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Ultrasound-assisted synthesis and antimicrobial activity of tetrazole-based pyrazole and pyrimidine derivatives

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Abstract

New tetrazole-based pyrazole and pyrimidine derivatives were synthesized by an ultrasound irradiation method. All compounds were characterized by infrared spectroscopy (IR), ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, mass spectrometry (MS) and elemental analysis and assessed *in vitro* for their efficacy as antimicrobial agents against four bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*) and two fungi (*Candida albicans*, *Aspergillus niger*). Compounds **8a**, **8e**, **9a**, **9b** and **9e** show potent activity against the tested strains compared to the reference drugs chloramphenicol and clotrimazole.

Keywords: antimicrobial; pyrazole; pyrimidine; tetrazole; ultrasound

Introduction

Ultrasound-accelerated chemical reactions proceed via the formation and adiabatic collapse

of transient cavitation bubbles. Ultrasound irradiation has become an alternative energy source for organic reactions ordinarily accomplished by heating. Many reactions can be conducted smoothly by sonication to provide improved yields and increased selectivity [1].

Life-threatening invasive infections caused by microorganisms have increased to alarming levels all over the world [2]. In the past decades, antibiotics have been used as antimicrobial drugs. However, many human pathogens no longer respond to these antibiotics. Therefore, it is necessary to find new and efficacious drugs to treat these problems. The substitution of fluorine in potential drug molecules can improve their therapeutic efficacy through hydrogen bonding interactions at the active sites of the enzyme [3]. Thus, fluorine substitution remains an important aspect in the development of more active and selective drug candidates.

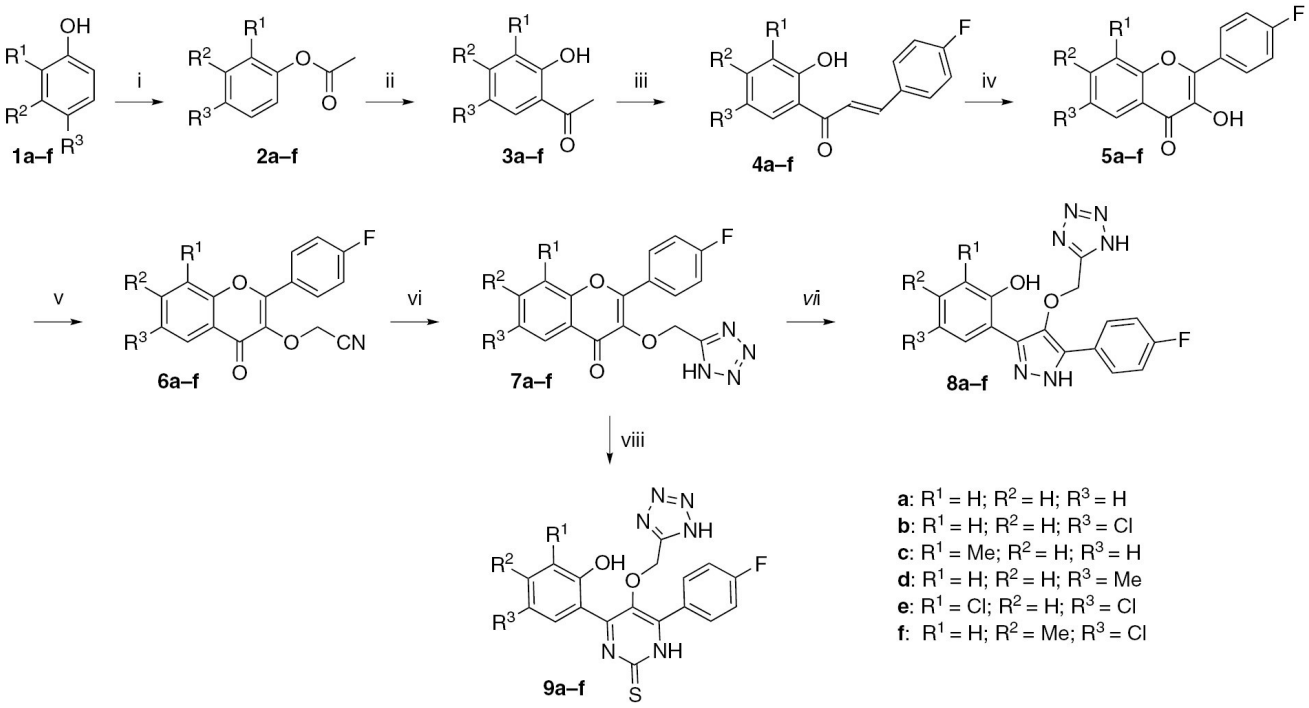
The synthetic versatility of tetrazole is due to its widespread applications in the field of medicinal chemistry. Tetrazole is considered a carboxylic acid analog because of their similar pK_a values and planar delocalized systems. An advantage of tetrazole derivatives over carboxylic acids is that they are resistant to various biological degradation processes contributing to longer bioavailability of drugs [4, 5]. These factors, prime in the potential applications of tetrazole derivatives, are responsible for anticancer [6], antifungal [7], antitubercular [8], anti-HIV [9], antioxidant [10] and hormonal properties of the bioactive agents [11]. Pyrazole derivatives have showed significant biological activities, such as antimicrobial [12], analgesic [13], anti-inflammatory [14] and anticancer activities [15]. Pyrimidines also occupy a distinct and unique place in medicinal chemistry [16]. Pyrimidine derivatives are of interest due to their pharmacological properties such as antitumor [17], antimalarial [18], antiviral [19], antifungal [20], antibacterial [21], anti-inflammatory [22], analgesic [23] and antiprotozoal [24] activities. In continuation of our earlier work on design and synthesis of pharmacologically significant heterocycles [25], herein we report an efficient green synthesis and antimicrobial activity of new tetrazole containing pyrazole and pyrimidine derivatives.

Results and discussion

Chemistry

New tetrazole-based pyrazole derivatives **8a–f** and tetrazole-based pyrimidine derivatives **9a–f** were synthesized using conventional heating and ultrasound irradiation methods (Scheme 1). The precursors **4a–f** were prepared in good yield according to the literature procedure [26, 27]. Compounds **4a–f** were converted into corresponding substituted 3-hydroxychromones **5a–f** by oxidative cyclization using hydrogen peroxide in ethanol. The hydroxychromones **5a–f** were alkylated with 2-chloroacetonitrile in the presence of K_2CO_3 in *N*,*N*′-dimethylformamide (DMF) at room temperature to afford the substituted 2-(2-(4-fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy)acetonitriles **6a–f**. The reaction of **6a–f** with sodium azide and zinc bromide in water at 100°C furnished 3-[(1*H*-tetrazol-5-yl)methoxy)-2-(4-fluorophenyl]-4*H*-chromen-4-one derivatives **7a–f**. Treatment of **7a–f**

with hydrazine hydrate in ethanol under ultrasound irradiation yielded the desired pyrazoles **8a–f**. In addition, treatment of compounds **7a–f** with thiourea in ethanolic KOH under ultrasound irradiation furnished the desired pyrimidines **9a–f**. All synthesized compounds were characterized by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, mass spectrometry (MS) and elemental analysis.



Scheme 1

Reagents and conditions: (i) acetic anhydride, pyridine, 100°C, 3–4 h; (ii) $AlCl_3$, 150°C, 3–4 h; (iii) 4-fluorobenzaldehyde, KOH, EtOH, room temperature (rt), 2–3 h; (iv) H_2O_2, NaOH, 0°C to rt, 2–3 h; (v) 2-chloroacetonitrile, K_2CO_3, DMF, rt, 3–4 h; (vi) sodium azide, zinc bromide, H_2O, reflux, 100°C, 4–5 h; (vii) hydrazine hydrate, ethanol,))))), 65°C, 45–55 min; (viii) thiourea, KOH, ethanol,))))), 65°C, 15–25 min.

Antimicrobial activity

All compounds were evaluated for antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (NCIM 2079) and *Bacillus subtilis* (NCIM 2920) and Gram-negative bacteria *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200) and for antifungal activity against *Candida albicans* (NCIM 3471) and *Aspergillus niger* (NCIM 596). The minimum inhibitory concentration (MIC) values were determined by the micro-broth dilution method. The antibacterial drug chloramphenicol and antifungal drug clotrimazole were used as reference antibiotics. The results are shown in Table 1. Of interest are high activities of compounds **9a** and **9e** against *A. niger*. Compound **9e** is also active against *C. albicans*. The activities of **8e**, **9a** and **9b** against *S. aureus* exceed those of the reference drug chloramphenicol.

Table 1

Antimicrobial screening results of bioactive compounds **8** and **9** using the micro-broth dilution method.

No.	Minimum inhibitory concentration (μg/mL)					
	Bacteria				Fungi	
	Sa	***Bs***	***Ec***	***Pa***	***Ca***	***An***
8e	25	200	50	200	75	50
9a	25	200	175	150	75	25
9b	25	75	125	100	100	50
9e	50	50	75	75	50	12.5
Chloramphenicol	50	25	50	50	–	–
Clotrimazole	–	–	–	–	50	25

Sa, *Staphylococcus aureus*; *Bs*, *Bacillus subtilis*; *Ec*, *Escherichia coli*; *Pa*, *Pseudomonas aeruginosa*; *Ca*, *Candida albicans*; *An*, *Aspergillus niger.*

Conclusion

Newly synthesized compounds **8e**, **9a**, **9b** and **9e** are highly potent antimicrobial agents.

Experimental

The progress of each reaction was monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} plates and visualized with ultraviolet (UV) light and iodine. Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded on a Carry 600 Series Fourier-transform infrared spectroscopy (FT-IR) spectrophotometer using KBr pellets. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AVANCE II 400 NMR spectrometer in $CDCl_3$ or DMSO-d_6. Mass spectra were recorded on a Waters quadrupole time-of-flight (Q-TOF) micro instrument equipped with an electrospray ionization (ESI) source. Elemental analysis was performed on a Perkin-Elmer EAL-240 elemental analyzer.

General procedure for synthesis of compounds 5a–f

A mixture of (*E*)-3-(4-fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**4a–f**, 1 mmol), ethanol (15 mL), NaOH (10%, 5 mL) and hydrogen peroxide (30%, 1.5 mL) was stirred vigorously for 30 min and then kept for 2–3 h in ice. The progress of the reaction was monitored by TLC using ethyl acetate/hexane as an eluent. After completion of the reaction, the mixture was poured into ice water and acidified with 1 M hydrochloric acid (HCl). The

precipitate was collected by filtration, washed with water and crystalized from chloroform/ethanol to afford a pure product.

2-(4-Fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (5a)

This compound was synthesized from (*E*)-3-(4-fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**4a**); a yellow solid; yield 74%; mp 152–154°C; IR: 3297 (OH), 2925 (ArH), 1618 (C=O, pyrone), 1572 cm^{-1} (C=C); ^1H NMR ($CDCl_3$: δ 7.11 (br s, 1H, ArH), 7.22 (t, 2H, *J*=8.5 Hz, ArH), 7.42 (m 1H, ArH), 7.58 (d, 1H, *J*=8.5 Hz, ArH), 7.71 (m, 1H, ArH), 8.24 (d, 1H, *J*=1.8 Hz, ArH), 8.26 (s, 1H, OH), 8.27–8.30 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 115.7, 115.9, 118.2, 120.7, 124.6, 125.5, 127.3, 129.9, 133.7, 138.2, 144.1, 155.3, 162.3, 164.8, 173.4. ESI-MS. Calcd for $C_{15}H_9FO_3$ $(M+H)^+$: *m/z* 257.0. Found: *m/z* 257.0.

6-Chloro-2-(4-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (5b)

This compound was synthesized from (*E*)-1-(5-chloro-2-hydroxyphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**4b**); a yellow solid; yield 77%; mp 213–215°C; ^1H NMR (DMSO-d_6): δ 7.08–7.25 (m, 2H, ArH), 7.68–7.82 (m, 2H, ArH), 8.12–8.23 (m, 2H, ArH), 8.51 (s, 1H, ArH), 9.08 (br s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 114.1, 117.5, 120.6, 125.4, 127.4, 128.4, 129.7, 130.2, 133.6, 148.5, 155.4, 157.6, 160.8, 162.5, 172.9. ESI-MS. Calcd for $C_{15}H_8ClFO_3$ $(M+H)^+$: *m/z* 291.0224. Found: *m/z* 291.2223.

2-(4-Fluorophenyl)-3-hydroxy-8-methyl-4*H*-chromen-4-one (5c)

This compound was synthesized from (*E*)-3-(4-fluorophenyl)-1-(2-hydroxy-3-methylphenyl)prop-2-en-1-one (**4c**); a yellow solid, yield 69%; mp 160–162°C; ^1H NMR (DMSO-d_6): δ 2.54 (s, 3H, CH_3), 7.24–7.29 (m, 3H, ArH), 7.53 (d, 1H, *J*=7 Hz, ArH), 7.93 (d, 1H, *J*=7.7 Hz, ArH), 8.26 (m, 2H, ArH), 9.38 (s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 15.3, 115.1, 115.3, 121, 122.3, 123.6, 127.1, 127.9, 129.6, 133.7, 138.7, 143.5, 152.8, 161.3, 163.7, 173. ESI-MS. Calcd for $C_{16}H_{11}FO_3$ $(M+H)^+$: *m/z* 271.077. Found: *m/z* 271.2154.

2-(4-Fluorophenyl)-3-hydroxy-6-methyl-*4H*-chromen-4-one (5d)

This compound was synthesized from (*E*)-3-(4-fluorophenyl)-1-(2-hydroxy-5-methylphenyl)prop-2-en-1-one (**4d**); a yellow solid, yield 73%; mp 171–173°C; ^1H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 7.27 (t, 2H, *J*=8.6 Hz, ArH), 7.54 (s, 2H, ArH), 7.91 (s, 1H, ArH),

8.27–8.30 (m, 2H, ArH), 9.31 (s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 20.5, 115, 115.2, 117.7, 120.9, 123.8, 127.7, 129.8, 130.6, 134.5, 138.7, 143.9, 152.8, 161.3, 163.8, 172.8. ESI-MS. Calcd for $C_{16}H_{11}FO_3$ $(M+H)^+$: *m/z* 271.0770. Found: *m/z* 271.0234.

6,8-Dichloro-2-(4-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (5e)

This compound was synthesized from (*E*)-1-(3,5-dichloro-2-hydroxyphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**4e**); a yellow solid; yield 75%; mp 203–205°C; ^{1}H NMR (DMSO-d_6): δ 7.25 (t, 2H, *J*=8.8 Hz, ArH), 7.82 (d, 1H, *J*=2.6 Hz, ArH), 7.96 (d, 1H, *J*=2.2 Hz, ArH), 8.29 (m, 2H, ArH), 9.81 (s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 115.2, 115.4, 122.8, 123, 123.7, 127.1, 128.8, 129.8, 132.6, 139.2, 144.5, 148.4, 161.5, 164, 171.3. ESI-MS. Calcd for $C_{15}H_7Cl_2FO_3$ $(M+H)^+$: *m/z* 324.9835. Found: *m/z* 325.0267.

6-Chloro-2-(4-fluorophenyl)-3-hydroxy-7-methyl-*4H*-chromen-4-one (5f)

This compound was synthesized from (*E*)-1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**4f**); a yellow solid, yield 79%; mp 222–224°C; ^{1}H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 7.28 (m, 2H, ArH), 7.64 (br s, 1H, ArH), 8.03 (s, 1H, ArH), 8.27 (br s, 2H, ArH), 9.54 (s, 1H, OH); ^{13}C NMR (DMSO-d_6): δ 20.3, 115.1, 115.3, 120.1, 120.5, 124, 127.5, 129.9, 130.1, 138.8, 141.8, 144.3, 152.8, 161.4, 163.9, 171.8. ESI-MS. Calcd for $C_{16}H_{10}ClFO_3$ $(M+H)^+$: *m/z* 305.0381. Found: *m/z* 305.0251.

General procedure for synthesis of compounds 6a–f

To a stirred solution of **5a–f** (1 mmol) in DMF was added potassium carbonate (2 mmol) and portion-wise 2-chloroacetonitrile (1 mmol) at room temperature for about 3–4 h. The mixture was quenched with crushed ice and the resultant precipitate was filtered and crystallized from ethanol.

2-(2-(4-Fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy)acetonitrile (6a)

This compound was prepared from **5a**; a white solid; yield 78%; mp 140–142°C; IR: υ 3012 (Ar-H), 2965 (C-H), 2346 (C≡N), 1634 (C=O), 1604 (C=C), 1238 (C-F) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.11 (s, 2H, OCH_2), 7.20–2.27 (m, 2H,ArH), 7.45 (m, 1H, ArH), 7.57 (dd, 1H, *J*=1.1, 8.6 Hz, ArH), 7.74 (ddt, 1H, *J*=1.5, 7.8, 8.0 Hz, ArH), 8.08 (m, 2H, ArH), 8.25 (dd, 1H, *J*=1.5, 7.8 Hz, ArH); ^{13}C NMR ($CDCl_3$): δ 56.1, 115.1, 115.9, 116.1, 118.1, 123.8, 125.4, 125.8, 126.1, 131.3, 134.2, 137.5, 155.3, 156.4,

163.1, 165.6, 174.1. Anal. Calcd for $C_{17}H_{10}FNO_3$: C, 69.15; H, 3.41; N, 4.74. Found: C, 69.07; H, 3.35; N, 4.68.

2-(6-Chloro-2-(4-fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy)acetonitrile (6b)

This compound was prepared from **5b**; a white solid, yield 86%; mp 168–170°C; IR: υ 3069 (Ar-H), 2952 (C-H), 2360 (C≡N), 1628 (C=O), 1600 (C=C), 1144 (C-F) 755 (C-Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.14 (s, 2H, OCH_2), 7.34 (t, 2H, *J*=8.0 Hz, ArH), 7.73 (d, 1H, *J*=8.0 Hz, ArH), 7.79 (dd, 1H, *J*=2.6, 9.2 Hz, ArH), 8.07 (d, 1H, *J*=2.6 Hz, ArH), 8.13 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 56.4, 115.5, 115.7, 120.4, 124, 124.3, 125.7, 130.3, 131.1, 131.2, 134.1, 137.6, 153.1, 155.4, 162.4, 165, 172.1. Anal. Calcd for $C_{17}H_9ClFNO_3$: C, 61.93; H, 2.75; N, 4.25. Found: C, 61.98; H, 2.82; N, 4.33.

2-(2-(4-Fluorophenyl)-8-methyl-4-oxo-4*H*-chromen-3-yloxy)acetonitrile (6c)

This compound was prepared from **5c**; a white solid; yield 71%; mp 176–178°C; IR: υ 3032 (Ar-H), 2915 (C-H), 2360 (C≡N), 1633 (C=O), 1604 (C=C), 1193 (C-F) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.58 (s, 3H, CH_3), 5.12 (s, 2H, OCH_2), 7.23–7.27 (m, 2H, ArH), 7.34 (t, 1H, *J*=7.0 Hz, ArH), 7.57 (d, 1H, *J*=7.0 Hz, ArH), 8.07–8.13 (m, 3H, ArH); ^{13}C NMR ($CDCl_3$): δ 15.8, 56, 115.1, 115.9, 116.2, 123.4, 123.8, 125.1, 126.5, 127.6, 131.3, 135, 137.4, 153.8, 155.8, 163.1, 165.6, 174.4. Anal. Calcd for $C_{18}H_{12}FNO_3$: C, 69.90; H, 3.91; N, 4.53. Found: C, 69.96; H, 3.94; N, 4.56.

2-(2-(4-Fluorophenyl)-6-methyl-4-oxo-4*H*-chromen-3-yloxy)acetonitrile (6d)

This compound was prepared from **5d**; a white solid; yield 74%; mp 162–164°C; IR: υ 3087 (Ar-H), 2974 (C-H), 2369 (C≡N), 1632 (C=O), 1603 (C=C), 1187 (C-F) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.53 (s, 3H, CH_3), 5.15 (s, 2H, OCH_2), 7.36 (s, 2H, ArH), 7.61 (s, 2H, ArH), 7.90 (s, 1H, ArH), 8.12 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6); δ 20.5, 56.4, 115.5, 115.7, 118, 122.9, 124.1, 126.2, 131.1, 131.1, 134.8, 135.4, 136.3, 137.5, 153, 154.9, 162.3, 173.1. Anal. Calcd for $C_{18}H_{12}FNO_3$: C, 69.90; H, 3.91; N, 4.53. Found: C, 69.82; H, 3.95; N, 4.66.

2-(6,8-Dichloro-2-(4-fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy)acetonitrile (6e)

This compound was prepared from **5e**; a white solid; yield 77%; mp 177–179°C; IR: υ 3064 (Ar-H), 2916 (C-H), 2361 (C≡N), 1625 (C=O), 1601 (C=C), 1180 (C-F) 717 (C-Cl) cm^{-1}; ^1H NMR

($CDCl_3$): δ 5.12 (s, 2H, OCH_2), 7.26 (t, 2H, *J*=8.6 Hz, ArH), 7.77 (d, 1H, *J*=2.2 Hz, ArH), 8.10 (d, 1H, *J*=2.2 Hz, ArH), 8.18 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 56.1, 114.8, 116.2, 116.4, 123.8, 124.6, 125.5, 131.2, 131.5, 131.6, 134.2, 137.6, 149.4, 156.3, 163.5, 166, 172.5. Anal. Calcd for $C_{17}H_8Cl_2FNO_3$: C, 56.07; H, 2.21; N, 3.85. Found: C, 56.18; H, 2.24; N, 3.79.

2-(6-Chloro-2-(4-fluorophenyl)-7-methyl-4-oxo-4*H*-chromen-3-yloxy)acetonitrile (6f)

This compound was prepared from **5f**; a white solid, yield 82%; mp 170–172°C; IR: υ 3013 (Ar-H), 2915 (C-H), 2360 (C≡N), 1599 (C=O), 1552 (C=C), 1203 (C-F) 750 (C-Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6): 2.54 (s, 3H, CH_3), 5.14 (s, 2H, OCH_2), 7.35 (s, 2H, ArH), 7.70 (s, 1H, ArH), 8.04 (s, 1H, ArH), 8.12 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 20.3, 56.4, 115.5, 115.6, 115.7, 120.3, 122.4, 124.2, 125.9, 125.9, 131.1, 131.1, 137.5, 142.9, 153.1, 155.1, 167.4, 172.1. Anal. Calcd for $C_{18}H_{11}ClFNO_3$: C, 62.89; H, 3.23; N, 4.07. Found: C, 62.93; H, 3.28; N, 4.11.

General procedure for synthesis of compounds 7a–f

To a mixture of sodium azide (1.5 mmol) and zinc bromide (1.5 mmol) in water (20 mL) was added compound **6a–f** (1 mmol). The mixture was then heated at reflux for 4–5 h with vigorous stirring. After completion of the reaction, as monitored by TLC using chloroform/methanol as an eluent, the mixture was quenched with crushed ice, and the resultant solid was filtered and crystallized from ethanol.

3-[(1*H*-Tetrazol-5-yl)methoxy]-2-(4-fluorophenyl)-4*H*-chromen-4-one (7a)

This compound was prepared from **6a**; a white solid, yield: 81%; mp 200–202°C; IR: υ 3444 (N-H), 3030 (Ar-H), 2917 (C-H), 1606 (C=O), 1554 (C=C), 1238 (C-F) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.47 (s, 2H, OCH_2), 7.26 (t, 2H, *J*=9.0 Hz, ArH), 7.48–7.52 (m, 1H, ArH), 7.70 (d, 1H, *J*=8.0 Hz, ArH), 7.82 (ddd, 1H, *J*=8.5, 7.1, 1.7 Hz, ArH), 8.01–8.05 (m, 2H, ArH), 8.16 (dd, 1H, *J*=8.0, 1.7 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 61.8 (O-CH_2), 115.3, 115.6, 118.3, 123.4, 124.9, 125.1, 126.2, 130.9, 131, 134.1, 138.2, 154.7, 154.9, 162 (tetrazole C), 164.5 (C-F), 173.5 (C=O). Anal. Calcd for $C_{17}H_{11}FN_4O_3$: C, 60.36; H, 3.28; N, 16.56. Found: C, 60.29; H, 3.25; N, 16.51.

3-[(1*H*-Tetrazol-5-yl)methoxy]-6-chloro-2-(4-fluorophenyl)-4*H*-chromen-4-one (7b)

This compound was prepared from **6b**; a white solid; yield 85%; mp 203–205°C; IR: υ 3435 (N-H), 3035 (Ar-H), 2992 (C-H), 1622 (C=O), 1605 (C=C), 1197 (C-F), 718 (C-Cl) cm^{-1}; ^{1}H NMR

(DMSO-d_6): δ 5.42 (s, 2H, OCH_2), 7.17 (t, 2H, *J*=9.0 Hz, ArH), 7.64 (d, 1H, *J*=9.0 Hz, ArH), 7.72 (dd, 1H, *J*=9.0, 2.6 Hz, ArH), 7.98 (m, 2H, ArH), 8.04 (d, 1H, *J*=2.6 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 61.7 (O-CH_2), 115.4, 115.6, 120.7, 123.8, 124.5, 125.9, 125.9, 129.9, 131, 134, 138.2, 153.2, 155.2, 162.1 (tetrazole C), 164.6 (C-F), 172.5 (C=O). Anal. Calcd for $C_{17}H_{10}ClFN_4O_3$: C, 54.78; H, 2.70; N, 15.03. Found: C, 54.84; H, 2.72; N, 15.23.

3-[(1*H*-Tetrazol-5-yl)methoxy]-2-(4-fluorophenyl)-8-methyl-4*H*-chromen-4-one (7c)

This compound was prepared from **6c**; a white solid; yield 73%; mp 198–200°C; IR: υ 3435 (N-H), 3035 (Ar-H), 2992 (C-H), 1622 (C=O), 1605 (C=C), 1197 cm^{-1} (C-F); ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 5.61 (s, 2H, OCH_2), 6.97 (s, 1H, ArH), 7.10–7.22 (m, 2H, ArH), 7.53 (s, 1H, ArH), 7.69–7.80 (m, 3H, ArH), 8.41 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 15.1 (CH_3), 63.5 (O-CH_2), 114.9, 115.2, 122.3, 123, 124.3, 126.6, 127.1, 128.2, 130.4, 134.2, 139, 149.5, 152.6, 161.5 (tetrazole C), 164 (C-F), 173.8 (C=O). Anal. Calcd for $C_{18}H_{13}FN_4O_3$: C, 61.36; H, 3.72; N, 15.90. Found: C, 61.45; H, 3.69; N, 15.98.

3-[(1*H*-Tetrazol-5-yl)methoxy]-2-(4-fluorophenyl)-6-methyl-4*H*-chromen-4-one (7d)

This compound was prepared from **6d**; a white solid; yield 75%; mp 202–204°C; IR: υ 3423 (N-H), 3021 (Ar-H), 2918 (C-H), 1602 (C=O), 1554 (C=C), 1174 cm^{-1} (C-F); ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 5.47 (s, 2H, OCH_2), 7.27 (t, 2H, *J*=9.0 Hz, ArH), 7.57–7.63 (m, 2H, ArH), 7.90 (s, 1H, ArH), 8.00 (dd, 2H, *J*=9.0, 5.3 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 20.4 (CH_3), 61.8 (O-CH_2), 115.3, 115.5, 118, 123.1, 124.1, 126.3, 126.3, 130.9, 134.7, 135.2, 138.2, 153, 154.7, 162 (tetrazole C), 164.5 (C-F), 173.4 (C=O). Anal. Calcd for $C_{18}H_{13}FN_4O_3$: C, 61.36; H, 3.72; N, 15.90. Found: C, 61.34; H, 3.65; N, 15.81.

3-[(1*H*-Tetrazol-5-yl)methoxy]-6,8-dichloro-2-(4-fluorophenyl)-4*H*-chromen-4-one (7e)

This compound was prepared from (**6e**); a white solid; yield 80%; mp 194–196°C; IR: υ 3423 (N-H), 3040 (Ar-H), 2917 (C-H), 1660 (C=O), 1603 (C=C), 1158 (C-F), 760 cm^{-1} (C-Cl); ^{1}H NMR (DMSO-d_6): δ 5.50 (s, 2H, OCH_2), 7.24 (t, 2H, *J*=8.4 Hz, ArH), 7.33–7.37 (m, 1H, ArH), 8.00 (d, 2H, *J*=5.5 Hz, ArH), 8.08 (dd, 1H, *J*=8.4, 5.5 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 62 (O-CH_2), 115.6, 115.8, 123, 123.8, 125.2, 129.6, 131, 133.5, 138.6, 139.4, 148.9, 154.6, 162.2 (tetrazole C), 164.7 (C-F), 172 (C=O). Anal. Calcd for $C_{17}H_9Cl_2FN_4O_3$: C, 50.15; H, 2.23; N, 13.76. Found: C, 50.33; H, 2.32; N, 13.87.

3-[(1*H*-Tetrazol-5-yl)methoxy]-6-chloro-2-(4-fluorophenyl)-7-methyl-4*H*-chromen-4-one (7f)

This compound was prepared from **6f**; a white solid; yield 78%; mp 178–180°C; IR: υ 3495 (N-H), 3077 (Ar-H), 2924 (C-H), 1638 (C=O), 1618 (C=C), 1166 (C-F) 771 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 5.45 (s, 2H, OCH_2), 7.30 (t, 2H, *J*=9.0 Hz, ArH), 7.73–7.76 (m, 1H, ArH), 7.92–7.96 (m, 3H, ArH); ^{13}C NMR (DMSO-d_6): δ 20.1 (CH_3), 61.8 (O-CH_2), 115.4, 115.7, 120.6, 122.5, 123.9, 126.1, 130.6, 130.9, 131, 138.1, 142.7, 153, 154.9, 162 (tetrazole C), 164.5 (C-F), 172.4 (C=O). Anal. Calcd for $C_{18}H_{12}ClFN_4O_3$: C, 55.90; H, 3.13; N, 14.49. Found: C, 55.97; H, 3.21; N, 14.58.

General procedure for synthesis of compounds 8a–f

Conventional method

Hydrazine hydrate (1 mmol) was added to a solution of **7a–f** (1 mmol) in ethanol (10 mL) and the mixture was heated for 5–6 h. After completion of the reaction, as monitored by TLC, 10 mL of water was added, and the resultant precipitate was filtered, dried under reduced pressure and crystallized from ethanol.

Ultrasound-assisted method

Hydrazine hydrate (1 mmol) was added to a solution of **7a–f** (1 mmol) in ethanol (10 mL) and the mixture was ultrasonicated for 45–55 min at 65°C. After completion of the reaction, as monitored by TLC, 10 mL of water was added, and the resultant precipitate was filtered, dried and crystallized from ethanol.

2-[4-((1*H*-Tetrazol-5-yl)methoxy)-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]phenol (8a)

This compound was prepared from **7a**; a yellow solid; conventional method time 340 min, yield 70%; ultrasound-assisted method time 51 min, yield 88%; mp 269–271°C; IR: υ 3943 (OH), 3352 (NH), 1884 (C=N), 1246 (C-F) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.08 (s, 2H, OCH_2), 6.89 (t, 1H, *J*=7.3 Hz, ArH), 6.96 (d, 1H, *J*=8.1 Hz, ArH), 7.19–7.23 (m, 3H, ArH), 7.63–7.88 (m, 3H, ArH), 10.80 (br s, 1H, OH), 12.69 (br s, 1H, NH), 13.54 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 64.0, 99.5, 115.4, 115.6, 116.1, 119.2, 119.3, 127.7, 127.8, 129.0, 129.1, 136.5, 153.0, 153.1, 154.9, 160.5, 162.9. Anal. Calcd for $C_{17}H_{13}FN_6O_2$: C, 57.95; H, 3.72; N, 23.85. Found: C, 57.92; H, 3.69; N, 23.76.

2-[4-((1*H*-Tetrazol-5-yl)methoxy)-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-4-chlorophenol (8b)

This compound was prepared from **7b**; a yellow solid; conventional method time 315 min, yield 75%; ultrasound-assisted method time 45 min, yield 93%; mp 228–230°C; IR: υ 3905 (OH), 3387 (NH), 1852 (C=N), 1216 (C-F) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.08 (s, 2H, OCH_2), 6.97 (d, 1H, *J*=8.4 Hz, ArH), 7.24–7.30 (m, 3H, ArH), 7.58–7.89 (m, 3H, ArH), 10.72 (br s, 1H, OH), 12.86 (s, 1H, NH), 13.68 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 64.3, 99.5, 115.7, 117.3, 117.6, 123.0, 126.5, 126.6, 127.7, 127.8, 128.5, 136.7, 153.4, 153.7, 153.8, 160.6, 163.0. Anal. Calcd for $C_{17}H_{12}ClFN_6O_2$: C, 52.79; H, 3.13; N, 21.73. Found: C, 52.63; H, 3.10; N, 21.68.

2-[4-((1*H*-Tetrazol-5-yl)methoxy)-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-6-methylphenol (8c)

This compound was prepared from **7c**; a yellow solid; conventional method time 350 min, yield 77%; ultrasound-assisted method time 54 min, yield 84%; mp 250–252°C; IR: υ 3918 (OH), 3346 (NH), 1815 (C=N), 1245 (C-F) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.57 (s, 3H, CH_3), 5.14 (s, 2H, OCH_2), 6.91–6.98 (m, 2H, ArH), 7.36 (t, 1H, *J*=7.7 Hz, ArH), 7.59 (d, 1H, *J*=7.7 Hz, ArH), 7.65–7.69 (m, 3H, ArH), 10.79 (br s, 1H, OH), 12.80 (s, 1H, NH), 13.71 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 15.8, 63.9, 99.8, 115.1, 115.9, 116.2, 119.4, 119.5, 127.6, 127.7, 129.5, 129.7, 137.0, 153.4, 153.6, 155.3, 160.7, 163.1. Anal. Calcd for $C_{18}H_{15}FN_6O_2$: C, 59.01; H, 4.13; N, 22.94. Found: C, 59.13; H, 3.98; N, 22.81.

2-[4-((1*H*-Tetrazol-5-yl)methoxy]-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-4-methylphenol (8d)

This compound was prepared from **7d**; a yellow solid; conventional method time 320 min, yield 79%; ultrasound-assisted method time 48 min, yield 91%; mp 240–242°C; IR: υ 3955 (OH), 3346 (NH), 1865 (C=N), 1258 (C-F) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.52 (s, 3H, CH_3), 5.19 (s, 2H, OCH_2), 6.35–6.42 (m, 2H, ArH), 6.92–6.94 (m, 2H, ArH), 7.64–7.89 (m, 3H, ArH), 10.84 (br s, 1H, OH), 12.78 (br s, 1H, NH), 13.73 (br s, 1H, NH); ^{13}C NMR (DMSO-*d*): δ 16.0, 64.2, 99.6, 115.5, 115.7, 116.5, 119.8, 119.9, 127.6, 127.7, 129.7, 129.8, 136.6, 154.2, 154.9, 155.8, 161.1, 163.6. Anal. Calcd for $C_{18}H_{15}FN_6O_2$: C, 59.01; H, 4.13; N, 22.94. Found: C, 58.96; H, 4.27; N, 22.98.

2-[4-((1*H*-Tetrazol-5-yl)methoxy]-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-4,6-dichlorophenol (8e)

This compound was prepared from **7e**; a yellow solid; conventional method time 330 min,

yield 71%; ultrasound-assisted method time 45 min, yield 95%; mp 225–227°C; IR: υ 3936 (OH), 3386 (NH), 1855 (C=N), 1282 (C-F) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.15 (s, 2H, OCH_2), 6.42 (t, 2H, *J*=8.8 Hz, ArH), 6.91 (d, 1H, *J*=2.6 Hz, ArH), 7.69 (d, 1H, *J*=2.6 Hz, ArH), 7.90 (dd, 2H, *J*=8.8, 5.2 Hz, ArH), 10.81 (br s, 1H, OH), 12.73 (br s, 1H, NH), 13.82 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 64.6, 99.9, 115.3, 115.4, 116.8, 120.1, 120.2, 127.9, 128.0, 129.4, 129.6, 137.3, 154.7, 155.0, 156.2, 161.3, 163.8. Anal. Calcd for $C_{17}H_{11}Cl_2FN_6O_2$: C, 48.47; H, 2.63; N, 19.95. Found: C, 48.55; H, 2.61; N, 19.98.

2-[4-((1*H*-Tetrazol-5-yl)methoxy]-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-4-chloro-5-methylphenol (8f)

This compound was prepared from **7f**; a yellow solid; conventional method time 330 min, yield 72%; ultrasound-assisted method time 45 min, yield 92%; mp 278–280°C; IR: υ 3945 (OH), 3335 (NH), 1852 (C=N), 1273 (C-F) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.52 (s, 3H, CH_3), 5.19 (s, 2H, OCH_2), 6.52 (s, 1H, ArH), 6.89 (s, 2H, ArH), 7.72–7.75 (m, 2H, ArH), 7.93 (s, 1H, ArH), 10.86 (br s, 1H, OH), 12.71 (br s, 1H, NH), 13.88 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 16.4, 64.8, 99.4, 115.9, 116.0, 116.8, 119.6, 119.7, 127.9, 128.0, 129.5, 129.7, 136.8, 154.4, 155.1, 155.9, 160.8, 163.5. Anal. Calcd for $C_{18}H_{14}ClFN_6O_2$: C, 53.94; H, 3.52; N, 20.97. Found: C, 53.96; H, 3.50; N, 21.01.

General procedure for synthesis of compounds 9a–f

Conventional method

To a mixture of compound **7a–f** (1 mmol) and KOH (1.5 mmol) in ethanol (10 mL), thiourea (1.5 mmol) was added and the mixture was heated for 3–4 h. After completion of the reaction, as monitored by TLC, the reaction mass was cooled to room temperature, poured on crushed ice and acidified with concentrated HCl. The resultant solid was filtered and crystallized from ethanol.

Ultrasound-assisted method

To a mixture of compound **7a–f** (1 mmol) and KOH (1.5 mmol) in ethanol (10 mL), thiourea (1.5 mmol) was added and the reaction mass was ultrasonicated for 15–25 min at 65°C. After completion of the reaction, as monitored by TLC, the mixture was cooled to room temperature, poured on crushed ice and acidified with concentrated HCl. The resultant precipitate was filtered and crystallized from ethanol.

5-[(1*H*-Tetrazol-5-yl)methoxy]-6-(4-fluorophenyl)-4-(2-hydroxyphenyl)pyrimidine-2(1*H*)-thione (9a)

This compound was prepared from **7a**; a yellow solid; conventional method time 230 min, yield 73%; ultrasound-assisted method time 21 min, yield 87%; mp 163–165°C; IR υ: 3919 (OH), 3423 (NH), 3313 (NH), 3040 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.79 (s, 2H, OCH_2), 7.19 (t, 2H, *J*=8.8 Hz, ArH), 7.26–7.30 (m, 2H, ArH), 7.43–7.46 (m, 1H, ArH), 7.76 (m, 1H, ArH), 7.97 (dd, 2H, *J*=5.5, 8.8 Hz, ArH), 10.10 (br s, 1H, OH), 10.81 (br s, 1H, NH), 11.19 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 74.5, 115.2, 115.4, 116.5, 117.4, 119.1, 120.0, 120.9, 129.5, 130.1, 130.5, 131.7, 135.5, 155.8, 157.9, 159.6, 161.4, 198.7. Anal. Calcd for $C_{18}H_{13}FN_6O_2S$: C, 54.54; H, 3.31; N, 21.20; S, 8.09. Found: C, 54.47; H, 3.45; N, 21.06; S, 8.22.

5-[(1*H*-Tetrazol-5-yl)methoxy]-4-(5-chloro-2-hydroxyphenyl)-6-(4-fluorophenyl)pyrimidine-2(1*H*)-thione (9b)

This compound was prepared from **7b**; a yellow solid; conventional method time 220 min, yield 80%; ultrasound-assisted method time 15 min, yield 95%; mp 148–150°C; IR: υ 3975 (OH), 3424 (NH), 3383 (NH), 3067 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.81 (s, 2H, OCH_2), 6.90 (d, 1H, *J*=8.8 Hz, ArH), 7.19 (t, 2H, *J*=8.8 Hz, ArH), 7.25 (dd, 1H, *J*=8.8, 2.6 Hz, ArH), 7.32 (d, 1H, *J*=2.6 Hz, ArH), 8.00 (m, 2H, ArH), 10.10 (br s, 1H, OH), 10.81 (br s, 1H, NH), 11.19 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 63.5, 115.4, 115.6, 117.9, 122.5, 122.8, 129.4, 130.4, 130.9, 131.6, 131.7, 146.4, 157.9, 159.6, 161.7, 162.2, 164.7, 190.6. Anal. Calcd for $C_{18}H_{12}ClFN_6O_2S$: C, 50.18; H, 2.81; N, 19.51; S, 7.44. Found: C, 50.25; H, 2.87; N, 19.49; S, 7.48.

5-[(1*H*-Tetrazol-5-yl)methoxy]-6-(4-fluorophenyl)-4-(2-hydroxy-3-methylphenyl)pyrimidine-2(1*H*)-thione (9c)

This compound was prepared from **7c**; a yellow solid; conventional method time 220 min, yield 74%; ultrasound-assisted method time 24 min, yield 82%; mp 215–217°C; IR: υ 3935 (OH), 3475 (NH), 3364 (NH), 3057 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 4.85 (s, 2H, OCH_2), 6.95 (s, 1H, ArH), 7.18–7.20 (m, 2H, ArH), 7.69 (s, 1H, ArH), 7.92–7.96 (m, 3H, ArH), 10.16 (br s, 1H, OH), 10.87 (br s, 1H, NH), 11.21 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 15.8, 68.7, 115.7, 115.8, 118.1, 122.8, 122.9, 129.0, 130.2, 131.3, 131.8, 132.2, 146.9, 158.2, 159.3, 161.5, 161.9, 165.0, 195.2. Anal. Calcd for $C_{19}H_{15}FN_6O_2S$: C, 55.60; H, 3.68; N, 20.48; S, 7.81. Found: C, 55.57; H, 3.72; N, 20.39; S, 7.76.

5-[(1*H*-Tetrazol-5-yl)methoxy]-6-(4-fluorophenyl)-4-(2-hydroxy-5-methylphenyl)pyrimidine-2(1*H*)-thione (9d)

This compound was prepared from **7d**; a yellow solid; conventional method time 235 min, yield 75%; ultrasound-assisted method time 24 min, yield 94%; mp 314–316°C; IR: υ 3954 (OH), 3424 (NH), 3364 (NH), 3095 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 4.87 (s, 2H, OCH_2), 6.97 (t, 2H, *J*=8.8 Hz, ArH), 7.17–7.23 (m, 2H, ArH), 7.74 (s, 1H, ArH), 7.90 (dd, 2H, *J*=8.8, 5.5 Hz, ArH), 10.11 (br s, 1H, OH), 10.82 (br s, 1H, NH), 11.19 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 16.3, 68.4, 115.9, 116.0, 118.5, 123.1, 123.4, 128.6, 129.8, 131.2, 131.5, 132.6, 147.7, 158.4, 160.2, 161.6, 161.8, 165.3, 194.9. Anal. Calcd for $C_{19}H_{15}FN_6O_2S$: C, 55.60; H, 3.68; N, 20.48; S, 7.81. Found: C, 55.65; H, 3.61; N, 20.55; S, 7.74.

5-[(1*H*-Tetrazol-5-yl)methoxy]-4-(3,5-dichloro-2-hydroxyphenyl)-6-(4-fluorophenyl)pyrimidine-2(1*H*)-thione (9e)

This compound was prepared from **7e**; a yellow solid; conventional method time 195 min, yield 78%; ultrasound-assisted method time 18 min, yield 91%; mp 183–185°C; IR: υ 3935 (OH), 3453 (NH), 3390 (NH), 3054 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.83 (s, 2H, OCH_2), 6.24 (t, 2H, *J*=8.5 Hz, ArH), 7.37–7.39 (m, 1H, ArH), 7.78 (d, 2H, *J*=5.5 Hz, ArH), 7.92 (dd, 1H, *J*=8.5, 5.5 Hz, ArH), 10.09 (br s, 1H, OH), 10.87 (br s, 1H, NH), 11.24 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 67.1, 115.6, 115.9, 116.8, 117.7, 119.5, 120.2, 121.0, 129.8, 130.2, 130.7, 131.2, 136.1, 156.4, 157.5, 159.9, 162.0, 193.6. Anal. Calcd for $C_{18}H_{11}Cl_2FN_6O_2S$: C, 46.46; H, 2.38; N, 18.06; S, 6.89. Found: C, 46.40; H, 2.48; N, 18.01; S, 6.97.

5-[(1*H*-Tetrazol-5-yl)methoxy]-4-(5-chloro-2-hydroxy-4-methylphenyl)-6-(4-fluorophenyl)pyrimidine-2(1*H*)-thione (9f)

This compound was prepared from **7f**; a yellow solid; conventional method time 210 min, yield 76%; ultrasound-assisted method time 21 min, yield 92%; mp 277–279°C; IR: υ 3954 (OH), 3473 (NH), 3323 (NH), 3036 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 4.86 (s, 2H, OCH_2), 6.18 (t, 2H, *J*=8.8 Hz, ArH), 7.67–7.70 (m, 2H, ArH), 7.98–8.01 (m, 2H, ArH), 10.15 (br s, 1H, OH), 10.91 (br s, 1H, NH), 11.32 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 17.3, 67.5, 115.9, 116.0, 116.9, 117.3, 119.6, 120.6, 121.4, 130.3, 130.7, 131.5, 131.9, 137.4, 155.6, 157.2, 160.4, 162.6, 194.2. Anal. Calcd for $C_{19}H_{14}ClFN_6O_2S$: C, 51.30; H, 3.17; N, 18.89; S, 7.21. Found: C, 51.38; H, 3.22; N, 18.91; S, 7.13.

Antimicrobial activity

Antibacterial activity of the synthesized compounds was tested *in vitro* against Gram-positive bacteria *S. aureus* (NCIM 2079) and *B. subtilis* (NCIM 2920) and Gram-negative bacteria *E. coli* (NCIM 2065) and *P. aeruginosa* (NCIM 2200). The compounds were also screened for antifungal activity against *C. albicans* (NCIM 3471) and *A. niger* (NCIM 596). Compounds were diluted in dimethyl sulfoxide (DMSO) with 1 µg/mL concentrations for bioassay. Micro-broth

dilution method [28] was used to determine the MICs of compounds in 96-well microtiter plates. Test compounds were serially diluted in growth media. Plates were incubated at 30°C for fungi and 37°C for bacteria for 24 h. All experiments were carried out in triplicates.

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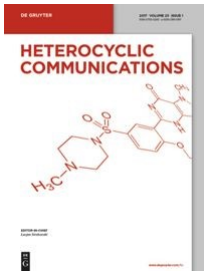

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