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SYNTHESIS OF SOME FUSED HETEROCYCLES BASED ON THIENO[2,3-*b*]PYRIDINE AND THEIR ANTIMICROBIAL ACTIVITY

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The reaction of 3-amino-4,6-dimethylthieno[2,3-b]pyridine-2-carbonitrile with ethylenediamine in the presence of a catalytic amount of carbon disulfide afforded 2-(4,5-dihydro-1H-imidazol-2-yl)-4,6-dimethylthieno-[2,3-b]pyridine-3-amine while its reaction with triethyl orthoformate followed by the reaction with hydrazine hydrate gave 4-imino-7,9-dimethylpyrido[3',2':4,5]thieno[3,2-d]pyrimidine-3(4H)-amine. These two derivatives underwent cyclocondensation reactions with commercially available reactants to afford new heterocycles containing the thieno[2,3-b]pyridine moiety. Some of the synthesized derivatives were tested for antimicrobial and antifungal activity.

Keywords: imidazole, pyridothienopyrimidine, 1,2,3,4-tetrazoles, 1,2,4-triazoles, antibacterial, antifungal activity, cyclocondensation.

The biological activities of condensed pyrimidines as sedatives, antibacterials, and antimalarials are well documented [1, 2]. Compounds containing a fused pyrimidine ring have attracted attention due to their wide range of biological activities, particularly in cancer and virus research [3]. Among these heterocycles the thienopyrimidine class is also of interest because some derivatives such as *Tiprinast* [4] have been shown to be clinically effective antiallergics. Several thieno[2,3-*b*]pyridine derivatives are known to possess antibacterial [5-7], antihypertensive [8], and gonadotropin releasing hormone antagonizing [9, 10] activity.

Many thienopyridines have been evaluated pharmacologically and have been found to show activity in diabetes mellitus [11-13] and also as analgesics and anti-inflammatory agents [14-16], sedatives [14], anticoagulants [16], and antiartherosclerotics [17-20]. Considerable attention has been drawn to the synthesis of condensed heterocyclic systems derived from 1,2,4-triazoles, pyrazoles, and pyridothienopyrimidine [21-25]. In continuation of our ongoing search for new heterocycles [26-29] we have prepared a series of compounds containing a pyridothienopyrimidine moiety and tested their antimicrobial properties.

3-Amino-4,6-dimethylthieno[2,3-*b*]pyridine-2-carbonitrile (**1**) [30] reacted with ethylenediamine in the presence of carbon disulfide to afford 2-(4,5-dihydro-1H-imidazol-2-yl)-4,6-dimethylthieno[2,3-*b*]pyridine-3-amine (**2**). Its IR spectrum showed the disappearance of the characteristic absorption band at 2190 cm^{-1} of the

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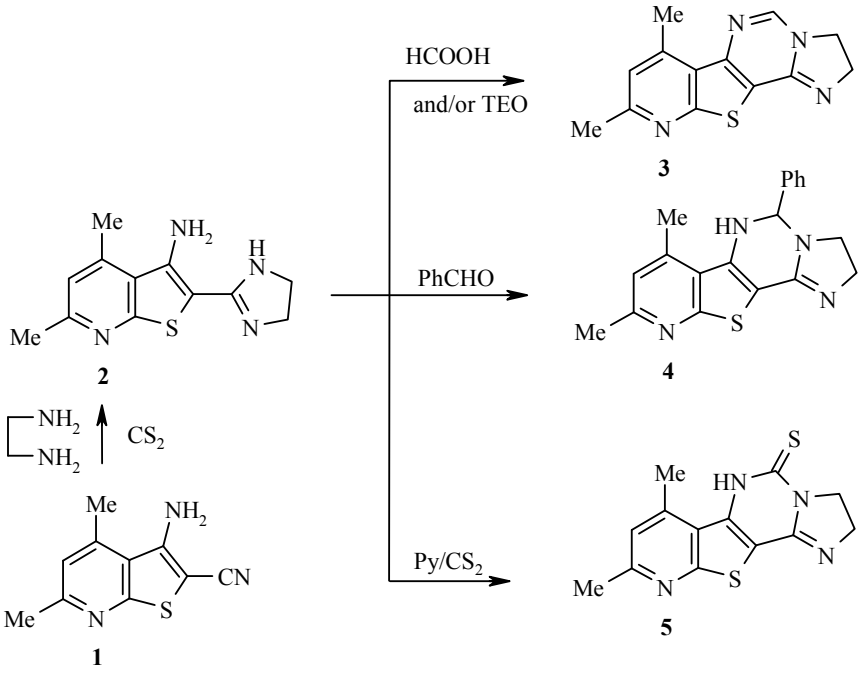
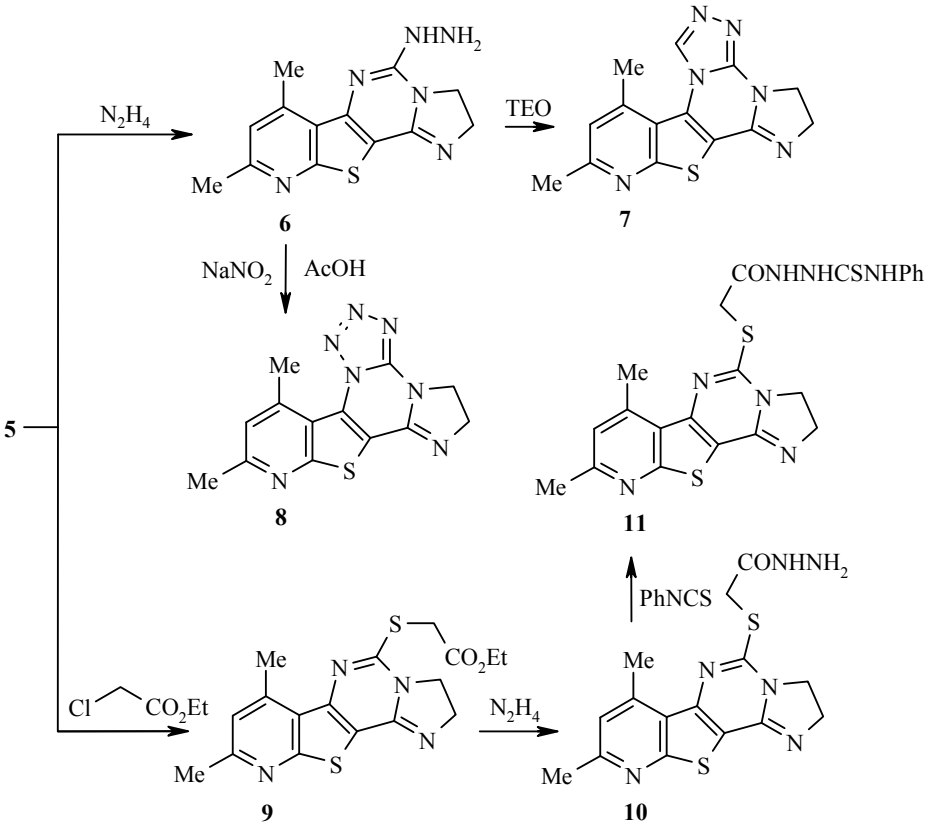
CN group, while the ^{1}H NMR spectrum showed the appearance of a characteristic signal of methylene protons at 3.50-3.98 ppm, as well as a singlet at 7.98 ppm for the NH group. Compound **2** was subjected to cyclization to tetracyclic imidazopyridothienopyrimidine systems in different ways. Treatment of compound **2** with triethyl orthoformate (TEO) gave 7,9-dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyrimidine (**3**), which showed a new signal in its ^{1}H NMR spectrum at δ 8.14 ppm due to H-5 of the pyrimidine ring. The reaction of compound **2** with benzaldehyde under conditions analogous to those reported in [31] led to the formation of 7,9-dimethyl-5-phenyl-2,3,5,6-tetrahydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyrimidine (**4**). Its ^{1}H NMR spectrum showed signals at δ 5.22 and 6.32 because of NH and H-5 of the cyclized pyrimidine ring. On the other hand, 7,9-dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyrimidine-5-thione (**5**) was obtained by heating compound **2** with CS_2 in boiling pyridine. The IR spectrum of compound **5** showed absorption bands at 3250 (NH) and 1220 cm^{-1} (C=S). Also its ^{1}H NMR spectrum showed a broad signal at δ 7.88 ppm due to the NH group. Mass spectra of these derivatives **3**, **4**, and **5** showed an [M^+] peak in agreement with their molecular weight.



Thione derivative **5** was readily converted into the corresponding 5-hydrazino-7,9-dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyrimidine (**6**) by treating with hydrazine hydrate at reflux temperature. Its ^{1}H NMR spectrum agreed with the structure, which showed a broad band at δ 4.41 and 7.20 ppm corresponding to the $NHNH_2$, and its mass spectrum revealed a molecular ion peak at 286, confirming the molecular weight of compound **6**. Cyclization of hydrazino derivative **6** by the action of TEO and sodium nitrite in acetic acid gave the fused 9,11-dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*][1,2,4]-triazolo[3,4-*a*]pyrimidine (**7**) and 10,12-dimethyl-5,6-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]tetrazolo[5,1-*a*]pyrimidine (**8**), respectively. The mass spectrum showed a molecular ion peak for each derivative at *m*/*z* 296 and 297, respectively. Alkylation of compound **5** with ethyl 2-chloroacetate under alkaline condition affords the S-alkylated compound **9**, which was readily converted into the corresponding acetohydrazide **10** after treatment with hydrazine hydrate in absolute ethanol. The structure of derivatives **9** was confirmed by IR spectrum, which showed absorption bands at 1729 cm^{-1} due to COOEt, besides its ^{1}H NMR spectrum, which showed signals as a triplet at δ 1.23, a quartet at δ 4.32 ppm due to the ester group, and a singlet at δ 4.98 for SCH_2.

Also derivative **10** was assigned by IR spectroscopy, which revealed the absorption band at 3427-3248 cm^{-1} due to $NHNH_2$, and its ^1H NMR spectrum showed the appearance of broad signals at δ 4.90 and 9.30 corresponding to the $NHNH_2$ group. The mass spectra of these derivatives **9** and **10** demonstrated the molecular ion peak for each compound at *m*/*z* 374 and 360 corresponding to their molecular weight. The latter acetohydrazide derivative **10** was treated with phenyl isothiocyanate in absolute ethanol to afford the corresponding thiosemicarbazide derivative **11**.



The thienopyridine derivative **1** reacted with TEO to give ethyl (2-cyano-4,6-dimethylthieno-[2,3-*b*]pyridin-3-yl)imidoformate (**12**). This compound was used as an intermediate for the preparation of compound **13** by cyclization with hydrazine hydrate. Its structure was elucidated using IR, MS, and ^1H NMR spectra, the latter showing two signals at δ 5.80 and 8.17 ppm corresponding to the vicinal amino and imino groups, respectively. 4-Imino-7,9-dimethylpyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-3(4H)-amine (**13**) is considered as a key intermediate for preparing fused heterocycles such as triazolo[1,5-*c*]pyrimidines, which may possess pharmacological properties similar to those of theophylline [32, 33]. 7,9-Dimethylpyrido[3',2':4,5]-thieno[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**14**) was prepared by cyclocondensation reaction of derivative **13** with neat formic acid (yield 33%), while it was prepared by heating the derivative **13** with the triethylorthoformate to afford the derivative **14** in good yield (83%). The structure of compound **14** was elucidated by the mass spectrum, which showed a molecular ion peak at *m/z* 255 corresponding to the molecular weight. 2,7,9-Trimethylpyrido[3',2':4,5]thieno[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**15**) was obtained by boiling compound **13** in glacial acetic acid. Its structure was elucidated, besides the mass spectrum, by the ^1H NMR spectrum, which showed a new signal corresponding to the methyl group of fused triazole ring at δ 3.06 ppm. Moreover, the interaction of diethylmalonate with compound **13** afforded ethyl (7,9-trimethylpyrido[3',2':4,5]-thieno[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)acetate (**16**) in good yield. Its structure was proven by the

^{1}H NMR spectrum, which showed a triplet at δ 1.23 ppm, a quartet at δ 4.25 ppm, and a singlet at δ 4.10 ppm corresponding to the ester and methylene groups, respectively, and the mass spectrum, which showed a molecular ion peak at *m/z* 269 and 270 corresponding to [M^+] and [M^++1], respectively.

Investigation of the antimicrobial activity of compounds **3-6**, **15**, and **16** demonstrated that imidazoline **3** was active against the Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* while derivative **5** showed moderate inhibition zones against *Bacillus cereus* only. The other derivatives **4**, **6**, **15**, and **16** were inactive against the two examined species of Gram-positive bacteria. Compounds **3**, **5**, and **16** showed strong to moderate activity against the fungi *Candida albicans*, *Trichophyton rubrum*, and *Chrysosporium tropicum*. The relatively high antifungal activity of compound **16** may be due to the presence of fused 1,2,4-triazoles in its molecule. The other derivatives showed no activity against the examined fungi species.

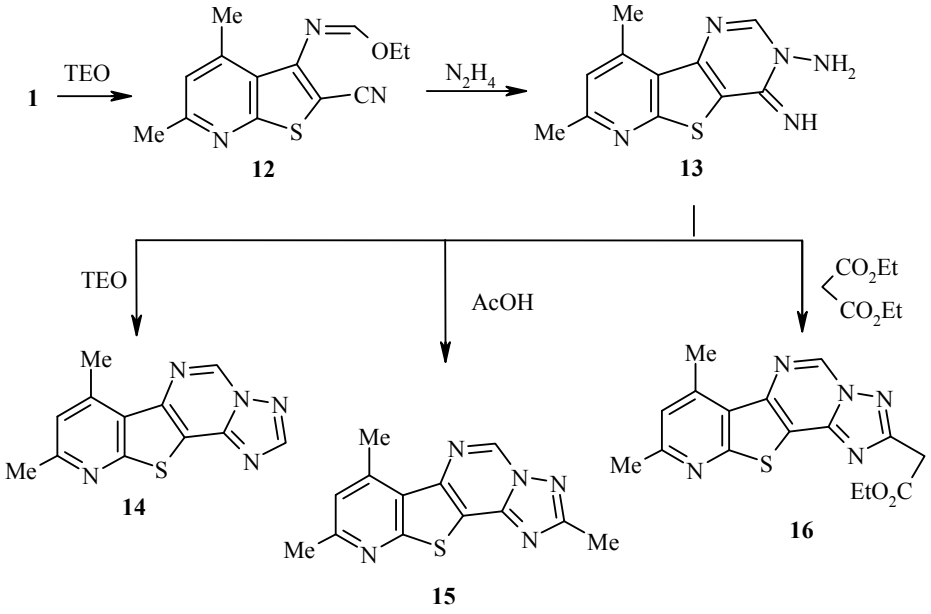


TABLE 1. Antimicrobial Activity of Derivatives **3-6**, **15**, and **16** (Inhibition Zone, mm)*

Compound	Gram-positive bacteria		Fungi		
	Bacillus cereus	*Staphylococcus aureus*	*Candida albicans*	*Trichophyton rubrum*	*Chrysosporium tropicum*
3	18	21	14	31	31
4	—	—	—	—	—
5	12	—	13	24	24
6	—	—	—	—	—
15	—	—	—	—	—
16	—	—	12	21	28
Reference*[2]	52	54	12	50	52

* Inhibition zone around the discs: 26-52 mm: very strong activity; 13-25 mm: strong activity; 0-7 mm: weak activity; dash denotes no activity.
*[2] Chloramphenicol (5%, antibacterial activity); terbinafine (5%, antifungal activity).

EXPERIMENTAL

IR spectra were recorded on a Nicolet FT-IR spectrophotometer on KBr discs. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Germini-2000 (300 and 75.5 MHz, respectively) and registered in DMSO-d_6 (compounds **4**–**11**) and $CDCl_3$ (compounds **2, 3, 12**–**16**), respectively. Mass spectra were measured on a Kratos 50 tc spectrometer. Microanalyses were performed in the microanalysis lab at Cairo University. Common reagent-grade chemicals were either commercially available and were used without further purification or prepared by standard literature procedures. All reactions were monitored by TLC carried out on 0.2 mm silica gel 60 F-254 (Merck) plates using UV light (254 and 360 nm) for detection. Melting points were determined on a Buchi melting point apparatus. The antimicrobial activity of the synthesized compounds was conducted at the Botany Department, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt.

2-(4,5-Dihydro-1H-imidazol-2-yl)-4,6-dimethylthieno[2,3-*b*]pyridine-3-amine (2). To a suspension of nitrile **1** (2.03 g, 10 mmol) in ethylenediamine (3 ml), carbon disulfide (1 ml) was added dropwise. The reaction mixture was heated on a water bath for 2 h. The precipitated solid was triturated with ethanol (10 ml), filtered off, and recrystallized from methanol to give a golden yellow crystals of compound **2** (2.22 g, 90%); mp 196-198ºC. IR spectrum, ν, cm^{-1}: 3447-3330 (NH) and (NH_2), 2917, 2860 (CH aliphatic), 1640 (C=N). ^{1}H NMR spectrum, δ, ppm: 2.59 (3H, s, CH_3); 2.75 (3H, s, CH_3); 3.50-3.98 (4H, m, CH_2 imidazoline); 6.38 (2H, s, NH_2); 6.86 (1H, s, Ar); 7.89 (1H, br. s, NH). Found, %: C 58.44; H 5.65; N 22.90. $C_{12}H_{14}N_4S$. Calculated, %: C 58.51; H 5.73; N 22.74.

7,9-Dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyrimidine (3). A mixture of compound **2** (0.27 g, 1.1 mmol), TEO (5 ml), and a catalytic amount of glacial acetic acid (0.2 ml) was heated for 4 h under reflux. The precipitate was filtered off and recrystallized from ethanol to afford pale-yellow crystals of compound **3** (0.24 g, 86%); mp 173-175ºC. IR spectrum, ν, cm^{-1}: 2915, 2860 (CH aliphatic), 1654 (C=N). ^{1}H NMR spectrum, δ, ppm: 2.53 (3H, s, CH_3); 2.77 (3H, s, CH_3); 3.89-4.15 (4H, m, CH_2 imidazoline); 7.14 (1H, s, H-8); 8.14 (1H, s, H-5). Mass spectrum, *m/z* (*I*, %): 256 $[M]^+$ (85), 255 (100). Found, %: C 60.51; H 4.65; N 21.74; S 12.44. $C_{13}H_{12}N_4S$. Calculated, %: C 60.91; H 4.72; N 21.86; S 12.51.

7,9-Dimethyl-5-phenyl-2,3,5,6-tetrahydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-e]pyrimidine (4). Concentrated HCl (0.1 ml) was added to a mixture of compound **2** (0.14 g, 0.55 mmol) and benzaldehyde (0.16 g, 1.5 mmol) in anhydrous ethanol (5 ml). The mixture was stirred under reflux for 8 h, cooled, and neutralized with an aqueous solution of sodium carbonate. The precipitate was filtered off and recrystallized from methanol to afford pale-yellow powder of compound **4** (0.15 g, 79%); mp 112-115ºC. IR spectrum, ν, cm^{-1}: 3250 (NH); 2934, 2864 (CH aliphatic); 1623 (C=N). ^{1}H NMR spectrum, δ, ppm: 2.52 (3H, s, CH_3); 2.62 (3H, s, CH_3); 3.60-3.97 (4H, m, CH_2 imidazoline); 5.22 (1H, s, H-5); 6.32 (1H, br. s, NH); 7.07 (1H, s, H-8); 7.23-7.86 (5H, m, Ph). ^{13}C NMR spectrum, δ, ppm: 20.48 (CH_3); 24.83 (CH_3); 50.90, 54.01 ($2CH_2$ imidazoline); 76.00 (C-5), 122.88, 124.00, 129.26, 129.33, 129.60, 129.73, 139.92, 144.42, 145.22, 146.97, 152.53, 156.05, 159.05, 161.76 (C Ar). Mass spectrum, *m/z* (*I*, %): 334 $[M]^+$ (20), 331 (70), 257 (100). Found, %: C 68.51; H 5.65; N 16.40. $C_{19}H_{18}N_4S$. Calculated, %: C 68.23; H 5.42; N 16.75.

7,9-Dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyrimidine-5-thione (5). A mixture of compound **2** (1.82 g, 7.4 mmol), CS_2 (10 ml), and anhydrous pyridine (20 ml) was heated for 13 h on water bath. The mixture was cooled and the precipitate was filtered off and recrystallized from methanol to give light-yellow crystals of compound **5** (1.80 g, 85%); mp 250-152ºC. IR spectrum, ν, cm^{-1}: 3123 (NH), 2950, 2857 (CH aliphatic), 1651 (C=N), 1220 (C=S). ^{1}H NMR spectrum, δ, ppm: 2.66 (3H, s, CH_3); 2.88 (3H, s, CH_3); 3.59-3.77 (4H, m, CH_2 imidazoline); 7.06 (1H, s, H-8); 7.88 (1H, br. s, NH). Mass spectrum, *m/z* (*I*, %): 288 $[M]^+$ (100). Found, %: C 54.25; H 5.13; N 19.40; S 22.43. $C_{13}H_{12}N_4S_2$. Calculated, %: C 54.14; H 4.19; N 19.43; S 22.24.

5-Hydrazino-7,9-dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyrimidine (6). A mixture of thione **5** (1.84 g, 6.4 mmol), hydrazine hydrate (10 ml), and absolute ethanol (20 ml) was refluxed

for 5 h. The precipitate was filtered off and recrystallized from DMF to give a yellow powder of compound **6** (1.51 g, 83%); mp 230-232ºC. IR spectrum, ν, cm^{-1}: 3400-3150 (NH_2NH), 2920, 2854 (CH aliphatic), 1646 (C=N). ^1H NMR spectrum, δ, ppm: 2.52, 2.56 (6H, 2s, CH_3); 3.82-3.95 (4H, m, CH_2 imidazoline); 4.41 (2H, s, NH_2); 7.10 (1H, s, H-8); 7.20 (1H, br. s, NH). Mass spectrum, *m/z* (*I*, %): 286 $[M]^+$ (60), 270 $[M^+ -NH_2]$ (100). Found, %: C 54.70; H 5.02; N 29.36; S 11.43. $C_{13}H_{14}N_6S$. Calculated, %: C 54.53; H 4.93; N 29.35; S 11.20.

9,11-Dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*][1,2,4]triazolo[3,4-*a*]pyrimidine (7). A mixture of compound **6** (0.72 g, 2.5 mmol) and TEO (10 ml) was heated under reflux for 5 h. After concentration and cooling at room temperature the reaction mixture was poured into ice-cold water. The resulting solid product was collected by filtration, washed with methanol, dried, and recrystallized from methanol to give compound **7** (0.51 g, 68%); mp 270–272ºC. IR spectrum, ν, cm^{-1}: 2925, 2840 (CH aliphatic), 1640 (C=N). ^1H NMR spectrum, δ, ppm: 2.55 (3H, s, CH_3); 2.67 (3H, s, CH_3); 3.77-3.98 (4H, m, CH_2 imidazoline); 7.43 (1H, s, H-11); 9.20 (1H, s, H triazole). Mass spectrum, *m/z* (*I*, %): 296 $[M]^+$ (100). Found, %: C 56.65; H 4.11; N 28.45. $C_{14}H_{12}N_6S$. Calculated, %: C 56.74; H 4.08; N 28.36.

10,12-Dimethyl-5,6-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]tetrazolo[5,1-*a*]pyrimidine (8). A solution of sodium nitrite (0.4 g, 5 mmol) in 5 ml H_2O was added dropwise under vigorous stirring at room temperature to a solution of compound **6** (0.46 g, 1.6 mmol) in acetic acid (10 ml). After the addition was complete, the reaction mixture was stirred for an additional 3 h and then neutralized with a sodium carbonate solution. The solid product was separated by filtration and recrystallized from ethanol to give compound **8** (0.32 g, 67%); mp 235–237ºC. IR spectrum, ν, cm^{-1}: 2930, 2822 (CH aliphatic), 1620 (C=N). ^1H NMR spectrum, δ, ppm: 2.60 (3H, s, CH_3); 2.69 (3H, s, CH_3); 3.80-3.96 (4H, m, CH_2 imidazoline); 7.50 (1H, s, H-11). Mass spectrum, *m/z* (*I*, %): 298 $[M^+ +1]$ (60), 297 $[M^+]$ (100). Found, %: C 52.35; H 3.55; N 32.63. $C_{13}H_{11}N_7S$. Calculated, %: C 52.51; H 3.73; N 32.97.

Ethyl [(7,9-Dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[3,2-*e*]pyrimidin-2-yl)sulfanyl]acetate (9). To a stirred suspension of thione **5** (1.44 g, 5 mmol) in dry DMF (10 ml), ethyl chloroacetate (0.92 g, 7.5 mmol) and K_2CO_3 (1.04 g, 7.5 mmol) were added. The reaction mixture was stirred at room temperature for 13 h and then poured into ice cold water with stirring. The obtained solid product was collected by filtration, washed with water, and recrystallized from ethanol to afford pale-yellow crystals of compound **9** (1.68 g, 90%); mp 176-178ºC. IR spectrum, ν, cm^{-1}: 2922, 2850 (CH aliphatic), 1729 (COOEt), 1653 (C=N). ^1H NMR spectrum, δ, ppm (*J*, Hz): 1.23 (3H, t, J = 7.1, $CH_2C\underline{H}_3$); 2.62 (3H, s, CH_3); 2.80 (3H, s, CH_3); 4.10-4.22 (4H, m, CH_2 imidazoline); 4.32 (2H, q, J = 7.0, $C\underline{H}_2CH_3$); 4.98 (2H, s, SCH_2); 7.31 (1H, s, H-8). ^{13}C NMR spectrum, δ, ppm: 15.13, 20.23 ($2CH_3$); 25.23 ($\underline{C}H_3CH_2$); 43.32 ($CH_3\underline{C}H_2$); 48.28, 49.99 ($2CH_2$ imidazoline); 62.71 (SCH_2); 109.10, 123.71, 124.23, 125.13, 147.58, 153.17, 157.17, 161.54, 163.25 (Ar); 168.83 (CO). Mass spectrum, *m/z* (*I*, %): 374 $[M]^+$ (100). Found, %: C 54.41; H 4.65; N 14.40; S 17.08. $C_{17}H_{18}N_4O_2S_2$. Calculated, %: C 54.52; H 4.84; N 14.96; S 17.13.

2-(7,9-Dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[3,2-*e*]pyrimidin-5-yl)sulfanylacetohydrazide (**10**). A solution of ester **9** (3.74 g, 10 mmol) in ethanol (30 ml) was refluxed with hydrazine hydrate (1.25 g, 25 mmol) for 4 h. After cooling to room temperature a pale-yellow solid appeared. It was recrystallized from methanol to afford hydrazide **10** (3.20 g, 89%); mp 295-296ºC. IR spectrum, ν, cm^{-1}: 3330-3140 (NH_2NH), 2939, 2820 (CH aliphatic), 1651 (CO). ^1H NMR spectrum, δ, ppm: 2.55 (3H, s, CH_3); 2.64 (3H, s, CH_3); 3.88-4.10 (4H, m, CH_2 imidazoline); 3.88 (2H, s, SCH_2); 4.90 (2H, br. s, $NHNH_2$); 7.40 (1H, s, H-8); 9.30 (1H, br. s, $N\underline{H}NH_2$). Mass spectrum, *m/z* (*I*, %): 360 $[M^+]$ (100). Found, %: C 49.65; H 4.20; N 23.16; S 17.59. $C_{15}H_{16}N_6OS_2$. Calculated, %: C 49.98; H 4.47; N 23.31; S 17.79.

1-(7,9-Dimethyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3',2':4,5]thieno[3,2-*e*]pyrimidin-5-yl)sulfanylacetyl-4-phenylthiosemicarbazide (**11**). To a solution of hydrazide **10** (1.80 g, 5 mmol) in absolute ethanol (10 ml) phenyl isothiocyanate (0.68 g, 5 mmol) was added. The reaction mixture was heated under reflux for 4 h. The product that separated on cooling was filtered off, washed with ethanol, and dried well to give compound **11** (2.10 g, 85%); mp 170-171ºC. IR spectrum, ν, cm^{-1}: 3313 (NH), 2929, 2815 (CH aliphatic), 1680

(CO). ^{1}H NMR spectrum, δ, ppm: 2.44, 2.54 (6H, 2s, CH_3); 3.90-4.30 (4H, m, CH_2 imidazoline); 4.53 (2H, s, SCH_2); 7.20-7.32 (6H, m, H-8, Ph); 9.60 (1H, br. s, PhN<u>H</u>); 9.71 (1H, br. s, CSNH); 10.17 (1H, br. s, CONH). Mass spectrum, *m/z* (*I*, %): 495 [M^+] (100). Found, %: C 53.18; H 4.09; N 19.56. $C_{22}H_{21}N_7OS_3$. Calculated, %: C 53.31; H 4.27; N 19.78.

Ethyl (2-Cyano-4,6-dimethylthieno[2,3-*b*]pyridin-3-yl)imidoformate (12). A mixture of nitrile **1** (0.89 g, 4.4 mmol), TEO (10 ml), and acetic anhydride (3 ml) was heated on a water bath at 60ºC for 4 h. The mixture was cooled and the precipitate was filtered off followed by recrystallization from ethanol to afford white crystals of compound **12** (0.96 g, 83%); mp 128–130ºC. IR spectrum, ν, cm^{-1}: 2199 (CN), 1644 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.45 (3H, t, *J* = 7.4, $OCH_2C\underline{H}_3$); 2.62 (3H, s, CH_3); 2.65 (3H, s, CH_3); 4.46 (2H, q, *J* = 7.4, $OC\underline{H}_2CH_3$); 6.99 (1H, s, H-5); 8.02 (1H, s, N=CH). Mass spectrum, *m/z* (*I*, %): 259 [M^+] (30), 258 (40), 203 (100). Found, %: C 60.12; H 4.96; N 16.12; S 12.23. $C_{13}H_{13}N_3OS$. Calculated, %: C 60.21; H 5.05; N 16.20; S 12.36.

4-Imino-7,9-dimethylpyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-3(4H)-amine (13). A mixture of compound **12** (5.70 g, 22 mmol) and hydrazine hydrate (8 ml, 80%) in ethanol (20 ml) was heated under reflux for 2 h. The white precipitate formed after cooling was filtered off and dried. Recrystallization from ethanol afforded white crystals of imine **13** (4.55 g, 84%); mp 218–220ºC. IR spectrum, ν, cm^{-1}: 3322–3278 (NH_2NH), 1624 (C=N). ^{1}H NMR spectrum, δ, ppm: 2.55 (3H, s, CH_3); 2.81 (3H, s, CH_3); 5.80 (2H, s, NH_2); 7.18 (1H, s, H-8); 8.17 (1H, s, NH). Mass spectrum, *m/z* (*I*, %): 245 [M^+] (20), 184 (100). Found, %: C 53.43; H 4.32; N 28.22; S 12.84. $C_{11}H_{11}N_5S$. Calculated, %: C 53.86; H 4.52; N 28.55; S 13.07.

7,9-Dimethylpyrido[3',2':4,5]thieno[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (14). A. A mixture of imine **13** (0.61g, 2.5 mmol) and formic acid was refluxed for 10 h and then cooled and poured into ice water to give a white precipitate, which was filtered off, washed several times with water, dried and recrystallized from ethanol to afford gray crystals of compound **14** (0.21 g, 33%); mp 244–245ºC. IR spectrum, ν, cm^{-1}: 2917, 2852 (CH aliphatic), 1618 (C=N). ^{1}H NMR spectrum, δ, ppm: 2.70 (3H, s, CH_3); 3.03 (3H, s, CH_3); 7.20 (1H, s, H-8); 8.50 (1H, s, H-5); 9.43 (1H, s, H triazole). Mass spectrum, *m/z* (*I*, %): 255 [M^+] (12), 69 (100). Found, %: C 56.23; H 3.33; N 27.24; S 12.36. $C_{12}H_9N_5S$. Calculated, %: C 56.45; H 3.55; N 27.43; S 12.56.

B. A mixture of imine **13** (0.61 g, 2.5 mmol) and TEO (2.2 g, 20 mmol) in DMF (10 ml) was refluxed for 7 h. After cooling and dilution with ice water (30 ml), the solid product formed was filtered off and recrystallized from ethanol to furnish gray crystals of compound **14** (0.52g, 83%). Mass spectrum, *m/z* (*I*, %): 255 [M^+] (12), 69 (100).

2,7,9-Trimethylpyrido[3',2':4,5]thieno[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (15). A mixture of imine **13** (0.25 g, 1 mmol) and acetic acid (15 ml) was refluxed for 10 h. After cooling and dilution with ice water (20 ml), the white precipitate formed was filtered off and recrystallized from ethanol to give white crystals of compound **15** (0.21 g, 78%); mp 236-238ºC. IR spectrum, ν, cm^{-1}: 2920, 2852 (CH aliphatic), 1621 (C=N). ^{1}H NMR spectrum, δ, ppm: 2.71 (3H, s, CH_3); 2.73 (3H, s, CH_3); 3.06 (3H, s, CH_3); 7.19 (1H, s, H-8); 9.32 (1H, s, H-5). Mass spectrum, *m/z* (*I*, %): 269 [M^+] (100). Found, %: C 57.77; H 4.09; N 25.86; S 11.66. $C_{13}H_{11}N_5S$. Calculated, %: C 57.97; H 4.12; N 26.00; S 11.91.

Ethyl (7,9-Trimethylpyrido[3',2':4,5]thieno[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)acetate (16). A suspension of imine **13** (0.25 g, 1 mmol) and diethyl malonate (5 ml) was heated under reflux over its boiling point for 2 h. The yellow solid product formed was triturated with ethanol, filtered off, and recrystallized from ethanol to afford yellow crystals of ester **16** (0.29 g, 83%); mp 184-186ºC. IR spectrum, ν, cm^{-1}: 2923, 2847 (CH aliphatic), 1714 (CO_2Et), 1619 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.23 (3H, t, *J* = 7.2, $C\underline{H}_3CH_2$); 2.72 (3H, s, CH_3); 3.05 (3H, s, CH_3); 4.10 (2H, s, CH_2); 4.25 (2H, q, *J* = 7.2, $CH_3C\underline{H}_2$); 7.20 (1H, s, H-8); 9.36 (1H, s, H-5). Mass spectrum, *m/z* (*I*, %): 341 [M^+] (40), 269 (100). Found, %: C 56.15; H 4.31; N 20.22; S 9.17. $C_{16}H_{15}N_5O_2S$. Calculated, %: C 56.29; H 4.43; N 20.51; S 9.39.

The Antimicrobial Activity of compounds **3-6**, **15**, and **16** was tested against the Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* and against the fungi *Candida albicans*, *Trichophyton*

rubrum, and *Chryosporium tropicum* using chloramphenicol (5%) and terbinafine (5%) as standards by the disc-diffusion technique [34, 35]. Samples were dissolved in DMSO to a concentration of 5%, and filter paper discs (Whatman No. 3, 5 mm in diameter) were impregnated with the solutions. The discs were placed on the surface of solidified nutrient agar dishes seeded by the test bacteria or Czapek's Dox agar dishes seeded by the test fungi. The inhibition zones were measured in millimeters by the end of the incubation period (48 h at 37ºC for bacteria and 28ºC for fungi). The results are collected in Table 1.

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