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Synthesis and Antimicrobial Activity of New 3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl) substituted- isoxazol-5-amine, 1-phenyl-1*H*-pyrazol-5-amine, and their Derivatives

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ABSTRACT New 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl) substituted aminoisoxazole, aminopyrazole, and their appropriate urea and amide analogs were prepared from thiazole substituted oxopropanenitrile. The structures of newly synthesized compounds were illustrated by infrared, nuclear magnetic resonance, and mass spectroscopic analysis and elemental analyses. The antimicrobial evaluation of analogs was carried out with Gram-positive bacteria **(***Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*). They showed moderate to good activity against both Gram-positive and Gram-negative bacteria.

KEYWORDS Antimicrobial activity, thiazole, isoxazole, pyrazole, oxopropanenitrile.

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INTRODUCTION

Over prescription and lack of awareness of antibacterial therapy cause to increase resistance power of microorganism over multiple antimicrobial agents available in market. Microorganisms develop resistance over antimicrobial agent using different installation mechanisms by natural way like to develop ability to neutralize their effect or pump out antibiotic before they effect. Some microorganism changes the site which effects due to inhibitor made it ineffective. It creates a serious problem for a patient suffering from an immunosuppressant disorder such as AIDs, tuberculosis, chronic disorders, cancer chemotherapy, and any type of surgery where microorganisms grow very fast and hence create a serious problem if the available microbial agents are unable to control particular infection. It leads go back to the pre-antibiotic era that time small injuries converted to serious one that causes death due to uncontrolled growth of microorganism. That's why it is a very big challenge in front of medicinal chemist and microbiologist to find out various novel inhibitors they may target either cell wall, folic acid or DNA synthesis, or any other innovative mechanism to control antimicrobial growth. To find out an alternative for treating resisted microorganisms is a very important and prime priority in the 21st century for the pharmaceutical industry and various government counsels to avoid situations like pre-antibiotic era.

Thiazole substituted heterocyclic compounds are seem to be very promising pharmacophore in antimicrobial research. The antibiotic cefdinir semisynthetic third-generation cephalosporin contains amino thiazole with

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ester and acid moiety. It is an orally administrative antibiotic which shows antibacterial activity against both Germ-positive and -negative bacteria, especially against *Staphylococcus* species. Furthermore, drug sulfathiazole the thiazole sulfonamide is an excellent antimicrobial agent and abafungin amino thiazole substituted pyrimidine is used as antibiotic.

In recent years, thiazole substituted with pyrazole, pyrimidine, pyridine, chalcones, and azo compounds have been extensively studied because of their magnificent pharmacological and therapeutic properties, particularly they show good antimicrobial activity[1-3] and promising bacterial DNA gyrase inhibitor.[3] Literature search reveals that thiazole substituted heterocyclic compounds are important pharmacophore scaffold due to their wide range of biological and industrial applications. These derivatives have been shown to possess antimicrobial,[4] anti-inflammatory,[5] antiviral,[6] anti-HIV,[7] anticancer,[8] antitumor,[9] antidiabetic,[10] anticonvulsant,[11] antioxidant,[12] and diuretic[13] activities.

Prompted by the aforementioned biological and pharmaceutical activities of thiazole substituted heterocyclic compounds, as a part of an ongoing program aiming to the synthesis of new heterocyclic compounds of pharmacological interest.[14-18] We describe herein an efficient procedure for the synthesis of a novel series of thiazole substituted pyrazole and isoxazole derivatives. The newly synthesized compounds were evaluated for their antimicrobial activity, particularly for antibacterial activity.

RESULTS AND DISCUSSION

Chemistry

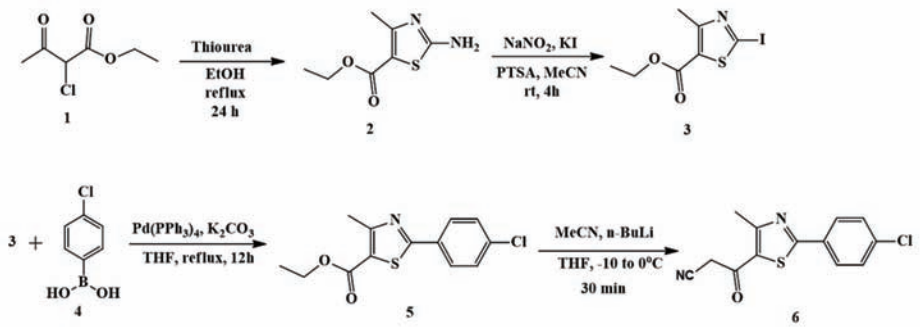
The reactions involved in the synthesis of target compounds are depicted in **Schemes 1-3**. The starting compound ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate **(5)** was prepared as per described in literature,[19-21] starting from ethyl 2-Chloroacetoacetate **(1)** and thiourea was refluxed in ethanol[19] to offer white solid product ethyl 2-amino-4-methylthiazole-5-carboxylate **(2)**, which was converted to ethyl 2-iodo-4- methylthiazole-5-carboxylate **(3)** using Sandmeyer reaction.[20] The iodine of compound **3** was replaced by 4-chlorophenyl using the Suzuki coupling reaction to obtain compound **5**.[21]

The key intermediate 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-3-oxopropanenitrile **(6)** was synthesized by the condensation of compound **5** with acetonitrile using n-BuLi in THF at −10°C. Compound **6** was confirmed by characteristic methylene protons singlet at δ 4.68 ppm in ^{1}H nuclear magnetic resonance (NMR) spectrum and carbonyl ketone stretching at 1676/cm with nitrile absorption band at 2263/cm^1 in infrared (IR) spectrum. The entire reaction sequence for synthesizing key intermediate compound **6** from compound **1** is outlined in **Scheme 1**.

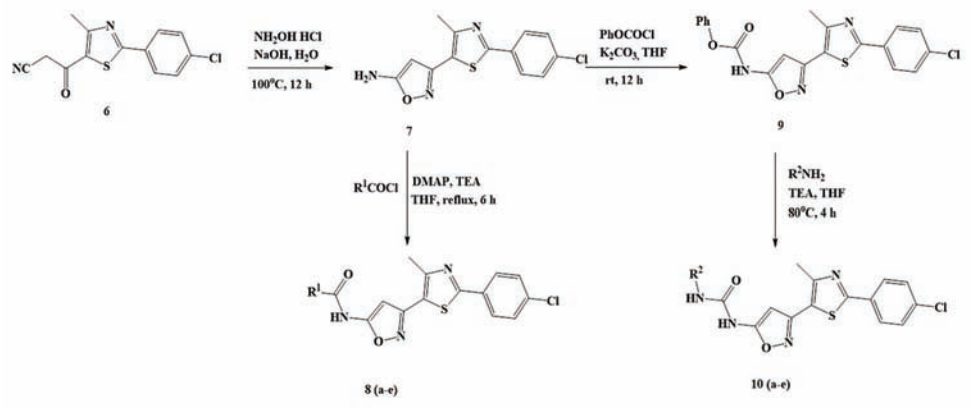
The compound 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-amine **(7)** was obtained from compound **6** by refluxing with hydroxylamine hydrochloride in NaOH and EtOH. Compound **7** was confirmed by singlet of isoxazole ring proton at δ 5.30 ppm and two exchangeable amine protons at δ 6.9 ppm in its ^{1}H NMR spectrum. This amine group of compound **7** was converted to different amide analogs $\mathbf{8}_{(a\text{-}e)}$ by refluxing with appropriate acid chlorides in TEA and THF. Amide analogs $\mathbf{8}_{(a\text{-}e)}$ were confirmed by exchangeable amide NH in ^{1}H NMR spectrum and carbonyl stretching of amide in IR spectrum.

The compound, phenyl (3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)carbamate **(9)**, was obtained from compound **7** by reacting with phenyl chloroformate in THF and potassium carbonate at room temperature. Compound **9** was confirmed by IR, ^{1}H NMR, and mass spectral data. This phenoxy carbamate **9** was converted to different urea analogs $\mathbf{10}_{(a\text{-}e)}$ by refluxing with corresponding amines in tetrahydrofuran (THF) and triethylamine (TEA). Urea analogs $\mathbf{10}_{(a\text{-}e)}$ were confirmed by two exchangeable NH of urea in ^{1}H NMR spectrum and carbonyl stretching of urea in IR spectrum. The entire reaction sequence for synthesizing amide $\mathbf{8}_{(a\text{-}e)}$ and urea $\mathbf{10}_{(a\text{-}e)}$ analogs from intermediate compound **6** is outlined in **Scheme 2**.

The intermediate compound **6** was converted to 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazol-5-amine **(11)** by refluxing of oxopropanenitrile with phenylhydrazine in ethanol and catalytic amount of acetic acid. ^{1}H NMR spectrum of compound **11** showed a singlet of pyrazole ring proton at δ 5.81 ppm and two exchangeable amine protons at δ 5.59 ppm. This amine **11** was converted to different amides analogs $\mathbf{12}_{(a\text{-}c)}$ using corresponding acid chlorides refluxing in THF and TEA with a catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction

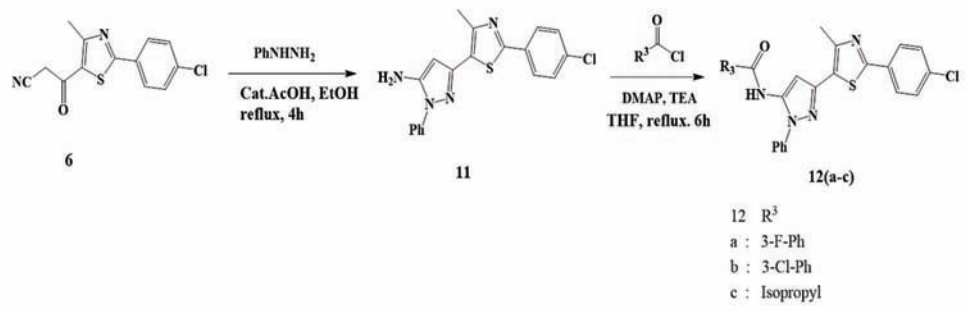


Scheme 1: Synthetic pathway for the formation of intermediate 6 from compound 1



8	R^1	10	R^2
a	3-F-Ph	**a**	3-CF_3-Ph
b	3-Cl-Ph	**b**	3-F-4-Cl-Ph
c	Isopropyl	**c**	3-CF_3-4-F-Ph
d	Tert-Butyl	**d**	4-OCH_3-Bz
e	Cyclohexyl	**e**	Isopropyl

Scheme 2: Synthetic pathway for the formation of aminoisoxazole 7 and its amides $8_{(a-e)}$ and ureas $10_{(a-e)}$ analogs from compound 6



Scheme 3: Synthetic pathway for the formation of aminopyrazole 11 and its amide $12_{(a-e)}$ analogs from compound 6

sequence for synthesizing amide $\mathbf{12}_{(a-c)}$ analogs from intermediate compound 6 is outlined in **Scheme 3**.

ANTIMICROBIAL ACTIVITY

The newly synthesized compounds, **7**, **8a-e**, **10a-e**, **11**, and **12a-c**, were screened to determine their antimicrobial activity *in vitro* against four bacterial strains, *Escherichia coli* (NCIM2645), *Salmonella typhi* (NCIM2501), *Bacillus subtilis* (NCIM2010), and *Staphylococcus aureus* (NCIM2079). The test cultures of bacterial strains were maintained in nutrient agar slants at 37°C. The plates containing 20 mL of nutrient agar were spread with 100 µL of seeded culture. The wells were made on these plates with the help of a borer of 7 mm diameter. The test compounds were loaded (100 µL of 1 mg/ml) into the well along with standard antibiotic ampicillin as positive control and dimethyl sulfoxide (DMSO) as a negative control. The bacterial plates were incubated in an incubator at 37°C for 24 h. Growth was evaluated visually by comparing a particular plate with the control plates.[22] All the experiments were performed in triplicate.

Results were determined for tested compounds as an average diameter of inhibition zone of bacterial growth in mm [**Table 1**].

The results summarized in **Table 1** clearly showed that thiazole substituted isoxazole and pyrazole analogs possess moderate to good activity against both Gram-positive and Gram-negative bacteria. The compounds **8a–10e** showed moderate activity against *B. subtilis* having an inhibitory zone of 11–13 mm diameter as compared to a 25 mm diameter of standard ampicillin. Isoxazole-amide **8a** showed good antibacterial activity 16.5 mm against *S. aureus*. The compound **10d** showed good inhibitory zone 17.5 mm as compared to standard (29 mm) in *E. coli* bacterial strain. Both isoxazole and pyrazole analogs showed a good inhibitory zone against *S. typhi*, 15.5–18 mm, as compared to 22 mm of ampicillin.

EXPERIMENTAL SECTION

All the chemicals and solvents used were dried and purified by standard literature procedures, and moisture was excluded from the glass apparatus using $CaCl_2$ drying

Table 1: Antimicrobial activity (Well diffusion method)

Compounds	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhi*
7	-	13.5	-	18
8a	12	16.5	11	15
8b	11	13.5	10	13.5
8c	11	10	10.5	15
8d	11	09	09	15
8e	12	11	09	17
10a	11	12	12	16.5
10b	13	11	12.5	17.5
10c	12.5	-	19	15.5
10d	13	-	17.5	14.5
10e	13	-	16	16
11	12	-	14	12
12a	-	8.5	08	13
12b	09	09	10	15.5
12c	-	09	-	17
Ampicillin	25	33	29	22

Diameter in mm calculated by Vernier Caliper, "-" means no zone of inhibition, NA: Not applicable

tubes. The melting points were determined in open capillary tubes with Gallenkamp melting point apparatus and are uncorrected. Fourier transform-IR (FT-IR) spectra were recorded on Bruker FTIR-TENSOR II spectrophotometer using platinum attenuated total reflection disks. ^{1}H 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 frequency in $CDCl_3$ or DMSO-*d6* using tetramethylsilane as internal standard. Chemical shifts were reported in δ ppm and multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Reactions were monitored by thin-layer chromatography (TLC), carried out on 0.2 mm silica gel 60 F254 (Merck) plates using ultraviolet light (254 and 366 nm) for detection. Common reagents grade chemicals were commercially available and used without further purification or prepared by standard literature procedures. All the compounds were prepared by conventional methods.

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

A solution of ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate (5) (4.6 g, 0.014 mol, 1 eq) and acetonitrile (1.10 mL, 0.021 mol, 1.5 eq) in dry THF (25 mL) was stirred under nitrogen atmosphere at −10°C. To this solution, n-BuLi (9.65 mL, 0.0154 mole, 9.65 mL, and 1.6 molar solution in hexane) was slowly added to maintain temperature up to −5°C. Then, reaction mixture was stirred for 30 min at same temperature, further quenched with 2N HCl (10 mL) at 0°C and extracted with ethyl acetate (4 × 100 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and evaporated to obtain yellow solid of compound 6. Yield (3.8 g, 84%), m.p. 153°C, IR (KBr) : ῡ = 3444/cm (CH), 2263/cm (CN), 1676/cm (C=O), ^{1}H NMR (DMSO-d6) δ 8.02–8.05 (d, J = 9 Hz, 2H, pCl-Ph), 7.60–7.63 (d, J = 9Hz, 2H, pCl-Ph), 4.68 (s, 2H, CH_2CN), 2.71 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 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), 278 (M+2) Anal.($C_{13}H_9ClN_2OS$, Exact Mass: 276.01). Elemental analysis: Calcd. C, 56.42; H, 3.28; N, 10.12%; Found: C, 56.61; H, 3.32; N, 10.22%.

3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-amine (7)

A mixture of compound 6 (1 g, 0.00362 mol, 1 eq), hydroxylamine hydrochloride (0.377 g, 0.00543 mol, 1.5 eq) and NaOH (0.289 g, 0.00724 mol, 2 eq saturated aq. solution) in water (10 mL) was heated at 100°C for 12 h. After the completion of reaction (as monitored by TLC), the reaction mixture was cooled to room temperature. The product thus separated was filtered, washed with water, dried, and recrystallized from ethanol to obtain pure solid of amine 7. Yield (0.750 g, 80%), m.p. 193°C, IR (KBr): ῡ = 3155.79/cm (NH_2), 3341.36/cm, 1647/cm, 1592.13/cm, (Ar-C=C). ^{1}H NMR (DMSO-d6) δ 7.982-7.960 (d, J = 8.8 Hz, 2H, p-ClPh), 7.592-7.570 (d, J = 8.8 Hz, 2H, p-ClPh), 5.304 (s, 1H, isoxazole-H), 6.982 (bs, 2H, NH_2) 2.580 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 171.685, 164.321, 156.231, 152.644, 135.585, 131.854, 129.803, 128.3, 121.340, 76.929, 17.314. MS: m/z 292.36 (M+H), 293.02 (M+2) Anal. ($C_{13}H_{10}ClN_3OS$, Exact Mass: 291.02). Elemental analysis: Calcd. C, 53.52; H, 3.45; N, 14.40%. Found: C, 53.53; H, 3.47; N, 14.49%.

3-(2(4-chlorophenyl)-4-methylthiazol-5-yl) isoxazole substituted amide analogs 8 a-e

General procedure

A mixture of compound 7 (0.1 g, 0.00034 mol, 1 eq) and substituted carbonyl chlorides (0.055 g, 0.00037 mol, 1.1 eq) was refluxed in THF (3 mL) for 6 h in the presence

of (0.2 mL) TEA and dimethylaminopyridine (0.008 g, 0.00067 mol, 0.25 eq). After the completion of reaction, as monitored on TLC, the reaction mixture was concentrated and dissolved in ethyl acetate (20 mL) and the solution was washed successively with 2 M HCl (2 × 10 mL), aq $NaHCO_3$ (10 mL), and brine (10 mL). The ethyl acetate phase was dried over sodium sulfate and concentrated. The crude product was recrystallized from ethanol to obtain 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl) isoxazole substituted amide analogs 8(a-e) with 81–89% yield.

N-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)-3-fluorobenzamide (8a)

Brown solid, yield 0.115 g, 81%, m.p. 197°C, IR (KBr) ῡ = 3336.20/cm (NH, amide), 1708.27/cm (C=O, amide), 1527.44/cm (Ar-C=C), 1599.54/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6) δ 12.377 (s, 1H, CONH), 8.009–8.031 (d, J = 8.8 Hz, 2H, p-ClPh), 7.866–7.925 (m, 2H, ArH), 7.641–7.656 (m, 1H, ArH), 7.601–7.623 (d, J = 8.8Hz, 2H, p-ClPh), 7.542–7.546 (m, 1H, ArH), 6.844 (s, 1H, isoxazole-H), 2.683 (s, 3H, CH_3). MS: m/z 412.31 (M-H), 415 (M+2) Anal. ($C_{20}H_{13}ClFN_3O_2S$, Exact mass: 413.04). Elemental analysis: Calcd. C, 58.04; H, 3.17; N, 10.15%. Found: C, 58.08; H, 3.19; N, 10.17%.

3-Chloro-N-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl) isoxazol-5-yl)-3-chlorobenzamide (8b)

Off white solid, yield 0.120 g, 81.6%, m.p. 211°C, IR (KBr) ῡ = 3305.20/cm (NH, amide), 1698.74/cm (C=O, amide), 1594.74/cm (Ar-C=C), 1523.38/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6) δ12.40 (s, 1H, CONH), 8.118–8.123 (s, 1H, Ar-H), 8.007–8.028 (d, J = 8.4 Hz, 2H, p-ClPh), 7.892–7.909 (m, 1H, ArH), 7.740–7.763 (m, 1H, Ar–H), 7.615–7.641 (d, J = 8.4 Hz, 2H, p-ClPh), 7.553–7.573 (m, 1H, ArH), 6.833 (s, 1H, isoxazole-H), 2.680 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 162.125, 156.974, 153.768, 136.576, 134.915, 134.211, 132.753, 130.132, 129.631, 129.294, 128.056, 127.805, 126.040, 119.762, 88.910, 17.135 MS: m/z 428.25 (M-H), 430.36 (M+1) Anal. ($C_{20}H_{13}Cl_2N_3O_2S$, Exact Mass: 429.01), elemental analysis: Calcd. C, 55.82; H, 3.05; N, 9.77%, found: C, 55.85; H, 3.09; N, 9.79%.

N-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)isobutyramide (8c)

Brown solid, yield 0.105 g, 86%, m.p. 191°C, IR (KBr) ῡ = 3222/cm (NH, amide), 1688/cm (C=O, amide), 1607/cm (Ar-C=C), 1433/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6) δ 11.82 (s, 1H, CONH), 7.99–8.01 (d, J = 8 Hz, 2H, p-ClPh), 7.60-7.58 (d, J = 8 Hz, 2H, p-ClPh), 6.61 (s, 1H, isoxazole-H), 2.63-2.6 (m, 1H, CH), 2.63 (s, 3H, CH_3), 1.14-1.12 (d, 6H, 2 × CH_3). MS: m/z 360.28 (M-H), 362.29 (M+1) Anal. ($C_{17}H_{16}ClN_3O_2S$, Exact Mass: 361.07), elemental analysis: Calcd C, 56.43; H, 4.46; N, 11.61%. Found: C, 56.51; H, 4.49; N, 11.63%.

N-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)pivalamide (8d)

Yellow solid, yield 0.109 g, 89%, m.p. 207°C, IR (KBr) ῡ = 3204.35/cm (NH, amide), 1708.91/cm (C=O, amide), 1603.02/cm (Ar-C=C), 1536.67/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6, 400 mHz), δ 11.394 (s, 1H, CONH), 7.987–8.008 (d, J = 8.4 Hz, 2H, p-ClPh), 7.588–7.609 (d, J = 8.4 Hz, 2H, p-ClPh), 6.647 (s, 1H, isoxazole-H), 2.693 (s, 3H, CH_3), 1.248 (s, 9H, 3 × CH_3). MS: m/z 374.27 (M-H), 377 (M+2) Anal. ($C_{18}H_{18}ClN_3O_2S$, Exact Mass: 375.08) Elemental analysis: Calcd C, 57.52; H, 4.83; N, 11.18%, found: C, 57.57; H, 4.49; N, 11.21%.

N-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)cyclohexanecarboxamide (8e)

Yellow solid, yield 0.110 g, 81.4%, m.p. 199°C, IR (KBr) ῡ = 3255.54/cm (NH, amide), 1681.61/cm (C=O of Amide), 1610.01/cm (Ar-C=C), 1529.82/cm (Ar-C=C). ^{1}HNMR (DMSO-d_6, 400 MHz), δ 11.775 (s, 1H, CONH), 7.992–8.013 (d, J = 8.4 Hz, 2H, p-ClPh), 7.612-7.591 (d, J = 8.4 Hz, 2H, p-ClPh), 6.613 (s, 1H, isoxazole-H), 2.638 (s, 3H, CH3), 2.513-2.504 (m, 1H, CH), 1.0645–1.856 (m, 4H, 2 × CH_2), 1.419-1.289 (m, 6H, 2 × CH_2). MS: m/z 400.36 (M-H), 402.41 (M+1) Anal. ($C_{20}H_{20}ClN_3O_2S$, Exact Mass: 401.10), elemental analysis: Calcd C, 59.77; H, 5.02; N, 10.46%, found: C, 59.79; H, 5.06; N, 10.48%.

Synthesis of phenyl (3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)carbamate (9)

A mixture of compound 7 (1 g, 0.0034 mol, 1 eq) and dried powdered K_2CO_3 (0.946 g, 0.0068 mol, 2 eq) in dry THF (10 ml) was stirred at room temperature for 1/2 h. Then, phenyl chloroformate (0.51 ml, 0.0041 mol, 1.2 eq) was added dropwise under the nitrogen atmosphere and the mixture was further stirred at the same temperature for 24 h. After the completion of reaction, as monitored on TLC, THF was evaporated and reaction mixture was poured in ice-cold water (20 mL). The product separated out was filtered, washed with water, and recrystallized from ethanol to offer yellow solid product **9**. Yield 1.2 g, 85%, m.p. 207°C, IR (KBr) ῡ = 3288.22/cm (NH, amide), 1756.24/cm (C=O), 1614.67/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ12.285 (s, 1H, CONH), 7.994–8.017 (d, J = 9.2 Hz, 2H, p-ClPh), 7.589–7.612 (d, J = 9.2 Hz, 2H, p-ClPh), 7.471–7.577 (m, 1H, Ar-H), 7.322–7.457 (m, 2H, Ar-H), 7.290–7.318 (m, 1H, Ar-H), 6.763–7.059 (m, 1H, Ar-H), 6.438 (s, 1H, isoxazole-H), 2.638 (s, 3H, CH_3). MS: m/z 410.02 (M-H), 413 (M+2) Anal. ($C_{20}H_{14}ClN_3O_3S$, Exact Mass: 411.04). Elemental analysis: Calcd C, 58.32; H, 3.43; N, 10.20%. Found: C, 58.39; H, 3.49; N, 10.26%.

Synthesis of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-amine substituted ureas $10_{(a-e)}$

General procedure

A mixture of compound **9** (0.1 g, 0.00024 mmol, 1 eq) and substituted amine (0.039 g, 0.00029 mmol, 1.2 eq) was refluxed in dry THF (5 mL) for 6 h in the presence of (0.2 mL) TEA. The reaction mixture was cooled at room temperature and poured into ice-cold water, stirring for 30 min. The solid was filtered, washed with water, and recrystallized from ethanol to obtain urea analogs $\mathbf{10}_{(a-e)}$ with 85–90% yield.

1-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)-3-(3-(trifluoromethyl)phenyl)urea (10a)

Off white solid, yield 0.095 g, 85%, m.p. 187°C, IR (KBr) ῡ=3409/cm (NH, urea), 2929.27/cm (NH, urea), 1734.14/cm (C=O, urea), 1611.22/cm (Ar-C=C), 1559.61/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.719 (bs, 1H, urea), 9.359 (s, 1H, urea), 8.045 (s, 1H, Ar-H), 7.987–8.010 (d, J=9.2Hz, 2H, p-ClPh), 7.549–7.668 (m, 4H, Ar-H), 7.591-7.549 (m, 1H, ArH), 7.399–7.419 (d, J=8Hz, 1H, Ar-H), 6.507 (s, 1H, isoxazole-H), 2.645 (s, 3H, CH_3). MS: m/z 479.27 (M+H), 480 (M+2) Anal. ($C_{21}H_{14}ClF_3N_4O_2S$, Exact Mass: 478.05). Elemental analysis: Calcd C, 52.67; H, 2.95; N, 11.70%. Found: C, 52.69; H, 2.99; N, 11.76%.

1-(4-chloro-3-fluorophenyl)-3-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)urea (10b)

Off white solid, yield 0.090 g, 81%, m.p. 206°C, IR (KBr) ῡ = 3387.38/cm (NH, urea), 3281.86/cm (NH, urea), 1667.71/cm (C=O, urea), 1610.12/cm (Ar-C=C), 1593.27/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ10.700 (bs, 1H, urea), 9.333 (s, 1H, urea), 7.987-8.010 (d, J = 9.2 Hz, 1H, p-ClPh), 7.673–7.708 (m, 1H, Ar-H), 7.595–7.618 (d, J = 9.2 Hz, 2H, p-ClPh), 7.504–7.590 (m, 1H, ArH), 7.263–7.291 (m, 1H, Ar-H), 6.479 (s, 1H, isoxazole-H), 2.649 (s, 3H, CH_3). MS: m/z 463.25 (M+H) Anal. ($C_{20}H_{13}Cl_2FN_4O_2S$, Exact Mass: 462.01), elemental analysis: Calcd C, 51.85; H, 2.83; N, 12.09%. Found: C, 51.91; H, 2.85; N, 12.11%.

1-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)-3-(4-fluoro-2-methylphenyl)urea (10c)

Off white solid, yield 0.093 g, 87%, m.p. 163°C, IR (KBr) ῡ = 3277.80/cm (NH, urea), 3134.85/cm (NH, urea), 1664.78/cm (C=O, urea), 1592.20/cm (Ar-C=C), 1561.06.27/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6, 400 MHz), δ 11.212 (bs, 1H, Urea), 8.752 (bs, 1H, Urea), 7.988–8.009 (d, J = 8 Hz, 1H, p-ClPh), 7.696–7.731 (m, 1H, Ar-H), 7.582–7.606 (d, J = 8 Hz, 2H, p-ClPh), 7.084–7.107 (m, 1H, ArH), 6.994-7.032 (m, 1H, Ar-H), 6.409 (s, 1H, isoxazole-H), 2.633 (s, 3H, CH_3), 2.275 (s, 3H, CH_3). MS: m/z 441.23 (M-H), 444(M+2) Anal. ($C_{21}H_{16}ClFN_4O_2S$, Exact Mass: 442.07), elemental analysis: Calcd C, 56.95; H, 3.64; N, 12.65%. Found: C, 56.98; H, 3.65; N, 12.68%.

1-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)-3-(4-methoxybenzyl)urea (10d)

Yellow solid, yield 0.096 g, 87%, m.p. 157°C, IR (KBr) ῡ = 3326.47/cm (NH, urea), 3254.67/cm (NH, urea), 1659.40/cm (C=O, urea), 1612.41/cm (Ar-C=C), 1551.10/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.469 (bs, 1H, urea), 9.333 (bs, 1H, urea), 8.008-7.987 (d, J=8.4Hz, 2H, p-ClPh), 7.587–7.608 (d, J = 8.4 Hz, 2H, p-ClPh), 7.230–7.252 (d, J = 8.8 Hz, 2H, Bz-H), 6.897–6.919 (d, J = 8.8 Hz, 2H, Bz-H), 6.361 (s, 1H, isoxazole-H), 4.258–4.272 (d, 2H, CH_2) 3.737 (s, 3H, OCH_3), 2.626 (s, 3H, CH_3). ^{13}C NMR (DMSO-d6) δ 164.948, 164.401, 158.764, 156.333, 153.412, 152.519, 135.780, 131.904, 131.707, 130.965, 129.847, 129.037, 128.786, 120.459, 114.222, 84.397, 55.657, 17.405. MS: m/z 455.42 (M+H), 456 (M+2) Anal. ($C_{22}H_{19}ClN_4O_3S$, Exact Mass: 454.09), elemental analysis: Calcd C, 58.08; H, 4.21; N, 12.32%. Found: C, 58.12; H, 4.27; N, 12.35%.

1-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)isoxazol-5-yl)-3-isopropylurea (10e)

Yellow solid, yield 0.080 g, 88%, m.p. 175°C, IR (KBr) ῡ = 3326.98/cm (NH, urea), 3260.26/cm (NH, urea), 1666.33/cm (C=O, urea), 1557.48/cm (Ar-C=C), 1607.86/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.169 (bs, 1H, urea), 8.005-7.984 (d, J = 8.4 Hz, 2H, p-ClPh), 7.604-7.583 (d, J = 8.4 Hz, 2H, p-ClPh), 6.337 (s, 1H, isoxazole-H), 6.462-6.446 (bs, 1H, NH-Urea), 3.816-3.765 (m, 1H, CH), 2.676 (s, 3H, CH_3), 1.135-1.119 (s, 6H, 2 × CH_3). MS: m/z 375.32 (M-H), 378 (M+2) Anal. ($C_{17}H_{17}ClN_4O_2S$, Exact Mass: 376.08), elemental analysis: Calcd C, 54.18; H, 4.55; N, 14.87%. Found: C, 54.21; H, 4.58; N, 14.92%.

Synthesis of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazol-5-amine (11)

To a mixture of compound **6** (0.5 g, 0.00181 mol, 1 eq) and phenylhydrazine (0.292 g, 0.00271 mol, 1.5 eq) in methanol (5 mL) was added catalytic amount of acetic acid (0.1 mL) and then all the contents were heated at 85°C for 12 h. The methanol was evaporated and residue was poured in to ice cold water, the solid was filtered and recrystallized from methanol to obtain brown solid product **11** with 65%yield, m.p. 211°C, IR (KBr): ῡ = 3430.87/cm (NH_2), 3211.91/cm, 1624.55/cm, 1599.01/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.911–7.939 (d, J = 8.4 Hz, 2H, p-ClPh), 7.603–7.629 (d, J = 8.4 Hz, 2H, p-ClPh), 7.479–7.546 (m, 4H, Ph-H), 7.326–7.375 (m, 1H, Ph-H), 5.816 (s, 1H, Pyrazole-H), 5.592 (bs, 2H, NH_2), 2.481 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 161.967, 149.498, 148.882, 143.667, 139.201, 134.980, 132.327, 129.716, 127.161, 126.772, 123.511, 89.046, 17.240, MS: m/z 367.25 (M+H), 368 (M+2) Anal. ($C_{19}H_{15}ClN_4S$, Exact Mass: 366.07, elemental analysis: Calcd C, 62.20; H, 4.12; N, 15.27%. Found: C, 62.27; H, 4.13; N, 15.29%.

Synthesis of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazol-5-substituted amides analogs $12_{(a-c)}$

General procedure

A mixture of compound **11** (0.1 g, 0.00027 mol, 1 eq) and substituted carbonyl chlorides (0.047 g, 0.0003 mol, 1.1 eq) was refluxed in THF (5 mL) for 6 h in the presence of (0.2 mL) TEA and DMAP (0.0082 g, 0.000067 mol, 0.25 eq). After the completion of the reaction, the reaction mixture was concentrated and dissolved in ethyl acetate (20 mL) and the solution washed successively with 2 M HCl (2 × 10 mL), aq $NaHCO_3$ (10 mL), and brine (10 mL). The ethyl acetate phase was dried over sodium sulfate and concentrated. The crude product was recrystallized from ethanol to offer $\mathbf{12}_{(a-c)}$ with 83-88% yield.

N-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1H-pyrazol-5-yl)-3-fluorobenzamide (12a)

Off white solid, yield 0.115 g, 83%, m.p. 235°C, IR (KBr) ῡ = 3441.60/cm (NH, amide), 1689.98/cm (C=O of Amide), 1548.79/cm (Ar-C=C), 1588.26/cm (Ar-C=C). ^{1}H NMR (DMSO-d6), δ10.7 (s, 1H, CONH), 7.981–8.002 (d, J = 8.4 Hz, 2H, p-ClPh), 7.407–7.974 (m, 11H, Ar-H), 6.936 (s, 1H, Pyrazole-H), 2.673 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 165.266, 163.607, 162.749, 161.170, 150.373, 143.802, 138.743, 137.673, 135.756, 135.689, 135.235, 132.179, 131.418, 131.338, 129.791, 128.400, 128.130, 125.614, 124.448, 123.953, 119.651, 115.146, 114.918, 103.182, 17.263, MS: m/z 489.31 (M+H), 490 (M+2) Anal. ($C_{26}H_{18}ClFN_4OS$, Exact Mass: 488.09), elemental analysis: Calcd C, 63.87; H, 3.71; N, 11.46 %. Found: C, 63.95; H, 3.78; N, 11.49%.

3-chloro-N-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1H-pyrazol-5-yl)benzamide (12b)

Yellow solid, yield 0.120 g, 87%, m.p. 215°C, IR (KBr) ῡ = 3440.14/cm (NH, amide), 1688.29/cm (C=O of Amide), 1546.78/cm (Ar-C=C), 1568.59/cm (Ar-C=C). ^{1}H NMR (DMSO-d_6) δ10.739 (s, 1H, CONH), 7.979–8.001 (d, J = 8.8 Hz, 2H, p-ClPh), 7.428–7.698 (m, 11H, Ar-H), 6.931 (s, 1H, Pyrazole-H), 2.671 (s, 3H, CH_3). MS: m/z 505.26 (M+H), 506.25 (M+2) Anal. ($C_{26}H_{18}C_{l2}N_4OS$. Exact Mass: 504.06). Elemental analysis: Calcd C, 61.79; H, 3.59; N, 11.09 %. Found: C, 61.86; H, 3.65; N, 11.11%.

N-(3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1H-pyrazol-5yl)isobutyramide (12c)

Yellow solid, yield 0.105 g, 88%, m.p. 249°C, IR (KBr) ῡ = 3235.83/cm (NH, amide), 1669.43/cm (C=O of Amide), 1593.88/cm (Ar-C=C), 1563.67/cm (Ar-C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ10.007 (s, 1H, CONH), 7.964–7.985 (d, J = 8.8 Hz, 2H, p-ClPh), 7.544–7.588 (m, 5H, ArH), 7.457 (m, 2H, Ar-H), 6.797 (s, 1H, Pyrazole-H), 2.644 (s, 3H, CH_3), 2.512 (m, 1H, CH), 1.050–1.033 (m, 6H, 2 × CH_3). MS: m/z 437.24 (M+H), 438 (M+2) Anal. ($C_{23}H_{21}ClN_4OS$, Exact Mass: 436.11) Elemental analysis: Calcd: C, 63.22; H, 4.84; N, 12.82%. Found: C, 63.29; H, 4.86; N, 12.86%.

CONCLUSIONS

In summary, we have designed and synthesized a new series of thiazole substituted aminoisoxazole, aminopyrazole, and their urea and amide analogs. The newly synthesized compounds, **7**, **8a-e**, **10a-e**, **11**, and **12a-c**, were screened for their antimicrobial activity *in vitro* against two pathogenic Gram-positive bacteria, namely, *B. subtilis* and *S. aureus*, two pathogenic Gram-negative bacteria, namely, *S. typhi* and *E. coli*. Our results showed that newly synthesized compounds exhibit moderate to good activity against both Gram-positive and Gram-negative bacteria. Therefore, it was concluded that thiazole substituted aminoisoxazole, amino-pyrazole, and their urea and amide derivatives could be developed as novel and promising antimicrobial agents.

REFERENCES

[1] Jason, B.C. Discovery of pyrazolopyridones as anovel class of Gyrase B inhibitors using structure guided design, *ACS Med. Chem. Lett*., **2016**, *7*, 374–378.

[2] Geronikaki, A. Thiazole based chalcones as potent antimicrobial agent, *Bioorg. Med. Chem.*, **2011**, *19*, 3135–3140.

[3] Steven, M.R. Discovery of pyrazolthiazoles as novel and potent inhibitors ofbacterial gyrase, *Bioorg. Med. Chem. Lett*., **2010**, *20*, 2828–2831.

[4] Abdel-Wahab, B.F., Abdel-Aziz, H.A., Ahmed, E.M. Synthesis and antimicrobial evaluation of 1- (benzofuran-2-yl)-4-nitro-3- arylbutan-1-ones and 3-(benzofuran-2-yl)-4,5-dihydro-5-aryl-1-[4- (aryl)-1,3-thiazol-2-yl]-1*H*-pyrazoles, *Eur. J. Med. Chem*., **2009**, *44*, 2632–2635.

[5] Holla, B.S., Malini, K.V., Rao, B.S., Sarojini, B.K., Kumari, N.S. Synthesis of some new 2, 4- disubstituted thiazoles as possible antibacterial and anti-inflammatory agent, *Eur. J Med. Chem*., **2003**, *38*, 313–318.

[6] Abdelrahaman, S.M., Mansoor, K.R., Kuhan, J., Mark, C., Design synthesis and biological evaluation of thaizole targeting flavivirus envelope protin, *J. Med. Chem*., **2011**, *54*, 1704–1714.

[7] Rawal, R.K., Tripathi, R., Katti, S.B., Pannecouque, C., Clercq, E.D. Design and synthesis of 2-(2,6-dibromophenyl)-3-heteroaryl-1, 3-thiazolidin-4- ones as anti-HIV agents, *Eur. J Med. Chem*., **2008**, *43*, 2800–2806.

[8] Hao, S., Shenhua, S., Shiliang, H., Alison, J.H. Substituted 4-(Thaizole-5-yl)-2- (phenylamino) pyrimidines are highly active CDK9 inhibitor: Synthesis, X-ray crystal structure, SAR, and anticancer activity, *J. Med. Chem*., **2013**, *56*, 640–659.

[9] Popsavin, M., Spaic, S., Svircev, M., Kojic, V., Bogdanovic, G., Popsavin, V. Synthesis and antitubercular activity of new tiazofurin analogues bearing a 2, 3-anhydrofunctionality in the furanose ring, *Bioorg. Med. Chem. Lett*., **2007**, *17*, 4123–4127.

[10] Iino, T., Hashimoto, N., Sasaki, K., Ohyama, S., Yoshimoto, R., Hosaka, H., Hasegawa, T., Chiba, M., Nagata, Y., Nishimura, J. Structure activity relationships of 3,5-disubstituted benzamides as glucokinase activators with potent *in vivo* efficacy, *Bioorg. Med Chem*., **2009**, *17*, 3800–3809.

[11] Satoh, A., Nagatomi, Y., Hirata, Y., Ito, S., Suzuki, G., Kimura, T., Maehara, S., Hikichi, H., Satow, A., Hata, M., Ohta, H., Kawamoto, H. Discovery and *in vitro* and *in vivo* profiles of 4-fluoro-N-[4-[6- (isopropylamino) pyrimidin-4-yl]-1,3-thiazol-2-yl]- N-methylbenzamide as novel class of an orally active metabotropic glutamate receptor 1 (mGluR1) antagonist, *Bioorg. Med. Chem*., **2009**, *19*, 5464–5468.

[12] Koufaki, M., Kiziridi, C., Nikoludaki, F., Alexis, M.N. Design and synthesis of 1,2-dithiolane derivatives and evaluation of their neuroprotective activity, *Bioorg. Med. Chem. Lett*., **2007**, *17*, 4223–4227.

[13] Andreani, A., Rambaldi, M., Mascellani, G., Rugarli, P. Synthesis and diuretic activity of imidazo [2,1-b]

thiazole acetohydrazones, *Eur. J Med. Chem*., **1987**, *22*, 19–22.

[14] Watpade, R., Bholay, A., Toche, R.B. Synthesis of new pyrano-fused quinolines as antibacterial and antimicrobial agents, *J. Heterocycl. Chem*., **2017**, *54*, 3434–3439.

[15] Toche, R.B., Nikam, P. Synthesis and evaluation of antimicrobial and antitubercular activity of arylidene hydrazines of indenothieno[2,3-d]pyrimidine, *Chem. Biol. Interface*, **2015**, *5*, 246–257.

[16] Kanawade, S.K., Toche, R.B., Rajani, D.P. Synthetic tactics of new class of 4-aminothieno[2,3-d]pyrimidine-6-carbonitrile derivatives acting as antimicrobial agents, *Eur. J. Med. Chem*., **2013**, *64*, 314.

[17] Patil, P.S., Toche, R.B. Synthesis of (E)-N'-(2-Hydroxy-3,5-dinitrobenzylidene)-2-cynocetohydrazide derivatives of effective antimicrobial agents, *Front. Drug Chem. Clin. Res*., **2018**, *1*, 1-3.

[18] Kanawade, S.K., Toche, R.B., Patil, S.P., Desai, A.E., Bhamare, S.S. Aminothiophenedicarboxamides and dicyanothiopheneacetamides as effective synthetic molluscicides against indoplanorbis exustus snail, *Eur. J. Med. Chem*., **2011**, *46*, 4682-4686.

[19] Karm, B.S., Madhav, J.V., Rajitha, B. Xanthane suphonic acid, an efficient bio-supported and recyclable solid acid catalyst for synthesis of 2-amino thiazole-5-carboxylate, *Lett. Org. Chem.*, **2011**, *8*, 549–553.

[20] Adams, L.A., Sharma, P., Mohanty, B., Martin, J. Application of fragment based screening to design of inhibitors of *E. coli*, *Angew Chem.*, **2015**, *7*, 2207–2212.

[21] Michael, L.S., Beneton, V., Anne, B., Robert, X.X., Pascal, M.P. Discovery of novel serese of potent HDLC raising agent, *J. Med Chem.*, **2007**, *50*,685–695.

[22] Espinel, I., Pfauler, M.A., Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L., Pfaller, M.A. Susceptibility test methods, yeasts and filaments fungi, in manual of clinical microbiology, *Clin Microbiol*, 2007,2, 1972-1986.

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