
Vikunjana K. Akbari, Pragnesh D. Patel and Keshav C. Patel*

Department of Chemistry, Veer Narmad South Gujarat University, Surat-395007, Gujarat, India.

*Corres. author: akbarivk@yahoo.co.in
Phone no: (+91) 9979735497

Abstract: Several new thieno[2,3-d]pyrimidine derivatives 2-([3-chloro-2-(substitutedphenyl)-4-oxoazetidin-1-yl]amino)-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide 6(a-j), 4-[(2E)-(2-substitutedbenzylidene)hydrazinyl]2-[(2Z)-2-(2,4-dichlorobenzylidene)hydrazinyl] thieno[2,3-d]pyrimidine 9(a-j) and 3-chloro-4-(substituted phenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one 14(a-j) were synthesized starting from 2-aminothiophene-3-carbonitrile 1. The characterization of the newly synthesized compounds was established by IR, 1H NMR, 13C NMR and Elemental analysis. The final compounds were screened for their antibacterial activity against Staphylococcus aureus and Streptococcus pyogenes from Gram positive group of bacteria and Escherichia coli and Pseudomonas aeruginosa from Gram negative group of bacteria and antifungal activity against Aspergillus niger.

Key words: Thieno[2,3-d]pyrimidine, antimicrobial activity, Schiff's base, azetidinone.

INTRODUCTION

Thienopyrimidine derivatives, which are structure analogues of purines, have been focus of great interest because of their large range of pharmacological activities1 as antibacterial,2-3 antifungal,4 analgesic,5-7 antipyretic,8,9 antiinflammatory,10 antihistaminic,11,12 anticancer,13 radioprotective.14,15 Many thieno[2,3-d]pyrimidine derivatives were reported as phosphodiesterase inhibitors,16 also exhibited good H1 receptor antagonistic activities,17 4-amino derivatives showed insecticidal, pesticidal and acaricidal activities.18 Numerous thieno[2,3-d]pyrimidines have been proved to use in case of cerebral ischemia, malaria, tuberculosis, Alzheimer’s and Parkinson’s diseases.19 This work aimed to synthesize some new thieno[2,3-d]pyrimidine derivatives starting with 2-aminothiophene-3-carbonitrile and to evaluate their biological activities.

EXPERIMENTAL

General Procedures:

Melting points were determined by open capillary method and are uncorrected. The structures of the compounds were confirmed by 1H and 13C nuclear magnetic resonance and Fourier transform infrared. 1H NMR spectra were recorded with Bruker Avance II 400 MHz NMR spectrometer at SAIF, Chandigarh, in CDCl3 or DMSO-d6 using TMS as internal standard and chemical shifts are expressed in δ ppm. 13C NMR spectra of the compounds were recorded with a Bruker Avance I 400 MHz NMR spectrometer at SAIF (Sophisticated Analytical Instrument Facilities), Chandigarh. The IR spectra were recorded with a Thermo Scientific Nicolet iS10 FTIR spectrophotometer at the Department of Chemistry, Veer Narmad South Gujarat University. Elemental analysis (C, H, N) were performed on Thermo Scientific Flash 2000 at G.N.F.C.
The progress of reactions and the purity of synthesized compounds were checked by TLC on E-Merck precoated 60 F<sub>254</sub> plates and the spots were examined under short-wave UV light. The synthesis of 2-[[3-chloro-2-(substitutedphenyl)-4-oxazetidin-1-yl]amino]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide 6(a-j) are shown in Scheme 1, 4-[(2E)-2-(substitutedbenzylidene)hydrazinyl]-2-[(2Z)-2-(2,4-dichlorobenzylidene) hydrazinyl] thieno[2,3-d]pyrimidine 9(a-j) are showm in Scheme 2 and 3-chloro-4-(substitutedphenyl)-1-(thieno[2,3-d]pyrimidin-4-yl)amino)azetidin-2-one 14(a-j) are shown in Scheme 3.
Scheme 2

Scheme 3
**Thieno[2,3-d]pyrimidin-4-amine (2)**

A mixture of compound 1 (0.01 mol) and formamide (10 mL) was heated under reflux for 4 h. After cooling, the reaction mixture was poured into ice cold water. The separated product was collected by filtration, dried and recrystallised from dioxane to give compound 2 (0.95g, 63.00%); mp 214-219°C. IR spectrum (KBr, ν, cm⁻¹): 3420-3313 (NH₂); ¹H NMR spectrum (CDCl₃, δ ppm): 7.49 (d, 1H, CH), 7.71 (d, 1H, CH), 6.85 (s, 2H, NH₂), 8.47 (s, 1H, CH). Anal. calcd. for C₇H₅N₂: C, 77.61; H, 4.63; N, 18.76; found: C, 77.42; H, 4.78; N, 18.65.

**2-Chloro-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (3)**

To a solution of compound 2 (0.01 mol) in chloroform (10 mL) and chloroacetyl chloride (0.01 mol) were refluxed in presence of K₂CO₃ (0.01 mol) for 9-10 h. The solvent was distilled on vacuum and residue was treated with 5% NaHCO₃ (10 mL) and then with water (15 mL). The product was collected, dried and recrystallised from methanol to furnish compound 3 (1.64g, 72.00%); mp 243-248°C. IR spectrum (KBr, ν, cm⁻¹): 3435 (NH), 1636 (CONH); ¹H NMR spectrum (CDCl₃, δ ppm): 4.31 (s, 2H, CH₂), 7.50 (d, 1H, CH), 7.70 (d, 1H, CH), 7.98 (s, 1H, CONH), 8.45 (s, 1H, CH). Anal. calcd. for C₇H₇ClN₂O: C, 55.67; H, 3.76; N, 18.76; S, 9.31; found: C, 55.48; H, 3.80; N, 18.69.

**2-Hydrazinyl-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (4)**

A mixture of compound 3 (0.01 mol) and hydrazine hydrate (99%) (5 mL) in methanol (10 mL) was refluxed for 8 h and then held for ~16h at room temperature. The separated product was filtered off, dried and recrystallised from ethanol to give compound 4 (1.49g, 67.00%); mp 177-182°C. IR spectrum (KBr, ν, cm⁻¹): 3455 (NH), 3350 (NHNH₂), 2888 (CH₂), 1648 (CONH); ¹H NMR (CDCl₃, δ ppm): 2.01 (brs, 2H, NH₂, D₂O exchangeable), 2.21 (brs, 1H, NH, D₂O exchangeable), 3.60 (s, 2H, CH₂), 7.32 (d, 1H, CH), 7.73 (d, 1H, CH), 8.01 (s, 1H, CONH), 8.51 (s, 1H, CH). Anal. calcd. for C₇H₇N₃O₂S: C, 56.29; H, 4.43; N, 31.89; found: C, 56.32; H, 4.38; N, 31.86.

**Synthesis of compounds 5(a-j)**

A solution of compound 4 (0.005 mol) in methanol (10 mL) and appropriate aromatic aldehyde (0.005 mol) containing 2-3 drops of glacial acetic acid, was boiled under reflux on a water bath for 8-9 h. The product that separated on cooling was filtered off, dried and recrystallised from ethanol to give 5(a-j).

2-[2-(2,4-dichlorobenzylidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5a)

Yield: 2.47g (65%); mp 219-224°C; IR spectrum (KBr, ν, cm⁻¹): 3265 (NH), 1648 (CONH), 1548 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 2.05 (s, 1H, NH), 3.54 (s, 2H, CH₂), 7.28-7.40 (m, 3H, Ar-H), 7.51 (d, 1H, CH), 7.77 (d, 1H, CH), 7.94 (s, 1H, CH), 8.2 (s, 1H, N=CH), 8.52 (s, 1H, CONH). Anal. calcd. for C₁₄H₁₁Cl₂N₄O₂S: C, 47.38; H, 2.92; N, 18.42; S, 8.43; found: C, 47.31; H, 3.01; N, 18.36; S, 8.39.

2-[2-(3,4-dihydroxybenzylidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5b)

Yield: 2.44g (71%); mp 197-202°C; IR spectrum (KBr, ν, cm⁻¹): 3495 (OH), 3264 (NH), 1651 (CONH), 1554 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 2.10 (s, 1H, NH), 3.56 (s, 2H, CH₂), 6.40-6.64 (m, 3H, Ar-H), 7.50 (d, 1H, CH), 7.76 (d, 1H, CH), 7.96 (s, 1H, N=CH), 8.18 (s, 1H, CH), 8.45 (s, 1H, CONH), 9.13, 9.39 (2brs, 2H, 2OH). Anal. calcd. for C₁₄H₁₂N₃O₂S: C, 52.47; H, 3.82; N, 20.40; S, 9.34; found: C, 52.39; H, 3.92; N, 20.46; S, 9.43.

2-[2-(3-methoxybenzylidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5c)

Yield: 2.39g (70%); mp 214-219°C; IR spectrum (KBr, ν, cm⁻¹): 3277 (NH), 1649 (CONH), 1551 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 2.12 (s, 1H, NH), 3.54 (s, 2H, CH₂), 3.85 (s, 3H, OCH₃), 6.65-7.09 (m, 4H, Ar-H), 7.56 (d, 1H, CH), 7.72 (d, 1H, CH), 7.96 (s, 1H, N=CH), 8.21 (s, 1H, CH), 8.51 (s, 1H, CONH). Anal. calcd. for C₁₄H₁₂N₂O₃S: C, 56.29; H, 4.43; N, 20.51; S, 9.39; found: C, 56.32; H, 4.38; N, 20.60; S, 9.51.

2-[2-(4-chlorobenzylidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5d)

Yield: 2.35g (68%); mp 229-234°C; IR spectrum (KBr, ν, cm⁻¹): 3262 (NH), 1645 (CONH), 1551 (N=CH); ¹H NMR (CDCl₃, δ ppm): 2.05 (s, 1H, NH), 3.52 (s, 2H, CH₂), 7.18-7.40 (m, 4H, Ar-H), 7.55 (d, 1H, CH), 7.75 (d, 1H, CH), 7.89 (s, 1H, N=CH), 8.10 (s, 1H, CH), 8.50 (s, 1H, CONH). Anal. calcd. for C₁₄H₁₂ClN₂O₂S: C, 52.10; H, 3.50; N, 20.25; S, 9.27; found: C, 52.20; H, 3.55; N, 20.18; S, 9.32.

2-[2-(4-fluorobenzylidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5e)

Yield: 2.17g (66%); mp 270-275°C; IR spectrum (KBr, ν, cm⁻¹): 3271 (NH), 1639 (CONH), 1550 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 2.11 (s, 1H, NH), 3.59 (s, 2H, CH₂), 7.07-7.13 (m, 4H, Ar-H), 7.50 (d, 1H, CH), 7.71 (d, 1H, CH), 7.94 (s, 1H, N=CH), 8.18 (s, 1H, CH), 8.47 (s, 1H, CONH).
2-[(2E)-2-(4-hydroxybenzimidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5f)

Yield: 2.39g (73%); mp 256-261 °C; IR spectrum (KBr, v cm⁻¹): 3489 (OH), 3263 (NH), 6.70-7.12 (m, 4H, Ar-H), 7.51 (d, 1H, CH), 7.73 (d, 1H, CH), 7.92 (s, 1H, N=CH), 8.14 (s, 1H, CH), 8.52 (s, 1H, CONH), 9.75 (s, 1H, OH). Anal. calcld. for C₁₉H₁₄N₄O₃S: C, 55.03; H, 4.00; N, 21.39; S, 9.79; found: C, 55.08; H, 4.10; N, 21.31; S, 9.86.

Synthesis of compounds 6(a-j)

Chloroacetyl chloride (0.005 mol) was added drop wise to a solution of Schiff’s base 5(a-j) (0.005 mol) and triethylamine (2-3 drops) in dry dioxane (10 mL) at 5-10°C. The reaction mixture was stirred for 3 hrs and then refluxed for 4-5 h. On cooling, the precipitate was obtained which was filtered, dried and recrystallised from dimethylformamide to produce 6(a-j).

2-[(3-chloro-2-(2,4-dichlorophenyl)-4-oxoazetidin-1-yl)amino]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (6a)

Yield: 3.06g (67%); mp 213-218 °C; IR spectrum (KBr, v cm⁻¹): 3259 (NH), 1657 (CONH), 1751 (CO, β-lactum); ¹H NMR (DMSO-d₆, δ ppm): 2.02 (s, 2H, CH₂), 3.45 (s, 1H, NH), 3.59 (s, 2H, CH₂), 7.00-7.35 (m, 4H, Ar-H), 7.59 (d, 1H, CH), 7.80 (d, 1H, CH), 7.90 (s, 1H, N=CH), 8.08 (s, 1H, CH), 8.42 (s, 1H, CONH). Anal. calcld. for C₁₇H₁₂Cl₂N₂O₃S: C, 58.29; H, 4.43; N, 20.51; S, 9.39; found: C, 58.36; H, 4.36; N, 20.45; S, 9.31

2-[(2E)-2-(4-methoxybenzimidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5g)

Yield: 2.28g (67%); mp 211-216 °C; IR spectrum (KBr, v cm⁻¹): 3269 (NH), 1643 (CONH), 1548 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 2.14 (s, 1H, NH), 3.57 (s, 2H, CH₂), 3.80 (s, 3H, OCH₃), 6.70-6.84 (m, 4H, Ar-H), 7.54 (d, 1H, CH), 7.76 (d, 1H, CH), 7.96 (s, 1H, N=CH), 8.20 (s, 1H, CH), 8.51 (s, 1H, CONH). Anal. calcld. for C₁₉H₁₄N₂O₃S: C, 56.29; H, 4.43; N, 20.51; S, 9.39; found: C, 56.36; H, 4.36; N, 20.45; S, 9.31

2-[(2E)-2-(4-methylbenzimidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5h)

Yield: 2.34g (72%); mp 262-267 °C; IR spectrum (KBr, v cm⁻¹): 3259 (NH), 1654 (CONH), 1544 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 2.09 (s, 1H, NH), 2.33 (s, 3H, CH₃), 3.57 (s, 2H, CH₂), 7.00-7.35 (m, 4H, Ar-H), 7.59 (d, 1H, CH), 7.80 (d, 1H, CH), 7.90 (s, 1H, N=CH), 8.08 (s, 1H, CH), 8.49 (s, 1H, CONH). Anal. calcld. for C₁₇H₁₄N₂O₃S: C, 59.06; H, 4.65; N, 21.52; S, 9.85; found: C, 59.16; H, 4.60; N, 21.49; S, 9.82.

2-[(2E)-2-(4-nitrobenzimidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5i)

Yield: 2.31g (65%); mp 259-264 °C; IR spectrum (KBr, v cm⁻¹): 3263 (NH), 1651 (CONH), 1549 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 2.04 (s, 1H, NH), 3.52 (s, 2H, CH₂), 7.07-7.45 (m, 4H, Ar-H), 7.51 (d, 1H, CH), 7.77 (d, 1H, CH), 7.91 (s, 1H, N=CH), 8.19 (s, 1H, CH), 8.44 (s, 1H, CONH). Anal. calcld. for C₁₇H₁₄N₂O₃S: C, 50.56; H, 3.39; N, 23.58; S, 9.00; found: C, C, 50.45; H, 3.45; N, 23.52; S, 9.06

2-[(2E)-2-(4-butylbenzimidene)hydrazinyl]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (5j)

Yield: 2.24g (61%); mp 271-276 °C; IR spectrum (KBr, v cm⁻¹): 3265 (NH), 1656 (CONH), 1543 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 1.01 (t, 3H, CH₃), 1.26-1.45 (m, 6H, 3CH₂), 2.10 (s, 1H, NH), 3.53 (s, 2H, CH₂), 7.07-7.45 (m, 4H, Ar-H), 7.55 (d, 1H, CH), 7.75 (d, 1H, CH), 7.97 (s, 1H, N=CH), 8.18 (s, 1H, CH), 8.49 (s, 1H, CONH). Anal. calcld. for C₁₉H₁₄N₂O₃S: C, 62.10; H, 5.76; N, 19.06; S, 8.73; found: C, .18; H, 5.70; N, 19.16; S, 8.69.

2-[[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl]-amino]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (6d)

Yield: 3.08g (73%); mp 229-234 °C; IR spectrum (KBr, v, cm⁻¹): 3252 (NH), 1656 (CONH), 1751 (CO, β-lactum); ¹H NMR (DMSO-d₆, δ ppm): 3.10 (s, 1H, NH), 3.53 (s, 2H, CH₂), 3.83 (s, 3H, OCH₃), 5.09 (d, 1H, CH-Ar), 5.45 (d, 1H, CH-Cl), 7.32-7.48 (m, 4H, Ar-H), 7.50 (d, 1H, CH), 7.73 (d, 1H, CH), 8.02 (s, 1H, CH), 8.52 (s, 1H, CONH); ¹³C NMR (DMSO-d₆, δ ppm): 53.1 (CH₂), 62.9 (CH), 63.3 (CH-Cl), 110.6-170.8 (thienopyrimidine aromatic carbon atoms), 163.3 (CO, β-lactam), 168.7 (CONH). Anal. calcld. for C₂₃H₁₉ClN₄O₄S: C, 51.74; H, 3.86; N, 16.76; S, 7.67; found: C, 51.68; H, 3.81; N, 16.71; S, 7.72.

2-[[3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl]-amino]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (6e)

Yield: 2.72g (67%); mp 214-219 °C; IR spectrum (KBr, v, cm⁻¹): 3252 (NH), 1656 (CONH), 1756 (CO, β-lactum); ¹H NMR (DMSO-d₆, δ ppm): 3.10 (s, 1H, NH), 3.55 (s, 2H, CH₂), 5.12 (d, 1H, CH-Ar), 5.48 (d, 1H, CH-Cl), 7.13-7.43 (m, 4H, Ar-H), 7.59 (d, 1H, CH), 7.80 (d, 1H, CH), 8.09 (s, 1H, CH), 8.47 (s, 1H, CONH); ¹³C NMR (DMSO-d₆, δ ppm): 53.7 (CH₂), 62.5 (CH), 63.2 (CH-Cl), 110.6-170.5 (thienopyrimidine aromatic carbon atoms), 164.3 (CO, β-lactam), 168.8 (CONH). Anal. calcld. for C₁₇H₁₂FClN₄O₄S: C, 50.31; H, 3.23; N, 17.26; S, 7.90; found: C, 50.37; H, 3.28; N, 17.16; S, 7.95.

2-[[3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl]-amino]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (6f)

Yield: 2.42g (60%); mp 204-209 °C; IR spectrum (KBr, v, cm⁻¹): 3291 (OH), 3246 (NH), 1659 (CONH), 1760 (CO, β-lactum); ¹H NMR (DMSO-d₆, δ ppm): 3.15 (s, 1H, NH), 3.58 (s, 2H, CH₂), 4.93 (s, 1H, OH), 5.16 (d, 1H, CH-Ar), 5.46 (d, 1H, CH-Cl), 7.08-7.41 (m, 4H, Ar-H), 7.52 (d, 1H, CH), 7.74 (d, 1H, CH), 8.04 (s, 1H, CH), 8.51 (s, 1H, CONH); ¹³C NMR (DMSO-d₆, δ ppm): 53.8 (CH₂), 62.4 (CH), 63.6 (CH-Cl), 110.3-170.6 (thienopyrimidine aromatic carbon atoms), 164.4 (CO, β-lactam), 168.4 (CONH). Anal. calcld. for C₁₇H₁₂ClN₄O₄S: C, 50.56; H, 3.49; N, 17.34; S, 7.94; found: C, 50.50; H, 3.54; N, 17.29; S, 7.90

2-[[3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl]-amino]-N-thieno[2,3-d]pyrimidin-4-yl)acetamide (6g)

Yield: 3.00g (72%); mp 244-249 °C; IR spectrum (KBr, v, cm⁻¹): 3259 (NH), 1657 (CONH), 1751 (CO, β-lactum); ¹H NMR (DMSO-d₆, δ ppm): 3.08 (s, 1H, NH), 3.59 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃), 5.11 (d, 1H, CH-Ar), 5.50 (d, 1H, CH-Cl), 7.30-7.51 (m, 4H, Ar-H), 7.76 (d, 1H, CH), 8.12 (s, 1H, CH), 8.54 (s, 1H, CONH); ¹³C NMR (DMSO-d₆, δ ppm): 53.5 (CH₂), 62.7 (CH), 63.9 (CH-Cl), 110.2-170.8 (thienopyrimidine aromatic carbon atoms), 163.7 (CO, β-lactam), 168.5 (CONH). Anal. calcld. for C₁₇H₁₂O₂N₄S: C, 51.74; H, 3.86; N, 16.76; S, 7.67; found: C, 51.69; H, 3.79; N, 16.82; S, 7.70.

2-[[3-chloro-2-(4-methylphenyl)-4-oxoazetidin-1-yl]-amino]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (6h)

Yield: 2.77g (69%); mp 250-255 °C; IR spectrum (KBr, v, cm⁻¹): 3252 (NH), 1658 (CONH), 1745 (CO, β-lactum); ¹H NMR (DMSO-d₆, δ ppm): 2.34 (s, 3H, CH₃), 3.01 (s, 1H, NH), 3.56 (s, 2H, CH₂), 5.24 (d, 1H, CH-Ar), 5.49 (d, 1H, CH-Cl), 7.25-7.44 (m, 4H, Ar-H), 7.54 (d, 1H, CH), 7.75 (d, 1H, CH), 8.05 (s, 1H, CH), 8.53 (s, 1H, CONH); ¹³C NMR (DMSO-d₆, δ ppm): 53.1 (CH₂), 62.8 (CH), 63.3 (CH-Cl), 110.9-170.5 (thienopyrimidine aromatic carbon atoms), 163.8 (CO, β-lactam), 168.8 (CONH). Anal. calcld. for C₁₇H₁₂ClN₄O₂S: C, 53.80; H, 4.01; N, 17.43; S, 7.98; found: C, 53.86; H, 4.09; N, 17.34; S, 7.91

2-[[3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl]-amino]-N-(thieno[2,3-d]pyrimidin-4-yl)acetamide (6i)

Yield: 3.07g (71%); mp 263-268 °C; IR spectrum (KBr, v, cm⁻¹): 3257 (NH), 1651 (CONH), 1753 (CO, β-lactum); ¹H NMR (DMSO-d₆, δ ppm): 3.09 (s, 1H, NH), 3.57 (s, 2H, CH₂), 5.21 (d, 1H, CH-Ar), 5.52 (d, 1H, CH-Cl), 7.12-7.49 (m, 4H, Ar-H), 7.53 (d, 1H, CH), 7.75 (d, 1H, CH), 8.01 (s, 1H, CH), 8.50 (s, 1H, CONH); ¹³C NMR (DMSO-d₆, δ ppm): 53.3 (CH₂), 62.6 (CH), 63.7 (CH-Cl), 110.4-170.3 (thienopyrimidine aromatic carbon atoms), 164.2 (CO, β-lactam), 168.3 (CONH). Anal. calcld. for C₁₇H₁₂ClN₄O₄S: C, 47.17; H, 3.03; N, 19.42; S, 7.41; found: C, 47.09; H, 3.11; N, 19.37; S, 7.48.
2-((3-chloro-2-(4-butylphenyl)-4-oxoacetimidoyl)amino)-N-(thieno[2,3-d]pyrimidin-4-y1)acetamide (6)

Yield: 3.20 g (72%); mp 297-302 °C; IR spectrum (KBr, ν, cm⁻¹): 3262 (NH), 1656 (CONH), 1758 (CO, β-lactum); ¹H NMR (DMSO-d₆, δ ppm): 1.05 (t, 3H, CH₃), 1.31-1.72 (m, 6H, 3CH₂), 3.04 (s, 1H, NH), 3.60 (s, 2H, CH₂), 5.19 (d, 1H, CH-AR), 5.45 (d, 1H, CH-Cl), 6.61-6.77 (m, 4H, Ar-H), 7.50 (d, 1H, CH), 7.72 (d, 1H, CH), 8.06 (s, 1H, CH), 8.54 (s, 1H, CONH); ¹³C NMR (DMSO-d₆, δ ppm): 53.6 (CH₂), 62.4 (CH), 63.1 (CH-Cl), 110.1-170.4 (thienopyrimidine aromatic carbon atoms), 163.9 (CO, β-lactum), 168.9 (CONH). Anal. calcd. for C₂₃H₂₅ClN₅O: C, 56.81; H, 4.99; N, 15.78; S, 7.22; found: C, 56.87; H, 5.05; N, 15.72; S, 7.18.

**Thieno[2,3-d]pyrimidine-2,4(1H,3H)-dithione (7)**

A mixture of compound 1 (0.01 mol) and carbon disulphide (0.01 mol) in pyridine (10 mL) were heated under reflux for 8 h. After completion of reaction, the reaction mixture was cooled at room temperature then poured into ice cold water (50 mL) and neutralized with hydrochloric acid. The separated product was collected by filtration, washed with water, dried and recrystallised from ethanol to give compound 7. Yield: 1.28 g (64%); mp 198-203°C; IR spectrum (KBr, ν, cm⁻¹): 3342, 3215 (2NH), 1340 (2C=S); ¹H NMR (CDCl₃, δ ppm): 7.50 (d, 1H, CH), 7.76 (d, 1H, CH), 8.76 (brs, 1H, NH, D₂O exchangeable), 12.45 (brs, 1H, NH, D₂O exchangeable). Anal. calcd. for C₁₃H₁₁N₂S₂: C, 55.98; H, 1.99; N, 13.98; S, 48.02; found: C, 36.09; H, 2.17; N, 13.88; S, 47.91.

2,4-dihydrazinylthieno[2,3-d]pyrimidine (8)

A solution of compound 7 (0.01 mol) in ethanol (20 mL) was treated with hydrazine hydrate (99%) (20 mL) and refluxed on water bath for 8 h and then held for ~16 h at room temperature. The separated product was filtered off, dried and recrystallised from dioxane to give compound 8. Yield: 1.37 g (70%); mp 173-178°C; IR spectrum (KBr, ν, cm⁻¹): 3434-3243 (2NH₂, 2NH); ¹NMR (CDCl₃, δ ppm): 4.17 (brs, 2H, NH₂, D₂O exchangeable), 4.66 (brs, 2H, NH₂, D₂O exchangeable), 5.73 (brs, 1H, NH, D₂O exchangeable), 6.52 (brs, 2H, NH, D₂O exchangeable), 7.48 (d, 1H, CH), 7.72 (d, 1H, CH). Anal. calcd. for C₁₃H₁₁N₅S₂: C, 36.72; H, 4.11; N, 42.83; S, 16.34; found: C, 36.65; H, 4.24; N, 42.74; S, 16.41.

**Synthesis of compounds 9(a-j)**

A mixture of compound 8 (0.005 mol) and appropriate aromatic aldehyde (0.005 mol) in 10 mL ethanol containing 2-3 drops of glacial acetic acid, was boiled under reflux on a water bath for 10-12 h. The product that separated on cooling was filtered off, dried and recrystallised from ethanol to give 9(a-j).

4-[(2E)-2-(2,4-dichlorobenzylidene)hydrazinyl]-2-[2Z]-2-(2,4-dichlorobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (9a)

Yield: 3.62 g (71%); mp 236-241°C; IR spectrum (KBr, ν, cm⁻¹): 3252 (NH), 1590 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 7.25-7.42 (m, 6H, Ar-H), 7.51 (d, 1H, CH), 7.73 (d, 1H, CH), 8.25 (s, 1H, N=CH), 9.10 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.2-167.8 (thienopyrimidine aromatic carbon atoms), 144.7 (CH), 151.2 (CH). Anal. calcd. for C₂₀H₁₃Cl₂N₅S: C, 47.08; H, 2.37; N, 16.47; S, 6.28; found: C, 47.17; H, 2.43; N, 16.36; S, 6.19.

4-[(E)-(2-[(2Z)-2-(3,4-dihydroxybenzylidene)]hydrazinyl]thieno[2,3-d]pyrimidin-4-yl]hydrazinyl]methyl]benzene-1,2-diol (9b)

Yield: 2.18 g (50%); mp 232-237°C; IR spectrum (KBr, ν, cm⁻¹): 3437 (OH), 3247 (NH), 1596 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 3.63 (brs, 4H, 4OH), 6.87-7.12 (m, 6H, Ar-H), 7.54 (d, 1H, CH), 7.77 (d, 1H, CH), 8.23 (s, 1H, N=CH), 9.23 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.8-167.6 (thienopyrimidine aromatic carbon atoms), 144.7 (CH), 151.6 (CH). Anal. calcd. for C₁₉H₁₈O₄N₅S: C, 55.04; H, 3.70; N, 19.26; S, 7.35; found: C, 55.11; H, 3.62; N, 19.19; S, 7.42.

4-[(2E)-2-(3-methoxybenzylidene)hydrazinyl]-2-[(2Z)-2-(2,4-dichlorobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (9c)

Yield: 3.41 g (79%); mp 245-250°C; IR spectrum (KBr, ν, cm⁻¹): 3246 (NH), 1602 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 3.75 (s, 6H, 2OCH₃), 6.98-7.36 (m, 8H, Ar-H), 7.50 (d, 1H, CH), 7.73 (d, 1H, CH), 8.28 (s, 1H, N=CH), 9.09 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.8-167.6 (thienopyrimidine aromatic carbon atoms), 145.4 (CH), 151.9 (CH). Anal. calcd. for C₂₀H₁₄O₂N₅S: C, 61.10; H, 4.66; N, 19.43; S, 7.41; found: C, 61.21; H, 4.58; N, 19.52; S, 7.34.

4-[(2E)-2-(4-chlorobenzylidene)hydrazinyl]-2-[(2Z)-2-(2,4-chlorobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (9d)

Yield: 3.18 g (72%); mp 271-276°C; IR spectrum (KBr, ν, cm⁻¹): 3250 (NH), 1595 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 7.24-7.48 (m, 8H, Ar-H), 7.49 (d, 1H, CH), 7.71 (d, 1H, CH), 8.30 (s, 1H, N=CH), 9.12 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.2-167.0 (thienopyrimidine aromatic carbon atoms), 144.7 (CH), 151.4 (CH). Anal. calcd. for
C_{3}H_{4}ClN_{2}S: C, 54.43; H, 3.20; N, 19.04; S, 7.27; found: C, 54.35; H, 3.29; N, 18.93; S, 7.35.

4-[(2E)-2-(4-fluorobenzylidene)hydrazinyl]-2-[(Z)-2-(4-fluorobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (9e)

Yield: 2.82g (69%); mp 252-257°C; IR spectrum (KBr, v cm⁻¹): 3445 (OH), 1599 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 8.20-7.41 (m, 8H, Ar-H), 7.51 (d, 1H, CH), 7.22 (d, 1H, CH), 8.23 (s, 1H, N=CH), 9.11 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.2-167.8 (thienopyrimidine aromatic carbon atoms), 145.5 (CH), 151.6 (CH). Anal. calcd. for C_{2}H_{4}ClN_{2}S: C, 58.81; H, 3.46; N, 20.58; S, 7.85; found: C, 58.76; H, 3.52; N, 20.50; S, 7.92.

4-[(E)-2-[(2Z)-2-(4-hydroxybenzylidene)hydrazinyl]thieno[2,3-d]pyrimdin-4-yl]phenylthieno[2,3-d]pyrimidine (9f)

Yield: 3.03g (75%); mp 209-214°C; IR spectrum (KBr, v cm⁻¹): 3445 (OH), 1351 (CH), 1594 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 4.55 (s, 2H, 2OCH), 6.94-7.21 (m, 8H, Ar-H), 7.48 (d, 1H, CH), 7.70 (d, 1H, CH), 8.23 (s, 1H, N=CH), 9.12 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.9-167.2 (thienopyrimidine aromatic carbon atoms), 144.9 (CH), 151.9 (CH). Anal. calcd. for C_{2}H_{4}N_{2}O_{2}S: C, 59.39; H, 3.99; N, 20.78; S, 7.93; found: C, 59.30; H, 4.09; N, 20.69; S, 8.01.

4-[(2E)-2-(4-methoxybenzylidene)hydrazinyl]-2-[(Z)-2-(4-methylbenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (9g)

Yield: 3.28g (76%); mp 245-250°C; IR spectrum (KBr, v cm⁻¹): 3245 (NH), 1603 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 3.78 (s, 6H, 2OCH), 7.33-7.48 (m, 8H, Ar-H), 7.50 (d, 1H, CH), 7.72 (d, 1H, CH), 8.25 (s, 1H, N=CH), 9.10 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.2-167.5 (thienopyrimidine aromatic carbon atoms), 145.2 (CH), 151.8 (CH). Anal. calcd. for C_{2}H_{3}O_{2}S: C, 61.10; H, 4.66; N, 19.43; S, 7.41; found: C, 61.01; H, 4.59; N, 19.54; S, 7.48.

4-[(2E)-2-(4-methylbenzylidene)hydrazinyl]-2-[(Z)-2-(4-methylbenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (9h)

Yield: 3.24g (81%); mp 248-253°C; IR spectrum (KBr, v cm⁻¹): 3256 (NH), 1598 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 2.25 (s, 6H, 2CH₃), 7.26-7.42 (m, 8H, Ar-H), 7.52 (d, 1H, CH), 7.71 (d, 1H, CH), 8.23 (s, 1H, N=CH), 9.07 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.6-167.7 (thienopyrimidine aromatic carbon atoms), 145.1 (CH), 151.5 (CH). Anal. calcd. for C_{2}H_{3}N_{2}S: C, 65.98; H, 5.03; N, 20.98; S, 8.01; found: C, 66.08; H, 5.11; N, 20.88; S, 7.94.

4-[(2E)-2-(4-nitrobenzylidene)hydrazinyl]-2-[(Z)-2-(4-nitrobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (9i)

Yield: 3.28g (71%); mp 278-283°C; IR spectrum (KBr, v cm⁻¹): 3259 (NH), 1593 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 7.33-7.45 (m, 8H, Ar-H), 7.48 (d, 1H, CH), 7.70 (d, 1H, CH), 8.29 (s, 1H, N=CH), 9.13 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 108.3-167.6 (thienopyrimidine aromatic carbon atoms), 144.6 (CH), 151.1 (CH). Anal. calcd. for C_{2}H_{4}N_{2}O_{2}S: C, 52.94; H, 3.05; N, 24.23; S, 6.93; found: C, 51.86; H, 3.15; N, 24.12; S, 7.01.

4-[(2E)-2-(4-butylnbenzylidene)hydrazinyl]-2-[(Z)-2-(4-butylnbenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (9j)

Yield: 3.54g (73%); mp 291-296°C; IR spectrum (KBr, v cm⁻¹): 3251 (NH), 1601 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 1.02 (t, 6H, 2CH₃), 1.31-1.72 (m, 12H, 6CH₃), 7.26-7.40 (m, 6H, Ar-H), 7.52 (d, 1H, CH), 7.75 (d, 1H, CH), 8.30 (s, 1H, N=CH), 9.11 (s, 1H, NH); ¹³C NMR (DMSO-d₆, δ ppm): 109.1-167.0 (thienopyrimidine aromatic carbon atoms), 144.8 (CH), 151.3 (CH). Anal. calcd. for C_{2}H_{4}N_{2}S: C, 69.39; H, 6.66; N, 17.34; S, 6.62; found: C, 69.30; H, 6.59; N, 17.43; S, 6.72.

Thieno[2,3-d]pyrimidine-4(3H)-one (10)

A mixture of compound 1 (0.01 mol) and formic acid (20 mL) was refluxed for 10 h. After the completion of reaction, the reaction mixture was allowed to cool and then poured into ice cold water. The solid thus obtained was filtered, washed with water, dried and crystallized from ethanol to give compound 10. Yield 0.86g (57%); mp 192-197°C; IR spectrum (KBr, v cm⁻¹): 3160 (NH), 1684 (C=O); ¹H NMR (CDCl₃, δ ppm): 7.57 (d, 1H, CH), 7.81 (d, 1H, CH), 8.15 (s, 1H, CH), 10.46 (brs, 1H, NH, D₂O exchangeable). Anal. calcd. for C_{18}H_{13}ClN_{3}O_{5}: C, 47.36; H, 2.65; N, 18.41; S, 21.07; found: C, 36.09; H, 2.17; N, 13.88; S, 47.91.

4-chlorothieno[2,3-d]pyrimidine (11)

A mixture of compound 10 (0.01 mol) and phosphorus oxychloride (20 mL) was refluxed for 12 h. The excess of phosphorus oxychloride was distilled off under reduced pressure and the residue thus obtained was treated with sodium bicarbonate solution (10%). The resulting solid was collected, washed with water, dried and recrystallized from ethanol to give compound 11. Yield 1.14g (67%); mp 259-264°C; ¹H NMR (CDCl₃, δ ppm): 7.50 (d, 1H, CH), 7.73 (d, 1H, CH), 8.23 (s, 1H, CH). Anal.
4-hyrazinylthieno[2,3-d]pyrimidine (12)
A mixture of compound 11 (0.01 mol) in hydrazine hydrate (99%) (10 mL) and ethanol were refluxed for 6 h. After cooling the separated product was filtered off, washed with water, dried and recrystallized from dioxane to give compound 12. Yield 1.12g (68%); mp 177-182 °C; IR spectrum (KBr, ν, cm⁻¹): 3416-3206 (NH, NH₂); 'H NMR (CDCl₃, δ ppm): 4.06 (brs, 2H, NH₃), 6.52 (brs, 1H, NH, D₂O exchangeable), 7.55 (d, 1H, CH), 7.79 (d, 1H, CH), 8.29 (1H, CH). Anal. calcd. for C₇H₆N₂S: C, 43.36; H, 3.64; N, 33.71; S, 19.29; found: C, 36.09; H, 2.17; N, 13.88; S, 47.91.

Synthesis of compounds 13(a-j)
A solution of compound 12 (0.005 mol) in ethanol (10 mL) and appropriate aromatic aldehyde (0.005 mol) containing 2-3 drops of glacial acetic acid, was boiled under reflux on a water bath for 8-9 h. The product that separated on cooling was filtered off, washed with water, dried and recrystallized from dioxane to give 13(a-j).

4-[2-(2,4-dichlorobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (13a)
Yield: 2.23g (69%); mp 168-173 °C; IR spectrum (KBr, ν, cm⁻¹): 3268 (NH), 1549 (N=CH); 'H NMR (DMSO-d₆, δ ppm): 7.32-7.50 (m, 3H, Ar-H), 7.55 (d, 1H, CH), 7.79 (d, 1H, CH), 8.05 (1H, N=CH), 8.29 (1H, CH), 8.92 (1H, NH). Anal. calcd. for C₁₅H₁₃Cl₂N₂S: C, 48.31; H, 2.49; N, 17.34; S, 9.9; found: C, 48.25; H, 2.55; N, 17.40; S, 9.82.

4-[2-(2-thieno[2,3-d]pyrimidin-4-yl)hydrazinylidene)methyl]benzene-1,2-diol (13b)
Yield: 1.49g (52%); mp 201-206 °C; IR spectrum (KBr, ν, cm⁻¹): 3486 (OH), 3260 (NH), 1543 (N=CH); 'H NMR (DMSO-d₆, δ ppm): 3.54 (s, 2H, 2OH), 6.88-7.22 (m, 3H, Ar-H), 7.51 (d, 1H, CH), 7.73 (d, 1H, CH), 8.06 (1H, N=CH), 8.27 (1H, CH), 8.99 (1H, NH). Anal. calcd. for C₁₅H₁₀O₃N₂S: C, 54.54; H, 3.52; N, 19.57; S, 11.20; found: C, 54.59; H, 3.60; N, 19.51; S, 11.11.

4-[2-(3-methoxybenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (13c)
Yield: 2.22g (78%); mp 213-218 °C; IR spectrum (KBr, ν, cm⁻¹): 3272 (NH), 1540 (N=CH); 'H NMR (DMSO-d₆, δ ppm): 3.80 (s, 3H, OCH₃), 7.23-7.35 (m, 4H, Ar-H), 7.53 (d, 1H, CH), 7.72 (d, 1H, CH), 8.09 (1H, N=CH), 8.23 (1H, CH), 8.93 (1H, NH). Anal. calcd. for C₁₄H₁₂N₂O₃S: C, 59.14; H, 4.25; N, 19.70; S, 11.28; found: C, 59.07; H, 4.32; N, 19.61; S, 11.35.

4-[2-(4-chlorobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (13d)
Yield: 2.11g (73%); mp 225-230 °C; IR spectrum (KBr, ν, cm⁻¹): 3276 (NH), 1551 (N=CH); 'H NMR (DMSO-d₆, δ ppm): 7.30-7.45 (m, 4H, Ar-H), 7.53 (1H, CH), 7.75 (d, 1H, CH), 7.99 (s, 1H, N=CH), 8.29 (1H, CH), 8.95 (1H, NH). Anal. calcd. for C₁₃H₁₁ClN₂S: C, 54.07; H, 3.14; N, 19.40; S, 11.10; found: C, 54.17; H, 3.19; N, 19.34; S, 11.01.

4-[2-(4-fluorobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (13e)
Yield: 1.77g (65%); mp 206-211 °C; IR spectrum (KBr, ν, cm⁻¹): 3275 (NH), 1549 (N=CH); 'H NMR (DMSO-d₆, δ ppm): 7.23-7.49 (m, 4H, Ar-H), 7.59 (1H, CH), 7.81 (d, 1H, CH), 8.05 (1H, N=CH), 8.30 (1H, CH), 8.95 (1H, NH). Anal. calcd. for C₁₃H₁₁FN₂S: C, 57.34; H, 3.33; N, 20.58; S, 11.78; found: C, 57.29; H, 3.28; N, 20.65; S, 11.83.

4-[2-(2-thieno[2,3-d]pyrimidin-4-yl)hydrazinylidene)methyl]phenol (13f)
Yield: 1.89g (70%); mp 217-222 °C; IR spectrum (KBr, ν, cm⁻¹): 3282 (NH), 1554 (N=CH); 'H NMR (DMSO-d₆, δ ppm): 3.94 (s, 1H, OH), 7.27-7.47 (m, 4H, Ar-H), 7.56 (d, 1H, CH), 7.80 (d, 1H, CH), 8.05 (1H, N=CH), 8.91 (1H, NH). Anal. calcd. for C₁₃H₁₀NO₂S: C, 57.76; H, 3.73; N, 20.73; S, 11.86; found: C, 57.65; H, 3.65; N, 20.83; S, 11.93.

4-[2-(4-methoxybenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (13g)
Yield: 2.10g (74%); mp 168-173 °C; IR spectrum (KBr, ν, cm⁻¹): 3276 (NH), 1556 (N=CH); 'H NMR (DMSO-d₆, δ ppm): 3.85 (s, 3H, OCH₃), 7.23-7.44 (m, 4H, Ar-H), 7.54 (d, 1H, CH), 7.77 (d, 1H, CH), 8.01 (1H, N=CH), 8.28 (1H, CH), 8.92 (1H, NH). Anal. calcd. for C₁₄H₁₂N₂O₃S: C, 59.14; H, 4.25; N, 19.70; S, 11.28; found: C, 59.23; H, 4.31; N, 19.63; S, 11.19.

4-[2-(4-methylbenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (13h)
Yield: 2.04g (76%); mp 225-230 °C; IR spectrum (KBr, ν, cm⁻¹): 3280 (NH), 1559 (N=CH); 'H NMR (DMSO-d₆, δ ppm): 2.27 (s, 3H, CH₃), 7.33-7.49 (m, 4H, Ar-H), 7.59 (d, 1H, CH), 7.81 (d, 1H, CH), 7.98 (1H, N=CH), 8.25 (1H, CH), 8.99 (1H, NH). Anal. calcd. for C₁₄H₁₂N₂S: C, 62.66; H, 4.51; N, 20.88; S, 11.95; found: C, 62.60; H, 4.57; N, 20.80; S, 12.04.
4-[2-(4-nitrobenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (13i)

Yield: 2.09g (70%); mp 202-207 °C; IR spectrum (KBr, v, cm⁻¹): 3275 (NH), 1562 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 7.28-7.47 (m, 4H, Ar-H), 7.52 (d, 1H, CH), 7.76 (d, 1H, CH), 7.99 (s, 1H, N=CH), 8.27 (s, 1H, CH), 8.92 (s, 1H, NH). Anal. calcd. for C₁₃H₈N₂O₂S: C, 52.17; H, 3.03; N, 23.40; S, 10.71; found: C, 52.26; H, 3.11; N, 23.34; S, 10.66.

4-[2-(4-butylnbenzylidene)hydrazinyl]thieno[2,3-d]pyrimidine (13j)

Yield: 2.20g (71%); mp 217-222 °C; IR spectrum (KBr, v, cm⁻¹): 3281 (NH), 1557 (N=CH); ¹H NMR (DMSO-d₆, δ ppm): 1.09 (t, 3H, CH₃), 1.34-1.77 (m, 6H, 3CH₃), 7.30-7.45 (m, 4H, Ar-H), 7.53 (d, 1H, CH), 7.74 (d, 1H, CH), 8.03 (s, 1H, N=CH), 8.25 (s, 1H, CH), 8.95 (s, 1H, NH). Anal. calcd. for C₁₅H₁₀N₂S: C, 65.78; H, 5.84; N, 18.05; S, 10.33; found: C, 65.72; H, 5.91; N, 17.95; S, 10.27.

Synthesis of compounds 14(a-j)

Chloroacetyl chloride (0.005 mol) was added drop wise to a solution of Schiff's base 13(a-j) (0.005 mol) and triethylamine (2-3 drops) in dry benzene (15 mL) at 5-10 °C. The reaction mixture was stirred for 3 h and then refluxed for 4-5 h. On cooling, the precipitate was obtained which was filtered, washed with water, dried and recrystallised from dimethylformamide to produce 14(a-j).

3-chloro-4-(2,4-dichlorophenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14a)

Yield: 2.20g (55%); mp 199-204 °C; IR spectrum (KBr, v, cm⁻¹): 3262 (NH), 1748 (C=O); ¹H NMR (DMSO-d₆, δ ppm): 4.46 (s, 1H, NH), 5.01 (d, 1H, CH=Ar), 5.49 (d, 1H, CH=Cl), 7.21-7.43 (m, 3H, Ar-H), 7.54 (d, 1H, CH), 7.75 (d, 1H, CH), 8.14 (s, 1H, CH); ¹³C NMR (DMSO-d₆, δ ppm): 63.9 (CH=Cl), 67.4 (CH), 110.4-167.3 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C₁₃H₁₁ClN₂O₂S: C, 49.66; H, 3.06; N, 15.44; S, 8.84; found: C, 49.61; H, 3.14; N, 15.39; S, 8.93.

3-chloro-4-(3-methoxyphenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14c)

Yield: 2.02g (56%); mp 198-203 °C; IR spectrum (KBr, v, cm⁻¹): 3271 (NH), 1759 (C=O); ¹H NMR (DMSO-d₆, δ ppm): 3.72 (s, 3H, OCH₃), 4.43 (s, 1H, NH), 4.99 (d, 1H, CH=Ar), 5.45 (d, 1H, CH=Cl), 7.34-7.49 (m, 4H, Ar-H), 7.58 (d, 1H, CH), 7.79 (d, 1H, CH), 8.13 (s, 1H, CH); ¹³C NMR (DMSO-d₆, δ ppm): 63.9 (CH=Cl), 67.4 (CH), 110.8-167.3 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C₁₃H₁₁ClN₂O₂S: C, 53.26; H, 3.63; N, 15.53; S, 8.89; found: C, 53.17; H, 3.56; N, 15.57; S, 8.95.

3-chloro-4-(4-chlorophenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14d)

Yield: 2.19g (60%); mp 207-212 °C; IR spectrum (KBr, v, cm⁻¹): 3262 (NH), 1748 (C=O); ¹H NMR (DMSO-d₆, δ ppm): 4.49 (s, 1H, NH), 5.03 (d, 1H, CH=Ar), 5.45 (d, 1H, CH=Cl), 7.08-7.17 (m, 4H, Ar-H), 7.52 (d, 1H, CH), 7.74 (d, 1H, CH), 8.09 (s, 1H, CH); ¹³C NMR (DMSO-d₆, δ ppm): 63.7 (CH=Cl), 67.3 (CH), 110.3-166.9 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C₁₃H₁₂ClN₂O₂S: C, 49.33; H, 2.76; N, 15.34; S, 8.78; found: C, 49.24; H, 2.69; N, 15.44; S, 8.83.

3-chloro-4-(4-fluorophenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14e)

Yield: 1.88g (54%); mp 216-221 °C; IR spectrum (KBr, v, cm⁻¹): 3273 (NH), 1753 (C=O); ¹H NMR (DMSO-d₆, δ ppm): 4.45 (s, 1H, NH), 5.01 (d, 1H, CH=Ar), 5.50 (d, 1H, CH=Cl), 7.39-7.49 (m, 4H, Ar-H), 7.51 (d, 1H, CH), 7.75 (d, 1H, CH), 8.12 (s, 1H, CH); ¹³C NMR (DMSO-d₆, δ ppm): 63.9 (CH=Cl), 67.5 (CH), 110.6-167.6 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C₁₃H₁₀FClN₂O₂S: C, 51.65; H, 2.89; N, 16.06; S, 9.19; found: C, 51.60; H, 2.93; N, 16.15; S, 9.08.

3-chloro-4-(4-hydroxyphenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14f)

Yield: 1.63g (47%); mp 192-197 °C; IR spectrum (KBr, v, cm⁻¹): 3465 (OH), 3263 (NH), 1745 (C=O); ¹H NMR (DMSO-d₆, δ ppm): 4.46 (s, 1H, NH), 4.55 (s, 1H, OH), 5.06 (d, 1H, CH=Ar), 5.56 (d, 1H, CH=Cl), 7.27-7.43 (m, 4H, Ar-H), 7.53 (d, 1H, CH), 7.74 (d, 1H, CH), 8.14 (s, 1H, CH); ¹³C NMR (DMSO-d₆, δ ppm): 63.3 (CH=Cl), 67.7 (CH), 111.2-167.4 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C₁₃H₁₀ClN₂O₃S: C, 51.95; H, 3.20; N, 16.16; S, 9.25; found: C, 51.89; H, 3.12; N, 16.26; S, 9.33.
3-chloro-4-(4-methoxyphenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14g)

Yield: 2.24g (62%); mp 183-188 °C; IR spectrum (KBr, v, cm\(^{-1}\)): 3269 (NH), 1754 (C=O); \(^1\)H NMR (DMSO-d\(_6\), δ ppm): 3.75 (s, 3H, OCH\(_3\)), 4.48 (s, 1H, NH), 5.05 (d, 1H, CH-Ar), 5.50 (d, 1H, CH-Cl), 7.11-7.39 (m, 4H, Ar-H), 7.56 (d, 1H, CH), 7.78 (d, 1H, CH); 13C NMR (DMSO-d\(_6\), δ ppm): 63.8 (CH-Cl), 67.4 (CH), 110.5-167.7 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C\(_{18}\)H\(_{18}\)ClN\(_2\)OS: C, 53.26; H, 3.63; N, 15.53; S, 8.89; found: C, 53.17; H, 3.56; N, 15.60; S, 8.97.

3-chloro-4-(4-methylphenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14h)

Yield: 2.24g (62%); mp 203-208 °C; IR spectrum (KBr, v, cm\(^{-1}\)): 3278 (NH), 1768 (C=O); \(^1\)H NMR (DMSO-d\(_6\), δ ppm): 2.23 (s, 3H, CH\(_3\)), 4.47 (s, 1H, NH), 5.03 (d, 1H, CH-Ar), 5.53 (d, 1H, CH-Cl), 6.97-7.34 (m, 4H, Ar-H), 7.52 (d, 1H, CH), 7.73 (d, 1H, CH), 8.11 (s, 1H, CH); 13C NMR (DMSO-d\(_6\), δ ppm): 63.3 (CH-Cl), 67.4 (CH), 110.2-167.6 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C\(_{17}\)H\(_{17}\)ClN\(_2\)OS: C, 55.73; H, 3.80; N, 16.25; S, 9.30; found: C, 55.67; H, 3.76; N, 16.31; S, 9.38.

3-chloro-4-(4-nitrophenyl)-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14i)

Yield: 2.03g (54%); mp 231-236 °C; IR spectrum (KBr, v, cm\(^{-1}\)): 3269 (NH), 1757 (C=O); \(^1\)H NMR (DMSO-d\(_6\), δ ppm): 4.42 (s, 1H, NH), 4.96 (d, 1H, CH-Ar), 5.47 (d, 1H, CH-Cl), 7.22-7.44 (m, 4H, Ar-H), 7.50 (d, 1H, CH), 7.72 (d, 1H, CH), 8.08 (s, 1H, CH); 13C NMR (DMSO-d\(_6\), δ ppm): 64.4 (CH-Cl), 67.5 (CH), 110.4-167.7 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C\(_{17}\)H\(_{17}\)ClN\(_2\)O\(_2\)S: C, 47.94; H, 2.68; N, 18.64; S, 8.53; found: C, 47.89; H, 2.61; N, 18.73; S, 8.61.

4-(4-butylphenyl)-3-chloro-1-(thieno[2,3-d]pyrimidin-4-ylamino)azetidin-2-one (14j)

Yield: 2.36g (61%); mp 245-250 °C; IR spectrum (KBr, v, cm\(^{-1}\)): 3266 (NH), 1759 (C=O); \(^1\)H NMR (DMSO-d\(_6\), δ ppm): 1.04 (t, 3H, CH\(_3\)), 1.29-1.50 (m, 6H, 3CH\(_3\)), 4.44 (s, 1H, NH), 5.02 (d, 1H, CH-Ar), 5.49 (d, 1H, CH-Cl), 7.19-7.32 (m, 4H, Ar-H), 7.51 (d, 1H, CH), 7.73 (d, 1H, CH), 8.15 (s, 1H, CH); 15C NMR (DMSO-d\(_6\), δ ppm): 63.2 (CH-Cl), 67.9 (CH), 110.6-167.7 (thienopyrimidine aromatic carbon atoms), 166.7 (CO). Anal. calcd. for C\(_{19}\)H\(_{19}\)ClN\(_2\)OS: C, 58.98; H, 4.95; N, 14.48; S, 8.29; found: C, 59.03; H, 4.90; N, 14.54; S, 8.22.

**BIOLOGICAL EVALUATION**

**Antimicrobial activity**

The prepared compounds were tested against *S.aureus* (ATCC-96) and *S.pyogenes* (ATCC-443) as Gram positive and *E.coli* (ATCC-442) and *P.aeruginosa* (ATCC-441) as Gram negative bacterial strains. Antifungal activities of the compounds were tested against *A.niger* (ATCC-282) as fungal strain. All the newly synthesized compounds were screened in vitro for their antibacterial and antifungal activities by broth dilution method (Table-1). The lowest concentration inhibiting growth of the organism is recorded as the MIC. DMSO was used as diluent. The stock 1000 g/ml was prepared. Serial dilutions were prepared in primary and secondary screening. Mueller Hinton Broth was used as nutrient medium to grow and dilute the drug suspension for the test bacteria, and sabourous dextrose broth used for fungal nutrition. Inoculum size for test strain was adjusted to 10\(^8\) CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The control tube containing no antibiotic is immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes are then incubated overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. The amount of growth from the control tube before incubation (which represents the original inoculum) is compared. Ampicillin and Chloramphenicol were used as standard antibacterial and Nystatin and Gresefulvin were used as standard antifungal drugs. Standard strains were procured from Institute of Microbial Technology, Chandigarh.
Table 1. Antimicrobial activity (MIC g/ml) of some selected synthesized compounds.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Minimum Inhibitory Concentration (µg/ml)</th>
<th>Antifungal</th>
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<tr>
<td></td>
<td>Gram +ve</td>
<td>Gram -ve</td>
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<tr>
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<tr>
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<tr>
<td>Nystatin</td>
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</table>

From the screening results, it can be seen that compound 6f showed excellent activity against Gram positive bacteria *S.aureus* and compound 14f showed excellent activity against Gram negative bacteria *E.coli*. Rest of the compounds showed good to moderate activity against other bacteria compared with the standard drugs.

**CONCLUSION**

In this work, we have prepared some new thieno[2,3-d]pyrimidine derivatives 6(a-j), 9(a-j) and 14(a-j) which were screened for their antibacterial and antifungal activities. The structures of all new synthesized compounds are confirmed successfully by IR, ¹H NMR, ¹³C NMR spectra and elemental analysis. Antibacterial activity of title compounds showed that hydroxyl group present at 4th position of phenyl ring in compound 6f could be responsible for increase activity against *S.aureus*. Compound 14f also contain hydroxyl group at 4th position in phenyl ring to show highest activity against *E.coli*.
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REFERENCES


