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Synthesis, antimicrobial activity and anti-biofilm activity of novel tetrazole derivatives

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Abstract: In the development of antimicrobial agents, we designed and synthesized novel tetrazole derivatives. The structures of compounds **6a–f** and **7a–f** were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. These compounds were tested for their antimicrobial activity against a series of strains *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* and for antifungal activity against the strains *Candida albicans*, *Candida glabrata*, and *Candida tropicalis*. Compounds **6e**, **6f**, **7a**, and **7f** exhibit potent antimicrobial activities compared to the reference drugs streptomycin and miconazole. Tetrazole derivatives **7a–f** also inhibit biofilm formation and compound **7f** exhibits best anti-biofilm activity with a biofilm inhibitory concentration (BIC) as low as 0.9 μM.

Keywords: antibiofilm; antimicrobial; flavones; tetrazole.

Introduction

Microbial infections become an increasingly serious and challenging problem for human health across the world. Such infections most commonly affect patients with decreased immunity, neoplastic disorders and undergoing organ transplantation [1]. This situation stimulates an urgent need to develop novel antimicrobial agents from newer classes of compounds. Fluorinated compounds have proved invaluable as antimicrobial agents, and have been used for the treatment of obesity and various diseases associated with the cardiovascular and central nervous systems [2]. The incorporation of fluorine into a potential drug molecule can improve the therapeutic efficacy due to hydrogen bonding interactions at the active sites of enzyme [3].

The naturally occurring flavones display biological activity (Figure 1) [4–10]. One of the recognized functions of flavonoids in plants is their protective role against microbial incursion [4]. These compounds play an important role in drug discovery processes.

The synthetic versatility of tetrazole is due to its widespread applications in medicinal chemistry. Tetrazole scaffold is part of highly effective drugs such as candesartan, pamiroplast and pranlukast (Figure 1) [11]. Tetrazole can be considered a carboxylic acid analogue because of similar pK_a values and planar delocalized systems. Tetrazole derivatives are resistant to various biological degradation processes, which contributes to bioavailability [12, 13]. These factors play a role in applications of tetrazole derivatives as anticancer [14], antifungal [15], antitubercular [16], anti-HIV [17], antioxidant [18] and hormonal agents [19].

It can be suggested that the presence of fluorine, flavone and tetrazole in a single molecular framework (Figure 2) would increase biological activity of the compound. In continuation of our earlier work on the synthesis of tetrazole derivatives [20], we now report the synthesis of such compounds and evaluation of their antimicrobial and anti-biofilm activities.

Results and discussion

Chemistry

Substituted 3-hydroxychromones **5a–f** were synthesized as described in our previous report [21] (Scheme 1). Claisen-Schmidt condensation of substituted 2-hydroxyacetophenones **3a–f** with 4-fluorobenzaldehyde in ethanolic solution of potassium hydroxide at room temperature furnished substituted chalcones **4a–f** which were converted into corresponding substituted 3-hydroxychromones **5a–f** upon oxidative cyclization using hydrogen peroxide in ethanol.

3-[(1*H*-tetrazol-5-yl)methoxy]-2-(4-fluorophenyl)-4*H*-chromen-4-ones **7a–f** were synthesized from the

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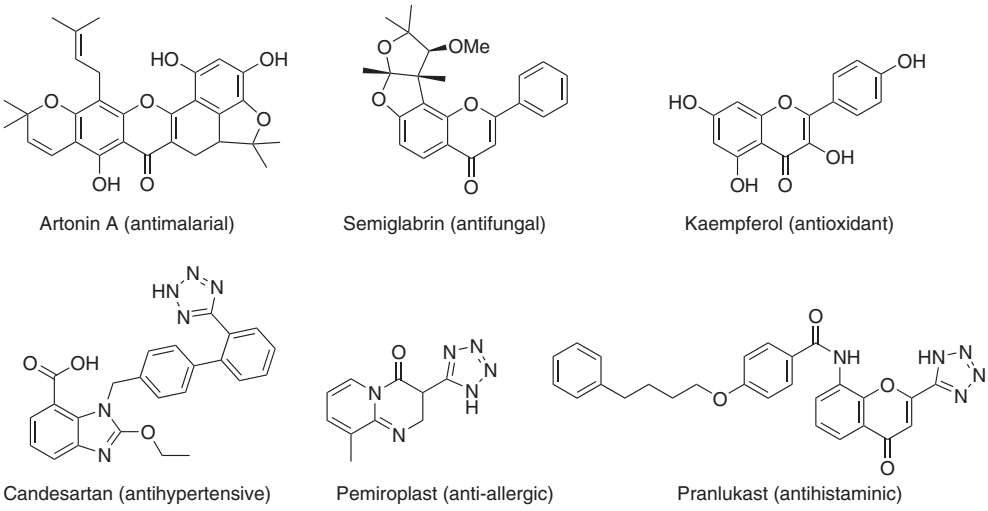


Figure 1 Naturally occurring flavones and pharmacologically important tetrazole drugs.

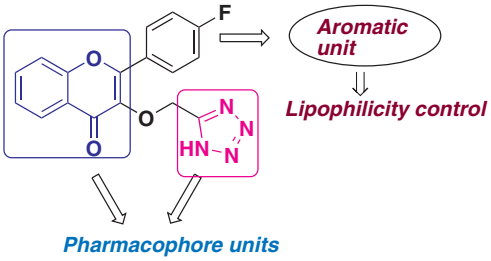
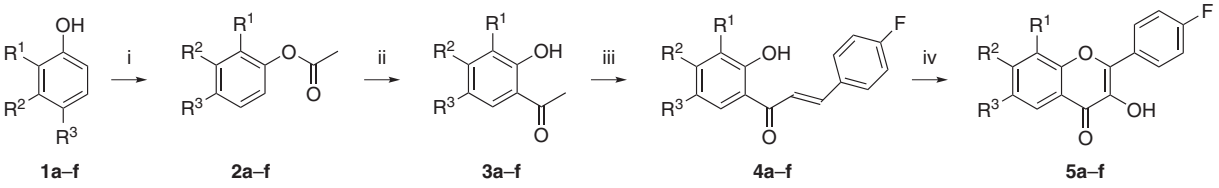
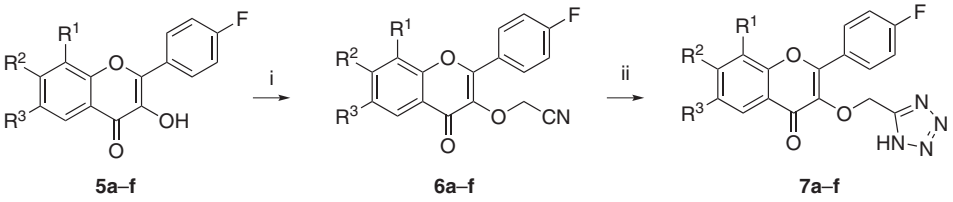


Figure 2 The designed scaffold containing fluorinated flavone and tetrazole as a main backbone.

corresponding 3-hydroxychromones **5a–f** (Scheme 2). First, compounds **5a–f** were alkylated with 2-chloroacetonitrile in the presence of K_2CO_3 in *N*,*N*′-dimethylformamide (DMF) at room temperature for 3–4 h to afford the substituted 2-(2-(4-fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy) acetonitriles **6a–f** in 71%–86% yield. Then, treatment of **6a–f** with sodium azide and zinc bromide in water at 100°C for 4–5 h furnished the final products **7a–f** in 73%–81% yield. All compounds were characterized by spectroscopic techniques of ^{1}H NMR, ^{13}C NMR and MS.



Scheme 1 Reagents and conditions: [i] acetic anhydride, pyridine, 100°C, 3–4 h, 80%–98%; [ii] $AlCl_3$, 150°C, 3–4 h, 70%–75%; [iii] 4-fluorobenzaldehyde, KOH, EtOH, room temp, 2–3 h, 60%–80%; [iv] H_2O_2, NaOH, 0°C to room temp, 2–3 h, 70%–80%.



a: R^1 = H; R^2 = H; R^3 = H
b: R^1 = H; R^2 = H; R^3 = Cl
c: R^1 = Me; R^2 = H; R^3 = H
d: R^1 = H; R^2 = H; R^3 = Me
e: R^1 = Cl; R^2 = H; R^3 = Cl
f: R^1 = H; R^2 = Me; R^3 = Cl

Scheme 2 Reagents and conditions: [i] 2-chloroacetonitrile, K_2CO_3, DMF, room temp, 3–4 h, 71%–86%; [ii] sodium azide, zinc bromide, H_2O, reflux, 4–5 h, 73%–81%.

Antimicrobial activity

The minimum inhibitory concentration (MIC) values were determined by micro-broth dilution method. The results were compared with activities of standard antibacterial drug streptomycin and antifungal drug miconazole (Table 1). All compounds **6a–f** and **7a–f** exhibit moderate to potent antibacterial and antifungal activity. It can be seen that compounds exhibit varying degrees of antifungal activity due to the nature of heterocyclic skeleton. Tetrazole derivatives **7a–f** are more active compared to **6a–f** analogues. Compounds **6f**, **7a** and **7f** are equipotent with miconazole against *Candida albicans*. Compound **7f** shows most potent activity against *Candida glabrata*. Compounds **6f**, **7a**, and **7c** exhibit more potent activity than **6e**, **7b**, **7d** and **7e** against *C. glabrata*. In addition, compounds **6a**, **6b**, **6c** and **6d** show excellent activity against *C. glabrata*. Compound **7a** shows highly potent activity against *Candida tropicalis*. Compounds **6f**, **7b** and **7f** show more potent activity than **6a**, **6e**, **7c**, **7d** and **7e** against *C. tropicalis*.

Table 1 Antimicrobial screening data of the compounds **6a–f** and **7a–f**.

Entry	Antimicrobial activity (MIC, μg/mL)						
	Antibacterial activity				Antifungal activity		
	Sa	*Bs*	*Ec*	*Pa*	*Ca*	*Cg*	*Ct*
6a	400	400	50	50	100	100	50
6b	400	400	100	100	100	200	200
6c	100	100	50	100	100	200	100
6d	400	400	50	50	50	100	100
6e	200	200	25	50	25	50	50
6f	100	100	100	100	12.5	25	25
7a	50	100	100	100	12.5	25	12.5
7b	100	200	100	50	25	50	25
7c	50	100	200	200	25	25	50
7d	200	200	100	50	50	50	50
7e	100	100	200	100	50	50	50
7f	50	50	400	200	12.5	12.5	25
Streptomycin	12.5	400	400	200	–	–	–
Miconazole	–	–	–	–	12.5	400	800

Sa, Staphylococcus aureus; Bs, Bacillus subtilis; Ec, Escherichia coli; Pa, Pseudomonas aeruginosa; Ca, Candida albicans; Cg, Candida glabrata; Ct, Candida tropicalis.

Biofilm inhibitory activity

Biofilm is surface-adhered bacterial area implanted in an extracellular matrix responsible for the growing resistance in bacteria to antibiotics [22]. Several pathogenic bacteria are found in environments that form biofilm which are structured microbial communities embedded with complex associations with each other. Many serious chronic infections are transmitted to humans via biofilms [23]. Developing new molecules having the ability to inhibit biofilm formation could be one of the solutions for the growing antibiotic resistance. In this regard, the synthesized flavones possessing tetrazole moiety **7a–f** were evaluated for anti-biofilm activity against three bacterial strains, namely *Staphylococcus aureus* NCIM 2178, *Bacillus subtilis* NCIM 2250, *Escherichia coli* NCIM 2137 and *Pseudomonas aeruginosa* NCIM 2036. The results shown in Table 2 reveal that these compounds exhibit good to excellent anti-biofilm activity against all four strains tested. Particularly, compounds **7a**, **7e** and **7f** demonstrate potent efficacy in inhibiting biofilm formation with IC_{50} values of 1.3, 1.4, and 0.9 μM, respectively, against *P. aeruginosa* NCIM 2036.

Conclusions

New tetrazole derivatives were synthesized and evaluated for antibacterial activity, antifungal activity, and bio-film

Table 2 Biofilm inhibition assay of flavone-tetrazole conjugates **7a–f**.

Compound	IC_{50} values (μM)			
	Staphylococcus aureus NCIM 2178	*Bacillus subtilis* NCIM 2250	*Escherichia coli* NCIM 2137	*Pseudomonas aeruginosa* NCIM 2036
7a	7.8 ± 0.09	2.6 ± 0.32	4.6 ± 0.04	1.3 ± 0.72
7b	4.5 ± 0.41	3.6 ± 0.08	4.3 ± 0.09	2.1 ± 0.04
7c	7.9 ± 0.36	5.6 ± 0.05	8.7 ± 0.02	2.3 ± 0.24
7d	8.6 ± 0.21	5.2 ± 0.08	9.1 ± 0.06	6.2 ± 0.15
7e	5.8 ± 0.25	4.8 ± 0.11	3.3 ± 0.31	1.4 ± 0.26
7f	7.1 ± 0.12	2.9 ± 0.04	4.5 ± 0.09	0.9 ± 0.03
Ciprofloxacin	1.56 ± 0.14	1.56 ± 0.02	1.56 ± 0.23	0.78 ± 0.13

inhibition. All compounds show moderate to potent activity compared to the standard drugs streptomycin and miconazole. Compounds **6e**, **6f**, **7a** and **7f** are potent antimicrobial agents. Compounds **7a–f** which contains tetrazole ring are more potent antimicrobial agents compared to **6a–f** series. Further, these compounds are also effective inhibitors of biofilm formation which may contribute to the development of antibiotic resistance in bacteria. Compound **7f** is the most active against *P. aeruginosa* NCIM 2036. It can be concluded that incorporation of the tetrazole ring into fluorinated flavones enhances the biological effect.

Experimental

All reagents were purchased from commercial suppliers and used without purification. The progress of each reaction was monitored by ascending thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} plates and visualized using UV light and iodine vapor. Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded on a Carry 600 Series 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 spectrometer in $CDCl_3$ or DMSO-d_6. Mass spectra were recorded on a Waters Q-TOF micro mass instrument equipped with an electro-spray ionization (ESI) source. Elemental analysis was performed on a Perkin-Elmer EAL-240 elemental analyzer.

General procedure for the synthesis of substituted 2-(2-(4-fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy)-acetonitriles 6a–f

A mixture of 2-(4-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one **5a–f** (1 mmol), potassium carbonate (2 mmol) and 2-chloroacetonitrile (1 mmol) and DMF was stirred at room temperature for about 3–4 h and analyzed by TLC using petroleum ether/ethyl acetate as an eluent. Then the mixture was quenched with crushed ice and the precipitated solid was collected by filtration and crystallized from ethanol.

2-(2-(4-Fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy)acetonitrile (6a) This compound was obtained from **5a** as 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 (ddt, 1H, *J*=8 Hz, 8 Hz and 1.2 Hz, ArH), 7.57 (dd, 1 H, *J*=9 Hz and 1.1 Hz, ArH), 7.74 (ddt, 1H, *J*=8 Hz, 8 Hz and 1.5 Hz, ArH), 8.08 (dd, 2H, *J*=9 Hz, ArH), 8.25 (dd, 1H, *J*=8 Hz and 1.5 Hz, ArH); ^{13}C NMR ($CDCl_3$): δ 56.1 (O-CH_2), 115.1, 115.9, 116.1 (C≡N), 118.1, 123.8, 125.4, 125.8, 126.1, 131.3, 134.2, 137.5, 155.3, 156.4, 163.1, 165.6 (C-F), 174.1 (C=O). ESI-HRMS. Calcd. for $C_{17}H_{10}FNO_3$ (M+H)$^+$: *m/z* 296.0723. Found: 296.0372. 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 obtained from **5b** as 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}; ^{1}H NMR (DMSO-d_6): δ 5.14 (s, 2H, OCH_2), 7.34 (t, 2H, *J*=9 Hz, ArH), 7.73 (d, 1H, *J*=9 Hz, ArH), 7.79 (dd, 1H, *J*=9 Hz and 2.6 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 (O-CH_2), 115.5, 115.7, 120.4 (C≡N), 124, 124.3, 125.7, 130.3, 131.1, 131.2, 134.1, 137.6, 153.1, 155.4, 162.4, 165 (C-F), 172.1 (C=O); ESI-MS: *m/z* 330 (M+H)$^+$, *m/z* 352 (M+Na)$^+$. 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 obtained from **5c** as 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}; ^{1}H 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 Hz, ArH), 7.57 (d, 1H, *J*=7 Hz, ArH), 8.07–8.13 (m, 3H, ArH); ^{13}C NMR ($CDCl_3$): δ 15.8 (CH_3), 56, 1 (O-CH_2), 115.1, 115.9, 116.2 (C≡N), 123.4, 123.8, 125.1, 126.5, 127.6, 131.3, 135, 137.4, 153.8, 155.8, 163.1, 165.6 (C-F), 174.4 (C=O); ESI-MS: *m/z* 310 (M+H)$^+$, *m/z* 332 (M+Na)$^+$. 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 obtained from **5d** as 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}; ^{1}H 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 (CH_3), 56.4 (O-CH_2), 115.5, 115.7, 118 (C≡N), 122.9, 124.1, 126.2, 131.1, 131.1, 134.8, 135.4, 136.3, 137.5, 153, 154.9, 162.3 (C-F), 173.1 (C=O); ESI-MS: *m/z* 310 (M+H)$^+$, *m/z* 332 (M+Na)$^+$. 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 obtained from **5e** as 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}; ^{1}H NMR ($CDCl_3$): δ 5.12 (s, 2H, OCH_2), 7.26 (t, 2H, *J*=9 Hz, ArH), 7.77 (d, 1H, *J*=2.6Hz, ArH), 8.10 (d, 1H, *J*=2.6Hz, ArH), 8.18 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 56.1 (O-CH_2), 114.8, 116.2, 116.4 (C≡N), 123.8, 124.6, 125.5, 131.2, 131.5, 131.6, 134.2, 137.6, 149.4, 156.3, 163.5, 166 (C-F), 172.5 (C=O); ESI-MS: *m/z* 364 (M+H)$^+$, *m/z* 386 (M+Na)$^+$. 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 obtained from **5f** as 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): δ 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 (CH_3), 56.4 (O-CH_2), 115.5, 115.6, 115.7 (C≡N), 120.3, 122.4, 124.2, 125.9, 125.9, 131.1, 131.1, 137.5, 142.9, 153.1, 155.1, 167.4 (C-F), 172.1 (C=O); ESI-MS: *m/z* 344 (M+H)$^+$, *m/z* 366 (M+Na)$^+$. 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 3-((1*H*-tetrazol-5-yl) methoxy)-2-(4-fluorophenyl)-4*H*-chromen-4-ones 7a–f

To a mixture of sodium azide (1.5 mmol) and zinc bromide (1.5 mmol) in water (20 mL) was added substituted 2-(2-(4-fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy)acetonitrile **6a–f** (1 mmol). The mixture was

then heated under reflux for 4–5 h with vigorous stirring. After completion of the reaction, as evident by TLC analysis using chloroform/methanol as an eluent, the mixture was quenched with crushed ice and the precipitated solid was collected by filtration and crystallized from ethanol.

3-((1*H*-Tetrazol-5-yl)methoxy)-2-(4-fluorophenyl)-4*H*-chromen-4-one (7a) This compound was obtained from **6a** as 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}; ^1H NMR (DMSO-d_6): δ 5.47 (s, 2H, OCH_2), 7.26 (t, 2H, *J*=9 Hz, ArH), 7.48–7.52 (m, 1H, ArH), 7.70 (d, 1H, *J*=8 Hz, ArH), 7.82 (ddd, 1H, *J*=9 Hz, 7 Hz and 1.7 Hz, ArH), 8.01–8.05 (m, 2H, ArH), 8.16 (dd, 1H, *J*=8 Hz and 1.7 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 61.8 ($O\text{-}CH_2$), 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); ESI-MS: *m/z* 339 $(M+H)^+$ and *m/z* 361 $(M+Na)^+$. 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 obtained from **6b** as 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}; ^1H NMR (DMSO-d_6): δ 5.42 (s, 2H, OCH_2), 7.17 (t, 2H, *J*=9 Hz, ArH), 7.64 (d, 1H, *J*=9 Hz, ArH), 7.72 (dd, 1H, *J*=9 Hz and 2.6 Hz, ArH), 7.98 (dd, 2H, *J*=9 Hz and 5.5 Hz, ArH), 8.04 (d, 1H, *J*=2.6 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 61.7 ($O\text{-}CH_2$), 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); ESI-MS: *m/z* 395 $(M+Na)^+$. 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 obtained from **6c** as 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); ^1H 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 ($O\text{-}CH_2$), 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); ESI-MS: *m/z* 353.3142 $(M+H)^+$, *m/z* 375.2781 $(M+Na)^+$. 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 obtained from **6d** as 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); ^1H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 5.47 (s, 2H, OCH_2), 7.27 (t, 2H, *J*=8.8 Hz, ArH), 7.57–7.63 (m, 2H, ArH), 7.90 (s, 1H, ArH), 8.00 (dd, 2H, *J*=9.0 Hz and 5.3 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 20.4 (CH_3), 61.8 ($O\text{-}CH_2$), 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); ESI-MS: *m/z* 353.0987 $(M+H)^+$. 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 obtained from **6e** as 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); ^1H 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 Hz and 5.5 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 62.0 ($O\text{-}CH_2$), 115.6, 115.8, 123, 123.8, 125.2, 129.6, 131.0, 133.5, 138.6, 139.4, 148.9, 154.6, 162.2 (tetrazole C), 164.7 (C-F), 172.0 (C=O); ESI-MS: *m/z* 407.2 $(M+H)^+$. 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 obtained from **6f** as 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 ($O\text{-}CH_2$), 115.4, 115.7, 120.6, 122.5, 123.9, 126.1, 130.6, 130.9, 131, 138.1, 142.7, 153.0, 154.9, 162.0 (tetrazole C), 164.5 (C-F), 172.4 (C=O);. ESI-MS: *m/z* 409.2642 $(M+Na)^+$. 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.

Antimicrobial activity

In vitro antibacterial activity of the synthesized compounds was tested against Gram-positive bacteria *S. aureus* (NCIM 2178), *B. subtilis* (NCIM 2250) and Gram-negative bacteria *E. coli* (NCIM 2137), *P. aeruginosa* (NCIM 2036). The compounds were also screened for antifungal activity against *C. albicans* (MTCC 277), *C. glabrata* (NCIM 3236), *C. tropicalis* (NCIM 3110). Compounds were diluted in DMSO with 1 μg/mL concentrations for bioassays. Micro-broth dilution method was used to determine MIC values of compounds in 96-well micro-titre plates [24]. Test compounds were serially 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 reported.

Biofilm inhibition assay

The flavone–tetrazole conjugates **7a–f** were screened in sterile 96 well polystyrene micro-titre plates using the modified bio-film inhibition assay [25] against a panel of pathogenic bacterial strains *S. aureus* NCIM 2178, *B. subtilis* NCIM 2250, *E. coli* NCIM 2137 and *P. aeruginosa* NCIM 2036, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). The test compounds of predetermined concentrations ranging from 0 to 200 μg/mL were mixed with the bacterial suspensions having an initial inoculums concentration of 5×105 cfu mL^{-1}. Aliquots of 100 μL were distributed in each well and then incubated at 37°C for 24 h under static conditions. Then medium was discarded and washed with phosphate buffered saline to remove the non-adherent bacteria. Micro-titre plate well was stained with 100 μL of 0.1% crystal violet solution followed by 30-min incubation at room temperature. Afterwards the crystal violet solution from the plates was discarded, thoroughly washed with distilled water 3–4 times and air dried at room temperature. The crystal violet stained biofilm was solubilized in 95% ethanol (100 μL) and the absorbance was recorded at 540 nm using a TRIAD multimode reader (Dynex Technologies, USA). All experiments were carried out in triplicates and the values are indicated as mean ± S.D.

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