HETEROCYCLES, Vol. 100, No. 11, 2020, pp. 1785 - 1798. © 2020 The Japan Institute of Heterocyclic Chemistry
Received, 7th July, 2020, Accepted, 4th September, 2020, Published online, 14th September, 2020
DOI: 10.3987/COM-20-14316

SYNTHESIS OF NOVEL (THIAZOL-5-YL)PYRAZOLES AND THEIR ANTIMICROBIAL EVALUATION

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

[1*] Department of Chemistry, KRT Arts, BH Commerce & AM Science College, Nashik 422002, India. Email: sanjay_deshmukh08@rediffmail.com [2†] Dadasheb Bidkar Arts, Science & Commerce College, Peth, Nashik 422208, 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

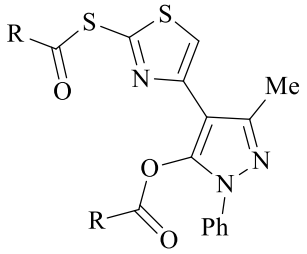
Abstract – A series of novel (thiazol-5-yl)pyrazoles were designed and synthesized. All the synthesized compounds were characterized by spectroscopic analysis and were evaluated for their antimicrobial activity *in vitro* against Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria, *Salmonella abony* and *Escherichia coli* and fungi, *Aspergillus flavus* and *Fusarium oxysporum*.

INTRODUCTION

Among the different sulfur containing heterocycles, thiazoles play an important role in medicinal chemistry and subsequently have emerged as a pharmacophore. The thiazoles structural motifs have attracted a great deal of interest because of their ready accessibility, diverse chemical reactivity, and wide gamut of biological activities like anticancer agents,[1] antidiabetic agents,[2] anti-Alzheimer's agents,[3] and antimicrobial agents.[4]

Pyrazoles are widely used in agrochemicals, particularly in crop protection (fungicides, herbicides, and insecticides)[5] and as insecticides/pesticides.[6] Masumoto[7] demonstrated significant biomedical activities such as antifungal activity via DNA cleavage mechanism of 2-thioacylated thiazoles containing pyrazole moiety. DNA is an important cellular receptor and many chemicals exerts their antitumor effects through binding to DNA thereby changing the replication of DNA and inhibiting the growth of tumor cells. The mechanism of compounds cleaving and/or binding to DNA possesses significant meaning. Substituted pyrazoles **(I)** were tested for *in vitro* DNA cleavage activity and antifungal activity against *Candida*

albicans and *Saccharomyces cerevisiae*. Substituted pyrazoles **(I)** exhibited high DNA cleavage activity *in vitro* with Cu^{2+} and good to excellent antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae*.



(I)

R = H, Me, Ph, 2-Me-C_6H_4, 4-MeO-C_6H_4, 4-Cl-C_6H_4, 2-thienyl

In the recent years, thiazolylpyrazoles have been extensively studied because of their magnificent pharmacological and therapeutic properties, such as epidermal growth factor receptor (EGFR, also known as HER1) and human epidermal growth factor receptor (HER2) inhibitors,[8,11] super oxidase inhibitors,[9] and their promising antioxidant,[10] and antitumor activities.[11]

Prompted by the aforementioned biological and pharmaceutical activities of thiazolylpyrazole derivatives, and as a part of an ongoing program aiming at the synthesis of new heterocyclic compounds of pharmacological interest,[12–16] we describe herein an efficient procedure for the synthesis of a novel series of thiazolylpyrazoles. The newly synthesized compounds were evaluated for their antimicrobial activity, particularly for antibacterial activity and antifungal activity.

RESULTS AND DISCUSSION

Chemistry

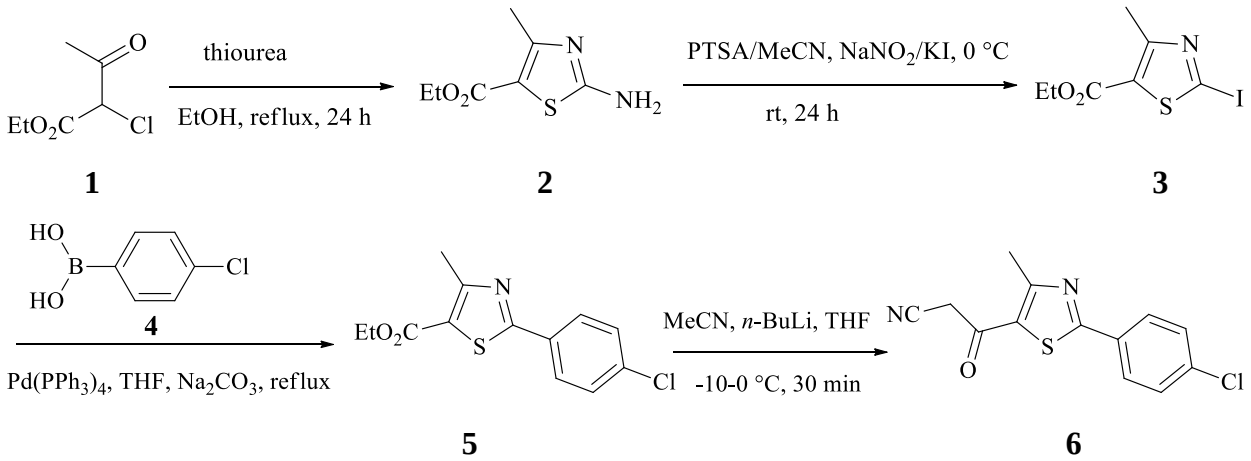
For the purpose of this study, the synthetic approach was confined to three reaction schemes to obtain the key intermediate compound **6** (Scheme 1), thiazolylpyrazoles **8a-e** (Scheme 2) and thiazolylpyrazol-4-amides **11a-d** (Scheme 3). The first starting compound, ethyl 2-amino-4-methylthiazole-5-carboxylate (**2**) was prepared in excellent yield (84%) via heating ethyl 2-chloroacetoacetate (**1**) with thiourea in ethanol. Structure **2** was confirmed by elemental analysis as well as IR, NMR, and mass spectral analyses. IR spectrum showed absorption bands at 3373 cm^{-1} and 1673 cm^{-1} due to NH_2 group and CO group, respectively. ^1H NMR spectrum exhibited signals at δ 7.70 ppm and δ 2.37 ppm corresponding to NH_2 and Me protons of thiazole **2**, respectively.

Ethyl 2-iodo-4-methylthiazole-5-carboxylate (**3**) was synthesized from thiazole **2** in 72% yield via Sandmeyer reaction. Thiazole **2** was diazotized with sodium nitrite/acid at 0 °C and coupled with potassium iodide in water at 0 °C followed by stirring for 24 h at room temperature. Structure **3** was

confirmed by elemental analysis as well as IR, NMR and mass spectral analyses. IR spectrum showed absorption band at 1711 cm^{-1} due to CO group and absence of absorption band due to NH_2 group. ^1H NMR spectrum exhibited signal at δ 2.62 ppm corresponding to Me protons and no signal corresponding to NH_2 of thiazole **3**.

Ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate (**5**) was prepared from thiazole **3** in 70% yield via Suzuki coupling. Thiazole **3** and (4-chlorophenyl)boronic acid (**4**) in THF were reacted in the presence of $Pd(PPh_3)_4$ under nitrogen atmosphere to afford thiazole **5**. Structure **5** was confirmed by elemental analysis as well as IR, NMR, and mass spectral analyses.

The key intermediate, 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-3-oxopropanenitrile (**6**) was synthesized by condensing thiazole **5** with acetonitrile in THF using *n*-BuLi under nitrogen atmosphere below -10 °C. Structure **6** was confirmed by elemental analysis as well as IR, NMR and mass spectral analyses. IR spectrum showed absorption bands at 2263 cm^{-1} and 1676 cm^{-1} due to CN and CO, respectively. The ester carbonyl stretching of compound **5** at 1709 cm^{-1} has now shifted in compound **6** at 1676 cm^{-1}. Further, there is prominent absorption band at 2263 cm^{-1} due to cyano group. ^1H NMR spectrum exhibited distinct singlet signal at δ 4.68 ppm corresponding to two methylene, CH_2 protons of compound **6**. The entire reaction sequence for synthesizing key intermediate compound **6** from compound **1** is provided in Scheme 1.

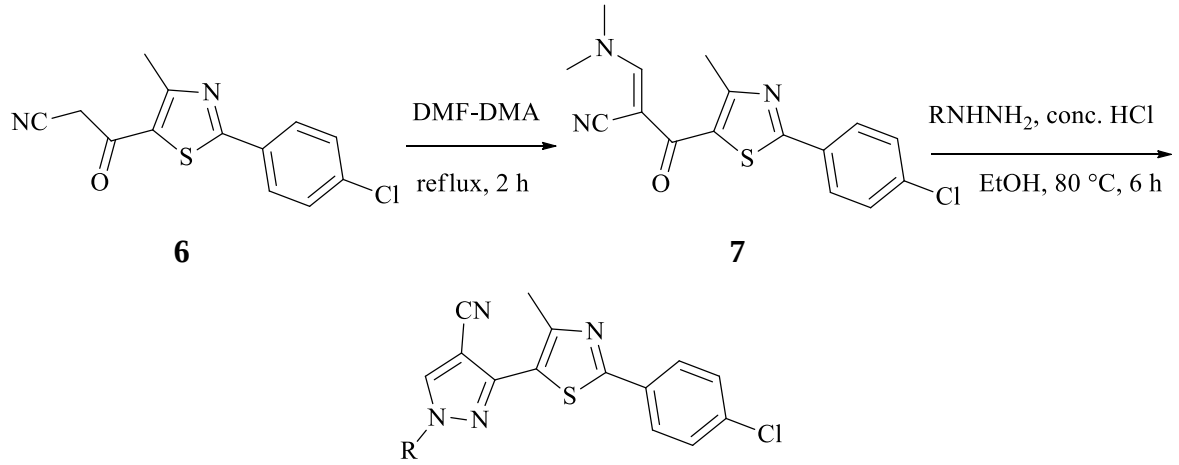


Scheme 1

The compound, 2-(2-(4-chlorophenyl)-4-methylthiazole-5-carbonyl)-3-(dimethylamino)acrylonitrile (**7**) was prepared in excellent yield (92%) via heating thiazol-5-yl-3-oxopropanenitrile **6** with dimethylformamide dimethyl acetal (DMF-DMA). Structure **7** was confirmed by elemental analysis as well as IR, NMR and mass spectral analyses. IR spectrum showed absorption band at 1633 cm^{-1} due to

C=C, enamine group. ^{1}H NMR spectrum exhibited singlet signals at δ 3.528 ppm and δ 3.558 ppm corresponding to six protons (2N-Me) and no signal corresponding to CH_2CN of thiazole **7**.

The compounds, 1-substituted-3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)pyrazole-4-carbonitriles (**8a-e**) were prepared in high yields (68-84%) via heating thiazole **7** in ethanol and catalytic amount conc. HCl with corresponding substituted hydrazines. Structures **8a-e** were confirmed by elemental analysis as well as IR, NMR and mass spectral analyses. The entire reaction sequence for synthesizing compounds **8a-e** from key intermediate compound **6** is provided in Scheme 2.



6 **7**

8a-e
8a: R = Ph
8b: R = Bz
8c: R = 4-F-Ph
8d: R = 3-F-Ph
8e: R = Me

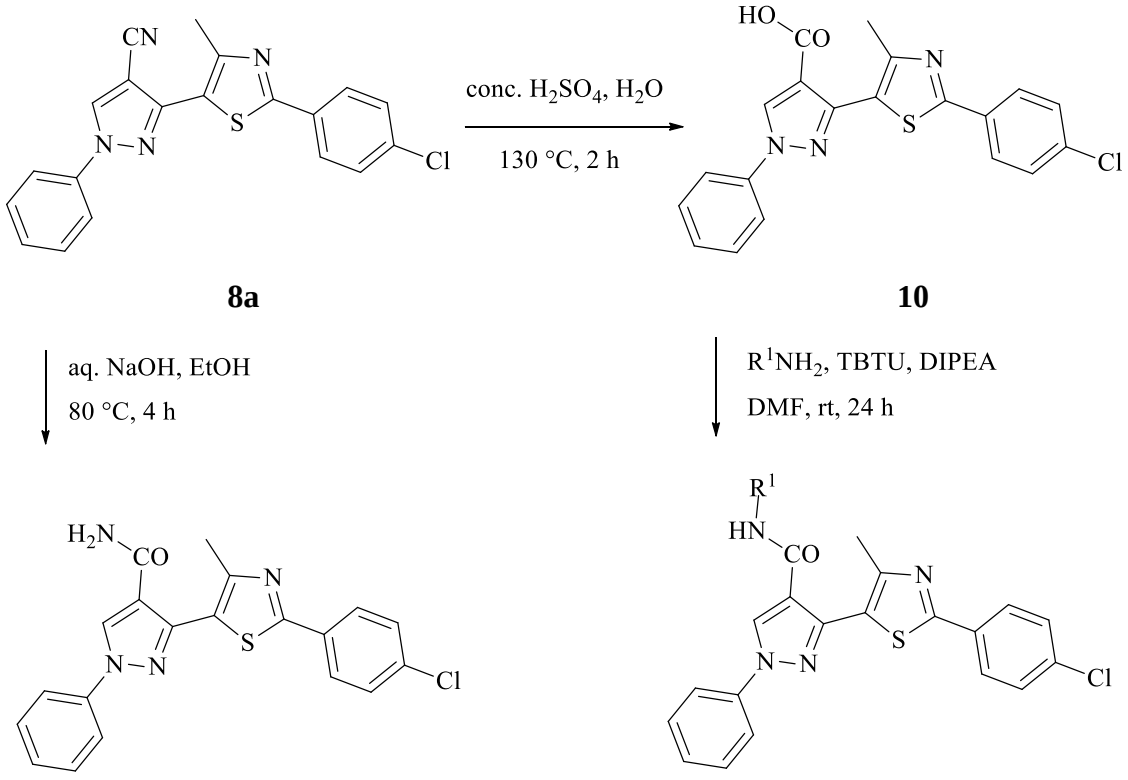
Scheme 2

The compound, 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**9**) was synthesized in high yield (90%) via heating thiazol-5-yl-1*H*-pyrazole-4-carbonitrile **8a** in ethanol with 10% aq. NaOH solution. Structure **9** was confirmed by elemental analysis as well as IR, ^{1}H NMR, ^{13}C NMR and mass spectral analyses. IR spectrum showed absorption bands at 3196 cm^{-1} and 1660 cm^{-1} due to NH_2 of amide group and due to CO of amide group, respectively and absence of absorption band at 2228 cm^{-1} cyano group. ^{1}H NMR spectrum exhibited signals at δ 7.716 ppm and δ 7.254 ppm corresponding to $CONH_2$ protons in compound **9**.

The compound, 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**10**) was synthesized in high yield (85%) via heating thiazol-5-yl-1*H*-pyrazole-4-carbonitrile **8a** in water and conc. H_2SO_4 at 130 °C. Structure **10** was confirmed by elemental analysis as well as IR, ^{1}H NMR, ^{13}C NMR and mass spectral analyses. IR spectrum showed absorption band at 3420 cm^{-1} and 1684 cm^{-1}

due to OH and CO of acid group, respectively and absence of absorption band at 2228 cm^{-1} due to cyano group. ^1H NMR spectrum exhibited signals at δ 12.585 ppm corresponding to CO_2H proton in compound **10**.

The compounds, 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carboxamide analogs (**11a-d**) were prepared in high yields (82-89%) via stirring at room temperature thiazol-5-yl-pyrazole-4-carboxylic acid **10** in *N*,*N*-dimethylformamide (3 mL), TBTU and *N*,*N*-diispropylethylamine with substituted amines. Structures **11a-d** were confirmed by elemental analysis as well as IR, NMR and mass spectral analyses. The entire reaction sequence for synthesizing compounds **9**, **10** and **11a-d** from compound **8a** is provided in Scheme 3.



Scheme 3

Biology

Antimicrobial activity

The newly synthesized compounds, **8a-e**, **9**, **10** and **11a-d** 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 reference drugs were Ciprofloxacin for antibacterial and Luliconazole for antifungal tests, respectively.

Antibacterial assay

Antibacterial activity 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. For the analysis, agar diffusion method was carried out. For the solid cultural media 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 cultures were inoculating in broth media and grown at 37 °C for 18 h and revived. The above stock solution poured in the petri plates and wells were made in the plate. For 18 h old cultures (100 μL 10^4 CFU) inoculated for each plate and spread consistently on the plates. The wells were filled after 20 min containing different concentrations of samples and antibiotic. After that at 37 °C all the plates incubated and zone of inhibition in diameter (mm) noted after 24 h.[17,18]

Antifungal assay

Antifungal activity was studied against two fungal strains *Aspergillus flavus* and *Fusarium oxysporum* using Luliconazole as standard. The Agar diffusion method was used for studying the results against the fungi *Aspergillus flavus* and *Fusarium oxysporum*. The solid culture media Czapek-Dox Agar prepared which is the composition of 30 g sucrose, 2 g sodium nitrite, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4$, and 20 g agar dissolved in 1000 mL of distilled water. This stock culture is inoculated in broth media and grown at 27 °C for 48 h and revived. The above stock solution poured in the petri plate and wells were made in each petri plate. For 48 h old cultures (100 μL 10^4 CFU) inoculated for each plate and spread consistently on the plate. The wells were filled after 20 min 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 h.[17,18]

Based on the values of the inhibition zone diameter tabulated in Table 1, it could be investigated that most of the evaluated thiazolylpyrazole compounds produced moderate to significant broad-spectrum antimicrobial activity comparing to the used reference drugs.

It has been found that the compound **8a-e** exhibited moderate antibacterial activity against Gram-positive *S. aureus* and Gram-negative *S. abony* with the reference drug Ciprofloxacin, while its antifungal activity against fungi *F. oxysporum* was negative except compound **8e**, which showed potent antifungal activity when compared to that of the reference drug Luliconazole.

Further, the compound **9** exhibited moderate antibacterial activity against Gram-negative *S. abony* of inhibition zone 11 mm/mg vs 40 mm/mg of the reference drug Ciprofloxacin, while its antifungal activity

against fungi *F. oxysporum* was potent producing inhibition zone 13 mm/mg when compared to that of the reference drug Luliconazole 17 mm/mg.

Furthermore, the compound **10** exhibited potent to moderate antibacterial activity against Gram-positive *B. subtilis*, *S. aureus* and Gram-negative *S. abony* of inhibition zones 13, 16, 13 mm/mg vs 30, 23, 40 mm/mg of the reference drug Ciprofloxacin, while its antifungal activity against fungi *A. flavus*, and *F. oxysporum* was potent producing inhibition zones 17, 13 mm/mg when compared to that of the reference drug Luliconazole 23, 17 mm/mg. Compound **10** is the most promising candidate for antibacterial activity and antifungal activity in this study. Thus, the carboxylic group contributes most to the antibacterial and antifungal activity followed by the amide group and further, followed by the cyano group.

Further, the compound **11a-d** exhibited potent to moderate antibacterial activity against Gram-positive *S. aureus* and Gram-negative *S. abony* with reference drug Ciprofloxacin, while its antifungal activity against fungi *F. oxysporum* was potent when compared to that of the reference drug Luliconazole.

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*
8a	-	12	12	-	-	-
8b	-	13	-	-	-	-
8c	-	14	10	-	-	-
8d	-	-	12	-	-	-
8e	-	-	11	-	-	12
9	-	-	11	-	-	13
10	13	16	13	-	17	13
11a	-	12	-	-	-	-
11b	-	14	11	-	-	11
11c	-	14	10	-	-	11
11d	-	14	11	-	-	11
Ciprofloxacin	30	23	40	26	N/A	N/A
Luliconazole	N/A	N/A	N/A	N/A	23	17

In conclusion, we have designed and synthesized a novel series of (thiazol-5-yl)pyrazoles. The newly synthesized compounds, **8a-e**, **9**, **10** and **11a-d** were screened for 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*. Our results showed that compounds **8a-e**, **9** and **11a-d** exhibited moderate to good activity against Gram-positive bacteria, *Staphylococcus aureus*, Gram-negative bacteria, *Salmonella abony* and fungus, *Fusarium oxysporum*. However, compound **10** is the most promising candidate for antibacterial activity and antifungal activity in this study. Therefore, it was concluded that (thiazol-5-yl)pyrazoles could be developed as novel and promising antimicrobial agents.

EXPERIMENTAL

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 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. 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. Common reagent grade chemicals were commercially available and used without further purification or prepared by standard literature procedures.

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

A mixture of ethyl 2-chloroacetoacetate (20 g, 0.125 mol), thiourea (11.09 g, 0.145 mol) in EtOH (100 mL) was heated under reflux for 24 h (monitored by TLC). The EtOH 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 EtOH to give **2** as white solid, yield 84%; mp 175 °C. IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 3373 (NH_2), 1673 (C=O, ester); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.70 (bs, 2H, NH_2), 4.17- 4.10 (q, J = 7.1 Hz, 2H, CH_2), 2.37 (s, 3H, CH_3), 1.24-1.19 (t, J = 7.1 Hz, 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.

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 MeCN (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 metabisulphate and was extracted with EtOAc (5 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent evaporated in vacuum which gave pale yellow solid, yield 72%; mp 175 °C. IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 1711 (C=O); ^{1}H NMR (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.

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

To a solution of ethyl 2-iodo-4-methylthiazole-5-carboxylate (9.3 g, 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 EtOAc (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% EtOAc-petroleum ether mixture as eluent to afford **5** as white solid, yield 70%; mp 67 °C. IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 2988 (CH), 1709 (C=O), 1522 (Ar-C=C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.02-8.00 (d, J = 9.0 Hz, 2H, Ar-H), 7.60-7.57 (d, J = 9.0 Hz, 2H, Ar-H), 4.32 (q, 2H, CH_2), 2.69 (s, 3H, CH_3), 1.33 (t, 3H, CH_3); MS *m/z* (%): 438 (M^+), 437.04 (100.0), 439.04 (68.6), 436.05 (24.8), 438.05 (21.7), 440.04 (18.2), 438.04 (18.2), 441.04 (14.5), 439.05 (6.7), 437.05 (5.4), 442.04 (3.0), 441.05 (1.5); Anal. Calcd (%) for $C_{19}H_{18}Cl_2NO_4S$: C, 52.09; H, 4.14; N, 3.20. Found: C, 52.27; H, 4.19; N, 3.12.

Synthesis of 2-(4-chlorophenyl)-4-methyl-β-oxo-5-thiazolepropanenitrile (6)

A solution of ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate (4.6 g, 0.014 mol) and MeCN (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, 0.0154 mole, 9.65 mL, 1.6 molar solution in hexane) 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 EtOAc (2 x 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent evaporated in vacuum which gave pale yellow solid, yield 84%; mp 153 °C. IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 3444 (CH), 2263 (CN), 1676 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.04-8.02 (d, J = 9.0 Hz, 2H, Ar-H), 7.63-7.60 (d, J = 9.0 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.

Synthesis of *(E)*-2-(4-chlorophenyl)-α-[(dimethylamino)methylene]-4-methyl-β-oxo-5-thiazolepropanenitrile (7)

A mixture of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-3-oxopropanenitrile (1 g, 0.0036 mol) and dimethylformamide dimethyl acetal (DMF-DMA) (5 mL) was heated under reflux for 2 h (monitored by TLC). The DMF-DMA was evaporated under reduced pressure and the crude product was washed by *n*-pentane to gave **7** as yellow solid, yield 92%; mp 127 °C. IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 2191 (CN), 1698 (C=O, ketone), 1633 (C=C, enamine), 1592, 1571 (Ar-C=C); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.988 (s, 1H, olefin-H), 7.94-7.927 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.448-7.426 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 3.528 (s, 3H, N-CH_3), 3.558 (s, 3H, N-CH_3), 2.725 (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.

General procedure for synthesis of 1-*N*-substituted-3-(thiazol-5-yl)pyrazole-4-carbonitrile (8a-e)

To a solution of 2-(2-(4-chlorophenyl)-4-methylthiazole-5-carbonyl)-3-(dimethylamino)acrylonitrile (0.1 g, 0.003 mol) in EtOH (5 mL), was added substituted hydrazine (0.356 g, 0.0033 mol) and catalytic amount conc. HCl (0.1 mL) and was heated under reflux for 6 h (monitored by TLC). The EtOH 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 EtOH to gave pure solids, yields 68-84%.

3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carbonitrile (8a)

Yellow solid; yield 84%; mp 169 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 2228, 1595-1573; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.546 (s, 1H, pyrazole-H), 7.933-7.912 (d, *J* = 8.4 Hz, 2H, *p*-Cl-Ph), 7.579-7.558 (d, *J* = 8.4 Hz, 2H, *p*-Cl-Ph), 7.508-7.413 (m, 5H, Ph-H), 2.195 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 168.210, 155.159, 143.810, 138.944, 138.355, 136.183, 131.277, 130.017, 129.837, 128.442, 125.539, 116.063, 113.636, 96.183, 16.452; MS *m/z*: 377.13 $(M+1)^+$, 378 $(M+2)^+$; Anal. Calcd (%) for $C_{20}H_{13}ClN_4S$: C, 63.74; H, 3.48; N, 14.87. Found: C, 63.62; H, 3.41; N, 14.91.

1-Benzyl-3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1*H*-pyrazole-4-carbonitrile (8b)

Brown solid; yield 68%; mp 175 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 2226, 1592-1573; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.357 (s, 1H, pyrazole-H), 7.981-7.959 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.623-7.601 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.318-7.295 (m, 3H, Ph-H), 7.055-7.031 (m, 2H, Ph-H), 5.407 (s, 2H, Bz-CH_2), 2.219 (s, 3H, CH_3); MS *m/z*: 391 $(M+1)^+$; Anal. Calcd (%) for $C_{21}H_{15}ClN_4S$: C, 64.53; H, 3.87; N, 14.33. Found: C, 64.44; H, 3.86; N, 14.36.

3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-1-(4-fluorophenyl)-1*H*-pyrazole-4-carbonitrile (8c)

Off-white solid; yield 73%; mp 117 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 2237, 1593-1579; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.357 (s, 1H, pyrazole-H), 7.937-7.916 (d, *J* = 8.4 Hz, 2H, *p*-Cl-Ph), 7.579-7.558 (d, *J* = 8.4 Hz, 2H, *p*-Cl-Ph), 7.537-7.334 (m, 4H, Ph-H), 2.227 (s, 3H, CH_3); MS *m/z*: 395 $(M+1)^+$, 396 $(M+2)^+$; Anal. Calcd (%) for $C_{20}H_{12}ClFN_4S$: C, 60.84; H, 3.06; N, 14.19. Found: C, 60.78; H, 3.11; N, 14.21.

3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-1-(3-fluorophenyl)-1*H*-pyrazole-4-carbonitrile (8d)

Pale yellow solid; yield 68%; mp 183 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 2228, 1593; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.6 (s, 1H, pyrazole-H), 7.951-7.928 (d, *J* = 9.2 Hz, 2H, *p*-Cl-Ph), 7.595-7.572 (d, *J* = 9.2 Hz, 2H, *p*-Cl-Ph), 7.454-7.258 (m, 4H, Ph-H), 2.224 (s, 3H, CH_3); MS *m/z*: 395.16 $(M+1)^+$; Anal. Calcd (%) for $C_{20}H_{12}ClFN_4S$: C, 60.84; H, 3.06; N, 14.19. Found: C, 60.80; H, 3.08; N, 14.17.

3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-1-methyl-1*H*-pyrazole-4-carbonitrile (8e)

Pale yellow solid, yield 79%; mp 121 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 2229, 1637, 1648; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.262 (s, 1H, pyrazole-H), 8.029-8.009 (d, *J* = 8 Hz, 2H, *p*-Cl-Ph), 7.632-7.612 (d, *J* = 8 Hz, 2H, *p*-Cl-Ph), 3.847 (s, 3H, N-CH_3), 2.383 (s, 3H, CH_3); MS *m/z*: 315.16 $(M+1)^+$; Anal. Calcd (%) for $C_{15}H_{11}ClN_4S$: C, 57.23; H, 3.52; N, 17.80. Found: C, 57.42; H, 3.55; N, 17.85.

Synthesis of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carboxamide (9)

To a solution of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carbonitrile (0.1 g, 0.0025 mol) in EtOH (3 mL), was added 10% aq. NaOH (0.4 g, 0.01 mol) and was heated under reflux for 4 h (monitored by TLC). The EtOH 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 EtOH to give **9** as white solid; yield 90%; mp 165 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 3196, 1660, 1613, 1594; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.329 (s, 1H, pyrazole-H), 7.716 (s, 1H, $CONH_2$), 7.254 (s, 1H, $CONH_2$), 7.912-7.890 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.567-7.545 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.459-7.300 (m, 5H, Ph-H), 1.993 (s, 3H, CH_3); MS *m/z*: 395.81 $(M+1)^+$; Anal. Calcd (%) for $C_{20}H_{15}ClN_4OS$: C, 60.83; H, 3.83; N, 14.19. Found: C, 60.75; H, 3.84; N, 14.21.

Synthesis of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carboxylic acid (10)

To a solution of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carbonitrile (1 g, 0.026 mol) in H_2O (5 mL), was added conc. H_2SO_4 (1 mL) and was heated at 130 °C for 2 h (monitored by TLC). The reaction mixture was cooled to room temperature and added to ice-water mixture. The resultant precipitate was subjected to filtration, dried, and recrystallized from MeOH to afford **10** as white solid; yield 85%; mp 151 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 3420, 1684, 1595, 1579; ^{1}H NMR (DMSO-d_6, δ, ppm): 12.585 (bs, 1H, COOH), 8.254 (s, 1H, pyrazole-H), 7.917-7.895 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.569-7.547 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.469-7.261 (m, 5H, Ph-H), 2.051 (s, 3H, CH_3); MS *m/z*:

396.26 $(M+1)^+$; Anal. Calcd (%) for $C_{20}H_{14}ClN_3O_2S$: C, 60.68; H, 3.56; N, 10.61. Found: C, 60.71; H, 3.54; N, 10.65.

General procedure for synthesis of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carboxamide analogs (11a-d)

To a solution of 3-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-1*H*-pyrazole-4-carboxylic acid (0.1 g, 0.00025 mol) in DMF (3 mL), was added 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylammonium tetrafluoroborate (TBTU) (0.121 g, 0.00037 mol) and *N*,*N*-diispropylethylamine (0.2 mL) and was stirred at room temperature for 1 h, followed by addition of substituted amine (0.03 g, 0.000275 mol) and was further stirred at room temperature overnight until the reaction was complete (monitored by TLC). The reaction mixture was added to ice-water mixture. The resultant precipitate was subjected to filtration, dried, and recrystallized from MeOH to give **11** as pure solids, yields 82-89%.

3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-*N*-(4-fluorophenyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (11a)

Off-brown solid; yield 85%; mp 163 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 3269, 1640, 1545, 1506; ^{1}H NMR (DMSO-d_6, δ, ppm): 10.02 (s, 1H, CONH), 8.55 (s, 1H, pyrazole-H), 7.919-7.897 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.703-7.667 (m, 2H, *p*-F-Ph), 7.567-7.545 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.486-7.367 (m, 5H, Ph-H), 7.164-7.142 (m, 2H, *p*-F-Ph), 2.027 (s, 3H, CH_3); MS *m/z*: 489.44 $(M+1)^+$; Anal. Calcd (%) for $C_{26}H_{18}ClFN_4OS$: C, 63.87; H, 3.71; N, 11.46. Found: C, 63.91; H, 3.74; N, 11.48.

3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-1-phenyl-*N*-(3-(trifluoromethyl)benzyl)-1*H*-pyrazole-4-carboxamide (11b)

Off-white solid; yield 82%; mp 173 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 3266, 1655, 1585, 1549; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.905 (s, 1H, CONH), 8.448 (s, 1H, pyrazole-H), 7.894-7.874 (d, *J* = 8.0 Hz, 2H, *p*-Cl-Ph) 7.732-7.714 (m, 2H, Ar-H), 7.685-7.665 (d, *J* = 8.0 Hz, 2H, *p*-Cl-Ph), 7.556-7.331 (m, 7H, Ar-H), 4.599 (d, 2H, CH_2), 1.997 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 166.610, 161.866, 154.329, 140.412, 139.116, 138.062, 125.579, 134.060, 133.135, 131.722, 129.835, 129.760, 128.646, 128.094, 127.808, 126.372, 126.300, 126.195, 125.351, 119.564, 119.045, 39.351, 16.220; MS *m/z*: 553.45 $(M+1)^+$; Anal. Calcd (%) for $C_{28}H_{20}ClF_3N_4OS$: C, 60.81; H, 3.65; N, 10.13. Found: C, 60.88; H, 3.66; N, 10.14.

3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-*N*-cyclopentyl-1-phenyl-1*H*-pyrazole-4-carboxamide (11c)

Brown solid; yield 89%; mp 172 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 3316, 1644, 1594, 1582; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.063-8.045 (d, 1H, CONH), 8.338 (s, 1H, pyrazole-H), 7.910-7.888 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph) 7.568-7.546 (d, *J* = 8.8 Hz, 2H, *p*-Cl-Ph), 7.459-7.298 (m, 5H, Ar-H), 4.127-4.110 (m, 1H, CH), 1.991 (s, 3H, CH_3), 1.676-1.647 (m, 2H, CH_2), 1.530-1.468 (m, 2H, CH_2), 1.451-1.096 (m, 4H, $2CH_2$); MS *m/z*:

463.42 $(M+1)^+$; Anal. Calcd (%) for $C_{25}H_{23}ClN_4OS$: C, 64.85; H, 5.01; N, 12.10. Found: C, 64.88; H, 5.05; N, 12.14.

3-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-*N*-isopropyl-1-phenyl-1*H*-pyrazole-4-carboxamide (11d)

Off-white solid; yield 86%; mp 153 °C; IR (ATR, cm^{-1}): $\bar{\upsilon}$ = 3316, 1644, 1594, 1582; ^1H NMR (DMSO-d_6, δ, ppm): 8.019-8.001 (d, 1H, CONH), 8.334 (s, 1H, pyrazole-H), 7.912-7.890 (d, J = 8.8 Hz, 2H, *p*-Cl-Ph), 7.567-7.545 (d, J = 8.8 Hz, 2H, *p*-Cl-Ph), 7.460-7.299 (m, 5H, Ar-H), 3.997-3.978 (m, 1H, CH), 1.992 (s, 3H, CH_3), 1.138-1.122 (d, 6H, $2CH_3$); MS *m/z*: 437.44 $(M+1)^+$; Anal. Calcd (%) for $C_{23}H_{21}ClN_4OS$: C, 63.22; H, 4.84; N, 12.82. Found: C, 63.24; H, 4.86; N, 12.84.

ACKNOWLEDGEMENTS

Authors thank to SPPU Pune for financial support and for spectral analysis and KTHM College, Nashik for all types of support to do research work.

REFERENCES

1. P. C. Sharma, K. K. Bansal, A. Sharma, D. Sharma, and A. Deep, *Eur. J. Med. Chem.*, 2020, **188**, 112016.
2. R. Mishra, P. K. Sharma, P. K. Verma, I. Tomer, G. Mathur, and P. K. Dhakad, *J. Heterocycl. Chem.*, 2017, **54**, 2103.
3. C. B. Mishra, S. Kumari, and M. Tiwari, *Eur. J. Med. Chem.*, 2015, **92**, 1.
4. S. J. Kashyap, V. K. Garg, P. K. Sharma, N. Kumar, R. Dudhe, and J. K. Gupta, *Med. Chem. Res.*, 2012, **21**, 2123.
5. C. Lamberth, *Heterocycles*, 2007, **71**, 1467.
6. L. Zhong, F. Zhang, T. Jiang, X. Lu, T. Xu, C. Ding, J. Chen, J. Yuan, and C. Tan, *Heterocycles*, 2019, **98**, 637.
7. E. Masumoto, N. Kashige, H. Nagabuchi, F. Okabe-Nakahara, and H. Maruoka, *Heterocycles*, 2019, **98**, 1736.
8. B. Sever, M. D. Altintop, M. O. Radwan, A. Ozdemir, M. Otsuka, M. Fujita, and H. I. Ciftci, *Eur. J. Med. Chem.*, 2019, **182**, 111648.
9. G. G. Mandawad, B. S. Dawne, S. D. Beedkar, C. N. Khobragade, and O. S. Yemul, *Bioorg. Med. Chem.*, 2013, **21**, 365.
10. H. E. Gaffer, S. Abdel-Fattah, H. A. Etman, and E. Abdel-Latif, *J. Heterocycl. Chem.*, 2017, **54**, 331.
11. P.-C. Lv, D.-D. Li, Q.-S. Li, X. Lu, Z.-P. Xiao, and H.-L. Zhu, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5374.

12. R. Watpade, A. Bholay, and R. Toche, *J. Heterocycl. Chem*., 2017, **54**, 3434.
13. R. B. Toche and P. Nikam, *Chem. Biol. Interface*, 2015, **5**, 246.
14. S. K. Kanawade, R. B. Toche, and D. P. Rajani, *Eur. J. Med. Chem*., 2013, **64**, 314.
15. P. S. Patil and R. B. Toche, *Front. Drug, Chem. Clin. Res*., 2018, **1**, 1.
16. S. K. Kanawade, R. B. Toche, S. P. Patil, A. E. Desai, and S. S. Bhamare, *Eur. J. Med. Chem*., 2011, **46**, 4682.
17. E. J. Threlafall, I. S. T. Fisher, L. R. Ward, H. Tschape, and P. Gerner-Smidt, *Microb. Drug Resist*., 1999, **5**, 195.
18. J. F. Prescott, J. D. Baggot, and R. D. Walker, "Antimicrobial Therapy in Veterinary Medicine," 3rd ed., Iowa State University Press, Ames, 2000, 12.