



Research Article

Thienopyrimidine-thiazolidinone Scaffolds: Synthesis, Characterization and Targeting Various Bioactivities

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Abstract

A new set of 5,6-dimethyl-2-(4-oxo-2-(substitutedphenyl)thiazolidin-3-ylamino)thieno[2,3-*d*]pyrimidin-4(3*H*)-one compounds (5a-5j) was successfully synthesized using a rational multi-step synthetic strategy starting from ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate. The synthetic route started with the formation of the thienopyrimidinone scaffold, followed by hydrazinolysis to give the corresponding hydrazine intermediate. The Schiff base reaction with various substituted aromatic aldehydes gave the key imine intermediates, which underwent cyclocondensation with thioglycolic acid to give the desired thiazolidinone hybrids. The protocol used proved efficient, as the desired compounds were obtained in good to excellent yields. All the newly synthesized compounds were fully characterized using elemental analysis and various spectroscopic techniques, including IR, ¹H NMR, ¹³C NMR, and mass spectrometry, thus confirming the proposed molecular structures. The characteristic spectral data provided evidence for the successful incorporation of both the thienopyrimidine and thiazolidinone pharmacophores. The synthesized compounds were tested for their multi-target biological properties, including antibacterial, antifungal, and antioxidant activities. Antibacterial activity was tested against representative Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, while antifungal activity was tested against *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*. In addition, antioxidant activity was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Several compounds showed significant antimicrobial activity, especially those with electron-withdrawing substituents, indicating enhanced interaction with microbial targets. However, compounds with electron-donating substituents showed better antioxidant activity, indicating favorable radical scavenging capacity. The results suggest that thienopyrimidine–thiazolidinone hybrids constitute a promising class of multifunctional heterocycles, meriting further investigation for potential therapeutic applications.

Keywords

Thieno[2, 3-*d*]Pyrimidine, Thiazolidinone, Antibacterial Activity, Antifungal Activity, Antioxidant Activity

1. Introduction

Heterocyclic compounds play a pivotal role in modern medicinal chemistry due to their structural diversity and broad spectrum of biological activities. Among nitrogen- and sulfur-containing het-

erocycles, thienopyrimidine derivatives have gained significant attention as privileged scaffolds because of their structural similarity to naturally occurring purine bases, enabling effective interaction

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with a variety of biological targets. Numerous studies have reported thieno [2, 3-*d*] pyrimidine derivatives to possess antibacterial, antifungal, anticancer, anti-inflammatory, antioxidant, and enzyme inhibitory activities, highlighting their importance in drug discovery programs [1-8].

Likewise, thiazolidin-4-one derivatives represent another important class of bioactive heterocycles known for their antimicrobial, antifungal, antitubercular, antiviral, antioxidant, and anticancer activities. The presence of a thiazolidinone ring provides favorable pharmacokinetic properties and multiple sites for structural modification, which contributes to enhanced biological activity against pathogenic microorganisms [9, 10]. Several thiazolidinone-based compounds have shown potent activity against both Gram-positive and Gram-negative bacteria, as well as clinically relevant fungal strains [11].

The concept of molecular hybridization, which involves combining two or more biologically active pharmacophores into a single molecular framework, has emerged as an effective strategy to improve biological efficacy and overcome antimicrobial resistance. Hybrid molecules containing both thienopyrimidine and thiazolidinone moieties have demonstrated synergistic biological effects and multi-target activity profiles, making them attractive candidates for the development of novel therapeutic agents [12, 13].

Infectious diseases caused by bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as fungal pathogens including *Candida albicans* and *Aspergillus* species, remain a serious global health concern due to the increasing emergence of drug-resistant strains. In addition, oxidative stress has been implicated in the pathogenesis of numerous chronic diseases, emphasizing the importance of compounds exhibiting antioxidant activity [14]. In this context, the design and synthesis of new heterocyclic hybrids with antibacterial, antifungal, and antioxidant properties is of considerable scientific and therapeutic interest.

2. Materials and Synthetic Methods

2.1. Materials

All the chemicals used for this research project were of general-purpose purity and obtained from standard chemical suppliers. No further purification of any of these compounds was required. When needed, the solvents used for this analysis were dried using conventional methods. Thin-layer chromatography, employing silica gel plates, with UV detection at 254 nm, 365 nm, and iodine vapor, was used to monitor the

progress of the reaction. IR analysis was performed with KBr discs, covering a wavelength range between 4000 and 400 cm^{-1} . The ^1H & ^{13}C NMR spectra were obtained using a BRUKER AVANCE II NMR spectrometer, with 400 MHz for ^1H and 100 MHz for ^{13}C spectra, employing DMSO- d_6 as the solvent and TMS as the reference compound. LC-MS was used for the mass spectra.

2.2. Synthetic Methods

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

A mixture of ethyl 2-amino-4, 5-dimethylthiophene-3-carboxylate (1) (0.01 mol) and potassium thiocyanate (1.5 g, 0.015 mol) was dissolved in 1, 4-dioxane (10 mL). Concentrated hydrochloric acid (2 mL) was added dropwise with stirring, and the reaction mixture was heated under reflux for 15 hours. Upon completion of the reaction, the mixture was allowed to cool to room temperature and then carefully poured into ice-cold water with continuous stirring. The precipitated pale-yellow solid was collected by filtration, washed thoroughly with water, dried, and recrystallized from ethanol to afford compound (2). Yield 52% and M. P. 268-270 °C. M. F.: $\text{C}_8\text{H}_8\text{N}_2\text{OS}_2$; Molecular weight: 212.29. Elemental analysis: Calcd. (%) C 45.26, H 3.80, N 13.20, O 7.54; Found (%) C 45.11, H 3.72, N 13.05, O 7.40. IR (KBr, cm^{-1}): 3235 (N-H), 2550-2595 (S-H), 2962-2874 (aliphatic C-H), 1682 (C=O), 1608 (C=N), 1518 (C=C), 742-705 (C-S). ^1H NMR (400 MHz, DMSO- d_6 , δ ppm): 2.18 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 10.12 (br s, 1H, N-H), 12.74 (br s, 1H, S-H). ^{13}C NMR (100 MHz, DMSO- d_6 , δ ppm): 15.5, 16.2 ($2 \times \text{CH}_3$), 117.9, 124.3, 137.1 (thienopyrimidine ring carbons), 158.6 (C=N), 163.2 (C=O). Mass spectrum (ESI): m/z 229 $[\text{M}+\text{H}]^+$.

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

Compound (2) (0.01 mol) was dissolved in methanol (10 mL), followed by the addition of hydrazine hydrate (1.6 g, 0.05 mol). The reaction mixture was heated under reflux for 8 hours, after which it was allowed to stand at ambient temperature for approximately 16 hours to ensure completion of the transformation. The resulting solid product was collected by filtration, thoroughly washed with water to remove residual reagents, dried, and purified by recrystallization from ethanol, yielding compound (3). Yield 68% and M. P. 299-301 °C. M. F.: $\text{C}_8\text{H}_{10}\text{N}_4\text{OS}$; Molecular weight: 210.26.

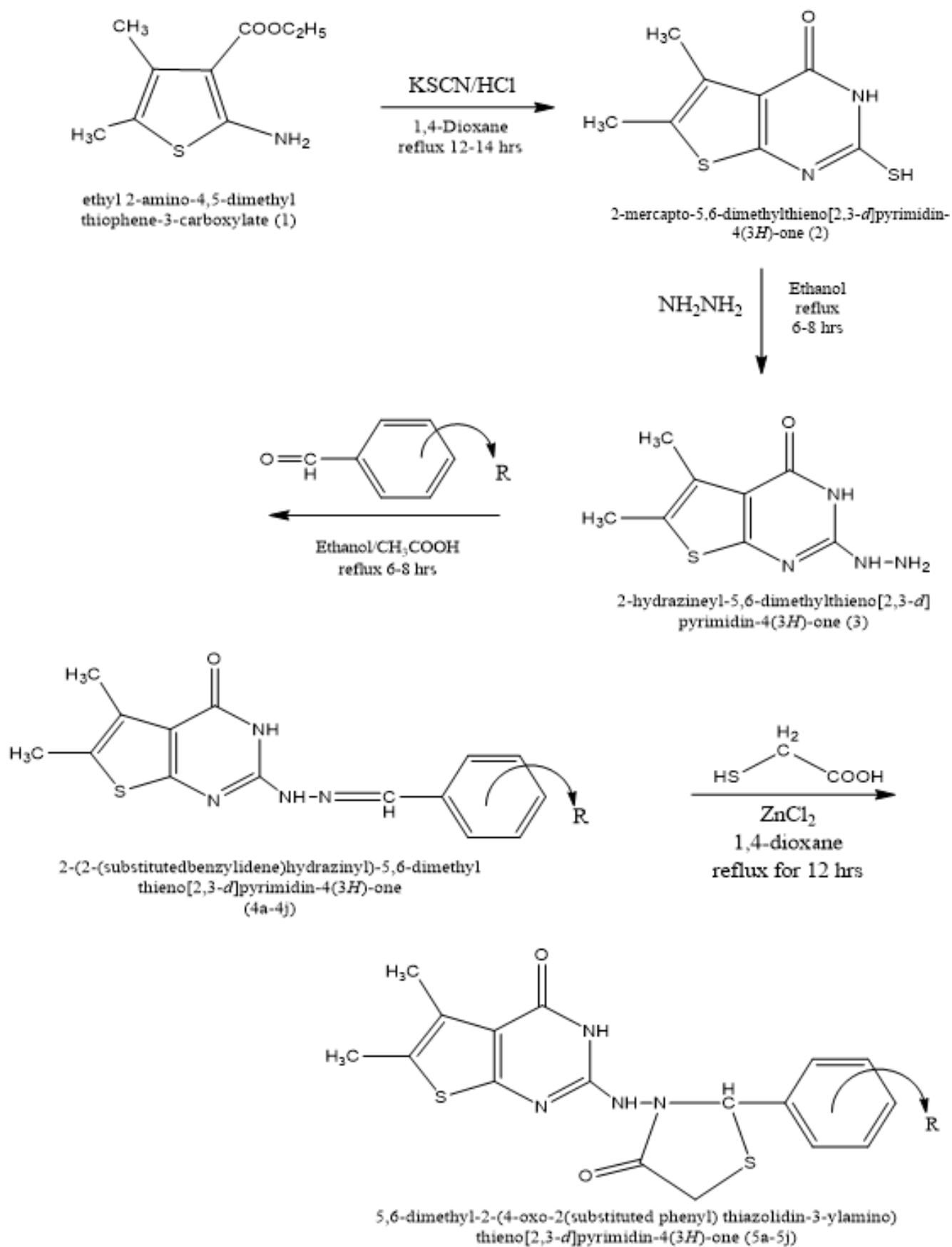


Figure 1. Synthesis of Thienopyrimidine-Thiazolidinone scaffolds.

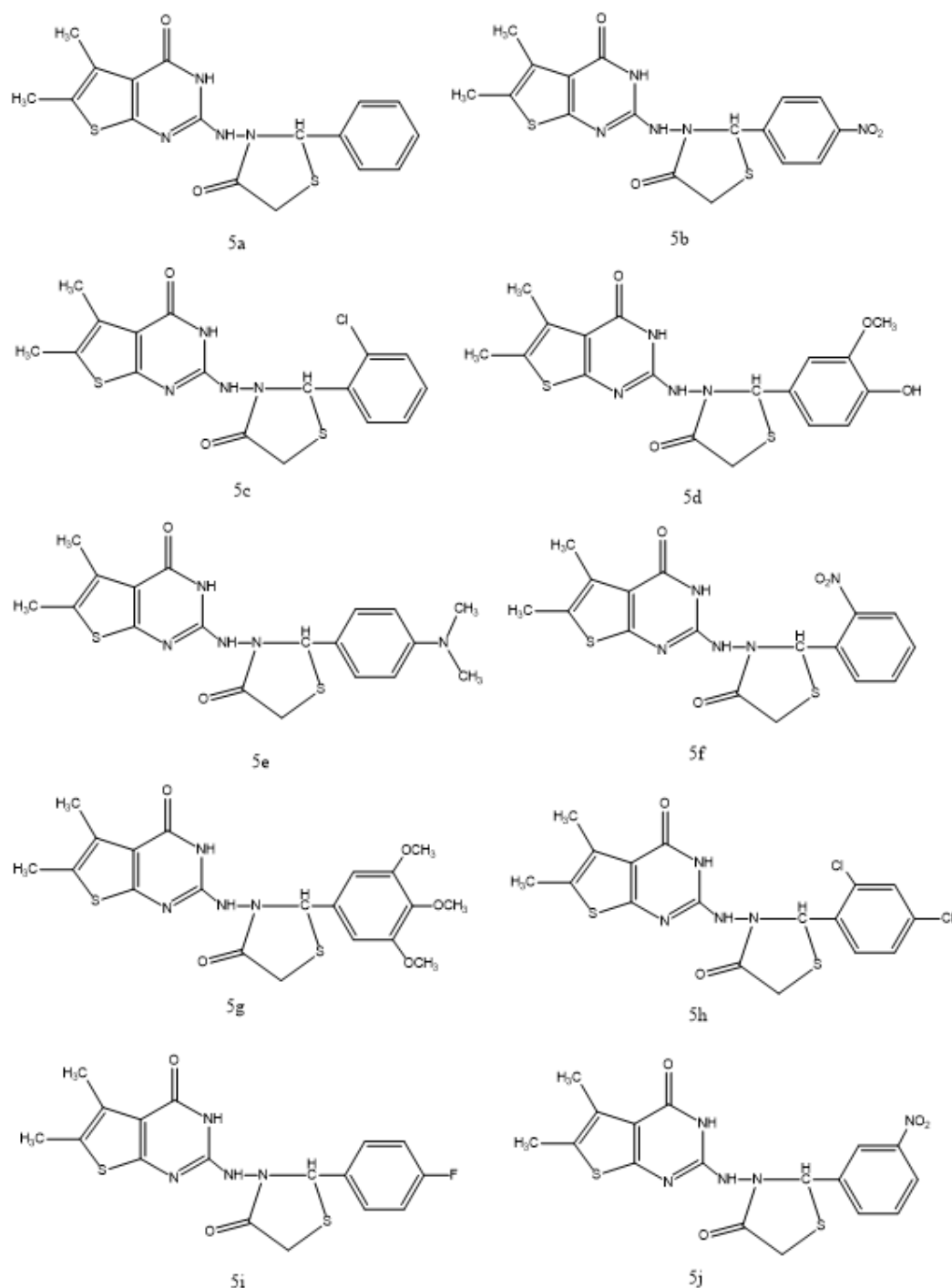


Figure 2. Structure of Thienopyrimidine-Thiazolidinone scaffolds (5a-5j).

Elemental analysis: Calcd. (%) C 45.70, H 4.79, N 26.65, O 7.61; Found (%) C 45.55, H 4.70, N 26.50, O 7.45. IR (KBr, cm^{-1}): 3330-3210 (NH, NH_2), 2965-2875 (aliphatic C-H), 1685 (C=O stretching), 1605 (C=N stretching), 755-705 (C-S stretching). ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ ppm): 2.20 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 4.62 (br s, 2H, NH_2), 9.82 (br s, 1H, NH), 10.42 (br s, 1H, NH). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, δ ppm): 15.3, 16.1 (2 $\times$ CH_3), 117.7, 125.2, 137.2 (thienopyrimidine carbons), 156.7 (C=N), 163.1 (C=O). Mass spectrum (ESI): m/z 211 $[\text{M}+\text{H}]^+$.

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

Compound (3) (0.01 mol) was dissolved in methanol (10 mL), and the corresponding substituted aromatic aldehyde (0.01 mol) was added. The reaction mixture was acidified with a few drops of glacial acetic acid and heated under reflux on a water bath for approximately 8-9 hours. The advancement of

the reaction was periodically monitored by thin-layer chromatography (TLC). Upon completion, the mixture was allowed to cool to ambient temperature, leading to the formation of a solid precipitate. The separated product was collected by filtration, washed with cold methanol, dried, and further purified by recrystallization from ethanol, yielding the desired Schiff base derivatives (4a-4j) in satisfactory yields.

2.2.4. Synthesis of 5,6-dimethyl-2-((4-oxo-2-(substitutedphenyl)thiazolidin-3-ylamino)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5a-5j)

Equimolar quantities of the appropriate Schiff base intermediates (4a-4j) (0.01 mol) and thioglycolic acid (1.39 g, 0.015 mol) were dissolved in 1, 4-dioxane (20 mL). A catalytic amount of anhydrous zinc chloride was added, and the reaction mixture was heated under reflux for 12 hours. The progress of the cyclization was monitored periodically by thin-layer chromatography (TLC). Upon completion of the reaction, the mixture was allowed to cool and then carefully poured into ice-cold water with continuous stirring. The resulting solid product was collected by filtration, thoroughly washed with water to remove residual reagents, and dried. Final purification was achieved by recrystallization from an acetone-alcohol mixture, affording the desired thiazolidinone derivatives (5a-5j) in good yields.

(i). 5,6-dimethyl-2-((4-oxo-2-phenylthiazolidin-3-yl)amino)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5a)

Yield 72% and M. P. 228-230°C. M. F.: $C_{17}H_{16}N_4O_2S_2$; Molecular weight: 372.46. Elemental analysis: Calcd. (%) C 54.82, H 4.33, N 15.04, O 8.59; Found (%) C 54.65, H 4.26, N 14.92, O 8.41. IR (KBr, cm^{-1}): 3280 (NH), 3062 (Ar-CH), 2960-2870 (aliphatic C-H), 1715 (C=O, thiazolidinone), 1680 (C=O, pyrimidinone), 1610 (C=N), 1518 (C=C), 748 (C-S). 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 2.18 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.32 (s, 2H, CH_2 , thiazolidinone), 4.05 (s, 1H, CH, thiazolidinone), 7.22-7.56 (m, 5H, Ar-H), 9.68 (br s, 1H, NH), 10.44 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO- d_6 , δ ppm): 15.6, 16.4 ($2\times CH_3$), 33.8 (CH_2), 58.2 (CH), 118.4, 124.6, 136.9 (thienopyrimidine C), 127.5-134.8 (aromatic C), 149.1 (C=N), 165.2 (C=O, pyrimidinone), 172.8 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 373 $[M+H]^+$.

(ii). 5,6-dimethyl-2-((2-(4-nitrophenyl)-4-oxothiazolidine-3-yl) amino)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5b)

Yield 70% and M. P. 242-244°C. M. F.: $C_{17}H_{15}N_5O_4S_2$; Molecular weight: 417.45. Elemental analysis: Calcd. (%) C 48.91, H 3.62, N 16.78, O 15.33; Found (%) C 48.75, H 3.54, N 16.62, O 15.10. IR (KBr, cm^{-1}): 3276 (NH), 3060 (Ar-CH), 2955-2865 (aliphatic C-H), 1714 (C=O, thiazolidinone), 1676 (C=O, pyrimidinone), 1612 (C=N), 1528 and 1348 (NO_2 asymmetric and symmetric), 746 (C-S). 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 2.19 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.34

(s, 2H, CH_2 , thiazolidinone), 4.08 (s, 1H, CH, thiazolidinone), 7.68 (d, 2H, Ar-H), 8.12 (d, 2H, Ar-H), 9.72 (br s, 1H, NH), 10.48 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO- d_6 , δ ppm): 15.6, 16.5 ($2\times CH_3$), 33.9 (CH_2), 58.4 (CH), 118.6, 124.8, 137.1 (thienopyrimidine C), 124.5-148.9 (aromatic C), 149.6 (C=N), 165.4 (C=O, pyrimidinone), 173.0 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 418 $[M+H]^+$.

(iii). 2-((2-(2-chlorophenyl)-4-oxothiazolidin-3-yl) amino)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (5c)

Yield 71% and M. P. 236-238°C. M. F.: $C_{17}H_{15}ClN_4O_2S_2$; Molecular weight: 406.90. Elemental analysis: Calcd. (%) C 50.18, H 3.72, N 13.76, O 7.87; Found (%) C 50.02, H 3.65, N 13.60, O 7.70. IR (KBr, cm^{-1}): 3282 (NH), 3060 (Ar-CH), 2960-2870 (aliphatic C-H), 1716 (C=O, thiazolidinone), 1680 (C=O, pyrimidinone), 1611 (C=N), 1516 (C=C), 762 (C-Cl), 748 (C-S). 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 2.18 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.33 (s, 2H, CH_2 , thiazolidinone), 4.06 (s, 1H, CH, thiazolidinone), 7.30-7.65 (m, 4H, Ar-H), 9.70 (br s, 1H, NH), 10.46 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO- d_6 , δ ppm): 15.6, 16.5 ($2\times CH_3$), 33.9 (CH_2), 58.3 (CH), 118.5, 124.7, 137.0 (thienopyrimidine C), 126.4-138.9 (aromatic C), 149.3 (C=N), 165.3 (C=O, pyrimidinone), 172.9 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 408 $[M+H]^+$.

(iv). 2-((2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)amino)-5,6-dimethyl thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5d)

Yield 69% and M. P. 248-250°C. M. F.: $C_{18}H_{18}N_4O_4S_2$; Molecular weight: 418.49. Elemental analysis: Calcd. (%) C 51.66, H 4.34, N 13.39, O 15.29; Found (%) C 51.48, H 4.26, N 13.22, O 15.05. IR (KBr, cm^{-1}): 3365 (O-H), 3275 (NH), 3060 (Ar-CH), 2955-2865 (aliphatic C-H), 1712 (C=O, thiazolidinone), 1676 (C=O, pyrimidinone), 1610 (C=N), 1515 (C=C), 1248 (Ar-O- CH_3), 748 (C-S). 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 2.18 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.33 (s, 2H, CH_2 , thiazolidinone), 3.78 (s, 3H, OCH_3), 4.04 (s, 1H, CH, thiazolidinone), 6.72-6.95 (m, 3H, Ar-H), 9.62 (br s, 1H, NH), 9.88 (s, 1H, OH), 10.44 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO- d_6 , δ ppm): 15.6, 16.4 ($2\times CH_3$), 33.8 (CH_2), 56.1 (OCH_3), 58.2 (CH), 112.4-147.8 (aromatic C), 118.3, 124.6, 136.8 (thienopyrimidine C), 149.0 (C=N), 165.2 (C=O, pyrimidinone), 172.6 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 419 $[M+H]^+$.

(v). 2-((2-(4-(dimethylamino)phenyl)-4-oxothiazolidin-3-yl)amino)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (5e)

Yield 73% and M. P. 220-222°C. M. F.: $C_{19}H_{21}N_5O_2S_2$; Molecular weight: 415.53. Elemental analysis: Calcd. (%) C 54.92, H 5.09, N 16.86, O 7.70; Found (%) C 54.78, H 4.98, N 16.70, O 7.52. IR (KBr, cm^{-1}): 3278 (NH), 3058 (Ar-CH), 2962-2870 (aliphatic C-H), 1714 (C=O, thiazolidinone), 1678

(C=O, pyrimidinone), 1608 (C=N), 1520 (C=C), 1350 (C-N), 748 (C-S). ¹H NMR (400 MHz, DMSO-d₆, δ ppm): 2.17 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 2.92 (s, 6H, N(CH₃)₂), 3.31 (s, 2H, CH₂, thiazolidinone), 4.03 (s, 1H, CH, thiazolidinone), 6.68 (d, 2H, Ar-H), 7.38 (d, 2H, Ar-H), 9.60 (br s, 1H, NH), 10.42 (br s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆, δ ppm): 15.5, 16.4 (2×CH₃), 33.7 (CH₂), 40.3 (N(CH₃)₂), 58.1 (CH), 112.6-149.2 (aromatic C), 118.2, 124.5, 136.7 (thienopyrimidine C), 148.8 (C=N), 165.1 (C=O, pyrimidinone), 172.7 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 416 [M+H]⁺.

(vi). 5,6-dimethyl-2-((2-(2-nitrophenyl)-4-oxothiazolidin-3-yl)amino)thieno[2,3-d]pyrimidin-4(3H)-one (5f)

Yield 68% and M. P. 240-242°C. M. F.: C₁₇H₁₅N₅O₄S₂; Molecular weight: 417.45. Elemental analysis: Calcd. (%) C 48.91, H 3.62, N 16.78, O 15.33; Found (%) C 48.72, H 3.55, N 16.60, O 15.12. IR (KBr, cm⁻¹): 3274 (NH), 3062 (Ar-CH), 2958-2868 (aliphatic C-H), 1716 (C=O, thiazolidinone), 1677 (C=O, pyrimidinone), 1611 (C=N), 1526 and 1346 (NO₂ asymmetric and symmetric), 748 (C-S). ¹H NMR (400 MHz, DMSO-d₆, δ ppm): 2.18 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 3.34 (s, 2H, CH₂, thiazolidinone), 4.07 (s, 1H, CH, thiazolidinone), 7.45-8.10 (m, 4H, Ar-H), 9.70 (br s, 1H, NH), 10.46 (br s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆, δ ppm): 15.6, 16.5 (2×CH₃), 33.9 (CH₂), 58.3 (CH), 118.5, 124.8, 137.1 (thienopyrimidine C), 123.8-148.7 (aromatic C), 149.4 (C=N), 165.3 (C=O, pyrimidinone), 173.0 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 418 [M+H]⁺.

(vii). 5,6-dimethyl-2-((4-oxo-2-(3,4,5-tri methoxy phenyl)thiazolidin-3-yl)amino)thieno[2,3-d]pyrimidin-4(3H)-one (5g)

Yield 67% and M. P. 214-216°C. M. F.: C₂₀H₂₂N₄O₅S₂; Molecular weight: 462.54. Elemental analysis: Calcd. (%) C 51.94, H 4.79, N 12.11, O 17.29; Found (%) C 51.78, H 4.68, N 11.98, O 17.05. IR (KBr, cm⁻¹): 3276 (NH), 3060 (Ar-CH), 2956-2864 (aliphatic C-H), 1712 (C=O, thiazolidinone), 1676 (C=O, pyrimidinone), 1609 (C=N), 1516 (C=C), 1252-1218 (Ar-O-CH₃), 748 (C-S). ¹H NMR (400 MHz, DMSO-d₆, δ ppm): 2.18 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 3.33 (s, 2H, CH₂, thiazolidinone), 3.70 (s, 6H, 2×OCH₃), 3.82 (s, 3H, OCH₃), 4.05 (s, 1H, CH, thiazolidinone), 6.58 (s, 2H, Ar-H), 9.62 (br s, 1H, NH), 10.44 (br s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆, δ ppm): 15.6, 16.4 (2×CH₃), 33.8 (CH₂), 55.7, 56.2 (OCH₃), 58.2 (CH), 105.6 (Ar-CH), 118.3, 124.6, 136.8 (thienopyrimidine C), 138.4-153.6 (aromatic C), 149.0 (C=N), 165.2 (C=O, pyrimidinone), 172.7 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 463 [M+H]⁺.

(viii). 2-((2-(2,4-dichlorophenyl)-4-oxothiazolidin-3-yl)amino)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (5h)

Yield 70% and M. P. 252-254°C. M. F.: C₁₇H₁₄Cl₂N₄O₂S₂;

Molecular weight: 441.34. Elemental analysis: Calcd. (%) C 46.26, H 3.20, N 12.70, O 7.25; Found (%) C 46.10, H 3.12, N 12.56, O 7.08. IR (KBr, cm⁻¹): 3278 (NH), 3060 (Ar-CH), 2960-2870 (aliphatic C-H), 1716 (C=O, thiazolidinone), 1678 (C=O, pyrimidinone), 1611 (C=N), 1518 (C=C), 760 and 730 (C-Cl), 748 (C-S). ¹H NMR (400 MHz, DMSO-d₆, δ ppm): 2.18 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 3.34 (s, 2H, CH₂, thiazolidinone), 4.07 (s, 1H, CH, thiazolidinone), 7.45-7.95 (m, 3H, Ar-H), 9.72 (br s, 1H, NH), 10.48 (br s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆, δ ppm): 15.6, 16.5 (2×CH₃), 33.9 (CH₂), 58.3 (CH), 118.5, 124.8, 137.1 (thienopyrimidine C), 126.6-141.2 (aromatic C), 149.4 (C=N), 165.3 (C=O, pyrimidinone), 173.0 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 442 [M+H]⁺.

(ix). 2-((2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)amino)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (5i)

Yield 72% and M. P. 230-232°C. M. F.: C₁₇H₁₅FN₄O₂S₂; Molecular weight: 390.45. Elemental analysis: Calcd. (%) C 52.30, H 3.87, N 14.35, O 8.20; Found (%) C 52.12, H 3.78, N 14.20, O 8.02. IR (KBr, cm⁻¹): 3279 (NH), 3062 (Ar-CH), 2960-2870 (aliphatic C-H), 1714 (C=O, thiazolidinone), 1679 (C=O, pyrimidinone), 1610 (C=N), 1517 (C=C), 1150-1125 (C-F), 748 (C-S). ¹H NMR (400 MHz, DMSO-d₆, δ ppm): 2.18 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 3.33 (s, 2H, CH₂, thiazolidinone), 4.06 (s, 1H, CH, thiazolidinone), 7.05-7.55 (m, 4H, Ar-H), 9.68 (br s, 1H, NH), 10.45 (br s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆, δ ppm): 15.6, 16.5 (2×CH₃), 33.9 (CH₂), 58.3 (CH), 116.2-163.0 (Ar-C-F), 118.4, 124.7, 137.0 (thienopyrimidine C), 149.2 (C=N), 165.3 (C=O, pyrimidinone), 172.9 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 391 [M+H]⁺.

(x). 5,6-dimethyl-2-((2-(3-nitrophenyl)-4-oxothiazolidin-3-yl)amino)thieno[2,3-d]pyrimidin-4(3H)-one (5j)

Yield 69% and M. P. 238-240°C. M. F.: C₁₇H₁₅N₅O₄S₂; Molecular weight: 417.45. Elemental analysis: Calcd. (%) C 48.91, H 3.62, N 16.78, O 15.33; Found (%) C 48.70, H 3.55, N 16.60%. IR (KBr, cm⁻¹): 3276 (NH), 3060 (Ar-CH), 2958-2866 (aliphatic C-H), 1715 (C=O, thiazolidinone), 1677 (C=O, pyrimidinone), 1611 (C=N), 1525 and 1347 (NO₂ asymmetric and symmetric stretching), 748 (C-S). ¹H NMR (400 MHz, DMSO-d₆, δ ppm): 2.18 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 3.34 (s, 2H, CH₂, thiazolidinone), 4.07 (s, 1H, CH, thiazolidinone), 7.55-8.20 (m, 4H, Ar-H), 9.70 (br s, 1H, NH), 10.47 (br s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆, δ ppm): 15.6, 16.5 (2×CH₃), 33.9 (CH₂), 58.3 (CH), 118.5, 124.8, 137.1 (thienopyrimidine C), 123.6-149.0 (aromatic C), 149.5 (C=N), 165.3 (C=O, pyrimidinone), 173.0 (C=O, thiazolidinone). Mass spectrum (ESI): m/z 418 [M+H]⁺.

2.3. Biological Activities

2.3.1. Antibacterial Activities

The newly synthesized derivatives (5a-5j) were screened for their *in vitro* antibacterial activity against representative Gram-positive bacterial strains, namely *S. aureus* and *S. pyogenes*, as well as Gram-negative bacteria, including *E. coli* and *P. aeruginosa*. The evaluation was performed employing the broth dilution method, following established procedures described in the literature [15, 16]. Ampicillin, chloramphenicol, and ciprofloxacin served as reference standards for comparison. The antibacterial potency of the tested compounds was determined in terms of minimum inhibitory concentration (MIC, $\mu\text{g/mL}$) values, and the results are summarized in Table 1.

2.3.2. Antifungal Activities

The synthesized compounds (5a-5j) were assessed for their *in vitro* antifungal activity against selected fungal strains, including *C. albicans*, *A. niger*, and *A. clavatus*. The antifungal evaluation was carried out using the broth dilution technique in accordance with reported methodologies. The antifungal

potential of the tested compounds was quantified by determining their minimum fungicidal concentration (MFC, $\mu\text{g/mL}$) values. Nystatin and griseofulvin were used as standard reference drugs for comparative analysis. The obtained results are presented in Table 2.

2.3.3. Antioxidant Activities

The antioxidant potential of the synthesized derivatives (5a-5j) was investigated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method, following a reported protocol with slight modifications. A freshly prepared methanolic DPPH solution (0.1 mM) was employed for the assay. Test samples at different concentrations were prepared in methanol and subsequently treated with the DPPH reagent. The resulting reaction mixtures were kept in the dark at ambient temperature for 30 minutes to ensure completion of the radical scavenging process. The reduction in absorbance was then recorded spectrophotometrically at 517 nm. Ascorbic acid and butylated hydroxytoluene (BHT) were used as standard antioxidant references for comparison. The experimental results are compiled in Table 3.

Table 1. Antibacterial Activity (MIC, $\mu\text{g/mL}$) of Compounds (5a-5j).

Compounds	Minimal Inhibition Concentration ($\mu\text{g/mL}$)			
	Gram +ve		Gram -ve	
	<i>S. aureus</i>	<i>S. pyogenes</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
5a	62.5	62.5	125	250
5b	31.25	31.25	62.5	125
5c	31.25	62.5	62.5	125
5d	62.5	62.5	125	250
5e	31.25	31.25	62.5	125
5f	31.25	31.25	62.5	125
5g	62.5	62.5	125	250
5h	15.62	31.25	31.25	62.5
5i	31.25	31.25	62.5	125
5j	31.25	31.25	62.5	125
Ampicillin	10	10	25	50
Chloramphenicol	12.5	12.5	12.5	25
Ciprofloxacin	6.25	6.25	6.25	12.5

Table 2. Antifungal Activity (MFC, $\mu\text{g/mL}$) of compounds (5a-5j).

Compounds	Minimal Fungicidal Concentration ($\mu\text{g/mL}$)		
	<i>C. albicans</i>	<i>A. niger</i>	<i>A. clavatus</i>
5a	125	250	250
5b	62.5	125	125
5c	62.5	125	125
5d	125	250	250
5e	62.5	125	125
5f	62.5	125	125
5g	125	250	250
5h	31.25	62.5	62.5
5i	62.5	125	125
5j	62.5	125	125
Nystatin	10	20	20
Greseofulvin	25	50	50

Table 3. Antioxidant Activity of Compounds (5a-5j) (DPPH Assay).

Compounds	IC ₅₀ ($\mu\text{g/mL}$)
5a	78.5
5b	65.2
5c	70.8
5d	32.4
5e	48.6
5f	60.3
5g	28.9
5h	58.7
5i	54.2
5j	62.8
Ascorbic acid	21.5
BHT	26.3

3. Results and Discussion

A set of 5, 6-dimethyl-2-(4-oxo-2-(substitutedphenyl) thiazolidin-3-ylamino) thieno [2, 3-*d*] pyrimidin-4 (3*H*)-one derivatives (5a-5j) was prepared through a multi-step procedure starting from ethyl 2-amino-4, 5-dimethylthiophene-3-carboxylate. All derivatives were prepared in satisfactory yields, and their structures were characterized by elemental analysis,

IR, ¹H NMR, ¹³C NMR, and mass spectrometry. The spectroscopic data provided conclusive evidence for the formation of the thienopyrimidine-thiazolidinone hybrid system.

The antibacterial screening results of compounds (5a-5j) revealed notable variations in inhibitory potency against both Gram-positive and Gram-negative bacterial strains (Table 1). Overall, the synthesized derivatives exhibited moderate to good antibacterial activity, with several compounds demonstrating enhanced effectiveness depending on substitution patterns. Among the tested molecules, compound 5h emerged as the most active derivative, displaying MIC values of 15.62 $\mu\text{g/mL}$ against *S. aureus* and 31.25 $\mu\text{g/mL}$ against *S. pyogenes*. This activity, although lower than the reference standards ciprofloxacin (6.25 $\mu\text{g/mL}$) and ampicillin (10 $\mu\text{g/mL}$), indicates promising antibacterial potential for a newly synthesized scaffold. Additionally, compounds 5b, 5c, 5e, 5f, 5i, and 5j exhibited consistent MIC values of 31.25-62.5 $\mu\text{g/mL}$, suggesting favorable interaction with bacterial targets. In general, the compounds demonstrated greater sensitivity toward Gram-positive bacteria compared to Gram-negative strains. This observation is commonly associated with the more complex outer membrane structure of Gram-negative bacteria, which restricts penetration of many heterocyclic compounds. The reduced activity observed against *P. aeruginosa* further supports this explanation. Comparison with standard drugs confirms that while the synthesized compounds are less potent than ciprofloxacin and chloramphenicol, several derivatives possess biologically relevant antibacterial effects worthy of further optimization.

The antifungal evaluation (Table 2) demonstrated that the synthesized derivatives exhibit moderate to promising fungicidal activity against *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*. Similar to antibacterial results, biological response was strongly dependent on structural variation. Compound 5h again showed superior activity, with MFC values of 31.25 $\mu\text{g/mL}$ against *C. albicans* and 62.5 $\mu\text{g/mL}$ against both *A. niger* and *A. clavatus*. Although less active than nystatin (10-20 $\mu\text{g/mL}$), its performance is notable for a synthetic heterocyclic derivative. Compounds 5b, 5c, 5e, 5f, 5i, and 5j also demonstrated appreciable antifungal activity, with MFC values ranging from 62.5-125 $\mu\text{g/mL}$. The comparatively higher resistance of filamentous fungi (*Aspergillus* spp.) relative to yeast (*Candida albicans*) is consistent with reported literature, reflecting inherent differences in fungal cell wall architecture.

The antioxidant assessment using the DPPH radical scavenging assay (Table 3) revealed that several derivatives possess significant free radical scavenging ability. Lower IC₅₀ values indicate stronger antioxidant potential. Compounds 5g (IC₅₀ = 28.9 $\mu\text{g/mL}$) and 5d (IC₅₀ = 32.4 $\mu\text{g/mL}$) exhibited the most promising activity among the synthesized series, approaching the potency of standard antioxidants BHT (26.3 $\mu\text{g/mL}$) and ascorbic acid (21.5 $\mu\text{g/mL}$). Other derivatives demonstrated moderate radical scavenging effects, suggesting

that substitution patterns influence hydrogen-donating capacity.

Overall, the data clearly demonstrate a structure-activity relationship, Electron-withdrawing substituents enhanced antibacterial and antifungal activity, likely due to improved interaction with microbial enzymes or membrane targets. Electron-donating substituents favored antioxidant activity, consistent with increased electron density facilitating radical stabilization. The thiazolidinone ring appears to contribute positively to biological performance, supporting previous medicinal chemistry findings. This suggests that thienopyrimidine-thiazolidinone hybrids are promising multi-target bioactive molecules.

4. Conclusions

A novel set of thienopyrimidine-thiazolidinone hybrid derivatives (5a-5j) was synthesized and their structures were confirmed using standard analytical and spectroscopic techniques. Evaluation of their biological properties demonstrated that several compounds possess noteworthy antibacterial, antifungal, and antioxidant activities. The observed activity patterns indicated a clear influence of substituent effects, where derivatives bearing electron-withdrawing groups showed improved antimicrobial performance, while compounds containing electron-donating substituents exhibited enhanced antioxidant behavior. Collectively, the results suggest that the thienopyrimidine-thiazolidinone molecular framework represents a valuable scaffold with multifunctional pharmacological potential, meriting further exploration and optimization in medicinal chemistry studies.

Abbreviations

M. F.	Molecular Formula
M. P.	Melting Point
TLC	Thin Layer Chromatography
UV	Ultra Violet
IR	Infrared
NMR	Nuclear Magnetic Resonance
MHz	Megahertz
DMSO	Dimethyl Sulfoxide
LC-MS/MS	Liquid Chromatography-tandem Mass Spectrometry
TMS	Tetramethylsilane
MIC	Minimal Inhibition Concentration
MFC	Minimal Fungicidal Concentration

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Author Contributions

Himanshu Patel: Conceptualization, Methodology, Investigation, Data curation, Formal Analysis, Validation, Resources, Visualization, Writing – original draft

Conflicts of Interest

The author declares no conflicts of interest.

Appendix

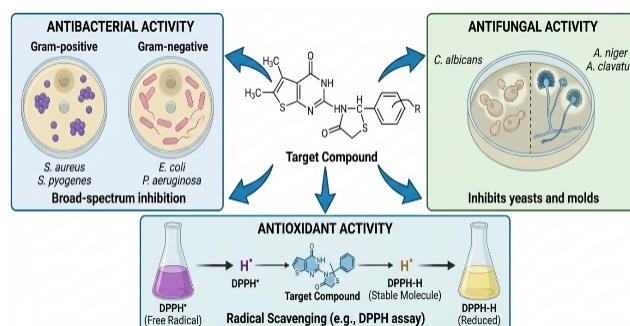


Figure A1. Graphical Abstract.

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