(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 November 2006 (09.11.2006)

PCT

(10) International Publication Number
WO 2006/117165 A2

(51) International Patent Classification:
A61K 31/16 (2006.01) *A61K 31/19* (2006.01)
A61K 31/166 (2006.01) *A61K 31/44* (2006.01)
A61K 31/167 (2006.01) *A61P 9/10* (2006.01)

(21) International Application Number:
PCT/EP2006/004022

(22) International Filing Date: 28 April 2006 (28.04.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
05009614 2 May 2005 (02.05.2005) EP

(71) Applicant *(for all designated States except US)*: **FRIEDRICH-ALEXANDER-UNIVERSITÄT ERLANGEN-NÜRNBERG** [DE/DE]; Schlossplatz 4, 91054 Erlangen (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BLÜMCKE, Ingmar** [DE/DE]; Mühlweg 7, 91080 Uttenreuth (DE). **HAHNEN, Eric** [DE/DE]; Geilenkirchner Strasse 5, 52525 Heinsberg (DE). **SIEBZEHNRÜBL, Florian** [DE/DE]; Ritzerstrasse 10, 91054 Erlangen (DE). **BUSLEI, Rolf** [DE/DE]; Löhestr. 12, 91054 Erlangen (DE). **EYÜPOGLU, Ilker, Yasin** [DE/DE]; Hauptstr. 68, 91054 Erlangen (DE).

(74) Agent: **VOSSIUS & PARTNER**; Siebertstrasse 4, 81675 Munich (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/117165 A2

(54) **Title:** MEANS AND METHODS FOR THE TREATMENT OF HEAD INJURIES AND STROKE

(57) **Abstract:** The present invention relates to the use of HDAC inhibitors for the preparation of a pharmaceutical composition for the amelioration, treatment or prevention of malignancies related to ischemic events or brain damage, whereby said HDAC inhibitor is selected from the group consisting of SAHA, M344, MS-275, CBHA and SBHA and whereby said HDAC inhibitor is most preferably SAHA.

Means and methods for the treatment of head injuries and stroke

The present invention relates to the use of HDAC inhibitors for the preparation of a pharmaceutical composition for the amelioration, treatment or prevention of malignancies related to ischemic events or spinal cord or brain damage, whereby said HDAC inhibitor is selected from the group consisting of SAHA, M344, MS-275, CBHA and SBHA and whereby said HDAC inhibitor is most preferably SAHA.

Ischemia, intracerebral bleeding as well as traumatic injury result in neuronal cell death of the affect brain region. The area of irreversible brain damage increases, however, with time from the moment of injurious incidence on and exceed into adjacent areas not directly hit by trauma or ischemia (so-called penumbra). One pathomechanism responsible for the enlargement of compromised brain tissue is propagated by excitotoxic glutamate release of damaged neurons.

Stroke is caused by deficient oxygen supply of the brain, either due to arterial stenosis, thrombo-embolic occlusion or bleeding. Hypertension, vessel malformations, cardiac arrhythmias, tumors, diabetes, nicotine abuse or blood-thinning medication increase the risk of stroke. Any resulting infarction is within the perfusion territory of the affected artery and clinical symptoms depend on the various size and localization. In case that the primary motor cortex or pyramidal fiber tract is affected, a person may be left with a hemiplegia. A causal therapy of stroke aims towards immediate reconstruction of blood and/or oxygen supply, i.e. thrombolysis during the first three to six hours after the insult.
In Germany, approx. 200.000 people suffer from stroke each year. Within the EU-25 1 Mio. people and in USA 700.000 people are affected. Every fifth patient dies from stroke accounting for the third most common cause of death in the Western world. For those patients who survive, stroke is the most common cause for disability (Jansen, 2002).

Non-missile or blunt head injury lead to acceleration or deceleration forces to the head that cause brain movement within the skull. These pathophysiological movements usually result in multiple injuries, i.e. "coup–contre–coup" lesions at opposite sides. Intracranial or epidural bleeding as well as focal damage (infarction) and traumatic axonal injury are seen as a consequence of severe head injuries. The severity of head injury is usually assessed using the Glasgow Coma Scale.
Head injury, whether accidental, criminal or suicidal, is the leading cause of death in people under 45 years of age in developed countries. It accounts for 1 % of all death, 30 % of death from trauma, and 50 % of death due to road traffic accidents. In Germany, an estimated 10.000 individuals each year sustain severe head injury, and 180/100.000 of the population have a persisting handicap. The number of severe head injuries each year in the UK is approx. 50.000; these account for 20 % of death between the ages of 5 and 45. In the USA, an estimated 700.000 individuals each year suffer from severe head injury (Ellison, 2004).

Prolonged ischemia, intracerebral bleeding or traumatic brain injury lead to neuronal cell death and tissue necrosis. Macrophages invade the site of the lesion and phagocyte cell debris. After weeks or months, the initial site of the injury is converted into a pseudocyst. This pseudocyst is, however, often larger than the primary site of the injury. Mechanisms of excitotoxicity appear to play a role during disease progression. Unaffected brain tissue bordering the lesion site is compromised by molecular and structural reorganization processes targeting neuronal as well as glial elements. As a consequence, excessive glutamate concentrations secreted by compromised neurons may not be efficiently cleared from synaptic sites and intraneuronal calcium concentration can reach cytotoxic levels (excitotoxicity), thereby inducing progressive neuronal cell death within bordering brain parenchyma.

To prevent a progressive neuronal cell death due to ischemia, current clinical treatment strategies aim to increase oxygen and blood supply, such as blood thinning medication (Albers, 2004) and thrombolysis therapy (Kwiatkowski, 1999). As an alternative, it was proposed to decrease the metabolism of neurons by hypothermia (Georgiadis, 2005). However, therapeutic strategies employed up to now have not

proven to be completely satisfactory and have not successfully employed epigenetic mechanisms to manipulate ischemia-associated molecular signalling pathways.

A number of inhibitors of histone deacetylases have been discovered and well studied including Trichostatin A (TSA), Valproic acid (VPA), Apicidin and Phenylbutyrate. From this group of compounds, only VPA has reached clinical significance for the treatment of neurological disorders (epilepsy). It has been shown, however, that maximal drug concentrations within the brain tissue are in the order of 500 μmol, which appear not to significantly inhibit HDAC enzymes, see appended examples. Alternative actions of valproic acid including propagation of proteosomal degradation of the HDAC 2 enzyme (Kramer, 2003; Göttlicher, 2004).

A group of novel, highly specific HDAC inhibitors have been described, some of which are already in clinical phase II trials for the treatment of cancer (SAHA, MS-275) (Marks, 2004). Data from recently published phase I clinical trials report furthermore about good oral bioavailability and little side effects (Kelly, 2003).

Ren (2004) has described experiments, wherein valproic acid at a concentration of 300mg/kg was employed in rats. Ren (2004) proposes that VPA (valproic acid) is neuroprotective in a rat cerebral ischemia model and suggests that the protective mechanism may involve HDAC inhibition. However, VPA has only moderate HDAC inhibitory efficacy and requires high milimolar concentrations in vivo. Furthermore, other mechanisms than HDAC inhibition play a role in neuroprotective effects of VPA, i.e. HDAC2 enzyme degradation as mentioned above (Kramer, 2003). The person skilled in the art is aware of the fact that first, a dosage of 20 mg VPA per kg body weight should not be exceeded due to considerable increase of side effects and second, that neuroprotective effects of VPA are most likely not due to HDAC inhibition. Accordingly, the skilled artisan would not consider the use of HDAC inhibitors in the treatment of ischemias of human patients. This is in particular the case, since the HDAC inhibiting action of VPA requires rather extensive concentrations (as documented herein). Furthermore, VPA has a well documented clinical risk to increase intracerebral haemorrhage by inducing factor XIII deficiency (Pohlmann-Eden, 2003; see also: Rote Liste, 2003). Thus, administration of the

HDAC inhibitor VPA is not a reasonable treatment option in humans suffering from stroke or head injury.

As pointed out herein above, the prior art has provided several injury treatment regimes for head injuries and also for ischemic event, i.e. stroke, however, the treatment regimes provided are not satisfactory and have certain down sides Major disadvantages of current treatment modalities necessitate the search for novel strategies. There is only a narrow time window for successful thrombolytic therapy in stroke. Several studies indicate that reconstitution of cerebral blood flow has to be achieved within three to six hours after impairment (Albers, 2004). Blood thinning medication increases the risk for intracerebral bleeding and requires intensive care observation of patients. Although induction of hypothermia appears to reduce mortality in patients with cerebral infarction, severe side effects, intensive medical treatment and a prolonged stay in the neurologic intensive care unit have to be considered. It is reported, therefore, that use of hypothermia should be restricted to specialized units (Georgiadis, 2005).

Accordingly, the technical problem underlying the present invention is to provide for means and methods for the medical intervention in head injuries or brain or spinal cord damages, in particular of traumatic event as well as ischemia/stroke. The above-identified problem is solved by the embodiments as provided herein and as characterized in the appended claims.

Therefore, the present invention relates to the use of HDAC inhibitors for the preparation of a pharmaceutical composition for the amelioration, treatment or prevention of malignancies related to ischemic events or brain or spinal cord damage, whereby said HDAC inhibitor is selected from the group consisting of SAHA, M344, MS-275, CBHA and SBHA .

The terms “SAHA”, “M344”, “MS-275”, “CBHA”, and “SBHA” are well known in the art. SAHA is also known as suberoylanilide hydroxamic acid and is also described in WO 03/032921. Also CBHA is known in the art from WO 03/032921 and relates to m-carboxycinnamic acid bishydroxamate. In WO 03/032921 the use of SAHA or CBHA

is proposed in the treatment of cancer of the brain and neurodegenerative diseases. It was suggested that up-regulation of the cell-cycle control gene p21/WAF1 is helpful to block neoplastic cell growth (Richon, 2000). Other prior art focussed on Huntington's disease. SAHA treatment in a transgenic animal model of the trinucleotide repeat disease indicates amelioration of clinical motor symptoms, although the authors were not able to clarify the underlying molecular action (Hockly, 2003). Yet, now it was surprisingly found that in particular SAHA is useful in the treatment and/or amelioration of stroke or (severe) head or spinal cord injury,like mechanic head injury or trauma. Also preventive use of the herein described HDAC inhibitors, in particular SAHA, is described. As documented in the appended examples, SAHA leads to a significant reduced TTC-derived infarct size in an experimental stroke model and, furthermore, compared to controls, neurological scores were in HDAC-inhibitor-treated (in particular SAHA-treated) animals significantly smaller.

In addition and in context of this invention, it could surprisingly be shown that HDAC-inhibitor treated animals, in particular SAHA-treated animals in a trauma model show significantly better co-ordination of (limb) movement and significantly better neurological scores could be obtained in the HDAC-inhibitor treated group.

M344 is a hybrid of hydroxamic acids and benzamides, and also known as 4-dimethylamino-N-5-hydroxy-carbamoyl-hexyl-benzamide, see, inter alia, Zhang (2004b).

MS-275, N-2-aminophenyl-4-N-pyridine-3-yl-methoxycarbonyl)aminomethyl-benz-amide, is also known in the art as HDAC inhibitor and is, inter alia, used in Glaser (2004).

Also SBHA, suberoyl bishydroxamic acid, is a known HDAC inhibitor and was proposed in the treatment of cancerous disorders, see, inter alia, Zhang (2004a).

As documented in the appended example, this invention is based on the surprising finding that the specific HDAC inhibitors selected from the group consisting of SAHA,

M344, MS-275, CBHA andSBHA are useful in the modulation of gene expression patterns associated with neuroprotection, i.e. excitatory aminoacid transporter 2 (EAAT2) (Shashidharan, 1994). Accordingly, these compounds are particularly useful in the treatment of stroke and brain or neurological damage following trauma, i.e. brain and/or head injuries as well as spinal cord injuries. The invention accordingly provides for a method for modulating EAAT2 gene expression in a subject, preferably a human patient. Yet, it is of note that the medical interventions provided herein, i.e. the use of HDAC inhibitors, in particular SAHA in the treatment, amelioration and/or prevention of neurological injuries, i.e. spinal cord or head injuries or of ischemic events in the brain (like stroke) is not to be limited to the scientific explanation provided herein. These explanations are given without being limited by theory. The inventive methods and uses comprise, inter alia, administering to the subject an efficient amount of SAHA, M344, MS-275, CBHA or SBHA sufficient to increase the expression level of EAAT2 protein in brain tissue. The efficient amount of these specified HDAC inhibitors to be administered to the subject, preferably the human patient, may be administered in doses of 150 - 300 mg/m² body surface in case of SAHA, M344 and MS-275, and in doses of 300 - 900 mg/m^2 body surface in case of CBHA and SBHA (see also: Kelly, 2003). Preferably, said doses are administered daily, however also other administration schemes are envisaged and may be easily deduced by the attending physician. For example, said doses are administered for 3, 4, 5, 6 or 7 days/week, e. g. over a period of time of 1, 2, 3 or 5 weeks.

In context of the present invention, the EAAT2 proteins, the expression level of which is to be modulated, preferably increased, may be from human, mouse or rat. Such EAAT2 proteins are well known in the art. For instance, such EAAT2 proteins, and/or nucleotide sequences encoding the same, are described in Pines (1992), Kirschner (1994) and Hoogland (2004) or are accessible via the accession numbers NM_004171, NP_004162 or AY066021 (all human), P43006 or NM_011393 (both mouse) or NM_017215 (rat). In context of the present invention, it is preferably envisaged that the EAAT2 protein, the expression level of which is to be modulated, preferably increased, is the human protein. Most preferably, said human EAAT2 protein is the one as described in Hoogland (2004), the one as reffered to by accession number NP_004162, the one as characterized by the amino acid

sequence as depicted in SEQ ID NO 2 or the one as encoded by the nucleotide sequence as referred to by the accession number NM_004171 or as depicted in SEQ ID NO 1. However, also corresponding variants, for example allelic variants, are envisaged.

Further EAAT2 proteins to be employed in context of the present invention may be EAAT2 proteins as characterized by the amino acid sequence as depicted in SEQ ID NO 4 and 6 or as encoded by the nucleotide sequence as depicted in SEQ ID NO 3 and 5. Again, it is of note that, nevertheless, the inventive uses and methods provided herein for the medical intervention of spinal cord or head injuries or ischemic events in the brain (like stroke) are not limited by the scientific theory provided herein.

In accordance with this invention, five compounds (SAHA, M344, MS-275, CBHA and SBHA) were found which transcriptionally activate the EAAT2 gene, previously shown to be neuroprotective by increasing glutamate up-take from the synaptic cleft (Shashidharan, 1994). These substances act via inhibition of histone deacetylases (HDAC), an epigenetic chromatin and transcription regulation mechanism. Surprisingly, the use of the HDAC inhibitors as defined herein in the treatment and/or prevention of stroke and the treatment of brain or spinal cord injury does not require immediate reconstruction of blood and oxygen supply. As surprisingly documented in the appended examples, an administration of micromolar concentrations of the defined HDAC inhibitors (1 – 8 μmol) significantly increase EAAT2 gene transcript and protein. The appended examples also document the successful use of HDAC inhibitors (in particular SAHA) in the medical intervention of stroke as well as in neurological trauma events. As mentioned above, the efficient concentrations of the specified HDAC inhibitors to be administered to a subject, preferably a human patient, amounts to a (daily) administration of 150 - 900 mg/m² body surface (see also Kelly 2003). Valproic acid is an already approved drug for the treatment of epilepsies (Rote Liste, 2003). Pharmacological mechanisms of antiepileptic action of VPA remain, however, obscure. Only recently, the HDAC inhibitory property of VPA was described (Kramer, 2003). Figure 5 reveals, however, that HDAC inhibiting concentrations of VPA are in the milimolar range, which cannot be achieved in human patients without risk of malignant side effects, i.e. bleeding or cytotoxic liver

failure. In contrast, the new generation of HDAC inhibitors, namely SAHA, M344, MS-275, CBHA and SBHA is by far more potent (approx. 200-fold) and low micro- and nanomolar concentrations are sufficient to inhibit the HDAC enzyme complex (Figure 5). In addition, clinical trials of cancer treatment have shown, that these concentrations can be achieved in human subjects and do not cause severe side effects (SAHA; Kelly, 2003). In a most preferred embodiment of the present invention, SAHA is to be employed in the medical intervention of stroke and/or head or spinal cord injuries.

The experiments of the present invention show that SAHA, M344, MS-275, CBHA and SBHAare capable to significantly increase EAAT2 protein levels, which makes each compound a candidate drug for the pharmacological treatment of stroke and head injury. In contrast to VPA, all compounds are – regarding the concentration of each compound needed to significantly increase EAAT2 protein levels in the experimental settings – by far more efficient than VPA, which was previously published as candidate drug for the treatment of stroke (Ren, 2004).

In order to characterize HDAC inhibitors as a novel treatment option in neurological disorders, the passage of pharmacological substances through the blood-brain-barrier (BBB) was deduced and therapeutic concentrations without risk for severe cytotoxicity dose could be defined. Only two of the second generation HDAC inhibitors appear to cross the BBB, i.e. SAHA (Hockly, 2003) and MS-275 (see Figure 8). Furthermore, in the experimental part, organotypic hippocampal slice cultures were employed to characterize the neurotoxic propensity of the second generation of HDAC inhibitors, such as CBHA, SBHA, SAHA, MS-275 and M344 (Figure 7). Most astonishingly, only SAHA and MS-275 showed a therapeutic window in the range of 1-80 μM (SAHA) and 1-40 μM (MS-275; Figure 7). All other compounds appear to compromise brain tissue at therapeutic concentrations above 10-20 μM. This differential behaviour of compounds, even obtained from a similar pharmacological class, i.e. hydroxamic acids SAHA and CBHA, virtually exclude a common application in neurological disorders.

The pharmacokinetics of MS-275 has been determined in patients with hematologic malignancies. The elimination half-life of MS-275 was 29.9 hours, while no dose limiting toxicity was detected (Ryan, 2003; Wisinski, 2003). Our experiments indicate that MS-275 is able to cross the blood-brain-barrier (Figure 8) and can be regarded thereby as potential drug for the treatment of neurological disorders.

The most preferred HDAC inhibitor to be employed in context of the present invention is SAHA, a compound well known in the art. In 2001 and 2002, clinical phase I data of SAHA tested in eight cancer patients were published. Patients received up to 900mg/m^2 body surface SAHA daily, 3 days per week, for 21 days. SAHA was well tolerated for 3 weeks with no grade 3 to 4 toxicities and fatigue as the most common adverse event (Kelly, 2003). SAHA also has shown to have excellent oral bioavailability (Reports on phase I trial of SAHA, Press Release 2002, Aton Pharma). In March 2003, clinical data on SAHA have been published. The phase II trials consisted of over 100 patients with several different types of cancer. No toxicity was observed at significant doeses with good penetration of the BBB (Ognjenovic,2003).

This invention is based on the surprising finding that the compounds SAHA, M344, MS-275, CBHA and SBHA, in particular SAHA have been identified as being useful in the modulation of EAAT2 gene expression. Accordingly, these compounds, and in particular SAHA, are useful as neuroprotective drugs in the treatment of ischemic events, like stroke and or in brain damage, particular brain damages caused by trauma, head injuries, head accidents and/or brain surgery or corresponding injuries or traumas of the spinal cord. The compounds MS-275 and SAHA are under development in single-agent clinical phase II trials for cancer treatment. The clinical data published so far indicate that MS-275 and SAHA have minor in vivo toxicities, a good biovailability and a good penetration of the BBB.

Accordingly, the invention also provides for a method for the amelioration, prevention and/or treatment of head injuries or brain or spinal cord damages, in particular of traumatic event as well as ischemia/stroke said method comprising administering to a patient in need of such an amelioration, prevention or treatment a pharmaceutically active amount of a HDAC inhibitor selected from a group consisting of SAHA, M344,

MS-275, CBHA and SBHA. The pharmaceutically active amount of a HDAC inhibitor to be employed within the present invention is well known in the art, can be derived from the art (e. g. Kelly, 2003) or can be determined by methods well known in the art. In particularly, said pharmaceutically active amount of a HDAC inhibitor may be in the range of 0,1 – 10^4 mg/m² body surface (e. g. via subcutaneous or oral application), more preferably in the range of 1 – 10^4 mg/m² body surface and more preferably in the range of 10 – 10^4 mg/m² body surface. Even more preferably said pharmaceutically active amount of a HDAC inhibitor is below the dose limiting cytotoxicity, which, for instance, is at 900 mg/m² body surface.

In particular, for the therapeutic administration of SAHA, M344 and MS-275, said pharmaceutically active amount may be in the range of 50 – 900 mg/m² body surface and more preferably in the range of 150 – 300 mg/m² body surface. Furthermore, a pharmaceutically active amount in the range of 200 – 250 mg/m² body surface or 220 – 230 mg/m² body surface may also be employed in particular with respect to SAHA, M344 and MS-275.

In particular for therapeutic administration of CBHA and SBHA, said pharmaceutically active amount may be in the range of 100 – 2700 mg/m² body surface and more preferably in the range of 300 – 900 mg/m² body surface. Furthermore, a pharmaceutically active amount in the range of 450 – 750 mg/m² body surface or 575 – 625 mg/m² body surface may also be employed, in particular with respect to CBHA and SBHA.

Again, in the medical uses provided herein, SAHA is most preferred. SAHA is also shown in appended Figure 2. An also preferred HDAC inhibitor in context of the present invention is MS-275 which is documented in the appended examples to cross the blood brain barrier (BBB); see also appended Figure 8.

The HDAC inhibitors to be used in the medical context of the present invention are preferably to be administered to a human patient, however, it is also envisaged that animals are treated by the administration of the specific HDAC inhibitors provided herein. When a human patient is to be treated, preferably the HDAC inhibitor is to be administered in a dose as mentioned herein above or, e. g., as can be derived by a skilled person from the *in vitro* dosages as exemplified in the appended examples as well as from the in vivo dosage provided in appended examples 6 and 7. The HDAC

inhibitors provided herein, and in particular SAHA, are to be employed in the treatment and/or amelioration of ischemic events, like stroke as well as in the treatment and/or amelioration of brain damage, i.e. brain or spinal cord damage caused by head injuries, head accidents, spinal cord injuries, spinal cord accidents or also caused during, e.g. brain or spinal cord surgeries. It is also envisaged that the HDAC inhibitors selected from the group consisting of SAHA, M344, MS-275, CBHA and SBHA be employed in the prevention of trauma, e.g. before surgical measures on the brain or on the spinal cord are taken.

The person skilled in the art is aware how a pharmaceutical composition may be produced. In particular, the HDAC inhibitors described herein are all well known in the art and, are also already in clinical use and/or assessment, like SAHA or MS-275 for cancer treatment.

It will be appreciated by the person of ordinary skill in the art that the compounds of the invention and the additional therapeutic agent may be formulated in one single dosage form, or may be present in separate dosage forms and may be either administered concomitantly (i.e. at the same time) or sequentially.

The pharmaceutical compositions prepared and to be administered in accordance with the present invention may be in any form suitable for the intended method of administration.

The compounds of the present invention may be administered orally, parenterally, such as subcutaneously, intravenously, intramuscularly, intraperitoneally, intrathecally, transdermally, transmucosally, subdurally, locally or topically via iontopheresis, sublingually, by inhalation spray, aerosol or rectally and the like in dosage unit formulations optionally comprising conventional pharmaceutically acceptable excipients.

Excipients that may be used in the formulation of the pharmaceutical compositions comprising the HDAC inhibitors comprise carriers, vehicles, diluents, solvents such as monohydric alcohols such as ethanol, isopropanol and polyhydric alcohols such

as glycols and edible oils such as soybean oil, coconut oil, olive oil, safflower oil cottonseed oil, oily esters such as ethyl oleate, isopropyl myristate; binders, adjuvants, solubilizers, thickening agents, stabilizers, disintergrants, glidants, lubricating agents, buffering agents, emulsifiers, wetting agents, suspending agents, sweetening agents, colourants, flavours, coating agents, preservatives, antioxidants, processing agents, drug delivery modifiers and enhancers such as calcium phosphate, magnesium state, talc, monosaccharides, disaccharides, starch, gelatine, cellulose, methylcellulose, sodium carboxymethyl cellulose, dextrose, hydroxypropyl-ß-cyclodextrin, polyvinylpyrrolidone, low melting waxes, ion exchange resins.
Other suitable pharmaceutically acceptable excipients are described in Remington`s Pharmaceutical Sciences, 15th Ed., Mack Publishing Co., New Jersey (1991).

Dosage forms for oral administration include tablets, capsules, lozenges, pills, wafers, granules, oral liquids such as syrups, suspensions, solutions, emulsions, powder for reconstitution.
Dosage forms for parentral administration include aqueous or olageous solutions or emulsions for infusion, aqueous or olageous solutions, suspensions or emulsions for injection pre-filled syringes, and/or powders for reconstitution.
Dosage forms for local/topical administration comprise insufflations, aerosols, metered aerosols, transdermal therapeutic systems, medicated patches, rectal suppositories, and/or ovula.

The amount of the compound of the present invention that may be combined with the excipients to formulate a single dosage form will vary upon the host treated and the particular mode of administration.

The pharmaceutical compositions of the invention can be produced in a manner known per se to the skilled person as described, for example, in Remington's Pharmaceutical Sciences, 15th Ed., Mack Publishing Co., New Jersey (1991).

The Figures show:

Figure 1: Acetylation and deacetylation of histones alter the chromatin structure.

Lysin residues (small, dark spheres) of core histones (large, brigth spheres) can be acetylated (Ac –sickles) by the histone acetyltransferase complex (HAT). Due to increased positive charges of acetylated lysine residues, neighbouring histones repel and the chromatin structure opens (relax). Histone deacetylase (HDAC) has an opposite effect and the chromatin structure condenses after cleavage of acetyl groups from lysine residues. The propensity to activate gene transcription is reduced in the compacted state of chromatin (transcription repression). HDAC inhibitors can block the cleavage of acetyl groups from lysine residues and the chromatin structure remain in a relaxed condition permissive for gene transcription.

Figure 2: Formula of hydroxamic acid SAHA (suberoylanilide hydroxamic acid).

Figure 3: Protein structure and distribution pattern of EAAT2

Figure 4: Close relation between glia cell processes and neuronal synapsis.

Fine astrocytic processes (arrow heads) enwrapping a synapse. Electron microscopical image following Lucifer Yellow injection into an astrocyte of rat hippocampus. Scale bar = 2μm. Dend – postsynaptic dendrite. Modified from (Blümcke, 1995).

Figure 5: HDAC inhibitors have a differential inhibitory efficacy for HDAC activity.

HDAC inhibition effect was determined for different compounds. Whereas the class of fatty acids, i.e. VPA, butyrate and phenybutyrate have only little impact on HDAC activity although using high concentrations above 1 mM, hydroxamic acids and benzamide block HDAC activities already at nano- and micromolar concentrations (SAHA, CBHA, SBHA und M344). Alexis HDAC Fluorometric Assay Kit (ALX-850-290).

Figure 6: HDAC inhibitors increase EAAT2-protein levels in brain tissue of rat hippocampus.

A significant increase of EAAT2 protein levels could be observed following a single application of VPA (2mM), SAHA (8 μM) and M344 (8μM). A monoclonal antibody directed against EAAT2 was used (Novocastra, Klon 1H8; EAAT2 – approx. 70 kDa; EAAT2' – a second band appear with a size of 180 kDa, most likely as complex bound or dimeric molecules). All slices were immediately snap-frozen in liquid nitrogen and proteins were extracted following standard protocols. Equal protein loading is achieved with respect to similar band intensities of ß-actin. Chemiluminescence detection method (Amersham).

Figure 7: Cytotoxicity and dose escalation studies of HDAC inhibitors.

Propidium iodide (PI, red color, left panel) enters only nuclei of damaged cells and was used to unravel irreversible cell damage (cytotoxicity). Organotypic slice cultures of rat hippocampus (Stoppini, 1991, Eyupoglu, 2005) were incubated with Apicidin (10μM), MS-275 (4μM), VPA (2mM), TSA (500 nM), CBHA (160 μM), SBHA (160 μM), SAHA (8μM) and M344 (4μM). Of particular note is the significant increase of PI accumulation in compromised brain tissue following CBHA and SBHA treatment. Dose escalation studies of specified HDAC inhibitors were systematically applied in organotypic slice cultures (right panel). These data confirm the remarkable heterogeneity between used compounds, with SAHA being least harmful and, accordingly, the most preferred compound to be employed in the medical uses provided herein.

Figure 8: MS-275 penetrates the blood-brain-barrier.

The inhibition of HDAC by MS-275 was confirmed by immunoblotting of acid-extracted proteins against acetylated versus none-acetylated histone H3 obtained from mouse brain 4h after intraperitoneal (i.p.) injection of 2 mg or 4 mg MS-275 (dissolved in 150 μl DMSO).

The examples illustrate the invention.

Example 1: HDAC inhibitors increase gene expression levels

HDAC inhibitors challenge the molecular machinery which regulates the functional state of the chromatin structure (Figure 1 and corresponding figure legend).

In context of the present invention, the following HDAC inhibitors have been tested:

Class	Compound	Concentration	Source
Cyclic tetrapeptides	Apicidin	1-10 μM	Sigma
Benzamides	MS275	1-8 μM	Calbiochem
Fatty acids	Valproat	1-100 mM	Sigma
	Butyrat	1-100 mM	Sigma
	Phenylbutyrat	1-100 mM	Sigma
Hydroxamic acids	SAHA	1-8 μM	Alexis
	Trichostatin A	10-500nM	Sigma
	M344	1-8 μM	Calbiochem
	CBHA	10-160 μM	Calbiochem
	SBHA	10-160 μM	Alexis

The concentration required to inhibit 90% of HDAC enzyme activity (IC_{90}) have been calculated from a commercially available fluorometric assay (see also Figure 5). In additional tests, these concentration was corroborated for inhibition of tumour cell growth and induction of tumour cell differentiation, both well established physiological actions of these HDAC inhibitors.

Example 2: EAAT2 as target gene of neuroprotection

The amino acid glutamate is the major neurotransmitter in the brain. To reduce the time of glutamate action at the synaptic cleft, several clearance pathways have been established. One of them belong to fine astrocytic processes embedding the synapsis (Figure 4). These fine processes harbor transporter proteins, such as the

glial excitatory amino acid transporter EAAT2 (Figure 3) (Huang and Bergles, 2004). EAAT2 belong to the family of membrane-bound proteins, which bind glutamate and actively transport glutamate in exchange to sodium ions through the cellular membrane. Altered expression and/or distribution patterns of EAAT2 proteins may crucially account for several neurological disorders but also constitute a pharmacological target structure for neuroprotection (Su, 2003).

In accordance with this invention, it was analyzed, whether HDAC inhibitors increase protein levels of EAAT2 by administering different HDAC inhibitors (SAHA, MS-275, CBHA, SBHA, M344 and valproic acid (VPA)) onto organotypic slice cultures of the rat hippocampus (Scheffler, 2003) (Figure 6). All tested compounds increased the EAAT2 protein level after single application (48 hours, diluted in DMSO), although different concentrations of the drug were necessary to achieve these effects (Figure 5, see also Example 1). It is of particular note that, for instance, in case of VPA, the required concentration to reduce HDAC activity is much higher as in the case of SAHA, MS-275, CBHA, SBHA or M344 (see Figure 5).

Example 3: HDAC inhibitors display differential neurotoxicity

In a second set of experiments, the neurotoxic potency of used compounds (Figure 7) was investigated. The results clearly show that increased levels of HDAC inhibitors damage brain tissue (Figure 7). There was considerable heterogeneity between used HDAC inhibitors with CBHA, SBHA and M344 being harmful already at low micromolar concentrations. The exception was suberoylanilide hydroxamic acid (SAHA), for which a doses escalation experiment above the 20-fold IC_{90} (IC90 = 4 μM) do not damage the brain slice (Figures 5, 7). Accordingly, SAHA is the most preferred compound in context of the present invention. The experiments were performed as mentioned herein below (see Example 5).

Example 4: Administration schemes for HDAC inhibitors

Several application forms of HDAC inhibitors can be achieved. Earlier studies report about the oral availability, subcutaneous injection as well as parenteral application

forms for SAHA, which also cross the BBB. In context of this invention MS-275 was injected into mouse peritoneum and achieved acetylation of Histone isolated from brain tissue after 4 and 6 hours (Fig. 8). This experiment proves the availability of MS-275 in brain tissue after parenteral application.

Example 5: Therapeutical evaluation of HDAC inhibitors

In accordance with the present invention, the compounds SAHA (suberoylanilide hydroxamic acid), M344 (4-dimethylamino-N-6-hydroxy-carbamoyl-hexyl-benzamide), MS-275 ((N-2-aminophenyl-4-N-pyridine-3-yl-methoxycarbonyl)aminomethyl-benzamide), CBHA (m-carboxycinnamic acid bis-hydroxamide) and SBHA (suberoyl bishydroxamic acid) were evaluated in terms of their therapeutic effect, in particular in light of their effect on EAAT2 expression levels. This assessment was carried out using hippocampal slice cultures obtained from 6 day old male Wistar rats. Slice cultures were exposed to SAHA (8μM), M344 (4μM), MS-275 (4 μM), CBHA (160 μM), SBHA (160 μM), or VPA (2000 μM) for 48h. Compared to time- and solvent matched controls, all compounds significantly increased EAAT2 protein levels (relative to the reference protein ß-actin) as shown by Western blot analysis (Figure 6).

The effects were compared with that of VPA, which has recently been described as a potential therapeutic option in stroke (Ren, 2004). There is a considerable difference concerning the concentration used to increase EAAT2 expression levels between VPA and SAHA, CBHA, SBHA, M344 and MS-275 (Figure 5). The HDAC inhibitory action of VPA is almost 200-fold less effective than SAHA, CBHA or SBHA and up to 2000 μM of VPA is required to increase EAAT2 protein levels. Yet, VPA concentrations in brain tissue will not exceed 600 μM and lead to considerable side effects. In accordance with the data provided herein, with regard to its HDAC inhibitory function, in particular in context of increasing EAAT2 protein levels, VPA is not useful as neuroprotective drug.

Neuroprotective drugs have to access neuronal tissue thru the BBB. Recently, passage of SAHA through the BBB has been established (Hockly, 2003).

Experiments of the inventors and provided herein prove that the benzamid MS-275 penetrates also through the BBB. Adult C57/B6 mice (Charles River, Sulzfeld, Germany) have been either injected with 2 or 4 mg of MS-275 (dissolved in 150µl of DMSO) or only with the DMSO vehicle. Two and four hours later, the brains were removed and proteins were extracted using acid protection of acetylated histones. Specific antibodies were applied to identify the amount of acetylated versus non-acetylated histone proteins in brain tissue (Figure 8). The significant increase of acetylated histones clearly indicate, that the compound MS-275 has passed thru the BBB and inhibited HDAC activity in neural tissue.

The organotypic slice culture model was further applied to test the neurotoxic potential of SAHA, M344, MS-275, CBHA and SBHA in nervous tissue at given therapeutic concentrations (Figure 7). The organotypic slice cultures were prepared following Stoppini (1991) and Eyupoglu (2005). Said preparation is described in the following:

Seven-day-old Wistar rats were used for explantation. After decapitation, brains were rapidly removed aseptically and placed into ice-cold preparation medium containing Hank's balanced salt solution (Gibco, Germany) with 10% normal horse serum (Biochrom, Berlin, Germany). After dissection of the frontal pole of the hemispheres and the cerebellum, the brains were cut in 350 µm thick horizontal slices using a vibratome (Technical Products International, St. Louis, MO, USA) in preparation medium as described above. The slices were transferred into culture plate insert membrane dishes (Becton Dickinson, Franklin Lakes, NJ, USA; pore size 0.4 µm) and subsequently transferred into six-well culture dishes (Becton Dickinson) containing 1.2 ml culture medium (MEM–HBSS, 2:1, 25% normal horse serum, 2% L-glutamine, 2.64 mg/ml glucose, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 10 µg/ml insulin-transferrin-sodium selenite supplement, and 0.8 µg/ml vitamin C), according to the interface technique described by Stoppini (1991). The slices were cultivated in a humidified atmosphere at 35°C and 5% CO2. The medium was changed 1 day after preparation and every second day thereafter. Experiments started six days after preparation. To analyze tumor induced neurotoxicity slices were incubated with 1 µg/ml propidium iodide for 20 min followed by complete medium exchange.

In dose escalation experiments, neurotoxic effects using a 20-fold increase of IC_{90} of SAHA (80 μM; Figure 7) were not observed. MS-275 had no neurotoxic effect up to 40 μM (respective 10-fold dose escalation) and SAHA had no neurotoxic effect up to 80 μM, whereas CBHA, SBHA and M344 revealed neurotoxic side effects already at low micromolar concentrations (Figure 7).

Example 6: HDAC-inhibitor-treatment in an experimental stroke model

Materials and Methods

The study was approved by the Institutional Review Board. Male Wistar rats weighting 250-350g were used in this study. Food and water were provided freely before and after surgery.

Animal preparation

Anaesthesia was induced by inhalative isoflurane and maintained by intramuscular injection of ketamine 10% and xylazine 2% (7:3) at a dose of 0.1ml/100g bodyweight (b.w.). Animals breathed spontaneously. The right femoral artery was catheterised to monitor the mean arterial blood pressure (MABP), heart rate, PaO_2 and $PaCO_2$ during animal preparation. Body temperature was controlled rectally and maintained normothermic at 37.5±0.5°C by applying external heat as needed using a heating pad.

Focal cerebral ischemia was introduced using an intraluminal suture occlusion model of the middle cerebral artery (MCAO) as described by Longa (1989). Briefly, the external carotid artery was ligated, the internal carotid artery (ICA) was isolated and the pterygopalatine artery was ligated. A 4-0 monofilament nylon suture, whose tip was coated with silicone, was introduced transvascularily via an arteriotomy into the common carotid artery and gently advanced through the ICA into the origin of the anterior cerebral artery, thus occluding its origin.

Study design

Group 1 (SAHA-Group): 7 rats were allocated to this treatment group based on the infarct size measured with diffusion-weighted MRI. SAHA-administration was performed immediately after the MR-scan at 1 hour after MCAO. Hereby, 200 mg per

kg body weight were administered intracutaneously. The dose was chosen according to Hockly (2003).

Group 2 (Controls): 7 rats were also allocated on this group based on the MR-derived infarct size. Animals of the control group received equivolumetric saline at 1 hour after MCAO.

Determination of infarct size

Twenty-four hours after MCAO animals were sacrificed, brains rapidly removed and 2mm coronal brain slices were incubated for 30 minutes in a 4 % solution of 2.3.5 triphenyl-tetrazolium-chloride (TTC) at 37°C and fixed by immersion in 4.5% buffered formalin solution according to Bedersen (1986b). Brain slices were photographed from both sides, the area of infarction was quantified using IMAGE 1.41 (NIH, Bethesda Md, USA) by an observer blinded to the animals' experimental group. Measurement and calculation of infarct size were done by an investigator blinded to the treatment groups. To avoid overestimation of infarct size by edema, the corrected infarct size was calculated according to Lin at al. (Lin 1993).

Determination of the neurological score

At day 7, all surviving animals were examined neurologically using an established scoring system according to Bedersen (1986a) and Menzies (1992).

Score	Evaluation
0	No apparent deficits
1	Contralateral forelimb flexion
2	Decreased grip of the contralateral forelimb while pulled by tail
3	Spontaneous movement in all directions; contralateral circling only if pulled by tail
4	Spontaneous contralateral circling
5	Death

Magnetic resonance imaging

To confirm MCAO and to match SAHA-treated animals and controls, MRI was performed at 55 minutes after MCAO on a 1.5T whole body MR imager (Siemens

Sonata, Erlangen, Germany) equipped with high-performance gradients (40mT/m, 200mT/m/ms). Anaesthesia was maintained. The standard body coil was used for RF transmission; the signal was detected with a temporo-mandibular joint surface coil (Ø=9cm). Diffusion-weighted spin-echo EPI images (TR=2000ms, TE=77ms, matrix 128x128, field of view=93x93cm, slice thickness=2mm) were acquired with the middle slice positioned at the level of the optic chiasm. Animals without successful MCAO were excluded from further data analysis. Using the Siemens-built-in software, the infarct size was calculated according to Lin (1993) and based on the result, animals were matched to the two groups.

Data analysis

For statistical analysis of all results, commercial software (Analyse-it® for Excel, General and Clinical Laboratory statistics, Version 1.68) was used. Infarction volumes were described by an analysis of variance (ANOVA). In order to conclude two given groups significantly different, a Fisher`s least significant difference test was performed when the overall differences were significant by the ANOVA test ($p<0.05$). For statistical analysis of the neurological score the Kruskal-Wallis-test was used, and a probability value of $P<0.05$ was considered to be significant. All values were expressed as mean ± standard deviation (SD).

Results

Physiologic parameters

No statistically significant differences were noted in animals among the two groups for any of the intraoperative physiological parameters. Throughout the surgical preparation the average body temperature for all animals was 37.2 ±0.4°C. Arterial blood gases ($pO2$ = 108±17mmHg, $pCO2$ = 37±4mmHg, pH = 7.37±0.03), hematocrit (41.2±2.2%) and mean arterial blood pressure (107±22mmHg) remained stable.

Infarction size

In MRI all fourteen animals showed infarction. There was no significant difference in infarct size in animals undergoing SAHA-treatment compared to controls (179 +/- 52 mm^3 compared to 184 +/- 47 mm^3, respectively).

Compared to controls (274 +/- 41 mm^3), the TTC-derived infarct size at 24 hours after MCAO of animals undergoing SAHA-treatment was significantly reduced with 164 +/- 67 mm^3 ($P<0.01$), i.e. infarct size was reduced by approximately 40% compared to controls.

Neurological score

Compared to controls (3.2 +/- 0.6), neurological scores measured at 24 hours after MCAO of animals undergoing SAHA-treatment were significantly smaller, i.e. better, with 2.4 +/- 0.7.

Example 7: HDAC inhibitor treatment in an experimental trauma model

Materials and Methods

The study was approved by the Institutional Review Board and guidelines for animal care. All experiments were carried out in accordance with the European Council Directive (86/609/EEC). All efforts were made to minimize the number of animals used, as well as their suffering. Female Wistar rats weighing 200-250g were used in this study. Food and water were provided freely before and after surgery.

Surgical Procedure

The surgical procedure was recently described by Weber ((2006) Am. J. Neuroradiol. 27, 598-604). Briefly, animals underwent anesthesia made up of a mixture of ketamine (62.5 mg/kg body weight; WDT, Garbsen, Germany), xylazine (3.175 mg/kg body weight; WDT), and acepromazine (0.625 mg/kg body weight, Sanofi-Ceva, Düsseldorf, Germany) in 0.9% sterile saline solution. Rats received spinal cord contusion injuries by using the Infinite Horizon (IH) Impactor spinal cord injury device (Precision Systems & Instrumentation, Lexington, Ky). A laminectomy was performed at the thoracal segment T10 to expose the dorsal portion of the spinal cord. The animals were suspended by attaching Adson forceps to the rostral T9 and caudal T11 vertebral bodies. Particular care was taken to align the exposed spinal cord perpendicular to the axis of the Impactor. The 2.5-mm stainless steel impounder tip was lowered to approximately 3–4 mm above the surface of the exposed spinal cord. The contusion injury was finally induced by applying an impact force of 2 Newton

(equal to 200 kilodyne) to the exposed spinal cord at a velocity of 130 mm/s. Overlying muscle layers were sutured and the skin was closed. Postoperatively, animals were kept warm, placed on beds of sawdust, and given manual bladder evacuation twice a day for a period of 7 days as necessary and received intramuscular injections of 10 mg Cotrimoxazol (Ratiopharm, Ulm, Germany) once daily for a period of 7 days.

Study design

Group 1 (SAHA-Group): 3 rats were allocated to this treatment group. SAHA-administration was performed 1 hour after spinal cord impact. Hereby, 200 mg per kg body weight were administered intracutaneously. A second injection was applied two days later. The dose was chosen according to Hockly (2003).

Group 2 (Controls): 3 rats were allocated to this group. Animals of the control group received equivolumetric DMSO vehicle 1 hour after spinal cord impact as well as two days later.

Determination of spinal cord trauma size

Seven days after spinal cord contusion, all animals were sacrificed, and the vertebrate column was removed en bloc from segment C5 – L5. Tissue specimens were fixed for 2 days in 10% formalin, the spinal cord removed, and post-fixed over night. Following photographic documentation, the spinal cord was dissected at the medio-sagittal plane and embedded into liquid paraffin. Serial HE-stained sections were obtained to microscopically determine the size of spinal cord trauma.

Determination of the neurological score

At day 6, all animals were alive and neurologically examined using video-monitoring of their movement ability. All animals had to pass an approx. 30 cm long passageway and spontaneous as well as coordinated movement of the hind limbs were analyzed according to the following evaluation score:

Score for neurological examination

Score	Evaluation
0	Death
1	No spontaneous movement of any hind limb
2	Spontaneous movement of one hind limb
3	Spontaneous movement of both hind limbs
4	Coordinated movement of one hind limb during walk
5	Normal walk of animal

Results

Microscopical Determination of Spinal trauma

All six animals developed pseudocystically transformed spinal trauma due to contusion (posttraumatic syringomyelia; de Girolami (1997), Greenfield's Neuropathy 6th Edition (ed. Graham and Lantos), Chapter 18, 1095-1121)). Histopathologically, the lesion was invaded by macrophages and already demarcated by reactive astrocytes. There was no meaningful difference between both study groups with respect to lesion size or invading hematopoetic cell populations within the affected area of the spinal cord.

Neurological score

Video monitoring demonstrates in all SAHA treated animals better coordination of hind limb movement (see below). Compared to controls, neurological scores measured 7 days after spinal contusion were significantly better in animals with SAHA-treatment (3.3 +/- 0.4 compared to 1,6 +/- 0.4), as documented in the following table:

Evaluation of spinal cord contusion model

Animal	Movement score	Histopathology
Control 1	1	syringomyelia
Control 2	2	syringomyelia
Control 3	2	syringomyelia
SAHA 1	3	syringomyelia
SAHA 2	4	syringomyelia
SAHA 3	3	syringomyelia

Despite similar structural lesions observed in the spinal cord, the data is compatible with SAHA administration having a significant improvement of the neurological status (movement of hind limbs) in the trauma model. It is concluded that neuroprotective mechanisms adjacent to the trauma to be involved, i.e. up-regulation of EAAT2 glutamate transporter.

The present invention refers to the following nucleotide and amino acid sequences:

Nucleotide sequence (cDNA) encoding for *Homo sapiens* solute carrier family 1 (glial high affinity glutamate transporter), member 2 (SLC1A2), EAAT2:

```
   1 caccctcgga gccccggag ctccccgcca agcgccatcc ccgcgggcgg aggggagcgc
  61 gggtcgcgcg ccgtggagag ccgggacgcg gattagcgcc cgcaggagcc tcctgcgccc
 121 gttgaggcgc taaagggctt accccggagg cgggtggaag ggcgggcaga ggctcctctt
 181 aaataccgct cccggccgca cttcgcgctc accccggcgt ccgctttctc cctcgcccac
 241 agctgccgga tagtgctgaa gaggaggggg cgttccccag accatggcat ctacggaagg
 301 tgccaacaat atgcccaagc aggtggaagt gcgaatgcac gacagtcatc ttggctcaga
 361 ggaacccaag caccggcacc tgggcctgcg cctgtgtgac aagctgggga agaatctgct
 421 gctcaccctg acggtgtttg gtgtcatcct gggagcagtg tgtggagggc ttcttcgctt
 481 ggcatctccc atccaccctg atgtggttat gttaatagcc ttcccagggg atatactcat
 541 gaggatgcta aaaatgctca ttctccctct aatcatctcc agcttaatca cagggttgtc
 601 aggcctggat gctaaggcta gtggccgctt gggcacgaga gccatggtgt attacatgtc
 661 cacgaccatc attgctgcag tactgggggt cattctggtc ttggctatcc atccaggcaa
 721 tcccaagctc aagaagcagc tggggcctgg gaagaagaat gatgaagtgt ccagcctgga
 781 tgccttcctg gaccttattc gaaatctctt ccctgaaaac cttgtccaag cctgctttca
 841 acagattcaa acagtgacga agaaagtcct ggttgcacca ccgccagacg aggaggccaa
 901 cgcaaccagc gctgttgtct ctctgttgaa cgagactgtg actgaggtgc cggaggagac
 961 taagatggtt atcaagaagg gcctggagtt caaggatggg atgaacgtct taggtctgat
1021 agggtttttc attgcttttg gcatcgctat ggggaagatg ggagatcagg ccaagctgat
1081 ggtggatttc ttcaacattt tgaatgagat tgtaatgaag ttagtgatca tgatcatgtg
1141 gtactctccc ctgggtatcg cctgcctgat ctgtggaaag atcattgcaa tcaaggactt
1201 agaagtggtt gctaggcaac tggggatgta catggtaaca gtgatcatag gcctcatcat
1261 ccacgggggc atctttctcc ccttgattta ctttgtagtg accaggaaaa accccttctc
1321 ctttttgct ggcattttcc aagcttggat cactgccctg ggcaccgctt ccagtgctgg
1381 aactttgcct gtcacctttc gttgcctgga agaaaatctg gggattgata agcgtgtgac
```

```
1441 tagattcgtc cttcctgttg gagcaaccat taacatggat ggtacagccc tttatgaagc
1501 ggtggccgcc atctttatag cccaaatgaa tggtgttgtc ctggatggag gacagattgt
1561 gactgtaagc ctcacagcca ccctggcaag cgtcggcgcg gccagtatcc ccagtgccgg
1621 gctggtcacc atgctcctca ttctgacagc cgtgggcctg ccaacagagg acatcagcct
1681 gctggtggct gtggactggc tgctggacag gatgagaact tcagtcaatg ttgtgggtga
1741 ctcttttggg gctgggatag tctatcacct ctccaagtct gagctggata ccattgactc
1801 ccagcatcga gtgcatgaag atattgaaat gaccaagact caatccattt atgatgacat
1861 gaagaaccac agggaaagca actctaatca atgtgtctat gctgcacaca actctgtcat
1921 agtagatgaa tgcaaggtaa ctctggcagc caatggaaag tcagccgact gcagtgttga
1981 ggaagaacct tggaaacgtg agaaataagg atatgagtct cagcaaattc ttgaataaac
2041 tccccagcgt atcctatggt aactgatggt ataaacaagc tttctttaaa aaggaaaaaa
2101 atgcgtatat ttctatgttt acttaatctg ttagccgagg cttagaggag ctcttctgag
```

Nucleotide sequence (cDNA) encoding for *Mus musculus* solute carrier family 1 (glial high affinity glutamate transporter), member 2 (Slc1a2), EAAT2:

```
   1 cagaagttgg aagccagtgc acttctacag ctgagagaat ggtcagtgcc aacaatatgc
  61 ccaagcaggt agaagtgcgc atgcacgaca gccacctcag ctccgatgag ccaaagcacc
 121 gaaacctggg catgcgcatg tgcgacaagc tggggaaaaa tctcctgctc tcactgactg
 181 tgtttggtgt catcctggga gcagtgtgtg gcgggctgct tcgcttggca tcgcccatcc
 241 accctgatgt ggtcatgttg atagccttcc cgggggacat actcatgagg atgctgaaga
 301 tgctcatcct ccctcttatc atctccagtt taatcacagg gttgtcaggc ctggatgcta
 361 aagccagcgg ccgcctaggc acgagagcta tggtgtatta catgtccacg accatcattg
 421 ccgccgtgct gggggtcatc ctggtgttgg ccatccaccc aggcaatccc aaactcaaga
 481 agcagctagg gcccgggaag aagaacgacg aggtgtctag cctggatgcc ttcctggatc
 541 tcattagaaa tctcttcccg gagaacctgg tgcaagcctg tttccagcag attcagacag
 601 tgacaaagaa agttctggtg gcacctccat ctgaggaggc caataccacc aaggcggtca
 661 tctccatgtt gaatgaaacc atgaacgagg cccctgaaga aactaagatc gttatcaaga
 721 agggcctgga gttcaaggac gggatgaatg tcttaggtct gatcggattc tttattgctt
 781 tcggcattgc catggggaag atgggtgaac aggccaagct gatggtggag ttcttcaaca
 841 ttctgaatga gatcgtgatg aagttagtga tcatgatcat gtggtactcc cctctgggta
 901 tcgcctgctt gatttgtggg aagatcatcg ccatcaagga cttagaagtg gttgctaggc
 961 agctggggat gtacatgatc accgtgatcg tgggcctcat cattcacggg ggcatctttc
1021 tccccttgat ttactttgta gtgaccagaa aaaatccatt ctccttttt gctggcatat
1081 tccaagcctg gatcactgct ctgggaactg cttccagtgc tggaactttg cctgttacct
1141 tccgttgctt ggaagataat ctagggattg acaagcgtgt gaccagattc gtcctcccag
1201 tcggagcaac cattaacatg gatggcacag ccctttacga ggctgtggca gccattttca
1261 tagcccaaat gaatggggtc atcttggatg gaggtcagat tgtgactgta agccttacag
1321 ccaccctggc aagcattggt gcagccagta ttccaagcgc cgggctggtc accatgctcc
1381 tcattctcac agctgtgggc ctgccaacgg aggatatcag tctgctggtg gcggtggact
1441 ggctgctgga tagaatgaga acttcagtca atgtggtggg cgattctttt ggggctggga
1501 ttgtctatca cctttccaag tctgagctgg acaccattga ctcccaacac cgaatgcagg
1561 aagacatcga aatgaccaag acgcagtcca tttacgacga caagaaccac agggaaagca
1621 actctaatca gtgtgtctat gccgcacaca actctgtcgt aatagatgag tgcaaggtac
1681 ctttcccatt cctggatatc gagacctgca tatgaatagt gcatgctgga ctccttaaaa
1741 aaaaatcaca acccccttgca tccattctta ccatgctttg taaggaatca tgtcacccaa
1801 gctcctgtgg ggcccagacc atctcactct gcatccagca tggtgggaca tcagcaagct
1861 gacaccaaaa ggaccaatca gttgcttgct tcaacagatt cccacatcca cctcacccct
1921 ctaatttggt caatattctt cactctttgg gccaagaatt ggtggttgtc agaactttaa
1981 tggccttcag atattctctt cctcaaagga agggtcagaa aaatgtagac caaatattat
2041 tgactttata tagcatgagt attccgcgct gcgctgtctt acaaattgct ttttaaaagc
2101 tcaaataaaa gtttaattgg ctgtcaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
2161 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa
```

Nucleotide sequence (cDNA) encoding for *Rattus norvegicus* solute carrier family 1 (glial high affinity glutamate transporter), member 2 (Slc1a2), EAAT2:

```
   1 cctgcccgtt aaataccgct ccccgccgca ctccgggctc acccagctcg tcgccactgt
  61 ctccagacgt gcccgggagg aggggcgttc cccacgccat ggcatcaacc gagggtgcca
 121 acaatatgcc caagcaggtg gaagtgcgca tgcacgacag ccacctcagc tccgaggagc
 181 caaagcaccg aaacctgggc atgcgcatgt gcgacaagct ggggaagaac ctcctgctct
 241 cactgactgt gtttggtgtc atcctgggag cagtatgtgg cgggctgctt cgcttggcgg
 301 ctcccatcca ccctgatgtg gtcatgttga tagccttccc gggggacatc ctcatgagga
 361 tgctgaagat gctcatcctc cctctcatca tttccagtct catcacaggg ctgtcagggc
 421 tggatgctaa agccagcggc cgcctaggca cgagagccat ggtatattac atgtccacga
 481 ccatcattgc cgctgtgctg ggggtcatcc tggtgttggc catccaccct gggaatccca
 541 aactcaagaa gcagctgggg cctgggaaga agaacgacga ggtgtccagc ctggatgcct
 601 tcctggatct cattagaaat ctcttcccgg agaacctggt acaagcctgt ttccaacaga
 661 ttcagactgt gacaaagaaa gttctggtgg cacctccatc cgaggaggcc aatacaacca
 721 aggcagtcat ctccctgttg aatgagacca tgaatgaggc ccctgaagaa actaagatcg
 781 ttatcaagaa gggcctggag ttcaaggacg ggatgaatgt cttaggtctg attggattct
 841 ttattgcttt cggcattgcc atggggaaga tgggtgtagc aggccaagct gatggtggag
 901 ttcttcaaca ttctgaacga gattgtcatg aagttagtga tcatgatcat gtggtattcc
 961 ccgctggtat cgcctgcttg atctgtggga agatcatcgc catcaaggac ttagaagtgg
1021 ttgctaggca gctggggatg tacatgatca cagtgatcgt gggcctcatc attcatgggg
1081 gcatctttct ccccttgatt tactttgtag tgaccagaaa aaacccattc tccttttttg
1141 ctggcatttt ccaagcctgg atcactgccc tgggaaccgc ttccagtgct ggaactttgc
1201 ctgtcacctt ccgttgcttg gaagataatc tagggattga caagcgtgtg accagattcg
1261 tcctcccagt cggagcaacc attaacatgg atggtacagc cctttacgaa gccgtggcag
1321 ccatcttcat agcccagatg aatggggtca tcctggatgg aggtcagata gtgactgtaa
1381 gccttacagc aactctggcg agcattggtg cagccagtat tcccagcgcc gggttggtca
1441 ccatgctcct cattctcaca gctgtgggcc tgccgacaga ggacatcagt ctgctggtgg
1501 cagtggactg gctgctggat agaatgagaa cttcggtcaa tgtagtgggc gattcttttg
1561 gggctgggat tgtctatcac ctttccaagt ccgagctgga caccattgac tcccaacacc
1621 gaatgcacga agacatcgaa atgaccaaga cgcagtccgt ttatgacgac acgaagaacc
1681 acagggaaag caactctaat cagtgtgtct atgccgcaca caactctgtc gtaatagatg
1741 agtgcaaggt aactctggcg gccaatggaa agtcagctga ctgcagtgtt gaggaagaac
1801 cttggaaacg tgaaaaataa tgacccgatt ctcagccaat tcttgaataa ctccccagcg
1861 tatcttatgg taaaagatgc tctaaacagg cttccttt
```

Amino acid sequences of the excitatory amino acid transporter 2 (EAAT2; EAAT2b; Sodium-dependent glutamate/aspartate transporter 2; GLUT-B; GLT-1; GLT-1a; GLT-1b) from human (*Homo sapiens,* SEQ ID NO: *4*) and rat (*Rattus norvegicus,* SEQ ID NO: 6):

```
Human    1 mastegannm pkqvevrmhd shlgseepkh rhlglrlcdk lgknllltlt vfgvilgavc
Rat      1 mastegannm pkqvevrmhd shlsseepkh rnlgmrmcdk lgknlllslt vfgvilgavc

Human   61 ggllrlaspi hpdvvmliaf pgdilmrmlk mlilpliiss litglsglda kasgrlgtra
Rat     61 ggllrlaapi hpdvvmliaf pgdilmrmlk mlilpliiss litglsglda kasgrlgtra

Human  121 mvyymsttii aavlgvilvl aihpgnpklk kqlgpgkknd evssldafld lirnlfpenl
Rat    121 mvyymsttii aavlgvilvl aihpgnpklk kqlgpgkknd evssldafld lirnlfpenl

Human  181 vqacfqqiqt vtkkvlvapp pdeeanatsa vvsllnetvt evpeetkmvi kkglefkdgmn
Rat    181 vqacfqqiqt vtkkvlvapp seeanttkav isllnetmne apeetkivik kglefkdgmn

Human  241 vlgligffi afgiamgkmg dqaklmvdff nilneivmkl vimimwyspl giaclicgkii
Rat    241 vlgligffia fgiamgkmge qaklmveffn ilneivmklv imimwysplg iaclicgkii

Human  301 aikdlevva rqlgmymvtv iigliihggi flpliyfvvt rknpfsffag ifqawitalgt
Rat    301 aikdlevva rqlgmymitv ivgliihggi flpliyfvvt rknpfsffag ifqawitalgt

Human  361 assagtlpv tfrcleenlg idkrvtrfvl pvgatinmdg talyeavaai fiaqmngvvld
Rat    361 assagtlpvt frclednlgi dkrvtrfvlp vgatinmdgt alyeavaaif iaqmngvild
```

```
Human    421 ggqivtvslt atlasvgaas ipsaglvtml liltavglpt edisllvavd wlldrmrtsv
Rat      421 ggqivtvslt atlasigaas ipsaglvtml liltavglpt edisllvavd wlldrmrtsv

Human    481 nvvgdsfga givyhlskse ldtidsqhrv hediemtktq siyddmknhr esnsnqcvya
Rat      481 nvvgdsfgag ivyhlsksel dtidsqhrmh ediemtktqs vyddtknhre snsnqcvya

Human    541 ahnsvivdec kvtlaangks adcsveeepw krek
Rat      541 ahnsvvidec kvtlaangks adcsveeepw krek
```

Amino acid sequences of Slc1a2 (GLT1, Eaat2, GLT-1, MGLT1, 1700091C19Rik) from mouse (*Mus musculus*)

```
MVSANNMPKQVEVRMHDSHLSSDEPKHRNLGMRMCDKLGKNLLL
SLTVFGVILGAVCGGLLRLASPIHPDVVMLIAFPGDILMRMLKMLILPLIISSLITGL
SGLDAKASGRLGTRAMVYYMSTTIIAAVLGVILVLAIHPGNPKLKKQLGPGKKNDEVS
SLDAFLDLIRNLFPENLVQACFQQIQTVTKKVLVAPPSEEANTTKAVISMLNETMNEA
PEETKIVIKKGLEFKDGMNVLGLIGFFIAFGIAMGKMGEQAKLMVEFFNILNEIVMKL
VIMIMWYSPLGIACLICGKIIAIKDLEVVARQLGMYMITVIVGLIIHGGIFLPLIYFV
VTRKNPFSFFAGIFQAWITALGTASSAGTLPVTFRCLEDNLGIDKRVTRFVLPVGATI
NMDGTALYEAVAAIFIAQMNGVILDGGQIVTVSLTATLASIGAASIPSAGLVTMLLIL
TAVGLPTEDISLLVAVDWLLDRMRTSVNVVGDSFGAGIVYHLSKSELDTIDSQHRMQE
DIEMTKTQSIYDDKNHRESNSNQCVYAAHNSVVIDECKVPFPFLDIETCI
```

Additional references cited

Albers (2004) The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 126:483S 512S

Bedersen (1986a) Stroke 17: 472-476

Bedersen (1986b) Stroke 17: 1304-1308

Blümcke (1995) Glia 15:131-140

Brichta (2003) Hum Mol Genet 12:2481-2489

Ellison (2004) Head and spinal injuries. In: Neuropathology, pp 241-269. Edinburgh: Mosby

Eyupoglu (2005) Malignant gliomas induce neuronal cell death in an organotypic glioma invasion model. J Neurosurgery (in press)

Georgiadis (2005) Curr Treat Options Neurol, Mar;7(2):119-127

Girolami (1997), In: Greenfield's Neuropathology 6th Edition Chapter 18, pp:1095-1121

Glaser (2004) Biochem. Biophys. Res. Com. 325, 683 to 690

Göttlicher (2004) Ann Hematol 83 Suppl 1:S91-92

Hockly (2003) Proc Natl Acad Sci U S A 100:2041-2046

Hoogland (2004) Epilepsy Res. 59: 75-82

Huang (2004) Curr Opin Neurobiol 14:346-352

Jansen (2002) Dtsch Arztebl 99:A1361-1370

Kelly (2003) Clin Cancer Res 9:3578-3588

Kirschner (1994) Genomics 24: 218-224

Kramer (2003) Embo J 22:3411-3420

Lin (1993) Stroke 24: 117-121

Longa (1989) Stroke 20: 84-91

Kwiatkowski (1999) N Engl J Med. 340:1781-7

Menzies (1992) Neurosurgery 31: 100-107

Marks (2004) Adv Cancer Res 91:137-168

Ognjenovic (2003) Americal Chemical Societey, 225th National Meeting, New Orleans, LA, USA

Pines (1992) Nature 360: 464-467

Pohlmann-Eden (2003) Acta Neurol. 108(2):142-5

Ren (2004) J Neurochem 89:1358-1367

Rote Liste (2003) ECV, Editio Cantor Verlag, Aulendorf: 15-0051/0052

Richon (2000) Proc Natl Acad Sci U S A 97:10014-9

Ryan (2003) ASCO Annual Meeting. Chicago, IL

Scheffler (2003) Development 130:5533-5541

Shashidharan (1994) *Biochim. Biophys. Acta* 1191: 393-396

Stoppini (1991) J neurosci Meth 37: 191-198

Su (2003) Proc Natl Acad Sci U S A 100:1955-1960

Weber, M (2006) Am. J. Neuroradiol. 27: 598 - 6

Wisinski (2003) Proc. Am. Soc. Clin. Oncol

Zhang (2004a) Mol. Cancer Ther. 3, 425 to 435

Zhang (2004b) Radiat. Res. 161, 667 to 674

Claims

1. Use of HDAC inhibitors for the preparation of a pharmaceutical composition for the amelioration, treatment or prevention of malignancies related to ischemic events or brain damage, whereby said HDAC inhibitor is selected from the group consisting of SAHA, M344, MS-275, CBHA and SBHA.

2. A method for the amelioration, prevention and/or treatment of head injuries or brain damages, in particular of traumatic event as well as ischemia/stroke said method comprising administering to a patient in need of such an amelioration, prevention or treatment a pharmaceutically active amount of a HDAC inhibitor selected from a group consisting of SAHA, M344, MS-275, CBHA and SBHA.

3. The use of claim 1 or the method of claim 2 wherein said HDAC inhibitor is SAHA.

4. The use of claim 1 or 3 or the method of claims 2 or 3, whereby said HDAC inhibitor is to be administered to a human patient.

5. The use or the method of claim 4, wherein said HDAC inhibitor is to be administered in a concentration of 150 to 300 mg/m^2 body surface in the case of SAHA, M344 and MS-275 or in a concentration of 300 to 900 mg/m^2 body surface in the case of CBHA or SBHA.

6. The use or the method of any one of claims 1 to 5, wherein said ischemic event is stroke.

7. The use or the method of any one of claims 1 to 5, wherein said brain damage is or is caused by head injury, head accident, trauma and/or is caused during brain surgery.

8. The use or the method of any one of claims 1 to 5, wherein said brain damage is or is caused by a spinal cord injury, spinal cord accident and/or is caused during surgery on the spinal cord.

FIGURE 1:

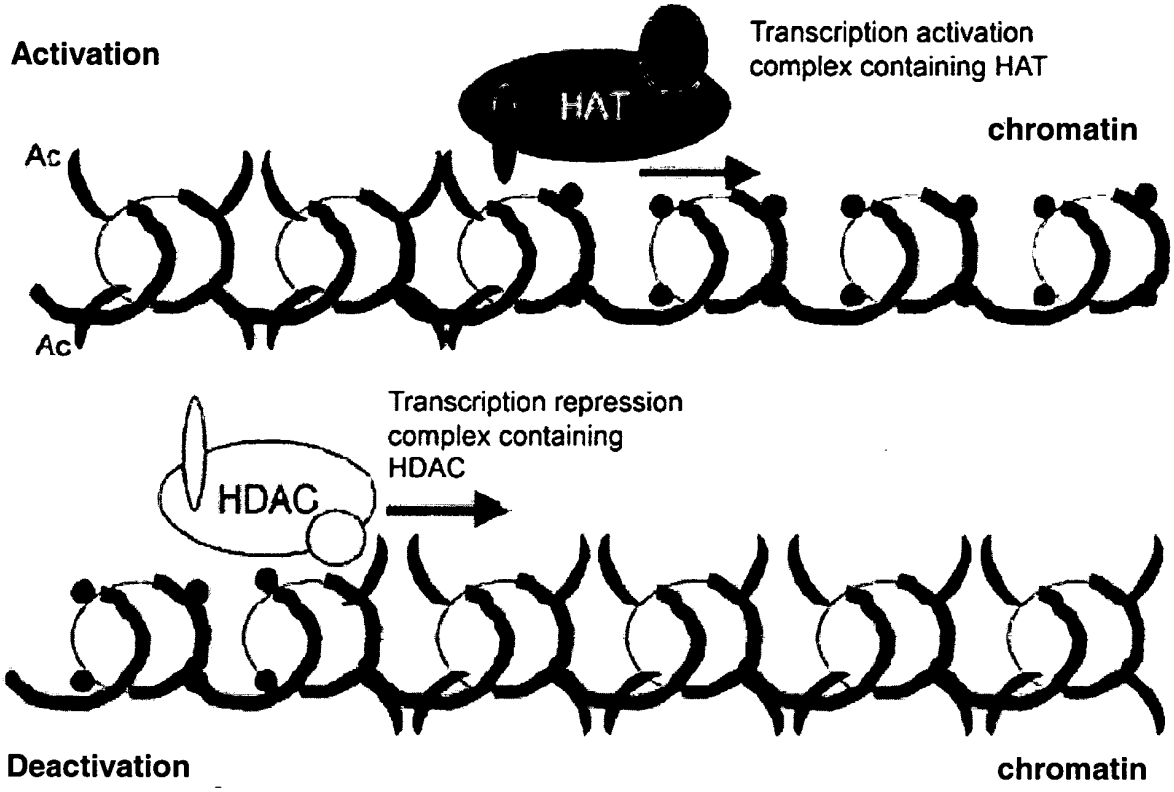
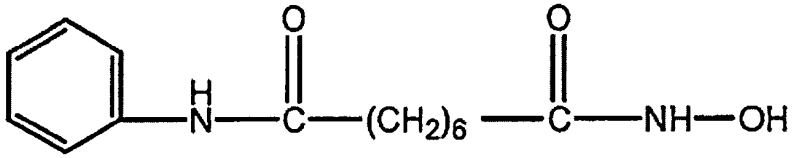
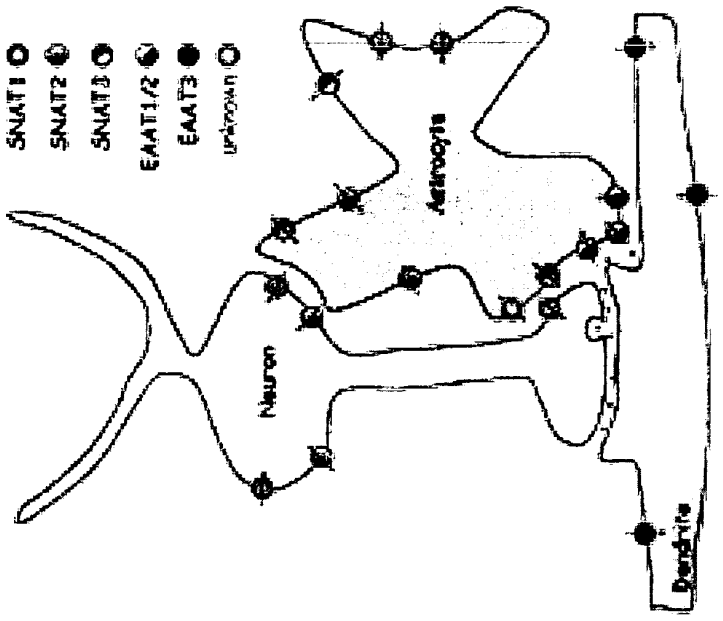


FIGURE 2:




SNAT1
SNAT2
SNAT3
EAAT1/2
EAAT3
unknown
Neuron
Astrocyte
Dendrite

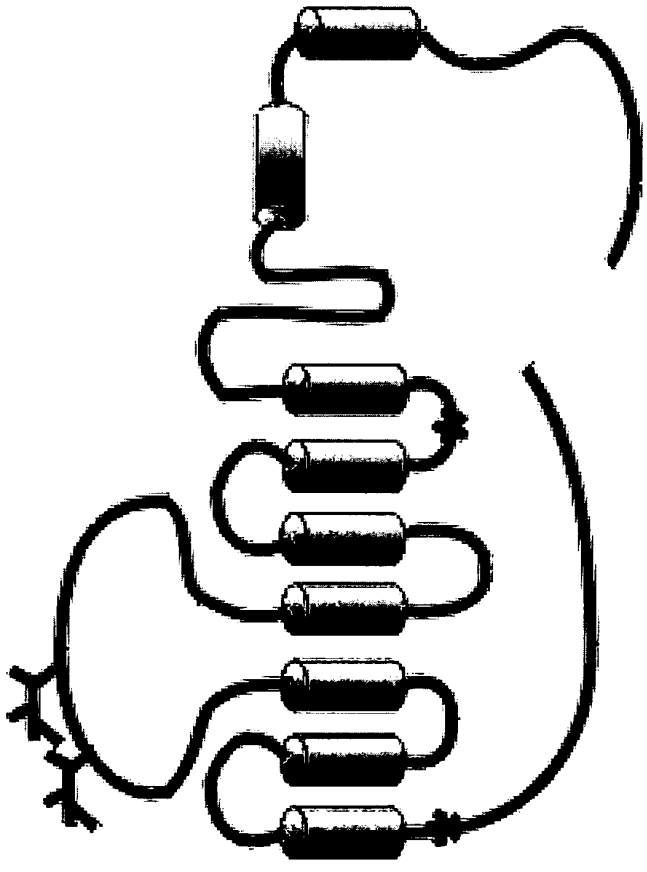


FIGURE 3:

FIGURE 4:

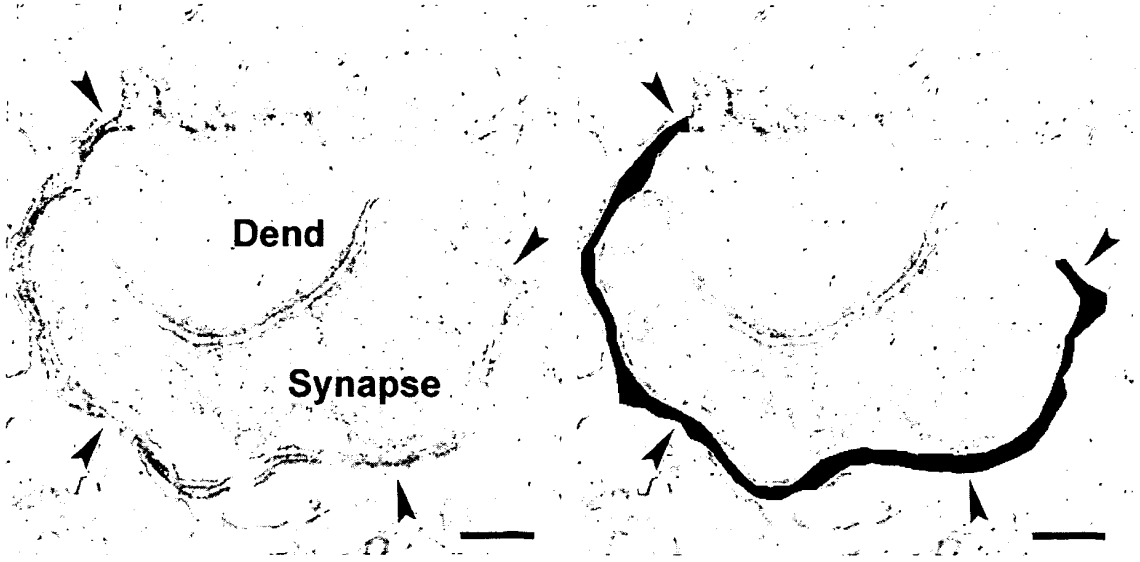


FIGURE 5:

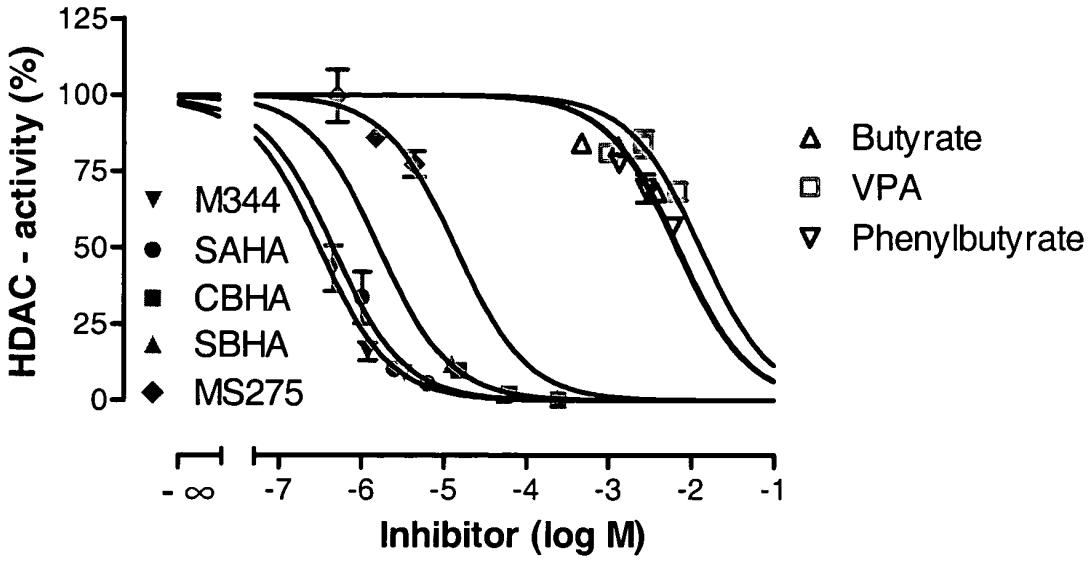


FIGURE 6:

Rat Hippocampal Slice

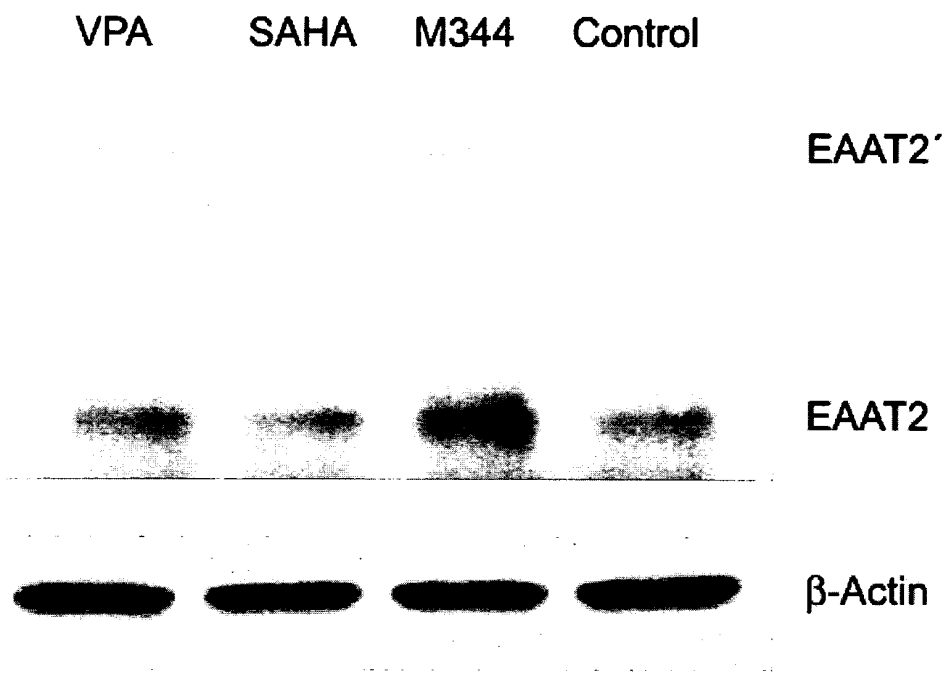


FIGURE 7: A

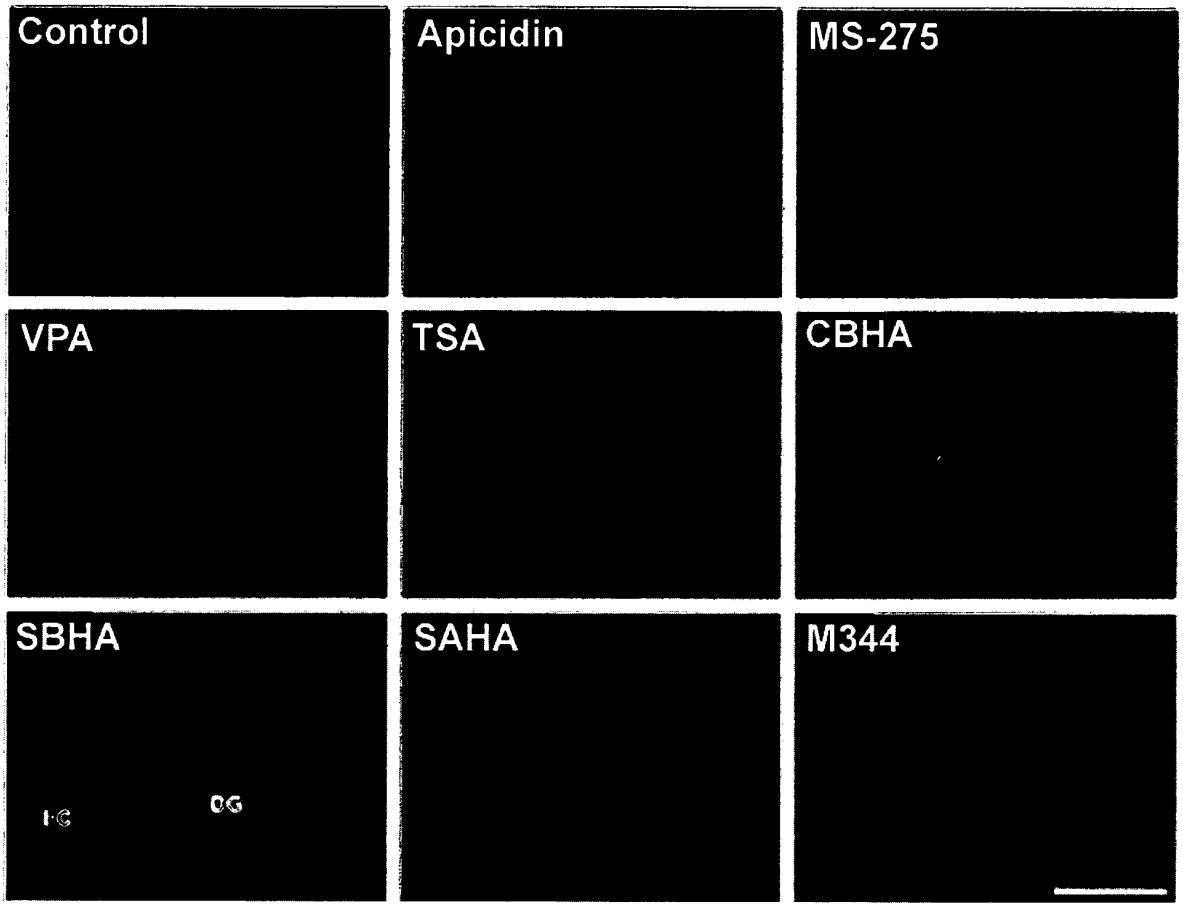


FIGURE 7 (cont.): B

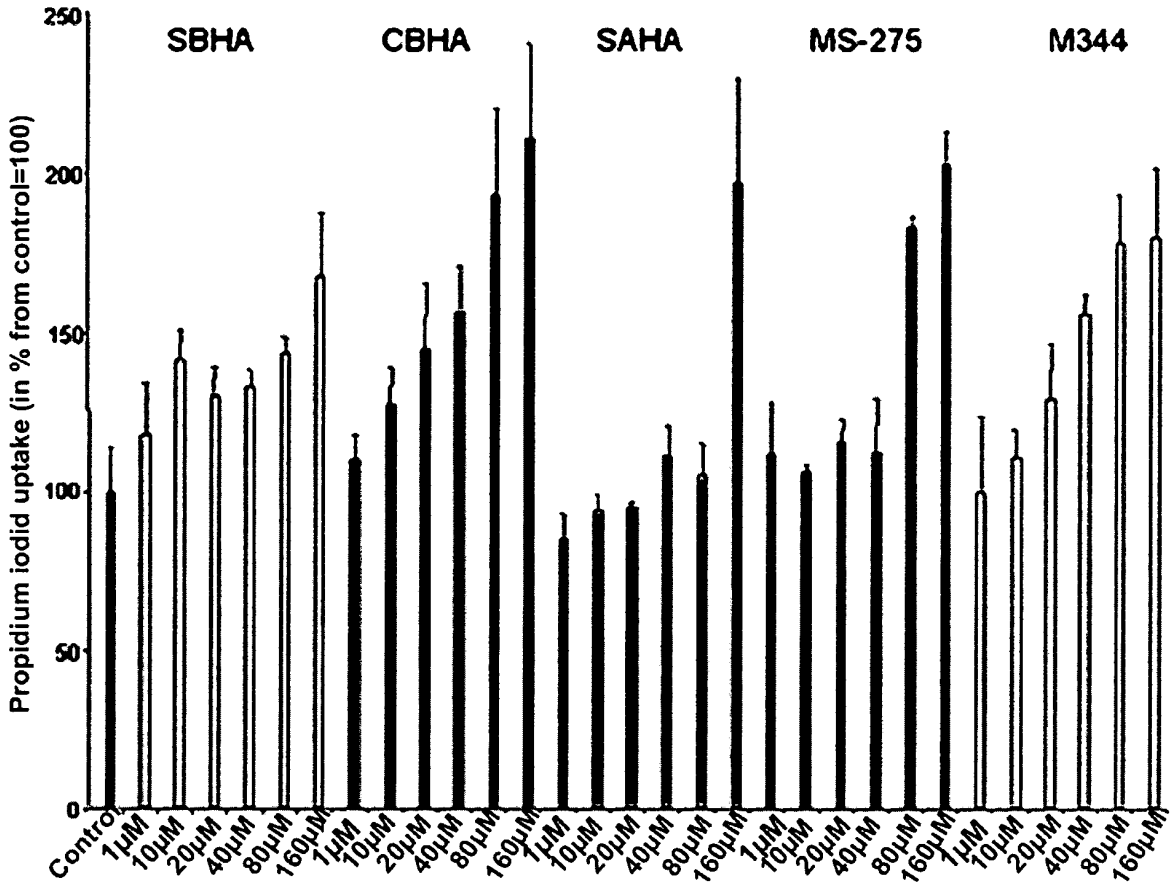
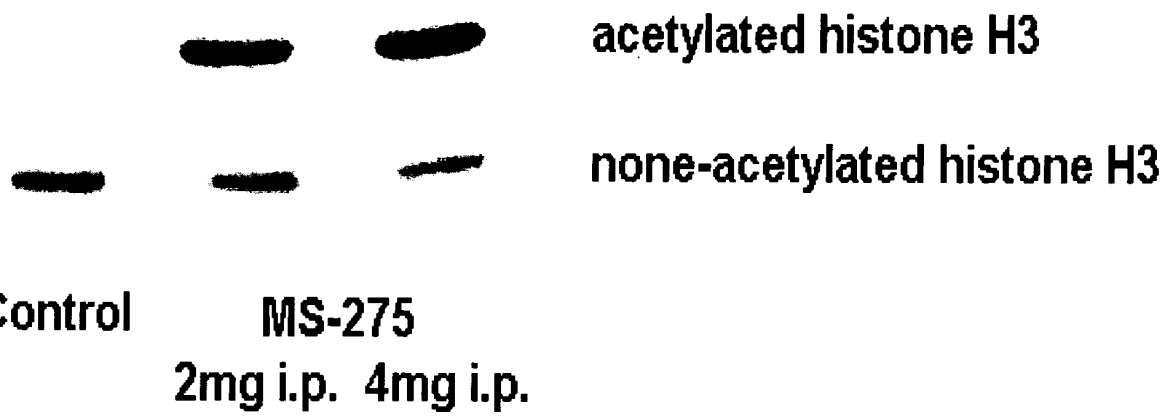


FIGURE 8:


acetylated histone H3
none-acetylated histone H3
Control
MS-275
2mg i.p.
4mg i.p.

SEQUENCE LISTING

<110> Friedrich-Alexander-Universität Erlangen-Nürnberg

<120> Means and methods for the treatment of head injuries and stroke

<130> K2769 PCT S3

<160> 6

<170> PatentIn version 3.3

<210> 1
<211> 2160
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (284)..(2005)

<400> 1
caccctcgga gccccggag ctccccgcca agcgccatcc ccgcgggcgg aggggagcgc 60

gggtcgcgcg ccgtggagag ccgggacgcg gattagcgcc cgcaggagcc tcctgcgccc 120

gttgaggcgc taaagggctt accccggagg cgggtggaag ggcgggcaga ggctcctctt 180

aaataccgct cccggccgca cttcgcgctc accccggcgt ccgctttctc cctcgcccac 240

agctgccgga tagtgctgaa gaggaggggg cgttccccag acc atg gca tct acg 295
Met Ala Ser Thr
1

gaa ggt gcc aac aat atg ccc aag cag gtg gaa gtg cga atg cac gac 343
Glu Gly Ala Asn Asn Met Pro Lys Gln Val Glu Val Arg Met His Asp
5 10 15 20

agt cat ctt ggc tca gag gaa ccc aag cac cgg cac ctg ggc ctg cgc 391
Ser His Leu Gly Ser Glu Glu Pro Lys His Arg His Leu Gly Leu Arg
25 30 35

ctg tgt gac aag ctg ggg aag aat ctg ctg ctc acc ctg acg gtg ttt 439
Leu Cys Asp Lys Leu Gly Lys Asn Leu Leu Leu Thr Leu Thr Val Phe
40 45 50

ggt gtc atc ctg gga gca gtg tgt gga ggg ctt ctt cgc ttg gca tct 487
Gly Val Ile Leu Gly Ala Val Cys Gly Gly Leu Leu Arg Leu Ala Ser
55 60 65

ccc atc cac cct gat gtg gtt atg tta ata gcc ttc cca ggg gat ata 535
Pro Ile His Pro Asp Val Val Met Leu Ile Ala Phe Pro Gly Asp Ile
70 75 80

ctc atg agg atg cta aaa atg ctc att ctc cct cta atc atc tcc agc 583
Leu Met Arg Met Leu Lys Met Leu Ile Leu Pro Leu Ile Ile Ser Ser
85 90 95 100

tta atc aca ggg ttg tca ggc ctg gat gct aag gct agt ggc cgc ttg 631

```
Leu Ile Thr Gly Leu Ser Gly Leu Asp Ala Lys Ala Ser Gly Arg Leu
                105                 110                 115

ggc acg aga gcc atg gtg tat tac atg tcc acg acc atc att gct gca      679
Gly Thr Arg Ala Met Val Tyr Tyr Met Ser Thr Thr Ile Ile Ala Ala
            120                 125                 130

gta ctg ggg gtc att ctg gtc ttg gct atc cat cca ggc aat ccc aag      727
Val Leu Gly Val Ile Leu Val Leu Ala Ile His Pro Gly Asn Pro Lys
        135                 140                 145

ctc aag aag cag ctg ggg cct ggg aag aag aat gat gaa gtg tcc agc      775
Leu Lys Lys Gln Leu Gly Pro Gly Lys Lys Asn Asp Glu Val Ser Ser
    150                 155                 160

ctg gat gcc ttc ctg gac ctt att cga aat ctc ttc cct gaa aac ctt      823
Leu Asp Ala Phe Leu Asp Leu Ile Arg Asn Leu Phe Pro Glu Asn Leu
165                 170                 175                 180

gtc caa gcc tgc ttt caa cag att caa aca gtg acg aag aaa gtc ctg      871
Val Gln Ala Cys Phe Gln Gln Ile Gln Thr Val Thr Lys Lys Val Leu
                185                 190                 195

gtt gca cca ccg cca gac gag gag gcc aac gca acc agc gct gtt gtc      919
Val Ala Pro Pro Pro Asp Glu Glu Ala Asn Ala Thr Ser Ala Val Val
            200                 205                 210

tct ctg ttg aac gag act gtg act gag gtg ccg gag gag act aag atg      967
Ser Leu Leu Asn Glu Thr Val Thr Glu Val Pro Glu Glu Thr Lys Met
        215                 220                 225

gtt atc aag aag ggc ctg gag ttc aag gat ggg atg aac gtc tta ggt     1015
Val Ile Lys Lys Gly Leu Glu Phe Lys Asp Gly Met Asn Val Leu Gly
    230                 235                 240

ctg ata ggg ttt ttc att gct ttt ggc atc gct atg ggg aag atg gga     1063
Leu Ile Gly Phe Phe Ile Ala Phe Gly Ile Ala Met Gly Lys Met Gly
245                 250                 255                 260

gat cag gcc aag ctg atg gtg gat ttc ttc aac att ttg aat gag att     1111
Asp Gln Ala Lys Leu Met Val Asp Phe Phe Asn Ile Leu Asn Glu Ile
                265                 270                 275

gta atg aag tta gtg atc atg atc atg tgg tac tct ccc ctg ggt atc     1159
Val Met Lys Leu Val Ile Met Ile Met Trp Tyr Ser Pro Leu Gly Ile
            280                 285                 290

gcc tgc ctg atc tgt gga aag atc att gca atc aag gac tta gaa gtg     1207
Ala Cys Leu Ile Cys Gly Lys Ile Ile Ala Ile Lys Asp Leu Glu Val
        295                 300                 305

gtt gct agg caa ctg ggg atg tac atg gta aca gtg atc ata ggc ctc     1255
Val Ala Arg Gln Leu Gly Met Tyr Met Val Thr Val Ile Ile Gly Leu
    310                 315                 320

atc atc cac ggg ggc atc ttt ctc ccc ttg att tac ttt gta gtg acc     1303
Ile Ile His Gly Gly Ile Phe Leu Pro Leu Ile Tyr Phe Val Val Thr
325                 330                 335                 340

agg aaa aac ccc ttc tcc ttt ttt gct ggc att ttc caa gct tgg atc     1351
```

```
Arg Lys Asn Pro Phe Ser Phe Phe Ala Gly Ile Phe Gln Ala Trp Ile
                345                 350                 355

act gcc ctg ggc acc gct tcc agt gct gga act ttg cct gtc acc ttt     1399
Thr Ala Leu Gly Thr Ala Ser Ser Ala Gly Thr Leu Pro Val Thr Phe
            360                 365                 370

cgt tgc ctg gaa gaa aat ctg ggg att gat aag cgt gtg act aga ttc     1447
Arg Cys Leu Glu Glu Asn Leu Gly Ile Asp Lys Arg Val Thr Arg Phe
        375                 380                 385

gtc ctt cct gtt gga gca acc att aac atg gat ggt aca gcc ctt tat     1495
Val Leu Pro Val Gly Ala Thr Ile Asn Met Asp Gly Thr Ala Leu Tyr
    390                 395                 400

gaa gcg gtg gcc gcc atc ttt ata gcc caa atg aat ggt gtt gtc ctg     1543
Glu Ala Val Ala Ala Ile Phe Ile Ala Gln Met Asn Gly Val Val Leu
405                 410                 415                 420

gat gga gga cag att gtg act gta agc ctc aca gcc acc ctg gca agc     1591
Asp Gly Gly Gln Ile Val Thr Val Ser Leu Thr Ala Thr Leu Ala Ser
                425                 430                 435

gtc ggc gcg gcc agt atc ccc agt gcc ggg ctg gtc acc atg ctc ctc     1639
Val Gly Ala Ala Ser Ile Pro Ser Ala Gly Leu Val Thr Met Leu Leu
            440                 445                 450

att ctg aca gcc gtg ggc ctg cca aca gag gac atc agc ctg ctg gtg     1687
Ile Leu Thr Ala Val Gly Leu Pro Thr Glu Asp Ile Ser Leu Leu Val
        455                 460                 465

gct gtg gac tgg ctg ctg gac agg atg aga act tca gtc aat gtt gtg     1735
Ala Val Asp Trp Leu Leu Asp Arg Met Arg Thr Ser Val Asn Val Val
    470                 475                 480

ggt gac tct ttt ggg gct ggg ata gtc tat cac ctc tcc aag tct gag     1783
Gly Asp Ser Phe Gly Ala Gly Ile Val Tyr His Leu Ser Lys Ser Glu
485                 490                 495                 500

ctg gat acc att gac tcc cag cat cga gtg cat gaa gat att gaa atg     1831
Leu Asp Thr Ile Asp Ser Gln His Arg Val His Glu Asp Ile Glu Met
                505                 510                 515

acc aag act caa tcc att tat gat gac atg aag aac cac agg gaa agc     1879
Thr Lys Thr Gln Ser Ile Tyr Asp Asp Met Lys Asn His Arg Glu Ser
            520                 525                 530

aac tct aat caa tgt gtc tat gct gca cac aac tct gtc ata gta gat     1927
Asn Ser Asn Gln Cys Val Tyr Ala Ala His Asn Ser Val Ile Val Asp
        535                 540                 545

gaa tgc aag gta act ctg gca gcc aat gga aag tca gcc gac tgc agt     1975
Glu Cys Lys Val Thr Leu Ala Ala Asn Gly Lys Ser Ala Asp Cys Ser
    550                 555                 560

gtt gag gaa gaa cct tgg aaa cgt gag aaa taaggatatg agtctcagca       2025
Val Glu Glu Glu Pro Trp Lys Arg Glu Lys
565                 570

aattcttgaa taaactcccc agcgtatcct atggtaactg atggtataaa caagctttct   2085
```

ttaaaaagga aaaaaatgcg tatatttcta tgtttactta atctgttagc cgaggcttag 2145

aggagctctt ctgag 2160

<210> 2
<211> 574
<212> PRT
<213> Homo sapiens

<400> 2

Met Ala Ser Thr Glu Gly Ala Asn Asn Met Pro Lys Gln Val Glu Val
1 5 10 15

Arg Met His Asp Ser His Leu Gly Ser Glu Glu Pro Lys His Arg His
20 25 30

Leu Gly Leu Arg Leu Cys Asp Lys Leu Gly Lys Asn Leu Leu Leu Thr
35 40 45

Leu Thr Val Phe Gly Val Ile Leu Gly Ala Val Cys Gly Gly Leu Leu
50 55 60

Arg Leu Ala Ser Pro Ile His Pro Asp Val Val Met Leu Ile Ala Phe
65 70 75 80

Pro Gly Asp Ile Leu Met Arg Met Leu Lys Met Leu Ile Leu Pro Leu
85 90 95

Ile Ile Ser Ser Leu Ile Thr Gly Leu Ser Gly Leu Asp Ala Lys Ala
100 105 110

Ser Gly Arg Leu Gly Thr Arg Ala Met Val Tyr Tyr Met Ser Thr Thr
115 120 125

Ile Ile Ala Ala Val Leu Gly Val Ile Leu Val Leu Ala Ile His Pro
130 135 140

Gly Asn Pro Lys Leu Lys Lys Gln Leu Gly Pro Gly Lys Lys Asn Asp
145 150 155 160

Glu Val Ser Ser Leu Asp Ala Phe Leu Asp Leu Ile Arg Asn Leu Phe
165 170 175

Pro Glu Asn Leu Val Gln Ala Cys Phe Gln Gln Ile Gln Thr Val Thr
180 185 190

```
Lys Lys Val Leu Val Ala Pro Pro Pro Asp Glu Glu Ala Asn Ala Thr
        195                 200                 205

Ser Ala Val Val Ser Leu Leu Asn Glu Thr Val Thr Glu Val Pro Glu
    210                 215                 220

Glu Thr Lys Met Val Ile Lys Lys Gly Leu Glu Phe Lys Asp Gly Met
225                 230                 235                 240

Asn Val Leu Gly Leu Ile Gly Phe Phe Ile Ala Phe Gly Ile Ala Met
                245                 250                 255

Gly Lys Met Gly Asp Gln Ala Lys Leu Met Val Asp Phe Phe Asn Ile
            260                 265                 270

Leu Asn Glu Ile Val Met Lys Leu Val Ile Met Ile Met Trp Tyr Ser
        275                 280                 285

Pro Leu Gly Ile Ala Cys Leu Ile Cys Gly Lys Ile Ile Ala Ile Lys
    290                 295                 300

Asp Leu Glu Val Val Ala Arg Gln Leu Gly Met Tyr Met Val Thr Val
305                 310                 315                 320

Ile Ile Gly Leu Ile Ile His Gly Gly Ile Phe Leu Pro Leu Ile Tyr
                325                 330                 335

Phe Val Val Thr Arg Lys Asn Pro Phe Ser Phe Phe Ala Gly Ile Phe
            340                 345                 350

Gln Ala Trp Ile Thr Ala Leu Gly Thr Ala Ser Ser Ala Gly Thr Leu
        355                 360                 365

Pro Val Thr Phe Arg Cys Leu Glu Glu Asn Leu Gly Ile Asp Lys Arg
    370                 375                 380

Val Thr Arg Phe Val Leu Pro Val Gly Ala Thr Ile Asn Met Asp Gly
385                 390                 395                 400

Thr Ala Leu Tyr Glu Ala Val Ala Ala Ile Phe Ile Ala Gln Met Asn
                405                 410                 415

Gly Val Val Leu Asp Gly Gly Gln Ile Val Thr Val Ser Leu Thr Ala
            420                 425                 430
```

```
Thr Leu Ala Ser Val Gly Ala Ala Ser Ile Pro Ser Ala Gly Leu Val
        435                 440                 445

Thr Met Leu Leu Ile Leu Thr Ala Val Gly Leu Pro Thr Glu Asp Ile
    450                 455                 460

Ser Leu Leu Val Ala Val Asp Trp Leu Leu Asp Arg Met Arg Thr Ser
465                 470                 475                 480

Val Asn Val Val Gly Asp Ser Phe Gly Ala Gly Ile Val Tyr His Leu
                485                 490                 495

Ser Lys Ser Glu Leu Asp Thr Ile Asp Ser Gln His Arg Val His Glu
            500                 505                 510

Asp Ile Glu Met Thr Lys Thr Gln Ser Ile Tyr Asp Asp Met Lys Asn
        515                 520                 525

His Arg Glu Ser Asn Ser Asn Gln Cys Val Tyr Ala Ala His Asn Ser
    530                 535                 540

Val Ile Val Asp Glu Cys Lys Val Thr Leu Ala Ala Asn Gly Lys Ser
545                 550                 555                 560

Ala Asp Cys Ser Val Glu Glu Glu Pro Trp Lys Arg Glu Lys
                565                 570
```

<210> 3
<211> 2198
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (39)..(1712)

<400> 3

```
cagaagttgg aagccagtgc acttctacag ctgagaga atg gtc agt gcc aac aat     56
                                          Met Val Ser Ala Asn Asn
                                          1               5

atg ccc aag cag gta gaa gtg cgc atg cac gac agc cac ctc agc tcc      104
Met Pro Lys Gln Val Glu Val Arg Met His Asp Ser His Leu Ser Ser
            10                  15                  20

gat gag cca aag cac cga aac ctg ggc atg cgc atg tgc gac aag ctg      152
Asp Glu Pro Lys His Arg Asn Leu Gly Met Arg Met Cys Asp Lys Leu
        25                  30                  35
```

```
ggg aaa aat ctc ctg ctc tca ctg act gtg ttt ggt gtc atc ctg gga      200
Gly Lys Asn Leu Leu Leu Ser Leu Thr Val Phe Gly Val Ile Leu Gly
    40                  45                  50

gca gtg tgt ggc ggg ctg ctt cgc ttg gca tcg ccc atc cac cct gat      248
Ala Val Cys Gly Gly Leu Leu Arg Leu Ala Ser Pro Ile His Pro Asp
55                  60                  65                  70

gtg gtc atg ttg ata gcc ttc ccg ggg gac ata ctc atg agg atg ctg      296
Val Val Met Leu Ile Ala Phe Pro Gly Asp Ile Leu Met Arg Met Leu
                75                  80                  85

aag atg ctc atc ctc cct ctt atc atc tcc agt tta atc aca ggg ttg      344
Lys Met Leu Ile Leu Pro Leu Ile Ile Ser Ser Leu Ile Thr Gly Leu
            90                  95                  100

tca ggc ctg gat gct aaa gcc agc ggc cgc cta ggc acg aga gct atg      392
Ser Gly Leu Asp Ala Lys Ala Ser Gly Arg Leu Gly Thr Arg Ala Met
        105                 110                 115

gtg tat tac atg tcc acg acc atc att gcc gcc gtg ctg ggg gtc atc      440
Val Tyr Tyr Met Ser Thr Thr Ile Ile Ala Ala Val Leu Gly Val Ile
    120                 125                 130

ctg gtg ttg gcc atc cac cca ggc aat ccc aaa ctc aag aag cag cta      488
Leu Val Leu Ala Ile His Pro Gly Asn Pro Lys Leu Lys Lys Gln Leu
135                 140                 145                 150

ggg ccc ggg aag aag aac gac gag gtg tct agc ctg gat gcc ttc ctg      536
Gly Pro Gly Lys Lys Asn Asp Glu Val Ser Ser Leu Asp Ala Phe Leu
                155                 160                 165

gat ctc att aga aat ctc ttc ccg gag aac ctg gtg caa gcc tgt ttc      584
Asp Leu Ile Arg Asn Leu Phe Pro Glu Asn Leu Val Gln Ala Cys Phe
            170                 175                 180

cag cag att cag aca gtg aca aag aaa gtt ctg gtg gca cct cca tct      632
Gln Gln Ile Gln Thr Val Thr Lys Lys Val Leu Val Ala Pro Pro Ser
        185                 190                 195

gag gag gcc aat acc acc aag gcg gtc atc tcc atg ttg aat gaa acc      680
Glu Glu Ala Asn Thr Thr Lys Ala Val Ile Ser Met Leu Asn Glu Thr
    200                 205                 210

atg aac gag gcc cct gaa gaa act aag atc gtt atc aag aag ggc ctg      728
Met Asn Glu Ala Pro Glu Glu Thr Lys Ile Val Ile Lys Lys Gly Leu
215                 220                 225                 230

gag ttc aag gac ggg atg aat gtc tta ggt ctg atc gga ttc ttt att      776
Glu Phe Lys Asp Gly Met Asn Val Leu Gly Leu Ile Gly Phe Phe Ile
                235                 240                 245

gct ttc ggc att gcc atg ggg aag atg ggt gaa cag gcc aag ctg atg      824
Ala Phe Gly Ile Ala Met Gly Lys Met Gly Glu Gln Ala Lys Leu Met
            250                 255                 260

gtg gag ttc ttc aac att ctg aat gag atc gtg atg aag tta gtg atc      872
Val Glu Phe Phe Asn Ile Leu Asn Glu Ile Val Met Lys Leu Val Ile
        265                 270                 275
```

```
atg atc atg tgg tac tcc cct ctg ggt atc gcc tgc ttg att tgt ggg      920
Met Ile Met Trp Tyr Ser Pro Leu Gly Ile Ala Cys Leu Ile Cys Gly
    280                 285                 290

aag atc atc gcc atc aag gac tta gaa gtg gtt gct agg cag ctg ggg      968
Lys Ile Ile Ala Ile Lys Asp Leu Glu Val Val Ala Arg Gln Leu Gly
295                 300                 305                 310

atg tac atg atc acc gtg atc gtg ggc ctc atc att cac ggg ggc atc     1016
Met Tyr Met Ile Thr Val Ile Val Gly Leu Ile Ile His Gly Gly Ile
                315                 320                 325

ttt ctc ccc ttg att tac ttt gta gtg acc aga aaa aat cca ttc tcc     1064
Phe Leu Pro Leu Ile Tyr Phe Val Val Thr Arg Lys Asn Pro Phe Ser
            330                 335                 340

ttt ttt gct ggc ata ttc caa gcc tgg atc act gct ctg gga act gct     1112
Phe Phe Ala Gly Ile Phe Gln Ala Trp Ile Thr Ala Leu Gly Thr Ala
        345                 350                 355

tcc agt gct gga act ttg cct gtt acc ttc cgt tgc ttg gaa gat aat     1160
Ser Ser Ala Gly Thr Leu Pro Val Thr Phe Arg Cys Leu Glu Asp Asn
    360                 365                 370

cta ggg att gac aag cgt gtg acc aga ttc gtc ctc cca gtc gga gca     1208
Leu Gly Ile Asp Lys Arg Val Thr Arg Phe Val Leu Pro Val Gly Ala
375                 380                 385                 390

acc att aac atg gat ggc aca gcc ctt tac gag gct gtg gca gcc att     1256
Thr Ile Asn Met Asp Gly Thr Ala Leu Tyr Glu Ala Val Ala Ala Ile
                395                 400                 405

ttc ata gcc caa atg aat ggg gtc atc ttg gat gga ggt cag att gtg     1304
Phe Ile Ala Gln Met Asn Gly Val Ile Leu Asp Gly Gly Gln Ile Val
            410                 415                 420

act gta agc ctt aca gcc acc ctg gca agc att ggt gca gcc agt att     1352
Thr Val Ser Leu Thr Ala Thr Leu Ala Ser Ile Gly Ala Ala Ser Ile
        425                 430                 435

cca agc gcc ggg ctg gtc acc atg ctc ctc att ctc aca gct gtg ggc     1400
Pro Ser Ala Gly Leu Val Thr Met Leu Leu Ile Leu Thr Ala Val Gly
    440                 445                 450

ctg cca acg gag gat atc agt ctg ctg gtg gcg gtg gac tgg ctg ctg     1448
Leu Pro Thr Glu Asp Ile Ser Leu Leu Val Ala Val Asp Trp Leu Leu
455                 460                 465                 470

gat aga atg aga act tca gtc aat gtg gtg ggc gat tct ttt ggg gct     1496
Asp Arg Met Arg Thr Ser Val Asn Val Val Gly Asp Ser Phe Gly Ala
                475                 480                 485

ggg att gtc tat cac ctt tcc aag tct gag ctg gac acc att gac tcc     1544
Gly Ile Val Tyr His Leu Ser Lys Ser Glu Leu Asp Thr Ile Asp Ser
            490                 495                 500

caa cac cga atg cag gaa gac atc gaa atg acc aag acg cag tcc att     1592
Gln His Arg Met Gln Glu Asp Ile Glu Met Thr Lys Thr Gln Ser Ile
        505                 510                 515
```

```
tac gac gac aag aac cac agg gaa agc aac tct aat cag tgt gtc tat     1640
Tyr Asp Asp Lys Asn His Arg Glu Ser Asn Ser Asn Gln Cys Val Tyr
    520                 525                 530

gcc gca cac aac tct gtc gta ata gat gag tgc aag gta cct ttc cca     1688
Ala Ala His Asn Ser Val Val Ile Asp Glu Cys Lys Val Pro Phe Pro
535                 540                 545                 550

ttc ctg gat atc gag acc tgc ata tgaatagtgc atgctggact ccttaaaaaa    1742
Phe Leu Asp Ile Glu Thr Cys Ile
                555

aaatcacaac cccttgcatc cattcttacc atgctttgta aggaatcatg tcacccaagc   1802

tcctgtgggg cccagaccat ctcactctgc atccagcatg gtgggacatc agcaagctga   1862

caccaaaagg accaatcagt tgcttgcttc aacagattcc cacatccacc tcacccctct   1922

aatttggtca atattcttca ctctttgggc caagaattgg tggttgtcag aactttaatg   1982

gccttcagat attctcttcc tcaaaggaag ggtcagaaaa atgtagacca aatattattg   2042

actttatata gcatgagtat tccgcgctgc gctgtcttac aaattgcttt ttaaaagctc   2102

aaataaaagt ttaattggct gtcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa   2162

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa                             2198
```

<210> 4
<211> 558
<212> PRT
<213> Mus musculus

<400> 4

```
Met Val Ser Ala Asn Asn Met Pro Lys Gln Val Glu Val Arg Met His
1               5                   10                  15

Asp Ser His Leu Ser Ser Asp Glu Pro Lys His Arg Asn Leu Gly Met
            20                  25                  30

Arg Met Cys Asp Lys Leu Gly Lys Asn Leu Leu Leu Ser Leu Thr Val
        35                  40                  45

Phe Gly Val Ile Leu Gly Ala Val Cys Gly Gly Leu Leu Arg Leu Ala
    50                  55                  60

Ser Pro Ile His Pro Asp Val Val Met Leu Ile Ala Phe Pro Gly Asp
65                  70                  75                  80

Ile Leu Met Arg Met Leu Lys Met Leu Ile Leu Pro Leu Ile Ile Ser
                85                  90                  95
```

```
Ser Leu Ile Thr Gly Leu Ser Gly Leu Asp Ala Lys Ala Ser Gly Arg
            100                 105                 110

Leu Gly Thr Arg Ala Met Val Tyr Tyr Met Ser Thr Thr Ile Ile Ala
        115                 120                 125

Ala Val Leu Gly Val Ile Leu Val Leu Ala Ile His Pro Gly Asn Pro
    130                 135                 140

Lys Leu Lys Lys Gln Leu Gly Pro Gly Lys Lys Asn Asp Glu Val Ser
145                 150                 155                 160

Ser Leu Asp Ala Phe Leu Asp Leu Ile Arg Asn Leu Phe Pro Glu Asn
                165                 170                 175

Leu Val Gln Ala Cys Phe Gln Gln Ile Gln Thr Val Thr Lys Lys Val
            180                 185                 190

Leu Val Ala Pro Pro Ser Glu Glu Ala Asn Thr Thr Lys Ala Val Ile
        195                 200                 205

Ser Met Leu Asn Glu Thr Met Asn Glu Ala Pro Glu Glu Thr Lys Ile
    210                 215                 220

Val Ile Lys Lys Gly Leu Glu Phe Lys Asp Gly Met Asn Val Leu Gly
225                 230                 235                 240

Leu Ile Gly Phe Phe Ile Ala Phe Gly Ile Ala Met Gly Lys Met Gly
                245                 250                 255

Glu Gln Ala Lys Leu Met Val Glu Phe Phe Asn Ile Leu Asn Glu Ile
            260                 265                 270

Val Met Lys Leu Val Ile Met Ile Met Trp Tyr Ser Pro Leu Gly Ile
        275                 280                 285

Ala Cys Leu Ile Cys Gly Lys Ile Ile Ala Ile Lys Asp Leu Glu Val
    290                 295                 300

Val Ala Arg Gln Leu Gly Met Tyr Met Ile Thr Val Ile Val Gly Leu
305                 310                 315                 320

Ile Ile His Gly Gly Ile Phe Leu Pro Leu Ile Tyr Phe Val Val Thr
                325                 330                 335
```

```
Arg Lys Asn Pro Phe Ser Phe Phe Ala Gly Ile Phe Gln Ala Trp Ile
            340                 345                 350

Thr Ala Leu Gly Thr Ala Ser Ser Ala Gly Thr Leu Pro Val Thr Phe
        355                 360                 365

Arg Cys Leu Glu Asp Asn Leu Gly Ile Asp Lys Arg Val Thr Arg Phe
    370                 375                 380

Val Leu Pro Val Gly Ala Thr Ile Asn Met Asp Gly Thr Ala Leu Tyr
385                 390                 395                 400

Glu Ala Val Ala Ala Ile Phe Ile Ala Gln Met Asn Gly Val Ile Leu
                405                 410                 415

Asp Gly Gly Gln Ile Val Thr Val Ser Leu Thr Ala Thr Leu Ala Ser
            420                 425                 430

Ile Gly Ala Ala Ser Ile Pro Ser Ala Gly Leu Val Thr Met Leu Leu
        435                 440                 445

Ile Leu Thr Ala Val Gly Leu Pro Thr Glu Asp Ile Ser Leu Leu Val
    450                 455                 460

Ala Val Asp Trp Leu Leu Asp Arg Met Arg Thr Ser Val Asn Val Val
465                 470                 475                 480

Gly Asp Ser Phe Gly Ala Gly Ile Val Tyr His Leu Ser Lys Ser Glu
                485                 490                 495

Leu Asp Thr Ile Asp Ser Gln His Arg Met Gln Glu Asp Ile Glu Met
            500                 505                 510

Thr Lys Thr Gln Ser Ile Tyr Asp Asp Lys Asn His Arg Glu Ser Asn
        515                 520                 525

Ser Asn Gln Cys Val Tyr Ala Ala His Asn Ser Val Val Ile Asp Glu
    530                 535                 540

Cys Lys Val Pro Phe Pro Phe Leu Asp Ile Glu Thr Cys Ile
545                 550                 555
```

<210> 5
<211> 1898
<212> DNA
<213> Rattus norvegicus

<220>
<221> CDS
<222> (99)..(1817)

<400> 5
cctgcccgtt aaataccgct ccccgccgca ctccgggctc acccagctcg tcgccactgt 60

ctccagacgt gcccgggagg aggggcgttc cccacgcc atg gca tca acc gag ggt 116
Met Ala Ser Thr Glu Gly
1 5

gcc aac aat atg ccc aag cag gtg gaa gtg cgc atg cac gac agc cac 164
Ala Asn Asn Met Pro Lys Gln Val Glu Val Arg Met His Asp Ser His
10 15 20

ctc agc tcc gag gag cca aag cac cga aac ctg ggc atg cgc atg tgc 212
Leu Ser Ser Glu Glu Pro Lys His Arg Asn Leu Gly Met Arg Met Cys
25 30 35

gac aag ctg ggg aag aac ctc ctg ctc tca ctg act gtg ttt ggt gtc 260
Asp Lys Leu Gly Lys Asn Leu Leu Leu Ser Leu Thr Val Phe Gly Val
40 45 50

atc ctg gga gca gta tgt ggc ggg ctg ctt cgc ttg gcg gct ccc atc 308
Ile Leu Gly Ala Val Cys Gly Gly Leu Leu Arg Leu Ala Ala Pro Ile
55 60 65 70

cac cct gat gtg gtc atg ttg ata gcc ttc ccg ggg gac atc ctc atg 356
His Pro Asp Val Val Met Leu Ile Ala Phe Pro Gly Asp Ile Leu Met
75 80 85

agg atg ctg aag atg ctc atc ctc cct ctc atc att tcc agt ctc atc 404
Arg Met Leu Lys Met Leu Ile Leu Pro Leu Ile Ile Ser Ser Leu Ile
90 95 100

aca ggg ctg tca ggg ctg gat gct aaa gcc agc ggc cgc cta ggc acg 452
Thr Gly Leu Ser Gly Leu Asp Ala Lys Ala Ser Gly Arg Leu Gly Thr
105 110 115

aga gcc atg gta tat tac atg tcc acg acc atc att gcc gct gtg ctg 500
Arg Ala Met Val Tyr Tyr Met Ser Thr Thr Ile Ile Ala Ala Val Leu
120 125 130

ggg gtc atc ctg gtg ttg gcc atc cac cct ggg aat ccc aaa ctc aag 548
Gly Val Ile Leu Val Leu Ala Ile His Pro Gly Asn Pro Lys Leu Lys
135 140 145 150

aag cag ctg ggg cct ggg aag aag aac gac gag gtg tcc agc ctg gat 596
Lys Gln Leu Gly Pro Gly Lys Lys Asn Asp Glu Val Ser Ser Leu Asp
155 160 165

gcc ttc ctg gat ctc att aga aat ctc ttc ccg gag aac ctg gta caa 644
Ala Phe Leu Asp Leu Ile Arg Asn Leu Phe Pro Glu Asn Leu Val Gln
170 175 180

gcc tgt ttc caa cag att cag act gtg aca aag aaa gtt ctg gtg gca 692
Ala Cys Phe Gln Gln Ile Gln Thr Val Thr Lys Lys Val Leu Val Ala
185 190 195

```
cct cca tcc gag gag gcc aat aca acc aag gca gtc atc tcc ctg ttg      740
Pro Pro Ser Glu Glu Ala Asn Thr Thr Lys Ala Val Ile Ser Leu Leu
    200                 205                 210

aat gag acc atg aat gag gcc cct gaa gaa act aag atc gtt atc aag      788
Asn Glu Thr Met Asn Glu Ala Pro Glu Glu Thr Lys Ile Val Ile Lys
215                 220                 225                 230

aag ggc ctg gag ttc aag gac ggg atg aat gtc tta ggt ctg att gga      836
Lys Gly Leu Glu Phe Lys Asp Gly Met Asn Val Leu Gly Leu Ile Gly
                235                 240                 245

ttc ttt att gct ttc ggc att gcc atg ggg aag atg ggt gta gca ggc      884
Phe Phe Ile Ala Phe Gly Ile Ala Met Gly Lys Met Gly Val Ala Gly
            250                 255                 260

caa gct gat ggt gga gtt ctt caa cat tct gaa cga gat tgt cat gaa      932
Gln Ala Asp Gly Gly Val Leu Gln His Ser Glu Arg Asp Cys His Glu
        265                 270                 275

gtt agt gat cat gat cat gtg gta ttc ccc gct ggt atc gcc tgc ttg      980
Val Ser Asp His Asp His Val Val Phe Pro Ala Gly Ile Ala Cys Leu
    280                 285                 290

atc tgt ggg aag atc atc gcc atc aag gac tta gaa gtg gtt gct agg     1028
Ile Cys Gly Lys Ile Ile Ala Ile Lys Asp Leu Glu Val Val Ala Arg
295                 300                 305                 310

cag ctg ggg atg tac atg atc aca gtg atc gtg ggc ctc atc att cat     1076
Gln Leu Gly Met Tyr Met Ile Thr Val Ile Val Gly Leu Ile Ile His
                315                 320                 325

ggg ggc atc ttt ctc ccc ttg att tac ttt gta gtg acc aga aaa aac     1124
Gly Gly Ile Phe Leu Pro Leu Ile Tyr Phe Val Val Thr Arg Lys Asn
            330                 335                 340

cca ttc tcc ttt ttt gct ggc att ttc caa gcc tgg atc act gcc ctg     1172
Pro Phe Ser Phe Phe Ala Gly Ile Phe Gln Ala Trp Ile Thr Ala Leu
        345                 350                 355

gga acc gct tcc agt gct gga act ttg cct gtc acc ttc cgt tgc ttg     1220
Gly Thr Ala Ser Ser Ala Gly Thr Leu Pro Val Thr Phe Arg Cys Leu
    360                 365                 370

gaa gat aat cta ggg att gac aag cgt gtg acc aga ttc gtc ctc cca     1268
Glu Asp Asn Leu Gly Ile Asp Lys Arg Val Thr Arg Phe Val Leu Pro
375                 380                 385                 390

gtc gga gca acc att aac atg gat ggt aca gcc ctt tac gaa gcc gtg     1316
Val Gly Ala Thr Ile Asn Met Asp Gly Thr Ala Leu Tyr Glu Ala Val
                395                 400                 405

gca gcc atc ttc ata gcc cag atg aat ggg gtc atc ctg gat gga ggt     1364
Ala Ala Ile Phe Ile Ala Gln Met Asn Gly Val Ile Leu Asp Gly Gly
            410                 415                 420

cag ata gtg act gta agc ctt aca gca act ctg gcg agc att ggt gca     1412
Gln Ile Val Thr Val Ser Leu Thr Ala Thr Leu Ala Ser Ile Gly Ala
        425                 430                 435
```

```
gcc agt att ccc agc gcc ggg ttg gtc acc atg ctc ctc att ctc aca     1460
Ala Ser Ile Pro Ser Ala Gly Leu Val Thr Met Leu Leu Ile Leu Thr
    440                 445                 450

gct gtg ggc ctg ccg aca gag gac atc agt ctg ctg gtg gca gtg gac     1508
Ala Val Gly Leu Pro Thr Glu Asp Ile Ser Leu Leu Val Ala Val Asp
455                 460                 465                 470

tgg ctg ctg gat aga atg aga act tcg gtc aat gta gtg ggc gat tct     1556
Trp Leu Leu Asp Arg Met Arg Thr Ser Val Asn Val Val Gly Asp Ser
                475                 480                 485

ttt ggg gct ggg att gtc tat cac ctt tcc aag tcc gag ctg gac acc     1604
Phe Gly Ala Gly Ile Val Tyr His Leu Ser Lys Ser Glu Leu Asp Thr
            490                 495                 500

att gac tcc caa cac cga atg cac gaa gac atc gaa atg acc aag acg     1652
Ile Asp Ser Gln His Arg Met His Glu Asp Ile Glu Met Thr Lys Thr
        505                 510                 515

cag tcc gtt tat gac gac acg aag aac cac agg gaa agc aac tct aat     1700
Gln Ser Val Tyr Asp Asp Thr Lys Asn His Arg Glu Ser Asn Ser Asn
    520                 525                 530

cag tgt gtc tat gcc gca cac aac tct gtc gta ata gat gag tgc aag     1748
Gln Cys Val Tyr Ala Ala His Asn Ser Val Val Ile Asp Glu Cys Lys
535                 540                 545                 550

gta act ctg gcg gcc aat gga aag tca gct gac tgc agt gtt gag gaa     1796
Val Thr Leu Ala Ala Asn Gly Lys Ser Ala Asp Cys Ser Val Glu Glu
                555                 560                 565

gaa cct tgg aaa cgt gaa aaa taatgacccg attctcagcc aattcttgaa        1847
Glu Pro Trp Lys Arg Glu Lys
            570

taactcccca gcgtatctta tggtaaaaga tgctctaaac aggcttcctt t            1898


<210>  6
<211>  573
<212>  PRT
<213>  Rattus norvegicus

<400>  6

Met Ala Ser Thr Glu Gly Ala Asn Asn Met Pro Lys Gln Val Glu Val
1               5                   10                  15


Arg Met His Asp Ser His Leu Ser Ser Glu Glu Pro Lys His Arg Asn
            20                  25                  30


Leu Gly Met Arg Met Cys Asp Lys Leu Gly Lys Asn Leu Leu Leu Ser
        35                  40                  45


Leu Thr Val Phe Gly Val Ile Leu Gly Ala Val Cys Gly Gly Leu Leu
```

```
    50                  55                  60

Arg Leu Ala Ala Pro Ile His Pro Asp Val Val Met Leu Ile Ala Phe
65                  70                  75                  80

Pro Gly Asp Ile Leu Met Arg Met Leu Lys Met Leu Ile Leu Pro Leu
                85                  90                  95

Ile Ile Ser Ser Leu Ile Thr Gly Leu Ser Gly Leu Asp Ala Lys Ala
            100                 105                 110

Ser Gly Arg Leu Gly Thr Arg Ala Met Val Tyr Tyr Met Ser Thr Thr
        115                 120                 125

Ile Ile Ala Ala Val Leu Gly Val Ile Leu Val Leu Ala Ile His Pro
    130                 135                 140

Gly Asn Pro Lys Leu Lys Lys Gln Leu Gly Pro Gly Lys Lys Asn Asp
145                 150                 155                 160

Glu Val Ser Ser Leu Asp Ala Phe Leu Asp Leu Ile Arg Asn Leu Phe
                165                 170                 175

Pro Glu Asn Leu Val Gln Ala Cys Phe Gln Gln Ile Gln Thr Val Thr
            180                 185                 190

Lys Lys Val Leu Val Ala Pro Pro Ser Glu Glu Ala Asn Thr Thr Lys
        195                 200                 205

Ala Val Ile Ser Leu Leu Asn Glu Thr Met Asn Glu Ala Pro Glu Glu
    210                 215                 220

Thr Lys Ile Val Ile Lys Lys Gly Leu Glu Phe Lys Asp Gly Met Asn
225                 230                 235                 240

Val Leu Gly Leu Ile Gly Phe Phe Ile Ala Phe Gly Ile Ala Met Gly
                245                 250                 255

Lys Met Gly Val Ala Gly Gln Ala Asp Gly Gly Val Leu Gln His Ser
            260                 265                 270

Glu Arg Asp Cys His Glu Val Ser Asp His Asp His Val Val Phe Pro
        275                 280                 285

Ala Gly Ile Ala Cys Leu Ile Cys Gly Lys Ile Ile Ala Ile Lys Asp
```

290 295 300

Leu Glu Val Val Ala Arg Gln Leu Gly Met Tyr Met Ile Thr Val Ile
305 310 315 320

Val Gly Leu Ile Ile His Gly Gly Ile Phe Leu Pro Leu Ile Tyr Phe
325 330 335

Val Val Thr Arg Lys Asn Pro Phe Ser Phe Phe Ala Gly Ile Phe Gln
340 345 350

Ala Trp Ile Thr Ala Leu Gly Thr Ala Ser Ser Ala Gly Thr Leu Pro
355 360 365

Val Thr Phe Arg Cys Leu Glu Asp Asn Leu Gly Ile Asp Lys Arg Val
370 375 380

Thr Arg Phe Val Leu Pro Val Gly Ala Thr Ile Asn Met Asp Gly Thr
385 390 395 400

Ala Leu Tyr Glu Ala Val Ala Ala Ile Phe Ile Ala Gln Met Asn Gly
405 410 415

Val Ile Leu Asp Gly Gly Gln Ile Val Thr Val Ser Leu Thr Ala Thr
420 425 430

Leu Ala Ser Ile Gly Ala Ala Ser Ile Pro Ser Ala Gly Leu Val Thr
435 440 445

Met Leu Leu Ile Leu Thr Ala Val Gly Leu Pro Thr Glu Asp Ile Ser
450 455 460

Leu Leu Val Ala Val Asp Trp Leu Leu Asp Arg Met Arg Thr Ser Val
465 470 475 480

Asn Val Val Gly Asp Ser Phe Gly Ala Gly Ile Val Tyr His Leu Ser
485 490 495

Lys Ser Glu Leu Asp Thr Ile Asp Ser Gln His Arg Met His Glu Asp
500 505 510

Ile Glu Met Thr Lys Thr Gln Ser Val Tyr Asp Asp Thr Lys Asn His
515 520 525

Arg Glu Ser Asn Ser Asn Gln Cys Val Tyr Ala Ala His Asn Ser Val

```
    530                 535                 540

Val Ile Asp Glu Cys Lys Val Thr Leu Ala Ala Asn Gly Lys Ser Ala
545                 550                 555                 560

Asp Cys Ser Val Glu Glu Glu Pro Trp Lys Arg Glu Lys
                565                 570
```