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

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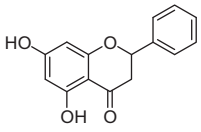
ABSTRACT

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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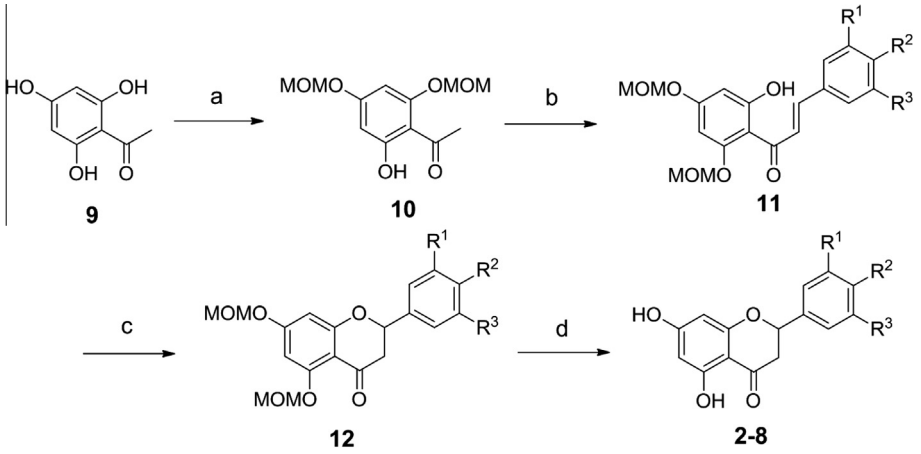

Scheme 1. Reagents and conditions: (a) Methoxymethyl (MOM) chloride, *N*,*N*-diisopropylethylamine, DCM, 0 °C, 12 h, 95%; (b) KOH, substituted benzaldehyde, MeOH, 16 h; (c) NaOAc, MeOH, reflux, 24 h; (d) 6 N HCl, MeOH, 60 °C, 42–56% yield over 3-steps.

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.