption bands at 3421 and 3301 cm^{-1} for the amino group confirmed the proposed structure. Furthermore, ^1H NMR spectrum showed signals at δ 2.50 (s, 3H, CH_3), δ 6.81 (s, 1H, 5-H of the pyridinethione ring), broad signal at δ 6.90–8.10 (m, 7H, Ar-H) and δ 13.90 (s, 2H, NH_2) and the disappearance of the signal at δ 4.2 (s, 2H, CH_2). Based on these facts, the structure of compound **9** was assigned as 1-(3-amino-6-(4-chlorophenyl)-4-(2-furyl)thieno[2,3-b]pyridine-2-yl)ethanone.

On the other hand, reaction of **3** with methyl iodide gave the methyl sulfide derivative **10**, as evident from its elemental analysis and spectral data, in a very good yield. Treatment of **10** with hydrazine hydrate under reflux for 12 h gave compound **11** in almost a quantitative yield (Scheme 2).

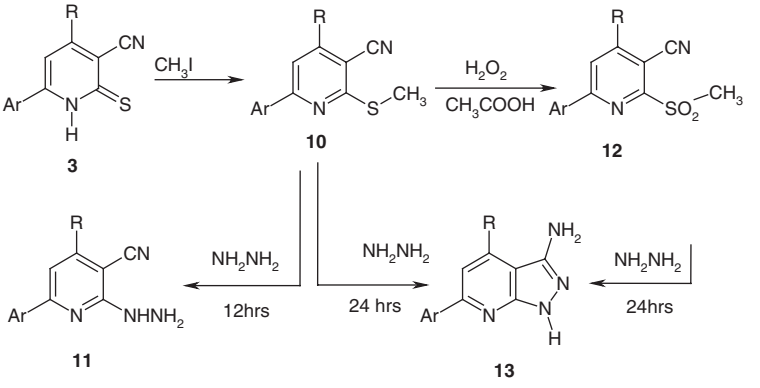


Scheme 1. Reactions of pyridinethione with chloroacetonitrile, ethyl bromoacetate and chloroacetone.

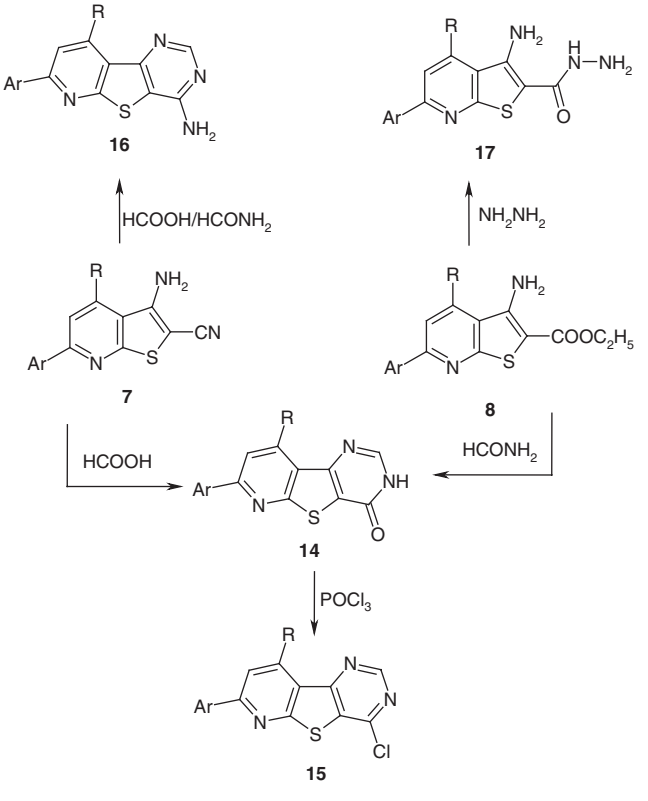
The IR spectrum of compound **11** revealed an absorption bands at 2210 cm^{-1} for the cyano group, 3436 and 3339 cm^{-1} for $NHNH_2$ group and its mass spectra showed the molecular ion peak at m/z 310. Based on the above data, structure **11** was assigned as 6-(4-chlorophenyl)-4-(2-furyl)-2-hydrazinylpyridine-3-carbonitrile. Meanwhile, treatment of **10** with hydrazine hydrate under reflux for 24 h gave compound **13** which can also be prepared by the reaction of **12** with hydrazine hydrate under the same conditions (Scheme 2). The IR spectrum of compound **13** revealed absorption bands at 3190 and 3100 cm^{-1} for the amino group and no absorption was detected for the cyano group.

Compound **7** as a typical enaminonitrile derivative reacted with formic acid upon heating for several hours to yield 7-(4-chlorophenyl)-9-(2-furyl) pyrido[3′, 2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (**14**) (Scheme 3). The absence of cyano absorption and the appearance of the NH and carbonyl absorption bands at 3434 and 1650 cm^{-1} in the IR spectrum confirmed the proposed structure **14**. Its mass spectrum showed a peak at m/z 379 (M^+). Compound **14** was alternatively obtained by heating compound **8** in formamide under reflux for 12 h (Scheme 3).

4-Chloro-7-(4-chlorophenyl)-9-(2-furyl)pyrido[3′,2′:4,5]thieno-[3,2-d]pyrimidine (**15**) was obtained by the reaction of **14** with $POCl_3$ under reflux for 1 h. Compound **7** reacted with



Scheme 2. Conversion of S-methyl derivative **10** to pyrazolopyridine derivative **13**.



Scheme 3. Reactions of thienopyridines (**7**, **8**) with formic acid, formamide and hydrazine.

a mixture of $HCOOH/HCONH_2$ under reflux for several hours to give 7-(4-chlorophenyl)-9-(2-furyl)-pyrido[3′,2′: 4,5]thieno[3,2-d]pyrimidin-4-amine (**16**). Finally, compound **8** reacted with hydrazine hydrate under reflux for 24 h to give the corresponding hydrazide derivative **17** (Scheme 3). Structures of compounds **16** and **17** have been determined by elemental analyses and spectral data studies. The IR spectrum of **16** revealed absorption bands at 3421 and 3301 cm^{-1} for the amino group, while its mass spectrum showed a peak at m/z 378 (M^+). On the other hand,

the IR spectrum of **17** revealed absorption bands at 3468 and 3373 cm^{-1} for the amino group and its mass spectrum showed a peak at m/z 384 (M^+).

2.2. *Antimicrobial activity*

The antibacterial and antifungal activities were carried out in the Microbiology Division of the Microanalytical Center at Cairo University, using the diffusion plate method (*20–22*). A bottomless cylinder containing a measured quantity (1 ml, 20 mg/ml) of the sample is placed on a plate (7 scm diameter) containing a solid bacterial medium (nutrient agar broth) or a fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the test organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism (% inhibition = sample inhibition zone (cm)/plate diameter $\times$ 100). All measurements were done in DMSO as a solvent which has zero inhibition activity. The obtained results were compared with some reference antibiotics that were purchased from Egyptian markets. As shown in Table 1, all the tested compounds were found to exhibit moderate to high activity against both *Escherichia coli* and *Staphylococcus aureus* microorganisms with respect to the used reference tetracycline. The antifungal activity of compounds **7**, **8** and **12** were found to be much higher than the used reference Amphotericin B.

2.3. *Minimum inhibitory concentration determination (by serial dilution method)*

Stationary-phase cultures of bacteria were prepared at 37°C and used to inoculate fresh 5.0 ml culture to an OD_{600} of 0.05. The 5.0 ml cultures were then incubated at 37°C until an OD_{600} of 0.10 was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6×10^{-5} colony forming units (CFUs)/ml. Serial dilutions from the treatments (0–320 μg/ml) were prepared and mixed with 5.0 ml of the standardized bacteria suspension and then added to the plates and incubated for 24 h at 37°C. The CFUs were counted for each dilution (*23*).

Table 1. Antibacterial and antifungal activities of some of the synthesized compounds.

Sample	*E. coli* inhibition (%)	*S. aureus* inhibition (%)	*Candida albicans* inhibition (%)
Control: DMSO	0.0	0.0	0.0
3	42.4	50.0	0.0
4	36.4	46.7	0.0
7	36.4	43.3	52.6
8	36.4	50.0	63.2
9	30.3	43.3	0.0
12	30.3	33.3	47.4
13	36.4	40.0	0.0
14	36.4	36.7	0.0
15	33.3	36.7	0.0
16	30.3	40.0	0.0
17	30.3	33.3	0.0
Tetracycline	33.0	30.0	–
Amphotericin B	–	–	19.0

Minimum inhibitory concentration (MIC) determination

	MIC (μg/ml)	
Sample	*E. coli* (G^-)	*S. aureus* (G^+)
3	214	198
Standard: tetracycline antibacterial agent	86	116

3. Experimental

Melting points were measured on an Electrothermal Melting Point Apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. The ^{1}H NMR spectra were determined in DMSO-d_6 at 300 MHz on a Varian mercury VX 300 NMR spectrometer using TMS as an internal standard. ^{13}C spectra were run at 75.46 MHz in DMSO-d_6. Chemical shifts are quoted in δ and were related to that of the solvent. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University.

3.1. *Synthesis of 3*

A mixture of **1** (11.6 g, 50 mmol) and cyanothioacetamide **2** (5 g, 50 mmol) was heated under reflux in ethoxide solution (1.15 g of Na in 50 ml ethanol) for 4 h. The reaction mixture was cooled; the so-formed solid was filtered and recrystallized from methanol/DMF.

3.1.1. *6-(4-Chlorophenyl)-4-(2-furyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile* (***3***)

Deep red crystals, m.p. = 228–232°C; yield: 23%. IR (KBr): 2217 and 3143 cm^{-1} (CN, NH); ^{1}H NMR (δ ppm): 6.88 (s, 1H, 5-H of pyridinethione ring), 7.33–8.15 (m, 7H, Ar-H), 14.10 (b, 1H, NH). ^{13}C NMR: δ = 102.6 (CH), 110.2 (C), 111.4 (CH), 112.7 (CH), 115.9 (C), 127.8 (2CH), 128.8 (CH), 128.8 (2CH), 132.4 (C), 133.5 (C), 149.9 (C), 162.8 (C), 169.3 (C), 170.6 (C=S). MS (m/z): 312 (M^+). Anal. Calcd. for $C_{16}H_9ClN_2OS$ (312.78): C, 61.44; H, 2.90; N, 8.96; S, 10.25%; found: C, 61.50; H, 3.00; N, 8.99; S, 10.00%.

3.2. *General procedure for the synthesis of 4, 5, 6 and 10*

A mixture of **3** (3.1 g, 10 mmol) and potassium hydroxide (0.62 g, 11 mmol) in DMF (10 ml) was stirred for 2 h at room temperature. Each of chloroacetonitrile, ethyl bromoacetate or chloroacetone and methyl iodide (10 mmol each) was added and stirring was continued for 2 h. The resulting solid was collected and recrystallized from the proper solvent to give **4**, **5**, **6** and **10**, respectively.

3.2.1. *6-(4-Chlorophenyl)-2-(cyanomethylthio)-4-(2-furyl)nicotinonitrile* (***4***)

Pink crystals, m.p. = 260–263°C; yield: 99% (DMF). IR (KBr): 2193 and 2215 cm^{-1}, 2(CN). ^{1}H NMR (δ ppm): 4.48 (s, 2H, CH_2), 6.80 (s, 1H, 5-H of the pyridinethione ring), 7.60–8.35 (m, 7H, Ar-H). MS (m/z): 351 (M^+). Anal. Calcd for $C_{18}H_{10}ClN_3OS$ (351.82): C, 61.54; H, 2.87; N, 11.94; S, 9.11%; found: C, 61.65; H, 2.90; N, 12.00; S, 9.35%.

3.2.2. *Ethy{[6-(4-chlorophenyl)-3-cyano-4-(2-furyl)pyridine-2-yl]-thio}acetate* (***5***)

Brown crystals, m.p. = 172–175°C; yield: 90% (EtOH/DMF). IR (KBr): 2210 and 1736 cm^{-1} (CN, CO ester). ^{1}H NMR (δ ppm): 1.55 (t, 3H, CH_3), 4.08 (s, 2H, CH_2), 4.23 (q, 2H, CH_2), 6.68 (s, 1H, 5-H of the pyridinethione ring), 7.27–8.06 (m, 7H, Ar-H); MS (m/z): 398 (M^+). Anal. Calcd. for $C_{20}H_{15}ClN_2O_3S$ (398.87): C, 60.22; H, 3.79; N, 7.02; S, 8.04%; found: C, 60.00; H, 3.89; N, 7.15; S, 8.00%.

3.2.3. *6-(4-Chlorophenyl)-4-(2-furyl)-2-[(2-oxopropylthio)]nicotinonitrile* (***6***)

Brown crystals, m.p. = 192–194°C; yield: 88% (ETOH/DMF). IR (KBr): 2218 and 1710 cm^{-1} (CN, CO). ^{1}H NMR (δ ppm): 2.28 (s, 3H, CH_3), 4.37 (s, 2H, CH_2), 6.80 (s, 2H, CH_2), 6.80 (s, 1H, 5-H of pyridinethione ring), 7.60–8.20 (m, 7H, Ar-H). ^{13}C NMR: δ = 29.8 (CH_3), 47.7 (CH_2), 103.1 (C), 105.0 (C), 107.2 (CH), 117.7 (CH), 129.0 (CH), 129.4 (2CH), 132.9 (C), 134.4 (C), 142.9 (CH), 149.3 (CH), 154.0 (C), 158.9 (C), 164.2 (C), 200.4 (C=O). MS (m/z): 368 (M^+). Anal. Calcd. for $C_{19}H_{13}ClN_2O_2S$ (368.84): C, 61.87; H, 3.55; N, 7.60; S, 8.69%; found: C, 61.90; H, 3.65; N, 7.85; S, 8.70%.

3.2.4. *6-(4-Chlorophenyl)-4-(2-furyl)-2-(methylthio)nicotinonitrile* (***10***)

White crystals, m.p. = 158–160°C; yield: 98% (ETOH/DMF). IR (KBr): 2210 cm^{-1} (CN). ^{1}H NMR (δ ppm): 2.70 (s, 3H, CH_3), 6.62 (s, 1H, 5-H of pyridinethione ring), 7.20–8.11 (m, 7H, Ar-H). MS (m/z): 326 (M^+). Anal. Calcd. for $C_{17}H_{11}ClN_2OS$ (326.81): C, 62.48; H, 3.39; N, 8.57; S, 9.81%; found: C, 62.50; H, 3.45; N, 8.55; S, 9.71%.

3.3. *General procedure for the synthesis of 7, 8 and 9*

Each of **4** (3.5 g, 10 mmol), **5** (4.0 g, 10 mmol) and **6** (3.7 g, 10 mmol) was refluxed in sodium ethoxide solution (0.23 g, 10 ml) for 6 h. The so-formed solid was collected by filtration and recrystallized from the proper solvent to give **7**, **8** and **9**, respectively.

3.3.1. *3-Amino-6-(4-chlorophenyl)-4-(furyl)thieno[2,3-b]pyridine-2-carbonitrile* (***7***)

Orange crystals, m.p. = 259–262°C, yield: 43% (DMF). IR (KBr): 2193, 3450 and 3379 cm^{-1} (CN, NH_2). ^{1}H NMR (δ ppm): 6.80 (s, 1H, 5-H of the pyridinethione ring), 7.33–8.20 (m, 7H, Ar-H), 10.50 (s, 2H, NH_2). ^{13}C NMR: δ = 82.9 (C), 105.0 (C), 107.0 (CH), 111.2 (C), 121.1 (C), 121.7 (CH), 129.0 (CH), 129.4 (2CH), 132.9 (C), 134.4 (C), 142.9 (CH), 144.9 (C), 148.1 (C), 154.0 (C), 155.2 (C), 157.1 (C). MS (m/z): 351 (M^+). Anal. Calcd. for $C_{18}H_{10}ClN_3OS$ (351.82): C, 61.45; H, 2.87; N, 11.94; S, 9.11%; found: C, 61.55; H, 2.85; N, 11.75; S, 9.00%.

3.3.2. *[3-Amino-6-(4-chlorophenyl)-4-(2-furyl)thieno[2,3-b]pyridine-2yl](ethoxy) methanol* (***8***)

Green crystals, m.p. = 117–120°C; yield: 96% (ETOH/DMF). IR (KBr): 1751, 3448 and 3348 cm^{-1}, (CO, NH_2). ^{1}H NMR (δ ppm): 1.43 (t, 3H, CH_3), 4.41 (q, 2H, CH_2), 6.80 (s, 1H, 5-H of the pyridinethione ring), 6.96–8.08 (m, 7H, Ar-H), 14.01 (s, 2H, NH_2). ^{13}C NMR: δ = 14.1 (CH_3), 60.9 (CH_2), 105.0 (CH), 107.2 (CH), 118.6 (C), 121.2 (C), 121.7 (CH), 129.0 (2CH), 129.4 (2CH), 132.9 (C), 134 (C), 134.4 (C), 142.9 (CH), 144.9 (C), 154.0 (C), 155.2 (C), 156.5 (C), 160.6 (C=O ester). MS (m/z): 398 (M^+). Anal. Calcd. for $C_{20}H_{15}ClN_2O_3S$ (398.87): C, 60.22; H, 3.79; N, 7.02; S, 8.04%; found: C, 60.10; H, 3.57; N, 7.00; S, 8.20%.

3.3.3. *1-(3-Amino-6-(4-chlorophenyl)-4-(2-furyl)thieno[2,3-b]pyridine-2-yl)ethanone (**9**)*

Orange crystals, m.p. = 208–210°C; yield: 97% (ETOH/DMF). IR (KBr): 1616, 3421 and 3301 cm^{-1} (CO, NH_2); ^{1}H NMR (δ ppm): 2.50 (s, 3H, CH_3), 6.81 (s, 1H, 5-H of the pyridinethione ring), 6.90–8.10 (m, 7H, Ar-H), 13.90 (s, 2H, NH_2). ^{13}C NMR: δ = 27.8 (CH_3), 105.0 (CH), 107.2 (CH), 121.3 (C), 121.7 (CH), 129.0 (2CH), 129.4 (2CH), 133 (C), 134 (C), 134.4 (C), 142.9 (CH),

144.9 (C), 146 (C), 154.0 (C), 155.2 (C), 157.3 (C), 190.5 (C=O). MS (*m/z*): 368 (M^+). Anal. Calcd. for $C_{19}H_{13}ClN_2O_2S$ (368.84): C, 61.87; H, 3.55; N, 7.60; S, 8.69%; found: C, 62.00; H, 3.35; N, 7.80; S, 8.56%.

3.4. *Synthesis of 11*

A mixture of **10** (1 g, 3 mmol) and excess of hydrazine hydrate was refluxed for 12 h. The reaction mixture was cooled, and the so-formed solid was filtered, dried and recrystallized from ethanol to give **11**.

3.4.1. *6-(4-Chlorophenyl)-4-(2-furyl)-2-hydrazinylpyridine-3-carbonitrile (**11**)*

Yellow crystals, m.p. = 245–247°C; yield: 99%. IR (KBr): 2210, 3421, 3301 and 3143 cm^{-1} (CN, NH_2, NH). ^1H NMR (δ ppm): 6.80 (s, 1H, 5-H of the pyridinethione ring), 7.00–7.93 (m, 7H, Ar-H), 10.45 (b, 1H, NH), 13.90 (s, 2H, NH_2). MS (*m/z*): 310 (M^+). Anal. Calcd. for $C_{16}H_{11}ClN_4O$ (310.75): C, 61.84; H, 3.57; N, 18.03%; found: C, 61.75; H, 3.50; N, 18.00%.

3.5. *Synthesis of 12*

To a stirred mixture of **10** (1.6 g, 5 mmol) in glacial acetic acid (10 ml) was added 30% H_2O_2 solution (15 ml) and the mixture was heated under reflux for 3 h. After cooling, the so-formed solid was collected and recrystallized from DMF.

3.5.1. *6-(4-Chlorophenyl)-4-(2-furyl)-2-(methylsulfonyl)thieno[2,3-b]pyridine-3-carbonitrile (**12**)*

Brown crystals, m.p. = 263–266°C; yield: 66%. IR (KBr): 2209 cm^{-1} (CN). ^1H NMR (δ ppm): 3.40 (s, 3H, CH_3), 6.88 (s, 1H, 5-H of the pyridinethione ring), 6.99–8.20 (m, 7H, Ar-H). MS (*m/z*): 358 (M^+). Anal. Calcd. for $C_{17}H_{11}ClN_2O_3S$ (358.81): C, 56.91; H, 3.09; N, 7.81, S, 8.94%; found: C, 57.00; H, 3.10; N, 7.99; S, 9.00%.

3.6. *Synthesis of 13*

A mixture of **10** or **12** (1 g, 3 mmol) and excess of hydrazine hydrate was refluxed for 24 h. The reaction mixture was cooled; the so-formed solid was filtered, dried and recrystallized from acetic acid to give **13**.

3.6.1. *6-(4-Chlorophenyl)-4-(furyl)-1H-pyrazolo[3,4-b]pyridine-3-amine (**13**)*

Yellow crystals, m.p. = 285–288°C; yield: 95%. IR (KBr): 3462, 3378 and 3100 cm^{-1} (NH_2, NH). ^1H NMR (δ ppm): 6.80 (s, 1H, 5-H of the pyridinethione ring), 7.00–8.20 (m, 7H, Ar-H), 13.90 (s, 1H, NH), 14.20 (s, 2H, NH_2). MS (*m/z*): 310 (M^+). Anal. Calcd. for $C_{16}H_{11}ClN_4O$ (310.75): C, 61.84; H, 3.57; N, 18.03%; found: C, 61.90; H, 3.75; N, 18.20%.

3.7. *Synthesis of 14*

A mixture of **7** (1.8 g, 5 mmol) and formic acid (20 ml) or a mixture of compound **8** (2 g, 5 mmol) and formamide (20 ml) was heated under reflux for 12 h. After cooling, the reaction mixture was poured on ice and the formed solid was collected and recrystallized from DMF to give **14**.

3.7.1. *7-(4-Chlorophenyl)-9-(2-furyl) pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one* (***14***)

Orange crystals, m.p. > 300°C; yield: (80%). IR (KBr):1650 and 3110 cm^{-1} (CO, NH); ^{1}H NMR (δ ppm): 6.86 (s, 1H, 5-H of the pyridinethione ring), 7.00–8.10 (m, 7H, Ar-H), 8.90 (s, 1H, NH). ^{13}C NMR: δ = 105.0 (CH), 107.2 (CH), 121.7 (CH), 125.6 (C), 129.0 (CH), 129.4 (2CH), 132.9 (C), 134.4 (C), 137 (C), 144.9 (C), 145.6 (CH), 146 (C), 154.0 (C), 155.2 (C), 158.9 (C), 160.1 (C=O amide). MS (m/z): 379 (M^+). Anal. Calcd. for $C_{19}H_{10}ClN_3O_2S$ (379.83): C, 60.08; H, 2.65; N, 11.06; S, 8.44%; found: C, 60.10; H, 2.50; N, 11.00; S, 8.22%.

3.8. *Synthesis of 15*

Compound **14** (1.1 g, 3 mmol) reacted with $POCl_3$ (20 ml) under reflux for 1 h. The reaction mixture was poured over ice, and the resulting solid was collected by filtration and recrystallization from DMF to give **15**.

3.8.1. *4-Chloro-7-(4-chlorophenyl)-9-(2-furyl)pyrido[3′,2′:4,5]thieno-[3,2-d]pyrimidine* (***15***)

Brown crystals, m.p. = 260–263°C; yield: (88%). ^{1}H NMR (δ ppm): 6.80 (s, 1H, 5-H of the pyridinethione ring), 7.00–8.12 (m, 7H, Ar-H), 9.46 (s, 1H, 2-H of the pyrimidine ring). MS (m/z): 412 (M^+). Anal. Calcd. for $C_{19}H_9Cl_2N_3S_2$ (412.96): C, 55.08; H, 2.19; N, 10.14; S, 15.48%; found: C, 55.00; H, 2.10; N, 10.00; S, 15.45%.

3.9. *Synthesis of 16*

A mixture of **7** (1.8 g, 5 mmol), formamide (0.5 ml) and formic acid (20 ml) was heated under reflux for 11 h. After cooling, the reaction mixture was poured on ice and the formed solid was collected and recrystallized from DMF to give **16**.

3.9.1. *7-(4-Chlorophenyl)-9-(2-furyl)-pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine* (***16***)

Orange crystals, m.p. > 300°C; yield: (82%). IR (KBr): 3421 and 3301 cm^{-1} (NH_2). MS (m/z): 378 (M^+). Anal. Calcd. for $C_{19}H_{11}ClN_4S$ (378.84): C, 60.24; H, 2.93; N, 14.79; S, 8.46%; found: C, 60.00; H, 3.00; N, 14.90; S, 8.66%.

3.10. *Synthesis of 17*

A mixture of **8** (4.0 g, 10 mmol) and hydrazine hydrates (4 ml, 85% solution, 4 mmol) in absolute ethanol (20 ml) for 24 h was heated under reflux. The reaction mixture was cooled, and the resulting solid was collected and washed with ethanol/water and recrystallized from acetic acid to give **17**.

3.10.1. *3-Amino-6-(4-chlorophenyl)-4-(2-furyl)thieno[2,3-b]pyridine-2-carbohydrazide* (***17***)

Orange crystals, m.p. = 230–233°C; (96%); IR (KBr): 3468 and 3373 cm^{-1} (NH_2). ^{13}C NMR: δ = 105.0 (CH), 107.2 (CH), 121.7 (CH), 121.6 (C), 129.0 (CH), 129.4 (2CH), 134.4 (C), 137 (C), 142.9 (CH), 144.9 (C), (CH), 155.2 (C), 158.9 (C), 160.7 (C=O amide). MS (m/z): 384 (M^+). Anal. Calcd. for $C_{18}H_{13}ClN_4O_2S$ (384.85): C, 56.18; H, 3.40; N, 14.56; S, 8.33%; found: C, 56.20; H, 3.50; N, 14.75; S, 8.20%.

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