Synthesis of Novel Thiazol-5-ylpyrimidine Derivatives and Their Antimicrobial Evaluation

[1]Sanjay U. Deshmukh,[1] Raghunath B. Toche,[2]* Sushama J. Takate,[3] Supriya P. Salve,[3] Ram W. Sabnis[4]

[1]Department of Chemistry, KRT Arts, BH Commerce & AM Science College, Nashik 422002, India

[2†]Dadasheb Bidkar Arts, Science & Commerce College, Peth, Nashik422208, India

[†]Affiliated to SPP University, Pune, India

[3]Department of Chemistry, New Art, Commerce and Science College, Ahmednagar 414001 India

[4]Georgia-Pacific LLC, 133 Peachtree Street NE, Atlanta, GA 30303, USA

Correspondence

Raghunath B. Toche, Dadasheb Bidkar Arts, Science & Commerce College, Peth, Nashik422208, India, Email: raghunath_toche@rediffmail.com

ABSTRACT

Novel thiazol-5-ylpyrimidine derivatives were designed and synthesized. The chemical structures of all new synthesized compounds were assigned by studying their elemental analyses and spectral data (FT-IR, ^{1}HNMR, ^{13}C NMR and MS). The target compounds, **8** and **9a-9d** were evaluated for their antimicrobial activity *in vitro* against Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria, *Salmonella abony* and *Escherichia Coli* and fungi, *Aspergillus flavus* and *Fusarium oxysporum*. In particular, compounds **9a-9c** exhibited moderate to good activity against Gram-positive bacteria, *Staphylococcus aureus*, Gram-negative bacteria, *Salmonella abony* and fungus, *Fusarium oxysporum* in comparison with reference drugs.

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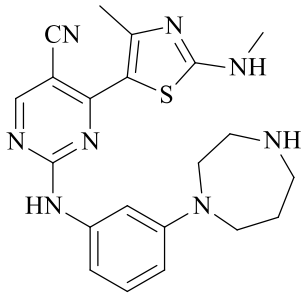
1 INTRODUCTION

The sulfur containing heterocyclic compounds, thiazoles exhibited key role in medicinal chemistry and subsequently materialised as a pharmacophore. The thiazoles moiety have attracted a great interest due to their ready accessibility, varied chemical reactivity, and wide range of biological activities like anticancer agents,[1] Antidiabetic agents,[2] antitubercular agents,[2] anti-Alzheimer's agents,[3] antifungal agents,[4] antiviral agents,[4] and antimicrobial agents.[4]

Pyrimidines are being building blocks of nucleic acids such as DNA and RNA, which make life possible. DNA damaged by exogenous physical and chemical agents is the most common cause of cancer. Conversely, some of the commonly anticancer drugs can kill malignant cell by damaging their DNA. Number of substituted pyrimidine derivatives are reported in literature having anticancer activity. Broprimine,[5] the 2-amino-5-bromo-6-phenylpyrimidin-4(3*H*)-one, is a well-known antiviral and antineoplastic agent.

Recently, thiazolylpyrimidines were extensively studied because of their magnificent pharmacological and therapeutic properties, such as cyclin dependent kinase (CDK) inhibitors,[6,10] Jak2 inhibitors,[7] spleen tyrosine kinase (SYK) inhibitors,[8] and their promising antibacterial,[9] antitumor,[10] and antihyperglycemic activities.[11] Further, cancer cells often have a high demand for antiapoptic proteins in order to resist programmed cell death. CDK9 inhibition selectively targets survival proteins and reinstates apoptosis in cancer cells. Thiazol-5-ylpyrimidine derivative **I** inhibits CDK9 with IC_{50} = 7 nM and shows over 80-fold selectivity for CDK9 over CDK2. X-ray crystal structure of compound **I** bound to CDK9 and CDK2 provide

insights into the binding modes. Compound **I** demonstrates potent anticancer activity against primary chronic lymphocytic leukemia cells with a therapeutic window 31- and 107-fold over those of normal B- and T-cells.[10]



I

Inspired by the above-mentioned potential biological activities of thiazolylpyrimidines, and our ongoing work aims for the synthesis of new molecules of pharmacological interest.[12-16] In this paper, we disclosed an efficient method towards the synthesis of new series of thiazolylpyrimidine derivatives and were screened for their antimicrobial activity.

2 RESULTS AND DISCUSSION

2.1 Chemistry

The synthetic method has been described in two reaction schemes to synthesize the key intermediate compound **6** (Scheme 1) and final thiazolylpyrimidines **9a-9d** (Scheme 2). The entire reaction sequence for synthesizing key intermediate compound **6** from compound **1** is provided in Scheme 1. The first starting compound, ethyl 2-amino-4-methylthiazole-5-carboxylate[17] **2** was prepared in excellent yield (84%) via heating 2-chloro ethylacetoacetate **1** with thiourea in ethanol. The structure of compound **2** was established by studying elemental analysis, FTIR, ^{1}H NMR and mass spectral analyses. IR spectrum showed absorption bands at 3373.24 cm^{-1} and 1673.46 cm^{-1} due to NH_2 group and CO group, respectively. ^{1}H NMR spectrum

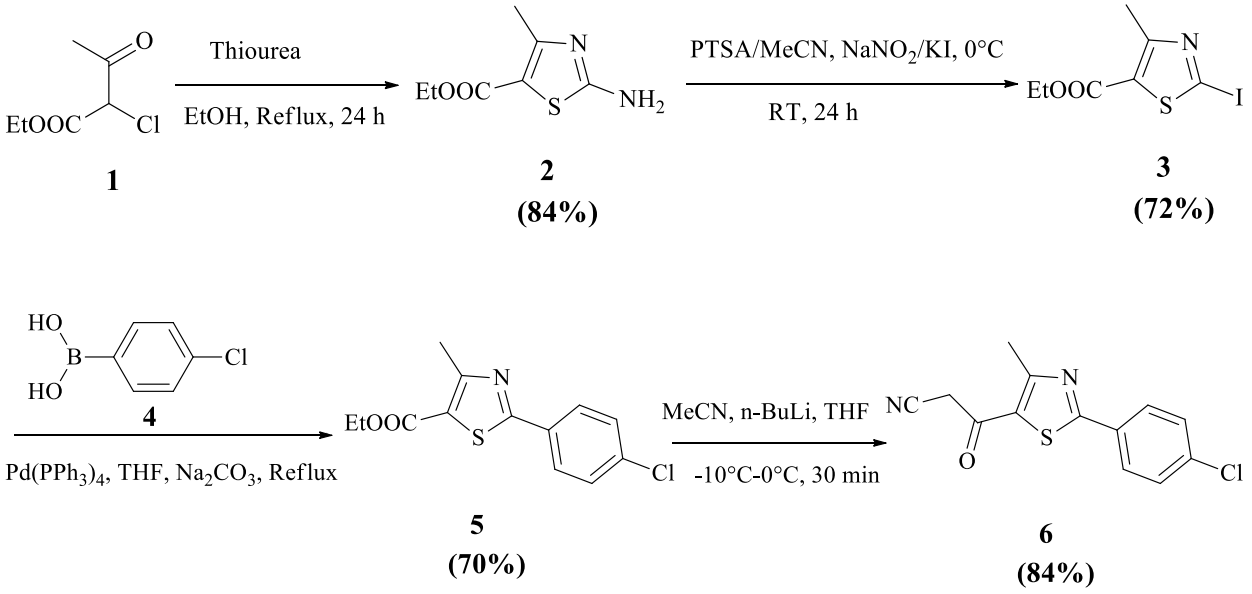
exhibited signals at δ 7.70 ppm and δ 2.37 ppm for NH_2 and CH_3 protons of thiazole **2**, respectively`.

Ethyl 2-iodo-4-methylthiazole-5-carboxylate[18] **3** was synthesized from ethyl 2-amino-4-methylthiazole-5-carboxylate **2** via Sandmeyer reaction, by performing diazotization of ethyl 2-amino-4-methylthiazole-5-carboxylate with sodium nitrite/acid at 0°C and coupled with potassium iodide at 0°C in water followed by continuous stirring for 24 h at room temperature. Structure of compound **3** was elucidated using elemental analysis data, FTIR, ^{1}H NMR and mass spectral analyses. The IR spectrum showed stretching frequency at 1711 cm^{-1} because of CO group and showed absence of band for NH_2 group. ^{1}H NMR spectrum exhibited singlet at δ 2.62 ppm due to CH_3 protons and disappearance of singlet, which corresponds to NH_2 of thiazole **2**.

Ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate[19] **5** was prepared from ethyl 2-iodo-4-methylthiazole-5-carboxylate **3** by Suzuki coupling reaction. Ethyl-2-iodo-4-methylthiazole-5-carboxylate **3** and (4-chlorophenyl)boronic acid **4** and $Pd(PPh_3)_4$ in THF under nitrogen atmosphere afforded ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate **5**. Structure **5** was elucidated by analysing elemental analysis, FT-IR, ^{1}H NMR and mass spectral analyses.

The key intermediate, 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-3-oxopropanenitrile **6** was synthesized by condensing 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate **5** with acetonitrile in THF using n-BuLi under nitrogen atmosphere below -10°C. Structure **6** was illustrated using elemental analysis data, FT-IR, ^{1}H NMR and mass spectral analyses. The IR spectrum showed stretching band at 2263 cm^{-1} and 1676 cm^{-1} for CN and CO. The ester carbonyl stretching frequency of compound **5** at 1709 cm^{-1} has now shifted in compound **6** at 1676 cm^{-1}. Further, there is prominent stretching frequency at 2263 cm^{-1}due to nitrile group. ^{1}H NMR

spectrum exhibited distinct singlet at δ 4.68 ppm corresponding to two CH_2 protons of compound **6**.



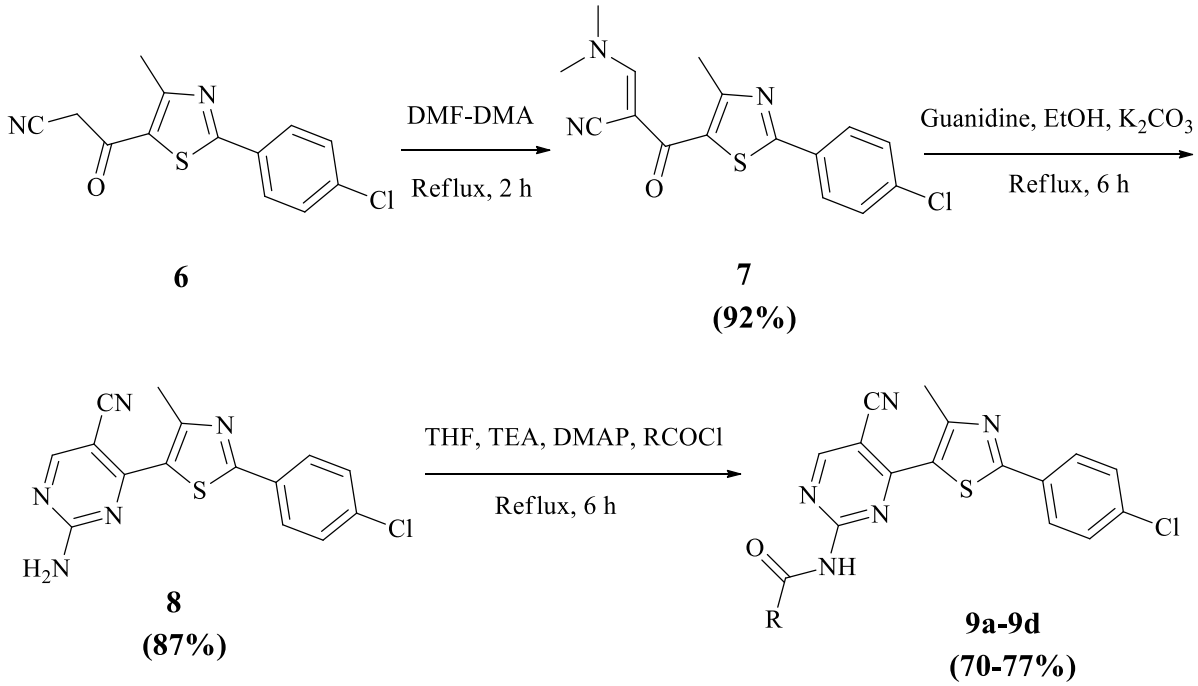
Scheme 1: Synthesis compound **6** as key intermediate.

The entire reaction sequence for synthesizing compounds **9a-9d** from key intermediate compound **6** is provided in Scheme 2. Compound, 2-(2-(4-chlorophenyl)-4-methylthiazole-5-carbonyl)-3-(dimethylamino) acrylonitrile **7** was prepared in excellent yield (92%) via heating 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-3-oxopropanenitrile **6** with dimethylformamide-dimethylacetal (DMF-DMA). Structure **7** was established by elemental analysis data, FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral analyses. IR spectrum showed stretching at 1633 cm^{-1} for C=C, enamine group. ^{1}H NMR spectrum exhibited singlet at δ 3.528 ppm and δ 3.558 ppm corresponding to six protons of 2N-CH_3 and no singlet corresponding to CH_2CN of thiazole **7**.

Compound, 2-amino-4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)pyrimidine-5-carbonitrile **8** was synthesized in high yield (87%) via heating 2-(2-(4-chlorophenyl)-4-methylthiazole-5-carbonyl)-3-(dimethylamino) acrylonitrile **7** in ethanol with guanidine and

anhydrous potassium carbonate. The Structure of the compound **8** was elucidated by elemental analysis data, FT-IR, ^{1}H NMR, ^{13}C NMR and Mass spectral analyses. IR spectrum showed stretching at 3402 cm^{-1} and 3177 cm^{-1} for the presence of NH_2 group and absence of stretching due to C=C, enamine group. ^{1}H NMR spectrum exhibited singlet at δ 8.782 ppm and δ 7.970 ppm corresponding to pyrimidine-H and NH_2 protons, respectively and no proton signals corresponding to six 2N-CH_3 protons in compound **8**.

The compounds, amide analogs of 2-amino-4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)pyrimidine-5-carbonitrile **9a-9d** were prepared in high yields (70-77%) via heating 2-amino-4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)pyrimidine-5-carbonitrile **8** in THF, triethylamine and 4-dimethylaminopyridine with corresponding acid chlorides. Structures **9a-9d** were illustrated by elemental analysis as well as FT-IR, ^{1}H NMR and mass spectral analyses.



6

7
(92%)

8
(87%)

9a-9d
(70-77%)

9a: R = 3-F-Ph

9b: R = 4-F-Ph

9c: R = cyclohexyl

9d: R = isobutyl

Scheme 2: General synthetic route of the target compounds **9a-9d**

2.2 Biology

2.2.1 Antimicrobial activity

The newly synthesized compounds, **8** and **9a-9d** were screened to determine their antimicrobial activity *in vitro* against two pathogenic Gram-positive bacteria viz. *Bacillus subtilis* and *Staphylococcus aureus*, two pathogenic Gram-negative bacteria viz *Salmonella abony* and *Escherichia Coli* and fungal culture of *Aspergillus flavus* and *Fusarium oxysporum*. The drugs used as reference were Ciprofloxacin for antibacterial and Luliconazole for antifungal tests, correspondingly.

2.2.2 Antibacterial assay

The antibacterial activity of the synthesized compounds was studied against two Gram-positive strains *Bacillus subtilis* and *Staphylococcus aureus*, two Gram-negative strains *Salmonella abony* and *Escherichia Coli*using Ciprofloxacin as standard by employing the Agar diffusion method. The media prepared using solid culture such as 10 g peptone, 10 g sodium chloride, 5 g yeast extract and 20 g agar were dissolved in 1000 ml of distilled water. The stock solution of culture was inoculated in broth media and grown at 37°C for 18 hours and was revived. This stock solution was added in the petridishes and bores were made in the plate. For 18 hours old cultures were (100 μL 10^{-4} cfu) inoculated for each plate and spread consistently on the plates. The bores were filled after 20 minutes containing different concentrations of samples and antibiotic. After that at 37°C all the dishes were incubated and zone of inhibition in diameter (mm) noted after 24 hours.[20,21]

2.2.3 Antifungal assay

The antifungal activity of the synthesized compounds was studied against two fungal strains *Aspergillus flavus* and *Fusarium oxysporum*using Luliconazole by using Agar diffusion method. The media Czapek-Dox Agar was prepared using solid culture composition of 30 g sucrose, 2 g sodium nitrite, 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4$, and 20 g agar dissolved in 1000 mL of distilled water. This stock culture was inoculated in broth media and grown at 27°C for 48 hours and revived. This stock solution was poured in the petri plate and wells were made in each petri plate. For 48 hours old cultures (100 μL 10^4 CFU) inoculated for each plate and spread consistently on the plate. The wells were filled after 20 minutes containing different concentrations of samples and antibiotic. After that at 27°C all the plates incubated and zone of inhibition noted in diameter (mm) after 96 hours.[20,21]

It was observed that the compound **8** exhibited moderate antibacterial activity against Gram-negative *S. abony* of inhibition zone 13 mm/mg vs 40 mm/mg as compared to reference drug Ciprofloxacin. The antifungal activity of compound **8** against fungi *F. oxysporum* was comparable inhibition zone 12 mm/mg when compared to that of the reference drug Luliconazole 17 mm/mg.

Further, the compound **9a** exhibited good to moderate antibacterial activity against Gram-positive *S. aureus* and Gram-negative *S. abony* of inhibition zones 14, 11 mm/mg vs 23, 40 mm/mg as compared to reference drug Ciprofloxacin, while its antifungal activity against fungi *F. oxysporum* was good producing inhibition zone 13 mm/mg when compared to that of the reference drug Luliconazole 17 mm/mg.

Also, the compound **9b** exhibited good to moderate antibacterial activity against Gram-positive *S. aureus* and Gram-negative *S. abony* of inhibition zones 15, 10 mm/mg vs 23, 40

mm/mg of the reference drug Ciprofloxacin, while its antifungal activity against fungi *F. oxysporum* was potent producing inhibition zone 11 mm/mg when compared to that of the reference drug Luliconazole 17 mm/mg.

Further, the compound **9c** exhibited good to moderate antibacterial activity against Gram-positive *S. aureus* and Gram-negative *S. abony* of inhibition zones 14, 12 mm/mg vs 23, 40 mm/mg of the reference drug Ciprofloxacin, while its antifungal activity against fungi *F. oxysporum* was potent producing inhibition zone 11 mm/mg when compared to that of the reference drug Luliconazole 17 mm/mg.

Furthermore, the compound **9d** exhibited good antibacterial activity against Gram-positive *S. aureus* of inhibition zone 14 mm/mg vs 23 mm/mg of the reference drug Ciprofloxacin, while it represented weak to complete loss of antifungal potency. Additional modification and optimization are needed to get new candidates of more significant antimicrobial activity against various types of bacteria and fungi.

TABLE 1 Antimicrobial activity of the samples against Gram-positive bacteria, Gram-negative bacteria, and Fungi

Sample	**Antibacterial activity**				**Antifungal activity**	
	B. subtilis	*S. aureus*	*S. abony*	*E. coli*	*A. flavus*	*F. oxysporum*
8	-	-	13	-	-	12
9a	-	14	11	-	-	13
9b	-	15	10	-	-	11
9c	-	14	12	-	-	11
9d	-	14	-	-	-	-
Ciprofloxacin	30	23	40	26	N/A	N/A
Luliconazole	N/A	N/A	N/A	N/A	23	17

3 MATERIALS AND METHODS

The chemicals and solvents used were dried and purified by using standard literature procedures and moisture was removed from the glass apparatus using $CaCl_2$ drying tubes. The melting points were determined in open capillary tubes with Gallenkamp melting point apparatus and are uncorrected. FT-IR spectra were recorded on Bruker FTIR-TENSOR II spectrophotometer using Platinum ATR discs. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Varian Mercury 300 NMR spectrophotometer at 300 MHz frequency and Bruker 400 NMR spectrophotometer at 400 MHz and 100 MHz frequency in $CDCl_3$ or dimethyl sulfoxide (DMSO-d_6) using tetramethylsilane (TMS) as internal standard. Chemical shifts were recorded in δ ppm and multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Mass spectra were recorded on a Shimadzu LC-MS QP 2020A mass spectrometer with an ionization potential of 70 eV. Elemental analyses were performed on Thermo Quest Flash 1112 Series EA analyzer. Reactions were monitored by thin layer chromatography, carried out on 0.2 mm silica gel 60 F_{254} (Merck) plates using UV light (254 and 366 nm) for detection. Regular reagent grade chemicals were commercially available and used without further purification or prepared by standard literature procedures. All the compounds were prepared by conventional methods.

4. EXPERIMENTAL

4.1 Synthesis of ethyl 2-amino-4-methylthiazole-5-carboxylate (2)

A mixture of 2-chloro ethylacetoacetate (20g, 0.125mol), thiourea (11.09g, 0.145mol) in EtOH (100mL) was reflux for 24 h (monitored by TLC). The solvent was evaporated under reduced pressure and the residue was stirred in ice-water mixture. The resultant precipitate was subjected

to filtration, dried and recrystallized from ethanol as white solid, yield 84%; mp 172-175°C. IR (KBr, cm^{-1}): $\bar{\upsilon}$ = 3373.24 (NH_2), 1673.46 (C=O, ester); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.70 (bs, 2H, NH_2), 4.17-4.10 (q, J = 7.1Hz, 2H, CH_2), 2.37 (s, 3H, CH_3), 1.24-1.19 (t, J = 7.1Hz, 3H, CH_3); MS *m/z* (%): 185.43 (M-1), 186.05 (100.0), 187.05 (8.6), 188.04 (4.5); Anal. Calcd. (%) for $C_7H_{10}N_2O_2S$: C, 45.15; H, 5.41; N, 15.04. Found: C, 45.37; H, 5.48; N, 15.12.

4.2 Synthesis of ethyl 2-iodo-4-methylthiazole-5-carboxylate (3)

To a solution of ethyl 2-amino-4-methylthiazole-5-carboxylate (10 g, 0.05 mol) in acetonitrile (100 mL) was added p-toluenesulfonic acid monohydrate (20.4 g, 0.107 mol) and the reaction mixture was stirred at room temperature for 4 h. The mixture was cooled to 0°C, followed by addition of aqueous solution of $NaNO_2$ (5.5 g, 0.080 mol) and KI (13.36 g, 0.080 mol) in water (25 mL), maintaining temperature below 0°C. The reaction mixture was further stirred for 24 h at room temperature followed by addition of aqueous sodium meta bisulphate and was extracted with ethyl acetate (5 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent evaporated in vacuum, dried and recrystallized from ethanol as pale yellow solid, yield 72%; mp 175-177°C. IR (KBr, cm^{-1}): $\bar{\upsilon}$ =1711 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.29-4.22 (q, J = 7.1 Hz, 2H, CH_2) 2.62 (s, 3H, CH_3), 1.29-1.24 (t, J = 7.1 Hz, 3H, CH_3); MS *m/z* (%): 297 (M^+), 296.93 (100.0), 297.94 (7.7), 298.93 (4.6), 297.93 (1.2); Anal. Calcd. (%) for $C_7H_8INO_2S$: C, 28.30; H, 2.71; N, 4.71. Found: C, 28.53; H, 2.77; N, 4.79.

4.3 Synthesis of ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate (5)

To a solution of ethyl-2-iodo-4-methylthiazole-5-carboxylate(9.3g, 0.03 mol) and (4-chlorophenyl)boronic acid(5.87 g, 0.037 mol) in dry THF (50 mL), was added $Pd(PPh_3)_4$ (903 mg, 25 mol%) under nitrogen atmosphere and the mixture was stirred at room temperature for 30 min. Aqueous Na_2CO_3 (10%) solution was added to the mixture and was heated under reflux

for 2 days. The THF was evaporated under reduced pressure, the residue was stirred in ice-water mixture and was extracted with ethyl acetate (5 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent evaporated in vacuum. The crude compound was purified by silica gel column chromatography, using 2% ethyl acetate-petroleum ether mixture as eluent afforded as white solid, yield 70%; mp 165-167°C. IR (KBr, cm^{-1}): $\bar{\upsilon}$ = 2988(CH), 1709(C=O), 1522(Ar-C=C); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.02-8.00 (d, J = 9 Hz, 2H, Ar-H), 7.60-7.57 (d, J = 9 Hz, 2H, Ar-H), 4.31-4.29 (q, J = 6.9 Hz, 2H, CH_2), 2.69(s, 3H, CH_3), 1.33-1.30 (t, J = 6.9 Hz, 3H, CH_3); MS *m/z* (%): 282.53 $(M+H)^+$, 281.03 (100.0), 283.02 (36.5), 282.03 (15.1), 284.03 (5.2), 283.03 (1.5), 285.02 (1.5); Anal. Calcd. (%) for $C_{13}H_{12}ClNO_2S$: C, 55.42; H, 4.29; N, 4.97. Found: C, 55.47; H, 4.31; N, 4.98.

4.4 Synthesis of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-3-oxopropanenitrile (6)

A solution of ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate(4.6 g, 0.014 mol) and acetonitrile (1.10 mL, 0.021 mol) in dry THF (25 mL) was stirred under nitrogen atmosphere below -10°C. To this solution, n-BuLi (9.65 mL, 1.6 molar solution in hexane, 0.0154 mol) was slowly added maintaining the temperature below -5°C. The reaction mixture was stirred for 30 min followed by addition of 2N HCl (10 mL) below 0°C and was extracted with ethyl acetate (2 x 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent evaporated in vacuum, dried and recrystallized from methanol as pale yellow solid, yield 84%; mp 151-153°C. IR (KBr, cm^{-1}): $\bar{\upsilon}$ = 3444 (CH), 2263 (CN), 1676 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.04-8.02 (d, J = 9 Hz, 2H, Ar-H), 7.63-7.60 (d, J = 9 Hz, 2H, Ar-H), 4.68 (s, 2H, CH_2CN), 2.71 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 182.93, 168.89, 160.29, 136.90, 131.10, 130.00, 128.84, 115.57, 33.38, 18.71; MS *m/z* (%): 277.41 $(M+1)^+$, 276.01 (100.0), 278.01 (36.6), 277.02 (14.2), 279.01 (5.7), 280.01 (1.7), 277.01 (1.5), 278.02 (1.3);

Anal. Calcd. (%) for $C_{13}H_9ClN_2OS$: C, 56.42; H, 3.28; N, 10.12. Found: C, 56.67; H, 3.12; N, 10.22.

4.5 Synthesis of 2-(2-(4-chlorophenyl)-4-methylthiazole-5-carbonyl)-3-(dimethylamino)acrylonitrile(7)

A mixture of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-3-oxopropanenitrile (1g, 0.0036 mol) and dimethylformamide-dimethylacetal (DMF-DMA) (5mL) was heated under reflux for 2 h (monitored by TLC). The DMF-DMA was evaporated under reduced pressure and the crude product was washed with n-pentane gave as yellow solid, yield 92%; mp 125-127°C. IR (KBr, cm^{-1}): $\bar{\upsilon}$ = 2191 (CN), 1698 (C=O, ketone), 1633 (C=C, enamine), 1592, 1571 (Ar-C=C); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.98 (s, 1H, olefin-H), 7.94-7.92 (d, J = 8.8 Hz, 2H, p-Cl-Ph), 7.44-7.42 (d, J = 8.8 Hz, 2H, p-Cl-Ph), 3.52 (s, 3H, N-CH_3), 3.55 (s, 3H, N-CH_3), 2.72 (s, 3H, CH_3); MS *m/z*: 332.40 $(M+1)^+$; Anal. Calcd. (%) for $C_{16}H_{14}ClN_3OS$: C, 57.91; H, 4.25; N, 12.66. Found: C, 57.75; H, 4.18; N, 12.61.

4.6 Synthesis of 2-amino-4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)pyrimidine-5-carbonitrile (8)

To a solution of 2-(2-(4-chlorophenyl)-4-methylthiazole-5-carbonyl)-3-(dimethylamino) acrylonitrile (1g, 0.003 mol) in ethanol (10 mL), was added guanidine (0.214 g, 0.0036 mol) and anhy. K_2CO_3 (1.2 g, 0.009 mol) and was heated under reflux for 6 h (monitored by TLC). The ethanol was evaporated under reduced pressure, the residue was stirred in ice-water mixture and acidified with 2N dil. HCl. The resultant precipitate was subjected to filtration and recrystallized from ethanol as brown solid, yield 87%; mp 253-255°C. IR (KBr, cm^{-1}): $\bar{\upsilon}$ = 3402, 3177 (NH_2, amine), 2215 (CN), 1658, 1579 (Ar-C=C); ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.78 (s, 1H, pyrimidine-H), 8.02-8.00 (d, 2H, J = 8.8 Hz, p-Cl-Ph), 7.97 (bs, 2H, NH_2), 7.62-7.60 (d, J = 8.8

Hz, 2H, p-Cl-Ph), 2.58 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 166.57, 164.257, 163.392, 161.657, 155.058, 136.101, 131.518, 129.929, 128.542, 128.165, 117.762, 94.545; MS *m/z*: 328.28 $(M+1)^+$; Anal. Calcd. (%) for $C_{15}H_{10}ClN_5S$: C, 54.96; H, 3.07; N, 21.37. Found: C, 54.91; H, 3.09; N, 21.32.

4.7 General Procedure for synthesis of amide analogs of 2-amino-4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl) pyrimidine-5-carbonitrile (9a-9d)

To a solution of 2-amino-4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)pyrimidine-5-carbonitrile (0.1 g, 0.0003 mol) in THF (5 mL), was added triethylamine (0.3 mL), catalytic amount of 4-dimethylaminopyridine (0.036 g, 0.00003 mmol) and acid chloride(0.057 g, 00036 mol) and was heated under reflux for 6 h (monitored by TLC). The reaction mixture was stirred in ice-water mixture and was extracted with ethyl acetate (2 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent evaporated in vacuum. The crude compound was washed by diethyl ether and n-pentane gave pure solids, yields 70-77%.

4.7.1 N-(4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-5-cyanopyrimidin-2-yl)-3-fluoro-benzamide (9a)

Yellow solid, yield 70%; mp 209-211°C. IR (KBr, cm^{-1}): $\bar{\upsilon}$ = 3494 (NH, amide), 2227 (CN), 1716 (C=O, amide), 1602 (Ar-C=C); ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.74 (s, 1H, CONH), 9.20 (s, 1H, pyrimidine-H), 8.06-8.04 (m, 4H, p-F-Ph), 7.81-7.79 (d, J = 8 Hz, 2H, p-Cl-Ph), 7.78-7.49 (d, J = 8 Hz, 2H, p-Cl-Ph), 2.76 (s, 3H, CH_3); MS *m/z*: 450.41$(M+1)^+$; Anal. Calcd. (%) for $C_{22}H_{13}ClFN_5OS$: C, 58.73; H, 2.91; N, 15.57. Found: C, 58.76; H, 2.95; N, 15.56.

4.7.2 N-(4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-5-cyanopyrimidin-2-yl)-4-fluoro-benzamide (9b)

Off white solid, yield 77%; mp 201-203°C. IR (KBr, cm^{-1}): ῡ = 3494 (NH_2 amide), 2227 (CN), 1716 (C=O, amide), 1602 (Ar-C=C); ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.30 (s, 1H, pyrimidine-H), 7.99-7.93 (m, 4H, p-F-Ph), 7.63-7.61 (d, J = 8.8 Hz, 2H, p-Cl-Ph), 7.41-7.36 (d, J = 8 Hz, 2H, p-Cl-Ph), 2.39 (s, 3H, CH_3); MS *m/z*: 448.33(M-1); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.101, 168.266, 166.952, 165.235, 164.434, 161.591, 160.184, 158.478, 136.638, 132.939, 132.842, 131.089, 130.032, 128.697, 126.091, 115.831, 103.841, 18.547; Anal. Calcd. (%) for $C_{22}H_{13}ClFN_5OS$: C, 58.73; H, 2.91; N, 15.57. Found: C, 58.76; H, 2.95; N, 15.56.

4.7.3 N-(4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-5-cyanopyrimidin-2-yl)cyclohexane carboxamide (9c)

Pale yellow solid, yield 72%; mp 219-221°C. IR (KBr, cm^{-1}): ῡ = 3422 (NH_2 amide), 2220 (CN), 1677 (C=O, amide), 1578, 1500 (Ar-C=C); ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.12 (s, 1H, CONH), 9.18 (s, 1H, pyrimidine-H), 8.05-8.03 (d, J = 8.8 Hz, 2H, p-Cl-Ph), 7.63-7.61 (d, J = 8.8 Hz, 2H, p-Cl-Ph), 2.73 (s, 3H, CH_3), 2.67-2.61 (m, 1H, CH), 1.85-1.39 (m, 10H, cyclohexyl); MS *m/z*: 438.20 $(M+1)^+$; Anal. Calcd. (%) for$C_{22}H_{20}ClN_5OS$: C, 60.34; H, 4.60; N, 15.99. Found: C, 60.41; H, 4.61; N, 15.96.

4.7.4 N-(4-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-5-cyanopyrimidin-2-yl)isobutyramide (9d)

Off white solid, yield 70%; mp 237-239°C. IR (KBr, cm^{-1}): ῡ = 3422 (NH_2 amide), 2220 (CN), 1677 (C=O, amide), 1578, 1500 (Ar-C=C); ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.18 (s, 1H, CONH), 9.18 (s, 1H, pyrimidine-H), 8.05-8.03 (d, J = 8.8 Hz, 2H, p-Cl-Ph), 7.63-7.61 (d, J = 8.8 Hz, 2H, p-Cl-Ph), 2.73 (s, 3H, CH_3), 2.90-2.85 (m, 1H, CH), 1.11-1.10 (m, 6H, $2CH_3$); MS *m/z*: 396.56 (M-1); Anal. Calcd. (%) for $C_{19}H_{16}ClN_5OS$: C, 57.35; H, 4.05; N, 17.60. Found: C, 57.39; H, 4.06; N, 17.63.

5 CONCLUSIONS

We have designed and synthesized a novel series of thiazolylpyrimidine derivatives. All new synthesized compounds, **8** and **9a-9d** were screened for antimicrobial activity *in vitro* against two pathogenic Gram-positive bacteria viz. *Bacillus subtilis* and *Staphylococcus aureus*, two pathogenic Gram-negative bacteria viz. *Salmonella abony* and *Escherichia Coli* and fungal culture of *Aspergillus flavus* and *Fusarium oxysporum*. Our results showed that compounds **9a-9c** exhibited moderate to good activity against Gram-positive bacteria, *Staphylococcus aureus*, Gram-negative bacteria, *Salmonella abony* and fungus, *Fusarium oxysporum*. Therefore, it was concluded that thiazolylpyrimidne derivatives could be developed as novel and promising antimicrobial agents.

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ORCID

Raghunath B. Toche: https://orcid.org/0000-0001-9555-9277

Ram W. Sabnis: https://orcid.org/0000-0001-7289-0581

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