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Methoxylated flavones, a superior cancer chemopreventive flavonoid subclass?

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Abstract

Dietary flavonoids and other polyphenols show great potential as cancer chemopreventive agents in cell culture studies. This does not translate well into in vivo activity, because of extensive conjugative metabolism of these compounds in the intestine and liver. This paper presents a review of a flavonoid subclass in which all hydroxyl groups are capped by methylation. This results in dramatically increased metabolic stability and membrane transport in the intestine/liver, thus improving oral bioavailability. The methoxyflavones also show increased cancer chemopreventive properties. At the cancer initiation stage, bioactivation of polyaromatic hydrocarbon carcinogens and binding to DNA are markedly diminished through effects on CYP1A1/1B1 transcription but also through direct interactions with the proteins. At the cancer promotion stage, the proliferation of cancer cells, but not normal cells, is inhibited with greater potency than with the unmethylated flavones. Limited mechanistic experiments, such as of effects on cell cycle regulation, indicate that the mechanisms of methoxyflavone activities are unique, including aromatase inhibition. The cancer preventive effects and mechanisms of the polymethoxyflavones, such as tangeretin and nobiletin, are discussed in comparison. It is concluded that the methoxyflavones have properties that may make them particularly useful as cancer chemopreventive agents.

Keywords

Methoxyflavones; Flavonoids; Chemoprevention; Cancer prevention; Bioavailability

1. Introduction

Dietary flavonoids and other polyphenolic food components have over many years been suggested to have preventive properties both at the initiation and the promotion stages of chemically-induced carcinogenesis [1-3]. At the cancer initiation stage, mainly in cell culture studies, polyphenols have clearly been shown to affect many of the carcinogen bioactivating steps necessary for the covalent binding of the carcinogen to cellular DNA, including the major bioactivating CYP1A1 enzyme. Whereas some polyphenols have been shown to act as inducers of CYP1A1 by being agonists of the arylhydrocarbon receptor (AhR), others have been shown to be inhibitors by being AhR antagonists [4-6], although recent studies show that this may be a too simplistic view (see below). At the promotion stage, cell culture studies have revealed a wide variety of biochemical mechanisms for the effects of polyphenols on human cancer cells.

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This most recently includes effects on VEGF and HIF-1 expression via PI3K/AKT pathways [7] or via ARNT [8], inactivation of EGFR [9] and inhibition of thioredoxin reductase [10], thymidylate synthase [11] and the MDM2 oncogene [12] as well as effects on cancer cell resistance by targeting the molecular chaperone glucose-regulated protein 78 [13].

However, *in vivo* cancer chemoprevention studies in animals and especially in humans, using modest, clinically tolerable doses of flavonoids or other polyphenols, have been mostly disappointing. This can be explained by the very poor oral bioavailability of the polyphenols, i.e. their inability to pass intact through the dual intestinal/hepatic barrier into the systemic circulation. In humans, this lack of bioavailability after oral doses has been shown directly for chrysin [14], quercetin [14,15], curcumin [16] and resveratrol [17,18]. Although the tea flavonoids [19-21] and the isoflavonoids, such as genistein [21-24], have some oral bioavailability, it is still low.

This review will present recent observations on a subclass of flavonoids, i.e., the methoxylated flavones, which may have chemopreventive properties superior to the more common unmethylated flavonoids or polyphenols. The focus will in first hand be on their oral bioavailability and in second hand on their potential cancer chemopreventive properties at both the initiation and the promotion stages of chemically induced carcinogenesis. Effects of the polymethoxyflavones will be discussed for comparison.

2. Lack of Oral Bioavailability of Polyphenols

The ability of nutritional small molecules as well as drugs to produce a biological effect depends in first hand on their ability to enter the target cell. For example, when bronchial epithelial cells were exposed to two dimethoxylated flavones and resveratrol, within five minutes all accumulated 30- to 50-fold in the cells compared to the surrounding buffer [25]. This situation seems to be similar for most flavonoids and other polyphenols. Thus, these compounds easily penetrate all cultured cells, including those from peripheral organs, such as the heart, lungs, breast, prostate, liver, kidneys and brain. Except for hepatic and intestinal cells, cultured cells usually also have low expression of enzymes capable of metabolizing dietary compounds as well as drugs. That includes enzymes such as the cytochrome P450 (CYP) system, the UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs). Thus, for most cell culture studies with dietary polyphenol aglycones, in contrast to their glycosides, there is no limitation in cellular uptake due to poor membrane penetration or possible efflux transporters and/or metabolic instability.

However, when the polyphenols are administered orally to animals or humans, very little or none of these compounds appears in the systemic circulation. The reason is the very high expression of in particular the UGTs and SULTs in the small intestine and liver, through which all of the oral dose must pass. This results in a very low oral bioavailability. Additionally, most dietary flavonoids and other polyphenols are present in the food as glycosides, further decreasing the bioavailability.

3. Metabolic Resistance of Methoxylated Flavones

As clearly demonstrated for the flavonoid chrysin (5,7-dihydroxyflavone), but also for many other polyphenols, glucuronidation and sulfation, and much less so oxidation, are critical for the very rapid metabolism of this compound in the human intestine and liver [26], resulting in essentially zero bioavailability *in vivo* in humans [14]. However, if the two hydroxyl groups in chrysin are methylated, as in 5,7-dimethoxyflavone (5,7-DMF) (structures, see Fig. 1), the situation is entirely different. Using the human liver 9,000g supernatant (S9 fraction), which contains the microsomal as well as cytosolic enzymes, with the cofactors for glucuronidation (UDPGA), sulfation (PAPS) and oxidation (NADPH), chrysin was rapidly metabolized, with

no parent compound remaining after a 20-min incubation. In contrast, 5,7-DMF was metabolically stable over the whole 60-min time-course studied [27,28]. Quercetin and resveratrol, two common unmethylated dietary polyphenols, as well as many others behaved very similarly to chrysin, i.e., were rapidly metabolized.

In further experiments, the transport of 5,7-DMF compared with chrysin was examined in Caco-2 cells cultured as monolayers on permeable membrane support, a well established human intestinal transport model [29,30]. The transcellular transport of 5,7-DMF was about 10-fold higher than for chrysin [28]. The reason for this difference is likely not due to differences in transport *per se* but rather due to differences in metabolic stability, similar to that seen with the hepatic S9 fraction (see Fig. 4). Similarly, 7-methoxyflavone, 7,4′-dimethoxyflavone and 5,7,4′-trimethoxyflavone were much more metabolically stable and had higher transport rate than the corresponding unmethylated flavones [28].

Based on the combined observations with the human hepatic S9 fraction and the human Caco-2 cell system, it could be predicted that the oral bioavailability would be much greater for the methoxyflavones than for the unmethylated flavones. This was tested directly *in vivo* in the rat, an animal model known to have much greater metabolic capacity than humans, thus, a stringent test [31]. 5,7-DMF and chrysin were co-administered by oral gavage at 5 mg/kg, which is a common dose when chrysin is used as a dietary supplement in humans. Only 5,7-DMF was detectable in the plasma, peaking at 2.3 μM at 1 hr (Fig. 2A). 5,7-DMF was also easily detectable in liver, lung and kidney tissue at quite high concentrations compared to plasma (Fig. **2B**). Chrysin was not detect*ed* in any tissue but started to appear in the fecal pellets in the intestinal lumen after 2 hr (Fig. **2C**). This is likely the result of intestinal absorption, enzymatic conjugation, MRP2-mediated export of the conjugates, enzymatic hydrolysis back to chrysin and fecal excretion, as described previously both *in vitro* [32] and *in vivo* in humans [14].

The oral bioavailability, based on tissue measurements, was also determined for 5,7-DMF and chrysin in a small fish model, the Atlantic killifish [33]. The fishes (about 4-6 g) were exposed to these flavones in the water at a concentration of 5 μM, a low human dietary concentration, for 8 h prior to sacrifice. The 5,7-DMF concentrations greatly exceeded those of chrysin in all tissues, most notably in the brain. The findings are very similar to those in the rat study. It should be noted that the killifish, being a saltwater species, ingests foreign chemicals by swallowing the compounds, i.e. similarly to oral administration in mammals. The very high 5,7-DMF concentration in the brain (5,7-DMF/chrysin tissue concentration ratio 150) is of potential importance for treating tumors within the CNS, in general a difficult task.

To our knowledge, very few studies exist on the oral bioavailability of other methoxyflavones or polymethoxyflavones. One is the citrus flavonoid nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone), which was administered together with the unmethylated luteolin (5,7,3′,4′-tetrahydroxyflavone) to rats at 25 mg/kg by intubation [34]. The nobiletin content in the liver and kidneys ranged from 2 to 6 μM, i.e. somewhat less than for 5,7-DMF, but the luteolin levels were much lower, more similar to chrysin. On the other hand, another polymethoxyflavone, the citrus flavonoid tangeretin (5,6,7,8,4′-pentamethoxyflavone), was administered to hamsters as 1% of their diet for 35 days. There was evidence of considerable intestinal absorption of tangeretin based on the urinary excretion of several metabolites in these animals. However, no unchanged tangeretin was detected in the circulating plasma [35].

These data taken together indicate that the methoxylated flavones have a great advantage over the nonmethylated flavones regarding oral bioavailability. It also appears that the compounds containing only one or two methoxy groups may be more metabolically stable than the polymethoxylated flavones, although more studies in this area are needed.

4. Inhibition of Cancer Initiation

It is well recognized that most types of cancers are due, at least in part, to exposure to environmental chemicals, including tobacco smoke, but also to similar contaminants in our diet [36]. The largest class of such chemicals is the polyaromatic hydrocarbons (PAHs) with benzo[*a*]pyrene (BaP) considered a model compound. The bioactivation of BaP to the form that ultimately binds to DNA to start the carcinogenic process, i.e. cancer initiation, has been well established [36].

It is also clear that BaP potently induces both of the major bioactivating enzymes CYP1A1 and CYP1B1 via activation of the AhR. However, cell context is highly important for determining which of the isoforms is predominantly expressed. Thus, among human cell types after BaP induction, hepatocytes express exclusively CYP1A1 [37], lung epithelial cells mainly CYP1A1 but also some CYP1B1 [25], oral epithelial cells preferentially CYP1B1 [38] and esophageal epithelial cells exclusively CYP1B1 [39].

The effects of flavonoids and other polyphenols on these two bioactivating oxidative enzymes have been studied thoroughly in cell culture models and are highly complex. Some may be AhR agonists, whereas others are antagonists [4-6]. However, it appears that responses very much depend on cell type. With regard to oral bioavailability, most unmethylated polyphenols studied in the past, e.g., quercetin, kaempferol, diosmetin, curcumin and resveratrol, would not be expected to reach internal organs, i.e. beyond sites along the gastrointestinal tract.

The orally bioavailable methoxyflavones have considerably greater potential to reach target tissues to exert biological effects *in vivo*. Their potential to inhibit the bioactivation of BaP *in vitro* has been shown for 3′,4′-DMF [25,38-40] and 5,7-DMF [25,37-39] and may also include additional methoxyflavones [41]. 5,7-DMF appears to be highly effective in inhibiting BaP bioactivation and binding to DNA in cell culture. Thus, after exposure of human hepatoma Hep G2 cells to a low concentration of BaP, the BaP-DNA binding was dramatically induced, second to CYP1A induction [37]. 5,7-DMF dramatically reduced the catalytic activity of CYP1A1 (Fig. 3A) and both CYP1A1 protein (Fig. **3B**) and mRNA (Fig. **3C**) were clearly reduced. In addition, 5,7-DMF was shown to be a highly potent direct inhibitor of recombinant CYP1A1 protein activity (Fig. **3D**). Thus, 5,7-DMF has two modes of action on CYP1A1 at quite low (1-2 μM) concentrations. It is important to note that these 5,7-DMF concentrations can easily be reached in tissues, as shown after oral dosing in the rat (Fig. 3B). Thus, studies of the effectiveness of 5,7-DMF treatment *in vivo* seem to be the next important step.

Similarly, both 5,7-DMF and 3′,4′-DMF were potent inhibitors of BaP-induced CYP1A1 protein expression in human bronchial epithelial cells [25]. As shown in Fig. 3B, oral 5,7-DMF administration in the rat resulted in considerable concentrations in the lungs (6 μM), suggesting potential *in vivo* effectiveness of 5,7-DMF in the chemoprevention also of lung cancer. Thus, studies of 5,7-DMF in animal models are urgently needed.

5,7-DMF appeared to be an inhibitor of CYP1B1 transcription in the human esophageal HET-1A cells [39] but not in the human oral SCC-9 cells [38]. This demonstrates that CYP1B1 regulation is different from that of CYP1A1, at least as far as 5,7-DMF is concerned, but also that cell context is highly important [42,43]. In contrast, the AhR antagonist 3′,4′-DMF [40] was an inhibitor of CYP1B1 both at the mRNA and protein levels in both esophageal and oral cells. From these observations it is clear that more mechanistic studies of the methoxyflavones are needed.

The mechanism(s) by which the methoxyflavones affect the transcription of CYP1A1/1B1 is complex. 3′,4′-DMF has been shown to be an AhR antagonist in human breast cancer cells, directly binding to the receptor [40]. However, whereas 5,7-DMF apparently inhibits CYP1A1

transcription in Hep G2 [37], SCC-9 [38] and BEAS-2B [25], in neither case was there evidence for an effect on AhR nuclear translocation (unpublished). The mechanism of the 5,7-DMF effect on CYP1A1 transcription thus remains to be elucidated. It may include effects on regulators of AhR, such as hsp90, as shown for the tea flavonoid EGCG [44], or ARNT, as shown for the yellow spice curcumin [8] or maybe the stability of the CYP1A1 mRNA [45].

5. Inhibition of Cancer Promotion

Central to protective effects by dietary flavonoids and other polyphenols at the promotion stage of chemically-induced carcinogenesis is the ability to inhibit cell proliferation. The damage that the carcinogens have inflicted on cellular DNA during the initiation stage is being propagated into a new cell population. This machinery, i.e. clonal expansion, is highly complex, geared towards giving the cells immortality by stimulating mitogenesis and/or decreasing cell death by inhibiting apoptosis. Protective effects at this stage are critically important. This has been demonstrated in cell culture with unmethylated flavonoids and other polyphenols, as discussed briefly in the Introduction, affecting numerous signal transduction pathways.

Some of the polymethoxylated citrus flavonoids have also in preliminary studies demonstrated antiproliferative properties [46,47] (see Section 6.). However, the effect of methylation of the flavonoids on their antiproliferative effects has not been clarified. This is in particular true for the smaller methoxylated flavones. Many studies in the past have assumed that the free hydroxyl groups of the flavonoids and other polyphenols are necessary for biological effects. The long held belief that their antioxidant properties were responsible for the antiproliferative effects has now been found not to be true [48].

The antiproliferative effects of methoxylated versus hydroxylated flavones were directly compared in SCC-9 human oral squamous carcinoma cells. The effects of treatment with 5,7,4′-trimethoxyflavone (5,7,4′-TMF) versus 5,7,4′-trihydroxyflavone (apigenin), one of the most thoroughly studied unmethylated flavones, are shown in Fig. 4. 5,7,4′-TMF was about 8 times more potent than apigenin with an IC_{50} value of 5 μM. The IC_{50} value of 40 μM for apigenin agrees with most previous studies of this flavone in various human cancer cell lines [49-51]. Very similar results as with 5,7,4′-TMF were obtained for 5,7-dimethoxyflavone (5,7-DMF) compared to its unmethylated analog 5,7-dihydroxyflavone (chrysin) [31]. The greater potency of the two methoxylated versus the two hydroxylated flavones could conceivably be due to greater cell uptake of the methoxylated flavones. However, after incubation of SCC-9 cells for up to 24 hr with 25 μM 5,7-DMF or chrysin, the uptake was rapid and virtually identical for the two compounds [31].

A small number of additional methoxylated flavones have been investigated for antiproliferative effects in the SCC-9 cells [31]. The calculated IC_{50} values were 36.5 μM (7-MF), 24.2 μM (7,4′-DMF), and 19.3 μM (tangeretin). In addition, 5,4′-DMF, 5,3′-DMF and 7,8-DMF showed weaker effects. In MCF-7 human breast cancer cells treated with a small number of hydroxy- and methoxy-substituted flavonoids, the 5-, 7-, and 5,7-methoxyflavanones, i.e., without the C-ring double bond, showed growth inhibitory effects with IC_{50} values around 35 μM, as measured by the MTT assay [52]. 5-Methoxyflavone was almost as potent as the flavanone and 7-methoxyflavone was more potent than its hydroxyl analog.

To determine whether the growth inhibitory effect was accompanied by cell cycle arrest, SCC-9 cells were treated with two pairs of flavones for 72 h. These experiments showed that apigenin caused a distinct increase in the G2/M phase population, as has previously been shown in several studies [50,51]. In contrast, 5,7,4′-TMF caused a dose-dependent increase in the G1 phase, significant already at 5 μM, with a concomitant decrease in the S phase and no change in the G2/M population [31]. Identical results were obtained for 5,7-DMF compared to its

unmethylated analog chrysin. The results seen by flow cytometry were thus very similar to those seen in the cell proliferation assay.

To determine if the potent antiproliferative effects observed by the methoxylated flavones on the SCC-9 cells were selective for cancer vs. noncancer cells, the effects of 5,7-DMF and chrysin in two additional cancer cell lines were compared with those in two noncancer cell lines. In the FaDu human larynx SCC cells, both 5,7-DMF and chrysin showed similar potency as 5,7-DMF in the SCC-9 cells (IC_{50} 8-10 μM). In the MCF-7 human breast cancer cells, both compounds again had similar but slightly lower potency with IC_{50} values of 10-20 μM. In contrast, two normal but transformed human cell lines, i.e. the HET-1A esophageal cells [53] and the BEAS-2B bronchial epithelial cells [54], were much less sensitive to both 5,7-DMF and chrysin with IC_{50} values > 100 μM.

The mechanism of the potent antiproliferative effects of 5,7,4′-TMF and 5,7-DMF compared to their unmethylated analogs has not yet been addressed. However, recent thinking [55,56] suggests that the effect of these methoxyflavones on the AhR-mediated expression, in particular of CYP1A1, may have a direct effect on cell cycle regulation. This is too early to conclude, but may provide a novel set of mechanisms to pursue, connecting cancer initiation with promotion, including the protective effects of the methoxyflavones.

A specific cancer protective mechanism against hormone-sensitive cancers, which has received much interest lately, is through downregulation of estrogen concentrations. Thus, two methoxylated flavones, 7-methoxyflavone and 7,4′-dimethoxyflavone, have very recently been shown to be potent inhibitors of aromatase, the enzyme responsible for converting testosterone to estradiol (IC_{50} values of 2-9 μM) [57]. Some flavonoids, notably chrysin, have been shown to be potent aromatase inhibitors *in vitro* [58]. However, due to lack of bioavailability [14, 27,28], their claims of therapeutic efficacy have never been substantiated. With the methoxylated flavones, this clinical application may be realistic.

6. Effects of Polymethoxylated Flavones

In SCC-9 oral cancer cells the citrus polymethoxyflavone tangeretin had an antiproliferative IC_{50} value of about 20 μM [31]. This is lower than in some other studies of this flavone in a colorectal carcinoma cell line (37 μM) [59] as well as in lung and breast carcinoma cell lines (≈ 100 μM) [60] [[61]. A very recent study reported IC_{50} values of 30-40 μM for breast and colon cell lines with slightly higher values for nobiletin [62]. However, two additional studies of tangeretin in a variety of human cancer cell lines and in promyelocytic leukemia cells reported much more potent effects [47,63]. These highly variable results may be related to both cell type, length of exposure and methodology for assessing effects.

With the citrus polymethoxylated flavones, as with most flavonoid or polyphenol potential cancer preventive agents, many different mechanisms for their effects have been suggested. G1 cell cycle arrest was induced by tangeretin in human colorectal carcinoma cells [59] and by tangeretin and nobiletin in breast and colon cancer cells [62], just as 5,7,4′-TMF in the oral cells [41]. This was shown to depend on modulation of the activities of several key G1 regulatory proteins, such as Cdk2 and Cdk4, and/or the Cdk inhibitors p21 and p27 [59]. In extended studies, tangeretin was shown to have anti-inflammatory properties in lung epithelial carcinoma cells [60]. Thus, pretreatment of cells with tangeretin inhibited IL-1β-induced p38 MAPK, JNK and AKT phosphorylation and the downstream activation of NF-κB, leading to COX-2 downregulation. Interestingly, while ERK was not affected in this study, a study in estrogen-treated mammary ductal carcinoma cells demonstrated inhibition of ERK phosphorylation by tangeretin [61]. COX-2 inhibition by nobiletin was seen in murine macrophages [64]. The same study showed significant inhibition of dimethylbenz[*a*] anthracene-induced skin cancer by topically applied nobiletin. Finally, in another recent study,

nobiletin inhibited the growth of several prostate cancer cell lines with IC_{50} values around 100 μM and significantly increased G_0/G_1 phase arrest. Most interesting, nobiletin administered in the feed for 15 weeks inhibited developing adenocarcinomas of the prostate in a transgenic rat model. This may be the first study indicating *in vivo* anticancer activity of an orally administered polymethoxyflavone [65].

Several groups have tested nobiletin as an anti-metastatic agent. As overexpression of matrix metalloproteinases (MMPs) is associated with cancer metastasis, this was one target protein. MMP-1 and -9 expression was suppressed by nobiletin in fibrosarcoma cells with associated increase in tissue inhibitors of MMPs [66]. MMP-7 was downregulated in colorectal cells [67] and proMMP-9 activity was inhibited in gastric cell lines [68]. The latter study also showed significantly decreased peritoneal dissemination of stomach cancer nodules, when nobiletin was administered subcutaneously to mice through an osmotic minipump.

7. Where Can the Methoxylated Flavones Be Found?

The polymethoxyflavones, including tangeretin, sinensetin and nobiletin, which have been known for a long time, can be found in high concentrations in the peel of various *Citrus* species whereas the many hydroxylated flavones [69,70] clearly predominate in the juice. These polymethoxyflavones contain from 5 to 7 methoxy groups. The various *Citrus* species show very high variability in their content of these polymethoxyflavones.

The smaller methoxyflavones, with one to three methoxy groups and without any hydroxyl groups, have been much less studied. The reasons are probably two: first, the flavonoids, have been thought to owe their biological activities to the antioxidant properties conferred by free hydroxyl groups. Second, these methoxyflavones are present in plants that are less utilized commercially for human consumption compared to the polymethoxyflavones. For example, 5,7,4′-TMF, although present in a *Citrus* species [71], also is present in other plants used in folk medicine [72,73]. 7,4′-DMF has been identified in fruits and leaves from neotropical nutmeg species [74,75] as well as from propolis [76]. 5,7-DMF is highly abundant in pepper tree leaves [77]. While none of the methoxylated flavones examined in this study are abundant in the common human diet, the mounting evidence of protective properties of these flavones may lead to increased use of their natural sources.

However, a most important aspect of the small methoxylated flavones is their availability in synthetic form. These molecules have apparently been made as building blocks in organic synthesis of potential novel anticancer drugs. Thus, further future studies of methoxyflavones should therefore be greatly facilitated.

8. Future Studies

A very recent epidemiological study provides strong support for the methoxylated flavones as potential cancer chemopreventive dietary agents. This prospective study in 42,311 men in the Health Professionals Follow-up Study [78] convincingly showed that histologically diagnosed oral premalignant lesions were suppressed by consumption of citrus fruits and citrus fruit juices (a 30 to 40% lower risk), whereas, surprisingly, vegetables provided no protection. This is the first epidemiological study providing evidence of protective effects of a subclass of dietary fruits and vegetables. What first comes to mind from these findings is the presence of high concentrations of methoxylated flavones in the essential oils of citrus fruit peel. This includes compounds such as sinensetin, nobiletin, tangeretin and heptamethoxyflavone [69,70] but most likely many others, including the simpler methoxyflavones similar to those described in this chapter. This should provide a particular incentive for further studies of these dietary chemicals.

There are two general approaches to the use of flavonoids or other polyphenols as cancer chemopreventive agents. One is to choose diets rich in such compounds, including verification of their possible effectiveness followed by attempts to deduce potential mechanisms. Examples of this whole food approach include herbal teas [21], soy incorporation in the diet [24] as well as a berry approach [79]. The other more common and simplest approach is to identify dietary components with potential cancer chemopreventive properties through testing of individual compounds first *in vitro* and then *in vivo*. The ultimate goal here is to arrive at food supplements, which may serve to reinforce the dietary contribution. The new methoxyflavones may fit into either or both of these categories.

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Abbreviations

AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; BaP, benzo[*a*]pyrene; Cdk, cyclin-dependent kinase; COX-2, cyclooxygenase-2; CYP, cytochrome P450; DMF, dimethoxyflavone; EGFR, epidermal growth factor receptor; EGCG, epigallocatechin gallate; HIF-1, hypoxia-inducible factor 1; hsp90, heat shock protein 90; IL, interleukin; MAPK, mitogen-activated protein kinase; MDM2, murine double minute-2; MF, methoxyflavone; MMP, metalloproteinase; MRP, multidrug resistance-associated protein; NF-κB, nuclear factor-κB; PAH, polyaromatic hydrocarbon; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PI3K/AKT, phosphatidylinositol 3-kinase/AKT; S9, 9 000 *g* supernatant; SULT, sulfotransferase; TMF, trimethoxyflavone; UDPGA, uridine 5′-diphosphoglucuronic acid; UGT, UDP-glucuronosyltransferase; VEGF, vascular endothelial growth factor A.

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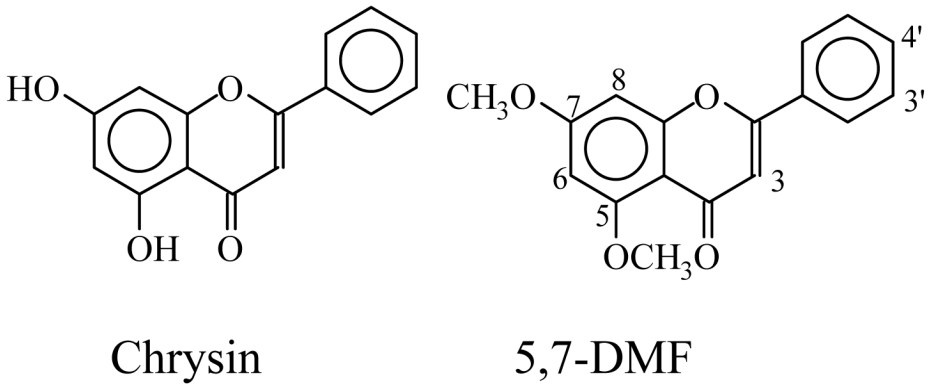

Fig 1.
Structures of 5,7-dihydroxyflavone (chrysin) and 5,7-dimethoxyflavone (5,7-DMF).

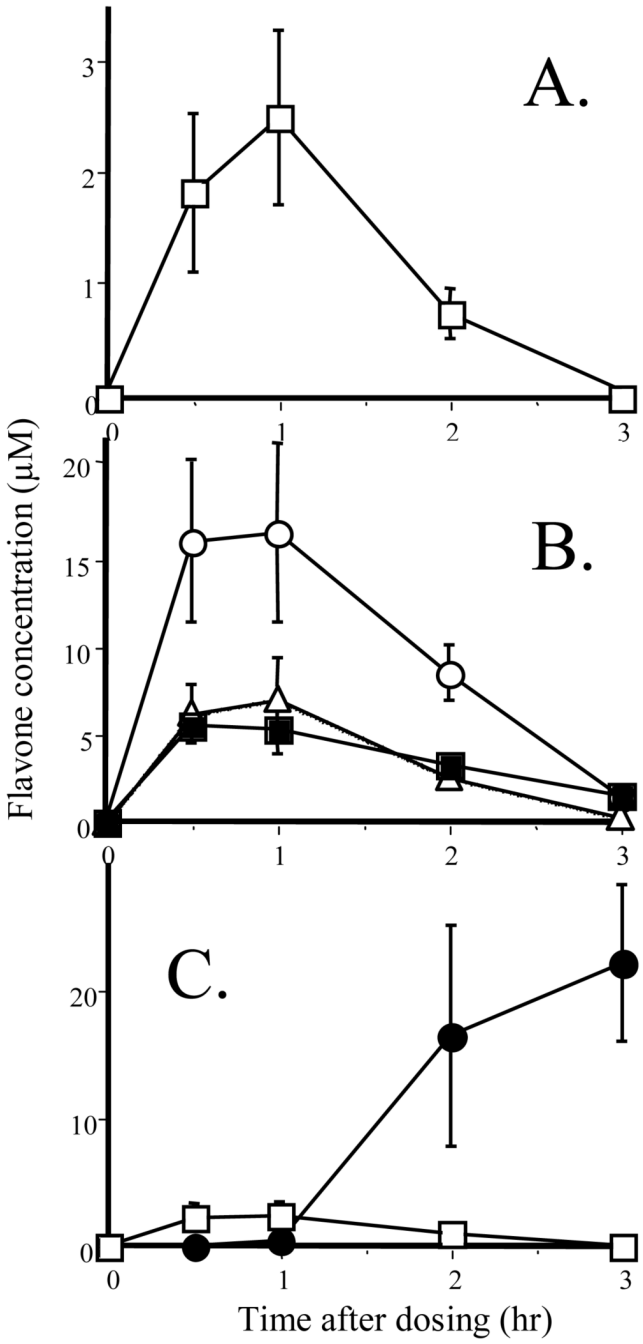

Fig 2.

Plasma and tissue levels of 5,7-DMF and chrysin after oral administration of 5 mg/kg in rats; (A) Plasma 5,7-DMF (no chrysin could be detected at any time-point); (B) 5,7-DMF in post-absorption tissues, liver (○), lung (■) and kidney (Δ); (C) 5,7-DMF (□) and chrysin (●) in the proximal 2 cm of the colon with the associated fecal pellet. The data represent the mean ± SEM of 5 animals at each time-point. From [31].

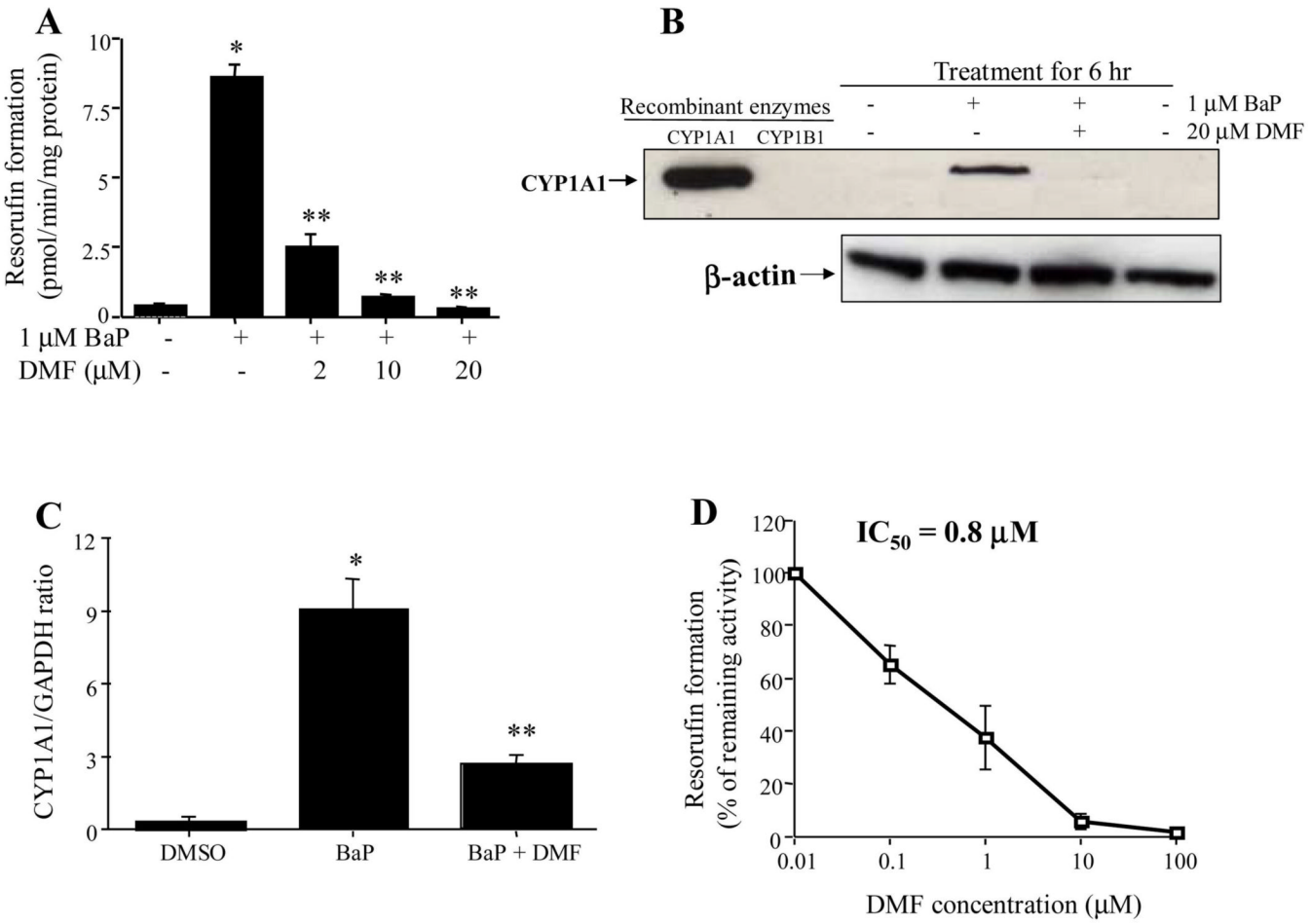

Fig 3.

Effects of 5,7-DMF on BaP-induced CYP1A1 activity (A), CYP1A1 protein expression (B) and CYP1A1 mRNA level (C) in the Hep G2 cells and on recombinant CYP1A1 activity (D). A-C: Hep G2 cells were exposed for 6 hr to different concentrations of 5,7-DMF in the presence or absence of 1 μM BaP. D: Recombinant CYP1A1 was incubated with various concentrations of 5,7-DMF [37].

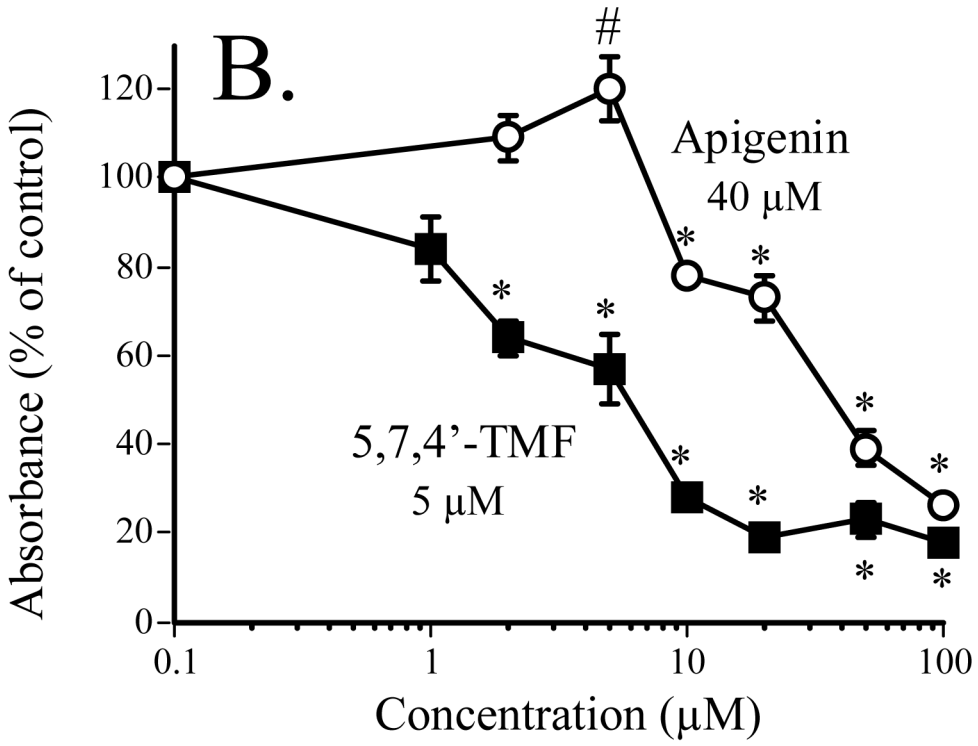

Fig 4.

Effect of 5,7,4′-TMF compared to the unmethylated analog apigenin on SCC-9 cell proliferation. Cell proliferation, expressed as percent of control (DMSO-treatment), was measured as BrdU incorporation into cellular DNA after a 24-h exposure of the cells to the flavones [31]. The numbers shown in the figure are the calculated IC_{50} values. From [31]. * significantly lower than control, $P < 0.05$. # significantly higher than control, $P < 0.05$.