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Synthesis, crystal studies and biological evaluation of flavone derivatives

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Abstract: Three substituted flavone derivatives have been synthesized from substituted O-hydroxy acetophenones and 4-trifluoromethyl benzaldehyde in good yield. These compounds were characterized by NMR spectroscopy and single crystal X-ray Diffraction. Compound F1 and F3 were re-crystallized from their concentrated solutions in chloroform ethyl acetate mixture while F2 was re-crystallized in ethyl acetate *n*-hexane mixture. Compound F1 and F3 are monoclinic (space group $P2_1/c$) with lattice parameters: [*a*, *b, c* (Å) / β (°)] = 13.332 (2), 15.616 (2) / 6.2898 (8) and 13.9716 (15), 7.1868 (7), 13.6912 (14) / 91.113(6) respectively. Compound F2 is Triclinic (space group *P*-1) and has lattice parameters: [*a*, *b, c* (Å) / α, β, γ (°)] = 6.5002 (6), 8.3801 (9), 13.5989 (14) / 89.348(5), 85.141(4), 84.521(5). Antioxidant, antibacterial and cytotoxic profile was investigated. The compounds showed moderate to less activity on 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Hydrogen peroxide (H_2O_2) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) models of radical scavenging activity while promising antibacterial potentials were recorded. Furthermore, these molecules can also be used as potential candidates for new antitumor agents.

Keywords: Flavone, x-ray diffraction, antioxidant, antibacterial, cytotoxic.

INTRODUCTION

Human body has an inborn antioxidative mechanism that is responsible for loads of biological properties like antiaging, anticancer and antimutagenic responses (Gulcin 2012, Gocer *et al.,* 2011). Generally, the antioxidants deactivate or stabilize free radicals before attacking targets in biological system (Nunes *et al.,* 2012). Recently, desire in natural antioxidants has increased to a great deal for its use in cosmetics, food and pharmaceutical products (Djeridane *et al.,* 2006, Wannes *et al.,* 2010). The function of free radicals in many acute and chronic disorders, like diabetes, cancer, aging and inflammation in human beings is well established (Harman 1998).

Infectious diseases are among the main cause of morbidity and mortality worldwide. Progression in the innovation of antimicrobial agents has shielded 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 antimicrobial agents (Ozçelik *et al.,* 2011). The emergence of hitherto unidentified microbes that causes infections poses a colossal health concern regarding the combat towards infectious diseases (Iwu, *et al.,* 1999). The need for new, valuable and inexpensive drugs for the treatment of infectious diseases in the developing world is one of the major issues confronting global health today and consequently, has created a new dimension in the search for new drugs (Shah, 2005). Structural modification of anti-infective agents has confirmed to be an effective way of enhancing the lifespan of these agents (Poole, 2001).

Cancer is the second leading cause of death around the globe after heart diseases (Huang *et al.,* 2010; Reddy *et al.,* 2003). Despite momentous advancement and investment in the cancer chemotherapeutics, limited progress in the survival of patient has been achieved. Natural or semisynthetic compounds may be used to treat or prevent cancers (Reddy *et al.,* 2003). The brine shrimp lethality tool test is used as predictive study in order to reveal cytotoxicity and search for new anticancer agents. In this connection for the hunt a Taxol TM was isolated naturally from Taxus that served as new antitumor drug for treatment of ovarian, breast and lung carcinomas (Nonita and Mylene, 2010).

Flavonoids are phenolic compounds that are mostly present in fruits, vegetables and tea that are an integral part of human diet (Mohammad *et al.,* 2005; Harleen *et al.,* 2011). Their functions in human have been the core

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subject of deep research and have been reported to acquire numerous biological functions (Sebastian *et al.,* 2006). Synthetic as well as naturally occurring flavonoid derivatives have been known for a longtime to exert diverse biological effects both in animals and humans including vasodilatory actions (Maikai, 2011), antihepatotoxic and anti-ulcer actions (Bors 1990, Colerige *et al.,* 1988), antioxidant and radical scavenging activity (Zhang *et al.,* 2006), anticancer (Moon *et al.,* 2006), antiviral (Asres *et al.,* 2005), antimicrobial (Cushnie *et al.,* 2005), anti-inflammatory (Kim *et al.,* 2004), antiulcer (Wightman, 2004), antiallergenic (Middleton and Kandaswami, 1992) and analgesic (Ahmed *et al.,* 2007) properties. Most of the scientific reports are on the natural flavonoids and acquire a special place in natural and heterocyclic chemistry because of its 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 the flavonoid derivatives and an attempt to portray how the structure activity relationships (SAR) are made.

Keeping in view the pharmacological and chemical importance of natural and synthetic flavonoid derivatives, herein we report synthesis, characterization, crystal structures and antioxidant, antibacterial and cytotoxic potentials of three flavone derivatives. The synthesized compounds were structurally characterized in solid state by X-ray crystallography and Infra-red spectroscopy.

MATERIALS AND METHODS

Chemicals and instruments

All chemicals and preparative work was handled in normal atmosphere. Substituted ketones (o-hydroxy acetophenones) and benzaldehyde (4-trifluoro-mehtyl benzaldehyde) used were purchased from Sigma Aldrich Chemical Company. TLC plates were of Merck 60 F_{254}, Darmstadt Germany. Solvents and chemicals like ethanol, n-hexane, ethyl acetate were of extra pure analytical grade and were purchased from E. Merck.

^{1}H-NMR was recorded in deutrated chloroform (CDCl3) on Bruker SF spectrometers operating at 300 and 75 megahertz (MHz) frequencies respectively. Chemical shifts values are expressed in δ (ppm) downfield relative to TMS which was used as an internal standard. Infrared spectra were recorded on Thermo scientific USA (Nickolet 6700), and Infrared spectrometer on KBr disk method. All melting points are uncorrected and were taken in open capillary tubes using Electrothermal 9100 apparatus (Barnstead UK). Reaction extents and final products purities were checked on TLC plates (Merck 60 F_{254}, Darmstadt Germany) and spots were visualized under UV Lamp (180-365 nm) with subsequent staining with iodine vapours.

General procedure for the synthesis of flavone derivatives (F1, F2 and F3)

Three flavone derivatives were synthesized according to reported procedure (Mohammad *et al.,* 2015a). Briefly, equimolar quantities (15 mili mol) of 4-trifluoro-mehtyl benzaldehyde and substituted o-hydroxy acetophenones were taken in 20 ml ethanol and 5ml of 50% ethanolic KOH was added drop wise to this stirring solution. The reaction was kept on stirring for 16 hours and monitored with TLC. Upon completion of reaction the mixture was poured into ice cold water and neutralized with 1 N HCl. Solid products were obtained, filtered and washed thoroughly with excess of water.

In next step, the chalcones were then cyclized to respective flavones derivatives in dimethyl sufoxide (DMSO) at 120˚C (1 hour) in the presence of Iodine. The final products were purified with column chromatography in EtOAc *n*-hexane (3:7) and recrystalized in chloroform ethyl acetate mixture.

Bruker kappa APEXII CCD diffractometer was used for X-rays diffraction data at 296+2 K using graphite-monochromator Mo-Ka radiation (k = 0.71073 Å). Multi-scan absorption correction and ω-scan was used for collection of data. Final refinement on F^2 was carried out by full-matrix least-squares techniques. Structure refinements and solutions were carried out with WinGx (Farrugia, 1999), SHELXL-97 (Sheldrick, 2007), PLATON (Spek, 2009) and SAINT (Bob *et al.,* 2004).

Biological evaluation

Antibacterial activity

Antibacterial screening of flavones derivatives mentioned above were tested against gram-positive and gram-negative bacterial strains using agar well diffusion method. 20ml of sterile Mueller-Hinton Agar was poured in sterile glass Petri plates and allowed to solidify. The sterile cotton swab was dipped into the bacterial culture (10^6 to 10^8CFU/ml) and the agar plates were evenly inoculated by swabbing followed by wells formation using sterile cork-borer (6mm diameter). Each labeled well was filled with 100 µl of various concentrations of flavones derivatives and allowed to diffuse under cold condition for 30min. The plates were then incubated at 37ºC for 24 hrs. Plates in triplicate were prepared for each sample and the average zone of inhibition excluding well was recorded. DMSO was used as negative control. The antibacterial potential in the form of zone of inhibition in millimeters (mm) was compared with standard antibiotic ampicillin and ciprofloxacin (Olutiola *et al.,* 1991; Colle *et al.,* 1989; Mujeeb *et al.,* 2014).

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 broth dilution technique. Briefly, stock solution of each compound was prepared in DMSO and serial dilutions were prepared to achieve the desired concentrations range. To each of pre-identified sterile test tube containing specific concentration of test compound, a prescribed 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 hrs and examined for turbidity. A control tube containing no antimicrobial agent was also included and ciprofloxacin was used as standard. The lowest concentration required to stop the growth of bacteria was regarded as minimum inhibitory concentration (MIC) (Husain *et al.,* 2012).

In-vitro antioxidant activity

The free-radical scavenging activity of flavone derivatives was assessed using DPPH, H_2O_2 and ABTS methods.

DPPH radical scavenging activity

The antioxidant activity of the synthesized compounds, gallic acid, rutin and quercetin was measured with slight modifications using DPPH. 2% methanolic solution of DPPH was freshly prepared and 1ml from this solution was added to each 1ml different concentrations of the tested flavone derivatives ranging from 12.5-250µg/ml.

After 30 minutes of incubation, the absorbance was measured at 517 nm. Gallic acid, rutin and quercetin were used as a positive control. The scavenging ability was calculated (in triplicate) by the formula and IC_{50} was calculated graph pad prism software 5 version 5.01 software (Mohammad *et al.,* 2015b).

%scavenging = {(Abs control – Abs sample)/ (Abs control)} × 100

Where

$A_{control}$ = absorbance of DPPH +methanol

A_{sample} = absorbance of DPPH +sample/standard

Hydrogen peroxide scavenging activity

The antioxidant activity of the synthesized compounds, gallic acid, rutin and quercetin was measured with the slight modifications using hydrogen peroxide. A 2mM solution of hydrogen peroxide was prepared in phosphate buffer (50mM, pH 7.4). 0.1ml of flavone derivatives (12.5-250µg/ml) was transferred into test tubes and their volumes were made up to 0.4 ml with phosphate buffer or solvent. After careful addition of hydrogen peroxide solution (0.6 ml), tubes were then incubated for 10 minutes and were determined against a blank (50 mM phosphate buffer). Gallic acid, rutin and quercetin were used as a positive control (Rahmat *et al.,* 2012).

The scavenging ability was calculated (in triplicate) by the formula and IC_{50} was calculated graph pad prism software 5 version 5.01 software. %scavenging activity = {(Abs control – Abs sample)/ (Abs control)} × 100

Where

$A_{control}$ = absorbance of H_2O_2+methanol

A_{sample} = absorbance of H_2O_2+sample/standard

ABTS Radical Scavenging activity

The antioxidant activity of the synthesized compounds, gallic acid and quercetin was measured with the slight modifications using ABTS. Briefly, an ABTS solution was prepared by mixing solutions of 7 mM ABTS and 2.45 mM potassium persulphate and incubated in dark at 25 ˚C for 16 hrs. Prior to addition test samples of flavone derivatives, sufficient methanol was added to ABTS solution to acquire an absorbance of 0.700 ± 0.02 at 734 nm. Sample solutions of synthesized flavone derivatives were prepared in concentration range of 12.5-250µg/ml. The sample and standard solutions (0.1ml) was mixed with 1.9 ml of ABTS solution and after incubation of 3-5 minutes absorbance was measured at 734 nm. Experiment was performed in triplicate manner and IC_{50} was calculated graph pad prism software 5 version 5.01 software (Kumar *et al.,* 2014).

Brine shrimp lethality assay

The eggs from Brine shrimp (Artemia salina) were hatched in artificial seawater aerated with the help of an air pump at 27-30°C. A light source was left on the eggs and within 48h the nauplii hatched and were collected. The synthesized pure compounds and the standard were dissolved in appropriate solvent (DMSO) to get a stock solution. From the stock solution, different concentrations (µg/ml) were achieved by serial dilution and each concentration of 500µl was transferred into clean vials using pipette. Seawater having 10-20 nauplii was added. After 24h, check count was performed by counting the number of survivors and compared with survival in control solvent and reference cytotoxic drug in vials. The LC_{50} of synthesized compounds and standard drug was calculated (Mayer *et al.,* 1982; Niaz *et al.,* 2015).

Supplementary materials

The crystallographic data corresponding to compound F1, F2 and F3 have been deposited at the Cambridge Crystallographic Data Centre (CCDC) as supplementary publications Nos. CCDC 1038699, CCDC 1038700 and CCDC 1038701 respectively. This data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/ retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK

STATISTICAL ANALYSIS

Data are presented as mean ± SEM, (n=3) with GraphPad prism 5 version 5.01 software.

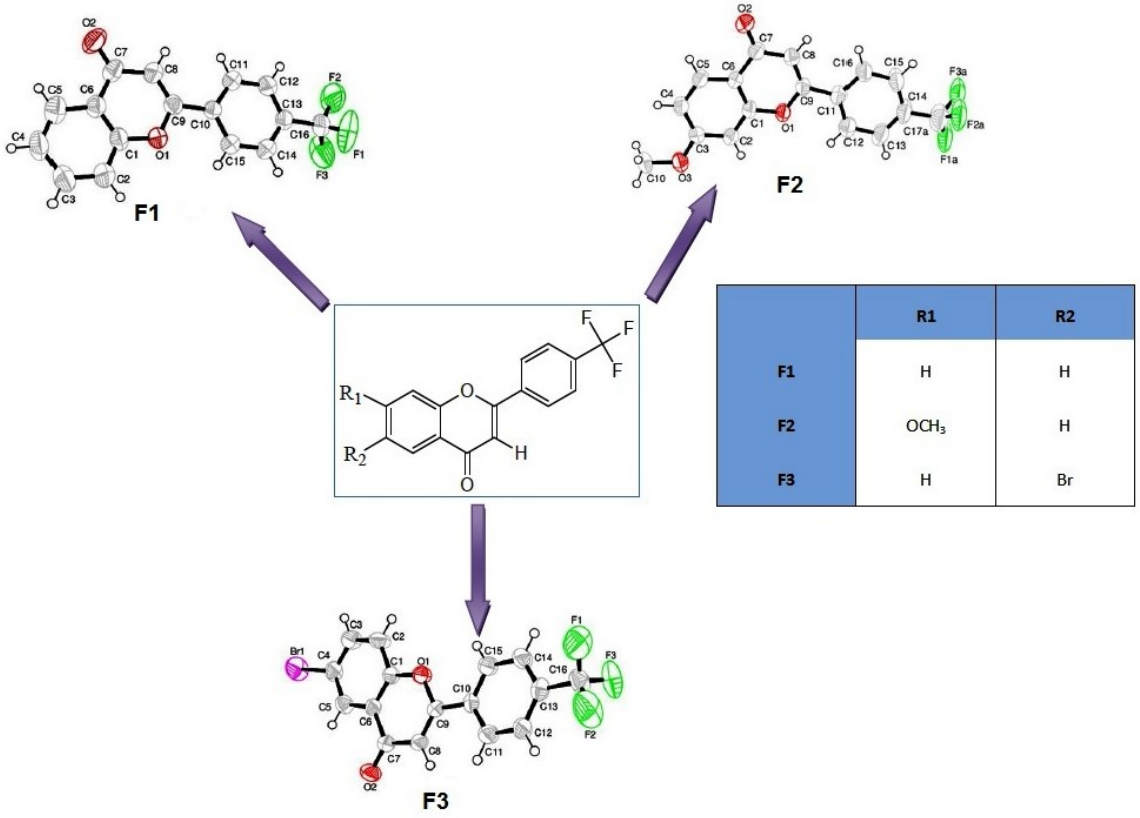
RESULTS

Spectroscopic analysis

The physical parameters of flavones derivatives are given in fig. 1 and table 1 while the spectroscopic parameters are given in experimental section of this study. The analytical data of reported flavones are compared with standard publications and is in accordance with the reported results. The compounds F1, F2 and F3 showed characteristic peaks for methene proton at C-3 of the chromone ring at variable of 6.88, 6.83 and 6.90 ppm respectively relative to TMS as singlets. This range is reported for many such flavonoid derivatives as well. Moreover, the methoxy group of F2 attached to C-7 of the chromone ring gave singlet of 3 protons at 3.97 ppm. ^{13}C NMR of all flavones show signals for the C=O group at 178.10, 177.56 and176.88cm^{-1}. The IR absorption peaks and elemental analysis data are listed in the spectral data of each compound.

***2-(4-(trifluoromethyl)phenyl)-4H-chromen-4-one* (F1).**

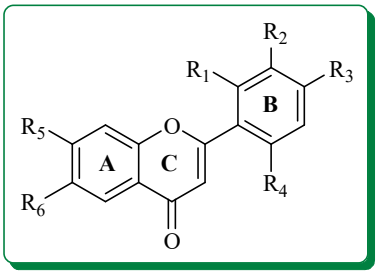
The compound was obtained from stirring equimolar amount of 2′-hydroxyacetophenone and 4-(trifluoromethyl) benzaldehyde in ethanol and then



	R1	R2
F1	H	H
F2	OCH_3	H
F3	H	Br

Fig. 1: Crystal structures of the synthesized flavone derivatives

Table 1: Physical parameters of 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	CF_3	H	H	H	85.3	Monoclinic crystals	0.63	134-137
F2	H	H	CF_3	H	OCH_3	H	83.4	Triclinic crystals	0.57	167-170
F3	H	H	CF_3	H	H	Br	85.7	Monoclinic crystals	0.71	171–173

Table 2: Summary of crystallographic data and structural parameters of flavone derivatives

Formula	F1	F2	F3
	$C_{16}H_9F_3O_2$	$C_{17}H_{11}F_3O_3$	$C_{16}H_8BrF_3O_2$
M_r	290.23	320.26	369.13
Crystal Size (mm)	0.40 × 0.20 × 0.18	0.42 × 0.24 × 0.20	0.34× 0.22 × 0.18
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	$P2_1/c$	P-1	$P2_1/c$
a, b, c (Å)	13.332 (2), 15.616 (2), 6.2898 (8)	6.5002 (6), 8.3801 (9), 13.5989 (14)	13.9716 (15), 7.1868 (7), 13.6912 (14)
α, β, γ (°)	90, 92.927 (9), 90	89.348(5), 85.141(4), 84.521(5)	90, 91.113(6), 90
V (Å)^3	1307.7 (3)	734.73(13)	1374.5 (2)
Z	4	2	4
$\Delta\rho$max., min (e.Å^{-3})	0.24, -0.19	0.18, -0.20	0.32, -0.27
Mo$K\alpha$ radiation, λ (Å)	0.71073	0.71073	0.71073
D_{calc},[g cm^{-1}]	1.474	1.448	1.784
F(000)	592	328	728
μ (mm^{-1})	0.13	0.12	3.03
Diffractometer	Bruker KAPPA APEX II	Bruker KAPPA APEX II	Bruker KAPPA APEX II
Data collection range	h = −16→16, k = −18→19, l = −7→7	h = −8→5, k = −10→10, l = −16→16	h = −17→16, k = −8→8, l = −16→16
No. of reflections	2561	2881	2701
No of parameters	190	237	199
Temperature (K)	296(2)	296(2)	296(2)
R_{int}	0.068	0.028	0.062
No. of measured, independent and observed [I>2 σ(I)] reflections	10055, 2561, 1010	10673, 2881, 1593	10574, 2701, 1453
GOF	0.997	1.009	1.026
R[F^2 > 2σ(F^2)], wR(F^2)	0.063, 0.161	0.055, 0.168	0.046, 0.115

Table 3: H-atom bonding parameters of flavone derivatives

Flavone	D—H···A	D—H	H···A	D···A	D—H···A
F1	C8—H8···O2^{i}	0.93	2.47	3.356 (5)	160
	C11—H11···O2^{i}	0.93	2.46	3.361 (5)	163
F2	C2—H2···O3^{i}	0.93	2.52	3.441 (3)	172.3
	C8—H8···O2^{ii}	0.93	2.53	3.455 (3)	175.0
F3	C2—H2···O2^{i}	0.93	2.62	3.381 (5)	139.4
	C8—H8···O2^{ii}	0.93	2.49	3.345 (5)	152.7

Symmetry code F1: (i) −x, −y+1, −z+2.
Symmetry codes F2: (i) −x, −y+1, −z; (ii) −x+2, −y+2, −z.
Symmetry codes F3: (i) x, −y+3/2, z+1/2; (ii) −x, −y+1, −z.

sodium hydroxide solution was added with continuous stirring to get a desire chalcone. In 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 (Klier *etal.,* 2012; Zhao *etal.,* 2013).

***2-(4-(trifluoromethyl)phenyl)-7-methoxy-4H-chromen-4-one* (F2).**

The compound was obtained from stirring equimolar amount of 2′-hydroxy-4′ methoxyacetophenone and 4-(trifluoromethyl)benzaldehyde in ethanol and then sodium hydroxide solution was added with continuous stirring to get a desire chalcone. In 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,

Table 4: Radical scavenging activity of flavone derivatives

Test Sample	Radical Scavenging activity IC_{50}(µg/ml)		
	(DPPH)	(H_2O_2)	(ABTS)
F1	174.15±2.14	205.53±1.93	196.43±1.48
F2	110.58±1.64	176.27±1.68	136.38±1.85
F3	103.39±1.38	108.47±1.83	168.86±1.43
Gallic Acid	28.46±0.73	10.35±0.89	12.16±1.02
Rutin	11.69±0.69	25.41±0.64	--
Quercetin	9.60±0.58	15.94±0.85	11.35±0.87

All the values were expressed as mean ±SEM (n=3).

Table 5: The antibacterial activity (Zone of inhibition) of flavone derivatives

Flavones	Concentration (µg/ml)	Zone of inhibition (mm)		
		Gram-positive Bacteria		Gram-negative bacteria
		B. subtilis	*S. aureus*	*P. aeruginosa*
F1	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
F2	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
F3	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	10 µg/disc	31±1.02	35±0.93	32±1.05
Ampicillin	10 µg/disc	29±0.87	38±1.15	33±0.96

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

Table 6: The antibacterial activity (Minimum inhibitory concentration) of flavone derivatives

Flavone	MIC (µg/ml)		
	Gram-positive Bacteria		Gram-negative bacteria
	B. subtilis	*S. aureus*	*P. aeruginosa*
F1	12.5	25	25
F2	50	50	37.5
F3	12.5	6.25	6.25
Ciprofloxacin	6.25	6.25	6.25

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 (Ghani *et al.,* 2013).

***6-bromo-2-(4-(trifluoromethyl)phenyl)-4H-chromen-4-one* (F3).**

The compound was obtained from stirring equimolar amount of 2′-hydroxy-5′ bromoacetophenone and 4-(trifluoromethyl)benzaldehyde in ethanol and then sodium hydroxide solution was added with continuous stirring to get a desire chalcone. In 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 (Ghani *et al.,* 2013).

Single crystal X-ray crystallography analysis

In X-ray crystallographic structures, the F1 molecule adopts a planar conformation, in which the O1, O4 and C16 atoms lie in the plane of the corresponding chromone and phenyl ring and the dihedral angle between the two rings is 4.55˚. The molecules of F1 are dimerised due to

C-H---O type of H-bonds interactions and form $R_2^2(8)$ and $R_2^1(7)$ loops. The crystal packing is stabilized through repeated weak C8-H and C11-H---O2 interactions. Similarly, the F2 and F3 molecule adopt a near planar conformation and the dihedral between chromone and phenyl rings are 5.75˚ and 9.17˚ respectively. The dihedral angle (7.16˚) between C3, O3, C10 (terminal methoxy group) and chromone/ phenyl ring of F2 also adopts a planer conformation and its molecules are dimerised and interlinked with each other through repeated C3-H---O3 and C8-H, C16-H---O2 interactions and form $R_2^2(8)$ and $R_2^1(7)$ loops while the molecules of F3 are dimerised due to C-H---O type of H-bonds interactions between C8-H---O2 interactions as shown in fig. 1. Summarized crystallographic data, structural and H-atom bonding parameters of F1, F2 and F3 are given in table 2 and table 3.

Biological evaluation

Antioxidant activity

In present study, all the synthesized flavone derivatives were found to possess moderate to less antioxidant activities when assessed on DPPH, H_2O_2 and ABTS free radical scavenging methods.

The results in IC_{50} are given in table 4. The DPPH free radical scavenging activity of the compound F1 and F2 showed good activity with IC_{50} value of 174.15±2.14 and 110.58±1.64µg/ml respectively while compound F3 showed better activity with IC_{50} value of 103.39±1.38µg/ml in comparison to gallic acid, rutin and quercetin as standard with IC_{50} values of 28.46±0.73, 11.69±0.69 and 9.60±0.58 respectively in DPPH model.

Similarly in H_2O_2 free radical antioxidant activity the compound F1 and F2 showed antioxidant activity with IC_{50} value of 205.53±1.93 and 176.27±1.68µg/ml respectively, while F3 showed considerably good activity with IC_{50} value of 108.47±1.83 µg/ml in comparison to gallic acid, rutin and quercetin as standard with IC_{50} values of 10.35±0.89, 25.41±0.64 and 15.94±0.85 respectively. Similar type of results was obtained in ABTS analysis in comparison to gallic acid and quercetin.

Table 7: The brine shrimp lethality of flavone derivatives

Flavone	LC_{50} (µg/ml)
F1	91.65±3.96
F2	47.42±6.24
F3	75.12±4.19

All the values were expressed as mean ±SEM (n=3).

Antibacterial activity

Results of the antibacterial activity of synthetic flavones in respect to its zone of inhibition are given in table 5. 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. The replacement of methoxy at ring A decreases the response in comparison to other trifluoromethyl group at ring B in flavone derivatives. The standard antibiotics ciprofloxacin and ampicillin showed a significant response on both Gram positive and Gram negative bacteria.

Table 6 illustrates the *Minimum inhibitory concentrations* MIC (µg/ml) of synthetic flavones derivatives against Gram positive and Gram negative bacteria. It is observed that substituted flavone at both rings (F3) possess inhibitory potentials 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 standard ciprofloxacin. It is assumed that the addition of halogen and trifluoromethyl moiety enhances the potentials in comparison with simple flavones and other derivatives. The MICs in µg/ml of the tested flavones derivatives are given in table 6.

Brine shrimp lethality test

The brine shrimp bioassay is a simplest, less expensive and easily achievable method replacing cell lines bioassay in order to determine the toxicity of samples by the estimation of their medium lethality concentration LC_{50}. Results of the brine shrimp lethality of synthetic flavones in respect to its LC_{50} are given in table 7. It is concluded from the results that substitution in the ring A and ring B may increase or decrease the lethality response.

Results from the study reveal that addition of methoxy (OCh_3) at ring A and trifluoromethyl at ring B in flavones (F2) enhances the lethality response against shrimps with LC_{50}=47.42±6.24. Moreover, the addition of halogen (Br) at ring A and trifluoromethyl at ring B (F3, LC_{50}=75.12±4.19) also enhances the potentials in comparison to F1 (LC_{50}=91.65±3.96).

DISCUSSION

Reactive oxygen species (ROS) and free radicals can cause in the oxidative damage to various biomolecules including proteins, DNA, lipids and cell membranes (Farber, 1994) that leads to the development of cancer, coronary heart diseases, hypertension, diabetes and neurodegeneration (Willcox *et al.,* 2004, Puntel *et al.,* 2006). The compounds having potentials to scavenge the free radicals acquire great capabilities in ameliorating these pathological conditions (Di and Esposito 2003, Behera *et al.,* 2006). Flavonoids are probably the largest of natural phenolic compounds (Shimoi *et al.,* 1996) that have antioxidant properties (Urquiaga and Leighton 2000, Mladenovic *et al.,* 2011) and act as effectual scavenger of harmful free radicals and ROS (Halliwell *et al.,* 1995,

Wang) and produce several biological responses including anticancer, antiaging and anti-inflammatory (Steinmetz *et al.,* 1996).

The antioxidant response depends on the location and number of hydroxyl groups of the flavone ring, the relationship between absorbing capacity of peroxyl radical and the number of hydroxyl groups (Cao *et al.,* 1997). The low activity is partly due to lack of hydroxyl substituents on both rings of the compounds and partly due to unavailability of the proton donor specie in their structures (Qin *et al.,* 2008). However the inclusion of methoxy and halogen increased the activity from moderate to good.

The activity decreased in the order of F3 (IC_{50}=103.39±1.13) >F2 (IC_{50}=110.58±1.64) >F1 (IC_{50}=174.15±2.14) in DPPH model. Similar results were also produced in H_2O_2 and ABTS models. Based upon the reported results, it is concluded that due to lack of hydroxyl and proton donor groups in the synthesized flavones derivatives, the compounds showed poor antioxidant activities.

Several antibacterial mechanisms of action have been assigned to flavonoids. Among these, the possible mechanisms of action can be inhibition of nucleic acid synthesis of bacteria by the inhibition of enzymes involved in replication including topoisomerase and DNA gyrase; causing pores in membrane or reduction in fluidity; damage in cytoplasmic membrane, inhibition of cellular metabolism, resulting from 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 (Cushnie and Lamb, 2005; Cushnie and Lamb, 2011; Gordon Wareham, 2010; Wu *et al.,* 2008).

Based upon the findings of this study, it can be concluded that presence of the halogen group at ring A and trifluoromethyl group at ring B produce flavones (F1, F2 and F3) with potent antibacterial activity against selected bacterial strains. Moreover, these molecules can also serve as potential candidate for its further validation against cell lines. These findings may help future research to look for promising anticancer agents.

CONCLUSION

Researchers are constantly designing and synthesizing the new molecules as antioxidant, antiinfective and antitumor agents worldwide. The present study is an effort to assess flavones derivatives as potential drug candidates for lead molecules. The SAR study of flavone derivatives with biological potentials revealed a correlation between the presence of additional functional groups at different positions of the flavone ring A and B structure and antibacterial activity. Flavones F1, F2 and F3 included in this study were found to be the most active compounds and can act as future potential candidates to develop newer synthetic antioxidant, antiinfective and antitumor agents. These new molecules can either be used alone or in combination with other molecules to combat infections and other life threatening diseases and would reduce the effective dose to be administered.

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