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Recent Advances in Anti-inflammatory Flavonoid Research since 2004

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Abstract - Certain flavonoids possess anti-inflammatory activity. Besides their antioxidative property, the cellular action mechanisms of flavonoids include an inhibition of arachidonate metabolizing enzymes such as cyclooxygenases and lipoxygenases, and a down-regulation of proinflammatory gene expression such as cyclooxygenase-2, inducible nitric oxide synthase and tumor necrosis factor-α. In this review, the recent findings of anti-inflammatory flavonoid research since 2004 were summarized. And the cellular mechanisms on signal transduction pathways were also discussed.

Key words □ flavonoid, anti-inflammation, cyclooxygenase, nitric oxide synthase, gene expression, transcription factor

INTRODUCTION

Flavonoids (Fig. 1) from plants possess various biological/pharmacological activities. In particular, flavonoids show anti-inflammatory activity in vitro and in vivo. They have multiple action mechanisms for exerting anti-inflammation. For example, certain flavonoids were found to affect arachidonic acid (AA) metabolism reducing eicosanoid production such as proinflammatory prostaglandins (PG) and leukotrienes (LT). The enzymes involved are phospholipase A_2 (PLA_2), cyclooxygenases (COX) and lipoxygenases (LOX). Recent investigations have also shown that some anti-inflammatory flavonoids, especially flavones and flavonols, were modulators of proinflammatory gene expression such as COX-2, inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), etc. Their cellular mechanisms of the transcriptional modulation include a regulation of cellular kinase activities such as mitogen-activated protein kinase (MAPK) and an inhibition of transcription factor activation like nuclear transcription factor-κB (NF-κB) or activator protein-1 (AP-1). These findings of flavonoids were previously summarized (Middleton *et al.*, 2000; Kim HP *et al.*, 2004). The present review summarizes the recent advances of anti-inflammatory flavonoid research during 2004 - 2006.

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THE EFFECTS ON AA/NO METABOLIZING ENZYMES

AA, a precursor of eicosanoids, is released mostly from membrane lipids in cells. The major enzyme responsible for this release is PLA_2. At least 19 PLA_2 isoforms are identified in mammals, among which secretory PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$) and calcium-independent PLA_2 ($iPLA_2$) have been involved in eicosanoid synthesis (Murakami and Kudo, 2004). Each of them has the different role in varieties of cells and tissues. Sometimes they are coupled with COXs to produce PGs. COX is an enzyme to convert arachidonic acid to PGH_2, which is further converted to thromboxanes (TX) and PGs by the respective enzymes. In this pathway, COX is a rate-limiting enzyme. Major isoforms of COX are COX-1 and -2 (Parente and Perretti, 2003). While COX-1 is a constitutive enzyme in most of cells, COX-2 is largely an inducible one in certain cells and tissues like macrophages and kidney. In inflamed sites, COX-2 expression increases and proinflammatory PGs are synthesized. The active site pocket of COX-2 is slightly larger than that of COX-1, rendering the COX-2 selective inhibitors made possible. Although some COX-2 selective inhibitors were withdrawn from the market due to the deleterious side effects on cardiovascular system, COX-2 inhibition is still valuable for anti-inflammation. On the other hand, 5-LOX among several isoforms of LOX is responsible for the biosynthesis of LTs involved in allergic disorders. In addition, NO is also involved in several inflammatory conditions and synthesized from arginine by NOS (Nathan, 1992). Among three different isoforms of NOS (eNOS, nNOS, iNOS), iNOS is an inducible

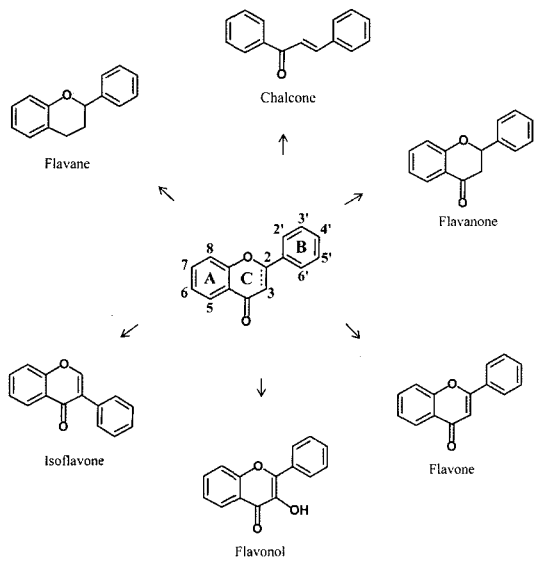


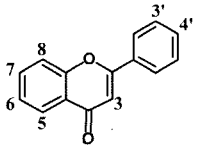
Fig. 1. Basic chemical structures of natural flavonoids

one and produces large amount of NO in the inflammatory lesions. Therefore, the impacts on these proinflammatory enzymes may produce anti-inflammatory action. Numerous flavonoid derivatives have been previously found to inhibit these enzyme activities and the investigations up to 2004 were summarized (Kim HP *et al.*, 2004). The followings include several deleted reports in the previous review and the additional findings by recent studies.

The flavone, nepetin (Table I) was found to inhibit PLA_2 from *Naja naja* venom ($sPLA_2$-IIB) (Sala *et al.*, 2000). We have previously shown that the prenylated flavonoid, papyriflavonol A, was a $sPLA_2$-IIA inhibitor (Kwak *et al.*, 2003). The PLA_2 inhibitory potential of the prenylated flavonoids was demonstrated in abyssinone V (Fig. 2), erycristagallin and 4'-hydroxy-6,3',5'-triprenylisoflavanone (Hegde *et al.*, 1997). Recently, it was also found that 2',4',7-trimethoxyflavone was a PLA_2 inhibitor without COX-2 inhibitory activity (Han *et al.*, 2005). These compounds are added to the list of PLA_2 inhibitory flavonoids.

The prenylated flavonoids, sanggenon C, D, E, O and moracin M, inhibited both COX-1 and COX-2, having higher inhibitory action on COX-2 (Rollinger *et al.*, 2005). This report is well correlated with our previous findings that sanggenon B, D and morusin possess COX-1 and COX-2 inhibitory activity (Chi *et al.* 2001b). Sanggenon B, D and morusin, however, showed the higher inhibition against COX-1 than against COX-2. The differences between these two reports may be due to the different enzyme assay systems used. It is interesting to

Table I. Some flavones and flavonols mentioned in this review



Compounds	3	5	6	7	8	3'	4'	5'
Flavone	-	-	-	-	-	-	-	-
Chrysin	-	OH	-	OH	-	-	-	-
Apigenin	-	OH	-	OH	-	-	OH	-
Wogonin	-	OH	-	OH	OCH_3	-	-	-
Luteolin	-	OH	-	OH	-	OH	OH	-
Diosmetin	-	OH	-	OH	-	OH	OCH_3	-
Nepetin	-	OH	OCH_3	OH	-	OH	OH	-
Nobiletin	-	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	-
Flavonol	OH	-	-	-	-	-	-	-
Kaempferol	OH	OH	-	OH	-	-	OH	-
Quercetin	OH	OH	-	OH	-	OH	OH	-
Myricetin	OH	OH	-	OH	-	OH	OH	OH
Tiliroside	O-c-g[a]	OH	-	OH	-	-	OH	-
Ayanin	OCH3	OH	-	OCH_3	-	OH	OCH_3	-
Santin	OCH_3	OH	OCH_3	OH	-	-	OCH_3	-
Centaureidin	OCH_3	OH	OCH_3	OH	-	OH	OCH_3	-
Eupalitin-3-O-galactoside	O-gal	OH	OCH_3	OCH_3	-	OH	OH	-

[a]O-6''-p-coumarylglucoside

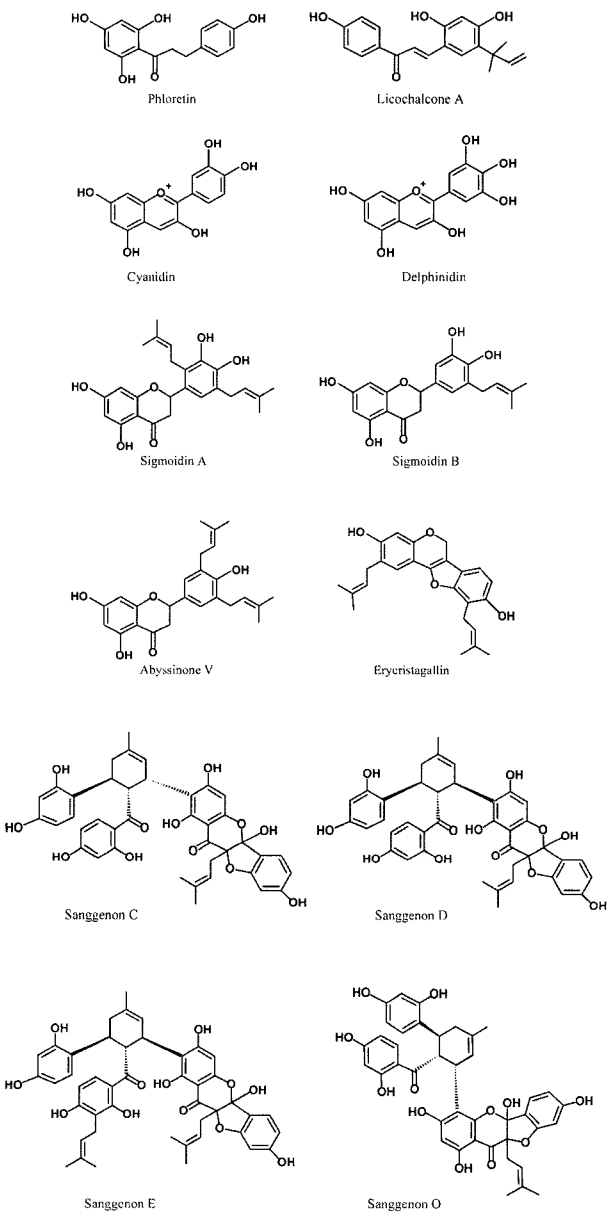


Fig. 2. Some chalcones, cyanidins and prenylated flavonoids mentioned in this review

find that quercetin and quercetin 3'-sulfate inhibited COX-2 (O'Leary *et al.*, 2004). Since quercetin 3'-sulfate is one of the metabolites found in the blood after quercetin ingestion in man, it is possible that quercetin-rich diet may really affect COX-2 activity in the body, but there is no evidence yet. It was also reported that santin, ermanin, centaureidin, and 5,3'-dihydroxy-4'-methoxy-7-methoxycarbonylflavonol inhibited PGE_2 and NO production from LPS-induced mouse peritoneal macrophages at least in part by COX-2 and iNOS inhibition (Abad *et al.*, 2004). However, these flavonoids are to be further elucidated using COX-2 and iNOS enzymes to clearly establish their inhibitory activity. Licochalcone A from Glycyrrhiza species was demonstrated to inhibit COX-2-dependent PGE_2 production, but not COX-1-dependent PGE_2 production from human skin fibroblasts. This compound has no effect on COX-2 expression (Furuhashi *et al.*, 2005). Using synthetic approach, many flavone derivatives were prepared and their effects on LPS-induced PGE_2 production (COX-2-catalyzed) from RAW 264.7 cells were compared (Dao *et al.*, 2004; Jang *et al.*, 2005; Park *et al.*, 2005). And several derivatives were found to show potent inhibition of PGE_2 production, but COX-2 inhibitory potential or down-regulating potential of COX-2 induction has not been established.

The several prenylated flavonoids were previously found to be 5-LOX inhibitors, thereby reducing LT formation. They include sophoraflavanone G and kenusanone A (Chi *et al.*, 2001b). Two other prenylated flavanones, sigmoidin A and B from *Erythria sigmoidea* were recently found to be 5-LOX inhibitors without COX-1 inhibitory activity (Njamen *et al.*, 2004). As noted above, some prenylated flavonoids possess the inhibitory properties against AA metabolizing enzymes. Further exploration may be needed to get clear structure-activity relationships of the prenylated flavonoids regarding as inhibitors of PLA_2, COX and LOX.

There have been only a few flavonoid derivatives having direct iNOS inhibitory activity. An example is echinoisosophoranone, but the inhibitory potency was not high (Cheon *et al.*, 2000). Recently, it was reported that a kaempferol glycoside, tiliroside, inhibited NO production from LPS/IFN-treated macrophages at least in part by direct iNOS inhibition (Rao *et al.*, 2005). Santin, ermanin, centaureidin, and 5,3'-dihydroxy-4'-methoxy-7-methoxycarbonylflavonol inhibited PGE_2 and NO production from LPS-induced mouse peritoneal macrophages (Abad *et al.*, 2004). Authors claimed that these flavonoids directly inhibited iNOS activity. But it is necessary to measure iNOS activity using enzyme homogenate for clearly establishing iNOS inhibitory activity. On the other hand, it is interesting that genistein was found to induce a late but sustained activation of the endothelial NOS system in vitro probably via the increased expression of eNOS (Rathel *et al.*, 2005). In general, flavonoids are not efficient NOS inhibitors. The effects of flavonoids on the constitutive iNOSs are to be elucidated further.

All these findings have clearly shown that certain flavonoids including the prenylated ones possess direct inhibitory activity on AA/NO metabolizing enzymes. These properties certainly contribute to anti-inflammatory activity of flavonoids. As men-

tioned above, during recent 2 - 3 years, only a few reports were available concerning new findings of flavonoids having inhibitory properties against AA/NO metabolizing enzymes. Meanwhile, many investigations of flavonoids showing inhibition of proinflammatory gene expression have been reported and they are summarized as below.

THE EFFECTS ON THE EXPRESSION OR PRODUCTION OF PROINFLAMMATORY MOLECULES

Flavonoids are now known as nature's transcriptional modulators of proinflammatory gene expression. In most cases, flavonoids were found to down-regulate proinflammatory gene expression such as COX-2, iNOS, TNF-α, etc. (Kim HP *et al.*, 2004). During 2004 - 2006, many investigations have repeatedly demonstrated that certain flavonoids are inhibitors of proinflammatory gene expression. Table II represents these recent findings. Delphinidin, cyanidin, eupalitin-3-*O*-galactoside, ayanin, diosmetin, phloretin, quercetin metabolites and some more derivatives are newly added to the lists.

In an effort to find the optimized structures for proinflammatory gene down-regulation, synthetic flavone derivatives were evaluated for NO inhibition from LPS-induced RAW cells. From the results, it was found that B-ring 3',4'-hydroxyl or hydroxymethoxyl substitutions were favorable. 5,7,3',4'-Tetrahydroxyflavone (luteolin), 5,7,3'-trihydroxy-4'-methoxyflavone (diosmetin) and 5,7,4'-trihydroxy-3'-methoxyflavone (chrysoeriol) were the most inhibitory against iNOS induction (Kim SJ *et al*, 2004). The potencies of these synthetic flavones for reducing NO production were comparable with that of wogonin. It is significant to mention that quercetin metabolites inhibit mRNA of lymphocytes in culture, but an intake of high content of onion as a quercetin source had no effect in human study (de Pascual-Teresa *et al.*, 2004).

Table II. The effects of flavonoids on the expression or production of proinflammatory molecules

Compounds	Target cells	Agonist	Target genes inhibited	References
Quercetin, quercetin-3-glucuronide, quercetin 3'-sulfate				
	Caco-2	IL-1β	COX-2	O'Leary *et al.* (2004)
Quercetin metabolites	lymphocyte	-	COX-2	de Pascual-Teresa *et al.* (2004)
Luteolin, luteolin-7-*O*-glucoside	RAW 264.7	LPS	COX-2, iNOS	Hu and Kitts (2004)
Nobiletin	human keratinocyte UVB		COX-2	Tanaka *et al.* (2004)
Delphinidin, cyanidin	RAW 264.7	LPS	COX-2	Hou *et al.* (2005)
Apigenin	HaCaT	TPA	COX-2	Van Dross *et al.* (2005)
Epicatechin	RINm5F, rat pancreatic islet			
		IL-1β	iNOS	Kim M-J *et al.* (2004)
Baicalein	rat primary microglia, BV-2, RAW 264.7			
		LPS	iNOS	Chen C-J *et al.* (2004)
Chrysin, galangin, kaempferol, quercetin	J774A.1	LPS	iNOS, IL-1β	Blonska *et al.* (2004)
Quercetin	hepatocyte	IL-1β	iNOS	Martinez-Florez *et al.* (2005)
Luteolin	BV-2	LPS	iNOS	Kim *et al.* (2006)
Luteolin	HT29	TNF-α	IL-8	Kim J-A *et al.* (2005)
Apigenin	HUVEC	hypoxia	VEGF	Osada *et al.* (2004)
Eupalitin-3-*O*-β-D-galactosylpyranoside	human PBMC	PMA, LPS	IL-2, TNF-α	Pandey *et al.* (2005)
Myricetin	RAW 264.7, macrophage			
		LPS	IL-12 (not IL-6)	Kang *et al.* (2005)
EGCG	HUVEC	TNF-α	VCAM-1	Ludwig *et al.* (2004)
Apigenin, kaempferol	J774.2	LPS	IL-1β, TNF-α	Kowalski *et al.* (2005a)
Kaempferol	J774.2	LPS	MCP-1	Kowalski *et al.* (2005b)
Apigenin	EL4, primary lymph node			
		KLH	IL-4	Park *et al.* (2006)
Ayanin, luteolin, apigenin, diosmetin, fisetin, scutellarein and many more				
	KU812	A23187+TPA	IL-4	Hirano *et al.* (2006)
Phloretin	HUVEC	TNF-α, IL-1β	ICAM-1, VCAM-1, E-selectin	
				Stangl *et al.* (2005)

HUVEC: Human umbilical vein endothelial cell, PBMC: peripheral blood mononuclear cell,
MCP-1: monocyte chemotactic protein-1, VCAM-1: vascular cell adhesion molecule-1

MECHANISMS OF MODULATING PROINFLAMMATORY GENE EXPRESSION

Quercetin (flavonol), a most widely distributed flavonoid, was shown to inhibit the expression of proinflammatory molecules. But, its cellular target molecules are different depending on the cells and the agonists used. For example, quercetin was reported to inhibit TNF-α- or TPA-induced intercellular adhesion molecule-1 (ICAM-1) expression by attenuating c-Jun NH_2-terminal kinase (JNK) pathway and AP-1 activation (Kobuchi *et al.*, 1999). In contrast, recent study has shown that quercetin inhibited iNOS expression by inhibition of NF-κB activation, but not by JNK inhibition (Comalada *et al.*, 2005). Inhibition of NF-κB activation by quercetin resulting in iNOS down-regulation was also found in rat hepatocytes (Martinez-Florez *et al.*, 2005). Other kinases or transcription factors were also affected by quercetin. Quercetin inhibited extracellular signal-regulated kinase (ERK) activation and AP-1 activation (Kim AR *et al.*, 2005). Repeatedly, quercetin was shown to inhibit iNOS expression from BV-2 microglia by inhibition of IκB kinase (IKK), NF-κB and signal transducer and activator of transcription (STAT1), and this inhibition was depending on heme oxygenase-1 (HO-1) induction (Chen *et al.*, 2005). Thus the cellular targets for quercetin are different depending on the cell types studied and the stimuli involved.

This notion also applied to the action mechanisms of other flavonoids. Luteolin and apigenin (flavone) were reported to act via down-regulation of NF-κB pathway in macrophages, thereby, inhibiting LPS-induced iNOS and COX-2 expression, and TNF-α and IL-6 release (Liang *et al.*, 1999; Xagorari *et al.*, 2001). It was recently described that luteolin inhibited IL-8 production from HT29 cells by inhibiting MAPKs, followed by inhibition of NF-κB activation (Kim J-A *et al.*, 2005). This compound inhibited p38 kinase, ERK, IκB degradation and NF-κB activation. Furthermore, the role of AP-1 and NF-κB concomitantly was examined and the greater inhibition of kaempferol, chrysin, apigenin and luteolin on AP-1 activity was found resulting in the attenuation of ICAM-1 expression (Chen C-C *et al.*, 2004). Luteolin also inhibited phosphatidylinositol 3'-kinase (PI3K), resulting in inhibition of vascular endothelial growth factor (VEGF)-induced angiogenesis (Bagli *et al.*, 2004). On the other hand, many flavone and flavonol derivatives including luteolin and apigenin among 37 flavonoids tested inhibited IL-4 expression from basophils (KU812) stimulated with A23187 and TPA. This inhibition by luteolin was not mediated via MAP kinase pathway, instead it inhibited c-jun and DNA binding of AP-1 (Hirano *et al.*, 2006). It is worth to mention that apigenin inhibited heme-induced induction of HO-1 from mouse embryonic fibroblasts (Abate *et al.*, 2005). Therefore, it was repeatedly found that certain flavonoids inhibited the activation of the different transcription factors such as NF-κB or AP-1 depending on the cells and the stimuli.

Some other flavonoids were also demonstrated to affect cellular signal transduction pathways. While the flavonols such as kaempferol and quercetin inhibited iNOS expression by inhibiting ERK activation and AP-1 activation (DNA binding activity), genistein (isoflavone) inhibited NF-κB and C/EBPβ, activation from LPS-induced RAW 264.7 cells (Kim AR *et al.*, 2005). Another flavonol, myricetin, has been also found to inhibit NF-κB activation (Kang *et al.*, 2005). Apigenin inhibited COX-2 expression from TPA-treated HaCaT cells by inhibiting Akt kinase (protein kinase B) activation but not by inhibiting MAP kinase pathway (Van Dross *et al.*, 2005). Apigenin was recently demonstrated to inhibit IL-4 production in activated T-cells by inhibition of nuclear factor for activated T-cells (NF-AT) DNA binding (Park *et al.*, 2006). Considering that apigenin inhibited NF-κB activation from macrophages in an earlier report (Liang *et al.*, 1999), these results also prove that the same flavonoid affects different cellular machinery depending on the cell types.

Previously, wogonin (flavone) was most potent to inhibit COX-2 and iNOS expression from LPS-induced RAW cells (Kim *et al.*, 1999; Chi *et al*, 2001a). Moreover, wogonin was shown to inhibit COX-2 expression from TPA-, TNF-α or IL-1β-induced NIH/3T3 fibroblasts (Chi and Kim, 2005). And the inhibition of AP-1 or NF-κB activation was suggested as cellular inhibition mechanism (Chang *et al.*, 2001; Kim *et al.*, 2001). It was repeatedly reported that wogonin is neuroprotective (Son *et al.*, 2004; Cho and Lee, 2004). The oral wogonin treatment reduced colitis-disease and modulated cytokine productivity. Wogonin reduced IgE production while it increased IgA production, hence this compound is an immune modulator (Lim, 2004). Structurally similar baicalein (flavone) also inhibited iNOS expression from LPS-treated rat primary microglia, BV-2 and RAW 264.7 cells by inhibition of DNA binding activity of NF-IL6. But the inhibition potency of NF-κB and AP-1 activation was not high and no inhibition of ERK1/2 was observed (Chen C-J *et al.*, 2004). In contrast, it is interesting to note that baicalein, baicalin and chrysin upregulated TGF-β1 expression from RAW 264.7 cells, but not by wogonin (Chuang *et al.*, 2005).

Eupalitin-3-*O*-galactoside was shown to inhibit IL-2 and

TNF-α expression from PBMC and it inhibited activation of DNA binding of NF-κB and AP-1 (Pandey *et al.*, 2005). Epicatechin was found to inhibit iNOS expression by inhibiting NF-κB activation (Kim M-J *et al.*, 2004). An important finding was observed that delphinidin and cyanidin (anthocyanin) inhibited COX-2 expression from LPS-treated RAW cells and delphinidin was shown to inhibit MAP kinase pathway including p38 kinase, JNK and ERK, and NF-κB, AP-1 and C/EBPδ activation (Hou *et al.*, 2005). Phloretin (dihydrochalcone) inhibited adhesion molecule expression from cytokine-activated HUVECs by inhibition of interferone regulatory factor 1, but not by inhibition of NF-κB activation (Stangl *et al.*, 2005). Naringenin (1 mM) inhibited NF-κB activation from LPS-treated RAW cells resulting in down-regulation of iNOS, TNF-α, COX-2 and IL-6 induction (Kanno *et al.*, 2006).

CONCLUDING REMARKS

Although the signal transduction pathways affected by flavonoids vary depending on the flavonoids, the cells studied and the agonists used, main cellular mechanisms are inihibition of cellular kinase(s) in signal transduction pathway involved and subsequent inhibition of target transcription factor(s) activation. Some flavonoids may also affect the DNA binding capacity of transcription factor(s) directly to each responsive regions of DNA. The continuous efforts will unveil the detailed cellular behaviors of flavonoids and a new class of anti-inflammatory agents based on the flavonoid molecule may be developed.

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