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UNITED STATES DISTRICT COURT
CENTRAL DISTRICT OF CALIFORNIA

NANJING NUTRABUILDING BIO-
TECH CO., LTD.,

Plaintiff,

vs.

BONERGE LIFESCIENCE (HUNAN)
CO., LTD., and DOES 1-9,
INCLUSIVE,

Defendants.

Case No. 5:25-cv-00271-JGB-SPx

[Hon. Jesus G. Bernal]

**AMENDED JOINT CLAIM
CONSTRUCTION AND
PREHEARING STATEMENT**

JOINT CLAIM CONSTRUCTION AND PREHEARING STATEMENT

Plaintiff, Nanjing Nutrabuilding Bio-Tech Co., Ltd. (“Plaintiff” or “NNB”) and Defendant Bonerge Lifescience (Hunan) Co., Ltd. (“Defendant” or “Bonerge”) hereby submit the following Amended Joint Claim Construction and Prehearing Statement, pursuant to the Court’s March 3, 2026 Order (Dkt 36) .

Following the parties’ exchange of proposed claim constructions, the parties met and conferred through videoconference on January 9, 2026. This statement identifies the agreed and disputed terms and constructions for U.S. Patent Nos. 10,278,961 (the “’961 Patent” or the “Patent-in-Suit”).

The Court’s Order Granting Stipulation to Correct and Amend Scheduling Order (Dkt. 29) sets a Claim Construction hearing date for April 21, 2026 (or as soon thereafter as possible). The parties propose that the Claim Construction hearing take place at 9:00 a.m. on April 21, 2026; although, the parties understand that this date and time may be adjusted or rescheduled by the Court.

Agreed Upon Constructions

Claim(s)	Claim Term	Agreed Upon Construction
1	A method of managing glucose tolerance in an individual, the method comprising:	The parties agree that the preamble of claim 1 should be construed as limiting (i.e., that it adds one or more elements to claim 1). The parties do <i>not</i>, however, agree on the nature or scope of the preamble’s limitation of claim 1, which will be addressed below.

Disputed Constructions

Claim	Claim Term	NNB Proposed Construction	Bonerge Proposed Construction
1	A method of managing glucose tolerance in an individual, the method comprising:	The preamble is limiting: “Regulating or controlling fasting glucose levels or the body’s ability to metabolize ingested glucose” <u>Support:</u> ‘961 Patent at Abstract; Col. 2:18-23; Col. 3:55-56; Col. 10:19-22; Claim 2; Provisional Patent Appl. at ¶¶ 3, 11, 13, 15, 17, 34.	The preamble is limiting: “enhancing an individual’s ability to process glucose that is measurable via a glucose tolerance test” <u>Support:</u> ’961 Patent at Col. 1:44-64; Col. 2:18-23; Col. 4:60-62; Col. 4:66-5:12; Col. 9:12-15; Col. 9:29-32; Col. 9:34-39; Col. 10:19-22; ’961 File History at May 24, 2018 Office Action p. 3; <i>see also</i> Exhs. G (at pp. 1415 - 1417 therein numbered as BON00688 - BON00690), H (at pp. 1, 3, 13, 20 - 21 therein

			numbered as BON00790, BON00792, BON00802, BON00809 - BON00810), and I (at pp. 381 – 382 therein numbered as BON01230 – 1231).
1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16
17	18	19	20
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25	26	27	28
1	administering, to an individual, a pharmaceutically effective amount of dihydroberberine	Plain and ordinary meaning (does not require construction). In the alternative, if plain and ordinary meaning is not adopted, then construe as “administering, to a human, an amount of dihydroberberine sufficient to achieve a therapeutic effect.” <u>Support:</u> ’961 Patent at Abstract; Col. 1:44-60; Col. 3:51-65; Col. 4:60-62	“Administering to an individual an amount of dihydroberberine that improves the individual’s ability to process glucose that is measurable via a glucose tolerance test” <u>Support:</u> ’961 Patent at Col. 1:44-64; Col. 2:30-37; Col. 2:57-61; Col. 4:6-10; Col. 4:60-5:12; Col. 7:63-8:4; Col. 8:27-32; Col. 10:25-28.

1			
2	1	approximately 25	Plain and ordinary
3		mg to	meaning (does not
4		approximately 800	require construction).
5		mg of	
6		dihydroberberine	In the alternative, if
7			plain and ordinary
8			meaning is not
9			adopted, then: “about
10			25 mg to about 800
11			mg of
12			dihydroberberine”
13			
14			<u>Support:</u>
15			’961 Patent at Claim
16			1; Col. 2:30-33; Col.
17			4:60-64; <i>see also</i>
18			Exhs. A (at p. 1
19			therein numbered as
20			NNB002027), B (at p.
21			1 therein numbered as
22			NNB002034), C (at
23			pp. 1-2 therein
24			numbered as
25			NNB002042-43), and
26			D (at p. 1 therein
27			numbered as
28			NNB002053).
			Indefinite.
			<u>Support:</u>
			’961 Patent at Col.
			2:30-33; Col. 4:60-64;
			Col. 8:12-15, ’961 File
			History at November
			20, 2018 Amendment
			and Reply to Office
			Action Mailed May 24,
			2018 at p. 7; Patent
			Owner’s Preliminary
			Response for IPR2025-
			01593 at pp. 27 and 39
			– 40.

1			
2	2	reduces fasting	Plain and ordinary
3		glucose levels	meaning (does not
4			require construction).
5			
6			In the alternative, if
7			plain and ordinary
8			meaning is not
9			adopted, then:
10			“lowering glucose
11			levels after not eating
12			or drinking anything
13			other than water for
14			approximately 8 or
15			more hours”
16			
17			<u>Support:</u>
18			‘961 Patent at
19			Abstract; Col. 2:18-
20			26; Col. 3:51-56; Col.
21			10:19-22; <i>see also</i>
22			Exhs. E (at p. 3
23			therein numbered as
24			NNB002011) and F
25			(at p. 3 therein
26			numbered as
27			NNB002018).
28			

1
2 **Identification of Terms Most Significant (up to 10)**

3 The parties have limited the terms in dispute to below ten (10). The following
4 terms may be dispositive depending on the nature of the construction given:

5 (1) “A method of managing glucose tolerance in an individual, the method
6 comprising:”

7 (2) “administering, to an individual, a pharmaceutically effective amount of
8 dihydroberberine” and

9 (3) “approximately 25 mg to approximately 800 mg of dihydroberberine.”
10

11 **Anticipated Length of Time Necessary for Claim Construction Hearing**

12 The parties presently anticipate that the Claim Construction Hearing may last
13 for approximately two (2) hours.
14

15 **Anticipated Witnesses**

16 The parties do not intend to call any witnesses, lay, or expert.
17

18 **Necessity of Any Fact Finding From the Claim Construction Hearing**

19 Should the Court choose to rely on any extrinsic evidence (evidence not from
20 the ‘961 Patent or its file history), the Court may need to determine if the extrinsic
21 evidence stands for, or supports, the propositions and matters for which they may be
22 cited by the parties.
23

24 **Other Issues for any Prehearing Conference**

25 The parties do not believe the patented technology is sufficiently complex that
26 a tutorial is needed; although, if the Court disagrees, the parties are glad to provide a
27 tutorial to the Court. The parties will likely include a brief discussion of the
28

1 technology at the outset of their respective presentations at the Claim Construction
2 Hearing.

3
4 Respectfully submitted:

5
6 SCHEEF & STONE LLP

7 By: /s/Mark D. Nielsen
8 Mark D. Nielsen

9 CISLO & THOMAS LLP
10 Daniel M. Cislo
11 C. Wook Pak

12 Attorneys for Plaintiff,
13 NANJING NUTRABUILDING BIO-
14 TECH CO., LTD.

15 Dated: March 4, 2026
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Respectfully submitted:

SCIENBIZIP, P.C.

By: /s/Hua Chen (with permission)
Hua Chen

Attorney for Defendant,
BONERGE LIFESCIENCE (HUNAN)
CO., LTD.

Dated: March 4, 2026

CERTIFICATE OF SERVICE

I hereby certify that on March 5, 2026, I electronically filed the foregoing document with the clerk of court for the U.S. District Court, Central District of California, using the electronic case filing system of the court.

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Attorneys for Plaintiff,
Nanjing Nutrabuilding Bio-Tech Co., Ltd.

I declare, under penalty of perjury under the laws of the United States of America that the foregoing is true and that I am employed in the office of a member of the Bar of this Court at whose direction the service was made.

Executed on March 5, 2026, at Los Angeles, California.

/s/ Hua Chen
Hua Chen



Search a word or phrase



View synonyms for [approximately](#) ↗

American

British

approximately 

[uh-**prok**-suh-mit-lee]

☒ Phonetic (Standard) ☐ IPA

adverb

[about](#); [roughly](#); [more or less](#).

It's approximately 25 miles from here to El Paso.

Add approximately 2 cups of milk or water.

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Word History and Origins

Origin of [approximately](#)¹

First recorded in 1845–50; [approximate](#) ([def.](#)) + [-ly](#) ([def.](#))

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Example Sentences

ⁱ Examples are provided to illustrate real-world usage of words in context. Any opinions expressed do not reflect the views of Dictionary.com.

At approximately age 32, the adult epoch begins.

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Harbour Energy said a reduction of "approximately 100 offshore roles" was expected following a consultation period, which is now under way.

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Targa said it expects Stakeholder to “generate unlevered adjusted free cash flow of approximately \$200 million annually with minimal capital needs, very low integration costs and attractive acreage with a stable volume profile.”

Read more on [The Wall Street Journal](#)

Newell Brands employed approximately 23,700 people worldwide as of the end of last year, according to its most recent annual filing with the Securities and Exchange Commission.

Read more on [The Wall Street Journal](#)

Coupang slumps following a data leak that exposed approximately 34 million accounts at the South Korean retailer.

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WORD OF THE DAY

DECEMBER 02, 2025

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[puh-ley-dee-uhn]

[Meaning and examples](#)

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penne

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Merriam-Webster

approximately

Q

Definition

Synonyms

Example Sentences

Word History

Rhymes

Entries Near

approximately adverb

ap·prox·i·mate·ly (ə-ˈpräk-sə-mət-lē ◀▶)

Synonyms of *approximately* >

- : in an **approximate** manner
 - | an event in ancient history that can only be *approximately* dated
- used to indicate that a stated number, amount, or value is an approximation
 - | *Approximately* [=around, about] 2,000 people attended the rally.
 - | arrived at *approximately* 11:30 in the morning
 - | During a lifetime, a person will take *approximately* 500 million breaths.
 - Arthur S. Slutsky, M.D., et al.

Synonyms of *approximately*

Relevance

- roughly

about
- around

See All Synonyms & Antonyms in Thesaurus >

Examples of *approximately* in a Sentence



Definition

Synonyms

Example Sentences

Word History

Rhymes

Entries Near

i Examples are automatically compiled from online sources to show current usage. [Read More](#)

However, the discount was **approximately** 95 percent, the incident was caught on camera, and the football players were subsequently arrested for grand theft.

— Dan Pompei, *New York Times*, 2 Dec. 2025

In the areas north of Route 50, including Baltimore Washington International (BWI) airport, a wintry mix may accumulate to **approximately** one-half inch before turning completely to rain.

— Kaicey Baylor, *CBS News*, 1 Dec. 2025

The New York Post reported **approximately** 3,000 were currently in flight at the time of the announcement.

— Colson Thayer, *PEOPLE*, 1 Dec. 2025

Such a revocation would put at risk **approximately** 700 Somali nationals nationwide who had TPS status as of March this year, as most Somalis in the state are American nationals.

— Chad De Guzman, *Time*, 1 Dec. 2025

[See All Example Sentences for *approximately* >](#)

Word History

First Known Use

1742, in the meaning defined [above](#)

Time Traveler

The first known use of *approximately* was in 1742



Definition

Synonyms

Example Sentences

Word History

Rhymes

Entries Near

Rhymes for *approximately*

proximately

[See All Rhymes for *approximately* >](#)

Browse Nearby Words

approximate

approximately

approximation

[See All Nearby Words >](#)

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Style

MLA



"Approximately." *Merriam-Webster.com Dictionary*, Merriam-Webster, <https://www.merriam-webster.com/dictionary/approximately>. Accessed 2 Dec. 2025.

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Definition

Synonyms

Example Sentences

Word History

Rhymes

Entries Near

Kids Definition

approximately adverb

ap·prox·i·mate·ly -mət-lē

: reasonably close to

| *approximately* 5 miles

| at *approximately* six o'clock

More from Merriam-Webster on *approximately*

English: [Translation of *approximately* for Spanish Speakers](#)

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MERRIAM-WEBSTER UNABRIDGED



Definition

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2 gyroscope

3 larvae

4 region

5 adaptable

6 churlish

7 content



WORD OF THE DAY

cajole 

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Play

Blossom

Pick the best words!

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Definition

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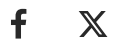


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Meaning of **approximately** in English



approximately

adverb

UK /əˈprɒk.sɪ.mət.li/ US /əˈprɑːk.sə.mət.li/

Add to word list

B1

close to a particular number or time although not exactly that number or time:

- *The job will take approximately three weeks, and cost approximately £1,000.*

Synonyms

about

around (APPROXIMATELY)

roughly

+ Thesaurus: synonyms, antonyms, and examples

+ More examples

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Cambridge
Dictionary



approximately | BUSINESS ENGLISH

approximately

adverb

UK /əˈprɒksɪmətli/ US

Add to word list

close to a particular number or time although not exactly that number or time:

- *In December, the company reduced its workforce by approximately 5%.*
- *It will take approximately two weeks to collect the data.*

(Definition of **approximately** from the Cambridge Business English Dictionary © Cambridge University Press)

EXAMPLES of approximately

approximately

In the first 5 months of 1995, 172 plans were surveyed and *approximately* one-third (34%) were granted full, 3-year accreditation.

From the Cambridge English Corpus

There are *approximately* 700 phonetic radicals and about 140 semantic radicals currently in use.

From the Cambridge English Corpus

Approximately one-quarter experienced a hearing limitation or a learning, developmental or psychological disability.

From the Cambridge English Corpus

Subsequent step sizes *approximately* halved the difference between the prior two reversals or starting points.

From the Cambridge English Corpus

Approximately two to three requests are received every 4 months.

From the Cambridge English Corpus

The joint width of zones 2, 3, and 4 is *approximately* 20% of the spot radius.





Cambridge
Dictionary



The questionnaire was mailed to the study teachers approximately one week before the interview.

From the [Cambridge English Corpus](#)



The average susceptibility of hosts at birth in our model remains *approximately* constant with time.

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According to this report, although most married couples sired *approximately* six to seven children, only one or two survived.

From the [Cambridge English Corpus](#)



Approximately 3 kg of fresh litter obtained from the removal-treatment plots and the surrounding area was added to the addition-treatment plots.

From the [Cambridge English Corpus](#)



Each of the sessions took two days to complete and lasted *approximately* 40 minutes per day.

From the [Cambridge English Corpus](#)



Then the water composition *approximately* oscillates at each point of the aquifer in phase with the water velocity.

From the [Cambridge English Corpus](#)



The procedures used during the two visits were identical, with each session lasting *approximately* 2 hours.

From the [Cambridge English Corpus](#)



Approximately one third of the syllables were from multiword utterances.

From the [Cambridge English Corpus](#)



That is, related and unrelated utterances were easily distinguished using a temporal criterion of *approximately* 4 seconds.

From the [Cambridge English Corpus](#)



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What is the pronunciation of *approximately*? >

Translations of **approximately**

in Chinese (Traditional)



in Chinese (Simplified)
大约, 大概, 左右...
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in Portuguese
aproximadamente...
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approximant
approximate
approximated
approximately
approximating
approximation
appurtenance
APR



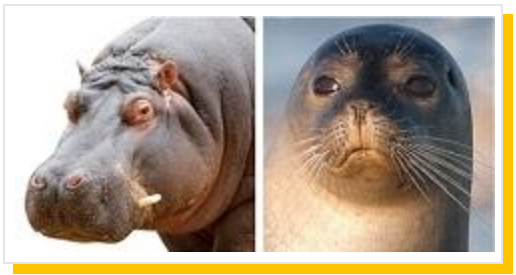


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More meanings of *approximately*

— All

approximately *adverb*, at approximate

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WORD OF THE DAY

vacuum

uk  /'væk.ju:m/ us  /'væk.ju:m/

to use a vacuum cleaner to collect dust, dirt, etc.



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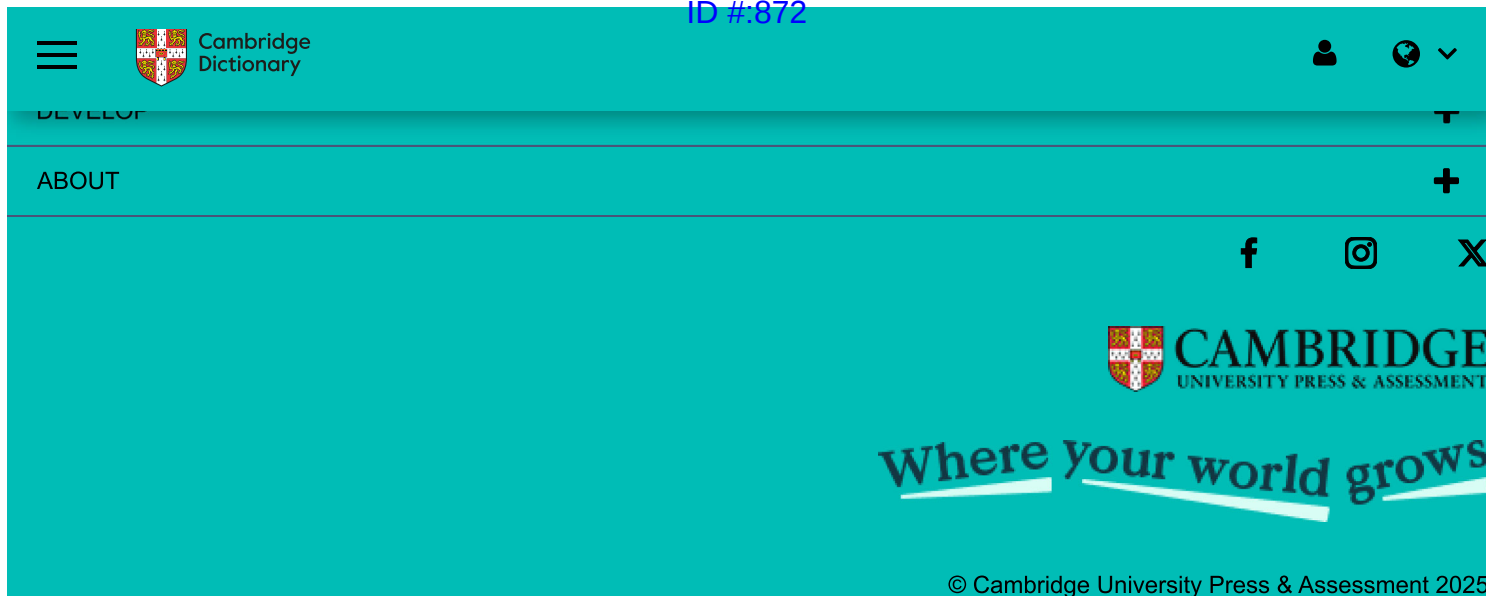






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Word: Approximately Definition Find

Approximately

Webster's 1913 Dictionary

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Ap`prox`i`mate`ly
adv. 1. With approximation; so as to approximate; nearly.

WordNet Dictionary

Adv. 1. approximately - (of quantities) imprecise but fairly close to correct; "lasted approximately an hour"; "in just about a minute"; "hold your stand"; "we meet about once a month"; "some forty people came"; "weighs around a hundred pounds"; "roughly \$3,000"; "hold party"
Synonyms: about, close to, more or less, or so, roughly, some, just about, around

Related Words

about, all but, all in all, almost, almost entirely, approaching, approximatively, around, by and large, chiefly, circa, close to, effectually, especially, in round numbers, in the main, mainly, more or less, most, mostly, much, nearabout, nearly, nigh, on balance, on the whole, roughly, roundly, say, some, substantially, virtually, well-nigh

Translation

Translate **Approximately** to Spanish, Translate **Approximately** to German, Translate **Approximately** to French

Definitions Index: # A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

-70%

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-77%

\$441.00

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-79%

\$363.35

-74%

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ABOUT DIABETES

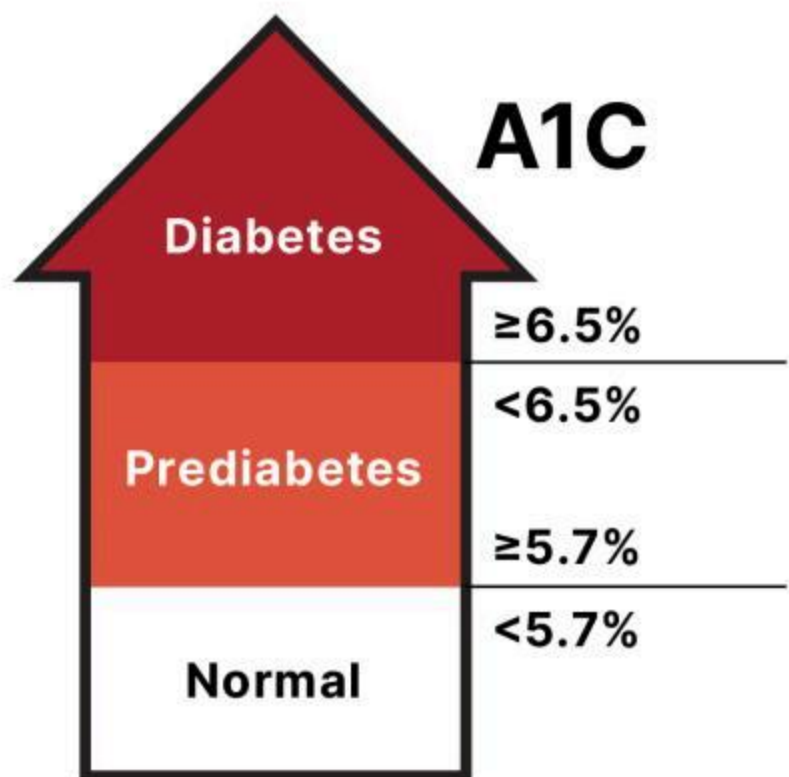
Diabetes Diagnosis

There are several ways to diagnose diabetes. Each way usually needs to be repeated on a second day to diagnose diabetes.

Testing should be carried out in a health care setting (such as your doctor's office or a lab). If your doctor determines that your blood glucose (blood sugar) level is very high, or if you have classic symptoms of high blood glucose in addition to one positive test, your doctor may not require a second test to diagnose diabetes.

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A1C

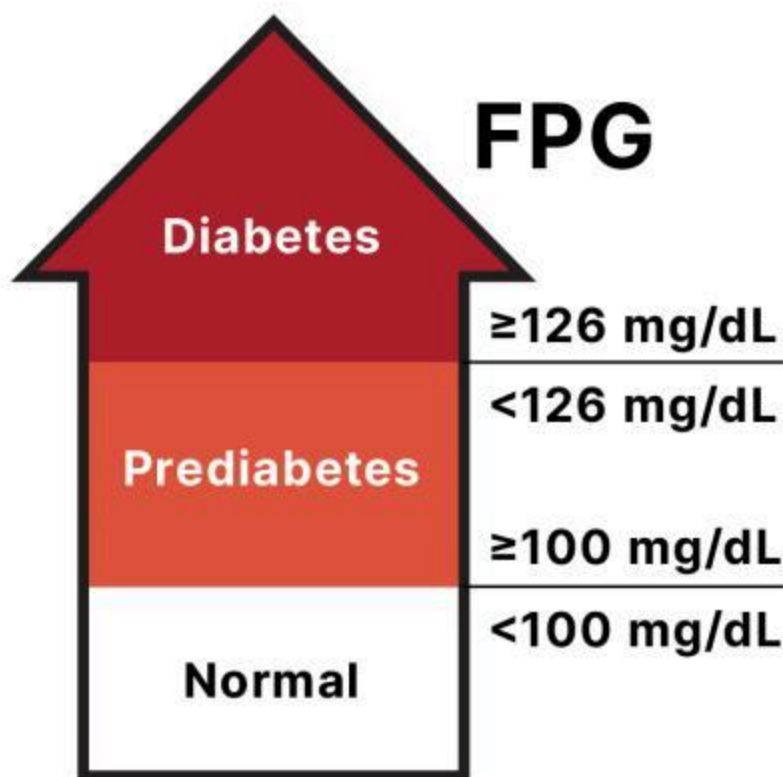


The A1C test measures your average blood glucose for the past two to three months. The advantages of being diagnosed this way are that you don't have to fast or drink anything.

Diabetes is diagnosed at an A1C of greater than or equal to 6.5%

Result	A1C
Normal	less than 5.7%
Prediabetes	5.7% to 6.4%
Diabetes	6.5% or higher

Fasting Plasma Glucose (FPG)

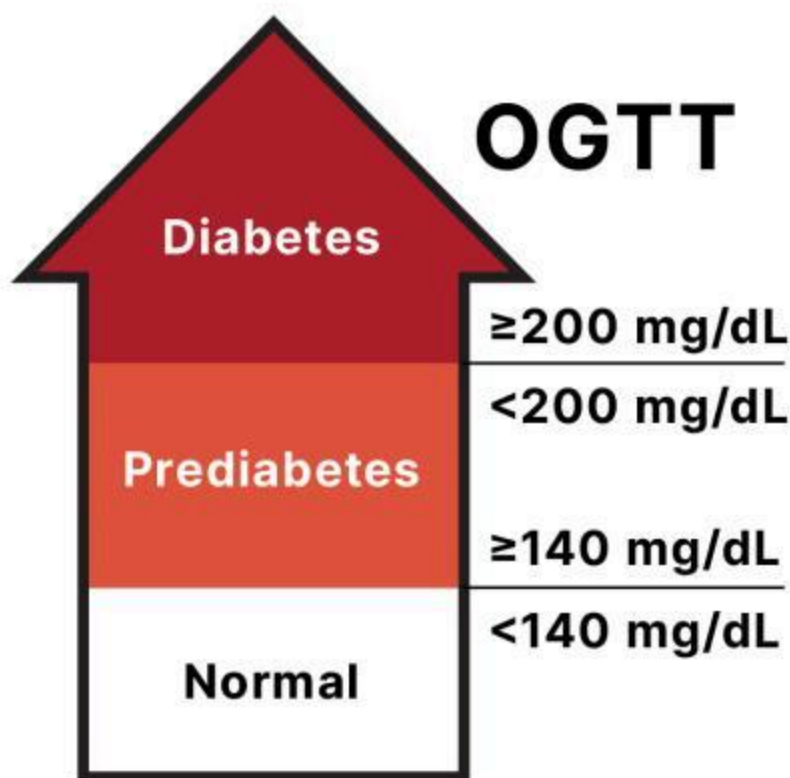


This test checks your fasting blood glucose levels. Fasting means after not having anything to eat or drink (except water) for at least 8 hours before the test. This test is usually done first thing in the morning, before breakfast.

Diabetes is diagnosed at fasting blood glucose of greater than or equal to 126 mg/dl

Result	Fasting Plasma Glucose (FPG)
Normal	less than 100 mg/dL
Prediabetes	100 mg/dl to 125 mg/dL
Diabetes	126 mg/dL or higher

Oral Glucose Tolerance Test (OGTT)



The OGTT is a two-hour test that checks your blood glucose levels before and two hours after you drink a special sweet drink. It tells the doctor how your body processes sugar.

Diabetes is diagnosed at two-hour blood glucose of greater than or equal to 200 mg/dl

Result	Oral Glucose Tolerance Test (OGTT)
Normal	less than 140 mg/dL

Result	Oral Glucose Tolerance Test (OGTT)
Prediabetes	140 to 199 mg/dL
Diabetes	200 mg/dL or higher

Random (also called Casual) Plasma Glucose Test

This test is a blood check at any time of the day when you have severe diabetes symptoms.

Diabetes is diagnosed at blood glucose of greater than or equal to 200 mg/dl

What is Prediabetes?

Before people develop type 2 diabetes, they almost always have prediabetes—blood glucose levels that are higher than normal but not yet high enough to be diagnosed as diabetes.

Doctors sometimes refer to prediabetes as impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), depending on what test was used when it was detected. This condition puts you at a higher risk for developing type 2 diabetes and cardiovascular disease.

Symptoms

There are no clear symptoms of prediabetes, so you may have it and not know it.

Some people with prediabetes may have some of the symptoms of diabetes or even problems from diabetes already. You usually find out that you have prediabetes when being tested for diabetes.

If you have prediabetes, you should be checked for type 2 diabetes every one to two years.

Results indicating prediabetes are:

- An A1C of 5.7–6.4%
- Fasting blood glucose of 100–125 mg/dL
- An OGTT two-hour blood glucose of 140–199 mg/dL

Preventing Type 2 Diabetes

You will not develop type 2 diabetes automatically if you have prediabetes. For some people with prediabetes, early treatment can actually return blood glucose levels to the normal range.

Research shows that you can lower your risk for type 2 diabetes by 58% by:

- Losing 7% of your body weight (or 15 pounds if you weigh 200 pounds)
- Exercising moderately (such as brisk walking) 30 minutes a day, five days a week

Don't worry if you can't get to your ideal body weight. Losing even 10 to 15 pounds can make a huge difference.



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Glucose tolerance test



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Overview

The glucose tolerance test measures the body's response to sugar, also called glucose. Another name for this test is the oral glucose tolerance test.

This test can be used to screen for type 2 diabetes or prediabetes before you have symptoms of either condition. Or it can help find out whether diabetes is causing existing symptoms. More often, a version of the test is used to check for diabetes that happens during pregnancy. That condition is called gestational diabetes.

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Why it's done

The glucose tolerance test finds problems with the way the body handles sugar after a meal. As you eat, your body breaks down food into sugar. The sugar enters your blood, and the body uses the sugar for energy. But with prediabetes and diabetes, the sugar level in the blood becomes too high.

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Risks

Case 5:25-cv-00271-JGB-SP Document 37-6 Filed 03/05/26 Page 3 of 11 Page ID
#883
The risks linked with having a blood sample taken are small. After your blood is taken, you may have bruising or bleeding. You also might feel dizzy or lightheaded. Rarely, an infection can happen after the procedure.

How you prepare

Food and medicines

Eat and drink as you usually would in the days leading up to the glucose tolerance test. Let your healthcare team know if you become sick or if you take any medicines. These factors can affect the results of your test.

What you can expect

Before the procedure

For eight hours before the test, you won't be able to eat or drink anything. This is called fasting. You might schedule the test for early in the morning and fast the night before.

During the procedure

You take the test at your healthcare professional's office, or in a clinic, hospital or lab. The test includes a few steps. First, a member of your healthcare team takes a sample of blood from a vein in your arm. This blood sample is used to measure your fasting blood sugar level.

Type 2 diabetes and prediabetes

Next, if you're being tested for type 2 diabetes or prediabetes:

- You drink a syrupy glucose solution that has 75 grams of sugar.



- Case 5:25-cv-00271-JGB-SP Document 37-6 Filed 03/05/26 Page 4 of 11 Page ID #:884
- One hour later and two hours later, samples of your blood are taken. The blood sugar levels are measured.

This often is called a two-hour glucose tolerance test.

Gestational diabetes

Most pregnant people are screened for gestational diabetes with a different version of the glucose tolerance test. Testing usually is done between 24 and 28 weeks of pregnancy.

Most often, screening includes a two-step process. The first involves the following test. You don't need to fast before this test:

- A sample of your blood is taken, and the blood sugar level is measured.
- Then you drink a glucose solution that has 50 grams of sugar.
- One hour later, another blood sample is taken, and the blood sugar level is checked.

You might hear this called a one-hour glucose tolerance test. If the result shows that your blood sugar is high by a certain amount, your healthcare team will likely suggest another test. This involves taking a longer, three-hour version of the test. For the three-hour test:

- You fast for eight hours first. Afterward, a sample of your blood is taken, and the blood sugar level is measured.
- Then you drink a glucose solution that has 100 grams of sugar.
- Your blood sugar level is tested again one, two and three hours after you drink the solution.

During the three-hour test, you'll likely stay in your healthcare professional's office, or in the clinic or lab. You wait there for your blood sugar levels to be tested.

Case 5:25-cv-00271-JGB-SP Document 37-6 Filed 03/05/26 Page 5 of 11 Page ID
#885
Less often, a single-step glucose tolerance test is used to screen for gestational diabetes. It's a two-hour test that involves fasting before you drink a glucose solution that has 75 grams of sugar.

Whether you take the one- or two-step glucose tolerance test, the timing of when you get tested could vary. Your healthcare professional may recommend taking it before 24 weeks of pregnancy if you have risk factors for gestational diabetes.

After the procedure

After the glucose tolerance test, you can get back to your usual activities right away. But call your healthcare professional if:

- You have serious pain.
- You have a fever.
- Your skin becomes swollen, changes color or oozes fluid in the area where blood was taken.

Results

Results of the glucose tolerance test are given in milligrams per deciliter (mg/dL) or millimoles per liter (mmol/L).

Type 2 diabetes or prediabetes

If you're being tested for type 2 diabetes or prediabetes, two hours after drinking the glucose solution:

- A healthy blood glucose level is lower than 140 mg/dL (7.8 mmol/L).
- A blood glucose level between 140 and 199 mg/dL (7.8 and 11 mmol/L) is considered prediabetes. If you have prediabetes, you're at risk of developing

type 2 diabetes. You're also at risk of heart disease, even if you don't get diabetes.

- A blood glucose level of 200 mg/dL (11.1 mmol/L) or higher suggests diabetes.

If your results suggest diabetes or prediabetes, the test may be repeated on another day. Or another blood test may be used to confirm whether you have diabetes or prediabetes. Various factors can affect how accurate the glucose tolerance test is. These include illness, your activity level and some medicines.

Gestational diabetes

If you're getting screened for gestational diabetes, your healthcare professional considers the results of each blood sugar test.

After the one-hour test, a blood sugar level of 190 milligrams per deciliter (mg/dL), or 10.6 millimoles per liter (mmol/L), or higher indicates gestational diabetes. A blood sugar level below 140 mg/dL (7.8 mmol/L) usually is thought to be within the standard range for the test. But this may vary by clinic or lab.

If your blood sugar level is higher than expected, your healthcare professional likely will recommend the three-hour test.

If you need the follow-up three-hour test:

- A healthy fasting blood sugar level is 95 mg/dL (5.3 mmol/L) or lower.
- One hour after you drink the glucose solution, an expected blood sugar level is 180 mg/dL (10 mmol/L) or lower.
- Two hours after you drink the glucose solution, an expected blood sugar level is 155 mg/dL (8.6 mmol/L) or lower.
- Three hours after you drink the glucose solution, an expected blood sugar level is 140 mg/dL (7.8 mmol/L) or lower.



If one of the results is higher than expected, your healthcare professional might:

- Suggest that you talk about your diet with a nutritionist.
- Advise that you get diabetes screening tests at least every three years.
- Encourage you to lose extra weight after you have your baby.

If two or more of the results are higher than expected, you'll likely be told that you have gestational diabetes.

It's less common, but some pregnant people may receive a standalone two-hour test. You'll likely be told you have gestational diabetes if you have any of the following:

- A fasting blood sugar level of 92 mg/dL (5.1 mmol/L) or higher.
- A blood sugar level of 180 mg/dl (10.0 mmol/L) or higher one hour after you drink the glucose solution.
- A blood sugar level of 153 mg/dL (8.5 mmol/L) or higher two hours after you drink the glucose solution.

If you learn that you have gestational diabetes, your risk of other medical problems is higher. But it's likely that you can prevent them. The key is to control your blood sugar throughout the rest of your pregnancy.

Clinical trials

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May 03, 2024

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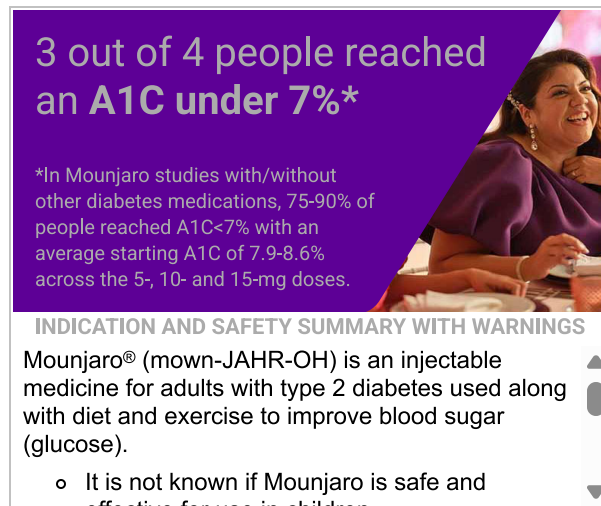
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3 out of 4 people reached an A1C under 7%*

*In Mounjaro studies with/without other diabetes medications, 75-90% of people reached A1C<7% with an average starting A1C of 7.9-8.6% across the 5-, 10- and 15-mg doses.

INDICATION AND SAFETY SUMMARY WITH WARNINGS

Mounjaro® (mown-JAHR-OH) is an injectable medicine for adults with type 2 diabetes used along with diet and exercise to improve blood sugar (glucose).

- It is not known if Mounjaro is safe and effective for use in children.

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INDICATION AND SAFETY SUMMARY WITH WARNINGS

Mounjaro® (mown-JAHR-OH) is an injectable medicine for adults with type 2 diabetes used along with diet and exercise to improve blood sugar (glucose).

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Language: English ▼

Feedback

Berberine and Its More Biologically Available Derivative, Dihydroberberine, Inhibit Mitochondrial Respiratory Complex I

A Mechanism for the Action of Berberine to Activate AMP-Activated Protein Kinase and Improve Insulin Action

Nigel Turner,¹ Jing-Ya Li,^{1,2} Alison Gosby,¹ Sabrina W.C. To,¹ Zhe Cheng,² Hiroyuki Miyoshi,³ Makoto M. Taketo,³ Gregory J. Cooney,¹ Edward W. Kraegen,¹ David E. James,¹ Li-Hong Hu,² Jia Li,² and Ji-Ming Ye¹

OBJECTIVE—Berberine (BBR) activates AMP-activated protein kinase (AMPK) and improves insulin sensitivity in rodent models of insulin resistance. We investigated the mechanism of activation of AMPK by BBR and explored whether derivatization of BBR could improve its *in vivo* efficacy.

RESEARCH DESIGN AND METHODS—AMPK phosphorylation was examined in L6 myotubes and *LKB1*^{-/-} cells, with or without the Ca²⁺/calmodulin-dependent protein kinase kinase (CAMKK) inhibitor STO-609. Oxygen consumption was measured in L6 myotubes and isolated muscle mitochondria. The effect of a BBR derivative, dihydroberberine (dhBBR), on adiposity and glucose metabolism was examined in rodents fed a high-fat diet.

RESULTS—We have made the following novel observations: 1) BBR dose-dependently inhibited respiration in L6 myotubes and muscle mitochondria, through a specific effect on respiratory complex I, similar to that observed with metformin and rosiglitazone; 2) activation of AMPK by BBR did not rely on the activity of either LKB1 or CAMKK β , consistent with major regulation at the level of the AMPK phosphatase; and 3) a novel BBR derivative, dhBBR, was identified that displayed improved *in vivo* efficacy in terms of counteracting increased adiposity, tissue triglyceride accumulation, and insulin resistance in high-fat-fed rodents. This effect is likely due to enhanced oral bioavailability.

CONCLUSIONS—Complex I of the respiratory chain represents a major target for compounds that improve whole-body insulin

sensitivity through increased AMPK activity. The identification of a novel derivative of BBR with improved *in vivo* efficacy highlights the potential importance of BBR as a novel therapy for the treatment of type 2 diabetes. *Diabetes* 57:1414–1418, 2008

Insulin resistance is a major metabolic abnormality leading to type 2 diabetes, and, as such, there is considerable interest in the discovery of insulin-sensitizing agents to aid in the treatment of this disease. AMP-activated protein kinase (AMPK), a heterotrimeric protein that plays a key role in regulation of whole-body energy homeostasis, is one attractive drug target. Two classes of commonly used insulin-sensitizing drugs, thiazolidinediones and biguanides, exert their beneficial effects, at least in part, by activating AMPK (1,2).

Natural products have been a rich resource for the development of novel therapeutics used to treat a variety of human diseases. We have recently reported that berberine (BBR) displays insulin-sensitizing properties in rodent models of insulin resistance and diabetes (3). BBR is commonly used as a nonprescription oral drug in China to treat gut infections and diarrhea with few side effects, and its therapeutic potential for the treatment of diabetes (4) and dyslipidemia (5) in humans has been reported. These beneficial effects are related in part to the ability of BBR to activate AMPK (3,6,7). Here we show that, similar to metformin and rosiglitazone, BBR activates AMPK via inhibition of respiratory complex I of the mitochondrion.

Despite its potent stimulation of AMPK in cell-based assays, an important issue arising from our previous work (3) is that considerably large oral doses (380–560 mg · kg⁻¹ · day⁻¹) of BBR were required for beneficial metabolic effects in rodents. Aiming to improve its therapeutic efficacy, we designed a number of BBR derivatives and show that one such derivative, dihydroberberine (dhBBR), has markedly improved *in vivo* efficacy in the treatment of insulin-resistant rodents.

RESEARCH DESIGN AND METHODS

Preparation of dihydroberberine. Details of the procedures used to prepare dhBBR are included in a supplemental file available in an online appendix at <http://dx.doi.org/10.2337/db07-1552>.

Cell experiments. Cell culture conditions for *LKB1*^{-/-} mouse embryonic fibroblasts (MEFs) and L6 myotubes were as described previously (3,8). [³H]-2-deoxyglucose uptake was measured in L6 cells according to Cheng et al. (7). For immunoblotting experiments, cell lysates were resolved by SDS-

From the ¹Diabetes and Obesity Research Program, Garvan Institute of Medical Research, Darlinghurst, Australia; the ²Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China; and the ³Department of Pharmacology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

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Additional information for this article can be found in an online appendix at <http://dx.doi.org/10.2337/db07-1552>.

N.T., J.-Y.L., and A.G. contributed equally to this work.

ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside; AMPK, AMP-activated protein kinase; BBR, berberine; CAMKK, Ca²⁺/calmodulin-dependent protein kinase kinase; dhBBR, dihydroberberine; HFD, high-fat diet; MEF, mouse embryonic fibroblast.

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PAGE, transferred to polyvinylidene difluoride membranes, and immunoblotted with antibodies specific for LKB1 (Santa Cruz Biotechnology, Santa Cruz, CA), AMPK, pThr172-AMPK, acetyl-CoA carboxylase (ACC), and pSer79-ACC (Cell Signaling Technology, Beverly, MA).

Animals. Male C57BL/6J mice (6–8 weeks old) and male Wistar rats (250 g) purchased from the Animal Resources Centre (Perth, Australia) were kept in a temperature-controlled room ($22 \pm 1^\circ\text{C}$) on a 12-h light/dark cycle with free access to water. Animals were randomly assigned to receive either standard control rodent diet or a high-fat diet (HFD) to generate insulin resistance (9,10). Mice and rats were fed for 10 weeks and 4 weeks, respectively, and based on pilot testing for dhBBR in mice, BBR and dhBBR were provided in the HFD at a dose of $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for the final 2 weeks of feeding. All experiments were carried out with the approval of the Garvan Institute Animal Experimentation Ethics Committee, following guidelines issued by the National Health and Medical Research Council of Australia.

Muscle mitochondrial isolation and respiration measurements. Methods describing the mitochondrial isolation and respiration measurements are provided in the supplemental file.

In vivo glucose metabolism. Glucose tolerance tests (2 g/kg glucose i.p.) in mice and euglycemic-hyperinsulinemic clamps in rats (insulin infusion $0.25 \text{ units} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) were conducted as previously described (9,10). Tissue triglyceride content was determined using a colorimetric assay kit (Roche Diagnostics, Indianapolis, IN).

Pharmacokinetic analysis. Rats were fasted (12 h) and gavaged with 20 mg/kg BBR or dhBBR, and blood samples were obtained over the subsequent 24 h. Plasma concentrations of BBR and dhBBR were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (11).

Statistical analysis. Results are presented as means $\pm$ SE. One-way ANOVA with Fisher's protected least-square difference post hoc test was used to assess statistical significance between groups. For respiration experiments, the effect of drug treatments are given as a percentage of intra-individual basal values (100%), with P values calculated by paired two-tailed t tests. $P \leq 0.05$ was regarded as statistically significant.

RESULTS

Role of LKB1 and Ca^{2+} /calmodulin-dependent protein kinase kinase (CAMKK) in the action of BBR. LKB1 and CAMKK β are two important upstream kinases for AMPK. To determine if activation of AMPK by BBR involves either of these kinases, we first examined the effects of the relatively specific CAMKK inhibitor STO-609 (12) on BBR activation of AMPK in L6 myotubes. The Ca^{2+} ionophore ionomycin increased phosphorylation of AMPK and its downstream target ACC, and this effect was blocked by STO-609 (Fig. 1A). However, the increase in phosphorylation of AMPK and ACC by BBR was unaffected by STO-609, suggesting that CAMKK is not the major AMPK kinase regulated by BBR in L6 cells (Fig. 1A). To examine the role of LKB1 in the activation of AMPK by BBR, we used *LKB1*^{−/−} MEFs (Fig. 1B). BBR caused a robust increase in AMPK and ACC phosphorylation in cells lacking LKB1, as did the AMPK activator AICAR (Fig. 1C). Interestingly, treatment of *LKB1*^{−/−} MEFs with STO-609 blocked the activation of AMPK by ionomycin, 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR), and BBR (Fig. 1C).

BBR inhibits mitochondrial respiration. We recently reported that BBR increases the AMP-to-ATP ratio in L6 myotubes (7). To investigate whether these changes in nucleotide levels may result from inhibition of cellular respiration, oxygen consumption was examined in L6 myotubes. BBR dose-dependently inhibited respiration in L6 myotubes, with an $\sim 50\%$ inhibition at $15 \mu\text{mol/l}$ (Supplementary Fig. 1). Metformin and rosiglitazone also dose-dependently inhibited respiration in L6 myotubes, with rosiglitazone displaying similar potency to BBR, while metformin was substantially less potent (Supplementary Fig. 1). We next examined whether the inhibitory effect of BBR was manifest at the level of the mitochondria. BBR produced a dose-dependent inhibition of oxygen consumption in isolated muscle mitochondria with complex I–I-

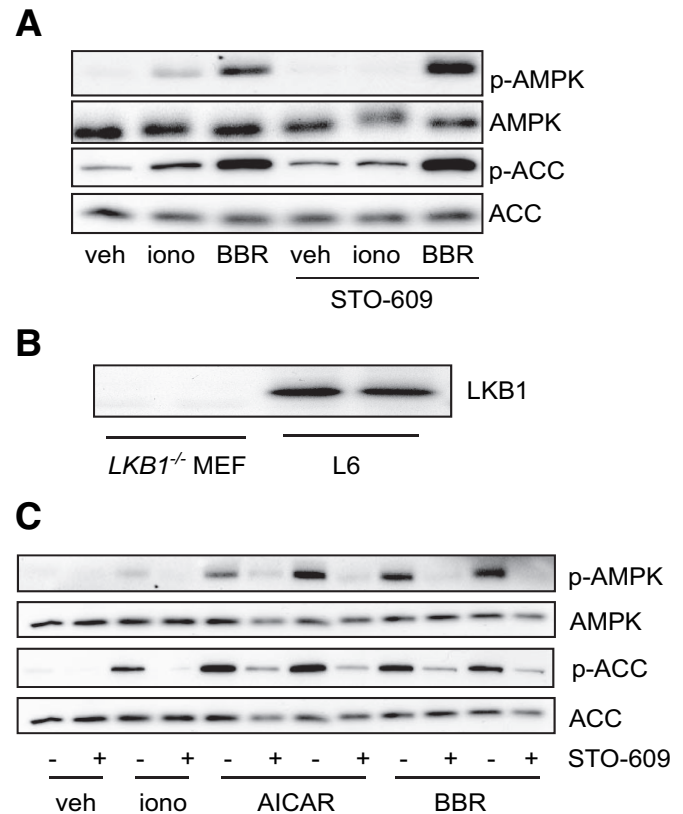


FIG. 1. Effect of CAMKK inhibition and LKB1 deficiency on the activation of AMPK by BBR. **A:** L6 myotubes were incubated for 30 min with vehicle (veh) $1 \mu\text{mol/l}$ ionomycin (iono) or $10 \mu\text{mol/l}$ BBR, with or without preincubation for 1 h with STO-609 ($10 \mu\text{g/ml}$). **B:** LKB1 expression in L6 myotubes and *LKB1*^{−/−} MEFs. **C:** *LKB1*^{−/−} MEFs were incubated for 30 min with $1 \mu\text{mol/l}$ ionomycin, 2 mmol/l AICAR, or $10 \mu\text{mol/l}$ BBR, with or without preincubation for 1 h with STO-609 ($10 \mu\text{g/ml}$). Cells were lysed in $4 \times$ SDS sample buffer, and equal amounts of lysates were resolved by SDS-PAGE and immunoblotted with antibodies specific for LKB1, phospho-AMPK- α (Thr172), total AMPK- α , phospho-ACC (Ser79), and total ACC.

linked substrate (pyruvate), but did not have a substantial effect on complex II-linked respiration using succinate as the substrate (Fig. 2A). A similar inhibitory pattern was also observed in mitochondria for metformin and rosiglitazone (Fig. 2).

dhBBR displays improved in vivo efficacy compared with BBR. Aiming to improve the in vivo efficacy of BBR, we prepared a panel of BBR derivatives and initially tested the effects of these derivatives on glucose uptake and AMPK activation in L6 cells (7). Five of six derivatives had no effect on glucose uptake (Supplementary Fig. 2) or AMPK activation (data not shown). Strikingly, one derivative (dhBBR) displayed similar potency to BBR to stimulate both glucose uptake and AMPK (Supplementary Fig. 2), as well as inhibit respiration in myotubes (Supplementary Fig. 1) and mitochondria (Fig. 2B). A structure-based analysis (using Chemdraw ultra 10 software, <http://www.cambridgesoft.com/services>) suggested that dhBBR would likely display improved in vivo efficacy compared with BBR because of its higher logP value (logP: 3.88 for dhBBR and -0.92 for BBR) (13), and hence its effects were examined in rodent models of insulin resistance.

In mice fed an HFD, treatment with dhBBR ($100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) markedly reduced adiposity and improved glucose tolerance, compared with HFD controls (Fig. 3). At the same

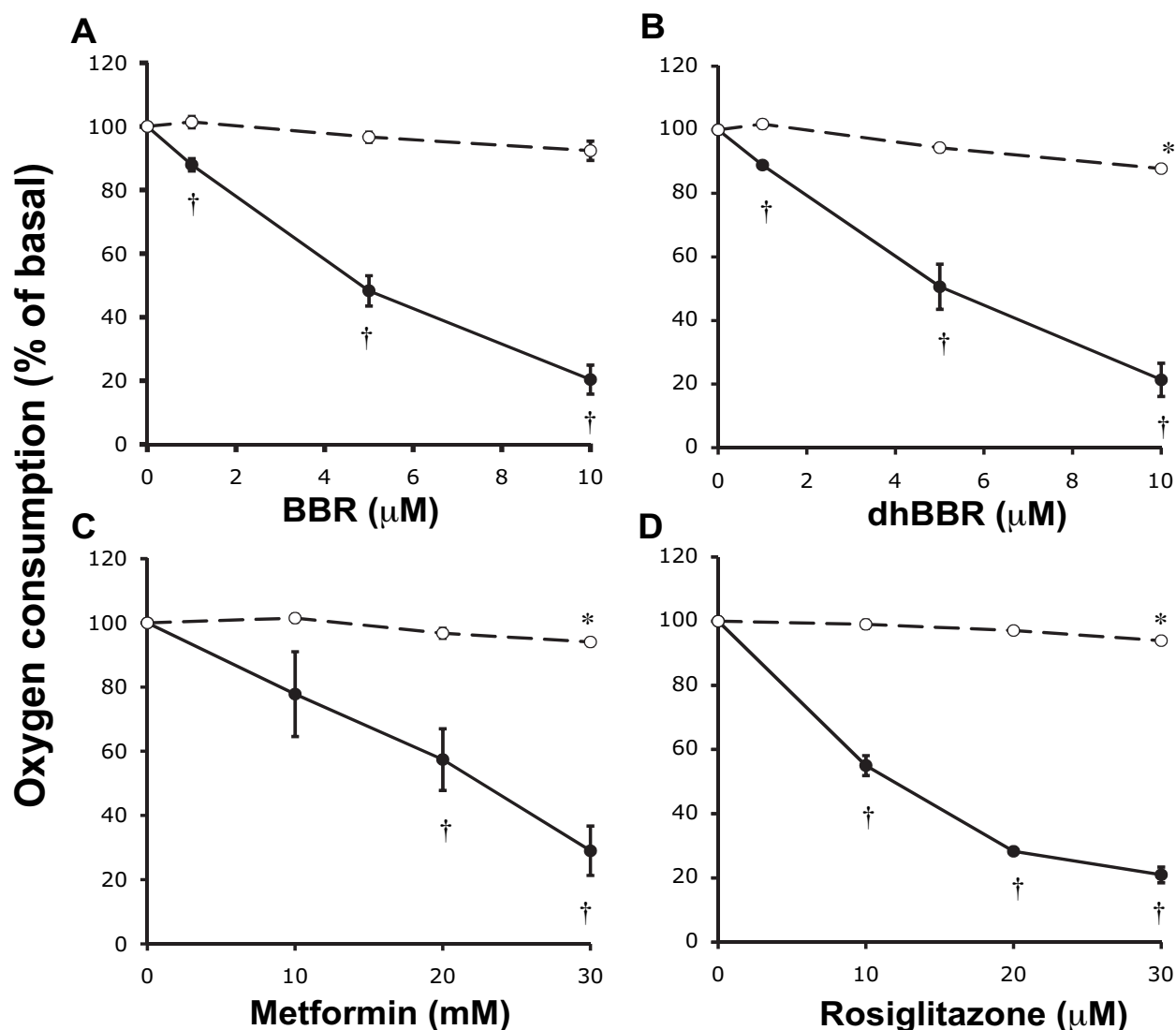


FIG. 2. Inhibition of oxygen consumption in isolated rat muscle mitochondria by BBR (A), dhBBR (B), metformin (C), and rosiglitazone (D). Oxygen consumption rates were measured in mitochondria at 37°C using substrate combinations targeting respiratory complex I (5 mmol/l pyruvate plus 2 mmol/l malate, ●) or complex II (10 mmol/l succinate plus 4 μmol/l rotenone, ○). Data represent means ± SE ($n = 3-6$ per group) and are expressed as a percentage of the basal value (100%). * $P < 0.05$, † $P < 0.01$ vs. basal levels.

dose, BBR had no effect on adiposity or glucose tolerance (Fig. 3), whereas at a dose of $560 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (data not shown), we observed the expected effects of BBR (3).

In HFD rats, treatment with dhBBR resulted in reduced fat pad mass and tissue triglyceride levels compared with HFD controls (Table 1). Whole-body insulin sensitivity, measured as the glucose infusion rate during a hyperinsulinemic-euglycemic clamp, was 44% ($P < 0.01$) higher in dhBBR-treated HFD rats compared with HFD controls, although it was not fully restored to control levels (Table 1). Using glucose tracers, we observed that improvements in whole-body insulin sensitivity in dhBBR-treated HFD rats were largely due to improved uptake of glucose into peripheral tissues, as evidenced by increased R_d and tissue-specific uptake of [^3H]-2-deoxyglucose in several tissues (Table 1).

Pharmacokinetic analyses were conducted in rats to determine if enhanced oral bioavailability underpinned the improved in vivo efficacy of dhBBR over BBR. After oral administration of 20 mg/kg BBR, we were unable to detect BBR in the plasma. In contrast, dhBBR at the same oral

dose was rapidly detected in the plasma (Supplementary Fig. 2), displaying a half-life ($t_{1/2}$) of 3.5 ± 1.3 h and a maximum concentration (C_{max}) of 2.8 ± 0.5 ng/ml. Interestingly, in rats gavaged with dhBBR, BBR was also present in the plasma, displaying a longer $t_{1/2}$ (9.6 ± 2.1 h, $P < 0.05$) and a greater C_{max} (12.6 ± 2.4 ng/ml, $P < 0.01$).

DISCUSSION

Here we show that BBR has a similar effect to metformin and rosiglitazone to inhibit respiratory complex I, consistent with previous studies (14–18). These data highlight the importance of complex I as a diabetes target. Inhibition of complex I is likely the main mechanism by which BBR activates AMPK, since we did not observe selective activation of either CAMKKβ or LKB1 by BBR. These findings are consistent with a recent model proposing a major role for AMPK regulation at the level of the AMPK phosphatase in response to metabolic stress (19,20). Finally, we have also identified a novel BBR derivative that

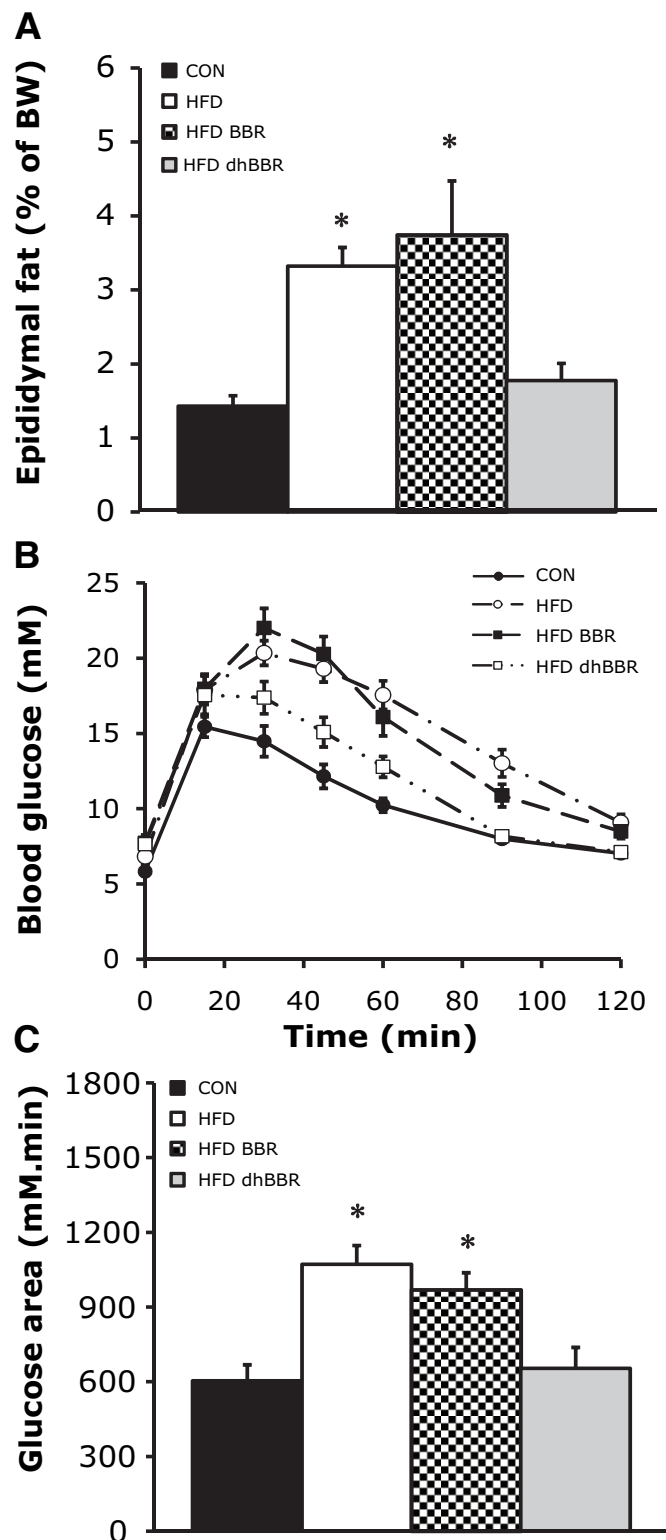


FIG. 3. Effect of BBR and dhBBR on epididymal fat mass and glucose tolerance in mice fed an HFD. BBR or dhBBR were provided in the HFD for 2 weeks at a dose of $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. A: Epididymal fat mass expressed as a percentage of body mass. BW, body weight. B: Glucose levels during an intraperitoneal glucose tolerance test (2 g/kg). C: Incremental area under the glucose curve. Data represent the means $\pm$ SE ($n = 5\text{--}15$ mice per group). * $P < 0.01$ vs. control (CON) and dhBBR.

displays improved in vivo efficacy, thus paving a path for future drug development in this area.

The use of $LKB1^{-/-}$ MEFs and the CAMKK inhibitor STO-609 has provided novel insights into the actions of BBR. We observed robust activation of AMPK in $LKB1^{-/-}$ MEFs by BBR, an effect that could be blocked by pretreatment with STO-609. Conversely in L6 myotubes, which express both LKB1 and CAMKK β , we found that STO-609 was unable to block the activation of AMPK by BBR. These findings suggest that in response to BBR, AMPK can be activated via either LKB1 or CAMKK β . We also observed activation of AMPK in $LKB1^{-/-}$ MEFs by the adenosine analog, AICAR, with this effect also abrogated by STO-609. Recently, it was proposed that in addition to a direct allosteric effect, elevated AMP concentrations activate AMPK indirectly by preventing dephosphorylation of Thr172 by phosphatases (19,20). Hence, this model predicts a permissive and/or redundant role for upstream AMPK kinases in metabolic stress-induced AMPK activation, consistent with the present findings for BBR. Interestingly, metformin and rosiglitazone appear to act in an analogous manner (Fig. 2; 16–18). The therapeutic advantage of diverse compounds targeting respiratory complex I is not yet fully appreciated; however, it is noteworthy, in this context, that BBR and metformin have been observed to be beneficial for a broad spectrum of human diseases, including diarrhea, cancer, inflammation, and diabetes (2–4,21–24). We suspect that disturbances in cellular energy homeostasis may be an early event contributing to the therapeutic actions of these compounds.

A potential disadvantage of BBR as an in vivo compound for treatment of diabetes is that we (3) and others (14) have previously reported the need for relatively high doses in rodents to achieve beneficial metabolic effects. In this regard, our identification of a novel derivative that works with substantially improved potency to BBR is a major step forward. At $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, dhBBR reduced adiposity and improved glucose tolerance in HFD mice, whereas we observed no effects of BBR at this dose. Furthermore, in HFD rats, the beneficial effects of dhBBR at $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ were of a similar magnitude to those previously reported for $380 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of BBR (3). Examination of the structure of BBR reveals that it possesses an extremely flat configuration, which is likely to have limited absorption across the intestinal epithelia. However, derivatization to dhBBR was predicted to open up the structure, making it more amenable to uptake. Our pharmacokinetics data were consistent with this prediction, with dhBBR displaying improved absorption compared with BBR. Intriguingly, our data also revealed that once absorbed, dhBBR was rapidly converted back to BBR, highlighting the fact that this is likely the active moiety. These findings indicate that dhBBR is in essence an effective vehicle for delivering BBR to the circulation, substantially reducing the oral dose required for beneficial metabolic effects. As BBR has been shown to improve clinical symptoms in patients with type 2 diabetes (4) and dyslipidemia (5), and undesirable side effects have been reported for several popular anti-diabetic drugs (25), dhBBR represents an attractive potential therapy for the treatment of type 2 diabetes and other components of the metabolic syndrome.

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TABLE 1

Fat pad mass, tissue triglyceride levels, and metabolic parameters from hyperinsulinemic-euglycemic clamps in rats

	Control	HFD	HFD dhBBR
Body mass (g)	349 ± 7	367 ± 5*	361 ± 6
Epididymal fat pad (%)	1.05 ± 0.04	1.94 ± 0.11†	1.48 ± 0.10†§
Inguinal fat pad (%)	1.04 ± 0.07	2.28 ± 0.16†	1.79 ± 0.11†§
Muscle triglyceride (μmol/g)	3.0 ± 0.3	5.1 ± 1.0*	3.1 ± 0.2‡
Liver triglyceride (μmol/g)	4.8 ± 0.2	17.1 ± 1.9†	9.4 ± 1.4*
Glucose infusion rate (mg · kg ⁻¹ · min ⁻¹)	40.2 ± 2.2	19.0 ± 0.4†	27.4 ± 1.9†§
R _d (mg · kg ⁻¹ · min ⁻¹)	38.4 ± 1.9	23.2 ± 0.5†	30.3 ± 0.6†§
Hepatic glucose output (mg · kg ⁻¹ · min ⁻¹)	-1.7 ± 0.5	4.2 ± 0.6†	2.8 ± 1.7†
R _g ['] : red gastrocnemius	35.6 ± 3.3	19.8 ± 1.7†	34.9 ± 7.9‡
R _g ['] : white gastrocnemius	15.2 ± 2.7	5.7 ± 0.7†	8.5 ± 1.5*
R _g ['] : epididymal fat	1.8 ± 0.2	1.3 ± 0.1	2.1 ± 0.4‡

Data are means ± SE (*n* = 5–12 per group). dhBBR was provided in the HFD (100 mg · kg⁻¹ · day⁻¹) for the final 2 weeks of high-fat feeding. Fat pad weights are expressed as a percentage of body mass. Plasma levels of glucose and insulin were similar among the three groups during the clamp (data not shown). R_g['], insulin-stimulated glucose uptake during the clamp (μmol/100 g · min). **P* < 0.05, †*P* < 0.01 vs. control; ‡*P* < 0.05, §*P* < 0.01 vs. HFD.

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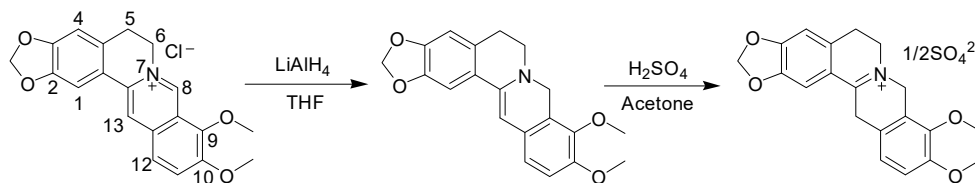
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Supplementary Methods

Preparation of Dihydroberberine.



LiAlH₄ (1.9 g, 50 mmol) was slowly added into a suspension of berberine (3.7 g, 10 mmol) in 100 mL of dry tetrahydrofuran (THF) at 0 °C. The mixture was stirred at room temperature for 3 h and then quenched cautiously by the subsequent addition of H₂O (2 ml), 5 N NaOH (2 ml) and H₂O (6 ml). The mixture was then filtered and the filtrate was evaporated under reduced pressure to remove THF. The residue was re-crystallized with a mixture of methanol and dichloromethane (Shanghai Chemical Plant, Shanghai, P. R. China) to form dihydroberberine (2.4 g, yield 65%). 2.5 ml of 2 N H₂SO₄ was then added into a solution of dihydroberberine (3.4 g, 10 mmol) in 200 ml of acetone over 10 min. The mixture was stirred for 20 min then cooled to 0 °C for 1 h. The mixture was filtered and the filter cake washed with acetone and water respectively. Finally, the filter cake was dried overnight to give dihydroberberine sulfate [5,6-Dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium sulfate] (2.9 g, yield 75%).

Muscle Mitochondrial Isolation. For mitochondrial isolation, red quadriceps muscle obtained from control rats was placed in ice-cold mitochondrial isolation medium (in

mM): 100 sucrose, 100 KCl, 50 Tris-HCl, 1 KH₂PO₄, 0.1 EGTA, 0.2% fatty acid free BSA, pH 7.0. Muscle was chopped finely and incubated with shaking at 4°C in isolation medium containing protease (Nagarse, 1.5 mg/g tissue) for 2 min. The nagarse was then diluted 4-fold and samples were immediately homogenized with a Polytron® Homogenizer (Kinematica, Lucerne, Switzerland), for 15 sec at the lowest speed. The homogenate was centrifuged at 800 g at 4°C for 5 min to remove nuclei and cell debris. The supernatant was removed and centrifuged at 10,500 g at 4°C for 10 min. The pellet was washed and recentrifuged at 10,500 g at 4°C for 10 min and the resulting pellet, containing mitochondria, was resuspended in respiration medium (see below) at a concentration of 2 ml per milligram of original tissue. Protein content in mitochondria and all cell preparations was measured using the Bradford method.

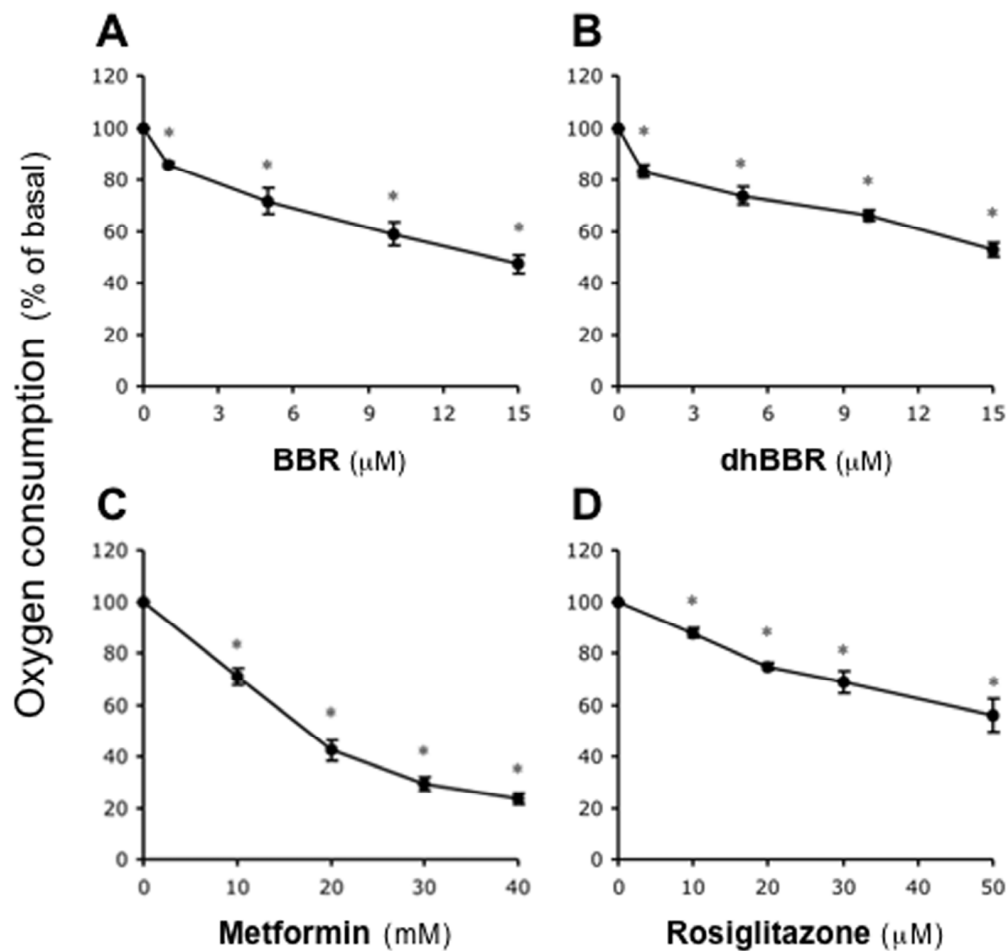
Respiration measurements. Respiration measurements in L6 myotubes and isolated mitochondria were conducted at 37°C in a Clark type oxygen electrode (Strathkelvin Instruments, Motherwell, Scotland). For L6 myotubes (1 – 2 mg protein) the respiration medium consisted of 25 mM glucose, 1 mM pyruvate, 2% BSA in PBS, pH 7.4. Cells were transferred to the electrode chamber and following attainment of a steady rate of oxygen consumption, BBR, dhBBR, metformin (MP Biomedicals, Irvine, CA, USA) or rosiglitazone (a gift from Novo Nordisk, Måløv, Denmark) were added dose-dependently and their effect on oxygen consumption recorded. For mitochondria, the respiration medium contained (in mM): 225 mannitol, 75 sucrose, 10 Tris-HCl, 10 KH₂PO₄, 10 KCl, 0.8 MgCl₂, 0.1 EDTA, 0.3% fatty acid free BSA,

pH 7.0 and the respiratory control ratios (state III/state IV respiration) determined with 5 mM pyruvate as substrate, were approximately 5, indicating well coupled mitochondria. Dose-response effects of the different compounds on mitochondrial respiration were determined in the presence of excess ADP (2.4 mM), using substrate combinations targeting either Complex I (5 mM pyruvate plus 2 mM malate) or Complex II (10 mM succinate plus 4 μ M rotenone) of the respiratory chain.

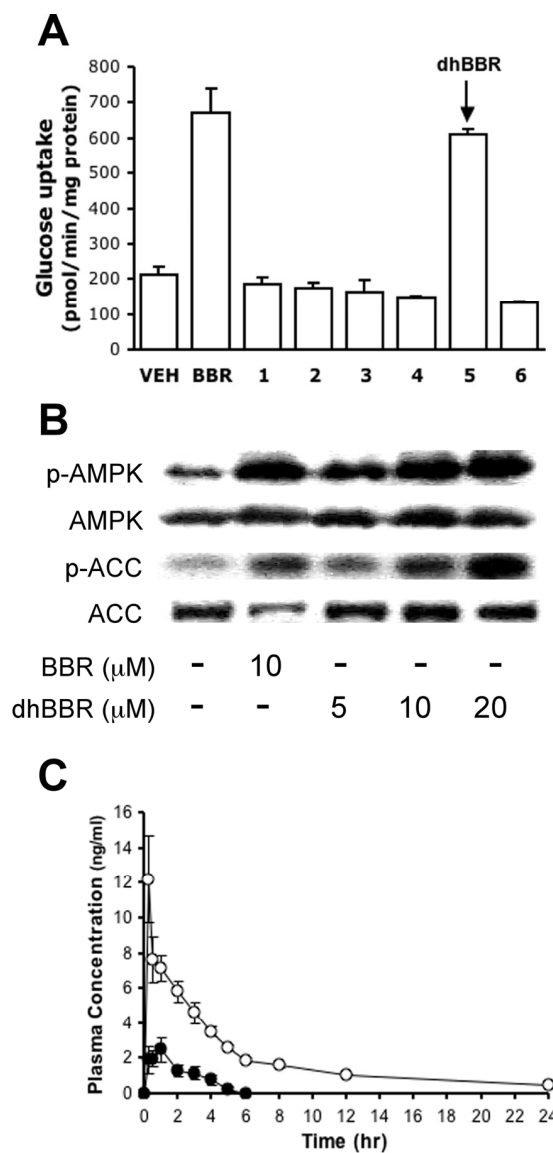
Supplementary Figure Legends

Supplementary Fig 1. Inhibition of oxygen consumption in L6 myotubes by (A) BBR, (B) dhBBR, (C) metformin and (D) rosiglitazone. Oxygen consumption rates were measured in myotubes and are expressed as a percentage of the basal value (=100%). Data represent means $\pm$ SE (n = 6-8 per group). *P < 0.01 vs. basal levels.

Supplementary Fig 2. Stimulation of glucose uptake and AMPK phosphorylation in L6 myotubes by dhBBR and pharmacokinetic analysis of dhBBR in rats. **A)** Glucose uptake measured in L6 myotubes pretreated for 3 hr with 5 μ M BBR and a range of BBR derivatives (compounds 1-6). Data represents mean $\pm$ SE of 2 independent experiments **B)** L6 myotubes were incubated for 30 min with BBR or dhBBR at the indicated concentrations, lysed in 4 x SDS sample buffer and equal amounts of lysates were resolved by SDS PAGE and immunoblotted with antibodies specific for phospho-AMPK- α (Thr172), total AMPK- α , phospho-ACC (Ser79) and total ACC. **C)** Plasma concentration of dhBBR (closed circles) and BBR (open circles) in rats following oral gavage of 20 mg/kg dhBBR. Data represent means $\pm$ SE (n=4).



Supplementary Fig 1



Supplementary Fig 2



US 20100113494A1

(19) **United States**(12) **Patent Application Publication****Hu et al.**(10) **Pub. No.: US 2010/0113494 A1**(43) **Pub. Date: May 6, 2010**(54) **13,13A-DIHYDROBERBERINE DERIVATIVES
FOR USE IN PHARMACEUTICAL
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(2), (4) Date:**Jan. 4, 2010**(30) **Foreign Application Priority Data**

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Publication Classification(51) **Int. Cl.****A61K 31/4375** (2006.01)(52) **U.S. Cl.** **514/280**; 514/279; 514/287(57) **ABSTRACT**

The present invention provides 13,13a-dihydroberberine derivatives or their physiologically acceptable salts represented by the following formula, pharmaceutical compositions comprising the same, and uses thereof. The 13,13a-dihydroberberine derivatives have an activity of promoting glucose absorption in muscle cells, and the whole animal tests show that the present compounds have effects on improving glucose-tolerance and insulin-resistance, facilitating weight loss, relieving fatty liver and the like. Thus, the present compounds can be used in treating diabetes mellitus, adiposity, fatty liver and complications thereof induced by insulin resistance.

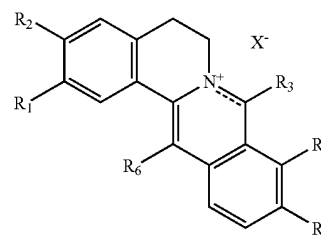


FIG.1

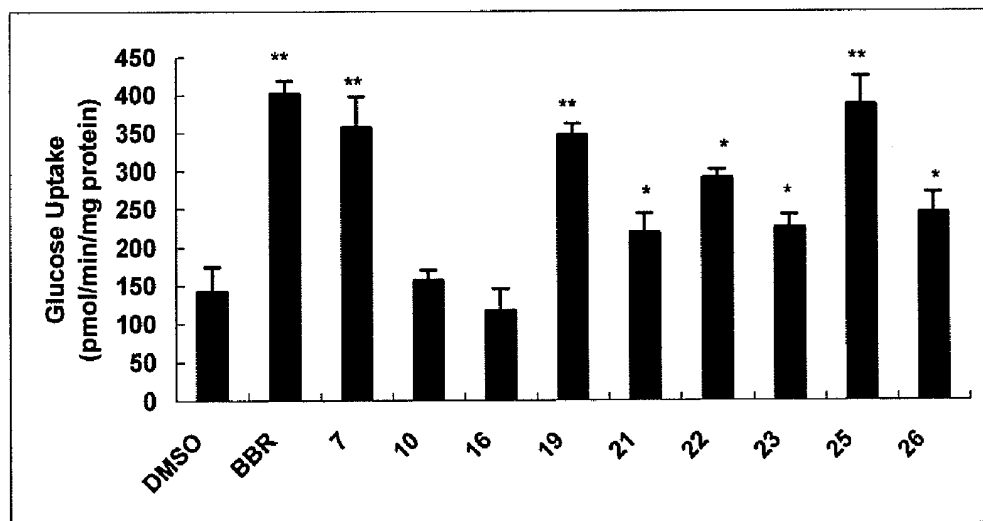


FIG.2

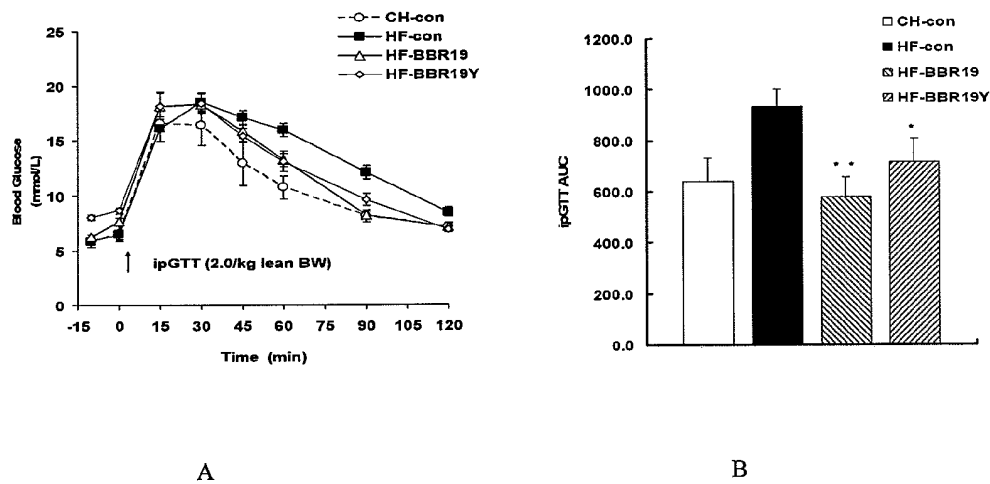


FIG.3

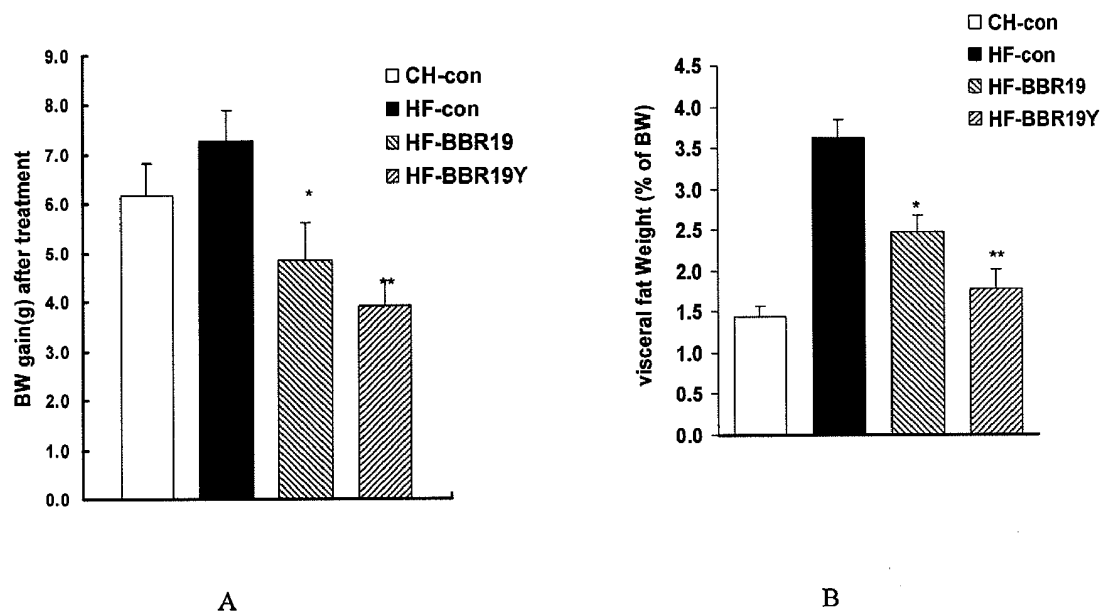
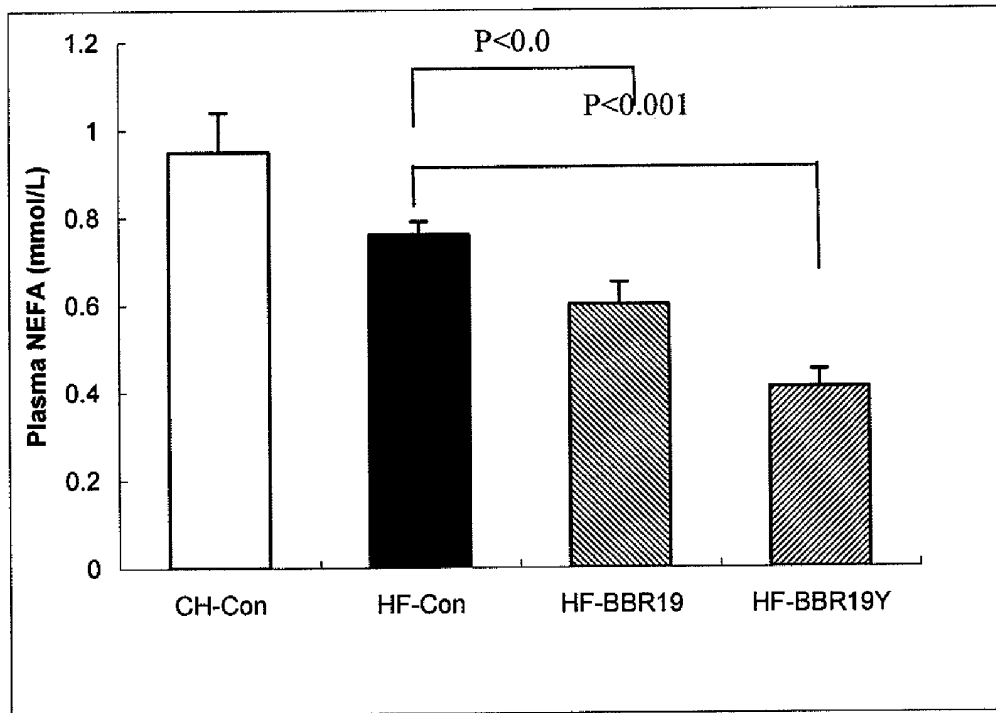
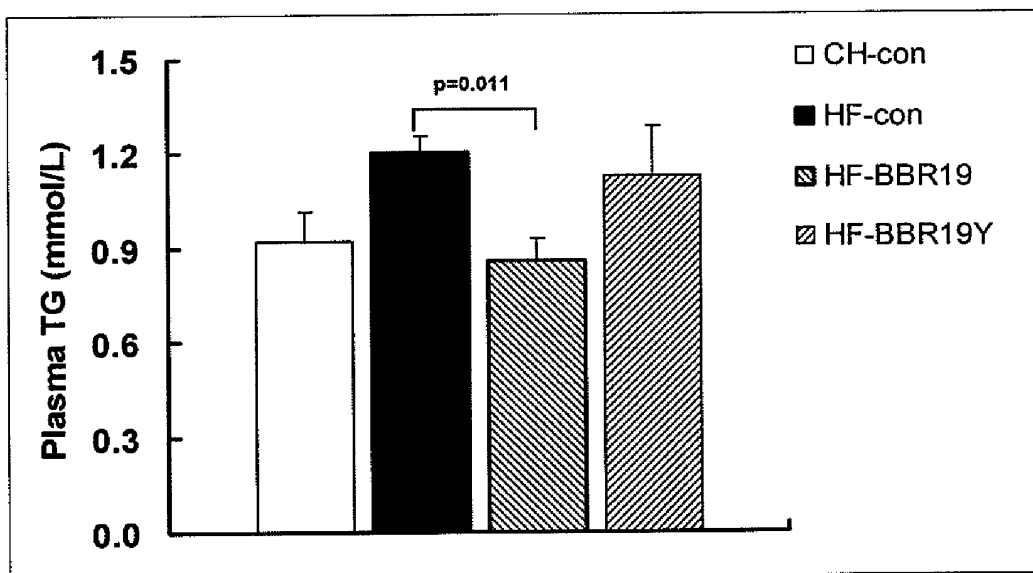


FIG.4



A



B

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13,13A-DIHYDROBERBERINE DERIVATIVES FOR USE IN PHARMACEUTICAL COMPOSITIONS

FIELD OF THE INVENTION

[0001] The present invention generally relates to the fields of pharmaceutical chemistry and therapeutic treatment of diseases. More particularly, the present invention relates to pharmaceutical compositions of 13,13a-dihydroberberine derivatives and the use thereof as an insulin sensitizer.

BACKGROUND OF THE INVENTION

[0002] Diabetes mellitus is a group of clinical metabolic diseases induced by the genetic and environmental interactions, and involves a series of disorders of carbohydrate, protein, fat, water and electrolyte metabolisms due to absolute or relative deficiencies in insulin secretion or reduced sensitivity of the target tissues to insulin. Clinically, diabetes mellitus is mainly characterized by hyperglycemia and may lead to damages to multiple organic systems as time goes on, and acute metabolic disorder such as ketoacidosis etc. may occur in serious conditions and stress. The incidence of severe complications such as cardiovascular diseases, kidney damage, diabetic angiopathy and peripheral vascular disease leading to blindness and gangrene etc. in patients suffering from diabetes mellitus is significantly higher than that in persons free of diabetes mellitus. Therefore, diabetes mellitus and complications thereof are the cause of serious public health problems that threaten human health throughout the world.

[0003] Diabetes mellitus is mainly classified into two types, namely type 1 diabetes mellitus (insulin-dependent diabetes mellitus, IDDM) and type 2 diabetes mellitus (non-insulin-dependent diabetes mellitus, NIDDM). Presently, over 95% of diabetic patients suffer from type 2 diabetes mellitus. With the improvement of living conditions, the incidence of diabetes mellitus is increasing due to aging, obesity, and participating in unhealthy life style choices such as eating an unhealthy diet and lacking exercise. According to the statistics, the worldwide number of the diabetic patients has exceeded 190 million, and it is estimated that this figure will rise to 330 million in 2025.

[0004] Type 1 diabetic patients have abnormal response to stimuli of environment factors, in particular such as viral infection or chemical toxic substance, due to genetic susceptibility determined by HLA-D gene in the short arm of chromosome 6, which destroys the pancreatic beta-cells through direct or indirect autoimmune response and hence results in insulin deficiency. These patients have the clinical features of quick onset, obvious symptoms such as polyphagia, polyuria, polydipsia and weight loss etc., and the tendency of having ketoacidosis, and have to rely on insulin therapy to maintain their lives.

[0005] Type 2 diabetes mellitus is also strongly affected by genetic and environmental factors, and shows significant heterogeneity, that is, its pathogenesis is varied and complicated, and there are great differences among type 2 diabetic patients. Generally, the reasons that cause type 2 diabetes mellitus can be attributed to the relative deficiencies in insulin secretion and/or insulin resistance. A series of studies on type 2 diabetic patients, in particular obese diabetic patients, have identified that the insulin resistance is a key factor during the occurrence and development of type 2 diabetes mellitus. Therefore, according to the results obtained from the studies on the

insulin signaling pathway in adipose and muscle cells, it is currently a focus and a major tendency of the development of new drugs for type 2 diabetes mellitus to design and develop insulin sensitizer so as to improve the condition of insulin resistance.

[0006] At present, the oral drugs for diabetes treatment in clinic can be classified into three main types including insulin secretagogues, insulin sensitizers and carbohydrate modulator.

[0007] Sulfonylureas insulin secretagogues: Sulfonylureas lower blood glucose levels in human body by stimulating the release of insulin from the pancreatic beta-cells. Such type of drugs includes Glibenclamide, Glipizide, Glucodone, Glucalazide, Glimepiride etc. Sulfonylureas are the first-line medications for non-obese type 2 diabetic patients. However, all of sulfonylureas run the risk of causing hypoglycemia, i.e. abnormally low and dangerous levels of blood glucose.

[0008] Non-sulfonylureas insulin secretagogues: Non-sulfonylureas that are highly related in terms of mechanism of action are the rapid-acting prandial insulin releasers. More particularly, they stimulate insulin secretion from the pancreatic beta-cells by increasing the concentration of Ca^{2+} in the cells through Ca^{2+} inflowing caused by closing the ATP depended K^{+} channels on the membranes of the pancreatic beta-cells. Such drugs including Repaglinide, Nateglinide etc. are mainly used as a diet regulator to adjust high blood glucose levels after meals. However, they can not improve insulin resistance, and may cause the patients to have temporal anaphylactic response.

[0009] Thiazolidinediones insulin sensitizers: Thiazolidinediones (TZDs) are a class of high selective peroxisome proliferator activated receptor γ (PPAR γ) agonists, which may improve insulin resistance and correct abnormal glucose and lipid metabolism. The mechanism of action of TZDs is to control blood glucose levels of human body effectively by increasing insulin sensitivity in its targeted tissues. Such drugs include Troglitazone hydrochloride (it has been withdrawn from market because of safety problems in term of hepatotoxicity, and the reason for causing such a side effect is still not found out), Rosiglitazone, Pioglitazone. The main side effects of the drugs are weight gain, edema and so on.

[0010] Biguanides insulin sensitizers: Biguanides cannot promote insulin secretion. Instead, their mechanism of action is to lower blood glucose levels by increasing glucose uptake of the peripheral tissues such as muscle, promoting the anaerobic glycolysis of tissues, improving the glucose absorption of tissues such as muscle, suppressing hepatic gluconeogenesis and decreasing the incidence of hyperglycemia in the case of diabetes mellitus. Biguanides can improve glycometabolism and cause weight loss without affecting blood serum insulin levels.

[0011] Therefore, there is no risk of lowering blood glucose levels after administration of Biguanides for the patients with normal blood glucose levels. Thus, Biguanides are the first-line drugs for obese diabetic patients. Such drugs include Phenethylbiguanide (which has been withdrawn from market due to safety problems), Metformin hydrochloride, Metformin hydrochloride sustained-release tablets etc. However, a large dose of Biguanides is required to be administrated to improve the insulin sensitivity. For example, the oral dose of Metformin hydrochloride is 1500 mg per day (mg/d), which is the upper limit of dose in China. The accompanying side effects are mainly gastrointestinal symptoms such as nausea,

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diarrhea and cramps etc., and the old with impaired heart or renal function are at a risk of lactic acidosis when taking such drugs.

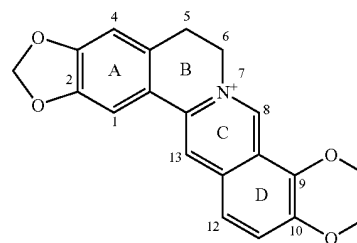
[0012] Drugs for reducing the absorption of carbohydrates are mainly α -glucosidase inhibitors and aldose reductase inhibitors. α -Glucosidase inhibitors may prevent the increase of blood glucose levels after meals by suppressing the decomposition and digestion of carbohydrates and disaccharides, and delaying and reducing the absorption of glucose in the upper segment of small intestine through competitively suppressing the activity of α -glucosidase which take part in the degradation of carbohydrates in the villi of small intestines. They may be used as the first-line drugs with an appropriate diet and exercise, or they may be used in combination with sulfonylureas, biguanides and/or insulin. This type of drugs includes Acarbose, Voglibose and Miglitol etc. Their main adverse effects are gastrointestinal upsets such as abdominal discomfort and flatulence etc. Aldose Reductase (AR) is a key rate-limiting enzyme in the polyol pathway. AR inhibitors have been a new focus in the research fields of diabetic therapy since 1970s.

[0013] A large number of animal experiments and clinical researches indicate that AR inhibitors can normalize effectively the impaired polyol pathway in organisms so as to prevent and/or delay diabetic complications. Tolrestat is a drug for diabetic therapy which was developed by Wyeth Pharmaceuticals and marketed in Ireland in 1989. However, it was not approved by the Food and Drug Administration (FDA) because of causing visual impairment and renal failure, and was withdrawn from market in 1996. Epalrestat is another drug for diabetic therapy which is marketed in Japan recently. However, it also has some similar adverse effects.

[0014] Therefore, the current drugs for the treatment of type 2 diabetes mellitus cannot sufficiently control blood glucose levels and are not effective for all diabetic patients.

Moreover, each type of drugs has more or less adverse effects. Thus, it is still a research highlight currently to develop new drugs which have new mechanism and are safe and effective for the treatment of type 2 diabetes mellitus.

[0015] Berberine, also called umbellatine, is an isoquinoline alkaloid represented by the following formula, which is a major active component in such plants as *Coptidis chinensis* Franch., *Berberis amurensis* Rupr., *Berberis sargentiana* Schneid and *Nandina domestica* etc. Clinically, berberine is mainly used to treat diarrhea caused by bacteria with the most important advantage of having little side effects. Berberine has been used as a broad spectrum antibiotic for many years, and is effective on inhibition and elimination of various Gram-positive and Gram-negative bacteria, fungi, moulds, viruses, protozoa and nematode.



[0016] Structure of Berberine

[0017] Since 1950s, a large number of animal experiments and clinical trials have been carried out by some specialists, researchers and clinicians with berberine hydrochloride which is widely used in clinic, and the results showed that berberine are effective on the therapy of diabetes mellitus and their complications besides being antibiotic. Some research literatures are summarized in the following Table I:

TABLE I

Summary of animal experiments and clinical trials on treating diabetes mellitus with berberine		
Authors	Results of animal studies and clinical trials	Literature
Qiming CHEN, Mingzhi XIE (Institute of Materia Medica, Chinese Academy of Medical Sciences)	Berberine possesses advantages of both sulfonylureas and biguanides. It can lower blood glucose levels of both normal mice and alloxan-induced diabetic mice, and antagonize the increase of blood glucose levels induced by exogenous glucose or epinephrine. It has powerful effect on lowering blood glucose levels and long action time lasting up to 5-6 hours. Berberine can also improve abnormal blood coagulation and prevent atherosclerosis. It had a significant effect on lowering blood glucose at a dose of 40 mg/kg.	Acta Pharmaceutica Sinica, 1986, 21(6): 401-406.
Yanxie NI, et. al. (The 208th hospital of PLA)	60 cases of type 2 diabetic patients were treated with berberine and the treatment was lasted for 1-3 months. The results showed that the fasting blood glucose decreased averagely by 5.1 mmol/L and the efficiency was 90%. It was found by pathologic examinations that berberine could promote the regeneration and functional recovery of pancreatic beta-cells. No abnormality was observed for all patients in the hepatic and renal functions and hemogram during the treatment and no obvious adverse effect was found.	Journal of Integrated Traditional and Western Medicine, 1988, 8(12): 711-713.
Guoying LIU (Pengze traditional Chinese medical hospital, Jiujiang, Jiangxi)	30 cases of type 2 diabetic patients were treated with berberine and the efficiency was 90%. Berberine was significantly effective for the patients with light or moderate diabetes mellitus. In addition, it also showed effects on lowering blood pressure and blood fat, and preventing infection and the occurrence of complication etc.	New use of old drug, 1992, 1: 3-4.

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TABLE I-continued

Summary of animal experiments and clinical trials on treating diabetes mellitus with berberine		
Authors	Results of animal studies and clinical trials	Literature
Mianrong YU, et. al. (Builder hospital of Beijing)	75 cases of type 2 diabetic patients were treated with berberine and the efficiency was 69.33%. Three diabetic symptoms, i.e. polyuria, polydipsia and polyphagia, disappeared for all patients within two weeks after administration of berberine accompanying the decrease of blood glucose. The diabetic patients have been treated with berberine and observed for 4 years, and none of them was observed with adverse effects or hypoglycemia regardless of dose. In addition, 11 patients had normal blood glucose with reduced dose more than 3 years.	Beijing Medical Journal, 1994, 16(2): 117.
Faguang HU, et. al. (The Shanxuan people's hospital, Henan)	60 cases of type 2 diabetic patients were treated with berberine and the efficiency was 90%. Berberine had a mild and lasting effect on lowering blood glucose, making the blood glucose start to lower after about 2 weeks, and achieving its maximum curative effect within 8 weeks. For all patients, berberine made the diabetic symptoms disappearing or being relieved and showed good effects on common diabetic complications. In addition, 4 patients with overweight recovered their normal weights. No toxic and/or adverse effects were observed.	The Practical Journal of Integrating of Chinese with Modern Medicine, 1995, 8(6): 358-359.
Jiaqing ZHANG (Changhai hospital, the 2nd military surgeon college)	Berberine has a certain effect on increasing insulin sensitivity and can improve insulin resistance with an insulin action index (IAI) similar to metformin.	Liaoning Journal of Practical Diabetology, 1999, 1(4): 56-57.
Changshan LIU, et al. (Weifang medical college, Shandong)	Renal failure often occurs to most of diabetic patients with the deterioration of conditions. Berberine, a traditional Chinese drug, has a significant effect on reversing diabetic renal failure and inhibiting the activities of the polyol and kidney aldose reductases of diabetic mice to lower urine protein.	Chinese Journal of Diabetes, 1996, 4(3): 163-166.
Jinhua TONG (Sanitation School of Fengtai, Anhui)	49 cases of type 2 diabetic patients were treated with berberine and the efficiency was 63.3%. Berberine can suppress glyconeogenesis and glycogenolysis, slow down insulin metabolism and promote the sensitivity of peripheral tissue cells to insulin by lowering the activity of the sympathetic nerve and inhibiting the adrenal cortical function. It can also promote the regeneration and functional recovery of pancreatic cells.	New Journal of Traditional Chinese Medicine, 1997, 29(3): 33-34.
Huading YU, Laiwen YAN (Shanghai seaman hospital)	The effect of berberine on treating non-insulin dependent diabetic patients was evaluated. The patients were divided randomly into 2 groups, and each group had 30 persons. One group took berberine and another took Diamicon. After 4 weeks treatment, the variations of the concentrations of blood glucose and blood serum insulin were compared between the two groups, and the results showed that the two groups had similar curative effects and no severe adverse effects were observed.	Medical Journal of Communications, 1995, 9(4): 45.
Yunfei ZHANG (Hangzhou sanatorium of Air Force)	For 20 cases of type 2 diabetic patients, their conditions were not improved significantly after they took oral anti-diabetic drugs for lowering blood glucose such as D860 and glibenclamide etc. Then, they were treated with berberine. The fasting blood glucose levels of all patients were lowered to normal levels 3 months later. The effect of berberine on lowering blood glucose was dependent on dose, and no side effects such as hypoglycemia were observed.	Chinese Journal of Integrated Traditional and Western Medicine, 1999, 19(9): 567.
Hong ZHU, Wei DAI (Affiliated hospital of Jining medical college)	112 cases of type 2 diabetic patients were treated with berberine and the efficiency was 90%, which showed that berberine had a good effect on lowering blood glucose. Specifically, berberine had mild and lasting effect of lowering blood glucose without severe toxicity, side effects and damages to liver and renal function, and had good curative effect on common complications of diabetes mellitus.	Journal of Jining Medical College, 1999, 22(3): 67.
Litian SHI, et al. (Taiyuan commercial staff hospital)	68 cases of type 2 diabetic patients were treated with berberine. The blood glucose levels of the patients were lowered significantly, and the total efficiency was 86.8%. The action mechanism of berberine on lowering blood sugar was multiple. The blood serum insulin levels of the patients increased significantly after the treatment.	Shanxi Clinical Medicine, 2000, 9(3): 181-182.
Dongkou WANG (Taizhou Health center, Jiangsu)	57 cases of type 2 diabetic patients were treated with a second generation sulfonylureas drug alone or in combination with metformin. However, secondary failure occurred 3 years later. Thus, berberine was taken additionally. The blood glucose levels of the patients were lowered more or less 1 month later, and total efficiency was 81%.	Zhejiang Journal of Integrated Traditional Chinese and Western Medicine, 2002, 12(1): 50.

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TABLE I-continued

Summary of animal experiments and clinical trials on treating diabetes mellitus with berberine		
Authors	Results of animal studies and clinical trials	Literature
Jiandong JIANG et al.	Berberine can interact with insulin receptors and increase significantly the expressions of insulin receptor genes and peroxisome proliferator activated receptor genes. Therefore, berberine can be used as an insulin sensitizer to treat type 2 diabetes mellitus alone or in combination with insulin or other type 2 diabetic drugs.	CN01121906.8
Kaimin WU	Highly soluble berberines such as berberine sulfate and berberine phosphate etc. are easily dissolved in water, which may improve the absorption of the drugs in organism and thus enhance significantly their curative effect. Thus, they can be used to treat and prevent diabetes mellitus or its complications.	WO03090749A1, CN1771944A

[0018] Recently, berberine has been widely used to treat type 2 diabetes mellitus in clinic. It was found in an initial research that the antihyperglycemic activity of berberine was in association with its activities of resisting hyperglycemic hormones and promoting the regeneration and functional recovery of pancreatic beta-cells (Hongyan ZHENG, Weiren XU, Chinese herbal medicine. 2004, 35: 708-711). Such research showed that 5-100 μmol of berberine could increase the glucose consumption in HepG2 cells by 32-60%, but it did not stimulate βTC3 cells to secrete insulin. So it was concluded that the antihyperglycemic activity of berberine acted by increasing the glucose consumption of liver cells instead of stimulating to secrete insulin, i.e., the antihyperglycemic activity of berberine acted through liver cells without depending on insulin.

[0019] Juan WANG (Juan WANG, China and foreign medical Journal. 2004, 2(12): 65-66) reported that it was observed clinically that the blood glucose levels decreased and the blood serum insulin levels increased when berberine was used to treat diabetes mellitus, which indicated that berberine could promote the regeneration and functional recovery of pancreatic beta-cells besides its resistance to the hyperglycemic hormones. Berberine can also inhibit gluconeogenesis and improve glycolysis, thereby lowering the blood glucose. Thus, the antihyperglycemic activity of berberine belongs to the field of insulin sensitizer. Berberine also has positive effects on preventing diabetes mellitus complications because of its activities of anti-hypertension, anti-hyperlipidemia and anti-infection.

[0020] Up to now, the antidiabetic mechanism of berberine has not been definitely expounded, although it has a significant effect on treating diabetes mellitus. Recently, Guangde YANG et al. from Xi'an Jiaotong University reported that palmatine hydrochloride, an analogue of berberine, could be used to treat type 2 diabetes mellitus (CN1582930A). They also synthesized various analogues of berberine and tetrahydro-berberine, and evaluated their antihyperglycemic activity using cell-membrane chromatography and alloxan-induced diabetic mice (Bioorganic & Medicinal Chemistry Letters, 2006, 16: 1380-1383).

[0021] It was found in our research that berberine had a similar effect to clinical diabetic drugs of Biguanides, and could improve the uptake and absorption of glucose in peripheral tissues of the human body (e.g. muscles) so as to lower blood glucose levels of diabetic patients and achieve the treatment purpose. So, berberine belongs to non-insulin dependent glucose absorption enhancer in the peripheral tissues of the human body. It has been reported in the literatures (Klip, A., et al., Endocrinology 1992, 130, 2535-2544; Yama-

moto, N. et al, Anal. Bio. 2006, 351, 139-145) that metformin hydrochloride could significantly increase the glucose uptake in human body muscle tissues in a model test to evaluate glucose uptake activity in L6 muscle cells. In a model test on the glucose transport in L6 muscle cells, the transport rate of 3-methyl glucose in L6 muscle cells increased by about 0.5 times with 400 μM metformin hydrochloride. Berberine hydrochloride could achieve the same effect with less dosage than metformin in a model test on the glucose transport in L6 muscle cells in vitro.

[0022] In our study, the glucose transport rate in L6 muscle cells increased by about 3 times with 10 μM berberine hydrochloride. In a further glucose absorption model test in L6 muscle cells, the glucose absorption in L6 muscle cells increased by more than 2 times with 5 berberine hydrochloride in 20 mM glucose medium. It was found that berberine had little effect on insulin signaling pathway, but significantly reinforced the activities of AMPK and p38 MAPK kinase with an immunoblotting assay of L6 muscle cells. The improvement in glucose absorption induced by insulin was inhibited obviously, while that induced by berberine was not affected after the treatment with wortmannin, a specific inhibitor of insulin signaling pathway. The glucose absorption induced by berberine was obviously lowered after the treatment with AMPK and p38 MAPK specific inhibitors (Compound C and SB202190, respectively).

[0023] The results of a further immunoblotting test showed that AMPK was located in the upstream of p38 MAPK in the cell signaling pathway affected by berberine. The results of the above tests indicated that AMPK was a key target protein by which berberine improved the glucose absorption. Through high performance liquid chromatography, it was found that the AMP:ATP ratio in cells increased significantly, indicating a variation of the energy metabolism levels in cells, which is the key factor of AMPK activation. To sum up, our research showed that berberine improved the glucose absorption by changing the energy metabolism level in cells and thus activating AMPK protein instead of affecting insulin signaling pathway.

[0024] In general, berberine has a significant activity of treating diabetes mellitus. However, it has some disadvantageous features such as poor solubility and low oral bioavailability, etc., which restrict the further use of berberine on treating diabetes mellitus. Thus, it is necessary to extensively investigate the relationship between the structure and antidiabetic activity of berberine, and find out berberine derivatives having better activity and absorption in vivo.

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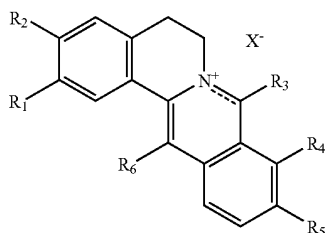
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SUMMARY OF THE INVENTION

[0025] The present invention is provided to solve the above problems.

[0026] An object of the present invention is to provide 13,13a-dihydroberberine derivatives or their physiologically acceptable salts represented by following Formula 1:



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[0027] Wherein, “—” is a double bond or single bond;

[0028] R₁ and R₂ are each independently H, OH, C₁-C₄ alkoxy or C₁-C₄ acyloxy, or R₁ and R₂ are combined into —O—CH₂—O—;

[0029] R₃ is H, OH, C₁-C₄ alkoxy, C₁-C₄ acyloxy, C₁-C₄ alkyl, C₁-C₄ haloalkyl or an aryl;

[0030] R₄ and R₅ are each independently H, OH, C₁-C₄ alkoxy or C₁-C₄ acyloxy, or R₄ and R₅ are combined into —O—CH₂—O—;

[0031] R₆ is H, OH, C₁-C₄ alkyl, C₁-C₄ haloalkyl, an aryl, C₁-C₄ alkoxy or C₁-C₄ acyloxy.

[0032] Preferably, the 13,13a-dihydroberberine derivatives of the present invention are compounds 2-18 shown in the following Table II:

TABLE II

compound	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	X
2	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	CH ₃	I
3	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	CH ₃ CH ₂	Br
4	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	CH ₃ CH ₂ CH ₂	I
5	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	CH ₃ CH ₂ CH ₂ CH ₂	I
6	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	ICH ₂ CH ₂ CH ₂	I
7	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	C ₆ H ₅ CH ₂	Cl
8	—O—CH ₂ —O—	H	OCH ₃		OCH ₃		Cl
9	—O—CH ₂ —O—	H	OCH ₃		OCH ₃		Br
10	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	OH	Cl
11	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	OCH ₃	I
12	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	OCH ₂ CH ₃	Br
13	—O—CH ₂ —O—	H	OCH ₃		OCH ₃	OCOCH ₃	Cl
14	—O—CH ₂ —O—	H	OH		OCH ₃	H	Cl
15	—O—CH ₂ —O—	H	OCOCH ₃		OCH ₃	H	Cl
16	—O—CH ₂ —O—	H	OCOCH ₂ CH ₃		OCH ₃	H	Cl
17	OH	OH	H	OCH ₃	OCH ₃	H	Cl
18	OH	OH	H	OCH ₃	OCH ₃	CH ₃ CH ₂	Cl

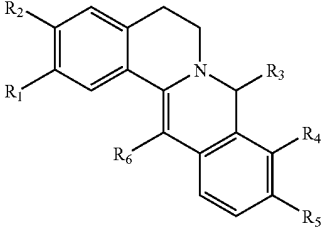
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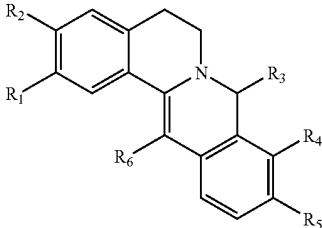
[0033] Preferably, the 13,13a-dihydroberberine derivatives of the present invention are compounds 19-41 shown in the following Table III:

TABLE III



com- pound	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
19	—O—CH ₂ —O—	H		OCH ₃	OCH ₃	H
20	—O—CH ₂ —O—	CH ₃ CH ₂		OCH ₃	OCH ₃	H
21	—O—CH ₂ —O—	PhCH ₂		OCH ₃	OCH ₃	H
22	—O—CH ₂ —O—	HO		OCH ₃	OCH ₃	H
23	—O—CH ₂ —O—	CH ₃ O		OCH ₃	OCH ₃	H
24	—O—CH ₂ —O—	CH ₃ CH ₂ O		OCH ₃	OCH ₃	H
25	—O—CH ₂ —O—	CCl ₃		OCH ₃	OCH ₃	H
26	—O—CH ₂ —O—	CH ₃ COCH ₂		OCH ₃	OCH ₃	H
27	—O—CH ₂ —O—	H		OCH ₃	OCH ₃	CH ₃
28	—O—CH ₂ —O—	H		OCH ₃	OCH ₃	CH ₃ CH ₂
29	—O—CH ₂ —O—	CH ₃		OCH ₃	OCH ₃	H
30	—O—CH ₂ —O—	CH ₃		OCH ₃	OCH ₃	CH ₃

TABLE III-continued



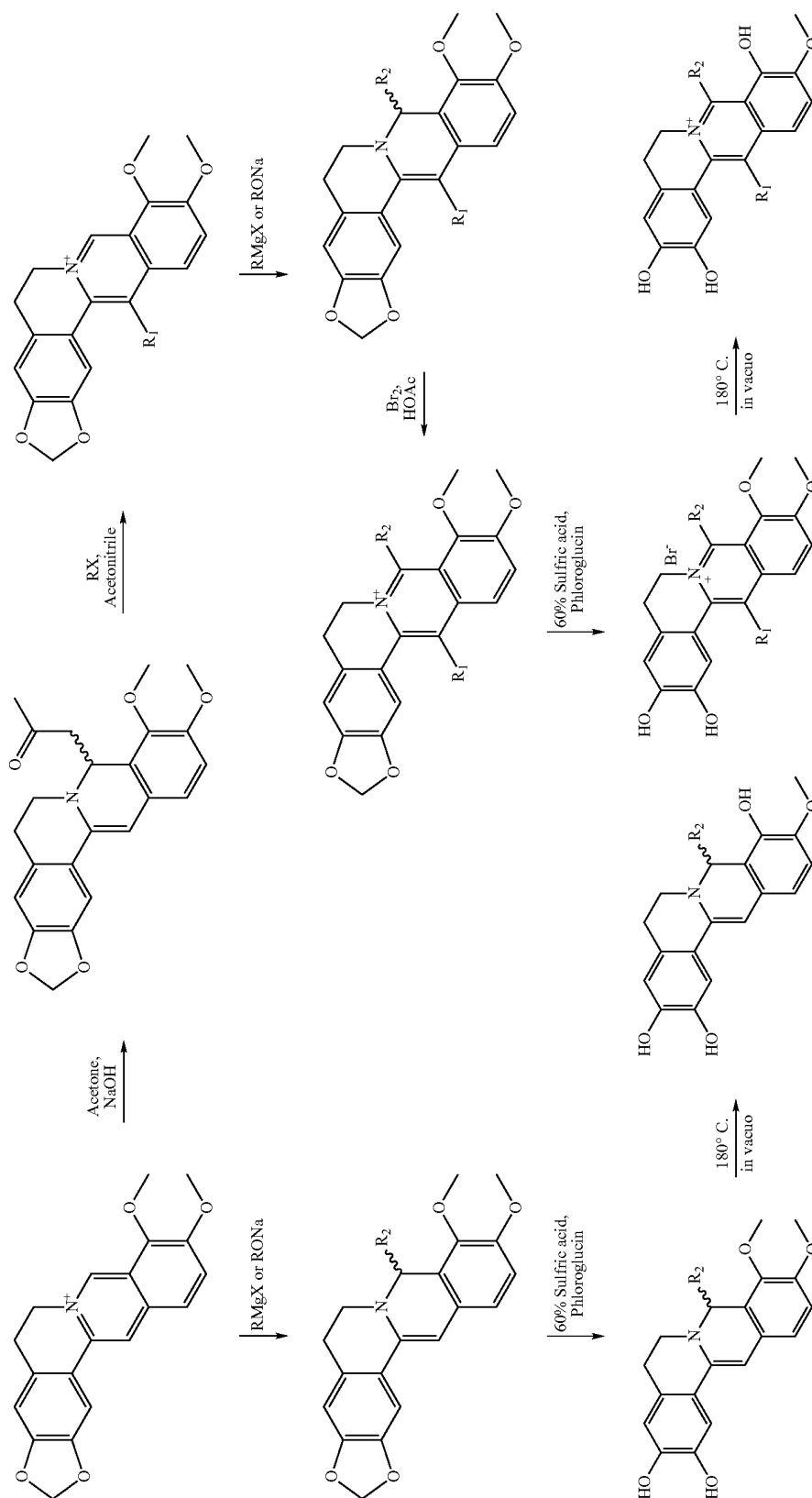
com- pound	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
31	—O—CH ₂ —O—	CH ₃ CH ₂		OCH ₃	OCH ₃	CH ₃ CH ₂
32	OH	OH	H	OCH ₃	OCH ₃	H
33	OH	OH	H	OH	OCH ₃	H
34	OH	OH	CH ₃	OCH ₃	OCH ₃	H
35	OH	OH	H	OCH ₃	OCH ₃	CH ₃
36	OH	OH	CH ₃	OCH ₃	OCH ₃	CH ₃
37	OAc	OAc	H	OCH ₃	OCH ₃	H
38	OAc	OAc	H	OAc	OAc	H
39	OCH ₃	OCH ₃	H	OCH ₃	OCH ₃	H
40	OCH ₃	OCH ₃	H	OCH ₃	OCH ₃	CH ₂ CH ₃
41	—O—CH ₂ —O—	H		—O—CH ₂ —O—	H	

[0034] The 13,13a-dihydroberberine derivatives or their physiologically acceptable salts of the present invention can be synthesized by the following process:

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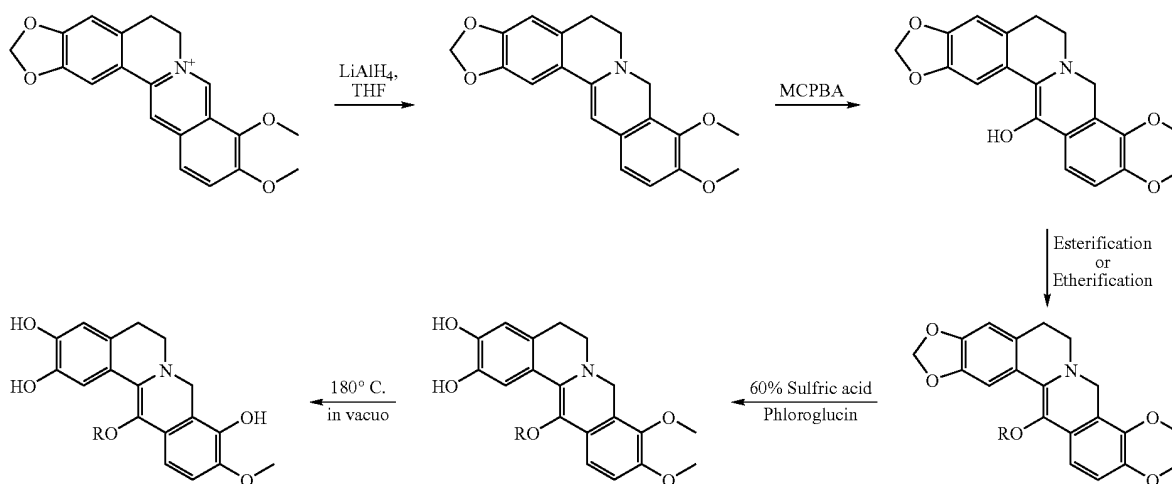
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[0035] Berberine hydrochloride is dissolved in acetone and treated with a base such as NaOH to obtain acetonilberberine, wherein the 8-site of berberine is attacked by the α -site of acetone. Then, acetonilberberine reacts with a halo-hydrocarbon under heating to obtain 13-alkylberberine. 13-alkylberberine then reacts with a Grignard reagent to produce a 8,13-dialkyldihydroberberine derivatives. 13-alkylberberine can also react with sodium alcoholate anion under a strong base condition to form 8-alkoxy-13-alkyldihydroberberine derivatives.

pounds can be used to treat diabetes mellitus, adiposity, fatty liver and their complications caused by insulin resistance. The compounds of present invention are easy to prepare and synthesize, and the materials thereof are abundant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] FIG. 1 is a graph illustrating the promotion effect of the compound according to the present invention at 5 M on glucose transport in L6 muscle cells.



[0036] Berberine is reduced into a dihydroberberine with lithium-aluminum hydride in anhydrous tetrahydrofuran. The dihydroberberine is then oxidized with m-chloroperoxybenzoic acid (MCPBA) to obtain a 13-hydroxydihydroberberine. After oxidation, the 13-hydroxydihydroberberine is etherified or esterified to produce a series of 13-alkoxy or 13-alkanoyloxy dihydroberberines.

[0037] Another object of the present invention is to provide a pharmaceutical composition containing a therapeutically effective amount of 13,13a-dihydroberberine derivatives or their physiologically acceptable salts.

[0038] Still another object of the present invention is to provide uses of the above 13,13a-dihydroberberine derivatives or their physiologically acceptable salts in preparing medicaments for treating diabetes mellitus, adiposity, fatty liver and their complications caused by insulin resistance.

[0039] Still another objection of the present invention is to provide uses of the pharmaceutical composition containing a therapeutically effective amount of 13,13a-dihydroberberine derivatives or their physiologically acceptable salts in preparing medicaments for treating diabetes mellitus, adiposity, fatty liver and their complications caused by insulin resistance.

ADVANTAGEOUS EFFECTS

[0040] The present invention designs and synthesizes a series of 13,13a-dihydroberberine derivatives, which may promote glucose absorption in muscle cells. The animal tests showed that this series of compounds had effects on improving glucose tolerance and insulin resistance, facilitating weight loss and relieving fatty liver etc. Thus, these com-

[0042] FIG. 2A is a graph showing the intraperitoneal glucose tolerance test (ipGTT) after treating the obese mice with dihydroberberine derivative 19 and its sulfate salt for 2 weeks.

[0043] FIG. 2B is a graph showing the incremental areas under the intraperitoneal glucose tolerance test (ipGTT) curves after treating the obese mice with dihydroberberine derivative 19 and its sulfate salt for 2 weeks.

[0044] FIG. 3A is a graph showing the changes in the body weight of mice after the treatment with dihydroberberine derivative 19 and its sulfate salt for 2 weeks.

[0045] FIG. 3B is a graph showing the changes in visceral fat/body weight ratio of mice after the treatment with dihydroberberine derivative 19 and its sulfate salt for 2 weeks.

[0046] FIG. 4A is a graph showing the changes in the content of free fatty acid in blood plasma of mice after the chronic treatment with dihydroberberine derivative 19 and its sulfate salt for 2 weeks.

[0047] FIG. 4B is a graph showing the changes in the content of triglyceride in blood plasma of mice after the chronic treatment with dihydroberberine derivative 19 and its sulfate salt for 2 weeks.

DETAILED DESCRIPTION

Best Mode for Carrying Out the Invention

[0048] Hereinafter, the present invention will be described in more detail through the following examples. However, the present invention is not limited to or by the examples.

[0049] In the following preparation examples, $^1\text{H-NMR}$ measurement was carried out on a Varian Mercury AMX300 instrument, and MS determination was performed using a VG

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ZAB-HS or VG-7070 tape as well as Esquire 3000 plus-01005 instruments. All solvents were redistilled before use, and the used anhydrous solvents were subjected to being dried using standard methods. Unless otherwise specified, all reactions were carried out under argon atmosphere and tracked by TLC, and each product was washed with saturated sodium chloride solution and dried over anhydrous magnesium sulfate at post-processing. Unless otherwise stated, all products were purified by column chromatography using silica gels, and the used silica gel is GF₂₅₄ with a particle size of 200-300 mesh, which is commercially available from Qingdao Haiyang Chemical Co. Ltd or Yantai Yuanbo Silica Gel Co.

Preparation Examples

Preparation of Compound 2

[0050] 8-acetonyl dihydroberberine (3 g) and methyl iodide were dissolved in 100 mL dichloromethane. The obtained reaction solution was heated to 100° C. under pressure and allowed to react for 3 hours. After the reaction finished, the solid by-product was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized in methanol to obtain compound 2 (1.53 g, 48%).

[0051] Compound 2, C₂₁H₂₀INO₄, MW: 477, a yellow crystal, easily dissolved in a mixed solvent of chloroform and methanol.

[0052] ¹H NMR (300 MHz, DMSO-d₆): δ 9.89 (1H, s, H-8), 8.20 (1H, d, J=9.0 Hz, H-12), 8.19 (1H, d, J=9.0 Hz, H-11), 7.48 (1H, s, H-1), 7.15 (1H, s, H-4), 6.18 (2H, s, —OCH₂O—), 4.80 (2H, m, H-6), 4.10 (3H, s, —OCH₃), 4.09 (3H, s, —OCH₃), 3.15 (2H, m, H-5), 2.92 (3H, s, —CH₃).

[0053] Preparation of Compound 3

[0054] 8-acetonyl dihydroberberine (1.5 g) and ethyl bromide were dissolved in 100 mL dichloromethane. The obtained reaction solution was heated to 100° C. under pressure and allowed to react for 5 hours. After the reaction finished, the solid by-product was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized in methanol to obtain compound 3 (0.83 g, 53%).

[0055] Compound 3, C₂₂H₂₂INO₄, MW: 491, a white crystal, easily dissolved in a mixed solvent of chloroform and methanol.

[0056] ¹H NMR (300 MHz, DMSO-d₆): δ 9.90 (1H, s, H-8), 8.21 (2H, ABq, J=9.0 Hz, H-11 and 12), 7.30 (1H, s, H-1), 7.17 (1H, s, H-4), 6.19 (2H, s, —OCH₂O—), 4.80 (2H, m, H-6), 4.10 (3H, s, —OCH₃), 4.09 (3H, s, —OCH₃), 3.36 (2H, q, J=7.5 Hz, H-1'), 3.09 (2H, m, H-5), 1.47 (3H, t, J=7.5 Hz, H-2').

[0057] Preparation of Compound 4

[0058] 8-acetonyl dihydroberberine (0.5 g) and 1-propyl iodide (0.43 g) were dissolved in 50 mL dioxane. The obtained reaction solution was refluxed and allowed to react for 5 hours. After the reaction finished, the solid by-product was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified through a silica gel column (methanol:dichloromethane=1:10) to obtain compound 4 (0.25 g, 52%).

[0059] Compound 4, C₂₃H₂₄INO₄, MW: 505, a yellow crystal, easily dissolved in a mixed solvent of chloroform and methanol.

[0060] ¹H NMR (300 MHz, DMSO-d₆): δ 9.96 (1H, s, H-8), 8.20 (2H, ABq, J=9.0 Hz, H-11 and 12), 7.23 (1H, s, H-1), 7.19 (1H, s, H-4), 6.21 (2H, s, —OCH₂O—), 4.80 (2H,

m, H-6), 4.10 (3H, s, —OCH₃), 4.09 (3H, s, —OCH₃), 3.08 (2H, m, H-5), 1.83 (2H, m, H-1'), 1.10 (2H, m, H-2'), 1.05 (3H, t, J=7.0 Hz, H-3').

[0061] Preparation of Compound 5

[0062] 8-acetonyl dihydroberberine (1.0 g) and 1-butyl iodide (9.2 g) were dissolved in 50 mL acetonitrile. The obtained reaction solution was refluxed and allowed to react for 5 hours. After the reaction finished, the solid by-product was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified through silica gel column (methanol:chloroform=1:9) to obtain compound 5 (0.42 g, 43%).

[0063] Compound 5, C₂₄H₂₆INO₄, MW: 519, a yellow powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0064] ¹H NMR (300 MHz, DMSO-d₆): δ 9.89 (1H, s, H-8), 8.23 (2H, ABq, J=9.0 Hz, H-11 and 12), 7.31 (1H, s, H-1), 7.20 (1H, s, H-4), 6.11 (2H, s, —OCH₂O—), 4.80 (2H, m, H-6), 4.01 (6H, s, —OCH₃), 3.31 (2H, m, H-1'), 3.14 (2H, m, H-5), 1.82 (2H, m, H-2'), 1.47 (2H, m, H-3'), 0.95 (3H, t, J=7.5 Hz, H-4').

[0065] Preparation of Compound 6

[0066] 8-acetonyl dihydroberberine (2.65 g) and 1,3-diiodopropane (7.4 mL) were dissolved in 75 mL acetonitrile. The obtained reaction solution was refluxed and allowed to react for 6 hours. After reaction finished, the solid by-product was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified through silica gel column (methanol:chloroform=1:9) to obtain compound 6 (0.37 g, 8.7%).

[0067] Compound 6, C₂₃H₂₃I₂NO₄, MW: 631, a yellow powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0068] ¹H NMR (300 MHz, CDCl₃): δ 10.26 (1H, s, H-8), 8.00 (1H, d, J=9.0 Hz, H-11), 7.89 (1H, d, J=9.0 Hz, H-12), 7.09 (1H, s, H-1), 6.89 (1H, s, H-4), 6.10 (2H, s, —OCH₂O—), 5.08 (2H, m, H-6), 4.37 (3H, s, —OCH₃), 4.07 (3H, s, —OCH₃), 3.51 (2H, t, J=8 Hz, CH₂—Ar), 3.32 (2H, t, J=6 Hz, H-5), 3.23 (2H, t, J=5 Hz, CH₂—I), 2.30 (2H, m, CH₂(propyl)).

[0069] Preparation of Compound 7

[0070] 8-acetonyl dihydroberberine (4 g) and sodium iodide (1.87 g) were dissolved in 50 mL of acetonitrile followed by adding 2 mL of benzyl chloride. The obtained reaction solution was heated to 80° C. and refluxed for 6 hours. Then, the reaction mixture was filtered and the filter cake was washed with acetonitrile. The combined filtrate was evaporated to remove acetonitrile under reduced pressure. The residual was purified through silica gel column (CHCl₃/CH₃OH=20:1) to obtain compound 7 (1.83 g, 45%).

[0071] Compound 7, C₂₇H₂₄ClNO₄, MW: 461, a brownish red amorphous powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0072] ¹H NMR (300 MHz, CDCl₃): δ 10.10 (1H, s, H-8), 7.63 (2H, dd, J=15.0 and 9.0 Hz, H-3' and 5'), 7.32-7.23 (3H, m, H-1', 4' and 5'), 7.05 (2H, d, J=7.0 Hz, H-11 and 12), 6.90 (1H, s, H-1), 6.84 (1H, s, H-4), 5.93 (2H, s, —OCH₂O—), 4.98 (2H, m, H-6), 4.27 (3H, s, —OCH₃), 3.96 (3H, s, —OCH₃), 3.18 (2H, m, H-5).

[0073] Preparation of Compound 8

[0074] 8-acetonyl dihydroberberine (4 g) and sodium iodide (1.87 g) were dissolved in 50 mL acetonitrile followed by adding 2 mL of ethyl chloroformate. The obtained reaction solution was heated to 80° C. and refluxed for 6 hours. Then,

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the reaction mixture was filtered and the filter cake was washed with acetonitrile. The combined filtrate was evaporated to remove acetonitrile under reduced pressure. The residual was purified through silica gel column ($\text{CHCl}_3/\text{CH}_3\text{OH}=20:1$) to obtain compound 8 (1.61 g, 40%).

[0075] Compound 8, $\text{C}_{23}\text{H}_{22}\text{ClNO}_6$, MW: 443, a yellow amorphous powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0076] ^1H NMR (300 MHz, CDCl_3): δ 10.82 (1H, s, H-8), 7.88 (1H, d, $J=9.0$ Hz, H-12), 7.73 (1H, d, $J=9.0$ Hz, H-11), 7.21 (1H, s, H-1), 6.89 (1H, s, H-4), 6.13 (2H, s, $-\text{OCH}_2\text{O}-$), 5.38 (2H, m, H-6), 4.40 (2H, q, $J=7.5$ Hz, H-2'), 4.08 (3H, s, $-\text{OCH}_3$), 4.02 (3H, s, $-\text{OCH}_3$), 3.38 (2H, m, H-5), 1.19 (3H, t, $J=7.5$ Hz, H-3').

[0077] Preparation of Compound 9

[0078] 8-acetonyl dihydroberberine (4 g) was dissolved in 30 mL acetonitrile followed by adding 2 mL of ethyl chloroacetate. The obtained reaction solution was heated to 80°C and refluxed for 6 hours. Then, the reaction mixture was filtered and the filter cake was washed with acetonitrile. The combined filtrate was evaporated to remove acetonitrile under reduced pressure. The residual was purified through silica gel column ($\text{CHCl}_3/\text{CH}_3\text{OH}=20:1$) to obtain compound 9 (1.34 g, 35%).

[0079] Compound 9, $\text{C}_{24}\text{H}_{24}\text{ClNO}_6$, MW: 457, a yellow crystal, easily dissolved in a mixed solvent of chloroform and methanol.

[0080] ^1H NMR (300 MHz, CDCl_3): δ 10.00 (1H, s, H-8), 7.84 (1H, d, $J=9.0$ Hz, H-2), 7.70 (1H, d, $J=9.0$ Hz, H-11), 7.13 (1H, s, H-1), 6.86 (1H, s, H-4), 6.0 (2H, s, $-\text{OCH}_2\text{O}-$), 4.28 (2H, m, H-3'), 4.26 (2H, m, H-6), 4.23 (3H, s, $-\text{OCH}_3$), 4.01 (3H, s, $-\text{OCH}_3$), 3.62 (2H, s, H-1'), 3.10 (2H, m, H-5), 1.28 (3H, t, $J=7.2$ Hz, H-4').

[0081] Preparation of Compound 10

[0082] Dihydroberberine (337 mg) was dissolved in 35 mL of dichloromethane. Under argon atmosphere, the temperature of the system was kept at -25°C – -30°C , and 8 mL of dichloromethane solution containing 258 mg MCPBA (1.5 mmol) was dropwise added slowly. After the addition, the temperature was kept and the reaction was carried out under agitation for 1 hour. Then, the temperature was raised to 0°C and 250 mg (2.0 mmol) of sodium sulfite was added therein, followed by stirred at room temperature for 1 hour. The reaction was stopped and the reaction mixture was filtered. The filtrate was evaporated to remove the solvent under reduced pressure and the residue was purified through silica gel column chromatography ($\text{CHCl}_3/\text{CH}_3\text{OH}=10:1$) to obtain compound 10 (280 mg, 80%).

[0083] Compound 10, $\text{C}_{20}\text{H}_{18}\text{NO}_5$, MW: 352, a yellow powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0084] ^1H NMR (300 MHz, CDCl_3): δ 8.83 (1H, s, H-8), 8.28 (1H, d, $J=8.1$ Hz, H-11), 7.78 (1H, s, H-1), 7.29 (1H, d, $J=8.1$ Hz, H-12), 6.52 (1H, s, H-4), 5.88 (2H, s, $-\text{OCH}_2\text{O}-$), 4.44 (2H, t, $J=6.3$ Hz, H-6), 3.95 (3H, s, OMe-9 or 10), 3.94 (3H, s, OMe-10 or 9), 2.95 (2H, t, $J=6.3$ Hz, H-5).

[0085] Preparation of Compound 11

[0086] Compound 10 (1.0 g) and potassium carbonate (200 mg) were dissolved in 20 mL of acetone followed by adding 0.2 mL of methyl iodide. The obtained reaction solution was heated and refluxed for 3 hours. Then, the reaction mixture was filtered and the filtrate was evaporated under reduced

pressure. The residual was purified through silica gel column ($\text{CHCl}_3/\text{CH}_3\text{OH}=20:1$) to obtain compound 11 (0.41 g, 37%).

[0087] Compound 11, $\text{C}_{21}\text{H}_{20}\text{INO}_5$, MW: 493, a brown powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0088] ^1H NMR (300 MHz, CDCl_3): δ 8.97 (1H, s, H-8), 8.33 (1H, d, $J=8.1$ Hz, H-11), 7.91 (1H, s, H-1), 7.45 (1H, d, $J=8.1$ Hz, H-12), 6.76 (1H, s, H-4), 5.97 (2H, s, $-\text{OCH}_2\text{O}-$), 4.32 (2H, t, $J=6.3$ Hz, H-6), 4.01 (3H, s, $-\text{OMe}$), 3.94 (3H, s, $-\text{OMe}$), 3.73 (3H, s, $-\text{OMe}$), 2.95 (2H, t, $J=6.3$ Hz, H-5).

[0089] Preparation of Compound 12

[0090] Compound 10 (1.0 g) and potassium carbonate (200 mg) were dissolved in 20 mL of acetone followed by adding 0.25 mL of ethyl bromide. The obtained reaction solution was heated and refluxed for 3 hours. Then, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residual was purified through silica gel column ($\text{CHCl}_3/\text{CH}_3\text{OH}=20:1$) to obtain compound 12 (0.37 g, 34%).

[0091] Compound 12, $\text{C}_{22}\text{H}_{22}\text{BrNO}_5$, MW: 459, a brown yellow powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0092] ^1H NMR (300 MHz, CDCl_3): δ 9.01 (1H, s, H-8), 8.45 (1H, d, $J=8.1$ Hz, H-11), 7.99 (1H, s, H-1), 7.63 (1H, d, $J=8.1$ Hz, H-12), 6.81 (1H, s, H-4), 6.08 (2H, s, $-\text{OCH}_2\text{O}-$), 4.35 (2H, t, $J=6.3$ Hz, H-6), 4.11 (3H, s, $-\text{OMe}$), 4.01 (3H, s, $-\text{OMe}$), 3.92 (2H, m, H-1'), 2.95 (2H, t, $J=6.3$ Hz, H-5), 1.35 (3H, t, $J=7.5$ Hz, H-2').

[0093] Preparation of Compound 13

[0094] Compound 10 (34 mg, 0.1 mmol) was dissolved in a mixed solution of 1 mL of pyridine and 1 mL of acetic anhydride. The mixture was allowed to react under agitation at room temperature overnight. Then, the reaction mixture was evaporated to remove the solvent under reduced pressure. The residual was purified through Sephadex LH-20 chromatography ($\text{CHCl}_3/\text{CH}_3\text{OH}=10:1$) to obtain compound 13 (28 mg, 80%).

[0095] Compound 13, $\text{C}_{22}\text{H}_{20}\text{NO}_6$, MW: 394, a yellow powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0096] ^1H NMR (300 MHz, CDCl_3): δ 8.81 (1H, s, H-8), 8.27 (1H, d, $J=8.1$ Hz, H-11), 7.76 (1H, s, H-1), 7.29 (1H, d, $J=8.1$ Hz, H-12), 6.56 (1H, s, H-4), 5.89 (2H, s, $-\text{OCH}_2\text{O}-$), 4.45 (2H, t, $J=6.3$ Hz, H-6), 3.95 (3H, s, OMe-9 or 10), 3.94 (3H, s, OMe-10 or 9), 2.95 (2H, t, $J=6.3$ Hz, H-5), 2.10 (3H, s, OAc).

[0097] Preparation of Compound 14

[0098] Berberine (4.5 g, 12.1 mmol) was added into a 50 mL round bottom flask. The reaction system was kept under a reduced pressure (20–30 mmHg) using an oil pump, and heated to 190°C followed by reacting for 40 minutes. The vacuum pump was switched off after the temperature dropped to room temperature. The reaction product was purified through silica gel column chromatography ($\text{CHCl}_3/\text{CH}_3\text{OH}=15:1$ and $10:1$, eluting until no compound was observed in the eluent) to obtain compound 14 (3.3 g, 85%).

[0099] Compound 14, $\text{C}_{19}\text{H}_{15}\text{NO}_4$, MW: 321, a brownish red amorphous powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0100] ^1H NMR (300 MHz, CDCl_3): δ 9.06 (1H, s, H-8), 8.60 (1H, s, H-13), 8.18 (1H, d, $J=8.1$ Hz, H-11), 8.10 (1H, d, $J=8.1$ Hz, H-12), 7.57 (1H, s, H-1), 6.87 (1H, s, H-4), 6.10

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(2H, s, —OCH₂O—), 4.90 (2H, t, J=6.3 Hz, H-6), 4.63 (3H, s, OMe-10), 3.20 (2H, t, J=6.3 Hz, H-5).

[0101] Preparation of Compound 15

[0102] Compound 14 (33 mg, 0.1 mmol) was dissolved in a mixed solution of 1 mL of pyridine and 1 mL of acetic anhydride. The obtained solution reacted under agitation at room temperature overnight. Then, the reaction mixture was evaporated to remove solvent under reduced pressure. The residual was purified through Sephdex LH-20 chromatography (CHCl₃/CH₃OH=10:1) to obtain compound 15 (32 mg, 88%).

[0103] Compound 15, C₂₁H₁₈NO₅, MW: 364, a red powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0104] ¹H NMR (300 MHz, CDCl₃+CD₃OD): δ 9.16 (1H, s, H-8), 8.64 (1H, s, H-13), 8.18 (1H, d, J=8.1 Hz, H-11), 8.04 (1H, d, J=8.1 Hz, H-12), 7.55 (1H, s, H-1), 6.87 (1H, s, H-4), 6.09 (2H, s, —OCH₂O—), 4.99 (2H, t, J=6.3 Hz, H-6), 4.03 (3H, s, OMe-10), 3.20 (2H, t, J=6.3 Hz, H-5), 2.10 (3H, s, OAc).

[0105] Preparation of Compound 16

[0106] Compound 14 (360 mg, 1.0 mmol) and triethylamine (1.08 g) were dissolved in 50 mL of anhydrous dichloromethane under agitation at room temperature. Then, 3 mL dichloromethane solution containing ethyl chlorformate (1.2 g, 11.1 mmol) was dropwise added slowly. After the addition, the reaction was performed for another 30 min. Then, the reaction mixture was evaporated to remove solvent under reduced pressure. The residual was purified through Sephdex LH-20 chromatography (CHCl₃/CH₃OH=1:1) to obtain compound 16 (202 mg, 88%).

[0107] Compound 16, C₂₂H₂₀NO₆, MW: 394, a yellow amorphous powder, easily dissolved in chloroform and methanol.

[0108] ¹H NMR (300 MHz, CDCl₃): δ 9.18 (1H, s, H-8), 8.60 (1H, s, H-13), 8.12 (1H, d, J=8.1 Hz, H-11), 8.06 (1H, d, J=8.1 Hz, H-12), 7.54 (1H, s, H-1), 6.57 (1H, s, H-4), 6.04 (2H, s, —OCH₂O—), 4.49 (2H, t, J=6.3 Hz, H-6), 4.25 (2H, q, J=6.0 Hz, H-2'), 4.13 (3H, s, OMe-10), 3.20 (2H, t, J=6.3 Hz, H-5), 1.10 (3H, t, J=6.0 Hz, H-3').

[0109] Preparation of Compound 17

[0110] Berberine (370 mg, 1.0 mmol) and 1,3,5-trihydroxybenzene (504 mg, 4.0 mmol) were dissolved in 10 mL of 60% sulphuric acid solution (volume ratio). Then, the obtained solution was heated to 80° C. and reacted under refluxing for 1 hour. The reaction mixture was cooled down, and 15 mL of sodium iodide solution (30 mg/mL) was added therein under ice bath. The reaction mixture was filtered, and the filter cake was dissolved in 2 mL of 1% potassium hydroxide solution. pH of the solution was adjusted to 4~5 with sodium bisulfite. The mixture was filtered again, and the filter cake was purified by Sephdex LH-20 chromatography (CHCl₃/CH₃OH=10:1) to obtain compound 17 (108 mg, 33%).

[0111] Compound 17, C₁₉H₁₈ClNO₄, MW: 359, a yellow powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0112] ¹H NMR (300 MHz, DMSO), δ 9.83 (1H, s, H-8), 8.75 (1H, s, H-13), 8.18 (1H, d, J=8.1 Hz, H-11), 8.04 (1H, d, J=8.1 Hz, H-12), 7.53 (1H, s, H-1), 6.84 (1H, s, H-4), 4.89 (2H, t, J=6.3 Hz, H-6), 4.08 (3H, s, OMe-9 or 10), 4.05 (3H, s, OMe-10 or 9), 3.11 (2H, t, J=6.3 Hz, H-5).

[0113] Preparation of Compound 18

[0114] Compound 2 (490 mg, 1.0 mmol) and 1,3,5-trihydroxybenzene (504 mg, 4.0 mmol) were dissolved in 10 mL of 60% sulphuric acid solution (volume ratio). The obtained solution was heated to 80° C. and reacted under refluxing for 1 hour. The reaction mixture was cooled down, and 15 mL of sodium iodide solution (30 mg/mL) was added therein under ice bath. The reaction mixture was filtered, and the filter cake was dissolved in 2 mL of 1% potassium hydroxide solution. pH of the solution was adjusted to 4~5 with sodium bisulfite. The mixture was filtered again, and the filter cake was purified through Sephdex LH-20 chromatography (CHCl₃/CH₃OH=10:1) to obtain compound 18 (123 mg, 31%).

[0115] Compound 18, C₂₁H₂₂ClNO₄, MW: 387, a yellow powder, easily dissolved in a mixed solvent of chloroform and methanol.

[0116] ¹H NMR (300 MHz, DMSO-d₆), δ 9.96 (1H, s, H-8), 8.91 (1H, s, H-13), 8.23 (1H, d, J=8.1 Hz, H-11), 8.11 (1H, d, J=8.1 Hz, H-12), 7.72 (1H, s, H-1), 6.91 (1H, s, H-4), 4.98 (2H, t, J=6.3 Hz, H-6), 4.23 (3H, s, OMe-9 or 10), 4.15 (3H, s, OMe-10 or 9), 4.01 (2H, m, H-1'), 3.27 (2H, t, J=6.3 Hz, H-5), 1.45 (3H, t, J=7.5 Hz, H-2').

[0117] Preparation of Compound 19

[0118] Berberine (370 mg, 1.0 mmol) was dissolved in 10 mL of anhydrous tetrahydrofuran followed by adding 190 mg of LiAlH₄ (5.0 mmol). The reaction was performed at room temperature under agitation for 2 hours. After the reaction finished, the reaction mixture was evaporated to remove the solvent under reduced pressure, and 0.2 mL of water, 0.2 mL of 30% sodium hydroxide solution and 0.6 mL of water were sequentially added therein. The reaction solution was then filtered, and the filtrate was extracted with ethyl acetate (10 mL×3). The obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residual was purified through silica gel column chromatography (CHCl₃/CH₃OH=50:1, eluting until no compound was observed in the eluent) to obtain compound 19 (240 mg, 65%).

[0119] Compound 19, C₂₀H₁₉NO₄, MW: 337, a yellow amorphous powder, easily dissolved in chloroform and acetone.

[0120] ¹H NMR (300 MHz, CDCl₃), δ 7.18 (1H, d, J=8.7 Hz, H-11), 6.73 (2H, m, H-12 and H-1), 6.56 (1H, s, H-4), 5.95 (1H, s, H-13), 5.94 (2H, s, —OCH₂O—), 4.32 (2H, s, H-8), 3.84 (6H, s, OMex2), 3.20 (2H, t, J=8.1 Hz, H-6), 2.90 (2H, t, J=8.1 Hz, H-5).

[0121] Preparation of Compound 20

[0122] Magnesium strip (240 mg, 10 mmol) and ethyl bromide (1.08 g, 10 mmol) were dissolved in 15 mL of anhydrous diethyl ether under argon atmosphere. The reactant mixture was refluxed for another 2 hours after the violent reaction quieted down. The reaction solution was cooled down to 0° C., and berberine (370 mg, 1.0 mmol) was slowly added in batch. The ice bath was removed, and the reaction was carried out at room temperature overnight. Then, the reaction solution was injected into ice water (20 mL), and pH of the mixture was adjusted to 5 with a 2 N hydrochloric acid solution. The water phase was separated from the diethyl ether phase, cooled down, adjusted to have a pH of 11~12 with a concentrated ammonia solution, and then extracted with chloroform (20 mL×3). The obtained organic phase was dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The obtained oil was recrystallized in diethyl ether to obtain compound 20 (220 mg, 59%).

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[0123] Compound 20, $C_{22}H_{23}NO_4$, MW: 365, a yellow powder crystal, easily dissolved in chloroform and acetone.

[0124] ^1H NMR (300 MHz, $CDCl_3$): δ 7.12 (1H, s, H-1), 6.80 (2H, m, H-11 and 12), 6.57 (1H, s, H-4), 6.00 (2H, s, $-OCH_2O-$), 5.89 (1H, s, H-13), 3.85 (6H, s, OMe $\times$ 2), 3.32 (2H, m, H-6), 3.06 (1H, t, $J=6.0$ Hz, H-8), 2.81 (2H, m, H-5), 1.72 (2H, m, H-1'), 0.94 (3H, t, $J=6.3$ Hz, H-2').

[0125] Preparation of Compound 21

[0126] Magnesium strip (240 mg, 10 mmol) and benzyl bromide (1.7 g, 10 mmol) were dissolved in 15 mL of anhydrous diethyl ether under argon atmosphere. The reaction mixture was refluxed for another 2 hours after the violent reaction quieted down. The reaction solution was cooled to 0° C., and berberine (370 mg, 1.0 mmol) was slowly added in batch. The ice bath was removed, and the reaction was carried out at room temperature overnight. The reaction solution was injected into ice water (20 mL), and pH of the mixture was adjusted to 5 with a 2 mol/L hydrochloric acid solution. The water phase was separated from the diethyl ether phase, adjusted to have a pH of 11–12 with a concentrated ammonia solution, and then extracted with chloroform (20 mL $\times$ 3). The obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The obtained oil was recrystallized in diethyl ether to give compound 21 (280 mg, 59%).

[0127] Compound 21, $C_{27}H_{25}NO_4$, MW: 427, a yellow powder crystal, easily dissolved in chloroform and acetone. ^1H NMR (300 MHz, $CDCl_3$): δ 8.80 (1H, s, H-1), 8.53 (1H, d, $J=8.7$ Hz, H-11), 7.39 (1H, d, $J=8.7$ Hz, H-12), 7.08–7.34 (6H, m), 6.53 (1H, s, H-13), 6.00 (2H, s, $-OCH_2O-$), 4.35 (2H, t, $J=6.0$ Hz, H-6), 3.95 (3H, s, OMe), 3.93 (1H, m, H-8), 3.81 (1H, m, H-113), 3.43 (3H, s, OMe), 2.75 (3H, m, H-5 and 1' α); ESIMS m/z 428.2 ($[M+H]^+$).

[0128] Preparation of Compound 22

[0129] Berberine (370 mg, 1.0 mmol) was dissolved in 10 mL of ethanol (analytical grade) under argon atmosphere, followed by adding 1.5 g of sodium ethylate. The reaction was carried out under agitation at room temperature overnight. The reaction mixture was evaporated to remove the solvent under reduced pressure, followed by adding 10 mL of water (which had been alkalified with sodium hydroxide). The mixture was then extracted with ethyl acetate (10 mL $\times$ 3), and the obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The obtained yellow powder was recrystallized in methanol to give a needle like crystal 22 (94 mg, 27%).

[0130] Compound 22, $C_{20}H_{19}NO_5$, MW: 353, a yellow needle like crystal, easily dissolved in chloroform and acetone.

[0131] ^1H NMR (300 MHz, $CDCl_3$): δ 7.16 (1H, s, H-1), 6.97 (1H, d, $J=8.7$ Hz, H-11), 6.88 (1H, d, $J=8.7$ Hz, H-12), 6.62 (1H, s, H-4), 6.10 (1H, s, H-13), 5.95 (2H, s, $-OCH_2O-$), 5.64 (1H, s, H-8), 3.88 (3H, s, OMe-9 or 10), 3.86 (3H, s, OMe-10 or 9), 3.86 (1H, m, H-6 β), 3.72 (1H, m, H-6 α), 3.36 (1H, m, H-5 β), 2.76 (1H, m, H-5 α).

[0132] Preparation of Compound 23

[0133] Berberine (370 mg, 1.0 mmol) was dissolved in 10 mL of methanol (analytical grade) under argon atmosphere, followed by adding 1.5 g of sodium methylate. The reaction was carried out under agitation at room temperature overnight. The reaction mixture was evaporated to remove the solvent under reduced pressure, followed by adding 10 mL of water (which had been alkalified with sodium hydroxide). The mixture was then extracted with ethyl acetate (10 mL $\times$ 3),

and the obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The obtained yellow powder was recrystallized in methanol to give a needle like crystal 23 (114 mg, 30%).

[0134] Compound 23, $C_{21}H_{21}NO_5$, MW: 367, a yellow needle like crystal, easily dissolved in chloroform and acetone.

[0135] ^1H NMR (300 MHz, $CDCl_3$): δ 7.17 (1H, s, H-1), 6.96 (1H, d, $J=8.7$ Hz, H-11), 6.89 (1H, d, $J=8.7$ Hz, H-12), 6.64 (1H, s, H-4), 6.11 (1H, s, H-8), 6.03 (1H, s, H-13), 6.00 (2H, s, $-OCH_2O-$), 3.88 (3H, s, OMe-9 or 10), 3.86 (3H, s, OMe-10 or 9), 3.64 (1H, m, H-6 α), 3.52 (1H, m, H-6 β), 3.05 (3H, s, OMe-8), 2.89 (2H, m, H-5).

[0136] Preparation of Compound 24

[0137] Metallic sodium (0.85 g) was slowly added into 10 mL of anhydrous ethanol. After the addition, berberine 370 mg (1.0 mmol) was added under argon atmosphere. The reaction was carried out under agitation at room temperature overnight. Then, the reaction mixture was evaporated to remove the solvent under reduced pressure, followed by adding 10 mL of water (which had been alkalified with sodium hydroxide). The mixture was extracted with ethyl acetate (10 mL $\times$ 3), and the obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The obtained yellow powder was recrystallized in ethanol to give a yellow needle like crystal 24 (130 mg, 34%).

[0138] Compound 24, $C_{22}H_{23}NO_5$, MW: 381, a yellow needle like crystal, easily dissolved in chloroform and acetone.

[0139] ^1H NMR (300 MHz, $CDCl_3$): δ 7.17 (1H, s, H-1), 6.95 (1H, d, $J=8.7$ Hz, H-11), 6.86 (1H, d, $J=8.7$ Hz, H-12), 6.63 (1H, s, H-4), 6.13 (1H, s, H-8), 6.03 (1H, s, H-13), 6.01 (2H, s, $-OCH_2O-$), 3.88 (3H, s, OMe-9 or 10), 3.85 (3H, s, OMe-10 or 9), 3.60 (1H, m, H-6 α), 3.51 (1H, m, H-6 β), 3.25 (2H, q, $J=6.3$ Hz, H-1'), 2.87 (2H, m, H-5), 1.00 (3H, t, $J=6.3$ Hz, H-2').

[0140] Preparation of Compound 25

[0141] Berberine (370 mg, 1.0 mmol) was dissolved in 10 mL of chloroform (analytical grade) under argon atmosphere, followed by adding 0.5 g of sodium hydride. The reaction was carried out under agitation at room temperature overnight. Then, the reaction mixture was evaporated to remove the solvent under reduced pressure, followed by adding 10 mL of water (which had been alkalified with sodium hydroxide). The mixture was then extracted with ethyl acetate (10 mL $\times$ 3), and the obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The obtained yellow powder was recrystallized in methanol to give a yellow needle like crystal 25 (80 mg, 21%).

[0142] Compound 25, $C_{21}H_{18}Cl_3NO_4$, MW: 453, a yellow needle like crystal, easily dissolved in chloroform and acetone.

[0143] ^1H NMR (300 MHz, $CDCl_3$): δ 7.16 (1H, s, H-1), 6.97 (1H, d, $J=8.7$ Hz, H-11), 6.86 (1H, d, $J=8.7$ Hz, H-12), 6.61 (1H, s, H-4), 6.10 (1H, s, H-13), 6.00 (2H, s, $-OCH_2O-$), 5.64 (1H, s, H-8), 3.94 (3H, s, OMe-9 or 10), 3.88 (3H, s, OMe-10 or 9), 3.87 (1H, m, H-6 α), 3.70 (1H, m, H-6 β), 2.89 (2H, m, H-5).

[0144] Preparation of Compound 26

[0145] Berberine (1 g) was dissolved in a 5 mol/L NaOH aqueous solution (5 mL), and acetone (2 mL) was dropwise added therein under agitation. The reaction was performed at room temperature for another 1 hour. The reaction mixture

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was filtered, and the filter cake was washed with methanol to give a yellow solid powder 26 (780 mg, 78%).

[0146] Compound 26, $C_{23}H_{23}NO_5$, MW: 393, a yellow amorphous powder, easily dissolved in chloroform and acetone.

[0147] ^1H NMR (300 MHz, $CDCl_3$): δ 7.13 (1H, s, H-1), 6.78 (2H, m, H-11 and 12), 6.57 (1H, s, H-4), 5.94 (2H, s, $-OCH_2O-$), 5.89 (1H, s, H-13), 5.32 (1H, dd, $J=6.9$, 15.3 Hz, H-8), 3.85 (6H, s, $OMex \times 2$), 3.32 (2H, m, H-6), 3.06 (1H, dd, $J=3.9$, 6.9 Hz, H-1' α), 2.81 (2H, m, H-5), 2.42 (1H, dd, $J=3.9$, 15.3 Hz, H-1' β), 2.04 (3H, s, H-3').

[0148] Preparation of Compound 27

[0149] Compound 2 (480 mg) was dissolved in 20 mL of anhydrous tetrahydrofuran followed by adding $LiAlH_4$ (120 mg) slowly. The reaction was carried out at room temperature for 3 hours. Water (0.1 mL), 5N sodium hydroxide solution (0.1 mL) and water (0.9 mL) were dropwise added into the reaction solution sequentially. Then, the reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized in dichloromethane-methanol mixed solvent to give compound 27 (300 mg, 61%).

[0150] Compound 27, $C_{21}H_{21}NO_4$, MW: 351, a yellow powder crystal, easily dissolved in chloroform and acetone.

[0151] ^1H NMR (300 MHz, $CDCl_3$): δ 7.17 (1H, s, H-1), 7.03 (1H, d, $J=8.4$ Hz, H-11), 6.84 (1H, d, $J=8.4$ Hz, H-12), 6.68 (1H, s, H-4), 5.98 (2H, s, $-OCH_2O-$), 4.33 (2H, s, H-8), 3.86 (6H, s, $-OCH_3$), 3.13 (2H, m, H-6), 2.83 (2H, m, H-5), 2.78 (3H, s, $-CH_3$).

[0152] Preparation of Compound 28

[0153] Compound 3 (490 mg) was dissolved in 20 mL of anhydrous tetrahydrofuran followed by adding $LiAlH_4$ (120 mg) slowly. The reaction was carried out at room temperature for 3 hours. Water (0.1 mL), 5N sodium hydroxide solution (0.1 mL) and water (0.9 mL) were dropwise added into the reaction solution sequentially. Then, the reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized in dichloromethane-methanol mixed solvent to give compound 28 (320 mg, 63%).

[0154] Compound 28, $C_{22}H_{23}NO_4$, MW: 365, a yellow powder crystal, easily dissolved in chloroform and acetone.

[0155] ^1H NMR (300 MHz, $CDCl_3$): δ 7.19 (1H, s, H-1), 7.09 (1H, d, $J=8.4$ Hz, H-11), 6.77 (1H, d, $J=8.4$ Hz, H-12), 6.69 (1H, s, H-4), 6.01 (2H, s, $-OCH_2O-$), 4.23 (2H, s, H-8), 3.78 (6H, s, $-OCH_3$), 3.13 (2H, m, H-6), 2.83 (2H, m, H-5), 2.78 (2H, m, H-1'), 1.34 (3H, t, $J=7.5$ Hz, H-2').

[0156] Preparation of Compound 29

[0157] Magnesium strip (240 mg, 10 mmol) and methyl iodide (1.40 g, 10 mmol) were dissolved in 15 mL of anhydrous diethyl ether under argon atmosphere. The reaction mixture was refluxed for another 2 hours after the violent reaction quieted down. The reaction solution was cooled down to 0° C., and berberine (370 mg, 1.0 mmol) was slowly added in batch. The ice bath was removed, and the reaction was carried out at room temperature overnight. Then, the reaction solution was injected into ice water (20 mL), and pH of the mixture was adjusted to 5 with a 2 N hydrochloric acid solution. The water phase was separated from the diethyl ether phase, cooled down, adjusted to have a pH of 11~12 with a concentrated ammonia solution, and then extracted with chloroform (20 mL $\times$ 3). The obtained organic phase was dried over anhydrous sodium sulfate, and evaporated under

reduced pressure. The obtained oil was recrystallized in diethyl ether to give compound 29 (245 mg, 61%).

[0158] Compound 29, $C_{21}H_{21}NO_4$, MW: 351, a yellow powder crystal, easily dissolved in chloroform and acetone.

[0159] ^1H NMR (300 MHz, $CDCl_3$): δ 7.17 (1H, s, H-1), 6.85 (2H, m, H-11 and 12), 6.62 (1H, s, H-4), 6.05 (2H, s, $-OCH_2O-$), 5.94 (1H, s, H-13), 3.90 (6H, s, $OMex \times 2$), 3.37 (2H, m, H-6), 3.11 (1H, t, $J=6.0$ Hz, H-8), 2.86 (2H, m, H-5), 1.31 (3H, t, $J=7.0$ Hz, H-2').

[0160] Preparation of Compound 30

[0161] Magnesium strip (240 mg, 10 mmol) and methyl iodide (1.40 g, 10 mmol) were dissolved in 15 mL of anhydrous diethyl ether under argon atmosphere. The reaction mixture was refluxed for another 2 hours after violent reaction quieted down. The reaction solution was cooled down to 0° C., and compound 2 (480 mg, 1.0 mmol) was slowly added in batch. The ice bath was removed, and the reaction was carried out at room temperature overnight. Then, the reaction solution was injected into ice water (20 mL), and pH of the mixture was adjusted to 5 with a 2 N hydrochloric acid solution. The water phase was separated from the diethyl ether phase, cooled down, adjusted to have a pH of 11~12 with a concentrated ammonia solution, and then extracted with chloroform (20 mL $\times$ 3). The obtained organic phase was dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The obtained oil was recrystallized in diethyl ether to give compound 30 (232 mg, 43%).

[0162] Compound 30, $C_{22}H_{23}NO_4$, MW: 365, a yellow powder crystal, easily dissolved in chloroform and acetone.

[0163] ^1H NMR (300 MHz, $CDCl_3$): δ 7.09 (1H, s, H-1), 6.81 (2H, m, H-11 and 12), 6.52 (1H, s, H-4), 6.01 (2H, s, $-OCH_2O-$), 3.73 (6H, s, $OMex \times 2$), 3.21 (2H, m, H-6), 3.08 (1H, m, H-8), 2.86 (2H, m, H-5), 1.83 (3H, s, H-1'), 1.38 (3H, d, $J=6.3$ Hz, H-2').

[0164] Preparation of Compound 31

[0165] Magnesium strip (240 mg, 10 mmol) and ethyl bromide (1.08 g, 10 mmol) were dissolved in 15 mL of anhydrous diethyl ether under argon atmosphere. The reaction mixture was refluxed for another 2 hours after the violent reaction quieted down. The reaction solution was cooled down to 0° C., and compound 3 (490 mg, 1.0 mmol) was slowly added in batch. The ice bath was removed, and the reaction was carried out at room temperature overnight. Then, the reaction solution was injected into ice water (20 mL), and pH of the mixture was adjusted to 5 with a 2 N hydrochloric acid solution. The water phase was separated from the diethyl ether phase, cooled down, adjusted to have a pH of 11~12 with a concentrated ammonia solution, and then extracted with chloroform (20 mL $\times$ 3). The obtained organic phase was dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The obtained oil was recrystallized in diethyl ether to give compound 31 (220 mg, 43%).

[0166] Compound 31, $C_{24}H_{27}NO_4$, MW: 393, a yellow powder crystal, easily dissolved in chloroform and acetone.

[0167] ^1H NMR (300 MHz, $CDCl_3$): δ 7.07 (1H, s, H-1), 6.75 (2H, m, H-11 and 12), 6.51 (1H, s, H-4), 5.98 (2H, s, $-OCH_2O-$), 3.85 (6H, s, $OMex \times 2$), 3.32 (2H, m, H-6), 3.06 (1H, t, $J=6.0$ Hz, H-8), 2.98 (2H, m, H-3'), 2.81 (2H, m, H-5), 1.72 (2H, m, H-1'), 1.33 (3H, t, $J=6.0$ Hz, H-4'), 0.94 (3H, t, $J=6.3$ Hz, H-2').

[0168] Preparation of Compound 32

[0169] Compound 19 (337 mg, 1.0 mmol) and 1,3,5-trihydroxybenzene (504 mg, 4.0 mmol) were dissolved in 10 mL of 60% sulphuric acid solution (volume ratio). The obtained

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solution was then heated to 80° C., and reacted under refluxing for 1 hour. The reaction mixture was cooled down and extracted with ethyl acetate. The obtained organic phase was distilled to dryness using a rotary evaporator, and the residue was purified through a silica gel column (CHCl₃:MeOH=10:1) to obtain compound 32 (108 mg, 33%).

[0170] Compound 32, C₁₉H₁₉NO₄, MW: 325, a brown powder, easily dissolved in chloroform and methanol.

[0171] ¹H NMR (300 MHz, CDCl₃): δ 6.87 (1H, s, H-1), 6.52 (1H, s, H-4), 6.75 (1H, d, J=8.1 Hz, H-11), 6.47 (1H, d, J=8.1 Hz, H-12), 5.88 (1H, s, H-13), 4.27 (2H, s, H-8), 3.76 (6H, s, —OCH₃), 3.01 (2H, t, J=6.3 Hz, H-6), 2.54 (2H, t, J=6.3 Hz, H-5).

[0172] Preparation of Compound 33

[0173] Compound 14 (321 mg, 1.0 mmol) and 1,3,5-trihydroxybenzene (504 mg, 4.0 mmol) were dissolved in 10 mL of 60% sulphuric acid solution (volume ratio). The obtained mixture was heated to 80° C., and reacted under refluxing for 1 hour. The reaction mixture was cooled down and extracted with ethyl acetate. The obtained organic phase was distilled to dryness using a rotary evaporator, and the residue was purified through a silica gel column (CHCl₃:MeOH=10:1) to obtain compound 33 (98 mg, 31%).

[0174] Compound 33, C₁₈H₁₇NO₄, MW: 311, a brown powder, easily dissolved in chloroform and methanol.

[0175] ¹H NMR (300 MHz, CDCl₃): δ 6.92 (1H, s, H-1), 6.57 (1H, s, H-4), 6.70 (1H, d, J=8.0 Hz, H-11), 6.41 (1H, d, J=8.0 Hz, H-12), 5.83 (1H, s, H-13), 4.33 (2H, s, H-8), 3.87 (3H, s, —OCH₃), 3.11 (2H, t, J=6.3 Hz, H-6), 2.33 (2H, t, J=6.3 Hz, H-5).

[0176] Preparation of Compound 34

[0177] Compound 29 (351 mg, 1.0 mmol) and 1,3,5-trihydroxybenzene (504 mg, 4.0 mmol) were dissolved in 10 mL of 60% sulphuric acid solution (volume ratio). The obtained mixture was heated to 80° C., and reacted under refluxing for 1 hour. The reaction mixture was then cooled down and extracted with ethyl acetate. The obtained organic phase was distilled to dryness using a rotary evaporator, and the residue was purified through a silica gel column (CHCl₃:MeOH=10:1) to obtain compound 34 (103 mg, 32%).

[0178] Compound 34, C₂₀H₂₁NO₄, MW: 339, a brown powder, easily dissolved in chloroform and methanol.

[0179] ¹H NMR (300 MHz, CDCl₃): δ 6.78 (1H, s, H-1), 6.47 (1H, s, H-4), 6.31 (1H, d, J=8.0 Hz, H-11), 6.23 (1H, d, J=8.0 Hz, H-12), 4.37 (2H, s, H-8), 3.73 (6H, s, —OCH₃), 3.11 (2H, t, J=6.3 Hz, H-6), 2.43 (2H, t, J=6.3 Hz, H-5), 1.79 (3H, s, —CH₃).

[0180] Preparation of Compound 35

[0181] Compound 27 (351 mg, 1.0 mmol) and 1,3,5-trihydroxybenzene (504 mg, 4.0 mmol) were dissolved in 10 mL of 60% sulphuric acid solution (volume ratio). The obtained mixture was heated to 80° C., and reacted under refluxing for 1 hour. The reaction mixture was then cooled down and extracted with ethyl acetate. The obtained organic phase was distilled to dryness using a rotary evaporator, and the residue was purified through a silica gel column (CHCl₃:MeOH=10:1) to obtain compound 35 (107 mg, 33%).

[0182] Compound 35, C₂₀H₂₁NO₄, MW: 339, a brown yellow powder, easily dissolved in chloroform and methanol.

[0183] ¹H NMR (300 MHz, CDCl₃): δ 6.77 (1H, s, H-1), 6.45 (1H, s, H-4), 6.29 (1H, d, J=8.0 Hz, H-11), 6.21 (1H, d, J=8.0 Hz, H-12), 4.51 (2H, s, H-8), 3.71 (6H, s, —OCH₃), 3.13 (2H, t, J=6.3 Hz, H-6), 2.41 (2H, t, J=6.3 Hz, H-5), 1.34 (3H, s, —CH₃).

[0184] Preparation of Compound 36

[0185] Compound 30 (365 mg, 1.0 mmol) and 1,3,5-trihydroxybenzene (504 mg, 4.0 mmol) were dissolved in 10 mL of 60% sulphuric acid solution (volume ratio). The obtained mixture was heated to 80° C., and reacted under refluxing for 1 hour. The reaction mixture was then cooled down and extracted with ethyl acetate. The obtained organic phase was distilled to dryness using a rotary evaporator, and the residue was purified through a silica gel column (CHCl₃:MeOH=10:1) to obtain compound 36 (123 mg, 37%).

[0186] Compound 36, C₂₁H₂₃NO₄, MW: 353, a brown yellow powder, easily dissolved in chloroform and methanol.

[0187] ¹H NMR (300 MHz, CDCl₃): δ 6.81 (1H, s, H-1), 6.55 (1H, s, H-4), 6.32 (1H, d, J=8.0 Hz, H-11), 6.22 (1H, d, J=8.0 Hz, H-12), 4.43 (2H, s, H-8), 3.70 (6H, s, —OCH₃), 3.34 (2H, t, J=6.3 Hz, H-6), 2.58 (2H, t, J=6.3 Hz, H-5), 1.79 (3H, s, —CH₃), 1.35 (3H, s, —CH₃).

[0188] Preparation of Compound 37

[0189] Compound 32 (325 mg) was dissolved in a mixture solution of 1 mL of pyridine and 1 mL of acetic anhydride. The reaction was carried out under agitation at room temperature overnight. The reaction mixture was then evaporated to remove the solvent under reduced pressure. The residue was purified through silica gel column chromatography (CHCl₃/CH₃OH=20:1) to obtain compound 37 (132 mg, 37%).

[0190] Compound 37, C₂₃H₂₃NO₆, MW: 409, a brown yellow powder, easily dissolved in chloroform and methanol.

[0191] ¹H NMR (300 MHz, CDCl₃): δ 7.15 (1H, s, H-1), 6.77 (1H, d, J=8.1 Hz, H-11), 6.52 (1H, s, H-4), 6.47 (1H, d, J=8.1 Hz, H-12), 5.93 (1H, s, H-13), 4.42 (2H, s, H-8), 3.84 (6H, s, —OCH₃), 3.13 (2H, t, J=6.3 Hz, H-6), 2.87 (2H, t, J=6.3 Hz, H-5), 2.08 (6H, s, —OCOCH₃).

[0192] Preparation of Compound 38

[0193] 2,3,9,10-tetrahydroxy dihydropalmatine (297 mg) was dissolved in a mixture solution of 1 mL of pyridine and 1 mL of acetic anhydride. The reaction was carried out under agitation at room temperature overnight. The reaction mixture was then neutralized with a saturated sodium bicarbonate solution and extracted with ethyl acetate. The obtained organic phase was evaporated under reduced pressure, and the residue was purified through silica gel column chromatography (CHCl₃/CH₃OH=25:1) to obtain compound 38 (108 mg, 31%).

[0194] Compound 38, C₂₃H₂₃NO₆, MW: 409, a brown yellow powder, easily dissolved in chloroform and methanol.

[0195] ¹H NMR (300 MHz, CDCl₃): δ 7.21 (1H, s, H-1), 6.79 (1H, d, J=8.1 Hz, H-11), 6.55 (1H, s, H-4), 6.47 (1H, d, J=8.1 Hz, H-12), 5.91 (1H, s, H-13), 4.51 (2H, s, H-8), 3.17 (2H, t, J=6.3 Hz, H-6), 2.84 (2H, t, J=6.3 Hz, H-5), 2.08 (6H, s, —OCOCH₃), 2.06 (6H, s, —OCOCH₃).

[0196] Preparation of Compound 39

[0197] Palmatine hydrochloride (390 mg, 1.0 mmol) was dissolved in 10 mL anhydrous tetrahydrofuran followed by adding 190 mg of LiAlH₄ (5.0 mmol). The reaction was carried out under agitation at room temperature for 2 hours. After the reaction finished, the reaction mixture was evaporated to remove the solvent under reduced pressure, and 0.2 mL of water, 0.2 mL of 30% sodium hydroxide solution and another 0.6 mL of water were sequentially added therein. The reaction solution was then filtered, and the filtrate was extracted with ethyl acetate (10 mL×3). The obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified through silica gel column chromatography (CHCl₃/

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CH₃OH=50:1, eluting until no compound was observed in the eluent) to obtain compound 39 (253 mg, 67%).

[0198] Compound 39, C₂₁H₂₃NO₄, MW: 353, a yellow amorphous powder, easily dissolved in chloroform and acetone.

[0199] ¹H NMR (300 MHz, CDCl₃), δ 7.21 (1H, s, H-1), 7.08 (1H, d, J=8.4 Hz, H-12), 6.91 (1H, d, J=8.4 Hz, H-11), 6.67 (1H, s, H-4), 5.76 (1H, s, H-13), 4.33 (2H, s, H-8), 3.90 (6H, s, —OMe x 2), 3.86 (3H, s, —OMe), 3.83 (3H, s, —OMe), 3.18 (2H, t, J=7.5 Hz, H-6), 2.91 (2H, t, J=7.5 Hz, H-5).

[0200] Preparation of Compound 40

[0201] Palmatine hydrochloride (415 mg, 1.0 mmol) was dissolved in 10 mL anhydrous tetrahydrofuran followed by adding 190 mg of LiAlH₄ (5.0 mmol). The reaction was carried out under agitation at room temperature for 2 hours. After the reaction finished, the reaction mixture was evaporated to remove the solvent under reduced pressure, and 0.2 mL of water, 0.2 mL of 30% sodium hydroxide solution and another 0.6 mL of water were sequentially added therein. The reaction solution was then filtered, and the filtrate was extracted with ethyl acetate (10 mL×3). The obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified through silica gel column chromatography (CHCl₃/CH₃OH=50:1, eluting until no compound was observed in the eluent) to obtain compound 40 (223 mg, 61%).

[0202] Compound 40, C₂₃H₂₇NO₄, MW: 381, a yellow amorphous powder, easily dissolved in chloroform and acetone.

[0203] ¹H NMR (300 MHz, CDCl₃), δ 7.17 (1H, s, H-1), 7.03 (1H, d, J=8.4 Hz, H-12), 6.84 (1H, d, J=8.4 Hz, H-11), 6.68 (1H, s, H-4), 4.27 (2H, s, H-8), 3.92 (3H, s, —OMe), 3.91 (3H, s, —OMe), 3.88 (3H, s, —OMe), 3.85 (3H, s, —OMe), 3.08 (2H, m, H-6), 2.81 (4H, m, H-5 and 1'), 1.34 (3H, t, J=7.5 Hz, H-2').

[0204] Preparation of Compound 41

[0205] Coptisine hydrochloride (355 mg, 1.0 mmol) was dissolved in 10 mL anhydrous tetrahydrofuran followed by adding 190 mg of LiAlH₄ (5.0 mmol). The reaction was carried out under agitation at room temperature for 2 hours. After reaction finished, the reaction mixture was evaporated to remove the solvent under reduced pressure, and 0.2 mL of water, 0.2 mL of 30% sodium hydroxide solution and another 0.6 mL of water were sequentially added therein. The reaction solution was then filtered, and the filtrate was extracted with ethyl acetate (10 mL×3). The obtained organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified through silica gel column chromatography (CHCl₃/CH₃OH=50:1, eluting until no compound was observed in the eluent) to obtain compound 41 (223 mg, 61%).

[0206] Compound 41, C₁₉H₁₅NO₄, MW: 321, a yellow amorphous powder, easily dissolved in chloroform and acetone.

[0207] ¹H NMR (300 MHz, CDCl₃), δ 7.13 (1H, s, H-1), 7.02 (1H, d, J=8.4 Hz, H-12), 6.83 (1H, d, J=8.4 Hz, H-11), 6.64 (1H, s, H-4), 5.98 (2H, s, —OCH₂O—), 5.96 (2H, s, —OCH₂O—), 4.23 (2H, s, H-8), 3.11 (2H, t, J=7.5 Hz, H-6), 2.81 (2H, t, J=7.5 Hz, H-5).

Experimental Examples

Experimental Example 1

[0208] The effects of some compounds according to the present invention on glucose absorption were preliminarily evaluated in vitro using the glucose uptake model in L6 muscle cells.

[0209] Experimental Steps:

[0210] The completely differentiated L6 muscle cells which were cultured in 24-well plate were washed with PBS one time, starved in a high glucose DMEM culture medium containing 0.2% of BSA for 2 hours, and incubated in a high glucose DMEM culture medium containing 5 μM of the dihydroberberine derivatives of the present invention and 0.2% of BSA for 2.5 hours. They were then washed twice with a HBS solution containing 5 μM of the dihydroberberine derivatives, and incubated for another 0.5 hour in a HBS solution containing 5 μM of the dihydroberberine derivatives.

[0211] ³H-labeled 2-deoxyglucose (dissolved in HBS or KRP to form a 1 mM, 5 μCi/mL temporary working solution) was added into a HBS solution to obtain a solution with a final concentration of 100 μM and 0.5 μCi/mL of the isotope. The cells were incubated at 37° C. for 10 min. The cell incubation solution was then removed quickly, and the cells were placed on ice, rinsed with ice-cold PBS three times, and dried in an oven at 42° C. 200 μL of 0.1% TritonX-100 was added therein, and the mixture was mildly vibrated at 4° C. for 45 min to lyse the cells. 150 μL of lysate was taken, and 1.1 mL of scintillation fluid was added therein to perform scintillation counting. 10 μL of lysate was diluted by 10 times to measure the protein concentration using Bradford method. Final result is presented with a unit of pmol/min/mg protein.

[0212] Evaluation Standard:

[0213] The compound to be measured was dissolved in DMSO. When the concentration is 5 μM, if the calculated glucose intake in the test using the compound of the present invention is higher than that in DMSO blank control and there is significant difference in statistics between the data of the two groups, it can be concluded that the compound of the present invention can promote glucose absorption.

[0214] Experimental Result:

[0215] FIG. 1 shows the effects of some compounds of the present invention at 5 μM on promoting glucose transport in 5.0 mM glucose medium in glucose intake model in L6 muscle cells, wherein DMSO is blank control, and BBR is berberine.

[0216] It has been found, by screening at cellular level, that several compounds have stronger activity of promoting glucose uptake than berberine. Depending on the difficult level of synthesis, we selected dihydroberberine derivative 19 and its sulfate salt to perform the pharmacodynamical test at the whole animal level.

Experiment Example 2

[0217] The in vivo antidiabetic activity of the compounds according to the present invention is evaluated.

[0218] Experimental Steps:

[0219] Normal male C57BL/6J mice were fed by high-fat diet for 10 weeks to generate obvious symptom of insulin resistance, and their ability of glucose tolerance decreased significantly. Ten mice per group were used to perform the efficacy study of the compounds. Dihydroberberine derivative 19 or its sulfate salt was mixed into the high-fat diet, and administered at a dose of 100 mg/kg/day for 2 weeks. After the mice were starved overnight, basal blood sugar value (0 min) was measured by taking blood from tail vein. Then the mice were injected glucose intraperitoneally at a dose of 2 g/kg according to the body weight of the mice in control group which were fed by normal diet followed by measuring blood sugar value at 15, 30, 45, 60, 90 and 120 min respectively and calculating the area under curve (AUC). The body

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weight and visceral fat of the mice were weighted, and the contents of fatty acid and triglyceride in the blood plasma of the mice were determined.

[0220] Experimental Result:

[0221] In FIGS. 2-4, CH-con means the normal mouse, HF-con refers to the obese mouse, HF-BBR19 is the obese mouse treated with dihydroberberine derivative 19 for 2 weeks, and HF-BBR19Y is the obese mouse treated with the sulfate salt of dihydroberberine derivative 19 for 2 weeks.

[0222] FIG. 2 shows the intraperitoneal glucose tolerance test (ipGTT) curves of the mice and the areas under curve. The mice which had been treated with dihydroberberine derivative 19 or its sulfate salt for 2 weeks and the mice in control group were intraperitoneally injected glucose at 2 g/kg after being starved overnight. The blood glucose value was then measured at 0, 15, 30, 45, 60, 90 and 120 min respectively and the area under curve (AUC) was calculated (*, $P < 0.05$; **, $P < 0.01$). It can be seen from FIG. 2 that the obese mice have a significantly improved glucose tolerance after being treated with dihydroberberine derivative 19 or its sulfate salt for 2 weeks.

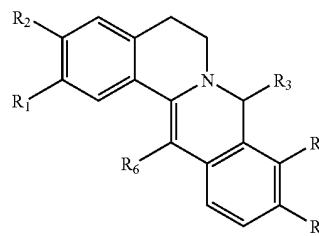
[0223] FIG. 3 shows the increase in body weight of the obese mice and their visceral fat/body weight ratio after treatment with dihydroberberine derivative 19 or its sulfate salt for 2 weeks (*, $P < 0.05$; **, $P < 0.01$). It can be seen from FIG. 3 that the increase in body weight of the mice which had been treated with dihydroberberine derivative 19 or its sulfate salt for 2 weeks is significantly less than that of the mice in the control group, and the ratio of intra-abdominal fat to body weight is lowered significantly comparing with the control group. Such results indicate that the compounds of the present invention have the effects on preventing the increase in body weight of the mice and fat accumulation induced by high-fat diet. Thus they may have a potential effect on treating obesity.

[0224] FIG. 4 shows the changes in the contents of free fatty acids (i.e. non-esterified fatty acid, NEFA) and triglyceride (TG) in the blood plasma of obese mice which have been treated with dihydroberberine derivative 19 or its sulfate salt for 2 weeks (*, $P < 0.05$; **, $P < 0.01$). It can be seen from FIG. 4 that the contents of free fatty acid (NEFA) (HF-Con vs HF-BBR19, 0.76 ± 0.03 mmol/l vs 0.6 ± 0.05 mmol/l) and triglyceride TG (HF-Con vs HF-BBR19, 1.20 ± 0.05 mmol/l vs 0.86 ± 0.08 mmol/l) in blood plasma of obese mice are both lowered significantly after the mice have been treated with dihydroberberine derivative 19 or sulfate thereof for 2 weeks.

EXPERIMENTAL CONCLUSION

[0225] Dihydroberberine derivative 19 and its sulfate salt have significant effects on improving glucose tolerance and insulin resistance, facilitating weight loss, lowering the contents of free fatty acids and triglyceride in blood plasma and relieving fatty liver in the mouse models with insulin resistance and obesity induced by a high-fat diet.

1. A method of treating diabetes mellitus in a patient comprising administering to the patient an effective amount of a 13,13a-dihydroberberine derivative or its physiologically acceptable salt represented by Formula 1:



Wherein,

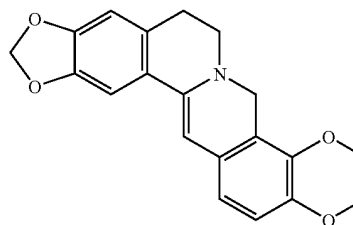
R_1 and R_2 are each independently H, OH, C_1 - C_4 alkoxy or C_1 - C_4 acyloxy, or R_1 and R_2 are combined into $-O-CH_2-O-$;

R_3 is H, OH, C_1 - C_4 alkoxy, C_1 - C_4 acyloxy, C_1 - C_4 alkyl, C_1 - C_4 haloalkyl or an aryl;

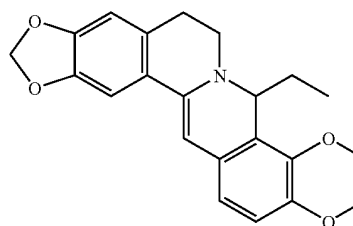
R_4 and R_5 are each independently H, OH, C_1 - C_4 alkoxy or C_1 - C_4 acyloxy, or R_4 and R_5 are combined into $-O-CH_2-O-$; and

R_6 is H, OH, C_1 - C_4 alkoxy, C_1 - C_4 acyloxy, C_1 - C_4 alkyl, C_1 - C_4 haloalkyl or an aryl.

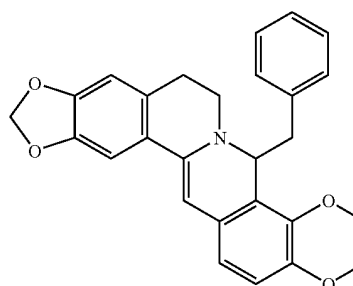
2. The method according to claim 1, wherein the compound of Formula 1 is one selected from the following compounds represented by Formulae 19 to 41:



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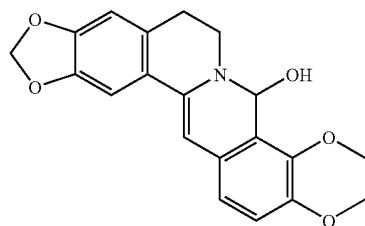
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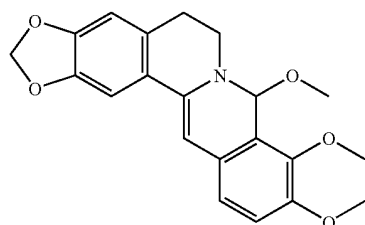
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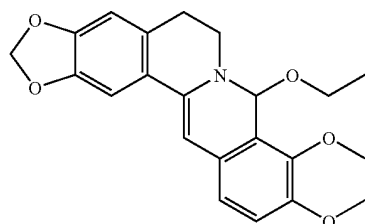
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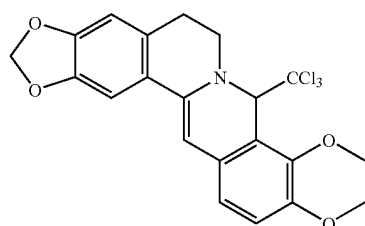
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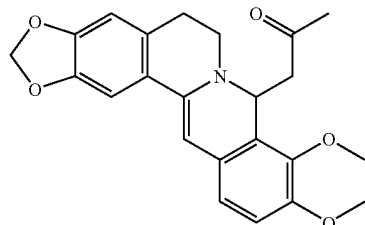
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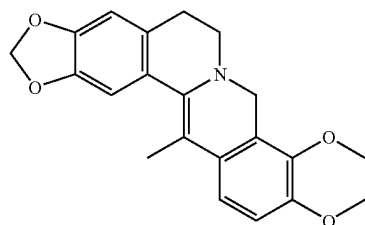
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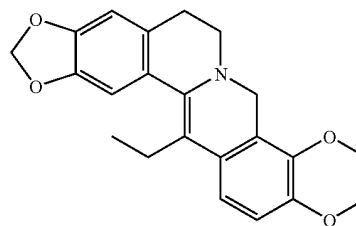


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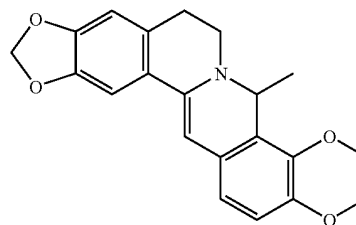


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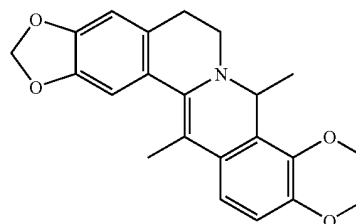
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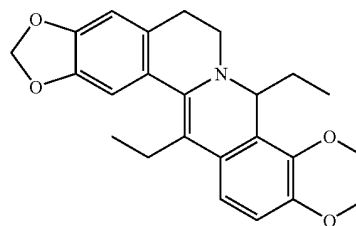
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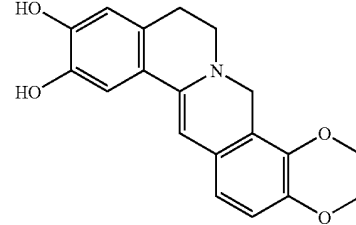
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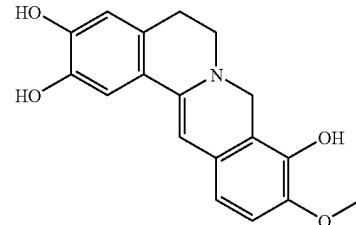
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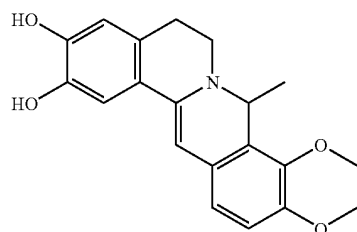
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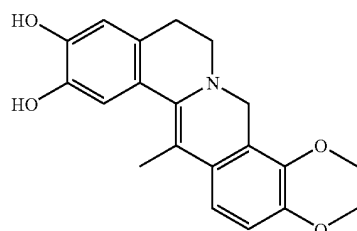
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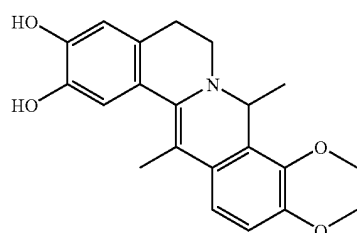
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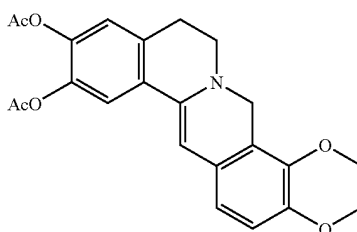
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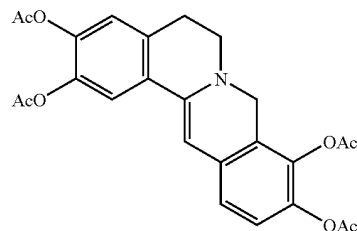
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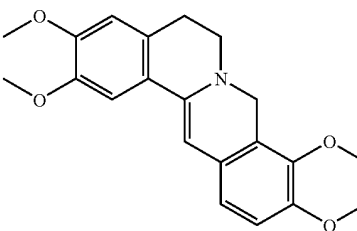
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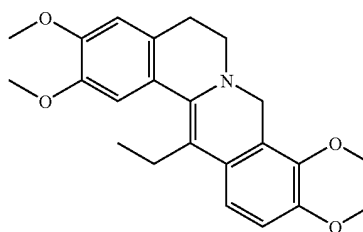


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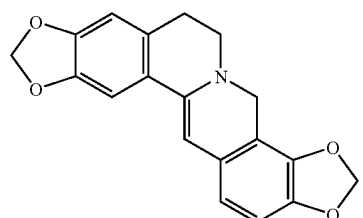


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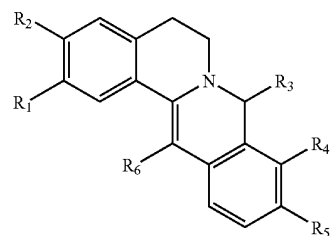


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3. A method of treating a patient suffering from insulin resistance and complications thereof comprising administering to the patient an effective amount of a 13,13a-dihydroberberine derivative or its physiologically acceptable salt represented by Formula 1:



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Wherein,

R₁ and R₂ are each independently H, OH, C₁-C₄ alkoxy or C₁-C₄ acyloxy, or R₁ and R₂ are combined into —O—CH₂—O—;

R₃ is H, OH, C₁-C₄ alkoxy, C₁-C₄ acyloxy, C₁-C₄ alkyl, C₁-C₄ haloalkyl or an aryl;

R₄ and R₅ are each independently H, OH, C₁-C₄ alkoxy or C₁-C₄ acyloxy, or R₄ and R₅ are combined into —O—CH₂—O—; and

R₆ is H, OH, C₁-C₄ alkoxy, C₁-C₄ acyloxy, C₁-C₄ alkyl, C₁-C₄ haloalkyl or an aryl.

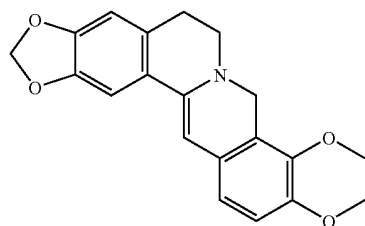
4. The method according to claim 3, wherein the complication includes diabetes mellitus, adiposity, and fatty liver.

5. The method according to claim 3, wherein the compound of Formula 1 is one selected from the following compounds represented by Formulae 19 to 41:

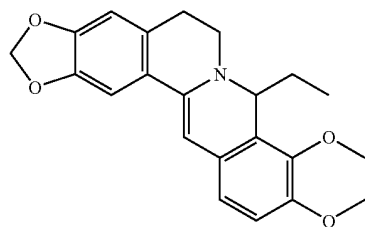
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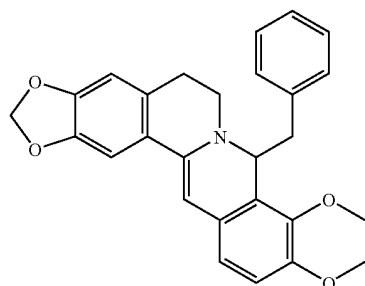
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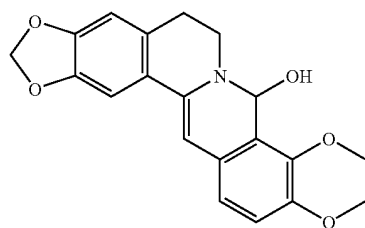
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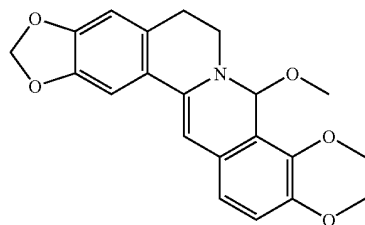
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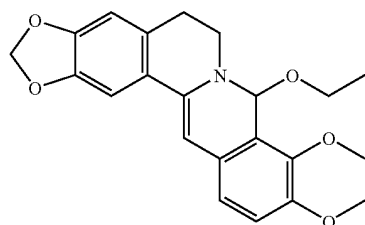
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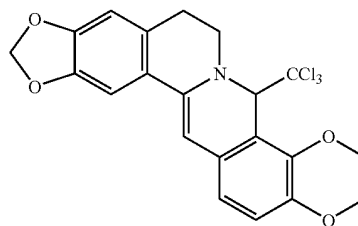


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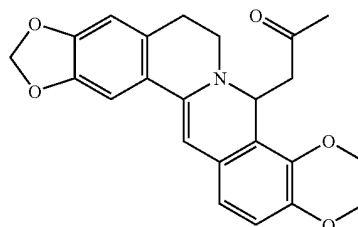


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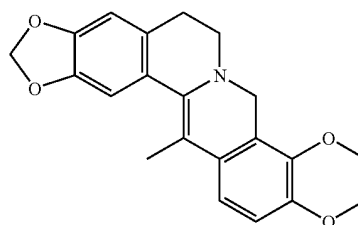
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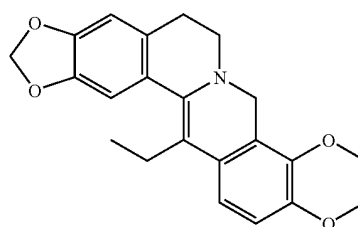
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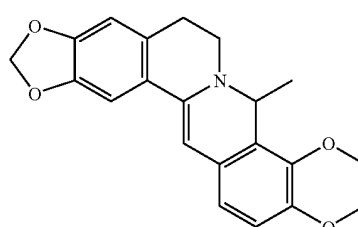
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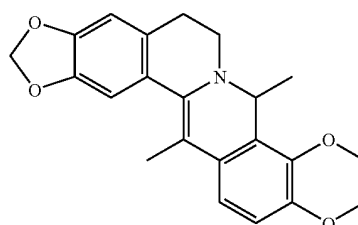
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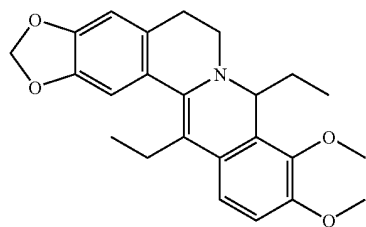
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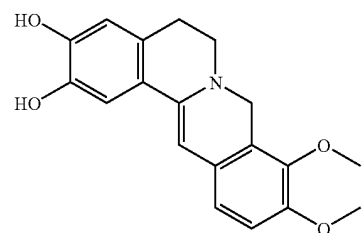
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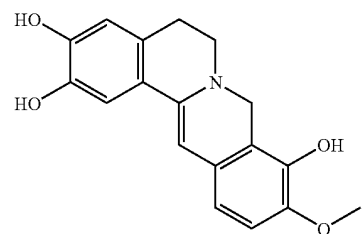
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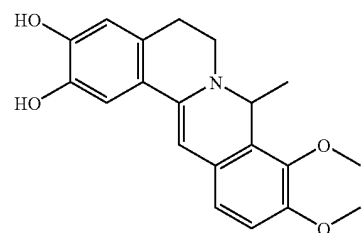
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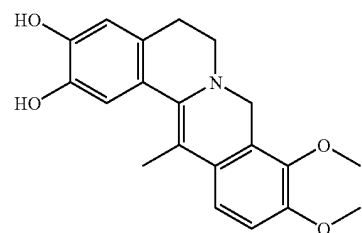
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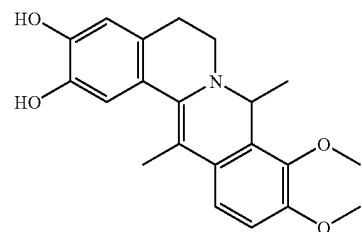
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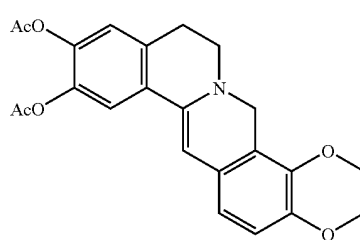


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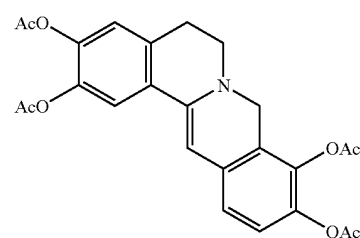


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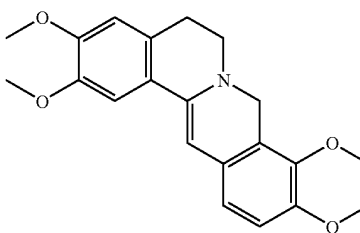
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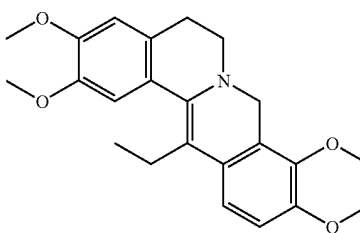
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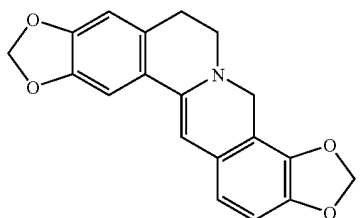
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Glucose Tolerance

B Åhrén, Lund University, Lund, Sweden

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Glossary

Hepatic glucose output A sum of glucose leaving the liver and the net effect of glucose uptake and glucose production.

IFG (impaired fasting glucose) A condition where fasting glucose is elevated above the normal limit but not reaching the threshold for type 2 diabetes.

IGT (impaired glucose tolerance) A condition when 2-h glucose after OGTT is elevated above the normal

limit but does not reaching the threshold for type 2 diabetes.

IVGTT (intravenous glucose tolerance test) Standardized test where glucose is given intravenously and blood samples are taken regularly during the following hour.

OGTT (oral glucose tolerance test) Standardized test where glucose is given orally and blood samples taken at 2 h.

Definition and Impact of Glucose Tolerance

The prevalence of type 2 diabetes is steadily increasing and it has been estimated that the prevalence will further increase during the coming years. It has been assumed that within 25 years, if the trend is not altered, more than 25% of the global adult population older than 65 years of age will be affected by diabetes. The prevalence also shows ethnic differences, with prevalence ranging by a factor of 10 between different populations. The high prevalence of diabetes also implies increasing prevalence of diabetic complications, resulting in high and significant morbidity. Diabetes is also a major risk factor for cardiovascular diseases, which are responsible for the majority of deaths in diabetes. Altogether, this makes diabetes a major burden for global health and health economy.

A most important factor underlying the morbidity in diabetes, its complications and concurrent cardiovascular diseases, is hyperglycemia. Importantly, even at such a low degree as not to reach the limit criteria for diabetes, hyperglycemia is related to morbidity. Lifestyle changes and pharmacological interventions to reduce or even normalize the hyperglycemia exist, and consistent adherence to such regimen will reduce the morbidity. However, hyperglycemia is initially without symptoms and therefore usually remains undetected for a long period of time. Therefore, it is important to have reliable methods for the detection of hyperglycemia in its initial stages for proper actions to be taken. Such detection relies on analysis of the circulating glucose in the fasting state or after a challenge. These detections imply that hyperglycemia is subdivided into two different entities. The first entity is fasting hyperglycemia. This is mainly due to inappropriate release of glucose from the liver, which is caused by excessive glucagon levels in combination with low insulin levels or deficient action of insulin to restrain glucose release from the liver. The second entity of hyperglycemia is postchallenge hyperglycemia, which occurs after meal or glucose ingestion. This is called 'glucose intolerance' and is equivalent to an impairment to dispose glucose after a challenge. Several modes to diagnose glucose intolerance exist but the gold standard for its diagnosis is the oral glucose tolerance test (OGTT). This article describes this test, its advantages and limitations, and its potential role

for early detection of patients with increased risk for developing type 2 diabetes and cardiovascular diseases. The article also summarizes the basic mechanisms determining glucose tolerance as well as epidemiological and clinical aspects of glucose intolerance, including the potential of treating the condition for prevention of diabetes and cardiovascular diseases.

Glucose Tolerance Tests

History and Definition of Oral Glucose Tolerance Tests

A major breakthrough in the understanding of glucose intolerance as a risk factor for development of type 2 diabetes and cardiovascular diseases was the introduction of a worldwide standardization of the OGTT in the 1970s. By this introduction, glucose tolerance became a standardized entity, which enabled studies in metabolism, physiology, and clinical medicine with detection of risk factors as well as progressive follow-up studies using a standard recognized worldwide. At the same time, and also of significant importance for the generation of present-day knowledge within the field, was the introduction of the clinical entity impaired glucose tolerance (IGT), which replaced the then commonly used term 'borderline' diabetes. IGT as an entity was introduced simultaneously with the suggestion that glucose tolerance in a clinical test should be determined following ingestion of 75 g glucose, with a blood sample for the measure of glucose to be taken after 2 h.

The evaluation of the standardized OGTT in the clinical setting thus relies on a single 2-h glucose value. This value during the 75-g OGTT usually displays a normal distribution slightly skewed to the right. **Figure 1** shows the distribution pattern of 2-h glucose levels obtained from 802 Caucasian subjects in Malmö, Sweden. From this distribution, normal values may be defined statistically from mean and variance values for statistical definition of the distribution. The mean value is $\approx 7 \text{ mmol l}^{-1}$ and standard deviation is $\approx 1 \text{ mmol l}^{-1}$. By defining reference values as 95% confidence intervals, the cutoff value for normality would be approximately

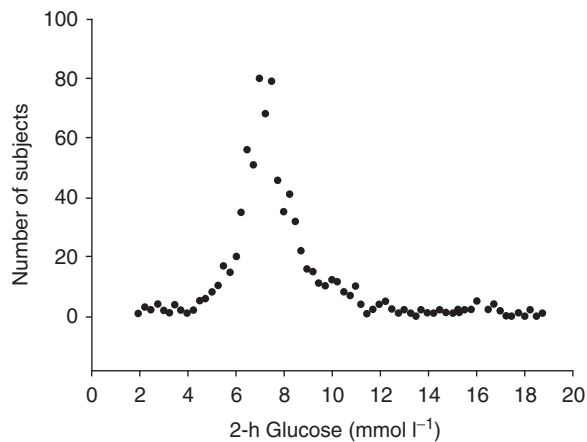


Figure 1 Distribution of the 2-h glucose value in OGTT performed in 802 Caucasian women, all aged 58 years, in Malmö, Sweden.

9 mmol l⁻¹ and, hence, values higher than 9 mmol l⁻¹ would indicate diabetes. By using such a definition of diabetes, a large number of subjects would have the disease, the clinical relevance of which is doubtful. Therefore, the definition of diabetes has instead been based on prospective studies evaluating the risk for microvascular disease and the cutoff-levels have been defined as levels substantially increasing this risk. Therefore, a cutoff value of 11.1 mmol l⁻¹ glucose has been used for the definition of type 2 diabetes.

OGTT was frequently used during the 1980s and 1990s for the clinical diagnosis of type 2 diabetes and in epidemiological studies, which markedly increased our knowledge of these conditions. By the end of the 1990s, however, definitions of IGT and clinical tests to be performed were again discussed. This resulted in revised cutoff levels and the introduction of a new entity called impaired fasting glycemia (IFG), which is defined as high fasting glucose. Current cutoff values for the 2 h glucose after 75 g glucose is 7.8–11.0 mmol l⁻¹ for IGT and ≥ 11.1 mmol l⁻¹ for type 2 diabetes. It was also suggested that fasting glucose was sufficient for the diagnosis of glucose intolerance and type 2 diabetes. The suggestion that a fasting sample is sufficient for the diagnosis of abnormal glycemia has been questioned, however, mainly because studies have shown that such a strategy will reduce the numbers at risk who are diagnosed and detected. This is because a large proportion of subjects with IGT have a normal fasting glucose but an elevated 2-h glucose value. In fact, there are populations with IFG alone, IGT alone, and IFG and IGT together, and these populations may represent different risks for diabetes and cardiovascular diseases. Consequently, those having a high 2-h glucose value but a normal fasting glucose, who also have increased risk for cardiovascular diseases, will be missed by the suggested strategy. A study from Sweden identified this dilemma because it was demonstrated that out of 414 subjects with abnormal fasting or 2-h glucose values during an OGTT, only 140 (34%) had elevation of both values. The largest group comprised subjects with high 2-h glucose values but normal fasting glucose values (i.e., IGT but not IFG), which were seen in 235 subjects (57%), whereas only 39 subjects (9%) had high fasting but normal 2-h glucose values (i.e., true IFG). The individual subgroups were shown to have similar

risk factor patterns in terms of degree of obesity, blood pressure, and lipid levels. Therefore, a proper strategy to detect early cases at risk for diabetes and cardiovascular diseases, involves an OGTT.

Procedures and Evaluation of the Oral Glucose Tolerance Test

Glucose tolerance is defined as the ability to dispose of a glucose load, and therefore glucose intolerance is defined as an impaired ability for glucose disposal. The gold standard technique is to challenge with an oral glucose load, with measurement of circulating glucose before and after the challenge – the OGTT. As routinely performed, this test determines the ability to dispose of glucose after oral administration of 75 g glucose. The test is standardized such that it is performed in the morning after a 12-h overnight fast and blood samples are taken before the glucose load and after 2 h. Furthermore, the diet during the 3 days preceding the test should contain at least 250 g carbohydrates per day and the subjects should rest during the test in a semirecumbent position without smoking. The glucose given should be dissolved in 250–300 ml fluid, and sometimes fruit-flavored water is used to improve the taste. There has been much debate about how to take the blood sample. The original diagnostic criteria used values obtained from plasma derived from blood taken venously in tubes containing additives for prevention of coagulation. However, valid results are also obtained when glucose is measured in whole blood and when capillary samples are taken, although cutoff levels need to be adjusted for the different glucose concentrations in these samples. Arterial samples are also possible but rarely, if ever, used. Sometimes, mainly for research purposes, more frequent samples are taken and the test may last 3 h; however, for clinical purposes, the routine OGTT lasts 2 h, with a sample taken at that time point.

As shown in Figure 2, in a normal person, circulating levels of glucose increase within the first 15 min after the oral ingestion of glucose to reach a peak after 30 min. Thereafter, a progressive decline occurs, with the 2-h value usually approximately 25% higher than the fasting value. Usually, it takes 3 h for a return to baseline glucose levels. In subjects with IGT, there is usually also a peak at 30 min, albeit at a higher level than in normal subjects, but the main difference versus normal subjects is that the glucose disposal is impaired, which results in a higher 2-h glucose value. In diabetics, there is usually not a peak at 30 min but a continuous rise throughout the 2-h study period.

Limitations of the Oral Glucose Tolerance Test

An important limitation of the OGTT is the variability in results when the test is repeated. The coefficient of variance (CV) is $\approx 15\%$ which is higher than that for most other clinical tests. This high variance is not dependent on CV in the measurement of glucose, which is a procedure with very small error and CVs ($< 3\%$). Instead, biological variation explains the high CV in OGTT. Such factors, which are poorly understood, may be preceding diet, exercise, emotions, stress, drugs

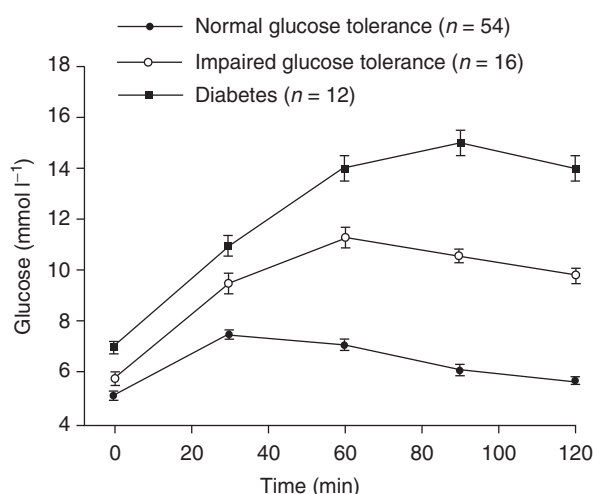


Figure 2 Venous plasma glucose levels during OGTT in subjects with normal or impaired glucose tolerance or type 2 diabetes who are treated with lifestyle modifications. Means $\pm$ SEM are shown.

taken for various diseases, and gender, which are all factors influencing gastric emptying, carbohydrate absorption, islet hormone secretion, hepatic glucose production, and peripheral glucose uptake. Because of the high variability in the 2-h glucose value, a diagnosis of IGT or diabetes, particularly if intervention is planned, should not be based on a single OGTT. Instead, a clinical recommendation is to perform two OGTTs and use the mean of the two 2-h glucose values as the diagnostic value. The time interval between the two OGTTs should not exceed 3 months.

Differential Tests for Glucose Intolerance

As an alternative to OGTT, glucose tolerance may also be determined by administering glucose intravenously. In the intravenous glucose tolerance test (IVGTT), glucose is injected intravenously, usually at a dose of 0.3, 0.5, or 1 g kg⁻¹, and circulating glucose is determined before and 8, 10, 15, 20, 30, 40, 50, 60, and 80 min after injection. Glucose tolerance is estimated from the elimination rate, where a glucose elimination constant (kg) is calculated. The theory behind this is that the glucose elimination after intravenous glucose displays an exponential function (i.e., after logarithmic transformation of the data, the elimination is linear). kg is thus calculated as the slope for the glucose curve following logarithmic transformation of the individual glucose values and is calculated from the formula $kg = (0.693 \times 100)/t_{1/2}$ where $t_{1/2}$ is the half-time of glucose elimination (in minutes). The unit for kg is percentage of glucose decay per minute. Figure 3 shows this variable. Before OGTT was routinely used, IVGTT was undertaken more frequently. Unless very specific questions are asked, it is currently not used in clinical practice because it is more cumbersome to perform and it identifies only some of the metabolic processes underlying glucose tolerance, mainly insulin secretion, insulin sensitivity, and glucose uptake. Thus, other important aspects, such as glucagon secretion, release of incretin hormones, and hepatic glucose output, which are involved in the overall glucose tolerance and included in the

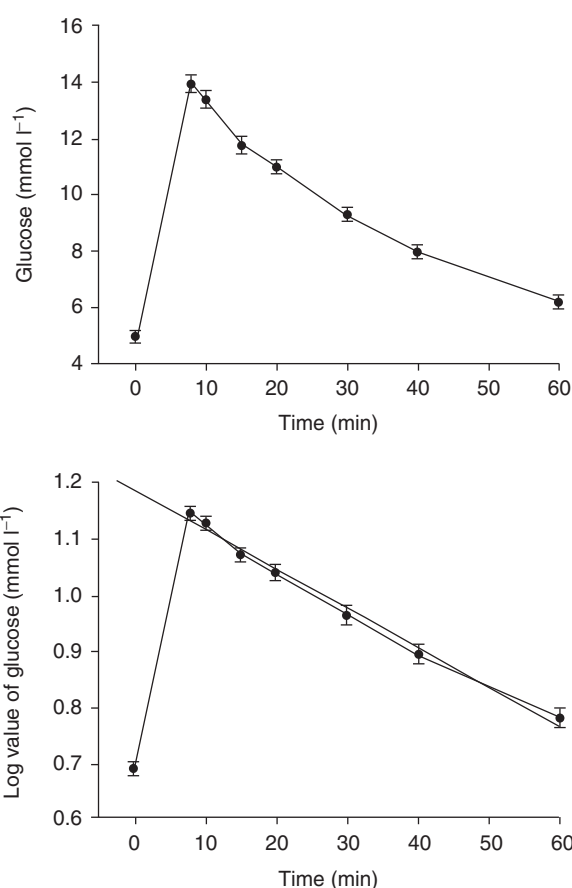


Figure 3 Glucose levels during IVGTT in 41 healthy subjects with normal glucose tolerance. Glucose (0.3 g kg⁻¹) was injected intravenously at time 0. Linear regression curve for the logarithmic values from minutes 8 to 60 is shown; the kg value in these subjects is $1.61 \pm 0.08\% \text{ min}^{-1}$. Means $\pm$ SEM are shown.

2-h glucose value after OGTT, contribute only marginally to the kg after IVGTT.

It has also been suggested that measurement of HbA1c (i.e., the fraction of glycosylated hemoglobin) may be used for the diagnosis of type 2 diabetes. The rationale is that hemoglobin is irreversibly glycosylated in proportion to the glucose level, and therefore the level of HbA1c should reflect the mean of the glucose concentrations during the preceding 2 or 3 months. However, although this theoretical assumption is true, replacing diagnostic tools based on glucose with those based on HbA1c needs further analysis.

Metabolic Basis for Oral Glucose Tolerance

Oral ingestion of glucose initiates a series of metabolic perturbations, which comprise the 2-h glucose value. These metabolic perturbations are complex and involve glucose entering the bloodstream, changes in neural activity and incretin and islet hormone secretion, suppression of hepatic glucose production, and stimulation of peripheral glucose uptake. From a quantitative standpoint, of most importance with regard to the 2-h value are the changes in islet hormone secretion, which

include stimulation of insulin secretion and inhibition of glucagon secretion, and the suppression of hepatic glucose production. In fact, there is an inverse linear relation between the inhibition of hepatic glucose production and the 2-h glucose value and, similarly, a linear inverse relation between stimulation of the early (first 30 min) insulin secretion and 2-h glucose. A first series of events in the OGTT is initiated during the anticipation of the oral glucose ingestion, through olfactory stimuli and through receptors located in the oral cavity. This response is called the cephalic phase and activates sensory nerves. These give input to the central nervous system. This information is integrated in the hypothalamus for initiation and adjustment of a vagal nerve response to release insulin from the pancreatic islets. Therefore, when analyzed in detail, there is an increase in circulating insulin after glucose or meal ingestion already before glucose levels become elevated.

After passage of glucose through the oral cavity, glucose passes to the stomach and through a regulated mechanism is delivered into the gut. Because glucose is a monosaccharide, it is readily absorbed in the small intestine and reaches the splanchnic venous drainage. Glucose then passes to the portal vein and the liver. In the portal vein, glucose activates glucosensitive receptors, which through afferent sensory nerves send signals centrally to the brain for further integration with the previous signals in the hypothalamus for adjustment of efferent nerve activity. Furthermore, glucose in the liver inhibits hepatic glucose production, which is high after the overnight fast. Then, glucose passes to the general circulation to reach the pancreatic islets and the peripheral cells.

The glucose load to the gut also stimulates the release of the intestinal incretin hormones, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagonlike peptide-1 (GLP-1). These hormones both activate local sensory nerves and pass through the circulation to reach the pancreas, where they stimulate insulin secretion and, in the case of GLP-1, inhibit glucagon secretion. In the pancreatic islets, vagal activation, intestinal hormones, and glucose stimulate insulin secretion, and glucose, GLP-1, and insulin inhibit glucagon secretion. These islet responses are of major importance for a normal glucose tolerance, and defects in these islet responses are major determinants of IGT and type 2 diabetes. Following passage of insulin into the venous drainage of the pancreas, the islet hormones reach the portal vein and the liver, and a main function of insulin is to potently suppress hepatic glucose production.

The suppression of hepatic glucose production by insulin is a major process with regard to the degree of hyperglycemia during the test; in subjects with inappropriately high hepatic glucose production, the glucose level after oral glucose is high. This explained why subjects with inappropriate insulin secretion have prandial hyperglycemia. This suppression of hepatic glucose production is augmented by the reduction in circulating levels of glucagon, which is initiated by direct and indirect actions of glucose and GLP-1 on the glucagon-producing cells and also by the action of insulin to inhibit glucagon secretion. After the liver, glucose and insulin reach the peripheral circulation and peripheral cells, where glucose is transported across the cell membranes and therefore leaves the circulation. In most cells, the insulin sensitivity of the cell is of major importance for the delivery rate of glucose. However, insulin-independent mechanisms also exist, even in tissues, which are also insulin

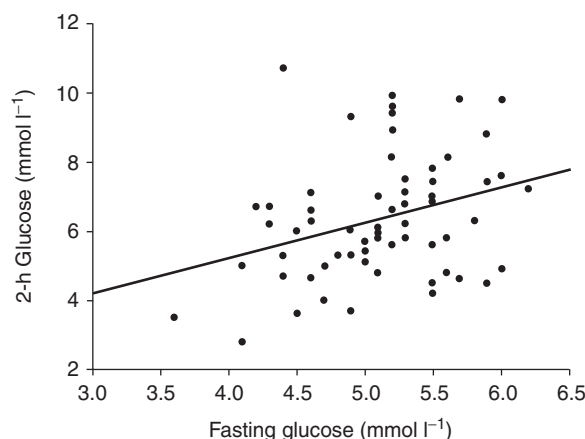


Figure 4 Correlation between fasting glucose and 2-h glucose during an OGTT in nondiabetic subjects ($r=0.32$; $P=0.008$).

sensitive, and glucose uptake is thus also dependent on glucose. Of most importance for glucose disposal after oral glucose is the muscle cells, which have a high capacity for glucose uptake. From all these processes, the glucose level at 2 h can be determined.

The metabolic processes underlying glucose tolerance are different from those underlying the fasting glucose value. Fasting glucose is mainly determined by hepatic glucose delivery, which in turn is governed by the ability to maintain normal basal insulin and glucagon levels. Therefore, mechanisms underlying IFG include defective insulin secretion, defective suppression of glucagon secretion, defective sensitivity in the liver for the action of insulin, and defective peripheral glucose disposal at low glucose levels, which is a sign of insulin resistance. Although mechanisms underlying fasting and 2-h glucose values differ, there is a high correlation between fasting and 2-h glucose values in normal subjects, as shown in **Figure 4**. Nevertheless, there is a limited overlap between IGT and IFG in a population; in fact, most subjects with IGT have normal fasting glucose, and most subjects with IFG have a normal 2-h glucose value. This suggests that different pathophysiological processes underlie IGT and IFG, which in turn suggests that OGTT should be undertaken more frequently than performed today.

Clinical Aspects of IGT

Epidemiology of IGT

After the introduction of the OGTT procedure in clinical practice and as a research tool in the 1980s, several studies on the prevalence of IGT and type 2 diabetes were performed in different populations. It became apparent that the prevalence of these conditions varied markedly among different populations. Studies during the past 15 years have further increased our knowledge because they have included additional populations and demonstrated that the prevalence of IGT and type 2 diabetes is steadily increasing over time. Also, an ethnic difference seems to exist, with extremely high rates in some Pacific island and North American Indian populations and

a low prevalence in South American Indian and Bantu populations.

Clinical Consequences of IGT

IGT is an important risk factor for development of type 2 diabetes. In general, the risk of transition of IGT into type 2 diabetes ranges from 1–2% to 5% and as high as 15–20% per year. The risk is higher for those older than 50 years of age. There is also evidence that hyperglycemia, even at levels not reaching the threshold for type 2 diabetes, is associated with a substantial risk for the development of cardiovascular diseases. One explanation for this is that glucose initiates metabolic perturbations of importance for developing angiopathy, such as tissue peroxidation, production of plasminogen activation inhibition-1, and impairment of endothelial function, such as nitric oxide production. Another explanation is that hyperglycemia is associated with a number of risk factors for cardiovascular diseases, such as high blood pressure, hyperinsulinemia, dyslipidemia, and microalbuminuria. In fact, if hyperglycemia is present, the risk for developing cardiovascular diseases for each of the other risk factors is augmented. Attempts to define cutoff values of glucose for cardiovascular risks have been problematic, however, probably due to the fact that the risk is continuously increased across the glucose ranges. Hence, the use of defined cutoff values is more a convenient practical issue, which is important in a clinical setting, but offers limitations from a theoretical standpoint. Furthermore, prospective studies have shown that an individual with IGT has an increased risk not only for type 2 diabetes but also for cardiovascular diseases and hence mortality. This indicates that attempts should be made to prevent IGT from progressing to cardiovascular diseases and type 2 diabetes.

Treatment of IGT

During recent years, the issue of whether IGT may be treated to prevent progression of type 2 diabetes or cardiovascular diseases has gained considerable interest. On the one hand, it has been argued that it is important to prevent progression of IGT. On the other hand, it has been argued that treating such a large population group as those with IGT would be risky. More fundamentally, the optimal preventive intervention for IGT is not known. The intervention may include lifestyle changes, notably increased physical activity and dietary regulations. Such interventions have been shown to be efficient in highly motivated populations and study centers. However, whether generalization of these results to the general population is possible is not known. Another mode of intervention is pharmacological treatment using compounds to stimulate insulin secretion and inhibit glucagon secretion, suppress hepatic glucose production, or enhance insulin sensitivity. These two strategies are not mutually exclusive, however, and introducing pharmacological intervention without giving lifestyle advice is not appropriate in a clinical setting.

Data from large population studies on the prevention of progression of IGT have been emerged. Two studies, the Finnish diabetes prevention study and the Diabetes Prevention Program, have shown that lifestyle changes (i.e., individualized diet and exercise counseling) in subjects with IGT reduced the incidence of diabetes by more than 50%. In

addition, in the Diabetes Prevention Program, it was shown that metformin (which reduces glucose output from the liver) reduces the risk by approximately 30%. This suggests that pharmacological treatment of IGT prevents development of type 2 diabetes. Several large studies are ongoing and results are expected within a few years.

Whether interventional programs on IGT are valid also for the prevention of cardiovascular diseases is not clearly established, mainly because long-term studies have not been performed. The Study to Prevent Non-insulin-dependent Diabetes Mellitus (STOP-NIDDM), however, showed that acarbose, which reduces glucose absorption from the gut, reduced cardiovascular events by more than 30% during a 3-year study period. This suggests that cardiovascular diseases may be prevented by treating IGT. It should be noted, however, that for prevention of cardiovascular diseases and mortality, more studies and longer follow-up periods are required.

Conclusion

Glucose intolerance increases the risk of developing type 2 diabetes and cardiovascular diseases, and it could be diagnosed with an OGTT. Lifestyle changes reduce the risk for progression of the condition.

See also: Diabetes Mellitus: Classification and Chemical Pathology; Etiology and Epidemiology. Glucose: Metabolism and Maintenance of Blood Glucose Level

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