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Aromatase inhibition by bioavailable methylated flavones

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Abstract

Previous studies have shown chrysin, 7-hydroxyflavone and 7,4'-dihydroxyflavone to be the most potent flavonoid inhibitors of aromatase. However, very poor oral bioavailability is a major limitation for the successful use of dietary flavonoids as chemopreventive agents. We have recently shown that methylated flavones, including 5,7-dimethoxyflavone, 7-methoxyflavone and 7,4'-dimethoxyflavone, are much more resistant to metabolism than their unmethylated analogs and have much higher intestinal absorption. In this study, we examined these fully methylated flavones as potential aromatase inhibitors for the prevention and/or treatment of hormone-dependent cancers. Whereas 5,7-dimethoxyflavone had poor effect compared to its unmethylated analog chrysin, 7-methoxyflavone and 7,4'-dimethoxyflavone were almost equipotent to their unmethylated analogs with IC₅₀ values of 2 to 9 μ M. Thus, some fully methylated flavones appear to have great potential as cancer chemopreventive/chemotherapeutic agents.

Keywords

Aromatase inhibition; Flavonoids; Methylated flavones; Methoxyflavones

1. Introduction

The enzyme aromatase (cytochrome P450 (CYP)19), an important regulator of estrogen hormone availability, has become a target for new drug synthesis of inhibitors attempting to treat estrogen hormone-dependent cancers, which in addition to breast cancer [1] now also includes lung cancer [2].

Some naturally-occurring flavonoids, in particular chrysin (5,7-dihydroxyflavone), have also been shown in vitro to be aromatase inhibitors [3]. This gave rise to claims of chrysin as a booster of testosterone levels, leading to its marketing by health food stores and use by body builders. However, there is no support for its effectiveness in vivo. A clinical study demonstrated that the oral bioavailability of chrysin was much too low for any biological activity [4]. Another clinical study did not show any effect of chrysin on urinary testosterone levels [5]. Similar findings were made in a rat study [6].

In contrast, we have recently described high metabolic stability in the human liver as well as high intestinal transport of fully methylated flavones compared to the unmethylated analogs [7,8] to predict high oral bioavailability. These methylated compounds, thus, have the potential to be effective aromatase inhibitors in humans in vivo.

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In the present study, we therefore determined the aromatase inhibitory activity of selected methylated flavones (see structures in Fig. 1). We compared the effects of the methylated versus the corresponding unmethylated analogs, the latter previously investigated by Ibrahim and Abul-Hajj [9]. The results suggest that some of these metabolically stable flavones may be effective aromatase inhibitors in humans *in vivo*.

2. Materials and methods

Chrysin was obtained from Sigma Chemical Co. (St. Louis, MO). 5,7-Dimethoxyflavone (5,7-DMF), 7,4'-dimethoxyflavone (7,4'-DMF), 7,4'-dihydroxyflavone (7,4'-DHF), 7-methoxyflavone (7-MF) and 7-hydroxyflavone (7-HF) (structures, see Fig. 1) were obtained from Indofine Chemical Co., Inc. (Hillsborough, NJ).

The inhibition of aromatase (cytochrome P450 19) by the test flavones was investigated using a kit from Gentest (Woburn, MA) with recombinant CYP19 Supersomes as the enzyme source and dibenzylfluorescein as the substrate [10,11] in a 96-well format. Serial dilutions of flavones (0.05-100 μM final concentrations) were preincubated at 37°C for 10 min with an NADPH generating system with control protein (0.1 mg/ml) in phosphate buffer. The enzymatic reaction was then carried out in the presence of 4 nM aromatase and 0.4 μM substrate for 30 min while shaking. The reaction was terminated with NaOH and the fluorescence was read 2 hr later in a plate reader with excitation at 485 nm and emission at 520 nm. Each flavone concentration was assayed in triplicates with appropriate background subtraction and controls.

Data were expressed as means \pm SEM. Statistical significance of differences between samples were calculated by ANOVA with Dunnett multiple comparison post-test. $P < 0.05$ was considered significant. The IC_{50} values were calculated using Prism 4 (GraphPad Software, San Diego, CA).

3. Results and Discussion

The effect of the flavones in this study on aromatase activity used recombinant CYP19 as the enzyme source and a substrate that showed fluorescence upon metabolism.

Chrysin was a potent aromatase inhibitor with an IC_{50} of 4.2 μM (Fig. 2A), consistent with previous studies showing values of 0.5 to 2.6 μM [3]. The methylated analog, 5,7-DMF, showed very poor effect with an estimated IC_{50} of 123 μM (Fig. 2A). The flavone with the single hydroxyl group in the 7-position (7-HF) had previously been shown to be the most potent flavone inhibitor (IC_{50} 0.50 μM) [9]. We found identical potency for 7-HF (IC_{50} 0.51 μM) (Fig. 2B). In contrast to 5,7-DMF, 7-MF, i.e. the methylated analog of 7-HF, was only slightly less potent than 7-HF (Fig. 2B) with an IC_{50} value of 1.9 μM . 7,4'-DHF had an IC_{50} value of 3.2 μM , similar to the previously reported value of 2.0 μM [9], while its methylated analog 7,4'-DMF had an IC_{50} value of 9.0 μM .

The critical finding in this study is that two methylated flavones, 7,4'-DMF and especially 7-MF, were only slightly less potent than 7,4'-DHF and 7-HF, previously shown to be the two most potent flavone inhibitors of aromatase [9]. The importance of this finding lies in the fact that these methylated flavones are highly stable against human hepatic metabolism. In contrast, the unmethylated analogs, like chrysin, are very rapidly metabolized by sulfate and glucuronic acid conjugation [8]. In addition, in a human intestinal transport model, both 7-MF and 7,4'-DMF demonstrated high transport capacity compared to 7-HF and 7,4'-DHF [8]. The high metabolic resistance together with high rate of intestinal absorption would predict the two methylated flavones to be orally bioavailable in humans and thus capable of inhibiting aromatase *in vivo*. Further support for this contention is that 5,7-DMF but not chrysin has high oral bioavailability in rats [12].

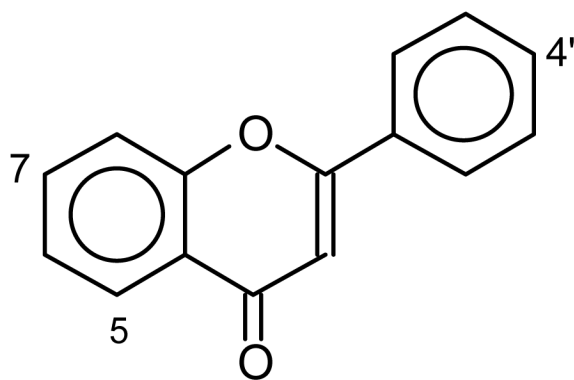
Both 7-MF and 7,4'-DMF used in this study were synthetic compounds which could be used as food supplements or potentially as drugs. However, both are also found in plants. Thus, 7-MF has been found in extracts from Meliaceae and Rutaceae plants [13] and 7,4'-DMF has been identified in fruits and leaves from neotropical nutmeg species [14,15] as well as from propolis [16].

Acknowledgements

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Flavone	Substituent		
	5	7	4'
5,7-DMF	OCH ₃	OCH ₃	H
Chrysin	OH	OH	H
7-MF	H	OCH ₃	H
7-HF	H	OH	H
7,4'-DMF	H	OCH ₃	OCH ₃
7,4'-DHF	H	OH	OH

Fig 1.
Structures of flavones used in this study.

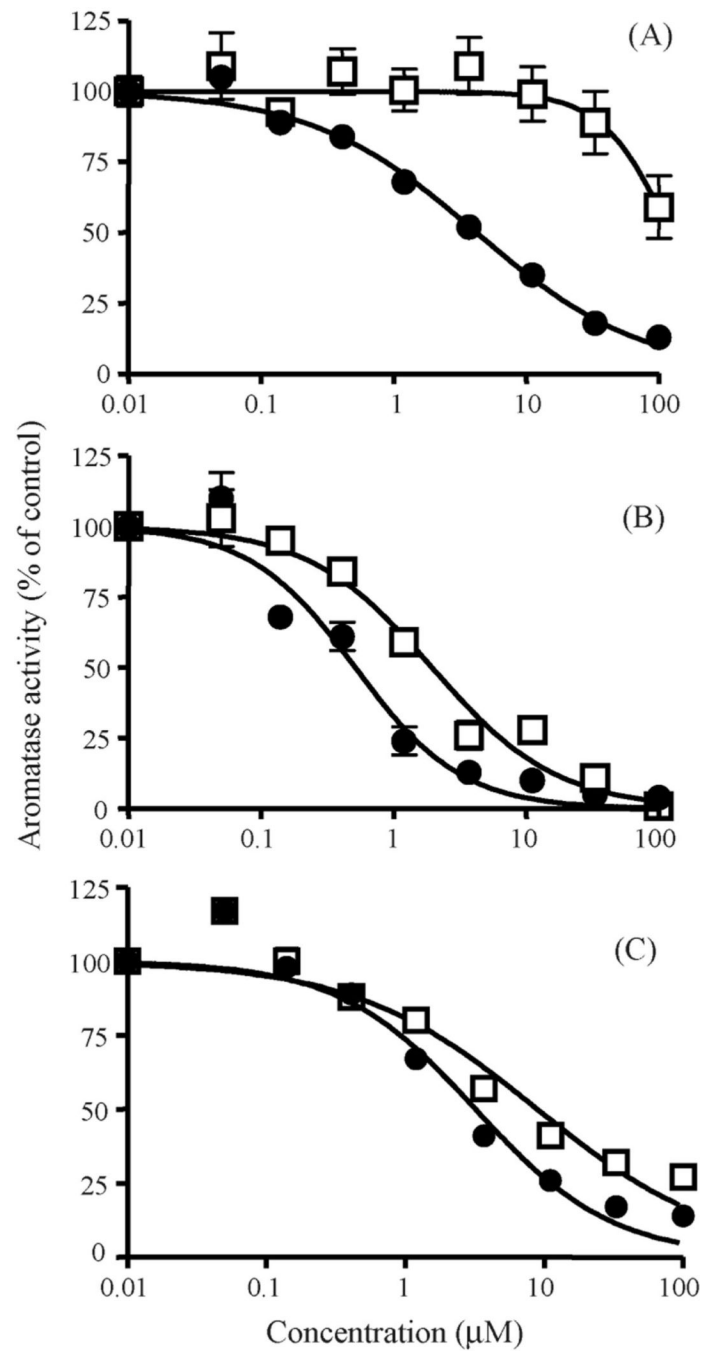


Fig 2. Effect of 5,7-DMF compared to chrysin (A), 7-MF compared to 7-HF (B), and 7,4'-DMF compared to 7,4'-DHF (C) on aromatase activity. □, methylated flavones; ●, unmethylated flavones. The data are expressed as percent of control. Mean values \pm SEM (in many cases smaller than the symbols) are shown ($n = 4 - 10$).