Ultrasound-Mediated Synthesis of Novel 1,2,3-Triazole-Based Pyrazole and Pyrimidine Derivatives as Antimicrobial Agents

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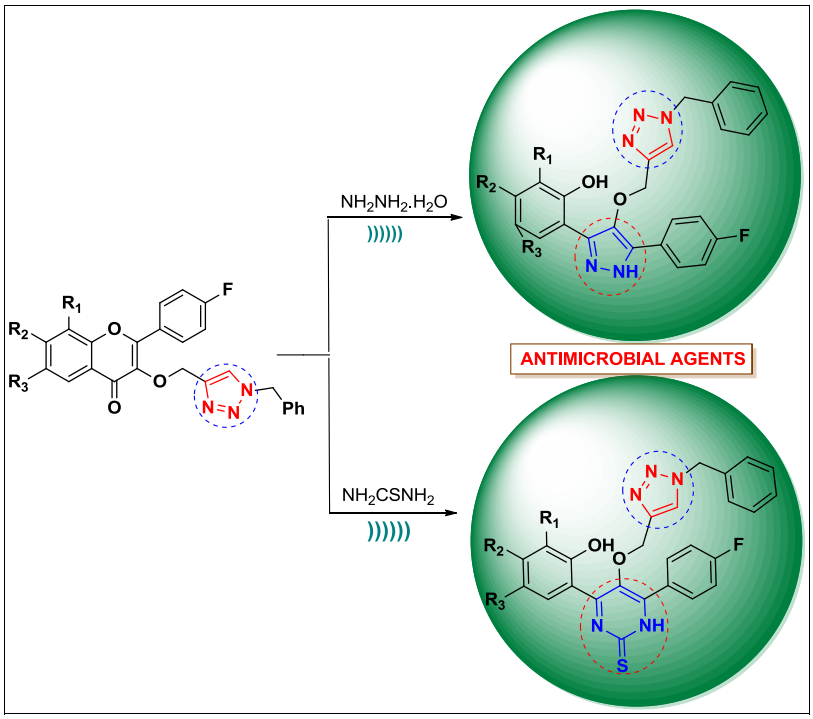


In the development of novel antimicrobial agents, we synthesized novel 1,2,3-triazole-based pyrazole and pyrimidine derivatives **6(a–f)** and **7(a–f)** by ultrasound-assisted method. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. All compounds were assessed *in vitro* for their efficacy as antimicrobial agents against four bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and two fungi (*Candida albicans* and *Aspergillus niger*). In particular, compounds **6a**, **6e**, **7a**, **7c**, and **7e** exhibited highly potent antimicrobial activity.

INTRODUCTION

Microbial infections are major problems around the world. There is a need for development of new antimicrobial agents which will be more selective, potent, and less toxic compared to the existing drugs to treat these life-threatening invasive infections [1].

Heterocycles containing an azole ring system are found to exhibit a wide spectrum of biological activities, including antibacterial and antifungal properties. Pyrazole derivatives have showed significant biological activities, such as antimicrobial [2], antianalgesic [3], anti-inflammatory [4], and anticancer activities [5]. This gave a great force to the search for potential pharmacologically active drugs carrying pyrazole substituents. Triazole nucleus is an important pharmacophore that appears extensively in various types of pharmaceutical agents and bioactive molecules, such as anti-HIV [6], antitubercular [7], anti-inflammatory, anticancer [8], antimicrobial [9], anticonvulsant [10], antimalarial [11], analgesic [12], antiviral [13], antidiabetic [14], and antimycobacterial [15]. The biological activity of these compounds is enhanced probably due to their high aromatic stabilization, high dipole moment, and high bonding capacity. On the other hand, pyrimidines occupy a distinct and unique place in medicinal chemistry due to their presence in several biologically active compounds [16]. Pyrimidine derivatives are of interest due to their pharmacological

properties such as antitumor [17], antifilarial [18], antiviral [19], antifungal [20], antibacterial [21], anti-inflammatory [22], analgesic [23], anti-HIV [24], antiprotozoal [25], antihypertensive [26] activity.

A survey of literatures showed that ultrasound-accelerated chemical reactions have attracted much attention during the past few years. Ultrasound irradiation has been considered as a green and efficient technique used as an alternative energy source for organic reactions [27,28]. Keeping in view the importance of greener protocol, we designed and synthesized 1,2,3-triazole-based pyrazole and pyrimidine derivatives under ultrasound irradiation and evaluated for antimicrobial activity.

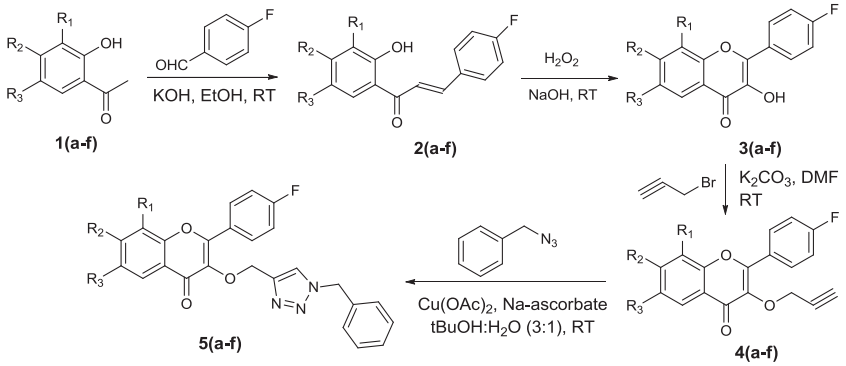
RESULTS AND DISCUSSION

Chemistry. The key starting material, 3-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-fluorophenyl)-4*H*-chromen-4-one **5(a–f)**, was synthesized in our previous report [29] (Scheme 1). We have synthesized novel triazole-based pyrazole derivatives **6(a–f)** and triazole-based pyrimidine derivatives **7(a–f)** under conventional method as well as ultrasound irradiation method.

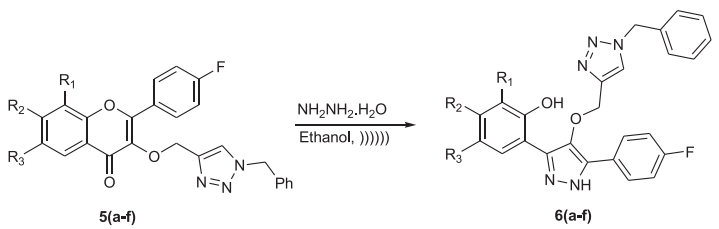
The compounds **5(a–f)** have been treated with hydrazine hydrate in ethanol at reflux condition to furnish the desired product **6(a–f)** (Scheme 2). The compounds **5(a–f)** have been treated with thiourea in ethanol by using KOH at reflux condition to furnish the desired product **7(a–f)** (Scheme 3). Moreover, these reactions were performed under ultrasound irradiation to afford substituted 2-(4-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl)phenol **6(a–f)** and 5-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(4-fluorophenyl)-4-(2-hydroxyphenyl)pyrimidine-2(1*H*)-thione **7(a–f)**.

The synthesized compounds **6a** and **7a** were confirmed by IR, MS, ^{1}H NMR, ^{13}C NMR, and elemental analysis. In the mass spectrum of **6a**, the $[M + H]^+$ observed at 442.1632. In the ^{1}H NMR spectrum of compound **6a**, the two methylene groups attached to nitrogen and oxygen showed singlets at δ 4.81 and 5.51 ppm, respectively, and proton of triazole ring showed singlet at δ 7.95 ppm. In addition to this, hydroxy proton showed singlet at δ 10.91 ppm and the pyrazole proton showed singlet at δ 13.44 ppm. In the ^{13}C NMR spectrum of compound **6a**, the signal at 52.8 and 66.3 ppm indicates the presence of methylene carbon attached to the nitrogen of the triazole ring and oxygen to the phenyl ring, respectively. In the mass spectrum of **7a**, the $[M + H]^+$ observed at 486.53. In the ^{1}H NMR spectrum of compound **7a**, the two methylene groups attached to nitrogen and oxygen showed singlets at δ 4.66 and 5.38 ppm, respectively, and proton of triazole ring showed singlet at δ 7.99 ppm. In addition to this, hydroxy proton showed singlet at δ 10.11 ppm and the pyrazole proton showed singlet at δ 11.68 ppm. In the ^{13}C NMR spectrum of compound **7a**, the signal at 52.7 and 63.6 ppm indicates

Scheme 1. Synthesis of triazole derivatives **5(a–f)**.



Scheme 2. Synthesis of triazole-based pyrazole derivatives **6(a–f)**.



Scheme 3. Synthesis of triazole-based pyrimidine derivatives **7(a–f)**.

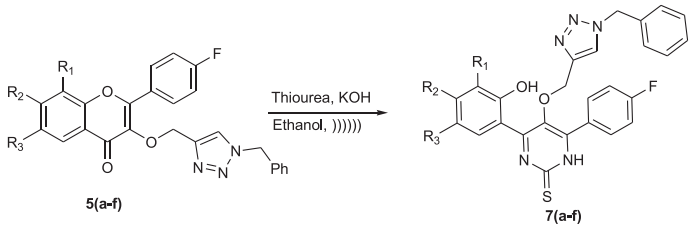


Table 1

Ultrasound-promoted synthesis of pyrazole and pyrimidine derivatives **6(a–f)** and **7(a–f)**.

					Conventional method		Ultrasound method	
Comp.	R_1	R_2	R_3	M. P. (°C)	Yield[a] (%)	Time (min)	Yield[a] (%)	Time (min)
6a	H	H	H	186–188	60	360	85	51
6b	H	H	Cl	180–182	65	310	86	48
6c	CH_3	H	H	208–210	61	320	89	54
6d	H	H	CH_3	152–154	69	330	92	54
6e	Cl	H	Cl	182–184	72	290	96	43
6f	H	CH_3	Cl	187–189	71	310	90	45
7a	H	H	H	170–172	69	225	89	21
7b	H	H	Cl	288–290	71	195	91	18
7c	CH_3	H	H	105–107	68	215	87	27
7d	H	H	CH_3	85–87	72	210	94	24
7e	Cl	H	Cl	238–240	77	190	93	15
7f	H	CH_3	Cl	200–202	79	205	91	18

[a]Isolated yields.

the presence of methylene carbon attached to the nitrogen of the triazole ring and oxygen to the phenyl ring, respectively. Furthermore, the signal at 199.8 ppm indicates the presence of pyrimidine carbon. Similarly, all other derivatives **6(b–f)** and **7(b–f)** was satisfactory in good conformity with the spectroscopic properties (Table 1).

Antimicrobial activity. In the screening assay studies, all the compounds were evaluated for antibacterial activity against Gram-positive bacteria viz. *Staphylococcus aureus* (NCIM 2079) and *Bacillus subtilis* (NCIM 2920) and Gram-negative bacteria *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200) and antifungal activity against *Candida albicans* (NCIM 3471) and *Aspergillus niger* (NCIM 596) (Table 2). The minimum inhibitory concentration (MIC) values were determined by microbroth dilution method. The result of antibacterial screening was compared with the standard antibacterial drug Chloramphenicol, and antibacterial screening was compared with the standard antibacterial drug Clotrimazole. Dimethylsulfoxide (DMSO) was used as solvent control.

From the antimicrobial screening of newly synthesized compounds, compound **7f** showed equipotent activity against *S. aureus* and compounds **6d** and **7a** showed equipotent activity against *E. coli* and compounds **6a** and **7f** showed equipotent activity against *P. aeruginosa* compared with Chloramphenicol MIC 50 μg/mL.

Table 2

Antimicrobial screening data of the compounds **6(a–f)** and **7(a–f)**.

	Antimicrobial activity[a] (μg/mL)					
	Antibacterial activity				Antifungal activity	
Entry	*Sa*	*Bs*	*Ec*	*Pa*	*Ca*	*An*
6a	12.5	12.5	25	50	25	50
6b	200	100	75	200	50	150
6c	25	125	100	100	75	125
6d	25	50	50	100	75	50
6e	25	50	75	75	50	12.5
6f	150	175	75	125	50	25
7a	25	25	50	75	25	50
7b	100	100	175	150	75	125
7c	12.5	50	25	75	50	50
7d	100	125	150	175	75	100
7e	75	12.5	25	75	50	25
7f	50	50	75	50	75	25
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*.
[a]Minimum inhibitory concentration (μg/mL) against the pathological strains based on microbroth dilution method.

Compound **7a** showed equipotent activity against *B. subtilis* compared with Chloramphenicol MIC 25 μg/mL. Compound **6a** has no substituent, and **7c** has *methyl*

group with MIC 12.5 μg/mL and showed potent activity against *S. aureus*. Compounds **6a** and **7e** with MIC 12.5 μg/mL exhibited potent activity against *B. subtilis*. Compounds **6a**, **7c**, and **7e** with MIC 25 μg/mL exhibited potent activity against *E. coli*.

Compounds **6b**, **6e**, **7c**, and **7e** showed equipotent activity with Clotrimazole MIC 50 μg/mL against *C. albicans*. Compounds **6f**, **7e**, and **7f** showed equipotent activity with Clotrimazole MIC 25 μg/mL against *A. niger*. Compounds **6a** and **7a** has no substituent and showed potent activity with MIC 25 μg/mL against *C. albicans*. Compound **6e** has *dichloro* substituent and exhibited highly potent antifungal activity against *A. niger* with MIC 12.5 μg/mL.

CONCLUSIONS

Ultrasonic irradiation was used to promote the synthesis of triazole-based pyrazole and pyrimidine derivatives **6** (**a**–**f**) and **7**(**a**–**f**) in good yields with more purity in short reaction time as compared to the conventional method. The synthesized compounds were evaluated for antimicrobial activity. It can be concluded that the compounds **6a**, **6e**, **7a**, **7c**, and **7e** were identified as highly potent antimicrobial agents.

EXPERIMENTAL

Material and methods. All the solvents and reagents used in synthesis were obtained from commercial sources and were used without further purification. Melting points were recorded by the open tube capillary method and are uncorrected. The purity of compounds and completion of the reaction was checked by thin-layer chromatography (TLC). Infrared spectra were recorded on Carry 600 Series (Agilent, Santa Clara, CA) FT-IR spectrophotometer by using KBr pellets. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Bruker AVANCE II 400 NMR spectrometer (Japan) in $CDCl_3$/ DMSO-d_6 solution. Tetramethylsilane was used as an internal standard. Chemical shift values are given in ppm relative to TMS as internal reference and the coupling constant (*J*) in hertz. The splitting pattern abbreviations are assigned as singlet (s), doublet (d), triplet (t), broad singlet (brs), doublet of doublets (dd), and multiplet (m). Mass spectra were recorded on a WATERS, Q-TOF micro mass (Milford, MA, United States) equipped with an Electron Spin Impact (ESI) Source. Elemental analysis was performed on a Perkin-Elmer EAL-240 elemental analyzer (Germany). For ultrasonic irradiation, Bandelin Sonorex (Berlin, Germany) (frequency 40 MHz, power 100 W) ultrasound bath was used, and the reaction flask was located in the ultrasonic bath containing water.

General procedure for the synthesis of 2-(4-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl)phenols 6(a–f). ***Conventional method.*** Hydrazine hydrate (1.0 mmol) was added to a solution of 3-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-fluorophenyl)-4*H*-chromen-4-ones **5(a–f)** (1.0 mmol) in ethanol (10 mL). The reaction mixture was heated for 5–6 h. After completion of the reaction (monitored by TLC), 10 mL cold water was added and the product was filtered, dried under vacuum, and crystallized from ethanol.

Non conventional method. Hydrazine hydrate (1.0 mmol) was added to a solution of 3-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-fluorophenyl)-4*H*-chromen-4-ones **5(a–f)** (1 mmol) in ethanol (10 mL). The reaction mixture was irradiated under ultrasonication for 40–55 min at 65°C. After completion of reaction (monitored by TLC), 10 mL cold water was added and the product was filtered, dried under vacuum, and crystallized from ethanol.

2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-5-(4-fluorophenyl)-1H-pyrazol-3-yl)phenol (6a). IR (KBr, cm^{-1}): 3655, 3126, 3036. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 4.81 (s, 2H, NCH_2), 5.51 (s, 2H, OCH_2), 6.88–6.94 (m, 2H, ArH), 6.99 (d, 1H, *J* = 8.1 Hz, ArH), 7.16–7.22 (m, 4H, ArH), 7.27 (t, 1H, *J* = 9.0 Hz, ArH), 7.31–7.33 (m, 2H, ArH), 7.35 (s, 1H, ArH), 7.85–7.89 (m, 1H, ArH), 7.95 (s, 1H, triazole-H), 8.04–8.05 (m, 1H, ArH), 10.91 (s, 1H, OH), 13.44 (brs, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 52.8, 66.3, 115.2, 115.6, 115.9, 116.3, 119.1, 124.3, 124.7, 126.5, 127.7, 128.0, 128.6, 128.7, 129.0, 129.1, 129.1, 129.2, 131.9, 135.6, 142.2, 154.3, 155.3, 160.6, 163.1. LCMS *Anal*. Calcd. for $C_{25}H_{20}FN_5O_2$ $[M + H]^+$: 442.1679. Found: 442.1632. *Anal*. calcd. for $C_{25}H_{20}FN_5O_2$: C, 68.02; H, 4.57; N, 15.86. Found: C, 68.08; H, 4.47; N, 15.82.

2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-5-(4-fluorophenyl)-1H-pyrazol-3-yl)-4-chlorophenol (6b). IR (KBr, cm^{-1}): 3680, 3136, 3087. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 4.85 (s, 2H, NCH_2), 5.49 (s, 2H, OCH_2), 6.89 (d, 1H, *J* = 8.4 Hz, ArH), 7.13 (dd, 2H, *J* = 2.4, 8.6 Hz, ArH), 7.19–7.23 (m, 3H, ArH), 7.29–7.32 (m, 3H, ArH), 7.86 (s, 2H, ArH), 7.92 (s, 1H, triazole-H), 7.97 (s, 1H, ArH), 10.95 (s, 1H, OH), 13.51 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 53.0, 66.4, 115.6, 115.8, 117.6, 117.7, 123.0, 124.1, 125.6, 127.7, 128.0, 128.1, 128.5, 132.0, 132.3, 135.2, 135.3, 136.5, 136.6, 140.2, 142.1, 153.9, 154.0, 160.7, 163.2. ESI-MS *Anal*. Calcd. for $C_{25}H_{19}ClFN_5O_2$ $[M + H]^+$: 476.13. Found: 476.06. *Anal*. calcd. for $C_{25}H_{19}ClFN_5O_2$: C, 63.09; H, 4.02; N, 14.72. Found: C, 63.01; H, 3.91; N, 14.70.

2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-5-(4-fluorophenyl)-1H-pyrazol-3-yl)-6-methylphenol (6c). IR (KBr, cm^{-1}): 3629, 3139, 3032. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 2.51 (s, 3H, CH_3), 4.85 (s, 2H, NCH_2), 5.48 (s, 2H, OCH_2), 6.80–6.84 (m, 3H, ArH), 7.18–7.24 (m, 3H, ArH), 7.26–7.29 (m, 3H, ArH), 7.86–7.88 (m, 2H, ArH), 7.91 (s, 1H,

triazole-H), 7.96–7.98 (m, 1H, ArH), 10.94 (s, 1H, OH), 13.49 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 15.3, 52.9, 66.3, 115.5, 115.9, 117.4, 117.8, 122.7, 123.8, 124.9, 127.5, 128.0, 128.6, 128.9, 132.3, 132.6, 134.6, 135.4, 136.3, 136.7, 139.6, 141.6, 154.7, 154.9, 161.3, 163.6. ESI-MS *Anal*. Calcd. for $C_{26}H_{22}FN_5O_2$ $[M + H]^+$: 456.18. Found: 456.22. *Anal*. calcd. for $C_{26}H_{22}FN_5O_2$: C, 68.56; H, 4.87; N, 15.38. Found: C, 68.65; H, 4.97; N, 15.32.

2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-5-(4-fluorophenyl)-1H-pyrazol-3-yl)-4-methylphenol (6d). IR (KBr, cm^{-1}): 3628, 3136, 3032. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 2.47 (s, 3H, CH_3), 4.85 (s, 2H, NCH_2), 5.50 (s, 2H, OCH_2), 6.81–6.83 (m, 2H, ArH), 7.20–7.23 (m, 3H, ArH), 7.26–7.31 (m, 4H, ArH), 7.88–7.89 (m, 1H, ArH), 7.91 (s, 1H, triazole-H), 7.94–7.97 (m, 2H, ArH), 10.92 (s, 1H, OH), 13.51 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 20.4, 52.8, 66.1, 115.5, 115.9, 117.6, 117.9, 123.0, 123.4, 125.2, 127.9, 128.4, 128.7, 129.3, 131.9, 132.4, 134.7, 135.0, 135.8, 136.5, 139.2, 140.9, 154.2, 155.1, 161.2, 163.8. ESI-MS *Anal*. Calcd. for $C_{26}H_{22}FN_5O_2$ $[M + H]^+$: 456.18. Found: 456.14. *Anal*. calcd. for $C_{26}H_{22}FN_5O_2$: C, 68.56; H, 4.87; N, 15.38. Found: C, 68.53; H, 4.76; N, 15.35.

2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-5-(4-fluorophenyl)-1H-pyrazol-3-yl)-4,6-dichlorophenol (6e). IR (KBr, cm^{-1}): 3629, 3136, 3064. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 4.83 (s, 2H, NCH_2), 5.45 (s, 2H, OCH_2), 7.18 (d, 2H, J = 2.4 Hz, ArH), 7.21–7.25 (m, 2H, ArH), 7.28–7.31 (m, 3H, ArH), 7.83–7.86 (m, 2H, ArH), 7.90 (s, 1H, triazole-H), 7.92–7.94 (m, 2H, ArH), 10.91 (s, 1H, OH), 13.55 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 53.3, 66.5, 115.3, 115.9, 117.7, 117.9, 123.2, 123.3, 126.4, 127.9, 128.2, 128.6, 129.0, 131.8, 132.4, 135.2, 135.6, 136.1, 136.3, 139.7, 142.3, 153.6, 154.4, 160.4, 163.7. ESI-MS *Anal*. Calcd. for $C_{25}H_{18}Cl_2FN_5O_2$ $[M + H]^+$: 510.09. Found: 510.12. *Anal*. calcd. for $C_{25}H_{18}Cl_2FN_5O_2$: C, 58.84; H, 3.56; N, 13.72. Found: C, 58.76; H, 3.64; N, 13.85.

2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-5-(4-fluorophenyl)-1H-pyrazol-3-yl)-4-chloro-5-methylphenol (6f). IR (KBr, cm^{-1}): 3680, 3136, 3087. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 2.46 (s, 3H, CH_3), 4.82 (s, 2H, NCH_2), 5.47 (s, 2H, OCH_2), 6.83 (s, 1H, ArH), 7.15–7.18 (m, 2H, ArH), 7.21–7.25 (m, 3H, ArH), 7.30–7.34 (m, 4H, ArH), 7.96 (s, 1H, triazole-H), 8.16 (s, 1H, ArH), 10.94 (s, 1H, OH), 13.50 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 20.3, 53.1, 66.5, 115.4, 115.6, 117.7, 117.8, 123.3, 124.5, 124.9, 127.5, 127.9, 128.4, 128.6, 131.9, 132.2, 135.4, 135.7, 137.0, 137.3, 140.3, 142.5, 152.9, 154.6, 160.8, 163.5. ESI-MS *Anal*. Calcd. for $C_{26}H_{21}ClFN_5O_2$ $[M + H]^+$: 490.14. Found: 490.07. *Anal*. calcd. for $C_{26}H_{21}ClFN_5O_2$: C, 63.74; H, 4.32; N, 14.29. Found: C, 63.62; H, 4.27; N, 14.25.

General procedure for the synthesis of 5-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(4-fluorophenyl)-4-(2-hydroxyphenyl) pyrimidine-2(1*H*)-thiones 7(a–f). ***Conventional method.*** Thiourea (1.5 mmol) was added to a mixture of substituted 3-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-fluorophenyl)-4*H*-chromen-4-ones **5(a–f)** (1.0 mmol) and KOH (1.5 mmol) in ethanol (10 mL), and the reaction mixture was heated for 3–4 h. After completion of the reaction (monitored by TLC), reaction mass was cooled to the room temperature and poured on crushed ice, and acidified with Conc. HCl to get yellow solid. The solid was filtered off and crystallized from ethanol.

Non conventional method. Thiourea (1.5 mmol) was added To a mixture of substituted 3-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(4-fluorophenyl)-4*H*-chromen-4-ones **5(a–f)** (1.0 mmol) and KOH (1.5 mmol) in ethanol (10 mL), and the reaction mass was irradiated under ultrasonication at 65°C. After completion of the reaction (monitored by TLC), reaction mass was cooled to the room temperature and poured on crushed ice, and acidified with Conc. HCl to get yellow solid. The solid was filtered off and crystallized from ethanol.

5-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-6-(4-fluorophenyl)-4-(2-hydroxyphenyl)pyrimidine-2(1H)-thione (7a). IR (KBr, cm^{-1}): 3914, 3660, 3070, 1603. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 4.66 (s, 2H, NCH_2), 5.38 (s, 2H, OCH_2), 6.96–7.01 (m, 9H, ArH), 7.54 (dd, 1H, J = 1.5, 8.1 Hz, ArH), 7.76 (dd, 3H, J = 5.5, 8.8 Hz, ArH), 7.99 (s, 1H, triazole-H), 10.11 (brs, 1H, OH), 11.68 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 52.7, 63.6, 115.3, 115.5, 117.5, 118.9, 119.2, 120.1, 121.7, 124.3, 124.4, 127.9, 127.9, 128.1, 128.2, 128.7, 129.7, 131.7, 135.5, 141.8, 144.0, 146.8, 155.7, 159.6, 160.8, 199.8. ESI-MS *Anal.* Calcd. for $C_{26}H_{20}FN_5O_2S$ $[M + H]^+$: 486.14. Found: 486.53. *Anal.* calcd. for $C_{26}H_{20}FN_5O_2S$: C, 64.32; H, 4.15; N, 14.42; S, 6.60. Found: C, 64.38; H, 4.17; N, 14.47, S, 6.56.

5-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-4-(5-chloro-2-hydroxyphenyl)-6-(4-fluorophenyl)pyrimidine-2(1H)-thione (7b). IR (KBr, cm^{-1}): 3917, 3670, 3071, 1639. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H ppm): 4.54 (s, 2H, NCH_2), 5.38 (s, 2H, OCH_2), 6.87 (d, 1H, ArH), 7.54 (dd, 1H, J = 8.8 Hz, ArH), 7.12 (s, 1H, ArH), 7.14 (s, 1H, ArH), 7.16 (d, 1H, J = 2.2 Hz, ArH), 7.21 (dd, 1H, J = 2.6, 8.8 Hz, ArH), 7.29–7.30 (m, 3H, ArH), 7.41 (s, 1H, ArH), 7.51 (d, 1H, J = 2.6 Hz, ArH), 8.02 (dd, 2H, J = 5.5, 8.8 Hz, ArH), 8.08 (s, 1H, triazole-H), 10.69 (s, 1H, OH), 10.73 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 52.9, 65.9, 115.1, 115.3, 118.2, 122.0, 122.6, 124.0, 127.8, 128.1, 128.5, 129.5, 130.5, 130.6, 131.1, 131.6, 131.7, 134.9, 141.6, 146.3, 155.1, 158.4, 159.3, 161.0, 162.3, 194.7. ESI-MS *Anal*. Calcd. for $C_{26}H_{19}ClFN_5O_2S$ $[M + H]^+$: 520.10. Found: 520.20. *Anal*. calcd. for $C_{26}H_{19}ClFN_5O_2S$: C, 60.06; H, 3.68; N, 13.47; S, 6.17. Found: C, 60.11; H, 3.72; N, 13.42; S, 6.23.

5-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-6-(4-fluorophenyl)-4-(2-hydroxy-3-methylphenyl)pyrimidine-2(1H)-thione (7c). IR (KBr, cm^{-1}): 3981, 3629, 3064, 1625. ^1H NMR (400 MHz, $CDCl_3$, δ_H ppm): 2.56 (s, 3H, CH_3), 4.74 (s, 2H, NCH_2), 5.52 (s, 2H, OCH_2), 6.71–6.76 (m, 3H, ArH), 7.21–7.25 (m, 4H, ArH), 7.32–7.35 (m, 2H, ArH), 7.79–7.82 (m, 2H, ArH), 7.94–7.96 (m, 1H, ArH), 8.02 (s, 1H, triazole-H), 10.87 (s, 1H, OH), 11.57 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 16.1, 53.3, 65.9, 115.7, 115.9, 117.9, 118.0, 123.1, 123.9, 125.9, 127.9, 128.2, 128.9, 129.5, 132.7, 133.1, 134.9, 135.8, 137.1, 137.5, 140.4, 142.2, 155.3, 155.6, 161.6, 164.5, 196.3. ESI-MS *Anal*. Calcd. for $C_{27}H_{22}FN_5O_2S$ $[M + H]^+$: 500.16. Found: 500.11. *Anal*. calcd. for $C_{27}H_{22}FN_5O_2S$: C, 64.91; H, 4.44; N, 14.02; S, 6.42. Found: C, 64.86; H, 4.38; N, 13.96; S, 6.38.

5-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-6-(4-fluorophenyl)-4-(2-hydroxy-5-methylphenyl)pyrimidine-2(1H)-thione (7d). IR (KBr, cm^{-1}): 3905, 3628, 3099, 1618. ^1H NMR (400 MHz, $CDCl_3$, δ_H ppm): 2.55 (s, 3H, CH_3), 4.82 (s, 2H, NCH_2), 5.43 (s, 2H, OCH_2), 6.70–6.74 (m, 3H, ArH), 7.27–7.30 (m, 2H, ArH), 7.35–7.38 (m, 3H, ArH), 7.92–7.98 (m, 2H, ArH), 8.08 (s, 1H, triazole-H), 8.16–8.18 (m, 2H, ArH), 10.95 (s, 1H, OH), 11.62 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 21.1, 52.2, 65.2, 114.8, 115.0, 116.7, 116.9, 123.2, 123.6, 125.9, 128.4, 128.8, 128.9, 129.6, 132.5, 132.8, 134.6, 135.3, 136.3, 136.7, 139.9, 141.6, 153.5, 155.4, 161.8, 164.5, 194.6. ESI-MS *Anal.* Calcd. for $C_{27}H_{22}FN_5O_2S$ $[M + H]^+$: 500.16. Found: 500.09. *Anal*. calcd. for $C_{27}H_{22}FN_5O_2S$: C, 64.91; H, 4.44; N, 14.02; S, 6.42. Found: C, 64.94; H, 4.42; N, 14.01; S, 6.35.

5-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-4-(3,5-dichloro-2-hydroxyphenyl)-6-(4-fluorophenyl)pyrimidine-2(1H)-thione (7e). IR (KBr, cm^{-1}): 3981, 3734, 3069, 1682. ^1H NMR (400 MHz, $CDCl_3$, δ_H ppm): 4.62 (s, 2H, NCH_2), 5.35 (s, 2H, OCH_2), 7.26 (d, 2H, J = 2.4 Hz, ArH), 7.35–7.38 (m, 2H, ArH), 7.49–7.51 (m, 3H, ArH), 7.98–8.01 (m, 2H, ArH), 8.13 (s, 1H, triazole-H), 8.16–8.19 (m, 2H, ArH), 10.74 (s, 1H, OH), 11.23 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 51.4, 68.1, 115.6, 115.7, 118.1, 118.3, 123.7, 123.9, 127.4, 127.7, 128.8, 128.9, 130.1, 132.2, 132.7, 135.7, 136.0, 136.2, 136.9, 140.3, 144.0, 154.5, 154.8, 160.7, 164.2, 191.5. ESI-MS *Anal*. Calcd. for $C_{26}H_{18}Cl_2FN_5O_2S$ $[M + H]^+$: 554.06. Found: 554.12. *Anal*. calcd. for $C_{26}H_{18}Cl_2FN_5O_2S$: C, 56.32; H, 3.27; N, 12.63; S, 5.78. Found: C, 56.41; H, 3.32; N, 12.61; S, 5.84.

5-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-4-(5-chloro-2-hydroxy-4-methylphenyl)-6-(4-fluorophenyl)pyrimidine-2(1H)-thione (7f). IR (KBr, cm^{-1}): 3985, 3630, 3062, 1680. ^1H NMR (400 MHz, $CDCl_3$, δ_H ppm): 2.50 (s, 3H, CH_3), 4.77 (s, 2H, NCH_2), 5.54 (s, 2H, OCH_2), 6.91 (s, 1H, ArH), 7.24–7.27 (m, 3H, ArH), 7.35–7.37 (m, 2H, ArH), 7.61–7.64 (m, 4H, ArH), 7.89 (s, 1H, ArH), 8.06 (s, 1H, triazole-H), 10.98 (s, 1H, OH), 11.81 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C ppm): 21.3, 56.2, 64.5, 115.8, 115.9, 117.3, 117.6, 124.5, 124.6, 124.8, 127.9, 128.5, 128.7, 128.9, 132.3, 132.6, 135.9, 136.0, 137.4, 137.6, 141.8, 143.4, 152.6, 155.0, 161.2, 164.3, 190.2. ESI-MS *Anal*. Calcd. for $C_{27}H_{21}ClFN_5O_2S$ $[M + H]^+$: 534.12. Found: 534.08. *Anal*. calcd. for $C_{27}H_{21}ClFN_5O_2S$: C, 60.73; H, 3.96; N, 13.11; S, 6.00. Found: C, 59.68; H, 3.92; N, 13.08; S, 6.04.

Antimicrobial activity. *In vitro* antibacterial activity of the synthesized compounds was tested against Gram-positive bacteria viz. *Staphylococcus aureus* (NCIM 2079) and *Bacillus subtilis* (NCIM 2920) and Gram-negative bacteria *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200). The compounds were also screened for antifungal activity against *Candida albicans* (NCIM 3471) and *Aspergillus niger* (NCIM 596). Compounds were diluted in DMSO with 1 μg/mL concentrations for bioassay. Microbroth dilution method [30] used to determined *in vitro* MIC of compounds in 96-well microtiter plates. National Committee for Clinical Laboratory Standards defined the method against a panel of human pathogenic strains. Test compounds were serially double diluted in growth medium. Plates were incubated at 30°C for fungi and 37°C for bacteria for 24 h. All experiments were carried out in triplicates, and mean values are represented.

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