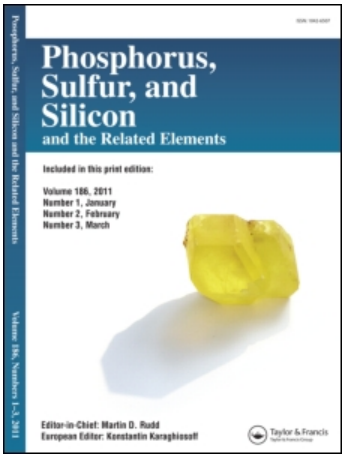

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Utility of the Enaminonitrile Moiety in the Synthesis of Some Biologically Active Thienopyrimidine Derivatives

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Utility of the Enaminonitrile Moiety in the Synthesis of Some Biologically Active Thienopyrimidine Derivatives

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*Because of its broad spectrum of biological activities, a novel series of thienopyrimidines and fused thienopyrimidines have been synthesized by reacting the enaminonitrile **1** with different reagents. Thus, reaction of **1** with urea, thiourea, formic acid and/or formamide afforded pyrimidine derivatives **2, 3, 4,** and **5**, respectively. Compound **4** reacted with $POCl_3$/PCl_5 giving the chloro-derivative **7** which upon treatment with thiourea, hydrazine, piperidine and/or thiols, produced 4-thio, 4-hydrazino, 4-piperidino- and 4-S-substituted pyrimidine derivatives **8–11**, respectively. Treatment of **9** with ethyl chloroformate, sodium nitrite/hydrochloric acid, benzoyl chloride, acetic and/or formic acid, benzaldehyde/piperidine and CS_2/pyridine provided the carbazate **13**, tetrazole **14**, triazoles **15,** and **16a**, **b**, Schiff's base **17** and triazole **18**, respectively. Some of the new synthesized compounds were screened for antibacterial activity. The structures of these compounds were confirmed by FT-IR, ^1H NMR and correct elemental analysis.*

Keywords Carbazate; *t*-Butyltetrahydrobenzo[b]thiophene; tetrazole; thienopyrimidine; triazole

INTRODUCTION

Thienopyrimidines are a class of heterocyclic compounds which possess antiallergic,[1] antiatherosclerotic,[2] antibacterial,[3] antiviral,[4] antihypertensive,[5] antihistaminic activities.[6] In addition to the above mentioned activities, thienopyrimidine derivatives have

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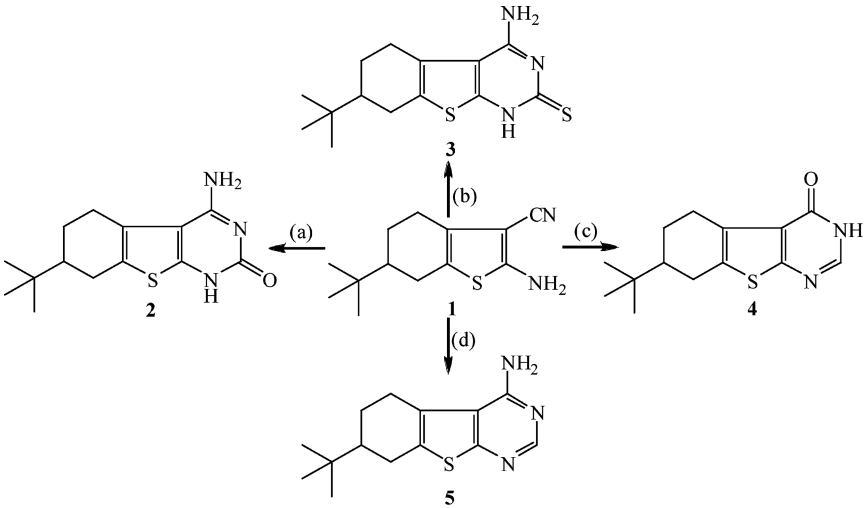
antimicrobial,[7–9] spasmolytic,[10] antitumor,[11,12] and anti-inflammatory properties.[13,14] They are also used as receptor antagonists.[15,16]

In continuation of our efforts[17–21] on the development of simple synthetic routes for the synthesis of heterocyclic compounds having biological activity, the enaminonitrile **1**, which was prepared by a previously reported method,[22] was used as a key starting material for the synthesis of the target thienopyrimidine derivatives. Some of the new synthesized compounds showed antibacterial activity. The spectroscopic data were in accordance with the proposed structures.

RESULTS AND DISCUSSION

Enaminonitrile **1**, were fused with urea and thiourea,[23] providing 4-aminopyrimidin-2(1H)-one **2** and 4-aminopyrimidin-2(1H)-thione **3**, respectively. The structures of **2** and **3** were confirmed by the spectral data, where the IR spectra showed the disappearance of C≡N band, and the presence of the C=O and C=S bands 1655 and 1234 cm^{-1}, respectively. Also, the ^1H NMR spectra of both compounds indicated the presence of the NH proton at 11.23 and 11.12, respectively (Scheme 1).



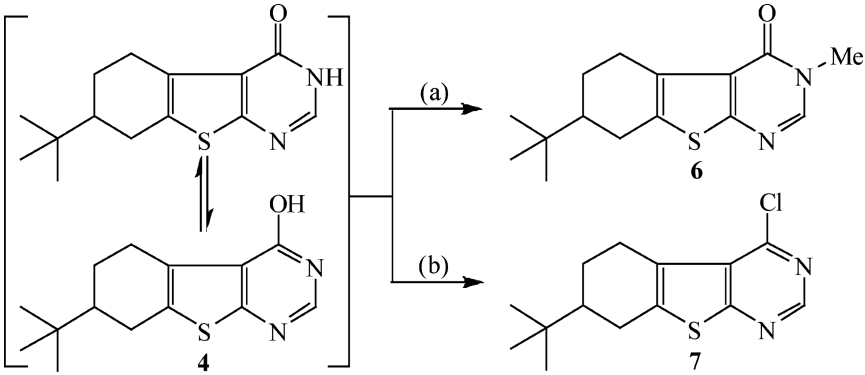
Reaction conditions:a) H_2NCONH_2/fusion, b) H_2NCSNH_2/fusuion, c) HCO_2H/Δ, d) $HCONH_2/\Delta$

SCHEME 1

In the other hand, refluxing compound **1** with formic acid and/or formamide[24] afforded pyrimidin-4(3H)-one **4** and 4-aminopyrimidine **5** derivatives, respectively (Scheme 1). The IR spectrum of **4** showed the absence of the NH_2 and C≡N absorption bands and the presence

of C=O band. The proposed structures got a further support from ^{1}H NMR spectra which showed a peak at δ 8.47 and 8.35 corresponding to the H of the pyrimidine ring.

Compound **4** underwent an N-methylation reaction through treatment with MeI in DMF, in the presence of anhydrous potassium carbonate, to furnish the 3N-methyl derivative **6**. While, the enol-form of **4** was chlorinated upon reaction with a mixture of $PCl_5/POCl_3$[25] to afford chloropyrimidine derivative **7** (Scheme 2). The structures of **6** and **7** were elucidated from their spectral data, where the IR showed the absence of the NH and the C=O bands (for compound **7**) and the ^{1}H NMR for compound **6** displayed peak at δ 3.56 for N–CH_3 and the absence of the NH peak.



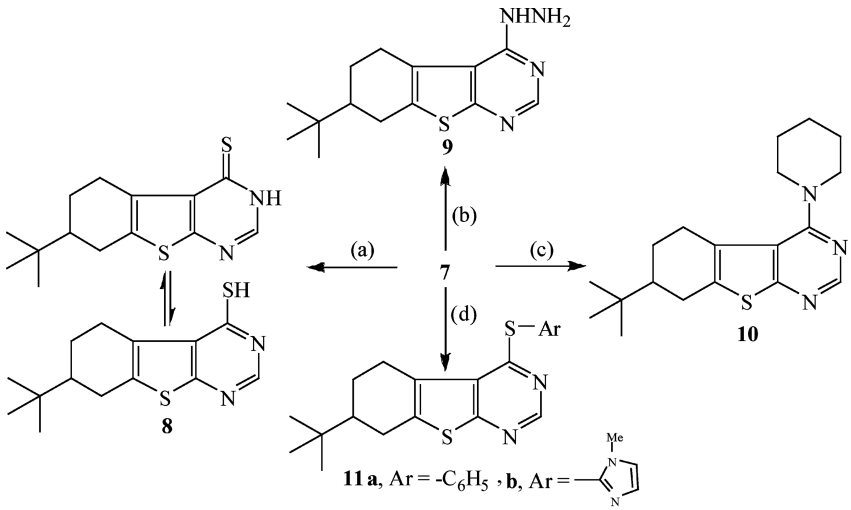
Reaction conditions: a) MeI/DMF/K_2CO_3 anhyd., b)$PCl_5/POCl_3$

SCHEME 2

The chloropyrimidine derivative **7** underwent nucleophilic substitution reactions to provide several substituted thienopyrimidine derivatives. Thus, reflux **7** with thiourea in n-butanol [26], gave the corresponding pyrimidin-4(3H)-thione **8**. The presence of NH peak at 3197 and a signal at δ 12.22 was in accord with the proposed structure.

Reaction of compound **7** with hydrazine hydrate (98%) and/or piperidine in ethanol furnished 4-hydrazinopyrimidine **9** and pipepiridopyrimidine **10**, respectively. The IR of compound **9** showed absorption bands at 3417–3198 cm^{-1} and the ^{1}H NMR displayed peaks at δ 3.62 and 6.48 (D_2O exchangeable) corresponding to NH_2 and NH. While the ^{1}H NMR of **10** displayed peaks at δ 1.91–3.09 (several m, 17H, cyclohexane, piperidine rings) which indicated the presence of piperidine moiety attached to the pyrimidine ring.

When compound **7** was allowed to react with thiophenol and/or 1-methylimidazol-2-thiol, in refluxing pyridine, the

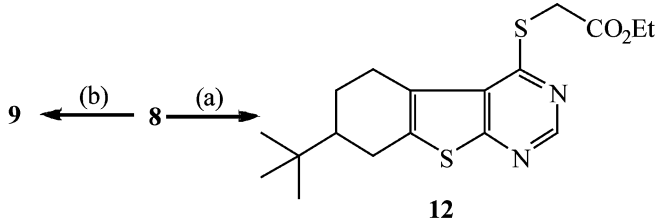


Reaction conditions: a) thiourea/n-butanol, b) N_2H_4/EtOH, c) pipepiridine/EtOH, d) thiphenol and/or 1-methylimidazol-2-thiol

SCHEME 3

4-S-substitutedthiopyrimidines **11a**, **b** were produced, respectively (Scheme 3). The ^{1}H NMR spectra of compounds **11a**, **b** displayed the following signals at δ 1.00 (s, 9H, tert-butyl group), 1.82–2.86 (m, 7H, cyclohexane ring), 3.61 (s, 3H, N—CH_3 of the imidazole ring), 7.23–7.42 (2d, 2H, imidazole ring), 8.44 (s, 1H, of the pyrimidine ring) for compound **11a**, and at δ 0.86 (s, 9H, tert-butyl group), 1.82–2.98 (several m, 7H, cyclohexane ring), 7.39–7.61 (m, 5H, benzene ring), and 8.45 (s, 1H, pyrimidine ring). This indicates the displacement of the chlorine atom by 1-methylimidazol and phenyl rings, respectively.

Furthermore, compound **8** reacted with ethyl chloroacetate, in the presence of anhydrous sodium acetate, and/or hydrazine hydrate in ethanol producing ethyl acetate derivative **12** and 4-hydrazinopyrimidine **9**, respectively (Scheme 4). The structure of the



Reaction conditions :a) $ClCH_2CO_2Et$/anhydrous CH_3CO_2Na/Δ , b) N_2H_4/EtOH/Δ

SCHEME 4

ester **12** was fully supported by its spectral data, where the IR spectrum showed absorption band at 1745 corresponding to the carbonyl of the ester group, the ^{1}H–NMR displayed signals at δ 1.3 (t, 3H, –OCH$_2$<u>CH$_3$</u>), 4.05 (s, 2H, S–CH$_2$–CO), 4.25 (q, 2H, –O<u>CH$_2$</u>CH$_3$), which revealed the presence of the $CH_2CO_2CH_2CH_3$ group attached to the sulfur atom.

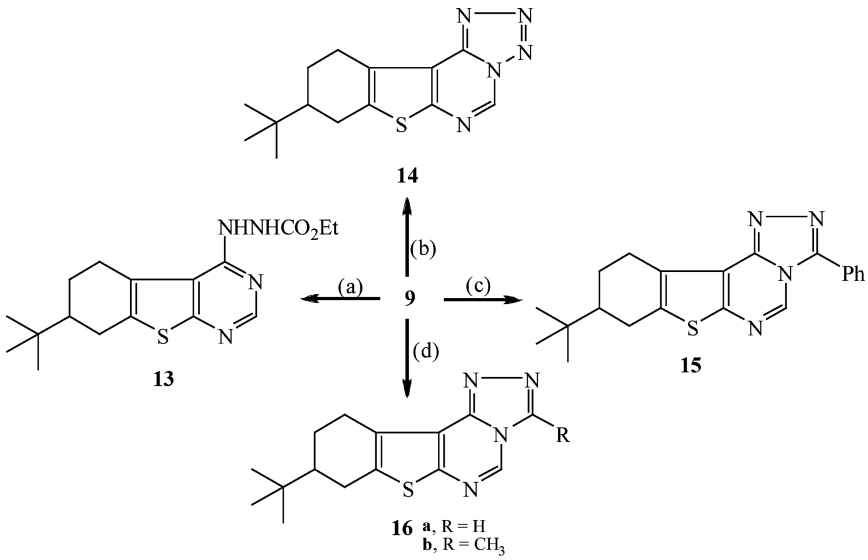
The hydrazine derivative **9** was used as a key starting material for the preparation of novel fused thienopyrimidine systems. Thus, when compound **9** was refluxed with ethyl chloroformate, in dry pyridine, the carbazate derivative **13** was obtained in a good yield. The structure of **13** was elucidated from its spectral data, where its IR showed a strong absorption band at 1741 corresponding to the carbonyl of the ester group and the ^{1}H NMR displayed signals at δ 1.25 (t, 3H, –OCH$_2$<u>CH$_3$</u>), 4.25 (q, 2H, –O<u>CH$_2$</u>CH$_3$).

On the other hand, when concentrated hydrochloric acid was added dropwise (at 0–5C) to a mixture of hydrazine **9** and sodium nitrite solution, the tetrazolo-derivative **14** was produced in a fair yield. The structure of **14** was supported by the absence of any absorption bands for the NH and NH$_2$ groups in the IR, and also the absence of their signals in the ^{1}H NMR spectra.

An interesting reaction was carried by reacting compound **9** with benzoyl chloride then the non-identified residue was treated with $POCl_3$ to furnish the triazolpyrimidine[27] derivative **15**. Its ^{1}H NMR indicated the presence of the aromatic protons at δ 7.24–7.46. Also, when the hydrazine derivative **9** was refluxed with acetic acid and/or formic acid the triazol derivatives **16a**, **b** were provided. The structures of compounds **16a, b** were supported by the ^{1}H NMR spectra, which displayed signals at δ 8.18 for the proton of the triazole ring for **16a**, and at 3.4 (3H) for the CH_3 group in compound **16b** (Scheme 5).

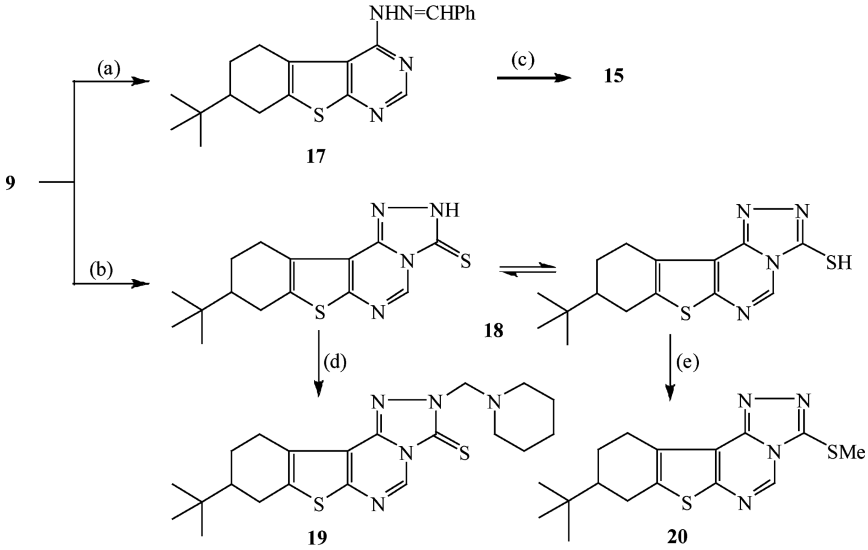
The investigation was extended to study the reactivity of compound **9** towards benzaldehyde, in the presence of piperidine, which produce the Schiff's base **17**. The ^{1}H NMR of **17** indicate the presence of 5H of the benzene ring at 7.33–7.67, 1H, N=CH at 8.08, and 1H, NH–N= (D_2O exchangeable) at 8.91. Refluxing compound **17** with thionyl chloride[28] led to the formation of compound **15**. This reaction can be considered as a chemical support for the proposed structure of compound **15**.

In the meantime, **9** was treated with carbon disulphide in dry pyridine to afford triazole derivative **18** which underwent Mannich reaction through the treatment with piperidine (as secondary amine) to furnish compound **19**. ^{1}H NMR of **19** showed the absence of NH signal and the presence of two signals at δ 1.51–3.05 (several m, 17H, cyclohexane and piperidine rings), 3.9 (s, 2H, –N–CH$_2$–N). The Methylation of **18** produced the S-methyl derivative **20**, which was supported by its ^{1}H NMR spectrum, which displayed a signal at 3.27 (3H, –S–CH$_3$), (Scheme 6).



Reaction conditions :a) $ClCO_2Et$/pyridine/Δ, b)$NaNO_2$/HCl , 0-5°C,
c) i-PhCOCl/dioxan /Δ; ii-$POCl_3$/Δ,d) RCO_2H/Δ

SCHEME 5



Reaction conditions :a) PhCHO/piperidine/Δ, b) CS_2/pyridine/Δ, c) $SOCl_2$/Δ ,
d) CH_2O/ piperidine, e) MeI/CH_3CO_2Na

SCHEME 6

ANTIBACTERIAL ACTIVITY

The new synthesized compounds were screened in vitro for their antibacterial activities against two strains of bacteria *Bacillus subtillis* (NCTC 10400) and *Escherichia coli* (NCTC 10416) by using the serial agar dilution method[29] to select the most potent compounds and sensitive bacterial species for further investigation.

One mg of each compound was dissolved in dimethyl sulfoxide (DMSO, 1 mL) then made up to 10 mL with sterile water to give a concentration of 100 μg/mL. Serial dilution was done to determine the MIC for each compound.

The bacteria was maintained on nutrient agar media. DMF showed no inhibition zones. The agar media was incubated with different microorganisms culture tested. After 180 min. of incubation at 30°C, the diameter of inhibition zone was measured. The minimal inhibitory concentration (MIC) of some of the tested compounds was measured by a two-fold serial dilution method.[30] The results are presented in Table I.

Table I showed that most of the tested compounds had an inhibition activity at concentration more than 100 μg/mL, while compounds 7 and 9 showed a minimum inhibitory effect against *Bacillus subtillis* (gram +ve) at concentrations 50 and 12.5 μg/mL, respectively. Compound 9 had inhibitory effect against *Escherichia coli* (gram +ve) at concentrations 50 μg/mL. From these results, it could be concluded that compounds **7** and **9** had the highest antibacterial activity among the tested compounds.

TABLE I Antibacterial Activities of the Newly Synthesized Compounds

Test organism / Compound number	MIC* (μg/ml)	
	B.subtilis	*E.coli*
1	>100	>100
2	>100	>100
3	>100	>100
7	50	>100
8	>100	>100
9	12.5	50
11b	>100	>100
13	>100	>100

*MIC = minimum inhibitory concentration.

Extraction of Bacillus Subtilis Intracellular Component

Acid soluble phosphorus was extracted from treated and untreated *B. subtilis* cells using 5% ice cold TCA, proteins were solublized in 1 N KOH at 37°C for 20 h; RNA was extracted by 10% TCA after addition of 6N HCl and DNA was hydrolyzed by 5% TCA in boiling water bath for 5 minutes.[31]

Acid soluble phosphorus was assessed according to the method of Toribarn et al.[32] Total proteins was determined using the method of Daughaday,[33] RNA was measured as described by Merchant et al.[34] and DNA as in Dische et al.[35]

After cell fractionation and extraction of acid soluble phosphorus, total proteins, RNA and DNA content of *B.subtilis* cells (at MIC level) treated with compounds **7** and **9**, the percentage of inhibition was calculated from the equation:

$$\%\ \text{of inhibition} = \frac{\text{Value of untreated B.subtilis} - \text{Value of treated B.subtilis}}{\text{Value of untreated B.subtilis}} \times 100 \quad (1)$$

The results are presented in Table II.

Table II showed the effect of either **7** or **9** on different *B. subtilis* cell targets at MIC level after 180 minutes incubation. Data revealed that **9** had a more inhibitory effect than **7**. Inhibition at 180 min was −72.55 % and −31.37% for acid soluble phosphorus; −45.32% and −11.78% for total proteins; −37.59% and −9.02 for RNA; and −45.22% and −28.10 for DNA content in **9** and **7** treated cells, respectively.

TABLE II Effect of 7 and 9 on Acid Soluble Phosphorus, Total Proteins, RNA and DNA Content of *B.subtilis* Cells at MIC Level

	mg/g wt			
Parameter	Acid soluble phosphorus	Total proteins	RNA	DNA
Treatment				
Untreated *B.subtilis*	0.51	33.1	13.3	1.15
B.subtilis treated with compd.	0.35	29.2	12.1	0.82
7 % of inhibition	−31.37	−11.78	−9.02	−28.70
B.subtilis treated with compd.	0.14	18.10	8.3	0.63
9 % of inhibition	−72.55	−45.32	−37.59	−45.22

Incubation period was 180 min at 37°C.

Acid soluble phosphorus, proteins, RNA and DNA contents of *B.subtilis* cells were significantly decreased by **7** and **9** at MIC concentration. The results indicated that the inhibitory effect of **9** on bacterial cells macromolecules was more pronounced than that of **7**.

These results indicate that compounds **7** and **9** inhibited protein and nucleic acid synthesis in bacterial cells due to increased destruction of ATP in the drug-treated cells. In conclusion, these compounds may be used as antibacterial drugs after further studies to investigate their toxicity and biochemical effect on living animals.

CONCLUSION

Novel thienopyrimidine and fused thienopyrimidine compounds were prepared via a simple route methodology using commercially available compounds. Some of the new synthesized compounds have antibacterial activity against gram +ve and gram –ve bacteria and also have inhibitory effect for acid soluble phosphorus, total proteins, RNA and DNA content in treated cells.

EXPERIMENTAL

All melting points reported are uncorrected and were determined on a Stuart electric melting point apparatus. The microanalysis were within ±0.3% of theoretical values and were measured at the microanalytical unit of the Faculty of Science, Cairo University. The IR spectra were measured on a Perkin-Elmer 1600 FT-IR using the KBr wafer technique. The ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions on a Bruker 200 MHz instrument (University of Regina, Regina, Saskatchewan, Canada) using TMS as internal standard with chemical shifts expressed in ppm. TLC was performed on ready-to-use silica gel plates Merck 60 to monitor the reactions and test the purity of the new synthesized compounds.

2-Amino-3-cyano-6-t-butyl-tetrahydrobenzo[b]thiophene (1)

This compound were prepared using the previously reported method.[22] Yellowish green solid, m.p.: 199–201°C, (from ethanol), yield : 90%; IR: ν cm^{-1}: 3418, 3320, 3214 (NH_2), 2950, 2826 ($CH_{al.}$), 2203 (C≡N), 1610 (C=C); ^{1}H NMR (DMSO–d_6): δ 0.89 (s, 9H, tert-butyl group), 1.87–3.05 (several m, 7H, cyclohexane), 7.73 (bs, 2H, NH_2); Anal. calcd. for $C_{13}H_{18}N_2S$ (234) : C, 66.67; H, 7.69; N, 11.97; found: C, 66.86; H, 7.79; N, 12.11.

4-Amino-7-t-butyltetrahydrobenzo[b]thieno[2,3-d]pyrimidin-2(1H)-one (2) and 4-Amino-7-t-butyltetrahydrobenzo[b]thieno [2,3-d]pyrimidin-2(1H)-thione (3)

Compound **1** (5 mmol, 1.17 g) and urea (5 mmol, 0.3 g) or thiourea (5 mmol, 0.36 g) were fused at 200°C in a sand bath for 3 h. The reaction mixture was left to cool, washed with water, and triturated with methanol (5 mL). The solid deposited was filtered off and recrystallized from a suitable solvent.

Compound 2

Yellow solid , m.p.: 233–235°C (from ethanol), yield: 0.82 g (72%); IR, ν cm^{-1}: 3421–3195 (NH_2,NH), 1655 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 0.93 (s, 9H, tert-butyl group), 1.89–3.09 (several m, 7H, cyclohexane), 7.95 (bs, 2H, NH_2, D_2O exchangeable), 11.23 (bs, 1H, NH, D_2O exchangeable); Anal. calcd. for $C_{14}H_{19}N_3OS$ (277): C, 60.65; H, 6.86; N, 15.16; found: C, 60.83; H, 7.01; N, 15.31.

Compound 3

Pale brown solid, m.p.: 245–247°C (from dioxane-water), yield: 0.98 g (69%). IR: ν cm^{-1}: 3413–3205 (NH_2,NH), 1234 (C=S); ^1H NMR (DMSO–d_6): δ 0.91 (s, 9H, tert-butyl group), 1.83–3.06 (several m, 7H, cyclohexane), 7.85 (bs, 2H, NH_2, D_2O exchangeable), 11.12 (bs, 1H, NH, D_2O exchangeable); Anal. calcd. for $C_{14}H_{19}N_3S_2$ (293) : C, 57.34; H, 6.48; N, 14.33; found: C, 57.17; H, 6.39; N, 14.15.

7-t-Butyltetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one (4)

A solution of **1** (10 mmol, 2.34 g) in formic acid (400 mmol, 15 mL,) was refluxed for 6 h. The reaction mixture was poured into ice/H_2O. The solid that precipitated was filtered off and recrystallized from ethanol to produce **4** as pale yellow crystals, m.p.: 268–270°C, yield: 2.34 g (95%); IR, ν cm^{-1}: 3211 (NH), 1659 (C=O); ^1H NMR ($CDCl_3$): δ 0.91 (s, 9H, tert-butyl group), 1.88–2.98 (several m, 7H, cyclohexane), 8.47 (s, 1H, pyrimidine), 10.90 (bs, 1H, NH, D_2O exchangeable); Anal. calcd. for $C_{14}H_{18}N_2OS$ (262) : C, 64.12; H, 6.87; N, 10.69; found: C, 64.30; H, 7.00; N, 10.85.

4-Amino-7-t-butyltetrahydrobenzo[b]thieno[2,3-d]pyrimidine (5)

A mixture of **1** (5 mmol, 1.17 g) and formamide (504 mmol, 20 mL) was refluxed for 4h. The solid that precipitated on cooling was recrystallized

from ethanol to give **5** as pale yellow crystals, m.p.: 155–157°C, yield: 2.34 g (95%); IR, ν cm^{-1}: 3419–3221 (NH_2), ^1H NMR ($CDCl_3$): δ 0.92 (s, 9H, tert-butyl group), 1.87–3.09 (several m, 7H, cyclohexane), 7.92 (bs, 2H, NH_2, D_2O exchangeable), 8.35 (s, 1H, pyrimidine); Anal. calcd. for $C_{14}H_{19}N_3S$ (261): C, 64.37; H, 7.28; N, 16.09; found: C, 64.20; H, 7.10; N, 15.90.

7-t-Butyl-3-methyltetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4-one (6)

Compound **4** (5 mmol, 1.31 g) was added methyl iodide (20 mmol, 2.86 g), DMF (20 mL) and anhydrous K_2CO_3 (73 mmol, 1 g), the reaction mixture was stirred for 2 h then poured into ice/water. The solid that deposited was collected by filtration, dried and recrystallized from *n*-hexane to produce the title compound **6** as a white powder, m.p.: 255–257°C; yield: 0.88g (64%); IR, ν cm^{-1}: 1663 (C=O); ^1H NMR ($CDCl_3$): δ 0.89 (s, 9H, tert-butyl group), 1.80–2.99 (several m, 7H, cyclohexane), 3.56 (s, 3H, N–CH_3) 8.25 (s, 1H, pyrimidine); Anal. calcd. for $C_{15}H_{20}N_2OS$ (276): C, 65.22; H, 7.25; N, 10.14; found: C, 65.40; H, 7.37; N, 10.30.

7-t-Butyl-4-chlorotetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4-one (7)

Compound **4** (10 mmol, 2.62 g), PCl_5 (12 mmol, 2.5 g) and $POCl_3$ (75 mmol, 7 mL) were heated on a water bath for 3 h. When the reaction mixture was poured carefully into ice and ammonia solution a precipitate was formed, that was filtered off and dried. Recrystallization from *n*-hexane afforded **7** as yellow crystals, m.p.: 170–172°C, yield: 2g (71%); IR, ν cm^{-1}: 1630 (C=N); ^1H NMR ($CDCl_3$): δ 0.87 (s, 9H, tert-butyl group), 1.85–2.94 (m, 7H, cyclohexane), 8.38 (s, 1H, pyrimidine); Anal. calcd. for $C_{14}H_{17}ClN_2S$ (280.5): C, 59.89; H, 6.06; N, 9.98; Cl, 12.66; found: C, 60.01; H, 6.18; N, 10.10; Cl, 12.85.

7-t-Butyltetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-thione (8)

Thiourea (7 mmol, 0.5 g) was added to a solution of **7** (5 mmol, 1.4 g) in dry methanol (15 mL) and refluxed for 2 h. The solid that deposited after cooling was filtered off and recrystallized from ethanol to give **8** as orange crystals, m.p.: 248–250°C, yield: 1.14 (82%); IR, ν cm^{-1}: 3197 (NH), 1242 (C=S); ^1H NMR (DMSO-d_6): δ 0.92 (s, 9H, tert-butyl group), 1.89–2.95 (several m, 7H, cyclohexane), 8.42 (s, 1H, pyrimidine); 12.22

(bs, 1H, NH, D_2O exchangeable); Anal. calcd. for $C_{14}H_{18}N_2S_2$ (278): C, 60.43; H, 6.47; N, 10.07; found: C, 60.30; H, 6.37; N, 10.30.

7-t-Butyl-4-hydrazinotetrahydrobenzo[b]thieno[2,3-d] pyrimidin-4-one (9)

A mixture of **7** (10 mmol, 2.8 g) and hydrazine hydrate (100 mmol, 3.2 mL) in ethanol (30 mL) was refluxed for 2 h. When the reaction mixture was allowed to cool, a solid product was separated out which upon recrystallized from ethanol produced a yellow crystals of the title compound **9**, m.p.: 193–195°C, yield: 2.6 g (94%); IR, ν cm^{-1}: 3417–3198 (NH_2, NH); ^1H NMR ($CDCl_3$): δ 1.06 (s, 9H, tert-butyl group), 1.90–3.03 (several m, 7H, cyclohexane), 3.62 (s, 2H, NH_2, D_2O exchangeable), 6.48 (bs, 1H, NH, D_2O exchangeable), 8.36 (s, 1H, pyrimidine); Anal. calcd. for $C_{14}H_{20}N_4S$ (276): C, 60.87; H, 7.25; N, 20.29; found: C, 61.02; H, 7.37; N, 20.49.

Conversion of 8 to 9

When a mixture of **8** (5 mmol, 1.38 g) and hydrazine hydrate (50 mmol, 1.6 mL) in ethanol (20 mL) was refluxed for 3 h; yellow crystals precipitated after cooling. The solid product was filtered off and dried. This solid was identified as **9** by m.p., mixed m.p., TLC, and FT-IR.

7-t-Butyl-4-piperidinotetrahydrobenzo[b]thieno[2,3-d] pyrimidin-4-one (10)

Compound **7** (5 mmol, 1.4 g) in ethanol (20 mL) was added piperidine (10 mmol, 1 mL) and the reaction mixture was refluxed for 2 h. The solid deposited after cooling was filtered off, dried and recrystallized from ethanol affording **10** as pale yellow crystals, m.p.: 128–130°C, yield: 0.92 g (56%); IR, ν cm^{-1}: 1620 (C=N); ^1H NMR ($CDCl_3$): δ 1.1 (s, 9H, tert-butyl group), 1.91–3.09 (several m, 17H, cyclohexane, piperidine rings), 8.48 (s, 1H, pyrimidine); Anal. calcd. for $C_{19}H_{27}N_3S$ (329): C, 69.36; H, 8.21; N, 12.77; found: C, 69.19; H, 8.07; N, 12.59.

7-t-Butyl-4-thiophenyl and thio(1-methylimidazol-2-yl) tetrahydrobenzo[b]-thieno[2,3-d]-pyrimidin-4-one (11a,b)

Thiophenol (5 mmol, 0.51 mL) and/or 1-methylimadazol-2-thiol (5 mmol, 0.57) were added to **7** (5 mmol, 1.4 g) in pyridine (10 mL) and refluxed for 3–4 h. After cooling, the reaction mixture was poured onto ice/HCl. The solid separated out was collected by filtration, washed with water, dried then recrystallized from the appropriate solvent.

Compound 11a

Pale brown solid, m.p.: 160–162°C (from *n*-hexane), yield: 1.11 g (62%); IR, ν cm^{-1}: 1623 (C=N); ^{1}H NMR ($CDCl_3$): δ 1.00 (s, 9H, tert-butyl group), 1.82–2.86 (m, 7H, cyclohexane), 3.61 (s, 3H, N–CH_3), 7.23-7.42 (2d, 2H, imidazole ring, $J = 8$, $J = 9$ Hz), 8.44 (s, 1H, pyrimidine); Anal. calcd. for $C_{18}H_{22}N_4S_2$ (358): C, 60.34; H, 6.15; N, 15.64; found: C, 60.46; H, 6.27; N, 15.79.

Compound 11b

Pale brown solid, m.p.: 140–142°C, (from n-hexane), yield: 1.27 g (72%); IR, νcm^{-1}: 3067 (CH_{ar}), 1625 (C=N); ^{1}H NMR ($CDCl_3$): δ 0.86 (s, 9H, tert-butyl group), 1.82–2.98 (several m, 7H, cyclohexane), 7.39–7.61 (m, 5H, benzene ring), 8.45 (s, 1H, pyrimidine); Anal. calcd. for $C_{20}H_{22}N_2S_2$ (354): C, 67.80; H, 6.21; N, 7.91; found: C, 68.00; H, 6.37; N, 8.09.

Ethyl 2-[7-t-butyltetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4-yl-thio]acetate (12)

Compound **8** (5 mmol, 1.39 g) in ethanol (20 mL) was added fused anhydrous sodium acetate (12 mmol, 1 g) and ethyl chloroacetate (10 mmol, 1.22 g). The reaction mixture was refluxed for 6 h, cooled. and the solid deposited was filtered off, washed with water, dried and then recrystallized from ethanol producing **12** as pale brown crystals, m.p.: 133–135°C, yield: 1.29 g (71%); IR, ν cm^{-1}: 1745 (C=O), 1620 (C=N); ^{1}H NMR ($CDCl_3$): δ 0.90 (s, 9H, tert-butyl group), 1.3 (t, 3H, –$OCH_2\underline{CH_3}$, J = 6.1 Hz), 1.9–2.96 (m, 7H, cyclohexane), 4.05 (s, 2H, S–CH_2–), 4.25 (q, 2H, –$O\underline{CH_2}CH_3$,J = 8.2 Hz), 8.35 (s, 1H, pyrimidine); Anal. calcd. for $C_{18}H_{24}N_2O_2S_2$ (364): C, 59.34; H, 6.59; N, 7.69; found: C, 59.42; H, 6.77; N, 7.89.

Ethyl [7-t-butyltetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4-yl] carbazate (13)

A mixture of **9** (5 mmol, 1.38 g) and ethyl chloroformate (20 mmol, 2 g) in dry pyridine (10 mL) was refluxed for 6 h. After cooling, the reaction mixture was poured onto ice/HCl, the solid that separated out was filtered off, washed with water several times, dried, and then recrystallized from ethanol to give **13** as pale yellow crystals, m.p.: 244–246°C, yield: 1.44 g (83%); IR, ν cm^{-1}: 3355, 3248 (NH), 1741 (C=O), 1609 (C=N); ^{1}H NMR (DMSO-d_6): δ 0.92 (s, 9H, tert-butyl group), 1.25 (t, 3H, –$OCH_2\underline{CH_3}$, J = 6.2 Hz), 1.89–2.99 (several m, 7H, cyclohexane), 4.25 (q, 2H, –$O\underline{CH_2}CH_3$, J = 8.2 Hz), 8.4 (s, 1H, pyrimidine), 8.6 (bs,

1H, NH—NH—CO, D_2O exchangeable), 9.25 (bs, 1H, NH—NH—CO, D_2O exchangeable); Anal. calcd. for $C_{17}H_{24}N_4O_2S$ (348): C, 58.62; H, 6.90; N, 16.09; found: C, 58.42; H, 6.77; N, 15.91.

9-t-Butyltetrahydrobenzo[b]thieno[2,3-d]tetrazolo[5,1-f] pyrimidine (14)

To a mixture of compound **9** (5 mmol, 1.38 g) and 7 mL of 5%$NaNO_2$ (3.45 g in 100 mL water), conc. HCl (37%, 5 mL) was added dropwise at 0°C for 30 min with stirring. The solid thus formed was filtered off, washed with water, dried, and recrystallized from ethanol affording the title compound **14** as brown crystals, m.p.: 145–147°C, yield: 0.83 g (58%); IR, ν cm^{-1}: 1615 (C=N), 1597 (N=N); ^1H NMR ($CDCl_3$): δ 0.91 (s, 9H, tert-butyl group), 1.82–2.98 (several m, 7H, cyclohexane), 8.42 (s, 1H, pyrimidine); Anal. calcd. for $C_{14}H_{17}N_5S$ (286): C, 58.74; H, 5.94; N, 24.48; found: C, 58.62; H, 5.77; N, 24.29.

9-t-Butyl-3-phenyltetrahydrobenzo[b]thieno[2,3-d]1,2,4-triazolo[5,4-f]-pyrimidine (15)

Benzoyl chloride (5 mmol, 0.7 g) was added dropwise to a solution of **9** (5 mmol, 1.38 g) in dry dioxane (10 mL), and the reaction mixture was refluxed for 8 h. After cooling, the volatile components were removed under reduced pressure and $POCl_3$ (53.6 mmol, 5 mL) was added to the residue. The reaction mixture was heated to 100°C for 1 h, then the excess $POCl_3$ was removed under reduced pressure, and the residue poured onto cooled concentrated NH_4OH. The precipitated was collected by filtration and recrystallized from ethanol to produce **15** as pale yellow crystals m.p.: < 300°C, yield: 0.92 g (51%); IR, νcm^{-1}: 1612 (C=N), 1599 (N=N); ^1H NMR ($CDCl_3$): δ 0.91 (s, 9H, tert-butyl group), 1.86–2.94 (several m, 7H, cyclohexane), 7.24–7.46 (m, 5H, benzene ring), 8.42 (s, 1H, pyrimidine); Anal. calcd. for $C_{21}H_{22}N_4S$ (362): C, 69.61; H, 6.08; N, 15.47; found: C, 69.40; H, 5.89; N, 15.28.

9-t-Butyltetrahydrobenzo[b]thieno[2,3-d]1,2,4-triazolo[5,4-f]pyrimidine (16a) and 3-methyl-9-t-butyltetrahydrobenzo[b] thieno[2,3-d]1,2,4-triazolo[5,4-f]pyrimidine (16b)

Compound **9** (5 mmol, 1.38 g) and formic acid (266 mmol, 10 mL) and/or acetic acid (174 mmol, 10 mL) were refluxed for 3 h. After cooling, the reaction mixture was poured onto ice/water with stirring, the solid deposited was collected by filtration, washed with water, dried and crystallized from ethanol to give **16a**, **b**, respectively.

Compound 16a

Yellowish white solid, m.p.: < 300°C, yield: 0.77 g (54%); IR, ν cm^{-1}: 1614 (C=N), 1600 (N=N); ^1H NMR ($CDCl_3$): δ 0.91 (s, 9H, tert-butyl group), 1.88–2.97 (several m, 7H, cyclohexane), 8.18 (s, 1H, triazole), 8.53 (s, 1H, pyrimidine); Anal. calcd. for $C_{15}H_{18}N_4S$ (286): C, 62.94; H, 6.29; N, 19.58; found: C, 63.12; H, 6.47; N, 19.75.

Compound 16a

16b, pale yellow solid, m.p.: < 300°C, yield: 0.75 g (50%); IR, ν cm^{-1}: 1616 (C=N), 1595 (N=N); ^1H NMR ($CDCl_3$): δ 0.91 (s, 9H, tert-butyl group), 1.88–2.99 (m, 7H, cyclohexane), 3.4 (s, 3H, CH_3), 8.38 (s, 1H, pyrimidine); Anal. calcd. for $C_{16}H_{20}N_4S$ (300): C, 64.00; H, 6.67; N, 18.67; found: C, 63.82; H, 6.53; N, 18.75.

[7-t-Butyltetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4-yl] benzalhydrazone (17)

Benzaldehyde (10 mmol, 1.06 g) and catalytic amount of piperidine (5 drops) were added to **9** (10 mmol, 2.76 g) in ethanol (25 mL) and the reaction mixture was refluxed for 4 h. The solid so formed on cooling was filtered off, dried, and crystallized from ethanol producing **17** as yellow crystals, m.p.: 255–256°C, yield: 2.84 g (78%); IR, ν cm^{-1}: 3203 (NH); ^1H NMR ($CDCl_3$): δ 0.90 (s, 9H, tert-butyl group), 1.83–2.94 (several m, 7H, cyclohexane), 7.33–7.67 (m, 5H, benzene ring), 8.08 (s, 1H, N=CH), 8.35 (s, 1H, pyrimidine), 8.91 (bs, 1H, NH-N=, D_2O exchangeable); Anal. calcd. for $C_{21}H_{24}N_4S$ (364): C, 69.23; H, 6.59; N, 15.38; found: C, 69.00; H, 6.77; N, 15.19.

Conversion of 17 into 15

$SOCl_2$ (137 mmol, 10 mL) was added to **17** (5 mmol, 1.82 g) and the reaction mixture was refluxed for 3 h. After cooling, the excess $SOCl_2$ was removed by distillation under reduced pressure. Cold saturated sodium bicarbonate was added to the residue The precipitated was collected by filtration and recrystallized from ethanol to produce **15**.

9-t-Butyl-3-thioxotetrahydrobenzo[b]thieno[2,3-d]1,2,4-triazolo[5,4-f]-pyrimidine (18)

A mixture of **9** (10 mmol, 2.76 g) in dry pyridine (15 mL) and CS_2 (83 mmol, 5 mL) was refluxed for 4 h. The reaction mixture was cooled neutralized by ice/HCl, the solid precipitated was filtered off, washed with water several times and crystallized from ethanol to furnish **18** as deep yellow crystals, m.p.: 274–277°C, yield: 2.35 g (74%); IR, ν cm^{-1}:

3226 (NH), 1618 (C=N), 1597 (N=N); ^{1}H NMR (DMSO–d_6): δ 0.89 (s, 9H, tert-butyl group), 1.86–2.97 (m, 7H, cyclohexane), 8.40 (s, 1H, pyrimidine), 8.6-8.8 (two s, 1H, -NH–C=S $\rightleftharpoons$ –N=C–SH); Anal. calcd. for $C_{15}H_{18}N_4S_2$ (318): C, 56.60; H, 5.66; N, 17.61; found: C, 56.77; H, 5.77; N, 17.79.

9-t-Butyl-2-[(piperidin-1-yl)methyl]-3-thioxotetrahydrobenzo [b]thieno[2,3-d]-1,2,4-triazolo-[5,4-f]pyrimidine (19)

To a suspension of **18** (5 mmol, 1.59 g) in ethanol (20 mL), formalin solution (37%, 1 mL) and piperidine (5 mmol, 0.5 mL) were added with stirring for 1 h. and left overnight at room temperature. The solid so formed was filtered off, washed with water and crystallized from ethanol to give the title compound **19** as canary yellow crystals, m.p.: 294–297°C, yield: 1.16 g (56%); IR, ν cm^{-1}: 1620 (C=N), 1596 (N=N); ^{1}H NMR (DMSO-d_6): δ 0.87 (s, 9H, tert-butyl group), 1.51–3.05 (several m, 17H, cyclohexane and piperidine rings), 3.9 (s, 2H, –N–CH_2–N), 8.43 (s, 1H, pyrimidine); Anal. calcd. for $C_{21}H_{29}N_5S_2$ (415): C, 60.72; H, 6.99; N, 17.61; found: C, 60.87; H, 7.17; N, 17.83.

9-t-Butyl-3-methylthiotetrahydrobenzo[b]thieno[2,3-d]1,2,4-triazolo[5,4-f]-pyrimidine (20)

Compound **18** (5 mmol, 1.59 g) and methyl iodide (10 mmol, 1.43 g) were stirred in dry ethanol (20 mL), in the presence of fused anhydrous sodium acetate (24 mmol, 2 g), for 8 h. at room temperature. The reaction mixture was diluted with water, the solid separated was collected by filtration, dried and crystallized from ethanol affording **20** as yellow crystals, m.p.: 190–193°C, yield: 1.31 g (79%); IR, ν cm^{-1}: 1617 (C=N), 1599 (N=N); ^{1}H NMR (DMSO-d_6): δ 0.90 (s, 9H, tert-butyl group), 1.81–2.95 (m, 7H, cyclohexane), 3.27 (s, 3H, –S–CH_3), 8.46 (s, 1H, pyrimidine); Anal. calcd. for $C_{16}H_{20}N_4S_2$ (332): C, 57.83; H, 6.02; N, 16.87; found: C, 58.01; H, 6.17; N, 17.09.

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