

Description

USE OF *N*-CARBAMOYL ASPARTIC ACID COMPOUNDS IN THE PREPARATION OF MEDICAMENTS FOR TREATING COCCIDIOSIS IN ANIMALS

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Technical Field

Provided herein is the use of an *N*-carbamoyl aspartic acid compound or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising the same in the preparation of a medicament for preventing and/or treating coccidiosis in an animal, as well as a pharmaceutical composition comprising the above compound as an anticoccidial active ingredient, and methods of their use for preventing and/or treating coccidiosis in an animal.

Background Art

Coccidiosis is a common and widespread disease caused by protozoan parasites of the genus *Eimeria*. Among poultry coccidiosis, the more important of these types are those caused by coccidia selected from *E. maxima*, *E. acervulina*, *E. tenella*, *E. necatrix*, *E. brunetti*, *E. praecox* and *E. mitis*. In turkeys, *E. meleagridis* and *E. adenoides* are also causative organisms of coccidiosis. In addition to chicken coccidiosis, which has existed for a long time and is well known by people, in recent years, the incidence of pig coccidiosis and dairy cow coccidiosis and others has been increasing year by year. Coccidiosis has become the main cause of diarrhea and death in suckling and weaned piglets. *Eimeria* is a direct life history parasitic protozoa, and its biological characteristics determine that it is difficult to eliminate. As long as there is intensive breeding of pigs, chickens and the like, coccidiosis infection and the prevalence of coccidiosis are inevitable. The serious consequences of such disease are poor animal weight gain, reduced feed efficiency and high mortality. It is an animal disease that seriously harms the production of breeding industry. Chicken coccidiosis alone costs billions of dollars annually worldwide. At present, the control of animal coccidiosis mainly relies on anticoccidial agents, and the long-term and widespread use of anticoccidial agents has led to the occurrence of drug resistance. Therefore, successful prevention and treatment of coccidiosis is of great significance.

Summary of the Invention

Accordingly, provided herein is the use of an *N*-carbamoyl aspartic acid compound in the preparation of a medicament for preventing and/or treating coccidiosis in an animal.

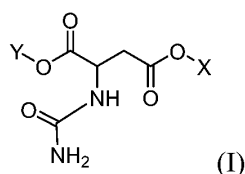
Also provided is a pharmaceutical composition comprising an *N*-carbamoyl aspartic acid compound.

Further provided is a method for preventing or treating coccidiosis in an animal.

In order to achieve the above objects of the disclosure, the following technical solutions are employed:

In one aspect, provided herein is the use of an *N*-carbamoyl aspartic acid compound of Formula (I), or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or

pharmaceutically acceptable salt thereof in the preparation of a medicament for preventing and/or treating coccidiosis in an animal:



wherein each of X and Y is independently H, or linear or branched C₁-C₁₈ alkyl.

- 5 In some embodiments, each of X and Y is independently H, or linear or branched C₁-C₄ alkyl.

In some embodiments, the pharmaceutically acceptable salt is an alkali or divalent metal salt where X and Y is independently H, preferably a sodium, potassium, calcium, zinc, or copper salt.

- 10 In some embodiments, the animal is a farmed animal susceptible to infection with coccidia, comprising poultry, a domestic and pet animal. In some instances, the poultry comprises chicken, turkey, duck, goose, and quail; the domestic animal comprises a pig, cattle, sheep, and rabbit; and the pet animal comprises a cat and dog.

- 15 In some embodiments, the coccidiosis is a disease caused by coccidia selected from the species of: *E. maxima*, *E. mitis*, *E. tenella*, *E. necatrix*, *E. acervulina*, *E. auburnensis*, *E. bovis*, *E. zuernii*, *E. deblicieki*, *E. stiedai*, *E. perforans*, *E. canis*, or *E. brunetti*, preferably *E. maxima*, *E. mitis*, *E. acervulina*, or *E. brunetti*.

- 20 In another aspect, provided herein is a pharmaceutical composition comprising an *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

In some embodiments, the pharmaceutical composition further comprises an additional anticoccidial agent.

- 25 In further embodiments, the pharmaceutical composition is in a water-dispersible powder.

In further embodiments, the pharmaceutical composition is in an aqueous solution.

- 30 In another aspect, provided herein is an *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising the same for use in preventing or treating coccidiosis in an animal.

- 35 In another aspect, provided herein is a method for preventing or treating coccidiosis in an animal, comprising administering a therapeutically effective amount of an *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising the same to the animal susceptible to infection with coccidia.

In some embodiments, the therapeutically effective amount is from 100 to 2000 ppm.

In some embodiments, the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically

acceptable salt thereof is administered in a pharmaceutical composition as described herein to an animal susceptible to infection with coccidia through admixing into feed or drinking water.

5 In another aspect, provided herein is an *N*-carbamoyl aspartic acid compound of Formula (I), or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising the same for use in preventing or treating coccidial infection in an animal.

10 In another aspect, provided herein is the use of an *N*-carbamoyl aspartic acid compound of Formula (I), or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as a medicament. In some embodiments, the *N*-carbamoyl aspartic acid compound or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof is for use in a method of treating or preventing coccidiosis in an animal, comprising administering a therapeutically effective amount of the *N*-carbamoyl aspartic acid compound or a
15 racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof to the animal.

The technical effects in relation to the present invention are:

20 The *N*-carbamoyl aspartic acid compound or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein has shown significant inhibition against coccidia and exhibits an obvious dose response. Specifically, in some embodiments, the *N*-carbamoyl aspartic acid compound or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof described herein exhibits the efficacy of a low-potency anticoccidial agent when the therapeutically effective amount is from 100 to 500 ppm,
25 in other embodiments, exhibits the efficacy of a moderate-potency anticoccidial agent when the therapeutically effective amount is 1000 ppm, and in still other embodiments, exhibits the efficacy of a high-potency anticoccidial agent when the therapeutically effective amount is from 2000 to 5000 ppm.

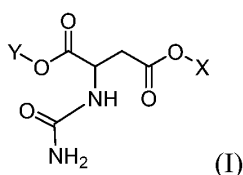
30 The foregoing described herein merely outlines certain aspects of the present invention, but is not limited to these aspects. The above aspects and others will be described in more detail below.

DETAILED DESCRIPTION

35 The detailed description below is intended to enable those skilled in the art to implement various embodiments. Descriptions of specific devices, techniques, use or applications are provided as examples only. The disclosure intends to encompass all alternatives, modifications and equivalents, which are all within the scope as defined by the appended claims herein. In addition, certain technical features disclosed herein, which are, for clarity, described separately in multiple independent embodiments, can also be provided in combination in an individual embodiment or in any suitable
40 subcombination.

Compounds

Provided herein is an *N*-carbamoyl aspartic acid compound of Formula (I), or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof:



wherein

each of X and Y is independently H, or linear or branched C₁-C₁₈ alkyl;

wherein the pharmaceutically acceptable salt is an alkali or divalent metal salt.

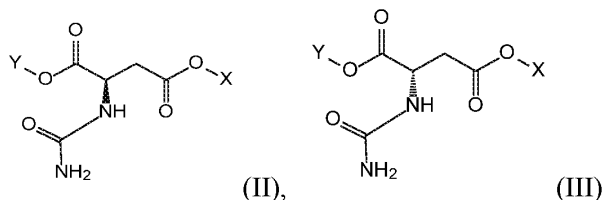
- 5 The term “pharmaceutically acceptable” means that the substance or composition must be chemically or toxicologically suitable in relation to the medicament of which it is composed.

- 10 The *N*-carbamoyl aspartic acid compound of Formula (I), or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein can be prepared as disclosed in Chinese Patent Publication No. CN110225706A or a modification thereof.

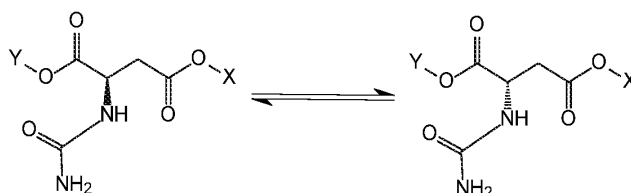
The *N*-carbamoyl aspartic acid compound as described herein comprises *N*-carbamoyl aspartic acid (NCA), and/or an alkyl ester thereof, and/or an alkali metal salt thereof, and/or a divalent metal salt thereof.

- 15 As used herein, “C_a-C_b alkyl” refers to a linear or branched, saturated alkyl group containing a to b carbons, such as methyl, ethyl, propyl, or isopropyl, or the like. In some embodiments, “C₁-C₅ alkyl” refers to a linear or branched, saturated alkyl group containing 1 to 5 carbons.

- 20 The *N*-carbamoyl aspartic acid compound as described herein has a chiral center and contains different stereoisomers. The stereoisomers are selected from the group consisting of L-(-)-*N*-carbamoyl aspartic acids of Formula (II), D-(+)-*N*-carbamoyl aspartic acids of Formula (III), and racemic DL-(±)-*N*-carbamoyl aspartic acids.



- 25 In some embodiments, the stereoisomers of the *N*-carbamoyl aspartic acids can undergo stereoconfiguration interconversion under appropriate conditions, for example, the conformational conversion of *N*-carbamoyl aspartic acid compound into each other will result in the simultaneous existence of two tautomers. The interconversion is as shown in the scheme below:



The above stereoisomers and tautomers are also encompassed in the scope of the present invention.

As used herein, “stereoisomer” refers to a compound that has the same chemical structure but different arrangements of atoms or groups in space, including enantiomers, diastereomers, conformational isomers, geometric isomers, and atropisomers, and the like. “Enantiomer” as used herein refers to two isomers of a compound that are non-superimposable and are mirror images of each other. “Diastereomer” as used herein refers to stereoisomers that have two or more chiral centers and whose molecules are not mirror images of each other, with different physical properties such as melting points, boiling points, spectral properties, and reactivity. Diastereomeric mixtures can be isolated by high-resolution analytical techniques such as electrophoresis or chromatography. “Tautomer” as used herein refers to structural isomers with different energies that can convert into each other through crossing a low energy barrier.

In some embodiments, the *N*-carbamoyl aspartic acid compound provided herein is *N*-carbamoyl aspartic acid where each of X and Y is independently H.

In some embodiments, the *N*-carbamoyl aspartic acid compound provided herein is an *N*-carbamoyl aspartate alkyl ester obtained from *N*-carbamoyl aspartic acid under esterification conditions, wherein each of X and Y is independently linear or branched C₁-C₁₈ alkyl.

In specific embodiments, the *N*-carbamoyl aspartate alkyl ester is obtained by the esterification reaction of *N*-carbamoyl aspartic acid with a linear or branched C₁-C₁₈ alkyl alcohol in the presence of an inorganic acid.

In specific embodiments, the *N*-carbamoyl aspartic acid compound is an *N*-carbamoyl aspartate alkyl ester where each of X and Y is independently linear or branched C₁-C₄ alkyl. In preferred embodiments, each of X and Y is independently methyl, ethyl, propyl, isopropyl, butyl, or isobutyl.

In further embodiments, the *N*-carbamoyl aspartic acid compound is propyl *N*-carbamoyl aspartate (NCA propyl ester) where each of X and Y is independently propyl.

In some embodiments, the pharmaceutically acceptable salt of the *N*-carbamoyl aspartic acid compound provided herein where each of X and Y is independently H is an alkali metal salt (in a molar ratio of 1:2) formed by contacting *N*-carbamoyl aspartic acid with a hydroxide, bicarbonate or carbonate of the alkali metal in an aqueous solution.

In specific embodiments, the hydroxide, bicarbonate or carbonate of the alkali metal is preferably potassium hydroxide, sodium hydroxide, potassium carbonate, potassium bicarbonate, sodium carbonate, or sodium bicarbonate; in particular, the alkali metal salt of *N*-carbamoyl aspartic acid is sodium *N*-carbamoyl aspartate (1:2) (hereinafter referred to as “NCA-Na”), or potassium *N*-carbamoyl aspartate (1:2) (hereinafter referred to as “NCA-K”).

In some embodiments, the pharmaceutically acceptable salt of the *N*-carbamoyl aspartic acid compound provided herein where each of X and Y is independently H is a divalent metal salt (in a molar ratio of 1:1 or 2:1) formed by contacting *N*-carbamoyl aspartic acid with a hydroxide, bicarbonate or carbonate of the divalent metal in an aqueous solution.

In specific embodiments, the hydroxide, bicarbonate or carbonate of the divalent metal is preferably a hydroxide, bicarbonate or carbonate of calcium, magnesium, zinc, copper or iron, and the divalent metal salt of *N*-carbamoyl aspartic acid comprises calcium *N*-carbamoyl aspartate (in a molar ratio of 1:1 or 2:1, hereinafter referred to as “NCA-Ca (1:1)” or “NCA-Ca (2:1)” respectively), magnesium *N*-carbamoyl aspartate (in a molar ratio of 1:1 or 2:1, hereinafter referred to as “NCA-Mg (1:1)” or “NCA-Mg (2:1)” respectively), copper *N*-carbamoyl aspartate (in a molar ratio of 1:1 or 2:1, hereinafter referred to as “NCA-Cu (1:1)” or “NCA-Cu (2:1)” respectively), zinc *N*-carbamoyl aspartate (in a molar ratio of 1:1 or 2:1, hereinafter referred to as “NCA-Zn (1:1)” or “NCA-Zn (2:1)” respectively), or iron *N*-carbamoyl aspartate (in a molar ratio of 1:1 or 2:1, hereinafter referred to as “NCA-Fe (1:1)” or “NCA-Fe (2:1)” respectively).

In some embodiments, the *N*-carbamoyl aspartic acid compound provided herein is a pharmaceutically acceptable salt of *N*-carbamoyl aspartic acid and is a non-toxic acid addition salt formed from a basic amino group therein with an organic or inorganic acid.

In some embodiments, the pharmaceutically acceptable salt of the *N*-carbamoyl aspartic acid compound provided herein is a salt formed from an organic acid, including but not limited to acetate, maleate, succinate, mandelate, fumarate, malonate, malate, 2-hydroxypropionate, pyruvate, oxalate, glycolate, salicylate, glucuronate, galacturonate, citrate, tartrate, aspartate, glutamate, benzoate, *p*-toluate, cinnamate, *p*-toluenesulfonate, benzenesulfonate, methanesulfonate, ethanesulfonate, or triflate, or a combination thereof.

In some embodiments, the pharmaceutically acceptable salt of the *N*-carbamoyl aspartic acid compound provided herein is a salt formed from an inorganic acid, including but not limited to hydrochloride, hydrobromide, phosphate, sulfate, or nitrate, or a combination thereof.

The *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein has been shown a significant inhibitory activity against coccidia, especially intestinal coccidia, in coccidial inhibition assays, and as the dosage increases, the inhibitory activity against coccidia becomes stronger, showing an obvious dose response. The intestinal coccidia include, but are not limited to, *E. maxima*, *E. mitis*, *E. tenella*, *E. necatrix*, *E. acervulina*, *E. auburnensis*, *E. bovis*, *E. zuernii*, *E. deblickei*, *E. stiedai*, *E. perforans*, *E. canis*, or *E. brunetti*, preferably *E. maxima*, *E. mitis*, *E. acervulina*, or *E. brunetti*.

As used herein, “inhibition” and “inhibitory” both refer to a relative reduction in a specific response of a given activity such as coccidial viability, in the presence of the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein. Inhibition of coccidial viability can be determined by those assays described herein.

The *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein can effectively inhibiting coccidial activity and can be used as an agent to treat, prevent or ameliorate conditions in farmed animals susceptible to infection with coccidia, for use in treating, preventing, or ameliorating all diseases, disorders or symptoms associated with coccidiosis.

As used herein, “treat” or “treating” or “treatment” refers to the complete or partial alleviation of a disorder, disease or condition, or one or more symptoms associated with the disorder, disease or condition, or the mitigation or cessation of the progression or worsening of those symptoms, or the alleviation or elimination of one or more causes of the disorder, disease or condition itself. In specific embodiments, the disorder, disease or condition refers to coccidiosis or coccidioid infection.

In some embodiments, “treat” or “treating” or “treatment” refers to administering to a diseased animal a therapeutically effective amount of an agent disclosed herein to ameliorate the disease or disorder (i.e., mitigate, prevent or alleviate the progression of the disease or at least one clinical symptom thereof). In other embodiments, “treat” or “treating” or “treatment” refers to administering to a diseased animal a therapeutically effective amount of an agent disclosed herein to alleviate or ameliorate at least one physical parameter, including physical parameters that may not be perceived by the subject. In still other embodiments, “treat” or “treating” or “treatment” refers to administering to a diseased animal a therapeutically effective amount of an agent disclosed herein to modulate a disease or disorder physically (e.g., stabilizing perceptible symptoms) or physiologically (e.g., stabilizing body parameters), or both. In yet other embodiments, “treat” or “treating” or “treatment” refers to administering to a diseased animal a therapeutically effective amount of an agent disclosed herein to prevent or retard the onset, progression, or worsening of the disease or disorder.

As used herein, “prevent” or “preventing” or “prevention” refers to a method of retarding and/or preventing the full or partial onset, recurrence or spread of a disorder, disease or condition, preventing a farmed animal from developing a disorder, disease or condition, or reducing the risk of a disorder, disease or condition in a farmed animal. In specific embodiments, the disorder, disease or condition refers to coccidiosis or coccidioid infection.

As used herein, “coccidioid infection” refers to an infection caused by coccidia. The infection caused by coccidia is coccidiosis in a farmed animal in which part of the body is infected with coccidia. The term “coccidiosis” refers to an infection or disease caused by coccidia.

As used herein, “animal” refers to a farmed animal that is unable to convert inorganics into organics, and can only use organics as food for life activities such as ingestion, digestion, absorption, respiration, circulation, excretion, sensation, movement, and reproduction. The farmed animal comprises livestock (aka “domestic animals”) at various growth stages, poultry, or other animals that are artificially raised and legally captured, including pet animals.

In further embodiments, the livestock (aka “domestic animal”) includes but is not limited to pigs, cattle, sheep, horses, rabbits, minks or donkeys, preferably pigs, cattle, sheep and rabbits. The poultry includes but is not limited to chickens, turkeys, ducks, geese, or quails, preferably broilers, laying hens, ducks, geese and quails. The pet animal includes but is not limited to cats or dogs of various subspecies.

Provided herein is a method for treating a disease caused by coccidia or coccidioid infection.

In some embodiments, the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein is used in veterinary medical therapy of farmed animals, especially in the treatment of coccidiosis or coccidial infection. In some

embodiments, the therapy comprises administering an *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein to an animal suffering from a disease caused by coccidia or susceptible to infection with coccidia to prevent or treat coccidiosis in the animal. The *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein is administered to a farmed animal through admixing into feed or drinking water. A therapeutically effective amount of 100 to 2000 ppm can satisfactorily control coccidiosis or coccidial infection, based on the total amount of feed or liquid intake of a farmed animal.

The term “therapeutically effective amount” as used herein refers to an amount of a compound that, when administered to a diseased animal to treat a disease, is sufficient to be effective in treating the disease and will vary with the compound, the disease and severity, as well as the condition, age, weight, and gender of the animal to be treated.

In an embodiment, provided herein is a method for treating or preventing coccidial infection or coccidiosis, comprising administering to an animal a therapeutically effective amount of an *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein.

In an embodiment, provided herein is a method for treating or preventing coccidial infection or coccidiosis, comprising administering to a subject a therapeutically effective amount of a combination of two or more anticoccidial agents, for example, the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein in combination with one or more additional anticoccidial agents. In one embodiment of a combination of anticoccidial agents administered for the treatment of coccidiosis or coccidial infection, the additional anticoccidial agent comprises amprolium, nicarbazine, glycineamide, 3,5-dinitrobenzamide, and 2-methyl-3,5-dinitrobenzamide.

Also provided is a pharmaceutical composition comprising an *N*-carbamoyl aspartic acid compound of Formula (I), or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof.

The pharmaceutical composition provided herein serves as an effective anticoccidial agent. The *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof is uniformly dispersed in or mixed with suitable pharmaceutically acceptable excipients, and its effective amount in the pharmaceutical composition can be at a level that will exert the desired effect. The amount of pharmaceutical composition used industrially per ton of feed depends on the desired end-use level.

As used herein, the terms “comprise” and “include” can be used interchangeably and both shall be construed as specifying the presence of the claimed feature or element mentioned but not excluding the presence or addition of one or more other features or elements, and are intended to include instances encompassed by “consisting of.” The term “consisting of” means that the subject matter has at least 90%, 95%, 97%, 98% or 99% of the features or elements in the claimed composition, and can also be understood as excluding any other features or elements from the scope of any subsequent elaboration, except those that are not necessary for the technical effect to be achieved.

As used herein, the term “or” shall be understood as an inclusive “or” meaning any one or any combination. As such, “A, B, or C” refers to any one of: “A”; “B”; “C”; “A and B”; “A and C”; “B and C”; and “A, B and C”. Exceptions to this definition only arise when a combination of elements, functions, steps, or procedures is inherently mutually exclusive in some way.

The pharmaceutical composition refers to a compound set or collection comprising one or more than one compound. In some embodiments, the pharmaceutical composition can be used to prepare as a medicament for preventing or treating coccidial infection or coccidiosis in an animal, and can also be used to supplement animal feed or prepare anticoccidial animal feed to prevent or treat coccidial infection or coccidiosis in an animal.

The pharmaceutical composition is a formulation that is prepared by routine methods using conventional pharmaceutically acceptable excipients.

The excipient used in the pharmaceutical composition shall be a vehicle in which the anticoccidial agent is stable, which is compatible with the feed and which can be safely administered to an animal. The pharmaceutical composition comprises relatively large amounts of an anticoccidial agent, which is mixed with or into the feed. In order to ensure that the coccidiostat is evenly distributed in the feed, an intermediate dilution step is usually employed, in which the pharmaceutical composition is mixed with a portion of the feed, and then the resulting “intermediate mixture” is added to the remaining feed and mixed thoroughly.

The pharmaceutically acceptable excipient is selected from one or more of a pharmaceutically acceptable carrier, diluent, excipient, binder, disintegrant, wetting agent, flavoring agent, preservative, stabilizer, suspending agent, dispersant, cosolvent, buffer, copolymer, base wax, and vehicle.

“Carrier” as used herein, refers to a pharmaceutically acceptable substance that can carry active ingredients, improve their dispersion, and have good chemical stability and adsorption properties, including but not limited to, those carriers that can be used in the formulation of pharmaceutically acceptable compositions and well-known methods of preparation thereof.

“Diluent” as used herein, refers to a substance that distributes the starting additive evenly in the material, and dilutes the high-concentration additive into a low-concentration premix, which can separate trace components from each other and reduce the interaction between active ingredients to increase the stability of active ingredients without affecting the physicochemical properties of related substances.

“Excipient” as used herein, refers to a wetting agent that induces the inherent viscosity of the substance, a binder that binds the substance together, a disintegrant that breaks the entire sheet of the substance into many fine particles, a retention aid that reduces friction between particles, or an anti-sticking agent that prevents material sticking, including but not limited to, cellulose, calcium carbonate, mannitol, sorbitol, magnesium stearate, talc, vegetable oil, magnesium lauryl sulfate, starch, starch slurry, water, inorganic salts, dextrin, or powdered sugar, or the like.

“Vehicle” as used herein, refers to a solvent required to dissolve or disperse solids, including but not limited to, water, glycerin, or ethanol, or the like.

As used herein, “binder” comprises cellulose, methylcellulose, hydroxymethylcellulose, polypropylpyrrolidone, polyvinylpyrrolidone, gelatin, gum arabic, polyethylene glycol, sucrose, or starch.

5 As used herein, “disintegrant” comprises starch, hydroxymethylcellulose, hydroxypropyl starch, low-substituted hydroxypropylcellulose, ammonium bicarbonate, calcium phosphate, or calcium citrate.

As used herein, “wetting agent” comprises magnesium stearate, light anhydrous silicic acid, talc, or sodium lauryl sulfate.

10 As used herein, “flavoring agent” comprises citric acid, menthol, glycine, or wheat starch.

As used herein, “preservative” comprises sodium benzoate, sodium bisulfite, methylparaben, or propylparaben.

As used herein, “stabilizer” comprises citric acid, sodium citrate, or acetic acid.

15 As used herein, “suspending agent” comprises methylcellulose, polyvinylpyrrolidone, or aluminum stearate.

As used herein, “dispersant” comprises hydroxypropyl methylcellulose.

As used herein, “cosolvent” comprises propylene glycol, or glycofurol.

20 In specific embodiments, the pharmaceutically acceptable excipient includes, but is not limited to, ion exchangers, aluminum, aluminum stearate, lecithin, serum protein, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated plant fatty acids, water, salts or electrolytes, sodium dihydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silicon, magnesium trisilicate, polyvinylpyrrolidone, polyacrylates, waxes, polyoxyethylene-polyoxypropylene-block polymers, lanolin; sugars such as lactose, 25 glucose, and sucrose; starches such as corn starch, and potato starch; cellulose and its derivatives such as sodium carboxymethylcellulose, ethylcellulose, and cellulose acetate; gum powder; malt; gelatin; talc; excipients such as cocoa butter, and suppository wax; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; glycols such as propylene glycol, and polyethylene glycol; esters such as 30 ethyl oleate, and ethyl laurate; agar; buffers such as magnesium hydroxide, and aluminum hydroxide; alginates; pyrogen-free water; isotonic salts; Ringer's solution; ethanol, phosphate buffered solution, and other non-toxic suitable lubricants such as sodium laurate and magnesium stearate, coloring agents, release agents, coating materials, sweeteners, flavorings, fragrances, preservatives, and antioxidants.

35 In further embodiments, excipients commonly used in the pharmaceutical composition can be solid orally acceptable animal feed additives, such as distiller's dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat middling, wheat shorts, molasses solubles, corn cob meal, corn gluten feed, corn germ meal, edible vegetable substances, soybean meal, dehulled soya flour, soybean mill feed, antibiotic mycelia, crushed limestone, and soya grits, and the like. Preferably, although not 40 required, the excipients are nutritious.

45 In further embodiments, the pharmaceutical composition further comprises at least one additional anticoccidial agent that is effective against coccidia and/or nematodes, and in many cases the efficacy of such combination is greater than what would be expected from the anticoccidial agent alone. The additional anticoccidial agent includes, but is

not limited to, amprolium, nicarbazine, glycineamide, 3,5-dinitrobenzamide, and 2-methyl-3,5-dinitrobenzamide.

The specific formulation in relation to a typical pharmaceutical composition comprising the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein is:

Formulation 1: distiller's dried grains, and *N*-carbamoyl aspartic acid;

Formulation 2: corn gluten feed, and calcium *N*-carbamoyl aspartate;

Formulation 3: soybean meal, and sodium *N*-carbamoyl aspartate; or

Formulation 4: wheat middling, and propyl *N*-carbamoyl aspartate.

A typical pharmaceutical composition comprising the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein is in a water-dispersible powder obtained by uniformly dispersing the *N*-carbamoyl aspartic acid compound or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof in glucose or sucrose at a concentration of about 0.3% to about 25% by weight.

A typical pharmaceutical composition comprising the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein is in an aqueous solution obtained by uniformly dispersing the *N*-carbamoyl aspartic acid compound or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof in a glucose or sucrose aqueous solution at a concentration of about 0.3% to about 25% by weight.

The method of using the animal medicament provided herein will be described below. The method for treating diarrhea in an animal is to administer a therapeutically effective amount of an *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising the same to a diseased animal. The pharmaceutical composition provided herein can be manufactured according to conventional methods disclosed in the art. The pharmaceutical composition is in a specific target dosage form suitable for therapeutic purpose. The *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof is administered in admixture with a suitable pharmaceutical diluent, excipient, or carrier or others selected according to the route of administration and conventional pharmaceutical practice, for example, in an oral dosage form via a suitable carrier.

The pharmaceutical composition provided herein is prepared into a dosage unit according to the above formulation to maintain consistency in dosage and dose. Each unit dose of the formulation suitable for administration may contain 1 to 100 mg of the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein. In these dosage forms, the active ingredient generally constitutes from 0.5 wt% to 95 wt% of the total composition.

As used herein, the term “unit dose” refers to a physically discrete unit of an agent required for the appropriate treatment of an organism.

The pharmaceutical composition is for use in treating diarrhea in an animal. The oral dosage form includes but is not limited to oral enteric forms of tablets, pills or capsules.

- 5 Breeders or veterinarians can administer the oral dosage form comprising the pharmaceutical composition provided herein to a diseased animal by gavage or mixing it with feed.

- 10 The dosing schedule of the pharmaceutical composition provided herein will vary depending on various known factors, such as pharmacokinetic profile of a specific agent and its dosage form and route of administration; species, age and weight of the diseased animal; nature and severity of symptoms; types of agents for concurrent treatment; frequency of treatment; route of administration; and desired efficacy. A veterinarian can make the determination and prescribe an effective amount of an agent to prevent, cease, alleviate, or retard the progression of symptoms associated with the disease.

- 15 The preferred dosage level of the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof required for preventing and/or treating coccidiosis in an animal will vary to some extent depending on the specific compound used, the type and severity of the coccidiosis, as well as the weight of the animal, it will be judged by a
20 veterinarian or breeder as appropriate.

In any case, the amount of the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof administered will also depend on, for example, the solubility of the active ingredient, the formulation used, and the route of administration.

- 25 In some embodiments, the therapeutically effective amount of the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof is from 100 to 500 ppm, exhibiting the efficacy of a low-potency anticoccidial agent.

- 30 In some embodiments, the therapeutically effective amount of the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof is 1000 ppm, exhibiting the efficacy of a moderate-potency anticoccidial agent.

- 35 In some embodiments, the therapeutically effective amount of the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof is from 2000 to 5000 ppm, exhibiting the efficacy of a high-potency anticoccidial agent.

- 40 The *N*-carbamoyl aspartic acid compound as described herein comprises *N*-carbamoyl aspartic acid, and/or an alkyl ester thereof, and/or an alkali metal salt thereof, and/or a divalent metal salt thereof, in inhibiting the growth of coccidia, a suitable *N*-carbamoyl aspartic acid compound and a pharmaceutically acceptable salt thereof can be selected according to the method of their use and animal species.

- 45 In some embodiments, *Eimeria tenella* infection can be well controlled using *N*-carbamoyl aspartic acid, i.e. by feeding poultry feed supplemented with *N*-carbamoyl aspartic acid at a level of about 100 to 5000 ppm. However, for best results, it is preferred to administer *N*-carbamoylaspartic acid at a level of 1000 to 5000 ppm. Those skilled in the art will appreciate that in most cases the lowest levels consistent with

complete and adequate control of coccidiosis and the development of immunity will be used to eliminate as far as possible the risk of possible side effects induced by feeding unnecessarily high doses of agent for long periods of time. The term “feed” refers to a product that is industrially processed and produced for animal consumption. Unless
5 otherwise stated, the term “feed” or “animal feed” comprises a feedstuff and one or more additives for an animal feed, including carbohydrates, proteins, fats, vitamins, minerals, and other nutrients commonly used in commercial animal feeding.

In some embodiments, a similar dose of the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or
10 pharmaceutically acceptable salt thereof as described herein is administered to poultry to achieve similar effects in controlling coccidial infection in an anti-coccidiosis assay for poultry. In further embodiments, the *N*-carbamoyl aspartic acid compound comprises *N*-carbamoyl aspartic acid, and/or an alkyl ester thereof, and/or an alkali metal salt thereof, and/or a divalent metal salt thereof, and its pharmaceutical acceptable salt
15 includes a salt formed with an organic or inorganic acid.

The *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof as described herein can also be used as an anticoccidial agent when administered to a diseased animal through admixing into drinking water. Preferred dosage levels in
20 drinking water are generally slightly lower than those used in solid feed, for example, poultry drink approximately twice as much water as the feed they receive. It is advantageous to add such anticoccidial compound to drinking water when the compound is used therapeutically. The compound can be prepared as a water-dispersible powder so that it is uniformly dispersed in a suitable carrier such as glucose or sucrose
25 at a concentration of about 0.3% to about 25% by weight, so that an animal breeder can readily add the powder to drinking water.

BIOLOGICAL EXAMPLES

In order to make the aspects, technical solutions and advantages of the present invention more clear, the compounds, compositions and methods of their use provided herein are
30 further described in detail below through examples. It should be understood that the specific embodiments described herein are only used to illustrate the description herein and are not intended to limit the present invention.

Example 1 Assay for the inhibitory effect of *N*-carbamoyl aspartic acid compound on *Eimeria tenella* in chickens

35 One-day-old fast-growing yellow-feathered broilers were raised in a coccidia-free environment. At 18 days of age, no coccidian oocysts were found in the feces under a microscope, and then the broilers were divided into groups for testing. Removal of thin chickens or overweight chicks, healthy chickens with individual weight differences within 20 g were selected and divided into 10 groups, with 10 test chickens per group.
40 Groups 1 and 2 are a negative control group with neither agent-administration nor coccidia challenge and a positive control group infected with coccidia but without agent-administration, respectively. Groups 3 to 8 are test groups with the administration of test agents. On the day of the test grouping, different test agents were admixed into the feed according to Table 2, and food and water were freely taken during the experiment. Except for Group 1, each test chicken in each test group was given 7.5×10^4
45 sporulated oocysts of *Eimeria tenella* through the crop 24 hours after the start of this assay, and it ended on day 8 after coccidia challenge. On day 5 after coccidial infection,

- bloody stools in each test group were scored. During the experiment, the chickens' mental condition, appetite, drinking water, defecation, status, and number of deaths were observed and recorded every day. From day 4 after infection to the end of the experiment, the number of bloody stools in the feces was counted every 12 hours. On day 8 after infection, all test chickens were weighed and necropsied, cecal lesions were observed, cecal contents were taken, oocysts in cecum was counted, and the Anticoccidial Index (ACI) was calculated.

Efficacy evaluation criteria: Following the Anticoccidial Index (ACI), it is highly effective when the ACI is above 180; it is moderately effective when the ACI is between 160 and 180; and it is low effective when the ACI is between 120 and 160. The equation for calculating ACI is: $ACI = (\% \text{ survival} + \% \text{ relative weight gain}) - (\text{lesion value} + \text{oocyst value})$, wherein:

- (1) % Survival: $(\text{Total chickens per test group} - \text{Number of chickens that died due to coccidiosis during the experiment}) / \text{Total chickens per test group} \times 100\%$;
- (2) % Relative weight gain: $(\text{Average weight gain of chickens in administration group or in infected and non-administration group} / \text{Average weight gain of chickens in non-infected and non-administration group}) \times 100\%$. % Relative weight gain of chickens in the non-infected and non-administration group was 100%;
- (3) Bloody stool score: score 0 represents no bloody stools; score 1 represents less than 25% of stools contain blood; score 2 represents 25-50% of stools contain blood; score 3 represents 50-75% of stools contain blood; and score 4 represents more than 75% of stools contain blood;
- (4) Lesion score: A scale of 0 to 5 indicates the severity of lesion, where score 0 represents no lesion and score 5 represents the most severe lesion;
- (5) Lesion value: $\text{Average lesion score per group} \times 10$;
- (6) Average oocysts per chicken: After scraping the cecal contents and digesting them with 10% sodium hypochlorite for 15 min, oocysts were counted using a hemocytometer and further converted to the number of oocysts in the cecum; and
- (7) Oocyst value: The conversion relationship between the number of oocysts and the oocyst value is shown in Table 1.

Table 1 Conversion relationship between oocyst number and oocyst value

Oocysts ($N \times 10^6$)	≤ 0.1	0.1-1	1-2	2-5	5-6	6-10	10-11	≥ 11
Oocyst value	0	1	5	10	15	20	30	40

The results are as shown below:

- For the positive control group, on day 3 after infection, the chickens' feed intake decreased and they were depressed. On day 4, they had bloody stools, and a total of 3 chickens died. An autopsy of the dead chicken showed that the cecum was highly edematous and filled with blood clots, and a large number of bleeding spots were seen on the intestinal mucosa. The surviving chickens had loose feathers, drooped wings, and a few chickens shrank their necks and closed their eyes.

For the negative control group, chickens had normal appetite and no abnormalities such as bloody stools.

As for the test groups with administration, the *N*-carbamoyl aspartic acid compound in each group showed a significant inhibitory effect against *Eimeria tenella* in chickens, and showed an obvious dose response.

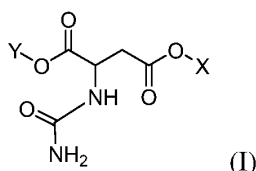
Table 2 Results of anticoccidial efficacy of each test group

Group	Test chickens	Agent/ Dose (ppm)	Average weight gain (g/chicken)	% Relative weight gain	Blood y stool score	% Survival	Lesio n value	Oocysts (N×10 ⁶)	Oocys t value	ACI
1	10	-	191	100	0	100	0	0	0	200
2	10	-	119	62.30	4	68	33	25.84	40	57.30
3	10	NCA/100	158	82.72	3	100	31	10.26	30	121.72
4	10	NCA/500	173	90.58	3	100	30	9.23	20	140.58
5	10	NCA/1000	178	93.19	2	100	16	5.82	15	162.19
6	10	NCA/2000	185	96.86	0	100	7	1.87	5	184.86
7	10	NCA/5000	192	100.52	0	100	5	1.13	5	190.52
8	10	NCA propyl ester/1000	180	94.24	2	100	16	5.79	15	163.24
9	10	NCA-Na (1:2)/1000	172	90.05	2	100	13	5.35	15	162.05
10	10	NCA-Ca (1:1)/1000	175	91.62	2	100	14	5.13	15	162.62

While particular embodiments of the present invention have been described in the foregoing, it is to be understood that other embodiments are possible within the scope of the invention and are intended to be included herein. It will be clear to any ordinary skill in the art that several modifications and adjustments can be made without departing from the concept of the present invention, and these are all within the scope of the present invention. The invention is therefore to be considered limited solely by the scope of the appended claims.

CLAIMS

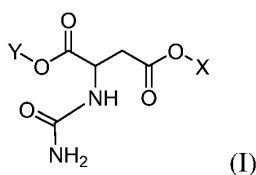
1. Use of an *N*-carbamoyl aspartic acid compound of Formula (I), or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof in the preparation of a medicament for preventing and/or treating coccidiosis in an animal:



wherein

each of X and Y is independently H, or linear or branched C₁-C₁₈ alkyl.

2. The use of claim 1, wherein the pharmaceutically acceptable salt is an alkali or divalent metal salt, preferably a sodium, potassium, calcium, zinc, or copper salt.
3. The use of claim 1, wherein the animal is a farmed animal susceptible to infection with coccidia.
4. The use of claim 1, wherein the animal is poultry susceptible to infection with coccidia, preferably chicken, turkey, duck, goose, or quail.
5. The use of claim 1, wherein the animal is a domestic animal susceptible to infection with coccidia, preferably a pig, cattle, sheep, or rabbit.
6. The use of claim 1, wherein the animal is a pet animal susceptible to infection with coccidia, preferably a cat, or dog.
7. The use of claim 1, wherein the coccidiosis is a disease caused by coccidia selected from the species of: *E. maxima*, *E. mitis*, *E. tenella*, *E. necatrix*, *E. acervulina*, *E. auburnensis*, *E. bovis*, *E. zuernii*, *E. deblickei*, *E. stiedai*, *E. perforans*, *E. canis*, or *E. brunetti*, preferably *E. maxima*, *E. mitis*, *E. acervulina*, or *E. brunetti*.
8. A pharmaceutical composition comprising an *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient:



wherein

each of X and Y is independently H, or linear or branched C₁-C₁₈ alkyl;

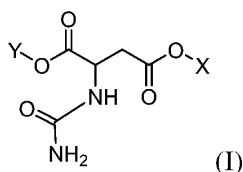
- wherein the pharmaceutically acceptable salt is an alkali or divalent metal salt, preferably a sodium, potassium, calcium, zinc, or copper salt.

9. The pharmaceutical composition of claim 8, wherein the pharmaceutical composition is in a water-dispersible powder.

10. The pharmaceutical composition of claim 8, wherein the pharmaceutical composition is in an aqueous solution.

5 11. The pharmaceutical composition of claim 8, further comprising an additional anticoccidial agent.

12. A method for preventing or treating coccidiosis, comprising administering a therapeutically effective amount of an *N*-carbamoyl aspartic acid compound of Formula (I), or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or
10 pharmaceutically acceptable salt thereof to an animal susceptible to infection with coccidia:



wherein

each of X and Y is independently H, or linear or branched C₁-C₁₈ alkyl;

15 wherein the pharmaceutically acceptable salt is an alkali or divalent metal salt, preferably a sodium, potassium, calcium, zinc, or copper salt.

13. The method of claim 12, wherein the therapeutically effective amount is from 100 to 2000 ppm.

14. The method of claim 12, wherein the *N*-carbamoyl aspartic acid compound of Formula (I) or a racemate, stereoisomer, geometric isomer, tautomer, solvate, or
20 pharmaceutically acceptable salt thereof is administered in a pharmaceutical composition according to any one of claims 8 to 11 through admixing into feed or drinking water.