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Synthesis and biological evaluation of 5,7-dihydroxyflavanone derivatives as antimicrobial agents

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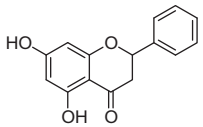
ABSTRACT

A series of 5,7-dihydroxyflavanone derivatives were efficiently synthesized. Their antimicrobial efficacy on Gram-negative, Gram-positive bacteria and yeast were evaluated. Among these compounds, most of the halogenated derivatives exhibited the best antimicrobial activity against Gram-positive bacteria, the yeast *Saccharomyces cerevisiae*, and the Gram-negative bacterium *Vibrio cholerae*. The cytotoxicities of these compounds were low as evaluated on HepG2 cells using a cell viability assay. This study suggests that halogenated flavanones might represent promising pharmacological candidates for further drug development.

Flavanones are an important class of flavonoids containing a 2-phenyl-benzopyran-4-one skeleton, and are commonly found in fruits and vegetables.[1–3] These natural products possess a variety of biological activities such as antitumor and anti-inflammatory properties that are closely related to the skeleton and substitution patterns.[2–7] The ability to manipulate flavonoid activity through structural variation motivates research on the synthesis of flavanone derivatives and evaluation of their bioactivity.[8–16]

Pinocembrin (5,7-dihydroxyflavanone) **1**, is one of the primary flavanones which is abundant in propolis and can be extracted from plants, fruits, vegetables, seeds, flowers and teas (Fig. 1).[17] A vast range of biological/pharmacological activities for pinocembrin have been reported, including antimicrobial, anti-inflammatory, anticancer and antioxidant, as well as neuroprotective potential.[17,18] Pinocembrin is a small molecular weight natural compound and is a biologically active constituent of honey. Its presence in food suggests its safety for long-term administration, making it an excellent chemical template for the design and synthesis of new compounds for pharmaceutical research. Recently, we reported the antimicrobial efficacy of synthetic flavanone



Pinocembrin (**1**)

Figure 1. Chemical structure of Pinocembrin (**1**).

derivatives.[19] Derivatives were identified that impede growth of a series of organisms. For instance, 4-chloro-flavanone, when combined with the inhibitor Phe-Arg-β-naphthylamide, exhibits MIC's of 30, 30 and 70 μg mL^{-1} for *Saccharomyces cerevisiae*, *Cryptococcus neoformans* and *Escherichia coli*, respectively.[19] These results led us to hypothesize that halide substitution of flavanones might show enhanced antimicrobial activity. In addition, there is increased interest in bioactive halogenated compounds.[20–23] Here, we use an improved synthetic approach to obtain a series of mostly halogenated 5,7-dihydroxyflavanone derivatives that were assessed for antimicrobial efficacy and mammalian cytotoxicity.

A modified synthetic approach was developed based on a conventional route to efficiently obtain a small library of flavanones.[24] Synthesis commenced with partial methoxymethyl (MOM) protection of 2,4,6-trihydroxy acetophenone monohydrate to produce

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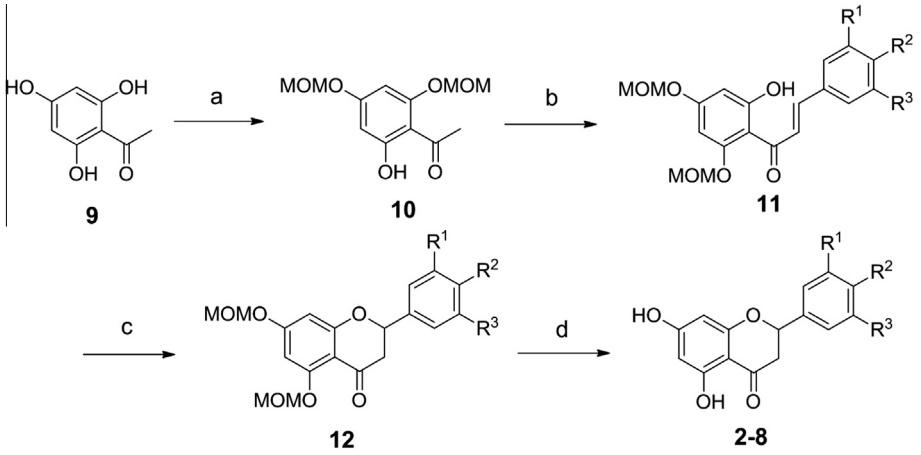

Scheme 1. Reagents and conditions: (a) Methoxymethyl (MOM) chloride, *N*,*N*-diisopropylethylamine, DCM, 0 °C, 12 h, 95%; (b) KOH, substituted benzaldehyde, MeOH, 16 h; (c) NaOAc, MeOH, reflux, 24 h; (d) 6 N HCl, MeOH, 60 °C, 42–56% yield over 3-steps.

Table 1
Target compounds

Compd	R^1	R^2	R^3	Yield[a] (%)
2[25]	H	F	H	45
3	F	F	H	56
4	F	F	F	52
5	F	Cl	H	45
6[26]	Cl	Cl	H	47
7	OH	OH	OH	42
8[27]	H	MeO	H	45

[a] Isolated yield from compound **10** over 3 steps.

phenol **10** (Scheme 1). A series of substituted benzaldehydes were reacted with phenol **10**, through a Claisen–Schmidt condensation, to prepare a series of chalcone intermediates **11** with different substituents on their B-ring. The corresponding chalcones **11** were cyclized with sodium acetate to provide the protected flavanones **12**. Finally, acidic hydrolysis of the MOM groups afforded the flavanone derivatives **2**–**8** (Table 1). Compared to the conventional synthetic method, the efficiency of this synthesis is demonstrated by only one column chromatography step being required for the conversion of phenol **10** to the target flavanones **2**–**8**. The structures of flavanone derivatives **2**–**8** were confirmed from their spectral (^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) and high resolution-mass spectrometry (MS)) properties (see the Supplementary data).

The antimicrobial activities of 5,7-dihydroxyflavanone derivatives **2**–**8** were evaluated by measuring their inhibitory effect against a series of Gram-negative and Gram-positive bacteria and yeast and their minimum inhibitory concentration (MIC) values were determined.

Natural flavanones as bacteriostatic agents have been investigated in the past, and have shown no inhibition against *E. coli*,[19] which is a Gram-negative bacterium. However, natural flavanones did show inhibition against *Bacillus subtilis*,[19] which is a Gram-positive organism. Normally, compounds show greater potency against Gram-positive than Gram-negative bacteria, since the Gram-negative bacteria have both an outer and an inner membrane making the cell permeability of an antimicrobial agent much more difficult.

Results herein are consistent with a previous report on the antimicrobial activity of natural flavanones.[19] Non-natural flavanones **2**–**8** were ineffective at inhibiting growth of the Gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa* (Table 2).

However, halogenated derivatives **2**, **3**, **5** and **6** showed significant activities (30–40 μg mL^{-1}) against *Vibrio cholera*, a Gram-negative organism. To the best of our knowledge, this is one of the rare examples of the inhibition of Gram-negative bacteria by flavanones.

Next, the activity of flavanones **2**–**8** was tested against the following Gram-positive bacteria: *Bacillus subtilis* 1012, *Bacillus anthracis*, *Bacillus cereus* 10987 and *Staphylococcusaureus* ATCC 33807. The lowest MIC values observed were obtained for the

Table 2
Non-natural flavanone MIC values in μg mL^{-1}(μM)

Organism	Compounds						
	2	**3**	**4**	**5**	**6**	**7**	**8**
Gram-negative bacteria							
Escherichia coli BW25113	>140[a](500)[b]	>290(1000)	>310(1000)	>80(250)	>40(125)	>300(1000)	>290(1000)
Pseudomonas aeruginosa PA01	>140(500)	>290(1000)	>310(1000)	>80(250)	>40(125)	>300(1000)	>290(1000)
*Vibrio cholerae*0395N1	30(125)	40(125)	>310(1000)	40(125)	40(125)	>300(1000)	>290(1000)
Gram-positive bacteria							
Bacillus subtilis 1012	30(125)	40(125)	>310(1000)	40(125)	20(62.5)	>300(1000)	>290(1000)
Bacillus anthracis delta sterne	30(125)	20(62.5)	>310(1000)	20(62.5)	10(31.25)	>300(1000)	>290(1000)
Bacillus cereus 10987	30(125)	40(125)	>310(1000)	30(93.75)	10(31.25)	>300(1000)	>290(1000)
Staphylococcus aureus ATCC 33807	30(125)	40(125)	>310(1000)	40(125)	20(62.5)	>300(1000)	>290(1000)
Eukaryote							
Saccharomyces cerevisiae INVSc1	60(250)	>290(1000)	40(125)	80(250)	10(31.25)	>300(1000)	>290(1000)

[a] Concentration unit is μg mL^{-1}.
[b] Concentration unit is μM.

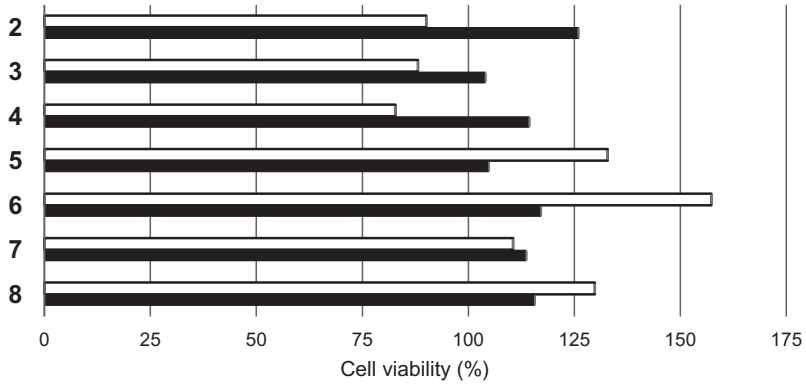

Figure 2. Cytotoxicity of 5,7-dihydroxyflavanone derivatives on HepG2 cells. Cell viability was measured after incubating HepG2 cells with 50 μM of flavanone derivative by MTS assay. Cell viability was normalized based on the negative control (100% growth medium) and positive control (100% DMSO). Flavonone **3** shows least toxicity out of the compounds that were tested. Open bars correspond to 24 h and closed bars correspond to 6 h. A single experiment was performed with three replicate determinations. The error bars included in the figure are too small to be seen.

flavanones **2**, **5** and **6**, which contain halide substituents. The dichlorinated flavanone **6** provided the greatest inhibition of the growth of all the Gram-positive strains tested, exhibiting MIC values ranging from 10 to 20 μg mL^{-1}. Flavanones without halogen substituents did not inhibit the growth of Gram-positive strains, and not all flavanones having halogens inhibited microbial growth. For example, the trifluorinated flavanone **4** had no effect on growth for Gram-positive bacteria, while the monofluorinated and difluorinated flavanone, **2** and **3**, afforded low MIC values. Inhibition of microbial growth was observed for trifluorinated compound **4** when tested against *S. cerevisiae*, affording a MIC of 40 μg mL^{-1}. Most importantly, the MIC values for some of the flavanones tested were better than that of their natural analogs, approaching MIC values observed for traditional antibiotics. All of the antimicrobial results were confirmed in triplicate using a 96-well plates bioassay.

We next used a MTS (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoly)-3-(4-sulfophenyl) tetrazolium inner salt) cell viability assay to evaluate mammalian cytotoxicity of 5,7-Dihydroxyflavanone derivatives in HepG2 cells. HepG2 is a well-established human hepatocellular carcinoma cell line. Approximately 50,000 cells were seeded and incubated for 24 h before addition of 5,7-dihydroxyflavanone to samples and to the controls. MTS reagent was added at 6 and 24 h of incubation and cell viability was determined by measuring Abs_{490}. The poor water solubility of many flavanones limited the range of concentrations that could be evaluated. The 5,7-dihydroxyflavanone derivatives generally had low water solubility, ranging from 50 to 2000 μM in growth medium containing 1% dimethylsulfoxide (DMSO). The maximal solubility for compounds **6** and **7** was 63 μM, for compounds **4** and **5** was 500 μM, and for compound **2** and **3**, 1000 μM. Methoxy-flavanone **8** had the highest solubility at 2000 μM. All of the flavanones showed dose-dependent toxicity towards HepG2 cells. Because of the limited solubility of the flavanones, it was only possible to obtain an IC_{50} value for compound **4** of 95 μM after 24 h. At a concentration of 50 μM (14–16 μg mL^{-1}), all the derivatives were completely soluble and exhibited low to no cytotoxicity towards HepG2 cells (Fig. 2). At this concentration, the order of their relative cytotoxicity to HepG2 cells is as follows: compound **4** > compound **3** > compound **2** > compound **5** > compound **8** > compound **7** > compound **6**. Toxicity increased with greater number of fluorine substituents, while OMe, OH, and Cl substituents promoted the proliferation of HepG2 cells. The dichlorinated flavanone **6** which has the highest antibacterial activity against all of the microorganisms tested, showed the lowest level of cytotoxicity and promoted proliferation of HepG2 cells by 17% at 6 h, and 57% at 24 h over that obtained in cell culture growth medium.

In summary, a series of 5,7-dihydroxyflavanone derivatives **2**–**8** were designed and efficiently synthesized. Their antimicrobial activity on Gram-negative, Gram-positive bacteria and yeast were evaluated as well as their cytotoxicity in HepG2 cells. In this study, most of the halogenated compounds showed greater antimicrobial activity (lower MIC values) than their natural analogs. Further discovery through target design and synthesis, as well as mechanistic studies are underway.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.003.

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