Novel Daidzein Analogs and Their *in Vitro* Anti-Influenza Activities

by **Shu-Ting Chung**[a]), **Yi-Ting Huang**[b]), **Hsin-Yi Hsiung**[b]), **Wen-Hsin Huang**[a])[b]), **Chen-Wen Yao**[c]), and **An-Rong Lee***[a])[b])

[a]) Institute of Medical Sciences, National Defense Medical Center, 161 Minchuan East Rd. Sec. 6, Taipei, 11490, Taiwan

[b]) School of Pharmacy, National Defense Medical Center, 161 Minchuan East Rd. Sec. 6, Taipei, 11490, Taiwan

[c]) Deparment of Pathology and Parasitology, Tri-Service General Hospital, National Defense Medical Center, 161 Minchuan East Rd. Sec. 6, Taipei, 11490, Taiwan

(phone: +886-2-87923100 ext. 18873; fax: +886-2-87923169; e-mail: lar@mail.ndmctsgh.edu.tw)

A series of novel isoflavonoids were synthesized based on structural modifications of daidzein, an active ingredient of traditional Chinese medicine (TCM) and evaluated for their anti-influenza activity, *in vitro*, against H1N1 *Tamiflu*-resistant (H1N1 TR) virus in the MDCK cell line. Among them, 4-oxo-4*H*-1-benzopyran-8-carbaldehydes **11a**–**11g** were most promising, and they demonstrated better activities and selectivities comparable to those the reference ribarivin, a nucleoside antiviral agent. 3-(4-Bromophenyl)-7-hydroxy-4-oxo-4*H*-1-benzopyran-8-carboxaldehyde (**11c**) displayed the best inhibitory activity (EC_{50}, 29.0 μM) and selectivity index ($SI > 10.3$). Analysis of the structure–activity relationships (SAR) indicated that both the non-naturally-occurring Br-substituted *B*-ring and appropriate CHO and OH groups on the *A*-ring might be critical for the activity and selectivity against H1N1 TR influenza viruses.

Introduction. – Prompted by the success of oseltamivir ('*Tamiflu*') and zanamivir ('*Relenza*'), two major neuraminidase (NA) inhibitors, drug designs for novel anti-H1N1 viral agents in recent decades have been focused on the transition-state analogs of substrate molecules. Since the structures of these compounds resemble the transition state of the sialoside substrate molecule in NA-catalyzed hydrolysis, they would bind more tightly to the enzyme than the actual substrate, and thus may be potent competitive inhibitors of neuraminidase [1][2]. Unfortunately, such potent NA inhibitors are always associated with inevitable adverse effects [3]. Recent studies also indicate that, owing to the widespread and indiscriminate use of oseltamivir, strains of pathogenic oseltamivir-resistant pandemic H1N1 viral isolates have gradually emerged, mainly resulting from the mutation of a single amino acid (His274Tyr in N1) in NA [4], which may lead to increasing fatality in human beings [5][6].

Recently, there have been several interesting reports concerning the anti-influenza activity of flavonoids, which possess three aromatic rings designated as ring *A*, *B*, and *C* based on the sequence of their biogenesis (*Fig.*) [7–12]. According to their structure–activity relationships (SAR), substituents capable of forming a H-bonding at C(8) on the *A* ring reportedly benefits NA inhibitory activity (*e.g.*, **1**, IC_{50}, 40.91 μM) [13]. While flavonoids have a 2-phenyl-4*H*-1-benzopyran-4-one backbone, isoflavonoids possess a 3-phenyl-4*H*-1-benzopyran-4-one backbone, resulting from the

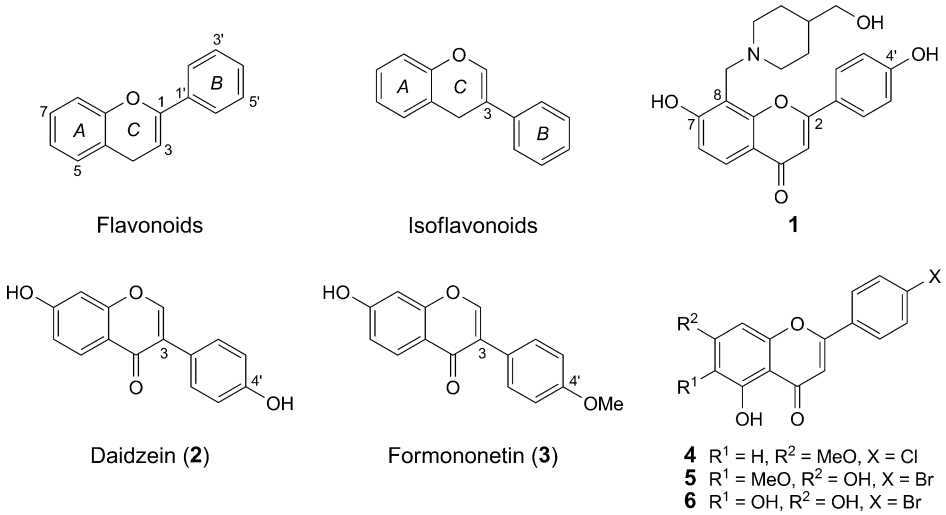


Figure. *The structures of flavonoids, isoflavonoids,* **1***, daidzein* (**2**)*, formononentin* (**3**)*, and* **4**–**6**

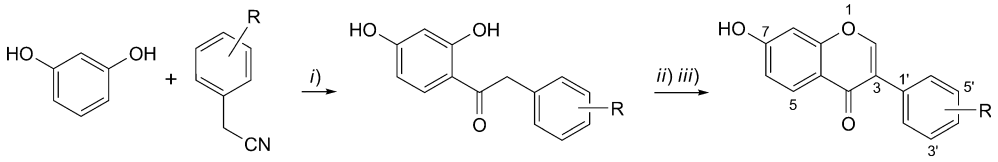
flavonoid biogenesis pathway. One of the important traditional Chinese medicine (TCM) components is daidzein (**2**), an isoflavone isolated from Kudzu (*Pueraria lobata* (WILD.) OHWI) [14] and abundant in soybeans (*Glycine max* (L.) MERRILL), which are a major dietary supplement in many Asian countries. This isoflavone has been reported to display significant antiviral and antidiabetic features [15–18]. A recent study indicated that two structurally similar isoflavones, daidzein (**2**; 4′-OH in ring *B*) and formononetin (**3**; 4′-MeO in ring *B*), isolated from beans had anti-influenza virus activity through inhibiting NA [19]. Although the anti-influenza virus activity of **2** ($IC_{50} > 787$ μM) is reportedly more potent than that of **3** (IC_{50} not determined), neither of their potencies is strong enough to be considered as a candidate for further development, and therefore they need optimization.

We have already reported that flavonoids with a *B* ring bearing properly positioned Cl-atoms, *e.g.*, compound **4**, exhibited significantly improved antiviral activity and selectivity [20]. We later showed that, after further modification of baicalein, a flavone ingredient found in the TCM *Scutellaria baicalensis* GEORGI, some synthetic products showed extremely potent *in vitro* activities against H1N1 *Tamiflu*-resistant (TR) and seasonal H3N2-infected influenza viruses with preserved favorable selectivity [21]. In this study, non-naturally-occurring Br-substituted *B*-rings and the OH group on the *A*-rings (*e.g.*, **5** and **6**) might both have critical effects on the activity and selectivity against H1N1-*Tamiflu*-resistant influenza viruses. To the best of our knowledge, however, no studies have been systematically conducted on the SAR on the isoflavonoid framework of daidzein (**2**) analogs. Therefore, in this study we applied the above mentioned approaches to structural modification on daidzein (**2**), especially on the *A* and *B* rings, to ameliorate the capability of the compound to inhibit the influenza virus. Again, some of our synthetic products displayed more potent *in vitro* activities and higher selectivities against the H1N1 TR virus than the reference ribavirin. The essential functional groups and moieties are discussed herein.

Results and Discussion. – *Design.* Reports on the weakly active daidzein (**2**), the active ingredient of the popular TCM *Puerariae Radix*, prompted this study of structural optimization on isoflavonoids. By employing similar strategies as in our previous reports, we envisaged that it would be possible to improve the antiviral activity and selectivity of the isoflavonoids *via* subjecting their *A* and *B* ring to bear appropriately positioned electron-donating or -withdrawing groups [20][21]. Herein, we designed the synthetic routes for preparation of three isoflavone derivatives, 2-[4-oxo-4*H*-1-benzopyran-7-yl)oxy]acetates **9**, [4-oxo-4*H*-1-benzopyran-7-yl)oxy]acetonitriles **10**, and 4-oxo-4*H*-1-benzopyran-8-carbaldehydes **11**. The aim was to use this new compound library to establish the SAR in anti-influenza virus activity.

Synthesis. As outlined in *Scheme 1*, the isoflavonoid daidzein (**2**) analogs were synthesized by a modified *Houben–Hoesch* approach [22][23]. The yields of the intermediates **7a**–**7g** obtained by reaction of resorcinol (= benzene-1,3-diol) and various substituted phenylacetonitriles were consistently within the range of 30–50%, *i.e.*, apparently much lower than the yields of (*i.e.*, >80%) obtained when using phloroglucinol (= benzene-1,3,5-triol) and $ClCH_2CN$ as starting materials [22]. The low yields are likely attributable to the electron-donating effects of the starting phenolic compounds on reactivity, *i.e.*, the higher the electron density on the benzene ring of phenols, the higher the reactivity in this electrophilic acylation. In contrast to phloroglucinol, resorcinol contains two OH groups on its benzene ring, which may explain its lower reactivity compared to phloroglucinol which possesses three OH groups. However, other subtle effects on yields such as the moisture of HCl and its flow rate could not be excluded, since we tended to observe variations of the yields in the repetition of the same reactions. During the synthesis of intermediate **7g**, an unexpected side product with a lower polarity was repeatedly detected. This side product, examined under scrutiny, turned out to be ethyl 2-(4-nitrophenyl)acetate, the structure of which was established by ^{1}H-NMR spectroscopy ((D_6)DMSO): 1.18 (*t*, *J* = 7.2, 3 H), 3.86 (*s*, 2 H), 4.09 (*q*, *J* = 7.2, 2 H), 7.55 (*d*, *J* = 8.7, 2 H), 8.18 (*d*, *J* = 8.7, 2 H)) and mass spectrometry (*m*/*z* 209 (M^+)). We speculate that, before the *Houben–Hoesch* reaction, some starting material 2-(4-nitrophenyl)acetonitrile, highly activated by the NO_2 group, might be first attacked by Et_2O, catalyzed by the acids, on the C-atom of the

Scheme 1



7a R = 4'-MeO
7b R = 3',4',5'-$(MeO)_3$
7c R = 4'-Br
7d R = 4'-Cl
7e R = 3'-OCH_2O-4'
7f R = 4'-F
7g R = 4'-NO_2

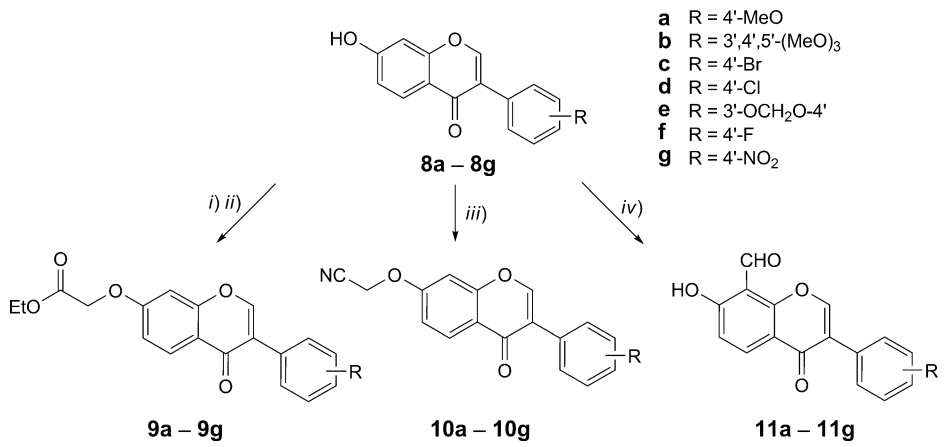
8a R = 4'-MeO
8b R = 3',4',5'-$(MeO)_3$
8c R = 4'-Br
8d R = 4'-Cl
8e R = 3'-OCH_2O-4'
8f R = 4'-F
8g R = 4'-NO_2

i) $ZnCl_2$, HCl, Et_2O, 12 h. *ii*) $BF_3 \cdot Et_2O$, DMF, 50°. *iii*) $MeSO_2Cl$, DMF, 100°, 1 h.

CN group, and undergoes subsequent hydrolysis to form the final ester product. The key intermediates **7a**–**7g** were converted smoothly to isoflavones **8a**–**8g** in tolerably good yields. Esterification on HO–C(7) of **8a**–**8g** with $ClCH_2COOEt$ afforded **9a**–**9g** with fairly high yields (65–95%; *Scheme 2*). Although simple filtration and washing generally provided sufficient purity, their preparations were time-consuming (*ca.* 12 h) and required dry acetone as the reaction solvent. Reaction of **8a**–**8g** with $BrCH_2CN$ under basic conditions produced **10a**–**10g** with yields ranging from 68 to 98%. While the syntheses of **10a**–**10g** proceeded smoothly with satisfactory purities and yields, preparation of **11a**–**11g** by formylation at C(8) of **8a**–**8g** was consistently difficult. Attempts to synthesize **11a**–**11g** by reacting **8a**–**8g** with various reagents, such as *Vilsmeier–Haack* reagent (*N*,*N*-dimethylaniline/$POCl_3$), paraformaldehyde/$MgCl_2$, or methenamine/CF_3COOH, consistently failed, even under reflux conditions. The desired products could only be obtained by *Duff* reaction [24] with methenamine (= hexamethylenetetramine = 1,3,5,7-tetrazatricyclo[3.31.1^{3,7}]decane; 1.1 equiv.) in glacial AcOH at 100°. The reaction required 6 h to furnish **11a**–**11g** (*Scheme 2*). Compounds **9a**–**9g** could be further hydrolyzed [25] smoothly under acidic conditions. The best results were obtained by hydrolysis with a solution of H_2SO_4 in glacial AcOH (1:3) for 12 h followed by neutralization by 1N NaOH to give the end products **12a**–**12g** with the desired carboxylic acid functional groups (*Scheme 3*). The yields of the **12a**–**12g** were significantly influenced by pH values; the optimal pH value turned out to be 2 or below. Residual **9a**–**9g** that were carried over to the end products was effortlessly removed by simply partitioning the mixture with AcOEt and H_2O. After acidifying the H_2O layer to pH 2 or below with 1N HCl, the precipitated products were collected. Higher pH values usually led to co-precipitation with unidentifiable impurities.

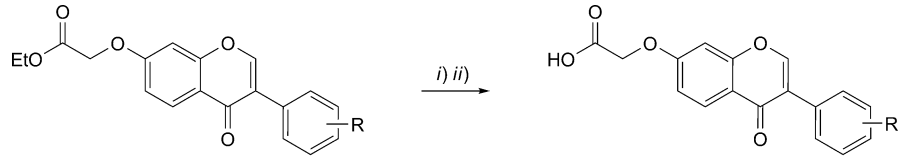
In most cases, crystallization was performed for purification. The structures of synthetic intermediates and end products were elucidated by various spectroscopic studies and by high-resolution mass spectra.

Scheme 2



i) K_2CO_3, acetone, r.t., 1 h. *ii*) $ClCH_2COOEt$, acetone, 75°, 12 h. *iii*) $BrCH_2CN$, K_2CO_3, acetone, reflux, 5–6 h. *iv*) Methenamine, glacial AcOH, 100°, 6 h.

Scheme 3



9a R = 4'-MeO
9b R = 3',4',5'-$(MeO)_3$
9c R = 4'-Br
9d R = 4'-Cl
9e R = 3'-OCH_2O-4'
9f R = 4'-F
9g R = 4'-NO_2

12a R = 4'-MeO
12b R = 3',4',5'-$(MeO)_3$
12c R = 4'-Br
12d R = 4'-Cl
12e R = 3'-OCH_2O-4'
12f R = 4'-F
12g R = 4'-NO_2

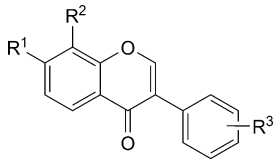
i) H_2SO_4, glacial AcOH, 12 h. *ii*) 1N NaOH, 2 h.

In Vitro *Anti-Influenza Virus Assay.* Activities of the daidzein (isoflavone) analogs in this study were expressed as 50% effective concentration (EC_{50}) against H1N1 *Tamiflu*-resistant virus in the MDCK cell line. The CC_{50} means the 50% cell cytotoxic concentration after treatment with the testing compounds, wherein selectivity index (*SI*) was expressed as CC_{50}/EC_{50} and employed in the SAR analysis (*Table*). In terms of potency of anti-H1N1 *Tamiflu*-resistant virus activity, the synthetic isoflavonoids were ranked as **11** > **9** > **12** > **10**. All our synthetic products showed moderate-to-significant antiviral activities except series **10**, which were inactive with both EC_{50} and CC_{50} higher than 300 μM. The hydrolyzed series **12** generally exhibited much lower cytotoxicities compared to their parents **9**, but revealed neither superior inhibitory effects nor more selective indexes. Compounds **9a**, **9e**, and **9g** displayed inhibitory activities in the range EC_{50} 75.6–135.7 μM; the most potent of these was **9f** with an inhibitory activity of 36.5 μM, but the cellular survival rate decreased to a concentration of 292.1 μM. Conjugation of bulkier groups, *e.g.*, $EtOCOCH_2O$-, $NCCH_2O$, and $HOCOCH_2O$, with a OH group at C(7) on the *A*-ring of isoflavonoids was scarcely advantageous to the anti-H1N1 TR virus activity.

Series **11** exhibited potent anti-H1N1 TR virus activity in MDCK cells (*SI*, 8.5–10.3) and were generally more active than the reference ribavirin. Both 7-OH and 8-CHO groups were essential for H1N1 TR virus inhibitory activity. Interestingly, the structure of the most active compound **11c** (*SI* > 10.3) bears a Br-atom on the *B* ring and a free OH group on the *A* ring, a reminiscence of the *A*- and *B*-ring substitutions in extremely potent anti-H1N1 TR flavonoids [21]. This indicates that this lipophilic substituent, 4′-Br on the *B* ring, might contribute tremendously to anti-H1N1 TR virus activity.

Conclusion. – We have synthesized a novel series of daidzein analogs and demonstrated that certain products have inhibiting effects on influenza virus. Notably, synthetic isoflavonoids with a Br-substituent on the *B* ring and a CHO and a free OH group on the *A* ring consistently revealed potent activities and highly selective indexes. This report, which presents the first systematic investigation of the use of the effects of daidzein analogs against H1N1 TR influenza virus in the MDCK cell line, led to the

Table. In Vitro *Anti-Influenza Activities of Isoflavonoids* **9**, **11**, *and* **12** *in MDCK Cells, Determined by CPE Assay*[a])[b])



Compound	R^1	R^2	R^3	H1N1 *Tamiflu*-resistant virus		
				EC_{50} [μM]	CC_{50} [μM]	*SI*
9a	$EtOCOCH_2O$	H	4′-MeO	70.63 ± 0.01	> 300	> 4.0
9b	$EtOCOCH_2O$	H	3′,4′,5′-$(MeO)_3$	60.32 ± 0.02	120.74 ± 0.03	2.0
9c	$EtOCOCH_2O$	H	4′-Br	> 300	124.02 ± 0.05	< 0.4
9d	$EtOCOCH_2O$	H	4′-Cl	> 300	139.41 ± 0.08	< 0.5
9e	$EtOCOCH_2O$	H	3′-OCH_2O-4′	135.71 ± 0.04	> 300	> 2.2
9f	$EtOCOCH_2O$	H	4′-F	36.54 ± 0.01	292.13 ± 0.03	8.0
9g	$EtOCOCH_2O$	H	4′-NO_2	135.41 ± 0.09	> 300	> 2.2
11a	OH	CHO	4′-MeO	33.82 ± 0.04	> 300	> 8.9
11b	OH	CHO	3′,4′,5′-$(MeO)_3$	33.34 ± 0.06	> 300	> 9.0
11c	OH	CHO	4′-Br	29.01 ± 0.03	> 300	> 10.3
11d	OH	CHO	4′-Cl	33.32 ± 0.01	> 300	> 9.0
11e	OH	CHO	3′-OCH_2O-4′	32.21 ± 0.07	> 300	> 9.3
11f	OH	CHO	4′-F	35.24 ± 0.02	> 300	> 8.5
11g	OH	CHO	4′-NO_2	32.13 ± 0.04	> 300	> 9.3
12a	$HOCOCH_2O$	H	4′-MeO	306.51 ± 0.06	> 300	> 1.0
12b	$HOCOCH_2O$	H	3′,4′,5′-$(MeO)_3$	258.82 ± 0.03	> 300	> 1.2
12c	$HOCOCH_2O$	H	4′-Br	> 300	> 300	ND[c])
12d	$HOCOCH_2O$	H	4′-Cl	> 300	> 300	ND
12e	$HOCOCH_2O$	H	3′-OCH_2O-4′	293.93 ± 0.05	73.52 ± 0.03	0.3
12f	$HOCOCH_2O$	H	4′-F	> 300	300	> 1.0
12g	$HOCOCH_2O$	H	4′-NO_2	300.02 ± 0.04	> 300	> 1.0
Ribavirin				40.94 ± 0.02	> 300	> 7.3

[a]) EC_{50}, Mean value the 50% effective concentration; CC_{50}, mean value the 50% cell cytotoxic concentration; *SI*, selective index, CC_{50}/EC_{50}. [b]) *In vitro* anti-H1N1 influenza virus activity of oseltamivir ('*Tamiflu*'): $EC_{50} = 32.0$ μM, $CC_{50} = 320.1$ μM, $SI = 10$; oseltamivir is void of activity against H1N1 *Tamiflu*-resistant virus. [c]) ND, Not determined. [d]) All results are expressed as mean ± SD ($n = 6$).

discovery of the 4′-Br-substituted product **11c** as the most promising. The Br-atoms might also facilitate the suppression of viral resistance. Considering their facile preparation, series **11** might be superior to commercially available drugs such as oseltamivir ('*Tamiflu*') for clinical treatment (prophylaxis and/or therapy) of H1N1-TR influenza. These results may provide a basis for further design and development of new anti-H1N1-TR agents. Detailed pharmacological studies are underway and will be reported subsequently.

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Experimental Part

General. All the chemicals were purchased from *Aldrich–Sigma* (St. Louis, MO, USA), and *Alfa–Aesar* (Heysham, LA32XY, England), and used without further purification. TLC: *F254* silica-gel plates (*Merck*). Column chromatography (CC): silica gel (70–230 mesh; *Merck*). M.p.: *Büchi-530* melting-point apparatus, uncorrected. UV/VIS Spectra: *Shimadzu UV-160A* spectrophotometer; λ_{max} ($\log \varepsilon$) in nm. IR Spectra: *Perkin-Elmer FTIR 1610* series infrared spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Varian Gemini-300* NMR spectrometer; (D_6)DMSO; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-EI-MS: *Finnigan MAT-95XL* spectrometer; in *m*/*z*.

General Procedure for the Synthesis of Isoflavones **8a**–**8g**. Resorcinol (= benzene-1,3-diol; 11.0 g, 100 mmol) and $ZnCl_2$ (5.0 g) in Et_2O (100 ml) were placed in a two-neck round bottle fitted with an inlet *Teflon* tube, through which dry HCl was passed into the stirring mixture. After two liquid layers had formed in the mixture, a soln. of a substituted phenylacetonitriles (30–80 mmol) was added dropwise to the mixture. Stirring was continued during the introduction of HCl at least for 12 h. The mixture was further stirred overnight. The Et_2O layer was decanted from the yellow solids formed, and H_2O was then added to half-volume of the flask (*ca.* 150 ml). The yellow imine hydrochloride was hydrolyzed by heating to reflux for 1 h. After cooling the mixture overnight, the precipitates were filtered and collected to obtain the corresponding 1-(2,4-dihydroxyphenyl)-2-(substituted phenyl)ethanones **7a**–7**g** (24–68%). To the respective ethanones benzylketones **7a**–**7g** (1.0 equiv.) in dry DMF (20 ml) was added dropwise $BF_3 \cdot Et_2O$ (5 equiv.), and then the mixture was warmed to 50°. To the mixture was slowly added a soln. of MsCl (3 equiv.) in dry DMF (5 ml). The resulting mixture was heated to 100° for 1 h. After cooling, it was poured into H_2O (100 ml) and left overnight to precipitate. After filtration, the precipitates were collected to give the corresponding isoflavones **8a**–**8g**: *7-hydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one* (**8a**; 70%), *7-hydroxy-3-(3,4,5-trimethoxyphenyl)-4*H*-1-benzopyran-4-one* (**8b**; 62%), *3-(4-bromophenyl)-7-hydroxy-4*H*-1-benzopyran-4-one* (**8c**; 54%), *3-(4-chlorophenyl)-7-hydroxy-4*H*-1-benzopyran-4-one* (**8d**; 59%), *3-(benzo[d][1,3]dioxol-5-yl)-7-hydroxy-4*H*-1-benzopyran-4-one* (**8e**; 56%), *3-(4-fluorophenyl)-7-hydroxy-4*H*-1-benzopyran-4-one* (**8f**; 83%), *7-hydroxy-3-(4-nitrophenyl)-4*H*-1-benzopyran-4-one* (**8g**; 87%).

General Procedure for the Synthesis of Isoflavones **9a**–**9g**. Various isoflavones derivatives **8a**–**8g** (1.0 equiv.) and anh. K_2CO_3 (3 equiv.) were placed in dry acetone (100 ml), and then the mixture was stirred for 1 h. $ClCH_2COOEt$ (1.5 equiv.) was added dropwise to the mixture, which was heated to 75° and maintained at this temp. for 12 h. Then, the mixture was evaporated under vacuum to give solid residues, to which H_2O (100 ml) was poured. The precipitates were filtered off and washed with cool H_2O to get the corresponding esters. Recrystallization from EtOH afforded pure **9a**–**9g** (65–95%).

*Ethyl 2-{[3-(4-Methoxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetate* (**9a**). Yield: 81%. M.p. 140–142°. R_f (hexane/AcOEt 1:1) 0.38. UV (MeOH): 263 (4.77). IR (KBr): 2996, 1746, 1622, 1252, 1180, 832. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 (*t*, *J* = 7.2, 3 H); 3.78 (*s*, 3 H); 4.19 (*q*, *J* = 7.2, 2 H); 4.98 (*s*, 2 H); 6.99 (*dd*, *J* = 6.9, 2.1, 2 H); 7.12 (*dd*, *J* = 9.0, 2.7, 1 H); 7.18 (*d*, *J* = 2.7, 1 H); 7.52 (*dd*, *J* = 6.9, 2.1, 2 H); 8.04 (*d*, *J* = 9.0, 1 H); 8.42 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 14.2; 55.5; 61.6; 66.2; 102.4; 114.5; 115.4; 119.8; 125.3; 125.4; 128.3; 131.0; 153.6; 158.6; 160.7; 163.4; 168.7; 175.7. HR-EI-MS: 354.1100 (M^+, $C_{20}H_{18}O_6^+$; calc. 354.1103).

*Ethyl 2-{[4-Oxo-3-(3,4,5-trimethoxyphenyl)-4*H*-1-benzopyran-7-yl]oxy}acetate* (**9b**). Yield: 67%. M.p. 127–129°. R_f (hexane/AcOEt 1:1) 0.18. UV (MeOH): 220 (4.46). IR (KBr): 2986, 1764, 1634, 1198, 1124, 835. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 (*t*, *J* = 7.2, 3 H); 3.68 (*s*, 3 H); 3.80 (*s*, 6 H); 4.19 (*q*, *J* = 7.2, 2 H); 4.99 (*s*, 2 H); 6.91 (*s*, 2 H); 7.13 (*dd*, *J* = 8.7, 2.4, 1 H); 7.20 (*d*, *J* = 2.4, 1 H); 8.02 (*d*, *J* = 8.7, 1 H); 8.50 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 13.9; 56.2; 60.1; 61.3; 65.8; 102.0; 107.6; 115.0; 119.4; 125.0; 127.9; 128.2; 139.3; 153.8; 153.9; 158.1; 163.0; 168.3; 175.1. HR-EI-MS: 414.1308 (M^+, $C_{22}H_{22}O_8^+$; calc. 414.1315).

*Ethyl 2-{[3-(4-Bromophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetate* (**9c**). Yield: 65%. M.p. 163–164°. R_f (hexane/AcOEt 1:1) 0.50. UV (MeOH): 254 (4.55). IR (KBr): 3069, 1760, 1637, 1196, 1062, 824. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 (*t*, *J* = 7.2, 3 H); 4.19 (*q*, *J* = 7.2, 2 H); 4.99 (*s*, 2 H); 7.13 (*dd*, *J* = 9.0, 2.4, 1 H); 7.21 (*d*, *J* = 2.4, 1 H); 7.55 (*dd*, *J* = 6.6, 2.1, 2 H); 7.63 (*dd*, *J* = 6.6, 2.1, 2 H); 8.05 (*d*, *J* = 9.0, 1 H); 8.52 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 14.3; 61.7; 66.2; 102.5; 115.6; 119.7; 122.4; 124.5;

128.3; 131.8; 132.1; 132.6; 154.5; 158.7; 163.7; 168.7; 175.2. HR-EI-MS: 402.0101 (M^+, $C_{19}H_{15}{}^{79}BrO_5^+$; calc. 402.0103), 404.0101 (M^+, $C_{19}H_{15}{}^{81}BrO_5^+$; calc. 404.0082).

*Ethyl 2-{[3-(4-Chlorophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetate* (**9d**). Yield: 89%. M.p. 135–137°. R_f (hexane/AcOEt 1:1) 0.50. UV (MeOH): 206 (4.56). IR (KBr): 2991, 1755, 1628, 1198, 1090, 850. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 (*t*, *J* = 7.2, 3 H); 4.19 (*q*, *J* = 7.2, 2 H); 4.99 (*s*, 2 H); 7.13 (*dd*, *J* = 9.0, 2.4, 1 H); 7.21 (*d*, *J* = 2.4, 1 H); 7.50 (*d*, *J* = 8.7, 2 H); 7.62 (*d*, *J* = 8.7, 2 H); 8.05 (*d*, *J* = 9.0, 1 H); 8.52 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 14.3; 61.7; 66.2; 102.5; 115.6; 119.8; 124.5; 128.3; 129.1; 131.5; 132.2; 134.3; 154.6; 158.7; 163.7; 168.7; 175.3. HR-EI-MS: 358.0607 (M^+, $C_{19}H_{15}ClO_5^+$; calc. 358.0608).

*Ethyl 2-{[3-(1,3-Benzodioxol-5-yl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetate* (**9e**). Yield: 73%. M.p. 164–167°. R_f (hexane/AcOEt 1:1) 0.40. UV (MeOH): 205 (4.78). IR (KBr): 2990, 1755, 1644, 1194, 1099, 832. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 (*t*, *J* = 7.2, 3 H); 4.19 (*q*, *J* = 7.2, 2 H); 4.98 (*s*, 2 H); 6.04 (*s*, 2 H); 6.98 (*d*, *J* = 8.1, 1 H); 7.06 (*dd*, *J* = 8.1, 1.8, 1 H); 7.11 (*dd*, *J* = 8.7, 2.4, 1 H); 7.15 (*d*, *J* = 1.8, 1 H); 7.18 (*d*, *J* = 2.4, 1 H); 8.03 (*d*, *J* = 8.7, 1 H); 8.42 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 14.3; 61.7; 66.2; 102.10; 102.5; 108.8; 110.5; 115.5; 119.9; 122.3; 123.3; 125.4; 127.1; 128.4; 148.6; 153.9; 158.7; 163.6; 168.7; 175.6. HR-EI-MS: 368.0894 (M^+, $C_{20}H_{16}O_7^+$; calc. 368.0896).

*Ethyl 2-{[3-(4-Fluorophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetate* (**9f**). Yield: 90%. M.p. 131–133°. R_f (hexane/AcOEt 1:1) 0.48. UV (MeOH): 248 (4.50). IR (KBr): 3599, 2988, 1755, 1633, 1223, 1068, 825. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 (*t*, *J* = 7.2, 3 H); 4.19 (*q*, *J* = 7.2, 2 H); 4.99 (*s*, 2 H); 7.13 (*dd*, *J* = 9.0, 2.4, 1 H); 7.20 (*d*, *J* = 2.4, 1 H); 7.27 (*t*, *J* = 8.7, 2 H); 7.63 (*dd*, *J* = 8.7, 5.4, 2 H); 8.05 (*d*, *J* = 9.0, 1 H); 8.49 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 13.8; 60.7; 65.2; 101.8; 114.8; 114.9; 115.1; 118.1; 122.9; 127.1; 128.2; 128.2; 130.9; 131.0; 154.2; 157.3; 162.2; 168.0; 174.4. HR-EI-MS: 342.0907 (M^+, $C_{19}H_{15}FO_5^+$; calc. 342.0904).

*Ethyl 2-{[3-(4-Nitrophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetate* (**9g**). Yield: 95%. M.p. 192–193°. R_f (hexane/AcOEt 1:1) 0.38. UV (MeOH): 219 (4.34). IR (KBr): 3002, 1752, 1629, 1351, 1199, 851. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 (*t*, *J* = 7.2, 3 H); 4.19 (*q*, *J* = 7.2, 2 H); 5.00 (*s*, 2 H); 7.16 (*dd*, *J* = 9.0, 2.4, 1 H); 7.24 (*d*, *J* = 2.4, 1 H); 7.91 (*dd*, *J* = 7.2, 2.1, 2 H); 8.07 (*d*, *J* = 9.0, 1 H); 8.29 (*dd*, *J* = 7.2, 2.1, 2 H); 8.68 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 14.3; 61.3; 65.7; 102.4; 115.7; 118.6; 122.5; 123.7; 127.7; 130.5; 139.6; 147.5; 156.3; 157.8; 162.9; 168.6; 174.5. HR-EI-MS: 369.0856 (M^+, $C_{19}H_{15}NO_7^+$; calc. 369.0849).

General Procedure for the Synthesis of Nitriles **10a**–**10g**. The respective isoflavone **8a**–**8g** (1.0 equiv.) with anh. K_2CO_3 (3 equiv.) was placed in dry acetone (100 ml). $BrCH_2CN$ (1.1 equiv.) was added dropwise to the mixture, which was heated to reflux for 5–6 h and then evaporated under vacuum to give solid residues, to which H_2O (100 ml) was poured the precipitates were filtered off to obtain the corresponding **10a**–**10g** (68–98%) after recrystallization from EtOH.

*2-{[3-(4-Methoxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetonitrile* (**10a**). Yield: 89%. M.p. 203–204°. R_f (hexane/AcOEt 1:1) 0.43. UV (MeOH): 330 (3.01). IR (KBr): 3074, 2963, 2942, 2841, 1638. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.72 (*s*, 3 H); 5.37 (*s*, 2 H); 7.00 (*d*, *J* = 8.7, 2 H); 7.21 (*dd*, *J* = 9, 2.4, 1 H); 7.39 (*d*, *J* = 2.4, 1 H); 7.54 (*d*, *J* = 9, 2 H); 8.14 (*d*, *J* = 9, 1 H); 8.48 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 54.7; 55.8; 102.9; 114.4; 115.4; 116.7; 119.7; 124.4; 124.7; 128.3; 130.8; 1584.4; 157.8; 159.6; 161.3; 175.4. HR-EI-MS: 307.0842 (M^+, $C_{18}H_{13}NO_4^+$; calc. 307.0845).

*2-{[4-Oxo-3-(3,4,5-trimethoxyphenyl)-4*H*-1-benzopyran-7-yl]oxy}acetonitrile* (**10b**). Yield: 90%. M.p. 196–198°. R_f (hexane/AcOEt 1:1) 0.22. UV (MeOH): 330 (3.04). IR (KBr): 3070, 1643, 1621, 1578. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.69 (*s*, 3 H); 3.81 (*s*, 6 H); 5.38 (*s*, 2 H); 6.93 (*s*, 2 H); 7.22 (*dd*, *J* = 8.7, 2.4, 1 H); 7.41 (*d*, *J* = 2.4, 1 H); 8.12 (*d*, *J* = 8.7, 1 H); 8.56 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 54.7; 56.7; 60.7; 102.9; 107.2; 107.5; 115.5; 116.7; 119.7; 124.5; 127.9; 128.3; 138.5; 153.2; 153.4;. 155.2; 157.8; 161.3; 175.2. HR-EI-MS: 367.1056 (M^+, $C_{20}H_{17}NO_6^+$; calc. 367.1056).

*2-{[3-(4-Bromophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetonitrile* (**10c**). Yield: 69%. M.p. 217–219°. R_f (hexane/AcOEt 1:1) 0.63. UV (MeOH): 332 (2.18). IR (KBr): 3063, 2952, 1936, 1902, 1847, 1642. ^{1}H-NMR (300 MHz, (D_6)DMSO): 5.38 (*s*, 2 H); 7.23 (*dd*, *J* = 8.7, 2.4, 1 H); 7.41 (*d*, *J* = 2.4, 1 H); 7.54 (*d*, *J* = 8.4, 2 H); 7.65 (*d*, *J* = 8.4, 2 H); 8.12 (*d*, *J* = 8.7, 1 H); 8.58 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 54.8; 103.1; 115.6; 116.7; 119.7; 121.9; 123.6; 128.3; 131.7; 131.8; 155.4; 157.9; 161.4; 174.9. HR-EI-MS: 354.9841 (M^+, $C_{17}H_{10}{}^{79}BrNO_3^+$; calc. 354.9844), 356.9822 (M^+, $C_{17}H_{10}{}^{81}BrNO_3^+$; calc. 356.9793).

*2-{[3-(4-Chlorophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetonitrile* (**10d**). Yield: 92%. M.p. 169–170°. R_f (hexane/AcOEt 1:1) 0.49. UV (MeOH): 332 (3.22). IR (KBr): 3073, 2956, 1773. ^{1}H-NMR (300 MHz, (D_6)DMSO): 5.39 (*s*, 2 H); 7.23 (*dd*, *J* = 9, 2.1, 1 H); 7.42 (*d*, *J* = 2.1, 1 H); 7.51 (*d*, *J* = 8.4, 2 H); 7.64 (*d*, *J* = 8.7, 2 H); 8.12 (*d*, *J* = 9, 1 H); 8.58 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 42.7; 52.6; 65.8; 102.5; 115.7; 118.9; 123.4; 127.9; 128.9; 131.4; 131.6; 155.2; 158.0; 162.9; 169.3; 175.0. HR-EI-MS: 311.0348 (M^+, $C_{17}H_{10}ClNO_3^+$; calc. 311.0349).

*2-{[3-(1,3-Benzodioxol-5-yl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetonitrile* (**10e**). Yield: 78%. M.p. 236–238°. R_f (hexane/AcOEt 1:1) 0.43. UV (MeOH): 330 (3.16), 206 (2.61). IR (KBr): 3078, 2952, 2892, 1618, 1590, 1500, 1246, 1196. ^{1}H-NMR (300 MHz, (D_6)DMSO): 5.37 (*s*, 2 H); 6.06 (*s*, 2 H); 6.99 (*d*, *J* = 8.1, 1 H); 7.08 (*dd*, *J* = 8.1, 1.5, 1 H); 7.17 (*s*, *J* = 1.8, 1 H); 7.21 (*dd*, *J* = 9, 2.4, 1 H); 7.38 (*d*, *J* = 2.4, 1 H); 8.11 (*d*, *J* = 9, 1 H); 8.48 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 54.7; 101.8; 102.9; 108.8; 110.1; 115.5; 116.7; 119.7; 123.2; 124.4; 126.1; 128.3; 147.8; 154.7; 157.8; 161.3; 157.3. HR-EI-MS: 321.0635 (M^+, $C_{18}H_{11}NO_5^+$; calc. 321.0637).

*2-{[3-(4-Fluorophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetonitrile* (**10f**). Yield: 98%. M.p. 175–177°. R_f (hexane/AcOEt 1:1) 0.63. UV (MeOH): 332 (2.51), 206 (2.54). IR (KBr): 3073, 2956, 2362, 1762. ^{1}H-NMR (300 MHz, (D_6)DMSO): 6.88 (*d*, *J* = 2.1, 1 H); 6.94 (*dd*, *J* = 8.7, 2.1, 1 H); 7.25 (*t*, *J* = 9, 2 H); 7.61 (*dd*, *J* = 8.7, 5.7, 2 H); 7.97 (*d*, *J* = 8.7, 1 H); 8.39 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 54.7; 102.9; 115.5; 115.8; 116.6; 119.6; 123.7; 128.2; 128.8; 128.8; 131.6; 131.7; 155.1; 157.9; 161.1; 161.3; 164.3; 175.1. HR-EI-MS: 295.0643 (M^+, $C_{17}H_{10}FNO_3^+$; calc. 295.0645).

*2-{[3-(4-Nitrophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetonitrile* (**10g**). Yield: 68%. M.p. 224–225°. R_f (hexane/AcOEt 1:1) 0.50. UV (MeOH): 334 (3.78), 206 (2.96). IR (KBr): 3067, 1650, 1625, 1568. ^{1}H-NMR (300 MHz, (D_6)DMSO): 5.39 (*s*, 2 H); 7.26 (*dd*, *J* = 9, 2.4, 1 H); 7.46 (*d*, *J* = 2.4, 1 H); 7.93 (*d*, *J* = 9, 2 H); 8.15 (*d*, *J* = 9, 1 H); 8.32 (*d*, *J* = 9, 2 H); 8.74 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 50.7; 70.4; 103.1; 115.8; 116.6; 119.6; 122.8; 123.9; 128.3; 130.6; 139.6; 147.75; 156.6; 157.8; 161.6; 174.7. HR-EI-MS: 322.0588 (M^+, $C_{17}H_{10}N_2O_5^+$; calc. 322.0590).

General Procedure for the Synthesis of Carbaldehydes **11a**–**11g**. Compound **8a**–**8g** (1.0 equiv.) with methenamine (= hexamethylenetetramine = 1,3,5,7-tetraaza[3.3.1.1^{3,7}]decane; 1.1 equiv.) was placed in glacial AcOH (100 ml), and then the mixture was heated to 100° for 6 h. The resulting hot reddish brown liquid was treated with HCl (1:1, 100 ml) and again heated to 100° for 5 min. After dilution with H_2O (150 ml), it was allowed to stand overnight. The yellow precipitates were collected, washed with H_2O, and dried. The dry solids were extracted with hot benzene (2 × 100 ml) and the residues were discarded. The benzene soln. was evaporated under reduced presure to obtain the crude solids. Recrystallization from EtOH afforded pure **11a**–**11g** (11–65%).

*7-Hydroxy-3-(4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran-8-carboxaldehyde* (**11a**). Yield: 33%. M.p. 160–163°. R_f (hexane/AcOEt 1:1) 0.50. UV (MeOH): 334 (4.00). IR (KBr): 2923, 1657. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.79 (*s*, 3 H); 7.00 (*d*, *J* = 8.7, 2 H); 7.11 (*d*, *J* = 8.7, 1 H); 7.53 (*d*, *J* = 8.7, 2 H); 8.25 (*d*, *J* = 9, 1 H); 8.48 (*s*, 1 H); 10.49 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 55.8; 110.5; 114.4; 116.6; 117.2; 124.2; 124.9; 130.8; 153.7; 157.6; 160.0; 166.7; 174.7; 190.8. HR-EI-MS: 296.0681 (M^+, $C_{17}H_{12}O_5^+$, calc. 296.0685).

*7-Hydroxy-4-oxo-3-(3,4,5-trimethoxyphenyl)-4*H*-1-benzopyran-8-carboxaldehyde* (**11b**). Yield: 51%. M.p. 172–176°. R_f (hexane/AcOEt 1:1) 0.73. UV (MeOH): 336 (3.85). IR (KBr): 3434, 2941, 1643. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.69 (*s*, 3 H); 3.79 (*s*, 6 H); 6.94 (*s*, 2 H); 7.13 (*dd*, *J* = 9, 1 H); 8.26 (*d*, *J* = 9, 1 H); 8.59 (*s*, 1 H); 10.50 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 54.7; 55.8; 102.9; 114.4; 115.4; 116.7; 119.7; 124.3; 124.6; 128.3; 130.8; 154.5; 157.8; 159.9; 161.2; 175.4. HR-EI-MS: 356.0891 (M^+, $C_{19}H_{16}O_7^+$; calc. 356.0896).

*3-(4-Bromophenyl)-7-hydroxy-4-oxo-4*H*-1-benzopyran-8-carboxaldehyde* (**11c**). Yield: 65%. M.p. 102–105°. R_f (hexane/AcOEt 1:1) 0.62. UV (MeOH): 392 (2.63), 333 (3.34). IR (KBr): 3067, 2248, 1638. ^{1}H-NMR (300 MHz, (D_6)DMSO): 7.13 (*d*, *J* = 9, 1 H); 7.57 (*d*, *J* = 8.4, 2 H); 7.65 (*d*, *J* = 8.4, 2 H); 8.26 (*d*, *J* = 9, 1 H); 8.59 (*s*, 1 H); 10.50 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 22.48; 110.61; 116.89; 117.19; 119.47; 121.51; 112.15; 131.04; 131.45; 131.58; 131.68; 131.88; 132.56; 131.23; 154.66; 166.81; 174.31; 190.72. HR-EI-MS: 343.9682 (M^+, $C_{16}H_9{}^{79}BrO_4^+$; calc. 343.9684), 345.9663 (M^+, $C_{16}H_9{}^{81}BrO_4^+$; calc. 345.9664).

*3-(4-Chlorophenyl)-7-hydroxy-4-oxo-4*H-*1-benzopyran-8-carboxaldehyde* (**11d**). Yield: 14%. M.p. 158–163°. R_f (hexane/AcOEt 1:1) 0.62. UV (MeOH): 336 (3.74). IR (KBr): 3074, 1638. ^{1}H-NMR (300 MHz, (D_6)DMSO): 7.13 (*d*, *J* = 9, 1 H); 7.51 (*d*, *J* = 8.7, 2 H); 7.63 (*d*, *J* = 8.4, 2 H); 8.25 (*d*, *J* = 8.7, 1 H); 8.58 (*s*, 1 H); 10.49 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 110.6; 116.8; 117.1; 124.1; 128.9; 131.0; 133.6; 134.2; 154.7; 57.6; 166.8; 174.3; 190.6. HR-EI-MS: 300.0185 (M^+, $C_{16}H_9ClO_4^+$; calc. 300.0189).

*3-(1,3-Benzodioxol-5-yl)-7-hydroxy-4-oxo-4*H-*1-benzopyran-8-carboxaldehyde* (**11e**). Yield: 49%. M.p. 242–243°. R_f (hexane/AcOEt 1:1) 0.46. UV (MeOH): 334 (3.53). IR (KBr): 3069, 1643. ^{1}H-NMR (300 MHz, (D_6)DMSO): 6.06 (*s*, 1 H); 6.99 (*d*, *J* = 8.1, 1 H); 7.08 (*dd*, *J* = 8.1, 1.5, 1 H); 7.12 (*d*, *J* = 9, 1 H); 7.16 (*d*, *J* = 1.5, 1 H); 8.25 (*d*, *J* = 9, 1 H); 8.50 (*s*, 1 H); 10.49 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 101.8; 108.8; 110.0; 116.7; 117.2; 123.2; 124.9; 125.7; 130.4; 134.3; 147.5; 147.9; 154.0; 166.7; 190.7. HR-EI-MS: 310.0477 (M^+, $C_{17}H_{10}O_6^+$; calc. 310.0477).

*3-(4-Fluorophenyl)-7-hydroxy-4-oxo-4*H-*1-benzopyran-8-carboxaldehyde* (**11f**). Yield: 11%. M.p. 163–165°. R_f (hexane/AcOEt 1:1) 0.57. UV (MeOH): 336 (3.70). IR (KBr): 3076, 1642. ^{1}H-NMR (300 MHz, (D_6)DMSO): 7.13 (*d*, *J* = 9, 1 H); 7.29 (*t*, *J* = 9, 2 H); 7.64 (*dd*, *J* = 8.7, 5.4, 2 H); 8.25 (*d*, *J* = 8.7, 1 H); 8.56 (*s*, 1 H); 10.49 (s, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 110.6; 115.6; 115.9; 116.8; 117.2; 124.4; 128.5; 131.7; 131.8; 134.3; 154.4; 157.7; 166.8; 174.6; 190.9. HR-EI-MS: 284.0485 (M^+, $C_{16}H_9FO_4^+$; calc. 284.0485).

*7-Hydroxy-3-(4-nitrophenyl)-4-oxo-4*H-*1-benzopyran-8-carboxaldehyde* (**11g**). Yield: 59%. M.p. 234–236°. R_f (hexane/AcOEt 1:1) 0.43. UV (MeOH): 332 (4.21). IR (KBr): 3077, 1652. ^{1}H-NMR (300 MHz, (D_6)DMSO): 7.15 (*d*, *J* = 8.7, 1 H); 7.91 (*d*, *J* = 9, 5.4, 2 H); 8.25–8.31 (*m*, 3 H); 8.74 (*s*, 1 H); 10.49 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 110.7; 117.0; 123.4; 123.9; 130.7; 134.1; 139.2; 147.7; 155.9; 157.5; 166.9; 174.0; 190.4. HR-EI-MS: 311.0427 (M^+, $C_{16}H_9NO_6^+$; calc. 311.0430).

General Procedure for the Synthesis of Acids **12a**–**12g**. The respective ester **9a**–**9g** was added in a mixture of conc. H_2SO_4 (4 ml) and glacial AcOH (12 ml), and stirred for 12 h. A soln. of 1N NaOH (20 ml) was then added dropwise to this mixture, which was for 2 h, and the precipitate was filtered off to obtain the corresponding acid. Recrystallization from EtOH afforded pure **12a**–**12g** (48–95%).

*2-{[3-(4-Methoxyphenyl)-4-oxo-4*H-*1-benzopyran-7-yl]oxy}acetic Acid* (**12a**). Yield: 55%. M.p. 224–226°. R_f (MeOH/CH_2Cl_2 1:5) 0.48. UV (MeOH): 262 (4.84). IR (KBr): 2903, 1738, 1623, 1249, 1182, 827. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.83 (*s*, 3 H); 4.90 (*s*, 2 H); 6.99 (*d*, *J* = 9.0, 2 H); 7.05 (*d*, *J* = 2.4, 1 H); 7.15 (*dd*, *J* = 9.0, 2.4, 1 H); 7.51 (*d*, *J* = 8.7, 2 H); 8.16 (*s*, 1 H); 8.27 (*d*, *J* = 9.0, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 55.5; 65.8; 102.4; 114.5; 115.4; 119.8; 125.3; 125.5; 128.3; 131.0; 153.6; 158.7; 160.7; 163.5; 169.4; 175.7. HR-EI-MS: 326.0811 (M^+, $C_{18}H_{14}O_6^+$; calc. 326.0790).

*2-{[4-Oxo-3-(3,4,5-trimethoxyphenyl)-4*H-*1-benzopyran-7-yl]oxy}acetic Acid* (**12b**). Yield: 95%. M.p. 152–155°. R_f (MeOH/CH_2Cl_2 1:5) 0.54. UV (MeOH): 219 (4.46). IR (KBr): 3448, 2944, 1741, 1625, 1197, 1130, 833. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.68 (*s*, 3 H); 3.79 (*s*, 6 H); 4.28 (*s*, 2 H); 6.88 (*d*, *J* = 2.4, 1 H); 6.91 (*s*, 2 H); 7.00 (*dd*, *J* = 9.0, 2.4, 1 H); 7.98 (*d*, *J* = 8.7, 1 H); 8.46 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 56.7; 60.7; 69.3; 70.4; 101.8; 107.6; 116.0; 117.7; 124.1; 127.1; 128.3; 138.5; 153.4; 154.6; 158.0; 164.8; 170.0; 175.2. HR-EI-MS: 386.1008 (M^+, $C_{20}H_{18}O_8^+$; calc. 386.1002).

*2-{[3-(4-Bromophenyl)-4-oxo-4*H-*1-benzopyran-7-yl]oxy}acetic Acid* (**12c**). Yield: 92%. M.p. 241–242°. R_f (MeOH/CH_2Cl_2 1:5) 0.63. UV (MeOH): 252 (4.55). IR (KBr): 3065, 1711, 1624, 1248, 1197, 828. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.95 (*s*, 2 H); 7.10 (*d*, *J* = 2.1, 1 H); 7.14 (*dd*, *J* = 8.7, 2.4, 1 H); 7.60 (*s*, 4 H); 8.13 (*d*, *J* = 8.7, 1 H); 8.35 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 65.5; 102.2; 115.5; 116.0; 122.2; 124.3; 128.1; 131.6; 131.9; 132.4; 154.4; 158.5; 163.5; 169.2; 175.1. HR-EI-MS: 373.9789 (M^+, $C_{17}H_{11}{}^{79}BrO_5^+$; calc. 373.9790), 375.9767 (M^+, $C_{17}H_{11}{}^{81}BrO_5^+$; calc. 375.9769).

*2-{[3-(4-Chlorophenyl)-4-oxo-4*H-*1-benzopyran-7-yl]oxy}acetic Acid* (**12d**). Yield: 48%. M.p. 233–234°. R_f (MeOH/CH_2Cl_2 1:5) 0.53. UV (MeOH): 206 (4.50). IR (KBr): 2907, 1709, 1624, 1249, 1197, 1090, 828. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.96 (*s*, 2 H); 7.11 (*d*, *J* = 2.1, 1 H); 7.15 (*dd*, *J* = 8.7, 2.4, 1 H); 7.46 (*d*, *J* = 8.7, 2 H); 7.67 (*d*, *J* = 8.7, 2 H); 8.14 (*d*, *J* = 8.4, 1 H); 8.35 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 65.5; 102.2; 115.3; 119.4; 124.2; 128.0; 128.7; 131.2; 131.9; 133.9; 154.2; 158.4; 163.4; 169.1; 175.0. HR-EI-MS: 330.0298 (M^+, $C_{17}H_{11}ClO_5^+$; calc. 330.0295).

*2-{[3-(1,3-Benzodioxol-5-yl)-4-oxo-4*H-*1-benzopyran-7-yl]oxy}acetic Acid* (**12e**). Yield: 90%. M.p. 241–243°. R_f (MeOH/CH_2Cl_2 1:5) 0.45. UV (MeOH): 206 (4.40). IR (KBr): 3504, 2906, 1639, 1249,

1050, 818. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.90 (*s*, 2 H); 5.99 (*s*, 2 H); 6.87 (*d*, *J* = 8.1, 1 H); 7.02 (*dd*, *J* = 8.1, 1.8, 1 H); 7.04 (*d*, *J* = 2.4, 1 H); 7.09 (*d*, *J* = 1.8, 1 H); 7.15 (*dd*, *J* = 9.0, 2.4, 1 H); 8.15 (*s*, 1 H); 8.26 (*d*, *J* = 9.0, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 65.5; 101.8; 102.1; 108.5; 110.2; 115.2; 119.4; 123.0; 125.0; 126.8; 128.0; 148.2; 153.7; 158.4; 163.3; 169.3; 175.4. HR-EI-MS: 340.0578 (M^+, $C_{18}H_{12}O_7^+$; calc. 340.0583).

*2-{[3-(4-Fluorophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetic Acid* (**12f**). Yield: 92%. M.p. 209–211°. R_f (MeOH/CH_2Cl_2 1:5) 0.38. UV (MeOH): 238 (4.43). IR (KBr): 3523, 3078, 1644, 1248, 1191, 817. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.89 (*s*, 2 H); 7.05 (*d*, *J* = 2.4, 1 H); 7.15 (*dd*, *J* = 9.0, 2.4, 1 H); 7.16 (*t*, *J* = 8.7, 2 H); 7.59 (*dd*, *J* = 8.7, 5.4, 2 H); 8.19 (*s*, 1 H); 8.27 (*d*, *J* = 9.0, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 65.5; 102.2; 115.3; 115.3; 115.6; 119.5; 124.4; 128.0; 129.3; 131.6; 131.7; 154.0; 158.4; 163.4; 169.1; 175.2. HR-EI-MS: 314.0592 (M^+, $C_{17}H_{11}FO_5^+$; calc. 314.0591).

*2-{[3-(4-Nitrophenyl)-4-oxo-4*H*-1-benzopyran-7-yl]oxy}acetic Acid* (**12g**). Yield: 95%. M.p. 273–274°. R_f (MeOH/CH_2Cl_2 1:5) 0.35. UV (MeOH): 220 (4.49). IR (KBr): 3588, 3076, 1746, 1624, 1246, 851. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.90 (*s*, 2 H); 7.15 (*dd*, *J* = 9.0, 2.4, 1 H); 7.22 (*d*, *J* = 2.7, 1 H); 7.91 (*d*, *J* = 8.7, 2 H); 8.07 (*d*, *J* = 9.0, 1 H); 8.29 (*d*, *J* = 9.0, 2 H); 8.68 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 65.8; 102.5; 115.9; 118.6; 122.6; 123.9; 127.8; 130.6; 139.8; 147.6; 156.4; 158.0; 163.3; 170.1; 174.7. HR-EI-MS: 341.0540 (M^+, $C_{17}H_{11}NO_7^+$; calc. 341.0536).

Cytopathic Effect (CPE) Assay. The antiviral activities of flavonoids were by the CPE assay and using ribavirin as a positive control. The CPE inhibition assays used in this study were performed as described [26]. The influenza virus strains, namely *Tamiflu*-resistant 2009 pandemic influenza A (H1N1) virus, which contained the H275Y mutation (N1 numbering) in neuraminidase, was provided by *Centers for Disease Control* (*CDC*), Taiwan, and used for evaluating the *in vitro* antiviral activities of the synthetic flavonoids.

In brief, virus at 100 $TCID_{50}$ (tissue culture infectious dose) were inoculated onto near confluent MDCK cell monolayers (1×10^5 cells/well) for 1 h. After incubation at 37° for 2 h, the virus soln. was removed, and 100 µl of sequential twofold serial dilutions of the respective flavonoids and reference compound ribavirin were added to each well of the 96-well culture plates, using the maximal non-cytotoxic concentration (*MNCC*, *i.e.*, 90% viable cells) as the highest concentration. An infection control without flavonoids was also included. The plates were incubated at 37° in a 75% humidity of 5% CO_2 atmosphere for 24 h, and then the CPE was observed. The virus-induced CPE was scored as follows: scores: 0, 0% CPE; 1, 0–25% CPE; 2, 25–50% CPE; 3, 50–75% CPE; and 4, 75–100% CPE. The reduction in virus multiplication was calculated as a percentage of the virus control (% virus control = CPE(exp)/CPE(virus control) × 100). The IC_{50} value of the CPE with respect to virus control was estimated using the *Reed–Muench* method and was expressed in µM. The selectivity index (*SI*) was calculated from the ratio CC_{50}/IC_{50}.

Statistical Analysis. Statistical calculations were carried out with *Microsoft Excel 2007* version. Results are expressed as the mean ± SD of six independent experiments.

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