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Modulation of Ionotropic GABA Receptors by Natural Products of Plant Origin

I. Chapter Overview

There is now an impressive array of natural products of plant origin that are known to influence the function of ionotropic receptors for GABA. The major chemical classes of such natural products are flavonoids, terpenoids, phenols, and polyacetylenic alcohols. While it was the interaction of flavonoids with benzodiazepine modulatory sites on $GABA_A$ receptors that led to the great interest in flavonoids as positive modulators of such receptors, many of the interactions between flavonoids and $GABA_A$ receptors do not involve classical flumazenil-sensitive benzodiazepine sites. There are significant synergistic interactions between some of these positive modulators, for example, between substances isolated from *Valeriana officinalis*. Thus, the sleep inducing effects of hesperidin are potentiated by 6-methylapigenin, while the sedating and sleep inducing effects of valerenic acid are dramatically potentiated when co-administered with the flavonoid glycoside linarin.

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The discovery of second order positive modulators adds an exciting new dimension to the concept of allosteric modulation of $GABA_A$ receptors. Second order positive modulators act only in conjunction with a specific first order positive modulator. The dietary flavonoids apigenin and (−)-epigallocatechin gallate, under conditions in which they have no direct action on the activation of $GABA_A$ receptors by GABA, have been shown to enhance the first order positive modulatory action of diazepam. The complexity of the interactions between active constituents of herbal preparations indicates that functional assays are vital for the quality control of such preparations. Understanding such complexity is likely to provide greater insight into the mechanisms underlying the allosteric modulation of ionotropic GABA receptors.

II. Introduction

Natural products of plant origin represent a rich diversity in chemical structures that has led to the discovery of important therapeutic agents. There is now an impressive array of natural products that are known to influence the function of ionotropic receptors for GABA, the major inhibitory neurotransmitter in the brain. Many of the chemicals first used to study ionotropic GABA receptors are of plant origin including the antagonists bicuculline (from *Dicentra cucullaria*) (Curtis *et al.*, 1970) and picrotoxin (from *Anamirta cocculus*) (Jarboe *et al.*, 1968) and the agonist muscimol (from *Amanita muscaria*) (Johnston *et al.*, 1968). We now know that a wide range of plant-derived flavonoids, terpenes, and related substances modulate the function of ionotropic GABA receptors. Such GABA modulators have been found in fruit (e.g., grapefruit), vegetables (e.g., onions), various beverages (including tea, red wine, and whiskey), and in herbal preparations (such as *Ginkgo biloba* and *Ginseng*). These substances are known to cross the blood–brain barrier and are thus able to influence brain function.

There is increasing community acceptance of herbal medicines and functional foods. The occurrence of substances that could modulate GABA receptor function in such preparations may underlie some of the actions that herbal medicines and functional foods may have on brain function. There is a widely held view that "natural substances" are inherently safer than "unnatural substances," that is, synthetic chemicals. In fact, many of the most toxic chemicals are natural products and the majority of therapeutic agents are synthetic. It is the molecular structure and dose that determine the effects of substances on human health, not whether they are of natural or synthetic origin (Topliss *et al.*, 2002). In the present context, it is the chemical diversity of natural substances and their effects on brain function that are important both to provide a rational basis for the understanding of the effects of dietary chemicals and herbal

products and to lead to the development of new therapeutic agents and strategies.

This review is directed toward the effects of some natural products of plant origin on the function of ionotropic GABA receptors. GABA itself is an important plant constituent, widely studied as a metabolite involved in responses to stress (Bouche and Fromm, 2004), but it may also have a role as a signaling molecule (Bouche *et al.*, 2003) and in regulating pollen tube growth and guidance (Yang, 2003). The presence of GABA can be a confounding factor in the bioactivity-guided fractionation of extracts of traditional medicinal plants using GABA/benzodiazepine binding assays (Misra, 1998). Furthermore, the use of benzodiazepine binding assays appears unwise in view of the discovery of an increasing number of agents that modulate ionotropic GABA receptors independently of classical flumazenil-sensitive benzodiazepine sites (Johnston, 2005).

III. Ionotropic GABA Receptors

Ionotropic receptors for the inhibitory neurotransmitter GABA are found on most, if not all, neurons in the central nervous system (CNS) (Chebib and Johnston, 2000). They are ligand-gated ion channels that mediate fast neurotransmission via a central pore constituted by five surrounding protein subunits that on activation by GABA is permeant to chloride ions. They belong to the nicotinicoid superfamily of ligand-gated ion channels (Le Novere and Changeux, 2001) that includes nicotinic acetylcholine, strychnine-sensitive glycine, and $5HT_3$ receptors. The family of ionotropic GABA receptors is divided into two subfamilies, $GABA_A$ and $GABA_C$ receptors on the basis of their ability to form endogenous functional heteromeric and homomeric receptors, respectively, and differences in their physiological and pharmacological properties (Chebib and Johnston, 2000).

The heteromeric $GABA_A$ receptors are made up of different protein subunits (e.g., a common makeup involves two $\alpha 1$, two $\beta 2$, and one $\gamma 2$ subunits). There are 16 different subunits comprising the $GABA_A$ receptor family: $\alpha 1{-}\mathbf{6}$, $\beta 1{-}3$, $\gamma 1{-}3$, δ, ϵ, π, and θ (Whiting, 2003). In addition, there are splice variants of many of these subunits. While the potential structural diversity of $GABA_A$ receptors is huge, studies of native $GABA_A$ receptors suggest that there may be fewer than 20 widely occurring $GABA_A$ receptor subtype combinations (McKernan and Whiting, 1996; Whiting, 2003). There is less diversity in the homomeric $GABA_C$ receptors in that they are made up exclusively of either $\rho 1$, $\rho 2$, or $\rho 3$ subunits, although "pseudoheteromeric" $GABA_C$ receptors have been described (Johnston *et al.*, 2003).

A. $GABA_A$ Receptors as Therapeutic Targets

Enhancing the action of GABA on $GABA_A$ receptors is a key property of several classes of important therapeutic agents including the benzodiazepines, barbiturates, and many general anesthetics. With advances in our understanding of the molecular diversity of $GABA_A$ receptors, there is an urgent need for the development of agents acting on subtypes of these receptors. The potential therapeutic market for subtype selective modulators of $GABA_A$ receptors is huge with particular emphasis on the treatment of anxiety, cognitive disorders, epilepsy, insomnia, and schizophrenia.

Heritable mutations are known to occur across the nicotinicoid superfamily of ligand-gated ion channels including $GABA_A$ receptors (Vafa and Schofield, 1998). For example, Angelman syndrome, a neurodevelopmental disorder characterized by severe mental retardation, epilepsy, and delayed motor development, has been associated with deletions of $GABA_A$ receptor $\beta 3$ subunits (Holopainen *et al.*, 2001). Heritable mutations in $GABA_A$ receptor subunits are strongly implicated in idiopathic generalized epilepsies (Jones-Davis and Macdonald, 2003). GABA systems are known to play an important role in sleep, and modulators of $GABA_A$ receptors are widely used to promote restful sleep (Gottesmann, 2002).

The subunit composition of $GABA_A$ receptors influences the effects of modulators. The therapeutically useful properties of benzodiazepines (anxiolytic, anticonvulsant, sedative, and muscle-relaxant effects) may result from actions on different $GABA_A$ receptor subtypes. Studies of mice deficient in particular α subunits suggest that the $\alpha 1$-$GABA_A$ subunit is responsible for the sedative properties of benzodiazepines, while the $\alpha 2$-$GABA_A$ subunit is responsible for the anxiolytic properties (McKernan *et al.*, 2000). The δ subunit has been shown to confer significantly increased sensitivity to ethanol at $GABA_A$ receptors (Wallner *et al.*, 2003).

Agents that enhance the action of GABA on $GABA_A$ receptors are known as positive modulators (Johnston, 1996) and are generally considered to involve action at allosteric sites on $GABA_A$ receptors remote from the GABA recognition sites (orthosteric sites). Such allosteric sites are highly valued as targets for the development of subtype specific drugs, since there is generally greater diversity between receptor subtypes in amino acid sequence at allosteric sites than at orthosteric sites (Christopoulos, 2002). Agents that reduce the action of GABA on $GABA_A$ receptors are known as negative allosteric modulators (once known as "inverse agonists," since they have the opposite actions to those of the classical benzodiazepines). Agents that block the actions of both positive and negative allosteric modulators are known as neutralizing allosteric modulators, for example, the classical benzodiazepine "antagonist" flumazenil (Johnston, 1996).

The discovery of second order positive modulators adds an exciting new dimension to the concept of allosteric modulation of $GABA_A$ receptors

(Campbell *et al.*, 2004). While it is known that some first order positive modulators interact positively with each other, for example, ethanol and neurosteroids (Akk and Steinbach, 2003), second order positive modulators act only in conjunction with a specific first order positive modulator. The dietary flavonoids apigenin and (−)-epigallocatechin gallate, under conditions in which they have no direct effect on the activation of $GABA_A$ receptors by GABA, have been shown to enhance the first order positive modulatory action of diazepam. The second order modulatory action of these flavonoids appears to be specific for first order benzodiazepine modulators as it is not observed with first order modulators such as allopregnanolone or pentobarbitone. Such second order modulation may result from alteration in the coupling of benzodiazepine allosteric sites with the orthosteric GABA sites on $GABA_A$ receptors. The second order modulation of a primary modulator may represent a novel form of drug action that is unlikely to be restricted to the modulation of $GABA_A$ receptors (Campbell *et al.*, 2004). In addition to offering increased chemical diversity of agents acting on $GABA_A$ receptors, second order allosteric modulators offer further possibilities in that they could influence the action of endogenous first order modulators and also offer the opportunity of reducing the dose needed of a drug acting as a first order modulator such as a benzodiazepine.

B. $GABA_C$ Receptors as Therapeutic Targets

There is evidence for functional $GABA_C$ receptors in the retina, spinal cord, superior colliculus, pituitary, and gastrointestinal tract. Given the lower abundance and less widespread distribution of $GABA_C$ receptors in the CNS compared to $GABA_A$ receptors, $GABA_C$ receptors may be a more selective drug target than $GABA_A$ receptors (Johnston *et al.*, 2003). The major indications for drugs acting on $GABA_C$ receptors are in the treatment of visual, sleep, and cognitive disorders. Agents acting on $GABA_C$ receptors may be useful for the treatment of myopia (Froestl *et al.*, 2004). A study has linked $GABA_C$ receptors to Alzheimer's disease by providing evidence that the stimulation of $GABA_C$ receptors has a neuroprotective action against amyloid β protein (Liu *et al.*, 2005).

IV. Flavonoids

Flavonoids are responsible for many of the brilliant colors of fruits and vegetables and are important constituents of red wine, green tea, and many herbal preparations. Fruits, vegetables, and beverages, such as tea and red wine, are major sources of flavonoids in our diet (Aherne and O'Brien, 2002). It has been estimated that the average daily intake of flavonoids is 1–2 g (Havsteen, 2002). Many flavonoids are polyphenolic and are thus

strongly antioxidant (Heim *et al.*, 2002). They have a wide variety of biological activities and are being studied intensively as anticancer agents (Le Marchand, 2002) and for their effects on the vascular system (Woodman and Chan, 2004). More than 5000 different flavonoids have been described.

Flavonoids have a range of activities on $GABA_A$ receptors (Marder and Paladini, 2002) and have been described as a "new family of benzodiazepine receptor ligands" (Medina *et al.*, 1997). They were first linked to $GABA_A$ receptors when three isoflavans isolated from bovine urine were shown to inhibit diazepam binding to brain membranes (Luk *et al.*, 1983). The most potent compound was 3′,7-dihydroxyisoflavan (Fig. 1) with an IC_{50} of 45 μM. These isoflavans were most probably derived from plant sources in the bovine diet. Subsequently many flavonoids directly isolated from plants were shown to influence benzodiazepine binding (Marder and Paladini, 2002).

A low-affinity benzodiazepine site is emerging as a possible target for the modulatory action of some flavonoids. This site is insensitive to flumazenil and has been described on a wide range of $GABA_A$ receptors including those made up of only $\alpha 1\beta 2$ subunits (Walters *et al.*, 2000).

A. Amentoflavone

The biflavonoid amentoflavone (Fig. 1) has one of the most potent actions of any plant-derived flavonoid in displacing benzodiazepine binding to rat brain membranes with a nM affinity comparable to that of diazepam (Nielsen *et al.*, 1988). Further studies on amentoflavone, however, illustrate the difficulties in investigating flavonoid actions—the variety of effects, the lack of selectivity, the need for functional assays, and the mismatch between *in vitro* and *in vivo* findings.

Amentoflavone was isolated from Karmelitter Geist®, an alcoholic tincture of various plants used to treat anxiety and epilepsy. However, it was concluded that amentoflavone could not be responsible for any pharmacological effects of the plant extract as amentoflavone did not influence flunitrazepam binding in the brain *in vivo* following i.v. administration to mice (Nielsen *et al.*, 1988). It was suggested that amentoflavone was either rapidly metabolized or did not cross the blood–brain barrier, but a study does indicate that amentoflavone does cross the blood–brain barrier (Gutmann *et al.*, 2002).

Amentoflavone occurs in a variety of herbal preparations including St John's wort (Baureithel *et al.*, 1997). It can be extracted from *Ginkgo biloba* but is removed from herbal preparations of *Ginkgo* such as EGb 761 (Hanrahan *et al.*, 2003). A comprehensive battery of *in vitro* binding assays has shown that amentoflavone influences a variety of G-protein–coupled receptors for serotonin, dopamine, and opioids at nM concentrations while

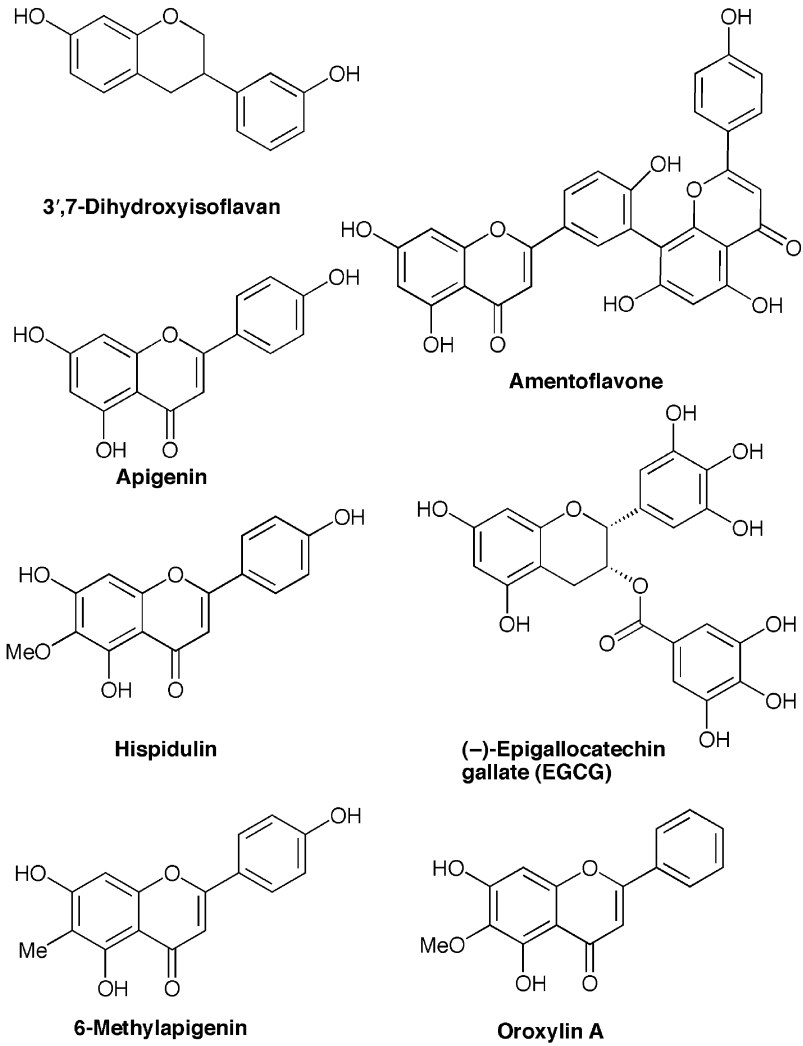


FIGURE 1 Some representative flavonoids that have been shown to influence benzodiazepine binding to brain membranes (3′,7-dihyroxyisoflavan, amentoflavone, apigenin, 6-methylapigenin, and oroxylin A), to act at $GABA_A$ receptor as positive modulators (hispidulin) or negative modulators (amentoflavone, apigenin), or at $GABA_C$ receptors as negative modulators (apigenin). In addition, apigenin and (−)-epigallocatechin gallate have been found to have a novel second order modulatory action on the first order modulation of $GABA_A$ receptors by diazepam.

having no effect on the binding of the $GABA_A$ agonist muscimol to $GABA_A$ receptors (Butterweck *et al.*, 2002). Using a functional assay employing recombinant $\alpha 1\beta 2\gamma 2L$ $GABA_A$ receptors expressed in oocytes, amentoflavone has been shown to be a relatively weak (EC_{50} 4 μM)

negative allosteric modulator of GABA action acting independently of classical flumazenil-sensitive benzodiazepine modulatory sites (Hanrahan *et al.*, 2003). It may be that amentoflavone has different effects on other $GABA_A$ receptor subtypes.

B. Apigenin—the Concept of Second Order Positive Modulation

Apigenin (5,7,4′-trihydroxyflavone) (Fig. 1) is a common flavonoid found in a range of plants, including chamomile (*Matricicaria recutita*). The traditional use of chamomile tea as a treatment for insomnia and anxiety led to investigations of its active constituents including apigenin. Apigenin was found to have anxiolytic properties, and it competitively inhibited the binding of flunitrazepam to brain membranes without influencing the binding of muscimol to $GABA_A$ receptors (Viola *et al.*, 1995). Apigenin was described as having "a clear anxiolytic effect in mice in the elevated plus maze without evidencing sedation or muscle relaxation effects at doses similar to those used for classical benzodiazepines" while being devoid of anticonvulsant effects (Viola *et al.*, 1995). These findings are in contrast to a later study in rats where apigenin was shown to reduce the latency of onset of picrotoxin-induced convulsions and to reduce locomotor activity but was devoid of anxiolytic or muscle relaxant activities (Avallone *et al.*, 2000). This later study showed that apigenin could reduce GABA-activated chloride currents in cultured cerebellar granule cells, an action that could be blocked by flumazenil and thus likely to involve classical benzodiazepine allosteric sites on $GABA_A$ receptors. The inhibitory action of apigenin on locomotor behavior, however, could not be blocked by flumazenil and thus could not "be ascribed to an interaction with $GABA_A$-benzodiazepine receptors but to other neurotransmitter systems" (Avallone *et al.*, 2000). Another study from the same group reported that apigenin exerted sedative effects on locomotor activity in rats in a flumazenil-insensitive manner, whereas chrysin, a structurally related flavonoid lacking the 4′-hydroxy substituent of apigenin, showed a clear flumazenil-sensitive anxiolytic effect in addition to the flumazenil-insensitive sedation (Zanoli *et al.*, 2000). The apparent discrepancy between the behavioral effects of apigenin on mice (Viola *et al.*, 1995) and rats (Avallone *et al.*, 2000) may be due to mice having higher baseline levels of anxiety. Flumazenil-insensitive effects of flavonoids on $GABA_A$ receptors have been extensively described (Hall *et al.*, 2004).

Studies on human recombinant receptors in oocytes have shown that apigenin inhibited the activation of $\alpha1\beta1\gamma2S$ $GABA_A$ receptors in a flumazenil-insensitive manner and had a similar effect on $\rho1$ $GABA_C$ receptors (Goutman *et al.*, 2003). Other studies on recombinant $\alpha1\beta2\gamma2L$ $GABA_A$ receptors also found an inhibitory effect of apigenin on GABA responses

and, in addition an enhancement of the diazepam-induced positive allosteric modulation of GABA responses by lower concentrations of apigenin, described as a second order modulation by apigenin of the first order modulation by diazepam (Campbell *et al.*, 2004).

The novel second order modulation by apigenin of the maximum first order modulatory action of diazepam of the activation by GABA of $GABA_A$ receptors observed in these studies may result from apigenin altering the coupling of the benzodiazepine allosteric sites with the orthosteric GABA sites on $GABA_A$ receptors (Campbell *et al.*, 2004). There is evidence from binding studies that the nexus between the benzodiazepine and GABA sites on $GABA_A$ receptors is complex and involves other factors, such as phospholipids, that can be removed from brain membranes by detergent extraction (Skerritt *et al.*, 1982).

The flumazenil-sensitive anxiolytic effects of apigenin may be the result of apigenin enhancing a subthreshold effect of an endogenous benzodiazepine system (Baraldi *et al.*, 2000). Evidence for physiologically relevant endozepines has come from the discovery of a mutant $GABA_A$ receptor in childhood absence epilepsy and febrile seizures that has diminished sensitivity to benzodiazepines with no other apparent alteration in functioning (Wallace *et al.*, 2001).

Overall, it seems that the effects of apigenin on $GABA_A$ receptors are complex and involve both flumazenil-sensitive and flumazenil-insensitive components and that other receptors could be involved in the behavioral effects of apigenin. Like most flavonoids, apigenin is known to have a wide variety of biological actions including effects on adenosine receptors (Jacobson *et al.*, 2002) and progestational activity (Zand *et al.*, 2000). Of particular interest are the findings that apigenin at concentrations at which it inhibits $GABA_A$ and $GABA_C$ receptors also inhibits NMDA receptors (Losi *et al.*, 2004); such an action could contribute to flumazenil-insensitive sedative actions of apigenin.

C. Hispidulin and Related Flavonoids

Hispidulin (4′,5,7-trihydroxy-6-methoxyflavone, i.e., the 6-methoxy derivative of apigenin) (Fig. 1) was isolated together with apigenin from *Salvia officinalis* (sage) using a benzodiazepine binding assay-guided fractionation (Kavvadias *et al.*, 2003). Hispidulin was some 30 times more potent than apigenin in displacing flumazenil binding. Preparations of sage have been used in herbal medicine to assist memory (Perry *et al.*, 1999a, 2000), and an extract of *Salvia lavandulaefolia* (Spanish sage) has been shown to enhance memory in healthy young volunteers (Tildesley *et al.*, 2003). Unlike apigenin, hispidulin has been shown to act as a positive allosteric modulator of $\alpha 1,3,5,6\beta 2\gamma 2S$ $GABA_A$ receptor subtypes showing little subtype selectivity, although being a little more potent at $\alpha 1,2,5\beta 2\gamma 2S$

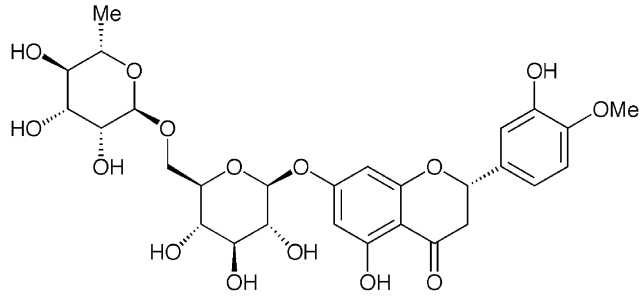
subtypes than at $\alpha 3,6\beta 2\gamma 2S$ subtypes (Kavvadias *et al.*, 2004). The positive modulatory action of 10-μM hispidulin at $\alpha 1\beta 2\gamma 2S$ receptors was reduced from 47% to 17% by flumazenil, indicating that sites other than classical flumazenil-sensitive benzodiazepine sites were involved in the action of hispidulin. As hispidulin did not influence the action of GABA on $\alpha 1\beta 2$ $GABA_A$ receptors, this indicates that it does not interact with low-affinity flumazenil-insensitive benzodiazepine sites (Walters *et al.*, 2000) in contrast to other flavonoids such as 6-methylflavone (Hall *et al.*, 2004). Of significance is the ability of hispidulin to act as a positive modulator at $\alpha 6\beta 2\gamma 2L$ $GABA_A$ receptors unlike diazepam; 10-μM hispidulin enhanced the action of GABA at these receptors by 65%, this action being reduced by 1-μM flumazenil to 37% (Kavvadias *et al.*, 2004). Hispidulin was shown to have an anticonvulsant action in seizure prone Mongolian gerbrils and to pass the blood–brain barrier (Kavvadias *et al.*, 2004).

Flavonoids structurally related to hispidulin that influence benzodiazepine binding have been isolated from *Scutellaria baicalensis*, an important herb in traditional Chinese medicine (Wang *et al.*, 2002). Oroxylin A (5,7-dihydroxy-6-methoxyflavone, i.e., hispidulin lacking the 4′-hydroxy group) (Fig. 1) inhibits flunitrazepam binding at 1 μM and on oral administration acts as a neutralizing allosteric modulator blocking the anxiolytic, myorelaxant, and motor incoordination effects but not the sedative and anticonvulsant effects elicited by diazepam (Huen *et al.*, 2003b). 6-Methylapigenin (4′,5,7-dihydroxy-6-methylflavone) (Fig. 1) isolated from *Valeriana wallichii*, a known sedative herb, influences benzodiazepine binding at 0.5 μM in manner suggesting it may be a positive modulator of $GABA_A$ receptors (Wasowski *et al.*, 2002). 6-Methylapigenin has anxiolytic properties and is able to potentiate the sleep-enhancing properties of hespiridin, a flavanone glycoside also isolated from *Valeriana officinalis* (Marder *et al.*, 2003).

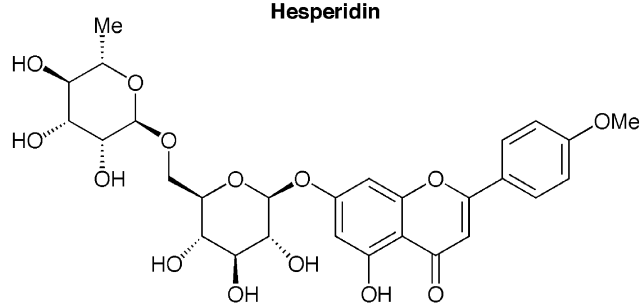
Thus, flavones substituted in the 6-position with a methoxy or methyl substituent have interesting effects on $GABA_A$ receptor function and may contribute to the properties of some herbal preparations. Natural and synthetic 2′-hydroxy–substituted flavones are also of interest (Huen *et al.*, 2003a). Several flavonoid glycosides including goodyerin (Du *et al.*, 2002), linarin, and hesperidin (Fig. 2) (Fernandez *et al.*, 2004) are also being studied as sedative and anticonvulsant agents likely to interact with $GABA_A$ receptors.

D. (−)-Epigallocatechin Gallate—a Potent Second Order Modulator

Green tea polyphenols are being considered as therapeutic agents in well-controlled epidemiological studies, aimed to alter brain aging processes and to serve as possible neuroprotective agents in progressive neurodegenerative



Hesperidin



Linarin

FIGURE 2 Two flavonoid glycosides found in *Valeriana officinalis* that show synergistic effects with other positive modulators of $GABA_A$ receptor function.

disorders such as Parkinson's and Alzheimer's diseases (Weinreb *et al.*, 2004).

(−)-Epigallocatechin-3-gallate (EGCG) (Fig. 1) is the major polyphenol in green tea (*Camellia sinensis*). EGCG was found to have an inhibitory action on the activation by GABA of bovine recombinant $\alpha1\beta1$ $GABA_A$ (Hossain *et al.*, 2002b). However, in studies on $\alpha1\beta2\gamma2L$ $GABA_A$ receptors, apigenin at low concentrations (0.1 μM) showed a potent second order modulatory action on the first order modulation by diazepam, while inhibiting the action of GABA at higher concentrations (>1 μM) (Campbell *et al.*, 2004). EGCG was thus an order of magnitude more potent than apigenin in acting as a second order modulator. EGCG and apigenin may serve as lead compounds for the development of more selective agents for the second order modulation of benzodiazepine enhancement of the action of GABA on $GABA_A$ receptors (Campbell *et al.*, 2004).

There is much interest in the anticancer and antitumor properties of EGCG associated with the consumption of green tea (Lambert and Yang, 2003). EGCG has anticancer effects on ovarian carcinoma cell lines (Huh *et al.*, 2004), is a selective inhibitor of COX-2 expression (Hussain *et al.*, 2005), and induces apoptosis in monocytes (Kawai *et al.*, 2005). Little is

known about the CNS actions of this flavan, but it is found in the brain after gastric administration to mice (Suganuma *et al.*, 1998) and is neuroprotective in rats on i.p. injection after focal ischemia (Choi *et al.*, 2004) and in a mouse model of Parkinson's disease (Levites *et al.*, 2002). This neuroprotective action may be associated with its antioxidant properties, but enhancement of $GABA_A$ mediated synaptic inhibition could also contribute (Campbell *et al.*, 2004). In addition, EGCG is known to reduce glutamate-induced cytotoxicity via intracellular calcium ion modulation suggesting that other neurotransmitters systems may be involved (Lee *et al.*, 2004).

V. Terpenoids

Terpenoids are widespread in plants, especially in what are known as essential oils that can be extracted from plants, and have a wide range of uses from perfume constituents to paint thinners. Terpenoids are oxygenated products formally derived from C5 isoprene units and are classified by the number of C5 units in their structure. Thus, monoterpenoids have 2 × C5 units, sesquiterpenoids 3 × C5 units, diterpenoids 4 × C5 units, and triterpenoids 6 × C5 units. The most widely used terpenoid in studies on $GABA_A$ receptors is the sesquiterpenoid lactone picrotoxinin (Fig. 4), a noncompetitive antagonist at $GABA_A$ receptors (Chebib and Johnston, 2000). A number of other terpenoids, however, are of interest for their actions on $GABA_A$ receptors.

A. Monoterpenoids—α-Thujone, Thymol, Thymoquinone, Borneol

The monoterpenoid α-thujone (Fig. 3) is a psychoactive component of absinthe, a liqueur popular in France in the nineteenth and early twentieth centuries. It is found in extracts of wormwood (*Artemisia absinthium*) and some other herbal medicines and beverages since ancient Egyptian times (Deiml *et al.*, 2004). α-Thujone is a convulsant that acts as a negative allosteric modulator of $GABA_A$ receptors (Hold *et al.*, 2000). It also acts as an antagonist of $5HT_3$ receptors by influencing agonist-induced desensitization (Deiml *et al.*, 2004).

The structurally related substance thymol (Fig. 3), a constituent of thyme essential oil, is a flumazenil-insensitive positive allosteric modulator of $GABA_A$ receptors (Priestley *et al.*, 2003). At higher concentrations, thymol had a direct action on $GABA_A$ receptors similar to that of the anesthetic propofol and other phenols (Mohammadi *et al.*, 2001). The anticonvulsant effects of thymoquinone (Fig. 3), the major constituent of *Nigella sativa* seeds, may be due to positive modulation of $GABA_A$ receptors (Hosseinzadeh and Parvardeh, 2004).

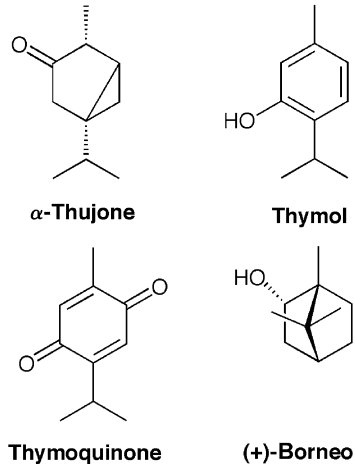


FIGURE 3 Monoterpenoids (α-thujone, thymol, thymoquinone, and (+)-borneol) that act as positive modulators of $GABA_A$ receptor function.

(+)-Borneol (Fig. 3), a monoterpenoid found in many essential oils, is a flumazenil-insensitive positive allosteric modulator of human recombinant $\alpha1\beta2\gamma2L$ $GABA_A$ receptors of low affinity (EC_{50} 250 μM) but very high efficacy producing a 10-fold enhancement of the action of 10-μM GABA at a concentration of 450 μM (Granger *et al.*, 2005). (−)-Borneol showed similar positive modulatory properties to (+)-borneol, while isoborneol, (−)-bornyl acetate, and camphor (a known convulsant) were much less active. The relatively rigid cage structure of these bicyclic monoterpenes and their high efficacy may aid in a greater understanding of the molecular aspects of positive modulation. (+)-Borneol is found in high concentrations in extracts of *Valeriana officinalis* that are widely used to reduce the latency of sleep onset, the depth of sleep, and the perception of well-being. Extracts of *Valeriana* are known to contain a large number of constituents including flavonoids and terpenoids, many of which are considered to be active at $GABA_A$ receptors.

B. Sesquiterpenoids—Bilobalide, Picrotoxinin, Valerenic Acid, and Isocurcumenol

Bilobalide (Fig. 4), a sesquiterpenoid lactone from *Ginkgo biloba* that bears some structural similarities to picrotoxinin, including a lipophilic side chain and a hydrophilic cage, is an antagonist at $GABA_A$ receptors (Huang *et al.*, 2003). Both bilobalide and picrotoxinin appear to act at sites in the chloride channel of $GABA_A$ receptors and are thus negative allosteric modulators, although their mode of action is complex. They also

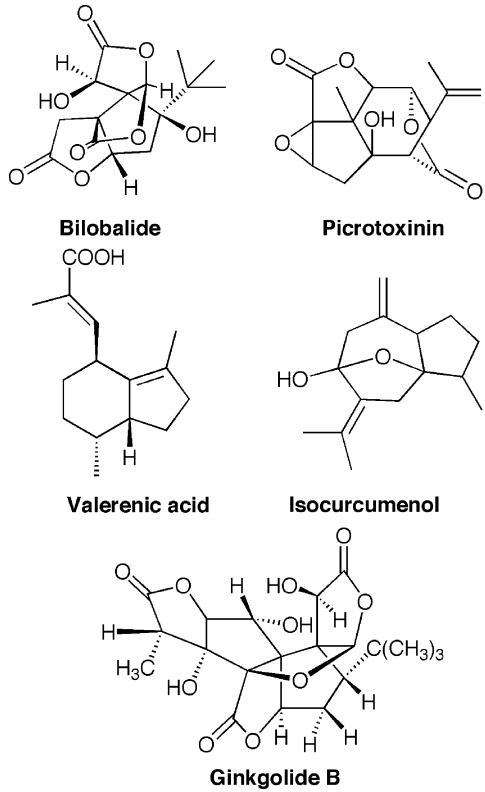


FIGURE 4 Sesquiterpenoids and a related compound that act as negative modulators of $GABA_A$ and $GABA_C$ receptor function (bilobalide, picrotoxinin, and ginkgolide B) or as positive modulators of $GABA_A$ receptor function (valerenic acid, isocurcumenol).

act similarly on $\rho 1$ $GABA_C$ receptors (Huang *et al.*, 2006; Qian *et al.*, 2005).

The cognition-enhancing effects of *Ginkgo* extracts may be partly mediated by bilobalide acting to enhance hippocampal pyridamidal neuronal excitability (Sasaki *et al.*, 1999b). While picrotoxinin is a convulsant, bilobalide is an anticonvulsant (Sasaki *et al.*, 1999a,b). The lack of convulsant action in an agent that reduces GABA action may be important for enhancement of cognition. The lack of convulsant action of bilobalide may result from inhibition of glutamate release (Jones *et al.*, 2002). Bilobalide has a neuroprotective action in a variety of models (DeFeudis, 2002). The structurally related ginkgolides, especially ginkgolide B (Fig. 4), also act as negative modulators at $GABA_A$ receptors (Huang *et al.*, 2004; Ivic *et al.*, 2003). They also inhibit strychnine-sensitive glycine receptors and platelet activating factor (Chatterjee *et al.*, 2003; Ivic *et al.*, 2003). Bilobalide and

the ginkgolides reduce barbiturate-induced sleeping time in mice, an effect perhaps relevant to the clinically observed "vigilance-enhancing" and antidepressant-like actions of *Ginkgo* extracts (Brochet *et al.*, 1999).

The sesquiterpenoid valerenic acid (Fig. 4) has a direct partial agonist action on $GABA_A$ receptors (Yuan *et al.*, 2004). Valerian extract and valerenic acid are partial agonists of the $5\text{-}HT_{5a}$ receptor *in vitro* (Dietz *et al.*, 2005). Valerenic acid is often assumed to be the most important active component of valerian extracts used in herbal medicine, but valerenic acid is only present in *Valeriana officinalis* and not in other active species widely used like *V. wallichii* and *V. edulis* (Fernandez *et al.*, 2004). The sedating and sleep-inducing effects of valerenic acid are dramatically potentiated when co-administered with the flavonoid glycoside linarin (Fig. 2) that is also found in *Valeriana officinalis* (Fernandez *et al.*, 2004).

Isocurcumenol (Fig. 4), a sesquiterpenoid from *Cyperus rotundus*, was found to inhibit flumazenil binding and enhance flunitrazepam binding in the presence of GABA in a manner consistent with it acting as a positive allosteric modulator (Ha *et al.*, 2002).

C. Diterpenoids

The diterpenoid quinone miltirone (Fig. 5), from the Chinese medicinal herb *Salvia miltriorrhiza*, inhibited flunitrazepam binding at 0.3 μM and was orally active in animal models as a tranquilliser without muscle relaxant properties (Lee *et al.*, 1991). Structure–activity studies on miltirone led to the development of a synthetic compound that was much more potent than miltirone on flunitrazepam binding (IC_{50} 0.05 μM) (Chang *et al.*, 1991). The diterpenoid lactone galdosol (Fig. 5) from the common sage *Saliva officinalis*, inhibited flumazenil binding at 0.8 μM (Kavvadias *et al.*, 2003).

The structurally related diterpenoids carnosic acid and carnosol (Fig. 5) extracted from *Salvia officinalis*, while not influencing diazepam or muscimol binding, did inhibit TBPS binding (Rutherford *et al.*, 1992). This suggests that, like flavonoids, diterpenoids can influence $GABA_A$ receptors in a manner independent of classical benzodiazepine sites and could be missed in benzodiazepine binding assays. The structures of galdosol, carnosic acid, and carnosol (Fig. 5) contain the *o*-isopropylphenolic moiety that is present in thymol (Fig. 3) and the anesthetic agent propofol.

D. Triterpenoids

Ginsenosides, triterpenoid glycosides that are the major active constituents of *Panax ginseng*, are known to negatively modulate nicotinic and NMDA receptor activity. Of a series of ginsenosides, ginsenoside Rc

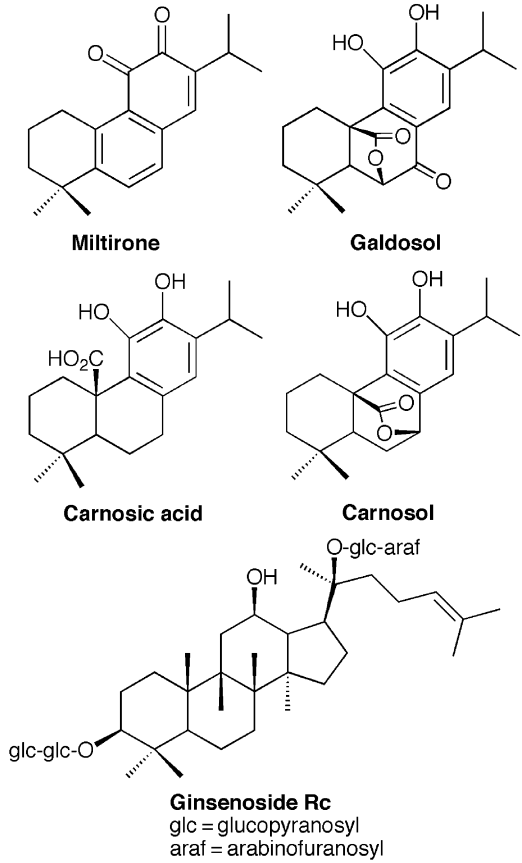


FIGURE 5 Diterpenoids and a triterpenoid that inhibit benzodiazepine binding (miltirone, galdosol), act as a positive modulator of $GABA_A$ receptor function (ginsenoside Rc) or inhibit TBPS binding without influencing benzodiazepine binding (carnosic acid, carnosol).

(Fig. 5) was the most potent (EC_{50} 53 μM) in enhancing the action of GABA on recombinant $\alpha 1\beta 1\gamma 2S$ $GABA_A$ receptors expressed in oocytes (Choi *et al.*, 2003).

VI. Other Phenolic Compounds

A. Honokiol

The bark of the root and stem of various *Magnolia* species has been used in Traditional Chinese Medicine to treat a variety of disorders including anxiety. The CNS muscle relaxant and depressant actions of the

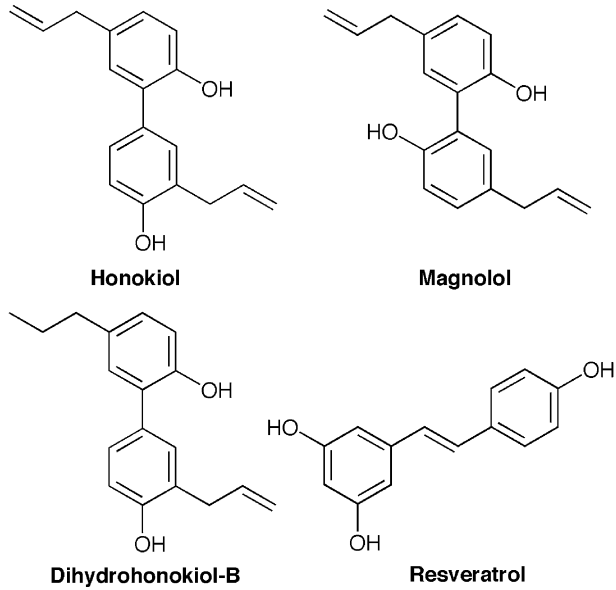


FIGURE 6 Phenolic compounds that act as positive modulators of $GABA_A$ receptor function particularly those that contain α2 subunits (honokiol, magnolol), or at $GABA_C$ receptors as positive (dihydrohonokiol-B) or negative modulators (resveratrol).

biphenols, honokiol and magnolol (Fig. 6), and related compounds extracted from *Magnolia officinalis* have been known for sometime (Watanabe *et al.*, 1975, 1983). Of these compounds honokiol was the most potent. At much lower doses than those that produce sedation, honokiol shows anxiolytic activity in mice in the elevated plus maze (Kuribara *et al.*, 1999). The anxiolytic effect of honokiol was inhibited by the benzodiazepine antagonist flumazenil and by the $GABA_A$ receptor antagonist bicuculline. In contrast to diazepam, honokiol selectively induces an anxiolytic effect with less liability of eliciting motor dysfunction or sedation suggesting that honokiol may act selectively on a subset of $GABA_A$ receptors. At anxiolytic doses, honokiol was less likely than diazepam to induce physical dependence, central depression, and amnesia (Kuribara *et al.*, 1999).

Studies with recombinant $GABA_A$ receptors expressed in the Sf-9/baculovirus system showed that honokiol potently enhanced the binding of muscimol to recombinant receptors containing the α2 subunit producing a fourfold enhancement (Ai *et al.*, 2001). This resulted from an increase in the number of muscimol binding sites. Honokiol preferentially increased muscimol binding to rat brain membranes prepared from hippocampus compared to those from cortex or cerebellum. The apparent increase in the number of muscimol binding sites may be due to honokiol allosterically

increasing the affinities of low-affinity muscimol binding sites (Squires *et al.*, 1999). The preferential effect of honokiol on α2 subunit-containing $GABA_A$ receptors is consistent with such receptors being associated with the anxiolytic rather than the sedative actions of diazepam (McKernan *et al.*, 2000).

Honokiol protects rat brain from focal cerebral ischemia-reperfusion injury by inhibiting neutrophil infiltration and the production of reactive oxygen species (Liou *et al.*, 2003b), consistent with its antiplatelet aggregation, anti-inflammatory, and antioxidant properties (Liou *et al.*, 2003a). Honokiol has been described as having anticancer properties inducing apoptosis through activating caspase cascades (Watanabe *et al.*, 1975) and as acting on calcium channels to inhibit muscle contraction (Lu *et al.*, 2003). Furthermore, it has been found to have neurotrophic activity in promoting neurite outgrowth in fetal rat cortical neuronal cultures (Fukuyama *et al.*, 2002).

Dihydrohonokiol-B (Fig. 6) was significantly more effective than honokiol in producing anxiolysis (Kuribara *et al.*, 2000). While the anxiolytic activity of dihydrohonokiol-B could be blocked by flumazenil, it was insensitive to bicuculline suggesting that it acted differently to honokiol (Kuribara *et al.*, 2000). Further studies on dihydrohonokiol-B showed that it inhibited ammonia-induced increases in intracellular chloride ion concentration in hippocampal neuronal cultures and that this action was insensitive to bicuculline but was inhibited by the $GABA_C$ receptor antagonist TPMPA (Irie *et al.*, 2001). This study suggests a possible role of $GABA_C$ receptors in protection against potentially pathological accumulations of chloride ions in neurons. Subsequent studies on amyloid *β* protein-induced neurotoxicity in hippocampal neuronal cultures showed that dihydrohonokiol-B protected against amyloid *β*-induced elevation of intracellular chloride ions in a TPMPA-sensitive manner indicating the involvement of $GABA_C$ receptors (Liu *et al.*, 2005). The authors suggest that dihydrohonokiol-B and $GABA_C$ receptor agonists can be one of the therapeutic and/or preventative strategies in Alzheimer's disease patients.

The exact role of $GABA_C$ receptors in the neuroprotective action of dihydrohonokiol-B is unclear. The presence of *ρ*1, *ρ*2, and *ρ*3 $GABA_C$ receptor subunits has been demonstrated in rat hippocampus by RT-PCR, and the $GABA_C$ agonist *cis*-4-aminocrotonic acid has been shown to suppress ammonia-induced apoptosis in hippocampal neurons in a TPMPA-sensitive manner (Yang *et al.*, 2003). It is not known whether or not dihydrohonokiol-B, or honokiol, have a positive modulatory action on $GABA_C$ receptors. Furthermore, in other tissues it has been shown that $GABA_A$ and $GABA_C$ receptors have opposing roles (Gibbs and Johnston, 2005) and the interplay between $GABA_A$ and $GABA_C$ receptors may be important in the actions of dihydrohonokiol-B and honokiol.

B. Resveratrol

The relatively low incidence of coronary heart diseases in France, despite intake of a high-fat diet—the "French Paradox"—has been attributed to the consumption of red wine containing high levels of polyphenolic compounds (Mojzisova and Kuchta, 2001; Sun *et al.*, 2002). Resveratrol (3,4′,5-trihydroxystilbene) (Fig. 6) is one of the most interesting polyphenolic compounds found in red wine. It has been shown to have estrogenic (Turner *et al.*, 1999) and neuroprotective effects (Bastianetto *et al.*, 2000). Resveratrol and related compounds are found in a variety of plants and herbs. Major dietary sources include grapes, wine, peanuts, and soy (Burns *et al.*, 2002). These compounds are also found in Itadori tea, which has long been used in Japan and China as a traditional remedy for heart disease and stroke (Burns *et al.*, 2002).

Resveratrol (Fig. 6) shows some structural similarity to apigenin (Fig. 1). It acts as a noncompetitive inhibitor of the effects of GABA (1 μM) at human $\rho 1$ recombinant $GABA_C$ receptors with an IC_{50} of 72 μM, while having no significant effect at doses up to 100 μM on the effects of GABA (40 μM) at $\alpha 1\beta 2\gamma 2L$ $GABA_A$ receptors (Campbell and Johnston, 2003). Resveratrol did not influence the positive modulation of $GABA_A$ receptors by diazepam, unlike apigenin (Campbell *et al.*, 2004). Resveratrol has been patented for the treatment of mild cognitive impairment based on its ability to increase the expression of soluble amyloid precursor protein (Wurtman and Lee, 2002). The $GABA_C$ antagonist effect of resveratrol may contribute to its effects on memory as other known $GABA_C$ antagonists have been shown to influence memory (Gibbs and Johnston, 2005; Johnston *et al.*, 2003). This is interesting in view of the evidence for an association between the neurotoxic effects of amyloid β protein and $GABA_C$ receptors (Liu *et al.*, 2005).

VII. Polyacetylenic Alcohols

The polyacetylene compounds that occur in plants are generally toxic. Cunaniol (Fig. 7), from the leaves of *Clibadium sylvestre* used as a fish poison by South American Indians (Clark, 1969), is a potent convulsant (Quilliam and Stables, 1969) that acts as a $GABA_A$ receptor antagonist (Curtis and Johnston, 1974). Water hemlock, *Cicuta virosa*, is well known as a toxic plant responsible for lethal poisonings in humans as well as animals, causing tonic and clonic convulsions and respiratory paralysis. The active constituent is the C(17) polyacetylene cicutoxin (Fig. 7), which was shown to be a potent inhibitor of the binding of the GABA channel blocker EBOB to ionotropic GABA receptors in rat brain cortex consistent with cicutoxin acting as a GABA antagonist and a convulsant (Uwai *et al.*,

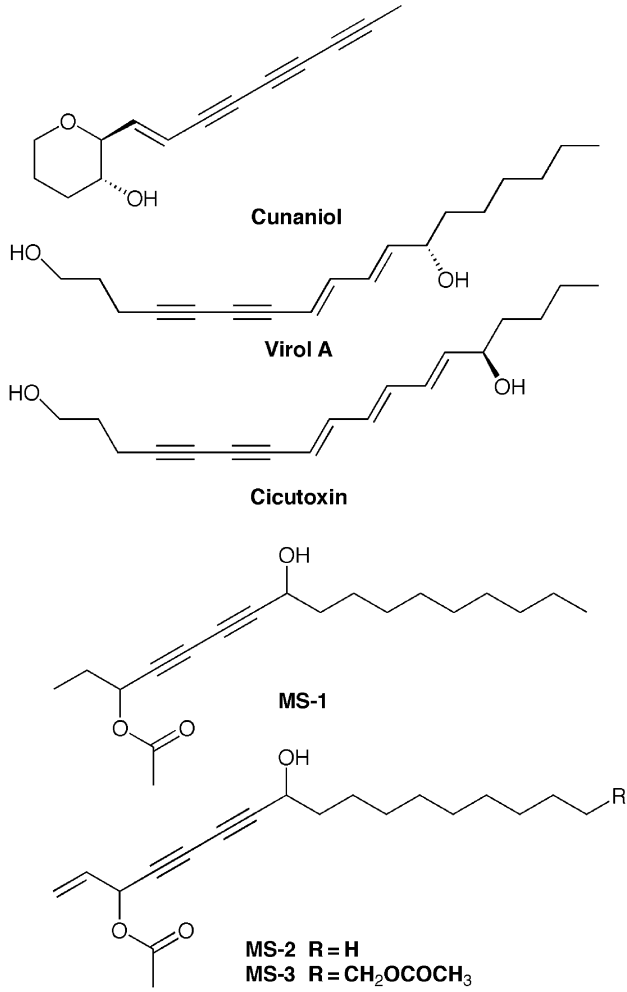


FIGURE 7 Polyacetylenic alcohols that may act as negative modulators of $GABA_A$ receptor function (cunaniol, cicutoxin, virol A) or as positive modulators of $GABA_A$ receptor function particularly those that contain $\beta 2$ subunits.

2000). A structurally related toxic polyacetylenic alcohol, virol A (Fig. 7), has been shown to inhibit GABA-induced chloride currents in acutely dissociated rat hippocampal CA1 neurons (Uwai *et al.*, 2001).

In contrast to these polyacetylenic alcohols that act as GABA receptor antagonists, some new polyacetylenic alcohols have been described that act as positive modulators of $GABA_A$ receptors that show novel subtypes specificity (Baur *et al.*, 2005). These substances (MS-1, MS-2, and MS-3) (Fig. 7) were isolated from the East African medicinal plant *Cussonia*

zimmermannii, which is used in Kenya and Tanzania to treat epilepsy and as a remedy for labor pain. They act independently of the classical benzodiazepine modulatory sites on $GABA_A$ receptors in that their actions are insensitive to flumazenil and are observed in the absence of the γ subunit. Half maximum stimulation was observed at 1–2 μM, and the maximum enhancement ranged from 110% to 450% depending on the subunit composition of the $GABA_A$ receptors. The positive modulation by MS-1 was dependent on the presence of the $\beta 2$ subunit and varied with the nature of the α subunit. The three substances differed in their relative subunit specificity. These substances represent a new lead structure for the development of subunit selective positive modulators of $GABA_A$ receptors.

VIII. Alcoholic Beverages Containing GABA Receptor Modulators

Alcoholic beverages are widely consumed. Ethanol has long been known to influence the activation of ionotropic GABA receptors, along with receptors for other neurotransmitters and ion channels (Narahashi *et al.*, 2001; see also Koob and Boehn *et al.*, this volume). $GABA_A$ receptors containing a δ subunit are particularly susceptible to the positive modulating effects of ethanol (Wallner *et al.*, 2003). Reproducible ethanol enhancement of GABA responses occurred at 3 mM, that is, concentrations that are reached with moderate ethanol consumption producing blood-ethanol levels well below the legal limit for driving in most countries. Ethanol influences the functioning of a variety of other receptors at concentrations in excess of 50 mM. This had been true for recombinant $GABA_A$ receptors (Harris, 1996) until studies on δ subunit containing receptors. The δ subunits appear to associate almost exclusively with $\alpha 4$ and $\alpha 5$ subunits forming functional receptors that are 50-fold more sensitive to GABA and desensitise more slowly than receptor subtypes that do not contain δ subunits (Jones *et al.*, 1997; Sur *et al.*, 1999). The δ subunit protein is expressed in brain regions expressing $\alpha 4$ (high in thalamus, dentate gyrus, striatum and outer cortical layers and low in hippocampus) and $\alpha 6$ subunit proteins (cerebellum) and appears to be associated with extrasynaptic rather than synaptic $GABA_A$ receptors (Hanchar *et al.*, 2004; Peng *et al.*, 2002). Knocking out the δ subunit gene in mice reduces their sensitivity to neurosteroids (Mihalek *et al.*, 1999) and increases their susceptibility to seizures (Spigelman *et al.*, 2003). As discussed previously, red wine contains the polyphenol resveratrol that acts as a noncompetitive inhibitor of the effects of GABA at human $\rho 1$ recombinant $GABA_C$ receptors (Campbell and Johnston, 2003).

Volatile components of alcoholic drinks, such as whiskey, wine, sake, brandy, and shochu potentiate GABA responses to varying degrees (Hossain *et al.*, 2002a). Although these fragrant components are present in alcoholic

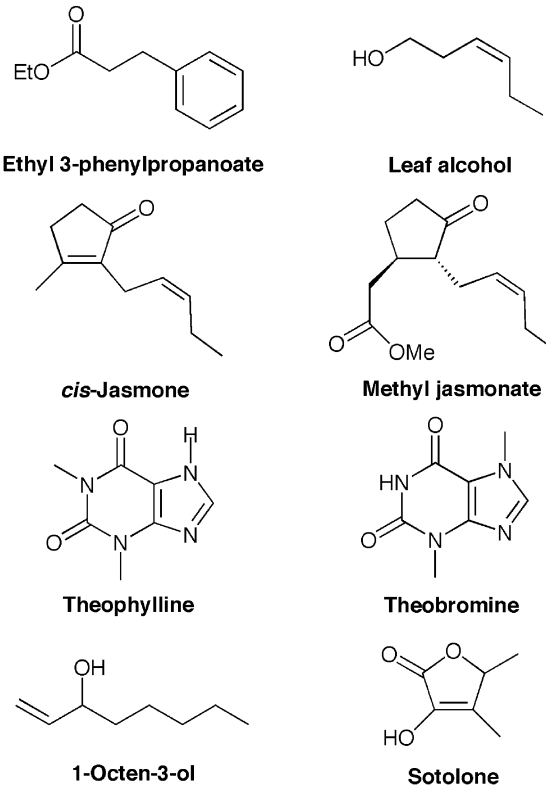


FIGURE 8 Positive (ethyl 3-phenylpropanoate, leaf alcohol, *cis*-jasmone, methyl jasmonate, 1-octen-3-ol, and sotolone) and negative (theophylline, theobromine) modulators of $GABA_A$ receptor function found in a variety of beverages.

drinks at low concentrations (extremely small quantities compared with ethanol), they may also modulate the mood or consciousness through the potentiation of $GABA_A$ responses after absorption into the brain, because these hydrophobic fragrant compounds are easily absorbed into the brain through the blood–brain barrier and are several thousands times as potent as ethanol in the potentiation of $GABA_A$ receptor-mediated responses (Hossain *et al.*, 2002a).

Many components in the fragrance of whiskey, in particular ethyl 3-phenylpropanoate (Fig. 8), strongly enhanced $\alpha 1\beta 1$ $GABA_A$ receptor responses (Hossain *et al.*, 2002a). When applied to mice through respiration, ethyl 3-phenylpropanoate delayed the onset of convulsions induced by pentylenetetrazole. The aging of whiskey results in enhanced potency of the fragrance in potentiating $GABA_A$ responses and in prolonging pentobarbitone-induced sleeping time in mice (Koda *et al.*, 2003). Sotolone (Fig. 8) is

a key component in the "nutty" and "spicy-like" aroma of oxidative aged port wine (Ferreira *et al.*, 2003) that enhances $GABA_A$ responses (Hossain *et al.*, 2003).

IX. GABA Receptor Modulators in Tea and Coffee

Tea and coffee contain a range of chemicals in addition to GABA that have been shown to influence recombinant bovine $\alpha1\beta1$ $GABA_A$ receptors. Extracts of green, oolong, or black tea contained catechins, especially (−)-epicatechin gallate and (−)-epigallocatechin gallate, that inhibited GABA responses and alcohols, such as leaf alcohol and linalool (Fig. 6), that enhanced GABA responses at concentrations of 1 mM (Hossain *et al.*, 2002b). Major components of green ((−)-epigallocatechin gallate) and chamomile teas (apigenin) have been shown to have an additional second order modulatory action on $\alpha1\beta2\gamma2L$ $GABA_A$ receptors that may contribute to the sedative properties of these teas (Campbell *et al.*, 2004). Fragrances of oolong tea have been shown to enhance the responses of recombinant bovine $\alpha1\beta1$ $GABA_A$ receptors to GABA, the most active constituents being *cis*-jasmone and methyl jasmonate (Fig. 8) (Hossain *et al.*, 2004). Leaf alcohol (Fig. 8) is one of a number of 6-carbon aliphatic alcohols and aldehydes found in the so-called "green odor" emanating from green leaves and which has been associated with attenuation of a variety of stress-induced effects such as elevation in plasma ACTH levels (Nakashima *et al.*, 2004); as stress is known to induce changes in GABA receptors (Akinci and Johnston, 1997), GABA mechanisms may contribute to the effects of "green odor." Coffee extracts contained theophylline (Fig. 8), which inhibited GABA responses in a noncompetitive mechanism (Ki 0.55 mM), and theobromine (Fig. 8), which inhibited in a competitive manner (Ki 3.8 mM), while a number of compounds including 1-octen-3-ol and sotolone (Fig. 8) enhanced GABA responses (Hossain *et al.*, 2003). When 1-octen-3-ol (100 mg/kg) was orally administered to mice prior to intraperitoneal administration of pentobarbitone, the sleeping time of mice induced by pentobarbital increased significantly (Hossain *et al.*, 2003).

X. Plant Sources of GABA Receptor Modulating Substances: Implications for Herbal Medicines

The widespread occurrence in plants of agents that are capable of modulating the function of ionotropic GABA receptors in the brain means that most plant extracts will contain a number of active substances. The assessment of the likely effects of such mixtures on brain function is a difficult task especially given the known interactions between many of

these substances and the variation in the relative proportions of the active substances in such plant extracts. Ideally such assessment should involve quantitative analytical data on the active constituents and functional assays of the effects of the extract on the biological targets of interest, in the present case ionotropic GABA receptors. This has important implications for the quality control of herbal medicines.

Sage is a good example of the complexity concerning active ingredients. It has been used widely to treat memory deficits and extracts of *Salvia lavandulaefolia* (Spanish sage) have been shown to enhance memory in healthy young volunteers (Tildesley *et al.*, 2003). $GABA_A$ and $GABA_C$ receptors are known to be important in many aspects of memory (Gibbs and Johnston, 2005), and there is a variety of agents found in sage that are known to influence these receptors. Such agents include the flavonoids apigenin, hispidulin, and linarin (Figs. 1 and 2) (Campbell *et al.*, 2004; Fernandez *et al.*, 2004; Kavvadias *et al.*, 2003) and the terpenoids galdosol, miltirone, carnosic acid, and carnosol (Fig. 5) (Chang *et al.*, 1991; Kavvadias *et al.*, 2003; Lee *et al.*, 1991).

Sage also contains α-thujone (Fig. 3), a known $GABA_A$ receptor antagonist as noted previously, which may influence the GABA enhancing effects of the other agents in sage extracts (Johnston and Beart, 2004). The levels of α-thujone in individual sage plants are known to vary considerably (Perry *et al.*, 1999b).

The interactions between active constituents in herbal preparations is a further complicating factor but of great interest with respect to our understanding of the modulation of GABA receptor activation. In addition to agents that are positive modulators of the action of GABA on $GABA_A$ receptors, that is, first order modulators, we now have second order modulators like apigenin and (−)-epigallocatechin gallate (Fig. 1) than modulate only in the presence of a first order modulator (Campbell *et al.*, 2004). Furthermore, we now have examples of positive modulators that appear to enhance the activity of other positive modulators, for example, ethanol and neurosteroids (Akk and Steinbach, 2003). The synergistic action of some positive modulators in herbal preparations is best illustrated with some active substances from *Valeriana officinalis*. The sleep-inducing effects of hesperidin (Fig. 2) are potentiated by 6-methylapigenin (Fig. 1) (Marder *et al.*, 2003), while the sedating and sleep-inducing effects of valerenic acid (Fig. 4) are dramatically potentiated when co-administered with the flavonoid glycoside linarin (Fig. 2) (Fernandez *et al.*, 2004). The specificity of these synergistic actions is particularly interesting. In addition, there are other powerful positive modulators that are found in valerian extract, such as (+)-borneol (Fig. 3), that have yet to be examined for possible synergistic effects with other agents (Granger *et al.*, 2005). We have much to learn as to the key substances in valerian extracts that are used in herbal medicines.

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References

Aherne, S. A., and O'Brien, N. M. (2002). Dietary flavonols: Chemistry, food content, and metabolism. *Nutrition* **18**, 75–81.

Ai, J., Wang, X., and Nielsen, M. (2001). Honokiol and magnolol selectively interact with $GABA_A$ receptor subtypes *in vitro*. *Pharmacology* **63**, 34–41.

Akinci, M. K., and Johnston, G. A. R. (1997). Sex differences in the effects of gonadectomy and acute swim stress on $GABA_A$ receptor binding in mouse forebrain membranes. *Neurochem. Int.* **31**, 1–10.

Akk, G., and Steinbach, J. H. (2003). Low doses of ethanol and a neuroactive steroid positively interact to modulate rat $GABA_A$ receptor function. *J. Physiol.* **546**, 641–646.

Avallone, R., Zanoli, P., Puia, G., Kleinschnitz, M., Schreier, P., and Baraldi, M. (2000). Pharmacological profile of apigenin, a flavonoid isolated from Matricaria chamomilla. *Biochem. Pharmacol.* **59**, 1387–1394.

Baraldi, M., Avallone, R., Corsi, L., Venturini, I., Baraldi, C., and Zeneroli, M. L. (2000). Endogenous benzodiazepines. *Therapie* **55**, 143–146.

Bastianetto, S., Zheng, W. H., and Quirion, R. (2000). Neuroprotective abilities of resveratrol and other red wine constituents against nitric oxide-related toxicity in cultured hippocampal neurons. *Br. J. Pharmacol.* **131**, 711–720.

Baur, R., Simmen, U., Senn, M., Sequin, U., and Sigel, E. (2005). Novel plant substances acting as beta subunit isoform-selective positive allosteric modulators of $GABA_A$ receptors. *Mol. Pharmacol.* **68**, 787–792.

Baureithel, K. H., Büter, K. B., Engesser, A., Burkard, W., and Schaffer, W. (1997). Inhibition of benzodiazepine binding *in vitro* by amentoflavone, a constituent of various species of *Hypericum*. *Pharm. Acta Helv.* **72**, 153–157.

Bouche, N., and Fromm, H. (2004). GABA in plants: Just a metabolite? *Trends Plant Sci.* **9**, 110–115.

Bouche, N., Lacombe, B., and Fromm, H. (2003). GABA signaling: A conserved and ubiquitous mechanism. *Trends Cell Biol.* **13**, 607–610.

Brochet, D., Chermat, R., DeFeudis, F. V., and Drieu, K. (1999). Effects of single intraperitoneal injections of an extract of Ginkgo biloba (EGb 761) and its terpene trilactone constituents on barbital-induced narcosis in the mouse. *Gen. Pharmacol.* **33**, 249–256.

Burns, J., Yokota, T., Ashihara, H., Lean, M. E. J., and Crozier, A. (2002). Plant foods and herbal sources of resveratrol. *J. Agric. Food Chem.* **50**, 3337–3340.

Butterweck, V., Nahrstedt, A., Evans, J., Hufeisen, S., Rauser, L., Savage, J., Poedak, B., Ernsberger, P., and Roth, B. L. (2002). *In vitro* receptor screening of pure constitutents of St. John's wort reveals novel interactions with a number of GPCRs. *Psychopharmacology* **162**, 193–202.

Campbell, E. L., and Johnston, G. A. R. (2003). Resveratrol is a $GABA_C$ receptor antagonist. *Proc. Aust. Neurosci. Soc.* **14**, P136.

Campbell, E. L., Chebib, M., and Johnston, G. A. R. (2004). The dietary flavonoids apigenin and (−)-epigallocatechin gallate enhance the positive modulation by diazepam of the activation by GABA of recombinant $GABA_A$ receptors. *Biochem. Pharmacol.* **68**, 1631–1638.

Chang, H. M., Chui, K. Y., Tan, F. W., Zhong, Z. P., Lee, C. M., Sham, H. L., and Wong, H. N. (1991). Structure-activity relationship of miltirone, an active central benzodiazepine ligand isolated from *Salvia miltiorrhiza* Bunge (Danshen). *J. Med. Chem.* **34**, 1675–1692.

Chatterjee, S. S., Kondratskaya, E. L., and Krishtal, O. A. (2003). Structure-activity studies with Ginkgo biloba extract constituents as receptor-gated chloride channel blockers and modulators. *Pharmacopsychiatry* **36**, S68–S77.

Chebib, M., and Johnston, G. A. R. (2000). GABA-activated ligand gated ion channels: Medicinal chemistry and molecular biology. *J. Med. Chem.* **43**, 1427–1447.

Choi, S. E., Choi, S., Lee, J. H., Whiting, P. J., Lee, S. M., and Nah, S. Y. (2003). Effects of ginsenosides on $GABA_A$ receptor channels expressed in *Xenopus* oocytes. *Arch. Pharm. Res.* **26**, 28–33.

Choi, Y. B., Kim, Y. I., Lee, K. S., Kim, B. S., and Kim, D. J. (2004). Protective effect of epigallocatechin gallate on brain damage after transient middle cerebral artery occlusion in rats. *Brain Res.* **1019**, 47–54.

Christopoulos, A. (2002). Allosteric binding sites on cell-surface receptors: Novel targets for drug discovery. *Nature Rev. Drug Discov.* **1**, 198–210.

Clark, J. B. (1969). Effect of a polyacetylenic fish poison on the oxidative phosphorylation of rat liver. *Biochem. Pharmacol.* **18**, 73–83.

Curtis, D. R., and Johnston, G. A. R. (1974). Convulsant alkaloids. *Neuropoisons* **2**, 207–248.

Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R. (1970). Bicuculline and central GABA receptors. *Nature* **228**, 676–677.

DeFeudis, F. V. (2002). Bilobalide and neuroprotection. *Pharmacol. Res.* **46**, 565–568.

Deiml, T., Haseneder, R., Zieglgansberger, W., Rammes, G., Eisensamer, B., Rupprecht, R., and Hapfelmeier, G. (2004). α-Thujone reduces 5-HT_3 receptor activity by an effect on the agonist-induced desensitization. *Neuropharmacology* **46**, 192–201.

Dietz, B. M., Mahady, G. B., Pauli, G. F., and Farnsworth, N. R. (2005). Valerian extract and valerenic acid are partial agonists of the 5-HT_{5a} receptor *in vitro*. *Brain Res. Mol. Brain Res.* **138**, 191–197.

Du, X. M., Sun, N. Y., Takizawa, N., Guo, Y. T., and Shoyama, Y. (2002). Sedative and anticonvulsant activities of goodyerin, a flavonol glycoside from *Goodyera schlechtendaliana*. *Phytother. Res.* **16**, 261–263.

Fernandez, S., Wasowski, C., Paladini, A. C., and Marder, M. (2004). Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*. *Pharmacol. Biochem. Behav.* **77**, 399–404.

Ferreira, A. C. S., Barbe, J.-C., and Bertrand, A. (2003). 3-Hydroxy-4,5-dimethyl-2(5H)-furanone: A key odorant of the typical aroma of oxidative aged port wine. *J. Agric. Food Chem.* **51**, 4356–4363.

Froestl, W., Markstein, R., Schmid, K. L., and Trendelenburg, A.-U. (2004). $GABA_C$ antagonists for the treatment of myopia. *PCT Int. Appl.* 20030627, 8 pp.

Fukuyama, Y., Nakade, K., Minoshima, Y., Yokoyama, R., Zhai, H., and Mitsumoto, Y. (2002). Neurotrophic activity of honokiol on the cultures of fetal rat cortical neurons. *Bioorg. Med. Chem. Lett.* **12**, 1163–1166.

Gibbs, M. E., and Johnston, G. A. R. (2005). Opposing roles for $GABA_A$ and $GABA_C$ receptors in short-term memory formation in young chicks. *Neuroscience* **131**, 567–576.

Gottesmann, C. (2002). GABA mechanisms and sleep. *Neuroscience* **111**, 231–239.

Goutman, J. D., Waxemberg, M. D., Donate-Oliver, F., Pomata, P. E., and Calvo, D. J. (2003). Flavonoid modulation of ionic currents mediated by $GABA_A$ and $GABA_C$ receptors. *Eur. J. Pharmacol.* **461**, 79–87.

Granger, R. E., Campbell, E. L., and Johnston, G. A. R. (2005). (+)- and (−)-borneol: Efficacious positive modulators of GABA action at human recombinant alpha1beta2-gamma2L $GABA_A$ receptors. *Biochem. Pharmacol.* **69**, 1101–1111.

Gutmann, H., Bruggisser, R., Schaffner, W., Bogman, K., Botomino, A., and Drewe, J. (2002). Transport of amentoflavone across the blood-brain barrier *in vitro*. *Planta Med.* **68**, 804–807.

Ha, J. H., Lee, K. Y., Choi, H. C., Cho, J., Kang, L. S., Lim, J. C., and Lee, D. U. (2002). Modulation of radioligand binding to the $GABA_A$-benzodiazepine receptor complex by a new component from *Cyperus rotundus*. *Biol. Pharm. Bull.* **25**, 128–130.

Hall, B. J., Chebib, M., Hanrahan, J. R., and Johnston, G. A. R. (2004). Flumazenil-independent positive modulation of γ-aminobutyric acid by 6-methylflavone at human recombinant $\alpha1\beta2\gamma2$L and $\alpha1\beta2$ $GABA_A$ receptors. *Eur. J. Pharmacol.* **491**, 1–8.

Hanchar, H. J., Wallner, M., and Olsen, R. W. (2004). Alcohol effects on gamma-aminobutyric acid type A receptors: Are extrasynaptic receptors the answer? *Life Sci.* **76**, 1–8.

Hanrahan, J. R., Chebib, M., Davucheron, N. M., Hall, B. J., and Johnston, G. A. R. (2003). Semisynthetic preparation of amentoflavone: A negative modulator at $GABA_A$ receptors. *Bioorg. Med. Chem. Lett.* **13**, 2281–2284.

Harris, R. A. (1996). Transfected cells for study of alcohol actions on GABA(a) receptors. *Addict. Biol.* **1**, 157–163.

Havsteen, B. H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacol. Ther.* **96**, 67–202.

Heim, K. E., Tagliaferro, A. R., and Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.* **13**, 572–584.

Hold, K. M., Sirisoma, N. S., Ikeda, T., Narahashi, T., and Casida, J. E. (2000). α-Thujone (the active component of absinthe): γ-Aminobutyric acid type A receptor modulation and metabolic detoxification. *Proc. Natl. Acad. Sci. USA* **97**, 3826–3831.

Holopainen, I. E., Metsahonkala, E. L., Kokkonen, H., Parkkola, R. K., Manner, T. E., Nagren, K., and Korpi, E. R. (2001). Decreased binding of [^{11}C]flumazenil in Angelman syndrome patients with $GABA_A$ receptor $\beta3$ subunit deletions. *Ann. Neurol.* **49**, 110–113.

Hossain, S. J., Aoshima, H., Koda, H., and Kiso, Y. (2002a). Potentiation of the ionotropic GABA receptor response by whiskey fragrance. *J. Agric. Food Chem.* **50**, 6828–6834.

Hossain, S. J., Hamamoto, K., Aoshima, H., and Hara, Y. (2002b). Effects of tea components on the response of $GABA_A$ receptors expressed in *Xenopus* oocytes. *J. Agric. Food Chem.* **50**, 3954–3960.

Hossain, S. J., Aoshima, H., Koda, H., and Kiso, Y. (2003). Effects of coffee components on the response of $GABA_A$ receptors expressed in *Xenopus* oocytes. *J. Agric. Food Chem.* **51**, 7568–7575.

Hossain, S. J., Aoshima, H., Koda, H., and Kiso, Y. (2004). Fragrances in oolong tea that enhance the response of $GABA_A$ receptors. *Biosci. Biotechnol. Biochem.* **68**, 1842–1848.

Hosseinzadeh, H., and Parvardeh, S. (2004). Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. *Phytomedicine* **11**, 56–64.

Huang, S. H., Duke, R. K., Chebib, M., Sasaki, K., Wada, K., and Johnston, G. A. R. (2003). Bilobalide, a sesquiterpene trilactone from *Ginkgo biloba*, is an antagonist at recombinant alpha(1)beta(2)gamma(2L) $GABA_A$ receptors. *Eur. J. Pharmacol.* **464**, 1–8.

Huang, S. H., Duke, R. K., Chebib, M., Sasaki, K., Wada, K., and Johnston, G. A. (2004). Ginkgolides, diterpene trilactones of *Ginkgo biloba*, as antagonists at recombinant alpha1beta2gamma2L $GABA_A$ receptors. *Eur. J. Pharmacol.* **494**, 131–138.

Huang, S. H., Duke, R. K., Chebib, M., Sasaki, K., Wada, K., and Johnston, G. A. R. (2006). Mixed antagonistic effects of bilobalide at $\rho1$ $GABA_C$ receptors. *Neuroscience* **137**, 607–617.

Huen, M. S., Hui, K. M., Leung, J. W., Sigel, E., Baur, R., Wong, J. T., and Xue, H. (2003a). Naturally occurring 2′-hydroxyl-substituted flavonoids as high-affinity benzodiazepine site ligands. *Biochem. Pharmacol.* **66**, 2397–2407.

Huen, M. S., Leung, J. W., Ng, W., Lui, W. S., Chan, M. N., Wong, J. T., and Xue, H. (2003b). 5,7-Dihydroxy-6-methoxyflavone, a benzodiazepine site ligand isolated from *Scutellaria baicalensis* Georgi, with selective antagonistic properties. *Biochem. Pharmacol.* **66**, 125–132.

Huh, S. W., Bae, S. M., Kim, Y. W., Lee, J. M., Namkoong, S. E., Lee, I. P., Kim, S. H., Kim, C. K., and Ahn, W. S. (2004). Anticancer effects of (−)-epigallocatechin-3-gallate on ovarian carcinoma cell lines. *Gynacol. Oncol.* **94**, 760–768.

Hussain, T., Gupta, S., Adhami, V. M., and Mukhtar, H. (2005). Green tea constituent epigallocatechin-3-gallate selectively inhibits COX-2 without affecting COX-1 expression in human prostate carcinoma cells. *Int. J. Cancer* **113**, 660–669.

Irie, T., Miyamoto, E., Kitagawa, K., Maruyama, Y., Inoue, K., and Inagaki, C. (2001). An anxiolytic agent, dihydrohonokiol-B, inhibits ammonia-induced increases in the intracellular Cl^- of cultured rat hippocampal neurons via $GABA_C$ receptors. *Neurosci. Lett.* **312**, 121–123.

Ivic, L., Sands, T. T., Fishkin, N., Nakanishi, K., Kriegstein, A. R., and Stromgaard, K. (2003). Terpene trilactones from *Ginkgo biloba* are antagonists of cortical glycine and $GABA_A$ receptors. *J. Biol. Chem.* **278**, 49279–49285.

Jacobson, K. A., Moro, S., Manthey, J. A., West, P. L., and Ji, X. D. (2002). Interactions of flavones and other phytochemicals with adenosine receptors. *Adv. Exp. Med. Biol.* **505**, 163–171.

Jarboe, C. H., Porter, L. A., and Buckler, R. T. (1968). Structural aspects of picrotoxinin action. *J. Med. Chem.* **2**, 729–731.

Johnston, G. A. R. (1996). $GABA_A$ receptor pharmacology. *Pharmacol. Ther.* **69**, 173–198.

Johnston, G. A. R. (2005). $GABA_A$ receptor channel pharmacology. *Curr. Pharm. Design* **11**, 1867–1885.

Johnston, G. A. R., and Beart, P. M. (2004). Flavonoids: Some of the wisdom of sage? *Br. J. Pharmacol.* **142**, 809–810.

Johnston, G. A. R., Curtis, D. R., De Groat, W. C., and Duggan, A. W. (1968). Central actions of ibotenic acid and muscimol. *Biochem. Pharmacol.* **17**, 2488–2489.

Johnston, G. A. R., Chebib, M., Hanrahan, J. R., and Mewett, K. N. (2003). $GABA_C$ receptors as drug targets. *Curr. Drug Targets CNS Neurol. Disord.* **2**, 260–268.

Jones, A., Korpi, E. R., Mckernan, R. M., Pelz, R., Nusser, Z., Makela, R., Mellor, J. R., Pollard, S., Bahn, S., Stephenson, F. A., Randall, A. D., Sieghart, W., *et al.* (1997). Ligand-gated ion channel subunit partnerships - $GABA_A$ receptor alpha(6) subunit gene inactivation inhibits delta subunit expression. *J. Neurosci.* **17**, 1350–1362.

Jones, F. A., Chatterjee, S. S., and Davies, J. A. (2002). Effects of bilobalide on amino acid release and electrophysiology of cortical slices. *Amino Acids* **22**, 369–379.

Jones-Davis, D. M., and Macdonald, R. L. (2003). $GABA_A$ receptor function and pharmacology in epilepsy and status epilepticus. *Curr. Opin. Pharmacol.* **3**, 12–18.

Kavvadias, D., Monschein, V., Sand, P., Riederer, P., and Schreier, P. (2003). Constituents of sage (*Salvia officinalis L.*) with *in vitro* affinity to human brain benzodiazepine receptor. *Planta Med.* **69**, 113–117.

Kavvadias, D., Sand, P., Youdim, K. A., Qaiser, M. Z., Rice-Evans, C., Baur, R., Sigel, E., Rausch, W.-D., Riederer, P., and Schreier, P. (2004). The flavone hispidulin, a benzodiazepine receptor ligand with positive allosteric properties, traverses the blood-brain barrier and exhibits anti-convulsive effects. *Br. J. Pharmacol.* **142**, 811–820.

Kawai, K., Tsuno, N. H., Kitayama, J., Okaji, Y., Yazawa, K., Asakage, M., Sasaki, S., Watanabe, T., Takahashi, K., and Nagawa, H. (2005). Epigallocatechin gallate induces apoptosis of monocytes. *J. Allergy Clin. Immunol.* **115**, 186–191.

Koda, H., Hossain, S. J., Kiso, Y., and Aoshima, H. (2003). Aging of whiskey increases the potentiation of $GABA_A$ receptor response. *J. Agric. Food Chem.* **51**, 5238–5244.

Kuribara, H., Stavinoha, W. B., and Maruyama, Y. (1999). Honokiol, a putative anxiolytic agent extracted from magnolia bark, has no diazepam-like side-effects in mice. *J. Pharm. Pharmacol.* **51**, 97–103.

Kuribara, H., Kishi, E., Kimura, M., Weintraub, S. T., and Maruyama, Y. (2000). Comparative assessment of the anxiolytic-like activities of honokiol and derivatives. *Pharmacol. Biochem. Behav.* **67**, 597–601.

Lambert, J. D., and Yang, C. S. (2003). Mechanisms of cancer prevention by tea constituents. *J. Nutr.* **133**, 3262S–3267S.

Le Marchand, L. (2002). Cancer preventive effects of flavonoids: A review. *Biomed. Pharmacother.* **56**, 296–301.

Le Novere, N., and Changeux, J. P. (2001). The ligand gated ion channel database: An example of a sequence database in neuroscience. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **356**, 1121–1130.

Lee, C. M., Wong, H. N., Chui, K. Y., Choang, T. F., Hon, P. M., and Chang, H. M. (1991). Miltirone, a central benzodiazepine receptor partial agonist from a Chinese medicinal herb *Salvia miltiorrhiza*. *Neurosci. Lett.* **127**, 237–241.

Lee, J. H., Song, D. K., Jung, C. H., Shin, D. H., Park, J., Kwon, T. K., Jang, B. C., Mun, K. C., Kim, S. P., Suh, S. I., and Bae, J. H. (2004). (−)-Epigallocatechin gallate attenuates glutamate-induced cytotoxicity via intracellular Ca modulation in PC12 cells. *Clin. Exp. Pharmacol. Physiol.* **31**, 530–536.

Levites, Y., Amit, T., Youdim, M. B., and Mandel, S. (2002). Involvement of protein kinase C activation and cell survival/cell cycle genes in green tea polyphenol (−)-epigallocatechin 3-gallate neuroprotective action. *J. Biol. Chem.* **277**, 30574–30580.

Liou, K. T., Lin, S. M., Huang, S. S., Chih, C. L., and Tsai, S. K. (2003a). Honokiol ameliorates cerebral infarction from ischemia-reperfusion injury in rats. *Planta Med.* **69**, 130–134.

Liou, K. T., Shen, Y. C., Chen, C. F., Tsao, C. M., and Tsai, S. K. (2003b). Honokiol protects rat brain from focal cerebral ischemia-reperfusion injury by inhibiting neutrophil infiltration and reactive oxygen species production. *Brain Res.* **992**, 159–166.

Liu, B., Hattori, N., Zhang, N. Y., Wu, B., Yang, L., Kitagawa, K., Xiong, Z. M., Irie, T., and Inagaki, C. (2005). Anxiolytic agent, dihydrohonokiol-B, recovers amyloid beta protein-induced neurotoxicity in cultured rat hippocampal neurons. *Neurosci. Lett.* **384**, 44–47.

Losi, G., Puia, G., Garzon, G., de Vuono, M. C., and Baraldi, M. (2004). Apigenin modulates GABAergic and glutamatergic transmission in cultured cortical neurons. *Eur. J. Pharmacol.* **502**, 41–46.

Lu, Y. C., Chen, H. H., Ko, C. H., Lin, Y. R., and Chan, M. H. (2003). The mechanism of honokiol-induced and magnolol-induced inhibition on muscle contraction and Ca^{2+} mobilization in rat uterus. *Naunyn-Schmiedebergs Arch. Pharmacol.* **368**, 262–269.

Luk, K. C., Stern, L., Weigele, M., O'Brien, R. A., and Spirst, N. (1983). Isolation and identification of "diazepam-like" compounds in bovine brain. *J. Nat. Prod.* **46**, 852–861.

Marder, M., and Paladini, A. C. (2002). $GABA_A$ receptor ligands of flavonoid structure. *Curr. Top. Med. Chem.* **2**, 853–867.

Marder, M., Viola, H., Wasowski, C., Fernandez, S., Medina, J. H., and Paladini, A. C. (2003). 6-Methylapigenin and hesperidin: New valeriana flavonoids with activity on the CNS. *Pharmacol. Biochem. Behav.* **75**, 537–545.

McKernan, R. M., and Whiting, P. J. (1996). Which $GABA_A$-receptor subtypes really occur in the brain? *Trends Neurosci.* **19**, 139–143.

McKernan, R. M., Rosahl, T. W., Reynolds, D. S., Sur, C., Wafford, K. A., Atack, J. R., Farrar, S., Myers, J., Cook, G., Ferris, P., Garrett, L., Bristow, L., *et al.* (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the $GABA_A$ receptor alpha(1) subtype. *Nat. Neurosci.* **3**, 587–592.

Medina, J. H., Viola, H., Wolfman, C., Marder, M., Wasowski, C., Calvo, D., and Paladini, A. C. (1997). Overview—flavonoids—a new family of benzodiazepine receptor ligands. *Neurochem. Res.* **22**, 419–425.

Mihalek, R. M., Banerjee, P. K., Korpi, E. R., Quinlan, J. J., Firestone, L. L., Mi, Z. P., Lagenaur, C., Tretter, V., Sieghart, W., Anagnostaras, S. G., Sage, J. R., Fanselow, M. S., *et al.* (1999). Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. *Proc. Natl. Acad. Sci. USA* **96**, 12905–12910.

Misra, R. (1998). Modern drug development from traditional medicinal plants using radioligand receptor-binding assays. *Med. Res. Rev.* **18**, 383–402.

Mohammadi, B., Haeseler, G., Leuwer, M., Dengler, R., Krampfl, K., and Bufler, J. (2001). Structural requirements of phenol derivatives for direct activation of chloride currents via $GABA_A$ receptors. *Eur. J. Pharmacol.* **421**, 85–91.

Mojzisova, G., and Kuchta, M. (2001). Dietary flavonoids and risk of coronary heart disease. *Physiol. Res.* **50**, 529–535.

Nakashima, T., Akamatsu, M., Hatanaka, A., and Kiyohara, T. (2004). Attenuation of stress-induced elevations in plasma ACTH level and body temperature in rats by green odor. *Physiol. Behav.* **80**, 481–488.

Narahashi, T., Kuriyama, K., Illes, P., Wirkner, K., Fischer, W., Muhlberg, K., Scheibler, P., Allgaier, C., Minami, K., Lovinger, D., Lallemand, F., Ward, R. J., *et al.* (2001). Neuroreceptors and ion channels as targets of alcohol. *Alcohol. Clin. Exp. Res.* **25**, 182S–188S.

Nielsen, M., Frokjaer, S., and Braestrup, C. (1988). High affinity of the naturally-occurring biflavonoid, amentoflavone, to brain benzodiazepine receptors *in vitro*. *Biochem. Pharmacol.* **37**, 3285–3287.

Peng, Z., Hauer, B., Mihalek, R. M., Homanics, G. E., Sieghart, W., Olsen, R. W., and Houser, C. R. (2002). $GABA_A$ receptor changes in delta subunit-deficient mice: Altered expression of alpha 4 and gamma 2 subunits in the forebrain. *J. Comp. Neurol.* **446**, 179–197.

Perry, E. K., Pickering, A. T., Wang, W. W., Houghton, P. J., and Perry, N. S. (1999a). Medicinal plants and Alzheimer's disease: From ethnobotany to phytotherapy. *J. Pharm. Pharmacol.* **51**, 527–534.

Perry, N. B., Anderson, R. E., Brennan, N. J., Douglas, M. H., Heaney, A. J., McGimpsey, J. A., and Smallfield, B. M. (1999b). Essential oils from Dalmation sage (*Salvia officinalis l.*): Vaiations among individuals, plant parts, seasons, and sites. *J. Agric. Food Chem.* **47**, 2048–2054.

Perry, N. S., Howes, M.-J., Houghton, P., and Perry, E. (2000). Why sage may be a wise memory remedy: Effects of salvia on the nervous system. *Med. Arom. Plants Indust. Profiles* **14**, 207–223.

Priestley, C. M., Williamson, E. M., Wafford, K., and Sattelle, D. B. (2003). Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human $GABA_A$ receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. *Br. J. Pharmacol.* **140**, 1363–1372.

Qian, H., Pan, Y., Zhu, Y., and Khalili, P. (2005). Picrotoxin accelerates relaxation of $GABA_C$ receptors. *Mol. Pharmacol.* **67**, 470–479.

Quilliam, J. P., and Stables, R. (1969). Convulsant effects of cunaniol, a polyacetylenic alcohol isolated from the plant *Clibadium sylvestre*, on frogs and mice. *Pharmacol. Res. Comm.* **1**, 7–14.

Rutherford, D. M., Nielsen, M. P. C., Hansen, S. K., Witt, M. R., Bergendorff, O., and Sterner, O. (1992). Isolation from *Salvia officinalis* and indentification of two diterpenes which inhibit t-butylbicyclophosphoro[^{35}S]thionate binding to chloride channel of rat cerebrocortical membranes *in vitro*. *Neurosci. Lett.* **135**, 224–226.

Sasaki, K., Hatta, S., Haga, M., and Ohshika, H. (1999a). Effects of bilobalide on gamma-aminobutyric acid levels and glutamic acid decarboxylase in mouse brain. *Eur. J. Pharmacol.* **367**, 165–173.

Sasaki, K., Oota, I., Wada, K., Inomata, K., Ohshika, H., and Haga, M. (1999b). Effects of bilobalide, a sesquiterpene in *Ginkgo biloba* leaves, on population spikes in rat hippocampal slices. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **124**, 315–321.

Skerritt, J. H., Willow, M., and Johnston, G. A. R. (1982). Diazepam enhancement of low affinity GABA binding to rat brain membranes. *Neurosci. Lett.* **29**, 63–66.

Spigelman, I., Li, Z., Liang, J., Cagetti, E., Samzadeh, S., Mihalek, R. M., Homanics, G. E., and Olsen, R. W. (2003). Reduced inhibition and sensitivity to neurosteroids in hippocampus of mice lacking the $GABA_A$ receptor delta subunit. *J. Neurophysiol.* **90**, 903–910.

Squires, R. F., Ai, J., Witt, M. R., Kahnberg, P., Saederup, E., Sterner, O., and Nielsen, M. (1999). Honokiol and magnolol increase the number of [^{3}H] muscimol binding sites three-fold in rat forebrain membranes *in vitro* using a filtration assay, by allosterically increasing the affinities of low-affinity sites. *Neurochem. Res.* **24**, 593–602.

Suganuma, M., Okabe, S., Oniyama, M., Tada, Y., Ito, H., and Fujiki, H. (1998). Wide distribution of [^{3}H](−)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* **19**, 1771–1776.

Sun, A. Y., Simonyi, A., and Sun, G. Y. (2002). The "French paradox" and beyond: Neuroprotective effects of polyphenols. *Free Radic. Biol. Med.* **32**, 314–318.

Sur, C., Farrar, S. J., Kerby, J., Whiting, P. J., Atack, J. R., and McKernan, R. M. (1999). Preferential coassembly of alpha 4 and delta subunits of the gamma-aminobutyric $acid_A$ receptor in rat thalamus. *Mol. Pharmacol.* **56**, 110–115.

Tildesley, N. T., Kennedy, D. O., Perry, E. K., Ballard, C. G., Savelev, S., Wesnes, K. A., and Scholey, A. B. (2003). *Salvia lavandulaefolia* (Spanish sage) enhances memory in healthy young volunteers. *Pharmacol. Biochem. Behav.* **75**, 669–674.

Topliss, J. G., Clark, A. M., Ernst, E., Hufford, C. D., Johnston, G. A. R., Rimoldi, J. M., and Weimann, B. J. (2002). Natural and synthetic substances related to human health - (IUPAC Technical Report). *Pure Appl. Chem.* **74**, 1957–1985.

Turner, R. T., Evans, G. L., Zhang, M. Z., Maran, A., and Sibonga, J. D. (1999). Is resveratrol an estrogen agonist in growing rats? *Endocrinology* **140**, 50–54.

Uwai, K., Ohashi, K., Takaya, Y., Ohta, T., Tadano, T., Kisara, K., Shibusawa, K., Sakakibara, R., and Oshima, Y. (2000). Exploring the structural basis of neurotoxicity in C(17)-polyacetylenes isolated from water hemlock. *J. Med. Chem.* **43**, 4508–4515.

Uwai, K., Ohashi, K., Takaya, Y., Oshima, Y., Furukawa, K., Yamagata, K., Omura, T., and Okuyama, S. (2001). Virol A, a toxic trans-polyacetylenic alcohol of *Cicuta virosa*, selectively inhibits the GABA-induced Cl^- current in acutely dissociated rat hippocampal CA1 neurons. *Brain Res.* **889**, 174–180.

Vafa, B., and Schofield, P. R. (1998). Heritable mutations in the glycine, $GABA_A$, and nicotinic acetylcholine receptors provide new insights into the ligand-gated ion channel superfamily. *Int. Rev. Neurobiol.* **42**, 285–332.

Viola, H., Wasowski, C., Levi de Stein, M., Wolfman, C., Silvera, R., Medina, A. E., and Paladini, A. C. (1995). Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects. *Planta Med.* **61**, 213–216.

Wallace, R. H., Marini, C., Petrou, S., Harkin, L. A., Bowser, D. N., Panchal, R. G., Williams, D. A., Sutherland, G. R., Mulley, J. C., Scheffer, I. E., and Berkovic, S. F. (2001). Mutant $GABA_A$ receptor gamma 2-subunit in childhood absence epilepsy and febrile seizures. *Nat. Genetics* **28**, 49–52.

Wallner, M., Hanchar, H. J., and Olsen, R. W. (2003). Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. *Proc. Natl. Acad. Sci. USA* **100**, 15218–15223.

Walters, R. J., Hadley, S. H., Morris, K. D. W., and Amin, J. (2000). Benzodiazepines act on $GABA_A$ receptors via two distinct and separable mechanisms. *Nat. Neurosci.* **3**, 1274–1281.

Wang, H. Y., Hui, K. M., Chen, Y. J., Xu, S. X., Wong, J. T. F., and Xue, H. (2002). Structure-activity relationships of flavonoids, isolated from *Scutellaria baicalensis*, binding to benzodiazepine site of $GABA_A$ receptor complex. *Planta Med.* **68**, 1059–1062.

Wasowski, C., Marder, M., Viola, H., Medina, J. H., and Paladini, A. C. (2002). Isolation and identification of 6-methylapigenin, a competitive ligand for the brain $GABA_A$ receptors, from Valeriana wallichii. *Planta Med.* **68**, 934–936.

Watanabe, K., Watanabe, H. Y., Goto, Y., Yamamoto, N., and Yoshizaki, M. (1975). Studies on the active principles of magnolia bark. Centrally acting muscle relaxant activity of magnolol and honokiol. *Jpn. J. Pharmacol.* **25**, 605–607.

Watanabe, K., Watanabe, H., Goto, Y., Yamaguchi, M., Yamamoto, N., and Hagino, K. (1983). Pharmacological properties of magnolol and honokiol extracted from *Magnolia officinalis*: Central depressant effects. *Planta Med.* **49**, 103–108.

Weinreb, O., Mandel, S., Amit, T., and Youdim, M. B. (2004). Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J. Nutr. Biochem.* **15**, 506–516.

Whiting, P. J. (2003). $GABA_A$ receptor subtypes in the brain: A paradigm for CNS drug discovery? *Drug Discov. Today* **8**, 445–450.

Woodman, O. L., and Chan, E. C. (2004). Vascular and anti-oxidant actions of flavonols and flavones. *Clin. Exp. Pharmacol. Physiol.* **31**, 786–790.

Wurtman, R. K., and Lee, R. K. K. (2002). Compositions and methods for treatment of mild cognitive impairment. *PCT Int. Appl.* **2002**, WO 02/28141 A2..

Yang, L., Omori, K., Otani, H., Suzukawa, J., and Inagaki, C. (2003). $GABA_C$ receptor agonist suppressed ammonia-induced apoptosis in cultured rat hippocampal neurons by restoring phosphorylated BAD level. *J. Neurochem.* **87**, 791–800.

Yang, Z. (2003). GABA, a new player in the plant mating game. *Devel. Cell* **5**, 185–186.

Yuan, C. S., Mehendale, S., Xiao, Y., Aung, H. H., Xie, J. T., and Ang-Lee, M. K. (2004). The gamma-aminobutyric acidergic effects of valerian and valerenic acid on rat brainstem neuronal activity. *Anesth. Analg.* **98**, 353–358.

Zand, R. S. R., Jenkins, D. J. A., and Diamandis, E. P. (2000). Steroid hormone activity of flavonoids and related compounds. *Breast Cancer Res. Treat.* **62**, 35–49.

Zanoli, P., Avallone, R., and Baraldi, M. (2000). Behavioural characterisation of the flavonoids apigenin and chrysin. *Fitoterapia* **71**, S117–S123.