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Synthetic flavone derivatives. An antibacterial evaluation and structure-activity relationship study

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Biologically active flavone derivatives with antibacterial potentials were synthesized *via* Claisen-Schmidt condensation of ketones with different aldehydes with good yields. The structures were established by different spectroscopic techniques like ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. The findings showed that some of the substituted flavones possess higher antibacterial potentials than simple flavones as regards their minimum inhibitory concentration (MIC) and are potential candidates for the treatment of a wide range of infectious diseases.

Keywords: Flavone derivatives, antibacterial, MIC, infectious diseases.

INTRODUCTION

Infectious diseases are among the main causes of mortality and morbidity worldwide. Advancement in the innovation of antimicrobial agents has safeguarded the way for human wellbeing. However, effectiveness of antibiotics in future is to some extent in doubtful condition due to the development of resistance to these agents in an unavoidable manner [1]. The emergence of hitherto unidentified microbes that cause infections poses a colossal health concern regarding the combat towards infectious diseases [2]. The need for new, effective and affordable drugs to treat microbial diseases in the developing world is one of the major issues facing global health today and consequently, this has created a new dimension in the search for new drugs [3]. Structural modification of anti-infective agents has confirmed to be an effective way of enhancing the lifespan of these agents [4]. Natural products have been reported to be a potential source of anti-infective agents and the prime examples are penicillin and tetracycline. Generally, polyphenolic compounds like flavonoids (natural compounds) have been reported for a wide range of pharmacological actions [5]. At present, this class of natural products is the area of interest in medical research and is known to possess pharmacological actions including antiallergic [6], anti-inflammatory [7], antiviral [8], antithrombotic [9], antimutagenic [10], antioxidant [11], antidiabetic [12], anticancer [13], hepatoprotective [14] and antimicrobial [15-18] effects.

Most of the scientific reports are on natural flavonoids and acquire a special place in natural and heterocyclic chemistry because of their structural ornamentation in many pharmacologically active compounds. In view of the above facts regarding the significance of flavonoids in nature, it was considered worthwhile to synthesize flavonoid derivatives and to attempt to portray the structure-activity relationships (SAR).

EXPERIMENTAL

Materials and Methods

Substituted ketones and benzaldehyde were purchased from Sigma Aldrich Chemical Company. TLC plates were Merck 60 F254, Darmstadt Germany. Solvents and chemicals like ethanol, n-hexane, ethyl acetate, DMSO of extra pure analytical grade were purchased from Merck. Mueller-Hinton agar and nutrient broth were purchased from Oxoid, UK. Ciprofloxacin and ampicillin were gifted by local pharmaceutical industries.

^{1}H-NMR and ^{13}C NMR spectra were recorded in deutrated chloroform ($CDCl_3$) on a Bruker SF spectrometer operating at 300 and 75 MHz frequencies, respectively. Chemical shift values are expressed in δ (ppm) downfield relative to TMS which was used as an internal standard. Infrared spectra were recorded on Thermoscientific, USA (Nicolet 6700) infrared spectrometer by the KBr disk method. All melting points are uncorrected and

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were taken in open capillary tubes using an Electrothermal 9100 apparatus (Barnstead, UK). Reaction extents and final products purities were checked on TLC plates (Merck 60 F254) and spots were visualized under UV lamp (180-365 nm) by staining with iodine vapor.

Synthesis and characterization

General procedure for the synthesis of flavone derivatives

To an ethanolic solution of substituted 2-hydroxy acetophenone (15 mM), sodium hydroxide (10 ml, 40% ethanolic) was dropwise added at room temperature. Then the corresponding benzaldehyde derivatives (15 mM) were dropwise added to this mixture and stirred for 24-48 h at room temperature (25 ± 2ºC). The reaction was monitored by TLC and upon completion, the contents were poured into crushed ice and neutralized with 1N HCl solution resulting in yellow precipitates of the corresponding chalcones. The latter were filtered and washed with water to remove the impurities.

In the next step, the respective chalcones were separately cyclized to flavone derivatives in 15 ml DMSO in the presence of iodine (375 mg) at 140 ºC for 1 h. Upon completion of the reaction, the mixtures were cooled to room temperature and poured into water followed by extraction with ethyl acetate (25 ml×3), treated with sodium thiosulfate solution (20%), brine solution and dried over sodium sulfate. The final products (a mixture of flavone and chalcone) were subjected to column chromatography using n-hexane: ethyl acetate (9:1) to purify the flavone derivatives (Scheme 1) [19, 20].

2-Phenyl-4H-chromen-4-one (F1)

The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and benzaldehyde in ethanol, and adding sodium hydroxide solution with continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine as described above.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.22 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.75 – 7.53 (m, 5H), 7.50 – 7.37 (m, 4H), 6.85 (s, 1H).^{13}C NMR (75 MHz, $CDCl_3$) δ 178.48, 163.42, 156.26, 133.79, 131.77, 131.61, 129.04, 126.66, 125.71, 125.24, 123.95, 118.09, 107.58 IR (KBr), *υ, cm^{-1}*, 1635.4, 1463.3, 1372.4, 766.0. Found, %: C 81.07; H 4.54. $C_{15}H_{10}O_2$. Calculated, %: C 81.19; H 4.60 [20-22].

2-(4-(Dimethylamino)phenyl)-4H-chromen-4-one (F2)

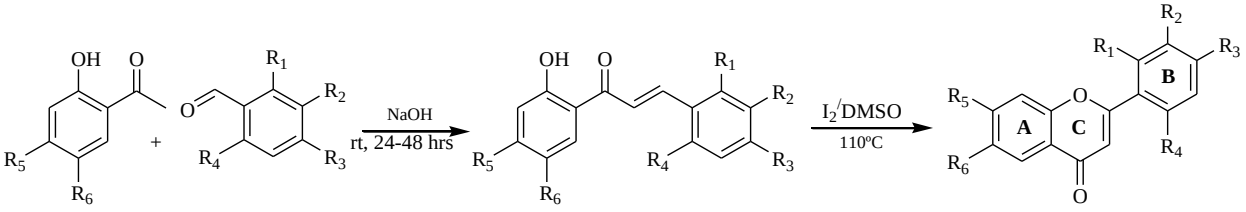
The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and 4-(dimethylamino) benzaldehyde in ethanol, and adding sodium hydroxide solution under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.24 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.89 – 7.81 (m, 2H), 7.68 (ddd, *J* = 8.7, 7.1, 1.7 Hz, 1H), 7.55 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.40 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 6.80 – 6.76 (m, 2H), 6.73 (s, 1H), 3.10 (s, 6H). ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm= 178.20, 163.7, 156.50, 152.60, 133.22, 127.75, 125.58, 124.81, 124.03, 117.84, 111.66, 104.39, 40.10. IR (KBr) *υ, cm^{-1}*, 2919.4 (CH) 1730.3(C=O), 1197.8 and 1363.2 (C-N), 1558.1(C=C), 3311.5 (=C-H) 1127.2 (C-0). Found, %: C 76.96; H 5.70; N 5.28. $C_{17}H_{15}NO_2$. Calculated, %,: C 76.59; H 5.60; N 5.60 [20].

2-(p-Tolyl)-4H-chromen-4-one (F3)

The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and 4-methylbenzaldehyde (p-tolylaldehyde) in ethanol, and adding sodium hydroxide solution under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.23 (dd, J = 7.9, 1.7 Hz, 1H), 7.85 (d, J = 8.3 Hz, 2H), 7.71 (td, J = 8.4, 7.2, 1.2 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.39 (td, J = 7.9, 0.8 Hz, 1H), 7.27 (d, 2H), 6.92 (s, 1H), 2.39 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.1, 162.7, 156.2, 142.5, 134.1, 129.6, 128.4, 126.5, 125.6, 125.2, 123.5, 118.2, 106.5, 104.1, 21.5. IR (KBr) *υ, cm^{-1}*, 1640, 1465, 817 [21, 22].



Scheme 1. Synthesis of flavone derivatives.

2-(4-Chlorophenyl)-4H-chromen-4-one (F4).

The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and 4-chlorobenzaldehyde in ethanol, and adding sodium hydroxide solution under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.24 (dd, J = 8.0, 1.6 Hz, 1H), 7.83 (d, 2H), 7.72 (td, J = 8.7, 7.1, 1.7 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.47 (d, 2H), 7.40 (t, J = 7.6 Hz, 1H), 6.75 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 162.1, 156.1, 137.8, 133.9, 130.2, 129.3, 127.5, 125.7, 125.3, 123.9, 118.0, 107.6. IR (KBr) *υ, cm^{-1}*, 1662, 1374, 1092, 827, 754 [21, 22].

2-(2,4-Dichlorophenyl)-4H-chromen-4-one derivative (F5)

The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and 2,4-dichlorobenzaldehyde in ethanol and adding sodium hydroxide solution under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*, ppm) δ 8.27 (dd, *J* = 8.0, 1.7 Hz, 1H, H-3`), 7.74 (ddd, *J* = 8.7, 7.1, 1.7 Hz, 1H, 5`-H), 7.64 – 7.56 (m, 2H, 5-H, 6`-H), 7.55 – 7.40 (m, 3H, 6-H, 7-H, 8-H), 6.68 (s, 1H, H-3).$^{13}$C NMR (75 MHz, $CDCl_3$, ppm) δ 177.98 (C-4), 161.51 (C-2), 156.54 (C-8a), 137.43(C-2`), 134.06 (C-4`), 133.81 (C-6`), 131.42 (C-5`), 130.77 (C-3`), 130.40 (C-1`), 127.58 (C-7), 125.82 (C-6), 125.50 (C-5), 123.81 (C-4a), 118.18 (C-8), 113.16(C-3). IR (KBr) V_{max} *cm^{-1}*: 3066.5, 2920.6, 1734.1, 1645.4, 1221.1, 748.2. Anal. Calcd. for $C_{15}H_8Cl_2O_2$: C, 61.88; H, 2.77. Found: C, 60.99; H, 2.28 [20].

2-(2,3-Dichlorophenyl)-4H-chromen-4-one (F6)

The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and 2,3-dichlorobenzaldehyde in ethanol and then sodium hydroxide solution was added under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.32 – 8.17 (m, 1H, H4`), 7.86 – 7.61 (m, 2H, H5`,6`), 7.56 – 7.21 (m, 4H, H-5,6,7,8), 6.67 (s, 1H, H3). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 178.39(C4), 162.62(C2), 156.58(C8a), 134.60(C7), 134.26(C3`), 134.08(C1`), 132.62(C5), 129.80(C4`), 128.93(C5`), 127.65(C2`), 125.85(C6`), 125.61(C4a), 123.69(C6), 118.23(C8), 112.98(C3). IR (KBr) V_{max} *cm^{-1}*: 3048.9, 2918.5, 1714.7, 1659.3, 1191.3, 747.8. Anal. Calcd. for $C_{15}H_8Cl_2O_2$: C, 61.88; H, 2.77. Found: C, 62.02; H, 2.46 [20].

2-(3,4-Dichlorophenyl)-4H-chromen-4-one (F7)

The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and 3,4-dichlorobenzaldehyde in ethanol and adding sodium hydroxide solution with continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.24 (dd, *J* = 8.0, 1.7 Hz, 1H), 8.04 (d, *J* = 2.1 Hz, 1H), 7.74 (tq, *J* = 7.0, 2.2 Hz, 2H), 7.65 – 7.56 (m, 2H), 7.46 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 6.80 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.03, 160.82, 156.08, 135.96, 134.09, 133.70, 131.69, 131.11, 128.06, 125.78, 125.55, 125.25, 123.87, 118.06, 108.19. IR (KBr) *υ, cm^{-1}*, 1659.3, 1413.7, 1378.8, 750.04, 747.8. Found, %: C 61.92; H 2.53. $C_{15}H_8Cl_2O_2$. Calculated, %,: C 61.88; H 2.77 [20].

2-(2,6-Dichlorophenyl)-4H-chromen-4-one (F8)

The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and 2,6-dichlorobenzaldehyde in ethanol and adding sodium hydroxide solution under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.30 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.83 – 7.68 (m, 1H), 7.58 – 7.36 (m, 5H), 6.47 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.06, 162.03, 156.96,, 133.65, 133.39, 128.48, 126.58, 125.75, 125.50, 124.89, 123.43, 120.38, 117.58. IR (KBr) *υ, cm^{-1}*, 2918.5, 1714.7, 1659.3, 1191.3, 747.8. Found, %: C 61.94; H 2.49. $C_{15}H_8Cl_2O_2$. Calculated, %,: C 61.88; H 2.77.

2-(4-(Trifluoromethyl)phenyl)-4H-chromen-4-one (F9)

The compound was obtained by stirring equimolar amounts of 2′-hydroxyacetophenone and 4-(trifluoromethyl) benzaldehyde in ethanol and adding sodium hydroxide solution under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.25 (dd, *J* = 8.0, 1.7 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 2H), 7.85 – 7.69 (m, 3H), 7.61 (dd, *J* = 8.4, 1.1 Hz, 1H), 7.47 (m, 1H), 6.88 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.14 , 161.57 , 156.17 , 135.16, 134.10 , 133.77 , 132.90 , 126.62 , 126.02, 125.77 , 125.54 , 123.91 , 118.11 , 108.71. IR (KBr) *υ, cm^{-1}*, 3073.3 (=C-H),

1641.8 (C=O), 1316.8 (C-F), 1165.3 (C-O), 849.8 (C-F). ESI: m/z ($C_{16}H_9F_3O_2$) H+: calculated, 291.0627, found: 291.0629 [23, 24].

2-(4-(Trifluoromethyl)phenyl)-7-methoxy-4H-chromen-4-one (F10)

The compound was obtained by stirring equimolar amounts of 2′-hydroxy-4′ methoxyacetophenone and 4-(trifluoromethyl) benzaldehyde in ethanol and adding sodium hydroxide solution under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.16 (d, *J* = 8.7 Hz, 1H), 8.10 – 8.00 (m, 2H), 7.80 (d, *J* = 8.3 Hz, 2H), 7.09 – 6.97 (m, 2H), 6.83 (s, 1H), 3.97 (s, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 177.56, 164.45, 161.23, 157.98, 135.27, 132.77, 127.18, 126.51, 126.08, 125.98, 125.93, 117.82, 114.76, 108.76, 55.91. IR (KBr) *υ, cm^{-1}*, 3130.4 (=C-H), 2815.3 (C-H), 1650.4 (C=O), 1381.4 (C-F), 1111.7 (C-O), 836.4 (C-F). HRMS (ES^+) m/z $C_{34}H_{22}F_6O_6Na$ requires 663.1213; Found 663.1234 [25].

6-Bromo-2-(4-(trifluoromethyl)phenyl)-4H-chromen-4-one (F11)

The compound was obtained by stirring equimolar amounts of 2′-hydroxy-5′ bromoacetophenone and 4-(trifluoromethyl) benzaldehyde in ethanol and adding sodium hydroxide solution under continuous stirring to get the desired chalcone. In the next step, the chalcone was cyclized to flavone by refluxing in DMSO in the presence of catalytic iodine.

^{1}H NMR (300 MHz, chloroform-*d*) δ 8.38 (d, *J* = 2.5 Hz, 1H), 8.06 (d, *J* = 8.1 Hz, 2H), 7.88 – 7.78 (m, 3H), 7.58 – 7.45 (m, 1H), 6.90 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.88, 161.93, 154.95, 137.13, 134.13 128.47, 126.72, 126.18, 126.13, 125.21, 124.32, 120.13, 119.07, 108.73. IR (KBr) *υ, cm^{-1}*, 3090.4 (=C-H), 1633.7 (C=O), 1316.3 (C-F), 1173.2, 828.4 (C-F), 632.1 (C-Br). HRMS (ES^+) m/z C_{32} H_{16} $Br_2F_6O_4Na$ requires 760.9212; found 760.9164 [25].

Antibacterial Activity

Antibacterial screening of the flavone derivatives mentioned above was performed against Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. Briefly, about 20 ml of sterile Mueller-Hinton Agar was poured in sterile petri plates and allowed to solidify. The sterile cotton swab was dipped into the bacterial culture (10^6 to 10^8 CFU/ml) and the agar plates were evenly inoculated by swabbing followed by the wells formation using a sterile cork-borer (6 mm diameter). Each prelabeled well was filled with 100 µl of various concentrations of flavone derivatives and allowed to diffuse by refrigerating for 30 min. The plates were then incubated at 37ºC for 24 h. Triplicate plates were prepared for each treatment and the average zone of inhibition excluding well was recorded. DMSO was used as a negative control. The antibacterial potential in the form of zone of inhibition in millimeters (mm) was compared with the standard antibiotics ampicillin and ciprofloxacin [26-28].

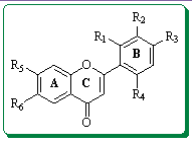
Minimum inhibitory concentration (MIC)

Compounds inhibiting growth of one or more of the above microorganisms were again tested for their minimum inhibitory concentration values (MIC). The MIC values were determined by the broth dilution technique. Briefly, a stock solution of each compound was prepared in dimethylsulfoxide (DMSO) and serially diluted to achieve the desired concentration range. To each of the preidentified sterile test tubes containing a specific concentration of the test compound, a standard volume of nutrient broth medium was added. The inoculum consisting of an overnight broth culture of microorganisms was added to each tube. The tubes were incubated at 37ºC for 24 h and examined for turbidity. A control tube containing no antimicrobial agent was also included and ciprofloxacin was used as a standard. The lowest concentration required to stop the growth of bacteria was regarded as MIC [29].

RESULTS AND DISCUSSION

The general structure and physical parameters of the flavone derivatives are given in Scheme 1 and Table 1 while the spectroscopic parameters are given in the experimental section of this study. Results of the antibacterial activity of synthetic flavones in respect to their zone of inhibition are given in Table 2. It is reported that substitution in the ring A and ring B may increase or decrease the antibacterial response. Results from the study reveal that addition of halogen (Br) at ring A and trifluoromethyl at ring B enhances the antibacterial response against Gram positive and Gram negative bacteria.

Table 1. Physical parameters of the flavone derivatives



Flavone	R_1	R_2	R_3	R_4	R_5	R_6	Yield	Appearance	R_f	M.P (ºC)
F1	H	H	H	H	H	H	68.7%	Creamy white solid	0.58	96-98
F2	H	H	$N(CH_3)_2$	H	H	H	73.6%	Brick red solid	0.67	107-109
F3	H	H	CH_3	H	H	H	77.8%	Off white solid	0.64	154-158
F4	H	H	Cl	H	H	H	84.2%	White crystals	0.69	178-181
F5	Cl	H	Cl	H	H	H	87.0%	White solid	0.57	190-193
F6	Cl	Cl	H	H	H	H	81.0%	White solid	0.64	188-191
F7	H	Cl	Cl	H	H	H	79.3%	White solid	0.61	195-197
F8	Cl	H	H	Cl	H	H	81.6%	White solid	0.63	182-185
F9	H	H	CF_3	H	H	H	85.3%	Monoclinic crystals	0.63	134-137
F10	H	H	CF_3	H	OCH_3	H	83.4%	Triclinic crystals	0.57	167-170
F11	H	H	CF_3	H	H	Br	85.7%	Monoclinic crystals	0.71	171–173

Table 2. Antibacterial activity (Zone of inhibition) of compounds F1–F11.

Flavones	Concentration (µg/ml)	Zone of inhibition (mm)		
		Gram-positive bacteria		Gram-negative bacteria
		B. subtilis	*S. aureus*	*P. aeruginosa*
F1	25	19.4±1.67	25.2±1.06	23.1±0.91
	50	23.6±2.13	29.8±0.81	25.4±1.33
	100	21.1±1.28	28.3±1.27	29.2±1.72
F2	25	13.5±0.97	16.2±0.84	15.3±1.41
	50	15.3±1.71	16.8±1.27	16.1±2.08
	100	19.6±2.25	18.1±1.15	21.4±1.14
F3	25	18.0±1.67	20.3±1.31	21.1±1.47
	50	19.2±2.08	18.6±1.01	19.4±1.07
	100	21.4±1.27	19.1±2.17	23.8±1.31
F4	25	12.2±0.87	10.1±1.15	13.3±1.54
	50	15.8±1.19	13.5±1.52	18.1±1.73
	100	16.3±1.65	15.8±1.37	17.4±0.87
F5	25	10.6±1.07	6.0±1.48	7.1±2.13
	50	13.4±1.12	8.1±0.85	8.3±1.04
	100	14.7±1.89	8.4±1.14	8.8±1.26
F6	25	10.3±1.38	8.1±0.91	9.3±1.66
	50	13.5±1.25	10.3±1.12	11.0±0.83
	100	13.1±1.87	10.8±1.54	11.3±1.24
F7	25	13.2±1.09	12.3±1.76	13.1±0.88
	50	13.0±1.18	15.5±2.08	18.0±1.34
	100	17.1±1.05	17.3±1.43	16.2±1.02
F8	25	9.7±1.31	5.4±3.04	5.2±1.07
	50	12.5±1.14	7.1±2.14	6.3±1.51
	100	14.8±1.07	7.7±1.87	6.4±1.25
F9	25	20.6±1.62	28.3±2.11	25.1±1.04
	50	23.2±0.74	31.1±1.33	26.4±0.79
	100	23.1±0.93	33.5±2.05	29.3±0.94
F10	25	15.3±1.61	22.4±0.87	19.2±1.17
	50	17.0±0.91	15.2±1.13	17.2±0.86
	100	18.4±1.18	23.8±1.25	21.8±1.21
F11	25	23.6±1.37	33.1±1.38	27.4±1.02
	50	25.3±0.74	35.0±1.15	29.2±1.14
	100	25.7±1.31	34.6±1.07	29.5±0.87
Ciprofloxacin		31±1.02	35±0.93	32±1.05
Ampicillin		29±0.87	38±1.15	33±0.96

All values are taken as mean±SEM (n=3)

The replacement of the methoxy group at ring A decreases the response in comparison to other trifluoromethyl groups at ring B in the flavone derivatives. The standard antibiotics ciprofloxacin and ampicillin showed a significant response on both Gram positive and Gram negative bacteria.

On the other hand, the simple flavone and the methyl containing flavones at ring B also showed significant response in comparison to other flavone derivatives. It is also worth mentioning that a resistance was observed by *S. aureus and P. aeruginosa* in some of the halogenated flavones that showed the ineffectiveness of these compounds. The order of response with respect to the zone of inhibition **is F11, F9, F10, F1, F3, F2, F4, F7, F6, F5, F8.**

Table 2 illustrates the minimum inhibitory concentrations MIC (µg/ml) of the synthetic flavone derivatives against Gram positive and Gram negative bacteria. It is observed that flavone substituted at both rings (F11) possesses an inhibitory potential at low concentration against all tested bacteria which is reported to be 12.5, 6.25 and 6.25 (µg/ml), respectively, that is almost equal to the response of the standard ciprofloxacin. This attested that the introduction of halogen and trifluoromethyl moiety enhances the potential in comparison with simple flavones and other derivatives. The MICs in µg/ml of the tested flavones derivatives are given in Table 3.

The plants have an unlimited capability to produce aromatic substances. Generally, these aromatic compounds are phenols or oxygen-substituted molecules that naturally serve as a defensive tool against attacks by insects, herbivores and microorganisms [30]. An in-depth literature survey reported the significance of natural and synthetic flavonoid derivatives as regards antibacterial activity [31–35]. In the present study, the antibacterial activities of synthetic flavone derivatives were compared with respect to zone of inhibition and minimum inhibitory concentration (MIC) values. From the results it is evident that there is a significant co-relationship between the presence of functional groups and the antibacterial response of the compounds. The present study illustrates an effort to predict the SAR of flavone derivatives with good activity against Gram-positive and Gram-negative bacteria.

Based upon the findings of this study, it can be concluded that the presence of a halogen group at ring A and a trifluoromethyl group at ring B produces flavones (**F9, F10** and **F11**) with potent activity.

Apart from simple flavone (**F1**), addition of methyl and dimethylamino groups at ring B (**F3** and **F2**) also showed promising results against all bacteria. On the other hand, halogenated substituted flavones at ring B (**F4-F8**) showed almost minimum activity.

Table 3. Antibacterial activity (minimum inhibitory concentration) of compounds F1–F11.

Flavone	MIC (µg/ml)		
	Gram-positive bacteria		Gram-negative bacteria
	B.subtilis	*S.aureus*	*P.aeruginosa*
F1	25	37.5	25
F2	75	75	62.5
F3	62.5	50	50
F4	87.5	87.5	100
F5	100	100	100
F6	125	125	100
F7	75	87.5	87.5
F8	>125	>125	>125
F9	12.5	25	25
F10	50	50	37.5
F11	12.5	6.25	6.25
Ciprofloxacin	6.25	6.25	6.25

Several antibacterial mechanisms of action have been assigned to flavonoids.

Among those, the possible mechanisms of action are inhibition of nucleic acid synthesis by inhibition of the enzymes topoisomerase and DNA gyrase; causing pores in the membrane or reduction in fluidity; damage of the cytoplasmic membrane, inhibition of the cellular metabolism, resulting from the inhibition of the enzyme NADH-cytochrome C reductase; inhibition of cell membrane synthesis; inhibition of cell wall synthesis caused by D-alanine/D-alanine ligase inhibition and aggregation of bacterial cells [36-39].

CONCLUSIONS

Researchers are constantly designing and synthesizing new anti-infective molecules

worldover. The dilemma of resistance to these agents is on the rise and there is a dire necessity for the discovery of new agents with antibiotic activity against the resistant bacterial strains. The present study is an effort to assess flavone derivatives as potential drug candidates for antibacterial activity. The SAR study of flavone derivatives with antibacterial potentials revealed a correlation between the presence of additional functional groups at different positions of the flavone ring A and B structure and the antibacterial activity. Of the compounds included in this study, **F9, F10** and **F11** were found to be the most active compounds. Thus, these three compounds can act as future potential candidates to develop newer synthetic antibacterial agents. These new molecules can either be used alone or in combination with other antibiotics to combat infections and would reduce the effective dose to be administered.

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СИНТЕТИЧНИ ФЛАВОНОВИ ПРОИЗВОДНИ. АНТИБАКТЕРИАЛНИ СВОЙСТВА И ВРЪЗКА МЕЖДУ АКТИВНОСТ И СТРУКТУРА

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(Резюме)

Синтезирани са с висок добив биологично активни флавонови производни с антибактериален потенциал от кетони с различни алдехиди чрез кондензация на Claisen-Schmidt. Структурите им са установени чрез различни спектроскопски методи: ^{1}H ЯМР, ^{13}C ЯМР, ИЧ и елементен анализ. Резултатите показват, че някои от заместените флавони проявяват по-висока антибактериална активност от простите флавони по отношение на тяхната минимална концентрация на инхибиране (MIC) и са потенциални кандидати за лечението на широк спектър от инфекциозни заболявания.