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Novel urease inhibitor

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ABSTRACT

Urease, also known as urea amide hydrolase, contains two metal nickel ion active centers that catalyze the decomposition of urea to ammonia and carbon dioxide. Urease is widely found in nature, including plants, animals, and microorganisms, and greatly affects the utilization of nitrogen-containing nutrients by plants and animals. At the same time, its content can also affect the normal physiological metabolism of microorganisms, which in turn affects the survival rate of microorganisms. It can be seen that the role of urease inhibitors is very important. Urease inhibitors, such substances, can affect or even inhibit the activity of urease by direct or indirect means. Guided by computer-aided drug design, the team developed two new urease inhibitors, which are low-cost, non-toxic to plants, animals and humans, and environmentally friendly urease inhibitors. After experimentally measuring the activity of urease inhibitors, the two urease inhibitors have stronger inhibition ability than the widely used acetohydroxamic acid on the market.

Keywords: Urease; Urease inhibitor; Animal; Crop; Helicobacter pylori; Acetohydroxamic acid

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1. Background

Urease is an oligomeric enzyme containing nickel, which is a relatively common substance in various plants and animals, and has different degrees of influence on different organisms. For plants, it can catalyze the hydrolysis of urea, which will decompose urea to produce carbon dioxide and ammonia. This will reduce the proportion of nitrogen absorbed by plants, and the effect of fertilization will become poor. At the same time, the carbon dioxide produced by decomposition will also increase global carbon. The total amount of emissions exacerbates the greenhouse effect, which greatly affects the living environment of humans and other living things on the planet. For large mammals such as cattle or sheep, the production of urease in their stomachs is very likely to cause Nitrogen is decomposed as soon as it is fully absorbed, which not only increases the cost of feeding, but also delays the development of cattle and sheep and cannot meet the production needs.

In 1981, physician Marshall worked with the pathologist Robin at the Royal Hospital. When they studied stomach diseases, they discovered *H. pylori* and proposed that *H. pylori* is the root cause of gastric ulcer and gastric cancer. However, doctors and scientists at the time did not believe that bacteria could survive in a very acidic stomach environment. Because of the large differences between humans and animals, Marshall's animal experiments did not achieve the expected results. And Marshall made a crazy move. He swallowed the culture fluid containing a large amount of *Helicobacter pylori* and tried to prove it with his own body. Soon, Marshall's body began to appear uncomfortable. At first, it was just a loss of appetite and difficulty in eating. Later, when Marshall gave herself a gastroscopy, she found that her stomach mucosa was

covered with *Helicobacter pylori*, which confirmed that *Helicobacter pylori* is the main cause of gastric ulcer. Therefore, in 2005, Dr. Marshall and Professor Robin jointly won the Nobel Prize in Physiology. We can see that *Helicobacter pylori* is extremely harmful to the human body, and it often leads to ferocious stomach diseases such as gastric ulcer and gastritis, and even stomach cancer. Studies have found that *H. pylori* contains a large amount of urease and relies on these ureases for survival and reproduction. More importantly, the clinical use of triple therapy or even quadruple therapy to treat stomach diseases caused by *Helicobacter pylori* is not only complicated but also costly. In addition, with triple therapy or quadruple therapy, it is easy to reduce the effect due to the resistance of one or more of the drugs, or even no effect at all. This will cause the patient's condition to recur and even worsen, not only increasing the cost of medication, but also increasing the suffering of the patient.

In this context, how to develop effective urease inhibitors is crucial. At present, there are many products of urease inhibitors, which have a certain effect on the improvement of crop yield and the increase of animal feeding value. However, it can be confirmed that the urease inhibitors currently on the market are far from the standard for clinical drugs. Then, how to develop a better urease inhibitor, which can be applied to animal husbandry, agriculture, and human gastric ulcer disease treatment is of great significance. Such a wide range of new high-efficiency urease inhibitors will have broad prospects and produce good economic, social and ecological benefits.

2. Introduction

There are three main mechanisms of action of urease inhibitors, which are different because of

the types of urease inhibitors, mainly in the following cases.

First, competitive inhibition, such as phosphoric acid and phosphate, which competitively bind urease with urea during the reaction, reduces the chance of urea binding to the urease active site. Therefore, urea decomposition is suppressed.

Second, the urease inhibitor denatures the urease. For example, the combination of heavy metal ions and urease changes the spatial structure of urease and loses the ability to decompose urea. The concentration of urease begins to decrease and the decomposition of urea decreases.

Third, the use of specific inhibitors, the urease production process is inhibited, the expression of the urease gene is blocked, and the urease concentration is effectively reduced.

3. Product main function

3.1 Application in large-scale lactation breeding

Microorganisms in the stomach of mammals that are capable of producing urease. Microorganisms in the rumen of ruminants such as urease and bovine sheep produce urease ^[1]. Urease rapidly breaks down proteins that enter the stomach. The rate of decomposition is much greater than the rate of protein absorption in the stomach, and the animals that are reared are not well developed. Therefore, appropriate measures need to be taken to reduce the concentration and activity of urease, so that the cultured animals can make full use of the protein in the feed, and fix the nitrogen element, which is transformed into the nutrients needed for development. In addition, urea decomposes to produce ammonia and carbon dioxide at a rate that is substantially the same as the rate at which the animal utilizes ammonia. Once the urea is de-

composed too fast, the concentration of ammonia will increase significantly. Animals that are kept will be poisoned because a large amount of ammonia cannot be eliminated from the body in time. Even if the ammonia content can be excreted from the body, the ammonia concentration in the shed and the surrounding environment will rise sharply, posing a life threat to people living nearby and other organisms. Urease inhibitors can effectively alleviate the decomposition rate of urea, and the concentration of ammonia in animals can be greatly reduced. This is also an effective method to control the ammonia concentration in the feeding environment and surrounding environment.

A common urease inhibitor on the market is acetohydroxamic acid. The addition of this type of urease inhibitor to daily feeds allows for better utilization of the proteins contained in the feed. Studies have shown that the addition of an appropriate amount of urease inhibitor in the feed can effectively improve the protein utilization rate of the livestock, which is manifested in the apparent increase in the protein content of the dairy product ^[2]. The selling price of livestock raised is greatly increased, and the utilization of protein in the feed is increased, which reduces the high cost of adding protein.

The new urease inhibitor developed by the team is called "Yuanqi Nitrogen". In contrast, the urease inhibitors developed by the team are more effective than acetohydroxamic acid and are more efficient to use. This new type of urease not only reduces urease activity, but also allows the animals to make full use of the protein in the feed. The protein utilization rate is increased by at least 6.8% over the original urease inhibitor. The team developed a new drug for the development of traditional drug resistance. According to experiments, urease inhibitors have a good

application effect in increasing production, improving feed utilization, and reducing greenhouse gas emissions.

	Performance	safety	scope of application
Current urease inhibitors	As a new additive in feed, the optimum ratio of ingredients is around 7%.	It usually has a small amount of toxicity, and frequent use can cause the accumulation of toxins to be harmful to the intended use.	Commonly used in dairy cows, raising, beef cattle and other ruminants.
Urease inhibitors produced by the company	The semi-inhibitory concentration is significantly improved, and the optimum ratio in feed is about 4%.	The use of cloud computing to screen out green, non-toxic medicines is highly safe.	Suitable for ruminants, because it is non-toxic and harmless, it can also be used in other animals to reduce nitrogen emissions.
Improvements	nearly double the efficiency	meet the medicinal standards, green, non-toxic, safer	more applicable

3.2 Application in crop growth

The nitrogen content of urea is about 45%, which is one of the most widely used nitrogen fertilizers in agriculture. Urea is poured into the field as a fertilizer and will soon be hydrolyzed. However, the rate of urea absorption by crops is too much different from the rate at which urea is decomposed in plants. Plants do not have time to absorb a large amount of nitrogen, and it is easy to increase the local salt concentration and cause damage to the roots of the seedlings [3]. In addition, the dissolved ammonia gas dissolves in the water, which raises the pH of the soil, prevents the conversion of nitrous acid to nitrate, and the nitrite begins to accumulate, further contaminating the groundwater [4]. Under the action of urease in plants and urease contained in soil, the rate of urea hydrolysis is too

fast. In the actual production process, a certain amount of urease inhibitor will be in the fertilizer, thereby achieving the purpose of inhibiting urease activity, and delaying the decomposition and conversion of urea. Urease inhibitors increase the effective use of nitrogen in the soil and increase crop yield. At the same time, it reduces ammonia and greenhouse gas emissions and is an important measure for green planting. The new urease inhibitors produced by our team are equally suitable for agricultural planting, and the cost is lower in the market comparison. In the production of stable fertilizers, urease and plant urease activity in plants can be reduced, urea hydrolysis can be weakened, and urease inhibitors can be used as nitrogen fertilizer synergists, which can effectively improve the utilization rate of fertilizer nitrogen.

	Environmental impact	effectiveness	scope of application
The current urease inhibitors	greatly affected by external factors such as soil and pH, and the effect is unstable.	As a synergist in fertilizers, the optimum ratio of ingredients is around 0.5%.	It is commonly used in straw and coarse grains such as corn and wheat.
The urease inhibitors produced by the team	affected by external factors such as environment and pH, but the adapted pH range becomes larger and the effect is more stable.	The half-inhibition concentration is significantly improved, and the optimum ratio in the fertilizer is about 0.3%.	It is suitable for straw and coarse grains such as corn and wheat, and it also has certain promotion effects for other agricultural products.
Improvements	stability is improved	performance is nearly doubled	the scope is wider.

3.3 In the treatment of *Helicobacter pylori*

Helicobacter pylori is mainly parasitic in the stomach of humans, and is also present in plants and animals. It is a highly infectious pathogen. The world's population is generally affected by *Helicobacter pylori*, in which developing countries, such as China, have the highest prevalence of infection. *Helicobacter pylori* is the main therapeutic factor for gastritis, gastric ulcer and duodenal ulcer. The detection rate of Hp in the general population in China is quite high, and the Hp infection rate is 55% [5]. *Helicobacter pylori* prefers a neutral living environment. Urease rapidly decomposes urea, generates a large amount of ammonia gas, and neutralizes gastric acid in the stomach to form a neutral environment for the survival and reproduction of *Helicobacter pylori*. In view of the living habits of *Helicobacter pylori*, it is believed in medicine and pharmacy that inhibition of urease can achieve the effect of inhibiting or even killing *Helicobacter pylori*. The new inhibitors invented by the team not only inhibit urease, but also inhibit the human proton pump, achieve a powerful treatment of gastric diseases caused by *Helicobacter pylori*, and also serve as an innovative way to treat *Helicobacter pylori*. In contrast, traditional triple and quadruple therapy take longer

to take drugs, which are prone to side effects such as physical discomfort and increased drug resistance. Repeated episodes of the disease increase the cost of medication for patients. As a new drug, it has little side effects and is not resistant to drugs.

Product Highlights

1, High-efficiency inhibition: It has a rapid inhibitory effect on urease, and the half-inhibition concentration is smaller than other urease inhibitors, and is higher than the most widely used acetohydroxamic acid on the market.

2, low cost: the use of big data cloud computing to quickly locate the screening range, virtual screening cloud computing, high-speed and accurate calculation of simulated big data, greatly saving manpower, material and financial resources, resource utilization has increased significantly. The treatment cycle is shortened and the cost of medication is reduced.

3, non-toxic: from natural products, the possibility of drug formation is large and stable under acidic conditions, non-toxic to animals, plants, human body, no side effects. Humans and animals can quickly absorb the peak plasma concentration after oral administration. They do not need to concentrate on the microcapsules and microtubules of the gastric parietal cells, and the

effect is faster, the effect of raising the pH is stronger, and the safety is better.

4, No pollution: reduce urease activity, increase urea utilization rate, reduce urea excretion in

dairy cow urine, reduce environmental ammonia concentration, improve air pollution status of dairy farms, and achieve the goal of controlling ecological environment pollution.

Ammonia emissions from cows without urease inhibitors Kg/(head/year)		27.41
Consumption of common urease inhibitors	Cow's ammonia emissions Kg (head/year)	13.32 (about 48.6%)
	Emission reduction Kg / (head/year)	14.09 (about 51.4%)
Consumption of inhibitors developed by the team.	Ammonia emissions from dairy cows Kg/(head/year)	10.50 (about 38.3%)
	Emission reduction Kg / (head/year)	16.91 (about 61.7%)

The ammonia in the greenhouse is mainly produced by hydrolysis of urea. Under the catalytic action of urease, urea is hydrolyzed to produce ammonia and carbon dioxide: $\text{CO}(\text{NH}_2)_2 + \text{H}_2\text{O} = \text{CO}_2\uparrow + 2\text{NH}_3\uparrow$. At 10°C or higher, urea is hydrolyzed completely in about 2 hours. When the ammonia concentration in the greenhouse reaches or exceeds a certain amount, it will have a bad influence on the body of the cow and the breeder, and the ammonia will eventually be discharged into the atmosphere, which will adversely affect the air and water in the ecosystem, and then It will cause serious consequences such as smog and eutrophication of water bodies.

According to relevant data, after adding the urease inhibitors commonly found in the market, the urea excretion in the urine of the cows was reduced by 49.5%, and the ammonia gas generation in the greenhouse was reduced by about 51.4%. After adding “yuanqi nitrogen” to the feed, the urea excretion in the cow's urine decreased by 56.2%, reducing the formation of ammonia gas in the greenhouse by about 61.7%. Compared with the addition of ordinary urease inhibitors, “energy nitrogen” can increa-

se the utilization rate of urea to a greater extent, thereby reducing the emission of ammonia by about 10.3%, more effectively reducing the concentration of ammonia in the greenhouse, and improving the cows. The odor of the air in the farm reduces the impact of excessive ammonia concentration on the growth and human health of the cows, while allowing less ammonia to be directly discharged into the atmosphere, reducing the pollution of the ecological environment.

5. Inactive chemical properties: The effectiveness of urease inhibitors in feed or soil is less affected by soil moisture, pH, urea, organic matter, climatic conditions, fertilization and fertilization. Therefore, it is not easy to be oxidized, and it does not easily affect other nutrients.

6, a wider range of applications: can be used in agricultural compound fertilizer, animal feed additives, biomedicine.

Cost Control

The basic principle of the team's control of marketing is to exchange the smallest output for maximum output or return. Urease inhibitor products for agriculture and animal husbandry, the team produces by means of production technology and product foundry. The cost of raw ma-

terials, equipment, etc. is not borne by the team. The team only needs to provide the agency fee to the company or factory that produces the product. Therefore, the main costs are foundry costs, transportation costs, and cost of sales. The team should calculate the cost of the sales team based on the cost of the sales team, the promotion expenses, the sales and transportation costs, and the establishment of the farmer's feed sales point. Applying drugs for the treatment of gastric ulcers, the team realized technology transfer, allowing larger pharmaceutical groups and companies to conduct phase III clinical trials of drugs, and the team can obtain high technology transfer fees. The company should calculate according to the operation mode of the product. The expenditure of each expense should be closely related to the actual operation, and detailed records should be made. This also ensures that the price of the product delivered to the customer is much lower.

Discuss

For new drugs, due to the wide range of market coverage, mature technology, good test results, and special characteristics of the pharmaceutical industry, the company adopts different sales methods. Sales require strong resilience and professional management, so professional personnel are introduced as the guidance of the sales team. The sales method can be as follows: 1, Establish Yichang as a divergent center and establish a farmer's feed purchase station.

As a new type of urease inhibitor for research and development of ruminant farmers such as cattle and sheep, and nitrogen fertilizer synergist for crop growers, functional feed is sold separately. Due to the separate sale and the large supply characteristics, the site selection does not need to be too strict, and the cost of the offline store is saved to some extent.

2, Direct supply of products to existing sellers of feeds under the line

The new functional feeds use the existing platform to help sell, extract the share, and expand the sales range. At the same time, we can also enhance the visibility of our products by leveraging the visibility of existing platforms and the trustworthiness of our sellers.

3, The sales platform is extensive and the core competitiveness is obtained.

Traditional feed can only be used in the feed market for sale. At present, the functional feed additive developed by our company can be applied to major agricultural towns and rural gathering points, and there is no need to set up a separate store in the fixed market.

4, online comprehensive publicity, expand product visibility

For feeds, farmers can be vigorously promoted through agricultural and rural broadcasting methods; drugs will be discussed with major pharmaceutical companies, and the company's research results and scientific and technological content will be actively promoted. Use the mass network user platform such as Taobao, Weidian, WeChat and Weibo to promote products.

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