

Synthesis and antimicrobial activity of some new thienopyrimidine derivatives

Mahmoud S. Tolba,^a Adel M. Kamal El-Dean,^b Mostafa Ahmed,^{a*} Reda Hassanien,^a and Mahmoud Farouk^a

^a Chemistry department, New Valley Faculty of Science, Assiut University, Assiut 72511, Egypt

^b Chemistry department, Faculty of Science, Assiut University, Assiut 71516, Egypt

E-mail: drmostafa@scinv.au.edu.eg

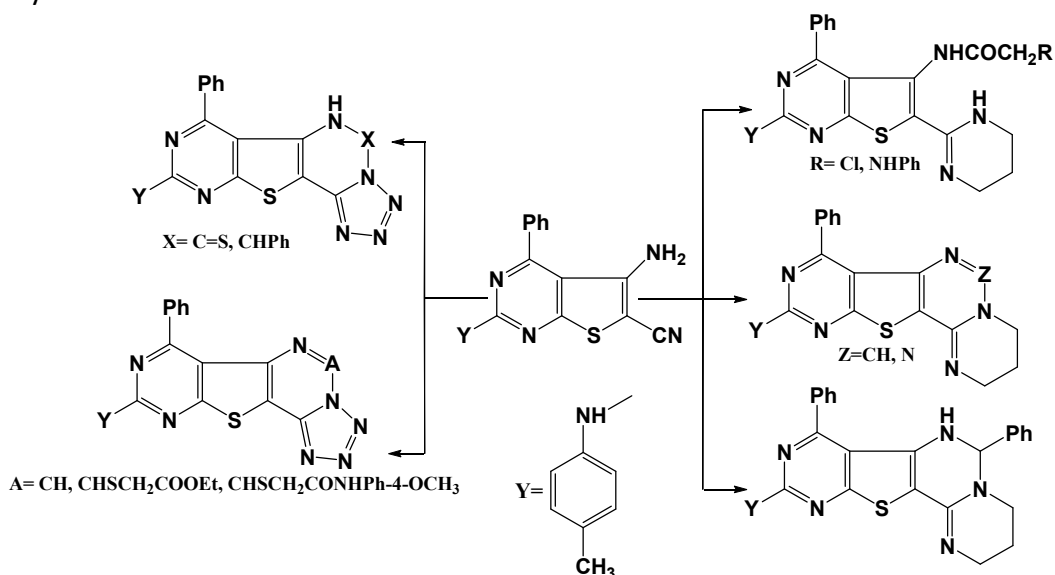
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Abstract

Due to the biological activities of pyrimidine and thienopyrimidine as antimicrobial agents, so in the present work, a series of new heterocyclic compounds containing thienopyrimidine moiety were synthesized such as, tetrazolo[1'',5'':1',6']pyrimido[4',5':4,5]thieno[2,3-d]pyrimidine derivatives (**8-12b**), pyrimido[3'',2'':1',6']pyrimido[4',5':4,5]thieno[2,3-d]pyrimidine derivatives (**14,16**) and pyrimido[5',4':4,5]thieno[2,3-e]pyrimido[1,2-c][1,2,3]triazine derivative (**15**) by using 5-amino-4-phenyl-2-(*p*-tolylamino)thieno[2,3-*d*]pyrimidine-6-carbonitrile (**7**) as starting material. All synthesized compounds were evaluated for their antimicrobial activity. And Compounds (**8-13**) exhibited high antibacterial activity. Also, compounds (**12b-18**) exhibited high antifungal activity.



Keywords: Antimicrobials, thienopyrimidine, pyrimidothienopyrimidine, fused tetrazoles

Introduction

Pyrimidine and thienopyrimidine derivatives display a broad variety of biological activities, such as anticancer,¹⁻⁶ antiviral,⁷⁻⁹ antitumor,¹⁰⁻¹⁵ anti-inflammatory,^{16,17} antimicrobial,¹⁸⁻¹⁹ antimalarial²⁰ and antioxidant activities.^{21,22} Also thienopyrimidines have long been the subject of chemical and biological research. Some thienopyrimidines show analgesic activity.²³ Recently, many important derivatives of the three basic thienopyrimidines, thieno[2,3-*d*]pyrimidine (I), thieno[3,2-*d*]pyrimidine (II), and thieno[3,4-*d*]pyrimidine (III) (Fig. 1) have been synthesized and studied for their pharmacological and biological applications.²⁴ In continuation of our program towards the synthesis of heterocyclic compounds containing a thienopyrimidine moiety,²⁵⁻³⁰ we describe here the synthesis of some new pyrimido[3'',2'':1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidines and novel related heterocyclic compounds and a study of their biological activities as antimicrobial compounds.

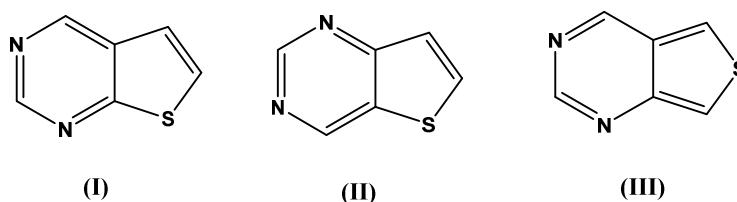
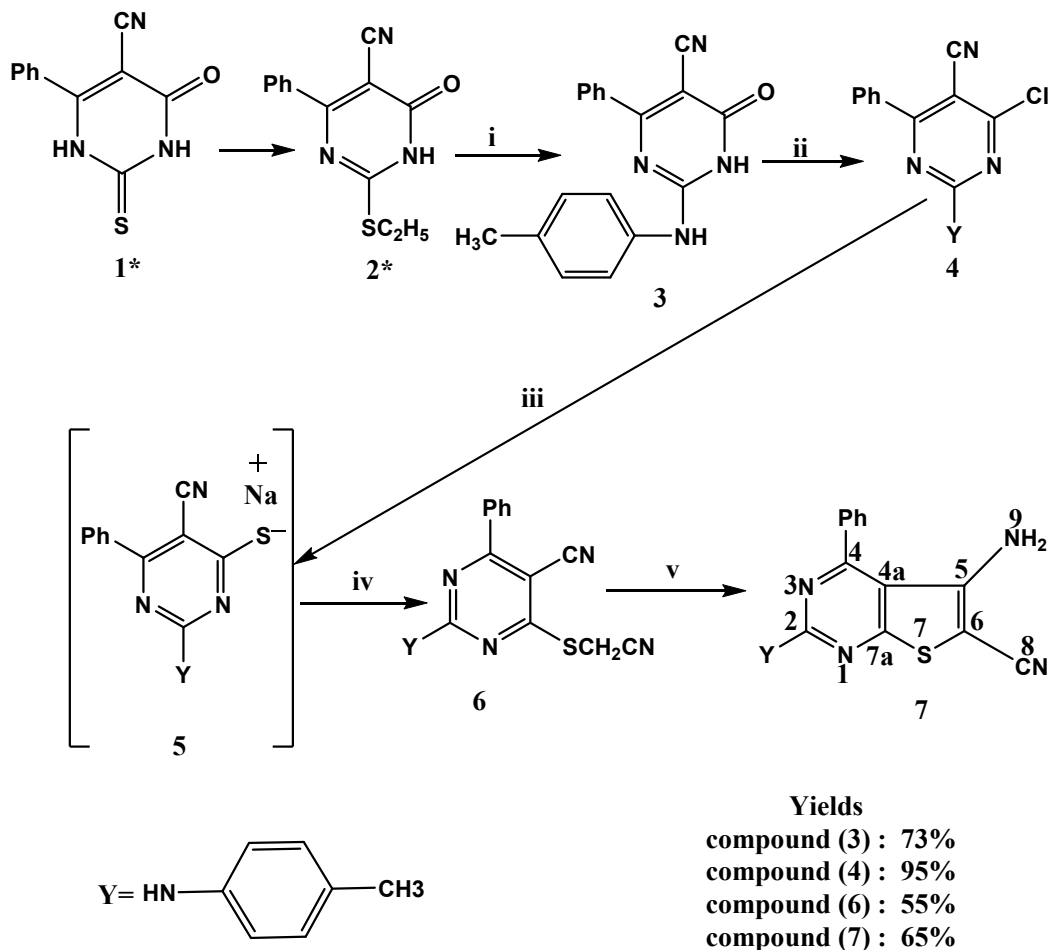


Figure 1. Structures of the three fundamental thienopyrimidines

Results and Discussion

2-(Ethylthio)-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitriles (**2**) synthesized from **1** according to a reported procedure,³³ when allowed to react with *p*-toluidine in ethanol under reflux gave 2-(*p*-tolylamino)-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**3**) (Scheme 1). Chlorination of **3**, by heating with an excess of phosphorus oxychloride, produced 4-chloro-2-(*p*-tolylamino)-6-phenyl-5-pyrimidinecarbonitrile (**4**) in good yield. Incorporation of sulfur into the pyrimidine ring was achieved by the reaction of **4** with elemental sulfur in the presence of sodium borohydride in ethanol via an in-situ sodium salt **5** that was subsequently treated with chloroacetonitrile to afford **6**. Thorpe-Ziegler cyclization of **6** upon heating with ethanolic sodium ethoxide afforded 5-amino-4-phenyl-2-(*p*-tolylamino)thieno[2,3-*d*]pyrimidine-6-carbonitrile (**7**) which was used as precursor for synthesizing novel fused heterocyclic compounds containing the thienopyrimidine moiety.



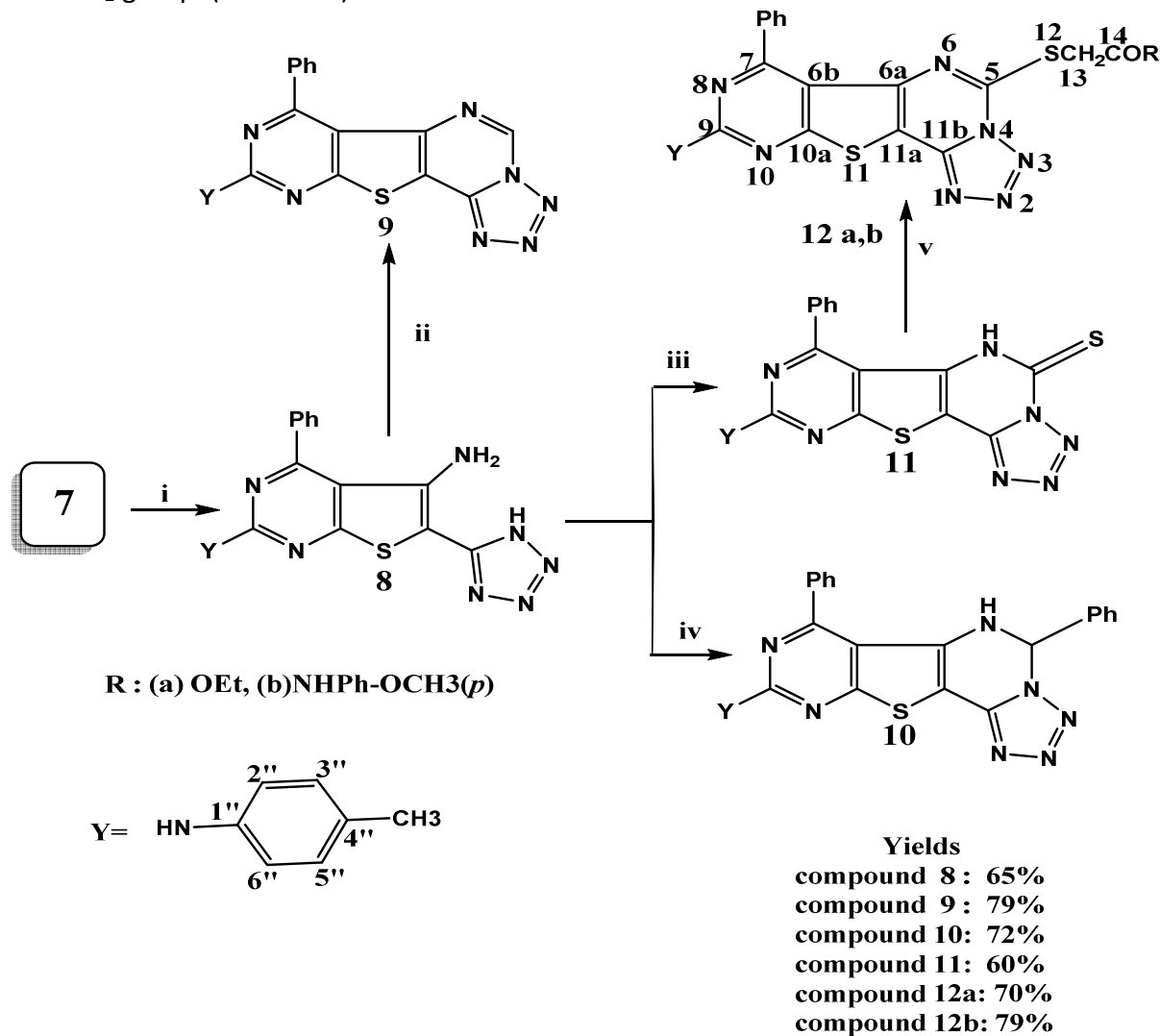
* Compound 1 prepared according to a reported procedure,³¹

** Compound 2 prepared according to a reported procedure,³³

Scheme 1. Reagents and conditions: (i) *p*-toluidine, EtOH, Reflux 6 h; (ii) POCl₃, reflux 4h; (iii) S, NaHB₄, EtOH (iv) ClCH₂CN, rt, over night; (v) EtONa, EtOH, reflux 0.5 h.

Treatment of 5-amino-2-(*p*-tolylamino)-4-phenylthieno[2,3-*d*]pyrimidine-6-carbonitrile **7** with sodium azide and ammonium chloride in DMF, followed by acidification, afforded the corresponding 5-amino-2-(*p*-tolylamino)-4-phenyl-6-(1*H*-tetrazol-5-yl)-thieno[2,3-*d*]pyrimidine (**8**). Aminotetrazolothienopyrimidine **8** was considered as precursor to the synthesis of other heterocyclic compounds, Thus, compound **8** was reacted with triethyl orthoformate in the presence of glacial acetic acid to afford the corresponding 9-*p*-tolylamino-7-phenyltetrazolo[1'',5'';1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine (**9**). Compound **8** reacted with benzaldehyde in refluxing ethanol in the presence of a few drops of piperidine to afford 5,7-diphenyl-9-(*p*-tolylamino)-5,6-dihydrotetrazolo[1'',5'';1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine **10**. Also, when **8** was allowed to react with carbon disulfide in the presence of pyridine on a steam bath for 3h. it gave the corresponding tetrazolopyrimidothienopyrimidinethione derivative **11**. Reaction of compound **11** with alkylating agents such as ethyl chloroacetate and *p*-methoxychloroacetanilid in refluxing ethanol in the presence of fused sodium acetate yielded the *S*-alkylated tetrazolopyrimidothienopyrimidine derivatives **12a,b**. The structures of the produced alkylthiotetrazolopyrimidothienopyrimidine derivatives were established by IR and ¹H NMR spectra. The IR spectrum of compound **12a** showed a peak at 1736 cm⁻¹ for C=O ester. The ¹H NMR spectrum showed

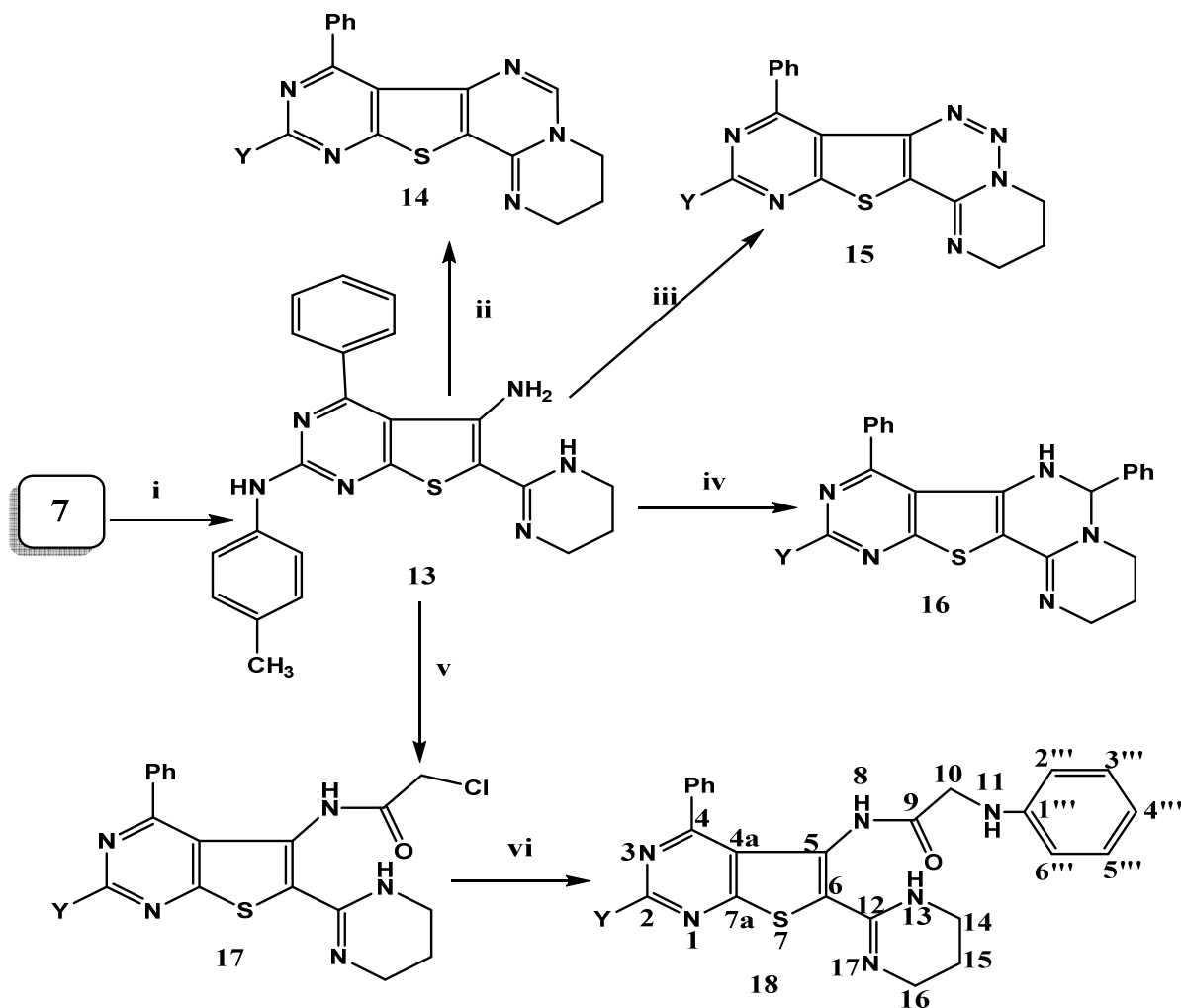
triplet and quartet signals at δ 1.34 for the CH₃ of the ester, 4.02 for the CH₂ of the ester and a singlet signal at δ 4.16 for the CH₂ group. (Scheme 2).



Scheme 2. Reagents and conditions: (i) NaN₃, NH₄Cl, DMF, 60 °C, 5h; (ii) CH(OEt)₃, AcOH, reflux 3h; (iii) CS₂, pyridine, 60 °C, 18h; (iv) PhCHO, EtOH, piperidine, 2h; (v) XCH₂COR, EtOH, CH₃COONa, 3h.

Incorporating a second pyrimidine ring in the thieno[2,3-*d*]pyrimidine system was achieved by the reaction of aminocarbonitrile compound **7** with 1,3-propanediamine in the presence of carbon disulfide to afford 5-amino-2-(*p*-tolylamino)-4-phenyl-6-(3,4,5,6-tetrahydropyrimidin-2-yl)thieno[2,3-*d*]pyrimidine (**13**). Treatment of compound **13** with triethyl orthoformate in the presence of a few drops of acetic acid gave compound **14**. Reaction of the tetrahydropyrimidylthienopyrimidine derivative **13** with HNO₂ yielded 8-phenyl-10-(*p*-tolylamino)-3,4-dihydro-2*H*-pyrimido[5',4':4,5]thieno[2,3-*e*]pyrimido[1,2-*c*][1,2,3]triazine (**15**). Also, compound **13** condensed with benzaldehyde in the presence of few drops of piperidine as a basic catalyst yielding compound **16**. Chloroacetylation of compound **13** with chloroacetyl chloride in dioxane on steam bath for 3h., gave the chloroacetylamino compound **17** which reacted with aniline to afford the corresponding phenylaminoacetamide derivative **18**. The IR spectrum of compound **17** revealed the disappearance of bands at 3468, 3363 cm⁻¹ characteristic for the NH₂ group and the appearance of an absorption band at 3291 cm⁻¹ for the NH and another at 1686 cm⁻¹ for the CO of the amide. ¹H NMR spectrum of compound **17** revealed the disappearance of a signal characteristic for NH₂ groups and the appearance of

signals at δ : 4.02 characteristic for the CH₂ and at 11.60 for the NH. Also compound **17** was confirmed by the mass spectrum which showed a molecular ion peak at m/z 491.21 (M^+ , 66 %). in agreement with the proposed structure (Scheme 3).



Yields
 compound **13** : 83%
 compound **14** : 75%
 compound **15** : 52%
 compound **16** : 72 %
 compound **17** : 58%
 compound **18** : 76%

Scheme 3. Reagents and conditions: (i) H₂N(CH₂)₃NH₂, CS₂, 60 °C, 20h; (ii) CH(OEt)₃, AcOH, reflux 3h; (iii) NaNO₂, HCl, AcOH, rt, 5h; (iv) PhCHO, EtOH, piperidine, 2h; (v) ClCH₂COCl, dioxane, 60 °C, 2h; (vi) PhNH₂, EtOH, 1h.

Antimicrobial activities

(i) **Antibacterial evaluation.** Using the agar well-diffusion method³², all of the synthesized compounds in this paper were screened in vitro for their antimicrobial activity against two pathogenic gram positive bacteria, *Staphylococcus aureus*, *Bacillus cereus* and two gram negative strains, *Escherichia coli* and *Pseudomonas*

aeruginosa. The inhibition zone (mm) was compared with a series of antibiotics according to the sensitivity of each bacteria type to the most effective antibiotic for it. (Table S1, fig. 2). The minimum inhibition concentrations (MICs) were recorded. All compounds exhibit significant antibacterial activities. Compounds **8**, **9**, **10**, **11**, **12b**, **13**, **14**, **15**, **16**, **17** and **18** revealed the existence of a remarkable activity against bacteria. Compound **13** showed the highest antibacterial activity against all strains of bacteria, with values almost similar to the corresponding reference antibiotics (Ofloxacin, Levofloxacin, Clindamycin and Nitrofurantoin, respectively). This highest activity due to the formation of 3,4,5,6-tetrahydropyrimidinyl ring. However, the presence of sulfanyl acetate group in ethyl 9-*p*-tolylamino-7-phenyltetrazolo[1'',5'':1',6']pyrimido[4',5':4,5]thieno[2,3-*d*] pyrimidine-5-ylsulfanyl] acetate **12a** decrease the antibacterial activity compared with other tested compounds especially compound **12b** which has higher antibacterial activity than compound **12a** due to the introduce the aromatic ring into the structure by sulfanylmethoxyacetanilide group.

(ii) **Antifungal evaluation.** The tested compounds were also screened for their antifungal activities against four antifungal species, *Candida albicans*, *Geotrichum candidum*, *Aspergillus flavus* and *Trichophyton rubrum*. As shown in (Table S2, fig. 3) most of the tested compounds are active. Compounds **13**, **14**, **15**, **16**, **17** and **18** showed high antifungal activity against *Trichophyton rubrum*, *Aspergillus flavus* and *Geotrichum candidum*, while compounds **12b**, **13**, **15**, **18** showed the highest antifungal activity against *Geotrichum candidum*. Compounds **13**, **17** showed the highest antifungal activity against *Candida albicans*. However, compounds **7**, **8**, **9**, and **12b** showed moderate fungal activity, while compound **12a** showed low activity against all strain of fungi. From this results we showed that compounds **13**, **15** and **18** showed highest antifungal activity against most fungi species that due to the presence of 3,4,5,6-tetrahydropyrimidinyl ring. And when the triazino ring formed in compound **15** this increase the activity of compound. On the hand, the introduce the phenylaminoacetamide group to compound **13** the antifungal activity increasing.

Table S1: Antibacterial activity, (inhibition zone, mm) and MIC ($\mu\text{g mL}^{-1}$) of the synthesized compounds

No.	<i>Bacillus cereus</i> (Gram+)	<i>Staphylococcus aureus</i> (Gram+)	<i>Pseudomonas aeruginosa</i> (Gram-)	<i>Esherichia coli</i> (Gram-)
8	17 ^a (5.0) ^b	15(5.0)	19(4.0)	19(5.0)
9	16(5.0)	20(4.0)	17(5.0)	17(5.0)
10	18(4.0)	21(4.0)	16(5.0)	20(5.0)
11	17(5.0)	17(5.0)	15(5.0)	18(5.0)
12a	13(6.0)	12(4.0)	13(5.0)	12(5.0)
12b	14(7.0)	18(5.0)	18(6.0)	12(8.0)
13	20(4.0)	19(5.0)	17(5.0)	18(4.0)
14	19(5.0)	22(3.0)	19(3.0)	19(4.0)
15	21(4.0)	19(4.0)	18(3.0)	17(5.0)
16	13(4.0)	16(5.0)	21(4.0)	20(4.0)
17	20(4.0)	17(5.0)	18(4.0)	18(5.0)
18	18(5.0)	19(4.0)	18(5.0)	11(8.0)
Ref.	22(5.0) Ofloxacin	23(4.0) Levofloxacin	20 (4.0) Clindamycin	21(5.0) Nitrofurantoin

(a) Numbers out parentheses represent the diameter of inhibition zone in (mm) of compounds 8-18; (b) Numbers in parentheses represent the *MIC* (minimum inhibition concentration) in ($\mu\text{g mL}^{-1}$) of tested compounds; (c) (-), no activity.

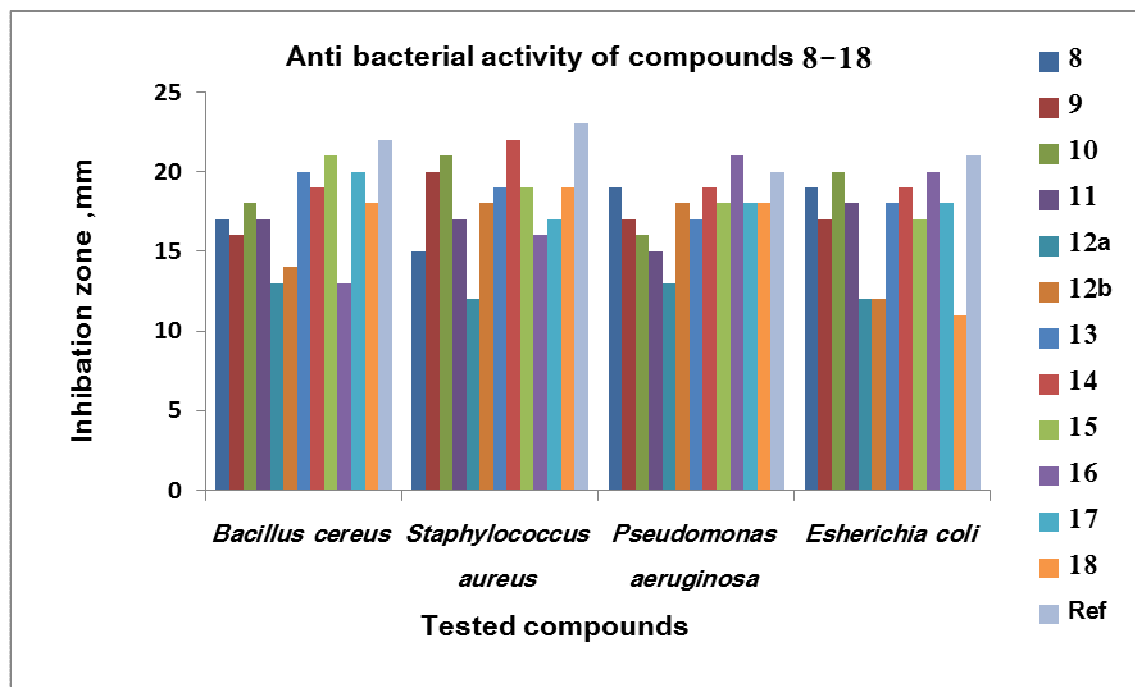


Figure 2. Comparison of (inhibition zone, mm) for anti-bacterial activity of the synthesized compounds 8-18 with standard Microorganisms.

Table S2. Antifungal activity, (inhibition zone, mm) and *MIC* ($\mu\text{g mL}^{-1}$) of the synthesized compounds

No.	<i>Geotrichum candidum</i>	<i>Candida albicans</i>	<i>Trichophyton rubrum</i>	<i>Aspergillus flavus</i>
8	12 ^b (4.0) ^c	17(5.0)	15(4.0)	17(5.0)
9	16(5.0)	21(5.0)	19(4.0)	15(6.0)
10	18(4.0)	–	23(4.0)	19(5.0)
11	17(4.0)	20(5.0)	19(4.0)	18(4.0)
12a	–	19(5.0)	22(4.0)	–
12b	20(4.0)	15(6.0)	–	18(5.0)
13	17(4.0)	22(5.0)	17(4.0)	22(5.0)
14	13(5.0)	19(5.0)	15(4.0)	14(6.0)
15	20(3.0)	17(6.0)	24(4.0)	–
16	17(4.0)	18(5.0)	19(4.0)	18(5.0)
17	19(4.0)	23(5.0)	27(4.0)	16(5.0)
18	20(4.0)	–	26(4.0)	21(5.0)
Ref ^a .	20(4.0)	23(5.0)	28(4.0)	22(5.0)

(a) Clotrimazole was used as antifungal standard; (b) (-), no activity, (c) Numbers out parentheses represent the diameter of inhibition zone in (mm) of compounds 8-18; (d) Numbers in parentheses represent the *MIC* (minimum inhibition concentration) in ($\mu\text{g mL}^{-1}$) of tested compounds.

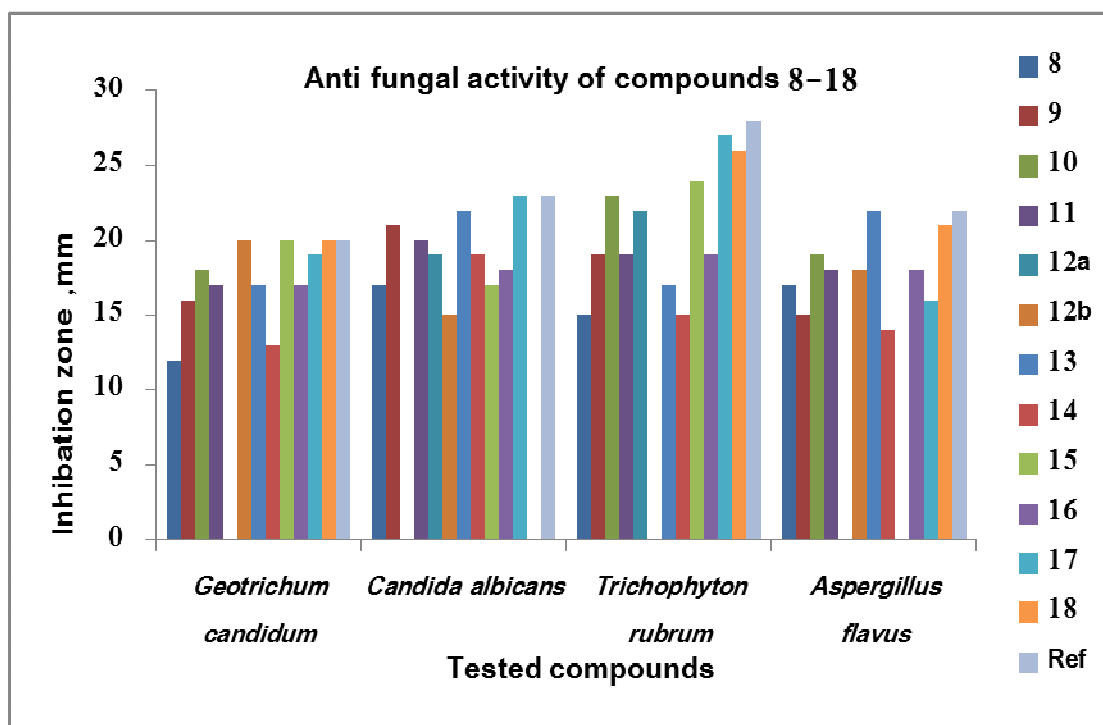


Figure 3. Comparison of (inhibition zone, mm) for anti-fungal activity of the synthesized compounds 8-18 with standard Microorganisms.

Conclusions

The objective of the present study was to synthesize, characterize and investigate antimicrobial activities of some new thieno[2,3-*d*]pyrimidine derivatives. The starting compound 5-amino-4-phenyl-2-(*p*-tolylamino)-thieno[2,3-*d*]pyrimidine-6-carbonitrile **7** was used to synthesize the target compounds. Compounds **8**, **9**, **10**, **12b**, **13**, **14**, **15**, **16**, **17** and **18** were found to be the most active compounds against all species of fungi and bacteria.

Experimental Section

General. All melting points are uncorrected and measured on a Fisher-John apparatus. Elemental analyses (C, H, N and S) were determined on an Elemental Analysis system GmbH-Vario EL V2.3 micro-analyzer in the central lab of Asyut University. Their results were found to be in good agreement ($\pm 0.2\%$) with the calculated values. IR spectra were recorded on a Pye-Unicam Sp-100 spectrophotometer using KBr wafer technique and values represented in cm^{-1} . ^1H NMR and ^{13}C NMR were carried out on Varian Gemini 300 MHz spectrophotometer at the Microanalytical Center, Cairo University, Cairo, Egypt, using tetramethylsilane (TMS) as internal standard in deuterated dimethyl sulfoxide (DMSO-d_6) and deuterated chloroform (CDCl_3) and the chemical shifts were recorded in ppm δ scale. The electron impact (EI) mass spectra were recorded on JEOL JMS- 600 spectrometer at Central unit for analysis and scientific service, National Research Center, Cairo, Egypt. Analytical thin layer chromatography (TLC) were carried out on silica gel plates (Fluka 70643-50EA.

Sigma-Aldrich, Germany) using UV light. All reactions were carried out under an air atmosphere. 5-Cyano-4-oxo-6-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine **1** was synthesized via a multicomponent reaction (MCR) by a one pot condensation reaction according to the literature;³¹ mp 298-300 °C (lit.³¹ m.p. 300-301 °C). Compound **2** was prepared according to literature procedure³³ in 85% yield, mp 238-240 °C (lit.³³ m.p. 242-246 °C).

2-(*p*-Tolylamino)-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (3). Compound **2** (30 g, 99 mmol) and *p*-toluidine (20 g) were fused for 1 h, then ethanol (50 ml) was added and the reflux was continued for 3 h. After cooling the crystals were filtered off, washed with ethanol, dried in air, and recrystallized from dioxane to give **3** as white crystals, 73% yield, mp 294-296 °C. IR (KBr): ν_{\max} (cm⁻¹) 3450 (NH), 3093 (CH aromatic), 2195 (CN), 1646 (C=O); ¹H NMR (300 MHz, CDCl₃) δ ppm: 2.32 (s, 3H, CH₃), 7.08-7.40 (m, 9H, Ar-H), 10.2 (s, 1H, NH), 11.69 (1H, s, NH); ¹³C NMR (75.4 MHz, CDCl₃) δ ppm: 21.65 (C9: CH₃ *p*-toluidine), 98.51 (C5:C-CN), 116.17 (C7: CN), 126.19 (C2', C6'), 127.45 (C3', C5'), 128.56 (C4'), 129.76 (C2", C6") , 130.67 (C3", C5"), 132.15 (C4"), 136.21 (C1"), 137.01 (C1'), 154.95 (C2), 167.56 (C4:CO), 173.16 (C6); MS: *m/z* 301.25 [M⁺-1, 85%]. Anal. calcd. for: C₁₈H₁₄N₄O (302.34): C, 71.51; H, 4.67; N, 18.53. Found: C, 71.47; H, 4.71; N, 18.45 %.

4-Chloro-2-(*p*-tolylamino)-6-phenyl-5-pyrimidine carbonitrile (4). A mixture of compound **3** (20 g, 66 mmol) in an excess amount of phosphorus oxychloride (60 ml) was heated under reflux on a water bath for 5 h. After cooling, the reaction mixture was poured into an ice-cooled water mixture (600 g), and then neutralized using sodium carbonate solution. The reaction mixture was stirred for half an hour, the formed precipitate was collected by filtration, washed several times with water, dried in air and recrystallized from ethanol to afford **4**; white crystals, 95% yield; mp 168-170 °C. IR (KBr): ν_{\max} (cm⁻¹) 3450 (NH), 3069 (CH aromatic), 2970 (C-H aliphatic), 2214 (CN); ¹H NMR (300 MHz, DMSO-d₆) δ ppm: 2.32 (s, 3H, CH₃), 6.96-7.74 (m, 9H, Ar-H), 11.24 (s, 1H, NH); ¹³C NMR (75.4 MHz, DMSO-d₆) δ ppm: 21.65 (C9: CH₃ *p*-toluidine), 98.76 (C5:C-CN), 117.54 (C7: CN), 119.83 (C2', C6'), 123.87 (C3', C5'), 126.55 (C4'), 128.98 (C2", C6") , 129.87 (C3", C5"), 133.78 (C4"), 137.65 (C1"), 138.89 (C1'), 164.66 (C4), 172.34 (C6), 176.87 (C2); Anal. Calcd. for C₁₈H₁₃ClN₄ (320.78): C, 67.42; H, 4.06; Cl, 11.05; N, 17.47. Found: C, 67.40; H, 4.07; Cl, 11.08; N, 17.45 %.

5-Cyano-6-phenyl-2-(*p*-tolylamino)pyrimidine-4-sulfanyl acetonitrile (6). A mixture of chloro compound **4** (2 g, 6.9 mmol) and elemental sulfur (0.64 mg, 20 mmol) was stirred in absolute ethanol (20 ml) in the presence of sodium borohydride (0.76 mg, 20 mmol) for 1 h. Then, the mixture was refluxed for 2h. After cooling, chloroacetonitrile (2 mmol) was added and kept stirring over night, The solid product which was formed on cooling and was filtered off, washed with water, dried and recrystallized from ethanol to give **6** as pale green crystals in 55% yield; mp 177-179 °C. IR (KBr): ν_{\max} (cm⁻¹) 3387 (NH), 3047 (CH aromatic), 2959 (CH aliphatic), 2187, 2216 (2CN). ¹H NMR (300 MHz- DMSO-d₆) δ ppm: 2.31 (s, 3H, CH₃), 4.63 (s, 2H, CH₂), 7.11-7.66 (9H, m, Ar-H) and 11.87 (1H, s, NH); ¹³C NMR (75.4 MHz, DMSO-d₆) δ ppm: 20.45 (C9:CH₂), 21.65 (C9: CH₃ *p*-toluidine), 96.56 (C5:C-CN), 117.92 (C7: CN), 118.86 (C10:CN), 121.73 (C2", C6"), 124.55 (C2', C6'), 126.37 (C4'), 128.98 (C3', C5') , 129.79 (C3", C5"), 132.66 (C4"), 136.72 (C1'), 137.88 (C1"), 167.02 (C6), 174.54 (C4), 178.17 (C2); MS: *m/z* 357.10 [M⁺, 54%]. Anal. Calcd. For C₂₀H₁₅N₅S (357.44): C, 67.25; H, 4.23; N, 19.55; S, 8.97. Found: C, 67.28; H, 4.25; N, 19.53; S, 8.94 %.

5-Amino-4-phenyl-2-(*p*-tolylamino)thieno[2,3-*d*]pyrimidine-6-carbonitrile (7). To a solution of 5-cyano-6-phenyl-2-*p*-tolylamino-pyrimidine-4-sulfanyl acetonitrile **6** (10 mmol) in absolute ethanol (20 mL), a few drops of sodium ethoxide (prepared by 0.5 g of finely divided sodium metal in absolute ethanol (20 mL)) were added. The reaction mixture was heated under reflux for 20 min. The precipitate that formed was collected and recrystallized from an ethanol-water mixture (1:1) to afford **7**, which was obtained as yellow crystals in 65 % yield; mp 208-210 °C. IR (KBr): ν_{\max} (cm⁻¹) 3477, 3387 (NH₂), 3047 (C-H aromatic), 2959 (C-H aliphatic), 2187

(CN). ^1H NMR (300 MHz, DMSO- d_6) δ ppm: 2.31 (s, 3H, CH₃), 6.47 (s, 2H, NH₂), 7.11-7.66 (m, 9H, ArH) and 11.87 (s, 1H, NH); ^{13}C NMR (75.4 MHz, DMSO- d_6) δ ppm: 21.13 (C11: CH₃ *p*-toluidine), 83.56 (C6:C-CN), 110.98 (C4a), 120.56 (C2", C6"), 125.97 (C3", C5"), 131.87 (C3', C5'), 139.67 (C2', C6'), 117.67 (C8: CN), 121.96 (C4"), 128.98 (C4'), 136.75 (C1'), 142.55 (C1"), 148.98 (C6), 154.77 (C2), 160.78 (C5: C-NH₂), 178.98 (C4); MS: *m/z* 357.12 (M⁺, 72%). Anal. Calcd. For C₂₀H₁₅N₅S (357.44): C, 67.25; H, 4.23; N, 19.55; S, 8.97. Found: C, 67.28; H, 4.25; N, 19.53; S, 8.94 %.

5-Amino-4-phenyl-6-(1*H*-tetrazol-5-yl)-2-(*p*-tolylamino)thieno[2,3-*d*]pyrimidine (8). A mixture of compound **7** (1.25 g, 3.33 mmol), sodium azide (0.4 g, 6 mmol), and ammonium chloride (0.32 g, 6 mmol) in DMF (15 ml) was heated on a steam bath for 5 h. The reaction was allowed to cool, diluted with water, and acidified with dilute acetic acid. The solid product was collected and crystallized from an ethanol- dioxane mixture (2:1) to give **8**, which was obtained as yellow crystals in 65 % yield; mp 248–250 °C. IR (KBr): ν_{max} (cm⁻¹) 3469, 3352 (NH₂), 3142 (NH), 3063 (CH aromatic) and 2984 (CH aliphatic); ^1H NMR (300 MHz, CDCl₃) δ ppm: 2.31 (s, 3H, CH₃), 6.37 (s, 2H, NH₂), 6.93-7.64 (m, 9H, Ar-H), 11.86 (s, 1H, NH) , 12.69 (s, 1H, NH); ^{13}C NMR (75.4 MHz, CDCl₃): δ 21.37 (C14: CH₃ *p*-toluidine), 119.57 (C2", C6"), 120.77 (C3", C5"), 122.45 (C4a), 125.77 (C3', C5'), 128.53 (C2', C6'), 130.67 (C4"), 132.35 (C1'), 132.73 (C4'), 135.97 (C1"), 138.36 (C6), 143.77 (C5), 147.55 (C8), 156.55 (C7a), 155.67 (C2), 171.55 (C4); MS: *m/z* 398.67 (M⁺-2, 100.00 %). Anal. Calcd. for C₂₀H₁₆N₈S (400.47): C, 59.99; H, 4.03; N, 27.98; S, 8.01. Found: C, 59.96; H, 4.05; N, 27.98; S, 8.01 %.

7-Phenyl-9-(*p*-tolylamino)tetrazolo[1",5":1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine (9). A mixture of compound **8** (0.5 g, 1.25 mmol) and triethyl orthoformate (5 ml) in the presence of a few drops of glacial acetic acid was heated under reflux for 3 h and was then allowed to cool. The solid precipitate which was formed on cooling, was collected and recrystallized from ethanol to give **9**, obtained as pale yellow crystals in 79 % yield; mp 294-296 °C. IR (KBr): ν_{max} (cm⁻¹) 3295 (NH), 3054 (CH aromatic), 2964, 2911, 2863 (C-H aliphatic), 1643 (C=N). ^1H NMR (300 MHz, DMSO- d_6) δ ppm : 2.31 (s, 3H, CH₃), 7.10-7.70 (m, 9H, ArH), 9.09 (s, 1H, CH pyrimidine), 10.74 (s, 1H, NH); ^{13}C NMR (75.4 MHz, DMSO- d_6) δ ppm: 21.13 (C13: CH₃ *p*-toluidine), 122.14 (C6b), 127.56 (C2", C6"), 128.96 (C3", C5"), 129.23 (C2', C6'), 129.67 (C3', C5'), 121.96 (C11a), 132.56 (C4'), 133.85 (C1'), 136.89 (C1"), 142.55 (C4"), 142.67 (C6a), 155.45 (C5), 156.78 (C9), 163.66 (11b), 172.78 (C7), 177.56 (C10a); MS: *m/z* 410.08 (M⁺, 77 %). Anal. Calcd. for C₂₁H₁₄N₈S (410.46): C, 61.46; H, 3.44; N, 27.30; S, 7.81. Found: C, 61.43; H, 3.45; N, 27.32; S, 7.79 %.

5,7-Diphenyl-9-(*p*-tolylamino)-5,6-dihydro-tetrazolo[1",5":1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine (10). A mixture of 5-amino-2-*p*-tolylamino-4-phenyl-6-(1*H*-tetrazol-5-yl)thieno[2,3-*d*] pyrimidine **8** (0.8 g, 2 mmol) and excess benzaldehyde were gently refluxed for 30 minutes at 100°C. Then absolute ethanol (15 ml) was added and reflux was continued for additional 2 h. The solid product which was formed after cooling, was collected and recrystallized from ethanol to afford **10** as yellow crystals, 72% yield; mp 342-344 °C. IR (KBr): ν_{max} (cm⁻¹) 3399, 3280 (2NH), 3057 (C-H aromatic) and 2918 (C-H aliphatic); ^1H NMR (300 MHz, DMSO- d_6) δ ppm: 2.33 (s, 3H, CH₃), 7.35 (s, 1H, CH pyrimidine), 6.90-7.57 (m, 14H, Ar-H), 9.36 (s, 1H, NH), 9.86 (s, 1H, NH); ^{13}C NMR (75.4 MHz, DMSO- d_6) δ ppm: 21.65 (C13: CH₃ *p*-toluidine), 85.98 (C5), 121.17 (C6a), 122.35 (C2", C6"), 124.55 (C6b), 125.72 (C4"), 126.82 (C2"', C6"'), 127.76 (C2', C6'), 128.95 (C3"', C5"'), 129.66 (C4'), 131.15 (C3', C5'), 132.72 (C3", C5"), 134.65 (C4"), 135.34 (C1'), 138.81 (C1"), 141.15 (C11a), 142.65 (C1"), 149.55 (C10a), 164.19 (C11b), 167.77 (C7), 172.88 (C9); MS: *m/z* 487.07 (M⁺-1, 54 %). Anal. Calcd. for C₂₇H₂₀N₈S (488.58): C, 66.38; H, 4.13; N, 22.93; S, 6.56. Found: C, C, 66.36; H, 4.15; N, 22.90; S, 6.54 %.

7-Phenyl-9-(*p*-tolylamino)tetrazolo[1",5":1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine-5(6*H*)-thione (11). A mixture of compound **8** (0.7 gm, 1.75 mmol) and carbon disulfide (4 ml) in pyridine (15 ml) was heated overnight under reflux on a water bath. After cooling, the solid that formed was collected and crystallized from an ethanol-water mixture (1:1) to afford **11** as yellow crystals in 60 % yield; mp 298–300 °C. IR (KBr): ν_{max}

(cm^{-1}) 3390 (NH), 3055 (C-H aromatic), 2958, 2852 (C-H aliphatic), 1212 (C=S); ^1H NMR (300 MHz, DMSO- d_6) δ ppm: 2.30 (s, 3H, CH_3), 6.91-7.65 (m, 9H, ArH), 11.97 (s, 1H, NH), 12.48 (s, 1H, NH); ^{13}C NMR (75.4 MHz, DMSO- d_6) δ ppm: 21.13 (C13: CH_3 *p*-toluidine), 113.45 (C6b), 116.35 (C2", C6"), 126.33 (C3", C5"), 129.11 (C3', C5'), 141.45 (C2', C6'), 120.23 (C11a), 128.89 (C4'), 131.30 (C11b), 132.45 (C4"), 136.74 (C1"), 140.02 (C1'), 151.46 (C10a), 154.22 (C9), 173.67 (C5), 181.09 (C7); MS: m/z 442.13 (M^+ , 54 %). Anal. Calcd. for $\text{C}_{21}\text{H}_{14}\text{N}_8\text{S}_2$ (442.52): C, 57.00; H, 3.19; N, 25.32; S, 14.49. Found: C, 57.04; H, 3.14; N, 25.28; S, 14.53 %.

Alkylation of 7-phenyl-9-(*p*-tolylamino)tetrazolo[1",5":1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine-5(6*H*)-thione (12a,b). A mixture of tetrazolothione derivative **11** (0.6 g, 1.3 mmol) and alkylating agent (2 mmol) in the presence of fused sodium acetate (0.85 g, 0.01 mmol) in ethanol (15 mL) was heated under reflux for 3 h. The white precipitate that formed was collected and recrystallized from ethanol to give **12a, b**.

Ethyl 7-phenyl-9-(*p*-tolylamino)tetrazolo[1",5":1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine-5-ylsulfanyl]-acetate (12a). White crystals, 70 % yield; mp 210-212 °C. IR (KBr): ν_{max} (cm^{-1}) 3349 (NH), 3055 (C-H aromatic) 2954 (C-H aliphatic), 1736 (C=O ester), 1655 (C=N). ^1H NMR (300 MHz, CDCl_3) δ ppm: 1.34 (*t*, *J* 7.2 Hz, 3H, CH_3 ester), 2.31 (s, 3H, CH_3), 4.16 (s, 2H, CH_2CO), 4.02 (q, *J* 6.3 Hz, 2H, CH_2 ester), 6.95-7.74 (m, 9H, Ar-H), and 10.00 (s, 1H, NH); ^{13}C NMR (75.4 MHz, CDCl_3) δ ppm: 13.09 (C18: CH_3 ester), 21.65 (C13: CH_3 *p*-toluidine), 36.87 (C13: CH_2), 62.77 (C17: CH_2 ester), 121.65 (C2", C6"), 126.14 (C11a), 127.45 (C6b), 128.16 (C2', C6'), 128.97 (C4'), 131.89 (C3', C5'), 132.87 (C3", C5"), 135.55 (C4"), 138.13 (C1'), 139.75 (C1"), 156.78 (C10a), 158.99 (C11b), 163.44 (C6a), 165.87 (C7), 167.19 (C14: CO), 169.82 (C5), 174.66 (C9); MS: m/z 528.32 (M^+ , 49 %). Anal. Calcd. for: $\text{C}_{25}\text{H}_{20}\text{N}_8\text{O}_2\text{S}_2$ (528.62): C, 56.80; H, 3.81; N, 21.20; S, 12.13. Found: C, 56.80; H, 3.81; N, 21.20; S, 12.13 %.

7-Phenyl-9-(*p*-tolylamino)tetrazolo[1",5":1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidin-5-ylsulfanyl]-*p*-methoxyacetanilide (12b). White crystals, 79% yield; mp 234-236 °C. IR (KBr): ν_{max} (cm^{-1}) 3420, 3334 (2NH), 2916, 2848 (C-H aliphatic), 1717 (C=O), 1625 (C=N); ^1H NMR (300MHz, CDCl_3) δ ppm: 2.32(s, 3H, CH_3 *p*-toluidine), 3.8 (s, 3H, *p*-methoxy acetanilide), 4.18 (s, 2H, CH_2CO), 6.90-7.54 (m, 13H, Ar-H), 9.33 (s, 1H, NH), 10.22 (s, 1H, NH); ^{13}C NMR (75.4 MHz, CDCl_3) δ ppm : 21.13 (C19: CH_3 *p*-toluidine), 32.93 (C13: CH_2CO), 56.04 (C17: OCH_3), 113.87 (C6b), 115.43 (C2", C6"), 118.22 (C3"', C5'''), 122.14 (C2"', C6'''), 126.44 (C3", C5"), 131.46 (C3', C5'), 144.44 (C2', C6'), 125.00 (C11a), 129.23 (C4'), 132.31 (C4"), 134.55 (C11b), 136.55 (C1'''), 139.89 (C1'), 142.45 (C6a), 143.44 (C1"), 152.21 (C10a), 155.01 (C9), 156.33 (C4''': C-O-CH_3), 160.55 (C5), 167.55 (C14: C=O), 182.72 (C7); MS : m/z 606.32 ($\text{M}^+ + 1$, 93 %). Anal. Calcd for: $\text{C}_{30}\text{H}_{23}\text{N}_9\text{O}_2\text{S}_2$ (605.70): C, 59.49; H, 3.83; N, 20.81; S, 10.59. Found: C, 59.53; H, 3.85; N, 20.83; S, 10.55 %.

5-Amino-4-phenyl-2-(*p*-tolylamino)-6-(3,4,5,6-tetrahydropyrimidin-2-yl)thieno[2,3-*d*]pyrimidine (13).

Aminocyan compound **7** (4 g, 10 mmol), 1,3-diaminopropane (5 ml, 59.9 mmol) and carbon disulfide (1ml) was heated on a water bath overnight. After cooling, the mixture was added to cold water (100 ml) and the product obtained was filtered and recrystallized from ethanol to give **13**, yellow crystals, 83 % yield; mp 238-240 °C. IR (KBr): ν_{max} (cm^{-1}) 3468, 3363 (NH_2), 3219 (NH), 3045 (C-H aromatic), 2953 (C-H aliphatic); ^1H NMR (300 MHz, CDCl_3) δ ppm: 1.6 (m, 2H, CH_2), 2.32(s, 3H, CH_3 *p*-toluidine), 3.35-3.5 (m, 4H, 2 CH_2), 6.11 (s, 2H, NH_2), 6.96-7.67 (m, 9H, Ar-H), 7.68 (s, 1H, NH), 12.25 (s, 1H, NH), ^{13}C NMR (75.4 MHz, CDCl_3): δ 21.13 (C16: CH_3 *p*-toluidine), 21.98 (C12), 42.22 (C11), 44.56 (C13), 113.03 (C4a), 115.65 (C6), 115.99 (C2", C6"), 126.02 (C3", C5"), 130.66 (C3', C5'), 141.45 (C2', C6'), 129.21 (C4'), 132.22 (C4"), 139.65 (C1'), 142.65 (C9), 143.25 (C1"), 145.67 (C5), 153.88 (C2), 156.43 (C10a), 178.33 (C4); MS: m/z 413.98 ($\text{M}^+ - 1$, 99%). Anal. Calcd. for $\text{C}_{23}\text{H}_{22}\text{N}_6\text{S}$ (414.54): C, 66.62; H, 5.35; N, 20.27; S, 7.75%. Found: C, 66.68; H, 5.28; N, 20.31; S, 7.73 %.

8-Phenyl-10-(*p*-tolylamino)-3,4-dihydro-2*H*-pyrimido[3",2":1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine (14). Compound **13** (0.5 g, 1.2 mmol) was heated under reflux for 3 h with triethyl orthoformate (5 ml) in the

presence of a few drops of glacial acetic acid. The precipitate that formed after cooling was collected and recrystallized from an ethanol-water mixture (2:1) to give **14** as pale yellow crystals, 75 % yield; mp >300 °C. IR (KBr): ν_{\max} (cm⁻¹) 3376 (NH), 3054 (C-H aromatic), 2953 (C-H aliphatic), ¹H NMR (300 MHz, DMSO-d₆) δ ppm: 1.90, 3.80, 4.1 (s, 6H, 3CH₂), 6.95-7.70 (m, 9H, Ar-H), 9.38 (s, 1H, CH pyrimidine), 12.00 (s, 1H, NH); ¹³C NMR (75.4 MHz, DMSO-d₆) δ ppm: 19.93 (C3), 21.65 (C14: CH₃ *p*-toluidine), 44.37 (C2), 46.93 (C4), 122.15 (C2'', C6''), 123.77 (C7b), 125.63 (C12a), 127.78 (C2', C6'), 130.35 (C4'), 130.98 (C3', C5'), 132.74 (C3'', C5''), 133.83 (C4''), 135.49 (C1'), 137.87 (C1''), 139.74 (C7a), 151.88 (C6), 152.55 (C11a), 159.35 (C12b), 166.48 (C8), 172.13 (C10); MS: *m/z* 425.06 (M⁺+1, 87%). Anal. Calcd. for C₂₄H₂₀N₆S (424.53): C, 67.90; H, 4.75; N, 19.80; S, 7.55. Found: C, 67.90; H, 4.75; N, 20.51; S, 7.64 %.

8-Phenyl-10-(*p*-tolylamino)-3,4-dihydro-2H-pyrimido[5',4':4,5]thieno[2,3-*e*]pyrimido[1,2-*c*][1,2,3]triazine (15).

To a suspension of compound **13** (0.46 g, 1.11 mmol) in a mixture of acetic acid (10 ml) and hydrochloric acid (2 ml), in an ice bath a solution of sodium nitrite (0.31 g, 4.7 mmol) in 5 ml H₂O was added during 5 minutes. After addition, the ice bath was removed and stirring was continued for 5 h. the mixture was diluted with water and the solid product obtained was collected and recrystallized from dioxane to afford **15** as yellow needles, 52% yield; mp 182-84 °C. IR (KBr): ν_{\max} (cm⁻¹) 3434 (NH), 3047 (C-H aromatic), 2964 (C-H aliphatic), 1635 (C=N); ¹H NMR (300 MHz, DMSO-d₆) δ ppm: 1.68 (m, 2H, CH₂), 3.93 (t, 2H, CH₂), 4.09 (q, 2H, CH₂), 6.94-7.69 (m, 9H, Ar-H), 11.66 (s, 1H, NH); ¹³C NMR (75.4 MHz, DMSO-d₆): δ 20.78 (C3), 21.89 (C14: CH₃ *p*-toluidine), 41.35 (C2), 46.46 (C4), 120.37 (C7a), 122.35 (C2'', C6''), 125.28 (C3'', C5''), 126.66 (C3', C5'), 127.98 (C2', C6'), 129.78 (C4'), 129.97 (C7b), 131.27 (C4''), 132.10 (C1'), 140.45 (C12a), 143.37 (C1''), 155.54 (C10), 156.66 (C12b), 157.35 (C11a), 176.66 (C8); MS: *m/z* 425.98 (M⁺-1, 99 %). Anal. Calcd. for C₂₃H₂₁N₇S (427.53): C, 64.66; H, 4.91; N, 22.91; S, 7.52. Found: C, 64.63; H, 4.89; N, 22.94; S, 7.54 %.

6,8-Diphenyl-10-(*p*-tolylamino)-3,4-dihydro-2H-pyrimido[3'',2'':1',6']pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine (16). A mixture of compound **13** (0.5 g, 1.2 mmol) and benzaldehyde (0.7 g, 6 mmol) in absolute ethanol (15 ml) was heated under reflux for 2 h. The solid product that formed after cooling was collected and recrystallized from ethanol to give **16** as yellow crystals, 72% yield; mp >300 °C. IR (KBr): ν_{\max} (cm⁻¹) 3434 (2NH), 3047 (C-H aromatic), 2964 (C-H aliphatic); ¹H NMR (300 MHz, CDCl₃) δ ppm: 1.57 (m, 2H, CH₂), 3.5 (q, 2H, CH₂), 3.85 (t, 2H, CH₂), 10.21 (s, 1H, CH pyrimidine), 6.91-7.62 (m, 14H, Ar-H), 5.25 (s, 1H, NH), 11.33 (s, 1H, NH); ¹³C NMR (75.4 MHz, CDCl₃) δ ppm: 21.13 (C14: CH₃ *p*-toluidine), 22.86 (C2), 42.43 (C3), 45.12 (C4), 71.45 (C6), 112.76 (C7b), 115.60 (C12a), 116.67 (C2'', C6''), 124.55 (C3''', C5'''), 126.44 (C2''', C6'''), 127.55 (C3'', C5''), 129.56 (C3', C5'), 142.44 (C2', C6'), 129.11 (C4'), 129.88 (C4'''), 132.21 (C4''), 133.00 (C4'), 135.91 (C1''), 138.55 (C7a), 139.22 (C1'), 146.55 (C1'''), 147.35 (C12b), 153.35 (C11a), 155.46 (C10), 178.33 (C8); MS: *m/z* 502.45 (M⁺, 77%). Anal. Calcd. for C₃₀H₂₆N₆S (502.65): C, 71.66; H, 5.23; N, 16.73; S, 6.38. Found: C, 71.68; H, 5.21; N, 16.75; S, 6.36 %.

2-Chloro-N-[4-phenyl-6-(1,4,5,6-tetrahydropyrimidin-2-yl)-2-(*p*-tolylamino)thieno[2,3-*d*]pyrimidin-5-yl]acetamide (17). A mixture of compound **13** (0.83 g, 2 mmole), and chloroacetyl chloride (0.4 ml, 3 mmole) in dioxane (20 ml) was heated on a water bath for 2h. The solid product which was obtained by pouring on dilute sodium carbonate solution was filtered off, dried and recrystallized from ethanol to give **17**: yellow crystals, 58% yield; mp 248-250 °C. IR (KBr): ν_{\max} (cm⁻¹) 3291 (3NH), 3106 (C-H aromatic), 2942 (C-H aliphatic), 1686 (CO amide); ¹H NMR (300 MHz, CDCl₃) δ ppm: 1.56 (m, 2H, CH₂), 3.32 (s, 3H, CH₃), 3.4 (t, 2H, CH₂), 3.46 (t, 2H, CH₂), 4.02 (s, 2H, COCH₂), 6.94-7.60 (m, 9H, Ar-H), 8.75 (s, 1H, NH tetrahydropyrimidine), 11.6 (s, 1H, NHCO), 12.36 (s, 1H, NH); ¹³C NMR (75 MHz, CDCl₃): δ 21.13 (C19: CH₃ *p*-toluidine), 22.86 (C11), 42.43 (C10), 45.12 (C16), 47.54 (C10), 114.41 (C4a), 115.96 (C2'', C6''), 126.02 (C3'', C5''), 131.66 (C3', C5'), 135.93 (C1''), 142.14 (C1'), 143.67 (C2', C6'), 128.75 (C4'), 131.93 (C4''), 132.33 (C6), 134.03 (C5), 150.83 (C7a), 152.99 (C2),

163.22 (C15: CO), 178.33 (C4); MS: m/z 491.21 (M^+ , 67 %). Anal. Calcd. for $C_{25}H_{23}ClN_6OS$ (491.02): C, 61.19; H, 4.68; Cl, 7.19; N, 17.11; S, 7.11. Found: C, 61.17; H, 4.70; Cl, 7.18; N, 17.12; S, 6.96 %.

2-Phenylamino-N-[4-phenyl-6-(1,4,5,6-tetrahydropyrimidin-2-yl)-2-(*p*-tolylamino)thieno[2,3-*d*]pyrimidin-5-yl]acetamide (18). A mixture of compound **17** (0.6 g, 1.22 mmol) and aniline (1 ml, 10 mmol) was gently refluxed for 15 minutes, then absolute ethanol (20 ml) was added. The solid product that formed was filtered off hot, dried and recrystallized from dioxane to afford **18**, white crystals, 76 % yield; mp 288-290 °C. IR (KBr): ν_{max} (cm^{-1}) 3400, 3380, 3290 (3NH), 3030 (C-H aromatic), 2900, 2850 (C-H aliphatic), 1682 (CONH); 1H NMR (300 MHz, DMSO- d_6) δ ppm: 1.6 (m, 2H, CH_2), 2.31 (s, 3H, CH_3), 3.40 (s, 4H, 2 CH_2), 6.55 (s, 2H, CO CH_2), 6.91-7.59 (m, 14H, Ar-H), 8.24 (s, 1H, NH tetrahydropyrimidine), 8.87 (s, 1H, NHPH), 11.12 (s, 1H, NHCO), 12.28 (s, 1H, NH); ^{13}C NMR (75.4 MHz, DMSO- d_6) δ ppm: 19.54 (C15), 21.65 (C19: CH_3 *p*-toluidine), 44.57 (C14), 46.87 (C16), 57.82 (C10:CH $_2$), 108.78 (C6), 115.84 (C2''',C6'''), 118.92 (C4a), 122.48 (C2',C6'), 123.67 (C4'''), 125.59 (C2',C6'), 127.67 (C4'), 130.54 (C3',C5'), 131.25 (C3''',C5'''), 132.68 (C3'',C5''), 133.80 (C4''), 135.54 (C1'), 137.72 (C1''), 139.65 (C5), 151.75 (C7a), 152.39 (C1'''), 159.19 (C12), 166.35 (C4), 172.26 (C9:CO), 176.55 (C2); MS: m/z 548.13 (M^+ , 100%). Anal. Calcd. for $C_{31}H_{29}N_7OS$ (547.69): C, 67.99; H, 5.34; N, 17.90; S, 5.81. Found: C, 67.97; H, 5.36; N, 17.86; S, 5.86 %.

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Supplementary Material

Tabulated data of the bactericidal and antifungal determinations of the compounds reported in this paper are given in the supplementary file, along with scanned spectral data of the compounds reported.

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