



# Interactions of Flavonoids with Ionotropic GABA Receptors

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## Abstract

In this overview, we highlight some recent advances in the interaction of natural and synthetic flavonoids with ionotropic GABA receptors. Examples of positive, negative, and neutralizing allosteric modulators as well as allosteric agonists are given. Flavonoids appear to act via multiple binding sites on GABA receptors. Unraveling these active sites remains a major task.

## ABBREVIATIONS

**2'MeO6MF** 2'-methoxy-6-methylflavone

**3-OH-2'MeO6MF** 3-hydroxy-2'-methoxy-6-methylflavone

**EGCG** epigallocatechin gallate

**Fa131** *trans*-(2S,3R)-3-acetoxy-4'-methoxyflavan

**Fa173** *cis*-(2S,3S)-3-acetoxy-3',4'-dimethoxyflavan

**GHB**  $\gamma$ -hydroxybutyric acid

**THIP** 4,5,6,7-tetrahydroisoxazolo-[5,4-*c*]-pyridin-3-ol

**TPMPA** 1,2,5,6-tetrahydro-pyridine-4-yl-methylphosphinic acid



## 1. INTRODUCTION

Flavonoids can act on ionotropic receptors for the inhibitory neurotransmitter GABA in many ways. They can act as positive, negative, and neutralizing allosteric modulators as well as agents that modulate other allosteric agonists. They appear to act at a variety of modulatory sites on GABA<sub>A</sub> receptors. Initially thought to act on classical benzodiazepine modulatory sites, it is clear that many flavonoid actions on GABA<sub>A</sub> receptors are insensitive to the classical benzodiazepine antagonist flumazenil. In this overview, we highlight some recent advances in the interaction of flavonoids with ionotropic GABA receptors since our 2011 review on this topic (Hanrahan, Chebib, & Johnston, 2011). We concentrate on flavonoids that have relatively specific action on subtypes of ionotropic GABA receptors. Furthermore, behavioral effects of some flavonoids are explored in terms of their effects on ionotropic GABA receptors. The emphasis is on relating chemical structure to activity.

Many investigators have noted structural similarities between certain flavonoids and benzodiazepines, such as diazepam, that are the most widely studied positive modulators of GABA<sub>A</sub> receptors. Benzodiazepines can act on these receptors via 'two distinct and separable mechanisms' (Walters, Hadley, Morris, & Amin, 2000). At nanomolar concentrations, benzodiazepines act in a classic flumazenil-sensitive manner to enhance the action of GABA, while at micromolar concentrations, benzodiazepines act in a flumazenil-insensitive manner. Flavonoids can act on GABA<sub>A</sub> receptors at low concentrations in either a flumazenil-sensitive or flumazenil-insensitive manner as modulators of these receptors (Hanrahan et al., 2011). Furthermore, many flavonoids act in a biphasic manner, potentiating GABA actions at low concentrations and inhibiting at high concentrations. In addition, some flavonoids have agonist actions on certain GABA receptors, directly gating the receptor in the absence of GABA. Clearly flavonoids can interact with a variety of specific active sites on ionotropic GABA receptors. Unraveling these active sites remains a major task.



## 2. 6-SUBSTITUTED FLAVONES

Previous studies have shown the 6-position on flavones as being relevant to determining the effects on recombinant GABA<sub>A</sub> receptors (Hall, Chebib, Hanrahan, & Johnston, 2004; Ren et al., 2010). A study of flavones

(Fig. 1), each varying only at position 6, were compared, including 6-fluoroflavone, 6-chloroflavone, 6-bromoflavone, 6-hydroxyflavone, and 6,2'-dihydroxyflavone demonstrated 6-bromoflavone to be a positive modulator at GABA<sub>A</sub> receptors acting through flumazenil-sensitive high-affinity benzodiazepine sites (Ren et al., 2011). In contrast, the other two 6-haloflavones and 2'-hydroxyflavone were neutralizing modulators, while 6,2'-dihydroxyflavone is a negative modulator of GABA<sub>A</sub> receptors.

In contrast, 6-methylflavone is known to be a flumazenil-insensitive positive modulator of GABA<sub>A</sub> receptors (Hall et al., 2004). The fact that flavone analogues differing only at position 6 showed drastically different pharmacological properties clearly points to 6-substitution being an important determinant of efficacy and binding sites.

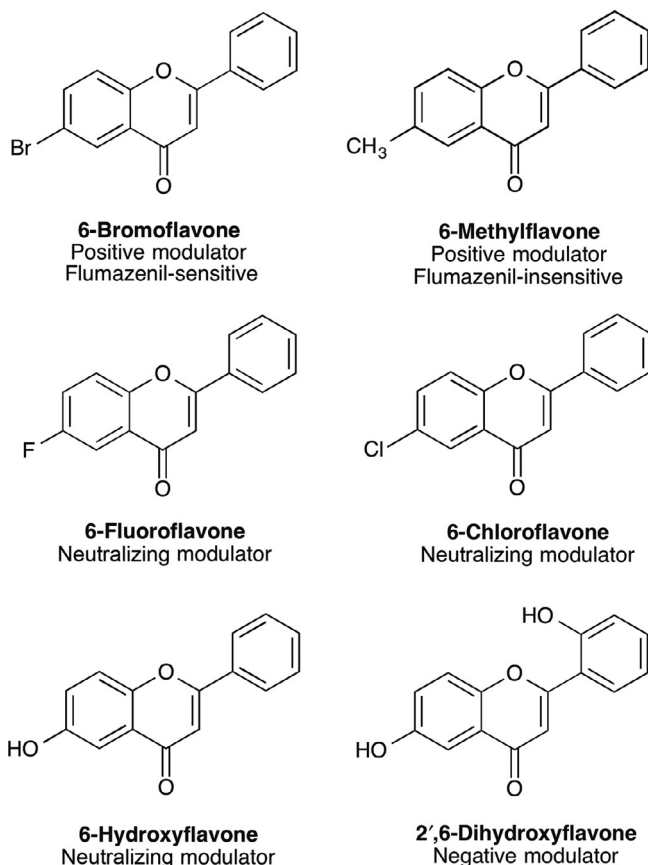
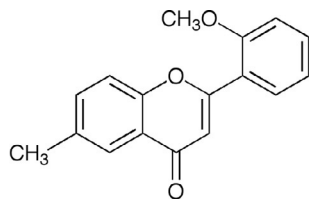


Figure 1 6-Substituted flavones.

2'-Methoxy-6-methylflavone (2'MeO6MF, Fig. 2) acts as a positive modulator at  $\alpha 2\beta 1\gamma 2L$  and all  $\alpha 1$ -containing GABA<sub>A</sub> receptor subtypes (Karim et al., 2012). In contrast, at  $\alpha 2\beta 2/3\gamma 2L$ , it directly activates these receptors without potentiating GABA. This activation is attenuated by bicuculline and gabazine but not flumazenil indicating a novel site. Mutation studies show that position 265 in the  $\beta 1/2$  subunit was key to whether 2'MeO6MF acts as an activator or a potentiator. In hippocampal neurons, 2'MeO6MF directly activated single-channel currents that showed the hallmarks of GABA<sub>A</sub> chloride currents. In the continued presence of 2'MeO6MF, the single-channel conductance increased and these high-conductance channels were disrupted by the  $\gamma(381-403)$  MA peptide, indicating that such currents are mediated by  $\alpha 2/\gamma 2$ -containing GABA<sub>A</sub> receptors. In mice, 2'MeO6MF displayed anxiolytic-like effects in two unconditioned models of anxiety: the elevated plus maze and light/dark tests. 2'MeO6MF induced sedative effects at higher doses in the hole board, actimeter, and barbiturate-induced sleep time tests. No myorelaxant effects were observed in the horizontal wire test (Karim et al., 2012).

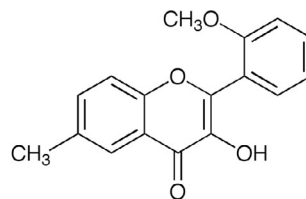
3-Hydroxy-2'-methoxy-6-methylflavone (3-OH-2'MeO6MF, Fig. 2) was found to potentiate GABA-induced currents at recombinant  $\alpha 1/2\beta$  and  $\alpha 1/2/4/6\beta 1-3\gamma 2L$  but not  $\alpha 3/5\beta 1-3\gamma 2L$  receptors (Karim et al., 2011). 3-OH-2'MeO6MF preferentially activated  $\beta 2/3$ - over  $\beta 1$ -containing receptors, with the highest efficacy observed at  $\alpha 2\beta 2/3\gamma 2L$ . In addition, this flavone acted as a potent bicuculline-sensitive allosteric agonist at  $\alpha 4\beta 2/3\delta$  receptors, as a partial agonist at  $\alpha 4\beta 1\delta$  receptors, and was devoid of potentiation effects at extrasynaptic  $\alpha 4\beta 2/3\delta$  receptors. The affinity of 3-OH-2'MeO6MF for  $\alpha 4\beta 2/3\delta$  receptors is 10-fold higher than at  $\alpha 4\beta 1\delta$  GABA<sub>A</sub> receptors. In mice, 3-OH-2'MeO6MF also induced anxiolytic-like effects in the elevated plus maze and light/dark paradigms (Karim et al., 2011). This 6-substituted flavone thus exhibited a unique profile at GABA<sub>A</sub> receptor subtypes.

6-Methoxyflavone and 6-methoxyflavanone (Fig. 2) both act as flumazenil-insensitive positive allosteric modulators of GABA responses at human recombinant  $\alpha 1\beta 2\gamma 2L$  and  $\alpha 2\beta 2\gamma 2L$  GABA<sub>A</sub> receptors. However, unlike 6-methoxyflavone, 6-methoxyflavanone was relatively inactive at  $\alpha 1\beta 2$  GABA<sub>A</sub> receptors. Both flavonoids were found to be inactive as modulators at  $\rho 1$ ,  $\rho 1I307S$ , and  $\rho 1W328M$  GABA receptors but acted as positive allosteric modulators of GABA at the benzodiazepine-sensitive  $\rho 1I307S/W328M$  GABA receptors. This double mutant retains  $\rho 1$  properties of being insensitive to bicuculline and antagonized by TPMPA and THIP.



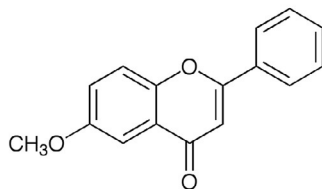
**2'-Methoxy-6-methylflavone**  
**2'-MeO6MF**

positive modulator at  $\alpha 1$  and  $\alpha 2\beta 1\gamma 2L$ , flumazenil-insensitive  
allosteric agonist at  $\alpha 2\beta 2/3\gamma 2L$

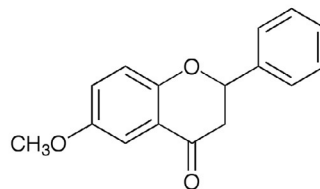


**3-Hydroxy-2'-methoxy-6-methylflavone**  
**3-OH-2'-MeO6MF**

positive modulator at  $\alpha 1/2\beta 2$ ,  $\alpha 1/2/4/6\beta 1-3\gamma 2L/3\gamma 2L$   
with highest efficacy at  $\alpha 2\beta 2/3\gamma 2L$ ,  
flumazenil-insensitive, inactive at  $\alpha 3/5\beta 1-3\gamma 2L$   
allosteric agonist at  $\alpha 4\beta 2/3\gamma 2L$



**6-Methoxyflavone**  
positive modulator at  $\alpha 1/2\beta 2\gamma 2L$ ,  $\alpha 1\beta 2$   
flumazenil-insensitive



**6-Methoxyflavanone**  
positive modulator at  $\alpha 1/2\beta 2\gamma 2L$   
flumazenil-insensitive

**Figure 2** Methoxy flavonoids.

Additionally, 6-methoxyflavanone was also a partial agonist at  $\rho 1W328$  M GABA receptors. The relative inactivity of 6-methoxyflavanone at  $\alpha 1\beta 2$  GABA<sub>A</sub> receptors and its partial agonist action at  $\rho 1W328$  M GABA receptors suggest that it exhibits a unique profile not matched by other flavonoids.

Further studies on 6-substituted flavones are needed to study the binding sites and the complex nature of the activation and modulation of GABA<sub>A</sub> receptor subtypes.

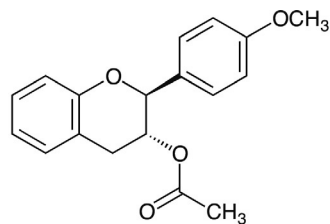


### 3. FLAVAN-3-OL ESTERS

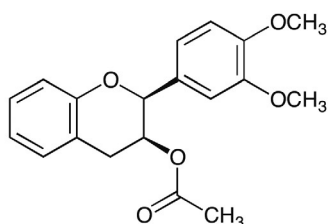
The flavan-3-ol ester Fa131 (*trans*-(2S,3R)-3-acetoxy-4'-methoxyflavan, Fig. 3) is a selective positive modulator of  $\alpha 2$ -containing GABA<sub>A</sub> recombinant receptors and an anxiolytic in mice without sedation (Fernandez, Mewett, Hanrahan, Chebib, & Johnston, 2008). The diastereoisomeric flavan-3-ol ester with an additional 3'-methoxy, Fa173 (*cis*-(2S,3S)-3-acetoxy-3',4'-dimethoxyflavan, Fig. 3) blocks the modulatory actions of Fa131 (Fernandez et al., 2012).

Fa173 also blocks the positive modulatory action of the anesthetic etomidate, the sedative anticonvulsant loreclezole, and selectively blocks the low-affinity effect of diazepam (100  $\mu$ M) at  $\alpha 1\beta 2\gamma 2L$  GABA<sub>A</sub> receptors, but not the high-affinity effect of diazepam (100 nM). Fa173 did not inhibit the positive modulation of GABA by the anesthetic propofol, barbiturate thiopental, or neuroactive steroid allopregnanolone. This suggested that Fa131, etomidate, loreclezole, and high doses of benzodiazepine all exert their positive modulatory effects via a common or overlapping binding site that can be blocked by the neutralizing modulator Fa173. Of these agents, Fa131 alone shows selectivity for  $\alpha 2$ -containing GABA<sub>A</sub> recombinant receptors.

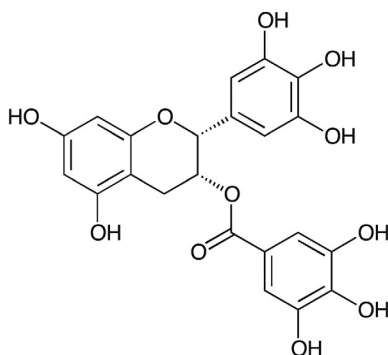
Fa131 and Fa173 were synthesized as simple analogues of the flavon-3-ol ester epigallocatechin gallate (EGCG, Fig. 3) found in green tea. EGCG acts as a negative modulator of GABA<sub>A</sub> receptors in high concentrations. At low concentrations, it has no direct effect on the action of GABA on GABA<sub>A</sub> receptors but potentiates the positive modulation by diazepam. This effect of modulating a modulator has been termed second-order modulation (Campbell, Chebib, & Johnston, 2004). Further evidence of the effects of EGCG on the modulation of GABA<sub>A</sub> receptors at the high-affinity



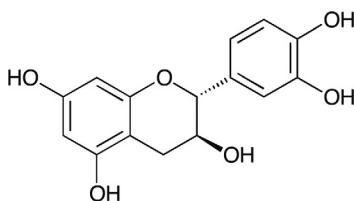
**Fa131**  
Positive modulator  
Flumazenil-insensitive  
Selective for  $\alpha 2$  over  $\alpha 1$ ,  $\alpha 3$ ,  $\alpha 5$   
Non-sedating anxiolytic



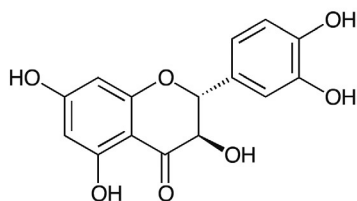
**Fa173**  
Blocks Fa131, etomidate, loreclezole and low but not high affinity diazepam.  
Inactive against propofol, thiopental and allopregnanolone



**(-)-Epigallocatechin gallate (EGCG)**  
Second order positive modulator of primary modulator diazepam  
Counteracts negative modulation by methyl  $\beta$ -carboline-3-carboxylate



**(+)-Catechin**  
Allosteric agonist for GHB  
at  $\alpha 4\beta 3\delta$



**(+)-Taxifolin**  
Negative modulator for GHB  
at  $\alpha 4\beta 3\delta$

**Figure 3** Flavan-3-ols.

benzodiazepine site come from studies on hippocampal neurones where the action of the negative modulator methyl  $\beta$ -carboline-3-carboxylate could be counteracted by EGCG (Vignes, 2013). The anxiolytic action of EGCG may involve a complex action on GABA<sub>A</sub> receptors.



#### 4. (+)-CATECHIN AND $\alpha 4\beta\delta$ GABA<sub>A</sub> RECEPTORS

The natural flavan-3-ol (+)-catechin (Fig. 3) is an allosteric agonist at recombinant  $\alpha 4\beta 3\delta$  receptors expressed in oocytes (Eghorn et al., 2014). (+)-Catechin appears to be a positive allosteric modulator for the high-affinity binding of  $\gamma$ -hydroxybutyric acid (GHB) on these receptors. This action is stereoselective in that (–)-catechin is much less active. Interestingly, the related (+)-taxifolin (Fig. 3), which is identical to (+)-catechin except that it has a ketone in the 4 position, was a negative modulator. (+)-Catechin has been reported to have no action on recombinant  $\alpha 1\beta 2\gamma 2L$  GABA<sub>A</sub> receptors (Campbell et al., 2004), although relatively weak in activity (+)-catechin may aid in further characterization of the GHB high-affinity sites that are likely to be present on certain GABA<sub>A</sub> receptors.



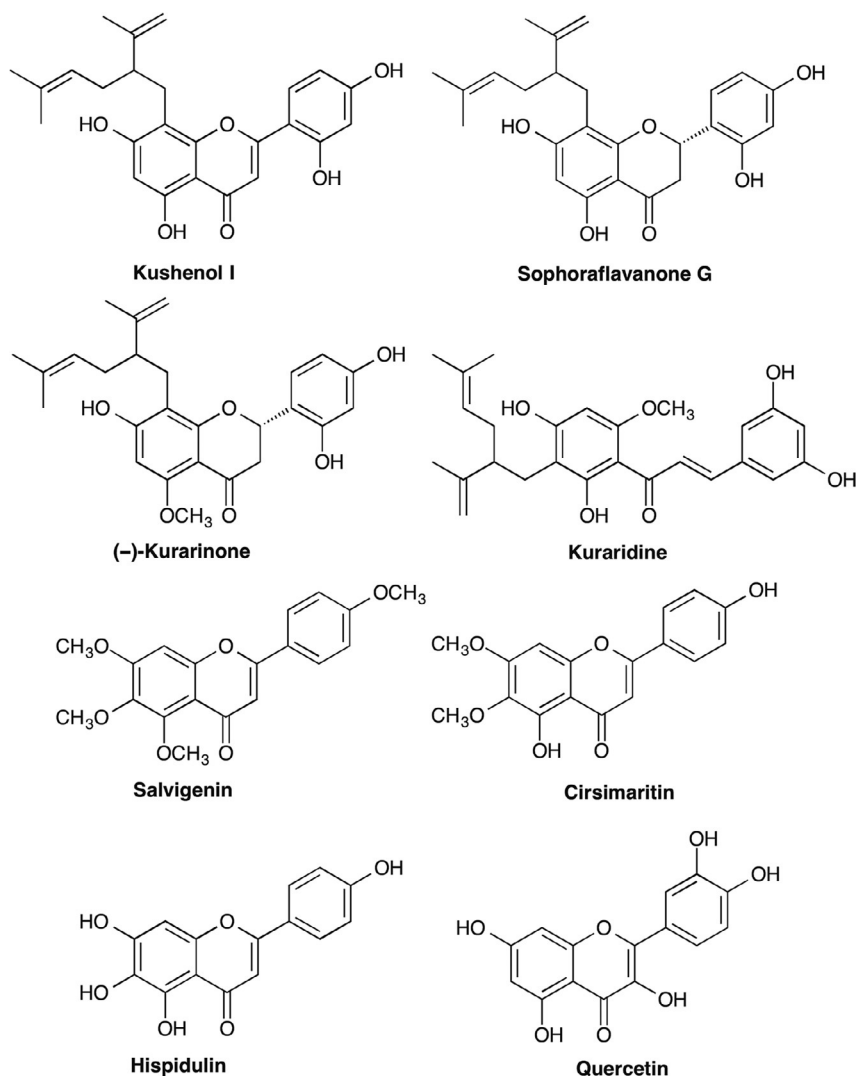
#### 5. NATURAL FLAVONOID AND RELATED COMPOUNDS

Many natural flavonoids have been identified as influencing ionotropic GABA receptors through bioassay-guided fractionation of plant extracts (Fig. 4). HPLC-based activity profiling of extracts the traditional Chinese herbal drug Kushen (*Sophora flavescens* root) led to the identification of the 8-lavandulyl flavonoids, kushenol I, sophoraflavanone G, and (–)-kuraridinone, and the related chalcone kuraridine as GABA<sub>A</sub> receptor modulators (Yang, Baburin, Plitzko, Hering, & Hamburger, 2011). The 8-lavandulyl flavonoids are first representatives of a novel scaffold for this target.

*Salvia* continues to be a rich source of GABA<sub>A</sub> modulators (Kavvadias, Monschein, Sand, Riederer, & Schreier, 2003; Kavvadias et al., 2004). *Salvia triloba*, traditionally known as Greek sage on bioassay-guided fractionation yielded a variety of flavonoids and terpenoids as modulators (Abdelhalim, Chebib, Aburjai, Johnston, & Hanrahan, 2004) (Fig. 4). The flavonoids salvigenin, cirsimaritin, and hispidulin acted as positive modulators when applied in the presence of low concentrations of GABA, but in the presence of high concentrations of GABA acted as negative modulators, demonstrating a biphasic action.

A range of natural flavonoids, including quercetin (Fig. 4), act as negative modulators of GABA<sub>C</sub> receptors containing  $\rho$ -subunits (Goutman, Waxemberg, Donate-Oliver, Pomata, & Calvo, 2003). The receptors are

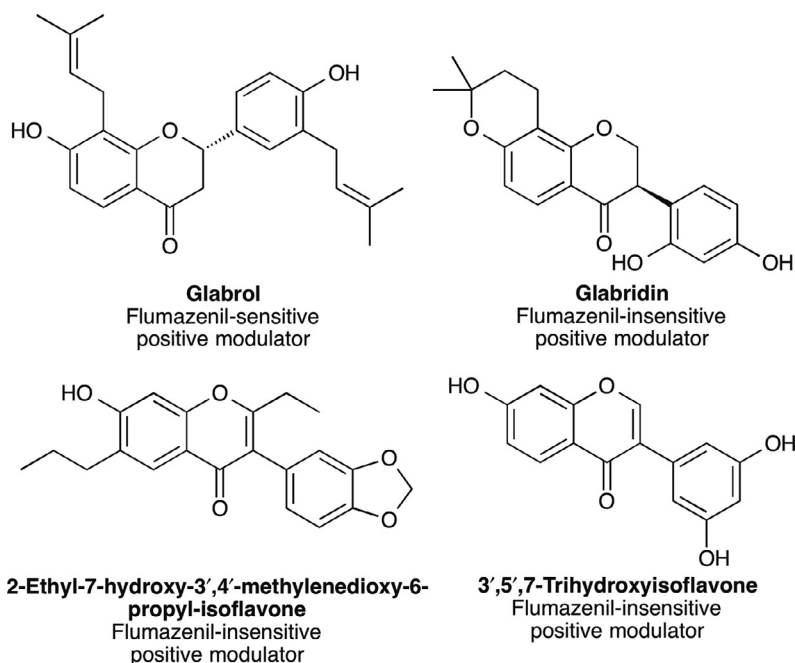




**Figure 4** Natural flavonoids.

insensitive to the classic flumazenil-sensitive high-affinity modulation by benzodiazepines. Further studies have shown that quercetin antagonizes GABA<sub>C</sub> receptors through a redox-independent allosteric mechanism that is prevented by ascorbic acid (Calero et al., 2013).

Glabrol (Fig 5), the major flavonoid in extracts of liquorice (*Glycyrrhiza glabra*, GG), is a flumazenil-sensitive positive modulator of GABA<sub>A</sub>



**Figure 5** Natural and synthetic flavonoids.

receptors (Cho et al., 2012). Glabrol increased sleep duration and decreased sleep latency in a dose-dependent manner. The molecular structure and pharmacophore modeling of glabrol indicate that the isoprenyl groups of glabrol may play a key role in its activity. Glabridin (Fig 5), another sedative-hypnotic flavonoid in extracts of liquorice, is a flumazenil-insensitive positive modulator of GABA<sub>A</sub> receptors in dorsal raphe neurons (Jin et al., 2013).

Glabrol is a flavanone, whereas glabridin is an isoflavan. A series of synthetic isoflavones have also been shown to act as modulators at recombinant GABA<sub>A</sub> receptors. 2-Ethyl-7-hydroxy-3',4'-methylenedioxy-6-propyl-isoflavone (Fig. 5) was the most potent and efficacious of the positive modulators, while 3',5',7-trihydroxyisoflavone (Fig. 5) was the most active of the negative modulators (Gavande, Karim, Johnston, Hanrahan, & Chebib, 2011). The actions of both compounds were flumazenil-insensitive. The variation in activity of these isoflavonoids suggests that further studies of subtype selectivity are warranted.



## 6. CONCLUSION

Understanding the structural determinants of flavonoid effects on ionotropic GABA receptors and developing agents specific for ionotropic GABA receptor subtypes remain a key challenge. Natural flavonoids are a significant part of our diet. As they may readily cross the blood–brain barrier, it is important that we understand how natural flavonoids might influence brain function. Synthetic flavonoids are attractive leads for drugs to treat brain dysfunction. They are useful for investigating the role of the modulatory sites at GABA<sub>A</sub> receptors, determining potential binding sites and the development of GABA<sub>A</sub> subtype selective agents. Significant progress has been made since our 2011 review (Hanrahan et al., 2011), but much remains to be done.

## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

## ACKNOWLEDGMENTS

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