

NEW COMPOUND, PREPARATION METHOD AND USE THEREOF

BACKGROUND

Field of the Invention

The present application relates to the field of biomedical chemistry, and specifically, to a new compound, a preparation method and use thereof.

Related Arts

Drugs refer to medicines that are used repeatedly and continuously for non-medical purposes and can cause dependence (that is, addiction). There is an increasing number of drug addicts, and their ages are getting younger. Only in China, there are more than 14 million drug addicts, while the number is more than 200 to 400 million in the whole world. Drugs are becoming more and more harmful to the world, and the types of drugs are becoming more and more diverse, from opium and marijuana to heroin, ketamine, methamphetamine, and the like. The proliferation of drugs can directly endanger physical and mental health of people, and brings a huge threat to economic development and social progress.

Detoxication refers to the withdrawal of drug addicts from taking and injecting drugs and the drug addiction. Detoxification treatment for drug addicts may generally include 3 stages: detoxification, rehabilitation, and counseling for re-entering society. Currently, there are 3 commonly used methods for detoxication: natural withdrawal, drug withdrawal, and non-drug withdrawal. The natural withdrawal method is a mandatory detoxication method and lacks humanity. The drug withdrawal method, also known as drug detoxification treatment, refers to a detoxication method by providing drug addicts with withdrawal drugs to relieve and reduce the withdrawal symptoms in a manner of replacement and decreasing dosage and gradually achieve detoxification, which is the most important detoxication method. Common detoxication drugs may also be classified into 3 categories: opioid chemicals, non-opioid chemicals, and traditional Chinese medicines. However, neither of those detoxication methods can help drug addicts to get rid of drugs physically and psychologically, and they are prone to lapse and relapse. To date, there is no good solutions to this. Therefore, drug detoxication and prohibition have become a worldwide problem. For this reason, people have been trying to find a safe detoxication drug with no dependence.

The traditional Chinese medicines have a long history in detoxication. Compared with the most commonly used replacement treatments such as methadone and naltrexone (both are opioid chemicals), it has the advantages of good compliance, no addiction, definite efficacy on protracted

symptoms, and high ethical rate (the proportion of drug addicts who do not relapse after detoxication). Currently, the traditional Chinese medicines used in detoxication are mostly compound preparations evolved on the basis of traditional detoxication recipes. They have many complex effective active ingredients, and their detoxication mechanism cannot be clearly determined. In addition, there are few researches on pharmacology and toxicology, thereby limiting the scientization, standardization, and internationalization of the traditional Chinese medicine compound preparations for detoxication. Therefore, the research on the active ingredients and detoxication mechanism of a single Chinese medicine is beneficial to the use and development of the Chinese medicine detoxication drugs.

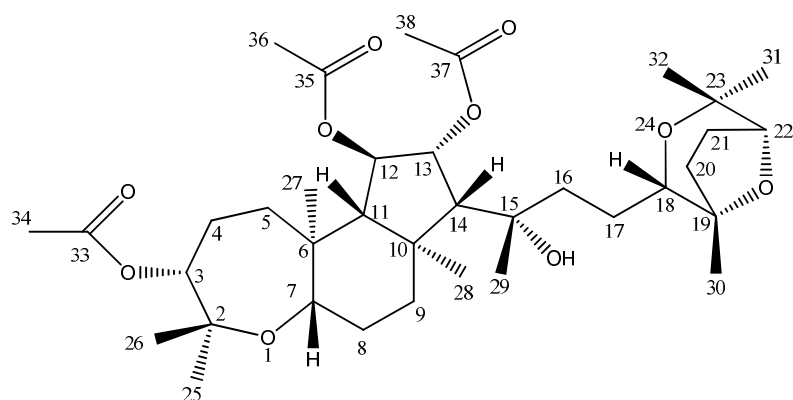
Limax is a pulmonate mollusk, belonging to the gastropod Gastropoda, Limacidae. Because its body surface is covered with mucus, it is commonly known as "sticky worm, slug, shellless snail, snail, and attached snail". From the perspective of Chinese Medicine, Limax is "salty and cold; acting in the lung meridian, the liver meridian, and the large intestine meridian". It has effects of expelling wind for relieving convulsion, and clearing away heat and removing toxic substances. It is mainly used for treating crooked eye and mouth due to apoplexy, tendon and vessel contracture, infantile convulsion, wheeze, pharyngeal swelling, sore throat, carbuncle, subcutaneous nodule, and swelling and pain due to hemorrhoid. The traditional Chinese medicine uses Limax to treat disorders such as tumors, asthma, and bronchitis. Existing studies have indicated that Limax contains various ingredients such as protein and polysaccharide. Furthermore, the pharmacological studies have found that Limax plays a role in anti-tumor, anti-asthma and the like. In recent years, studies on the efficacy of the Limax extract have mostly focused on anti-tumor and its effects on respiratory diseases. Among others, the researches on its anti-tumor effects have become a hot spot in domestic and foreign researches. In the researches on the folk prescriptions and secret recipes of Chinese herbal medicine, the inventor found that the Limax extract has the efficacy of detoxication treatment, and has been committed to this research. The related active ingredients have been extracted and separated from the Limax extract.

SUMMARY

An object of the present application is to provide a new compound and a preparation method thereof, which provides a new idea for the research and development of Chinese medicine detoxication products.

The present application uses the following technical solutions:

A new compound is provided, which has a structure of:



Further, the new compound has the chiral C configurations of: C3, R; C6, S; C7, S; C10, S; C11, R; C12, R; C13, R; C14, R; C15, S; C18, S; C19, S; and C22, R.

Further, the new compound has a molecular formula: $C_{36}H_{58}O_{10}$; molecular weight: 650; melting point: 228-229°C; and solubility: white needle-like crystal, insoluble in water, hardly soluble in acid and alkali, easily soluble in ethyl acetate and acetic acid, and soluble in methanol, ethanol, acetone, and chloroform.

Further, the new compound is prepared by extracting and separating from Limax, wherein the Limax comprises one or more of *Vaginulus alte* (Ferussac), *Limax maximus* L., *L. flavus* L., *Agriolimax agrestis* L., and *Phiolomycus bilineatus*.

A method for preparing the new compound as described above is provided, including a supercritical CO_2 extraction method and a solvent extraction method.

Further, in the method for preparing the new compound as described above, the supercritical CO_2 extraction method includes the following steps:

- R1. sorting Limax, removing impurities, and pulverizing, to obtain Limax powder;
- R2. putting the Limax powder into a supercritical CO_2 extractor for extraction to obtain an extract;
- R3. adding vegetable oil to the extract, heating and mixing evenly, cooling, standing, and filtering to obtain a precipitate, washing the precipitate with a washing solvent, and drying to obtain a dried precipitate; and
- R4. adding a crystallization solvent to the dried precipitate, heating to dissolve, cooling, standing to precipitate white needle-like crystals, and filtering to obtain the crystals, recrystallizing, and drying, to obtain the new compound.

Furthermore, in the method for preparing the new compound as described above, the pulverizing in R1 includes pulverizing into 10-30 meshes.

Furthermore, in the method for preparing the new compound as described above, the conditions for the supercritical CO₂ extraction in R2 include: pressure: 20-30 kPa, temperature: 60-70°C, flow: 400-500 PV, and extraction time: 3-5 h.

Furthermore, in the method for preparing the new compound as described above, the vegetable oil in R3 is tea seed oil, camellia seed oil, soybean oil, or olive oil;

the vegetable oil is added in a weight ratio of "extract: vegetable oil=2-6:1-3";

the heating is carried out at a temperature of 60-80°C;

the time for the standing is 7-10 days; and

the washing includes washing with n-hexane, petroleum ether, or 120#gasoline for 3-5 times, with an amount of 200-500 ml each time.

Furthermore, in the method for preparing the new compound as described above, the crystallization solvent in R4 is methanol, acetone, ethyl acetate, or chloroform added at an amount of 3-10 times the weight of the dried precipitate; and the time for the standing is 7-10 days.

Further, in the method for preparing the new compound as described above, the solvent extraction method includes the following steps:

S1. sorting Limax, removing impurities, and pulverizing, to obtain Limax powder;

S2. heating reflux extraction the Limax powder with a solvent, filtering, leaving a filtrate and recovering the solvent from the filtrate under reduced pressure until the recovery is complete, to obtain a thick paste;

S3. adding the thick paste to a silica gel chromatography column, eluting with an elution solvent, collecting an eluate, and recovering the elution solvent from the eluate under reduced pressure, to obtain a thick substance; and

S4. adding a dissolution solvent to the thick substance, heating to dissolve completely, cooling, and freezing to precipitate white crystals, filtering, leaving a filtrate, recovering the dissolution solvent from the filtrate under reduced pressure, and re-standing to precipitate white needle-like crystals, filtering, and drying, to obtain the crystals, recrystallizing, and drying, to obtain the new compound.

Furthermore, in the method for preparing the new compound as described above, the pulverizing in S1 includes pulverizing into 10-30 meshes.

Furthermore, in the method for preparing the new compound as described above, the solvent in S2 is one or more of ethanol, methanol, acetone, chloroform, 120#gasoline, n-hexane, petroleum ether, diethyl ether, and ethyl acetate; and

the heating reflux extraction includes heating reflux extraction 1-3 times with the solvent, with an amount of 5-15 times the weight of the Limax powder each time, for 1-3 h.

Furthermore, in the method for preparing the new compound as described above, the adding to the silica gel chromatography column in S3 specifically includes adding silica gel to the thick paste at an amount of 4-6 times the weight of the thick paste, mixing evenly, and adding to the chromatography column pre-filled with silica gel at an amount of 2-4 times the weight of the thick paste; and

the elution solvent is methanol, acetone, or ethyl acetate, and the amount of the elution solvent used is: the volume of the elution solvent: the total weight of the silica gel in the chromatography column=1.5-3:1-2.

Furthermore, in the method for preparing the new compound as described above, the dissolution solvent in S4 is methanol or ethanol added in an amount of 4-8 times the volume of the thick substance; the freezing is carried out at a temperature of 2-10°C for 24 hours; the recovering the solvent from the filtrate under reduced pressure comprises concentrating to 50-60% of the original volume; and the time for the re-standing is 7-10 days.

Furthermore, in the method for preparing the new compound as described above, before the eluting with an elution solvent in S3, a step of pre-elution with a pre-elution solvent is performed, wherein the pre-elution solvent is petroleum ether, n-hexane, or 120#gasoline, and the amount of the pre-elution solvent used is: the volume of the pre-elution solvent: the total weight of the silica gel in the chromatography column=1-3:0.5-1.5.

Use of the above-mentioned new compound in the preparation of medicines, health foods, and food for preventing or treating withdrawal or withdrawal-like symptoms is provided.

Use of the above-mentioned new compound in the preparation of medicines, health food, and food for inhibiting withdrawal symptoms in morphine-dependent animals is provided.

The new compound of the present application is prepared by separating from the extract of Chinese medicine Limax (scientific name: *Agriolima agrestis*) through a simple method, and named 3,12,13-triacetyl limaxol A after identification, with a chemical name of (3R,5aS,7aS,8R,9R,10R,10aR,10bS)-dodecahydro-8-((S)-2-hydroxy-4-((1S,2S,5R)-1,4,4-trimethyl-3,8-dioxa-bicyclo[3.2.1]octan-2-yl)butan-2-yl)-4,4,7a,10b-tetramethyl-1H-indeno[5,4-b]oxepine-3,9,10-triol triacetate. Pharmacological tests have proved that the compound has a significant inhibitory effect on withdrawal jumping symptoms in morphine-dependent animals 1 hour after intragastric administration, and still shows an inhibitory trend after 3 hours, indicating that the compound has

significant effects on physiological or psychological dependent detoxification or detoxication, and has potential application value in the preparation of medicines, health food, and food for detoxification, detoxication, or similar drug-dependent treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an X-ray crystal structure of the new compound according to the present application.

FIG. 2 to FIG. 5 show carbon (C) NMR spectra data and hydrogen (H) NMR spectra data of the new compound according to the present application.

FIG. 6 shows mass spectrometry (MS) data of the new compound according to the present application.

FIG. 7 shows infrared (IR) spectroscopy data of the new compound according to the present application.

FIG. 8 shows inhibition rates of methadone, Limax extract, and different doses of 3,12,13-triacetyl limaxol A on morphine-withdrawal jumping symptoms,

where: 1: methadone 20 mg/kg (1 h);

2: Limax crude extract 10 g/kg (1 h);

3: 3,12,13-triacetyl limaxol A 0.5 g/kg (1 h);

4: 3,12,13-triacetyl limaxol A 0.25 g/kg (1 h);

5: 3,12,13-triacetyl limaxol A 0.125 g/kg (1 h); and

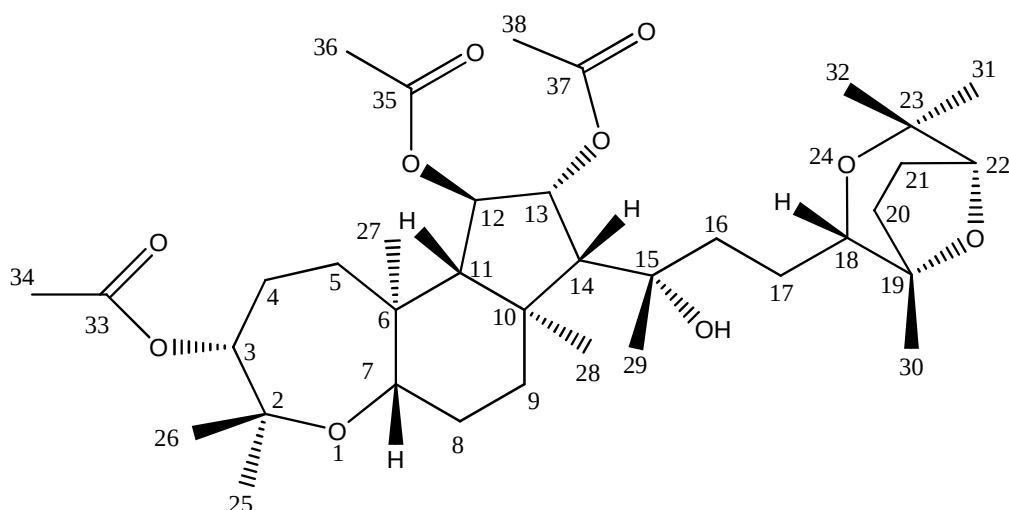
6: 3,12,13-triacetyl limaxol A 0.5 g/kg (3 h).

DETAILED DESCRIPTION

I. A New Compound

Example 1

The present application provides a new compound having a specific structure of:



The specific information of the compound is as follows:

(1) The compound is named "3,12,13-triacetyl limaxol A";

(2) Chiral C configurations of the compound include: C3, R; C6, S; C7, S; C10, S; C11, R; C12, R; C13, R; C14, R; C15, S; C18, S; C19, S; and C22, R with the crystal structure shown in FIG. 1;

(3) Physical and chemical properties and spectra data include:

- ① molecular formula: $C_{36}H_{58}O_{10}$; molecular weight 650;
- ② state: white needle-like crystals;
- ③ melting point: 228-229°C;
- ④ solubility: insoluble in water, hardly soluble in acid and alkali, easily soluble in ethyl acetate and acetic acid, and soluble in methanol, ethanol, acetone, and chloroform;
- ⑤ carbon (C) and hydrogen (H) NMR spectra data, as shown in Table 1 and FIG. 2 to FIG. 5;

Table 1. NMR data

MR2

¹³C and ¹H-NMR data (500 MHz, in CD₃OD)

NO	δ _C	δ _H (J in Hz)	NO	δ _C	δ _H (J in Hz)
1			20	37.7	1.66, 2.06 (m, 2H)
2	76.5		21	25.9	1.83, 2.14 (m, 2H)
3	81.0	4.82 (d, J = 9.8 Hz, 1H)	22	84.0	3.85 (d, J = 7.0 Hz, 1H)
4	27.5	2.01, 2.05 (m, 2H)	23	74.2	
5	42.1	1.36 (m, 2H)	24		
6	41.8		25	23.4	1.12 (s, 3H)
7	77.5	3.37 (dd, J = 11.6, 4.5 Hz, 1H)	26	24.8	1.17 (s, 3H)
8	28.7	1.51, 1.72 (m, 2H)	27	15.1	0.97 (s, 3H)
9	39.9	1.51, 1.98 (m, 2H)	28	18.2	1.36 (s, 3H)
10	46.3		29	26.7	1.28 (s, 3H)
11	60.6	1.50 (d, J = 11.5 Hz, 1H)	30	22.6	1.26 (s, 3H)
12	80.0	5.31 (dd, J = 11.5, 2.5 Hz, 1H)	31	27.0	1.04 (s, 3H)
13	83.4	5.42 (dd, J = 7.6, 2.5 Hz, 1H)	32	27.2	1.38 (s, 3H)
14	59.9	1.84 (d, J = 7.6 Hz, 1H)	33	171.6	
15	76.5		34	21.6	2.10 (s, 3H)
16	41.8	1.76-1.79 (m, 2H)	35	172.3	
17	27.2	1.57, 1.91 (m, 2H)	36	21.4	2.02 (s, 3H)
18	82.8	3.22 (dd, J = 10.8, 4.0 Hz, 1H)	37	172.3	
19	81.8		38	21.2	2.00 (s, 3H)

⑥ mass spectrometry (MS) data, as shown in FIG. 6; and

⑦ infrared (IR) spectroscopy data, as shown in FIG. 7.

II. A method for preparing a new compound

Example 2

A method for preparing a new compound (a supercritical CO₂ extraction method) includes the following steps:

R1. Limax, after sorting and removing impurities, was pulverized into 30 mesh powder for later use;

R2. 50 kg of Limax powder was put into a supercritical CO₂ extractor for extraction under the extraction conditions: pressure 20 kPa, temperature 60°C, flow 400-500 PV, and extraction time 3 h, obtaining 2.9 kg of ointment-like extract for later use;

R3. tea seed oil was added to the extract in a weight ratio of "extract: tea seed oil=2:1", that is, 2.9 kg of the extract and 1.45 kg of tea seed oil were put into a mixing tank; the mixture was heated at 60°C, mixed evenly, cooled and stood for 7 days to precipitate, and then filtered to obtain 198 g of precipitate; the precipitate was washed with petroleum ether (200 ml × 3) under heating, and then dried, to obtain 123 g of precipitate; and

R4. the precipitate was added to 800 ml of methanol, and dissolved by heating, cooled, and stood for 7 days to precipitate white needle-like crystals, filtered to obtain the crystals; an

appropriate amount of ethyl acetate was added to dissolve the crystals, filtered, and the filtrate was stood for 7 days to precipitate white needle-like crystals, filtered and dried to obtain 95.4 g of the new compound, 3,12,13-triacetyl limaxol A.

Example 3

A method for preparing a new compound (a supercritical CO₂ extraction method) includes the following steps:

R1. Limax, after sorting and removing impurities, was pulverized into 20 mesh powder for later use;

R2. 60 kg of Limax powder was put into a supercritical CO₂ extractor for extraction under the extraction conditions: pressure 25 kPa, temperature 65°C, flow 400-500 PV, and extraction time 4 h, obtaining 3.5 kg of ointment-like extract for later use;

R3. soybean oil was added to the extract in a weight ratio of "extract: soybean oil=4:2", that is, 3.5 kg of the extract and 1.75 kg of soybean oil were put into a mixing tank; the mixture was heated at 65°C, mixed evenly, cooled and stood for 8 days, and then filtered to obtain 235 g of precipitate; the precipitate was washed with n-hexane (300 ml × 3), and then dried, to obtain 151 g of precipitate; and

R4. the precipitate was added to 500 ml of ethyl acetate, and heated to dissolve, cooled, and stood for 8 days to precipitate white needle-like crystals, and filtered to obtain the crystals; an appropriate amount of ethyl acetate was added to dissolve the crystals, filtered, and the filtrate was stood for 8 days to precipitate white needle-like crystals, filtered and dried to obtain 116.0 g of the new compound, 3,12,13-triacetyl limaxol A.

Example 4

A method for preparing a new compound (a supercritical CO₂ extraction method) includes the following steps:

R1. Limax, after sorting and removing impurities, was pulverized into 20 mesh powder for later use;

R2. 100 kg of Limax powder was put into a supercritical CO₂ extractor for extraction under the extraction conditions: pressure 28 kPa, temperature 68°C, flow 400-500 PV, and extraction time 4.5 h, obtaining 5.9 kg of ointment-like extract for later use;

R3. olive oil was added to the extract in a weight ratio of "extract: olive oil=5:2.5", that is, 5.9 kg of the extract and 2.95 kg of olive oil were put into a mixing tank; the mixture was heated at 70°C, mixed evenly, cooled and stood for 9 days, and then filtered to obtain 393 g of precipitate; the

precipitate was washed with 120#gasoline (400 ml × 4), and then dried, to obtain 255 g of precipitate; and

R4. the precipitate was added to 2200 ml of acetone, and heated to dissolve, cooled, and stood for 9 days to precipitate white needle-like crystals, and filtered to obtain the crystals; an appropriate amount of ethyl acetate was added to dissolve the crystals, filtered, and the filtrate was stood for 9 days to precipitate white needle-like crystals, filtered and dried to obtain 196.1 g of the new compound, 3,12,13-triacetyl limaxol A.

Example 5

A method for preparing a new compound (a supercritical CO₂ extraction method) includes the following steps:

R1. Limax, after sorting and removing impurities, was pulverized into 10 mesh powder for later use;

R2. 150 kg of Limax powder was put into a supercritical CO₂ extractor for extraction under the extraction conditions: pressure 30 kPa, temperature 70°C, flow 400-500 PV, and extraction time 5 h, obtaining 8.7 kg of ointment-like extract for later use;

R3. camellia seed oil was added to the extract in a weight ratio of "extract: camellia seed oil=6:3", that is, 8.7 kg of the extract and 4.36 kg of camellia seed oil were put into a mixing tank; the mixture was heated at 80°C, mixed evenly, cooled and stood for 10 days, and then filtered to obtain 581 g of precipitate; the precipitate was washed with n-hexane (500 ml × 5), and then dried, to obtain 372 g of precipitate; and

R4. the precipitate was added to 5000 ml of chloroform, and heated to dissolve, cooled, and stood for 10 days to precipitate white needle-like crystals, and filtered to obtain the crystals; an appropriate amount of ethyl acetate was added to dissolve the crystals, filtered, and the filtrate was stood for 10 days to precipitate white needle-like crystals, filtered and dried to obtain 287.3 g of the new compound, 3,12,13-triacetyl limaxol A.

Example 6

A method for preparing a new compound (a solvent extraction method) includes the following steps:

S1. Limax, after sorting and removing impurities, was pulverized into 20 mesh powder for later use;

S2. 100 kg of Limax powder was put into a multi-functional extraction tank, and extracted at reflux with n-hexane for 2 times, firstly with an amount of 10 times the weight of the Limax powder

for 1.5 h, and then with an amount of 8 times the weight of the Limax powder for 1.0 h; each of the extracts was filtered, and the filtrates were combined, and the n-hexane was recovered under reduced pressure until the recovery was complete, to obtain 8.10 kg of an oily thick paste;

S3. 40.5 kg of silica gel was added to the thick paste and mixed evenly; the mixture was added to a chromatography column pre-filled with 24.3 kg of silica gel, and pre-eluted with petroleum ether in the ratio of "the volume of petroleum ether: the total weight of the silica gel in the chromatography column=2:1" (that is, 129.6 L of petroleum ether), and then eluted with ethyl acetate in the ratio of "the volume of ethyl acetate: the total weight of the silica gel in the chromatography column=2.25:1.5" (that is, 97.2 L of ethyl acetate), the ethyl acetate eluate was collected, and concentrated under reduced pressure to recover ethyl acetate, thereby obtaining 4.3 kg of a thick substance; and

S4. 27.0 L of methanol was added to the thick substance, heated to dissolve completely, and frozen at 6°C for 24 hours, to precipitate white crystals, filtered, and the filtrate was concentrated under reduced pressure to recover about 14.9 L of methanol, and then stood for 9 days to precipitate white needle-like crystals, filtered and dried to obtain 359 g of crystals, 1.0 L of ethyl acetate was added to the crystals, heated to dissolve, cooled, and stood for 9 days, to precipitate white needle-like crystals, filtered and dried to obtain 210.4 g of the new compound, 3,12,13-triacetyl limaxol A.

Example 7

A method for preparing a new compound (a solvent extraction method) includes the following steps:

S1. Limax, after sorting and removing impurities, was pulverized into 30 mesh powder for later use;

S2. 50 kg of Limax powder was put into a multi-functional extraction tank, and extracted at reflux with a mixed solvent (methanol: diethyl ether: ethyl acetate=1:1:1) at an amount of 15 times the weight of the Limax powder for 3 h, filtered, and the mixed solvent was recovered from the filtrate under reduced pressure until the recovery was complete, to obtain 3.98 kg of an oily thick paste;

S3. 23.8 kg of silica gel was added to the thick paste and mixed evenly; the mixture was added to a chromatography column pre-filled with 15.9 kg of silica gel, and pre-eluted with 120[#]gasoline in the ratio of "the volume of 120[#]gasoline: the total weight of the silica gel in the chromatography column=1:0.5" (that is, 79.4 L of 120[#]gasoline), and then eluted with methanol in the ratio of "the

volume of methanol: the total weight of the silica gel in the chromatography column=1.5:1" (that is, 59.5 L of methanol), the methanol eluate was collected, and concentrated under reduced pressure to recover methanol, thereby obtaining 2.07 kg of a thick substance; and

S4. 13.0 L of ethanol was added to the thick substance, heated to dissolve completely, and frozen at 10°C for 24 hours, to precipitate white crystals, filtered, and the filtrate was concentrated under reduced pressure to recover about 6.5 L of ethanol, and then stood for 7 days to precipitate white needle-like crystals, filtered and dried to obtain 178.1 g of crystals, 500 ml of ethyl acetate was added to the crystals, heated to dissolve, cooled, and stood for 7 days, to precipitate white needle-like crystals, filtered and dried to obtain 103.8 g of the new compound, 3,12,13-triacetyl limaxol A.

Example 8

A method for preparing a new compound (a solvent extraction method) includes the following steps:

S1. Limax, after sorting and removing impurities, was pulverized into 10 mesh powder for later use;

S2. 150 kg of Limax powder was put into a multi-functional extraction tank, and extracted at reflux with a mixed solvent (ethanol: acetone=1:1) for 3 times, firstly with an amount of 12 times the weight of the Limax powder for 2.5 h, secondly with an amount of 8 times the weight of the Limax powder for 1.5 h, thirdly with an amount of 5 times the weight of the Limax powder for 1 h; each of the extracts was filtered, and the filtrates were combined, and the mixed solvent was recovered under reduced pressure until the recovery was complete, to obtain 11.98 kg of an oily thick paste;

S3. 48.4 kg of silica gel was added to the thick paste and mixed evenly; the mixture was added to a chromatography column pre-filled with 24.3 kg of silica gel, and pre-eluted with n-hexane in the ratio of "the volume of n-hexane: the total weight of the silica gel in the chromatography column=3:1.5" (that is, 146.3 L of n-hexane), and then eluted with acetone in the ratio of "the volume of acetone: the total weight of the silica gel in the chromatography column=3:2" (that is, 110.8 L of acetone), the acetone eluate was collected, and concentrated under reduced pressure to recover acetone, thereby obtaining 6.3 kg of a thick substance; and

S4. 40.0 L of methanol was added to the thick substance, heated to dissolve completely, and frozen at 2°C for 24 hours, to precipitate white crystals, filtered, and the filtrate was concentrated under reduced pressure to recover about 24 L methanol, and then stood for 10 days to precipitate

white needle-like crystals, filtered and dried to obtain 534 g of crystals, 1.5 L of ethyl acetate was added to the crystals, heated to dissolve, cooled, and stood for 10 days, to precipitate white needle-like crystals, filtered and dried to obtain 311.2 g of the new compound, 3,12,13-triacetyl limaxol A.

Example 9

A method for preparing a new compound (a solvent extraction method) includes the following steps:

S1. Limax, after sorting and removing impurities, was pulverized into 20 mesh powder for later use;

S2. 70 kg of Limax powder was put into a multi-functional extraction tank, and extracted at reflux with a mixed solvent (chloroform: petroleum ether: 120#gasoline: n-hexane=1:2:1:6) for 2 times, firstly with an amount of 13 times the weight of the Limax powder for 2 h, and then with an amount of 6 times the weight of the Limax powder for 1.0 h; each of the extracts was filtered, and the filtrates were combined, and the mixed solvent was recovered under reduced pressure until the recovery was complete, to obtain 5.52 kg of an oily thick paste;

S3. 24.8 kg of silica gel was added to the thick paste and mixed evenly; the mixture was added to a chromatography column pre-filled with 13.8 kg of silica gel, and pre-eluted with petroleum ether in the ratio of "the volume of petroleum ether: the total weight of the silica gel in the chromatography column=2.4:1.2" (that is, 77.2 L of petroleum ether), and then eluted with ethyl acetate in the ratio of "the volume of ethyl acetate: the total weight of the silica gel in the chromatography column=1.8:1.2" (that is, 57.9 L of ethyl acetate), the ethyl acetate eluate was collected, and concentrated under reduced pressure to recover ethyl acetate, to obtain 2.90 kg of a thick substance; and

S4. 18.3 L of ethanol was added to the thick substance, heated to dissolve completely, and frozen at 8°C for 24 hours, to precipitate white crystals, filtered, and the filtrate was concentrated under reduced pressure to recover about 9.6 L of ethanol, and then stood for 8 days to precipitate white needle-like crystals, filtered and dried to obtain 252.0 g of crystals, 0.7 L of ethyl acetate was added to the crystals, heated to dissolve, cooled, and stood for 8 days, to precipitate white needle-like crystals, filtered and dried to obtain 148.2 g of the new compound, 3,12,13-triacetyl limaxol A.

III. Pharmacological tests

Example 10

Experiment on the effect of the Limax extract on morphine-induced physical dependence

1. Object of Experiment

This experiment aims to study the effect of the Limax extract on morphine-induced physical dependence symptoms.

2. Materials and Method

2.1 Materials

2.1.1 Test samples

The Limax extract was provided by Guangxi Jiufu Biotechnology Co., Ltd., the Limax crude extract was an ointment-like extract obtained by the supercritical CO₂ extraction in R2 in Example 3 of the present application, and the 3,12,13-triacetyl limaxol A was a sample prepared in Example 3 of the present application; the morphine hydrochloride injection was commercially available from Shenyang No.1 Pharmaceutical Factory of NORTHEAST PHARM; and the methadone was commercially available from Tianjin Central Pharmaceutical Industry Co., Ltd.

2.1.2 Experiment animals

Kunming mice, males, weighing 18-22 g, were commercially available from Shanghai SiJie Laboratory Animal Co., Ltd.

2.2 Method

2.2.1 Effect on morphine-induced physical symptoms

The animals were randomized into a normal saline group, a model group, a positive control group (methadone), and test medicine groups at different doses. Each group has at least 8 mice. The normal saline group was given normal saline every day. The animals in the morphine model group, the positive control group, and the test medicine groups were given morphine at increasing doses of 10, 20, 40, 80, 100 mg/kg by subcutaneous administration twice a day, with a 6-hour interval between each administration. From day 3, 100 mg/kg of morphine was administered for 7 consecutive days. On day 8, 4 mg/kg of naloxone was administered intraperitoneally 4 hours after the administration of morphine, and the number of jumpings within 30 minutes was recorded. At different time points (1 hour or 3 hours) before naloxone administration, the vehicle control, the positive control, and the test medicine were dosed intragastrically.

2.2.2 Statistical analysis

The experiment results were represented by Mean $\pm$ SEM, and differences between groups are tested by t-test. $P < 0.05$ showed a significant difference.

3. Results

Effect of the Limax extract on morphine-induced physical dependence symptoms

The experiment results were shown in Table 2 to Table 4. After chronic morphine treatment, naloxone was administrated to induce withdrawal responses, and the animals showed significant withdrawal jumping symptoms. The withdrawal jumping symptoms of the animals were significantly reduced 1 hour after the methadone (20 mg/kg) administration, 3 hours after the Limax crude extract (10 g/kg) administration, and 1 hour after the 3,12,13-triacetyl limaxol A (0.5 g/kg) administration. Table 5 showed that the withdrawal jumping symptoms of the animals tended to be reduced 3 hours after the 3,12,13-triacetyl limaxol A (0.5 g/kg) administration, but there was no significant difference from the model group. FIG. 8 showed inhibition rates of three drugs on morphine-withdrawal jumping symptoms, in an order of Limax crude extract (10 g/kg, -3 h)>3,12,13-triacetyl limaxol A (0.5 g/kg, -1 h)≈methadone (20 mg/kg, -1 h).

Table 2. Effect of methadone on morphine-withdrawal jumping symptoms

Group	Jumping times (Mean $\pm$ SEM)	% Inhibition of morphine-withdrawal jumping
Normal saline	0.3 $\pm$ 0.3	—
Morphine	62.4 $\pm$ 12.7***	—
Morphine + methadone 20 mg/kg	33.8 $\pm$ 3.3*	46.1 $\pm$ 5.2

The results in Table 2 showed that there was a significant difference between the morphine group and the control group (***P<0.001), indicating that naloxone administration after chronic morphine treatment can induce significant withdrawal jumping symptoms. There was a significant difference between the morphine + methadone group and the morphine group (*P<0.05), indicating that the withdrawal jumping symptoms induced by naloxone administration can be significantly reduced 1 hour after the methadone (20 mg/kg) administration.

Table 3. Effect of the Limax crude extract on morphine-withdrawal jumping symptoms

Group	Jumping times (Mean $\pm$ SEM)	% Inhibition of morphine-withdrawal jumping
Normal saline	0.0 $\pm$ 0.0	—
Morphine	54.8 $\pm$ 14.1**	—
Morphine + Limax crude extract 10 g/kg	14.3 $\pm$ 4.7*	73.9 $\pm$ 8.6

The results in Table 3 showed that there was a significant difference between the morphine group and the control group (**P<0.01), indicating that naloxone administration after chronic morphine treatment can induce significant withdrawal jumping symptoms. There was a significant difference between the morphine + Limax crude extract group and the morphine group (*P<0.05), indicating that the withdrawal jumping symptoms induced by naloxone administration can be significantly reduced 3 hours after the Limax crude extract (10 g/kg) administration.

Table 4. Effect of 3,12,13-triacetyl limaxol A on morphine-withdrawal jumping symptoms 1 hour after administration

Group	Jumping times (Mean $\pm$ SEM)	% Inhibition of morphine-withdrawal jumping
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Normal saline	1.3±0.8	—
Morphine	34.5±5.2***	—
Morphine + 3,12,13-triacetyl limaxol A 0.5 g/kg	18.5±2.4*	48.2±7.5
Morphine + 3,12,13-triacetyl limaxol A 0.25 g/kg	27.5±9.4	21.1±28.2
Morphine + 3,12,13-triacetyl limaxol A 0.125 g/kg	33.5±4.1	3.0±12.2

The results in Table 4 showed that there was a significant difference between the morphine group and the control group (***P<0.001), indicating that naloxone administration after chronic morphine treatment can induce significant withdrawal jumping symptoms. There was a significant difference between the morphine + high-dose 3,12,13-triacetyl limaxol A group and the morphine group (*P<0.05), indicating that the withdrawal jumping symptoms induced by naloxone administration can be significantly reduced 1 hour after the morphine + 3,12,13-triacetyl limaxol A (0.5 g/kg) administration.

Table 5. Effect of 3,12,13-triacetyl limaxol A on morphine-withdrawal jumping symptoms 3 hours after administration

Group	Jumping times (Mean ± SEM)	% Inhibition of morphine-withdrawal jumping
Normal saline	0.6±0.5	—
Morphine	29.8±9.5**	—
Morphine + 3,12,13-triacetyl limaxol A 0.5 g/kg	16.7±4.9	45.0±17.0

The results in Table 5 showed that there was a significant difference between the morphine group and the control group (***P<0.01), indicating that naloxone administration after chronic

morphine treatment can induce significant withdrawal jumping symptoms. There was no significant difference between the morphine + high-dose 3,12,13-triacetyl limaxol A group and the morphine group ($P>0.05$), indicating that the withdrawal jumping symptoms induced by naloxone administration tended to be reduced 3 hours after the morphine+3,12,13-triacetyl limaxol A (0.5 g/kg) administration, but there was no significant difference.

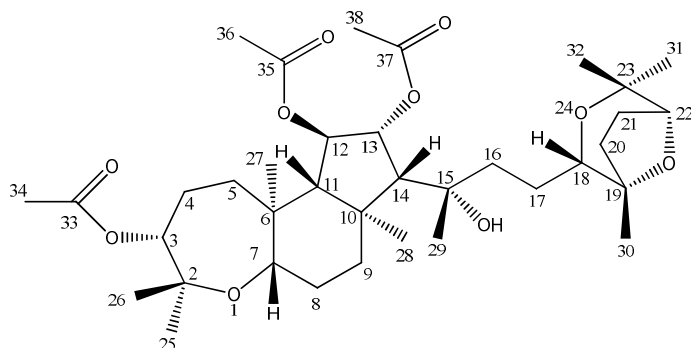
4. Conclusion

The Limax crude extract (10 g/kg) has a significant inhibitory effect on withdrawal jumping symptoms in morphine-dependent animals 3 hours after intragastric administration, while the 3,12,13-triacetyl limaxol A (0.5 g/kg) has a significant inhibitory effect on withdrawal jumping symptoms in morphine-dependent animals 1 hour after intragastric administration, and still shows an inhibitory trend after 3 hours.

CLAIMS

What is claimed is:

1. A new compound having a structure of:



2. The new compound according to claim 1, wherein the chiral C configurations of the new compound comprise: C3, R; C6, S; C7, S; C10, S; C11, R; C12, R; C13, R; C14, R; C15, S; C18, S; C19, S; and C22, R.

3. The new compound according to claim 1, wherein the new compound has a molecular formula: $C_{36}H_{58}O_{10}$; molecular weight: 650; melting point: 228-229°C; and solubility: white needle-like crystal, insoluble in water, hardly soluble in acid and alkali, easily soluble in ethyl acetate and acetic acid, and soluble in methanol, ethanol, acetone, and chloroform.

4. The new compound according to claim 1, wherein the new compound is prepared by extracting and separating from *Limax*.

5. The new compound according to claim 4, wherein the *Limax* comprises one or more of *Vaginulus alte* (Ferussac), *Limax maximus* L., *L. flavus* L., *Agriolimax agrestis* L., and *Phiolomycus bilineatus*.

6. A method for preparing the new compound according to claim 1, comprising a supercritical CO_2 extraction method and a solvent extraction method.

7. The method for preparing the new compound according to claim 6, wherein the supercritical CO_2 extraction method comprises the following steps:

- R1. sorting *Limax*, removing impurities, and pulverizing, to obtain *Limax* powder;
- R2. putting the *Limax* powder into a supercritical CO_2 extractor for extraction to obtain an extract;
- R3. adding vegetable oil to the extract, heating and mixing evenly, cooling, standing, and filtering to obtain a precipitate, washing the precipitate with a washing solvent, and drying to obtain a dried precipitate; and

R4. adding a crystallization solvent to the dried precipitate, heating to dissolve, cooling, standing to precipitate white needle-like crystals, and filtering to obtain the crystals, recrystallizing, and drying, to obtain the new compound.

8. The method for preparing the new compound according to claim 7, wherein the pulverizing in R1 comprises pulverizing into 10-30 meshes.

9. The method for preparing the new compound according to claim 7, wherein the conditions for the supercritical CO₂ extraction in R2 comprise: pressure 20-30 kPa, temperature 60-70°C, flow 400-500 PV, and extraction time 3-5 h.

10. The method for preparing the new compound according to claim 7, wherein the vegetable oil in R3 is tea seed oil, camellia seed oil, soybean oil, or olive oil;

the vegetable oil is added in a weight ratio of "extract: vegetable oil=2-6:1-3";

the heating is carried out at a temperature of 60-80°C;

the time for the standing is 7-10 days; and

the washing comprises washing with n-hexane, petroleum ether, or 120#gasoline for 3-5 times, with an amount of 200-500 ml each time.

11. The method for preparing the new compound according to claim 7, wherein the crystallization solvent in R4 is methanol, acetone, ethyl acetate, or chloroform added at an amount of 3-10 times the weight of the dried precipitate; and the time for the standing is 7-10 days.

12. The method for preparing the new compound according to claim 6, wherein the solvent extraction method comprises the following steps:

S1. sorting Limax, removing impurities, and pulverizing, to obtain Limax powder;

S2. heating reflux extraction the Limax powder with a solvent, filtering, leaving a filtrate and recovering the solvent from the filtrate under reduced pressure until the recovery is complete, to obtain a thick paste;

S3. adding the thick paste to a silica gel chromatography column, eluting with an elution solvent, collecting an eluate, and recovering the elution solvent from the eluate under reduced pressure, to obtain a thick substance; and

S4. adding a dissolution solvent to the thick substance, heating to dissolve completely, cooling, and freezing to precipitate white crystals, filtering, leaving a filtrate, recovering the dissolution solvent from the filtrate under reduced pressure, and re-standing to precipitate white needle-like crystals, filtering, and drying, to obtain the crystals, recrystallizing, and drying, to obtain the new compound.

13. The method for preparing the new compound according to claim 12, wherein the pulverizing in S1 comprises pulverizing into 10-30 meshes.

14. The method for preparing the new compound according to claim 12, wherein the solvent in S2 is one or more of ethanol, methanol, acetone, chloroform, 120[#]gasoline, n-hexane, petroleum ether, diethyl ether, and ethyl acetate.

15. The method for preparing the new compound according to claim 12, wherein the heating reflux extraction in S2 comprises heating reflux extraction 1-3 times with the solvent, with an amount of 5-15 times the weight of the Limax powder each time, for 1-3 h.

16. The method for preparing the new compound according to claim 12, wherein the adding to the silica gel chromatography column in S3 comprises adding silica gel to the thick paste at an amount of 4-6 times the weight of the thick paste, mixing evenly, and adding to the chromatography column pre-filled with silica gel at an amount of 2-4 times the weight of the thick paste.

17. The method for preparing the new compound according to claim 12, wherein the elution solvent in S3 is methanol, acetone, or ethyl acetate, and the amount of the elution solvent used is: the volume of the elution solvent: the total weight of the silica gel in the chromatography column=1.5-3:1-2.

18. The method for preparing the new compound according to claim 12, wherein the dissolution solvent in S4 is methanol or ethanol added in an amount of 4-8 times the volume of the thick substance; the freezing is carried out at a temperature of 2-10°C for 24 hours; the recovering the solvent from the filtrate under reduced pressure comprises concentrating to 50-60% of the original volume; and the time for the re-standing is 7-10 days.

19. The method for preparing the new compound according to claim 12, comprising a step of pre-elution with a pre-elution solvent before the eluting with an elution solvent in S3, wherein the pre-elution solvent is petroleum ether, n-hexane, or 120[#]gasoline, and the amount of the pre-elution solvent used is: the volume of the pre-elution solvent: the total weight of the silica gel in the chromatography column=1-3:0.5-1.5.

20. Use of the new compound according to claim 1 in the preparation of medicines, health food, and food for preventing or treating withdrawal or withdrawal-like symptoms.

21. Use of the new compound according to claim 1 in the preparation of medicines, health food, and food for inhibiting withdrawal symptoms in morphine-dependent animals.