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Synthesis, antibacterial and antifungal activity of some derivatives of 2-phenyl-chromen-4-one

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Abstract. Some derivatives of 2-phenyl-chromen-4-one (flavone ring) have been synthesized and tested for antibacterial and antifungal activities along with their chalcone precursors against four human pathogenic bacteria and five plant mould fungi. The structures of the synthesized compounds were elucidated by UV, IR and ^{1}H NMR spectroscopic techniques, and elemental analysis. The antibacterial and antifungal screens of the synthesized compounds were performed in vitro by the filter paper disc diffusion method and the poisoned food technique.

Keywords. Flavone; 2-phenyl-chromen-4-one; antibacterial and antifungal activity; inhibition zone.

1. Introduction

The flavone ring (2-phenyl-chromen-4-one**)** system is of considerable interest due to several biological effects including antibacterial[1] and antifungal activity.[2,3] A survey of the literature provides information that flavonoids containing methylenedioxy group (–O–CH_2–O–) widely occur in natural plant pigments.[4] The flavonoids are an group of natural products founds in fruits, vegetables, nuts, seeds and flowers as well as in teas and wines, and are an important constituent of human diet. They have been demonstrated to possess many biological and pharmacological activities such as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antimutagenic and antiallergic activities and inhibitory activities on several enzymes.[5,6] Our previous articles[7–10] have reported the antibacterial and antifugal effects of the flavone ring system (2-phenyl-chromen-4-one) containing furan, prenyl, methoxyl and hydroxyl group in various positions. This paper reports the syntheses of two derivatives of 2-phenyl-chromen-4-one (flavone) containing methylenedioxy group (**6** and **7**) from their corresponding chalcones (**4** and **5**) by using different DMSO/I_2, diphenyl sulphide and DDQ as oxidizing agents. Both the flavones and their corresponding chalcones were screened *in vitro* for their antibacterial and antifungal activity against four human pathogenic bacteria, viz., *Sarcina lutea* (G^+), *Bacilus subtillis* (G^+), *Shigella dysenteriae* (G^-), *Pseudomonas aeruginosa* (G^-) and five plant and mould fungi, viz. *Colletotrichum gloeosporioides* Penz., *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* sp.

2. Experimental

2.1 *Materials, methods and instruments*

Melting points were recorded on a Gallenkamp apparatus and are uncorrected. IR spectra (KBr) were measured using a Shimadzu, DR-8001 spectrophotometer, ^{1}H NMR spectra ($CDCl_3$) on a Brucker WH 400 MHz instrument with TMS as internal standard and UV spectra (MeOH) on a LKB 4053 spectrophotometer. The chemical used was purchased from Aldrich Chemical Company (Tokyo, Japan). Purity of the compounds was checked by TLC.

2.2 *Synthesis of 3-benzo[1,3]dioxol-5-yl-1-(2-hydroxy-phenyl)-propenone (2ℓ-hydroxy-3, 4-methylenedioxyhalcone, 4)*

A mixture of 2-hydroxyacetophenone (**1**, 20 mmol, 2·72 g) and 3,4-methylenedioxybenzaldehyde (**3**, 1·1 N, 3·30 g) in ethanolic solution of KOH (5%, 15 ml) was kept at room temperature for about 75 h. The reaction mixture was diluted with ice-cold water,

acidified with cold dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with benzene–acetone (10 : 1) and crystallized from ether as pale yellow crystals (4·09 g), yield 68%, m.p. 124–127°C, R_f 0·61 (benzene : acetone; 8 : 1).

Analysis (found) C, 71·92; H, 4·22%; (Calculated for $C_{16}H_{12}O_4$): C, 71·64; H, 4·51%.
UV λ_{max}^{MeOH}: 230, 265 and 355 nm.
IR ν_{max}^{KBr}: 3071, 2907, 2616, 1640, 1617, 1569, 1534, 1504, 1490, 1459, 1439, 1407, 1372, 1354, 1336, 1310, 1284, 1271, 1242, 1203, 1161, 1130, 1098, 1038, 1026, 975, 935, 916, 867, 816, 805, 759, 748, 721, 666, 623 cm^{-1}.
^{1}H NMR ($CDCl_3$): *d* 5·74 (*s*, 2H, –O–CH_2–O–), 6·65 (*d*, 1H, *J* = 8·7 Hz, C_5-H), 6·83 (*s*, 1H, C_2-H), 6·92 (*d*, 1H, *J* = 8·7 Hz, C_6-H), 7·01–7·07 (*m*, 1H, C_5'-H), 6·97 (*d*, 1H, *J* = 9 Hz, C_3'-H), 7·31–7·37 (*m*, 1H, C_4'-H), 7·41 (*d*, 1H, *J* = 16 Hz, C_a-H), 7·60 (*d*, 1H, *J* = 9 Hz, C_6'-H), 8·01 (*d*, 1H, *J* = 16 Hz, C_b-H), 11·58 (*s*, 1H, C_2-OH).

2.3 *Synthesis of 2-benzo[1,3]dioxol-5-yl-chromen-4-one (3¢, 4¢-methylenedioxyflavone, 6) using DMSO/I_2*

The chalcone (**4**, 1·5 mmol, 402 mg) was suspended in dimethyl sulphoxide (DMSO, 10 ml) and a crystal of iodine[11] was added to it. The mixture was refluxed for 20 min on a silicon oil bath and diluted with water. The solid obtained was filtered off and washed with 20% aq. sodium thiosulphate. It was purified by preparative TLC over silica gel GF_{254} using hexane–acetone (10 : 1) as developing solvent and crystallized from ether as yellow needles (247 mg), yield 61·50%, m.p. 131–132°C, R_f 0·59 (benzene–acetone; 10 : 1). It gave blue fluorescence in UV light and positive Mg/HCl test.

Analysis (found): C, 72·55; H, 3·51%; (Calculated for $C_{16}H_{10}O_4$): C, 72·18; H, 3·79%.
UV λ_{max}^{EtOH}: 232, 281 and 370 nm.
IR ν_{max}^{KBr}: 2922, 1647, 1597, 1569, 1544, 1473, 1446, 1430, 1381, 1346, 1326, 1296, 1266, 1245, 1219, 1126, 1151, 1110, 1025, 941, 918, 879, 862, 849, 833, 811, 778, 760, 747, 681 cm^{-1}.
^{1}H NMR ($CDCl_3$): *d* 5·81 (*s*, 2H, –O–CH_2–O–), 6·39 (*s*, 1H, C_3-H), 6·94 (*d*, 1H, *J* = 8.8 Hz, C_6'-H), 6·68 (*d*, 1H, *J* = 8·8 Hz, C_5'-H), 7·03–7·09 (*m*, 1H, C_6-H), 6·99 (*d*, 1H, *J* = 9 Hz, C_8-H), 6·81 (*s*, 1H, C_2'-H), 7·34-7·37 (*m*, 1H, C_7-H), 7·61 (*d*, 1H, J = 9 Hz, C_5-H).

2.4 *Synthesis of 2-benzo[1,3]dioxol-5-yl-chromen-4-one (3¢, 4¢-methylenedioxyflavone, 6) using Ph-S-S-Ph*

The chalcone (**4**, 1·5 mmol, 402 mg) was ground with diphenyl sulphide[12] (125 mg) in a mortar and the mixture was transferred to a 100-ml three-necked round-bottomed flask equipped with nitrogen inlet and outlet tubes. The central neck was closed with a glass stopper. The flask was then dipped into a silicon oil bath and heated at 265°C under nitrogen atmosphere until distilling of the thiols formed ceased through the other outlet tube (2·5 h). The reaction mixture was then cooled to room temperature and 20 ml chloroform was added. The organic layer was washed with water several times. It was dried over anhydrous sodium sulphate and the solvent removed by distillation. The product crystallized from ethanol as colourless needles (185 mg), yield 46%, m.p. 131–132°C, R_f 0·59 (benzene–acetone; 10 : 1). It gave blue fluorescence in UV light and positive Mg/HCl test. Spectral data of this flavone (**6**) were also similar to that of the product prepared by the DMSO/I_2 method.

2.5 *Synthesis of 2-benzo[1,3]dioxol-5-yl-chromen-4-one (3¢, 4¢- methylenedioxyflavone, 6) using DDQ*

DDQ (155 mg) was added to the chalcone (**4**, 1·5 mmol, 402 mg) in dry dioxane (50 ml) and the solution refluxed for 3 h. The product purified by preparative tlc over silica gel using petroleum spirit–benzene (1 : 2) as developing solvent. It crystallized from ethanol as colourless needles (231 mg), yield 57·50%, m.p. 131–132°C, R_f 0·59 (benzene–acetone; 10 : 1). It gave blue fluorescence in UV light and positive Mg/HCl test. Spectral data of this flavone (**6**) were also similar to that prepared by the DMSO/I_2 and diphenyl sulphide method.

2.6 *Synthesis of 3-benzo[1,3]dioxol-5-yl-1-(4-benzyloxy-2-hydroxy-phenyl)-propenone (2¢-hydroxy-4¢- benzyloxy-3, 4-methylenedioxychalcone, 5)*

The procedure is similar to the synthesis of **4**. The product is crystallized from alcohol as yellow crystals (2·67 g), yield 65·50%, m.p. 86–87°C [lit[13] m.p. 86°C], R_f 0·64 (benzene–acetone; 4 : 1).

Analysis (found): C, 73·42; H, 4·65%; (Calculated for $C_{23}H_{18}O_5$): C, 73·79; H, 4·81%.
UV l_{max}^{MeOH}: 225, 260 and 345 nm.
IR n_{max}^{KBr}: 1632, 1602, 1534, 1521, 1502, 1488, 1465, 1399, 1371, 1288, 1243, 1212, 1189, 1127, 1104, 1053, 1004, 977, 933, 914, 840, 808, 763, 739, 698, 651, 612 cm^{-1}.
^{11}H NMR ($CDCl_3$): *d* 5·22 (*s*, 2H, $-C\underline{H}_2-C_6H_5$), 5·79 (*s*, 2H, –O–CH_2–O–), 6·43 (*s*, 1H, C_3'-H), 6·90 (*s*, 1H, C_2-H), 6·72 (*d*, 1H, *J* = 8·6 Hz, C_5'–H), 6·81 (*d*, 1H, *J* = 9 Hz, C_5-H), 6·95 (*d*, 1H, *J* = 9 Hz, C_6-H), 7·40 (*d*, 1H, *J* = 16 Hz, C_a-H), 7·13–7·19 (*m*, 5H, $-CH_2-C_6\underline{H}_5$), 7·51 (*d*, 1H, *J* = 9 Hz, C_6'-H), 8·02 (*d*, 1H, *J* = 16 Hz, C_b-H), 12·12 (*s*, 1H, C_2-OH).

2.7 *Synthesis of 2-benzo[1,3]dioxol-5-yl-7-benzyloxy-chromen-4-one (7-benzyloxy-3¢, 4¢-methylenedioxyflavone, 7) using DMSO/I_2*

The method is similar to that for compound **6**. It was purified by preparative TLC over silica gel GF_{254} using hexane acetone (10 : 1) as developing solvent and crystallized from benzene as yellow crystals (364 mg), yield 69·40%, m.p. 103–104°C, R_f 0·63 (benzene–acetone; 12 : 1). It gives blue fluorescence in UV light and positive Mg/HCl test.

Analysis (found): C, 74·49; H, 4·77%; (Calculated for $C_{23}H_{16}O_5$) C, 74·19; H, 4·30%.
UV l_{max}^{EtOH}: 220, 270 and 365 nm.
IR n_{max}^{KBr}: 1647, 1617, 1607, 1591, 1569, 1516, 1501, 1484, 1450, 1417, 1399, 1380, 1343, 1313, 1294, 1209, 1156, 1139, 1110, 1102, 1083, 1071, 1028, 954, 919, 859, 844, 810, 761, 742, 711 cm^{-1}.
^{1}H NMR ($CDCl_3$): *d* 5·20 (*s*, 2H, $-C\underline{H}_2-C_6H_5$), 5·80 (*s*, 2H, –O–CH_2–O–), 6·67 (*s*, 1H, C_8-H), 6·94 (*s*, 1H, C_2'-H), 6·99 (*d*, 1H, *J* = 8·8 Hz, C_6-H), 6·86 (*d*, 1H, *J* = 9 Hz, C_5'-H), 6·44 (*s*, 1H, C_3-H), 6·76 (*d*, 1H, *J* = 9 Hz, C_6'-H), 7·05–7·10 (*m*, 5H, $-CH_2-C_6H_5$), 7·48 (*d*, 1H, *J* = 9 Hz, C_5-H).

2.8 *Synthesis of 2-benzo[1,3]dioxol-5-yl-7-benzyloxy-chromen-4-one (7-benzyloxy-3¢, 4¢-methylenedioxyflavone, 7) using Ph-S-S-Ph*

The method is similar to that mentioned earlier. The product crystallizes from ethyl acetate as pale yellow needles (267 mg), yield 51·00%, m.p. 103–104°C, R_f 0·63 (benzene–acetone; 12 : 1). It gives blue fluorescence in UV light and positive Mg/HCl test. Spectral data of this flavone (7) is also similar to that prepared by the DMSO/I_2 method.

2.9 *Synthesis of 2-benzo[1,3]dioxol-5-yl-7-benzyloxy-chromen-4-one (7-benzyloxy-3¢, 4¢-methylenedioxyflavone, 7) using DDQ*

The method is similar to that mentioned earlier. It was crystallized from ethyl acetate as pale yellow needles (325 mg), yield 62·00%, m.p. 103–104°C, R_f 0·63 (benzene–acetone; 12 : 1). It gives blue fluorescence in UV light and positive Mg/HCl test. Spectral data of this flavone (**7**) are also similar to that of flavone prepared by DMSO/I_2 and diphenyl sulphide method.

2.10 *Antibacterial screening*

Antibacterial activities of synthesized compounds **4**, **5**, **6** and **7** were studied against four human pathogenic bacteria, viz., *Shigella dysenteriae* (G^-), *Pseudomonas aeruginosa* (G^-), *Sarcina lutea* (G^+) and *Bacillus subtilis* (G^+). For the detection of antibacterial activities, the filter paper disc diffusion method[14,15] was used. Kanamycin was used as standard antibiotic for antibacterial activities. Nutrient agar (NA) was used as basal medium for test bacteria. The agar media were inoculated with 0.5 ml of 24 h liquid cultures containing 10^7 microorganisms/ml. Diffusion time was 24 h at 5°C for all bacteria, and incubation time was 12 h at 37°C. Discs with only DMSO were used as control. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones.

2.11 *Determination of the minimum inhibitory concentration (MIC)*

Minimal inhibitory concentration is defined as the lowest concentration that inhibits bacterial growth. To determine minimum inhibitory concentration (MIC), the serial dilution technique[16] was followed using nutrient broth medium. MIC values of the compound **6** and **7** were determined against *Pseudomonas aeruginosa* (G^-) and *Bacillus subtilis* (G^+).

2.12 *Antifungal screening*

Antifungal activities of compounds **4**, **5**, **6** and **7** towards five plant pathogenic and mould fungi were studied, viz., *Colletotrichum gloeosporioides* Penz.

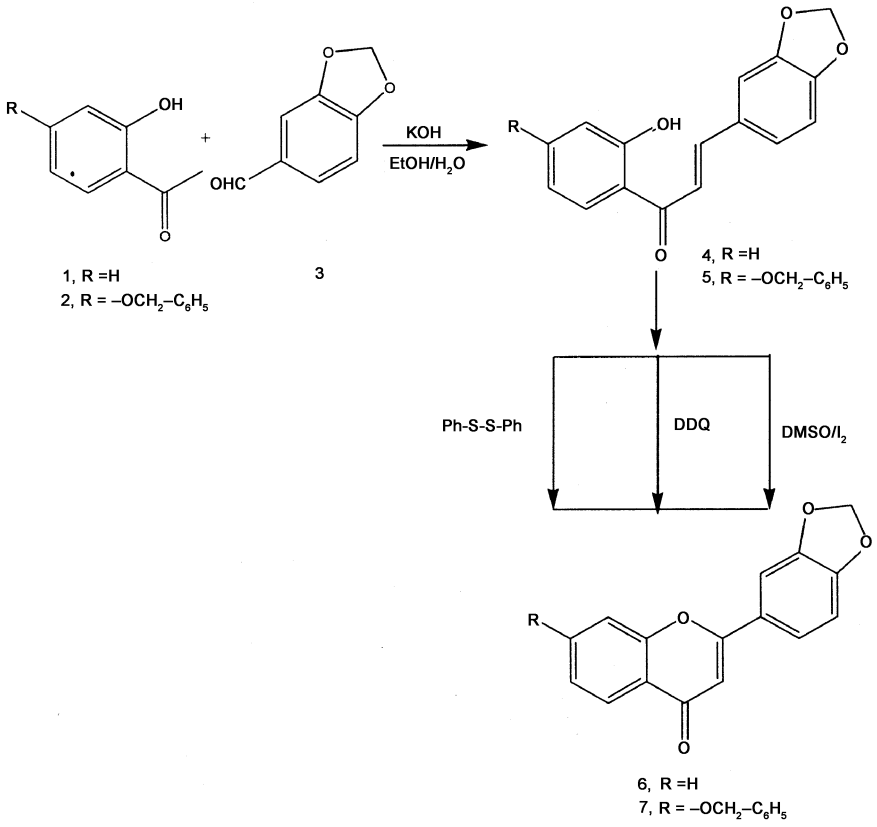
(plant pathogen), *Candida albicans* (human pathogen), *Aspergillus niger* (mould), *Aspergillus flavus* (mould) and *Penicillium* sp. (blue mould). Antifungal activity was assessed by the poisoned food technique,[17] in a modified condition.[18] Fluconazole (200 *m*g/disc) was used as standard fungicide. Potato dextrose agar (PDA) was used as basal medium for test fungi. Glass petridishes used were sterilized. Sterilized melted PDA medium (~ 45°C) was poured at the rate of 15 ml into each petridish (90 mm). After solidification of the medium, small portions of the mycelium of each fungus were spread carefully over the centre of each PDA plate with the help of sterilized needles. Thus, each fungus was transferred to a number of PDA plates, which were then incubated at (25 ± 2)°C and ready for use after five days of incubation. Prepared discs of samples were placed gently on solidified agar plates, freshly seeded with the test organisms with sterile forceps. A control disc was also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvent respectively. The plates were then kept in a refrigerator at 4°C for 24 h so that the materials had sufficient time to diffuse over a considerable area of the plates. After this, the plates were incubated at 37·5°C for 72 h. Dimethyl sulphoxide (DMSO) was used as solvent to prepare desired solutions (10 mg/ml) of the compounds initially and also to maintain proper control.

3. Results and discussion

3.1 *Synthesis of 2-benzo[1,3]dioxol-5-yl-chromen-4-one and 2-benzo[1,3]dioxol-5-yl-7-benzyloxy-chromen-4-one*

Syntheses of 3′,4′-methylenedioxyflavone (2-benzo-[1,3]dioxol-5-yl-chromen-4-one) and 7-benzyloxy-3′,4′-methylenedioxyflavone (2-Benzo[1,3]dioxol-5-yl-7-benzyloxy-chromen-4-one) were accomplished starting from 2-hydroxyacetophenone (**1**) and 2-hydroxy-4-benzyloxyacetophenone[19] (**2**) respectively, as shown in scheme 1.

Alkaline condensation of 2-hydroxyacetophenone (**1**) and 3, 4-methylenedioxybenzaldehyde (**3**) yielded 2′-hydroxy-3,4-methylenedioxychalcone (**4**). It was obtained as pale yellow crystals, m.p. 124–127°C. The structure of this chalcone (**4**) has been confirmed by spectral data and elemental analysis. The UV ab-



Scheme 1.

sorption band of **4** (l_{max} 230, 265 and 355 nm) suggests the presence of a chalcone skeleton. It gives brown color with alcoholic ferric chloride solution but no IR absorption band for hydroxyl group (–OH), at C-2 position.[20] This is supported by (i) a relatively weaker IR band at 1640 cm^{-1} (chelated >C=O), and (ii) an appropriately deshielded phenolic proton signal at *d* 11·58 (1H, *s*), exchangeable with D_2O. In the ^1H NMR spectrum, the methylene protons of the methylenedioxy group (–O–CH_2–O–) appear as a singlet at 5·74 integrating for two protons, the three aromatic protons of the B ring, which appear at *d* 6·65 (*d*, 1H, *J* = 8.7 Hz, C_5-H), 6·83 (*s*, 1H, C_2-H) and 6·92 (*d*, 1H, *J* = 8·7 Hz, C_6-H) integrating for one proton each. The four aromatic protons of the A ring appear as two double doublets and two multiplets at *d* 6·97 (*d*, 1H, *J* = 9 Hz), 7·60 (*d*, 1H, *J* = 9 Hz), 7·01–7·07 (*m*, 1H) and 7·31–7·37 (*m*, 1H) assigned to C_3', C_6', C_5' and C_4' protons respectively. The C_a-H and C_b-H protons of **4** appeared as two doublets at *d* 7·41 (*J* = 16 Hz) and 8·01 (*J* = 16 Hz) integrating for one proton each.

Oxidation of chalcone **4** into the corresponding flavone **6** was carried out differently by using DMSO/I_2, diphenyl sulphide and DDQ. Flavone **6** was obtained as yellow needles, m.p. 131–132°C. The structure of this flavone **6** has been confirmed by spectral data and elemental analysis. The UV absorption band of **6** (l_{max} 232, 281 and 370 nm) suggests the presence of a flavone skeleton. IR absorption frequency at *u* 1647 cm^{-1} shows the presence of a carbonyl group (>C=O) and absence of a hydroxyl group band confirms the oxidation of chalcone **4** into flavone **6**. The ^1H NMR spectrum of flavone **6** indicates the presence of a methylenedioxy group (–O–CH_2–O–) that appears as a singlet at *d* 5·81 integrating for two protons. The three aromatic protons of the B ring which appear at *d* 6·68 (*d*, 1H, *J* = 8·8 Hz, C_5'-H), 6·81 (*s*, 1H, C_2'-H) and 6.94 (*d*, 1H, *J* = 8.8 Hz, C_6'-H) integrate for one proton each. The other four aromatic protons of the A ring appear as two double doublets and two multiplets at *d* 6·99 (*d*, 1H, *J* = 9 Hz), 7·61 (*d*, 1H, *J* = 9 Hz), 7·03–7·09 (*m*, 1H) and 7·34–7·39 (*m*, 1H) and are assigned to C_8, C_5, C_6 and C_7 protons respectively. The C_3-H proton of the flavone nucleus appears as a singlet at *d* 6·39 integrating for one proton.

Base-catalysed aldol-condensation of 2-hydroxy-4-benzyloxyacetophenone (**2**) and 3,4-methylenedioxybenzaldehyde (**3**) gives 2′-hydroxy-4′-benzyloxy-3,4-methylenedioxy chalcone[13] (**5**). The chalcone **5** is obtained as yellow crystals, m.p. 86–87°C [Lit.[13] m.p. 86°C]. UV absorption maxima of **5** occur at 225, 260 and 345 nm. IR absorption peaks of **5** due to carbonyl group appear at 1632 cm^{-1} but no IR absorption band is seen for hydroxyl group (–OH), at C-2 position[20]. Spectral data (^1H NMR, UV and IR, see §2) and elemental analysis confirm the structure of compound **5** as 3-benzo[1,3]dioxol-5-yl-1-(4-benzyloxy-2-hydroxy-phenyl)-propenone (2′-hydroxy-4′-benzyloxy-3,4-methylenedioxychalcone).

Cyclization of chalcone **5** into the corresponding flavone **7** was also carried out differently by using DMSO/I_2, diphenyl sulphide and DDQ. Flavone **7** is obtained as yellow crystals, m.p. 103–104°C. The structure of this flavone **7** has been supported by spectral data and elemental analysis. The UV spectrum of this flavone **7** (l_{max} 220, 270 and 365 nm) suggests the presence of a flavone nucleus. IR absorption frequency at *u* 1647 cm^{-1} shows the presence of a carbonyl group (>C=O) and the absence of a hydroxyl group band, confirming the oxidation of chalcone **5** into flavone **7**, which is also supported by the ^1H NMR spectrum of flavone **7**. The ^1H NMR spectrum of **7** explains the presence of a methylenedioxy group (–O–CH_2–O–) that appear as a singlet at 5·80 integrating for two protons. Methylene and aromatic protons of a benzyloxy group indicated by a singlet at *d* 5·20 (2H, –O–C$\underline{H}_2$–C_6H_5) and a multiplet at *d* 7·05–7·10 (2H, –O–CH_2–$C_6\underline{H}_5$) integrating for two and five protons, respectively. The three aromatic protons of the B ring which appear at *d* 5·50 (*d*, 1H, *J* = 8·6 Hz, C_5'-H), 5·20 (*s*, 1H, C_2'-H) and 5·79 (*d*, 1H, *J* = 8·6 Hz, C_6'-H) integrating for one proton each respectively. The three aromatic protons of A ring appear as an ABC system at δ 6·67 (*s*, 1H, C_8–H), 6·99 (*d*, 1H, *J* = 8·8 Hz, C_6-H) and 7·48 (*d*, 1H, *J* = 8·8 Hz, C_5-H) integrating for one proton each. The flavone **7** also gives a characteristic singlet at *d* 6·44, assigned to the C_3–H proton.

Among the methods used for oxidation of chalcones (**4** and **5**) into their corresponding flavones (**6** and **7**), the DMSO/I_2 method gave the highest yield compared to the two other oxidizing methods, while the diphenyl sulphide method gave the lowest yield.

3.2 *Antibacterial activities*

The antibacterial activities of compounds **4**, **5**, **6**, and **7** have been assayed at the concentration of 100 *m*g/disc, 200 *m*g/disc and 300 *m*g/disc against four human pathogenic bacteria. Among them, two were

Table 1. Antibacterial screening for compounds **4**, **5**, **6** and **7**.

Name of the organisms	Concentration (*m*g/disc)	Diameter of the zone of inhibition (mm)				
		4	**5**	**6**	**7**	K-30*
Shigella dysenteriae	100	–	–	–	–	26
	200	–	–	–	–	
	300	–	–	6	6	
Pseudomonas aeruginosa	100	–	–	–	–	28
	200	–	–	–	–	
	300	–	–	11	11	
Sarcina lutea	100	–	–	–	–	34
	200	–	–	–	–	
	300	–	–	7	–	
Bacilus subtillis	100	–	–	–	–	30
	200	–	–	–	–	
	300	–	–	7	8	

*Kanamycin – 30 *m*g/disc

Table 2. Antifungal screening for compounds **4**, **5**, **6**, and **7**.

Name of the organisms	Concentration (*m*g/disc)	Diameter of the zone of inhibition (mm)				
		4	**5**	**6**	**7**	Fluconazole (200 *m*g/disc)
Penicillium sp.	100	–	–	–	–	–
	200	8	–	6	–	
	300	11	7	10	6	
Aspergillus niger	100	–	–	–	–	–
	200	–	–	–	–	
	300	–	–	–	–	
Aspergillus flavus	100	–	–	–	–	10
	200	–	–	–	–	
	300	–	–	–	–	
Candida albicans	100	–	–	–	–	–
	200	–	–	–	–	
	300	–	–	–	–	
Colletotrichum gloeosporioides	100	–	–	–	–	–
	200	–	–	–	–	
	300	–	5	–	7	

gram-positive and the other two were gram-negative. The inhibitory effects of compounds **4**, **5**, **6,** and **7** against these organisms are given in table 1.

The screening results indicate that compounds **4** and **5** do not show any antibacterial activity to the bacteria tested. Compound **6** has low antibacterial activity towards every bacteria tested, at a concentration of 300 *m*g/disc, while compound **7** also has low antibacterial activity against *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Bacilus subtillis* and no activity against *Sarcina lutea*. From the above result, it can be concluded that the antibacterial activities of compound **6** and **7** are due to the flavone skeleton.

3.3 *Minimum inhibitory activity*

The minimum inhibitory concentration of compounds **6** and **7** were determined against *Bacilus subtillis* and *Pseudomonas aeruginosa* by the serial dilution method. The MIC levels of both compounds **6** and **7** were found 256 *m*g/mL against *Bacilus subtillis* and *Pseudomonas aeruginosa*, respectively.

3.4 *Antifungal activities*

The antifungal activities of compounds **4**, **5**, **6**, and **7** have been assayed at concentrations of 100 *m*g/disc, 200 *m*g/disc and 300 *m*g/disc against five plant pathogens and mould fungi. The inhibitory effects of compounds **4**, **5**, **6**, and **7** against these organisms are given in table 2.

The screening results indicate that compounds **4** and **6** do not show any antifungal activities against *Aspergillus niger, Aspergillus flavus, Candida albicans* and *Colletotrichum gloeosporioides* while they show good antifungal activities at high concentration against only *Penicillium* sp. in comparison with standard fungicides, fluconazole. Whereas compounds **5** and **7** showed antifungal activities at high concentration against *Penicillium* sp. and *Colletotrichum gloeosporioides* whereas standard fungicide fluconazole did not show any antifungal activities against *Penicillium* sp. and *Colletotrichum gloeosporioides*.

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