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Design, synthesis and antimicrobial screening of some new thienopyrimidines

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Abstract: Heterocyclic compounds play an important role in our life due to their biological importance in the struggle of microorganisms. Herein, a series of novel hybrid compounds of thienopyrimidine with triazine and pyrimidine scaffolds were synthesized starting from difunctionalized compound 5-amino-4-phenyl-2-(p-tolylamino)thieno[2,3-d]pyrimidine-6-carbonitrile (1). Moreover, the diazotization of compound 1 with sodium nitrite in an acidic medium gave the chloro-triazine compound 2 which was subjected to the nucleophilic substitution of chlorine atom with different nucleophiles delivered compounds 3a-5c. Furthermore, the reaction of compound 1 with carbon disulfide led to the formation of dithione derivative 6 which was alkylated with ethyl chloroacetate to give compound 7, on the other hand, the reaction of compound 1 with phenyl isothiocyanate produced 4-imino-3,9-diphenyl-7-(*p*-tolylamino)-3,4-dihydropyrimido[4',5':4,5]thieno [2,3-*d*]pyrimidine-2(1H)-thione (8), while acylation of the amino group in compound 1 with acetic anhydride gave compound 9. All synthesized compounds were characterized by elemental and spectral analysis techniques (IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy). Furthermore, the synthesized compounds were tested for their antimicrobial activity against different strains of bacteria and fungi, and the results obtained showed good to moderate activity with almost all the strains.

Keywords: Thienopyrimidne; synthesis; antibacterial; antifungal; triazine.

1. Introduction

The building of new heterocyclic compounds containing nitrogen and sulfur atoms attracts the interest of many organic chemists due to their biological importance in various aspects[1,2]. Among heterocyclic compounds containing nitrogen and sulfur, thienopyrimidines play an important role in the drug research as a result of strong biological activity they exhibit[3–5] which may be attributed to the combination of thiophene and pyrimidine moieties which lead to potential bioactive molecules as they bear structural analogs and isoelectronic relations to purines[6,7].

Moreover, fused bioactive thienopyrimidine heterocycle is a versatile lead molecule finding many applications in the pharmaceutical field exhibiting a broad spectrum of activities such as antimicrobial[8–10],

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antimalarial[11–13], antiviral[14], analgesic[15,16], antioxidant[17,18], anticancer[9,19–21], anti-inflammatory[22–24], antihypertensive [25], anti-histaminic[24], antiproliferative [26,27], anticovalent[28], antihyperlipidemic[29,30], and anticancer activity[31–34].

Thienopyrimidine derivatives are considered good antimicrobial analog from the aspect of therapeutic activity[35]. Purines and pyrimidines derivatives make up the two groups of nitrogenos bases, including the two groups of nucleotide bases. These nucleotides are DNA and RNA building blocks, respectively. They also play an important role in many metabolic processes as potential nucleic acid antimetabolites[36].

Although the antimicrobial activity of thienopyrimidines has been extensively studied, the mode of action is still challengeable, a report has been conducted to study the mechanism of activity for some selected thienopyrimidines against different strains of microorganism[37]. Due to the versatile biological activity of thienopyrimidines and in continuation to our work in this research area[38-60], we report here a simple synthetic approach for the synthesis of some new thienopyrimidnes fused to triazine and other moieties as well as the screening of their antimicrobial activity.

2. Experimental

2.1. 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 Assiut University. The results were found to be in good agreement (±0.3%) with the calculated values. IR spectra were recorded on a Pye-Unicam Sp-100 spectrophotometer using the 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 an internal standard in deuterated dimethyl sulfoxide (DMSO-d6), 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 analyse and scientific service, National Research Center, Cairo, Egypt. Analytical thin-layer chromatography (TLC) was carried out on silica gel plates (Fluka 70643-50EA. Sigma-Aldrich, Germany) using UV light. All reactions were carried out under an air atmosphere. The structures of all synthesized compounds were drowned and named using computer Chem Draw professional 13.0. Compound **1** was prepared according to literature procedure[38] and compound **2** was also synthesized according to our reported procedure[45].

2.2. Procedures for the Synthesis of Proposed Compounds

*9-Phenyl-4-phenylamino-7-(p-tolylamino)pyrimido[5',4':4,5]thieno[3,2-d] [1,2,3]triazine (**3a**)*: A mixture of compound **2** (0.25 g, 6.17 mmol) and aniline (2 mmol) was gently fused for 5 min. then ethanol (10 mL) was added. The mixture was refluxed for 4 h. The solid precipitate which formed during reflux was filtered, dried and recrystallized from ethanol as yellow crystals in 75 % yield; mp 234-236 °C. IR (KBr): νmax (cm^{-1}) 3341, 3316 (2NH), 1618 (C=N). ^1H NMR (300 MHz, DMSO-d_6): 2.33 (s, 3H, CH_3), 6.90-7.75 (m, 14H, 3ArH), 9.26 (s, 1H, NH), 9.88 (s, 1H, NH). Anal. Calcd. For: $C_{26}H_{19}N_7S$ (461.55): C, 67.66; H, 4.15; N, 21.24; S, 6.95 %. Found: C, 67.63; H, 4.18; N, 21.21; S, 6.98 %.

*9-Phenyl-4,7-di-p-tolylamino-pyrimido[5',4':4,5]thieno[3,2-d][1,2,3]triazine (**3b**)* : A mixture of compound **2** (0.25 g, 6.17 mmol) and *p*-toluidine (2 mmol) was gently fused for 5 min. then ethanol (10 mL) was added. The mixture was refluxed for 3 h. The solid precipitate which formed during reflux was filtered, dried and recrystallized from ethanol to give buff fine in 92% yield; mp 244-246 °C. IR (KBr): νmax (cm^{-1}) 3461, 3339 (2NH), 3050 (CH aromatic), 2918, 2865 (C H aliphatic), 1592 (C=N). ^1H NMR (300 MHz, $CDCl_3$): 2.32 (s, 6H, $2CH_3$), 6.90-7.75 (m, 13H, 3ArH), 9.33 (s, 1H, NH), 10.05 (s, 1H, NH). Anal. Calcd. For: $C_{27}H_{21}N_7S$ (475.57): C, 68.19; H, 4.45; N, 20.62; S, 6.74%. Found: C, 68.16; H, 4.42; N, 20.59; S, 6.71%.

*9-Phenyl-4-(piperidin-1-yl)-7-(p-tolylamino)pyrimido[5',4':4,5]thieno[3,2-d] [1,2,3]triazine (**4a**)* : A mixture of compound **2** (0.25 g, 6.17 mmol) and piperidine (2 mmol) was gently fused for 10 min. then ethanol (10 mL) was added. The mixture was refluxed for 3h. The solid precipitate which formed during reflux was filtered, dried and recrystallized from dioxane-water (2:1) as faint brown crystals in 66 % yield; mp 198-200 °C. IR (KBr): νmax (cm^{-1}) 3138 (NH), 3061, 3019(CH aromatic), 2922, 2851 (C H aliphatic), 1612 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): 1.65 (m, 2H, CH_2), 1.68 (m, 4H, $2CH_2$-CH_2), 2.33 (s, 3H, CH_3), 3.30, 3.55 (quasi d, *J*= 3.6 Hz , 4H, $2CH_2$-N), 6.90-7.65 (m, 9H, 2ArH), 10.28 (s, 1H, NH). ^{13}C NMR (75.4 MHz, $CDCl_3$ 21.2, 32.6, 37.4, 59.0, 106.2, 122.2, 127.1, 128.6, 129.6, 132.99 ,133.8, 136.5, 139.0, 141.8, 151.7, 158.6, 168.2,172.7. Anal. Calcd. For: $C_{25}H_{23}N_7S$ (453.57): C, 66.20; H, 5.11; N, 21.62; S, 7.07%. Found: C, 66.25; H, 5.14; N, 21.58; S, 7.04%.

*4-(Morpholino-1-yl)-9-phenyl-7-(p-tolylamino)pyrimido[5',4':4,5]thieno[3,2-d] [1,2,3]triazine (**4b**)* : A mixture of compound **2** (0.25 g, 6.17 mmol) and morpholine (2 mmol) was gently fused for 10 min. then ethanol (10 mL) was added. The mixture was refluxed for 4h. The solid precipitate which formed during reflux was filtered, dried and recrystallized from dioxane as brown in 56 % yield; mp 228-230 °C. IR (KBr): ν (cm^{-1}) 3328 (NH), 2976, 2921 (C H aliphatic), 1587 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): 2.32 (s, 3H, CH_3), 3.45-3.60 (quasi d, J=3.9 Hz, 4H, $2CH_2$-N), 3.78-4.08 (quasi d, 4.0 Hz, 4H, $2CH_2$-O), 6.87-7.70 (m, 9H, 2ArH), 10.37 (s, 1H, NH). Anal. Calcd. For: $C_{24}H_{21}N_7OS$ (455.54): C, 63.28; H, 4.65; N, 21.52; S, 7.04%. Found: C, 63.25; H, 4.68; N, 21.48; S, 7.08%.

*4-((9-phenyl-7-(p-tolylamino)pyrimido[5',4':4,5]thieno[3,2-d][1,2,3]triazin-4-yl)amino)benzenesulfonamide (**5a**)* : A mixture of compound **2** (0.25 g, 6.17 mmol) and sulfanilamide (2 mmol) was gently fused for 5 min. Then ethanol (10 mL) was added. The mixture was refluxed for 3 h. The resultant solid product was filtered, washed with water, dried and recrystallized from ethanol as white crystals in 76% yield; mp 138-140 °C. IR (KBr): ν (cm^{-1}) 3330, 3217, (2NH, NH_2), 3080 (CH aromatic), 2923 (CH aliphatic),1642 (C=N),1444 (SO_2NH). ^{1}H NMR (300 MHz, DMSO-d_6): 2.32 (s, 3H, CH_3), 6.89-7.85 (m, 13H, 3ArH), 8.49 (s, 2H, NH_2), 9.71 (s, 1H, NH), 10.46 (s, 1H, NH) ppm. Anal. Calcd. For: $C_{26}H_{20}N_8O_2S_2$ (540.62): C, 57.76; H, 3.73; N, 20.73; S, 11.86 %. Found: C, 57.73; H, 3.76; N, 20.71; S, 11.83 %.

*N-Carbamimidoyl-4-((9-phenyl-7-(p-tolylamino)pyrimido[5',4':4,5]thieno[3,2-d] [1,2,3]triazin-4-yl)amino)benzenesulfonamide (**5b**)* : A mixture of compound **2** (0.25 g, 6.17 mmol) and sulphaguanidine (2 mmol) was gently fused for 5 min. Then ethanol (10 mL) was added. The mixture was refluxed for 4 h. The resultant solid product was filtered, washed with water, dried and recrystallized from ethanol as white crystals in 75 % yield; mp 266-268 °C. IR (KBr): ν (cm^{-1}) 3469, 3297, 3256, 3157 (NH_2, 4NH), 2998 (C H aliphatic), 1607 (C=N), 1333 (SO_2NH). ^{1}H NMR (300 MHz, DMSO-d_6): 2.32 (s, 3H, CH_3), 2.95 (s, 1H, NH), 6.29 (s, 2H, NH_2), 6.85-7.93 (m, 13H, 3Ar-H), 8.69 (s, 1H, NH), 9.42 (s, 1H, NH), 10.17 (s, 1H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): 21.1, 116.0, 124.0, 126.22, 128.6, 129.7, 142.3, 119.8, 129.4, 131.3, 134.3, 136.6, 144.4, 147.2, 149.4, 152.3, 154.7, 155.4, 159.9, 174.7. MS (m/z): 582.25 (M+,98%). Anal. Calcd. For: $C_{27}H_{22}N_{10}O_2S_2$ (582.66): C, 55.66; H, 3.81; N, 24.04; S, 11.00. Found: 55.69; H, 3.78; N, 24.07; S, 11.04%.

*N-[7-p-tolylamino-9-phenylpyrimido[5',4':4,5]thieno[3,2-d]triazine-4-yl]-sulfathiazole (**5c**)* : To a solution of compound **2** (0.25 g, 6.17 mmol) and sulphathiazole (2 mmol) was gently fused for 5 min. Then ethanol (10 mL) was added. The mixture was refluxed for 4 h. The solid product thus formed was filtered, washed with water, dried and recrystallized from dioxane: ethanol mixture (2:1) as brown crystals in 56 % yield; mp 203-205 °C. IR (KBr): ν (cm^{-1}) 3384 (3NH), 3060 (CH aromatic), 2923, 2852 (CH aliphatic), 1593 (C=N), 1404 (SO_2NH). ^{1}H NMR (300 MHz, DMSO-d_6): 2.32 (s, 3H, CH_3), 6.00 (s, 1H, NH), 6.87, 6,89 (s, 2H, 2CH thioazole), 7.06-7.86 (m, 13H, 3ArH), 9.06 (s, 1H, NH) and 10.50 (s, 1H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): 21.4, 107.9, 114.2, 116.8, 122.9, 124.43, 127.8, 130.6, 132.4, 134.8, 136.3, 137.5, 140.6, 145.7, 147.7, 152.4, 156.8, 158.9,174.1, 177.9. MS (m/z): 623.12 (M+, 97.56 %). Anal. Calcd. For: $C_{29}H_{21}N_9O_2S_3$ (623.73): C, 55.84; H, 3.39; N, 20.21; S, 15.42 %. Found: C, 55.87; H, 3.35; N, 20.24; S, 15.39 %.

*9-Phenyl-7-(p-tolylamino)pyrimido[4',5':4,5]thieno[2,3-d]pyrimidine-2,4(1H,3H)-dithione (**6**)* : Compound **2** (1.0 g, 2.79 mmol) was dissolved in 10% alcoholic sodium hydroxide solution (5 mL), and then refluxed with excess carbon disulphide on a water bath for 8 h. During the reflux time fresh carbon disulphide was added (two times), and then the product was separated, collected by filtration, and recrystallized from acetic acid as deep red crystals in 79% yield; mp >360 °C. IR (KBr): ν (cm^{-1}) 3475, 3406 and 3313 (3NH),1643 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): 2.33 (s, 3H, CH3), 6.92-7.63 (m, 9H, 2Ar-H), 8.79 (s, 1H, NH), 9.87 (s, 1H, NH), 11.16 (s, 1H, NH). Anal. Calcd. For: $C_{21}H_{15}N_5S_3$ (433.57): C, 58.18; H, 3.49; N, 16.15; S, 22.18%. Found: C, 58.14; H, 3.45; N, 16.12; S, 22.23 %.

*Diethyl2,2'-((9-phenyl-7-(p-tolylamino)pyrimido[4',5':4,5]thieno[2,3-d]pyrimidine-2,4-diyl) bis(sulfanediyl))diacetate (**7**)* : Compound **6** (0.5 g, 1.15 mmol) was refluxed with ethyl chloroacetate (2 mL, 18 mmol) in ethanol (20 mL) in the presence of anhydrous sodium acetate (2.0 g) for 2 h. The color of the reaction mixture changed from red to brown, then the brown product was precipitated, collected by filtration, and recrystallized from dioxane-ethanol mixture (2:1) as deep brown crystals in 63 % yield; mp 198-200 °C. IR (KBr): ν (cm^{-1}) 3365 (NH), 2974, 2929 (C H aliphatic), 1671(2CO ester), 1607 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): 1.36 (t, *J*=4.7 Hz, 3H, CH_3), 1.39 (t, *J*=4.7 Hz, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.96 (s, 2H, CH_2), 4.00 (s, 2H, CH_2), 4.14 (q, *J*=4.7 Hz, 2H, CH_2), 4.19 (q, *J*=4.7 Hz, 2H, CH_2), 6.89-7.64 (m, 9H, 2Ar-H), 9.59 (s, 1H, NH). Anal. Calcd. For $C_{29}H_{27}N_5O_4S_3$ (605.75): C, 57.50; H, 4.49; N, 11.56; S, 15.88. Found: C, 57.54; H, 4.53; N, 11.52; S, 15.92 %.

*4-Imino-3,9-diphenyl-7-(p-tolylamino)-3,4-dihydropyrimido[4',5':4,5]thieno [2,3-d]pyrimidine-2(1H)-thione (**8**)* : Compound **2** (1.0 g, 2.79 mmol) was dissolved in pyridine (5 mL), phenyl isothiocyanate (0.4 mL, 30 mmol) was added to the above solution. The reaction mixture was refluxed for 6 h, and then cooled, where by reddish brown crystals were separated out, collected by filtration, dried and recrystallized from ethanol in 63 % yield; mp 198-200 °C. IR (KBr): νmax (cm-1) 3433, 3317 (3NH), 2983 (C H aliphatic), 1626 (C=N), 1212 (C=S).^{1}H NMR (300 MHz, DMSO-d_6): 2.32 (s, 3H, CH_3), 6.89-7.66 (m, 14H, 3Ar-H), 7.01 (s, 1H, NH), 9.50 (s, 1H, NH), 10.71 (s, 1H, NH). Anal. Calcd. For: $C_{27}H_{20}N_6S_2$ (492.62): C, 65.83; H, 4.09; N, 17.06; S, 13.02%. Found: C, 65.80; H, 4.05; N, 17.09; S, 13.05 %.

*5-(Diacetylamino)-(6-cyano-4-phenyl-2-(p-tolylamino)thieno[2,3-d]pyrimidine (**9**)* : Compound **2** (0.5 g, 1.39 mmol) was refluxed with acetic anhydride (5 mL) for 3 h. The reaction mixture was cooled and then poured into cold water (100 mL), and then the product was precipitated, collected and recrystallized from benzene as yellow needles in 63 % yield; mp 174-176 °C. IR (KBr): ν (cm^{-1}) 3199 (NH), 2921 (CH aliphatic), 2216 (CN), 1676 (2CO), 1577 (C=N). ^{1}H NMR (300 MHz, $CDCl_3$): 2.33 (s, 3H, CH_3), 2.37(s, 6H, 2CH_3), 6.91-7.67 (m, 9H, 2Ar-H), 10.37 (s, 1H, NH). Anal. Calcd. For $C_{24}H_{19}N_5O_2S$ (441.51): C, 65.29; H, 4.34; N, 15.86; S, 7.26%. Found: C, 65.33; H, 4.37; N, 15.89; S, 7.29 %.

2.3. Antimicrobial Activities and Minimum Inhibitory Concentration (MIC)

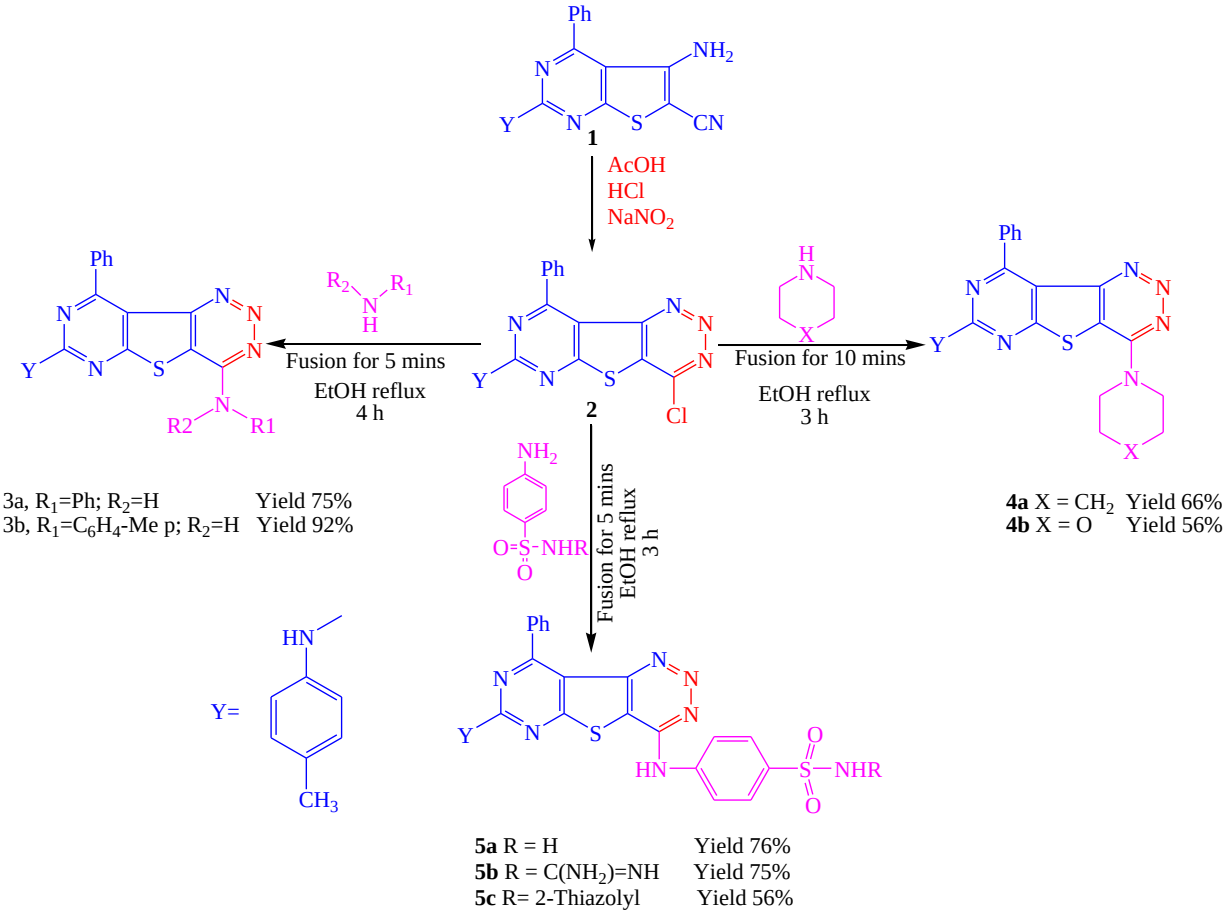
All the new synthesized compounds listed in Tables 1 and 2 were screened for their *in vitro* antimicrobial activity against model Gram-positive (*B. cereus* and *S. aureus*) and Gram-negative bacteria (*P. aruginose*, and *E. coli*). The antimicrobial activity and MIC were determined by the agar diffusion assay using the filter paper disc method[61]. The MICs of the synthesized compounds were determined against Gram-positive and Gram-negative bacteria and pathogenic fungi (*Geotrichum candidum*, *Candida albicans*, *Trichophyton rubrum*, and *Aspergillus flavus*). It was carried out by impregnation of different concentrations of synthesized compounds (50, 100, 150, 200 µg/mL) in DMSO as a solvent and placed on filter paper discs (5 mm). Nutrient agar and Sabouraud's dextrose agar media were used for the inoculation of bacteria and fungi, respectively. Standard antibiotic discs (Amoxicillin 50 mg, and fluconazole 50 mg) and blank discs (impregnated with DMSO) were used as positive and negative control. The plates were then incubated at 37 °C for 24 hr. for bacteria and 25 °C for 6 days for fungi. The zones of inhibition were measured in mm and recorded. The lowest concentration that inhibited the growth of the test organisms was recorded as the MIC. The biological activity as expressed by the growth inhibition zone

of the tested microorganism is listed in Tables 1and 2 and Figure S1 and Figure S2 in supporting information. The MICs were recorded.

3. Results and Discussion

3.1. Chemistry

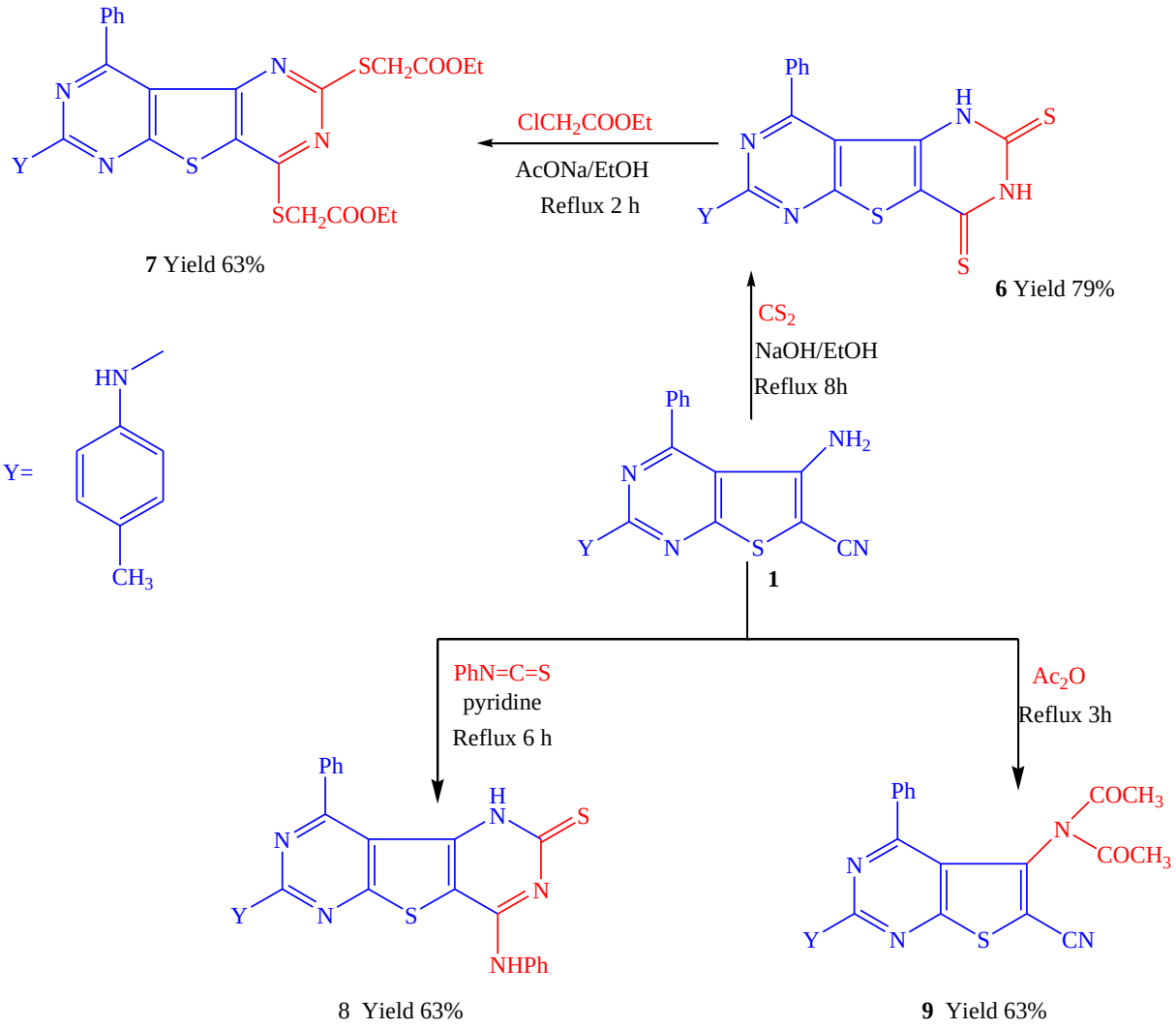
Diazotization of the bifunctionalized amino carbonitrile compound **1** with sodium nitrite solution (10 %) in the presence of a mixture of acetic acid and conc. HCl at room temperature was afforded the newly synthesized 4-chloro-9-phenyl-7-(p-tolylamino)pyrimido[5',4':4,5]thieno[3,2-d][1,2,3]triazine **(2)**. The chemical structure of compound **2** was confirmed based on the elemental and spectral data. Its IR and ^{1}H NMR spectra declared the disappearance of absorption bands at 3477, 3387, and 2187 cm^{-1} for NH_2 and CN groups in the starting material, the appearance of a new absorption band at 1600 cm^{-1} for C=N group, in addition to the disappearance of the singlet signal at δ 6.44 ppm for NH_2 in the ^{1}H NMR spectra. The chlorine atom in compound **2** underwent nucleophilic substitution reactions with various primary and secondary amines gave the corresponding *N*-substituted pyrimidothienotriazine derivatives **(3-5)**.



Scheme 1. Synthesis of triazine fused thienopyrimidine derivatives

Assignment of the chemical structures of the new compounds **(3-5)** was elucidated from their elemental and spectral analyses. The IR spectrum of compound **3a** showed two absorption bands at 3341 and 3316 cm^{-1} for NH groups. ^{1}H NMR spectrum of compound **3a** showed two singlet signals at δ 9.26 and 9.88 ppm for 2NH groups. In addition, the IR spectrum of piperidinyl compound **4a** showed new absorption bands at 3138, 3081, 2922, and 2851 cm^{-1} for NH, CH aromatic, and CH aliphatic groups respectively. ^{1}H NMR spectrum presented multiplet signals at δ 1.65-1.68 ppm for 3CH_2 aliphatic protons and triplet signals at δ 3.30 ppm characteristic of 2CH_2 aliphatic. In addition, the ^{13}C NMR spectrum of

compound **4a** showed signals at δ 32.56, 37.45, and 58.98 ppm for 5CH_2 groups respectively. Furthermore, the IR spectrum of benzenesulfonamide derivative **5c** revealed a broad absorption band at 3384 cm^{-1} for NH groups and an absorption band at 1403 cm-1 for the SO_2 group. 1H NMR spectrum revealed three singlet signals at δ 6.00, 9.60, and 10.50 ppm which attributed to NH groups. ^{13}C NMR spectrum showed the appearance of signals at δ 114.18, 137.49, and 177.87 for the carbon atom of the thiazolyl ring.



Scheme 2. Synthesis of pyrimidothienopyrimidine derivatives

Reaction of 5-amino-4-phenyl-2-(p-tolylamino)thieno[2,3-d]pyrimidine-6-carbonitrile **(1)** with carbon disulfide in pyridine on a steam bath yielded the corresponding 9-phenyl-7-(*p*-tolylamino)pyrimido[4',5':4,5]thieno[2,3-*d]*pyrimidine-2,4(1H, 3H)-dithione **(6)** which on subsequent alkylation with ethylchloro acetate afforded 2,4-bis(ethylacetatemercapto)-9-phenyl-7-(*p*-tolylamino)pyrimido[4',5':4,5]thieno[2,3-*d*]pyrimidine **(7)**. The chemical structures of compounds **(6, 7)** were elucidated by the elemental and the spectral analyses, the IR spectrum of the dithione compound **6** showed absorption bands at 3475, 3406, 3313, and 1176 cm^{-1} characteristic of NH and C=S groups respectively. IR spectrum of compound **7** revealed the appearance of absorption bands at 3365 and 1671 cm^{-1} characteristic of NH and CO ester groups respectively. In addition to the ^1H NMR spectrum of compound **6** stated the presence of three singlet signals at δ 8.79, 9.87, and 11.16 ppm characteristic of NH groups. The ^1H NMR spectrum of compound **7** showed triplet and quartet signals characteristic of CH_3 and CH_2 groups of ester at δ 1.36,1.39,4.14 and 4.19 ppm. In addition, the presence of two singlet signals at δ 3.96 and 4.00 ppm for 2CH_2 groups. Compound **1** was reacted with phenyl isothiocyanate in pyridine to

afford 9-phenyl-4-(phenylamino)-7-(*p*-tolylamino)pyrimido[4',5':4,5]thieno [2,3-*d*] pyrimidine-2(1H)-thione **(8)**. In addition, reaction of compound **1** with acetic anhydride afford 5-diacetylamino-4-phenyl-2-(*p*-tolylamino)thieno[2,3-*d*]pyrimidin-6-carbonitrile **(9)**. The chemical structures of compounds **8,** and **9** were elucidated by the elemental and spectral analyses, The IR spectrum of the iminothione compound **8** showed absorption bands at 3433, 3317, 1626, and 1212 cm^{-1} characteristic of NH, C=N, and C=S groups respectively. The IR spectrum of compound **9** revealed the presence of absorption bands at 3199, 2216, and 1676 cm^{-1} characteristic of NH, CN, and CO acetyl groups respectively. In addition, the ^1H NMR spectrum of compound **8** stated the presence of three singlet signals at δ 7.01, 9.50, and 10.74 ppm characteristic of NH groups and multiple signals at δ 6.89-7.66 ppm for 14 aromatic protons of 3 phenyl rings. ^1H NMR spectrum of compound **9** showed two singlet signals characteristic of two CH_3 groups at δ 2.33 and 2.37 ppm. In addition, the appearance of the singlet signal at δ 10.37 ppm for NH groups.

3.2. Antimicrobial and Antifungal Activities

3.2.1. Antibacterial Activity

From the data presented in Table 1, it can be concluded that all the investigated compounds showed remarkable antibacterial activity against Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aruginose*, *Escherichia coli*). From the inhibition zone values, compounds **3b, 4b, 5c, 6,** and **7** showed the highest antibacterial activity against almost all strains of bacteria, with values close to those of the corresponding reference antibiotics (Nitrofurantoin).

Table 1. Antibacterial activity of compounds (1-9)

No	*B. cereus*	*S. aureus*	*P. aruginose*	*E. coli*
1	12[a](9.0)[b]	14(8.0)	12(9.0)	13(11.0)
2	17(9.0)	16(8.0)	17(8.0)	17(8.0)
3a	18(8.0)	17(7.0)	19(8.0)	18(8.0)
3b	20(0.7)	19(7.0)	18(8.0)	19(7.0)
4a	16(8.0)	13(8.0)	18(7.0)	18(8.0)
4b	20(0.7)	19(8.0)	13(9.0)	14(8.0)
5a	15(9.0)	11(10.0)	-	12(9.0)
5b	17(9.0)	16(9.0)	19(8.0)	15(8.0)
5c	19(8.0)	16(8.0)	14(7.0)	17(8.0)
6	19(8.0)	17(9.0)	19(7.0)	19(8.0)
7	20(0.7)	15(8.0)	18(7.0)	19(8.0)
8	16(0.8)	13(9.0)	-	14(8.0)
9	-	14(8.0)	16(8.0)	15(8.0)
Nitrofurantoin	26(5.0)	25(4.0)	24(3.0)	20(0.5)

(a) Numbers out parentheses represent the dimer of inhibition zone in (mm) of compounds **1-9**
(b) Numbers in parentheses represent the MIC (minimum inhibition concentration) in (µg/mL) of tested compounds

While compounds **5a** and **9** showed the lowest antibacterial activity among all the tested compounds which suggested that the SO_2NH_2 group in **5a** has a low effect on the bacteria strains, unlike compound **5c** that exhibited strong activity which may be attributed to the existence of thiazolylsulfonyl moiety. Moreover, compounds **3b, 4b,** and **7** displayed excellent activity against *Bacillus cereus*, while the inhibition of *Staphylococcus aureus* was achieved by compounds **3b** and **4b**. Furthermore, compounds **2** and **6** demonstrated promising efficiency towards both gram-negative and gram-positive bacteria. In conclusion, the data obtained showed that the compound 9-phenyl-4,7-di-*p*-tolylamino-pyrimido[5',4':4,5]thieno[3,2-*d*][1,2,3]triazine **(3b)** containing pyrimidothienotriazine moiety with *p*-tolyl substituent and compound diethyl 2,2'-((9-phenyl-7-(*p*-tolylamino) pyrimido [4',5':4,5]thieno[2,3-*d*]pyrimidine-2,4diyl)bis(sulfanediyl))diacetate **(7)** which has pyrimidothienopyrimidne moiety with two ethyl acetate substituent exhibited the highest antibacterial activity against all bacteria strains. To our

delight that all the synthesized compounds showed antimicrobial activity higher than that of starting material which supports the utilization of this synthetic study.

3.2.2. Antifungal Activity

The antifungal activity results for all synthesized compounds against four fungal species are summarized in Table 2, the table shows that all the investigated compounds demonstrated remarkable antifungal activities against all the fungi (*Geotrichum candidum, Candida albicans, Trichophyton rubrum,* and *Aspergillus flavus*), the data showed that the compounds 9-phenyl-4,7-di-p-tolylamino-pyrimido[5',4':4,5]thieno[3,2-*d*][1,2,3]triazine **(3b)** and 9-phenyl-7-(ptolylamino)pyrimido[4',5':4,5] thieno[2,3-*d*]pyrimidine-2,4(1H,3H)-dithione **(6)**, have the highest antifungal activity against all the strains of fungi. From **Tables 1** and **2**, it could be stated that compounds **3b** and **6** have antimicrobial activity against different strains of gram-positive, gram-negative bacteria, and fungi with values close to those of the corresponding standers. Furthermore, all the investigated compounds showed good activity higher than that of the precursor which suggests the importance of this study and reflects the effect of derivatization on the enhancement of biological activity.

Table 2. Antifungal activity of compounds (**1-9**)

No	*Geotrichum candidium*	*Candida albicans*	*Trichophyton rubrum*	*Aspergillus flavus*
1	12[a](11.0)[b]	13(10.0)	14(9.0)	14(10.0)
2	14(10.0)	13(8.0)	13(9.0)	17(10.0)
3a	16(8.0)	16(8.0)	16(7.0)	18(9.0)
3b	18(8.0)	17(8.0)	22(7.0)	19(9.0)
4a	15(7.0)	17(8.0)	17(8.0)	16(8.0)
4b	12(8.0)	14(8.0)	15(9.0)	14(9.0)
5a	14(8.0)	17(7.0)	15(9.0)	12(9.0)
5b	12(7.0)	-	14(9.0)	17(8.0)
5c	15(7.0)	13(8.0)	11(8.0)	14(8.0)
6	18(7.0)	19(8.0)	17(9.0)	18(9.0)
7	-	13(8.0)	15(9.0)	16(8.0)
8	15(10.0)	11(7.0)	17(9.0)	-
9	17(10.0)	16(7.0)	10(9.0)	17(9.0)
Clotrimazole	20(4.0)	23(5.0)	28(4.0)	22(5.0)

a) Numbers out parentheses represent the dimer of inhibition zone in (mm) of compounds 1-9
(b) Numbers in parentheses represent the MIC (minimum inhibition concentration) in (µg/mL) of tested compounds

4. Conclusion

To sum up, in this work we have successfully synthesized a series of new potent heterocyclic compounds containing thienopyrimidine moiety, different spectral analysis techniques have utilized to confirm the structures of the designed products. All compounds were *in vitro* investigated for their antibacterial as well as antifungal activities against different strains of bacteria and fungi showing moderate to high activity and the obtained MIC values and inhibition zones for some synthesized derivatives were shown values close to the used reference drug. In the future, we will focus on the development of different series of thienopyrimidne for the investigation of other pharmaceutical activities such as anticancer and antitumor.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/organic-communications

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