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(57) Abstract

This invention relates to a particular group of chemical compounds for use as non–sedative anxiolytic drugs, and a method for the identification of chemical compounds with anxiolytic potential.

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CHEMICAL COMPOUNDS FOR USE AS ANXIOLYTIC AGENTS AND A METHOD FOR THE IDENTIFICATION OF ANXIOLYTIC COMPOUNDS

TECHNICAL FIELD

This invention relates to a particular group of chemical compounds for use as non-sedative anxiolytic drugs, and a method for the identification of chemical compounds with anxiolytic potential.

BACKGROUND ART

Receptors for the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA), are divided into two main classes, $GABA_A$ receptors and $GABA_B$ receptors.

$GABA_A$ receptors are members of a ligand-gated ion channel family, and are the most abundant inhibitory receptors in the mammalian brain. Each $GABA_A$ receptor complex comprises a chloride ion channel that controls the chloride flux across the neuronal membrane, along with multiple recognition sites for small modulatory molecules such as benzodiazepines, barbiturates, picrotoxin, and certain steroids. When GABA interacts with its receptor, the ion channel is opened, the chloride flux is enhanced, and the cell becomes less responsive to excitatory stimuli. This GABA induced ion current can be regulated by various agents, including agents that interact with the benzodiazepine receptor or recognition site.

Agents that bind or interact with the modulatory sites on the $GABA_A$ receptor complex, e.g. the benzodiazepine receptor, and have a positive modulatory effect on the action of GABA, are called benzodiazepine receptor agonists or partial agonists. Agonists generally produce muscle relaxant, hypnotic, sedative, anxiolytic, and/or anticonvulsant effects.

Benzodiazepine receptor ligands with negative modulatory effect on the action of GABA are termed inverse agonists, while benzodiazepine receptor ligands with no intrinsic activity are termed antagonists.

The present invention resides in the surprising discovery that a particular chemical compound, 5-acetyl-1-(3-(3-pyridyl)phenyl)benzimidazole O-ethyl oxime, in

addition to being a potent benzodiazepine receptor agonist, is also characterised by a remarkable capability to effectively potentiate responses evoked by lower concentrations of GABA, while inhibiting responses evoked by higher concentrations of GABA.

SUMMARY OF THE INVENTION

The present invention provides chemical compounds for use as non-sedative anxiolytic drugs, and pharmaceutical compositions comprising these anxiolytic compounds.

Moreover, the present invention provides methods for the screening of chemical compounds for anxiolytic potential. More specifically it is an object of the present invention to provide a convenient tool for the identification of benzodiazepine receptor agonists or partial agonists, capable of exerting a positive modulation of the $GABA_A$ receptor complex, which benzodiazepine receptor agonists or partial agonists are devoid the sedative effects often encountered with conventional benzodiazepine receptor agonists.

Accordingly, in its first aspect, the invention relates to the use of a chemical compound for the manufacture of a pharmaceutical composition for the treatment of anxiolytic disorders, which chemical compound potentiate responses evoked by lower concentrations of GABA, while inhibiting responses evoked by higher concentrations of GABA.

In another aspect the invention provides a method for the screening of chemical compounds for their potential as anxiolytic drugs, which method comprises the steps of determining, in the presence of different concentrations of GABA, the electrophysiological response evoked by GABA on a $GABA_A$ receptor; repeating the first step in the presence of a chemical compound to be screened; and selecting the chemical compounds that selectively potentiate responses evoked by GABA at lower concentrations.

In a further aspect, the invention provides chemical compounds capable of potentiating responses evoked by lower concentrations of GABA, while inhibiting responses evoked by higher concentrations of GABA, and the use of these compounds as non-sedative anxiolytic drugs.

Finally the invention provides pharmaceutical compositions comprising the chemical compound of the invention, and methods of treating anxiolytic disorders, by which methods a mammal, including a human, is treated with a chemical compound of the invention.

DETAILED DISCLOSURE OF THE INVENTION

The Chemical Compounds

The present invention resides in the surprising discovery that a particular chemical compound, 5-acetyl-1-(3-(3-pyridyl)phenyl)benzimidazole O-ethyl oxime, Compound 6 obtained as described in Ex. 1 (hereinafter referred to as Compound 6), in addition to being a potent benzodiazepine receptor agonist, is also characterised by a remarkable capability to potentiate the effect of GABA at low concentrations, while inhibiting the effect of GABA at higher concentrations. I.e. the effect of Compound 6 on a GABA evoked response was dependent on the GABA concentration used.

This effect is considered unexpected because Compound 6, while being a benzodiazepine receptor agonists, in itself has a positive modulatory effect on the action of GABA, and because of this positive modulatory effect would be expected to show an effect that is additive to the effect of GABA or even synergistic, no matter at what GABA concentration.

In particular it was noted that at low GABA concentrations (of from about 0.3 to about 1 μM), Compound 6 show a 4-5 fold potentiation of the GABA response. This potentiation is higher than for the classic benzodiazepines. However, at higher stimulus intensity (at GABA concentrations of more than about 3 μM), Compound 6 show little or no potentiation of GABA responses, and at very high GABA concentrations (of more than about 10 μM), Compound 6 actually blocked GABA responses.

In other words, Compound 6, like the classical benzidoazepines, possesses a potentiating efficacy at lower concentrations of GABA, while this compound, in contrast to the classical benzidoazepines, more quickly looses its potentiating efficacy at slightly higher concentrations of GABA, and ultimately, at high GABA concentrations, becomes an inhibitor of the GABA response.

The ability of Compound 6 to act as an efficient potentiator at relative low stimulus intensity while loosing its efficacy at higher stimulus intensity is a very attractive property, and in this way Compound 6 is considered a representative for an entire group of chemical compounds that is particularly useful as non-sedative anxiolytic drugs.

The group of chemical compounds contemplated according to this invention distinguish from the classical anxiolytic compounds and thus represents a novel and distinct group of anxiolytic compounds.

The chemical compounds of the invention is characterised by being a chemical compound that, like the classical benzidoazepines, possesses a potentiating efficacy at lower concentrations of GABA, while, in contrast to the classical benzidoazepines, loosing its potentiating efficacy at higher concentrations of GABA, and ultimately, at very high GABA concentrations, becomes an inhibitor of the GABA response.

In a preferred embodiment, the chemical compounds of the invention may be characterised by being any chemical compound, apart from Compound 6, which is capable of potentiating responses evoked by concentrations of GABA of below about 5 μM, preferably below about 3 μM, while inhibiting responses evoked by concentrations of GABA of above about 3 μM, preferably above about 5 μM.

A Method of Screening

Based on this finding, we now provide an efficient tool for use in the screening of new chemical compounds for their potential for use as anxiolytic drugs, in particular for use as non-sedative anxiolytic drugs.

According to the method of the invention the electrophysiological response evoked by GABA on a $GABA_A$ receptor is determined at different concentrations of GABA and in the presence and absence of the compound to be tested.

After the electrophysiological response has been recorded, the response evoked by GABA alone is compared to that recorded in the presence of the compound to be tested. According to the present invention, compounds capable of increasing the response evoked by GABA at lower concentrations of GABA are selected.

It is at present contemplated that the method shall be carried out at concentrations of GABA in the range of from about 0.001 μM to about 10,000 μM,

preferably in the range of from about 0.01 μM to about 1000 μM, more preferred in the range of from about 0.1 μM to about 500 μM, most preferred of from about 0.3 μM to about 300 μM.

Also it is contemplated that chemical compounds that selectively potentiate responses evoked by GABA at concentrations lower than 10 μM, preferably lower than 5 μM, most preferred lower than 3 μM, are selected.

Positive Modulatory Effect

As noted above, benzodiazepine receptor agonists or partial agonists are agents having a positive modulatory effect on the response on $GABA_A$ receptors evoked by the action of GABA. A positive modulatory response evoked on $GABA_A$ receptors by the action of GABA may be measured using known electrophysiological methods, in particular the well known patch clamp technology.

In a preferred embodiment the response evoked on $GABA_A$ receptors by the action of GABA may be measured using whole-cell patch clamp electrophysiological methods.

Cells

The whole-cell patch clamp method may be applied to any cell holding a $GABA_A$ receptor.

In a preferred embodiment, the cell applied in this method is a CHO cell, a HEK cell, or a HeLa cell.

$GABA_A$ Receptors

The $GABA_A$ receptors are structurally constituted macromolecular heteropentameric assemblies, containing a combinations of α, β, and γ/δ protein subunits. Several subunits of such $GABA_A$ receptors (α_{1-6}, β_{1-3}, δ and γ_{1-3}) have been characterised using techniques of modern molecular biology. By far the largest population (representing almost half of all $GABA_A$ receptors in the brain) is that containing the α1 subunit in combination with the β2 and γ2 subunits.

In a preferred embodiment, a receptor comprising an α1, an α2, an α3, an α5, or an α6 subunit; a β1, a β2, or a β3 subunit; and a γ2S or a γ2L subunit, or any combination hereof, is employed.

Also αβδ $GABA_A$ receptor subunits, or αβε $GABA_A$ receptor subunits may be employed.

According to the present invention the use of co-expression of the $GABA_A$ receptor subunits α1, β2 and γ2 is found to be very useful. Also the α1β2γ2L; the α1β3γ2L; the α2β2γ2L; the α2β3γ2L; the α3β2γ2L; the α3β3γ2L; the α5β2γ2L; the α5β3γ2L; the α6β2γ2L; and the α6β3γ2L $GABA_A$ receptors are found to be useful.

In a preferred embodiment, the whole-cell patch clamp method is applied to a CHO cell expressing the α1β2γ2 $GABA_A$ receptor.

Anxiolytic Drugs

In another aspect the invention relates to the use of the chemical compound of the invention for the manufacture of a pharmaceutical composition for the treatment of anxiolytic disorders.

As defined herein, anxiety disorders include generalised anxiety disorders, panic attacks, phobic disorders, obsessive-compulsive disorders, post-traumatic disorders, and mixed anxiety and depressive disorders.

Pharmaceutical Compositions

In another aspect the invention provides novel pharmaceutical compositions comprising a therapeutically effective amount of the chemical compound of the invention.

While a chemical compound of the invention for use in therapy may be administered in the form of the raw chemical compound, it is preferred to introduce the active ingredient, optionally in the form of a physiologically acceptable salt, in a pharmaceutical composition together with one or more adjuvants, excipients, carriers, buffers, diluents, and/or other customary pharmaceutical auxiliaries.

In a preferred embodiment, the invention provides pharmaceutical compositions comprising the chemical compound of the invention, or a pharmaceutically acceptable salt or derivative thereof, together with one or more pharmaceutically acceptable carriers therefor, and, optionally, other therapeutic and/or prophylactic ingredients, know and used in the art. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not harmful to the recipient thereof.

The pharmaceutical composition of the invention may be administered by any convenient route which suite the desired therapy. Preferred routes of administration include oral administration, in particular in tablet, in capsule, in dragé, in powder, or in liquid form, and parenteral administration, in particular cutaneous, subcutaneous, intramuscular, or intravenous injection. The pharmaceutical composition may be prepared by the skilled person using standard and conventional techniques appropriate to the desired formulation.

The actual dosage depend on the nature and severity of the disease being treated, and is within the discretion of the physician, and may be varied by titration of the dosage to the particular circumstances of this invention to produce the desired therapeutic effect. However, it is at present contemplated that the pharmaceutical composition of the invention contain, as the active compound, of from about 1 to about 1000 mg of the chemical compound of the invention per individual dose.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is further illustrated by reference to the accompanying drawing, in which:

Fig. 1 shows responses to 0.3, 3, 30 and 300 µM GABA, recorded from a CHO cell expressing $GABA_A$ $\alpha1\beta2\gamma2$ receptors;

Fig. 2 shows the full dose-response curve for GABA, recorded from a CHO cell expressing $GABA_A$ $\alpha1\beta2\gamma2$ receptors (the EC_{50}-value was calculated to 18.8 µM and the Hill coefficient to 1.74);

Fig. 3 shows responses to 6 µM GABA, 1 µM GABA, and 10 µM GABA, recorded in the absence and the presence of 10 µM of a test compound (Compound 6 of Ex. 1), recorded from a CHO cell expressing $GABA_A$ $\alpha1\beta2\gamma2$ receptors;

Fig. 4 shows the effect of 10 µM of a test compound (Compound 6 of Ex. 1) as a function of the GABA concentration, recorded from a CHO cell expressing $GABA_A$ $\alpha1\beta2\gamma2$ receptors;

Fig. 5 shows the effect of 1 µM of a test compound (Compound 6 of Ex. 1) as a function of the GABA concentration, and compared to 1 µM of a classical benzodiazepine agonist (Diazepam™), recorded from a CHO cell expressing $GABA_A$ $\alpha1\beta2\gamma2$ receptors; and

Fig. 6 shows the simultaneous effect of 10 μM of a test compound (Compound 6 of Ex. 1) and 3 μM of a classical benzodiazepine agonist (Diazepam™), compared the effect of 10 μM of the test compound alone, recorded from a CHO cell expressing $GABA_A$ α1β2γ2 receptors.

EXAMPLES

The invention is further illustrated with reference to the following examples which are not intended to be in any way limiting to the scope of the invention as claimed.

Example 1

Preparatory Example

5-Acetyl-1-(3-(3-pyridyl)phenyl)benzimidazole O-ethyl oxime (Compound 6)

4-Fluoro-3-nitroacetophenone (Compound 1): Concentrated sulphuric acid (200 ml) is cooled to 5°C. 4-Fluoroacetophenone (20 ml, 0.16 mol) is added keeping the temperature below 10°C. The mixture is cooled to 0-5°C and potassium nitrate (25 g, 0.25 mol) is added portionwise over 2 hours keeping the temperature within this range. Following the addition the mixture is stirred in the cold for additionally 2 hours. The mixture is poured on ice (600 g) and the crude product is filtered off. Column-chromatographic purification on silica gel using a mixture of ethyl acetate and petroleum ether (1:9) as the eluent affords pure Compound 1 (18.2 g, 60%). Mp 48-49°C.

3-(3-Pyridyl)aniline (Compound 2): A mixture of diethyl 3-pyridylborane (16.3 g, 0.11 mol), 3-bromoaniline (12.2 ml, 0.11 mol), potassium carbonate (45.8 g, 0.33 mol) and tetrakis(triphenylphosphine)palladium(0) (80 mg) in a mixture of water (40 ml) and dimethoxyethane (80 ml) is heated to 80°C under a stream of nitrogen over night. After cooling the mixture is diluted with water and ethyl acetate and filtered through a fluted filter paper. The layers are separated. The aqueous layer is extracted once with ethyl acetate. The combined organic phases are dried over sodium sulphate and concentrated under reduced pressure. The residue is dissolved in ethanol. Water is added and the mixture is evaporated to dryness. This residue crystallises upon trituration with ice-cold water. The crystals are collected, dried and washed with petroleum ether to afford pure Compound 2 (16.3g, 87%). Mp 75-76°C.

4-Acetyl-2-nitro-N-(3-(3-pyridyl)phenyl)aniline (Compound 3): A mixture of Compound 1 (5 g, 27.3 mmol) and Compound 2 (4.62 g, 27.2 mmol) in dry N-methyl-2-pyrrolidone (10 ml) is stirred at 40-50°C over night. The resulting solid reaction mixture is suspended in ice-water and rendered alkaline by addition of aqueous sodium carbonate (1M). The product is filtered off, washed with water and dried to yield 7.68 g (Compound 3) (85%). Mp 112-113°C.

5-Acetyl-2-(3-(3-pyridyl)phenylamino)aniline (Compound 4): Compound 3 (2 g, 6 mmol) is suspended in a mixture of ethanol (50 ml) and dichloromethane (10 ml) and is hydrogenated at ambient pressure using palladium (5% on activated carbon) as the catalyst. Filtration of the resulting solution through celite followed by evaporation of solvent leaves an oil. Trituration with a mixture of diethyl ether and petroleum ether (1:1) affords 1.46 g pure Compound 4 (80%). Mp 175-176°C.

5-Acetyl-1-(3-(3-pyridyl)phenyl)benzimidazole (Compound 5): A solution of Compound 4 (7 g, 23.1 mmol) in formic acid (20 ml) is stirred overnight at ambient temperature. The mixture is poured into water (750 ml) and rendered basic with concentrated aqueous ammonia. The precipitate is filtered off, washed with water and dried. The crude product is dissolved in refluxing ethanol. Water is added until onset of precipitation. The mixture is allowed to cool. The product is filtered off and dried to yield 4.3 (60%). Mp. 200 - 202°C.

5-Acetyl-1-(3-(3-pyridyl)phenyl)benzimidazole O-ethyl oxime (Compound 6): Compound 5 (5 g, 15.97 mmol) is suspended in absolute ethanol (50 ml) and heated to 70°C. O-Ethyl hydroxylamine hydrochloride (2.4 g, 24.61 mmol) is added and the mixture is heated to reflux for 1.5 hours. After cooling the solvent is removed by evaporation, and the residue is stirred with aqueous sodium hydroxide (50 ml, 1M). The crude product is filtered off. Column-chromatographic workup on silica gel using a mixture of ethyl acetate and ethanol (9:1) as the eluent affords pure Compound 6. Yield: 3.7g (65%). Mp 105-106°C.

Example 2

Preparation of CHO Cells expressing $GABA_A$ Receptors

Cloning: The cloning of $hGABA_A$ subunits was carried out as follows. Human brain hippocampus poly A^+ mRNA (Clontech) was reverse transcribed with oligo dT primer and MMLV reverse transcriptase (Pharmacia). 100 ng first strand

cDNA was subjected to PCR using Expand HF polymerase (Boehringer Mannheim) and specific primer sets spanning the full length cDNA sequence. The primer sets were:

1. $hGABA_A\alpha_1$: (s) 5'CCTTAGCTGCTCCAGCCCGCGATGAGGA3';
   (as) 5'TTCCCAGTGCAGAGGAACTGAACAACAGAATG3';
2. $hGABA_A\beta_2$: (s) 5'AAAACTAAAGGGATGTGGAGAGTGCGGAAAAG3';
   (as) 5'TCCAGTGGGAGGCCATGTTTTAGTTCACATAA3';
3. $hGABA_A\gamma_2$: (s) 5'CTGCAACCAAGAGGCAAGAGGCGAGAG3'; and
   (as) 5'CCATATCAGTAAAACCCATACCTCCTCACAGG3'.

PCR conditions were 1) 94 °C 60 seconds; 2) 15 times (94 °C for 60 seconds, 55°C for 60 seconds, 72°C for 120 seconds); 3) 20 times (94 °C for 60 seconds, 55°C for 60 seconds, 72°C for 180 seconds); 4) 72°C for 10 minutes. The amplified products were polished with *pfu* polymerase (Stratagene), purified on Quiaquick column (Qiagen) and subcloned into pCRScript (Stratagene). Maxiprep DNA (Qiagen) was prepared from positive clones and two to three individual clones of each subunits was sequenced bidirectionally on an ABI 377 sequencer using standard chain termination dyes (Perkin Elmer).

Subcloning: The α_1-subunit was subcloned into pUbi2z (a custom designed mammalian expression vector using a human ubiquitin promoter to drive the inserted cDNA and the cDNA for enhanced green fluorescence protein (EGFP) fused to the selection marker conferring Zeocin resistance), the β_2-subunit was subcloned into pNS1n (a custom designed mammalian expression vector using a CMV promoter to drive the inserted cDNA and kanamycin/neomycin selection marker), and the γ_{2s}-subunit was subcloned into pZeoSV (Invitrogen).

A single plasmid containing expression cassettes for both the β_2 subunit and the γ_{2s}-subunit was constructed as follows. The entire expression cassette for the γ_{2s}-subunit in pZeoSV (SV40 promoter, cDNA, SV40-polyA) was excised as a BamHI fragment and subcloned into the BglII site at position 1 in $pNS1n/hGABA_A\beta_2$. A clones in which the driving promoters SV40 and CMV were pointing in opposite directions was selected.

Transfection: For transient transfection experiments 1 µg of $pUbi2z/\alpha_1$ was mixed with 1µg of $pNS1n/\beta_2\gamma_{2s}$ and transfected into Chinese hamster ovary cells

(CHO, ATCC) using lipofectamine (Gibco) according to manufactures instructions. 24 hours after transfection, cells were plated into 35 mm Petri dishes containing 3.5 mm glass coverslips. Cells were kept at 37°C, in 5% CO_2/95% air and used for electrophysiological studies 2-50 hours after plating.

Example 3

Patch Clamp Experiments

Electrophysiology: All experiments were performed in voltage clamp using conventional whole cell patch clamp methods. The following salt solutions were used:

Extracellular solution (mM): NaCl (140), KCl (4), $CaCl_2$ (2), $MgSO_4$ (4), HEPES (10, pH =7.4);

Intracellular solution (mM): CsCl (120), CsF (20), $MgSO_4$ (4), EGTA (10), ATP (4), HEPES (10, pH = 7.2).

Electronics, programs and data acquisition: The amplifier used was an EPC-9 (HEKA-electronics, Lambrect, Germany) run by a Macintosh 7600/120 computer via an ITC-16 interface. Experimental conditions were set with the Pulse-software accompanying the amplifier. Data were low pass filtered and sampled directly to hard-disk at a rate of 3 times the cut-off frequency.

Pipettes and electrodes: Pipettes were pulled from borosilicate glass (Modulohm, Copenhagen, Denmark) using a horizontal electrode puller (Zeitz-Instrumente, Augsburg, Germany). The pipette resistance was 1.6 - 2.6 mW in the salt solutions used in these experiments. The pipette electrode was a chloridised silver wire, and the reference was a silverchloride pellet electrode (In Vivo Metric, Healdsburg, USA) fixed to the experimental chamber. The electrodes were zeroed with the open pipette in the bath just prior to sealing.

Experimental procedure: The patch clamp experiments were performed at room temperature (20 - 22 °C).

Coverslips were transferred to a 15 ml experimental chamber mounted on the stage of an inverted microscope (IMT-2, Olympus) supplied with Nomarski optics and a mercury lamp (Olympus). Cells were continuously superfused with extracellular saline at a rate of 2,5 ml/min. Cells chosen for experiments were spherical and emitted bright green fluorescence when exposed to UV-light from the mercury lamp. After

giga-seal formation (1-5 GW, success-rate ≈ 90 %) the whole cell configuration was attained by suction.

The cells were held at a holding potential of -60 mV and at the start of each experiment the current was continuously measured for 20 sec to ensure a stable baseline. GABA-containing solutions were delivered to the chamber through a custom-made gravity-driven flowpipe, the tip of which was placed approximately 50 µm from the cell. Application was triggered by compressing the tubing connected to the flow pipe with a valve controlled by the Pulse-software. In general, GABA was applied for 4 sec. every 30 sec. The sampling interval during application was 1 msec. Having obtained responses of a stable amplitude, the extracellular saline as well as the agonist containing solution were switched to solutions containing the compound (i.e. Compound 6 or Diazepam) to be tested. The compound was present until responses of a stable amplitude were achieved.

Currents were measured at the peak of the responses. Effect of compounds was calculated as the peak current at compound equilibrium divided by the current evoked by the pulse immediately before the compound was included.

The results of these experiments are presented in Figs. 1-6.

In Fig. 1 the cell was voltage clamped at -60 mV and exposed to GABA at varying concentrations ejected from the flowpipe. The time between pulses was 30 sec.

In Fig. 2 the magnitude of the peak currents were expressed as fractions of the peak current obtained with a maximal concentration of GABA. Symbols represent mean ±SEM, n=3-10. The curve is a fit to the Michaelis-Menten equation and the parameters EC50 and n were calculated from the equation.

In Fig. 3 the cell was voltage clamped at -60 mV and exposed to 4-sec pulses of 6 µM GABA every 20 sec. After assessment of the effect of Compound 6 (indicated by the horizontal bar, top panel), the GABA concentration was changed to 1 µM, and the effect of Compound 6 was evaluated (middle panel). Finally, the effect of Compound 6 on responses evoked by 10 µM GABA was evaluated (bottom) (please note the different scaling).

In Fig. 4 cells were voltage clamped at -60 mV and exposed to 4-sec pulses of GABA at 0.3-100 µM. After obtaining responses of a stable amplitude, Compound 6 (10 µM) was included in the bath-Ringer and in the GABA solutions. When responses

again were stable, solutions were switched back to control. Results are expressed as % of control, referring to the response obtained just before Compound 6 was included (100 % = no effect on peak amplitude). Data points are mean ±SEM (n=3-10).

In Fig. 5 the cells were voltage clamped at -60 mV and exposed to 4-sec pulses of GABA at 0.3-100 µM. After obtaining responses of a stable amplitude, Compound 6 (1 µM, closed symbols) or Diazepam (1 µM, open symbols) was included in the bath-Ringer and in the GABA solutions. When responses again were stable, solutions were switched back to control. Results are expressed as % of control, referring to the response obtained just before the drug in question was included (100 % = no effect on peak amplitude). Data points are mean ±SEM (n=3-5).

In Fig. 6 the cells were voltage clamped at -60 mV and exposed to 4-sec pulses of 0.3 µM GABA every 20 sec. After two control responses of the same size, Compound 6 (10 µM) and Diazepam (3 µM) were included in the solutions. After one minute (3 responses under the hatched bar), Diazepam was omitted from the solutions. After one minute (3 responses under the open bar only) solutions were changed back to control conditions.

CLAIMS

1. Use of a chemical compound for the manufacture of a pharmaceutical composition for the treatment of anxiolytic disorders, which chemical compound potentiate responses evoked by lower concentrations of GABA, while inhibiting responses evoked by higher concentrations of GABA.

2. The use according to claim 1, in which the chemical compound potentiate responses evoked by concentrations of GABA of below 3 μM, while inhibiting responses evoked by concentrations of GABA of more above 3 μM.

3. The use according to claim 1, in which the chemical compound potentiate responses evoked by concentration of GABA in the range of from about 0.001 to about 10 μM, more preferred in the range of from about 0.1 to about 5 μM, most preferred in the range of from about 0.3 to about 3 μM.

4. The use according to claim 1, in which the chemical compound inhibit responses evoked by concentrations of GABA of more than about 50 μM, more preferred of more than about 10 μM, yet more preferred of more than about 5 μM, most preferred of more than about 3 μM.

5. A method for the screening of chemical compounds for their potential as anxiolytic drugs, which method comprises the steps of
   (i) determining, in the presence of different concentrations of GABA, the electrophysiological response evoked by GABA on a $GABA_A$ receptor;
   (ii) repeating step (i) in the presence of a chemical compound to be screened; and
   (iii) selecting the chemical compounds that selectively potentiate responses evoked by GABA at lower concentrations.

6. The method according to claim 5, wherein, in steps (i) and (ii), the concentration of GABA range of from about 0.001 μM to about 10,000 μM, preferably of from

about 0.01 μM to about 1000 μM, more preferred of from about 0.1 μM to about 500 μM, most preferred of from about 0.3 μM to about 300 μM.

7. The method according to claim 5, wherein in step (iii) chemical compounds that selectively potentiate responses evoked by GABA at concentrations lower than 5 μM, preferably lower than 3 μM, are selected.

8. The method according to any of claims 5-7, wherein the determination of the electrophysiological response in step (i) and (ii) is performed by voltage clamp using whole cell patch clamp methods.

9. The method according to claim 8, wherein the $GABA_A$ receptor is located on a CHO cell, a HEK cell, or a HeLa cell.

10. The method according to claim 9, wherein the cell is expressing the $\alpha1\beta2\gamma2$; the $\alpha1\beta2\gamma2L$; the $\alpha1\beta3\gamma2L$; the $\alpha2\beta2\gamma2L$; the $\alpha2\beta3\gamma2L$; the $\alpha3\beta2\gamma2L$; the $\alpha3\beta3\gamma2L$; the $\alpha5\beta2\gamma2L$; the $\alpha5\beta3\gamma2L$; the $\alpha6\beta2\gamma2L$; or the $\alpha6\beta3\gamma2L$ $GABA_A$ receptor.

11. The method according to claim 9, wherein cell is a CHO cell expressing the $\alpha1\beta2\gamma2$ $GABA_A$ receptor.

12. A chemical compound having anxiolytic activity identified by the method according to any of claims 5-11.

13. A chemical compound capable of potentiating responses evoked by lower concentrations of GABA, while inhibiting responses evoked by higher concentrations of GABA.

14. The chemical compound of claim 13, which is capable of potentiating responses evoked by concentrations of GABA of below 3 μM, while inhibiting responses evoked by concentrations of GABA of more above 3 μM.

15. The chemical compound of either of claims 13-14 for use as a non-sedative anxiolytic drug.

16. A pharmaceutical composition comprising the chemical compound of any of claims 12-15.

17. A method of treating an anxiolytic disorder, by which method a mammal, including a human, is treated with a chemical compound according to any of claims 12-15.

Figure 1

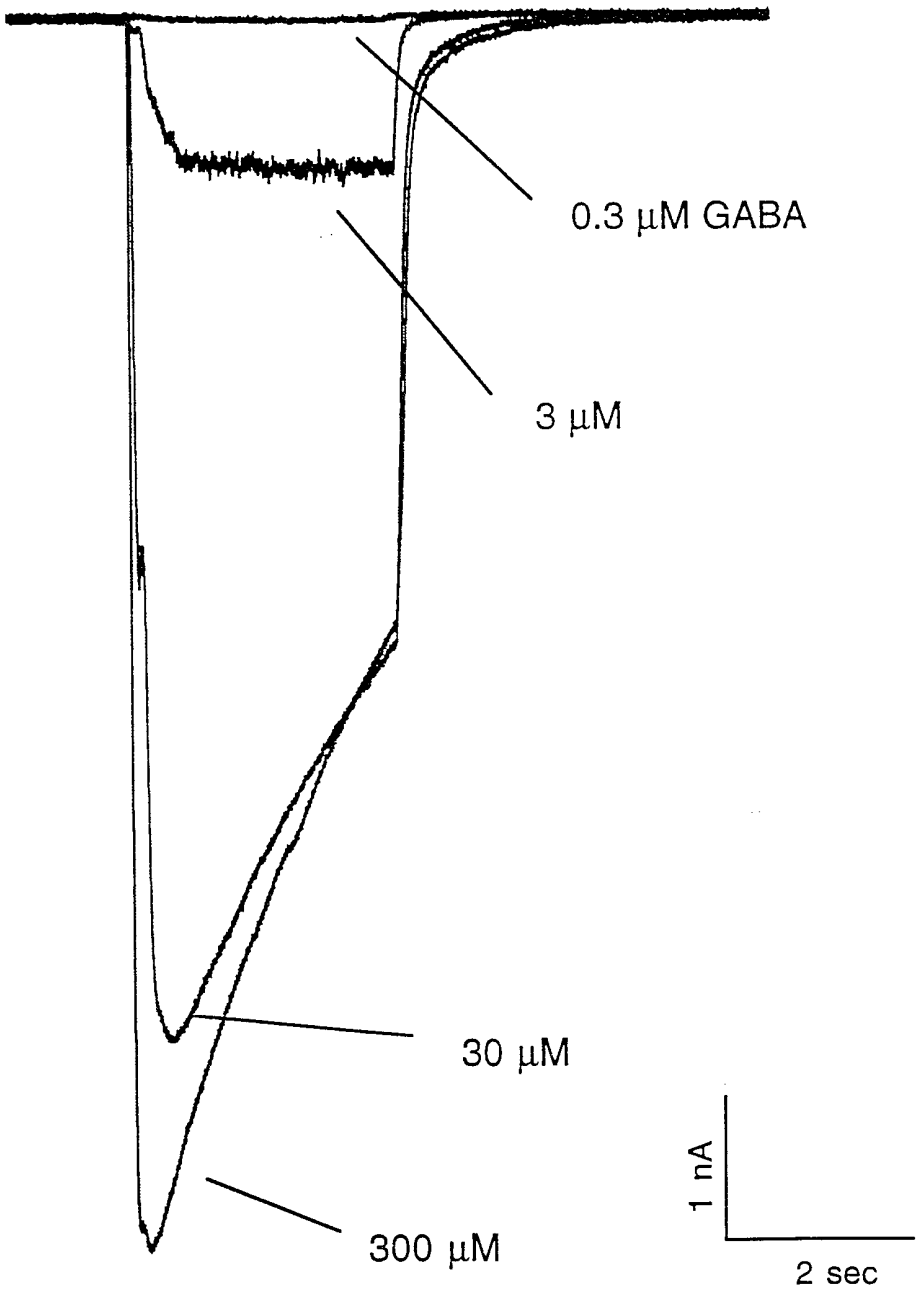

Figure 2

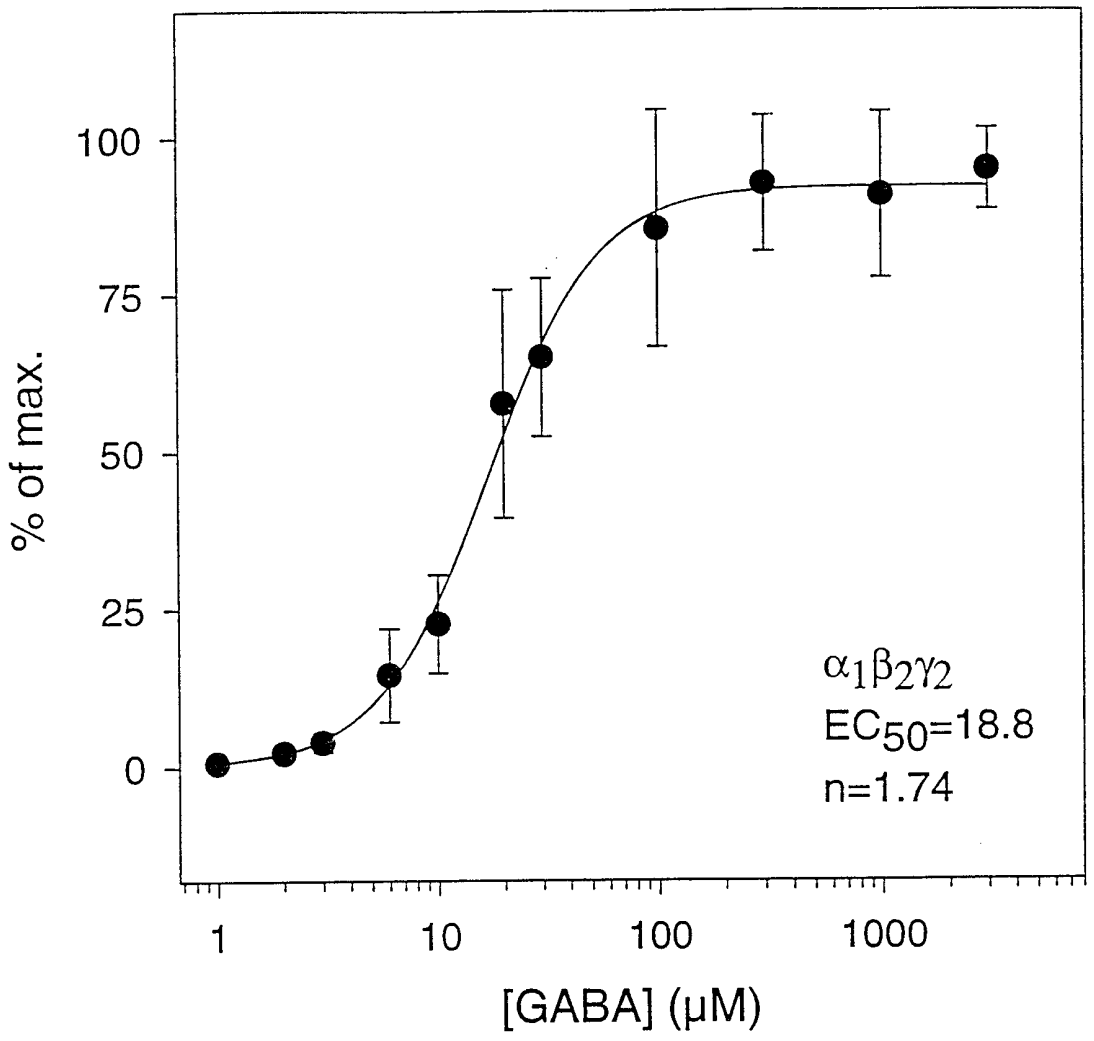
100
75
50
25
0
% of max.
1
10
100
1000
[GABA] (μM)
$\alpha_1\beta_2\gamma_2$
EC_{50}=18.8
n=1.74

Figure 3

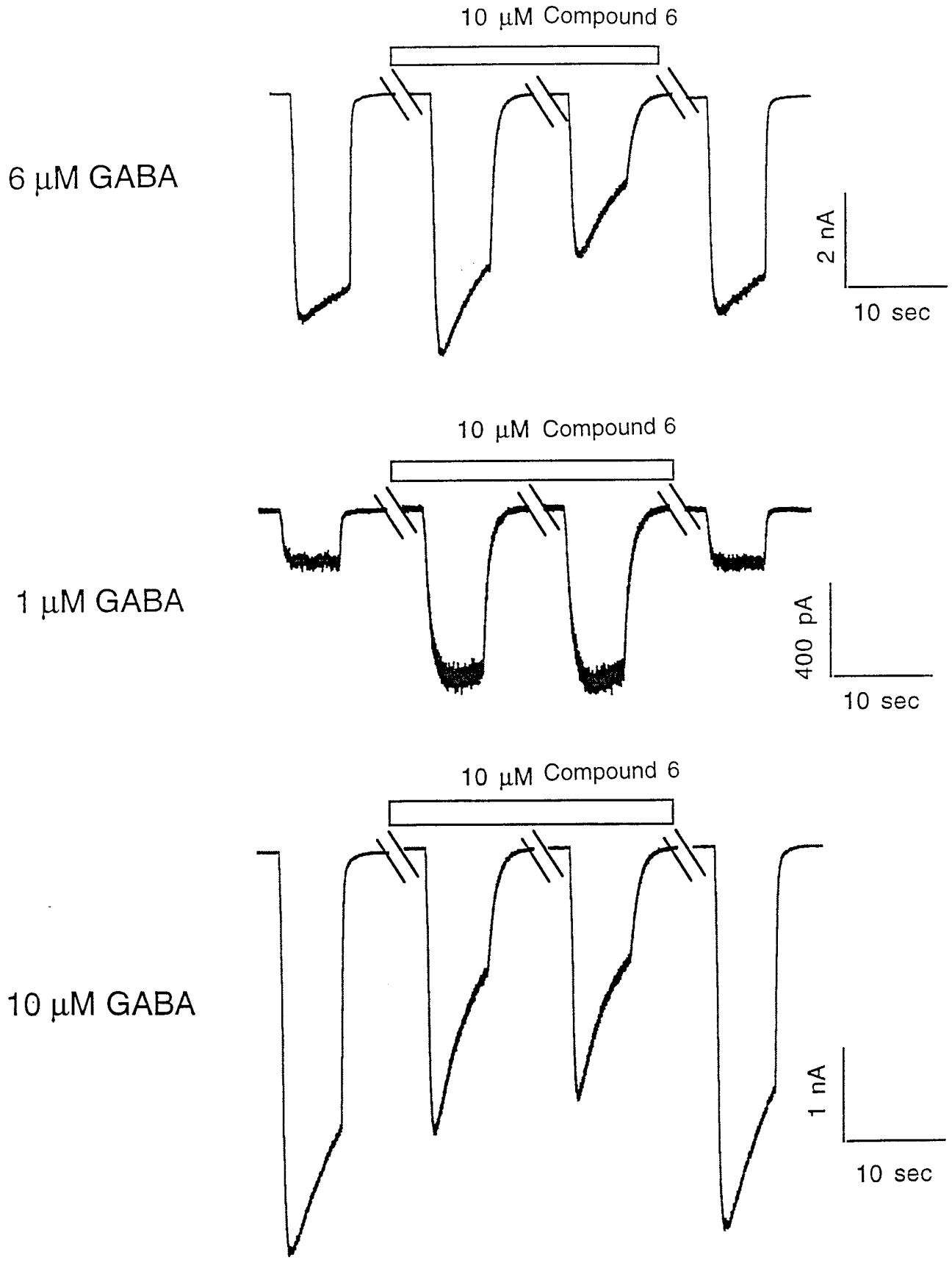
10 µM Compound 6
6 µM GABA
2 nA
10 sec
10 µM Compound 6
1 µM GABA
400 pA
10 sec
10 µM Compound 6
10 µM GABA
1 nA
10 sec

Figure 4

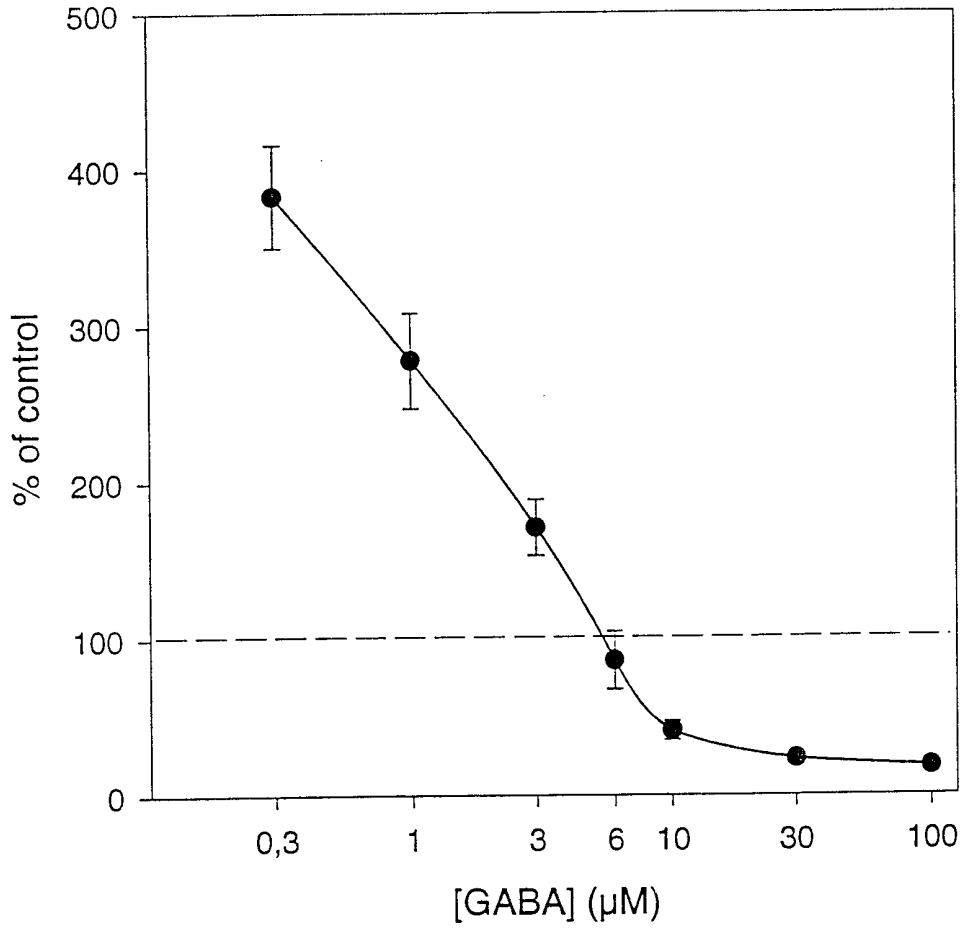

Figure 5

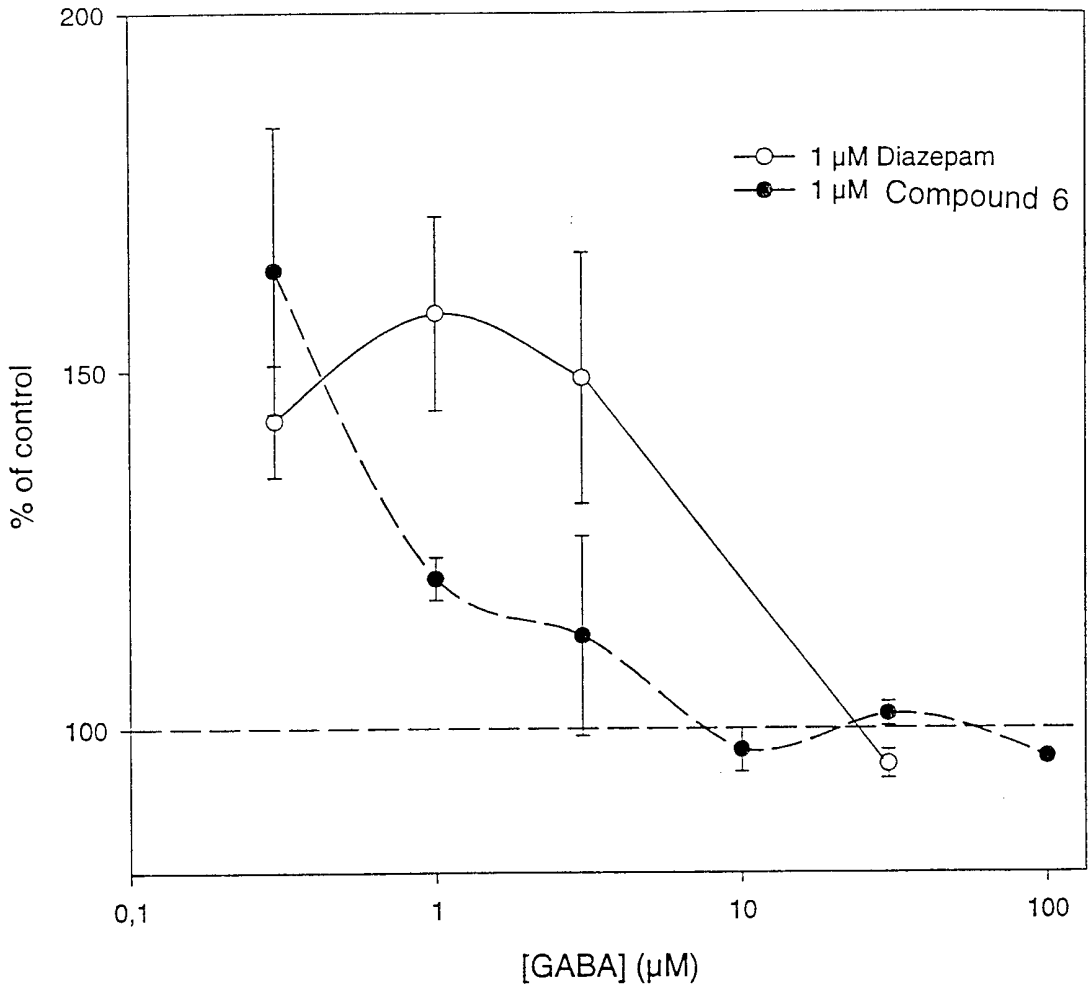
200
150
100
% of control
1 µM Diazepam
1 µM Compound 6
0,1
1
10
100
[GABA] (µM)

Figure 6