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(57) Abstract

Benzothiadiazide derivatives capable of increasing the synaptic response mediated by AMPA receptors are provided. The subject derivatives find use in a variety of applications in which an increase in AMPA receptor mediated synaptic response is desired, including methods of treating memory and learning disorders, schizophrenia, sexual dysfunctions, and the like.

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BENZOTHIADIAZIDE DERIVATIVES AND THEIR USE AS ALLOSTERIC UP-MODULATORS OF THE AMPA RECEPTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of United States application serial no. 60/026,951, filed September 17, 1997, the disclosure of which is herein incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to a novel class of benzothiadiazide derivatives

BACKGROUND OF THE INVENTION

The release of glutamate at synapses at many sites in the mammalian forebrain stimulates two classes of postsynaptic receptors. These classes are usually referred to as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/ quisqualate receptors and N-methyl-D-aspartic acid (NMDA) receptors. AMPA/quisqualate receptors mediate a voltage-independent fast excitatory post-synaptic current (the "fast epsc"), whereas NMDA receptors generate a voltage-dependent, slow excitatory current. Studies carried out in slices of hippocampus or cortex indicate that the AMPA receptor-mediated fast epsc is by far the dominant component at most glutamatergic synapses under most circumstances.

AMPA receptors are not evenly distributed across the brain, but instead are largely restricted to the telencephalon and cerebellum. These receptors are found in high concentrations in the superficial layers of the neocortex, in each of the major synaptic zones of the hippocampus, and in the striatal complex, as reported by Monaghan et al., in *Brain Research* 324:160-164 (1984). Studies in animals and

humans indicate that these structures organize complex perceptual-motor processes and provide the substrates for higher-order behaviors. Thus, AMPA receptors mediate transmission in those brain networks responsible for a host of cognitive activities.

For the reasons set forth above, drugs that enhance the functioning of the AMPA receptor have significant benefits for intellectual performance. Such drugs also facilitate memory encoding. Experimental studies, such as those reported by Arai and Lynch, *Brain Research*, 598:173-184 (1992), indicate that increasing the size of AMPA receptor-mediated synaptic response(s) enhances the induction of long-term potentiation (LTP). LTP is a stable increase in the strength of synaptic contacts that follows repetitive physiological activity of a type known to occur in the brain during learning. Compounds that enhance the functioning of the AMPA form of glutamate receptors facilitate the induction of LTP and the acquisition of learned tasks as measured by a number of paradigms. Granger et al., *Synapse* 15:326-329 (1993); Staubli et al., *PNAS* 91:77/-781 (1994); Arai et al., *Brain Res.* 638:343-346 (1994); Staubli a al., *PNAS* 91: 11158-11162 (1994); and Shors a al., Neurosci. Let. 186:153-156 (1995).

There is a considerable body of evidence showing that LTP is the substrate of memory. For example, compounds that block LTP interfere with memory formation in animals, and certain drugs that disrupt learning in humans antagonize the stabilization of LTP, as reported by del Cerro and Lynch, *Neuroscience* 49: 1-6 (1992). A possible prototype for a compound that selectively facilitates the AMPA receptor has recently been disclosed by Ito et al., *J. Physiol.* 424:533-543 (1990). These authors found that the nootropic drug aniracetam (N-anisoyl-2-pyrrolidinone) increases currents mediated by brain AMPA receptors expressed in Xenopus oocytes without affecting responses by γ-aminobutyric acid (GABA), kainic acid (KA) or NMDA receptors. Infusion of aniracetam into slices of hippocampus was also shown to substantially increase the size of fast synaptic potentials without altering resting membrane properties. It has since been confirmed that aniracetam enhances synaptic responses at several sites in hippocampus, and that it has no effect on NMDA receptor-mediated potentials. See, for example, Staubli et al., *Psychobiology* 18:377-381 (1990) and Xiao et al., *Hippocampus* 1:373-380 (1991). Aniracetam has also been found to have an extremely rapid onset and washout, and can be applied repeatedly with no apparent lasting

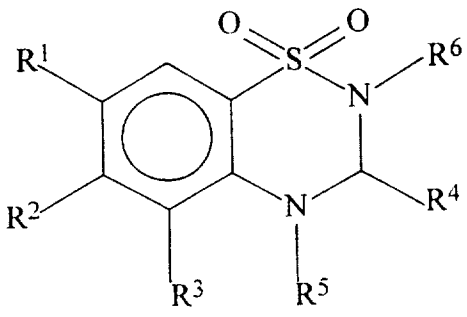
effects: these are valuable traits for behaviorally-relevant drugs. Unfortunately, the peripheral administration of aniracetam is not likely to influence brain receptors. The drug works only at high concentrations (about 1.0 mM), and Guenzi and Zanetti, *J. Chromatogr.* 530:397-406 (1990), report that about 80% of the drug is converged to anisoyl-GABA following peripheral administration in humans. The metabolite, anisoyl-ABA, has been found to have no aniracetam-like effects.

A class of compounds that do not display the low potency and inherent instability characteristic of aniracetam has recently been disclosed. These compounds, termed "ampakines," are disclosed in International Patent Application Publication No. WO 94/02475 (PCT/US93/06916), the disclosure of which is herein incorporated by reference. The ampakines are chemically more stable than aniracetam, and show improved bioavailability as judged by experiments performed by Positron Emission Tomography (PET) -- see, for example, Staubli et al., in PANS 91: 11158-11162 (1994). Additional ampakines in the form of benzoyl piperidines and pyrrolidines have since been discovered and are the subject of pending United States patent application serial no. 08/458,967, the disclosure of which is herein incorporated by reference. Moreover, additional ampakines in the form of benzoxazines have also been discovered and are the subject of pending United States patent application serial no. 08/624,335, the disclosure of which is herein incorporated by reference.

Although the foregoing compounds are quite useful, it would be advantageous to have other compounds that increase synaptic responses mediated by AMPA receptors. Such compounds are the subject of the present invention.

SUMMARY OF THE INVENTION

The present invention relates to a new class of benzothiadiazide derivatives having the general formula:



In the above formula, R^1 and R^2 are independently selected and are functional groups including, but not limited to, the following: hydrogen, alkyl, alkoxy,—SO_2NH_2,—NO_2, cyano and halogen. R^3 is a functional group including, but not limited to, the following: alkyl, alkoxy, halogen, hydroxy, acyl, aryl,—NO_2 and—SO_2NH_2. R^4 is a functional group including, but not limited to, the following: hydrogen, alkyl, acyl and aryl. R^5 is hydrogen or an alkyl, preferably a lower alkyl, more preferably methyl. R^6 is hydrogen or an alkyl, including a substituted alkyl, such as a heteroalkyl, e.g. hydroxy-alkyl, amino alkyl, thio-alkyl and the like, where when the heteroatom is a terminal heteroatom, it may be incorporated into a functional group, such as an ester, amide, ether, thio-ether and the like. In certain embodiments, R^3 and R^5 are connected, e.g. bonded to each other to form a third ring structure, preferably a third five membered ring, e.g. where R^5 is methyl and R^3 is ethyl and R^5 is connected to the first non-terminal carbon of R^3. It should be noted, however, that R^1, R^2, R^3 and R^4 are selected such that if R^3 is methyl, then R^1 is not—SO_2NH_2; if R^3 is halogen, then R^2 is—SO_2NH_2; if R^3 is—CF_3, then R_1 is not—SO_2NH_2.

It has been discovered that the compounds of the subject invention increase synaptic responses mediated by AMPA receptors. More particularly, it has been discovered that when R^3 is other than hydrogen, the resulting compounds have an increased ability to amplify the synaptic strength of excitatory synapses by potently and selectively attenuating AMPA receptor desensitization. In addition, it has been discovered that such compounds have the ability to increase neurotrophic factor expression and neurotrophic factor receptor expression, as described in a commonly assigned patent application serial no. 60/026,571 filed on September 17, 1996, the teachings of which are incorporated herein by reference.

The compounds of the invention primarily act, not by directly stimulating neural activation, but by up-modulating ("allosteric modulation") neural activation and transmission in neurons that contain glutamatergic receptors. Such compounds bind to the glutamate receptor at a site other than the glutamate binding site, but such binding does not by itself give rise to ion fluxes. However, when a glutamate molecule binds to a glutamate receptor that has bound to it a compound of the invention, the subsequent ion flux is of much longer duration. Thus, in the presence of the compounds of the present invention, postsynaptic neurons are activated by much lower concentrations of

glutamate than postsynaptic neurons that do not contain bound compounds.

As positive modulators of excitatory neuronal communication, the compounds of this invention have many applications in mammals and, in particular, in humans. For example, increasing the strength of excitatory synapses compensates for losses of synapses or receptors associated with aging and brain disease (e.g., Alzheimer's disease). Enhancing AMPA receptors causes more rapid processing by multisynaptic circuitries found in higher brain regions and, thus, can produce an increase in perceptual motor and intellectual performance. Moreover, because increasing AMPA receptor mediated responses facilitates synaptic changes of the type believed to encode memory, the compounds of this invention function as memory enhancers. Additional applications contemplated for the compounds of this invention include, but are not limited to, improving the performance of subjects with sensory-motor problems dependent upon brain networks utilizing AMPA receptors, improving the performance of subjects impaired in cognitive tasks dependent upon brain networks utilizing AMPA receptors, improving the performance of subjects with memory deficiencies, treating depression, alcoholism and schizophrenia, and improving the recovery of subjects suffering from trauma.

Accordingly, the compounds of this invention in suitable formulations can be employed for decreasing the amount of time needed to learn a cognitive, motor or perceptual task. Alternatively, these compounds can be employed for increasing the time for which cognitive, motor or perceptual tasks are retained. Still further, these compounds can be employed for decreasing the quantity and/or severity of errors made in recalling a cognitive, motor or perceptual task. Such treatment can prove especially advantageous in individuals who have suffered injury to the nervous system, or who have endured disease of the nervous system, especially injury or disease that affects the number of AMPA receptors in the nervous system. In addition, the compounds of this invention can serve, for example, as a research tool for studying the biophysical and biochemical properties of the AMPA receptor and the consequences of selectively enhancing excitatory transmission on the operation of neuronal circuitry. Moreover, because the compounds of this invention reach central synapses, they allow for testing of the behavioral effects of enhancing AMPA receptor currents.

As such, the compounds of this invention can be incorporated into a variety of

formulations for therapeutic administration. Examples include capsules, tablets, syrups, suppositories, and various injectable forms. Administration of the compounds can be achieved in various ways, including oral, bucal, rectal, parenteral and intraperitoneal administration. Dose levels can vary widely, and optimal dosages for any particular patient or condition are readily determinable by those of skill in the art. Typical dosages can range from milligrams to decigrams. Preferred formulations of the compounds are oral preparations, particularly capsules or tablets containing each from about 1 milligram up to about 1000 milligrams of active ingredient. Subjects contemplated for treatment with the compounds of the invention include humans, domesticated animals, laboratory animals and livestock.

In addition, kits containing the compositions of the present invention in the form of tablets or ampules or other suitable packaging means, formulated for controlled dosage administration, are also provided.

Other features, objects and advantages of the invention and its preferred embodiments will become apparent from the detailed description which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 illustrates the effects of D1 on AMPA receptor responses recorded in excised patches from hippocampal pyramidal cells.

Figure 2 illustrates the effects of D1 on synaptic responses.

Figure 3 illustrates the effects of D1 on [^{3}H]AMPA binding.

DEFINITIONS

"Cyano" refers to the group—CN.

"Halogen" refers to fluorine, bromine, chlorine, and iodine atoms.

"Hydroxy" refers to the group—OH.

"Thiol" or "mercapto" refers to the group—SH.

" Sulfamoyl" refers to the—SO_2NH_2.

"Alkyl" refers to a cyclic, branched or straight chain, alkyl group of one to eight carbon atoms. The term "alkyl" includes reference to both substituted and unsubstituted alkyl groups. This term is further exemplified by such groups as methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, i-butyl (or 2-methylpropyl),

cyclopropylmethyl, cyclohexyl, i-amyl, n-amyl, and hexyl. Substituted alkyl refers to alkyl as just described including one or more functional groups such as aryl, acyl, halogen, hydroxyl, amido, amino, acylamino, acyloxy, alkoxy, cyano, nitro, thioalkyl, mercapto and the like. These groups may be attached to any carbon atom of the lower alkyl moiety. "Lower alkyl" refers to C_1-C_6 alkyl, with C_1-C_4 alkyl more preferred. "Cyclic alkyl" includes both mono-cyclic alkyls, such as cyclohexyl, and bi-cyclic alkyls, such as [3.3.0] bicyclooctane and [2.2. l]bicycloheptane. "Fluoroalkyl" refers to alkyl as just described, wherein some or all of the hydrogens have been replaced with fluorine (e.g., $-CF_3$ or $-CF_2CF_3$).

"Aryl" or "Ar" refers to an aromatic substituent which may be a single ring or multiple rings which are fused together, linked covalently, or linked to a common group such as an ethylene or methylene moiety. The aromatic ring(s) may contain a heteroatom, such as phenyl, naphthyl, biphenyl, diphenylmethyl, 2,2-diphenyl-1-ethyl, thienyl, pyridyl and quinoxalyl. The term "aryl" or "Ar" includes reference to both substituted and unsubstituted aryl groups. If substituted, the aryl group may be substituted with halogen atoms, or other groups such as hydroxy, cyano, nitro, carboxyl, alkoxy, phenoxy, fluoroalkyl and the like. Additionally, the aryl group may be attached to other moieties at any position on the aryl radical which would otherwise be occupied by a hydrogen atom (such as 2-pyridyl, 3-pyridyl and 4-pyridyl).

The term "alkoxy" denotes the group—OR, where R is lower alkyl, substituted lower alkyl, aryl, substituted aryl, aralkyl or substituted aralkyl as defined below.

The term "acyl" denotes groups —C(O)R, where R is alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, amino and alkylthiol.

The term "amino" denotes the group NRR', where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl as defined below or acyl.

The term "amido" denotes the group —C(O)NRR', where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl as defined below or acyl.

The term "independently selected" is used herein to indicate that the two R groups, R^1 and R^2, may be identical or different (e.g., both R^1 and R^2 may be halogen or, R^1 may be halogen and R^2 may be hydrogen, etc.).

As used herein, "mammal" or "mammalian" means or relates to the class mammalia including the orders carnivore (e.g., dogs and cats), rodentia (e.g., mice, guinea pigs and rats) and primates (e.g., humans, chimpanzees and monkeys).

As used herein, "brain tissue" means individual or aggregates of cells from the brain. The cells may be obtained from cell culture of brain cells or directly from the brain.

As used herein, "allosteric upmodulator" means a compound which acts upon and increases the activity of an enzyme or receptor. An allosteric upmodulator of an AMPA receptor increases ligand induced current flow through the receptor, but has no effect until the receptor's ligand is bound.

As used herein, "α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid receptor" or "AMPA receptor" refers to the class of glutamatergic receptors which are present in cells, particularly neurons, usually at their surface membrane that recognize and bind to glutamate or AMPA. The binding of AMPA or glutamate to an AMPA receptor normally gives rise to a series of molecular events or reactions that result in a biological response. The biological response may be the activation or potentiation of a nervous impulse, changes in cellular secretion or metabolism, causing the cells to undergo differentiation or movement, or increasing the levels of nucleic acids coding for neurotrophic factors or neurotrophic factor receptors.

"Memory and learning disorders," as used herein, includes reference to the following: learning disorders in children, such as impairment in communication, imaginative activity and associated features, as well as attention disorders in children: learning and memory disorders resulting from aging, trauma, stroke, epilepsy and neurodegenerative disorders as described in greater detail hereinbelow; learning and memory disorders associated with senile dementia such as Alzheimer's disease; and memory and learning disorders associated with alcohol intoxication and neurotoxic agents such as PCP.

As used herein, treatment of "memory disorders or learning disorders" means a statistically significant increase in memory or learning assessed over time by the Randt Memory Test (Randt et al., *Clin. Neuropsychol.*, 1980, 2:184), Wechsler Memory Scale (*J. Psych.*, 19:87-95 (1945), Forward Digit Span test (Craik, *Age Differences in Human Memory*, in: Handbook of the Psychology of Aging, Birren, J., and Schaie, K.

(Eds.), New York, Van Nostrand (1977), Mini-Mental State Exam (Folstein et al., *J. of Psych. Res.* 12:189-192 (1975), or California Verbal Learning Test (CVLT) wherein such non-neurodegenerative pathological factors as aging, anxiety, fatigue, anger, depression, confusion, or vigor are controlled for. See, U.S. Patent No. 5,063,206. Methods for assessing and controlling for subjective factors not caused by a pathology which causes neurodegeneration is known in the art and determined by such standard clinical tests such as the BECK Depression Scale, Spielberger Trait State Anxiety test, and POMS test (Profile of Mood State). The time interval between administration of one or more tests of memory or learning is of sufficient length to detect the presence of a statistically significant decline in memory or learning (should one exist) beyond that resulting from factors not related to a neurodegenerative pathology (e.g., age).

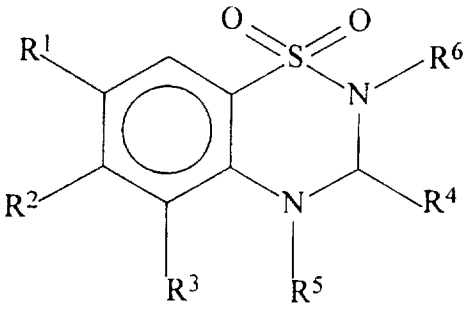
As used herein, "effective amount" or "amount effective to" or "therapeutically effective amount" means a dosage sufficient to produce a desired result. In one embodiment, the desired result is an increase in synaptic responses mediated by AMPA receptors. In another embodiment, the desired result is an increase in memory or learning.

As used herein, "contact" or "contacting" means to place in direct physical association. The terms "contact" and "contacting" are used herein interchangeably with the following: combined with, added to, mixed with, passed over, incubated with, flowed over, etc. Moreover, the compounds of the present invention may be "administered" by any conventional method, such as parenteral, oral, topical and inhalation routes.

As used herein, "blood-brain barrier permeant" or "blood-brain barrier permeable" means that at equilibrium the ratio of a compound's distribution in the cerebrospinal fluid (CSF) relative to its distribution in the plasma (CSF/plasma ratio) is greater than 0.01, generally at least 0.02, preferably at least 0.05, and most preferably at least 0.1.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

The present invention provides a new class of benzothiadiazide derivatives having the general formula:



where:

R^1 and R^2 are independently selected and are functional groups including, but not limited to, the following: hydrogen, alkyl, alkoxy,—SO_2NH_2,—NO_2, cyano and halogen;

R^3 is a functional group including, but not limited to, the following: alkyl, alkoxy, halogen, hydroxy, acyl, aryl,—NO_2 and—SO_2NH_2;

R^4 is a functional group including, but not limited to, the following: hydrogen, alkyl, acyl and aryl;

R^5 is hydrogen or an alkyl, preferably a lower alkyl, more preferably methyl;

R^6 is hydrogen or an alkyl, including a substituted alkyl substituted alkyl, such as a heteroalkyl, e.g. hydroxy-alkyl, amino alkyl, thio-alkyl and the like, where when the heteroatom is a terminal heteroatom, it may be incorporated into a functional group, such as an ester, amide, ether, thio-ether and the like;

where in certain embodiments, R^3 and R^5 are connected, e.g. covalently bonded to each other, to form a five membered ring, e.g. where R^5 is methyl and R^3 is ethyl and R^5 is connected to the non-terminal carbon of R^3.

It should be noted, however, that R^1, R^2, R^3 and R^4 are selected such that if R^3 is methyl, then R^1 is not—SO_2NH_2; if R^3 is halogen, then R^2 is—SO_2NH_2; if $R^3$is—NO_2, then R^2 is—SO_2NH_2; and if R^3 is —CF_3, then R^1 is not—SO_2NH_2.

One class of preferred compounds are those in which R^1 and R^2 are independently selected and are hydrogen, alkyl, alkoxy, —SO_2NH_2 or halogen. Another class of preferred compounds are those in which R^3 is alkyl, alkoxy, acyl or aryl. Another class of preferred compounds are those in which R^4 is alkyl, acyl or aryl. More preferred in some instances are embodiments in which R^1 is halogen; R^2 is hydrogen, —SO_2NH_2 or halogen; R^3 is alkyl, alkoxy, acyl and aryl; and R^4 is alkyl,

acyl or aryl. In other instances, more preferred are embodiments in which R^1 is halogen; R^2 is H; R^3 is alkyl and, in particular, lower alkyl; and R^4 is alkyl. In yet other instances, preferred are embodiments in which R^1 is hydrogen, alkyl or alkoxy; R^2 is hydrogen, alkyl or alkoxy; R^3 is alkyl, alkoxy or halogen; and R^4 is alkyl, acyl or aryl.

Specific compounds of interest include the following in which:

(1) R^1 is Cl, R^2 is H, R^3 is ethyl, and R^4 is methyl;

(2) R^1 is methyl, R^2 is H, R^3 is methyl, and R^4 is methyl;

(3) R^1 is H, R^2 is methyl, R^3 is methyl, and R^4 is methyl;

(4) R^1 is ethyl, R^2 is H, R^3 is methyl, and R^4 is methyl;

(5) R^1 is methoxy, R^2 is H, R^3 is methoxy, and R^4 is methyl;

(6) R^1 is methyl, R^2 is H, R^3 is Cl, and R^4 is methyl;

(7) R^1 is Cl, R^2 is H, R^3 is i-propyl, and R^4 is methyl; and

(8) R^1 is F; R^2 is H; R^3 is methyl; and R^4 is methyl;

where in all of the above specific compounds, R^5 and R^6 are H.

Other specific compounds of interest are those in which:

(9) R^1 is Cl, R^2 is H, R^3 is methyl, R^4 is methyl; R^5 is methyl and R^6 is H;

(10) R^1 is F, R^2 is H, R^3 is methyl, R^4 is methyl; R^5 is methyl and R^6 is H, wherein R^5 is connected to R^3 to form a five membered ring;

(11) R^1 is H, R^2 is H, R^3 is ethyl, R^4 is methyl; R^5 is methyl and R^6 is H;

(12) R^1 is Cl, R^2 is H, R^3 is ethyl, R^4 is methyl; R^5 is methyl and R^6 is H, wherein R^5 is connected to the non-terminal carbon of R^3 to form a five membered ring;

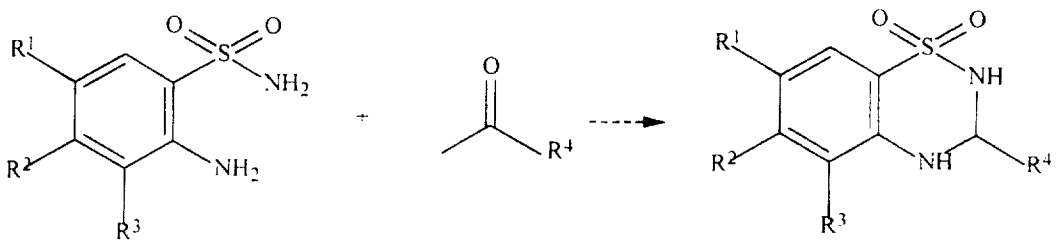
(13) R^1 is F, R^2 is H, R^3 is methyl, R^4 is methyl; R^5 is H and R^6 is $-C_2H_4OH$;

(14) R^1 is F, R^2 is H, R^3 is methyl, R^4 is methyl; R^5 is H and R^6 is $-C_2H_4OR$, where R is a terminal group.

Using the teachings of the present invention, one of skill in the art can prepare the subject compounds, screen such compounds for their ability to amplify, i.e., up-modulate, excitatory synaptic responses mediated by AMPA receptors and, in turn, use such compounds in a variety of methods to up-modulate or amplify synaptic responses mediated by AMPA receptors.

A. Preparation and Screening of the Subject Compounds

The compounds of the present invention can be synthesized in a variety of ways, using conventional synthetic chemistry techniques. Typically, the compounds of the present invention are prepared according to the following general reaction scheme



wherein R^1, R^2 R^3 and R^4 are as defined above. The use of appropriate organic solvents, temperature and time conditions for running the reactions are within the level of skill in the art. Reactions of this type are generally described in Werner, et al., *J. Am. Chem. Soc.*, 82:1161-1166 (1960); U.S. Patent No. 3,288,678 issued to de Stevens, et al.; U.S. Patent No. 3,40,150 issued to de Stevens, et al., the teachings of which are incorporated by reference.

Generally, the reaction is carried out as follows. A salt of the aniline derivative is used as the starting material and may be a salt with an alkali metal or an acid addition salt. Preferably, the aldehyde is reacted with the aniline derivative in about stoichiometric amounts and in the presence of a small amount of an acid, particularly a mineral acid, such as hydrohalic acid, e.g., hydrochloric or hydrobromic acid, or sulfuric acid, if desired, in anhydrous form. The aldehyde may also be given into the reaction medium in a form which yields the desired reactant in situ. Thus, for example, when formaldehyde is used as the reactant, it may be desirable to use it in the form of a polymer, such as paraformaldehyde or trioxane, or as an acetal, such as dimethoxymethane or diethoxymethane. Other aldehydes may be used as acetals, such as 1,1-di-methoxy-ethane or 1 ,1-diethoxy-ethane. The reaction may be carried out in the absence or preferably in the presence of a solvent, for example, an ether, e.g., p-dioxane or di-ethyleneglycol dimethylether, or a formamide, e.g., di-methylformamide. It may be completed at an elevated temperature, for example, at the boiling temperature of the solvent. Thus, the aldehyde reactant is, for example, added to a preheated solution of the aniline derivative in the solvent containing the

acid and heating may then be continued to complete the reaction. If necessary, the reaction may be performed under increased pressure or in the atmosphere of an inert gas, e.g., nitrogen.

Once prepared, the compounds of this invention can be screened for their ability to amplify (upmodulate) the activity of the natural stimulators of AMPA receptors, particularly by amplifying excitatory synaptic responses. A variety of accepted tests can be used to determine whether a given compound is an upmodulator of the AMPA receptor. The primary assay is measurement of the enlargement of the excitatory postsynaptic potential (EPSP) in in vitro brain slices, such as rat hippocampal brain slices.

In experiments of this kind, slices of hippocampus from a mammal, such as rat, are prepared and maintained in an interface chamber using conventional methods. Field EPSPs are recorded in the stratum radiatum of region CA1b and elicited by single stimulation pulses delivered once per 20 seconds to a bipolar electrode positioned in the Schaffer-commissural projections (see, Granger, R. et al., *Synapse*, 15:326-329 1993.; Staubli, U. et al., 1994a, *Proc. Nat. Acad. Sci.*, 91:777-781; and Staubli, V. et al., 1994b, *Proc. Nat. Acad. Sci.*, 91:11 158- 1 1 1 62; Arai, A. et al., 1994, *Brain Res.*, 638:343-346; Arai, A. et al., (submitted), "Effects of a centrally active drug on AMPA receptor kinetics"). The wave form of a normal EPSP is composed of an AMPA component, which has a relatively rapid rise time in the depolarizing direction (~5-10 msec) and which decays within ~20 msec.; an NMDA component (slow~30-40 msec rise time and slow~40-70 msec decay) (the NMDA portion will not appear in normal or artificial CSF (cerebro-spinal fluid) media, due to the voltage requirement for NMDA receptor channel activation, but in low magnesium media, an NMDA component may appear, a GABA (gamma-aminobutyric acid) component in the opposite (hyperpolarizing) direction as the glutamatergic (AMPA and NMDA) components, exhibiting a time course with a rise time of ~1-20 msec and very slow decay (~5-100 msec or more).

The different components can be separately measured to assay the effect of a putative AMPA receptor enhancing agent. This is accomplished by adding agents that block the unwanted components, so that the detectable responses are essentially only AMPA responses. For example, to measure AMPA responses, an NMDA receptor

blocker (e.g., AP-5 or other NMDA blockers known in the art) and/or a GABA blocker (e.g., picrotoxin or other GABA blockers known in the art) are added to the slice. To prevent epileptiform activity in the GABA-blocked slices, known agents such as tetrodotoxin may be used.

AMPA upmodulators useful in the present invention are substances that cause an increased ion flux through the AMPA receptor complex channels in response to glutamatergic stimulation. Increased ion flux is typically measured as one or more of the following non-limiting parameters: at least a 10% increase in decay time, amplitude of the waveform and/or the area under the curve of the waveform and/or a decrease of at least 10% in rise time of the waveform, for example in preparations treated to block NMDA and GABA components. The increase or decrease is preferably at least 25-50%; most preferably it is at least 100%. How the increased ion flux is accomplished (e.g., increased amplitude or increased decay time) is of secondary importance; upmodulation is reflective of increased ion fluxes through the AMPA channels, however achieved.

An additional and more detailed assay is that of excised patches, i.e., membrane patches excised from cultured hippocampal slices; methods are described in Arai et al., 1994. Outside-out patches are obtained from pyramidal hippocampal neurons and transferred to a recording chamber. Glutamate pulses are applied and data are collected with a patch clamp amplifier and digitized (Arai et al., 1994). Because no GABA is applied to the patch, GABAergic currents will not be elicited. Any NMDA currents can be blocked as above (e.g., with AP-5).

The central action of a drug can be verified by measurement of field EPSPs in behaving animals (see, Staubli et al., 1994a) and time course of biodistribution can be ascertained via injection and subsequent quantitation of drug levels in various tissue samples. Quantitation can be accomplished by methods known to those skilled in the art and will vary depending on the chemical nature of the drug.

B. Uses for the Compounds of The Present Invention

The compounds of the present invention have an increased ability to amplify the synaptic strength of excitatory synapses by potently and selectively attenuating AMPA receptor desensitization. As such, the compounds find use in methods of in

which it is desired to increase the synaptic responses mediated by AMPA receptors. Although how the increased ion flux of the increased synaptic response is accomplished is of secondary importance, it may nonetheless be desirable to selectively affect one of the components (e.g. amplitude, decay time) of the waveform depending on the particular situation in which the compound is employed.

For example, in certain situations it may be desirable to employ a compound that has a highly potent affect on the synaptic response. In such cases, compounds in which R^1 is F as opposed to Cl are preferred, where specific compounds of interest include the following: R^1=F, R^2=H, R^3=ethyl & R^4=methyl; R^1=F, R^2=H, R^3 =ethyl which is bonded to the N to form a third 5-membered ring fused to the first two rings & R^4=methyl; and R^1=F, R^2=H, R^3=methyl & R^4=methyl.Where one wishes to selectively increase the half-width of the synaptic response and thereby elongate the ESPS, compounds of interest are those in which R^3=ethyl, where specific compounds of interest include the following: R^1=F, R^2=H, R^3=ethyl & R^4=methyl; and R^1=Cl, R^2=H, R^3=ethyl & R^4=methyl. Where one wishes to selectively increase the amplitude of the synaptic response and raise the peak EPSP, compounds of interest are those in which R^3 = methyl, where specific compounds of interest include the following: R^1=F, R^2=H, R^3=methyl & R^4=methyl; and R^1=methyl, R^2=H, R^3=methyl & R^4=methyl. (Note that in all of the above compounds, R^5 and R^6 are H.)

Because the subject compounds increase the synaptic responses mediated by AMPA receptors, methods of their use as described herein provide therapeutic benefit to mammals afflicted with, or diagnosed as having, a neurodegenerative pathology. In particular, the compounds of the present invention are useful in the treatment of neurodegenerative pathologies including, but not limited to, those arising from a disease state and/or having an excitotoxic/ischemic mechanism.

Pathologies that would benefit from this invention include conditions (diseases and insults) leading to neuronal cell death and/or sub-lethal neuronal pathology including, for example, the following:

diseases of central motor systems including degenerative conditions affecting the basal ganglia (Huntington's disease, Wilson's disease, Striatonigral degeneration, corticobasal ganglionic degeneration), Tourettes syndrome, Parkinson's disease, progressive supranuclear palsy, progressive bulbar palsy, familial spastic paraplegia,

spinomuscular atrophy, ALS and variants thereof, dentatorubral atrophy, olivo-pontocerebellar atrophy, paraneoplastic cerebellar degeneration;

diseases affecting sensory neurons such as Friedreich's ataxia, diabetes, peripheral neuropathy, retinal neuronal degeneration;

diseases of limbic and cortical systems such as cerebral amyloidosis, Pick's atrophy, Retts syndrome;

neurodegenerative pathologies involving multiple neuronal systems and/or brainstem including Alzheimer's disease, AIDS-related dementia, Leigh's disease, diffuse Lewy body disease, epilepsy, Multiple system atrophy, Guillain-Barre syndrome, lysosomal storage disorders such as lipofuscinosis, late-degenerative stages of Down's syndrome, Alper's disease, vertigo as result of CNS degeneration;

pathologies arising with aging and chronic alcohol or drug abuse including, for example, with alcoholism the degeneration of neurons in locus coeruleus, cerebellum, cholinergic basal forebrain; with aging degeneration of cerebellar neurons and conical neurons leading to cognitive and motor impairments; and with chronic amphetamine abuse degeneration of basal ganglia neurons leading to motor impairments;

pathological changes resulting from focal trauma such as stroke, focal ischemia, vascular insufficiency, hypoxic-ischemic encephalopathy, hyperglycemia, hypoglycemia or direct trauma;

pathologies arising as a negative side-effect of therapeutic drugs and treatments (e.g., degeneration of cingulate and entorhinal cortex neurons in response to anticonvulsant doses of antagonists of the NMDA class of glutamate receptor).

Mammals displaying clinical manifestations of a neurodegenerative pathology and in need of the therapeutic benefit derived from an increase in synaptic strength of excitatory synapses mediated by AMPA receptors can be administered an allosteric modulator of the present invention according to the methods provided herein.

In addition, as a result of their increased ability to amplify the synaptic strength of excitatory synapses mediated by AMPA receptors, it has been determined that the compounds of the present invention can be used for the treatment of a subject suffering from schizophrenia in a manner similar to that described in pending United States patent application serial no. 08/458,967, the teachings of which are incorporated by reference for all purposes. Moreover, it has been determined that the compounds of the

present invention can be used for treating a sexual dysfunction in a subject in a manner similar to that described in pending United States patent application serial no. 60/010,778, the teachings of which are incorporated by reference for all purposes.

Therapeutic benefit includes any of a number of subjective or objective factors indicating a response of the condition being treated. This includes measures of increased neuronal survival or more normal function of surviving brain areas. For instance, some subjective symptoms of neurodegenerative disorders include pain, change in sensation including decreased sensation, muscle weakness, coordination problems, imbalance, neurasthenia, malaise, decreased reaction times, tremors, confusion, poor memory, uncontrollable movement, lack of affect, obsessive/compulsive behavior, aphasia, agnosia, visual neglect, etc. Frequently, objective signs, or signs observable by the physician or the health care provider, overlap with subjective signs. Examples include the physician's observation of signs such as decreased reaction time, muscle fasciculations, tremors, rigidity, spasticity, muscle weakness, poor coordination, disorientation, dysphasia, dysarthria, and imbalance. Additionally, objective signs can include laboratory parameters, such as the assessment of neural tissue loss and function by Positron Emission Tomography (PET) or functional Magnetic Resonance Imaging MRI), blood tests, biopsies and electrical studies such as electromyographic data.

In addition, macroscopic methods of evaluating the effects of the invention can be used which may be invasive or noninvasive. Further examples of evidence of a therapeutic benefit include clinical evaluations of cognitive functions including object identification, increased performance speed of defined tasks as compared to pretreatment performance speeds, and nerve conduction velocity studies.

Accordingly, the subject compounds find use in a variety of methods of treating disease conditions in mammals, where methods of particular interest include the administration of an effective amount of a compound according to the subject invention to: (a) enhance synaptic responses mediated by AMPA receptors; (b) treat memory disorders and learning disorders in a subject; (c) treat a subject suffering from schizophrenia; and (d) treat sexual dysfunction in a subject, and the like. Mammals that can be treated according to the subject methods include: rare and exotic animals, domestic animals, including cows, sheep, pigs, dogs, cats and the like, and humans.

C. Administration of the Compounds of The Present Invention

The compounds, i.e., allosteric upmodulators, of this invention can be incorporated into a variety of formulations for therapeutic administration. More particularly, the compounds of the present invention can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols. As such, administration of the compounds can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal,etc., administration. Preferably, the allosteric upmodulators will be sufficiently blood-brain permeable so that their administration into the systemic circulation will result in a therapeutically effective amount in the brain.

The compounds of the present invention can be administered alone, in combination with each other, or they can be used in combination with other known compounds (e.g., other memory or learning enhancing agents). In pharmaceutical dosage forms, the compounds may be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

For oral preparations, the compounds can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

The compounds can be formulated into preparations for injections by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher

aliphatic acids or propylene glycol: and if desired. with conventional additives such as solubilizers. isotonic agents. suspending agents. emulsifying agents. stabilizers and preservatives.

The compounds can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane. propane. nitrogen and the like.

Furthermore. the compounds can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The compounds of the present invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter. carbowaxes and polyethylene glycols. which melt at body temperature. yet are solidified at room temperature.

Unit dosage forms for oral or rectal administration such as syrups. elixirs. and suspensions may be provided wherein each dosage unit. for example. teaspoonful. tablespoonful. tablet or suppository. contains a predetermined amount of the composition containing one or more compounds of the present invention. Similarly. unit dosage forms for injection or intravenous administration may comprise the compound of the present invention in a composition as a solution in sterile water. normal saline or another pharmaceutically acceptable carrier.

The term "unit dosage form." as used herein. refers to physically discrete units suitable as unitary dosages for human and animal subjects. each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent. carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved. and the pharmacodynamics associated with each compound in the host.

The pharmaceutically acceptable excipients. such as vehicles. adjuvants. carriers or diluents. are readily available to the public. Moreover. pharmaceutically acceptable auxiliary substances. such as pH adjusting and buffering agents. tonicity adjusting agents. stabilizers. wetting agents and the like. are readily available to the public.

Preferred formulations of the compounds are oral preparations, particularly capsules or tablets containing each from about 10 milligrams up to about 1000 milligrams of active ingredient. The compounds are formulated in a variety of physiologically compatible matrixes or solvents suitable for ingestion or injection.

5

D. Preferred Dosages of the Compounds of the Present Invention

The compounds, i.e., allosteric upmodulators, of the present invention are administered at a dosage that increases synaptic responses mediated by AMPA receptors while minimizing any side-effects. It is contemplated that the composition 10 will be obtained and used under the guidance of a physician.

Typical dosages for systemic administration range from 0.1 to 50 milligrams per kg weight of subject per administration. A typical dosage may be one 10- 500 mg tablet taken once a day, or one time-release capsule or tablet taken once a day and containing a proportionally higher content of active ingredient. The time-release effect 15 may be obtained by capsule materials that dissolve at different pH values, by capsules that release slowly by osmotic pressure, or by any other known means of controlled release.

Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the 20 subject to side effects. Some of the specific compounds that stimulate glutamatergic receptors are more potent than others. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means. A preferred means is to measure the physiological potency of a given compound that is a candidate for administration by the method of Davis et al. (1996), submitted to *Behavioral* 25 *Neuroscience*. Briefly, excised patches and excitatory synaptic responses are measured in the presence of different concentrations of test compounds, and the differences in dosage response potency are recorded and compared. Davis, et al. found that one specific compound designated BDP-20 was about ten-fold more potent than another designated BDP-12 in a variety of behavioral (exploratory activity, speed of 30 performance) and physical (excised patches and excitatory synaptic responses) tests. The relative physiological potency was an accurate measure of their behavioral potency. Thus, excised patches and excitatory synaptic responses may be used to

gauge the relative physiological (and behavioral) potency of a given compound with regard to a known standard. (See also, Staubli, U. et al., 1994, *Proc. Nat. Acad. Sci.*, USE, 91:777-781 and Arai, A. et al., 1994, Brain Res., 638:343-346). A good correlation between physiological potency (increased AMPA currents) and behavioral effects has been observed. Thus, AMPA current modulation in vitro may be used to gauge the relative potency of a given compound for a biological response.

E. Kits

In addition, the present invention provides for kits with unit doses of AMPA up-modulating drugs either in oral or injectable doses. In addition to the containers containing the unit doses will be a informational package insert describing the use and attendant benefits of the drugs in treating neurodegenerative pathologies not significantly affecting memory or learning. Preferred compounds and unit doses are those described herein above.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

EXAMPLES

I. Preparation of Compound D1 and Activity Characterization

A. Preparation of Compound D1, i.e., 7-chloro-5-ethyl-3-methyl-3, 4--dihydro-2H-1,2,4 benzothiadiazine S,S-dioxide

1. Preparation of 5-chloro-3 ethyl-2-aminobenzenesulfonamide (D)

To a solution of 3-ethyl-2-aminobenzenesulfonamide (0.5043g, 2.52 mmol) in 10 mL acetonitrile was added *N*-chlorosuccinimide (0.3163g, 2.37 mmol). The mixture was stirred at reflux for 12.5 h, cooled, filtered, and concentrated *in vacuo*. Reaction was incomplete by ^{1}H NMR; the purple solids were redissolved in acetonitrile and additional NCS (0.1019g, 0.76 mmol) was added. The mixture was

refluxed for 15 minutes and worked up as before. Column chromatography on the mixture of succinimide and *D* (40% ethyl acetate:hexane) gave pure *D* (0.5224g, 94%). m.p. 134-135°C.

2. Preparation of 7-chloro-5-ethyl-3-methyl-3, 4-dihydro-2H-1,2,4 benzothiadiazine S,S-dioxide (*DI*)

To a 25 mL RB flask was added 5 chloro-3-ethyl-2 aminobenzenesulfonamide *D* (0.4074 g, 1.74 mmol), 8.5 mL acetonitrile, and 3Å molecular sieves. The stirring solution was cooled to 0°C and acetaldehyde (0.15 mL, 2.68 mmol) and (±) 10-camphor sulphonic acid (catalytic) were added. After 1 h at 0°C, the reaction was filtered through Celite and solvent was removed to give crude *DI*. The light brown solid was dissolved in EtOAc, passed through silica, and concentrated to give pure *DI* (0.4426 g, 98%), m.p. 181-183°C.

Those of skill in the art will readily appreciate that the foregoing protocol can be used, with only minor modifications, to prepare the other compounds of the present invention.

B. Effects of D1 on AMPA Receptor Responses Recorded in Patches Excised from Hippocampal Pyramidal Cells

Figure 1 illustrates the effects of D1 on AMPA receptor responses recorded in patches from hippocampal pyramidal cells. Figure 1D, the lower right panel of Figure 1, shows responses to application of 10 mM glutamate for 800 msec (upper set of traces) and 1 msec (lower traces). Each set of traces shows superimposed responses obtained from the same patch in the absence of D1 (uppermost trace in each set) and at increasing concentrations of D1 (30 to 240 μM). The upper set of traces illustrates that the AMPA receptor response quickly desensitizes in the absence of drug, i.e., the current declines within about 10-30 msec to a level that is less than 10% of the peak current. The drug D1 completely abolished this desensitization. Figure 1A, the upper left panel of Figure 1, shows the dose-response relation for this drug effect: the measure that has been plotted is the ratio between the steady-state current and the peak

current at each drug concentration.

The set of traces recorded in response to a one-millisecond glutamate pulse (lower set in the bottom right panel) provides information on the deactivation time constant, another important parameter of receptor kinetics. Deactivation represents the rate at which the response decays upon removal of the agonist. Since glutamate is present in the synaptic cleft for only about one millisecond, these traces provide the best predictor for the effect a drug will have on synaptic responses. The magnitude of the decay time constant of these responses (in msec) has been plotted against different drug concentrations in Figure 1C, the lower left panel of Figure 1.

Another relevant measure of receptor kinetics is the paired-pulse depression that is plotted in Figure 1B, the upper right panel of Figure 1. This depression is observed when two pulses of one-millisecond duration are applied in rapid succession (typically 20 - 100 msec apart). The explanation for this depression is that some of the receptors activated by the first pulse convert to the desensitized state and are therefore not available to respond when the second glutamate pulse arrival. As expected from the fact that D1 suppresses desensitization (Figure 1A, the upper left panel of Figure 1), it also completely abolishes the depression typically seen in the second response. This measure provides an alternative way to assess the effect of a drug on desensitization and to determine the drug potency.

C. Effect of D1 on Synaptic Responses

Figure 2 illustrates the results on synaptic responses obtained with D1. At 200 μM (Figure 2, left side), D1 produced a dramatic widening of the response, as illustrated by the traces at the top and by the 'half-width' plot underneath. The latter shows the time course of the effect, the x-axis is in minutes, drug onset and offset are indicated by horizontal bars. The effect is fully reversed after washing out the drug. D1 also produced some effects on the response amplitude (bottom graph): at higher concentrations, in particular, it tended to reduce the size of the response.

Qualitatively similar effects were obtained in three perfusion experiments in which a drug concentration of 5μM was used (Figure 2, right side). Amplitude effects in this case were variable, but the increase in response width was reliably seen in all three experiments.

The widening of the response most likely is related to the pronounced increase in the deactivation time constant measured in excised patches (i.e., to the slowing of the decay seen in responses to a one-millisecond glutamate application (see, above).

D. Effects of drug groups and of D1 on [^{3}H]AMPA binding

Binding experiments were carried out as an alternative avenue to determine drug potencies. It should be emphasized that drug effects on [^{3}H]AMPA binding are non-competitive in nature. The direction and the magnitude of the effect are thus not related in any simple way to the drug's effect on physiology (unless the effects are integrated in an appropriate kinetic receptor model). However, potent AMPA receptor drugs in general do have a pronounced effect on binding in one way or another. More importantly, it has been found that drug potencies established in binding experiments in general correlate reasonably well with those obtained for physiological measures.

Figure 3 illustrates a dose-response relation curve for the effects of D1 on [^{3}H]AMPA binding. In such experiments, binding of [^{3}H]AMPA to rat brain membranes was measured in the presence of 50 mM KSCN. The concentration of the radioligand was 50 nM, the incubation temperature was 25°C. Incubations were terminated by centrifugation. In Figure 3, data points and error bars represent means and s.d. of duplicate to quadruplicate determinations. Essentially identical data were obtained in two additional experiments of this kind (EC_{50} 20-26μM; Hill coefficient ~2).

II. Synthesis and Characterization of Additional Compounds

A. The following compounds were synthesized in a manner analogous to that described in 1A above:

DP-216: R^1=F, R^2=H, R^3=ethyl, R^4=methyl, R^5=H & R^6=H;

DP-171: R^1=Cl, R^2=H, R_3=i-propyl, R^4=methyl, R^5=H & R^6=H;

DP-240: R^1=Cl, R^2=H, R^3=ethyl, R^4=methyl, R^5=H & R^6=H;

DP-67: R^1=Cl, R^2=H, R^3=propyl, R^4=methyl, R^5=H & R^6=H;

DP-236: R^1=H, R^2=H, R^3=ethyl, R^4=methyl, R^5=H & R^6=H;

DP-60: R^1=Cl, R^2=H, R^3=methyl, R^4=methyl, R^5=H & R^6=H;

DP-75: R^1=F, R^2=H, R^3=ethyl, R^4=methyl, R^5=H & R^6=H;

DP-154: R^1=methyl, R^2=H, R^3=ethyl, R^4=methyl, R^5=H & R^6=H;

TK22: R^1=methyl, R^2=H, R^3=Cl & R^4=methyl, R^5=H & R^6=H;

DP-298-1: R^1=Cl, R^2=H, R^3=methyl, R^4=methyl, R^5= methyl & R^6=H, where R^3 and R^5 are bonded to each other to form a third five membered ring fused to the first two rings;

DP-2104: R^1=F, R^2=H, R^3=methyl R^4=methyl, R^5= methyl & R^6=H, wherein R^3 and R^5 are bonded to each other to form a third five membered ring fused to the first two rings;

DP-298: R^1=Cl, R^2=H, R^3=methyl R^4=methyl, R^5= methyl & R^6=H;

DP-2213: R^1=H, R^2=H, R^3=ethyl R^4=methyl, R^5= methyl & R^6=H;

DP-2215: R^1=Cl, R^2=H, R^3=ethyl R^4=methyl, R^5= methyl & R^6=H, wherein R^5 is bonded to the non-terminal carbon of R^3 to form a third five membered ring fused to the first two rings;

DP-2244: R^1=F, R^2=H, R^3=methyl R^4=methyl, R^5= H & R^6=C_2H_4OH; and

DP-2245: R^1=F, R^2=H, R^3=methyl R^4=methyl, R^5= H & R^6=C_2H_4OR, wherein R is a terminal group.

B. Characterization of Selected Compounds from 2A.

1. It was found that compounds with a fluoride in the 7 position, i.e. R_1, such as DP-216, DP-75 and DP-2104 tend to yield higher potency on synaptic responses in the hippocampal slice than those with a Cl in the same position, such as DP-171 and DP-60.

2. It was found that compounds with an ethyl in the 5 position, i.e. R_3, such as DP-216 and D-1, exert activity preferentially on the half-width of the synaptic responses, elongating EPSPs.

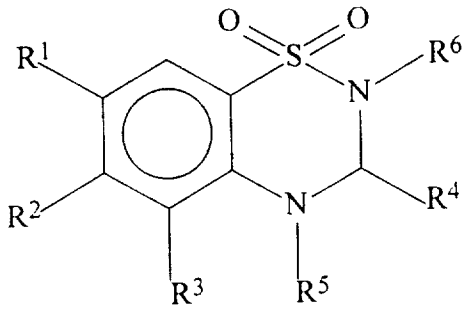
3. It was found that compounds with a methyl in the 5 position, i.e. R_3, such as DP-75 and DP-154, exert activity preferentially on the amplitude of the synaptic response, raising the peak of the EPSP.

4. It was found that compounds DP-298, DP-2104; DP-2213; DP-2215; DP-2244; and DP-2245 exhibit potent effects on AMPA receptors.

It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reading the above description. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent applications and publications, are incorporated herein by reference for all purposes.

WHAT IS CLAIMED IS:

1. A benzothiadiazide derivative having the formula:



where:

R^1 and R^2 are independently hydrogen, alkyl, alkoxy,—SO_2NH_2,—NO_2, cyano or halogen;

R^3 is alkyl, alkoxy, halogen, hydroxy, acyl, aryl,—NO_2 or—SO_2NH_2;

R^4 is hydrogen, alkyl, acyl or aryl;

R^5 is hydrogen or alkyl; and

R^6 is hydrogen, alkyl or substituted alkyl;

wherein certain embodiments, R^3 and R^5 may be joined to form a third ring;

with the provisos:

if R^3 is methyl, then R^1 is not—SO_2NH_2; if R^3 is halogen, then R^2 is—SO_2NH_2; if R^3 is—NO_2, then R^2 is—SO_2NH_2; and if R^3 is -CF_3, then R^1 is not—SO_2NH_2.

2. The benzothiadiazide derivative according to Claim 1, wherein R^1 is —SO_2NH_2 , hydrogen, alkyl, alkoxy or a halogen selected from the group consisting of Br, Cl, F and I.

3. The benzothiadiazide derivative according to Claims 1 or 2, wherein R^2 is a member selected from the group consisting of hydrogen and—SO_2NH_2.

4. The benzothiadiazide derivative according to Claims 1, 2 or 3, wherein R^3 is a member selected from the group consisting of alkyl, alkoxy, acyl and aryl.

5. The benzothiadiazide derivative according to any of the preceding claims, wherein R^4 is a member selected from the group consisting of alkyl, acyl and aryl.

6. The benzothiadiazide derivative according to any of the preceding claims, wherein said derivative is selected from the group consisting of D1, DP-216, DP-171, DP-240, DP-67, DP-236, DP-60, DP-75, DP-154, TK22, DP-298-1, DP-2104, DP-298, DP-2213, DP-2215, DP-2244 and DP-2245.

7. A composition useful for enhancing synaptic responses mediated by AMPA receptors, said composition comprising a compound in accordance with any of the preceding claims and a pharmaceutically acceptable carrier.

8. Use of an effective amount of a benzothiadiazide derivative according to any of the preceding claims to enhance the synaptic response mediated by AMPA receptors in a subject.

9. The use according to Claim 8, wherein said subject suffers from a disorder selected from the group consisting of: memory and learning disorders, schizophrenia, and sexual dysfunction in a subject.

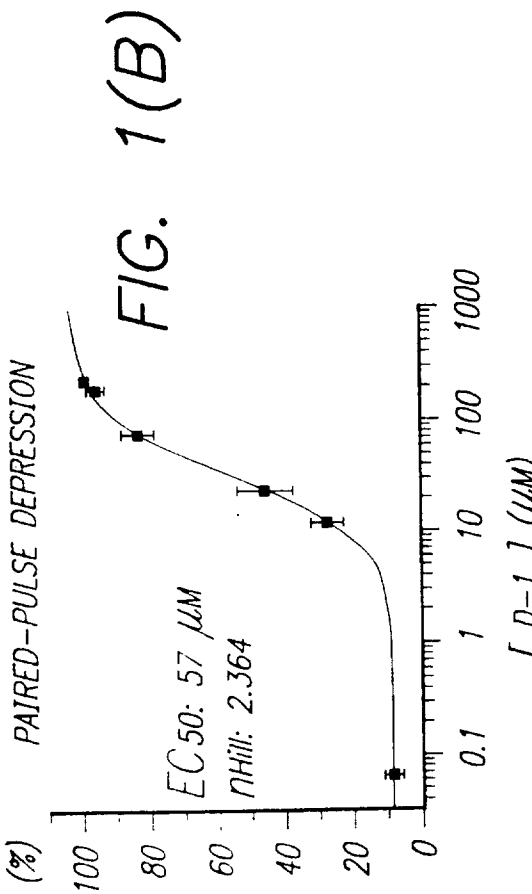


FIG. 1(A)

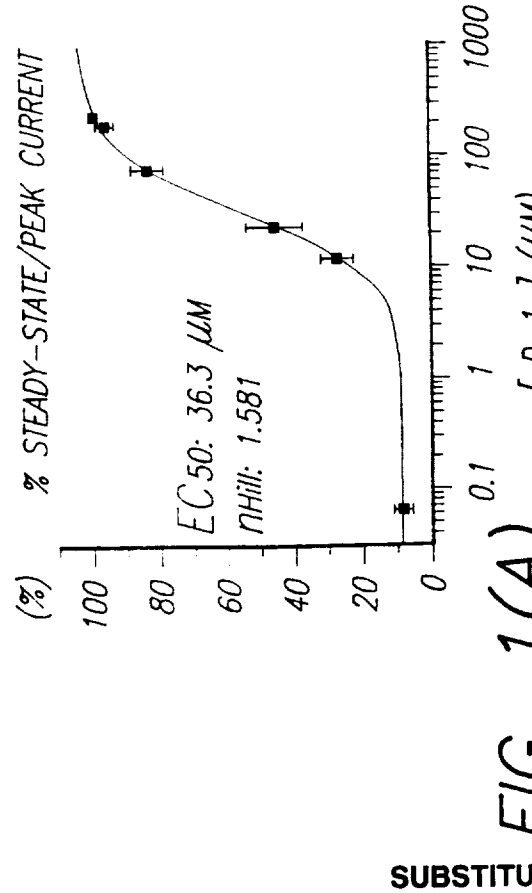


FIG. 1(B)

FIG. 1(C)

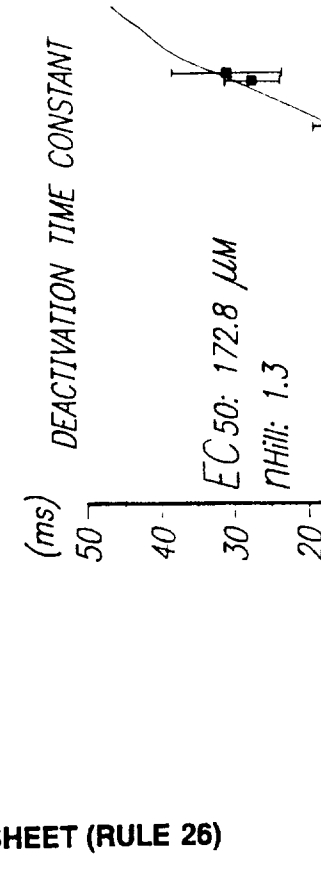
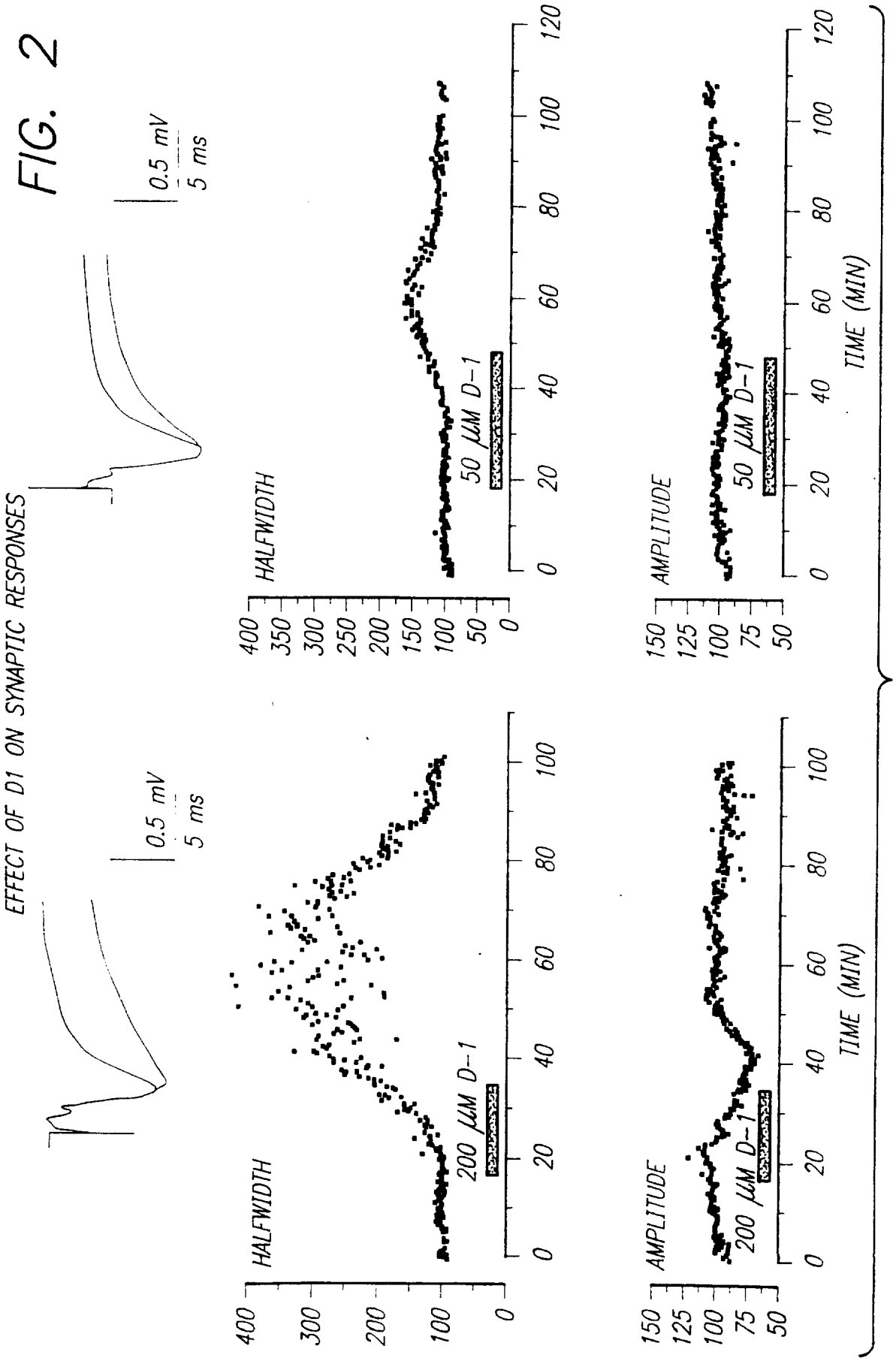


FIG. 1(D)

FIG. 2


EFFECT OF D1 ON SYNAPTIC RESPONSES
0.5 mV
5 ms
0.5 mV
5 ms
HALFWIDTH
200 µM D-1
AMPLITUDE
200 µM D-1
HALFWIDTH
50 µM D-1
AMPLITUDE
50 µM D-1
TIME (MIN)
TIME (MIN)

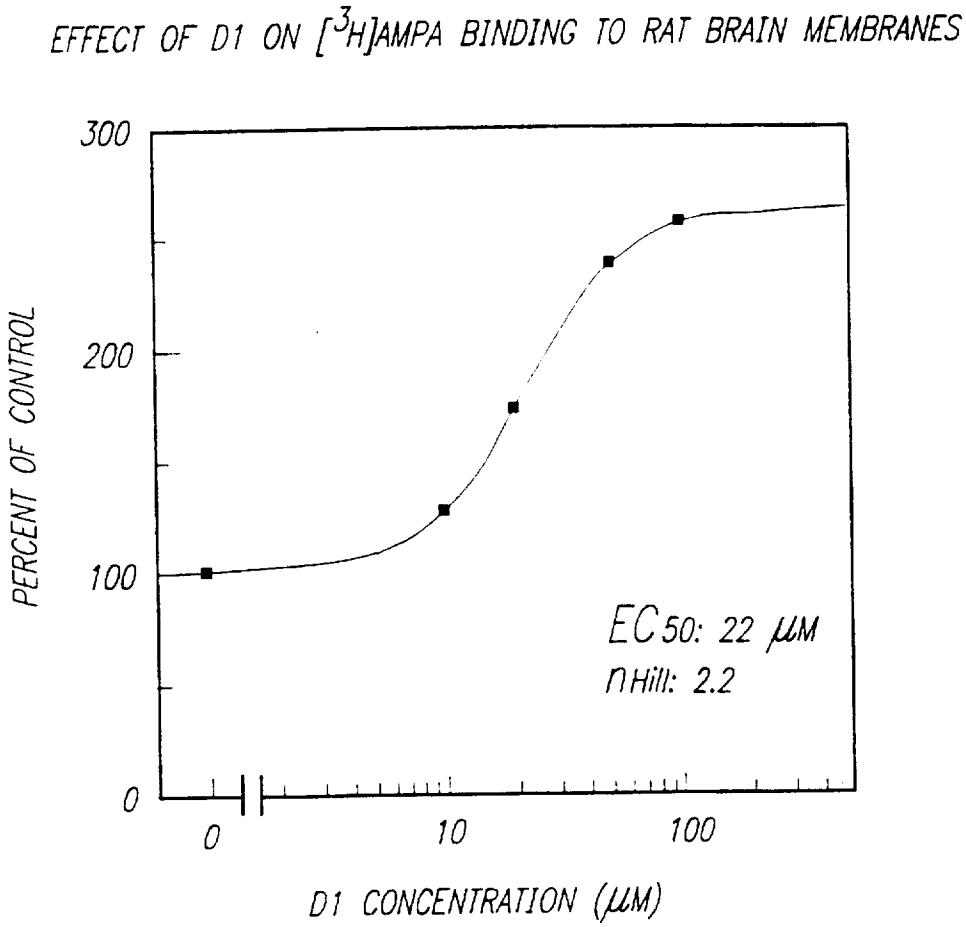

EFFECT OF D1 ON [3H]AMPA BINDING TO RAT BRAIN MEMBRANES
300
200
100
0
PERCENT OF CONTROL
EC50: 22 μM
nHill: 2.2
0
10
100
D1 CONCENTRATION (μM)

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16209

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07D 285/22; A61K 31/54
US CL :514/223.2; 544/12
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)

U.S. : 514/223.2; 544/12

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 practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,252,975 A (STEVENS et al.) 24 May 1966, column 1, lines 11-65.	1-3
Y	WO 95/07899 A1 (RHONE-POULENC RORER S.A.) 23 March 1995, see entire document.	1-3
Y	WO 93/21170 A1 (RHONE-POULENC RORER S.A.) 28 October 1993, see entire document.	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 24 NOVEMBER 1997	Date of mailing of the international search report 12 JAN 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MATTHEW V. GRUMBLING Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16209

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 8-9
because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [X] Claims Nos.: 4-9
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.