Activities of Wogonin Analogs and Other Flavones against *Flavobacterium columnare*

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In our on-going pursuit to discover natural products and natural product-based compounds to control the bacterial species *Flavobacterium columnare*, which causes columnaris disease in channel catfish (*Ictalurus punctatus*), we synthesized flavone and chalcone analogs, and evaluated these compounds, along with flavonoids from natural sources, for their antibacterial activities against two isolates of *F. columnare* (ALM-00-173 and *BioMed*) using a rapid bioassay. The flavonoids chrysin (**1a**), 5,7-dihydroxy-4′-methoxyflavone (**11**), isorhamnetin (**26**), luteolin (**27**), and biochanin A (**29**), and chalcone derivative **8b** showed strong antibacterial activities against *F. columnare* ALM-00-173 based on minimum inhibition concentration (*MIC*) results. Flavonoids **1a**, **8**, **11**, **13** (5,4′-dihydroxy-7-methoxy-flavone), **26**, and **29** exhibited strong antibacterial activities against *F. columnare BioMed* based upon *MIC* results. The 24-h 50% inhibition concentration (IC_{50}) results revealed that **27** and **29** were the most active compounds against *F. columnare* ALM-00-173 (IC_{50} of 7.5 and 8.5 mg/l, resp.), while **26** and **29** were the most toxic compound against *F. columnare BioMed* (IC_{50} of 9.2 and 3.5 mg/l, resp.). These IC_{50} results were lower than those obtained for wogonin against *F. columnare* ALM-00-173 and *F. columnare BioMed* (28.4 and 5.4 mg/l, resp.). However, based on *MIC* results, none of the compounds evaluated in this study were as active as wogonin (*MIC* 0.3 mg/l for each *F. columnare* isolate). Further modification of the wogonin structure to enhance antibacterial is of interest.

Introduction. – Columnaris disease is one of the most common worldwide bacterial fish diseases, causing heavy economic losses to various aquaculture industries [1]. In the Southeastern United States of America, epidemics of columnaris disease can occur in populations of pond-raised channel catfish (*Ictalurus punctatus*), resulting in high mortalities and subsequent large economic losses to producers in the channel catfish aquaculture industry. The bacterial species that causes columnaris disease is *Flavobacterium columnare*, a *Gram*-negative motile rod (2–10 μm in length). Most *F. columnare* isolates from diseased channel catfish can be designated as genomovars I (*F. columnare BioMed*) or II (*F. columnare* ALM-00-173), with genomovar II isolates appearing to be more pathogenic for channel catfish [2].

Catfish producers currently have several available management approaches to control columnaris disease including the application of medicated feeds (*e.g.*, *Aquaflor*®), attenuated vaccines [3], and non-antibiotic therapeutants such as 35% *Perox-Aid*® for external columnaris. One negative aspect of the use of medicated feeds

in aquaculture is public concern about the potential development of antibiotic-resistant strains of bacteria in the environment [4]. *Perox-Aid*® is not recommended for use in earthen ponds, unless water exchange can be implemented which is usually not practical in commercial-size (*e.g.*, 4 ha) catfish ponds. Although additional therapeutic agents such as copper sulfate pentahydrate ($CuSO_4 \cdot 5\ H_2O$) and potassium permanganate ($KMnO_4$) have been mentioned as potential treatments of columnaris [5], the efficacy of these therapeutants can be adversely impacted by certain water quality variables (*e.g.*, copper toxicity to fish is inversely correlated with dissolved organic matter, pH, and total alkalinity). Therefore, these compounds must be applied carefully due to their broad-spectrum toxicity towards non-target organisms (*e.g.*, channel catfish) [6].

Natural products have been explored as possible therapeutic agents for columnaris disease. For this purpose, a rapid 96-well microplate bioassay has been developed [7] and utilized to evaluate numerous natural and natural product-based compounds for antibacterial activity towards *F. columnare*. Some promising compounds have been identified using this assay such as tannic acid [8], and flavone and wogonin [9].

Flavone and wogonin (**1** and **2**, resp.; *Fig.*) were found to possess strong antibacterial activities towards two genomovars of *F. columnare*, ALM-00-173 (genomovar II) and *BioMed* (genomovar I). Both of these compounds belong to a class of natural products called flavones. Flavones possess a wide range of biological features such as anticancer [10–14], antiviral [15][16], anti-inflammatory [17], antioxidant [18], and antitumor activities [19][20]. In particular, flavone compounds in the diet may act as chemopreventive agents against the development of cancers of the alimentary tract [21]. These interesting properties render flavones a valuable tool for studying a number of important physiological processes.

To improve the antibacterial activity of wogonin, we synthesized wogonin analogs **3**–**5** and **14**–**21**, and other flavone analogs **6**–**10**, **12**, and **13**. The antibacterial activities of these flavonoids, three synthesized chalcone derivatives **7b**–**9b**, and 13 naturally-occurring flavonoids, **1a**, **2**, and **22**–**32**, were evaluated by a 96-well microplate bioassay. With the exception of wogonin (**2**), these natural and synthetic flavonoid analogs have not been previously tested for antibacterial activity against *F. columnare*. Herein, results, as well as synthesis of the flavonoids, are presented.

Results and Discussion. – Synthesis of the wogonin analogs was inspired by results from a previous study [9] that showed its strong antibacterial activity towards two isolates of *Flavobacterium columnare* (ALM-00-173 and *BioMed*). Acetylation, methylation, and benzoylation were performed envisioning an improvement of the effects of wogonin (**2**), as encouraged by studies that revealed that acetylation or methylation of the parent polyphenolic compound increased inhibitory activity against prostate cancer cells [22], and that benzoylation resulted in an analog with significant *in vitro* inhibitory activity against the malaria parasite comparable with the activity of the control, chloroquine diphosphate [23]. Acetylated, methylated, and benzoylated analogs, *i.e.*, **3** and **4**, **5**, and, **14**–**21**, respectively, were obtained, but these structure modifications resulted in weaker activities.

Other flavone analogs, **6**–**10**, **12**, and **13**, were also synthesized likewise triggered by the previous finding of a strong antibacterial activity of flavone [9]. Introduction of

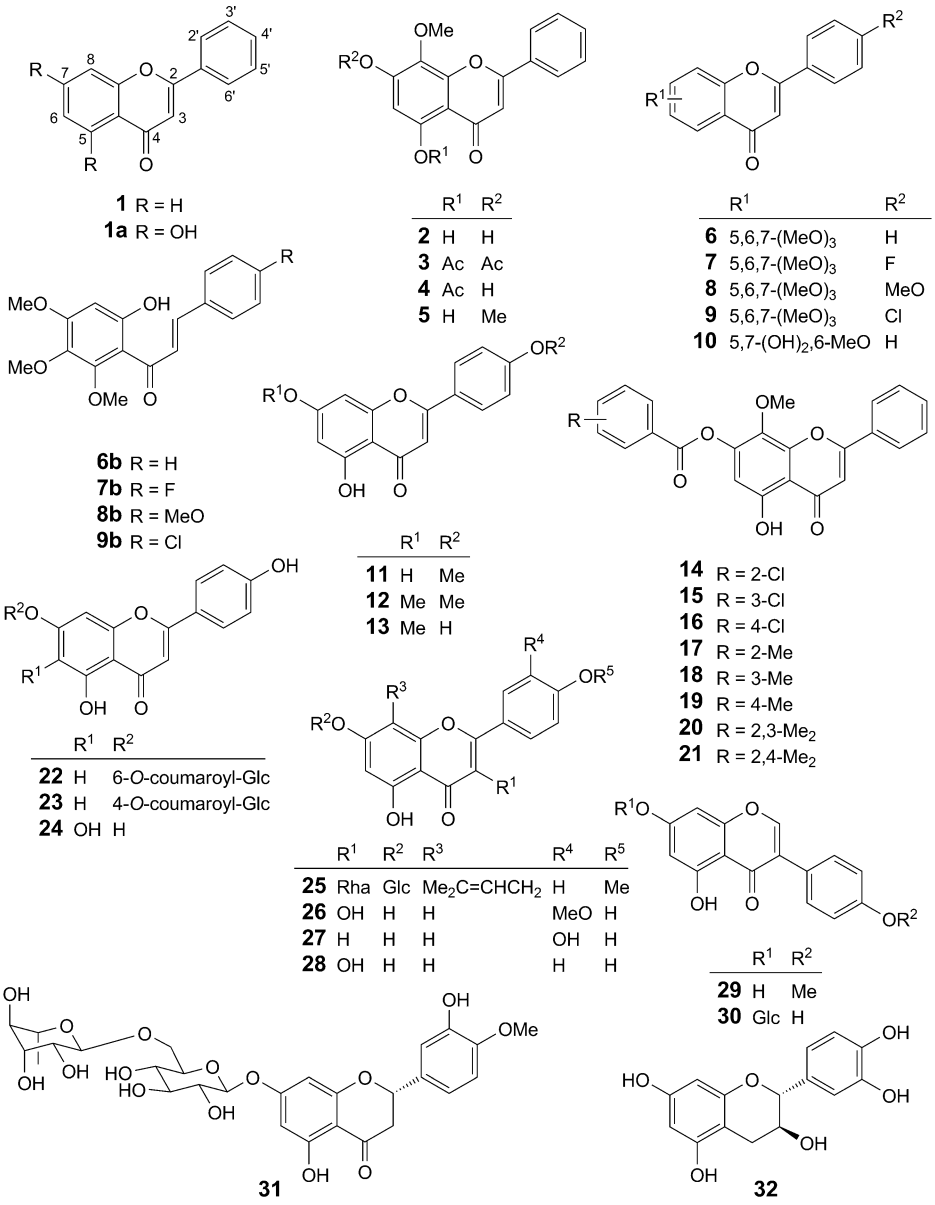

Figure. *Chemical structures of wogonin and flavone analogs, chalcones, and other flavonoids evaluated for their activities against* Flavobacterium columnare *ALM-00 and* Flavobacterium columnare *BioMed*

MeO substituents in ring *A* of flavone was performed as inspired by reports that showed P-glycoprotein inhibitory potency of methoxyflavones increased with increasing number of MeO groups and reversed multi-drug resistance in *in vitro* assays (*e.g.*, [24]). The synthetic flavones, along with synthetic chalcones, **7b**–**9b**, natural flavones,

1a and **22**–**25**, and other natural flavonoids, **29**–**32**, were evaluated for their activities against the two *F. columnare* isolates.

Chrysin (**1a**), 5,7-dihydroxy-4′-methoxyflavone (**11**), isorhamnetin (**26**), luteolin (**27**), and biochanin A (**29**) exhibited the strongest activities against *F. columnare* isolate ALM-00-173 based upon minimum inhibition concentration (*MIC*) results (2.5 ± 0, 1.6 ± 1.3, 3.2 ± 0, 2.9 ± 0, and 2.8 ± 0 mg/l, resp.; *Table 1*). Chalcone **8b**, which was an intermediate in the synthesis of compound **8**, also showed a strong activity against this isolate (*MIC* 3.4 ± 0 mg/l). Chalcones **7b** and **9b** showed only moderate inhibition activities, suggesting that a halogen substitution is not favorable. The *MIC* relative-to-drug control (RDC) values for the flavonoids **1a**, **11**, **26**, **27**, and **29** were also strong, with values ranging from 5.5 to 10.8 with respect to the two positive drug controls, florfenicol (F) and oxytetracycline (O). Compounds **27** and **29** had the lowest 24-h 50% inhibition concentration (IC_{50}) values of 7.5 ± 0.9 and 8.5 ± 2.8 mg/l, respectively, and these values indicated stronger activities than that of wogonin (**2**; 24-h IC_{50} 28.4 ± 0 mg/l). Based upon *MIC* results, none of the synthetic or natural flavonoids evaluated in this study were as active as **2** (*MIC* 0.3 ± 0 mg/l) towards *F. columnare* ALM-00-173. Among the active flavonoids, no common structural feature could be recognized as requirement for activity against this bacterial isolate. Notably, however, *ortho*-dihydroxy groups in the *B*-ring appears to confer selective activity towards *F. columnare* ALM-00-173; thereby, **27** was inhibitory to this isolate but not to *F. columnare BioMed*.

Evaluation of the flavone analogs for toxic activity towards the *F. columnare* isolate *BioMed* disclosed that **1a**, **8**, **11**, **13** (5,4′-dihydroxy-7-methoxyflavone), **26**, and **29** had strong antibacterial activities against *F. columnare BioMed* based on *MIC* results (*MIC* 2.5 ± 0, 3.4 ± 0, 3.0 ± 0, 2.8 ± 0, 3.2 ± 0, and 1.6 ± 1.3 mg/l, resp.; *Table 2*). The *MIC* RDCF, and RDCO values for these analogs were in the range of 1.0–10.8. Compound **29** had the lowest 24-h IC_{50} value of 3.5 ± 2.8 mg/l which was also lower than that of wogonin (**2**; 24-h IC_{50} 5.4 ± 0 mg/l), thereby indicating slightly higher activity. Based upon *MIC* results, none of the flavonoids evaluated in this study were as active as **2** (*MIC* 0.3 ± 0 mg/l) towards *F. columnare BioMed*. Of compounds **6**–**10**, only **8** showed strong inhibition, indicating that a MeO group at C(4′) is favorable over halogenation, similar to that observed with chalcones **7b** and **9b**. Of compounds **11**–**13**, **12** was only moderately inhibitory, suggesting that, for these congeners, an additional MeO group is an unfavorable substituent. No structural feature stands out as salient for activity towards this isolate. Curiously, **8** and **13**, both bearing a MeO at C(5), are inhibitory only to this isolate. It may be that this structural feature is important for selective activity towards *F. columnare BioMed*.

Four flavonoids, **1a**, **11**, **26**, and **29**, showed strong antibacterial activities towards both isolates. Other than the OH groups in *meta*-position to each other (at C(5) and C(7)) and the absence of a functional group at C(6), no other structural features could be identified as being essential for the activities of these four compounds. A *meta*-OH group is also present in **28**, but this analog had little or no antibacterial activity at the concentrations tested. Compound **28** only differs from **26** by lacking a MeO group at C(3′). Therefore, it appears that the MeO group conferred activity. Indeed, by examining the structure of wogonin (**2**), it is observable that the difference between **2** and these four active flavonoids is the presence of a MeO group at C(8) (see atom

Table 1. *Antibacterial Activities of Flavonoids towards* Flavobacterium columnare *ALM-00-173*

	24-h IC_{50}[a]) [mg/l]	*MIC*[b]) [mg/l]	24-h IC_{50}		*MIC*	
			RDCF[c])	RDCO[d])	RDCF[c])	RDCO[d])
1a	>25.4	2.5 (0)	>51.8	>49.3	10.0 (0)	10.8 (0)
2	28.4 (0)	0.3 (0)	51.8 (0)	49.3 (0)	1.0 (0)	1.1 (0)
3	>36.9	36.9 (0)	>51.8	>49.3	100.0 (0)	107.5 (0)
4	>32.6	32.6 (0)	>51.8	>49.3	100.0 (0)	107.5 (0)
5	>29.8	29.8	>51.8	>49.3	100.0 (0)	107.5 (0)
6	>31.2	31.2 (0)	>51.8	>49.3	100.0 (0)	107.5 (0)
7	>33.0	>33.0	>51.8	>49.3	>100.0	>107.5
8	10.1 (0.2)	34.2	15.3 (0.3)	14.6 (0.3)	100.0 (0)	107.5 (0)
9	18.8 (1.8)	34.7 (0)	28.0 (2.6)	26.6 (2.5)	100.0 (0)	107.5 (0)
10	>28.4	28.4 (0)	>51.8	>49.3	100.0 (0)	107.5 (0)
7b	>33.2	18.3 (15.0)	>51.8	>49.3	55.0 (45.0)	59.2 (48.4)
8b	>34.4	3.4 (0)	>51.8	>49.3	10.0 (0)	10.8 (0)
9b	>34.9	>34.9	>51.8	>49.3	>100.0	>107.5
11	>29.8	1.6 (1.3)	>51.8	>49.3	5.5 (4.5)	5.9 (4.8)
12	>28.4	28.4 (0)	>51.8	>49.3	100.0 (0)	107.5 (0)
13	>28.4	>28.4	>51.8	>49.3	>100.0	>107.5
14	>42.3	>42.3	>51.8	>49.3	>100.0	>107.5
15	>42.3	>42.3	>51.8	>49.3	>100.0	>107.5
16	>42.3	>42.3	>51.8	>49.3	>100.0	>107.5
17	>40.2	>40.2	>51.8	>49.3	>100.0	>107.5
18	>40.2	>40.2	>51.8	>49.3	>100.0	>107.5
19	>40.2	>40.2	>51.8	>49.3	>100.0	>107.5
20	>41.6	>41.6	>51.8	>49.3	>100.0	>107.5
21	>41.6	>41.6	>51.8	>49.3	>100.0	>107.5
22[e])	>57.9	>57.9	>51.8	>49.3	>100.0	>107.5
23	>57.9	>57.9	>51.8	>49.3	>100.0	>107.5
24	11.4 (0)	15.8 (12.9)	20.7 (0)	19.7 (0)	55.0 (45.0)	59.2 (48.4)
25	>67.7	>67.7	>51.8	>49.3	>100.0	>107.5
26	>31.6	3.2 (0)	>51.8	>49.3	10.0 (0)	10.8 (0)
27	7.5 (0.9)	2.9 (0)	13.5 (1.6)	12.8 (1.5)	10.0 (0)	10.8 (0)
28	>33.8	33.8 (0)	>51.8	>49.3	100.0 (0)	107.5 (0)
29	8.5 (2.8)	2.8 (0)	15.5 (5.2)	14.8 (4.9)	10.0 (0)	10.8 (0)
30	>41.6	>41.6	>51.8	>49.3	>100.0	>107.5
31	>61.1	>61.1	>51.8	>49.3	>100.0	>107.5
32	>29.0	>29.0	>51.8	>49.3	>100.0	>107.5

[a]) 24-h IC_{50}, 50% Inhibition concentration. [b]) *MIC*, Minimum inhibition concentration. [c]) Relative-to-drug control florfenicol; values closer to 1.0 indicate higher antibacterial activity compared to florfenicol. [d]) Relative-to-drug control oxytetracycline; values closer to 1.0 indicate higher antibacterial activity compared to oxytetracycline. [e]) Apigenin 7-*O*-*β*-D-(6-*O*-*p*-coumaroyl)glucoside. Values in parentheses are standard errors of the mean.

numbering in *Fig. 1*) in **2**. Interestingly, our observations are similar to those reported by other groups; *i.e.*, wogonin analogs without MeO group at C(8) are unable to inhibit prostaglandin E_2 production [25], and alkylation at C(5) and C(7) of wogonin (**2**) results in analogs with reduced inhibitory activity [26].

Table 2. *Antibacterial Activities of Flavonoids towards* Flavobacterium columnare BioMed

	24-h IC_{50}[a]) [mg/l]	*MIC*[b]) [mg/l]	24-h IC_{50}		*MIC*	
			RDCF[c])	RDCO[d])	RDCF[c])	RDCO[d])
1a	>25.4	2.5 (0)	>42.4	>58.8	10.0 (0)	10.8 (0)
2	5.4 (0)	0.3 (0)	8.1 (0)	11.2 (0)	1.0 (0)	1.1 (0)
3	>36.9	36.9 (0)	>42.4	>58.8	100.0 (0)	107.5 (0)
4	>32.6	32.6 (0)	>42.4	>58.8	100.0 (0)	107.5 (0)
5	>29.8	29.8 (0)	>42.4	>58.8	100.0 (0)	107.5 (0)
6	>31.2	31.2 (0)	>42.4	>58.8	100.0 (0)	107.5 (0)
7	>33.0	33.0 (0)	>42.4	>58.8	100.0 (0)	107.5 (0)
8	9.9 (0)	3.4 (0)	12.3 (0)	17.1 (0)	10.0 (0)	10.8 (0)
9	27.4 (3.1)	19.1 (15.6)	33.5 (3.8)	46.5 (5.3)	55.0 (45.0)	59.2 (48.4)
10	>28.4	28.4 (0)	>42.4	>58.8	100.0 (0)	107.5 (0)
7b	>33.2	18.3 (15.0)	>42.4	>58.8	55.0 (45.0)	59.2 (48.4)
8b	>34.4	18.9 (15.5)	>42.4	>58.8	55.0 (45.0)	59.2 (48.4)
9b	>34.9	19.2 (15.7)	>42.4	>58.8	55.0 (45.0)	59.2 (48.4)
11	>29.8	3.0 (0)	>42.4	>58.8	10.0 (0)	10.8 (0)
12	>28.4	28.4 (0)	>42.4	>58.8	100.0 (0)	107.5 (0)
13	>28.4	2.8 (0)	>42.4	>58.8	10.0 (0)	10.8 (0)
14	>42.2	42.3 (0)	>42.4	>58.8	>100.0	>107.5
15	>42.2	>42.2	>42.4	>58.8	>100.0	>107.5
16	>42.2	>42.2	>42.4	>58.8	>100.0	>107.5
17	>40.2	>40.2	>42.4	>58.8	>100.0	>107.5
18	>40.2	>40.2	>42.4	>58.8	>100.0	>107.5
19	>40.2	>40.2	>42.4	>58.8	>100.0	>107.5
20	>41.6	>41.6	>42.4	>58.8	>100.0	>107.5
21	>41.6	>41.6	>42.4	>58.8	>100.0	>107.5
22[e])	>57.9	>57.9	>42.4	>58.8	>100.0	>107.5
23	>57.9	>57.9	>42.4	>58.8	>100.0	>107.5
24	18.6 (1.4)	15.8 (12.9)	27.6 (2.2)	38.3 (3.0)	55.0 (45.0)	59.2 (48.4)
25	>67.7	>67.7	>42.4	>58.8	>100.0	>107.5
26	>31.6	3.2 (0)	>42.4	>58.8	10.0 (0)	10.8 (0)
27	9.2 (2.3)	15.8 (12.9)	13.6 (3.4)	18.8 (4.7)	55.0 (45.0)	59.2 (48.4)
28	>33.8	33.8 (0)	>42.4	>58.8	100.0 (0)	107.5 (0)
29	3.5 (2.8)	1.6 (1.3)	5.1 (4.2)	7.1 (5.8)	5.5 (4.5)	5.9 (4.8)
30	>41.6	>41.6	>42.4	>58.8	>100.0	>107.5
31	>61.1	>61.1	>42.4	>58.8	>100.0	>107.5
32	>29.0	>29.0	>42.4	>58.8	>100.0	>107.5

[a]) 24-h IC_{50}, 50% Inhibition concentration. [b]) *MIC*, Minimum inhibition concentration. [c]) Relative-to-drug control florfenicol; values closer to 1.0 indicate higher antibacterial activity compared to florfenicol. [d]) Relative-to-drug control oxytetracycline; values closer to 1.0 indicate higher antibacterial activity compared to oxytetracycline. [e]) Apigenin 7-*O*-*β*-D-(6-*O*-*p*-coumaroyl)glucoside. Values in parentheses are standard error of the mean.

Conclusions. – We have synthesized analogs of wogonin (**2**) and flavone (**1**) and, have evaluated their antibacterial activities towards two *F. columnare* isolates (ALM-00-173 and *BioMed*). While no analog was found to be more active than **2** based collectively on both 24-h IC_{50} and *MIC* results, **27** and **29** were more active than **2**

against *F. columnare* ALM-00-173, and **29** was more active than *F. columnare BioMed* based on 24-h IC_{50} results. Additionally, we have obtained valuable data for these synthetic and natural flavonoids, as well as the synthetic chalcones, regarding their activities against isolates of *F. columnare* for the first time, and our study showed that the presence of OH groups at C(5) and C(7) with a MeO group at C(8) appears to be essential and must be kept intact for the antibacterial activity to be maintained. Our study provides guidance in the synthesis of further analogs of **1** and **2**.

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Experimental Part

General. Chemicals and solvents were purchased from commercial sources and used without purification. Reactions were performed in a reaction flask with protection by N_2 and were monitored by TLC. Anal. and prep. TLC: silica gel *GF* plates (*Analtech* uniplate; 10×20 cm, 250 μm, and 20×20 cm, 250 μm, resp.); visualization by exposure to UV light. ^{1}H- and ^{13}C-NMR spectra: 400 and 100 MHz, resp., on an *AVANCE^{III} Bruker* spectrometer. HR-ESI-MS: *Waters Micromass Q-TOF Micro* and *JEOL AccuTOF* (*JMS-T100LC*) spectrometers.

Bactericide Bioassay. Two isolates of *Flavobacterium columnare* (*BioMed* (genomovar I) and ALM-00-173 (genomovar II)) were obtained from Dr. *Covadonga Arias* (Department of Fisheries and Allied Aquacultures, Auburn University, Alabama). To assure purity, cultures of *F. columnare* isolates were maintained separately on modified *Shieh* agar plates (pH 7.2–7.4) [27]. Prior to conducting the bioassay, single colonies of the test cultures were used to prepare the assay culture materials by culturing each genomovar isolate separately in 75 ml of modified *Shieh* broth (18 h for *BioMed* and 24 h for ALM-00-173) at $29 \pm 1°$ at 150 rpm on a rotary shaker (model *C24KC*; *New Brunswick Scientific*, Edison, New Jersey).

Compounds were evaluated for antibacterial activity using a rapid 96-well microplate bioassay as described in [7]. Florfenicol and oxytetracycline HCl, antibiotics that are utilized in medicated feed, were included as positive drug controls for each bioassay. In addition, control wells (no test compound added) and wells containing wogonin (**2**) were included in each assay. Technical-grade MeOH was used to dissolve the test compounds. Final concentrations of test compounds and drug control in the microplate wells were 0.01, 0.1, 1.0, 10.0, and 100.0 μM. Three replications were used for each dilution of each test compound and controls. Final results were converted to units of mg/l in order to allow comparison with previous studies.

For determination of the 24-h 50% inhibition concentration (IC_{50}) and minimum inhibition concentration (*MIC*), sterile 96-well polystyrene microplates (*Corning Costar Corp*., Acton, Massachusetts) with flat-bottom wells were used. Dissolved test compounds were added to microplate wells (10 μl/well), and solvent was allowed to completely evaporate, before 0.5 *MacFarland* bacterial culture (see [7]) was added to the microplate wells (200 μl/well). Microplates were incubated at 29° (*VWR* model 2005 incubator; *Sheldon Manufacturing, Inc*., Cornelius, Oregon). A *Packard* model *SpectraCount* microplate photometer (*Packard Instrument Company*, Meriden, Connecticut) was used to measure the absorbance (630 nm) of the microplate wells at time 0 and 24 h.

The means and standard deviations of absorbance measurements were calculated, graphed, and compared to controls to determine the 24-h IC_{50} and *MIC* value for each test compound (see [7]). The 24-h IC_{50} and *MIC* results for each compound tested were divided by the respective 24-h IC_{50} and *MIC* results obtained for the positive controls florfenicol and oxytetracycline to determine the relative-to-drug-control florfenicol (RDCF) and relative-to-drug-control oxytetracycline (RDCO) values.

Preparation and Synthesis of **3**–**5**. Compounds **3** and **4** were prepared by acetylation of wogonin (**2**; obtained from *AmplaChem Company*; Carmel, IN) [28], while **5** was prepared by methylation of **2** as described in [29].

*8-Methoxy-4-oxo-2-phenyl-4*H*-chromene-5,7-diyl Diacetate* (**3**) *and 7-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H*-chromen-5-yl Acetate* (**4**). The mixture of **2** (20 mg, 0.07 mmol), pyridine (3 ml), and Ac_2O (0.04 ml) in a sealed flask was stirred at r.t. for 30 h, then the reaction was quenched with cold H_2O, and the mixture was extracted with $CHCl_3$ (3×10 ml). The combined layers were washed with brine and dried ($MgSO_4$). After removal of the volatiles, the crude product was purified by open column chromatography (CC; SiO_2; AcOEt/hexane 40:60) to give **3** (3.0 mg, 13%) and **4** (16.8 mg, 65%) as white needles.

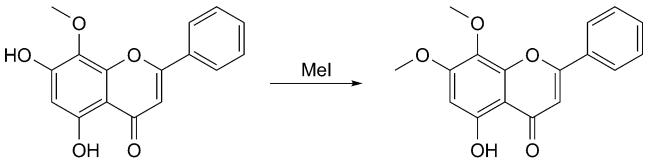
Data of **3**. ^{1}H-NMR ($CDCl_3$): 7.89 (*d*, *J* = 7.9, H–C(2′,6′)); 7.51–7.56 (*m*, H–C(3′,4′,5′)); 6.81 (*s*, H–C(6)); 6.68 (*s*, H–C(3)); 4.04 (*s*, MeO); 2.43 (*s*, C(7)AcO); 2.39 (*s*, C(5)AcO). ^{13}C-NMR ($CDCl_3$): 176.5 (C(4)); 169.7, 167.9 (CO(5)); CO(7)); 162.2 (C(2)); 151.5 (C(8a)); 146.8 (C(7)); 144.3 (C(5)); 139.1 (C(8)); 131.9 (C(1′)); 131.1 (C4′)); 129.2 (C(3′,5′)); 126.2 (C(2′,6′)); 116.0 (C(4a)); 114.3 (C(6)); 108.5 (C(3)); 61.8 (MeO); 21.0, 20.6 (C(5)AcO, C(7)AcO). HR-ESI-MS: 369.0974 ($[M+H]^+$, $C_{20}H_{17}O_7^+$; calc. 369.0959).

Data of **4**. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH); 7.89 (*d*, *J* = 8.0, H–C(2′,6′)); 7.53–7.58 (*m*, H–C(3′,4′,5′)); 6.70 (*s*, H–C(6)); 6.62 (*s*, H–C(3)); 4.11 (*s*, MeO); 2.43 (*s*, AcO). ^{13}C-NMR ($CDCl_3$): 178.5 (C(4)); 171.8 (AcO); 162.6 (C(2)); 152.5 (C(8a)); 151.3 (C(5)); 150.8 (C(7)); 136.1 (C(8)); 130.9 (C(1′)); 129.7 (C(4′)); 128.8 (C(3′,5′)); 127.2 (C(2′,6′)); 114.0 (C(4a)); 111.3 (C(6)); 105.5 (C(3)); 60.8 (MeO); 21.0 (AcO). HR-ESI-MS: 327.0854 ($[M+H]^+$, $C_{18}H_{15}O_6^+$; calc. 327.0868).

*5-Hydroxy-7,8-dimethoxy-2-phenyl-4*H*-chromen-4-one* (**5**). Compound **2** (30 mg, 0.11 mmol), MeI (7.5 µl, 0.12 mmol), and K_2CO_3 (30 mg, 0.22 mmol) were dissolved in DMSO (3 ml) (*Scheme 1*). The mixture was stirred overnight at r.t. (20°). The next day, 30 µl of double-dist. H_2O was added, and the white precipitate was collected by filtration and dried (Na_2SO_4) in $CHCl_3$. After evaporation, the residue was subjected to CC (SiO_2; AcOEt/hexane 20:80) to give **5** (27 mg, 82%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.6 (br. *s*, OH); 7.95 (*d*, *J* = 6.5, H–C(2′,6′)); 7.5–7.58 (*m*, H–C(3′,4′,5′)); 6.68 (*s*, H–C(3)); 6.44 (*s*, H–C(6)); 3.95 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 182.7 (C(4)); 162.7 (C(2)); 160.2 (C(7)); 154.9 (C(5)); 141.9 (C(8a)); 135.3 (C(8)); 131.6 (C(1′)); 130.3 (C(3′,5′)); 130.2 (C(2′,6′)); 126.3 (C(4′)); 116.2 (C(4a)); 116.0 (C(3)); 96.6 (C(6)); 61.9 (C(8)MeO); 56.1 (C(7)MeO). HR-ESI-MS: 299.0919 ($[M+H]^+$, $C_{17}H_{15}O_5^+$; calc. 299.0919).

*5,6,7-Trimethoxy-2-phenyl-4*H*-chromen-4-one* (**6**). 3,4,5-Trimethoxyphenol (1 g, 5.4 mmol), cinnamoyl chloride (0.9 g, 5.4 mmol), and $BF_3 \cdot Et_2O$ (50 µl) were dissolved in anh. toluene (10 ml) (*Scheme 2*). The mixture was stirred and heated to reflux for 2 h, then the reaction was quenched with excess of H_2O, and the mixture was extracted with toluene (3×10 ml). After removal of the volatiles, the crude product was purified by CC (SiO_2; AcOEt/hexane 30:70) to give *(2*E*)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)-3-phenylprop-2-en-1-one* (**6b**; 1 g, 86%). Yellow solid. ^{1}H-NMR ($CDCl_3$): 13.6 (br. *s*, OH); 7.95 (*d*, *J* = 15.6, C=*CH*–Ph); 7.83 (*d*, *J* = 15.6, *CH*=C–Ph); 7.65 (*dd*, *J* = 2.3, 2.3, H–C(2′,6′)); 7.39–7.44 (H–C(3′,4′,5′)); 6.31 (*s*, H–C(5)); 3.95 (*s*, MeO–C(2)); 3.91 (*s*, MeO–C(4)); 3.85 (*s*, MeO–C(3)). ^{13}C-NMR ($CDCl_3$): 192.9 (C(1)); 162.7, C(4)); 160.2, C(6)); 155.0 (C(2)); 143.1, (CH=*C*H–Ph); 135.5 (C(3)); 135.4 (C(1′)); 130.2 (*C*H=CH–Ph); 128.9 (C(3′,5′)); 128.4 (C(2′,6′)); 126.7 (C(4′)); 108.8 (C(1)); 96.6 (C(5)); 61.8 (MeO–C(2)); 61.2 (MeO–C(3)); 56.0 (MeO–C(4)). HR-ESI-MS: 315.1262 ($[M+H]^+$, $C_{18}H_{19}O_5^+$; calc. 315.1232).

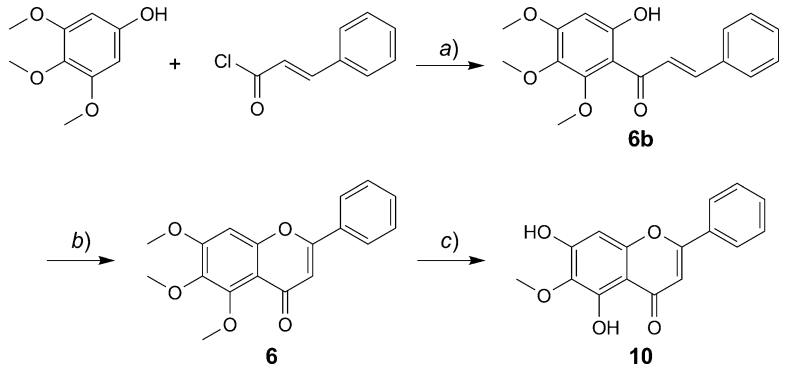
Scheme 1[a])



2 **5**

[a]) DMSO, K_2CO_3; wogonin/MeI 1:1.1.

Scheme 2

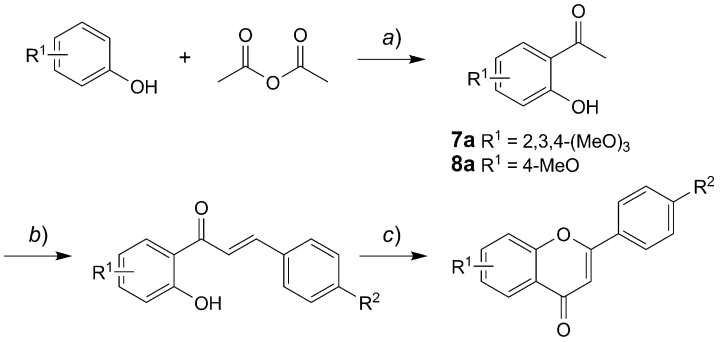


a) $BF_3 \cdot Et_2O$. *b*) I_2, DMSO, 2 h. *c*) 47% HBr/AcOH.

A mixture of **6b** (19.8 mg, 0.06 mmol) and I_2 (4.0 mg) in DMSO (4 ml) was stirred and heated at 100° for 2 h, and then poured into crushed ice/H_2O (20 ml). The precipitate was filtered and washed with 20% aq. Na_2SO_3 soln. The crude product was purified by CC (SiO_2; AcOEt/hexane 30:70) to give **6** (14 mg, 71%). Yellow solid. ^{1}H-NMR (MeOD): 7.98 (*dd*, *J* = 2.3, 2.3, H–C(2′,6′)); 7.52–7.57 (*m*, H–C(3′,4′,5′)); 7.11 (*s*, H–C(3)); 6.72 (*s*, H–C(8)); 4.01 (*s*, MeO–C(5)); 3.93 (*s*, MeO–C(6)); 3.87 (*s*, MeO–C(7)). ^{13}C-NMR (MeOD): 177.8 (C(4)); 162.3 (C(2)); 158.2 (C(7)); 153.9 (C(5)); 151.4 (C(8a)); 138.3 (C(6)); 131.4 (C(1′)); 129.3 (C(3′,5′)); 128.2 (C(2′,6′)); 125.9(C(4′)); 111.9 (C(4a)); 107.0 (C(3)); 96.5 (C(8)); 61.2 (MeO–C(5)); 60.4 (MeO–C(6)); 55.7 (MeO–C(7)). HR-ESI-MS: 313.1056 ($[M+H]^+$, $C_{18}H_{17}NO_5^+$; calc. 313.1076).

General Procedure for the Synthesis of Compounds **7**–**9**, **12**, *and* **13**. These compounds were synthesized as described in [30]. To a soln. of substituted phenol (10 mmol) in dry toluene (10 ml) were added Ac_2O (0.5 ml) and $BF_3 \cdot Et_2O$ (5 ml) (*Scheme 3*). The mixture was stirred at refluxing temp. for 2 h,

Scheme 3



	R^1	R^2
7b	2,3,4-(MeO)$_3$	F
8b	2,3,4-(MeO)$_3$	MeO
9b	2,3,4-(MeO)$_3$	Cl
12b	4-MeO	MeO
13b	4-MeO	OH

	R^1	R^2
7	5,6,7-(MeO)$_3$	F
8	5,6,7-(MeO)$_3$	MeO
9	5,6,7-(MeO)$_3$	Cl
12	5-OH,7-MeO	MeO
13	5-OH,7-MeO	OH

a) Toluene, $BF_3 \cdot Et_2O$. *b*) Arene-carb aldehydes, KOH, DMSO. *c*) I_2, DMSO, 2 h.

and then carefully poured into ice/H_2O (30 ml). The precipitate was filtered, washed with brine, and purified by flash CC (SiO_2; AcOEt/hexane 60:40) to afford **7a**–**9a**.

1-(6-Hydroxy-2,3,4-trimethoxyphenyl)ethanone (**7a**). Yield: 2.0 g (89%). Yellow solid. ^{1}H-NMR ($CDCl_3$): 13.4 (*s*, OH); 6.22 (*s*, H–C(5)); 3.98 (*s*, MeO–C(2)); 3.87 (*s*, MeO–C(3)); 3.77 (*s*, MeO–C(4)); 2.64 (*s*, AcO).

1-(2,6-Dihydroxy-4-methoxyphenyl)ethanone (**8a**). Yield: 1.8 g (81%). Yellow solid. ^{1}H-NMR ($CDCl_3$): 13.2 (*s*, OH); 6.31 (*s*, H–C(5)); 6.29 (*s*, H–C(3)); 3.73 (*s*, MeO); 2.60 (*s*, AcO).

Compounds **7b**–**9b**, **12b** and **13b** were synthesized by a base-catalyzed *Claisen–Schmidt* condensation reaction of substituted phenol and substituted aldehydes. To a DMSO soln. of **7a**–**9a** (1.0 equiv) and substituted aldehydes (1.0 equiv) was added 50% KOH (3.0 equiv). The mixture was stirred overnight at r.t., and then the pH was adjusted to 3–4 with 2M HCl. The precipitate was collected by filtration and purified by recrystallization in EtOH.

3-(4-Fluorophenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one (**7b**). Yield: 32 mg (9.6 mmol, 72%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 13.6 (br. *s*, OH); 7.86 (*d*, *J* = 15.6, CO–CH=C*H*–Ph); 7.83 (*d*, *J* = 15.6, CO–CH=C*H*); 7.65 (*d*, *J* = 7.8, H–C(2′,6′)); 7.08–7.13 (*m*, H–C(3′,5′)); 6.28 (*s*, H–C(5)); 3.92 (*s*, MeO–C(2)); 3.89 (*s*, MeO–C(3)); 3.82 (*s*, MeO–C(4)). ^{13}C-NMR ($CDCl_3$): 192.7 (CO); 165.1 (C(4)); 162.7 (C(4′)); 160.2 (C(6)); 154.9 (C(2)); 141.9 (CO–C=*C*H); 135.3 (C(3)); 131.6 (C(1′)); 130.3 (C(2′)); 130.2 (C(6′)); 126.3 (CO–*C*=CH); 116.2 (C(3′)); 116.0 (C(5′)); 108.7 (C(1)); 96.6 (C(5)); 61.9 (MeO–C(2)); 61.3 (MeO–C(3)); 56.1 (MeO–C(4)). HR-ESI-MS: 333.1111 ($[M+H]^+$, $C_{18}H_{18}FO_5^+$; calc. 333.0038).

1-(6-Hydroxy-2,3,4-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**8b**). Yield: 24 mg (7.0 mmol, 69%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 13.8 (br. *s*, OH); 7.84 (*dd*, *J* = 15.6, 15.6, CH=CH); 7.60 (*d*, *J* = 8.7, H–C(2′,6′)); 6.94 (*d*, *J* = 8.7, H–C(3′,5′)); 6.29 (*s*, H–C(5)); 3.93 (*s*, MeO–C(2)); 3.90 (*s*, MeO–C(3)); 3.86 (*s*, MeO–C(4)); 3.83 (*s*, MeO–C(4′)). ^{13}C-NMR ($CDCl_3$): 192.8 (CO); 162.6 (C(4)); 161.6 (C(6)); 159.9 (C(4′)); 155.0 (C(2)); 143.4 (CO–C=*C*H); 135.3 (C(3)); 130.2 (C(2′,6′)); 128.1 (C(1′)); 124.0 (CO–*C*=CH); 114.4 (C(3′,5′)); 108.8 (C(1)); 96.6 (C(5)); 61.9 (MeO–C(2)); 61.3 (MeO–C(3)); 56.1 (MeO–C(4)); 55.4 (MeO–C(4′)). HR-ESI-MS: 345.1319 ($[M+H]^+$, $C_{19}H_{21}O_6^+$; calc. 345.1338).

3-(4-Chlorophenyl)-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one (**9b**). Yield: 26 mg, (7.5 mmol, 72%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 13.6 (br. *s*, OH); 7.90 (*d*, *J* = 15.6, CO–CH=C*H*); 7.74 (*d*, *J* = 15.6, CO–CH=CH); 7.65 (*d*, *J* = 8.0, H–C(2′,6′)); 7.37 (*d*, *J* = 8.0, H–C(3′,5′)); 6.29 (*s*, H–C(5)); 3.92 (*s*, MeO–C(2)); 3.90 (*s*, MeO–C(3)); 3.83 (*s*, MeO–C(4)). ^{13}C-NMR ($CDCl_3$): 192.6 (CO); 162.7 (C(4)); 160.3 (C(6)); 154.9 (C(2)); 141.6 (CO–C=CH); 136.1 (C(3)); 135.3 (C(4′)); 133.9 (C(1′)); 129.5 (C(2′,6′)); 129.2 (C(3′,5′)); 127.0 (CO–*C*=CH); 108.7 (C(1)); 96.6 (C(5)); 61.9 (MeO–C(2)); 61.3 (MeO–C(3)); 56.1 (MeO–C(4)). HR-ESI-MS: 349.0826 ($[M+H]^+$, $C_{18}H_{17}ClO_5^+$; calc. 349.0842).

1-(2-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**12b**). Yield: 22.5 mg (7.4 mmol, 72%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 13.5 (br. *s*, OH); 7.86 (*dd*, *J* = 15.6, 15.6, CH=CH); 7.63 (*d*, *J* = 8.6, H–C(2′,6′)); 6.92 (*d*, *J* = 8.6, H–C(3′,5′)); 6.33 (*s*, H–C(5)); 6.30 (*s*, H–C(3)); 3.82 (*s*, MeO–C(4)); 3.79 (*s*, MeO–C(4′)). ^{13}C-NMR ($CDCl_3$): 192.4 (CO); 166.2 (C(4)); 162.4 (C(6)); 161.8 (C(2)); 159.7 (C(4′)); 143.2 (CO–C=CH); 130.4 (C(2′,6′)); 128.2 (C(1′)); 124.5 (CO–*C*=CH); 114.6 (C(3′,5′)); 108.5 (C(1)); 96.6 (C(5)); 96.4 (C(3)); 56.3 (MeO–C(4)); 55.2 (MeO–C(4′)). HR-ESI-MS: 301.1025 ($[M+H]^+$, $C_{17}H_{17}O_5^+$; calc. 301.1076).

1-(2-Hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**13b**). Yield: 21.6 mg (7.5 mmol, 67%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 13.7 (br. *s*, OH); 7.85 (*dd*, *J* = 15.4, 15.4, CH=CH); 7.58 (*d*, *J* = 8.6, H–C(2′,6′)); 6.74 (*d*, *J* = 8.6, H–C(3′,5′)); 6.30 (*s*, H–C(5)); 6.28 (*s*, H–C(3)); 3.82 (*s*, MeO–C(4)). ^{13}C-NMR ($CDCl_3$): 192.3 (CO); 166.1 (C(4)); 162.3 (C(6)); 161.7 (C(2)); 156.7 (C(4′)); 143.3 (CO–C=*C*H), 130.8 (C(2′,6′)); 128.5 (C(1′)); 124.4 (CO–*C*=CH); 115.3 (C(3′,5′)); 108.4 (C(1)); 96.3 (C(5)); 96.0 (C(3)); 56.2 (MeO–C(4)). HR-ESI-MS: 287.0943 ($[M+H]^+$, $C_{16}H_{15}O_5^+$; calc. 287.0919).

Compounds **7**–**9**, **12**, and **13** were prepared from **7b**–**9b**, **12b**, and **13b**, resp. A mixture of **7b**–**9b**, **12b**, or **13b** (0.06 mmol) and I_2 (4.0 mg) in DMSO (4 ml) was stirred and heated at 100° for 2 h, and then poured into crushed ice H_2O (20 ml). The precipitate was filtered and washed with 20% aq. Na_2SO_3 soln.

The crude product was purified by CC (SiO_2; AcOEt/hexane 30:70) to give corresponding compounds **7**–**9**, **12**, or **13** as a pale-yellow solid.

*2-(4-Fluorophenyl)-5,6,7-trimethoxy-4*H*-chromen-4-one* (**7**). Yield: 14.5 mg (4.8 mmol, 80%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 7.87 (*dd*, *J* = 5.3, 5.3, H–C(2′,6′)); 7.17–7.22 (*m*, H–C(3′,5′)); 6.80 (*s*, H–C(3)); 6.61 (*s*, H–C(8)); 3.98 (*s*, MeO–C(5), MeO–C(6)); 3.92 (*s*, MeO–C(7)). ^{13}C-NMR ($CDCl_3$): 177.1 (C(4)); 163.2 (C(2)); 162.3 (C(4′)); 159.8 (C(7)); 154.5 (C(8a)); 153.9 (C(5)); 138.1 (C(6)); 128.2 (C(2′,6′)); 127.8 (C(1′)); 116.3 (C(3′,5′); 111.9 (C(4a)); 105.0 (C(3)); 96.5 (C(8)); 62.2 (MeO–C(5)); 61.5 (MeO–C(6)); 56.3 (MeO–C(7)). HR-ESI-MS: 331.0945 ($[M+H]^+$, $C_{18}H_{16}FO_5^+$; calc. 331.0981).

*5,6,7-Trimethoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one* (**8**). Yield: 15.7 mg (4.6 mmol, 74%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 7.81 (*d*, *J* = 8.8, H–C(2′,6′)); 7.00 (*d*, *J* = 8.8, H–C(3′,5′)); 6.79 (*s*, H–C(3)); 6.59 (*s*, H–C(8)); 3.98 (*s*, MeO–C(5), MeO–C(6)); 3.91 (*s*, (MeO–C(7)); 3.87 (*s*, MeO–C(4′)). ^{13}C-NMR ($CDCl_3$): 177.2 (C(4)); 162.1 (C(2)); 161.2 (C(4′)); 157.6 (C(7)); 154.5 (C(8a)); 152.5 (C(5)); 140.3 (C(6)); 127.62 (C(2′,6′)); 123.8 (C(1′)); 114.4 (C(3′,5′)); 112.9 (C(4a)); 107.0 (C(3)); 96.2 (C(8)); 62.2 (MeO–C(5)); 61.5 (MeO–C(6)); 56.3 (MeO–C(7)); 55.5 (MeO–C(4′)). HR-ESI-MS: 343.1155 ($[M+H]^+$, $C_{19}H_{19}O_6^+$; calc. 343.1181).

*2-(4-Chlorophenyl)-5,6,7-trimethoxy-4*H*-chromen-4-one* (**9**). Yield: 16 mg (4.7 mmol, 74%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 7.81 (*d*, *J* = 8.6, H–C(2′,6′)); 7.48 (*d*, *J* = 8.6, H–C(3′,5′); 6.81 (*s*, H–C(3)); 6.65 (*s*, H–C(8)); 3.99 (*s*, MeO–C(5), MeO–C(6)); 3.92 (*s*, MeO–C(7)). ^{13}C-NMR ($CDCl_3$): 177.0 (C(4)); 160.0 (C(2)); 157.9 (C(4′)); 154.5 (C(7)); 152.6 (C(8a)); 140.5 (C(5)); 137.5 (C(6)); 130.0 (C(1′)); 129.3 (C(2′,6′)); 127.2 (C(3′,5′)); 111.9 (C(4a)); 108.5 (C(3)); 96.2 (C(8)); 62.2 (MeO–C(5)); 61.5 (MeO–C(6)); 56.3 (MeO–C(7)). HR-ESI-MS: 347.0661 ($[M+H]^+$, $C_{18}H_{15}ClO_5^+$; calc. 347.0686).

*5,7-Dihydroxy-6-methoxy-2-phenyl-4*H*-chromen-4-one* (**10**). A soln. of **6** (20 mg, 0.06 mmol) in 47% HBr (2 ml) and glacial AcOH (3 ml) was refluxed for 3 h, and then added to ice-water (30 ml). The pale-yellow precipitate was filtered and collected. The crude product was purified by CC (SiO_2; AcOEt/hexane 30:70) to give **10** (14 mg, 77%). Pale-yellow solid. ^{1}H-NMR ((D$_6$)DMSO): 12.5 (br. *s*, HO–C(5)); 7.98 (*d*, *J* = 6.3, H–C(2′,6′)); 7.53–7.57 (*m*, H–C(3′,4′,5′)); 6.70 (*s*, H–C(3)); 6.41 (*s*, H–C(8)); 3.85 (*s*, MeO). ^{13}C-NMR ((D$_6$)DMSO): 182.4 (C(4)); 163.3 (C(2)); 156.3 (C(7)); 153.6 (C(5)); 151.9 (C(8a)); 131.5 (C(6)); 130.6 (C(1′)); 128.3 (C(3′,5′)); 127.2 (C(2′,6′)); 127.0(C(4′)); 106.2 (C(4a)); 105.0 (C(3)); 96.1 (C(8)); 60.3 (MeO). HR-ESI-MS: 285.0765 ($[M+H]^+$, $C_{16}H_{13}O_5^+$; calc. 285.0763).

*5,7-Dihydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one* (=*5,7-Dihydroxy-4′-methoxyflavone*; **11**). This compound was generously supplied by Dr. *Hankui Wu*, School of Pharmacy, The University of Mississippi, Oxford, MS. ^{1}H-NMR ((D$_6$)DMSO): 13.0 (br. *s*, HO–C(5)); 7.95 (*d*, *J* = 8.9, H–C(2′,6′)); 6.91 (*d*, *J* = 8.8, H–C(3′,5′)); 6.84 (*s*, H–C(3)); 6.77 (*s*, H–C(8)); 6.37 (*s*, H–C(6)); 3.86 (*s*, MeO). ^{13}C-NMR ((D$_6$)DMSO): 182.0 (C(4)); 162.3 (C(7)); 161.2 (C(2)); 160.9 (C(5)); 158.3(C(4′)); 141.9 (C(8a)); 128.2 (C(2′,6′)); 121.6 (C(1′)); 116.3 (C(3′,5′)); 106.2 (C(4a)); 106.0 (C(3)); 98.6 (C(6)); 98.6 (C(8)); 56.1 (MeO). ESI-HR-MS: 285.0767 ($[M+H]^+$, $C_{16}H_{13}O_5^+$; calc. 285.0763).

*5-Hydroxy-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one* (=*5-Hydroxy-4′,7-dimethoxy-flavone*; **12**). Yield: 14 mg (4.6 mmol, 78%). Pale-yellow solid. ^{1}H-NMR ((D$_6$)DMSO): 12.9 (br. *s*, OH); 8.07 (*d*, *J* = 9.0, H–C(2′,6′)); 7.12 (*d*, *J* = 8.6, H–C(3′,5′)); 6.95 (*s*, H–C(3)); 6.81 (*s*, H–C(8)); 6.39 (*s*, H–C(6)); 3.87 (*s*, MeO–C(7)); 3.86 (*s*, MeO–C(4′)). ^{13}C-NMR ((D$_6$)DMSO): 182.2 (C(4)); 163.5 (C(7)); 162.7 (C(2)); 160.3 (C(5)); 159.83(C(4′)); 141.9 (C(8a)); 128.2 (C(2′,6′)); 122.5 (C(1′)); 114.3 (C(3′,5′)); 104.8 (C(4a)); 104.3 (C(3)); 98.5 (C(6)); 94.8 (C(8)); 56.8 (MeO–C(7)); 56.3 (MeO–C(4′)). HR-ESI-MS: 299.0919 ($[M+H]^+$, $C_{17}H_{15}O_5^+$; calc. 299.0919).

*5-Hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4*H*-chromen-4-one* (=*5,4′-Dihydroxy-7-methoxyflavone*, **13**). Yield: 12.8 mg (4.5 mmol, 75%). Pale-yellow solid. ^{1}H-NMR ((D$_6$)DMSO): 12.9 (br. *s*, HO–C(5)); 8.03 (*d*, *J* = 8.8, H–C(2′,6′)); 7.10 (*d*, *J* = 8.9, H–C(3′,5′)); 6.87 (*s*, H–C(3)); 6.50 (*s*, H–C(8)); 6.19 (*s*, H–C(6)); 3.85 (*s*, MeO). ^{13}C-NMR ((D$_6$)DMSO): 182.3 (C(4)); 163.3 (C(7)); 162.2 (C(2)); 160.5 (C(5)); 159.3(C(4′)); 141.7 (C(8a)); 128.0 (C(2′,6′)); 122.6 (C(1′)); 115.5 (C(3′,5′)); 106.2 (C(4a)); 106.0 (C(3)); 98.3 (C(6)); 94.6 (C(8)); 56.8 (MeO). HR-ESI-MS: 285.0762 ($[M+H]^+$, $C_{16}H_{13}O_5^+$; calc. 285.07630).

General Procedure for the Synthesis of Compounds **14**–**21**. Substituted benzoyl chloride and DMAP (1.05 mg, 0.01 mmol) were added to a soln. of **2** (15 mg, 0.053 mmol) in dry pyridine (2 ml; *Scheme 4*). The mixture was stirred at r.t. for 24 h and then carefully poured into ice H_2O (30 ml). The precipitate

was filtered and washed with brine. Purification was performed by flash CC (SiO_2; AcOEt/hexane 30:70).

*5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H*-chromen-7-yl 2-Chlorobenzoate* (**14**). Yield: 21.2 mg (4.9 mmol, 92%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH); 8.12 (*d*, *J* = 7.8, H–C(6″)); 7.96 (*d*, *J* = 7.4, H–C(2′,6′)); 7.52–7.58 (*m*, H–C(3′,5′,3″,4″,5″)); 7.40–7.44 (*m*, H–C(4′)); 6.79 (*s*, H–C(3)); 6.71 (*s*, H–C(6)); 4.00 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 183.0 (C(4)); 164.4 (CO); 161.9 (C(2)); 156.6 (C(5)); 150.8 (C(8a)); 149.1 (C(7); 134.8 (C(8)); 133.7 (C(4″)); 132.7 (C(2″)); 132.3 (C(1″)); 132.1 (C(6″)); 131.5 (C(1′)); 130.9 (C(3″)); 129.3 (C(3′,5′)); 128.3 (C(4′)); 126.9 (C(5″)); 126.4 (C(2′,6′)); 109.3 (C(4a)); 106.1 (C(6); 105.9 (C(3)); 62.0 (MeO). HR-ESI-MS: 423.06625 ($[M+H]^+$, $C_{23}H_{16}ClO_6$; 423.0635).

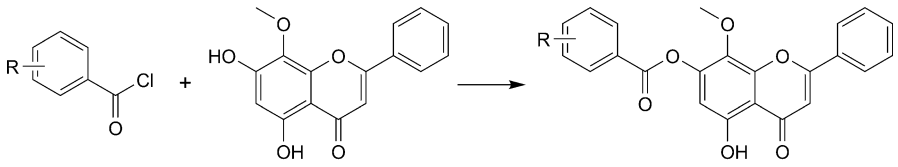
*5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H*-chromen-7-yl 3-Chlorobenzoate* (**15**). Yield: 19.4 mg (4.5 mmol, 87%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH), 8.22 (*s*, H–C(2″)); 8.12 (*d*, *J* = 7.8, H–C(6″)); 7.96 (*d*, *J* = 6.6, H–C(2′,6′)); 7.66 (*d*, *J* = 7.1, H–C(4″)); 7.51–7.56 (*m*, H–C(3′,4′,5′,3″,5″)); 6.79 (*s*, H–C(3)); 6.68 (*s*, H–C(6)); 3.96 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 183.0 (C(4)); 164.4 (CO), 162.9 (C(2)); 156.6 (C(5)); 149.9 (C(8a)); 149.3 (C(7)); 135.0 (C(8)); 134.1 (C(3″)); 132.6 (C(4″)); 132.3 (C(1″)); 130.9 (C(1′)); 130.4 (C(2″)); 130.3 (C(6″)); 129.3 (C(3′,5′)); 128.5 (C(4′)); 126.4 (C(2′,6′)); 109.7 (C(4a)); 106.1 (C(6)); 105.9 (C(3)); 62.0 (MeO). HR-ESI-MS: 423.0627 ($[M+H]^+$, $C_{23}H_{16}ClO_6^+$; calc. 423.06354).

*5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H*-chromen-7-yl 4-Chlorobenzoate* (**16**). Yield: 20.3 mg (4.7 mmol, 89%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH); 8.17 (*d*, *J* = 8.6, H–C(2″,6″)); 7.95 (*d*, *J* = 8.3, H–C(3″,5″)); 7.51–7.56 (*m*, H–C(2′,3′,4′,5′,6′)); 6.99 (*s*, H–C(3)); 6.88 (*s*, H–C(6)); 3.95 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 183.0 (C(4)); 164.4 (CO); 163.3 (C(2)); 156.6 (C(5)); 149.9 (C(8a)); 149.4 (C(7)); 140.7 (C(4″)); 132.6 (C(8)); 132.3 (C(1″)); 131.8 (C(2″,6″)); 130.9 (C(1′)); 129.3 (C(3″,5″)); 129.2 (C(3′,5′)); 127.0 (C(4′)); 126.4 (C(2′,6′)); 109.7 (C(4a)); 106.1 (C(6)); 105.9 (C(3)); 62.0 (MeO). HR-ESI-MS: 423.0650 ($[M+H]^+$, $C_{23}H_{16}ClO_6^+$; calc. 423.06354).

*5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H*-chromen-7-yl 2-Methylbenzoate* (**17**). Yield: 18.0 mg (4.5 mmol, 85%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH), 8.20 (*d*, *J* = 7.4, H–C(6″)); 7.95 (*d*, *J* = 6.6, H–C(2′,6′)); 7.55–7.59 (*m*, H–C(3′,5′,4″,5″)); 7.34–7.38 (*m*, H–C(4′,3″)); 6.78 (*s*, H–C(3)); 6.68 (*s*, H–C(6)); 3.98 (*s*, MeO), 2.70 (Me–C(2″)). ^{13}C-NMR ($CDCl_3$): 183.0 (C(4)); 164.5 (CO); 164.3 (C(2)); 156.5 (C(5)); 149.9 (C(8a)); 149.7 (C(7)); 141.7 (C(2″)); 133.2 (C(8)); 132.8 (C(4″)); 132.3 (C(1″)); 132.1 (C(3″)); 131.4 (C(1′)); 131.0 (C(6″)); 129.3 (C(3′,5′)); 127.7 (C(4′)); 126.4 (C(2′,6′)); 126.1 (C(5″)); 109.5 (C(4a)); 106.2 (C(6)); 106.0 (C(3)); 61.9 (MeO); 21.9 (Me–C(2″)). HR-ESI-MS: 403.1204 ($[M+H]^+$, $C_{24}H_{19}O_6^+$; calc. 403.1181).

*5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H*-chromen-7-yl 3-Methylbenzoate* (**18**). Yield: 17.3 mg (4.3 mmol, 81%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH), 8.02–8.06 (*m*, H–C(2″,6″)); 7.95 (*d*, *J* = 6.6, H–C(2′,6′)); 7.55–7.59 (*m*, H–C(3′,5′,4″)); 7.48–7.51 (*m*, H–C(5″)); 7.41–7.47 (*m*, H–C(4′)); 6.78 (*s*, H–C(3)); 6.68 (*s*, H–C(6)); 3.96 (*s*, MeO); 2.63 (Me). ^{13}C-NMR ($CDCl_3$): 183.0 (C(4)); 164.3 (CO); 164.3 (C(2)); 156.5 (C(5)); 149.9 (C(8a)); 149.7 (C(7)); 138.7 (C(3″)); 134.9 (C(8)); 132.8 (C(4″)); 132.3 (C(2″)); 131.0 (C(1′)); 130.9 (C(1″)); 129.3 (C(3′,5′)); 128.6 (C(5″)); 128.5 (C(4′));

Scheme 4[a])



2 **14 – 21**

[a]) 4-(Dimethylamino)pyridine (DMAP), pyridine; wogonin/substituted benzoyl chloride 1:1.1.

127.6 (C(6″)); 126.4 (C(2′,6′)); 109.5 (C(4a)); 106.08 (C(6)); 106.05 (C(3)); 62.0 (MeO), 21.3 (Me). HR-ESI-MS: 403.1190 ($[M+H]^+$, $C_{24}H_{19}O_6^+$; calc. 403.1181).

*5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H-*chromen-7-yl 4-Methylbenzoate* (**19**). Yield: 18.5 mg (4.6 mmol, 87%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH); 8.14 (*d*, *J* = 7.8, H–C(2′,6′)); 7.96 (*d*, *J* = 7.9, H–C(3″,5″)); 7.56–7.61 (*m*, H–C(3′,4′,5′)); 7.36 (*d*, *J* = 7.8, H–C(2″,6″)); 6.79 (*s*, H–C(3)); 6.70 (*s*, H–C(6)); 3.96 (*s*, MeO), 2.48 (Me). ^{13}C-NMR ($CDCl_3$): 183.0 (C(4); 164.3 (CO); 164.1 (C(2)); 156.5 (C(5)); 149.85 (C(8a)); 149.79 (C(7)); 145.0 (C(4″)); 132.8 (C(8)); 132.3 (C(1′)); 131.0 (C(1″)); 130.5 (C(2″,6″)); 129.5 (C(3″,5″)); 129.3 (C(3′,5′)); 126.4 (C(2′,6′)); 125.8 (C(4′)); 109.5 (C(4a)); 106.1 (C(6)); 106.0 (C(3)); 61.9 (MeO); 21.8 (Me). HR-ESI-MS: 403.1212 ($[M+H]^+$, $C_{24}H_{19}O_6^+$; calc. 403.1181).

*5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H-*chromen-7-yl 2,3-Dimethylbenzoate* (**20**). Yield: 16.6 mg (4.0 mmol, 75%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH); 7.96 (*d*, *J* = 7.2, H–C(2′,6′,6″)); 7.38–7.40 (*m*, H–C(3′,5′,4″)); 7.41–7.43 (*m*, H–C(4′)); 7.21–7.26 (*m*, H–C(5″)); 6.78 (*s*, H–C(3)); 6.68 (*s*, H–C(6)); 3.99 (*s*, MeO); 2.58 (Me–C(3″)); 2.39 (Me–C(2″)). ^{13}C-NMR ($CDCl_3$): 183.0 (C(4)); 165.5 (CO); 164.3 (C(2)); 156.5 (C(5)); 149.9 (C(8a)); 149.7 (C(7)); 139.2 (C(2″)); 138.4 (C(3″)); 134.4 (C(8)); 132.8 (C(4″)); 132.3 (C(1″)); 131.0 (C(1′)); 129.3 (C(3′,5′)); 128.9 (C(5″)); 128.6 (C(4′)); 126.4 (C(2′,6′)); 125.5 (C(6″)); 109.5 (C(4a)); 106.2 (C(6)); 106.0 (C(3)); 61.9 (MeO); 20.6 (Me–C(3″)); 16.7 (Me–C(2″)). HR-ESI-MS: 417.1361 ($[M+H]^+$, $C_{25}H_{21}O_6^+$; calc. 417.13381).

*5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H-*chromen-7-yl 2,4-Dimethylbenzoate* (**21**). Yield: 17.1 mg (4.1 mmol, 77%). Pale-yellow solid. ^{1}H-NMR ($CDCl_3$): 12.4 (br. *s*, OH); 8.11 (*d*, *J* = 8.4, H–C(6″)); 7.96 (*d*, *J* = 7.2, H–C(2′,6′)); 7.54–7.60 (*m*, H–C(3′,4′,5′)); 7.17 (*m*, H–C(3″,5″)); 6.78 (*s*, H–C(3)); 6.68 (*s*, H–C(6)); 3.96 (*s*, MeO); 2.67 (Me–C(2″)); 2.42 (Me–C(4″)). ^{13}C-NMR ($CDCl_3$): 183.0 (C(4)); 164.5 (CO); 164.3 (C(2)); 156.5 (C(5)); 149.9 (C(8a)); 149.8 (C(7)); 144.0 (C(2″)); 142.0 (C(4″)); 132.9 (C(8)); 132.8 (C(3″)); 132.2 (C(1′)); 131.6 (C(6″)); 131.0 (C(1″)); 129.3 (C(3′,5′)); 126.8 (C(4′)); 126.4 (C(2′,6′)); 124.7 (C(5″)); 109.5 (C(4a)); 106.3 (C(6)); 106.0 (C(3)); 61.9 (MeO), 21.9 (Me–C(2″)); 21.5 (Me–C(4″)). HR-ESI-MS: 417.1334 ($[M+H]^+$, $C_{25}H_{21}O_6^+$; calc. 417.1338).

The naturally-occurring flavonoids apigenin 7-*O*-*β*-D-(6-*O*-*p*-coumaroyl)glucoside (**22**), echinaticin (**23**), scutellarein (**24**), icariin (**25**), isorhamnetin (**26**), luteolin (**27**), quercetin (**28**), biochanin A (**29**), daidzin (**30**), hesperedin **(31)**, and epicatechin (**32**) were isolated from plants at the *National Center for Natural Products Research*, University of Mississippi, and have been chemically characterized. Their chemical structures and spectroscopic data were in agreement with those reported in the literature.

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