A method for the treatment or prevention of Restless Legs Syndrome (RLS) by administering clavulanic acid, or a pharmaceutically acceptable salt, ester or prodrug thereof.
CLAVULANIC ACID FOR TREATMENT OF RESTLESS LEGS SYNDROME

FIELD OF THE INVENTION

[0001] The invention relates to the use of clavulanic acid, or a pharmaceutically acceptable salt, ester or prodrug thereof for the treatment or prevention of Restless Legs Syndrome (RLS).

BACKGROUND OF THE INVENTION

[0002] Interestingly, it was recently reported that all antidepressant treatments increase dopamine in the frontal cortex as well as other areas of the brain through either direct or indirect mechanisms [12]. Lavergne, et al. [12] performed an extensive literature search of both chemical and non-chemical antidepressant treatments and found that all antidepressant treatments increase dopamine release in the prefrontal cortex consistently as well as other areas such as the limbic system, nucleus accumbens, striatum and other cortical regions. Therefore, enhancement of dopamine levels has been found to be is associated with antidepressant treatment.

[0003] Advances and scientific progress in psychiatric treatment for mood disorders, such as Major Depressive Disorder (MDD), have shown limitations. MDD affects an estimated 16% of the population [8]. Both genetic and non-genetic factors, such as trauma and stress, can contribute to depression [13]. The focal point of depression research has been directed towards the monoamine hypothesis, which entails the imbalance or deficiency of monoamine neurotransmitters (e.g., dopamine, serotonin, norepinephrine). Although the true cause of depression remains unknown, the introduction of monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs) has supported the monoamine hypothesis. These agents work by enhancing monoamine function [13,14]. The development of selective serotonin reuptake inhibitors (SSRIs) as functional antidepressants has added additional support to the monoamine hypothesis. Nevertheless, current treatments for depression are only effective in less than 50% of patients, indicating a discrepancy in the current knowledge of depression etiology and treatment [16]. Furthermore, antidepressants that alter monoamines have delayed therapeutic benefits, require chronic treatment and often have a variety of undesirable side effects. Therefore, the
molecular mechanism of depression may be far more complex and involve multiple
signaling pathways regulating neurotransmission.

[0004] Restless Legs Syndrome (RLS or Willis-Ekbom disease) is a sensorimotor
disorder in which an individual suffering from RLS has an unpleasant sensation in the legs at
rest (or during inactivity), causing what is often described as an irresistible desire to move,
and movement generally alleviates the discomfort. It is also considered a Parkinson like
neuronal disorder. In addition, individuals afflicted with RLS experience crawling sensations
in their legs that often occur at night and that are only relieved by moving the legs. RLS was
first described in 1945, with an estimated prevalence of 5%. More recent studies have
suggested prevalence rates of between 3% and 15%, although prevalence may be as high as
24% in certain patient groups. Many sufferers go undiagnosed and untreated, although the
introduction of standardized criteria for diagnosis of RLS (updated in 2003) has improved
this situation.

[0005] The clinical criteria for diagnosis, approved by the International Restless Legs
Syndrome Study Group, include sleep disturbance, involuntary movements in sleep or
wakefulness, a normal neurologic examination, a chronic clinical course, and, in some cases,
a positive family history. Clinically, RLS is indicated when the following four minimal
criteria for diagnosis are met: (1) desire to move the extremities, often associated with
paresthesias/dysesthesias; (2) motor restlessness to reduce sensations; (3) worsening of
symptoms at rest, or during inactivity, with at least temporary relief by activity, and (4)
worsening of symptoms in the evening or night. The related disorders share some of these
characteristics.

[0006] RLS may be subdivided into primary (idiopathic) and secondary RLS. Secondary
RLS is commonly associated with metabolic disorders or conditions that result in iron-
deficiency anemia, and may be treated by iron replacement therapy. Primary RLS is a
heterogeneous disease with multiple potential causes, but recent studies have suggested that
an underlying defect in dopaminergic function may be common to most forms of primary
RLS. In particular, there is evidence for a decrease in the number or affinity of dopamine D2
receptors in the striatum of RLS patients. The underlying cause of RLS and its related
disorders is not clearly known, but it has been observed that the frequency of occurrence
increases with age. In most individuals with RLS, diagnostic results of complete blood cell
counts and iron, ferritin, folate, and vitamin B12 levels do not indicate hematologic or
chemical abnormalities compared with those who do not have RLS. An evaluation of the
efficacy of certain drugs revealed that the dopaminergic, adrenergic and opiate systems may play a role in the pathogenesis of RLS.

[0007] Current treatments for RLS are limited and only five FDA-approved medications are available (the dopamine agonists ropinirole, pramipexole, pergolide and carbidopa/levodopa, and the GABA analog gabapentin enacarbil). Other treatment options commonly employed include the use of benzodiazepines (e.g., clonazepam), narcotics, clonidine and magnesium. However, existing dopaminergic therapies, the most commonly used agents, are often only partially effective, and subject to unpleasant side effects. For example, notable undesirable side effects include rebound (the return of symptoms as the medication wears off) and augmentation (a worsening of symptoms following initiation of therapy). Rebound is a particular problem with medications with a short plasma half-life, e.g., levidopa, and may occur in over 35% of patients. Augmentation is a more serious side effect, and is estimated to occur in 50-85% of patients taking levidopa, and 30-32% of patients using dopamine agonists. Benzodiazepines, opiates and anti-convulsants are not as uniformly effective as the dopamine agents, and have undesirable side effects including tolerance, dependency and GI disturbances. Pramipexole, another popular therapeutic agent, has been reported to cause major side effects including insomnia, dizziness, constipation, asthenia and hallucinations.

[0008] Therefore, there exists a need for an effective, alternative treatment and related treatment regimen options for individuals who are afflicted with RLS and/or its related disorders. More particularly, there exists a need for treatments that do not induce the unwanted effects observed in modern therapeutics for Restless Legs Syndrome (RLS) and related disorders.

SUMMARY OF THE INVENTION

[0009] This invention provides methods for the treatment or prevention of Restless Legs Syndrome (RLS) or its related disorders in a patient suffering from RLS or its related disorders with clavulanic acid and the pharmaceutically acceptable salts thereof or prodrug thereof.

[0010] Clavulanic acid increases the release of dopamine from neuronal cells in depolarizing conditions, so that clavulanic acid can provide an effective treatment or prevention for conditions such as Restless Legs Syndrome (RLS) and its related disorders, as well as other conditions potentially treatable by increasing dopamine release. In view of the
known safety profile of clavulanic acid and its freedom from side effects, it presents a new and useful modality for the treatment of Restless Legs Syndrome.

The invention is a method of treating or preventing Restless Legs Syndrome in a subject in need of treatment by administering a therapeutically or prophylactically effective amount of clavulanic acid, or a pharmaceutically acceptable salt, ester or prodrug. The amount administered is capable of enhancing dopamine neurosecretion in an amount effective to treat Restless Legs Syndrome. According to the invention, the clavulanic acid binds to or is capable of binding to Munc18-1 or Rab4, for example, in an amount effective to promote translocation of Munc18-1 or Rab4 from the cytoplasm to the plasma membrane. The invention is also a method of enhancing dopamine neurosecretion in a subject by administering a therapeutically or prophylactically effective amount of a clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, capable of enhancing dopamine release. In methods of the invention, the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is administered at a dose of about 1 µg/day to about 150 mg/day or at a dose of about 10 mg/day to about 20 mg/day. For example, the clavulanic acid can be administered in a dosage unit form containing an amount to deliver about 0.016 µg/kg/day to about 2 mg/kg/day.

In methods of the invention, the clavulanic acid can be administered in the form of clavulanic acid or a salt of clavulanic acid, such as sodium clavulanate or potassium clavulanate. Alternatively, the clavulanic acid can be administered as a clavulanate ester. The clavulanic acid can be administered once per day or in multiple doses each day.

The invention is also a pharmaceutical composition in oral dosage form, that includes clavulanic acid, or a pharmaceutically acceptable salt, ester or prodrug thereof, in an amount effective for the treatment of Restless Legs Syndrome. The pharmaceutical composition can include, for example, from about 0.5 µg to about 150 mg, from about 1.0 µg to about 75 mg, or from about 5 mg to about 20 mg of clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof. The pharmaceutical composition can include, as a source of clavulanic acid, clavulanic acid itself, a salt of clavulanic acid, such as sodium clavulanate or potassium clavulanate, or a clavulanate ester.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the increase of dopamine level in differentiated PC12 cells (1A) and differentiated SH-SY5Y cells (1C) after treatment with clavulanic acid. Tyrosine
hydroxylase expression in PC12 cells (IB) and upon treatment with clavulanic acid for 6 and 12 hours was also measured. (A) PC12 cells were treated with 100 µM clavulanic acid or vehicle control. Dopamine release and total dopamine levels were measured by ELISA at 6 and 12 h post treatment. (B) Tyrosine hydroxylase expression in PC12 cells upon treatment with clavulanic acid for 6 and 12 h was measured. (C) Differentiated SH-SY5Y cells were treated with 100 µM clavulanic acid or vehicle control. Dopamine release and total dopamine levels were measured by ELISA after 6 and 12 h post treatment. (D) Tyrosine hydroxylase expression in differentiated SH-SY5Y upon treatment with clavulanic acid for 6 and 12 h was measured. Values were expressed as mean ± S.D and * indicates values that are significantly different from control (p<0.05).

Figure 2 shows the binding of Clavulanic acid to Muncl 8-1 and Rab4. Clavulanic acid conjugated and immobilized onto affinity gel was incubated with rat brain homogenate. Proteins bound to the clavulanic acid conjugated gel were eluted and analyzed by gel electrophoresis and mass spectrometry. Muncl 8-1 and Rab4 were identified as a candidate protein that were present in the elution pool and was verified by western blotting. Brain homogenate alone, proteins eluted from the clavulanic acid conjugated to the affinity gel and proteins eluted from affinity gel alone (primary amine was protected with acetyl group, no clavulanic acid) were run on SDS/PAGE gel. Gels were stained to verify protein loading and then Western blot analysis was performed: 1st lane: brain homogenate (BH) alone probed for the presence of either Muncl 8-1 or Rab4; 2nd lane (-): control (BH elution pool of resin alone probed for either Muncl 8-1 or Rab4); 3rd lane (+): indicates the presence of either Muncl 8-1 or Rab4 from the eluted proteins collected from clavulanic acid conjugated gel.

Figure 3 shows that Clavulanic acid alters the subcellular localization of Rab4 in SH-SY5Y cells without (A) or with (B) 100 µM clavulanic acid for 1 hour. SH-SY5Y cells were treated in the absence (A) or presence (B) of 100 µM clavulanic acid for 1 h. Cells were fixed and stained with either Muncl 8-1 or Rab4 antibody followed by Rhodamine conjugated secondary antibodies. Cells were then visualized at 60X magnification using Zeiss confocal microscope. Corresponding light microscopy pictures were taken as well. White arrows indicate areas of altered Muncl 8-1 or Rab4 localization (to the plasma membrane). Similarly, cells were also treated with 10 µM simvastatin to inhibit protein prenylation, in the presence of clavulanic acid (C) and stained with Rab4. Images presented here are representative of four independent experiments.
This invention relates to the use of clavulanic acid for the effective treatment or prevention of the Restless Legs Syndrome.

Terms used herein have their normal meaning as would be understood by persons skilled in the art. By way of example, and not to contradict or alter the generally accepted meanings, certain terms are defined below for clarity.

As used herein, unless indicated otherwise by context, the terms "clavulanic acid" and "clavulanate" includes clavulanic acid per se (I), pharmaceutically acceptable clavulanic acid salts, salt compositions, prodrugs and derivatives, such as esters, in particular esters of clavulanic acid that hydrolyze in vivo to form the acid. Acceptable esters include those commonly used in the art. Examples of pharmaceutically acceptable clavulanic acid salts include alkaline metal salts, such as sodium clavulanate and potassium clavulanate, as well as organic salts such as amine salts. Potassium clavulanate may be supplied as a pure compound or as Clavitesse™, a 1:1 mixture of potassium clavulanate and microcrystalline cellulose or a 1:1 mixture of potassium clavulanate and silicon dioxide (available from DSM Anti-Infectives B.V., The Netherlands).

The term "oral administration" as used herein includes any form of delivery of a therapeutic agent or a composition thereof to a subject wherein the agent or composition is placed in the mouth of the subject, whether or not the agent or composition is swallowed. Thus "oral administration" includes buccal and sublingual as well as esophageal administration. Absorption of the agent can occur in any part or parts of the gastrointestinal tract including the mouth, esophagus, stomach, duodenum, ileum and colon.

As used herein, a "subject" to which a therapeutic agent or composition thereof can be administered includes a human patient of either sex and of any age, and also includes any nonhuman animal, particularly a domestic or companion animal, illustratively a cat, dog or horse.
The term "excipient" as used herein means any substance, not itself a therapeutic agent, used as a carrier or vehicle for delivery of a therapeutic agent to a subject or added to a pharmaceutical composition to improve its processing, handling, storage, disintegration, dispersion, dissolution, release or organoleptic properties or to permit or facilitate formation of a dose unit of the composition into a discrete article such as a capsule or tablet suitable for oral administration. Excipients can include, by way of illustration and not limitation, diluents, disintegrants, binding agents, adhesives, wetting agents, polymers, lubricants, glidants, substances added to mask or counteract a disagreeable taste or odor, flavors, dyes, fragrances, and substances added to improve appearance of the composition.

By "effective amount" is meant the amount of an agent required to ameliorate the symptoms in an untreated subject. The effective amount of an active therapeutic agent for the treatment varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending clinician will decide the appropriate amount and dosage regimen.

As used herein, the terms "prevent," "preventing," "prevention," "prophylactic treatment" and the like refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition.

As used herein, the terms "treat," "treating," "treatment," "therapeutic" and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

Although the causes of RLS are not particularly well understood, the most effective treatments for RLS involve dopamine agonists or increasing levels of dopamine or dopamine precursors in the brain. However, as described above, the primary treatment options for RLS that have been approved by the US FDA are dopamine agonists and dopamine precursors (particularly levodopa) and each suffer from drawbacks, notably rebound and augmentation. It has been found, as described further herein, that clavulanic acid, as well as its salts, esters and prodrugs, can increase neuronal release of dopamine without an increase in the overall production of dopamine. Without being bound by theory, it is proposed that an increase in dopamine release without a concomitant increase in dopamine production can alleviate or reduce at least one side effect of treatment such as rebound, augmentation and other undesirable or unpleasant side effects. Thus, the discovery herein
that clavulanic acid increases neuronal secretion of dopamine presents a new and different approach to treatment of RLS that has advantages over other dopamine based therapies.

[0027] As used herein, RLS includes both primary and secondary RLS. Whether primary or secondary, a form of RLS that can be treated according to the present invention is generally a form that is responsive to dopamine based therapy. In exemplary embodiments, the invention is a method of treating primary RLS with clavulanic acid.

[0028] Although clavulanic acid can be considered a dopaminergic agent, its mode of action is very different from other dopaminergic agents used for treatment of RLS. For example, Levodopa is the precursor to dopamine, while clavulanic acid increases the endogenous release of dopamine. Furthermore, clavulanic acid is not a dopamine agonist. Levodopa is an effective treatment for RLS but is a less desirable treatment because of its side effect profile. Side effects of Levodopa include augmentation and rebound with long-term use, and these side effects grow worse over time, regardless of disease phenotype, i.e., augmentation and rebound are commonly observed with levodopa therapies whether treating, for example, Parkinson's disease or RLS. Symptoms of augmentation include an increased intensity of RLS, shorter onset time at rest, and the involvement of other limbs. In one clinical study, 59% of patients experienced augmentation, with 37% requiring treatment cessation or alternative medication because of intolerable side effects (Sleep, Vol. 19, No.3, 1996). In contrast, in clinical trials, clavulanic acid has shown no augmentation after 8 weeks and had a very mild side effect profile. Mild side effects included gastrointestinal disorders, headache and muscle strain. Gastrointestinal related side effects were reported when dosing was in the range of 100 to 1,000 mg, and included nausea, stomachache, diarrhea, and vomiting. Some patients also reported tiredness and malaise when given consecutive high dose treatments. Additionally, clavulanic acid has a wide therapeutic range, an excellent safety profile, and is tolerated at doses up to 1,000 mg in humans. As described further herein, dosages for treatment of RLS in humans are generally significantly below this threshold.

[0029] In the present invention, the increase in dopamine levels resulting from clavulanic acid treatment has been shown in two dopaminergic neuronal cells lines, differentiated PC12 and SH-SY5Y cells. Furthermore, using affinity chromatography two proteins, Munc18-1 and Rab4, were identified that potentially bind to clavulanic acid and play a critical role in neurosecretion and the vesicle trafficking process. Consistent with this result, an increase in the translocation of Munc18-1 and Rab4 from the cytoplasm to the
plasma membrane was observed in clavulanic treated cells. Data show that clavulanic acid enhances dopamine levels in a mechanism involving Munc18-1 and Rab4 modulation. Thus, the enhancement of dopamine may be through a mechanism involving vesicle trafficking and fusion through the binding and regulation of Munc18-1 and Rab4 in early endosome recycling rather than through stimulating increased dopamine production.

[0030] Clavulanic acid was previously described as a non-competitive inhibitor of β-lactamase and augments other β-lactam family antibiotics, although the compound has negligible intrinsic antibacterial activity [18]. Recent studies have shown that clavulanic acid possesses strong CNS modulating effects; for example, Clavulanic acid decreases anxiety in rodent and primate models [9]. Clavulanic acid has been proposed to have anti-depressant activity and is currently entering Phase lib clinical trials for the treatment of Major Depressive Disorder (MDD). Further findings suggest that clavulanic acid is a neuroprotective agent in Parkinson's models in vivo [5]. Studies have also shown that clavulanic acid suppresses anxiety and enhances sexual functions in rodent and primate models by a mechanism involving central nervous system (CNS) modulation, although its detailed mechanism of action has yet to be elucidated [1]. Clavulanic acid easily crosses the blood-brain barrier, permitting its viable CNS drug properties. The distribution ratio of clavulanic acid between human cerebrospinal fluid and plasma is 0.25, suggesting considerably higher level of brain penetration than most other small molecules [15]. Clavulanic acid can also be administered as a pharmaceutically acceptable salts, or as a prodrug, for example an ester. Potassium clavulanate (C₆H₅N0₅K) is an exemplary compound that can be used in embodiments of the present invention.

[0031] The release of neurotransmitters into the synapse occurs by exocytosis of secretory vesicles. Intracellular membrane fusion events leading to the process of neurotransmitter release are controlled by four classes of proteins: SNARE-proteins, SM-proteins (Secl/Munc-18 proteins), Rab-proteins and Rab-effectors [6]. It is believed that Rab and Rab-effector proteins regulate and mediate upstream events (vesicle budding, transport, delivery and tethering) and the SNARE- and SM-proteins catalyze the fusion reaction of the secretory vesicle with the plasma membrane.

[0032] Among the SM family, Munc 8-1 is critical for regulation of vesicle fusion and exocytosis, since deletion of Munc 8-1 results in the loss of all synaptic vesicle fusion [25]. Although the exact role of Munc18-1 has yet to be determined it is proposed that Munc18-1 is a key regulator of neurosecretion and that the general function for Munc 8-1 and SM...
proteins is to directly promote SNARE complex assembly [4]. It has been shown that Munc 18-1 binds directly to the assembled SNARE complex [2] and can activate SNARE-mediated membrane fusion [20] and fusion between large vesicles and giant membranes [24]. The findings that clavulanic acid induces translocation of Munc 18-1 to the plasma membrane and enhances release of dopamine from neuronal cells strongly suggest that clavulanic acid-induced dopamine release may be via an interaction with Munc 18-1.

The Rab family, regulators of vesicular traffic (reviewed in [23]), consist of more than 60 Rab proteins in mammalian cells. Rab GTPases have been implicated in each step of vesicle formation, trafficking (vesicle budding, transport, delivery and tethering) and SNARE complex formation [21, 26]. Rab proteins switch between their active GTP-bound form which interacts with downstream effector proteins, and their inactive GDP-bound form. In their active GTP-bound form, Rab proteins recruit specific effector proteins onto the cytosolic face of membranes in order to regulate vesicle formation, movement and fusion through their effectors. Rab4 protein has been shown to be associated with early endosomes and regulates "short-loop" (fast) early endosome membrane recycling [22, 7]. Affinity binding studies showed that clavulanic acid binds to Rab4. Vesicle recycling plays a key role in maintaining homeostasis after vesicle exocytosis. Several mechanisms exist for synaptic vesicle recycling, including fast exocytosis/endocytosis and clathrin-mediated endocytosis. Regardless of the mechanism of endocytosis, all recycled vesicles are loaded with neurotransmitters before subsequent fusion [11]. Early endosomes have been shown to contain domains for Rab4 and Rab5 which are involved in endosome fusion and endocytic recycling. Recycled endosomes contain domains for Rab4 and Rabl 1 which are necessary for vesicle trafficking from the early endosome to the plasma membrane [23]. The findings of clavulanic acid-induced translocation of Rab4 to the plasma membrane and enhanced dopamine release from the cells suggest that dopamine secretion by clavulanic acid may be due to the interaction of clavulanic acid with Rab4, resulting in subsequent vesicle fusion and potentially endosomal recycling. Rab proteins are initially synthesized in the cytosol where they associate with Rab escort protein (REP) to undergo post-translational modification by the addition of one or two hydrophobic geranylgeranyl groups. This post-translational modification is required to allow for the attachment of the Rab proteins into the lipid bilayer [23]. The modified REP-associated Rab protein in its GDP-bound form is activated to its GTP-bound form upon membrane delivery. This exchange of GDP to GTP is catalyzed by a GDP/GTP exchange factor (GEF) and results in the release of REP. The
active membrane bound Rab is then able to carry out its various functions through binding of their effectors [3, 23]. Clavulanic acid enhances Rab4 localization to the plasma membrane via the above mechanism through post-translational modification. Upon treatment with simvastatin which is known to inhibit protein prenylation [17], clavulanic acid no longer enhanced Rab4 localization to the plasma membrane suggesting that clavulanic acid enhances dopamine release through a mechanism involving conventional Rab4 post-translational modification in the cytosol and subsequent movement to the plasma membrane.

Recently, it was demonstrated that clavulanic acid, at different effective dose ranges, possesses strong CNS modulating effects, including anti-anxiety effects in rodent and primate models [9], neuroprotective effects in Parkinson’s disease models in vivo [5] as well as enhanced sexual arousal in animal models through a proposed CNS-mediated mechanism [1]. These clavulanic acid-induced pharmacological activities may be due to the release of dopamine via clavulanic acid interaction with vesicle trafficking and fusion proteins Munc 18-1 and Rab4.

Because clavulanic acid has been found to enhance dopamine release in two neuronal cell lines, clavulanic acid can provide an effective treatment or prevention for conditions such as Restless Legs Syndrome (RLS) and its related disorders, as well as other conditions potentially treatable by dopamine agonists. Furthermore, in view of the known safety profile of clavulanic acid and its freedom from side effects, it presents a new and useful modality for the treatment of Restless Legs Syndrome. Furthermore, because clavulanic acid has been found to act through unique mechanisms that lead to an increase in release of dopamine without a significant overall increase in dopamine production, it possesses therapeutic advantages.

Disorders that have symptomology and causes in common with RLS can also be treated with clavulanic acid. Related disorders can include other disorders characterized by spasms or myoclonus, for example, Periodic limb movements of sleep (PLMS), Periodic Limb (or Leg) Movement Disorder (PLMD, also sometimes referred to as nocturnal myoclonus), stiff person syndrome (SPS, stiff-man syndrome or Moersch-Woltman Condition). Other disorders treatable with increasing dopamine release can also be treated without the side effects characteristic of dopamine agonists.

In order to elucidate the mechanism on how clavulanic acid enhances dopamine levels and possesses strong CNS-modulating effects, identification of potential binding
partners was investigated. Previous receptor based studies showed that clavulanic acid failed to bind to 63 well-known receptors and neurotransmitter-related targets, such as ion channels, second messengers, and other enzymes [9], implying that clavulanic acid acts through a novel mechanism. Affinity based studies were utilized to purify any proteins that may bind to clavulanic acid. In accordance with the present invention, clavulanic acid binds to Muncl8-1 and Rab4 and alters the subcellular localization of both proteins to the plasma membrane in neuronal cells. Indeed, both Muncl 8-1 and Rab4 protein are proteins involved with vesicle fusion/trafficking and recycling, respectively. Clavulanic acid has been found to bind to Muncl 8-1 and Rab4 enhancing neurosecretion of dopamine. Nevertheless, since clavulanic acid binds to Muncl 8-1 and Rab4 and alters the localization of these proteins, it is possible that clavulanic acid may have an effect on the release of other monoamines (5-HT, NA, NE). Previous studies have proposed that clavulanic acid enhances the release of both dopamine and 5-HT in vivo [9]. The dual enhancement of both dopamine and 5-HT by clavulanic acid may be a result of enhanced vesicle trafficking and fusion through a mechanism involving Muncl 8-1 and Rab4. Enhanced vesicle transport and fusion, potentially resulting in increased neurotransmitter release, may prove a novel and ideal therapy in MDD as well as other neurological disorders.

Conventional pharmaceutical preparations of the clavulanic acid can be used, e.g., consisting essentially of an inert pharmaceutical carrier and an effective dose of the active substance; e.g., plain or coated tablets, capsules, lozenges, powders, solutions, suspensions, emulsions, syrups, suppositories, transdermal patch, etc. Tablets are preferred. The inert carrier can include excipients known in the art, including but not limited to physiologically acceptable buffering agents, stabilizers (e.g. antioxidants), flavoring agents, agents to effect the solubilization of the compound, and the like.

In embodiments, the composition may be in any suitable form such as a solution, a suspension, an emulsion, an infusion device, or a delivery device for implantation or it may be presented as a dry powder to be reconstituted with water or another suitable vehicle before use. The composition may include suitable parenterally acceptable carriers and/or excipients.

In embodiments, the compositions may comprise an effective amount of a clavulanic acid in a physiologically-acceptable carrier. The carrier may take a wide variety of forms depending on the form of preparation desired for a particular route of administration. Suitable carriers and their formulation are described, for example, in
Remington's Pharmaceutical Sciences by E. W. Martin. In embodiments, the clavulanic acid may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for parenteral (e.g., subcutaneously, intravenously, intramuscularly, or intraperitoneally) or oral administration route. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

[0041] Clavulanic acid, its pharmaceutically acceptable salts, esters and prodrugs can be administered orally. Exemplary embodiments of orally administrable forms of clavulanic acid are described in U.S. Patent Application No. 12/258,062, the entire content of which are incorporated herein in its entirety for all purposes, including but not limited to particular forms of clavulanate as well as the dosage forms. The formulation described in U.S. Patent Application No. 12/258,062 is particularly useful in that it allows for a stable low dosage form for administration that does not readily hydrolyze on standing.

[0042] In embodiments, the clavulanic acid may be in a form suitable for administration by sterile injection by being dissolved or suspended in a parenterally acceptable liquid vehicle. Such formulations can include a sterile aqueous preparation of clavulanic acid, which preferably is isotonic with the blood of the recipient (e.g., physiological saline solution). Such formulations may include suspending agents and thickening agents and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose form.

[0043] In exemplary embodiments, the compositions may be in a form suitable for oral administration. In compositions in oral dosage form, any of the usual pharmaceutical media may be employed. Thus, for liquid oral preparations, such as, for example, suspensions, elixirs and solutions, suitable carriers and additives include water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like. For solid oral preparations such as, for example, powders, capsules and tablets, suitable carriers and additives include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. If desired, tablets may be sugar coated or enteric coated by standard techniques.
Compositions suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets, or lozenges, each containing a predetermined amount of the clavulanic acid as a powder or granules. Optionally, a suspension in an aqueous liquor or a non-aqueous liquid may be employed, such as a syrup, an elixir, an emulsion, or a draught. Formulations for oral use include tablets containing active ingredient(s) in a mixture with pharmaceutically acceptable excipients. Such formulations are known to the skilled artisan. Excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, macrocristalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

A syrup may be made by adding the clavulanic acid to a concentrated aqueous solution of a sugar, for example sucrose, to which may also be added any accessory ingredient(s). Such accessory ingredient(s) may include flavorings, suitable preservative, agents to retard crystallization of the sugar, and agents to increase the solubility of any other ingredient, such as a polyhydroxy alcohol, for example glycerol or sorbitol.

In addition to the aforementioned ingredients, compositions of the invention may further include one or more accessory ingredient(s) selected from encapsulants, diluents, buffers, flavoring agents, binders, disintegrants, surface active agents, thickeners, lubricants, preservatives (including antioxidants), and the like.

In some embodiments, compositions may be formulated for immediate release, sustained release, delayed-onset release or any other release profile known to one skilled in the art.

In some embodiments, the pharmaceutical composition may be formulated to release the clavulanic acid substantially immediately upon administration or at any predetermined time or time period after administration. The latter types of compositions are
generally known as controlled release formulations, which include (i) formulations that create a substantially constant concentration of the drug within the body over an extended period of time; (ii) formulations that after a predetermined lag time create a substantially constant concentration of the drug within the body over an extended period of time; (iii) formulations that sustain action during a predetermined time period by maintaining a relatively constant, effective level in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the active substance (sawtooth kinetic pattern); (iv) formulations that localize action by, e.g., spatial placement of a controlled release composition adjacent to or in the central nervous system or cerebrospinal fluid; (v) formulations that allow for convenient dosing, such that doses are administered, for example, once every one or two weeks; and (vi) formulations that target the site of a pathology. For some applications, controlled release formulations obviate the need for frequent dosing to sustain activity at a medically advantageous level.

Any of a number of strategies can be pursued in order to obtain controlled release in which the rate of release outweighs the rate of metabolism of the compound in question. In one example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the clavulanic acid is formulated with appropriate excipients into a pharmaceutical composition that, upon administration, releases the clavulanic acid in a controlled manner. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, molecular complexes, nanoparticles, patches, and liposomes.

The administration of clavulanic acid may be by any suitable means that results in a concentration that, combined with other components, is effective in preventing, treating, ameliorating, or reducing RLS and/or its associated symptoms.

Generally, the amount of administered agent of the invention will be empirically determined in accordance with information and protocols known in the art. In animal, i.e. mouse, models, dosages are about 0.1 μg/kg to about 10 mg/kg administered twice daily. Thus, using accepted conversion methodologies, a typical human dose is about 0.008 μg/kg to about 0.81 mg/kg administered twice per day or about 0.016 μg/kg/day to about 1.6 mg/kg/day. Thus, to account for wider range of therapeutic utility, dosages of clavulanic acid for the treatment of Restless Legs Syndrome can range from about 0.00001 mg/kg/day to 10 mg/kg/day. For example, the dosage can be about 0.00001 mg/kg/day, about 0.000015
mg/kg/day, about 0.0001 mg/kg/day, about 0.001 mg/kg/day, about 0.01 mg/kg/day, about 0.1 mg/kg/day, about 0.15 mg/kg/day, about 0.25 mg/kg/day, about 0.30 mg/kg/day, about 0.75 mg/kg/day, about 1.0 mg/kg/day, about 1.5 mg/kg/day, about 2.0 mg/kg/day, about 2.5 mg/kg/day, about 3.0 mg/kg/day, about 4.0 mg/kg/day, about 5.0 mg/kg/day, about 7 mg/kg/day or about 10 mg/kg/day. As will be appreciated, normal dosages can be within a range between any two of these recited dosages. For example, the dosage can be in the range of from about 0.0001 mg/kg/day to about 7 mg/kg/day, about 0.015 µg/kg/day to about 1.5 mg/kg/day, about 0.016 µg/kg/day to about 2 mg/kg/day, or about 0.01 mg/kg/day to about 1.5 mg/kg/day.

[0052] For a normal adult of about 70 kg, the effective dosage range of clavulanic acid for the treatment of Restless Legs Syndrome can be about 1.0 µg/day to about 150 mg/day, for example about 0.007 mg/day to about 700 mg/day, about 0.01 mg/day to about 500 mg/day, or about 1 to 100 mg/day. Dosages can be, for example, about 0.001 mg/day, about 0.01 mg/day, about 0.1 mg/day, about 1.0 mg/day, about 10 mg/day, about 15 mg/day, about 17.5 mg/day, about 20 mg/day, about 35 mg/day, about 50 mg/day, about 70 mg/day, about 100 mg/day, about 150 mg/day, about 175 mg/day, about 200 mg/day, about 250 mg/day, about 350 mg/day, about 500 mg/day, or about 700 mg/day. As will be appreciated, normal dosages can be within a range between any two of these recited dosages. For example, the dosage can be in the range of from about 0.007 mg/day to about 500 mg/day, about 1 µg/day to about 150 mg/day, about 1.12 µg/day to about 150 mg/day, or about 0.7 mg/day to about 100 mg/day. In some embodiments, more than about 1 mg will be administered to a patient per administration and per day, for example, about 10 to about 20 mg/day.

[0053] Clavulanate can be administered orally in one or more doses per day to achieve the above dosages. For example, clavulanate can be administered once per day, twice per day or three times per day. The timing of dosages depends on the form of the pharmaceutical composition administered and the underlying symptomology to be treated. For example, if an approximate steady state concentration of clavulanate is found to be beneficial, a controlled or sustained release dosage form can be used and administered with sufficient frequency to maintain the desired blood level of clavulanic acid. Alternatively, a steady state can be maintained by more frequently administering a more immediate release dosage form with a lower dosage. If it is found desirable for clavulanate levels to be increased at specific times, an immediate release form administered sufficiently in advance of the time for desired peak concentrations can be used. For example, if it is found that it is only necessary to
increase clavulanate levels at bedtime to relieve symptoms during sleep, a composition can be administered in advance of bedtime to assure peak levels at the desired times. A skilled clinician will be able to determine the most effective treatment regimen depending on symptomology and success of various treatments.

**EXAMPLES**

[0054] The invention may be further clarified by reference to the following Examples, which serve to exemplify some of the preferred embodiments, and not to limit the invention in any way.

[0055] **EXAMPLE 1**

[0056] **Cell Culture:** PC12 cells (adrenal gland; Pheochromocytoma) and SH-SY5Y cells (human neuroblastoma) were obtained from American Type Culture Collection (ATCC, VA). PC12 cells were maintained in poly-D-lysine coated dishes (BD Biocoat, MA) in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with heat-inactivated 10% horse serum (HS) (Gibco, MD) and 5% fetal bovine serum (FBS) (Gibco, MD), 100 units/ml penicillin, 100 µg/ml streptomycin in a water-saturated atmosphere of 5% CO₂ at 37 °C. SH-SY5Y cells were cultured in a medium containing DMEM, Hanks' balanced salt solution (HBSS), F-12 medium (2:1:1) with 10% heat inactivated fetal bovine serum (Invitrogen, Carlsbad, CA). SH-SY5Y cells were differentiated to a neuronal phenotype by adding 10 µM of retinoic acid to the culture medium for 3 days; then the media was removed and replaced with fresh media containing 80 nM of 12-0-tetradecanoyl-phorbol-13-acetate (TPA) for another 3 days.

[0057] **Dopamine measurements:** Measurement of dopamine in PC12 and differentiated SH-SY5Y cells was performed using commercially available dopamine ELISA kit (Rocky Mountain Diagnostics, CO). Cells were treated with 100 µM of clavulanic acid for 6 or 12 hours. After treatment, cells were harvested and lysed immediately. Cell homogenates were centrifuged at 10,000g for 20 min at 4°C and supernatant was used to measure dopamine as per the manufacture's instruction. Protein concentrations were determined using Bradford reagent (Bio-Rad). For dopamine release, cells were treated with 100 µM of clavulanic acid for 6 and 12 h and stimulated with 50 mM of K⁺ solution for depolarization. Samples were collected and dopamine level was measured immediately as per the manufacture's instruction.
Western blot analyses: Cells were harvested, lysed and protein concentrations were determined, using the Bradford reagent (Bio-Rad), 25 µg of lysates were resolved on NuPAGE 4-12% Bis-Tris gel (Invitrogen, Carlsbad, CA) followed by Western blotting using the desired antibodies as described [19].

Preparation of brain homogenate: Rat brain tissue (Pel-Freeze Biologicals, AZ) was homogenized in homogenization buffer (60 mM β-glycerophosphate, 15 mM p-nitrophenyl phosphate, 25 mM MOPS (pH 7.2), 15 mM EGTA, 15 mM MgCl₂, mM DTT, 1 mM Na₃VO₄, 1 mM NaF, 1 mM phenyl phosphate, 100 µM benzamidine) with the addition of IX protease inhibitor at 4°C. The homogenate was centrifuged at 14,000 rpm for 10 minutes at 4°C, and the supernatant was collected and analyzed for total protein concentration by BCA analysis.

Target binding affinity studies: Potassium clavulanate (DSM Anti-Infectives, Sweden) was covalently bound to CarboxylLink™ (Immobilized dianinodipropylamine) coupling gel (Pierce, IL) for affinity studies. Brain homogenate was incubated with clavulanic acid conjugated to the activated coupling gel for 2 hours with gentle shaking at 4°C. After washing the gel four times with washing buffer (50 mM Tris-HCl, pH 7.4, 5 mM NaF, 250 mM NaCl, 5 mM EDTA, 5 mM EGTA, 0.1 % Nonidet p-40, 100 µM benzamidine, IX protease inhibitors), bound proteins were released from the affinity gel by the addition of 2X Laemmli sample buffer (BioRad, CA) and heat denaturation at 95°C for 5 minutes. The denatured proteins were resolved by SDS/PAGE (4-12% gradient gels), and protein bands were visualized by staining or identified by protein mass spectrometry sequencing and identified proteins were verified by Western blotting with Munc18-1 or Rab4 antibody (Santa Cruz, CA).

Confocal Microscopy: For immunofluorescence, differentiated SH-SY5Y cells were grown on coverslips, washed with PBS and fixed for 30 minutes using 4% paraformaldehyde. The cells were then permeabilized using cold methanol and blocked for 1 hour with horse serum (5% in PBS). Fixed cells were incubated with either Munc18-1 or Rab4 antibody at 1:100 dilution, washed and labeled with rhodamine red-linked anti-rabbit secondary antibody (1:100 dilutions). Confocal images were collected using an MRC 1024-krypton/argon laser scanning confocal equipped with a Zeiss LSM 510 Meta photomicroscope.

Quantitation of data and statistical analysis: Data shown in this study were expressed as means ± S.D. Differences between experimental groups were considered
significant when $p < 0.05$ by Student's $t$-test. All statistical analyses were performed using GraphPad Prism software (GraphPad Software Inc., CA).

[0063] RESULTS

[0064] Clavulanic acid enhances dopamine release in neuronal cells

Dopamine levels were analyzed in PC12 and differentiated SH-SY5Y neuronal cells in the presence or absence of clavulanic acid. Quantitative dopamine levels were measured in both cell lines by enzyme-linked immunosorbent assay. As shown in Figure 1A, dopamine release was not affected in PC12 cells treated for 6 hours with clavulanic acid, but the dopamine level was increased ~1.8 fold in the medium after 12 hours of clavulanic acid treatment upon depolarization with K+. The increase in dopamine by clavulanic acid is attributed to increased release of intracellular dopamine since total amount of dopamine levels remained unchanged from control upon clavulanic acid treatment (Figure 1A). Additionally, dopamine release was increased ~2 fold and -2.5 fold in differentiated SH-SY5Y cells treated with clavulanic acid for both 6 and 12 hours, respectively. Total amount of intracellular dopamine remained unchanged, indicating that clavulanic acid enhanced release of dopamine after treatment (Figure 1C). Furthermore, clavulanic acid had no effect on the levels of tyrosine hydroxylase in either cell line (Fig 1B and ID). These results suggest that clavulanic acid does not affect the synthesis of dopamine, but rather increases the release of intracellular dopamine in depolarizing condition.

[0066] Identification of possible protein targets of Clavulanic acid.

[0067] The following study was performed to identify potential target proteins that bind to clavulanic acid and that are involved in neurotransmitter release. Earlier studies have shown that clavulanic acid does not bind to any well-known signaling receptors, transporters or ion channels involved in neurotransmission [9]. In this study, the eluted fraction of brain homogenate that was mixed with clavulanic acid conjugated gel was analyzed by gel electrophoresis and candidate proteins were selected, excised and identified by mass spectrometry. Munc18-1 and Rab4 were among those binding proteins. Western blotting was performed to verify the specificity of Munc18-1 and Rab4 and indicated that both proteins were specifically bound to clavulanic acid (Figure 2).
Clavulanic acid translocates Muncl8-1 and Rab4 from the cytoplasm to the membrane.

It is known that Muncl8-1 and Rab proteins are essential in the secretion of neurotransmitters from synaptic vesicles. Binding studies indicate clavulanic acid specifically binds to Muncl8-1 and Rab4 and since these proteins play a key role in membrane trafficking and fusion as well as vesicle recycling [3,4,22], we investigated the subcellular localization of Muncl8-1 and Rab4 in the presence or absence of clavulanic acid. In SH-SY5Y cells, both Muncl8-1 and Rab4 translocated from the cytoplasm to the plasma membrane in the presence of 100 µM clavulanic acid (Figure 3B) compared to vehicle treated control (Figure 3A). It is known that the movement of Rab proteins from the cytoplasm to the plasma membrane is regulated by prenylation, therefore we investigated if clavulanic acid induced translocation of Rab4 affected prenylation. Inhibition of prenylation by simvastatin, a known inhibitor of protein prenylation [17], decreased the effects of clavulanic acid on Rab4 translocation to the plasma membrane, suggesting that proper post-translational modification through prenylation of Rab4 must occur in order for Rab4 to localize to the plasma membrane by clavulanic acid. Overall, these results suggest that both Muncl8-1 and Rab4 may be a part of clavulanic acid-induced increase in dopamine release observed in Figure 1.

EXAMPLE 2

Iron deficiency has been shown to be correlated with RLS in humans. Postmortem studies have demonstrated that levels of iron and heavy chain ferritin are reduced in RLS [Connor et al. 2011]. Several preclinical studies have demonstrated that animals exposed to an iron-deficient diet show an increase in activity levels, especially during the hours that correspond to human sleep [F. Luo et al. / Sleep Medicine 12 (2011) 41-46; Movement Disorders Vol. 15, No. 1, 2000, pp. 154-158; Journal of Neuroscience Research 85:1065-1076 (2007)].

The alleviation of symptoms of RLS by clavulanic acid is tested in mice using a model of chronic dietary iron deficiency. Clavulanic acid is prepared in sterile buffered saline or other standard vehicle solution, and made fresh daily. Each animal is dosed orally with 100 microliters of a clavulanic acid solution at dosages ranging from 0.0001 mg/kg/dose to 10 mg/kg/dose to establish a dose-response curve. Animals are dosed orally two times per day. Parameters indicative of treatment are measured including distance
moved, total moving time, horizontal movements and vertical movements. These parameters increase in animals fed an iron-deficient diet compared to control diet.

[0073] RESULTS - Parameters in iron-deficient animals are normalized to control levels upon treatment with clavulanic acid.

[0074] While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention. It is intended, therefore, that the invention be defined by the scope of the claims that follow and that such claims be interpreted as broadly as is reasonable.

References


What is claimed is:

1. A method of treating or preventing Restless Legs Syndrome in a subject in need of treatment comprising administering to the subject a therapeutically or prophylactically effective amount of clavulanic acid, or a pharmaceutically acceptable salt, ester or prodrug thereof.

2. The method of claim 1, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is administered in an amount capable of enhancing dopamine neurosecretion, said amount capable of enhancing dopamine neurosecretion in an amount effective to treat Restless Legs Syndrome.

3. The method of claim 1, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is capable of binding to Munc18-1 or Rab4.

4. The method of claim 3, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is administered in an amount effective to enhance promote translocation of Munc18-1 or Rab4 from the cytoplasm to the plasma membrane.

5. The method of claim 1, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is administered in an amount effective to enhance dopamine neurosecretion, thereby treating Restless Legs Syndrome.

6. A method of enhancing dopamine neurosecretion in a subject comprising administering to the subject a therapeutically or prophylactically effective amount of a clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, capable of enhancing dopamine release.

7. The method of one of claims 1-6, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is administered at a dose of about 1 µg/day to about 150 mg/day.

8. The method of one of claims 1-6, wherein the clavulanic acid, or pharmaceutically
acceptable salt, ester or prodrug thereof, is administered at a dose of about 10 mg/day to about 20 mg/day.

9. The method of one of claims 1-6, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is administered in a dosage unit form containing about 0.016 µg/kg/day to about 2 mg/kg/day.

10. The method of one of claims 1-6, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is clavulanic acid or a salt of clavulanic acid.

11. The method of claim 10, wherein the clavulanic acid or salt of clavulanic acid, is sodium clavulanate or potassium clavulanate.

12. The method of any one of claims 1-6, wherein the clavulanic acid is administered as a clavulanate ester.

13. The method of any one of claims 1-6, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof is administered once per day.

14. The method of any one of claims 1-6, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof is administered in multiple doses each day.

15. A pharmaceutical composition in oral dosage form, comprising clavulanic acid, or a pharmaceutically acceptable salt, ester or prodrug thereof, in an amount effective for the treatment of Restless Legs Syndrome.

16. The pharmaceutical composition of claim 15, comprising from about 0.5 µg to about 150 mg of clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof.

17. The pharmaceutical composition of claim 15, comprising from about 1.0 µg to about 75 mg of clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof.
18. The pharmaceutical composition of claim 15, comprising from about 5 mg to about 20 mg clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof.

19. The pharmaceutical composition of one of claims 15-18, wherein the clavulanic acid, or pharmaceutically acceptable salt, ester or prodrug thereof, is clavulanic acid or a salt of clavulanic acid.

20. The pharmaceutical composition of claim 19, wherein the clavulanic acid or salt of clavulanic acid, is sodium clavulanate or potassium clavulanate.

21. The pharmaceutical composition of one of claims 15-18, wherein the clavulanic acid is administered as a clavulanate ester.
Figure 1

A

Dopamine release

![Graph showing dopamine release](image)

Total Dopamine

![Graph showing total dopamine](image)

B

TH

Actin

![Western blot showing TH and Actin](image)

C

Dopamine release

![Graph showing dopamine release](image)

Total Dopamine

![Graph showing total dopamine](image)

D

TH

Actin

![Western blot showing TH and Actin](image)