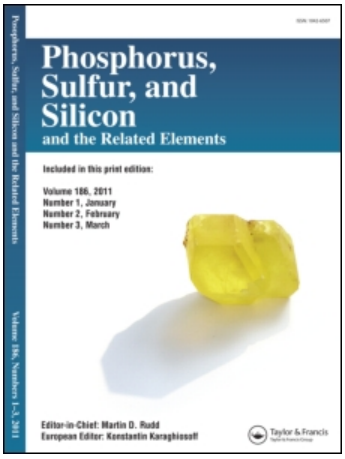

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Synthesis, Reactions, and Antimicrobial Activity of Some Fused Thieno[2,3-*d*]pyrimidine Derivatives

Mohamed I. Hegab[a]; Nasser A. Hassan[a]; Aymn E. Rashad[a]; Afaf A. Fahmy[a]; Farouk M. E. Abdel-Megeid[a]
[a] National Research Centre, Dokki, Cairo, Egypt

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Synthesis, Reactions, and Antimicrobial Activity of Some Fused Thieno[2,3-*d*]pyrimidine Derivatives

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National Research Centre, Dokki, Cairo, Egypt

*Some new indeno[1′,2′:4,5]thieno[2,3-d]pyrimidines were prepared from the reaction of 2-aminoindeno[2,1-b]thiophene-3-carbonitrile (**1**) with different reagents. Also, some indeno[1′,2′:4,5]thieno[3,2-e]tetrazolo[1,5-c]pyrimidines and indeno[1′,2′:4,5]thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidines and their isomeric [1,2,4]triazolo[1,5-c]pyrimidines were prepared. The antimicrobial evaluation of some prepared products showed that many of them revealed promising antimicrobial activity.*

Keywords Antimicrobial evaluation; indenothienopyrimidines; rearrangement; thienotetrazolopyrimidines; thienotriazolopyrimidines

INTRODUCTION

Thieno[2,3-*d*]pyrimidine derivatives represent one of the most active classes of compounds possessing a wide spectrum biological activity. They bear structure analogy and isoelectronic relation to purine.[1] Besides, some active compounds have been described when the thiophene moiety is fused to a carbocyclic ring,[2] also prominent biological activities have been reported for fused thienopyrimidine derivivatives.[3,4] So, this article is aimed at the synthesis of new compounds related to indeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidine derivatives, their fused [1′,2′:4,5]thieno[3,2-*e*]tetrazolo[1,5-*c*]pyrimidines,

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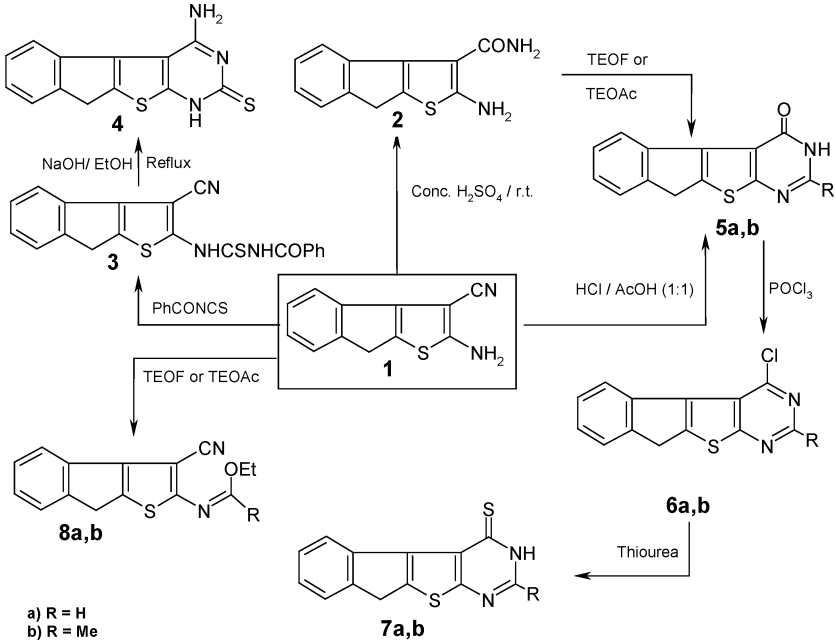
Address correspondence to Farouk Abdel-Megeid, National Research Centre, EL-Bhoos Street, 12622, Dokki, Cairo, Egypt. E-mail: faroukezat@yahoo.com

indeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine derivatives, and their isomeric [1,2,4]triazolo[1,5-*c*]pyrimidines, and the study of some of their reactions, as well as the evaluation of some of their antimicrobial activities.

RESULTS AND DISCUSSION

When a mixture of 2-aminoindeno[2,1-*b*]thiophene-3-carbonitrile (**1**)[6] and conc. sulfuric acid was stirred at room temperature or when heated in a mixture of polyphosphoric acid /orthophosphoric acid (1:1), hydrolysis of the cyano group took place to give 2-aminoindeno[2,1-*b*]thiophene-3-carboxylic acid amide (**2**) (Scheme 1).



SCHEME 1

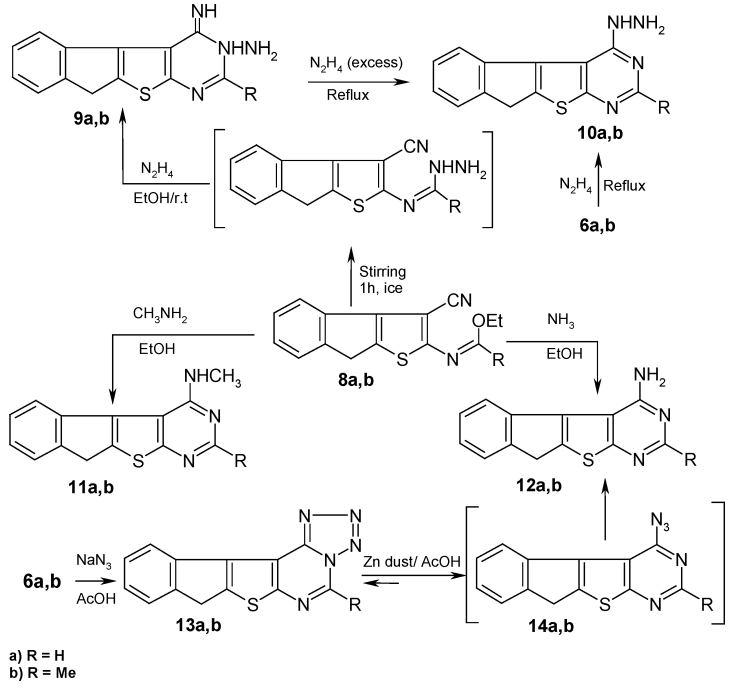
Refluxing of compound **1** with benzoyl isothiocyanate, led to the formation of 2-(3-benzoylthioureido)indeno[2,1-*b*]thiophene-3-carbonitrile (**3**), which was annulated to a pyrimidine derivative by refluxing its ethanolic sodium hydroxide solution to give the tetracyclic product, 4-aminopyrimidine-2(1*H*)-thione derivative **4** (Scheme 1). The 4-pyrimidinone derivatives were prepared by reacting compound **1** with formic acid or with acetic acid/conc. hydrochloric acid (1:1) under reflux temperature to give derivatives **5a** or **5b**, respectively (Scheme 1).

Products **5a** and **5b** were formed presumably via the intermediacy of the corresponding oxazinimine derivative,[2,7] which then rearranged under the conditions of the reaction. Besides, products **5a** and **5b** were also obtained by treating compound **2** with triethyl orthoformate or triethyl orthoacetate, respectively under reflux temperature.

Products **5a** and **5b** were converted into their corresponding 4-chloro derivatives **6a** and **6b**, respectively by treating with phosphorus oxychloride under reflux temperature (Scheme 1).

Compounds **6a** and **6b** were converted into their corresponding 4-thiono derivatives by refluxing their ethanolic solution with thiourea following the procedure of Shiba et al. [8] to give products **7a** and **7b**, respectively (Scheme 1).

When compounds **1a** or **1b** were refluxed with triethyl orthoformate or triethyl orthoacetate, they afforded derivatives **8a** or **8b**, respectively (Scheme 1). On the other hand, when the ethanolic solutions of **8a** or **8b** were stirred at room temperature with hydrazine hydrate, they afforded the 4-iminopyrimidin-3-ylamine derivatives **9a** or **9b**, respectively. However, the pyrimidin-4-ylhydrazine derivatives **10a** and **10b** were obtained by treatment of compounds **6a** or **6b** and **8a** or **8b** with hydrazine hydrate under reflux temperature (Scheme 2).



SCHEME 2

Also, compounds **9a** and **9b** were isomerized to their corresponding more stable 4-hydrazino derivatives **10a** and **10b**, respectively upon refluxing in ethanol in the presence of hydrazine hydrate. Actually, hydrazine hydrate acts as a base in this Dimroth type rearrangement, which involves a sequence of ring opening and ring closure reactions.[9,10]

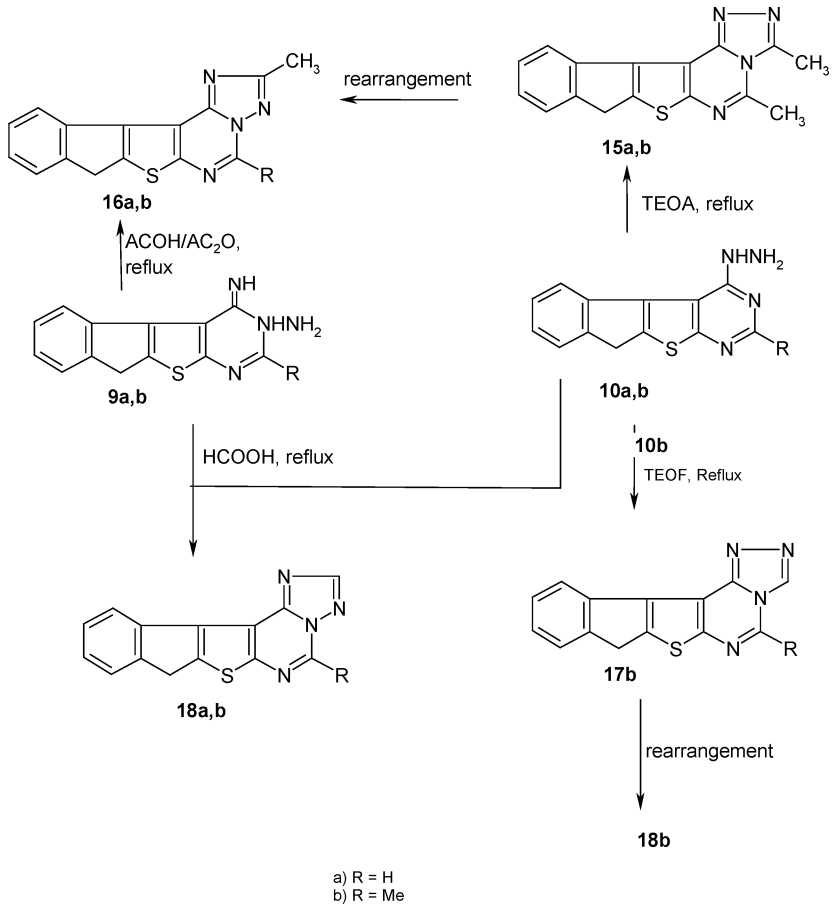
The synthesis of 4-amino pyrimidine derivatives was achieved by heating compound **8b** with methylamine to give the *N*-methylpyrimidin-4-ylamine derivative **11**, or by heating compounds **8a** or **8b** with ammonium hydroxide solution to give the pyrimidin-4-ylamine derivatives **12a** and **12b**, respectively.

The displacement of the 4-chlorine atom of both compounds **6a** or **6b** in glacial acetic acid with sodium azide, afforded the polycyclic tetrazolo[1,5-*c*]pyrimidine derivatives **13a** and **13b**, respectively as judged by the IR spectra, since both compounds did not show the characteristic azide absorption bands near the region at ν 2100–2200 cm^{-1}.[11–13]

The reduction of compounds **13a** or **13b** was accomplished by treatment of both compounds in glacial acetic acid with zinc dust, to afford the pyrimidin-4-ylamine derivatives **12a** and **12b**, respectively; presumably via the formation of the azido intermediates **14a** and **14b** (Scheme 2).

Many reports[14,15] have described the synthesis of [1,2,4]triazolo[4,3-*c*]pyrimidine derivatives and [1,2,4]triazolo[1,5-*c*]pyrimidine fused to different nitrogen containing heterocyclic moieties. In the meantime, the synthesis of [1,2,4]triazolo[4,3-*c*]pyrimidine derivatives and [1,2,4]triazolo[1,5-*c*]pyrimidine derivatives fused to a thiophene moiety have attracted the attention of many investigators.[3,10,16–18] Actually, previous observations revealed that thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidines can isomerizes under different suitable reaction conditions to the more stable thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines.[10,19] Nevertheless, this pattern of isomerization appears to have been overlooked by some workers.[14,16,20]

In this investigation, when compounds **10a** or **10b** were treated with triethyl orthoacetate (TEOA)] under reflux temperature, they afforded the polycyclic [1,2,4]triazolo[4,3-*c*]pyrimidine derivatives **15a** and **15b**, respectively. On the other hand, when the 4-imino-3-amino derivatives **9a** or **9b** were treated with a mixture of acetic acid/acetic anhydride (1:1) they gave the [1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **16a** and **16b**, respectively. In the meantime, compounds **16a** and **16b** could be obtained by isomerization of compounds **15a** and **15b**, respectively upon treatment with a base under reflux temperature (Scheme 3). It is noteworthy to mention that the ^{1}H NMR spectra, in general, revealed that the signals of the C_3-H, or C_3-CH_3 and C_5-H or C_5-CH_3 protons of the



SCHEME 3

thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine derivatives whenever they exist in this text are more deshielded and appeared at a more downfield when compared with the corresponding protons of the thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines derivatives (c.f. Exp.). These observations are in accordance with the results of Shishoo et al.[16,17] Actually, this type of isomerization to a more stable products in the presence of a catalytic amount of sodium ethoxide, was also described by some authors as a Dimroth type rearrangement,[10,21] which presumably involves a sequence of ring opening and ring closure reactions.

When compound **10b** was treated with triethyl orthoformate (TEOF) under reflux temperature it afforded the [1,2,4]triazolo[4,3-*c*]pyrimidine derivative **17b**.

Moreover, compounds **9a** or **9b** and **10a** or **10b** were treated with formic acid under reflux temperature, in both cases they gave one and the same identical product in all respects assigned the structure of the [1,2,4]triazolo[1,5-*c*]pyrimidine derivative **18a** or **18b**, respectively. In fact, the [4,3-*c*]triazolo products could not be isolated even when the reaction mixture was heated at low temperature (40–50°C) and at short time, since tlc monitoring revealed the formation of more than one spot during the reaction course until the reaction ended by formation of one spot giving the final product. It is noteworthy to mention that the [1,2,4]triazolo[4,3-*c*]pyrimidine **17b,** which was obtained before, rearranged into its isomeric[1,2,4]triazolo[1,5-*c*]pyrimidine derivative **18b** upon heating with formic acid under reflux temperature (Scheme 3).

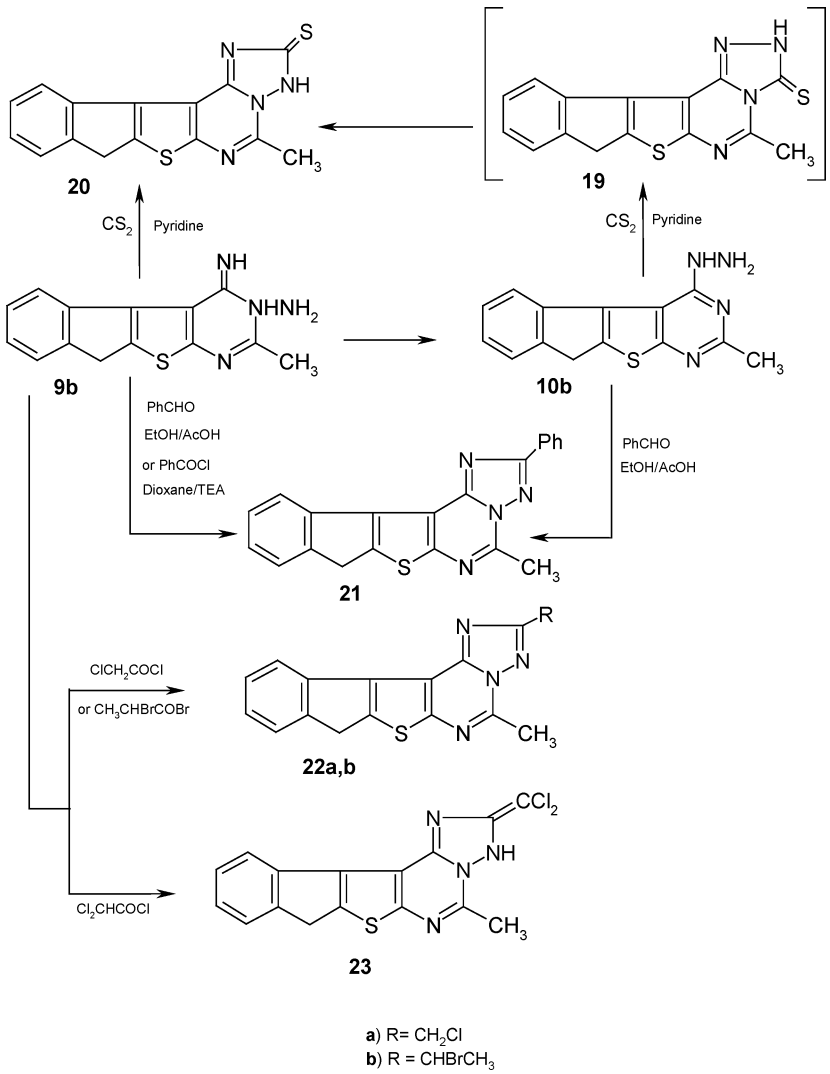
Also, the [1,2,4]triazolo[1,5-*c*]pyrimidine-2(1*H*)-thione derivative **20** was prepared by reacting compound **9b** or **10b** in dry pyridine with carbon disulfide under reflux temperature (presumably *via* the intermediacy of the triazolo[4,3-*c*] isomer **19** in case of the reaction of compound **10b**, which then rearranged under the conditions of the reaction to the triazolo[1,5-*c*] product **20**.

Moreover, a series of triazolo[1,5-*c*]pyrimidine derivatives was prepared by reacting compounds **9b** or **10b** with some bifunctional one-carbon donor cyclizing agents. So, when compounds **9b** or **10b**, in ethanol, were treated with benzaldehyde in the presence of few drops of acetic acid under reflux temperature or when compound **9b**, in dioxane, was treated with benzoyl chloride in the presence of a few drops of triethylamine under reflux temperature, they afforded in both cases one and the same product: the 2-phenyl-[1,2,4]triazolo[1,5-*c*]pyrimidine derivative **21**. Also, when compound **9b** was treated with chloroacetyl chloride, α-bromopropionyl bromide in the presence of few drops of triethyl amine (TEA), or dichloroacetyl chloride under reflux temperature, it gave the corresponding 2-substituted 5-methylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*] pyrimidine derivatives **22a,b**, and **23**, respectively (Scheme 4).

Compounds **2**, **4**, **6a**,**7a**,**7b**,**10a**,**21, 22b**, and **23** were selected to represent the newly synthesized compounds and tested for their antimicrobial activity. Streptomycin (S), Amoxicillin (Am), and Fusidic acid (FA) were used as reference drugs and the biological results can be summarized in Table I.

CONCLUSION

Starting from 2-aminoindeno[2,1-*b*]thiophene-3-carbonitrile (**1**), we have demonstrated a convenient procedure for the preparation of some



SCHEME 4

new indeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidines, indeno [1′,2′:4,5]thieno-[3,2-*e*]tetrazolo[1,5-*c*]pyrimidines and indeno[1′,2′:4,5]thieno[3,2-*e*]-[1,2,4]triazolo[4,3-*c*]pyrimidines and their isomeric [1,2,4]triazolo[1,5-*c*]pyrimidines were prepared. As shown in Table I, the 2-substituted-5-methyindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine

TABLE I Test of the Synthesized Compounds

The tested compounds[a]	C. albicans	S.aures	M.phlei	B.subtilis	E.coli
(S)[b]	++++	−	−	++++	++++
(Am)[c]	−	−	+	−	+++
(FA)[d]	−	+++	+	++++	−
2	−	−	−	+	+++
4	++	++	+	+	++
6a	+	−	−	−	−
7a	−	−	−	−	+
7b	++	−	+	−	+++
10a	+++	+	+	−	+
21	−	−	−	++	++
22b	−	−	−	++	++
23	++	+	++	+++	+++

[a] $\gamma = 2$ μg mL–1 in DMSO; [b] $\gamma = 25$ μg/mL^{-1}; [c] $\gamma_{-} = 4$ μg mL^{-1}; [d] $\gamma = 4$ μg/mL^{-1}; +++—Highly sensitive (inhibition zone = 21–25 mm); ++—Fairly sensitive (inhibition zone = 16–20 mm); +—Slightly sensitive (inhibition zone =10–15 mm); and- —Not sensitive (inhibition zone = 0–9 mm).

derivative **23** revealed the highest antimicrobial activity than the other tested compounds and some of the reference drugs.

EXPERIMENTAL

All melting points were uncorrected and measured using Electrothermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Shimadzu, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 180 spectrometer (Varian, UK), and chemical shifts were expressed as (ppm) values against TMS as internal reference. Mass spectra were recorded on Gc Ms-QP 1000 EX (Shimadzu, Japan). Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm).

Synthesis of 2-Aminoindeno[2,1-*b*]thiophene-3-carbonitrile (1)

Method A

A mixture of 1-indanone (1.32 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) in pyridine (20 mL) was heated under reflux temperature for 3 h, cooled, poured into ice-water with stirring for 1 h, neutralized with conc. hydrochloric acid, filtered,

dried off, and purified on silica gel using petroleum ether 60–80°C : ethyl acetate (4:1) to give compound **1**.

Method B

Step 1. A mixture of 1-indanone (1.32 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol), ammonium acetate (5 g), glacial acetic acid (20 mL) and dry benzene (50 mL) was heated under reflux temperature for 6 h using a Dean-Stark water separator, cooled, filtered, dried, and recrystallized from ethanol to give 1-indanylidenmalononitrile (1.50 g, 83%). IR; υ 2280, 2200 (NH_2), 2210 (CN). ^{1}H NMR: δ 3.75 (s, 2H, CH_2), 4.80 (s, 2H, NH_2, D_2O exchangeable), 7.20–7.60 (m, 3H, Ar H), 7.75 (d, J = 6.00 Hz, 1H, Ar H). MS: *m/z* 212 (M^+, 100), 196 (1.71), 184 (4.20).

Step 2. A mixture of 1-indanylidenmalononitrile (1.80 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) was heated under reflux temperature in 20 mL dry pyridine for 3 h, cooled, poured into ice-water with stirring for 1 h, neutralized with conc. hydrochloric acid, filtered, dried, and purified as in method A to give compound **1.** Compound **1** prepared by this method is identical in all respects (physical and spectral data) to those prepared by method A.

Synthesis of 2-Aminoindeno[2,1-*b*]thiophene-3-carboxylic acid amide (2)

Method A

Compound **1** (0.212 g, 1 mmol) was added portion wise to 20 mL conc. sulfuric acid, with stirring for 1 h. The reaction mixture was poured into ice-water, neutralized, filtered, dried, and recrystallized from DMF to give compound **2**. Yield 87%, M. p. 196–198°C. IR; υ 3470, 3396 ($CONH_2$), 3290, 3196 (NH_2), 1642 (C=O).^{1}H NMR: δ 3.60 (s, 2H, CH_2), 3.80–4.20 (br s, 2H, NH_2, D_2O exchangeable), 6.60 (s, 2H, $CONH_2$, D_2O exchangeable), 7.00–7.50 (m, 3H, Ar H), 7.80 (d, J = 10.80 Hz, 1H, Ar H). MS: *m/z* 230 (M^+, 81.80), 213(100), 185 (38.00).

Method B

Compound **1** (0.212 g, 1 mmol) was added to a mixture of 20 mL orthophosphoric acid:polyphosphoric acid (1:1) with stirring for 1 h at room temperature, then the reaction mixture was refluxed for 6 h, cooled, poured into water, filtered, dried, and recrystallized from DMF to give compound **2**. Compound **2** prepared by this method is identical in all respects (physical and spectral data) to those prepared by method A.

Synthesis of 2-(3-Benzoylthioureido)indeno[2,1-*b*]-thiophene-3-carbonitrile (3)

Compound **1** (0.212 g, 1 mmol) was added to 50 mL dry acetone containing 1 mmol of benzoyl isothiocyanate (prepared in situ from potassium isothiocyanate and benzoyl chloride) then the reaction mixture was refluxed for 2 h, cooled, filtered, dried, and recrystallized from DMF to give compound **3**. Yield 75%, M. p. 248–250°C. IR: υ 3292 (NH), 3245 (NH), 2211 (CN), 1664 (C=O). MS: *m/z* 375 (M^+, .87), 316 (43.79), 251 (10.50), 211(4.87), 195 (2.47). Anal. Calcd. For $C_{20}H_{13}N_3OS_2$ (375); C, 64.30; H, 3.60; N, 11.03; S, 17.30%. Found: C, 64.00; H, 3.46; N, 11.20; S, 17.06.

Synthesis of 4-Aminoindeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidine-2(1*H*)-thione (4)

Compound **3** (0.75 g, 2 mmol) was refluxed in 10 mL ethanol containing 5.5 ml in sodium hydroxide for 1 h, cooled, poured into water, and neutralized with drops of conc. hydrochloric acid, filtered, dried, and recrystallized from DMF to give compound **4**. yield 91%, M. p. 318–20°C. IR: υ 3501, 3402 (NH_2), 3187 (NH), 1677 (C=N). ^{1}H NMR: δ 3.90 (s, 2H, CH_2), 6.00–6.30 (s, 2H, NH_2, D_2O exchangeable); 7.10–7.60 (m, 4H, 3 Ar H, NH, D_2O exchangeable), 7.90 (d, J = 10.80 Hz, 1H, Ar H); ^{13}C NMR (DMSO-d_6, ppm): 35.11 (C-9), 120.66–126.65 (Ar C), 136.69 (C-4b), 137.10 (C-9a), 137.40 (C-10a), 145.90 (C-4a), 153.87 (C-4), 174.00 (C=S). MS: *m/z* 271(M^+, 35.46), 212 (23.5). Anal. Calcd. for $C_{13}H_9N_3S_2$ (271.36); C, 57.44; H, 3.16; N, 15.22%. Found: C, 57.53; H, 3.34; N, 15.48.

Synthesis of Indeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5a)

Method A

Compound **1** (0.212 g, 1 mmol) was heated under reflux temperature in 20 mL formic acid for 6 h. The reaction mixture was cooled, poured into water, filtered, dried, and the residue was recrystallized from dioxane/methanol (1:1) to give compound **5a**. Yield 92%, M. p. 310–312°C. IR: υ 3200 (NH), 1700 (C=O), 1590 (C=N). ^{1}H NMR: δ 4.00 (s, 2H, CH_2), 7.20–7.50 (m, 3H, Ar H), 8.10 (s, 1H, C_2-H), 8.40 (d, J = 9.45 Hz, 1H, Ar H), 12.60 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 35.30 (C-9), 118.90–126.74 (Ar-C), 138.27 (C-4a), 140.00 (C-4b), 140.23 (C-9a), 145.50 (C-10a), 168.50 (C=O). MS: *m/z* 240 (M^+, 100), 212 (5.67), 197(1.48), 185 (15.53). Anal. Calcd. for $C_{13}H_8N_2OS$ (240); C, 64.94; H, 3.40; N, 11.42; S, 13.63%. Found: C, 65.00; H, 3.30; N, 11.66; S, 13.33.

Method B

Compound **5a** was also obtained by treating compound **2** (0.23 g, 1 mmol) with 20 mL triethyl orthoformate in the presence of few drops of piperidine under reflux temperature for 4 h, then the reaction mixture was cooled, filtered, dried, and the residue was recrystallized from dioxane/methanol (1:1) to give compound **5a**. Compound **5a** prepared by this method is identical in all respects (physical and spectral data) to those prepared by method A.

Synthesis of 2-Methylindeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-4(3H)-one (5b)

Method A

A mixture of 20 mL glacial acetic acid/conc. hydrochloric acid (1:1) and compound **1** (0.212 g, 1 mmol) was heated under reflux temperature for 3 h, filtered while hot, washed with water, then methanol, dried, and recrystallized from DMF to give compound **5b**. Yield 79%, M. p. 290–292°C. IR: υ 3250 (NH), 1665 (C=O), 1590 (C=N). ^{1}H NMR (DMSO-d_6): δ 2.66 (s, 3H, C_2-CH_3), 3.90 (s, 2H, CH_2), 7.20–7.50 (m, 3H, Ar H), 8.50 (d, J = 12.15 Hz, 1H, Ar H), 11.60 (s, 1H, NH, D_2O exchangeable). MS: *m/z* 254 (M^+, 100), 213 (13.15), 195 (0.88), 183 (9.20). Anal. Calcd. for $C_{14}H_{10}N_2OS$ (254.31); C, 66.44; H, 4.10; N, 10.96; S, 12.61%. Found: C,66.14; H, 3.93; N, 11.02; S, 12.59.

Method B

Compound **5b** was also obtained by treating compound **2** (0.23 g, 1 mmol) with 20 mL triethyl orthoacetate in the presence of few drops of piperidine under reflux temperature for 3 h, then the reaction mixture was cooled, filtered, dried, and the residue was recrystallized from DMF to give compound **5b**. Compounds **5b** prepared by this method are identical in all respects (physical and spectral data) to those prepared by method A.

Synthesis of 4-Chloroindeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidine (6a)

Compound **5a** (0.24 g, 1 mmol) was heated under reflux temperature in 20 mL phosphorus oxychloride for 5 h, then the reaction mixture was poured into ice-water, stirred, filtered, and purified on silica gel using petroleum ether (40–60°C) : ethyl acetate (4 : 1) to give compound **6a**. Yield 89%, M. p. 152–154°C. ^{1}H NMR: δ 4.20 (s, 2H, CH_2), 7.20–7.60 (m, 3H, Ar H), 8.45 (d, J = 10.80 Hz, 1H, Ar H), 8.90 (s, 1H, C_2-H). MS:

m/z 260 (M^+, Cl^{37}, 47.50), 258 (M^+, Cl^{35}, 100), 223 (13.76), 195 (32.85). Anal. Calcd. for $C_{13}H_7ClN_2S$ (258.00); C, 60.54; H, 2.90; N, 10.59; S, 12.11%. Found: C, 60.46; H, 2.71; N, 10.85; S, 12.40.

Synthesis of 4-Chloro-2-methylindeno[1′,2′:4,5]-thieno[2,3-*d*]pyrimidine (6b)

Compound **5b** (0.254 g, 1 mmol) was heated under reflux temperature in 20 mL phosphorus oxychloride for 6 h, then the reaction mixture was cooled, poured into ice-water. The solid that formed was filtered off, dried, and purified on silica gel using petroleum ether (40–60°C) : ethyl acetate (4:1) as an eluent to give compound **6b**. Yield 55%, M. p. 168–170°C. ^{1}H NMR: δ 2.85 (s, 3H, C_2-CH_3), 4.00 (s, 2H, CH_2), 7.30–7.60 (m, 3H, Ar H), 8.50 (d, J = 10.80 Hz, 1H, Ar H). MS: *m/z* 274 (M^+, Cl^{37}, 39.91), 272 (M^+, Cl^{35}, 100), 237 (65.12), 30 (52.04), 10 (4.49), 195 (77.69). Anal. Calcd. for $C_{14}H_9ClN_2S$ (272.50); C, 61.76; H, 3.30; N, 10.29; S, 11.76%. Found: C, 61.76; H, 3.30; N, 10.29; S, 11.76.

Synthesis of Indeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidine-4(3*H*)-thione (7a)

A mixture of compound **6a** (0.258 g, 1 mmol) dissolved in 20 mL absolute ethanol, thiourea (2 mmol) was heated for 6 h, cooled, filtered, washed with a little amount of methanol, dried, and recrystallized from DMF to give compound **7a**. Yield 78%, M. p. 320–322°C. ^{1}H NMR: δ 4.00 (s, 2H, CH_2), 7.20–7.50 (m, 3H, Ar H), 8.10 (d, J = 10.80 Hz, 1H, Ar H), 9.40 (s, 1H, C_2-H), 13.90 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 35.20 (C-9), 124.40–129.60 (Ar C), 138.40 (C-4a), 140.50 (C-4b), 143.60 (C-9a), 144.18 (C-10a), 145.70 (C_2), 177.50 (C=S). MS: *m/z* 256 (M^+, 100), 240 (3.36), 228 (14.45), 196 (21.45). Anal. Calcd. for $C_{13}H_8N_2S_2$ (256.00); C, 61.30; H, 3.14; N, 11.00; S, 25.07%. Found: C, 60.93; H, 3.12; N, 10.93; S, 25.00.

Synthesis of 2-Methylindeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidine-4(3*H*)-thione (7b)

Compound **6b** (0.272 g, 1 mmol) was dissolved in 30 mL absolute ethanol, then thiourea (2 mmol) was added and the reaction mixture was refluxed for 4 h, cooled, filtered, washed with a little amount of water, then ethanol, dried, and recrystallized from DMF to give compound **7b**. Yield 93%, M. p. 324–326°C. ^{1}H NMR: δ 3.40 (s, 3H, C_2-CH_3), 4.00 (s, 2H, CH_2), 7.20–7.50 (m, 3H, Ar H), 9.40 (d, J = 10.80 Hz, 1H, Ar

H), 13.80 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): δ 20.50 (CH_3), 35.20 (C-9), 124.30–126.30 (Ar C), 133.00 (C-4a), 138.00 (C-4b), 140.00 (C-9a), 146.00 (C-10a), 154.00 (C-2), 178.00 (C=S). MS: *m/z* 270 (M^+, 71.70), 253 (1.45), 228 (100), 211 (22.15), 196 (21.19), 184 (4.50). Anal. Calcd. for $C_{14}H_{10}N_2S_2$ (270.00); C, 62.60; H, 3.45; N, 11.00; S, 10.23%. Found: C, 62.22; H, 3.70; N, 10.37.

Synthesis of Ethyl *N*-{3-cyanoindeno[2,1-*b*]thien-2-yl}-methanimidate (8a)

A mixture of compound **1** (0.212 g, 1 mmol) and 20 mL triethyl orthoformate was heated under reflux temperature for 4 h, then evaporated under reduced to give compound **8a**. Yield 93%, M. p. 134–136°C. IR (KBr): υ 3265 (NH).^1H NMR: δ1.40 (t, J = 8.45 Hz, 3H, CH_3), 3.80 (s, 2H, CH_2), 4.40 (q, J = 1.80 Hz, 2H, OCH_2), 7.20–7.50 (m, 3H, Ar H), 7.80 (d, J = 8.10 Hz, 1H, Ar H), 8.05 (s, 1H, N=CHOEt). MS: *m/z* 268 (M^+, 1.75), 252 (36.20), 254 (13.17).

Synthesis of 2-Ethyl *N*-{3-cyanoindeno[2,1-*b*]thien-2-yl}-ethanimidate (8b)

A mixture of compound **1** (0.212 g, 1 mmol) and 20 mL triethyl orthoacetate was heated under reflux temperature for 5 h, then evaporated under reduced pressure, and the residue was recrystallized from dioxane to give compound **8b**. Yield 89%, M. p. 140–142°C.^1H NMR: δ 1.40 (t, J = 8.50 Hz, 3H, CH_3), 3.20 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 4.35 (q, J = 8.20 Hz, 2H, OCH_2), 7.20-7.50 (m, 3H, Ar H), 7.80 (d, J = 8.10 Hz, 1H, Ar H). MS: *m/z* 282 (M^+, 1.16), 268 (100), 252 (33.80).

Synthesis of 4-Iminoindeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-3-ylamine (9a)

A mixture of compound **8a** (0.268 g, 1 mmol) dissolved in 20 mL of absolute ethanol and 3 mL of hydrazine hydrate (99%), was stirred for 1 h at room temperature. The solid that formed was filtered, washed with a little amount of methanol, dried, and recrystallized from ethanol to give compound **9a**. Yield 91%, M. p. 223–225°C. IR (KBr): υ 3250, 3200 (NH_2), 3100 (NH), 1645 (C=N). ^1H NMR: δ 3.95 (s, 2H, CH_2), 5.70 (s, 2H, NH_2, D_2O exchangeable); 7.20–7.50 (m, 4H, 3 Ar H, NH, D_2O exchangeable), 8.40 (d, J = 10.80 Hz, 1H, Ar H), 8.95 (s, 1H, C_2-H). MS: *m/z* 254 (M^+, 100), 238.10(13.70), 211 (42.70), 195 (2.34).

Synthesis of 2-Methyl-4-iminoindeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-3-ylamin (9b)

A mixture of compound **8b** (0.282 g, 1 mmol) dissolved in 20 mL absolute ethanol containing 3 mL of hydrazine hydrate (99 %), was stirred for 1 h at room temperature, the solid that formed was filtered, washed with a little amount of cold methanol, dried, and recrystallized from ethanol to give compound **9b**. Yield 93%, M. p. 324–326°C. IR (KBr): υ 3295, 3200 (NH_2), 3150 (NH), 1640 (C=N). ^{1}H NMR: δ 2.60 (s, 3H, C_2-CH_3), 3.80 (s, 2H, CH_2), 4.80 (s, 2H, NH_2, D_2O exchangeable), 7.20 (s, 1H, NH, D_2O exchangeable), 7.30–7.50 (m, 3H, Ar H), 7.95 (d, J = 10.85, 1H, Hz, Ar H). 268 (M^+, 100), 252 (36), 210 (38), 195 (10), 183 (8). Anal. Calcd. for $C_{14}H_{12}N_4S$ (268.00); C, 62.40; H, 4.35; N, 20.60%. Found: C, 62.68; H, 4.47; N, 20.89.

Synthesis of Indeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-4-ylhydrazine (10a)

Compound **8a** (0.258, g 1 mmol) was dissolved in 20 mL absolute ethanol, then 2 mL of hydrazine hydrate (99%) were added, and the reaction mixture was heated under reflux temperature for 3 h; it was then evaporated under reduced pressure, and the residue was recrystallized from dioxane to give compound **10a**. Yield 79%, M. p. 200–202°C.^{1}H NMR: δ 4.00 (s, 2H, CH_2), 5.00–5.50 (br. s, 2H, NH_2, D_2O exchangeable), 7.10–7.50 (m, 4H, 3 Ar H + NH, D_2O exchangeable), 8.05 (d, J = 10.80 Hz, 1H, Ar H), 8.40 (s, 1H, C_2-H); ^{13}C NMR (DMSO-d_6, δ ppm): 35.00 (C-9), 122.00–127.00 (Ar C), 130.00 (C-4a), 136.00 (C-4b), 138.00 (C-9a), 142.00 (C-10a), 145.00 (C-2), 160.00 (C-4). MS: *m/z* 254 (M^+, 60), 239 (60), 224 (10.70), 210 (50.35), 196 (14.15), 183 (11.89). Anal. Calcd. for $C_{13}H_{10}N_4S$ (254.00); C, 61.20; H, 4.14; N, 22.39; S, 12.40%. Found: C, 61.41; H, 3.93; N, 22.04; S, 12.59.

Synthesis of 2-Methylindeno[1′,2′:4,5]thieno[2,3-*d*]-pyrimidin-4-ylhydrazine (10b)

Compound **8b** (0.272 g, 1 mmol) was dissolved in 20 mL absolute ethanol, then 3 mL hydrazine hydrate (99%) were added then and reaction mixture was heated under reflux temperature for 2 h, evaporated under reduced pressure, and the residue was recrystallized from ethanol to give compound **10b**. Yield 75%, M. p. 213–215°C. ^{1}H NMR: δ 2.65 (s, 3H, C_2-CH_3), 3.90 (s, 2H, CH_2), 4.30 (s, 2H, NH_2, D_2O exchangeable), 6.60 (s, 1H, NH, D_2O exchangeable), 7.20–7.60 (m, 3H, Ar H), 7.90 (d, J = 10.80 Hz, 1H, Ar H). MS: *m/z* 268 (M^+, 62.10), 252 (14.73), 238

(6.79), 226 (4.13), 210 (100), 196 (34.61), 183 (23.67) Anal. Calcd. for $C_{14}H_{12}N_4S$ (268); C, 62.35; H, 4.50; N, 20.46%. Found: C, 62.68; H, 4.47; N, 20.89.

Isomerization of 9a and 9b to 10a and 10b

Compounds **9a** (0.254 g, 1 mmol) and **9b**, (0.268 g, 1 mmol) were dissolved in 20 mL ethanol and then drops of hydrazine hydrate were added, then the reaction mixture was heated under reflux temperature for 2 h, and evaporated under reduced pressure to give compounds. **10a** (0.24 g, 94%) and compound **10b** (0.25 g, 93 %), respectively. Products **10a** and **10b** obtained from these isomerizations are identical in all respects with compounds **10a** and **10b** obtained before.

Synthesis of *N*-Methyl-2-methylindeno[1′,2′:4,5] thieno[2,3-*d*]pyrimidin-4-ylamine (11)

A mixture of compound **8b** (0.28 g, 1mmol), 20 mL absolute ethanol, and 10 mL alcoholic methylamine was stirred on a water-bath for 5 h, the reaction mixture was cooled, filtered off, and the residue was recrystallized from dioxane to give compound **11**. Yield 94%, M.p. 168–170°C. IR (KBr): υ 3273 (NH), 1611 (C=N). ^{1}H NMR: δ 2.30 (d, 3H, $NHCH_3$), 2.40 (s, 3H, C_2-CH_3), 3.00 (s, 2H, CH_2), 3.40 (s, 1H, NH, D_2O exchangeable), 7.20–7.70 (m, 4H, Ar H).

Synthesis of Indeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-4-ylamine (12a)

A mixture of compound **8a** (0.268, g 1 mmol), in 20 mL absolute ethanol, and 10 mL ammonium hydroxide solution was stirred at room temperature for 10 min then, for 2 h on a water-bath, evaporated under reduced pressure, and the residue was recrystallized from ethanol to give indeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-4-ylamine (**12a**). Yield 80%, M.p. 202–204°C. ^{1}H NMR: δ 4.10 (s, 2H, CH_2), 7.10 (s, 2H, NH_2, D_2O exchangeable); 7.30–7.70 (m, 3H, Ar H), 8.40 (d, J = 11.60 Hz, 1H, Ar H), 8.90 (s, 1H, C_2-H). MS: *m/z* 239 (M^+, 100), 211 (21.40), 195 (10.34). Anal. Calcd. for $C_{13}H_9N_3S$ (239); C, 65.03; H, 4.00; N, 17.30%. Found: C, 65.20; H, 3.79; N, 17.50.

Synthesis of 2-Methylindeno[1′,2′:4,5]thieno[2,3-*d*]-pyrimidin-4-ylamine (12b)

A mixture of Compound **8b** (0.282 g, 1 mmol), dissolved in 20 mL absolute ethanol, and 10 mL ammonium hydroxide solution was stirred

at room temperature for 10 min, then for 2 h on a water-bath, evaporated under reduced pressure, and the residue was recrystallized from ethanol to give compound **12b**. Yield 87%, M.p. 260–262°C. IR: υ 3453, 418 (NH_2), 1661 (C=N).^{1}H NMR: δ 2.45 (s, 3H, C_2-CH_3), 4.30 (s, 2H, CH_2), 4.50-5.00 (br. s, 2H, NH_2, D_2O exchangeable), 7.20–7.70 (m, 4H, Ar H).

Synthesis of Indeno[1′,2′:4,5]thieno[3,2-*e*]tetrazolo[1,5-*c*]-pyrimidine (13a)

Compound **6a** (0.258 g, 1 mmol) was added to 20 mL glacial acetic acid containing sodium azide (0.13 g, 2 mmol) with stirring at 50–70°C for 2 h, then, the reaction mixture was cooled, filtered, dried, and the residue was recrystallized from dioxane to give compound **13a**. Yield 79%, M.p. 234–236°C. ^{1}H NMR: δ 4.25 (s, 2H, CH_2), 7.20–7.50 (m, 3H, Ar H), 8.80 (d, J = 10.80 Hz, 1H, Ar H), 9.50 (s, 1H, C_5-H). MS: *m/z* 265 (M^+, 35.00), 237(65.63), 236 (100), 210 (49.69).

Synthesis of 5-Methylindeno[1′,2′:4,5]thieno[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (13b)

Compound **6b** (0.272 g, 1 mmol) was added to 20 mL glacial acetic acid containing sodium azide (0.13 g, 2 mmol) with stirring at 50–70°C for 2 h, the reaction mixture was cooled filtered, dried, and the residue was recrystallized from dioxane to give compound **13b**. Yield 79%, M.p. 238–240°C. ^{1}H NMR: δ 3.20 (s, 3H, C_5-CH_3), 4.10 (s, 2H, CH_2), 7.20–7.60 (m, 3H, Ar H), 8.70 (d, J = 10.90 Hz, 1H, Ar H). MS: *m/z* 279 (M^+, 37.75), 250 (100), 224 (8.89), 210 (27.12). Anal. Calcd. for $C_{14}H_9N_5S$ (279); C60.50; H, 3.14; N, 25.42%. Found: C, 60.21; H, 3.22; N, 25.08.

Reduction of Tetrazopyrimidines 13a, and 13b to 12a and 12b

To a solution of both compounds **13a** (0.265 g, 1 mmol) and **13b** (0.279 g, 1 mmol) in 20 mL glacial acetic acid, zinc dust (0.24 g, 3 mmol) was added with stirring for ½ h at room temperature then refluxed for 10 h; the reaction mixture was cooled, poured into water, extracted with chloroform, the organic layer was evaporated under reduced pressure, and the residue was recrystallized from ethanol to give indeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-4-ylamine (**12a**) and 2-methylindeno[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-4-ylamine (**12b**), respectively. Products **12a** and **12b** are identical in all respects with compound **12a** and **12b** obtained before.

Synthesis of 3-Methylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine (15a)

A mixture of compound **10a** (0.254 g, 1 mmol) and 20 mL triethyl orthoacetate was heated under reflux temperature for 10 h. The reaction mixture was cooled, filtered, dried, and the residue was recrystallized from dioxane to give compound **15a**. Yield 43%, M.p. 298–300°C. ^{1}H NMR: δ 2.80 (s, 3H, C_3-CH_3), 4.10 (s, 2H, CH_2), 7.20–7.60 (m, 3H, Ar H), 8.70 (d, J = 11.6 Hz, 1H, Ar H), 9.70 (s, 1H, C_5-H). MS: *m/z* 278 (M^+, 100), 263 (0.54), 236 (9.91).

Synthesis of 3,5-Dimethylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine(15b)

Compound **10b** (0.268 g, 1 mmol) was heated under reflux in 20 mL triethyl orthoacetate for 10 h, then evaporated under reduced pressure and the residue was recrystallized from DMF to give compound **15b**. Yield 82%, M.p. 328–330°C. ^{1}H NMR: δ 2.95 (s, 3H, C_3-CH_3), 3.10 (s, 3H, C_5-CH_3), 4.20 (s, 2H, CH_2), 7.20–7.60 (m, 3H, Ar H), 8.80 (d, J = 12.15 Hz, 1H, Ar H). MS: *m/z* 292 (M^+, 100), 277 (1.00), 250 (5.25), 210 (20.70), 195 (4.62), 183 (8.20). Anal. Calcd. for $C_{16}H_{12}N_4S$ (292); C, 65.61; H, 4.42; N, 19.25%. Found: C, 65.75; H, 4.10; N, 19.17.

Synthesis of 2-Methylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (16a)

Compound **9a** (0.254 g, 1 mmol) was heated under reflux temperature in 20 mL glacial acetic acid/acetic anhydride (1:1) for 6 h; then, the reaction mixture was cooled, poured into water, filtered, dried, and recrystallized from ethanol to give compound **16a**. Yield 72%, M.p. 222–224°C. ^{1}H NMR: δ 2.60 (s, 3H, C_2-CH_3) 4.10 (s, 2H, CH_2), 7.20–7.60 (m, 3H, Ar H), 8.55 (d, J = 10.80 Hz, 1H, Ar H), 9.50 (s, 1H, C_5-H). MS: *m/z* 278 (M^+, 100), 263 (3), 236(4.9), 210 (33.82), 195 (1.69), 183 (8.20). Anal. Calcd. for $C_{15}H_{10}N_4S$ (278); C, 64.56; H, 3.44; N, 20.32%. Found: C, 64.74; H, 3.59; N, 20.14.

Synthesis of 2,5-Dimethylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (16b)

Compound **9b** (0.268 g, 1 mmol) was heated under reflux in 20 mL glacial acetic acid/acetic anhydride (1:1) for 6 h; the reaction mixture was cooled, filtered, dried, and the residue was recrystallized from ethanol to give compound **16b**. Yield 69%, M.p. 268–270°C. ^{1}H NMR: δ

2.75 (s, 3H, C_2-CH_3), 3.00 (s, 3H, C_5-CH_3), 4.00 (s, 2H, CH_2), 7.20–7.60 (m, 3H, Ar H), 8.70 (d, J = 11.00 Hz, 1H, Ar H). MS: *m/z* 292 (M^+, 100), 277 (2.69), 250 (2.28), 210 (28.66), 195 (2.39), 183 (5.58). Anal. Calcd. for $C_{16}H_{12}N_4S$ (292); C, 65.63; H, 4.32; N, 19.00%. Found: C, 65.75; H, 4.10; N, 19.17.

Isomerization of 15a and 15b to 16a and 16b

Compound **15a** (0.278 g, 1 mmol) and **15b** (0.292 g, 1 mmol) were heated under reflux temperature in 20 mL absolute ethanol containing few drops of base [piperidine or sodium ethoxide] for 6 h (compound **15a**) and 16 h (compound **15b**); then, the reaction mixture was evaporated under reduced pressure and the residue was recrystallized from ethanol to give products **16a** and **16b**, respectively, which are identical in all respects with compounds **16a** and **16b** obtained in the previous experiments.

Synthesis of 5-Methylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine (17b)

Compound **10b** (0.268 g, 1 mmol) was heated under reflux temperature in 20 mL triethyl orthoformate for 2 h; then, the reaction mixture was cooled, filtered, dried, and the residue was recrystallized from DMF to give compound **17b**. Yield 90%, M.p. 270–272°C. ^{1}H NMR: δ 2.90 (s, 3H, C_5-CH_3), 4.15 (s, 2H, CH_2), 7.30–7.60 (m, 3H, Ar H), 8.75 (d, J = 11.20 Hz, 1H, Ar H), 9.60 (s, 1H, C_3-H).

Synthesis of Indeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo-[1,5-*c*]pyrimidine (18a)

Method A

Compound **9a** (0.254 g, 1 mmol) was heated under reflux in 20 mL formic acid for 5 h; then, the reaction mixture was cooled, poured into water, filtered off, dried, and purified on silica gel using petroleum ether (40–60°C) : ethyl acetate (3:2) to give compound **18a** (0.19 g, 72 %), m.p. 256–258°C. ^{1}H NMR: δ 4.20 (s, 2H, CH_2), 7.20–7.60 (m, 3H, Ar H), 8.60 (d, J = 11.07 Hz, 1H, Ar H), 8.80 (s, 1H, C_2-H), 9.70 (s, 1H, C_5-H). MS: *m/z* 264 (M^+, 100), 236 (5), 210 (28.5), 195 (2.66). Anal. Calcd. for $C_{14}H_8N_4S$ (264); C, 63.80; H, 3.30; N, 21.10; S, 12.00%. Found: C, 63.63; H, 3.03; N, 21.21; S, 12.12.

Method B

The same as in method A but compound **10a** (0.254 g, 1 mmol) was refluxed for 8 h, to give compound **18a**.

Synthesis of 5-Methylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (18b)

Method A

A mixture of Compound **9b** (0.268 g, 1 mmol) and formic acid (20 mL) was heated under reflux temperature for 5 h, cooled, filtered, dried, and recrystallized from ethanol to give compound **18b**. Yield 76%, M.p. 255–257°C. ^{1}H NMR: δ 2.95 (s, 3H, C_5-CH_3), 4.25 (s, 2H, CH_2), 7.30–7.60 (m, 3H, Ar H), 8.70 (d, J = 11.00 Hz, 1H, Ar H), 8.80 (s, 1H, C_2-H). MS: *m/z* 278 (M^+, 100), 264 (4.0), 210 (72.0), 195 (6.0), 183 (16.0). Anal. Calcd. for $C_{15}H_{10}N_4S$ (278.00); C, 65.11; H, 3.35; N, 20.46%. Found: C, 64.74; H, 3.59; N, 20.14.

Method B

Compound **10b** (0.268 g, 1 mmol) was heated under reflux in 20 mL formic acid for 10 h; then, the reaction mixture was cooled, filtered, dried, and recrystallized from ethanol to give compound **18b**. Both products obtained in experiments A and B are identical with each other in all respects.

Isomerization of 17b to 18b

Compound **17b** (0.278 g, 1 mmol) was heated under reflux temperature in 20 mL formic acid for 4 h; then, the reaction mixture was cooled, poured into water, filtered, dried, and recrystallized from ethanol to give product **18b**. The product of this experiment is identical in all respects with compound **18b** obtained in the previous experiment.

Synthesis of 2-Methylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine-2(1*H*)-thione (20)

To a solution of compound **9b** or **10b** (0.268 g, 1 mmol) in 20 mL dry pyridine, carbon disulfide (10 mL) was added then the reaction mixture was refluxed for 5 h (compound **9b**) or 3 h (compound **10b**); the reaction mixture was cooled, poured into cold water, then 1 mL of concentrated hydrochloric acid was added, filtered, dried, and recrystallized from DMF to give in both cases one and the same identical product **20** (0.21 g, 68% from compound **9b** and 0.25 g, 81% from compound **10b**). M.p. 290–292°C. ^{1}H NMR: δ 3.20 (s, 3H, C_5-CH_3) 4.10 (s, 2H, CH_2), 7.30–7.60 (m, 3H, Ar H), 8.30 (d, J = 11.34 Hz, 1H, Ar H), 14.60 (s, 1H, NH, D_2O exchangeable). MS: *m/z* 310 (M^+, 100), 295 (46.17), 236(5.38), 2 10 (31.69).

Synthesis of 5-methyl-2-phenylindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (21)

Method A

To a solution of compound **9b** (0.268 g, 1 mmol) in 20 mL dry dioxane containing 5 drops of triethyl amine, benzoyl chloride (0.50 mL) was added, then the reaction mixture was heated under reflux temperature for 10 h, evaporated under reduced pressure, and the residue was recrystallized from dioxane to give compound **21** (0.31 g, 89%). M.p. 250–252°C. ^{1}H NMR: δ 3.00 (s, 3H, C_5-CH_3), 4.00 (s, 2H, CH_2), 7.20–8.80 (m, 10H, Ar H). MS: *m/z* 354 (M^+, 100), 277 (1.84), 210 (28.40), 195 (4.17), 183 (9.2). Anal. Calcd. for $C_{21}H_{14}N_4S$ (354); C, 71.13; H, 4.17; N, 15.69; S, 9.01%. Found: C, 71.18; H, 3.95; N, 15.81; S, 9.03.

Method B

To a solution of compound **9b** or **10b** (0.268 g, 1 mmol) in ethanol, benzaldehyde (1 mmol) was added followed by addition of 2–3 drops glacial acetic acid; then, the reaction mixture was heated under reflux temperature for 10 h (compound **9b**) and 4 h (compound **10b**), evaporated under reduced pressure to give in both cases one and the same product **21** (0.25 g, 71% from compound **9b** and 0.30 g, 85% from compound **10b**). Products **21** obtained from both method A and B are identical with each other in all respects.

Reaction of 9b with: $ClCH_2COCl$, $CH_3CHBrCOBr$, and $Cl_2CHCOCl$

To a solution of compound **9b** (0.268 g, 1 mmol) in 20 mL dry dioxane containing 5 drops of triethylamine, chloroacetyl chloride (3 mL), α-bromopropionyl bromide (1 mL), or dichloroacetyl chloride (1 mL) were added; then, the reaction mixture was heated under reflux temperature for 5, 5, and 9 h, respectively. The reaction mixture was then evaporated under reduced pressure, and the residue was recrystallized from dioxane to give the 2-substituted-5-methyindeno[1′,2′:4,5]thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **22a**, **22b**, and **23**, respectively.

22a: Yield 80%, M.p. 234–236°C. ^{1}H NMR: δ 3.00 (s, 3H, C_5-CH_3), 4.00 (s, 2H, CH_2), 4.90 (s, 2H, CH_2Cl), 7.20–7.60 (m, 3H, Ar H), 8.70 (d, J = 10.80 Hz, 1H, Ar H). MS: *m/z* 328 (M^+, Cl^{37}, 39.90), 326 (M^+, Cl^{35}, 100), 291.1 (14.1), 277 (7.5), 250 (8.2), 210 (32.6), 195 (13.5). Anal. Calcd. for $C_{16}H_{11}ClN_4S$ (326); C, 58.63; H, 3.45; N, 17.04; S, 9.60%. found: C, 58.80; H, 3.37; N, 17.17; S, 9.82.

22b: Yield 78%, M.p. 200–202°C. ^{1}H NMR: δ 2.30 (d, 3H, CH_3), 3.00 (s, 3H, C_5-CH_3), 3.95 (s, 2H, CH_2), 5.50 (q, J = 8.20 Hz, 1H, CH), 7.30–7.55 (m, 3H, Ar H), 8.70 (d, J = 10.80 Hz, 1H, Ar H). MS: *m/z* 386 (M^+, Br^{81}, 39), 384 (M^+, Br^{79}, 36), 305 (67.15), 264 (2.85), 210 (7.76), 195 (5.00), 183 (4.15). Anal. Calcd. for $C_{17}H_{13}BrN_4S$ (384); C, 52.90; H, 3.59; N, 14.41; S, 8.05%. Found: C, 53.12; H, 3.38; N, 14.58; S, 8.33.

23: Yield 83%, M.p. 262–264°C. ^{1}H NMR: δ 3.00 (s, 3H, C_5-CH_3), 4.00 (s, 2H, CH_2), 7.00–7.50 (m, 4H, Ar H), 11.60 (s, 1H, NH, D_2O exchangeable). MS: *m/z* 364 (M^+, $2Cl^{37}$, 14), 362 (M^+, Cl^{37}, Cl^{35} 69.29), 360 (M^+, $2Cl^{35}$, 100), 324 (18), 277 (9.84), 236 (2.38), 210 (9.30), 195 (4.78), 183 (3.08).

Antimicrobial Activity

The in vitro antimicrobial activity of the synthesized compounds was investigated against several pathogenic representative Gram-positive bacteria (*Staphylococcus aureus* ATCC 29231, *Bacillus subtilis,* ATCC 10783 and *Mycobacterium phlei,* ATCC 1014); Gram-negative bacteria (*Escherichia coli* ATCC 11105) and yeast (*Candida albicans* ATCC 10231). All microorganisms used were obtained from the culture collection of the Department of Natural and Microbial Products, National Research Centre, Dokki, Cairo, Egypt. Compounds were tested against *Escherichia coli* and*Staphylococcus aureus* in a nutrient broth, pH = 7.0, against *Bacillus subtilis, Mycobacterium phlei* in the Bacto brain heart infusion broth, pH = 7.0, and against *Candida albicans* in a broth containing 1% peptone, 2% dextrose, pH = 5.7. *Escherichia coli* of known antibiotic sensitivity served for control purposes. Media for disc sensitivity tests were the nutrient agar and Muller-Hinton agar (MHA) purchased from Difco (USA). Nonsterile powder of the tested compound was dissolved in sterile DMSO to yield 2.0 μg mL^{-1}, and passed through 0.2 μm membrane filters (Millipore Corp., USA). The filtrates were dispensed as 2 mL samples into sterile, small screw-capped vials, frozen and kept stored at −15°C. The vials were refrozen after thawing. Disc diffusion sensitivity test was done in the manner identical to that of Bauer et al.[5] DMSO showed no inhibition zones. Streptomycine, Amoxicilin (Bioanalyse, Turkey) and Fusidic acid (Sigma, Aldrich, USA) were used as reference substances.

REFERENCES

[1] Y. A. Ibrahim and A. H. M. Elwahy, *Adv. Heterocycl. Chem.*, **65,** 235–281 (1996).
[2] I. A. Shehata, H. I. El-Subbagh, A. M. Abdelal, M. A. El-Sherbeny, and A. A. Al-Obaid, *Med. Chem. Res*., **6,** 148–163 (1996).

[3] F. M. E. Abdel-Megeid, N. A. Hassan, M. A Zahran, and A. E. Rashad, *Sulfur Lett.*, **21,** 269–284 (1998).
[4] K. A. Ismail, O. M. Aboulwafa, and E. Koreish, *Farmaco*, **50,** 611–616, (1995).
[5] A. W. Bauer, M. M. Kirby, J. C. Sherris, and M. Turck, *Am. J. Clin. Pathol.*, **45,** 493–496 (1996).
[6] E. F. Elslager, P. Jacob, and L. M. Werbel, *J. Heterocycl. Chem.*, **9,** 775–784 (1972).
[7] F. M. Abdelrazek, and H. A. Ead, *Phosphorus, Sulfur, and Silicon*, **119,** 271–277 (1996).
[8] S. A. Shiba, A. A. El-Khamry, M. E. Shaban, and K. S. Atia, *Pharmazie*, **52,** 189–194 (1997).
[9] M. A. Mohamed, A. E. Rashad, M. E. A. Zaki, and S. S. Fatahala, *Acta Pharm.*, **55,** 237–249 (2005).
[10] A. E. Rashad, O. A. Heikal, A. O. H. El-Nezhawy, and F. M. E. Abdel-Megeid, *Heteroatom Chem.*, **16,** 226–234 (2005).
[11] M. Robba, M. Cugnon de Sevricourt, and J. M. Lecomte, *J. Heterocycl. Chem.*, **12,** 525–532 (1972).
[12] F. M. Abdel Razek and H. A. Ead, *J. Prakt. Chem.*, **330,** 585–589 (1988).
[13] C. J. Shishoo and K. S. Jain, *J. Heterocycl. Chem.*, **29,** 883–893 (1992).
[14] P. G. Baraldi, B. Cacciari, M. Viziano, S. Dionisotti, and E. Ongini, PCT Int. Appl. WO 9705, 138 (Cl. CO7D 487/14) 13 Feb. 1997; *Chem. Abstr* **126,** 212155z (1998).
[15] A. B. A. El-Gazzar, M. I. Hegab, S. A. Swelam, and A. S. Ali, *Phosphorus Sulfur and Silicon,* **177,** 123–136 (2002).
[16] C. J. Shishoo, M. B. Devani, G. V. Ullas, S. Ananthan, and V. S. Bhadti, *J. Heterocycl. Chem.,* **18,** 43–46 (1981).
[17] C. J. Shishoo, M. B. Devani, G. V. Ullas, S. Ananthan, and V. S. Bhadti, *J. Heterocycl. Chem.*, **22,** 825–832 (1985).
[18] A. M. Abdel-Fattah, A. S. Aly, F. A. Gad, N. A. Hassan, and A. B. A. El-Gazzar, *Phosphorus Sulfur and Silicon*, **163,** 1–27 (2000).
[19] G. G. Danagulyan, L. G. Saakyan, and M. G. Zalinyan, *Khim. Geterotsikl. Soedin.,* **2,** 225–227 (1992); *Chem. Abstr*, **117**: 233226c (1992).
[20] A. B. A. El-Gazzar, and N. A. Hassan, *Molecules,* **5,** 835–850 (2000).
[21] V. Nair, A. G. Lyons, and D. F. Purdy, *Tetrahedron*, **47,** 8949–8968 (1991).