Recent Advances in Anti-inflammatory Synthetic Flavonoids as Potential Drugs

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Abstracts – Flavonoids are well-known anti-inflammatory agents that exert their effects via a variety of mechanisms including antioxidative action, inhibition of eicosanoid metabolizing enzymes and regulation of the expression of proinflammatory molecules. In this review, synthetic approaches to obtain more useful flavonoid derivatives are summarized. Human clinical trials of flavonoid therapy are discussed. Through continual investigation to identify more potent and comparable flavonoids, new anti-inflammatory flavonoid therapy will be successfully launched, especially for the treatment of chronic inflammatory disorders.
Keywords – Synthetic flavonoid, Anti-inflammation, Clinical trial

Introduction

Flavonoids are natural plant constituents that have various biological and pharmacological activities (Havsteen, 1983). The flavonoid family includes chalcone, flavan-3-ol, flavanone, flavone, flavonol and various biflavonoids (Fig. 1). Some of these compounds exert anti-inflammatory activity in vitro and in vivo (Gabor, 1986; Middleton *et al.*, 2000). Generally, flavonoids have multiple cellular mechanisms of action that lead to anti-inflammation. In addition to their well-known anti-oxidative action, some flavonoids exert inhibitory activity against eicosanoid and nitric oxide (NO) metabolism. For example, some compounds inhibit phospholipase A_2s (PLA_2), cyclooxygenases (COX), lipoxygenases (LOX) and nitric oxide synthases (NOS). Some flavonoids also inhibit other proinflammatory enzymes such as matrix metalloproteinases (MMP). Furthermore, certain flavonoids, especially flavones, down-regulate the expression of many proinflammatory molecules. Specifically, these flavonoids inhibit the expression of the inducible forms of COX and NOS, interleukins, TNF-α and adhesion molecules in inflammatory cells and tissues. Many flavonoids and biflavonoids affect multiple points in the aforementioned pathways, which suggest that they are anti-inflammatory agents with multiple action mechanisms (Kim *et al.*, 2004a). The typical natural flavonoids that have been isolated from plant extracts include catechins, wogonin, quercetin, kaempferol and myricetin.

Many flavonoid derivatives were examined for their anti-inflammatory activity in a variety of animal models of inflammation. Some derivatives showed strong activity, while other derivatives showed only weak activity. However, it seems to be clear that flavonoids, in general, do not show activity high enough for a clinical trial when administered orally. The main problem may be the bioavailability. As many researchers found, most flavonoids show low bioavailability and rapid metabolism to inactive compounds when administered orally. To overcome this situation and to examine the drug potential, many varieties of flavonoids have been synthesized and their pharmacological activities were evaluated.

Development of anti-inflammatory agents from synthetic flavonoid libraries

The synthetic flavonoids with various pharmacological activities – Although there have been many trials conducted to synthesize the flavonoid libraries, this is not a comprehensive review covering all published work. Rather, selected papers showing significant progress were summarized here.

Many flavonoid derivatives have been synthesized for anti-inflammatory purposes as well as for other uses such as antiviral activity, antiplatelet aggregation and anticancer use. For instance, the effects of various synthetic and natural flavonoids on sialidase inhibitory activity were examined. The derivatives, mostly C-5, -6, -7 and -8-substituted flavones with hydroxyl, methoxyl, acetyl, benzyl, prenyl or glucose, more or less inhibited sialidase. The most active one was 4',5,7-trihydroxy-8-glucurony-

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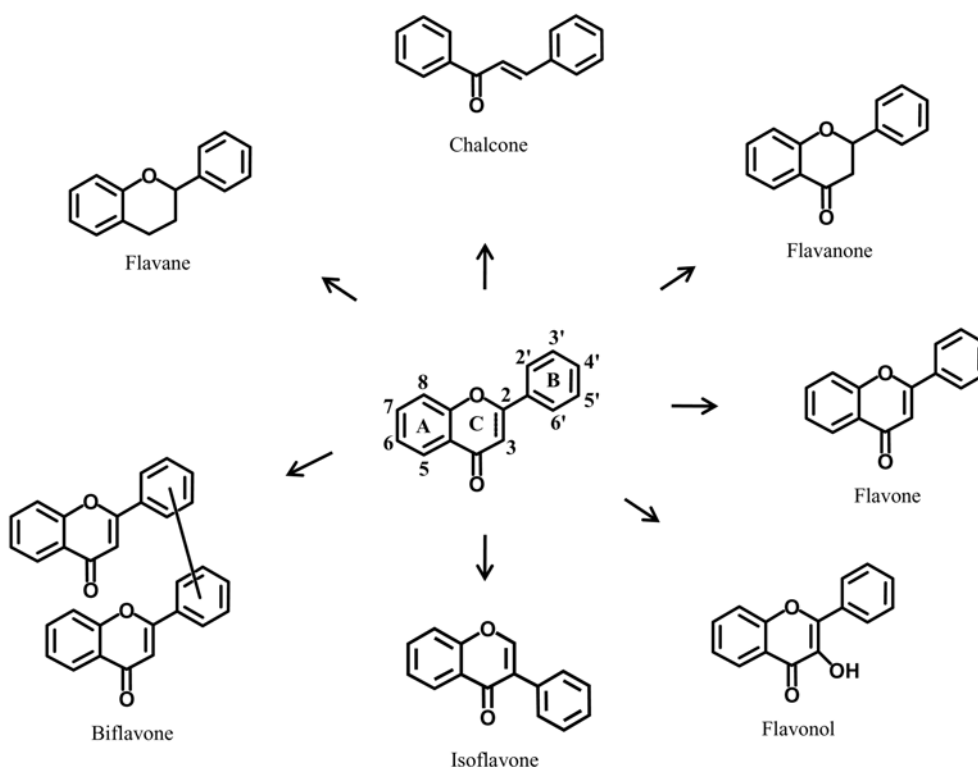 Tel: +82-33-250-6915; E-mail: hpkim@kangwon.ac.kr

Fig. 1. Classes of naturally-occurring flavonoids.

lflavone, suggesting its potential use for antiviral agent (Nagai *et al.*, 1989). In addition, many flavans and isoflavans having CN or amidino substituent (F1) (Fig. 2) were synthesized and their antirhinovirus activity was evaluated (Conti *et al.*, 1990).

It should be noted that the lipophilic flavonoid, 3-[2(E)-phytyl mercaptylmethylene-4'-fluoro-5,7-dihydroxyflavone] (F2), was synthesized and found to possess 15-times higher antioxidative activity than vitamin E (Beck *et al.*, 1990). Additionally, di-*tert*-butylhydroxylated flavones (F3) were prepared and found to have strong antioxidative activity (Lebeau *et al.*, 2000). Recently, 3-alkyl-3',4',5,7-tetrahydroxyflavones were prepared and their inhibitory action against oxidative damage to low density lipoprotein (LDL), RBCs and keratinocytes were compared (Filipe *et al.*, 2009). The activities varied depending on the length of the alkyl chain, and some of these compounds strongly protected against toxic oxidative effects in human keratinocytes. These compounds may be used for topical anti-inflammatory agents in dermatological drugs, as well as for cosmetic preparations.

It is well-known that some flavonoids show antiplatelet action, probably by inhibiting COX and/or cAMP-phosphodiesterase (PDE). Ertan *et al.* (1991) synthesized the interesting derivatives of flavone. They prepared 20 benzodioxane derivatives of flavone including N-[3-diethylamino]-1-propyl]-2,3-dihydro-6-(4-oxo-4H-1-benzopyran-2-yl)-1,4-benzodioxin-2-carboxamide (F4). Some of these derivatives potently inhibited platelet aggregation, with the ethoxycarbonyl derivative of 4',5'-benzodioxane having the most potent activity. Specifically, its IC_{50} values for human platelet aggregation induced by ADP, collagen and thrombin were 1, 2 and 24 μM, respectively. Furthermore, several 2'-*O*-aminopropanol flavone derivatives (F5) were synthesized, and these compounds also showed antiplatelet effects (Chung *et al.*, 2001).

There have been several synthetic approaches to identify anticancer flavonoids since some flavonoids are known to potently inhibit protein kinase C (PKC) or protein tyrosine kinase (PTK). In this respect, the new synthetic procedures for various flavones were established and the derivatives such as 5,7-dihydroxy or 5,7-dimethoxy-3'-nitro-4'-chloroflavones (F6) were prepared. Some of these compounds showed cytotoxic effects and PTK inhibitory activity as expected (Cunningham *et al.*, 1992). Generally, flavonoids having nitrogen-containing side chain are known to possess the increased anticancer activity. For example, flavopiridol [(cis)-5,7-dihydroxyphenyl-8-[4-(3-hydroxy-1-methyl)piperidinyl]-4H-1-benzopyran-4-one hydrochloride hemihydrate] (L868276) having C-8-

cyclic ring of nitrogen atom (F7) showed anticancer activity and cyclin-dependent kinase (CDK) inhibitory action (Losiewicz *et al.*, 1994). This compound has been carried out on human clinical trial (Phase 1 and 2). In addition, C-8 nitrogen-containing flavonoids were recently synthesized and they were found to be CDK1/cyclin B inhibitory (Liu *et al.*, 2007). Specifically, flavopiridol-mimicking chalcones and flavones having C-8 nitrogen-containing molecules such as dimethylamino and piperidinyl group (F8) showed potent inhibitory activities on CDK1/cyclin B, suggesting they may have anticancer activities. Moreover, B-ring substituted 5,6,7-trimethoxy-flavones (F9) were synthesized and some of them showed anticancer activity in vitro (Liao and Hu, 2004). In particular, 5,6,7-trimethoxy-4'-aminoflavone potently inhibited Hep G2 cell growth. Recently, 5-hydroxy-3',6,7,8-tetramethoxy-3'-aminoflavones/flavonols were prepared, some of which, including 3-chloro-3'-amino-4',6,7,8-
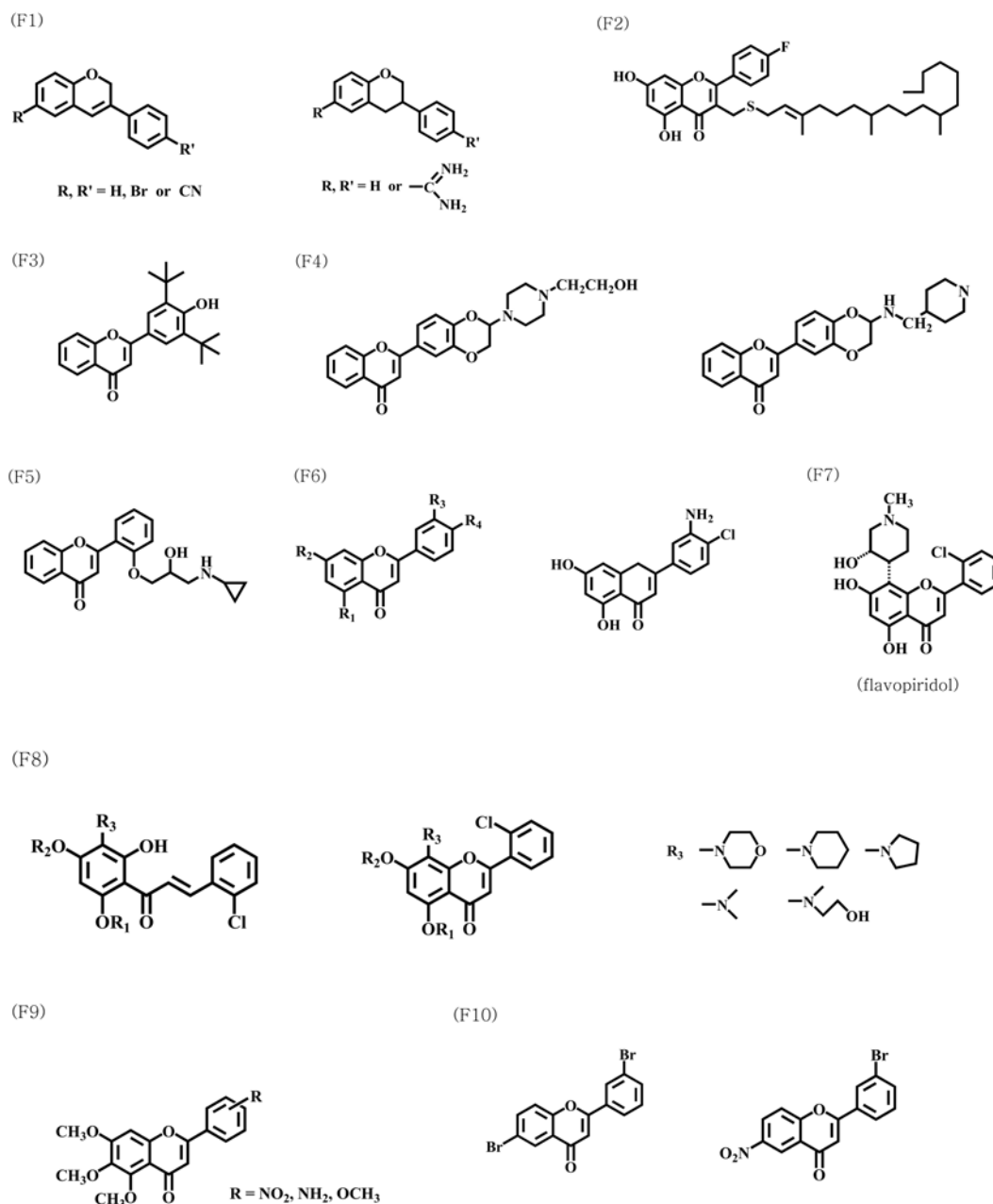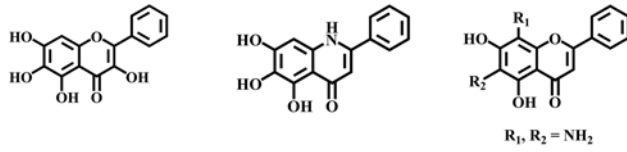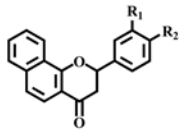

Fig. 2. The representative synthetic flavonoid derivatives mentioned in this study.

(F11)

R₁, R₂ = NH₂

(F12)

(F13)
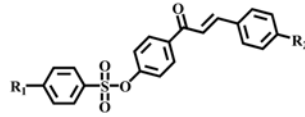
(F14)
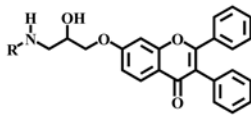
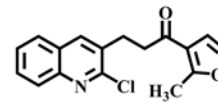

(F15)
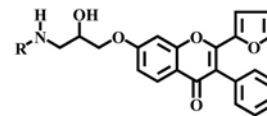
(F16)

(F17)

(F18)

(F19)

(F20)

(F21)

S-wogonin   TMF   DCMF
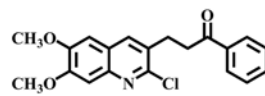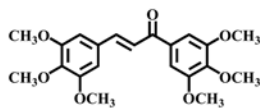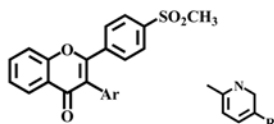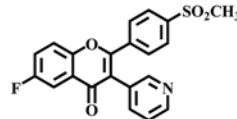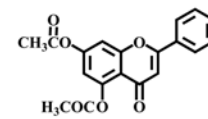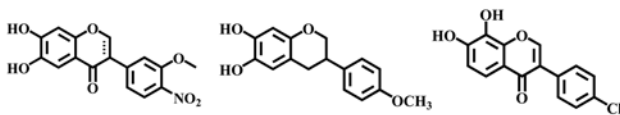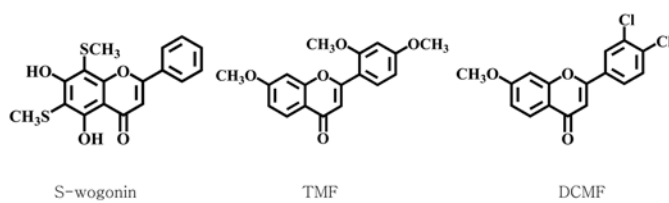
Fig. 2. continued

tetramethoxy-5-hydroxyflavone showed cytotoxic effects (Quintin et al., 2009).

It is also known that some flavonoids possess tranquilizing activity. In this respect, many halogen- and nitro-substituted flavone derivatives were synthesized and their anxiolytic activity was evaluated. Among the derivatives, 6,3'-dibromo- and 6-nitro-3'-bromoflavones (F10) showed potent activity (Viola et al., 2000). The synthetic derivatives having other biological activities were also demonstrated. 6-Hydroxyflavone derivatives and N-containing flavonoids (F11) were synthesized and found to have α-glucosidase inhibitory (Gao and Kawabata, 2005). Moreover, persulfated flavonoids were synthesized and some of them inhibited factor Xa (Gunnarsson et al., 2005). Interestingly, 3',4'-substituted-7,8-benzoflavanones and 3,4-substituted-2',3'-benzochalcones (F12) were synthesized and benzoflavanones were found to be potent aromatase inhibitors (Yahiaoui et al., 2008). In addition, the sulfonated chalcones (F13) were prepared and they were found to comprise a new class of voltage-dependent K^+ channel blocker (Yarishkin et al., 2008).

As summarized above, various flavonoid derivatives were prepared and their biological activities were evaluated. However, only a few compounds are being evaluated in clinical trials for anticancer drugs such as flavopiridol.

Anti-inflammatory synthetic flavonoids – Many efforts have also been carried out to develop anti-inflammatory flavonoids. For example, 3',4'-dihydroxy-C-5,6,7- and C-5,7,8-substituted flavones with alkoxy groups at C-5 or C-6 were initially prepared and their inhibitory activities against 5-LOX were evaluated. The results of these studies revealed that 5- or 6-substitution of these compounds with an alkoxy group having 5 – 10 carbons increased the 5-LOX inhibitory activity (Horie et al., 1986). The derivatives were relatively specific for 5-LOX, and they did not inhibit COX. Therefore, these compounds are potential antiallergic agents. Some mono-hydroxy- and monomethoxyflavones at C-5, 6 or C-7 were also synthesized (Hirano et al., 1989), and these compounds were generally capable of inhibiting T-cell-dependent lymphocyte proliferation.

3-Phenylflavone derivatives (F14) were synthesized and found to have antihypertensive activity (Wu et al., 1987). Based on these initial findings, some 2-alkyl-7-O-substituted isoflavones were synthesized, but their anti-inflammatory activities were not strong in rat CGN-induced paw edema (Wu et al., 1992). Nonetheless, it is worth to mention that among the derivatives, 2-furyl-7-O-aminoalkylisoflavone (F15) has a unique structure.

Synthetic flavonoids having cyclic substituents with hetero-atom may possess interesting biological activity. However, in this case, this compound only showed weak anti-inflammatory activity. Similarly, several 7-O-alkylated biochanin A (isoflavone) derivatives were prepared and their anti-inflammatory activities were examined by mouse ear edema assay, but their activities were not strong (Lee et al., 1994).

Chalcones also possess anti-inflammatory activity. Various hydroxy- and methoxy-substituted chalcones were synthesized and several derivatives containing 2',5'-dihydroxyl group showed COX inhibitory activity (Lin et al., 1997). Some novel chalcones were also found to possess anti-inflammatory activity. For instance, synthetic chalcones and their congeners, including 3,4,5,3',4',5'-hexamethoxychalcone (F16), down-regulated iNOS and COX-2 expression at micromolar concentrations (Herencia et al., 1999).

Flavonoid-like molecules were also synthesized and their activities were examined. Interestingly, 2,3-diaryl-benzopyrene derivatives were prepared. Flavonoids with 4'-methylsulfonyl and 2-phenyl substitutions (F17) were found to have potent COX-2 inhibitory activity (Joo et al., 2003). Among these compounds, 6-fluoro derivative (F18) showed potent anti-inflammatory activity and antiarthritic activity against λ-carrageenan (CGN)-induced paw edema and adjuvant-induced arthritis (AIA) in rats. Among chrysin derivatives synthesized, 5,7-diacetylflavone (F19) inhibited COX-2 (Cho et al., 2004). Additionally, Huang et al. (2005) synthesized the B-ring halogenated analogues of baicalein and found that compounds with 2'- or 4'-chloro substituents had higher NO inhibitory activity in RAW 264.7 cells. Recently, many flavonoid derivatives were prepared and their inhibitory activities against 12- and 15-LOXs were evaluated (Vasquez-Martinez et al., 2007). The groups of 3',4'-substituted-6,7-dihydroxyiso-flavones, -isoflavanones and 3',4'-substituted-7,8-dihydroxyiso-flavones (F20) inhibited human 12-LOX and 15-LOX.

To identify the optimum structures of anti-inflammatory flavonoid molecules, our group synthesized various types of flavones containing hydroxyl, methoxyl, halogen and nitro substituents. Some of these compounds have been found to have the capacity to inhibit proinflammatory enzymes and regulate the proinflammatory expression of molecules such as PLA_2, COX-2 and iNOS (Dao et al., 2003a and 2003b; Dao et al., 2004; Jang et al., 2005; Park et al., 2005). Particularly, some derivatives having 5,7-dihydroxyl groups with 6,8-substituted moieties or 3',4'-dichloro substitution showed potent inhibitory activity against PGE_2 production from LPS-

treated macrophages. Their potencies were comparable with that of wogonin, which is the most potent natural flavone. The examples are 5,7-dihydroxy-6,8-dimethyl-sulfurylflavone (S-wogonin), 2',4',7-trimethoxyflavone (TMF) and 3',4'-dichloro-7-methoxyflavone (DCMF) (F21). From the cellular mechanism study, TMF was revealed as a PLA_2 inhibitor, inhibiting arachidonate release from lipopolysaccharide (LPS)-treated RAW 264.7 cells. IC_{50} value for PGE_2 inhibition was 0.6 µM. This compound neither inhibits COX-2 nor suppresses COX-2 induction (Han *et al.*, 2005).

The inhibitory activities of various synthetic flavone derivatives against iNOS-catalyzed NO production were examined using the LPS-treated macrophages, RAW 264.7. The results of these studies revealed that 3',4',5,7-hydroxyl groups were found to be favorable as in luteolin (Kim *et al.*, 2004b). Moreover, luteolin and wogonin strongly inhibited iNOS-mediated NO production, mainly via the down-regulation of iNOS. In addition, 41 chalcone derivatives were synthesized and their iNOS inhibitory activities were evaluated in LPS-treated RAW 264.7 cells. Among these derivatives, 2'-methoxy-3,4-dichlorochalcone and 2'-hydroxy-3-bromo-6'-methoxychalcone were

found to inhibit iNOS-mediated NO production at 1 - 10 µM, and these effects occurred partly via the suppression of iNOS induction. Further evaluation of the mechanism of these chalcone derivatives revealed that they down-regulated iNOS primarily by inhibiting nuclear factor-κB (NF-κB) activation in LPS-treated RAW cells (Kim *et al.*, 2007).

Anti-inflammatory flavonoids: Human clinical trials

Varieties of natural and synthetic flavonoid derivatives were evaluated for their anti-inflammatory activity in vitro and in vivo. There have been some pharmacokinetic studies of flavonoid ingestion as a dietary food or supplement. Although many clinical trials have been carried out for anti-cancer flavonoid therapy, only a few clinical investigations have been carried out for anti-inflammatory flavonoid drugs. Table 1 summarizes the clinical effects of pure flavonoid compounds that have been demonstrated to date, but only a few pharmaco-kinetic studies with mixture form of flavonoids are included here.

Table 1. Human clinical trials of flavonoids

Compounds	Dose	Results	References
Quercetin	100 mg (i.v.), 4000 mg (p.o.)	Pharmacokinetic study (low bioavailability by p.o.)	Gugler *et al.* (1975)
Quercetin	60 - 1700 mg/m^2 (i.v.)	Phase I trial (renal toxicity), lymphocyte tyrosine kinase inhibited	Ferry *et al.* (1996)
Quercetin ingestion		No effect on heart disease	Conquer *et al.* (1998)
Quercetin, naringenin, hesperetin diet		Lowered asthma incidence	Knekt *et al.* (2002)
High quercetin diet		No effect on COX-2 expression of lymphocytes	de Pascual-Teresa *et al.* (2004)
Genistein	30 - 300 mg	Phase I trial (safe)	Ullmann *et al.* (2005)
Scutellarin	60 mg	Pharmacokinetic study	Chen *et al.* (2006)
Tea catechin	7.4 mg × 3/day (7 days) (inhalation)	Reduced MRSA in sputum (no side effect)	Yamada *et al.* (2006)
Quercetin	500 mg × 3/day	Pharmacokinetic study	Moon *et al.* (2008)
Quercetin	365 × 2 mg/day (28 days)	Lowering blood pressure in hypertensive subjects	Edwards *et al.* (2007)
Quercetin ingestion	1000 mg/day (6 weeks)	No effect on exercise-induced oxidative stress and inflammation	McAnulty *et al.* (2008)
Quercetin (as a form of juice)	15 mg/day (4 weeks)	No effect	Boots *et al.* (2008)
α-Glucosylhesperidin	3000 mg/day (3 months)	Effective on RA	Kometani *et al.* (2008)
Quercetin, epicatechin	200 mg	Augmentation of NO status (improving endothelial function)	Loke *et al.* (2008)
Quercetin	150 mg/day (6 weeks)	Lowering systolic blood pressure	Egert *et al.* (2009)
Flavocoxid	500 × 2 mg/day (1 month)	Effective to manage OA	Levy *et al.* (2009)

All compounds were orally administered unless otherwise specified. MRSA: methicillin-resistant *Staphylococcus aureus*

These clinical investigations suggest that anti-inflammatory therapy with conventional flavonoids may be possible, but difficult. The following studies may show some promise for anti-inflammatory flavonoid therapy especially on chronic inflammatory diseases. Against human rheumatoid arthritis, α-glucosylhesperidin showed some effectiveness (Kometani *et al.*, 2008). And the oral administration of flavocoxid (90% baicalein + catechins) showed improvement for the signs and symptoms of osteoarthritis (OA) of the knee (Levy *et al.*, 2009). More phase II and III clinical trials may be needed to prove effectiveness of anti-inflammatory flavonoid therapy.

On the other hand, other pharmacological activities were observed in clinical trials. For example, quercetin, naringenin and hesperetin diets lowered the incidence of asthma (Knekt *et al.*, 2002) and oral quercetin lowered blood pressure (Edwards *et al.*, 2007). A reduction in systolic blood pressure in response to quercetin was also observed in overweight subjects in a study recently conducted by Egert *et al.* (2009). Furthermore, tea catechins administered by inhalation were found to reduce the occurrence of methicillin-resistance *Staphylococcus aureus* (MRSA) in sputum (Yamada *et al.*, 2006). In addition, isoflavones showed effectiveness on several forms of cancer and postmenopausal syndrome.

Perspectives

Although there has been some success in the use of flavonoids in anticancer and postmenopausal treatment in clinical trials, successful anti-inflammatory therapy using flavonoids have been rare to date. In fact, many flavonoids having various chemical structures were examined in vivo and found to have potencies that were not strong enough to justify clinical trials. Along with the continual search for more potent flavonoids from plants, the following alternatives may be pursued. One approach is the synthesis of more comparable flavonoids with new and meaningful substituent (s) as summarized above. Another approach is formulation studies designed to improve the oral bioavailability and/or metabolism of flavonoids. In the latter approach, it is important to keep in mind that i.v.-administration of quercetin showed renal toxicity in human.

Acknowledgements

References

Beck, G., Bergmann, K., Kebeler, K., and Wess, G., Synthesis of a new flavonoid-antioxidant. *Tetrahedron Lett.*, **31**, 7293-7296 (1990).

Boots, A.W., Wilms, L.C., Swennen, E.L.R., Kleinjans, J.C.S., Bast, A., and Haenen, G.R.M.M., In vitro and ex vivo anti-inflammatory activity of quercetin in healthy volunteers. *Nutr.*, **24**, 703-710 (2008).

Chen, X., Cui, L., Duan, X., Ma, B., and Zhong, D., Pharmacokinetics and metabolism of two flavonoid scutellarin in humans after a single oral administration. *Drug Metab. Dispos.*, **34**, 1345-1352 (2006).

Cho, H., Yun, C.-W., Park, W.-K., Kong, J.-Y., Kim, K.S., Park, Y., Lee, S., and Kim, B.-K., Modulation of the activity of proinflammatory enzymes, COX-2 and iNOS, by chrysin derivatives. *Pharm. Res.*, **49**, 37-43 (2004).

Chung, M.-I., Han, F.-W., Lin, H.-C., Liou, S.-S., Huang, P.-L., Ko, H.-H., Chang, Y.-L., Kang, J.-J., Teng, C.-M., and Lin, C.-N., Synthesis, antiplatelet and vasorelaxing effects of monooxygenated flavones and flavonoxypropanolamines. *J. Pharm. Pharmacol.*, **53**, 1601-1609 (2001).

Conquer, J.A., Maiani, G., Azzini, E., Raguzzini, A., and Holub, B.J., Supplementation with quercetin markedly increase plasma quercetin concentration without effect on selected risk factors for heart disease in healthy subjects. *J. Nutr.*, **128**, 593-597 (1998).

Conti, C., Desideri, N., Orsi, N., Sestili, I., and Stein, M.L., Synthesis and antirhinovirus activity of cyano and amidino substituted flavanoids. *Eur. J. Chem.*, **25**, 725-730 (1990).

Cunningham, B.D.M., Threadgill, M.D., Grounwater, P.W., Dale, I.L., and Hickman, J.A., Synthesis and biological evaluation of a series of flavones designed as inhibitors of protein tyrosine kinases. *Anti-Cancer Drug Design*, **7**, 365-384 (1992).

Dao, T.T., Chi, Y.S., Kim, J., Kim, H.P., Kim, S.H., and Park, H., Synthesis and PGE_2 inhibitory activity of 5,7-dihydroxyflavones and their *O*-methylated flavone analogs. *Arch. Pharm. Res.*, **26**, 345-350 (2003a).

Dao, T.T., Chi, Y.S., Kim, J., Kim, H.P., Kim, S., and Park, H., Synthesis and inhibitory activity against COX-2 catalyzed prostaglandin production of chrysin derivatives. *Bioorg. Med. Chem. Lett.*, **14**, 1165-1167 (2004).

Dao, T.T., Oh, J.W., Chi, Y.S., Kim, H.P., Sin, K.-S., and Park, H., Synthesis and PGE_2 inhibitory activity of vinylated and allylated chrysin analogues. *Arch. Pharm. Res.*, **26**, 581-584 (2003b).

de Pascual-Teresa, S., Johnston, K.L., DuPont, M.S., O'Leary, K.A., Needs, P.W., Morgan, L.M., Clifford, M.N., Bao, Y., and Williamson, G., Quercetin metabolites downregulate cyclooxygenase-2 transcription in human lymphocytes ex vivo but not in vivo. *J. Nutr.*, **134**, 552-557 (2004).

Edwards, R.L., Lyon, T., Litwin, S.E., Rabousky, A., Symons, J.D., and Jalili, T., Quercetin reduces blood pressure in hypertensive subjects. *J. Nutr.*, **137**, 2405-2411 (2007).

Egert, S., Bosy-Westphal, A., Seiberi, J., Kurbitz, C., Settler, U., Plachta-Danielzik, S., Wagner, A.E., Frank, J., Schrezenmeir, J., Rimbach, G., Wolffran, S., and Muller, M.J., Quercetin reduces systolic blood pressure and plasma oxidized low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. *Br. J. Nutr.*, **102**, 1065-1074 (2009).

Ertan, R., Goker, H., Ertan, M., Beretz, A., Cazenave, J.P., Haag, M., and Anton, R., Synthesis of some flavone derivatives: potent inhibitors of human platelet aggregation. *Chimie*, **26**, 735-738 (1991).

Ferry, D.R., Smith, A., Malkhandi, J., Fyfe, D.W., de Takats, P.G., Anderson, D., Baker, J., and Kerr, D.J., Phase I clinical trial of the flavonoid quercetin: Pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. *Clinical Cancer Research*, **2**, 659-668 (1996).

Filipe, P., Silva, A.M.S., Seixas, R.S.G.R., Pinto, D.C.G.A., Santos, A., Patterson, L.K., Silva, J.N., Cavaleir, J.A.S., Freitas, J.P., Maziere, J.-C., Santus, R., and Morliere, P., The alkyl chain length of 3-alkyl-3',4',5,7-tetrahydroxyflavones modulates effective inhibition of oxidative damage in biological systems: Illustration with LDL, red blood cells and human keratinocytes. *Biochem. Pharmacol.*, **77**, 957-964 (2009).

Gabor, M., Anti-inflammatory and anti-allergic properties of flavonoids, In Cody *et al.* (eds.), *Plant flavonoids in biology and medicine: Biochemical, pharmacological, and structure-activity relationships*, Alan R. Liss, New York, 1986, pp. 471-480.

Gao, H. and Kawabata, J., α-Glucosidase inhibition of 6-hydroxyflavones. Part 3: Synthesis and evaluation of 2,3,4-trihydroxybenzoyl-containing flavonoid analogs and 6-aminoflavones as α-glucosidase inhibitors. *Bioorg. Med. Chem.*, **13**, 1661-1671 (2005).

Gugler, R., Leschik, M., and Dengler, H.J., Disposition of quercetin in man after single oral and intraveneous doses. *Eur. J. Clin. Pharmacol.*, **9**, 229-234 (1975).

Gunnarsson, G.T., Riaz, M., Adams, J., and Desai, U.R., Synthesis of persulfated flavonoids using 2,2,2-trichloroethyl protecting group and their factor Xa inhibition potential. *Bioorg. Med. Chem.*, **13**, 1783-1789 (2005).

Han, C.K., Son, M.J., Chang, H.W., Chi, Y.S., Par, H., and Kim, H.P., Inhibition of prostaglandin production by a structurally-optimized flavonoid derivative, 2',4',7-trimethoxyflavone and cellular action mechanism. *Biol. Pharm. Bull.*, **28**, 1366-1370 (2005)

Havsteen, B., Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.*, **32**, 1141-1148 (1983).

Herencia, F., Ferrandiz, M.L., Ubeda, A., Gwillen, I., Dominguez, J.N., Charris, J.E., Lobo, G.M., and Alcaraz, M.J., Novel anti-inflammatory chalcone derivatives inhibit the induction of nitric oxide synthase and cyclooxygenase-2 in mouse peritoneal macrophages. *FEBS Lett.*, **453**, 129-134 (1999).

Hirano, T., Oka, K., Kawashima, E., and Akiba, M., Effects of synthetic and naturally occurring flavonoids on mitogen-induced proliferation of human peripheral-blood lymphocytes. *Life Sci.*, **45**, 1407-1441 (1989).

Horie, T., Tsukayama, M., Kourai, H., Yokoyama, C., Furukawa, M., Yoshimoto, T., Yamamoto, S., Waranabe-Kohno, S., and Ohata, K., Synthesis of 5,6,7- and 5,7,8-trioxygenated 3',4'-dihydroxyflavones having alkoxy groups and their inhibitory activities against arachidonate 5-lipoxygenase. *J. Med. Chem.*, **29**, 2256-2262 (1986).

Huang, W.-H., Lee, A.-R., Chien, P.-Y., and Chou, T.-Z., Synthesis of baicalein derivatives as potential antiaggregatory and anti-inflammatory agents. *J. Pharm. Pharmacol.*, **57**, 219-225 (2005).

Jang, J., Kim, H.P., and Park, H., Structure and anti-inflammatory activity relationships of wogonin derivatives. *Arch. Pharm. Res.*, **28**, 877-884 (2005).

Joo, A.H., Kim, J.K., Kang, S.-H., Noh, M.-S., Ha, J.-Y., Choi, J.K., Lim, K.M., Lee, C.H., and Chung, S., 2,3-Diarylbenzopyran derivatives as a novel class of selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.*, **13**, 413-417 (2003).

Kim, H.P., Son, K.H., Chang, H.W., and Kang, S.S., Anti-inflammatory flavonoids and cellular action mechanisms. *J Pharmacol. Sci.*, **96**, 229-245 (2004a).

Kim, S.J., Park, H., and Kim, H.P., Inhibition of nitric oxide production from lipopolysaccharide-treated RAW264.7 cells by synthetic flavones: Structure-activity relationship and action mechanism. *Arch. Pharm.*

Res., **27**, 937-943 (2004b).

Kim, Y.H., Kim, J., Park, H., and Kim, H.P., Anti-inflammatory activity of the synthetic chalcone derivatives: Inhibition of inducible nitric oxide synthase-catalyzed nitric oxide production from lipopolysaccharide-treated RAW 264.7 cells. *Biol. Pharm. Bull.*, **30**, 1450-1455 (2007).

Knekt, P., Kumpulainen, J., Jarvinen, R., Rissanen, H., Heliovaara, M., Reunanen, A., Hakulinen, T., and Aromaa, A., Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.*, **76**, 560-568 (2002).

Kometani, T., Fukuda, T., Kakuma, T., Kawaguchi, K., Tamura, W., Kumazawa, Y., and Nagata, K., Effects of α-glucosylhesperidin, a bioactive food material, on collagen-induced arthritis in mice and rheumatoid arthritis in humans. *Immunopharmacol. Immunotoxicol.*, **30**, 117-134 (2008).

Lebeau, J., Furman, C., Bernier, J.-U., Duriez, P., Teissier, E., and Cotelle, N., Antioxidant properties of di-tert-butylhydroxylated flavonoids. *Free Rad. Biol. Med.*, **29**, 900-912 (2000).

Lee, S.J., Baek, H.J., Lee, C.H., and Kim, H.P., Anti-inflammatory activity of isoflavonoids from Pueraria radix and biochanin A derivatives. *Arch. Pharm. Res.*, **17**, 31-35 (1994).

Levy, R.M., Saikovsky, R., Shmidt, E., Khokhlov, A., and Burnett, B.P., Flavocoxid is as effective as naproxen for managing the signs and symptoms of osteoarthritis of the knee in humans: a short-term randomized, double-blind pilot study. *Nutr. Res.*, **29**, 298-304 (2009).

Liao, H.-L. and Hu, M.-K., Synthesis and anticancer activities of 5,6,7-trimethylbaicalein derivatives. *Chem. Pharm. Bull.*, **52**, 462-465 (2004).

Lin, C.-N., Lee, T.-H., Hsu, M.-F., Wang, J.-P., Ko, F.-N., and Teng, C.-M., 2'.5'-Dihydroxychalcone as a potent chemical mediator and cyclooxygenase inhibitor. *J. Pharm. Pharmacol.*, **49**, 530-536 (1997).

Liu, T., Xu, Z., He, Q., Chen, Y., Yang, B., and Hu, Y., Nitrogen-containing flavonoids as CDK1/cyclin B inhibitors: Design, synthesis, and biological evaluation. *Bioorg. Med. Chem. Lett.*, **17**, 278-281 (2007).

Loke, W.M., Hodgson, J.M., Proudfoot, J.M., McKinly, A.J., Puddey, I.B., and Croft, K.D., Pure dietary flavonoids quercetin and (−)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am. J. Clin. Nutr.*, **88**, 1018-1025 (2008).

Losiewicz, M.D., Carlson, B.A., Kaur, G., Sausville, E.A., and Worland, P.J., Potent inhibition of CDC2 kinase activity by the flavonoid L86-8275. *Biochem. Biophys. Res. Comm.*, **201**, 589-595 (1994).

McAnulty, S.R., McAnulty, L.S., Nieman, D.C., Quindry, J.C., Hosick, P.A., Hudson, M.H., Still, L., Henson, D.A., Milne, G.L., Morrow, J.D., Dumke, C.L., Utter, A.C., Triplett, N.T., and Dibarnardi, A., Chronic quercetin ingestion and exercise-induced oxidative damage and inflammation. *Appl. Physiol. Nutr. Metab.*, **33**, 254-262 (2008).

Middleton, E., Kandaswami, C., and Theoharides, T.C., The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.*, **52**, 673-751 (2000).

Moon, Y.J., Wang, L., DiCenzo, R., and Morris, M.E., Quercetin pharmacokinetics in humans. *Biopharm. Drug. Dispos.*, **29**, 205-217 (2008).

Nagai, T., Miyaichi, Y., Tomimori, T., and Yamada, H., Inhibition of mouse liver sialidase by plant flavonoids. *Biochem. Biophys. Res. Comm.*, **163**, 25-31 (1989).

Park, H., Dao, T.T., and Kim, H.P., Synthesis and inhibition of PGE_2 production of 6,8-disubstituted chrysin derivatives. *Eur. J. Med. Chem.*, **40**, 943-948 (2005).

Quintin, J., Buisson, D., Thoret, S., Cresteil, T., and Lewin, G., Semisynthesis and antiproliferative evaluation of a series of 3'-aminoflavones. *Bioorg. Med. Chem. Lett.*, **19**, 3502-3506 (2009).

Ullmann, U., Metzner, J., Frank, T., Cohn, W., and Riegger, C., Safety, tolerability, and pharmacokinetics of single ascending doses of synthetic genistein (Bonistein) in healthy volunteers. *Adv. Ther.*, **22**,

65-78 (2005).

Vasquez-Martinez, Y., Ohri, R.V., Kenyon, V., Holman, T.R., and Sepulveda-Boza, S., Structure-activity relationship studies of flavonoids as potent inhibitors of human platelet 12-hLO, reticulocyte 15hLO-1, and prostate epithelial 15hLO-2. *Bioorg. Med. Chem*., **15**, 7408-7425 (2007).

Viola, H., Marder, M., Wasowski, C., Giorgi, O., Paladini, A.C., and Medina, J.H., 6,3'-Dibromoflavone and 6-nitro-3'-bromoflavone: New additions to the 6,3'-disubstituted flavone family of high-affinity ligands of the brain benzodiazepine binding site with agonistic properties. *Biochem. Biophys. Res. Comm*., **273**, 694-698 (2000).

Wu, E.S.C., Cole, T.E., Davidson, T.A., Blosser, J.C., Borrelli, A.R., Kingsolving, C.R., and Parker, R.B., Flavones. 1. Synthesis and antihypertensive activity of 3-phenylflavone-oxopropanolamines without β-adrenergic receptor antagonism. *J. Med. Chem*., **30**, 788-792 (1987).

Wu, E.S.C., Loch, J.T., Toder, B.H., Borrelli, A.R., Gawlak, D., Radov, L.A., and Gensmantel, N.P., Flavones. 3. Synthesis, biological activities, and conformational analysis of isoflavone derivatives and related compounds. *J. Med. Chem*., **35**, 3519-3525 (1992).

Yahiaoui, S., Fagnere, C., Pouget, C., Buxeraud, J., and Chulia, A.-J., New 7,8-benzoflavanones as potent aromatase inhibitors: Synthesis and biological evaluation. *Bioorg. Med. Chem*., **16**, 1474-1480 (2008).

Yamada, H., Tateishi, M., Harada, K., Ohashi, T., Shimizu, T., Atsumi, T., Komagata, Y., Iijima, H., Komiyama, K., Watanabe, H., Hara, Y., and Ohashi, K., A randomized clinical study of tea catechin inhalation effects on methicillin-resistant *Staphylococcus* aureus in disabled elderly patients. *J. Am. Med. Dir. Assoc*., **7**, 79-83 (2006).

Yarishkin, O.V., Ryu, H.W., Park, J.-Y., Yang, M.S., Hong, S.-G., and Park, K.H., Sulfonate chalcone as new class voltage-dependent K^+ channel blocker. *Bioorg. Med. Chem. Lett*., **18**, 137-140 (2008).