Synthesis, Structural Characterization, and Antimicrobial Evaluation of Newer Thiazolo[4,5-d]pyrimidine-2,5-dione Derivatives

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Abstract:

A new series of 3-(2-methoxybenzoyl)-7-substituted-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-diones (3a–m) were synthesized in a three-step procedure consisting of condensation, N-acylation and cyclization with urea. The synthesized compounds were characterized by IR, 1H NMR, 13C NMR, mass, and elemental analysis wherever possible. The compounds were screened for in vitro antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes and antifungal activity against Candida albicans, Aspergillus niger and Aspergillus clavatus by determining the minimum inhibitory concentration (MIC) values. The most promising antibacterial activity was observed for compounds 3j and 3k. Generally, the best antifungal profile was observed for 3m. The observed activity was dependent on the nature of the aryl and heteroaryl substituents, giving SAR information for future optimization. These results indicate that substitution at the 7-position influences antimicrobial activity and that thiazolo[4,5-d]pyrimidine-2,5-diones may provide useful scaffolds for further antimicrobial development.

1. Introduction:

Heterocyclic compounds have been among the most important classes of organic compounds in medicinal chemistry because of their structural diversity and broad spectrum of biological activities.[1] In particular, nitrogen- and sulfur-containing heterocycles have gained considerable attention in the development of biologically active molecules and therapeutic agents.[2, 3] Among these, thiazole, pyrimidine, and thiazolidine-2,4-dione-containing systems have been extensively investigated because their heteroatom-rich structures provide useful opportunities for molecular recognition and structural modification.[4-6] The thiazolidine-2,4-dione (TZD) nucleus is a particularly versatile scaffold that can be readily substituted at its 3- and 5-positions and has been associated with antimicrobial and several other pharmacological activities.[7, 8]

Pyrimidine derivatives and their fused analogues have also gained considerable attention because of their diverse pharmacological properties.[9] Fusion of pyrimidine with another heterocyclic ring represents an attractive strategy for generating structurally constrained molecules with new biological properties.[10, 11] In this context, thiazolo[4,5-d]pyrimidines have emerged as an important fused heterocyclic class and have been described as privileged scaffolds in medicinal chemistry because of their structural resemblance to purine systems.[12, 13] Several thiazolo[4,5-d]pyrimidine derivatives have been investigated for antibacterial, antifungal, antiviral, anticancer and other biological activities.[14, 15]

There are several reported examples which justify the biological potential of this fused heterocyclic system. For instance, Rida et al. reported thiazolo[4,5-d]pyrimidine derivatives with different functional groups and evaluated their antibacterial, antifungal, anti-HIV and anticancer activities. Several derivatives exhibited promising antifungal activity.[16] Urgun et al. synthesized thiazolo[4,5-d]pyrimidine derivatives and evaluated the compounds for antibacterial and antifungal activity . One of the synthesized derivatives exhibited remarkable activity against Gram-positive bacteria and yeast-like fungi.[17] More recently, thiazolopyrimidine derivatives have continued to demonstrate antimicrobial potential against Staphylococcus aureus, Bacillus cereus, Escherichia coli and fungal pathogens including Candida albicans and Aspergillus species.[18] These findings indicate that modification of the thiazolo[4,5-d]pyrimidine nucleus can provide useful chemical space for the development of new antimicrobial candidates.

The emergence of antimicrobial resistant microorganisms has raised the demand for structurally diverse molecules with the potential activity against bacterial and fungal pathogens.[19] Therefore, the development of novel heterocyclic scaffolds and systematic modification of known pharmacophores are still important strategies in medicinal chemistry.[20] The combination of the thiazolidine-2,4-dione-derived carbonyl system with a fused thiazolo[4,5-d]pyrimidine framework therefore provides an attractive platform for exploring antimicrobial activity.

In the present study, we designed and synthesized a series of 3-(2-methoxybenzoyl)-7-substituted-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-diones (3a–m). The series incorporates electronically and structurally diverse aryl and heteroaryl substituents at the 7-position, including fluoro, dimethylamino, methyl, bromo, nitro, chloro, methoxy, phenyl, trimethoxy, pyridyl, thienyl, and furyl groups.

The synthesized compounds were characterized by appropriate analytical and spectroscopic techniques and evaluated for their in vitro antibacterial and antifungal activities. The structural diversity introduced within the 3a–m series was intended to examine the influence of substituent variation on antimicrobial response and to obtain preliminary structure–activity relationship information. Thus, the present work describes the synthesis, characterization and antimicrobial evaluation of 3a–m, with particular emphasis on identifying structurally promising derivatives for further investigation.

2. Results and Discussion

2.1 Chemistry

In Scheme 1. the synthetic route used for the synthesis of the target 3-(2-methoxybenzoyl)-7-substituted-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-diones (3a–m) is demonstrated. The synthesis was carried out by three-step sequence involving the formation of substituted benzylidene-thiazolidine-2,4-diones, N-acylation with a 2-methoxybenzoyl moiety and subsequent cyclisation with urea to give the fused thiazolo[4,5-d]pyrimidine-2,5-dione nucleus.

Initially, 1,3-thiazolidine-2,4-dione was condensed with the appropriate substituted aromatic or heteroaromatic aldehydes in the presence of piperidine and ethanol under reflux conditions to afford the corresponding 5-substituted benzylidene-thiazolidine-2,4-diones (1a–m). The reaction was carried out for 16 h and the products were isolated after acidification and recrystallization. The remaining benzylidene derivatives were prepared by following the same procedure.

In the second step, 2-methoxybenzoic acid was converted into its corresponding acid chloride using thionyl chloride in dioxane. The resulting intermediate was subsequently reacted with the appropriate benzylidene-thiazolidine-2,4-dione in dry DMF in the presence of anhydrous potassium carbonate to furnish the corresponding 3-(2-methoxybenzoyl)-5-substituted benzylidene-thiazolidine-2,4-diones (2a–m). The reaction mixture was stirred overnight, followed by quenching with ice water, filtration, and drying of the resulting solid.

Finally, the prepared intermediates (2a–m) were subjected to cyclization with urea in the presence of P_2O_5 and ethanolic HCl under reflux conditions to construct the fused thiazolo[4,5-d]pyrimidine system. As a representative example, intermediate 2a was refluxed with urea and P_2O_5 in ethanolic HCl for 10 h to give 3a in 70% yield with a melting point of 172–174 °C. The remaining target compounds (3b–m) were synthesized under analogous conditions.

The final series comprised thirteen derivatives containing diverse substituents at the 7-position, including 4-F, 4-$N(CH_3)_2$, 4-CH_3, 4-Br, 2-NO_2, 3-Cl, 4-OCH_3, phenyl, 3-Br, 2,4,6-trimethoxyphenyl, 2-pyridyl, 2-thienyl and 2-furyl groups. The reported isolated yields for the final series were in the 59–80% range, demonstrating that the cyclization strategy was applicable to both substituted aromatic and heteroaromatic substrates.

The structures of the synthesized compounds were further supported by their IR, 1H NMR, 13C NMR, mass spectrometric and elemental-analysis data, as available. The analytical

data were consistent with the proposed structures of the synthesized thiazolo[4,5-d]pyrimidine-2,5-dione derivatives.

2.2 In vitro antibacterial activity and antifungal activity

The antimicrobial screening data are shown in Table 1. Results revealed that final compound 3k exhibited significant bacterial inhibition at MIC value of 62.5 µg/ml with compared to ampicillin (100 µg/ml MIC value) against E. coli. Compounds 3a and 3j displayed significant activity at MIC value of 62.5 µg/ml, with compared to ampicillin (100 µg/ml MIC value); against P. aeruginosa. Compounds 3j and 3k (62.5 µg/ml MIC value) possessed significant bacterial inhibition with compared to ampicillin (250 µg/ml MIC value) against S. aureus.

The antifungal screening data are shown in Table 2. Results revealed that final compounds 3d (250 µg/ml MIC value), 3a (200 µg/ml MIC value) showed significant fungal inhibition with compared to Griseofulvin (500 µg/ml MIC value) and comparble activity with compared to Nystatin (100 µg/ml MIC value) against C. Albicans. Compounds 3j possessed moderate activity at MIC value of 125 µg/ml with compared to Nystatin (100 µg/ml MIC value) and Griseofulvin (100 µg/ml MIC value) against A.niger. Compounds 3m possessed also comparable activity at MIC value of 100 µg/ml with compared to Nystatin (100 µg/ml MIC value) and Griseofulvin (100 µg/ml MIC value) against A. clavatus.

Table 1. In vitro antibacterial activity of compounds 3a–m

Compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenes*
3a	500	**62.5**	250	500
3b	500	500	200	250
3c	250	250	500	200
3d	500	250	500	500
3e	200	250	250	125
3f	125	200	250	250
3g	200	500	200	500
3h	100	125	200	250
3i	125	200	250	125
3j	100	**62.5**	**62.5**	100
3k	**62.5**	100	**62.5**	100
3l	100	125	250	250
3m	200	200	250	250

Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

Table 2. In vitro antifungal activity of compounds 3a–m

Compound	*C. albicans*	*A. niger*	*A. clavatus*
3a	**200**	500	500
3b	500	1000	1000
3c	1000	500	500
3d	**250**	250	1000
3e	1000	500	500
3f	500	500	250
3g	500	250	200
3h	**100**	500	500
3i	500	200	250
3j	500	**125**	250
3k	1000	500	500
3l	500	250	1000
3m	**100**	200	**100**
Griseofulvin	500	100	100
Nystatin	100	100	100

2.3 Structure–Activity Relationship (SAR)

The antibacterial activity of compounds 3a–m was influenced by the nature and position of the substituent at the 7-position of the thiazolo[4,5-d]pyrimidine-2,5-dione nucleus. The most promising antibacterial profiles were obtained for the 2,4,6-trimethoxyphenyl substituted series 3j and the 2-pyridyl substituted series 3k. The MIC values of 3j were found to be 100, 62.5, 62.5 and 100 µg/mL against E. coli, P. aeruginosa, S. aureus and S. pyogenes, respectively. The MIC values of 3k were found to be 62.5, 100, 62.5 and 100 µg/mL against the same organisms. Also significant activity against P. aeruginosa (62.5 µg/mL) was observed for the 4-fluorophenyl derivative 3a. In contrast, the methyl-, methoxy-, dimethylamino- and several halogen-substituted analogues generally exhibited moderate activity, suggesting that antibacterial potency was dependent on the overall structural and electronic characteristics of the substituent rather than simply on the presence of an electron-withdrawing or electron-donating group.

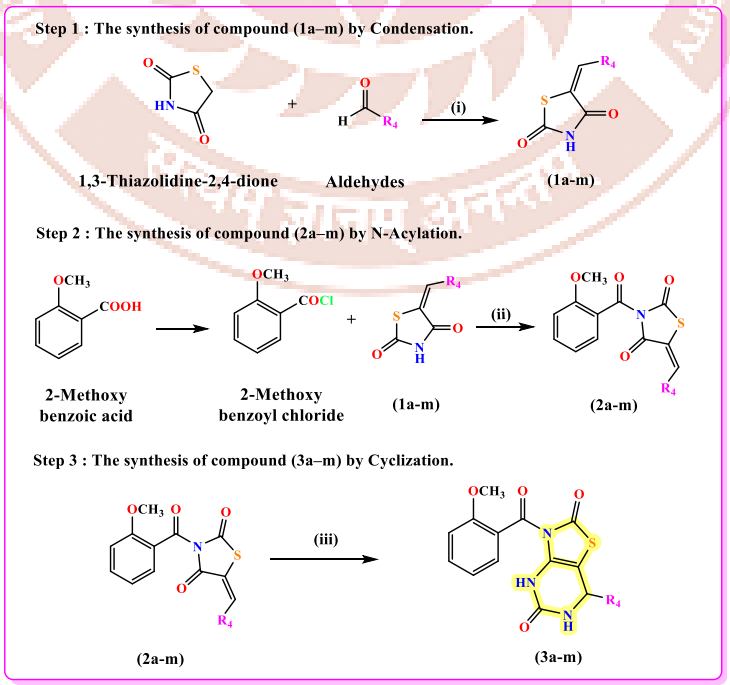
A different activity pattern was observed in the antifungal evaluation. 3m, containing a 2-furyl substituent, displayed the most favorable overall antifungal profile, with MIC values of 100, 200 and 100 µg/mL against C. albicans, A. niger and A. clavatus, respectively. 3h showed good activity against C. albicans with MIC of 100 µg/mL while 3g showed comparatively better activity against A. clavatus (200 µg/mL). The difference between the antibacterial and antifungal profiles indicates that the nature of the 7-substituent may influence interaction with bacterial and fungal biological systems differently. Overall, 3j and 3k can be considered the principal antibacterial leads, while 3m represents the most promising antifungal derivative of the present series. These observations provide preliminary SAR information for further optimization of the thiazolo[4,5-d]pyrimidine-2,5-dione scaffold

3. Experimental

3.1 Materials and methods

All chemicals and reagents used in the synthesis were of analytical grade and were used as received unless otherwise specified. The progress of the reactions was monitored by thin-layer chromatography (TLC) using silica gel plates, and the synthesized products were purified by appropriate crystallization/recrystallization procedures. Melting points were determined using the reported laboratory procedure. The synthesized compounds were characterized by spectroscopic and analytical techniques, including IR, 1H NMR, 13C NMR, mass spectrometry and elemental analysis, where applicable.

Scheme 1: General procedure for the synthesis of compounds (3 a-m).



Where: (i) 1,3-Thiazolidine-2,4-dione, substituted aldehyde, Piperidine/ethanol, reflux, 16 h Refluxed, 10 h; (ii) DMF, overnight stirring; (iii) NH_2CONH_2, P_2O_5, ethanolic HCl, 8-12 h

Here R4 = -C_6H_4-4-F, -C_6H_4-4-N(CH_3)$_2$, -C_6H_4-4-CH_3, -C_6H_4-4-Br, -C_6H_4-2-NO_2, -C_6H_4-3-Cl, -C_6H_4-4-OCH_3, -C_6H_5, -C_6H_4-3-Br, -C_6H_2-(OCH_3)$_3$, 2-pyridinyl, 2-thiophenyl, 2-furyl

3.2 General procedure for the synthesis of compound (1a–m).

A mixture of 1,3-thiazolidine-2,4-dione (0.085 mol), 4-fluoro benzaldehyde (0.085 mol), piperidine (0.085 mol) and ethanol (250 ml) was refluxed with stirring for 16h. The reaction was monitored by TLC on silica gel using ethyl acetate : toluene (2.5:7.5) as a mobile phase. After completion of the reaction, the reaction mixture was poured into crushed ice and acidified with acetic acid to give 5-(4-fluoro-benzylidene)-thiazolidine-2,4-dione (1a) as a solid, which was recrystallized from methanol. Other benzylidene-thiazolidine-2,4-diones (1b-m) have been prepared by the same method.

3.3 General procedure for the synthesis of compound (2 a-m).

A mixture of 2-methoxy-benzoic acid (0.012 mol), thionyl chloride (0.21 mol) and anhydrous dioxane (35 ml) was refluxed for 10 h. The mixture was protected from humidity with CaCl2 tube. Then the solvent was distilled off and the residue cooled to room temperature. To this residue was added 100 ml of dry chloroform, and the mixture was stirred for 10 min and evaporated to dryness. The semisolid material obtained was directly used in the forward step. The resulting compound (I) was dissolved in dry DMF (10 ml) and the solution was cooled in an ice-bath.

A solution of 5-(4-fluoro-benzylidene)-thiazolidine-2,4-dione (1a) (0.012 mol) in dry DMF (30 ml) was taken and cooled to 0–5°C in an ice bath. Anhydrous potassium carbonate (0.024 mol) was added to the cold mixture and stirred for 10 min. To the stirred solution was successively added in small portions the resulting cold solution of 2-methoxybenzoyl chloride in DMF. The resulting mixture was stirred overnight at room temperature. Reaction completion was monitored through TLC using toluene : ethyl acetate (7:3) as mobile phase. After completion of the reaction, the reaction mixture was quenched with ice water and stirred for 1 h. The solid product (2a) was filtered, dried, and, as such taken for the next step. (Scheme 1) Other respective thiazolidinediones (2b-m) have been prepared by the same method.

3.4 General procedure for the synthesis of compound (3a-m).

A mixture of 5-(4-fluoro-benzylidene)-3-(2-methoxy-benzoyl)-thiazolidine-2,4-dione (2a) (0.0058 mol), urea (0.012 mol), P_2O_5 (0.2 g) and ethanolic HCl (100 ml) was refluxed with stirring for 10 h. The reaction was monitored by TLC on silica gel using methanol:toluene (2.5:7.5) as a mobile phase. After the completion of the reaction, the reaction mixture was

cooled to room temperature and allowed to stand overnight; the separated solid was isolated, washed with ethanol, and recrystallized from ethanol to form 3a (Scheme 1). Yield: 70%; m.p.: 172-174ºC. Other final respective thiazolo-pyrimidinediones (3b-m) have been prepared by the same method.

3.5 Antibacterial and antifungal assay

The synthesized compounds 3a–m were evaluated for their in vitro antibacterial and antifungal activities by determination of the minimum inhibitory concentration (MIC) using the broth dilution method. The bacterial strains used for evaluation were Escherichia coli (MTCC 442), Pseudomonas aeruginosa (MTCC 441), Staphylococcus aureus (MTCC 96), and Streptococcus pyogenes (MTCC 443). The fungal strains used were Candida albicans (MTCC 227), Aspergillus niger (MTCC 282), and Aspergillus clavatus (MTCC 1323). The microbial strains were collected from the Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial assay nutrient medium was Mueller–Hinton broth. DMSO was used as diluent/vehicle in the preparation of compound solutions. The inoculum concentration was adjusted to approximately 108 CFU/mL by turbidity comparison. The assay was done with proper controls like drug, vehicle, organism, and standard-drug controls.

The stock solutions of the synthesized compounds were prepared at a concentration of 2000 µg/mL for MIC determination. In primary screening, compound concentrations of 500, 250 and 125 µg/mL were tested. Compounds active in the primary screening were further subjected to secondary screening at 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 µg/mL. Serial dilutions were performed, and the tubes were inoculated with the respective test organisms and incubated overnight. The MIC was defined as the lowest concentration of the test compound that inhibited the visible growth of the microbe. The assay was read as at least 99% inhibition. The comparison was made with standard antibacterial and antifungal drugs as reference controls.

3.6 Characterization of synthesized compounds

3-(2-Methoxybenzoyl)-7-(4-fluorophenyl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3a).

IR (KBr), νmax (cm^{-1}): 3318, 3246 (N–H), 1719, 1654, 1636 (C=O); 1H NMR: (300 MHz, DMSO-d_6) δ 9.93 (s, 1H, NH), 9.58 (s, 1H, NH), 7.59 (s, 1H, CH), 7.98–6.64 (m, 8H, Ar-H), 3.22 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 62.5, 84.6, 112.7, 115.4, 116.7, 119.5, 123.5, 127.3, 128.5, 135.7, 138.9, 150.3, 157.7, 161.1, 162.8, 163.3; mass: m/z (M+) 399.

3-(2-Methoxybenzoyl)-7-[4-(dimethylamino)phenyl]-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3b).

IR (KBr), νmax (cm^{-1}): 3319, 3244 (N–H), 1718, 1655, 1635 (C=O); 1H NMR: (300 MHz, DMSO-d_6) δ 9.845 (s, 1H, NH), 9.452 (s, 1H, NH), 7.523 (s, 1H, CH), 7.891–6.691 (m, 8H, Ar-H), 3.735 (s, 3H, OCH_3), 3.236 (s, 6H, $N(CH_3)_2$); 13C NMR: (100 MHz, DMSO-d_6) δ 44.4, 61.6, 85.5, 112.6, 113.9, 116.8, 119.7, 123.5, 127.7, 128.4, 133.3, 135.6, 149.2, 150.3, 157.1, 162.4, 163.8; mass: m/z (M+) 424.

3-(2-Methoxybenzoyl)-7-(4-methylphenyl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3c).

IR (KBr), νmax (cm^{-1}): 3317, 3245 (N–H), 1719, 1656, 1634 (C=O); 1H NMR: (300 MHz, DMSO-d_6) δ 9.991 (s, 1H, NH), 9.621 (s, 1H, NH), 7.652 (s, 1H, CH), 8.010–6.76 (m, 8H, Ar-H), 3.295 (s, 3H, OCH_3), 2.234 (s, 3H, CH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 13.8, 61.5, 85.5, 112.2, 116.2, 119.0, 123.1, 126.8, 128.1, 128.9, 135.2, 136.3, 137.3, 150.7, 157.5, 162.6, 163.2; mass: m/z (M+) 395.

3-(2-Methoxybenzoyl)-7-(4-bromophenyl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3d).

IR (KBr), νmax (cm^{-1}): 3320, 3245 (N–H), 1722, 1650, 1632 (C=O); 1H NMR: (300 MHz, DMSO-d_6) δ 10.133 (s, 1H, NH), 9.725 (s, 1H, NH), 7.654 (s, 1H, CH), 7.936–6.626 (m, 8H, Ar-H), 3.655 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 123.2, 121.1, 150.2, 157.4, 54.8, 142.3, 118.2, 131.4, 129.1, 111.5, 128.5, 133.1, 121.1, 164.6, 55.4; mass: m/z (M+) 458.

3-(2-Methoxybenzoyl)-7-(2-nitrophenyl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3e).

IR (KBr), νmax (cm^{-1}): 3322, 3242 (N–H), 1711, 1654, 1633 (C=O), 1527, 1323 (NO_2); 1H NMR: (300 MHz, DMSO-d_6) δ 9.915 (s, 1H, NH), 9.553 (s, 1H, NH), 7.518 (s, 1H, CH), 7.830–6.60 (m, 8H, Ar-H), 3.325 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 123.2, 150.2, 157.4, 50.2, 147.2, 140.5, 118.2, 111.5, 124.7, 127.8, 128.5, 133.1, 127.6, 130.9, 121.1, 164.6, 55.4; mass: m/z (M+) 425.

3-(2-Methoxybenzoyl)-7-(3-chlorophenyl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3f).

IR (KBr), νmax (cm^{-1}): 3316, 3240 (N–H), 1715, 1659, 1635 (C=O), 758 (C–Cl); 1H NMR: (300 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.61 (s, 1H, NH), 5.56 (s, 1H, CH), 7.15–8.06 (m, 8H, Ar-H), 3.93 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0,

123.2, 134.1, 50.2, 157.4, 54.3, 144.7, 118.2, 126.8, 126.7, 111.5, 125.0, 128.5, 133.1, 129.9, 121.1, 164.6, 55.4; mass: m/z (M+) 415.

3-(2-Methoxybenzoyl)-7-(4-methoxyphenyl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3g).

IR (KBr), νmax (cm^{-1}): 3317, 3242 (N–H), 1716, 1656, 1635 (C=O), 1245 (C–O–C); 1H NMR: (300 MHz, DMSO-d_6) δ 9.821 (s, 1H, NH), 9.528 (s, 1H, NH), 7.675 (s, 1H, CH), 7.882–6.842 (m, 8H, Ar-H), 3.565 (s, 3H, OCH_3), 3.419 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 123.2, 150.2, 157.4, 158.6, 54.8, 135.6, 118.2, 114.1, 125.7, 111.5, 128.5, 133.1, 121.1, 164.6, 55.4, 55.8; mass: m/z (M+) 410.

3-(2-Methoxybenzoyl)-7-phenyl-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3h).

IR (KBr), νmax (cm^{-1}): 3319, 3244 (N–H), 1718, 1655, 1634 (C=O); 1H NMR: (300 MHz, DMSO-d_6) δ 9.676 (s, 1H, NH), 9.275 (s, 1H, NH), 7.264 (s, 1H, CH), 7.838–6.648 (m, 9H, Ar-H), 3.479 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 123.2, 150.2, 157.4, 54.8, 143.3, 118.2, 126.9, 111.5, 126.9, 128.5, 133.1, 128.5, 126.7, 121.1, 164.6, 55.4; mass: m/z (M+) 380.

3-(2-Methoxybenzoyl)-7-(3-bromophenyl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3i).

IR (KBr), νmax (cm^{-1}): 3315, 3238 (N–H), 1717, 1656, 1639 (C=O); 1H NMR: (300 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.61 (s, 1H, NH), 5.56 (s, 1H, CH), 7.54–7.07 (m, 8H, Ar-H), 3.93 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 123.2, 122.9, 150.2, 157.4, 54.1, 145.5, 118.2, 129.6, 131.7, 111.5, 125.9, 128.5, 133.1, 129.3, 121.1, 164.6, 55.4; mass: m/z (M+) 458.

3-(2-Methoxybenzoyl)-7-(2,4,6-trimethoxyphenyl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3j).

IR (KBr), νmax (cm^{-1}): 3321, 3243 (N–H), 1714, 1657, 1634 (C=O), 1248, 1215, 1168 (C–O–C) ; 1H NMR: (300 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.61 (s, 1H, NH), 5.56 (s, 1H, CH), 6.29 (s, 2H, Ar-H), 7.23–8.06 (m, 5H, Ar-H), 3.72, 3.81 and 3.93 (s, 3H each, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 123.2, 150.2, 158.5, 157.4, 158.5, 162.0, 43.0, 105.9, 118.2, 92.5, 92.5, 111.5, 128.5, 133.1, 121.1, 164.6, 56.1, 55.4, 56.1, 55.8; mass: m/z (M+) 471.

3-(2-Methoxybenzoyl)-7-(pyridin-2-yl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3k).

IR (KBr), νmax (cm^{-1}): 3316, 3241 (N–H), 1717, 1655, 1636 (C=O), 1598 (C=N); 1H NMR: (300 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.61 (s, 1H, NH), 5.56 (s, 1H, CH), 8.48, 7.75, 7.46 and 7.22 (m, 4H, pyridyl-H), 7.23–8.06 (m, 4H, Ar-H), 3.93 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 123.2, 150.2, 157.4, 155.4, 54.8, 148.6, 118.2, 120.9, 111.5, 121.9, 128.5, 133.1, 136.2, 121.1, 164.6, 55.4; mass: m/z (M+) 381.

3-(2-Methoxybenzoyl)-7-(thiophen-2-yl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3l).

IR (KBr), νmax (cm^{-1}): 3318, 3243 (N–H), 1716, 1656, 1635 (C=O), 1408 (C–S); 1H NMR: (300 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.61 (s, 1H, NH), 5.56 (s, 1H, CH), 7.34, 6.93 and 6.89 (m, 3H, thiophenyl-H), 7.23–8.06 (m, 4H, Ar-H), 3.93 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 139.4, 125.5, 123.2, 126.7, 127.0, 150.2, 157.4, 55.5, 118.2, 111.5, 128.5, 133.1, 121.1, 164.6, 55.4; mass: m/z (M+) 387.

3-(2-Methoxybenzoyl)-7-(furan-2-yl)-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione (3m).

IR (KBr), νmax (cm^{-1}): 3317, 3242 (N–H), 1715, 1655, 1635 (C=O), 1105, 1068 (C–O–C); 1H NMR: (300 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.61 (s, 1H, NH), 5.79 (s, 1H, CH), 7.56, 6.39 and 6.29 (m, 3H, furyl-H), 7.23–8.06 (m, 4H, Ar-H), 3.93 (s, 3H, OCH_3); 13C NMR: (100 MHz, DMSO-d_6) δ 161.7, 80.0, 152.5, 142.1, 123.2, 106.7, 110.6, 150.2, 157.4, 56.0, 118.2, 111.5, 128.5, 133.1, 121.1, 164.6, 55.4; mass: m/z (M+) 371.

4. Conclusion

In conclusion, a series of 3-(2-methoxybenzoyl)-7-substituted-6,7-dihydro-3H,4H-thiazolo[4,5-d]pyrimidine-2,5-dione derivatives (3a–m) was synthesized and evaluated for their in vitro antimicrobial activity. The synthesized compounds showed variable antibacterial and antifungal activities depending on the nature of the substituent at the 7-position. Among the synthesized derivatives, 3j and 3k exhibited the most promising antibacterial activity, whereas 3m showed the most favorable overall antifungal activity, particularly against Candida albicans and Aspergillus clavatus. Compound 3h also demonstrated notable activity against C. albicans. These results suggest that the antimicrobial activity can be affected by structural modification of the thiazolo[4,5-d]pyrimidine-2,5-dione nucleus and give useful preliminary SAR information. Overall, the present 3a–m series represents a promising starting point for further structural optimization and antimicrobial investigation.

5. Credit author statement:

Amitkumar C. Purohit: Validation, formal analysis, synthesis work, conceptualization, review & editing, biological activity, and supervision; Bhadreshkumar N. Parmar: Data curation, writing—original draft; Mahima A. Rathod: Review & editing; Dipali M. Maisuriya: Review & editing; Dhanji P. Rajani: Biological activity; Navin B. Patel: Methodology, conceptualization, resources, and supervision.

6. Declaration of competing interest:

There are no potential conflicts of interest reported by the authors.

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References:

1. Sharma, A., et al., *Heterocyclic Molecules as an Active Pharmaceutical Ingredient: An Introduction.* Heterocyclic Compounds as Pharmaceutical Ingredients, 2026: p. 1-25.
2. Sharma, P.K., A. Amin, and M. Kumar, *A review: Medicinally important nitrogen sulphur containing heterocycles.* The open medicinal chemistry journal, 2020. **14**(1).
3. Drakontaeidi, A., I. Papanotas, and E. Pontiki, *Multitarget pharmacology of sulfur–nitrogen heterocycles: anticancer and antioxidant perspectives.* Antioxidants, 2024. **13**(8): p. 898.
4. Petrou, A., M. Fesatidou, and A. Geronikaki, *Thiazole ring—A biologically active scaffold.* Molecules, 2021. **26**(11): p. 3166.
5. Nammalwar, B. and R.A. Bunce, *Recent advances in pyrimidine-based drugs.* Pharmaceuticals, 2024. **17**(1): p. 104.
6. Jain, V.S., D.K. Vora, and C. Ramaa, *Thiazolidine-2, 4-diones: Progress towards multifarious applications.* Bioorganic & medicinal chemistry, 2013. **21**(7): p. 1599-1620.

7. Kajal, K., et al., *The medicinal perspective of 2, 4-thiazolidinediones based ligands as antimicrobial, antitumor and antidiabetic agents: a review.* Archiv der Pharmazie, 2022. **355**(9): p. 2100517.
8. Gupta, S., et al., *Medicinal Perspective of 2, 4-Thiazolidinediones Derivatives: An Insight Into Recent Advancements.* ChemistryOpen, 2024. **13**(11): p. e202400147.
9. Joshi, G., et al., *Pyrimidine-fused derivatives: Synthetic strategies and medicinal attributes.* Current Topics in Medicinal Chemistry, 2016. **16**(28): p. 3175-3210.
10. Tamer, M., et al., *Pyrimidine Scaffolds in Modern Medicinal Chemistry: A Review of Biological Activities and Therapeutic Potential.* Al-Farahidi Biomedical Journal, 2026. **2**(1): p. 3.
11. Islam, M.W., et al., *A review on pyrimidine-based derivatives: Synthesis and their biological application.* Journal of Heterocyclic Chemistry, 2024. **61**(7): p. 1159-1179.
12. Kuppast, B. and H. Fahmy, *Thiazolo [4, 5-d] pyrimidines as a privileged scaffold in drug discovery.* European Journal of Medicinal Chemistry, 2016. **113**: p. 198-213.
13. Nazir, M.S., et al., *Recent updates on the synthesis of thiazolopyrimidines derivatives.* Synthetic Communications, 2023. **53**(15): p. 1173-1206.
14. Chaban, T., et al., *Thiazolo [5, 4-d] pyrimidines and thiazolo [4, 5-d] pyrimidines: A review on synthesis and Pharmacological importance of their derivatives.* Pharmacia, 2018. **65**(2): p. 54-70.
15. Khator, R. and V. Monga, *Thiazolopyrimidine, a privileged scaffold: Recent updates on synthetic and pharmacological perspective in drug discovery.* Archiv der Pharmazie, 2025. **358**(3): p. e2400870.
16. Habib, N., et al., *Condensed thiazoles, ii: synthesis of 7-substituted thiazolo [4, 5-d] pyrimidines as possible anti-HIV, anticancer, and antimicrobial agents.* Monatshefte für Chemie/Chemical Monthly, 1996. **127**(11): p. 1209-1214.
17. Urgun, H., A. Balkan, and M. Oezalp, *New thiazolo [4, 5-d] pyrimidine derivatives as potential antimicrobial agents.* Arzneimittelforschung, 2000. **50**(12): p. 1115-1119.
18. Abd Elhameed, A.A., et al., *Synthesis and biological screening of new thiazolo [4, 5-d] pyrimidine and dithiazolo [3, 2-a: 5', 4'-e] pyrimidinone derivatives as antimicrobial, antiquorum-sensing and antitumor agents.* Bioorganic Chemistry, 2018. **81**: p. 299-310.
19. Sharma, S., et al., *Emerging challenges in antimicrobial resistance: implications for pathogenic microorganisms, novel antibiotics, and their impact on sustainability.* Frontiers in microbiology, 2024. **15**: p. 1403168.

20. Ahmed, S.A., et al., *A review on the role of heterocyclic scaffolds in drug discovery.* Journal of Pharma Insights and Research, 2025. **3**(5): p. 394-402.