Flavonoid and biflavonoid derivatives, their pharmaceutical compositions, their anxiolytic activity

Certain flavonoids, notably derivatives of flavone, chrysin and apigenin together with dimers thereof such as ametoflavone, have been found to possess anxiolytic properties (i.e. anxiety reducing properties) without exhibiting a sedative effect. The compounds are defined by general formula (I), wherein R¹, R², R³, R⁴, R⁵ and R⁶ are independently selected from H, OH, R, NO₂, halo, OR, NH₂, NHR, NR₂, COOR, COOH, CN or a sugar group; R² and R⁷ are both H, or R⁶ and R⁸ together form a single bond; R is C₁₋₄ alkyl or alkenyl; or the administration of an effective non-toxic amount of a biflavonoid which is a dimer of a compound of general formula (I). Novel compounds and pharmaceutical formulations are also described.
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FLAVONOID AND BIFLAVONOID DERIVATIVES, THEIR PHARMACEUTICAL COMPOSITIONS, THEIR ANXIOLYTIC ACTIVITY

The present invention relates to certain flavonoids which have been found to have anxiolytic properties (i.e. anxiety reducing) without corresponding depression of the central nervous system which is commonly also found in known sedatives such as benzodiazepines.

Some compounds of the invention are novel; other compounds are known but no pharmaceutical uses have previously been described.

Flavone is a known compound which is described in the Merck Index (entry 4030). Chrysin (2261) and apigenin (763) are other known flavonoids. Chrysin has been described as having binding properties for benzodiazepine receptors and anticonvulsant properties in Medina J.H. et al. Biochem. Pharmacol; 40: 2227-2232, 1990. This reference also suggests that chrysin may possess myorelaxant (i.e. muscle relaxant) action.

Flavone, 2-phenyl-4H-1-benzopyran-4-one has the formula

![Flavone Structure](image-url)

Chrysin is 5,7-dihydroxyflavone
Apigenin is 4', 5,7- trihydroxyflavone.
The present invention relates to a method of treatment of anxiety in a patient which comprises administering to the patient an effective non-toxic amount of a flavonoid compound of general formula (I):

![Chemical Structure](image)

where \( R^1, R^2, R^3 \) and \( R^4, R^5 \) and \( R^8 \) are independently selected from H, OH, R, NO₂, halo, OR, NH₂, NHR, NR₂, COOR, COOH, CN, or a sugar group;

\( R^6 \) and \( R^7 \) are both H, or \( R^6 \) and \( R^7 \) together form a single bond;

\( R \) is C₁₋₆ alkyl or alkenyl;

or the administration of an effective non-toxic amount of a biflavonoid which is a dimer of a compound of general formula (I) and wherein \( R^1 \) to \( R^8 \) and \( R \) have the meanings given for general formula (I).

It is found that the compounds of the present invention have anxiolytic properties without the associated depression of the central nervous system (e.g. sedative and muscle relaxant effects) commonly found with benzodiazepines. This may allow patients to be treated
for anxiety without inducing sedative or myorelaxant side-effects.

It is found further that compounds of the present invention may not show an anti-convulsant activity commonly found with benzodiazepines.

The compounds of general formula (I) wherein R¹, R², R³, R⁴ and R⁵ may independently be H, OH or halo (including F, Cl, Br or I) are preferred. The preferred halo substituent is Br, F or Cl.

The following compounds of general formula (I) are particularly preferred wherein R¹, R³, and R⁵ may be hydroxy. Also wherein R¹, and R³ are hydroxy and R⁵ is halo. Alternatively wherein R³ is hydroxy and R¹ is hydrogen, or wherein R³ and R¹ are both hydroxy is preferred. More preferably R² and R⁴ are both halo. More preferably R⁵ is OH or halo.

When R⁵ is halo it is particularly preferred that the compounds of general formula (I) are substituted at the 2' position.

The compounds of general formula (I) which are flavone, chrysin, apigenin and the derivatives 2'-chlorochrysin, 2'-fluorochrysin, 6,8-dibromochrysin and 7-bromoflavone are particularly preferred.

Compounds where R⁶ and R⁷ together form a single bond are flavone derivatives, whereas compounds where R⁶ and R⁷ are both H are flavonone derivatives.

The sugar group may be any of the known sugars, including monosaccharides, disaccharides and
polysaccharides; and may in particular be glycosyl, galactopyranosyl or mannopyranosyl.

The biflavonoid is a dimer of two covalently bonded moieties which are each of general formula (I) as set out above. Bonding between the two moieties generally occurs at the 3'-position of one moiety and the 8-position of the other moiety. The preferred biflavonoid has general formula (II) wherein \( R^1 \) to \( R^8 \) and \( R \) have the same meanings as for general formula (I).

![Diagram of the biflavonoid structure (II)](image)

The compounds of general formula (II) wherein \( R^1, R^3 \) and \( R^5 \) in each of the dimer moieties of general formula (I) are hydroxy or methoxy are preferred.

The compounds of general formula (II) wherein the compounds are amentoflavone, ginkgetin or isoginkgetin are preferred.

Pharmaceutical formulations include at least one compound of general formula (I) or (II) together with at least one pharmaceutically acceptable carrier or excipient. Each carrier must be "pharmaceutically
acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

It should be understood that the flavonoid compounds of the present invention can be administered in the form of pharmaceutically acceptable salts or esters thereof. Salts are usually acid addition salts (e.g. with hydrohalogen acids) or acceptable metal salts (e.g. Na, Ca, Mg).

Formulations include those adapted for oral, rectal, nasal, vaginal and parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention adapted for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous
or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethylcellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethylcellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide the desired release profile.

Formulations for rectal administration may be represented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulation for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes,
foams or spray formulations containing in addition to the
active ingredient such carriers as are known in the art to
be appropriate.

Formulations for parenteral administration include
aqueous and non-aqueous isotonic sterile injection
solutions which may contain anti-oxidants, buffers,
bacteriostats and solutes which render the formulation
isotonic with the blood of the intended recipient; and
aqueous and non-aqueous sterile suspensions which may
include suspending agents and thickening agents. The
formulations may be presented in unit-dose or multi-dose
sealed containers, for example, ampoules and vials, and
may be stored in a freeze-dried (lyophilized) condition
requiring only the addition of the sterile liquid carrier,
for example water for injections, immediately prior to
use. Extemporaneous injection solutions and suspensions
may be prepared from sterile powders, granules and tablets
of the kind previously described.

The dose will depend on a number of factors known to
the skilled physician including the severity of the
conditions, the identity of the recipient; and also the
efficacy and toxicity of the particular compound of
general formula (I) which is being administered.
Generally doses in the range 0.1-100 mg/kg body weight may
be used, particularly 1-10 mg/kg. The frequency of
administration will vary depending on the rate of
metabolism or excretion of the administered compound, but
may be repeated daily, optionally as two or more sub-doses. Unit doses of 20 to 500mg, preferably 100 to 400mg may be used.

The present invention further relates to a flavonoid compound of general formula (I)

![Chemical Structure](image)

wherein R₁, R₂, R₃, R₄ and R₅ are independently selected from H, OH and halo.

R⁶ and R⁷ are both H, or R⁶ and R⁷ together form a single bond; and

R is C₁-6 alkyl or alkenyl.

Embodiments of the invention will now be described by way of example only.

**EXAMPLE 1** (Preparation of synthetic flavonoids)

1. Preparation of halogenated chrysin

2′-fluorochrysin and 2′-chlorochrysin were prepared by the Floc’h Lefevre synthesis (Tetrahedron Lett. 27, 5503-5504, 1985) by reaction of ortho-fluoro or chlorobenzoyl chlorides with the ylid obtained from 2,4,6-trihydroxyphenacylidene-triphenylphosphorane. Other compounds including 6,8 dibromo chrysin were prepared from...

2. Preparation of 7-bromo flavone

Bromine was added to a solution of flavanone in carbon tetrachloride at 0°C. The ratio of bromine/flavanone was 1.3 in molar terms. The temperature of the solution was raised to 30°C and kept there for 1 hour. The temperature of the solution was then raised to 65°C and kept there for 45 minutes.

The reaction mixture was then extracted with an equal volume of a saturated solution of sodium metabisulphite and then dried with anhydrous sodium sulphate. The product was then recovered by evaporation of the solvent.

This gave a mixture of brominated flavones, in which the only active ingredient was identified by NMR analysis as 7-bromo flavone (as shown in Figure 19).

EXAMPLE 2 (Experimental effect of flavonoids)

Animals

Male CF1 mice from our breeding stock weighing 28-35g were used. The animals were placed in groups of 10-12 with free access to water and food, and maintained on a 12h/12h day-night cycle.

Drugs:

Diazepam (DZ; Hoffman-La Roche) and the flavonoids were dissolved in DMSO 40%, NaOH 0.1N (7:3: v/v) at pH 8.2 Control animals were injected with the same vehicle
(VEH). RO 15-1788 (Hoffmann-La Roche) was suspended in DMSO 10%, propyleneglycol 10% in distilled water.

**Experimental Devices**

(a) **Elevated Plus Maze:** consisted of 4 perpendicularly disposed wood arms (20 x 5cm; two had 35cm high wood walls, and two were open) linked by a central 10 x 10 cm square. The maze was suspended 50cm from the room floor. Animals were placed on the central part of the maze facing a closed arm. This test has been widely validated to measure anxiety in rodents. The number of entries into and the time spent in the open and closed arms were counted during 5 min. A selective increase in the parameters corresponding to open arms reveals an anxiolytic effect. Total exploratory activity (number of entries in both arms) was also determined.

(b) **Holeboard Test:** consisted of a wood box (60 x 60 x 30cm) with four 2cm diameter holes equidistant in the floor. The number of head-dips and the time spent head-dipping were counted during 5 min. An increase in the number and the time spent head-dipping implies a greater exploratory activity. A decrease of both parameters reveals a sedative behavior.

(c) **Locomotor Activity Test:** we used an OPTO-VARIMEX apparatus consisting of a glass box (36 x 15 x 20cm) and two lateral bars with 15 light beams (0.32 cm diameter, beam spacing 2.65 cm). The apparatus detects
automatically all the mouse movements, and discriminates between total and ambulatory activity. The locomotor activity (number of movements across the beams) was counted during 5 min. An increase in the number of transitions through the beams reflects an augmented locomotor activity.

(d) **Horizontal Wire Test**: It consisted of an horizontally strung wire (1mm diameter, 15cm long), placed at 20cm from the table. Mice were lifted by the tail, allowed to grasp the wire with their forepaws and released. The number of mice that did not grasp the wire with their forepaws or actively grasped the wire with at least one hindpaw within 3 sec was determined. After two trials, performed at 5min. intervals, the test took place. A myorelaxant drug, like diazepam at high doses, will impair the ability of mice to grasp the wire. Generally, this state of muscle relaxation is commonly associated with sedation.

**General Experimental Procedure**

The general procedure for all the tests is as follows: mice were injected with vehicle or the drug solution 20min before the beginning of the test and put into another home cage. Ro 15-1788, a specific BZD receptor antagonist was injected 10min before the tested drug. All the injections were given intraperitoneally (i.p.). Control mice were tested in each session, in
parallel with those animals receiving the test drug or
diazepam. Testing was carried out 'blind'. All data were
submitted to analysis of variance (ANOVA). Post-hoc
comparisons between individual treatments and controls
were made using Dunnett's t-test.

Results

The results of experiments in devices (a) to (d) are
summarised in the Figures 1-18.

Figure 1 shows ambulatory locomotor activity counts
during a 5min test session in an OPTO-Varimex apparatus,
20min after IP injection with DZ (0.3-3 mg/kg), or chrysin
(CHRY, 0.6-10mg/kg). Data are expressed as medians
(interquartile range) of (n) number of animals. *p< 0.05,
**p< 0.02, ***p< 0.002 significantly different from
controls (Mann Whitney test).

Figure 2 shows mean (±S.E.M.) percentage of open arm
entries (hatched bars) and percentage of time (sec) spent
in the open arms (closed bars) in mice given a 5min test
in the elevated plus-maze, 20 min after i.p. injection
with DZ (0.3 and 0.6 mg/kg), or CHRY (0.1-10 mg/kg). *p<
0.01, significantly different from controls (two-tailed
Dunnett's t-test after analysis of variance).

Figure 3 shows mean (±S.E.M.) percentage of open arm
entries (hatched bars) and percentage of time (sec) spent
in the open arms (closed bars) in mice given a 5min test
in the elevated plus-maze, 20min after i.p. injection with
VEH, CHRY (1mg/kg) or CHRY + RO 15-1788 (3 mg/kg) administered i.p. 10min before chrysin. * p< 0.01, significantly different from controls (two-tailed Dunnett’s t-test after analysis of variance). No significant differences were found in the total arm entries (F(2,49) = 3.18).

Figure 4 shows mean (± S.E.M.) number of head-dips (closed bars) and time (sec) spent head-dipping (hatched bars) for mice given a 5min test in the holeboard, 20 min after an i.p. injection with DZ (0.3-6 mg/kg) or CHRY (1-10 mg/kg). *p< 0.05, **p< 0.01, significantly different from controls (two-tailed Dunnett’s t-test after analysis of variance).

Figure 5 shows the performance of mice in the wire test 20min after an i.p. injection with DZ (3 and 6 mg/kg) or CHRY (0.6-30 mg/kg). The test took place after two trials, executed after a 5 min interval.

Figure 6 shows mean (± S.E.M.) of total entries, percentage of open arm entries (% Nr open) and percentage of time (sec) spent in the open arms (% T open) in mice given a 5 min test in the elevated plus-maze, and ambulatory activity in mice given a 5min test in an OPTO-VARIMEX apparatus, 20min after i.p. injection with vehicle and apigenin (1, 3, 10 mg/kg).

Figure 7 shows mean (± S.E.M.) number of head-dips and time (sec) spent head-dipping and rearings for mice given a 5 min test in the holeboard, and performance to
grasping the wire test, 20min after an i.p. injection with vehicle and apigenin 3 and 10 mg/kg; and

Figure 8 shows noradrenaline levels in locus coeruleus nucleus after a session of immobilization stress alone or with pretreatments as indicated in the figure (noradrenaline is expressed as per cent of control value) Apigenin blocked almost completely the noradrenaline decrease provoked by stress - first bar. (Chrysin was almost equipotent with diazepam).

Figure 9 shows total entries, percentage of open arm entries (% number open) and percentage of time (sec) spent in the open arms (% time open) in mice given a 5 min test in the elevated plus-maze, and ambulatory activity in mice given a 5min test in an OPTO-VARIMEX apparatus, 20 min after i.p. injection with vehicle and flavone (1,3 mg/kg).

Figures 10, 11, 12, 13 and 14 show mean (± S.E.M.) of total entries (hatched box), percentage of open arm entries (closed box) and percentage of time (sec) spent in the open arms (cross hatched box) in mice given a 5 min test in the elevated plus-maze, 20 min after i.p. injection with vehicle and a mixture of brominated flavone (0.6, 1.3 mg/kg) in Figure 10; 2’ chlorinated chrysosin (1,3 mg/kg) in Figure 11 and 2’ fluorinated chrysosin (3 mg/kg) in Figure 12; 6, 8 dibromo chrysosin (1mg/kg) in Figure 13; 7-bromo flavone (0.5mg/kg) in Figure 14.
* p<0.01, ** p<0.02, *** p<0.05, significantly different from the controls (student’s t-test after analysis of variance).
Figures 15, 16, 17 and 18 show ambulatory locomotor activity counts during a 5 min. test session in an OPTO-Varimex apparatus, 20 min. after i.p. injection with a mixture of brominated flavones (0.6,1,3 mg/kg), Figure 15; 2′ chlorinated chrysine (1,3 mg/kg); Figure 16; 2′ fluorinated chrysine (3 mg/kg), Figure 17; 6,8 dibromoflavone, Figure 18. Data are expressed as medians (interquartile range) of (n) number of animals. No significant difference was observed in comparison to the controls (Mann Whitney test). No significant change was observed in ambulatory activity when 7-bromo flavone was compared against a control (data not shown).

In all the experiments, diazepam DZ (0.3-6 mg/kg) was used as reference drug. Figure 1 shows the typical pharmacological profile of increasing locomotor activity by DZ. Similarly, there was a significant increase in locomotor activity with equipotent doses of chrysine (0.6-1 mg/kg). Figure 2 shows the performance of mice following i.p. administration of vehicle, DZ or chrysine on the elevated plus maze. DZ (0.3 and 0.6 mg/kg) increased the percentage of entries in the open arms (p< 0.01) and the percentage of the time spent on the open arms (p< 0.01). Chrysine (1 mg/kg) produced also an increase in both parameters (p< 0.01). No differences were observed in the total arm entries (Table 1). Thus both DZ and CHRY displayed an anxiolytic effect. The effect of chrysine (1 mg/kg) on the number of entries into and the time spent on
the open arms was prevented by the prior administration of Ro 15-1788, a central BZD receptor antagonist (Figure 3).

As shown in Figure 4 in the holeboard DZ (0.3 mg/kg) increased the number of head-dips (p< 0.05) and at 1 mg/kg increased the time spent head-dipping (p< 0.01). As expected, DZ (6 mg/kg) induced a decrease in both the number of head-dips and in the time spent head-dipping (p< 0.01) which indicates sedation.

Chrysin (3 mg/kg) produced a significant increase in the time spent head-dipping but did not elicit sedative effects at high doses (10 mg/kg).

Figure 5 shows that at 6 mg/kg DZ significantly decreased the percentage of animals grasping the horizontal wire, indicating a muscle relaxant effect. On the other hand, chrysin (0.6-30 mg/kg) was ineffective in the same test and produced no muscle relaxant effect.

Thus, chrysin had anxiolytic effects in the elevated plus maze test but did not exhibit sedation or muscle relaxation.

Figures 6 and 7 show analogous results for apigenin (API.) Apigenin administered interperitonealy shows anxiolytic effects in the elevated plus maze test (Figure 6); but did not induce any effect in the holeboard test (Figure 7) or the horizontal wire test (Figure 7) indicating no sedative or muscle relaxant activity.

Figure 8 shows that both chrysin and apigenin dampened the noradrenaline decrease in the locus coeruleus
provoked by immobilisation stress. In comparison to
diazepam, apigenin showed the most potent effect.

The figure shows per cent change (related to
controls) of locus coeruleus noradrenaline levels after
stress and different treatment (as indicated in the
figure). Rats were submitted to a 90-minute
immobilization stress session in a plastic cylinder. The
locus coeruleus was dissected out after killing by
decapitation and noradrenaline assessed by HPLC with
electrochemical detection. Doses were 1mg/kg for each
substance, injected i.p., 30 minutes before the stress
session.

Figure 9 shows the performance of mice following i.p.
administration of vehicle, D2 or flavone on the elevated
plus-maze. Flavone (1mg/kg) increased the percentage of
entries and time spent in the open arms (p<0.002)
Mann-Whitney test.

Figure 10 shows that at a concentration of 1 mg/kg a
mixture of brominated flavones results in a significant
increase in the number of entries into the open arms in
comparison to the control. The corresponding ambulatory
locomotion test (Figure 14) shows no significant decrease
in activity. Thus, the increased entries in the elevated
plus-maze test show a reduction in anxiety without a
significant decrease in activity (i.e. without a
significant sedative effect).

A similar effect can be seen for 1 mg/kg administered
2' chlorinated chrysin (Figures 11 & 16), for 3 mg/kg administered 2' fluorinated chrysin (Figures 12 & 17), for 1mg/kg administered 6,8 dibromo chrysin (Figures 13 & 18) and for 0.5mg/kg administered 7-bromo flavone (Figure 14).

**EXAMPLE 3** (Competitive inhibition of $^{3}$H-flunitrazepam to benzodiazepine receptor)

This experiment was carried out as a general screen for compounds exhibiting benzodiazepine-like activity, in order to identify compounds for testing for specific anxiolytic activity.

Binding of $^{3}$H-flunitrazepam (0.7nM) to the benzodiazepine receptor was carried out in extensively washed cerebral cortical membranes.

$^{3}$H-flunitrazepam has a $K_i$ of 3 micromole to the benzodiazepine receptor.

IC$_{50}$ values were obtained using flavonoids at different concentrations (10$^{-10}$ to 10$^{-3}$M).

Table II shows the results of the tests. Flavone, chrysin, apigenin and 6,8 dibromochrysin (IC$_{50}$=0.7-3um) show benzodiazepine ligand behaviour similar to that of $^{3}$H-flunitrazepam. 7-bromo flavone and amentaflavone show a far higher affinity for the benzodiazepine receptor (IC$_{50}$=0.05um and 0.01um respectively) than $^{3}$H-flunitrazepam.

The other flavonoids show weak benzodiazepine ligand behaviour.
EXAMPLE 4 (Induced seizures in mice)

Benzodiazepines in general show an anxiolytic effect, a sedative effect and an anticonvulsant effect. In order to assess the present compounds for anticonvulsant activity the following experiment was carried out.

The method of Medina et al Biochem. Pharm. 40 p2227-2231, (1990) was followed (with the exception that injections were carried out intraperitoneally) and the mice observed for seizures.

Table III shows that apigenin has no anticonvulsant activity. This differs from the benzodiazepines and shows that the flavonoids have a more selective and specific mode of action, being limited to anxiolytic activity.
TABLE I

Total number of arm entries made by mice during 5 min test in the elevated plus-maze, 20 min after drug injection.

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<th>DRUGS (mg/kg)</th>
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<td>VEH</td>
<td>(53)</td>
<td>8.7 ± 0.6</td>
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<tr>
<td>DZ 0.3</td>
<td>(21)</td>
<td>10.5 ± 0.8</td>
</tr>
<tr>
<td>DZ 0.6</td>
<td>(22)</td>
<td>12.4 ± 1.9</td>
</tr>
<tr>
<td>CHRYSIN 1</td>
<td>(36)</td>
<td>9.9 ± 0.8</td>
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<tr>
<td>CHRYSIN 3</td>
<td>(21)</td>
<td>8.7 ± 1.1</td>
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<tr>
<td>CHRYSIN 10</td>
<td>(15)</td>
<td>10.2 ± 1.1</td>
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Data are expressed as means ± S.E.M. of n=number of animals.
Analysis of variance F(5,160) = 2.27, p< 0.05
TABLE II

Structure - activity relationships of several flavonoids on the $^3$H Flunitrazepam binding to bovine brain membranes.

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<th>FLAVONOID</th>
<th>$^3$H Flunitrazepam binding IC$_{50}$(uM)</th>
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<tbody>
<tr>
<td>Flavone</td>
<td>1</td>
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<tr>
<td>Apigenin</td>
<td>3</td>
</tr>
<tr>
<td>Isoquercetin</td>
<td>10</td>
</tr>
<tr>
<td>6,8 dibromochrysins</td>
<td>0.7</td>
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<tr>
<td>Isoquercitrin</td>
<td>80</td>
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<tr>
<td>5 hydroxy 7 methoxyflavone</td>
<td>46</td>
</tr>
<tr>
<td>Rutin</td>
<td>60</td>
</tr>
<tr>
<td>2' fluorochrysins</td>
<td>8</td>
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<tr>
<td>2' chlorochrysins</td>
<td>9</td>
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<tr>
<td>chrysins</td>
<td>2</td>
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<td>flavanone</td>
<td>40</td>
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<tr>
<td>7-bromoflavone</td>
<td>0.05</td>
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<td>Amentoflavone</td>
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<td>Ginkgetin</td>
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<tr>
<td>Isoginkgetin</td>
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TABLE III

EFFECTS OF IP ADMINISTRATION OF APigenin ON PTZ INDUCED SEIZURES IN MICE.

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<th>Doses (mg/kg)</th>
<th>Number of animals with clonic convulsions</th>
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<tr>
<td>PTZ</td>
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</tr>
<tr>
<td>Diazepam 3 + PTZ</td>
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</tr>
<tr>
<td>Apigenin 3 + PTZ</td>
<td>8/8</td>
</tr>
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<td>Apigenin 20 + PTZ</td>
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</tr>
<tr>
<td>Apigenin 40 + PTZ</td>
<td>12/14</td>
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<tr>
<td>Apigenin 80 + PTZ</td>
<td>11/11</td>
</tr>
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The convulsant doses of pentylenetetrazole are between 50-80 mg/kg in different experiments carried out in 6 independent days.
5,7-Dihydroxy-2'-fluoroflavone (2'-fluorochrysin). Light tan prisms, mp 273-276°C. 
\(^1\)H NMR δ (DMSO-\(d_6\)) 6.24 (1H, \(d, J = 1.8 \text{ Hz}, \text{ H-6}\)), 6.47 (1H, \(d, J = 1.8 \text{ Hz}, \text{ H-8}\)), 6.69 (1H, s, H-3), 7.44 (2H, m, H-3'/-6'), 7.67 (1H, \(d, J = 6.1, J'' = 1.3 \text{ Hz}, \text{ H-4' or -5'}\)), 7.98 (1H, \(d, d, J = J' = 7.4, J'' = 1.2 \text{ Hz}, \text{ H-5' or -4'}\)), 12.67 (1H, s, C-5-OH). \(^{13}\)C NMR δ (DMSO-\(d_6\)) 94.29 (C-8), 99.33 (C-6), 104.05 (C-4a), 109.84 (d, \(J = 10.6 \text{ Hz}, \text{ C-3}\)), 117.10 (d, \(J = 21.9 \text{ Hz}, \text{ C-6'}\)), 119.38 (d, \(J = 10.6 \text{ Hz}, \text{ C-1'}\)), 125.42 (d, \(J = 3.8 \text{ Hz}, \text{ C-3'}\)), 129.72 (C-5'-or -4'), 134.05 (d, \(J = 36 \text{ Hz}, \text{ C-4' or -5'}\)), 157.72 (C-8a), 158.71 (d, \(J = 75 \text{ Hz}, \text{ C-2'}\)), 159.21 (C-2), 161.60 (C-5), 164.82 (C-7), 181.63 (C-4).

5,7-Dihydroxy-2'-chloroflavone (2'-chlorochrysin). Yellow granular powder, sublimes from 225°C, melts 273-275°C. \(^1\)H NMR δ (DMSO-\(d_6\)) 6.35 (1H, \(d, J = 1.8 \text{ Hz}, \text{ H-6}\)), 6.50 (1H, \(d, J = 1.8 \text{ Hz}, \text{ H-8}\)), 6.66 (1H, s, H-3), 7.63 (1H, \(d, d, J = J' = 7.5, J'' = 1.3 \text{ Hz}, \text{ H-5' or -4'}\)), 7.70 (1H, \(d, d, J = J' = 7.6, J'' = 1.3 \text{ Hz}, \text{ H-4' or -5'}\)), 7.76 (1H, \(d, d, J = 7.8, J'' = 1.8 \text{ Hz}, \text{ H-6' or -3'}\)), 7.78 (1H, \(d, d, J = 7.4, J'' = 1.2 \text{ Hz}, \text{ H-3' or -6'}\)), 12.67 (1H, s, C-5-OH). \(^{13}\)C NMR δ (DMSO-\(d_6\)) 94.35 (C-8), 99.54 (C-6), 103.96 (C-4a), 110.76 (C-3), 127.97 (C-5'-or -4'), 130.69 (C-6' or 3'), 131.13 (C-1), 131.50 (C-3' or 6'), 131.81 (C-2'), 132.88 (C-4' or -5'), 158.02 (C-8a), 161.75 (C-5), 162.86 (C-7), 165.26 (C-2), 181.58 (C-4).

6,8-Dibromo-5, 7-dihydroxyflavone (6,8-dibromochrysin). Prepared by bromination of chrysin at room temperature with excess bromine in acetic acid. Very fine, light yellow needles, subliming from 265°C to give prisms, mp (309) 320°C. \(^1\)H NMR δ (DMSO-\(d_6\)) 7.17 (s, H-3), 7.59 (dd, \(J = J' = 6.5 \text{ Hz}, \text{ H-3'/5'}\)), 7.62 (dd, \(J = J' = 6.5 \text{ Hz}, \text{ H-4'}\)), 8.12 (d, \(J = 6.4 \text{ Hz}, \text{ H-2'/6'}\)), 13.71 (s, C-5-OH). \(^{13}\)C NMR δ (DMSO-\(d_6\)) 88.37 (C-8), 94.47 (C-6), 105.03 (C-4a), 126.0 (C-3), 126.37 (C-2'/6'), 129.14 (C-3'/5'), 130.13 (C-1'), 132.35 (C-4'), 152.18 (C-7), 165.97 (C-8a or -5), 157.34 (C-5 or 8a), 163.41 (C-2), 181.44 (C-4).

Tectochrysin (5-hydroxy-7-methoxyflavone). Prepared from chrysin by warming with one equivalent of dimethyl sulphate in DMF with finely ground K₂CO₃. Fine, light yellow needles, mp 175-180°C (lit. 163°C). \(^1\)H NMR δ (DMSO-\(d_6\)) 3.87 (s, OCH₃), 6.39 (d, \(J = 1.7 \text{ Hz}, \text{ H-6}\)), 6.80 (d, \(J = 1.7 \text{ Hz}, \text{ H-8}\)), 7.02 (s, H-3), 7.58 (dd, \(J = J' = 7.8 \text{ Hz}, \text{ H-3'/5'}\)), 7.60 (t, \(J = 7.8 \text{ Hz}, \text{ H-4'}\)), 8.09 (d, \(J = 7.8 \text{ Hz}, \text{ H-2'/6'}\)), 12.80 (s, C-5-OH).
5,7-Dimethoxyflavone (chrysirn 5,7-di-O-methyl ether). Prepared similarly to the previous compound, but with excess dimethyl sulphate. Light tan powder, mp 147-149 °C. $^1$H NMR $\delta$ (CDCl$_3$) 3.91 (s, OCH$_3$), 3.96 (s, OCH$_3$), 6.37 ($d, J=2.0$ Hz, H-6), 6.57 ($d, J=2.2$ Hz, H-8), 6.68 (s, H-3), 7.50 ($dd, J=3.5'5'$), 7.51 ($tt, H-4'$), 7.87 ($d, J=5.3$ Hz, H-2'6').

2'-Chloro-5-hydroxy-7-methoxyflavone (2'-chlorotectochrysin). Prepared from 2'-chlorochrysin by warming with one equivalent of dimethyl sulphate in DMF with finely ground K$_2$CO$_3$. Light yellow needles, sublimes from 160°C, melts 183-185°C. $^1$H NMR $\delta$ (DMSO-d$_6$) 3.88 (s, OCH$_3$), 6.47 ($br s$, H-6), 6.66 (s, H-3), 6.72 ($br s$, H-8), 7.6 ($m$, H-3'/4'/5'), 7.82 ($br d$, $J=6.9$ Hz, H-6'), 12.64 (s, C-5-OH).

2'-Chloro-6,8-dibromo-5,7-dihydroxyflavone (2'-chloro-6,8-dibromochrysin). Prepared from 2'-chlorochrysin by bromination with excess bromine in acetic acid. Very fine, pale yellow needles, sublimes from 250°C, melts 285-290°C.

$^1$H NMR $\delta$ (DMSO-d$_6$) 6.83 (s, H-3), 7.6 (3H, m, H-3'/4'/5'), 7.84 ($d, J=6.2$ Hz, H-6'), 13.59 (s, C-5-OH). $^{13}$C NMR $\delta$ (DMSO-d$_6$) 88.66 (C-8), 94.97 (C-6) 105.20 (C-4a), 111.02 (C-3), 128.02 (C-5'), 130.50 (C-6'), 130.90 (C-1'), 131.72 (C-3'), 131.91 (C-4'), 133.24 (C-2'), 152.99 (C-7), 157.26 (C-8a or -8), 157.88 (C-5 or -8a), 163.30 (C-2), 181.40 (C-4).
CLAIMS

1. A method of treatment of anxiety in a patient which comprises administering to the patient an effective non-toxic amount of a flavonoid compound of general formula (I)

\[
\text{R}^1, \text{R}^2, \text{R}^3, \text{R}^4, \text{R}^5 \text{ and } \text{R}^8 \text{ are independently selected from } \text{H, OH, R, NO}_2, \text{ halo, OR, NH}_2, \text{ NHR, NR}_2, \text{ COOR, COOH, CN or a sugar group; } \\
\text{R}^6, \text{ and } \text{R}^7 \text{ are both } \text{H, or } \text{R}^6 \text{ and } \text{R}^7 \text{ together form a single bond; } \\
\text{R} \text{ is } C_{1-6} \text{ alkyl or alkenyl; } \\
\text{or the administration of an effective non-toxic amount of a biflavonoid which is a dimer of a compound of general formula (I).}
\]

2. A method according to claim 1 wherein \( \text{R}^6 \) and \( \text{R}^7 \) are both \( \text{H} \).

3. A method according to claim 1 wherein \( \text{R}^6 \) and \( \text{R}^7 \) together form a single bond.
4. A method according to claim 3 wherein R\(^1\), R\(^2\), R\(^3\), R\(^4\) and R\(^5\) are independently selected from H, OH, and halo.

5. A method according to claim 4 wherein R\(^1\) and R\(^3\) are both hydroxy.

6. A method according to claim 5 wherein the compound of general formula (I) is chrysin.

7. A method according to claim 4 wherein R\(^1\), R\(^3\) and R\(^5\) are all hydroxy.

8. A method according to claim 7 wherein the compound of general formula (I) is apigenin.

9. A method according to claim 4 wherein R\(^5\) is selected from OH and halo.

10. A method according to claim 5 wherein R\(^5\) is halo.

11. A method according to claim 10 wherein the compound of general formula (I) is selected from 2'-chlorochrysin and 2'-fluorochrysin.

12. A method according to claim 5 wherein R\(^2\) and R\(^4\) are both halo.
13. A method according to claim 12 wherein the compound of general formula (I) is 6,8-dibromochrysin.

14. A method according to claim 5 wherein $R^3$ is halo.

15. A method according to claim 14 wherein the compound of general formula (I) is 7-bromochrysin.

16. A method according to claim 1 wherein the biflavonoid dimer has the general formula (II).

\[
\begin{align*}
\text{wherein: } & \quad R^1 \text{ to } R^8 \text{ and } R \text{ have the meanings given in claim 1.} \\
\end{align*}
\]

17. A method according to claim 16 wherein $R^1$, $R^3$ and $R^5$ in each of the dimer moieties of general formula (I) are hydroxy or methoxy.

18. A method according to claim 17 wherein the biflavonoid dimer is amentoflavone, ginkgetin or isoginkgetin.
19. A method according to any preceding claim wherein the treatment reduces anxiety without exerting any substantial sedative effect.

20. A pharmaceutical formulation which comprises a flavonoid compound of general formula (I) as set out in claim 1 or a dimer thereof in admixture with a pharmaceutically acceptable carrier; with the proviso that R¹ to R⁸ are not all H;
R¹ and R³ are not both OH when R² and R⁴ to R⁸ are H;
and R¹ and R³ are not both OH and R⁵ is not 4’hydroxy, when R⁴ and R⁶ to R⁸ are H.

21. A formulation according to claim 20 wherein R¹, R², R³, R⁴ and R⁵ are independently selected from H, OH and halo.

22. A formulation according to claim 21 wherein R³ is halo, and R¹, R² and R⁴ and R⁵ are H.

23. A formulation according to claim 21 wherein R⁵ is halo, and R¹ and R³ are both OH.

24. A formulation according to claim 23 wherein the compound of general formula (I) is 2’-chlorochrysin or 2’-fluorochrysin.
25. A formulation according to claim 21 wherein $R^2$ and $R^4$ are both halo.

26. A formulation according to claim 25 wherein the compound of general formula (I) is 6,8-dibromochrysirin.

27. A formulation according to claim 21 wherein $R^3$ is halo.

28. A formulation according to claim 27 wherein the compound of general formula (I) is 7-bromochrysirin.

29. A formulation according to claim 20 wherein the biflavonoid dimer has the general formula (II).

![Chemical Structure](image)

wherein: $R^1$ to $R^8$ and $R$ have the meanings given in claim 20.

30. A formulation according to claim 29 wherein $R^1$, $R^3$ and $R^5$ in each of the dimer moieties of general formula (I) are hydroxy or methoxy.
31. A formulation according to claim 30 wherein the biflavonoid dimer is amentoflavone, ginkgetin or isoginkgetin.

32. A flavonoid compound of general formula (I)

![Chemical Structure](image)

wherein $R^1$, $R^2$, $R^3$, $R^4$ and $R^5$ are independently selected from H, OH and halo.

$R^6$ and $R^7$ are both H, or $R^6$ and $R^7$ together form a single bond; and

$R$ is $C_{1-6}$ alkyl or alkenyl. with the proviso that at least one of $R^1$, $R^2$, $R^3$, $R^4$ and $R^5$ is halo.

33. A flavonoid compound according to claim 32 which is selected from

- 2'-chlorochrysin
- 2'-fluorochrysin
- 6,8-dibromochrysin, and
- 7-bromoflavone.
34. The use of a flavonoid compound of general formula (I) as defined in claim 1 or a biflavonoid which is a dimer thereof for the preparation of a medicament for the treatment of anxiety in a patient.
FIG. 4
FIG. 5
% of control value

0 20 40 60 80 100
Stress Stress Stress Stress
Diazepam + Chrysin + Apigenin

FIG. 8
FIG. 9
FIG. 15
FIG. 17

VEH 3 mg/kg

(5) (7)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<td>MEDINA ET AL 'Chrysin (5,7-di-OH-Flavone), a Naturally Occuring Ligand for</td>
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<td>Benzodiazepine Receptors, with Anticonvulsant Activity' cited in the application</td>
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Further documents are listed in the continuation of box C.

| Patent family members are listed in annex. |

X

* Special categories of cited documents:
  - 'A' document defining the general state of the art which is not considered to be of particular relevance
  - 'B' earlier document but published on or after the international filing date
  - 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - 'O' document referring to an oral disclosure, use, exhibition or other means
  - 'P' document published prior to the international filing date but later than the priority date claimed
  - 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  - 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
  - 'Z' document member of the same patent family

Date of the actual completion of the international search

9 January 1995

Date of mailing of the international search report

27 Oct. 95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax (+31-70) 340-3016

Authorized officer

Uiber, P

Form PCT/ISA/210 (second sheet) (July 1992)
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<td>BIOCHEMICAL PHARMACOLOGY, vol.37, 1988 pages 3285 - 87 NIELSEN ET AL 'High Affinity of the Naturally Occurring Biflavonoid, Amentoflavon, to Brain Benzodiazepine Receptors In Vitro' see page 3286, column 2, line 25 - page 3287, column 2, line 23</td>
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<td>PLANTA MEDICA, vol.43, 1981 pages 64 - 70 CHAKRAVARTHY ET AL 'Isolation of Amentoflavone from Selaginella Rupesstris and its Pharmacological Activity on Central Nervous System, Smooth Muscles and Isolated Frog Heart Preparations' see the whole document</td>
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<td>ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol.36, 1992 pages 1890 - 93 HAYASHI ET AL 'Mechanism of Action of the Antiherpesvirus Biflavone Ginkgetin' see the whole document</td>
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<td>INDIAN J. MED. RES., vol.88, 1988 pages 192 - 96 GOEL ET AL 'Mechanism of Anti-Ulcerogenic Effect of Amentoflavone' Abstract see the whole document</td>
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<td>EXPERIENTIA, vol. 47, 1991, pages 195 - 199; CHOLBI ET AL 'Inhibitory Effects of Phenolic Compounds on CCl4-Induced Microsomal Lipid Peroxidation'</td>
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