IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 19, Issue 1 Ser. I (January. 2026), PP 05-11
www.iosrjournals.org

Development Of Novel Thienopyrimidine Hybrids: Synthesis, Characterization And Multi-Target Biological Evaluation

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Abstract:
A new array of thienopyrimidine-based Schiff bases, specifically 2-(2-(substitutedbenzylidene) hydrazinyl)-5,6-dimethylthieno[2,3-d] pyrimidin-4(3H)-one (4a–j), was prepared by a multistep convergent approach. Synthesis was initiated with a Gewald reaction involving ethyl methyl ketone, ethyl cyanoacetate, and elemental sulfur to afford ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate. Further cyclization with potassium thiocyanate gave the critical 2-mercaptothieno[2,3-d] pyrimidin4(3H)-one intermediate. The target Schiff bases were obtained by hydrazinolysis followed by acid-catalyzed condensation with diverse substituted aromatic aldehydes. All the newly synthesized compounds were fully characterized using melting point measurement, elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. This novel scaffold affords a highly privileged template aptly suited for biological assessments against multiple targets and medicinal chemistry studies.

Keywords: *Thieno[2,3-d]pyrimidine, Schiff bases, Gewald reaction, Heterocyclic synthesis, Drug discovery.*

Date of Submission: 10-01-2026 Date of Acceptance: 20-01-2026

I. Introduction

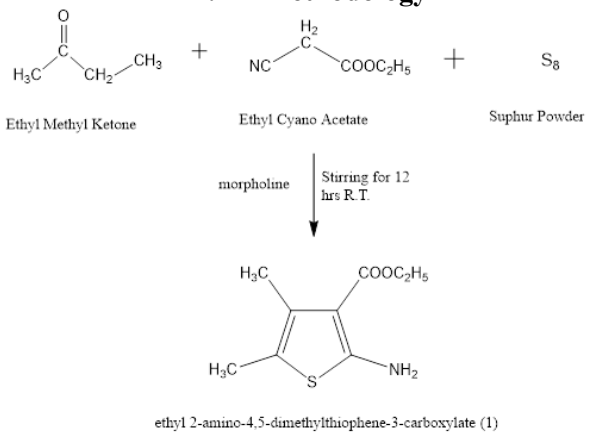
Thienopyrimidine, a heterocyclic compound including a fused thiophene and pyrimidine ring system, has gained significant attention within medicinal chemistry due to the structure similarity it holds with purines. This positions it as an attractive scaffold for a range of pharmacological applications [1]. Among nitrogen- and sulfur-containing heterocycles, thienopyrimidines hold an important place in the research field of drugs due to the presence of potent biological activities within them. They are also noted for similarity in structure to purine bases like adenine and guanine, making them popular scaffolds for medicinal chemistry [2]. This structural mimicry allows the thienopyrimidines to act as potential antimetabolites of nucleic acids, contributing to a wide array of biological activities [3]. This has facilitated intense research in their synthesis and evaluation for a variety of therapeutic indications, which include antimicrobial, analgesic, and antioxidant properties [4-7]. Integration of different pharmacophores into the thienopyrimidine moiety would be expected to generate novel entities with improved biological activity, thus expanding its therapeutic scope. For instance, some thienopyrimidine derivatives have exhibited potential as antimicrobial, anticancer, and anti-inflammatory agents [8]. Many pyridothienopyrimidine derivatives were synthesized and showed outstanding pharmacological activities such as antimicrobial, anticancer, and protein kinase inhibitory activities [9]. The variation in substituents on the pyrimidine rings of thienopyrimidine derivatives can significantly affect the biological activity of these molecules, rendering them highly tunable for drug discovery applications [10] The rest of the general biological activities, the thienopyrimidine derivatives have been shown to possess a wide range of specific activities such as analgesic and ulcerogenic action, antitubercular and antimalarial, besides functioning as EGFR inhibitors or adenosine receptor antagonists [11].

II. Materials And Methods

2-(2-(substitutedbenzylidene) hydrazinyl)-5,6-dimethylthieno[2,3-*d*] pyrimidin-4(3*H*)-one (4a–j), was synthesized from the key compound ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate by via the Gewald reaction following the reported procedure with minor modifications [12]. Solvents were dried and distilled according to standard practices. Melting points were measured using a digital capillary melting point apparatus and are uncorrected. Thin Layer Chromatography was performed on Merck aluminum-backed silica gel 60 F254 plates; the spots were viewed under UV light (254 nm and 366 nm) and by iodine vapors. The spectroscopic data were obtained as follows: Infrared spectra were measured using KBr pellets (range 4000-400 cm^{-1}). NMR spectra were recorded on BRUKER AVANCE II 400 MHz and 100 MHz NMR spectrometer using DMSO-d_6 as solvent

and TMS as internal standard. Mass spectra were recorded on an LC-MS/MS instrument Synthetic pathways are as follows;

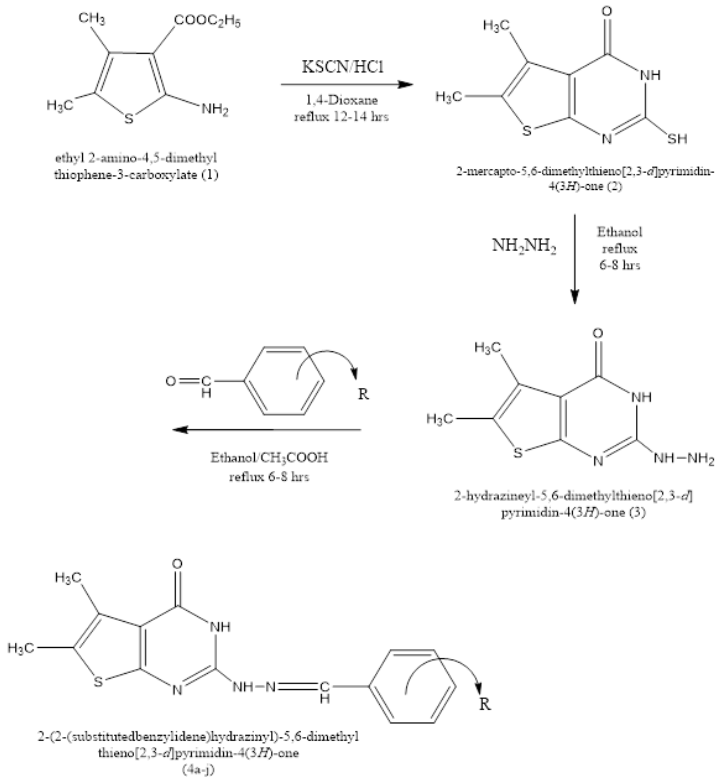
III. Methodology



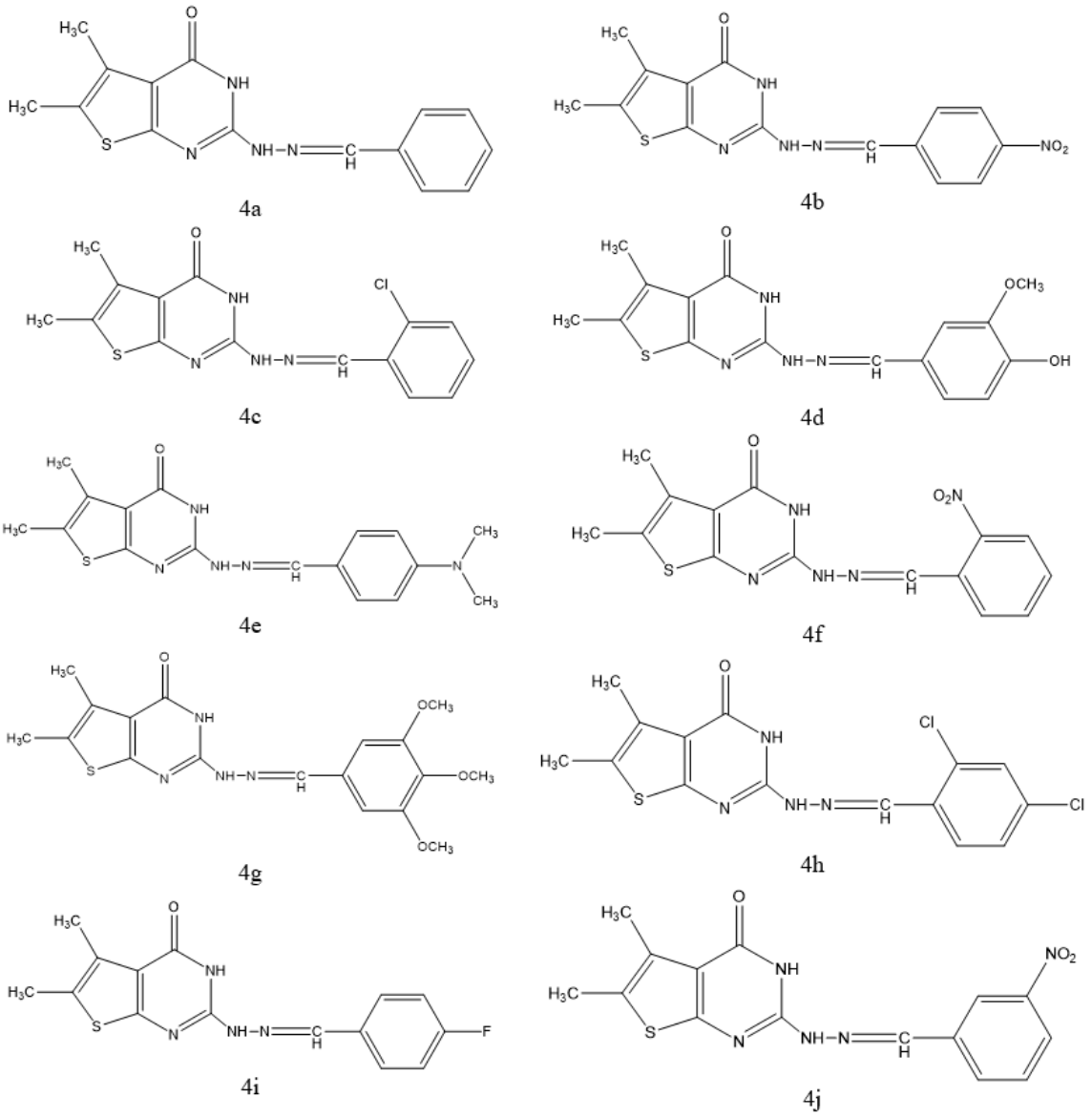
Synthetic method

Synthesis of ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate (1)

Ethyl cyanoacetate (0.01 mol) was dissolved in 10 mL of methanol and ethylmethylketone (0.01 mol) and powdered Sulphur (0.01 mol) were added to the same solution, then morpholine (0.01 mol) and 10 g of potassium carbonate were added as catalyst to the resulting solution. This heterogeneous mixture was stirred at room temperature for 12 hrs. After the completion of the reaction as monitored by TLC, the reaction mixture was poured into 150 mL of ice-cold water. The pale-yellow solid product got precipitated out, which was filtered, washed with water, dried and recrystallised from ethanol. Yield 85%. (M.P. 88˚C), Elemental analysis calculated for $C_9H_{13}O_2S$ (MW 215.27 g·mol^{-1}): C, 50.20; H, 6.09; N, 6.51; O, 14.86%; found: C, 50.10; H, 6.02; N, 6.45; O, 14.80%, FT-IR (KBr, cm^{-1}): 3320–3180 (NH_2), 2980–2870 (aliphatic C–H), 1725 (ester C=O), 1585 (C=C, thiophene), 1260–1180 (C–O), 750–700 (C–S), ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 1.25 (t, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.20 (q, 2H, CH_2), 5.20 (br s, 2H, NH_2), ^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 14.3 (CH_3), 15.8, 16.4 (2× CH_3), 60.5 (CH_2), 108.2–137.6 (thiophene C), 165.8 (C=O), MS (ESI): m/z 216 $[M+H]^+$.



Compound (4a-j)



Synthesis of 2-mercapto-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (2)

A mixture of ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate (0.01 mol) and an excess of potassium thiocyanate (1.5 g, 0.015 mol) was combined in 10 mL of 1,4-dioxane, followed by the addition of 2 mL of concentrated hydrochloric acid. The reaction mixture was heated under reflux for 15 hours. After the completion of the reaction, the mixture was allowed to cool, then poured onto ice-cold water while stirring. The solid resulting from this was collected by filtration, washed with water, dried, and recrystallized from ethanol to furnish compound (2). Yield 52%. (M.P. 268-270˚C), Elemental analysis calcd for $C_8H_8N_2OS_2$: C 42.09, H 3.53, N 12.27, O 7.01%; found: C 41.95, H 3.48, N 12.15, O 6.95%. FT-IR (KBr, cm^{-1}): 3270 (NH), 2550–2600 (SH), 2960–2870 (aliphatic C–H), 1685 (C=O, pyrimidinone), 1605 (C=N), 750–700 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.20 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 10.20 (br s, 1H, NH), 12.80 (br s, 1H, SH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 15.6, 16.3 (2×CH_3), 118.4, 124.7, 136.8 (thienopyrimidine C), 158.9 (C=N), 163.5 (C=O). MS (ESI): m/z 229 $[M+H]^+$.

Synthesis of 2-hydrazinyl-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (3)

A mixture of compound (2) (0.01 mol) and concentrated hydrazine hydrate 1.6g (0.05 mol) in methanol (10 mL) refluxed for 8 hrs and then held for ~16 hrs at room temperature. The separated product was filtered off, washed with water, dried and recrystallized from ethanol to give compound (3). Yield 68%. (M.P. 299-301˚C) Elemental analysis calcd for $C_8H_{10}N_4OS$: C 45.70, H 4.79, N 26.65, O 7.61%; found: C 45.55, H 4.70, N 26.50, O 7.55%. FT-IR (KBr, cm^{-1}): 3320–3200 (NH, NH_2), 2960–2870 (aliphatic C–H), 1680 (C=O, pyrimidinone),

1600 (C=N), 750–700 (C–S). [1]*H NMR (400 MHz, DMSO-d_6, δ ppm):* 2.18 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.60 (br s, 2H, NH_2), 9.80 (br s, 1H, NH), 10.40 (br s, 1H, NH). [13]*C NMR (100 MHz, DMSO-d_6, δ ppm):* 15.4, 16.2 (2×CH_3), 117.8, 125.3, 137.1 (thienopyrimidine C), 156.8 (C=N), 163.2 (C=O). MS (ESI): m/z 211 $[M+H]^+$.

General procedure for Synthesis of 2-(2-(substitutedbenzylidene)hydrazinyl)-5,6-dimethyl thieno[2,3-d]pyrimidin-4(3H)-one (4a-j)

A solution of compound (3) (0.01 mol) and appropriate aromatic aldehyde (0.01 mol) in 10 mL methanol containing a few drops of glacial acetic acid, was heated under reflux on a water bath for 8-9 hrs. The products that separated on cooling was filtered off, dried and recrystallized from ethanol.

2-(2-benzylidenehydrazineyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (4a)

Yield: 78%; m.p.: 234 °C. Elemental analysis calcd for $C_{14}H_{14}N_4OS$: C 56.36, H 4.73, N 18.78, O 5.36%; found: C 56.20, H 4.65, N 18.60, O 5.30%. FT-IR (KBr, cm^{-1}): 3280 (NH), 2960–2870 (aliphatic C–H), 1685 (C=O), 1610 (C=N), 3050 (Ar–C–H), 750–700 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.19 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 7.20–7.65 (m, 5H, Ar–H), 8.38 (s, 1H, CH=N), 9.85 (br s, 1H, NH), 10.45 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 15.5, 16.3 (2×CH_3), 118.0, 124.8, 136.7 (thienopyrimidine C), 126.5–138.9 (aromatic C), 148.6 (C=N), 163.6 (C=O). *MS (ESI):* m/z 299 $[M+H]^+$.

5,6-dimethyl-2-(2-(4-nitrobenzylidene)hydrazineyl)thieno[2,3-d]pyrimidin-4(3H)-one (4b)

Yield 69 %; m.p. 220°C Elemental analysis calcd for $C_{14}H_{12}N_6O_3S$: C 46.15, H 3.32, N 23.06, O 13.17%; found: C 46.00, H 3.25, N 22.90, O 13.05%. FT-IR (KBr, cm^{-1}): 3275 (NH), 2960–2870 (aliphatic C–H), 1682 (C=O, pyrimidinone), 1615 (C=N, azomethine), 1525 and 1345 (NO_2 asymmetric and symmetric), 750–700 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.20 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.55–8.20 (m, 4H, Ar-H), 8.42 (s, 1H, CH=N), 9.90 (br s, 1H, NH), 10.60 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 15.6, 16.4 (2×CH_3), 118.2, 124.6, 136.9 (thienopyrimidine C), 123.5–147.8 (aromatic C), 149.2 (C=N), 163.8 (C=O). MS (ESI): m/z 365 $[M+H]^+$.

2-(2-(2-chlorobenzylidene) hydrazineyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (4c)

Yield: 75%**;** m.p.: 245 °C. Elemental analysis calcd for $C_{14}H_{13}ClN_4OS$**:** C 51.14, H 3.98, N 17.04, O 4.87%; found: C 51.00, H 3.90, N 16.90, O 4.80%**.** FT-IR (KBr, cm^{-1}): 3275 (NH), 2960–2870 (aliphatic C–H), 1683 (C=O, pyrimidinone), 1612 (C=N, azomethine), 3055 (aromatic C–H), 760–700 (C–Cl and C–S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.20 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.30–7.85 (m, 4H, Ar–H), 8.40 (s, 1H, CH=N), 9.90 (br s, 1H, NH), 10.50 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 15.6, 16.4 (2×CH_3), 118.1, 124.9, 136.8 (thienopyrimidine C), 127.4–138.5 (aromatic C), 149.0 (C=N), 163.7 (C=O). MS (ESI): m/z 333 $[M+H]^+$.

2-(2-(4-hydroxy-3-methoxybenzylidene)hydrazineyl)-5,6-dimethylthieno[2,3-d] pyrimidin-4(3H)-one (4d)

Yield: 72%; m.p.: 260 °C. Elemental analysis calcd for $C_{15}H_{16}N_4O_3S$: C 52.93, H 4.74, N 16.46, O 14.10%; found: C 52.80, H 4.65, N 16.30, O 14.00%. FT-IR (KBr, cm^{-1}): 3360 (phenolic O–H), 3270 (NH), 2960–2870 (aliphatic C–H), 1680 (C=O, pyrimidinone), 1608 (C=N, azomethine), 1510 (aromatic C=C), 1245 (Ar–O–CH_3), 750–700 (C–S). [1]*H NMR (400 MHz, DMSO-d_6, δ ppm):* 2.18 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.75–7.30 (m, 3H, Ar–H), 8.36 (s, 1H, CH=N), 9.20 (s, 1H, OH), 9.85 (br s, 1H, NH), 10.45 (br s, 1H, NH). [13]*C NMR (100 MHz, DMSO-d_6, δ ppm):* 15.5, 16.3 (2×CH_3), 55.8 (OCH_3), 112.4–149.5 (aromatic and thienopyrimidine C), 148.8 (C=N), 163.6 (C=O). MS (ESI): m/z 345 $[M+H]^+$.

2-(2-(4-(dimethylamino)benzylidene)hydrazineyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (4e)

*Yield: 70%**; m.p.: 244 °C.* Elemental analysis calcd for $C_{16}H_{19}N_5OS$**:** C 55.96, H 5.57, N 20.39, O 4.66%; found: C 55.80, H 5.48, N 20.20, O 4.55%. FT-IR (KBr, cm^{-1}): 3275 (NH), 2960–2870 (aliphatic C–H), 1682 (C=O, pyrimidinone), 1610 (C=N, azomethine), 1520 (aromatic C=C), 1340 (C–N, dimethylamino), 750–700 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.18 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.02 (s, 6H, $N(CH_3)_2$), 6.65–7.20 (m, 4H, Ar–H), 8.32 (s, 1H, CH=N), 9.80 (br s, 1H, NH), 10.40 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 15.4, 16.2 (2×CH_3), 40.6 ($N(CH_3)_2$), 111.8–149.8 (aromatic and thienopyrimidine C), 148.5 (C=N), 163.4 (C=O). MS (ESI): m/z 346 $[M+H]^+$.

2-(2-(2-nitrobenzylidene) hydrazinyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (4f)

Yield: 73%; m.p.: 250 °C. Elemental analysis calcd for $C_{14}H_{12}N_6O_3S$**:** C 46.15, H 3.32, N 23.06, O 13.17%; found: C 46.00, H 3.25, N 22.90, O 13.05%. FT-IR (KBr, cm^{-1}): 3280 (NH), 2960–2870 (aliphatic C–H), 1684 (C=O, pyrimidinone), 1612 (C=N, azomethine), 1528 and 1348 (NO_2 asymmetric and symmetric), 750–700 (C–S). [1]*H NMR (400 MHz, DMSO-d_6, δ ppm):* 2.19 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.45–8.10 (m, 4H, Ar–

H), 8.48 (s, 1H, CH=N), 9.95 (br s, 1H, NH), 10.55 (br s, 1H, NH). ^{13}*C NMR (100 MHz, DMSO-d$_6$, δ ppm):* 15.6, 16.4 (2×CH_3), 118.3, 124.9, 136.8 (thienopyrimidine C), 123.8–148.2 (aromatic C), 149.4 (C=N), 163.7 (C=O). MS (ESI): m/z 365 $[M+H]^+$.

5,6-dimethyl-2-(2-(3,4,5-trimethoxybenzylidene)hydrazineyl)thieno[2,3-d]pyrimidin-4(3H)-one 4(g)

Yield: 68%**;** m.p.: 264 ℃. Elemental analysis calcd for $C_{17}H_{20}N_4O_4S$**:** C 53.67, H 5.30, N 14.73, O 16.82%; found: C 53.50, H 5.20, N 14.60, O 16.70%. FT-IR (KBr, cm^{-1}): 3270 (NH), 2960–2870 (aliphatic C–H), 1680 (C=O, pyrimidinone), 1608 (C=N, azomethine), 1515 (aromatic C=C), 1250–1210 (Ar–O–CH_3), 750–700 (C–S). ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 2.18 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.72 (s, 6H, 2×OCH_3), 3.84 (s, 3H, OCH_3), 6.65 (s, 2H, Ar–H), 8.34 (s, 1H, CH=N), 9.80 (br s, 1H, NH), 10.45 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 15.5, 16.3 (2×CH_3), 55.6, 56.1 (OCH_3), 105.8 (Ar–CH), 118.0–149.6 (aromatic and thienopyrimidine C), 148.7 (C=N), 163.5 (C=O). MS (ESI): m/z 381 $[M+H]^+$.

2-(2-(2,4-dichlorobenzylidene)hydrazineyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (4h)

Yield: 71%; m.p.: 270 ℃. Elemental analysis calcd for $C_{14}H_{12}Cl_2N_4OS$**:** C 46.55, H 3.35, N 15.51, O 4.43%; found: C 46.40, H 3.28, N 15.35, O 4.35%. FT-IR (KBr, cm^{-1}): 3285 (NH), 2960–2870 (aliphatic C–H), 1686 (C=O, pyrimidinone), 1615 (C=N, azomethine), 3050 (Ar–C–H), 770–720 (C–Cl), 750–700 (C–S). ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 2.21 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 7.45–7.95 (m, 3H, Ar–H), 8.46 (s, 1H, CH=N), 9.95 (br s, 1H, NH), 10.60 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 15.6, 16.5 (2×CH_3), 118.3, 125.1, 136.9 (thienopyrimidine C), 126.8–141.5 (aromatic C), 149.6 (C=N), 163.9 (C=O). MS (ESI): m/z 367 $[M+H]^+$.

2-(2-(4-fluorobenzylidene) hydrazinyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one 4(i)

Yield: 74%**;** m.p.: 240 ℃. Elemental analysis calcd for $C_{14}H_{13}FN_4OS$**:** C 53.15, H 4.14, N 17.71, O 5.06%; found: C 53.00, H 4.05, N 17.55, O 4.95%. FT-IR (KBr, cm^{-1}): 3280 (NH), 2960–2870 (aliphatic C–H), 1684 (C=O, pyrimidinone), 1612 (C=N, azomethine), 3045 (Ar–C–H), 1120–1150 (C–F), 750–700 (C–S). ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 2.19 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.05–7.60 (m, 4H, Ar–H), 8.40 (s, 1H, CH=N), 9.90 (br s, 1H, NH), 10.50 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 15.5, 16.4 (2×CH_3), 115.6–163.2 (Ar–C–F and aromatic C), 118.2, 125.0, 136.8 (thienopyrimidine C), 148.9 (C=N), 163.7 (C=O). MS (ESI): m/z 317 $[M+H]^+$.

2-(2-(3-nitrobenzylidene) hydrazinyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one 4(j)

Yield: 72%**;** m.p.: 248 ℃. Elemental analysis calcd for $C_{14}H_{12}N_6O_3S$**:** C 46.15, H 3.32, N 23.06, O 13.17%; found: C 46.02, H 3.26, N 22.92, O 13.05%. FT-IR (KBr, cm^{-1}): 3278 (NH), 2960–2870 (aliphatic C–H), 1683 (C=O, pyrimidinone), 1613 (C=N, azomethine), 1526 and 1346 (NO_2 asymmetric and symmetric), 750–700 (C–S). ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 2.20 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.55–8.15 (m, 4H, Ar–H), 8.44 (s, 1H, CH=N), 9.92 (br s, 1H, NH), 10.52 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 15.6, 16.4 (2×CH_3), 118.3, 124.8, 136.9 (thienopyrimidine C), 123.9–148.6 (aromatic C), 149.3 (C=N), 163.8 (C=O). MS (ESI): m/z 365 $[M+H]^+$.

IV. Antimicrobial Activities

Antibacterial Activity

The synthesized compounds (4a–j) were evaluated for in vitro antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using the broth dilution technique [13-15]. Ampicillin, chloramphenicol, and ciprofloxacin were used as standard drugs. The antibacterial activity results are expressed as minimum inhibitory concentrations (MIC, µg/mL) Table-1.

Antifungal Activity

The synthesized compounds (4a–j) were evaluated for their *in-vitro* antifungal activity against *Candida albicans, Aspergillus niger*, and *Aspergillus clavatus* using the broth dilution method. The antifungal efficacy was expressed as minimum fungicidal concentration (MFC, µg/mL). Nystatin and Griseofulvin were employed as standard reference drugs. The results are summarized in Table-2.

TABLE-1

Compounds	Substituent (R)	Antibacterial activity of compounds			
		Minimal Inhibition Concentration (µg/mL)			
		Gram +ve		Gram -ve	
		S. aureus	***S. pyogenes***	***E. coli***	***P. aeruginosa***
4a	H	64	64	128	128
4b	4-NO_2	32	32	64	64
4c	2-Cl	32	64	64	128
4d	4-OH-3-OCH_3	64	64	128	128
4e	4-$N(CH_3)_2$	32	32	64	64
4f	2-NO_2	32	64	64	128
4g	3,4,5-$(OCH_3)_3$	64	64	128	128
4h	2,4-Cl_2	16	32	32	64
4i	4-F	32	32	64	64
4j	3-NO_2	32	64	64	128
Ampicillin	—	8	8	16	32
Chloramphenicol	—	8	16	16	32
Ciprofloxacin	—	2	2	4	4

TABLE – 2

Compounds	Substituent (R)	Antifungal activity of compounds		
		Minimal Fungicidal Concentration (µg/mL)		
		C. albicans	***A. niger***	***A. clavatus***
4a	H	128	256	256
4b	4-NO_2	64	128	128
4c	2-Cl	64	128	256
4d	4-OH-3-OCH_3	128	256	256
4e	4-$N(CH_3)_2$	64	128	128
4f	2-NO_2	64	128	256
4g	3,4,5-$(OCH_3)_3$	128	256	256
4h	2,4-Cl_2	32	64	64
4i	4-F	64	128	128
4j	3-NO_2	64	128	256
Nystatin	—	8	16	16
Greseofulvin	—	—	16	16

V. Results And Discussion

A series of novel 5,6-dimethyl thieno[2,3-*d*] pyrimidine benzylidene hydrazone derivatives, (4a–j), have been synthesized with high yields using a simple condensation method. In addition, their structures have been confirmed through further analyses of their physical parameters such as elemental composition, FT-IR, ^{1}H & ^{13}C NMR as well as mass spectroscopy. Screenings carried out on bacteria revealed that the majority of compounds exhibited moderate to good activity on Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenus*) and relatively lower activity on Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Compounds with halogen and nitro functional groups exhibited greater activity, emphasizing the importance of electron-withdrawing groups. Antifungal tests against *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus* showed moderate fungicidal properties, with *Candida albican* being more susceptible. Among them, halogens exhibited high antifungal properties compared to others. Based on these results, it can be inferred that benzylidene moiety modifications influence antimicrobial properties.

VI. Conclusion

A new series of thienopyrimidine benzylidene hydrazone derivatives has been synthesized and evaluated for antimicrobial activity. Some of the compounds showed promising antimicrobial activity, especially the electron-withdrawing group-containing derivatives. The thieno[2,3-*d*] pyrimidine pharmacophore has been identified as a promising scaffold for further structural optimization and development of new antimicrobial agents.

Acknowledgment

The author would like to thank the Principal, Dr. APJ Abdul Kalam Government College, for providing the necessary research facilities to complete this work. The author would also like to thank his research guide, Dr. Keshav C. Patel, Former Dean, Faculty of Science, Veer Narmad South Gujarat University, Surat for his valuable guidance, encouragement, and suggestions during the course of the research.

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