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Synthesis and Antimicrobial Activity of Novel Thienopyrimidine Linked Rhodanine Derivatives

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Abstract:

The current work states that the preparation of a new series of *N*-(substituted phenyl)-2-(4-oxo-5-(4-(thieno[2,3-*d*]-pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)acetamide derivatives (**8a-l**). A condensation reaction of thienopyrimidin-2-thioxothiazolidin-4-one derivative (**5**) with various 2-chloro-*N*-phenylacetamides (**7a-l**) was employed to afford the new thienopyrimidine tagged rhodanine derivatives under acetone solvent in the presence of potassium carbonate (K_2CO_3). All the novel target molecules were characterized by IR, ^{1}H NMR, ^{13}C NMR, and LC-MS spectral analyses and were screened for their *in vitro* antimicrobial activity by using broth dilution method. Compounds **8c, 8g** and **8h** found to have antibacterial potency against E. coli, B. subtilis, B. cereus and K. pneumonia with MIC values 3.25 to 6.25 µg/mL compare with the standard Gentamicin. Compounds **8c** and **8f** demonstrated better antifungal potency (MIC = 3.25 – 6.25 µg/mL) against *A. flavus, A. niger, P. marneffei,* and *C. albicans* when compared with Fluconazole.

Keywords: Synthesis; Thieno[2,3-*d*]pyrimidinones; Rhodanine; *N*-phenylacetamide; Antimicrobial activity.

Introduction

Recently, microbial infection is one of the most complex health problems in worldwide and facing various types of microbial (bacterial and fungal) contaminations in humans and animals.[1,2] The control of infective illnesses still scraps a significant and interesting problem because of a mixture of influences counting an emergent infective diseases and the growing number of multidrug resilient bacteriological, fungicidal pathogens.[3-5] In spite of a huge amount of antibiotics and chemotherapeutics accessible for medicinal usage,[6] on the other hand an occurrence of old and innovative antibiotic resistance formed in the past periods exposed a considerable therapeutic necessity for new classes of antimicrobial agents.[7] There is essential for the finding of novel molecules endowed with antimicrobial activity, feasibly performing via mechanisms of action, which are distinctive from those of eminent stages of bacterial proxies to which several scientifically related pathogens are now resilient.[6-8] From this point of view, it is a vital to enhance most effective antimicrobial agents.

Although, numerous thienopyrimidine frameworks have reported with different wide-range of pharmacological activities like antimicrobial,[9] analgesic,[10] anti-inflammatory[11] and antitumor.[12] Further, these activities also inhibit various protein kinase as CK2 involved in a specific anticancer activity.[13] Thus, the segment displays an essential role in medicinal chemistry always since it is a communal moiety in numerous structures of biologically active compounds which approves a remarkable array of activities. Furthermore, rhodanine comprising hybrids displayed several biological activities such as antidiabetic,[14,15] antimicrobial[16-19] and antiviral agents[20] and also medicinally important rhodanine drug Epalrestat is a clinical used for the treatment of diabetic problems.[21] Therefore, the improvement of such hybrid molecules with *N*-phenylacetamide framework, which can readily combine with a range enzymes and receptors in

organisms during forming hydrogen bonding interactions, has been discovered to be a reasonable antibacterial strategy. With our continuous concern in improving various synthetic approaches for biologically novel heterocyclic compounds[22-27] and synthesis of heterocycles via one-pot, multicomponent reactions under green conditions,[28-32] we herein describing to study the influence of thienopyrimidine moiety and 2-thioxothiazolidine-3-yl-N-phenylacetamide scaffold combination molecules which were subsequently displayed synergistic antimicrobial effect.

Results and Discussions

Chemistry

The preparation of target *N*-(substituted phenyl)-2-(4-oxo-5-(4-(thieno[2,3-*d*] pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)acetamide derivatives (**8a-l**) was represented in scheme-1. Initially, the key starting material 2-aminothiophene-3-carbonitrile (**1**) was synthesized using by Gewald method via multi-component condensation between elemental Sulphur, carbonyl compound and malanonitrile in ethanol solvent, triethyl amine as a catalyst.[20] Further, cyclisation of amino and cyano functionality of compound (**1**) on treatment with formic acid and sulphuric acid under reflux at 110 °C resulted in the formation of thieno[2,3-d] pyrimidin-4(3H)-one (**2**). Next, compound **2** reacted with phosphorous oxychloride to obtain 4-chlorothieno[2,3-*d*]pyrimidine (**3**). Then, the reaction of compound **3** with p-hydroxy benzaldehyde in the presence of potassium carbonate as a catalyst in acetonitrile solvent produced the corresponding 4-(thieno[2,3-*d*]pyrimidin-4-yloxy)benzaldehyde (**4**). Subsequent condensation of **4** using with rhodamine in the presence of sodium acetate as catalyst in acetic acid system to furnished 5-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-4-one (**5**). Simultaneously, another key intermediate aromatic *N*-phenylacetamide derivatives (**7a-l**) were prepared from substituted anilines and chloro acetyl chloride in the presence of TEA

as catalyst in acetone at 0 ^{o}C. Finally, the title compounds, (**8a-l**) were synthesized by the involving of two intermediates such as compound **5** and **7a–l** in acetone in the presence of K_2CO_3 as catalyst at reflux condition. All the synthesized molecules were characterized and analyzed by ^{1}HNMR, ^{13}CNMR, LC-MS and IR spectra. The conversion of thieno[2,3-*d*]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-4-one (**5**) to thieno[2,3-*d*]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)-*N*-phenylacetamide derivatives (**7a-l**) were established by the appearance of a singlet at δ 4.82-5.28 ppm due to the resonance of CH_2 of the *N*-phenylacetamide ring. ^{1}H NMR spectra of compounds 8a-l exhibited a singlet around 8.72-9.16 ppm for pyrimidine CH proton, broad singlet range 4.82–5.26 ppm corresponding to or NH proton and another singlet peak range at 4.82-5.26 due to CH_2 group.

Biological Evaluation

All the target molecules (**8a-l**) were confirmed for their *in vitro* antimicrobial activities. The antibacterial strains viz., *Escherichia coli* and *Klebsiella pneumonia* for Gram-negative and *Bacillus subtilis and Bacillus cereus for* Gram-positive and the fungal strains *Aspergillus flavus, Aspergillus niger, Penicillium marneffei* and *Candida albicans* were used. The minimum inhibitory concentration (MIC) was determined by the broth dilution approach using Mueller-Hinton nutrient broth medium and Gentamicin and Fluconazole were used as reference drugs. The MIC (lowest concentration (highest dilution) of the compound) values were found more potent with either less or equal as compared to standard drugs and were represented in **Table 1** and **Table 2**.

The MIC of the assayed compounds (8a-l) against bacterial pathogens ranged from 1.56 to 100 µg/mL. Of the three compounds **8c**, **8g** and **8h** showed most potent activity against all the bacterial strains. The hybrid **8c** having electron withdrawing 4-nitro substituted N-

phenylacetamide was found to be the excellent activity with MIC 1.56 µg/mL against *E. coli* and 3.12 µg/mL against *B. subtilis*, *B. cereus* and *K. pneumonia* compare with the standard Gentamicin (MIC = 6.25 µg/mL). Although, the electron donating substituted (methoxy and methyl) compounds **8g** and **8h** exhibited good antibacterial activity with MIC values of 6.25 µg/mL against *B. subtilis*, *E. coli* and *K. pneumonia*, respectively, with these MIC values equal to the standard Gentamicin (MIC = 6.25 µg/mL). Further, all other reaming synthesized derivatives (**8a-b, 8d-f** and **8i-l**) displayed moderate to weak activity with ranging from 25µg/mL to 100 µg/mL or >100 µg/mL against all the tested bacterial strains.

Moreover, the antifungal activity revealed that the compounds **8c**, **8e, 8f** and **8l** exhibited considerable activity against all the tested fungal strains with MIC values ranging from 3.12-6.25 µg/mL due to the effect of electron withdrawing groups (NO_2 and Cl) substituted on N-phenylacetamide ring. The compounds **8a**, **8d, 8g** and **8k** displayed comparatively good activity against all the fungal strains with MIC 6.25-25 µg/mL. All the remaining analogues were sighted with low effects at MIC level around 100 µg/mL, where as some were devoid of potency with >100 µg/mL of MIC against the mentioned panel of fungal strains.

It is noticeable from the investigation of antibacterial activity results that electron withdrawing group (nitro) and electron donating groups such as methoxy, methyl groups irrespective of position in the *N*-phenylacetamide ring derivative showed strong effect against four bacterial strains. Further, in presence of electron withdrawing groups (nitro and chloro) substituted on *N*-phenylacetamide ring derivatives displayed remarkably activity against four fungal strains.

Conclusion

In this study, synthesis of a new series of *N*-(substituted phenyl)-2-(4-oxo-5-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)acetamide derivatives (**8a-l**) were described. The *in vitro* antimicrobial activity of these compounds were evaluated by broth dilution method. The electron withdrawing group (nitro) compound **8c** showed excellent activity and electron donating group compounds methoxy and methyl (**8g** & **8h)** were exhibited good activity against all bacterial and fungal strains. The remaining compounds displayed moderate to poor antimicrobial activity.

Experimental

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized with UV light. Merck silica gel (60-120 mesh) was used for column chromatography. The IR spectra were recorded on a Perkin-Elmer BX1 FTIR Spectrophotometer as KBr pellets and the wave numbers were given in cm^{-1}. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer in $CDCl_3$/DMSO-d_6 solution using TMS as an internal standard.The mass spectra were recorded on Agilent 1100 LC/MSD instrument with method API-ES at 70 eV. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. Chemicals were purchased from Sigma Aldrich and used without further purification.

Procedure for preparation of thieno[2,3-*d*]pyrimidin-4(3H)-one (2)

2-Aminothiophene-3-carbonitrile (**1**) (6 mmol) was treated with formic acid (36 mmol) for 4 h at 120 ˚C. After exploit of reaction, the mixture was cooled and washed with water. Next, the crude was purified by recrystallization with ethanol.

Yield: 96%; mp 186-188 ˚C; IR (KBr) (ν_{max}/cm^{-1}): 3379, 2884, 1541; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 6.86 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.09 (s, 1H, NH); 7.21 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 8.85 (s, 1H, pyrimidine-CH), ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 119.26, 129.36, 137.24, 146.19, 151.16, 162.36; LC-MS (70 eV): m/z = 153 $(M+H)^+$.

Procedure for preparation of 4-chlorothieno[2,3-*d*]pyrimidine (3)

To a mixture of compound **2** (10 mmol) and phosphorus oxychloride (15 mmol) was added and the reaction substances were refluxed for 5 h. After completion of the reaction, then the reaction mixture was cooled to room temperature and poured into ice cold water. The obtained crude product was separated by filtration, washed with ice cold water, dried well and recrystallized with ethanol to obtain pure compound.

Yield: 92%; mp 191-193 ˚C; IR (KBr) (ν_{max}/cm^{-1}): 2905, 1526, 768; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 6.88 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.05 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 9.07 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 124.11, 132.37, 140.31, 147.26, 150.47, 159.91; LC-MS (70 eV): m/z = 170 $(M+H)^+$.

General procedure for preparation of 4-(thieno[2,3-*d*]pyrimidin-4-yloxy)benzaldehyde (4)

A mixture of 4-chlorothieno[2,3-*d*]pyrimidine 3 (6 mmol) and p-hydroxy benzaldehyde (6 mmol) dissolved in acetonitrile (15 mL) in the presence of anhydrous potassium carbonate (24 mmol) stirred at 40 ^{0}C for 5 h. After completion of the starting compounds, then the mixture was cooled to room temperature, poured and washed with ice cold water. Then the crude product was dried and recrystallized with ethanol to obtain in good yields.

Yield: 95%; mp 170-172 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2871, 1721, 1613, 1528; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 6.97 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.12 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.36 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.64 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.72 (s, 1H,

pyrimidine-CH), 9.46 (s, 1H, CHO); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 119.16, 123.26, 127.28, 133.01, 141.07, 146.17, 152.46, 156.19, 158.09, 162.39, 169.71; LC-MS (70 eV): m/z = 257 $(M+H)^+$.

General procedure for synthesis of 5-(4-(thieno[2,3-*d*]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-4-one (5)

A combination of compound (**4**) (1 mmol), rhodanine (1 mmol) and sodium acetate (3 mmol) in acetic acid (5 mL) was stirred and refluxed for 6 h at 90 ^{o}C. The reaction mixture was poured and washed with cold water. The resulted crude product was recrystallized by ethanol. Yield: 97%; mp 192-194 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2852, 1701, 1620, 1530; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 6.93 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.09 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.28 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.36 (s, 1H, NH), 7.59 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.81 (s, 1H, CH), 9.08 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 114.16, 121.86, 124.35, 127.48, 134.18, 138.06, 141.73, 147.24, 150.10, 154.24, 158.46, 161.20, 166.35; LC-MS (70 eV): m/z = 371 $(M+H)^+$.

General procedure for preparation of thienopyrimidine-*N*-phenylacetamides (8a-l)

To the solution of thienopyrimidine-2-thioxothiazolidin-4-one (**5**) (1 mmol) in acetone (10 mL) solvent was added potassium carbonate (1.5 mmol) followed by substituted *N*-phenylacetamides isothiocyanate (7a-l) (1 mmol). The reaction mixture was refluxed for 2 h at 78 °C after completion of the reaction, cooled to room temperature and evaporated under reduced pressure. Then the resulted mixture was washed by water and dried. Further, the crude was purified by column chromatography in good yield.

{2-(4-Oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)-N-phenylacetamide} (8a)

Yield: 88%; mp 210-212 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2837, 1708, 1670, 1626, 1541; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 4.82 (s, 2H, CH_2), 6.92 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.16 (bs, 1H, NH), 7.19 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.30 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.41-7.67 (m, 5H, Ar-H), 7.76 (s, 1H, CH), 8.01 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.86 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 52.14, 109.46, 112.47, 118.52, 121.04, 124.20, 125.41, 128.40, 132.81, 135.86, 138.75, 140.67, 148.41, 149.63, 150.91, 155.83, 158.01, 164.32, 168.19; LC-MS (70 eV): m/z = 505 $(M+H)^+$.

{N-(4-chlorophenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxo thiazolidin-3-yl)acetamide} (8b)

Yield: 92%; mp 194-196 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2851, 1709, 1667, 1621, 1547; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 5.16 (s, 2H, CH_2), 6.99 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.08 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.22 (bs, 1H, NH), 7.48 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.62 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.79 (s, 1H, CH), 8.16 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.34 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 9.05 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 56.48, 106.35, 114.33, 120.23, 121.25, 126.02, 127.30, 128.21, 134.02, 136.42, 137.11, 144.37, 146.08, 149.19, 151.74, 156.30, 160.27, 161.06, 167.46; LC-MS (70 eV): m/z = 539 $(M+H)^+$.

{N-(4-nitrophenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxo thiazolidin-3-yl)acetamide} (8c)

Yield: 91%; mp 221-223 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2841, 1716, 1673, 1618, 1528; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 5.12 (s, 2H, CH_2), 6.79 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 6.98 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.24 (d, J_{HH} = 12.0 Hz, 2H, Ar-H), 7.31 (s, 1H, NH), 7.58 (d, J_{HH} = 12.0 Hz, 2H, Ar-H), 7.63 (s, 1H, CH), 7.94 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.20 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.72 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 54.01, 108.11,

119.84, 121.16, 121.33, 125.26, 127.19, 129.72, 133.07, 136.14, 139.82, 143.22, 148.26, 149.33, 152.26, 158.26, 159.27, 162.27, 166.14; LC-MS (70 eV): m/z = 548 $(M-H)^-$.

{N-(2-chlorophenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxo thiazolidin-3-yl)acetamide} (8d)

Yield: 89%; mp 232-234 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2843, 1714, 1668, 1624, 1534; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 5.28 (s, 2H, CH_2), 6.81 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 6.96 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.29 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.42 (s, 1H, NH), 7.56-7.82 (m, 4H, Ar-H), 7.86 (s, 1H, CH), 8.14 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.91 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 50.26, 110.46, 118.70, 119.02, 124.44, 124.89, 126.31, 128.07, 129.69, 132.07, 137.06, 138.37, 140.01, 145.76, 147.03, 148.42, 150.31, 159.19, 159.74, 161.18, 165.22; LC-MS (70 eV): m/z = 539 $(M+H)^+$.

{N-(3-nitrophenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxo thiazolidin-3-yl)acetamide} (8e)

Yield: 97%; mp 244-246 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2868, 1710, 1674, 1622, 1536; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 5.01 (s, 2H, CH_2), 6.98 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.16 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.29 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.36 (s, 1H, Ar-H), 7.49 (s, 1H, NH), 7.71 (s, 1H, CH), 7.79-7.91 (m, 3H, Ar-H), 8.09 (d, J_{HH} = 12.0 Hz, 2H, Ar-H), 9.08 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 49.31, 107.19, 112.42, 118.02, 120.39, 126.48, 128.00, 128.46, 130.33, 135.18, 137.27, 139.41, 143.28, 146.19, 146.58, 149.07, 153.22, 157.42, 158.16, 160.22, 164.49; LC-MS (70 eV): m/z = 550 $(M+H)^+$.

{N-(2-nitrophenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxo thiazolidin-3-yl)acetamide} (8f)

Yield: 90%; mp 228-230 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2836, 1711, 1678, 1620, 1537; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 5.14 (s, 2H, CH_2), 6.86 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 6.93 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.21 (s, 1H, NH), 7.38 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.48-7.69 (m, 4H, Ar-H), 7.86 (s, 1H, CH), 8.03 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.79 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 56.40, 109.70, 111.33, 117.48, 119.37, 123.73, 125.07, 126.32, 129.41, 131.23, 136.30, 137.83, 139.08, 141.22, 144.39, 148.14, 150.24, 158.23, 159.18, 162.27, 165.38; LC-MS (70 eV): m/z = 550 $(M+H)^+$.

{N-(4-methoxyphenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxo thiazolidin-3-yl)acetamide} (8g)

Yield: 87%; mp 236-238 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2847, 1713, 1676, 1619, 1540; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 3.96 (s, 3H, OCH_3), 5.18 (s, 2H, CH_2), 6.91 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.12 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.24 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.32 (s, 1H, NH), 7.41 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.69 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.81 (s, 1H, CH), 8.12 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.91 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 54.30, 55.14, 108.20, 110.57, 118.30, 120.26, 124.22, 125.21, 129.27, 130.36, 134.75, 139.46, 140.28, 143.03, 146.28, 150.21, 156.07, 158.43, 159.09, 163.26; LC-MS (70 eV): m/z = 535 $(M+H)^+$.

{2-(4-Oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)-N-(p-tolyl)acetamide} (8h)

Yield: 85%; mp 198-200 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2878, 1711, 1668, 1612, 1539; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 2.18 (s, 3H, CH_3), 5.26 (s, 2H, CH_2), 6.86 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 6.98 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.19 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.29 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.42 (s, 1H, NH), 7.76 (s, 1H, CH), 7.89 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.06

(d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.86 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 26.38, 54.26, 110.14, 116.26, 119.43, 123.31, 124.10, 127.73, 129.30, 133.41, 138.09, 140.32, 140.96, 145.53, 148.63, 152.48, 155.27, 159.39, 160.28, 166.37; LC-MS (70 eV): m/z = 519 $(M+H)^+$.

{N-(4-bromophenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxo thiazolidin-3-yl)acetamide} (8i)

Yield: 84%; mp 242-244 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2868, 1714, 1673, 1609, 1552; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 5.07 (s, 2H, CH_2), 6.82 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 6.97 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.23 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.31 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.38 (s, 1H, NH), 7.62 (s, 1H, CH), 7.93 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.27 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 8.97 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 49.35, 112.35, 114.68, 117.27, 119.48, 120.26, 126.32, 128.41, 130.38, 137.26, 139.30, 142.37, 146.23, 148.67, 150.37, 156.30, 159.87, 160.39, 165.37; LC-MS (70 eV): m/z = 582 $(M+H)^+$.

{2-(4-Oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)-N-(o-tolyl)acetamide} (8j)

Yield: 90%; mp 238-240 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2851, 1709, 1670, 1618, 1548; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 2.26 (s, 3H, CH_3), 5.19 (s, 2H, CH_2), 6.88 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 6.95 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.26 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.30 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.38 (s, 1H, NH), 7.52 (dd, J_{HH} = 4.0 Hz and 8.0 Hz, 2H, Ar-H), 7.73 (s, 1H, CH), 7.98 (m, 2H, Ar-H), 8.76 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 23.41, 53.43, 108.39, 113.32, 117.43, 119.19, 120.23, 124.47, 127.36, 129.47, 131.32, 136.49, 139.57, 140.08, 142.97, 143.28, 149.37, 150.33, 154.37, 159.41, 164.35, 167.86; LC-MS (70 eV): m/z = 517 $(M-H)^-$.

{N-(2-methoxyphenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)acetamide} (8k)

Yield: 86%; mp 218-220 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2856, 1710, 1672, 1621, 1540; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 3.86 (s, 3H, OCH_3), 5.20 (s, 2H, CH_2), 6.83 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 6.96 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.20 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.29 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.43 (s, 1H, NH), 7.56-7.78 (m, 4H, Ar-H), 7.86 (s, 1H, CH), 8.89 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 51.41, 58.48, 108.87, 114.23, 116.24, 119.75, 121.28, 124.67, 128.36, 129.07, 133.22, 136.58, 139.14, 140.73, 143.79, 146.24, 149.09, 151.27, 155.70, 158.37, 165.41, 168.71; LC-MS (70 eV): m/z = 535 $(M+H)^+$.

{N-(3,4-dichlorophenyl)-2-(4-oxo-5-(4-(thieno[2,3-d]pyrimidin-4-yloxy)benzylidene)-2-thioxothiazolidin-3-yl)acetamide} (8l)

Yield: 91%; mp 223-225 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2891, 1710, 1668, 1608, 1536; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 5.16 (s, 2H, CH_2), 6.96 (d, J_{HH} = 4.0 Hz, 1H, thiophene-CH), 7.09 (d, J_{HH} = 8.0 Hz, 1H, thiophene-CH), 7.31 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.46 (s, 1H, NH), 7.58 (d, J_{HH} = 8.0 Hz, 2H, Ar-H), 7.69 (s, 1H, CH), 7.81 (s, 1H, Ar-H), 8.23 (d, J_{HH} = 12.0 Hz, 2H, Ar-H), 9.16 (s, 1H, pyrimidine-CH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 56.19, 109.26, 115.18, 118.40, 119.38, 120.46, 123.22, 127.81, 130.18, 133.37, 135.73, 138.22, 141.71, 147.20, 149.20, 150.09, 154.70, 157.21, 158.09, 162.28, 166.34; LC-MS (70 eV): m/z = 572 $(M+H)^+$.

Biological Evaluation

Antibacterial and antifungal assays

All the synthesized compounds were assessed for their antimicrobial (antibacterial and antifungal) activities. For antibacterial studies were screened by the *in vitro* minimum inhibitory concentrations (MICs) of the target compounds using with NCCLS broth dilution method in 96-

well micro test plates conferring to the National Committee for Clinical Laboratory Standards.[33,34] For antifungal tests were evaluated by MIC approach using with serial plate dilution method[35,36] and Gentamicin and Fluconazole were used as standard drugs.

Antibacterial assay

The synthesized target molecules were assessed for their antibacterial activity against *Escherichia coli* and *Klebsiella pneumonia* as Gram-negative Bacteria and *Bacillus cereus* and *Bacillus subtilis* as Gram-positive bacteria. The strain suspension was attuned with hygienic saline to a concentration of 1.0×10^5 c.f.u/mL. The tested molecules were dissolved using with DMSO (10 µL). It was added carefully in duplicate onto the surface of the pre immunized plate and allowed to dry before incubation. Further, the solvent to synthesis the standard solutions and also standard drugs were synthesized using with Mueller Hinton broth method by serial dilution to afford the various prerequisite concentrations such as 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µg/mL. All the dilutions were incubated for 24 h at 37 ˚C. To endorse that the solvent had no effect on bacterial growth and a control test was exhibited with test standard accompanied with DMSO at the same dilutions as used in the experiment. The MIC was perceived by detecting the lowermost concentration of the test compounds at which there was no visible progress in the presence of Gentamicin as standard.

Antifungal assay

For antifungal evaluating, the target molecules and Fluconazole (reference drug) solution was synthesized in DMSO to make an efficient various concentrations (3.12, 6.25, 12.5, 25.0, 50.0 and 100 µg/mL). Isolated fungal strains such as *Aspergillus flavus, Aspergillus niger, Penicillium marneffei* and *Candida albicans* were choosen and also Sabouraud dextrose agar was prepared at pH 5.7. A loopful of specific fungal strain was taken into saline (3 mL) to make a

suspension of following species. Next, 20 mL of agar media were poured into each Petri dish. Then, excess suspension was transferred and petri dishes were dried by placing in an incubator at 37 ^{0}C for 1 h. An agar sock was utilized to prepare wells and MICs of the test molecules in dimethylsulfoxide (DMSO) were added into each marked well. A control was prepared for the plates in the same way using solvent DMSO (10 µL). All the dishes were made in triplicate and preserved for 3-4 days at 37 ˚C. The activity was measured by calculating the MIC values.

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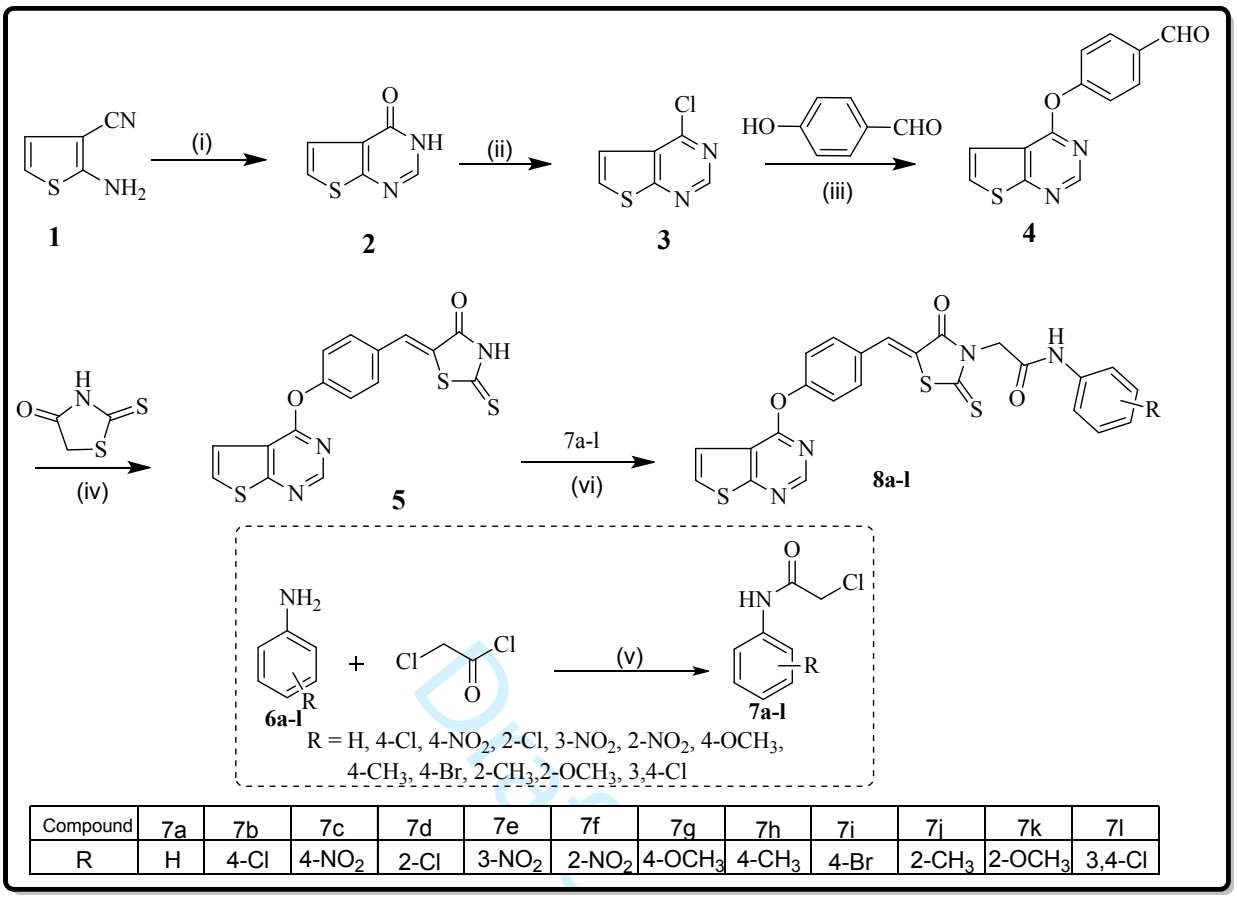
Scheme 1: Synthesis of thienopyrimidin-2-thioxothiazolidin-3-yl)acetamide derivatives (**8a-l**)



Compound	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	7k	7l
R	H	4-Cl	4-NO_2	2-Cl	3-NO_2	2-NO_2	4-OCH_3	4-CH_3	4-Br	2-CH_3	2-OCH_3	3,4-Cl

Reagents and conditions:

(i) HCOOH, 4 h, Reflux at 120 ˚C.

(ii) $POCl_3$, 5 h, Reflux.

(iii) K_2CO_3, Acetonitrile, 5 h, 30-40 °C;

(iv) NaOAc, AcOH, 6 h, Reflux at 90 °C.

(v) Et_3N, Acetone, 0-10 °C

(vi) Acetone, K_2CO_3, 3 h, Reflux.

Table 1: Antibacterial activities of the title compounds (8a-l)

Compound	MIC in µg/mL			
	Gram-possitive		Gram-negative	
	B. cereus	*B. subtilis*	*E. coli*	*K. pneumonia*
8a	12.5	12.5	25	12.5
8b	25	25	25	25
8c	3.12	1.56	1.56	3.12
8d	25	100	50	–
8e	6.25	12.5	12.5	6.25
8f	6.25	25	12.5	12.5
8g	6.25	12.5	6.25	6.25
8h	3.12	6.25	6.25	6.25
8i	50	–	25	25
8j	–	50	100	–
8k	50	100	50	50
8l	50	100	–	50
Gentamicin [a]	6.25	6.25	6.25	6.25

–Indicates bacteria is resistant to the compounds at >100 µg/mL

[a]Gentamicin was used as a standard

Table 2: Antifungal activities of the title compounds (8a-1)

Compound	MIC in µg/mL			
	A. flavus	***A. niger***	***P. marneffei***	***C. albicans***
8a	12.5	25	25	12.5
8b	–	25	25	50
8c	3.12	3.12	3.12	3.12
8d	12.5	12.5	6.25	12.5
8e	6.25	12.5	6.25	6.25
8f	3.12	3.12	6.25	3.12
8g	6.25	6.25	12.5	6.25
8h	25	–	25	50
8i	50	25	100	–
8j	25	–	–	50
8k	25	12.5	25	25
8l	6.25	6.25	12.5	6.25
Fluconazole[a]	6.25	3.12	6.25	6.25

–Indicates fungus is resistant to the compounds at >100 µg/mL

[a]Fluconazole was used as a standard