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Synthesis, stereochemical assignments, and biological activities of homoisoflavonoids

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Abstract—A series of four naturally occurring homoisoflavonoids and eight analogs have been synthesized starting from an appropriately substituted phenol through chroman-4-one, in four steps. The products were assigned as *E*-isomers based on NMR spectroscopic data. The *E*-isomers were converted into *Z*-isomers by photoisomerization. The *E*- and *Z*-isomers showed distinct chemical shifts and the differences between (*E*) and (*Z*)-homoisoflavonoids in the proton NMR spectra afford a useful method for ascertaining the stereochemistry. The antioxidant activity of homoisoflavonoids was determined by superoxide (NBT) and DPPH free radical scavenging methods. The analog 7-hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene]chroman-4-one displayed excellent activity followed by sappanone A in both the methods and were several times potent than the commercial antioxidants like BHA, BHT, etc. These compounds were evaluated in vitro for their inhibitory activities against 5-lipoxygenase (5-LOX) enzyme. The analog 7-hydroxy-3-[(*N*,*N*-dimethylaminophenyl)methylene]chroman-4-one was found to possess potent inhibitory activity and was comparable to that of the standard, nordihydroguiaretic acid. These results suggest that these homoisoflavonoids, with their potent antioxidant and 5-LOX inhibitory activities, may have useful applications as antioxidants and lead compounds for asthma and inflammatory diseases.

1. Introduction

Homoisoflavonoids (3-benzylidene-4-chromanones) are related to flavonoids and occur as natural products and exhibit biological activity.[1] These compounds have been reported to possess antifungal,[2] hypocholesterolemic,[3] antimutagenic,[4] and antiviral activities.[5] Synthesis of these compounds is based usually on the condensation of 4-chromanones with aromatic aldehydes in the presence of acidic or basic catalyst.[6,7] However, Baylis–Hillman reaction[8] was utilized to obtain homoisoflavonoids, recently. Bonducellin was isolated from *Caesalpinia bonducella*[9] and *Caesalpinia pulcherrima*.[10] Very recently isobonducellin, the *Z*-isomer of bonducellin, and 2′-methoxybonducellin (Fig. 1) were also isolated from *C. pulcherrima*.[11,12] Sappanone A (Fig. 1) was isolated from the heartwood of *Caesalpinia sappan* L.[13] and *Caesalpinia japonica Sieb*.[14] Antioxidant activity of flavonoids has been well established, but to the best of our knowledge, these homoisoflavonoids have not been studied for their antioxidative activity. In view of the importance of dietary antioxidants in

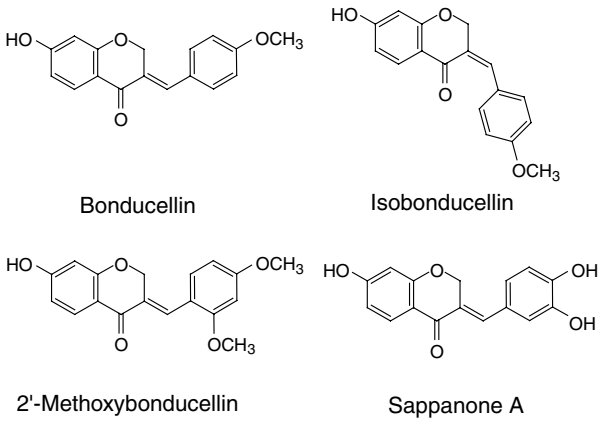

Figure 1. Chemical structures of natural homoisoflavonoids.

Keywords: Homoisoflavonoids; Synthesis; Stereochemical assignments; Antioxidant activity; 5-LOX.
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chemoprevention of degenerative diseases, such as cancer, Alzheimer's, Parkinson's, and cardiovascular diseases, we have synthesized for the first time bonducellin, isobonducellin, 2′-methoxybonducellin, and sappanone A along with eight new structural analogs. We have also screened these compounds for 5-lipoxygenase enzyme inhibitory activity (5-LOX). 5-Lipoxygenase is the key enzyme for the biosynthesis of leukotrienes, the important mediators for inflammatory, allergic, and obstructive processes. 5-LOX inhibitors have potential in treating ashthma and various inflammatory disorders[15,16] In this paper, we report the details of synthesis, stereochemical assignments, and antioxidative and 5-LOX activities of several homoisoflavonoids.

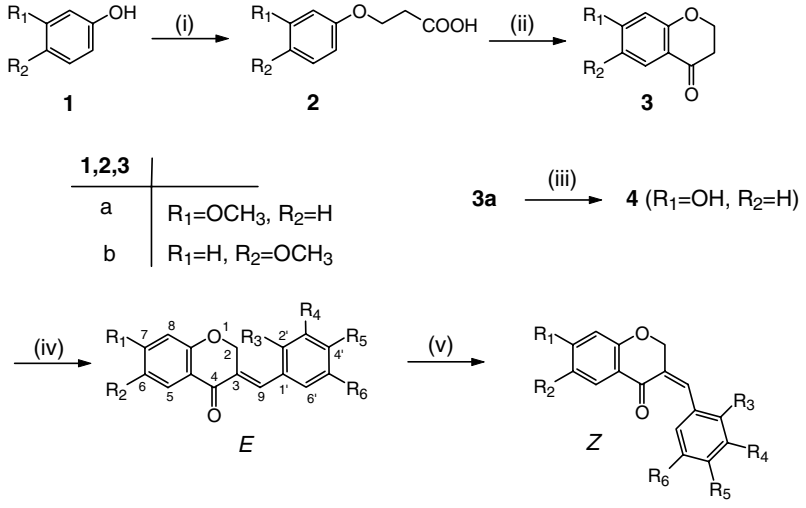
2. Results and discussion

2.1. Synthesis

The reaction of 3-methoxyphenol (**1a**) with 3-bromopropionic acid using sodium hydride as base furnished 3-phenyloxypropionic acid (**2a**), which was cyclized using polyphosphoric acid[17] to give 7-methoxychroman-4-one (**3a**). Demethylation of **3a** using pyridine hydrochloride[18] gave **4**, in 63% yield, a key intermediate in the synthesis. Base catalyzed condensation of **4** with 4-methoxybenzaldehyde, 2,4-dimethoxybenzaldehyde, and 3,4-dihydroxybenzaldehyde afforded bonducellin (**5***E*),2′-methoxybonducellin (**6***E*), and sappanone A (**7***E*) in 65%, 64%, and 58% yields, respectively (Scheme 1). In order to assign the stereochemistry unambiguously and to get isobonducellin, **5***E* was photoisomerized to **5***Z* using medium pressure mercury lamp.[19] The spectral data (IR, NMR, and MS) of **5***E*, **5***Z*, **6***E*, and 7*E* were identical with those of natural products.[9,11–13] The other analogs (**8**–**14**) were synthesized using the appropriately substituted benzaldehydes as shown above (Scheme 1). In all cases, a single stereo isomer (*E*) was obtained and some of them were converted into *Z*-isomers by irradiation.

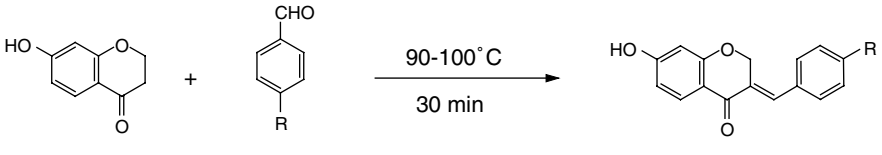
Due to poor yields in base catalyzed condensation of **4** with 4-chlorobenzaldehyde, a simple method under solvent-free conditions catalyzed by perchloric acid supported on silica gel was developed (Scheme 2).

The condensation reaction of **4** with 4-chlorobenzaldehyde using catalytic $HClO_4$–SiO_2[20] under stirring at 90–100 °C gave homoisoflavonoid (**15**) in 71% yield.



5	R_1= OH, R_5= OCH_3, R_2=R_3=R_4=R_6=H	**10**	R_1= OH, R_5= $N(CH_3)_2$, R_2=R_3=R_4=R_6=H
6	R_1= OH, R_3=R_5=OCH_3, R_2=R_4=R_6=H	**11**	R_1=R_5=OH, R_2=R_3=R_4=R_6=H
7	R_1=R_4=R_5=OH, R_2=R_3=R_6= H	**12**	R_1=R_4=R_5=R_6= OH, R_2= R_3=H
8	R_1=R_5= OCH_3, R_2=R_3=R_4=R_6=H	**13**	R_1=R_5= OH, R_4=COOH, R_2=R_3=R_6=H
9	R_1= OH, R_4=R_5=R_6=OCH_3, R_2=R_3=H	**14**	R_2=OCH_3, R_5=OH, R_1=R_3=R_4=R_6=H

Scheme 1. Reagents and conditions: (i) Bromopropionic acid, NaH, DMF, 70–80 °C, rt, 16 h, 30%; (ii) PPA, 80 °C, 4 h, 46%; (iii) Py·HCl, 180–190 °C, 2 h, 62%; (iv) substituted benzaldehyde, piperidine, 70–80 °C, 2 h, 43–72%; (v) hν, benzene, 4 h, 32–54%.



Scheme 2. Synthesis under solvent-free conditions.

The reaction was completed in 30 min and the product was isolated by filtration through silica gel. It is an environment-friendly and cost-effective process. The generality of the reaction was checked with 4-methoxybenzaldehyde and obtained bonducellin (**5***E*) in 68% yield.

2.2. Stereochemical assignments

The proton NMR data of *E* and *Z* homoisoflavonoids are presented in Table 1. In α,β-unsaturated carbonyl compounds, the olefinic proton *cis* to the carbonyl exhibits a chemical shift lower than that of the corresponding *trans* proton and this difference could be used for assignment of configuration.[21–23] The present data also reveal that the olefinic proton is useful to distinguish between the two isomers in homoisoflavonoids. The olefinic proton (=CH) in *E*-isomers appeared as singlet in the range of δ 7.74 and 7.81, while the same proton in *Z*-isomers appeared in the range of δ 6.78 and 7.07. The protons (CH_2) adjacent to double bond are also useful in distinguishing the isomers. In *E*-isomers, a singlet appeared in the range of δ 5.20–5.36 and in *Z*-isomers these protons were appeared in the range of δ 4.93–4.98. In addition to these, in all *Z*-isomers, the protons (H-2′ and H-6′) of pendant aryl unit appeared as a doublet at a much lower field than the corresponding protons in *E*-isomers and these chemical shift differences are significant and could be used to distinguish the isomers. Thus, 2′,6′-protons in *Z*-isomers appeared as doublet at about δ 7.86, while in *E*-isomers the same protons appeared in the range of δ 7.02–7.27. These differences lead to unambiguous assignment of stereochemistry at the double bond.

2.3. Antioxidant activity

In the present study, two commonly used antioxidant evaluation methods, superoxide radical scavenging (NBT method) and the DPPH free radical scavenging methods, were chosen to determine the antioxidant potential of the compounds. Both the methods measure the efficacy of a hydrogen atom transfer from a phenol to a radical.

2.3.1. Superoxide radical scavenging activity. Superoxide radicals were generated in vitro by non-enzymatic system and determined spectrophotometrically (560 nm) by nitro blue tetrazolium (NBT) photoreduction method of McCord and Fridovich.[24,25] The antioxidant activity of homoisoflavonoids (**5***E*, **5***Z*, **6***E*, and **7***E*) and their analogs was expressed as 50% inhibitory concentration (IC_{50} in μM) and the values are incorporated in Table 2. From the superoxide scavenging activity data, the analog, 7-hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene]chroman-4-one (**12***E*, IC_{50}: 6.5 μM), having pyrogallol moiety showed highest activity followed by sappanone A (**7***E*, IC_{50}: 26.3 μM) having catechol moiety and showed several fold potent activity in comparison with those of the commercially available antioxidants like vitamin C (IC_{50}: 852 μM), vitamin E acid succinate (IC_{50}: 726 μM), BHA (IC_{50}: 966 μM, lit.[26] IC_{50}: >1000 μM), and BHT (IC_{50}: 381 μM). The superior scavenging ability of these compounds (**7***E* and **12***E*) lends further support to the fact that the catechol or pyrogallol system enhances the antioxidant activity.[27]

Table 1. ^{1}H NMR data of *E* and *Z* homoisoflavonoids

Compound	*E*			*Z*		
	H-2	H-9	H-2′,6′	H-2	H-9	H-2′,6′
5	5.32	7.74	7.26	4.93	6.82	7.86
6	5.20	7.78	7.02	4.98	7.07	7.86
8	5.36	7.81	7.27	4.94	6.78	7.87

Table 2. Antioxidant activity of homoisoflavonoids

Compound	NBT superoxide scavenging activity (IC_{50} in μM)*	DPPH free radical scavenging activity (IC_{50} in μM)*
Bonducellin (**5***E*)	>100	>100
Isobonducellin (**5***Z*)	>100	>100
2′-Methoxy-bonducellin (**6***E*)	>100	>100
Sappanone A (**7***E*)	26.3	8.3
8	>100	>100
9	>100	>100
10	>100	>100
11	>100	>100
12	6.5	3.8
13	>100	>100
14	>100	>100
15	>100	>100
Vitamin C	852	25.1
Vitamin E acid succinate	726	>100
BHA	966	34
BHT	381	22.5

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.
The lower the IC_{50} values, the higher the antioxidant activity.
* This indicates the significant difference at the level of $P < 0.05$, $n = 3$.

2.3.2. DPPH radical scavenging activity. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of homoisoflavonoids was determined by the method described by Lamaison et al.,[28] based on the reduction of methanolic solution of the colored DPPH radical. From the IC_{50} values (Table 2), again 7-hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene]chroman-4-one (**12***E*, IC_{50}: 3.8 μM) and sappanone A (**7***E*, IC_{50}: 8.3 μM) were found to be powerful scavengers of DPPH free radicals in comparison with the commercially available antioxidants [vitamin C IC_{50}: 25.1 μM (lit.[29] IC_{50}: 26.5 μM)].

2.4. 5-Lipoxygenase enzyme inhibitory activity

The homoisoflavonoids (**5**–**15**) were screened for their 5-lipoxygenase enzyme inhibitory potential using colorimetric method,[30,31] at different concentrations. From the inhibitory values (Table 3), the homoisoflavonoids, 7-hydroxy-3-[(*N*,*N*-dimethylaminophenyl)methylene]chroman-4-one (**10***E*, 39% inhibition at 10 μM), 7-hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene]-chroman-4-one (**12***E*, 45% inhibition at 50 μM), and 2′-methoxybonducellin (**6***E*, 44% inhibition at 50 μM), displayed good activity. Interestingly, the inhibitory activity of **10***E* was comparable with that of NDGA (37% inhibition at 10 μM).

Table 3. 5-Lipoxygenase inhibitory activity of homoisoflavonoids

Compound	Concentration (μM)	% inhibition
Bonducellin (**5***E*)	50	28.8
Isobonducellin (**5***Z*)	50	19.0
2′-Methoxy-bonducellin (**6***E*)	50	44.0
Sappanone A (**7***E*)	50	21.3
8	50	28.0
9	50	19.1
10	10	39.2
11	50	21.2
12	50	45.4
13	50	24.9
14	50	—
15	50	30.9
NDGA	10	36.9

NDGA, nordihydroguiaretic acid.

3. Conclusions

In conclusion, we have accomplished the synthesis of four natural homoisoflavonoids, bonducellin (**5***E*), isobonducellin (**5***Z*), 2′-methoxybonducellin (**6***E*), and sappanone A (**7***E*), along with eight analogs (**8**–**15**) in three steps from substituted phenol. Some of the *E*-isomers were converted into *Z*-isomers and assigned the stereochemistry at the double bond based on proton NMR data. The analog **12***E* and sappanone A exhibited excellent antioxidative activity and were several fold more potent than the commercial antioxidants like vitamin C, vitamin E acid succinate, BHA, and BHT. The analogs **10***E* and **12***E* exhibited potent 5-LOX inhibitory activities and were comparable with that of the standard drug, nordihydroguiaretic acid. Homoisoflavonoid **12***E* with its potent antioxidative and 5-LOX inhibitory activities may find applications as an antioxidant and to be useful for asthma and inflammatory diseases.

4. Experimental

4.1. General

Melting points were recorded with a Mel-Temp melting point apparatus, in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer BX1 FTIR Spectrophotometer. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) spectra were recorded on a Joel JNM λ-300 spectrometer using TMS as internal reference, the values for chemical shifts (δ) being given in ppm and coupling constants (J) in Hertz (Hz). In the ^{13}C NMR spectra, the nature of the carbons (C, CH, CH_2 or CH_3) was determined using DEPT-135 and is given in parentheses. Mass spectra were recorded on an Agilent 1100 LC/MSD and elemental analysis on a Vario El Elementar instrument. Acme silica gel G and silica gel (100–200 mesh) were used for analytical TLC and column chromatography, respectively. Nitro blue tetrazolium (NBT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and vitamin E acid succinate were purchased from Sigma–Aldrich, and other chemicals and solvents were of commercial grade and used without further purification. 6-Methoxychroman-4-one (**3b**) was prepared by the literature procedure.[32]

4.2. 3-(3-Methoxyphenyl)-propionic acid (2a)

To a mixture of sodium hydride (5 g, 50%, 104 mmol) in DMF (40 mL) was added 3-methoxyphenol (5 g, 40 mmol) in DMF (10 mL) at 10–15 °C and the mixture was stirred at rt for 0.5 h. Then a solution of 3-bromopropionic acid (7.4 g, 48 mmol) in DMF (10 mL) was added and the mixture was stirred at rt for 14 h. The reaction mixture was diluted with methanol (10 mL), acidified with dil HCl, and extracted with ethyl acetate (2× 100 mL). The combined ethyl acetate layer was washed with water (50 mL), brine (30 mL), and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using mixtures of petroleum ether and ethyl acetate (75:25) as eluent to give the title product (2.4 g, 30%), mp 60–62 °C (lit.[33] mp 80–81 °C).

4.3. 7-Methoxychroman-4-one (3a)

A mixture of 3-(3-methoxyphenyl)-propionic acid (0.5 g) and polyphosphoric acid (2 g) was stirred at 70–75 °C for 1.5 h and at rt for 2 h. The mixture was diluted with ice-cold water and extracted with ether (2× 50 mL). The combined ethereal solution was washed with aq. sodium carbonate (10%, 20 mL), water (50 mL), brine (30 mL), and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using mixtures of petroleum ether and ethyl acetate (85:15) as eluent to give **3a** (0.21 g, 46%), which was crystallized from chloroform–hexane, mp 46–48 °C (lit.[33] mp 52–54 °C), IR (neat): 2942, 1681, 1610, 1259, 1234, 1159, 1119, 1024, 839 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.76 (2H, t, J = 6.5 Hz, H-3), 3.84 (3H, s, Ar-OCH_3), 4.52 (2H, t, J = 6.5 Hz, H-2), 6.41 (1H, d, J = 2.4 Hz, H-8), 6.58 (1H, dd, J = 2.4, 8.8 Hz, H-6), 7.84 (1H, d, J = 8.8 Hz, H-5); LCMS (positive scan): m/z 179 $(M+H)^+$.

4.4. 7-Hydroxychroman-4-one (4)

A mixture of **3a** (0.35 g) and pyridine hydrochloride (3.5 g) was stirred in N_2 atmosphere at 180–190 °C for 2 h. The cooled mixture was diluted with water (100 mL), acidified with dil HCl, and extracted with ethyl acetate (3× 50 mL). The combined ethyl acetate solution was washed with water (30 mL), brine (30 mL), and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using mixtures of petroleum ether and ethyl acetate (80:20) as eluent to give **4** (0.2 g, 62%), mp 146–148 °C (lit.[34] mp 145–146 °C), IR (KBr): 3271, 2924, 1650, 1602, 1244, 1164, 1127, 1035 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.65 (2H, t, J = 6.5 Hz, H-3), 4.45 (2H, t, J = 6.5 Hz, H-2), 6.28 (1H, d, J = 2.1 Hz, H-8), 6.44 (1H, dd, J = 2.1, 8.6 Hz, H-6), 7.61 (1H, d, J = 8.6 Hz, H-5), 8.16 (1H, br s, Ar-OH); LCMS (negative scan): m/z 163 $(M-H)^-$.

4.5. General procedure for the preparation of *E*-homoisoflavonoids

A mixture of chroman-4-one (1.22 mmol), substituted benzaldehyde (1.76 mmol), and piperidine (5 drops) was heated at 70–80 °C for 2 h. The cooled reaction mixture was diluted with water (50 mL), acidified with dil HCl, and extracted with ethyl acetate (3× 50 mL). The combined EtOAc layer was washed with water (30 mL), brine (30 mL), and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using mixtures of petroleum ether and ethyl acetate (80:20) as eluent to give homoisoflavonoids (43–72% yield).

4.5.1. 7-Hydroxy-3-[(4-methoxyphenyl)methylene]chroman-4-one (bonducellin, 5*E*). Crystallized from chloroform–methanol as a pale yellow powder (225 mg, 65%), mp 206–208 °C (lit.[10] mp 208 °C), IR (KBr): 3256, 1656, 1603, 1289, 1260, 1240, 1157, 1028 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.85 (3H, s, Ar-OCH_3), 5.32 (2H, d, J = 1.8 Hz, H-2), 6.37 (1H, d, J = 2.1 Hz, H-8), 6.57 (1H, dd, J = 2.1, 8.6 Hz, H-6), 6.96 (2H, d, J = 8.7 Hz, H-3′,5′), 7.26 (2H, d, J = 8.7 Hz, H-2′,6′), 7.74 (1H, s, H-9), 7.85 (1H, d, J = 8.6 Hz, H-5); ^{13}C NMR ($CDCl_3$+DMSO-d_6) δ 180.2 (C-4), 164.4 (C-7), 162.5 (C-8a), 160.0 (C-4′), 135.5 (C-9), 131.4 (C-2′,6′), 129.2 (C-5), 128.7 (C-3), 126.6 (C-1′), 114.4 (C-4a), 113.8 (C-3′,5′), 110.9 (C-6), 102.4 (C-8), 67.3 (C-2), 54.9 (–OCH_3); LCMS (negative scan): m/z 281 $(M-H)^-$.

4.5.2. 7-Hydroxy-3-[(2,4-dimethoxyphenyl)methylene]-chroman-4-one (2′-methoxybonducellin, 6*E*). Crystallized from chloroform–methanol as a pale yellow amorphous powder (245 mg, 64%), mp 210–212 °C (lit.[11] mp not given), IR (KBr): 3174, 1628, 1267, 1163, 1033 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.85 (3 H, s, Ar-OCH_3), 3.87 (3H, s, Ar-OCH_3), 5.20 (2H, s, H-2), 6.30 (1H, d, J = 2.1 Hz, H-8), 6.52 (1 H, dd, J = 2.1, 8.7 Hz, H-6), 6.57 (1H, d, J = 8.2 Hz, H-5′), 6.58 (1H, s, H-3′), 7.02 (1H, d, J = 8.2 Hz, H-6′), 7.75 (1H, d, J = 8.7 Hz, H-5), 7.78 (1H, s, H-9), 10.53 (1H, br s, Ar-OH); ^{13}C NMR ($CDCl_3$+DMSO-d_6) δ 179.6, 164.1, 162.2, 161.8, 158.9, 131.1, 130.8, 128.8, 128.4, 115.3, 114.1, 110.5, 104.4, 102.1, 97.8, 67.4, 55.1, 54.9; LCMS (negative scan): m/z 311 $(M-H)^-$.

4.5.3. 7-Hydroxy-3-[(3,4-dihydroxyphenyl)methylene]-chroman-4-one (sappanone A, 7*E*). Crystallized from chloroform–methanol as a pale yellow powder (200 mg, 58%), mp 220–222 °C (lit.[13] mp 220–221 °C), IR (KBr): 3401, 1651, 1615, 1585, 1264, 1215, 1162, 1109 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 5.34 (2H, s, H-2), 6.35 (1H, s, H-8), 6.55 (1H, d, J = 8.1 Hz, H-5′), 6.70–6.89 (1H, m, H-6), 6.80–6.89 (2H, m, H-2′,6′), 7.66 (1H, s, H-9), 7.82 (1H, d, J = 7.8 Hz, H-5), 8.5–9.0 (2H, br s, 2 × Ar-OH), 9.8–10.2 (1H, br s, Ar-OH); LCMS (negative scan): m/z 283 $(M-H)^-$.

4.5.4. 7-Methoxy-3-[(4-methoxyphenyl)methylene]chroman-4-one (8*E*). Crystallized from chloroform–hexane as a colorless powder (245 mg, 68%), mp 130–132 °C (lit.[35] mp 124–125 °C), IR (neat): 2966, 1660, 1605, 1589, 1259, 1107, 1028, 829 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.84 (3H, s, Ar-OCH_3), 3.86 (3H, s, Ar-OCH_3), 5.36 (2H, s, H-2), 6.40 (1H, d, J = 2.1 Hz, H-8), 6.63 (1H, dd, J = 2.1, 8.9 Hz, H-6), 6.96 (2H, d, J = 8.8 Hz, H-3′,5′), 7.27 (2 H, d, J = 8.8 Hz, H-2′,6′), 7.81 (1H, s, H-9), 7.96 (1H, d, J = 8.9 Hz, H-5); LCMS (positive scan): m/z 297 $(M+H)^+$.

4.5.5. 7-Hydroxy-3-[(3,4,5-trimethoxyphenyl)methylene]chroman-4-one (9*E*). Crystallized from chloroform–hexane as a colorless powder (280 mg, 67%), mp 198–200 °C, IR (KBr): 3233, 2925, 1650, 1618, 1583, 1278, 1158, 1126, 1009 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.80 (3H, s, Ar-OCH_3), 3.86 (6H, s, 2 × Ar-OCH_3), 5.37 (2H, s, H-2), 6.32 (1H, d, J = 2.0 Hz, H-8), 6.53 (1H, dd, J = 2.0, 8.8 Hz, H-6), 6.60 (2H, s, H-2′,6′), 7.65 (1H, s, H-9), 7.76 (1H, d, J = 8.8 Hz, H-5), 10.37 (1H, br s, Ar-OH); LCMS (negative scan): m/z 341 $(M-H)^-$. Elemental analysis, found: C, 66.62; H, 5.34. Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30.

4.5.6. 7-Hydroxy-3-[(*N*,*N*-dimethylaminophenyl)methylene]chroman-4-one (10*E*). Crystallized from chloroform–methanol as a colorless powder (190 mg, 53%), mp 264–266 °C, IR (KBr): 3435, 1650, 1599, 1298, 1243, 1199, 1169, 1106 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.05 (6H, s, –$N(CH_3)_2$), 5.38 (2H, s, H-2), 6.36 (1H, s, H-8), 6.55 (1H, d, J = 8.6 Hz, H-6), 6.73 (2H, d, J = 8.4 Hz, H-3′,5′), 7.23 (2H, d, J = 8.4 Hz, H-2′,6′), 7.72 (1H, s, H-9), 7.83 (1H, d, J = 8.6 Hz, H-5), 9.95 (1H, br s, Ar-OH); LCMS (positive scan): m/z 296 $(M+H)^+$. Elemental analysis, found: C, 73.19; H, 5.86. Calcd. for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80.

4.5.7. 7-Hydroxy-3-[(4-hydroxyphenyl)methylene]chroman-4-one (11*E*). Crystallized from chloroform–methanol as a colorless powder (200 mg, 61%), mp 220–222 °C, IR (KBr): 3279, 1651, 1623, 1604, 1291, 1261, 1232, 1161, 1112 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 5.26 (2H, s, H-2), 6.30 (1H, s, H-8), 6.49 (1H, d, J = 8.4 Hz, H-6), 6.84 (2H, d, J = 8.2 Hz, H-3′,5′), 7.10 (2H, d, J = 8.2 Hz, H-2′,6′), 7.67 (1H, s, H-9), 7.79 (1H, d, J = 8.4 Hz, H-5), 9.32 (1H, s, Ar-OH), 9.81 (1H, s, Ar-OH); LCMS (negative scan): m/z 267 $(M-H)^-$. Elemental analysis, found; C, 71.61; H, 4.55. Calcd. for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51.

4.5.8. 7-Hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene]chroman-4-one (12*E*). Crystallized from chloroform–methanol as a colorless powder (160 mg, 43%), mp 286–290 °C, IR (KBr): 3436, 1650, 1604, 1285, 1235, 1200, 1102, 1041 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.32 (2H, s, H-2), 6.30 (1H, d, J = 1.9 Hz, H-8), 6.36 (2H, s, H-2′,6′), 6.51 (1H, dd, J = 1.9, 8.1 Hz, H-6), 7.42 (1H, s, H-9), 7.70 (1H, d, J = 8.1 Hz, H-5), 8.78 (1H, br s, Ar-OH), 9.17 (2H, br s, 2 × Ar-OH), 10.59 (1H, br s, Ar-OH); LCMS (negative scan): m/z 299 $(M-H)^-$. Elemental analysis, found; C, 63.97; H, 4.09. Calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03.

4.5.9. 7-Hydroxy-3-[(3-carboxy-4-hydroxyphenyl)methylene]chroman-4-one (13*E*). Crystallized from chloroform–methanol as a colorless powder (200 mg, 52%), mp 236–238 °C, IR (KBr): 3433, 3062, 1657, 1602, 1292, 1253, 1200, 1169, 1106, 1038 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.36 (2H, s, H-2), 6.31 (1H, s, H-8), 6.52 (1H, d, J = 8.3 Hz, H-6), 6.74 (1H, d, J = 7.8 Hz, H-5′), 7.30 (1H, s, H-6′), 7.56 (1H, s, H-2′), 7.71 (1H, d, J = 8.3 Hz, H-5), 7.72 (1H, s, H-9), 8.46 (1H, br s, Ar-OH), 10.30 (1H, br s, Ar-OH); LCMS (negative scan): m/z 311 $(M-H)^-$. Elemental analysis, found; C, 65.37; H, 3.89. Calcd. for $C_{17}H_{12}O_6$: C, 65.39; H, 3.87.

4.5.10. 3-[(4-Hydroxyphenyl)methylene]-6-methoxy-chroman-4-one (14*E*). Crystallized from chloroform–hexane as a colorless powder (250 mg, 72%), mp 184–185 °C; IR (neat): 3410, 1648, 1610, 1280, 1161, 1022 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.84 (3H, s, Ar-OCH_3), 5.33 (2H, d, J = 1.5 Hz, H-2), 5.36 (1H, s, Ar-OH), 6.91 (2H, d, J = 8.8 Hz, H-3′,5′), 6.92 (1H, d, J = 8.7 Hz, H-8), 7.10 (1H, dd, J = 2.3, 8.7 Hz, H-7), 7.24 (2H, d, J = 8.8 Hz, H-2′,6′), 7.44 (1H, d, J = 2.3 Hz, H-5), 7.83 (1H, s, H-9); LCMS (positive scan): m/z 283 $(M+H)^+$. Elemental analysis, found; C, 72.29; H, 5.04. Calcd. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00.

4.6. Preparation of $HClO_4$–SiO_2

Perchloric acid (1.25 g, 12.5 mmol, as a 70% aqueous solution) was added to the suspension of silica gel (23.75 g, 200–400 mesh) in diethyl ether. The mixture was concentrated and the residue was heated at 100 °C for 72 h under vacuum to afford $HClO_4$–SiO_2 reagent, as a free-flowing powder.

4.7. 7-Hydroxy-3-[(4-chlorophenyl)methylene]chroman-4-one (15*E*)

A mixture of 7-hydroxychroman-4-one (328 mg, 2 mmol), 4-chlorobenzaldehyde (393 mg, 2.8 mmol), and a catalytic amount of $HClO_4$–SiO_2 ($\sim$ 50 mg) was heated under stirring at 90–100 °C for 30 min. After completion of the reaction, the residue was chromatographed over silica gel column using mixtures of hexane and ethyl acetate as eluents to give **15** (407 mg, 71%), which was crystallized from chloroform–methanol as a colorless powder, mp 196–198 °C, IR (KBr): 3224, 1653, 1619, 1591, 1284, 1221, 1157, 1115, 1017 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 5.28 (2H, s, H-2), 6.32 (1H, d, J = 1.7 Hz, H-8), 6.54 (1H, dd, J = 1.7, 8.8 Hz, H-6), 7.33 (2H, d, J = 8.3 Hz, H-3′,5′), 7.45 (2H, d, J = 8.3 Hz, H-2′,6′), 7.67 (1H, s, H-9), 7.77 (1H, d, J = 8.8 Hz, H-5); LCMS (negative scan): m/z 285, 287 $(M-H)^-$. Elemental analysis, found: C, 67.01; H, 3.89. Calcd. for $C_{16}H_{11}ClO_3$: C, 67.03; H, 3.87.

4.8. General procedure for the preparation of *Z*-homoisoflavonoids

A solution of *E*-homoisoflavonoids (1 mmol) in benzene (100 mL) was irradiated using a medium pressure mercury lamp for 4 h and the solvent was evaporated. The residue was chromatographed over silica gel column to give the *Z*-isomers (32–54% yield).

4.8.1. 7-Hydroxy-3-[(4-methoxyphenyl)methylene]chroman-4-one (isobonducellin, 5*Z*). Crystallized from chloroform–hexane as a pale yellow powder (120 mg, 42%), mp 152–154 °C (lit.[11] mp 156–158 °C), IR (KBr): 3130, 1639, 1602, 1572, 1248, 1181, 1159, 1124, 1028 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.81 (3 H, s, Ar-OCH_3), 4.93 (2 H, s, H-2), 6.35 (1H, d, J = 2.0 Hz, H-8), 6.52 (1H, dd, J = 2.0, 8.8 Hz, H-6), 6.82 (1H, s, H-9), 6.85 (2H, d, J = 8.6 Hz, H-3′,5′), 7.78 (1H, d, J = 8.8 Hz, H-5), 7.86 (2H, d, J = 8.6 Hz, H-2′,6′); ^{13}C NMR ($CDCl_3$+DMSO-d_6) δ 181.6 (C-4), 164.4 (C-7), 162.6 (C-8a), 160.2 (C-4′), 138.6 (C-9), 132.5 (C-2′,6′), 129.2 (C-5), 127.3 (C-3), 126.7 (C-1′), 115.6 (C-4a), 112.9 (C-3′,5′), 110.7 (C-6), 102.2 (C-8), 75.1 (C-2), 54.9 (–OCH_3); LCMS (negative scan): m/z 281 $(M-H)^-$.

4.8.2. 7-Hydroxy-3-[(2,4-dimethoxyphenyl)methylene]-chroman-4-one (6*Z*). Crystallized from chloroform as a colorless powder (100 mg, 32%), mp 172–174 °C, IR (KBr): 3156, 1653, 1613, 1268, 1160, 1112, 1037 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.78 (6H, s, 2 × Ar-OCH_3), 4.98 (2H, s, H-2), 6.31 (1H, d, J = 2.0 Hz, H-8), 6.46–6.54 (3H, m, H-3′,5′,6), 7.07 (1H, s, H-9), 7.67 (1H, d, J = 8.8 Hz, H-5), 7.86 (2H, d, J = 8.3 Hz, H-2′), 10.61 (1H, br s, Ar-OH); LCMS (negative scan): m/z 311 $(M-H)^-$.

4.8.3. 7-Methoxy-3-[(4-methoxyphenyl)methylene]chroman-4-one (8*Z*). Colorless oil (160 mg, 54%), IR (neat): 2927, 1663, 1606, 1246, 1179, 1158, 1104, 1029, 968 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.80 (6H, s, 2 × Ar-OCH_3), 4.94 (2H, s, H-2), 6.39 (1H, d, J = 2.1 Hz, H-8), 6.59 (1H, dd, J = 2.1, 8.8 Hz, H-6), 6.78 (1H, s, H-9), 6.87 (2H, d, J = 8.8 Hz, H-3′,5′), 7.87 (2H, d, J = 8.8 Hz, H-2′,6′), 7.93 (1H, d, J = 8.8 Hz, H-5); LCMS (positive scan): m/z 297 $(M+H)^+$.

4.9. Superoxide free radical scavenging activity

The superoxide free radical scavenging activity of homoisolfavonoids (**5**–**15**) was determined by the NBT method.[24,25] The assay mixture contained EDTA (6.0 μM), NaCN (3 μg), riboflavin (2 μM), NBT (50 μM), various concentrations of the test substances in ethanol, and a phosphate buffer (58 mM, pH 7.8) in a final volume of 3 mL. The tubes were shaken well, and the absorbance was measured at 560 nm and mean control OD is 0.8247. The test tubes were uniformly illuminated with an incandescent lamp for 15 min, after which the absorbance was measured again at 560 nm. The percent inhibition of superoxide radical generation was measured by comparing the mean absorbance values of control tubes and those of the test substances. IC_{50} values were obtained from the plot drawn of concentration in μg versus percent inhibition and were converted into μM. All the tests were run in triplicate and data were analyzed using one-way ANOVA.

4.10. DPPH free radical scavenging activity

DPPH radical scavenging activity was measured based on the reduction of methanolic solution of the colored DPPH.[28] Free radical scavenging ability of the test drug in ethanol added to the methanolic solution of DPPH is inversely proportional to the difference in initial and final absorption of DPPH solution at 517 nm and mean control OD is 0.8998. Drug activity is expressed as the 50% inhibitory concentration (IC_{50}). The reaction mixture contained 1×10^{-4} mM methanolic solution of DPPH and various concentrations of test drugs and kept in a dark area for 50 min. The absorbance of the samples was measured on a spectrophotometer against a blank. The percent inhibition was determined by comparing the mean absorbance values of control tubes and those of the test substances. IC_{50}values were obtained from the plot drawn of concentration in μg verses percent inhibition. All the tests were run in triplicate and data were analyzed using one-way ANOVA.

4.11. 5-Lipoxygenase enzyme inhibitory activity

The four synthetic natural homoisoflavonoids and their analogs were screened for their 5-LOX inhibitory potential using colorimetric method.[30] The assay mixture contained 50 mM phosphate buffer, pH 6.3, 5-lipoxygenase, various concentrations of test substances in dimethylsulfoxide, and linoleic acid (80 mM) in a total volume of 0.5 mL, after 5 min incubation of the above reaction mixture, 0.5 mL ferric xylenol orange reagent (in perchloric acid) was added and absorbance was measured after two minutes at 585 nm on a spectrophotometer. Controls were run along with test in a similar manner, except using vehicle instead of test substance solution. Percent inhibition was calculated by comparing the absorbance values of the test solution with that of control. All the tests were run in triplicate and averaged.

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References and notes

1. Lockhart, I. M. In *The Chemistry of Heterocylic Compounds; Chromenes, Chromanones and Chromones*; Ellis, G. P., Ed.; John Wiley & Sons: New York, 1977.
2. A1 Nakib, T.; Bezjak, V.; Meegan, M. J.; Chandy, R. *Eur. J. Med. Chem.* **1990**, *25*, 455.
3. Kirkiacharian, B. S.; Gomis, M.; Koutsourakis, P. *Eur. J. Med. Chem.* **1989**, *24*, 309.
4. Wall, M. E.; Wani, M. C.; Manikumar, G.; Taylor, H.; McGivney, R. *J. Nat. Prod.* **1989**, *52*, 774.
5. Desideri, N.; Olivieri, S.; Stein, M. L.; Sgro, R.; Orsi, N.; Conti, C. *Antiviral Chem. Chemother.* **1997**, *8*, 545.
6. Malhotra, S.; Sharma, V. K.; Parmar, V. S. *J. Chem. Res. (S)* **1988**, 179.
7. Farkas, L.; Gottsegen, A.; Nogradi, M. *Tetrahedron* **1970**, *26*, 2787.
8. Basavaiah, D.; Bakthadoss, M.; Pandiaraju, S. *J. Chem. Soc., Chem. Commun.* **1998**, 1639.
9. Purushothaman, K. K.; Kalyani, K.; Subramaniam, K.; Shanmughanathan, S. P. *Indian J. Chem.* **1982**, *21B*, 383.
10. McPherson, D. D.; Cordell, G. A.; Soejarto, D. D.; Pezzuto, J. M.; Fong, H. H. S. *Phytochemistry* **1983**, *22*, 2835.
11. Srinivas, K. V. N. S.; Rao, Y. K.; Mahender, I.; Das, B. *Phytochemistry* **2003**, *63*, 789.
12. Zhao, P.; Iwamoto, Y.; Kouno, I.; Egami, Y.; Yamamoto, H. *Phytochemistry* **2004**, *65*, 2455.
13. Saitoh, T.; Sakashita, S.; Nakata, H.; Shimokawa, T.; Kinjo, J.-E.; amahara, J.; Yamasaki, M.; Nohara, T. *Chem. Pharm. Bull.* **1986**, *34*, 2506.
14. Namikoshi, M.; Nakata, H.; Nuno, M.; Ozawa, T.; Saitoh, T. *Chem. Pharm. Bull.* **1987**, *35*, 3568.
15. Samuelsson, B. *Science* **1983**, *220*, 568.
16. Ghosh, J.; Myers, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13182.
17. Parham, W. E.; Huestis, L. D. *J. Am. Chem. Soc.* **1962**, *84*, 813.
18. Venkateswarlu, S.; Panchagnula, G. K.; Guraiah, M. B.; Subbaraju, G. V. *Tetrahedron* **2005**, *61*, 3013.
19. Levai, A.; Nemes, C.; Patonay, T. *Heterocycl. Commun.* **1999**, *5*, 441.
20. Chakraborti, A. K.; Gulhane, R. *J. Chem. Soc., Chem. Commun.* **2003**, 1896.
21. Bevan, J. A.; Gagnon, P. E.; Rae, I. D. *Can. J. Chem.* **1965**, *43*, 2612.
22. Kevill, D. N.; Weiler, E. D.; Cromwell, N. H. *J. Org. Chem.* **1964**, *29*, 1276.
23. Bohler, P.; Tamm, Ch. *Tetrahedron Lett.* **1967**, 3479.
24. McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049.
25. Venkateswarlu, S.; Raju, M. S. S.; Subbaraju, G. V. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 2236.
26. Badami, S.; Prakash, O.; Dongre, S. H.; Suresh, B. *Indian J. Pharmacol.* **2005**, *37*, 251.
27. Wang, M.; Jin, Y.; Ho, C.-T. *J. Agric. Food Chem.* **1999**, *47*, 3974.
28. Lamaison, J. I.; Ptijean-Freytet, C.; Carnet, A. *Pharm. Acta Helv.* **1991**, *66*, 185.
29. Kang, S. S.; Lee, J. Y.; Choi, Y. K.; Kim, G. S.; Han, B. H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2261.
30. Gay, C. A.; Gebicki, J. M. *Anal. Biochem.* **2002**, *304*, 42.
31. Komoda, T.; Yoshida, K.; Abe, N.; Suguyama, Y.; Imachi, M.; Hirota, H.; Koshino, H.; Hirota, A. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 104.
32. Morsingh, F.; Ong, S. H. *Tetrahedron* **1969**, *25*, 361.
33. Loudon, J. D.; Razdan, R. K. *J. Chem. Soc.* **1954**, *7*, 4299.
34. Narkhede, D. D.; Iyer, P. R.; Iyer, C. S. R. *Tetrahedron* **1990**, *46*, 2031.
35. Evans, D.; Lockhart, I. M. *J. Chem. Soc. (C)* **1966**, 711.