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Synthesis and Characterization of Variably Halogenated Chalcones and Flavonols and Their Antifungal Activity

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Three series of mono and di-halogenated chalcones and flavonols were synthesized according to the Claisen-Schmidt and Algar-Flynn-Oyamada reactions, respectively. Halogenated chalcones were prepared by condensing equimolar quantities of halogenated hydroxyacetophenones with halogen-substituted benzaldehydes, in the presence of aqueous alcoholic alkali. The resultant chalcones were immediately reacted with H_2O_2 in the presence of a mixture of methanol and aqueous sodium hydroxide to obtain the corresponding halogenated flavonols. The synthesized halogenated flavonoids were characterized on the basis of their physical properties and spectroscopic data. All the synthesized compounds were tested for their antifungal activity against a number of test organisms. Most of the flavonoids were found to be active against the tested fungi and in some cases even stronger than the standard antifungal drugs. Increased activity was found in those flavonoids in which the B-ring is halogenated. The antifungal activity increased as the ring is substituted with more electronegative halogens such as fluorine. However, it was noticed that chalcones having a phenolic hydroxyl in ring A at 2' position, showed more activity than that of its corresponding flavonol, in which the 2'-OH is lost on cyclization of C-ring.

Key Words: Halogenated, Chalcones, Flavonols, Antifungal activity.

INTRODUCTION

Flavonoids isolated from natural sources have been reported to possess a wide range of biological activities. The pharmaceutical importance of these compounds resides in the fact that they can be effectively utilized as antibacterial, antifungal, anti-inflammatory, antiviral, antioxidant, anti-allergic, antimalarial and many other agents[1-7]. Amongst the major classes of flavonoids, chalcones have been reported to be very active compounds and some have been reported to be responsible for inhibiting aldose reductase (ALR_2) enzymes[8]. Some other chalcones have pharmaceutical use in diagnosis and treatment of cancer and inflammation[9]. Schiff

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bases of chalcones have also shown strong antibacterial activity against *Escherichia coli, Phylococcus aureus*[10]. 4-Carboxyvinylene chalcone showed antifungal activity[11] and a related chalcone isolated from *Didymocarpus pedicellate* inhibited the spore germination of fungi by inhibiting the phytopathogenic bacterium[12]. Some other chalcones have shown strong antibacterial activity against *Symphoricarpos albus in vitro*[13]. Similarly, flavonols form another very important class of flavonoids and have a wider range of biological activities. For example quercetagetin-3,7,3'-trimethylether is an antiviral agent[14] while quercetin exhibits strong radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anions and hydroxyl radical[15]. Flavonols also inhibit the development of intestinal carcinoma and are administered to patients with uncerative colitis, who have an elevated risk of colorectal carcinogenesis[16]. Kaempferol and its glycosides show antiviral activity against human cytomegalovirus (HCMV) and some derivatives of quercetin are also used to inhibit the activity of cyclin dependent kinase[17]. Natural flavonoids are commonly substituted at variable positions, mainly by hydroxyl, methoxyl, isoprenyl and glycosyl groups. It is believed that the source of biological activities of chalcones and flavonols is due to the presence of these substituents. On the other hand it has also been found that halogenated organic compounds also show strong biological activities[18]. Since halogenated chalcones and flavonols are not found in nature, it was considered appropriate to synthesize these two classes of flavonoids with halogen substituents and to study the effect of halogenation on potential of their biological activities.

EXPERIMENTAL

All melting points were determined in open capillaries using Gallenkemp melting point apparatus and are uncorrected. R_f values were recorded by using pre-coated silica gel aluminium backed plates Kieselgel 60 F_{254} Merck (Germany), in ethyl acetate: pet-ether (40-60ºC) (1:9). The FTIR spectra were recorded on Bio-red spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded on Bruker (400 MHz) AM-250 in CD_3OD solution using TMS as internal standard. EIMS was recorded on MAT-311-A machine. Antifungal tests were performed at the HEJ Research Institute of Chemistry, University of Karachi, Pakistan.

Procedure for the synthesis of 1-(5'-chloro-2'-hydroxyphenyl)-3-(4-bromophenyl)-2-propen-1-one ($5C_1$) and 2-(4'-bromophenyl)-6-chloro-3-hydroxy-4-oxo-4*H*-1-benzopyran ($6F_1$)

30 g (768 mmol) *p*-Chlorophenol (**1a**) was added to a mixture of 75 mL acetic anhydride (768 mmol) and 65 mL pyridine (816 mmol) under

inert atmosphere of nitrogen. The mixture was heated at 100ºC for 3 h. To the cooled reaction mixture, water was added and acidified with dilute HCl and extracted with diethyl ether. The organic fraction was washed with saturated aqueous solution of $NaHCO_3$, dried on anhydrous $MgSO_4$ and the solvent was evaporated to give *O*-acetyl-*p*-chloro phenol (**2e**) as colourless oil. *O*-Acetyl-*p*-chlorophenol (**2e**) (34 g, 199 mmol) was added dropwise on anhydrous $AlCl_3$ 40 g (293 mmol) and the mixture was heated at 130ºC for 3 h. To the cooled reaction mixture crushed ice was added slowly and the resulting solution was extracted with diethyl ether. The organic fraction was dried over anhydrous $MgSO_4$ and solvent was evaporated to obtain oil. Distillation under reduced pressure gave 25 g of 5-chloro-2-hydroxyacetophenone (**3i**). 1.7 g (0.01 mol) 5-chloro-2-hydroxyacetophenone (**3i**) and 1.85 g (0.01 mol) *p*-bromobenzaldehyde (**4n**) were added in 10 mL of absolute ethanol. The reaction mixture was heated up to 50ºC and 5 mL of 50 % aq NaOH solution was added slowly with shaking. The reaction mixture was kept at room temperature for 8 h. Then crushed ice was added to the solid mass and neutralized with dil HCl. The product was obtained as yellow precipitate, which was filtered and recrystallized from aqueous EtOH. The chalcone *i.e.* 1-(5'-chloro-2'-hydroxyphenyl)-3-(4-bromophenyl)-2-propen-1-one (**5C$_1$**) was thus obtained as yellow needles. 1-(5'-Chloro-2'-hydroxyphenyl)-3-(4-bromophenyl)-2-propen-1-one (**5C$_1$**) (1 g) was added to a mixture of MeOH (20 mL) and 20 % aqueous sodium hydroxide (3 mL). The reaction mixture was kept in an ice bath at 0ºC and 18% H_2O_2 (3 mL) was added dropwise with constant stirring. After 24 h of refrigeration, it was acidified with cold dilute aqueous HCl. The precipitates formed were filtered off and yellow needles of the flavonol 2-(4'-bromophenyl)-6-chloro-3-hydroxy-4-oxo-4*H*-1-bezopyran (**6F$_1$**) were obtained after recrystallization from aqueous EtOH.

Antifungal activity

Chalcones (**5C$_1$-C$_{15}$**) and flavonols (**6F$_1$-F$_{15}$**) were tested by agar tube dilution protocol[19] for their *in vitro* fungicidal bioassay. Results were reported as linear growth inhibition (LGI) against some human pathogens, *e.g. Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata*. Linear growth inhibition results of halogenated chalcone and flavonols are given in Table-7. For this purpose nine tubes are taken, three each for control, reference drug and the sample to be tested for antifungal activity. In each set of tubes, 4 mL of the sterilized sabaraud dextrose agar media (adjusted to pH 5.6) was poured. 200 µg of the sample and the reference drug (in DMSO) were mixed, respectively in the sample and the reference drug set of tubes. At this stage, all the tubes were placed in the slant position and were allowed

to solidify. 4 × 4 mm pieces of fungus culture were removed from the petri dish, previously grown on sabaraud dextrose agar media and were placed in the center of all the three set of tubes. The tubes were then incubated at 28º ± 1ºC for 48 h. Linear growth inhibition (LGI) was measured by vernier caliper.

Antifungal activity analysis of A and B ring di-halogenated chalcones and flavonols show significant activity against *T. longifusus, A. flavus* and *M. canis*, but do not exhibit significant activity against *C. albicans, F. solani* and *C. glaberata*. Chalcone **5C$_1$** having chloro atom at A-ring and bromo atom at B-ring, the linear growth inhibition against *T. longifusus* is 52.6 % whereas standard drug miconazole shows 70 % inhibition. However, when bromo in ring B is replaced by fluoro in **5C$_4$** and chloro in **5C$_8$**, inhibition is increased to 84.2 and 68.4 %, respectively. When the chalcone **5C$_1$** is converted into its respective flavonol **6F$_1$**, percentage inhibition increases from 52.6 to 60.3 %. This increase in activity is probably due to the formation of C-ring of flavonol. In case of chalcones, it is observed that percentage inhibition increases as the electronegativity of the halogen of B-ring increases. Other factor may be due to the size of the halogen atom. Smaller the size of halogen atom, higher is the percentage inhibition against *T. longifusus*.

The chalcones **5C$_1$** and **5C$_4$** are inactive against *A. flavus* but **5C$_8$** show an inhibition of 50.5 %, as compared to the standard drug amphotericin B (20 %). The respective flavonol **6F$_1$** shows 40.7 % inhibition. The chalcone **5C$_1$**, **5C$_4$**, **5C$_8$** and flavonol **6F$_1$** show significant inhibition against *M. canis* as compared to the standard drug miconazole, which has a value of 98.4 %. Other A and B-ring di-halogenated chalcones and flavonols did not show any significant antifungal activity against any of the strains.

Amongst the A-ring mono-halogenated chalcones and flavonols (**5C$_{10}$-C$_{12}$** and **6F$_{10}$-F$_{12}$**), only **5C$_{10}$** and **6F$_{10}$** showed significant activity against *T. longifusus* and *M. canis*, but were inactive against *C. albicans, F. solani, A flavus* and *C. glaberata*. From Table-7 it is noted that chalcone **5C$_{10}$** show linear growth inhibition of 89.4 % and its respective flavonol **6F$_{10}$** show moderate activity (30%) against *T. longifusus*. Similarly **5C$_{10}$** and **6F$_{10}$** show activities of 85.2 and 65.2%, respectively against *M. canis* as compared to the standard drug miconazole that is 98.4 %. In this case, when the B-ring is non-halogenated, flavonol show less inhibition as compared to its corresponding chalcone. However, it is observed that a di-halogenated flavonol show higher inhibition with respect to its chalcone. It can thus be concluded that B-ring halogen increases the activity of the flavonol; only C-ring is not responsible for its enhanced antifungal activity. Other A ring di-halogenated chalcones and flavonols did not show any significant antifungal activity against any of the strains.

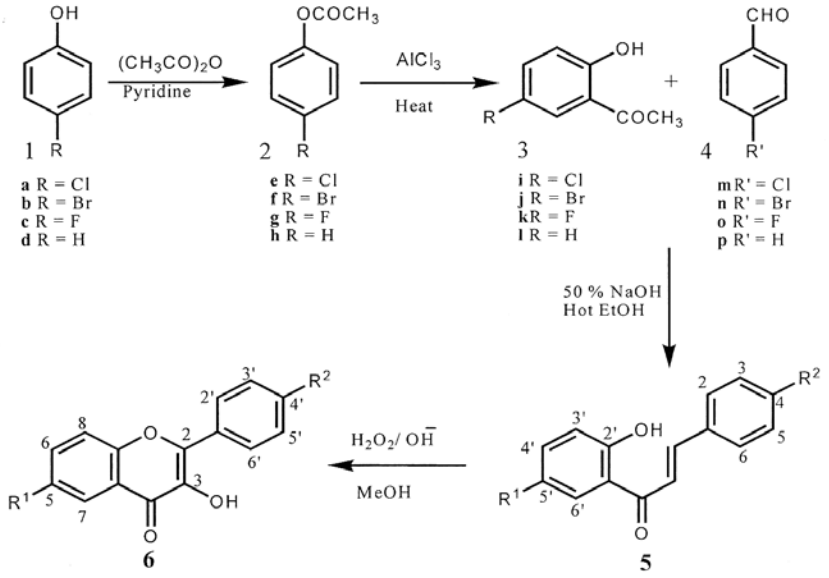
Table-7 also indicates that chalcones halogenated only in B-ring (**$5C_{13}$-C_{15}**) show linear growth inhibition of 89.4, 71.5 and 84.2 %, respectively. Similarly flavonols **$6F_{14}$** and **$6F_{15}$** both show higher inhibition against *T. longifusus* than standard antifungal drug miconazole. Further more, **$5C_{13}$**, **$5C_{14}$** and **$5C_{15}$** show significant activity of 90.2, 65.4 and 75.3 %, respectively agsinst *M. canis*. Whereas flavonols **$6F_{14}$** and **$6F_{15}$** show moderate inhibition as compared to the standard drug miconazole (98.4 %) against the same fungus. It is observed that the fluorinated chalcone in this series is most active against both the fungi but no such change is observed in flavonols. Chalcone **$5C_{14}$** is inactive but its respective flavonol **$6F_{14}$** shows 85.2% inhibition against *A. flavus* as compared to the standard drug amphotericin B (20%). This increase in active may also be due to the cyclization of the C-ring of flavonol. All other B-ring di-halogenated chalcones and flavonols remained inactive against any of the strains.

RESULTS AND DISCUSSION

In this paper syntheses of nine (**$5C_1$-C_9**) A and B ring di-halogentated, three (**$5C_{10}$-C_{12}**) A ring mono-halogenated and three (**$5C_{13}$-C_{15}**) B ring mono-halogenated chalcones as well their fifteen (**$6F_1$-F_{15}**) corresponding flavonols are described. A and B-ring di-halogenated chalcones were prepared by acetylating *p*-halophenols (**1a-c**) to *O*-acetyl-*p*-halophenols (**2e-g**) that in turn were converted to 5-halo-2-hydroxyacetophenones (**3i-k**) by Fries rearrangement. The latter intermediates on reaction (one by one) with *p*-halobenzaldehydes (**4m-o**) gave nine 1-(5'-halo-2'-hydroxy phenyl)-3-(4-halophenyl)-2-propen-1-ones or chalcones (**$5C_1$-C_9**). For A and B-ring mono-halogenated chalcones (**$5C_{10}$-C_{12}** and **$5C_{13}$-C_{15}**), 5-halo-2-hydroxyacetophenones (**3i-k**) were reacted with simple benzaldehyde (**4p**) and 2-hydroxyacetophenones (**3I**) with *p*-halobenzaldehydes (**4m-o**), respectively. The corresponding flavonols (**$6F_1$-F_{15}**) were prepared by oxidizing all the fifteen chalcones with H_2O_2 in the presence of methanol and NaOH solution (**Scheme-1**).

The chalcone (**$5C_1$**) *i.e.* 1-(5'-Chloro-2'-hydroxyphenyl)-3-(4-bromophenyl)-2-propen-1-one was synthesized by treating 5-chloro-2-hydroxyacetophenone and *p*-bromobenzaldehyde in hot ethanol under basic condition. The molecular formula and molecular weight of this compound are $C_{15}H_{10}BrClO_2$ and 336, respectively. The FTIR spectrum showed stretching frequency at 1637, 1563 and 3445 cm^{-1} which are characteristics of (C=O), (C=C) and (OH), respectively. The mass spectrum of (**$5C_1$**) showed chalcone and flavanone type of molecular ion peaks $[M]^+$, $[M+2]^+$ and $[M+4]^+$ at m/z 336, 338 and 340, respectively with relative isotopic abundances of Cl and Br which are found in good agreement with molecular weight. The peaks at m/z 335, 337 and 339 appeared due to loss

of hydrogen radical from B-ring and its cyclization with oxygen of the carbonyl group. The characteristics peaks at m/z 257 and 259 appeared due to loss of Br radical from the molecular ion. The base peak in the mass spectrum appeared at m/z 154 which resulted from retro Diels-Alder ring fission of flavanone type of molecular ion. Other peaks at 181 and 183 are formed from flavanone type molecular ion due to loss of B-ring.



	R^1	R^2
F_1 =	Cl	Br
F_2 =	F	Cl
F_3 =	F	Br
F_4 =	Cl	F
F_5 =	Br	F
F_6 =	Br	Cl
F_7 =	F	F
F_8 =	Cl	Cl
F_9 =	Br	Br
F_{10} =	F	H
F_{11} =	Cl	H
F_{12} =	Br	H
F_{13} =	H	F
F_{14} =	H	Cl
F_{15} =	H	Br

	R^1	R^2
C_1 =	Cl	Br
C_2 =	F	Cl
C_3 =	F	Br
C_4 =	Cl	F
C_5 =	Br	F
C_6 =	Br	Cl
C_7 =	F	F
C_8 =	Cl	Cl
C_9 =	Br	Br
C_{10} =	F	H
C_{11} =	Cl	H
C_{12} =	Br	H
C_{13} =	H	F
C_{14} =	H	Cl
C_{15} =	H	Br

Scheme-1

^{1}H NMR spectrum of (**5C$_1$**) showed pair of doublets at δ 7.76 ppm and 8.01 with large coupling constant J = 15.6 and 15.6 Hz, respectively, which are characteristics of *trans* olefinic protons (α and β). The protons at position 2,6 and 3,5 showed doublets with ortho coupling at δ 7.87 ppm and 7.65, respectively, which are evident by J = 8.5 and 8.4 Hz. The δ values, multiplicity and J values of all protons of 1-(5'-chloro-2'-hydroxyphenyl)-3-(4-bromophenyl)-2-propen-1-one (**5C$_1$**) are presented in Table-1.

TABLE-1
^{1}H NMR DATA OF CHALCONES

Compd.	δ(ppm), Multiplicity, J (Hz), ^{1}H
C_1	δ7.87 (d, J = 8.5 Hz, 2H, H-2, H-6), 7.65 (d, J = 8.4 Hz, 2H, H-3, H-5), 7.76 (d, J = 15.6 Hz, 1H, H-α), 8.01 (d, J = 15.6 Hz, 1H, H-β), 7.03 (d, J = 8.8 Hz, 1H, H-3´), 7.57 (dd, J = 8.58 Hz, 2.5 Hz, 1H, H-4´), 8.23 (d, J = 2.5 Hz, 1H, H-6´)
C_2	δ7.82 (d, J = 7.8 Hz, 2H, H-2, H-6), 7.22 (d, J = 8.1 Hz, 2H, H-3, H-5), 7.72 (d, J = 15.5 Hz, 1H, H-α), 8.15 (d, J = 15.5 Hz, 1H, H-β), 7.42 (d, J = 8.1 Hz, 1H, H-3´), 7.21 (dd, J = 8.7 Hz, 5.3 Hz, 1H, H-4´), 7.85 (d, J = 2.5 Hz, 1H, H-6´)
C_3	δ7.89 (d, J = 8 Hz, 2H, H-2, H-6), 7.62 (d, J = 8.3 Hz, 2H, H-3, H-5), 7.53 (d, J = 15.2 Hz, 1H, H-α), 7.93 (d, J = 15.2 Hz, 1H, H-β), 7.45 (d, J = 8.5 Hz, 1H, H-3´), 7.22 (dd, J = 8.6 Hz, 5.4 Hz, 1H, H-4´), 7.83 (d, J = 2.4 Hz, 1H, H-6´)
C_4	δ8.1 (d, J = 8.6 Hz, 2H, H-2, H-6), 7.31 (d, J = 8.8 Hz, 2H, H-3, H-5), 7.81 (d, J = 15.5 Hz, 1H, H-α), 7.95 (d, J = 15.5 Hz, 1H, H-β), 7.00 (d, J = 8.8 Hz, 1H, H-3´), 7.57 (dd, J = 8.8 Hz, 2.5 Hz, 1H, H-4´), 8.24 (d, J = 2.5 Hz, 1H, H-6´)
C_5	δ7.99 (d, J = 8.4 Hz, 2H, H-2, H-6), 7.34 (d, J = 8.8 Hz, 2H, H-3, H-5), 7.82 (d, J = 15.4 Hz, 1H, H-α), 7.94 (d, J = 15.4 Hz, 1H, H-β), 6.97 (d, J = 8.7 Hz, 1H, H-3´), 7.66 (dd, J = 8.8 Hz, 2.1 Hz, 1H, H-4´), 8.33 (d, J = 2.4 Hz, 1H, H-6´)
C_6	δ7.85 (d, J = 8.9 Hz, 2H, H-2, H-6), 7.24 (d, J = 8.4 Hz, 2H, H-3, H-5), 7.84 (d, J = 15.0 Hz, 1H, H-α), 7.89 (d, J = 15.0 Hz, 1H, H-β), 7.01 (d, J = 9.1 Hz, 1H, H-3´), 7.61 (dd, J = 8.5 Hz, 2.8 Hz, 1H, H-4´), 8.27 (d, J = 2.6 Hz, 1H, H-6´)
C_7	δ7.91 (d, J = 8.4 Hz, 2H, H-2, H-6), 7.12 (d, J = 8.5 Hz, 2H, H-3, H-5), 7.70 (d, J = 15.6 Hz, 1H, H-α), 8.13 (d, J = 15.6 Hz, 1H, H-β), 7.41 (d, J = 8.1 Hz, 1H, H-3´), 7.24 (dd, J = 8.3 Hz, 5.6 Hz, 1H, H-4´), 7.83 (d, J = 2.4 Hz, 1H, H-6´)
C_8	δ7.82 (d, J = 7.8 Hz, 2H, H-2, H-6), 7.22 (d, J = 8.5 Hz, 2H, H-3, H-5), 7.56 (d, J = 15.2 Hz, 1H, H-α), 7.91 (d, J = 15.2 Hz, 1H, H-β), 7.08 (d, J = 8.0 Hz, 1H, H-3´), 7.36 (dd, J = 8.2 Hz, 1.4 Hz, 1H, H-4´), 8.21 (d, J = 2.3 Hz, 1H, H-6´)
C_9	δ7.89 (d, J = 8.1 Hz, 2H, H-2, H-6), 7.62 (d, J = 8.3 Hz, 2H, H-3, H-5), 7.74 (d, J = 15.4 Hz, 1H, H-α), 7.99 (d, J = 15.4 Hz, 1H, H-β), 6.96 (d, J = 8.6 Hz, 1H, H-3´), 7.59 (dd, J = 8.9 Hz, 2.7 Hz, 1H, H-4´), 8.31 (d, J = 2.1 Hz, 1H, H-6´)
C_{10}	δ7.27-7.43 (m, 5H, H-2, H-3, H-4, H-5, H-6), 7.79 (d, J = 15.3 Hz, 1H, H-α), 8.01 (d, J = 15.3 Hz, 1H, H-β), 6.78 (d, J = 8.1 Hz, 1H, H-3´), 7.06 (dd, J = 7.9 Hz, 1.8 Hz, 1H, H-4´), 7.21 (d, J = 2.1 Hz, 1H, H-6´)
C_{11}	δ7.36-7.53 (m, 5H, H-2, H-3, H-4, H-5, H-6), 7.84 (d, J = 15.4 Hz, 1H, H-α), 7.93 (d, J = 15.4 Hz, 1H, H-β), 6.97 (d, J = 8.9 Hz, 1H, H-3´), 7.75 (dd, J = 6.9 Hz, 1.8 Hz, 1H, H-4´), 8.12 (d, J = 2.1 Hz, 1H, H-6´)
C_{12}	δ7.21-7.30 (m, 5H, H-2, H-3, H-4, H-5, H-6), 7.81 (d, J = 15.7 Hz, 1H, H-α), 7.96 (d, J = 15.7 Hz, 1H, H-β), 6.95 (d, J = 8.1 Hz, 1H, H-3´), 7.68 (dd, J = 8.8 Hz, 2.5 Hz, 1H, H-4´), 8.31 (d, J = 2.3 Hz, 1H, H-6´)
C_{13}	δ7.95 (d, J = 8.6 Hz, 2H, H-2, H-6), 7.23 (d, J = 8.8 Hz, 2H, H-3, H-5) 7.84 (d, J = 15.2 Hz, 1H, H-α), 8.03 (d, J = 15.2 Hz, 1H, H-β), 7.31-7.93 (m, 4H, H-3´, H-4´, H-5´, H-6´)
C_{14}	δ7.93 (d, J = 8.5 Hz, 2H, H-2, H-6), 7.53 (d, J = 8.5 Hz, 2H, H-3, H-5) 7.81 (d, J = 15.4 Hz, 1H, H-α), 8.01 (d, J = 15.3 Hz, 1H, H-β), 7.01-8.10 (m, 4H, H-3´, H-4´, H-5´, H-6´)
C_{15}	δ7.85 (d, J = 8.3 Hz, 2H, H-2, H-6), 7.61 (d, J = 8.4 Hz, 2H, H-3, H-5) 7.51 (d, J = 15.2 Hz, 1H, H-α), 7.91 (d, J = 15.5 Hz, 1H, H-β), 7.21-8.10 (m, 4H, H-3´, H-4´, H-5´, H-6´)

Oxidative cyclization of 1-(5'-chloro-2'-hydroxyphenyl)-3-(4-bromophenyl)-2-propen-1-one (**5C_1**) afforded corresponding flavonol (**6F_1**) *i.e.* 2-(4'-bromophenyl)-6-chloro-3-hydroxy-4-oxo-4*H*-1-benzopyran or (6-chloro-4'-bromofavonol). The flavonol (**6F_1**) was found to have molecular formula of $C_{15}H_8ClBrO_3$ and molecular weight 350. The stretching

TABLE-2
^{1}H NMR DATA OF FLAVONOLS

Compd.	Δ(ppm), Multiplicity, J (Hz), ^{1}H
F_1	δ8.16 (d, J = 8.5 Hz, 2H, H-2′, H-6′) 7.77 (d, J = 8.6 Hz, 2H, H-3′, H-5′), 7.76, (d, J = 8.6 Hz, 1H, H-8), 8.15 (dd, J = 8.2 Hz, 2.5 Hz, 1H, H-7), 8.23 (d, J = 2.8 Hz, 1H, H-5)
F_2	δ8.20 (d, J = 8.7 Hz, 2H, H-2′, H-6′) 7.50 (d, J = 8.0 Hz, 2H, H-3′, H-5′), 7.10, (d, J = 8.2 Hz, 1H, H-8), 7.24 (dd, J = 8.2 Hz, 2.6 Hz, 1H, H-7), 7.45 (d, J = 2.8 Hz, 1H, H-5)
F_3	δ8.12 (d, J = 8.7 Hz, 2H, H-2′, H-6′) 7.41 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.15, (d, J = 8.4 Hz, 1H, H-8), 7.26 (dd, J = 7.9 Hz, 2.8 Hz, 1H, H-7), 7.48 (d, J = 2.5 Hz, 1H, H-5)
F_4	δ8.26 (d, J = 8.4 Hz, 2H, H-2′, H-6′) 7.39 (d, J = 8.1 Hz, 2H, H-3′, H-5′), 7.38, (d, J = 8.5 Hz, 1H, H-8), 8.25 (dd, J = 8.4 Hz, 2.5 Hz, 1H, H-7), 8.01 (d, J = 2.5 Hz, 1H, H-5)
F_5	δ8.27 (d, J = 8.5 Hz, 2H, H-2′, H-6′) 7.38 (d, J = 8.8 Hz, 2H, H-3′, H-5′), 7.35, (d, J = 8.7 Hz, 1H, H-8), 8.27 (dd, J = 8.5 Hz, 2.4 Hz, 1H, H-7), 8.12 (d, J = 2.5 Hz, 1H, H-5)
F_6	δ7.86 (d, J = 8.2 Hz, 2H, H-2′, H-6′) 7.21 (d, J = 8.1 Hz, 2H, H-3′, H-5′), 7.22, (d, J = 8.6 Hz, 1H, H-8), 7.51 (dd, J = 8.7 Hz, 2.5 Hz, 1H, H-7), 7.43 (d, J = 2.5 Hz, 1H, H-5)
F_7	δ8.25 (d, J = 8.5 Hz, 2H, H-2′, H-6′) 7.41 (d, J = 8.6 Hz, 2H, H-3′, H-5′), 7.13, (d, J = 8.5 Hz, 1H, H-8), 7.26 (dd, J = 9.2 Hz, 2.5 Hz, 1H, H-7), 7.89 (d, J = 2.2 Hz, 1H, H-5)
F_8	δ8.20 (d, J = 8.6 Hz, 2H, H-2′, H-6′) 7.54 (d, J = 8.1 Hz, 2H, H-3′, H-5′), 7.35, (d, J = 8.8 Hz, 1H, H-8), 8.24 (dd, J = 8.4 Hz, 2.4 Hz, 1H, H-7), 8.21 (d, J = 2.5 Hz, 1H, H-5)
F_9	δ8.01 (d, J = 8.0 Hz, 2H, H-2′, H-6′) 7.47 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.39, (d, J = 8.9 Hz, 1H, H-8), 7.98 (dd, J = 8.0 Hz, 2.3 Hz, 1H, H-7), 8.22 (d, J = 2.6 Hz, 1H, H-5)
F_{10}	δ7.21-7.40 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 7.42 (d, J = 8.8 Hz, 1H, H-8, 7.49 (dd, J = 8.0 Hz, 2.5 Hz, 1H, H-7), 7.61 (d, J = 2.7 Hz, 1H, H-5)
F_{11}	δ7.22-7.42 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 6.89 (d, J = 7.9 Hz, 1H, H-8, 7.28 (dd, J = 8.0 Hz, 2.5 Hz, 1H, H-7), 7.55 (d, J = 2.4 Hz, 1H, H-5)
F_{12}	δ7.24-7.46 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 6.80 (d, J = 8.5 Hz, 1H, H-8, 7.65 (dd, J = 8.1 Hz, 1.5 Hz, 1H, H-7), 7.91 (d, J = 2.4 Hz, 1H, H-5)
F_{13}	δ8.26 (d, J = 8.3 Hz, 2H, H-2′, H-6′), 7.64 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.43-8.12 (m, 4H, H-8, H-7, H-6, H-5
F_{14}	δ8.24 (d, J = 8.6 Hz, 2H, H-2′, H-6′), 7.64 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.43-8.10 (m, 4H, H-8, H-7, H-6, H-5
F_{15}	δ8.16 (d, J = 8.1 Hz, 2H, H-2′, H-6′), 7.64 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.40-8.15 (m, 4H, H-8, H-7, H-6, H-5

frequencies in FTIR spectrum at 3273 and 1630 cm^{-1} are characteristics of hydroxyl and ketone group of the flavonol. The mass spectrum of (**F_1**) showed molecular ion peaks $[M]^+$, $[M+2]^+$ and $[M+4]^+$ at m/z 350, 352 and 354, respectively. The molecular ion M+2 at m/z 352 is the base peak. The peak at m/z 349, 351 and 353 is due to loss of hydrogen radical from molecular ion. The peak at m/z 271 and 273 are due to loss of Br radical. The characteristic fragmentation pattern of flavonol showed peaks at m/z 154, 156, 183 and 185, which are due to retro Diels-Alder ring fission. The peaks at m/z 243, 245, 215 and 217 are formed by successive loss of CO from M-Br and M+2-Br fragments, respectively. ^{1}H NMR spectrum of 2-(4'-bromophenyl)-6-chloro-3-hydroxy-4-oxo-4*H*-1-benzopyran (**$6F_1$**) showed a singlet at δ 8.03 due to hydrogen at position 5, which did not show *meta* coupling due to the presence of chloro at position 6. The *ortho* coupled protons at 2', 6' and 3', 5' showed characteristics doublets at δ 8.16

ppm and 7.77, respectively. The doublets of H-7 and H-8, which are *ortho* coupled and are overlapped on the doublets of 2', 6' and 3', 5' protons due to same chemical shifts. The δ values, multiplicity and J values of all protons of 2-(4'-bromophenyl)-6-chloro-3-hydroxy-4-oxo-4*H*-1-benzopyran (**6F$_1$**) are presented in Table-2.

TABLE-3
FTIR AND EIMS SPECTRAL DATA OF CHALCONES

Compd.	FTIR (cm^{-1})	EIMS (m/z) %
C_1	3448, 1637, 1563, 667, 640	336** [M^+, 28], 335(16), 257(06), 182(45), 181(57), 155(33), 154(100), 133(16), 127(12).
C_2	3370, 1652, 1572, 1160, 705	276* [M^+, 52], 275(35), 259(11), 241(08), 165(23), 139(13), 138(100), 137(01), 111(9), 110(18).
C_3	3442, 1658, 1575, 1167, 660	320* [M^+, 31], 319(25), 303(08), 241(12), 209(10), 182(61), 181(25), 165(06), 155(20), 139(15), 138(100), 111(07), 110(20).
C_4	3375, 1643, 1578, 1167, 720	276* [M^+, 37], 275(23), 259(05), 241(05), 181(31), 155(28), 154(100), 149(20), 127(12), 126(18), 122(57), 121(22), 95(12).
C_5	3390, 1645, 1575, 11167, 660	320* [M^+, 45], 319(24), 303(05), 241(09), 225(22), 199(24), 198(100), 171(18), 170(21), 149(35), 122(71), 121(27), 95(26).
C_6	3442, 1643, 1560, 718, 667	336**[M^+, 26], 335(24), 308(18), 257(23), 225(32), 199(86), 198(100), 171(11), 170(08), 165(25), 138(07), 137(08), 111(10).
C_7	3470, 1647, 1581, 1157	260 [M^+, 259], 243(05), 165(23), 149(47), 139(100), 138(36), 122(58), 121(22), 111(07), 110(13), 95(05).
C_8	3317, 1642, 1587, 715	292** [M^+, 47], 291(12), 275(09), 257, 181(91), 165(81), 155(08), 154(100), 138(07), 137(16), 127(43), 126(41), 111(35).
C_9	3382, 1634, 1558, 668	380** [M^+, 32], 379(17), 363(13), 301(47), 255(11), 209(07), 199(16), 198(100), 182(64), 181(22), 171(51), 170(56), 155(18).
C_{10}	3412, 1658, 1568, 1169	242 [M^+, 30], 241(10), 225(08), 165(10), 139(100), 138(63), 131(05), 111(12), 110(12), 104(07), 103(21), 77(12).
C_{11}	3485, 1644, 1577, 725	258* [M^+, 73], 257(52), 241(07), 223(13), 181(58), 155(25), 154(100), 131(16), 127(19), 126(21), 104(57), 103(42), 77(41).
C_{12}	3366, 1642, 1579, 665	302* [M^+, 37], 301(16), 285(12), 245(18), 223(12), 199(31), 171(14), 170(04), 131(12), 104(05), 103(29), 77(19).
C_{13}	3470, 1643, 1599, 1135	242 [M^+, 24], 241(02), 225(41), 149(49), 147(14), 122(53), 121(100), 120(67), 95(13), 93(31), 92(05).
C_{14}	3399, 1663, 1581, 715	258* [M^+, 62], 257(61), 241(11), 223(10), 165(13), 147(100), 138(29), 137(12), 121(73), 120(95), 111(11), 93(29), 92(26).
C_{15}	3482, 1643, 1585, 669	302* [M^+, 40], 301(04), 285(09), 223(05), 209(22), 182(10), 181(10), 155(05), 147(14), 121(95), 120(81), 93(28), 92(43).

*M+ 2 peaks for isotopes of ^{37}Cl with intensity 1/3, *M+2 peaks for isotopes of ^{81}Br with intensity 1/1, ** M+2 and M+4 peaks for the isotopes of ^{37}Cl / ^{79}Br, ^{81}Br / ^{35}Cl and ^{37}Cl / ^{81}Br with intensities 2/3 and 1/3.

Structures of all other chalcones and corresponding flavonols were confirmed on the basis of their ^{1}H NMR, FTIR and EIMS data. ^{1}H NMR data of all chalcones and flavonols is listed in Tables 1 and 2, whereas FTIR and mass data is presented in Tables 3 and 4, respectively. Moreover, molecular formulae, melting points, yield and R_f values of chalcones (**5C$_1$-C$_{15}$**) and flavonols (**6F$_1$-F$_{15}$**) are listed in Tables 5 and 6, respectively.

TABLE-4
FTIR AND EIMS SPECTRAL DATA OF FLAVONOLS

Compd.	FTIR (cm^{-1})	EIMS (m/z) %
F_1	3272, 1610, 1568, 765, 621	350** [M^+, 77], 349(31), 271(14), 243(19), 183(05), 155(18), 154(19), 126(08), 76(75).
F_2	3275, 1654, 1575, 1163, 715	290* [M^+, 100], 289(11), 255(29), 227(04), 139(24), 111(54), 110(18), 76(55).
F_3	3241, 1659, 1573, 1165, 662	334* [M^+, 100], 333(47), 255(36), 227(09), 183(12), 155(47), 139(10), 110(09), 76(42).
F_4	3270, 1642, 1579, 1166, 725	290* [M^+, 100], 289(52), 255(18), 227(06), 155(06), 126(08), 123(07), 95(36).
F_5	3290, 1645, 1577, 1170, 664	334* [M^+, 100], 333(30), 255(20), 227(11), 199(07), 170(15), 123(07), 95(61).
F_6	3292, 1643, 1565, 720, 670	350** [M^+, 74], 349(43), 271(09), 243(25), 199(15), 170(09), 139(24), 111(43), 76(07).
F_7	3280, 1645, 1582, 1158	274 [M^+, 100], 273(17), 246(21), 139(08), 123(56), 95(44).
F_8	3220, 1645, 1585, 718	306** [M^+, 76], 305(05), 278(35), 271(10), 243(20), 154(19), 139(49), 111(27), 76(24).
F_9	3282, 1634, 1560, 675	394** [M^+, 72], 393(23), 366(42), 315(13), 287(08), 199(14), 183(59), 155(36), 76(07).
F_{10}	3414, 16586, 1570, 1165	256 [M^+, 100], 255(12), 139(18), 110(17), 105(23), 77(37).
F_{11}	3245, 1645, 1574, 724	272* [M^+, 100], 271(52), 244(05), 237(09), 209(22), 155(08), 126(04), 105(27), 77(20).
F_{12}	3268, 1648, 1576, 669	316* [M^+, 100], 315(64), 288(12), 237(51), 209(38), 199(07), 170(22), 105(66), 77(31).
F_{13}	3275, 1645, 1597, 1131	256 [M^+, 100], 255(47), 228(09), 123(23), 121(11), 95(37), 92(15).
F_{14}	3290, 1660, 1587, 725	272* [M^+, 100], 271(52), 244(05), 237(09), 209(23), 139(10), 121(08), 111(20), 92(12).
F_{15}	3295, 1647, 1585, 665	316* [M^+, 100], 315(18), 288(08), 237(21), 209(15), 183(29), 155(22), 121(61), 92(17).

*M+ 2 peaks for isotopes of ^{37}Cl with intensity 1/3, *M+2 peaks for isotopes of ^{81}Br with intensity 1/1, ** M+2 and M+4 peaks for the isotopes of ^{37}Cl / ^{79}Br, ^{81}Br / ^{35}Cl and ^{37}Cl / ^{81}Br with intensities 2/3 and 1/3.

TABLE-5
MOLECULAR FORMULAE, MOLECULAR WEIGHT, MELTING POINTS, YIELDS, RECRYSTALLIZATION SOLVENT AND R_f VALUES OF CHALCONES

Compd.	m.f.	m.w.	m.p. (ºC)	Yield (%)	Chemical Analysis (%)				Recrystallization solvent / R_f x 100
					Calcd.		Found		
					C	H	C	H	
C_1	$C_{15}H_{10}ClBrO_2$	336.5	178-179	73	53.37	2.99	53.11	2.77	Ethanol/73
C_2	$C_{15}H_{10}FClO_2$	276.5	152-154	72	64.89	3.40	64.89	3.40	Ethanol/75
C_3	$C_{15}H_{10}FBrO_2$	320.0	160-161	77	55.74	3.42	55.74	3.42	Ethanol/67
C_4	$C_{15}H_{10}FClO_2$	276.5	181-182	76	65.01	3.43	65.01	3.43	Ethanol/78
C_5	$C_{15}H_{10}BrFO_2$	320.0	162-164	74	55.82	3.47	55.82	3.47	Ethanol/72
C_6	$C_{15}H_{10}BrClO_2$	336.5	179-180	80	53.03	2.69	53.03	2.69	Ethanol/74
C_7	$C_{15}H_{10}F_2O_2$	260.0	154-155	88	68.92	3.70	68.92	3.70	Ethanol/71

Compd.	m.f.	m.w.	m.p. (ºC)	Yield (%)	Chemical Analysis (%)				Recrystallization solvent / R_f x 100
					Calcd.		Found		
					C	H	C	H	
C_8	$C_{15}H_{10}Cl_2O_2$	293.0	170-172	81	61.30	3.32	61.30	3.32	Ethanol/81
C_9	$C_{15}H_{10}Br_2O_2$	380.0	180-182	78	47.31	2.77	47.31	2.77	Ethanol/73
C_{10}	$C_{15}H_{11}FO_2$	242.0	82-84	74	74.80	4.49	74.80	4.49	Ethanol/65
C_{11}	$C_{15}H_{11}ClO_2$	258.5	99-100	73	71.15	4.42	71.15	4.42	Ethanol/70
C_{12}	$C_{15}H_{11}BrO_2$	302.0	94-96	82	59.80	3.09	59.80	3.09	Ethanol/80
C_{13}	$C_{15}H_{11}FO_2$	242.0	130-131	74	74.73	4.50	74.73	4.50	Ethanol/71
C_{14}	$C_{15}H_{11}ClO_2$	258.5	142-144	74	70.34	4.53	70.34	4.53	Ethanol/74
C_{15}	$C_{15}H_{11}BrO_2$	302.0	138-139	74	59.78	3.11	59.78	3.11	Ethanol/76

TABLE-6
MOLECULAR FORMULAE, MOLECULAR WEIGHT, MELTING POINTS, YIELDS, RECRYSTALLIZATION SOLVENT AND R_f VALUES OF FLAVONOLS

Compd.	m.f.	m.w.	m.p. (ºC)	Yield (%)	Chemical Analysis (%)				Recrystallization solvent / R_f x 100
					Calcd.		Found		
					C	H	C	H	
F_1	$C_{15}H_8BrClO_3$	350.0	255-256	74	51.24	2.29	51.45	2.12	Ethanol/87
F_2	$C_{15}H_8FClO_3$	290.5	276-277	80	61.98	2.77	61.79	2.82	Ethanol/83
F_3	$C_{15}H_8FBrO_3$	335.0	291-292	83	53.76	2.41	53.32	2.57	Ethanol/85
F_4	$C_{15}H_8Cl_2O_3$	290.5	201-203	74	61.98	2.77	61.80	2.80	Ethanol/78
F_5	$C_{15}H_8BrFO_3$	335.0	188-190	70	53.76	2.41	53.41	2.55	Ethanol/78
F_6	$C_{15}H_8BrClO_3$	351.5	172-173	85	51.24	2.29	51.48	2.16	Ethanol/87
F_7	$C_{15}H_8F_2O_3$	274.0	289-290	68	56.70	2.94	65.16	2.80	Ethanol/80
F_8	$C_{15}H_8ClO_3$	307.0	175-177	68	58.66	2.63	58.32	2.49	Ethanol/82
F_9	$C_{15}H_8Br_2O_3$	396.0	233-235	77	45.49	2.04	45.77	2.16	Ethanol/91
F_{10}	$C_{15}H_8FO_3$	256.0	160-161	66	70.31	3.54	70.57	3.32	Ethanol/86
F_{11}	$C_{15}H_8ClO_3$	272.5	166-168	87	66.07	3.33	66.29	3.07	Ethanol/81
F_{12}	$C_{15}H_8BrO_3$	317.0	180-181	73	56.81	2.86	56.45	3.01	Ethanol/89
F_{13}	$C_{15}H_9FO_3$	256.0	148-150	76	70.31	3.54	70.56	3.34	Ethanol/79
F_{14}	$C_{15}H_9ClO_3$	272.5	198-200	73	66.07	3.33	66.31	3.09	Ethanol/82
F_{15}	$C_{15}H_9BrO_3$	317.0	170-171	77	56.81	2.86	56.51	2.99	Ethanol/88

TABLE-7
ANTIFUNGAL ACTIVITY ANALYSIS OF HALOGENATED CHALCONES AND FLAVONOLS

Compd.	Linear Growth Inhibition (%)											Std. Drug 200 µg/mL of DMSO	Inhibition %
Fungus	**$5C_1$**	**$5C_4$**	**$5C_8$**	**$5C_{10}$**	**$5C_{13}$**	**$5C_{14}$**	**$5C_{15}$**	**$6F_1$**	**$6F_{10}$**	**$6F_{14}$**	**$6F_{15}$**		
Trichophyton longnifusus	52.6	84.2	68.4	89.4	89.4	71.5	84.2	60.3	30.1	85.5	85.5	Miconazole	70.0
Candida albicans	*	*	*	*	*	*	*	*	*	*	*	Miconazole	110.8
Asergillus flavus	*	*	50.5	*	*	*	*	40.7	*	85.2	*	Amphotericin (B)	20.0
Microsporum canis	70.0	85.6	80.1	85.2	90.2	65.4	75.3	42.5	65.2	30.6	35.2	Miconazole	98.4
Fusarium solani	*	*	*	*	*	*	*	*	*	*	10.4	Miconazole	73.3
Candida glaberata	*	*	*	*	*	*	*	*	*	*	*	Miconazole	110.8

*No inhibition.

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